Wood Saponins. Part I. A Preliminary Investigation of the Saponins from Morabukea [Mora gonggrijpii (*Kleinh.*) *Sandwith*].

By R. A. Laidlaw.

[Reprint Order No. 4714.]

Acid hydrolysis of morabukea saponin gave oleanolic acid (30%) and a mixture of sugars (70%) containing D-galactose (8·4%), D-glucose (56·1%), and D-xylose (35·5%). Acetylation of the saponin, methylation, and hydrolysis yielded methyl oleanolate and a mixture of methylated sugars containing tetra-*O*-methyl-D-glucopyranose (62 parts), tetra-*O*-methyl-D-galactopyranose (3 parts), 2 : 4 : 6-tri- (12 parts) and 3 : 4-di-*O*-methyl-D-glucose (2 parts), tri-*O*-methyl-D-galactoses (9 parts), 2 : 3- (4 parts) and 2 : 4-di-*O*-methyl- (2 parts), and 2- (20 parts) and 3-*O*-methyl-D-xylose (20 parts), along with traces of other methylated derivatives and of xylose. Since no single structural formula could account for such a complex mixture, the material must comprise several distinct components.

The crude saponin isolated from morabukea (Farmer and Campbell, *Nature*, 1950, **165**, 237) had a small ash content and contained traces of free galactose and arabinose in admixture. Acid hydrolysis liberated oleanolic acid, D-galactose, D-glucose, and D-xylose.

Our saponin was purified by acetylation and deacetylation. Hydrolysis of the product gave oleanolic acid (30%) and quantitative examination on the paper chromatogram (Duff and Eastwood, *Nature*, 1950, **165**, 848) of the mixture of sugars produced showed galactose 8·4%, glucose 56·1%, and xylose 35·5%. These results, taken in conjunction with hypoiodite estimations of the total reducing sugars liberated, indicated the oleanolic acid : galactose : glucose : xylose ratio to be approximately 1 : 0·4 : 3 : 2.

Methylation of the acetate with methyl sulphate and sodium hydroxide followed by several treatments with methyl iodide and silver oxide gave a methylated material (OMe, 29·2%, not raised by further methylation). Hydrolysis gave methyl oleanolate and a mixture of sugars which by partition on a column of cellulose (Hough, Jones, and Wadman, *J.*, 1949, 2511) gave the following substantially pure materials: crystalline 2 : 3 : 4 : 6-tetra-*O*-methyl-D-glucose (51·7%; this and the following percentages are based on the weight of material recovered from the column), 2 : 4 : 6-tri-*O*-methyl-D-glucose (9·2%), crystalline 2-*O*-methyl- (6·4%) and 2 : 3- (2·2%) and crystalline 2 : 4-di-*O*-methyl-β-D-xylose (1·4%), 3 : 4-di-*O*-methyl-D-glucose (1·4%), and a small amount of 3-*O*-methyl-D-xylose. The trimethylglucose was identified by its R_G value, methoxyl content, specific rotation, and demethylation to glucose, by its failure to yield a methyl furanoside with cold methanolic hydrogen chloride or formaldehyde with periodate, and by the negative Weerman test given by the derived amide. The 2 : 3-di-*O*-methyl-D-xylose and 3 : 4-di-*O*-methyl-D-glucose were identified by their R_G values, methoxyl contents, and failure to yield formaldehyde with periodate.

In addition the following less pure fractions were obtained:

(*a*) A mixture (22·8%) of 2- and 3-*O*-methyl-D-xylose, from which the above-mentioned quantities of the pure monoethers were isolated. The whole mixture gave only xylose on hydrolysis. Ionophoresis of the non-crystalline portion at pH 10 (cf. Foster, *J.*, 1953, 982) indicated the presence of the two ethers in a proportion of *ca.* 1 : 2; the derived lactone mixture had $[\alpha]_D^{18}$ +84° ⟶ 51° (in 500 hr. in H_2O) which, from the known rotations of the pure lactones (Percival and Willox, *J.*, 1949, 1608; Laidlaw and Percival, *J.*, 1950, 528) and on the assumption of equal ease of lactonisation, again indicated a 1 : 2 ratio. The lactones were converted into the amides which with alkaline hypochlorite and semicarbazide, yielded 20% of hydrazodicarbonamide; since 3-*O*-methyl-D-xylonamide gives a *ca.* 33% yield under similar conditions (Laidlaw and Percival, *J.*, 1949, 1600), the content of this amide can be calculated to be about 61%, which again gives the 1 : 2 ratio. These results, with the yields of crystalline materials isolated, indicate that the mixture (*a*) contained about equal parts of the two ethers.

(*b*) A mixture (7·2%) on demethylation (Hough, Jones, and Wadman, *J.*, 1950, 1702)

gave galactose and a small amount of glucose. The galactose derivatives were all trimethyl ethers, as shown by analysis of the aniline derivatives; the 2 : 3 : 6-trimethyl ether was absent (failure to give a methyl furanoside under the conditions mentioned above), as was the 3 : 4 : 6-trimethyl ether (failure of the derived amides to give a Weerman test), and the derived lactones were of δ-type, so that the fraction contained mainly 2 : 3 : 4- and 2 : 4 : 6-tri-*O*-methyl-D-galactose; the proportions of these cannot be calculated—the formaldehyde–dimedone derivative (30%) obtained by use of periodate, etc., was impure. The glucose component, was, on the basis of its R_G value, 2 : 3-di-*O*-methyl-D-glucose, and this accords with the low methoxyl content of the total fraction.

(*c*) A tetramethyl ether fraction (2·6%) also gave galactose with a little glucose on demethylation; identification of a crystalline aniline derivative showed the main constituent to be 2 : 3 : 4 : 6-tetra-*O*-methyl-D-galactose, and the R_G value indicated that the contaminant was 2 : 3 : 4-tri-*O*-methylglucose.

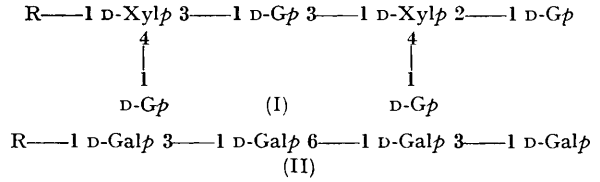
(*d*) A small fraction (0·5%) had the R_G value of 2 : 3 : 6-tri-*O*-methylglucose but was not further investigated.

Traces of free xylose were also identified among the hydrolysis products. This material, however, almost certainly arose from the demethylation known to occur when mono-*O*-methylxyloses are heated with acids (cf. Laidlaw and Percival, *J.*, 1949, 1600), while the dimethylxyloses may be cleavage products of degraded methylated saponin. A trial hydrolysis carried out with tetra-*O*-methyl-D-glucopyranose showed that no appreciable demethylation was effected under the conditions used in the hydrolysis of the methylated saponin and, therefore, the partially methylated glucose derivatives found could not have arisen by destruction of the fully methylated material. Oxidation of the free saponin with sodium periodate gave a product which on hydrolysis yielded xylose along with small amounts of glucose. Since glucose residues linked through $C_{(1)}$ or through $C_{(1)}$, $C_{(2)}$, and $C_{(6)}$ would be split by the reagent, it follows that the glucose found must have been derived from residues linked through $C_{(1)}$ and $C_{(3)}$; the origin of the small amounts of methylated glucoses other than the 2 : 4 : 6-derivative which have been found is obscure, though some of these are probably artifacts arising by incomplete methylation.

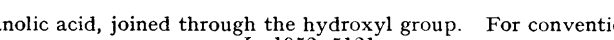
While it is evident that the starting product is a mixture, no fractionation of the components has been achieved so far.

Partial hydrolysis of the saponin yielded a substance containing oleanolic acid and xylose only, indicating that, in one component of the mixture at least, the sapogenin is linked directly to xylose. Attempts to isolate a disaccharide containing xylose residues only from the saponin degradation products were unsuccessful. The alkali-stability of the saponin and its non-reducing properties indicate that the terminal reducing groups of the oligosaccharide chains are joined to the sapogenin through the hydroxyl group of the latter.

In view of the heterogeneous nature of the material, no single structural formula can be ascribed to the product; a structure such as (I), one of several possible variants, would explain many of the properties of the saponin.



(I)



(II)

Where R = oleanolic acid, joined through the hydroxyl group. For conventional symbols, see *J.*, 1952, 5121.

The role of the galactose in the saponin molecule cannot be decided as yet. While it may form part of a " mixed " saponin, it is also possible that it may be derived from a compound containing only oleanolic acid and galactose residues, such as (II).

The amounts of periodate reduced and formic acid liberated on periodate oxidation of the saponin agree with the methylation evidence that the molecule is highly branched.

Experimental

Evaporations were conducted under diminished pressure. Temperatures recorded are bath-temperatures. Fractions from the cellulose column were evaporated to dryness; solutions of the residues in water were digested with charcoal and filtered hot, then evaporated to dryness; these residues were exhaustively extracted with boiling acetone, and the extracts were evaporated to dryness.

The saponin, prepared from the heartwood of morabukea [*Mora gonggrijpii* (Kleinh.) Sandwith; Farmer and Campbell, *loc. cit.*], was kindly supplied by Dr. R. H. Farmer.

The product gave 3·0% of ash (as sulphate; magnesium, potassium, and sodium were identified). The aqueous solution did not reduce boiling Fehling's solution.

The sapogenin has been identified as oleanolic acid (R. H. Farmer, personal communication). Hydrolysis of the saponin with ethanolic hydrogen chloride (Marker, Wagner, Ulshafer, Wittbecker, Goldsmith, and Ruop, *J. Amer. Chem. Soc.*, 1947, **69**, 2167) yielded the crystalline acid which, after purification *via* the acetate, m. p. 266—267°, $[\alpha]_D^{20}$ +73·5° (*c*, 2·2 in $CHCl_3$), had m. p. 299—301°, $[\alpha]_D^{20}$ +74° (*c*, 2·0 in $CHCl_3$). Mixed m. p. determinations with authentic specimens showed no depression.

Acetylation.—The saponin (20 g.) was dissolved in pyridine (200 c.c.), acetic anhydride (140 c.c.) was added with stirring, and the whole was left overnight at room temperature. The acetate was precipitated with water, filtered off, and washed free from pyridine; its solution in chloroform was dried (Na_2SO_4), filtered, and evaporated to small volume. The purified acetate was precipitated by the addition of light petroleum (b. p. 60—80°; 2 l.). Attempts to fractionate the product by stepwise precipitation or dissolution were ineffective. The acetate (23 g.) had $[\alpha]_D^{17}$ −13° (*c*, 2·03 in $CHCl_3$) and OAc 35·9%.

Deacetylation.—The acetate (7 g.) was treated with 0·4N-sodium hydroxide (500 c.c.) at room temperature for 24 hr. The resulting solution was de-ionised with resins ("Zeocarb 215" and "Deacidite E"), and evaporated to small volume, decyl alcohol being used to minimise frothing. Ethanol (150 c.c.) was added and the mixture evaporated to dryness. The residue was redissolved in ethanol, the solution filtered and evaporated to small volume, and excess of acetone added. The precipitated saponin was removed at the centrifuge, washed with acetone, and dried in a vacuum-desiccator ($CaCl_2$); it had $[\alpha]_D^{19}$ −4° (*c*, 2·1 in H_2O) (yield, 3 g.).

Hydrolysis with Sulphuric Acid.—The purified saponin (2 g.) was heated with N-sulphuric acid (50 c.c.) at 100°. $[\alpha]_D^{19}$ were +9° (1 hr.), +22° (6 hr., constant). After neutralisation and filtration the filtrate was evaporated to a syrup. Quantitative chromatography (*loc. cit.*) indicated the presence in the syrup of only galactose 8·4%, glucose 56·1%, and xylose 35·5%.

The syrup partly crystallised. Trituration with glacial acetic acid and recrystallisation of the residue from ethanol yielded α-D-glucopyranose, m. p. and mixed m. p. 140—142°, $[\alpha]_D^{18}$ +91° (5 min.), +77° (1 hr.), +52° (24 hr.). Evaporation of the acetic acid washings gave a syrup in which D-xylose was identified as the di-*O*-benzylidene dimethyl acetal (Breddy and Jones, *J.*, 1945, 738), m. p. and mixed m. p. 207—209°, $[\alpha]_D^{19}$ −7° (*c*, 2·1 in $CHCl_3$). Treatment of a portion of the syrup with methylphenylhydrazine gave D-galactose methylphenylhydrazone, m. p. and mixed m. p. 180° (cf. Hirst, Jones, and Woods, *J.*, 1947, 1048).

The purified saponin (0·7767 g.) was hydrolysed as above. The solution was cooled and filtered through a tared alundum crucible. The residue was washed thoroughly with water and dried at 105° to constant weight (0·2594 g., 33·4%). The filtrate and washings were combined and made up to 200 c.c. The reducing sugars present were estimated on aliquot portions by oxidation with alkaline hypoiodite. Assuming that the sugar mixture has the composition found above, the analysis indicated the presence of 0·448 g. of anhydro-sugars. Total recovery = *ca.* 90%.

Methylation.—The acetate (14 g.) in acetone (200 c.c.) was methylated four times with methyl sulphate and sodium hydroxide at room temperature. After each methylation the acetone was removed in a partial vacuum, at 75°, and the solid methylated material was removed by filtration and washed with hot water. After the fourth methylation the product was dissolved in chloroform, and the solution was dried (Na_2SO_4) and evaporated to a syrup (11·5 g.; OMe, 27·6%) which was methylated five times with the Purdie reagents; the methylated saponin was obtained as a brittle glass {10·5 g.; $[\alpha]_D^{19}$ −9° (*c*, 3·02 in $CHCl_3$); OMe, 29·2%}. The properties of this product were unchanged by further methylation, even by the sodium–methyl iodide method (Pacsu and Trister, *J. Amer. Chem. Soc.*, 1939, **61**, 2442).

Methanolysis, Hydrolysis, and Fractionation.—The methylated saponin (7·3 g.) was heated with methanolic hydrogen chloride (3%; 300 c.c.) under reflux for 5 hr., neutralised with silver

carbonate, and filtered. The solution gradually deposited crystalline methyl oleanolate (m. p. 198° after recrystallisation from methanol). The mother-liquors were evaporated to dryness and the residue heated with N-sulphuric acid (200 c.c.) for 11 hr. The solution was filtered free from methyl oleanolate (2 g.) (Found: OMe, 5·8. Calc. for $C_{31}H_{50}O_3$: OMe, 6·6%), and the filtrate was neutralised with barium carbonate. Filtration, evaporation, and extraction of the residue with boiling acetone gave mixed methylated sugars (4·2 g.) which were heated with nitric acid (2%; 100 c.c.) at 100° for 2 hr. (rotation constant). The product (3·80 g.) was recovered in the usual manner and fractionated on a column of cellulose (25″ × 1·5″) (Hough, Jones, and Wadman, *loc. cit.*) with 2 : 3 butanol–light petroleum (b. p. 100—120°), saturated with water, as eluant. This gave fractions (1) 1·599 g., (2) 0·082 g., (3) 0·420 g., (4) 0·221 g., (5) 0·069 g., and (6) 0·705 g. (recovery, 3·096 g., 81·5%). Traces of xylose were also found.

Fractions (3) and (5) were mixtures. Fraction (3) was refractionated on cellulose as above, to yield (3*a*) 0·013 g., (3*b*) 0·247 g., (3*c*) 0·007 g., (3*d*) 0·060 g., and (3*e*) 0·038 g. Fraction (5) was fractionated on Whatman's 3 MM paper with butanol–ethanol–water (4 : 1 : 5) as solvent, to give (5*a*) 0·029 g. and (5*b*) 0·016 g.; the latter was combined with fraction (6).

Fraction 1. This had R_G 1·00 and crystallised completely, to give 2 : 3 : 4 : 6-tetra-*O*-methyl-D-glucose, which, on recrystallisation from ether–light petroleum (b. p. 40—60°), had m. p. and mixed m. p. 94°, $[\alpha]_D^{18}$ +97° (2 min.), +93° (20 min.), +89° (50 min.), +81° (24 hr., constant) (*c*, 1·95 in H_2O) (Found: C, 50·8; H, 8·5; OMe, 53·4. Calc. for $C_{10}H_{20}O_6$: C, 50·8; H, 8·5; OMe, 52·5%).

Fraction (2). This travelled at the same rate on the chromatogram as tetra-*O*-methyl-galactopyranose and had OMe 49·3%. Treatment of the syrup with aniline in ethanol gave tetra-*O*-methyl-*N*-phenyl-D-galactopyranosylamine, m. p. and mixed m. p. 194°, $[\alpha]_D^{18}$ −77° (*c*, 0·21 in acetone). A small portion of the aniline derivative failed to crystallise. This syrupy material was decomposed with sulphuric acid and the liberated free sugars were demethylated with hydrobromic acid (46—48%) (Hough, Jones, and Wadman, *loc. cit.*). Chromatography, with butanol–pyridine–water–benzene (5 : 3 : 3 : 1) as solvent, indicated the presence of both glucose and galactose.

Fraction (3*a*). This had the same R_G value as 2 : 3 : 6-tri-*O*-methylglucopyranose, and was not further investigated.

Fraction (3*b*). This had the same R_G value as 2 : 4 : 6-tri-*O*-methylglucopyranose (Found: OMe, 40·8. Calc. for $C_9H_{18}O_6$: OMe, 41·8%) and $[\alpha]_D^{18}$ +84° (*c*, 1·25 in H_2O). Fraction (3*b*) (0·2075 g.) was dissolved in methanolic hydrogen chloride (1%; 11 c.c.); $[\alpha]_D^{17}$ (+87·5°) was unchanged after 24 hr. at room temperature. The solution was neutralised (silver carbonate), filtered, and evaporated to dryness, and the residue heated with N-sulphuric acid, to give a reducing syrup. This (10 mg.), on demethylation with hydrobromic acid, gave glucose only, identified on the paper chromatogram.

Oxidation of fraction (3*b*) with periodate (Reeves, *loc. cit.*) gave no formaldehyde. Bromine oxidation yielded a syrupy lactone which was converted into the amide (negative Weerman test).

Fraction (3*c*). This was a mixture of (3*b*) and (3*d*).

Fraction (3*d*). This had the same R_G value as 2 : 3-di-*O*-methylxylose (Found: OMe, 32·0. Calc. for $C_7H_{14}O_5$: OMe, 34·8%) and had $[\alpha]_D^{19}$ +21° (*c*, 0·5 in H_2O). Demethylation gave xylose only, and periodate oxidation gave formaldehyde, identified as the dimedone compound, m. p. and mixed m. p. 186—188°.

Fraction (3*e*). This travelled on the chromatogram at the same rate as 2 : 4-di-*O*-methyl-xylose, and crystallised completely, to give 2 : 4-di-*O*-methyl-β-D-xylose, m. p. and mixed m. p. 108° (Found: C, 46·8; H, 7·75; OMe, 33·0. Calc. for $C_7H_{14}O_5$: C, 47·2; H, 7·9; OMe, 34·8%).

Fraction (4). This did not crystallise (Found: OMe, 37·5. Calc. for $C_9H_{18}O_6$: OMe, 41·8%) and had $[\alpha]_D^{18}$ +121° (*c*, 1·5 in H_2O). Demethylation gave galactose along with small amounts of glucose.

Syrup (4) (*ca.* 120 mg.) was heated with aniline in ethanol, and on removal of the solvent by gradual evaporation in a vacuum-desiccator the following crops of crystals were obtained: (i) 7·4 mg., m. p. 101° with previous softening (Found: N, 5·35; OMe, 27·7%), (ii) 15 mg., m. p. 119°, (iii) 50 mg., m. p. 120°, unchanged on admixture with crop (ii) (Found: C, 59·7; H, 7·5; N, 4·9; OMe, 29·8. Calc. for $C_{14}H_{21}O_5N$: C, 57·6; H, 7·5; N, 4·95; OMe, 21·9. Calc. for $C_{15}H_{23}O_5N$: C, 60·6; H, 7·8; N, 4·7; OMe, 31·3%).

Treatment of fraction (4) with 1% methanolic hydrogen chloride at room temperature caused a change of $[\alpha]_D^{18}$ +72° to +120° (24 hr.). Oxidation with bromine water yielded the lactone which was distilled at 150°/0·10 mm. The syrupy product had $[\alpha]_D^{18}$ +125° (20 min.),

+88° (1½ hr.), +25° (4½ hr.), +3° (24 hr., constant) (*c*, 1·5 in H_2O). Oxidation of the lactone (16·9 mg.) with periodate gave formaldehyde, identified as the dimedone compound (8·0 mg., m. p. *ca.* 160°). The lactone was recovered from the polarimetric solution by evaporation and was redistilled as before. The product crystallised immediately, but in quantity too small for further investigation. The lactone was converted into the amide by methanolic ammonia and the partly crystalline product gave a negative Weerman test.

Fraction (5*a*). This travelled on the chromatogram at the same rate as 3 : 4-di-*O*-methylglucose (Found: OMe, 26·1. Calc. for $C_8H_{16}O_6$: OMe, 29·8%). Demethylation yielded glucose, along with traces of galactose. Oxidation with periodate gave formaldehyde in small yield (dimedone compound, m. p. and mixed m. p. 186°). The fraction travelled at a slightly different rate from 2 : 4-di-*O*-methylglucose on the chromatogram and was much slower than 2 : 3-di-*O*-methylglucose.

Fraction (6). The syrup (OMe, 17·5%) partly crystallised during several days. The crystals were separated on a tile and triturated with light petroleum (b. p. 40—60°)–acetone (3 : 1). The very small quantity of hygroscopic crystalline material had m. p. 95—97°, unchanged on admixture with 3-*O*-methyl-D-xylose. The tile was exhaustively extracted with boiling acetone and the extracts were combined with the light petroleum mother-liquors and evaporated to dryness. The residue was dissolved in ethanol, and the solution was evaporated slowly to small volume in a vacuum-desiccator. The crystals which separated were removed by filtration and washed with ethanol–light petroleum (b. p. 60—80°) (1 : 3). The yield was 0·15 g., the m. p. 137—138°, unchanged on admixture with an authentic specimen of 2-*O*-methyl-D-xylose, and $[\alpha]_D^{18}$ −21° (3 min.), +1° (11 min.), +27° (30 min.), +34° (2 hr., constant value) (*c*, 0·73 in H_2O) (Found: C, 44·2; H, 7·3; OMe, 19·5. Calc. for $C_6H_{12}O_5$: C, 43·9; H, 7·4; OMe, 18·9%). Evaporation of the combined filtrates and washings gave a syrup (OMe, 17·0%) which was examined by paper ionophoresis (cf. Foster, *loc. cit.*) on Whatman's No. 1 filter sheet at 400 v in borate buffer (pH 9·97). Both 2- and 3-*O*-methylxylose were detected (the complexes travelled 6·8 and 13·6 cm. respectively in 3¾ hr.).

The syrup did not crystallise further. Oxidation of this product (0·3 g.) with bromine, and distillation of the mixture of lactones at 145—155°/0·01 mm., yielded a syrup (0·19 g.) having $[\alpha]_D^{19}$ +84° (20 min.), +80° (22 hr.), +65° (166 hr.), +57° (333 hr.), +51° (500 hr., constant) (*c*, 1·39 in H_2O) (10·8 mg. required 6·03 c.c. of 0·01135N-sodium hydroxide for neutralisation. Calc. for $C_6H_{10}O_5$: 5·87 c.c.).

Treatment of the lactones with methanolic ammonia gave the corresponding amides. The syrupy mixture (60 mg.) with alkaline hypochlorite and semicarbazide hydrochloride (Weerman, *Rec. Trav. chim.*, 1917, **37**, 16) yielded hydrazodicarbonamide (7·6 mg., 20%), m. p. and mixed m. p. 256°.

Periodate Oxidation Experiments.—These were carried out on portions of the saponin purified by acetylation and deacetylation.

Formic acid. The saponin was oxidised with potassium periodate (Halsall, Hirst, and Jones, *J.*, 1947, 1427), and the formic acid liberated was determined with 0·01N-sodium hydroxide, *viz.*: 0·728 (2 days), 0·914 (4 days), 1·14 (6 days, constant) × 10^{-3} mole of formic acid per g. of saponin.

Periodate uptake. To the saponin (*ca.* 0·2 g.) in water (35 c.c.) sodium metaperiodate (15 c.c.; 0·3M) was added. The periodate content, determined in portions (5 c.c.) by the arsenite method, was 5·14 (4 days), 5·79 (6 days, constant) × 10^{-3} mole of periodate consumed per g. of saponin.

Formaldehyde production. The saponin (0·5 g.) was dissolved in 0·3M-sodium metaperiodate (15 c.c.) and left at room temperature for 76 hr. No formaldehyde was produced.

Identification of free sugars among the hydrolysis-products of periodate-oxidised saponin. The saponin (0·5 g.) in water (30 c.c.) was treated with 0·3M-sodium metaperiodate (30 c.c.) at room temperature for 5 days. Iodate and periodate were destroyed by the addition of sodium arsenite (30 c.c.; 1·2M). Ions were removed by columns of resins, and the solution was evaporated to dryness. The residue was hydrolysed by sulphuric acid (2N; 20 c.c.) at 100° for 5 hr. Neutralisation, filtration, and evaporation left a syrup which on the chromatogram was shown to contain xylose along with smaller amounts of glucose. Traces of galactose appeared to be present also.

Oleanolic acid. Recrystallised oleanolic acid (0·5 g.), suspended in water and oxidised as above, did not take up periodate.

Hydrolysis of the Saponin with Acetic Acid.—The crude saponin (2 g.) was heated at 100° with acetic acid (20%; 100 c.c.) for 4½ hr. Some solid material separated and was removed

by filtration. Complete hydrolysis of this product with 4N-sulphuric acid and examination of the products on the paper chromatogram indicated the presence of xylose along with very small amounts of other sugars.

The filtrate was neutralised and evaporated to dryness. Examination on the chromatogram showed the presence of glucose, a little galactose, and some oligosaccharides. Only very small amounts of xylose were detected.

Acetolysis of the Saponin.—The saponin (10 g.) was heated in boiling acetic anhydride (50 c.c.) containing zinc chloride (2 g.) under reflux for 1 hr., then poured into ice-water; the acetate was separated by filtration and washed with water, then deacetylated with sodium methoxide in chloroform (Zemplen and Pacsu, *Ber.*, 1929, **62**, 1613). Examination of the product on the chromatogram showed the presence of a range of oligosaccharides, each of which gave mixtures of free sugars on hydrolysis.

Other Fractionations.—Effective fractionation could not be obtained by fractionation on a charcoal column (Whistler and Durso, *J. Amer. Chem. Soc.*, 1950, **72**, 677); the product eluted in low yield by 50% ethanol had the same properties as the starting material and gave similar acetylated and methylated derivatives. Attempts to separate the components of the saponin mixture by fractional dissolution in ethanol were unsuccessful. In an effort to split off methyl oleanolate without disrupting the methylated oligosaccharide chain, several methods of hydrolysis were tried: in both aqueous and non-aqueous solvents the strength of acid necessary to cleave the sapogenin linkage also caused considerable breakdown of the oligosaccharide residue.

The author thanks Dr. R. H. Farmer for a supply of saponin and for helpful comment, and Professor E. L. Hirst, F.R.S., for his interest and for use of the ionophoresis apparatus. Thanks are also expressed to Mr. R. Mahomed for performing the ionophoresis experiment and to Drs. E. E. Percival and D. J. Bell for specimens of methylated sugars. This work forms part of the current research programme of the Forest Products Research Board, and is published by permission of the Department of Scientific and Industrial Research.

SECTION OF CHEMISTRY, FOREST PRODUCTS RESEARCH LABORATORY,
PRINCES RISBOROUGH, AYLESBURY, BUCKS. [*Received, October 10th*, 1953.]