288. *A Determination of the Relationship between Refractive Index and Specific Rotation in Mixtures of 2 : 3 : 4 : 6-Tetramethyl α- and β-Methyl-*d*-galactosides.*

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The graphical relationship between the refractive index and the specific rotation of mixtures of α- and β-forms of 2 : 3 : 4 : 6-tetramethyl methyl-*d*-galactoside has been found to be a straight line. This establishes a criterion of homogeneity useful, for example, in polysaccharide investigations where assays of tetramethyl galactopyranose are concerned.

HIRST and YOUNG (J., 1938, 1247) established criteria of homogeneity with respect to the parent sugar for mixtures of 2 : 3 : 4 : 6-tetramethyl α- and β-methyl-*d*-glucosides. They determined the refractive index and the specific rotation in water of the sample of mixed glucosides and compared these constants with the straight-line graphical relationship between $n_D^{16°}$ and $[\alpha]_D$ for authentic tetramethyl methylglucoside mixtures in various α : β proportions. In the present communication the same procedure is applied to the analogous galactosides, with the object of aiding researches on galactose derivatives.

For our work it was first decided to prepare, in as pure a state as possible, specimens of both 2 : 3 : 4 : 6-tetramethyl α- and β-methyl-*d*-galactosides, and the specimens finally obtained had the following properties :

Isomer.	M. p.	$[\alpha]_D$ in water.	$n_D^{16°}$.	Increment in n_D for fall of 1°.
α [1]	Syrup	+190°	1·45035	0·00034
β [2]	48—49°	+ 18·7	1·44805	0·00034

[1] Micheel and Littmann (*Annalen*, 1928, **466**, 115) give $[\alpha]_D$ +188·5°; Irvine and Cameron (J., 1904, **85**, 1078) give $[\alpha]_D$ +143·4°.

[2] Schlubach and Moog (*Ber.*, 1923, **56**, 1957) give m. p. 47—48°; $[\alpha]_D$ +19·6°, $n_D^{20°}$ 1·4420; * Micheel and Littmann (*loc. cit.*) give m. p. 46—47°, $[\alpha]_D$ +20·7°; Irvine and Cameron (*loc. cit.*) give m. p. 44—45°, $[\alpha]_D$ +30·7°.

* This low value of $n_D^{20°}$ for the β-galactoside was never approached in the present experiments despite different methods of preparing the crystalline material; 1·4480—1·4481 was always found at 16°.

Differences between the glucose and galactose methylglycosides were found in both refractive index and ease of separation by fractional distillation. The difference between $n_D^{16°}$ for the α- and the β-form of tetramethyl methylglucopyranoside is 0·0040 (Hirst and Young, *loc. cit.*), whereas the corresponding difference for the galactose derivatives is only 0·0023, which makes the galactose measurements much less sensitive, especially when compared with the differences between the specific rotations of the α- and the β-members in the two series, *viz.*, 147° and 171° respectively. It is well known that in distillation of a mixture of tetramethyl α- and β-methylglucopyranosides, the latter, having a lower boiling point, tends to be concentrated in the distillate; no such fractionation could be observed with the galactose derivatives (cf. " Mixtures A, B, and C," below).

Starting with pure tetramethylgalactose, three mixtures of the α- and β-methylglycosides, in different proportions, were prepared. These, termed " A," " B," and " C," had constants which very closely approached to the straight-line relationship between $n_D^{16°}$ and $[\alpha]_D$ fixed by the constants of pure 2 : 3 : 4 : 6-tetramethyl α- and β-methyl-*d*-galactosides. Final confirmation of this relationship was afforded by obtaining points relating to ten artificial mixtures of the α- and β-derivatives prepared by mixing weighed quantities of the two substances.

EXPERIMENTAL.

Measurements of optical activity were made on approximately 5% solutions in water, the same volumetric apparatus and 2 dm. polarimeter tube being used for all experiments. Investigations showed that concentration of solute had no appreciable effect on the value of α. The error in polarimetric readings was of the order of ± 0·1° in $[\alpha]_D$ for 5% solutions.

Refractometric observations were made with a Hilger refractometer of the Abbé type, the temperature of the instrument being read to 0·25°. Calibration against carbon dioxide-free water was done with every batch of material examined. The error in reading was ± 0·00015.

2 : 3 : 4 : 6-*Tetramethyl* β-*Methyl*-d-*galactoside*.—Pure 2 : 3 : 4 : 6-tetramethyl *d*-galactose, prepared according to Schlubach and Moog (*loc. cit.*), was treated with Purdie's reagents until the reaction mixture no longer reduced Fehling's solution. On evaporation of the filtered solution, the crude product crystallised spontaneously. The crisp needles obtained after draining on porous tile were recrystallised from light petroleum (b. p. below 40°) until the specific rotation in water and the refractive index were constant. The purest material thus prepared had $n_D^{29\cdot50^\circ}$ 1·4435; $n_D^{17\cdot5^\circ}$ 1·4476; $n_D^{16^\circ}$ 1·44805 (by extrapolation) (see also p. 1543).

A second preparation, from pure α-methyl-*d*-galactoside (cf. Ault, Haworth, and Hirst, J., 1935, 1012) by methylation first by methyl sulphate and alkali and finally by Purdie's reagents, was fractionally distilled, hydrolysed, and the resulting free sugar treated with Purdie's reagents. The crude product crystallised, and was recrystallised as above; $[\alpha]_D^{20^\circ}$ + 18·9°; $n_D^{36^\circ}$ 1·4411; $n_D^{29\cdot5^\circ}$ 1·4434; $n_D^{16^\circ}$ 1·4480 (by extrapolation).

2 : 3 : 4 : 6-*Tetramethyl* α-*Methyl*-d-*galactoside*.—α-Methyl-*d*-galactoside hydrate, prepared according to Ault, Haworth, and Hirst (*loc. cit.*), had m. p. 108° (constant), $[\alpha]_D^{22^\circ}$ + 176·8° in water (constant). Micheel and Littmann (*loc. cit.*) quote $[\alpha]_D$ + 175·5°, E. Fischer (*Ber.*, 1895, **28**, 1155) gives + 179·3°, and Irvine and Cameron (*loc. cit.*) + 178°. After methylation as for the second preparation above, the crude product was twice fractionally distilled, the middle fractions being collected each time and the others rejected, and n_D and $[\alpha]_D$ being the criteria. The material was again distilled, giving three fractions having the following properties :

Fraction.	$n_D^{11^\circ}$.	$n_D^{30^\circ}$.	Increment in n_D per 1° fall.	$n_D^{16^\circ}$.	$[\alpha]_D^{20^\circ}$.
A	1·4521	1·4456	0·00034	1·4503	+189·2°
B	1·4521	1·4457	0·00034	1·4504	+189·2
C	1·4522	1·4458	0·00034	1·4505	+190·1

Fraction B was taken to be pure 2 : 3 : 4 : 6-tetramethyl α-methyl-*d*-galactoside (Found : C, 52·7; H, 8·9; OMe, 61·5. Calc. for $C_{11}H_{22}O_6$: C, 52·8; H, 8·8; OMe, 62·0%).

"*Mixture A.*" Pure 2 : 3 : 4 : 6-tetramethyl galactose was boiled with methyl alcohol containing 1% of hydrogen chloride until no reduction of Fehling's solution was observed (1 hour). After a preliminary vacuum distillation, the galactoside mixture, isolated as usual, was distilled very slowly at 0·001 mm. Five fractions were collected :

Fraction.	1.	2.	3.	4.	5.
$n_D^{16^\circ}$	1·4497	1·4496	1·4496	1·4496	1·4497
$[\alpha]_D^{20^\circ}$	+144·8°	—	+149·7°	—	+150·9°

No appreciable fractionation had thus taken place. The increment in n_D for a fall of 1° was 0·00034.

"*Mixture B.*" Pure 2 : 3 : 4 : 6-tetramethyl galactose was methylated with Purdie's reagents Isolated in the usual way, the crude product crystallised. All but a small residue was distilled through a vacuum-jacketed column at 0·001 mm., three fractions being collected. It was not possible to observe the refractive index below 17·5° owing to the tendency of the supercooled material to crystallise. The fractions were essentially identical :

	$n_D^{36\cdot25^\circ}$.	$n_D^{30^\circ}$.	$n_D^{17\cdot5^\circ}$.	$n_D^{16^\circ}$.	$[\alpha]_D^{20^\circ}$.
Fraction 1	1·4418	1·4438	1·4481	1·4484	+37·0°
Fraction 2	1·4418	1·4438	1·4481	1·4484	+37·4
Fraction 3	1·4419	1·4440	1·4481	1·4484	+38·1

The increment in n_D for a fall of 1° was 0·00034.

"*Mixture C.*" 2 : 3 : 4 : 6-Tetramethyl galactose was dissolved in methyl alcohol containing 1% of hydrogen chloride and kept at room temperature for 48 hours. The solution reduced Fehling's solution. The crude product was next treated with Purdie's reagents until it became non-reducing. Again, fractional distillation of the mixed galactosides led to no separation :

Fraction.	1.	2.	3.	4.	5.
$n_D^{16^\circ}$	1·4494	1·4495	1·4495	1·4496	1·4495
$[\alpha]_D^{20^\circ}$	+126·8°	—	—	—	+126·4°

Artificial Mixtures.—Ten mixtures were prepared by mixing weighed quantities of pure tetramethyl α- and β-methyl-*d*-galactosides. Only one showed $n_D^{16°}$ which differed from the calculated value by more than the experimental error (0·00015).

α, %.	$[\alpha]_D$.	$n_D^{16°}$, found.	$n_D^{16°}$, calc.	Diff. in n_D.	α, %.	$[\alpha]_D$.	$n_D^{16°}$, found.	$n_D^{16°}$, calc.	Diff. in n_D.
21·2	+ 57·8°	1·4484	1·44855	−0·00015	25·7	+ 62·2°	1·4488	1·4486	+0·00020
11·4	+ 38·6	1·4482	1·4483	−0·00010	91·5	+176·4	1·4500	1·45015	−0·00015
42·9	+ 93·9	1·4492	1·44905	+0·00015	16·6	+ 47·0	1·4485	1·4584	+0·00010
58·1	+120·7	1·4495	1·44945	+0·00005	27·8	+ 65·8	1·4487	1·44865	+0·00005
14·1	+ 44·0	1·4484	1·44835	+0·00005	55·1	+112·7	1·4493	1·44935	−0·00005

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