22-Dihydrostigmasterol from *Saussurea lappa* Clarke

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The identity of a natural product, erroneously reported as a diterpene alcohol from *Saussurea lappa* Clarke, has been established as 22-dihydrostigmasterol.

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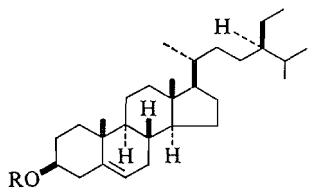
Paul and his co-workers (1) had reported the isolation of a diterpene alcohol, $C_{20}H_{34}O$, m.p. 135–136 °C, $[\alpha]_D$ — 40.55°, from hexane extract of the roots of *Saussurea lappa* Clarke. As a part of our projected program on diterpenoids,[2] we were interested in elucidating the structure of this diterpene alcohol. By essentially following the reported procedure, we isolated the same alcohol, m.p. 137.5–138 °C, $[\alpha]_D$ — 34.34°. The nuclear magnetic resonance (n.m.r.) spectrum of this alcohol described in sequel, at first sight, indicated that it was characteristic of a steroid rather than a diterpenoid skeleton. This led us to reexamine its elementary composition prior to elucidation of its structure. High resolution mass measurement on the parent alcohol as well as on its several derivatives (see Table I) clearly established its molecular formula as $C_{29}H_{50}O$.

Sterol **1** contains one double bond as it absorbs one mole of hydrogen during catalytic hydrogenation. It gives positive coloration with tetranitromethane and Liebermann–Burchard reaction. The trisubstituted nature of the olefinic linkage was revealed by a pair of bands at 797 and 834 cm^{-1} in its infrared (i.r.) spectrum, further supported by its ultraviolet (u.v.) spectrum (λ_{max} 200.4 mμ, ε 5930, ε_{210} 1986, ε_{215} 552, and ε_{220} 135) typical of Δ^5-steroids (2). The broad signal due to olefinic proton at τ 4.56 had disappeared in the n.m.r. spectrum of dihydro compound **4**. The i.r. spectrum of **1** exhibited absorption bands at 3440 and 1053 cm^{-1} due to the hydroxyl group, confirmed by an acetate **2** (i.r. spectrum: 1727, 1255, and 1039 cm^{-1}) and a benzoate **3** (i.r. spectrum: 1712, 1276, 1256, and 1112 cm^{-1}). The n.m.r. spectrum of **1** shows an 18-proton multiplet ranging from τ 9.02 to 9.30 which accounts for six methyl groups. The hydroxyl proton resonates as a sharp singlet at τ 8.46 which is removed by exchange with D_2O.

The mass spectrum of the parent alcohol **1** was very informative. The principal peak in the mass range 205–245 corresponded to *m/e* 213 ($\Sigma_{40} = 0.84\%$) which led to the formulation of $C_{10}H_{21}$ side chain at C-17 in D ring (3). This also eliminated the possibility of an unsaturation in the side chain. The presence of an intense peak at *m/e* 119 ($\Sigma_{40} = 0.98\%$) suggested it to be Δ^5-3β-sterol (4). In addition, fragments characteristic of stigmastane skeleton (5, 6) were observed at *m/e* 273 (M — side chain), 255 (M — side chain — H_2O), 231 [M — side chain — 42 (ring D fragment)], and 213 (231 — H_2O). These fragments shifted by two units in the mass spectrum of dihydro compound **4**. It is worth mentioning that the molecular ion peaks for acetate **2** and benzoate **3** constitute only 4.4% and 0.8% respectively of the base peak at *m/e* 396, even when using direct inlet system. All



1, R = —H

2, R = —C(=O)—CH_3

3, R = —C(=O)—C_6H_5

these chemical and spectroscopic properties can be accounted for if the parent alcohol has structure **1**, which then should be identical with 22-dihydrostigmasterol. This was confirmed by comparing its i.r., n.m.r., and mass spectra

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[2]R. J. Striha and T. C. Jain. Unpublished results.

TABLE I

Exact masses and composition of ions

	m/e		
	Observed	Calculated	Composition
Sterol **1**	414.3865	414.3862	$C_{29}H_{50}O$
Acetate **2**	456.3968	456.3967	$C_{31}H_{52}O_2$
Benzoate **3**	396.3756	396.3756	$C_{29}H_{48}$ ($M - C_6H_5COOH$)
Dihydro compound **4**	416.4018	416.4018	$C_{29}H_{52}O$

with the corresponding spectra of 22-dihydrostigmasterol and they were identical in all respects. Besides this, R_f values of **1**, **2**, **3**, and **4** in several thin-layer chromatographic systems (silica gel, silica gel – $AgNO_3$ and alumina) were comparable with those of corresponding authentic specimens.

Experimental

Melting points were determined on a Kofler hot-stage microscope and are uncorrected. Infrared (i.r.) spectra were recorded in KBr pellets on a Perkin–Elmer model 337 grating spectrophotometer calibrated with polystyrene film; ultraviolet (u.v.) spectrum was measured in cyclohexane on a Unicam model SP-700 spectrophotometer. Nuclear magnetic resonance (n.m.r.) spectra were obtained in $CDCl_3$ with Varian A-60 spectrometer using TMS as an internal standard. Unless otherwise stated, specific rotations were determined in chloroform on a Perkin–Elmer model 141 polarimeter. The mass spectra were obtained with an AEI-MS9 mass spectrometer, using direct insertion technique. The ionizing energy was maintained at 70 eV.

Sterol 1

This was isolated from the hexane extract of the roots of *Saussurea lappa* Clarke following essentially the procedure described in the literature (1); overall yield, 0.4% based on crude extract. Crystallization from ether–ethanol afforded sterol **1** as colorless plates, m.p. 137.5–138 °C, $[\alpha]_D^{31}$ – 34.34° (*c*, 1.03); dark-yellow coloration with tetranitromethane; Liebermann–Burchard reaction (7) positive; u.v. spectrum: λ_{max} 200.4 mμ, ε 5930, ε_{210} 1986, ε_{215} 552, and ε_{220} 135; i.r. spectrum: ν_{max} 3440, 1053 cm^{-1} (equatorial hydroxyl group (8)), 1665, 834, and 797 cm^{-1} (trisubstituted olefinic linkage); n.m.r. spectrum: τ 9.02–9.30 (multiplet, 18H, 6x—C*H*$_3$), τ 8.46 (sharp singlet, —O*H*, disappeared after exchange with D_2O), τ 4.56 (broad multiplet, 1H, $>C=\underset{|}{C}-H$); mass spectrum[3]: *m/e* (%Σ_{40})[4]: 43 (4.79, base peak), 119 (0.98), 231 (0.21, M – side chain – 42 [ring *D* fragment]), 213 (0.84, 231 – H_2O), 255 (1.26, M – side chain – H_2O), 273 (0.33, M – side chain), 381 (0.84, M – CH_3 – H_2O), 396 (4.30, M – H_2O), 399 (0.42, M – CH_3), and 414 (1.90, M^+).

Anal. Calcd.[5] for $C_{29}H_{50}O \cdot 1/2H_2O$: C, 82.24; H, 12.13. Found: C, 81.99; H, 12.06.

Acetate 2

A solution of compound **1** (20.5 mg) dissolved in anhydrous pyridine (5 ml) was treated with acetic anhydride (0.5 ml) and was allowed to stand overnight at room temperature. The mixture was then decomposed with water and the solvent was removed *in vacuo* and dried over Na_2SO_4. The crystalline material, obtained after removal of solvent, was chromatographed on neutral alumina (500 mg). Elution with petroleum ether gave acetate **2**, crystallized from ether–ethanol as white rectangular plates, m.p. 127–129 °C, $[\alpha]_D^{30}$ –39.90° (*c*, 1.06); i.r. spectrum: ν_{max} 1727 cm^{-1} (aliphatic ester carbonyl), 1255, and 1039 cm^{-1} (C—O—C stretching of ester). The i.r. spectrum was superimposable with that of authentic specimen. Mass spectrum: *m/e* (%Σ_{40}): 255 (1.95, M – CH_3COOH – side chain), 381 (1.04, M – CH_3COOH – CH_3), 396 (7.58, base peak, M – CH_3COOH), 456 (0.30, M^+).

Benzoate 3

Sterol **1** (20 mg), dissolved in dry pyridine (5 ml), was treated with freshly distilled benzoyl chloride (0.25 ml) and the mixture was stirred magnetically overnight. It was worked up in the same manner as described above for acetate. Crystallization of the product from ethanol–ether furnished shining flakes of benzoate, m.p. 144–146 °C; i.r. spectrum: ν_{max} 1712 cm^{-1} (aromatic ester carbonyl), 1276, 1256, and 1112 cm^{-1} (C—O—C stretching of ester). It was superimposable with the i.r. spectrum of authentic specimen. Mass spectrum: *m/e* (%Σ_{186}): 255 (4.49, M – C_6H_5COOH – side chain), 381 (2.78, M – C_6H_5COOH – CH_3), 396 (23.90, base peak, M – C_6H_5COOH), and 518 (0.19, M^+).

Dihydro Compound 4

Sterol **1** (50 mg) was added to a prereduced PtO_2 catalyst (25 mg) in glacial acetic acid (100 ml). The solution was stirred magnetically at room temperature whereupon it absorbed one mole equivalent of hydrogen

[3]For the sake of brevity, peaks relevant to structural assignment are described. Complete mass spectra will be presented in the M.Sc. Thesis of C. M. Banks, University of Victoria.

[4]The symbol Σ_{40} denotes the percentage of total ionization over the range *m/e* 40 to M^+.

[5]Crystals with $\frac{1}{2}H_2O$ have been observed by various investigators (9, 10).

in 12 h. The reaction mixture was worked up in the customary manner to give a solid (46 mg). This was chromatographed on neutral alumina (2.0 g). Elution with petroleum ether gave a trace of oily material and further elution with petroleum ether – benzene (1:1) afforded dihydro compound **4**, which crystallized from ether–ethanol as plates, m.p. 139–140 °C, $[\alpha]_D^{31}$ +20.70° (*c*, 0.57); no coloration with tetranitromethane; i.r. spectrum: ν_{max} 3406, 1043 cm^{-1} (equatorial hydroxyl), no band in 790–840 cm^{-1} region. It was identical with the infrared spectrum of authentic sample. The n.m.r. spectrum had τ 9.03–9.33 (multiplet, 18H, 6x—C*H*$_3$), τ 8.52 (sharp singlet, —O*H*), and no signal in olefinic region. Mass spectrum (%Σ_{40}): 233 (3.69, M − side chain − 42 (ring D fragment)), 215 (3.33, 233 − H_2O), 257 (0.32, M − side chain − H_2O), 275 (0.12, M − side chain), 383 (0.58, M − CH_3 − H_2O), 398 (0.18, M − H_2O), 401 (1.42, M − CH_3), and 416 (5.08, M^+, base peak).

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Added in proof—Because of the unavailability of the original sample described in reference (1), a direct comparison with our sample was not possible. We thank Professor Bhattacharyya for this information.

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