to the benzoylation mixture, afforded the fully blocked compound in 96% yield. The compound melted at 101–103° as reported.[2]

1,4,5-Tri-O-benzoyl-D-arabitol. A solution of 1 g. of 1,4,5-tri-*O*-benzoyl-2,3-*O*-isopropylidene-D-arabitol in 80% acetic acid was heated on the steam bath until a constant optical rotation was obtained. Acetic acid and water were removed *in vacuo* yielding 0.6 g. (65%) of a crystalline material which could be recrystallized from ether-heptane; m.p. 93–94°; $[\alpha]_D^{23}$ −7.6° (*c*, 0.88 in dry methanol).

Anal. Calcd. for $C_{28}H_{24}O_8$ (464.45): C, 67.23; H, 5.21; C_6H_5CO, 67.5. Found: C, 67.58; H, 5.68; C_6H_5CO, 66.3.

1,4,5-Tri-O-benzoyl-D-arabitol (allotropic form). A solution of 1 g. of 1,4,5-tri-*O*-benzoyl-2,3-*O*-benzylidene-D-arabitol in 80% acetic acid was heated on the steam bath until a constant optical rotation was obtained. The acetic acid and water were removed *in vacuo* yielding 0.62 g. of a crystalline material which melted at 104° after recrystallization from ether–petroleum ether (b.p. 60–70°); $[\alpha]_D^{23}$ −7.3° (*c*, 0.96 in dry methanol). If an ether–petroleum ether solution of this compound was seeded with the tribenzoate melting at 93° the crystalline material obtained melted at 93–94°. If the conditions were reversed, the compound which crystallized melted at 104°. The infrared spectra of the two compounds differ when taken in potassium bromide, but are identical in chloroform solution.

Anal. Calcd. for $C_{28}H_{24}O_8$ (464.45): C, 67.23; H, 5.21. Found: C, 67.58; H, 5.28.

2,3-O-Isopropylidene-D-arabitol. To a sample (1 g.) of 1,5-di-*O*-benzoyl-2,3-*O*-isopropylidene-D-arabitol dissolved in 50 ml. of dry methanol was added 0.5 ml. of 0.8*N* barium methoxide. After 18 hr. at room temperature the methanol was removed *in vacuo* and the amorphous residue was dissolved in water and chromatographed, ascendingly, on large sheets of Whatman 3MM paper. The upper phase of a 4:1:5 (v./v.) mixture of *n*-butyl alcohol, ethanol, and water was the developing solvent. The sugar, which was chromatographed as a single component, was eluted with water and concentrated to dryness *in vacuo*. After 3 days of drying under high vacuum, a crystalline substance was obtained which consumed 0.94 mole of periodate per mole of sugar when oxidized in ammonium acetate buffer *p*H 5.8. The crystalline material melted near room temperature and was very hygroscopic.

Anal. Calcd. for $C_8H_{16}O_6$ (192.21): C, 49.99; H, 8.39. Found: C, 48.8; H, 8.5.

BERKELEY 4, CALIF.

[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY OF THE OHIO STATE UNIVERSITY]

The Composition of Pyrodextrins. III. Thermal Polymerization of Levoglucosan[1]

M. L. WOLFROM, A. THOMPSON,[2] R. B. WARD,[2] D. HORTON,[2] AND R. H. MOORE[2,3]

Received April 11, 1961

The di- and trisaccharide portions of the thermal polymers of 1,6-anhydro-β-D-glucopyranose were isolated as acetates by carbon and silicate chromatography and the peracetates of 4-*O*-α-D-glucopyranosyl-1,6-anhydro-β-D-glucopyranose (1,6-anhydromaltose), 4-*O*-β-D-glucopyranosyl-1,6-anhydro-β-D-glucopyranose (1,6-anhydrocellobiose), 2-*O*-α-D-glucopyranosyl-1,6-anhydro-β-D-glucopyranose (1,6-anhydrokojibiose), 2-*O*-β-D-glucopyranosyl-1,6-anhydro-β-D-glucopyranose (1,6-anhydrosophorose), and three peracetylated anhydrotrisaccharides were identified therein. All save the maltose and cellobiose derivatives are hitherto undescribed.

The thermal polymerization of 1,6-anhydro-β-D-glucopyranose was studied[4] by Pictet, Pringsheim and Schmalz, Irvine, and Oldham, and more recently by da Silva Carvalho, Prins, and Schuerch.[5] Wolfrom, Thompson, and Ward[1] studied the thermal polymerization of 1,6-anhydro-β-D-glucopyranose and its possible relation to reactions taking place during the roasting of starch to produce pyrodextrins. They isolated gentiobiose, isomaltose, maltose, cellobiose, sophorose, and 1,6-anhydro-β-D-glucopyranose as their acetates from the fragments present in the partial hydrolyzate of the ethanol-insoluble portion of the thermal polymer. The isolation of these hydrolytic fragments indicates the presence of 6-*O*-α-(and β)-D-, 4-*O*-α-(and β)-D-, 2-*O*-β-D-linkages, and 1,6-anhydro-β-D-glucopyranose end groups in the polymer.

We wish to describe herein a study of the ethanol-soluble portion of the thermal polymer of 1,6-anhydro-β-D-glucopyranose, which contains low molecular weight carbohydrates with 1,6-anhydro-β-D-glucopyranose end groups. These substances were isolated by carbon column elution chromatography and further refractionation of the acetylated fractions by silicate column chromatography to give the individual sugar acetates. Four anhydrodisaccharides, 4-*O*-α-D- and 4-*O*-β-D-, 2-*O*-α-D-, and 2-*O*-β-D-glucopyranosyl-1,6-anhydro-β-D-glucopyranose were isolated as their hexaacetates, the latter pair of substances for the first time. 4-*O*-(2,3,4,6-Tetra-*O*-acetyl-β-D-glucopyranosyl)-2,3-di-*O*-acetyl-1,6-anhydro-β-D-glucopyranose (1,6-anhydrocellobiose hexaacetate) was obtained in two crystalline forms: m.p.

(1) Previous communication in this series: M. L. Wolfrom, A. Thompson, and R. B. Ward, *J. Am. Chem. Soc.*, 81, 4623 (1959). Preliminary communication: *Abstracts Papers Am. Chem. Soc.*, 138, 5D (1960).

(2) Postdoctoral Fellows of the Corn Industries Research Foundation (O. S. U. Proj. 11168-5241).

(3) R. H. Moore gratefully acknowledges a travel grant from The Wellcome Trust, 52 Queen Anne St., London W 1.

(4) A. Pictet, *Helv. Chim. Acta*, 1, 226 (1918); H. Pringsheim and K. Schmalz, *Ber.*, 55, 3001 (1922); J. C. Irvine and J. W. H. Oldham, *J. Chem. Soc.*, 127, 2903 (1925).

(5) J. da Silva Carvalho, W. Prins, and C. Schuerch, *J. Am. Chem. Soc.*, 81, 4054 (1959).

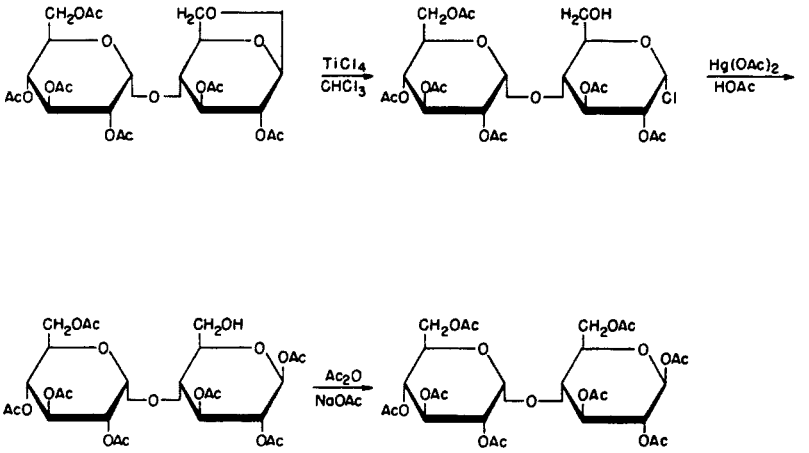


Figure 1

94–96° and 145–146°. These dimorphs exhibited different x-ray powder diffraction patterns and the lower-melting form was convertible to the higher-melting form upon recrystallization with proper nucleation. The identities of the anhydrodisaccharide acetates were determined by ring cleavage with titanium tetrachloride[6–8] in technical chloroform, followed by acetate replacement with anomeric inversion by mercuric acetate in acetic acid, and completion of the acetylation with acetic anhydride to produce the corresponding fully acetylated β-D-disaccharide of known structure (Figure 1).

No 3-*O*-D-glucopyranosyl anhydrodisaccharides were encountered in this study, nor were any 3-*O*-D-glucosyl linked substances found among the hydrolytic products of the more complex thermal polymers of 1,6-anhydro-β-D-glucopyranose.[1] In the *acid-catalyzed* polymerization of 1,6-anhydro-β-D-glucopyranose, Abe and Prins[9] postulate that the initial reaction involves predominant formation of (1→6)-linked dimers. However, the absence of primary hydroxyls in the unhydrolyzed 1,6-anhydro-β-D-glucopyranose precludes the formation of (1→6)-linked 1,6-anhydrodisaccharides, and indeed in our work no (1→6)-linked disaccharides were encountered in the dimeric stage of the *thermal* polymerization. The experimental procedure utilized would have revealed even minute quantities of gentiobiose.

Three anhydrotrisaccharides were isolated as their acetates: I, m.p. 256–256.5°, $[\alpha]_D^{25}$ +20° (chloroform); II, m.p. 209–209.5°, $[\alpha]_D^{24}$ +26° (chloroform); III, m.p. 230–230.5°, $[\alpha]_D^{24}$ −47° (chloroform). These substances were further characterized by carbon-hydrogen analyses, molecular weight determinations, and x-ray powder diffraction patterns. Their exact structures are unknown.

EXPERIMENTAL

Conversion of 4-O-(2,3,4,6-tetra-O-acetyl-α-D-glucopyranosyl)-2,3,-di-O-acetyl-1,6-anhydro-β-D-glucopyranose (1,6-anhydromaltose hexaacetate) to β-maltose octaacetate. 1,6-Anhydromaltose hexaacetate[10] (200 mg.) was dissolved in 5 ml. of chloroform containing 0.4 g. of titanium tetrachloride[6] and a trace of ethyl acetate[6d] and refluxed for 3 hr. The mixture was poured into ice and water, and extracted with chloroform. The chloroform solution was washed successively with water, aqueous sodium bicarbonate solution, and water, dried over anhydrous sodium sulfate, and evaporated to a sirup. The sirup was dissolved in a solution of 200 mg. of mercuric acetate in 2 ml. of acetic acid and allowed to stand for 2 hr. The solution was diluted with 50 ml. of chloroform and washed successively with water, aqueous sodium bicarbonate solution, and water, dried with sodium sulfate, and evaporated to a sirup which was dried by repeated concentration of its solution in methanol, under reduced pressure. The sirup was boiled for 1 min. with 5 ml. of acetic anhydride containing 250 mg. of sodium acetate, then cooled, poured into 30 ml. of ice and water, and stirred for several hours. The mixture was extracted with three 15-ml. portions of chloroform. The combined extract was washed successively with water, aqueous sodium bicarbonate solution, and water, dried with sodium sulfate, and evaporated under reduced pressure to a sirup; yield 195 mg. This material was dissolved in benzene and chromatographed[11] on a column (230 × 45 mm.) of Magnesol[12]: Celite[13] (5:1 by wt.) using 1 l. of benzene:2-methyl-2-propanol (100:1 by vol.). The extruded column was streaked with 1% potassium permanganate in 10% sodium hydroxide. Two zones appeared 15–45 mm. and 55–75 mm. from the column top. The zones were cut out, extracted with acetone and evaporated to sirups which were crystallized from ethanol. The

(6) (a) G. Zemplén and Z. Csürös, *Ber.*, **62**, 993 (1929); (b) G. Zemplén, H. Gerecs, and J. Erdelyi, *Ber.*, **64**, 1545 (1931); (c) G. Zemplén, H. Gerecs, and Hedwig Flesch, *Ber.*, **7**, 774 (1938); (d) Z. Csürös, G. Deak, and Frau M. Haraszthy, *Acta Chim. Acad. Sci. Hung.*, **21**, 181, 193 (1959).

(7) L. Asp and B. Lindberg, *Acta Chem. Scand.*, **6**, 941 (1952).

(8) A. Thompson, M. L. Wolfrom, and M. Inatome, *J. Am. Chem. Soc.*, **77**, 3160 (1955).

(9) H. Abe and W. Prins, *Makromol. Chem.*, **42**, 216 (1960).

(10) P. Karrer and L. Kamienski, *Helv. Chim. Acta*, **15**, 739 (1932).

(11) A. Thompson in *Methods in Carbohydrate Chemistry*, Vol. I, R. L. Whistler and M. L. Wolfrom, eds., Academic Press, New York, *in press*.

(12) A product of the Westvaco Chemical Division of Food Machinery and Chemical Corp., South Charleston, W. Va.

(13) A product of Johns-Manville, New York, N. Y.

lower zone yielded 7.5 mg. of β-maltose octaacetate; m.p. 157–158°, x-ray powder diffraction pattern identical with that of authentic material. The top zone produced 31 mg. of unchanged 1,6-anhydromaltose hexaacetate.

Polymerization of 1,6-anhydro-β-D-glucopyranose and fractionation of the product. 1,6-Anhydro-β-D-glucopyranose (100 g.) was heated at 250° for 20 min. in an oil bath, cooled and dissolved in 500 ml. of water. This solution was placed on a column (900 × 75 mm.) of Nuchar C Unground[14] and the column was developed successively with 20 l. of water (Fraction I), 20 l. of 3% ethanol (Fraction II), 20 l. of 5% ethanol (Fraction III), and 20 l. of 10% ethanol (Fraction IV). Each fraction was concentrated to a sirup by evaporation under reduced pressure; yields: Fraction I, 20 g.; Fraction II, 12.9 g.; Fraction III, 5.4 g.; Fraction IV, 9.6 g. Paper chromatographic examination using the upper phase of a butanol:ethanol:water (4:1:5 parts by vol.) solvent system revealed that Fraction I contained D-glucose and 1,6-anhydro-β-D-glucopyranose and this fraction was not further investigated; Fraction II produced one nonreducing spot with R_g 1, Fraction III, two nonreducing spots with R_g 1 and 0.5, Fraction IV, nonreducing spots R_g 0.5 and base line ($R_g = R_{glucose}$).

4-O-(2,3,4,6-Tetra-O-acetyl-α-D-glucopyranosyl)-2,3-di-O-acetyl-1,6-anhydro-β-D-glucopyranose (1,6-anhydromaltose hexaacetate). Fraction II (12.9 g.) was acetylated by heating to boiling with 67 g. of sodium acetate and 130 ml. of acetic anhydride. The reaction mixture was cooled, poured into 1 l. of water, and stirred overnight. The product was extracted from the aqueous solution with chloroform, the extract was washed successively with water, aqueous sodium bicarbonate solution, and water, dried with anhydrous sodium sulfate, and evaporated to a sirup under reduced pressure; yield 17.3 g. The sirup was dissolved in ethanol which deposited crystalline material after standing at room temperature for several days; yield 3.25 g., m.p. 177–179°. Recrystallization from ethanol produced pure material; m.p. 183°, $[\alpha]_D^{25}$ +49.5° (*c* 2, chloroform), x-ray powder diffraction pattern[15]: 9.18 vs(1), 8.31w, 7.76 s(2), 7.15vw, 6.30vw, 5.93m, 5.18m, 4.82s(3), 4.63vw, 4.48s, 4.15w, 4.06-vw, 3.95m, 3.69w, 3.51vw, 3.37w, 3.30vw, 3.04w. The values for the melting point and specific rotation agree with those recorded[7,10] for 1,6-anhydromaltose hexaacetate.

2-O-(2,3,4,6-Tetra-O-acetyl-α-D-glucopyranosyl)-3,4-di-O-acetyl - 1,6 - anhydro - β - D - glucopyranose (1,6 - anhydro-kojibiose hexaacetate). The mother liquor from the crystallization of 1,6-anhydromaltose hexaacetate, above, was evaporated under reduced pressure to a sirup, and dissolved in benzene. Two aliquots containing about 3.5 g. of the sirup were chromatographed[11] on two Magnesol[12]-Celite[13] columns (235 × 75 mm.) with 1% 2-methyl-2-propanol in benzene (2 l.) as the developer. The columns were extruded and the zone materials indicated by streaking with 1% potassium permanganate in 10% sodium hydroxide. Four zones appeared: 1, 155–200 mm.; 2, 70–110 mm.; 3, 20–45 mm.; and 4, 4–15 mm. from the column top. The zone material was extracted from each with acetone and evaporated under reduced pressure to sirups. The sirups from zones 1, 3, and 4 crystallized from ethanol. The product from zone 1 was tri-O-acetyl-1,6-anhydro-β-D-glucopyranose, m.p. 109–110°; the products from zones 2, 3, and 4 will be discussed below. The combined material (3.8 g.) from zone 2 of the two columns failed to crystallize and was further purified by a second chromatographic treatment on Magnesol-Celite as described above; $[\alpha]_D^{30}$ +58° (*c* 3, chloroform). This sirup (500 mg.) was determined to be crude 1,6-anhydrokojibiose hexaacetate by converting it to β-kojibiose octaacetate by the titanium tetrachloride ring splitting procedure described above for 1,6-anhydromaltose hexaacetate; yield after chromatography on Magnesol-Celite, 91 mg., m.p. 122–123° undepressed on admixture with known β-kojibiose octaacetate, $[\alpha]_D^{20}$ +112° (*c* 0.8, chloroform). The infrared absorption curve was identical with that of the known sample kindly furnished by Dr. K. Aso,[16] x-ray powder diffraction pattern[15]: 12.77m, 11.37s (1) 8.29m(2), 8.01m, 6.45vw, 5.87m(3), 5.16w, 4.84w, 4.58m, 4.30w, 4.06w, 3.87w, 3.65m.

2-O-(2,3,4,6-Tetra-O-acetyl-β-D-glucopyranosyl)-3,4-di-O-acetyl - 1,6 - anhydro - β - D - glucopyranose (1,6 - anhydrosophorose hexaacetate). Zone 3, obtained from the same Magnesol-Celite chromatographic separation (above) which produced kojibiose hexaacetate from the mother liquor of the maltosan hexaacetate crystallization, crystallized from ethanol; combined yield 925 mg., m.p. 170–171°. Pure material was obtained on further recrystallization from ethanol; m.p. 170–171.5°, $[\alpha]_D^{29}$ −40.6° (*c* 1.4, chloroform), x-ray powder diffraction pattern[15]: 12.49m, 8.17vs(2), 6.24s(3), 5.96m, 5.71w, 5.19m, 4.99vw, 4.71m, 4.53vw, 4.37vs(1), 4.20w, 4.02vw, 3.69w, 3.61s, 3.51w, 3.37m, 3.26w, 3.13vw, 3.01vw, 2.93w.

Anal. Calcd. for $C_{24}H_{32}O_{16}$: C, 50.00; H, 5.56. Found: C, 49.93; H, 5.75.

Ring opening of this anhydro compound (200 mg.) by the procedure described above for 1,6-anhydromaltose hexaacetate yielded 58 mg. of β-sophorose octaacetate, m.p. 187° unchanged on admixture with known material. The x-ray powder diffraction pattern was identical with that of known material.[17] This evidence proves that the acetylated anhydro sugar is 1,6-anhydrosophorose hexaacetate.

4-O-(2,3,4,6-Tetra-O-acetyl-β-D-glucopyranosyl)-2,3-di-O-acetyl - 1,6 - anhydro - β - D - glucopyranose (1,6 - anhydro-cellobiose hexaacetate). Zone 4, obtained in the Magnesol-Celite chromatographic separation of the mother liquor from the crystallization of 1,6-anhydromaltose hexaacetate, described in the isolation of 1,6-anhydrokojibiose hexaacetate above, produced material which crystallized from ethanol; yield 590 mg., m.p. 88–94°. Rechromatography effected in the same manner followed by crystallization from ethanol resulted in pure material; m.p. 94–96°, $[\alpha]_D^{23}$ −52° (*c* 3, chloroform), x-ray powder diffraction pattern[15]: 16.67w, 14.55s, 11.16vs(2), 8.76vw, 8.27s(3), 7.93w, 7.24m, 7.08w, 6.51vw, 6.07w, 5.56m, 5.20m, 4.93vw, 4.70w, 4.50s, 4.35m, 4.20vs(1), 3.98m, 3.85w.

The product (90 mg., m.p. 94–96°) was recrystallized from ethanol by nucleation with a known sample (m.p. 145–146°) of 1,6-anhydrocellobiose hexaacetate kindly furnished by Dr. N. K. Richtmyer[18]; yield 50 mg., m.p. 142° unchanged on admixture with the known sample, x-ray powder diffraction pattern[15]: 11.93vw, 10.78vs(2), 9.94vw. 9.16w, 6.35w, 5.75w, 5.24vs(1), 4.95w, 4.63s(3), 4.36w, 4.17m, 4.04w, 3.95m, 3.74vw, 3.59vw, 3.37w, 3.22w, 3.08w, 2.77w, 2.59vw, 2.47vw. Anhydrocellobiose hexaacetate is thus shown to exist in two crystalline forms, the less stable form being converted upon recrystallization with proper nucleation to the more stable form.

Isolation of anhydrotrisaccharide nonaacetates. Fraction III (5.4 g.) from the carbon column was acetylated by the procedure described for the acetylation of Fraction II, above; yield 12 g. The acetylated material was dissolved in benzene and chromatographed in 3 portions on Magnesol-Celite columns (250 × 75 mm.), developing with 3 l. of benzene:2-methyl-2-propanol (100:1 by vol.). Five zones appeared upon streaking with the permanganate indicator; 1, 77–150 mm.; 2, 37–77 mm.; 3, 15–35 mm.; 4, 4–15 mm. and 5, 0–4 mm. from the column top. The acetylated material was extracted

(14) A product of West Virginia Pulp and Paper Co., New York 17, N. Y.

(15) Interplanar spacing, Å, CuK_α radiation. Relative intensity, estimated visually: s, strong; m, medium; w, weak; v, very. Parenthetic numerals indicate order of three most intense lines; 1, most intense.

(16) K. Aso and A. Sato, *Nature*, **180**, 984 (1957).

(17) A. Thompson, Kimiko Anno, M. L. Wolfrom, and M. Inatome, *J. Am. Chem. Soc.*, **76**, 1309 (1954).

(18) Edna M. Montgomery, N. K. Richtmyer, and C. S. Hudson, *J. Am. Chem. Soc.*, **65**, 1848 (1943).

from the zones with acetone and the extracts were evaporated under reduced pressure to sirups. Zone 1 material crystallized from ethanol-methanol; yield 82 mg. of anhydrotrisaccharide nonaacetate I. Further crystallization from ethanol produced pure material; m.p. 256–256.5°, $[\alpha]_D^{25}$ +20° (*c* 0.3, chloroform), x-ray powder diffraction pattern[15]: 13.81s(3), 10.98m, 10.02m, 9.21w, 6.78vw, 6.08w, 5.44s, 4.86vs(1), 4.63w, 4.56w, 4.33s(2), 4.12vw, 3.74m, 3.59vw, 3.40w, 3.26vw, 3.14vw, 3.02vw.

Anal. Calcd. for $C_{36}H_{48}O_{24}$: C, 50.00; H, 5.59; mol. wt., 864.7. Found: C, 49.93; H, 5.86; mol. wt. (Rast), 820.

Zone 2 material failed to crystallize. Zone 3 material crystallized from ethanol; yield 110 mg. of anhydrotrisaccharide nonaacetate II. Pure material was obtained upon recrystallization from ethanol; m.p. 209–209.5°, mixed m.p. with anhydrotrisaccharide nonaacetate I, 206–240°, $[\alpha]_D^{24}$ +26° (*c* 3, chloroform), x-ray powder diffraction pattern[15]: 11.67 vw, 10.62vs(1), 9.85m, 7.90m, 6.69s, 6.47vw, 6.15m, 5.99s, 5.68m, 5.32s(3), 4.93w, 4.64vs(2), 4.18s, 4.03s, 3.87m, 3.72w, 3.54w, 3.48m, 3.32m, 3.22vw.

Anal. Calcd. for $C_{36}H_{48}O_{24}$: C, 50.00; H, 5.59; mol. wt., 864.7 Found: C, 49.94; H, 5.57; mol. wt. (Rast), 721.

Zones 4 and 5 produced material which crystallized from methanol-ethanol; yield 162 mg. of anhydrotrisaccharide nonaacetate III. Further crystallization from ethanol produced pure material, m.p. 230–230.5°, mixed m.p. with anhydrotrisaccharide nonaacetate I, 224–228°, mixed m.p. with anhydrotrisaccharide nonaacetate II, 204–215°, $[\alpha]_D^{24}$ −46.9° (*c* 0.8, chloroform), x-ray powder diffraction pattern[15]: 13.70vw, 10.68w, 10.08m. 9.36s, 8.54w, 6.47vw, 5.48-vs(2), 5.33vw, 5.04m, 4.75w, 4.44vs(3), 4.12vs(1), 3.80vw, 3.57m, 2.94w, 2.51vw, 2.40vw, 1.99vw.

Anal. Calcd. for $C_{36}H_{48}O_{24}$: C, 50.00; H, 5.59; mol. wt., 864.7. Found: C, 50.38; H, 5.56; mol. wt. (Rast), 874.

COLUMBUS 10, OHIO

[CONTRIBUTION FROM THE DEPARTMENT OF ENTOMOLOGY, UNIVERSITY OF CALIFORNIA CITRUS EXPERIMENT STATION]

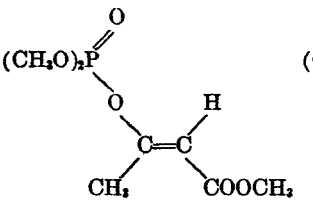
Configuration of the α-and β-Isomers of Methyl 3-(Dimethoxyphosphinyloxy)-crotonate (Phosdrin®)[1,2]

T. R. FUKUTO, E. O. HORNIG, R. L. METCALF, AND M. Y. WINTON

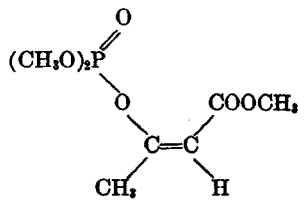
Received February 27, 1961

From proton NMR spectra and enzyme inhibition data the α- and β-isomers of methyl 3-(dimethoxyphosphinyloxy) crotonate (Phosdrin®) have been assigned the *cis*-crotonate and *trans*-crotonate configuration, respectively. The higher rate of inhibition of fly-brain cholinesterase by the α-isomer has been attributed to steric factors.

The assignment of configuration of the α- and β-isomers of methyl 3-(dimethoxyphosphinyloxy)-crotonate (hereafter referred to as Phosdrin®) is of interest because of the widely differing biological properties exhibited by the two forms. The technical isomeric mixture, prepared through the condensation of trimethyl phosphite and methyl 2-chloroacetoacetate, is being used extensively as a wide spectrum insecticide of short residual action. That technical Phosdrin consists primarily of *cis-trans* isomers was first demonstrated by Casida[3] who was able to separate an α- and β-form by column chromatography. He also found when either the α- or β-fractions were irradiated with ultraviolet light a mixture of approximately 30% α- and 70% β-isomers was obtained. On the assumption that ultraviolet irradiation should result in a predominance of the more stable isomer, the α-fraction was assigned the *trans*-crotonate (II) configuration and the β-fraction the *cis*-crotonate (I) configuration since II with two bulky groups on one side of the olefinic bond would be expected to be the thermodynamically less stable form.



I. *cis*-crotonate



II. *trans*-crotonate

It was also found that the α-form was considerably more active as a cholinesterase inhibitor, less stable to hydrolytic splitting of the P—O—C bond, and more toxic to mammals and insects.

To assign configurations upon results obtained from ultraviolet irradiation may lead to erroneous conclusions. In fact, ultraviolet irradiation of the stable isomer often results in the formation of the labile form and is often used as preparative method for the unstable isomer. For example, fumaric acid is transformed into maleic acid upon exposure to ultraviolet light.[4] Although, for thermodynamic reasons, I may be considered the more stable form in reactions involving the olefinic bond, the difference in reactivity of the P—O—C ester bond, particularly in the case of enzyme inactivation, may more likely be attributed to steric factors. For these reasons it was decided that further investigation was needed and this paper reports the application of NMR spectrometry and enzyme

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(1) Paper No. 1304, University of California Citrus Experiment Station.

(2) Supported in part by a research grant from the U. S. Public Health Service, No. RG-5433(C3).

(3) J. E. Casida, *Science*, 122, 597 (1955).

(4) R. Stoermer, *Ber.*, 42, 4865 (1909).