The Chemistry of Extractives from Hardwoods. Part XXIV. A Saponin Constituent of Makoré* (Mimusops heckelii).

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From the ethanol extract of makoré, the wood of *Mimusops heckelii*, an amorphous saponin has been isolated which yields bassic acid on hydrolysis with aqueous-alcoholic acid. With aqueous acid a monoglucoside of bassic acid is obtained. Also present in the aqueous-alcoholic acid hydrolysate are D(?)-glucose, L-rhamnose, and D-xylose in the molar ratio of 1 : 2 : 2 to one molecule of triterpene. The saponin contains a sulphato-ester group, and there is evidence that this constituent is attached to the 6-position of the glucose residue.

Mimusops heckelii (family, *Sapotaceae*), a large tree occurring in the forests of West Africa, produces a resistant wood commonly termed makoré which in colour and texture superficially resembles mahogany. Apart from a quantitative estimation of its pentosan, cellulose, and lignin contents by Marmasse (Association colonies-sciences et comité national des bois coloniaux, Paris, 1931, *Chem. Abs.*, 1932, **26**, 3281), no chemical examination of the heartwood has been reported. It is, however, readily procured from commercial sources and in a further investigation of the timber the nature of some of its principal extractable constituents has been determined. Two distinct samples of makoré were purchased, as well as a quantity of wood marketed as balata—a description usually applied to a related species of West Indian origin, *viz.*, *M. balata*—but on examination at the D.S.I.R. Forest Products Research Laboratory, all three were found to be derived from *M. heckelii*, and, when treated with solvents, each afforded approximately equal quantities of similar products.

By successive extractions of the granulated wood with boiling light petroleum and with ether, comparatively small amounts of semi-solid substances were isolated and these were not further examined. Refluxing the wood with ethanol then yielded a viscous solution from which a solid was slowly deposited. This was purified by dissolution in water and treatment with lead acetate to precipitate tannins. Removal of soluble lead with hydrogen sulphide and evaporation, an operation which was complicated by foaming, yielded the friable colourless saponin; with a further crop of inferior quality obtained by concentrating the original alcoholic extract and processing in the manner already indicated, the yield amounted to 3% of the wood.

The product could not be obtained crystalline, and when incinerated it left an ash containing sodium, magnesium, and sulphate ions. Zeisel determinations indicated the presence of a persistent alkoxy-content, but in view of the purification of the saponin through alcohol it is uncertain whether this constituent is an integral part of the molecule. An aqueous solution of the saponin gave no sulphate reaction, but positive sulphate tests were obtained after short hydrolysis. The action of boiling aqueous-alcoholic mineral acid caused the precipitation of bassic acid, $C_{30}H_{46}O_5$, already shown by Heywood and Kon (*J.*, 1940, 713) to be present in the seeds of several plants belonging to the family *Sapotaceae*, including certain *Bassia* and *Mimusops* species. Its identity was confirmed by the preparation of the known methyl ester, *iso*propylidene derivative, and bromo-lactone.

Monosaccharides were detected in the aqueous-alcoholic hydrolysate from which the triterpene had been collected. By means of paper chromatography (Partridge, *Biochem. J.*, 1948, **42**, 239) the presence of glucose, rhamnose, and xylose was established. Subsequently, crystals of L-rhamnose monohydrate separated from the concentrated solution, and the preparation of L-rhamnose benzoylhydrazone and of di-*O*-benzylidene-D-xylose dimethyl acetal gave further evidence of their identity.

Hydrolysis of the saponin with *aqueous* mineral acid gave a crystalline prosapogenin consisting of bassic acid monoglucoside, which was resolved into its constituents by heating it with aqueous-ethanolic acid.

* Part XXIII, preceding paper.

Indications of the point of attachment of the sulphato-group were obtained by alkali treatment of the saponin followed by hydrolysis under acid conditions. The resulting mixed carbohydrates were examined chromatographically whereupon a fourth product was detected. From its higher R_F value this was identified as an anhydro-sugar, and direct comparison with 3 : 6-anhydro-D-glucose prepared from a specimen of methyl 3 : 5-anhydro-D-glucoside, kindly supplied by Professor M. Stacey, gave conclusive proof of its constitution. It therefore follows (cf. Peat, *Adv. Carbohydrate Chem.*, 1948, **2**, 38) that the sulphato-group is attached to the 6-position of the glucose unit. The acidic functions of the saponin are neutralised in the natural product, as is shown by the pH of its solution and by the presence of sodium and magnesium ions in the purified substance. The saponin-acid was obtained by precipitation of a solution of the natural material with basic lead acetate, the resulting lead salt being dissolved in aqueous acetic acid and freed from lead by passage through a column of ion-exchange resin.

Many experiments were made to ascertain the homogeneity of the saponin, *e.g.*, by dialysis and by chromatography. Analysis of the fractions thus obtained invariably showed the presence of glucose, rhamnose, and xylose in each—as far as could be estimated visually, in approximately the same relative proportions. All fractions also liberated sulphate ions on alkali treatment.

The relative amounts of the three sugars in the saponin were determined by the paper chromatography procedure of Flood, Hirst, and Jones (*J.*, 1948, 1679). It was necessary to use aqueous-ethanolic acid for the hydrolysis, afterwards neutralising the solution with an ion-exchange resin. The sugars were then separated and estimated by the Somogyi copper reagent (Flood *et al.*, *loc. cit.*). From the percentage of sulphur present in the saponin an estimate of the sulphato-groups was possible. The combined results suggest that the saponin contains one molecule of triterpene, one of glucose, two of rhamnose, two of xylose, and one sulphato-ester group. It cannot, of course, be supposed with any certainty that the saponin is a single chemical entity; nevertheless, the inability of chromatographic techniques to resolve it into different fractions, and the approximate whole-number ratio of the constituent parts, imply that if more than one compound is present all are of very similar composition. It is of interest to compare these results with those of investigations by Van der Haar (*Rec. Trav. chim.*, 1929, **48**, 1155, 1166) on the bassic acid saponins of *M. elengi* and *Achras sapota*, which show that arabinose, rhamnose, and glucose are present in the molecular ratio of approximately 2 : 2 : 1.

Information on the distribution of the carbohydrate residues was sought by the method of methylation, using methyl sulphate–aqueous sodium hydroxide followed by methyl iodide–methanol–silver oxide. The amorphous product was then hydrolysed and the triterpene fraction esterified with diazomethane. Treatment with benzene left a crystalline solid, $C_{30}H_{44}O_3(OMe)_2$, insoluble in alcoholic alkali and therefore regarded as a mono-methyl triterpene ester. The benzene solution contained a non-crystalline material, obtained by distillation in a high vacuum as a glass, which was isomeric with the less soluble product $C_{32}H_{50}O_3$. One triterpenoid alcoholic group is therefore unsubstituted in the original saponins, but owing to the possible operation of steric factors it does not necessarily follow that both remaining hydroxyl groups are glycosidically linked in the parent compound.

Experimental

Extraction of the Saponin from Makoré.—Powdered heartwood of *Mimusops heckelii* (3 kg.) was extracted with boiling light petroleum (b. p. 60—80°) for 12 hr. When the solvent was evaporated a soft orange-yellow wax (12·3 g., 0·4%) remained. Afterwards, similar treatment with ether gave a red-brown wax (4 g., 0·13%). Thereafter, only ether was used for the preliminary treatment. The wood was then extracted for several hours with boiling ethanol (8 l.), and the dark red solution left in the refrigerator for 24 hr. The brown solid (72 g.) then deposited was collected and thoroughly washed with cold alcohol, care being taken to minimise exposure of the very deliquescent material to the air. However, after being washed with ether and dried in a vacuum-desiccator it formed a moisture-stable powder.

A solution of the crude product in hot water was, after filtration, treated with a saturated solution of lead acetate until precipitation was complete. The mixture was filtered and the last traces of tannins removed by boiling the filtrate with a small quantity of litharge insufficient for complete neutralisation of the acetic acid liberated during the precipitation. Excess of lead was removed as sulphide, its separation being assisted by the addition of charcoal and by filtration through diatomaceous earth. The colourless solution was evaporated to dryness with as much reduction in pressure as was possible without excessive frothing. Dissolution of the residue in absolute methanol removed insoluble inorganic matter (*ca.* 1 g.), and by evaporation the saponin was recovered as a white, friable amorphous solid (34 g.). When further purified by separation from a hot saturated solution in ethanol it was dried for analysis *in vacuo* at room temperature (Found: C, 46·2, 46·7; H, 7·4, 7·3; S, 1·9, 2·0; OMe, 2·0, 1·7; Ash, 3·4, 3·1. $C_{59}H_{93}O_{29}SNa,10H_2O$ requires C, 47·2; H, 7·5; S, 2·1; OMe, 2·1%).

By evaporation of the original alcoholic solution, finally to dryness in an open vessel, a dark resinous powder (150 g.) was obtained, from which by extraction with hot water and purification by the addition of lead acetate in the manner already outlined, an additional quantity of the *glycoside* (55 g.) was recovered as a light brown powder. Although less pure than the first fraction, the product was suitable for the investigation of its hydrolysis products.

The saponin, decomp. 180° to 220° according to the rate of heating, was very readily soluble in water and methanol, but much less soluble in ethyl alcohol. It showed no reaction with Fehling's solution or ammoniacal silver nitrate, and gave a precipitate with barium chloride only after hydrolysis with acids or alkalis.

Bassic Acid.—The saponin (50 g.) in aqueous-ethanolic (1 : 1) 2N-sulphuric acid (300 c.c.) was boiled under reflux on a steam-bath for 12 hr. The brown solid which separated from the hot solution was collected from the cooled mixture and washed with 50% aqueous ethanol and finally digested with absolute ethanol. The sandy residue consisted of bassic acid (7 g., 14%) which crystallised (charcoal) either from aqueous acetic acid as the monohydrate, needles, m. p. 290° (decomp.) (Found: C, 71·7; H, 9·7. Calc. for $C_{30}H_{46}O_5,H_2O$: C, 71·3; H, 9·5%), or from aqueous 2-ethoxyethanol as anhydrous prisms, m. p. 320° (decomp.), $[\alpha]_D$ +79° (in pyridine) (Found: C, 74·3; H, 9·5. Calc. for $C_{30}H_{46}O_5$: C, 74·0; H, 9·5%). Heywood, Kon, and Ware (*J.*, 1939, 1124) give for bassic acid, m. p. 320°, $[\alpha]_D$ +82·4—82·9°.

The methyl ester prepared with diazomethane crystallised from aqueous methanol as needles, m. p. 212°, $[\alpha]_D$ +56° (in MeOH) (Found: C, 74·1; 74·2; H, 9·6, 9·8; OMe, 6·2. Calc. for $C_{31}H_{48}O_5$: C, 74·4; H, 9·7; OMe, 6·2%). Heywood *et al.* (*loc. cit.*) give for α-methyl bassate, m. p. 213°, $[\alpha]_D$ +64° and for β-methyl bassate, m. p. 210°, $[\alpha]_D$ +55·5°.

The characteristic *iso*propylidene derivative of the methyl ester was prepared in acetone with a trace of hydrochloric acid, forming flat prisms, m. p. 206° (lit., 206°) (Found: C, 75·1, 75·3; H, 9·3, 9·5; OMe, 5·4. Calc. for $C_{34}H_{52}O_5$: C, 75·4; H, 9·7; OMe, 5·8%). The acid with bromine in acetic acid gave the bromo-lactone, colourless needles (from aqueous acetic acid), m. p. 220° (lit., 220°) (Found: C, 63·1; H, 7·7; Br, 13·4. Calc. for $C_{30}H_{45}O_5Br$: C, 63·7; H, 8·0; Br, 14·1%).

Bassic Acid Glucoside.—The saponin (5 g.) was hydrolysed on a steam-bath with 2N-sulphuric acid (25 c.c.) for 5 hr., the original gelatinous precipitate having then coagulated. The solid was collected and washed repeatedly with hot water to remove mineral acid and finally dried (desiccator). The pale yellow powdery product (2 g.) was taken up in cold ethanol (25 c.c.), and the solution filtered and allowed to evaporate at room temperature to a third of its bulk; the crystalline deposit (0·5 g.) of the *monoglucoside* then readily crystallised from aqueous ethanol as needles, m. p. 225°, $[\alpha]_D$ +40° (*c*, 0·37 in EtOH) (Found: C, 66·9; H, 9·0. $C_{34}H_{56}O_{10}$ requires C, 66·7; H, 8·6%). The prosapogenin gave a crystalline sodium salt from aqueous-alcoholic sodium hydroxide, but the acetate was amorphous. The *methyl ester*, prepared with diazomethane, crystallised from aqueous methanol as long colourless needles, m. p. 141—142°, $[\alpha]_D$ +45° (*c*, 0·64 in EtOH) (Found: C, 66·8; H, 9·1; OMe, 5·1. $C_{37}H_{58}O_{10}$ requires C, 67·1; H, 8·8; OMe, 4·7%).

Treatment of the prosapogenin with 4N-sulphuric acid at 100° (sealed tube) for 48 hr. gave bassic acid and D-glucose (identified by chromatography on paper) as its sole hydrolysis products.

Carbohydrate Constituents of the Saponin.—(a) *Identification.* The soluble hydrolysate and washings from the aqueous-alcoholic hydrolysis of the saponin were neutralised with barium carbonate, and the mixture was filtered. The filtrate was evaporated under reduced pressure and the residue redissolved in boiling methanol. After removal of a small amount of insoluble material, evaporation of the filtrate gave the sugars as a syrupy mass. Paper partition chromatography in butanol–water, butanol–ethanol–water (4 : 1 : 5), and butanol–acetic acid–

water (4 : 1 : 5) gave three spots identified by the R_F values and by direct comparison with authentic specimens as being due to glucose, xylose, and rhamnose.

A portion of the syrupy sugar mixture slowly deposited L-rhamnose monohydrate, m. p. 94°, $[\alpha]_D$ +8·3° (in H_2O) (Found : C, 39·3; H, 7·8. Calc. for $C_6H_{12}O_5,H_2O$: C, 39·6; H, 7·7%). This was further characterised as the benzoylhydrazone (Hirst, Jones, and Wood, *J.*, 1947, 1048), m. p. and mixed m. p. 180°. The crude sugar mixture with benzaldehyde and hydrogen chloride in methanol slowly deposited di-*O*-benzylidene-D-xylose dimethyl acetal (Breddy and Jones, *J.*, 1945, 738), m. p. and mixed m. p. 211° (Found : C, 67·4; H, 6·5; OMe, 15·9. Calc. for $C_{27}H_{24}O_6$: C, 67·7; H, 6·5; 2OMe, 16·7%).

(b) *Estimation.* Samples of the saponin of *ca.* 0·1 g. were hydrolysed in 2N-hydrochloric acid in 30% aqueous ethanol in a sealed tube for 8 hr. After neutralisation of the cooled solution with a basic ion-exchange resin (Amberlite IR.4B) the relative proportions of the sugars present were determined by the method of Flood *et al.* (*loc. cit.*). The mean of three determinations gave the molecular ratios of glucose : xylose : rhamnose to be 1 : 2·1 : 2·0.

Alkali Hydrolysis of the Saponin.—The saponin (1 g.) was hydrolysed with boiling saturated aqueous barium hydroxide for 1 hr., and the solution so obtained was acidified with sulphuric acid and then neutralised with barium carbonate. The filtrate from this operation was examined for the presence of sugars by paper chromatography in butanol–ethanol–water (4 : 1 : 5) for development. In addition to spots due to glucose, xylose, and rhamnose, aniline phthalate revealed a fourth faster-running spot identified by its position on the paper compared with that of an authentic specimen as due to 3 : 6-anhydro-D(?)-glucose.

The Saponin-acid.—A solution of the saponin salt (5 g.) in water (25 c.c.) was treated with an excess of saturated basic lead acetate. The lead salt of the saponin was collected and thoroughly washed and then dissolved in dilute acetic acid, and the solution freed from lead by passage down a column of acid ion-exchange resin (Zeocarb 215). The lead-free eluate was evaporated at 30—40° by drawing a stream of dust-free air over the surface and finally in a vacuum-desiccator to give a pale yellow glassy powder, very soluble in water.

Fractionation of the Saponin.—(a) *Dialysis.* The saponin acid (5 g.) was dissolved in water (50 c.c.) and the solution was placed in a seamless "Visking" tube and rotated slowly in a beaker containing water (350 c.c.). A total of 12 fractions were collected, the first 5 after 12 hr. and the remainder after 24 hr. The first 2 fractions after evaporation gave coloured gums, the remainder giving colourless products. The twelve fractions contained a total of 3·9 g. of solid, each fraction giving sulphate on brief alkali hydrolysis, and on acid hydrolysis bassic acid and a solution containing glucose, xylose, and rhamnose, the proportions, so far as could be estimated visually from the size and intensity of the spots obtained on paper chromatography, being the same in each case.

(b) *Chromatography.* Large-scale paper chromatography of the saponin acid by Novellie's method (*Nature*, 1952, **169**, 672) on wide strips of paper for prolonged periods during which the developing liquid (butanol–methanol–water, 4 : 1 : 5) was allowed to drip from the end of the paper gave a chromatogram, a test strip from the edge of which was developed by one of three methods, namely (i) thermal preferential charring of the saponin at 200°, (ii) preferential charring of the saponin by concentrated sulphuric acid, and (iii) spraying with bromocresol-green. In every case the saponin was spread over a considerable length of paper, but only in one of several experiments was any positive separation indicated. Horizontal strips were cut from the main chromatogram at places spaced along the area occupied by the saponin and these were extracted completely with water. Each fraction was thus shown to give sulphate on alkaline hydrolysis and glucose, xylose, and rhamnose on acid hydrolysis.

Methylation of the Saponin.—The saponin (5 g.) in water (10 c.c.) was methylated at 70° by the dropwise addition of aqueous sodium hydroxide (25 g. of 30%) and methyl sulphate (28·5 g.) at such a rate that the solution remained alkaline. During the reaction the product separated as a rubbery mass which was collected and washed free from alkali with cold saturated sodium sulphate solution. The dried product was further treated in boiling methanol for 5 hr. with silver oxide (5 g.) and methyl iodide (10 c.c.), this being followed by the addition of more silver oxide (5 g.) and methyl iodide (10 c.c.) and further heating for 15 hr. Silver salts were then removed and the filtrate was evaporated. The residue was dissolved in boiling methyl iodide (30 c.c.), silver oxide (10 g.) was added, and heating continued for 48 hr., a further portion of silver oxide (10 g.) being added after 24 hr. The silver salts were then removed and the filtrate was evaporated to give the methylated saponin as a pale yellow friable amorphous solid (3·3 g.).

Methyl O-Monomethylbassate.—The methylated saponin (3·3 g.) was hydrolysed by boiling with aqueous-methanolic (1 : 1) 2N-hydrochloric acid (50 c.c.) for 4 hr. Evaporation of the methanol

led to the separation of the aglycone which was collected and further hydrolysed in methanol (35 c.c.) with boiling 6N-hydrochloric acid (15 c.c.) for 9 hr. The aglycone isolated after evaporation of the methanol was then methylated with diazomethane, giving *methyl O-methylbassate* which separated from benzene in colourless needles (0·13 g.), m. p. 245—246° (Found: C, 74·7; H, 9·2; OMe, 7·3. $C_{32}H_{50}O_5$ requires C, 74·8; H, 9·7; 2OMe, 10·7%). The ester was not readily hydrolysed by 10% methanolic potassium hydroxide and did not react under the conditions in which methyl bassate gives an *iso*propylidene derivative.

High-vacuum distillation of the residue from the benzene mother-liquors of the above ester gave a glassy non-crystallisable solid, b. p. 200—210° (air-bath)/10^{-4} mm., apparently isomeric with the crystalline ester (Found: C, 74·8; H, 9·6%). It failed to give a crystalline *iso*propylidene derivative with acetone–hydrochloric acid.

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