J. Indian Chem. Soc.,
Vol. 81, January 2004, pp. 73-76

NOTE

Chemical constituents of *Lasia spinosa*, *Mussaenda incana* and *Wendlandia tinctoria*

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Manuscript received 1 November 2002, revised 3 April 2003, accepted 11 July 2003

Isolation of β-sitosterol acetate, stigmasterol and its acetate from the rhizome of *Lasia spinosa*; two new triterpene esters-3-palmitoyllupeol 1 and 3-benzoylepibetulin 2 along with β-sitosterol from the stem of *Mussaenda incana*; and a new aliphatic myricyl stearate 3 along with stearic acid, *d*-mannitol, geniposidic acid 4, β-sitosterol and stigmasterol from the stem of *Wendlandia tinctoria* are being reported.

The rhizome of *Lasia spinosa* Thw. syn. *L. aculeata* Lour. (Fam. : Araceae) is used in the treatment of piles and throat affections[1]. The undershrub *Mussaenda incana* Wall. (Rubiaceae) was grown wild in the forest lands of North Eastern States of India[1]. The stem of *Wendlandia tinctoria* (Roxb.) DC (Rubiaceae) is used by the tribal people as antidote of snakebite[1]. The pharmacologically active parts of *L. spinosa*, *M. incana* and *W. tinctoria* were chemically investigated for the first time to find out the active principles. Here we report the isolation and characterization of β-sitosterol acetate, stigmasterol and its acetate from the rhizome of *L. spinosa*; 3-palmitoyllupeol **1**, 3-benzoylepibetulin **2** and β-sitosterol from the stem of *M. incana*; myricyl stearate **3**, stearic acid, *d*-mannitol, geniposidic acid **4**, β-sitosterol and stigmasterol from the stem of *W. tinctoria*.

Results and discussion

The EtOAc extract of *L. spinosa* rhizome was subjected to column chromatography (CC) over silica gel. Petroleum ether (PE)-PhH (9 : 1) eluate gave a white solid of two compounds, which was rechromatographed over silica gel. Elution of the column with PE-PhH (8 : 1) gave colourless crystals (50 mg), $C_{31}H_{50}O_2$ (M^+ 454), m.p. 145°, responded positive Liebermann-Burchard test for steroids, ν_{max} (KBr) 1730 (ester) cm^{-1}; LR FAB$^+$ MS *m/z* 477 $[M + Na]^+$ (50%); EIMS *m/z* 394 $[M\text{-}HOAc]^+$ (100%); identified as stigmasterol acetate by comparison of its spectral data with those of authentic sample[2]. PE-PhH (7 : 1) eluate gave colourless crystals (15 mg), $C_{31}H_{52}O_2$ (M^+ 456), m.p. 126°; responded positive LB test for steroids; identified as β-sitosterol acetate by comparison of ^1H and MS spectral data with literature as well as by co-TLC with authentic sample. PE-PhH (5 : 1) eluate of the main column gave colourless crystals (30 mg), $C_{29}H_{48}O$ (M^+ 412), m.p. 168°; gave positive LB test for steroids; identified as stigmasterol by comparative spectral stu dies[3] and by preparation of its acetate, m.p. 146°.

The MeOH extract of *M. incana* stem was dissolved in minimum quantity of H_2O and was partitioned successively with PhH, EtOAc and *n*-BuOH. The PhH extract (2.2 g) was chromatographed on silica gel. Elution of the column with PE-EtOAc (9 : 1) gave a residue of three components, which on re-CC on silica gel afforded amorph. 3-Palmitoyllupeol (**1**) (35 mg) and a residue which on *p*-TLC afforded 3-benzoylepibetulin (**2**) (28 mg). Elution of the column with PE-EtOAc (6 : 1) gave colourless crystals (20 mg), $C_{29}H_{50}O$ (M^+ 414), m.p. 137°, responded to LB test for steroids and was identified as β-sitosterol.

Compound **1**, m.p. 110°, $C_{46}H_{80}O_2$ gave positive LB test for triterpenoid and quasimolecular ion peak at *m/z* 665 (calcd. *m/z* 665.6179 for $C_{46}H_{81}O_2$) in FAB-MS (positive mode). IR spectrum (KBr) showed bands for ester (1730 cm^{-1}) and terminal olefinic (1660 and 880 cm^{-1}) functions. ^1H NMR spectrum ($CDCl_3$) exhibited signals for six singlet methyls [δ0.78 (6H, H_3-26,28), 0.83 (3H, H_3-25), 0.85 (3H, H_3-23), 0.94 (3H, H_3-27) and 1.03 (3H, H_3-24)], a palmitoyl oxymethine group [δ 0.89 (3H, t), 1.20–1.28 (24H, brs), 1.63 (2H, m), 2.30 (2H, t, *J* 7.0 Hz), 4.47 (1H, m)], an isopropenyl group [δ 1.68 (3H, s), 4.56 and 4.68 (each 1H, d, *J* 1.5 Hz)] and a typical lyupene H_β-19 proton signal (δ 2.39, m) suggesting 3-palmitoyllupeol structure (**1**) of the compound[3]. The FAB-MS (positive mode) in addition to quasimolecular ion, $[M + H]^+$ (*m/z* 665, 3%) showed some characteristics mass peaks at *m/z* 425 [M-palmitoyl group $(C_{16}H_{31}O)]^+$ (10), 409 [M-palmitic acid + H]$^+$ (100), 394

[409-Me]$^+$ (12), 257 [palmitic acid + H]$^+$ (8), 218 (21), 206 (23) and 189 (37). Compound **1** on heating with 5% methanolic HCl gave lupeol, $C_{30}H_{50}O$ (M^+ 426), m.p. 215° and methyl palmitoate, $C_{17}H_{34}O_2$ (M^+ 270), semi solid mass[4]. Thus, the structure of compound **1** was confirmed as 3-palmitoyllupeol, which was supported by ^{13}C NMR with DEPT experiments (Table 1). Compound **2**, m.p. 65°, gave positive LB test for triterpenoids. The FAB-MS (positive mode) gave a quasimolecular ion, [M + H]$^+$ at *m/z* 547, consistent with the molecular formula $C_{37}H_{54}O_3$ (calcd. *m/z* 547.8397 for $C_{37}H_{55}O_3$). IR spectrum (KBr) showed bands for aromatic ester (1720 cm^{-1}) and terminal olefinic (1660 and 880 cm^{-1}) functions. ^1H NMR spectrum ($CDCl_3$) displayed five singlet methyl groups [δ 0.76 (3H, H_3-26), 0.82 (3H, H_3-25), 0.83 (3H, H_3-23), 0.97 (3H, H_3-27), 1.00 (3H, H_3-24)], a benzoyl oxymethine group [δ 4.60 (1H, m), (obscured with olefinic proton), 7.50–8.30 (5H, m)], an isopropenyl group [δ 1.68 (3H, s), 4.56 and 4.68 (1H each, d, *J* 1.5 Hz)], a hydroxymethyl group [δ 3.32 and 3.80 (each 1H, d, *J* 11.0 Hz)] suggesting 3-benzoylbetulin structure for the compound. The FAB-MS (positive mode) of **2** recorded quasimolecular ion at *m/z* 547 (10%), and other significant mass ions at *m/z* 441 [M-benzoyl group (C_7H_5O)]$^+$ (19), 425 [MH-benzoic acid]$^+$ (85), 410 [425-Me]$^+$ (79), 392 [410-H_2O]$^+$ (13), 218 (58), 205 (27), 203 ((44), 123 [benzoic acid + H] (48), 105 [benzoyl]$^+$ (100) also corroborated the said skeletal structure which was supported by ^{13}C NMR data (Table 1). The upfield chemical shift value of C-3 carbon (δ 79.0 ppm) compared to usual value (δ 80.9 ppm) suggested C-3 alpha conformation of benzoate group. Compound **2** on heating with 5% methanolic HCl gave 3-epibetulin, $C_{30}H_{50}O_2$ (M^+ 442), m.p. 205° (lit[5]. m.p. 200°) and methyl benzoate, $C_8H_8O_2$ (M^+ 136), oily mass, both of which, were identified by co-TLC with authentic samples. Thus compound **2** was characterised as 3-benzoylepibetulin. This is the first report of lupene derivative from genus *Mussaenda* and to the best of our knowledge, both **1** and **2** are new natural products.

Table 1. ^{13}C NMR spectral data of compounds **1** and **2** (75 MHz, $CDCl_3$) in δ ppm*

Carbon	1	2	Carbon	1	2
1	38.4 (t)	38.5 (t)	21	29.8 (t)	29.7 (t)
2	23.8 (t)	23.7 (t)	22	40.0 (t)	34.7 (t)
3	80.6 (d)	79.0 (d)	23	28.0 (q)	28.0 (q)
4	38.06 (s)	38.0 (s)	24	16.0 (q)	16.0 (q)
5	55.4 (d)	55.3 (d)	25	16.2 (q)	16.1 (q)
6	18.2 (t)	18.3 (t)	26	16.6 (q)	16.8 (q)
7	34.2 (t)	34.3 (t)	27	14.5 (q)	14.7 (q)
8	41.0 (s)	40.9 (s)	28	18.0 (q)	60.4 (t)
9	50.3 (d)	50.4 (d)	29	109.4 (t)	109.8 (t)
10	37.1 (s)	37.2 (s)	30	19.3 (q)	19.3 (q)
11	20.9 (t)	20.9 (t)	1′	173.8 (s)	129.3 (s)
12	25.2 (t)	25.2 (t)	2′	34.2 (t)	127.7 (d)
13	37.6 (d)	37.6 (d)	3′	25.1 (t)	127.7 (d)
14	42.8 (s)	42.7 (s)	4′–13′/4′*	29.2–29.8 (t)	*131.5 (d)
15	27.4 (t)	27.4 (t)	14′/7′*#	32.0 (t)	*#166.8 (s)
16	29.2 (t)	29.4 (t)	15′	22.7 (t)	
17	42.9 (s)	46.8 (s)	16′	14.2 (q)	
18	48.3 (d)	48.6 (d)			
19	48.0 (d)	47.7 (d)			
20	150.9 (s)	150.6 (s)			

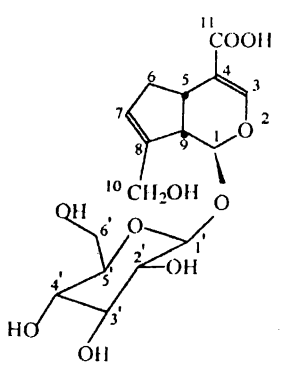
*Multipliciteis in parentheses.

The MeOH extract of *W. tinctoria* stem was dissolved in minimum quantity of water and was extracted with EtOAc and *n*-BuOH, successively. The EtOAc fraction was concentrated and chromatographed over silica gel. Elution of the column with PE-PhH (9 : 1) gave colourless crystals (20 mg) of compound **3**. Elution of the column with PE-PhH (5 : 1) gave colourless crystals (10 mg), $C_{29}H_{48}O$ (M^+ 412), m.p. 167°; responded positive LB test; identified as stigmasterol by direct comparison with an authentic sample. Elution of the column with PE-PhH (3 : 1) afforded colourless crystals (40 mg), m.p. 138°, identified as β-sitosterol. Elution of the column with PhH-$CHCl_3$ (9 : 1) gave granular crystals (25 mg), $C_{18}H_{36}O_2$ (M^+ 284), m.p. 72°, ν_{max} (KBr) 1705 (-CO_2H) cm^{-1}; identified as stearic acid from its spectral data as well as by comparison of the retention time of its methyl ester with that of authentic sample in gas chromatography. Elution of the column with EtOAc-MeOH (9 : 1) gave colourless crystals (50 mg), $C_6H_{14}O_6$, m.p. 168°, ν_{max} (KBr) 3400 cm^{-1} (OH); FAB-MS (negative mode) *m/z* 181 [M-H]$^-$ (92%); identified as *d*-mannitol by comparison of its spectral data[6] and co-TLC and mixed m.p. with an authentic sample.

Compound **3**, $C_{48}H_{96}O_2$ ([M + H]$^+$, *m/z* 705), m.p. 98°, showed IR band (KBr) for ester (1730 cm^{-1}) function. Its ^1H NMR spectrum ($CDCl_3$) displayed signals for one methyl [δ 0.88 (3H, t, *J* 7.0 Hz)], methylene protons [δ 1.26 (84H, brs, CH_2 × 42), 1.63 (2H, quintet, β-CH_2), 2.35 (2H, t, *J* 7.0 Hz, CH_2-CO), 4.05 (2H, t, *J* 7.0 Hz, OCH_2)] suggesting an aliphatic ester structure for the compound. FAB-MS (positive mode) recorded mass peaks at *m/z* 705 [M + H]$^+$ (15%), 285 [stearic acid + H]$^+$ (100), 284 (35), 57 (95), 43 (60) suggesting it to be an ester of stearic acid. The compound on saponification with 1 *N* methanolic KOH

under N_2 atmosphere gave myricyl alcohol (1-triacontanol), $C_{30}H_{62}O$, m.p. 86° (lit[7]. 88°) and stearic acid, $C_{18}H_{36}O_2$, m.p. 72° (lit[7]. 72°). Hence, the structure of compound **3** was deduced as myricyl stearate. To the best of our knowledge it is a new natural product.

The *n*-BuOH fraction of the MeOH extract was chromatographed over Diaion HP-20. Elution of the column with H_2O gave a colourless residue which was chromatographed over silica gel. Elution of the column with $CHCl_3$-MeOH (9 : 1) gave colourless crystals (50 mg), m.p. 155°, $C_{16}H_{22}O_{10}$ [HR-FAB-MS (negative), *m/z* 373.1169 $(M-H)^-$, calcd. for $C_{16}H_{21}O_{10}$ *m/z* 373.2819], ν_{max} (MeOH) 240 (log ε, 3.44)nm; ν_{max} (KBr) 3440 (OH), 1680 (conjugated CO_2H), 1630 (C=C) cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6) δ 5.06 (d, *J* 8.0 Hz, H-1), 7.39 (d, *J* 1.0 Hz, H-3), 3.02 (ddd, *J* 7.0, 7.0, 7.0 Hz, H-5), 2.03 (dd, *J* 15.0, 7.0 Hz, H_A-6), 2.67 (dd, *J* 15.0, 7.0 Hz, H_B-6), 5.66 (brs, H-7), 2.58 (t, *J* 7.0 Hz, H-9), 3.95 and 4.12 (each d, *J* 15.0 Hz, H-10), 4.52 (d, *J* 8.0 Hz, H-1′), 2.97 (t, *J* 9.0 Hz, H-2′), 3.14 (t, *J* 9.0 Hz, H-3′), 3.03 (t, *J* 9.0 Hz, H-4′), 3.10 (ddd, *J* 9.0, 4.5, 2.0 Hz, H-5′), 3.41 (dd, *J* 11.5, 5.5 Hz, H_A-6′), 3.64 (dd, *J* 11.5, 2.0 Hz, H_B-6′), 11.95 (brs, exchangeable with D_2O, HO_2C-4); ^{13}C NMR (100 MHz, DMSO-d_6) δ95.7 (d, C-1), 151.0 (d, C-3), 111.5 (s, C-4), 34.6 (d, C-5), 38.1 (t, C-6), 125.5 (d, C-7), 144.2 (s, C-8), 45.8 (d, C-9), 59.4 (t, C-10), 168.0 (s, C-11), 98.5 (d, C-1′), 73.3 (d, C-2′), 77.2 (d, C-3′), 70.0 (d, C-4′), 76.6 (d, C-5′), 61.0 (t, C-6′); (all NMR chemical shifts values were assigned on the basis of decoupling, DEPT, HMQC and HMBC experiments); HR-FAB-MS (negative mode) *m/z* 373.12 $[M-H]^-$ (100%); LR-FAB-MS (negative mode) *m/z* 373 (100%), 355 $[373-H_2O]^-$ (4), 211 $[M-C_6H_{11}O_5]^-$ (44), 179 [glucose-H]$^-$ (18), 107 (37). On the basis of spectral data, it was identified as geniposidic acid **4**. Although it was isolated earlier from *Wendlandia formosa*[8] and *Ixora*[9] and *Genipa*[10] spp, but detailed ^1H, ^{13}C NMR and MS data are being reported for the first time.



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Experimental

All organic solvents were distilled before use. Petroleum ether (PE) used had b.p. 60–80°. Electronic spectra were recorded on a Spectronic 21 spectrometer, IR spectra on a Perkin-Elmer 781 spectrophotometer, ^1H and ^{13}C NMR spectra on a Bruker AM-300 or Varian XL400 spectrometer using TMS as internal reference. GC was carried out on Pye Unicam 104 gas chromatograph and EI-MS and FAB-MS on a JEOL JMS-AX505H, JEOL JMS-700 Mstaion or JEOL X102/DA-6000 mass spectrometer. Extracts were column chromatographed on silica gel (Qualigen, 60–120 mesh) and thin layer chromatographed on silica gel G (Merck).

The rhizome of *L. spinosa* and stems of *M. incana* and *W. tinctoria* were collected from Belonia Sub-Division of Tripura. The plant materials were identified in Department of Botany, M. B. B. College, Agartala, and the voucher specimens were preserved in Botanical Garden of India, Shibpur, India.

The air-dried powdered rhizome (2 kg) of *L. spinosa* was extracted with EtOAc in a soxhlet for ~48 h and the extract was then evaporated under reduced pressure to obtain a brown residue (12.4 g). The air-dried powdered stem (2 kg) of *M. incana* was extracted with MeOH (6 L) at RT three times (24 h each time), and the extract was then concentrated under reduced pressure to a dark brown residue (22.6 g). The air-dried powdered stem (2 kg) of *W. tinctoria* was extracted with MeOH at room temperature to get a brown residue (18.2 g).

Acknowledgement

The authors thank Dr. Rina Ghosh, Jadavpur University, Kolkata, for IR spectra, R.S.I.C., Lucknow, for mass spectra of some samples and R.S.I.C., Shillong for NMR spectra of some samples. One of the authors (S.D.) is thankful to C.S.I.R., New Delhi, for the award of Senior Research Fellowship.

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