Antimycobacterial agent, (E)-phytol and lauric amide from the plant *Lagascea mollis*

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Chemical examination of aerial parts of the plant, *Lagascea mollis* has resulted in the isolation of two compounds, an acyclic diterpene alcohol which has been identified as (E)-phytol 1 and lauric amide 3. Their structures have been elucidated by spectral data and chemical transformations. This is the first report of isolation of both these compounds from this plant. It is noteworthy that compound 1 has been found to be a potent antimycobacterial agent and thus, *L. mollis* could be exploited as an alternative source.

Keywords: *Lagascea mollis*, diterpene, amide, structure elucidation, chemical transformation

The plant, *Lagascea mollis* (family: Asteraceae) is a common weed and grows throughout Western Ghats. Earlier phytochemical work on the roots of *L. mollis* had resulted in the isolation of polyene ketone and dehydrofalcarione. El-Naggar *et al* reported flavonol glycosides, patulitrin and acetyl patulitrin from the aerial parts of *L. mollis*. This research group has been interested in naturally occurring bioactive secondary metabolites and hence got interested to chemically examine the plant, *L. mollis*. Herein is reported the isolation and characterization of an acyclic diterpene alcohol, (E)-phytol 1 and lauric amide 3 (Figure 1). This is the first report for the isolation of a diterpene and lauric amide from the plant, *L. mollis*.

**Results and Discussion**

A portion of DCM extract (9.0 g) was chromatographed over silica gel (400 g, 100-200 mesh) using petroleum ether, petroleum ether-dichloromethane (1:1), dichloromethane and dichloromethane-MeOH (1-100%) to provide six broad fractions: A (1.1 g), B (300 mg), C (280 mg), D (2.2 g), E (3.8 g) and F (1.2 g). The fraction B (300 mg) on silica gel column chromatography followed by flash chromatography (Isco Teledyne, US, RediSep™ silica gel column, 12 g) using petroleum ether with increasing amounts of dichloromethane, resulted in the isolation of a compound (80 mg), structure of which was elucidated by its spectral data. The presence of allylic hydroxyl- methylene and the *trans*-geometry of the double bond was assigned on the basis of comparison of spectral data of (E)-phytol 1 and its acetate derivative 2 with the reported literature. Isolation of (E)- and (Z)-phytol has been reported from various sources.

Interestingly, (E)-phytol 1 has been found to be a naturally occurring antimycobacterial agent having minimum inhibitory concentration (MIC) of 2 µg/mL against *M. tuberculosis* H$_{37}$Rv strain radiorespirometrically. Therefore, *L. mollis* could also be exploited as an alternative source for the isolation of (E)-phytol for development of antitubercular agents from natural sources.

The fraction C (280 mg) was flash chromatographed (Isco Teledyne, US, RediSep™ silica gel column, 12 g) using petroleum ether and mixtures of ethyl acetate in petroleum ether. The fractions eluting with 3% ethyl acetate in petroleum ether yielded...
viscous liquid (13 mg) whose structure was established as compound 3 on the basis of its spectral data. Lauric amide 3 has been reported from French fern, A. capillus-veneris.

**Experimental Section**

**General experimental procedures.** Thin layer chromatography (TLC) was performed using pre-coated silica gel F254 aluminium sheets, obtained from Merck, Germany. Chromatography refers to purification by column chromatography using silica gel (100-200 mesh size; Merck, India). IR spectra were recorded on a Perkin-Elmer Spectra One FT-IR spectrophotometer. \(^1\)H and \(^{13}\)C NMR spectra were recorded at 200 MHz and 50 MHz, respectively, on a Varian Mercury spectrometer. Elemental analysis was performed on Carlo-Erba CHNS-O EA 1108 elemental analyzer. Flash chromatography was performed on CombiFlash Companion (Isco Teledyne, US) using RediSep™ columns (silica gel columns, 12 g).

**Plant material.** The aerial parts of the plant, L. mollis was collected and identified by Dr. M. M. Jana, Head, Horticulture Section, CSIR-NCL from the CSIR-NCL Campus, Pune in the month of October 2005. A voucher specimen is being maintained in this laboratory.

**Extraction and fractionation.** Dried and powdered aerial parts of the plant material (800 g) were repeatedly extracted with petroleum ether (8 × 4 L) at RT. After removal of the solvent at reduced pressure, petroleum ether extract (10.75 g) was obtained. The plant material was repeatedly extracted with EtOH (12 × 4 L). After removal of the solvent at reduced pressure, \(\text{H}_2\text{O} (800 \text{ mL})\) was added and the aqueous phase successively extracted with DCM, EtOAc and \(n\)-BuOH affording the corresponding subfractions. A portion of DCM extract (9.0 g) was chromatographed over silica gel (400 g, 100-200 mesh) using petroleum ether, petroleum ether-DCM (1:1), DCM and MeOH-DCM (1:100%) to provide six broad fractions: A (1.1 g), B (300 mg), C (280 mg), D (2.2 g), E (3.8 g) and F (1.2 g).

**Isolation process.** (E)-Phytol acetate 2: To a solution of 1 (20 mg) in dry pyridine (1.0 mL), Ac\(_2\)O (2.0 mL) was added and the reaction mixture was left at RT for overnight. The reaction mixture was diluted with \(\text{H}_2\text{O} (100 \text{ mL})\) and the aqueous phase extracted with \(\text{CH}_2\text{Cl}_2 (3 \times 150 \text{ mL})\), dried (anhydrous \(\text{Na}_2\text{SO}_4\)) and concentrated under vacuum. The traces of pyridine were removed by co-distillation with toluene to afford a residue which was further purified by flash chromatography with RediSep™ silica gel column (12 g) using petroleum ether and mixtures of dichloromethane in petroleum ether. The fractions eluting with 10% dichloromethane in petroleum ether yielded compound 2 as oil (19 mg), R\(_f\): 0.8 (\(\text{CH}_2\text{Cl}_2\)-MeOH, 99:1); IR (CHCl\(_3\)): 3020, 2928, 2868, 1733, 1673, 1592, 1459, 1383, 1216, 1023, 758 cm\(^{-1}\); \(^1\)H NMR (200 MHz, CDCl\(_3\)): \(\delta\) 0.83-0.88 (12H, m, \(4 \times \text{CH}_3\)), 1.02-1.40 (21H, m), 1.69 (3H, s, \(\text{CH}_3\)), 2.00 (2H, t, \(J = 7.3 \text{ Hz}, \text{CH}_2\)), 2.05 (3H, s, \(\text{CH}_3\)), 4.58 (2H, d, \(J = 7.1 \text{ Hz}, \text{CH}_2\text{OAc}\)), 5.34 (1H, m, CDCH\(_2\)OH); \(^{13}\)C NMR (50 MHz, CDCl\(_3\)): \(\delta\) 16.4 (\(\text{CH}_2\)), 19.7 (\(\text{CH}_3\)), 19.7 (\(\text{CH}_3\)), 21.0 (\(\text{CH}_3\)), 22.6 (\(\text{CH}_3\)), 22.7 (\(\text{CH}_3\)), 24.5 (\(\text{CH}_2\)), 24.8 (\(\text{CH}_2\)), 25.1 (\(\text{CH}_2\)), 28.0 (\(\text{CH}_2\)), 32.7 (\(\text{CH}_2\)), 32.8 (\(\text{CH}_2\)), 36.7 (\(\text{CH}_3\)), 37.3 (\(\text{CH}_3\)), 37.4 (\(\text{CH}_3\)), 37.4 (\(\text{CH}_3\)), 39.4 (\(\text{CH}_2\)), 39.9 (\(\text{CH}_3\)), 59.3 (\(\text{CH}_3\)), 123.1 (\(\text{CH}\)), 140.2 (\(\text{C}\)); ESI-MS: \(m/z\) 361 (M + Na). Analog. Calcld for C\(_{22}\)H\(_{42}\)O\(_2\): C, 81.01; H, 13.60. Found: C, 80.91; H, 13.24%.

**Lauric amide 3: Fraction C (280 mg) on flash chromatography with RediSep™ silica gel column (12 g) using petroleum ether and mixtures of ethyl acetate in petroleum ether yielded pure compound 3 as viscous liquid (13 mg). R\(_f\): 0.2 (\(\text{CH}_2\text{Cl}_2\)-MeOH, 99:1); IR (CHCl\(_3\)): 2918, 2850,
2358, 2331, 1701, 1689, 1456, 1296, 908, 733 cm⁻¹; 
¹H NMR (200 MHz, CDCl₃): δ 0.88 (3H, t, J = 6.0 Hz, CH₃), 1.26 (16H, brs), 1.63 (2H, m, CH₂CH₂CO), 2.35 (2H, t, J = 7.3, 7.6 Hz, CH₂CO); 
¹³C NMR (50 MHz, CDCl₃): δ 14.1 (CH₃), 22.7 (CH₂), 24.7 (CH₂), 29.1 (CH₂), 29.3 (CH₂), 29.4 (CH₂), 29.5 (CH₂), 29.6 (CH₂), 29.7 (CH₂), 31.9 (CH₂), 34.0 (CH₂), 179.9 (CO); ESI-MS: m/z 222 (M + Na)⁺. Anal. Calcd for C₁₂H₂₅NO: C, 72.31; H, 12.64. Found: C, 71.92; H, 12.40%.

Conclusion

In summary, herein is reported the chemical examination of the aerial parts of the plant, Lagascea mollis which resulted in the isolation of two compounds, an acyclic diterpene alcohol which was identified as (E)-phytol 1 and lauric amide 3. Their structures were elucidated by spectral data and chemical transformations. This is the first report of isolation of both these secondary metabolites from this plant. It is pertinent to mention here that compound 1 has been found to be a potent antimycobacterial agent and thus, L. mollis could be further exploited as an alternative source.

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References