(6) **2,3,4,6-Tetra-*O*-methyl-D-mannose.**—This chromatographically pure component (75 mg.) was obtained as a sirup $[\alpha]^{22}D$ +29.2° in water (*c* 3.9). Although chromatographically pure, the possibility existed that this component could be a mixture of 2,3,4,6-tetra-*O*-methyl-D-glucose and 2,3,4,6-tetra-*O*-methyl-D-mannose since it has been shown that a variety of chromatographic solvents failed to separate these two compounds.[6]

The aniline derivative of this component was prepared and the crude crystals were separated from the adhering sirup by use of a porous tile. These crystals were fractionally crystallized from ether–petroleum ether mixture into four fractions, all of which had melting points and mixed melting points of 143–146°. The four fractions were combined and had $[\alpha]^{22}D$ −8.4° in methanol (*c* 1.5) (lit.[31] m.p. 144–145°, $[\alpha]^{15}D$ −7.5° (in methanol)). From the preceding data, and from the values given in the literature for the aniline derivative of 2,3,4,6-tetra-*O*-methyl-D-glucose, m.p. 137–138°,[32] it was concluded that only 2,3,4,6-tetra-*O*-methyl-D-mannose was originally present in this component.

Acknowledgment.—The authors wish to express their thanks to Mr. J. E. Jeffery under whose supervision the intrinsic viscosity determinations, sugar and methoxyl analyses were carried out. We also wish to acknowledge the capable assistance of Mr. R. G. Rogerson. The authors are also indebted to Dr. F. Smith, University of Minnesota, for authentic specimens of crystalline 3,6-di-*O*-methyl-D-glucose, 2,6-di-*O*-methyl-D-glucose and 2,3-di-*O*-methyl-D-mannonic acid.

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[31] W. N. Haworth, R. L. Heath and S. Peat, *J. Chem. Soc.*, 833 (1941).

[32] M. L. Wolfrom and W. L. Lewis, THIS JOURNAL, **50**, 837 (1928).

SHELTON, WASH.

[CONTRIBUTION FROM THE DEPARTMENT OF BIOCHEMISTRY, PURDUE UNIVERSITY]

Alkaline Degradation of Alginates[1]

BY ROY L. WHISTLER AND J. N. BEMILLER

RECEIVED JUNE 1, 1959

Alginates are readily degraded by alkaline solutions at 80–120° with the formation of new 6-carbon, dicarboxylic saccharinates, 3-deoxy-2-C-hydroxymethyl-pentarates.

Vollmert reported that methyl alginate is depolymerized in alkaline solutions while sodium alginate is relatively more stable.[2] However, both esterified and ionic alginates are degraded in alkaline solutions and form salts of saccharinic-type acids by the ordinary process of sequential elimination of the reducing end unit and its subsequent transformation into a saccharinate (Fig. 1).[3] The alkaline degradation reaction is more pronounced in high concentrations of alkalies and at high temperatures.

Alginic acids are heteroglycuronoglycans of D-mannopyranosyluronic acid units and L-guluronic acid units.[4] Alginic acid from *Macrocystis pyrifera* contains mainly D-mannopyranosyluronic acid units and 20–40% of L-guluronic acid units.[4c] The D-mannopyranosyluronic acid units are connected with β-(1→4)-linkages.[5–7] The position on the L-guluronic acid units to which the glycosidic linkages are attached is not known, but periodate oxidation[8] gives no indication of linkages at either C2 or C3. If all uronic acid units are connected with (1→4)-linkages, the saccharinates formed would be the isosaccharinate type[9] with a carboxylate group at the C5 position (C6 of the original uronic acid unit). Because of the presence of both D- and L-hexuronic acid units, this new acid, 3-deoxy-2-C-hydroxymethyl-pentaric acid, would be produced in both D- and L-isomeric forms. Experience has shown that the process of alkaline degradation forms a saccharinic-type acid with predominantly one configuration of C2.

The isomeric, 6-carbon, dicarboxylic saccharinic acids have been isolated in the course of the present work. Structures are assigned to these acids on the basis of the currently accepted mechanism for the formation of products from (1→4)-linked D-hexo- and D-pentoglycans.[2] Supporting evidence for the proposed structures is afforded by the equivalent weight (detd. 100, calcd. 97), the production of formaldehyde upon periodate oxidation (0.5 mole per acid equivalent), and by comparison of the nitric acid oxidation products with those of α-D-isosaccharinic acid. Nitric acid oxidation of α-D-isosaccharinic acid (I) produces a carboxyl group from the primary alcohol group (C2′) so that the asymmetry of C2 is destroyed. A tricarboxylic acid, 2-C-carboxy-3-deoxy-D-*glycero*-pentaric acid (III), is formed in which only the penultimate carbon atom is asymmetric. Nitric acid oxidation of the two 3-deoxy-2-C-hydroxymethyl-pentaric acids produces two tricarboxylic acids, one which has the same rotation as that of the known 2-C-carboxy-3-deoxy-D-*glycero*-pentaric acid and one which has an opposite rotation. The isolated 3-deoxy-2-C-hydroxymethyl-pentarate whose nitric acid oxidation product has the same rotation as that of the known 2-C-carboxy-3-

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[1] Journal Paper No. 1438 of the Purdue Agricultural Experiment Station, Lafayette, Ind.

[2] B. Vollmert, *Angew. Chem.*, **61**, 329 (1949); *Makromol. Chem.*, **5**, 110 (1950).

[3] For a general discussion of saccharinic acid formation see: R. L. Whistler and J. N. BeMiller, *Advances in Carbohydrate Chem.*, **13**, 289 (1958).

[4] (a) F. G. Fischer and H. Dörfel, *Hoppe-Seyler's Z. physiol. chem.*, **302**, 186 (1955); (b) D. W. Drummond, E. L. Hirst and Elizabeth Percival, *Chemistry & Industry*, 1088 (1958); (c) R. L. Whistler and K. W. Kirby, *Hoppe-Seyler's Z. physiol. chem.*, **314**, 46 (1959).

[5] G. Lunde, E. Heen and E. Öy, *Kolloid-Z.*, **83**, 196 (1938); E. Heen, *Tids. Kjemi Bergvesen*, **17**, 127 (1937); *C. A.*, **32**, 5792 (1938).

[6] E. L. Hirst, J. K. N. Jones and W. O. Jones, *Nature*, **143**, 857 (1939); *J. Chem. Soc.*, 1880 (1939).

[7] S. K. Chanda, E. L. Hirst, E. G. V. Percival and A. G. Ross, *ibid.*, 1833 (1952).

[8] H. J. Lucas and W. T. Stewart, THIS JOURNAL, **62**, 1792 (1940).

[9] For the action of alkali on 4-*O*-substituted sugars see: W. M. Corbett and J. Kenner, *J. Chem. Soc.*, 2245 (1953); 1789 (1954); J. Kenner and G. N. Richards, *ibid.*, 1810 (1955).

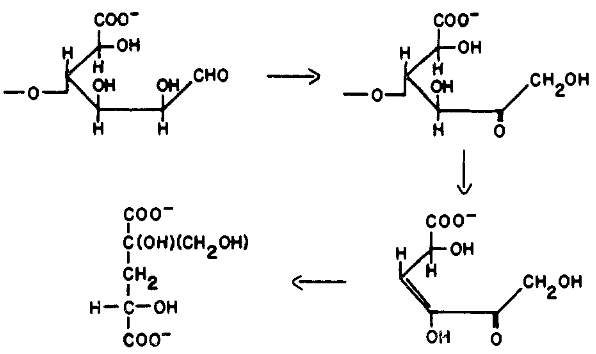


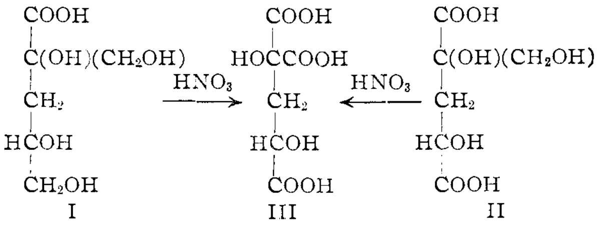
Fig. 1.—The formation of 3-deoxy-2-C-hydroxymethyl-D-*erythro*(or D-*threo*)-pentaric acid from 4-*O*-substituted-D-mannuronate.

deoxy-D-*glycero*-pentaric acid has been designated D-*erythro*(or D-*threo*). The other whose oxidation product has an opposite rotation has been designated L-*erythro*(or L-*threo*).

The combined evidence presented here that the two saccharinic-type acids which have been isolated are D- and L-isomers of 3-deoxy-2-C-hydroxymethyl-pentaric acid is an indication that both the D-mannuronic acid units and the L-guluronic acid units are linked through the 1- and 4-positions.

It has been shown that β-(1→4)-linked D-mannoglycans form β-D-isosaccharinates when treated with alkaline solutions.[10] Therefore, it would be predicted that the acid formed by the alkaline degradation of a β-(1→4)-linked D-mannuronan would have the same configuration at C2 as that of β-D-isosaccharinic acid. In contrast, the action of alkalies on 4-*O*-substituted-D-glucose forms α-D-isosaccharinates,[9] and, therefore, it would be expected that a 3-deoxy-2-C-hydroxymethyl-pentaric acid of the D-series with the α-configuration of C2 would be formed from 4-*O*-methyl-D-glucuronate.[11] In partial support of this view it is observed that the two brucine salts of the designated 3-deoxy-2-C-hydroxymethyl-pentaric acids of the D-series from alginates and 4-*O*-methyl-D-glucuronate have different X-ray diffraction patterns.

3-Deoxy-2-C-hydroxymethyl-pentarates are formed in good yields from either alginate esters or salts. Rates of production of alcohol-soluble fragments indicate that alkaline fragmentation of alginate esters is more rapid than that of salts. This increase in depolymerization rate is very likely due to a β-alkoxy carbonyl mechanism with the formation of an α,β-unsaturated acid.[3] It has been proposed that the proton on C5 which is in the α-position to the ester group is removed. The free pair of electrons which is left on C5 then shifts to form a double bond between C4 and C5. This process ejects the portion of the molecule attached to C4 as a glycoxy anion and breaks the polymer chain. This reaction is competitive with de-esterification and, although it takes place only to a slight extent, the exposure of a few reducing ends in each polymer chain would greatly increase the rate of the normal alkaline degradation reaction. Alkali-treated pectin and pectic acid show similar behavior.[2,12,13] It is likely that 3-deoxy-2-C-hydroxymethyl-pentarates could also be produced by alkaline treatment of dinitrogen tetroxide oxidized starch or cellulose. Although the alkaline degradation of glycuronoglycans is slow at temperatures of 20–40°, it can be completed in several hours at 120°.



$$\begin{array}{ccccc} \mathrm{COOH} & & \mathrm{COOH} & & \mathrm{COOH} \\ \mathrm{C(OH)(CH_2OH)} & & \mathrm{HOCCOOH} & & \mathrm{C(OH)(CH_2OH)} \\ \mathrm{CH_2} & \xrightarrow{\mathrm{HNO_3}} & \mathrm{CH_2} & \xleftarrow{\mathrm{HNO_3}} & \mathrm{CH_2} \\ \mathrm{HCOH} & & \mathrm{HCOH} & & \mathrm{HCOH} \\ \mathrm{CH_2OH} & & \mathrm{COOH} & & \mathrm{COOH} \\ \mathrm{I} & & \mathrm{III} & & \mathrm{II} \end{array}$$

Experimental

Chromatography.—Chromatographic separations were done at 25° using the following irrigants: A, 1-butanol–ethanol–3 *N* ammonium hydroxide (4:1:5 v./v.); B, ethyl acetate–acetic acid–formic acid–water (18:3:1:4 v./v.); C, ethyl acetate–pyridine–water (10:4:3 v./v.); D, 1-butanol–ethanol–water (40:11:19 v./v.). The components were detected with silver nitrate and sodium hydroxide.[14]

Degradation.—Several methods of degradation were used; four typical experiments are given here. Sodium alginate (10 g.) was dispersed in 500 ml. of oxygen-free *N* sodium hydroxide solution, and the dispersion was kept at 37° for 28 days. After removal of sodium ions with Amberlite IR-120(H), 0.4 g. of material which was insoluble in 75% ethanol and 6.1 g. of 75% ethanol-soluble, non-volatile acids were obtained. The latter were converted to their calcium salts (3.8 g.) which had a $R_{\mathrm{gluconate}}$ value of 0.81 with irrigant A and $[\alpha]^{25}\mathrm{D}$ −11.6° (*c* 1.0, water).

In another procedure, 1 g. of alginic acid was dissolved in 500 ml. of oxygen-free water, and an 100% excess (0.360 g.) of calcium hydroxide was added. The mixture was autoclaved at about 120° for 65 hr., cooled, decationized with Amberlite IR-120(H) cation exchange resin, and concentrated under reduced pressure. No material which was insoluble in 70% ethanol was present. The acids were reconverted to their calcium salts with calcium carbonate. These salts (0.6 g.) had a $R_{\mathrm{gluconate}}$ value of 0.81 with irrigant A and $[\alpha]^{25}\mathrm{D}$ −12.8° (*c* 1.0, water).

Kelcoloid O[15] (8.1 g.) was dispersed in 500 ml. of oxygen-free *N* sodium hydroxide solution and kept at 37° for 7 days. After removal of sodium ions with Amberlite IR-120(H), 2.3 g. of material which was insoluble in 70% ethanol and 4.9 g. of 70% ethanol-soluble, non-volatile acids were obtained. The latter were converted to their calcium salts, 0.5 g., which had a $R_{\mathrm{gluconate}}$ value of 0.81 with irrigant A and $[\alpha]^{25}\mathrm{D}$ −12.9° (*c* 1.0, water).

Thirty-five grams of sodium alginate was dispersed in 3.5 l. of oxygen-free water at 80° with stirring. After 12 hr., 10 g. of calcium hydroxide was added, and the dispersion of insoluble calcium alginate was stirred at 80° for 10 days. At the end of this period, the solution, which had become clear, was decationized by passing it through a column of Amberlite IR-120(H) and concentrated under reduced pressure to a thick sirup. A portion of this sirup was converted to calcium salts and a portion was converted to brucine salts. To make the brucine salt, a solution of the free acid was treated with a slight excess of brucine for 18 hr. at 50°. The volume of the solution was sufficient to dissolve all the brucine. Excess brucine was removed by chloroform extraction. The aqueous solution was concentrated to dryness, and the salts were crystallized out of ethanol.

Equivalent Weight.—The weighed, dried calcium salts were dissolved in water, and cations were removed from the

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[10] R. L. Whistler and J. N. BeMiller, unpublished data.

[11] R. L. Whistler and G. N. Richards, THIS JOURNAL, **80**, 4888 (1958).

[12] H. Neukom and H. Deuel, *Chemistry & Industry*, 683 (1958).

[13] P. Garrick, *ibid.*, 1430 (1958).

[14] W. E. Trevelyan, D. P. Procter and J. S. Harrison, *Nature*, **166**, 444 (1950).

[15] An 80% propylene glycol ester of alginic acid of the Kelco Co., New York, N. Y.

solution with Amberlite IR-120(H). The equivalent weight was determined by titration of the acidic sirup obtained upon removal of water with standard sodium hydroxide solution. The value obtained was 100 compared to a calculated equivalent weight of 97 for 3-deoxy-2-C-hydroxymethyl-pentaric acid.

Resolution.—An ethanol solution of brucine salts was concentrated to dryness, and the resulting powder was shaken with a little water at 0°. The mixture was then stored for 24 hr. at 0° and centrifuged at 0°. This process was repeated until all the remaining glass was soluble in water at 0°. The crystalline residue which was insoluble in water at 0° had a block m.p. of 163–188° and a X-ray powder diffraction pattern which differed from that of the brucine salt of the acid formed by the action of lime water on a 4-*O*-methyl-D-glucuronate.[11]

3-Deoxy-2-C-hydroxymethyl-L-*erythro*(or L-*threo*)-pentaric Acid.—The above crystalline, but slightly soluble, brucine salt was converted to the free acid with Amberlite IR-120(H), and the sirup obtained by concentration of the solution under reduced pressure at 35–40° was further purified by paper chromatography using the descending method and irrigant B. A component was obtained which appeared as a single component with irrigant to B, C and D. This acid had a R_{ga}[16] value of 3.1–3.4 and a R_l[17] value of 1.2–1.3. The purified sirupy, free acid thus obtained (1.46 g.) had 6.4×10^{-3} acid equivalents per gram and $[\alpha]^{25}D$ +5.6° (*c* 1.0, water).

When a portion of this sirup was oxidized with periodate according to the method of Sowden[18] and the formaldehyde was determined by the method of Reeves,[19] 0.5 mole of formaldehyde per equivalent of acid was obtained.

Another portion of the acidic sirup was converted to its brucine salt which was crystallized from ethanol. The crystals had a block m.p. of 192–193°, a capillary m.p. of 177–186° and $[\alpha]^{25}D$ −18.0° (*c* 3.4, water).

Anal. Calcd. for $C_{52}H_{62}O_{15}N_4$: C, 63.6; H, 6.4; N, 5.7. Found: C, 58.2; H, 6.5; N, 5.78, 5.67.

A third portion (0.1 g.) was oxidized with nitric acid according to the method of Kiliani,[20] that is, by treatment with nitric acid (1.4 d.) for 24 hr. at 35°, for 24 hr. at 45°, and then for 24 hr. at 50°. The reaction product was diluted with water, and, after standing for 12 hr., the mixture was filtered. The filtrate was concentrated under reduced pressure at 35–40° and purified by paper chromatography with irrigant B. A chromatographically pure sirup was obtained (53 mg.) which had R_{ga}[16] and R_l[17] values of 2.9 and 1.2, respectively, in irrigant B and a $[\alpha]^{25}D$ −0.3° (*c* 2.7, water). This sirup had chromatographic flow rates in irrigants B, C and D which were identical to those of the nitric acid oxidation product of α-D-isosaccharinic acid.

Oxidation of α-D-Isosaccharinic Acid.—Calcium α-D-isosaccharinate (3.5 g.) was oxidized with nitric acid by Kiliani's method.[20] The oxidized product was isolated by paper chromatography with irrigant B; R_{ga}[16] and R_l[17] values of the product in irrigant B were, respectively, 2.9 and 1.2. The yield was 0.74 g. of sirup which contained 1.2×10^{-3} acid equivalents per gram and had $[\alpha]^{25}D$ +1.2° (*c* 7.4, water). This acid is 2-C-carboxy-3-deoxy-D-*glycero*-pentaric acid.

3-Deoxy-2-C-hydroxymethyl-D-*erythro*(or D-*threo*)-pentaric Acid (II).—The cold-water-soluble brucine salt from the above resolution was converted to the free acid by stirring its solution with Amberlite IR-120(H). When this acidic solution was concentrated under reduced pressure at 35–40°, a sirup was obtained which was purified by paper chromatography. The final sirup (0.325 g.) moved as a single component with irrigants B, C and D and had R_{ga}[16] and R_l[17] values of 3.1–3.4 and 1.2–1.3, respectively, with irrigant B. It contained 2.0×10^{-3} acid equivalents per gram and had $[\alpha]^{25}D$ +3.0° (*c* 1.3, water).

A portion of the sirup was converted to its brucine salt which, when crystallized from ethanol and placed in a capillary, began to melt at 162° and decomposed at about 250°.

Anal. Calcd. for $C_{52}H_{62}O_{15}N_4$: C, 63.6; H, 6.4; N, 5.7. Found: C, 61.7; H, 6.4; N, 5.28, 5.53.

Another portion (0.1 g.) was oxidized with nitric acid by the method of Kiliani[20] and purified chromatographically with irrigant B. A sirup was obtained which had a R_{ga}[16] value of 2.8 and a R_l[17] value of 1.2 with irrigant B and a $[\alpha]^{25}D$ +1.2° (*c* 4.3, water). This acid had chromatographic flow rates with irrigants B, C and D which were identical to those of 2-C-carboxy-3-deoxy-D-*glycero*-pentaric acid produced by nitric acid oxidation of α-D-isosaccharinic acid.

Acknowledgment.—The authors gratefully acknowledge the grant from the Kelco Co. and from the Department of Health, Education, and Welfare which helped to support this work.

LAFAYETTE, IND.

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(16) R_{ga} is the rate of movement relative to that of D-gluconic acid.

(17) R_l is the rate of movement relative to that of D-glucono-1,4-lactone.

(18) J. C. Sowden, Mary G. Blair and Dorothy J. Kuenne, THIS JOURNAL, **79**, 6450 (1957).

(19) R. E. Reeves, *ibid.*, **63**, 1476 (1941).

(20) H. Kiliani, *Ber.*, **18**, 631 (1885).

[CONTRIBUTION FROM AVERY LABORATORY, UNIVERSITY OF NEBRASKA]

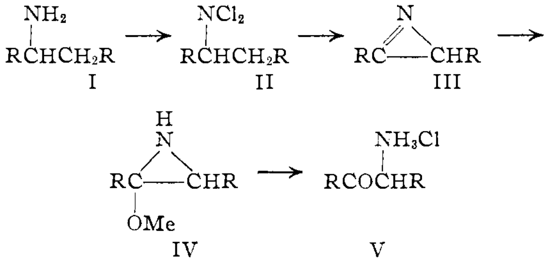
Reactions of Amines. V. Synthesis of α-Amino Ketones[1,2]

BY HENRY E. BAUMGARTEN AND JAMES M. PETERSEN

RECEIVED JULY 13, 959

The treatment of *sec*-alkyl amines (I) with *t*-butyl hypochlorite in benzene solution followed by successive treatments of the unisolated intermediates II–IV with sodium methoxide and with dilute hydrochloric acid gave good yields of the corresponding α-amino ketone hydrochlorides V. The infrared spectra of the α-amino ketone hydrochlorides resembled to some extent the spectra of α-amino acid hydrochlorides.

In the first paper[3] of this series a new rearrangement of N,N-dichloro *sec*-alkyl amines II, which led to the formation of the corresponding α-amino ketone hydrochlorides V, was described. The present communication reports an improved procedure for the conversion of *sec*-alkyl amines I to α-amino ketone hydrochlorides *via* II and offers some measure of the scope of the reaction.



In the original work the amine was converted into the N,N-dichloroamine II using aqueous

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(1) Paper IV, THIS JOURNAL, **81**, 2132 (1959).

(2) This work was supported in part by grant G-3689 of the National Science Foundation.

(3) H. E. Baumgarten and F. A. Bower, THIS JOURNAL, **76**, 4561 (1954).