TRITERPENOIDS OF *ACHRAS SAPOTA* (SAPOTACEAE)

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Abstract

Four free triterpene acids from *Achras sapota* wood have been identified as hederagenin, bayogenin, polygalacic acid, and protobassic acid, and hederagenin and bayogenin have been obtained by hydrolysis of the saponins.

Achras sapota L. belongs to the family Sapotaceae and is commonly known as sapodilla or chicle wood. It is of South American origin and widely cultivated. Door lintels of temples and carvings made from the wood in the Mayan period have resisted termite and fungus attack for more than 1200 years and the durability of the timber has been attributed to the presence of saponins which on hydrolysis afforded a hydroxy- and a dihydroxy-oleanolic acid.[1] These sapogenins were incompletely characterized and remained unidentified.[1] The recorded mass spectrometric data[1] are consistent with triterpenoid acid structures in which all the hydroxy groups are situated on the A and B rings, but the correct allocation of the oleanane skeletons to the sapogenins, which was not established until the present study, must be regarded as having been fortuitous.

Extraction of the wood of *A. sapota* has now given a mixture of triterpene acids and a mixture of saponins. Four major constituents of the triterpene acids have been separated, fully characterized as their methyl esters and identified as 3β,23-dihydroxyolean-12-en-28-oic acid (hederagenin), 2β,3β,23-trihydroxyolean-12-en-28-oic acid (bayogenin), 2β,3β,16α,23-tetrahydroxyolean-12-en-28-oic acid (polygalacic acid), and 2β,3β,6β,23-tetrahydroxyolean-12-en-28-oic acid (protobassic acid). Of these triterpene acids hederagenin occurs relatively commonly, and bayogenin is a constituent of *Castanospermum australe* (Leguminosae).[2] Polygalacic acid, which was first isolated from *Polygala paenea* (Polygalaceae) and originally assigned a β-configuration for the C16 hydroxy group,[3] has recently been isolated from *Platycodon grandiflorum* (Campanulaceae) and shown conclusively to have a C16α hydroxy group.[4] Protobassic acid was recently isolated from seed kernels of *Madhuca longifolia* (Sapotaceae).[5] Other triterpene acids of *A. sapota* were present in amounts

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[1] Sandermann, W., and Funke, H., *Naturwissenschaften*, 1970, **57**, 407.

[2] Eade, R. A., Simes, J. J. H., and Stevenson, B., *Aust. J. Chem.*, 1963, **16**, 900.

[3] Rondest, J., and Polonsky, J., *Bull. Soc. chim. Fr.*, 1963, 1253.

[4] Akiyama, T., Tanaka, O., and Shibata, S., *Chem. pharm. Bull., Tokyo*, 1972, **20**, 1945.

[5] Kitagawa, I., Inada, A., Yosioka, I., Somanathan, R., and Sultanbawa, M. U. S., *Chem. pharm. Bull., Tokyo*, 1972, **20**, 630.

too small for convenient isolation. Examination by thin-layer chromatography indicated that there was probably a trace of oleanolic acid present, and that there are other minor constituents which may have more than four hydroxy groups.

The triterpene acids from hydrolysis of the saponin fraction had largely two constituents which were separated after conversion into their methyl esters, and identified as hederagenin and bayogenin. Traces of a number of other constituents could be detected by thin-layer chromatography, and although polygalacic acid appeared to be present as a minor constituent, there was relatively much more hederagenin than in the free triterpene acids. Bassic acid was not isolated as its methyl ester although it has been shown that protobassic acid undergoes dehydration to give bassic acid by elimination of the 6β-axial hydroxy group under the conditions of the hydrolysis of the saponins.[5] The sugars from the saponins have not been identified.

Hederagenin and bayogenin are respectively a hydroxyoleanolic acid and a dihydroxyoleanolic acid, and to this extent the conclusions of the present study are in accordance with the mass spectrometric data of Sandermann and Funke.[1] There is a discrepancy however between the melting points reported by Sandermann and Funke for the methyl esters and those now obtained. The methyl esters of hederagenin and bayogenin melt at 236–238° and 248–250° respectively, whereas Sandermann and Funke reported m.p. 247–248° for the methyl ester of sapogenin A_2 ($C_{30}H_{48}O_4$, isomeric with hederagenin methyl ester) and m.p. 233–235° for sapogenin A_1 ($C_{30}H_{48}O_5$, isomeric with bayogenin methyl ester).

Concurrent collaborative studies are in progress in the hope of establishing the role, if any, of the saponins of *A. sapota* in resistance of the wood to fungal attack.

Experimental

Extraction of Achras sapota *Wood*

Milled, dry wood of *A. sapota* (300 g) was extracted with diethyl ether in a Soxhlet apparatus for 6 hr. Evaporation of the extract gave a residue (1·43 g).

The wood was then extracted with methanol for 5 hr and on removal of the methanol a residue (6·68 g) remained. This residue consisted largely of phenolic material and afforded only traces of saponins. The wood was then extracted with ethanol containing 20% water for 5 hr. Evaporation of the solution to dryness left a residue (1·97 g) which was dissolved in methanol (10 ml). Addition of water (50 ml) precipitated some insoluble material (0·16 g) which was removed by filtration. The largely aqueous solution was then extracted repeatedly with n-butanol until little further material was being extracted. Evaporation of the combined n-butanol extracts under reduced pressure yielded crude saponins (1·19 g).

Separation of the Triterpenes

The fraction extracted from the wood by diethyl ether was suspended in methanol and a solution of diazomethane in diethyl ether added. Examination of the product on t.l.c. plates (Kieselgel G) which were developed twice in the solvent system chloroform–methanol (9 : 1) showed four strong spots (R_F 0·73, hederageni methyl ester; 0·65, bayogenin methyl ester; 0·57, methyl polygalacate; 0·43, methyl protobassate; and a weak spot at R_F 0·85 which corresponds to methyl oleanolate). A partial separation of the crude esters into two main fractions was achieved by chromatography on neutral alumina. Elution with 50% chloroform in benzene gave fractions which contained mainly esters of hederagenin and bayogenin, and fractions eluted by chloroform contained

all four esters. Individual triterpene esters were isolated by preparative t.l.c. in the system given above.

Characterization of the Triterpene Esters

The methyl esters of the triterpene acids were identified in each instance by comparison of their i.r. spectra (KBr discs) and mass spectra with those of authentic reference specimens, and by the absence of any m.p. depression on admixture with authentic reference samples, which in each instance had the same m.p. as the sample to be identified. The methyl esters were crystallized from methanol and the following physical constants were determined: hederagenin methyl ester, m.p. 236–238°, $[\alpha]_D$ +54° (*c*, 0·4 in $CHCl_3$); bayogenin methyl ester, m.p. 248–250°, $[\alpha]_D$ +68° (*c*, 0·5 in $CHCl_3$); methyl polygalacate, m.p. 250–252°, $[\alpha]_D$ +40° (*c*, 0·3 in $CHCl_3$); methyl protobassate, m.p. 198–201°, $[\alpha]_D$ +44° (*c*, 0·2 in $CHCl_3$).

Separation of the Sapogenin Methyl Ester

The crude saponin fraction was dissolved in a mixture of water (40 ml), methanol (40 ml), and concentrated hydrochloric acid (10 ml). The solution was refluxed for 6 hr. The solution was partly concentrated to remove methanol, and dark insoluble material containing the sapogenins was separated by filtration. The crude sapogenins (0·31 g) were suspended in methanol and converted into methyl esters. Preliminary examination by t.l.c. indicated a relatively greater proportion of hederagenin and bayogenin esters than were present in the esters derived from the free triterpene acids. Chromatography on alumina and preparative t.l.c. gave hederagenin methyl ester, m.p. 236–238°, and bayogenin methyl ester, m.p. 248–250°, identical with the esters obtained above. There was insufficient material to isolate methyl esters of other constituents but t.l.c. showed spots at R_F 0·85 and 0·57, which correspond in R_F with the esters of oleanolic and polygalacic acid respectively.

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