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Euphane triterpenoid and lipid constituents from *Butea monosperma*☆

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Abstract

Besides stigmasterol, stigmasterol-ßD-glucopyranoside and nonacosanoic acid, two new compounds isolated from the stems of *Butea monosperma* have been characterised as 3α-hydroxyeuph-25-ene and 2,14-dihydroxy-11,12-dimethyl-8-oxo-octadec-11-enylcyclohexane by spectral data and chemical studies.

Keywords: *Butea monosperma*; Fabaceae; 3α-Hydroxyeuph-25-ene; Lipids; Steroids

1. Introduction

Butea monosperma (Fabaceae) is a medium sized tree native of the mountainous regions of India and Burma and now grows wild throughout India (Anonymous, 1988). The bark is reported to possess antitumour and antiulcer properties (Anonymous, 1988), while stem bark possess antifungal activity (Bandara et al., 1989; 1990). The root bark is used as an aphrodisiac, analgesic and anthelmintic (Anonymous, 1988), whereas the leaves possess antimicrobial property (Zaffar et al., 1989). The flowers are used for the treatment of liver disorders (Wagner et al., 1986) and the seeds as anthelmintic (Lal et al., 1978). Flavonoids from flowers (Gupta et al., 1970), stem bark (Bandara et al., 1990) and nitrogenous compounds from the seeds (Guha et al., 1990; Mehta and Bokadia, 1981) have been reported earlier.

During the course of investigation on the yellow dye from the flowers, it was of interest to work on the stems as relatively few compounds have been reported from it. This report describes the characterisation of a new euphane and a new lipid derivative.

2. Results and discussion

Compounds **1–5** were isolated from the stems of *B. monosperma*. Compound 1 was obtained as colourless needles. Its IR spectrum bands corresponded to OH (3480 cm^{-1}), terminal unsaturated CH_2 (1610, 880 cm^{-1}) and gem dimethyl (1380, 1360 cm^{-1}) functions. An $[M]^+$ at m/z 428 suggested the molecular formula as $C_{30}H_{52}O$. Its ^1H NMR spectrum exhibited signals due to five quaternary methyls (δ 0.79, 0.83, 0.94, 0.96, 1.04), a vinylic methyl (δ 1.62), and a secondary methyl (δ 1.08) indicative of a tetracyclic triterpene skeleton (Hemmert et al., 1966). Two broad singlets at δ 4.56 and 4.68, each integrating for one proton, corresponded to the methine protons of an olefinic methylene group (Jagodzinska et al., 1985) which in conjunction with a broad singlet at δ 1.62 suggested the existence of a isopropylidene group. This was also substantiated by the appearance of signals at δ 151.0 and 109.30 in the ^{13}C NMR spectrum for $>C{=}CH_2$ grouping (Schneider and Agrawal, 1984). A multiplet

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at δ 3.34 ($W^{1/2} = 7$ Hz) was assigned to a methine proton with an axially oriented C-3 OH group (Jolad et al., 1981; Nakanishi et al., 1986). There is a remarkable similarity in the ^{13}C NMR chemical shifts of euphenol and lanosterol except for those of C-12, C-15, C-16 and C-19 (Knight, 1974). This excludes **1** to be included under lanosterol series. Such difference is also seen in euphane and tirucallane triterpenoids (Jolad et al., 1981; Bhakuni et al., 1987; Singh et al., 1989). The chemical shift range of the methyl protons except for vinylic CH_3 suggested a euphane or tirucallane skeleton (differing C-20 stereochemistry) in **1**, and a (+) optical rotation 37.8° of **1** indicated that it belonged to euphane rather than tirucallane series (Itoh et al., 1976).

In the MS of **1**, the loss of CH_3 and H_2O from $[M]^+$ was seen at *m/z* 413 and 410. The formation of a fragment ion at *m/z* 316 [M-side chain (C_8H_{15})–H]$^+$ suggested the presence of a monounsaturated side chain (Wyllie and Djerassi, 1968) and the existence of OH group in the tetracyclic system.

Compound **1** on treatment with Ac_2O/pyridine afforded an acetate (**1a**), $C_{32}H_{54}O_2$, whose ^1H NMR spectrum was similar to **1** except for the appearance of a new signal at δ 2.04 and downfield shifted C-3 methine by 1.11 ppm. In the ^{13}C NMR spectrum of **1**, the appearance of C-18, C-20 and C-21 at 15.4, 35.6 and 19.3 also established the euphane skeleton (Wehrli and Nishida, 1979; Knight, 1974). The ^{13}C NMR spectral data of **1** (Table 1) were analysed by analogy with the reported values for euphane and other related triterpenoids (Wehrli et al., 1979; Knight, 1974; Singh et al., 1989). These data led to characterise **1** as 3α-hydroxyeuph-25-ene.

Table 1
^{13}C NMR spectral data for compound **1** (75 MHz, δ, $CDCl_3$)

C	δ
1	35.6
2	27.4
3	79.0
4	38.0
5	50.4
6	19.3
7	29.8
8	38.8
9	48.0
10	42.8
11	25.1
12	34.3
13	43.0
14	48.3
15	34.3
16	29.7
17	55.3
18	15.4
19	18.3
20	35.6
21	19.3
22	34.3
23	28.0
24	37.2
25	151.0
26	109.3
27	20.9
28	14.5
29	25.1
30	18.0

Compound **4** showed IR bands at 3438 (OH), 1730 (CO), 1670 (tetrasubstituted) (Nakanishi and Solomon, 1977), 1386 (Me) and 720 cm^{-1} (straight chain). It was assigned the molecular formula $C_{26}H_{48}O_3$ ($[M]^+$ = 408 and elemental analysis). Successive loss of two H_2O molecules was seen at *m/z* 390 and 372, whereas the location of these OH groups was assigned at C-2 and C-14 since significant α-fission ions were appeared at *m/z* 311, 281, 127, 97 and 351, 321, 87, 57, respectively. The other diagnostic α-fission ions at *m/z* 225, 211, 197, 183 together with ß-fission ions involving McLafferty rearrangement (Budzikiewicz et al., 1964) at *m/z* 226 and 182 located the keto group at C-8. The abundant ions at *m/z* 83 and 325 indicated the presence of a cyclohexyl moiety at the end carbon atom (Bhakuni and Shukla, 1992). The tetrasubstituted double bond along with the two methyl groups were assigned at C-11,12 since significant ions were seen at *m/z* 307, 253, 155 and 101. These data suggested the structure of **4** as 2,14-dihydroxy-11,12-dimethyl-8-oxooctadec-11-enylcyclohexane which was also in full accord with its ^1H NMR (see Section 3).

Compounds **2**, **3** and **5** were identified as nonacosanoic acid, stigmasterol and stigmasterol-ßD-glucopyranoside, respectively, by comparison with authentic samples. The straight chain alcohols having a cyclohexane moiety are not commonly found in nature.

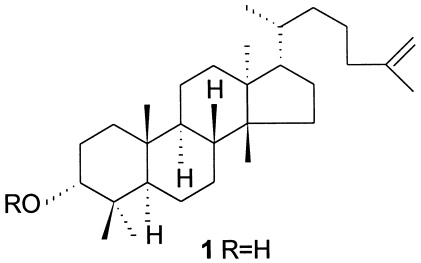
3. Experimental

3.1. General

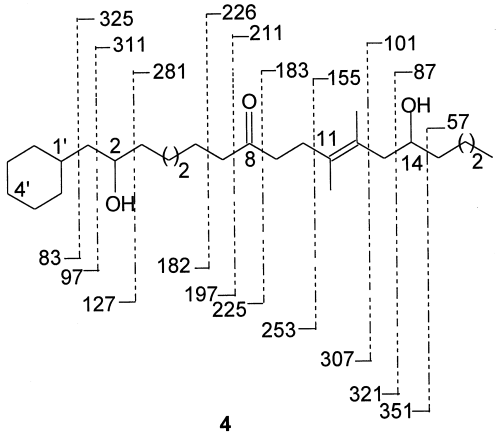
Mps: uncorrected; IR: KBr; ^1H NMR: Bruker (200 MHz) in $CDCl_3$, chemical shifts in δ (ppm) with TMS as internal standard; ^{13}C NMR: 75 MHz, $CDCl_3$; EIMS: 70 ev; TLC: silica gel G; CC: silica gel (Merk, 60–120 mesh). Spots were detected by exposure to I_2 vapour. The homogeneity of the isolates was checked on TLC in at least two solvent systems.

3.2. Plant material

The stems of *B. monosperma* were collected from the local area and identified in our Botany Department where a voucher specimen has been maintained.



1 R=H
1a R=Ac



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3.3. Extraction and isolation

Dried and powdered stems (2.8 kg) were extracted with MeOH (5 × 11 l) and the combined extract concentrated to 500 ml. After the addition of water (500 ml), the extract was fractionated with *n*-hexane (5 × 200 ml, 7.33 g), EtOAc (5 × 200 ml, 13.34 g) and *n*-BuOH (5 × 200 ml, 33.2 g). A portion (6.1 g) of the hexane fraction was chromatographed over silica gel (300 g), eluting with varying proportions of hexane, EtOAc and MeOH to provide compounds **1** (50 mg), **2** (61 mg), **3** (68 mg), **4** (25 mg) and **5** (46 mg). The fractions collected were 100 ml each and monitored by TLC.

3.4. 3α-Hydroxyeuph-25-ene (*1*)

Removal of solvent from the fractions 65–68 of hexane–EtOAc (95:5) gave a residue, mp 180–82°C, $[\alpha]_D$ +37.8° ($CHCl_3$). IR: γ_{max} cm^{-1}: 3480, 2930, 1610, 1450, 1360, 1244, 1110, 1014, 880; ^1H NMR: δ 0.79 (3H, *s*, 19-H_3), 0.83 (3H, *s*, 18-H_3), 0.94 (3H, *s*, 29-H_3), 0.96 (3H, *s*, 28-H_3), 1.04 (3H, *s*, 30-H_3), 1.08 (3H, *d*, $J = 6$ Hz, 21-H_3), 1.62(3H, *s*, 27-H_3), 1.90 (1H, *m*, 20-H), 2.36 (2H, *m*, 24-H_2), 3.34 (1H, *m*, $W^{1/2} = 7$ Hz, 3ß-H), 4.56, 4.68, (2H, each *br s*, 26-H_2); MS *m/z* (rel. int.): 428 $[M]^+$ ($C_{30}H_{52}O$, 67), 413 $[M - Me]^+$(19), 410 $[M - H_2O]^+$(5), 395 $[M - Me–H_2O]^+$(5), 387 $[M - 41]^+$(6), 317 $[M - sc]^+$(15), 316 $[M - sc–H]^+$(18), 301 $[316 - Me]^+$(5), 298 $[316 - H_2O]^+$(5), 286 $[301 - Me]^+$(5), 196 (5), 194 (8), 178 $[196 - H_2O]^+$(18), 179 $[194 - Me]^+$(10), 161 (12), 128 (5), 111 (20), 110 (15), 95 (100), 81 (80), 69 (75).

3.5. Monoacetate (*1a*) of *1*

Compound **1** (25 mg) was acetylated with C_5H_5N–Ac_2O (1 ml each), overnight at room temperature. The usual work-up yielded **1a**, mp 205°C, 20 mg. IR γ_{max} cm^{-1}: 2940, 2856, 1738, 1640, 1460, 1374, 1245, 1020, 978, 880; ^1H NMR: δ 0.79 (3H, *s*, 19-H_3), 0.84 (3H, *s*, 18-H_3), 0.94 (3H, *s*, 29-H_3), 0.97 (3H, *s*, 28-H_3), 1.03 (3H, *s*, 30-H_3), 1.08 (3H, *d*, $J = 6$ Hz, 21-H_3), 1.68 (3H, *s*, 27-H_3), 1.90 (1H, *m*, 20-H), 2.04 (3H, *s*, OAc), 2.36 (2H, *m*, 24-H_2), 4.45 (1H, *m*, $W^{1/2} = 7$ Hz, 3ßH), 4.56, 4.68 (2H, each *br s*, 26-H_2); MS *m/z* (rel. int.): 470 $[M]^+$ ($C_{32}H_{54}O_2$, 11), 455 (4), 429 (5), 410 (2), 359 (5), 257 (5), 238 (5), 236 (4), 188 (100), 176 (15), 161 (17), 160 (21), 111 (20), 110 (15), 95 (60), 81 (80), 69 (40), 55 (50), 43 (98).

3.6. Nonacosanoic acid (*2*)

Fractions 71–75 of hexane–EtOAc (95:5) eluates yielded a residue, mp 68–70°C, identified by co-TLC, MS, ^1H NMR.

3.7. Stigmasterol (*3*)

Fractions 90–95 of hexane–EtOAc (95:5) eluates when freed of the solvent provided **3**, mp 160°C, identified by co-TLC, MS, IR, ^1H NMR.

3.8. 2,14-Dihydroxy-11,12-dimethyl-8-oxooctadec-11-enylcyclohexane (*4*)

Elimination of solvent from the fractions 221–226 of hexane–EtOAc (1:1) eluates furnished **4**, mp 83–84°C. IR γ_{max} cm^{-1}: 3438, 2920, 2850, 1730, 1670, 1450, 1386, 1180, 1110, 1052, 720; ^1H NMR: δ 0.88 (3H, *t*, $J = 6$ Hz, 18-H_3), 1.25 (10H, *br s*, 5 × CH_2), 1.50 (10H, *br s*, cyclohexyl $(CH_2)_5$), 1.64 (1H, *m*, 1′-H), 1.60 (6H, *s*, 11-H_3, 12-H3), 1.90 (4H, *m*, 3-H_2, 15-H_2), 2.02 (2H, *m*, 1-H_2), 2.35 (4H, *t*, $J = 6$ Hz, 7-H_2, 9-H_2), 2.45 (2H, *t*, $J = 6$ Hz, 10-H_2), 2.48 (2H, *d*, $J = 5$ Hz, 13-H_2), 3.64 (1H, *m*, 2-H), 3.94 (1H, *m*, 14-H); MS *m/z* (rel. int.): 408 $[M]^+$ ($C_{26}H_{48}O_3$, 2), 390$[M - H_2O]^+$(7), 372 $[M - 2H_2O]^+$(7), 393 $[M - Me]^+$(3), 378 $[M - 2 \times Me]^+$(3), 351 (4), 325 (5), 321 (3), 311 (4), 307 (5), 281 (4), 253 (5), 226 (6), 225 (5), 211 (7), 197 (6), 183 (10), 182 (7), 155 (4), 127 (19), 101 (6), 97 (90), 87 (12), 83 (65), 57 (100) (Found: C, 76.40; H, 11.82. $C_{26}H_{48}O_3$ requires: C, 76.47; H, 11.76%).

3.9. Stigmasterol-βD-glucopyranoside (5)

Removal of solvent from 290–301 fractions of MeOH–EtOAc (2:98) eluates gave **5**, mp 263–65°C, identified by MS, ^{1}H NMR and hydrolysis products.

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