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RUBRAGENIN, CHENOGENIN AND WALLOGENIN, STEROIDAL SAPOGENINS FROM *CORDYLINE RUBRA*

MING-HE YANG, GERALD BLUNDEN, ASMITA PATEL, TREVOR A. CRABB* and WILLIAM J. GRIFFIN†

School of Pharmacy & Biomedical Sciences; *Department of Chemistry, Portsmouth Polytechnic, King Henry I Street, Portsmouth, PO1 2DZ, U.K., †Department of Pharmacy, University of Queensland, St. Lucia, Queensland 4067, Australia

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Key Word Index—*Cordyline rubra*; Agavaceae; steroidal sapogenins; (22*S*,25*R*)-5α-furostan-22,25-epoxy-1β,3α,26-triol; (22*R*,25*R*)-5α-furostan-22,25-epoxy-1β,3α,26-triol; (22*R*,25*R*)-5α-spirostane-1β,3α,25-triol.

Abstract—Three new steroidal sapogenin triols have been isolated from the leaves of *Cordyline rubra*. NMR spectroscopic and mass spectrometric data prove the compounds to be (22*S*,25*R*)-5α-furostan-22,25-epoxy-1β,3α,26-triol (rubragenin), (22*R*,25*R*)-5α-furostan-22,25-epoxy-1β,3α,26-triol (wallogenin), and (22*R*,25*R*)-5α-spirostane-1β,3α,25-triol (chenogenin). Wallogenin is epimeric at C-22 to all the previously reported 'furanose' F-ring sapogenins.

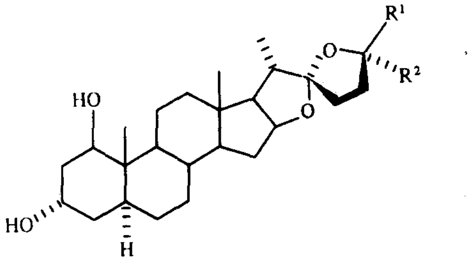
INTRODUCTION

In a previous study of the steroidal sapogenins produced after acid hydrolysis of the saponins extracted from the leaves of *Cordyline rubra*, (25*R*)-5α-spirostane-1β,3α-diol (cannigenin) was isolated and characterized. Evidence was obtained also for the presence of (25*R*)-5α-spirostan-3α-ol (3-*epi*-tigogenin) and its 25*S*-epimer (3-*epi*-neotigogenin), (25*R*)-5α-spirostan-3β-ol (tigogenin), (25*R*)-spirost-5-en-3β-ol (diosgenin) and its 25*S*-epimer (yamogenin), (25*R*)-5α-spirostane-1β,3β-diol (brisbagenin), and (25*R*)-spirost-5-ene-1β,3β-diol (ruscogenin) [1]. Other compounds believed to be sapogenins were detected by TLC, but insufficient material was available to enable these compounds to be identified. We now report the isolation and characterisation of some of these, which include three new steroidal sapogenins.

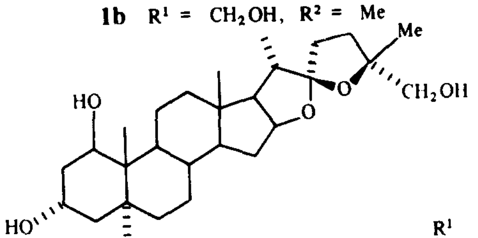
RESULTS AND DISCUSSION

The saponins of *Cordyline rubra* leaves were extracted, hydrolysed and the sapogenins separated and isolated by column chromatography and preparative TLC. Two dihydroxysapogenins and five trihydroxysapogenins were obtained and their structures elucidated from ^{1}H and ^{13}C NMR spectroscopic and mass spectral data. The two dihydroxy compounds were characterised as cannigenin and its 25*S*-epimer (cordylagenin). Two of the trihydroxysapogenins were shown to be (22*S*,25*S*)-5α-furostan-22,25-epoxy-1β,3α,26-triol (strictagenin; **1a**) and (22*R*,25*S*)-5α-spirostane-1β,3α,25-triol (pompeygenin; **3a**), both of which have been recorded previously for *C. stricta* [2]. The other three sapogenins are new compounds.

Rubragenin (**1b**), $C_{27}H_{44}O_5$ (M^+ at *m*/*z* 448.3258; calculated 448.3477), crystallizes from acetone as white needles, mp 248–250°. The base peak in the mass spectrum is observed at *m*/*z* 155, indicating a hydroxy substituent in ring F [3]. Additional F-ring fragments are detected at *m*/*z* 142 and 131. Major ions are observed at *m*/*z* 418 and 417, both of which have been reported for F-ring hydroxylated spirostanes [4, 5]. Other ions are observed at *m*/*z* 360, 318 and 289, showing the presence of two hydroxy groups in rings A to D [3]. The ^{13}C NMR spectrum of **1b** shows absorptions for C-22 and C-25 at δ120.1 and 84.1, respectively, diagnostic of C-26 hydroxylated F-ring 'furanose' sapogenins. The corresponding values for F-ring 'pyranose' sapogenins with C-25 hydroxylation are δ109 and 67 [6]. The close similarity of the ^{13}C shifts of **1b** and **1a** (Table 1), with the exception of



1a R^1 = Me, R^2 = CH_2OH
1b R^1 = CH_2OH, R^2 = Me



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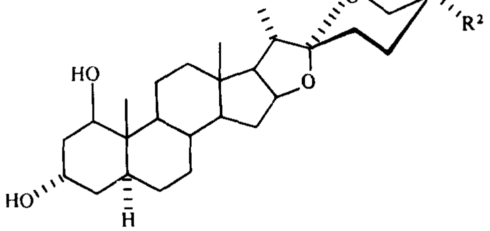

3a R^1 = OH, R^2 = Me
3b R^1 = Me, R^2 = OH

Table 1. ^{13}C NMR chemical shifts ($CDCl_3$) of F-ring hydroxylated steroidal sapogenins isolated from *Cordyline rubra*

						C							
Compound	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Strictagenin (**1a**)	73.9	40.4	66.2	36.1	37.4	28.3	32.0	35.6	55.0	42.6	24.4	40.4	40.3	56.3
Rubragenin (**1b**)	74.0	40.3	66.7	35.8	38.2	28.1	32.1	35.5	54.6	42.5	24.4	39.8	40.1	56.2
Wallogenin (**2**)	74.0	40.4	66.7	35.8	38.2	28.1	32.2	35.4	54.7	42.5	24.4	39.8	40.7	55.6
Pompeygenin (**3a**)	74.1	40.0	66.9	35.9	38.2	28.1	32.2	35.5	54.7	42.5	24.5	40.3	40.0	56.3
Chenogenin (**3b**)	74.0	40.2	66.4	36.0	38.3	28.2	32.2	35.6	54.8	42.5	24.4	40.4	40.0	56.4

						C							
Compound	15	16	17	18	19	20	21	22	23	24	25	26	27
Strictagenin (**1a**)	30.7	81.1	62.1	16.2	5.9	38.3	14.7	120.2	32.2	33.8	85.8	68.9	23.9
Rubragenin (**1b**)	31.9	80.6	62.0	16.3	5.6	38.5	14.8	120.1	32.8	34.1	84.1	69.5	25.3
Wallogenin (**2**)	29.7*	79.2	62.1	16.6	5.6	37.5	16.7	120.4	30.0*	32.8	84.7	68.0	24.0
Pompeygenin (**3a**)	31.9	81.3	62.0	16.4	5.6	41.6	14.5	109.1	27.0	32.7	66.7	69.0	24.7
Chenogenin (**3b**)	31.8	81.0	62.2	16.4	5.8	41.0	14.4	109.0	29.6	34.9	67.0	69.1	24.0

*Signals could be reversed.

C-15 ($\Delta\delta$1.2), C-25 ($\Delta\delta$1.7) and C-27 ($\Delta\delta$1.4), show identical A–E ring substitution in the two compounds. The C-15, C-25 and C-27 differences in shifts indicate an epimeric relationship at C-25, and this is confirmed by the downfield shift of the C-27 methyl protons in the ^{1}H NMR spectrum of **1b** (δ1.36 in **1b** and 1.14 in **1a**) due to the proximity of the ring E oxygen. These results show that **1b** is the 25*R*-epimer of strictagenin.

Wallogenin (**2**), $C_{27}H_{44}O_5$ ($[M]^+$ at *m/z* 448.3341; calculated 448.3477) was obtained in low yield. The base peak at *m/z* 417, along with major peaks at *m/z* 155 and 418, show that the compound is F-ring hydroxylated [4, 5]. The ^{13}C NMR spectrum of **2** (Table 1), with the exception of the absorptions of C-15, C-16, C-21, C-23, C-24 and C-26, is very similar to those of strictagenin and rubragenin, showing identical ring A–D substitutions in the three compounds. Resonances for the quaternary C-22 and C-25 are observed at δ120.4 and 84.7, respectively, showing that the sapogenin has a 'furanose' F-ring. Thus wallogenin must be a C-22 isomer of **1a** and **1b**. The ^{13}C NMR shift differences between **2** and **1a** and **1b**, noted above, are in accord with this 'rotation' about C-22. In the ^{1}H NMR spectrum of **2** the upfield shift of the C-16H (δ4.16) relative to that in the spectra of rubragenin and strictagenin (δ4.45) confirms the stereochemical inversion. The orientation at C-25 may be determined from a comparison of the C-27 Me shifts of **1b** (δ1.36; *cis*-Me to ring-E O) and **1c** (δ1.16; *trans*-Me to ring-E O). The observed value for wallogenin (δ1.14) confirms the structure **2**. To our knowledge this is the first record of a naturally occurring furospirostane which is epimeric at C-22 to all previously reported compounds of this type. We have named the compound in honour of Monroe E. Wall, who has made such a major contribution to steroidal sapogenin research.

Chenogenin (**3b**), $C_{27}H_{44}O_5$ (M^+ at *m/z* 448.3262; calculated 448.3477) was obtained as white, granular crystals from chloroform, mp 278–280°. The mass spectrum of the compound is very similar to that of pompeygenin (**3a**), both containing features of F-ring hydroxylated sapogenins. The ^{13}C NMR spectrum of **3b**, like that of **3a**, shows absorption for C-22 characteristic of a spirostane with a 'pyranose' F-ring, and, in each case, the quaternary carbon signals at δ66.7 in **3a** and 67.0 in **3b** indicate disubstitution at C-25. The close similarity of ^{13}C shifts, with the exception of C-23, C-24 and C-27, for **3a** and **3b** indicate structures that are epimeric at C-25. The relative orientations of the methyl group in ring-F is indicated by the ^{1}H NMR downfield shift of the C-27 methyl in **3b** (δ1.30) compared with **3a** (δ1.11). In addition, $J_{26a,26e}$ in **3a** is -12 Hz compared with -10 Hz in **3b**. This may be due to the intra-molecular H-bonding in **3a** (between hydroxyl and ring-F oxygen) withdrawing electrons away from the oxygen atom. This leads to a negative contribution to J_{gem} in **3a**. This observation provides evidence for the C-25 configuration in both **3a** and **3b**. Thus **3b** is the 25*R*-epimer of pompeygenin; this new compound has been named chenogenin in honour of Professor Chen Yan-yong, who has contributed so much to the steroid industry in China.

EXPERIMENTAL

Cordyline rubra leaves (voucher specimen HN. VKM 928) were collected from Mount Glorious, Queensland, Australia in 1980. The air-dried leaves (3 kg) were powdered and extracted by the method used by Griffin *et al.* [7]. This involved hot percolation with 85% aq. EtOH. The extract was concd to 3 l under red. pres., H_2O (1 l) was added and the mixture was shaken with 2 × 2 l of Et_2O. To the aq. extract combined with 2 × 1 l aq. washings of the mixed Et_2O extracts, an equal volume of EtOH was added and the mixture concd under reduced pressure to 1 l. NaCl (100 g) was added, the pH adjusted to 4.5 by adding 1 M H_2SO_4, and the mixture extracted 3 × with equal volumes of *n*-BuOH satd with H_2O. To the combined *n*-BuOH extracts was added an equal volume of H_2O and the mixture was concd to 800 ml. HCl was added to make the soln 4 M, the mixture was refluxed for 4 hr and filtered. The insoluble material, after drying, was dissolved in toluene (1 l) and refluxed with 20% methanolic KOH soln (250 ml) for 1 hr before filtration. The insoluble material was washed with hot toluene and the filtrates combined. The toluene soln, after washing with 1 l H_2O, was evapd to dryness to yield a mixture of crude sapogenins.

Table 2. ^{1}H NMR spectral features of F-ring hydroxylated steroidal sapogenins isolated from *Cordyline rubra* ($CDCl_3$)

	18-Me	19-Me	21-Me	27-Me	1_a-H	3_e-H	16-H	26-H	26-H′
Strictagenin (**1a**)	0.78 (*s*)	0.84 (*s*)	0.78 (*d*) $J=7$	1.16 (*s*)	3.82 (*dd*) $J=11, 5$	4.12 (*br s*)	4.45 (*q*) $J=7.5$	3.35 (*d*) $J=11$	3.50 (*d*) $J=11$
Rubragenin (**1b**)	0.77 (*s*)	0.83 (*s*)	0.99 (*d*) $J=7$	1.36 (*s*)	3.88 (*dd*) $J=11, 5$	4.10 (*br s*)	4.45 (*q*) $J=7.5$	3.35 (*d*) $J=11$	3.44 (*d*) $J=11$
Wallogenin (**2**)	0.88 (*s*)	0.82 (*s*)	1.00 (*d*) $J=7$	1.14 (*s*)	3.80 (*dd*) $J=11, 5$	4.10 (*br s*)	4.15 (*q*) $J=7.5$	3.35 (*d*) $J=11$	3.57 (*d*) $J=11$
Pompeygenin (**3a**)	0.77 (*s*)	0.83 (*s*)	1.04 (*d*) $J=7$	1.11 (*s*)	3.81 (*dd*) $J=11, 5$	4.09 (*br s*)	4.39 (*q*) $J=7.5$	3.25 (*dd*) $J_{26e,26a}=-12$ $J_{26e,24e}=2$	3.74 (*d*) $J_{26a,26e}=-12$
Chenogenin (**3b**)	0.76 (*s*)	0.83 (*s*)	0.97 (*d*) $J=7$	1.30 (*s*)	3.80 (*dd*) $J=11, 5$	4.09 (*br s*)	4.39 (*q*) $J=7.5$	3.23 (*dd*) $J_{26e,26a}=-10$ $J_{26e,24e}=2$	3.59 (*d*) $J_{26a,26e}=-10$

a = axial; e = equatorial.

Half of the crude sapogenin mixture was chromatographed on a column of silica gel (5 × 60 cm) using successively $CHCl_3$–MeOH (99:1 1 l), (98.5:1.5 1 l), (98:2 1 l), (97.5:2.5 2 l), (97:3 2 l), (96.5:3.5 2 l), (96:4 1 l), (95:5 1 l) and (92:8 2 l). The collected fractions were screened for sapogenins by TLC using air-dried layers of silica gel G, and *n*-hexane–EtOAc (4:1) and $CHCl_3$–MeOH (9:1) as development solvents. The compounds were located by spraying the layers with 50% aq. H_2SO_4 and heating at 100° until the characteristic colours had developed. The sapogenins present in the $CHCl_3$–MeOH (24:1) and (19:1) eluants were separated further by prep. TLC on silica gel G layers (500 μm) using 4-fold development in $CHCl_3$–MeOH (19:1). The separated compounds were detected by spraying the layers with H_2O. After drying, the sapogenin bands were dried, separately removed and the compounds eluted with $CHCl_3$. The sapogenins obtained were purified further by repeated prep. TLC and crystallization. In this way, cannigenin, cordylagenin, strictagenin (3.3 mg), pompeygenin (13.5 mg), rubragenin (4.7 mg; ν_{max}^{KBr} cm^{-1}: 3350 (hydroxyl), 995, 938, 923, 900, 892, 870 and 840), chenogenin (5 mg; ν_{max}^{KBr} cm^{-1}: 3420 (hydroxyl), 991, 974, 959, 941, 921, 895, 871 and 848) and wallogenin (approx. 1 mg; ν_{max}^{KBr} cm^{-1}: 3420 (hydroxyl), 990, 975, 960, 942, 920, 908, 890, 868 and 840) were obtained; cannigenin and cordylagenin were obtained as a mixture (100 mg).

^{1}H NMR (270 MHz) and ^{13}C NMR (68 MHz) spectra were obtained in $CDCl_3$ and EIMS (probe) were recorded at 70 eV.

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