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COUMARIN SULPHATES OF *SESELI LIBANOTIS*

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Key Word Index—*Seseli libanotis* subsp. *eu-libanotis*; Umbelliferae; dihydrofurocoumarins; dihydropyranocoumarins; coumarin sulphates; sulphate ester salts; FDMS; ^{13}C NMR.

Abstract—Three new sulphate ester salts derived from known coumarin alcohols—one of them tertiary—have been obtained from roots of *Seseli libanotis* subsp. *eu-libanotis*. Their structures were established as (2′*S*)-rutaretin-1″-sulphate, (3′*R*)-lomatin-3′-sulphate and (3′*R*,4′*R*)-khellactone-3′-sulphate. They were together with their parent alcohols characterized by ^{13}C NMR spectroscopy. It is the first report on coumarin sulphates in plants.

INTRODUCTION

Seseli libanotis (L.) Koch is a perennial herb, which in its typical form, subspecies *eu-libanotis* Thellung, is widespread in Europe. It has earlier been shown to be a rich source of coumarins [1, 2] in particular diesters of (+)-*cis*-khellactone, well known for their properties as coronary vasodilators [3, 4].

In continuation of our earlier studies on the chemistry of Umbelliferae, and in a search for new biologically active coumarins, we have initiated a study of polar coumarins of this plant. In the present communication we describe the isolation and strùcture elucidation of three coumarin sulphate ester salts **1**–**3** from a water-soluble extract of the roots. The occurrence of sulphated coumarins in plants has not been described before.

RESULTS AND DISCUSSION

The coumarin sulphates, **1**–**3**, obtained in this work were found to be strongly retained by chromatography of the crude water-soluble root extract on polyamide, using a water–methanol gradient, but were quickly eluted upon addition of a small percentage of ammonium carbonate to the eluent. Thus, they were easily separated from most other polar constituents, including glycosides. Primarily chromatography on Sephadex LH 20 was utilized for further separation and purification. The major coumarin sulphate, **1**, crystallized readily in the form of its potassium salt, whereas the salts of **2** and **3** were obtained only as amorphous powders. Upon hydrolysis with aqueous hydrochloric acid they all, in addition to sulphate ion, afforded known coumarin alcohols. Thus (−)-rutaretin, **1a** [5, 6], (+)-lomatin, **3a** [7, 8], and a mixture of epimeric alcohols, (+)-*cis*-khellactone, **2a** and (−)-*trans*-khellactone, **2b** [9, 10], were obtained from **1**, **3** and **2** respectively. Epimerization at the benzylic carbon of **2**, during acid hydrolysis was not unexpected, as epimerization is known to occur also during alkaline hydrolysis and acid methanolysis of khellactone carboxylic esters [9]. It was found, however, that mild solvolysis of the acid form of the sulphate, **2**, in wet ethyl acetate as commonly used for cleavage of steroid sulphates [11], proceeded quickly and without epimerization, affording only (+)-*cis*-khellactone, **2a**. The presence of a single sulphate group in **1** and in **2** followed from their sulphur content, which was analysed with some difficulty due to their hygroscopicity and their propensity to retain solvents. Additionally, monosulphation in **1** and **2** was inferred from the similarity of the electrophoretic mobilities of **1**, **2** and **3** [12].

The possibility that cyclization reactions might have taken place during hydrolysis of the sulphates could be rejected, as comparisons of ^{1}H NMR as well as ^{13}C NMR spectra (Table 1) of **1**–**3** with those of their respective hydrolysis products **1a**–**3a**, showed subtle differences, fully explicable in terms of their structural relationships as sulphate esters and parent alcohols. Thus, **3** must be (3′*R*)-lomatin-3′-sulphate. Furthermore a singlet at δ9.6 in the ^{1}H NMR spectrum of **1** confirmed the presence in this compound of a free phenolic group, as had been suggested already by shifts induced in its UV spectrum by addition of sodium acetate. As this pointed to the tertiary oxygen function as the site of sulphation, **1** must be (2′*S*)-rutaretin-1″-sulphate. Similarly, 3′-*O*-sulphation in **2** was evident from the coupling observed in its ^{1}H NMR spectrum between H-4′ and the hydroxylic proton. Accordingly **2** must be (3′*R*,4′*R*)-khellactone-3′-sulphate.

The sulphation shifts deduced from the spectral data were 0.6 ppm downfield for the α-hydrogens in **2a** and **3a**, in similarity to data reported earlier for carbohydrate and steroid sulphates [13, 14], and 0.2–0.4 ppm downfield for β-hydrogens in **1a**–**3a**. Consistently, the sulphation shifts deduced for the α-carbons of **1a**–**3a** were downfield and those of the β-carbons slightly upfield. This observation and the magnitudes of the α-shifts (+5 ppm) deduced for **2a** and **3a** are concordant with data earlier reported for sulphates of secondary (and primary) alcohols [13]. A more dramatic sulphation shift (+9 ppm) was observed for the α-carbon of the tertiary alcohol, **1a**, which may possibly be seen as a parallel to the large α-shifts (+10 ppm) generally observed upon acetylation of tertiary alcohols.

Although some sulphate ester salts have been described

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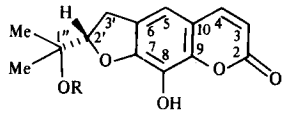
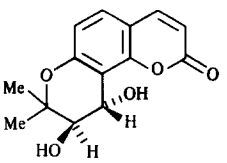
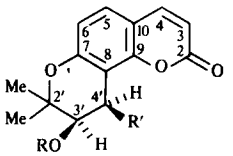


R
1 SO_3K
1a H



2b



2 R = SO_3K, R' = OH
2a R = H, R' = OH
3 R = SO_3K, R' = H
3a R = H, R' = H

Table 1. ^{13}C NMR data [δ-values (ppm)] of coumarin sulphates, 1–3, and parent alcohols, 1a–3a (22.7 MHz, DMSO-d_6)

	1	1a	2	2a	3*	3a
C-2	160.3	160.2	159.9	160.2	160.4	160.3
C-3	110.9	110.8	111.8†	111.7†	111.8†	111.5
C-4	145.1	144.9	144.5	144.5	144.8	147.7
C-5	114.0	113.9	128.9	128.8	126.9	126.8
C-6	125.2	125.2	113.8	113.8	113.7	113.5
C-7	151.2	151.3	155.4	155.7	156.2	156.1
C-8	128.1	128.0	111.2†	111.2†	107.5	107.9
C-9	143.8	143.6	153.8	153.9	153.2	152.9
C-10	113.0	112.7	111.8†	111.7†	111.7†	111.5
C-2'	89.4	90.5	77.6	78.8	77.3	78.2
C-3'	29.8	29.4	76.5‡	71.1	71.3‡	66.6
C-4'	—	—	58.8	60.1	23.5	25.1
C-1''	79.1‡	69.9	—	—	—	—
Me	22.9	25.9	27.0	26.7	24.5	25.1
Me	21.4	24.5	21.7	21.1	22.9	21.1

*Recorded at 67.9 MHz.
†Assignments, which may be interchanged.
‡Site of sulphate group.

to afford cluster ions upon FDMS [15], we were not successful in this respect. In contrast, the acid forms of 1–3 afforded FDMS spectra which were structurally informative, showing either $[M]^+$, $[M+1]^+$ or $[M-18]^+$ ions, although formation of the latter type of ion from 1 may be difficult to explain.

The occurrence of sulphate ester salts in higher plants, for a long time largely overlooked, has received much attention lately. Especially, a large number of flavonoids and other types of phenolics are now known to occur in higher plants in sulphated form [12], most often with sulphate linked to phenol groups but occasionally with sulphated sugars. Recent examples of other classes of natural sulphates are the alkaloid, corynoline-11-sulphate [16], and the cyanogenic glycoside, cardiospermin-5-sulphate [17], both containing sulphated alcohol groups.

It appears that sulphate esters often occur in plants growing under saline conditions and it has been suggested [12] that flavonoid sulphates have a role to play in salt uptake and metabolism. Actually, *Seseli libanotis* subsp. *eu-libanotis* in Denmark, where plant material for this study was collected, is confined to sandy seashores.

The sulphate ester, 2, derived from (+)-*cis*-khellactone, 2a, was one of the substances tested as part of a recent study on coronary vasodilatory, spasmolytic and cAMP-phosphodiesterase inhibitory properties of dihydropyranocoumarins and dihydrofuranocoumarins [4]. Compound 2 did not show significant activity in any of these tests in contrast to carboxylic esters of (+)-*cis*-khellactone, 2a, of which some showed activities comparable to those of papaverine.

EXPERIMENTAL

All mps are corr. ^{13}C NMR data were obtained from proton noise decoupled spectra and assigned by consideration of data published for related coumarins [18, 19] and additivity rules [20]. MS spectra were obtained by means of a combined FD/FI/EI ion source using a benzonitrile activated tungsten emitter. Detection of SO_4^{2-} was by precipitation with $BaCl_2$ and by a $BaCl_2$–$KMnO_4$ spray test [21]. The R_f values relate to silica gel plates using EtOAc–MeCOEt–HCO_2H–H_2O (5:3:1:1) as solvent and detection by fluorescence under long wave UV. The reported electrophoretic mobilities are relative to 4-methylumbelliferone sulphate potassium salt on paper in: (a) HOAc–HCO_2H–H_2O (3:1:16, pH 1.6) at 87 V/cm, and (b) aq. ammonium carbonate (0.1%) at 90 V/cm. Polyamide-6 (Macherey, Nagel & Co.) for CC was washed according to ref. [22] prior to use.

Plant material. Seseli libanotis subsp. *eu-libanotis* Thellung was collected at the seashore of Reerso, Denmark.

Extraction and isolation. Dried and ground roots (1600 g) were defatted with petrol and extracted with MeOH. An aq. soln of the MeOH concentrate was washed with EtOAc and evaporated. The residue (105 g) was chromatographed on polyamide with a H_2O–MeOH gradient affording after a first fraction composed mainly by sugars, further fractions containing glycosides (still under study). Blue and yellow fluorescent bands retained on the column were eluted with 0.1% ammonium carbonate in 60% MeOH affording 0.5 g of crude sulphate esters. Further separation and purification was by CC on polyamide with 0.1% ammonium carbonate in 10% MeOH and on Sephadex LH 20 with water as eluents. Complete conversion to potassium salts was ensured by passage through Dowex 50 × 4 (K^+) prior to final purification on Sephadex and characterization. Yields: 1, 150 mg; 2, 110 mg; 3, 24 mg. Just prior to FDMS analysis the salts were converted to acid forms by treatment of MeOH solns with Lewatit SP 1080 (H^+) (Merck).

2'(S)-Rutaretin-1''-(hydrogen sulphate), potassium salt, 1. Mp 128–130°; $[\alpha]_D^{23}$ −46.2, $[\alpha]_{436}^{23}$ −164 (MeOH; *c* 0.4); (Found: S, 8.3%. $C_{14}H_{13}O_8SK$ requires: S, 8.4%); FDMS of acid form *m/z*: 324 $[M-18]^+$. R_f 0.48 (yellow fluorescent spot); electrophoretic mobility: (a) 0.76, (b) 1.16; UV λ_{max}^{MeOH} nm (log ε): 230 sh (4.07), 257 sh (3.59), 266 (3.66), 335 (4.14), shift to 226 (4.3), 284 (4.0), 340 (4.1) upon addition of NaOAc; IR ν_{max}^{KBr} cm^{-1}: 1250 strong and broad (S=O); ^1H NMR (90 MHz, DMSO-d_6): δ9.64 (1H, *s*, phenolic H), 7.90 (1H, *d*, *J* = 9.5 Hz, H-4), 7.01 (1H, *s*, H-5), 6.19 (1H, *d*, *J* = 9.5 Hz, H-3), 4.93 (1H, *t*, *J* = 8 Hz, H-2'), 3.23 (2H, *d*, *J* = 8 Hz, H-3'), 1.49 (3H, *s*, Me), 1.34 (3H, *s*, Me).

Acid hydrolysis of 1. Hydrolysis with 0.05 N HCl at 95° was complete after 1.5 hr. Extraction with EtOAc afforded (*S*)-rutaretin, 1a, mp 192–193°; $[\alpha]_D^{23}$ −30.5 (EtOH; *c* 0.3); ^1H NMR (90 MHz, DMSO-d_6) partial: δ4.68 (1H, *t*, *J* = 8 Hz, H-2'), 1.16 (6H, *s*, gem-dimethyls). Compound 1a was identified by comparison with a sample obtained by acid hydrolysis of authentic rutarin. In the concd aq. washings, SO_4^{2-} was detected.

3'(R)4'(R)-Khellactone-3'-(hydrogen sulphate), potassium salt, 2. Non-crystalline, $[\alpha]_D^{23}$ −51, $[\alpha]_{436}^{23}$ −88 (MeOH; *c* 0.3);

(Found: S, 7.8%. $C_{14}H_{13}O_8SK$ requires 8.4%); FDMS of acid form *m/z*: 324 $[M-18]^+$, 262 (M^+ of parent alcohol); R_f 0.60 (blue fluorescent spot); electrophoretic mobility: (a) 0.86, (b) 0.88; UV λ_{max}^{MeOH} nm (log ε): 219 sh (4.10), 247 (3.47), 257 (3.43), 299 sh (3.86), 325 (4.09); IR ν_{max}^{KBr} cm^{-1}: 1250 strong and broad (S=O); ^1H NMR (90 MHz, DMSO-d_6): δ7.98 (1H, *d*, *J* = 9.5 Hz, H-4), 7.55 (1H, *d*, *J* = 8.6 Hz, H-5), 6.78 (1H, *d*, *J* = 8.6 Hz, H-6), 6.28 (1H, *d*, *J* = 9.5 Hz, H-3), 5.32 (1H, *t*, H-4'), 4.93 (1H, *d*, *J* = 4.2 Hz, 4'-OH) δ4.18 (1H, *d*, *J* = 4.4 Hz, H-3'), 1.40 (6H, *s*, gem-dimethyls).

Solvolysis of acid form of **2**. An aq. soln of **2** (30 mg) was passed through a column of Lewatit SP1080 (H^+). The aq. eluate was concd to 2 ml at red. pres., dissolved in EtOAc (80 ml) and left at 50° for 1.5 hr. Washing with H_2O and evaporation afforded (+)-*cis*-khellactone, **2a**, mp 171–172°; $[\alpha]_D^{23}$ +79.5 ($CHCl_3$; *c* 0.3); ^1H NMR (90 MHz, DMSO-d_6 + 0.5% CF_3COOD) partial: δ4.92 (1H, *d*, *J* = 4.6 Hz, H-4'), 3.62 (1H, *d*, *J* = 4.6 Hz, H-3'), 1.36 (6H, *s*, gem-dimethyls). Compound **2a** was identified by comparison with an authentic sample. In the concd aq. washing, SO_4^{2-} was detected.

(3'R)-*Lomatin-3'-(hydrogen sulphate), potassium salt*, **3**. Non-crystalline; $[\alpha]_D^{23}$ +27, $[\alpha]_{436}^{23}$ +92 (MeOH; *c* 0.2); FDMS of acid form *m/z*: 327 $[M+1]^+$, 326 $[M]^+$, 246 (M^+ of parent alcohol); R_f 0.65 (blue fluorescent spot); electrophoretic mobility: (a) 0.90, (b) 0.92; UV λ_{max}^{MeOH} nm (log ε): 220 sh (4.1), 246 sh (3.5), 257 (3.5), 325 (4.0); IR ν_{max}^{KBr} cm^{-1}: 1250 strong and broad (S=O); ^1H NMR (90 MHz, DMSO-d_6): δ7.97 (1H, *d*, *J* = 9.5 Hz, H-4), 7.46 (1H, *d*, *J* = 8.6 Hz, H-5), 6.77 (1H, *d*, *J* = 8.6 Hz, H-6), 6.26 (1H, *d*, *J* = 9.5 Hz, H-3), 4.34 (1H, *t*, H-3'), 3.18 (1H, *dd*, *J* = 3.9 and 17.8 Hz, H-4'$_a$), 3.01 (1H, *dd*, *J* = 4.9 and 17.8 Hz, H-4'$_b$), 1.35 (3H, *s*, Me), 1.27 (3H, *s*, Me).

Solvolysis of acid form of **3**. As for **2** this afforded (+)-lomatin, **3a**, mp 180–182°; $[\alpha]_D^{23}$ +51 (EtOH; *c* 0.2); ^1H NMR (90 MHz, DMSO-d_6 + 0.5% CF_3COOD) partial: δ3.75 (1H, *dd*, *J* = 4.9 and 6.8 Hz, H-3'), 2.98 (1H, *dd*, *J* = 4.9 and 17.3 Hz, H-4'$_a$), 2.68 (1H, *dd*, *J* = 6.8 and 17.3 Hz), 1.30 (3H, *s*, Me), 1.25 (3H, *s*, Me). Compound **3a** was identified by comparison with an authentic sample. In the concd aq. washing, SO_4^{2-} was detected.

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