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# AUSTRALIGENIN, A NEW STEROIDAL SAPOGENIN FROM *CORDYLINE AUSTRALIS* FRUITS

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ABSTRACT.—From the fruits of *Cordyline australis*, the new steroidal sapogenin australigenin [5α-spirost-25(27)-ene-1β,3β-diol] has been isolated and characterized from ir, pmr, and mass spectral data. Thirteen other steroidal sapogenins have been identified, including polygenin, 1β-hydroxycrabbogenin, and sceptrumgenin.

The steroidal sapogenins yielded by the leaves of many *Cordyline* species (Agavaceae) have been recorded (1-8), but no work has been published on these compounds from the fruits and seeds of species within the genus. In this paper, we report the steroidal sapogenins found in extracts of fruits of *Cordyline australis* Hook. f.; one of these compounds, australigenin, is a new dihydroxyspirostane.

## MATERIALS AND METHODS

EXTRACTION OF SAPOGENINS.—*C. australis* fruits were collected on the Island of Tresco, Scilly Isles, Cornwall, UK, in August 1980, and from Victoria Park, Portsmouth, UK, in September, 1981. The plant material was oven dried at 60° and powdered, and then the sapogenins were extracted by the method of Blunden, *et al.* (9). This involved soaking the powdered fruit (200 g) in $H_2O$ for 24 h at room temperature before refluxing the suspension with 2 N HCl for 2 h. The acid-insoluble material was removed by filtration and washed with $H_2O$, $NH_4OH$ solution, and again with $H_2O$ until neutral. The dried, acid-insoluble residue was extracted with $CHCl_3$ for 6 h.

DETECTION AND ISOLATION OF SAPOGENINS.—The crude sapogenin extract was examined by two-dimensional tlc on air-dried silica gel G layers (250 mμ) as described by Blunden *et al.* (6,7). $CH_2Cl_2$-MeOH-formamide (93:6:1) was used in the first direction (system I) and twofold development in cyclohexane-EtOAc-$H_2O$ (600:400:1) in the second (system II). The steroidal compounds were detected by spraying with 50% $H_2SO_4$ and heating at 100° until the characteristic colors developed (10).

The individual sapogenins were separated by preparative tlc. Using air-dried layers of silica gel G (500 mμ) and threefold development in *n*-hexane-EtOAc (4:1) (system III), the sapogenins were divided into two monohydroxy fractions, A and B, and one dihydroxy fraction. The chromatograms were sprayed with distilled $H_2O$ to locate the sapogenins as opaque bands. The bands were dried, each one removed separately, and the sapogenins eluted with $CHCl_3$.

Fraction B was subdivided further into six fractions (B1-B6) on layers of silica gel G impregnated with $AgNO_3$ using threefold development in $CH_2Cl_2$-$Me_2CO$ (49:1) (system IV). The layers (air dried) had been prepared by soaking them for 90 sec in a solution of $AgNO_3$ (13.5 g) in EtOH (390 ml) and acetonitrile (60 ml). After draining, the layers were activated at 100° for 30 min prior to use.

The dihydroxy sapogenins were separated into fractions C and D on air-dried layers of silica gel G (500 mμ) using twofold development in $CHCl_3$-EtOH (95:5) (system V). Fraction D was divided further into D1 and D2 by preparative tlc using threefold development in system II. The components of C and D2 were acetylated separately by dissolving them in pyridine and refluxing with $Ac_2O$ for 15 min. The acetylated sapogenins of C were fractionated into C1 and C2, and those of D2 into D2-1, D2-2, and D2-3 by preparative tlc using single development in system IV. The sapogenin acetates were refluxed with 5% methanolic KOH for 15 min to yield the respective sapogenins.

## RESULTS AND DISCUSSION

The saponins of *C. australis* fruits were hydrolyzed and the liberated sapogenins extracted. The extract, on examination by two-dimensional tlc, produced at least five spots. By preparative tlc, the extract was divided into two monohydroxysapogenin frac-

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tions, A and B, and one dihydroxysapogenin fraction. Tlc examination of fraction A using $AgNO_3$-impregnated layers and system IV showed the presence of four components, one of which had identical tlc characteristics to *epi*-tigogenin and one to *epi*-neotigogenin. The other two components, both of which produced a yellow color with the $H_2SO_4$-locating reagent, did not match any of the available reference compounds. All four substances were present in very small amounts, and further characterization was not possible.

Fraction B was subdivided into six components (B1-B6) by preparative tlc. B1-B3 produced yellow with $H_2SO_4$, while B4-B6 produced purple. From mp, mmp, ir, pmr, and tlc data, B1 proved to be tigogenin; B2, neotigogenin; B4, diosgenin, and B5, yamogenin. Neotigogenin and yamogenin were the major compounds; B3 was a very minor component and could not be identified. B6 was also a minor component; its pmr spectrum (270 MHz, $CDCl_3$) shows resonances at $\delta$[2] 0.79 (3H, s; C-18 Me), 0.94 (3H, d, $J \simeq 6.5$ Hz; C-21 Me), 1.00 (3H, s; C-19 Me), 3.44 (1H, m; C-3$_\alpha$H), 3.77 (1H, d, $J_{26\beta,26\alpha} \simeq 12.5$ Hz; C-26$_\alpha$H), 4.22 (1H, d, $J_{26\alpha,26\beta} \simeq 12.5$ Hz; C-26$_\beta$H), 4.31 (1H, q, $J \simeq 7.5$ Hz; C-16 H), 4.64 (1H, s, $J_{gem\ AB} \simeq 0$ Hz; C-27$_A$H), 4.68 (1H, s, $J_{gem\ BA} \simeq 0$ Hz; C-27$_B$H) and 5.24 (1H, d, $J \simeq 4.2$ Hz; C-6 H). The shape of the signals and the chemical shifts of the C-18, C-19, and C-21 methyl groups and of the C-16, C-6, and C-3$_\alpha$ protons are very similar for diosgenin, yamogenin, and B6. However, the spectrum of B6 lacks the doublet observed for the C-27 methyl groups of diosgenin and yamogenin, and the C-26 protons appear as an AB quartet. These properties show the presence of an exocyclic methylene group at C-25. The geminal protons (C-27$_{A\&B}$) of the C-25 methylene group are resolved as two singlets, the coupling constant of approximately 0 Hz being characteristic of an exocyclic methylene (8). From this information, B6 was concluded to be sceptrumgenin. The pmr results are consistent with those recorded for sceptrumgenin acetate (11). Unfortunately, the quantity of B6 isolated was insufficient to obtain further information.

By preparative tlc, the dihydroxysapogenins were divided into two fractions, C and D. The sapogenins of C had identical tlc characteristics to cordylagenin and cannigenin. After acetylation, the components of C were fractionated into C1 (major) and C2 (minor) by preparative tlc using $AgNO_3$-impregnated layers. C1 was identified as a mixture of cordylagenin and cannigenin diacetates from tlc and pmr spectral data (3,5). The pattern and chemical shifts of the C-26 protons and the secondary methyl resonances in the pmr spectrum showed the presence of both 25R- and 25S-epimers (7). C2 did not separate from 1$\beta$-hydroxycrabbogenin diacetate on tlc examination, and the two had identical pmr spectral features (8). Further verification of the identity of C2 as 1$\beta$-hydroxycrabbogenin was not possible because of the small quantity isolated. This is only the second record of this compound, which was isolated originally from the leaves of *Cordyline stricta* (8).

Fraction D was divided into D1 and D2 by preparative tlc. D1 was a very minor component. It had tlc characteristics identical to those of ruscogenin, gave the same brownish color with $H_2SO_4$, and produced the same color under uv light after spraying with $H_2SO_4$. Further study of D was not possible.

After acetylation, the sapogenins of D2 were fractionated into D2-1, D2-2, and D2-3 by preparative tlc using $AgNO_3$-impregnated layers. From mp, ir, pmr, and tlc data (4), D2-1 proved to be brisbagenin diacetate. After hydrolysis, the sapogenin had an identical mp, ir and pmr spectral features, and tlc characteristics to brisbagenin.

D2-2, $C_{31}H_{48}O_6$ ($M^+$, *m/z* 516.3441) was obtained as an oil. Its ir spectrum ($CHCl_3$) shows absorption at 1725 ($CH_3COO$), 986, 915, 894, and 862 $cm^{-1}$

[2]All chemical shifts are given in $\delta$-values.

(spiroketal moiety), with the absorption at 915 being of greater intensity than that at 894 $cm^{-1}$ (25S-spirostane). The pmr spectrum of D2-2 (270 MHz, $CDCl_3$) shows resonances at δ 0.74 (3H, s; C-18 Me), 0.95 (3H, d, $J \simeq 6.5$ Hz; C-21 Me), 0.97 (3H, s; C-19 Me), 1.06 (3H, d, $J \simeq 6.5$ Hz; C-27 Me), 1.96 (3H, s; $OCOCH_3$), 1.98 (3H, s; $OCOCH_3$), 3.23 (1H, d, $J_{26\beta,26\alpha} \simeq 11$ Hz and $J_{26\beta,25\alpha} \simeq 0$ Hz; C-$26_\beta$H), 3.87 (1H, dd, $J_{26\beta,26\alpha} \simeq 11$ Hz and $J_{26\alpha,25\alpha} \simeq 2.5$ Hz; C-$26_\alpha$H), 4.30 (1H, q, $J \simeq 7.5$ Hz; C-16 H), 4.53 (1H, dd, $J_{1\alpha,2\beta} \simeq 12$ Hz and $J_{1\alpha,2\alpha} \simeq 5$ Hz; C-$1_\alpha$H), and 4.63 (1H, m; C-$3_\alpha$H). The spectrum of D2-2 is very similar to that of brisbagenin diacetate. However, the doublet observed at δ 0.79 for the C-27 methyl group of brisbagenin diacetate is seen at δ 1.06 in the spectrum of D2-2. Also, the triplet and the double doublet of doublets at δ 3.31 and δ 3.42, respectively, produced by the C-$26_\alpha$ and C-$26_\beta$ protons of brisbagenin diacetate are seen as a doublet of doublets and a doublet, respectively, at δ 3.87 and δ 3.23. These differences in the two spectra indicate strongly that D2-2 is the 25*S*-epimer of brisbagenin diacetate (polygenin diacetate) (7).

Hydrolysis of D2-2 yielded the sapogenin, $C_{27}H_{44}O_4$ ($M^+$, *m/z* 432.3239; calculated 432.3239), mp 212° [lit. value (12) for polygenin 215°]. The base peak in the mass spectrum is at *m/z* 139, which is strong evidence for a spirostane without substitution in either the E or F rings (13). The ir spectrum ($CHCl_3$) shows absorption at 862, 896, 918, and 985 $cm^{-1}$ (spiroketal moeity), with the absorption at 918 more intense than that at 896 $cm^{-1}$ (25*S*-spirostane). The pmr spectrum (270 MHz, $CDCl_3$) shows signals at δ 0.75 (3H, s; C-18 Me), 0.84 (3H, s; C-19 Me), 0.97 (3H, d, $J \simeq 6.5$ Hz; C-21 Me), 1.06 (3H, d, $J \simeq 6.5$ Hz; C-27 Me), 3.24 (1H, d, $J \simeq 11$ Hz; C-$26_\beta$H), 3.41 (1H, dd, $J \simeq 12$ Hz and 5 Hz; C-$1_\alpha$H), 3.59 (1H, m; C-$3_\alpha$H), 3.89 (1H, dd, $J \simeq 11$ Hz and 2.5 Hz; C-$26_\alpha$H), and 4.32 (1H, q, $J \simeq 7.5$ Hz; C-16 H). All the information available proves the isolated sapogenin to be polygenin. This compound has been reported once previously, when it was isolated from *Solanum polyadenium* (12). Characterization was based mainly on cmr spectral results, and no pmr spectral data were reported.

Although we succeeded in separating brisbagenin and polygenin diacetates on $AgNO_3$-impregnated layers of silica gel, we were unable to separate the diacetates of cordylagenin and cannigenin with the same procedure.

D2-3 was obtained as an oil. Its ir spectrum ($CHCl_3$) is very similar to that of 1β-hydroxycrabbogenin diacetate (8). The characteristic bands of the spirostane ring are absent, but absorption at 918 $cm^{-1}$ indicates the presence of an exocyclic methylene group (14,15); strong absorption is observed at 1730 $cm^{-1}$ (carbonyl). The pmr spectrum of D2-3 is very similar to those of brisbagenin and polygenin diacetates. However, the doublet associated with the C-27 methyl group is missing. Also, the signals produced by the C-$26_\alpha$ and C-$26_\beta$ protons in the spectra of brisbagenin and polygenin diacetates are missing but are seen as an AB quartet at δ 3.79 (C-$26_\alpha$H) and δ 4.22 (C-$26_\beta$H). These features, also seen in the spectrum of sceptrumgenin, are indicative of an exocyclic methylene group at C-25. As in the case of sceptrumgenin, crabbogenin and 1β-hydroxycrabbogenin (8), the geminal protons (C-$27_{A\&B}$) of the C-25 methylene group are resolved as two singlets at δ 4.66 and δ 4.70. The signal for the C-$3_\alpha$ proton is hidden under the signals of the C-$27_{A\&B}$ protons.

Hydrolysis of D2-3 yields the sapogenin, $C_{27}H_{42}O_4$ ($M^+$, *m/z* 430). The base peak in the mass spectrum is at *m/z* 137, which is indicative of an exocyclic methylene group attached to C-25 of a spirostane (16,17) and shows the lack of substitution in both the E and F rings (13). The ir spectrum ($CHCl_3$) closely resembles that of D2-3 but lacks the carbonyl absorption at 1730 $cm^{-1}$. The pmr spectrum of the sapogenin is very similar to those of brisbagenin and polygenin, except for the differences recorded for the respective sapogenin diacetates for the C-$26_{\alpha\&\beta}$ and the C-$27_{A\&B}$ protons. Signals are ob-

served at δ 0.76 (3H, s; C-18 Me). 0.84 (3H, s; C-19 Me), 0.94 (3H, d, $J \simeq 6.5$ Hz; C-21 Me), 3.39 (1H, dd, $J \simeq 12$ Hz and 5 Hz; C-$1_{\alpha}$H), 3.57 (1H, m; C-$3_{\alpha}$H), 3.78 (1H, d, $J \simeq 12.5$ Hz; C-$26_{\alpha}$H), 4.21 (1H, d, $J \simeq 12.5$ Hz; C-$26_{\beta}$H), 4.34 (1H, q, $J \simeq 7.5$ Hz; C-16 H), 4.63 (1H, s; C-$27_{A}$H), and 4.68 (1H, s; C-$27_{B}$H). From all the data available, the sapogenin has been concluded to be 5α-spirost-25(27)-ene-1β,3β-diol, which, to our knowledge, is the first record of this compound; the trivial name australigenin has been given to the compound. The quantity isolated was insufficient to obtain accurate values for optical rotation.

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