[CONTRIBUTION FROM THE DEPARTMENT OF BIOCHEMISTRY, UNIVERSITY OF CALIFORNIA, BERKELEY]

Methyl Glycosides of D-*Manno*hexodialdose

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The hexose dialdehyde with the D-*manno* configuration, D-*manno*hexodialdose (6-aldo-D-mannose), has been treated with methanol and hydrogen chloride under the conditions of the Emil Fischer glycoside synthesis. Although a number of products resulted, the only crystalline material isolated (20% yield) was the dimethyl diglycoside of the difuranose form of D-*manno*hexodialdose. If the anomeric configuration on each end is assigned independently by its relation to the most distant asymmetric HCOH group, the diglycoside probably has the α,α-configuration.

The methyl glycosides have been of continued use in carbohydrate chemistry for the purpose of stabilizing the cyclic forms of the aldoses and ketoses, thereby facilitating study of the ring forms in which the sugars may exist. We have recently described the preparation of a new hexose dialdehyde, D-*manno*hexodialdose,[1] and are presenting in this paper a description of some of the products formed when the dialdehyde is treated with dry methanol containing hydrogen chloride under the conditions of the classical Fischer glycoside synthesis.[2]

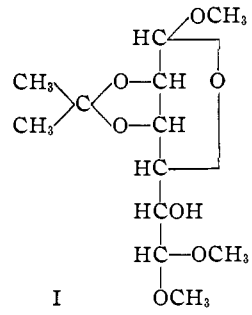
Although several hexodialdoses have been prepared in the past,[3] their conversion to glycosides has not been reported. As the ordinary hexoses, for example, glucose and galactose exist in ring modifications, it is to be expected that the dialdehydes could also form cyclic hemiacetal structures. However, in a manner comparable to the formation of dilactones by saccharic acids, the dialdehydes should be able to form rings from both ends. Because glycoside formation may take place on either end or both ends of the molecule, and in α and β anomeric configurations as well as furanose and pyranose forms, the number of structural isomers possible is unusually large. D-*Manno*hexodialdose is theoretically capable of forming ten dimethyl D-*manno*hexodialdosides: three difuranosides, α-α, α-β, β-β; three dipyranosides, α-α, α-β, β-β; and furanosidopyranosides, α-α, α-β, β-α, β-β. In addition to the ten diglycosides, the possibility exists for sixteen methyl monoglycosides.

The difficult problem of nomenclature which arises when one attempts to describe precisely the structure of these new "two-headed" glycosides stems from the necessity to define the nature of the two rings and the anomeric configurations of the two end carbons. The convention for assignment of anomeric configuration to aldoses relates to the asymmetric HCOH furthest removed from the reducing end of the molecule. On this basis, each end of the diglycoside would be related anomerically to a different HCOH. Since D-*manno*hexodialdose possesses the same configuration at either end, the nomenclature of this compound and its glycosides offers the simplest model from which to devise a congruous convention.

We propose to call these glycosides *mannohexodialdosides* (from the class name dialdose).[1] The designation of ring form and anomeric configuration of a monoglycoside follows readily, *i.e.*, methyl α-D-*manno*furanohexodialdoside. A typical diglycoside, with two anomeric ends and with two ring forms, would be dimethyl-α<1,4>β<6,3>D-*manno*hexodialdoside.

Methyl glycosides of D-*manno*hexodialdose were prepared by treating under reflux either the free dialdehyde or its diacetone compound with dry methanol containing 2% hydrogen chloride. The mixture of products was separated by chromatography on a column of cellulose.[4] When 2,3-4,5-diisopropylidene-D-*manno*hexodialdose was used as the starting material, the product obtained could be resolved into four major components with R_f values 0.89, 0.65, 0.44 and 0.13, in a benzene solvent (see Experimental part). When the free dialdehyde was used as the starting material, only three major components were found: R_f 0.65, 0.44 and 0.13.

Fraction I (R_f 0.89), which did not crystallize, was found on analysis to contain one acetone and three methoxyl groups per hexodialdehyde. The following structure is proposed, resulting apparently from incomplete alcoholysis of the acetone groups (methyl-2,3-isopropylidene-α-D-*manno*furanohexodialdoside 6-dimethyl acetal). This supposition



I

is supported by the fact that I was not formed when the starting material was the free dialdehyde. The acid hydrolysis of I (Fig. 1) indicates that the two reducing groups are freed at different rates, although both with values characteristic of furanosides. The significance of the complex change in optical rotation during hydrolysis of I is not known. (Fig. 2).

(1) C. E. Ballou and H. O. L. Fischer, THIS JOURNAL, **75**, 3673 (1953).

(2) E. Fischer, *Ber.*, **26**, 2400 (1893).

(3) See reference 1.

(4) L. Hough, J. K. N. Jones and W. H. Wadman, *J. Chem. Soc.*, 2511 (1949).

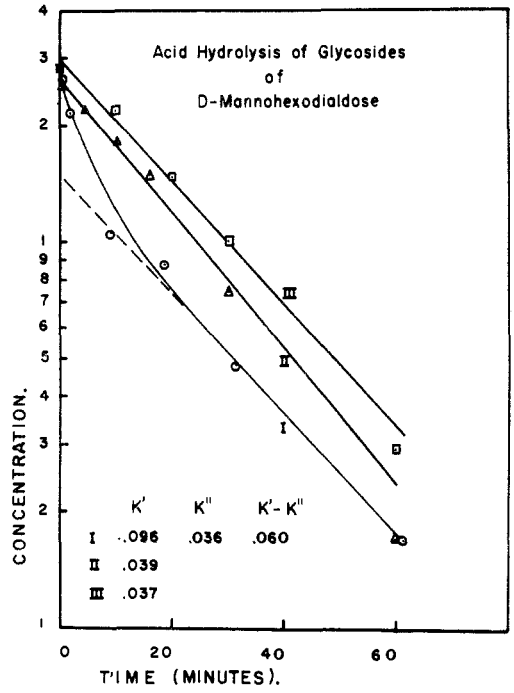


Fig. 1.

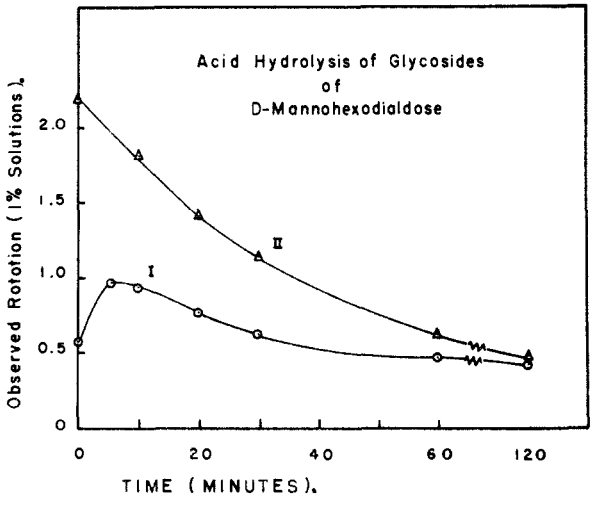
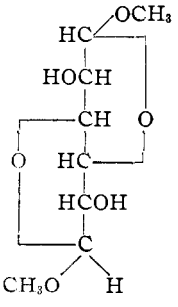


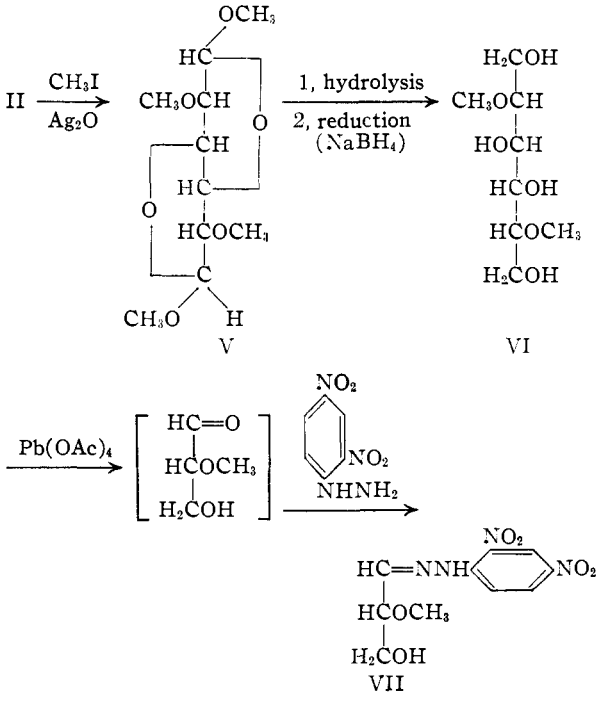
Fig. 2.

Fraction II (R_f 0.65) was obtained crystalline, and has been characterized as the dimethyl α,α-difuranoside of D-*manno*hexodialdose (dimethyl-α_5<1,4>α_2<6,3>D-*manno*hexodialdoside).[5] The structure of II was assigned on the basis of



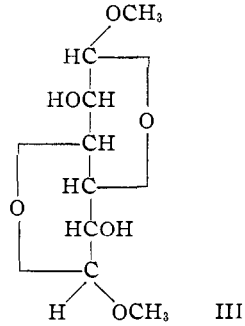
II

elemental analysis, strong dextrorotation (+262°) indicative of two α-glycosidic linkages, a rate of hydrolysis characteristic of furanosides, and methylation studies which show that only positions 2 and 5 are unsubstituted.



The structure of VI was established by definitive synthesis and by the formation of 2-methyl-D-glyceraldehyde following the consumption of one mole of oxidant.

Fraction III (R_f 0.44) was obtained as a non-reducing sirup which analyzed correctly for an isomer of II. Its acid hydrolysis is characteristic of a difuranoside, and the specific rotation (+50°) would be reasonable for the compound with an α- and β-configuration.[6] However, the structure and purity of III must remain in question.



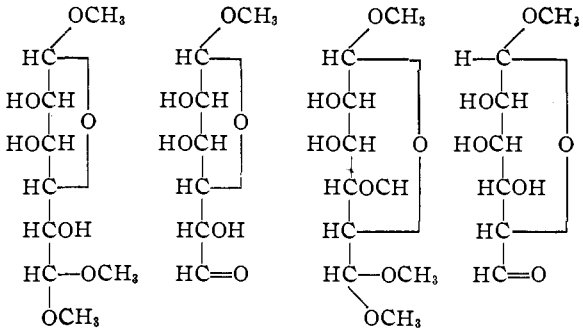
III

Fraction IV (R_f 0.13), also obtained only as a sirup, was, however, slightly reducing to hot Benedict solution. It readily consumed periodate

(5) The subscripts denote the carbon atom to which the anomeric configuration is related.

(6) The molecular rotation of methyl α-D-mannofuranoside (+22,000°, water) and of compound II (+54,000°, chloroform) indicates approximate additive character of the anomeric asymmetry arising from glycoside formation on both ends of D-*manno*hexodialdose. Since methyl β-D-mannofuranoside has a molecular rotation of −20,800° in water, the dimethyl diglycoside III with one α- and one β-linkage might be expected to have a very small rotation due to the cancellation (+22,000° − 20,800° = +1200°). The actual molecular rotation of III is +10,300° in chloroform.

indicating some vicinal glycol groups. The results indicate that IV is a mixture of substances, possibly represented by the formulas



Experimental

Preparation of Methyl Glycosides from Diisopropylidene-D-*manno*hexodialdose.—A mixture of 13.75 g. of 1,2-5,6-diisopropylidene-D-inositol[1] and 23.8 g. of lead tetraacetate in 400 ml. of dry benzene was triturated with a glass rod. After 30 minutes, when all of the oxidant was consumed, the lead acetate was removed by filtration and washed with benzene. The combined benzene filtrate was concentrated *in vacuo* to a dry crystalline material.

The crude diisopropylidene-D-*manno*hexodialdose was dissolved in 200 ml. of dry methanol, and 4.0 g. of dry hydrogen chloride was dissolved in the solution. The mixture was refluxed for three hours, then cooled to room temperature and the acid was neutralized by addition of silver carbonate. The silver chloride was removed by filtration, and the filtrate was concentrated *in vacuo* to a thick sirup, which was dissolved in 25 ml. of ether and filtered a second time. The ether filtrate was concentrated to a thick, slightly reducing sirup that weighed 13.0 g. and had a specific rotation of +140° (chloroform). The methoxyl content was 30.7%.

Chromatographic Resolution of Crude Methyl Glycosides.—A paper chromatogram of the above sirup (using the organic layer of the solvent mixture, benzene:ethanol:water, 170:47:15 (v./v./v.) showed four major spots with the aniline oxalate spray. The R_f and spot colors were: 0.89 (green), 0.65 (green), 0.44 (green) and 0.13 (brown).

The sirup obtained above was resolved (2.0-g. portions at a time) by chromatography on a column of cellulose (4 cm. × 25 cm.) with the benzene solvent.[4] The eluate, collected in 6-ml. fractions, was analyzed by papergrams. Tubes 40–50 contained pure substance R_f 0.89, designated fraction I. Fraction II (R_f 0.65) appeared in tubes 56–65, fraction III (R_f 0.44) in tubes 90–120 and fraction IV (R_f 0.13) in tubes 200–300. The fractions were obtained in the following approximate proportions: 2 parts I, 2 parts II, 1 part III, 3 parts IV.

Properties of Fraction I.—The substance, obtained as a sirup from the column, was dissolved in water and filtered from a small amount of gummy material. The water was removed by distillation and the sirupy residue was distilled at 0.2 mm. pressure (b.p. 93–97°). The pure substance obtained was chromatographically homogeneous. It was non-reducing to hot Fehling solution, but became strongly reducing following mild acid hydrolysis. There was no reaction with sodium metaperiodate. The substance gave $[\alpha]^{21}D$ +58.3° (*c* 1, chloroform).

Anal. Calcd. for $C_{12}H_{22}O_7$ (278.1): C, 51.8; H, 7.9; OCH_3, 33.4; acetone, 20.9. Found: C, 51.5; H, 7.8; OCH_3, 30.5; acetone, 21.5.

The methoxyl and acetone content of I indicate that it results from incomplete deacetonation during the reaction with methanolic hydrogen chloride.

Properties of Fraction II.—On concentration of the eluate from the column, fraction II crystallized readily. It was recrystallized from a benzene and petroleum ether mixture, and melted at 79–80°. Following sublimation at 0.2 mm. and 60–70°, the m.p. was 79–81°. The pure, colorless needles were non-reducing, but following acid hydrolysis, readily reduced Fehling solution. The substance did not react with metaperiodate. The specific rotation was $[\alpha]^{20}D$ +262° (*c* 1.4, chloroform). The analysis and properties are those of a dimethyl diglycoside of D-*manno*hexodialdose.

Anal. Calcd. for $C_8H_{14}O_6$ (206): C, 46.6; H, 6.8; OCH_3, 30.5. Found: C, 46.7; H, 6.9; OCH_3, 29.1.

Properties of Fraction III.—This substance was obtained as a sirup following concentration of the eluate from the column. It was non-reducing prior to acid hydrolysis, but readily reducing after. The optical rotation was $[\alpha]^{20}D$ +50 (*c* 5, chloroform). The analysis corresponded to that of an isomer of II.

Anal. Calcd. for $C_8H_{14}O_6$ (206): C, 46.6; H, 6.8; OCH_3, 30.5. Found: C, 46.0; H, 7.1; OCH_3, 28.6.

Properties of Fraction IV.—This substance had a wide spread in the chromatographic separation. After concentration of the eluate to a thick sirup, the material could not be further purified. It was slightly reducing, and gave $[\alpha]^{20}D$ +45.4 (*c* 3, chloroform). The methoxyl content was 34.6%, and the material consumed 1 mole of periodate per 120 g.

Acid Hydrolysis of Fractions I, II and III.—The rates of acid hydrolysis were determined in an effort to assign ring forms. The hydrolyses, carried out in 0.01 *N* HCl at 95°, were followed polarimetrically and by reducing sugar determinations. The results are presented graphically.

Methylation of Glycoside II.—A sample of the dimethyl diglycoside II (0.35 g.) was dissolved in methyl iodide (14.2 ml.) and ether (25 ml.), and shaken overnight at room temperature with silver oxide (11.6 g.). The silver salt was removed by filtration and washed well with methanol. After removal of the solvent by distillation the product, V, crystallized immediately. It weighed 0.37 g. and melted at 120–122°. This was recrystallized twice from ether to give 0.28 g. of long white needles with m.p. 123–124°, and $[\alpha]^{19}D$ +255° (*c* 1, chloroform).

Anal. Calcd. for $C_{10}H_{18}O_6$ (234.1): C, 51.3; H, 7.7; OCH_3, 53.0. Found: C, 51.0; H, 7.9; OCH_3, 52.6.

Hydrolysis of the methylated product was effected by refluxing 0.2 g. in 1 *N* hydrochloric acid for two hours. The cooled solution was deionized, then treated with an excess of sodium borohydride and left for two hours. It was then acidified with hydrochloric acid and again deionized by the use of the appropriate exchange resins. The neutral water solution was concentrated to dryness, and the sirup obtained was extracted with boiling benzene. The benzene extracts, on cooling, deposited 0.10 g. of hygroscopic needles, which melted over a range, 65–80°. The melting point could not be improved. This dimethylmannitol, VI, showed $[\alpha]^{20}D$ −20° (*c* 1, water).

Anal. Calcd. for $C_8H_{18}O_6$: OCH_3, 29.5. Found: OCH_3, 28.1.

Periodate oxidation of VI was rapid and terminated with an uptake of 1 g. mole per 195 g. of the dimethylmannitol; theory for 2,5-dimethylmannitol is 1 g. mole periodate per mole (210 g.) of the substance.

A dinitrophenylhydrazone prepared from the products of lead tetraacetate oxidation melted at 147–149°, and when mixed with 2-methyl-D-glyceraldehyde dinitrophenylhydrazone (m.p. 147–149°), the m.p. was not depressed.

Preparation of Authentic 2,5-Dimethyl-D-mannitol.—Methylation of 1,6-dibenzoyl-3,4-isopropylidene-D-mannitol (m.p. 94–96°)[7] with silver oxide and methyl iodide in refluxing ethyl ether solution gave a thick sirup which could not be crystallized and did not distil at 0.02 mm. and 185°. It analyzed for 12.7% acetone and 11% methoxyl; theory for dimethyldibenzoylisopropylidenemannitol is 12.5% acetone and 13.5% methoxyl. The specific rotation was +17.5° (*c* 2, chloroform).

Three grams of the methylated derivative was refluxed one hour with 50 ml. of methanol containing 1 ml. of 30% sodium hydroxide to remove the benzoyl groups. The methanol was distilled off, 20 ml. of water added and the aqueous alkaline solution boiled 30 minutes. This solution was then acidified with 6 *N* hydrochloric acid and refluxed for one hour. The cooled solution was filtered to remove most of the benzoic acid, then extracted with ether, and finally neutralized. The neutral water solution was concentrated *in vacuo* to dryness. The residue was extracted several times with 25-ml. portions of boiling benzene. On

(7) H. Ohle, H. Erlbach, H. Hepp and G. Toussaint, *Ber.*, **62**, 2982 (1929).

cooling, the benzene extracts deposited 1.2 g. of crystalline 2,5-dimethyl-D-mannitol (VI). This was recrystallized from benzene and from an acetone–benzene mixture. The material was hygroscopic, melted over a range 65–80° and had $[\alpha]^{21}D$ −18° (*c* 2, water).

Anal. Calcd. for $C_8H_{18}O_6$ (210): C, 45.7; H, 8.6; OCH_3, 29.5. Found: C, 45.8; H, 8.6; OCH_3, 29.1.

Periodate oxidation proceeded with an uptake of 1 g. mole per 200 g. of the dimethylmannitol; theory requires 1 g. mole per 210 g. for 2.5-dimethylmannitol.

Although both 2,5-dimethyl- and 2,4-dimethylmannitol could consume 1 mole of periodate per mole, the products would be different, *i.e.*, 2-methyl-D-glyceraldehyde in one case, and 2,4-dimethyl-D-arabinose in the latter. Therefore, 100 mg. of dimethylmannitol was treated with 1 mole equivalent, 0.22 g., of lead tetraacetate in dry benzene. After one hour, the precipitated lead acetate was removed by filtration and the benzene filtrate was concentrated to dryness. The resulting sirup, dissolved in 10 ml. of water, was treated with 8.5 ml. of perchloric acid–dinitrophenylhydrazine reagent[8] containing 0.24 g. of the precipitant.

After two hours, a light orange crystalline product (100 mg.) (VII) was collected and recrystallized from absolute ethanol. It melted at 145–147°, after slight softening at 141°.

Anal. Calcd. for $C_{10}H_{12}O_6N_4$ (286): N, 19.5; OCH_3, 10.8. Found: N, 19.8; OCH_3, 10.7.

Acknowledgment.—The authors wish to express their gratitude to the Nutrition Foundation, Inc., for the generous support of this work.

(8) C. Neuberg, A. Grauer and P. V. Pisha, *Anal. Chem. Acta*, **7**, 238 (1952).

BERKELEY, CALIFORNIA

[CONTRIBUTION FROM THE DEPARTMENT OF ORGANIC CHEMISTRY, INDIAN ASSOCIATION FOR THE CULTIVATION OF SCIENCE]

Terpenoids. I. Synthesis of the Gross Structure of Zingiberene[1]

BY S. M. MUKHERJI[2] AND N. K. BHATTACHARYYA

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A synthesis of the gross structure proposed by Eschenmoser and Schinz for the monocyclic sesquiterpene hydrocarbon, zingiberene, has been achieved by the application of the Birch reduction. The synthetic product, which appears to be a mixture of bond isomers has been shown to be comparable with the natural zingiberene of Eschenmoser and Schinz, thus supporting their invalidation of the long-accepted structure suggested by Ruzicka and van Veen.

On the basis of degradative evidence Ruzicka and van Veen[3] suggested the structure I for zingiberene, a monocyclic sesquiterpene hydrocarbon, the main constituent of ginger oil from the rhizomes of *Zingiber officinale*, Roscoe. The assignment of the two double bonds in the side-chain was largely based on the assumption of 1,2-reduction of the conjugated system of double bonds in zingiberene by sodium and alcohol to give dihydrozingiberene which must have the structure II. The assumption of 1,2-reduction of the conjugated double bonds in I is not unwarranted in view of the observation by one of us[4] that 2-methyl-2,4-hexadiene on reduction with sodium and alcohol in liquid ammonia gave 2-methyl-2-hexene as the major product as shown by oxidation to acetone and *n*-butyraldehyde. However, the possibility of formation of dihydrozingiberene from such a structure as III was not considered by Ruzicka and van Veen.[3] Eschenmoser and Schinz[5] by a consideration of cationotropic cyclization experiments in the light of the structure of isozingiberene,[6] reinvestigated the structure of zingiberene and represented it by III. Their views were confirmed by ultraviolet and infrared absorption spectra and through condensation with dimethyl acetylenedicarboxylate.

Ruzicka's structure I was synthesized by one of us[7] and the similarity of its properties with those of natural zingiberene as exhibited in refractive indices and dehydrogenation experiments led to the conclusion of gross structural identity between synthetic I and the natural substance. The production of cadalene on sulfur dehydrogenation of I is not surprising in view of the fact that dihydrozingiberene (II) on similar treatment yielded some cadalene.[3] However, in view of Eschenmoser and Schinz's brilliant experiments we undertook the synthesis of the new structure III proposed by them.

The starting material, 6-(4-keto-1′-cyclohexenyl)-2-methyl-2-heptene (VI) was prepared according to Birch and Mukherji[8,9] with the modification that two-stage reduction of 6-(*p*-methoxyphenyl)-2-methyl-2,5-heptadiene (IV) was employed. The ketone VI was isomerized to the corresponding α,β-unsaturated ketone VII by means of sodium ethoxide in ethanol under nitrogen. No solid derivative of VII could be prepared in spite of several attempts. However, the ultraviolet absorption spectrogram (Fig. 1) compares very well with that of cryptone[10] which is unequivocally an α,β-unsaturated ketone of analogous structure. Moreover, the data conform to Woodward's rule for mono-alkyl substituted α,β-unsaturated ketones.[11]

Reaction of methylmagnesium iodide on VII proceeded smoothly to give the corresponding carbinol VIII which was dehydrated with oxalic acid solution[12] to give 6-(4′-methyl-2′,4′-cyclohexadienyl)-2-methyl-2-heptene (III). The hydrocarbon thus obtained proved to be comparable with natural zingiberene[5] in its ultraviolet absorption spectra (Fig. 2).

(1) A preliminary report of this work appeared in *Science and Culture*, **16**, 269 (1950).

(2) Punjab University, Hoshiarpur, East Panjab, India.

(3) L. Ruzicka and van Veen, *Ann.*, **468**, 143 (1929).

(4) S. M. Mukherji, unpublished work.

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(7) S. M. Mukherji, *J. Indian Chem. Soc.*, **25**, 155 (1948).

(8) A. J. Birch, *J. Chem. Soc.*, 430 (1944); 809 (1945); 593 (1946); 102, 1642 (1947).

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(12) A. S. Galloway, J. Dewar and J. Read, *J. Chem. Soc.*, 1595 (1936).