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FURTHER MONOTERPENE 5-METHYLCOUMARINS AND AN ACETOPHENONE DERIVATIVE FROM *ETHULIA CONYZOIDES*

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Key Word Index—*Ethulia conyzoides*; Vernonieae; Asteraceae; monoterpene coumarins; monoterpene acetophenone derivative.

Abstract—Re-investigation of the aerial parts of *Ethulia conyzoides* from Egypt afforded two new monoterpene 5-methylcoumarins, namely 5′-epi-isoethuliacoumarin B and 5′-epi-isoethuliacoumarin A, and a new monoterpene acetophenone derivative, ethuliaconyzophenone, in addition to the five known compounds, ethuliacoumarin, cycloethuliacoumarin, isoethuliacoumarin A, isoethuliacoumarin B and 4-hydroxy-5-methylcoumarin-4-*O*-β-D-glucopyranoside. © 1998 Elsevier Science Ltd. All rights reserved

INTRODUCTION

The genus *Ethulia* with ca 19 species is widely distributed in tropical Africa and less commonly in tropical Asia [1]. Among this genus, *E. conyzoides* L var. gracillis Asch, and Scheweinf, a wild growing Egyptian plant, is used in folklore medicine as an antihelminthic for round worms and for abdominal disorders [2, 3]. Only two species, *E. conyzoides* and *E. vernonioides*, have been chemically studied. Terpenoid 5-methylcoumarins [4–9] and some flavonoid glycosides were isolated [10]. In continuation of our studies on the chemical constituents of the genus *Ethulia* [11], we have reinvestigated the aerial parts of the Egyptian species *E. conyzoides* L.

RESULTS AND DISCUSSION

The methylene chloride–methanol (1 : 1) extract of the dried aerial parts of *E. conyzoides* afforded two new monoterpene 5-methylcoumarins, namely 5′-epi-isoethuliacoumarin B (**3a**) and 5′-epi-isoethuliacoumarin A (**4a**), a new monoterpene acetophenone derivative (ethuliaconyzophenone) (**5**) and the known compounds ethuliacoumarin (**1**) [4], cycloethuliacoumarin (**2**) [4] isoethuliacoumarins B and A (**3** and **4**) [5] and 4-hydroxy-5-methylcoumarin-4-*O*-β-D-glucopyranoside [6].

The molecular formula of **3a**, $C_{20}H_{22}O_5$, was deduced from the EI mass spectrum and confirmed by the ^{13}C NMR data. The MS fragmentation pattern was similar to that of isoethuliacoumarin B (**3**) previously isolated from the same species [5]. Two fragments at *m/z* 284 $[M-Me_2CO]^+$ and 270 $[M-Me_2C{=}CHOH]^+$ supported the presence of an oxetane ring in **3a** and indicated that it was an isomer of **3**. The ^1H NMR spectra of **3** and **3a** (Table 1) were very similar. However, the H-6′ and H-2′ signals at δ 4.57 and 6.26 in **3**, were shifted upfield to δ 4.28 and 6.05, respectively, in **3a**. These shift differences together with the downfield shift of the aromatic methyl H-9 (δ 2.85) indicated epimeric configuration at C-5′ [4, 5, 8]. The stereochemistry at C-5′ and C-6′ in **3** and **3a** was proved by NOE experiments with inspection of the molecular model. Thus, in the case of **3**, clear effects were observed between H-6′, H-8′ and the aromatic methyl H-9 as well as between H-9, H-6 and H-6′ while in **3a**, irradiation of H-6′ gave clear effects with H-9′ and H-4′α and no effect with H-9. Further NOEs were obtained in **3a** between H-9′, H-6′ and H-8′ and between H-10′, H-4′β and H-2′ as well as between H-8′, H-9 and H-9′. The ^{13}C NMR spectral data of **3** and **3a** (Table 2) supported the structures. Therefore, **3a** was identified as 5′-epi-isoethuliacoumarin B. Some closely related epimers were reported from an Indian collection of *E. conyzoides* [8]. The structure and absolute configuration of one of these epimers have been established by X-ray analysis.

The EIMS spectrum of compound **4a** showed a $[M]^+$ peak at *m/z* 342 corresponding to $C_{20}H_{22}O_5$ (confirmed by ^{13}C NMR). Again, this molecular formula, together with the fragmentation pattern, indicated that we were dealing with an isomer of isoethuliacoumarin A (**4**) [5]. Comparison of the ^1H

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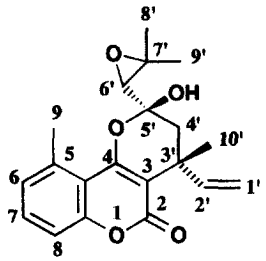
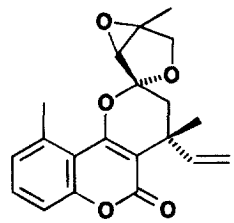


1



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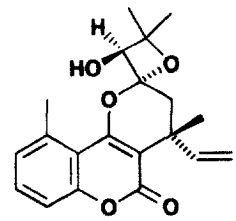
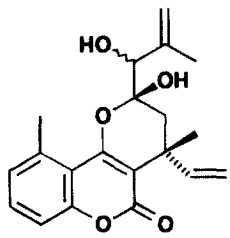


3

3a: 5',6'-epi



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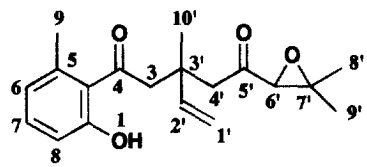
4a: 5'-epi



5

Table 1. ^{1}H NMR spectral data of compounds **3a**, **4a** and **5** (400 MHz, $CDCl_3$, δ Values)

H	3a	4a	5*
3	—	—	3.29 *s*
6	7.05 *br d*	6.99 *br d*	6.70 *br d*
7	7.36 *dd*	7.34 *dd*	7.21 *dd*
8	7.16 *br d*	7.12 *br d*	6.75 *br d*
9	2.85 *br s*	2.76 *br s*	2.52 *br s*
1'c	5.14 *d*	5.22 *m*	5.05 *d*
1't	5.04 *d*	5.22 *m*	5.00 *d*
2'	6.05 *dd*	6.21 *dd*	6.01 *dd*
$4'_1$	2.26 *d*	2.23 *d*	2.96 *s*
$4'_2$	2.12 *d*	2.08 *d*	2.96 *s*
6'	4.28 *d*	4.29 *d*	3.37 *s*
8'	1.52 *s*	1.89 *br s*	1.43 *s*
9'	1.48 *s*	5.18, 5.22 *br s*	1.23 *s*
10'	1.61 *s*	1.59 *s*	1.24 *s*
OH	2.32 *d*†	2.50 *d*‡	10.50 *s*

* Assignment were confirmed by ^{1}H-^{13}C COSY.

J (Hz): 6, 7 = 7, 8 = 8 Hz, $4'_1$, $4'_2$ = 14 Hz, 1't, 2' = 17 Hz, 1'c, 2' = 10 Hz.

† 6', OH = 8.5 Hz.

‡ 6', OH = 3.5 Hz.

NMR spectra of **4** and **4a** (Table 1) showed some characteristic differences. In the ^{1}H NMR spectrum of **4a**, H-1' and H-2' signals were shifted downfield to δ 5.22 and 6.21, respectively, while H-10' was shifted upfield to δ 1.59 compared with δ 1.73 in **4**. The signals of H-$4'_1$ (δ 2.23) and H-$4'_2$ (δ 2.08) in **4a** were also significantly downfield from those in **4**. These shift differences clearly indicated 5' α-OH [4, 5, 8]. The ^{13}C NMR spectra of **4** and **4a** (Table 2) also supported the structures. Finally, oxidation of **4a** by Jones reagent, gave the lactone previously obtained [4] from oxidation of isoethuliacoumarin A (**4**). Thus **4a** was 5'-epi-isoethuliacoumarin B.

The ^{13}C NMR spectrum of **5** (Table 2) displayed 19 carbon signals, instead of 20 as in **1–4**. This was supported by the high-resolution positive ion FAB-mass spectrum which showed a $[M+1]^+$ peak at *m/z* 317.4755 corresponding to $C_{19}H_{25}O_4$. While the aromatic signals (H-6, H-7, H-8 and H-9) in the ^{1}H NMR spectrum of **5** (Table 1) were similar to those in **1–4**, the ^{13}C NMR spectrum (Table 2) indicated the absence of C-2. Furthermore, the IR and ^{1}H NMR spectral data (Table 1 and Experimental) showed the presence of a chelated hydrogen bond between a phenolic hydroxyl group (3500 cm^{-1} and δ_H 10.50) and an aromatic ketone (1632, 1608 cm^{-1} and δ_C 205.7). These differences indicated that **5** was a monoterpene linked with 2-hydroxy-6-methylacetophenone. The presence of trisubstituted oxirane ring at C-6' and C-7' was inferred from the H-6' signal at δ_H 3.37 (1H, *s*) and δ_C 65.9 (*s*) while C-7' resonated at δ 61.8. Whereas, the tertiary methyl H-8' (δ 1.43) and H-9' (δ 1.23), correlated with C-7' in the COLOC spectrum. The two (CH_2) singlet signals [(δ_H 3.29, δ_C 51.5 *t*) and (δ_H 2.96, δ_C 48.6)] were assigned to the methylene groups C-3 and C-4', respectively, while the vinyl group signals were similar to those in **1–4**. The tertiary methyl group [δ_H 1.24 (*s*), δ_C 25.7 (*q*)] was assigned to C-10'. The assignments of all proton and carbon signals in **5** were established from the results of 2D ^{1}H-^{13}C COSY together with COLOC experiments which also confirmed the connection between the monoterpene part

Table 2. ^{13}C NMR spectral data of compounds **1–5** (100.6 MHz, $CDCl_3$ δ-values)

C	1	2	3	3a	4	4a	5*
2	159.9 *s*	159.8 *s*	159.8 *s*	160.1 *s*	159.7 *s*	160.5 *s*	—
3	106.3 *s*	107.5 *s*	106.4 *s*	106.1 *s*	107.6 *s*	104.8 *s*	51.5 *t*
4	160.0 *s*	160.1 *s*	160.5 *s*	160.9 *s*	160.6 *s*	161.2 *s*	205.7 *s*
4a	114.5 *s*	114.4 *s*	114.5 *s*	114.1 *s*	114.8 *s*	114.7 *s*	124.0 *s*
5	137.0 *s*	136.3 *s*	137.0 *s*	136.4 *s*	136.7 *s*	137.0 *s*	137.9 *s*
6	127.4 *d*	127.5 *d*	127.5 *d*	127.6 *d*	127.3 *d*	127.6 *d*	123.2 *d*
7	130.7 *d*	130.9 *d*	130.0 *d*	131.0 *d*	130.6 *d*	131.1 *d*	133.5 *d*
8	114.3 *d*	115.1 *d*	115.0 *d*	115.0 *d*	114.7 *d*	114.1 *d*	115.9 *d*
8a	153.7 *s*	154.0 *s*	154.1 *s*	154.0 *s*	153.8 *s*	154.0 *s*	159.7 *s*
9	23.8 *q*	23.6 *q*	23.9 *q*	22.2 *q*	24.0 *q*	23.7 *q*	23.7 *q*
1′	112.4 *t*	112.5 *t*	112.0 *t*	113.3 *t*	111.9 *t*	111.8 *t*	112.2 *t*
2′	145.0 *d*	143.1 *d*	142.8 *d*	143.1 *d*	146.0 *d*	144.4 *d*	145.0 *d*
3′	35.9 *s*	35.4 *s*	35.0 *s*	37.4 *s*	35.4 *s*	35.7 *s*	39.3 *s*
4′	41.7 *t*	39.8 *t*	40.5 *t*	45.2 *t*	40.8 *t*	38.6 *t*	48.6 *t*
5′	97.4 *s*	105.3 *s*	107.5 *s*	106.5 *s*	99.7 *s*	101.6 *s*	207.9 *s*
6′	66.4 *d*	63.5 *d*	78.6 *d*	76.4 *d*	80.0 *d*	78.7 *d*	65.9 *d*
7′	59.2 *d*	61.5 *s*	82.8 *s*	87.4 *s*	141.9 *s*	142.5 *s*	61.8 *s*
8′	24.9 *q*	69.9 *t*	28.6 *q*	28.6 *q*	17.9 *q*	20.3 *q*	24.7 *q*
9′	17.6 *q*	13.3 *q*	21.9 2 *q*	22.2 *q*	117.5 *t*	115.9 *t*	18.3 *q*
10′	25.0 *q*	25.6 *q*	25.8 *q*	25.2 *q*	24.1 *q*	23.7 *q*	25.7 *q*

* Assignments were confirmed by ^1H-^{13}C COSY and COLOC experiments.
Multiplicities were deduced from DEPT experiments.

with the acetophenone moiety. The most important correlations were observed between H-3 and C-4a, between H-10′ and C-3, C-2′, C-4′ as well as between H-6′ and C-4′, C-8′, C-9′.

EXPERIMENTAL

Plant material

The aerial parts of *E. conyzoides* were collected on the Cairo–Alexandria road in May, 1996. A voucher sample is deposited in the Department of Botany, Faculty of Science, El-Minia University, Egypt.

Extraction and isolation

The air-dried aerial parts (4 kg) were powered and extracted with CH_2Cl_2–MeOH (1 : 1) at room temp. The extract (340 g) was prefractionated as reported [12] by CC on silica gel using gradient mixture of (petrol–Et_2O–MeOH) with increase polarity into five frs [fr. 1 : petrol, fr. 2 : petrol–Et_2O (9 : 1), fr. 3 petrol–Et_2O (1 : 1), fr. 4 petrol–Et_2O (1 : 9) and fr. 5 Et_2O–MeOH (9 : 1.5)]. Purification of fr. 2 by prep TLC (Et_2O–petrol 1 : 3) gave 2-hydroxy-6-methylacetophenone (50 mg). Fr. 3 was further separated by CC on silica gel (Et_2O–petrol 1 : 1) to afforded **1** (1 g), **2** (40 mg). One of subfr. 2 was further separated by CC on Sephadex LH-20 (hexane–CH_2Cl_2–MeOH) and purified by TLC eluted with (Et_2O–petrol 1 : 1) to give **5** (8 mg). Repeated separation of fr. 4 on CC silica gel and Sephadex LH-20 gave a mixt. of **3** and **3a** (25 mg), and of **4** and **4a** (100 mg). Further separation of the two mixts. using CC on Sephadex LH-20 (hexane–CH_2Cl_2–MeOH 7 : 4 : 0.5) followed by TLC (petrol–ether 1 : 1) afforded **3** (8 mg), **3a** (6 mg), **4** (75 mg) and **4a** (40 mg). Fraction 5 was further separated by CC on silica gel (hexane–CH_2Cl_2–MeOH) to afforded 4-hydroxy-5-methylcoumarin-4*O*-*β*-D-glucopyranoside (2 g).

5′-Epi-isoethuliacoumarin B (**3a**). White powder. IR ν_{max}^{KBr} cm^{-1}: 3480 (OH), 1680 (C=O), 1600, 1560 (aromatic), 1460, 1200, 920, 860, 780; UV λ^{max} ($CHCl_3$, nm): 325 *sh*, 312, 292, 282; EIMS (direct inlet) 70 eV, *m/z* (rel. int.): 342 $[M]^+$ ($C_{20}H_{22}O_5$) (5), 324 $[M-H_2O]^+$ (2), 284 $[M-Me_2C{=}O]^+$ (3), 270 $[M-Me_2C{=}CHOH]^+$ (35), 228 $[270-C_2H_2O]^+$ (50), 177 (30), 135 (80), 95 (100); $[\alpha]D^{26}+33$ (*c* 1.127, $CHCl_3$).

5′-Epi-isoethuliacoumarin A (**4a**). White powder. IR (max) cm^{-1}: 3470 (OH), 1690 (C=O), 1605, 1555 (aromatic), 1465, 1150, 910, 850, 780; UV λ^{max} ($CHCl_3$, nm): 322 *sh*, 308, 292, 280; EIMS (direct inlet) 70 eV, *m/z* (rel. int.): 342 $[M]^+$ ($C_{20}H_{22}O_5$) (2), 324 $[M-H_2O]^+$ (3), 271 $[M-MeC(=CH_2)CHOH]^+$ (55), 229 $[271-C_2H_2O]^+$ (50), 177 (30), 135 (80), 95 (100).

Ethuliaconyzophenone (**5**). Oil. IR ($CDCl_3$) cm^{-1}: 3550 (OH), 1708 (C=O), 1632, 1608 (aromatic ketone, hydrogen bridge), 1576, 1454, 1226, 924; UV λ^{max} ($CHCl_3$, nm): 240 *sh*, 265, 291 *sh*, 324; HR-FABMS 317.4755 (calc. for $C_{19}H_{24}O_4$, 317.4025); FABMS (direct inlet) *m/z* (rel. int.): 317 $[M+H]^+$ (15), 299 $[M-H_2O+H]^+$ (8), 245 $[M-Me_2C(O)CH{-}]^+$ (90), 229 (8), 203 245 $[M-Me_2C(O)CHCOCH_2{-}]^+$ (10), 185 (65), 135 (80); $[\alpha]D^{26}+32$ (*c* 0.125, $CHCl_3$).

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