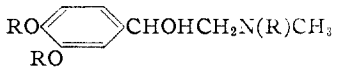
derivative, melting, when pure, at 94–95°. Its neutral nature and its failure to give a color reaction



Ia, R = H
Ib, R = CH_3CO

in the presence of ferric chloride demonstrate that acylation of the amino nitrogen and phenolic hydroxyl groups has occurred, and permit the identification of the product as O^3,O^4,N-triacetyl-(−)-epinephrine (Ib).[2] Its ultraviolet[3] and infrared[4] absorption spectra are shown in Figs. 1 and 2, respectively.

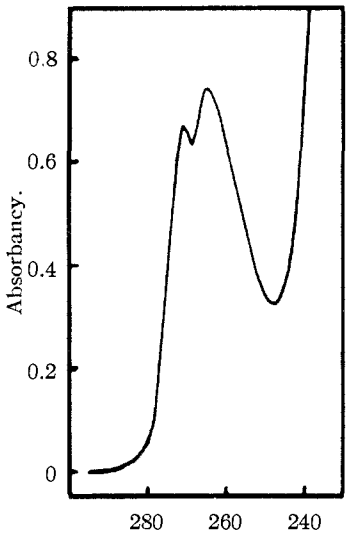


Fig. 1.—Ultraviolet absorption spectrum of triacetylepinephrine taken in 95% ethanolic solution (400 mg./l.) with a model 11 Carey spectrophotometer (1-cm. cell): ϵ_{271} 518, ϵ_{254} 575.

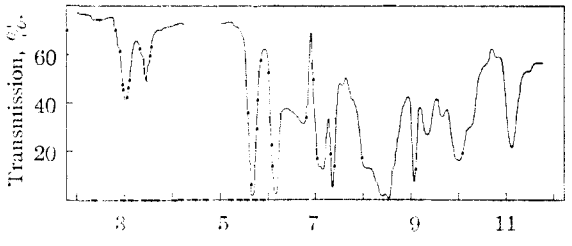


Fig. 2.—Infrared absorption spectrum of triacetylepinephrine taken in carbon tetrachloride (supersaturated 1% solu.) with a model 21 Perkin–Elmer recording spectrophotometer (1-mm. cell).

In contrast to Ia, Ib is quite stable, and easily soluble in chloroform. These characteristics of the substance, and the ease with which it is formed, suggest that the acetylation reaction may be employed to advantage in the isolation, identification and estimation of epinephrine.

Resistance of the alcoholic hydroxyl group of ephedrine and epinephrine toward acetylation under the experimental conditions appears noteworthy. In the presence of as much as 15 to 30 molecular proportions of anhydride, this functional group remains virtually, if not literally, intact while the amino and phenolic functions are quantitatively acetylated.

(2) The racemic substance, along with other products, including the tetracetyl derivative, results from the action of acetyl chloride on racemic epinephrine: see H. Bretschneider, *Monatsh.*, **76**, 355 (1947).

(3) Determination by Lee S. Harrow, Division of Cosmetics, Food and Drug Administration.

(4) Determination by Jonas Carol, Division of Pharmaceutical Chemistry, Food and Drug Administration.

Experimental

Finely powdered (−)-epinephrine[5] (2.00 g., 0.0109 mole) was added to a mixture of 60 g. of sodium bicarbonate and 200 cc. of water in a 1-l. erlenmeyer flask. While the system was vigorously stirred by a motor-driven wire stirrer, 30 cc. (0.32 mole) of reagent grade acetic anhydride was added cautiously in 4 equal portions. Violent foaming was controlled by the addition of a few drops of ether. When the evolution of carbon dioxide had ceased after the last addition of anhydride, the mixture was allowed to stand about five minutes then extracted with six 100-cc. portions of chloroform. The filtered extracts were concentrated on the steam-bath to an oil which was induced to crystallize by adding ether and triturating. The yield of almost white product was quantitative (3.35 g.); m.p. 91–93° (cor.). It was recrystallized with 90% recovery by dissolving it in acetone (1.3 cc./g.) and adding 7 volumes of ether. Thrice-recrystallized material had m.p. 94–95° (cor.), $[\alpha]^{21}D$ −94.7° (U.S.P. $CHCl_3$, *c* 1.01).

Anal. Calcd. for $C_{15}H_{19}NO_6$: C, 58.24; H, 6.19; N, 4.53. Found: C, 58.17, 58.28; H, 6.10, 6.08; N (Kjeldahl),[6] 4.45, 4.53.

From acetone–ether, the substance crystallizes as platy or tabular prisms: *refractive indices*, α = 1.516, β = 1.527, γ = 1.572 (all ± 0.002); *optic sign*, biaxial positive; *optic angle*, $2V$ = 52° (from microscopic observation of interference figure and calculated from refractive indices); *extinction*, both parallel and inclined; *system*, probably monoclinic.[7]

(5) Prepared from Winthrop-Stearns synthetic (−)-epinephrine bitartrate by precipitation with ammonia: $[\alpha]^{24}D$ −51.1° (*c* 2 in 0.5 *N* hydrochloric acid).

(6) Determinations by Charles Graichen, Division of Cosmetics, Food and Drug Administration.

(7) Optical crystallographic properties determined by Wm. V. Eisenberg and A. H. Tillson, Division of Microbiology, Food and Drug Administration.

DIVISION OF PHARMACEUTICAL CHEMISTRY
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NEW COMPOUNDS

Aryl Esters of Pivalic Acid

Five new esters of pivalic acid were prepared by the method given below.

One-quarter mole of pivalyl chloride was added to a solution of one-quarter mole of the phenol in 50 ml. of pyridine, and the solution was heated on the steam-bath for three hours. The solution was poured into a large excess of water and the aqueous mixture was extracted with ether. The ether extracts were washed with 10% sodium hydroxide solution to remove pivalic acid or pivalyl chloride, washed with 5% hydrochloric acid to remove pyridine, and finally washed with water. The ether solution was dried and concentrated, and the ester was then distilled under reduced pressure through a Vigreux column.

TABLE I

[a]ARYL ESTERS OF PIVALIC ACID, $(CH_3)_3CCOOAr$

Ar	Yield, %	B.p., °C.	Mm.	$n^{20}D$	Formula	Carbon, % Calcd.	Carbon, % Found	Hydrogen, % Calcd.	Hydrogen, % Found
Phenyl	78	105	17	1.4809	$C_{11}H_{14}O_2$	74.16	73.90	7.86	7.70
2-Methoxyphenyl	70	133	15	1.4913	$C_{12}H_{16}O_3$	69.23	69.00	7.69	7.45
3-Methoxyphenyl	75	142	15	1.4918	$C_{12}H_{16}O_2$	69.23	69.46	7.69	7.80
4-Methoxyphenyl	70	135	11	1.4905	$C_{12}H_{16}O_3$	69.23	69.27	7.69	7.68
3-Methylphenyl	62	120	15	1.4828	$C_{12}H_{16}O_2$	75.00	74.60	8.33	8.12

[a] Boiling points are uncorrected. Analyses by Mrs. G. L. Sauvage.

DEPARTMENT OF CHEMISTRY
UNIVERSITY OF ROCHESTER WARREN A. RECKHOW
ROCHESTER, NEW YORK D. S. TARBELL

RECEIVED MAY 9, 1952

6-Chloro-9-[2-(2′-hydroxyethylamino)-ethylamino]-2-methoxyacridine

The reaction of 6,9-dichloro-2-methoxyacridine with N-(2-hydroxyethyl)-ethylenediamine was carried out in a phenol melt after the method employed for quinacrine.[1] An 83.5% yield of 6-chloro-9-[2-(2′-hydroxyethylamino)-ethylamino]-2-methoxyacridine dihydrochloride was isolated; after crystallization from water the yellow microcrystalline solid melted 289–292° dec. This compound ("Neo-acranil") has been tested for antibacterial activity.[2]

Anal. Calcd. for $C_{18}H_{20}ClN_3O \cdot 2HCl$: base, 82.6; HCl, 17.4; Cl (total), 25.4. Found[3,4]: base,[5] 82.3; HCl, 17.7; Cl (total), 25.2.

The dihydrochloride was converted to the base with aqueous ammonia; it separated from aqueous ethanol as golden needles, m.p. 184–184.5° (cor.).

Anal. Calcd. for $C_{18}H_{20}ClN_3O$: Cl, 10.75; N, 12.74. Found[3]: Cl, 10.30; N, 12.92.

(1) F. Mietzsch and H. Mauss, U. S. Patent 2,113,357.

(2) G. R. Goetchius and C. A. Lawrence, *J. Lab. Clin. Med.*, **29**, 134 (1944).

(3) Analyses by Mr. M. E. Auerbach.

(4) Dry basis: sample contained 6.2% moisture.

(5) Method of M. E. Auerbach, *J. Amer. Pharm. Assoc., Sci. Ed.*, **26**, 231 (1937).

STERLING–WINTHROP RESEARCH INSTITUTE
JOHN T. SHEEHAN
RENSSELAER, NEW YORK EDGAR A. STECK

RECEIVED MAY 9, 1952

Derivatives of 3-Methoxy-4-nitrobenzoic Acid and 3-Carbomethoxy-4-nitrobenzoic Acid

3-Methoxy-4-nitrobenzoyl chloride was prepared by treating the acid[1] with phosphorus pentachloride on a steam-bath for about 30 minutes. After removing the phosphorus oxychloride by vacuum distillation, the acid chloride was recovered by extracting the crude product with hot petroleum ether, from which the acid chloride crystallized, on cooling, in light yellow needles; m.p. 63–63.5°.

Anal. Calcd. for $C_8H_6O_4Cl$: Cl, 16.47; Found: Cl, 16.45.

The anilide was prepared by treating a benzene solution of the acid chloride with an excess of aniline. After several recrystallizations from ethanol and water, the flat, white plates melted at 162–163°.

Anal. Calcd. for $C_{14}H_{12}O_4N_2$: N, 10.30. Found: N, 10.43.

The amide was prepared by adding an excess of ammonia to a benzene solution of the acid chloride. The product was recrystallized several times from ethanol, forming large crystals which softened at 189° and melted at 193–196°.

Anal. Calcd. for $C_8H_8O_4N_2$: N, 14.30. Found: N, 14.42.

(1) Prepared by oxidation of 3-methoxy-4-nitrotoluene by method adapted from "Organic Syntheses," Coll. Vol. II, John Wiley and Sons, Inc., New York, N. Y., 1943, p. 135.

3-Nitro-4-carbomethoxybenzoyl chloride, formed as before from the acid[2] and phosphorus pentachloride, crystallized in small, white crystals on cooling the petroleum ether extraction; m.p. 91–94°.

Anal. Calcd. for $C_9H_6O_5NCl$: N, 5.75. Found: N, 5.92.

The anilide crystallized from methanol and water in fine, white needles which melted at 140–142° after several recrystallizations.

Anal. Calcd. for $C_{15}H_{12}O_5N_2$: N, 9.34. Found: N, 9.54.

The amide crystallized from methanol as flat, diamond-shaped crystals melting at 196–198.5°.

Anal. Calcd. for $C_9H_8O_5N_2$: N, 12.50. Found: N, 12.58.

(2) Prepared from dimethyl 4-nitroisophthalate according to method of P. Axer, *Monatsh.*, **41**, 153 (1920).

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RECEIVED MAY 12, 1952

4-Methylglutamic Acid

A new amino acid amide, isolated from peanut plants, is believed to be the amide of either an unsaturated 2-amino adipic acid or of an unsaturated 4-methylglutamic acid.[1] Done and Fowden mention that 4-methylglutamic acid was not available for comparison. We prepared this amino acid in 1945 from acetamidomalonic and acetamidocyanoacetic esters. Loss of a carbethoxy group in the preparation of ethyl 2-acetamido-4-carbomethoxyvalerate was to be expected.[2]

Ethyl 2-Acetamido-4-carbomethoxyvalerate.—To a solution of 24 ml. of methyl methacrylate and 43.5 g. of acetamidomalonic ester in 100 ml. of dry ethanol was added a solution of 0.4 g. of sodium in 30 ml. of ethanol. The resulting solution was refluxed for five hours and concentrated to dryness. The residue was recrystallized from benzene, filtering from sodium ethylate. The solution was cooled to room temperature, filtered and washed with Skellysolve A to remove the yellow color; yield 33.8 g. melting at 105–108°. Cooling the filtrate gave 5.8 g. melting at 100–104°. Concentration of the filtrate to small volume gave 5.2 g. of lower melting material. A sample for analysis, recrystallized twice from benzene, melted at 108°.

Anal. Calcd. for $C_{11}H_{19}NO_5$: N, 5.71. Found: N, 5.73.

4-Methylglutamic Acid.—In the above manner, methyl methacrylate (12 ml.) was condensed with acetamidocyanoacetic ester (17 g.) to give 19.3 g. of viscous liquid. This was refluxed for four hours with 80 ml. of concentrated hydrochloric acid and then concentrated to dryness *in vacuo*. The residue was dissolved in a minimum amount of warm water, brought to *p*H 3 with ammonium hydroxide, treated with charcoal, filtered and chilled overnight to give about 8 g. of crude product. Recrystallization from 21 ml. of water gave 2 g. of amino acid. Recrystallization from 6 ml. of water then gave 1.1 g. melting at 168–169°.

Anal. Calcd. for $C_6H_{11}NO_4$: N, 8.69. Found: N, 8.69.

The amino acid was obtained in better yield by hydrolyzing the intermediate cyano compound with sulfuric acid

(1) J. Done and L. Fowden, *Biochem. J.*, **49**, XX (1951).

(2) *Cf.* F. H. McMillan and N. F. Albertson, THIS JOURNAL, **70**, 3778 (1948).

(and working up with barium hydroxide to remove ammonia in the usual manner), but the crude amino acid melted at 151–164°. When recrystallized from water to constant melting point, 166–168°, the yield was 10.4 g. of amino acid from one mole of acetamidocyanoacetic ester. This is essentially the same over-all yield as reported above. No attempt was made to isolate the second DL-stereoisomer which is presumably present.

5-Carboxy-3-methyl-2-pyrrolidone.—The pyrrolidone was obtained in an attempt to prepare the hydantoin. A solution of 10.4 g. of 4-methylglutamic acid in 100 ml. of water was brought to *p*H 7 with sodium hydroxide and heated with 7.1 g. of potassium cyanate on the steam-bath for one hour. It was then acidified with hydrochloric acid and heated for three hours more. The solvent was removed *in vacuo* and the residue extracted with hot alcohol. The product, recrystallized from water, melted at 173°.

Anal. Calcd. for $C_6H_9NO_3$: C, 50.43; H, 6.34; N, 9.79. Found: C, 49.92; H, 6.23; N, 9.53.

STERLING-WINTHROP RESEARCH INSTITUTE
RENSSELAER, N. Y. JEANNE L. FILLMAN
NOEL F. ALBERTSON

RECEIVED APRIL 29, 1952

COMMUNICATIONS TO THE EDITOR

A NEW PROCEDURE FOR THE DETERMINATION OF THE FINE STRUCTURE OF POLYSACCHARIDES

Sir:

In a previous communication[1] it was reported that the dialdehydes obtained from simple glycosides by periodate oxidation could readily be reduced to the corresponding alcohols in almost quantitative yield.

We wish to report here that periodate oxidation followed by reduction with either hydrogen and a Raney nickel catalyst between 60 and 100° under pressure or with sodium borohydride in aqueous solution represents a general procedure which can also be applied to polysaccharides. Now, whereas the periodate-oxidized polysaccharides or "polyaldehydes" usually undergo profound decomposition when hydrolyzed even in the cold, the corresponding new "polyalcohols" can be subjected to hydrolysis with boiling dilute mineral acid with little or no decomposition to give cleavage products which can be separated by partition chromatography and determined quantitatively. Results obtained in this manner provide information concerning the nature and amount of glycosidic linkages in a polysaccharide.

Glucose residues linked so that free OH groups are present at C_2 and C_3 will give rise to erythritol and glycolic aldehyde when subjected to periodate oxidation followed by reduction and hydrolysis; this applies to residues linked through positions 1 and 4 or 1, 4, and 6. Glucose residues with free hydroxyl groups at C_3 and C_4 such as those in terminal positions and those joined through positions 1 and 6 or 1, 2 and 6 will provide glycerol instead of erythritol. However, any glucose residue linked so that no pair of adjacent hydroxyl groups is present will not be affected by periodate oxidation and will therefore appear as free glucose after the final hydrolysis step. Similar considerations, which are clearly not restricted to polyglucosans, will also apply to polysaccharides composed wholly or in part of furanose residues.

The deductions that can be made from the results of an examination of the polyalcohol produced from a given polysaccharide do not necessarily permit a clear cut solution to a structural problem but taken in conjunction with other experimental results such as, for example, those of methylation it is feasible to restrict greatly the number of structural possibilities.

The few typical examples given below will serve to illustrate the usefulness of the proposed new procedure. In the case of the branched chain polysaccharides amylopectin and glycogen which are composed of glucopyranose residues joined by 1,4 bonds and have branches at certain C_6 positions, the non-reducing terminal unit will give rise to glycerol while the glucose units of the main chain joined through positions 1 and 4 and those at which branching occurs with linkages at positions 1, 4 and 6 will all give erythritol. Hence the molecular ratio of glycerol to erythritol, as determined by the chromotropic acid procedure,[2] should equal the molecular ratio of terminal to non-terminal glucose residues. For glycogen a ratio of 1:10 has been found for the glycerol/erythritol ratio. This is in good agreement with the value of 1:11 for the molecular ratio of tetramethyl- to the sum of the trimethyl- and dimethyl-glucose components derived from methylation studies. The result is also in good agreement with the figure of 1:11 for the ratio of terminal to non-terminal residues as determined from the amount of formic acid liberated by periodate oxidation of glycogen itself.[3] Similar correlations have been obtained with amylopectin.

In a polysaccharide composed of hexopyranose residues joined by 1,6 and 1,4 linkages the ratio of the number of these two types of linkages should correspond to the mole ratio of the glycerol to the erythritol obtained from the corresponding polyalcohol by hydrolysis. By application of the new procedure reported herein to one type of dextran,[4] produced by *Leuconostoc mesenteroides* NRRL-B-512, followed by chromatographic separation and determination of glycerol and erythritol,[2] the ratio of 1,6 to 1,4 linkages has been found to be approximately 45:1.

Paper partition chromatographic investigation of

(1) Bertha Lewis, R. Montgomery, F. Smith and J. Van Cleve, 121st A.C.S. Meeting, Milwaukee, Wisconsin, April, 1952.

(2) Marguerite Lambert and A. C. Neish, *Can. J. Res.*, **B28**, 83 (1950).

(3) M. Abdel-Akher and F. Smith, THIS JOURNAL, **73**, 994 (1951).

(4) The authors thank Dr. Allene Jeanes of the Northern Regional Research Laboratory, Peoria, for the sample of dextran.