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Isolation of a new keto steroid stigmast-4, 20(21), 23-trien-3-one and a new alcohol tricontan-10 α -ol from the roots of *Albizia lebbek* Benth

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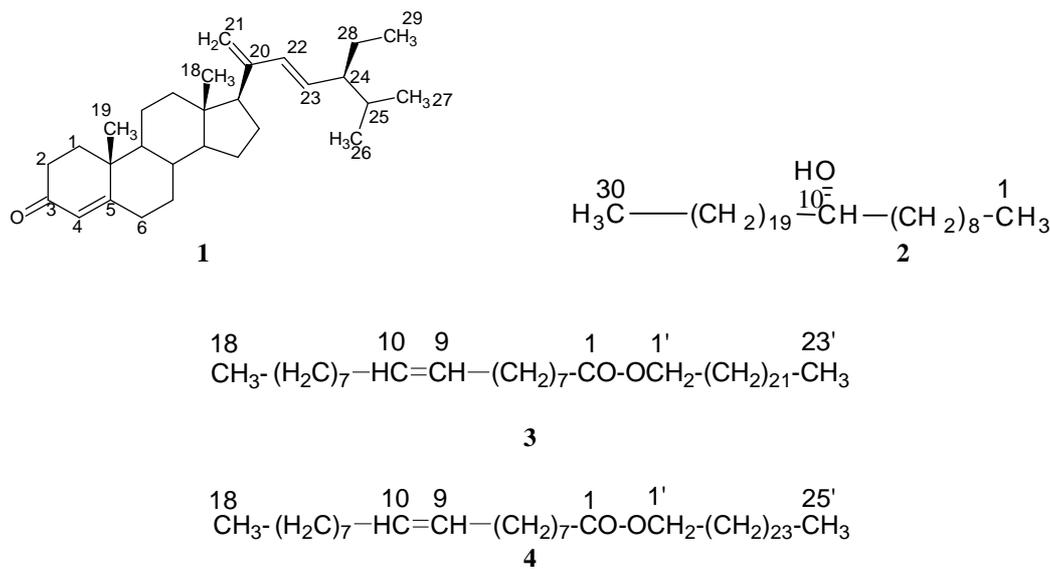
ABSTRACT

Phytochemical investigation of the methanolic extract of the roots of *Albizia lebbek* Benth. (Fabaceae) led to the isolation of a new keto steroid characterized as stigmast-4, 20 (21), 23-trien-3-one (lebbeksterone) and an alcohol tricontan-10 α -ol (Isotriacontanol), along with tricosanyloctadec-9-en-1-oate and pentacosanyloctadec-9-en-1-oate. The structures of all these phytoconstituents have been elucidated on the basis spectral data analysis and chemical reactions.

Keywords: *Albizia lebbek*, Fabaceae, roots, lebbeksterone, isotriacontanol.

INTRODUCTION

A. lebbek Benth (Fabaceae), commonly known as sirish is a large, erect, deciduous tree, available across India, ascending to 1200 m on the Himalayas, widely cultivated and naturalized in other tropical and subtropical regions [1-3]. The stem bark of the plant is used in eczema, leprosy, itching, cough, ulcer, oedema, asthma, urticaria, blood disorders, sinusitis, malaria fever, rhinitis, snake bite, worm infection [2]. The root bark is used as dental powder for strengthening the gums [1]. The saponins and flavonoids isolated from different parts are: echinocystic acid glycosides, albiziasaponins A, B and C from the bark [4,5]; 3', 5 dihydroxy 4', 7 dimethoxy flavone and N-Benzoyl L phenyl alaninol and a triterpenoid sapogenin, albigenic acid from the pods [6,7] ; tri-o-glycoside flavonols kaempferol and quercetin from the leaves [8]; Albzziahexoside, saponins from leaves [9]; N-demethyl budmunchamines from seeds [10] and tannins from [11]. Recently, four new phytoconstituents, 2' α - hydroxy octyl hexadecanoate (hydroxyoctyl palmitate), salicylic acid-2-O- β -D-glucofuranosyl-6'-octadec-9"-enoate (salicylic acid arabinosyl oleate) along with a fatty acid phytoconstituents, docos-3-en-1-oic acid (docosenoic acid), and two esters tricosanyl hexadecanoate and hexacosanyl octadec-9-en-1-oate (hexacosanyl oleate) have been isolated from the methanolic root extract of the plant [12]. In continuation of our research, the present paper describes the isolation and characterization of one new keto steroid stigmast-4, 20 (21), 23-trien-3-one (1) and one new aliphatic alcohol tricontan-10 α -ol (2) and two known fatty acid esters tricosanyloctadec-9-en-1-oate (3), and pentacosanyloctadec-9-en-1-oate (4) from the roots of *A. lebbek*.

Structures of compounds **1-4**

MATERIALS AND METHODS

3. Experimental

3.1 General experimental procedure

The melting points were determined on a Perfit apparatus and are uncorrected. The IR spectra were recorded in KBr pellet on Win IR FTS 135 instrument (Biorad, USA). ^1H (300 MHz) and ^{13}C (75 MHz) NMR spectra were run by Bruker spectropin NMR instrument in CDCl_3 , using TMS as internal standard. FAB MS were scanned at 70 eV on a Jeol D-300 instrument. Column chromatography was performed on silica gel (Merck, 60-120 mesh) and thin-layer chromatography on silica gel G coated TLC plates (Merck). Spots were visualized by exposure to iodine vapours, UV radiation and by spraying reagent.

3.2 Plant Material

The roots of *A. lebeck* were collected from West Champaran, Bihar, (India) and identified by Dr. H. B. Singh, Scientist F and Head, Raw Materials, Herbarium and Museum, National Institute of Science Communication and Information Resources (NISCAIR), New Delhi, India. A voucher specimen (No. NISCAIR/RHMD/Consult/-2008-09/1114/145) was deposited in The Herbarium of NISCAIR, New Delhi.

3.3 Extraction and isolation

The roots (1.6 kg) were shade dried, coarsely powdered and extracted exhaustively with methanol. The methanolic extract was concentrated under reduced pressure in a Buchi rotavapor to obtain a dark green viscous mass. Small portion of the extract was analyzed chemically to determine the presence of different chemical constituents. The viscous mass was dissolved in little amount of methanol and adsorbed on silica gel (60-120 mesh) for column for preparation of slurry. The slurry (85.5 g) was air-dried and chromatographed over silica gel column packed in petroleum ether. The column was eluted successively with petroleum ether, mixture of petroleum ether and chloroform (9:1, 3:1, 1:1 and 1:3), pure chloroform and finally the mixture of chloroform and methanol (99:1, 98:2, 96:4, 95:5, 97:3, 9:1). Various fractions were collected separately and matched by TLC to check homogeneity. Similar fractions (having same R_f values) were combined and crystallized. The isolated compounds were recrystallized to get the following compounds:

3.3.1 Lebbeksterone (1)

Elution of the column with petroleum ether-chloroform (1:3) afforded colourless crystals of **1**, recrystallized from acetone, 710 mg; R_f : 0.52 (CHCl_3); m.p: 252-253 °C; UV (MeOH) λ_{max} (log ϵ) 223 nm (5.5); IR ν_{max} (KBr) cm^{-1} : 2925, 2845, 1705, 1645, 1475, 1360, 1290, 1015 ; The ^1H NMR and ^{13}C NMR (CDCl_3), see Table 1; +ve ion FAB MS m/z (*rel. int.*): 408 [$\text{M}]^+$ ($\text{C}_{29}\text{H}_{44}\text{O}$) (27.1), 393 (21.6), 272 (32.8), 271 (18.2), 256 (19.9), 241 (15.7), 232 (14.2),

229 (13.1), 213 (21.6), 190 (20.3), 176 (19.3), 175 (18.1), 162 (26.7), 160 (3.1), 148 (31.2), 147 (41.3), 145 (11.5), 137 (21.5), 136 (38.5), 134 (40.6), 133 (34.2), 122 (49.9), 119 (52.1), 108 (75.8), 85 (56.1), 43 (33.1).

3.3.2 Isotriacontanol (2)

Elution of the column with petroleum ether-chloroform (3:1) yielded colourless crystals of **2**, recrystallized from chloroform-methanol (1:1), 150 mg (0.17% yield); R_f : 0.64 (CHCl₃-MeOH, 9:1); m.p: 88-89 °C; UV λ_{max} (MeOH): 225 nm (log ϵ 3.1); IR ν_{max} (KBr) cm⁻¹: 3446, 2923, 2854, 1641, 1462, 1112, 726; The ¹H NMR (CDCl₃): δ 4.05 (1H, m, $w_{1/2}$ = 6.6 Hz, H-10 β), 2.31 (2H, m, CH₂), 1.61 (6H, m, 3 \times CH₂), 1.25 (46H, brs, 23 \times CH₂), 0.88 (3H, t, J = 6.9 Hz, Me-1), 0.86 (3H, t, J = 6.6 Hz, Me-30); The ¹³C NMR (CDCl₃): δ 74.11 (C-10), 32.06 (CH₂), 29.57 (21 \times CH₂), 29.03 (CH₂), 29.01 (CH₂), 28.99 (CH₂), 26.13 (CH₂), 22.55 (CH₂), 14.17 (Me-1), 14.09 (Me-30); +ve ion FAB MS m/z (*rel. int.*): 438 [M]⁺ (C₃₀H₆₂O) (26.3), 423 (55.7), 420 (36.1), 408 (72.2), 311 (16.3), 293 (17.5), 281 (78.6), 157 (31.8), 127 (23.4).

3.3.3 Tricosanyl oleate (3)

Elution of the column with chloroform furnished colourless crystals of **3**, recrystallized from chloroform-methanol (1:1), 210 mg (0.25% yield); R_f : 0.69 (CHCl₃); m.p: 79-80 °C; UV λ_{max} (MeOH): 225 nm (log ϵ 3.7); IR ν_{max} (KBr) cm⁻¹: 2922, 2854, 1745, 1638, 1461, 1373, 1165, 723; ¹H NMR (CDCl₃): δ 5.35 (2H, m, H-9, H-10), 4.28 (1H, d, J = 6.6 Hz, H₂-1'a), 4.17 (1H, d, J = 6.6 Hz, H₂-1'b), 2.31 (2H, brs, H₂-2), 2.04 (2H, brs, H₂-8), 2.01 (2H, brs, H₂-11), 1.64 (6H, brs, 3 \times CH₂), 1.25 (58H, brs, 29 \times CH₂), 0.88 (6H, brs, Me-18, Me-23'); ¹³C NMR (CDCl₃): δ 173.26 (C-1), 129.98 (C-9), 128.04 (C-10), 62.07 (C-1'), 34.02 (CH₂), 31.90 (CH₂), 31.50 (CH₂), 29.67 (27 \times CH₂), 29.31 (CH₂), 27.17 (CH₂), 25.60 (CH₂), 24.84 (CH₂), 22.66 (CH₂), 14.08 (2 \times CH₃); +ve ion FAB MS m/z (*rel. int.*): 604 [M]⁺ (C₄₁H₈₀O₂) (28.3), 339 (100), 281 (13.5), 265 (41.9), 139 (11.8), 113 (15.1).

3.3.4 Pentacosanyl oleate (4)

Elution of the column with chloroform furnished colourless crystals of **4**, recrystallized from methanol, 270 mg (0.31% yield); R_f : 0.56 (CHCl₃); m.p: 86-90 °C; UV λ_{max} (MeOH): 223 nm (log ϵ 2.9); IR ν_{max} (KBr) cm⁻¹: 2923, 2854, 1745, 1639, 1460, 1374, 1164, 723; ¹H NMR (CDCl₃): δ 5.36 (1H, m, H-9), 5.34 (1H, m, H-10), 4.12 (2H, brs, H₂-1'), 2.34 (1H, d, J = 7.2 Hz, H₂-2a), 2.29 (1H, d, J = 7.2 Hz, H₂-2b), 2.06 (2H, m, H₂-8), 2.02 (2H, m, H₂-11), 1.78 (2H, m, CH₂), 1.54 (4H, m, 2 \times CH₂), 1.30 (2H, brs, CH₂), 1.04 (2H, m, CH₂), 0.88 (3H, t, J = 6.6 Hz, Me-18), 0.83 (3H, t, J = 7.8 Hz, Me-25'); ¹³C NMR (CDCl₃): δ 172.16 (C-1), 131.54 (C-9), 129.15 (C-10), 62.18 (C-1'), 34.55 (CH₂), 34.49 (CH₂), 31.95 (CH₂), 31.04 (CH₂), 30.47 (CH₂), 29.73 (24 \times CH₂'), 29.73 (CH₂), 29.25 (CH₂), 27.22 (CH₂), 25.64 (CH₂), 24.89 (CH₂), 22.31 (CH₂), 21.16 (CH₂), 19.01 (CH₂), 13.03 (Me-18), 13.01 (Me-25); +ve ion FAB MS m/z (*rel. int.*): 632 [M]⁺ (C₄₃H₈₄O₂) (11.3), 395 (31.9), 367 (32.8), 265 (23.7), 237 (11.5).

RESULTS AND DISCUSSION

Compound **1**, designated as lebbeksterone, was obtained as colourless crystals from petroleum ether: chloroform (1:3) eluants. It responded positively to Liebermann-Burchard test for steroids. Its IR spectrum showed characteristic absorption bands for carbonyl group (1705 cm⁻¹) and unsaturation (1645 cm⁻¹). Its +ve FAB mass spectrum displayed a molecular ion peak at m/z 408 consistent with a steroidal molecular formula, C₂₉H₄₄O. It indicated the presence of eight double-bond equivalents, four of them were adjusted in the steroidal carbon framework, three in olefinic linkages and one in a carbonyl group. The diagnostically important peaks were observed at m/z 108 [C_{5,6}-C_{9,10} fission]⁺, 122 [C_{6,7}-C_{9,10} fission]⁺, 272 and 136 [C_{7,8}-C_{9,10} fission]⁺ that indicated the saturated nature of ring B and the presence of olefinic linkage in ring A. The fragment ion peaks at m/z 162 [C_{8,14}-C_{9,11} fission]⁺, 176 [C_{8,14}-C_{11,12} fission]⁺, 190 [C_{8,14}-C_{12,13} fission]⁺ supported the saturated nature of ring C. The other important fragment peaks at m/z 137 [C₁₀H₁₇, side chain]⁺, 271 [M-side chain]⁺, 229 [271-ring D]⁺ and 256 [271-Me]⁺ suggested the presence of saturated ring D and a C-10 unsaturated side chain. The fragment ion peaks at m/z 134 [162-CO]⁺, 148 [176-CO]⁺, 162 [190-CO]⁺ and 213 [241-CO]⁺ supported the presence of carbonyl group in ring A that was placed at C-3 on biogenetic considerations. The ion fragments at m/z 43 [C₂₄-C₂₅ fission]⁺ and 85 [C₂₃-C₂₄ fission]⁺ indicated the presence of exocyclic vinylic linkages at C-20 and C-22 and an ethyl group in the side chain at C-24. The ¹H NMR spectrum of **1** exhibited three one-proton downfield broad signals at δ 5.55, 5.04 and 4.99 assigned correspondingly to H-4, H-21a and H-21b vinylic protons. A doublet and a double-doublet at δ 5.12 (J = 6.3 Hz) and 5.06 (J = 6.3, 6.1 Hz), each integrating for one proton, were ascribed correspondingly to H-22 and H-23 vinylic protons. Two doublets, three-protons each, at δ 0.87 (J = 4.2 Hz) and 0.84 (J = 5.0 Hz), were assigned to H-26 and H-27 secondary methyl protons. The tertiary methyl protons resonated as two three-proton broad

singlets at δ 1.02 (Me-19) and 0.65 (Me-18). The appearance of methyl protons in the range δ 1.02-0.65 suggested that these groups were attached to saturated carbons. The ^{13}C NMR spectrum of **1** displayed signals for carbonyl carbon at δ 212.11 (C-3) and olefinic carbons at δ 129.52 (C-4), 139.55 (C-5), 138.11 (C-20), 117.01 (C-21), 130.04 (C-22) and 129.58 (C-23). The ^{13}C NMR data were compared to β -sitosterol, lawsaritol and phyllanfraterusterol. The ^1H - ^1H COSY spectrum of **1** showed interactions of H-4 with H₂-2 and H₂-6; H₂-21 with H-17 and H-22; and H-23 with H-22 and H-24. The HMBC spectrum of **1** exhibited correlations of C-3 with H₂-2 and H-4; C-5 with H-4 and H₂-6; and C-20 with H₂-21, H-17 and H-22. On the basis of above discussion the structure of **1** was formulated as stigmast-4, 20 (21), 23-trien-3-one. This is a new ketosteroid isolated from a plant source.

Table 1. ^1H and ^{13}C NMR spectral values of Lebbeksterone (**1**)

Position	^1H NMR		^{13}C NMR
	α (alpha)	β (beta)	
1	1.34 m	2.27 m	38.19
2	2.31 m	2.15 m	31.95
3	--	---	212.11
4	5.55 brs	--	129.52
5	--	--	139.55
6	2.42 brs	2.77 brs	19.01
7	1.83 m	1.74 m	34.42
8	--	1.25 brs	30.06
9	1.50 m	--	51.26
10	--	--	39.33
11	2.02 m	1.47 m	21.71
12	1.10 brs	1.83 brs	40.07
13	--	--	41.27
14	1.53 m	--	55.85
15	1.10 brs	1.49 brs	24.66
16	1.61 m	1.51 m	28.53
17	1.43 m	--	55.03
18	0.65 brs	--	21.14
19	1.02 brs	--	21.14
20	--	--	138.11
21	5.04 brs	4.99 brs	117.01
22	5.12 d (6.3)	--	130.04
23	5.06 dd (6.3, 6.1)	--	129.58
24	1.19 m	--	48.04
25	1.57 m	--	29.40
26	0.87 d (4.2)	---	23.04
27	0.84 d (5.0)	--	21.40
28	1.25 brs	1.25 brs	28.53
29	0.81 d (6.1)	--	12.12

Coupling constants in Hertz are provided in parenthesis.

Compound **2**, named isotriacontanol, was obtained as a colourless crystalline mass from petroleum ether: chloroform (3:1) eluants. Its IR spectrum showed characteristic absorption bands for hydroxyl group (3446 cm^{-1}) and long aliphatic chain (726 cm^{-1}). The mass spectrum of **2** exhibited a molecular ion peak at m/z 438 corresponding to a molecular formula of a saturated aliphatic alcohol, $\text{C}_{30}\text{H}_{62}\text{O}$. The mass spectrum displayed $\text{C}_n\text{H}_{2n+2}$, C_nH_{2n} and $\text{C}_n\text{H}_{2n-1}$ fragmentation peaks and most of the fragments were separated by 14 mass units, that indicated straight chain nature of **2** (Ali and Ansari, 1991). The prominent ion peaks generated at m/z 127 $[\text{CH}_3(\text{CH}_2)_8]^+$, 311 $[\text{CH}_3(\text{CH}_2)_{19}\text{CHOH}]^+$ due to C_9 - C_{10} fission and at m/z 157 $[\text{CH}_3(\text{CH}_2)_8\text{CHOH}]^+$, 281 $[\text{CH}_3(\text{CH}_2)_8]^+$ due to C_{10} - C_{11} fission suggested the location of the hydroxyl group at C_{10} position. The fragment ion peaks at m/z 420 $[\text{M}-\text{H}_2\text{O}]^+$ and 293 $[311-\text{H}_2\text{O}]^+$ supported the presence of hydroxyl group in the compound **2**. The ^1H NMR spectrum of **2** showed a- one proton broad multiplet at δ 4.05 with half width of 6.6 Hz assigned to β -oriented H-10 carbinol proton. Four broad signals at δ 2.31 (2H), 1.61 (6H) and 1.25 (46H) were ascribed to the methylene protons. Two three-proton triplets at δ 0.88 ($J= 6.9$ Hz) and 0.86 ($J= 6.6$ Hz) were attributed to H-1 and H-30 primary methyl protons, respectively. The absence of any signal beyond δ 4.05 supported the saturated nature of the molecule. The ^{13}C NMR spectrum of **2** exhibited important signals for carbinol carbon C-10 at δ 74.11, methylene carbons between δ 32.06 to 22.25 and methyl carbons at δ 14.17 and 14.09. The absence of any signal beyond δ 74.11 supported the saturated nature of **2**. On the basis of foregoing discussion the structure of **2** has been elucidated as tricontan-10 α -ol. This is a new aliphatic alcohol.

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