NOTES

The Solubility of Ethyl Acetate in Water

By A. P. Altshuller[1] and H. E. Everson

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In view of some uncertainties in the literature[2-6] it was found necessary to redetermine the solubility of ethyl acetate at various temperatures for an investigation which was in progress.

The experimental results are given in Table I where t is the turbidity temperature in °C. and s is the solubility in grams of ethyl acetate per 100 g. of water.

Table I

t	s	t	s	t	s
19.2	8.34	25.9	7.93	31.2	7.60
20.4	8.31	26.0	7.93	31.8	7.58
20.5	8.29	26.3	7.94	31.9	7.58
21.3	8.26	27.0	7.89	31.9	7.57
22.0	8.22	28.0	7.80	32.0	7.51
22.6	8.14	28.1	7.81	32.8	7.49
22.7	8.12	28.4	7.82	33.0	7.46
23.0	8.11	28.7	7.81	33.8	7.40
23.4	8.10	29.7	7.71	34.0	7.38
24.7	8.05	29.9	7.71	36.8	7.32
25.1	8.03	30.0	7.72	37.8	7.25
25.4	8.02	30.0	7.69	39.9	7.15
25.4	8.03	30.1	7.69	39.9	7.15
25.4	8.00				

By the application of the method of least squares the following equation is calculated

$$s = (9.552 \pm 0.018) - (0.0618 \pm 0.0006)t$$

The results of this investigation are compared with those previously given in the literature in Table II.

Table II

Comparison of the Solubility Results for Ethyl Acetate in Water

Investigator	20°	25°	30°	35°	40°	45°	50°
This invest.	8.32	8.01	7.70	7.39	7.08	..	..
Schles. and Kub.[3]	8.42	8.03	7.69	7.41	7.18	7.00	6.88
Merriman[4]	8.53	8.08	7.70	7.38	7.10	..	..
Seidel[5]	9.02	8.58	8.24	7.98	7.72	7.53	7.31
Gl. and P.[6]	..	7.39	..	..	..	..	6.04
B. and Gl.[7]	8.40	..	..	..	6.97	..	..

While the earlier results of Glasstone and Pound[5] are far lower than the other results, the more recent values of Beech and Glasstone,[6] determined by a different method, are in better agreement although the value given at 40° is still somewhat low. The high results of Seidel[4] may be caused by appreciable amounts of water and alcohol being present in the ethyl acetate thus increasing the solubility above the correct value.

Consideration of the data above leads to the following average values for the solubility of ethyl acetate (in g. of ethyl acetate per 100 g. of water) as reliable: 20°, 8.42; 25°, 8.04; 30°, 7.70; 35°, 7.39; 40°, 7.12.

Experimental

The solubilities were determined by the synthetic, turbidimetric method.[7] The solubilities of ethyl acetate agree within ±0.1° when determined by heating and then cooling. The appearance of turbidity may be detected within about 0.2° range usually. Temperatures were determined with a $^1/_{20}$° thermometer which gave agreement within $^1/_{100}$° with the sodium sulfate transition point. Each solubility was redetermined two or three times.

The ethyl acetate employed was Mallinckrodt analytical reagent grade ethyl acetate. The percentage of water as determined by Karl Fischer reagent[8] was 0.06%. The ethyl acetate was redistilled from a column of 24 theoretical plates. No change in refractive index ($n^{24.3}$D 1.3697) was found.

(1) National Advisory Commitee for Aeronautics, Lewis Flight Propulsion Laboratory, Cleveland, Ohio.

(2) N. Schlesinger and W. Kubasowa, *Z. physik. Chem.*, **142**, 25 (1929).

(3) R. W. Merriman, *J. Chem. Soc.*, **103**, 1774 (1913).

(4) Landolt-Börnstein, "Physikalisch-chemische Tabellen," 5 Aufl., Bd. I, s. 752 (1923).

(5) S. Glasstone and A. Pound, *J. Chem. Soc.*, **127**, 2660 (1925).

(6) D. Beech and S. Glasstone, *ibid.*, 67 (1938).

(7) A. Weissburger, "Physical Methods of Organic Chemistry," Vol. I, Part I, Interscience Publishers, Inc., New York, N. Y., 1939, p. 319.

(8) K. Fischer, *Angew. Chem.*, **48**, 394 (1935).

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Determination of the Terminal Carboxyl Residues of Peptides and of Proteins[1]

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Recently, Fromageot, *et al.*,[3] and Chibnall and Rees[4] have described the reduction of the terminal carboxyl groups of insulin with subsequent identification of the resulting amino alcohols generated by the hydrolysis of the reduced protein. The present note reports a modification similar to that which Waley and Watson[5] have applied to insulin, of the method originally presented by Schlack and Kumpf[6] for the identification of the terminal carboxyl residues of peptides.

In outline, this method involves the creation of the thiohydantoin on the carboxyl end of the peptide chain in an acid medium, the amino end being blocked by acetylation. The thiohydantoin is then hydrolyzed from the peptide in an acid medium and isolated. The purified thiohydantoin is then hydrolyzed in an alkaline medium, thus producing the corresponding amino acid which is then identified and estimated.

(1) From a thesis submitted by V. H. Baptist to the Graduate School of Northwestern University in partial fulfillment of the requirements for the degree of Doctor of Philosophy, June, 1952.

(2) Biochemistry Department, State University of Iowa, Iowa City, Iowa.

(3) C. Fromageot, M. Jutisz, D. Meyer and L. Penasse, *Biochim. Biophys. Acta*, **6**, 283 (1950).

(4) A. C. Chibnall and M. W. Rees, *Biochem. J.*, **48**, xlvii (1951).

(5) S. G. Waley and J. Watson, *J. Chem. Soc.*, 2394 (1951).

(6) P. Schlack and W. Kumpf, *Z. physiol. Chem.*, **154**, 125 (1926).

Experimental

The general procedure for the production of the thiohydantoins and the final identification of the amino acids was as follows: Approximately 0.1 millimole of the synthetic peptide was treated with 1 to 1.5 millimoles of anhydrous, powdered ammonium thiocyanate, dissolved in 2 ml. of solvent consisting of 9 parts of acetic anhydride and 1 part of glacial acetic acid by volume and the reaction mixture maintained between 40 and 45° for 4 hours with stirring. Two ml. of 20% hydrochloric acid was then added dropwise with good agitation. After the hydrochloric acid had been added, the mixture was heated on a steam-bath for one hour and then taken to dryness under vacuum. Water was added and the mixture again taken to dryness. The dry mixture was dissolved in 10 ml. of 0.25 molar phosphate buffer at *p*H 6.5 and the solution extracted three times each with 10 ml. of ethyl acetate. The ethyl acetate extract was taken to dryness and the dry mixture hydrolyzed with 2.5 ml. of 1.25 *N* barium hydroxide at 140° in a sealed tube for 5 hours. The cooled hydrolysate was neutralized with carbon dioxide and the resulting solution heated for 10 minutes on a steam-bath to destroy the carbamic acids which may have formed. The resulting solution was made up to an appropriate volume and a quantitative estimate of the amino acids present made with filter paper chromatography as described by Bull, Hahn and Baptist.[7]

It was necessary to check the effectiveness of the various steps in the above procedure. For example, we must know the extent of hydrolysis of the thiohydantoin by the hydrochloric acid used to split the thiohydantoin from the peptide. To this end, 15 mg. of 5-methyl-2-thiohydantoin was hydrolyzed at 100° in a sealed tube with 2 ml. of 10% hydrochloric acid for different lengths of time. Even after 5 hours hydrolysis, the extent of conversion of this thiohydantoin to alanine amounted to less than 2%.

It was also necessary to test the completeness of the extraction of the thiohydantoin from the phosphate buffer with ethyl acetate. Thiohydantoins of alanine, leucine, methionine, phenylalanine, glycine, tyrosine and valine were prepared by the method of Johnson and Nicolet.[8] The partition coefficient between ethyl acetate and phosphate buffer ranged from 0.49 for 2-thiohydantoin to 1.0 for the thiohydantoin of phenylalanine.

It was found that lysine, arginine, alanine, leucine, cysteine, serine, glycine, methionine, phenylalanine, aspartic acid, glutamic acid, threonine, tyrosine and valine were not extracted in any detectable amounts from phosphate buffer by ethyl acetate.

TABLE I

OVER-ALL RECOVERIES OF AMINO ACIDS FROM PURE AMINO ACIDS AND FROM SYNTHETIC PEPTIDES

Starting material	Amino acid recovered	Recovery, %
Alanine	Alanine	63
Serine	Serine	12[a]
Threonine	Threonine	16[b]
Methionine	Methionine	48
Lysine	Lysine	0
Arginine	Arginine	0
Glutamine	Glutamic	0
Glutathione	Glutamic	0
Glutathione	Glycine	7
Leucylglycylglycine	Glycine	28
Glycylglycylalanine	Alanine	50
Glycyltyrosine	Tyrosine	33
Glycylleucine	Leucine	57
Dicarbobenzoxycystinylvaline	Valine	41
Glycylvaline	Valine	61
Glycylphenylalanine	Phenylalanine	39
Diphenylacetyllysine	Lysine	54
Benzoxyarginine	Arginine	>1

[a] Recovered as alanine. [b] Recovered as glycine (8%) and as α-amino-*n*-butyric acid (8%).

(7) H. B. Bull, J. W. Hahn and V. H. Baptist, THIS JOURNAL, **71**, 550 (1949).

(8) T. B. Johnson and B. H. Nicolet, *ibid.*, **33**, 1973 (1911).

Finally, the hydrolysis of 5-methyl-2-thiohydantoin with 1.25 *N* barium hydroxide at 140° for 5 hours produced a 75% conversion of this thiohydantoin to alanine indicating the effectiveness of the hydrolysis of the thiohydantoins by barium hydroxide.

The over-all recoveries of the terminal carboxyl residues as amino acids from synthetic peptides and from pure amino acids as starting materials are shown in Table I. In no case were other than carboxyl terminal amino acids from peptides detected.

The above method for the terminal carboxyl residues was applied to three purified proteins. 0.126 g. of crystalline pork insulin (Armour) was dissolved in 10 ml. of acetic anhydride–acetic acid–ammonium thiocyanate mixture and the reaction allowed to proceed overnight at room temperature and the reaction mixture treated as described above for synthetic peptides. 0.134 g. of beef insulin (Armour) was treated in the same way as for pork insulin. One gram of lyophilized crystalline egg albumin reacted with 0.3 g. of ammonium thiocyanate in 77 ml. of the acetic acid–acetic anhydride solvent. The amounts of the recovered amino acids were multiplied by recovery factors. The recovery factors were arrived at by adding a known amount of a given amino acid to a second sample of the protein previous to treatment and determining the percentage of the added amino acid recovered. The recovery factors together with the corrected amounts of the amino acids as terminal α-carboxyl residues are shown in Table II.

TABLE II

CORRECTED MICROMOLES OF AMINO ACIDS AS TERMINAL CARBOXYL GROUPS PER GRAM OF PROTEIN

Amino acid	Correction factor	Pork insulin	Beef insulin	Egg albumin
Alanine	2.7	77	59	..
Tyrosine	7.3	129	118	..
Phenylalanine	2.2	149	258	..
Alanine	5.0	...	...	17.7
Valine	3.6	...	...	21.4
Leucine	3.8	...	...	65.4

Discussion

As can be seen from Table I, the thiohydantoin method for the detection and estimation of the free α-carboxyl residues of peptides and of proteins is not a complete method. The thiohydantoin method fails to detect free α-carboxyl residues of glutamic acid, of aspartic acid, of lysine and of arginine. Although a 54% recovery of lysine was obtained using diphenylacetyllysine, it is believed that this high recovery resulted from the incomplete hydrolysis of the diphenylacetyllysine to lysine and thus the diphenylacetyl or monophenylacetyl lysine was extracted and subsequently hydrolyzed to lysine in the last step. The method as employed also leads to another ambiguity, because as pointed out by Bremner[9] and confirmed by us, serine is converted to alanine and threonine into glycine and into α-amino-*n*-butyric acid during alkaline hydrolysis. The destruction of threonine is not so serious, because α-amino-*n*-butyric acid does not ordinarily occur in proteins and, accordingly, this amino acid can be identified and assumed to arise from threonine.

It will be noted that the results shown in Table II are not in complete agreement with those of Fromageot, *et al.*,[3] who reported two alanine and two glycine residues for the free α-carboxyl residues per 12,000 g. of beef insulin, Chibnall and Rees[4] who reported two alanine and one glycine and one unknown; Harris[10] with the use of carboxypepti-

(9) J. Bremner, *Nature*, **168**, 518 (1951).

(10) J. I. Harris, THIS JOURNAL, **74**, 2944 (1952).

dase reported alanine for the B-fraction and asparagine for the A-fraction.

Our results agree with the results of Sanger,[11] Waley and Watson,[5] and all of the above-named authors that alanine does occur as a terminal α-carboxyl residue. It is true that insulin also yielded small amounts of terminal glycine residues for us, but the amounts were too little to be estimated by our technique. It has been shown above that if aspartic acid occurred as a free carboxyl terminal residue it could not be detected by the present modification of the 2-thiohydantoin method.

The method as modified shows that the terminal α-carboxyl residues of both pork and beef insulin apparently arise principally from alanine, tyrosine and phenylalanine. It is conceivable, however, that the tyrosine and phenylalanine residue could have arisen from impurities common to the three samples of crystalline insulin available to us.

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(11) F. Sanger and H. Tuppy, *Biochem. J.*, **49**, 463 (1951).

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The Base-Catalyzed Decomposition of N-Nitroso-N-cyclopentylurethan[1]

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A previous paper[3] has described the base-catalyzed decomposition of N-nitroso-N-cyclohexylurethan. In continuation of this work this paper reports the base-catalyzed decomposition of N-nitroso-N-cyclopentylurethan. N-Cyclopentylurethan, prepared from cyclopentylamine and ethyl chloroformate, was treated with excess nitrous acid to yield N-nitroso-N-cyclopentylurethan. This product was allowed to decompose in methanol which was in contact with a catalytic amount of potassium carbonate.

Nitrogen, carbon dioxide and methyl nitrite were evolved in the course of the reaction. Cyclopentene, ethanol, methyl ethyl carbonate, cyclopentanol, ethyl cyclopentyl carbonate and N-cyclopentylurethan were obtained by careful fractional distillation. These decomposition products are analogous to those obtained from the base-catalyzed decomposition of N-nitroso-N-cyclohexylurethan.

The formation of these compounds from N-nitroso-N-cyclopentylurethan can be rationalized in terms of a scheme already proposed for the decomposition of N-nitroso-N-cyclohexylurethan: (1) the solvolysis of N-nitroso-N-cyclopentylurethan by methanol or water (formed in the reaction) and (2) a displacement of cyclopentyldiazotate ion by methoxide or hydroxide ion. The five-membered carbon ring skeleton does not undergo contraction in this decomposition. This is in agreement with the work of Hückel, *et al.*,[4] who on diazotization of cyclopentylamine in aqueous solution observed that cyclopentanol and cyclopentene were obtained. The dearth of derivatives of cyclopentylamine in the chemical literature has prompted us to place several on record.

Experimental[5]

N-Cyclopentylurethan.—Following the method of Hartman and Brethen[6] for alkylurethans this compound was obtained in 83% yield, m.p. 7.0–8.5°, b.p. 125° (20 mm.), $n^{25}D$ 1.4605, d^{25}_4 1.0232.

Anal. Calcd. for $C_8H_{15}O_2N$: N, 8.91. Found: N, 9.12.

N-Nitroso-N-cyclopentylurethan.—Following the method of Bollinger, Hayes and Siegel[3] this compound was obtained in 98% yield, an orange oil, $n^{25}D$ 1.4656, d^{25}_4 1.1016. Unlike N-nitroso-N-methylurethan N-nitroso-N-cyclopentylurethan is not a skin irritant.

Anal. Calcd. for $C_8H_{14}O_3N_2$: N, 15.36. Found: N, 15.67.

Decomposition of N-Nitroso-N-cyclopentylurethan.—The decomposition of N-nitroso-N-cyclopentylurethan and the identification of gaseous and solid products were carried out by methods previously described.[3]

By a careful fractional distillation, cyclopentene, ethanol, methyl ethyl carbonate, cyclopentanol, ethyl cyclopentyl carbonate and N-cyclopentylurethan were obtained. Cyclopentene (crude yield 25%, purified yield 22%), b.p. 45–47°, $n^{20}D$ 1.4165 (lit.[7] b.p. 44.3–44.4° (761 mm.), $n^{20}D$ 1.42246) gave the 2-chlorocyclopentyl 2′,4′-dinitrophenyl sulfide derivative, m.p. and mixed m.p. with an authentic derivative 76.5–77°. In addition cyclopentene was brominated to yield *trans*-1,2-dibromocyclopentane, b.p. 98–102° (43 mm.), $n^{25}D$ 1.5417 (lit.[8] b.p. 105–105.5° (45 mm.), $n^{24}D$ 1.5444[9]). Ethanol (crude yield 78%, purified yield 23%), b.p. 73–76°, $n^{25}D$ 1.3653 (lit.[10] b.p. 78.5°, $n^{20}D$ 1.3610) gave an iodoform derivative, m.p. 119° (lit.[11] m.p. 119°). Methyl ethyl carbonate (crude yield 4%), b.p. 105–110°, $n^{20}D$ 1.3782 (lit.[12] b.p. 107.2–107.8°, $n^{20}D$ 1.3779) was not purified further. Cyclopentanol (crude yield 4%, purified yield 3%), b.p. 61–63° (19 mm.), $n^{25}D$ 1.4480 (lit.[13] b.p. 139°, $n^{20}D$ 1.4530) gave an α-naphthylurethan derivative, m.p. and mixed m.p. with an authentic derivative 118–119° (lit.[14] m.p. 118°). Ethyl cyclopentyl carbonate (crude yield 6%, purified yield 3%), b.p. 90–91° (19 mm.), $n^{25}D$ 1.4300, was identical with the compound made by independent synthesis. This was confirmed by infrared spectra of the two materials. N-Cyclopentylurethan (crude yield 7%, purified yield 4%), b.p. 122–125° (19 mm.), $n^{25}D$ 1.4605 was identical with the product previously prepared. This was confirmed by infrared spectra of the two materials.

2-Chlorocyclopentyl 2′,4′-Dinitrophenyl Sulfide.—This compound, m.p. 76.5–77°, was obtained from cyclopentene and 2,4-dinitrobenzenesulfenyl chloride by the method of Kharasch and Buess.[15]

(1) Abstracted in part from a dissertation submitted by Frederick W. Bollinger to the Graduate School of Illinois Institute of Technology in partial fulfillment of the requirements for the degree of Doctor of Philosophy.

(2) Merck & Co., Inc., Rahway, New Jersey.

(3) F. W. Bollinger, F. N. Hayes and S. Siegel, THIS JOURNAL, **72**, 5592 (1950).

(4) W. Hückel, E. Kamenz, A. Gross and W. Tappe, *Ann.*, **533**, 1 (1937).

(5) Melting points and boiling points are uncorrected. Microanalyses were performed by Micro-Tech Laboratories, Skokie, Ill.

(6) W. W. Hartman and M. R. Brethen, "Organic Syntheses," John Wiley and Sons, Inc., New York, N. Y., Coll. Vol. II, 1943, p. 278.

(7) A. I. Vogel, *J. Chem. Soc.*, 1323 (1938).

(8) J. Wislicenus and C. Gärtner, *Ann.*, **275**, 332 (1893).

(9) M. W. Lister, THIS JOURNAL, **63**, 145 (1941).

(10) F. H. Getman and V. L. Gibbons, *ibid.*, **37**, 1995 (1915).

(11) R. L. Shriner and R. C. Fuson, "The Systematic Identification of Organic Compounds," John Wiley and Sons, Inc., New York, N. Y., 3rd ed., 1948, p. 258.

(12) M. H. Palomaa, E. J. Salmi and K. Suoja, *Ber.*, **72**, 313 (1939).

(13) C. R. Noller and R. Adams, THIS JOURNAL, **48**, 1084 (1926).

(14) Reference 11, p. 226.

(15) N. Kharasch and C. M. Buess, THIS JOURNAL, **71**, 2724 (1949).

Anal. Calcd. for $C_{11}H_{11}ClO_4SN_2$: C, 43.64; H, 3.66. Found: C, 44.00; H, 3.80.

Ethyl Cyclopentyl Carbonate.—Following the method of Bollinger, Hayes and Siegel[3] for ethyl cycloalkyl carbonates this compound was obtained in 30% yield, b.p. 90–91° (19 mm.), $n^{20}D$ 1.4318, $n^{25}D$ 1.4300, d^{20}_4 1.0218, d^{25}_4 1.0182.

Anal. Calcd. for $C_8H_{14}O_3$: C, 60.74; H, 8.92. Found: C, 60.37; H, 9.19.

Saponification of ethyl cyclopentyl carbonate with solid sodium hydroxide followed by distillation gave ethanol, b.p. 78°, $n^{20}D$ 1.3635 (lit.[10] b.p. 78.5°, $n^{20}D$ 1.3610); α-naphthylurethan derivative m.p. 78° (lit.[11] m.p. 78°) and cyclopentanol, b.p. 138–139°, $n^{20}D$ 1.4524 (lit.[11] b.p. 139°, $n^{20}D$ 1.4530); α-naphthylurethan derivative, m.p. 118° (lit.[14] m.p. 118°). The solid residue remaining after distillation gave a positive test for carbonate.

Cyclopentanone Oxime.—Following the method of Bousquet[16] for oximes this compound was obtained in 91% yield, m.p. 56.5° (lit.[17] m.p. 56°), b.p. 96–98° (18 mm.).

Cyclopentylamine.—In the 300-ml. liner of a high pressure hydrogenator were placed 26 g. (0.26 mole) of cyclopentanone oxime, 50 ml. of absolute ethanol and a slurry consisting of 5 g. of Raney nickel in 10 ml. of ethanol. Hydrogenation was complete in 35 minutes during which time the temperature rose from 60 to 73°. The mixture was filtered and fractionated to obtain 12.5 g. (56%) of cyclopentylamine and 5.75 g. (29%) of dicyclopentylamine.[18,19]

Cyclopentylamine was allowed to stand over solid potassium hydroxide; redistillation provided a center cut, b.p. 107–108° (760 mm.), lit.[20] b.p. 108°), $n^{25}D$ 1.4472, d^{25}_4 0.8512. Cyclopentylamine was characterized by its thiocyanate salt, m.p. 94.5 (lit.[21] m.p. 93–94°). N-Cyclopentylbenzenesulfonamide, m.p. 68.5–69.5°, was prepared from cyclopentylamine and benzenesulfonyl chloride by the usual procedure.[22]

Anal. Calcd. for $C_{11}H_{15}O_2SN$: S, 14.23. Found: S, 14.01.

In like manner N-cyclopentyl-*p*-toluenesulfonamide, m.p. 84°, was obtained.

Anal. Calcd. for $C_{12}H_{17}O_2SN$: S, 13.40. Found: S, 13.22.

Cyclopentylamine was further characterized by its phthalimide and phthalamic acid derivatives. To phthalic anhydride was added an equimolar amount of cyclopentylamine. The mixture warmed spontaneously; it was then heated at 145° for 20 minutes. The melt, which solidified on cooling, was ground in a mortar, extracted with 5% sodium hydroxide and filtered. The residue, N-cyclopentylphthalimide (crude yield 65%, purified yield 53%), was recrystallized from 95% ethanol, m.p. 99–100°. This compound was identical with that obtained from the Gabriel synthesis, m.p. and mixed m.p. 99–100°.

Anal. Calcd. for $C_{13}H_{13}O_2N$: C, 72.54; H, 6.09; N, 6.51. Found: C, 72.28; H, 6.20; N, 6.74.

The alkaline extract from N-cyclopentylphthalimide was made acid to congo red. The precipitate, N-cyclopentylphthalamic acid, was washed, dried and recrystallized from 50% ethanol, m.p. 143–144°.

Anal. Calcd. for $C_{13}H_{15}O_3N$: C, 66.93; H, 6.48; N, 6.01. Found: C, 67.08; H, 6.37; N, 6.28.

N-Cyclopentylphthalamic acid and N-cyclopentylphthalimide were converted each to the other. The acid was converted to the imide by heating at 145° for 30 minutes. Recrystallization from 95% ethanol gave N-cyclopentylphthalimide, m.p. 99–100°. This material was identical with that obtained from the Gabriel condensation, m.p. and mixed m.p. 99–100°.

The imide was hydrolyzed for 9 hours with boiling, 47% hydrobromic acid. After the mixture was cooled and filtered, the precipitate was washed with water, dried and recrystallized from 50% ethanol. The product, N-cyclopentylphthalamic acid, was identical with that previously obtained, m.p. and mixed m.p. 143–144°.

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(16) E. W. Bousquet, "Organic Syntheses," Coll. Vol. II, John Wiley and Sons, Inc., New York, N. Y., 1943, p. 313.

(17) Reference 11, p. 262.

(18) Reductive ammonation of cyclopentanone and the Gabriel synthesis gave less favorable yields of cyclopentylamine.

(19) H. Adkins, "Reactions of Hydrogen," University of Wisconsin Press, Madison, Wis., 1937, p. 92, reported that the hydrogenation of cyclopentanone oxime in alcohol yields 80% cyclopentylamine and 10% dicyclopentylamine. This experiment was not found in the reference cited by Adkins nor could it be found elsewhere in periodical literature. A private communication from Adkins confirms the authors' search of the literature but expresses belief in the authenticity of the experiment.

(20) J. Wislicenus and W. Hentzschel, *Ann.*, **275**, 325 (1893).

(21) R. A. Mathes, F. D. Stewart, and F. Swedish, Jr., THIS JOURNAL, **70**, 3455 (1948).

(22) Reference 11, p. 91.

Magnetic and Spectroscopic Studies on Triphenylboron Sodium[1]

BY TING LI CHU[2]

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It has long been known that sodium reacts with triphenylboron to form a yellow addition compound having the chemical composition $(C_6H_5)_3BNa$ (I). Triphenylboron is not a free group and bears no resemblance to the triphenylmethyl group. However, its reduction product, the compound I, has an odd number of electrons and has been described as a free radical from its chemical behavior toward oxygen, iodine, triphenylmethyl, etc.[3] We might expect the compound I to have magnetic properties characteristic of the unpaired electron and it is the purpose of this work to carry out such investigations.

Triphenylboron was prepared by the reaction of phenylmagnesium bromide with boron trifluoride.[3,4] The product was sensitive toward oxygen, hence it was necessary to effect the purification by repeated distillation and recrystallization from ether under vacuum. A product with a melting point of 136° (uncor.) was obtained. I was then prepared by treating triphenylboron either in dry ether or dry tetrahydrofuran with excess of 40% sodium amalgam under high vacuum. This compound is yellow, only slightly soluble in ether but very soluble in tetrahydrofuran. To determine the composition of the product, aliquot portions of the tetrahydrofuran solution were analyzed for sodium and boron. Sodium was determined by hydrolyzing the solution and titrating liberated alkali with acid, boron was determined by the procedure of Fowler and Kraus.[5] The mole ratio Na/B was found by this analysis to be 0.97 ± 0.02.

Measurements of the magnetic susceptibility were made utilizing a Gouy balance. Measurements were made immediately after the preparation of a sample as a precaution against decomposition. A 10% solution of I in tetrahydrofuran was placed in a Pyrex tube (40 cm. length and 12 mm. diameter) and sealed off while under high vacuum. The sample was weighed in the magnetic field to give a result related to the algebraic sum of the paramagnetic susceptibility and the diamagnetic susceptibility of I as well as the diamagnetic susceptibility of the solvent. Corrections for the

(1) Assisted by the joint program of the ONR and AEC.

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(3) E. Krause and H. Polack, *Ber.*, **59**, 777 (1926).

(4) H. E. Bent and M. Dorfman, THIS JOURNAL, **57**, 1259 (1935).

(5) D. E. Fowler and C. A. Kraus, *ibid.*, **62**, 1143 (1940).

two diamagnetic contributions to the total susceptibility was made by making a second measurement on the same sample after chemically destroying the source of the paramagnetism. This measurement was effected by admitting air to the sample until decolorized and then repeating the weighing in the magnetic field. If we assume the diamagnetic contribution of I not to be changed appreciably by treatment with air, the net difference in the two weighings is proportional to the paramagnetic susceptibility of I. These measurements were carried out at several field strengths ranging from 6,000 to 10,000 oersteds and the results showed no paramagnetism.

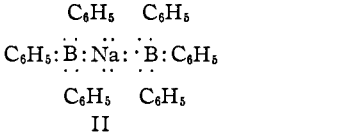
The paramagnetic resonance absorption results from the Larmor precession of the unpaired electron spin in an external magnetic field. The frequency of the radiation is

$$\nu = \frac{E_2 - E_1}{h} = \frac{g\beta H}{h}$$

where E_2 and E_1 are the energies of the two Zeeman levels of the unpaired electron spin, h is the Planck constant, g is the gyromagnetic ratio, β is the Bohr magneton and H is the magnetic field strength. The measurements were made on the apparatus constructed by Dr. J. Townsend of the Physics Department of Washington University,[6] it is sensitive for detecting 10^{-8} mole of free radicals. The frequency employed was about 9000 mc./sec., the measurement of resonance absorption was accomplished by modulating the magnetic field over a region which spans the resonant field for the applied radiofrequency. A tetrahydrofuran solution of I showed no absorption over a wide range of concentrations and magnetic field strengths; I also showed no absorption in the solid state. If the compound I had an unpaired electron spin, we would expect to observe the nuclear hyperfine structure arising from the interaction between the magnetic moments of the odd electron and the boron nucleus.

In a solvent of low dielectric constant, the compound I will exist as ion-pairs. The magnetic measurements indicate that the triphenylboron anion probably dimerizes to $(C_6H_5)_3B:B(C_6H_5)_3^-$ which is diamagnetic. There is evidence that trimethylgallium forms a colorless dimeric ion $Me_3Ga:GaMe_3^-$.[7] It is possible that there is an equilibrium between simple and dimeric triphenylboron anions which equilibrium, however, is shifted far toward the dimer under ordinary conditions.

Bent and Dorfman[4] observed the anomaly that when part of the sodium in I was removed by shaking with mercury or dilute amalgam, the yellow color entirely disappeared. The color change was reversible and they assumed that triphenylboron reacts with I to form a colorless addition product.



II

This observation has also been confirmed in this Laboratory from the color change during the reaction between triphenylboron and sodium. However, the solutions obtained from various ratios of triphenylboron and sodium are all diamagnetic from paramagnetic resonance absorption and static susceptibility measurements. Furthermore, assuming that the triphenylboron anions are simple, we have no knowledge of how the presence of the sodium ion in the ion-pair would affect the properties of I in solution. The structure of formula II seems highly improbable. The color change might be accounted for by the equilibrium between simple and dimeric triphenylboron anions.

If I exists in a dimeric form in the solid state, the replacement of sodium by a bulky group, like tetraphenylstibonium group, appeared likely to bring about a dissociation into free radicals. However, the reaction product of I and tetraphenylstibonium hydroxide is diamagnetic in the solid state.

The absorption and fluorescence spectra of I were also measured in order to compare with those of triphenylmethyl. An ether solution of approximately 2×10^{-5} M sealed off under vacuum in a 1-cm. Pyrex cell was used for absorption measurement. The spectrum obtained at room temperature with a Beckman model DU spectrophotometer is given in Fig. 1,[8] it is quite different from that of triphenylmethyl. Triphenylmethyl shows seven weak absorption bands of extreme sharpness in the visible region which represent the vibrational terms in a single electronic band.[9] This set of absorption bands has an oscillator strength of the order of magnitude of 10^{-6} and these transitions probably arise from the slight distortion of planar configuration of the molecule and the perturbation by asymmetric vibrations.[10] The remarkable fine structures are also observed in other triarylmethyls. Triphenylmethyl also shows an intense absorption band in the near ultraviolet[11] representing an allowed electronic transition. I shows two intense absorption bands in the violet and near ultraviolet

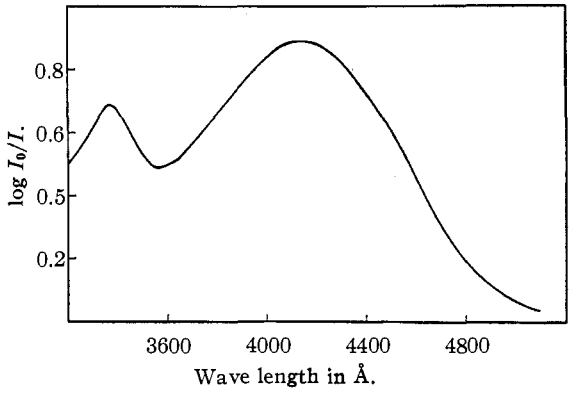


Fig. 1.—Absorption spectrum of triphenylboron sodium in tetrahydrofuran; approximate concentration 2×10^{-5} M.

(6) Washington University, First Progress Report on Paramagnetic Resonance of Free Radicals under ONR contract N6onr-20205, December, 1951, p. 5.

(7) C. A. Kraus and F. E. Toonder, THIS JOURNAL, 55, 3547 (1933).

(8) Because of the difficulty of measuring the exact concentration of I, optical density, instead of molar extinction coefficient, is plotted *versus* wave length.

(9) G. N. Lewis, D. Lipkin and T. T. Magel, THIS JOURNAL, 66, 1579 (1944).

(10) S. I. Weissman and T. L. Chu, in preparation.

(11) L. C. Anderson, THIS JOURNAL, 57, 1673 (1935).

with a separation of about 5,600 cm.$^{-1}$ between the peak maxima and the approximate oscillator strengths for these two transitions are 0.58 and 0.23, respectively. Even at low temperature, no fine structures could be observed within the experimental error. Most probably each absorption band represents a separate electronic transition.

A dilute solution of I in 4 parts ether and 1 part tetrahydrofuran[12] shows very strong fluorescence at 77°K. when illuminated with ultraviolet light. The fluorescence spectrum was photographed on a Steinheil spectrograph and the densitometer tracing of the spectral plate is reproduced in Fig. 2. The fluorescence of most organic molecules in condensed systems results from transitions from the fluorescent state down to the various vibrational levels of the ground state. Taking the normal state of I as zero, the position of the fluorescent level is 16,500 ± 50 cm.$^{-1}$ and the low lying vibrational levels are approximately located at 920 and 1,600 cm.$^{-1}$. The fluorescence of triphenylmethyl appears to be the mirror image of its absorption.[9]

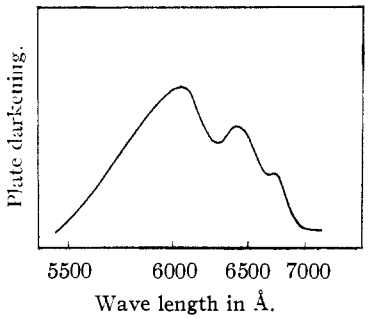


Fig. 2.—Fluorescence spectrum of triphenylboron sodium in 1:4 tetrahydrofuran–ether mixture.

Bent and Dorfman[13] prepared the disodium salt of tris-α-naphthylboron by treating tris-α-naphthylboron in ether solution with 40% sodium amalgam. It may be reported here that triphenylboron also forms dark green disodium salt by prolonged treatment with 40% sodium amalgam in tetrahydrofuran solution. The reversible color change similar to those reported for tris-α-naphthylboron disodium was also observed.

(12) This solvent mixture forms a rigid transparent medium at 77°K.

(13) H. E. Bent and M. Dorfman, THIS JOURNAL, **54**, 2132 (1932).

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Synthesis of Aureomycin Degradation Products. II

BY JAMES H. BOOTHE, R. G. WILKINSON, S. KUSHNER AND J. H. WILLIAMS

RECEIVED SEPTEMBER 29, 1952

Previous reports[1,2] have shown various phthalides and related compounds to be degradation products of aureomycin. These compounds have for the most part been synthesized for confirmation of structure. From a different portion of the aureomycin molecule some cyclopentane derivatives have been isolated,[3] two of which have been synthesized, namely, 1,3-cyclopentanedione and 1,2,4-cyclopentanetrione.

1,3-Cyclopentanedione was prepared from ethyl methyl β-ketoadipate[4] by cyclizing its ethylene ketal[5] with sodium ethoxide followed by hydrolysis and decarboxylation.

1,2,4-Cyclopentanetrione was prepared by treating diethyl oxalate and diethyl acetonedicarboxylate in the presence of sodium ethoxide to yield 3,5-dicarbethoxy-1,2,4-cyclopentanetrione. On hydrolysis and decarboxylation the triketone was obtained.

Richter[6] has described the preparation of 2-carbethoxy-1,3-cyclopentanedione. This compound was prepared in our laboratory and showed none of the properties expected. It could not be hydrolyzed and decarboxylated to the dione, it could not be oxidized to succinic acid, and it showed two C-methyl groups.

Ruggli and Maeder[7] have also reported the preparation of 2-carbethoxy-1,3-cyclopentanedione by the cyclization of methyl ethyl β-ketoadipate. They did not characterize the compound however and in our hands this reaction mixture after hydrolysis and decarboxylation did not yield 1,3-cyclopentanedione.

Ruggli and Doebel[8] prepared 2,4-dicarbethoxy-1,3-cyclopentanedione and have oxidized this with selenium dioxide to 3,5-dicarbethoxy-1,2,4-cyclopentanetrione. This compound, obtained in rather small amounts and not thoroughly purified or analyzed, melted approximately 20° below our best sample.

1,3-Cyclopentanedione and 1,2,4-cyclopentanetrione are quite acidic, the former having a pK_a of 4.5 and the latter a pK_a of 3.0. The ultraviolet absorption spectra of the dione show maxima at 257 mμ (E 29,400) in 0.1 N sodium hydroxide and at 242 mμ (E 20,700) in 0.1 N hydrochloric acid. The

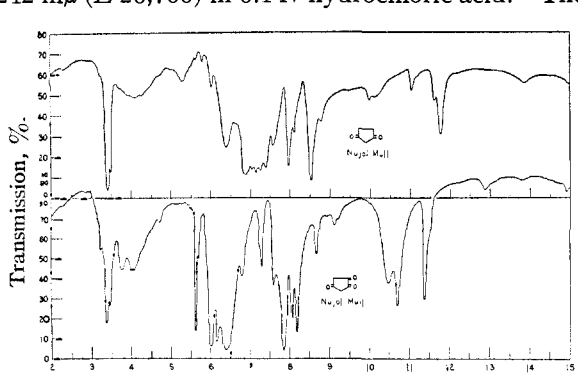

Fig. 1.—Infrared absorption spectra of 1,3-cyclopentanedione and 1,2,4-cyclopentanetrione.

(1) B. L. Hutchings, C. W. Waller, S. Gordon, R. W. Broschard, C. E. Wolf, A. A. Goldman and J. H. Williams, THIS JOURNAL, **74**, 3710 (1952).

(2) S. Kushner, J. H. Boothe, J. Morton II, J. Petisi and J. H. Williams, *ibid.*, **74**, 3710 (1952).

(3) C. W. Waller, *et al.*, *ibid.*, **74**, 4978 (1952).

(4) J. C. Bardhan, *J. Chem. Soc.*, 1848 (1936).

(5) H. Schintz and G. Schappi, *Helv. Chim. Acta*, **30**, 1488 (1947).

(6) R. Richter, *ibid.*, **32**, 1123 (1949).

(7) P. Ruggli and A. Maeder, *ibid.*, **26**, 1476 (1943).

(8) P. Ruggli and K. Doebel, *ibid.*, **29**, 600 (1946).

trione shows maxima at 310 mμ (E 13,450) in 0.1 N sodium hydroxide and at 267 mμ (E 10,850) in 0.1 N hydrochloric acid. The infrared absorption spectra of the two compounds are shown in Fig. 1.

Experimental

1,3-Cyclopentanedione.—A mixture of 5.8 g. of ethyl methyl β-ketoadipate, 2.53 g. of ethylene glycol, 0.01 g. of *p*-toluenesulfonic acid monohydrate and 10 cc. of dry benzene was refluxed four hours using a water separator. The solution was stirred with solid sodium bicarbonate and anhydrous sodium sulfate and decanted onto dry sodium ethoxide (from 0.69 g. of sodium) in a nitrogen atmosphere. About 20 cc. of dry benzene and 20 cc. of dry ether were used to wash the inorganic salts and the mixture was refluxed 45 minutes. After acidification with acetic acid and addition of 15 cc. of concentrated hydrochloric acid, the mixture was filtered and the organic solvents distilled off. The aqueous solution was refluxed 1.5 hours and concentrated to dryness *in vacuo*. The oily residue was evaporatively distilled at 120° (0.5 mm.), and the oily distillate partially crystallized from ethyl acetate. The product was sublimed at 120° (0.5 mm.) to yield 0.211 g. (7.5%) of 1,3-cyclopentanedione, m.p. 151.5–152.5°, which showed no depression upon admixture with the degradation product. *Anal.* Calcd. for $C_5H_6O_2$: C, 61.2; H, 6.2. Found: C, 61.0; H, 6.3. The ultraviolet and infrared absorption spectra were identical with those of the degradation product.

3,5-Dicarbethoxy-1,2,4-cyclopentanetrione.—A mixture of 2.5 cc. of ethyl oxalate, 3.45 cc. of diethyl acetonedicarboxylate and sodium ethoxide (from 0.8 g. of sodium), in 100 cc. of dry benzene and 25 cc. of dry ether was refluxed under nitrogen for 1.75 hours. The cooled solution was poured into 150 cc. of cold 10% sulfuric acid and the water layer was extracted with ethyl acetate. The combined organic layers were evaporated, and by crystallization from ethyl acetate and sublimation of the residue a total yield of 1.42 g. (28%) was obtained, m.p. 159–162° with gas. *Anal.* Calcd. for $C_{11}H_{12}O_7$: C, 51.6; H, 4.7. Found: C, 51.5; H, 4.7.

1,2,4-Cyclopentanetrione.—A mixture of 0.22 g. of the above diester was refluxed 90 minutes in 30 cc. of concentrated hydrochloric acid and the solution was concentrated to dryness. The residue was sublimed twice at 90° and 0.5 mm. giving 0.06 g. (62%) of a white solid, m.p. 172.5–173° with decomposition starting at 167°. There was no depression upon admixture with the degradation product from aureomycin. *Anal.* Calcd. for $C_5H_4O_3$: C, 53.6; H, 3.6. Found: C, 53.8; H, 3.9. The ultraviolet[3] and infrared spectra of the degradation product and 1,2,4-cyclopentanetrione were identical.

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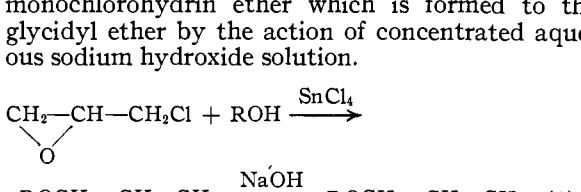
Synthesis of Polyglycidyl Ethers

BY SAUL G. COHEN AND HOWARD C. HAAS

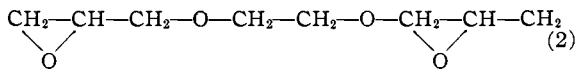
RECEIVED OCTOBER 13, 1952

Monoglycidyl ethers are generally prepared[1] by treating a large excess of an alcohol with epichlorohydrin in the presence of a small amount of stannic chloride and converting the glycerol α-monochlorohydrin ether which is formed to the glycidyl ether by the action of concentrated aqueous sodium hydroxide solution.



(1) Shell Chemical Corporation Technical Booklet SC: 49-35.

Although the reaction of epichlorohydrin with polyhydric alcohols is discussed, in the literature,[2-7] little information is available on the preparation and isolation of polyglycidyl ethers of the type



(2)

by this reaction. The use of excess epichlorohydrin in such a reaction is prohibited because of its rapid polymerization which is catalyzed by acids.

The addition of epichlorohydrin to a polyhydric alcohol in the presence of stannic chloride, one mole of epichlorohydrin per mole of hydroxyl group, led to polyglycidyl ethers in satisfactory yield. This was accomplished, however, only if the conversion of the polychlorohydrin to the polyepoxide was carried out in the presence of a water-immiscible solvent which extracted the polyglycidyl ether as it was formed. Otherwise a vigorous alkali-catalyzed polymerization occurred resulting in almost complete conversion of the polyepoxide to non-volatile products. An example of the procedure is the following preparation of 1,2-bis-(2,3-epoxypropoxy)-ethane, the diglycidyl ether of ethylene glycol.

Experimental

Ethylene glycol (distilled, 31 g., 0.5 mole) and 0.3 g. of stannic chloride were placed in a three-necked flask equipped with a stirrer, reflux condenser and dropping funnel. The solution was heated to 85–90° and 92.5 g. (1 mole) of epichlorohydrin (Shell) was added over a 16-hour period. Heating was continued for an additional 24 hours after which time no low boiling materials were distilled off under vacuum indicating that the epichlorohydrin had reacted completely. The reaction mixture was cooled and diluted with 100 ml. of ether. Eighty grams of 50% sodium hydroxide was added with vigorous stirring while the reaction was held at ice-bath temperature. The ether solution was decanted from the dense aqueous phase, dried over anhydrous sodium sulfate and fractionated through a 3 ft. column. After stripping off the ether, a small fraction was collected, b.p. 89–90° (10 mm.), $n^{24}D$ 1.4490. A second larger fraction was collected at 128–131° (10 mm.), $n^{23.8}D$ 1.4530. A considerable amount of non-volatile residues remained in the distillation flask. Both fractions were colorless oils which gave positive Beilstein tests indicating halogen impurities. Analysis of the second fraction for oxirane oxygen by hydrochloric acid consumption[8] gave a value of 97% diepoxide; analysis of fraction 1 indicated 88% monoepoxide. Fraction 2 was refractionated and yielded 18 g. of material, b.p. 119° (2–3 mm.), d^{30}_4 1.1182, $n^{30}D$ 1.4498, M_D 41.80. *Anal.* Calcd. for $C_8H_{14}O_4$: C, 55.15; H, 8.1. Found[9]: C, 54.80; H, 8.1.

By use of Eisenlohr's atomic refractivities and a value of 0.22 for the three-membered epoxide ring (calculated from M_D and d of epichlorohydrin), M_D was calculated for the diglycidyl ether and found to be 41.76.

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(2) J. Nivière, *Compt. rend.*, **156**, 1628 (1913).

(3) O. Loehr, German Patent 510,422 (October 18, 1930).

(4) O. Schmidt and E. Meyer, U. S. Patent 1,922,459 (August 15, 1933).

(5) W. Schneider and A. Fröhlich, U. S. Patent 2,280,722 (April 21, 1942).

(6) M. DeGroote and B. Keiser, U. S. Patent 2,411,029 (November 12, 1946).

(7) M. S. Kharasch and W. J. Nudenberg, *J. Org. Chem.*, **8**, 189 (1943).

(8) S. Siggia, "Quantitative Organic Analysis via Functional Groups," John Wiley and Sons, Inc., New York, N. Y., 1949, p. 108.

(9) Analyses were performed by Dr. C. K. Fitz, Boston, Massachusetts.

Synthesis and Reactions of 3-Butyne-1,2-diol

By V. A. Engelhardt and J. E. Castle

Received November 13, 1952

It has been found that 3-butyne-1,2-diol can be prepared from monovinylacetylene by the hydroxylation procedure of Swern and co-workers[1] using hydrogen peroxide in formic acid. This method offers a more direct route to the acetylenic glycol than an earlier four-step synthesis based on acetaldehyde.[2] The preferential attack of hydroxylating agents on the olefinic portion of a conjugated eneyne compound has been previously demonstrated in the oxidation of 2-pentene-4-yne-1-ol to 4-pentyne-1,2,3-triol by performic acid[3] and in the hydroxylation of 1-phenyl-3-ene-1-yne type compounds by peracetic acid.[4]

Derivatives of 3-butyne-1,2-diol such as the diacetate, dibenzoate, dicarbanilate and 3,5-octadiyne-1,2,7,8-tetrol have been prepared. The tetrol was prepared by oxidative coupling of 3-butyne-1,2-diol diacetate followed by methanolysis. An attempt to prepare the tetrol by oxidative coupling of 3-butyne-1,2-diol was unsuccessful due to the difficulty in isolating the water-soluble tetrol from an aqueous solution of ammonium and copper salts.

Experimental

3-Butyne-1,2-diol.—Formic acid (98–100%, 150 ml.), aqueous 30% hydrogen peroxide (151 g., 0.45 mole) and monovinylacetylene (15.6 g., 0.3 mole) were charged into each of nine pressure bottles at 0°. These bottles were capped and then placed in a rocker bath at 40° for 25 hours. It is necessary to carry out this reaction in a properly shielded water-bath, for a bottle that was allowed to stand at room temperature in the open air exploded within a short time. The combined products were passed through a steam-jacketed stripping still under 80 mm. pressure to remove the bulk of the formic acid. Distillation of the residual oil gave 119 g. of the monoformate, b.p. 70–115° (0.5–0.9 mm.), which distilled mainly at 71° (0.6 mm.). The distillate (119 g.) was placed in an ice-bath and aqueous 33% sodium hydroxide (77 ml.) was added portionwise with swirling to keep the temperature below 40°. After two hours at room temperature, water was removed from the solution at 70° (10 mm.). The residue was mixed with acetone (250 ml.) and filtered from sodium formate. After distillation of acetone, the residual oil was distilled to give 67.7 g. (29% over-all yield) of 3-butyne-1,2-diol, b.p. 64–66° (0.2 mm.), $n^{25}D$ 1.4723.

A sample of the glycol was recrystallized from an equal volume of absolute ether to give hygroscopic crystals which melted at 37.5–38.5°. A melting point of 39.5–40° was reported by Lespieau.[2]

Anal. Calcd. for $C_4H_6O_2$: C, 55.80; H, 7.02; mol. wt., 86; quant. hydrogenation, g. H_2/g., 0.0468. Found: C, 56.14; H, 7.15; mol. wt. (ebullioscopic in ethanol), 90; quant. hydrogenation, g. H_2/g., 0.0469.

3-Butyne-1,2-diol Dicarbanilate.—This compound, m.p. 134–135°, was prepared according to the directions of Lespieau.[2]

Anal. Calcd. for $C_{18}H_{16}O_4N_2$: C, 66.66; H, 4.97; N, 8.64. Found: C, 66.81; H, 5.20; N, 8.60.

3-Butyne-1,2-diol Dibenzoate.—3-Butyne-1,2-diol (0.7 g., 0.008 mole) and benzoyl chloride (2.0 g., 0.014 mole) were dissolved in pyridine (10 ml.). After the initial reaction had subsided, the solution was boiled for one minute and then poured into water (30 ml.). Decantation of the aqueous layer left an oil which was then triturated with water several times. After standing for two days, the oil crystallized. The solid was washed with aqueous 5% sodium bicarbonate and then recrystallized twice from ethanol to give 0.7 g. of product, m.p. 74–75°.

Anal. Calcd. for $C_{18}H_{14}O_4$: C, 73.46; H, 4.79. Found: C, 73.62; H, 4.90.

The presence of a terminal acetylenic group in this compound was shown by the formation of a precipitate with potassium mercuric iodide reagent.[5]

3-Butyne-1,2-diol Diacetate.—3-Butyne-1,2-diol (50 g., 0.58 mole) and freshly fused sodium acetate (25.9 g.) were dissolved in acetic anhydride (259 ml.). This solution was heated on a steam-bath for two hours and then poured with stirring into ice-water (1.7 l.). The aqueous mixture was neutralized with sodium carbonate (300 g.) and then extracted several times with ether. The combined ethereal extracts were washed with aqueous 5% sodium bicarbonate and then dried over magnesium sulfate. Evaporation of the ether left an oil which on distillation gave 86.0 g. (87% yield) of the diacetate, b.p. 54.5–55.5° (0.2 mm.), $n^{25}D$ 1.4351.

Anal. Calcd. for $C_8H_{10}O_4$: C, 56.46; H, 5.92; sapn. equiv., 85. Found: C, 56.96; H, 6.08; sapn. equiv., 85.

Although this diacetate failed to give a positive test for a terminal acetylene group with potassium mercuric iodide reagent,[6] the presence of such a group was indicated by infrared absorption bands at 3.04 and 4.70 μ and by formation of a white precipitate with ammoniacal aqueous 5% silver nitrate.

3,5-Octadiyne-1,2,7,8-tetrol.—3-Butyne-1,2-diol diacetate was oxidatively coupled using a procedure developed for the coupling of acetylenic alcohols.[6] The diacetate (48 g., 0.28 mole), cuprous chloride (55.5 g., 0.28 mole), ammonium chloride (90 g., 1.68 moles), ethanol (12 ml.), hydrochloric acid (0.6 ml.) and water (305 ml.) were stirred and heated at 55–60° for 6.5 hours while air was bubbled through the solution. After standing overnight the mixture was extracted with ether. The combined extracts were dried with magnesium sulfate, evaporated on the steam-bath, and heated at 126° (0.2 mm.), to give a residue of viscous oil (48 g.). A portion of this crude tetraacetate (14 g., 0.041 mole) was dissolved in absolute methanol (140 ml.) containing sodium (0.1 g.). This solution was allowed to stand overnight and was then refluxed for 3.5 hours. Volatile material was removed at 40° (2 mm.) leaving a tan, waxy solid. This was dissolved in hot ethanol and the solution was decolorized with Darco, diluted with an equal volume of benzene, and cooled to give 4.4 g. (62% yield) of a cream colored solid. The product was evidently a mixture of diastereoisomers for it melted over the range of 116–121° after repeated recrystallizations.

Anal. Calcd. for $C_8H_{10}O_4$: C, 56.46; H, 5.92; quant. hydrogenation, g. H_2/g., 0.0474; mol. wt., 170. Found: C, 55.89; H, 5.94; quant. hydrogenation, g. H_2/g., 0.0470; mol. wt. (thermoelectric method in water[7]), 166.

The ultraviolet spectrum of 3,5-octadiyne-1,2,7,8-tetrol is analogous to those of closely related diacetylenes[8] as shown in Table I.

TABLE I

Compound	Maximum I λ, Å.	Maximum I ε	Maximum II λ, Å.	Maximum II ε	Maximum III λ, Å.	Maximum III ε
3,5-Octadiyne-1,2,7,8-tetrol	2310	390	2430	392	2570	255
2,4-Hexadiyne-1,6-diol	2315	410	2440	440	2580	250
3,5-Octadiyne-1,8-diol	2285	450	2395	490	2540	330

Contribution No. 318, Chemical Department
Experimental Station, E. I. du Pont de Nemours & Co.
Wilmington 98, Delaware

(1) D. Swern, G. N. Billen, T. W. Findley and J. T. Scanlan, This Journal, **67**, 1786 (1945).

(2) R. Lespieau, *Bull. soc. chim.*, [4] **43**, 199 (1928).

(3) R. A. Raphael, *J. Chem. Soc.*, S-44 (1949).

(4) N. M. Malenok, *J. Gen. Chem. U.S.S.R.*, **9**, 1947 (1939); N. M. Malenok and J. V. Sologub, *ibid.*, **6**, 1904 (1936); **9**, 1947 (1939); **11**, 983 (1941).

(5) J. R. Johnson and W. L. McEwen, This Journal, **48**, 469 (1926).

(6) K. Bowden, I. Heilbron, E. R. H. Jones and K. H. Sargent, *J. Chem. Soc.*, 1579 (1947).

(7) G. B. Taylor and M. B. Hall, *Anal. Chem.*, **23**, 947 (1951).

(8) J. B. Armitage and M. C. Whiting, *J. Chem. Soc.*, 2005 (1952).

Possible Isomers for Coördination Compounds with Terdentate Ligands and Ligands of Higher Function

BY W. CONARD FERNELIUS AND BURL E. BRYANT

RECEIVED OCTOBER 17, 1952

In the consideration of any particular coördination compound, it is important to know what isomers are theoretically capable of existence. Main Smith[1] has presented tables showing (for both uni- and bidentate groups) the types, classes and isomeric forms theoretically expected for entities (compounds or ions) exhibiting coördination numbers four (square and tetrahedral) and six (octahedral). Marchi, Fernelius and McReynolds[2] similarly have worked out the possible isomeric forms for several classes of various likely configurations for entities exhibiting coördination number eight with uni- and bidentate groups. Recently we have had occasion to work out by means of models the isomeric forms for six-coördinate entities with terdentate groups and groups of higher function. These are given in Tables I–IV. Optical activity originating within the coördinating groups has been disregarded. Also the customary assumption has been made that adjoining points of attachment on the coördinating groups will always be in the closest adjacent positions (*cis*) in the coördination sphere of the metal.

Symbolism.—It is customary to use lower case letters to represent unidentate groups and pairs of capital letters, bidentate groups: *i.e.*, AA = a symmetrical group like ethylenediamine, the acetylacetonate ion, or $C_2O_4^=$ and AB = an unsymmetrical group like propylenediamine or glycine. In this paper the symbolism A_2 will be used instead of AA. For terdentate groups, A_3 = three equivalent points of attachment such as $HC(CH_2NH_2)_3$, A_2B = two equivalent positions and one different such as $HN(CH_2CH_2NH_2)_2$, and ABC = three non-equivalent positions (possible example is $HSCH_2CH(NH_2)COOH$). For quadridentate groups A_2B_2 would represent such groups as $(H_2NCH_2)_2CHCH(CH_2SH)_2$; ABBA, $H_2NCH_2CH_2NHCH_2CH_2NHCH_2CH_2NH_2$; and A(B)CD, $H_2NCH_2(HSCH)_2$-

TABLE I

ISOMERIC CLASSES AND FORMS FOR THE OCTAHEDRAL CONFIGURATION CONTAINING TERDENTATE GROUPS

Class symbol	Number of isomeric forms: Optically active *cis*	Optically active *trans*	Optically inactive *cis*	Optically inactive *trans*	Total
Type 5					
A_3 3b	..	..	1	1	2
A_3 2b c	..	..	1	2	3
A_3 b c d	2	..	..	3	5
A_2B 3c	..	..	1	1	2
A_2B 2c d	2	..	1	2	5
A_2B c d e	6	6	..	3	15
ABC 3d	2	..	..	1	3
ABC 2d e	6	2	..	1	9
ABC d e f	12	6	..	..	18
Total	30	14	4	14	62
Type 6					
A_3 B_2 c	..	..	1	1	2
A_3 BC d	2	..	..	2	4
A_2B C_2 d	2	..	1	1	4
A_2B CD e	6	..	..	2	8
ABC D_2 e	6	2	..	..	8
ABC DE f	12	4	..	..	16
Total	28	6	2	6	42
Type 7					
$2A_3$	..	..	1	1	2
$2A_2B$	2	..	1	1	4
2ABC[a]	10	2	..	..	12
A_3 B_3	..	..	1	1	2
A_3 B_2C	..	..	1	1	2
A_3 BCD	2	..	..	1	3
A_2B C_2D	4	..	..	1	5
A_2B CDE	6	..	..	1	7
ABC DEF	12	2	..	..	14
Total	36	4	4	7	51

[a] P. Pfeiffer and S. Saure [*Ber.*, 74B, 935 (1941)] have studied this class to some extent.

(1) J. D. Main Smith, "Chemistry and Atomic Structure," Ernest Benn, Ltd., London, 1924, p. 97.

(2) L. E. Marchi, W. C. Fernelius and J. P. McReynolds, THIS JOURNAL, 65, 329 (1943); L. E. Marchi, *ibid.*, 65, 2257 (1943); 66, 1984 (1944).

TABLE II

ISOMERIC CLASSES AND FORMS FOR AN OCTAHEDRAL CONFIGURATION CONTAINING QUADRIDENTATE GROUPS

Class symbol	Number of isomeric forms: Optically active *cis*	Optically active *trans*	Optically inactive *cis*	Optically inactive *trans*	Total
Type 8					
A_2B_2 2c	2	..	..	..	2
A_2B_2 c d	4	..	..	..	4
ABBA 2c[a]	4	..	..	1	5
ABBA c d	6	..	..	1	7
A_2B(C) 2d	4	..	..	..	4
A_2B(C) d e	8	..	..	..	8
A_2BC 2d	2	..	1	..	3
A_2BC d e	8	..	..	..	8
A(B)C(D) 2e	8	..	..	..	8
A(B)C(D) e f	16	..	..	..	16
A(B)CD 2e	6	..	..	..	6
A(B)CD e f	12	..	..	..	12
ABCD 2e	6	..	..	1	7
ABCD e f	12	2	..	..	14
Total	98	2	1	3	104
Type 9					
A_2B_2 C_2	2	..	..	..	2
A_2B_2 CD	4	..	..	..	4
ABBA C_2	4	..	..	..	4
ABBA CD	6	..	..	..	6
A_2B(C) D_2	4	..	..	..	4
A_2B(C) DE	8	..	..	..	8
A_2BC D_2	2	..	1	..	3
A_2BC DE	4	..	2	..	6
A(B)C(D) E_2	8	..	..	..	8
A(B)C(D) EF	16	..	..	..	16
A(B)CD E_2	6	..	..	..	6
A(B)CD EF	12	..	..	..	12
ABCD E_2	6	..	..	..	6
ABCD EF	12	..	..	..	12
Total	94	..	3	..	97

[a] The five isomers of this class have been isolated for diammine{α,α'-(*o*-phenylenediimino)-di-*o*-cresolato}cobalt(III) ion by G. T. Morgan and J. D. Main Smith [*J. Chem. Soc.*, 127, 913, 2030 (1925)].

$CHNHCH_2CH_2NH_2$. The meaning of the other symbols follows from these examples.

Use of Terms *cis* and *trans*.—For terdentate groups the words *cis* and *trans* are used in the same manner as they are used to designate the isomers of M 3a 3b; *cis* indicates that the three points of attachment of the polyfunctional ligand are on the same face of the octahedron; *trans*, that they are along a plane which passes through the coördination center. For entities with quadridentate groups, *cis* and *trans* refer to the position of the remaining monodentate ligands.

Probability of Realizing Theoretical Possibilities.—Some quadri-, quinque- and sexadentate groups can be postulated (and even prepared) which are not at all likely to coördinate to a common center. Certainly for $C(CH_2NH_2)_4$, only three of the nitrogens can coördinate to a common center; $(H_2NCH_2)_2CHCH(CH_2NH_2)$ probably will coördinate completely; while *cis*-1,2,3,4-cyclobutanetetramine awaits study to determine whether or not it will coördinate completely. Because of such complications, the classes containing the groups A_4 and A_3B are not considered here. If A_4 were of the type of phthalocyanine, no isomerism is possible for either M A_4 2b or M A_4 b c. Other groups which are difficult of realization or whose coördination to a common center may not be possible are A_5, A_4B, A_3B_2, A_3BC, A_2B_2C, A_6, A_5B, A_4B_2, A_4BC, A_3B_3, A_3B_2C, $A_2B_2C_2$ and A_2B_2CD. These have also been omitted.

TABLE III

ISOMERIC CLASSES AND FORMS FOR AN OCTAHEDRAL CONFIGURATION CONTAINING QUINQUEDENTATE GROUPS

Type	Class symbol	Optically active	Optically inactive	Total
10	A_2BC_2 d	..	1	1
	ABCBA d	8	..	8
	$A_2BC(D)$ e	2	..	2
	A_2BCD e	4	..	4
	A(B)CD(E) f	4	..	4
	A(B)CDE f	8	..	8
	ABCDE f	12	..	12
	Total	38	1	39

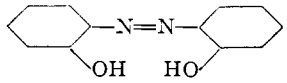
TABLE IV

ISOMERIC CLASSES AND FORMS FOR AN OCTAHEDRAL CONFIGURATION CONTAINING SEXADENTATE GROUPS

Type	Class symbol	Optically active	Optically inactive	Total
11	A_2BBA_2	2	..	2
	ABCCBA[a]	8	..	8
	A(B)CC(B)A	6	..	6
	A_2BCD_2	2	..	2
	$A_2BCD(E)$	4	..	4
	A_2BCDE	4	1	5
	A(B)CDE(F)	8	..	8
	ABCDEF	10	..	10
	Total	44	1	45

[a] An example of this class is being studied by F. P. Dwyer and co-workers, THIS JOURNAL, 69, 2917 (1947); **72**, 1545 (1950); **74**, 4188 (1952).

It seems likely that all groups will not show all the potentialities anticipated for the class to which they belong. Groups may show a preference for certain arrangements because of considerations of steric situations (size, arrangement of atoms, rigidity, etc.). Thus, $H_2NCH_2CH(NH_2)CH_2NH_2$ in contrast to $(H_2NCH_2CH_2)_2NH$ may never be able to coördinate *trans* and



may be sufficiently rigid that it will never coördinate *cis*. The experimental realization of some of the potentialities presented in the tables should not prove too difficult and would contribute greatly to our knowledge of coördination compounds.

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A Polarographic Study of the Zinc Thiocyanate Complexes[1]

BY RICHARD E. FRANK[2] AND DAVID N. HUME

RECEIVED OCTOBER 21, 1952

Although the thiocyanate complexes of cadmium and mercury are well known and have been extensively studied, very little attention has been paid to complex formation between zinc and thiocyanate ions. The existence of such complex ions is suggested by the fact that solids such as $K_2Zn(SCN)_4 \cdot 2H_2O$ have been isolated by Walden.[3] The only published account of an investigation of the ions in solution is the paper by Ferrell, Ridgion and Riley,[4] who obtained potentiometric data which suggested the existence of a $ZnSCN^+$ ion with a formation constant of the order of 50. Some unpublished polarographic measurements by DeFord[5] pointed to the existence of several complexes in solutions between 0.1 and 2.0 *M* thiocyanate ion, but the measurements were, unfortunately, not made at constant ionic strength, and since the shifts in half-wave potential observed were of the same order of magnitude as those sometimes observed due to ionic strength effects alone, no quantitative conclusions could be drawn from them. We have, therefore, measured the half-wave potential of zinc ion in potassium nitrate–potassium thiocyanate mixtures with thiocyanate concentrations ranging from 0.2 to 2.0 *M* at a constant ionic strength of 2.0 *M*.

Experimental

All measurements were made on a Sargent model XXI recording polarograph at a temperature of 30.0 ± 0.1°. The dropping electrode was made of marine barometer tubing and had a value of $m^{2/3}t^{1/6}$ of 1.355 at zero applied volts *vs.* the S.C.E. The working anode and reference potential was a saturated calomel electrode which was connected to the polarograph through a large diameter 2 *M* potassium nitrate agar bridge. All polarograms were started at −0.8 v. and run with a span voltage of 0.4 v. in order to spread out the wave for maximum accuracy of measurement. The initial

(1) This work was supported in part by the Atomic Energy Commission.

(2) On leave from the University of North Dakota, Grand Forks, North Dakota.

(3) P. Walden, *Z. anorg. Chem.*, **23**, 374 (1900).

(4) E. Ferrell, J. M. Ridgion and H. L. Riley, *J. Chem. Soc.*, 1121 (1936).

(5) D. D. DeFord, M.S. Thesis, University of Kansas, 1947.

and final span voltages were measured to ±0.1 mv. with a Rubicon portable potentiometer. The resistance of the cell system was 143 ohms and the polarograms were recorded with the recorder sensitivity set at 0.04 microampere/mm.

All chemicals were of "analytical reagent" grade. The zinc concentration was 0.001 M and each solution was also 0.0001 M in nitric acid. No maximum suppressor was found necessary; in all probability traces of agar from the salt bridge served in this capacity.

The waves were tested for polarographic reversibility by plotting the quantity log $[i/(i_d - i)]$ against E (corrected for iR drop) at eight or more points along the rising part of the curve. Good straight lines were obtained with slopes between 31.2 and 32.8 mv. per unit log term (average 32.1) and showing no trend in slope with change in thiocyanate concentration.

Half-wave potentials were estimated to the nearest 0.1 mv. using the same technique described in an earlier publication.[6] At least four independent measurements were made on each composition and the average of the concordant values taken. An idea of the precision achieved may be obtained from the eight determinations of the half-wave potential of zinc ion in 2 M potassium nitrate. The mean value was −0.9977 v. with a maximum range of 1.6 mv. and standard deviation of 0.6 mv.

At the same time, an alternative and less laborious method of estimating the half-wave potential was tried. The potential at which the current, corrected for residual current, was equal to one-half of the diffusion current, also corrected for residual current, was very carefully estimated from the polarogram. This, when corrected for iR drop, gave a value each time very close to that obtained by the longer method. Examination of the same eight polarograms of zinc ion in nitrate medium by this method gave again +0.9977 as the average value with a range of 2.1 mv. and a standard deviation of 0.8 mv. When the wave is well spread out by using a small span voltage, as in the present work, it would appear that the shorter method is capable of giving almost as good precision as the more elaborate type of measurement.

Results and Discussion

The resulting data were analyzed mathematically by the method developed by DeFord and Hume[7] and the results are summarized in Table I.

TABLE I

ANALYSIS OF $E^1/_2$ AS A FUNCTION OF THIOCYANATE CONCENTRATION

(SCN⁻), M	$E_{1/2}$, v.	i_d, μa.	$F_0(X)$	$F_1(X)$	$F_2(X)$	$F_3(X)$	$F_4(X)$
0.0	−0.9977	6.64	(1.00)	....	..	..	..
.2	1.0066	6.63	1.99	4.95	9.8	..	..
.5	1.0212	6.58	6.12	10.24	14.5	..	..
.8	1.0339	6.58	16.26	19.08	20.1	16.4	19
1.0	1.0430	6.46	33.35	32.35	29.4	22.4	21
1.2	1.0508	6.48	60.4	49.5	38.8	26.5	21
1.5	1.0599	6.51	121.1	80.0	51.4	29.6	19
1.8	1.0693	6.45	252	140	76	38	21
2.0	1.0739	6.44	357	178	88	40	20
				$K_1 = 3$	$K_2 = 7$	$K_3 = 1$	$K_4 = 20$

Figures 1 and 2 show the plots of the $F(X)$ values as a function of thiocyanate concentration. The extrapolated values for the formation constants are $K_1 = 3 \pm 0.5$, $K_2 = 7 \pm 3$, $K_3 = 1 \pm 1$ and $K_4 = 20 \pm 2$. The observed shifts in half-wave potential are small enough to create appreciable uncertainty in the numerical values of the formation constants, as indicated above, but the magnitudes of the values must certainly be correct. It seems clear that no complexes higher than $Zn(SCN)_4^=$ are formed in solutions up to 2.0 M in thiocyanate. The zinc complexes, as might be expected from a comparison of other zinc and cadmium complex systems, are much less stable than the corresponding cadmium complexes. It is striking, however,

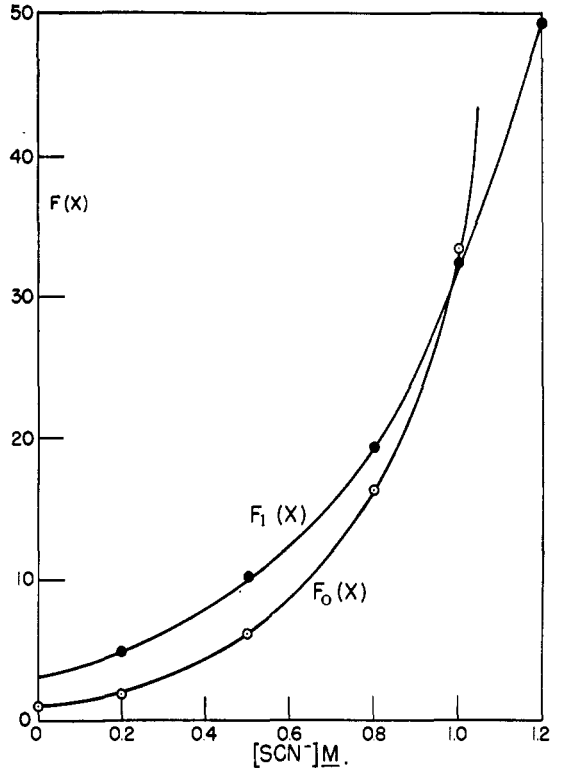

Fig. 1.—Plot of $F_0(X)$ and $F_1(X)$ and a function of thiocyanate concentration.

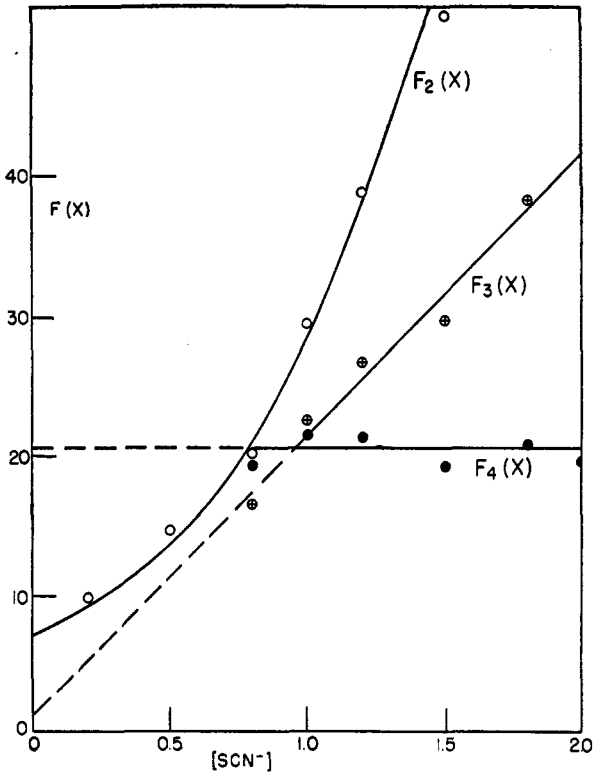

Fig. 2.—Plot of $F_2(X)$, $F_3(X)$ and $F_4(X)$ as a function of thiocyanate concentration.

(6) D. N. Hume, D. D. DeFord and G. C. B. Cave, THIS JOURNAL, 73, 5323 (1951).

(7) D. D. DeFord and D. N. Hume, *ibid.*, 73, 5321 (1951).

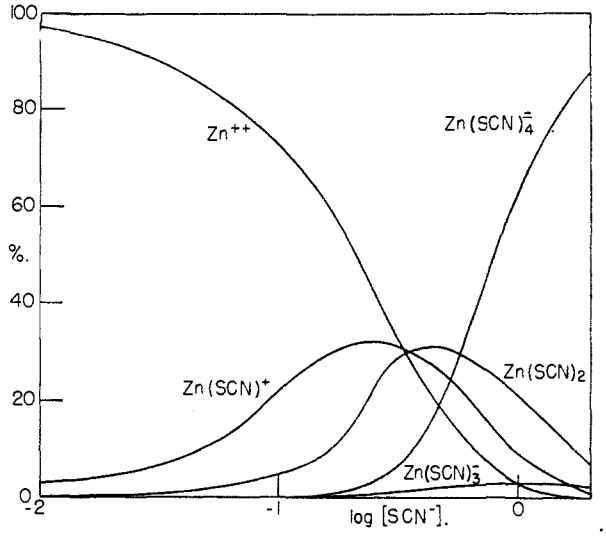

Fig. 3.—Percentage distribution of zinc in various forms as a function of free thiocyanate concentration.

that the relative stabilities of the ions in each series are very similar. The distribution of zinc among the several species as a function of equilibrium thiocyanate ion concentration shown in Fig. 3 is almost identical with the corresponding distribution for cadmium except that the whole family of curves has been displaced toward higher thiocyanate concentrations.

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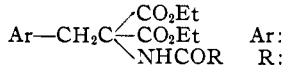
Synthesis of Isoquinolines by the Use of Acetamidomalonic Ester

BY ALEXANDER GALAT

RECEIVED NOVEMBER 21, 1952

Compounds of the formula



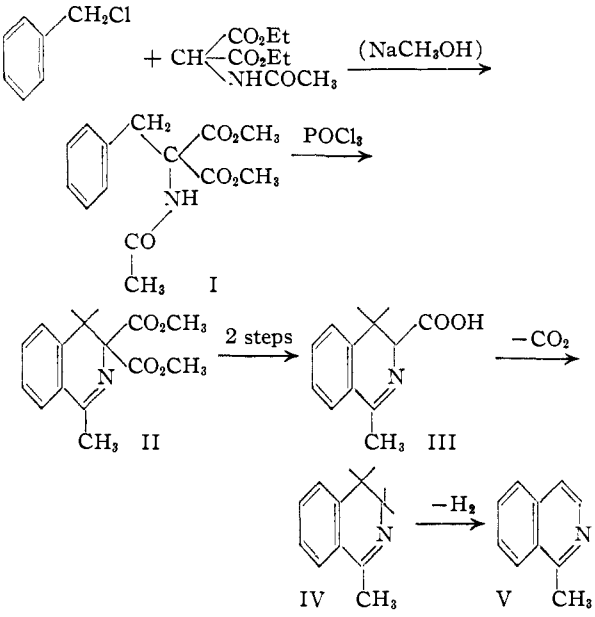
Ar: phenyl, indolyl, naphthyl
R: H, CH_3, phenyl

obtained by the condensation of the appropriate halides or Mannich bases with acylamidomalonic esters, have been used as intermediates in the synthesis of amino acids.[1] Since these compounds are readily accessible, it seemed of interest to investigate their behavior under the conditions of the Bischler–Napieralski reaction.

Methyl 2-acetamido-2-carbomethoxy-3-phenylpropionate (I), obtained by the condensation of benzyl chloride with ethyl acetamidomalonate in methanol, was used as a model substance in this study. It was readily cyclized with phosphorus oxychloride to the dihydroisoquinoline II which was degraded to 1-methylisoquinoline

This synthesis provides a route to a new group of isoquinoline derivatives (compound II) which would be difficult to prepare by any other method, as well as a simple method for the preparation of some of the known compounds. Since substituted benzyl chlorides are readily available, this synthesis should prove of interest for the preparation of a large variety of isoquinolines.

(1) C. E. Redeman and M. C. Dunn, *J. Biol. Chem.*, **130**, 341 (1939); N. F. Albertson and S. Archer, THIS JOURNAL, **67**, 308 (1945); E. E. Howe, A. J. Zambito, H. R. Snyder and M. Tishler, *ibid.*, **67**, 38 (1945); A. Galat, *ibid.*, **69**, 965 (1947).



Experimental

Methyl 2-acetamido-2-carbomethoxy-3-phenylpropionate (I) was prepared from benzyl chloride and commercial ethyl acetamidomalonate by the method of Albertson and Archer[1] except that the reaction was run in methanol.[2] The yield was 85%, m.p. 164–165°.

Anal. Calcd. for $C_{14}H_{17}O_5N$: N, 5.02. Found: N, 5.17.

1-Methyl-3,3'-dicarbomethoxy-3,4-dihydroisoquinoline (II).—Ten grams of I was dissolved in 40 ml. of phosphorus oxychloride and heated under reflux until the evolution of hydrogen chloride ceased (1–1.5 hours). The excess phosphorus oxychloride was removed *in vacuo* in a water-bath and the residue was treated with 100 ml. of cold water. The resulting mixture was stirred with charcoal, filtered and made alkaline with sodium carbonate. The product separated as an oil which soon solidified. The water-washed and air-dried product weighed 6.5 g. (70.5%). It was purified by dissolving in dilute hydrochloric acid, treating with charcoal and precipitating with ammonium hydroxide. It was finally recrystallized from methanol to give an analytically pure material, m.p. 94°.

Anal. Calcd. for $C_{14}H_{15}O_4N$: C, 64.4; H, 5.75; N, 5.37. Found: C, 64.8; H, 5.65; N, 5.50.

1-Methyl-3-carboxy-3,4-dihydroisoquinoline (III).—One gram of II was suspended in 10 ml. of boiling water and the mixture treated dropwise with 20% sodium hydroxide until the product went into solution and the alkaline reaction persisted. The hot solution was acidified with hydrochloric acid and kept in a bath of boiling water until the evolution of carbon dioxide ceased. The crystalline product which separated on cooling was filtered, washed with water and purified by recrystallization from water. There was obtained 0.65 g. of a product which melted at 160–165° with evolution of carbon dioxide and water. Analytical figures showed it to be a monohydrate.

Anal. Calcd. for $C_{11}H_{11}O_2N \cdot H_2O$: C, 63.8; H, 6.29; N, 6.77. Found: C, 64.1; H, 6.20; N, 6.90.

1-Methyl-3,4-dihydroisoquinoline (IV).—One-half gram of III was heated in a flask suspended in an oil-bath at 170–180° in an atmosphere of carbon dioxide. When the evolution of gas ceased, there remained a light-colored oil of floral odor. It was dissolved in methanol and treated with

(2) A previous study of similar types of compounds (A. Galat, *ibid.*, **73**, 3654 (1951)) showed that methyl esters give higher yields and purer products on cyclization with phosphorus oxychloride.

picric acid in methanol, giving a precipitate of a crystalline picrate, m.p. 190° (lit.[3] 190°).

1-**Methylisoquinoline** (V) was obtained by the dehydrogenation of IV in the presence of palladium–charcoal in tetralin or directly from III by a simultaneous decarboxylation and dehydrogenation, following the procedure reported previously[2] for an analogous compound. The crude base was converted to the picrate, m.p. 208–210° (lit.[4] 209–210°) and the sulfate, m.p. 248–250° (lit.[5] 246–247°).

(3) E. Spath, F. Berger and W. Kuntara, *Ber.*, **63**, 134 (1930).
(4) V. Krauss, *Monatsh.*, 11, 358 (1889).
(5) C. Pomerantz, *ibid.*, **15**, 299 (1893).

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Concentration Effect on Sedimentation Rate and its Use in Estimating Molecular Weights[1]

BY RICHARD H. GOLDER

RECEIVED JULY 31, 1952

Concentration Effect on Sedimentation Rate.—Many studies have been made relating sedimentation rate to the concentration of the sedimenting substance. The results have been found in almost all cases to follow the equation

$$S_0/S = 1 + Ac \qquad (1)$$

where S is the rate at concentration c, S_0 is the rate extrapolated to zero concentration, and A is an experimentally determined constant which may be called the specific sedimentation slope. This relation is in agreement with theoretical considerations of Burgers[2] as demonstrated by Schachman and Kauzmann,[3] and of Powell and Eyring.[4]

The common practice has been to plot the sedimentation rate against the concentration of the solution which prevails at the start of the centrifugation. During the course of the centrifugation, however, the concentration decreases continuously. This decrease, due to the sector shape of the cell and the increase in centrifugal force with increasing distance from the axis of rotation, was shown by Svedberg and Rinde[5] to follow the equation

$$c = c_1x_1^2/x^2 \qquad (2)$$

where c and c_1 are the colloid concentrations at the boundary distances, x and x_1, respectively, from the axis of rotation. The combined effects expressed by equations (1) and (2) result in a continuous increase in the sedimentation rate during each run. This increase was assumed by Sanigar, Krejci and Kraemer,[6] and was demonstrated experimentally by Lauffer.[7] The rate measured in the usual way is, therefore, a composite value, equaling the instantaneous rate at some time *after* the start of the centrifugation. The concentration at *that* particular time should be used for obtaining the correct relation between sedimentation rate and concentration; or, the rate at *any* given time, and the concentration which prevails at the same time, may be used. Kegeles and Gutter[8] related the concentrations at the mid-points between the first and last photographs of the runs to the over-all sedimentation rates. As will be shown, this is a very close approximation to the correct relation.

The correct relation between sedimentation rate and concentration may be found by substituting equations (1) and (2) into Svedberg's equation[9] defining sedimentation rate

$$S = k(\mathrm{d}x/\mathrm{d}t)/\omega^2x \qquad (3)$$

where $\mathrm{d}x/\mathrm{d}t$ is the velocity of the sedimenting boundary at the distance x from the axis of rotation, ω is the angular velocity of the rotor and k represents a constant which comprises the different corrections for reducing the observed rate to its value at the standard conditions of sedimentation in water at 20°. The combined equations may be written

$$S_0\mathrm{d}t = (k/\omega^2x)(1 + Ac_1x_1^2/x^2)\mathrm{d}x \qquad (4)$$

Taking $t = 0$ at x_1, and $t = t$ at x_2, and integrating, one obtains

$$S_0 = (k/\omega^2t)[\ln(x_2/x_1) + (Ac_1/2)(1 - x_1^2/x_2^2)] \qquad (5)$$

S_0 and A may be evaluated by the usual methods of solving simultaneous equations. Measurements from two runs at different initial concentrations would generally be best, but measurements from two intervals within a single run may be used if they are precise enough. The values of c_1 may be found in each case with the aid of equation (2). The greater the difference between the two values of c_1 used in equation (5), the more accurate will be the determination of A.

The specific sedimentation slope of rabbit myosin,[10] determined in the above manner, is about 1% lower than that determined by the method of Kegeles and Gutter. Since myosin has an unusually high change of sedimentation rate with concentration, most other substances should show even better agreement. The use of the method of Kegeles and Gutter is, therefore, fully justified for most work; further, because it is simpler, it is generally preferable to the more complicated procedure described above. When sedimentations are carried out over the full range of the cell, it is found that the concentrations at the midpoints are about 85% of the starting concentration.[11] This approximation may be used in calculating A by Kegeles and Gutter's method from ultracentrifuge studies in which more detailed information is not given. Of course, the value of S_0 obtained by either of these methods should be the same as that found by simply plotting $1/S$ against c.

Relation between Specific Sedimentation Slope and Intrinsic Viscosity.—Both theoretical[2,3,4,12] and

(1) This investigation was supported in part by a research grant from the National Cancer Institute of the National Institutes of Health, Public Health Service; and in part by an institutional grant from the American Cancer Society.

(2) J. M. Burgers, *Proc. Acad. Sci. Amsterdam*, **44**, 1045, 1177 (1941); **45**, 9, 126 (1942).
(3) H. K. Schachman and W. J. Kauzmann, *J. Phys. Colloid Chem.*, **53**, 150 (1949).
(4) R. E. Powell and H. Eyring, *Advances in Colloid Science*, 1, 183 (1942).
(5) T. Svedberg and H. Rinde, THIS JOURNAL, **46**, 2677 (1924).
(6) E. B. Sanigar, L. E. Krejci and E. O. Kraemer, *ibid.*, **60**, 757 (1938).
(7) M. A. Lauffer, *ibid.*, **66**, 1195 (1944).
(8) G. Kegeles and F. J. Gutter, *ibid.*, **73**, 3770 (1951).
(9) T. Svedberg and K. O. Pedersen, "The Ultracentrifuge," Oxford University Press, New York, N. Y., 1940.
(10) G. L. Miller and R. H. Golder, *Arch. Biochem. and Biophys.*, **41**, 125 (1952).
(11) G. L. Miller and R. H. Golder, *Arch. Biochem.*, **36**, 249 (1952).
(12) W. O. Kermack, A. G. McKendrick and E. Ponder, *Proc. Roy. Soc. Edinburgh*, **49**, 170 (1929).

TABLE I

SPECIFIC SEDIMENTATION SLOPES, INTRINSIC VISCOSITIES AND MOLECULAR WEIGHTS OF VARIOUS PROTEINS

Protein	A	$\frac{A-5}{0.75}$	$[\eta]$	Molecular weights calculated from equation (14) Using A	Using $\frac{A-5}{0.75}$	Using $[\eta]$
Carbon monoxide hemoglobin	6.4[8]	1.9	6.4[8]	68,000	43,000	68,000
Bovine plasma albumin	6.6[8]	2.1	5.8[8]	64,000	42,000	61,000
β-Lactoglobulin	7.6[11]	3.5	6.0[15]	41,000	30,500	37,000
Southern bean mosaic virus	8.9[16]	5.2	6.2[16]	7.6×10^6	6.2×10^6	6.6×10^6
Pyrophosphatase	11.9[17]	9.2	4.1[17]	89,000	81,000	60,000
Egg albumin	13.8[8]	11.7	5.3[8]	65,000	61,000	45,000
Lee influenza virus	15.8[18]	14.5	9.5[18]	6.3×10^8	6.2×10^8	5.3×10^8
Fibrinogen	23.5[19]	24.7	43.4[19]	220,000	220,000	270,000
Tobacco mosaic virus	48.6[7,a]	52.8	44.6[7]	34×10^6[a]	36×10^6	33×10^6
Myosin	193[10]	250	230[10]	400,000	440,000	420,000

[a] Calculated from two intervals in the single run of ref. 9.

experimental[7,8,10] investigations have demonstrated correlations between specific sedimentation slope and intrinsic viscosity, and these correlations have been subjected to criticism.[6,8,13,14] These relations have only been expressed implicitly, but the following explicit relations may be obtained from them. From Schachman and Kauzmann's derivations,[3] which are based on the hydrodynamic calculations of Burgers,[2] it can be deduced that

$$A \backsimeq 0.75[\eta] + 5 \qquad (6)$$

where $[\eta]$ is the intrinsic viscosity. Kermack, McKendrick and Ponder[12] indicate that for spherical and disc-shaped particles

$$A \approx [\eta] + 5.5 \qquad (7)$$

where $[\eta]$ is given a theoretical value of 1.6 for spheres and somewhat less for discs. From the thermodynamic calculations of Powell and Eyring,[4] it may be deduced that

$$A \approx [\eta] \qquad (8)$$

Lauffer[7] has presented experimental data supporting equation (8), but his data are not uniform enough to exclude (6).

In the second, third and fourth columns of Table I are shown values of A, $(A - 5)/0.75$, and $[\eta]$, respectively, for a number of proteins, which are comparatively rigid and compact, for which data are available in the literature. For these calculations, c is taken as the volume fraction. It is seen that Powell and Eyring's relation holds better for values of A less than 8, while Burgers' is generally better for values of A greater than 8. Although the agreement is not as close in either case as may be desired, it is clear that despite the preliminary nature of the theories involved, the specific sedimentation slope gives at least a first approximation of the viscosity.

It is not intended, of course, that the ultracentrifuge be generally used in place of a viscometer. The relations shown above have been brought out because of their theoretical interest, and their practical value in special cases like the following: (1) A sedimentation rate *versus* concentration curve is often obtained to find the extrapolated sedimentation constant or for other purposes, so that the data are often already available. (2) With highly precise data, like that reported by Lauffer, it is possible to estimate the viscosity and, as shown below, the molecular weight, from a single sedimentation experiment.

Calculation of Molecular Weight.—Methods of calculating molecular weight which are based on sedimentation data require also data on the partial specific volume and on either the diffusion rate or the intrinsic viscosity.

The approximate agreement between the specific sedimentation slope and the viscosity makes it possible, however, to estimate molecular weights from sedimentation rate and partial specific volume data alone. This is done by eliminating the viscosity factor from Lauffer's expression[20] for calculating molecular weights from intrinsic viscosity, sedimentation rate, and partial specific volume. Lauffer uses Simha's equation[21]

$$[\eta] = \frac{(b/a)^2}{15[\ln(2b/a) - 3/2]} + \frac{3(b/a)^2}{15[\ln(2b/a) - 1/2]} + \frac{14}{15} \qquad (9)$$

to get the axial ratio of elongated particles, a/b, from the intrinsic viscosity; and the equation of Perrin[22] and Herzog, Illig and Kudar[23]

$$\frac{f}{f_0} = \frac{(a/b)^{2/3}}{[1 - (a/b)^2]^{1/2}} \ln\left(\frac{1 + [1 - (a/b)^2]^{1/2}}{a/b}\right) \qquad (10)$$

to get the frictional factor, f/f_0, from the axial ratio. He then calculates the molecular weight from the equation

$$M^{2/3} = 6(f/f_0)S_0\eta^0_{20}\pi N(3V_{20}/4\pi N)^{1/3}/(1 - V_{20}\rho^0_{20}) \qquad (11)$$

where M is the molecular weight, η^0_{20} is the viscosity, in poises, of water at 20°, N is the Avogadro number, V is the partial specific volume of the sedimenting material at 20°, and ρ^0_{20} is the density of water at 20°.

We have combined equations (8) and (9) by graphical methods, and found that the intrinsic viscosity and frictional factor are related by the simple equation

$$[\eta] = 2.38\,(f/f_0)^4 \qquad (12)$$

It will be noted that for the special case of spherical,

(13) I. Jullander, *Arkiv. Kemi Mineral., Geol.*, **A21**, 8 (1945); *J. Polymer Sci.*, **2**, 329 (1947); **3**, 631 (1948).

(14) K. O. Pedersen, Dissertation, Univ. of Upsalla, 1945.

(15) J. W. Mehl, J. L. Oncley and R. Simha, *Science*, **92**, 132 (1940).

(16) G. L. Miller and W. C. Price, *Arch. Biochem.*, **10**, 467 (1946).

(17) H. K. Schachman, *J. Gen. Physiol.*, **35**, 451 (1952).

(18) G. L. Miller, *J. Biol. Chem.*, **169**, 745 (1947).

(19) V. L. Koenig and J. D. Perrings, *Arch. Biochem.*, **36**, 147 (1952).

(20) M. A. Lauffer, THIS JOURNAL, **66**, 1188 (1944).

(21) R. Simha, *J. Phys. Chem.*, **44**, 25 (1940).

(22) F. Perrin, *J. Phys. Rad.*, **5**, 497 (1934); **7**, 1 (1936).

(23) R. O. Herzog, R. Illig and H. Kudar, *Z. physik. Chem.*, **A167**, 329 (1933).

unhydrated particles, for which $f/f_0 = 1$, equation (12) is in good agreement with Einstein's theoretical value for these particles[24]

$$[\eta] = 2.5 \qquad (13)$$

Furthermore, equation (12) is supported in the more general case by the data shown in Table II.

TABLE II

FRICTIONAL FACTORS AND INTRINSIC VISCOSITIES OF VARIOUS PROTEINS

Protein	1 f/f^{0a}	2 $[\eta]$ calcd.[b]	3 $[\eta]$ obsd.[a]
Pepsin	1.08	3.2	5.2
Hemoglobin	1.16	4.3	5.3
Egg albumin	1.17	4.5	5.7
Helix pomatia hemocyanin	1.24	5.6	6.4
Serum albumin	1.25	5.8	6.5
Lactoglobulin	1.26	6.0	6.0
Homarus hemocyanin	1.27	6.2	6.4
Amandin	1.28	6.4	7.0
Octopus hemoglobin	1.38	8.7	9.0
Serum globulin	1.41	9.5	9.0
Thyroglobulin	1.43	10.0	9.9
Gliadin	1.60	15.5	14.6
Helix hemocyanin	1.89	30.4	18.0

[a] From a table compiled by Mehl, Oncley and Simha.[15] The values of f/f^0 were calculated from sedimentation and diffusion data by the method of Svedberg and Pedersen.[9] [b] Calculated from values in column 1 by use of equation (12).

Equations (11) and (12) may now be combined to give

$$M^{2/3} = 4.82[\eta]^{1/3} S_0 \eta^0_{20} \pi N (3V_{20}/4\pi N)^{1/3}/(1 - V_{20}\rho^0_{20}) \qquad (14)$$

$[\eta]$ may finally be replaced by its equivalent as given in equation (6) or (8). Molecular weights calculated with either substitution, and also with experimentally determined $[\eta]$, are shown in the last three columns of Table I. The last mentioned method is, of course, the equivalent of Lauffer's method.[20] It is seen that using equation (6) for values of A greater than 8, and equation (8) for values of A under 8, reasonably good approximations of molecular weights are obtained based only on sedimentation rate and partial specific volume data.

Acknowledgments.—The author wishes to thank Dr. Gail L. Miller for his help in preparing this paper, and Dr. A. L. Patterson for his advice on the mathematical expressions presented here.

(24) A. Einstein, *Ann. Physik*, **19**, 289 (1906); **34**, 591 (1911).

PHILADELPHIA, PENNA.

Utilization of Guanine by *Tetrahymena geleii*[1]

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Tetrahymena geleii has been shown to have an absolute requirement for guanine, although adenine will satisfy a portion of this requirement.[2] It has been assumed, therefore, that all purines of the organism are derived from these exogenous sources. Flavin and Graff showed combined nucleic acid purines to be derived from preformed purines in strain H by administration of labeled guanine[3] and adenine,[4] followed by isolation from the organisms of purines with approximately the same specific activity. Although these workers did not isolate the purines from the acid-soluble nucleotides, the copper precipitate from this fraction was reported to have low activity. This finding suggested the possibility of a synthesis of acid-soluble purines from non-purine precursors, as found by Abrams[5] in the case of a purine-requiring yeast. This was found not to be true for *Tetrahymena* in the studies described below.

Experimental

Guanine-8-C^{14} was prepared by a modification of the Traube[6] method. Three hundred mg. (0.0044 mole) of sodium formate[7] containing 0.6 millicurie of carbon-14 was mixed with 0.94 ml. (0.022 mole) of 90% formic acid. 475 mg. (0.0022 mole) of 2,4,5-triamino-6-hydroxypyrimidine dihydrochloride[8] was added, and the mixture refluxed for 24 hours (yields of approximately 75% were obtained after 12 hours). A trap containing sodium hydroxide pellets was placed at the top of the condenser. At the conclusion of the reaction, the excess radioactive formate was recovered by distillation.

The crude guanine was dissolved in N hydrochloric acid, partially decolorized with charcoal, and reprecipitated twice as the free base at *p*H 6, to give 315 mg. (95%) of partially purified product. The guanine was recrystallized twice as the sulfate and twice as the hydrochloride. The product gave a negative phosphotungstic acid test[9] before and after heating in dilute acid, indicating the absence of unreacted triaminohydroxypyrimidine and the intermediate formamidopyrimidine. The white guanine-8-C^{14} hydrochloride (225 mg., 54%, with more recoverable from supernatants) gave the ultraviolet absorption spectrum of pure guanine. Paper strip chromatograms in four solvent systems and ion-exchange chromatography[10] showed only one component, which also contained all the radioactivity.

Culture of Organisms.—*Tetrahymena geleii* W. was grown (4 days, 25°, in the dark) in sterile culture in one liter of medium A[11] modified as follows: Tween 80 (10 mg./ml.) was substituted for Tween 85, 12.4 mg. of guanine-8-C^{14}-HCl (10 μg. guanine/ml.) and 40 μg. uracil/ml. were used in place of the nucleic acid derivatives listed, concentrations of thioctic acid (= protogen) and the other eight vitamins were doubled. The culture was aerated by placing it in a 3.5-gallon Pyrex bottle which was rotated on its side at 10 r.p.m. by two motor-driven rollers. An air inlet and outlet, protected by sterile cotton plugs, were mounted in a swivel joint in the rubber stopper; air was supplied by an aquarium pump. Respiratory carbon dioxide was collected in 2 N sodium hydroxide and precipitated with barium chloride.

Isolations.—The procedures were similar to those previously described,[10] aliquots of various fractions being removed for radioactivity assay. After the medium had been removed from the organisms by settling in the cold, they were washed three times with 1% sucrose in the same manner, and once with water by centrifugation, wet weight *ca.* 10 g. Acid-soluble nucleotides were extracted with three 100-ml. portions of 5% trichloroacetic acid (TCA) in the Waring blendor at 0–5°, and centrifuged cold. This

(1) Supported by a grant from Research Corporation and by Contract No. AT(30-1)-1351 with the U. S. Atomic Energy Commission.

(2) G. W. Kidder and V. C. Dewey, *Proc. Natl. Acad. Sci. U. S.*, **34**, 566 (1948).

(3) M. Flavin and S. Graff, *J. Biol. Chem.*, **191**, 55 (1951).

(4) M. Flavin and S. Graff, *ibid.*, **192**, 485 (1951).

(5) R. Abrams, *Arch. Biochem. Biophys.*, **37**, 270 (1952).

(6) W. Traube, *Ber.*, **33**, 1371 (1900).

(7) Purchased from the U. S. Atomic Energy Commission.

(8) Kindly supplied by Dr. R. B. Angier, Lederle Laboratories, American Cyanamid Co.

(9) H. B. Lewis and B. H. Nicolet, *J. Biol. Chem.*, **16**, 369 (1914).

(10) M. R. Heinrich, V. C. Dewey, R. E. Parks, Jr., and G. W. Kidder, *ibid.*, **197**, 199 (1952).

(11) G. W. Kidder, V. C. Dewey and R. E. Parks, Jr., *Physiol. Zool.*, **24**, 69 (1951).

fraction was hydrolyzed with sulfuric acid, and copper purines precipitated.[12]

The TCA-extracted tissue was washed twice with cold ethanol, and lipides removed by refluxing three times with 150-ml. portions of 3:1 ethanol–ether, and once with ether; dry weight 385 mg. Combined nucleic acids were obtained by hot sodium chloride extractions, maintaining *p*H 7–8.[13] Nucleates were precipitated with 3 volumes of cold ethanol, and washed with ethanol and ether; weight 39.1 mg.

RNA and DNA were separated by hydrolysis in 2 ml. of *N* sodium hydroxide at room temperature, followed by precipitation of DNA.[14] The DNA precipitate was redissolved in 1 ml. of 0.1 *N* sodium hydroxide, immediately reprecipitated, and washed with TCA. DNA was extracted from the precipitate with hot TCA,[15] the solution made 1 *N* with hydrochloric acid, and hydrolyzed 1 hour at 100°. The solution was evaporated to dryness and a portion used for paper chromatography. The RNA contained in the DNA supernatants (0.1 ml. gave a negative cysteine–sulfuric acid test[16]) was hydrolyzed in *N* hydrochloric acid at 100° for 1 hour, and copper purines precipitated.

Copper purines of the ASN and RNA fractions were separately treated with hydrogen sulfide,[12] and the purine solutions evaporated *in vacuo* several times to remove excess acid. The residues were taken up in 0.5 *N* hydrochloric acid and separated on a column of Dowex-50.[10]

Radioactivity Assays.—All samples were counted with a thin mica window Geiger–Mueller tube. Aliquots of fractions obtained during the isolations were oxidized to carbon dioxide by wet combustion,[17] and barium carbonate plates prepared by filtration on paper. Purine fractions obtained in the ion-exchange separation were often not sufficiently pure for direct combustion and assay. The most satisfactory procedure was preliminary separation of purines on the ion-exchange column, followed by paper chromatography of portions of each purine on 3-cm. strips of Whatman No. 1 paper in Wyatt's[18] isopropyl alcohol–2 *N* hydrochloric acid solvent. Discs 14 mm. in diameter were cut from the purine spots with a sharp cork borer, counted, and eluted with 3 ml. of 0.1 *N* hydrochloric acid. Eluates were read in the Beckman DU spectrophotometer against eluates of discs cut from blank strips run at the same time. The quantity of purine present was calculated from the extinction coefficients of Wyatt,[18] correcting for any non-purine absorption by reading at 300 or 310 mμ.[19]

TABLE I

INCORPORATION OF GUANINE-8-C^{14} IN *T. geleii*

	Specific activity, c.p.m./mg. C[a]	c.p.m./μg. C[b]
Guanine administered	12,700	35.2
Respiratory CO_2, 4 days	0	
Whole cells	15	
Medium	1	
Acid-soluble compounds[c]	18	
Lipide-free tissue	186	
Combined nucleic acids	1000	
Nucleic acid-free tissue	2	
ASN guanine		33.8
ASN adenine		30.8
ASN hypoxanthine[d]		27.2
RNA guanine		31.2
RNA adenine		31.3
DNA guanine		31.2
DNA adenine		29.8

[a] Counts/min./mg. carbon, counted as barium carbonate, corrected to infinite thickness. [b] Counts/min./μg. carbon, counted on filter paper discs; this procedure results in higher specific activities than those obtained with barium carbonate plates of the same area. [c] Cold TCA extract after removal of TCA by ether extraction. [d] Arising principally by enzymatic deamination of adenine during isolation.

(12) G. H. Hitchings, *J. Biol. Chem.*, **139**, 843 (1941).
(13) R. B. Hurlbert and V. R. Potter, *ibid.*, **195**, 257 (1952).
(14) G. Schmidt and S. J. Thannhauser, *ibid.*, **161**, 83 (1945).
(15) W. C. Schneider, *ibid.*, **161**, 293 (1945).
(16) P. K. Stumpf, *ibid.*, **169**, 367 (1947).
(17) D. D. Van Slyke, J. Plazin and J. R. Weisiger, *ibid.*, **191**, 299 (1951).
(18) G. R. Wyatt, *Biochem. J.*, **48**, 584 (1951).

Results

The data of Table I show that guanine-8-C^{14} was utilized to the same extent for all the purines of the various fractions, within the accuracy of the method. It is evident that *T. geleii* W. utilizes exogenous purine exclusively, and does not synthesize purine from smaller precursors; such synthesis would dilute the activity of the guanine administered. As further evidence for lack of synthesis, no activity was found in ASN purines, total nucleic acids or on paper strips of hydrolyzed RNA and DNA after giving 30 mg. of sodium formate-C^{14} (31,000 counts/min./mg. carbon). Low activities were found in respiratory carbon dioxide during the five-day growth period.

The assistance of Miss Marjorie Anastasia is gratefully acknowledged.

(19) R. D. Hotchkiss, *J. Biol. Chem.*, **175**, 315 (1948).

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The Syntheses of 5- and 6-Chloroacetovanillone[1]

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During a study of the oxidation of chlorite lignin with nitrobenzene and alkali, 6-chlorovanillin and 6-chlorovanillic acid were isolated.[3] In anticipation of the isolation of chloro analogs of other products of the nitrobenzene oxidation of lignin, the synthesis of 5- and 6-chloroacetovanillone were undertaken.

Attempted condensation of a methyl Grignard reagent with 6-chlorovanillonitrile acetate was unsuccessful, as were the many attempted oxidations of 1-(6-chloro-4-hydroxy-3-methoxyphenyl)-1-ethanol, a compound prepared by the condensation of 6-chlorovanillin with methylmagnesium iodide. Recourse was then had to the diazomethane method for the synthesis of acetophenones from benzaldehydes.

Reaction of 6-chlorovanillin acetate with diazomethane yielded as the main product the β-hydroxyketone formed from two moles of the aldehyde with one mole of diazomethane, namely, 1,3-bis-(4-acetoxy-6-chloro-3-methoxyphenyl)-3-hydroxy-1-propanone (I). Deacetylation under mild alkaline conditions caused dehydration and yielded 6,6′-dichloro-4,4′-dihydroxy-3,3′-dimethoxychalcone (II). The β-hydroxyketone (I) was dehydrated to the diacetate of the chalcone (II) by means of acetic anhydride in pyridine. Complete hydrolysis of I yielded 6-chlorovanillin and the desired 6-chloroacetovanillone. 5-Chloroacetovanillone was prepared in similar fashion by hy-

(1) A portion of a thesis submitted to Lawrence College in partial fulfillment of the requirements of The Institute of Paper Chemistry for the degree of Doctor of Philosophy. This work was carried out under the direction of I. A. Pearl.
(2) Kimberly-Clark Corp., Neenah, Wisconsin.
(3) J. E. Jayne, Dissertation, The Institute of Paper Chemistry, 1953.

drolyzing directly the original reaction mixture obtained by treating 5-chlorovanillin acetate with diazomethane.

The mechanism of the formation of the chalcone as an intermediate in these reactions was proved by repeating the reactions with unchlorinated compounds to obtain previously known products.

Experimental

All melting points are uncorrected.

1-(6-Chloro-4-hydroxy-3-methoxyphenyl)-1-ethanol.—6-Chlorovanillin[4] was treated with methylmagnesium iodide essentially according to the procedure employed by Roberti, York and MacGregor for the preparation of analogous vanillin derivatives.[5] The carbinol, after crystallization from benzene–petroleum ether (b.p. 65–110°), melted at 128–128.5°.

Anal. Calcd. for $C_9H_{11}O_3Cl$: CH_3O, 15.3. Found: CH_3O, 15.4.

6-Chlorovanillin Acetate.—6-Chlorovanillin was treated with acetic anhydride essentially according to the preparation of vanillin acetate by Pisovschi.[6] The product was crystallized from absolute ethanol to give white needles melting at 97–97.5°.

Anal. Calcd. for $C_{10}H_9O_4Cl$: CH_3O, 13.6. Found: CH_3O, 13.7.

1,3-Bis-(4-acetoxy-6-chloro-3-methoxyphenyl)-3-hydroxy-1-propanone (I).—The diazomethane,[7] generated from 6.8 g. of nitrosomethylurea, was introduced beneath the surface of a solution of 8.6 g. of 6-chlorovanillin acetate in 250 ml. of ether kept at 0°. After 1.5 hours at 0° and 0.5 hour at 20°, the ether and excess diazomethane were removed by distillation leaving 9.0 g. of crude product melting at 115–145°. Repeated crystallization from ethanol raised the melting point to 157–158°.

Anal. Calcd. for $C_{21}H_{20}O_8Cl_2$: C, 53.52; H, 4.28; CH_3O, 13.2. Found: C, 53.72; H, 4.38; CH_3O, 13.2.

6,6'-Dichloro-4,4'-dihydroxy-3,3'-dimethoxychalcone (II).—I (4 g.) was warmed with 30 ml. of 5% sodium hydroxide solution. The deep orange solution was cooled, saturated with carbon dioxide and extracted with chloroform. Concentration of the chloroform extract yielded a crude product which, after several crystallizations from benzene–petroleum ether (b.p. 65–110°) and dilute ethanol, melted at 198.5–200° and weighed 150 mg.

Anal. Calcd. for $C_{17}H_{14}O_5Cl_2$: C, 55.30; H, 3.82; CH_3O, 16.8. Found: C, 55.38; H, 3.97; CH_3O, 16.5.

The residue from the chloroform-extracted solution was dissolved in 5% sodium hydroxide, acidified with sulfur dioxide and extracted with ether; the ether extract was concentrated to yield an additional 170 mg. of the 6-chlorochalcone melting at 194–195°.

The ether-extracted bisulfite solution was acidified and boiled; 0.4 g. of 6-chlorovanillin was obtained melting at 167–169°.

6-Chloroacetovanillone.—A solution of 2.1 g. of (I) in 80 ml. of 15% potassium hydroxide solution was refluxed for 20 hours. The solution was saturated with sulfur dioxide and the precipitate removed by filtration. The filtrate yielded a crop of white crystals during several hours under reduced pressure; the crystals weighed 150 mg. and melted from 100–105°. Crystallization from dilute ethanol raised the melting point to 109–110°.

Anal. Calcd. for $C_9H_9O_3Cl$: C, 53.88; H, 4.52; CH_3O, 15.5. Found: C, 53.70; H, 4.57; CH_3O, 15.8.

The 4,4'-Diacetoxy-6,6'-dichloro-3,3'-dimethoxychalcone (III).—Approximately 0.5 g. of I was dissolved in 2 ml. of hot pyridine, and 4 ml. of acetic anhydride was added. After standing for 20 hours, the mixture was poured onto ice. The oil which formed soon solidified; it melted at 129 to 133°. Several crystallizations from 95% ethanol raised the melting point to 137.5–138.5°.

Anal. Calcd. for $C_{21}H_{19}O_7Cl_2$: C, 55.64; H, 4.00; CH_3O, 13.7. Found: C, 55.61; H, 4.05; CH_3O, 13.7.

(4) L. C. Raiford and J. G. Lichty, THIS JOURNAL, **52**, 4576 (1930).

(5) P. C. Roberti, R. F. York and W. S. MacGregor, *ibid.*, **72**, 5760 (1950).

(6) I. J. Pisovschi, *Ber.*, **43**, 2139 (1910).

(7) F. Arndt, *Org. Syntheses*, **15**, 3 (1935).

III (180 mg.) was warmed on the steam-bath for 10 minutes with 5 ml. of ethanol and 3 ml. of 5% sodium hydroxide and then neutralized with dilute sulfuric acid. Upon cooling, a crop of yellow needles was formed which weighed 150 mg. and melted at 202–203.5°. No depression was observed in a mixed melting point with the previously described II.

5-Chlorovanillin Acetate.—5-Chlorovanillin acetate was prepared in a manner similar to that described for the 6-chloro compound. Crystallization from petroleum ether (b.p. 30–60°) gave a product melting at 63–64°.

Anal. Calcd. for $C_{10}H_9O_4Cl$: CH_3O, 13.6. Found: CH_3O, 13.8.

5-Chloroacetovanillone.—A large excess of diazomethane prepared from 45 g. of nitrosomethylurea was added to an ether solution of 7.15 g. of 5-chlorovanillin acetate at room temperature. The reaction mixture stood for several weeks. The residue was boiled briefly with 5% sodium hydroxide causing a portion of the oil to dissolve. Saturation of the alkaline solution with sulfur dioxide and extraction with ether gave an extract which was concentrated and applied to a column of acid-washed Magnesol.[8] The chromatogram was developed with 100:1 benzene–ethanol; elution of the major zone with acetone yielded a product melting at 122–125°. Its analysis and the analogy to the vanillin–diazomethane reaction showed it to be 5-chloroacetovanillone in 23% of the theoretical yield based on 5-chlorovanillin acetate. Treatment with charcoal and crystallization from dilute ethanol raised the melting point to 124–125°.

Anal. Calcd. for $C_9H_9O_3Cl$: C, 53.88; H, 4.52; CH_3O, 15.5. Found: C, 54.01; H, 4.53; CH_3O, 15.4.

6-Chlorovanillonitrile.—6-Chlorovanillonitrile acetate, (5.0 g.) was treated with methylmagnesium iodide; decomposition of the complex yielded 3.2 g. of crude 6-chlorovanillonitrile. Treatment with charcoal and crystallization from benzene gave a product melting at 151.5–152°.

Anal. Calcd. for $C_8H_6O_2ClN$: CH_3O, 16.9. Found: CH_3O, 16.7.

Acknowledgment.—The author wishes to thank Mr. Donald McDonnell and Miss Dorothy Dugas for the carbon and hydrogen analyses reported in this paper.

(8) I. A. Pearl and E. E. Dickey, THIS JOURNAL, **73**, 863 (1951).

(9) L. C. Raiford and D. J. Potter, *ibid.*, **55**, 1682 (1933).

THE INSTITUTE OF PAPER CHEMISTRY
APPLETON, WISCONSIN

Kinematic Viscosity of *n*-Heptane at Low Temperature

BY J. F. JOHNSON AND R. L. LETOURNEAU

RECEIVED OCTOBER 20, 1952

The kinematic viscosity of *n*-heptane has been measured at several temperatures below 0°. The results at the lower temperatures are very appreciably different from the values tabulated by the American Petroleum Institute Project 44,[1] which we understand represent a long extrapolation of the previously available data. Because there is increasing interest in low temperature viscosities, it is hoped that this note will be of interest.

Experimental

All viscosities were measured in the Zeitfuchs cross arm-type capillary viscometer.[2] Temperatures were measured by a platinum resistance thermometer and were constant to ±0.01°. Four samples of *n*-heptane were used. The purity was established as 99.8 mole % or better by cooling curves. Viscometers were calibrated using water at 20° with an assumed value for the viscosity of water at this tempera-

(1) American Petroleum Institute Research Project 44, Selected Values of Properties of Hydrocarbons Table 20c-E (Part 2).

(2) J. F. Johnson, R. L. LeTourneau and Robert Matteson, *Anal. Chem.*, **24**, 1505 (1952).

ture of 1.0068 centistokes. Two viscometers were used at each temperature, and the results were averaged. At 0, −40, −62° two completely independent sets of determination involving different samples, resetting the bath temperature, etc., were made. The standard deviation was less than 0.15% for all temperatures.

Results.—The results are summarized in Table I.

TABLE I

t, °C.	Viscosity, cs.	Number of detn.	Standard deviation
−17.78	0.936	14	0.00092
−28.88	1.100	6	.00074
−40.01	1.321	13	.0015
−51.09	1.637	6	.0022
−62.17	2.092	24	.0028
−73.25	2.823	5	.0033

CALIFORNIA RESEARCH CORPORATION
RICHMOND, CALIFORNIA

A Rapid Method for the Resolution of *s*-Butyl Alcohol[1]

BY SIMON W. KANTOR AND CHARLES R. HAUSER

RECEIVED NOVEMBER 28, 1952

In connection with another study we needed large quantities of optically pure *s*-butyl alcohol. The resolution of *s*-butyl alcohol through the acid phthalate ester has previously been a rather tedious process. In the usual procedure[2] the pure acid phthalate salt is dissolved in a large volume of refluxing acetone and an equimolar amount of brucine slowly added in increments, each increment being added only after the previous one has dissolved. On cooling the resulting solution, the first crop of the brucine salt of the acid phthalate ester is obtained in only relatively low degree of resolution and six to eight recrystallizations are usually required to give the pure diastereoisomer.

We have found that when equimolar quantities of the acid phthalate ester and brucine are mixed and acetone then added to the mixed solids, followed by 24 hours of refluxing the solid obtained on filtering the hot mixture is 82% optically pure. Only two or three recrystallizations of this solid are required to give the pure isomer in yields of 19–25%. Apparently continuous recrystallization takes place during the refluxing period to leave the relatively insoluble brucine salt of the *d*-isomer. It is possible that the method may be generally applicable.

Experimental

d-2-**Butanol.**—Redistilled Eastman Kodak Co. 2-butanol, b.p. 98–99.5°, was converted to the acid phthalate ester by the procedure of Pickard and Kenyon.[3] A mixture of 447 g. (2.01 moles) of *s*-butyl hydrogen phthalate (m.p. 58.5–59.5°) and 790 g. (2.0 moles) of brucine (m.p. 177–178°) was intimately mixed in a 6-l. erlenmeyer flask. Acetone (2 l.) was added and the mixture was refluxed for 24 hours. During this time, a definite change was observed in the physical appearance of the insoluble solid. Since the success of this procedure depends on all of the solid coming in contact with the acetone, care was taken to break up any solid cake that formed on the walls of the flask. The mixture was filtered hot to get the first crop of brucine salt. The solid weighed 306 g. (50%), $[\alpha]^{29}D$ −5.6° (*c* 4, ethanol); this corresponds to a salt of 82% optical purity. Two or three recrystallizations from 500 ml. of methanol gave the very pure brucine salt of the *d*-isomer in yields of 19–25%, $[\alpha]^{30}D$ −2.75° to −2.94° (*c* 4, ethanol). The filtrate of the first crop on cooling deposited more salt which was recrystallized in the usual manner[3] to give an additional 20–30% yield of the pure brucine salt.

The brucine salt was hydrolyzed by sodium hydroxide[4] to give the pure *d*-2-butanol, b.p. 98–99.5°, d^{30}_4 0.799, $n^{23}D$ 1.3955, $[\alpha]^{27}D$ +13.28° (reported d^{20}_4 0.7990, $n^{23}D$ 1.3955).[5] Since the highest value reported for *d*(+)-2-butanol is $[\alpha]^{27}D$ +13.52°,[5] our *d*-2-butanol was at least 98.2% optically pure.

(1) Supported by the Office of Naval Research.

(2) A. W. Ingersoll, "Organic Reactions," Vol. II, John Wiley and Sons, Inc., New York, N. Y., 1944, pp. 400-404.

(3) R. H. Pickard and J. Kenyon, *J. Chem. Soc.*, **99**, 45 (1911).

(4) See ref. 2, p. 402.

(5) J. Timmermans and F. Martin, *J. chim. phys.*, **25**, 431 (1928).

DEPARTMENT OF CHEMISTRY
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Reactions of Ketene Diethylacetal with 1,1,1-Trichloro-2-methyl-2-propene

BY DONALD G. KUNDIGER AND KENNETH H. FROMAN[1]

RECEIVED DECEMBER 4, 1952

A large number of publications[2] have dealt effectively with the chemistry of ketene diethylacetal and related ketene acetals. We have been interested particularly in the chemistry of 1,1,1-trichloro-2-methyl-2-propene (called herein TMP) because of its trichloromethyl structure coupled with its allylic placed bond, and interested in its allylic rearrangement to 1,1,3-trichloro-2-methyl-1-propene (called herein allyl TMP or II). Accordingly, it seemed of interest to investigate the reaction of TMP with ketene diethylacetal.

It was found that pure TMP did not react appreciably *as such* with ketene acetal when equimolar amounts were heated at 100° for 48 to 72 hours. This finding is in keeping with the fact that a common class in the inert halogen group consists of those halogenated aliphatic compounds with three or more halogens on the same carbon.[3] However, the TMP did undergo allylic rearrangement to the allyl TMP, and a 5.8% conversion to ethyl chloride *via* this allyl TMP was obtained.

At the same time a typical major portion of product in each of three runs was found as a co-distilling mixture of the allyl TMP *and* ethyl orthoacetate. This orthoester arose because either the allyl-TMP, the original TMP—or both—had caused the initial ketene acetal to polymerize accompanied by elimination of ethanol[4,5] from the various polymers. The ethanol then reacted at once with the ketene acetal still present as monomer to form the ethyl orthoacetate.

The co-distilling mixture of the allyl TMP and ethyl orthoacetate consisted of about 86% of the allyl TMP and (13–14%) of this orthoester. About 85% of the initial TMP was rearranged to the product, the allyl TMP. An over-all accounting of approximately 80% of the total initial chlorine was made in the form of obtained allyl TMP and of ethyl chloride.

(1) Work toward the M.S. degree by K. H. Froman.

(2) S. M. McElvain, in *Chem. Revs.*, **45**, 453 (1949).

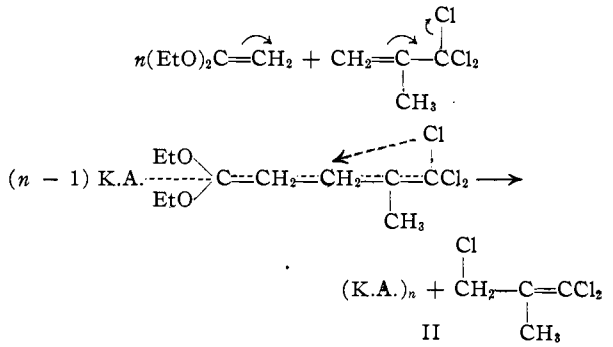
(3) S. M. McElvain, "The Characterization of Organic Compounds," The Macmillan Co., New York, N. Y., 1947, p. 90.

(4) S. M. McElvain and D. G. Kundiger, THIS JOURNAL, **64**, 254 (1942).

(5) P. R. Johnson, Barnes and McElvain, *ibid.*, **62**, 964 (1940).

It seems that the most significant finding here is the facile conversion of TMP *via* an allylic rearrangement over to the allyl TMP by means of a strong electron donor agent such as ketene acetal. Previously, conversion of TMP to this allylic isomer was brought about by heating 10 g. of TMP with 0.1 g. of hydrogen chloride at 200° overnight.[6]

The mechanism for ketene acetal causing facile conversion of TMP to the allylic isomer appears interesting. The following seems reasonable, and is postulated (K. A. stands for ketene acetal).



Experimental

Starting Materials.—1,1,1-Trichloro-2-methylpropene-2 (TMP) was prepared along the lines of the method of McElvain and Stevens[6] except that a longer reaction period of 20–30 hr. was used. TMP having b.p. 132–135° was used. *Ketene acetal* was prepared by the method of McElvain and Kundiger.[7] *Ethyl orthoacetate* was prepared for a control experiment by treating 5.8 g. (0.05 mole) of ketene acetal with 100% absolute alcohol (2.8 g., slightly more than 0.05 mole). These materials were heated under anhydrous conditions at 95–100° for 15 minutes; then distillation gave 6.5 g. of ethyl orthoacetate, observed b.p. 146–150°.

Reaction of 1,1,1-Trichloro-2-methylpropene-2 (TMP) with Ketene Diethylacetal. Formation of 1,1,3-Trichloro-2-methylpropene-1 (allyl TMP).—To a 100-ml. round-bottom flask fitted with a reflux condenser connected to a Dry Ice cooled trap, a mercury sealed mechanical stirrer and a dropping funnel, there was added TMP (35.5 g., 0.221 mole). Ketene acetal (25.8 g, (0.221 mole)) was added dropwise to the TMP (constant stirring), beginning at room temperature. There was spontaneous rise of temperature in the reaction mixture to only 40°. Then, the reaction mixture was stirred and held at 100° by a bath for 48 hours. Indication of deep red dealcoholated polymers of ketene acetal was obtained because the mixture became a clear deep red solution. Ethyl chloride, b.p. 12° (3.5 g.) collected in the Dry Ice trap. Distillation of the reaction mixture through a column fractionating setup gave the following significant fractions: (a) 3.1 g., b.p. 47–49° at a pressure of 145 mm. (This material redistilled at 71–78°, had a strong odor of ethyl acetate and contained admixed ethanol since it gave the proper ceric nitrate test and also gave the 3,5-dinitrobenzoate derivative of ethanol, m.p. 95–96°, no depression of m.p. on admixture with an authentic sample); (b) 3.2 g., b.p. 100–102° (145 mm.); (c) 26.8 g., b.p. 96–100° (125 mm.); (d) 5.6 g., b.p. 50–53° (2 mm.); (e) 3.5 g., b.p. 70–129° (2 mm.) and (f) 7.5 g., b.p. rising constantly above 129° (2 mm.).

The above fractions (b), (c) and (d) were found to be a mixture of allyl-TMP, b.p. 154° (Cl content 66.4%) and ethyl orthoacetate, b.p. 146°. These combined fractions (b), (c) and (d)—termed "product A"—redistilled at atmospheric pressure at b.p. 142–154° and 88% of it at b.p. 149–154°. Carius analyses of "product A" gave total Cl content: 52.5, 52.2, 52.7%. Based on these Carius analyses and the wt. of "product A" (35.4 g.), this mixture was 86.8% allyl TMP and 13.2% ethyl orthoacetate. Standard Zeisel semi-micro ethoxyl determinations were also carried out on "product A." Various samples of this product had 12.2 to 13.5% ethoxyl, also indicating the ethyl orthoacetate content.

Acid hydrolysis of "product A" to remove the ethyl orthoacetate and prove its presence was carried out thus: To a 100-ml. round-bottom 3-necked flask fitted with a reflux condenser, a mercury sealed stirrer, and a dropping funnel, there were added 20.5 g. of "product A"; and then with very rapid stirring throughout, 60 ml. of 5% hydrochloric acid was added from the dropping funnel, and the resulting mixture boiled vigorously for ten minutes. The mixture was cooled to 25°, the water-insoluble layer separated and, on distilling it, the lower fractions had no definite b.p. but had a strong odor of ethyl acetate; the major fraction came over at b.p. 153–155°, corresponding with the b.p. of the allyl TMP, *i.e.*, II. Carius analyses on this fraction gave 65.8% Cl as compared to 66.1% Cl obtained on known allyl TMP (calcd. Cl, 66.4).

Heating of 50 g. of TMP at 100° for 48 hr. resulted in practically no rearrangement to allyl TMP. The original TMP was recovered practically quantitatively.

Two other runs of TMP mixed with ketene acetal were made for 72 hr. and the methods and results checked those described above.

Acknowledgment.—We wish to thank Mr. H. Pledger, Jr., who aided materially in developing successful syntheses of TMP.

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(6) S. M. McElvain and C. L. Stevens, THIS JOURNAL, **69**, 2669 (1947).

(7) S. M. McElvain and D. G. Kundiger, *Org. Syn.*, **23**, 45 (1943).

The Dehydration of a Steroidal Δ^8-11α-Hydroxy-7-ketone

BY A. J. LEMIN,[1] G. ROSENKRANZ[2] AND CARL DJERASSI

RECEIVED NOVEMBER 13, 1952

In a recent synthesis of cortisone,[3] there was investigated the isomerization of the unsaturated ketol I to the corresponding saturated 7,11-dione and it was found that this could be accomplished in nearly quantitative yield by treatment with a strong base such as potassium *t*-butoxide. However, all attempts to employ acidic reagents resulted in dehydration to a dienone with ultraviolet absorption maxima at 226 and 298 mμ to which was assigned the structure of a $\Delta^{8,11}$-dien-7-one II. Since this dehydration product proved to be useless as far as the synthesis of 11-keto steroids was concerned, no further work was done with this substance.

Recently, Halsall, Jones and Lemin[4] in their degradation of the triterpene polyporenic acid A were able to demonstrate that the vinylogous β-acetoxy ketone V smoothly underwent deacetoxylation on boiling with acetic acid to afford a dienone VI with an ultraviolet absorption maximum at 318 mμ (log ϵ 3.88) in excellent agreement with the maximum (314 mμ, log ϵ 3.88) found for $\Delta^{2,4}$-cholestadien-6-one.[5] Since the chromophore in the last two mentioned substances is identical with that present in the proposed[3] structure of the dehydration product II, which, however, exhibited a maximum at 298 mμ, it was considered worthwhile to reinvestigate the structure of that substance.

(1) Syntex Postdoctorate Fellow at Wayne University, 1952–1953.

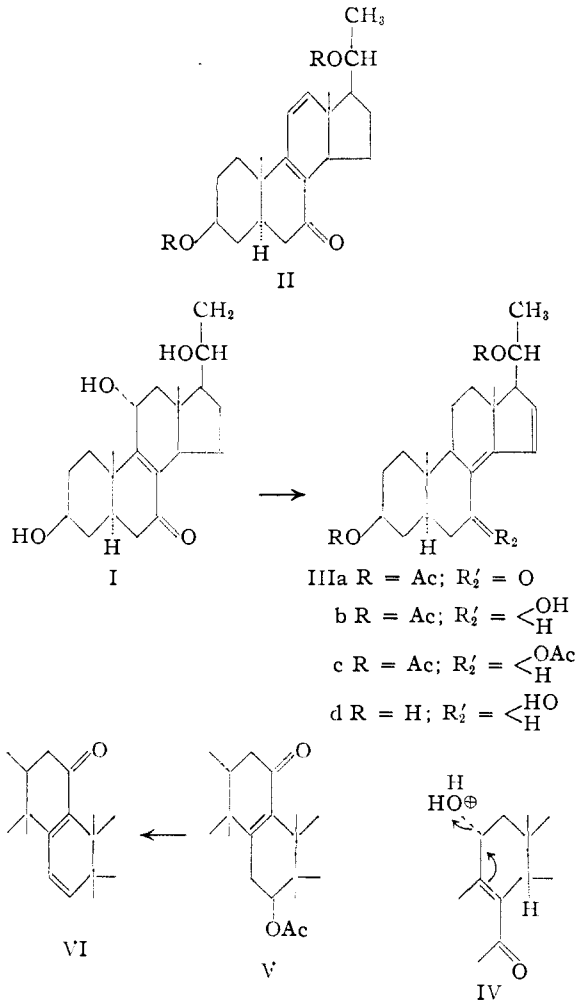
(2) Syntex, S.A., Laguna Mayran 413, Mexico City, D. F.

(3) J. Romo, G. Stork, G. Rosenkranz and C. Djerassi, THIS JOURNAL, **74**, 2918 (1952).

(4) T. G. Halsall, E. R. H. Jones and A. J. Lemin, *J. Chem. Soc.*, in press (1953).

(5) H. Reich, F. E. Walker and R. W. Collins, *J. Org. Chem.*, **16**, 1753 (1951).

If structure II were correct, then reduction of the 7-keto function[6] should afford a homoannular diene with a characteristic ultraviolet absorption maximum above 260 mμ. When the diacetate of the dehydration product of I was reduced with sodium borohydride, there was obtained an oil with an ultraviolet absorption maximum at 246 mμ. The acetylation product IIIc similarly failed to crystallize but the free triol IIId was obtained as a crystalline solid with an ultraviolet absorption maximum at 247 mμ, log ε 3.90. The position of this maximum completely eliminates a homoannular diene structure II from consideration and makes it appear most likely that the acidic dehydration of the Δ^{8}-11α-ol-7-one leads to a $\Delta^{8(14)15}$-dien-7-one IIIa. The positions of the ultraviolet absorption maxima (298 mμ for IIIa and 247 mμ for IIIb-d) are in excellent agreement with the proposed structures; the relatively low extinction (log ε *ca.* 3.9) is noteworthy. It is generally accepted now[7] that ionic 1,2-elimination in alicyclic systems proceeds most readily when both substituents are polar. Since the 11α-hydroxy group is equatorial and should thus be eliminated toward C-12 only with difficulty, the dehydration probably proceeds as indicated in IV, the initially formed $\Delta^{8(14),9(11)}$-diene rearranging in the acid medium to the thermodynamically more stable linear dienone structure IIIa.



(6) Such a procedure was employed by L. F. Fieser in the structure proof of a similar dienone in the cholesterol series (Abstracts of Ciba Foundation Conference, London, July 7-10, 1952).

(7) *Cf.* D. H. R. Barton and W. J. Rosenfelder, *J. Chem. Soc.*, 1048 (1951).

Experimental[8]

A mixture of 0.55 g. of Δ^{8}-allopregnene-3β,11α,20β-triol (I)[9] (m.p. 249–251°) was refluxed for one hour with 40 cc. of methanol and 1 cc. of concd. hydrochloric acid and then diluted with water. Filtration afforded 0.48 g. of colorless crystals with m.p. 220–225°, λ_{max}^{EtOH} 225 and 298 mμ, log ε 4.20 and 3.77, λ_{max}^{Nujol} 1665 cm.$^{-1}$ and free hydroxyl band. The physical constants are in good agreement with those reported earlier,[3] but the yield has been markedly improved. Acetylation produced **$\Delta^{8(14)15}$-allopregnadiene-3β,20β-diol-7-one diacetate** (**IIIa**) with m.p. 158–160°. λ_{max}^{EtOH} 225 and 298 mμ, log ε 4.27 and 3.80, $\lambda_{max}^{CHCl_3}$ 1730 and 1665 cm.$^{-1}$.

The above diacetate IIIa (0.175 g.) in 5 cc. of methanol and 1 cc. of dioxane was allowed to stand at room temperature for one hour with 0.01 g. of sodium borohydride. Dilution with water, extraction with ether, washing, drying and evaporation afforded 0.175 g. of an oil, IIIb, λ_{max}^{EtOH} 246 mμ, log ε 3.85. Acetylation similarly produced an oil λ_{max}^{EtOH} 246 mμ, log ε 3.90, but saponification of the triacetate (IIIc) with methanolic potassium hydroxide (2 hours refluxing) followed by recrystallization from acetone led to colorless crystals of **$\Delta^{8(14)15}$-allopregnadiene-3β,7,20β-triol** (**IIId**) with m.p. 198–200°, λ_{max}^{EtOH} 247 mμ, log ε 3.90.

Anal.[8] Calcd. for $C_{21}H_{32}O_3$: C, 75.86; H, 9.70. Found: C, 75.90; H, 10.17.

(8) Melting points are uncorrected and were taken on the Fisher block. Ultraviolet absorption spectra were determined in absolute ethanol solution; infrared spectra were measured with a Baird double beam infrared spectrometer.

(9) G. Stork, J. Romo, G. Rosenkranz and C. Djerassi, THIS JOURNAL, **73**, 3546 (1951).

DEPARTMENT OF CHEMISTRY
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Fractionation of an Enzyme by Foaming[1]

BY MORRIS LONDON, MARTIN COHEN AND PERRY B. HUDSON[2]

RECEIVED NOVEMBER 8, 1952

In this Laboratory a 15-fold purification of prostatic acid phosphatase[3] was obtained from an enzyme fraction already 300-fold purified on a wet tissue weight basis, by foaming off inactive protein. Several recent papers[4,5,6] have dealt with the foaming power and foam stability of protein solutions. Some authors have attempted to use these properties for the analysis of protein mixtures,[7] and two other investigators[8,9] used the method of foaming for preparing more or less pure proteins from a mixture of proteins.

This is a report on a series of experiments carried out on jackbean urease (Arlco brand) with the view of ascertaining the effect of various conditions on

(1) This research was supported by grants from the American Cancer Society, and the Damon Runyon Memorial Fund.

(2) Damon Runyon Memorial Fellow.

(3) The purification of this enzyme is the subject of another paper in preparation.

(4) H. Kimizuka and T. Sasaki, *Bull. Chem. Soc. Japan*, **24**, 230 (1951).

(5) F. Schütz, *Trans. Faraday Soc.*, **42**, 437 (1946).

(6) Wo. Ostwald and A. Siehr, *Kolloid-Z.*, **76**, 33 (1936).

(7) D. Peters, *ibid.*, **125**, 157 (1952).

(8) A. Dognon, "Surface Chemistry," Butterworth, London, 1949, page 253.

(9) F. Schütz, R. Bader and M. Stacey, *Nature*, **154**, 183 (1944).

the concentration of one of the components of a protein mixture (*viz.*, urease) in the foam.

The apparatus used consisted either of gas washing bottles (Corning) or of graduated cylinders fitted with fritted glass discs. The gas was supplied from a bottle partially filled with chips of Dry Ice (in the *p*H range investigated, carbon dioxide does not affect the *p*H appreciably). The foam was led from the top of the apparatus by glass tubing and a rubber hose, and collected in graduated tubes so that series of 2 to 3 ml. foam fractions could be collected.

The original material, the foam fractions ("froth") and the residue ("frothate") were analyzed for nitrogen after sulfuric acid digestion by direct nesslerization, and for urease by Sumner and Graham's method.[10] The ratio, urease/total nitrogen, was defined as "purity number." The ratio, purity number of fraction/purity number of original material, was defined as "purification."

The effect of protein concentration on the accumulation of urease in the froth was found by a series of experiments where solutions of varying protein concentrations were foamed under identical conditions. A distinct optimum, from the point of view of both purification and recovery, was found at a concentration of 0.16% (Table I).

TABLE I

EFFECT OF PROTEIN CONCENTRATION ON THE ACCUMULATION OF UREASE IN FROTH FRACTIONS[a]

Concn. % prepn.[b]	Av. purifn. (froth fractions only)	Max. purifn. (best fraction)	Activity recovd. in all fractions, %
0.125	4.1 (2)[c]	4.1	52
.160	7.2 (3)	9.7	>100[d]
.300	2.3 (4)	3.0	77
.500	1.9 (7)	2.5	72

[a] The jackbean urease was dissolved in 0.2 *M* acetate buffer *p*H 5.0. The volume foamed was 100 ml. The foaming was carried out in a gas washing bottle (Corning No. 31760, 250 ml.) with a "coarse" disk. [b] Urease preparation as weighed out was approximately 50% protein. [c] Number of froth fractions. [d] 71–78% of all urease recovered was found in the froth fractions and maximum purification, the best fraction, was usually found in a central froth fraction.

It was similarly found that an optimum *p*H of the foaming medium exists. This optimum was found to be close to the isoelectric point of urease for various protein concentrations (Table II). However, since so many variables affect this process, it is not proposed to draw any conclusions from this fact before other protein mixtures are investigated.

TABLE II

EFFECT OF THE *p*H OF THE FOAMING MEDIUM ON THE ACCUMULATION OF UREASE IN FROTH FRACTIONS[a]

*p*H	Av. purifcn. (froth fractions)	Max. purifcn. (best fraction)	Activity recovd. in all fractions, %
4.6	3.9	5.8	86
5.0	7.2	9.7	>100
5.2	3.9	3.9	55

[a] 0.16% jackbean urease was dissolved in 0.2 *M* acetate buffer. Other conditions as in Table I.

(10) J. B. Sumner and H. Graham, *Proc. Soc. Exptl. Biol. Med.*, **22**, 504 (1925). However, the material was incubated at 37° for 15 minutes and the urease was diluted with 1% bovine albumin dissolved in 0.2 *M* acetate buffer *p*H 5.0. The incubate was directly nesslerized.

The porosity of the foaming disc was also found to affect purification and recovery (Table III).

TABLE III

EFFECT OF THE POROSITY OF THE FOAMER ON THE ACCUMULATION OF UREASE IN FROTH FRACTIONS[a]

Disc designation (Corning)	Av. max. pore size (Corning), μ	Av. purifcn. (froth)	Max. purifcn.	Activity recovd. in all fractions, %
Extra coarse	160	2.6	2.7	47
Coarse	40	2.1	3.5	61
Medium	14	4.9	7.2	100

[a] 300 ml. of 0.16% jackbean urease dissolved in 0.2 *M* acetate buffer *p*H 5.0 were foamed in cylinders with liquid height of 12 cm. and foam height of 35 cm.

Acknowledgment.—The authors express gratitude to Dr. J. M. Reiner for advice and encouragement received during this work.

DEPARTMENTS OF BIOCHEMISTRY AND UROLOGY AND FRANCIS DELAFIELD HOSPITAL
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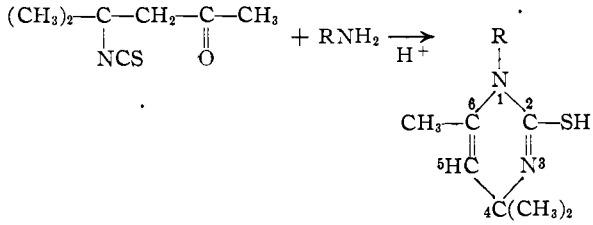
2-Pyrimidinethiols

BY ROGER A. MATHES

RECEIVED NOVEMBER 24, 1952

The preparation of 2-mercapto-1-substituted-4,-4,6-trimethyl-1H,4H-pyrimidines, obtained by reaction of 2-methyl-2-isothiocyano-4-pentanone with amines[1] and with amino acids,[2] was described in two previous papers. This series of pyrimidines has now been extended to include further examples derived from other types of amines and from hydrazines.[3]



The preparation[1] of "2-methyl-2-thiocyano-4-pentanone" used in the synthesis of 2-pyrimidinethiols was described previously. A further examination of this compound including both its chemical reactions and infrared absorption affords quite conclusive evidence that it is 2-methyl-2-isothiocyano-4-pentanone. Infrared absorption spectra measurements showed a band at about 4.9 μ, the characteristic broad band attributed to the isothiocyano group. 2-Methyl-2-isothiocyano-4-pentanone in its reaction with amines to form pyrimidines, which can be considered as cyclic thioureas, conforms to the well known reaction of isothiocyanates with amines to give thioureas. In a qualitative test for isothiocyanates,[4] 2-methyl-2-isothiocyano-4-pentanone when shaken with ammoniacal silver nitrate in aqueous alcohol gives silver sulfide readily.

(1) R. A. Mathes, F. D. Stewart and F. Swedish, Jr., THIS JOURNAL, **70**, 1452 (1948).

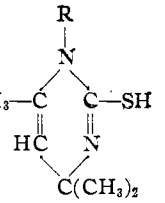
(2) R. A. Mathes and F. D. Stewart, *ibid.*, **72**, 1879 (1950).

(3) R. A. Mathes and F. D. Stewart, U. S. Patent 2,535,858 (Dec. 26, 1950).

(4) S. P. Mulliken, "Identification of Pure Organic Compounds," Vol. IV, J. Wiley and Sons, Inc., New York, N. Y., 1922, p. 17.

TABLE I

2-MERCAPTO-1-SUBSTITUTED-4,4,6-TRIMETHYL-1H,4H-PYRIMIDINES



Compd.[a]	R	Derived from	M.p., °C.[b]	Yield, %[c]	Formula	Carbon, % Calcd.	Carbon, % Found	Hydrogen, % Calcd.	Hydrogen, % Found	Nitrogen, % Calcd.	Nitrogen, % Found
1	Amino	Hydrazine	209–210	80	$C_7H_{13}N_3S$	49.09	49.11	7.65	7.70	24.54	24.57
2	*p*-Aminophenyl	Phenylhydrazine	170–171	95	$C_{13}H_{17}N_3S$	63.12	63.33	6.93	6.93	16.99	16.73
3	Methyl[d]	Methylamine	86–87	..	$C_8H_{14}N_2S$	56.43	56.47	8.29	8.17	16.46	16.42
4	Ethyl	Ethylamine	148–149	78	$C_9H_{16}N_2S$	58.65	58.75	8.75	8.87	15.20	15.13
5	*n*-Butyl	*n*-Butylamine	113–114	70	$C_{11}H_{20}N_2S$	62.21	62.28	9.50	9.57	13.19	13.11
6	Allyl[e]	Allylamine	129–130[f]	..	$C_{10}H_{16}N_2S$	61.18	61.10	8.22	8.28	14.27	14.11
7	3-Isopropoxypropyl	3-Isopropoxypropylamine	84–85	50	$C_{13}H_{24}N_2OS$	60.89	60.77	9.43	9.54	10.93	10.87
8	*p*-Nitrophenyl	*p*-Nitroaniline	201	75	$C_{13}H_{14}N_3O_2S$	56.30	56.41	5.45	5.53	15.15	14.70
9	2,4-Dichlorophenyl	2,4-Dichloroaniline	203–204	78	$C_{13}H_{14}Cl_2N_2S$	51.83	51.88	4.68	4.72	9.30	8.99
10	*o*-Mercaptophenyl	*o*-Aminobenzenethiol	172–173	79	$C_{13}H_{16}N_2S_2$	59.05	59.19	6.10	6.16	10.60	10.60
11	*p*-Hydroxyphenyl	*p*-Aminophenol	200	86	$C_{13}H_{16}N_2OS$	62.88	62.74	6.50	6.56	11.29	11.19
12	*p*-Anisyl	*p*-Anisidine	189	41	$C_{14}H_{18}N_2OS$	64.09	64.15	6.91	6.98	10.68	10.62
13	*p*-Acetylphenyl	*p*-Aminoacetophenone	189	78	$C_{15}H_{18}N_2OS$	65.66	65.65	6.61	6.46	10.21	10.11
14	Benzyl	Benzylamine	181–182	87	$C_{14}H_{18}N_2S$	68.25	68.35	7.36	7.40	11.37	11.20
15	Furfuryl	Furfurylamine	126–127	86	$C_{12}H_{16}N_2OS$	60.98	61.03	6.83	6.73	11.86	11.79
16	*p*-(α-Phenylisopropyl)-phenyl	*p*-(α-Phenylisopropyl)-aniline	173–175	76	$C_{22}H_{26}N_2S$	75.38	75.55	7.48	7.36	7.99	7.93

[a] Compounds 2 and 13 were recrystallized from benzene; 3,7 from hexane; all others from ethanol. [b] Melting points are for analytical samples and are uncorrected. [c] Yields are based on crude products. [d] The intermediate, 1-(1,1-dimethyl-3-oxobutyl)-3-methyl-2-thiourea, obtained in 93% yield, and melting at 161° (with decomposition) after recrystallization from hexane, was the initial product. *Anal.* Calcd. for $C_8H_{16}N_2OS$: C, 51.03; H, 8.57; N, 14.88. Found: C, 50.64; H, 8.48; N, 14.64. [e] The intermediate, 1-(1,1-dimethyl-3-oxobutyl)-3-allyl-2-thiourea, obtained in 83% yield, and melting at 138° after recrystallization from alcohol, was the initial product. *Anal.* Calcd. for $C_{10}H_{18}N_2OS$: C, 56.04; H, 8.46; N, 13.07. Found: C, 56.01; H, 8.39; N, 13.06. W. Traube and H. Lorenz, *Ber.*, **32**, 3156 (1899), reported a melting point of 138°. [f] W. Traube and H. Lorenz, *ibid.*, **32**, 3156 (1899), reported a melting point of 130°.

Experimental

2-Mercapto-1-substituted-4,4,6-trimethyl-1H,4H-pyrimidine. Typical Preparation.—Ethylamine (45 g., 1 mole) was added as a 25% aqueous solution,[5] over a period of 15 minutes, to a vigorously agitated mixture of 157 g. (1 mole) of 2-methyl-2-isothiocyano-4-pentanone, 300 ml. of water and 5 ml. of hydrochloric acid. The reaction mixture was heated to reflux and after cooling to room temperature, the product which precipitated was filtered, washed with water and dried.

In two instances (Table I, compounds 3 and 6) the products initially obtained were the intermediate thioureas. By heating these intermediates with an excess of 25% sulfuric acid, ring closure was effected to give the corresponding pyrimidines.

Acknowledgment.—The analyses of all compounds were made by J. R. Kubik and A. K. Kuder.

(5) Water dilution is advantageous in controlling the reaction rate when employing water soluble amines and hydrazines.

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Crystalline Sodium Lactobionate Monohydrate

BY GEORGE E. N. NELSON AND FRANK H. STODOLA

RECEIVED DECEMBER 4, 1952

The study and utilization of the bionic acids have been hampered by the lack of salts which can be readily purified by crystallization. After many attempts Isbell[1] was able to prepare crystalline calcium lactobionate, but unfortunately its gelling tendency made it of little use in purification. Recently we succeeded in preparing crystalline sodium lactobionate and find it to be readily obtainable in the pure state as a monohydrate which filters easily and is stable to heat.

(1) H. S. Isbell, *Bur. Stand. J. Res.*, 11, 713 (1933).

Experimental

Calcium lactobionate, prepared by the oxidation of lactose by *Pseudomonas graveolens*,[2] was converted to the sodium salt by reaction with the calculated amount of sodium oxalate. The filtered solution was brought to incipient cloudiness by the addition of alcohol and then stirred with seed crystals. More alcohol was then added and the crystals, which filtered rapidly, were washed successively with 70% alcohol, absolute alcohol and ether. For analysis, this product was recrystallized as follows: One gram was dissolved in the minimum amount of water (2.5 ml.) at room temperature. After addition of more water (0.5 ml.) 3 ml. of 95% alcohol was added gradually. Seed crystals were added and the solution stirred vigorously for several minutes. On standing at room temperature, clusters of bars crystallized out. After 1 day at room temperature and another day in the refrigerator, the crystals (810 mg.) were filtered off and washed as described above. After drying in a vacuum desiccator (20 mm., 25°) for a day, the compound lost no further weight at 78° (1 mm.); it was then analyzed.

Anal. Calcd. for $C_{12}H_{21}O_{12}Na \cdot H_2O$: C, 36.18; H, 5.82; Na, 5.77; H_2O, 4.51. Found: C, 36.1; H, 5.81; Na, 5.92; H_2O (Karl Fischer Method), 4.7.

A sample dried to constant weight at 140° (1 mm.) lost only 0.3% of its weight.

(2) F. H. Stodola and L. B. Lockwood, *J. Biol. Chem.*, **171**, 213 (1947).

Heated in a capillary tube, the monohydrate evolves gas rapidly at 175° (cor.) and increases in bulk about twenty-fold. It shows a rotation of $[\alpha]^{24}D$ +22.3° (c 5, H_2O).

FERMENTATION DIVISION
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(3) One of the laboratories of the Bureau of Agricultural and Industrial Chemistry, Agricultural Research Administration, U. S. Department of Agriculture. Article not copyrighted.

Acid-catalyzed Hydrolysis of Ethyl Acetate

BY OGDEN R. PIERCE AND GEORGE GORIN

RECEIVED NOVEMBER 10, 1952

In connection with other work done in this Laboratory, it was of interest to measure the rates of hydrolysis of ethyl acetate at 25° in aqueous acetone (70% by volume) with varying concentrations of hydrochloric acid as catalyst, and also to estimate the relative catalytic activities of trifluoroacetic and difluoroacetic acids. The results are reported in Table I, together with data from the literature from which interesting comparisons can be made. Each value determined in this investigation is the average of three or four determinations, and the degree of reproducibility is indicated by the indeterminate error of the mean.[1] The ester concentration was 0.2 M unless otherwise specified in the footnote.

TABLE I

Concn. catalyst, c, mole/l.	Medium	Cat. constant, k/c, $\times 10^5$, l. mole^{-1} sec.$^{-1}$
0.025 N HCl	70% acetone	4.24 ± 0.05
.05 N HCl[2]	70% acetone	4.27 ± .01
.1 N HCl	70% acetone	4.28 ± .04
.5 N HCl[3]	70% acetone	4.61
.1 N CF_3COOH	70% acetone	1.90 ± 0.02
.1 N HCl[4]	Water	10.8
.1 N CF_3COOH	Water	10.0 ± 0.1
.1 N CHF_2COOH	Water	6.09 ± 0.05
.1 N CCl_3COOH[5]	Water	10.7
.1 N $CHCl_2COOH$[6]	Water	6.30

It is seen that the reaction-rate constant divided by the concentration of catalyzing acid is constant within experimental error for 0.1, 0.05 and 0.025 N hydrochloric acid. This indicates that the rate is proportional to the concentration of catalyst within this concentration range. The catalytic constant is somewhat lower than the value previously found by Haskell and Hammett[3] in 0.5 N acid, and this is wholly analogous to the results reported for water, where the catalytic constant is 1.08×10^{-4} throughout the range 0.0002 to 0.1 N hydrochloric acid, and shows a 5% increase in 0.5 N acid.[4]

In 70% acetone at 0.1 N concentration, trifluoroacetic acid is less than half as effective as hydrochloric acid, and this must reflect the relative strength of these acids, although the relation is not linear. In water, a medium of much higher dielectric constant, the levelling effect of the solvent[7] makes the difference less marked; hydrochloric acid, trifluoroacetic acid and trichloroacetic acid all appear to be about equally strong. Even in water, however, difluoroacetic acid is weaker than any of these, and about as good a catalyst as dichloroacetic acid.

Experimental

Materials.—Ethyl acetate was a commercial C.P. product, which was purified according to Weissberger and Proskauer[8] and distilled through a 4-ft. helices-packed Todd column; the middle third boiled within 0.1° and was collected in three successive fractions which were employed interchangeably with no noticeable change in results. A portion of this product was purified again in the same way and a small center cut was collected at constant temperature. The results obtained with this preparation were the same as the rest within experimental error.

Trifluoroacetic acid was obtained from the Minnesota Mining and Mfg. Co. and distilled twice; a small center fraction was collected for use each time, and both preparations gave the same results: b.p. 70–71°, neut. equiv. 113.7 (calculated 114.0). Difluoroacetic acid was a laboratory preparation, redistilled from phosphorus pentoxide: b.p. 133.2°, neut. equiv. 95.6 (calculated 96.0).

Acetone was a C.P. product that was refluxed with potassium permanganate and distilled, the center four-fifths being retained for use. The "70% acetone" medium consisted of 70.0 ml. of acetone (54.9 ± 0.1 g.) per 100 ml. of solution.

Method.—Water and other reagents were first allowed to come to constant temperature. Two milliliters of ester was then placed in a weighed 100-ml. volumetric flask containing a little water, and the weight of the ester was determined by difference; the initial concentration of ester, a, was calculated from this weight. Water, acetone if desired, and appropriate amounts of acid were then added to the flask and the solution diluted to the mark. Immediately after mixing and at appropriate intervals thereafter 2.00-ml. aliquots were withdrawn, diluted to 20 ml. with water, and titrated with standard 0.01 N barium hydroxide to a phenolphthalein end-point. Zero time was taken as the instant at which the acid was added, and the titer at zero time was estimated by extrapolation. The concentration of catalyzing acid could thus be estimated directly, and the value compared to that calculated from the normality of the original solution and the dilution factor; an average of the two values was taken as the actual catalyst concentration. The amount of ester, x, hydrolyzed at any time, t, was calculated from the titer of barium hydroxide required in excess of the extrapolated zero-time titer. Measurements were made only in the interval to 50% reaction, so that the effect of the reverse reaction could be neglected. The reaction rate constant was then computed by means of the equation[9]

$$k = \frac{2.303}{t_2 - t_1} \log \frac{(a - x_1)}{(a - x_2)}$$

taking care to vary the intervals of time between successive measurements. Usually five or six values of k were obtained in each experiment, and the results averaged.

(1) F. Daniels, *et al.*, "Experimental Physical Chemistry," McGraw-Hill Book Co., Inc., New York, N. Y., 1949, p. 357.

(2) G. Davies and D. P. Evans, *J. Chem. Soc.*, 339 (1940), reported 4.47×10^{-5} at 24.8° and correction to 25.0° using their value for the energy of activation gives 4.55×10^{-5}. In their experiments the ester concentration was 0.05 M and the medium contained about 1.5 ml. more water per 100 ml. of solution: the higher water content is sufficient to account for the difference in rates. The rate found by the present authors for 0.1 M ester in a medium containing about 1 ml. more water was 4.60×10^{-5}.

(3) V. C. Haskell and L. P. Hammett, THIS JOURNAL, 71, 1284 (1949).

(4) H. M. Dawson and W. Lowson, *J. Chem. Soc.*, 2146 (1928).

(5) H. S. Taylor, *Medd. K. Vetenskapsakad. Nobelinst.*, 2, No. 37, 1–18 (1913).

(6) H. M. Dawson and W. Lowson, *J. Chem. Soc.*, 1217 (1929).

(7) L. P. Hammett, "Physical Organic Chemistry," McGraw-Hill Book Co., Inc., New York, N. Y., 1940, p. 256.

(8) A. Weissberger and E. Proskauer, "Organic Solvents," Oxford University Press, 1935, p. 150.

(9) W. E. Roseveare, THIS JOURNAL, 53, 1651 (1931). As pointed out in this article, the more popular alternative form of the equation, *i.e.*, $k = 2.303/t \log (a/a - x)$, gives greater statistical weight to the first measurements, which would be particularly undesirable in this case because the first titers are small differences between large numbers.

Acknowledgment.—The authors wish to express their appreciation of the assistance rendered by Mrs. Jane Hurt, who performed some of the rate measurements in aqueous solution. This work was sponsored by the Materials Laboratory, Wright Air Development Center, Dayton, Ohio.

DEPARTMENT OF CHEMISTRY
PURDUE UNIVERSITY
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N,N′-Dialkylethylenediamines by Reduction of Dialkyloxamides[1a,b]

BY LEONARD M. RICE, BERNARD H. ARMBRECHT, CHARLES H. GROGAN AND E. EMMET REID[2]

RECEIVED OCTOBER 28, 1952

Lithium aluminum hydride has proved to be an efficient agent for the reduction of acid amides to amines. It seemed to be of interest to try the reduction of N,N′-dialkyloxamides in which the two carbonyls are adjacent. This has been found to go smoothly and to give a good yield of a product which does not require extensive purification. There are several more or less satisfactory ways of preparing the N,N′-dialkylethylenediamines, but our method may prove useful in special cases, particularly for symmetrical alkyl-aryl compounds.

In order to check the identity and purity of our products we also prepared them by the reduction of the isomeric diacylethylenediamines. As in the case of the substituted oxamides, the reduction proceeded evenly to give good yields of pure dialkylethylenediamines.

$$\left.\begin{array}{l}\mathrm{RCH_2NHCOCONHCH_2R}\\ \mathrm{RCONHCH_2CH_2NHCOR}\end{array}\right\rangle \mathrm{RCH_2NHCH_2CH_2NHCH_2R}$$

This has been done with three pairs of amides with the results shown in Table I in which the boiling points, density and refractive index all checked. The diphenylurea derivatives correspond in each set.

Experimental

The Dialkyloxamides.—These were conveniently prepared by adding ethyl oxalate to a slight excess of the amine in water or in alcohol. The melting points of the products were 176° for diethyl, 154° for dibutyl, and 123° for didecyl, which were in agreement with recorded values.[3,4] The ethylene bis-amides were materials from a previous investigation.[5]

General Procedure for Reduction.—In a two-liter, three-necked flask, fitted with a dropping funnel, sealed stirrer and a long reflux condenser closed with a drying tube, a solution of 15 g. of lithium aluminum hydride in 600 ml. of anhydrous ether was prepared. The bis-amide, 30 g. in the form of a well mixed ether slurry, was added to the hydride solution at such a rate that the ether refluxed at a moderate rate.

In some cases it was found that the use of a soxhlet extractor was more convenient, although there was no appreciable increase in yield. In either case when all of the reactants had been brought together the contents were vigorously stirred under reflux for four hours and then allowed to stand overnight. The reaction mixture was decomposed by the dropwise addition of water regulated at such a rate that the capacity of the condenser was not exceeded. After reflux had ceased, a 10-ml. excess of water was added and the mixture stirred an additional hour. The suspension was filtered and the residue was washed three times with 50-ml. portions of ether. The ethereal filtrate was dried over potassium hydroxide and the ether stripped off. Distillation in vacuum over a pellet of potassium hydroxide yielded the products as colorless liquids. The decyl compound solidified in the receiver. As these liquids are strong absorbants of CO_2, it was advisable to purge the apparatus with nitrogen.

TABLE I

N,N′-DIALKYLETHYLENEDIAMINES, R—NH—CH_2CH_2—NH—R

R	Source	B.p. °C.	B.p. Mm.	Yield, %	d^{25}_4[e]	$n^{25}D$	Diphenylurea derivative, m.p., °C.
Ethyl[a]	Diacetate	148–150	760	50	0.804	1.4298	197–199[f]
Ethyl	Oxamide	148–150	760	53	.809	1.4296	197–199[f,g]
Butyl[b]	Dibutyrate	74–77	3	72	.811	1.4382	174–176[b,h]
Butyl	Oxamide	73–78	3	63	.811	1.4387	174–175[h]
Decyl[c]	Dicaprate	180–183	0.7	61	...	....	86–86.5[i,j]
Decyl[d]	Oxamide	184–190	1	56	...	....	85.5–86[i]

[a] W. R. Boon, *J. Chem. Soc.*, 307 (1947). [b] A. E. Frost, S. Chaberek and A. E. Martell, THIS JOURNAL, **71**, 3842 (1949). [c] F. Linsker and R. L. Evans, *ibid.*, **68**, 1432 (1946), recorded m.p. 0–2°, found, m.p. 28–30°. [d] Dihydrochloride. *Anal.* Calcd. for $C_{22}H_{48}N_2Cl_2$: C, 64.38; H, 11.70; N, 6.77; Cl, 17.15. Found: C, 63.71; H, 11.82; N, 6.79; Cl, 17.08. [e] ±0.005. [f] Mixed m.p. 196–197°. [g] *Anal.* Calcd. for $C_{20}H_{26}N_4O_2$: N, 15.81. Found: N, 16.02. [h] Mixed m.p. 174–176°. [i] Mixed m.p. 86–87°. [j] *Anal.* Calcd. for $C_{36}H_{58}N_4O_2$: N, 9.68. Found: N, 9.84.

(1) (a) Presented at the Meeting of the American Chemical Society, Medicinal Section, Atlantic City, N. J., September 15, 1952. (b) Supported in part by a grant from the Geschickter Fund for Medical Research, Inc.

(2) Professor Emeritus, Johns Hopkins University, Baltimore 18, Md.

(3) L. M. Rice, C. H. Grogan and E. E. Reid, THIS JOURNAL, **75**, 242 (1953).

(4) O. C. Dermer and J. W. Hutcheson, *Proc. Okla. Acad. Sci.*, **23**, 60 (1943).

(5) H. C. Chitwood and E. E. Reid, THIS JOURNAL, **57**, 2424 (1935).

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Aluminum Monochlorodilaurate, a Non-thickener for Hydrocarbons[1]

BY KAROL J. MYSELS AND DORIS MAY CHIN[2]

RECEIVED JUNE 26, 1952

Aluminum difatty acid soaps, depending on the nature of the third substituent, show marked and as yet unexplained differences in their ability to increase the viscosity of hydrocarbon solvents. The hydroxy compounds are excellent thickeners and widely used in greases and flame warfare, while the alcoxy,[3] the cresoxy[3] and perhaps the carboxy[4]

(1) Based upon the M.S. thesis of D.M. Chin, University of Southern California, Los Angeles 7, California, August, 1950, and presented in part before the Division of Colloid Chemistry during the 118th Meeting of the American Chemical Society at Chicago, Illinois.

(2) Colgate–Palmolive–Peet Fellow 1949–1950.

(3) V. R. Gray and A. E. Alexander, *J. Phys. Colloid Chem.*, **53**, 23 (1949).

(4) J. Glazer, T. S. McRoberts and J. R. Shulman, *J. Chem. Soc.*, 2082 (1950).

form solutions of very low viscosity. We have now found that the chloro compound can be prepared and is a non-thickener.

These results strongly suggest[1] that the thickening action is due to hydrogen bonding, but infrared absorption spectra[5] seem to show that no hydrogen bonds are present in these systems, which eliminates this explanation.

Experimental

The Reaction of Aluminum Chloride with Lauric Acid.—Weighed amounts of distilled anhydrous aluminum chloride and lauric acid were placed in a three-neck flask. Upon addition of cyclohexane and stirring, the reaction proceeded smoothly at room temperature. Its progress was followed by titrating the hydrogen chloride evolved. The product was frozen and the solvent removed by sublimation *in vacuo*. With two or more equivalents of lauric acid present, two equivalents of hydrogen chloride were evolved, showing that the reaction proceeded only to the formation of $AlClL_2$. Aluminum chloride gives a red color with tetralin. The products gave this same color with tetralin only when less than one equivalent of lauric acid was present. This suggests that an aluminum dichloromonolaurate as well as a monochlorodilaurate is formed. Because of the large solubility of reagents and products in hydrocarbons and their reactivity with polar solvents and traces of moisture, we have not been able to separate the components of the reaction mixture.

Aluminum Monochlorodilaurate.—Reaction of stoichiometric amounts yielded after lyophilization an extremely viscous liquid which froze at 2°. Due to this viscosity it stayed white for several days after being obtained by lyophilization. Upon standing for several weeks, or rapidly upon centrifugal compaction, it became transparent. Analysis indicated the presence of hydrolytic products and agreed with that expected if the product contained a small amount of the hydroxy dilaurate as shown by Table I.[6] It reacted rapidly with atmospheric moisture evolving hydrogen chloride and forming a white brittle coating, but the reaction did not go to completion due to protective action of the coating.

TABLE I

	Found	$AlClL_2$	Theoretical 89% $AlClL_2$ + 11% $AlOHL_2$
C	62.3	62.51	62.70
H	10.02	10.06	10.10
Al_2O_3	11.02	11.06	11.07
Cl	6.84	7.69	6.88

Solutions of Aluminum Chlorodilaurate.—Our product was very soluble in hydrocarbons and also soluble in acetone. Part of it reacted with any residual moisture present in dried acetone[9] forming an insoluble product and the remainder dissolved. The acetone solution gradually darkened due to the formation of condensation products by the solvent.

All solutions were fluid. A few relative viscosities were estimated either in an Ostwald viscometer or by the rate of rise of air bubbles, with the following results: in cyclohexane 1.3%, 1.05; 4.8%, 1.19; 27%, 4.5; in cetane 22%, 8. These increases in viscosity are negligible compared to those produced by the hydroxylaurate.[10]

The passage of moist air through hydrocarbon solutions caused the hydrolysis of only about one-half the chlorine present. Most of the remainder was removed during lyophilization of the hydrolyzed solution. During the hydrolysis of solutions, even of those containing 27% of the compound, we never observed any bulk gelation. Occasional small fragments of gel appeared but always redispersed. The viscosity of the solutions (as measured by the rate of rise of air bubbles) increased to about double the original value in early stages of hydrolysis and then decreased. This behavior is contrary to that observed by Condit[11] who studied a chlorine-containing product[12] of reaction of naphthenic acid with aluminum chloride and that reported for alcoxy and cresoxy soaps.[3,13]

(5) W. W. Harple, S. E. Wiberly and W. H. Bauer, *Anal. Chem.*, **24**, 635 (1952).

(6) The difficulty of obtaining really pure compounds of this class may be judged by the following per cent. deviations from theoretical values for some recent preparations: triacetate,[7] Al_2O_3: +5%; tripropionate,[7] Al_2O_3: +10%; trilaurate,[4] Al_2O_3: +20%; *s*-butoxy dilaurate,[8] L: −24 to −36%; *s*-butoxy stearate,[8] St: 0 to −14%; cresoxy dilaurate,[8] L: −15 to −30%; dihydroxymonolaurate,[8] Al_2O_3: −20 to +6%.

(7) G. C. Hood and A. J. Ihde, THIS JOURNAL, **72**, 2094 (1950).

(8) C. G. McGee, *ibid.*, **71**, 278 (1949).

(9) J. Timmermans and L. Gillo, *Roczniki Chem.*, **18**, 812 (1938); K. J. Mysels, *J. Phys. Colloid Chem.*, **51**, 708 (1947).

(10) K. J. Mysels, *J. Colloid Sci.*, **2**, 375 (1947); H. Sheffer, *Can. J. Research*, **B26**, 481 (1948); V. R. Gray and A. E. Alexander, *J. Phys. Colloid Chem.*, **53**, 9 (1949).

(11) D. H. Condit, U. S. Patent 2,321,463 (1943).

(12) D. H. Condit, private communication.

(13) T. S. McRoberts and J. H. Shulman, *Proc. Royal Soc. (London)*, **A200**, 136 (1949).

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The Magnetic Susceptibility of Adsorbed Paramagnetic Salts[1]

BY MAX T. ROGERS AND ROBERT VANDER VENNEN

RECEIVED DECEMBER 13, 1952

The only systematic study of the magnetic susceptibilities of adsorbed salts is that of Bhatnagar, Mathur and Kapur[2] who reported that salts of iron, cobalt, nickel and manganese lost their paramagnetism when adsorbed on charcoal, but not when adsorbed on silica gel. This behavior might be explained by postulating the formation of a covalent complex between the ion and the surface of the charcoal; the magnetic moments would then correspond to the diamagnetic, or weakly paramagnetic covalent complexes, rather than the strongly paramagnetic ionic complexes, in each case. However, the charcoal they used was itself paramagnetic so there is some question concerning the interpretation of their observations. Since data of this type should provide fundamental knowledge concerning the nature of the adsorption of ions we have reinvestigated this problem using adsorbents free of paramagnetic impurities.

Experimental

Apparatus.—Magnetic susceptibilities were measured by the Gouy method using a semi-micro balance. The electromagnet was constructed in the shop of the Chemistry Department; the rectangular yoke is built up from 2″ × 8″ mild steel bars with 4.5″ diam. pole pieces located centrally. The coils are each wound with 1440 turns of No. 8 d.c.c. magnet wire and double turns of wire are separated by spacers to allow the circulation of cooling oil through the windings. Current is supplied by a 7.5-kw. motor generator set and is controlled both by resistances in series with the windings and by varying the field resistance of the generator. Interchangeable pole pieces of various types are provided and the pole gap can be continuously varied. The field was calibrated after each change of pole gap using both 29.20% nickel chloride solution and distilled water as standards.[3] The iron was demagnetized between runs by rapidly reversing a continuously diminishing direct current through the windings. The glass susceptibility tubes were each divided by a septum and showed no change in weight with field strength. Measurements were always made at

(1) This work was performed under contract NRO57232, Nonr-02300 with the Office of Naval Research.

(2) S. S. Bhatnagar, K. N. Mathur and P. L. Kapur, *Ind. J. Phys.*, **3**, 53 (1928).

(3) P. W. Selwood, "Magnetochemistry," Interscience Publishers, Inc., New York, N. Y., 1943.

five field strengths, varying from 8,000 to 13,500 gauss, and the arithmetic mean of the values was used.

Method.—A weighed amount of adsorbent (about 7 g.) was agitated for about an hour with 10 ml. of a standard solution of the paramagnetic salt, the mixture filtered through sintered glass, the adsorbent washed several times with water, dried in an oven (110° for charcoal and 140° for silica gel) for two hours and transferred to the susceptibility tube for measurement. The filtrate and washings were combined and analyzed, the amount of salt adsorbed being determined by difference. The susceptibility of the pure adsorbent was determined in a separate experiment. When ferrous (or ferric) salts were studied the drying was carried out in an inert atmosphere and special analyses were made to determine whether any oxidation (or reduction) had occurred in handling.

Analytical methods used were as follows: (a) ferrous iron was determined by titration with cerate, (b) ferric ion was reduced in a silver reductor and the ferrous ion titrated with cerate, (c) nickel was determined gravimetrically with dimethylglyoxime, (d) cobalt was titrated potentiometrically with ferricyanide in ammonium citrate–ammonium hydroxide solution, (e) manganous ion was titrated potentiometrically with permanganate in a neutral pyrophosphate solution.

Materials.—Eimer and Amend Co. C.P. charcoal was treated with concd. sulfuric acid to remove materials which reduced cerate, washed several times with water and dried at 110° for two hours. Silica gel from Davisson Chemical Co. was used directly and showed no change in susceptibility after treatment with water and redrying at 140° for two hours. Both adsorbents were shown to be reasonably free of ferromagnetic impurities by the small change in susceptibility observed with varying field strength.

Results.—The gram susceptibilities of the adsorbed salts were calculated by the additivity rule from the observed gram susceptibilities of the mixtures, the analytical data and the susceptibility of the pure adsorbent. In each case measurements were made on several samples treated in various ways, and the values so obtained were averaged. The mean values of the gram atomic susceptibilities of the adsorbed paramagnetic metal atoms are shown in Table I along with the corresponding magnetic moments, in Bohr magnetons, calculated on the assumption that the molecular field constant Δ is zero. The range of magnetic moments commonly observed for the ion in solution or in simple salts is given for comparison, and an average value of the amount of metal ion adsorbed is shown for each ion studied. The diamagnetism of the anions has been neglected. The probable error in χ_A for the adsorbed salt is about 15% so the magnetic moments are, in general, reliable to only about ± 0.4; this precision is adequate for the purpose of the present work.

In a separate series of experiments it was shown that the charcoal used was a good adsorbent for base, a poor adsorbent for acids and, when equilibrated with a neutral salt solution (KCl), hydrogen ion was liberated from the charcoal but no change in the chloride ion concentration occurred. This indicates that the charcoal is a low-temperature air-activated material which behaves as though it were a cation exchanger.[4]

TABLE I

THE MAGNETIC SUSCEPTIBILITIES AND MAGNETIC MOMENTS OF ADSORBED IONS

Adsorbent	Adsorbate	Amount adsorbed[a]	χ_A, c.g.s. units $\times 10^{+6}$	μeff., adsorbed salt	μeff.[b] typical salts
Charcoal	$NiCl_2$	4.5	3,930	3.08	2.9–3.4
Charcoal	$CoCl_2$	4.6	10,100	4.96	4.4–5.2
Charcoal	$CoSO_4$	2.0	10,470	5.05	4.4–5.2
Charcoal	$MnSO_4$	4.5	15,450	6.13	5.2–5.96
Charcoal	$FeSO_4$	1.8	13,400	5.69	5.0–5.5
Charcoal	$Fe_2(SO_4)_3$	2.6	16,750	6.24	5.4–6.0
Charcoal	None	...	−0.462[c]	..	...
Silica gel	$NiCl_2$	7.2	4,660	3.32	2.9–3.4
Silica gel	$FeSO_4$	0.7	10,110	4.93	5.0–5.5
Silica gel	$Fe_2(SO_4)_3$	2.5	8,400	4.50	5.4–6.0
Silica gel	None	...	−0.317[c]	...	...

[a] Milligrams of metal per gram of adsorbent. [b] See ref. 3, pp. 79 and 99. [c] Gram susceptibility.

Discussion

The paramagnetic cations studied here evidently do not lose their paramagnetism when adsorbed on sugar charcoal or on silica gel. This is in qualitative agreement with the results of Bhatnagar, *et al.*,[2] for silica gel but is contrary to their findings in the case of charcoal. Since their charcoals were strongly paramagnetic and so must have contained paramagnetic (and possibly ferromagnetic) impurities, it seems possible that the losses in paramagnetism they observed resulted from desorption of impurities in the charcoal.

The magnetic moments observed here for the adsorbed ions are within the normal range of values observed for the corresponding ion in solution or in a simple salt,[3] with the exception of adsorbed ferrous and ferric ions. The origin of the discrepancies for these ions are not known but may be associated with the formation of surface oxides or hydroxides of iron. Since, in every case, the covalent complexes of the ions have much smaller magnetic moments (or are diamagnetic),[5] we conclude that the ions are held on the surface by essentially ionic bonds. This would be expected for cations adsorbed on silica gel since they presumably displace hydrogen ions from acidic hydroxyl groups on the silica gel surface. A similar ion-exchange mechanism for the adsorption of cations on charcoals which have been activated in air at low temperatures (<600°) has been postulated.[4] The charcoal surface would thus be covered by a surface oxide characterized by acidic hydroxyl groups which could exchange hydrogen ions with the cations in solution and the ions so adsorbed would be held by an essentially ionic bond to an oxygen atom of the surface.[6–8] Our results are in agreement with this hypothesis.

Acknowledgment.—We are indebted to the Office of Naval Research for support of this work.

(4) See, for example, B. Steenberg, "Adsorption and Exchange of Ions on Activated Charcoal," Almquist and Wiksells, Uppsala, 1944.

(5) L. Pauling, "Nature of the Chemical Bond," Second Ed., Cornell University Press, Ithaca, N. Y., 1940.

(6) H. L. Bennister and A. King, *J. Chem. Soc.*, 991 (1938).

(7) J. Wilson and T. R. Bolam, *J. Colloid Sci.*, **5**, 550 (1950).

(8) S. Weller and T. F. Young, THIS JOURNAL, **70**, 4155 (1948).

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Some Nitrogen Derivatives of Dibenzothiophene

BY EUGENE SAWICKI[1] AND H. S. GREENE

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It has been shown that 3-acetylaminodibenzothiophene and 3-acetylaminodibenzothiophene-5-oxide are carcinogenic to rats.[2] For this reason N-substituted derivatives of 3-aminodibenzothiophene have been prepared. The isomeric 2-substituted derivatives have also been synthesized for comparison.

(1) Cancer Research Laboratory, University of Florida, Gainesville, Fla.

(2) E. C. Miller, J. A. Miller, R. B. Sandin and R. K. Brown, *Cancer Research*, **9**, 504 (1949).

Experimental[3]

2-Benzenesulfonylaminodibenzothiophene.—To an ice-cold solution of 4 g. (0.02 mole) of 2-aminodibenzothiophene,[4] m.p. 132–133°, in 20 ml. of pyridine was added dropwise 3.0 ml. (0.024 mole) of benzenesulfonyl chloride. The violet solution was refluxed for one hour. The mixture was cooled and then poured into 200 ml. of dilute hydrochloric acid. A brown oil precipitated. Within two hours the oil solidified. Two crystallizations from acetic acid gave 5.1 g. (76%) of lustrous white crystals, m.p. 171–172°. The compound was soluble in alcohol, acetone, benzene, pyridine and acetonitrile. It was moderately soluble in chloroform and very slightly soluble in heptane.

Anal. Calcd. for $C_{18}H_{13}NO_2S_2$: N, 4.13; S, 18.9. Found: N, 4.03; S, 18.9.

3-Benzenesulfonylaminodibenzothiophene.—This compound was prepared from 3-aminodibenzothiophene,[5] m.p. 124–125°, by the procedure used for the 2-isomer. Crystallization from chlorobenzene and then alcohol gave 71% colorless needles, m.p. 198–199°.

Anal. Calcd. for $C_{18}H_{13}NO_2S_2$: N, 4.13; S, 18.9. Found: N, 4.06; S, 18.8.

3-(N⁴-Acetylsulfanilamido)-dibenzothiophene.—The same procedure was followed as for the other sulfonamines. Slightly more than a molar equivalent of pure *p*-acetylaminobenzenesulfonyl chloride[6] was used. Crystallization from aqueous acetic acid gave a 78% yield of colorless crystals, m.p. 269–270°.

Anal. Calcd. for $C_{20}H_{16}N_2O_3S_2$: N, 7.07. Found: N, 6.94.

2-(N⁴-Acetylsulfanilamido)-dibenzothiophene.—Crystallization from acetic acid gave a 58% yield of colorless crystals, m.p. 215–216°.

Anal. Calcd. for $C_{20}H_{16}N_2O_3S_2$: N, 7.07. Found: N, 6.93.

2-Sulfanilamidodibenzothiophene.—To 1.5 g. (0.0038 mole) of 2-(N⁴-acetylsulfanilamido)-dibenzothiophene suspended in 40 ml. of boiling alcohol was added slowly 15 ml. of concentrated hydrochloric acid. The clear solution was refluxed for one hour. Colorless needles precipitated. An almost quantitative yield of the hydrochloride was obtained, m.p. 252 dec. To the hydrochloride suspended in water an excess amount of dilute ammonium hydroxide was added. The gummy precipitate was crystallized out of methanol. Colorless crystals (1.2 g., 90%) were obtained, m.p. 175–176°.

Anal. Calcd. for $C_{18}H_{14}N_2O_2S_2$: N, 7.91. Found: N, 7.87.

3-Sulfanilamidodibenzothiophene.—To a suspension of 1.0 g. (0.0025 mole) of 3-(N⁴-acetylsulfanilamido)-dibenzothiophene in 40 ml. of boiling alcohol was added 15 ml. of concentrated hydrochloric acid. The mixture was refluxed an hour. Charcoal was added to the clear solution. The mixture was refluxed an additional half-hour and filtered hot. Dilute ammonium hydroxide was added to the clear hot solution until a definite turbidity was formed. The solution was allowed to cool. The crystals were collected on a buchner funnel and washed with water. Colorless microcrystals (0.80 g., 90%) were obtained, m.p. 254–255° dec.

Anal. Calcd. for $C_{18}H_{14}N_2O_2S_2$: N, 7.91. Found: N, 7.97.

***n*-Propyl N-2-Dibenzothienylcarbamate.**—To an ice-cold solution of 1.00 g. (0.005 mole) of 2-aminodibenzothiophene in 10 ml. of pyridine was added dropwise 1.85 g. (0.015 mole) of *n*-propyl chlorocarbonate. The mixture was allowed to stand at ice-water temperature for 30 minutes and was then poured into cold dilute hydrochloric acid. Crystallization from heptane gave 0.72 g. (51%) of colorless glistening needles, m.p. 117.5–119°.

Anal. Calcd. for $C_{16}H_{15}NO_2S$: S, 11.23. Found: S, 11.10.

***n*-Propyl N-3-Dibenzothienylcarbamate.**—This compound was prepared from 3-aminodibenzothiophene by the procedure used for the 2-isomer. Crystallization from heptane gave an 84% yield of colorless crystals, m.p. 103–104°.

Anal. Calcd. for $C_{16}H_{15}NO_2S$: S, 11.23. Found: S, 11.48.

2-(*o*-Carboxybenzoylamino)-dibenzothiophene.—To a suspension of 9.4 g. (0.064 mole) of phthalic anhydride in 80 ml. of warm xylene, a solution of 12 g. (0.06 mole) of 2-aminodibenzothiophene in 80 ml. of warm xylene was added. The mixture was warmed for half an hour and then allowed to stand at room temperature for half an hour. The pasty precipitate was collected on a buchner funnel and dissolved in 10% sodium hydroxide solution. The filtered solution was acidified with dilute hydrochloric acid. Crystallization of the precipitate from methanol gave 10 g. (48%) of colorless microcrystals, m.p. 208–210° dec.

Anal. Calcd. for $C_{20}H_{13}NO_3S$: S, 9.22. Found: S, 9.39.

3-(*o*-Carboxybenzoylamino)-dibenzothiophene.—This compound was prepared from 3.0 g. of 3-aminodibenzothiophene by the procedure used for the 2-isomer. Crystallization from methanol gave 2.9 g. (57%) of colorless microcrystals, m.p. 286–289° dec.

Anal. Calcd. for $C_{20}H_{13}NO_3S$: S, 9.22. Found: S, 9.42.

2-Benzalaminodibenzothiophene.—A solution of 0.20 g. (0.001 mole) of 2-aminodibenzothiophene in 3 ml. of alcohol was refluxed for 15 minutes with 0.10 ml. (0.001 mole) of benzaldehyde. Solidification took place when the cooled supersaturated solution was scratched or seeded. Crystallization from aqueous methanol gave 0.23 g. (80%) of colorless plates, m.p. 121–122°.

Anal. Calcd. for $C_{19}H_{13}NS$: S, 11.2. Found: S, 11.0.

3-Benzalaminodibenzothiophene.—This compound was prepared from 3-aminodibenzothiophene by the procedure used for the 2-isomer. Crystallization from hexane gave a 90% yield of yellow needles, m.p. 160°.

Anal. Calcd. for $C_{19}H_{13}NS$: S, 11.2. Found: S, 11.1.

3-(4′-Dimethylaminobenzalamino)-dibenzothiophene.—A solution of 0.40 g. (0.002 mole) of 3-aminodibenzothiophene in 8 ml. of alcohol was refluxed for one hour with 0.30 g. (0.002 mole) of *p*-dimethylaminobenzaldehyde. The Schiff base was precipitated by adding the cooled mixture to excess water. Two crystallizations from methyl cellosolve gave 0.53 g. (80%) of yellow crystals, m.p. 195–196°.

Anal. Calcd. for $C_{21}H_{18}N_2S$: S, 9.70. Found: S, 9.51.

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(3) All melting points are uncorrected.

(4) H. Gilman and J. F. Nobis, THIS JOURNAL, **71**, 274 (1949).

(5) R. K. Brown, R. G. Christiansen and R. B. Sandin, *ibid.*, **70**, 1748 (1948).

(6) S. Smiles and J. Stewart, "Organic Syntheses," Coll. Vol. I, John Wiley and Sons, Inc., New York, N. Y., 1948, p. 8.

Preparation of Lithium Fluoborate

BY I. SHAPIRO[1] AND H. G. WEISS[1]

RECEIVED MARCH 13, 1952

In the course of studying the reaction of lithium aluminum hydride with boron trifluoride etherate[2] it became necessary to prepare a quantity of pure lithium fluoborate. In addition to the well-known methods of preparing lithium fluoborate from aqueous solutions,[3] Baumgarten and Bruns[4] prepared lithium fluoborate in milligram quantity by the reaction of gaseous boron trifluoride with lithium carbonate heated at 300°.

$$3Li_2CO_3 + 9BF_3 \xrightarrow{300°} 6LiBF_4 + 3CO_2\uparrow + (BOF)_3\uparrow \quad (1)$$

The product was found to contain about 4% lithium fluoride. This impurity can be attributed to

(1) Research Department, Mathieson Chemical Corporation, Pasadena, California.

(2) I. Shapiro, H. G. Weiss, M. Schmich, Sol Skolnik and G. B. L. Smith, THIS JOURNAL, **74**, 901 (1952).

(3) Since fluoborate ion is known to hydrolyze, the lithium fluoborate that can be obtained from an aqueous solution is of questionable purity.

(4) P. Baumgarten and W. Bruns, *Ber.*, **72B**, 1753 (1939).

the dissociation of lithium fluoborate since at 300° the calculated dissociation pressure is *ca.* 675 mm.[5]

We have found it possible to prepare pure lithium fluoborate by using the same reactants given in eq. 1 but by carrying out the reaction in an ethereal solution at 35°. From measurements of the volume of gas evolved after each addition of small portions of boron trifluoride etherate to a slurry of lithium carbonate in anhydrous ethyl ether (Fig. 1), the stoichiometry of the reaction was ascertained to be

$$3Li_2CO_3 + 8BF_3 \xrightarrow{\text{ether}} 6LiBF_4 + 3CO_2\uparrow + B_2O_3 \quad (2)$$

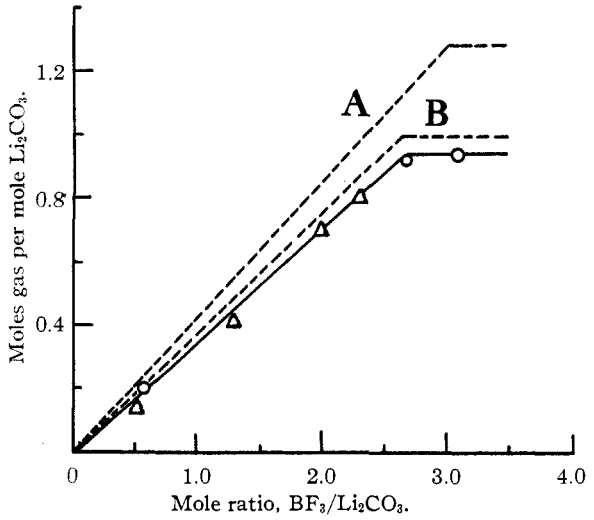

Fig. 1.—Gas evolution as a function of mole ratio of reactants: A, eq. (1); B, eq. (2).

From molecular weight determinations and vapor tension measurements (28 mm. at −111.8°) the gas was identified as (pure) carbon dioxide. The amount of boric oxide present as an end-product of the reaction was shown by titration to be in good agreement with that expected from eq. 2. Before dissolving the boric oxide all boron present as the fluoride complex was removed by ignition. The ignited solids were dissolved in water, the *p*H of the solution was adjusted to the phenolphthalein endpoint and the boric oxide was titrated with standard hydroxide after the addition of mannitol. Chemical analysis of the solid obtained by evaporation of the filtered ethereal solution indicated lithium fluoborate of 99.5% purity. The analysis consisted of measuring the loss in weight (boron trifluoride evolved) upon heating the solid, and then converting the resulting lithium fluoride to lithium sulfate.

The apparatus and techniques employed in this study have been described previously.[2] A typical experiment for preparing lithium fluoborate was as follows: To a slurry of 8.15 g. (0.110 mole) of lithium carbonate[6] in 400 ml. of dry ether was added dropwise 25 ml. (0.198 mole) of boron trifluoride etherate. The mixture was stirred vigorously and the ether refluxed during the addition of the boron trifluoride (0.5 hour) and for a period of three hours following the addition. After 20 minutes standing to permit settling of the solids, the supernatant liquid was transferred to a flask where the ether was evaporated under vacuum at room temperature. The lithium fluoborate was washed with a small quantity of ether and then dried, first, by passing dry nitrogen gas over the solid and, finally, by heating overnight in an oven at 80–90°. A further recovery of the lithium fluoborate remaining in the original solid was made by an extraction with ether. Six and one-half grams of lithium fluoborate was obtained from the original filtrate and one extraction. This quantity was only slightly less than that expected from the solubility of lithium fluoborate which was found by precipitation as nitron fluoborate[7] to be 1.3 g./100 ml. of ether at 25°.

The spacings and intensity of lines for powder diffraction data on lithium fluoborate are included here since these data apparently have not been published previously. The X-ray diffraction data were obtained with a cylindrical camera of 5.73 cm. radius using CuK_α radiation filtered through nickel foil. Line intensities were estimated visually as follows: 4.76 ms, 3.33 s, 3.19 s, 2.57 f, 2.39 s, 2.37 f, 2.27 f, 2.03 s, 1.89 vvf, 1.81 f, 1.73 f, 1.68 f, 1.59 f, 1.46 vvf, 1.43 vf, 1.36 vvf, 1.31 vvf, 1.28 vf, 1.22 vf, 1.18 vvf, 1.13 vvf, 1.06 vvf, 1.02 vvf, 0.994 vvf, 0.942 vvf, 0.931 vvf, 0.906 vvf, 0.849 vvf, 0.828 vvf.

Acknowledgment.—The authors are grateful to Dr. L. A. Burkardt for preparing and measuring the X-ray photographs.

(5) Calculated from the data of Klinkenberg as reported by H. S. Booth and D. R. Martin, "Boron Trifluoride and Its Derivatives," John Wiley and Sons, Inc., New York, N. Y., 1949, p. 98.

(6) The purification of all reagents has been described in detail in ref. 2.

(7) W. Lange, *Ber.*, **59**, 2107 (1926).

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Preparation of Isoasparagine by the Phthaloyl Method

BY STUART W. TANENBAUM[1]

RECEIVED SEPTEMBER 26, 1952

Recent methods developed for the syntheses of phthaloylglutamine[2] and of glutamine itself[3] based upon the γ-directive influence of the N-phthaloyl group coupled to the smooth procedures[4,5] for its removal, led us to attempt a similar approach for the aspartic acid homolog. This mode of asparagine formation has already been described in detail by King and Kidd.[6] Corollary to these experiments we had noted that the intermediary compound N-phthaloylaspartic anhydride (I), yields upon reaction with ammonia followed by dephthaloylation, asparagine (II), isoasparagine (III) or a mixture of the two; the direction of ring opening being dependent upon the nature of the solvent used during ammonolysis. It may be of some theoretical and practical interest to record conditions under which the different isomers are formed and to delineate the preparation and identification of isoasparagine.

The selective ring opening of phthaloylaspartic anhydride with ammonia in aqueous alcohol to give predominantly N-phthaloylisoasparagine is in contradistinction to what takes place with phthaloylglutamic anhydride under the same conditions.[2] Furthermore, ammonolysis in aqueous ether yields a mixture of N-phthaloylasparagine and N-phthal-

(1) Post-doctoral Fellow of the American Cancer Society recommended by the Committee on Growth of the National Research Council.

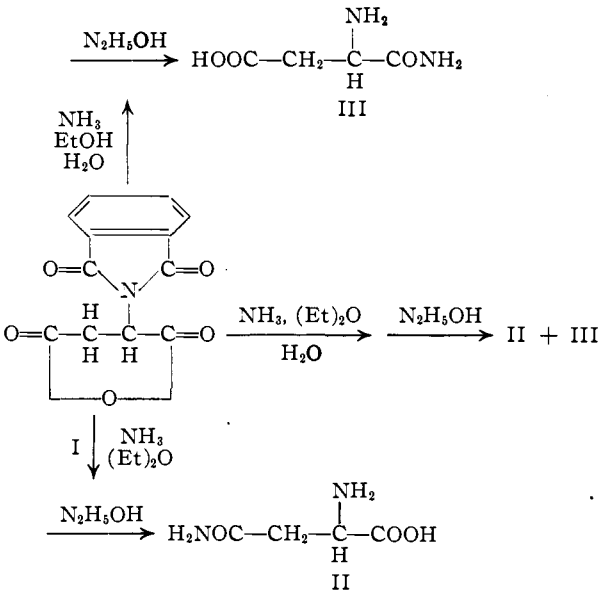
(2) J. C. Sheehan and W. E. Bolhofer, THIS JOURNAL, **72**, 2469 (1950).

(3) F. E. King and D. A. A. Kidd, *J. Chem. Soc.*, 3315 (1949).

(4) J. C. Sheehan and V. S. Frank, THIS JOURNAL, **71**, 1856 (1949).

(5) F. E. King and D. A. A. Kidd, *Nature*, **162**, 776 (1948).

(6) F. E. King and D. A. A. Kidd, *J. Chem. Soc.*, 2976 (1951).



oylisoasparagine, whereas the use of anhydrous ether produces the asparagine derivative almost exclusively.[6] A somewhat similar example of the effect of solvent upon ring opening with ammonia has come to light recently.[7] In this case, β-propriolactone reacted with amines in water to give the hydracrylamides, while in some circumstances ring opening in ether favored the formation of β-alanine derivatives. For the ammonolysis of phthaloylaspartic anhydride it would appear then that two competing reactions are also involved and that under the influence of a polar solvent α-amide formation predominates. The hypothesis is offered that in the presence of water or of aqueous alcohol an acid-catalyzed attack by hydrogen or ammonium ion occurs at the α-carbonyl, whereas base-catalyzed attack takes place at the opposite end of the molecule.

Asparagine can readily be distinguished from isoasparagine either microbiologically, by microscopic examination, or by means of paper partition chromatography. By far the most critical and sensitive method for the detection of one isomer in admixture with the other is the latter technique. The biological assay, however, offers the possibility of being the simplest method for the quantitative micro-estimation of asparagine.

Experimental

Phthaloylaspartic Anhydride.—A mixture of L-aspartic acid (13.3 g., 0.1 mole) and phthalic anhydride (14.8 g., 0.1 mole) in 200 ml. of pyridine was refluxed for two hours. The solvent was distilled off at reduced pressure leaving a glassy yellow residue. This sirup was triturated with acetic anhydride at room temperature which resulted in the precipitation of phthaloylaspartic anhydride. After several hours in the cold the crude anhydride was filtered off, washed with dry ether followed by anhydrous dioxane to give 18.7 g. (76% yield) of product, m.p. 224–225°. Several recrystallizations from dioxane–ether raised the m.p. to 227–228°.

Anal. Calcd. for $C_{12}H_7O_5N$: C, 58.8; H, 2.9. Found: C, 58.8; H, 2.9.

N^{α}-Phthaloylisoasparagine.—The addition of 90 ml. of 1 *N* alcoholic ammonia (0.09 mole) to 10 g. (0.041 mole) of phthaloylaspartic anhydride resulted in a slightly exothermic reaction to give a pale yellow solution. The alcohol and excess ammonia were removed by evaporation *in vacuo* at a bath temperature not over 40°. After dilution with water the dropwise addition of 6 *N* hydrochloric acid to congo red precipitated N^{α}-phthaloylisoasparagine, m.p. 215–216°. The product was recrystallized twice from water; final yield 8.6 g. (80%), m.p. 220–222°.

Anal. Calcd. for $C_{12}H_{10}O_5N_2$: C, 55.0; H, 3.8. Found: C, 55.0; H, 3.8.

Isoasparagine.—In individual trial experiments the phthaloyl grouping was removed from N^{α}-phthaloylisoasparagine by refluxing with alcoholic hydrazine[4] and by the procedure of dissolving the compound in aqueous sodium carbonate followed by reaction with hydrazine hydrate at room temperature.[3] Acidification with hydrochloric acid precipitated phthalhydrazide in each case, and the filtrates were analyzed by paper chromatography. For rapid analyses at this point the horizontal filter paper method of Rutter[8] proved most convenient. It was found in this manner that although a small amount of asparagine was present in these filtrates, the predominant reaction product was isoasparagine, irrespective of the method used for removal of the phthaloyl substituent.

One and seven-tenths grams (0.0065 mole) of N^{α}-phthaloylisoasparagine was added to 0.51 ml. of 64% hydrazine hydrate in 100 ml. of absolute alcohol and the mixture refluxed for one hour. After distillation *in vacuo*, the remainder was dissolved in water and 6 *N* hydrochloric acid was added dropwise to *p*H 2.6. The resultant precipitate of phthalhydrazide was removed, and the filtrate was treated with Amberlite IR-4B resin (sodium salt) in a batchwise manner until *p*H 6 was attained. The neutral solution was then decanted from the settled resin, combined with washings, and slowly passed through a column 1 × 15 cm. of the same resin. The eluates were concentrated under reduced pressure, and the isoasparagine was precipitated by the addition of absolute alcohol. This precipitate gave equivocal growth response when tested auxanographically with *Neurospora* mutant S1007, indicating that some asparagine was present as a contaminant. Additional recrystallization from water–alcohol and from water–acetone produced needles (0.35 g., 36%) of pure isoasparagine hydrate, m.p. browned at 235°, but did not melt up to 285°. This preparation was now biologically inactive with the above test organism, and could not be distinguished chromatographically from an authentic sample of isoasparagine kindly supplied by Dr. J. P. Greenstein. A specimen dried at 100° was analyzed.

Anal. Calcd. for $C_4H_8O_3N_2$: C, 36.4; H, 6.1; N, 21.2; amide N, 10.6. Found: C, 36.7; H, 5.8; N, 20.5; amide N, 10.6.

Mixture of the Amides; Asparagine.—Amidation of phthaloylaspartic anhydride in ether without the exclusion of water followed by working up as detailed above for isoasparagine afforded a 60% yield of the N^{α}-phthaloylamides, m.p. 219–224°. Chromatographic analyses of the aspartic amides obtained from this mixture indicated both asparagine and isoasparagine to be present in roughly equal quantity.

Strict adherence to the procedure of King and Kidd for the ammonolysis, followed by removal of the phthaloyl group and isolation of the amide as outlined above gave prisms of asparagine hydrate in 40% yield. It contained but a trace of isoasparagine when examined chromatographically and was fully biologically active.

Anal. Calcd. for $C_4H_8O_3N_2 \cdot H_2O$: H_2O, 12.0; α-amino N (after drying), 10.6. Found: H_2O, 11.8; α-amino N (after drying), 10.4.

The optical properties of the amides formed by these procedures were not examined. Conditions for the synthesis of stereochemically pure peptides by the phthaloyl method have recently been described by Sheehan and co-workers.[9]

Paper Chromatography.—Butanol–water–acetic acid mixture proved to be a satisfactory solvent for separating the two compounds (Table I). Both substances are ninhydrin positive, asparagine giving yellow to tan colored spots which turn purple after several days standing, while isoasparagine

(7) T. L. Gresham, J. E. Jansen, F. W. Shaver, R. A. Bankert and F. T. Fiedorek, THIS JOURNAL, **73**, 3168 (1951).

(8) L. Rutter, *Nature*, **161**, 435 (1948).

(9) J. C. Sheehan, D. W. Chapman and R. W. Roth, THIS JOURNAL **74**, 3822 (1952).

gives an immediate wine colored reaction. In this solvent system, excess or unreacted hydrazine appears as a bright yellow spot superimposed upon the isoasparagine spot following ninhydrin treatment.

TABLE I

R_f VALUES AND COLOR REACTIONS OF ASPARAGINE AND ISOASPARAGINE IN BUTANOL–WATER–ACETIC ACID (40:50:10) AND IN WATER-SATURATED PHENOL

18 hour ascending chromatograms; spray reagent 0.25% ninhydrin in butanol

	Phenol		Butanol–water–acetic acid	
	R_f	Color	R_f	Color
Isoasparagine	0.39	Wine[a]	0.14	Wine
Asparagine	.40	Yellow-tan	.095	Yellow-tan

[a] Unless the phenol is allowed to air dry for 24 hours, even pure samples of isoasparagine will show a tan-colored halo around the wine spot due to interaction with the residual phenol at 100°.

Biological Assay.—Since only asparagine is active in supporting growth of *Neurospora* mutant S1007, the auxanographic plate technique[10] is applicable for differentiating between asparagine and isoasparagine on a semi-micro scale. Here, the addition of a crystal of test substance to a minimal agar plate heavily seeded with the microörganism followed by incubation at 30° for eighteen hours indicates the presence of asparagine by a zone of growth. Both D- and L-isomers can serve to fulfill this nutritional requirement. The quantitative determination of asparagine by means of measuring growth of the organism in liquid cultures will be described elsewhere.

(10) M. J. Beijerinck, *Arch. Neerl. Sci.*, **23**, 367 (1889).

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Copolymerization of Vinyl Acetate with a Cyclic Disulfide

BY W. H. STOCKMAYER, R. O. HOWARD AND J. T. CLARKE

RECEIVED OCTOBER 24, 1952

In the course of an extensive study of chain transfer in the free-radical polymerization of vinyl acetate, the high reactivity of disulfides as compared with monosulfides was observed. For example, the following transfer constants C were obtained at 60°: di-*n*-butyl sulfide, 0.026; di-*n*-butyl disulfide, 1.0; diethyl dithioglycolate, 1.5. These figures are the slopes of plots[1] of reciprocal number-average polymerization degree P_n^{-1} against the mole ratio S/M of transfer agent to monomer, for low-conversion polymers initiated by benzoyl peroxide or azo-bis-isobutyronitrile. The values of P_n were obtained viscometrically with the aid of a viscosity–molecular weight relation to be described elsewhere.

It should be mentioned that the disulfides caused considerable retardation of the polymerization of vinyl acetate; for example, at a benzoyl peroxide concentration of 10^{-2} M the addition of 5.5×10^{-3} M and 2.7×10^{-2} M dibutyl disulfide reduced the polymerization rate to about 40 and 1.5%, respectively, of the value for pure vinyl acetate. Under such conditions, the true transfer constant C may be less than the slope of P_n^{-1} against S/M. At worst, however, this slope becomes $2C$, so that the values given above still display the high reactivity of the disulfides.

The above figures suggest that the transfer reaction in disulfides involves the scission of the disulfide link (compare the "induced decomposition" of benzoyl peroxide[2])

$$R'M_n\cdot + RSSR \longrightarrow R'M_nSR + RS\cdot \qquad (1)$$

The lability of this bond under more extreme conditions is of course well known. If reaction (1) correctly depicts the transfer process, a *cyclic* disulfide would be capable of copolymerization with vinyl monomers

$$R'M_n\cdot + \overset{R}{\underset{S}{\diagup\diagdown}}\!\!S \longrightarrow R'M_nSRS\cdot \qquad (2)$$

provided the resulting radical were sufficiently reactive to propagate the chain

$$R'M_nSRS\cdot + M \longrightarrow R'M_nSRSM\cdot, \text{ etc.} \qquad (3)$$

If this scheme holds, a cyclic disulfide would give an apparently *low* transfer constant by the molecular-weight method, but the polymer would contain a large amount of combined sulfur.

We have demonstrated that the reaction actually follows this course to a considerable extent, using for this purpose the cyclic disulfide 1-oxa-4,5-dithiacycloheptane,[3] $\boxed{SC_2H_4OC_2H_4S}$. An impure but adequate sample of this material was prepared[3] by cracking of the related disulfide polymer; from its properties (45.1% S, $n^{25}D$ 1.5711, d_{25}^{25} 1.261) the purity of the product is about 90% if the sole contaminant is the related monosulfide, *p*-oxathiane. The properties of a series of low-conversion vinyl acetate polymers prepared at 60° in the presence of various concentrations of the cyclic disulfide are given in Table I. The values of P_n^{-1} fall on a fair straight line against S/M, yielding an apparent transfer constant of about 0.25. However the high sulfur content (7.07% S) of the last polymer corresponds to a mole ratio 0.11 of disulfide to vinyl acetate in the polymer, and therefore to an actual transfer constant of about 2.5. Since P_n for this sample is about 90, there are on the average about nine disulfide units per polymer molecule, so that the predicted copolymerization is clearly substantiated. We may remark, in view of the impurity of our disulfide and of a retardation comparable to that found with dibutyl disulfide, that the transfer constants given above are only approximate.

TABLE I

POLYMERIZATION[a] OF VINYL ACETATE IN PRESENCE OF A CYCLIC DISULFIDE, $\boxed{SC_2H_4OC_2H_4S}$, AT 60°

S/M	$[\eta]^b$	P_n	% S[c]
0	1.24	2200	
0.010	0.31	310	
.023	.26	250	
.035	.15	110	
.045	.12	90	7.07

[a] Initiator, 5×10^{-3} M azo-bis-isobutyronitrile. [b] Limiting viscosity number of low-conversion polymer in acetone, 25°. [c] Weight per cent. of sulfur in the polymer.

Obviously this reaction could in principle be used to prepare certain "block" copolymers by polymerizing vinyl monomers in the presence of polymeric disulfides. However, retardation such as that evi-

(1) F. R. Mayo, THIS JOURNAL, **65**, 2324 (1943).

(2) K. Nozaki and P. D. Bartlett, *ibid.*, **68**, 1686 (1946).

(3) F. O Davis and E. M. Fettes, *ibid.*, **70**, 2611 (1948).

dent in our work would present a practical limitation.

Since completing these experiments, we learned[4] that Tobolsky and Baysal[5] have demonstrated this reaction in the case of styrene. We thank the American Chicle Company for a grant in aid of this investigation, Dr. D. H. Johnson for details of the cyclic disulfide preparation, and S. M. Nagy for the sulfur analysis.

(4) A. V. Tobolsky, private communication, July 7, 1952.

(5) A. V. Tobolsky and B. Baysal, THIS JOURNAL, **75**, 1757 (1953).

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The Reaction between Styrene and Ring Disulfides: Copolymerization Effected by the Chain Transfer Reaction

BY A. V. TOBOLSKY AND B. BAYSAL

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Disulfides such as dibutyl disulfide are active chain transfer agents in the polymerization of vinyl and diene monomers such as styrene and butadiene. The transfer reaction probably proceeds as

$$R'(CH_2CH\varphi)_xCH_2CH\varphi\cdot + RSSR \longrightarrow R'(CH_2CH\varphi)_xSR + RS\cdot$$

$$RS\cdot + CH_2 = CH\varphi \longrightarrow RSCH_2CH\varphi$$

It was therefore conceived that if a ring disulfide was present in a vinyl polymerization, the chain transfer process would result in the incorporation of the ring disulfide molecule in the growing polymer chain; *i.e.*, a copolymerization would be effected by the elementary reaction of chain transfer.

$$R'(CH_2CH\varphi)_xCH_2CH\varphi\cdot + \overset{\diagup R\diagdown}{S\text{—}S} \longrightarrow R'(CH_2CH\varphi)_xSRS\cdot$$

$$R'(CH_2CH\varphi)_xSRS\cdot + CH_2CH\varphi \longrightarrow R'(CH_2CH\varphi)_xSRSCH_2CH\varphi\cdot \xrightarrow{\text{etc.}}$$

The direct consequence of these considerations is that if a vinyl monomer is polymerized in the presence of a large amount of an open chain disulfide such as dibutyl disulfide, two sulfur atoms should be incorporated in every polymer chain. On the other hand, polymerization of a vinyl monomer in the presence of a ring disulfide such as diethyl ether disulfide,[1,2] should produce polymers with more than two sulfur atoms per polymer chain.

To test this hypothesis we polymerized styrene in the presence of varying amounts of dibutyl disulfide and diethyl ether disulfide. The polymerizations were carried out for 48 hours at 130° followed by 48 hours at 150° in the absence of catalysts. Oxygen was rigorously excluded from the system. The polymers were then twice precipitated in methanol and weighed. Sulfur analyses of the polymers were carried out, and the molecular weights of the polymers determined by measurement of the intrinsic viscosities, using the relation of Mayo, *et al.*[3] This relation was also verified by Pepper[4] for low molecular weight polymers. The use of this relation is only approximate for styrene polymers prepared in the presence of large amounts of disulfide, particularly if the ring disulfide is incorporated in the polymer chain.

The results of these experiments are shown in Table I. Two facts are especially noteworthy. The polymerizations effected in the presence of large amounts of ring disulfide gave a larger weight of polymer than the weight of styrene incorporated in the charge. Also, the number of sulfur atoms per chain in the case of these polymers was much larger than two, whereas in the case of polymers prepared in the presence of dibutyl disulfide the number of S atoms per chain was approximately two.

The results shown in Table I provide a clear indication that a significant difference results in the polymerizations carried out in the presence of chain and ring disulfides, which can only be accounted for by an effective copolymerization in the case of the ring disulfides.

TABLE I

Chain transfer agent (A)	Charge ratio, c.c.A:c.c. styrene	Total weight charge,[a] g.	Total weight polymer, g.	$[\eta]$
Dibutyl disulfide	1:3	3.64	2.64	0.239
Dibutyl disulfide	2:3	4.56	2.11	.145
Diethyl ether disulfide	2:3	5.26	4.37	.048
Diethyl ether disulfide	1:3	3.99	3.25	.094
Diethyl ether disulfide	0.5:3	3.35	2.86	.109
Diethyl ether disulfide	0.3:3	3.10	2.53	.175
Diethyl ether disulfide	0.1:1	2.85	2.56	.492

Chain transfer agent (A)	Charge ratio c.c.A:c.c. styrene	$\overline{Mn}$[b]	% S in polymer	% S in polymer/% S in charge	S atom per chain
Dibutyl disulfide	1:3	24600	0.35	0.038	2.69
Dibutyl disulfide	2:3	11800	1.04	.072	3.84
Diethyl ether disulfide	2:3	2570	9.20	.410	7.34
Diethyl ether disulfide	1:3	6560	5.43	.369	11.1
Diethyl ether disulfide	0.5:3	7950	2.72	.310	6.75
Diethyl ether disulfide	0.3:3	15200	0.96	.168	4.56
Diethyl ether disulfide	0.1:1	52500	1.05	.495	17.2

[a] Density of styrene at 20° = 0.905; density of dibutyl disulfide at 20° = 0.919; density of diethyl ether disulfide at 20° = 1.274. [b] Number average molecular weight.

We wish to thank Mr. F. O. Davis and the Analytical Department of the Tiokol Corporation for carrying out the sulfur analyses of the polymer samples.

(1) E. Fettes and F. O. Davis, THIS JOURNAL, **70**, 2611 (1948).

(2) A. V. Tobolsky, F. Leonard and G. P. Roeser, *J. Polymer Sci.*, **8**, 604 (1948).

(3) F. R. Mayo, R. A. Gregg and M. S. Matheson, THIS JOURNAL, **73**, 1691 (1951).

(4) D. C. Pepper, *J. Polymer Sci.*, **7**, 347 (1951).

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Synthesis of Radioactive Noradrenaline

BY RICHARD W. SCHAYER[1]

RECEIVED NOVEMBER 26, 1952

The synthesis of α-C^{14}-*dl*-noradrenaline (norepinephrine, arterenol) was accomplished by known procedures[2] modified for small scale use suitable for the preparation of high activity material.

Chloroacetylcatechol, 210 mg., was converted successively to noradrenalone, 93 mg., noradren-

(1) Supported in part by a research grant from the U. S. Public Health Service.

(2) W. Langenbeck and F. Fischer, *Pharmazie*, **5**, 56 (1950).

alone hydrochloride, 77 mg., and noradrenaline, 53 mg., a 28% yield. The activity was 8.5×10^4 c.p.m. per mg. Identity and purity of the noradrenaline were established by microanalysis, pharmacological activity and isotope dilution assay.

Full experimental details for this synthesis are available on microfilm.[3]

(3) For full experimental details of this synthesis order Document 3847 from American Documentation Institute, c/o Library of Congress, Washington 25, D. C., remitting $1.25 for microfilm (images 1 inch on standard 35-mm. motion picture film) or $1.25 for photostats readable without optical aid.

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Temperature Coefficients of Rotation of Some *o*- and *p*-Nitrophenyl Glycosides and their Polyacetates[1]

BY JACK A. SNYDER AND KARL PAUL LINK

RECEIVED NOVEMBER 15, 1952

Pigman[2] has suggested that the anomalous positive rotations of the *ortho*-substituted phenyl β-D-glycoside tetraacetates are due to "interactions between the acetyl and aglycon groups. Such interaction might take the form of weak bonds between these groups or it might operate through steric hindrance to free rotation of the aglycon group about the glycosidic linkage." This was postulated on the basis of the large temperature coefficients of rotation of these glycosides in contrast to those of their *m*- and *p*-isomers. We have determined the rotations of several *o*- and *p*-nitrophenyl glycosides and their polyacetates over the range 15–45°, and find that the *ortho* compounds have large temperature coefficients while the *para* compounds have normal coefficients. This indicates that the acetate groups are not directly concerned with the production of large temperature coefficients and favors their explanation on the basis of steric hindrance.

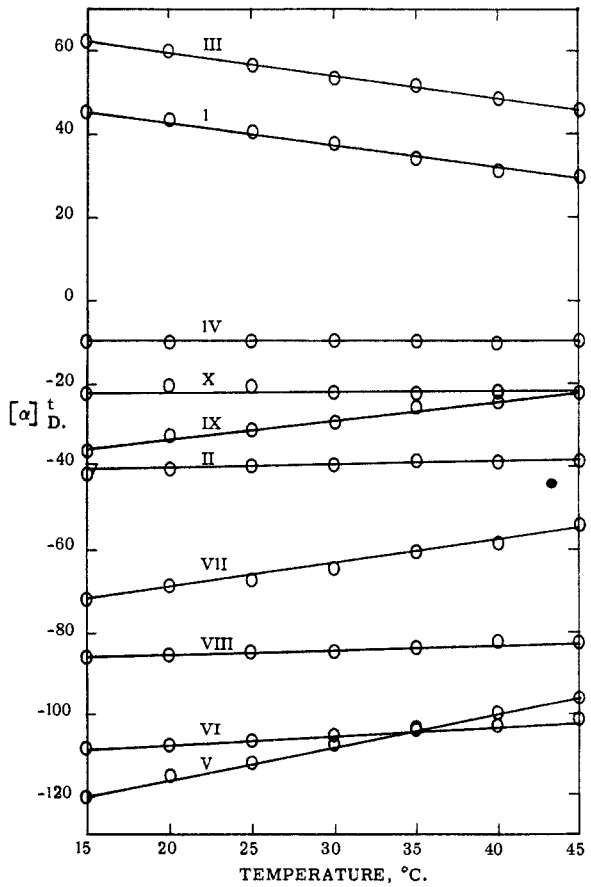

Fig. 1.—Influence of temperature on specific rotation of some *o*- and *p*-nitrophenyl glycosides and their polyacetates.

Experimental

Change of Specific Rotation with Temperature.—The method of preparation of the compounds studied has been reported previously.[3] Rotations were determined with a Schmidt and Haensch polarimeter No. 52-b with monochromator. A 2-dm. jacketed tube was used, with water, maintained at $t \pm 0.2°$ by means of a thermostatically controlled water-bath, as the circulating fluid. No correction was made for liquid density change with temperature.

TABLE I

SOLVENTS AND CONCENTRATIONS IN DETERMINATION OF CHANGE OF SPECIFIC ROTATION WITH TEMPERATURE

Compound	Solvent	Concn., %
o-Nitrophenyl β-D-glucoside tetraacetate (I)	Chloroform	1.966
p-Nitrophenyl β-D-glucoside tetraacetate (II)	Chloroform	1.884
o-Nitrophenyl β-D-galactoside tetraacetate (III)	Chloroform	1.865
p-Nitrophenyl β-D-galactoside tetraacetate (IV)	Chloroform	1.983
o-Nitrophenyl β-D-glucoside (V)	Water	0.828
p-Nitrophenyl β-D-glucoside (VI)	Water	0.987
o-Nitrophenyl β-D-galactoside (VII)	Water	1.065
p-Nitrophenyl β-D-galactoside (VIII)	Water	0.980
o-Nitrophenyl α-L-arabinoside (IX)	Water	.290
p-Nitrophenyl α-L-arabinoside (X)	Water	.265

(1) Published with the approval of the Director of the Wisconsin Agricultural Experiment Station. Supported in part by the Research Committee of the Graduate School from funds supplied by the Wisconsin Alumni Research Foundation.

(2) W. W. Pigman, *J. Research Natl. Bur. Standards*, **33**, 129 (1944).

(3) J. A. Snyder and K. P. Link, THIS JOURNAL, **74**, 1883 (1952).

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The Sedimentation Constant of Insulin in Acid Solution: A Re-examination[1]

BY FRANK TIETZE AND HANS NEURATH

RECEIVED NOVEMBER 1, 1952

On the basis of their observations on the sedimentation and diffusion constants of bovine insulin in acid solution, Fredericq and Neurath[2] concluded that the minimum molecular weight of this protein was about 6000. Although this conclusion has received support from the more recent work of Harfenist and Craig[3] on counter-current distribu-

(1) This work has been supported by the Lilly Research Laboratories, Eli Lilly and Company, Indianapolis, Indiana, to whom we are also indebted for the supply of crystalline insulin.

(2) E. Fredericq and H. Neurath, THIS JOURNAL, **72**, 2684 (1950).

(3) E. J. Harfenist and L. Craig, *ibid.*, **74**, 3087 (1952).

tion studies of insulin partially substituted by dinitrofluorobenzene, other investigations, notably osmotic pressure[4] and light scattering[5,6] measurements, have failed to yield a minimum molecular weight of less than 12,000.[7] In view of these discrepancies it was deemed of importance to re-examine the sedimentation behavior of insulin under conditions similar to those of Fredericq and Neurath.[2] These results of these more detailed measurements are the subject of the present communication.

In the present measurements, certain improvements in technique over those of the preceding study[2] were made in an effort to increase the precision of sedimentation analyses of low molecular weight portions. These have included (1) use of a Gaertner microcomparator for measurement of peak displacements and (2) use of the "synthetic boundary cell" of Harrington, Schachman and Pickels[8] in place of the conventional cell. With regard to (1), it may be remarked that the previous practice of determining peak displacements from enlarged projections of the photographic plates was found to lack precision particularly when low protein concentrations and slow sedimentation rates were involved, which give rise to rapid boundary blurring. As regards (2), use of the synthetic boundary cell served to eliminate one of the chief difficulties previously encountered with insulin, particularly in dilute solutions: when sufficient time was allowed to elapse for the boundary to separate from the meniscus so as to reveal the region of the maximum gradient, boundary spreading had occurred to such an extent as to impair the precise location of the boundary peak. With the synthetic boundary cell, however, the boundary becomes visible almost immediately after the centrifuge has attained full speed and photographs can be taken while the gradient curve is still sharp and its position well defined.

Preliminary experimentation with the synthetic boundary cell revealed that the rotor speed at which boundary formation occurred was a critical factor. Irregular results were obtained when boundary formation took place outside the limits of 5000 to 10,000 r.p.m. and hence all such data were discarded. While this procedure is admittedly arbitrary and influenced by the characteristics of the particular cell which has been used, it did serve to minimize scatter of the data.

All runs were performed in a Spinco Model E ultracentrifuge at 59,780 r.p.m. at an average rotor temperature of 24–27°. Each individual run was extended over a time not exceeding 1 hour during which the average temperature rise was not more than 1°. Sedimentation constants were calculated in the usual manner from the slope of a plot of log x *vs.* time, where x is the distance in cm. of the maximum ordinate of the gradient curve from the center of rotation. Correction of the observed sedimentation constants to standard conditions (20°, water as solvent) was carried out in the customary manner.[9]

Phosphate buffers, *p*H 2.6, of ionic strength 0.1, 0.2 and 0.4, respectively, were prepared by the addition of phosphoric acid to calculated quantities of potassium dihydrogen phosphate. Crystalline zinc insulin, Lot T-2842, was used since the supply of Lot T-2344, employed by Fredericq and Neurath,[2] was exhausted. These two lots, however, appear to be of the same degree of purity.

The results of sedimentation measurements in phosphate buffer of varying ionic strength are shown in Fig. 1. For comparison, the experimental data previously reported[2] are also included in Fig. 1. Although considerable scatter of the data is evident at 0.1 ionic strength, particularly at lower protein concentrations, this curve appears to lie convex relative to the axis of the abscissas. This somewhat anomalous behavior, previously observed,[4] is in accord with the suggestion that at a salt concentration of 0.1 ionic strength the sedimentation potential is not entirely suppressed[4] throughout the range of insulin concentrations employed here.[10] In higher insulin concentrations the resulting decrease in sedimentation rate is apparently overcome by the increased aggregation of the protein, for the slope of the curve relating sedimentation constant to protein concentration becomes positive beyond 0.8% insulin. In view of these observations, no linear extrapolation of these data to zero protein concentration was attempted.

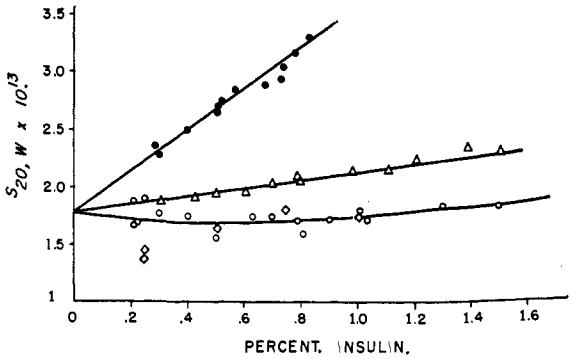

Fig. 1.—Concentration dependence of the sedimentation rate of insulin in phosphate buffers, *p*H 2.6; the ionic strength was: ●, 0.4; △, 0.2; ○, 0.1. The previous data of Fredericq and Neurath[3] are indicated by ◇.

At ionic strengths of 0.2 and 0.4 the sedimentation constants increase with increasing protein concentration throughout the entire range. The concentration dependence, moreover, appears to be linear in each case and permits unambiguous extrapolation to zero concentration. The extrapolated sedimentation constant is 1.78 Svedberg units (S). This value is considerably higher than that previously reported[3] (1.2 S) but is in fair agreement with that reported by Gutfreund[4] for a 0.25% solution of Boots insulin (1.65 S). While it is conceivable

(4) H. Gutfreund, *Biochem. J.*, **42**, 544 (1948); **50**, 564 (1952).

(5) F. Tietze and H. Neurath, *J. Biol. Chem.*, **194**, 1 (1952).

(6) P. Doty, M. Gellert and B. Rabinovitch, THIS JOURNAL, **74**, 2065 (1952); P. Doty and G. E. Myers, *Trans. Faraday Soc.*, in press.

(7) The sedimentation analyses of Ellenbogen and Oncley (J. L. Oncley, E. Ellenbogen, D. Gitlin and F. R. N. Gurd, *J. Phys. Chem.*, **56**, 85 (1952)) are likewise in agreement with a molecular weight of 12,000; however, these measurements were not performed under conditions of maximum dissociation of the protein and were not extended to as low a range of protein concentration as the other investigations previously cited.

(8) W. F. Harrington, H. K. Schachman and E. G. Pickels, Abstracts of the 122nd Meeting, American Chemical Society, 51C (1952); E. G. Pickels, W. F. Harrington and H. K. Schachman, *Proc. Nat. Acad. Sci., U. S.*, **38**, 943 (1952). We are indebted to Dr. E. G. Pickels for placing an experimental model of this cell at our disposal.

(9) T. Svedberg and K. O. Pedersen, "The Ultracentrifuge," Oxford University Press, Oxford, 1940.

(10) K. O. Pedersen, *Cold Spring Harbor Symposium Quant. Biol.*, **14**, 140 (1950).

that at ionic strengths 0.2 and 0.4 the measurements have not been extended to sufficiently low protein concentrations to exclude a further downward curvature to lower sedimentation constants, such a possibility is minimized by the behavior at 0.1 ionic strength. Although the latter data cannot be precisely extrapolated, they do show a trend toward a common intercept with the data obtained at the higher ionic strengths. It must be concluded, therefore, that in the presence of phosphate buffer *p*H 2.6, the extrapolated sedimentation constant of bovine insulin is in agreement with a molecular weight of 12,000.

Acknowledgment.—We are indebted to Mr. Roger M. Wade for the performance of sedimentation measurements.

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The Relative Catalytic Activity of Nickel Produced by the Reduction of Nickel(II) Bromide with Liquid Ammonia Solutions of Different Alkali Metals[1]

BY GEORGE W. WATT AND PEGGY I. MAYFIELD

RECEIVED NOVEMBER 1, 1952

Burgess and co-workers have reported marked differences in both the chemical and catalytic activity of silver and nickel precipitated by the reduction of salts with solutions of metals in liquid ammonia at its normal boiling temperature. Thus, the reduction of certain silver salts with solutions of potassium yielded silver far more active than that which resulted when sodium was employed.[2] Similar differences were observed in studies involving the reduction of silver salts with solutions of calcium[3] and in the reduction of nickel salts with sodium, potassium and calcium.[4] No explanation of these differences was proposed by Burgess, *et al.*, and since similar observations have been made in our laboratories it seemed worthwhile to carry out somewhat more definitive experiments.

In view of the presently accepted interpretation of the physical nature of solutions of metals in liquid ammonia,[5] it seems unlikely that differences in the properties of these reduction products are attributable to any inherent differences in the nature of the metal solutions. Rather it is more likely that both the chemical and catalytic activities of the reduction products are determined by rate factors and solubility relationships.

Although both the rates of solution of the alkali and alkaline earth metals in ammonia and the rates of the ensuing reactions with nickel(II) bromide are too rapid for accurate measurements, our experiments show qualitatively that both of these rates increase from lithium to cesium. Furthermore, the solubilities of the by-products (alkali bromides and amides) increase in the same direction. Thus, one obtains from the corresponding reactions, elemental nickel that is different in only one important respect, namely, surface area. This is shown by the fact that for the products obtained using lithium, sodium, potassium, rubidium and cesium as the reducing metals, catalytic activity per unit surface area is substantially constant. The relative insolubility of the by-products obtained using calcium obviated a rigorous comparison including this metal.

Burgess and Eastes[4] have attributed the pyrophoric character of the elemental nickel so-produced to the presence of adsorbed hydrogen. While all of the products prepared in our studies were pyrophoric in a degree that increased from lithium to cesium, the corresponding quantities of adsorbed hydrogen per unit weight of metal showed no consistent trend.

Experimental

Materials.—Hexamminenickel(II) bromide was prepared as described by Watt.[6] All other materials were commercial reagent grade chemicals.

Reduction Reactions.—The equipment and procedures employed were in all respects the same as those described previously[7] except that lithium was maintained in an atmosphere of nitrogen prior to addition to the solution and suspension of nickel(II) bromide, and that rubidium and cesium were added in fragile glass ampoules that were subsequently crushed.

When samples of hexamminenickel(II) bromide of the order of 2.5 g. in 15–20 ml. of liquid ammonia at −33.5° were treated with alkali metals (*ca.* 10% in excess of that required for complete removal of bromide ion), both the rates of solution of the alkali metals and the rates of the ensuing reactions with the bromide were quite evidently dependent upon the alkali metal employed. *Approximate* total times that elapsed between the addition of the alkali metal and the disappearance of the blue color characteristic of solutions of these metals in ammonia were as follows: Li, 5 min.; Na, 20 sec.; K, 10 sec.; Rb, < 10 sec.; Cs, << 10 sec. Following completion of the reactions, the ammonia-insoluble products were washed with liquid ammonia, with ethanol, and thereafter handled out of contact with the atmosphere and under strictly anhydrous conditions.

Properties of the Reduction Products.—By methods previously described,[7] the highly pyrophoric ammonia-insoluble products were analyzed for nickel, nitrogen, bromine and

TABLE I

PROPERTIES OF PRODUCTS FROM THE REDUCTION OF NICKEL (II) BROMIDE WITH ALKALI METALS IN LIQUID AMMONIA

	Ammonia-insoluble product				
Alkali metal	Ni, %	H_2, cc./g.	Surface area, $m.^2/g.$	Reaction rate	Rate/unit area
Li	82.3	17.6	30[a]	1.6	0.05
Na	93.6	7.5	27	3.1	.11
K	92.0	18.7	54	3.8	.07
Rb	90.4	10.4	105	8.8	.08
Cs	83.9	2.1	127	9.1	.07

[a] This value was determined using a sample washed with liquid ammonia but not with ethanol and involves a correction for an initial rapid uptake of ammonia during the surface area determinations. This was attributed to the ammonation of impurities present and the validity of this procedure was confirmed by a surface area estimate obtained from electron photomicrographs of an ethanol-washed product which showed an average particle radius of 88 Å. and led to a computed surface area of 38 $m.^2/g.$

(f) This work was supported, in part, by the Office of Naval Research, Contract N6onr-26610.

(2) W. M. Burgess and F. R. Holden, THIS JOURNAL, **59**, 459 (1937).

(3) W. M. Burgess and F. R. Holden, *ibid.*, **59**, 462 (1937).

(4) W. M. Burgess and J. W. Eastes, *ibid.*, **63**, 2674 (1941).

(5) W. C. Johnson and A. W. Meyer, *Chem. Revs.*, **8**, 273 (1931); *cf.* W. L. Jolly, *ibid.*, **50**, 351 (1952).

(6) G. W. Watt, "Inorganic Syntheses," Vol. II, McGraw-Hill Book Co., Inc., New York, N. Y., 1950, p. 194.

(7) G. W. Watt, W. F. Roper and S. G. Parker, THIS JOURNAL, **73**, 5791 (1951).

alkali metal after washing with ammonia, and usually only for nickel following washing with ethanol. In addition to surface area measurements, the quantities of hydrogen associated with the reduction products were determined. Catalytic activity was evaluated in terms of catalysis of the hydrogenation of allyl alcohol. The essential data are given in Table I, in which the catalytic activity of the nickel is expressed as the rate (in millimoles H_2 consumed/min./g. of catalyst) of the catalyzed hydrogenation reaction and the numerical values of which are taken from those portions of the corresponding rate curves over which the rates were substantially linear with time. In all cases this condition prevailed over at least three-fourths of the total reaction time.

Reduction Reactions Employing Calcium.—Similar reduction reactions employing excess calcium occurred at about the same rate as those involving lithium. Owing to the insolubility of calcium amide[8] and calcium bromide,[9] purification of the nickel by washing with liquid ammonia was ineffective. The composition of a typical ammonia-insoluble product was as follows: Ni, 17.4; Br, 44.0; N, 21.4; Ca, 11.7. Although 16% excess calcium was used in this particular case, unreacted hexamminenickel(II) bromide was present. Washing with ethanol was only partially effective as a means of purification and no means was found to purify the products without eliminating the catalytic activity of the elemental nickel present.

(8) F. W. Bergstrom, *Ann.*, **515**, 34 (1934).
(9) M. Linhard and M. Stephan, *Z. physik. Chem.*, **167**, 87 (1933).

DEPARTMENT OF CHEMISTRY
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The Synthesis of Aryl and Aralkyl Amidines of Pharmacologic Interest[1]

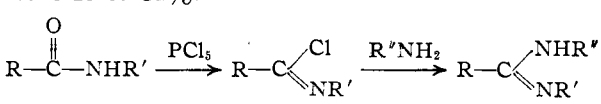
BY GEORGE L. WEBSTER AND JACOB S. RODIA[2]

RECEIVED AUGUST 14, 1952

In a previous paper from this Laboratory[3] it was shown that hydrochlorides of methoxysubstituted benzamidines possessed local anesthetic activity. The fact that all of these compounds produced sloughing of tissue at the site of injection suggested the desirability of preparing a new series of amidines in an effort to eliminate the undesirable toxicity.

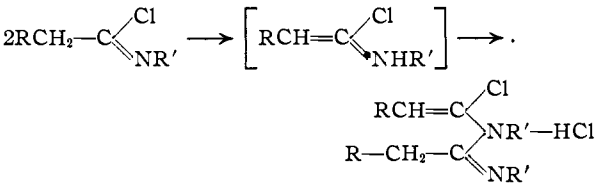
Since most local anesthetics require the use of a vasoconstrictor agent to increase the duration of anesthesia, it also appeared of interest to investigate whether the incorporation in the amidine molecule of the β-phenylethylamine skeleton would give rise to a substance having both local anesthetic and vasoconstrictor properties.

This paper deals with the synthesis of a number of new N,N′-disubstituted amidines. The amidines listed in Table I were prepared by a modification of the method of Hill and Cox[4] in yields ranging from 43 to 81%.



In the preparation of the amidines numbered 1, 2, 4, 5 and 6 in Table I, the phosphorus oxychloride formed by the interaction of the acylamino compound and phosphorus pentachloride was removed under reduced pressure prior to the addition of the amine. This procedure was found to be essential to the synthesis of the amidines (1 and 2), since their preparation required the use of an aminophenol with an unprotected phenolic group. In the other cases, this modification was adopted in order to shorten the time during which the β-phenylethylamide would be in contact with the phosphorus halide. It is known[5] that β-phenylethylamides are cyclized by phosphorus halides to the corresponding 3,4-dihydroisoquinoline derivatives.

Since the imidochlorides are very unstable and readily change into chlorovinylamidinium chlorides,[6] probably according to the reaction



it was necessary in the preparation of the disubstituted phenylacetamidines to conduct the conversion of the starting anilide into the imidochloride in the presence of the arylamine. By employing the method of Hill and Cox such a change was prevented and the expected disubstituted phenylacetamidine obtained in good yield.

In the preparation of the vanillamidines it was found that the carbethoxyl group proved to be satisfactory for the protection of the phenolic hydroxyl group. Its subsequent removal was easily accomplished with dilute alkali without affecting the amidine.

Experimental

Carbethoxyvanilloylanilide.—Vanillic acid, m.p. 207°, prepared from vanillin by the method described by Pearl,[7] was carbethoxylated according to the procedure given by Heap and Robinson.[8] A solution of 60 g. of carbethoxyvanillic acid in 150 ml. of thionyl chloride was boiled for 45 minutes or until active evolution of hydrogen chloride ceased. After removal of the excess reagent under reduced pressure, the residue was dissolved in 100 ml. of anhydrous ether, cooled and 45 ml. of aniline in 100 ml. of anhydrous ether was gradually added with stirring. The white solid which consisted of aniline hydrochloride and the desired anilide was successively washed with 100-ml. portions of water, dilute alkali, dilute acid and water. The product was recrystallized from 95% alcohol, filtered by suction and dried; yield 62.0 g., m.p. 132–133°.

Anal. Calcd. for $C_{17}H_{17}NO_5$: C, 64.75; H, 5.44; N, 4.44. Found: C, 64.70; H, 5.42; N, 4.39.

Preparation of the Amidines.—One and one-tenth molecular proportion of phosphorus pentachloride in 50 ml. of sodium-dried benzene was heated on a water-bath under reflux until active evolution of hydrogen chloride ceased. The solution was cooled and 0.03–0.05 mole of the anilide was added. In the preparation of amidines numbered 5 and 6, the imidochloride was formed in the absence of the solvent using one molecular proportion of phosphorus pentachloride. The reaction mixture was then heated for two hours on a water-bath, after which the solvent and the phosphorus oxychloride formed during the reaction were removed

(1) An abstract of a thesis submitted by J. S. Rodia to the Graduate College of the University of Illinois in partial fulfillment of the requirements for the Degree of Doctor of Philosophy.
(2) American Foundation for Pharmaceutical Education Fellow, 1951–1952.
(3) M. J. Sintov, *et al.*, THIS JOURNAL, **71**, 3990 (1949).
(4) A. J. Hill and M. V. Cox, *ibid.*, **48**, 3215 (1926)).
(5) R. Adams, "Organic Reactions," Vol. VI, John Wiley and Sons, Inc., New York, N. Y., 1951, p. 74.
(6) J. V. Braun, F. Jostes and A. Heymons, *Ann.*, **453**, 113 (1927).
(7) I. Pearl, THIS JOURNAL, **68**, 2180 (1946).
(8) T. Heap and R. Robinson, *J. Chem. Soc.*, 2341 (1926).

TABLE I

N,N'-DISUBSTITUTED AMIDINES AND THEIR SALTS

	Benzamidines	Formula	M.p., (uncor.), °C.	Nitrogen, % Calcd.	Nitrogen, % Found	Chlorine, % Calcd.	Chlorine, % Found
1	N-Phenyl-N'-*p*-hydroxyphenyl	$C_{19}H_{16}ON_2$[a]					
	hydrochloride	$C_{19}H_{17}ON_2Cl$	285	8.62	8.65	10.92	10.79
2	N-Phenyl-N'-*m*-hydroxyphenyl	$C_{19}H_{16}ON_2$	215	9.72	9.65		
	hydrochloride	$C_{19}H_{17}ON_2Cl$	271	8.62	8.58	10.92	10.86
3	N-Phenyl-N'-*p*-benzoxyphenyl	$C_{26}H_{20}O_2N_2$	131	7.14	7.08		
	hydrochloride	$C_{26}H_{21}O_2N_2Cl$	257	6.53	6.33	8.27	8.34
4	N-Phenyl-N'-homoveratryl	$C_{23}H_{24}O_2N_2$[b]	114	7.77	7.69		
	hydrochloride	$C_{23}H_{25}O_2N_2Cl$[c]					
5	N-Phenyl-N'-β-phenylethyl	$C_{21}H_{20}N_2$[d]	70	9.33	9.31		
	hydrochloride	$C_{21}H_{21}N_2Cl$	162–163	8.32	8.35	10.53	10.58
6	N,N'-Bis-β-phenylethyl	$C_{28}H_{24}N_2$	78	8.53	8.41		
	hydrochloride	$C_{28}H_{25}N_2Cl$[c]					
	sulfate	$C_{28}H_{26}O_4NS$	141	6.42	6.38		
	picrate	$C_{29}H_{27}O_7N_5$	169	12.56	12.46		
		Phenylacetamidines					
7	N-Phenyl-N'-*p*-phenetyl	$C_{22}H_{22}ON_2$	96	8.48	8.54		
	hydrochloride	$C_{22}H_{23}ON_2Cl$[c]					
8	N-Phenyl-N'-*p*-tolyl	$C_{21}H_{20}N_2$	93	9.33	9.23		
	hydrochloride	$C_{21}H_{21}N_2Cl$	155–156	8.32	8.41	10.53	10.50
		Vanillamidines					
9	N,N'-Bisphenyl 4-carbethoxy	$C_{23}H_{22}O_4N_2$[e]					
	hydrochloride	$C_{23}H_{23}O_4N_2Cl$	165	6.56	6.49	8.31	8.44
10	N-Phenyl-N'-*p*-phenetyl-4-carbethoxy	$C_{25}H_{26}O_4N_2$[e]					
	hydrochloride	$C_{25}H_{27}O_4N_2Cl$	184	6.16	6.27	7.79	7.50
11	N-Phenyl-N'-*p*-tolyl-4-carbethoxy	$C_{24}H_{24}O_4N_2$[e]					
	hydrochloride	$C_{24}H_{25}O_4N_2Cl$	167	6.36	6.42	8.04	8.15
12	N,N'-Bisphenyl	$C_{20}H_{18}O_2N$	187	8.80	8.57		
13	N-Phenyl-N'-*p*-phenetyl	$C_{22}H_{22}O_3N_2$	86	7.73	7.77		
14	N-Phenyl-N'-*p*-tolyl	$C_{21}H_{20}O_2N_2$	76	8.43	8.18		

[a] This amidine was also prepared by the method of Wagner and Holljes.[9] By both procedures the same hydrochloride and amidine were obtained. The amidine isolated in both cases possessed a melting point range 124–130°, and was found to be analytically impure. [b] Obtained in much better yield starting with N-(3,4-dimethoxyphenylethyl)-benzamide. [c] Could not be obtained pure. [d] Prepared in considerably better yield starting with N-(β-phenylethyl)-benzamide. [e] Isolated as an uncrystallizable oil.

under reduced pressure. In the preparation of the phenylacetamidines and the vanillamidines, the solvent and the phosphorus oxychloride were not removed prior to the addition of the amine. One molecular proportion of the amine (two in the cases of amidines numbered 4, 5 and 6) was added to the residue in 50 ml. of dry benzene and the mixture heated on a water-bath for three hours or until no more precipitation occurred.

When the amidine hydrochloride precipitated (compounds numbered 1, 2 and 3), it was filtered, dried and extracted with water until no more amidine could be obtained upon the addition of concentrated ammonia water to the extracts.

When the amidine did not precipitate as the hydrochloride, the solvent was removed under reduced pressure and the residue dissolved in a minimum amount of 95% alcohol. To the cooled alcoholic solution, a slight excess of strong ammonia was added slowly with stirring. When an oil was obtained, additional cooling and scratching of the sides of the vessel converted it into a crystalline material. The amidines were recrystallized from 95% alcohol.

In the cases of the 4-carbethoxyvanillamidines (compounds 9, 10 and 11) the amidine precipitated as an uncrystallizable oil which was extracted with ether and the extract dried over anhydrous sodium sulfate. After the evaporation of the ether, the residual gum was dissolved in an alcoholic hydrogen chloride solution and the hydrochloride precipitated by several additions of anhydrous ether. The gummy hydrochloride became crystalline upon cooling in an ice mixture and scratching the sides of the vessel. The hydrochloride was recrystallized from absolute methanol and anhydrous ether.

Hydrolysis of the 4-Carbethoxyvanillamidines.—One gram of the 4-carbethoxyvanillamidine hydrochloride and 10 ml. of a dilute potassium hydroxide solution were heated on a steam-bath until solution occurred (0.5 to 4 hr.). The solution was diluted, cooled and neutralized with concentrated hydrochloric acid. The yellow precipitate was filtered by suction, washed with water and dried in the air. The crude base was recrystallized from 95% alcohol with the addition of hot water. The yellow crystalline base which precipitated was filtered by suction and dried in the air.

Preparation of the Amidine Salts.—The amidine was dissolved in a minimum amount of an alcoholic hydrogen chloride solution and the hydrochloride precipitated by several small additions of anhydrous ether. The crude hydrochloride was filtered by suction, dried, and recrystallized from an absolute alcohol–anhydrous ether mixture.

The hydrochlorides of amidines numbered 1, 2 and 3 in Table I precipitated during the reaction. They were filtered by suction, dried and recrystallized from an absolute alcohol–anhydrous ether mixture.

The sulfate of N,N'-bis-β-phenylethylbenzamidine, which precipitated as an oil by the addition of a dilute solution of sulfuric acid to the pure base, was converted to a crystalline product upon cooling and scratching the sides of the vessel. It was recrystallized from an absolute alcohol–anhydrous ether mixture.

The picrate of the above amidine was obtained by warming an alcoholic solution of the base and picric acid. It was recrystallized from benzene.

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(9) E. C. Wagner and E. L. Holljes, *J. Org. Chem.*, **9**, 31 (1949).

Preparation of Some Organophosphorus Compounds[1]

BY DASU RAMASWAMI[2] AND ERNST R. KIRCH

RECEIVED SEPTEMBER 29, 1952

In a previous publication[3] we reported our studies on the effect of substituting the C=O group in phenylurethan by the P=O group. We wish to present the details of the synthesis of compounds.

Experimental

Dialkyl anilidophosphates were synthesized according to the following scheme reactions: Dialkyl chlorophosphates, the intermediates were prepared (in yields of 80–90%) from phosphorus trichloride and the respective alkanol according to the procedure of McCombie, *et al.*[4]

Chlorination in the second step was carried out according to the procedure of Atherton, *et al.*,[5] using sulfuryl chloride.

The dialkyl chlorophosphate was then treated with aniline in the molar ratio of 1:2. The end-product in each case was extracted with boiling benzene and separated from the solid aniline hydrochloride by filtration. Purification was effected by distillation under reduced pressure in the case of liquids or by repeated crystallization in the case of solids.

$$(RO)_2P(O)Cl + 2C_6H_5NH_2 \longrightarrow (RO)_2P(O)NHC_6H_5 + C_6H_5NH_2HCl$$

The following detailed procedure is a typical example for the series.—**Di-*n*-propyl Hydrogen Phosphite** (HO)P(O-*n*-propyl)$_2$: *n*-Propyl alcohol (54 g., 0.9 mole) was dissolved in 54 ml. of carbon tetrachloride in a flask immersed in ice-water. Phosphorus trichloride (41.5 g., 0.3 mole) dissolved in 15 ml. of carbon tetrachloride was added slowly with stirring. The mixture was allowed to stand for one hour and then distilled under reduced pressure in a claisen flask. The residue after removing hydrogen chloride and carbon tetrachloride distilled at 78–80° (15 mm.), yield 85%.

Di-*n*-propyl Chlorophosphate (*n*-propyl-O-)$_2$P(O)Cl.—Di-*n*-propyl hydrogen phosphite from the previous step (43 g., 0.28 mole) was stirred, keeping the flask in ice-water while sulfuryl chloride (37 g., 0.28 mole) was added dropwise maintaining the temperature at 35–40°. The mixture was stirred for a further 90 minutes and distilled under reduced pressure. The residue after removing sulfur dioxide and hydrogen chloride distilled at 87–88° (11 mm.), yield 87%.

Di-*n*-propyl Anilidophosphate (*n*-propyl-O)$_2$P(O)NHC$_6$H$_5$.—Diisopropyl chlorophosphate (10 g., 0.05 mole) was slowly added to aniline (9.3 g., 0.1 mole) the mixture being immersed in ice-water. The mixture was set aside for an hour, extracted with boiling benzene and filtered. The filtrate was evaporated to a sirup which solidified. The mass was crystallized from alcohol twice.

The rest of the chloro- and dichloro-compounds were prepared by a procedure represented by the following example: Ethyl benzene phosphorus oxychloride

$$H_5C_6\cdot P(O)Cl_2 + C_2H_5OH \longrightarrow H_5C_6\cdot P(O)(OC_2H_5)Cl$$

Benzene phosphorus oxydichloride (39 g., 0.2 mole) dissolved in 100 ml. of chloroform was kept in a freezing mixture and absolute alcohol (9.5 g., 0.2 mole) added dropwise with stirring keeping the temperature at 0° or below. The mixture was distilled under reduced pressure.

Table I summarizes the physical constants and analytical data of these compounds.

Acknowledgment.—We are indebted to Victor Chemical Works for a gift of some of the starting compounds.

TABLE I

No.	Compound	Carbon, % Calcd.	Carbon, % Found	Hydrogen, % Calcd.	Hydrogen, % Found	M.p. or b.p. °C. cor.	Mm.	$n^{20}D$
1	(*n*-Pr-O)$_2$P(OH)	43.36	43.67	9.09	9.29	93–95	11	1.4140
2	(*n*-Pr-O)$_2$P(O)Cl	35.93	36.20	7.04	7.30	95–97	2	1.4230
3	(*n*-Pr-O)$_2$P(O) (NHC$_6$H$_5$)	56.02	56.35	7.84	7.91	54.5–55.5		
4	(*n*-Bu-O)$_2$P(O) (NHC$_6$H$_5$)	58.92	58.58	8.48	8.35	191	1	1.4907
5	(*n*-Am-O)$_2$P(OH)	54.03	53.98	10.43	10.67	138–139	6	1.4300
6	(*n*-Am-O)$_2$P(O)Cl	46.78	47.09	8.65	8.85	141–142	6	1.4342
7	(*n*-Am-O)$_2$P(O) (NHC$_6$H$_5$)	61.74	62.10	8.41	8.23	201–203	2.5	1.4810
8	(*i*-Am-O)$_2$P(O) (NHC$_6$H$_5$)	61.74	62.01	8.41	8.53	191	2	1.4863
9	(H$_5$C$_2$O)$_2$P(S) (NHC$_6$H$_5$)	48.96	48.63	6.58	6.37	140–142	1.5	1.4950
10	(C$_6$H$_5$)(H$_5$C$_2$O):P(O)NHC$_6$H$_5$	64.36	64.08	6.17	6.20	130–132		
11	(C$_6$H$_5$)(H$_5$C$_2$O) P(S)NHC$_6$H$_5$	60.61	60.33	5.81	5.90	81–82		
12	(H$_5$C$_6$)(H$_5$C$_2$O) P(S)Cl	43.53	43.61	4.57	4.30	115–116	1.5	1.4950
13	H$_5$C$_6$·P(O) (OC$_2$H$_5$)Cl	46.96	46.50	4.94	5.05	120	2	1.5372

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(1) Experimental data taken in part from the thesis submitted by Dasu Ramaswami in partial fulfillment of the requirements for the degree of Doctor of Philosophy in Pharmaceutical Chemistry.

(2) D. R. is indebted to the University of Illinois for a fellowship.

(3) D. Ramaswami, E. R. Kirch and E. H. Jenney, *Science*, **116**, 58 (1952).

(4) H. McCombie, B. C. Saunders and G. J. Stacey, *J. Chem. Soc.*, 380 (1945).

(5) F. R. Atherton, H. T. Howard and A. R. Todd, *ibid.*, 1106 (1948).

Diethyl Vinyl Phosphate, Divinyl Benzenephosphonate and their Polymers

BY R. W. UPSON

RECEIVED NOVEMBER 21, 1952

Although unsaturated esters of phosphorus acids, such as allyl, methallyl and crotyl phosphates and phosphonates[1–4] have been reported, no reference has been made in the literature to vinyl esters of phosphorus acids. It was therefore of interest to prepare vinyl esters of typical phosphorus acids and to investigate their polymerization.

Diethyl vinyl phosphate and divinyl benzenephosphonate have been synthesized by dehydrohalogenation of the corresponding 2-chloroethyl esters.[5] The 2-chloroethyl esters were prepared by reaction of diethyl chlorophosphate and benzenephosphonyl dichloride with ethylene oxide by the procedure described by Daly and Lowe[6] for the preparation of tris-(2-chloroethyl) phosphate.

Copolymers of diethyl vinyl phosphate with styrene, methyl methacrylate and acrylonitrile were prepared. Attempts to form homopolymers

(1) L. N. Whitehill and R. S. Barker, U. S. Patent 2,394,829 (1946).

(2) A. D. F. Toy, THIS JOURNAL, **70**, 186 (1948).

(3) A. D. F. Toy, U. S. Patent 2,425,765 (1947).

(4) British Patent 534,826 (1941).

(5) R. W. Upson, U. S. Patent 2,557,805 (1951).

(6) A. J. Daly and W. G. Lowe, U. S. Patent 2,157,164 (1939).

from diethyl vinyl phosphate were unsuccessful. A soft, tacky homopolymer of divinyl benzenephosphonate was obtained. The vinyl ester group in diethyl vinyl phosphate is stable toward water and dilute hydrochloric acid solution.

Experimental

Diethyl Vinyl Phosphate.—Diethyl 2-chloroethyl phosphate used in the preparation of diethyl vinyl phosphate, was prepared by reaction of diethyl chlorophosphate with ethylene oxide by the general procedure of Daly and Lowe.[6] After this work was completed, Saunders, *et al.*,[7] reported the preparation of diethyl 2-chloroethyl phosphate by reaction of diethyl chlorophosphate with ethylene chlorohydrin.

To a stirred solution containing 387 g. (2.24 moles) of diethyl chlorophosphate and 3.9 g. (0.03 mole) of anhydrous aluminum chloride was added 114 g. (2.6 moles) of ethylene oxide over a period of 8 hours at room temperature. Distillation of the reaction mixture under reduced pressure gave 400 g. (82.5%) of diethyl 2-chloroethyl phosphate, b.p. 115–117° (5 mm.), $n^{25}D$ 1.4281.

Anal. Calcd. for $C_6H_{14}O_4ClP$: P, 14.32; Cl, 16.37. Found: P, 14.46; Cl, 16.48.

A solution containing 56.1 g. (1.0 mole) of potassium hydroxide, 1000 ml. of ethanol and 216 g. (1.0 mole) of diethyl 2-chloroethyl phosphate was refluxed for 11 hours. The potassium chloride that separated (59 g.) from the reaction solution during this time was removed by filtration. Distillation of the filtrate gave 93 g. (49%) of diethyl vinyl phosphate, b.p. 67° (2.5 mm.), $n^{25}D$ 1.4040.

Anal. Calcd. for $C_6H_{13}O_4P$: P, 17.20. Found: P, 16.80.

Diethyl vinyl phosphate was recovered unchanged after being refluxed in water and in 1% aqueous hydrochloric acid solution for 3–6 hours. There was no evidence that acetaldehyde or ethanol were liberated by these treatments.

Copolymers of Diethyl Vinyl Phosphate.—These were prepared as follows. A solution containing 1.8 g. of diethyl vinyl phosphate, 1.04 g. of styrene and 0.14 g. of benzoyl peroxide was heated under nitrogen at atmospheric pressure for 42 hours at 80°. A colorless, viscous liquid was formed from which 1.2 g. of a white, solid copolymer was obtained by precipitation of the polymerization product with 50 ml. of methanol. The diethyl vinyl phosphate/styrene copolymer softened at 68° and contained 2.52% phosphorus.

Diethyl vinyl phosphate/methyl methacrylate and diethyl vinyl phosphate/acrylonitrile copolymers containing 1.42% phosphorus and 0.91% phosphorus, respectively, were prepared by substitution of equivalent amounts of methyl methacrylate and acrylonitrile for the styrene in the above procedure. The copolymer with methyl methacrylate softened at 110° and the copolymer with acrylonitrile softened at 210–220°. Diethyl vinyl phosphate did not form a copolymer with vinyl acetate under these conditions.

Diethyl vinyl phosphate did not polymerize when heated for 42–70 hours at 80° in the presence of 2–5% α,α'-azodiisobutyronitrile or benzoyl peroxide, at 125° in the presence of di-(*t*-butyl) peroxide, or at −30° in the presence of 2–4% sodium in liquid ammonia. In each case the monomer was recovered almost quantitatively by distillation of the product.

Divinyl Benzenephosphonate.—Bis-(2-chloroethyl) benzenephosphonate was prepared in 77% yield by reaction of 195 g. (1.0 mole) of benzenephosphonyl dichloride with 132 g. (3.0 moles) of ethylene oxide in the presence of 1.95 g. of anhydrous aluminum chloride. The product was a colorless liquid, b.p. 160° (0.8 mm.), $n^{25}D$ 1.5235.

Anal. Calcd. for $C_{10}H_{13}O_3Cl_2P$: P, 10.94; Cl, 25.05. Found: P, 11.05; Cl, 25.63.

A mixture containing 87.3 g. (0.31 mole) of bis-(2-chloroethyl) benzenephosphonate and 65.6 g. (0.62 mole) of anhydrous sodium carbonate was heated for 1.5 hours at 105°, 2 hours at 155° and then for 3 hours at 200°. The sodium chloride that separated from the reaction solution was removed by filtration. Distillation of the filtrate gave 40 g. (61.5%) of divinyl benzenephosphonate, b.p. 174° (3.0 mm.), $n^{25}D$ 1.5258.

(7) B. C. Saunders, G. J. Stacey, F. Wild and I. G. E. Wilding, *J. Chem. Soc.*, 699 (1948).

Anal. Calcd. for $C_{10}H_{11}O_3P$: P, 14.74. Found: P, 14.96.

The non-volatile residue from the distillation was a viscous liquid polymer of divinyl benzenephosphonate and amounted to 15 g. (23.1%).

Anal. Calcd. for $C_{10}H_{11}O_3P$: P, 14.74. Found: P, 14.90.

Homopolymers.—These ranged from viscous liquids to soft, tacky solids and were obtained by heating divinyl benzenephosphonate at 80° in the presence of 1–3% benzoyl peroxide.

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Studies on the Chemistry of Heterocyclics. XXII. Investigations on the Mechanism of Reactions of 2-Thienyl Halides with Sodium Amide and Sodium Acetylide in Liquid Ammonia

BY ALEXANDER VAITIEKUNAS AND F. F. NORD

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In view of the growing importance of the reactions of sodium amide as well as sodium acetylide in liquid ammonia for the syntheses and elucidation of the structure of natural products[1,2,3] it appeared worthwhile to investigate this reaction in the heterocyclic series. It is known that sodium amide in liquid ammonia gave the corresponding acetylenic carbinols[2d] with furan and tetrahydrofuran derivatives and thiophene-2-aldehyde, 3-methyl-thiophene-2-aldehyde as well as β-2-thienylacrolein readily gave the corresponding acetylenic alcohols with sodium acetylide in liquid ammonia.[4] However, the alkylations carried out by this method are unsuccessful when applied to halides in the benzene series.[5] In view of the greater reactivity of the thiophene nucleus it appeared desirable to investigate the reaction of 2-thienyl halides with sodium acetylide in liquid ammonia to attempt the direct synthesis of 2-thienylacetylene.

Preliminary experiments have indicated that 2-chlorothiophene did not react and the starting material was recovered quantitatively while 2-bromothiophene did react vigorously.[5a] However, the main reaction product obtained was not the expected 2-thienylacetylene, but tetrabromothiophene, the monohalide being converted into a tetrahalo derivative. Subsequent investigations with 2-bromothiophene and 2-iodothiophene confirmed the results of the preliminary experiments and we obtained, in the case of 2-bromothiophene, tetrabromothiophene in a yield of 35 to 50%. In the case of 2-iodothiophene the tetraiodo derivative was obtained in a yield of 50% together with a small amount of di- and triiodothiophene.

It appears that in all the above reactions some electrophilic reagent such as NH_2Br or $CH{\equiv}CBr$

(1) J. Heilbron, *J. Chem. Soc.*, 386 (1948).

(2) (a) E. R. H. Jones, *ibid.*, 754 (1950); (b) J. B. Armitage, E. R. H. Jones and M. C. Whiting, *ibid.*, 1993 (1952); (c) J. B. Armitage, C. L. Cook and E. R. H. Jones, *ibid.*, 2010 (1952); (d) G. Eglinton, E. R. H. Jones and M. C. Whiting, *ibid.*, 2873 (1952).

(3) H. H. Schlubach and V. Franzen, *Ann.*, **573**, 105 (1951).

(4) A. Vaitiekunas, R. E. Miller and F. F. Nord, *J. Org. Chem.*, **16**, 1603 (1951).

(5) T. H. Vaughn, G. F. Hennion, R. R. Vogt and J. A. Nieuwland, *ibid.*, **2**, 9 (1938).

(5a) A. Vaitiekunas and F. F. Nord, *Nature*, **168**, 875 (1951).

might serve as an intermediary brominating agent, or that the reaction might proceed through complex formation, or that the amide ion initiates the reaction. To establish the more probable mechanism the following reactions have been studied: (1) ammonolysis of 2-thienyl halides at the boiling point of liquid ammonia; (2) reactions of 2-bromo-3-bromo- or 2-iodothiophene, 3-methyl-2-bromothiophene, 2,5-dibromothiophene and 2,3-dibromothiophene with sodium amide and sodium acetylide; (3) reactions of 2-bromothiophene and phenol mixtures with sodium amide and sodium acetylide in liquid ammonia; (4) the effect of non-ionizing solvent; and (5) the effect of ammonium halides.

Results

1. We have found that neither 2-bromothiophene nor 2-iodothiophene are affected by liquid ammonia at its boiling point where the original reactions were carried out. Both thienyl halides used were recovered quantitatively. They are both easily soluble in liquid ammonia at the concentrations used in the above reactions.

2. The reactions of 2,5-dibromothiophene and 2,3-dibromothiophene with sodium acetylide and sodium amide in liquid ammonia were carried out in a manner analogous to that applied to the monohalides. Tetrabromothiophene was obtained in a yield of 35%. Some 2-bromothiophene was isolated despite the fact that the 2,5-dibromothiophene was carefully purified. In the case of 2,3-dibromothiophene which was prepared by a method which excludes the formation of monobromothiophene, some 3-bromothiophene was obtained. When sodium amide alone was used the conversion of ϕ 2,5-dibromothiophene into tetrahalide gave a yield of 20%.

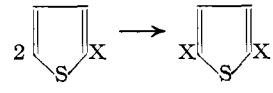
Reactions of 2-bromothiophene, 3-bromothiophene and 2-iodothiophene with sodium amide in liquid ammonia were carried out using freshly prepared sodium amide according to an earlier method.[6] Dibromothiophene and some tribromo product[7] were obtained which were identified by their elemental analysis, refractive indices and boiling points. The dibromothiophene was difficult to purify, probably due to the presence of small amounts of partially hydrogenated decomposition products of the free thiophene. The possibility that such hydrogenation of the thiophene nucleus may take place with decomposition has been recorded recently.[8] Applying the same method to 2-iodothiophene, no triiodothiophene was isolated. We found that using smaller amounts of sodium amide, *e.g.*, 0.2 mole of sodium amide to 1 mole of 2-thienyl halide, the yield of dihalide obtained was much smaller and 70% of the starting material was recovered as recorded in Table I. However, using greater amounts than 1 mole, *e.g.*, 2 moles, of sodium amide to 1 mole of 2-thienyl halide the amount of recovered monohalide was insignificant while the greatest part of monohalide was decomposed probably under partial dehydrohalogenation. No doubt the C–S bond is broken down. The 3-bromothiophene did not react.

TABLE I

EFFECT OF SODIUM AMIDE CONCENTRATION ON THE YIELDS OF THIENYL DIHALIDES

Thienyl halide	Quantity, mole	Sodium amide, moles	Yield in[a] thienyl dihalide, %	Recovered starting material, %
2-Bromothiophene	1	2	18	10
2-Bromothiophene	1	1	36	35
2-Bromothiophene	1	0.2	17	70
2-Iodothiophene	1	2	22	12
2-Iodothiophene	1	1	15	45
2-Iodothiophene	1	0.2	15	65

[a] Calculated according to the equation:



It appears that the initial reaction of 2-bromothiophene as well as of 2-iodothiophene proceeds in an analogous way as with sodium acetylide.

Reactions of 3-methyl-2-bromothiophene with sodium amide in liquid ammonia were performed in a manner analogous to that applied to the monohalides. The dibromo product was formed in a yield of 16.5% only. The methyl group functioning as an electron releasing group did not facilitate the conversion. Analogous results were obtained using sodium acetylide in liquid ammonia.

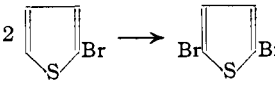
3. Reactions of mixtures of 2-bromothiophene and phenol with sodium acetylide and sodium amide in liquid ammonia: Nitrogen bromide or acetylene bromide might serve as electrophilic agents in these conversions. It is known that nitrogen iodide easily iodinates phenol thus giving the triiodo derivative.[9] When such a reagent serves as intermediary halogenating agent it would be conceivable to obtain some bromo derivative of phenol. However, using different molecular amounts of phenol as recorded in Table II, we were unable to isolate either a mono- or a tribromo derivative of phenol while a conversion of 2-thienyl halide into tetrahalide did take place. The latter reaction took place less readily.

TABLE II

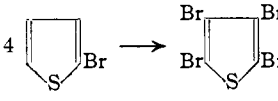
EFFECT OF PHENOL ON THE CONVERSION OF 2-THIENYL HALIDE TO TETRAHALIDE

2-Bromothiophene, mole	Sodium amide (sodium acetylide), mole	Phenol, mole	Recovered monohalide, %	Dihalide,[a] %	Tetrahalide,[b] %
0.1	0.2	0.1	37	50	10
.1	.2	.05	25	21	20
.1	.2	.01	6	12	35

[a] Calculated as dibromoproduct:



[b] Calculated according to the equation:



(6) T. H. Vaughn, R. R. Vogt and J. A. Nieuwland, THIS JOURNAL, **56**, 2120 (1934).

(7) H. D. Hartough, "The Chemistry of Heterocyclic Compounds, Thiophene and Its Derivatives," Interscience Publishers, Inc., New York, N. Y., 1952, pp. 208, 498.

(8) S. F. Birch and D. T. McAllan, *J. Chem. Soc.*, 2556 (1951).

(9) Beilstein, "Handbuch der Org. Chem.," Vol. 6, Springer, Berlin, 1923, p. 118.

These observations do not agree with the possible occurrence of intermediary brominating agents such as nitrogen bromide or acetylene bromide. The interference of phenol in this conversion is readily explained by the fact that phenol acting as an acid diminished the concentration of the amide as well as the acetylide ion.

4. While studying the effect of a non-ionizing solvent on the reaction we found that addition of absolute ether to the reaction mixture in amounts of 10% of the total solvent stopped the reaction. The starting materials were recovered and no tetrahalides were obtained.

5. Effect of ammonium halides on the reaction: These are known to react as acids in liquid ammonia and attack the sodium acetylide as well as the amide thus diminishing the acetylide as well as the amide ion concentrations following the equation

$$NaC{\equiv}CH + NH_4X \longrightarrow HC{\equiv}CH + NH_3 + NaX$$

Our present studies indicate that the addition of ammonium chloride as well as of ammonium bromide in amounts of 1 mole of ammonium halide to 1 mole of sodium acetylide or amide interrupts the reaction. In all cases the starting materials were recovered and no tetrahalides were obtained. However, it has to be noticed here that even when ammonium halides were added to the reaction mixture after 3 hours of continuous stirring and shortly prior to the decomposition of the reaction mixture with water no tetrahalides were obtained. This would support the view that the reaction proceeds through a complex formation which under decomposition would give rise to the tetrahalides. Attempts to isolate and to identify such a complex were, however, unsuccessful. It is to be concluded that the presence of sufficient amounts of acetylide as well as of amide ions are necessary for the reaction to proceed.

From the above results we may conclude that: 1, the presence of a sufficient concentration of amide ion is necessary; 2, by means of sodium amide, generally, only monohalides are converted to dihalides, the further conversion to tetrahalides being halted; 3, when dihalides are used as starting materials the conversion with sodium amide proceeds to tetrahalides; 4, the presence of sodium acetylide is indispensable for the conversion of monohalides into the tetrahalides.

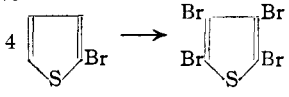
According to these conclusions it could be expected that mixtures of sodium amide and sodium acetylide are capable of converting the monohalides into tetrahalides more readily than sodium acetylide alone. When only the latter is present the amide ion concentration is not effective enough to remove the proton and thus to initiate the reaction. However, the presence of sodium acetylide prevents the decomposition of the thiophene nucleus. Our experiments with 2-bromothiophene confirmed this conclusion. The freshly prepared sodium amide was converted to sodium acetylide to the approximate extent of 25%, 50% and 75% of the total amount of the sodium amide present. The results obtained are shown in Table III.

TABLE III

EFFECT OF MIXTURES OF SODIUM AMIDE AND SODIUM ACETYLIDE ON THE YIELD OF TETRABROMOTHIOPHENE

Sodium amide, %	Sodium acetylide,[a] %	Tetrahalide yield,[b] %
25	75	90
50	50	65
75	25	35
..	100	35

[a] The approximate percentages were calculated by reducing the time required for 100% conversion. [b] Calculated according to the equation:



Discussion

It has been shown earlier[10] that monohalides of benzene, under analogous reaction conditions as outlined above, were converted in liquid ammonia with sodium amide and potassium amide to the corresponding amines. However, we have to consider the different reactivities of the thiophene and benzene nuclei. The former, due to the presence of its sulfur atom, is known to have a smaller effective nuclear charge and a greater electron mobility and is, therefore, capable of reacting in a quite different manner. It is known also that the corresponding thiopheneamines are unstable compounds and can be isolated only as salts. Under the present conditions, the isolation of the corresponding amines would, therefore, not be expected.

The failure of 2-chlorothiophene and 3-bromothiophene to undergo the above conversion reaction can easily be explained considering the higher bond energies of the carbon–chlorine bond or carbon–bromine bond in the 3-position. It is known that the bond energy of the bromine–carbon bond in 2- or 5-position of the thiophene nucleus is lower than that of the corresponding chlorine–carbon bond, *e.g.*, the typical Wurtz syntheses are not reported with the chlorothiophene, while 2-bromothiophene undergoes this reaction.[11]

3-Bromothiophene does not undergo Grignard formation with magnesium in ether[12] while 2-bromothiophene reacts vigorously.

It seems that when the amide ion concentration is too high the dehydrohalogenation reaction prevails, the C–S bond breaks and the decomposition proceeds to the thiol stage and beyond. It is true that in the course of our investigations we failed to isolate any free thiophene when monohalides were used as starting materials; however, we succeeded in isolating monohalides when dihalides served as starting materials. It is very probable that the free thiophene nucleus possessing a negative charge is unstable and before accepting the proton decomposes more readily.

That nitrogen bromide or acetylene bromide could serve as electrophilic brominating agents was disproved by the fact that attempts to isolate bromophenol derivatives were of no avail. It appears therefore that the conversion is initiated by the amide ion and may proceed either *via* formation of a complex which under decomposition with water (or more probably sodium hydroxide) gives

(10) F. W. Berson, R. E. Wright, Ch. Chandler and W. A. Gilkey, *J. Org. Chem.*, 1, 170 (1936).

(11) E. Schleicher, *Ber.*, **18**, 3015 (1885).

(12) W. Steinkopf, H. Jacob and H. Penz, *Ann.*, **515**, 135 (1934).

rise to the higher halogenated thiophene derivative. However, the results might also satisfy the consideration of an S_N2 nucleophilic displacement involving solvolysis of the ions taking part in the reaction.

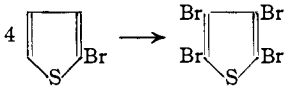
Experimental

Materials.—The 2-bromothiophene (partly) as well as 2-iodothiophene, 3-bromothiophene, 3-methyl-2-bromothiophene, 2,3-dibromothiophene and 2,5-dibromothiophene were prepared according to earlier descriptions[7] while sodium amide and sodium acetylide were obtained by known methods.[6] 2-Chlorothiophene, 2-bromothiophene (partly), phenol, absolute ether, 3-methylthiophene and the ammonium halides used were commercial products.

Ammonolysis of 2-Thienyl Halides at the Boiling Point of Liquid Ammonia.—To 750 ml. of liquid ammonia 0.5 mole of 2-thienyl halide was added dropwise under continuous stirring. The solution was stirred for 3–4 additional hours. Sufficient amount of water was added to the reaction mixture and the halides were extracted with ether. The thienyl halides used were recovered quantitatively.

General Procedure for the Reactions of 2-Thienyl Halides, 3-Bromothiophene, 3-Methyl-2-bromothiophene, 2,3- and 2,5-Dibromothiophene with Sodium Acetylide and Sodium Amide.—The procedure applied was essentially the same as recorded for 2-bromothiophene with sodium acetylide in liquid ammonia.

Reactions of 2-Bromothiophene with Sodium Acetylide in Liquid Ammonia.—To the prepared sodium acetylide (1 mole) in 1.5 l. of liquid ammonia, 1 mole of 2-bromothiophene was added dropwise. The reaction mixture was stirred for 3–4 additional hours and hydrolyzed with water. After extraction with ether there resulted a crystalline substance (45 g., yield 45%) when calculated according to the equation



0.25 mole of 2-bromothiophene was recovered and 6 g. of high boiling oil, a mixture of tri- and dibromothiophene, was obtained. The substance recrystallized from dilute ethanol gave white needles melting at 114°. The molecular weight determined by the Rast method was 380 ± 12. The product was identified as tetrabromothiophene in that it did not depress the melting point on admixture with an authentic specimen prepared according to the method of Volhard and Erdmann.[13]

Anal. Calcd. for C_4Br_4S: C, 12.02; Br, 80.00; S, 8.04. Found: C, 12.31; Br, 80.25; S, 8.44.

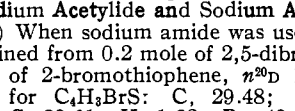
Reactions of 2-Iodothiophene with Sodium Acetylide in Liquid Ammonia.—Eighteen grams of crystalline substance was obtained from 50 g. (0.24 mole) of 2-iodothiophene; yield 50%. It melted at 198° and did not depress the m.p. on admixture with an authentic specimen prepared according to an earlier description.[14]

Anal. Calcd. for C_4I_4S: I, 86.38. Found: I, 86.33.

No starting material was recovered. However, there was isolated 2,5-diiodothiophene (3 g.), m.p. 38°, and probably 2,3,5-triiodothiophene. The latter had a m.p. of 98°.

Anal. Calcd. for $C_4H_2I_2S$: C, 14.33; H, 0.60; I, 75.77. Found: C, 14.5; H, 0.50; I, 75.8. Calcd. for C_4HI_3S: I, 82.42. Found: I, 82.3.

Reactions of 2,5-Dibromothiophene and 2,3-Dibromothiophene with Sodium Acetylide and Sodium Amide in Liquid Ammonia.—(a) When sodium amide was used as a reagent there was obtained from 0.2 mole of 2,5-dibromothiophene: (1) 0.02 mole of 2-bromothiophene, $n^{20}D$ 1.585, showing *Anal.* Calcd. for C_4H_3BrS: C, 29.48; H, 1.84; Br, 49.1. Found: C, 29.61; H, 1.82; Br, 49.52. (2) 3.5 g. (0.011 mole) tribromothiophene which melted at 29°. (3) 7 g. (0.017 mole) of tetrabromothiophene which melted at 114°. According to the equation



this amounts to a conversion of 18.5%.

(13) J. Volhard and H. Erdmann, *Ber.*, **18**, 454 (1890).

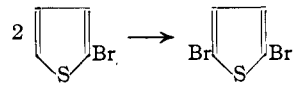
(14) W. Steinkopf and W. Hanske, *Ann.*, **527**, 247 (1937).

(b) When sodium acetylide was used alone there was obtained tetrabromothiophene (m.p. 113°) in a yield of 35% and an analogous amount of 2-bromothiophene to the above described which was identified by its refractive index ($n^{20}D$ 1.5864) and elemental analysis. *Anal.* Calcd. for C_4H_3BrS: Br, 49.1. Found: Br, 49.6.

(c) When sodium amide was used as a reagent there was obtained from 0.1 mole of 2,3-dibromothiophene: (1) 0.01 mole of 3-bromothiophene $n^{20}D$ 1.586, b.p. 157°. *Anal.* Calcd. for C_4H_3BrS: C, 29.48; H, 1.84; Br, 49.1. Found: 29.6; H, 1.82; Br, 49.5. (2) 0.5 g. of tribromothiophene which melted at 43° (probably 2,3,4-tribromothiophene). (3) 5 g. (0.013 mole) of tetrabromothiophene which melted at 113°. This amounts to a conversion of 13%.

(d) When sodium acetylide was used alone, the results were analogous to that of 2,5-dibromothiophene.

Reactions of 2-Bromo, 2-Iodo-, 3-Methyl-2-bromo-, 3-Bromothiophene with Sodium Amide.—(a) From 163.2 g. (1 mole) of 2-bromothiophene there was obtained: (1) 2-bromothiophene, 59 g. (0.36 mole), 35% recovery. (2) 2,5-dibromothiophene, 45 g. (0.188 mole), $n^{20}D$ 1.6380. This amounts to a conversion of 36% according to the equation



Anal. Calcd. for $C_4H_2Br_2S$: C, 19.76; H, 0.83; Br, 66.12. Found: C, 19.76; H, 0.88; Br, 66.40.

(b) Using 1 mole of 2-iodothiophene to 1 mole of sodium amide there was obtained 2,5-diiodothiophene in a yield of 15% which melted at 38° while 45% of 2-iodothiophene was recovered. *Anal.* Calcd. for $C_4H_2I_2S$: C, 14.33; H, 0.60; I, 75.77. Found: C, 14.20; H, 0.5; I, 75.8.

(c) Using 1 mole of 3-methyl-2-bromothiophene to 1 mole of sodium amide there was obtained 21 g. of 3-methyl-2,5-dibromothiophene (b.p. 228°, $n^{20}D$ 1.612). This amounts to a conversion of 16.5%. *Anal.* Calcd. for $C_5H_4Br_2S$: C, 23.6; H, 1.56; Br, 62.67. Found: C, 24.0; H, 1.23; Br, 63.2.

(d) The 3-bromothiophene was recovered quantitatively. When the sodium amide ion concentration was increased to 2 moles to 1 mole of 3-bromothiophene used some decomposition of the latter took place.

Reactions of 2-Bromothiophene with Sodium Amide and Sodium Acetylide Mixtures.—0.2 gram-atom of metallic sodium was dissolved in 450 ml. of liquid ammonia, 0.04 g. of ferric nitrate was added and the mixture stirred for 15 minutes. The dry acetylene gas was bubbled through during a period of 20 minutes. According to the standard procedure developed in other experiments approximately 75% of the sodium amide present was converted to sodium acetylide. 0.1 mole (16.3 g.) of 2-bromothiophene was added dropwise and stirred for 2.5 additional hours. After decomposition with water and extraction with ether there resulted 9.5 g. of tetrabromothiophene which when recrystallized from ethanol melted at 114°; yield 95%.

Reactions of Mixtures of 2-Bromothiophene and Phenol with Sodium Amide and Sodium Acetylide in Liquid Ammonia.—0.2 gram-atom of metallic sodium was converted to sodium amide in liquid ammonia. 0.1 mole of 2-bromothiophene and 0.1 mole of phenol were mixed and added dropwise to the sodium amide solution in liquid ammonia. The phenol was readily dissolved in the halide under absorption of heat. The reaction mixture was stirred for 3–4 additional hours. After decomposition with water the thienyl halides were extracted with ether while the phenol remained in solution as sodium phenolate. The solution of the latter was evaporated under diminished pressure and after acidification the phenol was recovered quantitatively. From the ethereal solution there was obtained 37% (6.05 g.) of recovered 2-bromothiophene and tetrabromothiophene (1 g.) in a yield of 10%.

Reactions of 2-Thienyl Halides with Sodium Acetylide in the Presence of Liquid Ammonia and Absolute Ether or Ammonium Halides.—The halides used were dissolved in absolute ether and then added dropwise to the sodium acetylide solution in liquid ammonia. Absolute ether in amounts of 10% of the total solvent stopped the progress of the reaction. The ammonium halides were added in form of a salt.

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Interconversion of Hexachlorocyclohexane Isomers

BY R. R. WHETSTONE, F. C. DAVIS AND S. A. BALLARD

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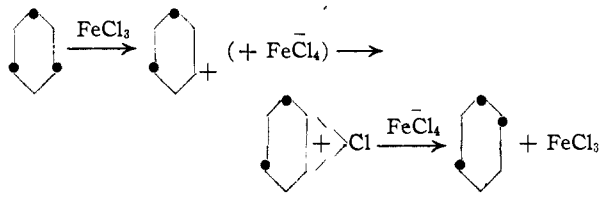
Because of the insecticidal properties of the γ-isomer, the 1,2,3,4,5,6-hexachlorocyclohexanes have received extensive investigation[1] but the interconversion of the isomers has apparently not been reported. A study of the pure α-, β-, γ- and δ-hexachlorocyclohexane isomers revealed that they are isomerized by heating with anhydrous ferric chloride in sealed tubes under nitrogen at temperatures of 140° or higher. Under these conditions the alpha was the most stable isomer and was isomerized slowly and in low conversion to a mixture of isomers from which delta was isolated and in which beta, gamma and epsilon were indicated by infrared spectrophotometric analysis. The β-isomer was converted readily and almost exclusively to alpha. Gamma was isomerized largely to the α- and δ-isomers; the change in composition of this product mixture with severity of heating suggested that delta was the primary product and was subsequently converted to alpha. Reaction of the pure delta was indeed found to give alpha with a lesser amount of gamma; however, only a small portion of the delta was isomerized. Other Friedel–Crafts type catalysts also promoted isomerization but were usually either less effective or like aluminum trichloride caused extensive degradation with formation of trichlorobenzene. Compositions of typical isomerization products are given in Table I.

The isomerization of the symmetrical β-isomer (135/246) configuration[2] to the α-isomer (124/356) requires the inversion of the positions of the chlorine atoms on any two adjacent carbon atoms. Such a change is highly suggestive of a displacement reaction with participation from a neighboring group as illustrated below.[3] In the formulas a dot represents a chlorine atom above the plane of the ring.



First, a ferric chloride molecule abstracts one of the three chlorine atoms on, for illustration, the top side of the cyclohexane ring. The electron deficient carbon atom then forms a cyclic ion with either of the two identical adjacent carbon atoms and the attached chlorine atom. This chlorine atom and hence the cyclic ion are necessarily on the under side of the cyclohexane ring. Approach to and return of a chlorine atom from the catalyst complex can then be only to the top side of the ring. Return of the chlorine atom to the original carbon atom reforms the β-isomer but return to the neighboring participating carbon atom results in the α-isomer.

Since all carbon atoms in the β-isomer are equivalent, isomerization by this mechanism can give only the α-isomer as a primary product. With the other known isomers in which all carbon atoms are not identical formation of two or more isomers would be expected. Since a carbon atom can participate with each of its two neighboring carbon atoms, four transformations are possible from each carbon atom or a total of 24 from all six. Many of these transformations will of course reform the starting isomer. In this mechanism the positions of both the abstraction and the return of the chlorine atom would be influenced by steric factors and selective formation of one or more isomers would be expected. The products predicted from each of the four isomers and the number of possible transformations giving rise to each product are shown in the table with the experimental results. In investigating the composition of the isomerization mixtures, no thorough attempt was made to isolate minor constituents. Since the spectra and indeed the

TABLE I

INTERCONVERSION OF HEXACHLOROCYCLOHEXANE ISOMERS

Isomer	Structure[2]	$FeCl_3$, % w. of isomer	Time, hr.	Temp.,[a] °C.	Composition, % w.[b] α	β	γ	δ	Total	Predicted products[e]
α	124/356	100	48	170	87	1.5	0.8	3.7[c]	93[d]	α(12),β(2),γ(4),δ(4),ε = 123/456(2)
β	135/246	8[f]	24	170	77.3	13.0	1.3	2.7	94.3	α(12),β(12)
		5	0.5	310	80.1[c]	10.3	1.0	1.3	92.7	
γ	1245/36	5	12	140	2.8	0.0	60.3	34.1	97.2	α(8),γ(8),δ(8)
		5	12	170	15	0	21	52[c]	88	
		20	122	170	50.0	2.3	4.6	44.0	100.9	
δ	1235/46	20	72	170	15.8	0.0	4.4	68.1	88.3	α(4),β(4),γ(4),δ(8),η = 1234/56(4)

[a] Heated in evacuated, sealed glass tube. [b] By infrared spectrophotometric analysis. [c] Presence confirmed by isolation. [d] Trace of ε-isomer indicated. [e] From displacement with participation from neighboring groups; figures in parentheses are the number of possible transformations out of the total of 24 which would give the indicated isomer. [f] $SbCl_3$, 61%, present as solvent.

(1) S. J. Cristol, N. L. Hause and J. S. Meek, THIS JOURNAL, **73**, 674 (1951).

(2) Following Cristol,[1] the numbers above the line indicate the positions of chlorine atoms which lie above a hypothetical planar cyclohexane ring and those below the line lie below the hypothetical planar cyclohexane ring.

(3) S. Winstein and E. Grunwald, THIS JOURNAL, **70**, 828 (1948).

existence of the η- and θ-isomers[4] were not known at the time of these experiments, these and possibly other isomers may have been formed in small amounts. Also, secondary isomerization of primary products undoubtedly affected the final composition both qualitatively and quantitatively; the β-isomer in particular if formed should have largely isomerized to alpha. In view of these considerations the agreement of the observed results with those predicted appears sufficiently good to support the described mechanism. The ϵ-, η- and θ-isomers have not been available to us for isomerization so that we have not been able to use them to test the suggested mechanism.

We gratefully acknowledge the aid of Messrs. W. R. Harp, Jr., and F. S. Mortimer in making the spectroscopic analyses, and the suggestions of Dr. A. G. Kridl on the mechanism of isomerization.

(4) A. J. Kolka, H. D. Orloff and M. E. Griffing, Abstracts of Papers, 121st Meeting of American Chemical Society, Buffalo, N. Y., March 24-27, 1952.

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Tracer-diffusion in Liquids. IV. Self-diffusion of Calcium Ion and Chloride Ion in Aqueous Calcium Chloride Solutions[1]

BY JUI H. WANG

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The salt diffusion of calcium chloride in its aqueous solutions has been the subject of experimental study of many workers in the past fifteen years. The results obtained by Harned and Levy[2] indicate that the measured salt diffusion coefficients of calcium chloride are lower than the values computed from the Onsager–Fuoss theory in solutions of concentration between 0.001 and 0.005 formula weight per liter. The salt diffusion coefficients of calcium chloride in concentrated aqueous solutions have been determined by Hollingshead and Gordon[3] and by Robinson and Chia[4] by means of the diaphragm cell method, and independently by Stokes and coworkers[5] and by Lyons and Riley[6] by means of the optical method. Their results show that in calcium chloride solutions of concentration above 0.2 formular weight per liter the salt diffusion coefficient increases with increasing salt concentration up to about 2.2 formular weight per liter. This phenomenon has generally been attributed to the continual decrease of the activity coefficient of the salt along the diffusion path. In self-diffusion, however, the chemical composition and hence the activity coefficient of the diffusing ions is constant along the diffusion path, the theoretical relationship between the diffusion coefficient and salt concentration becomes simpler and we may expect the shape of the D *vs.* $\sqrt{c}$ curves for self diffusion to be quite different from that for salt diffusion. Furthermore, it should be of interest to see how the self diffusion coefficients for different ions in the same solution vary as the salt concentration of the solution increases continually up to saturation. In the present work the self-diffusion coefficients of calcium ion and chloride ion in aqueous calcium chloride solutions at 25° have been measured by means of the improved capillary method.

Experimental

Radioactive Tracers.—Ca^{45} and Cl^{36} were used as tracers in the self-diffusion measurements. These were obtained from the Isotopes Division of the U. S. Atomic Energy Commission at Oak Ridge, Tennessee.

Diffusion Measurement.—The experimental techniques involved were already discussed in paper I of this series. The radioactivity of the diffusion samples was measured with a windowless, continuous flow counter. Since dry calcium chloride is hygroscopic in air, the calculated amount of sodium fluoride solution was added to each sample before evaporation to dryness under an infrared lamp. The samples so prepared contained approximately equal amounts of dry residue (solid sodium chloride and calcium fluoride) and were kept in a desiccator overnight before counting. Since in the calculations to obtain the diffusion coefficients only the ratio of the concentrations of radioactive tracers is involved, possible errors due to self-absorption of β-radiation are automatically eliminated.

Results.—The measured self-diffusion coefficients of Ca^{++} and Cl^- in aqueous calcium chloride solutions at 25° are listed in Table I. Each value listed in Table I is the average result of at least four measurements.

TABLE I

SELF-DIFFUSION COEFFICIENTS OF Ca^{++} AND Cl^- IN $CaCl_2$ (aq.) AT 25°

Concentration formular wt./liter	$D_{Ca^{++}} \times 10^5$, cm.²/sec.	$D_{Cl^-} \times 10^5$, cm.²/sec.
0.0100	0.778 ± 0.028	
.0705	.782 ± .015	1.89 ± 0.02
.282	.767 ± .008	1.72 ± .04
.803	.646 ± .025	1.60 ± .04
1.41	.560 ± .020	1.42 ± .03
2.68	.405 ± .015	0.907 ± .020
4.02	.225 ± .002	0.447 ± .015
5.36	.100 ± .009	0.159 ± .010

Discussion.—Using apropriate units the Onsager equation may be written as[7]

$$D_j = \frac{RT\lambda_j^0}{|Z_j|\mathfrak{F}^2}\frac{\lambda_j^0|Z_j|\mathfrak{F}}{3N\mathfrak{D}} \times 2.694 \times 10^{16} \times \sqrt{\frac{4\pi}{\mathfrak{D}RT}}\,[1 - d(\omega_j)]\sqrt{\sum_i c_i Z_i^2} \quad (1)$$

where D_j is the tracer-diffusion coefficient of ions of the jth kind in a salt solution, Z_i is the charge in electronic units and c_i the concentration in moles per liter of ion i, λ_j^0 the limiting equivalent conductance of ion j, $\mathfrak{D}$ the dielectric constant of the solvent k the Boltzmann constant, $\mathfrak{F}$ the Faraday constant, T the absolute temperature, and $d(\omega_j)$ a function given by

$$d(\omega_j) = \frac{1}{\sum_i c_i Z_i^2}\sum \frac{c_i|Z_i|\lambda_i^0}{(\lambda_i^0/|Z_i|) + (\lambda_j^0/|Z_j|)} \quad (2)$$

For the diffusion of tracer amount of ions of species 1 in salt solution containing ions of species 2 and 3, we have

(1) Contribution No. 1134 from the Department of Chemistry of Yale University: Paper I, THIS JOURNAL, **74**, 1182, 6317 (1952); paper II, **74**, 1611 (1952); paper III, **74**, 1612 (1952).

(2) H. S. Harned and A. L. Levy, *ibid.*, **71**, 2781 (1949).

(3) E. A. Hollingshead and A. R. Gordon, *J. Chem. Phys.*, **9**, 152 (1941).

(4) R. A. Robinson and C. L. Chia, THIS JOURNAL, **74**, 2776 (1952).

(5) Hall, Wishaw and Stokes, private communication.

(6) P. A. Lyons and J. F. Riley, private communication.

(7) See paper I or II of this series.

$$c_1 \cong 0$$
$$c_2|Z_2| = c_3|Z_3|$$

and hence (2) can be written as

$$d(\omega_1) = \frac{|Z_1|}{|Z_2| + |Z_3|}\left[\frac{|Z_2|\lambda_2^0}{|Z_2|\lambda_1^0 + |Z_1|\lambda_2^0}\right] + \frac{Z_3|\lambda_3^0}{|Z_3|\lambda_1^0 + |Z_1|\lambda_3^0} \quad (3)$$

If we take $\lambda^0_{Cl^-} = 76.36$, $\lambda^0_{Ca^{++}} = 59.4$, and using the relationship $\sum_i c_i Z_i^2 = 6c$ where c is the salt concentration in formular weight of $CaCl_2$ per liter of solution, we obtain by combining (1) and (3)

$$D_{Ca^{++}} \times 10^5 = 0.791 - 0.808\sqrt{c} \quad (4)$$

and

$$D_{Cl^-} \times 10^5 = 2.033 - 1.729\sqrt{c} \quad (5)$$

for the self-diffusion of Ca^{++} and Cl^-, respectively, in dilute calcium chloride solutions.

Values of $D_{Ca^{++}} \times 10^5$ and $D_{Cl^-} \times 10^5$ are plotted *vs.* $\sqrt{c}$ in Fig. 1. The two straight lines in the dilute concentration range represent equations (4) and (5), respectively.

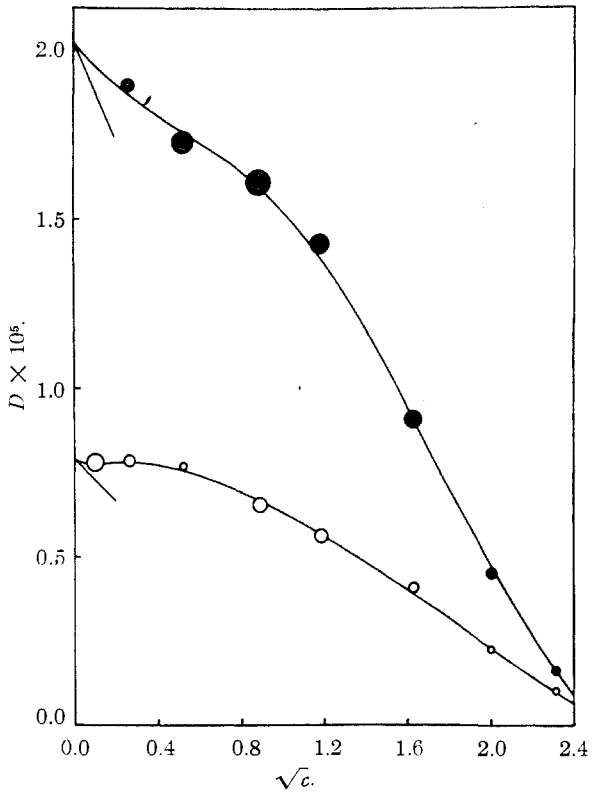

Fig. 1.—Self-diffusion coefficient of Ca^{++} and Cl^- in $CaCl_2$(aq.) at 25°: ●, self-diffusion of Cl^-; ○, self-diffusion of Ca^{++}.

It can be noticed from Fig. 1 that as the concentration of calcium chloride solution decreases continuously to zero the self-diffusion coefficients of both Ca^{++} and Cl^- appear to approach the Nernst's limiting values from above the two straight lines representing Onsager's equations (4) and (5). This tendency of approaching the limiting value from above Onsager's equation appears to be especially apparent in the self-diffusion of calcium ion. It is interesting to recall that an analogous behavior exists in the transference number data of calcium ion in dilute aqueous calcium chloride solutions.[8]

The general shapes of the curves in Fig. 1 are in apparent agreement with the general qualitative interpretation suggested in the three preceding papers of this series.[1] Thus because of the larger mean radius of the hydrated calcium ion and the fact that calcium chloride is a 2-1 electrolyte, all the previously mentioned "distortion-effect" on the structure of solvent water, "effect of sharing of hydration," etc., should become important at lower formular concentration of the salt for calcium chloride than for sodium or potassium chloride solutions.

It may also be noticed from the values listed in Table I that the ratio of the self-diffusion coefficient at infinite dilution to that in 5.36 formular wt. per liter calcium chloride solution is approximately 13 for Cl^- and 8 for Ca^{++}. The ratio of viscosity of 5.36 formular wt. per liter calcium chloride solution to that of pure water at 25° is about 11.

Acknowledgment.—This work was supported by contract AT(30-1)-1375 between the U. S. Atomic Energy Commission and Yale University. When preparing the present manuscript, the author benefited through discussion with Professor H. S. Harned,

(8) H. S. Harned and B. B. Owen, "Physical Chemistry of Electrolytic Solutions," 2nd Ed., Reinhold Publ. Corp., New York, N. Y., 1950, p. 164.

DEPARTMENT OF CHEMISTRY
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Local Anesthetics. III. Modifications of β′-4-Methylphenoxyethyl β-N-Methylbenzylaminoethyl Ether

BY HOWARD B. WRIGHT AND M. B. MOORE

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In papers I and II we have reported that the salts of aryl alkamine ethers $ArOR'NR_2$[1a] and aryloxyalkyl alkamine ethers $ArOR'OR''NR_2$[1b] in which Ar is an aryl, R′ and R″ are alkylene and R is an alkyl or aralkyl group, have been tested as local anesthetics. As a continuation of this work, we have now prepared a number of compounds related to β-4-methylphenoxyethyl β′-N-methylbenzylaminoethyl ether which was reported in paper II[1b] to produce good corneal anesthesia.

Table I lists the compounds synthesized, with pertinent physical and analytical data. Solutions of hydrochlorides or other salts of these ethers were tested for local anesthetic activity by Dr. R. K. Richards and his staff to whom we are indebted. All these ethers displayed corneal and wheal anesthesia but the activity was accompanied by some irritation.

Experimental

The *o*-diphenoxyethoxyethyl chloride was prepared in 47.5% yield by a method Bruson[2] developed for analogous compounds. The boiling point of an analytical sample was 162° (1.0 mm.), $n^{26}D$ 1.5792.

Anal. Calcd. for $C_{16}H_{27}ClO_2$: C, 69.43; H, 6.19; Cl, 12.81. Found: C, 69.51; H, 6.29; Cl, 12.81.

(1) (a) H. B. Wright and M. B. Moore, THIS JOURNAL, **73**, 2281 (1951); (b) **73**, 5525 (1951).

(2) H. A. Bruson, U. S. Patent 2,115,250, April 26, 1938.

TABLE I

ALKAMINE ETHERS, $ArO(CH_2)_2O(CH_2)_2NR_2$

No.	Compound Structural formula Ar	NR_2	Time, hr.	Method	Yield, %	B.p. °C.	Mm.	Formula	Carbon, % Calcd.	Carbon, % Found	Hydrogen, % Calcd.	Hydrogen, % Found
1	$2\text{-}C_6H_5C_6H_4$	$N(CH_3)CH_2C_6H_5$	4.5	B	[e]	218–220	1.0	$C_{24}H_{27}NO_2$	79.74	79.69	7.53	7.25
2	$2\text{-}C_6H_5C_6H_4$	$N(C_2H_5)CH_2C_6H_5$	24	A	[e]	210–211	0.45	$C_{25}H_{29}NO_2$	79.97	79.86	7.78	7.59
3	$4\text{-}CH_3C_6H_4$	$C_5H_{10}N$[a]	24	A	30	171	2.6	$C_{16}H_{25}NO_2$	72.97	72.77	9.57	9.48
4	$4\text{-}CH_3C_6H_4$	$C_6H_{12}N$[b]	24	A	39	180–181	3.1	$C_{17}H_{27}NO_2$	73.60	73.85	9.81	9.79
5	$4\text{-}CH_3C_6H_4$	$C_6H_{12}NS$[c]	24	A	57.5	190–191	1.1	$C_{17}H_{27}NO_2S$	65.98	66.17	8.79	8.67
6	$4\text{-}CH_3C_6H_4$	$N(C_4H_9)_2$	24	A	[e]	191–192	4.4	$C_{19}H_{33}NO_2$	74.22	74.36	10.82	10.56
7	$4\text{-}CH_3C_6H_4$	$NHCH_2CH(CH_3)_2$	24	A	14	156–158	2.4	$C_{15}H_{25}NO_2$	71.67	71.91	10.03	9.81
8	$4\text{-}CH_3C_6H_4$	$NH(CH_2)_4CH_3$	24	A	20	175	2.9	$C_{16}H_{27}NO_2$	72.41	73.03[d]	10.25	10.13[d]
9	$4\text{-}CH_3OC_6H_4$	$N(CH_3)CH_2C_6H_5$	3.5	B	[e]	204–205	1.4	$C_{19}H_{25}NO_3$	72.35	72.68[d]	7.99	8.05[d]

[a] 1-Piperidyl radical. [b] 2-Methyl-1-piperidyl radical. [c] 2,6-Dimethyl-4-thiomorpholinyl radical. [d] Average of two analyses. [e] Obtained in low yield.

The benzalethylimine prepared by the method of Cromwell[3,4] was obtained in 75% yield. The imine, boiling point 84–88° (10 mm.), $n^{26}D$ 1.5365, was hydrogenated at 1025 p.s.i. (80°) with Raney nickel for 1.5 hours in the absence of a solvent by G. R. Stone and Morris Freifelder. The N-ethylbenzylamine, b.p. 195–200° (747 mm.), n_D^{25} 1.5090, was obtained in 75% yield.[5]

(3) N. H. Cromwell, THIS JOURNAL, **65**, 313 (1943).

(4) H. Zaunschirm, *Ann.*, **245**, 279 (1888).

(5) (a) A. Mailke, *Bull. soc. chim.*, [4] **25**, 322 (1919); (b) O. Wallach, *Ann.*, **343**, 73 (1905); (c) F. Kraft, *Ber.*, **23**, 278 (1890).

Anal. Calcd. for $C_9H_{13}N$: C, 79.95; H, 9.69. Found: C, 80.22; H, 9.54.

All of the ethers in Table I were prepared by Method A or B described in paper II.[1b]

Acknowledgment.—We are indebted to E. F. Shelberg, Chief Microanalyst, and his staff for the analytical data.

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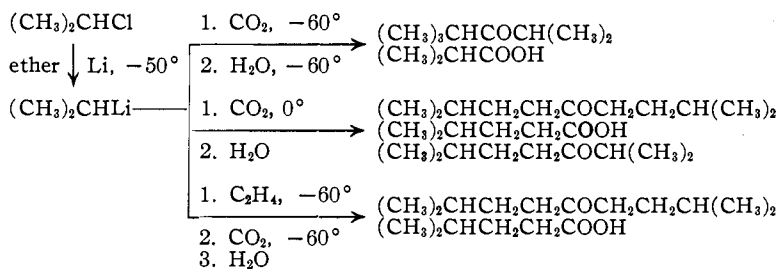
COMMUNICATIONS TO THE EDITOR

THE REACTION OF ISOPROPYLLITHIUM AND *t*-BUTYLLITHIUM WITH SIMPLE OLEFINS

Sir:

Isopropyllithium[1] and *t*-butyllithium[2] are advantageously manipulated in ether, being much more readily prepared in this solvent than in hydrocarbons. However, they show in enhanced degree the well known tendency of organolithium compounds to decompose ether.[3,4,5,6,7,8] When isopropyllithium is prepared and carbonated in ether at temperatures below −50°, hydrolysis of the resulting solution yields the normal products, diisopropyl ketone and some isobutyric acid. When however, a solution of isopropyllithium from one mole of isopropyl chloride was allowed to warm to room temperature, a reaction was observed which caused the ether to boil, and subsequent carbonation yielded only *diisoamyl ketone* (0.25 mole, b.p. 87° (8 mm.); calcd. for $C_{11}H_{22}O$: C, 77.78, H, 13.01; found: C, 77.80, H, 13.13) and 0.05 mole of isocaproic acid. Since these products could be formed only by the addition of isopropyllithium to ethylene (resulting from decomposition of ether), an experiment was performed in which ethylene was bubbled at −60° through a solution of isopropyllithium prepared at −50°. The ethylene was readily absorbed. This solution was carbonated without allowing the temperature to rise. Hydrolysis yielded diisoamyl ketone in addition to a smaller acid fraction and residue. The diisoamyl ketone (2,4-dinitrophenylhydrazone, m.p. 53.5–54°) was identified by Beckmann degradation to isoamylamine (phenylthiourea, m.p. 105–105.5°; no depression on mixture with an authentic specimen, m.p. 104–105°)[9] and isocaproic acid (anilide, m.p. 110.5–111°; *p*-phenylphenacyl ester, m.p. 70°), and by synthesis from isoamyl chloride by carbonation of the lithium derivative.



It is clear from these experiments that iso-

(1) H. Gilman, E. A. Zoellner, W. M. Selby and C. Boatner, *Rec. trav. chim.*, **54**, 584 (1935).

(2) P. D. Bartlett, C. G. Swain and R. B. Woodward, THIS JOURNAL, **63**, 3229 (1941).

(3) K. Ziegler and A. Colonius, *Ann.*, **479**, 135 (1930).

(4) A. Luttringhaus and G. von Saaf, *Angew. Chem.*, **51**, 915 (1938); *Ann.*, **557**, 25 (1947).

(5) A. Haubein, *Iowa State Coll. J. Sci.*, **18**, 48 (1943); *C.A.*, **38**, 716 (1944).

(6) H. Gilman and R. N. Clark, THIS JOURNAL, **69**, 1499 (1947).

(7) K. Ziegler and H. G. Gellert, *Ann.*, **567**, 185 (1950).

(8) R. L. Letsinger, A. W. Schnizer and E. Bobko, THIS JOURNAL, **73**, 5708 (1951).

(9) The melting point is given as 101–102° by M. L. Willard and M. Z. Jones, *ibid.*, **62**, 2876 (1940).