The Chemistry of Ribose and Its Derivatives. Part V. The Synthesis of Methyl Ethers by Partial Methylation.*

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By methods involving partial methylation, 2- and 3-*O*-methyl-D-ribose, and 2 : 5- and 3 : 5-di-*O*-methyl-D-ribose have been synthesised and characterised. The chromatographic behaviour of 4-*O*-methyl-D-ribose is also described.

OF the methyl ethers of ribose, 5-*O*-methyl-D-ribose (Levene and Stiller, *J. Biol. Chem.*, 1934, **102**, 187), 2 : 3-di-*O*-methyl-D-ribose (Barker and Smith, preceding paper), 2 : 3 : 5-tri-*O*-methyl-D-ribose (Barker, *J.*, 1948, 2035), and 2 : 3 : 4-tri-*O*-methyl-D-ribose (Levene and Tipson, *J. Biol. Chem.*, 1931, **93**, 623) have been fully characterised. As far as is known, no unambiguous route is available for the synthesis of other methyl ethers of this sugar. Since methylated riboses are obtained from methylated nucleotides and polynucleotides it is particularly desirable that 2- and 3-*O*-methyl-D-riqose, and 2 : 5- and 3 : 5-di-*O*-methyl-D-ribose should be available synthetically. Attempts have therefore been made to obtain these ethers of ribose by partial methyation and to develop techniques for their isolation from the mixtures so produced. Part of this work has already been briefly summarised (Barker and Smith, *Chem. and Ind.*, 1954, 19).

Brief treatment of methyl 5-*O*-trityl-D-ribofuranoside with methyl iodide and silver oxide gave, after removal of protective groups, a mixture which on paper chromatograms in a butanol–water system showed three spots corresponding to D-ribose, 2 : 3-di-*O*-methyl-D-ribose, and a mono-*O*-methyl-D-ribose fraction. On a preparative scale it was subsequently found more convenient to isolate the monomethylribose fraction by chromatography on neutral alumina before removal of the protecting groups. The mixed monomethylriboses were percolated through Dowex-1 ion-exchanger in the borate form (cf. Khym and Zill, *J. Amer. Chem. Soc.*, 1952, **74**, 2099). Elution with 0·01M-sodium tetraborate gave two fractions which were freed from sodium ions and concentrated to small volume. Chromatography of these two fractions on paper, with butanol–water, gave two distinct spots, the slower of which (component *A*) had the same R_F value as that previously observed for the whole mono-*O*-methylribose fraction. The faster-moving spot (component *B*) appeared to be contaminated with non-carbohydrate material since it had a concave rear boundary. By running chromatograms of boric acid alone, and by using the spray reagent of Buchanan, Dekker, and Long (*J.*, 1950, 3162), it was found that a spot probably containing boric acid was present between the two carbohydrate spots. It was then found that chromatography of the original mono-*O*-methyl-D-ribose fraction on paper impregnated with boric acid (cf. Rose and Sweigert, *J. Amer. Chem. Soc.*, 1951, **73**, 5903) also gave two spots corresponding to those given by the fractions obtained by ion-exchange chromatography. In this way, interference by local concentrations of boric acid was avoided. The two spots were believed to correspond to 2- and 3-*O*-methyl-D-ribose and in order to determine which spot corresponded to which isomer, the following series of experiments was undertaken.

Condensation of methyl D-ribopyranoside with acetone gave a syrupy methyl *iso*propylidene-D-riboside. Levene and Stiller (*J. Biol. Chem.*, 1934, **106**, 421) have shown that during this condensation a partial change in the size of the sugar ring takes place, and they considered that the product contained methyl 2 : 3-*O*-*iso*propylidene-D-ribofuranoside and methyl 2 : 3-*iso*propylidene-D-ribopyranoside. However, no evidence for the structure of the latter was provided and it appeared to us that methyl 3 : 4-*O*-*iso*propylidene-D-ribopyranoside might also be present. After methylation of the mixed product and removal of protecting groups, chromatography on Dowex-1 borate gave two fractions. Paper chromatography of fraction 2 in butanol–boric acid indicated that

* Part IV, preceding paper.

it was identical with component *A* obtained from methyl 5-*O*-trityl-D-ribofuranoside. Under the same conditions fraction 1 was resolved into two spots, one having the same R_F value as 5-*O*-methyl-D-ribose, the other having an R_F value different from any of those of methyl ethers of ribose so far encountered. Provided that acetone condenses only with adjacent hydroxyl groups, the only methylriboses which could arise from the mixed methyl *iso*propylidene-D-ribosides are 2-, 4-, and 5-*O*-methyl-D-ribose. On the other hand, methyl 5-*O*-trityl-D-ribofuranoside can give only 2- and 3-*O*-methyl-D-ribose. Thus 2-*O*-methyl-D-ribose is expected to be formed by both routes. From the chromatographic evidence, we therefore associate this compound with component *A* and fraction 2. Component *B* is thus tentatively identified as 3-*O*-methyl-D-ribose, and fraction 1 appears to consist of 5- and 4-*O*-methyl-D-ribose. This last compound was not studied further, but from the results now reported, it appears that not only is some methyl 2 : 3-*O*-isopropylidene-D-ribofuranoside formed in the condensation of acetone with methyl D-ribopyranoside, as suggested by Levene and Stiller (*loc. cit.*), but both methyl 2 : 3- and methyl 3 : 4-*O*-*iso*propylidene-D-ribopyranoside. This reaction is being studied further.

2- and 3-*O*-Methyl-D-ribose obtained by elution of appropriate portions of paper chromatograms were purified by conversion into the methyl furanosides which were sublimed *in vacuo* and reconverted into the free sugars. The material previously identified as 2-*O*-methyl-D-ribose gave D-ribose phenylosazone, with loss of methoxyl, and consumed two mols. of sodium metaperiodate and liberated one mol. of titratable acid. The material previously identified as 3-*O*-methyl-D-ribose gave an osazone identical with that derived from 2 : 3-di-*O*-methyl-D-ribose (Barker and Smith, preceding paper). The progress of the oxidation of this sugar with sodium metaperiodate was more complex than in the above case, but nevertheless consistent with its formulation as 3-*O*-methyl-D-ribose. It rapidly consumed 1 mol. of periodate with the liberation of no titratable acid, but thereafter slowly liberated 1 mol. of acid and consumed a further mol. of periodate. It is believed that this behaviour is due to the sugar's reacting initially with the periodate in one of its lactol forms (cf. Barker and Smith, *Chem. and Ind.*, 1952, 1035). The chromatographic identifications of 2- and 3-*O*-methyl-D-ribose are thus confirmed. It may also be pointed out that it is now possible to identify chromatographically all the monomethyl ethers of ribose, by use of a combination of the procedures outlined above. With these techniques, it was shown that methylation of benzylideneguanosine yielded, after hydrolysis, 5-*O*-methyl-D-ribose as the sole methylated sugar, thus confirming the conclusion of Brown, Haynes, and Todd (*J.*, 1950, 643). The same result was also obtained with the condensation product of benzaldehyde and methyl D-ribofuranoside, showing that condensation takes place with adjacent hydroxyl groups in this case also. This is to be expected since, whereas in methyl D-glucopyranoside, which condenses with benzaldehyde at the 4- and the 6-position, the $C_{(5)}$–$C_{(6)}$ and $C_{(4)}$–$O_{(4)}$ bonds can assume equatorial positions, this is not so in the case of ribofuranoside derivatives. This probably results in the oxygen atoms at the 3- and the 5-position being too far apart for condensation (cf. condensation products of xylose and acetone; Haworth and Porter, *J.*, 1928, 611). It is of interest to observe that the condensation product of benzaldehyde and methyl β-D-ribopyranoside, after methylation and hydrolysis, gave only 5-*O*-methyl-D-ribose. It is evident that in this condensation complete change of the size of the ring takes place instead of a partial change as observed by Levene and Stiller (*loc. cit.*) in the condensation with acetone.

The successful synthesis of 2- and 3-*O*-methyl-D-ribose suggested a similar route to 2 : 5- and 3 : 5-di-*O*-methyl-D-ribose from 5-*O*-methyl-D-ribose. Levene and Stiller (*J. Biol. Chem.*, 1932, **102**, 187) obtained 5-*O*-methyl-D-ribose by methylation of methyl 2 : 3-*O*-*iso*propylidene-D-ribofuranose. Since there is a possibility of partial change of ring size in this case also, it was necessary to modify the preparation of 5-*O*-methyl-D-ribose and the preparation and characterisation of a homogeneous material are described in the experimental section.

Methyl 5-*O*-methyl-D-ribofuranoside was methylated with methyl iodide and silver oxide for a short time, and the hydrolysis product shown by paper chromatography in *n*-butanol–water to contain 5-*O*-methyl-D-ribose, a di-*O*-methyl-D-ribose fraction, and 2 : 3 : 5-tri-*O*-methyl-D-ribose. In *n*-butanol–boric acid the di-*O*-methyl-D-ribose fraction was resolved

into two components, one having the same R_F value in the two solvent systems and one travelling more slowly in presence of boric acid. The latter component was tentatively identified as 3 : 5-di-*O*-methyl-D-ribose since it was evidently capable of complex formation with boric acid. These two fractions were obtained pure by chromatographing the hydrolysate of the crude methylated product twice on thirty to forty large sheets of paper, and their designation as 2 : 5- and 3 : 5-di-*O*-methyl-D-ribose was confirmed by their conversions into 5-*O*-methyl-D-ribose phenylosazone and 3 : 5-di-*O*-methyl-D-ribose phenylosazone, respectively.

After this work was completed, 2-*O*-methyl-, 3-*O*-methyl-, 2 : 5-di-*O*-methyl-, and 3 : 5-di-*O*-methyl-D-ribose were reported as fission products of methylated uridylic acid *b* (Brown, Magrath, and Todd, *J.*, 1954, 1422). The monomethyl sugars were obtained in amounts too small to allow of their full characterisation, but their behaviour on chromatography and on electrophoresis in presence of sodium borate was recorded. 3-*O*-Methyl-D-ribose was identified by the fact that it migrated electrophoretically at the same speed as 3 : 5-di-*O*-methyl-D-ribose, the assumption being that the two sugars form similar complexes with sodium borate. This would undoubtedly be true of complex-formation with boric acid. However, complex formation with sodium borate takes place more readily, and it is significant that while 2-*O*-methyl-D-ribose migrated electrophoretically in sodium borate, chromatography in presence of boric acid suggested that it does not form a complex under these conditions (Barker and Smith, *Chem. and Ind.*, 1954, 19). Complex-formation with boric acid is usually associated with a reduction of R_F value, but in a few cases, of which 3-*O*-methyl-D-ribose is one, the R_F value is increased. No explanation of this behaviour can be given, but it is believed that a different type of complex may be formed (cf. Bell and Northcote, *Chem. and Ind.*, 1954, 1328) with this sugar compared with others such as 3 : 5-di-*O*-methyl-D-ribose.

In the work of Brown, Magrath, and Todd (*loc. cit.*) 2 : 5- and 3 : 5-di-*O*-methyl-D-ribose were also separated by electrophoresis in presence of sodium borate but for purposes of identification, a mixture of the sugars was converted into a mixture of *p*-bromophenylosazones. We have found that removal of boric acid is less troublesome than the removal of sodium borate and we were able, by using partition chromatography, to isolate the components of a mixture for purposes of characterisation. Furthermore, removal of the last traces of boric acid is unnecessary before conversion into phenylosazones.

The work now described is in agreement with the identifications of methylated riboses described by Brown *et al.* (*loc. cit.*). The anomalous behaviour of 3-*O*-methyl-D-ribose, however, cannot yet be explained.

Experimental

M.p.s were determined on a Kofler block.

Paper Chromatography.—Two solvent systems were used : *n*-butanol saturated with water, and *n*-butanol saturated with both water and boric acid. Whatman No. 1 filter paper was used throughout, but for use with the *n*-butanol–boric acid systems the paper was first soaked in saturated aqueous boric acid and dried at room temperature.

2- *and* 3-O-*Methyl*-D-*ribose.*—Methyl 5-*O*-trityl-D-ribofuranoside (14 g.) was boiled with stirring for 10 min. with methyl iodide (50 c.c.) and silver oxide (8 g.). Silver oxide (8 g.) was added and heating continued for a further 10 min. After being cooled, silver salts were filtered off and washed with ether. The combined filtrate and washings were evaporated at 35° to a syrup (16·0 g.) which was hydrolysed and the product was resolved by paper chromatography (butanol–water) into fractions containing ribose, monomethylriboses, and 2 : 3-di-*O*-methylribose, and small quantities of the monomethylribose fraction were obtained by elution from the paper. The main part of the syrup was dissolved in benzene (300 c.c.) and run in three separate portions on neutral alumina (5 × 45 cm.). Elution with ether (1 l. each column) and evaporation of the solvent gave methyl 2 : 3-di-*O*-methyl-5-*O*-trityl-D-ribofuranoside (5 g.), which was rejected. Each column was then eluted with methanol (600 c.c. each), and evaporation gave a mixture of methyl 2- and methyl 3-*O*-methyl-5-*O*-trityl-D-ribofuranoside. The mixed product (10 g.) was heated on the steam-bath for 30 min. with 80% aqueous acetic acid

(100 c.c.) and cooled, and the triphenylmethanol filtered off. The filtrate was diluted with water (100 c.c.), filtered (charcoal), and extracted twice with chloroform (150 c.c.). The residual aqueous solution was evaporated under reduced pressure to a syrup from which ethanol (200 c.c.) was distilled three times to remove the last trace of acetic acid. The residual syrup was boiled for 1 hr. with 0·04N-hydrochloric acid, cooled, freed from chloride ions by being ground with silver oxide and filtered (charcoal), and evaporated under reduced pressure to a syrup (3 g.). Approximately one-third of this material was chromatographed on thirty large sheets of paper (butanol–boric acid system), and crude 2-*O*-methyl-D-ribose (Component *A*) (R_F, 0·3; 0·29 g.) and 3-*O*-methyl-D-ribose (Component *B*) (R_F, 0·55; 0·29 g.) were eluted with methanol from appropriate areas of the papers. After removal of the solvent under reduced pressure boric acid was removed by repeated distillation with methanol. The resulting sugars were separately converted into the methyl furanosides which were purified by sublimation at 0·01 mm. These were separately hydrolysed with 0·04N-hydrochloric acid as described above and yielded, respectively, 2-O-*methyl-D-ribose*, $[\alpha]_D^{20}$ −32° (*c*, 1·09 in MeOH) (Found: OMe, 18·9. $C_6H_{12}O_5$ requires OMe, 19·0%), and 3-O-*methyl-D-ribose*, $[\alpha]_D^{20}$ +7·5 (*c*, 0·6 in MeOH) (Found: OMe, 18·0%).

D-*Ribose Phenylosazone from* 2-O-*Methyl-D-ribose*.—2-*O*-Methyl-D-ribose (0·02 g.), 2N-acetic acid (3·5 c.c.), and phenylhydrazine (0·2 c.c.) were heated at 100° for 4·5 hr. After cooling, the solid was collected, dissolved in benzene (10 c.c.) and run on to neutral alumina. D-Ribose phenylosazone was obtained by elution with benzene–ether (1 : 1) and removal of the solvent. It had m. p. 160° (Found: N, 16·7. Calc. for $C_{17}H_{20}O_3N_4$: N, 17%).

3-O-*Methyl-D-ribose Phenylosazone*.—3-*O*-Methyl-D-ribose (0·035 g.), 2N-acetic acid (3·5 c.c.), and phenylhydrazine (0·2 c.c.) were heated at 100° for 2 hr. and the solid obtained as described above was eluted from neutral alumina with benzene containing 30% of ether. The product had m. p. 135°, not depressed by the osazone prepared from 2 : 3-di-*O*-methyl-D-ribose (Found: OMe, 7·7. Calc. for $C_{18}H_{22}O_3N_4$: OMe, 9·0%).

Methylation of Methyl iso*Propylidene-D-ribopyranoside*.—The crude condensation product of methyl D-ribopyranoside and acetone (Levene and Stiller, *loc. cit.*) was fractionally distilled and had b. p. 170—175° (bath temp.)/0·01 mm., n_D^{16} 1·4596 (Found: C, 51·1; H, 8·1; OMe, 14·6. Calc. for $C_9H_{16}O_5$: C, 52·4; H, 7·9; OMe, 15·2%). This material (0·64 g.) was stirred and boiled with methyl iodide (10 c.c.), and silver oxide (1 g.) was added every hour during 6 hr. The filtrate from silver salts was evaporated leaving a syrupy product having b. p. 70—80° (bath temp.)/0·02 mm., n_D^{17} 1·4478 (Found: C, 55·0; H, 8·2; OMe, 26·2. Calc. for $C_{10}H_{18}O_5$: C, 55·0; H, 8·3; OMe, 28·5%).

Chromatography of 2-, 4-, *and* 5-O-*Methyl-D-ribose*.—The mixed methylated products obtained from methyl *iso*propylidene-D-ribopyranoside were hydrolysed with 0·04-hydrochloric acid as described above and, after removal of chloride ions, were dissolved in 0·01M-sodium tetraborate (5 c.c.) and run on to Dowex-1 (1 × 10 cm.) in the borate form. The column was eluted with 0·01M-sodium tetraborate, and samples (10 c.c.) were analysed for pentose by a modification of Brown's orcinol method (*Arch. Biochem.*, 1946, **11**, 270). Combined solutions of fraction 1 and fraction 2 were separately freed from sodium ions as described by Khym and Zill (*J. Amer. Chem. Soc.*, 1951, **73**, 2399), and aliquot parts were chromatographed on paper (*n*-butanol–boric acid). Fraction 1 showed spots with R_F values 0·19 and 0·27. Fraction 2 showed a single spot having R_F 0·30.

Preparation and Methylation of Benzylidene Derivatives.—(a) *From methyl* D-*ribofuranoside*. Methyl D-ribofuranoside (6·8 g.), benzaldehyde (35 g.), and powdered anhydrous zinc chloride (8 g.) were shaken in a closed flask for 24 hr. The viscous mass was triturated with light petroleum to remove some benzaldehyde, taken up in chloroform and washed successively with water and aqueous sodium hydrogen carbonate, and then concentrated to a syrup which was heated at 60°/0·05 mm. The residue was steam-distilled *in vacuo* to remove the last trace of benzaldehyde and extracted with chloroform; the extract was washed with aqueous sodium hydrogen carbonate, dried ($MgSO_4$), and evaporated under reduced pressure to yield syrupy *methyl* 2 : 3-O-*benzylidene-D-ribofuranoside* (8·9 g.), b. p. 100° (bath temp.)/10^{-5} mm., n_D^{20} 1·5225, $[\alpha]_D^{20}$ −41·0° (*c*, 8·49 in $CHCl_3$) (Found: C, 61·3; H, 6·5; OMe, 11·7. $C_{13}H_{16}O_5$ requires C, 61·9; H, 6·35; OMe, 12·3%). During a few days, the compound undergoes slight decomposition resulting in the reappearance of the smell of benzaldehyde. This may be related to the phenomenon observed in the case of methyl 3 : 4-*O*-benzylidene-β-L-arabinoside (Oldham and Honeyman, *J.*, 1946, 986).

The methyl 2 : 3-*O*-benzylidene-D-ribofuranoside was methylated with methyl iodide and silver oxide in the usual way. After filtration from silver salts and removal of methyl iodide,

the residue (4 g.) was dissolved in ether and run on to alumina (Peter Spence, Grade H). Elution with ether gave, after removal of solvent, syrupy *methyl* 2 : 3-O-*benzylidene*-5-O-*methyl*-D-*ribofuranoside* (1·4 g.) which was sublimed at 100°/10^{-3} mm. It had $[\alpha]_D^{20}$ −55·6° (*c*, 4·1 in $CHCl_3$), n_D^{17} 1·5161 (Found: C, 62·9; H, 6·6; OMe, 22·4. $C_{14}H_{18}O_5$ requires C, 63·0; H, 6·8; OMe, 23·3%). A small sample, after hydrolysis with 0·04N-hydrochloric acid as described above, showed a single spot on paper chromatography, having R_F 0·39 and 0·19 in *n*-butanol–water and *n*-butanol–boric acid, respectively.

(b) *From methyl* D-*ribopyranoside*. Methyl β-D-ribopyranoside (Minsaas, *Annalen*, **1934**, **512**, 286; Barker and Smith, *J.*, **1954**, 2151) (1·03 g.) was condensed with benzaldehyde (45 g.) as described above. Sublimation of the syrupy product gave methyl 2 : 3-*O*-benzylidene-D-ribofuranoside (Found: C, 62·7; H, 6·9; OMe, 12·7%), which was methylated with methyl iodide and silver oxide. After hydrolysis of the syrupy product, a single spot was obtained on paper chromatography, having R_F 0·39 and 0·19 in *n*-butanol–water and *n*-butanol–boric acid, respectively.

(c) *From guanosine*. 2′ : 3′-*O*-Benzylideneguanosine (Bredereck and Berger, *Ber.*, **1940**, **73**, 1124) (2·8 g.) was methylated by the method described for purine nucleosides (Anderson, Barker, Gulland, and Lock, *J.*, **1952**, 369). Hydrolysis of the crude product was effected as described above and the hydrolysate was chromatographed on paper. In *n*-butanol–water, a spot (R_F 0·39) corresponding to 5-*O*-methyl-D-ribose was observed besides a faint spot (R_F 0·17) corresponding to D-ribose. In *n*-butanol–boric acid, a single spot (R_F 0·19) was observed, since the above two sugars have identical R_F values in this solvent system.

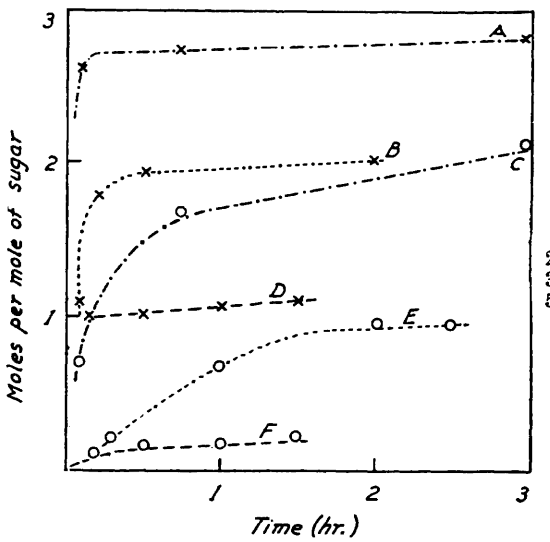
5-O-*Methyl*-D-*ribose*.—Crude methyl 5-*O*-methyl-2 : 3-*O*-*iso*propylidene-D-ribofuranoside (Levene and Stiller, *loc. cit.*) was hydrolysed and chromatographed in *n*-butanol–water; it yielded an appreciable quantity of unmethylated ribose which was not reduced in amount by further methylation. This is believed to be due to the presence of some *iso*propylideneribose anhydride (Levene and Stiller, *loc. cit.*). Chromatography of the hydrolysate in *n*-butanol–boric acid indicated the presence of small amounts of methylated products other than 5-*O*-methyl-D-ribose. The crude methyl 5-*O*-methyl-2 : 3-*O*-*iso*propylidene-D-ribofuranoside (11·4 g.) was therefore dissolved in benzene (100 c.c.) and run on to a column (diameter 5 cm.) of alumina (Peter Spence, grade H) (400 g.) and eluted with further quantities of benzene. Fractions (50 c.c.) were collected and samples were examined, after removal of the solvent and hydrolysis, by paper chromatography in *n*-butanol–water and in *n*-butanol–boric acid. Those fractions which yielded only 5-*O*-methyl-D-ribose were pooled, hydrolysed by boiling 0·1N-sulphuric acid (150 c.c.) for 1 hr., and after removal of sulphate ions with Amberlite IR-4B (carbonate), were concentrated under reduced pressure to yield syrupy 5-*O*-methyl-D-ribose (3·0 g.) (Found: C, 43·6; H, 7·1; OMe, 18·8. Calc. for $C_6H_{12}O_5$: C, 43·9; H, 7·3; OMe, 18·9%). It yielded a phenylosazone, m. p. 150° (Found: C, 63·2; H, 6·2; N, 15·6; OMe, 9·1. Calc. for $C_{18}H_{22}O_3N_4$: C, 63·1; H, 6·5; N, 16·4; OMe, 9·1%).

5-O-*Methyl*-D-*ribonolactone*.—5-*O*-Methyl-D-ribose (0·5 g.), water (10 c.c.), and bromine (1 c.c.) were kept in a stoppered flask for 4 days at room temperature. Excess of bromine was removed by aeration, bromide ions by grinding with silver oxide and filtration, and silver ions with hydrogen sulphide. The filtrate from silver sulphide was evaporated under reduced pressure to yield a syrupy *lactone* which crystallised (0·42 g.), and after recrystallisation from ethyl acetate had m. p. 109—110°, $[\alpha]_D^{18}$ +27·4° (initial value), +26·4° (36 min.), +25·2° (84 min.), +21·9° (180 min.), +20·0° (238 min.), +17·2° (324 min.), +15·3° (420 min.) (*c*, 8·5 in H_2O) (Found: C, 44·3; H, 6·3. $C_6H_{10}O_5$ requires C, 44·4; H, 6·2%).

2 : 5-*Di*-O-*methyl*-D-*ribose and* 3 : 5-*Di*-O-*methyl*-D-*ribose*.—A solution of chromatographically homogeneous 5-*O*-methyl-D-ribose (2·9 g.) in methanol (200 c.c.) containing 1% of hydrogen chloride was set aside at room temperature for 1·5 hr. After removal of chloride ions by grinding with silver carbonate and filtration, methanol was removed under reduced pressure. The remaining syrupy methyl 5-*O*-methyl-D-ribofuranoside was partially methylated as described for methyl 5-*O*-trityl-D-ribofuranoside and the product (3·1 g.) was boiled for 1·25 hr. with 0·1N-sulphuric acid (100 c.c.). Sulphate ions were removed by means of Amberlite IR-4B (carbonate), and the neutral solution was concentrated under reduced pressure to a syrup which was chromatographed (*n*-butanol–boric acid system) on 40 large sheets of paper. Appropriate areas of the paper were eluted with water and after removal of solvent and of boric acid by repeated distillation with methanol, the crude 2 : 5- and 3 : 5-di-*O*-methyl-D-ribose fractions were separately re-chromatographed in the same way on 30 sheets each. The two fractions were eluted from the chromatograms and freed from solvent and boric acid as before to give, respectively, syrupy 2 : 5-*di*-O-*methyl*-D-*ribose* (0·5 g.) (Found: C, 47·6; H, 7·8; OMe, 32·6. $C_7H_{14}O_5$ requires C,

47·2; H, 7·9; OMe, 34·8%) and 3 : 5-*di*-O-*methyl*-D-*ribose* (0·4 g.) (Found : C, 46·5; H, 7·6; OMe, 32·7%).

3 : 5-*Di*-O-*methyl*-D-*ribose Phenylosazone*.—3 : 5-Di-*O*-methyl-D-ribose (0·09 g.) was heated on the steam-bath for 10 min. with phenylhydrazine (0·2 c.c.) and 2N-acetic acid (5 c.c.). The solid was collected, dissolved in benzene (10 c.c.), and run on to neutral alumina (40 × 2 cm.). The column was washed with benzene (100 c.c.), and the osazone was then eluted with benzene–ether (1 : 1) (100 c.c.). After removal of the solvent, the osazone (0·05 g.) was crystallised from methanol. It had m. p. 161° (Found : C, 63·8; H, 7·2; N, 15·6; OMe, 16·3. Calc. for $C_{19}H_{24}O_3N_4$: C, 64·0; H, 6·7; N, 15·7; OMe, 17·4%).



D-Ribose derivative	HIO_4 consumed	Acid produced
2-*O*-Methyl	*B*	*E*
3-*O*-Methyl	*D*	*F*
5-*O*-Methyl	*A*	*C*

Preparation of the Osazone from 2 : 5-*Di*-O-*methyl*-D-*ribose*.—The osazone was prepared by the method described above except that heating was continued for 6 hr. and 500 c.c. of benzene were used for the elution of the osazone from the alumina. The 5-*O*-methyl-D-ribose phenylosazone crystallised from methanol and had m. p. 150°, not depressed by the osazone prepared from 5-*O*-methyl-D-ribose (Found : C, 62·9; H, 6·1; N, 15·6; OMe, 8·8. Calc. for $C_{18}H_{22}O_3N_4$: C, 63·1; H, 6·5; N, 16·4; OMe, 9·1%).

Periodate Oxidations.—2-, 3-, and 5-*O*-Methyl-D-ribose were oxidised by sodium metaperiodate and the reactions were followed as previously described (Barker and Smith, preceding paper). The rates of consumption of periodate and the released acid are shown in the Figure.

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