RESEARCH PAPER

Accepted 15 January 1999
Received 4 August 1998
Indian J. Pharm. Sci. 1999, 61 (2) 90-96

Four New Seco-Sterols of *Phyllanthus fraternus* Roots

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Four new seco-sterols, named phyllanthosterol, phyllanthosecosteryl ester, phyllanthostigmasterol and fraternusterol, have been isolated from the alcoholic extract of the roots of *Phyllanthus fraternus*. The structure of these sterols have been elucidated as 13, 14-seco-stigmaster-5,20(22)-diene-3α-ol, 9,10-seco-stigmater-5,9-diene-3β-yl-7,16'-dihydroxytetra-cosanoate, 13,14-seco-stigmaster-5,20(22)-diene-3β-ol and 24-β-methyl-5 (10), 13(14)-diseco-cholest-6,9(11)-diene-22-one-3 β-ol, respectively, on the basis of the spectral data analysis and chemical reactions.

Phyllanthus fraternus Webster (syn.*P. niruri* auct. non Linn. Family Euphorbiaceae) is a well known folklore medicinal plant. The fresh roots are an excellent remedy for jaundice. An infusion of the roots is good tonic and diuretic when taken cold in repeated doses. It is also useful in menorrhagia[1-4]. Lup-20, (20)-en-3 β-ol and its acetate, and an acyclic diterpene have reported from the roots of *P. niruri*[5,6]. This communication describes the isolation and characterization of four new seco-steroidal constitutents from this plant.

EXPERIMENTAL

Extraction

Air-dried and coarsely powdered material (1.0 kg), collected from the campus of Jamia Hamdard, was extracted exhaustively with ethanol (95%) in a Soxhlet apparatus. The combined extracts were dried under reduced pressure to obtain a brown-coloured viscous residue (55%). The dried ethanolic extract was dissolved in minimum amount of methanol and adsorbed on silica gel to form a slurry. The slurry was air-dried and subjected to silica gel column-chromatography. The column was eluted with petroleum ether, petroleum ether-$CHCl_3$ (9:1, 3:1, 1:1, 1:3 v/v), $CHCl_3$, $CHCl_3$-MeOH (99:1,95:5, 9:1, 3:1, 1:1) and MeOH to isolate the following compounds:

*For correspondence

Compound 1 :

Elution of the column with petroleum ether-$CHCl_3$ (1:1) afforded colourless white waxy solid 1, crystallized from petroleum ether-chloroform (1:1), 167 mg (0.0167%), m.p. 129-130°, R_f 0.27 (C_6H_6-$CHCl_3$,1:1), $[\alpha]_D$+33.33° (c 0.15,$CHCl_3$). UV ν_{max} ($CHCl_3$):259 nm (log ε 6.0). IR V_{max} (KBr) :3452, 2944, 1744, 1660, 1604, 1384, 1334, 1054, 972, 958, 838, 784, 724 cm^{-1}. ^{1}H-NMR (100 MHz, $CDCl_3$) : Table 1. EIMS m/z (rel.int.) 414 $[M]^+$($C_{29}H_{50}O$) (8.0), 399(2.2), 381(1.6), 329(3.1), 315(2.6), 301(3.6), 300(8.3), 274(1.4), 272(4.3), 256(1.7), 254(2.2), 218(2.6), 215(2.2), 214(3.7), 212(1.6), 200(1.5) 199(5.3), 188(1.1), 186(4.5), 178(1.9), 176(2.0), 174(5.8), 172(4.1), 164(1.7), 162(4.7), 161(1.7), 160(3.6), 158(10.9), 150(5.4), 146(8.4), 144(18.2), 139(2.2), 137(6.6), 136(1.4), 132(22.0), 123(13.4), 121(10.2), 120(11.5), 119(17.4), 111(4.0), 108(10.8), 106(13.2), 104(8.4), 98(1.4), 96(6.0), 92(12.0), 90(13.4), 83(12.0) 82(1.4),71(4.8), 69(25.4), 68(1.4), 65(1.4), 55(100), 53(2.4), 41(6.2). Elemental analysis : Calcd. C, 84.05 H 12.07; Found C, 83.69; H, 11.92.

Acetylation of 1

Compound **1** (10 mg) was treated with Ac_2O (3 ml) and pyridine (1 ml) to yield monoacetyl derivative **1a**, mp 110-111° different spot on TLC plate from that of the starting compound 1. IR γ_{max} (KBr) 1725 cm^{-1}.

Jones Oxidation of 1 :

To compound **1** (20 mg) in acetone, Jones reagent (2 ml) was added at 4°. After usual work up 3-oxo derivative (**1b**) was obtained, mp 94-95°. IR γ_{max}(KBr) 1705 cm^{-1}.

Compound **1b** (5 mg) in MeOH was reduced with $NaBH_4$ (50 mg) to yield compound **1**. mp, mmp and co-TLC comparable with **1**.

Compound 2 : Petroleum ether-$CHCl_3$ (1:1) eluants gave colurless amorphous mass of **2**, re-crystallized from $CHCl_3$-MeOH (1:1), 120 mg (0.012%), R_f 0.40 (C_6H_6-$CHCl_3$, 1:1), $[\alpha]_D$-15.87° (c 0.63, $CHCl_3$); mp 122-124°. UV λ_{MAX} (MeOH) : 213 nm (log ε 5.3). IR ν_{max}(KBr) :3460, 2925, 2860, 1740, 1635, 1595, 1460, 1375, 1160, 1055, 970, 800 cm^{-1}. ^{1}H-NMR (100 MHz, $CDCl_3$) : δ 5.34 (1H, brs, H-6), 5.14 (1H, m, H-9), 5.08 (1H, m H-11), 4.27 (1H, brm, w1/2=16.0 Hz, H-3α) , 3.97(1H, m, H-7') 3.47(1H, m, H-16'), 2.38 (2H, brs, CH_2), 2.30(2H, brs, CH_2), 2.23 (2H, brs CH_2), 2.07 (3H, brs, CH_2, CH), 2.05 (2H, brs, CH_2), 1.97 (1H, brs, CH), 1.79 (1H, brs, CH), 1.67(2H, brs CH_2), 1.59 (4H, brs, 2xCH_2), 1.57 (4H, brs, 2xCH_2), 1.25 (40H, brs, 20xCH_2), 1.01 (3H, d, J=6.00 Hz, Me-19), 0.94 (3H, d, J=6.50 Hz, Me-21), 0.87 (3H, d, J=6.50 Hz, Me-27), 0.84 (3H, d, J=7.0 Hz, Me-29), 0.80 (3H, d, J=6.0 Hz, Me-26), 0.78 (3H, t, J=7.5 Hz, Me-24), 0.68 (3H, brs, Me-18). EIMS m/z (rel.int.) : 413(32.0), 400(18.6), 399 (8.7), 396(12.1), 383(8.7), 281(7.6), 352(11.8), 329(11.2), 313(7.5), 303(11.5), 300, (19.0), 299(5.9), 289(10.8), 285(5.4), 273 (18.3), 272(17.5), 271(23.4), 255(37.8), 231(13.7), 213(22.2), 198(9.8), 189(10.4), 159(28.4), 148(79.1), 145(32.3), 143(10.9), 132(30.9), 122(31.3), 113(3.9), 109(28.8), 107(47.0), 95(44.4), 85(56.3), 83(56.4), 71(29.2),69(57.6), 57(75.3), 55(100). Elemental analysis : Calcd. C, 79.89; h, 12.06; Found c, 80.72; H, 11.85.

Hydrolysis of 2

Compound **2** (25 mg) was heated with 2N ethanolic KOH solution (5 ml) for 4 h. Water (10 ml) was added and the reaction mixture extracted with solvent ether (3x5 ml). The organic phase was washed with water (3x10 ml) and evaporated to get an aglycone **2a**, mp 131-132°C. IR ν_{max} (KBr) : 3450 cm^{-1}. The reaction mixture after extraction was neutralized with dilute HCl and re-extracted with solvent ether. After usual work-up the acid **2b** was obtained, mp 75 - 76°.

Compound 3: Elution of the column with petroleum ether-$CHCl_3$ (1:1) afforded colourless needle shaped crystals of **3**, crystallized from petroleum ether-chloroform (1:1), 145 mg (0.0145%), mp 134-136°, R_f0.43 (C_6H_6-$CHCl_3$, 1:1), $[\alpha]_D$-31.25° (c 0.32 $CHCl_3$). UV λ_{max} ($CHCl_3$) : 242 nm (log ε 5.6). IR ν_{max} (KBr) : 3260, 2950, 1610, 1390, 1340, 1056, 970, 960, 830, 800, 742, 728 cm^{-1}. ^{1}H-NMR (100 MHz, $CDCl_3$) : Table 1. EIMS m/z (rel.int.) : 414 $[M]^+$ ($C_{29}H_{50}O$) (16.9), 399(6.1), 396(2.2), 381(2.0), 353(5.4), 341(1.3), 329(4.2), 315(5.6), 301(10.0), 290(5.6), 276(1.4), 275(2.5), 274(6.3), 272(11.7), 260(2.8), 257(32.1), 250(1.2), 244(1.3), 242(5.3), 232(10.8), 220(2.1), 214(11.4), 204(3.6), 202(6.3), 199(9.4), 193(1.6), 192(1.3), 188(9.5), 186(8.8), 173(11.8), 164(10.4), 159(33.4), 145(16.8), 139(1.2), 133(21.6), 123(10.8), 119(16.8), 111(2.4), 107(26.4),105(16.8), 97(21.6), 96(2.4), 91(38.4), 71(12.0), 83(50.4), 69(36.0), 65(4.8), 55(100), 54(2.4). Elemental analysis : Calcd. C, 84.05; H 12.07, Found C, 84.26; H, 12.16.

Acetylation of 3 :

Compound **3** (10 mg) was treated with Ac_2O (30 ml) and pyridine (1 ml) yielded monoacetyl derivative **3a**, mp 120-122°; different spot on TLC plate from that of the starting compound **3**. IR ν_{max} (KBr) : 1725 cm^{-1}.

Jones Oxidation of 3 :

To compound **3** (20 mg) in acetone, Jones reagent (2 ml) was added at 4°. Water (10 ml) was added, the reaction mixture extracted with $CHCl_3$ (3x10 ml), dried (Na_2SO_4) and concentrated to yield 3-oxo derivative (**3b**), mp 107-108°. IR ν_{max} (KBr): 1705 cm^{-1}.

Compound 4: Elution of the column with petroleum ether-$CHCl_3$ (1:1) afforded light green crystalline powder (**4**), crystallized from petroleum ether-$CHCl_3$ (1:1), 185 mg (0.0185%), mp 113-115°, R_f, 0.42 ($CHCl_3$), $[\alpha]_D$+34.48 (C 0.29, $CHCl_3$). UV γ_{max} ($CHCl_3$) : 246, 320 nm (log ε 5.5, 6.7). IR γ_{max} (KBr) : 3444, 2940, 1716, 1606, 1386, 1334, 1054, 972, 960, 838, 800, 718, 686 cm^{-1}. ^{1}H-NMR (100 MHz, $CDCl_3$) : Table 2. EIMS m/z (rel.int) : 416$[M]^+$($C_{28}H_{48}O_2$) (14.2), 401(9.2), 398(8.7), 383(6.0), 331(12.8), 317(4.4), 303(4.0), 302(4.2), 276(3.7), 275(5.2), 257(29.9), 234(3.4), 220(2.3), 217(4.7), 216(12.2) 206(4.4), 204(4.7), 202(9.9), 196(5.0), 192(3.4), 190(6.4), 188(10.3), 187(3.6), 186(10.3), 178(5.7), 177(4.6), 161(17.0),

160(17.0), 159(13.5), 155(2.4), 145 (7.5), 141(3.2), 140(2.4), 135(18.3), 125(4.4), 122(6.5), 114(3.9), 111(4.4), 107(40.35), 97(17.4), 96(3.6), 84(34.3), 83(30.5), 81(69.6), 71(15.6), 69(52.2), 66(2.2), 58(36.2), 55(100), 53(3.6), 40(6.6). Elemental analysis : Calcd. C, 80.76; H, 11.53; Found C, 81.16; H, 11.88.

Acetylation of 4

Compound **4** (10 mg) was treated with Ac_2O (3 ml) and pyridine (1 ml) yielded mono acetyl derivative **1**, mp 119-120°, IRν_{max} (KBr) : 1725, 1720 cm^{-1}.

Jones oxidation of 4 gave 3-oxo derivative 4b, mp 98-99°C. IR ν_{max} (KBr) : 1705 cm^{-1}

$NaBH_4$ Reduction of 4 b yielded the original compound **4**.

RESULTS AND DISCUSSION

Compound **1**, designated phyllanthosterol, was obtained as a colourless white amorphous product from petroleum ether-chloroform (1:1) eluants. It responded positively to steroidal tests and showed molecular ion peak at m/z 414 corresponding to a molecular formula $C_{29}H_{50}O$. It indicated five double bond equivalents. Its IR spectrum demonstrated the presence of hydroxy group (3452 cm^{-1}), unsaturation (1604 cm^{-1}) and gem dimethyl/isopropyl group (1384, 1334 cm^{-1}). Its ^{1}H-NMR spectrum displayed a one-proton triplet at δ 5.088 (J=6.35 Hz) assignable to H-22 proton. A broad multiplet at δ 3.470 with $w^1/_2$=12.0 Hz was associated with 3 β-methine proton (equitorial) indicating that compound 1 was a 3-epimer of compound **3**.

Two three-proton each broad singlets at δ1.539 and 1.009 were attributed to C.21 methyl group attached to C-20 olefinic carbon and C-19 tertiary methyl group, respectively.Two doublets at δ 0.877 (J=7.32 Hz), and 0.767 (J=4.63 Hz) and a triplet at 0.848 (J=6.53 Hz) were ascribed to C-26 and C-27 secondary methyl groups and C-29 primary methyl functionality, respectively. The appearance of a three-proton doublets at δ 0.701 (J=6.59 Hz) of the C-18 methyl group reflected C-13, C-14 seconature of the steroidal nucleus. The remaining methine and methylene groups appeared in between δ 2.299-1.054 (Table 1).

The mass spectrum of compund 1 exhibited diagnostically important fragment ions at m/z 399 [M-Me]$^+$, 381 [399-H_2O]$^+$, 274 [M-side chain, $C_{10}H_{19}$, SC]$^+$, 272 [274-2H]$^+$, 256 [274-H_2O]$^+$, 254 [272-H_2O]$^+$ and 329 [M-85, C_6H_{13}]$^+$, suggesting that it was a C_{29} sterol with two double bonds, one in the carbocyclic framework, other in the side chain and one removable hydroxyl group. The other ion fragments appeared identical as observed in the mass spectrum of **3** indicating saturated nature of ring A, trisubstituted olefinic bond in the ring B at C-5 position and presence of hydroxyl group in ring A at C-3 position. The mass spectrum also revealed the presence of an ethyl group in the side chain and this was assigned to C-24 position on the basis of biogenetic analogy as well as close similarties in the chemical shifts of protons of the side chain with the related compounds[7,8,9].

Acetylation of **1** with acetic anhydride and pyridine yielded a monoacetyl derivative **1a**, confirming the existence of one acetylable hydroxyl function. Oxidation of 1 with Jones reagent formed 3-oxo-derivative **1b**. Sodium borohydride reduction of the oxidized product regenerated the compound **3** with β-oriented hydroxyl group thus confirming the α-orientation of the hydroxyl group in compound **1**.

On the basis of these evidences the structure of phyllanthosterol (**1**) was determined as 13,14-seco-stigmaster-5, 20(22)-diene-3α-ol. This is an unknown stigmasterol-type compound with 13,14-seco ring and the occurrence of such compound is being reported for the time in *P. fraternus.*

Compound **2**, named phyllanthosecosteryl ester, was obtained as colourless amorphous mass from petroleum ether-$CHCl_3$ (1:1) eluants. It responded positively to steroidal tests. Its IR spectrum exhibited absorption for hydroxyl (3460 cm^{-1}) and ester (1740 cm^{-1}) groups. Its mass spectrum had distinctive fragmentation pattern identical to that of a steroid and an aliphatic-hydrocarbon. Cleavage of ester linkage formed prominent ion peaks at m/z 383 [ion b]$^+$ due to acyl moiety and at m/z 413 [ion d]$^+$ due to an aglycone unit. Generation of intensified ion peaks at m/z 113 [CH_3 $[CH_2)_7]^+$, 143 [C_8H_{17} CHOH]$^+$, 255 [C_8H_{17}CH (OH) $(CH_2)_8]^+$ and 285 [C_8H_{17}CH(OH) $(CH_2)_2$ CHOH]$^+$ suggested 7,10-dihydroxy ocyl group attached to the steroidal moiety. Removal of mass unit 400 from the molecular ion (m/z 796; not observed) formed an ion fragment at m/z 396. The ion peaks of diagnostical

Table 1 - ^{1}H-NMR Spectral Chemical Shifts of Compounds 1 and 3

	1		3	
Position	Alpha	Beta	Alpha	Beta
1.	1.348 brs	2.299 brs	1.348 brs	2.301 brs
2.	2.056 brs	2.061 brs	2.024 brs	2.061 brs
3.	-	3.470 brm (w1/2=12.0 Hz)	3.480 brm (w1/2=16.0 Hz)	-
4.	1.255 brs	1.255 brs	1.031 brs	1.255 brs
6.	5.368 d (J=4.88 Hz)	-	5.375 d (J=4.93 Hz)	
7.	1.348 brs	1.054 brs	1.348 brs	1.137 m
8.	1.181 brs	-	1.176 m	-
9.	1.801 brs	-	1.759 brs	-
11.	2.230 brs	1.421 brs	2.230 brs	1.421 brs
12.	1.054 brs	1.941 brs	1.054 brs	1.870 brs
13.	1.421 brs	-	1.421 brs	-
14.	1.255 brs	1.255 brs	1.255 brs	1.255 brs
15.	1.181 brs	1.348 brs	1.107 brs	1.421 brs
16.	1.907 brs	1.801 brs	1.649 brs	1.448 brs
17.	1.421 brs	-	1.448 brs	-
18.	0.701 d (J=6.59 Hz)	-	0.701 d (J=7.09 Hz)	-
19.	1.009 brs	-	1.009 brs	-
21.	1.539 s	-	1.541 brs	-
22.	5.088 t (J=6.35 Hz)		5.088 t (J=6.35 Hz)	-
23.	1.255 brs	1.907 brs	1.255 brs	1.804 brs
24.	1.181 brs	-	1.176 brs	-
25.	1.421 brs	-	1.448 brs	-
26.	0.877 d (J=7.32 Hz)		0.877 d (J=7.32 Hz)	-
27.	0.767 d (J=4.63 Hz)	-	0.803 d (J=7.33 Hz)	-
28.	1.181 brs	1.255 brs	1.132 brs	1.220 brs
29.	0.848 t (J=6.53 Hz)	-	0.826 t (J=6.22 Hz)	-

Coupling constant are given in parenthesis in Hertz.

importance were appeared at m/z 272 [413-$C_{10}H_{21}$, SC]$^+$ 255 [396-SC]$^+$, 213 [255-ring D fission]$^+$ and 198 [213-Me]$^+$. The C_6-C_7 cleavage produced an ion fragment at m/z 289 which on elimination of the side chain formed an intensified ion peak at m/z 148 supporting C_9-C_{10} seco-nature of the molecule.

The ^{1}H-NMR of **2** displayed three one-proton each downfield signals at δ 5.34 (brs), 5.14 (m) and 5.08 (m) assigned to C-6, C-9 and C-11 olefinic protons, respectively. The carbinol protons appeared as one-proton each multiplets at δ 4.27 (w $^1/_2$=16.0 Hz), 3.97 and 3.47 associated correspondingly with H-3, H-7' and H-16'. The presence of a three-proton doublet at δ 1.01 (J=6.0 Hz), ascribed to C-19 methyl group, supported the C_9-C_{10} seco-nature. Another four doublets at δ 0.94 (J=6.50 Hz), 0.87 (J=6.5 Hz), 0.84 (J=7.0 Hz) and 0.80 (J = 6.0 Hz), integrating three-proton each, were associated respectively with C-21, C-27, C-29 and C-26 methyl functionalities. The C-18 tertiary methyl group appeared as a three-proton broad singlet at δ 0.68. A three-proton upfield triplet at δ 0.78 (J=7.5 Hz) was accounted to the terminal methyl group of the acyl group. A forty-proton broad signglet at δ1.25, accounted to 20 methylene groups, inducted aliphatic nature of the acyl group. The presence of all the methyl signals in the range δ 1.01-0.68 was indicative that all the functionalities were attached to the saturated carbons. Alkaline hydrolysis of **2** yielded the aglycone **2a** and the acid **2b**.

On the basis of these accunmulative data the structure of phyllanthosecosteryl ester (**2**) has been established as 9, 10-seco-stigmaster - 5,9-diene-3β yl -7,' 16' dihydroxy-tetracosanoate. This is a new seco-stigmasteryl ester and contributes the first report of occurrence of a 9, 10-secosterol in *P. fraternus.*

Compound **3**, named phyllanthostigmasterol, was obtained as a colourless needle shaped crystalline product from petroleum ether-chloroform (1:1) eluants of the Si-gel column. It responded positively to steroidal tests and showed molecular ion peak at m/z 414 corresponding to a molecular formula $C_{29}H_{50}O$. It indicated five degrees of unsaturation. The IR spectrum of **3** displayed characteristic bands for hydroxyl group (3260 cm^{-1}, unsaturation (1610 cm^{-1}), and gemdimethyl/isopropyl group (1390; 1340 cm^{-1}). Its ^{1}H-NMR spectrum displayed a one-proton doublet at δ 5.375 (J=4.92 Hz) assigned to

Table 2 - ^{1}H-NMR Chemical Shifts of Compund 4

Position	Alpha	Beta
1.	1.255 brs	2.301 brs
2.	1.801 brs	1.801 brs
3.	3.976 brm (w$^1/_2$=15.53)	-
4.	2.230 m	1.921 m
5.	1.801 brs	1.764 brs
6.	5.323 m	-
7.	5.323 m	-
8.	2.301	-
9.	-	-
10.	1.764 m	-
11.	5.083 t (J=6.35)	-
12.	1.764 m	1.801 brs
13.	1.519 brs	-
14.	1.255 brs	1.255 brs
15.	1.054 brs	1.519 brs
16.	1.764 brs	1.519 brs
17.	1.519 brs	-
18.	0.683 d (J=6.59)	-
19.	1.009 d (J=4.40)	-
20.	2.076 brs	-
21.	0.951 d (J=7.32)	-
22.	-	-
23.	2.230 m	230 m
24.	2.301 brs	-
25.	1.519 brs	-
26.	0.848 d (J=6.11)	-
27.	0.803 d (J=6.59)	-
28.	0.816 d (J=6.11)	-
29.	-	-

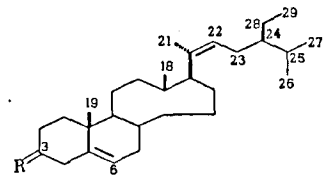
Coupling constants are given in parentesis in Hertz.

H-6 proton. A one-proton triplet at δ 5.088 (J=6.35Hz) was ascribed to H-22 proton. A Broad multiplet at δ 3.480 with $w^1/_2$=16.0Hz was associated with 3 α-methine proton (axial) interacting with C-2_{ax}, C-2_{eq}, C-4_{ax} and C-4_{eq} protons. Two three-proton each broad singlets at δ 1.541 and 1.009 were attributed to C-21 methyl group attached to C-20 olefinic carbon and C-19 tertiary methyl group, respectively. Two doublets at δ 0.877 (J=7.32 Hz), 0.803 (J=7.33 Hz) and a triplet 0.826 (J=6.22 Hz) were associated with C-26 and C-27 secondary methyl and C-29 primary methyl functionalities, respectively. Appearence of a three-proton doublet at δ 0.701 (J=7.09 Hz) of the C-18 methyl group reflected C-13, C-14 seco nature of the steroidal skeleton. The remaining methine and methylene protons resonated between δ 2.30-1.054 (Table 1).

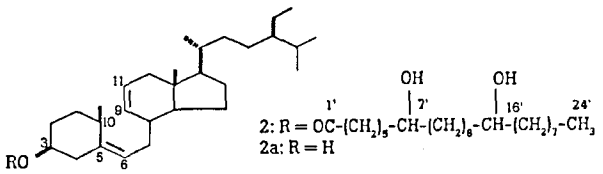
The electron impact mass spectrum of compound 3 exhibited diagnostically important fragment ions at m/z 399 $[M-Me]^+$, 396 $[M-H_2O]^+$, 381 $[396-Me]^+$, 329 [M-85, $C_6H_{13})^+$ 275 $[M-C_{10}H_{19}$, Side Chain SC$]^+$ 257 $[275-H_2O]^+$, 242 $[257-Me]^+$ and 214 [257-ring D]$^+$ suggesting that it was a C-29 sterol possessing two double bonds, one in the carbocyclic framework and one in the side chain and one removable hydroxyl group. The ion fragments at m/z 71, 341,123,290,69,83,54 $[71-H_2O]^+$, 105 $[123-H_2O]^+$, 65 $[83-H_2O]^+$ and 202 [341-SC]$^+$, indicated saturated nature of ring A, a trisubstituted olefinic bond in the ring B at C-5 and the presence of hydroxyl group in ring A which was placed at C-3 on the basis of biogenetic grounds. The ion fregments at 164,250,192,220 and 111 [250-SC]$^+$, supported the existence of another double bond in the side chain with ethyl group and saturated nature of rings C and D and seco-nature of ring C/D junction. The ethyl group was assigned to C-24 on the basis of biogenetic analogy as well as close similarities in the chemical shifts of the side chain protons with the related compounds[7,8,9].

Acetylation of **3** with AC_2O-pyridine at room temperature afforded a monoacetyl derivative **3a** (ν_{max}1725 cm^{-1}) confirming the existence of one acetylable hydroxyl function. Oxidation of **3** with Jones reagent formed 3-oxo-derivative (**3b**) (ν_{max}1705 cm^{-1}). Sodium borohydride reduction of the oxidized product regenerated the original compound **3** confirming the β - orientation of the hydroxyl group.

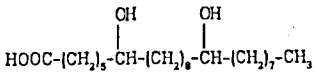
On the basis of these findings, the sterol **(3)** was identified as 13,14-seco-stigmasta-5,20(22)-diene-3β-o1.



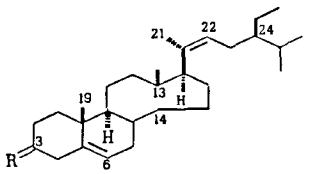
1: R = α-OH, H
1a: R = α-OAc, H
1b: R = O



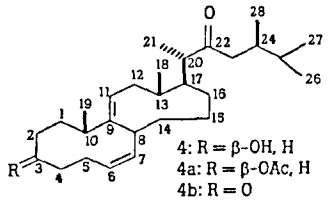
2: R = OC-$(CH_2)_5$-CH-OH-$(CH_2)_6$-CH-OH-$(CH_2)_7$-CH_3
2a: R = H



2b



3: R = β-OH, H
3a: R = β-OAc, H
3b: R = O



4: R = β-OH, H
4a: R = β-OAc, H
4b: R = O

This is a new stigmasterol type compound and the presence of 13,14-secoring of the sterol is being reported for the first time in the herb.

Compound **4**, named fraternusterol, was obtained as colourless crystalline powder from petroleum ether-chloroform (1:1) eluants. It responded positively to steroidal tests and showed molecular ion peak at m/z 416 consistent with the molecular formula $C_{28}H_{48}O_2$. It indicated five degrees of unsaturation. The IR spectrum of **4** showed characteristic absorption bands for hydroxyl groups (3444 $^{cm-1}$, carbonyl group (1716 $^{cm-1}$), unsaturation (1606 $^{cm-1}$) and gem dimethyl (1386, 1334 $^{cm-1}$). Its ^{1}H-NMR spectrum displayed a two-proton mutiplet at δ 5.323 assigned to H-6 and H-7 protons and one -proton triplet at δ 5.083 (J=6.35 Hz) ascribed to H-11. A one-proton broad mutiplet at δ 3.976 with $w^1/_2$ =15.53 Hz was attributed to H-3α methine proton. The six secondary methyl functionalities appeared as doublets intergrating three-protons each, at δ 0.683 (J=6.59 Hz),

1.009 (J=4.40 Hz), 0.951 (J=7.32 Hz), 0.848 (J=6.11 Hz), 0.803 (J=6.59 Hz) and 0.816 (J=6.11 Hz) due to Me-18, Me-26, Me-27 and Me-28, respectively and their existence in the upfield region (δ 1.009-0.683) reflected that these groups were located on saturated carbons. The remaining methine and methylene protons resonated in between δ 2.301-1.054 (Table 2).

The elctron impact mass spectrum of **4** exhibited diagnostically important ion fragments at m/z 401[M-Me]$^+$, 398 [M-H_2O]$^+$, 383 [398-Me]$^+$, 275 [M-141, side chain, SC]$^+$ and 257 [275-H_2O]$^+$ suggesting that it was a C_{28} di-seco-sterol possessing two double bonds and one removable hydroxyl group in the carboxylic framework, a C-9 saturated side chain ($C_9H_{17}O$) with carbonyl group. The ion fragments at m/z 114, 96 [114-H_2O]$^+$, 302, 161 [302-SC]$^+$, 140, 122 [140-H_2O]$^+$, 58 and 84 indicated seco-nature of rings A/B at C_5/C_{10}, the presence of the olefinic linkage at C-6(7) and the hydroxyl group in the ring A which was placed at C-3 on the basis of biogenetic grounds. The ion peak at m/z 276, 135 [276-SC]$^+$, 178, 160 [178-H_2O]$^+$, 220, 196, 155 and various other fragments generated due to cleavage of other linkages supported the existence of another double bond at C-9 (11) and the seco nature of the rings C/D at C13-C14. The appearance of the ion peaks at m/z 331 [M-C_6H_{13}]$^+$ and 303 [M-$C_6H_{13}CO$]$^+$ attested the presence of the carbonyl group at C-22. Acetylation of **4** with acetic anhydride and pyridine yielded a monoacetyl derivative 4a.

On the basis of these evidences the structure of fraternusterol (**4**) was formulated as 24 β-methyl-5(10),13(14)-diseco-cholest-6,9(11)-diene-22-one-3β-ol. This is an unknown natural product and the first report of the occurrence of a diseco-ergostanol type sterol in *P. fraternus.*

ACKNOWLEDGEMENTS

The authors are thankful to the instrumentation centres RRL, Jammu Tawi, CDRI, Lucknow and IIT, New Delhi for recording spectral data and to CSIR, New Delhi for awarding SRF to one of us (JG).

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