Acta Poloniae Pharmaceutica – Drug Research, Vol. 71 No. 1 pp. 205–212, 2014 ISSN 0001-6837
Polish Pharmaceutical Society

SHORT COMMUNICATION

NEW ABIETATRIENE-TYPE DITERPENES LINKED WITH LANOSTENES FROM OLEO-RESIN OF *PINUS ROXBURGHII* SARG.

MOHD. SHUAIB, MOHAMMED ALI* and KAMRAN J. NAQUVI

Phytochemical Research Laboratory, Department of Pharmacognosy and Phytochemistry, Faculty of Pharmacy, Jamia Hamdard, New Delhi-110062, India

Keywords: *Pinus roxburghii*, Pinaceae, colophony, triterpene-linked abietatrienic acids, structure elucidation

Pinus roxburghii Sarg., syn. *Pinus longifolia* Roxb. (Pinaceae), commonly known as chir pine, is a tall tree with a spreading crown found in the Himalayan from Kashmir to Bhutan, Afghanistan, Pakistan, China, Nepal and in southern Indian hills. It is also planted in the garden for ornamental purpose. The tapping of the stem produces a clear, transparent oleo-resin with the pungent and bitter taste. Distillation of the turpentine oil from the oleo-resin leaves faintly aromatic and transparent rosin (colophony). It is utilized in the manufacturing of fireworks, insecticides and disinfectants and enters into certain lubricating compositions, hair fixing and nail polishing preparations (1). It is used in preparation of ointments and plasters and in many products such as chewing gum, polishes, and varnishes, but is a common cause of contact allergy. The resin is applied to cure boils (2) and administered orally to combat gastric troubles (3). The rosin is useful as pharmaceutical aids in adhesives, printing ink, electric isolation, paper, soldering flux, varnish and matches. In printing ink industry rosin gives adhesiveness, surface smoothness, hardness, antiblocking and other properties. Rosin has a good electric isolation, being used as oil in cables for high voltage electricity. In soldering process, rosin is beneficial to get rid of oxide compounds in the surface of metal, synthetic rubber and chewing gums (4). Native Americans have used pine resin to treat rheumatism because of its anti-inflammatory properties. The resin acts to remove the joint inflammation caused by rheumatism, which helps to restore movement and to alleviate pain. The Costanoan Indians gained these benefits by chewing on the gum-like resin. A traditional use for pine resin has been as an external treatment for burns and sores. The pine resin has stimulant, diuretic and laxative properties. In China, the resin from a particular pine tree is used to treat abscesses. Resin from the spruce tree was used by colonial Americans as a cold and cough remedy, as well as straight from the tree as a cancer treatment. Physicians in colonial America also recommended tar water, or ground pine resin mixed with water, as a remedy for ulcers, smallpox, and syphilis (5). Different parts of the plant are prescribed to treat cough, colds, influenza, tuberculosis, bronchitis, as antiseptic, diaphoretic, diuretic, rubefacient, stimulant and febrifuge (6, 7). Rosin consists mainly a mixture of diterpenic acids. The principal acid is abietic acid (37.5%) followed by isopimaric (20.9%), neoabietic (15.1%), levopimaric acid (13.5%), pimaric and dihydroabietic acids. In this paper, we report the isolation and structure elucidation of four triterpenoic acids linked with dehydroabietic acid derivatives obtained from the colophony of *Pinus roxburghii* Sarg., collected from Haldwani (Uttarakhand).

EXPERIMENTAL

Melting points were determined on a Perfit melting apparatus (Ambala, Haryana, India) and are uncurrected. UV spectra were measured with a Lambda Bio 20 spectrophotometer (Perkin-Elmer-Rotkreuz, Switzerland) in methanol. Infra red spectra were recorded on Bio-Rad FTIR 5000 (FTS 135, Kawloon, Hong Hong) spectrophotometer using KBr pellets; ν_{max} values are given in cm^{-1}. ^{1}H and ^{13}C NMR spectra were screened on advance DRX 400, Bruker

* Corresponding author: email: maliphyto@gmail.com

spectrospin 400 and 100 MHz instrument in 5 mm spinning tubes at 27°C, respectively (Karlesruthe, Germany), using TMS as an internal standard. Mass spectra were scanned by effecting FAB ionization at 70 eV on a JEOL-JMS-DX 303 spectrometer (Japan) equipped with direct inlet probe system. Column chromatography was performed on silica gel (60–120 mesh; Qualigen, Mumbai, India). TLC was run on silica gel G (Qualigen). Spots were visualized by exposing to iodine vapours, UV radiation, and spraying with ceric sulfate solution.

Plant material

The oleo-resin was procured from a Rosin factory, Haldwani, Uttarakhand. The sample was identified on the basis of exomorphic characters, chemical reactions and reviews of literature by Dr. H.B. Singh, Taxonomist, NISCAIR, CSIR, New Delhi. A voucher specimen of the sample (No. N/R/C/2007/08/851/35) was deposited in the RHM Division, NISCAIR, New Delhi-110012.

Extraction and isolation

The air dried oleo-resin (220 g) was coarsely powdered and dissolved in methanol. The concentrated solution was adsorbed on silica gel particles. It was dried in the air and pulverized to get uniform particle size and chromatographed over silica gel (60–120 mesh) column packed in petroleum ether (b.p. 60–80°C). The column (1.6 m × 16 mm × 2 mm) was eluted successively with petroleum ether, mixture of petroleum ether and chloroform (9 : 1, 3 : 1, 1 : 1, and 1 :3, v/v), chloroform and finally the mixture of chloroform and methanol (99 : 1, 97 : 3, 19 : 1, 23 : 2, 9 : 1, 3 : 1, 1 : 1, 1 : 3, v/v). Various fractions were collected separately and matched by TLC to check homogeneity. Similar fractions having the same R_f values were combined and crystallized. The isolated compounds were recrystallized to get pure compounds. The following compounds were isolated.

Dehydroabietic acid (1)

Further elution of the column with petroleum ether-chloroform (3 : 1. v/v) produced light brown amorphous powder of **1**, recrystallized with methanol-acetone (1 : 1, v/v), 0.24 g (0.109% yield); R_f: 0.86 (chloroform-methanol; 3 : 1, v/v); m.p.: 295–297°C; IR ν_{max} (KBr, cm^{-1}): 3432, 3020, 1705, 1599, 1526; ^{1}H NMR (DMSO-d_6, δ, ppm): 7.63 (1H, m, H-14), 7.11 (1H, m, H-12), 7.06 (1H, d, *J* = 9.3 Hz, H-11), 2.48 (2H, m, H-7), 2.25 (2H, m, H-1), 2.19 (1H, m, H-15), 2.05 (1H, m, H-5), 1.52 (2H, m, H-2), 1.31 (2H, m, H-3), 1.29 (2H, m, H-6), 1.21 (3H, brs, H-19), 1.04 (3H, brs, Me-20), 0.83 (3H, d, *J* = 6.1 Hz, Me-16), 0.81 (3H, d, *J* = 6.3 Hz, Me-17); positive ion FAB MS m/z (rel. int.): 300 $[M]^+$ ($C_{20}H_{28}O_2$) (36.2).

12-Hydroxydehydroabietic acid (2)

Elution of the column with petroleum ether-chloroform (1 : 1, v/v) produced buff amorphous powder of **2**, recrystallized from methanol-acetone (3 : 1, v/v), 82 mg (0.037% yield); R_f: 0.73 (chloroform-methanol; 3 : 1, v/v); m.p.: 310–312°C; IR ν_{max} (KBr, cm^{-1}): 3518, 3455, 1704, 1525, 1424, 1216, 1044, 928; ^{1}H NMR ($CDCl_3$, δ, ppm): 7.13 (1H, brs, H-11), 7.04 (1H, brs, H-14), 1.16 (3H, brs, Me-20), 1.03 (3H, brs, Me-19), 0.97 (3H, d, *J* = 6.9 Hz, Me-17), 0.84 (3H, d, *J* = 8.4 Hz, Me-16), 2.74-1.26 (12H, m, 5×CH_2, H-5, H-15); ^{13}C NMR ($CDCl_3$, δ, ppm): 16.05 (C-19), 17.25 (C-20), 17.93 (C-6), 20.94 (C-17), 23.47 (C-16), 29.15 (C-2), 34.78 (C-7), 35.85 (C-3), 35.91 (C-15), 36.27 (C-1), 37.35 (C-10), 45.89 (C-4), 50.37 (C-5), 123.36 (C-14), 127.38 (C-11), 131.52 (C-8), 145.45 (C-9), 151.16 (C-13), 165.76 (C-12), 180.03 (C-18); positive ion FAB MS m/z (rel. int.): 316 $[M]^+$ ($C_{20}H_{28}O_3$) (24.8), 253 (36.1).

Pinusoic acid A (3)

Elution of the column with petroleum ether-chloroform (1 : 3, v/v) produced light yellow amorphous powder of **3**, recrystallized from acetone, 1.02 g (0.463% yield). R_f: 0.75 (petroleum ether-chloroform; 1 : 3, v/v), m.p.: 100–102°C; UV λ_{max} (MeOH): 219, 266, 301 nm (log ε 3.1, 5.2, 4.7); IR ν_{max} (KBr, cm^{-1}): 3450, 3360, 3019, 2939, 2852, 2360, 1697, 1645, 1386, 1216, 1043, 928; ^{1}H NMR ($CDCl_3$, δ, ppm): 7.13 (1H, d, *J* = 9.0 Hz, H-12'), 7.08 (1H, d, *J* = 9.0, H-11'), 5.21 (1H, d, *J* = 4.2 Hz, H-6), 5.03 (1H, dd, *J* = 5.3, 6.5 Hz, H-22), 4.92 (1H, m, $w_{1/2}$ = 9.6 Hz, H-23), 4.50 (1H, dd, *J* = 5.1, 9.2 Hz, H-3α), 3.36 (1H, brs, H-19'α), 3.32 (1H, brs, H-19'β), 1.27 (3H, d, *J* = 6.0 Hz, Me-27), 1.16 (3H, brs, Me-28), 1.15 (3H, brs, Me-20'), 1.13 (3H, brs, Me-30), 1.03 (3H, brs, Me-19), 1.01 (3H, brs, Me-29), 0.95 (3H, d, *J* = 6.9 Hz, Me-21), 0.93 (3H, d, *J* = 6.9 Hz, Me-17'), 0.84 (3H, d, *J* = 6.6 Hz, Me-16'), 0.76 (3H, brs, Me-18'), 2.74-1.34 (32 H, m, 13×CH_2, 6×CH); ^{13}C NMR ($CDCl_3$, δ, ppm): Table 1; positive ion FAB MS m/z (rel. int.): 770 $[M]^+$ ($C_{50}H_{74}O_6$) (1.6), 438 (1.1), 332 (25.2), 301 (64.5), 291 (18.1), 289 (20.5), 287 (21.3), 256 (36.2), 215 (23.8), 141 (59.9).

Pinusquinoic acid (4)

Elution of the column with chloroform-methanol (97 : 3, v/v) produced brown solid mass of **4**, recrystallized from methanol, 65 mg (0.0295% yield); R_f: 0.54 (chloroform-methanol 97 : 3, v/v); m.p.: 68–69°C; UV λ_{max} (MeOH): 212, 267, 310 nm

(log ε 3.2, 3.0, 2.1); IR ν_{max} (KBr): 3418, 2921, 2358, 1725, 1710, 1702, 1698, 1599, 1460, 1331, 1219 cm^{-1}; ^1H NMR (DMSO-d_6, δ, ppm): 9.22 (1H, brs, H-28), 9.02 (1H, brs, H-30), 6.71 (1H, brs, H-12), 5.75 (1H, d, *J* = 5.8 Hz, H-7), 5.71 (1H, d, *J* = 5.8 Hz, H-6), 5.03 (1H, m, $w_{1/2}$ = 9.2 Hz, H-22), 4.93 (1H, m, $w_{1/2}$ = 8.5 Hz, H-23), 4.69 (2H, brs, H_2-27), 4.39 (1H, dd, *J* = 5.5, 9.0 Hz, H-3α), 3.28 (1H, brs, H_2-19' α), 3.22 (1H, brs, H_2-19' β), 1.13 (3H, d, *J* = 5.9 Hz, Me-16'), 1.08 (3H, d, *J* = 6.0 Hz, Me-17'), 1.02 (3H, brs, Me-19), 0.97 (3H, brs, Me-20'), 0.91 (3H, d, *J* = 7.5 Hz, Me-21), 0.86 (3H, brs, Me-29), 0.63 (3H, brs, Me-18), 2.79–1.40 (27 H, m, 11×CH_2, 5×CH); ^{13}C NMR ($CDCl_3$, δ, ppm): Table 1; positive ion FAB-MS m/z (rel. int.): 832 $[M]^+$ ($C_{50}H_{72}O_{10}$) (2.1), 462 (19.6), 362 (23.4), 360 (18.3), 331 (8.9), 317 (29.5), 315 (36.3), 289 (51.8), 272 (32.0), 241 (28.2), 226 (22.1), 221 (19.8), 197 (41.5), 185 (20.2), 183 (24.8), 169 (31.6), 156 (43.0), 141 (35.7), 121 (73.2), 107 (75.2).

Pinusoic acid B (5)

Elution of the column with chloroform-methanol (9 : 1, v/v) produced brown solid mass of **5**, recrystallized from methanol, 72 mg (0.0327% yield); R_f: 0.74 (chloroform-methanol; 9 : 1, v/v); m.p.: 65–66°C; UV λ_{max} (MeOH): 217, 266, 301 nm (log ε 3.1, 4.7, 4.1); IR ν_{max} (KBr, cm^{-1}): 3405, 2939, 2850, 2361, 1710, 1700, 1650, 1449, 1385, 1216, 1041; ^1H NMR (DMSO-d_6, δ, ppm): 7.11 (1H, d, *J* = 3.0 Hz, H-14'), 7.00 (1H, dd, *J* = 3.0, 9.5 Hz, H-12'), 6.89 (1H, d, *J* = 9.5 Hz, H-10), 5.31 (1H, m, H-6), 5.23 (1H, m, $w_{1/2}$ = 9.3 Hz, H-22), 5.01 (1H, m, $w_{1/2}$ = 8.7 Hz, H-23), 4.13 (1H, dd, *J* = 5.5, 9.0 Hz, H-3α), 3.53 (1H, brs, H_2-19'α), 3.49 (1H, brs, H_2-19'β), 1.18 (3H, brs, Me-20'), 1.12 (3H, brs, Me-30), 1.06 (3H, brs, Me-29), 1.04 (3H, brs, Me-19), 1.01 (3H, brs, Me-28), 0.98 (3H, d, *J* = 6.3 Hz, Me-17'), 0.95 (3H, d, *J* = 6.3 Hz, Me-21), 0.82 (3H, d, *J* = 6.2 Hz, Me-16'), 0.80 (3H, d, *J* = 6.1 Hz, Me-27), 0.72 (3H, brs, Me-18), 2.73–1.45 (31H, m, 12×CH_2, 7×CH); ^{13}C NMR (DMSO-d_6, δ, ppm): Table 1; positive ion FAB MS m/z (rel. int.): 768 $[M]^+$ ($C_{50}H_{72}O_6$) (1.3), 439 (6.1), 410 (19.2), 394 (18.3), 330 (21.5), 299 (58.3), 298 (21.3), 287 (17.3), 272 (21.2), 141 (61.9).

Pinusoic acid C (6)

Elution of the column with chloroform-methanol (3 : 1, v/v) produced brown crystalline powder of **6**, recrystallized from methanol 100%, 55 mg (0.025% yield); R_f: 0.83 (chloroform-methanol; 3 : 1, v/v); m.p.: 80–81°C; UV λ_{max} (MeOH): 227, 269, 303 nm (log ε 3.1, 4.9, 4.2); IR ν_{max} (KBr, cm^{-1}): 3409, 3380, 3016, 2941, 2837, 1699, 1645, 1446, 1386, 1216, 1044; ^1H NMR (DMSO-d_6, δ, ppm): 7.39 (1H, brs, H-11'), 6.83 (3H, brs, H-14'), 5.33 (1H, d, *J* = 4.5 Hz, H-6), 5.09 (1H, m, $w_{1/2}$ = 9.5 Hz, H-22), 4.90 (1H, m, $w_{1/2}$ = 8.3 Hz, H-23), 4.43 (1H, dd, *J* = 5.1, 9.2 Hz, H-6), 3.40 (2H, brs, H_2-19'), 1.26 (3H, d, *J* = 6.3 Hz, Me-27), 1.16 (3H, brs, Me-30), 1.14 (3H, brs, Me-20'), 1.05 (3H, brs, Me-28), 1.03 (3H, brs, Me-29), 1.01 (3H, brs, Me-19), 0.93 (3H, d, *J* = 6.1 Hz, Me-21), 0.91 (3H, d, *J* = 6.4 Hz, Me-17'), 0.85 (3H, d, *J* = 6.5 Hz, Me-16'), 0.72 (3H, brs, Me-18), 2.48–1.32 (33H, m, 13×CH_2, 7×CH); ^{13}C NMR (DMSO-d_6, δ, ppm): Table 1; positive ion FAB MS m/z (rel. int.): 770 $[M]^+$ ($C_{50}H_{74}O_6$) (2.2), 438 (11.6), 334 (38.2), 317 (65.1), 303 (66.3), 297 (31.6), 291 (22.8), 289 (27.5), 141 (53.5), 134 (80.6), 109 (82.1), 95 (100).

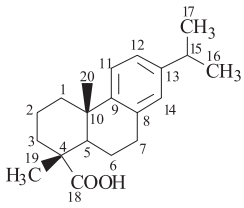
RESULTS AND DISCUSSION

Compound **1** and **2** are is the known phytoconstituents identified as dehydroabietic acid and 12-hydroxydehydroabietic acid, respectively (8–11).

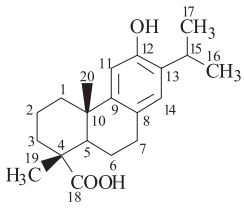
Compound **3**, designated as pinusoic acid A, was obtained as a light yellow amorphous powder from petroleum ether-chloroform (1 : 3, v/v) eluants. It produced effervescences with sodium bicarbonate solution and green color with ferric chloride solution. Its IR spectrum displayed characteristic absorption bands for hydroxyl group (3450 cm^{-1}), carboxylic group (3360, 1697 cm^{-1}) and unsaturation (1645 cm^{-1}). On the basis of ^{13}C NMR and positive FAB mass spectra, it displayed a molecular ion peak at m/z 770 consistent with the molecular formula of a triterpene linked with diterpene, $C_{50}H_{74}O_6$. The important ion peaks arose at m/z 438 $[C_{30}H_{46}O_2]^+$ and 332 $[C_{20}H_{28}O_4]^+$ due to the cleavage of triterpenic linkage attached to diterpenic moiety. The subsequent ion fragments of diterpenic acid moiety arising at m/z 287 $[332\text{-}COOH]^+$, 301 $[332\text{-}CH_2OH]^+$, 256 $[301\text{-}COOH]^+$, 289 $[332\text{-}C_3H_7]^+$ suggested the presence of carboxylic, oxygenated methylene and hydroxy groups in it. The fragments of the triterpenic moiety generating ions at m/z 141 $[C_8H_{13}O_2$; side chain, SC$]^+$ and 291 $[432\text{-}SC]^+$ supported the presence of a monounsaturated C_8-side chain with one carboxylic group located in a tetracyclic triterpenic moiety with one vinylic linkage. The ^1H NMR spectrum of **3** exhibited two one-proton doublets at δ 7.08 (*J* = 9.0 Hz) and 7.13 (*J* = 9.0 Hz) ppm assigned correspondingly to *ortho*-coupled H-11' and H-12' aromatic protons. Two one-proton broad signals at δ 3.36 and 3.32 ppm were ascribed to oxygenated H_2-19' methylene protons. Two doublets at δ 0.84 (*J* = 6.6 Hz) and 0.93 (*J* = 6.9 Hz) ppm and a broad singlet at δ 1.15 ppm, three-protons each, were attributed to secondary Me-

16', Me-17' and tertiary Me-20' methyl protons of the diterpene unit, respectively. A doublet at δ 5.21 (*J* = 4.2 Hz) ppm, a double doublet at δ 5.03 (*J* = 5.3 Hz, 6.5 Hz) ppm and a multiplet at δ 4.92 ($w_{1/2}$ = 9.6 Hz) ppm, were attributed correspondingly to *cis*-vinylic H-6, H-22 and H-23 protons of the triterpenic unit. A double-doublet at δ 4.50 (*J* = 5.1, 9.2 Hz) ppm was ascribed to α-oriented oxygenated methine H-3 proton. Five broad singlets at δ 0.76, 1.03, 1.16, 1.01 and 1.13 ppm were ascribed to tertiary C-18, C-19, C-28, C-29 and C-30 methyl protons, respectively, all attached to saturated carbons. Two three-proton doublets at δ 0.95 (*J* = 6.9 Hz) and 1.27 (*J* = 6.0 Hz) ppm were attributed to secondary C-21 and C-27 methyl protons, respectively. The ^{13}C NMR spectrum of **3** exhibited signals for carboxylic carbons at δ 178.85 (C-26) and 178.79 C-18') ppm; aromatic carbons between δ 123.66–165.76 ppm, vinylic carbons at δ 145.45 (C-5), 122.14 (C-6), 127.38 (C-22) and 123.36 (C-23) ppm, carbinol carbon at δ 70.03 ppm (C-3) and oxygenated methylene carbon at δ 65.21 ppm (C-19'). The shifting of oxygenated H-3 methine proton in the downfield region at δ 4.50 ppm and oxygenated C-19' methylene carbon at δ 65.21 ppm suggested linkage of triterpenoid with C-19' methylene carbon. The ^{1}H and ^{13}C NMR spectral data of **3** were compared with the values of the reported lanostene type triterpenoids (12–15). The spectral data of the abietatriene unit were compared with the reported values of the similar compounds (16–18). The ^{1}H-^{1}H COSY spectrum of **3** showed correlation of H-3 with H-2, Me-28 and H-19; H-6 with H-7; H-22 with H-20, Me-21 and H-23; H-11 with H-12 and Me 20; and H-14 with H-7 and H-12. The HMBC spectrum of **3** displayed interactions of H-2 and H-19 with C-3; H-6, Me-19 and Me 28 with C-5; H-20, Me 21 and H-23 with C-22; Me-27 with C-26; H-19 with C-18; and H-11, H-12 H-14, H-15, Me-16 and Me-17 with C-13. On the basis of above discussion the structure of **3** was elucidated as lanost-5,22-diene-26-oic acid-3β-olyl-(3→19')-dehydroabietic acid (Fig. 1). This is a new dimer form of lanostenoic acid linked with dehydroabietic acid.

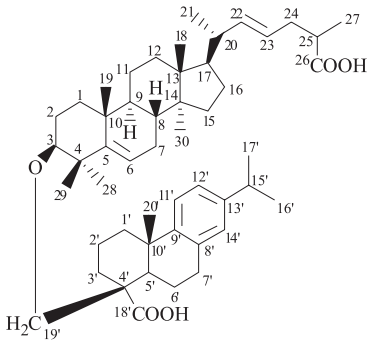
Compound **4,** designated as quinoroxburghianoic acid, was obtained as a brown solid mass from chloroform-methanol (97 : 3, v/v) eluants. It produced effervescences with sodium bicarbonate solution. Its IR spectrum displayed absorption bands for aldehydic group (1702 cm^{-1}), carboxylic function (3418, 1698 cm^{-1}) and oxo groups (1725, 1710 cm^{-1}). On the basis of mass and ^{13}C NMR spectra, its molecular weight was established at m/z 832 consistent with the molecular formula of a triterpene linked with abietaquinone, $C_{50}H_{72}O_{10}$. The important fragment ion peaks arose due to the cleavage of ether linkage at m/z 360 $[C_{20}H_{24}O_6]^+$ and 462 $[C_{30}H_{38}O_4]^+$. The subsequent fragment ions of the diterpenic unit arising at m/z 315 [360-COOH]$^+$, 272 [315-C_3H_7]$^+$, 241 [272-CH_2OH]$^+$ and 317 [360-C_3H_7]$^+$ indicated the presence of an oxygenated methylene, isopropyl chain and carboxylic group in the diterpenic moiety of **4**. The ion fragments of the triterpenic moiety generated at m/z 139 [$C_8H_{11}O_2$· side chain, SC]$^+$, 323 [462-SC]$^+$, 433 [462-CHO]$^+$, 404 [433-CHO]$^+$ and 447 [462-Me]$^+$ indicated the presence of two aldehydic groups and a C_8 side chain with a carboxylic group and two double bonds. The ^{1}H NMR spectrum of **4** exhibited a downfield one-proton broad signal at δ 6.71 ppm assigned to H-12' quinone proton. Two one-proton broad signals at δ 3.28 and 3.22 ppm were ascribed to oxygenated H_2-19' methylene protons of the diterpenic unit. Two doublets at δ 1.13 (*J* = 5.9 Hz) and 1.08 (*J* = 6.0 Hz) ppm and one broad singlet at δ 0.97 ppm, three-protons each, were attributed to secondary Me-16', Me-17' and tertiary Me-20' methyl protons, respectively. Two one-proton broad signals appearing in the downfield region at δ 9.22 and 9.01 ppm were due to aldehydic H-28 and H-30 protons, respectively. Two one-proton doublets at δ 5.71 (*J* = 5.8 Hz) and 5.75 (*J* = 5.8 Hz) ppm and two multiplets at δ 5.23 ($w_{1/2}$ = 9.2 Hz) and 4.93 ($w_{1/2}$ = 8.5 Hz) ppm were ascribed correspondingly to *cis*-oriented vinylic H-6, H-7, H-22 and H-23 protons. A two-proton broad singlet at δ 4.69 ppm was attributed to methylene H-27 protons. A double doublet at δ 4.39 (*J* = 5.5, 9.0 Hz) ppm was accounted to oxygenated methine H-3a proton. Three three-proton broad singlets at δ 0.63, 1.02 and 0.86 ppm were assigned to tertiary C-18, C-19 and C-29 methyl protons, respectively. A three-proton doublet at δ 0.91 (*J* = 6.3 Hz) ppm was due to secondary C-21 methyl protons of triterpene unit. The ^{13}C NMR spectrum of **4** displayed important signals for aldehydic carbons at δ 206.66 (C-28), 202.39 (C-30) ppm, oxo carbons at δ 199.60 (C-11'), 199.12 (C-14') and 194.40 (C-3') ppm, carboxylic carbons at δ 182.82 (C-26) and 181.35 (C-18') ppm, vinylic carbons between δ 151.91–106.48 ppm, oxygenated methine at δ 76.58 ppm (C-3) and oxygenated methylene carbon at δ 65.02 ppm (C-19'). The shifting of C-19' carbon signal in the deshielding region at δ 65.02 ppm supported its linkage to C-3 methine carbon through oxygen. The ^{1}H and ^{13}C NMR spectral data of **4** were compared with the values of the reported lanostene type triterpenoids (12–15) The spectral data of the abietatriene unit were compared with the reported values of the similar compounds (16–18). The ^{1}H-^{1}H COSY spectrum of **4** exhibited



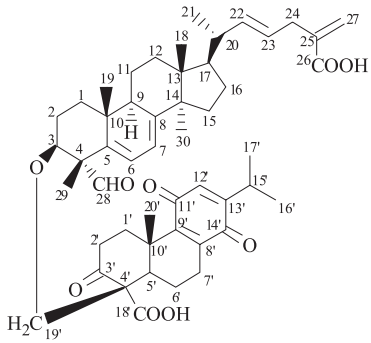
1



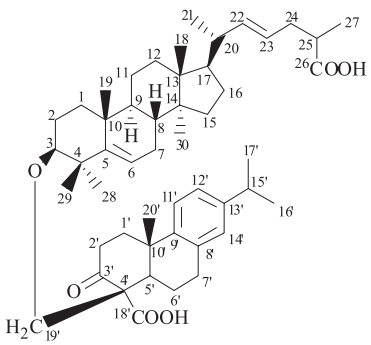
2



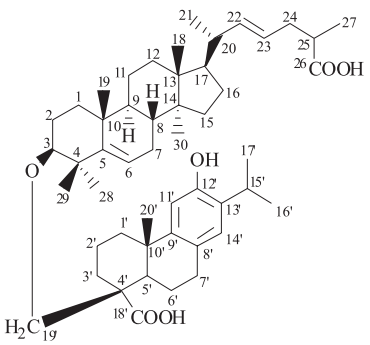
3



4



5



6

Figure. 1. Structures of compound **1**, **2**, **3**, **4**, **5** and **6**

correlations of H-3 with H-2, Me-28 and H-19; H-7 with H-6 and H-9; H-22 with H-20, Me-21 and H-23; H_2-27 with H-24; and H-12 with H-15. The HMBC spectrum of **4** displayed interactions of H-2, Me-28 and H-19 with C-3; H-6, H-7 and H-9 with C-8; H-20, Me-21 and H-23 with C-22; H-24 with C-26; and H-7 and H-12 with C-14. On the basis of the spectral data analysis, the structure of **4** has been formulated as lanost-5,7,22,25(27)-tetraen-26-oic acid-28,30-dial-3β-olyl-(3→19')-3-oxoabiet-11',14'-quinone-18'-oic acid (Fig. 1). This is a new triterpenic linked abietaquinoic acid.

Compound **5**, named pinusoic acid B, was obtained as a brown solid mass from chloroform-methanol (9 : 1, v/v) eluants. It produced effervescences with sodium bicarbonate solution. Its IR spectrum displayed absorption bands for carboxylic group (3405, 1700 cm^{-1}), keto group (1710 cm^{-1}), and unsaturation (1650 cm^{-1}). On the basis of ^{13}C NMR and positive FAB mass spectra, it displayed a

Table 1. ^{13}C NMR spectral values of **3**, **4**, **5** and **6**.

Position	^{13}C NMR **3**	^{13}C NMR **4**	^{13}C NMR **5**	^{13}C NMR **6**
1	36.72	36.84	36.75	36.32
2	26.25	26.47	26.21	26.08
3	70.03	76.58	71.52	70.21
4	42.35	44.52	42.87	42.51
5	145.45	139.99	141.46	145.36
6	122.14	124.69	124.30	121.89
7	31.53	123.49	30.53	31.51
8	45.89	146.48	46.23	45.94
9	45.29	49.37	46.56	46.30
10	38.72	38.68	39.03	38.68
11	23.47	23.26	23.04	23.69
12	29.15	28.62	29.58	31.15
13	43.61	45.79	43.36	44.53
14	50.37	51.33	50.69	50.41
15	31.53	31.12	31.85	30.72
16	32.62	30.17	31.26	31.89
17	51.29	49.58	49.83	51.06
18	13.62	14.30	14.36	13.65
19	17.45	17.68	17.89	17.69
20	41.48	41.84	41.58	40.37
21	20.94	16.04	20.16	20.23
22	127.38	132.18	133.25	128.06
23	123.36	130.39	128.15	123.42
24	45.29	47.06	45.53	44.65
25	39.51	143.80	39.57	39.23
26	178.85	182.82	179.46	179.15
27	16.05	106.48	16.18	16.90
28	25.21	206.66	25.16	25.19
29	23.47	19.38	22.01	23.39
30	16.01	202.39	14.77	17.63
1’	36.41	37.81	36.92	36.26
2’	29.07	34.22	28.07	29.15
3’	31.57	194.40	197.55	36.72
4’	43.52	46.57	45.21	41.30
5’	48.76	50.37	48.82	48.63
6’	16.15	18.14	16.76	16.90
7’	32.71	33.09	32.67	32.67
8’	137.81	134.45	133.67	137.76
9’	144.56	151.91	144.32	145.03
10’	37.83	38.71	37.95	38.05
11’	126.65	199.60	126.67	127.15
12’	123.66	147.78	123.66	164.53
13’	144.18	123.43	145.63	144.32
14’	165.76	199.12	125.68	126.51
15’	33.25	33.62	33.37	33.27
16’	24.55	24.81	24.23	22.89
17’	21.89	17.68	21.66	22.06
18’	178.79	181.35	179.22	178.87
19’	65.21	65.02	65.91	64.93
20’	20.98	20.46	20.94	21.08

molecular ion peak at m/z 768 consistent with the molecular formula of triterpenoid linked with diterpene, $C_{50}H_{72}O_6$. The important ion peaks generating at m/z 439 $[C_{30}H_{47}O_2]^+$ and 330 $[C_{20}H_{26}O_4]^+$ due to the cleavage of triterpenoid linkage with the diterpene indicated that compound **5** consists of triterpenic unit linked with diterpenic acid. The subsequent ion fragments of the diterpenic acid moiety arising at m/z 287 $[330\text{-}C_3H_7]^+$, 272 $[287\text{-}Me]^+$, 284 $[330\text{-}HCOOH]^+$ and 299 $[330\text{-}CH_2OH]^+$ suggested the presence of isopropyl, carboxylic, ketonic and oxygenated methylene groups in the diterpene unit. The ion fragments of triterpenic moiety generating at m/z 141 [$C_8H_{13}O_2$, side chain, SC]$^+$, 298 $[439\text{-}SC]^+$ and 394 $[439\text{-}COOH]^+$ supported the presence of a C_8-mono unsaturated side chain with carboxylic group. The ^{1}H NMR spectrum of **5** exhibited two downfield doublets at δ 6.89 (J = 9.5 Hz) and 7.11 (J = 3.0 Hz) ppm and a double-doublet at δ 7.00 (J = 3.0, 9.5 Hz) ppm, one proton each, assigned correspondingly to *ortho*-coupled H-11', *meta*-coupled H-14' and *ortho*-, *meta*-coupled H-12' aromatic protons. Two one-proton broad singlets at δ 3.53 and 3.49 ppm were ascribed to oxygenated H_2-19' methylene protons. Two doublets at δ 0.82 (J = 6.2 Hz) and 0.98 (J = 6.3 Hz) ppm and a broad singlet at δ 1.18 ppm were attributed to secondary Me-16', Me-17' and tertiary Me-20' methyl protons of the diterpene unit. Three one-proton multiplets at δ 5.31, 5.23 ($w_{1/2}$ = 9.3 Hz) and 5.01 ($w_{1/2}$ = 8.7 Hz) ppm were ascribed correspondingly to *cis*-oriented vinylic H-6, H-22 and H-23 protons of the triterpenic unit. A double-doublet at δ 4.13 (J = 5.5, 9.0 Hz) ppm was assigned to α-oriented oxygenated methine H-3 proton. The remaining methylene and methine protons resonated between δ 1.45–2.32 ppm. Five broad singlets at δ 0.72, 1.04, 1.01, 1.06, 1.12 ppm and two doublets at δ 0.95 (J = 6.3 Hz) and 0.80 (J = 6.1 Hz) ppm, three-protons each, were ascribed to tertiary C-18, C-19, C-28, C-29, C-30 methyl and secondary C-21 and C-27 methyl protons, respectively, all attached to saturated carbons. The ^{13}C NMR spectrum of **5** exhibited signals for ketonic carbon at δ 197.55 ppm (C-3'), carboxylic carbons at δ 179.46 ppm (C-26), 179.22 ppm (C-18'); aromatic and vinylic carbons between δ 123.66–141.46 ppm, oxygenated methine carbon at δ 71.52 ppm (C-3) and oxygenated methylene carbon at δ 65.91 ppm (C-19'), respectively. The shifting of C-19' methylene carbon in the downfield region at δ 65.91 ppm suggested linkage of the triterpenic unit at this carbon. The ^{1}H and ^{13}C NMR spectral data of **5** were compared with the values of the reported lanostene type triterpenoids (12–15). The spectral data of the abietatriene unit were compared with the reported values of the similar compounds (16–18). The ^{1}H-^{1}H COSY spectrum of **5** showed correlations of H-3 with H-2, Me-28 and H-19; H-6 with H-7; H-22 with H-20, Me-21 and H-23; and H-14 with H-7, H-17 and H-15. The HMBC spectrum of **5** exhibited interactions of H-2, Me-28 and H-19 with C-3; H-6, H-7 and Me-28 with C-5; H-20, Me-21 and H-23 with C-22; H-25 and Me-27 with C-26; H-2, H-5 and H-19 with C-3; and H-11, H-12, H-14, Me-17 and Me-16 with C-13. On the basis of above discussion the structure of **5** was formulated as lanost-5,22-dien-26-oic acid-3β-olyl-(3→19')-3-oxodehydroabietic acid (Fig. 1). This is a new lanostenoic acid linked with dehydroabietic acid.

Compound **6,** named pinusoic acid C, was obtained as a brown crystalline powder from chloroform-methanol (3 : 1, v/v) eluants. It produced effervescences with sodium bicarbonate solution and green color with $FeCl_3$ solution. Its IR spectrum exhibited absorption bands characteristic for hydroxyl (3409 cm^{-1}), carboxyl groups (3380, 1699 cm^{-1}) and unsaturation (1645 cm^{-1}). Its positive FAB mass and ^{13}C NMR spectra suggested a molecular ion peak at m/z 770 consistent with the molecular formula of a triterpenic unit linked with a diterpene, $C_{50}H_{74}O_6$. The important fragment ion peaks arose at m/z 438 $[C_{30}H_{46}O_2]^+$ and 334 $[C_{20}H_{28}O_4]^+$. The ion fragments of the diterpenic acid moiety produced at m/z 303 $[334\text{-}CH_2OH]^+$, 291 $[334\text{-}C_3H_7]^+$ and 289 $[334\text{-}COOH]^+$ suggested the presence of oxygenated methylene, isopropyl and carboxylic groups in it. The ion fragments of triterpenic unit generating at m/z 141 [$C_8H_{13}O_2$, side chain, SC]$^+$ and 297 $[438\text{-}SC]^+$ indicated the presence of an unsaturated C_8-side chain with carboxylic function in the compound. The ^{1}H NMR spectrum of **6** displayed two downfield one-proton singlets at δ 7.39 and 6.83 ppm assigned correspondingly to *para*-coupled H-11' and H-14' aromatic protons and supported the existence of the phenolic group at C-12'. A two-proton broad signal at δ 3.40 ppm was attributed to oxygenated H-19' methylene protons. Two doublets at δ 0.85 (J = 6.5 Hz) and 0.91 (J = 6.4 Hz) ppm and one broad singlet at δ 1.14 ppm, each integrating for three protons; were attributed to secondary C-16', C-17' and tertiary C-20' methyl protons, respectively. A one-proton doublet at δ 5.33 (J = 4.5 Hz) ppm and two one-proton multiplets at δ 5.09 ($w_{1/2}$ = 9.5 Hz) and 4.90 ($w_{1/2}$ = 8.3 Hz) ppm were ascribed correspondingly to *cis*-oriented vinylic H-6, H-22 and H-23 protons of triterpenic moiety. A one-proton double-doublet at 4.43 (J = 5.1, 9.2 Hz) ppm was

attributed to α-oriented oxygenated methine H-3 proton. Five three-proton broad singlets at δ 0.72, 1.01, 1.05, 1.03 and 1.16 ppm were ascribed to tertiary C-18, C-19, C-28, C-29 and C-30 methyl protons, respectively. Two three-proton doublets at δ 0.93 (J = 6.1 Hz) and 1.26 (J = 6.3 Hz) ppm were attributed correspondingly to secondary C-21 and C-27 methyl protons of the triterpenic moiety. The ^{13}C NMR spectrum of **6** displayed signals for carboxylic carbons at δ 179.15 (C-26), 178.87 (C-18') ppm, aromatic carbons at δ 127.15–164.53 ppm; vinylic carbons at δ 145.36 (C-5), 121.89 (C-6), 128.06 (C-22), 123.42 (C-23) ppm and carbinol carbon at δ 70.21 ppm (C-3). The shifting of C-19' methylene protons at δ 3.40 ppm in the ^{1}H NMR spectrum and the carbon signal at δ 64.93 ppm in ^{13}C NMR spectrum suggested the linkage of the triterpenic moiety at this carbon. The ^{1}H and ^{13}C NMR spectral data of **6** were compared with the values of the reported lanostene type triterpenoids (12–15). The spectral data of the abietatriene unit were compared with the reported values of the similar compounds (16–18). The ^{1}H-^{1}H COSY spectrum of **6** exhibited correlations of H-3 with H-2, Me-28 and H_2-19; H-6 with H-7; H-22 with H-20, Me-21 and H-23; H-14 with H-7; and Me-16 with Me-17. The HMBC spectrum of **6** showed interactions of H-2, Me-28 and H-19 with C-3; H-6, H-7, Me-28 and Me-19 with C-5; H-20, Me-21 and H-23 with C-22; H-19 and H-5 with C-18; and H-11 and H-14 with C-12. On the basis of above discussion, the structure of **6** was characterized as lanost-5,22-dien-26-oic acid-3β-olyl-(3→19')-dehydroabietic acid (Fig. 1). This is a new triterpenic acid linked with dehydroabietic acid.

REFERENCES

1. Anonymous: The Wealth of India, A Dictionary of Indian Raw Materials and Industrial Products, Raw Materials, p. 69. National Institute of Science Communication and Information Resources, CSIR, New Delhi 2003.
2. Rajbhandari K.R.: Ethnobotany of Nepal. Ethnobotanical Society of Nepal, Kathmandu 2001.
3. Manandhar N.P.: Plants and people of Nepal. Timber Press Inc., Portland, Oregon 2002.
4. Wiyono, B., Tachibana, S., Tinambunan, D.: J. Forestry Res., 3, 7 (2006).
5. Langenheim, L.H.: Plant resins: chemistry, evaluation, ecology and ethnobotany, p. 453, Timber Press, Auckland, New Zealand (2003).
6. Chopra R.N., Nayar S.L. Chopra I.C.: Glossary of Indian Medicinal Plant, CSIR, New Delhi 2003.
7. Puri A., Srivastava A.K., Singhal B., Mishra S.K., Srivastava S., Lakshmi V.: Med. Chem. Res., 20, 1589 (2011).
8. Zhu Y., Chen X., Chen Y., Zhong H., Chen Z., Tong Z.: J. Chem. Indust. Eng. 59, 920 (2008).
9. Demirbas A.: Holzforschung, 45, 337 (1991).
10. Wiyono B., Tachibana S., Tinambunan D.: J. Forestry Res. 3, 7 (2006).
11. Ramírez-Macías I., Marín C. Chahboun R., Olmo F., Messouri I., Huertas O., Rosales M.J. et al.: Mem. Inst. Oswaldo Cruz, Rio de Janeiro 107, 370 (2012).
12. Chung I.-M., Ali M., Yang Y.-M., Peebles C.A.M., Chun S.C., Lee S.J., San K.Y., Ahmad A.: Bull. Korean Chem. Soc. 28, 1294 (2007).
13. Chung I.-M, Ali M., Chun S.-C., Lee O.K., Ahmad A.: Asian J. Chem. 19, 1535 (2007).
14. Ansari S.H., Ali M., Naquvi K.J.: Indian Drugs 48, 28 (2011).
15. Ahmad A., Ali M., Tondon S.: Chinese J. Chem. 28, 2474 (2010).
16. Ali M.: Techniques in terpenoids identification, pp. 254–259, Birla Publications, Delhi (2001).
17. Chamy M.C., Piovano M., Garbarino J.A., Miranda C., Gambaro V., Rodriguez M.L., Ruiz-Perez C., Brito L.: Phytochemistry 30, 589 (1991).
18. Georges P., Legault J., Lavoie S., Grenon C., Pichette A.: Molecules 17, 9716 (2012).

Received: 23. 12 2012

Erratum

In the paper: Cytotoxic effects of *Potentilla reptans* L. rhizome and aerial part extracts, by Ana M. Radovanovic et al., Acta Pol. Pharm. Drug Res. 70, issue 5, page 851, the name of one of co-authors should be: SLOBODAN M. JANKOVIC instead of: SLOBODAN E. JANKOVIC. We apologize for this error.