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Two new terpenes from the lichen *Parmelia perlata*

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The lichen *Parmelia perlata* has yielded a new lanost-2-en type triterpene, named parmelanostene and a new labdane type diterpenoid, named permelabdone, identified on the basis of spectroscopic studies as 29, 30-nordimethyl-lanost-2-en **1** and 2′-methoxyphenyl-19-[4α, 8β, 10β-trimethyl-9-(13-methyl butyl)-decahydronapthalen-4-yl]-20-methoxybenzoate **2**, respectively which were also found to have antibacterial potential against *S. aureus* and *E. coli* bacterial strains.

Keywords: Lichen, labdane, diterpenoid, permelabdone, antibacterial, 29, 30-nordimethyl-lanost-2-en, 2′-methoxyphenyl-19-[4α, 8β, 10β-trimethyl-9-(13-methyl butyl)-decahydronapthalen-4-yl]-20-methoxybenzoate

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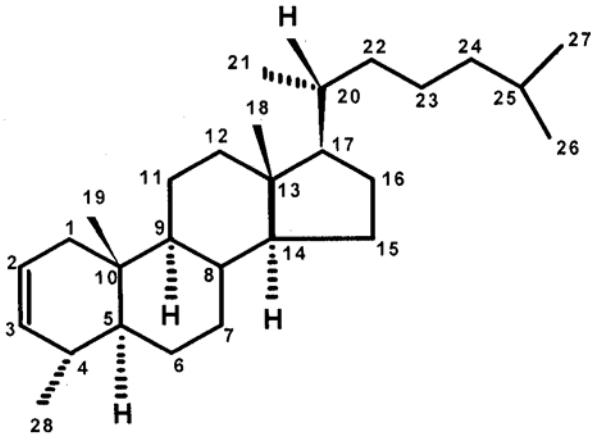
The lichen *Parmelia perlata* (Parmeliaceae) is an important crude drug used in Indian system of medicine as demulcent, tonic, febrifuge, diuretic and emollient[1]. It is also reported to have been used in stomach disorder, despepsia, vomiting, headache and pain in the liver or curing of wounds[1]. The presence of atranorin, lecanoric acid, usnic acid and chrysophanic acid has so far been reported[2-6] from this plant. Significant medicinal properties attributed to this plant prompted us to take up its phytochemical investigation. We herein report the isolation and characterization of a new lanost-2-en type triterpene **1**, and a new labdane type diterpenoid **2**.

Results and Discussion

Compound **1** obtained as colourless crystals exhibited a molecular ion peak $[M]^+$ at 384 in its EI-mass spectrum consistent with $C_{28}H_{48}$. In its IR spectrum **1** exhibited the presence of C-H stretching (2918, 2850), unsaturation (1673) cm^{-1} and showed no absorption bands for any other functional group. The EI-MS exhibited significant fragment ions at *m/z* 344, 316, 276, 194, 190, 68, 40 and 136, associated with retro-Diels-Alder cleavage of ring B for the tetracyclic nucleus [7], thereby suggesting the presence of a double bond at C-2. Both ^1H and ^{13}C NMR data were consistent with a lanostane type skeleton. The ^1H NMR spectrum of **1** showed signals for two olefinic protons at δ 5.80 and 4.94 assigned to C-2 and C-3 protons, respectively and a methyl doublet 1.10 (Me-28) of a tetracyclic triterpene. The ^1H NMR spectrum of **1** also indicated the presence of two tertiary methyls (δ 0.70, 3H, s, Me-18; 1.05, 3H, s, Me-19), two secondary methyl groups (δ 0.93, 3H, d, *J*= 6.5 Hz, Me-21; 1.10, 3H, s, Me-28) and gem-dimethyls (δ 0.98, 6H, d, *J*=6.6 Hz, Me-26, Me-27), suggesting a lanostane-type skeleton[8]. In the ^{13}C NMR spectrum, the olefenic carbons C-2 and C-3 appeared at δ 117.13 and 125.11, respectively. Two tertiary methyl carbons resonated at δ 14.12 (C-18) and 19.75 (C-19), while the secondary methyls (C-21), (C-28) and two gem-dimethyls (C-26, 27) appeared at 21.46, 22.71 and 21.06, 20.33, respectively. The above ^{13}C data was in agreement with the above assignments and the published data[9]. The mass spectrum also displayed the fragment ions at *m/z* 231 $[344\text{-side chain}]^+$, 203 $[316\text{-side chain}]^+$, 135 $[276\text{-side chain}]^+$ and 81 $[194\text{-side chain}]^+$, supporting the existence of an eight carbon saturated side chain. The ^1H and ^{13}C NMR resonances of **1** confirmed the saturated nature of the side chain. Further confirmation of the structure came from HMBC experiments. The correlations were observed



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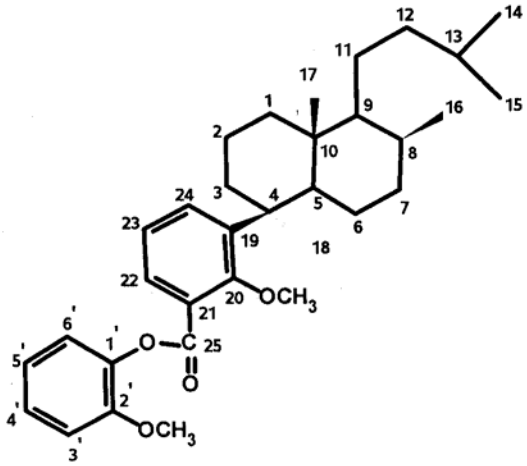

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between C-2, C-10, C-19 and H-1 protons. Olefinic proton H-2 correlated with C-1, C-3 while H-3 showed correlation with C-2, C-4 and C-28. In the HMBC spectrum correlations were also observed between C-28, C-2, C-3, C-5 and H-4. The methine proton at C-5 showed correlations with C-4, C-10 and C-28, similarly H-9 correlated with C-8, C-10 and C-19. The correlations were also observed between C-13, C-15 and H-14 methine proton. The secondary methyl protons at C-28 correlated with C-3 and C-4. The C-18 methyl protons were observed to show correlation with C-12, C-13, C-14 and C-17, similarly C-19 methyl protons correlated with C-1, C-5, C-9 and C-10 in the HMBC spectrum. These data indicated that **1** was 29,30-nordimethyl lanost-2-en. This constitutes the first report of the occurrence of triterpene in lichen *P. perlata*.

Compound **2** was obtained as colourless crystalline solid. The EI-mass spectrum of **2**, exhibited the molecular ion peak $[M]^+$ at *m/z* 506. The ^{13}C NMR data and EI-mass spectrum data were consistent with the molecular formula $C_{33}H_{46}O_4$. The ^1H and ^{13}C NMR spectra of **2** revealed the presence of two tertiary methyls (δ 1.22, s, Me-17; 0.83, s, Me-18) and three secondary methyls (δ 1.21, d, *J*=4.5 Hz, Me-16; 0.78, d, *J*=6.6 Hz, Me-14; 0.70, d, *J* =6.0 Hz, Me-15). These structural features strongly suggested a labdane-type skeleton[10] for this compound. The IR spectrum of **2** showed absorption for an ester group (1725 cm^{-1}) and aromatic ring, which was fully, supported by the ^1H and ^{13}C NMR spectra. In the ^1H NMR spectrum two, three-proton singlet peaks for two methoxyl groups were observed at δ 3.62 and 3.78, which appeared at δ 55.28 and 58.4, respectively in the ^{13}C NMR spectrum. The carboxylate carbon appeared at δ 168.90 while the aromatic carbons resonated at 156.71 (C-19), 165.05 (C-20), 159.93 (C-21), 156.7 (C-22), 100.59 (C-23), 104.93 (C-24), 168.90 (C-25), 160.77 (C-1′), 161.26 (C-2′), 102.71 (C-3′), 101.35 (C-4′), 120.42 (C-5′) and 121.36 (C-6′) in the ^{13}C NMR spectrum. The fragment ions at *m/z* 383 and 123 in the mass spectrum of **2** confirmed the presence of methoxy substituted phenyl benzoate in **2**. The absence of a tertiary methyl signal in the ^1H NMR spectrum indicated the attachment of this group with C-4 carbon atom[11]. The position of the methoxy groups was determined by ^1H NMR spectrum; the *o*-, *m*-coupled protons (δ 6.62, dd, *J*=7.5, 1.5 Hz, H-22; 6.74, dd, *J*=7.5, 1.5 Hz, H-24) and *o*-coupled proton (δ 7.45, dd, *J*= 7.5, 7.5 Hz, H-23) suggested the presence of methoxy group at C-20 in ring C. The coupled protons at δ 7.60 (d, *J*=8.5 Hz, H-3', H-6) and 6.87 (d, *J*=8.5 Hz, H-4', H-5') supported the presence of a second methoxy group at C-2'. These data led to formulate the structure of **2** as 2′-methoxyphenyl-19-[4α, 8β,10β-trimethyl-9-(13-methylbutyl)-decahydro-napthalen-4-yl]-20-methoxybenzoate. This constitutes the first report of the existence of a labdane-type diterpenoid in *P. perlata.*

The results of screening for antibacterial activity of compound **1** and compound **2** have been summarized in **Table I**. It is evident that both the compounds showed activity against gram-positive and gram-negative bacteria.

Experimental Section

Melting points are uncorrected. IR spectra were recorded in KBr pellet on a Perkin-Elmer 377 spectrometer. ^1H and ^{13}C NMR spectra were recorded

Table I—Antibacterial activity of cmpounds **1** and **2**

S No	Tested Organism	Compd **1** (0.4 mM)		Compd **2** (0.4 mM)		Control		Norfloxacin	
		ZI (mm)	% ZI	ZI (mm)	% ZI	ZI (mm)	% ZI	ZI (mm)	% ZI
1	*S. aureus*	12.3	49.2	23.0	92	00	00	25	100
2	*E. coli*	21.0	84.0	19.5	78	00	00	25	100

ZI-zone of inhibition
All the experiments were done in triplicate.

in $CDCl_3$ at 300 and 75 MHz on a Brucker spectrospin NMR instrument, respectively, using TMS as internal standard; 2D NMR experiments were conducted at 500 (^{1}H) and 125 MHz (^{13}C); EIMS spectra were scanned at 70 eV on a Jeol D-300 instrument. Silica gel (60-120 mesh) and silica gel G were used for performing column chromatography and TLC, respectively. The spots were visualized by spraying the TLC plates with Liebermann-Burchard reagent followed by heating at 105°C for 5 min.

Plant material. The lichen *P. perlata* was procured from Khari Baoli, New Delhi and authenticated by Dr M P Sharma, Reader, Botany Department, Jamia Hamdard. A voucher specimen has been deposited in the Phytochemical Research Laboratory of this university.

Extraction and isolation. The dried and coarsely powdered material (1.5 kg) was extracted successively with petroleum ether, chloroform and 95% ethanol in a soxhlet apparatus. Solvent was removed under reduced pressure in a Buchi rotavapour. The petroleum-ether extract was adsorbed on silica gel to form slurry and loaded on silica gel column packed in petroleum-ether. Elution was carried out with petroleum-ether and petroleum-ether-chloroform mixtures in different proportions. The fractions eluted with petroleum ether afforded compound **1**. It was recrystallized from petroleum-ether-ethyl acetate to give colourless crystals. The ethanolic extract on elution with petroleum-ether-chloroform (1:9) yielded compound **2**, which was crystallized from petroleum-ether-chloroform as crystalline colourless solid.

Compound 1. Colourless crystals; m.p. 81-82°C; UV-Vis (MeOH) λ_{max} nm (log ε): 205 (5.2); IR (KBr): 2918, 2850, 1673, 1630, 1463, 1376, 1168, 1110, 910, 824, 720 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$): δ 5.80 (1H, ddd, *J* = 10.24, 10.21, 7.17 Hz, H-2), 4.94 (1H, dd, *J* = 10.21, 16.13 Hz, H-3), 2.04 (1H, d, *J* = 6.9 Hz, H-1$_a$), 2.00 (1H, d, *J* = 7.17 Hz, H-1$_b$), 1.55 (1H, m, H-4), 1.49 (2H, m, H-5, H-17), 1.31 (2H, m, H-8, H-14), 1.29 (2H, m, H-9, H-20), 1.25 (14H, brs, 7 × CH_2), 1.19 (1H, m, H-25), 1.03 (2H, m, CH_2), 1.00 (2H, m, CH_2), 1.10 (3H, brs, Me-28), 0.93 (3H, d, *J* = 6.50 Hz, Me-21), 0.98 (6H, d, *J* = 6.66 Hz, Me-26 and Me-27), 1.05 (3H, brs, Me-19,), 0.70 (3H, brs, Me-18); ^{13}C NMR (75 MHz, $CDCl_3$): δ 37.47 (C-1), 117.13 (C-2), 125.11 (C-3), 27.15 (C-4), 46.41 (C-5), 26.65 (C-6), 32.81 (C-7), 37.00 (C-8), 45.97 (C-9), 37.14 (C-10), 24.31 (C-11), 33.83 (C-12), 33.49 (C-13), 45.56 (C-14), 27.35 (C-15), 29.72 (C-16), 45.52 (C-17), 14.12 (C-18), 19.75 (C-19), 30.07 (C-20), 21.46 (C-21), 29.39 (C-22), 28.98 (C-23), 24.50 (C-24), 31.95 (C-25), 21.06 (C-26), 20.33 (C-27), 22.71 (C-28); EIMS *m/z* (rel.int): 384 [M]$^+$ (3.6), 344 (3.4), 338 (3.8), 316 (2.1), 276 (2.3), 262 (2.4), 248 (2.0), 231 (3.6), 222 (2.5), 203 (6.0), 194 (3.4), 190 (7.0), 176 (8.6), 163 (9.1), 162 (10.1), 151 (12.8), 149 (11.7), 136 (11.0), 135 (14.4), 122 (23.6), 109 (17.8), 108 (32.1), 95 (41.8), 83 (38.3), 81 (38.2), 69 (61.3), 68 (47.7), 56 (91.2), 43 (71.6), 40 (100).

Compound 2. Colourless crystalline solid; m.p. 246-47°C; UV-Vis (MeOH) λ_{max} nm (log ε): 247 (5.6), 3.20 (4.8) nm; IR (KBr): 3360, 2955, 2862, 1725, 1691, 1604, 1566, 1373, 1264, 1194, 1159, 1055, 855 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.60 (2H, d, *J* = 8.5 Hz, H-6 ′, H-3′), 6.87 (2H, d, *J* = 8.5 Hz, H-4 ′, H-5′), 7.45 (1H, dd, *J* = 7.5,7.5 Hz, H-23), 6.62 (1H, dd, *J* = 7.5,1.5 Hz, H-22), 6.74 (1H, dd, *J* = 7.5,1.5 Hz, H-24), 3.62 (3H, brs, OMe), 3.78 (3H, brs, OMe), 2.03 (1H, m, H-8), 2.10 (1H, m, H-13), 1.43 (1H, m, H-3$_b$),1.20 (1H, dddd, *J* = 3.7, 3.7,13.2 Hz, H-3$_a$), 1.01 (1H, ddd, *J* = 3.4,3.4,13.4 Hz, H-1$_a$), 1.85 (1H, m, H-1$_b$), 1.54 (1H, m, H-6$_a$), 1.82 (1H, m,H-6$_b$), 1.65 (2H, m, H_2-12), 1.52 (2H, m, H_2-11), 1.46 (2H, m, H_2-2), 1.91(1H, m, H-9), 1.62 (2H, brs, H_2-7), 1.25 (dd, *J* = 4.9,12.3 Hz, H-5), 1.22 (3H, brs, Me-17), 1.21 (3H, d, *J* = 4.5 Hz, Me-16), 0.83 (3H, s, Me-18), 0.78 (3H, d, *J* = 6.6 Hz, Me-14), 0.70 (3H, d, *J* = 6.0 Hz, Me-15); ^{13}C NMR (75 MHz, $CDCl_3$): δ 38.82 (C-1), 22.77 (C-2), 37.86 (C-3), 40.28 (C-4), 55.70 (C-5), 24.50 (C-6), 30.68 (C-7), 39.37 (C-8), 55.23 (C-9), 39.09 (C-10), 22.83 (C-11), 30.10 (C-12), 32.40 (C-13), 21.94 (C-14), 21.94 (C-15), 26.01 (C-16), 28.60

(C-17), 13.50 (C-18), 156.71 (C-19), 165.05 (C-20), 159.93 (C-21), 156.7 (C-22), 100.59 (C-23), 104.93 (C-24), 168.90 (C-25), 160.77 (C-1′), 161.26 (C-2′), 102.71 (C-3′), 101.35 (C-4′), 120.42 (C-5′), 121.36 (C-6′) and 55.28 (OMe), 58.4 (OMe); EIMS (rel. int. %): *m/z* 506 $[M]^+$ (100), 480 (27.7), 463 (12.5), 383 (72.9), 365 (7.4), 262 (8.4), 205 (6.8), 149 (83), 123 (4.1), 71 (4.2), 43 (55.6).

Antibacterial activity

The antibacterial activity of compounds **1** and **2** was determined by the standardized disk method of Kirby and Bauer[12]. *Staphylococcus aureus* (NCTC 10418), *Escherecia coli* (NCTC 6571) were used as test bacteria. The results were reported as the diameter of the zone of inhibition around each disk (in mm). The results showed that compound **1** was active against the Gram-negative bacteria *E. coli* at a concentration level of 0.4 m mole but had a low inhibitory activity against Gram-positive bacteria *S. aureus.*

Compound **2** was found to have significant activity against *S. aureus* and a good activity against *E. coli*. The isolated compounds may be in part responsible for the antimicrobial potential of the plant.

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