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NOTE

Hentriacontanyl eicosanoate ester from *Parthenium hysterophorus* Linn. and its antimicrobial activity

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Abstract : Aerial parts of *Parthenium hysterophorus* Linn. on chemical investigation afforded a new aliphatic ester hentriacontanyl eicosanoate along with stigmasterol, parthenin, isoparthenin and coronopilin. The structures of isolated compounds were ascertained using various spectral (IR, ^1H, ^{13}C NMR, MS) techniques and hentriacontanyl eicosanoate has been evaluated for its antibacterial activity against *Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa* and antifungal activity against *Aspergillus niger, Candida albicans* and *Fusarium oxysporum*.

Keywords : *Parthenium hysterophorus* Linn., Asteraceae, hentriacontanyl eicosanoate.

Introduction

Parthenium hysterophorus Linn. (Asteraceae) is commonly called as congress grass, congress weed, carrot weed and wild feverfew. It is now considered as one of the most feared noxious weed[1]. The plant is used in the treatment of ulcerated sores, wounds, fever, anaemia and heart troubles. A decoction of the root finds use in treatment of dysentery[2]. The lower concentrations of extracts display antifungal properties[3]. It is applied externally on skin disorders and decoction of the plant is often taken internally as a remedy for a wide variety of ailments[4]. It is also reported as promising remedy against hepatic amoebiasis[5].

The present report deals with the isolation and characterization of a new compound hentriacontanyl eicosanoate (**1**) along with the known stigmasterol, parthenin, isoparthenin and coronopilin (**2-5**) from aerial parts of *Parthenium hysterophorus* Linn. Hentriacontanyl eicosanoate has been evaluated for its antibacterial activity against *Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa* and antifungal activity against *Aspergillus niger, Candida albicans, Fusarium oxysporum.*

Results and discussion

The leaves of *P. hysterophorus* were extracted with petroleum ether. The extract was chromatographed over silica gel and hentriacontanyl eicosanoate-**1** was obtained as white granules, m.p. 80 °C in petroleum ether-benzene, 2 : 2 fraction. The infrared spectrum indicated the presence of ester group (1740 cm^{-1}) and long chain of $(CH_2)_n$ where $n \geq 4$ (doublet at 735 cm^{-1} and 700 cm^{-1}).

The ^1H NMR spectrum of **1** displayed a triplet at δ 0.88 (6H, t, *J* 6.78 Hz) due to methyl groups, a broad singlet at δ 1.25 due to 45 (90H, s) methylene groups, a triplet at δ 2.30 (2H, t, *J* 7.5 Hz) due to methylene group adjacent to keto group and downfield triplet at δ 4.05 (2H, t, *J* 6.78 Hz) on account of oxymethylene moiety of an ester. The ^{13}C NMR spectrum exhibited signals at δ 64.04, δ 34.43 due to carbon of oxymethylene moiety of an ester and methylene group adjacent to keto group respectively. Signal at δ 31.95 was assigned to carbon of methylene group adjacent to oxymethylene group. A downfield ^{13}C signal at δ 174.05 confirmed its esteric nature. Both terminal methyl groups showed ^{13}C signal at δ 14.14. The compound displayed a molecular ion peak at *m/z* 746 $[M]^+$ in its FAB mass spectrum consistent with the molecular formula $C_{51}H_{102}O_2$. The molecular ion peak was very weak but the appearance of an abundant ion peak at *m/z* 303 on account of acylium ion $CH_3(CH_2)_{18}CO^+$ in its mass spectrum confirmed the acid moiety of an ester. The most characteristic peak at *m/z* 494 was due to Mc Lafferty rearrangement. It also showed a diagnostic peak at *m/z* 313 due to transfer of two hydrogen atoms to the fragment containing oxygen atoms. Peaks

at interval of 14 mass units were also depicted. From above data it was clear that compound is an ester of a long chain alcohol with a long chain fatty acid.

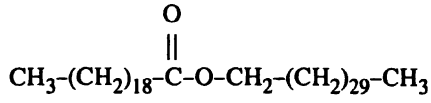


Fig. 1. Hentriacontanyl eicosanoate.

vity against *Aspergillus niger, Candida albicans, Fusarium oxysporum* by disk diffusion method[10]. Gentamycin and mycostatin were used as reference compounds for evaluating antibacterial and antifungal activities. The compound **1** showed good activity against *Staphylococcus aureus, Escherichia coli* and fungi *Candida albicans, Fusarium oxysporum*. The results obtained are presented in Tables 1 and 2.

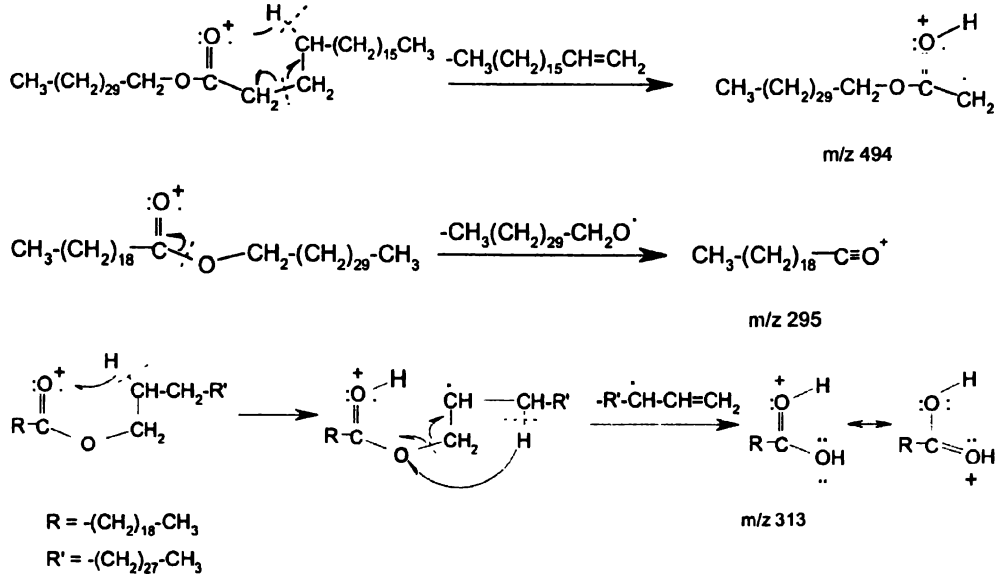


Fig. 2. Mass fragmentation of hentriacontanyl eicosanoate.

The compound **2** was obtained as colourless shining flakes, m.p.166–167 °C and also gave positive tests for sterols. It was identified as stigmasterol[6]. Compound **3** was isolated as colourless prismatic crystals, m.p.165–166 °C. Its analytical and spectral data led to its confirmation as parthenin[7]. Compound **4** was obtained as colourless gum. Its molecular formula $C_{15}H_{18}O_4$ was established from its mass spectrum and characterized as isoparthenin[8]. Compound **5** was isolated as colourless plates, m.p.177–178 °C and was identified as coronopilin on the basis of complete spectral analysis, comparison with literature data[9].

Hentriacontanyl eicosanoate (**1**) at four concentrations viz. 200, 400, 600, 800 ppm was also screened for antibacterial activity against *Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa* and antifungal acti-

Table 1. Antibacterial activity of hentriacontanyl eicosanoate

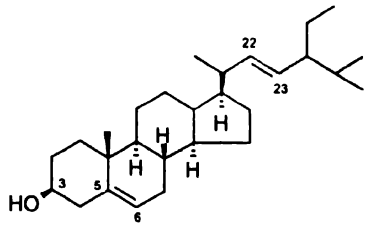
		Mean value of area of inhibition in mm IZ (AI))		
Bacteria →		*Staphylococcus aureus*	*Escherichia coli*	*Pseudomonas aeruginosa*
Gentamycin (Control) →		20	22	16
Extract Conc. (ppm) ↓				
1	200	8.5	8.0	5.5
		(0.43)	(0.36)	(0.34)
1	400	8.8	8.2	5.8
		(0.44)	(0.37)	(0.36)
1	600	9.0	8.5	6.0
		(0.45)	(0.38)	(0.38)
1	800	9.5	9.0	6.2
		(0.48)	(0.40)	(0.39)

Table 2. Antifungal activity of hentriacontanyl eicosanoate

Fungi →		Mean value of area of inhibition in mm IZ (AI)) *Candida albicans*	*Aspergillus niger*	*Fusarium oxysporum*
Mycostatin (Control) →		15	17	16
Extract Conc. (ppm) ↓				
1	200	8.5 (0.57)	5.5 (0.32)	8.2 (0.51)
1	400	8.7 (0.58)	6.0 (0.35)	8 4 (0 53)
1	600	9.0 (0.6)	6.0 (0 35)	9.0 (0.56)
1	800	9.2 (0.61)	6.5 (0 38)	9 5 (0 60)

automatic recording. Mass spectra were determined by Jeol SX-102 spectrometer.

Extraction of aerial parts of Parthenium hysterophorus : The aerial parts of *Parthenium hysterophorus* were collected from near by Kota region. The air dried and coarsely powdered leaves (3 kg) were exhaustively extracted with petroleum ether (b.p. 60–80 C°) on boiling water bath for 12 × 6 h. The resulting extract (30 g) was concentrated in vacuum, dissolved in methanol and left overnight and decolorized with activated charcoal. The methanol soluble part (2 g) was subjected to column chromatography over silica gel. Five fractions were obtained from the column : Fraction eluted with (petroleum ether-benzene, 3 : 1) afforded colourless shining needles (70 mg) as compound **2**. Fraction eluted with (petroleum ether-benzene, 2 : 2)



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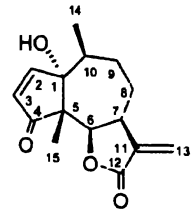

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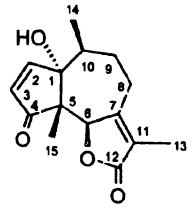

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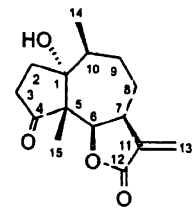

(5)

Fig. 3. Compounds **2-5** isolated from *Parthenium hysterophorus*.

Experimental

General : Melting points were determined in open glass capillary tube and were uncorrected. Column chromatography : silica gel. Qualitative and quantitative TLC was conducted on aluminium sheets Kieselgel $60F_{254}$ (E. Merck). IR spectra (cm^{-1}) were recorded on FTIR Nicolet Magna 550 and Shimadzu 8400 S spectrophotometer in KBr disc and noteworthy absorptions levels (cm^{-1}) are listed. ^{1}H and ^{13}C NMR spectra were recorded on Jeol AL 300 MHz FTNMR instrument. UV-Vis spectra were recorded in ethanol (95%) on Perkin-Elmer model 2R gave compound **1** as white solid (140 mg). Fraction eluted with (benzene-ethyl acetate, 3 : 1) revealed the presence of two compounds upon TLC. After repeated preparative TLC, two compounds were isolated, compound **3** as colourless prismatic crystals (250 mg), and compound **4** as colourless gum (100 mg). Fraction obtained after eluting (benzene-ethyl acetate, 2 : 2) afforded compound **5** as colourless needles (70 mg).

Hentriacontanyl eicosanoate (**1**) : White granules, 140 mg, m.p. 80 °C; IR ν_{max} (KBr) : 2910–2850 (C–H, stretch), 1740, 1205 (C=O), 735 and 725 $(CH_2)_n$ cm^{-1};

^{1}H NMR (300 MHz, $CDCl_3$, δ (ppm)) : 0.88 (6H, t, *J* 6.78 Hz, 2 × CH_3), 1.25 (90H, s br), 1.60 (2H, q, -CH_2-), 2.30 (2H, t, *J* 7.5 Hz, -CH_2-), 4.05 (2H, t, *J* 6.78 Hz, -OCH_2-); ^{13}C NMR (75.45 MHz, $CDCl_3$, δ) : 14.14 (2 × CH_3), 22.71, 25.05, 25.95, 28.66, 29.18, 29.30, 29.38, 29.50, 29.56, 29.63, 29.68, 29.72, 31.95, 64.43 (OCH_2), 174.05 (C); MS, *m/z* (relative intensity %) : 746 ($[M]^+$, $C_{51}H_{102}O_2$, 4), 494 (6), 313 (100), 295 (7.5).

Hydrolysis : 20 mg of compound-**1** was treated with methanolic KOH at 90 °C at boiling bath. It gave hentriacontanol as colourless gum and eicosanoic acid as colourless solid, m.p. 75 °C confirming its identity.

Stigmasterol (**2**) : Colourless shining needles, 70 mg, m.p. 166–167 °C; IR ν_{max} (KBr) : 3400–3200 (OH), 1460 (-CH_2 bending), 1380, 1360, 1260, 1050, 960, 800 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$, δ (ppm)) : 5.30 (1H, t br, H-6), 5.05 (1H, dd, *J* 16, 10 Hz, H-2), 5.15 (1H, dd, *J* 16, 10 Hz, H-23), 3.40 (1H, m, H-3), 0.86 (1H, t, H-29), 1.16 (1H, s, H-27), 1.00 (1H, d, *J* 7 Hz, H-21); MS, *m/z* (relative intensity %) : 412 ($[M]^+$, $C_{29}H_{48}O$, 41.40), 397 (8.90), 328 (5.10), 302 (12.8).

Parthenin (**3**) : Colourless prismatic crystals, 250 mg, m.p. 165–166 °C; IR ν_{max} (KBr) : 3450 (OH), 1760 (γ-lactone), 1720 (C=C-C=O), 1655, 1592 (C=C), 1350, 1290, 1040 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$, δ (ppm)) : 7.63 (1H, d, *J* 6 Hz, H-2), 6.15 (1H, d, *J* 6 Hz, H-3), 6.25 (1H, d, *J* 3 Hz, H-13), 5.61 (1H, d, *J* 3 Hz, H-13′), 5.02 (1H, d, *J* 7 Hz, H-6), 3.53 (1H, m, H-7), 3.30 (1H, s, OH), 1.6–2.5 (5H, m, H-8, H-8′, H-9, H-9′, H-10), 1.27 (1H, s, H-15); MS, *m/z* (relative intensity %) : 262 ($[M]^+$, $C_{15}H_{18}O_4$, 75.5), 244 (100), 215 (19.3), 200 (18.2), 146 (11.1), 145 (5.5).

Isoparthenin (**4**) : Colourless gum, 100 mg, IR ν_{max} (KBr) : 3400 (OH), 1760 (γ-lactone), 1720 (C=C- C=O), 1600 (C=C), 1350, 1290, 1040 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$, δ (ppm)) : 7.15 (1H, d, *J* 6 Hz, H-2), 6.05 (1H, d, *J* 6 Hz, H-3), 2.10 (1H, s br, H-13), 5.00 (1H, s br, H-6), 1.23 (1H, s, H-15), 1.06 (1H, d, *J* 7 Hz, H-14), 1.6–2.12 (3H, m, H-8, H-9, H-10); MS, *m/z* (relative intensity %) : 262 ($[M]^+$ $C_{15}H_{18}O_4$, 100), 244 (23.3), 215 (28.0), 200 (18.1), 146 (14.8), 145.

Coronopilin (**5**) : Colourless needles, 70 mg, m.p. 177–178 °C, IR ν_{max} (KBr) : 3400 (OH), 1750 (γ-lactone), 1720 (C=O), 1610 (C=C), 1350, 1300, 1040 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$, δ (ppm)) : 6.23 (1H, d, *J* 3 Hz, H-13), 5.59 (1H, d, *J* 3 Hz, H-13′), 4.92 (1H, d, *J* 7 Hz, H-6), 3.35 (m, H-7), 1.5–2.7 (9H, m, H-2, H-2′, H-3, H-3′, H-8, H-8′, H-9, H-9′, H-10), 1.20 (1H, d, *J* 7 Hz, H-14), 1.13 (1H, s, H-15); MS, *m/z* (relative intensity %) : 264 ($[M]^+$, $C_{15}H_{20}O_4$,18.3), 246 (40.2), 228 (8.9), 217 (30.3), 200 (8.9).

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