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Chemical constituents of *Piper mullesua*

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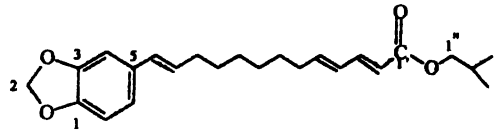
Chemical analysis of *Piper mullesua* led to the isolation of two new compounds isobutyl 13-(1,3-benzdioxolo-5)trideca-2*E*,4*E*,12*E*-trienoate (1) and *N*-isobutyl-16-phenylhexadeca-2*E*,4*E*-dienamide (2). *N*-Isobutyldeca-2*E*,4*E*-dienamide (3) has also been reported for the first time from this species.

Piper mullesua (syn. *P. brachystachyum*), popularly known as *Pahari peepal*, is indigenous to India which grows wild in the Himalayan hills from Himachal Pradesh in the west to Arunachal Pradesh in the east. It is also distributed in the Jayantia and Khasi hills located in the states of Meghalaya, Assam and Manipur in the north-east India, at altitudes of 600-1500 m. Although, *Piper* species are source of many important secondary metabolites, *P. mullesua* is not yet a commercially exploited species. We have reported earlier[1] volatile constituents, and some new 1,3-benzodioxanes from *P. mullesua* fruits. Of these compounds, myristicin and sesamin were found to possess potent insecticidal and antifeedant activities, respectively[2]. The present chemical analysis of *P. mullesua* led to the isolation of a new 1,3-benzodioxole designated as isobutyl 13-(1,3-benzdioxolo-5)trideca-2*E*,4*E*,12*E*-trienoate (**1**). The preparative HPLC has resulted in the isolation of minor amides, namely, *N*-isobutyl-16-phenylhexadeca-2*E*,4*E*-dienamide (**2**) and *N*-isobutyldeca-2*E*,4*E*-dienamide (**3**), of which **2** is new whereas **3** has been reported for the first time from this plant.



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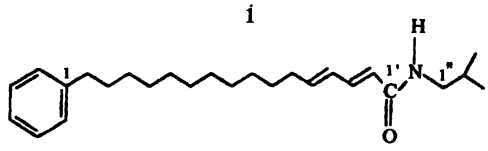

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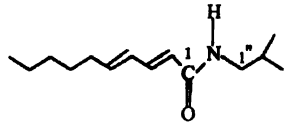

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Results and Discussion

IR bands[3,4] of compound **1** are in support of a methylenedioxy group, conjugated ester and a trisubstituted benzene in the molecule. The signal at δ 5.93 in its ^{1}H NMR[4] also supports the presence of methylenedioxy group. A set of olefinic protons[3,5] at δ 5.77, 7.26, 6.01 and 6.12 support the presence of a ketone in conjugation with a diene. One of the coupling constants (16 Hz) of all the olefinic protons suggests that both the double bonds had *E*-configuration[4]. Double bond, in conjugation with aromatic ring[3], appeared at δ 6.09 and 6.21. A sharp singlet at δ 6.89 was due to aromatic proton at carbon-4 whereas a broad singlet at δ 6.74 was attributable to aromatic protons at carbon-6 and 7. The signal at δ 2.18 was due to methylene protons adjacent to double bond. Signals at δ 0.92, 1.80 and 3.17 suggested the presence of an isobutyl ester group. Thus, the structure of compound **1** could be assigned as isobutyl 13-(1,3-benzdioxolo-5)trideca-2*E*,4*E*,12*E*-trienoate. ^{13}C NMR data were also in support of structure **1** (vide Experimental).

Compound **2** gave orange colour with Dragendroffs reagent. Its UV spectrum had bands at 260 and 218 nm of a conjugated dienamide chromophore[5,6] whereas IR showed bands characteristics of a NH function (3300 cm^{-1}) incorporated in a dienamide grouping[6] (1654, 1630 and 1010 cm^{-1}). All the five aromatic protons appeared as a broad singlet at δ 7.26. Protons, similar to **1** protons, on a conjugated diene appeared at δ 5.70, 7.26, 6.01 and 6.08. Signals at δ 0.92, 1.75 and 3.28 were for an isobutyl group in vicinity of an amide[6]. Methylene protons, adjacent to aromatic ring appeared at δ 2.31. Thus, the structure of compound **2** could be assigned as *N*-isobutyl-16-phenylhexadeca-2*E*,4*E*-dienamide.

Compound **3**, $[M]^+$ at *m/z* 223, $C_{14}H_{25}NO$, obtained by preparative HPLC gave orange colour with Dragendroffs reagent. The compound was identified as *N*-isobutyldeca-

2*E*,4*E*-dienamide from its spectral analysis. Although, the compound has been reported earlier from different *Piper* species[6,7], this is the first report on its isolation from *P. mullesua*.

Experimental

M.ps. were uncorrected (Electrothermal 1A 9000 apparatus). NMR spectra were recorded on a Bruker FT NMR spectrometer using TMS as internal standard, IR and UV on Perkin-Elmer 1710 FTIR and Pye-Unicam SPB-100 spectrophotometers, respectively, and mass spectras on a Jeol D-300 spectrometer. TLC was performed on silica gel G (Merck) plates and the spots were visualised by exposure to I_2 vapour or spraying with vanillin-H_2SO_4 reagent.

Air-dried and powdered inflorescence (1.5 kg) was extracted with ethanol (3×5 dm^3). The combined extract was concentrated under reduced pressure and fractionated to give hexane (44 g), chloroform (23 g) and butanol (2 g) soluble fractions. Compound **1** was isolated from the fractions of hexane-ethyl acetate (85 : 15) of hexane extract using Si-gel column chromatography whereas preparative HPLC afforded compounds **2** and **3** from the chloroform extract.

Isolation of **2** *and* **3** : Chloroform extract (23 g) was chromatographed over silica gel (500 g) using methanol in chloroform (increasing polarity). Fractions of $CHCl_3$-MeOH (95 : 5) were monitored by TLC (150 mg) and dissolved in methanol (2 ml) for the isolation of **2** and **3** using preparative HPLC method. A Shimadzu LC-8A gradient HPLC instrument, equipped with two LC-8A pumps, controlled by a CBM-10A interface module was used. A SPD-M10 AVP (Shimadzu) photodiode array detector was used for peak detection/peak purity test. Fractions were collected using FRC-10A (Shimadzu) automatic fraction collector. Compounds **2** and **3** were eluted at retention times of 8.8 and 16.1 min, respectively, in the following operating conditions : acetonitrile-water, 70 : 30 (v/v); flow rate, 17 ml min^{-1}; column temperature, 26°.

Isobutyl 13-(1,3-benzdioxolo-5)trideca-2E,4E,12E-trienoate (**1**) : Compound **1** was crystallized in hexane-acetone (90 mg), m.p. 113–115°; R_f 0.63 (benzene-acetone, 9 : 1); λ_{max} (MeOH) 263, 213 nm; ν_{max} (KBr) 3300, 1665, 1625, 1610, 1560, 1440, 1318, 1255, 1000, 945, 920 cm^{-1}; ^{1}H NMR δ ($CDCl_3$, 300 MHz) 0.92 (6H, d, 6 Hz, H-3″,4″), 1.33–1.45 (8H, m, H-7′,8′,9′,10′), 1.80 (1H, m, H-2″), 2.18 (4H, m, H-6′,11′), 3.17 (2H, m, H-1″), 5.77 (1H, d, 16 Hz, H-2′), 5.93 (s, -O-CH_2-O-), 6.01 (1H, dd, 16, 7 Hz, H-4′), 6.09 (1H, m, H-12′), 6.12 (1H, dd, 16, 11 Hz, H-5′), 6.21 (1H, d, 16 Hz, H-13′), 6.74 (2H, br s, H-6,7), 6.89 (1H, s, H-4), 7.26 (1H, dd, 16, 10 Hz, H-3′); ^{13}C NMR δ ($CDCl_3$, 75 MHz) 148.0 (C-1), 100.9 (C-2), 141.3 (C-3), 105.4 (C-4), 141.3 (C-5), 120.2 (C-6), 108.2 (C-7), 167.6 (C-1′), 121.7 (C-2′), 145.0 (C-3′), 128.3 (C-4′), 143.1 (C-5′), 32.8 (C-6′), 28.6–29.5 (C-7′,8′,9′,10′), 32.8 (C-11′), 129.3 (C-12′), 129 (C-13′), 46.9 (C-1″), 28.6 (C-2″), 20.1 (C-3″), 20.1 (C-4″); *m/z* (rel. int.) 384 [M]$^+$ (100), 248 (37.1), 180 (13.0), 161 (36.1), 152 (39.7), 131 (78.3).

N-Isobutyl-16-phenylhexadeca-2E,4E-dienamide (**2**) : Fractions of column chromatography in chloroform-methanol (95 : 5) were further purified by preparative HPLC to obtain **2** as viscous mass (10 mg), R_f 0.58 (benzene : acetone, 8 : 2); λ_{max} (MeOH) 260, 218 nm; ν_{max} (KBr) 3300, 2921, 1654, 1630, 1559, 1542, 1458, 1386, 1036, 1010, 669 cm^{-1}; ^{1}H NMR δ ($CDCl_3$, 200 MHz) 0.92 (6H, d, 6 Hz, H-3″,4″), 1.09–1.26 (16H, m, H-7′,8′,9′,10′, 11′,12′,13′,14′), 1.75 (1H, m, H-2″), 2.03 (2H, m, H-6′), 2.12 (2H, m, H-15′), 2.31 (2H, m, H-16′), 3.28 (2H, m, H-1″), 5.70 (1H, d, 16 Hz, H-2′), 6.01 (1H, dd, 16, 11 Hz, H-5′), 6.08 (1H, dd, 16, 7 Hz, H-4′), 7.20 (1H, dd, 16, 10 Hz, H-3′), 7.26 (5H, br s, H-2,3,4,5,6); *m/z* (rel. int.) 383 [M]$^+$ (5), 250 (16.9), 179 (37.0), 173 (85.3), 91 (100), 83 (20), 57 (15).

N-Isobutyldeca-2E,4E-dienamide[6,7] (**3**) : It was also obtained by preparative HPLC of the chloroform-methanol eluates (95 : 5) (column chromatography) and crystallised from hexane (50 mg), m.p. 87–89°, R_f 0.56 (benzene : acetone; 8 : 2).

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References

1. S. Srivastava, M. M. Gupta and S. Kumar, *J. Ess. Oil Res.*, 1999, **11**, 5636 ; S. Srivastava, M. M. Gupta, A. K Tripathi and S. Kumar, *Indian J. Chem., Sect. B*, 1999, in press; S. Srivastava, R. K. Verma, M. M. Gupta and S. Kumar, *J. Chromatogr. A*, 1999, **841**, 123.
2. S. Srivastava, M. M. Gupta, V. Prajapati, A. K. Tripathi and S. Kumar, *Pharm. Biol.*, 1999, communicated; *Phytother. Res.*, 1999, in press.
3. A. Kijjoa, M. M. M. Pinto, B. T. Wie and W. Herz, *Planta Med.*, 1989, **55**, 193.
4. A. Banerji and C. Das, *Phytochemistry*, 1989, **28**, 3039.
5. S. K. Koul, S. C. Taneja, V. K. Agarwal and K. L. Dhar, *Phytochemistry*, 1988, **27**, 1523.
6. S. Dasgupta and A. B. Ray, *Indian J. Chem., Sect. B*, 1979, **17**, 538.
7. V. S. Parmar, S. C. Jain, K. S. Bisht, R. Jain, P. Taneja, A. Jha, O. D. Tyagi, A. K. Prasad, J. Wengel, C. E. Olsen and P M. Boll, *Phytochemistry*, 1997, **46**, 597.