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A pseudoguaiane sesquiterpene xylopyranoside from *Echinops hussoni*

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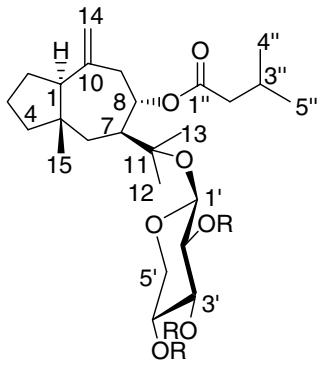
A new pseudoguaiane sesquiterpene xylopyranoside ester, named echusoside (**1**), was isolated from the aerial parts of *Echinops hussoni* Boiss. (Asteraceae) collected from the southeastern border of Egypt. The structure elucidation of echusoside was based primarily on 1D, 2D-NMR analyses and chemical derivatization. This is the first report for a pseudoguainane sesquiterpene in the genus *Echinops*.

1. Introduction

Plants of the genus *Echinops*, family Asteraceae, subtribe Echinopeae Cass., comprise about 120 species [1–3]. These plants display a range of secondary metabolites including thiophenes [3–4], quinoline alkaloids [5–7], sesquiterpene hydrocarbons [8], triterpenes [7], and flavonoids [9]. Many of *Echinops* species secondary metabolites or crude extracts exert bioactivity including: hepatoprotective [10], anti-inflammatory [10], antifungal [11], antifeedant [12], and nematocidal activities [13].
Echinops hussoni Boiss is a rare perennial desert thistles shrub grows in Elba Mountain and the Red Sea coastal region in the southeastern area of Egypt [2]. The present study represents the first phytochemical investigation of this plant species.

2. Investigations, results and discussion

Repeated flash chromatography on silica gel and final purification using C18 RP-HPLC of the petroleum ether extract of the aerial parts of *E. hussoni* afforded compound **1**. The EIMS of **1** displayed a molecular ion peak at *m/z* 454, suggesting the molecular formula $C_{25}H_{42}O_7$ and five degrees of unsaturation. The IR spectrum ($CHCl_3$) of **1** showed a broad absorption band at 3610–3220 cm^{-1}, suggesting the presence of hydroxy groups. It also showed an absorption band at 1730 cm^{-1}, consistent with the presence of a carbonyl ester functionality. The ^{13}C and ^{1}H NMR spectra of **1** (Table) were in agreement with a pseudoguaiane skeleton for **1** [14–15]. The narrow olefinic doublets (J = 1.0) resonating at δ 4.69 and 4.42 (Table), which correlated with the exomethylene carbon resonating at δ 105.4 are assigned H_2-14. This assignment was based on the observed LR-HETCOR coupling with the methine carbon resonating at δ 49.8 (C-1) and the allylic methylene carbon resonating at δ 36.8 (C-9). The monoglycosidic nature of **1** was suggested based on the presence of one anomeric methine carbon (C-1′) resonating at δ 96.7. Three oxygenated methine carbons (Table) at δ 70.5, 76.4 and 73.5 (C-2′, 3′ and 4′, respectively) and one oxygenated methylene carbon at δ 63.6 (C-5′), completed the pentose sugar carbon skeleton. The β-orientation of the glycosidic linkage was revealed from the high coupling constant of H-1′ (J = 7.7) [16]. The 11-*O*-glycosidation of **1** was deduced from the downfield shifting of C-11 (δ 80.6) and its LR-HETCOR couplings with H_3-12, H_3-13 and the anomeric proton H-1′. Mild acid hydrolysis of **1** and TLC analysis of the aqueous aliquot revealed β-D-xylose. Attempts to isolate the aglycone were unsuccessful, probably due to its decomposition. Acetylation of **1** afforded its corresponding tri-*O*-acetate (**2**), as suggested by physical and spectral analyses, which confirmed the presence of 3 secondary hydroxyl groups. The high coupling between H-3′ and H-4′ (J = 9.4, Table) in **2** confirmed the *trans*-diaxial relation between both protons and hence provided further support to assign the sugar as xylose rather than arabinose. The downfield proton signal resonating at δ 3.42 in **2** (Table) was assigned H-8 based on its COSY couplings with H-7 and H_2-9. Proton H-8 showed LR-HETCOR correlation with the carbonyl carbon absorbed at δ 173.5 (C-1″). The carbon C-1″ of **1** also showed ^{2}J-LR-HETCOR coupling with the H_2-2″ which in turn displayed LR-HETCOR couplings to both C-4″ and C-5″ methyl signals. Both methyl signals C-4″ and C-5″ also displayed COSY couplings to the proton multiplet absorbed at δ 2.05 (H-3″) which supported the assignment of the ester as a 3-methyl-butyroyl moiety. The splitting and coupling constant pattern of H-1 (brd, J = 12.1) suggested its axial and hence α-orientation (large $H\text{-}1_{axial}/H\text{-}2_{axial}$ and minor $H\text{-}1_{axial}/H\text{-}2_{equatorial}$ couplings) [17]. The relative stereochemistry of the chiral centers C-5 and C-7 was assigned β based on comparison of their ^{13}C NMR chemical shift values with related known compounds [15, 18]. The coupling constants (J) for the β-oriented H-8 in known related guaianes are 6, 6, and 8 Hz [19] while those reported for H-8α are 10, 8, and 5 Hz which better match the same values of H-8 in **2** (Table). Moreover, the reported ^{13}C NMR chemical shift value for β-C-8 is 66–68 ppm while those of α-C-8 is around 73 ppm in compounds containing the same ester moiety, which proved the α-orientation of H-8 in **1** [15, 18–20]. Therefore, the identity of compound **1** was established as 10(14)-pseudoguaien-8α-(3-methylbutyroyloxy)-11-*O*-β-D-xylopyranoside, a new natural product named echusoside.



	R
Echusoside (**1**)	H
Echusoside tri-*O*-acetate (**2**)	CH_3CO

Table: ^{13}C and ^{1}H NMR spectral data of 1 and 2[a]

Position	1		2	
	δ_C	δ_H	δ_C	δ_H
1	49.8, d	1.74, brd (12.1)	50.0, d	1.72, brd (12.1)
2	23.5, t	1.49, m 1.12, m	23.6, t	1.50, m 1.18, m
3	24.8, t	1.62, m 1.10, m	24.7, t	1.62, m 1.20, m
4	41.8, t	1.40, m 1.14, m	41.9, t	1.40, m 1.25, m
5	35.9, s	–	36.0, s	–
6	41.1, t	1.51, m 1.19, m	41.2, t	1.57, m 1.24, m
7	48.2, d	1.50, m	48.4, d	1.54, m
8	73.7, d	3.42, m	73.2, d	3.42, ddd (9.8, 5.6, 2.9)
9	36.8, t	2.30, m 1.98, m	36.9, t	2.31, m 1.99, m
10	151.1, s	–	150.9, s	–
11	80.6, s	–	80.7, s	–
12	24.3, q	1.22, 3 H, s	24.9, q	1.20, 3 H, s
13	23.6, q	1.21, 3 H, s	22.8, q	1.16, 3 H, s
14	105.4, t	4.69, d (1.0) 4.42, d (1.0)	105.3, t	4.69, d (1.0) 4.41, d (1.0)
15	16.4, q	0.68, 3 H, s	16.4, q	0.68, 3 H, s
1′	96.7, d	4.44, d (7.7)	95.2, d	4.68, d (7.9)
2′	70.5, d	3.37, m	71.5, d	4.97, dd (9.6, 7.9)
3′	76.4, d	3.56, dd (9.0, 8.8)	71.7, d	5.22, dd (9.5, 9.4)
4′	73.5, d	3.33, m	71.6, d	5.01, ddd (10.1, 9.2, 1.0)
5′	63.6, t	4.34, m	62.3, t	4.12, m
		4.27, m		4.14, m
1″	173.5, s	–	172.6, s	–
2″	43.3, t	2.20, 2 H, m	43.3, t	2.22, 2 H, m
3″	25.6, d	2.05, m	25.6, d	2.03, m
4″	22.3, q	0.94, 3 H, d (6.6)	22.3, q	0.95, 3 H, d (6.6)
5″	22.4, q	0.94, 3 H, d (6.6)	22.5, q	0.94, 3 H, d (6.6)
2′-Ac	–		20.7, q	1.99, s
			168.9, s	–
3′-Ac	–		20.8, q	2.01, 3 H, s
			169.3, s	–
4′-Ac	–		20.8, q	2.02, 3 H, s
			170.3, s	–

[a] In $CDCl_3$, at 300 MHz for ^{1}H and 75 MHz for ^{13}C. Carbon multiplicities were determined by DEPTGL experiment; s = quaternary, d = methine, t = methylene, q = methyl carbons, Coupling constants (J) are in Hz.

Echusoside did not show antimicrobial (against *Candida albicans*, *Staphyllococcus aureus* and *Pseudomonas aeruginosa*), insecticidal (against southern corn rootworm, *Diabrotica undecimpunctata* and tobacco budworm, *Heliothis virescens*) or antimalarial (against *Plasmodium falciparium* D6 and W2 clones) activities.

3. Experimental

3.1 General experimental procedure

The ^{1}H and ^{13}C NMR spectra were recorded on Varian VXR-300 or Bruker DRX-400 NMR spectrometers operating at 300 or 400 MHz for ^{1}H, and 75 or 100 MHz for ^{13}C NMR. The EIMS was measured using an E. I. Finnigan model 3200 (70 ev ionization potential) with INCOS data system. TLC analyses were carried out on precoated silica gel G_{254} 500 μm using $CHCl_3$-MeOH (95 : 5) as developing system. For silica gel 60, 40 μm was used. For HPLC, a Phenomenex semi-preparative 5 μ, Ultracarb C18 RP column (10 mm i.d. × 25 cm) was used at λ_{245}. An isocratic mixture of CH_3CN-H_2O (1 : 1) was used as an eluting system at a rate of 2 ml/min.

3.2. Plant material

Echinops hussoni was collected in January 1994, from Elba Mountain, Southeastern coast of Egypt. The plant was identified at the Faculty of Science, Mansoura University, Egypt. A voucher specimen has been deposited at the Herbarium of the Pharmacognosy Department, Faculty of Pharmacy, Mansoura, Egypt (94 Elba 10).

3.3. Extraction and isolation

The air-dried aerial parts of *E. hussoni* (1.1 kg) were blended with EtOH (90%, 3 l) and filtered. The extract was evaporated under reduced pressure. The residue (120 g) was dissolved in EtOH (1 l) and filtered. The filtrated was evaporated under reduced pressure. The dried EtOH extract (70 g) was partitioned between H_2O and petroleum ether (2 l, each). The petroleum ether-soluble residue (12.5 g) was column-chromatographed on silica gel 60 (200 g) using isocratic $CHCl_3$–MeOH (99 : 1) as eluent. Fractions were subjected to repeated flash column chromatography on silica gel and finally on C18-RP HPLC to afford **1** (270 mg, R_f 0.51).

3.4. Acetylation of **1**

To a solution of 30 mg of **1** in 1 ml Ac_2O, 0.4 ml of pyridine was added. The mixture was stirred for 24 h at room temperature. About 5 ml of brine solution was added to each reaction mixture and the solution was extracted with Et_2O (2 × 5 ml). The organic layers were washed with saturated $NaHCO_3$ solution and H_2O (2 × 5 ml), dried over anh. Na_2SO_4 and evaporated under reduced pressure. Preparative TLC on silica gel G_{254}, using the system: $CHCl_3$– MeOH (95 : 5) afforded **2** (22 mg, R_f 0.69).

3.5. Mild acid hydrolysis of **1**

A solution of 30 mg of **1** in 6 ml MeOH is treated with 1 ml 0.1 M H_2SO_4. The solution was stirred at 60° for 30 min and then diluted with 6 ml of H_2O. The whole solution was concentrated under reduced pressure to about 6 ml and extracted with Et_2O (2 × 5 ml). The combined ethereal extracts were washed with 5% $NaHCO_3$ solution and H_2O (2 × 5 ml), dried over anh. Na_2SO_4 and evaporated under reduced pressure. TLC analysis of the residue showed numerous spots.

3.6. TLC of the sugar moiety of **1**

The aqueous mother liquor from mild acid hydrolysis was neutralized with a saturated solution of $Ba(OH)_2$, filtered and evaporated under reduced pressure. The residue was dissolved in 0.4 ml pyridine and TLC analyzed along with authentic β-D-xylose on silica gel G_{254} using $CHCl_3$–MeOH–H_2O (18 : 3 : 1, lower layer).

Echusoside (**1**): Colorless needles from EtOH, mp 98–100°, +22.0 (*c* 0.1, $CHCl_3$); UV λ_{max} (log ε) (MeOH) 210 (1.85), 228 (1.65), 239 (1.85), 266 (1.39) nm; IR υ_{max} ($CHCl_3$) 3610–3220 (OH), 3050–2820, 1730 (C=O), 1643 (C=C), 1489, 1380, 1270, 1158, 880 cm^{-1}; ^{13}C and ^{1}H NMR, see Table; EI MS *m/z*: 454 $[M]^+$ calculated for $C_{25}H_{42}O_7$.

Echusoside triacetate (**2**): Colorless needles from MeOH, mp 121–122 °C, $[\alpha]_D^{20}$ +29 (*c* 0.05, $CHCl_3$); UV λ_{max} (log ε) (MeOH) 210 (1.88), 230 (1.89), 267 (1.40) nm; IR υ_{max} ($CHCl_3$) 3050–2820, 1730–1710 (C=O), 1640, 1480, 1240, 1158, 890 cm^{-1}; ^{13}C and ^{1}H NMR, see Table.

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