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ISOLATION OF A NATURAL STEROL AND AN ALIPHATIC ACID FROM *VERNONIA CINEREA*

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Key Word Index—*Vernonia cinerea*; Compositae; sterols; stigmast-5,17(20)-dien-3β-ol; stigmasterol; sitosterol; aliphatic acid; 26-methylheptacosanoic acid; isomontanic acid.

Abstract—From the roots of *Vernonia cinerea* a new natural sterol and a new aliphatic acid characterized as stigmast-5,17(20)-dien-3β-ol and 26-methylheptacosanoic acid, respectively, have been isolated together with stigmasterol and sitosterol.

INTRODUCTION

In continuation of our earlier work [1] we have isolated four more compounds from the roots of *Vernonia cinerea*. This paper deals with the isolation and structure elucidation of these compounds, two of which are new natural products.

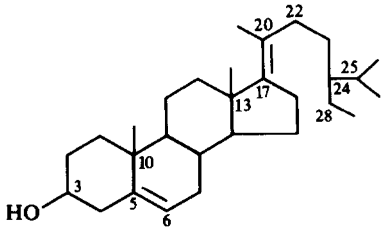
RESULTS AND DISCUSSION

A petrol extract (260 g) of air-dried and powdered roots was chromatographed over silica gel and the column was eluted successively with solvents of increasing polarity. Two compounds were isolated from the benzene eluate. The first component (**1**) afforded white fibrous crystals from hexane–benzene (3:1), mp 100–102° (lit. [2] 98–100°), $[\alpha]_D^{22}$ −49.3° ($CHCl_3$). It gave a violet colour with the Liebermann–Burchard reagent [3, 4], a yellow colour with tetranitromethane [5] and no colour with Noller's reagent [6], which indicated it to be an unsaturated steroid. In the mass spectrum the $[M]^+$ peak appeared at *m/z* 412. The MW and elemental analyses led to the molecular formula $C_{29}H_{48}O$. It showed IR absorptions for a hydroxyl (3370 cm^{-1}), unsaturation (1640 cm^{-1}) and isopropyl (1380, 1370 and 1140 cm^{-1}) functions in the molecule. The C–OH stretching band at 1040 cm^{-1} suggested the presence of an equatorial hydroxyl group located at the C-3 position of an A/B *trans*-steroid [7]. The appearance of a band at 840 cm^{-1} demonstrated that it contained a trisubstituted double bond [8, 9].

The ^1H NMR spectrum of the compound revealed that it was a steroid [10] and it displayed signals at δ 0.64 (3H, *s*, H-18) and 0.75 (3H, *s*, H-19) for the two tertiary methyl groups. A doublet for six protons at δ 0.87 (6H, *d*, $J = 6.5$ Hz) was assigned to an isopropyl group situated in the side chain [10]. A triplet centred at δ 0.95 (3H, *t*, $J = 6$ Hz) was attributed to a primary methyl group attached to C-28. A singlet at δ 1.82 (3H, *s*) for the C-21 protons showed the presence of a double bond either between C-17 and C-20 or C-20 and C-22. Moreover, the spectrum demonstrated signals at δ 3.40 (1H, *m*), 4.98 (1H, *m*) and 5.20 (1H, *m*) corresponding to a carbinolic proton, hydroxyl proton and an olefinic proton, respectively. In view of the above data, it was concluded that this compound was a sterol having a hydroxyl group at C-3, a trisubstituted double bond and another double bond located in the side chain.

The mass spectrum of the compound displayed a molecular ion peak at *m/z* 412 and other prominent peaks at *m/z* 397 $[M - Me]^+$, 394 $[M - H_2O]^+$, 379 $[M - Me - H_2O]^+$, 299 (base peak) and 271. The peak at *m/z* 271 was due to the ion formed by the loss of the side chain from the molecular ion together with one hydrogen atom from the charge-retaining portion. Formation of this fragment (*m/z* 271) is a characteristic feature in the mass spectral fragmentation of those sterols (Δ^5-3β-ol moiety) which have a double bond in the side chain [11]. Assignment of the Δ^5-double bond was confirmed by the absence of a characteristic peak at *m/z* 289 which is formed with 5α-sterols by fragmentation of ring B involving migration of the 5α-hydrogen [12]. The absence of a characteristic peak at *m/z* 314 resulting from McLafferty rearrangement and also of fragments at *m/z* 246, 232, 231, 180, 166, 95 and 81 produced by the cleavage of ring D ruled out the location of a double bond between C-20 and C-22 [2, 11–13]. The ions at *m/z* 397 and 394 resulted from the loss of one methyl group and a water molecule, respectively, from the molecular ion peak. In view of these observations and assignments, the compound was identified as stigmast-5,17(20)-dien-3β-ol (**1**).

Acetylation of the compound with acetic anhydride and pyridine at room temperature gave an acetate, mp 108–109° (lit. [2] 109–110°). Balakrishnan *et al.* [2] and



1

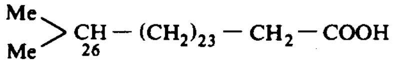
Ayanoglu *et al.* [14] obtained stigmast-5,17(20)-dien-3β-ol from the double-bond isomerization of stigmasterol with the help of *N*-lithioethylenediamine. The mp and spectral data of the isolated compound closely resembled those obtained by the above workers for the synthetic compound. Hence the identity of the newly isolated natural product as stigmast-5,17(20)-dien-3β-ol was finally established. A direct comparison with the authentic sample could not be made due to its non-availability from the sources quoted above.

The second compound of the benzene eluate was obtained as white needles after recrystallization from acetone, mp 170°, $[\alpha]_D^{22} - 51.0°$ ($CHCl_3$). It responded positively to tests [3–5] for an unsaturated steroid. The IR spectrum of the compound showed the presence of a hydroxyl group and double bond at 3360 and 1640 cm^{-1}, respectively. The absorption bands at 950 and 840 cm^{-1} indicated the presence of a *trans*-disubstituted double bond at C-22 [15] and a trisubstituted double bond [8, 9], respectively. Location of a Δ^{22}-double bond was also confirmed by the ^1H NMR spectrum. The compound was identified as stigmasterol by mmp and co-TLC examinations with an authentic sample. Its identification was confirmed by preparing the acetate derivative, mp 143–144° (lit. [16] 144–145°), $[\alpha]_D^{20} - 55.5°$ ($CHCl_3$).

The benzene–ethyl acetate (9 : 1) eluate gave a crude solid which afforded white needles from acetone, mp 136–138°, $[\alpha]_D^{22} - 34°$ ($CHCl_3$). It responded positively to colour tests [3–5] for an unsaturated steroid. The IR spectrum demonstrated the presence of hydroxyl (3240 cm^{-1}) and unsaturation (1640 cm^{-1}) functions in the molecule. It was identified as sitosterol by mmp and co-TLC examinations with an authentic sample and by the preparation of the acetate derivative, mp 130–131° (lit. [17] 134°).

The benzene–ethyl acetate (3 : 1 and 1 : 1) eluates yielded a brownish solid which on repeated crystallization from acetone afforded yellowish white crystals, mp 87–88° (lit. [18] 89.3°). Elemental analyses and MW (424) determination by mass spectrometry gave the molecular formula $C_{28}H_{56}O_2$. The IR spectrum revealed the presence of carboxyl (3480 and 1710 cm^{-1}), *gem*-dimethyl (1380, 1370 and 1140 cm^{-1}) and $-(CH_2)_n-$ (730 and 720 cm^{-1}).

In the ^1H NMR spectrum a six-proton doublet at δ 0.85 (6H, *d*, $J = 6.0$ Hz) showed the presence of an isopropyl group in a saturated hydrocarbon environment [19]. This was further confirmed by the appearance of a signal at δ 1.50 (1H, *m*, $>CH-$). A signal at δ 1.18 (46H, *s*, $-(CH_2)_{23}-$) indicated the presence of a straight chain of 23 carbon atoms. The spectrum displayed a signal at δ 2.20 (2H, *t*, $-CH_2-COOH$) for methylene protons attached to a carboxyl group. On the basis of the IR and NMR spectra it was inferred that this compound was an aliphatic acid having an isopropyl group at one end, a straight chain of a $-(CH_2)_{23}-$ moiety and a carboxyl group attached to a methylene unit at the other end. The isolated aliphatic acid was therefore identified as 26-methylheptacosanoic acid (**2**).



$$\overset{Me}{\underset{Me}{}}\!>\underset{26}{CH}-(CH_2)_{23}-CH_2-COOH$$

2

Mass spectral studies offered further support to the above assignment. The molecular ion peak at *m/z* 424 gave the molecular formula $C_{28}H_{56}O_2$. The base peak appeared at *m/z* 43 and was due to the formation of an isopropyl carbonium ion [20] from the molecular ion. The loss of an isopropyl radical from the parent ion gave an ion which appeared at *m/z* 381 and this ion underwent successive loss of $-CH_2-$ units to give ions appearing at odd mass numbers.

Weitkamp [18] isolated a sterol ester from the petrol extract of Degras (wool fat, wool wax) and saponified it to yield sterol and 26-methylheptacosanoic acid (isomontanic acid). This acid was later prepared [21] by anodic synthesis. The mp of the acid isolated by us closely resembled that of the synthesized product and hence the compound was identified as 26-methylheptacosanoic acid. Its direct comparison could not be done due to non-availability of the authentic sample. This is the first report of the isolation of 26-methylheptacosanoic acid in the free state from a natural source.

EXPERIMENTAL

All the reported mps are uncorr. ^1H NMR spectra were recorded at 90 MHz in $CDCl_3$ with TMS as int. standard. Silica gel G was used for TLC and spots were detected by (a) viewing under UV light, (b) I_2 vapour, or (c) heating the plates after spraying with 10% H_2SO_4.

Plant material. Plants of *V. cinerea* Less were collected locally in December 1981. The roots were separated, washed, air-dried and ground to a coarse powder.

Extraction and isolation of compounds. Air-dried and powdered roots (10 kg) were exhaustively extracted with petrol and the solvent was removed under red. pres. to give a dark brown residue (450 g). The extract (260 g) was chromatographed over silica gel (2.5 kg) and the column was eluted with solvents of increasing polarity. Elution was monitored by intermittent co-TLC examinations of 200 ml fractions. Chromatographically identical fractions were mixed and the solvent was removed under red. pres.

*Stigmast-*5,17(20)*-dien-*3β*-ol*(**1**). The C_6H_6 eluate gave a crude solid which on crystallization from hexane–C_6H_6 (3 : 1) yielded colourless, fibrous needles (150 mg), mp 100–102°, $[\alpha]_D^{22} - 49.3°$ ($CHCl_3$); IR ν_{max}^{KBr} cm^{-1}: 3370, 2960, 2940, 2900, 2880, 1640, 1460, 1380, 1370, 1330, 1240, 1230, 1200, 1140, 1100, 1090, 1060, 1050, 1040, 840, 800, 780, 740; ^1H NMR ($CDCl_3$): δ 0.64 (3H, *s*), 0.75 (3H, *s*), 0.87 (6H, *d*, $J = 6.5$ Hz), 0.95 (3H, *t*, $J = 6$ Hz), 1.82 (3H, *s*, H-21), 3.40 (1H, *m*, H-3α), 4.98 (1H, *m*, HO–), 5.20 (1H, *m*, H-6); MS *m/z* (rel. int.): 412 $[M]^+$ (20), 397 (4.0), 394 (20.0), 379 (4.5), 300 (10.0), 299 (100), 281 (22.0), 271 (17.5), 230 (7.5), 175 (7.5), 159 (36.2).

*Stigmast-*5,17(20)*-dien-*3β*-yl acetate.* A mixture of **1** (50 mg), Ac_2O and pyridine (2 ml each) was allowed to stand overnight at room temp. The mixture on usual work-up afforded the acetate as white needles (30 mg) from EtOH, mp 108–109°; IR ν_{max}^{KBr} cm^{-1}: 1724, 1380, 1370, 1325, 1260, 1240, 1125, 1030, 840.

Stigmasterol. The C_6H_6 eluate gave a compound which crystallized from Me_2CO into colourless crystals (200 mg), mp 170°, $[\alpha]_D^{22} - 51.0°$ ($CHCl_3$); IR ν_{max}^{KBr} cm^{-1}: 3350, 2960, 2940, 2880, 1640, 1460, 1380, 1370, 1330, 1260, 1240, 1100, 1050, 1040, 950, 840, 790; ^1H NMR ($CDCl_3$): δ 0.64 (3H, *s*), 0.70 (3H, *s*), 0.79 (3H, *d*, $J = 6$ Hz, H-21), 0.95 (6H, *d*, $J = 6$ Hz, H-26 and H-27), 0.95 (3H, *t*, $J = 6$ Hz, H-29), 3.30 (1H, *m*, H-3α), 4.95 (1H, *m*, HO–), 4.88 (2H, *m*, H-22 and H-23), 5.20 (1H, *m*, H-6).

Stigmasteryl acetate. The acetate was prepared in the usual way, mp 143–144°, $[\alpha]_D^{22} - 55.5°$ ($CHCl_3$); IR ν_{max}^{KBr} cm^{-1}: 1724, 1380, 1370, 1330, 1240, 1100, 1030, 950, 840, 790.

Sitosterol. The C_6H_6–EtOAc (9 : 1) fraction gave a solid which on repeated crystallization from Me_2CO afforded white needles (200 mg), mp 136–138°, $[\alpha]_D^{22} -34°$ ($CHCl_3$); IR ν_{max}^{KBr} cm^{-1}: 3240, 2910, 2825, 1640, 1450, 1380, 1370, 1360, 1060, 1040, 850, 810.

Sitosteryl acetate. Sitosterol (50 mg) gave an acetate, mp 130–131°; IR ν_{max}^{KBr} cm^{-1}: 2990, 2910, 2790, 1730, 1460, 1420, 1380, 1370, 1060, 1030, 850, 810.

26-Methylheptacosanoic acid. The C_6H_6–EtOAc (3 : 1 and 1 : 1) eluate yielded a brownish solid which on repeated crystallization from Me_2CO afforded yellowish white crystals (150 mg), mp 87–88°; IR ν_{max}^{KBr} cm^{-1}: 3480, 2920, 2860, 1710, 1470, 1460, 1380, 1370, 1140, 730, 720; ^{1}H NMR ($CDCl_3$): δ0.85 (6H, *d*, $J = 6.0$ Hz, isopropyl Me), 1.18 (46H, *s*, $-(CH_2)_{23}-$), 1.50 (1H, *m*, $-CHMe_2$), 2.20 (2H, *t*, $J = 7.0$ Hz, $-CH_2-COOH$); MS *m/z* (rel. int.): 424 $[M]^+$ (1.8), 353 (3.0), 351 (3.0), 339 (1.8), 325 (4.3), 323 (4.3), 311 (2.0), 297 (2.3), 283 (2.0), 269 (2.3), 241 (2.3), 227 (2.0), 185 (6.3), 183 (1.4), 171 (4.3), 157 (20.0), 155 (22.5), 143 (2.5), 141 (5.0), 129 (25.0), 127 (5.0), 115 (5.6), 113 (5.0), 109 (5.6), 99 (10.0), 87 (13.1), 85 (30.7), 73 (60.0), 71 (56.0), 59 (22.5), 57 (21.2), 45 (23.2), 43 (100).

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