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COUMARINS FROM *SESELI BOCCONI*

AURORA BELLINO, PIETRO VENTURELLA, MARIA LUISA MARINO, ORIETTA SERVETTAZ* and GIUSEPPE VENTURELLA†

Istituto di Chimica Organica dell'Università, Archirafi 20, 90123 Palermo, Italy; *Dipartimento di Biologia, Università di Milano, Italy; †Dipartimento di Scienze Botaniche, Università di Palermo, Italy

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Key Word Index—*Seseli bocconi*; Apiaceae; khellactones ester; bocconin.

Abstract—A new khellactone, bocconin, in addition to known compounds, have been isolated from *Seseli bocconi* subsp. *bocconi* and subsp. *praecox Gamisans*. Their structures were elucidated on the basis of spectral analyses and hydrolytic studies.

INTRODUCTION

The genus *Seseli* is widely distributed in Europe and many species of this genus are rich in coumarins, some of which are known for their medicinal properties [1–9]. Continuing our work on the metabolites from plants of Southern Italy [10, 11], we have examined *Seseli bocconi* Guss, a perennial herbaceous rocky plant endemic of the great Islands of the western Mediterranean and of many small islands, of which two subspecies are described. The subspecies *bocconi* Guss, which grows on limestone in Sicily (Monte Pellegrino, Marettimo and Favignana Islands), flourishes from September to October. The subspecies *praecox Gamisans*, which flourishes from June to July, grows on porphyry (Corsica), on limestone (Capo Caccia and Tavolara Islands) and on trachiti (S. Pietro Island, Sardinia).

In a preliminary study of the subspecies *bocconi* [12], we reported the isolation from the aerial parts of four crystalline compounds which were identified as osthol [13–15], imperatorin, bergapten [14–16] and mannitol (mmp, TLC). The occurrence of these compounds is very common in Apiaceae [17]. The composition of the essential oils from samples of subspecies *bocconi* and *praecox Gamisans* was also reported [18].

A new study of these subspecies has led to the isolation of 3',4'-diangeloyl-*cis*-khellactone (anomalin, **1**) already isolated from other species of *Seseli* and other plants [19–23] and of 3'-angeloyl-4'-acetyl-*cis*-khellactone (isopteryin, **2**) from subsp. *bocconi* [24–28]. The (+)-enantiomer of the latter coumarin is known as praeruptorin [29]. In addition, a new coumarin was isolated for which we propose the name bocconin and the structure of 3'-isobutyl-4'-acetyl-*cis*-khellactone (**3**).*

The subsp. *praecox Gamisans* contained **3** and 3'-angeloyl-*cis*-khellactone (**4**) (for which only mp [30] and ^{1}H NMR data had been reported [31]). In addition D-mannitol and lignoceric acid were isolated from both subspecies.

*A referee has suggested that an alternative structure for bocconin is one in which the acyl groups are interchanged.

RESULTS AND DISCUSSION

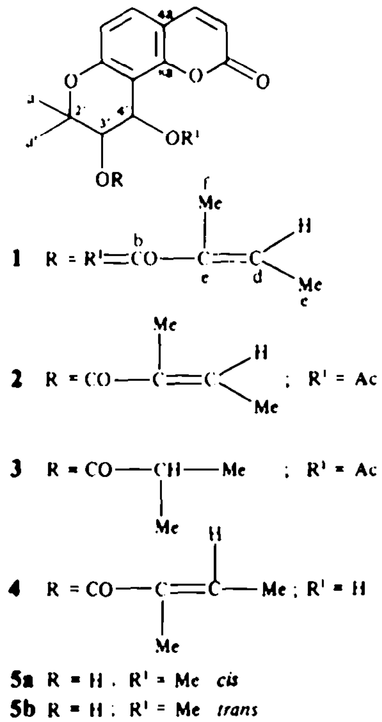
The structures of compounds **1**–**4** were unambiguously determined by means of their spectral properties (UV, IR, ^{1}H and ^{13}C NMR, and MS).

All the products had nearly identical UV spectra with maxima at 217 (sh), 245 (sh), 255, 297 (sh) and 320 nm indicative of the 7-oxycoumarin moiety [32]. IR bands at 1745, 1630, 1610, 1573 and 1488 cm^{-1} and the NMR spectra (Tables 2 and 3), suggested that the compounds were khellactone esters. The mass spectrum of each compound, the main fragmentation ions of which are given in Table 1, contained a coumarino-pyrilium ion showing that these compounds were dihydropyrano-coumarins [33, 34].

Alkaline hydrolysis (methanolic sodium hydroxide) of compounds **1**, **2** and **4** gave rise to a mixture of the glycols **5a** and **5b** and angelic acid (mp 45–47° [35]). The ^{1}H NMR spectrum of the acid showed the presence of protons of two *trans*-methyl groups (δ 2.02 and 1.90) and a vinyl proton (6.18, multiplet CH=), characteristic for angelic acid [36]. Alkaline hydrolysis of compound **3** gave the glycols **5a** and **5b** and 2-methylpropionic acid.

The physical properties of the isomeric diols **5a** and **5b** were the same as those of the previously known (+)-*cis*-methylkhellactone (mp 124–125°) and (−)-*trans*-methylkhellactone (mp 163–164°) [37, 38]. In particular, the appearance of a singlet at δ 3.80 (3H) showed that, as expected [37], the solvent had been incorporated into the system during the saponification step. The formation of a mixture of the diols was known to be a consequence of the epimerization at the benzylic 4'-position during alkaline hydrolysis of the natural khellactone esters.

The position of the acyl residue attached to the khellactones was established by the ^{1}H and ^{13}C NMR chemical shifts and MS. In particular, it was noted that the signal from H-3' was similar in all the compounds whereas the signal from H-4' in **4** (contains a hydroxyl) was shifted upfield by $\simeq$ 1.25 ppm (δ 5.26, J = 5.0 Hz). Likewise the ^{13}C NMR spectrum of **4** showed that the signal for C-4' was shifted downfield by 2.40 ppm. The presence of the secondary hydroxyl at 4' was obvious from the presence of a singlet at δ 5.82 which disappeared after exchange with D_2O. The presence of this group was confirmed by the



formation of an acetate whose NMR spectrum was the same as that of compound **2**. The relative *cis*-configuration of compounds were assigned on the basis of the coupling constant $J_{3',4'}$, which was between 2.4 and 3.0 Hz (*trans* compounds) and between 4.1 and 5.0 Hz (*cis* compounds). The dihydropropane gem-dimethyl signals generally appear as a separate doublet in *trans* isomers and as a rather broad singlet or two singlets close together in *cis* isomers [37, 38].

Therefore, compounds **1**, **2** and **4** were assigned, respectively, the structures 3',4'-diangeloyl-(−)-*cis*-khellactone (anomalin), 3'-angeloyl-4'-acetyl-(−)-*cis*-khellactone (isopteryxin) and 3'-angeloyl-(−)-*cis*-khellactone. Compound **3**, 3'-isobutyl-4'-acetyl-(+)-*cis*-khellactone, is a new natural coumarin for which we propose the trivial name bocconin.

The optical rotations for anomalin (**1**) {$[\alpha]_D^{20} = -50.2$ (*c* 1; 96% EtOH)}, isopteryxin (**2**) ($[\alpha]_D^{20} = -48.4$) and **4** ($[\alpha]_D^{20} = -89.8$) indicates the configuration 3'*R*, 4'*R*. The absolute configuration of **3** was not determined because the small amount of sample precluded an accurate evaluation of the optical rotations. The results obtained indicate that no chemotaxonomic relation exists between the two subspecies studies.

EXPERIMENTAL

Mps (Buchi 520): uncorr.; IR: nujol mull; MS: 75 eV; UV: EtOH; CC: silica gel (Merck, 005-0.02); TLC: silica gel F_{254} (Merck). All the products here reported gave satisfactory elemental analyses.

Plant material. *Seseli bocconi* Guss. was collected in Oct. 1980 (Pellegrino Mont, Sicily); *S. bocconi praecox Gamisans* in June 1982 (Capo Caccia and S. Pietro Island, Sardinia). The specimens were deposited in the Herbarium of the Botanic Garden, University of Palermo.

Table 1. Main fragmentation ions of compounds 1–4

Compound	*m/z* (first line) and relative intensity (second line)																		
1	426	327	311	243	229	213	191	162	134	83	55								
	0.33	12.64	13.50	5.09	25.52	6.09	1.14	1.10	1.38	100	96								
2	386	327	326	311	287	286	271	261	245	244	243	229	213	191	189	162	134	83	55
	4.98	0.79	0.71	3.84	5.07	11.59	4.62	3.23	11.97	35.51	9.56	96.7	7.09	18.60	7.42	8.31	6.95	100	81.09
3	374	315	314	299	287	286	271	261	245	244	243	229	213	191	189	162	134	83	55
	2.24	1.70	7.59	12.71	8.79	2.16	1.12	10.13	11.8	19.21	10.53	100	7.09	13.83	9.74	36.54	6.10	38	19.43
4	344	326	311	244	229	213	191	162	134	83	55								
	11.80	0.73	1.96	8.09	24.52	2.21	16.63	12.5	12.07	100	84.61								

Table 2. ^{1}H NMR data for compounds 1–4

H	1*	2†	3†	4†	*J* (Hz)
3	6.30	6.34	6.32	6.34	*d*, 10.0
4	7.60	8.03	8.02	8.04	*d*, 10.0
5	7.36	7.69	7.68	7.62	*d*, 8.0
6	6.82	6.93	6.90	6.88	*d*, 8.0
3'	5.45	5.37	5.29	4.95	*d*, 5.0
4'	6.69	6.48	6.42	5.26	*d*, 5.0
					t, 5.0 and 6.0
gem-dimethyls	1.46; 1.50	1.42; 1.45	1.39	1.41; 1.50	*s*
Me | CO-C=	1.90	1.84	—	1.92	*q*, 1.5
H : =C-Me	2.00	1.90	—	2.02	*dq*, 8.0 and 1.5
=C-Me	6.18	6.23	—	6.24	*q* (*br*), 8.0
H					
OAc	—	2.02	2.08	—	*s*
-CHMe$_2$	—	—	1.11 and 1.13	—	*dd*, 7.0

*80 MHz, $CDCl_3$, TMS as int. standard.
†200 MHz, DMSO, TMS as int. standard.

Table 3. ^{13}C NMR data for compounds 1–4 (20 MHz, $CDCl_3$, TMS as int. standard)

C	1	2	3	4
2	159.67	159.82	159.66	160.60 *s*
3	113.33	113.22	113.28	112.60 *d*
4	143.09	143.23	143.29	143.89 *d*
5	129.16	129.13	129.39	128.75 *d*
6	114.37	114.34	114.50	114.57 *d*
7	154.24	154.31	154.11	154.44 *s*
8	107.73	107.17	107.29	110.02 *s*
4a	156.83	156.81	156.67	156.07 *s*
8a	112.55	112.58	112.64	111.02 *s*
2'	77.55	77.76	77.33	77.64 *s*
3'	60.31	61.11	60.55	59.90 *d*
4'	70.31	69.89	70.54	72.70 *d*
a	22.57	22.99	22.20	22.57 *q*
a'	25.43	24.95	25.27	25.91 *q*
b	166.31; 166.51	166.48	175.84	166.94 *s*
c	127.18; 127.54	127.10	—	127.39 *s*
d	138.27; 139.62	139.57	—	139.29 *d*
e	15.54; 15.73	15.72	—	15.81 *q*
f	20.30	20.62	—	20.57 *q*
Ac	—	169.74	169.79	— *s*
	—	20.45	20.68	— *q*
-CHMe$_2$	—	—	34.18	— *d*
	—	—	18.87; 18.97	— *q*

All the assignments were confirmed by off-resonance experiments.

Isolation coumarins from S. bocconi. The dried plant (680 g) on extraction with Et_2O and subsequent evaporation of the solvent afforded an oily residue which was dissolved in 90% MeOH and then freed of lipids. The bulk of the chlorophylls was removed by extraction with petrol. The defatted extract was chromatographed on silica gel (10% H_2O) with petrol, petrol-Et_2O mixtures, EtOAc and MeOH as the eluents. A part of the material eluted was rechromatographed using the same conditions. Elution of the columns with EtOAc, EtOH and MeOH afforded further material which was not completely investigated. The fraction eluted with petrol–Et_2O (9:1) after crystallization from MeOH had mp 83–84°. Its blue fluorescence in UV light and R_f

(TLC) agreed with those of an authentic sample of osthol (mmp [13–15]). The fraction eluted by petrol Et_2O (5:1) yielded a product which was characterized as imperatorin, mp 102–103° (mmp and ^1H NMR [14 16]). The petrol–Et_2O (1:1) fraction yielded a yellow crystalline substance which after repeated crystallization from MeOH became colourless, mp 188 189°, fluorescence in UV light. It was identified as bergaptene (mmp, NMR [14 16]). The petrol–Et_2O (1:9) fraction was rechromatographed on silica gel (10% H_2O) when elution with hexane Et_2O (3:1) afforded a residue. Compounds **1** and **2** were isolated by HPLC on Micropak MCH-5 developed with MeOH (1.0 ml/min). The Et_2O–EtOAc (9:1) fraction was chromatographed several times on *ca* 100-fold amounts of silica gel (10% H_2O) Et_2O–EtOAc (increasing up to 10%) was used as eluent. This gave khellactone **3** and tetracosanoic acid (lignoceric acid).

Isolation of coumarins from S. bocconi praecox Gamisans. The dried and finely powdered plant (540 g) was extracted with Me_2CO (4 l.) at room temp. for 2 weeks. After filtration, the solvent was evaporated and the residue, worked up as described above, was subjected to CC over silica gel (10% H_2O). Elution with Et_2O–EtOAc gave compounds **3** and **4** and lignoceric acid.

3',4'-*Diangeloyl*-cis-*khellactone* (*anomalin*, **1**). Recrystallized from Et_2O–$CHCl_3$, mp 173 174°, gave analytical data concordant with the composition $C_{24}H_{26}O_7$: MS *m/z*: 426 $[M]^+$ [39, 40].

3'-*Angeloyl*-4'-*acetyl*-cis-*khellactone* (**2**) was obtained from petrol, mp 134–135° [24, 28]; MS *m/z*: 386 $[M]^+$ for $C_{21}H_{22}O_7$.

3'-*Isobutyl*-4'-*acetyl*-cis-*khellactone* (**3**) was crystallized from EtOAc, mp 147–148°; MS *m/z* 374 $[M]^+$, $C_{20}H_{22}O_7$; $[\alpha]_D^{20}$ = + 7.81 (*c* 1; 96% EtOH). Compound **3** was obtained in greater amounts as the racemate.

3'-*Angeloyl*-cis-*khellactone* (**4**), mp 157° from EtOAc; MS: *m/z* 344 $[M]^+$, $C_{19}H_{20}O_6$ [30, 31].

Acetylation of compound **4**. Compound **4**, on treatment with C_5H_5N–Ac_2O, as usual, gave a monoacetate, mp 134–135°, identical with compound **2** (mmp, co-TLC and NMR).

Treatment of **3** *with methanolic hydroxide*. A soln of **3** (100 mg) in 6 ml MeOH was mixed with 7 ml methanolic KOH and refluxed for 1.5 hr. The soln was acidified with H_2SO_4 and extracted with Et_2O. The Et_2O was backwashed with $NaHCO_3$ soln, dried and concd. The extract was chromatographed on silica gel with EtOAc containing increasing amounts of MeOH to give: (+)-cis-*Methylkhellactone* (**5a**), mp 124–125 from EtOAc $[\alpha]_D^{20}$ + 80.1 (*c* 0.3; $CHCl_3$) [37, 38]. ^1H NMR: δ 1.38 (*s* (*br*), 6H, 2-Me), 3.78 (*s*, –OMe), 3.80 (*d*, *J* = 5.4 Hz, H-4'), 4.68 (*d*, *J* = 5.4 Hz, H-3'), 6.22 (*d*, *J* = 9.6 Hz, H-3), 6.73 (*d*, *J* = 8.4 Hz, H-6), 7.30 (*d*, *J* = 8.4 Hz, H-5), 7.62 (*d*, *J* = 9.6 Hz, H-4). (–)-trans-*Methylkhellactone* (**5b**), mp 163 164° from Et_2O, $[\alpha]_D^{20}$ = –30 (*c* 0.4; $CHCl_3$), 65%. [37, 38] ^1H NMR: δ 1.48 and 1.43 (*s*, 6H, gem-dimethyls), 3.70 (*s*, 3H, OMe), 3.92 (*d*, *J* = 3.0 Hz, H-4'), 4.58 (*d*, *J* = 3.0 Hz, H-3'), 6.20 (*d*, *J* = 9.6 Hz, H-3), 6.77 (*d*, *J* = 9.6 Hz, H-6), 7.30 (*d*, *J* = 9.6 Hz, H-5), 7.58 (*d*, *J* = 9.6 Hz, H-4). The $NaHCO_3$ soln was acidified with 10% H_2SO_4 and extracted throughly with Et_2O. Preparative TLC of the residue with cyclohexane Et_2O (3:1) gave 2-methylpropionic acid as an oil (co-TLC and NMR).

Treatment of **1**, **2** *and* **4** *with methanolic* KOH. These were treated in the manner just described for **3**. Compounds **1** and **4** gave an equimolar mixture of *cis*- and *trans*-methyl-khellactones (**5a** and **5b**). Compound **2** gave 65% *trans*-khellactone. The isomers, after chromatographic separation, were identified by NMR spectra. The $NaHCO_3$ soln of the hydrolysis products, worked up as described above, yielded an acid, mp 42 44°, which was identified as 2-methyl-2-butenoic acid (angelic acid) [35].

Methanolic extract. The concd extract yielded a dark brown solid mass which was a mixture. All attempts to isolate the individual compounds in a pure state were unsuccessfull. CC on silica gel led to the isolation only of pure D-mannitol (mp and mmp 165–166°).

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