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BARBOURGENIN, A NEW STEROIDAL SAPOGENIN FROM *AGAVE SISALANA* LEAVES

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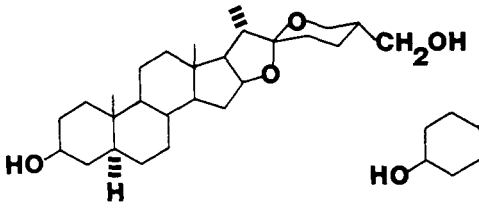
Hecogenin [(25*R*)-3β-hydroxy-5α-spirostan-12-one], an important precursor for the production of corticosteroids, is extracted from the sapogenin concentrate known as "coffee grounds," which is produced from the acid hydrolyzed juice of the leaves of *Agave sisalana* Perrine (Agavaceae). In addition to hecogenin, many different steroidal sapogenins have been isolated from either *A. sisalana* leaves or "coffee grounds" (1-3). From the latter we report the isolation of barbourgenin (**1**), which is a new dihydroxy spirostane.

"Coffee grounds," on extraction and separation by column chromatography, yielded a fraction that contained a relatively high proportion of dihydroxy sapogenins. In addition to the known compounds, rockogenin [(25*R*)-5α-spirostane-3β,12β-diol] and chlorogenin [(25*R*-5α-spirostane-3β,6α-diol], the novel compound barbourgenin (**1**) was isolated. This was shown to be a saturated dihydroxy spirostane from its mass spectrum (M^+, *m/z* 432.3246; calc. for $C_{27}H_{44}O_4$ 432.3239), which also located one of the hydroxy substituents in the F ring, as the base peak was observed at *m/z* 155 (4). The ^{1}H-nmr spectrum of **1** in pyridine-d_5 resembled that of tigogenin [(25*R*)-5α-spirostan-3β-ol] with resonances for the C-18, C-19, and C-21 methyl groups at δ 0.85 (s), δ 0.80 (s), and δ 1.14 (d), respectively. However, the C-27 methyl doublet observed in the spectrum of tigogenin at δ 0.68 was absent in that of **1** and was replaced by a two proton, eight line multiplet (AB part of ABX system, $J_{gem} = -10.7$, centered at δ 3.65), which is typical of -CH-CH_2OH protons. This, combined with the signals of the C-26 protons and the mass spectral data, showed that one of the hydroxy substituents is attached at C-27. The 25*S*-configuration (equatorial orientation of the CH_2OH group) of the molecule was indicated by the ^{1}H-nmr parameters of the C-26 protons (δ 3.86, t, $J_{26\,ax,\,26\,eq} = -11.0$ Hz, $J_{26\,ax,\,25\,ax} =$ 11.0 Hz; 26α-H and δ 4.12, dd, $J_{26\,eq,\,26\,ax} = -11.0$ Hz, $J_{26\,eq,\,25\,ax} = 3.8$ Hz; 26β-H) (5). These protons absorb downfield of those in the spectrum of tigogenin by 0.36 ppm and 0.53 ppm, respectively.

The close similarity between the chemical shifts of the C-18, C-19, and C-21 methyl protons of tigogenin and **1** strongly indicated that the latter, like the former, has a 3β- hydroxy substituent and a 5α configuration. Confirmation of the β-configuration of the 3-hydroxy substituent was obtained from the ^{1}H-nmr spectrum of barbourgenin diacetate in which a multiplet was observed at δ 4.66 (1H, 3α-*H*.OH). This characteristic 3α-proton multiplet was not observed clearly in the ^{1}H-nmr spectrum of **1** due to overlap of these signals with those of the C-26$_\alpha$ proton. Further evidence for the 3β-hydroxy group and the 5α-configuration of **1** came from the ^{1}H-nmr spectrum of isonarthogenin [(25*S*)-spirost-5-ene-3β,27-diol (**2**), which was isolated originally from *Metanarthecium luteo-viride* Maxim (Liliaceae) (6), but isolated by us from an impure sample of diosgenin. The C-21 methyl resonance was observed at δ 1.12 (d), but the C-18 and C-19 methyl sig-



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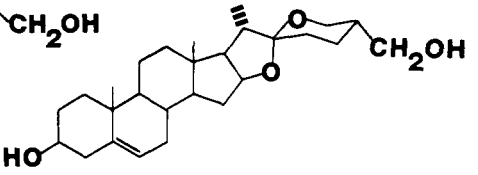

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nals were seen at δ 0.83 and δ 1.00, respectively. The differences between these chemical shifts and those of **1** were consistent with the latter having a 5α-configuration and **2** having a Δ^5 double bond (7). The spectrum of **2** in pyridine-d_5 exhibited a two proton, eight line multiplet at δ 3.65 similar to that observed in the spectrum of **1**, which again may be assigned to the -CH(25)H-CH_2OH protons. The base peak in the mass spectrum of **2** was seen at *m/z* 155, as expected (4). Catalytic hydrogenation of **2** over Adams' catalyst in MeOH gave **1** (^{1}H nmr).

From the data available, **1** was concluded to be (25*S*)-5α-spirostane-3β,27-diol. This compound, to our knowledge, has not been recorded previously, and we have named it in honor of Dr. John Barbour, who has contributed largely to the development of new production methods of "coffee grounds." The compound was present as a very minor component and was isolated in only small amount. As a result, no optical rotation values could be determined with any accuracy.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—^{1}H-nmr spectra were recorded in $CDCl_3$ and pyridine-d_5 on a Bruker WH-270 spectrometer and mass spectra were obtained using a VG Analytical ZAB-1F Mass spectrometer.

EXTRACTION AND ISOLATION.—Samples of "coffee grounds" produced on various plantations in Tanzania and Kenya, containing from 25-48% hecogenin and 1-5% tigogenin, were mixed together and 2×250 g samples were extracted with $CHCl_3$ in a Soxhlet apparatus. Each extract was concentrated, added to a column of silica (60-120 mesh; 250 g), and the sapogenins eluted with $CHCl_3$ (50 ml), $CHCl_3$-EtOH (97:3; 200 ml), $CHCl_3$-EtOH (95:5; 850 ml), $CHCl_3$-EtOH (90:10; 100 ml), and EtOH (100 ml). The $CHCl_3$-EtOH (90:10) and EtOH eluates were mixed, concentrated and the individual sapogenins isolated by preparative tlc using air-dried layers of silica gel G (500 mμ), $CHCl_3$-EtOH (95:5) as the developing solvent, and locating the sapogenins as opaque bands by spraying with distilled H_2O. The bands were dried, each one removed separately, and the compounds eluted with $CHCl_3$. Each isolated sapogenin, after recrystallization from MeOH, was examined by tlc along with suitable reference compounds, by ^{1}H-nmr, and ms.

Three sapogenins were isolated; two of these were rockogenin and chlorogenin (ms, ^{1}H nmr, and tlc). The third compound was barbourgenin (1.7 mg) (**1**), $C_{27}H_{44}O_4$ (M^+ *m/z* 432.3246; calculated 430.3239), mp 228-230°. In the ^{1}H-nmr spectra all coupling constants are in Hz and chemical shifts are in ppm from TMS, which was used as the internal standard; ($CDCl_3$) δ 0.74 (3H, s; C-18 Me), 0.81 (3H, s; C-19 Me), 0.96 (3H, d, $J_{21,20}$=6.5; C-21 Me), 3.46 (2H, m, J_{gem}=−10.7; C-27 H_2OH), 3.50 (1H, t, $J_{26\,ax,\,26\,eq}$=−11.0, $J_{26\,ax,\,25\,ax}$=11.0; C-26$_\alpha$H), =3.58 (1H, m; C-3$_\alpha$*H*.OH), 3.68 (1H, dd, $J_{26\,eq,\,26\,ax}$=−11.0, $J_{26\,eq,\,25\,ax}$=3.8; C-26$_\beta$H), 4.36 (1H, q, $J_{16,17}$=$J_{16,15\,ax}$=$J_{16,15\,eq}$=7.5; C-16 H); (pyridine-d_5) δ 0.78 (3H, s; C-19 Me), 0.85 (3H, s; C-18 Me), 1.14 (3H, d, $J_{21,20}$=6.5; C-21 Me), 3.65 (2H, m, J_{gem}=−10.7; C-27 H_2OH), =3.83 (1H, m; C-3$_\alpha$*H*.OH), 3.86 (1H, t, $J_{26\,ax,\,26\,eq}$=−11.0, $J_{26\,ax,\,25\,ax}$=11.0; C-26$_\alpha$H), 4.12 (1H, dd, $J_{26\,eq,\,26\,ax}$=−11.0, $J_{26\,eq,\,25\,ax}$=3.8; C-26$_\beta$H), 4.54 (1H, q, $J_{16,17}$=$J_{16,15\,ax}$=$J_{16,15\,eq}$=7.5; C-16 H). Eims *m/z* (rel. int. %) 432.3246 (M^+, 5, $C_{27}H_{44}O_4$), 347 (18, $C_{22}H_{35}O_3$), 302 (22, $C_{21}H_{33}O$), 287 (16, $C_{20}H_{30}O$; 302-CH_3), 273 (58, $C_{19}H_{29}O$), 255 (8, $C_{19}H_{27}$; 273-H_2O), 155 (100, $C_9H_{15}O_2$), 139 (48, $C_9H_{14}O$), 131 (21, $C_6H_{11}O_3$).

Barbourgenin was acetylated by dissolving in pyridine and refluxing with Ac_2O for 15 min.

An impure sample of diosgenin [(25*R*)-spirost-5-en-3β-ol], extracted from *Dioscorea floribunda* Mart. & Gal., was kindly supplied by Dr. R.K. Bammi. This material yielded, on preparative tlc as described above, diosgenin and isonarthogenin (**2**). The latter compound (M^+ *m/z* 430.3080;

calculated for $C_{27}H_{42}O_4$ 430.3083), produced resonances in the ^{1}H-nmr spectrum ($CDCl_3$) at δ 0.78 (3H, s; C-18 Me), 0.97 (3H, d, $J_{21,20}$=6.5; C-21 Me), 1.01 (3H, s; C-19 Me), 3.37-3.58 (3H, m; C-27 H_2OH and C-3$_\alpha$H.OH), 3.50 (1H, t, $J_{26\ ax,\ 26\ eq}$=−11.0, $J_{26\ ax,\ 25\ ax}$=11.0; C-26$_\alpha$H), 3.69 (1H, dd, $J_{26\ eq,\ 26\ ax}$=−11.0, $J_{26\ eq,\ 25\ ax}$=3.8; C-26βH), 4.40 (1H, q, $J_{16,17}$= $J_{16,15\ ax}$=$J_{16,15\ eq}$=7.5; C-16 H), 5.33 (1H, br d; C-6 H); (pyridine-d_5) 0.83 (3H, s; C-18 Me), 1.00 (3H, s; C-19 Me), 1.12 (3H, d, $J_{21,20}$=6.5; C-21 Me), 3.65 (2H, m, J_{gem}=−10.7; C-27 H_2OH), =3.80 (1H, m; C-3$_\alpha$H.OH), 3.85 (1H, t, $J_{26\ ax,\ 26\ eq}$=−11.0, $J_{26\ ax,\ 25\ ax}$=11.0; C-26$_\alpha$H), 4.11 (1H, dd, $J_{26\ eq,\ 26\ ax}$=−11.0, $J_{26\ eq,\ 25\ ax}$=3.8; C-26$_\alpha$H), 4.53 (1H, q, $J_{16,17}$= $J_{16,15\ ax}$=$J_{16,15\ eq}$=7.5; C-16 H), 5.34 (1H, br d; C-6 H).

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