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SESQUITERPENE LACTONE GLYCOSIDES FROM *CREPIS CAPILLARIS*

WANDA KISIEL

Institute of Pharmacology, Polish Academy of Sciences, Department of Phytochemistry, 31-343 Kraków, Poland

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Key Word Index—*Crepis capillaris*; Compositae; sesquiterpene lactone glycosides; guaianolides.

Abstract—Four new sesquiterpene lactone glucosides, in addition to known compounds, have been isolated from the roots of *Crepis capillaris* and their structures elucidated on the basis of spectral analysis and hydrolytic studies.

INTRODUCTION

In previous studies [1–3] on the sesquiterpene lactones from the aerial parts of *Crepis capillaris* the isolation of several guaianolides has been reported. In continuation of these studies, the roots of the plant were examined. This has resulted in the isolation of closely related sesquiterpene lactone glycosides **2**, **3**, **4** and **8a**, in addition to guaianolides **1** and **8**, previously found in the aerial parts of the plant [2, 3].

RESULTS AND DISCUSSION

The most polar compound **2** ($C_{21}H_{28}O_9$) was an amorphous powder. Its IR spectrum showed peaks for hydroxyl groups, a γ-lactone and double bonds. The ^{1}H NMR spectrum of **2** was quite similar to that of **1** except for the additional presence of signals characteristic for the sugar moiety. A glycosidic linkage was confirmed by a positive Molisch test and hydrolysis with β-glucosidase which permitted the identification of glucose by paper chromatography. The aglycone was identified as **1** by direct comparison with an authentic sample of 8-epidesacylcynaropicrin, previously isolated from the aerial parts of the same species [3]. Acetylation of **2** gave a pentaacetate **2a**. Its mass spectrum revealed peaks characteristic for tetraacetylglucose fragmentation. The presence of β-glucoside followed from the large coupling constant (7 Hz) of the anomeric proton doublet. The position of the sugar moiety will be discussed subsequently together with that of glycosides **3** and **4** which were isolated from less polar fractions of the chromatographic column.

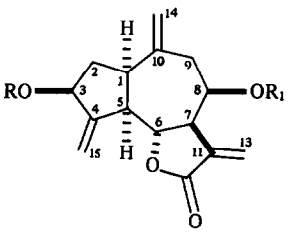
Compounds **3** and **4** could not be induced to crystallize and were relatively unstable at room temperature. The correlation of their spectral data with those of **2** suggested that **3** and **4** might be esters of **2**. That the sesquiterpene portions of the three compounds were identical was confirmed chemically by hydrolysis of **3** and **4** with β-glucosidase to give **5** ($C_{20}H_{24}O_7$) and **6** ($C_{21}H_{26}O_7$), respectively, and glucose. Subsequent hydrolysis of **5** and **6** with potassium carbonate in aqueous methanol afforded **7**. The compound was also prepared from **2** in the same manner [3].

The gross structures of the ester side chains in **3** and **4** were established by a combination of mass and ^{1}H NMR spectrometry of **5** and **6**. The compounds exhibited ^{1}H NMR spectral parameters nearly identical with those of **1** for the medium ring portion except that the H-8 signal was shifted downfield. In addition **5** showed two sharp three-proton singlets at δ2.19 and 1.49. These signals together with mass spectral data which showed elimination of water and the acid $C_5H_8O_4$, suggested the presence of an ester side chain of type A in **5**. Peaks at *m/z* 43 (100%) and 87 were attributed to the ions probably formed by fragmentation between C-2 and C-3, and C-1 and C-2, respectively, in A. A reasonably strong peak at *m/z* 69 was formed, probably by loss of water and 1,2 cleavage of the ester portion.

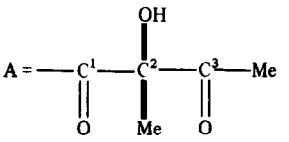
Ester side chain A has been found previously in the sesquiterpene lactone polydalin (C-2 Me: δ1.5, C-3 Me: δ2.19) [4, 5]. The molecular formula of **5** contained 14 mass units less than that of **6**. The ^{1}H NMR spectrum of **6** differed from that of **5** by the absence of the methyl singlet at δ1.49 which was replaced by a methyl triplet at 0.81 and a two-proton quartet at 1.85. These data were corroborated by diagnostic mass spectral peaks. Besides a weak M^+ at *m/z* 390, peaks at *m/z* 372 and 244 indicated the loss of water and the acid ($C_6H_{10}O_4$), respectively. Further peaks at *m/z* 57 and 43 (100%), indicating the presence of side chain B in **6**, were probably formed by fragmentation of B involving cleavage between C-1 and C-2, and C-2 and C-3. This type of ester group seems to be new.

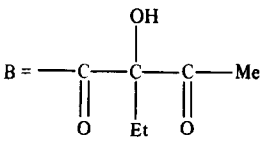
Acetylation of **3** and **5** afforded two pentaacetates **3a** and **3b**, and two diacetates **5a** and **5b**, respectively. The ^{1}H NMR spectra of the two pairs of acetates differed only in the chemical shifts of the side chain methyls (i.e. δ1.5 and 2.18 for the more polar compounds **3b** and **5b**, δ1.64 and 2.27 for the less polar compounds **3a** and **5a**) so they could be stereoisomeric in the ester side chains. Acetylation products of **4** and **6** were inseparable mixtures.

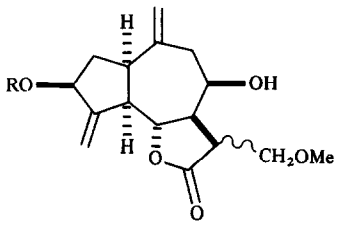
The remaining problem was the position of attachment of the glucose moiety in **2**, **3** and **4** since the ^{1}H NMR spectra of the glycosides were difficult to interpret. Conversion of **2**, **3** and **4** into the common derivative **7a** indicated that the glucose moiety was attached at C-3 in the three compounds. This was also confirmed by ^{1}H NMR studies of **6** involving Eu[fod]$_3$ shift reagent (mole ratio 0.3). The largest shift observed (Δ = 0.45 ppm) for the C-4 methylene protons as compared to the shifts of the C-11 (Δ = 0.17 ppm) and C-10 (Δ = 0.18 and

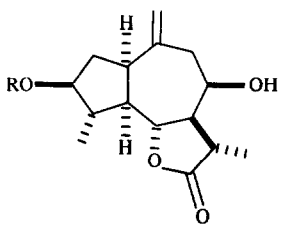


1 R = R_1 = H
2 R = Glc, R_1 = H
2a R = Glc $(Ac)_4$, R_1 = Ac
3 R = Glc, R_1 = A
3a, **3b** R = Glc $(Ac)_4$, R_1 = A—Ac
4 R = Glc, R_1 = B
5 R = H, R_1 = A
5a, **5b** R = Ac, R_1 = A—Ac
6 R = H, R_1 = B









7 R = H
7a R = Glc

8 R = H
8a R = Glc

0.26 ppm) methylene protons was compatible with the hydroxyl group at C-3.

Thus the three glucosides must be represented by formulae **2**, **3** and **4**, respectively.

Finally, the polar fractions contained, in addition to the main constituent **2**, the fourth sesquiterpene lactone glycoside which showed a chromatographic behaviour very similar to that of **2** and could only be separated with difficulty. Hydrolysis of the compound with β-glucosidase afforded lactone **8** (TLC, IR, mmp) and glucose. Though the small quantity of the material did not allow further investigation, the proposed structure **8a** for the compound seemed to be likely as all the other glycosides isolated from the plant had the glucose moieties at C-3.

Compounds **2**, **3** and **4** were also detected in the ethyl acetate–methanol (95:5) extract [2] of the aerial parts of the plant.

EXPERIMENTAL

Mps were uncorr. The ^{1}H NMR spectral assignments were confirmed by double irradiation or INDOR experiments. Known compounds were identified by comparison of their spectral data, TLC and mmp with those of authentic material. Silica gel 60 (70–230 mesh, Merck) was used for CC. A voucher specimen was deposited in the herbarium of the Department.

Extraction and isolation. The air-dried roots (421 g) of *C capillaris* collected in August, 1980, in the vicinity of Kraków, were exhaustively extracted with EtOH. After filtration the combined extracts were evaporated and the dark brown gum (68 g) was chromatographed on a silica gel (500 g) column packed in C_6H_6 and eluted with solvents of increasing polarity The following fractions (0.2 l. each) were collected and monitored by TLC: fr 1–37 (C_6H_6), fr 38–72 (C_6H_6–EtOAc, 9:1), fr. 98–121 (C_6H_6–EtOAc, 8.2), fr. 122–149 (C_6H_6–EtOAc, 1:1), fr 150–160 ($CHCl_3$), fr. 161–164 ($CHCl_3$–MeOH, 95:5), fr. 165–207 ($CHCl_3$–MeOH, 9:1). Fractions 1–121 afforded terpenoid and steroid compounds and contained no sesquiterpene lactones Fraction 139 afforded **8** (3 mg) and **1** (20 mg) identical with the material isolated from the aerial parts of the plant [2, 3] Fractions 172–173 (0 27 g), 174–177 (0.5 g) and 178–182 (0 35 g) contained **4**, mixtures of **3** and **4**, which showed two major spots on TLC, and **3**, respectively The residual gum from fractions 172–173 on further purification on a silica gel column eluted with

$CHCl_3$–MeOH (95:5) yielded **4** as brittle gum; IR ν_{max}^{KBr} cm^{-1}: 3450 (OH), 1770 (γ-lactone), 1740 (CO_2R), 1670, 1650, 940 (C=C). Similar purification of evaporated fractions 178–183 afforded **3** as brittle gum; IR ν_{max}^{KBr} cm^{-1}: 3450 (OH), 1770 (γ-lactone), 1740 (CO_2R), 1670, 1655, 1645, 935 (C=C). Fractions 192–203 (1.1 g) contained main constituent **2** contaminated with **8a**. Repeated CC on silica gel resulted in the separation of **2** (700 mg) and almost pure **8a** (8 mg) from the mixture. Compound **2**: amorphous powder; IR ν_{max}^{KBr} cm^{-1}: 3400 (OH), 1745 (γ-lactone), 1660, 1645, 940, 920 (C=C); MS *m/z* (rel. int.): 424 $[M]^+$ (0.97), 406 $[M-H_2O]^+$ (0.97), 388 $[M-2\times H_2O]^+$ (1), 262 $[C_{15}H_{18}O_4]^+$ (98), 244 $[262-H_2O]^+$ (32), 234 $[262-CO]^+$ (10), 226 $[262-2\times H_2O]^+$ (3), 166 (49), 136 (51), 60 (100).

Acetylation of **2** *and* **3**. The acetate was prepared from 50 mg of **2** and Ac_2O (1 ml) in pyridine (2 ml), under standard conditions. The product was crystallized from Et_2O to give **2a**, colourless needles, mp 167–170°; IR ν_{max}^{KBr} cm^{-1}: 1760, 1240 (γ-lactone, OAc), 1650, 960, 925 (C=C); MS *m/z* (rel. int.): 592 $[M-C_2H_2O]^+$ (0.02), 574 $[M-AcOH]^+$ (0.28), 514 $[M-2\times AcOH]^+$ (0.71), 454 $[M-3\times AcOH]^+$ (0.46), 394 $[M-4\times AcOH]^+$ (0.41), 331 [tetraacetylglucose]$^+$ (67), 289 (9), 245 $[C_{15}H_{17}O_3]^+$ (11), 229 (12), 227 $[C_{15}H_{15}O_2]^+$ (11), 169 (100), 109 (18). Acetylation of **3** (100 mg) was carried out similarly to that of **2**. The residue, which showed two spots on TLC, was subjected to CC eluted with C_6H_6–Me_2CO (9:1). The compound **3a** of higher R_f was an amorphous powder, IR ν_{max}^{KBr} cm^{-1}: 1760 (C=O), 1650, 940, 925 (C=C); MS *m/z* (rel. int.): 592 $[M-RCO+H]^+$ (0.44), 586 $[M-2\times AcOH-C_2H_2O]^+$ (0.4), 574 $[M-RCO_2H]^+$ (0.08), 331 [tetraacetylglucose]$^+$ (100), 289 (2), 245 $[C_{15}H_{17}O_3]^+$ (8), 227 $[C_{15}H_{15}O_2]^+$ (17), 169 (91), 157 $[RCO]^+$ (5), 115 $[RCO-C_2H_2O]^+$ (2), 109 (7), 97 $[RCO–AcOH]^+$ (1); ^1H NMR of **3a** is listed in Table 1. The non-crystalline compound **3b** of lower R_f revealed IR and MS data nearly identical with those presented above. Its ^1H NMR spectrum differed in the position of the ester side chain methyls (δ2 18 and 1 50).

Enzymatic hydrolysis of **2**, **3**, **4** *and* **8a**. To the aq. acetate buffer solution (pH 5) of **2** (150 mg) β-glucosidase was added, and the mixture kept at 37° for 2 days. Aglycone was extracted with EtOAc, mp 219–221°, and identified with an authentic sample of **1**. The aq. layer was spotted on the Whatman paper No 1 together with a number of representative hexoses and developed with different solvent systems. The spot of the hydrolysis mixture corresponded to that of glucose.

Compounds **3**, **4** and **8a** were hydrolysed in the manner described for **2**.

Hydrolysis of **3** afforded glucose and **5**, brittle gum, MS

Table 1. ^1H NMR spectral data* of compounds **2a**, **3a**, **5**, **5b** and **6**

H	2a	3a	5	5b	6
1	1.70–2.90 *m*	1.70–2.90 *m*	2.90 *m*	2.90 *m*	2.90 *m*
2a			2.20 *m*	2.40 *m*	2.20 *m*
2b			1.78 *ddd* (14, 7, 7)	1.80 *m*	1.80 *m*
3	4.55 *br t*	4 54 *t* (7.5)	4.57 *br t* (7 3)	5.45–5.60 *m*	4.58 *br t* (8)
5	1.70–2.90 *m*	1.70–2 90 *m*	2.90 *m*	2 90 *m*	2 90 *m*
6	4.47 *t* (9)	4.40 *t* (9)	4.49 *t* (9)	4.43 *t* (9)	4.52 *t* (9)
7	3.08 *m*	3.10 *m*	3.17 *m*	3.17 *m*	3.18 *m*
8	5.50 *m*	5 50 *m*	5.59 *m*	5.45–5.60 *m*	5.59 *m*
9a	1.70–2.90 *m*	1.70–2.90 *m*	2.90 *m*	2.90 *m*	2.90 *m*
9b			2.57 *m*	2.57 *m*	2.57 *m*
13a	6 27 *d* (3.5)	6.30 *d* (3.5)	6.27 *d* (3.5)	6 34 *d* (3.14)	6.27 *d* (3.5)
13b	5 50 *m*	5.50 *m*	5 53 *d* (3)	5 57 *d* (3)	5.53 *d* (3)
14a	4 80–5.30 *m*	5.10 *br s*	5.14 *s*	5.10 *s*	5.14 *s*
14b		5.02 *br s*	4.96 *s*	5.02 *s*	4 94 *s*
15a	5.50 *m*	5.50 *m*	5.45 *br s*	5.45–5.60 *m*	5.46 *br s*
15b	4.80–5.30 *m*	4.80–5.30 *m*	5.35 *br t*	5 32 *t* (2)	5.35 *br t*
1′†	4.73 *d* (7)	4.77 *d* (7)	—	—	—
2′	4.80–5 30 *m*	4.80–5 30 *m*	—	—	—
3′					
4′					
5′	3.75 *m*	3.70 *m*	—	—	—
6′	4.21 *m*	4.20 *m*	—	—	—
OCOR	—	2.27 *s*	2.19 *s*	2.18 *s*	2.19 *s*
	—	1 64 *s*	1.49 *s*	1.50 *s*	0.81 *t* (7)
					1.85 *q* (7)
OAc	2.02 *s*‡	1.98 *s*	—	2 10 *s*‡	—
	2.06 *s*‡	2.02 *s*‡	—		—
	2.10 *s*	2.06 *s*	—		—
		2.10 *s*	—		—

*Run at 80 MHz in $CDCl_3$ with TMS as internal standard, values in ppm (δ), coupling constants (Hz) in parentheses

†Glucosyl protons

‡Intensity six protons.

m/z (rel. int.): 376 $[M]^+$ (0.28), 358 $[M-H_2O]^+$ (0.11), 348 $[M-CO]^+$ (0.04), 334 $[M-C_2H_2O]^+$ (0.81), 316 $[M-H_2O-C_2H_2O]^+$ (1), 244 $[M-RCO_2H]^+$ (2), 227 $[M-H_2O-RCO_2]^+$ (5), 115 $[RCO]^+$ (4), 97 $[RCO-H_2O]^+$ (3), 87 $[R]^+$ (9), 69 $[87-H_2O]^+$ (6), 59 (9), 43 $[C_2H_3O]^+$ (100).

Hydrolysis of **4** gave glucose and **6**, brittle gum; MS *m/z* (rel. int.): 390 $[M]^+$ (0.46), 372 $[M-H_2O]^+$ (0.17), 362 $[M-CO]^+$ (0.01), 348 $[M-C_2H_2O]^+$ (1), 330 $[M-H_2O-C_2H_2O]^+$ (1), 244 $[M-RCO_2H]^+$ (2), 227 $[M-H_2O-RCO_2]^+$ (5), 129 $[RCO]^+$ (4), 101 $[R]^+$ (4), 57 $[C_2H_5CO]^+$ (14), 43 $[C_2H_3O]^+$ (100).

The hydrolysis mixture of **8a** afforded glucose as the sugar and an aglycone of mp 168–169° (MeOH) which was identified with an authentic sample of **8**.

Acetylation of **5**. Compound **5** on acetylation with Ac_2O–pyridine after the usual work-up, gave a mixture of two compounds (TLC) which were separated on a column eluted with C_6H_6–Me_2CO (9:1). ^1H NMR spectra of the two noncrystalline acetates differed in the positions of the ester side chain methyl singlets: δ1.62 and 2.26 for the less polar compound **5a** and δ2.19 and 1.50 for the more polar compound **5b** (Table 1).

Alkaline hydrolysis of **2**, **3**, **4**, **5** *and* **6**. A soln of 100 mg of **3** in 10 ml of MeOH and 500 mg of K_2CO_3 in H_2O (2 ml) was stirred until TLC indicated that all of **3** had reacted. The mixture was diluted with H_2O and extracted with EtOAc. The washed and dried extract was evaporated to give **7a**, amorphous solid, IR ν_{max}^{KBr} cm^{-1}: 3450 (OH), 1760 (γ-lactone), 1655, 1645, 930 (C=C); MS *m/z* (rel. int.): 426 $[M-CH_3O]^+$ (6), 414 $[M-C_2H_2O]^+$ (11), 411 $[M-MeOCH_2]^+$ (6), 396 $[M-C_2H_2O-H_2O]^+$ (12), 294 $[M-\text{glucose}]^+$ (12), 277 $[294-OH]^+$ (11), 177 (100), 105 (96), 45 $[MeOCH_2]^+$ (12). The compound was identical with the material prepared from **2** and **4** in the same manner.

Hydrolysis of 45 mg of **5** and **6** was carried out similarly with identical results, in each case **7** was obtained in crystalline form, mp 186–188°, and identified by direct comparison with the compound prepared from **1** [3]

REFERENCES

1. Barbetti, P., Casinovi, C. G., Santurbano, B. and Longo, R. (1979) *Collect. Czech. Chem. Commun.* **44**, 3123.
2. Kisiel, W. (1983) *Polish J. Chem.* (in press).
3. Kisiel, W. (1983) *Planta Med.* **49**, 246.
4. Herz, W. and Bhat, S. V. (1970) *J Org. Chem.* **35**, 2605
5. Harz, W. and Bhat, S. V. (1973) *Phytochemistry* **12**, 1737.