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TRITERPENOIDS AND PHENANTHRENES FROM LEAVES OF *BRYOPHYLLUM PINNATUM*

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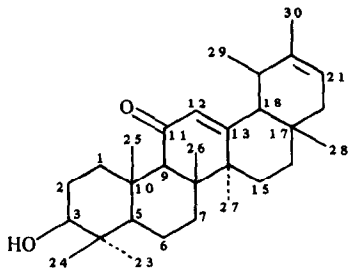
Key Word Index—*Bryophyllum pinnatum*; Crassulaceae; leaves; sterols; triterpenoids; bryophollenone; phenanthrene derivatives.

Abstract—From fresh leaves of *Bryophyllum pinnatum* three new constituents, bryophyllol, bryophollone and bryophollenone have been isolated and their structures elucidated by spectral and chemical studies. Three new compounds, bryophynol and two phenanthrene derivatives have also been identified in the mixture. 18α-Oleanane, ψ-taraxasterol, β-amyrin acetate and a new sterol, reported earlier as a hydrolysed product, have also been obtained, along with a mixture of α- and β-amyrins and their acetates.

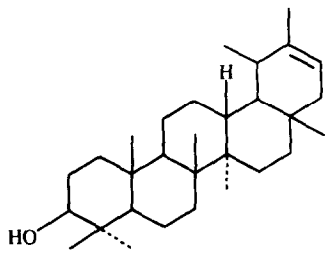
INTRODUCTION

Bryophyllum pinnatum syn. *B. calycinum* and *Kalanchoe pinnata* (Crassulaceae) commonly known as 'Zakham-hayat', is an ornamental plant which grows in Africa and Asia [1]. Its leaves, which are succulent in nature are known in folklore and traditional systems of medicine, in the treatment of cholera, bruises, contused wounds, urinary diseases and whitlow [2, 3]. In view of its importance in the indigenous system of medicine, various groups of workers have investigated the constituents of its leaves and reported the occurrence of organic acids [4, 5], hydrocarbons [6–8], phenolic components [9], flavonoids [4, 10] and sterols [7].

In the present studies, chemical investigations of fresh, *B. pinnatum* leaves were undertaken. The neutral fraction of the methanolic extract of the leaves was subjected to solvent fractionation, followed by column chromatography. As a result, seven components, showing single spots on TLC, were ultimately obtained, two of which provisionally named as bryophyllol (**5**) and bryophollone (**7**) are new compounds while constituent **6** characterized as 18α-oleanane has previously been reported as a synthetic product [11] and isolated only from petroleum oil [12]. Component **2** has been identified as α-amyrin acetate, and **1** was found to be a mixture of α- and β-amyrin acetates while component **3** was a mixture of α- and β-amyrins [13]. Fraction **4** was also a mixture of two triterpenoids tentatively identified as bryophynol (**4a**) and ψ-taraxasterol (**4b**) [14]. Bryophynol is hitherto unreported from any source whereas the isolation of α- and β-amyrin acetates and ψ-taraxasterol, from *B. pinnatum* has not been reported earlier. α- and β-Amyrins have been detected earlier by GC from the dried leaves of *B. pinnatum* [7]. The known constituents have been identified through comparison of their spectral data with those reported in literature for the corresponding constituents. In another working the neutral fraction of the ethanolic extract of the leaves of *B. pinnatum* was subjected to solvent fractionation and the major fraction was subjected to preparative TLC resulting in the isolation of two UV active zones (**9** and **10**) and one iodine active band (**12**). Component **10** has been characterized as a new oxygenated straight chain olefinic hydrocarbon named bryophollenone, while **12** has been identified as 24-ethyl, 25-hydroxycholesterol; the latter has previously been obtained following hydrolysis of a glycoside [15] and synthesis [16], but there is no report of its isolation in the free form. Component **9** is a mixture of two new phenanthrene derivatives characterized by GC-MS and high resolution mass spectrometry as 2(9-decenyl) phenanthrene (**9a**) and 2(9-undecenyl) phenanthrene (**9b**).



4a



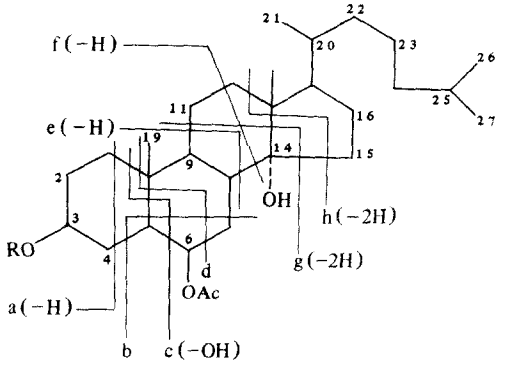
4b

RESULTS AND DISCUSSION

Bryophyllol (**5**) has a molecular formula $C_{29}H_{50}O_4$ (HR mass spectrometry) and gives positive sterolic tests. Its IR spectrum showed peaks at 3400 (OH), 2800 (aliphatic C–H), 1725 (ester carbonyl) and 1080 cm^{-1} (C–O). The molecular formula indicated five double bond equivalents, four were assigned to the four rings of the carbocyclic nucleus and the fifth to the carbonyl group of the ester function. The remaining two oxygen functions were ascribed to two hydroxyl groups, one of which was placed at C-3 on biogenetic grounds. The width at half height of the H-3 signal in ^{1}H NMR spectrum (δ 2.90, $W/2$ = 21.0 Hz) showed the equatorial position of the hydroxyl group. The quaternary nature of the second hydroxyl group was indicated by the absence of any other carbinylic proton in the ^{1}H NMR spectrum and the formation of only the monoacetyl derivative (**8**, Ac_2O–pyridine, $[M]^+$ *m/z* 504). This was placed at C-14 in the light of various fragments observed in the HR mass spectrum (see Experimental), including the typical fragments at *m/z* 307 and 289 for 14-hydroxylated steroids resulting from the cleavages of 13/17- and 14/15-bonds [17]. The position of the ester function of C-6 with axial orientation was suggested by the appearance of H-6 at δ 4.30 as a doublet of double doublets ($J_{6\alpha,5}$ = 7.0, $J_{6\alpha,7\alpha}$ = 7.0 and $J_{6\alpha,7\beta}$ = 4.0 Hz) in the ^{1}H NMR spectrum. This was supported by the appearance of H-5 at δ 2.30 as a doublet of double doublets ($J_{5,4\beta}$ = 13.8, $J_{5,6\alpha}$ = 7.0, $J_{5,4\alpha}$ = 7.0 Hz). Placement of various functionalities in the steroidal skeleton left a C_8H_{17} side chain and the appearance of two doublets at δ 0.86 (3H, J = 6.6 Hz, H-21) and δ 0.85 (6H, J = 7.1 Hz, H-26 and H-27) showed its composition as depicted in the structure. These observations led to the assignment of structure **5** to bryophyllol, which was substantiated by the HR mass spectrum. Apart from the $[M]^+$, it showed fragments at *m/z* 444.3594 ($C_{29}H_{48}O_3$), 402.3503 ($C_{27}H_{46}O_2$) and 384.3378 ($C_{27}H_{44}O$) resulting from the loss of water, acetic acid and acetic acid plus water, respectively.

18α-Oleanane (**6**), showed a $[M]^+$ at *m/z* 412.4054 corresponding to the molecular formula $C_{30}H_{52}$. Its IR spectrum exhibited absorptions at 2860 (aliphatic C–H), and 1380 cm^{-1}, (two peaks; *gem*-dimethyl) while its UV spectrum showed maxima at 190 and 207 nm. The calculations of double bond equivalents and the absence of an olefinic proton in the ^{1}H NMR spectrum suggested a pentacyclic triterpenoid skeleton. This was supported by eight singlets of three-protons each at δ 0.76, 0.82, 0.84, 0.87, 0.88, 0.92, 0.97 and 1.00. The appearance of these methyl protons as singlets in the ^{1}H NMR spectrum showed the oleanane skeleton while their chemical shifts suggested that it was 18α-oleanane [11]. The mass spectrum, apart from the $[M]^+$, showed fragments at *m/z* 122, 123 and 124 resulting from 9/10- and 5/6-fissions [18, 19].

The molecular formula, $C_{29}H_{42}O_3$, of bryophollone (**7**) was derived from exact mass measurement of the $[M]^+$. Its IR spectrum showed peaks at 3450 (OH of COOH), 3100, 2950, 2860 (C–H), 1720, *br* (carbonyls), 1610 (C=C), 1460 (C–H), 1380, 1385 (*gem*-dimethyl) and 1140 cm^{-1} (C–O). The triterpenoidal nature of **7** was indicated by the presence in the ^{1}H NMR spectrum of five three-proton singlets at δ 0.83, 0.85, 0.87, 0.90 and 0.92 and a doublet (J = 1.2 Hz) at δ 1.62 (H-29). The molecular formula showed nine double bond equivalents two of which were assigned to the carbonyl functions, five for the five rings of the carbocyclic nucleus, while the remaining two were taken for the two trisubstituted double bonds [δ 5.12 *t* (J = 3.5 Hz, H-12), and δ 5.37 *q* (J = 6.8 and 1.2 Hz, H-20)]. The highest intensity of the ion at *m/z* 218.2038 ($C_{16}H_{26}$, base peak) strongly suggested that this fragment results from RDA cleavages around ring C and led to the placement of one of the double bonds at C-9 (11) [20]. The fragments b and c (see structure) indicated that



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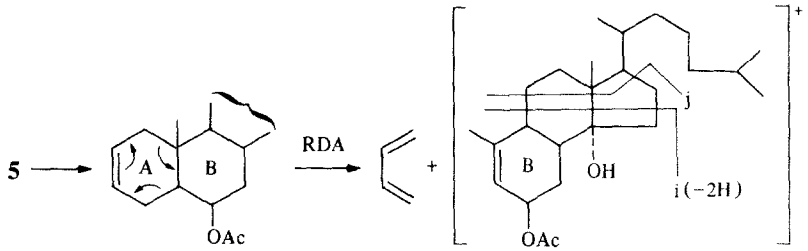
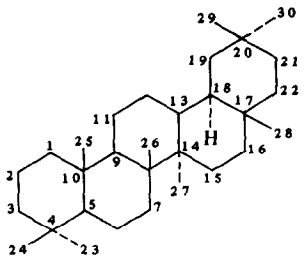
8 R = Ac





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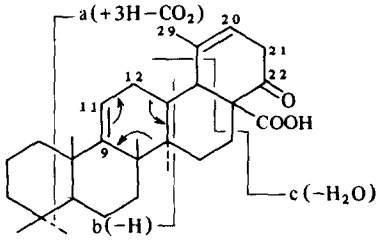
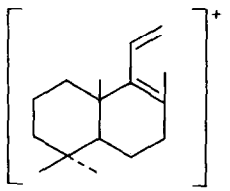


7

RDA

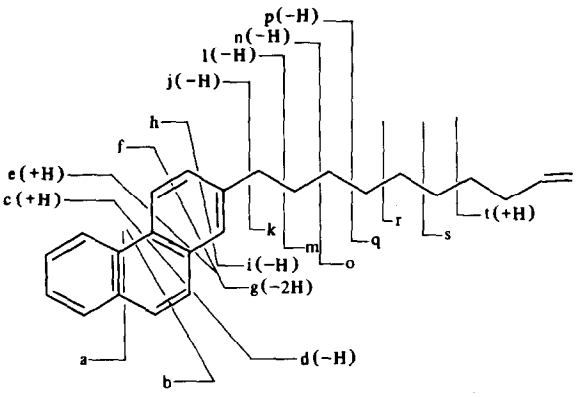


m/z 218.2083

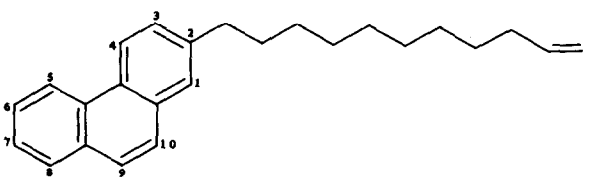
the carboxyl group is located at C-17. The placement of the other double bond at C-19 and the carbonyl function at C-22 could be deduced from double resonance experiments which revealed the presence of a $-C(Me)=CH-CH_2-CO-$ unit. Thus, irradiation at δ4.10 (*br d*, H-21) collapsed the quartet of triplet at δ 5.37 (J = 6.8 and 1.2 Hz, H-20) into a quartet (J = 1.2 Hz) while irradiation at δ5.37 changed the *br d* at δ4.10 (J = 6.8 Hz) into a broad singlet and the doublet at δ 1.62 (J = 1.2 Hz) to a sharp singlet. These observations along with the molecular formula showed that **7** is a 30-nortriterpenoid of the ursane series. In the light of these data structure **7** has been assigned to bryophollone. It is noteworthy in this context that, although there are many examples of C-22 oxygenated oleananes [21, 22], ursanes bearing an oxygen function at C-22 are rare [23, 24]. Moreover, **7** is also the first ursane triterpenoid with an isolated double bond between C_9 and C_{11} although a conjugated diene in ring C of ursane has been reported earlier from *Marsdenia formosana* [25].

The IR spectrum of fraction **4** showed bands at 3450 (OH), 2900, 2810 (C–H), 1690 (α,β-unsaturated ketone) and 1610 cm^{-1} (C=C). The mass spectrum of **4** gave two $[M]^+$ ions at *m/z* 438 and 426 with a difference of 12 mu. Exact mass measurements of these ions gave molecular formulae as $C_{30}H_{46}O_2$ (438.3467) and $C_{30}H_{50}O$ (426.3893), respectively. This observation along with the appearance of several peaks in the ^{1}H NMR spectrum, particularly in the upfield region, revealed that it is a mixture of two triterpenoids. The integration of the peaks in the ^{1}H NMR spectrum further indicated that they are in a ratio of 4:1. The peaks corresponding to the major component, provisionally named as bryophynol, appeared at δ3.46 (*t*, J = 6.5 Hz, H-3), 5.60 (*br s*, H-12), 1.68 (*br s*, H-30), 5.11 (*t*, J = 6.0 Hz, H-21), 0.89, 0.91, 0.98, 1.08, 1.12 and 1.14 (each 3H singlet) and 1.15 (*d*, J = 7.0 Hz) for seven C-methyls. In the light of these data structure **4a** has been tentatively assigned to bryophynol. On the other hand, peaks corresponding to the minor component appeared at δ3.20 (*t*, J = 6.0 Hz, H-3), 1.60 (*br s*, H-30), 5.09 (*t*, J = 6.0 Hz, H-21), 0.74, 0.80, 0.85, 0.95, 1.01 and 1.04 (each 3H singlet) and δ0.97 (*d*, J = 7.5 Hz, H-29). These data led to the tentative assignment of **4b** as ψ-taraxasterol. These values are moreover comparable with those reported in the lit. [26]. It should be noted that after several attempts towards their separation using various solvent systems, they could ultimately be resolved into two spots as their acetyl derivatives. However, with limited available quantities, further support of their structures through the spectral data of individual constituents could not be obtained.

The IR spectrum of fraction **9** showed peaks at 3100, 2900, 2950 (C–H), 1400–1590 (four peaks, benzene ring) and 1620 cm^{-1} (C=C), while its mass spectrum showed it to be a mixture of an homologous series of unsaturated hydrocarbons. The unsaturation was also indicated by its ^{1}H NMR spectrum which exhibited the presence of olefinic protons extending from δ4.05–4.31, a multiplet at δ5.26, and a multiplet extending from δ6.90–7.15 (aromatic protons). After GC-MS analysis it gave two peaks, the mass spectra of which showed $[M]^+$ at *m/z* 330 and 316. Exact masses of these peaks were measured from the HR mass spectrum of **9** as 330.2345 and 316.2208 giving molecular formulae of $C_{25}H_{30}$ and $C_{24}H_{28}$, respectively.



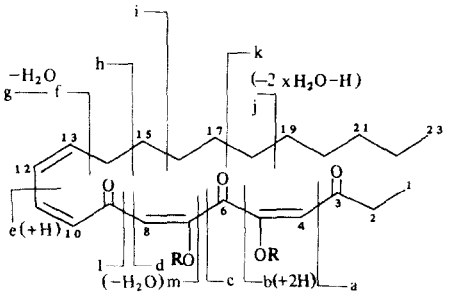
9a



9b

The double bond equivalents, UV absorptions at 208, 228, 260, 270, 292, 302 and 325 nm [27], appearance of olefinic and aromatic signals in the ^{1}H NMR spectrum of **9** and the characteristic fragments at *m/z* 76.0324 (C_6H_4, fragment a), 89.0391 (C_7H_5, fragment b) and 126.0511 ($C_{10}H_6$, fragment e) [28] suggested that they are phenanthrene derivatives. Apart from the $[M]^+$ at *m/z* 316.2208 for **9a**, the HR mass spectrum gave fragments at *m/z* 176.0636, and 139.1511 corresponding to the phenanthrene nucleus ($C_{14}H_8$, fragment j) and the side chain ($C_{10}H_{19}$, fragment k), respectively. Furthermore, the absence of any methyl signal in the ^{1}H NMR spectrum of **9** showed that the double bond, indicated by the ^{1}H NMR spectrum and the fragment $C_{10}H_{19}$, must be placed at a terminal carbon in the side chain. In the light of these observations, the structure of the side chain could be elucidated as depicted in structure **9a** which was also supported by successive increment of 14 mass units in the fragment j ($C_{14}H_8$). The second component **9b** was a homologue of **9a**, as indicated by the HR mass spectrum, the only difference being an increment of a CH_2 moiety. Finally, the fragments a–i (see structure **9a**) suggested that the side chain is located at C-2 or C-3. However, its exact location could not be ascertained.

Bryophollenone (**10**) was obtained as an oily constituent and its mass spectrum exhibited a $[M]^+$ at *m/z* 390.2346 corresponding to the molecular formula $C_{23}H_{34}O_5$. Its UV spectrum showed maxima at 212, 235, 260, 285, 300 and 327 nm, while the IR spectrum displayed peaks at 3450 (OH), 2850, 2950 (C–H), 1680–1710 (carbonyls) and 1600–1640 cm^{-1} (olefinic bonds). The ^{1}H NMR spectrum showed two terminal methyls at δ0.90 and 0.88 each as a triplet (J = 7.0 Hz) and a two-proton quartet at δ2.55 (J = 7.0 Hz) indicating a carbonyl group at its adjacent carbon and the partial structure $MeCH_2CO$– which was supported by a fragment at *m/z* 57.0338 (C_3H_5O, fragment a) in the HR mass spectrum (see Experimental). It formed a diacetate (**11**, Ac_2O–pyridine, $[M]^+$ *m/z* 474) showing the presence of two hydroxyl groups, while the remaining three oxygens were ascribed to the carbonyl groups. The calculation of double bond equivalents left four olefinic bonds, and as the ^{1}H NMR spectrum showed only six olefinic protons, two of the olefinic carbons must therefore be quaternary. The location of olefinic bonds, carbonyl functions and the hydroxyl groups as depicted in the structure (**10**) could be unequivocally decided by HR and EI mass spectrometry (see Experimental). The fragments f and g at *m/z* 249.0724 and 231.0625 corresponding to the formulae $C_{13}H_{13}O_5$ and $C_{13}H_{11}O_4$, indicated that all the oxygen functions and the double bonds are located between C-1 and C-13. Fragments a–d and m showed the substitution pattern between C-1 and C-7 (seven carbon unit) as provided in the structure, while fragments e–g and l indicated that the remaining oxygen function and the double bonds are present between C-8 and C-13. The appearance of a multiplet at δ7.10 (H-13), two double doublets at δ5.93 ($J_{12,11}$ = 11.0, $J_{12,13}$ = 8.0 Hz, H-12) and 5.41 ($J_{11,10}$ = 7.0, $J_{11,12}$ = 11.0 Hz, H-11) and a multiplet of three-protons at δ5.62 (H-4, H-8 and H-10) exhibited a partial structure H_2C–CH=CH–CH=CH–CO–CH– which could be joined with the seven-carbon unit. The rest of the molecule was taken to be a straight chain moiety, as no further functionalities or branching was indicated by the spectral data. This substitution pattern was further demonstrated by the fragments at *m/z* 135.0808, 178.0625, 245.1875 and 282.1250. The ion at *m/z* 282.1250 (fragment j) results from the successive losses of C_5H_{12} (C_{19}–C_{23}) and two molecules of water. The fragments at *m/z* 135.0808 and 178.0625 resulted from the cleavages of C_8–C_9/C_{17}–C_{18} bonds and C_5–C_6/C_{15}–C_{16} bonds, respectively, while the fragment at *m/z* 245.1875 corresponding to the formula $C_{17}H_{25}O$ resulted from the cleavage of the C_6–C_7 bond followed by the loss of a hydroxyl group. These data led to the assignment of the structure of bryophollenone as tricos-5,7-dihydroxy-3,6,9-trion-4,7,10,12-tetraene (**10**).



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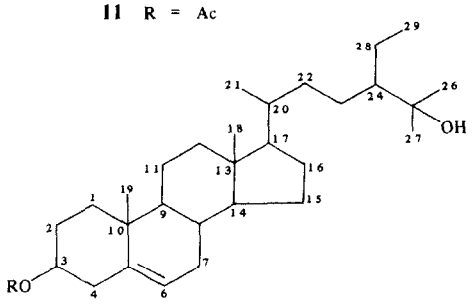
11 R = Ac



12 R = H
13 R = Ac

Compound **12** showed a $[M]^+$ at *m/z* 430.3756 corresponding to the molecular formula $C_{29}H_{50}O_2$. Its IR spectrum showed peaks at 3400 (OH), 2800 (C–H), 1610 (C=C) and 1100 cm^{-1} (C–O) while the UV spectrum had maxima at 208 and 228 nm. In the ^{1}H NMR spectrum three singlets were observed at δ1.30 (6H), 0.90 (3H) and 0.88 (3H), assignable to four quaternary methyls (H-27 and H-26, H-19 and H-18), indicating its steroidal nature. A doublet at δ0.86 (J = 7.0 Hz, H-21), a triplet at δ0.97 (J = 7.5 Hz, H-29) and a singlet at δ1.30 revealed the presence of a 24-ethyl-25-hydroxycholesteryl side chain. The ^{1}H NMR spectrum furthermore showed a one-proton multiplet at δ3.65 ($W/2$ = 21.5 Hz) and a one-proton doublet of doublets at δ5.34 (J = 5.5 and 3.3 Hz) related to H-3 and H-5, respectively. In the light of these spectral data the structure of **12** has been assigned as 24-ethyl-25-hydroxycholesterol, which was confirmed through various fragments observed in the mass and formation of the monoacetyl derivative (**13**), (see Experimental).

EXPERIMENTAL

General. Mps: uncorr. ^{1}H NMR spectra were determined at 300 MHz in $CDCl_3$. MS were recorded on a double focussing MS spectrometer connected to a PDP 11/34 computer system.

GC-MS was performed in EI mode using a 0.3 m capillary glass column SP2100. Temp. prog. was from 70 to 250° at 8°/min. Electron impact energy, emission current and ion source temp of the spectrometer were 80 eV, 0.8 mA and 270°, respectively. Silica gel 60 PF 254 coated on glass plates were used for analytical and prep. TLC.

Plant material. Leaves of *B. pinnatum* were identified by Prof. S. I. Ali (Department of Botany, University of Karachi), and a voucher specimen has been deposited in the herbarium of the department.

Extraction and isolation. Fr. leaves of *B. pinnatum* (5 kg) collected in the month of May from the Karachi region, were cut into small pieces and repeatedly extracted with MeOH at room temp. The residue obtained from the combined extracts was partitioned between H_2O and EtOAc. The EtOAc phase was shaken out with 4% Na_2CO_3 soln to separate acidic from neutral components. The neutral fr. was washed, dried, treated with charcoal and filtered. The residue obtained on removal of the solvent from the EtOAc filtrate was redissolved in petrol and shaken out with 90% MeOH. The lower phase was extracted with petrol–C_6H_6 (1:1) and C_6H_6, and the residue obtained on removal of the solvent from the combined extracts subjected to CC (silica gel 40, 70–230 mesh, petrol and petrol–EtOAc in order of increasing polarity). Petrol eluates afforded a mixt. of α- and β-amyrin acetates, α-amyrin acetate, a mixt. of α- and β-amyrins, a mixt. of bryophynol (**4a**) and ψ-taraxasterol (**4b**), bryophyllol (**5**) and 18α-oleanane (**6**), whilst petrol–EtOAc (24:1) eluates yielded bryophollone (**7**).

In a second extraction, leaves (1.5 kg) were collected in January from the same region and extracted with MeOH at room temp. The neutral EtOAc fr. obtained employing the procedure described above was treated with charcoal, filtered and the charcoal eluted with MeOH–C_6H_6 (1:1). The residue obtained on removal of solvent from the EtOAc filtrate and MeOH–C_6H_6 eluate was subjected to prep. TLC (silica gel, petrol) yielding three constituents **9**, bryophollenone (**10**) and **12**; **9** has been characterized by GC-MS as decenylphenanthrene (**9a**) and undecenylphenanthrene (**9b**).

Bryophyllol (**5**). Crystallization from MeOH–C_6H_6 (1:1), needles mp 272–273°. HRMS and EIMS *m/z* (formula, fragment) (rel. int.): 462.3693 $[M^+]$ (calc. for $C_{29}H_{50}O_4$: 462.3709) (3), 444.3594 $[M-H_2O]^+$ (3), 419.3516 $[M-C_2H_3O]^+$ (100), 402.3503 $[M-MeCOOH]^+$ (25), 401.3448 $[C_{27}H_{45}O_2]^+$ (2), 384.3378 $[M-MeCOOH-H_2O]^+$ (3), 307 (16), 289 (15), 279.1574 $[C_{16}H_{23}O_4]^+$ h, (15), 271 (16), 264 (14), 251.1266 $[C_{14}H_{19}O_4]^+$ g, (14), 237.1458 $[C_{14}H_{21}O_3]^+$ f, (10), 233.1235 $[C_{14}H_{17}O_3]^+$ g−H_2O, (2), 223.1300 $[C_{13}H_{19}O_3]^+$ e, (10), 218 (16), 217 (16), 209.1143 $[C_{12}H_{17}O_3]^+$ j, (3), 195.1027 $[C_{11}H_{15}O_3]^+$ i, (3), 177.0972 $[C_{11}H_{13}O_2]^+$ *i*−H_2O, (7), 157.0842 $[C_8H_{13}O_3]^+$ d, (2), 99.0487 $[C_5H_7O_2]^+$ b, (4), 71.0556 $[C_4H_7O]^+$ a, (46), and 68.0667 $[C_5H_8]^+$ c, (8). ^1H NMR: δ4.30 (1H, *ddd*, $J_{6,5}=7.0$, $J_{6,7\alpha}=7.0$, $J_{6,7\beta}=4.0$ Hz, H-6), 2.90 (1H, *m*, $W/2=21.0$ Hz, H-3), 2.30 (1H, *ddd*, $J_{5,4\beta}=13.8$, $J_{5,6}=7.0$, $J_{5,4\alpha}=7.0$ Hz, H-5), 2.01 (3H, *s*, OAc), 1.82 (1H, *d*, $J=3.3$ Hz, OH), 1.05 (3H, *s*, H-19), 0.86 (3H, *d*, $J_{21,20}=6.6$ Hz, H-21), 0.85 (6H, *d*, $J=7.1$ Hz, H-26 and H-27) and 0.84 (3H, *s*, H-18).

Acetylation of **5** *to* **8**. To a soln of **5** (30 mg) in pyridine (1 ml), Ac_2O (1 ml) was added and the reaction mixt. kept overnight at room temp. On usual work-up and crystallization from MeOH, **8** was obtained as needles, mp 240–241°. IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3355 (OH), 1720 *br* (OAc). EIMS *m/z* (rel. int.): 504 $[M]^+$ (2), 444 $[M-60]^+$ (6), 384 $[M-2\times60]^+$ (10), 43 (100). ^1H NMR: δ4.35 (1H, *ddd*, $J_{6,5}=6.5$, $J_{6,7\alpha}=6.5$, $J_{6,7\beta}=3.0$ Hz, H-6); 4.01 (1H, *m*, $W/2=18.0$ Hz, H-3), 2.32 (1H, *m*, H-5), 2.02 (3H, *s*, OAc), 2.01 (3H, *s*, OAc), 1.06 (3H, *s*, H-19), 0.87 (3H, *d*, $J_{21,20}=6.6$ Hz, H-21), 0.85 (6H, *d*, $J=7.1$ Hz, H-26 and H-27) and 0.84 (3H, *s*, H-18).

18α-*Oleanane* (**6**). Crystallization from C_6H_6–MeOH (2:1), rods mp 208–209°. EIMS *m/z* (rel. int.): 412.4054 $[M]^+$ (calc. for $C_{30}H_{52}$: 412.4069) (2), 397 (1), 369 (1), 354 (3), 353 (4), 340.3129 $[C_{25}H_{40}]^+$ (2), 290 (1), 124 (18), 123 (16), 122 (16), 97 (58), 84 (38), 83 (61) and 69 (100).

Bryophollone (**7**). Crystallized from MeOH–C_6H_6 (1:1), needles mp 210°. EIMS *m/z* (rel. int.): 438.3100 $[M]^+$ (calc. for $C_{29}H_{42}O_3$: 438.3134) (2), 410 (3), 314 (1), 313.2548 $[C_{22}H_{33}O]^+$ a, (3), 286 (3), 271 (2), 259 (2), 257.2262 $[C_{19}H_{29}]^+$ b, (5), 246 (4), 243 (4), 231 (3), 229 (3), 228 (3), 220 (10), 218.2038 $[C_{16}H_{26}]^+$ (100), 204 (8), 203 (20), 201 (4), 191 (6), 187 (6), 173 (4), 161 (4), 159 (4), 151 (1), 146 (12), 137 (12), 131 (2), 124 (8), 121.0289 $[C_7H_5O_2]^+$ c, (12), 84 (11), 81 (12), 69 (11), and 66 (8). ^1H NMR: δ10.09, (1H, *s*, –COOH), 5.37 (1H, *qt*, $J_{20,21}=6.8$, $J_{20,29}=1.2$ Hz, H-20), 5.12 (1H, *t*, $J=3.5$ Hz, H-12), 4.10 (2H, *br d*, $J_{21,20}=6.8$ Hz, H-21), 3.21 (1H, *br s*, H-18), 1.62 (3H, *br d*, $J_{29,20}=1.2$ Hz, H-29), 0.92, 0.90, 0.87, 0.85 and 0.83 (each 3H, *s*, 5 × Me).

2(9-*decenyl*) *Phenanthrene* (**9a**). HRMS *m/z* (formula, fragment) (rel. int.): 316.2208 $[M]^+$ (calc. for $C_{24}H_{28}$: 316.2191) (4), 245.1371 $[C_{19}H_{17}]^+$ (4), 231.1197 $[C_{18}H_{15}]^+$ (6), 218.1098 $[C_{17}H_{14}]^+$ p, (6), 213.1656 $[C_{16}H_{21}]^+$ d, (4), 204.0956 $[C_{16}H_{12}]^+$ n, (8), 190.0782 $[C_{15}H_{10}]^+$ l, (14), 176.0636 $[C_{14}H_8]^+$ j, (6), 175.1452 $[C_{13}H_{19}]^+$ g, (2), 163.1472 $[C_{12}H_{19}]^+$ i, (4), 152.0624 $[C_{12}H_8]^+$ h, (6), 139.1511 $[C_{10}H_{19}]^+$ k, (4), 139.0491, $[C_{11}H_7]^+$ f, (8), 127.0536 $[C_{10}H_7]^+$ (6), 126.0511 $[C_{10}H_6]^+$ e, (2), 125.1318 $[C_9H_{17}]^+$ m, (16), 111.1178 $[C_8H_{15}]^+$ o, (38), 103.0514 $[C_8H_7]^+$ c, (8), 102.0454 $[C_8H_6]^+$ (6), 98.1075 $[C_7H_{14}]^+$ (6), 97.1016 $[C_7H_{13}]^+$ q, (65), 89.0391 $[C_7H_5]^+$ b, (8), 84.0920 $[C_6H_{12}]^+$ (12), 83.0857 $[C_6H_{11}]^+$ r, (75), 76.0324 $[C_6H_4]^+$ a, (8), 70.0790 $[C_5H_{10}]^+$ (28), 69.0711 $[C_5H_9]^+$ s, (100) and 56.0625 $[C_4H_8]^+$ t, (20).

Bryophollenone (**10**). HRMS and EIMS *m/z* (formula, fragment) (rel. int.): 390.2346 $[M]^+$ (calc. for $C_{23}H_{34}O_5$: 390.2406) (4), 282.1250 $[C_{18}H_{18}O_3]^+$ j, (12), 277.1076 $[C_{15}H_{17}O_5]^+$ i, (4), 263.0919 $[C_{14}H_{15}O_5]^+$ h, (6), 249.0724 $[C_{13}H_{13}O_5]^+$ f, (3), 245.1875 $[C_{17}H_{25}O]^+$ m, (2), 243.0625 $[C_{14}H_{11}O_4]^+$ (4), 231.0625 $[C_{13}H_{11}O_4]^+$ g, (10), 224.0684 $[C_{11}H_{12}O_5]^+$ e, (3), 221 (1), (8), 178.0629 $[C_{10}H_{10}O_3]^+$ (2), 169 (d), (9), 135.0808 $[C_9H_{11}O]^+$ (20), 127.0382 $[C_6H_7O_3]^+$ c, (4), 101.0625 $[C_5H_9O_2]^+$ b, (1), 85.1017 $[C_6H_{13}]^+$ k, (30) and 57.0338 $[C_3H_5O]^+$ a, (100). ^1H NMR: δ7.10 (1H, *m*, H-13), 5.93 (1H, *dd*, $J_{12,11}=11.0$, $J_{12,13}=8.0$ Hz, H-12), 5.62 (3H, *m*, H-4, H-8 and H-10), 5.41 (1H, *dd*, $J_{11,12}=11.0$, $J_{11,10}=7.0$ Hz, H-11), 2.55 (2H, *q*, $J=7.0$ Hz, H-2), 0.90 (3H, *t*, $J=7.0$ Hz, H-1) and 0.88 (3H, *t*, $J=7.0$ Hz, H-23).

Acetylation of **10** *to* **11**. Ac_2O (2 ml) was added to a soln of **10** (20 mg) in pyridine (1 ml) and the reaction mixt. kept for 48 hr at room temp. After usual work-up, acetate (**11**) was obtained as an oily product. UV λ_{max}^{MeOH} nm: 210, 236, 265, 280, 290 and 310. IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 2845, 2960 (C–H), 1685 (α,β-unsat. ketone), 1740 (ester carbonyl) and 1650 (C=C). EIMS *m/z* (rel. int.): 474 $[M]^+$ (3), 431 $[M-43]^+$ (6), 388 $[M-2\times43]^+$ (3), 135 (3), 102 (4), 83 (4), 71 (10) and 57 (55).

24-*Ethyl*-25-*hydroxycholesterol* (**12**). Needles, mp 144°. EIMS *m/z* (rel. int.): 430.3756 $[M]^+$ (calc. for $C_{29}H_{50}O_2$: 430.3811) (2), 415 (3), 412 $[M-18]^+$ (2), 401 (1), 397 (3), 369 (4), 359 (5), 358 (4), 329 (8), 273 (10), 157 (12), 139 (16), 123 (20), 110 (38) and 58 (100). ^1H NMR: δ5.34 (1H, *dd*, $J_{6,7a}=5.5$, $J_{6,7b}=3.3$ Hz, H-6), 3.65 (1H, *m*, $W/2=21.5$ Hz, H-3), 2.78 (1H, *ddd*, $J_{gem}=16.1$, $J_{7a,8}=10.5$, $J_{7a,6}=5.5$ Hz, H-7a), 2.04 (1H, *m*, H-7b), 1.61 (1H, *m*, OH), 1.30 (6H, *s*, H-26 and H-27), 0.97 (3H, *t*, $J=7.5$ Hz, H-29), 0.90 (3H, *s*, H-19), 0.88 (3H, *s*, H-18) and 0.86 (3H, *d*, $J=7.0$ Hz, H-21).

Acetylation of **12** *to* **13**. Acetylation of **12** with Ac_2O (10 ml)/pyridine (1 ml) at room temp overnight followed by usual

work-up afforded the acetate (**13**) as needles, mp 160°. IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3450 (OH), 1735 (ester carbonyl) and 1240 (C–O). EIMS *m/z* (rel. int.): 472 $[M]^+$ (6), 430 (15), 412 (10), and 395 (8).

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