6. *The Chemistry of Ribose and its Derivatives. Part II. The Constitution of an Anhydro-D-ribose.*

By G. R. Barker and M. V. Lock.

An anhydroribose prepared by the method of Bredereck, Köthnig, and Berger (*Ber.*, 1940, **73**, 956) has been examined and found to be bimolecular. Oxidation of the anhydride with sodium metaperiodate has been investigated and a crystalline *tetramethyl di-D-ribose anhydride* has been prepared and hydrolysed to 2 : 3-dimethyl D-ribose. Of three possible structures for the anhydride that corresponding to 1 : 5′-5 : 1′-diribofuranose anhydride is the most probable.

In an attempt to prepare 1 : 2 : 3-triacetyl D-ribofuranose by removal of the triphenylmethyl (trityl) grouping from 1 : 2 : 3-triacetyl 5-trityl D-ribofuranose, Bredereck, Köthnig, and Berger (*Ber.*, 1940, **73**, 956) obtained a compound which they claimed was 1 : 5-anhydro-D-ribofuranose. This structure was based on the failure of the compound to reduce Fehling's solution until after hydrolysis and a positive test for adjacent *cis*-hydroxyl groups with copper sulphate and alkali. However, it must be borne in mind that a number of different types of non-reducing anhydro-sugars are known, some monomeric and some dimeric, and that the structure postulated for the anhydroribose is by no means a unique interpretation of the experimental evidence. Our doubts as to the truth of the constitution assigned were aroused by the melting point of the compound (229—230°), which is unexpectedly high for a monomeric anhydride, compared with the melting point of D-ribose itself, variously given as 86—87° (Levene and Jacobs, *Ber.*, 1909, **42**, 1198) and 95° (van Ekenstein and Blanksma, *Chem. Weekblad*, 1913, **10**, 664). It was therefore decided to examine further the properties of the anhydride.

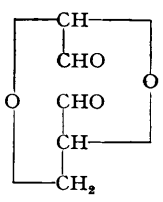
These studies have now reached a point at which it is clear that we have an anhydride not conforming in structure to that postulated by Bredereck and his co-workers. It will be seen from the results presented below that although it is probable we are dealing with the same compound we are unable to reproduce the specific rotation recorded previously. It is therefore considered desirable to place our observations on record in order that the question of the identity of the two materials may be considered by the German workers.

Trityl ribose and triacetyl trityl ribose were prepared and were found to correspond in properties with those previously recorded. By carrying out the acetylation without prior isolation of the trityl ribose the yield of the acetylated material was considerably improved. From triacetyl 5-trityl ribose prepared in either way were obtained an acetyl anhydroribose and an anhydroribose of which the melting points coincided with those recorded by Bredereck and his co-workers. The anhydroribose, however, had, in water, $[\alpha]_D^{19\cdot5}$ +11°, whereas this was previously recorded as +78°. All other properties of our materials were identical with those recorded by Bredereck.

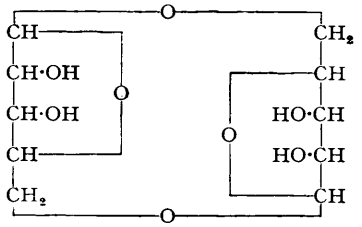
Oxidation of the anhydride by sodium metaperiodate resulted in the consumption of 1 mol. of oxidant per mol. of ribose and no formic acid was liberated. If the constitution put forward by Bredereck and his co-workers for the anhydroribose is correct, then the product of the above oxidation would be 2 : 4-diformyldioxolan (I), produced in a similar manner from *lævo*glucosan, D-altrosan, or D-mannosan. This compound is known to have $[\alpha]_D^{20}$ −15° (Jackson and Hudson, *J. Amer. Chem. Soc.*, 1940, **62**, 958; Richtmyer and Hudson, *ibid.*, p. 961; Knauf, Hann, and Hudson, *ibid.*, 1941, **63**, 1447), whereas the solution obtained after oxidising our anhydroribose with sodium metaperiodate had an observed rotation which corresponded to $[\alpha]_D^{13}$ −48°, calculated on the assumption of the presence of the above dialdehyde. It appeared improbable, therefore, that our anhydride had the structure claimed by Bredereck for his material. Furthermore, cryoscopic determination of the molecular weight of the anhydroribose in aqueous solution indicated that the compound was dimeric. The possibility that the compound was exhibiting an anomalous depression of freezing point in aqueous solution was excluded by the fact that cryoscopic determination of the molecular weight of the acetylated anhydride in benzene confirmed the dimeric nature of the material (cf. Jackson *et al.*, *J. Res. Nat. Bur. Stand.*, 1929, **3**, 27; 1930, **5**, 733; 1931, **6**, 709). It is concluded that the anhydroribose consists of two molecules of ribose united with loss of two molecules of water in such a way that both reducing groups are involved in the union and two pairs of adjacent hydroxyl groups are present in the molecule.

Hydrolysis of the acetylated anhydride with boiling aqueous acid, followed by examination of the hydrolysate on the paper chromatogram using Partridge's method (*Nature*, 1946, **158**, 270), indicated that ribose was the only fission product. The rotation of the solution was also in agreement with this conclusion. It appears therefore, that no ether bridge is present, but

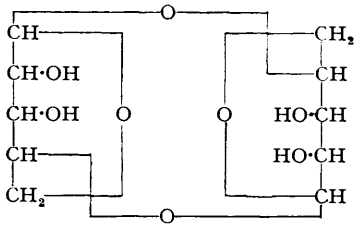
that the ribose residues are joined by two glycosidic linkages. In order to establish which hydroxyl groups of the ribose radicals are thus combined in the anhydride, the tetra-acetyl diribose anhydride was methylated with methyl sulphate following Haworth and Streight's method (*Helv. Chim. Acta*, 1932, **15**, 609, 693) and *tetramethyl di-D-ribose anhydride* was obtained which did not reduce Fehling's solution. This material was hydrolysed by boiling aqueous acid and the product, when examined on the paper chromatogram, moved as one component. In view of the observation of Hirst, Hough, and Jones (*J.*, 1949, 928) that certain isomeric methylated sugars can be separated by partition chromatography, it seemed likely from the above results that tetramethyl di-D-ribose anhydride is split by acid hydrolysis into two identical molecules. Evidence in agreement with this view was obtained by further studies of the methylated anhydride. No dimethyl ribose has yet been described and, since it seemed highly



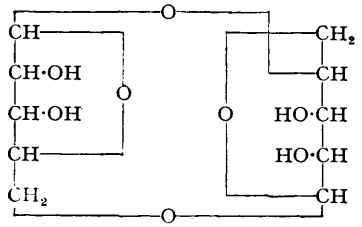
(I.)



(II.)



(III.)



(IV.)

probable that hydrolysis of the methylated anhydride would produce a dimethylated ribose, recourse was made to methods involving oxidation with the periodate ion. Moreover, a method was required whereby the homogeneity of the product of acid hydrolysis, suggested by partition chromatography, could be confirmed. Oxidation of the acid hydrolysate with periodic acid produced no formic acid, but 0·45 mol. of formaldehyde was produced as shown by the method of Jeanloz (*Helv. Chim. Acta*, 1944, **27**, 1509). Both results indicate that two molecules of 2 : 3-dimethyl ribose are produced from the methylated anhydride by acid hydrolysis. First, during the oxidation of methylated sugars with the periodate ion, formic acid is produced by oxidation of a reducing group adjacent to a free hydroxyl group or of a 1 : 2 : 3-trihydric alcohol system. It can be concluded, therefore, that in the acid hydrolysate from the tetramethyl di-D-ribose anhydride there can be no ribose derivative in which position 2 is unmethylated, no monomethyl ribose, and no free ribose, any of which would have yielded formic acid. Since there are the four original methoxyl groups of the methylated anhydride to be accounted for, and no ribose or monomethyl ribose is present, the methylated anhydride must be split into two molecules of dimethyl ribose, both of which are methylated at position 2. Furthermore, since the anhydroribose itself consumes two mols. of sodium metaperiodate it must contain two 1 : 2-glycol groupings and hence the tetramethyl derivative possesses two pairs of adjacent methoxyl groups. 2 : 3-Dimethyl D-ribose, therefore, is the sole product of hydrolysis of tetramethyl D-diribose anhydride. The production of formaldehyde during the oxidation by periodic acid is also in agreement with this conclusion. Formaldehyde is produced by periodate oxidation of a 1 : 2-glycol of which one hydroxyl group is primary and. of those sugars allowed by the other evidence discussed above, this condition is fulfilled only by 2 : 3-dimethyl ribose. If, however, 2 : 3-dimethyl ribose is the sole product of acid hydrolysis of tetramethyl di-D-ribose anhydride, the production of two mols. of formaldehyde would be expected during the oxidation of the hydrolysate of this compound with periodic acid. The low yield of formaldehyde obtained in these experiments is consistent with previous findings (Bell, *J.*, 1948, 992 : Jeanloz, *loc. cit.*) that some methylated sugars are incompletely oxidised by periodic acid and it has been stated

(Bell, *loc. cit.*) that, while the detection of formaldehyde is a useful qualitative test for the presence of a free primary hydroxyl group, it is not susceptible of quantitative use. The low yield of formaldehyde is not, therefore, considered to alter in any way the validity of the previous statement that 2 : 3-dimethylribose is the sole product of hydrolysis from tetramethyl di-D-ribose anhydride.

From the results of the experiments summarised above it follows that the anhydroribose possesses one of the structures (II), (III), and (IV). No convenient method of distinguishing between these structures is at present available but the formation of either (III) or (IV) from 1 : 2 : 3-triacetyl 5-trityl ribose would involve an interchange of furanose and pyranose forms, which although not impossible, is not suggested by any evidence so far obtained. We therefore consider that our anhydroribose is 1 : 5′-5 : 1′-diribofuranose anhydride (II). The configurations at the glycosidic centres of the two ribose residues are not known, but it seems possible that the discrepancy between the rotations of our material and that of Bredereck, Köthnig, and Berger is due to isomerism at these positions.

Experimental.

Acetyl groups were determined by Alicino's method (*Analyt. Chem.*, 1948, **20**, 590).

1 : 2 : 3-*Triacetyl* 5-*Trityl* D-*Ribofuranose*.—(*a*) 5-Trityl D-ribose was acetylated following the method of Bredereck *et al.* (*loc. cit.*) (Found : OAc, 25·0. Calc. for $C_{30}H_{30}O_8$: OAc, 24·9%).

(*b*) A solution of D-ribose (11·6 g.), dried in a vacuum over phosphoric oxide at 60°, and trityl chloride (21·6 g.) in pyridine (116 c.c.), distilled three times from phosphoric oxide, was maintained at 37° for 4 days, with the exclusion of moisture, and then, after being heated at 70° for 30 minutes, was cooled to 5°, and acetic anhydride (60 c.c.) and anhydrous pyridine (50 c.c.) were added. The solution was set aside for a further 24 hours and was then poured into ice-water (3 l.) containing one drop of saturated aqueous trisodium phosphate. This assisted the coagulation of the precipitate which, after 30 minutes, was collected by filtration and purified as described by Bredereck *et al.* (*loc. cit.*) (Found : C, 71·8; H, 5·8; OAc, 22·8. Calc. for $C_{30}H_{30}O_8$: C, 69·5; H, 5·8; OAc, 24·9%). The yield (30 g.) of this material was approx. three times that obtained by using the method of the German workers, and although it probably contained a small quantity of triphenylmethyl acetate it was quite satisfactory for subsequent experiments, contamination being removed as triphenylmethyl bromide.

*Tetra-acetyl Di-*D*-ribose Anhydride*.—1 : 2 : 3-Triacetyl 5-trityl D-ribofuranose, prepared by either method, yielded by use of the procedure of the German workers, tetra-acetyl di-D-ribose anhydride in the form of needles, m. p. 168·5—169·5° [Found : C, 50·0; H, 5·5; OAc, 39·6%; *M* (cryoscopic in benzene), 428. Calc. for $C_{18}H_{24}O_{12}$: C, 50·0; H, 5·6. OAc, 39·8%; *M*, 432·2]. This material (62·1 mg.) was heated at 97° with N/10-hydrochloric acid (3 c.c.) for 165 minutes by which time the solution had $[\alpha]_D^{18·5}$ −21·6° (calculated on the assumption of the presence of D-ribose), unchanged on further heating. Under similar conditions D-ribose had $[\alpha]_D^{18·5}$ −21·2°. The solution obtained from the above hydrolysis was examined on the paper chromatogram alongside a specimen of authentic D-ribose, following Partridge's method (*loc. cit.*), an R_F value of 0·23 being obtained in each case. Partridge records a value of 0·21 for D-ribose.

*Di-*D*-ribose Anhydride*.—Tetra-acetyl di-D-ribose anhydride was deacetylated as described by Bredereck *et al.* (*loc. cit.*) and gave di-D-ribose anhydride in the form of colourless plates, m. p. 229° after sintering from 224° [Found : C, 45·7; H, 5·8%; *M* (cryoscopic in water), 246. Calc. for $C_{10}H_{16}O_8$: C, 45·5; H, 6·1%; *M*, 264). In water the material had $[\alpha]_D^{19·5}$ +11° (*c*, 0·68). After 24 hours at 28° the compound had consumed 1·01 mols. of sodium metaperiodate, no formic acid being detected by titration with N/100-sodium hydroxide after destruction of periodate ions. The solution obtained after completion of this oxidation had $[\alpha]_D^{13}$ −48°, calculated on the assumed presence of 2 : 4-diformyldioxolan.

*Tetramethyl Di-*D*-ribose Anhydride*.—Tetra-acetyl di-D-ribose anhydride (0·091 g.) in acetone (10 c.c.) was stirred at 55°, and methyl sulphate (3·2 c.c.) and aqueous sodium hydroxide (8·6 c.c.; 30%) were added portionwise every 15 minutes during 2·5 hours. Further portions of acetone (5 c.c.) were added to aid mixing. Water (4 c.c.) was added and, after the temperature had been allowed to rise to 75° for 5 minutes, the reaction mixture was cooled to room temperature and extracted 12 times with chloroform (20 c.c. each). The combined chloroform extracts were dried ($MgSO_4$) and after distillation of the solvent the residual syrupy *tetramethyl di-*D*-ribose anhydride* crystallised when kept at 0°. It separated from light petroleum (b. p. 80—100°) in needles and plates (0·045 g.), m. p. 129·5—130° after sintering at 125° (Found : C, 52·9; H, 7·5; OMe, 37·4. $C_{14}H_{24}O_8$ requires C, 52·5; H, 7·5; OMe, 38·7%). The compound did not reduce Fehling's solution.

*Examination of the Products of Hydrolysis of Tetramethyl Di-*D*-ribose Anhydride*.—Tetramethyl di-D-ribose anhydride (10 mg.) was heated at 97° with N/10-hydrochloric acid (2 c.c.) for 40 minutes. This treatment had been found in a preliminary experiment to bring about complete hydrolysis of the material as shown by measurements of reducing power using Somogyi's method (*J. Biol. Chem.*, 1926, **70**, 599). After removal of chloride ions by grinding with silver oxide and filtration, the solution was examined on the paper chromatogram by Partridge's method (*loc. cit.*). One component was observed having an R_F value of 0·55. A further sample of the tetramethyl compound (4·946 mg.) was hydrolysed as above, N-sodium hydrogen carbonate (10 c.c.) and potassium periodate (6 c.c. of a 2·3% solution in N/3-sulphuric acid at 45°) were added (the pH of the solution was then 7·5), and the solution was set aside at room temperature for 24 hours. After destruction of excess of periodate ions, formaldehyde was determined by Jeanloz's method (*loc. cit.*). The formaldehyde–dimedon complex (2·03 mg.) had m. p. 188—190° alone or mixed with an authentic specimen. A third sample of tetramethyl di-D-ribose anhydride (4·076 mg.) was hydrolysed as before, and, after being kept with sodium metaperiodate for

50 hours at room temperature, no liberation of formic acid could be detected using the method of Halsall Hirst, and Jones (*J.*, 1947, 1427).

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