STEROIDAL SAPOGENINS FROM LEAVES OF *CORDYLINE* SPECIES

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ABSTRACT.—The steroidal sapogenins obtained by hydrolysis of the saponins found in the leaves of eight species of *Cordyline* have been studied. With the exception of *C. terminalis* var. *petiolaris*, the extracts of all species yielded 1,3-dihydroxy sapogenins, which were usually the predominant compounds. *C. terminalis* var. *petiolaris* differed from all the other species examined, not only because of its lack of dihydroxy sapogenins, but also because it yielded the 5β-spirostanes, sarsasapogenin and smilagenin. The extracts of all seven other species contained only 5α-sapogenins. Compounds detected were 3-*epi*-tigogenin, 3-*epi*-neotigogenin, tigogenin, neotigogenin, diosgenin, yamogenin, cannigenin, cordylagenin, brisbagenin, ruscogenin and 25*S*-ruscogenin.

The isolation has been reported of smilagenin {(25*R*)-5β-spirostan-3β-ol} from *Cordyline australis* (1) and sarsasapogenin {(25*S*)-5β-spirostan-3β-ol} from *C. neocaledonica* (2). The steroidal sapogenins yielded by *C. cannifolia* leaves have been extensively studied; and cordylagenin {(25*S*)-5α-spirostane-1β,3α-diol}, its 25*R* epimer cannigenin, brisbagenin {(25*R*)-5α-spirostane-1β,3β-diol}, yamogenin {(25*S*)-spirost-5-en-3β-ol}, 3-*epi*-neotigogenin {(25*S*)-5α-spirostan-3α-ol}, and its 25*R* epimer, 3-*epi*-tigogenin have been isolated (3–6). Chromatographic evidence indicated also the presence of ruscogenin {(25*R*)-spirost-5-ene-1β,3β-diol} and diosgenin {(25*R*)-spirost-5-en-3β-ol} (5, 6). Cordylagenin has been isolated also from the leaves of *C. stricta* (3). Leaves of a further eight species of *Cordyline* (Agavaceae) have been studied and the steroidal sapogenins yielded by them are reported in this paper.

MATERIALS AND METHODS

PLANT MATERIALS.[1]—Leaves of *C. manners-suttoniae* F. Muell.[a], *C. rubra* Hueg ex Kunth.[b], *C. stricta* (Sims) Engl.[c] and *C. terminalis* (L.) Kunth. var. *petiolaris* Domin.[d] were collected in Queensland, Australia. Leaves of *C. australis* Hook. f., *C. banskii* Hook. f. and *C. indivisa* Steud., authenticated by Professor A. Mark, Botany Department, University of Otago, were obtained from plants growing in Dunedin, New Zealand. *C. pumilio* Hook. f., authenticated by Mr. A. E. Esler, Botany Division, D.S.I.R., was collected from the Waitaker Range, Auckland, New Zealand. Samples of *C. australis*, *C. indivisa* and *C. stricta* were obtained also from plants growing in the United Kingdom at Portsmouth, the Logan Botanic Garden, Port Logan, and the Cambridge Botanic Gardens, respectively.

EXTRACTION OF SAPOGENINS.—The dried, powdered leaves (50 g) were initially depigmented with chloroform for 2 hr before the sapogenins were extracted by the method of Blunden *et al.* (7). This involved soaking the plant material in water at room temperature for 24 hr and refluxing the suspension with 2N hydrochloric acid for 2 hr. The acid-insoluble material was separated by filtration and washed with water, ammonium hydroxide solution, and water until neutral. The dried, acid-insoluble residue was then extracted with chloroform for 6 hr.

[1]Herbarium Australiense voucher numbers: [a]HN. VKM 1313, [b]HN. VKM 928, [c]HN. WTJ 3837, and [d]HN. WTJ 3821.

DETECTION AND ISOLATION OF SAPOGENINS.—The crude sapogenin extracts were examined by two-dimensional tlc on air-dried silica gel G layers (wet thickness 250mμ); dichloromethane-methanol-formamide (93:6:1) was used in the first direction (System I) and two-fold development in cyclohexane-ethyl acetate-water (600:400:1) in the second (System II). The steroidal compounds were located by spraying with 50% sulfuric acid and heating at 100° until the characteristic colors developed (8).

Initial separation of the steroidal sapogenins was achieved by preparative tlc on air-dried silica gel G layers, wet thickness 500 mμ, using System I. The chromatograms were sprayed with distilled water to locate the sapogenin bands, which were dried, separately removed, and eluted with chloroform. Each extract, if necessary, was purified further by preparative tlc, usually with chloroform-ethanol (95:5) (System III). This system was used, in particular, for the separation of the 5α-spirostane-1β,3α-diols from the 5α-spirostane-1β,3β-diols, whereas three-fold development in System II was used to isolate the saturated- from the unsaturated-1β,3β-diols. When 5α-spirostan-3α-ols and 5α-spirostan-3β-ols were present together, they were separated by three-fold development in n-hexane-ethyl acetate (4:1) (System IV). The 25*R*- and 25*S*-epimers of the monohydroxy sapogenins were separated from each other by preparative tlc on silica gel G containing 2% silver nitrate. The layers, 500 mμ wet thickness, had been activated at 100° for 1 hr before use. The chromatograms were developed three times in dichloromethane-acetone (49:1) (System V). The system was used also for the purification of the major dihydroxy sapogenin fraction of *C. stricta* leaves, after acetylation.

The sapogenins isolated were recrystallized from either methanol or acetone. Melting points were determined with a Kofler block and are uncorrected. Ir spectra were obtained from either potassium bromide discs or from chloroform solutions on a Perkin Elmer 377 Grating Infra-Red Spectrophotometer. Nmr spectra were determined in $CDCl_3$ on a Bruker 270 MHz machine. The mass spectra were recorded on a VG-Micromass 7070 F spectrometer at an ionizing potential of 70 eV.

RESULTS AND DISCUSSION

The steroidal saponins present in the dry, powdered leaf samples of *Cordyline* species were hydrolyzed, and the sapogenins produced were extracted and compared by tlc with suitable reference compounds before isolation by preparative tlc. When sufficient material was available, each isolated compound was examined by tlc with Systems I to V, by determination of its mp and mmp with the appropriate sapogenin, and on the basis of its ir, nmr and mass spectra.

C. stricta

Tlc examination of the extract of *C. stricta* indicated the presence of eight monohydroxy steroidal sapogenins. The major constituent of this mixture, $C_{27}H_{44}O_3$ (M^+, m/e 416), mp 249–50°, undepressed on admixture with 3-*epi*-tigogenin, crystallized as needles from acetone. Its tlc and ir, mass and nmr spectral characteristics were identical to those of 3-*epi*-tigogenin. In the mass spectrum, the water loss from the ions at *m/e* 347, 302, 287 and 273 was consistent with a 5α-spirostan-3α-ol (9). In the nmr spectrum, resonances were observed at δ 0.76 (3H, s; C-18 Me), δ 0.78 (3H, s; C-19 Me), δ 0.78 (3H, d, $J \simeq 6$Hz; C-27 Me), δ 0.95 (3H, d, $J \simeq 6.5$Hz; C-21 Me), δ 3.36 (1H, t, $J_{26\beta,26\alpha} = J_{26\alpha,25\beta} \simeq 10.5$Hz; C-26$_\alpha$ H), δ 3.47 (1H, d, dd, $J_{26\alpha,26\beta} \simeq 10.5$Hz and $J_{26\beta,25\beta} \simeq 4$Hz and $J_{26\beta,24\beta} \simeq 1.5$Hz; C-26$_\beta$ H), δ 3.99 (1H, broad s, $W_{1/2} \simeq 7.5$Hz; C-3$_\beta$ H) and δ 4.37 (1H, q, $J \simeq 7.5$Hz; C-16 H). All the data obtained showed that the compound was 3-*epi*-tigogenin. This steroid has been reported previously only in the leaves of *C. cannifolia* (6). However, in that earlier study insufficient material was isolated to characterize the compound fully.

A second monohydroxy sapogenin, $C_{27}H_{44}O_3$ (M^+, *m/e* 416), mp 194–195°, undepressed on admixture with 3-*epi*-neotigogenin, crystallized as needles from methanol. The isolated compound had tlc and ir, nmr and mass spectral features identical to 3-*epi*-neotigogenin, including the extent of water elimination from the ions at *m/e* 347, 302, 287 and 273 (9). In the nmr spectrum resonances were recorded at δ 0.75 (3H, s; C-18 Me), δ 0.78 (3H, s; C-19 Me), δ 0.98 (3H, d, $J \simeq 6.5$Hz; C-21 Me), δ 1.07 (3H, d, $J \simeq 6.5$Hz; C-27 Me), δ 3.28 (1H, d, $J_{26\beta,26\alpha} \simeq$

11Hz and $J_{26\beta,25\alpha} \simeq 0$; C-26$_\beta$ H), δ 3.93 (1H, dd, $H_{26\beta,26\alpha} \simeq 11$Hz and $J_{26\alpha,25\alpha} \simeq$ 2.5Hz; C-26$_\alpha$ H), δ 4.01 (1H, broad s, $W_{1/2} \simeq 7.5$Hz; C-3$_\beta$ H) and δ 4.38 1H, q, $J \simeq 7.5$Hz; C-16 H). All the information obtained showed that the compound was 3-*epi*-neotigogenin. Like 3-*epi*-tigogenin, this sapogenin has been isolated previously only from the leaves of *C. cannifolia* (6). However, here a more detailed account of the nmr spectral characteristics is given.

Examination of the 270 MHz nmr spectra of 3-*epi*-tigogenin and 3-*epi*-neotigogenin clearly shows a number of important features which aid the interpretation of structure of steroidal sapogenins in general (fig. 1). As pointed out in previous studies (10–12), the stereochemistry at C-25 can be deduced by the pattern and chemical shifts of the secondary methyl groups and C-26 protons. In the 25*R*-series the equatorial C-27 methyl group exhibits signals at a higher field ($\simeq$0.30 ppm) than in the 25*S*-series. A similar, but less striking, diamagnetic shift ($\simeq$0.03 ppm) was observed in this study for the C-21 methyl group also.

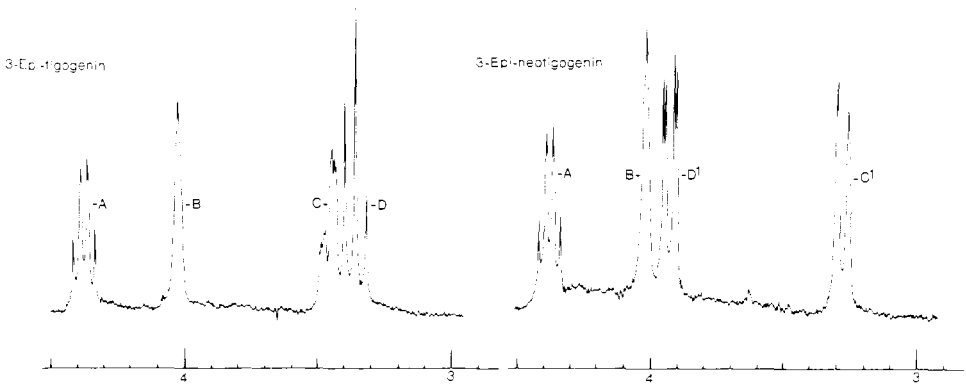

FIG. 1. Nmr spectra (270 MHz) of 3-*epi*-tigogenin and 3-*epi*-neotigogenin from δ 3.0 to δ 4.5 showing differences in the resonances of C-26 protons between 25*R*- and 25*S*-spirostanes. A = C-16 H (1H); B = C-3$_\beta$ H (1H); C = C-26$_\beta$ H (1H; "25*R*-"); C^1 = C-26$_\beta$ H (1H; "25 *S*-"); D = C-26$_\alpha$ H (1H; "25*R*-"); D^1 = C-26$_\alpha$ H (1H; "25*S*-").

The two protons at C-26, because of their attachment to a carbon bearing an oxygen atom, are deshielded enough for their signals to be well removed from the "methylene" envelope and easily recognized. As previously outlined (10, 12), the chemical shifts and splitting patterns of the C-26 protons are similar for sapogenins of the 25*R*-series, but quite distinct from those of members of the 25*S*-series. In the 25*R*-series a "triplet" ($\simeq \delta$ 3.36) and a double "double-doublet" ($\simeq \delta$ 3.47) pattern is seen (fig. 1). The former splitting pattern is assigned to the C-26$_\alpha$ proton, as expected (13, 14), and the latter to the C-26$_\beta$ proton. Long range coupling ($\simeq$1.5 Hz) is observed between the C-26$_\beta$ and the C-24$_\beta$ protons. However, in the case of an axially oriented C-27 methyl group (25*S*-series), the C-26$_\alpha$ proton suffers an interesting paramagnetic shift ($\simeq$0.57 ppm) and the resonance due to the C-26$_\alpha$ proton appears downfield from the C-26$_\beta$ signal. The splitting pattern is a "doublet of doublets" ($\simeq \delta$ 3.93) and a "doublet" ($\simeq \delta$ 3.28) for the C-26$_\alpha$ and C-26$_\beta$ protons respectively. The assignment of the downfield "doublet of doublets" to the C-26$_\alpha$ proton in the 25*S*-series is supported by Segre and Musher (15), who have observed similar "anomalous" chemical shifts in the nmr spectra of 1,3,5-trimethylcyclohexanes.

Of the other six monohydroxy steroidal sapogenins detected in the leaf extract of *C. stricta*, tlc evidence alone was obtained for the presence of tigogenin { (25*R*)-5α-spirostan-3β-ol}, neotigogenin { (25*S*)-5α-spirostan-3β-ol}, diosgenin and yamogenin. The other two compounds were not characterized, but one of them is probably the same as the unidentified compound A–2, recorded for the monohydroxy sapogenin fraction of *C. cannifolia* leaf extracts (6).

The principal sapogenin component of *C. stricta* failed to crystallize from aqueous methanol, but was obtained as a white, granular powder. This was purified by acetylation, followed by preparative tlc on silver nitrate impregnated layers of silica gel. In this way the material was separated into two components. The major one had tlc and mass and nmr spectral characteristics identical to the diacetates of both cordylagenin and cannigenin. The pattern and chemical shifts of the C–26 protons and the secondary methyl resonances in the nmr spectrum showed the presence of both 25*R*- and 25*S*-epimers. It was concluded, therefore, that the material examined was a mixture of both cordylagenin and cannigenin diacetates. Cordylagenin has been isolated previously from *C. stricta* leaves (3). The minor component that was separated from the cordylagenin and cannigenin diacetates by preparative tlc was obtained in trace amount and was not characterized.

In addition to cordylagenin and cannigenin, other dihydroxy sapogenins were detected, and tlc evidence was obtained for the presence of ruscogenin and brisbagenin.

On tlc examination of *C. stricta* leaf extract, at least six spots were detected with R_f values lower than those of the dihydroxy sapogenins. These spots were probably produced by trihydroxy sapogenins. Insufficient quantities of these compounds were isolated to characterize them. However, a large collection of *C. stricta* has been made and work is in progress on the elucidation of these compounds as well as of the unidentified, monohydroxy and dihydroxy sapogenins.

Very similar results were obtained for the extracts of *C. stricta* leaves collected in New Zealand and the United Kingdom.

C. indivisa

Only very small quantities of monohydroxy sapogenins were detected in the leaf extracts of *C. indivisa*. From tlc data alone these were identified as 3-*epi*-tigogenin and tigogenin.

The major sapogenin, $C_{27}H_{44}O_4$ (M^+, *m/e* 432), mp 198°, undepressed when mixed with brisbagenin, crystallized as plates from methanol. The compound had tlc and ir, nmr and mass spectral characteristics identical to brisbagenin. However, in the ir spectrum, the absorption at 899 cm^{-1} was of only slightly greater intensity than that at 921 cm^{-1}, thus indicating a possible mixture of 25*R*- and 25*S*-epimers (16). The nmr spectrum gave no indication of the presence of the 25*S*-epimer, so the isolated compound was concluded to be brisbagenin. The use of the comparative intensities of the absorptions at 899 and 921 cm^{-1} to assign the conformation of the C–27 methyl group in this compound is thus unreliable, as has been found previously for cordylagenin (3). On acetylation, the mp of the isolated sapogenin was not depressed when mixed with brisbagenin diacetate and the compound had tlc and ir and nmr spectral features identical to brisbagenin diacetate.

Four spots were detected with R_f values lower than brisbagenin when the leaf extract was examined by two-dimensional tlc. These spots were probably produced by trihydroxy sapogenins, but it was not possible to characterize them.

The same sapogenins were detected in the extracts of both the New Zealand and Scottish grown leaves.

C. pumilio

The predominant sapogenin in the extract of *C. pumilio* leaf was shown to be brisbagenin on the basis of mp, mass, ir and nmr spectral features, and of the tlc characteristics of both the isolated sapogenin and its diacetate. Tlc evidence was obtained also for the presence of tigogenin. Two other, probably monohydroxy, sapogenins were detected but could not be identified. Also four spots were detected on tlc examination with R_f values lower than that of brisbagenin. The components of the extract producing these spots had tlc characteristics identical to the four components with low R_f values present in the *C. indivisa* extract.

C. rubra

From tlc and ir spectral data, both 3-*epi*-tigogenin and tigogenin were identified in the extract of *C. rubra* leaves. Tlc results indicated also the presence of 3-*epi*-neotigogenin, diosgenin and yamogenin. Two other unidentified components were detected; both of them are probably monohydroxy sapogenins, but they separated from all the available reference compounds. One of the unidentified components showed the same tlc properties as compound A–2 isolated from *C. cannifolia* (6). All the monohydroxy sapogenins were present in small quantities.

The predominant sapogenin, $C_{27}H_{44}O_4$ (M^-, *m/e* 432), mp 219°, undepressed on admixture with cannigenin, crystallized as needles from methanol. The compound had tlc and ir, nmr and mass spectral features identical to cannigenin. The nmr spectrum gave no indication of the presence of the 25*S*-epimer, cordylagenin, and so the isolated compound was concluded to be cannigenin. This assignment was confirmed by conversion of the compound into its diacetate, which had tlc and spectral features identical to cannigenin diacetate.

Small quantities of compounds with tlc characteristics identical to brisbagenin and ruscogenin were detected as well as a number of components with low R_f values, which appeared similar to compounds detected in the leaf extracts of *C. stricta*, *C. indivisa* and *C. pumilio*.

C. australis

Extracts of the leaves of *C. australis* obtained from plants grown in New Zealand and in the United Kingdom gave low yields of steroidal sapogenins. The major compound was characterized from tlc and ir data as tigogenin, but only tlc results were obtained for the identification of neotigogenin, diosgenin, yamogenin and brisbagenin. The finding of these 5α-spirostanes in *C. australis* contrasts with the report of the 5β-spirostane, smilagenin, by Marker *et al.* (1).

C. manners-suttoniae

Only two sapogenins were isolated from the leaf extract of *C. manners-suttoniae*, but a number of other components with low R_f values were detected which had tlc characteristics very similar to the compounds with low R_f values detected in some of the other *Cordyline* leaves studied. The major sapogenin was proved to be brisbagenin from its mp, tlc and spectral characteristics. The second isolated component had tlc properties identical to those of cordylagenin and cannigenin, but it was obtained in insufficient quantity for characterization.

C. terminalis var. *petiolaris*

Only two sapogenins were detected on tlc examination of *C. terminalis* var. *petiolaris* leaf extract. The major compound, $C_{27}H_{44}O_3$ (M^+, *m/e* 416) gave

needle crystals from methanol, mp 194°; this value was unaltered on admixture with sarsasapogenin. The tlc characteristics and the ir, mass and nmr spectral details of the isolated compound were identical to those of sarsasapogenin. In the mass spectrum the water loss from the ions at *m/e* 347, 302, 287 and 273 was that expected from a 5β-spirostan-3β-ol (9). The data obtained showed that the compound was sarsasapogenin.

The minor compound, $C_{27}H_{44}O_3$ (M^+, *m/e* 416), crystallized from acetone as needles, mp 186°, undepressed when mixed with smilagenin. The tlc properties and the ir, nmr and mass spectral data were the same as those of smilagenin, and this identity was assigned to the compound.

C. banksii

The major sapogenin component of *C. banksii* leaf extract had tlc and ir spectral characteristics identical to ruscogenin but was not isolated in pure form. Resonances in the nmr spectrum, very similar to those described earlier under *C. stricta*, showed that the component was a mixture of 25*R*- and 25*S*-sapogenins; it was concluded that the isolated material was a mixture of ruscogenin and 25*S*-ruscogenin.

From tlc and ir spectral data yamogenin was also identified as a component of the extract, and from tlc results alone the presence of diosgenin and brisbagenin was indicated.

The sapogenins found in the leaves of the eight species of *Cordyline* studied are summarized in table 1.

The leaves of the species of *Cordyline* examined, with the exception of *C. terminalis* var. *petiolaris*, all yielded 1,3-dihydroxy sapogenins, these usually being the major compounds. This was the case also with *C. cannifolia* (3–5).

TABLE 1. Steroidal sapogenins detected in leaf extracts of *Cordyline* species.

Species	Steroidal sapogenins												
	3-*epi*-tigogenin	3-*epi*-neotigogenin	tigogenin	neotigogenin	diosgenin	yamogenin	smilagenin	sarsasapogenin	cannigenin	cordylagenin	brisbagenin	ruscogenin	25*S*-ruscogenin
Cordyline australis	−	−	+	±	±	±	−	−	−	−	±	−	−
C. banksii	−	−	−	−	±	+	−	−	−	−	±	+	+
C. indivisa	±	−	±	−	−	−	−	−	−	−	++	−	−
C. manners-suttoniae	−	−	−	−	−	−	−	−	±	±	++	−	−
C. pumilio	−	−	±	−	−	−	−	−	−	−	++	−	−
C. rubra	+	±	+	−	±	±	−	−	++	−	±	±	−
C. stricta	++	++	±	±	±	±	−	−	++	++	±	±	−
C. terminalis var. *petiolaris*	−	−	−	−	−	−	++	++	−	−	−	−	−

++ compound fully characterized
\+ compound incompletely characterized
± compound identified from tlc data only
− compound not detected

However, although *C. australis* extract contained the 1,3-dihydroxy compound, brisbagenin, it was not the major sapogenin. *C. terminalis* var. *petiolaris* differs from all the other species examined in this study, not only because no dihydroxy sapogenin was detected, but also because it yielded 5β-sapogenins; the extracts of all the other species studied contained only 5α-sapogenins. The 5β-compound sarsasapogenin has been reported also from *C. neocaledonica*, which was not included in this study.

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