Characterisation of Methyl 4 : 6-O-*Benzylidene-α-*D*-galactoside and of* 2 : 3-*Di*-O-*methyl*-D-*galactose.*

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Several derivatives of 2 : 3-di-*O*-methyl-D-galactose have been prepared; the aniline derivative, 2 : 3-di-*O*-methylgalactaro-1→4-lactone, and 2 : 3-di-*O*-methyl-D-galactonamide are suitable for its characterisation. The sugar and several of its derivatives had physical characteristics different from those previously reported. It is probable that only one form of methyl 4 : 6-*O*-benzylidene-α-D-galactoside has so far been obtained.

Five years ago we prepared 2 : 3-di-*O*-methyl-D-galactose, following the directions of Robertson and Lamb (*J.*, 1934, 1321), with the object of characterising more fully this non-crystallisable sugar. From the outset we were hindered by inability to prepare methyl 4 : 6-*O*-benzylidene-α-D-galactoside having the rotation reported by Robertson and Lamb (*loc. cit.*) ($[\alpha]_D$ +166·5° in $CHCl_3$) and later by Sorkin and Reichstein (*Helv. Chim. Acta*, 1945, **28**, 1) ($[\alpha]_D$ +162°). Although we used methyl α-D-galactoside with $[\alpha]_D$ close to the accepted value (Dale and Hudson, *J. Amer. Chem. Soc.*, 1930, **52**, 2534; Ault, Haworth, and Hirst, *J.*, 1935, 1012; Reeves, *J. Amer. Chem. Soc.*, 1949, **71**, 1737), and followed the procedure of Sorkin and Reichstein (*loc. cit.*), our product had $[\alpha]_D$ +144°. We were convinced that we were handling the pure substance, since we could regenerate it from its crystalline diacetate without change in $[\alpha]_D$. Further, a specimen prepared in this Laboratory by the late Mr. S. Williamson by the distillation method used by Robertson and Lamb (essentially that of Irvine and Scott, *J.*, 1913, 575) was found by us to have, after recrystallisation, the same melting point and rotation. About this time Reeves (*loc. cit.*) reported $[\alpha]_D$ +142°; and Dr. J. G. Buchanan of the Lister Institute (personal communication) informs us that he has been unable to prepare this substance with properties other than those found by Reeves and ourselves.

Fletcher, Diehl, and Ness recently (*J. Amer. Chem. Soc.*, 1954, **76**, 3029) also found +144°, and inferred the existence of two forms of this compound, differing through enantiomorphism of the benzylidene grouping. We therefore communicated with Professor Reichstein about this matter, with the result that Dr. Tamm, at Basle, re-examined Sorkin and Reichstein's original specimen finding m. p. 168—170° (Kofler block) and $[\alpha]_D^{21}$ +142·1° ± 1° in $CHCl_3$. Although our material underwent slight decomposition on storage, unless sealed with a trace of ammonia, a specimen from Basle shows no sign of breakdown. Professor Reichstein has kindly authorised us (August 28th, 1954) to make known these corrected values, and states : " The published figure must therefore be due to an error. It is impossible to trace the reason now." Robertson and Lamb's values must likewise be due to some error which cannot now be explained. The specimen labelled " 4 : 6-benzylidene-α-methyl galactoside " obtained from the St. Andrews laboratory through the kind co-operation of Professor J. Read, F.R.S., and Dr. E. S. Steele was found by us to be an impure specimen of the β-glycoside. We believe that only one form of methyl 4 : 6-*O*-benzylidene-α-D-galactoside has so far been prepared, but whether or not this is homogeneous with respect to a single " benzylidene enantiomorph " remains undecided.

In the preparation of 2 : 3-di-*O*-methylgalactose and its derivatives (see scheme annexed) we obtained other products with physical characteristics different from those given in the literature (see Table). In particular, (i) the sugar, which is very difficult to free from solvents, had a much higher $[\alpha]_D$ (*c* determined by hypoiodite reduction) than previously reported; (ii) methyl 2 : 3-di-*O*-methyl-α-galactoside had $[\alpha]_D$ lower than that given by Robertson and Lamb (*loc. cit.*), and not raised by regeneration of the compound from its 4 : 6-dinitrate (which itself had different characteristics from those reported by Robertson and Lamb); the refractive index was also different; and (iii) the aniline derivative of the sugar was quite unlike the substance described by Robertson and Lamb, which was said to have been crystallised from " alcohol "; our product was extremely soluble in ethanol.

Compound (α = methyl α-D-galactoside)	$[\alpha]_D$			M. p.	
	Solvent	Present work	Other work	Present work	Other work
4 : 6-*O*-Benzylidene α	$CHCl_3$	+144° (17—21°)	+166·5° (15°) [2] +162° (15°) [3] +142° (25°) [5] +144° (20°) [6]	169—170°	152° [1] 170—172° [2] 177—178° * [3] 170—172° * [4] 168—169° [5] 171° [6]
2 : 3-Di-*O*-acetyl-4 : 6-*O*-benzylidene α	$CHCl_3$	+211·5° (19°)	+198° (21°) [7]	117—118°	117—118° [7]
4 : 6-*O*-Benzylidene-2 : 3-di-*O*-methyl α	$CHCl_3$	+170° (18—22°)	+173·7° (15°) [2]	126·5—127°	123—124° [2] 128—129° [8] 125° [9] 120° [11]
2 : 3-Di-*O*-methyl α	$CHCl_3$	+167° (19°)	+173·7° (15°) [2]	—	—
2 : 3-Di-*O*-methyl α 4 : 6-di-*O*-nitrate	$CHCl_3$	+114° (19°)	+100·3° (15°) [2]	95—95·5°	88—90° [2]
2:3-Di-*O*-methylgalactose	Water	+116° (19°) †	+80·9° (22°) [10] −2° (17°) [9] +71° (23°) † [11] +78·5° (23°) † [11]	—	—
2:3-Di-*O*-methylgalactose aniline derivative	Ethanol	−56·8° (20 min.) → +12·1° (121 hr., equil.; 17°)	+119·4° (15°) [2]	154—155°	130—131° [2] 128—129° [10]

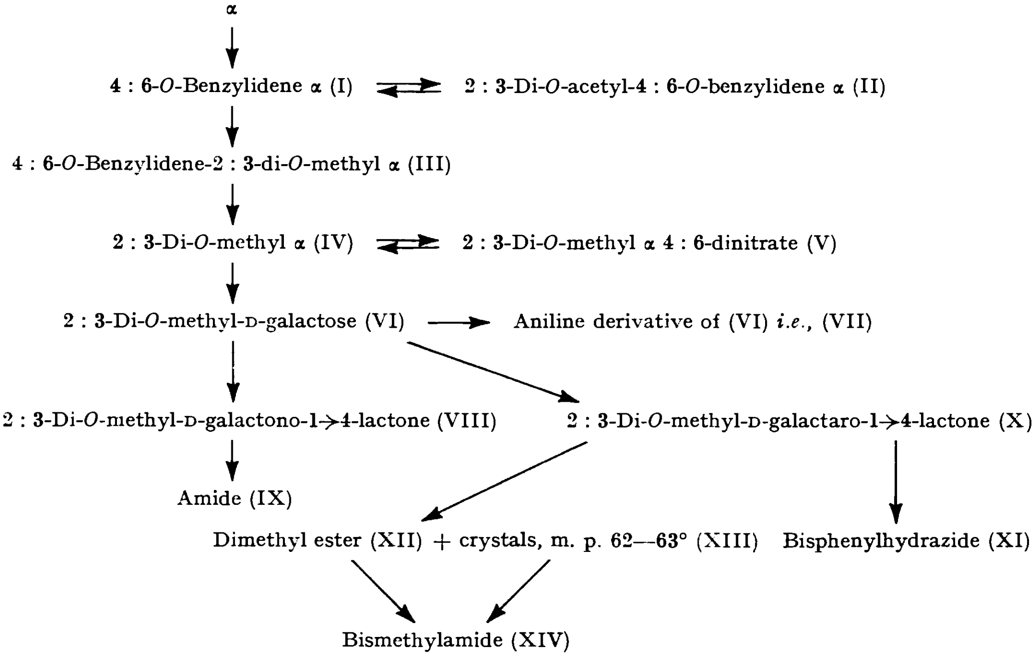
* Corrected. † *c* determined by hypoiodite reduction.

[1] Van Eckenstein and Blanksma, *Rec. Trav. chim.*, 1906, **25**, 153. [2] Robertson and Lamb, *loc. cit.* [3] Sorkin and Reichstein, *loc. cit.* [4] Tamm, *Helv. Chim. Acta*, 1949, **32**, 163. [5] Reeves, *loc. cit.* [6] Fletcher *et al.*, *loc. cit.* [7] Müller, Móricz, and Verner, *Ber.*, 1939, **72**, 745. [8] Reber and Reichstein, *Helv. Chim. Acta*, 1945, **28**, 1164. [9] Luckett and Smith, *J.*, 1940, 1106. [10] Pascu and Trister, *J. Amer. Chem. Soc.*, 1940, **62**, 2301. [11] Bolliger and Schmid, *Helv. Chim. Acta*, 1954, **37**, 888.

The aniline derivative is suitable for characterisation of the sugar. Crystalline 2 : 3-di-*O*-methylgalactono-1→4-lactone, obtained by oxidation of the sugar with bromine water, is extremely deliquescent but yields a satisfactory amide. We have also investigated 2 : 3-di-*O*-methylgalactaric acid derivatives. Beavan and Jones (*J.*, 1947, 1218) oxidised 2 : 3-di-*O*-methyl-D-galacturonic acid with bromine water and obtained a syrup, stated to be 2 : 3-di-*O*-methyl-D-galactaric acid (methoxyl content and equivalent determined). Luckett and Smith (*loc. cit.*) by a similar reaction prepared the acid barium salt (no analysis given); they also oxidised 2 : 3-di-*O*-methylgalactose (made according to Robertson and Lamb) with nitric acid to an acid product. Reeves (*J. Amer. Chem. Soc.*, 1948, **70**, 3963) hydrolysed and oxidised methyl 4 : 6-*O*-benzylidene-2 : 3-di-*O*-methyl-β-D-galactoside with nitric acid, but again the product was not analysed. All these workers esterified the oxidation product, obtaining, after high-vacuum distillation, 2 : 3-di-*O*-methyl-D-galactaric 1→4-lactone methyl ester. For this Luckett and Smith give m. p. 92°, Beavan and Jones 77—78°, raised to 96° by drying *in vacuo*, and Reeves 72—74°. Reeves gives no analysis, but suggests that his compound represents a different crystal modification from that of Luckett and Smith, since its melt, inoculated with their material, crystallised and then melted at 92°. Luckett and Smith converted the lactone-ester into the bis-methylamide, and Reeves obtained the same compound.

On oxidising 2 : 3-di-*O*-methylgalactose with nitric acid (more dilute than that used by Luckett and Smith) we obtained crystalline 2 : 3-di-*O*-methyl-D-galactaro-1→4-lactone. On esterification a crystalline product was obtained without distillation. A single recrystallisation, however, separated this into two fractions: (XII) the dimethyl ester of 2 : 3-di-*O*-methyl-D-galactaric acid, m. p. 95—96°, and (XIII) crystals, m. p. 62—63°. Elementary and methoxyl analyses suggested that (XIII) consisted of a mixture of the dimethyl ester and the ester-lactone. It is, however, noteworthy that these crystals were obtained in two separate preparations, and that their melting point was unaffected by further recrystallisation. Both (XII) and (XIII) gave the bismethylamide in good yield.

(" α " denotes methyl α-D-galactoside)



EXPERIMENTAL

M. p.s and rotations (measured in 2-dm. tubes unless otherwise stated) were determined on specimens dried over phosphoric oxide at *ca.* 0·01 mm. and room temperature. Evaporations were done under reduced pressure below 50°, and solvents distilled in glass used for recrystallisations. Equivalent weights of lactones were determined by treatment with aqueous sodium hydroxide in 25% excess for 4—6 hr. at room temperature and back-titration with hydrochloric acid, with exclusion of carbon dioxide throughout. pH was measured by the glass electrode. We thank Dr. D. H. Northcote for some of the methoxyl determinations.

Methyl 4 : 6-O-*Benzylidene-α-D-galactoside* (I).—In accord with the work by Dale and Hudson (*loc. cit.*) and Ault, Haworth, and Hirst (*loc. cit.*), crude methyl α-D-galactoside was purified through the tetra-*O*-acetyl derivative, $[\alpha]_D^{19}$ +133° (*c*, 6·1 in $CHCl_3$) (Dale and Hudson, *loc. cit.*, give +133°). After recrystallisation from ethanol and drying over phosphoric oxide at 90—95°/0·01 mm. the purified galactoside had $[\alpha]_D^{17}$ +195·1° (*c*, 1·8 in H_2O) and m. p. 123—125°. (The anhydrous galactoside readily forms the monohydrate in air, and pressing the material on porous plate lowered the m. p. considerably. Ault *et al.*, *loc. cit.*, give m. p. 116°.) It was converted into the benzylidene compound (I) by Freudenberg's method (*Ber.*, 1928, **61**, 1728), following Sorkin and Reichstein (*loc. cit.*); recrystallised from ethanol or ethanol–light petroleum (b. p. 60—80°) this had $[\alpha]_D^{20}$ +168° (*c*, 2·3 in MeOH); for m. p. and $[\alpha]_D$ in $CHCl_3$ (*c*, 1·8—2·1) see Table (Found: C, 59·7; H, 6·4. Calc. for $C_{14}H_{18}O_6$: C, 59·6; H, 6·4%).

Our material was somewhat unstable; after 3 months in a desiccator it smelt of benzaldehyde and had m. p. 165—167°. However, after 4 years in a sealed tube a sample smelt only faintly of benzaldehyde and had m. p. 168—169°, or sealed with a trace of ammonia had unchanged m. p. and no smell after the same period. To test the possibility that the high m. p.s and rotations given by some authors resulted from overheating of the preparation, a sample was heated for 2 hr. at 96—100°/0·3 mm.; it lost only 0·2% in weight, and the m. p. and $[\alpha]_D$ were not raised.

The 2 : 3-di-*O*-acetate (II), prepared from (I) by the procedure of Müller, Móricz, and Verner (*loc. cit.*), had, after three recrystallisations from aqueous ethanol, the m. p. and $[\alpha]_D$ (*c*, 1·5) given in the Table (not raised by further recrystallisation) (Found: C, 59·2; H, 6·15; Ac, 23·5. Calc. for $C_{18}H_{22}O_8$: C, 59·0; H, 6·0; Ac, 23·5%). The acetate (II) was reconverted into (I) by 30 minutes' boiling with ethanolic sodium hydroxide. The product (95% yield) had m. p. 168—169° and $[\alpha]_D^{18}$ +141° in $CHCl_3$, and after one recrystallisation from ethanol 169—170° and +143·5° respectively.

Methyl 2 : 3-*Di-*O*-methyl-α-*D*-galactoside* (IV).—The galactoside (I), dissolved in acetone, was treated with methyl sulphate and aqueous sodium hydroxide, giving methyl 4 : 6-*O*-benzylidene-2 : 3-di-*O*-methyl-α-D-galactoside (III) (77%), m. p. 124—125°, $[\alpha]_D^{22}$ +170° (*c*, 2·0 in $CHCl_3$) after one recrystallisation from ether. Further recrystallisations from acetone–light petroleum (b. p. 60—80°) and ether–acetone raised the m. p. to 126·5—127°, with no change in $[\alpha]_D$ (Found: C, 61·9; H, 6·85; OMe, 30·2. Calc. for $C_{16}H_{22}O_6$: C, 61·9; H, 7·1; OMe, 30·0%). The derivative (III) was converted into the methylgalactoside (IV) by Robertson and Lamb's method (*loc. cit.*) except that the product was extracted by anhydrous ether–absolute ethanol instead of acetone. It was distilled at 0·05 mm. and collected in three fractions (bath-temp. 150—173°) which had the following n_D^{28} and $[\alpha]_D^{19}$ in $CHCl_3$: (i) (10%) 1·4771, +165°; (ii) (53%) 1·4773, +167°; (iii) (28%) 1·4773, +167° [Found for fraction (iii): C, 49·0; H, 8·1. Calc. for $C_9H_{18}O_6$: C, 48·65; H, 8·1%]. Another preparation also had n_D^{28} 1·4773 (decrement for rise of 1°, 0·00029 from 17° to 30°). Our value for n_D^{15}, 1·4811, obtained by extrapolation from n_D^{17}, differs considerably from 1·4720 found by Robertson and Lamb (*loc. cit.*). $[\alpha]_D^{19}$ was +209° (*c*, 2·2 in H_2O).

The 4 : 6-di-*O*-nitrate (V), prepared from the galactoside (IV) by fuming nitric acid in chloroform had, after two recrystallisations from ethanol, the m. p. and $[\alpha]_D$ (*c*, 1·6) given in the Table (Found: C, 34·8; H, 5·3; N, 8·75. Calc. for $C_9H_{16}O_{10}N_2$: C, 34·6; H, 5·1; N, 8·95%). It was reconverted into (IV) with zinc and iron in glacial acetic acid; after distillation at 0·01 mm. with rejection of the first runnings, the regenerated galactoside was obtained in 62% yield, with n_D^{28} 1·4771, $[\alpha]_D^{19}$ +164° in $CHCl_3$ and +205° in H_2O.

Methyl 2 : 3-*Di-*O*-methyl-β-*D*-galactoside.*—The *β-galactoside* had been prepared in this Laboratory by the late Mr. S. Williamson from the 4 : 6-*O*-benzylidene derivative (Oldham and Bell, *J. Amer. Chem. Soc.*, 1938, **60**, 323). After distillation at 0·05 mm. it was a syrup, $[\alpha]_D^{18}$ +23·0° (*c*, 7·4 in H_2O), −10·7° (*c*, 6·8 in $CHCl_3$), n_D^{20} 1·4787 (decrement for rise of 1°, 0·00033 from 17° to 30°) (Found: C, 49·1; H, 8·1. $C_9H_{18}O_6$ requires C, 48·65; H, 8·1%).

2 : 3-*Di-*O*-methyl-*D*-galactose* (VI).—The galactoside (IV) was hydrolysed as directed by Robertson and Lamb (*loc. cit.*). The product was dissolved in dichloroethane, the solution filtered and evaporated, and the residue dried over phosphoric oxide at 43°/0·2 mm. for 3 hr. It gave a single compact spot on a paper chromatogram (butanol–water) and had $[\alpha]_D^{17}$ (*c*, 1·8 in H_2O) +76° (10 min.), +97° (6·5 hr., equil.). Further drying over phosphoric oxide at 70—75°/0·2 mm. for 3 hr. caused a 6·2% loss in weight; the sugar was now a colourless, hygroscopic, very viscous syrup, $[\alpha]_D^{18}$ (*c*, 1·5 in H_2O) +57° (5 min.), +74° (10 min.), +105° (140 min., equil.). The mutarotation is unusually large for a syrupy sugar. Another preparation (3·0 g.), which had been kept at 40—50°/0·2 mm. for 4 hr., was dissolved in water, the solution was evaporated to remove traces of organic solvents, and the residue was dissolved in water and filtered (charcoal). This process was repeated, the sugar being finally in 100 ml., and the rotation measured. Reduction of hypoiodite was determined under Macleod and Robison's conditions (*Biochem. J.*, 1929, **23**, 517) but on a larger scale (12 mg. of sugar); it was complete in 30 min. With *c* (2·43), thus determined, $[\alpha]_D^{19}$ was 116° in H_2O (*l*, 4). To test if this high $[\alpha]_D$ was due to presence of galactoside or anhydro-compounds the sugar was heated with N-hydrochloric acid at 95—100° for 2 hr. After working up and removal of traces of organic solvents, as before, $[\alpha]_D^{19}$ was +113° (*c*, 3·19 in H_2O, determined by hypoiodite; *l*, 4).

Aniline Derivative (VII).—After removal of impurities with ether–light petroleum (b. p. 40—60°), the derivative (VII) was crystallised from *n*-butanol–light petroleum (b. p. 40—60°), then twice from acetone; for m. p. and $[\alpha]_D$ (*c*, 3·6) see Table (Found: C, 59·2; H. 7·4; N, 4·95; OMe, 22·3. Calc. for $C_{14}H_{21}O_5N$: C, 59·4; H, 7·4; N, 4·95; OMe, 21·9%). At the end of the polarimetric observations the solution was allowed to evaporate; one recrystallisation of the crystalline residue from acetone gave an almost quantitative recovery of material, m. p. 153—154°. The changing rotation was therefore not due to decomposition.

2 : 3-*Di*-O-*methyl*-D-*galactono*-1→4-*lactone* (VIII).—The sugar (VI), in 6% solution in water, was treated with bromine for 43 hr. The product was distilled at 0·1 mm. (bath-temp. 180—190°). The crystalline distillate was spread on porous plate at −10°, and the plate transferred to a desiccator containing phosphoric oxide. The crystals were washed by decantation with anhydrous ether at room temperature and dissolved in boiling anhydrous ether, and the solution decanted or filtered, and allowed to crystallise at −10°. The *lactone*, highly deliquescent colourless needles, neutralised 1·03 equiv. of sodium hydroxide. $[\alpha]_D^{19}$ (*c*, 1·9 in H_2O; *l*, 1) −48·8° (18 min.), −46·2° (26 hr.), −41·2 (100 hr.), −36·2° (270 hr., equil.). The rate of hydrolysis indicates a γ-lactone. A 1% solution in water had the following pH values (21°): 4·17 (initially); 2·90 (2 days); 2·79 (25 days).

2 : 3-*Di*-O-*methyl*-D-*galactonamide* (IX).—The lactone (VIII), with ammonia in methanol for 4 days at room temperature, gave the *amide* in 53% yield after recrystallisation from ethanol–acetone; this had m. p. 140° (not raised by further recrystallisation from ethanol), $[\alpha]_D^{20}$ +13·1° (*c*, 2·0 in H_2O) (Found: C, 42·9; H, 7·35; N, 6·5; OMe, 27·6. $C_8H_{17}O_6N$ requires C, 43·0; H, 7·6; N, 6·3; OMe, 27·8%). The phenylhydrazide was unstable and could not be recrystallised.

2 : 3-*Di*-O-*methyl*-D-*galactaro*-1→4-*lactone* (X).—The sugar (VI) was oxidised with nitric acid (*d*, 1·15) on the boiling-water bath. After removal of the nitric acid, the product was crystallised twice from ether, giving the *lactone* (X) (50%) as colourless crystals, stable in air, m. p. 126—126·5° (not raised by further recrystallisation) (Found: C, 43·8; H, 5·3; OMe, 28·1%; equiv., 110. $C_8H_{12}O_7$ requires C, 43·6; H, 5·45; OMe, 28·2%; equiv., 110), $[\alpha]_D^{18}$ (*c*, 2·0 in H_2O) −55·4° (8 min.), −51·3° (8·75 hr.), −38·6° (49 hr.), −28·7° (101 hr.), −18·3° (198 hr.), −13·4° (275 hr.), −11·0° (370 hr., equil.). The rate of hydrolysis indicates a γ-lactone. A 1% solution in water had the following pH values (21°): 2·22 (initially); 2·22 (2 days); 2·30 (6 days); 2·31 (25 days).

2 : 3-*Di*-O-*methyl*-D-*galactaric Bisphenylhydrazide* (XI).—The bisphenylhydrazide, obtained by heating the lactone (X) under reflux with phenylhydrazine and enough ethanol to effect dissolution, was obtained, after removal of impurities with ether, as colourless crystals, m. p. 140° (decomp.), from ethanol–ether. The m. p. was not raised by further recrystallisation from ethanol–ether or ethyl acetate–ether, but a satisfactory elementary analysis could not be obtained, probably because of some decomposition during recrystallisation (Found: C, 55·7; H, 6·5; N, 12·8; OMe, 15·3. Calc. for $C_{20}H_{26}O_6N_4$: C, 57·4; H, 6·2; N, 13·4; OMe, 14·8%). The substance decomposes in ethanol solution at room temperature, but $[\alpha]_D^{20}$ in ethanol remains at approx. +17° for several hours.

Dimethyl 2 : 3-*Di*-O-*methyl*-D-*galactarate* (XII).—The lactone (X) (0·83 g.) was heated under reflux for 10 hr. with 2% (w/v) hydrogen chloride in methanol. The crystalline product was recrystallised from ether–light petroleum (b. p. 40—60°) with a little chloroform. The first crop (0·38 g.) consisted of the *dimethyl ester* (XII), m. p. 95—96° (not raised by further recrystallisation), $[\alpha]_D^{18}$ (*c*, 1·0 in H_2O) +12·7° (7 min.), +7·3° (20 hr.), +3·4° (69 hr.), +0·8° (123 hr.), −3·6° (292 hr., constant); pH of 1% solution in water (16—18°), 4·45 (initially), 3·00 (5 days), 2·75 (16 days) (Found: C, 45·2; H, 6·9; OMe, 46·8. $C_{10}H_{18}O_8$ requires C, 45·1; H, 6·8; OMe, 46·6%). The second (0·34 g.) and the third (0·12 g.) crop had m. p. 62—63° and 61—62° respectively; this material (XIII), on further recrystallisation from ether, yielded three crops, 0·38 g. in all, each of m. p. 62—63° (Found: C, 45·8; H, 6·4; OMe, 42·7. Calc. for the lactone-ester $C_9H_{14}O_7$: C, 46·1; H, 6·0; OMe, 39·7%. For $C_{10}H_{18}O_8$ see above); these had $[\alpha]_D^{18}$ (*c*, 1·1 in H_2O) −22·2° (7 min.), −20·6° (18 hr.), −15·7° (77 hr.), −13·3° (122 hr.), −5·4° (30 days), −4·0° (34 days). The solution, originally neutral, became acid to Congo-red. The polarimetric behaviour is consistent with the crystals' being a mixture of dimethyl ester and lactone-ester; for the latter Luckett and Smith (*loc. cit.*) found $[\alpha]_D^{19}$ −56° (initial), −4° (29 days, constant).

The ester (XII) (122 mg.) and the material (XIII) (125 mg.) were separately treated with 25 ml. of 1·5N-methylamine in methanol for 2 days at room temperature. The methylamine was generated from hydrochloride which had been recrystallised from *n*-butanol to remove ammonium chloride. After recrystallisation from ethanol–ether both specimens of bismethylamide (XIV) [105 mg. from (XII) and 99 mg. from (XIII)] had m. p. 181—182° (uncorr.) [183·5—184·5° (corr.)] (not raised by further recrystallisation), $[\alpha]_D^{18}$ −7·7° (*c*, 2·5 in H_2O). Luckett and Smith (*loc. cit.*) give m. p. 184°, $[\alpha]_D^{18}$ −7·5°.

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