

182. *Characterisation of 2:6-Dimethyl d-Galactose.*

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The constitution of this sugar (Oldham and Bell, *J. Amer. Chem. Soc.*, 1938, **60**, 324) has been verified by application of periodate oxidations to the crystalline sugar and to its β -methylgalactoside. To aid the identification of the sugar, its properties, and those of certain derivatives, have been investigated; it is most easily characterised in the form of its *aldonic acid phenylhydrazide*.

THE work described below was undertaken by arrangement with Dr. E. G. V. Percival as a result of investigations in progress in his laboratory which indicated the presence in a polysaccharide, obtained from the marine Alga, *Gigartina stellata*, of *d*-galactose radicals where the hydroxyl groups on positions 3 and 4 were substituted. From the methylated polysaccharide Dewar and Percival (*Nature*, in the press) isolated a crystalline dimethyl galactose which possessed the chemical characteristics of the 2:6-derivative, but had a m. p. lower than that (128°) recorded by Oldham and Bell (*loc. cit.*). As my original specimen had been destroyed by fire, fresh preparations of 2:6-dimethyl galactose by methylation of 3:4-isopropylidene β -methylgalactoside followed by stepwise elimination of the radicals substituting positions 3, 4, and 1 were undertaken. Despite fractional crystallisation from a variety of solvents, almost the entire amount of each preparation had a m. p. in the range of 105–108°. No higher-melting crystals were obtained; the final product had m. p. 106–108°. The mutarotation in water of the sugar gave values virtually identical with those found for the original preparation, namely +48.2° rising to a constant rotation of +88.0°. Oldham and Bell found (for crystals of m. p. 128°) figures of +46.8° and +87.5°, respectively. Dewar and Percival's substance melted at 119–120° and had $[\alpha]_D^{25} + 48^\circ$ rising to +87.1°. The discrepancy between the melting points recorded for various specimens of 2:6-dimethyl galactose is probably due to the presence of varying amounts of the α -form accompanying the preponderating β -form of the free sugar.

Oxidation of the new preparations of the sugar by sodium metaperiodate in phosphate buffer (pH 7.5) yielded no evidence of formaldehyde production, even when treatment was prolonged. We have found no instance, so far, of an aldohexose which does not yield formaldehyde under such conditions when positions 5 and 6 are unsubstituted; it must be pointed out, however, that the yields of formaldehyde are not in every instance quantitative. (Compare Reeves, *J. Amer. Chem. Soc.*, 1941, **63**, 1476; Jeanloz, *Helv. Chim. Acta*, 1944, **27**, 1509; Bell, Rees, and Williamson, unpublished work in this laboratory.) We therefore consider that periodate oxidation of our sugar affords strong evidence that it is methylated in position 6.

The dimethyl β -methylgalactoside obtained as an intermediate in the preparation of the sugar had m. p. 73–75° in place of the value 45.5° originally recorded for 2:6-dimethyl β -methylgalactoside. [Dewar and Percival (*loc. cit.*) found 72° for their material.] As an elementary analysis of Oldham and Bell's specimen has not been recorded we assume that it was impure. The new preparation consumed periodate in the proportion of one mole of reagent to one mole of glycoside, no formic acid being produced during the course of the oxidation. The galactoside therefore contains two, and not three, consecutive hydroxyl groups (cf. Jackson and Hudson, *J. Amer. Chem. Soc.*, 1937, **59**, 994; 1939, **61**, 1530; Lythgoe and Todd, *J.*, 1944, 592).

The results of the oxidations described above, coupled with the previously reported formation of 6-methyl galactosazone by the action of excess phenylhydrazine on the sugar (Oldham and Bell, *loc. cit.*) leave no doubt that positions 2 and 6 are etherified and that the sugar and its β -methylgalactoside are analytically pure.

The difficulty of using the m. p. of 2:6-dimethyl galactose as a criterion of identification led us to examine derivatives of the sugar as an aid to its characterisation. We prepared a crystalline anilide by the usual procedure, but it proved to be unstable and analysis indicated that it was not homogeneous, an observation confirmed by Dewar and Percival. A useful derivative was found in the *phenylhydrazide* of 2:6-dimethyl galactonic acid which was obtained in 90% overall yield from the free sugar; it has m. p. 139–140° and proved to be identical with material prepared from the sugar supplied by Dr. Percival.

EXPERIMENTAL.

(Glass-distilled solvents were used throughout this work. Substances were recrystallised to constant melting-point, and polarimetric observations were made in 2-dm. tubes. Solvents were evaporated under reduced pressure below 50°. Elementary analyses were performed by Drs. Weiler and Strauss, of Oxford.)

2:6-Dimethyl β -Methylgalactoside.—3:4-isoPropylidene β -methylgalactoside (8.0 g.) (Micheel, *Ber.*, 1929, **62**, 687) was twice methylated by Purdie's reagents. The product (7.5 g., 83.7%), recrystallised from ligroin (b. p. below 40°), had m. p. 55° and $[\alpha]_D^{25} - 4.5^\circ$ (c, 5.8; chloroform); Oldham and Bell (*loc. cit.*) found 56–57° and $[\alpha]_D - 4.46^\circ$. This material (7.0 g.) was dissolved in 5% aqueous acetic acid (80 ml.) and the solution heated at 95° until a constant polarimetric reading was observed. Evaporation of the solvent left a crystalline residue; as this proved difficult to recrystallise, it was distilled at 0.01 mm. and bath temp. 140°. The distillate set to a hard mass of crystals, which, after recrystallisation from chloroform–ligroin (b. p. 60–80°), was obtained in long soft needles, m. p. 73–75°, $[\alpha]_D^{25} - 24.0^\circ$ (c, 6.98; chloroform) and +2.0° (c, 10.8; water) (Found: C, 48.6; H, 8.0; OMe, 40.2. Calc. for C₉H₁₈O₆: C, 48.7; H, 8.1; OMe, 41.9%).

Treated with sodium metaperiodate solution under the conditions of Lythgoe and Todd (*loc. cit.*) the galactoside consumed the reagent as follows: (a) 31.6 mg. reduced 28.73 mg. of periodate (0.97 millimole); (b) 20.0 mg. reduced 20.18 mg. of periodate (1.04 millimole). No titratable acid was formed during these oxidations for which the optimum time was found to be 4–4.5 hours. Prolonged treatment (*e.g.*, 24 hours) results in a slow continuous uptake of periodate, beyond that required by theory; this has been observed with a number of different glycosides. The reason for this slow "periodate drift" is not apparent; we understand that it has also been observed by Hirst and Jones (private communication).

2:6-Dimethyl β -d-Galactose.—The preceding compound (3.0 g.) was dissolved in 5% hydrochloric acid (30 ml.) and the solution heated at 95° until a constant polarimetric reading was observed. The acid was neutralised by addition of barium carbonate, three volumes of acetone added to precipitate the bulk of the barium chloride and inorganic material removed by filtration on a bed of charcoal. The residue, obtained on evaporation of the filtrate, was exhaustively extracted with dry acetone and the resultant solution evaporated to a colourless syrup. This was dissolved in hot dry ethyl acetate and the solution filtered hot. On allowing the solvent to evaporate slowly in a desiccator several crops of needles (total, 2.6 g.) were obtained, m. p. 103–105.5°. Identical material, m. p. 106–108°, was obtained by fractional crystallisation of these crops, using as solvent ethyl or propyl acetates and mixtures of these with varying amounts of methanol, ethanol,

n- and *isopropanol*, acetone and chloroform (Found : C, 46.2; H, 7.6. Calc. for $C_8H_{16}O_6$: C, 46.2; H, 7.7%). A 5.4% solution of the sugar in water displayed the following rotation change :

| | | | | | | | | | |
|--------------------------------|--------|-------|-------|-------|-------|-------|-------|-------|------------------|
| Time (mins.) | 0 | 4 | 9 | 15 | 41 | 62 | 71 | 85 | 1030 |
| $[\alpha]_D^{21}$ (deg.) | +45.0* | +48.2 | +51.2 | +55.7 | +71.0 | +78.5 | +80.2 | +82.7 | +88.0 (constant) |

* By extrapolation.

The upward trend of the rotation indicates that the crystals of 2 : 6-dimethyl galactose are essentially of the β -configuration.

Oxidation of the sugar by sodium metaperiodate in a phosphate buffer (pH 7.5) for periods of from 2 to 48 hours, yielded no evidence of the production of formaldehyde (determined as the dimedon derivative). A crystalline amide was readily obtained by heating the sugar with two equivalents of pure aniline in ethanol for six hours. The substance rapidly decomposed despite recrystallisation from a variety of solvents, and the analytical results were erratic.

2 : 6-Dimethyl Galactonic Acid Phenylhydrazide.—A slight excess of undissolved bromine was maintained in a 10% aqueous solution of 2 : 6-dimethyl galactose (700 mg.) until no reduction of Fehling's solution was detectable (72 hrs.). The reaction product was isolated in the usual manner and lactonised by heating at 120° and 0.05 mm. for one hour. The substance, a colourless syrup (650 mg., 93.6%), had the general characteristics of a hexonolactone (Found : OMe, 30.1. Calc. for $C_8H_{14}O_6$: OMe, 30.9%). The crude lactone (600 mg.) was boiled for six hours with pure phenylhydrazine (0.5 ml.) dissolved in dry benzene (15 ml.) containing a trace of ethanol to promote initial solution of the reactants. On evaporation of the solvents a mass of fine needles remained. After washing with much ether, the phenylhydrazide (950 mg., 96%) melted sharply at 140°. This m. p. was not raised by crystallisation from a mixture of ethanol, ether, and ligroin (Found : C, 53.6; H, 7.0; N, 9.3; OMe, 19.6. $C_{14}H_{22}O_6N_2$ requires C, 53.5; H, 6.9; N, 9.0; OMe, 19.7%). It had $[\alpha]_D^{21}$ -44.8° (c, 2.25; ethanol).

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