Assuming that the ratio of the values of the solubility product and instability constants for the cobaltous hydroxide and hydroxy–cobalt complex ion, respectively, remain constant over the range of hydroxyl ion concentration involved, we may plot the logarithm of the absorbancy *versus* the logarithm of the hydroxyl ion activity. Hydroxyl ion activities[8] are used instead of molar concentrations because of the high concentrations used. We should obtain a straight line whose slope is $(n - 2)$, where n is the number of hydroxyl ions complexing the cobaltous ion to form the light-absorbing ion species in strongly alkaline solutions.

The data for the determination of $(n - 2)$ are presented in Fig. 4. The straight lines for the values at the two absorption peaks of 535 and 585 mμ were drawn from the equations determined by the method of least squares for the concentration range of 5 to 5 M. The corresponding slopes, which are both equal to 1.24, yield a value of 3.24 for n, the number of hydroxyl ions in the coördination sphere of the hydroxy-cobalt(II) complex ion. Above 5 M hydroxyl ion concentration the straight line veers off and forms another straight line which was similarly treated by the method of least squares. The values for their slopes were found to be 0.784 and 0.774, at 535 and 585 mμ, respectively. These values for $(n - 2)$ resulted in n being equal to 2.78 and 2.77, respectively. Again, they are approximately equal to a hydroxyl ion coördination value of three for the complex. Considering the ionic strengths and activities of these strongly alkaline solutions and their consequent departures from ideality, the experimentally determined value is indicative of the existence of the trihydroxy-

(8) R. A. Robinson and R. H. Stokes, *Trans. Faraday Soc.*, **45**, 612 (1949).

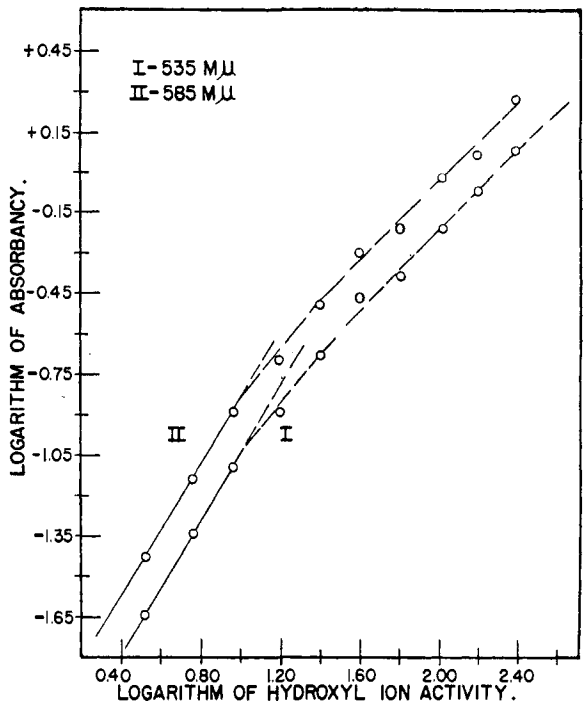


Fig. 4.—Logarithm of absorbancy as a function of hydroxyl ion activity in potassium hydroxide solutions saturated with cobalt(II); 1.00 cm. cells; 0.020 mm., slit width; 25°.

cobalt(II) complex ion in the alkali hydroxide solutions.

The reaction for the formation of this complex ion may be represented by the equation

$$Co(H_2O)_6^{++} + 3OH^- \rightleftharpoons Co(OH)_3(H_2O)_3^- + 3H_2O$$

OAK RIDGE, TENNESSEE

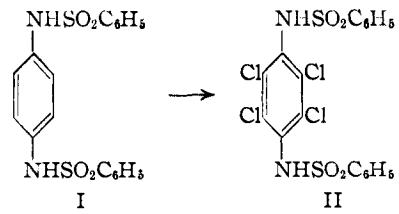
NOTES

Chlorination of Benzenesulfonyl Derivatives of Aromatic Amines

BY ROGER ADAMS AND B. H. BRAUN[1]

RECEIVED JANUARY 14, 1952

Chlorination of *p*-phenylenedibenzenesulfonamide (I) in nitrobenzene by means of chlorine resulted in the formation of a mixture of dichloro derivatives. The tetrachloro derivative (II), which was desired, has been prepared previously by the rather tedious route of successive oxidations and additions of hydrogen chloride to *p*-phenylenedibenzenesulfonamide.[2] By use of the solvent, dimethylformamide, the synthesis of the tetrachloro derivative (II) was readily achieved in a one step chlorination. This solvent is itself chlorinated with



I II

an exothermic reaction, but by keeping the reaction mixture below 60° through regulation of the flow of chlorine, the tetrachloro compound resulted in good yield. The amount of chlorine used was a critical factor and was determined empirically. Too large an excess of chlorine failed to give the compound desired.

In a similar manner, several other polychlorinated

(1) An abstract of a portion of a thesis submitted by Mr. B. H. Braun to the Graduate College of the University of Illinois, 1952, in partial fulfillment of the requirements of the Degree of Doctor of Philosopby.

(2) R. Adams, E. F. Elslager and K. F. Heumann, THIS JOURNAL, **74**, 2608 (1952).

products were synthesized which were not accessible by chlorination in customary solvents; 2-methyl-3,5,6-trichloro-*p*-phenylenedibenzenesulfonamide from 2-methyl-*p*-phenylenedibenzenesulfonamide; 3,4,5,6-tetrachloro-*o*-phenylenedibenzenesulfonamide from *o*-phenylenedibenzenesulfonamide; 4-methyl-3,5,6-trichloro-*o*-phenylenedibenzenesulfonamide from 4-methyl-*o*-phenylenedibenzenesulfonamide. The ortho compounds react less vigorously than the para and a larger excess of chlorine is required.

When the benzenesulfonyl derivative of aniline was chlorinated in nitrobenzene, the product was the benzenesulfonyl derivative of 2,4-dichloroaniline. With dimethylformamide as solvent, however, the 2,4,6-trichloro compound resulted.

The dibenzenesulfonyl derivative of 3,3′-dichlorobenzidine was also chlorinated in dimethylformamide to the 3,3′,5,5′-tetrachloro derivative when the use of other solvents failed. This is reported in another[2a] communication.

Experimental

All melting points are corrected.

Chlorination of Benzenesulfonanilide in Nitrobenzene: 1-Benzenesulfonamido-2,4-dichlorobenzene.—Into a solution of 10 g. of benzenesulfonanilide in 50 ml. of nitrobenzene (induced by prolonged agitation) a vigorous stream of chlorine was passed. The solution rapidly became warm and the flow of chlorine was regulated so that the temperature of the reaction mixture did not rise over 60°. After 15 minutes, the temperature dropped and did not rise even on increasing the rate of addition of chlorine. The gain in weight at this point was 5.7 g. The solvent was removed by steam distillation and the resulting heavy brown oil solidified on cooling. After one crystallization from 95% ethanol, 11.1 g. (86%) of pale brown crystals resulted. By recrystallization from the same solvent white needles were formed, m.p. 127–128° (lit. m.p. 128°).[3]

By heating 2.75 g. of the product with 10 ml. of concentrated sulfuric acid to 105–110° for 45 minutes, hydrolysis occurred. After pouring the reaction mixture into 100 ml. of water, the solution was basified with aqueous ammonia. The amine which separated was taken up in two 50-ml. portions of ether, the ether layers were combined, dried over potassium hydroxide, and the solvent distilled. The oily residue was dissolved in petroleum ether (b.p. 80–110°) and after partial evaporation and cooling, crystals formed. The balance of the solvent was removed at room temperature by an air stream. The yield of 2,4-dichloroaniline was 1.40 g. (93%) of product which on recrystallization from petroleum ether melted at 62.5–63° (lit. m.p. 63°).[4]

Chlorination of Benzenesulfonanilide in Dimethylformamide: 1-Benzenesulfonamido-2,4,6-trichlorobenzene.—Into a solution of 10 g. of benzenesulfonanilide in 40 ml. of dimethylformamide, chlorine was passed until the gain in weight was 16.3 g. The temperature of the reaction mixture was maintained below 60°. The mixture was poured into water, filtered, and the precipitate recrystallized from glacial acetic acid. The yield was 11.5 g. of needles and dilution of the mother liquor with water gave an additional 1.0 g. The total yield was 12.5 g. (85%). Three recrystallizations from glacial acetic acid gave a pure product, m.p. 152–154°.

Anal. Calcd. for $C_{12}H_8Cl_3NO_2S$: C, 42.81; H, 2.39; N, 4.16. Found: C, 43.08; H, 2.61; N, 4.26.

After heating 1.00 g. with 7 ml. of concentrated sulfuric acid to 110–115° for one hour, the solution was cooled and poured into water. The 2,4,6-trichloroaniline, weighing 0.55 g. (94%), precipitated. It was purified by crystallization from petroleum ether (b.p. 80–110°), m.p. 77.5–78° (lit. m.p. 78.5°).[5]

(2a) R. Adams and R. R. Holmes, *ibid.*, **74**, 3033 (1951).
(3) F. D. Chattaway, *J. Chem. Soc.*, **85**, 1181 (1904).
(4) F. Beilstein and A. Kurbatow, *Ann.*, **182**, 94 (1876).
(5) P. J. Montagne, *Rec. trav. chim.*, **21**, 376 (1902).

Chlorination of *p*-Phenylenedibenzenesulfonamide in Various Solvents: Acetic Acid.—No exothermic reaction occurred on passing chlorine into a glacial acetic acid solution of the *p*-phenylenedibenzenesulfonamide at 70°. Only low melting mixtures were isolated which obviously were lower chlorinated products.

Nitrobenzene: 2,5- and 2,3-Dichloro-*p*-phenylenedibenzenesulfonamide.—Into a suspension of 50 g. of *p*-phenylenedibenzenesulfonamide in 200 ml. of nitrobenzene containing a catalytic amount of ferric chloride, chlorine was passed with vigorous stirring at such a rate that the temperature was held between 52–56°. After 80 minutes the temperature dropped and did not rise even on increasing the rate of addition of chlorine. Addition of 200 ml. of carbon tetrachloride caused precipitation of 43.6 g. (74%) of almost white crystals which sintered at 173° and melted with much decomposition above 182°. A small portion was recrystallized from glacial acetic acid and then melted with only slight darkening at 187–229.5°, after sintering at 172°. This sample was submitted for infrared analysis which indicated it to be a mixture of 2,5- and 2,3-dichloro-*p*-phenylenedibenzenesulfonamide by comparison with a similar mixture which had been prepared previously in this Laboratory by another route.[2]

Dimethylformamide: 2,3,5,6-Tetrachloro-*p*-phenylenedibenzenesulfonamide.—Into a solution of 50 g. of *p*-phenylenedibenzenesulfonamide in 200 ml. of dimethylformamide, chlorine was passed at such a rate that the temperature did not rise above 60° until the gain in weight of the reaction mixture was 32.2 g. After pouring into water, the precipitate that separated was filtered by suction, then dissolved in the minimum amount of dimethylformamide at 100° (about 500 ml. was required). The color of the solution became a deep violet with a red fluorescence. Upon addition with stirring of 1 liter of hot acetic acid to the hot solution, white needles started to separate. After cooling, the precipitate weighed 46.5 g. (68%). From the mother liquors by dilution with water an additional 4 g. resulted. The product had an m.p. 278–281° (dec.) (lit. m.p. 273–274°).[2] This melting point with decomposition varies with rate of heating. By four additional recrystallizations from nitrobenzene, the product darkened at 303° and melted at 309–310° (dec.). For oxidation to the diimide, the product, m.p. 278–281°, is entirely satisfactory.

Use of less chlorine usually resulted in a mixture of tri- and tetrachloro derivatives. If saturated with chlorine, no solid products could be obtained from the reaction mixture. The use of a temperature of from 30 to 60° gave the same results, but at 80° a vigorous reaction occurred after which no solid products could be obtained. Ferric chloride was added in several chlorinations, but the yields were unaffected.

Chlorination of 2-Methyl-*p*-phenylenedibenzenesulfonamide: 2-Methyl-3,5,6-trichloro-*p*-phenylenedibenzenesulfonamide.—Into a solution of 10 g. of 2-methyl-*p*-phenylenedibenzenesulfonamide in 40 ml. of dimethylformamide, chlorine was passed at such a rate that the temperature was maintained below 60° until the gain in weight of the reaction mixture was 6.2 g. The product was isolated as previously described and once crystallized from dimethylformamide and glacial acetic acid. The yield was 8.5 g. (68%), m.p. 273–274° (dec.) (lit. m.p. 270–271°).[2] The melting point was unchanged after a second similar crystallization, but after four recrystallizations from nitrobenzene, the product darkened at 284° and melted at 292–293° (dec.). The infrared spectra of the crude sample, m.p. 273–274°, and of that recrystallized from nitrobenzene were identical. However, a sample of material made by another route with m.p. 270–271°,[2] had an extra band at 1027 cm.$^{-1}$. This disappeared after recrystallization from nitrobenzene which raised the melting point to that reported in this investigation.

Chlorination of *o*-Phenylenedibenzenesulfonamide: 3,4-5,6-Tetrachloro-*o*-phenylenedibenzenesulfonamide.—A solution of 5 g. of *o*-phenylenedibenzenesulfonamide in 20 ml. of dimethylformamide was cooled and chlorine passed in, keeping the temperature below 40°. After the gain in weight of the reaction mixture was 6.3 g., it was poured into water. A dark red oil separated which slowly turned pale and crystallized overnight. It was recrystallized by dissolving in a minimum of hot dimethylformamide and addition of twice the volume of hot glacial acetic acid. The yield was 4.8 g. (71%). An additional 1.2 g. (17%) was

obtained after dilution of the mother liquor with water. After two recrystallizations from glacial acetic acid the product melted at 231–232° (lit. m.p. 230–231°).[6]

Chlorination of 4-Methyl-*o*-phenylenedibenzenesulfonamide: 4-Methyl-3,5,6-trichloro-*o*-phenylenedibenzenesulfonamide.—Into a solution of 10 g. of 4-methyl-*o*-phenylenedibenzenesulfonamide in 40 ml. of dimethylformamide, chlorine was passed until the reaction mixture had gained 9.5 g. in weight, keeping the temperature below 50°. The mixture was worked up as described for the methyl-free product. The yield was 7.8 g. (62%). A sample was recrystallized for analysis from glacial acetic acid; white microcrystalline powder, m.p. 237–238°.

Anal. Calcd. for $C_{19}H_{15}Cl_3N_2O_4S_2$: C, 45.12; H, 2.95; N, 5.54. Found: C, 45.36; H, 3.20; N, 5.53.

Acknowledgment.—The authors are indebted to Miss Emily Davis, Mrs. Jean Fortney and Mrs. Katherine Pih for the microanalyses and to Miss Helen Miklas and Miss Elizabeth Petersen for the infrared spectra determinations.

(6) R. Adams and C. N. Winnick, THIS JOURNAL, **73**, 5687 (1951).

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Preparation and Hydrolysis of Optically Active 2-Butyl Acetal

BY ELLIOT R. ALEXANDER,[1] HIRSH M. BUSCH[2] AND GEORGE L. WEBSTER

RECEIVED FEBRUARY 11, 1952

O'Gorman and Lucas[3] have recently shown that hydrolysis with 5% aqueous phosphoric acid of D-(+)-2-octyl acetal leads to totally unracemized D-(+)-2-octanol. They concluded that the reaction does not proceed through a secondary octyl carbonium ion.

We have confirmed their conclusions using D-(+)-2-butyl acetal from D-(+)-2-butyl orthoformate.[4] The hydrolysis of this acetal to D-(+)-2-butanol gives alcohol of the same specific rotation as that used initially. It is, therefore, probable that none of the reactions employed in this cycle involves cleavage of the oxygen–butyl bond and that no secondary butyl carbonium intermediate is involved.

Experimental[5]

D-(+)-2-Butanol.—2-Butanol, Eastman Kodak Company White Label, was resolved by the method of Pickard and Kenyon[6] according to the modification of Sprung and Wallis[7] and had an observed rotation of +7.98 ± 0.02° in a one-decimeter tube at 25°.

D-(+)-2-Butyl Orthoformate.—The preparation of D-(+)-2-butyl orthoformate, $[\alpha]^{25}D$ +27.07 ± 0.02° (*l* 1, no solvent), was carried out as described by Alexander and Busch.[4]

D-(+)-2-Butyl Acetal.—To 24.0 g. (0.103 mole) of D-(+)-2-butyl orthoformate, 2.0 g. (0.025 mole) of granulated ammonium nitrate and 6.0 ml. of D-(+)-2-butanol was added 15.0 g. (0.34 mole) of freshly prepared acetaldehyde. After the mixture was refluxed for 90 minutes, 150 ml. of ether was added to the cooled solution. It was then washed with aqueous ammonium hydroxide (1:1) and distilled water. The ether solution was dried over anhydrous potassium carbonate. The ether was removed and the solution was distilled under vacuum through a five-inch column packed with glass helices. Active *s*-butyl acetal (11.5 g., 64.5%) was obtained as a colorless liquid, b.p. 66–68° (16 mm.); $n^{20}D$ 1.4050; d^{20}_4 0.8279, $[\alpha]^{25}D$ +25.40 ± 0.02° (*l* 1, no solvent).

Anal. Calcd. for $C_{10}H_{22}O_2$: C, 68.91; H, 12.73; *MR*, 51.68. Found: C, 68.62; H, 12.95; *MR*, 51.59.

Hydrolysis of Acetal.—A mixture of 11.5 g. (0.066 mole) of D-(+)-2-butyl acetal and 100 ml. of 5% phosphoric acid was refluxed for 1 hour. The cooled solution was saturated with potassium carbonate and extracted with ether. After drying over anhydrous potassium carbonate, the ether solution was distilled. Alcohol, (6.7 g., 71%) b.p. 97–98° with $[\alpha]^{25}D$ +8.00 ± 0.02° (*l* 1), was obtained.

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(1) Deceased.

(2) University of Illinois, College of Dentistry, Chicago, Illinois.

(3) J. M. O'Gorman and H. J. Lucas, THIS JOURNAL, **72**, 5489 (1950).

(4) E. R. Alexander and H. M. Busch, *ibid.*, **74**, 554 (1952).

(5) All boiling points are uncorrected.

(6) R. Pickard and J. Kenyon, *J. Chem. Soc.*, **103**, 1937 (1913).

(7) M. Sprung and E. Wallis, THIS JOURNAL, **56**, 1717 (1934).

The Addition of Fluorene to, and the Fine Structure of, Benzylidenefluorene

BY ERNST D. BERGMANN AND DAVID LAVIE

RECEIVED OCTOBER 29, 1951

Like all fulvenes,[1] benzylidenefluorene has a polar semicyclic double bond; the moment of the latter is directed toward the five-membered ring. It is, therefore, correct to regard the addition of fluorene to benzylidenefluorene, which has been observed by Pinck and Hilbert,[2] as a special case of the Michael condensation; the latter occurs only with polar unsaturated systems.[3]

The direction of the dipole moment in benzylidenefluorene demands that in the addition of fluorene the proton derived from the 9-hydrogen atom of fluorene combines with the 9-carbon atom of the benzylidene compound and the fluorenyl anion with the phenylated carbon atom, thus giving di-(9-fluorenyl)-phenylmethane (I), in analogy to the addition of lithium aluminum hydride in which the negative hydrogen ion[4] combines with the phenylated carbon atom, the $(LiAlH_3)^+$ ion with the central carbon atom of benzylidenefluorene.[5]

Pinck and Hilbert[2] have not decided whether their condensation product of m.p. 240° was (I) or the isomeric 9-benzyl-9,9′-difluorenyl (II), which would have resulted from the inverse addition of fluorene to benzylidenefluorene. (II)[6] has, moreover, the same melting point as Pinck and Hilbert's hydrocarbon.

It has now been shown that this hydrocarbon is not identical with (II); a mixture of the two compounds gives a strong melting point depression.[7] It is, therefore, concluded that formula (I) is correct. The Michael condensation of fluorene and benzylidenefluorene (I), is, thus, an additional proof for the direction of the moment in the latter which has been predicted by the theory.

(1) A. Pullman, G. Berthier and B. Pullman, *Bull. soc. chim. France*, 1097 (1950), and previous publications; G. W. Wheland and D. F. Mann, *J. Chem. Phys.*, **17**, 264 (1949); H. Lumbroso, A. Pacault and B. Pullman, *Bull. soc. chim. France*, 34 (1950); E. D. Bergmann and E. Fischer, *ibid.*, 1084 (1950).

(2) L. A. Pinck and G. E. Hilbert, THIS JOURNAL, **68**, 2014 (1946).

(3) E. D. Bergmann, D. Ginsburg and R. Pappo, in preparation.

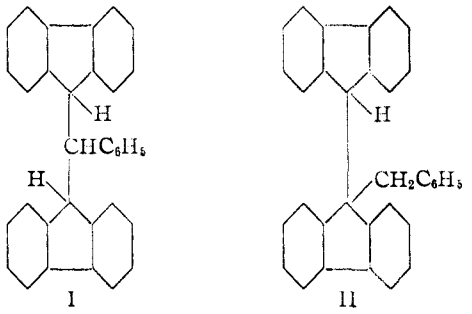
(4) L. W. Trevoy and W. G. Brown, THIS JOURNAL, **71**, 1675 (1949).

(5) D. Lavie and E. D. Bergmann, *Bull. soc. chim. France*, 250 (1951).

(6) R. C. Fuson and H. D. Porter, THIS JOURNAL, **70**, 895 (1948).

(7) The referee kindly informed the authors that he has made the same observation with the two original samples obtained by Pinck and Hilbert and by Fuson and Porter, respectively.

(I) was prepared by the method of Pinck and Hilbert,[2] (II) by the action of benzylmagnesium chloride on dibiphenylene-ethylene.[6] An attempt to prepare (I) from 2 moles of fluorenyllithium and 1 mole of benzal chloride did not lead to any identifiable product.



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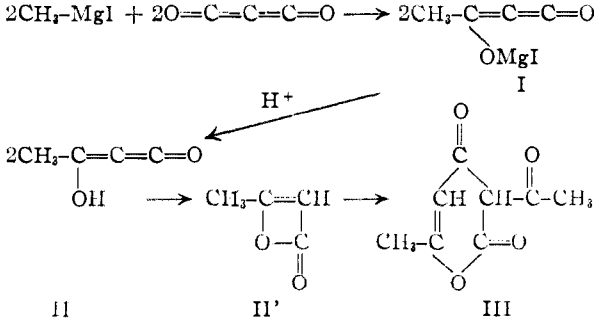
The Reactions of Carbon Suboxide with Grignard Reagents

BY JOHN H. BILLMAN[1] AND CARL M. SMITH

RECEIVED NOVEMBER 30, 1951

In an earlier publication[2] it was reported that methylmagnesium iodide reacts with carbon suboxide to produce 2,4,6-triacetylphloroglucinol in a 24% yield. In some subsequent experiments this phenol was obtained in yields as high as 39%.

Further examination of the reaction mixture has revealed the presence of another condensation product which upon identification proved to be dehydroacetic acid (III). The formation of this compound may be accounted for by the condensation of two molecules of acetylketene (II) which could be formed by the hydrolysis of the Grignard addition product (I) in which only one molecule of the Grignard reagent has been added to the carbon suboxide.[2]



Treatment of cyclohexylmagnesium bromide with carbon suboxide produced the expected phloroglucinol: 2,4,6-trihexahydrobenzoylphloroglucinol. Proof of the structure of the latter compound was obtained by analysis and by mixed melting points with a sample of 2,4,6-trihexahydrobenzoylphloroglucinol prepared by the Fries rearrangement of the trihexahydrobenzoate of phloroglucinol in the same way that the 2,4,6-triacetylphloroglucinol was synthesized.[2]

(1) Indiana University, Bloomington, Indiana.

(2) J. H. Billman and C. M. Smith, THIS JOURNAL, **61**, 457 (1939).

Since lithium methyl and methylmagnesium halides frequently undergo similar reactions, it seemed of interest to see if lithium methyl would react with carbon suboxide to produce 2,4,6-triacetylphloroglucinol. When this reaction was tried using identical conditions,[2] none of the expected product could be isolated from the reaction mixture.

Experimental

Dehydroacetic Acid.—A solution of 10.7 g. of carbon suboxide dissolved in 292 ml. of dry ether was added to a solution of methylmagnesium iodide, prepared from 28 g. of methyl iodide and 4.7 g. of magnesium, in a monomer similar to the procedure previously described.[2] After hydrolysis and extraction of the reaction mixture with ether, the ether solution was evaporated to a mush. This residue was treated with an excess of a saturated sodium bicarbonate solution and filtered. The residue yielded 3.4 g. of 2,4,6-triacetylphloroglucinol.

On adding dilute sulfuric acid to the bicarbonate extract, 1.5 g. of a yellow solid was obtained. A high boiling petroleum ether extract of the solid yielded 0.68 g. of dehydroacetic acid melting at 108–110°. A mixed melting point with some authentic dehydroacetic acid showed no depression. Its monoanilide, prepared according to the method of Oppenheim and Precht,[3] melted at 119–120° and did not depress the m.p. of an authentic sample. The identity of dehydroacetic acid was also confirmed by its solubility and its neutral equivalent.

2,4,6-Trihexahydrobenzoylphloroglucinol.—To an ether solution of cyclohexylmagnesium bromide, prepared from 85 ml. of cyclohexyl bromide, 10 g. of magnesium and 170 ml. of ether, was added 183 ml. of an ether solution containing 15 g. of carbon suboxide. The reaction was carried out as previously described.[2]

The reaction mixture was hydrolyzed with dilute sulfuric acid and extracted with ether. After extracting the ether layer with 3 × 50-ml. portions of a saturated bicarbonate solution, the ether layer was dried with anhydrous sodium sulfate and then evaporated to a paste. Extraction of the paste with 100 ml. of low boiling petroleum ether left a residue of 12.41 g. of crystals which when recrystallized from benzene melted at 195–196°, and did not depress the melting point of an authentic sample of 2,4,6-trihexahydrobenzoylphloroglucinol prepared from phloroglucinol by means of the Fries rearrangement.

Anal. Calcd. for $C_{27}H_{36}O_6$: C, 71.54; H, 7.96. Found: C, 71.01; H, 7.96.

(3) A. Oppenheim and H. Precht, *Ber.*, **9**, 1100 (1876).

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2-Methoxycyclohexylmercury Mandelate

BY ROBERT E. BUCKLES AND ROBERT J. SMITH

RECEIVED DECEMBER 17, 1951

Mercuric *dl*-mandelate reacted with cyclohexene in methanol to form 2-methoxycyclohexylmercury *dl*-mandelate. This compound was converted by the action of potassium bromide to the α-2-methoxycyclohexylmercury bromide described by Romeyn and Wright.[1] By this means the mandelate was shown to have the same configuration as the corresponding acetate and lactate prepared[1] before.

The use of mercuric (+)-mandelate in the addition reaction with cyclohexene gave rise to a mixture which was separated into two crude fractions. Each of these fractions reacted with potassium bromide to give the same α-2-methoxycyclohexyl-

(1) J. Romeyn and G. F. Wright, THIS JOURNAL, **69**, 697 (1947).

mercury bromide obtained from the addition with mercuric *dl*-mandelate. Thus, no effective synthesis of the optically active bromide was accomplished.

Experimental

Mercuric *dl*-Mandelate.—A solution of 30.4 g. (0.2 mole) of *dl*-mandelic acid in 250 ml. of water was mixed with a solution of 31.9 g. (0.1 mole) of mercuric acetate in 150 ml. of water. The precipitated product was removed by filtration, washed with water, and allowed to stand under 100 ml. of 95% ethanol for 24 hours. A yield of 45.6 g. (91%) of mercuric *dl*-mandelate, m.p. 182–183°, was obtained. The m.p. checks that listed by Hart and Andersen.[2]

Mercuric (+)-Mandelate.—A solution of 6.09 g. (0.04 mole) of (+)-mandelic acid,[3] m.p. 130–131°, $[\alpha]^{25}D$ 152.5° (2% solution in water), in 100 ml. of water was mixed with a solution of 6.37 g. (0.02 mole) of mercuric acetate in 50 ml. of water. The procedure of product isolation used for the *dl*-salt yielded 9.6 g. (95%) of mercuric (+)-mandelate, m.p. 172–174°, $[\alpha]^{25}D$ 102.5° (2% solution in 5% aqueous acetic acid).

Anal. Calcd. for $C_{16}H_{14}O_6Hg$: Hg, 39.9. Found: Hg, 39.5.

2-Methoxycyclohexylmercury *dl*-Mandelate.—To a suspension of 10.1 g. (0.020 mole) of mercuric *dl*-mandelate in 50 ml. of methanol was added 2.05 g. (0.025 mole) of cyclohexene. The reaction mixture was allowed to stand for 24 hours. The solid product which precipitated was recrystallized from a hexane fraction (60–70°) to give 3.6 g. (39%) of 2-methoxycyclohexylmercury *dl*-mandelate, m.p. 130–131°.

Anal. Calcd. for $C_{15}H_{20}O_4Hg$: Hg, 43.1. Found: Hg, 42.8.

Treatment of 1.0 g. of the mandelate in methanol with 5% aqueous potassium bromide yielded 0.70 g. (83%) of α-2-methoxycyclohexylmercury bromide, m.p. 112–113°. There was no lowering of the m.p. when this compound was mixed with the bromide prepared from the corresponding acetate.[1]

2-Methoxycyclohexylmercury (+)-Mandelate.—The reaction of 5.0 g. (0.010 mole) of mercuric (+)-mandelate with 1.0 g. (0.012 mole) of cyclohexene in 25 ml. of methanol for 48 hours yielded a clear solution. The solution was neutralized with base and concentrated under reduced pressure. The residue was crystallized from hexane (60–70°) to yield 2.5 g. (54%) of a mixture, m.p. 84–85°.

Anal. Calcd. for $C_{15}H_{20}O_4Hg$: Hg, 43.1. Found: Hg, 42.7.

Fractional crystallization of the mixture from acetone yielded two fractions: m.p.'s 112–115° and 96–99°. Each product when treated with 5% potassium bromide as described for the *dl*-mandelate yielded the α-bromide, m.p. 112–114°, which was optically inactive.

(2) M. C. Hart and H. P. Andersen, *ibid.*, **57**, 1059 (1935).

(3) L. Gatterman and H. Wieland, "Laboratory Methods of Organic Chemistry," The Macmillan Company, New York, N. Y., 1937, p. 228.

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4-Pyridylacetone

BY ALFRED BURGER, JAMES R. RECTOR AND A. CHANDLER SCHMALZ

RECEIVED OCTOBER 24, 1951

4-Pyridylacetone has been obtained by decarboxylative acylation[1] of 4-pyridylacetic acid with acetic anhydride in analogy to the preparation of the 3-isomer reported by Burger and Walter.[2]

(1) J. A. King and F. H. McMillan, THIS JOURNAL, **73**, 4911 (1951).

(2) A. Burger and C. R. Walter, Jr., *ibid.*, **72**, 1988 (1950); H. S. Mosher and J. E. Tessieri [*ibid.*, **73**, 4925 (1951)] working with 3-pyridylacetonitrile have stated that "none of the isomeric pyridylacetonitriles has previously been reported." The 3-isomer had served us as an intermediate in an alternative synthesis of 3-pyridylacetone.

4-Picolylthiomorpholide[3] used as an intermediate in the synthesis of 4-pyridylacetic acid could be desulfurized by the method of Kornfeld[4] to 1-(4-pyridyl)-2-morpholinoethane.

Experimental[5]

4-Pyridylacetone.—A mixture of 40 g. (0.23 mole) of 4-pyridylacetic acid hydrochloride, 31.4 g. (3.8 moles) of anhydrous sodium acetate and 56.4 g. (0.52 mole) of acetic anhydride was refluxed for 18 hours, the dark reaction mixture was hydrolyzed with 100 ml. of water, cleared with Darco, and concentrated under reduced pressure. It was then made carbonate alkaline and extracted exhaustively with ether. The oily pale yellow ketone boiled at 76.5–78° (0.5 mm.) and weighed 15.6 g. (50%).

Anal. Calcd. for C_8H_9NO: C, 71.09; H, 6.71. Found: C, 70.83; H, 6.55.

The **semicarbazone** crystallized from water, m.p. 188–189°.

Anal. Calcd. for $C_9H_{12}N_4O$: N, 29.15. Found; N, 29.20.

Methyl 4-Pyridylacetate.—This ester was prepared from 4-pyridylacetic acid hydrochloride with diazomethane in ether–methanol solution. The colorless oily product boiled at 103–105° (2–3 mm.).

4-Pyridylacetamide.—This amide was obtained in 97% yield from ethyl or methyl pyridylacetate by the procedure described for 3-pyridylacetamide,[2] and recrystallization from dioxane. The colorless crystals melted at 143.5–145°.

Anal. Calcd. for $C_7H_8N_2O$: N, 20.58. Found: N, 20.73.

1-(4-Pyridyl)-2-morpholinoethane.—A solution of 10 g. of 4-picolylthiomorpholide in 200 ml. of absolute ethanol was added to 70 g. of alcohol-moist Raney nickel, and the mixture was refluxed under an atmosphere of nitrogen for three hours. The nickel was filtered, most of the solvent removed under reduced pressure, the residue was treated with alkali and extracted with ether. The amine from the ether extracts boiled at 128–130° (0.7 mm.) and weighed 3.6 g. (41%). The free base was not stable enough to be analyzed.

The **dihydrochloride** melted at 215.5–217° after recrystallization from methanol–ethyl acetate.

Anal. Calcd. for $C_{11}H_{18}Cl_2N_2O$: N, 10.58. Found: N, 10.82.

The **yellow dipicrate** crystallized from water, m.p. 190–192°.

Anal. Calcd. for $C_{28}H_{22}N_8O_{15}$: N, 17.23. Found: N, 17.37.

(3) R. L. Malan and P. M. Dean, *ibid.*, **69**, 1797 (1947).

(4) E. C. Kornfeld, *J. Org. Chem.*, **16**, 131 (1951).

(5) All melting points are corrected.

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The Preparation of 1,5-Anhydro-D-mannitol (Styracitol) from D-Mannitol

BY HEWITT G. FLETCHER, JR., AND HARRY W. DIEHL

RECEIVED FEBRUARY 25, 1952

1,5-Anhydro-D-mannitol, originally discovered by Asahina[1] in the husks of the fruit of *Styrax obassia* and named styracitol, was first synthesized by Zervas[2] through the catalytic reduction of tetraacetyl-2-hydroxy-D-glucal. A recent communication[3] from this Laboratory described a more convenient synthesis based on the reduction of tetraacetyl-α-D-mannopyranosyl bromide with lithium

(1) Y. Asahina, *Arch. Pharm.*, **245**, 325 (1907); **247**, 157 (1909).

(2) L. Zervas, *Ber.*, **63**, 1689 (1930).

(3) R. K. Ness, H. G. Fletcher, Jr., and C. S. Hudson, THIS JOURNAL, **72**, 4547 (1950).

aluminum hydride. We wish now to report an even simpler and less expensive method based on the direct dehydration of D-mannitol.

Montgomery and Wiggins[4] investigated the complex mixture which results when D-mannitol is heated with concentrated hydrochloric acid and were able to demonstrate, through the isolation of dibenzylidenestyracitol, that 1,5-anhydro-D-mannitol was present. More recently Foster and Overend[5] have found that when D-mannitol is boiled with concentrated hydrochloric acid for 24 hours the chief product isolated (other than unchanged D-mannitol) is 1,4(=3,6)-anhydro-D-mannitol. We have confirmed Foster and Overend's observation but wish now to add that extension of the reaction time to 48 hours results in the isolation of 1,5- rather than 1,4-anhydro-D-mannitol. While the yield, 10.9%, is low, the starting material is cheap and the styracitol obtained is readily purified. The identity of the styracitol was confirmed through comparison with authentic material and through the preparation of its tetraacetate.

Experimental

In each experiment 50.0 g. of pure D-mannitol was dissolved in 200 ml. of concentrated hydrochloric acid and the solution boiled gently under reflux for the specified time (Table I). The reaction mixture was then concentrated *in vacuo* to a sirup, dissolved in 150 ml. of water, treated with decolorizing carbon and reconcentrated *in vacuo*. After the evaporation (*in vacuo*) of two successive 50-ml. batches of absolute alcohol from the residual sirup it was dissolved in 50 ml. of absolute alcohol. The properties of the various crops of crystalline products obtained thus are listed in Table I.

TABLE I

Run	Hr. reflux	No.	Wt., g.	Fractions M.p.,[a] °C.	$[\alpha]^{20}D$ (H_2O)[b]
I	24	1	4.4	120–130	−24.8°
		2	2.5	132–137	−35.8
		3	0.9	140–150	−43.6
II	36	1	4.4	146–154	−45.7
		2	0.7	118–122	−31.6
III	48	1	4.9	139–150	−45.6
IV	60	1	4.1	145–152	−46.5
V	72	1	4.2	145–150	−45.6
VI	96	1	3.6	145–151	−45.0

[a] Melting points are corrected. 1,4-Anhydro-D-mannitol melts at 147–148°; 1,5-anhydro-D-mannitol at 156–157°. [b] 1,4-Anhydro-D-mannitol shows $[\alpha]^{20}D$ −23.8° in water; 1,5-anhydro-D-mannitol shows $[\alpha]^{20}D$ −50.9° (H_2O).

A number of crops of crude 1,5-anhydro-D-mannitol (20.4 g., $[\alpha]^{20}D$ *ca.* −45°) were combined and recrystallized from seven parts of ethanol to give 16.3 g. of pure styracitol showing $[\alpha]^{20}D$ −50.3° in water (*c*, 1.06) and melting at 156–157° either alone or in admixture with authentic styracitol.

Three grams of styracitol, made as described above, was acetylated with acetic anhydride in pyridine solution to give 1.5 g. (25%) of its tetraacetate melting at 66–67° and showing in chloroform −42.4° (*c*, 0.826); a mixed melting point with authentic styracitol tetraacetate was undepressed. Fletcher and Hudson[6] reported a value of $[\alpha]^{20}D$ −42.0° for the rotation of 1,5-anhydro-D-mannitol tetraacetate in chloroform.

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(4) R. Montgomery and L. F. Wiggins, *J. Chem. Soc.*, 2204 (1948).
(5) A. B. Foster and W. G. Overend, *ibid.*, 680 (1951).
(6) H. G. Fletcher, Jr., and C. S. Hudson, THIS JOURNAL, **71**, 3682 (1949).

Guanidines Derived from N-Cyanotetrahydroquinolines

BY ROBERT D. GANO WITH R. L. MCKEE AND J. W. AGER, JR.

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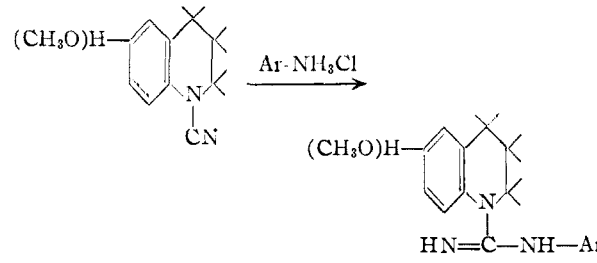
N-Cyano-1,2,3,4-tetrahydroquinoline has been prepared by v. Braun[1] through interaction of 1-alkyltetrahydroquinolines with cyanogen bromide. In the present work, this compound and its 6-methoxy and 6-chloro analogs have been prepared in excellent yield from corresponding 1,2,3,4-tetrahydroquinolines. The cyanamides thus prepared were condensed with aromatic amine hydrochlorides to form guanidines.[1]



Under the conditions employed (prolonged reflux in alcohol or hexanol-1 or heating without solvent to temperatures as high as 250°) methylammonium chloride failed to condense with the cyanamides. Other conditions involving heating 1-cyano-6-chlorotetrahydroquinoline with a mixture of molar quantities of diethylamine and its hydrochloride likewise failed to produce isolable amounts of the alkyl derivatives. A low yield of the desired product was obtained from diethylcyanamide and 6-chlorotetrahydroquinoline hydrochloride.

Experimental

The yields here reported are those obtained in a single experiment and are not considered to be the maximum obtainable.

1-Cyano-6-methoxy-1,2,3,4-tetrahydroquinoline.—To a solution of cyanogen bromide (52 g., 0.49 mole) in 240 cc. of benzene was added dropwise with stirring, a solution of 160 g. (0.98 mole) of thalline in 125 cc. of benzene, the temperature being maintained at 25° by external cooling. After standing overnight, the solution was filtered from the precipitated thalline hydrobromide (110 g., 92%), the benzene distilled at atmospheric pressure, and the product distilled *in vacuo* to yield 82 g. of a pale yellow oil boiling at 160–177° (4.0–4.5 mm.). Redistillation gave 73 g. (79% of the theoretical) of material boiling from 177–182° (4.0–4.5 mm.) which solidified to form white crystals melting at 44–47°. Neither recrystallization of this substance from petroleum ether nor redistillation produced any alteration in melting point.

Anal. Calcd. for $C_{11}H_{12}N_2O$: N, 14.89; CH_3O, 16.49. Found: N, 15.18; CH_3O, 16.73.

1-Cyano-1,2,3,4-tetrahydroquinoline.[1]—Cyanogen bromide (63.1 g., 0.595 mole) and tetrahydroquinoline (159.5 g., 1.19 moles) were allowed to react as above to form a 90% yield of the desired product boiling at 153.5–154.5° (7 mm.) and 169–172° (13 mm.).

1-Cyano-6-chloro-1,2,3,4-tetrahydroquinoline.—6-Chloro-1,2,3,4-tetrahydroquinoline was prepared from tetrahydroquinoline by acetylation, chlorination with sulfuryl chloride in carbon tetrachloride, and acid hydrolysis. It boiled at 125–127° at 3 mm., melted at 41°, and formed a hydrochloride which decomposed at 195° and a picrate melting at 151.5–152.5°.[2] The cyanamide was prepared from 21.2

(1) J. v. Braun, *Ber.*, **42**, 2219 (1909).
(2) J. v. Braun, A. Grabowski and M. Rawicz, *ibid.*, **46**, 3169 (1913).

g. of cyanogen bromide and 67 g. of 6-chlorotetrahydroquinoline. The yield of purified material boiling at 155° (2 mm.) and melting at 54–55° was 44%.

Anal. Calcd. for $C_{16}H_{13}ClN_2$: N, 14.53. Found: N, 14.43.

1-(*p*-Nitrophenylguanyl)-1,2,3,4-tetrahydroquinoline.—N-Cyanotetrahydroquinoline (13.0 g., 0.082 mole) and *p*-nitroaniline hydrochloride (14.3 g., 0.082 mole) were heated in the absence of a solvent by means of an oil-bath, the temperature being slowly raised to 200° (bath temperature). The resulting red solid was dissolved in 180 cc. of hot alcohol, poured into 200 cc. of water containing 8 g. of sodium hydroxide, and the solid material (24.9 g., m.p. 155–169°) which separated was removed by filtration. After crystallization once from 800 cc. of alcohol and once from 800 cc. of acetone, the material (9.1 g., m.p. 176.5–179.5°) was extracted with 600 cc. of boiling dioxane leaving a small residue melting from 162° to 275°. The dioxane solution was concentrated to 160 cc. and water was added to a faint turbidity while hot. On chilling 6.3 g. of an orange yellow solid melting at 187.5–188.5° was obtained. An additional 3.6 g. of less pure product (m.p. 183.5–187°) was recovered from the mother liquors.

Anal. Calcd. for $C_{16}H_{16}N_4O_2$: C, 64.83; H, 5.44; N, 18.91. Found: C, 64.78; H, 5.62; N, 18.88.

1-(*p*-Methoxyphenylguanyl)-1,2,3,4-tetrahydroquinoline hydrochloride was prepared similarly from 11.1 g. of N-cyanotetrahydroquinoline and 11.1 g. of *p*-anisidine hydrochloride. The substance was precipitated from an ether solution with hydrogen chloride and recrystallized three times from alcohol–ether to give 3.4 g. (15%) of white crystals melting at 185–186°.

Anal. Calcd. for $C_{17}H_{20}ClN_3O$: C, 64.10; H, 6.34; Cl, 11.16. Found: C, 64.10; H, 6.35; Cl, 11.13.

Bis-(1,2,3,4-tetrahydroquinolyl-1)-ketimine was prepared in 57% yield from 16.5 g. of cyanotetrahydroquinoline and 22.7 g. of tetrahydroquinoline hydrobromide in the absence of a solvent at 160°. Crystallization from alcohol–petroleum ether formed a white microcrystalline powder melting at 146.5–147.5°.

Anal. Calcd. for $C_{19}H_{21}N_3$: C, 78.31; H, 7.27; N, 14.42. Found: C, 78.35; H, 7.40; N, 14.62.

Bis-(6-methoxy-1,2,3,4-tetrahydroquinolyl-1)-ketimine was prepared as in the previous reaction from 9.8 g. of thalline hydrobromide and 7.5 g. of N-cyanothalline. Crystallization from aqueous alcohol gave a 23% yield of a white powder melting at 143.5–143.8°.

Anal. Calcd. for $C_{21}H_{25}N_3O_2$: C, 71.77; H, 7.67; N, 11.96; CH_3O, 17.67. Found: C, 71.62; H, 7.31; N, 12.21; CH_3O, 17.93.

1-(*p*-Nitrophenylguanyl)-6-methoxy-1,2,3,4-tetrahydroquinoline.—Seven grams of *p*-nitroaniline hydrochloride and 7.5 g. of N-cyanothalline treated as in the previous reaction gave a 23% yield of deep red crystals (from alcohol) melting at 132.5–134°.

Anal. Calcd. for $C_{17}H_{18}N_4O_3$: C, 62.62; H, 5.56; N, 17.17; CH_3O, 9.51. Found: C, 62.92; H, 5.68; N, 17.08; CH_3O, 10.08.

1-(1-Chlorophenylguanyl)-6-methoxy-1,2,3,4-tetrahydroquinoline.—Analogously, 13.1 g. of *p*-chloroaniline hydrochloride and 15.0 g. of N-cyanothalline formed 17.5% of white crystals melting (after crystallization from ethyl acetate) at 135.5–136.5°.

Anal. Calcd. for $C_{17}H_{18}ClN_3O$: C, 64.65; H, 5.74; N, 13.31; CH_3O, 10.2. Found: C, 64.81; H, 5.49; N, 13.96; CH_3O, 10.2.

1-Diethylguanyl-6-chlorotetrahydroquinoline Hydrochloride.—Seven grams of 6-chlorotetrahydroquinoline hydrochloride and 3.4 g. of diethylcyanamide were heated in absence of a solvent. At about 100°, an exothermic reaction took place, the temperature rising spontaneously to 165°. The melt was dissolved in water, adjusted to a *p*H of 8.5 and extracted (ether) to remove tetrahydroquinoline. The aqueous layer was made strongly basic and extracted thoroughly with ether after drying, dry hydrogen chloride was pressed through the solution to precipitate the hydrochloride (1 g.) which was crystallized to constant melting point, 207–210°, from ethyl acetate.

Anal. Calcd. for $C_{14}H_{21}Cl_2N_3$: C, 55.6; H, 6.96; N, 13.9. Found: C, 55.8; H, 6.73; N, 13.8.

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Reactions of Some Amines with Heterocyclic Ethers

BY HENRY GILMAN, IRVING ZAREMBER AND JOHN A. BEEL

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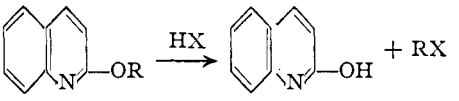
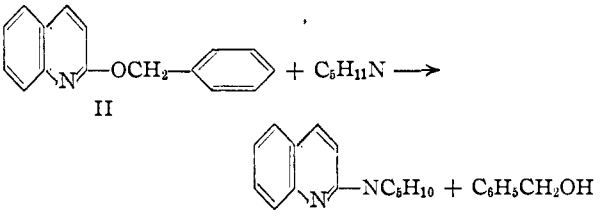
It has been shown that alkoxy groupings in the 2-position of quinoline are labile to reagents like hydrochloric acid,[1] thiophenols[2] and carboxylic acids.[3] The cleavage results in the formation of 2-hydroxyquinoline (I) and the alkylated cleaving reagent.



I X = Cl-, C_6H_5S-, RCOO-

These reactions suggested the possibility that 2-alkoxyquinolines might be cleaved by compounds containing the =NH grouping. Consequently in this work we have investigated the reactions between 2-benzyloxyquinoline (II) and certain aliphatic amines, aromatic amines and amides.

The aromatic amines, aniline, N-methylaniline, diphenylamine, carbazole and phenothiazine, reacted at relatively high temperatures like the acidic reagents to yield I and the alkylated amine. The aliphatic amines, piperidine and morpholine, showed this same cleavage at lower temperatures (boiling points of the amines). In addition, these cyclic aliphatic amines effected cleavage between the quinoline ring and the oxygen atom to yield the 2-aminoquinoline and benzyl alcohol. Dibenzylamine, acetanilide and N-benzylbenzenesulfonamide showed neither type of cleavage.



II

The reaction was also affected by the alkyl grouping, for experiments with diphenylamine and morpholine indicated that II was more reactive than 2-ethoxyquinoline. 2-Benzyloxybenzothiazole[3] did not react with diphenylamine at 115–125°.

Since phenothiazine reacted with II to form I in 90% yield, the other product which melted at 91–92° was thought to be N-benzylphenothiazine. The melting point corresponded with that reported by Desai[4] for N-benzylphenothiazine, but we were unable to prepare the compound by his method of heating sulfur and N-benzyldiphenylamine.

Finzi[5] reported a melting point of 132–134°

(1) P. Friedländer and H. Ostermaier, *Ber.*, **15**, 333 (1882).
(2) G. Illuminati and H. Gilman, THIS JOURNAL, **71**, 3349 (1949).
(3) H. Gilman, K. E. Lentz and J. A. Beel, *ibid.*, **74**, 1081 (1952).
(4) R. D. Desai, *J. Ind. Inst. Sci.*, **7**, 235 (1924).
(5) C. Finzi, *Gazz. chim. ital.*, **62**, 175 (1932).

TABLE I

REACTIONS OF N-HETEROCYCLIC ETHERS WITH —NH COMPOUNDS

Cleaving reagent	Reaction time, hr.	T., °C. (bath)	Products, %	Recovery of starting material, %
		Ether, 2-benzyloxyquinoline[a]		
Sodium hydroxide, aq. (20%)	7	Reflux	.....	2-Benzyloxyquinoline,[b] 94
Hydrochloric acid (6 *N*)	7	Reflux	2-Hydroxyquinoline,[c] 90 Benzyl chloride,[d] 46	.....
Aniline	48	170–180	2-Hydroxyquinoline,[c] 14 N-Benzylaniline,[e] 8	2-Benzyloxyquinoline,[b] 82 Aniline,[f] 62
N-Methylaniline	40	170–180	2-Hydroxyquinoline,[c] 76 N-Methyl-N-benzylaniline,[g] 41	.....
Diphenylamine	40	170–180	2-Hydroxyquinoline,[c] 84 N-Benzyldiphenylamine,[h] 58	Diphenylamine,[i] 58
Diphenylamine	48	100	No 2-hydroxyquinoline	.....
Diphenylamine	49	140[j]	No 2-hydroxyquinoline	.....
Carbazole	48	200–220[k]	2-Hydroxyquinoline,[c] 9.5	.....
Carbazole	48	140[j]	.....	Carbazole,[i] 93 2-Benzyloxyquinoline,[b] 88
Carbazole	48	165[l]	2-Hydroxyquinoline,[c] 16 N-Benzylcarbazole,[m] 7	Carbazole,[i] 80
Phenothiazine	48	170–210	2-Hydroxyquinoline,[c] 24	2-Benzyloxyquinoline,[b] 25
Phenothiazine	48	153[n]	2-Hydroxyquinoline,[c] 90 N-Benzylphenothiazine,[o] 34 (?)	.....
Di-*n*-butylamine	48	170–180	.....	Di-*n*-butylamine,[p] 76 2-Benzyloxyquinoline,[b] 84
Di-*n*-butylamine	48	170–180	2-Hydroxyquinoline,[c] 5	Di-*n*-butylamine,[p] 70 2-Benzyloxyquinoline,[b] 77
Dibenzylamine	48	180	.....	Dibenzylamine,[q] 53 2-Benzyloxyquinoline,[b] 66
Piperidine	46	Reflux	2-Hydroxyquinoline,[c] 15 N-Benzylpiperidine,[r] 22	.....
Piperidine	53	Reflux	2-Hydroxyquinoline,[c] 7 N-Benzylpiperidine,[r] 18 2-(N-Piperidino)-quinoline,[s] 2.5	2-Benzyloxyquinoline,[b] 76
Morpholine	53	Reflux	2-Hydroxyquinoline,[c] 35 N-Benzylmorpholine,[t] 19 Benzyl alcohol,[u] 28 2-(N-Morpholino)-quinoline,[v] 65	.....
Pyrrole	46	Reflux	.....	2-Benzyloxyquinoline,[b] 85
Acetanilide	48	125	.....	Acetanilide,[i] 82 2-Benzyloxyquinoline,[b] 62
N-Benzylbenzenesulfonamide	46	170	.....	2-Benzyloxyquinoline,[b] 70 N-Benzylbenzenesulfonamide,[i] 59
		Ether, 2-benzyloxybenzothiazole[w]		
Diphenylamine	26	115–125	.....	Diphenylamine,[i] 92 2-Benzyloxybenzothiazole,[x] 60
		Ether, 2-Ethoxyquinoline[y]		
Diphenylamine	48	170–180	.....	Diphenylamine,[i] 84 2-Ethoxyquinoline,[i] 82
Morpholine	48	Reflux	2-(N-Morpholino)-quinoline,[v] 3.4	Morpholine,[z] 94 2-Ethoxyquinoline,[i] 69

[a] See ref. 2. [b] M.p. 50°. Identified by mixed m.p. [c] M.p. 199°. Identified by mixed m.p. [d] B.p. 175–185°. Liquid gave a white precipitate with alc. silver nitrate. [e] Identified by m.p. (114–115°) of benzenesulfonamide and mixed m.p. [f] Converted to acetanilide and identified by mixed m.p. [g] Identified by m.p. (127–128°) of picrate and mixed m.p. See E. Wedekind, *Ber.*, **32**, 517 (1899). [h] Identified by m.p. (83.5–86.5°) and mixed m.p. See J. Forrest, D. A. Liddell and S. H. Tucker, *J. Chem. Soc.*, 454 (1946). [i] Identified by m.p. and mixed m.p. [j] Refluxed in xylene solution. [k] Extensive pyrolysis occurred. [l] Refluxed in mesitylene solution. [m] Identified by m.p. (117–119°) and mixed m.p. See B. Levy, *Monatsh.*, **33**, 177 (1912). [n] Refluxed in cumene solution. [o] M.p. 91–92°. *Anal.* Calcd. for $C_{19}H_{15}NS$: S, 11.1. Found: S, 10.7, 11.1. See refs. 4, 5. [p] Identified by m.p. (84–86°) of phenylthiourea and mixed m.p. [q] Isolated as N,N-dibenzylbenzamide (m.p. 112–114°) and identified by mixed m.p. [r] Identified by m.p. of the hydrochloride (m.p. 171–175°) and of the picrate (181°) and mixed m.p. See C. Schotten, *Ber.*, **15**, 421 (1882). [s] Identified by m.p. (174°) of the picrate and mixed m.p. See N. Luthy, F. W. Bergstrom and H. S. Mosher, THIS JOURNAL, **71**, 1109 (1949). [t] Identified by m.p. of hydrochloride (244–245°) and picrate (189–190°). See S. Gabriel and R. Stelzner, *Ber.*, **29**, 2381 (1896), Cerkovnikov and Stern, *Arkiv Kemi*, **18**, 12 (1946) [*C.A.*, **42**, 1942 (1948)], and J. P. Mason and M. Zief, THIS JOURNAL, **62**, 1450 (1940). [u] Converted to the α-naphthyl urethan (m.p. 132–133°) and identified by mixed m.p. [v] Identified by m.p. (91–92°) and mixed m.p. See L. Fullhart, Doctoral Dissertation, Iowa State College (1946). [w] See ref. 3. [x] Identified by m.p. (60–63°) and mixed m.p. [y] See ref. 1. [z] Converted to phenylthiourea (m.p. 132–134°) and identified by mixed m.p.

for the same compound which he prepared by heating benzyl chloride with phenothiazine. This compound was shown to be different from that obtained in our experiment by a mixed melting point determination. We were also unable to prepare N-benzylphenothiazine from N-lithiophenothiazine and benzyl chloride.

Experimental

In most of the experiments the reagents were stirred for 48 hours in a nitrogen atmosphere at a bath temperature of 170–180°. After cooling the mixture was shaken with ether, which precipitated most of the 2-hydroxyquinoline if it were present in appreciable amounts. The ether was then extracted with 5% aqueous sodium hydroxide to remove any residual 2-hydroxyquinoline which was recovered by acidification of these extracts with hydrochloric acid and concentration by evaporation. The ether layer was dried over sodium sulfate, and after filtration the ether was removed by distillation. From the residue the other cleavage products and unreacted starting materials were obtained by vacuum distillation or by recrystallization. In Table I we have listed the pertinent data for the various experiments.

Attempt to Prepare N-Benzylphenothiazine.—N-Lithiophenothiazine was prepared by adding 0.11 mole of phenyllithium in 20 ml. of dry ether to 5.0 g. (0.025 mole) of phenothiazine in 200 ml. of dry benzene. As this mixture gave a blue-green color which resembled Color Test I,[6] the time at which the phenyllithium was used up could not be noted, so the mixture was stirred at room temperature for 48 hours. A solution of 4.0 g. (0.03 mole) of benzyl chloride in 75 ml. of dry ether was then added, and the stirring was continued at room temperature for 24 hours. After refluxing for one hour, the volatile solvents were removed by distillation. This left a gummy residue which was extracted with 95% ethanol. No crystalline material could be obtained from these extracts.

Preparation of N-Benzylbenzenesulfonamide.—A modification of Hinsberg's method[7] was used in this preparation. The addition of 17.6 g. (0.1 mole) of benzenesulfonyl chloride and 40 ml. of 10% sodium hydroxide in small portions, with shaking, to 10.7 g. (0.1 mole) of benzylamine resulted in a brown oil. The mixture was shaken with an additional 400 ml. of 10% sodium hydroxide before filtering. After standing for two days it was filtered again and then acidified with hydrochloric acid. This yielded a white precipitate which was washed with water and dried. The yield was 17.3 g. (70%) of N-benzylbenzenesulfonamide (m.p. 87–89°). Attempts to recrystallize the material from aqueous ethanol, as recommended by Hinsberg, yielded a tan material with a lower melting point (about 80°).

Acknowledgment.—The authors are grateful to Dr. Gabriello Illuminati for generous assistance.

(6) H. Gilman and F. Schulze, THIS JOURNAL, **47**, 2002 (1925).
(7) O. Hinsberg, *Ann.*, **265**, 178 (1891).

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Steroid Mercaptols. II[1]

BY H. HAUPTMANN AND M. MOURA CAMPOS

RECEIVED DECEMBER 21, 1951

Some years ago[1] it was found that 7- and 12-ketosteroids form mercaptols only with dithiols, whereas the carbonyl groups in the 3-, 16[2] and 17-positions react also with monothiols. Recently, we examined cholestanol-3-one-6, whose keto group is generally more reactive than those in the 7- and 12-positions.[3] However, in the mercaptol formation under the usual conditions cholestanol-3-one-6 behaves in the same manner as the 7- and 12-ketosteroids in that it does not form a mercaptol with ethanethiol but reacts with ethanedithiol.

The 11-ketosteroids are known for their low reactivity. Accordingly ethyl 11-keto-3-hydroxyetiocholanate did not react with ethanedithiol[4] but was recovered almost completely. Similar observations have recently been made on 7,11-diketones derived from ergosterol and cholanic acid.[5]

A survey of the results obtained makes it obvious that all keto groups which form mercaptols with monothiols as well as with dithiols are in rings A and D, whereas those which give no mercaptols with monothiols are in rings B and C. Among these the 11-keto group shows a special behavior, since it is even inert to dithiols.

Studies on Stuart–Fischer–Hirschfelder models show that the 11-position is the only one in which a hemimercaptol cannot be constructed since there is not enough space around the carbon atom for both an alkylmercapto and a hydroxy group. This might explain why even with a dithiol no mercaptol is formed. However, more evidence will be necessary before a definite explanation of the behavior of the different keto groups can be given.

Acknowledgment.—We are indebted to the Rockefeller Foundation for a grant supporting this work. One of us (H. H.) wishes to express his thanks to Dr. L. F. Fieser for granting facilities at his laboratory and to the Rockefeller Foundation for a special fellowship.

Experimental

Treatment of Cholestanol-3-one-6 with Ethanethiol.—Dry hydrogen chloride was passed for two hours through a mixture of 450 mg. of cholestanol-3-one-6 and 4 ml. of ethanethiol cooled in an ice-bath. After standing overnight at room temperature, the mixture was kept in a vacuum desiccator over potassium hydroxide until all hydrogen chloride and ethanethiol had been removed. The residue was recrystallized twice from methanol, giving 400 mg., m.p. 142–144°, undepressed by admixture of starting material.

Reaction of Cholestanol-3-one-6 Acetate with Ethanedithiol.—Cholestanol-3-one-6 acetate (200 mg.) and 1.5 ml. of ethanedithiol were cooled in an ice-bath and treated with a stream of dry hydrogen chloride for two hours. After standing for several hours the reaction mixture was dissolved in ether, the ether solution washed with water, 5% sodium hydroxide and with water again, dried with calcium chloride and evaporated to dryness. The residue after several recrystallizations from acetone yielded the mercaptol, m.p. 148–151°, yield 190 mg. (80%).

Anal. Calcd. for $C_{31}H_{52}O_2S_2$: S, 12.31. Found: S, 12.13.

Treatment of 11-Keto-3-hydroxyetiocholanate with Ethanedithiol.—A stream of dry gaseous hydrogen chloride was allowed to pass through a mixture of 500 mg. of ethyl 11-keto-3-hydroxyetiocholanate and 2 ml. of ethanedithiol, cooled in an ice-bath. After 15 minutes the mixture was warmed to about 40° in order to dissolve the suspended ester. Passing of hydrogen chloride was continued at room temperature for half an hour, and 3 ml. of ethanedithiol was added when precipitation occurred again. After leaving the reaction mixture at room temperature for several hours the hydrogen chloride was removed in a vacuum desiccator over potassium hydroxide. The precipitate was separated by filtration, washed with petroleum ether and recrystal-

(1) H. Hauptmann, THIS JOURNAL, **69**, 562 (1947).
(2) M. N. Huffmann and M. H. Lott, *ibid.*, **69**, 1835 (1947).
(3) L. F. Fieser and M. Fieser, "Natural Products Related to Phenanthrene," Reinhold Publishing Corp., New York, N. Y., 1949.
(4) This experiment was performed in the Converse Memorial Laboratory, Harvard University.
(5) H. Heusser, Y. Eichenberger, P. Kurath, H. R. Dallenbach and O. Jeger, *Helv. Chim. Acta*, **34**, 2106 (1951).

lized from a mixture of benzene and ligroin, giving 465 mg. of colorless crystals, m.p. 159–161°, undepressed by admixture of starting material.

DEPARTAMENTO DE QUÍMICA DA
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N-Substituted Colchiceinamides

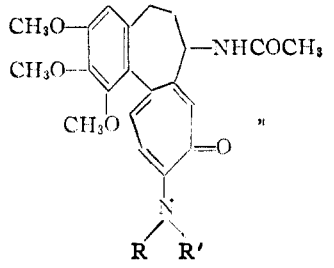
BY JONATHAN L. HARTWELL, M. V. NADKARNI[1] AND J. LEITER

RECEIVED JANUARY 24, 1952

Since certain N-substituted colchiceinamides[2] have been reported to inhibit cell mitosis[3] and growth[4] in certain tumors, we have prepared a series of these derivatives as listed in the table, for screening against Sarcoma 37 in mice. Six of the fourteen compounds are new; of the other eight, analyses are not given in the literature for six, the melting points for two are not listed, and the melting points reported for three others are widely different from those reported here. It was therefore thought desirable to bring all the characterizing data together.

With the exception of the β-chloroethyl derivative, all the compounds were prepared generally according to the method of Zeisel[5] by heating colchicine with a 10% alcoholic solution of the appropriate amine in 50% excess in a sealed tube at 120° (100° for colchiceinamide itself) for varying lengths of time depending on the amine. The reaction mixtures were evaporated to dryness and the products crystallized from suitable solvents.

N-SUBSTITUTED COLCHICEINAMIDES



Substituent	Reaction time, hr.	Appearance, crystallizing solvent	M.p., °C. cor.	Yield, % Crude	Yield, % Pure	Empirical Formula	Analyses,[k,l] % Methoxyl Calcd.	Methoxyl Found	Nitrogen Calcd.	Nitrogen Found
None[a,b]	4	Prisms, alc.	261–262	82	63	$C_{21}H_{24}N_2O_5$	24.2	24.0	7.3	7.0
Methyl[b,c]	20	Prisms, EtOAc	230–232 (softens 185)	85	66	$C_{22}H_{26}N_2O_5$	23.4	23.1	7.0	6.8
Ethyl[b,d]	20	Needles, EtOAc	200–210 (softens 94)	87	82	$C_{23}H_{28}N_2O_5$	22.6	22.2	6.8	6.8
n-Propyl[b,e]	18	Prisms, alc.	162–165	..	61	$C_{24}H_{30}N_2O_5$	21.8	22.5	6.6	6.3
n-Butyl[b,f]	18	Prisms, alc.	192–193	87	75	$C_{25}H_{32}N_2O_5$	21.1	20.7	6.4	6.3
n-Amyl	18	Prisms, alc.	189–194	..	98	$C_{26}H_{34}N_2O_5$	20.5	20.5	6.2	6.0
n-Hexyl	18	Needles, benz.	164–166 (softens 157)	90	..	$C_{27}H_{36}N_2O_5$	19.9	20.5	6.0	6.0
n-Heptyl	18	Amorphous[j]	131 (softens 94)	83	..	$C_{28}H_{38}N_2O_5$	19.3	19.4	5.8	5.8
n-Octyl	18	Amorphous[j]	121 (softens 85)	77	..	$C_{29}H_{40}N_2O_5$	18.7	18.6	5.6	5.8
β-Hydroxyethyl[g]	20	Prisms, EtOAc	225–226 (softens 185)	86	51	$C_{23}H_{28}N_2O_6$	21.7	21.2	6.5	6.5
β-Chloroethyl	..	Amorphous	...	94	..	$C_{23}H_{27}ClN_2O_5 \cdot 2H_2O$	18.3	18.8	[m]	
Dimethyl[h]	20	Amorphous	203–205 (foams 145)	89	68	$C_{23}H_{28}N_2O_5$	22.6	22.6	6.8	6.6
Diethyl[i]	26	Needles, EtOAc & pet. ether	209–211	86	75	$C_{25}H_{32}N_2O_5$	21.1	21.4	6.4	6.1
Bis-(β-hydroxy)-ethyl	26	Amorphous	...	47	34	$C_{25}H_{32}N_2O_7$	19.7	19.5	5.9	6.2

[a] Reference 1; dimorphic crystals from ethanol analyzing for 0.5 mole ethanol of crystallization. No m.p. given. [b] H. Lettré, *Naturwissenschaften*, **33**, 75 (1946). No m.p. or anal. given. [c] May and Baker, Ltd., *et al.*, British Patent 577,606 (1946); prisms from ethanol–ether, m.p. 173–174°. No anal. given. [d] See ref. [c]; prismatic needles from ether, m.p. 160–162°. No anal. given. [e] See ref. [c]; prisms from ether, m.p. 164°. No anal. given. [f] See ref. [c]; prisms from benzene–ether, m.p. 196°. No anal. given. [g] See ref. [c]; amorphous. No m.p. or anal. given. [h] See ref. [c]; micro crystals, m.p. 204–206°. No anal. given. More recently this compound has been described by H. Rapoport and A. R. Williams, THIS JOURNAL, **73**, 1896 (1951), as having m.p. 174–176°, and its constitution was confirmed by analysis. [i] See ref. [c]; prisms from alcohol–ether, m.p. 207°. No anal. given. [j] Further treatment by chromatography in chloroform solution over activated alumina did not yield a crystalline product. [k] By the Microanalytical Laboratory, National Institutes of Health, in charge of Dr. W. C. Alford. [l] Difficulty was experienced in burning most of these compounds in order to obtain C and H percentages. Since colchicine itself, which would be the expected impurity, has the calculated values OCH_3, 31.1 and N, 3.5, methoxyl and nitrogen analyses represent valid criteria of purity. [m] Chlorine analysis: calcd., 7.5; found 7.4. [n] This formula is based on what is regarded as the most likely structure for colchicine. As an alternative, the substituents in the "C" ring may be reversed, with appropriate shifts of the double bonds.

The β-chloroethyl derivative was prepared from the β-hydroxyethyl derivative by the action of thionyl chloride. While nearly all the compounds were obtained crystalline, it was found that these crystals gave erratic analytical results, probably due to retention of small amounts of solvent,

(1) Post-doctorate Research Fellow of the National Cancer Institute.

(2) Colchiceinamide has been more usually called colchicamide and colchicinamide. It seemed to us more logical to base the name on the "acid" colchiceine rather than on colchicic acid, a name which has been given to two compounds, or on the "ester" colchicine.

(3) H. Lettré, *Die Chemie*, **55**, 265 (1942); H. Lettré and H. Fernholz, *Z. physiol. Chem.*, **278**, 175 (1943).

(4) H. Lettré, *Z. Krebsforsch.*, **57**, 1 (1950).

(5) S. Zeisel, *Monatsh.*, **9**, 1 (1888).

whereas drying under vacuum at temperatures around 100° produced decomposition. Consequently, for analysis, the crystalline products were dissolved in chloroform and precipitated with light petroleum ether or *n*-hexane; this procedure yielded products apparently free of retained solvents. All the compounds were yellow in color, and all gave water-soluble hydrochlorides with the exception of the derivatives butyl through octyl.

Some activity against Sarcoma 37 in mice was exhibited by all of the compounds.[6]

Experimental

The colchicine used had a m.p. of 158–159° cor. and was purified[7] by us from a commercial product employing chromatography over activated alumina. The amines were the best grades of Eastman Kodak Co., Sharples Chemicals Inc., and Fischer Scientific Co. with the exception of hexylamine (Eastman Kodak Co., practical), and of diethanolamine (Carbon and Carbide Chemicals Corp., practical); methylamine, ethylamine and dimethylamine were used as the concentrated aqueous solutions.

N-(β-Chloroethyl)-colchiceinamide.—To a solution of 0.43 g. (0.001 mole) of N-(β-hydroxyethyl)-colchiceinamide in 200 cc. of dry, thiophene-free benzene, cooled to 20°, was added dropwise with vigorous stirring 0.1 cc. of purified[8] thionyl chloride. After standing overnight in the refrigerator, the supernatant liquid was decanted off, the residue washed with benzene by decantation, and the crude yellow product dried in a vacuum desiccator; yield 0.45 g. (94%). The compound was purified by dissolving in chloroform, precipitating with twenty volumes of absolute ether, washing the solid with ether and drying in vacuum at 55°. The amorphous substance is hygroscopic.

Attempts to prepare the N,N-bis-(β-chloroethyl) derivative from N,N-bis-(β-hydroxyethyl)-colchiceinamide in a similar fashion yielded an extremely hygroscopic gummy product which always contained less than the theoretical amount of chlorine and could not be satisfactorily purified.

(6) To be reported in the *Journal of the National Cancer Institute.*

(7) J. N. Ashley and J. O. Harris, *J. Chem. Soc.*, 677 (1944).

(8) L. F. Fieser, "Experiments in Organic Chemistry," 2nd edition, D. C. Heath and Co., Boston, Mass., 1941, p. 381.

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Amino Acid Composition of Crystalline Inorganic Pyrophosphatase Isolated from Bakers Yeast

BY WERNER HAUSMANN

RECEIVED JULY 11, 1951

Inorganic pyrophosphatase has recently been isolated by Dr. M. Kunitz in crystalline form.[1,2] It appeared of interest to investigate the amino acid composition of the new enzyme qualitatively and quantitatively by hydrolysis and chromatography of the hydrolysate.

Qualitative Determination.—A good qualitative picture of the amino acid spectrum of the protein was obtained by paper chromatography.

Five mg. of air-dried crystalline pyrophosphatase was hydrolyzed in 1 ml. of 6 *M* HCl for 24 hours at 110°, in a sealed, evacuated Pyrex glass tube. The hydrolyzate was then evaporated to dryness at 50° and 9 mm. pressure, redissolved in 1 ml. of distilled water and evaporated again in order to remove excess HCl. The remaining mixture of amino acid hydrochlorides was dissolved in 0.5 ml. of distilled water to give a concentration corresponding to 10 γ of original protein per μl.

Twenty μl. (200 γ) of this solution was subjected to two dimensional paper chromatography on Whatman No. 1 filter paper, using the ascending technique.[3] The solvent system used first for running along the longer edge of the paper consisted of 150 ml. of redistilled secondary butanol +60 ml. of 3% aqueous ammonia. This was done twice before turning the paper and running it once along the short paper edge in the second solvent system: 150 ml. of distilled secondary butanol +30 ml. of 88% aqueous formic acid +20 ml. of water. After drying, the paper was held in a horizontal position and sprayed with ninhydrin solution. After five minutes, when the paper looked dry, the cystine region was sprayed with Folin reagent.[4] The area of arginine was sprayed with Sakaguchi solution.[5] Control runs proved that these two specific color reactions were positive even after ninhydrin treatment, if applied immediately.

For comparison a synthetic mixture of 20 amino acid hydrochlorides was prepared, and chromatographed in exactly the same manner. The result is illustrated in Fig. 1. Proline and hydroxyproline give yellow spots. For more accurate comparison the hydrolyzate was run together with standard samples of the 16 amino acids indicated. No new spots appeared.

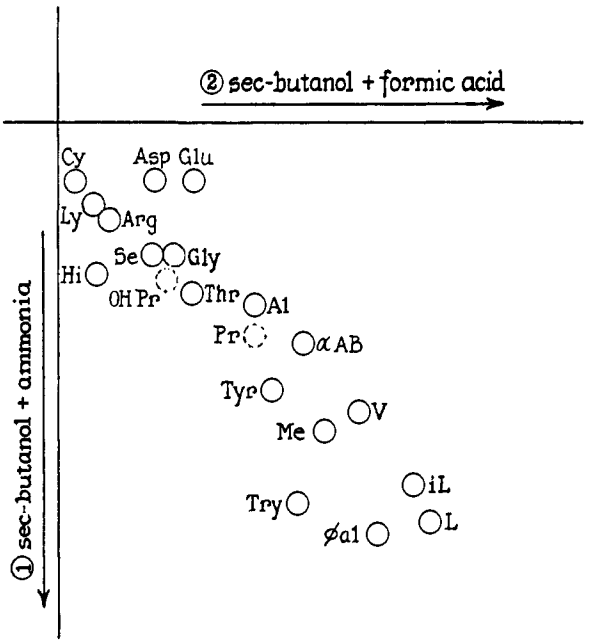

Fig. 1.—Synthetic mixture of amino acid hydrochlorides.

Cystine could not be detected either by ninhydrin or Folin treatment.

Reaction of the intact protein with *p*-dimethylaminobenzaldehyde[6] indicated the presence of tryptophan. This was confirmed by paper chromatography of a $Ba(OH)_2$ hydrolyzate.

Paper chromatography has revealed that crystalline inorganic pyrophosphatase is a protein containing the following 17 amino acids: aspartic acid, glutamic acid, lysine, arginine, histidine, serine, threonine, proline, methionine, tyrosine, tryptophan, glycine, alanine, valine, leucine, isoleucine and phenylalanine. Cystine and hydroxyproline could not be detected.

Quantitative Determination.—The quantitative amino acid composition was determined on the above mentioned hydrolyzate in HCl by chromatography on Dowex 50 columns,[7] and the fractions were analyzed by the colorimetric ninhydrin method.

Figures 2 and 3 represent patterns obtained by the 100-cm. and the 15-cm. columns, respectively. The qualitative

(1) M. Kunitz, THIS JOURNAL, **73**, 1387 (1951).

(2) M. Kunitz, *J. Gen. Physiol.*, **35**, 423 (1952).

(3) R. J. Williams, and H. Kirby, *Science*, **107**, 481 (1948).

(4) O. Folin and J. M. Looney, *J. Biol. Chem.*, **51**, 421 (1922).

(5) E. Jorpes and S. Thoren, *Biochem. J.*, **26**, 1504 (1932).

(6) J. R. Spies and D. C. Chambers, *Anal. Chem.*, **21**, 1249 (1949).

(7) S. Moore and W. H. Stein, *J. Biol. Chem.*, **192**, 663 (1951).

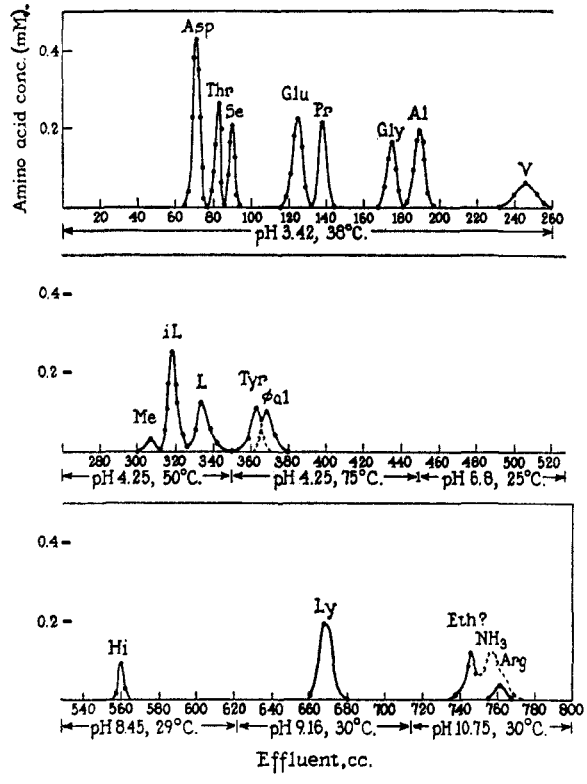

Fig. 2.

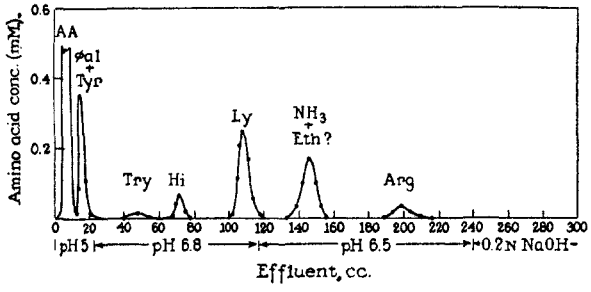

Fig. 3.

composition found by paper chromatography was confirmed and an as yet unidentified ninhydrin positive peak which seemed to be differentiated from the ammonia peak, appeared near the ethanolamine position on the 100-cm. column. On the short column this possible component may be included in the ammonia peak and the ammonia figure is therefore questionable. Cystine was found to be present in traces only, or not at all. The ammonia–arginine doublet was analyzed by evaporating the ammonia from every second tube.

Table I gives the amounts of the single amino acids in per cent. amino acid residue and per cent. nitrogen in the original protein, dried for 3 hours at 100°.

TABLE I

Amino acid	G. amino acid residue per 100 g. protein	Nitrogen, %
Aspartic acid	12.1	1.5
Alanine	4.9	1.0
Ammonia[a]	1.5	1.2
Arginine	3.3	1.2
Glutamic acid	9.7	1.1
Glycine	2.9	0.7
Histidine	2.2	0.7
Isoleucine	8.9	1.1
Leucine	6.0	0.8
Lysine	10.9	2.4
Methionine	1.3	0.1
Phenylalanine	6.2	.6
Proline	6.4	.9
Serine	3.1	.5
Threonine	4.8	.7
Tryptophan[b]	3.6	.5
Tyrosine[c]	6.0	.5
Valine	4.1	.6
Total	97.9	16.1
Found N (Kjeldahl)		16.2

[a] Tentative figure, perhaps including an additional component. [b] Determined spectrophotometrically by Kunitz. 2.5% was found chromatographically in the acid hydrolyzate. [c] This value checks closely with that of Kunitz, determined by the ultraviolet absorption method.

Acknowledgment.—I wish to thank Dr. M. Kunitz for providing the material for this investigation and Miss E. A. Jacobs for her technical assistance.

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The Effect of α-Fluorine Atoms on S_N1 Reactivity[1]

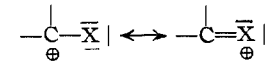
BY JACK HINE AND DONALD E. LEE

RECEIVED JANUARY 2, 1952

It has been previously shown that in comparison to hydrogen, both chlorine and bromine atoms increase the S_N1 reactivity[2] of other halogen atoms attached to the same carbon atom.[3] It is now found that the corresponding effect of α-fluorine atoms is very much smaller than that of α-chlorine or bromine and that it may even be of a deactivating nature.

In 50% aqueous acetone, benzodifluorochloride solvolyzes less than one-fifth as fast as benzyl chloride. If benzyl chloride, under the conditions used, hydrolyzes entirely by the S_N1 (carbonium ion) mechanism, then α-fluorine atoms do decrease the S_N1 reactivity. However, there are several facts which suggest that this reaction may be at least partly S_N2 (bimolecular displacement). One is the great reactivity of benzyl chloride in reactions known to be S_N2 in mechanism. Another is the fact that while the replacement of the first chlorine of benzotrichloride by hydrogen causes a 33-fold decrease in reactivity, the replacement of the second causes only a 5-fold decrease in reaction rate (the situation with benzyl bromide is even more striking).[3] Hence the effect of α-fluorine on S_N1 reactivity in comparison to that of α-hydrogen, while probably not large, is not discernible from the present work.

Although increasing ease of double bond formation and the resultant increased carbonium ion



(1) From an M.S. thesis submitted by Donald E. Lee to the Graduate School of the Georgia Institute of Technology.

(2) For the significance of the terms, S_N1 and S_N2, see I. Dostrovsky, E. D. Hughes and C. K. Ingold, *J. Chem. Soc.*, 173 (1946), and earlier papers.

(3) J. Hine and D. E. Lee, THIS JOURNAL, **73**, 22 (1951).

stability may overrule the slight increase in electronegativity found on going from bromine (2.8 on Pauling's scale) to chlorine (3.0), it does not appear capable of surmounting the large increase in electronegativity found in fluorine (4.0).

Experimental

The benzodifluorochloride used had the following properties: $n^{24.7}D$ 1.4622, $d^{26.2}_4$ 1.2397; molar refractivity calcd. 35.59, found 36.03.

First order rate constants for hydrolysis in 50% (by volume) aqueous acetone, determined as described previously,[3] yielded the following results

at 36°, $k = (0.0830 \pm 0.0023) \times 10^{-4}$ min.$^{-1}$
at 45°, $k = (0.2232 \pm 0.0023) \times 10^{-4}$ min.$^{-1}$
at 60°, $k = (0.9880 \pm 0.0048) \times 10^{-4}$ min.$^{-1}$
at 30°, $k = 0.0419 \times 10^{-4}$ min.$^{-1}$ (extrapolated from the data above to facilitate comparisons with Table I, reference 3)

The average activation energy was 21.2 kcal./mole.

Acknowledgment.—The authors wish to express their thanks to The Hooker Electrochemical Company for the gift of the benzodifluorochloride used in this investigation.

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The Determination of the Relative Affinities of a Series of Specific Substrates from their Respective K_S and k_3 Values[1]

BY DAVID S. HOGNESS AND CARL NIEMANN[2]

RECEIVED JANUARY 5, 1952

For the systems

$$E_f + S_{1f} \underset{k_{2.1}}{\overset{k_{1.1}}{\rightleftharpoons}} ES_1 \xrightarrow{k_{3.1}} E_f + P_f \qquad (1.1)$$

$$E_f + S_{2f} \underset{k_{2.2}}{\overset{k_{1.2}}{\rightleftharpoons}} ES_2 \xrightarrow{k_{3.2}} E_f + P_f \qquad (1.2)$$

where $K_{S_1} = (k_{2.1} + k_{3.1})/k_{1.1}$ and $K_{S_2} = (k_{2.2} + k_{3.2})/k_{1.2}$ it has been argued[3-5] that $k_{2.1}/k_{1.1} > k_{2.2}/k_{1.2}$ when $K_{S_1} > K_{S_2}$ and $k_{3.1} < k_{3.2}$, thus implying that in certain cases it is possible to order the relative affinities of a series of specific substrates solely on the basis of their respective K_S and k_3 values. While this conclusion was at one time accepted by workers in this Laboratory[6] it is now recognized that the argument is fallacious and that an unambiguous ordering of affinities cannot be achieved by the above procedure. That this latter conclusion is correct is apparent from the fact that $k_2/k_1 = K_S(1 + k_3/k_2)^{-1}$ and that

$$\frac{k_{2.1}/k_{1.1}}{k_{2.2}/k_{1.2}} = \frac{K_{S_1}}{K_{S_2}}\left(\frac{1 + k_{3.2}/k_{2.2}}{1 + k_{3.1}/k_{2.1}}\right) \qquad (2)$$

Thus if no restriction is placed upon the values of $k_{2.1}$ and $k_{2.2}$, except that they be positive, then $k_{2.1}/k_{1.1}$ can be less than, equal to, or greater than $k_{2.2}/k_{1.2}$ regardless of the relative values of K_{S_1} and K_{S_2} and of $k_{3.1}$ and $k_{3.2}$. Since the determination of K_S and k_3 does not give any information relative to the magnitude of k_2 it follows that the evaluation of only these two constants, *i.e.*, K_S and k_3, for two or more specific substrates cannot under any circumstance lead to an unambiguous ordering of their respective enzyme–substrate dissociation constants.

CONTRIBUTION NO. 1653 FROM THE
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An Improved Synthesis for α-Methyl-Δ,α,β-butenolide[1,2]

BY WILLIAM H. HOUFF AND HAROLD M SELL

RECEIVED DECEMBER 22, 1951

Introduction

In a study on the effects of inhibition of respiration in germinating seeds by α,β-unsaturated lactones it was necessary to synthesize α-methyl-Δ,α,β-butenolide. The reported method of synthesis,[3] which involves the condensation of formaldehyde and acetone, could not be employed because of the great tendency for butanol-1-one-3 to polymerize to yield other compounds. This note is a report of a modified method which will produce α-methyl-Δ,α,β-butenolide in good yields.

Experimental

1-Chlorobutanone-3.—Into a 3-necked flask equipped with a dropping funnel, stirrer, and downward condenser was placed 32.5 g. (0.3 mole) of 1-chlorobutanol-3. The solution was heated to 125° and a mixture of 60 g. of potassium dichromate in 300 ml. of dilute 1–1 sulfuric acid was added dropwise until the temperature increased and the oxidizing mixture no longer gave the green color of chromous ion. The ketone, as it was formed, was permitted to distil over with the water. The two layers were separated by means of a separatory funnel and the aqueous portion was extracted three times with ether. The product was combined with the ether extracts and the solution was dried over anhydrous sodium sulfate for 12 hours. The ether was distilled off and the fraction distilling at 52–53° at 15 mm. was collected. The yield was 24 g. or 80%.

α-Hydroxy-α-methylbutyrolactone.—To 24 g. (0.22 mole) of 1-chlorobutanone-3 in 100 ml. of water was added 100 ml. of a solution containing 20 g. of potassium cyanide. The solution was cooled to 15° and the 100 ml. of a 25% solution of hydrochloric acid was added slowly with stirring. An additional quantity of 200 ml. of concentrated hydrochloric acid was added and the solution stirred vigorously for four hours. The reaction mixture was extracted continuously with ether in a liquid–liquid extraction unit for eight hours. After drying the ethereal extract over anhydrous sodium sulfate, the ether was removed under vacuum. The crude product distilled with an evolution of hydrogen chloride. The yield of the fraction distilling at 109–110° at 6 mm. was 14 g. or 54%; m.p. of the *p*-nitrobenzoate of the hydroxylactone 161–161.5°, reported[3] m.p. 162°.

α-Methyl-Δ,α,β-butenolide.—The same procedure described by Cavallito, *et al.*,[3] was used. The product distilling at 82° at 7 mm. was collected. The yield was 3.5 g. or 30%; $n^{20}D$ was 1.465.

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(1) Supported in part by a grant from Eli Lilly and Company.
(2) To whom inquiries regarding this article should be addressed.
(3) H. Neurath and G. W. Schwert, *Chem. Revs.*, **46**, 69 (1950).
(4) J. E. Snoke and H. Neurath, *Arch. Biochem.*, **21**, 351 (1949).
(5) J. E. Snoke and H. Neurath, *J. Biol. Chem.*, **181**, 789 (1949).
(6) H. T. Huang and C. Niemann, THIS JOURNAL, **73**, 1541 (1951).

(1) Published as Journal Article No. 1308 of the Michigan Agricultural Experiment Station.
(2) The research was supported by a grant from the Rockefeller Foundation.
(3) C. J. Cavallito and T. H. Haskell, THIS JOURNAL, **68**, 2332 (1946).

Synthesis of Radioactive Parathion Using S^{35}[1,2]

BY JENS A. JENSEN AND GEORGE W. PEARCE

RECEIVED FEBRUARY 1, 1952

The insecticidal compound parathion, O,O-diethyl-O-*p*-nitrophenyl thiophosphate has been prepared and radioactively labeled with S^{35}. The following steps were used

$$S^{35} + PCl_3 \xrightarrow{AlCl_3} PS^{35}Cl_3 \quad (1)$$

$$PS^{35}Cl_3 + 2C_2H_5ONa \longrightarrow (C_2H_5O)_2\text{-}P(S^{35})\text{-}Cl + 2\,NaCl \quad (2)$$

$$(C_2H_5O)_2\text{-}P(S^{35})\text{-}Cl + NaO\text{-}C_6H_4\text{-}NO_2 \longrightarrow (C_2H_5O)_2\text{-}P(S^{35})\text{-}O\text{-}C_6H_4\text{-}NO_2 + NaCl \quad (3)$$

Phosphorus thiochloride was obtained by the method described by Knotz.[3] The cooled liquid was not washed but distilled directly. Reactions (2) and (3) followed those published by Fletcher, *et al.*[4-5] Seventy-five millimoles of S containing approximately 16 millicuries of activity yielded 44.4 millimoles of parathion (59%); sp. gr. 1.26 ± 0.01; $n^{25}D$ 1.5380; specific activity, 220 microcuries per millimole.

(1) From the Communicable Disease Center, Public Health Service, Federal Security Agency, Savannah, Georgia.

(2) For detailed paper order Document 3566 from American Documentation Institute, 1719 N Street, N.W., Washington 6, D. C., remitting $1.00 for microfilm (images 1 inch high on standard 35 mm. motion picture film) or $1.00 for photocopies (6 × 8 inches) readable without optical aid.

(3) Østerr F. Knotz, *Chem. Z.* **50**, 128 (1949).

(4) J. H. Fletcher, *et al.*, *ibid.*, **70**, 3943 (1949).

(5) J. H. Fletcher *et al.*, *ibid.*, **72**, 2461 (1950).

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The Solubility of Rubidium Iodate

BY W. D. LARSON AND J. J. RENIER

RECEIVED JANUARY 28, 1952

No data for the solubility of rubidium iodate at 25° were found in the literature. Measurements of its solubility in water and in potassium nitrate solutions were undertaken to provide these data, and to determine the activity solubility product of the salt. The solubility of this salt in dilute nitric acid solutions was also measured.

Experimental

Rubidium iodate was prepared by the addition of an excess of iodic acid solution to rubidium carbonate solution; the precipitate was washed with cold water. The iodic acid was prepared by adding 35 g. of Mallinckrodt A.R. I_2O_5 to 25 ml. of water; the rubidium carbonate solution was prepared by adding 25 g. of the salt (from Fairmount Chemical Company) to 25 ml. of water. These two were slowly mixed with mechanical stirring which was continued for 30 minutes. The salt was washed by decantation three times, and finally collected on a coarse sintered glass filter, where it was given a final washing. It was air-dried and stored over anhydrous calcium chloride. The samples of rubidium iodate were found to contain over 99.9% of the theoretical amount of IO_3^-. Potassium nitrate and nitric acid were C.P. grade chemicals. Potassium nitrate was dried for several hours at 150° before weighing. Conventional volumetric methods were used. In the density determinations, buoyancy corrections were made. Potassium iodate, carefully recrystallized, was the primary standard.

The solubility of rubidium iodate in water was determined in glass bottles coated with paraffin wax and in uncoated glass bottles. The results were identical. This was taken to indicate that no significant amounts of Rb^+ exchanged with alkali metal ions in the glass, since the IO_3^- concentration was the same in either coated or uncoated bottles.

The temperature was constant at 25.0° (as read from a thermometer certified by the U.S. Bureau of Standards) to within less than 0.05°. The bottles were rotated end-over-end for at least 12 hours; experiments had shown that eight hours ensured saturation.

At least two independent experiments were performed for each concentration of potassium nitrate or nitric acid; two or more samples were taken for analysis from each saturated solution. The analyses agreed with each other to within about one part in eight or nine hundred.

The data are summarized in Tables I and II. In the tables, m, c and x refer to the molality, molarity and mole fraction, respectively. The ionic strength and density are represented by μ and d.

TABLE I

SOLUBILITY OF $RbIO_3$ IN AQUEOUS KNO_3 SOLUTIONS AT 25.0°

m_{KNO_3}	c_{RbIO_3}	$d_{satd.soln.}$	$\mu^{1/2}$	$10^3 x_{Rb^+}$
0	0.0919	1.0160	0.3031	1.665
0.05001	.0972	1.0196	.3830	1.747
.1004	.1018	1.0236	.4482	1.851
.1511	.1054	1.0285	.5044	1.917
.2021	.1070	1.0318	.5530	1.949
.2547	.1118	1.0370	.6017	2.037
.2940	.1143	1.0396	.6345	2.084
.4069	.1192	1.0475	.7187	2.179
.5025	.1220	1.0538	.7813	2.235

TABLE II

SOLUBILITY OF $RbIO_3$ IN AQUEOUS HNO_3 SOLUTIONS AT 25.0°

m_{HNO_3}	c_{RbIO_3}	$d_{satd.soln.}$	c_{HNO_3}
0	0.0919	1.0160	0
0.05020	.1051	1.0216	0.04977
.1006	.1175	1.0250	.09941
.1511	.1297	1.0290	.1490
.2016	.1415	1.0337	.1985
.2516	.1533	1.0385	.2473
.2933	.1626	1.0414	.2878
.4056	.1546	1.0472	.3984
.5006	.1495	1.0506	.4908

Aqueous KNO_3 Solutions.—When the Debye–Hückel equation is applied to solubility data, it is convenient to write it in the form[1]

$$\mu_1^{1/2}\mu_2^{1/2}A^2 + (\mu_1^{1/2} + \mu_2^{1/2})\,A + 1 - \frac{B(\mu_2^{1/2} - \mu_1^{1/2})}{\log (L_2/L_1)^{1/2}} = 0$$

$A = 0.329 \times 10^8\, a$, where a is the mean distance of closest approach; $B = 0.509$ at 25°: $L_1^{1/2}$ and $L_2^{1/2}$ are the mole fractions of Rb^+ ion in the two solutions; and μ_1 and μ_2 are the ionic strengths in these same solutions. Values of A were obtained by solving the equation with data from pairs of solutions which had quite different ionic strengths. Using fourteen pairs of data, this procedure yields a value of $A = 0.674 \pm 0.026$. This value of A leads to a value of x_0, the mole fraction of Rb^+ when the square root of the ionic strength is zero, of $1.237 \pm 0.004 \times 10^{-3}$, and a corresponding value of $c_0 = 0.06834$ mole/liter of solution. The mole fraction and molarity activity coefficients

(1) F. H. MacDougall, "Thermodynamics," 3rd ed., John Wiley and Sons, Inc., New York, N. Y., 1939, p. 316.

f_x and γ_c can be calculated from these values and the relations

$$f_x = x_0/x \text{ and } \gamma_c = \frac{C_0}{C}$$

Aqueous HNO_3 Solutions.—The existence of a maximum in the solubility of rubidium iodate in the nitric acid solutions seems surprising. Great care was taken to make sure that this is not due to an analytical error. It may be that one or both of the acid iodates[2] is part of the solid phase.

(2) Mellor, "Comprehensive Treatise on Inorganic Chemistry," Vol. II, Longmans, London, 1927, pp. 337–338, gives the formulas $RbIO_3 \cdot HIO_3$ and $RbIO_3 \cdot 2HIO_3$.

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Reactions of 1-Methylvinyl Acetate

BY CHARLES D. HURD AND LEON L. GERSHBEIN

RECEIVED FEBRUARY 20, 1952

Marvel[1] reported the formation of 4-acetoxy-2,5-dimethyl-1,3-dioxane by interaction of vinyl acetate and acetaldehyde, pretreated with metallic sodium. The same cyclic acetal apparently was made by Spaeth[2] a few years later by acetylating the adduct of aldol and acetaldehyde. Marvel noted that vinyl acetate was ineffective in the reaction with other aldehydes such as propionaldehyde, *n*- and isobutyraldehyde. A comparable inertness was found in the present work with 1-methylvinyl acetate and acetaldehyde. Aldol was the only product isolated.

Methylvinyl acetate serves as an acetylating agent toward benzene in the presence of aluminum chloride. Acetophenone was the major product.

Experimental

1-Methylvinyl acetate, b.p. 96–98°, was prepared by the reaction of ketene with acetone in the presence of sulfuric acid.[3]

Non-reaction with Acetaldehyde.—A total of 25 g. of methylvinyl acetate was shaken into 11 g. of acetaldehyde previously treated with a small amount of sodium.[1] Absolute ethanol (25 ml.) was then introduced. After two days at 25°, the alcohol and ester were distilled off under reduced pressure, and 6.35 g. of aldol, b.p. 80–90° (34 mm.), was obtained. On redistillation, it boiled at 94.5–95° (33 mm.), 80.5° (20 mm.), $n^{20}D$ 1.4532, d^{20}_{20} 1.090. Analytical figures (C, 55.4; H, 9.01) confirmed the identity of aldol (calcd.: C, 54.5; H, 9.09).

Reaction with Benzene. Run 1.—To a stirred mixture of 27 g. of anhydrous aluminum chloride in 120 ml. of benzene was added a total of 20 g. of 1-methylvinyl acetate in 40 ml. of benzene over a period of 15 minutes. Some heat was liberated; hence the reaction was moderated by tap cooling. After 20 minutes at 25°, the contents were refluxed for 2 hours, cooled and poured onto ice. From the benzene layer, after washing and drying, were obtained these fractions at 30 mm.: (1) boiling range 94–104°, $n^{20}D$ 1.5335, 5.75 g.; (2) 117–137°, $n^{20}D$ 1.5515, 0.75 g. of green oil. Redistillation of (1) yielded colorless acetophenone, b.p. 97–98° (25 mm.). It formed a semicarbazone which after two recrystallizations from aqueous ethanol melted at 203.5–204° (uncor.) and which remained unchanged on admixture with an authentic sample of acetophenone semicarbazone. The absence of ester was ascertained by the saponification test.

Run 2.—When 88 g. (0.66 mole) of anhydrous aluminum chloride in 80 ml. of benzene was added to 20 g. of the acetate in 40 ml. of benzene and the mixture refluxed for 15 hours on the steam-bath, the acetophenone fraction at 87–94° (17 mm.) weighed 17.7 g. A small forerun (0.45 g.) was obtained. Of the viscous residue (5.1 g.), 3.2 g. of amber-orange oil boiled at 134–167° (4 mm.).

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(1) C. S. Marvel, J. Harmon and E. H. Riddle, *J. Org. Chem.*, **4**, 252 (1939).

(2) E. Spaeth, R. Lorenz and E. Freund, *Ber.*, **76**, 57 (1943).

(3) B. H. Gwynn and E. F. Degering, THIS JOURNAL, **64**, 2216 (1942).

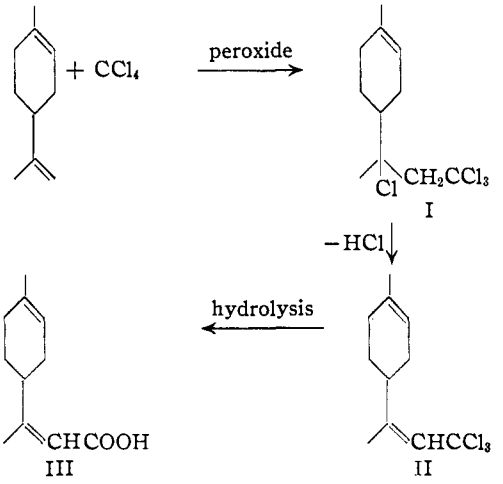
The Peroxide-catalyzed Addition of Carbon Tetrachloride to *d*-Limonene

BY SHALOM ISRAELASHVILI AND ERICH DIAMANT

RECEIVED AUGUST 23, 1951

The peroxide-catalyzed addition of polyhalogenated methane to a terminal carbon–carbon double bond has been reported by Kharasch and his co-workers.[1] In the terpene series, it has been shown that the peroxide-catalyzed addition of carbon tetrachloride to β-pinene[2] and nopinene[3] gave good yields of a one-to-one addition product. In this paper we wish to report the applicability of these reactions to *d*-limonene.

We have studied the radical addition reaction of chloroform, bromoform and carbon tetrachloride to *d*-limonene. It was found that *d*-limonene reacts with carbon tetrachloride in the presence of four mole per cent of benzoyl peroxide to give a good yield (60–70%) of a one-to-one addition product, whereas chloroform and bromoform failed to react under the same conditions. However, the addition did not take place readily, even with the relatively large amount of peroxide used. The addition product obtained (II) is optically active $[\alpha]^{13}D$ + 15.8°. Bromine titration reveals the presence of two double bonds and according to analysis it contains three chlorine atoms. It is believed that the initial adduct (I) is converted to product (II) by elimination of one molecule of hydrogen chloride from carbon atoms 8 and 9 as



(1) M. S. Kharasch, E. V. Jensen and W. H. Urry, THIS JOURNAL, **69**, 1100 (1947).

(2) D. M. Oldroyd, G. S. Fisher and L. A. Goldblatt, *ibid.*, **72**, 2407 (1950).

(3) G. Dupont, R. Dulou and G. Clement, *Compt. rend.*, **230**, 2027 (1950).

The alternate elimination of hydrogen chloride from carbon atoms 8 and 4 would have given an optically inactive product. The structure of (II) receives additional support from the properties of the carboxylic acid (III) obtained by alkaline hydrolysis of (II). In ultraviolet absorption spectrum (Fig. 1) the carboxylic acid (III) shows a peak at 248–250 mμ, which is indicative of a chromophore involving a conjugation of the carboxylic group with a double bond between carbon atoms.[4]

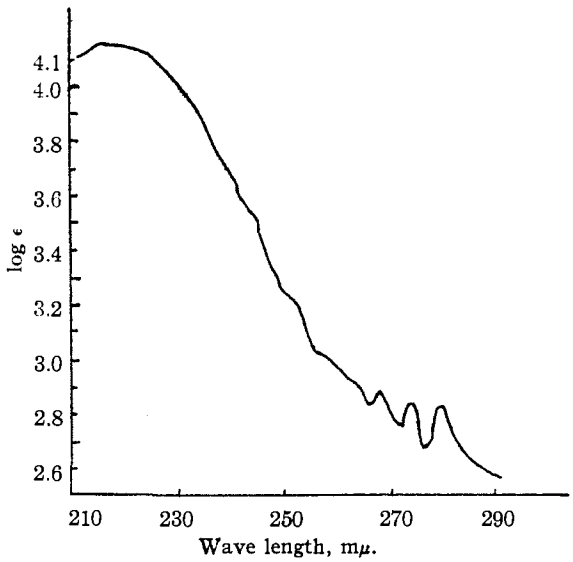


Fig. 1.—Ultraviolet absorption spectrum of 9-carboxy-1,8-(9)-*p*-menthadiene in 95% ethanol.

Experimental[5]

d-Limonene.—*d*-Limonene was obtained by fractional distillation of commercial *d*-limonene. The fraction used had the following constants: b.p. 171–172° (680 mm.), d^{20}_4 0.8404, $n^{20}D$ 1.4407, $[\alpha]^{20}D$ +124°.

Addition of **Carbon Tetrachloride to *d*-Limonene.**—In a three-necked, 500-cc. flask fitted with a reflux condenser, thermometer, dropping funnel and gas inlet-tube, a mixture of *d*-limonene (14 g., 0.1 mole) and C.P. carbon tetrachloride (154 g., 1.0 mole) was refluxed for 24 hours in a continuous stream of dry nitrogen. Benzoyl peroxide (1 g., 0.004 mole) in carbon tetrachloride (20 cc.) was added in four equal portions at intervals of six hours. During refluxing the temperature gradually rose to 88°. The brown reaction product was then washed with 10% sodium carbonate solution and with water, and dried over anhyd. sodium sulfate. From the sodium carbonate washings 0.6 g. of benzoic acid was obtained upon acidification. From the dry reaction mixture, the excess of unreacted carbon tetrachloride was removed by distillation at atmospheric pressure. The residue obtained weighed 19 g., and gave upon fractional distillation at reduced pressure 7 g. of unreacted *d*-limonene, 1.1 g. of intermediate and 8.7 g. of yellow oil (II), b.p. 165–170° (19 mm.), $n^{20}D$ 1.5260, $[\alpha]^{13}D$ +15.8° (*ca.* 10% in ethanol).

The adduct (II) represents a yield of about 30% on the basis of the *d*-limonene used or 60% on the basis of the *d*-limonene consumed.

Anal. of adduct. Calcd. for $C_{11}H_{15}Cl_3$: C, 52.4; H, 5.95; Cl, 41.6. Found: C, 51.9; H, 6.1; Cl, 41.2.

A higher yield of adduct (70% on the basis of *d*-limonene consumed) is obtained when the same mixture is heated in a sealed tube at 120–130° for 12 hours.

Hydrolysis of (II).—The adduct (II) is not readily hydrolyzed by aqueous, methanolic or ethanolic alkali. However, it is hydrolyzed by aqueous alcoholic alkali when heated in a sealed tube at 140°. For the preparation of the carboxylic acid (III), a mixture of 7 g. of (II), 50 cc. of ethanol and 6.5 g. of potassium hydroxide dissolved in 15 cc. of water was heated for 15 hours at 140–150° in a sealed tube. The reaction mixture was diluted with 100 cc. of water, the ethanol removed by distillation and the alkaline residue extracted with ether. The alkaline solution was acidified with dilute sulfuric acid and again extracted with ether. After removal of the ether, 2 g. of crude acid was obtained. Recrystallization from dilute acetic acid yielded 1.5 g. (30%) of colorless long prismatic rods, m.p. 94–95°. The acid absorbs two moles of bromine (titration with 0.1 *N* solution of KBr–$KBrO_3$[6]).

Anal. Calcd. for $C_{11}H_{16}O_2$: C, 73.3; H, 8.9; neut. equiv., 180. Found: C, 73.2; H, 9.0; neut. equiv., 179.8.

The same yield of the saponification product was obtained, when a mixture of 20 g. of (II) and 10 g. of potassium hydroxide dissolved in 75 cc. of dry ethanol was heated for 24 hours at 150–160° in a sealed tube (see, Gätzki and Stammbach,[7] Grummitt, *et al.*[8]).

Ethyl Ester of (III).—The ethyl ester of (III) was obtained when 1 g. of the acid was dissolved in absolute ethanol, saturated with dry hydrogen chloride, and refluxed for two hours. The ethanol was removed by distillation, and the residue was washed with water, dried and distilled at reduced pressure. A colorless oil of very pleasant odor was obtained; b.p. 125° (25 mm.), $n^{20}D$ 1.4925, yield 58%.

Anal. Calcd. for $C_{13}H_{20}O_2$: C, 75.0; H, 9.6; sapn. equiv., 208. Found: C, 74.8; H, 9.5; sapn. equiv., 208.5.

Saponification of the ester yielded a pure specimen of the carboxylic acid, m.p. 95.5°.

Ultraviolet spectrum was obtained with a Beckman quartz spectrophotometer. The spectrum was measured in ethanol at concentration of 0.001 molar.

(4) K. W. **Hausser**, R. Kuhn, A. **Smakula** and M. Hoffer, *Z. physik. Chem.*, **B29**, 371 (1935); H. Mohler and H. Lohr, *Helv. Chim. Acta*, **21**, 485 (1938).

(5) All b.ps. and m.ps. are uncorrected.

(6) H. J. Lucas and D. Pressman, *Ind. Eng. Chem., Anal. Ed.*, **10**, 140 (1938).

(7) K. Gätzki and W. Stammbach, *Helv. Chim. Acta*, **29**, 563 (1946).

(8) O. Grummitt, *et al.*, THIS JOURNAL, **67**, 156 (1946).

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High Temperature Heat Contents of Magnesium Orthotitanate and Magnesium Dititanate

BY R. L. ORR AND J. P. COUGHLIN

RECEIVED JANUARY 31, 1952

An earlier paper[1] from this Laboratory reported high temperature heat content values for the metatitanates of calcium, iron and magnesium. The present paper contains similar data for magnesium orthotitanate and magnesium dititanate.

Materials.—The magnesium titanates were prepared from reagent-grade magnesium oxide and pure titania (99.8% TiO_2, after ignition). Stoichiometric quantities of the oxides were thoroughly mixed, compressed (15,000 lb./sq. in.) into pellets, and heated for prolonged periods at 1300 to 1500°. At intervals, the products were cooled, ground to −100 mesh, analyzed, adjusted in composition, re-formed into pellets, and reheated, until reaction was complete.

The magnesium orthotitanate analyzed 49.53% TiO_2 and 0.21% SiO_2, as compared with the theoretical 49.77% TiO_2. Tests for free magnesia showed only a negligible amount. The X-ray diffraction pattern gave no evidence of impurities.

The magnesium dititanate analyzed 79.63% TiO_2 and 0.16% SiO_2, as compared with the theoretical 79.85% TiO_2. Tests for free magnesia were negative. The X-ray diffraction pattern agreed with that of Jander and Bunde,[2] and gave no evidence of impurities.

(1) B. F. Naylor and O. A. Cook, THIS JOURNAL, **68**, 1003 (1946).

(2) W. Jander and K. Bunde, *Z. anorg. Chem.*, **239**, 418 (1938).

Measurements and Results.—The heat content measurements were made with previously described[3] apparatus and techniques. The substances were enclosed during the measurements in platinum–rhodium capsules. Corrections for the heat contents of these capsules were determined by separate experiments. The results are expressed in defined calories (1 cal. = 4.1833 int. joules), and molecular weights accord with the 1949 International Atomic Weights.

The measured heat content data are listed in Table I. The precision uncertainty, considering all the measurements for each substance, is less than 0.1%, although an occasional determination may deviate from a smooth curve by as much as 0.5%. Both substances show regular behavior, there being no evidence of any transformation or region of anomalous heat capacity. The heat content of the orthotitanate, the heat content of the dititanate is less than the sum for the metatitanate and rutile by 6.4% at 400°K., and greater than this sum by 1.7% at 1800°K. No previous high temperature heat content data for either the orthotitanate or dititanate were found in the literature.

TABLE I

HEAT CONTENTS ABOVE 298.16°K. (CAL./MOLE)

T, °K.	$H_T - H_{298.16}$	T, °K.	$H_T - H_{298.16}$	T, °K.	$H_T - H_{298.16}$
Mg_2TiO_4 (mol. wt. 160.54)					
392.4	3,080	1086.6	31,260	1197.9	36,420
493.9	6,780	1092.1	31,590	1286.8	40,490
592.6	10,560	1092.7	31,410	1286.8	40,550
695.3	14,640	1097.9	31,910	1295.4	41,000
789.2	18,500	1106.5	32,260	1392.1	45,650
894.8	22,910	1109.8	32,420	1490.4	50,290
999.3	27,370	1113.1	32,420	1597.2	55,400
1004.7	27,530	1113.6	32,610	1705.9	60,750
1006.0	27,550	1114.0	32,530	1792.4	64,860
1027.8	28,670	1124.6	33,050	1817.8	66.510
1074.2	30,700				
$MgTi_2O_5$ (mol. wt. 200.12)					
396.8	3,650	901.9	26,330	1389.5	50,910
492.7	7,590	998.8	30,950	1500.5	56,920
587.8	11,750	1084.4	35,120	1601.9	62,440
695.5	16,660	1182.4	40,050	1696.2	67,790
795.0	21,190	1287.9	45,440	1812.1	74,160

TABLE II

HEAT CONTENTS (CAL./MOLE) AND ENTROPIES (CAL./DEG. MOLE) ABOVE 298.16°K.

	Mg_2TiO_4		$MgTi_2O_5$	
T, °K.	$H_T - H_{298.16}$	$S_T - S_{298.16}$	$H_T - H_{298.16}$	$S_T - S_{298.16}$
400	3,340	9.61	3,780	10.87
500	6,990	17.74	7,910	20.08
600	10,850	24.77	12,290	28.06
700	14,840	30.92	16,830	35.06
800	18,930	36.38	21,470	41.25
900	23,120	41.31	26,200	46.82
1000	27,430	45.86	31,010	51.89
1100	31,910	50.13	35,910	56.56
1200	36,510	54.13	40,930	60.92
1300	41,200	57.88	46,090	65.05
1400	45,960	61.41	51,410	69.00
1500	50,760	64.72	56,850	72.75
1600	55,600	67.84	62,370	76.31
1700	60,470	70.79	67,940	79.68
1800	65,370	73.59	73,530	82.88

Table II contains heat content and entropy increments above 298.16°K. at even 100° intervals, for use by those who prefer the tabular method of thermodynamic calculations. The entropy increments have been calculated to match the heat contents by the method of Kelley.[4]

The heat contents are represented, to within the average deviation indicated in parentheses, by the equations

Mg_2TiO_4: $H_T - H_{298.16} = 35.96T + 4.27 \times 10^{-3}T^2 + 6.89 \times 10^5T^{-1} - 13{,}412$; (298 – 1800°K.; 0.3%)

$MgTi_2O_5$: $H_T - H_{298.16} = 40.68T + 4.60 \times 10^{-3}T^2 + 7.35 \times 10^5T^{-1} - 15{,}003$; (298 – 1800°K.; 0.3%)

(3) K. K. Kelley, B. F. Naylor and C. H. Shomate, U. S. Bur. Mines Tech. Paper, 686 (1946).

(4) K. K. Kelley, *U. S. Bur. Mines Bull.*, 476 (1949).

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The Identity of Neamine and Neomycin A

BY BYRON E. LEACH AND CHARLOTTE M. TEETERS

RECEIVED FEBRUARY 11, 1952

The hydrolysis of the antibiotic neomycin with mineral acid yields a crystalline biologically active base which has been named neamine.[1] Peck and co-workers[2] had previously reported the isolation of neomycin A hydrochloride from the fermentation broths of *Streptomyces fradiae*. An exchange of samples with Dr. R. L. Peck[3] has revealed that neamine is identical with neomycin A.

Neomycin A hydrochloride was converted to the free base and crystallized from ammoniacal methanol. The melting point was 256° (dec.), and showed no depression in melting point when mixed with neamine. The infrared absorption spectra, measured in liquid petrolatum (Nujol) suspension, of the hydrochloride and the crystalline free base of neomycin A were identical with neamine hydrochloride and its crystalline free base, respectively. Paper chromatograms using wet *n*-butanol containing 2% *p*-toluenesulfonic acid monohydrate[4] and also *n*-butanol–acetic acid–water (2:1:1) systems showed no differences in R_f values for these two substances; the slopes of the *B. subtilis* bioassay curves were also identical.

Hydrolysis of neamine with boiling 48% hydrobromic acid yielded the hydrobromide of an optically inactive base. The analytical data obtained for this compound are in good agreement with those calculated for the dihydrobromide of 1,3-diamino-4,5,6-trihydroxycyclohexane which Kuehl, *et al.*,[5]

(1) B. E. Leach and C. M. Teeters, THIS JOURNAL, **73**, 2794 (1951).

(2) R. L. Peck, C. E. Hoffhine, Jr., P. Gale and K. Folkers, *ibid.*, **71**, 2590 (1949).

(3) We are grateful to Dr. R. L. Peck, Research Laboratories, Merck and Co., Rahway, N. J., for the sample of neomycin A hydrochloride.

(4) D. H. Peterson and L. M. Reineke, THIS JOURNAL, **72**, 3598 (1950).

(5) F. A. Kuehl, Jr., M. N. Bishop and K. Folkers, *ibid.*, **73**, 881 (1951).

reported as a degradation product of neomycin A. Our previously proposed empirical formula[1] of $C_6H_{12-14}N_2O_3$ for neamine appeared, from the molecular weight data, to represent the molecular formula. Unless the cyclohexane degradation product arises merely from racemization or rearrangement, the molecular weight data are anomalous and the molecular formula of neamine would now appear to be a multiple of C_6.

Experimental

Crystalline Neomycin A.—A 15.9-mg. sample of neomycin A hydrochloride[3] was suspended in 1 ml. of commercial methanol and the mixture was saturated with ammonia gas. The neomycin A hydrochloride dissolved completely in the ammoniacal methanol and after standing at room temperature for thirty minutes, neomycin A free base crystallized. The crystals were collected on a filter stick and washed twice with 0.5-ml. portions of methanol. The dried crystals weighed 8.0 mg. The compound decomposed in a capillary tube at 256° and showed no depression in the decomposition point when mixed with neamine.

Neamine Hydrochloride.—A 100-mg. sample of crystalline neamine prepared as described previously[1] was dissolved in 10 ml. of water and titrated to *p*H 4.5 with *N* hydrochloric acid. The solution was freeze-dried to give a quantitative yield of neamine hydrochloride.

Hydrolysis of Neamine with 48% Hydrobromic Acid.—A 5.0-g. sample of neamine was dissolved in 150 ml. of 48% hydrobromic acid and heated under reflux for 18 hours. The reaction mixture became colored rather quickly. The solution was evaporated *in vacuo* to dryness, 50 ml. of water was added and again evaporated to dryness. This process was repeated twice to insure complete removal of the excess hydrobromic acid. The residue was treated with 50 ml. of boiling methanol and filtered. The methanol insoluble fraction weighed 4.59 g. It was dissolved in 50 ml. of water, treated with 10 g. of Darco G-60, filtered and the solution concentrated *in vacuo* until crystals appeared. After refrigerating overnight, the crystals were collected, washed with 0.5 ml. of ice water and dried to yield approximately 2.5 g. of crystals. These crystals decompose at 280° (micro-block) and show no optical activity.

Anal. Calcd. for $C_6H_{14}N_2O_3 \cdot 2HBr$: C, 22.24; H, 4.98; N, 8.65; Br, 49.33, eq. wt., 162. Found: C, 22.58; H, 4.95; N, 8.64; Br, 48.58, eq. wt., 156.

The analytical data for this product are in good agreement with those calculated for the dihydrobromide of 1,3-diamino-4,5,6-trihydroxycyclohexane which has been reported by Kuehl, *et al.*,[5] to be a degradation product of neomycin A.

The methanolic extract of the hydrolysate above yielded a small amount of ammonium bromide and other unidentified degradation products.

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Preparation of Anhydrous Alcohol

BY HAKON LUND

RECEIVED MARCH 5, 1952

The method of Lund and Bjerrum[1] for the preparation of absolute alcohol by means of magnesium seems now to be in general use and is described in several books on organic syntheses[2] in the original form using iodine as a catalyst for the initiation of the reaction. It might be useful to point out that small amounts of aliphatic halogen compounds are better catalysts than iodine.[3] If traces of the halogen compound are harmless in the alcohol obtained, chloroform or carbon tetrachloride may serve, but when halogen compounds have to be strictly excluded ethyl bromide can be used. In that case the catalyst is removed with the first few cc. of the distillate.

AARHUS, DENMARK

(1) Lund and Bjerrum *Ber.*, **64**, 210 (1931).

(2) For instance L. F. Fieser, "Experiments in Organic Chemistry," and David A. Shirley, "Preparation of Organic Intermediates."

(3) *Ber.*, **37**, 936 (1934).

Reaction of Vanillin and Its Derived Compounds. XV.[1] 3-Ethoxy-4-hydroxybenzoic Acid and Some of Its Esters.[2]

BY IRWIN A. PEARL AND DONALD L. BEYER

RECEIVED JANUARY 16, 1952

The treatment of disseminated histoplasmosis with ethyl vanillate has been reported recently by Christie, Middleton, Peterson, and McVicker.[3] These investigators found that ethyl vanillate is the only known effective therapeutic agent for disseminated and progressive histoplasmosis, but that the margin between effective therapeutic levels and those which produce toxic manifestations is only about 25 to 30%, a margin of safety too small for a desirable therapeutic agent. These results led to the investigation of the effect of changes in the ethyl vanillate molecule on the therapeutic activity of the compound. The present paper reports the preparation of the related 3-ethoxy-4-hydroxybenzoic acid and representative esters prepared therefrom.

Larsson[4] has recently prepared 3-ethoxy-4-hydroxybenzoic acid from the corresponding aldehyde by a number of different procedures. We have now prepared it by the oxidation of 3-ethoxy-4-hydroxybenzaldehyde with silver oxide in aqueous alkaline solution. The low temperature caustic fusion procedure used so successfully for the preparation of vanillic acid from vanillin[5] when applied to ethylvanillin yielded only protocatechuic acid and unchanged ethylvanillin indicating that, under the conditions of caustic fusion, the ethoxyl group of ethylvanillin is more susceptible to dealkylation than is the aldehyde group to oxidation.

The desire to derive 3-ethoxy-4-hydroxybenzoic acid from our basic raw material, vanillin, led to a study of its preparation from protocatechuic acid, a compound easily prepared by caustic fusion of vanillin at temperatures above 240°.[5] Following the procedure employed by Bertram[6] for the preparation of vanillin from protocatechualdehyde, ethyl protocatechuate was treated with one mole of ethyl bromide and two moles of potassium carbonate in boiling ethanol. In addition to the desired ethyl 3-ethoxy-4-hydroxybenzoate, chromatographic separation of the reaction product yielded the ethyl

(1) For paper XIV of this series, see THIS JOURNAL, **74**, 1357 (1952).

(2) This paper represents a portion of the results obtained in the research program sponsored by the Sulphite Pulp Manufacturers' Research League and conducted for the League by The Institute of Paper Chemistry. Acknowledgment is made by the Institute for permission on the part of the League to publish these results.

(3) A. Christie, J. G. Middleton, J. C. Peterson and D. L. McVicker, *Pediatrics*, **7**, 7 (1951).

(4) E. Larsson, *Trans. Chalmers Univ. Technol. Gothenberg*, No. **59**, 21 (1947).

(5) I. A. Pearl, THIS JOURNAL, **68**, 2180 (1946).

(6) J. Bertram, German Patent 63,007 (Aug. 19, 1890); *Ber.*, **25**, R823 (1892).

TABLE I

ESTERS OF 3-ETHOXY-4-HYDROXYBENZOIC ACID

Ester	Yield,[a] %	B.p. °C.	B.p. Mm.	M.p.,[b] °C.	Formula	Analyses, % Carbon Calcd.	Carbon Found	Hydrogen Calcd.	Hydrogen Found	Inhibiting concn.,% *Bacillus mycoides*
Ethyl	92	142	2.0	64	$C_{11}H_{14}O_4$	62.84	62.83	6.71	6.69	0.09
Propyl	93	138	2.0	75–76	$C_{12}H_{16}O_4$	64.27	64.41	7.19	7.27	> .21
Isobutyl	80	151	2.5	93	$C_{13}H_{18}O_4$	65.53	65.68	7.61	7.72	.09
s-Butyl	78	139	0.6	61–62	$C_{13}H_{18}O_4$	65.53	65.51	7.61	7.63	> .21
Amyl	88	157	1.1	42	$C_{14}H_{20}O_4$	66.64	66.58	7.99	7.97	.009
Octyl	74	182	1.5	32–33	$C_{17}H_{26}O_4$	69.36	69.39	8.90	8.89	.0009
Decyl	75	197	1.5	35–36	$C_{19}H_{30}O_4$	70.77	70.67	9.38	9.21	.15

[a] Yields are of purified products. [b] All esters except octyl and decyl were recrystallized from petroleum ether (b.p. 65–110°); octyl and decyl esters recrystallized from petroleum ether (b.p. 30–60°).

esters of 4-ethoxy-3-hydroxybenzoic acid and 3,4-diethoxybenzoic acid.

The ethyl, propyl, isobutyl, *s*-butyl, amyl, octyl and decyl esters of 3-ethoxy-4-hydroxybenzoic acid were prepared by procedures employed for the preparation of analogous vanillic acid esters.[7,8] Data for these esters are given in Table I. The ultraviolet absorption spectra of 3-ethoxy-4-hydroxybenzoic acid and its esters were determined in purified 95% ethanol with a Beckman spectrophotometer. The absorption spectra for all the esters are the same as that for the acid except for actual extinction values. They are all characterized by peaks at 293, 263 and 222 mμ and are almost identical with the spectra of the corresponding esters of vanillic acid.

The inhibiting concentrations of these esters were determined for four representative aerobic microorganisms—namely, non-sporeforming (*Aerobacter aerogenes*) and sporeforming (*Bacillus mycoides*) bacteria and two molds (*Aspergillus niger* and *Penicillium expansum*). Because of the insolubility of these compounds in dilute aqueous solutions containing one equivalent of sodium hydroxide, it was necessary to employ 80% ethanol as a solvent. Except for ethyl 3-ethoxy-4-hydroxybenzoate, none of the esters of 3-ethoxy-4-hydroxybenzoic acid inhibited *Aerobacter aerogenes* or *Aspergillus niger* at concentrations as high as 0.21%. The ethyl ester inhibited these two organisms at 0.09%. *Penicillium expansum* was inhibited by the ethyl ester at 0.09%, the propyl ester at 0.15%, and the *s*-butyl ester at 0.21%. The other esters were ineffective at concentrations as high as 0.21%. The inhibiting concentrations for *Bacillus mycoides* are given in Table I. It is noteworthy that octyl 3-ethoxy-4-hydroxybenzoate is more toxic toward *Bacillus mycoides* than any ester tested to date in our studies on the esters of vanillic acid and related acids.

Experimental

All melting points are uncorrected.

3-Ethoxy-4-hydroxybenzoic Acid.—Ethylvanillin (Monsanto) was oxidized with silver oxide and alkali by the procedure reported earlier for the preparation of vanillic acid,[9] but the silver oxide and alkali ratios were doubled. The yield was quantitative. The ratios employed for vanillin gave only 50–60% yields with ethylvanillin. This was also true when only the silver oxide ratio was doubled. Silver oxide recovered by the permanganate procedure[9] was found to be inoperative in this reaction with ethylvanillin.

Lower Alkyl Esters.—All the esters except the octyl and decyl esters were prepared by boiling under reflux a mixture of 3-ethoxy-4-hydroxybenzoic acid in a fivefold excess of the esterifying alcohol for five hours, removing most of the excess alcohol under reduced pressure, diluting with water, neutralizing with sodium bicarbonate, and extracting with ether. The ether was dried and distilled to leave the crude ester which was then distilled under reduced pressure.

Higher Alkyl Esters.—The octyl and decyl esters were prepared by the iminoether synthesis employed in the past for long chain alkyl vanillates.[8]

3-Ethoxy-4-hydroxybenzonitrile.—A mixture of 156 g. of 4-acetoxy-3-ethoxybenzonitrile[4] and 450 cc. of concentrated hydrochloric acid was heated at 60–70° until all the solid dissolved. The solution was cooled, diluted with 3000 ml. of cold water, and neutralized to *p*H 4–5 with dilute sodium hydroxide. The mixture was extracted with ether, and the ether was dried and distilled to dryness. The crude 3-ethoxy-4-hydroxybenzonitrile melted at 66° and weighed 93 g. (75%). Recrystallization from petroleum ether (b.p. 65–110°) yielded white needles melting at 66°.

Anal. Calcd. for $C_9H_9O_2N$: C, 66.24; H, 5.56. Found: C, 66.39; H, 5.74.

Octyl 3-Ethoxy-4-hydroxybenzimidate Hydrochloride.—A mixture of 50 g. of 3-ethoxy-4-hydroxybenzonitrile, 41 g. of octyl alcohol and 150 cc. of absolute ether was treated with anhydrous hydrogen chloride for one hour while cooling in an ice-bath. The flask was stoppered with a calcium chloride tube and allowed to stand at 20° for 24 hours. The heavy precipitate was filtered, washed with ether, and air dried to yield 69 g. (69%) of crude octyl 3-ethoxy-4-hydroxybenzimidate hydrochloride melting at 154–155°. Purification by solution in chloroform and precipitation with ether yielded tiny white crystals melting at 155°.

Anal. Calcd. for $C_{17}H_{28}O_3NCl$: C, 61.90; H, 8.56; N, 4.25. Found: C, 61.86; H, 8.60; N, 4.45.

Decyl 3-Ethoxy-4-hydroxybenzimidate Hydrochloride.—This compound was prepared in an identical manner and was obtained in 81% yield as white crystals melting at 155–156°.

Anal. Calcd. for $C_{19}H_{32}O_3NCl$: C, 63.76; H, 9.01; N, 3.91. Found: C, 63.82; H, 9.02; N, 4.11.

Ethylation of Ethyl Protocatechuate.—A mixture of 18.2 g. (0.1 mole) of ethyl protocatechuate, 11 g. (0.1 mole) of ethyl bromide, 27.6 g. (0.2 mole) of anhydrous potassium carbonate and 100 cc. of absolute ethanol was boiled under reflux for 7 hours. The ethanol was removed under reduced pressure and was finally replaced with water. The resulting aqueous solution was extracted with ether. The ether was dried and distilled to yield 16.35 g. of viscous oil. The aqueous solution was acidified with dilute sulfuric acid and extracted with ether to yield 4.0 g. of product which, upon recrystallization from petroleum ether (b.p. 65–110°), yielded crystals of ethyl protocatechuate melting at 126–127°.

The viscous oil (2.0 g.) was chromatographed from petroleum ether (b.p. 65–110°) on a column (44 mm. in diameter and 240 mm. long) of acid washed Magnesol[10] and developed with 375 cc. of 50:1 petroleum ether–ethanol. Three bands were indicated by streaking with alkaline permanganate.[10] The leading band on elution with acetone yielded a product which, on crystallization from petroleum ether, gave colorless needles of ethyl 3,4-diethoxybenzoate melting at 52–53°.

(7) I. A. Pearl and J. F. McCoy, THIS JOURNAL, **69**, 3071 (1947).
(8) I. A. Pearl and D. L. Beyer, *ibid.*, **73**, 4091 (1951).
(9) I. A. Pearl, *ibid.*, **68**, 429 (1946).
(10) I. A. Pearl and E. E. Dickey, *ibid.*, **73**, 863 (1951).

Anal. Calcd. for $C_{13}H_{18}O_4$: C, 65.53; H, 7.61. Found: C, 65.38; H, 7.59.

Hydrolysis with ethanolic sodium hydroxide yielded 3,4-diethoxybenzoic acid which, upon recrystallization from water, was obtained as fluffy white needles melting at 162–163°.

Anal. Calcd. for $C_{11}H_{14}O_4$: C, 62.84; H, 6.71. Found: C, 62.74; H, 6.71.

Herzig[11] treated the tetraethyl ether of quercitin with alcoholic potassium hydroxide and obtained 3,4-diethoxybenzoic acid melting at 165–166°. He prepared the ethyl ester and recorded a melting point of 56–57°.

The other two chromatographic bands were combined and eluted. The acetone eluate, upon removal of solvent, yielded crystals and oil. The crystals were removed and recrystallized from petroleum ether (b.p. 65–110°) to give ethyl 4-ethoxy-3-hydroxybenzoate as slightly yellow crystals melting at 77–78° and not depressing the melting point of a mixture with ethyl 4-ethoxy-3-hydroxybenzoate prepared from authentic 4-ethoxy-3-hydroxybenzoic acid.[12]

Anal. Calcd. for $C_{11}H_{14}O_4$: C, 62.84; H, 6.71. Found: C, 62.89; H, 6.79.

The oil removed from the crystals of ethyl 4-ethoxy-3-hydroxybenzoate was boiled with dilute sodium hydroxide solution, cooled and acidified with dilute sulfuric acid. The solid obtained was filtered, washed with water, and recrystallized from dilute methanol to yield 3-ethoxy-4-hydroxybenzoic acid as white needles melting at 164–165° and not depressing a mixed melting point with authentic 3-ethoxy-4-hydroxybenzoic acid.

The approximate yields obtained in this experiment were: ethyl 4-ethoxy-3-hydroxybenzoate, 15%; ethyl 3,4-diethoxybenzoate, 20%; and 3-ethoxy-4-hydroxybenzoic acid, 30%.

Acknowledgment.—The authors wish to thank Mr. Donald McDonnell for the analyses and Mr. John Carlson for the microbiological data reported in this paper.

(11) J. Herzig, *Monatsh.*, **5**, 81 (1884).

(12) H. King, *J. Chem. Soc.*, 1157 (1939).

APPLETON, WIS.

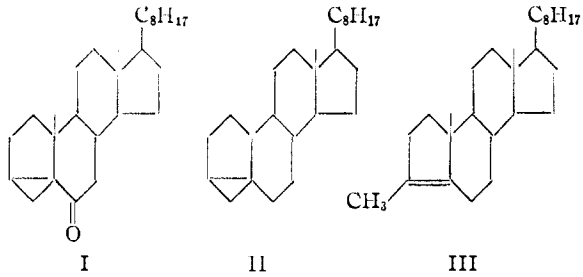
Reductions of *i*-Cholestan-6-one[1]

BY FRANKLIN S. PROUT AND BYRON RIEGEL

RECEIVED JANUARY 17, 1952

We have confirmed the preparation of *i*-cholestane (II) by the Wolff–Kishner reduction of *i*-cholestan-6-one (I)[2] recently reported by Schmid and Kagi,[3] although we have used the Huang-Minlon[4] modification of this reaction. The properties of this hydrocarbon agree with those of *i*-cholestane reported by Schmid and Kagi[3] and by Schmid and Karrer who prepared this hydrocarbon by action of lithium aluminum hydride on cholesteryl *p*-toluenesulfonate.[5]

i-Cholestane (II) failed to add hydrogen under atmospheric pressure using Raney nickel (W-4)[6] in dioxane or prereduced platinum oxide in glacial acetic acid. In both cases *i*-cholestane was recovered in 88% yield by direct crystallization of the hydrocarbon. In contrast hydrogenation of *i*-cholestane in the presence of platinum oxide and acetic acid by Schmid and Kagi[3] was followed by



I II III

treatment of the hydrocarbon with cold concentrated sulfuric acid (−10°) to give a hydrocarbon having a m.p. 43.5–44.5°, $[\alpha]^{15}D$ + 54.4° (chloroform). This compound actually appears to be an impure rearrangement product of *i*-cholestane, since *i*-cholestane was converted to compound III by shaking with concentrated sulfuric acid at 0–10°.

i-Cholestane (II) when treated with bromine according to the directions of Hauptmann[7] absorbed some bromine. The absorption was apparently random and incomplete since the only product obtained was a small amount (36%) of starting material.

Using hydrobromic acid in acetone,[8] we have confirmed the acid-catalyzed rearrangement of *i*-cholestane (II) described by Schmid and Kagi.[3] The resulting hydrocarbon (III), brilliantly characterized by these workers,[3] has been further characterized by its reaction with bromine.[7] Here the addition of bromine was accompanied by liberation of hydrogen bromide and furnished an allylic monobromide not obtained by Schmid and Kagi.[3]

In another sequence *i*-cholestan-6-one was reduced quantitatively with aluminum isopropoxide to give the *i*-cholestan-6-ol as an oil.[9] This oil was converted to cholesteryl acetate in boiling acetic acid with zinc acetate in an over-all yield of 94%.

Acknowledgment.—The authors wish to express their appreciation for a grant from the American Cancer Society on the recommendation of the Committee on Growth of the National Research Council for the support of part of this work.

Experimental[10]

The Conversion of *i*-Cholestan-6-one (I) to Cholesteryl Acetate.—One gram (2.62 mmoles) of *i*-cholestan-6-one[11] was converted quantitatively to *i*-cholestan-6-ol by reduction with aluminum isopropoxide and isopropyl alcohol.[9] Half of this crude oil (1.27 mmoles) was heated under reflux in a mixture of 25 cc. of acetic acid, 1 g. of zinc acetate dihydrate and 1 cc. of acetic anhydride for six hours.[12] The mixture was diluted with water to give 511 mg. (94%) of

(1) Presented before the Organic Division of the American Chemical Society, 115th Meeting, March 27 to April 1, 1949, San Francisco, California.

(2) O. Windaus and A. Dalmer, *Ber.*, **52B**, 162 (1919).

(3) H. Schmid and K. Kagi, *Helv. Chim. Acta*, **33**, 1582 (1950).

(4) Huang-Minlon, THIS JOURNAL, **68**, 2487 (1946).

(5) H. Schmid and P. Karrer, *Helv. Chim. Acta*, **32**, 1371 (1949).

(6) A. A. Pavlic and H. Adkins, THIS JOURNAL, **68**, 1471 (1946).

(7) *Cf.* H. Hauptmann, *ibid.*, **69**, 562 (1947).

(8) *Cf.* B. Riegel, G. P. Hager and B. L. Zenitz, *ibid.*, **68**, 2562 (1946).

(9) I. M. Heilbron, J. Hodges and F. S. Spring, *J. Chem. Soc.*, 759 (1938).

(10) All melting points are uncorrected, except for one. The analyses were performed by Misses Margaret Hines and Virginia Gibbs of Northwestern University and by Micro-Tech Laboratories, Skokie, Illinois. Analyses for carbon and hydrogen content of *i*-cholestane (II), Compound III and the oxide of III were determined but are not reported.

(11) The *i*-cholestan-6-one (m.p. 97–98°) was prepared for us by Drs. Frank A. Vingiello and William L. Hartop according to the procedure of Windaus and Dalmer (Ref. 2).

(12) *Cf.* J. H. Beynon, I. M. Heilbron and F. S. Spring, *J. Chem. Soc.*, 406 (1937).

solid which on systematic recrystallization from butanone, alcohol and ethyl acetate furnished 434 mg. of cholesteryl acetate; m.p. 112.7–114.7°, no depression on mixing with an authentic sample.

i-**Cholestane** (II).[3,5]—A mixture of 3 g. of sodium, 60 cc. of diethylene glycol, 5.00 g. (13.0 mmoles) of *i*-cholestan-6-one[11] and 10 cc. of 85% hydrazine hydrate was heated under reflux for two hours.[4] The condenser was removed and the boiling solution was evaporated until the pot temperature had reached 205°. Boiling of the two-phase mixture was then continued for four hours under reflux. The reaction mixture was diluted with water and extracted with ligroin (Skellysolve B, b.p. 60–70°). The ligroin solution after washing and drying was chromatographed on a column of 20 g. of alumina (Fisher adsorption alumina, 80–200 mesh). Elution with ligroin furnished 3.80 g. of *i*-cholestane, m.p. 76–78°, upon removal of the solvent. Further elution of the column with benzene gave 879 mg. of the azine (see below) respresenting 17.7% of the starting material, m.p. 220–239°.

The *i*-cholestane was crystallized from acetone to give 3.48 g. (72.4%); m.p. 77.4–79.1°. Two more crystallizations from acetone gave the purified hydrocarbon as plate-like crystals: m.p. 78.4–79.1° (cor.); $[\alpha]^{26}D$ +78.5° (20.0 mg. of hydrocarbon made up to 2 cc. with chloroform, $\alpha^{26}D$ +1.57°, *l*, 2 dm.).

Schmid and Kagi[3] give m.p. 80–80.5° and $[\alpha]^{20}D$ +79.6°.

The azine fraction was crystallized five times from butanone to give the purified product as needles: m.p. 239.8–243.5° (dec.) after softening at 220°; $[\alpha]^{26}D$ +121° (42.3 mg. made up to 5 cc. with benzene, $\alpha^{25}D$ +2.05°, *l*, 2 dm.).

Anal. Calcd. for $C_{27}H_{46}N_2$ (hydrazone): C, 81.34; H, 11.63; N, 7.03. Calcd. for $C_{54}H_{88}N_2$ (azine): C, 84.75; H, 11.59; N, 3.66. Found: C, 84.89; H, 11.55; N, 3.65.

Rearrangement of *i*-Cholestane (II) to Compound III.[3]—*i*-Cholestane (0.49 g., 1.32 mmoles) was heated under reflux for eight hours with 0.5 cc. of 48% hydrobromic acid in 15 cc. of acetone. This mixture was diluted with 2–3 cc. of water and allowed to crystallize at 5° as heavy blades: 0.45 g.; m.p. 51–61°. One recrystallization from acetone gave 0.32 g.; m.p. 61.5–64.5°; $[\alpha]^{20}D$ +61.2° (50.9 mg. of hydrocarbon was dissolved up to 4.94 cc. with chloroform, $\alpha^{20}D$ +1.26°, *l*, 2 dm.).

Schmid and Kagi[3] give m.p. 64.5–65° and $[\alpha]^{19}D$ +57.9° for this compound. Chromatography of this hydrocarbon effected no change in properties but the resulting product was more stable on storage. Hydroiodic acid in acetone was an effective rearrangement catalyst. A ligroin (b.p. 40–42°) solution of *i*-cholestane was shaken with concentrated sulfuric acid at 0–10° to effect this rearrangement. Sulfuric acid and acetic acid at 100°[13] also effected the reaction though in reduced yield. Hydrochloric or sulfuric acids in acetone catalyzed the reaction slowly. The rearrangement failed to occur with hydrobromic acid in ethanol.

The oxide of III was prepared using perbenzoic acid in chloroform on III. After chromatography and crystallization from ethyl acetate the purified oxide was obtained: m.p. 96.5–97.5°; $[\alpha]^{26}D$ +43° (33.4 mg. dissolved up to 1.96 cc. with chloroform, $\alpha^{26}D$ +0.73°, *l*, 1 dm.). The literature[3] reports m.p. 97.5–98.5°; $[\alpha]^{19}D$ +40.8°.

Bromination of Compound III.—Compound III (154 mg., 0.416 mmole, m.p. 62.5–64°) was dissolved in 2 cc. of ether and treated with 3 cc. of acetic acid containing 67 mg. of bromine.[7] The brown color was discharged immediately and on cooling the solution in ice and salt there was obtained 90 mg. (48%) of a white crystalline compound: m.p. 106–109°; $[\alpha]^{27}D$ −101° (16.2 mg. made up to 1.96 cc. in chloroform, $\alpha^{27}D$ −0.82°, *l*, 1 dm.).

Anal. Calcd. for $C_{27}H_{45}Br$: C, 72.13; H, 10.09; Br, 17.78. Calcd. for $C_{27}H_{46}Br_2$: C, 61.13; H, 8.74; Br, 30.13. Found: C, 72.63, 72.93; H, 10.41, 10.37.

In cold alcoholic silver nitrate this bromide reacts rapidly to form a white precipitate, suggesting an allylic bromide structure. No way was found to purify the compound.

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(13) E. Kaiser and J. J. Svarz, THIS JOURNAL, **71**, 517 (1949).

A Substance with Rh Activity. A Correction

BY CHARLES C. PRICE AND GIANCARLO BERTI[1]

RECEIVED APRIL 24, 1952

In a previous communication,[2] the isolation of a crystalline substance, m.p. 157°, from blood lipids was reported. Many attempts to duplicate this work have been unsuccessful. Further investigation of the remaining 40 mg. of the material previously isolated has established its identity as Amytal (5-ethyl-5-isoamylbarbituric acid).[3] It was evidently incorporated in some of the early blood liquid samples by accidental contamination.

Reëxamination of the original material by sodium fusion, contrary to previous work, showed the presence of nitrogen. Combustion gave analytical figures in excellent agreement for Amytal.

Anal. Calcd. for $C_{11}H_{18}O_3N_2$: C, 58.39; H, 8.02; N, 12.38. Found: C, 58.50; H, 7.95; N, 12.15.

The melting point of the lipid material, an authentic sample of amytal and a mixture of the two, were all 156–157°.

A methylation product, m.p. 87–88.5°, obtained by Read[4] by treatment with alkali and methyl sulfate, is evidently N,N'-dimethyl ethylisoamylmalonamide.

Anal. Calcd. for $C_{12}H_{24}O_2N_2$: C, 63.12; H, 10.6. Found: C, 63.35; H, 10.9.

Veronal is reported to react in this way on alkaline methylation to yield N,N'-dimethyl diethylmalonamide.[5]

(1) Eli Lilly and Company Fellow, 1951–1952.

(2) C. C. Price, D. H. Read, T. J. Bardos and C. Chen, THIS JOURNAL, **70**, 3527 (1948).

(3) H. A. Shonle, *ibid.*, **45**, 243 (1923); M. M. Tiffeneau, *Bull. soc. chim.*, **33**, 183 (1923).

(4) D. H. Read, Ph.D. Dissertation, University of Notre Dame, 1949.

(5) R. Cohn, *Pharm. Z.*, **53**, 29 (1912).

DEPARTMENT OF CHEMISTRY
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Enzymatic Dephosphorylation of Casein

BY GERTRUDE E. PERLMANN

RECEIVED MARCH 26, 1952

Phosphoproteins such as casein have thus far been considered to be resistant toward the action of purified phosphatases from mammalian tissues.[1–3] Since casein, however, is a mixture of at least two proteins which differ in solubility, phosphorus content[4,5] and electrophoretic behavior[5,6] it seemed possible that differences also may exist in the action of phosphatases on the various fractions. It has now been found that one of these fractions, α-casein, is readily dephosphorylated in the *p*H range of 5.6 to 6.6 by prostate phosphatase with liberation of about 42% of the phosphorus. The enzyme has no effect on β-casein, whereas on prolonged exposure, to the enzyme, of "unfractionated" casein about 12% of phosphorus is set free.

The casein preparations used in these experiments are similar to those described by Warner[5]

(1) Rimington and Kay, *Biochem. J.*, **20**, 777 (1926).

(2) Schmidt and Thannhauser, *J. Biol. Chem.*, **149**, 369 (1943).

(3) Anagnostopoulos, Pacht, Bourland and Grabar, *Bull. soc. chim. Biol.*, **33**, 699 (1951).

(4) Linderstrøm-Lang, *Compt. rend. Lab. Carlsberg*, **17**, No. 9 (1929).

(5) Warner, THIS JOURNAL, **66**, 725 (1944).

(6) Mellander, *Biochem. Z.*, **300**, 240 (1939).

and were kindly provided by Dr. Thomas L. McMeekin of the Eastern Regional Laboratory. One-ml. samples containing 5 mg. of protein in veronal acetate buffer of *p*H 6.4 were incubated with 0.05 mg. of prostate phosphatase for 6 and 24 hours, respectively. Preliminary to the estimation of the inorganic phosphate that is released by the enzyme, one ml. of 20% trichloroacetic acid was added and the protein precipitate removed by centrifugation. The results with the three preparations are summarized in Table I.

TABLE I

ACTION OF PROSTATE PHOSPHATASE ON CASEIN FRACTIONS

Protein	Phosphorus content, %	Time of incubation at 37° in hours	Phosphorus released by enzyme, % of total phosphorus
"Unfractionated"	0.8	6	0
casein		24	12.5
α-Casein	1.0	6	24.0
		24	42.0
β-Casein	0.6	6	0
		24	0

During the dephosphorylation of α-casein the solubility of the protein decreases. Simultaneously several new components appear in the electrophoretic pattern, as is shown in Fig. 1. Here the full curve is the tracing of the pattern of α-casein whereas the dashed line is that of the protein after 20% of the phosphorus had been liberated.

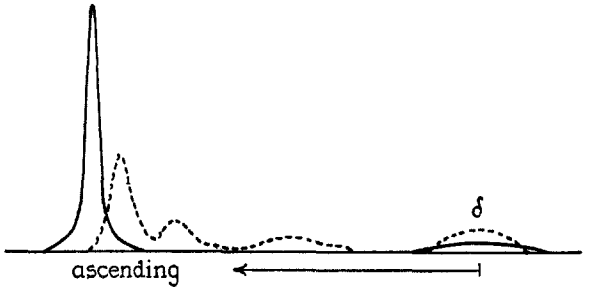

Fig. 1.—Superimposed tracings of electrophoretic patterns of α-casein, ——, and partially dephosphorylated α-casein, - - - - -. Patterns recorded after electrophoresis of 0.5% protein solutions in 0.1 ionic strength sodium phosphate buffer of *p*H 6.8 at a potential gradient of 6 volts per cm. for 8200 seconds.

In experiments in which α-casein and β-casein are remixed in different proportions, if the total concentration of the β-component exceeds 30%, the enzyme reaction is partially inhibited, the degree of inhibition being proportional to the concentration of the β-casein. From these results it emerges that the failure of previous investigators to dephosphorylate crude casein without a preceding transformation to phosphopeptone may be due to the inhibiting action of β-casein on the dephosphorylation of the α-form.

I wish to express my sincere thanks to Dr. Gerhard Schmidt of the Boston Dispensary for a generous sample of prostate phosphatase.

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NEW YORK, N. Y.

Reduction of N-Nitrosodiphenylamine to *unsym*-Diphenylhydrazine by Lithium Aluminum Hydride[1]

BY R. H. POIRIER AND F. BENINGTON

RECEIVED FEBRUARY 11, 1952

The reduction of nitrosodimethylamine to *unsym*-dimethylhydrazine by adding the nitrosamine to an excess of lithium aluminum hydride has recently been described by Schueler and Hanna.[2] Their attempt to apply this procedure to the preparation of *unsym*-diphenylhydrazine by the reduction of N-nitrosodiphenylamine yielded only diphenylamine. We have found, however, that by using equimolar quantities of the reactants, *unsym*-diphenylhydrazine is obtained in 73% yield, along with approximately 20% of diphenylamine. Moreover, the yield of hydrazine is increased to more than 90% by an "inverse" order of addition, that is, by adding a solution of lithium aluminum hydride to N-nitrosodiphenylamine. The course of reaction is best expressed by the equation

$$2Ph_2NNO + 2LiAlH_4 \longrightarrow (Ph_2NN)_2AlLi + LiAlO_2 + 2H_2$$
$$\downarrow 2H_2O$$
$$2Ph_2NNH_2 + LiAlO_2$$

Experimental

To 9.9 g. (0.05 mole) of N-nitrosodiphenylamine[3] in 50 ml. of dry ether at 10° was slowly added 57 ml. of a 0.97 molar solution of lithium aluminum hydride (0.055 mole) in ether. A precipitate, presumably $LiAlO_2$, appeared during the addition of the hydride to the nitrosamine. After standing at 10° for one hour, excess hydride and the product complex were decomposed by adding 25 ml. of wet ether followed by 100 ml. of a 30% solution of potassium sodium tartrate. The aqueous phase was separated and extracted with four 100-ml. portions of ether. Upon treating the combined ether solutions successively with water, brine solution, and ether previously equilibrated with concentrated hydrochloric acid, 10.85 g. of crude *unsym*-diphenylhydrazine hydrochloride precipitated. The crude product decomposed at 140–145°. Recrystallization from absolute ethanol gave 9.9 g. (90%) of silvery gray needles which began to decompose at 140°.[4]

Anal. Calcd. for $C_{12}H_{13}N_2Cl$: N, 12.7. Found: N, 12.2.

This product gave a mono-acetyl derivative which, after recrystallization from ethanol, melted at 188.5°,[5] and did not depress the melting point of an authentic sample.

Anal. Calcd. for $C_{14}H_{14}N_2O$: N, 12.4. Found: N, 11.9.

(1) This is a part of the research supported by the United States Air Force under Contract AF 33(038)-12656.

(2) F. W. Schueler and C. Hanna, THIS JOURNAL, **73**, 4996 (1951).

(3) S. Wexman, *Farm. Chilena*, **20**, 299 (1946).

(4) All decomposition and melting points are uncorrected.

(5) D. Vorländer and G. Bittins, *Ber.*, **68B**, 2269 (1935), reported 186° as the melting point for N-monoacetyldiphenylhydrazine, and 125° for N,N-diacetyldiphenylhydrazine.

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Infrared Spectrum of Cyclobutene. A Correction

BY JOHN D. ROBERTS AND C. W. SAUER

RECEIVED FEBRUARY 22, 1952

Reëxamination of the infrared spectra reported for cyclobutene[1] has revealed that the samples were heavily contaminated with carbon dioxide (strong absorption at 2350 $cm.^{-1}$). The infrared spectrum of carbon dioxide-free cyclobutene pre-

(1) J. D. Roberts and C. W. Sauer, THIS JOURNAL, **71**, 3925 (1949).

pared by the decomposition of cyclobutyldimethylamine oxide[1] is shown in Fig. 1.

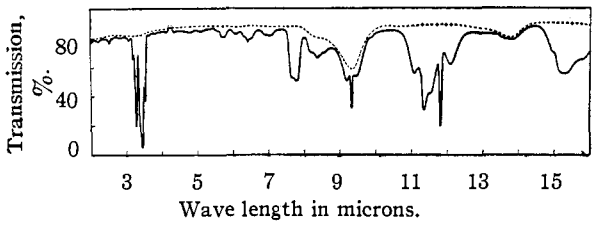

Fig. 1.—Infrared spectrum of cyclobutene at about 100 mm. pressure in 5-cm. cell with NaCl windows determined with Baird Spectrograph with NaCl prism. The solid line is the sample curve and the dashed line is the curve for the empty cell. The reference cell compartment contained a NaCl slab.

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The Structure of Methylenedihydrofuran

BY HAROLD L. RICE[1]

RECEIVED JANUARY 24, 1952

In the course of studies on the decomposition of hydrazones Kishner found[2] that furfural hydrazone half hydrate, when decomposed with heat in small portions over platinized clay plate and potassium hydroxide, gave, in addition to 2-methylfuran, a product which boiled at 78–80°. Two possible structures, I and II, were proposed for this material

I II

on the basis of its conversion by a trace of acid into methylfuran and by analogy to the isomerization of methylenecyclohexane to methylcyclohexene.[3] Structure I, in which the exo- and endocyclic double bonds are not conjugated, was preferred by Kishner because the molecular refraction was in better agreement, and because a normal condensation product with maleic anhydride was not formed.[4] Levulinic anhydride and pentene-2-ol-1-one-4 were formed when the methylenedihydrofuran was hydrated, and this suggested that the material could be a mixture of I and II.

The present spectral investigation has, on the other hand, led to the conclusion that structure II is more likely. Non-aromatic conjugation is indicated by the strength and position of the absorption maximum; λ_{max}, 239 mμ; ϵ_{max}, 6300 mμ.

Comparison of the position of this maximum with those of β-phellandrene (232 mμ), 7-methylenecholesterol (236 mμ), $\Delta^{4,6}$-cholestadienol (238 mμ), and 9-oxycholestadienol (248 mμ) would indicate that there is conjugation.[5] That this conjugation is not aromatic can be seen from Fig. 1

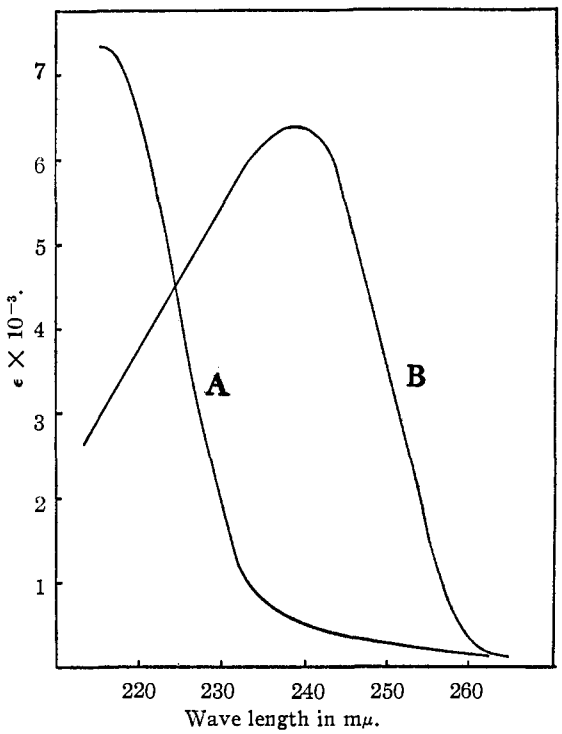

Fig. 1.—Absorption spectrum of 2-methylfuran (A) and 2-methylene-2,5-dihydrofuran (B) in absolute methanol.

wherein the maximum for methylfuran is at about 217 mμ The strength of the maximum in B is of the expected order of magnitude[6] when compared to those of vinylcyclohexene (8500) and cyclopentadiene (2,500).

The procedure which was reported by Kishner for the preparation of methylenedihydrofuran was followed closely here, but with somewhat variable results. The ratio of methylenedihydrofuran to methylfuran varied from 1.7 down to 0.43 in different preparations; ratios of about 1.0 were the most common. In order to improve the yield of methylenedihydrofuran and to decrease variability in the ratio of the isomers obtained, several different procedures were tried. The amount of hydrazone, of potassium hydroxide, and of platinized clay plate was changed independently with either no improvement in the constancy of the ratio of isomers or adverse effect on the yield. The time for each decomposition also was varied, again with no constant effect. The best yield of the unstable isomer was 29%, which was comparable to that reported by Kishner[2] (32%).

Experimental

Furfural Hydrazone Half Hydrate.—This compound was prepared in 89% yield by the method of Kishner.[2]

2-Methylene-2,5-dihydrofuran.—Furfural hydrazone half hydrate (530 g.) was added in 10–15-ml. portions to a mixture of 1.5 g. of potassium hydroxide pellets and 1.5 g. of platinized clay chips.[7] After each portion was added, the mixture was heated gently until the decomposition became self-sustaining. Because reaction was vigorous, a long and

(1) du Pont Post-doctoral Fellow, University of Illinois, 1949. Present address, E. I. du Pont de Nemours & Co., Inc., Electrochemicals Department, Niagara Falls, New York.

(2) N. Kishner, *J. Gen. Chem.* (*U.S.S.R.*), **1**, 1212 (1931).

(3) A. Favorskii and I. Borgmann, *Ber.*, **40**, 4871 (1907).

(4) N. Kishner, *J. Gen. Chem.* (*U.S.S.R.*), **3**, 198 (1933).

(5) R. B. Woodward, THIS JOURNAL, **64**, 72 (1942).

(6) E. A. Braude, *Ann. Reports, Chem. Soc.*, 111 (1946).

(7) In the preparation of the catalyst 15 g. of clay chips was soaked in 15 ml. of 5% chloroplatinic acid, the water evaporated on a steam-bath and the material was reduced in a stream of hydrogen. The temperature was raised to 150° over a two-hour period. After cooling the system, the hydrogen was displaced by carbon dioxide and the chips were stored, for later use, under an atmosphere of carbon dioxide.

efficient condenser was required to condense the vapors; those vapors which were not trapped in an ice-cooled collection flask were caught in a Dry Ice trap at the end of the system.

The products of decomposition were washed with three 50-ml. portions of water and then steam distilled. The distillate was washed with three 50-ml. portions of water and the distillate dried with anhydrous potassium carbonate. The yield was 207 g. (56.6%). The mixture was subjected to fractional distillation at 690 mm.; results are noted below:

Fraction	Boiling range, °C.	Weight, g.	$n^{25}D$
1	57 -63	13.1	1.4328
2	63 -65.5	47.9	1.4342
3	65.5-68.5	25.2	1.4369
4	68.5-71.5	10.4	1.4415
5	71.5-75	17.0	1.4468
6	75 -77	81.4	1.4526

Redistillation of 35 g. of fraction six from barium oxide yielded 16.2 g. (46%) of material; b.p. 76.5–77°; $n^{24}D$ 1.4538; d^{25}_4 0.9316. Kishner[2] reported: b.p. 78.5–79°; $n^{18}D$ 1.457; d^{13}_4 0.9406.

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PULLMAN, WASHINGTON

3,3-Dialkylpiperidines

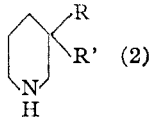
BY R. C. SCHREYER

RECEIVED DECEMBER 17, 1951

Several methods[1] for the synthesis of piperidines by ring closure at the nitrogen atom have been described. However, preparation by reductive amination of 4-cyanobutyraldehydes has not been previously reported. It has now been found in this Laboratory that 3,3-dialkylpiperidines can be synthesized from 2,2-dialkyl-4-cyanobutyraldehydes *via* the two-step process

$$RR'CHCHO + CH_2 = CHCN \longrightarrow RR'C(CHO)CH_2CH_2CN \quad (1)$$

$$RR'C(CHO)CH_2CH_2CN + H_2 + NH_3 \longrightarrow$$



(2)

Isobutyraldehyde, 2-methylbutyraldehyde and cyclohexanecarboxaldehyde were employed as the aldehydic components. Cyanoethylation of isobutyraldehyde[2,3,4] has been disclosed in the patent literature. The reaction of acrylonitrile with cyclohexanecarboxaldehyde was not exothermic in contrast to the other aldehydes used.

A novel spirane derivative, 2-azaspiro(5.5)hendecane, was obtained from 1-(2′-cyanoethyl)-cyclohexanecarboxaldehyde.

Experimental

2-Ethyl-2-methyl-4-cyanobutyraldehyde.—A charge of 86 g. of 2-methylbutyraldehyde and 159 g. of acrylonitrile was added slowly to 5 g. of a 50% sodium hydroxide solution over a one-hour period. The mixture was stirred throughout the addition and the temperature was maintained at 35–50° by external cooling. The solution was neutralized with 25% sulfuric acid and the oil layer distilled under vacuum to give 43 g., b.p. 66–70° (0.3–0.35 mm.), $n^{25}D$ 1.4441. *Anal.* Calcd. for $C_8H_{13}NO$: C, 69.06; H, 9.35; N, 10.07. Found: C, 68.78; H, 9.52; N, 9.99.

1-(2′-Cyanoethyl)-cyclohexanecarboxaldehyde.—A mixture of 106 g. of acrylonitrile, 102 g. of cyclohexanecarboxaldehyde and 2 g. of 50% sodium hydroxide solution was refluxed 30 minutes. The solution was cooled and 250 cc. of ether added. The ether solution was washed with 50 cc. of 10% hydrochloric acid, 50 cc. of 5% sodium bicarbonate solution and 50 cc. of water, and then distilled directly under vacuum to give 51 g., b.p. 103–105° (1 mm.), $n^{26}D$ 1.4750. *Anal.* Calcd. for $C_{10}H_{15}NO$: C, 72.72; H, 9.09; N, 8.48. Found: C, 72.56; H, 9.13; N, 8.54.

3,3-Dimethylpiperidine.[5]—A mixture of 55 g. of 2,2-dimethyl-4-cyanobutyraldehyde (b.p. 59–60° (0.6 mm.), $n^{23}D$ 1.4355) 119 g. of ammonia and 15 g. of Raney nickel was hydrogenated at 100–110° and 700 atmospheres pressure for one hour in a stainless steel shaker tube. The product was filtered to remove the catalyst and distilled under vacuum to give 12.5 g., b.p. 45–46° (33 mm.), $n^{25}D$ 1.4470. *Anal.* Calcd. for $C_7H_{15}N$: C, 74.34; H, 13.27; N, 12.39; neut. equiv., 113. Found: C, 74.58; H, 13.11; N, 11.91; neut. equiv., 115.

3-Ethyl-3-methylpiperidine.—A mixture of 50 g. of 2-ethyl-2-methyl-4-cyanobutyraldehyde, 102 g. of ammonia and 15 g. of Raney nickel was hydrogenated at 100–110° and 700 atmospheres for one hour in a silver shaker tube. The product was filtered to remove the catalyst and vacuum distilled to give 9 g., b.p. 67–69° (25 mm.), $n^{25}D$ 1.4565. *Anal.* Calcd. for $C_8H_{17}N$: neut. equiv., 127; N, 11.02. Found: neut. equiv., 126; N, 11.13.

2-Azaspiro(5.5)hendecane.—A charge of 50 g. of 1-(2′-cyanoethyl)-cyclohexanecarboxaldehyde, 119 g. of ammonia and 15 g. of Raney nickel was hydrogenated at 120–129° and 700 atmospheres pressure for one hour in a stainless steel shaker tube. The product was filtered to remove the catalyst and distilled under vacuum to give 17 g., b.p. 91–93° (10 mm.), $n^{25}D$ 1.4891. *Anal.* Calcd. for $C_{10}H_{19}N$: C, 78.43; H, 12.42; N, 9.15; neut. equiv., 153. Found: C, 78.15; H, 12.46; N, 9.21; neut. equiv., 149.

(1) R. C. Elderfield, Editor, "Heterocyclic Compounds," Vol. I, John Wiley and Sons, Inc., New York, N. Y., 1950, pp. 642–655.

(2) H. A. Bruson and T. W. Reiner, U. S. Patent 2,353,687.

(3) J. F. Walker, U. S. Patent 2,409,086.

(4) I. G. Farbenindustrie A. G., French Patent 886,846.

(5) Dunlop, *J. Chem. Soc.*, **107**, 1112 (1915). has prepared 3,3-dimethylpiperidine by the reduction of 2,2-dimethylglutarimide with sodium and amyl alcohol.

E. I. DU PONT DE NEMOURS & CO.
POLYCHEMICALS DEPARTMENT
EXPERIMENTAL STATION
WILMINGTON, DELAWARE

Carbon Dioxide Production in the Browning Reaction[1]

BY F. H. STADTMAN, C. O. CHICHESTER AND G. MACKINNEY

RECEIVED NOVEMBER 26, 1952

Non-enzymatic browning reactions in certain natural systems and interactions of sugars and nitrogenous compounds have been thoroughly reviewed, the former by Stadtman in 1948,[2a] the latter by Danehy and Pigman[2b] in 1950. Many types of compounds can be involved. Haas and Stadtman[3] for example showed that brown pigments could be formed by combining and heating any two of the three fractions (anion, cation and neutral) that were obtained from apricot sirups by ion-exchange treatment. It is frequently assumed, however, in natural systems where both amino acids and carbohydrates are present, that

(1) Presented at the XII International Chemical Congress, New York, 1951.

(2) (a) E. R. Stadtman, *Advances in Food Research*, **1**, 325 (1948). (b) J. P. Danehy and W. W. Pigman, *ibid.*, **3**, 241 (1951).

(3) V. A. Haas and E. R. Stadtman, *Ind. Eng. Chem.*, **41**, 983 (1949).

browning is limited to interactions between these two classes of compounds. The reaction between amino acids and reducing sugars was first studied by Maillard.[4] His work has been responsible for the direction of much of the subsequent thinking. Maillard observed that when an amino acid and a reducing sugar such as glucose were heated together, considerable quantities of carbon dioxide were evolved, and at the same time brown-colored substances were formed.

He deduced that the carbon dioxide was produced by decarboxylation of the amino acid. When he compared the weight of the dried reaction mixture with the original dry weight of the reactants, he found a weight loss not accounted for as carbon dioxide. He concluded that this loss was due to water formed as a result of condensations. A weight balance on this basis indicated that twelve mols of water were formed for each mol of amino acid decarboxylated. Thus a maximum of twelve mols of sugar might be involved for each mol of carbon dioxide. These conclusions neglect the possibilities of losses due to escape of other volatile fragments and of carbon dioxide from sources other than the amino acid carboxyl.

Recent browning studies[5] here have utilized C^{14}-labeled sugars. Glucose and fructose were incorporated into apricot sirups as radioactive tracers. These sirups were then browned at 55°. Carbon dioxide formed during the reaction was trapped as barium carbonate and its specific activity determined. It was found that appreciable quantities of carbon from sugar sources were present in the carbon dioxide evolved (Fig. 1). Consequently, the possibility of carbon dioxide formed from sugar sources in the Maillard reaction cannot be disregarded. A simple glucose–glycine system was therefore studied by use of radioactive tracer techniques to determine whether some carbon dioxide also comes from the sugar. This should ultimately permit evaluation of Maillard reaction CO_2 in natural systems.

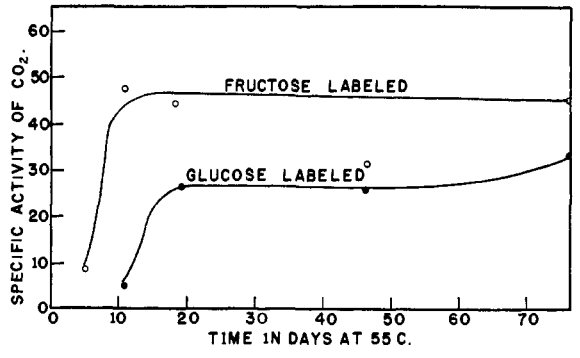


Fig. 1.—Specific activities of carbon dioxide from fructose- and glucose-labeled apricot sirups, as a function of time.

Experimental

Glucose–glycine browning reactions were carried out with the same concentrations of reactants as those used in Maillard's original experiment. The reaction was studied at two temperatures; at 100° to approximate the reflux temperatures used by Maillard, and at 56.5°, the temperature of a boiling acetone-bath, to duplicate reaction temperatures used by us in apricot browning studies. To check possible participation of both glucose and glycine as sources of carbon dioxide production, two experiments were run at each temperature. In one, uniformly labeled glucose-C^{14} reacted with unlabeled glycine, and in the other, carboxyl-labeled glycine reacted with unlabeled glucose. The pigment concentration as measured at 440 mμ in an Evelyn colorimeter, the quantity and specific activity of carbon dioxide produced, and the *p*H of the reaction mixtures were determined as a function of time. Reactions at 100° were followed for two hours, approximately the time used in Maillard's experiments, while the reactions at 56.5° were continued for over 450 hours, for comparison with long-time reactions in browning of apricot sirups.

For the tracer experiments, the reaction chamber (3 ml. in volume) was immersed in a bath of water or acetone according to the temperature desired, under independent reflux. A weighed amount of reactant mixture (*ca.* 2 ml. in volume) was inserted in the reaction chamber and maintained under its own reflux to prevent loss of water. The CO_2 produced was allowed to diffuse from the solution into a stream of nitrogen (CO_2-free, humidified) and swept into a sodium hydroxide trap. All-glass connections were used, and the apparatus was tested for leaks.

For measurement of total CO_2 and pigment, a similar train was used with a larger reaction chamber (25 ml.) and an exact weight (*ca.* 20 ml. in volume) of reactants introduced.

The reagents were anhydrous C.P. glucose, C.P. glycine and CO_2-free water. The weight ratios for the three were 4:1:6, representing a 2.4 to 1 mol ratio for glucose to glycine.

Results.—During the course of the reaction, the *p*H of the reaction mixture decreased from *p*H 4.83 to 4.15. This acid condition of the reaction mixture is not unlike that existing in browning fruit products and some comparisons should be possible. However, one should be cautious when comparing these results with those obtained at much lower or higher *p*H levels.

The amount of pigment produced as a function of time is shown in Fig. 2. The shape of the curve is the same for the reaction at 100 and at 56.5°. The data for both curves may be represented by third degree polynomials. Browning proceeds at a much slower rate at the lower temperature, 250 hours being required to produce the same degree of browning as is reached at the higher temperature in two hours.

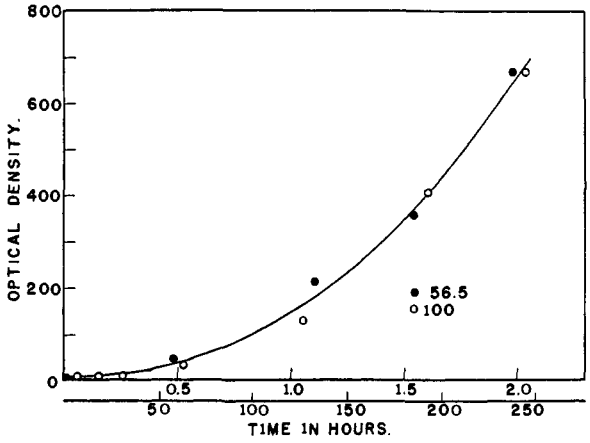


Fig. 2.—Pigment production as a function of time: optical density (calculated per gram solids) as a measure of pigment concentration *vs.* upper scale, time at 100°, lower scale, time at 56.5°.

The curve for CO_2 production is of a similar form, but when the amount of CO_2 produced per gram of

(4) L. C. Maillard, *Compt. rend.*, **154**, 66 (1912); **155**, 1554 (1912).
(5) Unpublished data.

solids is plotted against the optical density at 440 mμ (Fig. 3) it is clear that far more carbon dioxide is produced per unit optical density at 100° than at 56.5°. The carbon dioxide-producing reaction therefore may not follow the same course at the two different temperatures, though the color-producing parts of the two reactions appear to be similar. This suggests the carbon dioxide liberation in amino acid–sugar reactions may not be essential for color production, which is substantiated by the fact that brown pigments retained after dialysis[6] contain less carboxyl carbon when browning occurs at 100° than when it takes place at 56.5°. It is significant that considerable amounts of carboxyl carbon from glycine become associated with the brown pigment produced.[5] CO_2 liberation may therefore take place subsequent to pigment formation.

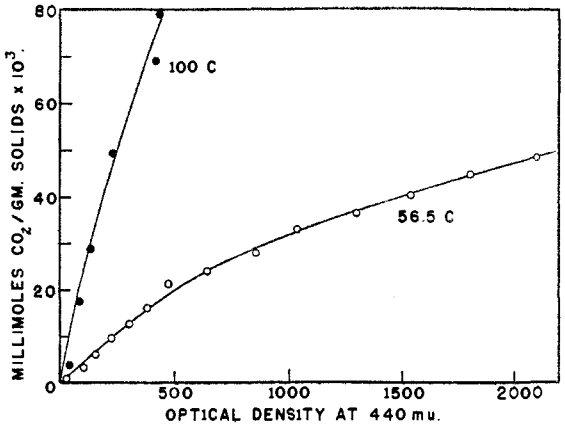


Fig. 3.—Carbon dioxide production in relation to pigment production at the two temperatures.

The results obtained with C^{14}-labeled glucose show that some CO_2 is derived from the sugar as well as the amino acid, Fig. 4. The major portion,

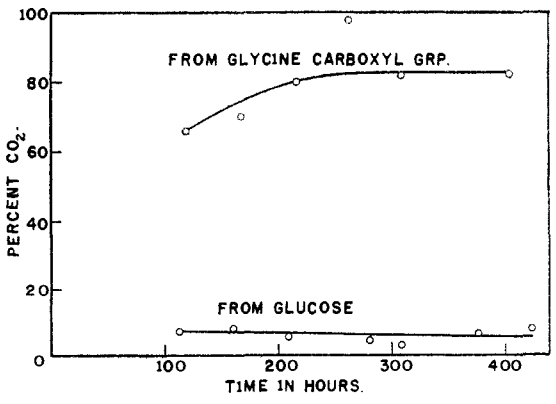


Fig. 4.—The percentages of carbon dioxide derived from glucose and glycine as a function of time.

well over 80%, is derived from the carboxyl group of the amino acid, as Maillard suggested, and somewhat less than 10% comes from the sugar. The rather unlikely possibility that the methyl carbon of the glycine contributes to the CO_2 will be checked, and the fate of the amino acid residue will be determined, through the use of methyl-labeled glycine.

(6) C. O. Chichester, F. H. Stadtman and G. Markinney, THIS JOURNAL, **74**, in press (1952).

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N-Methyl-6,7,8-trimethoxy-1,2,3,4-tetrahydroisoquinoline

BY E. P. TAYLOR

RECEIVED MARCH 11, 1952

In an attempt to synthesize trichocereine, *i.e.*, N-dimethylmescaline, by methylation of mescaline with formic acid–formaldehyde, Reti and Castrillón[1] found that the product was a tetrahydroisoquinoline base. This has now been identified by Castrillón[2] as N-methyl-6,7,8-trimethoxy-1,2,3,4-tetrahydroisoquinoline. Oxidation of the hydrochloride to the known N-methyl-3,4,5-trimethoxyphthalimide confirms the structure of the base, which is claimed as a new compound. May I point out that this substance has already been prepared and described[3]: its constitution has been established by a Bischler–Napieralski reaction upon N-formylmescaline, followed by reduction of the methiodide of the resultant 6,7,8-trimethoxy-3,4-dihydroisoquinoline. In addition, reduction of the ethiodide of this base yielded the corresponding N-ethyl-1,2,3,4-tetrahydroisoquinoline.

(1) L. Reti and J. A. Castrillón, THIS JOURNAL, **73**, 1767 (1951).
(2) J. A. Castrillón, *ibid.*, **74**, 558 (1952).
(3) E. P. Taylor, *J. Chem. Soc.*, 1153 (1951).

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The Reaction of Silyl Bromide with Magnesium

BY E. R. VAN ARTSDALEN[1] AND JEROME GAVIS

RECEIVED FEBRUARY 5, 1952

The preparation of compounds of the type SiH_3MgX, analogous to organic Grignard reagents, should offer many interesting new possibilities for syntheses of compounds containing the silyl group. Emeleus, Maddock and Reid[2] in conjunction with their studies on the synthesis and properties of silyl iodide, SiH_3I, describe a reaction between SiH_3I and magnesium in diisoamyl ether. The reaction yielded hydrogen, silane and silicon but no stable addition compound. Complete reaction took place. These results were attributed to the formation of an unstable Grignard-type compound.

This note describes an attempt to prepare a Grignard compound using silyl bromide. It was hoped that use of the bromide would impart greater stability to the desired product, which was then to be used in synthesis of other silyl compounds.

We were unable to obtain any stable Grignard compound, nor did any easy reaction of the type found by Emeleus, Maddock and Reid take place. Indeed, no reaction occurred between Mg and

(1) Chemistry Division, Oak Ridge National Laboratory, Oak Ridge, Tennessee.
(2) H. J. Emeleus, A. G. Maddock and C. Reid, *Nature*, **144**, 328 (1939); *J. Chem. Soc.*, 353 (1941).

SiH_3Br beyond the evolution of small amounts of silane and a non-condensable gas, presumably hydrogen, and the deposition of a small amount of silicon. Eighty-five to 90% of the SiH_3Br could be recovered after the reaction, while the amounts of SiH_4 produced varied between 4 and 6% of the SiH_3Br originally present in the reaction mixture. These results are consistent with the interpretation that between 8 and 12% of the original amount of SiH_3Br reacted with Mg according to the over-all equation

$$2SiH_3Br + Mg \longrightarrow SiH_4 + H_2 + Si + MgBr_2$$

Thus our work, as well as that of Emeleus, Maddock and Reid,[2] suggests that reaction between silyl radicals leads to drastic disproportionation rather than recombination. Presumably this means that the free energy of formation of SiH_4 (−9.4 kcal./mole[3]) is more favorable than that of Si_2H_6.

A possible explanation for the great difference between the bromide and iodide in their ease of reaction with magnesium may be had by a consideration of bond strengths (dissociation energies). In carbon systems Grignard reagents are in general more difficult to prepare with chlorides than with the corresponding bromides which latter have lower bond strengths. While data are not available from which to compute the silicon–halogen bond strengths in SiH_3Br and SiH_3I, one can estimate from data tabulated by the Bureau of Standards[3] that the average Si–Br bond strength in $SiBr_4$ is 70.2 kcal. while the corresponding Si–I bond strength is 51.4 kcal., a difference of 19 kcal. Although these values are not directly applicable, we would certainly predict on their basis a much lessened reactivity in the case of SiH_3Br compared with SiH_3I.

Experimental

All experimental work was carried out in a high vacuum apparatus, using glass stopcocks greased with Apiezon N which was stable toward SiH_3Br at moderately low pressures.

Preparation of Silane.—Although the greater part of the SiH_4 used was supplied through the courtesy of the General Electric Company, supplementary amounts were prepared by the method of Finholt, Bond, Wilzbach and Schlesinger[4] which employs reduction of $SiCl_4$ by $LiAlH_4$ in ethyl ether solution. Purity was controlled by checking vapor pressures at two different temperatures and comparing with the values given by Stokland.[5]

Preparation of SiH_3Br.—The method used was essentially that of Stock and Somieski.[6] Silane was heated for two hours at 100–110° with a 15% excess of dry HBr in the presence of $AlBr_3$ in a previously evacuated flask immersed in an oil-bath. Purification was accomplished, after removal of non-condensable gases, by a distillation at −126° to remove unreacted SiH_4 and then several distillations at −96° to separate the SiH_3Br from higher bromides. When the vapor pressures checked at two temperatures with those reported by Stock and Somieski,[6] the SiH_3Br was considered pure.

Since SiH_3Br tends to disproportionate readily, it was kept at Dry Ice temperatures till needed, closed off by a stopcock. There was no detectable deterioration for periods of several weeks as verified by vapor pressure measurements.

Reaction of SiH_3Br with Mg.—A small shaving of Mg, burnished with a knife and washed with ether, was placed in a 0.6 × 5 cm. tube which was sealed into the vacuum apparatus, evacuated, and well flamed to remove surface adsorbed water. C.P. dibutyl ether was distilled over sodium into the tube. The SiH_3Br was then distilled into the tube and frozen with liquid nitrogen. The ratio of bromide to ether was varied in the different runs. The amounts of SiH_3Br varied from about 0.0005 mole (0.055 g.) to about 0.001 mole (0.11 g.) in quantities of ether ranging from about 0.1 to 0.5 ml. No correlation was evident between the ratio of halide to ether and extent of reaction.

The mixture was allowed to warm to room temperature, whereupon the SiH_3Br dissolved in the ether to form a clear solution. Loss of volatile SiH_3Br to the vapor state was minimized by using short lengths of small bore tubing, and closing the reaction tube with a stopcock. Gradually, over a period of 3 or 4 hours a dark deposit began to form on the Mg. Except for a small amount of bubbling, which could have been caused by vaporization into the vacuum above, there was no other evidence of reaction. In a few trials the mixture was allowed to stand overnight, but no further reaction could be observed.

To separate the products, the mixture was frozen with liquid nitrogen, and the non-condensable gas (H_2) removed. Distillation at −126° removed SiH_4, and the unreacted SiH_3Br was recovered by distilling at −63° and later purified by redistillation at −96°. There was no evidence of disilane. Four to 6% of SiH_4 was found on the basis of the original SiH_3Br and 85–90% of the SiH_3Br was recovered.

Acknowledgment.—We are indebted to Dr. A. E. Newkirk, of the General Electric Company, Schenectady, New York, for his advice and for generously providing the silane with which most of this investigation was carried out.

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(3) Natl. Bur. Standards, "Selected Values of Chemical Thermodynamic Properties," Series I, 1949.

(4) A. E. Finholt, A. C. Bond, K. E. Wilzbach and H. I. Schlesinger, THIS JOURNAL, **69**, 2692 (1947).

(5) K. Stokland, *Kgl. Norske Videnskab. Selskabs, Forh.*, **12**, 122 (1939).

(6) A. Stock and C. Somieski, *Ber.*, **50**, 1739 (1917).

Fluorene Analog of Trypan Blue[1] (2,7-Bis-(8′-amino-3′,6′-disulfo-1′-hydroxy-2′naphthylazo)-fluorene)

BY SIEGFRIED WOISLAWSKI

RECEIVED NOVEMBER 5, 1951

In continuation of previous work[2] on compounds useful in cancer research, 2,7-diaminofluorene dihydrochloride has been tetrazotized coupled with H-acid to form an analog of Trypan Blue. Novelli[3] reported the preparation of this compound but gave no details of yield or purity. When prepared by his method the compound was not homogenous. Both Trypan Blue and the fluorene analog have an absorption maximum at 590 mμ, Fig. 1.

Experimental

To a solution of 5.38 g. (0.02 mole) of 2,7-diaminofluorene dihydrochloride in 100 ml. of water was added 4.5 ml. (0.06 mole) of concentrated hydrochloric acid (sp. gr. 1.18) and 50 g. of ice. After cooling to 2°, agitation was started and 90% of 2.8 g. (0.04 mole) of sodium nitrite (95%) in 40 ml. of water was added rapidly, and the balance dropwise until a distinct test for nitrous acid was obtained. This excess was maintained for 0.5 hour. Throughout the tetrazotization tests were made for an excess of acid by means of congo red paper. When the tetrazotization was completed, excess nitrous acid was eliminated by adding 0.3 g. of urea. The brown tetrazonium chloride may form an emulsion that can easily be broken up by adding ice or a few drops of capryl alcohol. The temperature during the tetrazotization was kept at 0–5°.

(1) Supported by Grant C-1356 from the National Cancer Institute of the National Institutes of Health, U. S. Public Health Service.

(2) H. S. Block and F. E. Ray, *J. Nat. Cancer Inst.*, **7**, 61 (1946).

(3) A. Novelli, *Anales Soc. espan. fis. quim.*, **28**, 362 (1930).

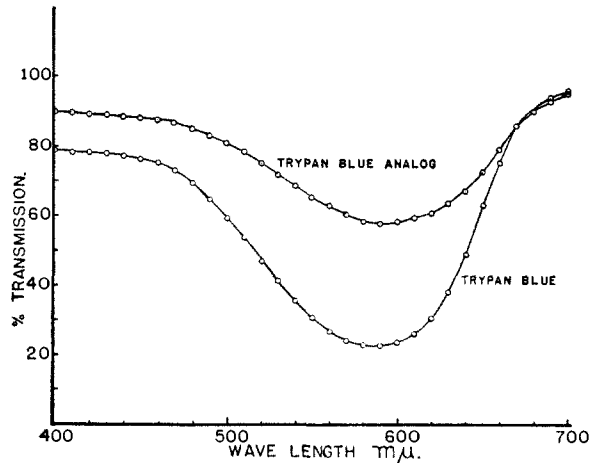

Fig. 1.

Since technical H-acid contains an impurity that forms a red dye with the tetrazonium chloride, it was purified by dissolving 27 g. of H-acid in 200 ml. of hot water and filtering. After reheating to 70°, it was salted out by the addition of 60 g. of sodium chloride. This procedure was repeated twice and the final product dried in a vacuum desiccator. This purification reduced the amount of the impurity but did not eliminate it entirely. It is therefore possible that it might be caused by mono-coupling.

Then 13.72 g. (0.04 mole) of H-acid was dissolved in 100 ml. of water containing 0.72 g. of sodium hydroxide (filtering if necessary). The solution was thus left faintly acid until just before coupling. This solution was cooled to 18°, 3.4 g. of sodium bicarbonate was added, and the tetrazonium chloride was run rather rapidly into the vigorously stirred alkaline solution of H-acid. It is important that the coupling mixture be kept alkaline, adding more sodium bicarbonate if necessary. Agitation was continued for 2 hours to ensure complete coupling. Tests were done for excess tetrazonium chloride and H-acid. The mixture was heated to 85°, 5 g. of decolorizing carbon was added, and the solution stirred for 15 minutes and filtered. The filtrate was reheated to 85° with agitation, and 27 g. of hydrated sodium acetate for each 100 ml. of solution was added slowly in four or five portions. The mixture, while still warm, was centrifuged, and the supernatant decanted. This procedure was repeated twice, and although it makes the filtration easier, it still was not possible to remove the red dye completely. For further purification, the dye was refluxed four times with 300-ml. portions of 95% ethyl alcohol which removes both the red dye and sodium acetate. To test for sodium acetate, a few drops of concentrated sulfuric acid were added to 10 ml. of filtrate until no turbidity was formed on cooling with ice. The absence of red dye was proven by the capillary test and by chromatography, using equal amounts of Hyflo-Super-cel and adsorptive magnesia. The dye was thus free of organic impurities; yield 42%.

Solubilities of the Dye.—Soluble in water, methyl alcohol, glacial acetic acid and methyl cellosolve; difficultly soluble in ethyl alcohol; insoluble in acetone, benzene, chloroform, dioxane, ether and petroleum ether.

Anal. Calcd. for $C_{33}H_{20}N_6O_{14}S_4Na_4$: N, 8.9; S, 13.6. Found: N, 8.9; S, 13.2.

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COMMUNICATIONS TO THE EDITOR

GERMBUDINE, ISOGERMIDINE AND VERATETRINE THREE NEW HYPOTENSIVE ALKALOIDS FROM VERATRUM VIRIDE

Sir:

Recent studies[1,2] have disclosed the isolation of the hypotensive ester alkaloids germitrine, neogermitrine and germidine from *Veratrum viride*. Fried, White and Wintersteiner have also shown that the triester germitrine can be partially hydrolyzed to the hypotensive diester germerine.

We wish to report that present investigations in our laboratories, on the alkaloids extractable from the ground roots and rhizomes of commercial *Veratrum viride*, have yielded germerine together with three new, highly potent, ester alkaloids for which we propose the names germbudine, isogermidine and veratetrine.

The benzene-extractable alkaloids, obtained by the procedure of Jacobs and Craig,[3] were separated into a crystalline non-ester alkaloid fraction and an amorphous fraction which contained the bulk of the hypotensive activity. This amorphous material was subjected to a 24-plate Craig counter-current distribution between benzene and 2 *M* acetate buffer at *p*H 5.5. The known triester neogermitrine was obtained by crystallizing the material in tubes 12–20 from acetone. Crystallization of the material in tubes 4–11 from benzene gave germerine (m.p. 203–205°; $[\alpha]^{26}D$ − 14.2° (*c*, 1 in pyridine), +6° (*c*, 1 in chloroform); the sample was identified further by comparison of its infrared spectrum, and by mixed melting point with authentic germerine kindly provided by Dr. J. Fried). In a personal communication, the latter disclosed that Dr. D. R. Walters of Squibb and Sons has also isolated germerine from *Veratrum viride*.

The alkaloids in tubes 0–3 were given a 72-plate Craig distribution between benzene and 2 *M* acetate at *p*H 6.5 and three fractions from this distribution, A, B and C, were crystallized from benzene to give three new alkaloids.

Fraction A (tubes 1–3), yielded germbudine (m.p. 158–160°, $[\alpha]^{27}D$ − 8.4° (*c*, 1 in pyridine), + 10.7° (*c*, 1 in chloroform)). Analytical data indicate the empirical formula $C_{37}H_{59}O_{13}N$ (calcd. C, 61.2; H, 8.20; eq. wt., 726; found: C, 61.0; H, 8.21; eq. wt., 732). Volatile acid determination, found: 0.91 equivalent of acid. Alkaline hydrolysis of germbudine afforded the alkamine germine and an acid fraction. The acids were converted to their *p*-phenylphenacyl esters which were separated chromatographically into the ester of α-meth-

(1) J. Fried, H. L. White and O. Wintersteiner, THIS JOURNAL, **71**, 3260 (1949); **72**, 4621–4630 (1950).

(2) J. Fried and P. Numerof, Abst. 119th Meeting A.C.S., Cleveland, Ohio, April, 1951.

(3) W. A. Jacobs and L. C. Craig, *J. Biol. Chem.*, **160**, 555 (1945).