Rare Eugenol- and *Z*-Coniferyl Alcohol Derivatives in Roots of Three *Coreopsis* Species

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Roots of *Coreopsis tinctoria*, *Coreopsis lanceolata*, *Coreopsis grandiflora*, Compositae, Phenylpropanoids

Roots of three species of the genus *Coreopsis* (Compositae), *C. tinctoria*, *C. lanceolata* and *C. grandiflora* were investigated for the occurrence of phenylpropanoids. In addition to phenylpropanoids already known from other *Coreopsis* species some new derivatives of eugenol- and *Z*-coniferyl alcohol were found. In this report we present their structures together with their ^{1}H NMR, ^{13}C NMR and MS data. Two aromatic acetylenes – one of them not previously described for *Coreopsis* species – were identified as well.

Introduction

The genus *Coreopsis* has already been examined for phenylpropanoids and other lipophilic compounds by several authors [1–5]. For the purpose of comparative work on the accumulation of phenylpropanoids in intact plants and derived tissue cultures, we have investigated the three species *C. tinctoria*, *C. grandiflora* and *C. lanceolata* for their occurrence.

Results and Discussion

According to examinations of Bohlmann *et al.* [1–5] the essential oil of different *Coreopsis* species consists mainly of polyines, terpens with chinoid bisabolene derivatives predominating, and phenylpropanoids. The latter compounds are mainly derivatives of 1′-hydroxy-eugenol and of epoxy-*Z*-coniferyl-alcohol accumulated principally in the roots. Only traces of these phenylpropanoids were found in the aerial parts of the plants.

Roots of the three species *Coreopsis tinctoria*, *C. grandiflora* and *C. lanceolata* have hitherto not been investigated with respect to their lipophilic phenylpropanoids. In order to isolate pure constituents of this class of compounds they were localized in the crude root extracts by TLC, prepurified by column chromatography and finally isolated in the pure state by preparative HPLC using UV detection. As directed by the aim of this work, the UV detector was adjusted to 278 nm, where the compounds of interest have their UV maximum.

Five of the isolated compounds were identified as structure **1**, **2**, **5**, **6** and **7** (Fig. 1) already described in other *Coreopsis* species [1, 2]. Identification was carried out by ^{1}H NMR spectroscopy (Table I) with corresponding proton decoupling and mass spectrometry. Our results were in accordance with data

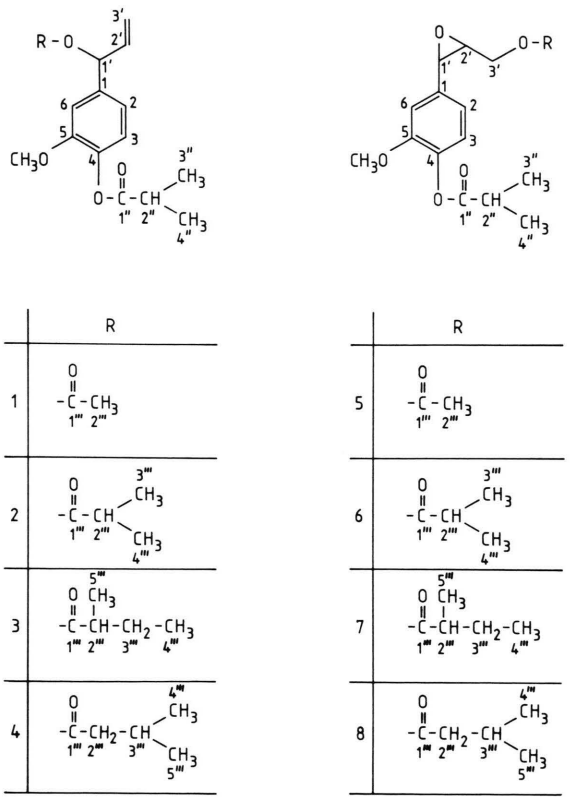

Fig. 1. Phenylpropanoids from *Coreopsis* species.

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Table I. ^{1}H NMR data of compounds **1–8** (250 MHz, $CDCl_3$, TMS int. stand.).

^{1}H No.	**1**	**2**	**3**	**4**
2	6.92 dd (8.5; 2)	6.93 dd (8.5; 2)	6.86 dd (8.5; 2)	
3	6.98 d (8.5)	6.99 d (8.5)	6.93 d (8.5)	
6	6.92 d (2)	6.93 d (2)	6.86 d (2)	
1′	6.25 ddd (5.5; 1.5; 1.5)		6.19 ddd (5.5; 1.5; 1.5)	
2′	5.99 ddd (17; 11; 5.5)	5.98 ddd (17; 11; 5.5)	5.92 ddd (17; 11; 5.5)	
3′a	5.27 ddd (11; 2; 1.5)		5.18 ddd (11; 2; 1.5)	
3′b	5.31 ddd (17; 2; 1.5)	5.29 ddd (17; 2; 1.5)	5.24 ddd (17; 2; 1.5)	5.21 ddd (17; 2; 1.5)
2″	2.85 septett (7)	2.89 septett (7)	2.76 septett (7)	2.77 septett (7)
3″				
4″	1.33 d (7)	1.32 d (7)	1.25 d (7)	1.26 d (7)
2‴	2.13 s	2.62 qq (7; 7)	2.38 sextett (7)	2.19 m
3‴	–	1.18 d (7)*	1.34–1.70 m	1.96–2.16 m
4‴	–	1.21 d (7)*	0.80 t (7.5)	0.89 d (7)
5‴	–	–	1.11 d (7)	
OMe	3.83 s		3.75 s	

^{1}H No.	**5**	**6**#	**7**	**8**
2	6.90 dd (8; 2)	6.92 dd (8; 2)	6.92 dd (8; 2)	6.93 dd (8; 2)
3	7.0 d (8)	7.01 d (8)	7.01 d (8)	7.02 d (8)
6	$	6.94 br d (2)	$	$
1′	4.14 d (4.5)	4.15 br d (4.5)	4.17 d (4.5)	4.17 d (4.5)
2′	3.46 dt (7; 4.5)	3.45 dt (7; 4.5)	3.47 dt (7; 4.5)	
3′a	4.07 dd (12.5; 4)	4.07 dd (4.5; 12.5)	4.10 dd (12.5; 4)	
3′b	3.84 dd (12.5; 7)	3.89 dd (12.5; 7)	3.88 dd (12.5; 7)	
2″	2.81 septett (7)	2.83 septett (7)	2.84 septett (7)	
3″				
4″	1.30 d (7)	1.32 d (7)	1.32 d (7)	1.35 d (7)
2‴	2.06 s	2.57 qq (7; 7)	2.41 sextett (7)	2.23 m
3‴	–	1.170 d (7)*	1.42–1.76 m	2.02–2.20 m
4‴	–	1.166 d (7)*	0.89 t (7.5)	–
5‴	–	–	1.14 d (7)	0.96 d (7)
OMe	3.80 s	3.82 s	3.84 s	

* Assignments may be reversed;
# at 500 MHz;
$ the signal of 6-H was overlapped by the signal of 2-H (200 MHz).
Chemical shifts in -values (ppm); numbers in parentheses are coupling constants in Hz; all signals are interpreted according to first order.

from literature [1, 2]. The ^{13}C NMR data presented here have not been published before (Table II).

Additionally we succeeded in proving the existence of three new compounds: 1′-(2-methylbutyryloxy)-eugenol-isobutyrate (**3**), 1′-isovaleroyloxy-eugenol-isobutyrate (**4**), 1′,2′-epoxy-*Z*-coniferyl-alcohol-3′-isovaleroyl-4-isobutyrate (**8**). Showing comparable properties (R_F values on TLC, blue-violet colour after detection with anisaldehyde-sulphuric acid, weak fluorescence quenching at 254 nm and similar retention times in HPLC), the unidentified compounds seemed to belong to the group of phenylpropanoids already known from *Coreopsis* species. This assumption was confirmed by common ^{1}H NMR properties: signals of three aromatic protons (δ 6.83–7.02 ppm) with a coupling pattern supporting an asymmetric aromatic substitution, the singulett of an –OMe group and signals of an isopropyl group. Their mass spectra indicated that this isopropyl group was part of an isobutyric acid moiety (M^+ –70, loss of isobutyryl as ketene). The isomers **3** and **4** were difficult to separate due to their almost identical chromatographic properties. Their ^{1}H NMR spectra showed signals belonging to a vinyl group with one proton strongly shifted downfield (δ 6.19 ppm) induced by a vinyl-, phenyl- and an

Table II. ^{13}C NMR data of compounds **1–7** (50 MHz, $CDCl_3$, TMS int. stand.).

^{13}C No.	**1**	**2**	**3**	**4**	**5**	**6**	**7**
1	137.43	137.72	138.07	137.60	132.78	132.84	132.88
2	119.60	119.39	119.71	119.51	118.45	118.45	118.49
3	122.72	122.71	122.98	122.67	122.76	122.73	122.77
4	139.84	139.73	140.00	139.68	139.72	139.67	140.00
5	151.16	151.15	151.43	151.09	151.20	151.16	151.22
6	111.53	111.30	111.61	111.35	110.21	110.20	110.28
1′	75.71	75.30	75.55	75.38	56.25	56.18	56.23
2′	136.01	136.21	136.50	136.10	55.97	56.06	56.11
3′	116.97	116.84	117.25	117.00	62.26	62.09	61.99
1″	175.13	175.14	175.44	175.18	175.10	175.08	175.11
2″	33.96	34.16$	34.27	33.95	33.94	33.93$	33.96
3″	19.00	19.00*	19.31	19.01	18.98	18.97*	19.00
4″							
1‴	169.86	175.82	175.76	171.98	170.67	176.78	176.40
2‴	21.22	33.97$	41.48	43.59	20.71	33.82$	40.89
3‴	–	18.90*	27.04	25.78	–	18.91*	26.76
4‴	–		11.83	22.40	–		11.55
5‴	–	–	16.80		–	–	16.51

*, $ Assignments may be reversed.
Chemical shifts in -values (ppm). For compounds **1** and **6** assignments are based on DEPT experiments.

acetoxy group. Thus we assigned them to the eugenol derivatives. Both eugenols had the same molecular weight (M^+ 334,1786; $C_{19}H_{26}O_5$) and almost identical mass spectra. The difference of 84 mass units between *m/z* 264 (= M^+ −70, loss of isobutyryl as ketene) and *m/z* 180 in connection with a fragment *m/z* 57 was in accordance with an esterified 2-methyl-butyric acid (**3**) and with an esterified isovaleric acid moiety (**4**), respectively. The data of their ^1H NMR agree with this interpretation (Table I).

From of its ^1H NMR spectrum the third unidentified compound was compatible with the structure of an esterified *Z*-epoxy-coniferyl alcohol. The *Z*-configuration of the epoxy structure was derived from the coupling constant of 1′H and 2′H: 4.5 Hz [6] (the *E*-isomer should have about 2 Hz). Based on the same molecular weight and a similar mass spectrum (exp.) this compound seemed to be an isomer of compound **7**. By evaluation of the ^1H NMR spectrum supporting an isovaleroyl moiety we established structure **8**. In analogy to the eugenols **3** and **4** the corresponding isomers also existed in the case of the coniferyl alcohols.

For compounds **1–7** the accordance of the aromatic substitution pattern with the eugenol- and coniferyl alcohol type (1-alkyl-4-acyloxy-5-methoxy substitution) had to be confirmed additionally, since their ^1H NMR spectra also would have been in accordance with an pseudoisoeugenol (1-alkyl-2-acyloxy-5-methoxy) substitution pattern [7]. Proof of this was furnished by NOE-difference spectra showing only one single aromatic proton (H-6) on irradiation of OMe as well as by ^{13}C NMR spectroscopy. The chemical shifts observed were in good accordance with calculated values as well as with literature data [7–9].

To discriminate the acid moiety of the phenolic and the alcoholic ester the respective ^1H NMR spectrum was compared with that of epoxy-eugenol-isobutyrate synthesized by ourselves [7] and with spectral data of synthesized compound **2** and **5** (Bohlmann [1]). As a result the downfield shifted signals of an isopropyl group (H-2″, H-3″, H-4″, Table I) occurring in all spectra could be assigned to a phenolically-bound isobutyric acid.

Aromatic acetylenes

As a result of the selected detection methods mentioned above we isolated two aromatic polyines: 1-acetoxy-7-phenyl-hepta-4,6-diin-2(t)-ene from *C. tinctoria* and *C. lanceolata* already described in *Coreopsis* species [10] and 2-(2′-phenylethinyl)-5-acetoxymethyl-thiophene from *C. grandiflora*. Their

structures were derived from mass spectrometry and ^{1}H NMR. With regard to the thiophene derivative we note that only the corresponding aldehyde has already been isolated from *C. grandiflora* [11]. Signals of a methylene group (δ 5.21) and of an acetate group (δ 2.11) – instead of a signal belonging to an aldehyde proton – proved together with the mass fragment *m/z* 197 (M′ – OAc) the structure of an acetylated alcohol. Spectral data of the synthesized alcoholic thiophene derivative [1] agree with our results.

Experimental

Plants

Fruits of *Coreopsis tinctoria* and *C. grandiflora* were purchased from Samen Wagner in Heidelberg and sown in the Botanical Garden of the University of Heidelberg. *C. lanceolata* already grew in this garden. The plants were harvested in blossom.

Extraction and separation of phenylpropanoids

The roots (800 g of *C. tinctoria*, 500 g of *C. grandiflora* and 20 g of *C. lanceolata*) were extracted twice with acetone at room temperature overnight. The combined extracts were concentrated *in vacuo*, diluted with water and extracted three times with dichloromethane. The resulting solution was concentrated and chromatographed over Si-gel using *n*-hexane/CH_2Cl_2 10/90, CH_2Cl_2 and EtOAc/CH_2Cl_2 from 5 to 10% EtOAc. The resulting phenylpropanoid fractions were concentrated and used for HPLC separation.

HPLC equipment (LDC/Milton Roy)

2 pumps Constametric I and III; injector: Rheodyne 7125, 20- and 200-µl loop; detector: Spectro-Monitor D, 278 nm. Columns: LiChrospher 100 CH18/2, 5 µm, 4 × 250 mm, flow 1.2 ml/min (analytically) and LiChrospher RP-18, 7 µm, 10 × 250 mm, flow 7 ml/min. (preparatively); solvent: MeOH/water 60:40 to 80:20.

Phenylpropanoid yields

C. tinctoria: 20 mg (1), 120 mg (2), 3 mg (3), 3 mg (4), 2 mg (5), 18 mg (6), 2 mg (7), 0.5 mg (8); *C. grandiflora:* 20 mg (1), 50 mg (2), 2 mg (3), 10 mg (5), 16 mg (6), 4 mg (7); *C. lanceolata:* 14 mg (1), 50 mg (2), 1 mg (3), 1 mg (5), 2 mg (6). Each isolated compound was identified by ^{1}H NMR.

^{1}H NMR data from acetylenic compounds (250 MHz)

1-Acetoxy-7-phenyl-hepta-4,6-diin-2(t)-ene: 2.01 (s, OAc), 4.65 (dd, J = 6 Hz, 1.5 Hz, H-1), 5.87 (dt, J = 6 Hz, 16 Hz, H-3), 6.35 (dt, J = 1.5 Hz, 16 Hz, H-2), 7.51 (m, H-2′, 6′), 7.35 (m, H-3′, 4′, 5′).

2-(2′-Phenylethinyl)-5-acetoxymethyl-thiophene: 2.11 (s, OAc), 5.21 (s, $-CH_2-$), 6.98 (d, J = 4 Hz, H-4), 7.14 (d, J = 4 Hz, H-3), 7.35 (m, H-3′, 4′, 5′), 7.51 (m, H-2′, 6′).

Mass spectral data (EI/MS, 100 eV, direct inlet) in the format m/z (rel. int.)

1′-Acetoxy-eugenol-isobutyrate (**1**): 292.1317 $[M]^+$ (11) ($C_{16}H_{20}O_5$, requires: 292.1311), 222.0892 $[M - O{=}C{=}CMe_2]^+$ (62) ($C_{12}H_{14}O_4$, requires: 222.0892), 180.0789 $[M - O{=}C{=}CMe_2 - O{=}C{=}CH_2]^+$ (79) ($C_{10}H_{12}O_3$, requires: 180.0786), 162.0680 (67) ($C_{10}H_{10}O_2$, requires: 162.0681), 131 (34), 43 (100).

1′-Isobutyryloxy-eugenol-isobutyrate (**2**): 320.1623 $[M]^+$ (8) ($C_{18}H_{24}O_5$, requires: 320.1624), 250 $[M - O{=}C{=}CMe_2]^+$ (43), 180 $[M - 2(O{=}C{=}CMe_2)]^+$ (100), 162 (53), 131 (21).

1′-(2-Methylbutyryloxy)-eugenol-isobutyrate (**3**): 334.1786 $[M]^+$ (10) ($C_{19}H_{26}O_5$, requires: 334.1780), 264.1386 $[M - O{=}C{=}CMe_2]'$ (39) ($C_{15}H_{20}O_4$, requires: 264.1362), 180 $[M - O{=}C{=}CMe{-}Et - O{=}C{=}CMe_2]^+$ (100), 162 (61), 131 (31), 85 (12), 71 (21), 57 (32), 43 (54).

1′-Isovaleroyloxy-eugenol-isobutyrate (**4**): 334.1786 $[M]^+$ (11) ($C_{19}H_{26}O_5$, requires: 334.1780), 264 (37), 180 (100), 162 (82), 131 (31), 85 (13), 71 (23), 57 (29), 43 (65).

1′,2′-Epoxy-*Z*-coniferylalcohol-3′-acetyl-4-isobutyrate (**5**): 308.1258 $[M]^+$ (20) ($C_{16}H_{20}O_6$, requires: 308.1260), 238 $[M - O{=}C{=}CMe_2]^+$ (54), 195 (22), 179 $[M - O_2CCH_3 - O{=}C{=}C{=}Me_2]^+$ (100), 153 (42), 137 (19).

1′,2′-Epoxy-*Z*-coniferylalcohol-3′-isobutyryl-4-isobutyrate (**6**): 336.1582 $[M]'$ (7) ($C_{18}H_{24}O_6$, requires: 336.1573), 266 $[M - O{=}C{=}CMe_2]^+$ (14), 223 (15), 179 (64), 153 (15), 137 (7), 71 (60), 43 (100).

1′,2′-Epoxy-*Z*-coniferylalcohol-3′-(2-methylbutyryl)-4-isobutyrate (**7**): 350.1582 $[M]^+$ (12) ($C_{19}H_{26}O_6$, requires: 350.1730), 280 $[M - O{=}C{=}CMe_2]^+$ (22), 237 (31), 179 (100), 153 (20), 137 (10), 85 (84), 71 (27), 57 (50), 43 (62).

1′,2′-Epoxy-*Z*-coniferylalcohol-3′-isovaleroyl-4-isobutyrate (**8**): 350.1734 $[M]^+$ (13) ($C_{19}H_{26}O_6$, requires: 350.1730), 280 (27), 237.1127 $[M - O{=}C{=}CMe_2 - O{=}C{-}CH_3]^+$ (33) ($C_{13}H_{17}O_4$, requires: 237.1127), 179 (100), 153 (39), 137 (17), 85 (76), 71 (38), 57 (51), 43 (82).

1-Acetoxy-7-phenyl-hepta-4,6-diine-2(t)-ene: 224.0838 $[M]^+$ (70) ($C_{15}H_{12}O_2$, requires: 224.0837), 181.0646 $[M - Ac]^+$ (68) ($C_{13}H_9O$, requires: 181.0653), 165 (28), 153 $[M - OAc{-}CO]^+$ (42), 139 (17), 105 (7), 43 (100).

5-Acetoxy-2-phenylethinyl-thiophene: 256.0520 $[M]^+$ (66) ($C_{15}H_{12}O_2S$, requires: 256.0660), 214 $[M - O{=}C{=}CH_2]^+$ (6), 197.0414 $[M - OAc]^+$ (100) ($C_{13}H_9S$, requires: 197.0527), 152 $[M - OAc{-}SCH]^+$ (31), 145 $[PhC{\equiv}C{-}C{=}S]^+$ (7), 139 (15), 43 (34).

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