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Phytochemical investigation of the aerial parts of *Protasparagus falcatus* (L.) Oberm

Mohammed Ali and Shahnaz Sultana

Abstract
Protasparagus falcatus (L.) Oberm., syn. *Asparagus falcatus* L. (Asparagaceae), is a large, thorny, climbing plant indigenous to African countries, Sri Lanka, Mediterranean region and other countries. The plant is considered as an anthelmintic and antisyphilitic, used to treat constipation, burns, wounds and swellings. Phytochemical investigation of a methanolic extract of the aerial parts of *P. falcatus* resulted in the isolation of two new chemical constituents characterized as 4,4,8,10β-tetramethyl-9β-[(13-methylene), 20α,34-; 24α,35-diolide-24-furanyl]-tetradecanyl-*trans*-decalin (furanosesquaterpene diolide, 3) and dilup-20 (29)-enyl labd-7″-en-19″,20″-dioate (dilupenyl labdandioate, 4) along with the known compounds 1-hexacosanol (1) and lupenyl palmitate (2). The structures of all the isolated phytoconstituents have been established on the basis of spectral data analysis and chemical reactions.

Keywords: *Protasparagus falcatus*, aerial parts, chemical constituents, isolation, characterization

1. Introduction
Protasparagus falcatus (L.) Oberm., syn. *Asparagus falcatus* L. (Asparagaceae), known as large forest asparagus or imblekazama, is a thorny, rapidly growing, climbing plant. It is distributed from south-western Ethiopia to South Africa, Arabian Peninsula, India, Sri Lanka, Canary Islands and Mediterranean region [1, 2]. It serves as a very good safety hedge when planted along a fence and is often grown as a security hedge in African and other countries [3, 4]. Its roots are antiseptic, antispasmodic, aphrodisiac and nerve tonic; taken orally to relieve constipation. The leaf ashes are used to heal burns and wounds. The plant is considered as an anthelmintic and antisyphilitic. Its leaves and stems are used as a poultice to treat swellings [5]. An extract of the plant inhibited progression of hepatic injury in mice induced by acetaminophen [6]. The plant contained carotenoids [7, 8], phytoecdysteroids, steroid sapogenins, eupalitin and a caryophyllene type sesquiterpene lactone aspfalcolide [9-13]. The paper describes isolation and characterization of chemical constituents from the aerial parts of *P. falcatus.*

2. Materials and Methods
2.1 General procedure
Melting points were determined on a Perfit apparatus without correction. The infrared (IR) spectra were measured in KBr pellet on a Bio‑Rad Fourier transform‑IR spectrometer (Spectra Lab Scientific Inc., Ontario, Canada). Ultraviolet (UV) spectra were obtained in methanol with a Lambda Bio 20 spectrometer (Perkin‑Elmer, Rotkreuz, Switzerland). ^{1}H (400 MHz) and ^{13}C (100 MHz) nuclear magnetic resonance (NMR) spectra were recorded on Bruker spectrospin spectrometer (Bruker AXS, Karlsruhe, Germany). $CDCl_3$ (Sigma-Aldrich, Bengaluru, India) was used as a solvent and TMS as an internal standard. Electrospray ionization mass spectrometry (ESI MS) analyses were performed on a Waters Q‑TOF Premier (Micromass MS Technologies, Manchester, UK) mass spectrometer. Column chromatography separations were carried out on silica gel (Merck, 60–120 mesh, Mumbai, India). Precoated silica gel plates (Merck, Silica gel 60 F_{254}) were used for analytical thin layer chromatography and visualized by exposure to iodine vapors and UV radiations.

2.2. Plant material
The aerial parts of *P. falcatus* were collected from Ethiopia and identified by Dr. H. B. Singh, In-charge, Raw Materials Herbarium and Museum, National Institute of Science Communication and Information Resources, New Delhi.

2.3. Extraction and isolation

The air dried coarsely powdered aerial parts of *P. falcatus* (500 g) were extracted with methanol using a Soxhlet apparatus for 18 h. The extract was concentrated in vacuum to yield a brown semisolid mass (112 g). A small portion of the extract was analyzed chemically to determine the presence of different chemical constituents. The extract (100 g) was dissolved in minimum amount of methanol to adsorb on silica gel (60–120 mesh) for preparation of a slurry. The slurry was dried in air and subjected to silica gel column loaded in petroleum ether. The column was eluted with petroleum ether, chloroform and methanol in order of increasing polarity to isolated the following compounds 1-4:

2.4. 1- Hexacosanol (1)

Elution of the column with petroleum ether - chloroform (1:1) gave colorless powder of **1,** recrystallized from acetone, 84 mg, m.p. 79-81 °C; UV λmax (MeOH): 208 nm (log ε 3.3); IR √max (KBr): 3433, 2927, 2857, 1462, 1373, 1262, 1082, 723 cm^{-1}; ^1H NMR ($CDCl_3$): δ 3.56 (2 H, t, J = 12.3 Hz, H_2-1), 1.55 (2 H, m, H_2-2), 1.23 (4H, brs, 2 x CH_2), 1.19 (42 H, brs, 21 x CH_2), 0.83 (3 H, t, J = 6.5 Hz, Me-25); ^{13}C NMR ($CDCl_3$): δ 63.11 (C-1), 32.86 (C-2), 31.91 (C-3), 29.71 (13 x CH_2), 29.59 (CH_2), 29.57 (CH_2), 29.55 (CH_2), 29.53 (CH_2), 29.44 (CH_2), 29.33 (CH_2), 27.76 (CH_2), 24.71 (CH_2), 22.68 (CH_2), 14.15 (Me-25); ESI MS *m/z* (rel. int.): 382 $[M]^+$ ($C_{26}H_{54}O$) (5.5).

2.5. Lupenyl palmitate (2)

Elution of the column with chloroform furnished colorless powder of 2, recrystallized from acetone, 312 mg (0.71% yield), R_f 0.61 (petroleum ether – chloroform, 1:1), m. p. 136-137 ^{0}C, UV λ_{max} (MeOH): 207 nm (log ε 3.8); IR λ_{max} (KBr): 2924, 2852, 1734, 1645, 1461, 1379, 1247, 1026, 981 cm^{-1}; ^1H NMR ($CDCl_3$): δ 4.99 (1H, brs, H_2-29a), 4.65 (1H, brs, H_2-29b), 4.15 (1H, dd, J = 5.5, 9.3 Hz, H-3α), 1.76 (3H, brs, Me-30), 1.13 (3H, brs, Me-23), 1.01 (3H, brs, Me-24), 0.78 (3H, brs, Me-25), 0.63 (3H, brs, Me-26), 0.70 (3H, brs, Me-27), 0.57 (3H, brs, Me-28), 2.07 – 1.26 (28H, m, 12 x CH_2; 4 x CH), 2.26 (2H, t, J = 7.2 Hz, H_2-2′), 1.30 (16H, brs, 8 x CH_2), 1.22 (6H, brs, 3 x CH_2), 0.81 (3H, t, J = 6.0 Hz, Me-16′); ^{13}C NMR ($CDCl_3$): δ 39.20 (C-1), 27.58 (C-2), 80.99 (C-3), 38.42 (C-4), 55.37 (C-5), 18.17 (C-6), 34.36 (C-7), 41.07 (C-8), 50.33 (C-9), 37.78 (C-10), 21.28 (C-11), 27.87 (C-12), 37.82 (C-13), 42.32 (C-14), 29.33 (C-15), 34.16 (C-16), 48.28 (C-17), 48.69 (C-18), 47.98 (C-19), 150.92 (C-20), 31.90 (C-21), 36.68 (C-22), 27.93 (C-23), 21.60 (C-24), 16.48 (C-25), 16.32 (C-26), 14.68 (C-27), 17.68 (C-28), 109.34 (C-29), 20.93 (C-30), 171.01 (C-1′), 42.16 (C-2′), 37.01 (C-3′), 35.56 (C-4′), 29.67 (C-5′), 29.67 (C-6′), 29.67 (C-7′), 29.67 (C-8′), 29.67 (C-9′), 29.65 (C-10′), 29.33 (C-11′), 29.30 (C-12′), 27.02 (C-13′), 23.68 (C-14′), 22.69 (C-15′), 14.08 (C-16′). +ve ESI MS *m/z* (rel.int.): 664 $[M]^+$ ($C_{46}H_{80}O_2$) (21.8).

2.6. Furanosesquaterpene diolide (3)

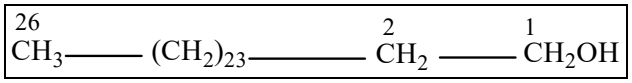
Elution of the column with chloroform – methanol (49:1) afforded colorless crystals of **3,** 110.5 mg; UV λ_{max} (MeOH)**:** 214, 283, 304 nm (log ε 4.1, 5.1, 4.5); IR $\sqrt{}_{max}$ (KBr): 2925, 2837, 1725, 1635, 1545, 1463, 1351, 1258, 1033, 930 cm^{-1}; ^1H NMR ($CDCl_3$): δ 6.93 (1H, d, J = 8.5 Hz, H -28), 6.81 (1H, dd, J = 8.5, 7.5 Hz, H-27), 6.76 (1H, d, J = 7.5 Hz, H-26), 5.10 (1H, m, H-7), 4.97 (1H, s, H_2-33a), 4.94 (1H, s H_2-33b), 4.65 (1H, ddd, J = 4.4, 7.5, 4.3 Hz, H-20α), 4.60 (1H, dd, J = 5.2, 10.8 Hz, H-24α), 2.83 (1 H, J = 4.3, 4.5 Hz, H-9α), 2.33 (1H, m, H-17), 2.29 (1H, J = 3.6, 5.6 Hz, H-5α), 2.17 (1 H, m, H-21), 1.71 (3H, s, Me-31), 1.05 (3H brs, Me-32), 0.97 (3H, brs, Me-30), 0.93 (3H, brs, Me-29), 2.51 – 1.36 (26 H, m, 13 x CH_2); ^{13}C NMR ($CDCl_3$): δ 39.83 (C-1), 21.09 (C-2), 26.42 (C-3), 33.46 (C-4), 48.89 (C-5), 24.38 (C-6), 114.16 (C-7), 131.63 (C-8), 54.85 (C-9), 33.17 (C-10), 27.18 (C-11), 43.96 (C-12), 130.49 (C-13), 41.16 (C-14), 26.41 (C-15), 25.37 (C-16), 40.92 (C-17), 26.45 (C-18), 25.41 (C-19), 69.85 (C-20), 39.89 (C-21), 29.97 (C-22), 41.19 (C-23), 71.75 (C-24), 150.61 (C-25), 146.13 (C-26), 146.15 (C-27), 147.61 (C-28), 22.82 (C-29), 22.73 (C-30), 21.16 (C-31), 24.37 (C-32), 113.71 (C-33), 172.33 (C-34), 172.62 (C-35); ESI MS *m/z* (*rel. int.*): 550 $[M]^+$ ($C_{35}H_{50}O_5$) (2.5).

2.7. Dilupenyl labdandioate (4)

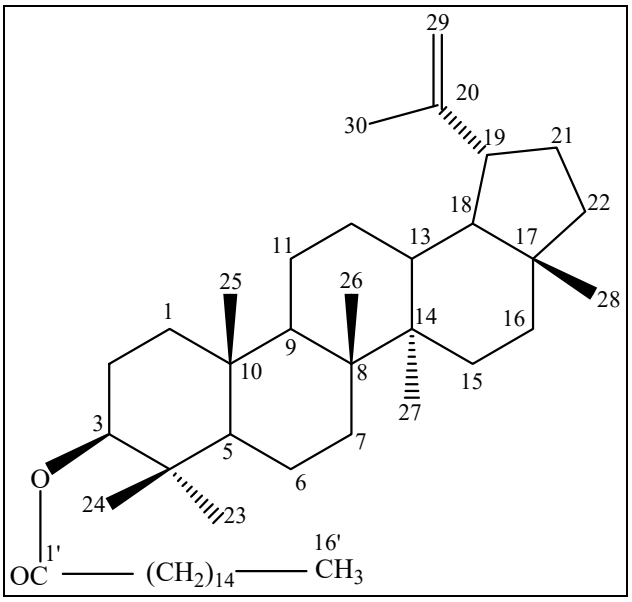
Elution of the column with chloroform – methanol (49:2) yielded colourless powder of 4, recrystallized from acetone, 132 mg, m. p. 159-160 ^{0}C, UV λ_{max} (MeOH): 217 nm (log ε 3.8); IR $\sqrt{}_{max}$ (KBr): 2927, 2842, 1723, 1645, 1471, 1367, 1109, 1036, 931 cm^{-1}; ^1H NMR ($CDCl_3$): δ 4.99 (1H, brs, H_2-29a), 4.69 (1H, brs, H_2-29b), 4.48 (1H, dd, J = 5.5, 8.9 Hz, H-3α), 1.76 (3H, brs, Me-30), 0.99 (3H, brs, Me-23), 0.95 (3H, brs, Me-24), 0.93 (3H, brs, Me-27), 0.89 (3H, brs, Me-26), 0.87 (3H, brs, Me-25), 0.83 (3H, brs, Me-28); 4.79 (1H, brs, H_2-29a′), 4.65 (1H, brs, H_2-29b′), 4.41 (1H, dd, J = 5.3, 8.3 Hz, H-3α′), 1.71 (3H, brs, Me-30′), 1.02 (3H, brs, Me-23′), 0.97 (3H, brs, Me-24′), 0.91 (3H, brs, Me-27′), 0.85 (6H, brs, Me-25′, Me-26′), 0.80 (3H, brs, Me-28′); 5.33 (1H, m, H-7″), 1.67 (3H, brs, Me-18″), 1.05 (3H, brs, Me-16″), 0.84 (3H, brs, Me-17″), 0.75 (3H, t, J = 6.5 Hz, Me-15″); 2.41 – 1.13 (67H, m, 27 x CH_2; 13 x CH); ^{13}C NMR ($CDCl_3$): δ 40.03 (C-1), 27.17 (C-2), 80.97 (C-3), 40.85 (C-4), 55.47 (C-5), 18.23 (C-6), 34.22 (C-7), 42.08 (C-8), 50.46 (C-9), 37.17 (C-10), 21.62 (C-11), 25.17 (C-12), 34.45 (C-13), 42.82 (C-14), 27.43 (C-15), 35.61 (C-16), 42.23 (C-17), 48.35 (C-18), 48.79 (C-19), 150.79 (C-20), 29.83 (C-21), 38.40 (C-22), 27.91 (C-23), 15.92 (C-24), 16.35 (C-25), 16.31 (C-26), 14.73 (C-27), 17.68 (C-28), 109.35 (C-29), 19.42 (C-30); 38.96 (C-1′), 26.65 (C-2′), 80.92 (C-3′), 40.92 (C-4′), 55.43 (C-5′), 18.25 (C-6′), 34.25 (C-7′), 42.10 (C-8′), 50.41 (C-9′), 37.13 (C-10′), 21.52 (C-11′), 25.66 (C-12′), 34.58 (C-13′), 42.37 (C-14′), 27.63 (C-15′), 35.76 (C-16′), 43.05 (C-17′), 48.05 (C-18′), 48.76 (C-19′), 150.61 (C-20′), 29.69 (C-21′), 38.33 (C-22′), 27.97 (C-23′), 15.95 (C-24′), 16.42 (C-25′), 16.02 (C-26′), 14.70 (C-27′), 17.62 (C-28′), 109.17 (C-29′), 19.24 (C-30′); 38.45 (C-1″), 36.37 (C-2″), 26.18 (C-3″), 39.22 (C-4″), 39.37 (C-5″), 20.97 (C-6″), 119.91 (C-7″), 139.83 (C-8″), 34.15 (C-9″), 38.06 (C-10″), 23.72 (C-11″), 34.24 (C-12″), 39.25 (C-13″), 25.44 (C-14″), 14.52 (C-15″), 17.71 (C-16″), 18.15 (C-17″), 22.53 (C-18″), 170.92 (C-19″), 170.65 (C-20″); +ve ESI MS *m/z* (rel.int.): 1152 $[M]^+$ ($C_{80}H_{128}O_4$) (2.8), 727 (5.3), 425 (11.6), 408 (18.2), 218 (13.6), 207 (21.8).

3. Results and Discussion

Compounds 1 and 2 were the known phytoconstituents characterized as 1-hexacosanol [14, 15] and lupenyl palmitate [16, 17], respectively.

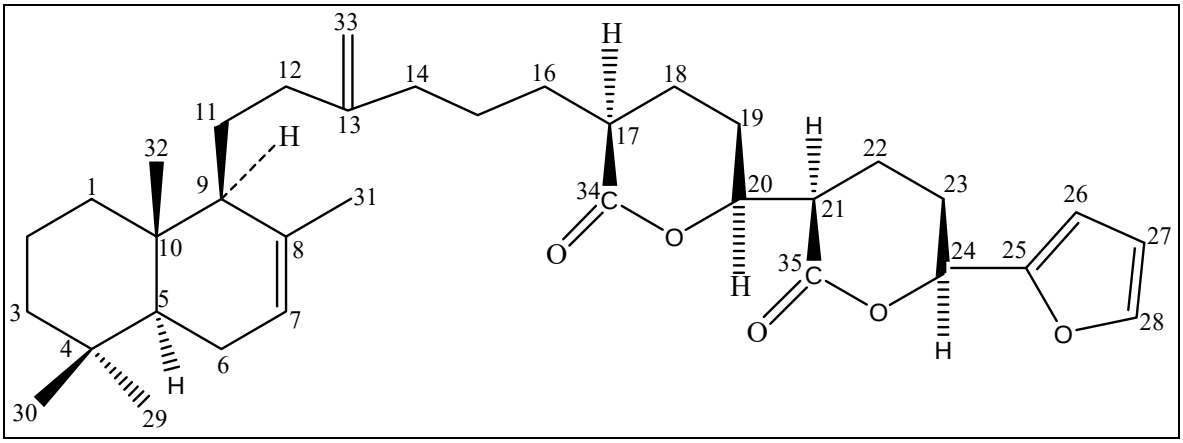


1-Hexacosanol (**1**)



Lupenyl palmitate (2)

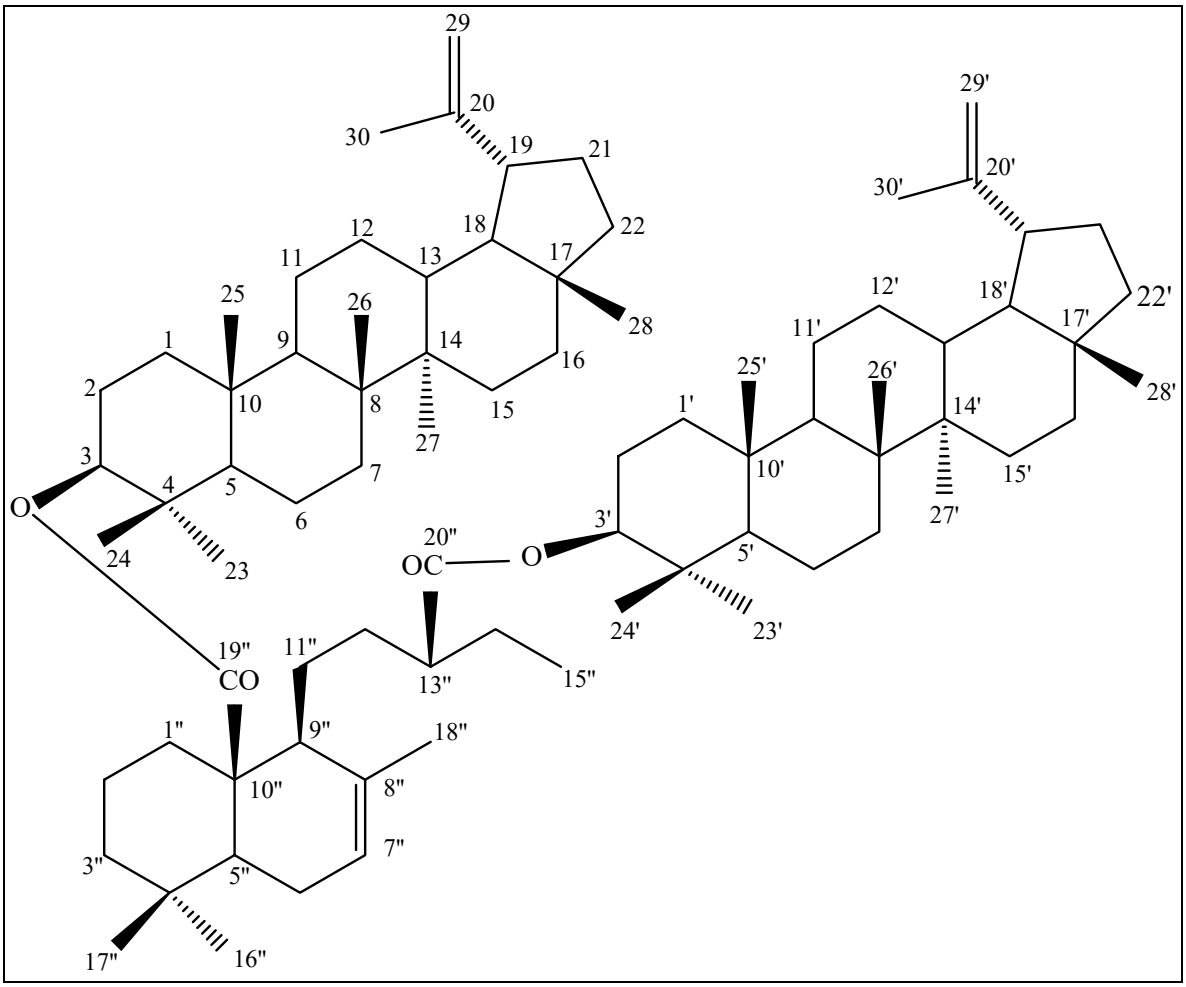
Compound 3, named furanosesquaterpene diolide, showed IR absorption bands for δ-lactone (1725 cm $^{-1}$) and unsaturation (1635 cm^{-1}). Its mass spectrum displayed a molecular ion peak at *m/z* 550 $[M]^+$ consistent with the molecular formula of a sesquaterpenoid, $C_{35}H_{50}O_5$. The ^{1}H NMR spectrum of 3 showed two one-proton doublets at δ 6.93 (J= 8.5 Hz) and 6.76 (J = 7.5 Hz) and a one-proton double doublet at δ 6.81 (J = 8.5, 7.5 Hz) assigned to furanic H-28, H-26 and H-27 protons, respectively. A one-proton multiplet at δ 5.10 was accounted to vinylic H-7 proton. Two one-proton broad singlets at δ 4.97 and 4.94 were ascribed to unsaturated methylene H_2-33. A one-proton doublet of double doublet at δ 4.65 (J = 4.4, 7.5, 4.3 Hz) and a one-proton double doublet at δ 4.60 with coupling interactions of 5.2 and 10.8 Hz were attributed correspondingly to α-oriented H-20 and H-24 oxygenated methine protons. Two one-proton double doublets at δ 2.29 (J = 3.6, 5.6 Hz) and 2.83 (J = 4.3, 4.5 Hz) and two one-proton multiplets at δ 2.33 and 2.17 were associated with α-oriented H-5, H-9, H-17 and H-21 methine protons, respectively. Four three-proton broad singlets at δ 0.93, 0.97, 1.05 and 1.71 were assigned to tertiary C-29, C-30, C-32 and C-31 methyl protons, respectively. The remaining methine and methylene protons appeared from δ 2.51 to 1.36. The ^{13}C NMR spectrum of 3 displayed signals for δ-lactone carbons at δ 172.33 (C-34) and 172.62 (C-35), furanic carbons from δ 150.61 to 146.13, vinylic carbons at δ 131.63 (C-8), 130.49 (C-13), 114.16 (C-7) and 113.71 (C-33), oxygenated methine carbons at δ 69.85 (C-20) and 71.75 (C-24), methyl carbons at δ 22.82 (C-29), 22.73 (C-30), 21.16 (C-31) and 24.37 (C-32), methine carbons at δ 48.89 (C-5), 54.84 (C-9), 40.92 (C-17) and 39.89 (C-21) and methylene carbons between δ 41.19 – 21.09. The degree of protonation of each carbon was determined by DEPT experiment which showed the presence of four methyl, fourteen methylene, ten methine and seven quaternary carbons. The ^{1}H – ^{1}H COSY spectrum of 3 exhibited correlations of H-24 with H_2-23 and H-26; H-27 with H-26 and H-28; H-20 with H-21 and H_2-19; and Me-31 with H-7 and H-9. On the basis of these evidences the structure of 3 has been elucidated as 4,4,8,10β-tetramethyl-9β-[(13-methylene), 20α,34-; 24α,35-diolide-24-furanyl]-tetradecanyl-*trans*-decalin. This is a new sesquaterpenic compound.



Furanosesquaterpene diolide (3)

Compound 4, named dilupenyl labdandioate, showed IR characteristic absorption bands for ester groups at 1723 cm $^{-1}$ and unsaturation at 1645 cm^{-1}. On the basis of mass and ^{13}C NMR spectra its molecular ion peak was determined at *m/z* 1152 consistent with a molecular formula of a dilupenyl diterpenic dioic acid ester, $C_{80}H_{128}O_4$. The ion peaks arising at *m/z* 425 [$C_{30}H_{49}O$, $C_{19''}$-O fission]$^+$, 725 [M – 425]$^+$, 408 [425 -- OH]$^+$ and 218, 207 [$C_{8,14}$ – $C_{9,11}$ fission]$^+$ indicated that two lupenyl units were linked to a labdenedioic acid. The ^{1}H NMR spectrum of 4 exhibited four one-proton singlets at δ 4.58, 4.55 and 4.72, 4.65 assigned to vinylic methylene H_2-29 and H_2-29′ protons, respectively. A one-proton multiplet at δ 5.33 was ascribed to vinylic methine H-7″ proton. Two one-proton double doublets at δ 4.48 (J = 5.5, 8.6 Hz) and 4.41 (J = 5.3, 7.9 Hz) were attributed to α-oriented oxygenated methine H-3 and H-3′ protons, respectively, and their deshielding nature suggested location of these protons at the ester functions. Three three-proton broad singlets at δ 1.70, 1.71 and 1.67 were accounted to C-30, C-30′ and C-18″ methyl protons attached to the vinylic carbons. A three-proton triplet at δ 0.75 (J = 6.5 Hz) was due to C-15″ primary methyl protons. The remaining tertiary methyl protons resonated between δ 1.05 – 0.80. The ^{13}C NMR spectrum of 4 displayed 80 carbon signals and the important signals appeared for ester carbons at δ 170.92 (C-19″) and 170.65 (C-20″), vinylic carbons at δ 150.79 (C-20), 150.61 (C-20′), 139.83 (C-8″), 109.35 (C-29),

107.19 (C-29′) and 119.91 (C-7″), oxygenated methine carbons at 80.97 (C-3) and 80.75 (C-3′) and methyl carbons between δ 27.97 -14.52. The ^{1}H and ^{13}C NMR values of the triterpenic units of 4 were compared with the related lupenol-type compounds [18-20]. The multiplicity of each carbon was determined by DEPT spectrum which showed the presence of 18 methyl, 29 methylene and 16 methine carbons. The ^{1}H-^{1}H COSY spectrum of 4 showed interactions of H-3 with H_2-1, H_2-2 and Me-23; H_2-29 with H-19 and Me-30; H-3′ with H_2-1′, H_2-2′ and Me-23′; H_2-29′ with H-19′ and Me-30′; H-7″ with H-5″, H_2-6″, H-9″ and Me-18″; and Me-15″ with H-13″ and H_2-14″. On the basis of the foregoing account the structure of 4 has been formulated as dilup-20 (29)-enyl labd-7″-en-19″, 20″-dioate. This is a new dilupene-type triterpenic ester.



Dilupenyl labdandioate (4)

4. Conclusion

Phytochemical investigation of a methanolic extract of the aerial parts of *P. falcatus* yielded two new chemical constituents furanosesquaterpene diolide and dilupenyl labdandioate along with the known compounds 1-hexacosanol and lupenyl palmitate. These compounds may be used as chromatographic markers for standardization of the *P. falcatus* plant parts.

5. Acknowledgements

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