379. *Studies on Agar-agar. Part II. The Isolation of Derivatives of 3 : 6-Anhydro-*l*-galactose from Agar and the Synthesis of their Enantiomorphs.*

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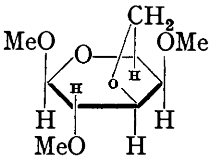
Details are given of investigations (some of which were reported briefly in *Nature*, 1938, **142**, 797, 1076) constituting independent proof of the work of Hands and Peat (*ibid.*, p. 797). The synthesis of 2 : 4-dimethyl 3 : 6-anhydro-β-methyl-*d*-galactoside is described and this compound and other derivatives of the parent sugar are shown to be enantiomorphs of the corresponding derivatives prepared from methylated agar. It is concluded that the 3 : 6-anhydro-*l*-galactose units are preformed in agar.

In Part I (J., 1937, 1615) Percival and Somerville, by the hydrolysis of methylated agar, isolated 2 : 4 : 6-trimethyl α-*d*-galactose, and the correctness of the constitution assigned to this substance has since been proved by its synthesis by Bell and Williamson (J., 1938, 1196). At the same time a fraction of higher boiling point was isolated which on account of its strong ketose colour reactions was considered to be a dimethyl methylketoside. By methylation Somerville (Thesis, Edinburgh, 1938) isolated a crystalline substance, hereafter called X, m. p. 82°, $[\alpha]_D^{20^\circ}$ + 75° in water, + 85° in chloroform, which he demonstrated to be a dimethyl anhydromethylhexoside ($C_9H_{16}O_5$) and for which, on account of its extreme ease of hydrolysis, he proposed the structure of a dimethyl 3 : 6-anhydro-ketofuranoside. On applying the method used by Peat and Wiggins (J., 1938, 1088) for the hydrolysis of a dimethyl 3 : 6-anhydromethylglucoside, it was found that the glycosidic group was smoothly eliminated to yield a free dimethyl anhydro-sugar, $[\alpha]_D^{17^\circ}$ − 23°. Since, if the Seliwanoff reaction were disregarded, the possibility existed that the anhydro-sugar might be a galactose derivative, and since 3 : 6-anhydro-α-methyl-*d*-galactoside had been described by Ohle and Thiel (*Ber.*, 1933, **66**, 525), it was decided to prepare the methylated derivative of this substance for comparison.

By the direct methylation of Ohle and Thiel's compound 2 : 4-*dimethyl* 3 : 6-*anhydro-α-methyl*-d-*galactoside* was obtained as an oil almost identical in boiling point and rotation ($[\alpha]_D^{20^\circ}$ + 87° in chloroform) with X. Both this oil and 3 : 6-anhydro-α-methylgalactoside gave strong Seliwanoff reactions, so this test is clearly not specific for a ketose. The oil would not crystallise, and that it was essentially different from the crystalline material X was shown by the fact that, although hydrolysis with N-sulphuric acid was complete in 24 hours, the equilibrium specific rotation was + 22° instead of − 23°. 3 : 6-Anhydro-*d*-galactose could not therefore be the parent of X. The possibility remained, however, that *l*-galactose might be concerned, since the rates of hydrolysis of the two substances were so similar, and the fact that the specific rotations of the free dimethyl anhydro-sugar from agar and of 2 : 4-dimethyl 3 : 6-anhydro-*d*-galactose were approximately equal but opposite in sign, although possibly fortuitous, would have a significance if the natural substance were a derivative of 3 : 6-anhydro-*l*-galactose. Pirie (*Biochem. J.*, 1936, **30**, 369) had demonstrated the presence of *l*-galactose in agar (his isolation of hepta-acetyl *dl*-galactose having since been confirmed in this laboratory), which also lent colour to this view. These conclusions were announced briefly in *Nature*, 1938, **142**, 797.



X.

This hypothesis could be tested by the synthesis of the only possible enantiomorph, *viz.*, 2 : 4-dimethyl 3 : 6-anhydro-β-methyl-*d*-galactoside. At the time this work was proceeding Hands and Peat (*Chem. and Ind.*, 1938, **57**, 937) showed by the isolation of the enantiomorphous 2 : 4-dimethyl 3 : 6-anhydrogalactoses that this hypothesis appeared to be correct, and further support for this view was found by us as follows.

3 : 6-*Anhydro-β-methyl*-d-*galactoside* was obtained from the 2 : 3 : 4-triacetyl 6-tosyl galactose-α-1-bromide of Ohle and Thiel (*loc. cit.*) by treatment with silver carbonate in methyl-alcoholic solution, followed by deacylation. The crystalline product had m. p. 118°, $[\alpha]_D^{20^\circ}$ − 114° in water, and the method of preparation, coupled with the specific rotation of the product and that of the intermediate *triacetyl* 6-*tosyl methylgalactoside*, left no doubt that the substance was the β-form. On methylation 2 : 4-*dimethyl* 3 : 6-*anhydro-β-methyl*-d-*galactoside* was obtained, which crystallised spontaneously; it was identical with X in appearance, melting point, and analytical composition. A mixed melting point determination showed a depression and the sign of the specific rotation was reversed. It was concluded that the two substances were enantiomorphs and that X must accordingly be described as 2 : 4-*dimethyl* 3 : 6-*anhydro-β-methyl*-l-*galactoside.* It was found possible to isolate from the corresponding free sugars enantiomorphous *anilides*, and from the sugar acids, *amides* and *esters*, the properties of which are included in the table.

The conversion of the syrupy 2 : 4-dimethyl 3 : 6-anhydro-α-methyl-*d*-galactoside into the β-form was found to be surprisingly easy in methyl-alcoholic hydrogen chloride solution. This has also been observed, and more extensively studied, by Jackson, Haworth, and Smith, in a letter which appeared in *Nature* (1938, **142**, 1075) at the same time as our communication (*ibid.*, p. 1076). These authors also announced the synthesis directly from β-methyl-*d*-galactoside of the corresponding 2 : 4-dimethyl 3 : 6-anhydro-derivative and the isolation of the corresponding anilide, amide, and acid.

The yield of 2 : 4-dimethyl 3 : 6-anhydro-β-methyl-*l*-galactoside was recorded as 16% in our first communication (*Nature, loc. cit.*), but it has proved impossible since to isolate such a quantity of the crystalline material, the yield of which appears to vary between

10 and 13%, a precise separation of X from the oil which accompanies it being difficult. If the highest figure for 2 : 4 : 6-trimethyl methylgalactoside is taken, *viz.*, 65% (this only refers to crystalline material and leaves out of account any syrupy β-form), and 15% is allowed for the anhydride, only 80% of the products of hydrolysis of methylated agar are thus accounted for. The oil (*ca.* 20% of the hydrolysis products) from which X was separated still contained some of this substance in solution (*ca.* 16%). Hydrolysis with cold acid, extraction with ether, and distillation effected a partial separation. The remaining syrup on hydrolysis and treatment with aniline yielded several crops of a crystalline anilide, but this proved to be tetramethyl *d*-galactose anilide, and a rigorous search of the products from many experiments failed to reveal the presence of any other recognisable substance.

Natural Product.	*Synthetic Product.*
2 : 4-Dimethyl 3 : 6-anhydro-β-methyl-*l*-galactoside, m. p. 82°.	2 : 4-Dimethyl 3 : 6-anhydro-β-methyl-*d*-galactoside, m. p. 82°, mixed m. p. 65°.
$[\alpha]_D^{14^\circ}$ +75° in water (*c*, 5·6)	$[\alpha]_D^{20^\circ}$ −77° in water (*c*, 0·6)
$[\alpha]_D^{20^\circ}$ +85° in chloroform (*c*, 2·1)	$[\alpha]_D^{20^\circ}$ −86° in chloroform (*c*, 0·5)
$[\alpha]_D^{19^\circ}$ −23° in N-sulphuric acid after 24 hrs. (*c*, 2·8)	$[\alpha]_D^{19^\circ}$ +22° in N-sulphuric acid after 24 hrs. (*c*, 1·5)
Methyl 2 : 4-dimethyl 3 : 6-anhydro-*l*-galactonate, m. p. 48—49°.	Methyl 2 : 4-dimethyl 3 : 6-anhydro-*d*-galactonate, m. p. 49—50°, mixed m. p. 38°.
$[\alpha]_D^{10^\circ}$ −72·5° in chloroform (*c*, 0·6)	$[\alpha]_D^{16^\circ}$ +73° in chloroform (*c*, 0·6)
$[\alpha]_D^{16^\circ}$ −64° in water (*c*, 0·6)	$[\alpha]_D^{16^\circ}$ +63° in water (*c*, 0·6)
2 : 4-Dimethyl 3 : 6-anhydro-*l*-galactonamide, m. p. 151°.	2 : 4-Dimethyl 3 : 6-anhydro-*d*-galactonamide, m. p. 150°, mixed m. p. 139°.
$[\alpha]_D^{19^\circ}$ −74° in water (*c*, 0·7)	$[\alpha]_D^{20^\circ}$ +75° in water (*c*, 1·0)
2 : 4-Dimethyl 3 : 6-anhydro-*l*-galactose anilide, m. p. 117°.	2 : 4-Dimethyl 3 : 6-anhydro-*d*-galactose anilide, m. p. 118°, mixed m. p. 106°.

There is no evidence that there is a ketose present in the hydrolysis products, since the oil seems to be mainly tetramethyl methylgalactoside. At the same time, since syrups are involved and there is a notable loss in yield on hydrolysis, caution is clearly necessary in the interpretation of this result and small quantities of other sugars, not necessarily ketoses, may be present. The presence of fully methylated galactose could be due either to an incomplete separation of the trimethyl methylgalactoside, or in view of the methoxyl content of the syrup before methylation, to the presence of dimethyl galactose derivatives or to both.

The production of lævulic acid in quantity by the treatment of methylated agar with 6% sulphuric acid (Percival and Somerville, *loc. cit.*) was thought to be connected with the presence of a ketose, but it is now shown that the 3 : 6-anhydromethylgalactosides, whether methylated or not, on similar treatment undergo drastic decomposition with the formation of lævulic acid. It is significant that the yield of lævulic ester (16%) produced by the hydrolysis of methylated agar is of the same order as that estimated for the anhydro-*l*-galactose.

It is important to decide whether the anhydro-ring is preformed in agar or whether it is produced as the result of side reactions during methylation and hydrolysis. The washed agar used in this work contained only a trace of sulphur (cf. Neuberg and Schwietzer, *Monatsh.*, 1937, **71**, 1, 46), so the anhydride could not be produced by the hydrolysis of a sulphuric ester during methylation. Hands and Peat (*Chem. and Ind.*, *loc. cit.*) also emphasised this point. Furthermore, the low acetyl and methoxyl content of agar acetate and methylated agar (attempts to raise the methoxyl content by the use of sodium in liquid ammonia having failed) may be adduced as evidence that the anhydro-*l*-galactose residues pre-exist in the agar molecule. These residues must be attached by the reducing group and by one other position, since the fully methylated anhydro-sugar cannot be isolated until the products of hydrolysis are remethylated. The predominant 1 : 3-linkage is also absent in this case, but further study is necessary to decide how and in what form the 3 : 6-anhydro-*l*-galactose is incorporated in the agar molecule.

EXPERIMENTAL.

The Isolation of 2 : 4-*Dimethyl* 3 : 6-*Anhydro*-β-*methyl*-l-*galactoside from Methylated Agar.*—In a typical experiment methylated agar [12 g.; prepared from powdered agar (B.D.H.) *via* the acetate as described by Percival and Somerville (*loc. cit.*)], $[\alpha]_D^{20^\circ}$ − 85° in chloroform (*c*, 0·9) (OMe, 32·4%), was treated for 19 hours at 70° with 2% methyl-alcoholic hydrogen chloride. After neutralisation with silver carbonate and removal of solvent a syrup (10·6 g.), $[\alpha]_D^{20^\circ}$ + 32° in chloroform (*c*, 1) (OMe, 39%), was obtained which yielded the following fractions on distillation (all the temperatures recorded in this paper are bath temperatures) : (1) 5·8 g., b. p. 125—145°/0·01 mm., crystalline; (2) 1·6 g., b. p. 145—165°/0·01 mm., partly crystalline, $[\alpha]_D^{19^\circ}$ + 14° in chloroform (*c*, 1); (3) 1·1 g., b. p. 165—175°/0·01 mm., syrup, $[\alpha]_D^{20^\circ}$ − 15° in chloroform (*c*, 0·6); (4) 2·2 g., residue, $[\alpha]_D^{20^\circ}$ − 12° in chloroform (*c*, 0·7). Fraction 4 was submitted to a further treatment with methyl-alcoholic hydrogen chloride and worked up as before to yield (5) 1·5 g., b. p. 135—145°/0·01 mm., crystalline, and (6) 0·6 g., b. p. 160—175°/0·01 mm., $[\alpha]_D^{20^\circ}$ ± 0° in chloroform.

Fractions 2, 3, and 6 gave strong ketose tests (Seliwanoff). Fractions 1 and 5 were combined and gave on recrystallisation from light petroleum crystalline 2 : 4 : 6-trimethyl methylgalactoside (αβ-mixture) (6·2 g.), m. p. 65°, representing together with a further 0·6 g. obtained from fraction 2 a yield of 65% of the hydrolysed syrup. A syrupy residue (0·9 g.) remained which gave a positive Seliwanoff test. This was combined with similar fractions from other experiments, extracted with light petroleum, and methylated as described below; 7·0 g. thus yielded 1·0 g. of the crystalline dimethyl anhydromethylhexoside (X), m. p. 82°, corresponding to a yield of 0·13 g. from the experiment under consideration.

After removal of the crystalline material from fraction 2 the residual syrup (1·0 g.) together with fractions 3 and 6 were extracted several times with hot light petroleum (b. p. 60—80°), glass beads being used to increase the syrup–solvent interface, and the hot supernatant liquid (A) decanted until the residue (2·3 g.), which gave very strong ketose colour reactions, was of constant rotation, $[\alpha]_D^{18^\circ}$ − 22° in chloroform (*c*, 1·1) (OMe, 40·4%). An attempt at further separation by fractionation at 0·03 mm. was unsuccessful.

The syrup (3·5 g.) obtained by combining similar products from three experiments was dissolved in methyl iodide (40 c.c.) and methylated three times in the usual way with silver oxide (52 g.). The product (3·6 g.) gave the following fractions on distillation : (1) 1·5 g., b. p. 90—95°/0·01 mm., $n_D^{18^\circ}$ 1·4497, $[\alpha]_D^{17^\circ}$ + 27° in chloroform; (2) 1·7 g., b. p. 95—100°/0·01 mm., $n_D^{18^\circ}$ 1·4471; (3) 0·4 g., b. p. 100—105°/0·01 mm.

On standing at 0° crystals (0·45, 0·35, and 0·1 g. respectively) were deposited in each of these fractions and were removed on porous tile. The residual oil was remethylated and redistilled to give a further quantity of X (0·15 g.), the total yield (1·05 g.) representing 0·69 g. from the experiment concerned.

Another source of X was found to be the syrup (0·45 g.) obtained on evaporation of the light petroleum extracts (A), for on methylation and distillation 0·15 g. was obtained. The oily fully methylated residue (2·5 g.) after the removal of X was shown later to contain *ca.* 16% of X in solution, representing the presence of a further 0·4 g., making the total yield 11·5%.

The Properties of X.—X was isolated in colourless plates, m. p. 82°, b. p. 85—90°/0·05 mm., $n_D^{69^\circ}$ 1·4402, $[\alpha]_D^{20^\circ}$ + 85° in chloroform (*c*, 2·1), $[\alpha]_D^{14^\circ}$ + 75° in water (*c*, 5·6); it was non-reducing to Fehling's solution and gave a positive Seliwanoff reaction (Found : C, 52·5; H, 8·1; OMe, 44·0. $C_9H_{16}O_5$ requires C, 52·9; H, 7·9; OMe, 45·6%). The following changes in specific rotation were observed in N-sulphuric acid at 19° (*c*, 2·8) : $[\alpha]_D^{19^\circ}$ + 71·2° (initial); 22° (6 hours); − 11° (16 hours); − 23° (24 hours, constant value). The solution was then strongly reducing, but the free sugar which was isolated in the usual way failed to crystallise.

Preparation of 2 : 4-*Dimethyl* 3 : 6-*Anhydro*-α-*methyl*-d-*galactoside from* 6-p-*Toluenesulphonyl Diacetone Galactose.*—The latter was prepared by the method of Freudenberg and Hixon (*Ber.*, 1923, **56**, 2123) and converted into the 6-tosyl galactose (αβ-mixture) of Ohle and Thiel (*loc. cit.*), which showed $[\alpha]_D^{20^\circ}$ + 60° ⟶ + 27° after 4 days in pyridine (*c*, 0·9). The corresponding α-methylgalactoside, m. p. 162°, $[\alpha]_D^{20^\circ}$ + 106° in pyridine (*c*, 0·2), was obtained from this by treatment with methyl-alcoholic hydrogen chloride, and treatment with N-sodium hydroxide as described by Ohle and Thiel (*loc. cit.*) yielded 3 : 6-anhydro-α-methyl-*d*-galactoside, m. p. 141°, $[\alpha]_D^{20^\circ}$ + 78° in water (*c*, 0·5). This substance gave a strong Seliwanoff reaction.

This substance (2·4 g.) in acetone (10 c.c.) was methylated with methyl iodide (40 c.c.) and silver oxide (45 g.). Two further methylations were carried out without the addition of acetone. The *product* distilled at 90—95°/0·01 mm. as a colourless oil (2·3 g.) showing a strong Seliwanoff reaction; $n_D^{18^\circ}$ 1·4641, $n_D^{70^\circ}$ 1·4412, $[\alpha]_D^{20^\circ}$ + 87° in chloroform (*c*, 0·9), $[\alpha]_D^{19^\circ}$ + 75° in water (*c*, 0·5) (Found : OMe, 44·2. $C_9H_{16}O_5$ requires OMe, 45·6%). In a later experiment the improved method of Bell and Williamson (*loc. cit.*) for the direct preparation of 6-tosyl α-methylgalactoside from 6-tosyl diacetone galactose was used.

The following changes in specific rotation in N-sulphuric acid (*c*, 1·2) were noted: $[\alpha]_D^{18^\circ}$ + 72° (initial); + 24° (8 hours); + 22° (24 hours, constant value). The free sugar isolated in the usual way failed to crystallise. It showed $[\alpha]_D^{20^\circ}$ + 14° in chloroform (*c*, 1·3), + 21° in water (*c*, 1·2) (Found : OMe, 32·5. Calc. for $C_8H_{14}O_5$: OMe, 32·6%).

3 : 6-*Anhydro*-β-*methyl*-d-*galactoside*.—2 : 3 : 4-Triacetyl 6-tosyl α-*d*-galactosyl 1-bromide, m. p. 147°, $[\alpha]_D^{20^\circ}$ + 151° in chloroform (*c*, 0·7), was prepared according to Ohle and Thiel (*loc. cit.*). The above compound (2 g.) was shaken with anhydrous methyl alcohol (75 c.c.) and silver carbonate (12 g.) for 15 hours. After filtration and evaporation a glass (1·5 g.), $[\alpha]_D^{20^\circ}$ − 3° in chloroform (*c*, 1·5), was obtained (Found : OMe, 10·5. $C_{20}H_{26}O_{11}S$ requires OMe, 10·75%).

The *triacetyl* 6-*tosyl* β-*methyl*-d-*galactoside* (5 g.) prepared as described above was dissolved in alcohol (100 c.c.) and titrated during 2½ hours with N-sodium hydroxide until a permanent pink colour remained to phenolphthalein. Exhaustive extraction of the product remaining after evaporation under diminished pressure with ethyl acetate, yielded a crystalline *substance* (3 g.), which was recrystallised from ethyl acetate–light petroleum (b. p. 60—80°). It had m. p. 118° and $[\alpha]_D^{20^\circ}$ − 114° in water (*c*, 0·7) (Found : C, 47·5; H, 7·0; OMe, 17·0. $C_7H_{12}O_5$ requires C, 47·7; H, 6·9; OMe, 17·6%).

2 : 4-*Dimethyl* 3 : 6-*Anhydro*-β-*methyl*-d-*galactoside*.—By methylation of the above substance (2·6 g.) as described for the corresponding α-methylgalactoside a *product* (2·5 g.) was obtained which crystallised completely on removal of solvent; m. p. 80° (crude) and 82° after recrystallisation from light petroleum (b. p. 60—80°); mixed m. p. with X 65°; $[\alpha]_D^{20^\circ}$ − 77° in water (*c*, 0·6), − 86° in chloroform (*c*, 0·5) (Found : C, 52·8; H, 8·1; OMe, 44·5. $C_9H_{16}O_5$ requires C, 52·9; H, 7·9; OMe, 45·6%).

Conversion of 2 : 4-*Dimethyl* 3 : 6-*Anhydro*-α-*methyl*-d-*galactoside into the* β-*Form*.—The α-compound (0·28 g.), dissolved in anhydrous methyl alcohol, had $[\alpha]_D^{20^\circ}$ + 98°. To this solution (7 c.c.) was added 7% methyl-alcoholic hydrogen chloride (3 c.c.) and the following rotations were observed: $[\alpha]_D^{20^\circ}$ + 42° (5 mins.); + 26° (60 mins.); + 26° (2 hours, constant value). Silver carbonate was then added and after filtration and removal of solvent the syrup crystallised spontaneously; m. p. 81°, mixed m. p. with X 65°. A mixture with 2 : 4-dimethyl 3 : 6-anhydro-β-methyl-*d*-galactoside showed no depression. $[\alpha]_D^{20^\circ}$ − 76° in water (*c*, 1·0).

2 : 4-*Dimethyl* 3 : 6-*Anhydro*-d-*galactose Anilide*.—2 : 4-Dimethyl 3 : 6-anhydro-β-methyl-*d*-galactoside (0·5 g.) was dissolved in N-sulphuric acid and kept at 18° for 24 hours. After neutralisation with barium carbonate and evaporation to dryness at 35° the residual reducing syrup (0·4 g.) was dissolved in alcohol (3 c.c.) and treated with aniline (0·2 g.) at 90° for 1½ hours. The needles which appeared on standing (0·15 g.) were recrystallised from ethyl alcohol and showed m. p. 118°. $[\alpha]_D^{20^\circ}$ + 100° in alcohol (initial); + 72° (30 mins.); + 61° (12 hours); + 56° (1 day, constant value) (Found : OMe, 21·5; N, 5·7. $C_{14}H_{19}O_4N$ requires OMe, 23·4; N, 5·3%).

A small quantity of the corresponding compound prepared from X in a similar manner had m. p. 117°, mixed m. p. with the synthetic substance 106°.

2 : 4-*Dimethyl* 3 : 6-*Anhydro*-d-*galactonamide*.—Syrupy 2 : 4-dimethyl 3 : 6-anhydro-*d*-galactose (0·8 g.) in water (8 c.c.) was treated with bromine (0·8 c.c.) for 24 hours at 35° and 24 hours at 20°. Removal of the bromine, neutralisation with silver carbonate, and precipitation with hydrogen sulphide, followed by evaporation at 40°/15 mm. with a subsequent heating at 100°/0·01 mm. for 2 hours, yielded a yellow syrup (0·6 g.). Distillation at 140°/0·05 mm. gave a product, $n_D^{19^\circ}$ 1·4607, $[\alpha]_D^{20^\circ}$ + 22° in water (*c*, 0·9) (initial), + 26° (1 day), + 27° (2 days). The solution was then heated to 80° and an equilibrium value of + 55° was reached in 24 hours, titration showing the presence of 84% of free acid at this stage. It was found impossible to obtain a product containing more than 70% of lactone (by titration).

Methyl-alcoholic ammonia (3 c.c.) was added to the impure lactone and after 24 hours removal of solvent left a crystalline *amide*, m. p. 150°, $[\alpha]_D^{20^\circ}$ + 75° in water (*c*, 1·0) (Found : OMe, 29·3; N, 6·9. $C_8H_{15}O_5N$ requires OMe, 30·2; N, 6·8%).

Methyl 2 : 4-*Dimethyl* 3 : 6-*Anhydro*-d-*galactonate*.—The impure lactone (0·4 g.) was treated with 3% methyl-alcoholic hydrogen chloride (60 c.c.) at 70° for 6 hours. After neutralisation with silver carbonate and removal of solvent the *ester* was distilled at 125°/0·02 mm. (0·3 g.), $n_D^{16^\circ}$ 1·4634. The oil crystallised in small prisms which on recrystallisation from acetone had

m. p. 49—50°, $[\alpha]_D^{16^\circ}$ + 63° in water (*c*, 0·6), + 73° in chloroform (*c*, 0·6) (Found : C, 48·9; H, 7·4. $C_9H_{16}O_6$ requires C, 49·1; H, 7·3%). The corresponding amide, m. p. 150°, was obtained readily from this ester.

2 : 3-*Dimethyl* 3 : 6-*Anhydro*-l-*galactonamide*.—The lactone was prepared from the free sugar obtained from X by treatment with N-sulphuric acid as described for the synthetic derivative. The syrupy lactone distilled at 135°/0·01 mm. and contained 30% of the free acid, $[\alpha]_D^{19^\circ}$ − 27° in water (*c*, 1·0) (initial value), − 34° (1 day), − 37° (7 days), reaching − 54° on heating at 80° for 24 hours. The equilibrium solution contained 20% of lactone.

The corresponding *amide* was prepared from the lactone and the corresponding ester in the usual way. It had m. p. 151°, mixed m. p. with the synthetic amide 139°; $[\alpha]_D^{16^\circ}$ − 74° in water (*c*, 0·7) (Found : OMe, 28·8; N, 6·7. $C_8H_{15}O_5N$ requires OMe, 30·2; N, 6·8%).

Methyl 2 : 4-*Dimethyl* 3 : 6-*Anhydro*-l-*galactonate*.—This *ester* was isolated as described for the synthetic compound. It distilled at 125°/0·02 mm., had $n_D^{19^\circ}$ 1·4615, and the crystals were identical in appearance with the synthetic ester, m. p. 48—49° on recrystallisation from acetone; m. p. on admixture with the synthetic ester 38°. $[\alpha]_D^{16^\circ}$ − 64° in water (*c*, 0·6), $[\alpha]_D^{10^\circ}$ − 72·5° in chloroform (*c*, 0·6) (Found : C, 48·9; H, 7·3; OMe, 40·7. $C_9H_{16}O_6$ requires C, 49·1; H, 7·2; OMe, 42·3%).

Isolation of Tetramethyl Galactopyranose Anilide from the Fully Methylated Oil after the Removal of X.—The oil (8·1 g.), $[\alpha]_D^{20^\circ}$ + 10° in chloroform (OMe, 58%), collected from several experiments was kept at 20° with N-sulphuric acid (50 c.c.) for 24 hours. After neutralisation with barium carbonate and removal of solvent the residue was extracted with dry ether. A white crystalline solid (0·7 g.), m. p. 116° (OMe, 32·8%), was separated which showed $[\alpha]_D^{15^\circ}$ − 21° in water (*c*, 1·3) and was undoubtedly the 2 : 4-dimethyl 3 : 6-anhydro-*l*-galactose of Hands and Peat (*loc. cit.*) which we were unable to crystallise on former occasions. After removal of the ether from the solution the syrup was distilled to give the following fractions : (1) 0·9 g., b. p. 85—90°/0·01 mm.; (2) 2·75 g., b. p. 95—105°/0·01 mm.; (3) 1·4 g., b. p. 105—115°/0·01 mm.; (4) 1·0 g., b. p. 115—125°/0·01 mm.; (5) residue, 1·1 g.

Fractions 1, 2, and 3 were non-reducing and 3, 4, and 5 gave the Seliwanoff reaction. Fractions 1, 2, and 3 were combined and remethylated with silver oxide and methyl iodide, and the product distilled : (1) 3·8 g., b. p. 90—110°/0·01 mm., $n_D^{11^\circ}$ 1·4520 (OMe, 56%), $[\alpha]_D^{16^\circ}$ + 60° in chloroform (*c*, 0·5); (2) 0·5 g., b. p. 110—125°/0·01 mm., $n_D^{10^\circ}$ 1·4556, $[\alpha]_D^{18^\circ}$ + 45° in chloroform (*c*, 0·4). 1·75 G. of (1) were hydrolysed for 4 hours with N-sulphuric acid at 80° and the reducing syrup (1·6 g.) obtained from it was subjected to anilide formation. Tetramethyl galactopyranose anilide (0·4 g.) was isolated, m. p. 197—198° after recrystallisation from alcohol, unchanged on admixture with an authentic specimen, $[\alpha]_D^{16^\circ}$ − 70° in acetone (*c*, 0·5) (Found : OMe, 38·7; N, 4·5. Calc. for $C_{16}H_{25}O_5N$: OMe, 39·9; N, 4·5%). The syrup obtained by evaporation of the mother-liquor was heated with sulphuric acid (3%) at 90° for 8 hours, the solution neutralised with barium carbonate, and the aniline removed by extraction with ether. The brown reducing syrup (1 g.) was again methylated and distilled (for purification) at 90—100°/0·01 mm. to yield an oil which gave no " ketose " colour tests; $n_D^{12^\circ}$, 1·4545 (OMe, 57·0%). This oil (0·8 g.) was hydrolysed with 8% hydrochloric acid for 3 hours, and the free sugar treated with aniline. The hydrolysis had evidently been insufficient on the first occasion, for four crops of crystals (0·28 g.) were isolated, all of which proved to be tetramethyl galactopyranose anilide. Similar experiments on other specimens of the oil accompanying X led to the separation of the same anilide and no other crystalline derivatives could be isolated.

Lævulic Acid Formation.—3 : 6-Anhydro-α-methyl-*d*-galactoside and 2 : 4-dimethyl 3 : 6-anhydro-β-methyl-*l*-galactoside on treatment with 6% sulphuric acid for 3 hours, followed by neutralisation with barium carbonate, yielded a small quantity of a resin, similar to that obtained when methylated agar is so treated, and a barium salt (10—20%) which gave an immediate precipitate of iodoform in the cold with sodium hypoiodite.

Thanks are expressed to the Earl of Moray Endowment and to Imperial Chemical Industries Ltd. for grants and to the Carnegie Trust for the award of a Teaching Fellowship (to E. G. V. P.).

KING'S BUILDINGS, UNIVERSITY OF EDINBURGH. [*Received, September* 20*th*, 1939.]