## 421. *Polysaccharides. Part XVIII. The Constitution of Xylan.*

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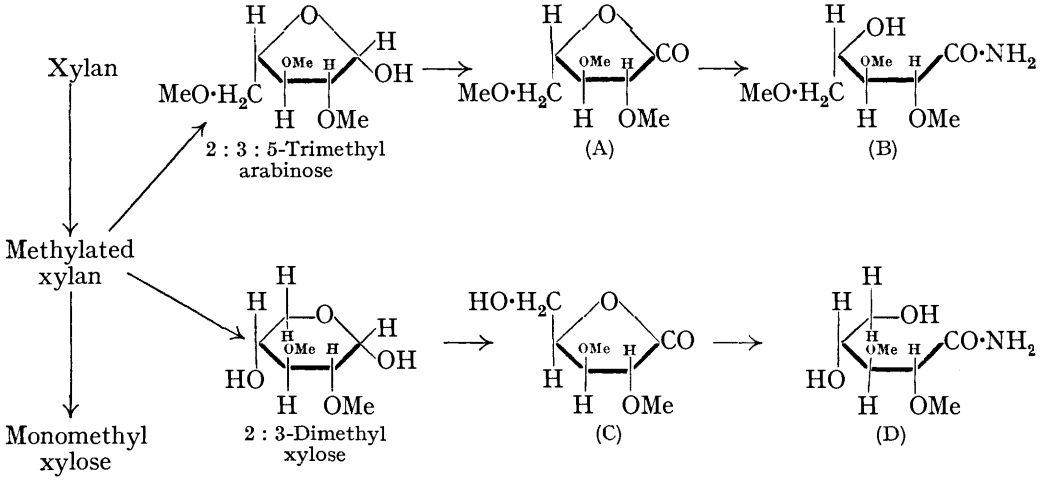
Our previous work on the structure of xylan (J., 1929, 1739; 1931, 2850) revealed that this polysaccharide was composed of chains of xylopyranose units linked together through positions 1 and 4 of the pentose molecule. The glycosidic linkages were known to be β-in type and in many ways xylan presented close structural analogies with cellulose, from which it could conceivably be derived by oxidation of the terminal $CH_2$·OH group, followed

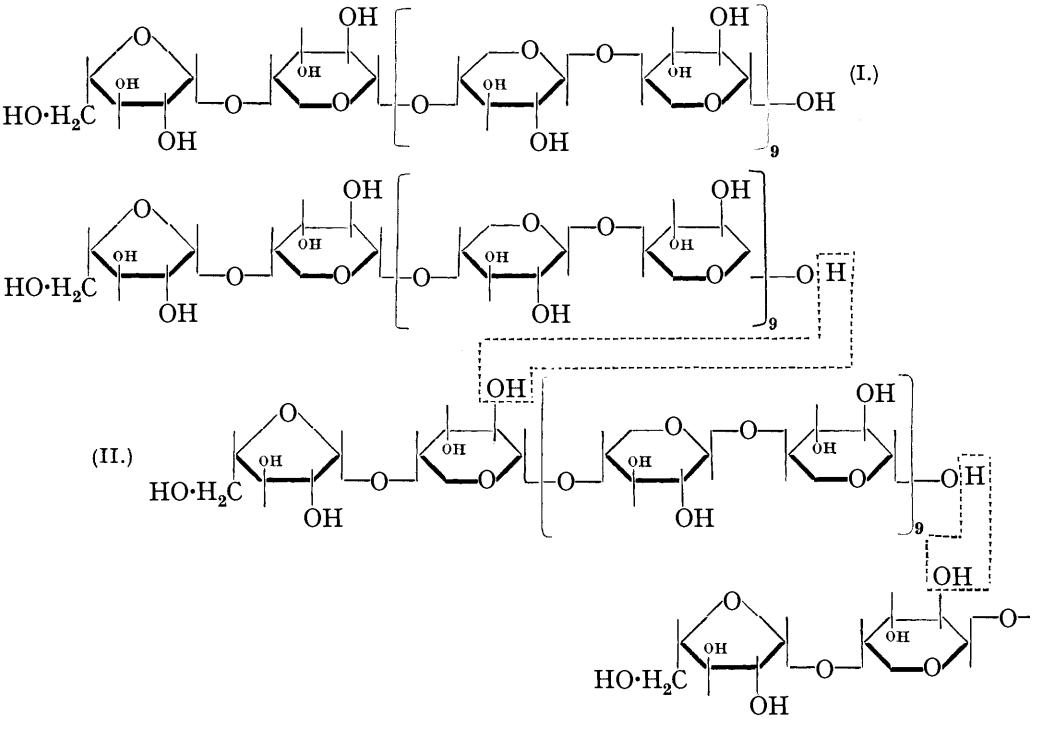
by decarboxylation. Hitherto we had been able to isolate 93% of the theoretical amount of xylose required to satisfy this view. The present work is an illustration of the fact that even so narrow a gap in the theoretical requirements of a hypothesis needs to be considered with due care before such a hypothesis can be substantiated. The results now communicated advance our knowledge of the intimate structure of xylan in a novel and unexpected direction and present the possibility that xylan may be related more closely to the plant gums than to normal cellulose. We find, for instance, that xylan prepared from esparto celluloses of different origin contains, in addition to the xylopyranose residues, a fixed and constant proportion of combined *l*-arabinose in the furanose form. This *l*-arabofuranose unit is retained during methylation and the methylated xylan gives on hydrolysis 2 : 3-dimethyl xylose (90%) accompanied by about 6% of 2 : 3 : 5-trimethyl *l*-arabofuranose. The latter was recognised by its physical and chemical properties and by its quantitative conversion by oxidation into the corresponding crystalline lactone, *l*-2 : 3 : 5-trimethyl $\gamma$-arabonolactone (A), from which the crystalline amide (B) was prepared. The methylated xylan was rigorously fractionated before hydrolysis and it was found that each of the fractions (which differed but slightly among themselves except that certain of them had a high ash content) gave rise to the same proportion of 2 : 3 : 5-trimethyl arabinose. Confirmation of this evidence of the occurrence of arabofuranose units was sought by a repetition of the whole of the lengthy series of operations with xylan prepared from esparto celluloses of different origin. In every instance quantitative estimations revealed the presence of about 6% of arabinose. Separation of this small amount of 2 : 3 : 5-trimethyl arabinose was accomplished by fractional distillation of the products obtained by hydrolysis of methylated xylan after their conversion into methyl-pentosides. The effective separation of the constituents required special care in the choice of distilling flasks and fractionating columns and in view of the highly volatile nature of the




trimethyl methylpentosides, the distillations were carried out at a pressure not lower than about 14 mm.

The experimental evidence now shows indubitably that arabinose forms an integral part of the xylan molecule. Xylan is thus clearly differentiated from cellulose despite the former hypothesis of structural similarity. It is especially significant that methylated xylan should give rise on hydrolysis to trimethyl arabofuranose, inasmuch as it follows at once that the arabinose residue must be present in the furanose condition in xylan and that it forms a terminal group attached to a chain of consecutive xylopyranose residues. So far as we are aware, this is the first occasion on which the natural occurrence of arabofuranose has been observed. The quantitative results show that one arabinose unit is associated with 18—20 xylopyranose units, the latter being identified to the extent of 86% as 2 : 3-dimethyl xylose, which was transformed into 2 : 3-dimethyl $\gamma$-xylonolactone

(C) and into the crystalline amide (D). The simplest interpretation of these observations is that the molecular structure (I) of xylan is expressed by the continuous linking of xylose units in a chain which is terminated at one end by an arabofuranose unit. The principal




difficulties centre round the nature of the other terminal group (if any) situated at the end of the chain remote from the arabinose residue. This question will be more fully discussed in subsequent papers, but it may now be stated that xylan is non-reducing and that we have been unable to confirm the evidence advanced by Schmidt (*Cellulosechem.*, 1932, **13**, 129) in favour of the termination of the chain by a carboxyl group although we have repeated his experiments. The approximate numerical agreement between our results and those of Schmidt must therefore be regarded as merely fortuitous, inasmuch as the presence of any unit possessing a carboxyl group has no foundation in fact.

It is significant that we have so far been unable to detect the presence of trimethyl xylose in the cleavage products of methylated xylan and it seems evident that the trimethyl arabofuranose takes the place of this as a terminal group in what is otherwise a series of xylopyranose units. What may be significant also is that we were able to isolate about 5% of a monomethyl xylose even after hydrolysis of the purest and most exhaustively methylated xylan. On the basis of the structure expressed by formula (I) this would be explicable by the view that, despite every effort to effect complete methylation of the available hydroxyl groups, a very small proportion escapes protection. This is not an unreasonable hypothesis, since methylated xylan is not of such a nature that it can be purified as rigidly as a substance which is capable of recrystallisation. The alternative view should, however, be advanced, namely, that a monomethyl xylose has significance in the sense that one xylose unit in every 18 or 20 residues is combined in the xylan molecule at two points of the three hydroxyl positions. This conception is expressed by the formula (II) for xylan, which indicates the linking of consecutive chains by the union, whether in the manner of a co-ordinated link or an ordinary covalency link produced by dehydration, connecting the potential reducing end of one chain with another xylose unit of a succeeding chain. At this stage it is not possible to present a precise picture of the

position of the xylose units involved in this type of union, but further experiments which are in progress may make this clear. Until the orientation of the monomethyl xylose has been decided this and other points must be in suspense, assuming that the molecular structure (II) is ultimately found to be the best expression for the experimental facts. Yet a third formulation which must equally be considered is that xylan consists of a long chain of xylopyranose units linked through the positions 1 and 4 with arabofuranose residues attached as side chains at intervals of about every 18 xylose residues. This chain may be considerably extended in length or it may equally be continuous in the form of a loop. Viscosity measurements of methylated xylan in *m*-cresol have a value corresponding with an average of 75 to 80 $C_5$ units. This calculation of the viscosity measurements is based on the same assumptions as were made by Staudinger in his determination of the chain length of cellulose, but the factor involved may not apply to pentose units and at the present stage it is not proposed to attach great importance to these values.

## Experimental.

*Preparation and Properties of Xylan.*—Three different samples of esparto cellulose were used for the preparation of xylan. The first was specially prepared from Oran esparto grass by Mr. A. J. C. Aikman, B.Sc., in the laboratories of the Guardbridge Paper Company, Ltd., and gave xylan (115 g.) from esparto cellulose (500 g.). The second and third batches were different samples of esparto " half-stuff " prepared by Messrs. Alex. Pirie & Sons, Ltd., of Aberdeen. To both these donors of material we express our thanks. Esparto " half-stuff " (500 g.) gave xylan (70 g.). The properties of the three batches of xylan (which was extracted and purified by the method already described; J., 1929, 1739) were identical with those previously recorded. Furthermore, each sample gave the same yield of arabinose on hydrolysis, and after hydrolysis of the corresponding dimethyl derivative each sample of xylan gave the same yield of trimethyl methylarabinoside. For this reason it is necessary to describe only one complete series of experiments. The details given below refer to experiments made with Messrs. Pirie's esparto " half-stuff." Exactly similar results were obtained with the other two series of investigations.

*Hydrolysis of Xylan.*—Xylan (60 g.) was hydrolysed with 3% nitric acid (Hampton, Haworth, and Hirst, *loc. cit.*), giving crystalline xylose (51 g.). Concentration of the mother-liquors yielded a syrup (2·5 g.), from which no more xylose crystallised. This syrup was shown to contain arabinose in the following way : To the syrup (0·5 g.), dissolved in 75% alcohol (6 c.c.), α-benzylphenylhydrazine (0·5 g.) was added. On standing over-night, arabinose benzylphenylhydrazone (0·2 g.) was precipitated and after recrystallisation from alcohol the crystals had m. p. 172°, alone or when mixed with an authentic sample of the same m. p.

*Methylation of Xylan and Properties of Methylated Xylan.*—Xylan was methylated according to Hampton, Haworth, and Hirst (*loc. cit.*), the following modifications being used in the quantities of reagents. Xylan (12·5 g.) was mixed to a smooth paste with water (300 c.c.) and potassium hydroxide (675 g.) was then added, its heat of solution being effective in dissolving the xylan. The solution was further diluted with water (525 c.c.), and methyl sulphate (750 c.c.) added during 4 hours at room temperature with mechanical stirring. The solution was then treated in the usual way and the partially methylated xylan obtained was remethylated with potassium hydroxide (675 g. in 725 c.c. of water) and methyl sulphate (500 c.c.). The crude methylated xylan (22 g.) was a white fibrous powder with no action on boiling Fehling's solution. M. p. 198°, $[\alpha]_D^{20°}$ −90·6° in chloroform (*c*, 0·49) (Found : OMe, 37·4. Calc. for $C_7H_{12}O_4$ : OMe, 38·9%).

Table I.

| Methylated xylan. | % Total. | % Ash content. | $[\alpha]_D^{20}$ in $CHCl_3$ (*c*, 0·5). | % OMe. |
|---|---|---|---|---|
| Fraction I | 52 | 13·87 | not readable | 40·1 |
| Fraction II | 44 | 1·8 | −96·8° | 39·3 |
| Fraction IA | 27 | 15·9 | not readable | 39·4 |
| Fraction IB | 24 | 3·77 | −99·4 | 38·6 |
| Fraction IIA | 19 | 2·43 | −97·9 | 38·3 |
| Fraction IIB | 24 | 1·46 | −98·3 | 40·0 |

*Purification and Fractionation of Methylated Xylan.*—Methylated xylan (120 g.) which had been dried for 4 hours at 100°/12 mm. dissolved slowly in chloroform (2·5 l.), swelling to form a gel. The chloroform solution was centrifuged and to the clear solution ether (3·5 l.)

was added with mechanical stirring, causing the precipitation of a brownish gel (Fraction I). When the precipitate had settled, the supernatant liquor (A) was decanted, and the precipitate (Fraction I) triturated with ether. An excess of light petroleum was then added to the decanted liquor (A) to precipitate the remaining methylated xylan (Fraction II). Fractions I and II were refractionated as before, being dissolved in chloroform and giving fractions IA and IIA by precipitation with ether and fractions IB and IIB by further addition of light petroleum.

Another sample corresponding to fraction IIB was further fractionated until a pure ash-free sample of methylated xylan was obtained. This had $[\alpha]_D^{20°}$ — 98·3° in chloroform (*c*, 0·525) (Found : C, 52·5; H, 7·4; OMe, 38·9. Calc. for $C_7H_{12}O_4$ : C, 52·5; H, 7·6; OMe, 38·75%). The viscosity of a 4% solution of the purified ash-free methylated xylan in *m*-cresol was determined in an Ostwald viscometer by the Staudinger method : mean time of flow of solution, 373 seconds at 20°; mean time of flow of *m*-cresol at 20°, 287 seconds ($\eta_{sp.}$ = 1·30).

Upon subsequent hydrolysis and fractionation the same yield of trimethyl methylarabinoside (calculated on ash-free material) was obtained from each fraction. The only noticeable difference in the fractions was that of ash content.

*Hydrolysis of Methylated Xylan.*—Each fraction (see above) of methylated xylan was hydrolysed by boiling with methyl-alcoholic hydrogen chloride (Hampton, Haworth, and Hirst, *loc. cit.*). The mixture of methylated methylpentosides obtained was distilled under diminished pressure. The more volatile portion (5—6 g., A of Table II) was submitted to further fractionation through an efficient column. Control experiments showed that the whole of the trimethylarabinoside present in the products of hydrolysis was contained in these distillates. Yields are summarised in Table II.

TABLE II.

*Fractionation of Distillate A.*

| Origin of distillate. | | Bath temp. | B. p./14 mm. | $n_D^{19°}$. | $n_D^{19°}$ (last drop). | Yield, g. | % OMe. |
|---|---|---|---|---|---|---|---|
| Methylated xylan, fraction IA | (a) | 139—140° | 116—118° | 1·4415 | 1·4405 | 1·67 | 60·3 |
| | (b) | 150 | 118—130 | 1·445 | 1·4513 | 0·95 | 54·41 |
| Fraction IB | (a) | 145—150 | 116—118 | 1·442 | 1·442 | 1·52 | 58·41 |
| | (b) | 150 | 120 | 1·447 | 1·4485 | 0·69 | 53·6 |
| Fraction IIA | (a) | 138—142 | 116—118 | 1·442 | 1·4435 | 1·24 | 60·6 |
| | (b) | 145—150 | 118—122 | 1·445 | 1·450 | 0·75 | 53·0 |
| Fraction IIB | (a) | 139 | 115—118 | 1·4400 | 1·439 | 1·22 | 60·61 |
| | (b) | 140—141 | 116—118 | 1·441 | 1·439 | 0·53 | 59·67 |
| | (c) | 145 | 126 | 1·4433 | 1·447 | 0·6 | 54·98 |

In every case pure dimethyl methylxyloside was obtained when the distillation was carried beyond the stage given by the above table.

TABLE III.

*Summary of Yields of Hydrolysis Products from the Fractions IA, IB, IIA, IIB.*

| | Methylated xylan, g. | Methylated methylpentosides, g. | Trimethyl methylarabinoside, g. | Dimethyl methylxyloside, g. |
|---|---|---|---|---|
| Fraction IA..... | 33·48 | 33·57 | 2·15 | 29·0 |
| Fraction IB..... | 29·21 | 33·21 | 1·79 | 29·5 |
| Fraction IIA ..... | 21·64 | 23·48 | 1·53 | 20·2 |
| Fraction IIB ..... | 29·79 | 32·9 | 2·09 | 28·6 |

*Identity of Dimethyl Methylxyloside.*—The dimethyl methylxyloside gave OMe, 48·4 (Calc. for $C_8H_{16}O_5$ : OMe, 48·4%).

(a) Dimethyl methylxyloside (8·0 g.) was methylated by methyl iodide and silver oxide, and the product distilled, yielding a colourless mobile syrup (7·0 g.). A portion of the distillate (1·02 g.) was hydrolysed by heating with 3% nitric acid (25 c.c.) for 1 hour. The acid was neutralised with barium carbonate, the solution evaporated to dryness, and the residue extracted with boiling ether. Evaporation of the ether left a solid crystalline mass of 2 : 3 : 4-trimethyl xylopyranose (0·86 g.).

(b) A solution of dimethyl methylxyloside (5·0 g.) in 3% hydrobromic acid (70 c.c.) was heated for 1 hour at 85° and then maintained at 75° during the addition of bromine (5·5 c.c.). After 16 hours the reducing action towards Fehling's solution had disappeared. The solution

was aerated to remove bromine and extracted with chloroform, the extracts being dried over magnesium sulphate and then concentrated. The product on distillation gave a colourless mobile syrup (4·33 g.), b. p. 120°/0·05 mm., $n_D^{19°}$ 1·464. The phenylhydrazide was prepared in the usual way and had m. p. 105°.

The lactone (4·0 g.) was dissolved in methyl alcohol (4·0 c.c.) saturated with ammonia at 0°. The solution, after being left over-night, was concentrated in a vacuum desiccator. A solid crystalline mass (4·2 g.) was obtained. This was recrystallised from ethyl acetate and gave m. p. 134°. $[\alpha]_D^{20°}$ +46·6° in water (*c*, 0·75) (Found : C, 43·8; H, 7·6; N, 7·2; OMe, 32·4. $C_7H_{15}O_5N$ requires C, 43·5; H, 7·7; N, 7·25; OMe, 32·1%).

The undistillable portion of the hydrolysis product which remained after the separation of the whole of the dimethyl methylxyloside had OMe, 36·7 (Calc. for $C_7H_{14}O_5$ : OMe, 34·8%). These residues (7 g. from 114 g. of methylated xylan) were methylated three times with Purdie's reagents in the usual way and on distillation of the product a mobile colourless syrup (6·5 g.) ($n_D^{20°}$ 1·4408) was obtained. This syrup (1·65 g.) on hydrolysis with 2% nitric acid gave crystalline trimethyl xylose (1·25 g.), m. p. 87° alone or when mixed with an authentic specimen. The residues consisted, therefore, almost exclusively of monomethyl methylxyloside.

*Proof of the Structure of* 2 : 3 : 5-*Trimethyl* γ-*Methylarabinoside.*

The various fractions containing trimethyl methylarabinoside referred to above were collected together and redistilled, giving the pure trimethyl derivative, b. p. 116—118°, $n_D^{19°}$ 1·4400, $[\alpha]_D^{20°}$ — 29° in chloroform (*c*, 0·083), $[\alpha]_D^{20°}$ — 36·2° in water (*c*, 0·083) (Found : C, 52·4; H, 8·8; OMe, 60·6. Calc. for $C_9H_{18}O_5$ : C, 52·4; H, 8·7; OMe, 60·2%).

*Hydrolysis of the Trimethyl Methylarabinoside.*—The syrup (5·8 g.) was dissolved in 2% nitric acid (150 c.c.) and heated at 100° until the rotation was constant. $[\alpha]_D^{20°}$ —39° (initial value), — 33·8° (15 mins.), — 31·8° (25 mins.), — 24·4° (40 mins.), — 21·3° (60 mins.), — 14·3° (90 mins.), — 14·3° (110 mins.). The trimethylarabinose (4·9 g.) was isolated in the usual way.

*Oxidation of Trimethyl Arabinose with Concentrated Nitric Acid.*—Trimethyl methylarabinoside (1 g.) was dissolved in concentrated nitric acid (10 c.c.) and heated to 65°; a vigorous reaction then occurred. After 15 minutes the temperature was raised to 90—95° until the oxidation ceased. The solution was diluted with an equal volume of water and distilled at 40°/12 mm., water being continuously added until 1 litre of water had passed through the flask. Methyl alcohol was similarly passed through the distillation flask to dry the syrup. The syrup obtained was dissolved in 3% methyl-alcoholic hydrogen chloride (12 c.c.) and heated under reflux for 6 hours. After neutralisation and concentration, the syrup was dissolved in ether, filtered to remove any mineral residue, then concentrated, and distilled, giving a colourless syrup (0·3 g.), b. p. 116—120°/0·06 mm. (bath temp.), $n_D^{19°}$ 1·4400; $[\alpha]_D^{19°}$ + 46·2° in methyl alcohol (*c*, 0·77). The distillate gave on treatment with methyl-alcoholic ammonia, *d*-dimethoxysuccinamide, m. p. 270° (decomp.), $[\alpha]_D^{20°}$ + 94° in water (*c*, 0·53) (Found : C, 41·3; H, 7·0. Calc. for $C_6H_{12}O_4N_2$ : C, 40·9; H, 6·8%).

2 : 3 : 5-*Trimethyl* γ-*Arabonolactone.*—The trimethyl arabinose (4·9 g.) was dissolved in water (25 c.c.), and bromine (5 c.c.) added. The solution was left at room temperature overnight and then heated for 2 hours at 40° to complete the oxidation. The solution was aerated to remove bromine and extracted with chloroform, the extracts being dried over magnesium sulphate and then concentrated. The product on distillation gave (a) 2·55 g. of syrup, b. p. 100—105°/0·05 mm. (bath temp.), (b) 0·73 g. of syrup, b. p. 110—135°/0·05 mm. (bath temp.). The first fraction was a colourless mobile oil which crystallised completely and consisted entirely of 2 : 3 : 5-trimethyl γ-arabonolactone, m. p. 30° alone or when mixed with an authentic sample; $[\alpha]_D^{20°}$ — 42° in water (*c*, 1·33; initial value); — 40° (10 hours); — 38° (64 hours); — 37° (88 hours) (Found : C, 50·3; H, 7·35; OMe, 48·2. Calc. for $C_8H_{14}O_5$ : C, 50·5; H, 7·4; OMe, 49·0%). The lactone (0·24 g.) gave on treatment with methyl-alcoholic ammonia the corresponding crystalline amide (Humphreys, Pryde, and Waters, J., 1931, 1298), which after recrystallisation from ethyl alcohol had m. p. 137° alone or when mixed with an authentic specimen; $[\alpha]_D^{20°}$ + 23° in ethyl alcohol (*c*, 1·8) (Found : C, 46·1; H, 8·2; N, 6·4; OMe, 43·8. Calc. for $C_8H_{17}O_5N$ : C, 46·4; H, 8·2; N, 6·8; OMe, 44·9%).

The second fraction of the trimethyl γ-arabonolactone was contaminated with some 50% by weight of 2 : 3-dimethyl xylonolactone; $[\alpha]_D^{20°}$ + 53° in water (*c*, 2·261). This rotation is midway between that of 2 : 3 : 5-trimethyl γ-arabonolactone (— 44°) and 2 : 3-dimethyl xylonolactone (+ 97°). The mixed amides were prepared and upon nucleation with 2 : 3-dimethyl xylonamide a crystalline mass was obtained. Since 2 : 3-dimethyl xylonamide was found to be

less soluble in ethyl acetate than 2 : 3 : 5-trimethyl γ-arabonamide, the crude crystalline amide was recrystallised from this solvent. The product was pure 2 : 3-dimethyl xylonamide, m. p. 133·5° alone or when mixed with an authentic specimen, $[\alpha]_D^{20°}$ + 45° in water (*c*, 0·47).

It was concluded that the second fraction was a mixture of 2 : 3-dimethyl xylonolactone and 2 : 3 : 5-trimethyl γ-arabonolactone, due to a small amount of dimethyl methylxyloside distilling over with the trimethyl methylarabinoside during fractionation.

The authors express gratitude to the Government Grant Committee of the Royal Society for financial assistance.

UNIVERSITY OF BIRMINGHAM, EDGBASTON. [*Received, November 15th*, 1934.]