557. *The Constituents of the Wood of* Castanospermum australe *Cunn. et Fras. Part I. The Isolation of a New Sapogenin, Castanogenin.*

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Extraction of the wood of *Castanospermum australe* gives a crude saponin which on hydrolysis yields a mixture of sapogenins, one of which, castanogenin, has been characterized as a new sapogenin having the formula $C_{28}H_{42}(OH)_2(CO_2H)_2$.

In an investigation of some Australian woods for saponin content, it was noted that *Castanospermum australe* gave strongly positive tests, and the present work was undertaken to investigate the saponin present in the wood. This large tree, more commonly known as "Black Bean" and sometimes called the "Moreton Bay Chestnut," occurs in the brush forests of northern New South Wales and Queensland. The seeds of the tree are reported to be

poisonous to stock, although on analysis no poisonous principle was detected (see Hurst, " The Poison Plants of New South Wales," Sydney, 1942, p. 153).

Extraction of the wood (see Experimental section for details) gave fats, phenols, sugars, and sapogenins. It was difficult to obtain crystalline material from the crude sapogenin; the method eventually adopted was to carry out a fractionation by long extraction with ether, whereafter the ether-soluble portion could be crystallized from methanol. This was resolved by fractional crystallization from methanol into a more soluble sapogenin, castanogenin, and a less soluble one not yet investigated.

Analyses of castanogenin and its derivatives are consistent with the molecular formula $C_{30}H_{46}O_6$ for castanogenin, and indicate the presence of two carboxyl and two hydroxyl groups. Attempts to reduce castanogenin, its diacetate, and its dimethyl ester with hydrogen using Adams's catalyst at room temperature were unsuccessful. With tetranitromethane castanogenin itself gives no colour; its more soluble derivatives, the diacetate and dimethyl ester, however, give deep yellow colours, indicating the presence of a carbon–carbon double bond which is evidently of the unreactive type present in triterpenes. The results of the investigation so far carried out agree with the classification of castanogenin as a new member of the triterpenoid acid class of sapogenins.

Experimental.

(M. p.s are uncorr.)

Isolation of the Saponin.—The dried wood (25 kg.) of *Castanospermum australe* was reduced to a fine saw-dust and steeped in ethanol. The red-brown alcoholic extract was drained off after 24 hours and replaced with fresh ethanol; this process was repeated 7 times, the wood being then practically exhausted. The combined alcoholic extracts were evaporated, finally in an open dish on a steam-bath, until most of the solvent had been removed leaving a black viscous mass. Addition of ether yielded a precipitate which was triturated with fresh portions of ether until it became a friable solid. Evaporation of the solvent from the ethereal extract left an intractable black material on the surface of which accumulated a small amount of a yellow fatty substance. The black material gave a red-brown colour in the Liebermann–Burchard test, and a green colour with neutral ferric chloride solution; the fatty substance gave no colour in either test. The crude saponin precipitated by ether became moist on exposure to the atmosphere and difficult to handle; it was thoroughly dried at 105° and then was no longer hygroscopic; the yield was 700 g. The crude saponin was readily soluble in water and ethanol, leaving a small residue in each case, and its aqueous solution on agitation gave a very large froth stable for up to 2 days. It gave a deep red colour, changing to purple, in the Liebermann–Burchard test. At a dilution of 1 in 20,000 in 0·9% saline solution it required 5 minutes to hæmolyse completely a 1% suspension of sheep-blood corpuscles at 28°.

Hydrolysis of the Saponin.—A solution of crude saponin (50 g.) in hot water (1·5 l.) was freed from a small amount of insoluble matter by filtration with kieselguhr. It was then heated to boiling and sufficient 10N-hydrochloric acid added to make the solution approx. N. with respect to hydrochloric acid; a gelatinous precipitate immediately commenced to form and the mixture was boiled under reflux for 30 minutes, with vigorous stirring in the latter stages to overcome the frothing caused by the heavy gelatinous precipitate; the mixture was filtered hot. A sample of the filtrate was boiled for 4 hours with stronger acid and gave only a small amount of further precipitate, indicating that hydrolysis was virtually complete. The filtrate gave a strong test for reducing sugars with Fehling's solution; when left in the refrigerator for 2 days it deposited pale yellow needles of a substance which gave a green colour with neutral ferric chloride solution. The precipitate of sapogenins was extracted with boiling water until the extract no longer gave the ferric chloride colour (this process also removed the last traces of hydrochloric acid; this was found essential, as residual hydrochloric acid in the sapogenin caused considerable darkening on drying). The sapogenin was air-dried at 105°; yield (from 50 g. of saponin), 22 g. The crude sapogenin was a dark brown amorphous powder, insoluble in water, and soluble in ethanol and in aqueous and alcoholic sodium hydroxide solutions. It gave a deep red colour, changing to purple in the Liebermann–Burchard test.

Separation of the Sapogenins.—The crude sapogenin was easily soluble in many organic solvents but no crystalline material could be obtained from these solutions. Sublimation at 200—300/10^{-4} mm. produced some amorphous sublimate, containing a little crystalline material, with much decomposition. Attempts to purify the crude sapogenin through the sodium and potassium salts were also unsuccessful. Though the crude sapogenin was very sparingly soluble in ether, very extended extraction with ether in a Soxhlet apparatus removed a fraction which separated spontaneously from the ethereal extract and was much lighter in colour. 75 G. of material was extracted from 300 g. of crude sapogenin in 200 hours and even at this stage some sapogenin was still extractable; during the extraction 55 g. of light-brown amorphous solid (fraction *A*) separated spontaneously from the boiling ether, leaving 20 g. dissolved in the ethereal extract. Fraction *A* was boiled under reflux for 1 hour with methanol (300 ml.) and filtered hot, leaving a colourless residue consisting principally of the less soluble sapogenin. The methanolic solution was concentrated, causing crystallization to occur. In all 15 g. of crystals were obtained from this solution, which on further evaporation set to a gel. These were crystallized again from methanol and then from dioxan (which was the most satisfactory solvent for recrystallization) to the constant m. p. 380—382° (decomp.). An additional amount of this sapogenin, castanogenin, was also obtained by refrigeration of the ethereal solution remaining after filtering off fraction *A*. The total yield from 300 g. of crude sapogenin was 9 g.

Castanogenin.—*Castanogenin* was moderately soluble in methanol, ethanol, or dioxan, slightly soluble in ether, and practically insoluble in chloroform, light petroleum, or benzene. It crystallized as prisms from methanol and as needles from ethanol, dioxan, or ether, both forms having the same m. p. It gave a deep red colour, changing to purple, in the Liebermann–Burchard test and had $[\alpha]_D^{23}$ +107° (*c*, 1·06 in absolute ethanol) [Found: C, 71·7; H, 9·1; active H, 0·75%; equiv., 256. $C_{28}H_{42}(OH)_2(CO_2H)_2$ requires C, 71·7; H, 9·2; active H, 0·8%; equiv., 251] [the equiv. was determined by titration of an ethanolic solution with 0·1N-sodium hydroxide (phenolphthalein)].

Ethanolic sodium hydroxide solution was added to an ethanolic solution of castanogenin, giving a gelatinous precipitate of the *disodium* salt, very soluble in water, and slightly soluble in ethanol from which it crystallized in fine needles (Found: Na, 8·5. $C_{30}H_{44}O_6Na_2$ requires Na, 8·4%).

Diacetate. Castanogenin (2·5 g.), acetic anhydride (30 ml.), and anhydrous sodium acetate (2 g.) were boiled under reflux for 2 hours and then poured into water, yielding a white solid which recrystallized from methanol in colourless plates, which softened at 220°, formed a clear glass at 226°, and flowed at 239—240°, and had $[\alpha]_D^{27}$ +75° ±2° (*c*, 0·166 in chloroform; *l* = 4 dm.) and gave a yellow solution with tetranitromethane in chloroform [Found: C, 70·0; H, 8·5%; sap. val., 374; *M* (camphor), 561. $C_{34}H_{50}O_8$ requires C, 69·6; H, 8·6%; sap. val., 382; *M*, 586].

Dimethyl esters. Castogenin and diazomethane in ether gave the *dimethyl* ester as a crystalline material, soluble in methanol, ethanol, dioxan, or ether, recrystallizing from aqueous methanol in plates, m. p. 224—225°, $[\alpha]_D^{27}$ +80° ±4° (*c*, 0·084 in chloroform), and giving a yellow colour with tetranitromethane in chloroform [Found: C, 72·2; H, 9·5%; *M* (camphor), 518. $C_{32}H_{50}O_6$ requires C, 72·4; H, 9·5%; *M*, 530].

Castanogenin diacetate gave, as above, the *diacetate dimethyl* ester which crystallized from methanol in plates, m. p. 238·5—239°, $[\alpha]_D^{27}$ +90° ±3° (*c*, 0·127 in chloroform) (Found: C, 70·0; H, 9·1. $C_{36}H_{54}O_8$ requires C, 70·3; H, 8·9%).

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