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p-Coumaric acid esters from *Tanacetum longifolium*☆

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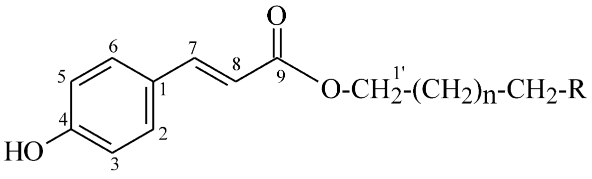
Abstract

Two new long chain alkyl *p*-coumaric acid esters (**2**–**3**) along with eicosanyl *trans*-*p*-coumarate (**1**) were isolated from chloroform extract of the roots of *Tanacetum longifolium*. The structures of new compounds were assigned as 21′-hydroxyheneicosanyl-4-hydroxy-(*cis* and *trans*) *p*-coumarate (**2a**, **2b**) and 27′-hydroxy heptacosanyl-*cis*-*p*-coumarate (**3**) by extensive chromatographic and spectroscopic analysis and by comparison with literature data of known compounds.

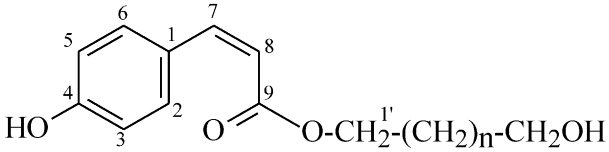
Keywords: *Tanacetum longifolium*; Asteraceae; Roots; *p*-Coumaric acid ester; Eicosanyl; 21′-Hydroxy heneicosanyl; 27′-Hydroxy heptacosanyl-*p*-coumarate

1. Introduction

Tanacetum longifolium (Asteraceae) is an annual hairy herb growing wild at an altitude of 3000–4500 m from Uttranchal to Kashmir in India (Anon., 1962). In our previous investigation we reported the essential oil composition of roots and aerial parts (Kaul et al., 1993), a new irregular "non-head to-tail" sesquiterpene, tanacetene, and a new long chain alkyl ester alongwith known compounds (Mahmood et al., 2002). In continuation to our work on chemical investigation of this plant we now report two new *p*-coumaric acid esters **2**, **3** alongwith eicosanyl-*trans*-*p*-coumarate **1**. These compounds which were previously isolated (Mahmood et al., 2002) as complex mixtures from $CHCl_3$ extract have now been separated by preparative HPLC and their structures elucidated by spectroscopic analysis.



1 R=H, n=18
2b R=OH, n=19



2a n=19
3 n=25

2. Results and discussion

Compound **1** was identified as eicosanyl-*trans*-*p*-coumarate by comparing it with literature data, IR, ^{1}H NMR and MS (Keriko et al., 1997). The earlier authors reported it as a mixture of cis and *trans* isomers but in our finding we found only trans isomer. This is the first report of isolation of this compound from *T. longifolium*. This compound is also reported to have plant growth inhibitor activity.

Compound **2** showed [M^+] ion at *m*/*z* 474, corresponding to molecular formula $C_{30}H_{50}O_4$ which is also supported by NMR. A strong peak at *m*/*z* 147 and a base peak at *m*/*z* 164 corresponded to hydroxy cinnamaldehyde and hydroxy cinnamic acid ions (Sakushima et al., 1985). Other MS fragmentation pattern is also

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similar to **1**, thus clearly indicating *p*-coumaric acid moiety in **2**. Its ^{1}H NMR spectra showed prominent signals for cis-p-coumarate moiety at δ 7.65 and δ 6.81 (both 2H, d, $J = 8.4$, Hz H2 for H-2/H-6 and H-3/H-5 respectively) and δ 6.86 and δ 5.83 (both 1H, d, $J = 13$ Hz, for C-7 and C-8 respectively) the coupling constant of 13 Hz between H-7 and H-8 confirm the *cis* geometry (Hashidoko et al., 1992). The 21′-hydroxy heneicosanyl moiety was confirmed by observing at δ 4.11 ($J = 6.5$ Hz) for methylene adjacent to oxycarbonyl function (CH_2-1′) and other at δ 3.65 ($J = 6.5$ Hz) for terminal methylene (27′) attached to hydroxy group (Dobhal et al., 1999) in place of CH_3 as found in **1**. A broad singlet at δ 1.24 (34H) was observed for side chain methylene protons thus confirming 21′-hydroxyheneicosanyl moiety which was further confirmed by ^{1}H–^{1}H-COSY experiments. The final structure was confirmed as 21′-hydroxy heneicosanyl-*p*-coumarate **2a**.

On close observation of minor signals of **2** it also showed the presence of *trans* isomer (**2b**) as well. Low intensity signal in ^{1}H-NMR of **2** at δ 7.44 and 6.82 (both 2H, d, $J = 8.4$ Hz for H-2/H-6 and H-5/H-6 and δ 7.63 and δ 6.31 (both 1H, d, $J = 17$ Hz for H-7 and H-8) the coupling constant between H-7 and H-8 of 17 Hz showed clearly trans geometry and a triplet at δ 4.18 for CH_2-1′ which is very similar to **1**. Final structural of **2b** was confirmed by ^{1}H–^{1}H COSY experiments. Thus the structure of **2b** was determined 21′-hydroxy heneicosanyl-*trans*-*p*-coumarate.

On the basis of signal intensities of ^{1}H NMR of both isomers the relative ratio of **2a** and **2b** was found to be 3:1. To the best of our knowledge **2a** and **2b** are new *p*-coumaric acid esters. It is also likely that **2a** may have partially converted into **2b** due to photo-isomerisation.

Compound **3** showed [M$^+$] ion peak at m/z 558 corresponding to molecular formula $C_{36}H_{62}O_4$. Its MS fragmentation pattern was similar to **1** and **2** thus confirming *p*-coumarate moiety. ^{1}H NMR spectra of **3** was almost identical with that of **2a** (Table 1) having a terminal hydroxy group in its side chain thus confirming it as 27′-hydroxy heptacosanyl-*cis*-*p*-coumarate. This is the first report of isolation of this compound from natural source.

3. Experimental

3.1. General

IR spectra (KBr) were recorded on Jasco FT/IR-5300. NMR spectra (^{1}H, ^{13}C, 1D, 2D) were recorded in $CDCl_3$ on Bruker DRX-300 MHz and 75 MHz (^{13}C) instrument using TMS as Internal standard and the chemical shifts reported in δ (ppm) units relative to TMS signal and coupling constants (J) in Hz. EIMS were recorded on a Finnigan Mat 900 S spectrometer with HR mode. HPLC analysis was carried out on waters HPLC systems (600 gradient pump, 7725 rheodyne injector 996 PDA detector), under conditions as follows: Column Lichrospher RP-18e, 5 μm, 250×4mm with 4×4 mm guard column, flow rate 1 ml/min, uv detection at λ 230 nm, temp. 30 °C, eluent MeCN/H_2O) 60:40, Preparative HPLC on Prep LC, 4000 system, Waters 2487 with dual absorbance detector (millennium version 1.65.1), column Bondapak C18, 15–20 μm, 200×25mm, flow rate 30 ml/min. detection at 230 nm, eluent MeCN–H_2O) 50:50.

3.2. Plant material

Aerial parts and roots of *T. longifolium* wall were collected from Dhauladhar hills at a height of 3000 m in Himachal Pradesh, India. An authenticated voucher specimen (No. 298 BKS) has been deposited in the herbarium of IHBT at Palampur.

3.3. Extraction and isolation:

Air-dried roots of *T. longifolium* (1 kg) were extracted with $CHCl_3$ (cold extraction) and concentrated to

Table 1
^{1}H (300 MHz) NMR spectral data for **1**, **2a**, **2b** and **3** ($CDCl_3$)[a]

	1	**2a**	**2b**	**3**
1	–			–
2, 6	7.42 (2H, *d*, $J = 8.2$ Hz)	7.65 (*d*, $J = 8.4$ Hz)	7.44 (*d*, $J = 8.4$ Hz)	7.63 (2H *d*, $J = 8.4$ Hz)
3, 5	6.83 (2H, *d*, $J = 8.2$ Hz)	6.81 (*d*, $J = 8.4$ Hz)	6.82 (*d*, $J = 8.4$ Hz)	6.79 (2H *d*, $J = 8.4$ Hz)
4	–			–
7	7.62 (1H, *d*, $J = 17$ Hz)	6.86 (*d*, $J = 13$ Hz)	7.63 (*d*, $J = 17$ Hz)	6.84 (2H *d*, $J = 13$ Hz)
8	6.30 (1H, *d*, $J = 17$ Hz)	5.83 (*d*, $J = 13$ Hz)	6.31 (*d*, $J = 17$ Hz)	5.85 (2H *d*, $J = 13$ Hz)
9	–			–
1′	4.18 (2H, *t*, $J = 6.5$ Hz)	4.11 (*t*, $J = 6.5$ Hz)	4.18 (*t*, $J = 6.5$ Hz)	4.11 (2H, *t*, $J = 6.5$ Hz)
2′	1.68 (2H, *m*)	1.64 (*m*)	–	1.64 (*m*)
3-n	1.25 (34H, *brs*)	1.24 (34 H, *brs*)	–	1.24 (50H, *brs*)
n′	0.87 (3H, *t*, $J = 6.5$ Hz)	3.64 (*t*, $J = 6.5$)	3.64 (2H, *t*, $J = 6.5$ Hz)	3.65 (2H, *t*, $J = 6.5$ Hz)

[a] Assignments based on 2D ^{1}H–^{1}H COSY experiments.

dryness to afford residue (10 g) by evaporation. The residue on drying in vacuum was subjected to normal phase column chromatography as described previously (Mahmood et al., 2002). The hexane–EtOAc (85:15) fraction (26 mg) was further analyzed by HPLC which showed the presence of four compounds having R_t 7.62, 8.52, 13.03 and 13.79 min, respectively in isocratic mode. Individual compounds were purified by prep HPLC ODS in isocratic mode. Compound **3** (4 mg) was first isolated followed by **2** (1.5 mg), β-sitosterol and compound **1** (6 mg) as the last.

3.4. Eicosanyl-trans-p-coumarate 1

Colourless amorphous powder, IR $\nu_{\mathrm{Max}}^{\mathrm{KBr}}\mathrm{cm}^{-1}$: 3400 (br, OH) 1705 (C=O) 1610, 1467, 1167, 908, 733, ^{1}H NMR (see Table 1), ^{13}C NMR (75 MHz, $CDCl_3$, DEPT) δ 167.44 (C-9), 157.72 (C-4), 144.24 (C-7), 130.68 (C-2, C-6), 125.84 (C-1), 115.70 (C-3, C-5), 114.83 (C-8), 64.65 (C-1′), 31.92 (C-18′), 28.75 (C-2′), 29.35-29.69 (C-3′ C-17′), 22.68 (C-19′), 14.11 (C-20′); EIMS, probe (70 ev) *m/z* (rel int.) 444 $[\mathrm{M}]^+$ (0.30), 167 (17.8), 166 (32.54), 164 (100), 147 (65.25), 125 (9.47), 111 (16.21), 107 (9.55), 83 (15.4) 69 (25.12), 57 (50.81), 43 (62.46).

3.5. 21′-Hydroxyheneicosanyl-p-coumarate 2

Amorphous powder, IR $\nu_{\mathrm{Max}}^{\mathrm{KBr}}\mathrm{cm}^{-1}$: 3420, 1710, 1612, 1460, 1155, 735 cm^{-1} ^{1}H NMR (see Table 1) EIMS, *m/z* (rel. int) 474 $[\mathrm{M}^+]$, (0.9) 219 (0.51) 164 (100), 147 (78.24), 120 (12.54), 107 (28.59), 83 (14.36), 69 (21.50), 57 (43.68), 43 (59.15).

3.6. 27′-Hydroxy Heptacosanyl-cis-p-coumarate 3

Amorphous powder IR $\nu_{\mathrm{Max}}^{\mathrm{KBr}}\mathrm{cm}^{-1}$: 3422, 1712, 1608, 1462, 1167, 733, ^{1}H NMR (see Table 1) ^{13}C NMR (75 MHz, $CDCl_3$) δ 166.23 (C-9), 157.32 (C-4), 143.51 (C-7), 132.80 (C-2, C-6), 127.98 (C-1), 119.54 (C-8), 115.48 (C-3, C-5), 64.52 (C-1′), 62.58 (C-27′), 28.70 (C-2′, C-26′), 29.27-29.72 (C-3′ to C-25′); EIMS, (70 ev) *m/z* rel. int: 558 $[\mathrm{M}]^+$ (1.35), 219 (0.30) 166 (51.51) 164 (100), 147 (79.85), 120 (27.21), 107 (26.41), 83 (16.84), 69 (22.6), 57 (48.84), 43 (60.65).

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