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Research Article

Phytochemical Investigations of *Heliotropium eichwaldi* Stued. Ex Dc. Roots

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ABSTRACT
In the present study the methanol was used to extract of dried root of *Heliotropium eichwaldi* giving four compounds whose names are glyceryl-1,2,3-triarachidate, stigmast-5,23-dien-3β-ol, n-tetracont-17-enoic acid and n-undecanyl-n-docosanoate. The structures of the compounds were established on the basis of spectroscopic analysis.

Keywords: *Heliotropium eichwaldi*, Borganiaceae, glyceryl-1,2,3-triarachidate, stigmast-5,23-dien-3β-ol, n-tetracont-17-enoic acid, n-undecanyl-n-docosanoate.

INTRODUCTION

Today estimate that about 80% of people in developing countries still relays on traditional systems of medicine for their primary health care. Plants form the main ingredients of medicines in traditional systems of medicines and have been the source of inspiration for many researcher in field of medicine. The use of plants as medicines has involved the isolation of active compounds, beginning with the isolation of morphine from opium in the early 19th century[1] and subsequently led to the isolation of early drugs such as cocaine, codeine, digitoxin and quinine[2,3]. Isolation and characterization of pharmacologically active compounds from medicinal plants continue today[4-10]. Investigation into the chemical composition of medicinal plants and especially secondary metabolites is a dynamic research field worldwide and is the base for Drug discovery. The present study is aimed to isolate and characterize few phytoconstituents from the methanolic extract of *Heliotropium eichwaldi* Stued. ex Dc. roots.

Experimental: The melting points were determined on a Perfit apparatus. The IR spectra were recorded on KBr pellet using a Jasco FT/IR-5000 instrument (FTS 135, Hongkong).The ^{1}H NMR (400 MHz) and ^{13}C NMR (100 MHz) spectra were scanned on Avance DRX 400, Bruker spectrospin 400 MHz instrument (Rheinstetten, Germany) using $CDCl_3$ as solvent and TMS as internal standard. ESI MS was scanned at 70 eV on a Jeol D-300 instrument (Jeol, USA). Column (450×4×0.2 cm) chromatography was performed on silica gel (60-120 mesh, Qualigens, Mumbai, India) and thin layer chromatography on silica gel G-coated TLC plates (Merck). Spots were visualized

Table 1: ^{13}C-NMR ($CDCl_3$) of Compound HE-2

Position	δc	Position	Δc
1	37.59	16	28.23
2	31.80	17	56.14
3	83.04	18	11.86
4	42.32	19	19.22
5	149.71	20	36.50
6	123.83	21	18.73
7	31.89	22	36.19
8	38.80	23	123.82
9	49.94	24	138.50
10	36.77	25	28.03
11	21.05	26	22.83
12	39.53	27	22.58
13	39.68	28	27.38
14	56.65	29	23.84
15	24.28		

ESI MS *m/z* (relative intensity): 412$[M]^+$ ($C_{29}H_{48}O$) (8.1)

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by exposure to iodine vapours, UV radiation and by spraying with ceric sulphate solution.

Plant Material: The roots of the plant *Heliotropium eichwaldi* Stued. ex DC. were collected from waste land of Dist. Hisar and Sirsa, Haryana (India), in the months of September and October 2009.

The plants were taxonomically identified and authenticated by Dr. H.B. Singh, Head, Raw Materials Herbarium and Museum Division of National Institute of Science Communication and Information Resources (Ref. no. NISCAIR/RHMD/Consult/-2009-10/1406/04). The voucher specimens have been deposited in the herbarium section of the Pharmacognosy Division, Department of Pharmaceutical Sciences, Guru Jambheshwar University of Science and Technology, Hisar for further reference. The roots were dried under shade and pulverised using a mechanical grinder and stored in an air tight container for further use.

Extraction and isolation: The powdered roots were extracted exhaustively with methanol in a soxhlet apparatus. The methanolic extract was concentrated under reduced pressure to yield blackish brown, viscous syrupy mass (94.5g, 2.7 %). It was dissolved in minimum amount of methanol and adsorbed on silica gel (60-120 mesh) for preparation of slurry. The slurry was dried in air and chromatographed over silica gel column packed in petroleum ether. The column was eluted with petroleum ether, petroleum ether-chloroform (9:1, 3:1, 1:1, and 2:3 *v/v*), chloroform and chloroform-methanol (19:1, 9:1, 17:3, 3:1, 1:1 *v/v*) in order of increasing polarity to isolate the following compounds:

1. Glyceryl-1,2,3-triarachidate (HE-1): Elution of column with petroleum ether (60-80°C)-chloroform (3:1) (fraction nos. 51-75) furnished colourless amorphous mass of compound HE-1, 78 mg (0.0031%), R_f 0.48 (petroleum ether-chloroform, 3:1), m.pt. 63-65°C. IR ν_{max} (KBr): 2924, 2854, 1723, 1395, 1274, 1084, 1054, 1002, 725 cm^{-1}. ^{1}H-NMR ($CDCl_3$): δ 4.27 (1H, m H-2), 4.12 (2H, m, H_2-1), 4.05 (2H, m, H_2-3), 2.33 (2H, t, *J*= 7.2 Hz, H_2-2′), 2.29 (2H, t, *J*= 7.6 Hz, H_2-2″), 2.25 (2H, t, *J*= 7.2 Hz, H_2-2‴), 1.59 (2H, m, CH_2), 1.57 (2H, m, CH_2), 1.22 (98 H, br s, 49 × CH_2), 0.86 (3H, t, *J*= 6.0 Hz, Me-18′), 0.84 (3H, t, *J*= 6.0 Hz, Me-18″), 0.82 (3H, t, *J*= 6.0 Hz, Me-18‴). ^{13}C-NMR ($CDCl_3$): δ174.36 (C-1′), 173.99 (C-1″), 171.68 (C-1‴), 68.24 (C-2), 64.99 (C-1), 63.29 (C-3), 34.77 (CH_2), 34.11 (CH_2), 31.93 (CH_2), 29.71 (16×CH_2), 29.48 (16×CH_2), 29.37 (10×CH_2), 29.27 (7×CH_2), 21.14 (CH_2), 24.89 (CH_2), 22.69 (CH_2), 14.10 (Me-18′, Me-18″ Me-18‴). ESI MS *m/z* (relative intensity): 975$[M+H]^+$($C_{63}H_{123}O_6$) (11.6), 283 (81.5).

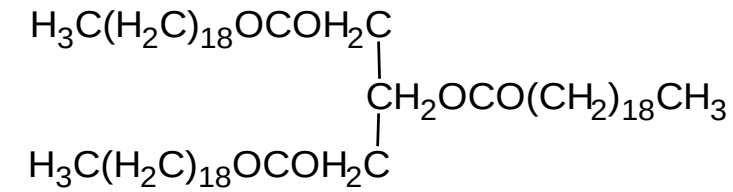
2. Stigmast-5,23-dien-3β-ol (HE-2): Elution of column with petroleum ether (60-80°C) – Chloroform (1:1) (fraction nos. 76-100) afforded colourless amorphous mass of compound HE-2, 107 mg (0.0043%), R_f 0.48 (petroleum ether-chloroform, 2:3), m.pt. 119-120°C. IR ν_{max} (KBr): 3398, 2935, 2825, 1645, 1461, 1374, 1056, 956, 837 cm^{-1}. ^{1}H-NMR ($CDCl_3$): δ 5.41 (1H, m H-23), 5.39 (1H, m, H-6), 4.66 (1H, brm, $W_{1/2}$ = 16.4 Hz, H-3 α), 2.01 (2H, m, H_2-4), 1.99 (2H, m, H_2-7), 1.97 (2H, m, H_2-22), 1.01 (3H, br s, Me-19), 0.93 (3H, d, *J*= 6.4 Hz, Me-21), 0.86 (3H, d, *J*= 6.0 Hz, Me-26), 0.84 (3H, d, *J*= 6.1 Hz, Me-27), 0.82 (3H, d, *J*= 6.3 Hz, Me-29), 0.66 (3H, br s, Me-18).

3. n-Tetracont-17-enoic acid (HE-3): Elution of column with chloroform-methanol (19:1) (fraction nos. 126-150) yielded pale yellow powder of compound HE-3, 54 mg (0.0022%), R_f 0.52 (chloroform-methanol, 9:1), m.pt. 63-65°C. IR ν_{max} (KBr): 3085, 2925, 2855, 1710, 1645, 1461, 1376, 1280, 939, 723 cm^{-1}. ^{1}H-NMR ($CDCl_3$): δ 5.35 (1H, m H-17), 5.33 (1H, m, H-18), 2.76 (2H, t, *J*= 7.2 Hz, H_2-2), 2.33 (2H, m, H_2-16), 2.30 (2H, m, H_2-19), 2.24 (2H, m, CH_2), 2.01 (2H, m, CH_2), 1.92 (2H, m, CH_2), 1.64 (4H, m, 2× CH_2), 1.25 (40H, br s, 20× CH_2), 1.94 (16H, br s, 8× CH_2), 0.85 (3H, t, *J*= 6.5 Hz, Me-40). ^{13}C-NMR ($CDCl_3$): δ180.37 (C-1), 130.22 (C-17), 127.95 (C-18), 34.15 (CH_2), 31.97 (CH_2), 31.58 (CH_2), 29.73 (15 × CH_2), 29.41 (6 × CH_2), 29.31 (2×CH_2), 29.12 (6×CH_2), 27.24 (CH_2), 25.57 (CH_2), 24.71 (CH_2), 22.72 (CH_2), 14.12 (Me-40). ESI MS *m/z* (relative intensity): 591$[M+H]^+$($C_{40}H_{79}O_2$) (6.1), 309 (14.3).

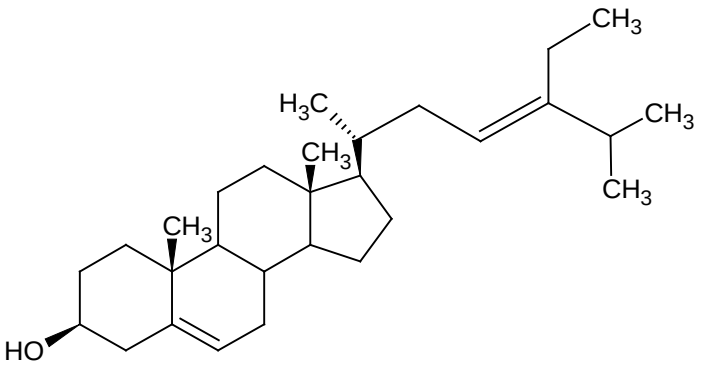
4. n-Undecanyl-n-docosanoate (HE-4): Elution of column with chloroform-methanol (9:1) furnished pale yellow powder of compound HE-4, 51 mg (0.0020%), R_f 0.44 (Chloroform-methanol, 9:1), m.pt. 88-90°C. IR ν_{max} (KBr): 2925, 2840, 1721, 1641, 1465, 1278, 1179, 1054, 813 cm^{-1}. ^{1}H-NMR ($CDCl_3$): δ 4.06 (2H, t, *J*= 9.6 Hz, H_2-1′), 2.30 (2H, t, *J*= 7.2 Hz, H_2-2), 1.99 (2H, m, CH_2), 1.52 (8H, br s, 4× CH_2), 1.25 (46H, br s, 23 CH_2), 0.85 (3H, t, *J*= 6.5 Hz, Me-22), 0.82 (3H, t, *J*= 6.1 Hz, Me-11′). ^{13}C-NMR ($CDCl_3$): δ 172.79 (C-1), 60.21 (C-1′), 34.40 (CH_2), 31.95 (CH_2), 29.73 (CH_2), 29.41 (CH_2), 29.30 (CH_2), 29.20 (CH_2), 27.87 (CH_2), 25.94 (CH_2), 23.13 (CH_2), 22.71 (CH_2), 14.30 (Me-22), 14.13 (Me-11′). ESI MS *m/z* (relative intensity): 494$[M]^+$($C_{33}H_{66}O_2$) (92.8), 339 (11.2), 323 (71.6).

DISCUSSIONS

1. Compound HE-1*:* Compound HE-1, triarachidin, was obtained as colourless amorphous mass from petroleum ether- chloroform (3:1) eluant. Its IR spectrum showed absorption bands for ester group (1723 cm-^{1}) and long aliphatic chain (725 cm-^{1}). It had a molecular ion peak at *m/z* 975 $[M+H]^+$ in its mass spectrum corresponding to the molecular formula of a glyceryl triester, $C_{63}H_{123}O_6$. The ion peak arising at *m/z* 283 $[CH_3(CH_2)_{18}COO]^+$ suggested that arachidic acid was esterified with glycerol



On the basis of these evidences the structure of HE-1 has been determined as glyceryl-1,2,3 triarachidate (1).



These data led to formulate the structure of HE-2 as stigmast-5,23-dien-3β-ol (2). This is a new sterol. (2)

$$CH_3(CH_2)_{21}CH{=}CH(CH_2)_{15}\ COOH$$

On the basis of above discussion the structure of HE-3 has been characterized as n-tetracont-17-enoic acid (3).

$$CH_3(CH_2)_{20}COOCH_2(CH_2)_9\ CH_3$$

*On the basis of above discussion the the structure of **HE-4** has been elucidated as n-undecanyl-n-docosanoate (4).*

unit. The ^{1}H-NMRspectrum of HE-1 displayed a one-proton multiplet at δ 4.27 and two multiplet at δ 4.12 and 4.05 integrating for two protons each assigned correspondingly to oxygenated C-1and C-2 methylene protons; three two-proton triplets at δ 2.33 (*J*= 7.2 Hz), 2.29 (*J*= 7.6 Hz) and 2.25 (*J*= 7.2 Hz) were attributed to methylene H_2-2′, H_2-2″ and H_2-2‴ protons, respectively, adjacent to the ester functions. The other methylene protons appeared as multiplets at 1.59 (2H) and 1.57 (2H) and as a broad singlet at δ1.22 (98H). Three triplets at δ 0.86 (*J*= 6.0 Hz), 0.84 (*J*= 6.0 Hz), 0.82 (*J*= 6.3 Hz), integrating for three protons each, were accounted to C-18′, C-18″, C-18‴ primary methyl protons, respectively. The 13 C-NMR spectrum of HE-1 displayed signals for ester carbons at δ 174.36 (C-1′), 173.99 (C-1″) and 171.68 (C-1‴), oxygenated methane carbon at δ 68.24 (C-2), oxygenated methylene carbons at δ 64.99 (C-1) and 63.29 (C-3), other methylene carbons from δ 34.77 to 22.69 and methyl carbons at δ 14.10 (C-18′, C-18″, C-18‴).

2. Compound HE-2: Compound HE-2 designated as 23-epistigmasterol, was obtained as a colorless amorphorus powder for petroleum ether–chloroform (1:1) eluent. It gave positive Lebermann-Burchard test for sterols. Its IR spectrum showed characteristic absorption bands for hydroxyl groups (3398 cm-^{1}) and unsaturation (1645 cm-^{1}). On the basis of mass and ^{13}C-NMR spectra the molecular ion peak of HE-2 was established at m/z 412 consistent with molecular formula of a sterol $C_{29}H_{48}O$. The ^{1}H-NMRspectrum of HE-2 exhibited two one-proton multiplets at δ 5.41 and 5.39 assigned to vinylic H-23 and H-6 protons, respectively. A one-proton broad multiplet at δ 4.66 with half width of 16.4 Hz was exhibited to α-oriented carbinol H-3 proton. Two three-proton broad singlets at δ 1.01 and 0.66 and four three-proton doublets at δ 0.93 (J= 6.0 Hz), 0.86 (J= 6.1 Hz) and 0.82 (J= 6.3 Hz) were attributed correspondingly to tertiary C-19 and C-18, secondary C-21, C-26 and C-27 and primary C-29 methyl protons, all attached to saturated carbons. The ^{13}C-NMR spectrum of HE-2 displayed signals for vinylic carbons at δ 149.71 (C-5), 123.83 (C-6), 123.82 (C-3) and 138.50 (C-24), carbinol carbon at δ δ 83.04 (C-3) and methyl carbons at δ11.86 (C-18), 19.22 (C-5), 18.73 (C-21), 22.83 (C-26), 22.58 (C-27) and 23.84 (C-29). The ^{1}H and ^{13}C-NMR spectral data of HE-2 were compared with the reported data of the related steroids.

3. Compound HE-3: Compound **HE-3**, a fatty acid, was obtained as a pale yellow powder from chloroform-methanol (19:1) eluent. It gave effervescence with sodium bicarbonate solution. Its IR spectrum displayed absorption bands for carboxylic group (3085, 1710 cm-^{1}), unsaturation (1645 cm-^{1}) and long aliphatic chain (723 cm-^{1}). On the basis of mass and ^{13}C-NMR spectra, its molecular ion peak was determined at *m/z* 519 $[M + H]^+$ consistent with molecular formula of an unsaturated fatty acid $C_{40}H_{79}O_2$. An ion peak arising at *m/z* 309 $[CH_3(CH_2)_{21}]^+$ suggested the presence of the vinylic linkage at C-17. The ^{1}H-NMRspectrum of HE-3 exhibited two one-proton multiplets at δ 5.35 and 5.33 assigned to vinylic H-17 and H-18 protons, respectively. A two-proton triplet at δ 2.76 (*J*= 7.2 Hz) was ascribed to methylene H_2-2 protons adjacent to the carboxylic function. The other methylene protons resonated between δ 2.33 – 1.94. A three-proton triplet at δ 0.85 (*J*= 6.5 Hz) was accounted to C-40 primary methyl protons. The ^{13}C-NMR spectrum of HE-3 showed signals for carboxylic carbon at δ 180.37 (C-1), vinylic carbons at δ 130.22 (C-17) and 127.95 (C-18) methylene carbons between δ 34.15 – 22.72 and methyl carbon at δ14.12 (C-40).

4. Compound HE-4: Compound HE-4, a fatty acid ester, was obtained as a pale yellow powder from chloroform-methanol (9:1) eluent. Its IR spectrum displayed absorption bands for ester group (1721 cm-^{1}) and long aliphatic chain (813 cm-^{1}). It had a molecular ion peak at *m/z* 494 in mass spectrum corresponding to the molecular formula of the ester $C_{33}H_{66}O_2$. The ion peak generating at

Table 2: Physical constants and nomenclature of the phytoconstituents isolated from *Heliotropium eichwaldi* Stued. ex DC. roots

Code no.	Name	Molecular formula/ M. wt.	Column fraction; R_f value	% Yield	Melting point (°C)
HE-1	Glyceryl-1,2,3-triarachidate	$C_{63}H_{123}O_6$/ 975	Petroleum ether-chloroform (3:1); R_f = 0.48 (Petroleum ether-Chloroform, 3:1)	0.0031	63-65°C
HE-2	Stigmast-5,23-dien-3β-ol (New)	$C_{29}H_{48}O$/ 412	Petroleum ether – chloroform (1:1); R_f = 0.48 (Petroleum ether-Chloroform, 2:3)	0.0043	119-120°C
HE-3	n-Tetracont-17-enoic acid	$C_{40}H_{79}O_2$/ 591	Chloroform-methanol (19:1); R_f = 0.52 (Chloroform-methanol, 9:1)	0.0022	56-58°C
HE-4	n-Undecanyl-n-docosanoate	$C_{33}H_{66}O_2$/ 494	Chloroform-methanol (9:1); R_f = 0.44 (Chloroform-methanol, 9:1)	0.0020	88-90°C

m/z 339 $[CH_3 (CH_2)_{20}CO]^+$ indicated that C_{22} fatty acid was esterified with undecanol. The ^{1}H-NMRspectrum of HE-4 exhibited two two-proton triplets at δ 4.06 (*J*= 9.6 Hz) and 2.30 (*J*= 7.2 Hz) assigned to oxygenated methylene H_2-1′ and methylene H_2-2 nearby the ester function. The other methylene protons resonated as a multiplet at δ 1.99 (2H) and as broad singlets at 1.52 (8H) and 1.25 (46H). Two three-proton triplets at δ 0.85 (*J*= 6.5 Hz) and δ 0.82 (*J*= 6.1 Hz) were accounted to C-22 and C-11′ primary methyl protons. The ^{13}C-NMR spectrum of HE-4 displayed signals for ester carbon at δ 172.82 (C-1), oxygenated methylene carbon at δ 60.21 (C-1′), other methylene carbons between δ 34.40 – 22.71 and methyl carbon at δ14.13 (C-11′).

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