## 449. *Caulosapogenin and its Identity with Hederagenin.*

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Caulosapogenin, the sapogenin obtainable by hydrolysis of an ethanolic extract from the roots and rhizomes of *Caulophyllum thalictroides* Linn. Michaux (Berberidaceae), and from the flowering branches of *Clematis vitalba*, Linn. (Ranunculaceae), is shown to have the formula $C_{30}H_{48}O_4$ and not $C_{42}H_{66}O_6$ as suggested by Power and Salway.[1] Caulosapogenin is identical with hederagenin.

Caulosapogenin was first obtained by Power and Salway [1] from the roots and rhizomes of *Caulophyllum thalictroides*, Linn. Michaux (Berberidaceae), by acid hydrolysis of an aqueous-ethanolic solution of the constituent saponin, caulosaponin, which previously had been known as leontin.[2] It was also isolated in the following year by Tutin and Clewer [3] in a somewhat similar manner from the flowering branches of *Clematis vitalba*, Linn. (Ranunculaceae), the well-known "Traveller's Joy" or "Old Man's Beard." Power and Salway assigned to caulosapogenin the molecular formula $C_{42}H_{66}O_6$, based upon elementary analysis and molecular-weight determination of the sapogenin and a number of its derivatives, of

[1] Power and Salway, *J.*, 1913, **103**, 191.
[2] Lloyd, *Proc. Amer. Pharm. Assoc.*, 1887, 151.
[3] Tutin and Clewer, *J.*, 1914, **105**, 1845.

which a di- and a tetra-acetate, a di- and a tetra-benzoate, and a methyl ether were described. Some doubt as to the correctness of this formulation was expressed by Tutin and Clewer [3] based on their analytical data for the so-called methyl ether and its benzoate. This is discussed more fully below.

Our interest in *C. thalictroides* arose through the identification of hederagenin as the sapogenin present in a closely related species, *Leontice leontopetalum*, Linn. (Berberidaceae).[4] Hederagenin has also been identified in the Clematis species *Clematis paniculata*.[5] Consideration of the analytical data given by Power and Salway for caulosapogenin suggested that it could in fact be formulated as a triterpenoid $C_{30}H_{48}O_4$ and the present investigation was undertaken to examine this hypothesis.

An authentic sample of Caulophyllum, B.P.C. 1934, consisting of the roots and rhizomes of *C. thalictroides*, was extracted with hot ethanol and the crude saponins were precipitated by ether. Recrystallisation of a small fraction gave caulosaponin as a white crystalline powder as described by Power and Salway. No attempt was made to fractionate the bulk of the crude saponins; instead, they were hydrolysed directly to a colourless crystalline sapogenin, $C_{30}H_{48}O_4$, identical with an authentic sample of hederagenin. The presence of two hydroxyl groups was established by conversion into a diacetate identical with hederagenin diacetate, and by formation of an *iso*propylidene derivative from the corresponding methyl ester. The sapogenin gave a yellow colour with tetranitromethane, and an ultraviolet absorption maximum at 210 mμ (ε 2860), as for hederagenin, confirmed the presence of a trisubstituted double bond. Presence of the carboxyl group was proved by the formation of a sodium salt and methyl ester, the latter identical with hederagenin methyl ester.

Power and Salway also reported the isolation of a second saponin, caulophyllosaponin, from *Caulophyllum thalictroides*, which on hydrolysis yielded a second sapogenin, caulophyllosapogenin, $C_{55}H_{88}O_9$. Careful examination of the mother-liquors remaining after the separation of hederagenin in the present experiments failed to reveal a second sapogenin.

Identification of caulosapogenin as hederagenin establishes the identity of most of the derivatives, which were described by Power and Salway, and by Tutin and Clewer. Caulosapogenin tetrabenzoate from its melting point and analysis must be regarded as almost certainly hederagenin dibenzoate. On the other hand, the so-called caulosapogenin tetraacetate, described as an amorphous solid of m. p. 120°, bears no relation to either the di- or the mono-acetate of hederagenin, whilst caulosapogenin diacetate, although described as having m. p. 160—162° and phenolic properties, is readily identifiable as hederagenin diacetate (m. p. 173—174°). The phenolic properties ascribed to this compound were based solely on the formation of a sodium salt, the analytical figures of which, like those of the parent diacetate, are in excellent agreement with the calculated figures for the corresponding hederagenin derivatives. The melting-point anomaly is explained by Jacobs's observation [6] that hederagenin diacetate sintered to a vitreous mass at 156—159°, but did not melt completely until 170—175°, as we have ourselves observed. Similarly the method of preparation, the description, and the accompanying analytical data for caulosapogenin methyl ether and its benzoate leave no doubt that these were in fact hederagenin methyl ester and its benzoate.

## Experimental

Rotations were determined in 95% EtOH (unless otherwise stated) in a 1 dm. tube. Ultraviolet absorption spectra were determined in absolute EtOH on a Hilger Uvispek photoelectric spectrophotometer. We are indebted to Mr. W. McCorkindale and Dr. A. C. Syme for the microanalyses, and to Mr. W. Gardiner for technical assistance.

*Material.*—This was authentic Caulophyllum (B.P.C. 1934) obtained through the usual commercial channels, and consisting of the dried rhizomes and roots of *Caulophyllum thalictroides* (Linn.) Mich. (Berberidaceae).

*Isolation of the Crude Saponins.*—The drug (350 g.) in No. 80 powder was exhausted by boiling absolute ethanol (2 l.). The ethanol extract was filtered, and the saponin precipitated from the

[4] Unpublished work.
[5] Ishiwatari, Nakano, and Shinkawa, *J. Pharm. Soc. Japan*, 1944, **64**, 34.
[6] Jacobs, *J. Biol. Chem.*, 1925, **63**, 621.

cold solution as a sticky mass by ether. The product, recrystallised from ethanol, gave the crude saponin (8·2 g.) as a light tan powder, m. p. 191—196° (decomp.). Treatment of the filtrate with ether as before yielded a further 3 g. of crude saponin, m. p. 190—198° (decomp.). Recrystallisation from absolute ethanol gave caulosaponin, m. p. 254—255°. Power and Salway gave m. p. 250—255° for caulosaponin.

*Hydrolysis of Crude Saponin.*—(*a*) Crude saponin (10 g.) was heated with ethanol (200 ml.) and dilute hydrochloric acid (60 ml.) under reflux for 5 hr. The gelatinous precipitate which first separated gradually crystallised, and was separated after cooling. Recrystallisation from absolute ethanol (charcoal) gave crystals, m. p. 332—333° (slight sintering at 319°) ($\varepsilon_{max.}$ 2860 at 210 mμ), $[\alpha]_D^{20}$ +78° (*c* 0·10), $[\alpha]_D^{20}$ +79° (*c* 0·10 in pyridine) {Jacobs [6] gives m. p. 332—334°, $[\alpha]_D$ +81° (*c* 2·009 in pyridine)}, for hederagenin. Mixed m. p. of sapogenin with authentic hederagenin, 332—333° (Found: C, 76·3; H, 10·3%; equiv., 474·9. Calc. for $C_{30}H_{48}O_4$: C, 76·2; H, 10·2%; equiv., 472·7). Power and Salway found for caulosapogenin, m. p. 315° (decomp.), C, 75·7 and H, 10·1%. Tutin and Clewer [3] found for caulosapogenin, m. p. 323°, C, 75·8 and H, 10·2%.

(*b*) Crude sapogenin (6·22 g.) from 578 g. of root yielded, after two recrystallisations, hederagenin (5·808 g., 93·4%), m. p. 333—333·5°.

*Derivatives.*—The sapogenin (0·5 g.), refluxed with acetic anhydride (5 ml.) for 1 hr., gave sapogenin diacetate (from 1 : 1 aqueous ethanol), m. p. 173—174° (sintering at 157—159°), $[\alpha]_D^{20}$ +64° (*c* 0·312) {Jacobs [6] gives for hederagenin diacetate, m. p. 172—174°, $[\alpha]_D$ +64° (*c* 1·0)}, mixed m. p. with hederagenin diacetate, 172—174° [Found: C, 73·6; H, 9·6%; equiv. (by hydrolysis), 553. Calc. for $C_{34}H_{52}O_6$: C, 73·3; H, 9·4%.: equiv., 556·7]. Power and Salway found for caulosapogenin diacetate, m. p. 160—162°, C, 73·4 and H, 9·4%; Tutin and Clewer found for the sodium salt, Na, 3·7% (Calc. for $C_{34}H_{51}O_6Na$: Na, 3·9%).

The sapogenin (1 g.), refluxed with benzoyl chloride in pyridine, gave sapogenin dibenzoate, m. p. 290—291°, $[\alpha]_D^{20}$ +114° (*c* 0·112 in $CHCl_3$) (Jacobs [6] gives for hederagenin dibenzoate, m. p. 290—291°), mixed m. p. with hederagenin dibenzoate, 290—291° (Found: C, 77·4; H, 8·4. Calc. for $C_{44}H_{56}O_6$: C, 77·6; H, 8·3%). Power and Salway found for caulosapogenin tetrabenzoate, m. p. 288°, $[\alpha]_D$ +111° (in $CHCl_3$), C, 77·3, and H, 7·9%). Tutin and Clewer found for caulosapogenin tetrabenzoate, m. p. 282°, C, 77·4, and H, 8·3%.

The sapogenin (1·005 g.) with excess of diazomethane gave the methyl ester, m. p. 236—237° (from aqueous ethanol, 1 : 1), $[\alpha]_D^{20}$ +76° (*c* 0·498 in $CHCl_3$) {Jacobs [6] gave for hederagenin methyl ester, m. p. 238—240°, $[\alpha]_D^{23}$ +76° (in $CHCl_3$)}, mixed m. p. with hederagenin methyl ester, 235—236·5° (Found: C, 75·9; H, 10·5. Calc. for $C_{31}H_{50}O_4$: C, 75·9; H, 10·6; OMe, 6·4%). Power and Salway found for caulosapogenin methyl "ether," m. p. 235°, $[\alpha]_D$ +74·4° (in $CHCl_3$), C, 75·2, H, 10·3, and OMe, 5·5%. Tutin and Clewer found (from *Caulophyllum thalictroides*), m. p. 229°, C, 76·5, H, 10·5, and OMe, 7·5%, and (from *Clematis vitalba*), m. p. 229°, $[\alpha]_D$ +73·3°, C, 76·5, H, 10·5, and OMe, 7·8%.

The methyl ester (287 mg.), refluxed with acetic anhydride (5 ml.), yielded the methyl ester diacetate as needles, m. p. 192—193°, $[\alpha]_D^{20}$ +63° (*c* 0·564) {Van der Haar [7] gives for hederagenin methyl ester diacetate, m. p. 193°, $[\alpha]_D$ +62° (in EtOH)}, mixed m. p. with hederagenin methyl ester diacetate, 192—193° (Found: C, 74·0; H, 9·7. Calc. for $C_{35}H_{54}O_6$: C, 73·6; H, 9·5%).

A solution of the methyl ester (300 mg.) in acetone (4 ml.) with 2 drops of concentrated hydrochloric acid slowly deposited platelets of the acetonyl sapogenin methyl ester, m. p. 250—251° (from absolute ethanol) alone or mixed with *iso*propylidenehederagenin methyl ester (Found: C, 77·5; H, 10·3. Calc. for $C_{34}H_{54}O_4$: C, 77·5; H, 10·3%).

Tutin and Clewer [3] found m. p. 187—188°, C, 77·8, H, 8·6, and Bz (hydrolysis) for caulosapogenin methyl "ether" benzoate, 31·1% (calc. for hederagenin methyl ester dibenzoate: C, 77·5; H, 8·4; Bz, 27·9%).

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[7] Van der Haar, *Ber.*, 1921, **54**, 3142.