[CONTRIBUTION FROM THE DIVISION OF APPLIED BIOLOGY, NATIONAL RESEARCH COUNCIL OF CANADA]

Structure of an Aldotriouronic Acid Isolated from Jute Fiber Hemicellulose[1,2]

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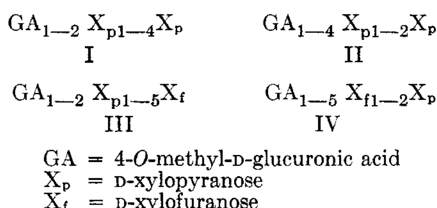
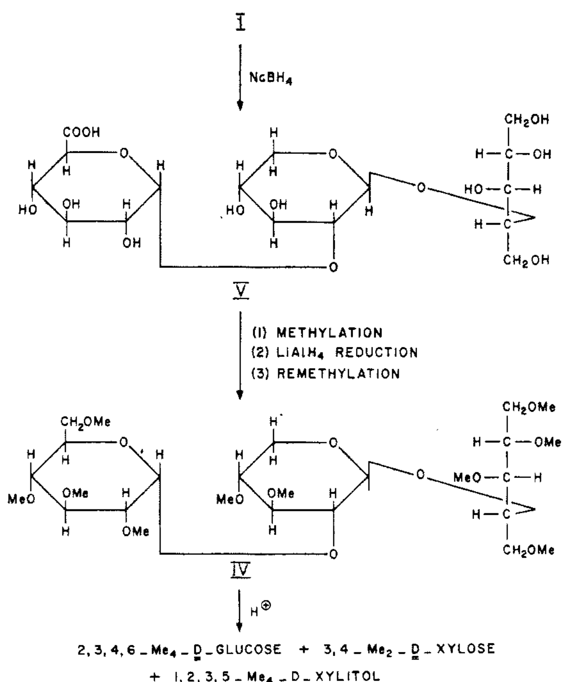
Received March 27, 1961

Reduction of the aldotriouronic acid followed by methylation, reduction, and remethylation yielded the methylated trisaccharide alcohol. Hydrolysis of the latter produced (a) 2,3,4,6-tetra-*O*-methyl-D-glucose, (b) 3,4-di-*O*-methyl-D-xylose, and (c) 1,2,3,5-tetra-*O*-methyl-D-xylitol. The structure of the aldotriouronic acid is therefore established as *O*-α-4-*O*-methyl-D-glucuronosyl-(1 → 2)-*O*-β-D-xylopyranosyl-(1 → 4)-D-xylose. Preparation of 1,2,3,5-tetra-*O*-methyl-D-xylitol from xylotriose is described.

A previous communication[4] from these laboratories described partial depolymerization of the jute fiber hemicellulose and the isolation and identification of three acidic components, namely, 4-*O*-methyl-D-glucuronic acid, 2-*O*-(4-*O*-methyl-D-glucuronosyl)-D-xylose, and an aldotriouronic acid.

Although the constitution of the first two compounds was fully established, the structure of the aldotriouronic acid (I) was not proved beyond doubt. It was shown in the earlier work[4] that the methyl ester methyl glycoside of I gave upon reduction a neutral trisaccharide methyl glycoside, which on methylation and hydrolysis afforded equimolecular proportions of 2,3,4,6-tetra-*O*-methyl-D-glucose, 3,4-di-*O*-methyl-D-xylose, and 2,3-di-*O*-methyl D-xylose. On the basis of these results, I was assigned, tentatively, the structure *O*-α-4-*O*-methyl-D-glucuronosyl-(1 → 2)-*O*-β-D-xylopyranosyl-(1 → 4)-D-xylose. However, it will be apparent that the above results could be obtained from structures II–IV as well as from I. This communication is concerned with definitive proof of the structure of the aldotriouronic acid.





The aldotriouronic acid was reduced to the corresponding triouronic acid alcohol (V). The latter was methylated, reduced, and remethylated to produce *O*-α-2,3,4,6-tetra-*O*-methyl-D-glucosyl-(1 → 2)-*O*-β-3,4,-di-*O*-methyl-D-xylosyl-(1 → 4)-1,2,-3,5-tetra-*O*-methyl-D-xylitol (VI). Methanolysis of VI followed by acid hydrolysis and separation of the methylated fragments gave (a) 3,4-di-*O*-methyl-D-xylose and (b) a mixture of 2,3,4,6-tetra-*O*-methyl-D-glucose and 1,2,3,5-tetra-*O*-methyl-D-xylitol (1,3,4,5-tetra-*O*-methyl-L-xylitol) which could not be resolved either by column or paper chromatography. The mixture was oxidized with bromine water and then passed over Amberlite IR-45 (OH) to adsorb the 2,3,4,6-tetra-*O*-methyl-D-gluconic acid; the 1,2,3,5-tetra-*O*-methyl-D-xylitol was recovered from the effluent.

The identification of 3,4-di-*O*-methyl-D-xylose as a cleavage product of methylated triitol derived from the aldotriouronic acid rules out structures II and IV, and on the basis of identification of 1,2,3,5-tetra-*O*-methyl-D-xylitol, structure III is discarded. The aldotriouronic acid is therefore assigned the structure I as proposed earlier.[4,5]

The configurations assigned to the glycosidic linkages were based on work by Gorin and Perlin[6]

(1) Issued as N.R.C. No. 6436.

(2) Presented in part before the Division of Carbohydrate Chemistry at the 136th National Meeting of the American Chemical Society held in September 1959, at Atlantic City, N. J.

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(4) H. C. Srivastava and G. A. Adams, *J. Am. Chem. Soc.*, **81**, 2409 (1959).

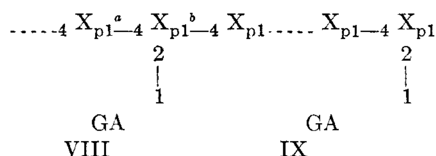
(5) J. Kelvin Hamilton and Norman S. Thompson, *J. Am. Chem. Soc.*, **79**, 6464 (1957).

(6) P. A. J. Gorin and A. S. Perlin, *Can. J. Chem.*, **36**, 999 (1958).

and by Aspinall and Das Gupta.[7] Thus the former authors provided direct chemical proof of the configuration of the 4-*O*-methyl-D-glucuronosidic linkage; the latter workers showed that the D-xylose residues in jute hemicellulose were joined by β 1 → 4 glycosidic bonds.

1,2,3,5-Tetra-*O*-methyl-D-xylitol was prepared in the following manner. Xylotriose was reduced to xylotriitol, which was methylated and hydrolyzed to yield 2,3,4-tri-*O*-methyl-D-xylose, 2,3-di-*O*-methyl-D-xylose, and 1,2,3,5-tetra-*O*-methyl-D-xylitol. This mixture was oxidized with bromine water, sugar acids were adsorbed on a cation exchange column, and the 1,2,3,5-tetra-*O*-methyl-D-xylitol was recovered as a sirup.

Aspinall and Das Gupta[7] have shown that jute hemicellulose is made up of a backbone of 1,4-linked β-D-xylopyranose residues with approximately every seventh residue carrying a terminal 4-*O*-methyl-D-glucuronic acid unit (VIII). Partial degradation of such a polymer with acid is expected to give two isomeric aldotriouronic acids, I and IX. Our attempts to isolate aldotriouronic acid IX have



not met with success. Hamilton and Thompson[5] also made a search, without success, for the aldotriouronic acid IX in the hydrolyzate of a xylan polyuronide which gave aldotriouronic acid of structure I. It would appear therefore that in the structure VIII, the bond *a* is more sensitive to acid hydrolysis than the bond *b*. This is in conformity with the hypothesis of Marchessault and Rånby[8] who suggest that the glycosidic bond in 4-*O*-β-D-glucopyranosyl-D-glucuronic acid (pseudocellobiouronic acid) is considerably more sensitive to acid than the linkages in cellobiouronic acid and cellobiose.

EXPERIMENTAL

The following solvents were used for paper chromatography: A: ethyl acetate–water–acetic acid–formic acid (20:4:3:1). B: butanone–water azeotrope. C: benzene–ethanol–water–ammonia (200:47:14:1). *p*-Anisidine hydrochloride in ethanol was used for the detection of sugars and their methylated derivatives. All evaporations were carried out under reduced pressure below 40°.

Reduction of aldotriouronic acid. Aldotriouronic acid (400 mg.), m.p. 180–183°; $[\alpha]^{25}_{D}$ + 58° in water, was neutralized with 0.1*N* sodium hydroxide (equiv. weight calcd. for $C_{16}H_{25}O_{14}$: $(OCH_3)\cdot 3\ H_2O$, 526. Found: 536) and reduced with solid potassium borohydride (150 mg.).[9] The product, *O*-α-4-*O*-methyl-D-glucuronosyl-(1 → 2)-*O*-β-D-xylopyranosyl-(1 → 4)-D-xylitol (V), a white glass, showed $[\alpha]^{26}_{D}$ + 62.5° in water. Paper chromatography of the sirup using solvent A showed that the xylose end of the aldotriouronic acid was completely reduced.

O-α-2,3,4,6-Tetra-O-methyl-D-glucosyl-(1 → 2)-O-β-3,4-di-O-methyl-D-xylosyl-(1 → 4)-1,2,3,5-tetra-O-methyl-D-xylitol. The triouronic acid alcohol (V) (414 mg.) was methylated,[10] esterified by diazomethane, and the sirupy product (405 mg.) was reduced by lithium aluminum hydride[11] (1 g.) in tetrahydrofuran (40 ml.). Complete methylation of the partially methylated triitol by Purdie's[12] reagents yielded *O*-α-2,3,4,6-tetra-*O*-methyl-D-glucosyl-(1 → 2)-*O*-β-3,4-di-*O*-methyl-D-xylosyl-(1 → 4)-1,2,3,5-tetra-*O*-methyl-D-xylitol (VI), a colorless sirup (380 mg.), $[\alpha]^{25}_{D}$ + 35.6° in ethanol (*c*, 3.3). The product had a methoxyl content of 47.2% (calcd. for $C_{26}H_{50}O_4$: OCH_3, 52.9) and showed no hydroxyl peak in the infrared. The low methoxyl content was probably due to the presence of inorganic salts.

Hydrolysis of VI. The methylated triitol (325 mg.) was methanolyzed by refluxing for 7 hr. in methanolic hydrogen chloride (12 ml., 4%). Methanol was removed by distillation and the residue, after dissolution in 0.8*N* hydrochloric acid (5 ml.), was heated on a steam bath for 8 hr. Chromatography of the sirup (280 mg.) in solvents B and C showed the presence of 2,3,4,6-tetra-*O*-methyl-D-glucose and 3,4-di-*O*-methyl-D-xylose. The latter could be distinguished from 2,3-di-*O*-methyl-D-xylose by ionophoresis in borate buffer as well as by paper chromatography for 16 hr. in solvent C.

The mixture was examined by gas-liquid partition chromatography using conditions previously described (apiezon M column).[13,14] A single peak corresponding to 1,2,3,5-tetra-*O*-methyl-D-xylitol was obtained. When an authentic sample of 1,2,3,5-tetra-*O*-methyl-D-xylitol was added to the mixture of methylated fragments which were then examined again by gas chromatography only one peak having the retention volume of 1,2,3,5-tetra-*O*-methyl-D-xylitol was observed. Sugars with free reducing groups do not pass through the gas liquid partition chromatogram under the conditions used and hence were not detected.

Separation of components. The mixture of the methylated fragments (260 mg.) was chromatographed on a cellulose column with solvent B. First to emerge from the column was a mixture of 2,3,4,6-tetra-*O*-methyl-D-glucose and 1,2,3,5-tetra-*O*-methyl-D-xylitol. These were not separable by paper chromatography either. Later fractions yielded 3,4-di-*O*-methyl-D-xylose (38 mg., $[\alpha]^{27}_{D}$ + 28.8° in methanol *c*, 1.2) which was identified by converting it into 3,4-di-*O*-methyl-D-xylose-δ-lactone,[15] m.p. and mixed m.p. 67°; $[\alpha]^{24}_{D}$ − 47° → −25° (constant value in water).

Separation of 2,3,4,6-tetra-O-methyl-D-glucose and 1,2,3,5-tetra-O-methyl-D-xylitol. The mixture of the two methylated fragments was oxidized with bromine water for 48 hr. Examination of the product by paper chromatography showed that no unoxidized tetra-*O*-methyl-D-glucose was left. The solution, after removal of excess bromine, was passed through a column of Amberlite IR-45(OH). The effluent was evaporated to a light yellow sirup (107 mg.). Distillation of the latter *in vacuo* at 38–45°/0.02 mm. gave a colorless mobile sirup.

Anal. Calcd. for $C_9H_{20}O_5$: OCH_3, 59.6. Found: OCH_3, 56.0.

(7) G. O. Aspinall and P. C. Das Gupta, *J. Chem. Soc.*, 3627 (1958).

(8) R. H. Marchessault and B. G. Rånby, *Svensk Papperstidn.*, **62**, 230 (1959).

(9) M. Abdel-Akher, J. K. Hamilton, and F. Smith, *J. Am. Chem. Soc.*, **73**, 4691 (1957).

(10) W. N. Haworth, *J. Chem. Soc.*, **107**, 8 (1915).

(11) B. Lythgoe and S. Trippett, *J. Chem. Soc.*, 1983 (1950).

(12) T. Purdie and J. C. Irvine, *J. Chem. Soc.*, **83**, 1021 (1903).

(13) A. G. McInnes, D. H. Ball, F. P. Cooper, and C. T. Bishop, *J. Chromatog.*, **1**, 556 (1958).

(14) C. T. Bishop and F. P. Cooper, *Can. J. Chem.*, **38**, 388 (1960).

(15) Sybil P. James and F. Smith, *J. Chem. Soc.*, 739 (1945).

The infrared spectrum of the sirup was identical with authentic 1,2,3,5-tetra-*O*-methyl-D-xylitol. When examined by gas-liquid partition chromatography, the sirup had the same retention volume as that of the authentic 1,2,3,5-tetra-*O*-methyl-D-xylitol.

4-O-p-Nitrobenzoyl-tetra-O-methyl-D-xylitol. The tetra-*O*-methylxylitol (8 mg.) was converted into its *p*-nitrobenzoate by the procedure of Rebers and Smith.[16] The crystals gave the same X-ray diffraction pattern as that from the authentic 4-*O*-p-nitrobenzoyl-tetra-*O*-methyl-D-xylitol.

Preparation of 1,2,3,5-tetra-O-methyl-D-xylitol. Beechwood xylan[17] (10 g.) was hydrolyzed with pectinase[18] and the cleavage products were removed by dialysis according to the procedure of Painter.[19] In this way 4.75 g. of monosaccharides and oligosaccharides was obtained. The mixture was resolved by charcoal: Celite chromatography.[20] Elution of the column with 5% aqueous ethanol gave two fractions: I, 341 mg., composed of approximately equal amounts of xylose, xylobiose, and xylotriose; and II, 197 mg., predominantly xylotriose.[21]

(16) P. A. Rebers and F. Smith, *J. Am. Chem. Soc.*, **76**, 6097 (1954).
(17) G. A. Adams, *Can. J. Chem.*, **35**, 556 (1957).
(18) A product of Rohm and Haas Co., Philadelphia, Pa.
(19) T. J. Painter, *Can. J. Chem.*, **37**, 497 (1959).
(20) R. L. Whistler and D. F. Durso, *J. Am. Chem. Soc.*, **72**, 677 (1950).

Xylotriose (m.p. 204–205°C., $[\alpha]_D^{25}$ −44°, in water) was reduced with sodium borohydride[9] and the product was methylated successively by the Haworth,[10] Purdie,[11] and Kuhn[22] procedures. Absence of OH absorption in the infrared spectrum showed that the product was fully methylated.

The methylated xylotriitol was hydrolyzed with *N*-hydrochloric acid at 97° for 12 hr. and the hydrolyzate was oxidized directly with bromine for 48 hr. The sugar acids were adsorbed on Amerlite IR-45(OH) and the effluent was extracted continuously with chloroform to give 1,2,3,5-tetra-*O*-methyl-D-xylitol, a sirup which failed to crystallize. The latter was *p*-nitrobenzoylated as described previously to afford crystalline 4-*O*-*p*-nitrobenzoyltetra-*O*-methyl-D-xylitol which was recrystallized from cyclopentane, m.p. 187–189° with preliminary softening or change in crystal form at 165–167°; $[\alpha]_D^{25}$ ± 0° (*c*, 0.8 in methanol).

Anal. Calcd. for $C_{16}H_{23}O_8N$: C, 53.8; H, 6.49. Found: C, 53.4; H, 6.51.

Acknowledgment. Thanks are due to F. P. Cooper for assistance in the gas liquid chromatography and to A. E. Castagne for microanalyses.

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(22) R. Kuhn, H. Trischmann, and I. Löw, *Angew. Chem.*, **67**, 32 (1955).

[CONTRIBUTION FROM THE AGRICULTURAL RESEARCH DIVISION, AND EMERYVILLE RESEARCH CENTER, SHELL DEVELOPMENT CO., A DIVISION OF SHELL OIL CO.]

Preparation, Physical Properties, and Configuration of the Isomers in PHOSDRIN® Insecticide

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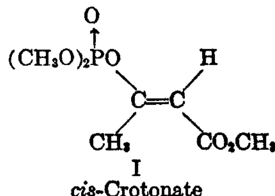
Received March 20, 1961

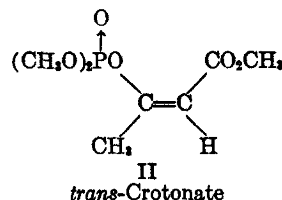
Phosdrin® Insecticide has been separated into its two geometrical isomers I and II by a combination of low temperature fractional crystallization and chromatography. The isomer which predominates in Phosdrin and has the greater toxicity has been assigned the *cis*-crotonate structure on the basis of NMR and infrared absorption spectra as well as other physical evidence. Assignment of structure is also made to a related pair of chlorovinyl phosphates (2-chlorovinyl dimethyl phosphate, V).

Phosdrin® insecticide is prepared by the reaction of trimethyl phosphite with methyl 2-chloroacetoacetate[1] and is essentially a mixture of the two geometrical isomers of methyl 3-(dimethoxyphosphinyloxy)crotonate, I and II.



I
cis-Crotonate

Partition chromatography with hydrated silica gel and chloroform, carbon tetrachloride and hexane was used by Casida[2] to separate these isomers.



II
trans-Crotonate

The isomer partitioning in favor of the organic phase was removed first and designated as the α-isomer. The isomer partitioning in favor of the water phase, when carbon tetrachloride and hexane were the organic phases, was then designated as the β-isomer. Casida[2,3] showed that the α-isomer was the more toxic compound to insects and mammals and predominated over the β-isomer in

(1) A. R. Stiles, U. S. Pat. **2,685,552**, to Shell Development Co.
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