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A CYCLOHEXENONE AND A CYCLOHEXADIENONE GLYCOSIDE FROM *ERYNGIUM CAMPESTRE*

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Key Word Index—*Eryngium campestre*; Umbelliferae; monoterpene glycosides; 3-(β-D-glucopyranosyloxymethyl)-2,4,4-trimethyl-2,5-cyclohexadien-1-one; 3-(β-D-glucopyranosyloxymethyl)-2,4,4-trimethyl-2-cyclohexen-1-one; INKA C13 NMR data system.

Abstract—The structures of two new monoterpene glycosides of the cyclohexenone type, isolated from *Eryngium campestre*, have been elucidated on the basis of spectral data as 3-(β-D-glucopyranosyloxymethyl)-2,4,4-trimethyl-2,5-cyclohexadien-1-one and 3-(β-D-glucopyranosyloxymethyl)-2,4,4-trimethyl-2-cyclohexen-1-one.

INTRODUCTION

The genus *Eryngium*, belonging to the Umbelliferae subfamily Saniculoideae, is known to contain acetylenes [1], flavonoids [2] and triterpene saponins [3]. The presence of coumarins in some *Eryngium* species has also been reported [4, 5]. In this paper, we describe the isolation and structure elucidation of two new monoterpene glycosides of the cyclohexenone type. Their occurrence in *Eryngium campestre* is of interest since they are related to terpene aldehyde esters isolated from several *Eryngium* species by Bohlmann *et al.* [6] and Drake and Lam [1].

RESULTS AND DISCUSSION

The dried, ground *Eryngium campestre* roots were first extracted with chloroform to remove lipophilic components. The TLC chromatogram of the methanolic extract showed several UV active spots and some minor constituents with blue fluorescence at 366 nm. The extract was prefractionated with silica gel CC to obtain four fractions A–D. Craig distribution of fraction B and subsequent semipreparative HPLC (RP-18) afforded three compounds. One was identified by its spectral data and in comparison with an authentic sample as umbelliferone.

In the ^{13}C NMR spectra of **1** and **2** a singlet at δ187.1 and 201.9, respectively, indicated a keto function for both compounds. This was confirmed by strong IR absorptions at 1660 and 1655 cm^{-1}, respectively. The ^{1}H NMR spectrum of **1** showed two olefinic protons, which appeared as an AB-system (J_{AB} = 9.9 Hz), three methyl groups, two methylene protons and an anomeric proton. One of the methyl groups was concluded to be attached to an unsaturated carbon atom due to the downfield shift of the signal at δ1.98. The anomeric proton at δ4.35 (J = 7.7 Hz) suggested a β-D-glucoside. The chemical shifts of the remaining glucosidic protons were as expected. The ^{1}H NMR spectrum of the acetylated compound **1a** showed four aliphatic acetyl groups and clearly the shifts and splittings for an acetylated β-D-glucoside.

In the ^{13}C NMR spectra of **1** and its acetate **1a** a singlet in the δ40 region indicated a quaternary carbon atom (C-4). Two methyl groups of the aglycone moiety were found at δ25.4 and 25.5. This was consistent with a geminal dimethyl grouping at C-4 of the molecule. Two olefinic doublets at δ125.9 and at 157.2 were in agreement with the two olefinic protons found in the ^{1}H NMR spectrum. Two further olefinic carbons appeared as singlets, at δ136.2 and 152.7, most probably linked with the remaining methyl group (at C-2) and with the methylene group (at C-3). The signals of the glucose moiety were found as expected.

The ^{1}H NMR signals of compound **2** were similar to those of **1**, except the signals of H-5 and H-6. In the same way the C-5 and C-6 ^{13}C NMR signals of **2** differed from those of **1**. In the ^{1}H NMR spectrum the protons at C-5 and C-6 appeared as an A_2B_2-system with triplets at δ1.84 ($J_{5,6}$ = 6.8 Hz) and 2.48 ($J_{6,5}$ = 6.9 Hz). Analogously, the ^{13}C NMR spectrum of **2** showed two triplets for C-5 and C-6 at δ35.2 and 38.4. The EIMS of the tetraacetates **1a** and **2a** revealed the molecular ion peaks $[M]^{+\cdot}$ at m/z 496 and $[M+H]^{+}$ at m/z 499, respectively. Further fragments were observed at m/z 150 (aglycone moiety of **1**)

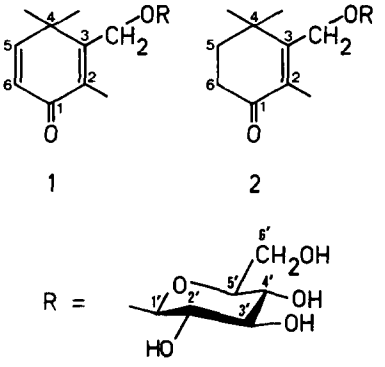

Table 1. ^{13}C NMR data of compounds **1**, **1a**, **2**, **2a** and estimated shifts for **1** and **2** by the INKA system

	1 (CD_3OD)	**1** (estimated)	**1a** ($CDCl_3$)	**2** (CD_3OD)	**2** (estimated)	**2a** ($CDCl_3$)
C-1	187.1	186.2	186.3	201.9	199.6	199.2
C-2	*	133.6	136.2	135.4	134.1	134.9
C-3	*	156.3	152.7	160.0	156.3	155.6
C-4	41.3	39.5	39.6	36.5	35.4	35.4
C-5	160.5	148.2	157.2	35.2	34.2	34.3
C-6	126.1	126.1	125.9	38.4	37.4	37.4
Me at C-4	25.5	27.8	25.4	26.8	26.6	26.5
Me at C-4	25.5	27.8	25.5	27.0	26.6	26.7
Me at C-2	11.6	11.6	11.2	11.9	11.4	11.4
CH_2 at C-3	66.3	69.3	64.9	66.5	69.3	65.4
C-1′	104.2	99.7	99.6	104.0	99.7	99.6
C-2′	75.0	72.3	71.2	75.0	72.3	71.3
C-3′	78.1	75.4	72.8	78.1	75.4	72.9
C-4′	71.7	70.4	68.5	71.7	70.4	68.6
C-5′	78.1	76.0	72.0	78.0	76.0	72.0
C-6′	62.9	61.8	62.0	62.8	61.8	61.1
OAc			20.5			20.7
			169.1			169.1
			169.4			169.4
			170.2			170.2
			170.5			170.5

*Signals not evident.

and at *m/z* 152 (aglycone moiety of **2**). For both compounds characteristic peaks of the tetraacetylglucose moiety were found at *m/z* 331, 169 and 109.

The structures of compounds **1** and **2** have been confirmed by estimation of the ^{13}C NMR chemical shifts expected on the basis of the postulated structures, with the help of the INKA C13 NMR data system [7]. A good agreement between measured and estimated values was found. The ^{13}C NMR assignments of **1** and **2** are given in Table 1 together with the estimated shifts. The D-configuration of the glucose moieties of **1** and **2** was deduced from the optical rotation of their tetraacetates compared to literature data [8].

The structures of **1** and **2** are thus established as 3-(β-D-glucopyranosyloxymethyl)-2,4,4-trimethyl-2,5-cyclohexadien-1-one (**1**) and 3-(β-D-glucopyranosyloxymethyl)-2,4,4-trimethyl-2-cyclohexen-1-one (**2**). Both are new naturally occurring glycosides.

EXPERIMENTAL

General procedures. EIMS were recorded at 70 eV. ^1H and ^{13}C NMR spectra [δ (ppm), *J* (Hz)] were obtained at 300.13 MHz using a Bruker WM 300 Spectrospin instrument and at 25.2 MHz using a Varian XL 100 spectrometer, with TMS as an internal standard. Silica gel 60 (70–230 mesh, Merck) was used for CC and silica gel 60 F_{254} (Merck) prepared plates for TLC. Semiprep. HPLC was carried out with a Waters M6000 pump and a U6K injector. The column used was a Knauer RP-18, 7 μm (25 cm × 16 mm i.d.). The system was equipped with a Zeiss spectrophotometer PMQ3 (254 nm) and a W + W recorder.

Extraction and isolation. Dried, ground roots of *Eryngium campestre* L. (obtained from Dixa AG, St. Gallen, Switzerland, Lot. No. 12825, 1.7 kg) were defatted with petrol and extracted with MeOH (1 × 4 l., 2 × 2 l.) to obtain 170 g of extract after evaporation. The MeOH extract (140 g) was prefractionated over silica gel CC ($CHCl_3$–MeOH, 8:2). Four fractions were collected (A–D). Fraction B (20.7 g) was subjected to Craig distribution ($CHCl_3$–MeOH–*n*-PrOH–H_2O, 5:6:1:4) and three fractions were obtained (I–III). Fraction II (1.8 g) was filtered through silica gel ($CHCl_3$–MeOH, 9:1) to give 212 mg of residue. Subsequent semiprep. HPLC (MeOH–H_2O, 25:75, flow rate 7.8 ml/min) yielded 23.8 mg **1** and 43.8 mg **2**.

Umbelliferone. The spectral data were identical with those of an authentic sample (from Fluka).

3-(β-D-Glucopyranosyloxymethyl)-2,4,4-trimethyl-2,5-cyclohexadien-1-one (**1**). IR ν_{max}^{KBr} cm^{-1}: 3390 (OH), 2960, 2920, 2870, 1660 (C=O), 1620 (C=C), 835. ^1H NMR (300.13 MHz, CD_3OD): δ 1.98 (3H, *s*, Me at C-2), 1.30/1.32 (2 × 3H, 2*s*, 2 × gem. Me at C-4), 6.96 (1 H, *d*, $J_{5,6}$ = 9.9 Hz, H-5), 6.17 (1H, *d*, $J_{6,5}$ = 9.9 Hz, H-6), 4.34/4.80 (2H, 2*d*, J_{gem} = 11.2 Hz, CH_2 at C-3), 4.35 (1H, *d*, $J_{1',2'}$ = 7.7 Hz, H-1′), 3.18 (1H, *dd*, $J_{2',1'} = J_{2',3'}$ = 7.9 Hz, H-2′), 3.28–3.37 (3H, H-3′, H-4′, H-5′), 3.71 (1H, *dd*, $J_{6',5'}$ = 6.0 Hz, $J_{6'a,6'b}$ = 10.4 Hz, H_a-6′), 3.92 (1H, *d*, $J_{6'b,6'a}$ = 10.4 Hz, H_b-6′). ^{13}C NMR: see Table 1.

Tetraacetate **1a**. $[\alpha]_D^{20}$ −20.8° ($CHCl_3$; *c* 0.481). EIMS (70 eV) *m/z*: $[M]^+$ 496 (0.4), 331 (15.9), 169 (49.8), 150 (43.6), 149 (28.1), 121 (17.3), 109 (30.3), 57 (27.3), 43 (100). ^1H NMR (300.13 MHz, $CDCl_3$): δ 1.93 (3H, *s*, Me at C-2), 1.24/1.25 (2 × 3H, 2*s*, 2 × gem. Me at C-4), 6.74 (1H, *d*, $J_{5,6}$ = 9.9 Hz, H-5), 6.21 (1H, *d*, $J_{6,5}$ = 9.9 Hz, H-6), 4.32/4.61 (2H, 2*d*, J_{gem} = 11.2 Hz, CH_2 at C-3), 4.35 (1H, *d*, $J_{1',2'}$ = 8.0 Hz, H-1′), 5.00 (1H, *dd*, $J_{2',1'} = J_{2',3'}$ = 8.0 Hz, H-2′), 5.21 (1H, *t*, $J_{3',2'} = J_{3',4'}$ = 9.4 Hz, H-3′), 5.09 (1H, *t*, $J_{4',3'} = J_{4',5'}$ = 9.6 Hz, H-4′), 3.71 (1H, *m*, H-5′), 4.23 (2H, *m*, H-6′), 1.99, 2.00, 2.03, 2.09 (12H, 4 × aliphatic OAc). ^{13}C NMR: see Table 1.

3-(β-D-Glucopyranosyloxymethyl)-2,4,4-trimethyl-2-cyclohexen-1-one (**2**). IR ν_{max}^{KBr} cm^{-1}: 3390 (OH), 2960, 2920, 2870, 1655 (C=O). ^1H NMR (300.13 MHz, CD_3OD): δ1.98 (3H, *s*, Me at C-2), 1.22/1.23 (2 × 3H, 2*s*, 2 × gem. Me at C-4), 1.84 (2H, *t*, $J_{5,6}$ = 6.8 Hz, H-5), 2.48 (2H, *t*, $J_{6,5}$ = 6.9 Hz, H-6), 4.25/4.67 (2H, 2*d*, J_{gem} = 10.9 Hz, CH_2 at C-3), 4.31 (1H, *d*, $J_{1',2'}$ = 7.8 Hz, H-1'), 3.18 (1H, *dd*, $J_{2',1'} = J_{2',3'}$ = 7.8 Hz, H-2'), 3.28–3.40 (3H, H-3', H-4', H-5'), 3.70 (1H, *dd*, $J_{6',5'}$ = 5.0 Hz, $J_{6'a,6'b}$ = 10.4 Hz, H_a-6'), 3.90 (1H, *d*, $J_{6b',6a'}$ = 10.4 Hz, H_b-6'). ^{13}C NMR: see Table 1.

Tetraacetate **2a**. $[\alpha]_D^{20}$ −21.4° ($CHCl_3$; *c* 0.499). EIMS (70 eV) *m/z*: $[M+H]^+$ 499 (0.4), 331 (18.5), 169 (56.8), 152 (34.6), 151 (10.1), 123 (11.8), 109 (33.0), 81 (12.6), 43 (100). ^1H NMR (300.13 MHz, $CDCl_3$): δ1.80 (3H, *s*, Me at C-2), 1.15/1.16 (2 × 3H, 2*s*, 2 × gem. Me at C-4), 1.82 (2H, *t*, $J_{5,6}$ = 6.2 Hz, H-5), 2.49 (2H, *t*, $J_{6,5}$ = 6.9 Hz, H-6), 4.23/4.53 (2H, 2d, J_{gem} = 10.9 Hz, CH_2 at C-3), 4.54 (1H, *d*, $J_{1',2'}$ = 7.9 Hz, H-1'), 5.00 (1H, *dd*, $J_{2',1'} = J_{2',3'}$ = 8.0 Hz, H-2'), 5.21 (1H, *t*, $J_{3',2'} = J_{3',4'}$ = 9.4 Hz, H-3'), 5.09 (1H, *t*, $J_{4',3'} = J_{4',5'}$ = 9.4 Hz, H-4'), 3.70 (1H, *m*, H-5'), 4.22 (2H, *m*, H-6'), 1.99, 2.00, 2.03, 2.08, (12H, 4 × aliphatic OAc). ^{13}C NMR: see Table 1.

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GERMACRANOLIDES FROM *CHAENACTIS DOUGLASII*

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Key Word Index—*Chaenactis douglassi*; Compositae; new germacranolide; ^{13}C NMR investigation.

Abstract—The investigation of *Chaenactis douglasii* yielded one known germacranolide and a new oxidized germacranolide.

To date, *Chaenactis douglasii* has yielded only one reported germacranolide, eupatoriopicrin [1]. I wish to report the isolation of other germacranolides from *Chaenactis*. The ethyl acetate extract from the dried aerial parts of *Chaenactis douglasii* (Hook.) H. et A. has yielded two major germacranolides. These compounds were purified by column chromatography followed by HPLC.

The ^1H NMR and ^{13}C NMR spectra of the more polar component were identical with the known germacranolide eupaformosanin (**1**), which has been isolated from *Eupatorium formosanum* [2].

The less polar component (**2**) had a molecular formula of $C_{20}H_{24}O_7$ (HRMS). The IR spectrum of **2** indicated a large OH stretch and three C=O at 1760 (lactone), 1720 (ester) and 1680 cm^{-1} (unsaturated acid). The IR spectrum along with the ^1H NMR resonance at δ5.95 (*dd*) indicated the C-14 methyl had been oxidized to a carboxylic acid. The chemical shift of this proton indicated a *Z* alkene. This stereochemistry is in contrast to other oxidized germacranolides with the *E* configuration around this double bond [3]. The chemical shift of the protons with this configuration are at much lower field (δ7.0–7.2). Resonances at 1.87 (*br s*), and 6.85 (*br t*) and 1.80 (*d*) indicated that this germacranolide was esterified with a tiglic acid moiety [3]. The positions and relative stereochemistry of the hydroxyl and ester groups on the ring were established by analysis of the ^1H NMR spectrum of **2**, the acetate (**3**) (Table 1) and NOE experiments done on the acetate. The two broad singlets at δ4.48 (H-9) and 6.02 (H-8) in the parent compound appeared as a doublet at δ5.45 (H-9, J = 9.6 Hz) and a broad doublet at δ6.67 (H-8, J = 9.6 Hz) in the acetate. The H-8 proton