liquid (0.5 g.), b.p. (bath temp.) 115–120° (0.005 mm.), $n^{25}D$ 1.4694, $[\alpha]^{26}D$ +30.5° in methanol (*c* 1.2). *Anal.* Calcd. for $C_7H_{14}O_5$: OCH_3, 34.8. Found: OCH_3, 34.0.

Action of Hydrogen Bromide on 2,5-Anhydro-L-arabinose Dimethyl Acetal.—2,5-Anhydro-L-arabinose dimethyl acetal (22 mg.) was exposed to an atmosphere of hydrogen bromide for 30 seconds during which time the sirup became very dark. It was dissolved in acetone and the solution neutralized with silver carbonate. After filtration, the solution was evaporated to leave a light brown sirup (18 mg.) which showed $[\alpha]^{26}D$ +80° in methanol (*c* 0.4).

Synthesis of 2,5-Anhydro-D-arabitol. 2,5-Anhydro-3,4-*O*-isopropylidene-D-arabitol.—3,6-Anhydro-4,5-*O*-isopropylidene-D-mannitol (VI), 6.7 g., m.p. 84°, prepared by the method of Foster and Overend,[9] was treated with 0.46 *N* sodium periodate (500 ml.) at 5° in the dark for approximately 48 hours, after which time 1.0 mole of periodate per mole of material had been consumed. To the resulting solution was added 1 *M* barium chloride (120 ml.) and the precipitate filtered. The filtrate was evaporated in the presence of strontium carbonate under reduced pressure to about 50 ml., the resulting mixture extracted with acetone and the aqueous acetone solution evaporated to dryness. The solid residue was extracted six times with boiling ethyl acetate and the combined extracts were evaporated to give sirupy 2,5-anhydro-3,4-*O*-isopropylidene-D-arabinose (VII), 4.29 g. A solution of the latter in thiophene-free benzene (100 ml.) showed the following changes in optical rotation: $[\alpha]^{20}D$ −126° (after 5 min.), −154° (20 min.), −160° (40 min.), −164° (46 hr.), −170° (51 hr.), −176° (73 hr., mutarotation incomplete). The benzene solution was evaporated to a sirup which was purified by extraction with ice-cold ethanol.

The solution of the sirupy 2,5-anhydro-3,4-*O*-isopropylidene-D-arabinose, in ethanol (100 ml.), was reduced using Raney nickel catalyst and 1000 lb. per square inch pressure of hydrogen at 120° for 7 hours. The resulting solution was evaporated under reduced pressure to give a crystalline residue which readily sublimed. Recrystallization of the material from benzene–ether–petroleum ether (b.p. 30–60°) at 5° gave 2,5-anhydro-3,4-*O*-isopropylidene-D-arabitol (VIII) as long colorless needles, m.p. 75–76°, $[\alpha]^{23}D$ −40.5° in water (*c* 5.4). *Anal.* Calcd. for $C_8H_{14}O_4$: C, 55.2; H, 8.1. Found: C, 55.0; H, 8.5.

2,5-Anhydro-3,4-*O*-isopropylidene-1-*O*-*p*-toluenesulfonyl-D-arabitol, prepared from the corresponding alcohol in the usual way, gave on recrystallization from aqueous ethanol, crystals, m.p. 67–68°, $[\alpha]^{23}D$ −33° in ethanol (*c* 1.6). *Anal.* Calcd. for $C_{15}H_{20}O_6S$: C, 54.9; H, 6.1. Found: C, 55.1; H, 6.3.

2,5-Anhydro-D-arabitol (IX).—2,5-Anhydro-3,4-*O*-isopropylidene-D-arabitol (0.1 g.), dissolved in *N* sulfuric acid (10 ml.), was heated on a boiling water-bath for two hours. The acid was neutralized with barium hydroxide, the solution filtered and the filtrate evaporated to dryness. The residue was extracted four times with boiling ethyl acetate and the combined extracts evaporated to a sirup (0.08 g.), which on distillation gave 2,5-anhydro-D-arabitol (IX), b.p. (bath temp.) 125–135° (0.24 mm.), $n^{23}D$ 1.4941, $[\alpha]^{23}D$ −1.4 ± 0.5° in water (*c* 0.9). *Anal.* Calcd. for $C_5H_{10}O_4$: C, 44.8 H, 7.5. Found: C, 44.4; H, 7.7.

2,5-Anhydro-1,3,4-tri-*O*-*p*-toluenesulfonyl-D-arabitol, prepared from the 2,5-anhydro-D-arabitol in the usual way, had m.p. 128–129°, $[\alpha]^{23}D$ +27.4° in chloroform (*c* 6.4) (after recrystallization from aqueous ethanol). When mixed with the L-isomer, the melting point was depressed to 110–111°.

ST. PAUL, MINNESOTA

[CONTRIBUTION FROM THE CHEMISTRY DEPARTMENT, BRISTOL UNIVERSITY]

Methylene Derivatives of L-Rhamnose[1]

BY P. ANDREWS, L. HOUGH AND J. K. N. JONES[2]

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The acid-catalyzed condensation of formaldehyde with L-rhamnose has yielded at least six methylene derivatives which have been separated and identified as 3,4-*O*-dimethyleneoxy-L-rhamnose, 2,3-*O*-dimethyleneoxy-L-rhamnose, 3,5-mono-*O*-methylene-L-rhamnose, 1,2;3,5-di-*O*-methylene-L-rhamnose, and *O*-dimethyleneoxy-mono-*O*-methylene-L-rhamnose and 2,3-mono-*O*-methylene-L-rhamnose. The first five crystallized.

Crystalline mono-*O*-methylene-L-rhamnose (m.p. 76°)[3] and mono-*O*-methylene-L-rhamnonolactone[4] (m.p. 151–152°) have been described but their structures are unknown. When L-rhamnose reacted with paraformaldehyde in the presence of sulfuric acid, at least six compounds were produced as indicated by paper chromatography. After neutralization the mixture was fractionated on a column of hydrocellulose,[5] giving five different crystalline methylene derivatives. None was identical with the compound (m.p. 76°) of Lobry de Bruyn and Alberda van Ekenstein.[3]

Three of the crystalline compounds (I, X, IX) reduced Fehling solution, sodium hypoiodite and sodium metaperiodate, whereas the other two (VIII, XII) did not. All were readily hydrolysed by acid to L-rhamnose and formaldehyde.

The main product of the condensation was 3,4-*O*-dimethyleneoxy-L-rhamnose (I). On hydrolysis it gave two moles of formaldehyde. It consumed one mole of metaperiodate with oxidative rupture to give one mole each of formic acid and of 5-deoxy-2,3-*O*-dimethyleneoxy-L-arabinose (II), thus showing the existence of an α-hydroxycarbonyl group in I. This was confirmed by methylation of I, followed by hydrolysis which gave 2-*O*-methyl-L-rhamnose (IV). The position of the *O*-dimethyleneoxy group in I was determined by reduction with sodium borohydride[6] to 3,4-*O*-dimethyleneoxy-L-rhamnitol (V). Methylation of V afforded the 1,2,5-tri-*O*-methyl derivative which on hydrolysis gave 1,2,5-tri-*O*-methyl-L-rhamnitol (VI). VI consumed one mole of metaperiodate, thus proving, in conjunction with the above evidence, that the *O*-dimethyleneoxy group was situated at C_3 and C_4 of L-rhamnopyranose.

2,3-*O*-Dimethyleneoxy-L-rhamnose (X) moved at a slower rate on the paper chromatogram than the 3,4-isomer I. It also yielded two moles of formaldehyde on hydrolysis but, on oxidation with meta-

(1) Paper presented before the Division of Carbohydrate Chemistry at the 125th Meeting of the American Chemical Society at Kansas City, Mo., March, 1954.

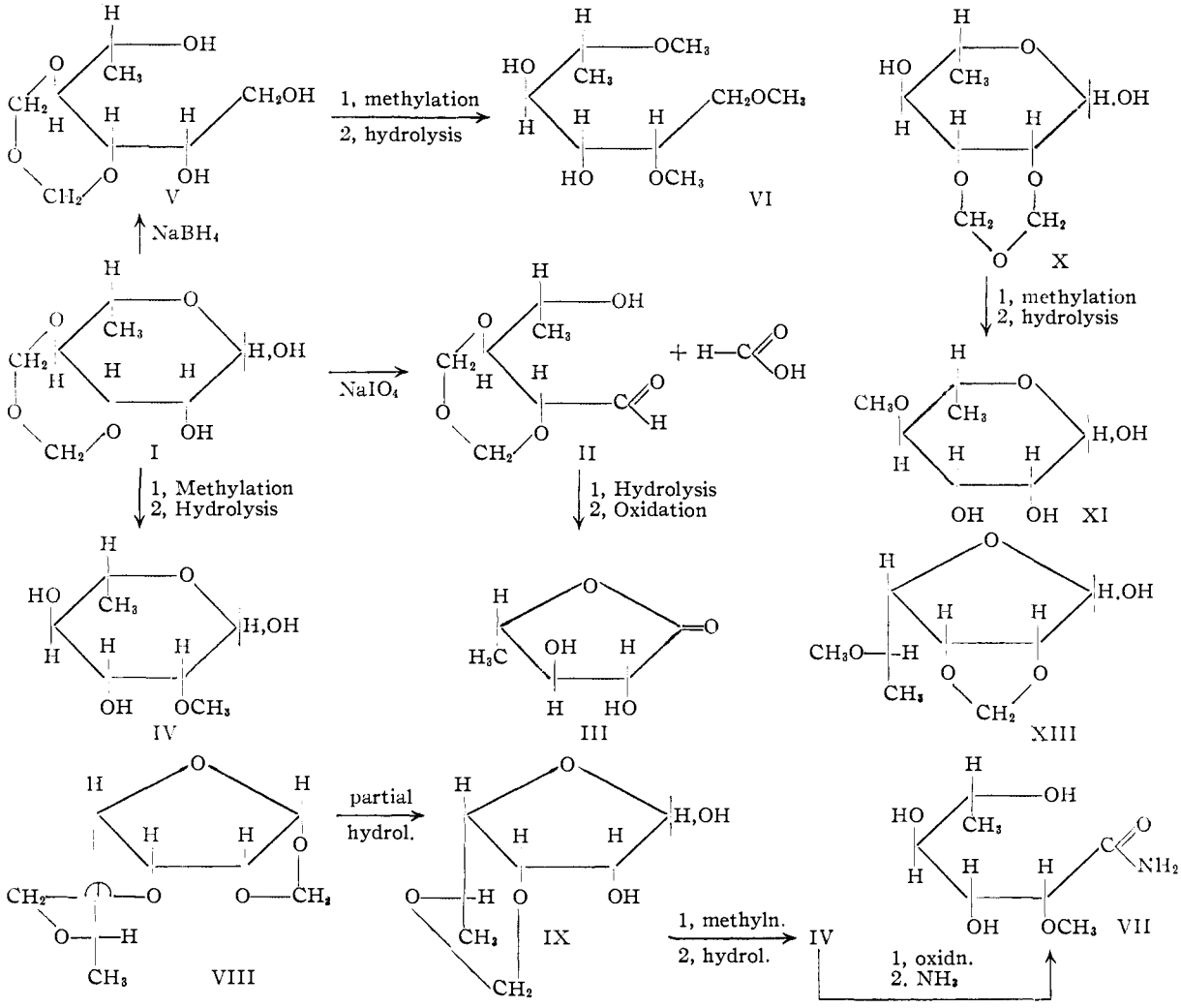
(2) Queen's University, Kingston, Ontario, Canada.

(3) C. A. Lobry de Bruyn and W. Alberda van Ekenstein, *Rec. trav. chim.*, **22**, 159 (1903).

(4) K. Weber and B. Tollens, *Ann.*, **299**, 323 (1898).

(5) L. Hough, J. K. N. Jones and W. H. Wadman, *J. Chem. Soc.*, 1702 (1950).

(6) B. Abdel-Akher, J. K. Hamilton and F. Smith, THIS JOURNAL, **73**, 4691 (1951).



periodate, it reacted only slowly and without the production of formic acid. The slow oxidation is believed to be due to the stability of the cyclic hemiacetal structure of L-rhamnose, as already has been observed with the methyl ether derivatives.[7] Methylation of X produced first the methyl β-L-rhamnoside and then the methyl 4-*O*-methyl-β-L-rhamnoside which on hydrolysis yielded 4-*O*-methyl-L-rhamnose. Therefore X possesses an *O*-dimethyleneoxy group at C_2 and C_3. These *O*-dimethylenoxy compounds, which possess a seven-membered trihetero ring, are similar to the *O*-diethylideneoxy-D-glucose derivatives prepared by Appel, Haworth, Cox and Llewellyn[8] and Helferich and Porck.[9]

The derivative which moved slowest on the paper chromatogram is probably 3,5-mono-*O*-methylene-L-rhamnose (IX), but the possibility of it being the 3,4-isomer is not positively excluded. Hydrolysis of IX gave one mole of formaldehyde. On oxidation with periodate it consumed one mole of the reagent with the formation of one mole of formic acid. Its high negative rotation ($[\alpha]_D$ −64°) is suggestive of a furanose ring. Methylation of IX formed the methyl glycoside-2-methyl ether, which, on hydrolysis, gave 2-*O*-methyl-L-rhamnose. Oxidation of IX with yellow mercuric oxide gave a lactone with the properties of a furanolactone. However, this evidence is inconclusive as lactone rules may not hold[10] when another ring system is present, in this case due to the methylene bridge The compound IX thus possesses free hydroxyls on C_1, C_2 and, probably, C_4.

The non-reducing crystalline compound VIII could not be detected on the paper chromatogram and gave on hydrolysis L-rhamnose and two moles of formaldehyde. Partial hydrolysis gave in low yield crystalline IX. If IX is 3,5-mono-*O*-methylene-L-rhamnose and not the 3,4-isomer, then VIII is 1,2;3,5-di-*O*-methylene-L-rhamnose. Both D-xylose[11,11a] and D-glucose[12] give 1,2;3,5-di-*O*-methylene derivatives.

Complete hydrolysis of the fifth crystalline compound XII, which was also non-reducing, gave

(7) F. Brown, L. Hough and J. K. N. Jones, *J. Chem. Soc.*, 1125 (1950).

(8) H. Appel, W. N. Haworth, E. G. Cox and F. J. Llewellyn, *J. Chem. Soc.*, 793 (1938).

(9) B. Helferich and A. Porck, *Ann.*, **582**, 225 (1953).

(10) F. Smith, personal communication.

(11) O. T. Schmidt, *Angew. Chem.*, **60A**, 252 (1948).

(11a) O. T. Schmidt and Gertrud Nieswandt, *Chem. Ber.*, **82**, 1 (1949).

(12) O. T. Schmidt, *ibid.*, **21**, 741 (1953).

three moles of formaldehyde per mole of L-rhamnose. It appears to be an *O*-dimethyleneoxy-mono-*O*-methylene derivative, but it could not be related to I, X or IX, none of the products of partial hydrolysis corresponding to any one of these compounds on the paper chromatogram.

Formalin and hydrochloric acid with L-rhamnose yielded VIII, IX and, after purification until it gave only one spot on the paper chromatogram, a sirupy mono-*O*-methylene-L-rhamnose (E). Hydrolysis gave rather less than one mole of formaldehyde. It reduced Fehling solution and consumed on oxidation with alkaline hypoiodite one mole of iodine. With sodium metaperiodate after three hours, 0.16 mole of formic acid was liberated with consumption of 0.18 mole of periodate, and, after 186 hours, the formic acid yield was substantially the same, but the periodate uptake had risen to 0.9 mole. The evidence suggests that sirup E is a mixture containing a little 3,4- or 3,5-mono-*O*-methylene compound, but mainly another mono-*O*-methylene isomer in which carbon atom 2 is substituted and in which before oxidation with periodate the relatively stable hemiacetal ring must open (*cf.* 7). If IX is 3,5-mono-*O*-methylene-L-rhamnose, as indicated above, the sirup E contains the 3,4-isomer since these two isomers are widely separated on the paper chromatogram (R_g 0.67 and 0.92, respectively). The main constituent is either 2,3- or 2,5-mono-*O*-methylene-L-rhamnose on the periodate evidence. Methylation of the sirup E gave a sirupy methyl-*O*-methyl-*O*-methylene-L-rhamnoside. Hydrolysis gave a partially crystalline material from which crystalline *N*-phenyl-L-rhamnosylamine 2-methyl ether (derived from the 3,4(?)-isomer) and 5-*O*-methyl-2,3-*O*-methylene-L-rhamnose (XIII) were isolated. The latter compound is extremely stable to acid hydrolysis. However, on oxidation with bromine water it was oxidized with loss of the methylene group to 5-*O*-methyl-L-rhamnono-γ-lactone. These observations suggest that the sirupy E contained a mixture of 2,3-mono-*O*-methylene-L-rhamnose (*ca.* 80%) and 3,4-(or 3,5-) mono-*O*-methylene-L-rhamnose (*ca.* 20%). Reduction of sirup (E) with sodium borohydride[6] gave a mixture of at least two mono-*O*-methylene rhamnitols as indicated by paper chromatography.

Experimental

Unless stated otherwise: (i) Chromatography was carried out by the descending method[13] on Whatman No. 1 filter paper, using *n*-butyl alcohol–ethanol–water (40:11:19 parts v./v.) as the mobile phase. (ii) Sugars and derivatives were located on the chromatograms with either ammoniacal silver nitrate[13] or an *ca.* 3% solution of *p*-anisidine hydrochloride in *n*-butyl alcohol–water containing a little stannous chloride.[5] (iii) The rate of movement of compounds on the chromatogram is quoted relative to that of tetra-*O*-methyl-D-glucopyranose (*i.e.*, R_g value). (iv) Hydrolyses were performed with *N* sulfuric acid at 100° and after 8 *N* cooling the acid was removed with Amberlite IR4B anion-exchange resin. (v) Optical rotations were determined in aqueous solution at 20°. (vi) "Light petroleum" refers to the fraction, b.p. 60–80°. (vii) Solutions were concentrated under reduced pressure.

Preparation of the Methylene Derivatives. (a) With Paraformaldehyde and Sulfuric Acid.—L-Rhamnose hydrate (20 g.) was dissolved in water (10 cc.), sulfuric acid (5 drops) and paraformaldehyde (30 g.) were added, and the mixture heated in a stoppered flask on the boiling water-bath. After 2 hours the solution was cooled, diluted to *ca.* 50 cc., neutralized with barium carbonate and filtered. The filtrate was extracted with chloroform (2 × 50 cc.), giving a colorless sirup (A) (0.5 g.), easily soluble in chloroform. Further continuous extraction of the filtrate with chloroform for 18 hours yielded a second colorless chloroform-soluble sirup (B) (7.2 g.). The aqueous solution then contained rhamnose only (detected chromatographically).

(b) **With Formalin and Hydrochloric Acid.**—L-Rhamnose hydrate (20 g.) was dissolved in 40% formalin (40 cc.), concentrated hydrochloric acid (40 cc.) was added, and the solution heated on the boiling water-bath for 0.5 hour. After cooling, the dark-brown solution was neutralized with lead carbonate, filtered, treated with hydrogen sulfide, filtered then neutralized with silver carbonate, filtered, treated again with hydrogen sulfide and again filtered. The resultant solution was extracted continuously with chloroform for 18 hours, after which time it contained only rhamnose. Evaporation of the chloroform extract gave a dark brown sirup (6.9 g.) which was dissolved in water, and the solution shaken with chloroform (3 × 50 cc.). Evaporation of this extract gave a brown sirup (C) (1.6 g.), and evaporation of the aqueous layer gave a brown sirup (D) (5.3 g.), less soluble in chloroform than (C).

Examination of the Chloroform-soluble Sirups.—(A) When triturated with ethanol, this sirup crystallized. Recrystallization from ethanol afforded colorless crystals of **1,2;3,5-di-*O*-methylene-L-rhamnofuranose** (VIII) (0.2 g.), m.p. 100°, $[\alpha]_D$ +3 ± 1° (*c* 2.3); $[\alpha]_D$ −27° (*c* 2.5 in MeOH).

Anal. Calcd. for $C_8H_{12}O_5$: C, 51.1; H, 6.4. Found: C, 50.75; H, 6.4.

A second crop of crystals (*ca.* 0.2 g.) from the mother liquors had m.p. 87–92°. They were redissolved in ethanol and the solution allowed to evaporate slowly at 0°; a further crop of VIII, admixed with some colorless cubes, remained. The latter (30 mg.) were picked out by hand, and after washing with ethanol had m.p. 144–146° and $[\alpha]_D$ +3 ± 2° (*c* 0.6), being apparently an **O-dimethyleneoxy-O-methylene-L-rhamnose (XII).**

Anal. Calcd. for $C_9H_{14}O_6$: C, 49.5; H, 6.4. Found: C, 49.8; H, 6.5.

Neither VIII nor XII could be detected on the paper chromatogram with either of the spray reagents.

(B) This sirup smelled strongly of formaldehyde; trioxane sublimed from it when it was heated under reduced pressure. Chromatographic examination showed that it contained compounds with R_g 0.8–0.95 (mainly) and 0.67, and a little rhamnose (R_g 0.41). This mixture (*ca.* 7 g.) was fractionated by chromatography on a column of powdered hydrocellulose[5] (25 × 5 cm.) using light petroleum (b.p. 100–120°)-*n*-butyl alcohol mixtures (70:30 changed in stages to 40:60 parts v./v. as the mobile phase); finally ethanol was added to elute the rhamnose. After paper chromatographic examination of the effluent, the portion containing rhamnose (*ca.* 1 g.) was discarded, and the remainder divided into six fractions. On evaporation they all yielded sirups, and of these fractions five contained more than one methylene derivative. Some began to crystallize after keeping at 30° for several days, and eventually yielded, after fractional crystallizations from chloroform–light petroleum, two crystalline products. **3,4-*O*-Dimethyleneoxy-β-L-rhamnose (I)** (2 g.) was obtained as colorless prisms, m.p. 132–133°, $[\alpha]_D$ +31° (10 min., *c* 1.6) → +17.5° (4 hours) → + 14° (24 hours, equil. value), with R_g 0.89.

Anal. Calcd. for $C_8H_{14}O_6$: C, 46.6; H, 6.8. Found: C, 46.9; H, 6.8.

The other derivative, **2,3-*O*-dimethyleneoxy-α-L-rhamnose (X)** (0.3 g.) gave rosettes of colorless blunt needles, m.p. 143–144°, $[\alpha]_D$ +65° (5 min., *c* 1.1) → +72° (18 hours, equil. value) with R_g 0.84.

Anal. Calcd. for $C_8H_{14}O_6$: C, 46.6; H, 6.8. Found: C, 46.6; H, 6.8.

Evaporation of the sixth fraction of the column effluent gave crystalline **3,5-*O*-methylene-L-rhamnose (IX)** (0.4 g.) (which crystallized from ethanol as small colorless needles, m.p. 146°, $[\alpha]_D$ −64° (10 min., *c* 1.0), unchanged on adding a trace of ammonia; with R_g 0.67.

Anal. Calcd. for $C_7H_{12}O_5$: C, 47.7; H, 6.8. Found: C, 47.7; H, 6.9.

(13) S. M. Partridge, *Biochem. J.*, **42**, 238 (1948).

Paper chromatographic examination of the portion of B remaining after isolation of the above compounds indicated that it consisted mainly of material with R_g *ca.* 0.9, together with traces of a substance with R_g 0.75. The sirup crystallized very slowly giving a small yield of I with m.p. 130–132°, R_g 0.89. The residual sirup was methylated with Purdie reagents, and the resultant sirup hydrolyzed for 3 hours. The solution was neutralized with barium carbonate, filtered and concentrated. Paper chromatographic examination of the partly crystalline product (1.2 g.) indicated that it consisted largely of two compounds, with R_g 0.98 (crystalline) and 0.66 (sirupy), respectively. The crystalline material (*ca.* 0.5 g.) was extracted from the mixture with boiling cyclohexane, and shown to be 5-*O*-methyl-2,3-*O*-methylene-β-L-rhamnose (XIII) with m.p. 75–77° after recrystallization from the same solvent, by mixed m.p. (76–78°) with a specimen of proved structure (see below). The sirup contained 2-*O*-methyl-L-rhamnose characterized as its aniline derivative (see below).

(C) This sirup did not crystallize spontaneously, nor did it contain compounds which could be detected on the paper chromatogram with either of the spray reagents. When seeded with 1,2;3,5-di-*O*-methylene-L-rhamnose (VIII) it yielded a small crop of this compound, m.p. and mixed m.p. 100°. The remaining sirup was submitted to a partial hydrolysis (*N* sulfuric acid at 100° for 30 min.) and the products had rates of movement on the paper chromatogram identical with those of the compounds in (D); the material with R_g 0.92 was present in greatest amount.

(D) Chromatographic examination indicated that this sirup consisted mainly of material with R_g *ca.* 0.9, together with smaller amounts of 3,5-*O*-methylenerhamnose (IX), R_g 0.67, and rhamnose. The mixture (4.6 g.) was fractionated on a hydrocellulose column (28 × 4 cm.), using as eluant benzene–ethanol mixtures (commencing with 5% ethanol, and increasing this percentage as the separation proceeded) half saturated with water. Appropriate subdivision of the effluent and removal of the solvent gave: (1) rhamnose (*ca.* 0.5 g.); (2) 3,5-*O*-methylene-L-rhamnose (IX) (0.35 g.), which after recrystallization from ethanol had m.p. and mixed m.p. 146°; (3) a sirup (E) (2.5 g.), $[\alpha]_D$ +21° (*c*, 1.0) which gave only one spot, R_g 0.92, on the paper chromatogram, but which failed to crystallize. (4) Sirup (*ca.* 1.2 g.) consisting mainly of material R_g 0.92; it was not further examined.

Anal. Calcd. for mono-*O*-methylenerhamnose (E), $C_7H_{12}O_5$: C, 47.7; H, 6.8. Found: C, 47.9; H, 6.6.

Hydrolysis of the Methylene Derivatives.—All the crystalline compounds were completely hydrolyzed in *N* sulfuric acid at 100° within one hour, giving rhamnose (detected chromatographically) and formaldehyde. The same products were given by sirup E, but after 4 hours the hydrolysis was still incomplete. In each case, formaldehyde liberated was identified and estimated as follows: as the dimedon derivative (FD) (m.p.'s sharp, between 187° and 190° in each case). The sugar (5–15 mg.) was hydrolyzed in a sealed tube with acid (1 cc.) for 1 hour (5 hours for E). The tube was then cooled, and shaken vigorously for *ca.* 1 minute to dissolve any gaseous formaldehyde it might contain (omission of the shaking gave varying low results). The contents were then centrifuged to one end of the tube, which was opened and the contents immediately washed into water (10 cc.). The directions of Bell[14] were then followed. The results are collected in Table I.

TABLE I

Compound	IX	I	X	VIII	XII	E
Molecular weight (calcd.)	176	206	206	188	218	176
Weight taken, mg.	12.3	10.5	7.3	9.6	6.5	13.2
Yield of FD, mg.	18.4	28.6	20.6	28.9	24.9	17.2
Yield of formaldehyde, moles	0.90	1.92	1.99	1.94	2.86	0.79

The progress of the hydrolysis of 3,4-*O*-dimethyleneoxy-L-rhamnose (I) in 0.01 *N* sulfuric acid at 100° was followed by paper chromatography. The reaction was roughly half complete in 3–4 hours, and after 9 hours only a trace of the compound remained. For similar experiments on VIII and XII see below.

(14) D. J. Bell, *J. Chem. Soc.*, 992 (1948).

Reducing Power of the Methylene Derivatives.—Compounds IX, I, X and the material E all reduced Fehling solution; their reducing power was estimated with alkaline hypoiodite.[15] To the sugar (*ca.* 5 mg.) in water (10 cc.) were added sodium hydroxide–phosphate buffer[16] (*p*H 11.4) (4 cc.) and iodine (0.1 *N*, 2 cc.). After 18 hours, each reaction mixture was acidified with 2 *N* sulfuric acid (4 cc.) and the liberated iodine titrated with 0.02 *N* sodium thiosulfate. The results are collected in Table II.

TABLE II

Compound	IX	I	X	E
Molecular weight (calcd.)	176	206	206	176
Weight taken, mg.	3.1, 3.5	5.0, 5.05	4.65	6.2, 4.1
Iodine consumed (cc. 0.01 *N*)	3.45, 4.14	3.16, 3.36	4.37	6.90, 4.37
Iodine consumed (moles)[a]	0.98, 1.04	0.65, 0.69	0.97	0.98, 0.94

[a] Under similar conditions, L-rhamnose consumed iodine equivalent to 0.82 mole of iodine.

Periodate Oxidations. (a) **Of IX, I and X.**—To each compound (5–10 mg.) in water (5 cc.) was added 0.2 *N* sodium metaperiodate (2 cc.). After 3 hours, to the reaction mixtures were added either (i) saturated sodium hydrogen carbonate solution (20 cc.), 0.1 *N* sodium arsenite (5 cc.) and excess potassium iodide, and the excess arsenite titrated with 0.01 *N* iodine, or (ii) ethylene glycol (1 cc.), and the formic acid titrated with 0.01 *N* sodium hydroxide. The results are given in Table III.

TABLE III

	Compound	IX	I	X
	Molecular weight (calcd.)	176	206	206
(i)	Weight taken, mg.	6.6	8.5	6.65
(i)	Periodate uptake (cc. 0.01 *N*)	8.02	8.48	1.00
(i)	Periodate uptake, moles	1.07	1.03	0.15
(ii)	Weight taken, mg.	7.2	7.6	6.8
(ii)	Formic acid yield (cc. 0.01 *N*)	3.91	3.10	Nil
(ii)	Formic acid yield, moles	0.96	0.84	Nil

The formyl esters which were produced by the oxidation of I and IX underwent a slow hydrolysis in aqueous solution at *p*H[6] at room temperature. Since this hydrolysis was incomplete after 24 hours, the formic acid titrations were completed on the hot solutions.

(b) **Of E.**—This sirup was oxidized by metaperiodate as above, and the extent of the reaction determined at intervals (Table IV).

TABLE IV

Weight taken, mg.	Period of oxidation, hr.	Periodate uptake: Cc. 0.01 *N*	Periodate uptake: Moles/176 g. of (E)
4.5	3	0.91	0.18
7.1	22	2.55	.315
5.0	71	3.68	.65
6.4	186	6.39	.90
		Formic acid yield: Cc. 0.01 *N*	Moles/176 g. of (E)
7.6	3	0.70	0.16
4.85	186	.51	.18

Proof of the Structures of the Methylene Derivatives. 3,4-*O*-Dimethyleneoxy-L-rhamnose (I).—The compound (300 mg.) was oxidized in 0.3 *N* sodium metaperiodate solution (30 cc.) and after 17 hours the sirupy product (280 mg.) (II) was isolated by continuous extraction of the reaction mixture with chloroform. This product was hydrolyzed with acid (10 cc.) for one hour, the cooled solution was neutralized, filtered and concentrated, giving sirupy 5-deoxy-L-arabinose (170 mg.) with $[\alpha]_D$ −3° (*c* 3.4) and R_g 0.68. When oxidized with bromine water in the usual way, this yielded 5-deoxy-L-arabono-γ-lactone (150 mg.) (III), which crystallized from a large volume of chloroform in colorless needles several cm. long, m.p. 125°, $[\alpha]_D$ −39° (init. value, *c* 0.7) → −34° (7 days).

(15) E. L. Hirst, L. Hough and J. K. N. Jones, *ibid.*, 928 (1949).

(16) O. G. Ingles and G. C. Israel, *ibid.*, 810 (1948).

Anal. Calcd. for $C_5H_8O_4$: C, 45.4; H, 6.1. Found: C, 45.5; H, 5.9.

The compound I (400 mg.) was dissolved in acetone (2 cc.) and methylated with silver oxide and methyl iodide, giving **3,4-di-*O*-methyleneoxy-2-*O*-methyl-L-rhamnoside** (400 mg.), which crystallized from ether in colorless needles, or from light petroleum (b.p. 40–60°) as colorless cubes, m.p. 121°, $[\alpha]_D$ +88° (*c* 1.2).

Anal. Calcd. for $C_8H_{12}O_4(OCH_3)_2$: C, 51.3; H, 7.7; OMe, 26.5. Found: C, 51.1; H, 7.5; OMe, 26.2.

The rhamnoside (250 mg.) was hydrolyzed in acid (5 cc.) for 3 hours; evaporation of the neutralized solution gave sirupy 2-*O*-methyl-L-rhamnose (IV) (200 mg.) with $[\alpha]_D$ +24° (*c* 3.8).

Anal. Calcd. for $C_6H_{11}O_4(OCH_3)$: OMe, 17.4. Found: OMe, 17.4.

It had R_g 0.66 and gave an orange-brown spot on the paper chromatogram with *p*-anisidine hydrochloride. 3-*O*-Methylrhamnose had R_g 0.62 and gave a gray-brown spot, and 4-*O*-methyl-rhamnose had R_g 0.65 and gave a yellow-brown spot. The sirupy monomethylrhamnose, when heated under reflux with aniline in ethanol for one hour, gave ***N*-phenyl-L-rhamnosylamine 2-methyl ether**, which crystallized from ether as white needles, m.p. 152°, $[\alpha]_D$ + 43° (init. value, *c* 0.9 in pyridine).

Anal. Calcd. for $C_{12}H_{16}O_5N(OCH_3)$: C, 61.7; H, 7.5; N, 5.5; OMe, 12.3. Found: C, 61.5; H, 7.4; N, 5.8; OMe, 13.4.

When oxidized with bromine water in the usual way, IV gave **2-*O*-methyl-L-rhamnono-γ-lactone**, which crystallized from chloroform as tiny colorless needles, m.p. 116–117° with previous softening from 114°, $[\alpha]_D$ −62° (init. value, *c* 1.2 in a 1 dm. tube) → −64° (117 hours).

Anal. Calcd. for $C_6H_9O_4(OCH_3)$: C, 47.7; H, 6.8; OMe, 17.6. Found: C, 48.0; H, 6.4; OMe, 17.0.

The derived **amide VII**, prepared by treating the lactone with methanolic ammonia, crystallized only after impurities had been removed from it by separation on a wide (20 cm.) paper chromatogram (the amide was detected with ninhydrin). When recrystallized from acetone–light petroleum it formed colorless crystals, m.p. 117–118°.

Anal. Calcd. for $C_7H_{15}O_5N$: C, 43.5; H, 7.8; N, 7.25. Found: C, 43.7; H, 7.7; N, 6.9.

It gave a negative Weerman test,[17] proving the absence of an α-hydroxy amide group.

Reduction of (I).—(I) (500 mg.) was dissolved in water (10 cc.) and a solution of sodium borohydride (150 mg.) in water (10 cc.) added. After 18 hours, the solution was neutralized with 0.2 *N* sulfuric acid and evaporated to dryness. The residue was extracted with boiling chloroform, from which the soluble material (485 mg.) crystallized and gave on recrystallization **3,4-*O*-dimethyleneoxy-L-rhamnitol (V)** (380 mg.), m.p. 119°, $[\alpha]_D$ +9.4° (*c* 1.2), with R_g 0.67 (detected with ammoniacal silver nitrate).

Anal. Calcd. for $C_8H_{16}O_6$: C, 46.15; H, 7.7. Found: C, 46.35; H, 7.8.

Hydrolysis of a portion (50 mg.) gave L-rhamnitol, which was crystallized from acetone and had m.p. and mixed m.p. 124–125°, $[\alpha]_D$ −11° (*c* 0.7). The rhamnitol derivative V (160 mg.) was methylated three times with Purdie reagents and gave sirupy **3,4-*O*-dimethyleneoxy-1,2,5-tri-*O*-methyl-L-rhamnitol** (146 mg.), soluble in light petroleum.

Anal. Calcd. for $C_8H_{13}O_3(OCH_3)_3$: OMe, 37.2. Found: OMe, 39.3.

Hydrolysis of this product for one hour gave a sirup (80 mg.) which consisted mainly of material with R_g 0.98. Purification on a paper chromatogram gave the main component, **1,2,5-tri-*O*-methyl-L-rhamnitol (VI)** (50 mg.) as a sirup with $[\alpha]_D$ +29° (*c* 1.5).

Anal. Calcd. for $C_6H_{11}O_3(OCH_3)_3$: OMe, 44.7. Found: OMe, 43.9.

When oxidized with sodium metaperiodate as described above, this compound (6.4 mg.) reduced periodate (0.01 *N*, 6.36 cc.) equivalent to 1.03 moles per mole of tri-*O*-methylrhamnitol, thus proving the presence of one α-glycol group in the molecule.

2,3-*O*-Dimethyleneoxy-L-rhamnose (X).—This compound (160 mg.) when methylated once with Purdie reagents gave mainly methyl **2,3-*O*-dimethyleneoxy-β-L-rhamnoside** which crystallized from ether–light petroleum as tiny colorless needles (80 mg.), m.p. 155°, $[\alpha]_D$ +129° (*c* 1.2).

Anal. Calcd. for $C_8H_{13}O_5(OCH_3)$: C, 49.1; H, 7.3; OMe, 14.1. Found: C, 49.3; H, 7.2; OMe, 14.6.

On hydrolysis this compound gave only rhamnose (detected chromatographically). Remethylation (twice) of the above product, together with the material in the mother liquors from which it crystallized, gave methyl **2,3-*O*-dimethyleneoxy-4-*O*-methyl-β-L-rhamnoside** which from ether–light petroleum formed colorless needles (88 mg.), m.p. 137°, $[\alpha]_D$ +115° (*c* 1.0).

Anal. Calcd. for $C_8H_{12}O_4(OCH_3)_2$: C, 51.3; H, 7.7; OMe, 26.5. Found: C, 51.3; H, 7.7; OMe, 26.5.

Hydrolysis of the latter (60 mg.) for 3 hours gave 4-*O*-methyl-L-rhamnose (XI) (40 mg.) with $[\alpha]_D$ +26° (*c* 1.0) and R_g 0.65. This sugar did not crystallize satisfactorily, and so was characterized by conversion into 4-*O*-methyl-L-rhamnono-δ-lactone, which after recrystallization from chloroform–light petroleum had m.p. and mixed m.p. 80–81° and $[\alpha]_D$ −140° (init. value, *c* 0.5) → −112° (18 hours, equil. value).

3,5-*O*-Methylene-L-rhamnose (IX).—This compound (170 mg.) was dissolved in acetone (5 cc.) and methylated with Purdie reagents. The product, a sirup (140 mg.) soluble in light petroleum, was hydrolyzed for one hour, then the solution was neutralized, filtered and concentrated, giving sirupy 2-*O*-methyl-L-rhamnose (IV) (90 mg.) with $[\alpha]_D$ +26° (*c* 1.8) and R_g 0.66.

Anal. Calcd. for $C_6H_{11}O_4(OCH_3)$: OMe, 17.4. Found: OMe, 16.4.

When boiled under reflux in ethanolic solution for one hour with one equivalent of aniline, it yielded *N*-phenyl-L-rhamnosylamine-2-*O*-methyl ether, with m.p. 151–152° (from ether), undepressed on admixture with the above sample of this compound, and $[\alpha]_D$ +42° (*c* 0.8 in pyridine).

An attempt to reduce IX to the corresponding rhamnitol derivative with sodium borohydride gave only a very small yield of a crystalline product. Attempts to prepare a lactone from IX by oxidizing it with bromine water were likewise unsuccessful, even when the oxidation was carried out in the presence of barium benzoate; L-rhamnono-γ-lactone was the only product that could be isolated, or detected on the paper chromatogram. The oxidation was effected with yellow mercuric oxide. 3,5-*O*-Methylene-L-rhamnose (IX) (20 mg.) was heated on the water-bath with freshly prepared yellow mercuric oxide (*ca.* 3 g.) and sufficient water to keep the material moist. After 8 hours, more water was added and the mixture filtered; the filtrate was treated with hydrogen sulfide, filtered and concentrated. The product (15 mg.) crystallized and, after washing with chloroform, the crystals (12 mg.) had m.p. 164–167° after shrinking at *ca.* 150°; attempts at recrystallization were unsuccessful. The crystals (6 mg.) in water (5 cc.) required 3.45 cc. of 0.01 *N* sodium hydroxide for neutralization (using screened methyl red as indicator), giving equivalent weight 172 ($C_7H_{10}O_5$ requires equiv. wt. 174). It showed $[\alpha]_D$ −31° (init. value, *c* 0.6) → −35° (44 hours). This lactone had R_g 0.68, and could be detected on the paper chromatogram with ammoniacal silver nitrate, but not with *p*-anisidine hydrochloride.

1,2;3,5-Di-*O*-methylene-L-rhamnose (VIII).—The compound (150 mg.) was heated at 100° in 0.1 *N* sulfuric acid (5 cc.) for 1.5 hours and, after removal of the acid, the products of the hydrolysis were examined on the paper chromatogram; rhamnose and a compound with R_g 0.67 corresponding to IX were detected. The latter compound (35 mg.) was separated on a large paper chromatogram, and characterized as 3,5-*O*-methylene-L-rhamnose (IX) with m.p. 146°, undepressed on admixture with the above material, and $[\alpha]_D$ −60° (*c* 0.5).

***O*-Dimethyleneoxy-*O*-methylene-L-rhamnose (XII).**—The compound (10 mg.) was heated at 100° in 0.1 *N* sulfuric acid (2 cc.). After a half-hour, the solution contained both rhamnose and a compound with R_g 0.74 in roughly equal proportions; after 1 hour the proportions were *ca.* 5:1, respectively. The compound with R_g 0.74 had a rate of movement similar to that of a compound detected in small amounts in sirup B.

Sirup E.—The sirup (250 mg.) was methylated with Purdie reagents, giving a sirupy product (230 mg.).

(17) R. A. Weerman, *Rec. trav. chim.*, **37**, 16 (1917).

Anal. Calcd. for methyl *O*-methyl-*O*-methylenerhamnoside, $C_7H_{10}O_3(OCH_3)_2$: OMe, 30.4. Found: OMe, 28.5.

Hydrolysis of this material for one hour gave a partially crystalline material which was dissolved in water, and the solution extracted continuously with ether. Evaporation of the residual aqueous solution gave a sirup (25 mg.) with $[\alpha]D$ +24° (*c* 1.0) and R_g 0.66. After reaction with ethanolic aniline, it yielded *N*-phenyl-L-rhamnosylamine 2-methyl ether, m.p. and mixed m.p. 150° (from ether). The ethereal extract gave crystalline **5-*O*-methyl-2,3-*O*-methylene-L-rhamnose (XIII)** (130 mg.), which after recrystallization from cyclohexane had m.p. 77–79° and $[\alpha]D$ +6.4° (init. value, *c* 1.4) → +4.5° (46 hours, equil. value).

Anal. Calcd. for $C_7H_{11}O_4(OCH_3)$: C, 50.5; H, 7.4; OMe, 16.3. Found: C, 50.2; H, 7.4; OMe, 16.3.

Prolonged heating of this compound in *N* sulfuric acid at 100° failed to remove the methylene group. It was therefore oxidized with bromine water for 3 days, and after the usual treatment yielded a crystalline product. Recrystallization from chloroform–light petroleum afforded **5-*O*-methyl-L-rhamnono-γ-lactone** as colorless stubby needles, m.p. 164–166°, $[\alpha]D$ −36 ± 4° (10 min., showing no significant change in 60 hours; *c* 0.6).

Anal. Calcd. for $C_6H_9O_4(OCH_3)$: C, 47.7; H, 6.8; OMe, 17.6. Found: C, 47.7; H, 6.6; OMe, 16.5.

The lactone (10 mg.) in water (5 cc.) required 5.58 cc. of 0.01 *N* sodium hydroxide for neutralization (screened methyl red), hence equiv. wt. 179 (equiv. wt. of $C_7H_{12}O_5$, 176).

Oxidation of this sodium salt with sodium metaperiodate failed to give any detectable acetaldehyde.[18]

One of us (P.A.) thanks the Directors of Monsanto Chemicals Ltd. for the award of a Fellowship.

(18) B. H. Nicolet and L. A. Shinn, THIS JOURNAL, **63**, 1456 (1941).

BRISTOL, ENGLAND

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Ouabagenin. II. The Hydroxyl Groups of the A/B Ring System[1]

BY R. P. A. SNEEDEN AND RICHARD B. TURNER

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Of the eight oxygen atoms of the cardiac aglycone, ouabagenin, two are accounted for by an α,β-unsaturated lactone side chain, and one by a tertiary hydroxyl group at C_{14}. The remaining five oxygen functions are all present as hydroxyl groups, of which four can be acetylated by the use of acetic anhydride and pyridine. Selective oxidation of 20,21-dihydroöuabagenin with platinum and oxygen, followed by treatment with base, results in aromatization of ring A with the simultaneous loss of an angular hydroxymethyl group as formaldehyde. Methylation of the phenolic product thus formed yields a phenolic methyl ether, which on subsequent dehydration and rearrangement furnishes a compound possessing typical β-methoxynaphthalenoid absorption in the ultraviolet. Interpretation of these conversions permits assignment of hydroxyl groups at C_1, C_3, C_5 and C_{19} in ouabagenin.

In 1942, Mannich and Siewert[2] suggested structure I[3] for the cardiac glycoside, ouabain. This proposal was based upon the following observations. Ouabain gives a positive Legal test and is converted into an iso compound by the action of methanolic potassium hydroxide.[4] Such behavior suggests that ouabain, like other members of the heart poison group, possesses an α,β-butenolide side chain at C_{17} and a tertiary hydroxyl group at C_{14}, conclusions that have been amply verified by the ultraviolet absorption measurements and degradative studies of Reichstein and his associates.[5,6] On acetolysis, the tetrahydro derivative of heptaacetylanhydroouabain undergoes aromatization with loss of a carbon atom as formaldehyde.[4] This change has been interpreted by Fieser and Newman[7] as evidence for the presence of a hydroxymethyl group at an angular position, probably C_{10}.

Whereas ouabain is cleaved by vigorous acid hydrolysis to rhamnose and resinous conversion products of the genin,[8] treatment with acetone and small amounts of hydrochloric acid under mild conditions affords the aglycone, ouabagenin, in good yield as a monoacetonide derivative.[2] The monoacetonide yields a diacetate, and is readily convertible in weakly acidic media into free ouabagenin, from which a tetraacetyl derivative may be obtained. Since it has been demonstrated by lead tetraacetate titration that no two hydroxyl groups in ouabagenin can occupy adjacent positions,[2] the monoacetonide was formulated as a derivative of a 1,3-glycol, in which both hydroxyl groups are acylable. These groups were tentatively assigned to C_1 and C_3. Formation of a tetraacetate from ouabagenin further suggests the presence in this molecule of a second tertiary hydroxyl group, which was placed at C_5 on the basis of analogy to other cardiotonic substances. The remaining hydroxyl group has been provisionally assigned to C_{11} for reasons that are discussed in a later paragraph.

Since the occurrence of an oxygen function at C_1 in a steroid of natural origin is without precedent,[9] we have undertaken an investigation of ouabagenin with a view toward establishing the details of its structure in an unambiguous manner. In order to avoid complications resulting from epimerization at C_{17}, or from isomerization of the side chain structure in the presence of base, we employed 20,21-dihydroöuabagenin (*II*)[2] as starting material.

After a number of abortive attempts at partial

(1) This investigation was supported by a research grant, H-1084, from the National Heart Institute, of the National Institutes of Health, Public Health Service. A preliminary account of this work appeared in *Chem. & Ind.*, 1235 (1954). For paper I see R. P. A. Sneeden and R. B. Turner, THIS JOURNAL, **75**, 3510 (1953).

(2) C. Mannich and G. Siewert, *Ber.*, **75**, 737 (1942).

(3) The original formulation of Mannich and Siewert contained a β,γ-butenolide side chain at C_{17}. In consideration of more recent work of Reichstein noted below [*cf.* W. D. Paist, E. R. Blout, F. C. Uhle and R. C. Elderfield, *J. Org. Chem.*, **6**, 273 (1941)], an α,β-unsaturated structure has been incorporated in formula I.

(4) W. A. Jacobs and N. M. Bigelow, *J. Biol. Chem.*, **96**, 647 (1932); **101**, 15 (1933).

(5) A. Meyrat and T. Reichstein, *Helv. Chim. Acta*, **31**, 2104 (1948).

(6) R. F. Raffauf and T. Reichstein, *ibid.*, **31**, 2111 (1948).

(7) L. F. Fieser and M. S. Newman, *J. Biol. Chem.* **114**, 705 (1936).

(8) A. Arnaud, *Compt. rend.*, **126**, 346, 1208 (1898).

(9) The possibility that acovenoside A and abomonoside may possess such a function has been discussed by T. Reichstein and his associates, *Helv. Chim. Acta*, **34**, 1224 (1951); **35**, 2202 (1952).