Pergamon

PII: S0031-9422(97)00687-0

Phytochemistry, Vol. 48, No. 1, pp. 159–163, 1998
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Printed in Great Britain
0031-9422/98 $19.00+0.00

DITERPENE ACIDS FROM *CONYZA INCANA*

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(*Received* 2 *June* 1997)

Key Word Index—*Conyza incana*; Compositae; diterpene acids; *ent*-labdane; NMR; MS.

Abstract—The aerial parts of *Conyza incana* yielded three new diterpene acids: the two alicyclic furano diterpenes, namely, *E,E* and *E,Z*-incanic acids and an *ent*-labdane succinate ester. The structures of these compounds were elucidated by spectroscopic methods. © 1998 Elsevier Science Ltd. All rights reserved

INTRODUCTION

More than 1200 diterpenes have been identified from approximately 550 Compositae taxa. The genus *Conyza* (tribe Asterae) comprises about 50 species, which are mainly distributed in tropical and subtropical areas. The diterpenes of the genus *Conyza* are mainly of labdane, clerodane, *seco*-clerodane and alicyclic types [1]. *C. incana* Willd was studied for its essential oil by GC/MS [2]. In addition, the flavonoids chrysophenol B and penduletin [3] were isolated from its aerial parts. As a continuation of the photochemical investigation of diterpene-containing plants of Saudi flora, the aerial parts of *C. incana* have been investigated, resulting in the isolation of three diterpenes, which are the subject of this paper.

RESULTS AND DISCUSSION

The CH_2Cl_2 extract of the aerial parts of *C. incana* afforded three diterpenes: the two furano alicyclic acids **1** and **5** and the *ent*-labdane succinate ester **6**. The structure of compound **1** was deduced from its molecular formula $C_{20}H_{26}O_5$ (high resolution MS), fragmentation pattern (see Experimental) and from the ^1H NMR and ^{13}C NMR data (Tables 1 and 2). The presence of a β-substituted furan ring followed from the presence of a fragment ion at m/z 81 (C_5H_5O, pyrylium ion) in its EIMS and from the characteristic pattern of the downfield signals in its ^1H NMR spectrum (δ 6.27, 7.24, 7.35) (Table 1). The fast methylation of **1** to **1a** using diazomethane indicated the presence of two carboxylic groups (two methoxy groups at δ 3.74 and 3.72 in the ^1H NMR). The ^1H NMR and ^{13}C NMR spectra of **1** were consistent with an alicyclic diterpene acid with a terminal furan ring. The structure of **1** and its methyl ester **1a** showed close resemblance to those of centipedic acid (**2**), a diterpene isolated from *Centipeda orbicularis* [4] and microglossic acid (**3**), a diterpene acid isolated from *Microglossa zeylanica* [5]. Also, the unsaturated side chain of **1** appeared to be identical in composition and stereochemistry to the alkyl group on C-3 of oleaxillaric acid (**4**), a diterpene acid isolated from *Olearia axillaris* [6]. This fact was ascertained by comparing their relevant proton chemical shift values, which were almost indistiguishable (Table 1). The chemical shift values of H-6 and H-10 (δ 6.99 and 6.89, respectively) clearly indicated an *E* configuration for both double bonds as in **4** and related compounds with *E* configurations [5–7]. The name *E,E*-incanic acid is now used to describe compound **1**.

Compound **5** ($C_{20}H_{26}O_5$) showed very similar spectral data (Table 1, 2) to those of **1**. The main difference was the upfield shift of H-10 in **1** by 0.99 ppm, which suggested a reversed stereochemistry around the C-10/11 double bond from *E* in **1** to *Z* in **5** [6]. This was further confirmed by comparison with compounds having a similar configuration [4, 7–9] and also by noting the deshielded chemical shift value of C-12 (δ 34.53) compared to δ 28.21 in the isomeric compound **1**. The relative deshielding of C-12 can be attributed to the lack of steric compression at this position due to the *Z* configuration around the C-10/11 double bond [10]. Therefore, compound **5** was identified as the *E,Z* isomer of **1** and is named *E,Z*-incanic acid.

Compound **6**, $C_{24}H_{36}O_6$ (HRMS), was found to

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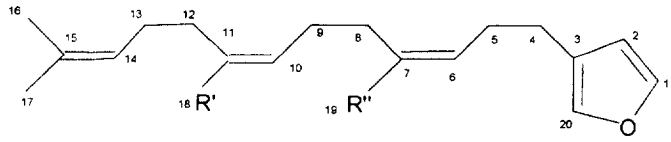
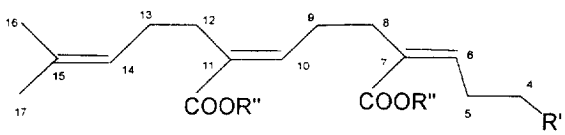
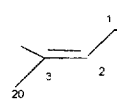


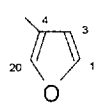
1 R' = R'' = COOH, 1a R' = R'' = COOCH3

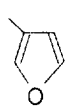
2 R' = COOH, R'' = H

3 R' = R'' = COOH, 6,7 dihydro



4 R' =  , R'' =CH_3

5 R' =  , R'' = CH_3

5a R' =  , R'' = CH_3

have a β-substituted butanolide ring as indicated by a positive reaction with Raymond reagent [11], which was further confirmed by the presance of an absorption band at 1775 cm^{-1} in the IR spectrum. The IR spectrum also showed absorption bands at 3090, 1640 and 890 cm^{-1}, suggesting the presence of an exocyclic methylene group [11], whose presence was further substantiated from the ^1H NMR data (Table 1). The ^1H NMR spectrum of **6** showed signals at δ 5.85 (*d*, $J = 1.3$, H-14) and two *dd* at δ 4.69 and 4.75 ($J = 17,1.3$,H-16), which are consistent with the values of a β-substituted butanolide ring [11], similar to that of the steroidal cardenolides [12]. The ^1H NMR spectrum of **6** also showed a close resemblance to those of the minor lactone alcohol **7** isolated from *Xanthocephalum linearifolium* [13] and daniellol succinate (**8**) isolated from *C. welwitschii* [14]. The EIMS showed the mass ion at *m/z* 318 $[M-100]^+$ indicating the loss of a succinic acid moiety. The presence of the latter was further confirmed by the multiplet signal at δ 2.63 (^1H NMR) assigned for the two CH_2 groups and the two carboxyl signals at δ 177.63 and 172.2 (^{13}C NMR). The presence of the succinyl group at C-19 caused a downfield shift of the respective protons (δ 4.27 and 3.87) relative to those of the alcohol **7** [13] and similar values to those of **8** [14]. The stereochemistry of **6** was ascertained as an *ent*-labdane based on its negative optical rotation [14]. Therefore, compound **6** was identified as the 19-succinate ester of **7**.

EXPERIMENTAL

General. Mps: uncorr.; IR: KBr and neat; ^1H and ^{13}C NMR were: $CDCl_3$ using a JEOL GNM-GX400 spectrophotometer (400 and 100 MHz, respectively); HRMS and EIMS: JEOL JMS D-300. $[\alpha]_D$: at amb. temp.; MPLC: 40 g of RP C18 silica gel (25–40 μ, 2.5 X22.5 cm), flow rate 5 ml/min; TLC: silica gel 60 F_{254}, CH_2Cl_2-MeOH (10:1) as solvent system; visualization using *p*-anisaldehyde/H_2SO_4 as spray reagent.

Plant material. The aerial parts of *C. incana* Willd were collected in Abha, Saudi Arabia in August 1995.

Table 1. ^{1}H NMR spectral data of the diterpenes isolated from *C. incana* and oleaoxillaric acid (**4**)

	1	**1a**	**4**	**5**	**5a**	**6**	**6a**
1	7.35 *t*	7.35 *t*	4.58 *d*	7.36 *t*	7.38 *t*	*β* 1.08 *ddd*	1.07 *ddd*
						α 1.80 *m*	1.80 *m*
2	6.27 *dd*	6.26 *dd*	5.36 *br.d*	6.27 *dd*	6.31 *dd*	1.50–1.52 m	1.50–1.52 *m*
3	—	—	—	—	—	1.02 *m*	1.05 *m*
						1.78 *m*	1.76 *m*
4	2.60 *t*	2.57 *t*	2.15 *br.t*	2.55 *t*	2.40 *t*	—	—
5	2.49 *br.q*	2.45 *br.q*	2.29 *m*	2.41 *br.q*	2.46 *br.q*	1.25 *dd*	1.25 *dd*
6	6.99 *t*	6.82 *t*	6.76 *t*	6.93 *t*	6.81 *t*	*α* 1.36 *dq*	1.35 *dq*
	—	—	—	—	—	*β* 1.88 *m*	1.85 *m*
7	—	—	—	—	—	*β* 1.94 *ddd*	1.94 *m*
	—	—	—	—	—	*α* 2.42 *br.d*	2.43 *ddd*
8	2.43 br t	2.42 t	3.41 *t*	2.44 *t*	2.57 *t*	—	—
9	2.29 *br.q*	2.26 *br.q*	2.29 *m*	2.64 *br.q*	2.48 *br.q*	1.65 *br.s*	1.62 *br.s*
10	6.89 *t*	6.67 *t*	6.71 *br.t*	5.90 *t*	5.80 *t*	—	—
11	—	—	—	—	—	1.65–1.8 *m*	1.7–1.8 *m*
12	2.33 *br.t*	2.28 *t*	2.29 *m*	2.24 *t*	2.24 *t*	A 2.26 *m*	2.25 *m*
						B 2.56 *m*	2.55 *m*
13	2.08 *q*	2.05 *q*	2.05 *dt*	2.10 *q*	2.08 *q*	—	—
14	5.11 *t*	5.12 *t*	5.10 *br.t*	5.08 *t*	5.10 *t*	5.85 *br.s*	5.85 *t*
16	1.66 *br.s*	1.66 *br.s*	1.66 br.s	1.67 *br.s*	1.70 *br.s*	A 4.69*dd*	4.68 *dd*
						B 4.75 *dd*	4.75 *dd*
17	1.58 b. s	1.57 br.s	1.57 *br.s*	1.57 *br.s*	1.50 *br.s*	A 4.47 *br.s*	4.47 *br.s*
						B 4.89 *br.s*	4.88 *br.s*
18	—	—	—	—	—	0.97 *s*	0.96 *s*
19	—	—	—	—	—	A 3.87 *d*	3.87 *d*
	—	—	—	—	—	B 4.27 *d*	4.26 *d*
20	7.24 *br.s*	7.23 *t*	1.71 *br.s*	7.23 *t*	7.27 *t*	0.70 *s*	0.70 *s*
Succinic acid moiety:							
$(CH_2)_2$	—	—	—	—	—	2.63 *m*	2.62 *m*
OCH_3	—	3.74 *s*	—	3.74 *s*	3.73 *s*	—	3.7 *s*
		3.72 *s*		3.71 *s*	3.71 *s*	—	—
OAc	—	—	2.04 *s*	—	—	—	—

J [Hz]; Compound **1**, **1a**, **5** and **5a**: 4,5 = 5,6 = 8,9 = 9,10 = 12,13 = 13,14 = ~ 7; 1,2 = 1,20 = 1.8; Compound **6** and **6a**: 1*α*, 1*β* = 1*β*, 2*α* = 12.0; 1*α*, 2*α* = 1*β*, 2*β* = 5; 5, 6*α* = 12.8; 5, 6*β* = 1.8; 6*α*, 7*α* ~4; 6*α*, 6*β* = 6*α*, 7*β* = 12.5; 6*β*, 7*β* = 5; 14,16A = 14,16*β* ~ 1.3; 16A, 16B = 17.4; 19A, 19B = 11.

A voucher specimen (# 12959) was deposited in the herbarium of the College of Pharmacy, King Saud University, Riyadh, Saudi Arabia.

Isolation procedure. The dried ground aerial parts (1 kg) of *C. incana* were percolated with CH_2Cl_2 (6 l) at room temp then evaporated to give 63 g of a dark green extract. A portion of the CH_2Cl_2 extract (50 g) was partitioned between MeCN and *n*-hexane to give 35 g and 9 g, respectively. The MeCN fraction (4 g) was dissolved in EtOAc (75 ml) and extracted with 2% $NaHCO_3$ soln (3 × 100 ml). The combined alkaline extracts were adjusted to pH 4 using HOAc and the acidic soln was shaken with EtOAc (3 × 100 ml). The combined EtOAc extracts were washed with H_2O and filtered on anhydrous Na_2SO_4 then evaporated under vacuum (1.3 g). In a typical run, the EtOAc extract (500 mg) was subjected to MPLC over RP C18 using MeOH-MeCN-H_2O (5:2:3) as eluent, to afford compound **1** (140 mg, R_f 0.36), followed by compound **5** (73 mg, R_f 0.47).

The EtOAc phase left after shaking with $NaHCO_3$ soln, was dried over anhydrous Na_2SO_4 to leave 1.7 g. The residue was subjected to silica gel CC using CH_2Cl_2-MeOH (9.5:0.5) as the solvent system, followed by MLPC rechromatography using RP C18 and MeOH-MeCN-H_2O (2:1:1) as eluent (flow rate 3 ml/min), to give 113 mg of compound **6**.

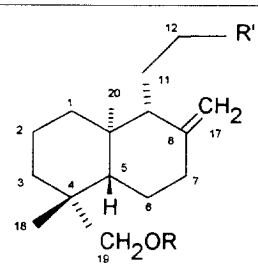
E,E-*Incanic acid* (**1**). White amorphous powder, mp 81–83°, $[\alpha]_D$ +6.7° (*c* 0.102; MeOH). IR ν_{max} (KBr) cm^{-1}: 3420 br (COOH), 1670 (CO), 1630, 1425; ^{1}H and ^{13}C NMR: Tables 1 and 2; EIMS *m/z* (rel. int.): 346 $[M]^+$ (6), 328 $[M-H_2O]^+$ (35), 277 $[M-C_5H_9]^+$ (15), 179 $[M-C_{10}H_{15}O_2]^+$ (10), 95 $[M-C_{14}H_{19}O_4]^+$ (18), 81 $[C_5H_5O]^+$ (85) and 69 $[C_5H_9]^+$ (100).

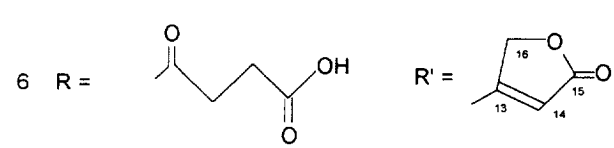
E,Z-*Incanic acid methyl ester* (**1a**). Colourless oil, $[\alpha]_D$ +10.3° (*c* 0.07; MeOH). ^{1}H and ^{13}C NMR: Tables 1 and 2; EIMS *m/z* (rel. int.): 374 $[M]^+$ (15), 342 $[M-MeOH]^+$ (57), 310 $[M-2XMeOH]^+$ (32),229 (41),179 $[M-C_{10}H_{15}O_2]^+$ (22), 81 $[C_5H_5O]^+$ (100) and 69 $[C_5H_9]^+$ (96).

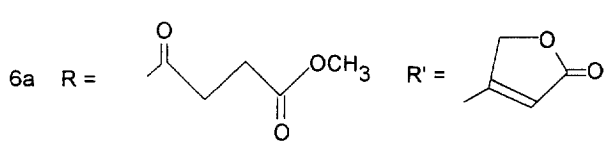
E,Z-*Incanic acid* (**5**). Colourless oil, $[\alpha]_D$ +8.9° (*c* 0.092; MeOH). IR ν_{max} (neat) cm^{-1}: 3500 br

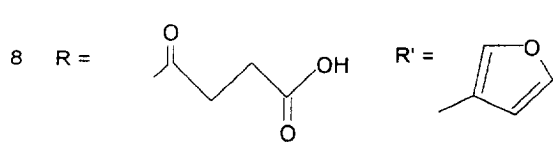
Table 2. ^{13}C NMR spectral data of diterpenes isolated from *C. incana*

	1	1a	5	5a	6	6a
1	143.03 *d*	141.99	142.97	42.93	38.87 *t*	38.93
2	110.76 *d*	110.75	110.76	110.81	18.80 *t*	18.86
3	123.48 *s*	123.84	123.32	123.45	36.01 *t*	36.04
4	23.90 *t*	24.02	24.06	26.90	37.38 *s*	37.41
5	29.49 *t*	29.24	29.46	29.03	56.11 *d*	56.08
6	145.28 *d*	142.63	144.06	142.39	24.35 *t*	24.42
7	132.40 *s*	132.49	131.10	131.88	38.32 *t*	38.35
8	25.63 *t*	26.08	25.94	24.08	147.00 *s*	147.06
9	26.60 *t*	26.90	28.54	29.09	56.01 *d*	56.17
10	144.06 *d*	141.46	141.23	140.51	39.56 *s*	39.63
11	131.04 *s*	132.28	133.13	132.28	21.32 *t*	21.35
12	28.21 *t*	28.00	34.53	34.70	27.37 *t*	27.56
13	27.70 *t*	27.70	27.76	27.70	170.98 *s*	170.83
14	123.69 *d*	123.59	123.27	123.38	115.16 *d*	115.28
15	132.07 *s*	131.99	132.46	132.28	174.26 *s*	174.08
16	25.63 *q*	25.60	25.66	25.70	73.14 *t*	73.08
17	17.62 *q*	17.60	17.71	17.61	106.96 *t*	106.99
18	173.42 *s*	168.49	174.48	168.34	27.48 *q*	27.39
19	173.05 *s*	167.86	173.54	168.04	67.01 *t*	66.92
20	139.08 *d*	139.05	139.02	139.05	15.10 *q*	15.16
Succinic acid moiety:						
$(CH_2)_2$	—	—	—	—	28.94 *t*	29.21
	—	—	—	—	28.94 *t*	28.94
COOH	—	—	—	—	177.63 *s*	172.72
	—	—	—	—	172.20 *s*	172.32
CH_3O	—	51.77 *q*	—	51.74 *q*	—	51.83
	—	51.70 *q*	—	51.13 *q*	—	—









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E,E-*Incanic acid methyl ester* (**5a**). Colourless oil, $[\alpha]_D+8.8°$ (*c* 0.082; MeOH). ^{1}H and ^{13}C NMR: Tables 1 and 2; EIMS *m/z* (rel. int.): 374 $[M]^+$ (5), 342 [M–MeOH]$^+$ (28), 310 [M–2XMeOH]$^+$ (25), 229 (40),179 $[M-C_{10}H_{15}O_2]^+$ (16), 107 (30), 81 $[C_5H_5O]^+$ (100) and 69 $[C_5H_9]^+$ (96).

Compound **6**. Colourless oil, $[\alpha]_D-25.33°$ (*c* 0.107; MeOH). IR ν_{max} (neat) cm^{-1}: 3450 br (COOH), 3090, 1640, 890 (exocyclic methylene group), 1775 ($^{\alpha,\beta}$butanolide ring); ^{1}H and ^{13}C NMR: Tables 1 and 2; EIMS *m/z* (rel. int.): 418 $[M]^+$ (4), 400 $[M-H_2O]^+$ (6), 318 [M–succinate]+(13), 300 $[M-H_2O$–succinate$]^+$ (45), 287 $[M-CH_2OCO(CH_2)_2COOH]^+$ (38), 203 (75),135 (86), 119 (57), 98 $[C_5H_6O_2]^+$ (100) and 69 (85).

Compound **6a**. Colourless oil, $[\alpha]_D-26.9°$ (*c* 0.086; MeOH). ^{1}H and ^{13}C NMR: Tables 1 and 2; EIMS *m/z* (rel. int.): 432 $[M]^+$ (5), 318 [M–methyl succinate]$^+$ (4), 300 $[M-H_2O$–methyl succinate$]^+$ (66), 287 $[M-CH_2OCO(CH_2)_2\ COOCH_3]^+$ (54), 203 (88),135 (99), 115 (100), 98 [C5H6O2]$^+$ (92) and 55 (82).

Acknowledgement—The authors thank Dr. Sultan-Ul-Abedin for the identification of plant materials.

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