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Sesquiterpenes from *Centaurea aspera*

J. Alberto Marco [a,*], Juan F. Sanz-Cervera [a], Alberto Yuste [a],
Félix Sancenón [a], Miguel Carda [b]

[a] *Departamento de Química Orgánica, Universidad de Valencia, E-46100 Burjassot, Valencia, Spain*
[b] *Departamento de Química Inorgánica y Orgánica, Universidad Jaume I, E-12071 Castellón, Spain*

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Abstract

The aerial parts of two subspecies of *Centaurea aspera* L. (Asteraceae) yielded the germacranolides **1a**–**h**, **2**, **3**, **4** and **5**, the elemane derivatives **6d** and **6f**, the lignan matairesinol, the degraded terpene loliolide, and the onopordopicrin–valine dimeric adduct **7**. From these, compounds **1e**, **3** and **6d** are natural products. The chemical composition of the two subspecies is very similar, a circumstance which does not support a taxonomic subdivision of the species.

Keywords: *Centaurea aspera*; Compositae; Structural elucidation; Synthesis; Sesquiterpene lactones; Germacranolides; Elemanes; Chemotaxonomy

1. Introduction

Within our phytochemical investigations related to members of the Compositae family, we here present the results of our investigations of two subspecies of *Centaurea aspera* L.: *C. aspera* L. subsp. *aspera* and *C. aspera* L. subsp. *stenophylla* (Dufour) Nyman (syn. *C. stenophylla* Dufour). Both subspecies are the only reported for *C. aspera* in two recent books (Mateo-Sanz and Crespo-Villalba, 1990; Bolòs and Vigo, 1995) even though the authors admit that the morphological differences are small and report the existence of "transition forms", so that their taxonomic separation is questionable. Our research aims at establishing whether or not the chemical data lend support to this separation.

Prior to our work, some chemical investigations on *C. aspera* have been published. For instance, *C. aspera* subsp. *stenophylla* has yielded flavonoids, waxes and triterpenes and several sesquiterpene lactones: the elemanolides melitensin and 11,13-dehydromelitensin, and the germacranolides stenophyllolide **4** and its 11,13-dihydroderivative **5** (Ferreres and Tomás, 1980; Picher et al., 1984, 1985). Another subspecies, *C. aspera* L. subsp. *subinermis* DC. (Cadevall and Sallent, 1919–1929), was found to contain various known and new sesquiterpene lactones as well as flavonoids (Cardona et al., 1991, 1992; Fernández et al., 1993). More recently, another investigation of the chemical composition of three subspecies of *C. aspera* has been reported (Barrero et al., 1995).

2. Results and discussion

The extraction of the aforementioned plant material followed by chromatographic separation of the extracts yielded the germacranolides salonitenolide **1a** (Suchý et al., 1967), **1b** (Zdero and Bohlmann, 1987), **1c** (Tsankova and Ognyanov, 1985), **1d** (Huneck et al., 1986), **1e**, onopordopicrin **1f** (Drożdż et al., 1968), cnicin **1g** (Seaman, 1982), **1h** (Jakupovic et al., 1986), **2** (Marco et al., 1992), **3**, **4** and **5** (Picher et al., 1984), the elemane deriv-

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[*] Corresponding author. Tel.: +34 96 3544337; fax: +34 96 3544328.
E-mail address: alberto.marco@uv.es (J.A. Marco).

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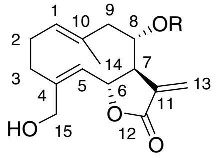
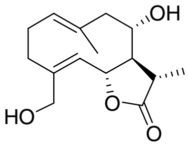
atives **6d** and elemacarmanin **6f** (Rustaiyan et al., 1986), the lactone–valine dimeric adduct **7** (Marco et al., 1991), the lignan matairesinol (Ayres and Loike, 1990), and the degraded terpene loliolide (Behr et al., 1979). Both subspecies gave similar products, as detailed in Section 3. All compounds were identified with the aid of spectroscopic analyses, aided in one case by a chemical correlation. From the aforementioned products, compounds **1e**, **3** and **6d** have not previously been reported in the bibliography.

The spectral data of **1e** suggested that it was a 8-*O*-acyl derivative of **1a**. Strong bands of hydroxyl (3450 cm^{-1}), γ-lactone (1765 cm^{-1}) and ester carbonyl groups (1745, 1720 cm^{-1}) were marked features of the IR spectrum (Section 3). The ^{1}H and ^{13}C NMR spectra (Tables 1 and 2) were very similar to those of germacranolides of general structure **1**, except for specific differences in the signals of the ester side chain. Comparison with literature data (Buděšínský and Šaman, 1987) revealed that the latter was a 4′-acetoxyangeloyl residue, a conclusion supported by spin decoupling experiments. In the mass spectrum, the highest mass peak at *m*/*z* 246 was attributed to the loss of the side chain acid from the molecular ion.



1a-h



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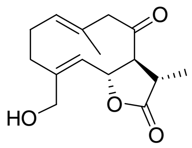

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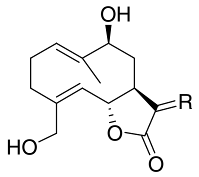

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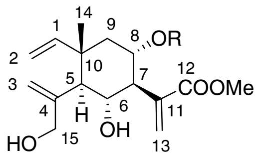
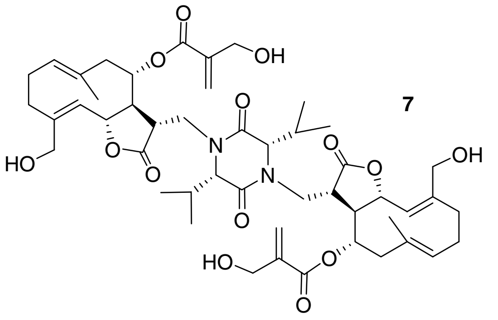
5 R = H,αMe



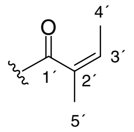
6d, **6f**



7

a R = H

b R =



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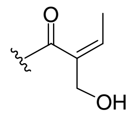

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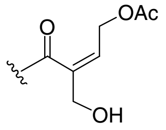

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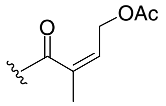
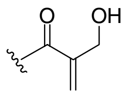
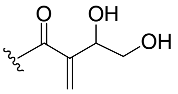


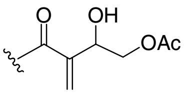
f R =



g R =



h R =



The NMR spectra of compound **6d** (Tables 1 and 3) had clear similarities with those of elemacarmanin **6f**, the only differences being attributable to the presence of a different ester side chain. Comparison with the data of lactone **1d** and with literature data (Buděšínský and Šaman, 1987) indicated that the side chain was a 4′-acetoxy-5′-hydroxyangelate residue, which manifested itself in the peak at *m*/*z* 278 in the mass spectrum (loss of the side chain acid from the molecular ion, see Section 3). This acid moiety has often been found attached to hydroxyl groups of sesquiterpenes from *Onopordon* species (Rustaiyan et al., 1986).

Compound **3** had strong hydroxyl (3450 cm^{-1}), γ-lactone (1760 cm^{-1}) and ketone carbonyl bands (1690 cm^{-1}) in its IR spectrum. ^{13}C NMR (Table 3) and mass spectral data indicated the molecular formula $C_{15}H_{20}O_4$. The four oxygen atoms were accounted for with one hydroxyl, one ketone and one lactone group. The ^{13}C NMR data further indicated the presence of two C=C bonds and two carbonyl groups. In view of the molecular formula, it was concluded that the molecule contained one carbocycle. The ^{1}H NMR spectrum (Table 1) displayed clear similarities to those of the other germacranolides isolated from the plant. Signals

Table 1
^{1}H NMR data of compounds **1e**, **3** and **6d**[a]

H	**1e**[b]	**3**[c]	**6d**[b,d]
1	5.15 *m*[e]	5.20 *br dd* (10; 3.5)	5.68 *dd* (17.3, 10.7)
2α	2.60–2.20 *m*[e]	2.35 *m*	4.94 *br d* (10.7)
2β			4.91 *br d* (17.3)
3α		2.11 *ddd* (12, 12, 6)	5.37 *br s*
3β		2.61 *ddd* (12, 5.5, 2)	5.02 *br s*[e]
5	4.85 *br d* (10)	5.06 *br d* (10.5)	2.11 *d* (10.5)
6	5.10 *dd* (10, 8)	4.92 *dd* (10.5, 9)	4.20 *dd*[e] (10.8, 10.5)
7	3.07 *dddd* (8, 8, 3.5, 3)	3.10 *m*[e]	2.70 *dd* (10.8, 10.8)
8	5.00 *m*[e]	–	5.45 *ddd* (11.5, 10.8, 4.5)
9α	2.60–2.50 *m*[e]	3.32 *br d* (10)	1.60 *dd* (12.5, 11.5)
9β		2.98 *br d* (10)	1.95 *dd* (12.5, 4.5)
11	–	3.10 *m*[e]	–
13	6.29 *d* (3.5)	1.21 *d* (6.7)	6.30 *br s*
	5.69 *d* (3)		5.76 *br s*
14	1.50 *br s*	1.48 *br s*	1.17 *s*
15	4.28 *dd* (14, 1)	4.16 *d* (13.7)	4.05 *d* (13)
	4.09 *d* (14)	3.86 *d* (13.7)	3.93 *d* (13)
3′	6.09 *tq* (5.5, 1.5)	–	6.25 *br t* (5)
4′	5.05 *dd* (15, 5.5)	–	5.02 *br s*[e]
	5.00 *dd* (15, 5.5)		
5′	1.95 *br d* (1.5)	–	4.20 *br s*[s]

[a] At 200 MHz in $CDCl_3$, unless otherwise stated. Chemical shifts are followed by coupling constants in parenthesis.
[b] OAc: 2.07 *s*.
[c] At 400 MHz.
[d] COOMe: 3.76 *s*.
[e] Overlapped signals.

Table 2
^{13}C NMR data for lactones **1a**–**h**[a]

C	**1a**	**1b**	**1c**	**1d**	**1e**	**1f**[b]	**1g**[b]	**1h**
1	128.7[c]	129.5	129.8	129.8	129.8	129.8	129.8	129.9
2	26.3	26.3	26.3	26.1	26.3	26.3	26.1	26.3
3	35.0	34.7	34.7	34.6	34.6	34.7	34.6	34.7
4	143.6	143.8	143.9	144.3	143.9	144.2	144.6	144.1
5	128.5[c]	128.8	128.6	128.2	128.6	128.4	128.0	128.4
6	76.5	76.7	76.6	76.9	76.7	76.6	77.1	76.4
7	54.7	53.0	53.0	52.7	52.9	53.0	53.0	53.0
8	70.9	72.2	72.8	73.3	73.0	73.1	73.1	73.3
9	52.5	49.0	48.9	48.7	48.8	48.7	48.6	48.6
10	134.0	132.7	132.4	132.1	132.4	132.4	132.1	132.3
11	136.3	135.6	135.5	135.4	135.6	135.4	135.3	135.4
12	171.1	169.8	169.8	170.2	169.7	169.9	170.5	169.8
13	127.0	125.1	125.3	125.3	125.0	125.5	125.5	125.3
14	16.9	16.9	16.8	16.8	16.8	16.8	16.7	16.8
15	60.9	61.5	61.5	60.8	61.5	61.3	60.7	61.5
1′		166.8	166.1	164.8	165.7	165.1	165.1	164.6
2′		127.3	131.6	131.7	128.2	139.4	139.7	138.8
3′		139.5	141.7	140.3	140.1	126.3	127.1	127.8
4′		15.9	15.9	62.8	62.8	62.1	70.8	69.5
5′		20.5	64.9	62.3	19.7		65.8	67.3
OAc				171.1	170.8			171.3
				20.8	20.9			20.8

[a] At 50 MHz in $CDCl_3$, unless otherwise stated.
[b] In $CDCl_3/CD_3OD$ 5.1.
[c] Interchangeable signals.

from hydrogen atoms geminal to ester functions as well as signals from ester side chains were absent, however. All spectral data were explained by assuming the presence of a ketone function at C-8, as depicted in structure **3**. This conclusion was further supported by the results of extensive spin decoupling experiments.

Table 3
^{13}C NMR data for compounds **3** and **6d**[a]

C	**3**	**6d**[b]
1	132.3	146.2
2	24.8	112.1
3	33.4	115.0
4	145.1	146.3
5	128.3	55.3
6	75.3	71.0
7	64.4	54.8
8	203.6	71.4
9	57.4	43.6
10	126.5	40.3
11	40.5	138.0
12	177.1	167.2
13	13.6	128.7
14	16.0	18.3
15	60.1	67.8

[a] At 50 MHz in $CDCl_3$.

[b] Other signals: 164.9 (C-1′), 131.4 (C-2′), 140.8 (C-3′), 63.9 (C-4′), 62.9 (C-5′), 52.1 (OMe), 170.8, 20.9 (OAc).

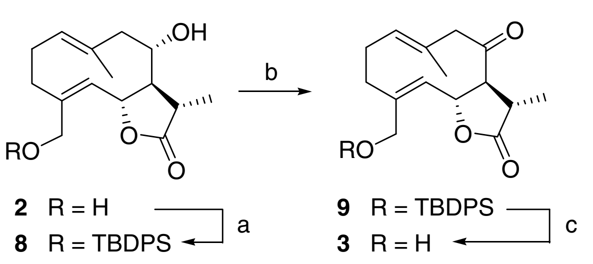
The definitive confirmation of structure **3** was given by the chemical conversion of 11β,13-dihydrosalonitenolide **2** into **3** (Scheme 1). Selective protection of the primary hydroxyl group of **2** was performed by means of treatment with *t*-butyldiphenylsilyl chloride (Greene and Wuts, 1999). The monosilylated derivative **8** was then oxidized with pyridinium dichromate (PDC) to yield ketone **9**. Finally, cleavage of the silyl group with tetra-*n*-butylammonium fluoride (Greene and Wuts, 1999) yielded a keto lactone identical in its chromatographic and spectral properties to natural product **3**.

The onopordopicrin–valine dimeric adduct **7**, which we described in a preliminary communication (Marco et al., 1991), is a very unusual type of natural product. A few addition products of aminoacids to the conjugated double bond of sesquiterpene lactones have been reported in *Saussurea* species (Li and Jia, 1989; Matsuda et al., 2000) and found later to display various pharmacological properties (Yoshikawa et al., 2000; Matsuda et al., 2003). However, this the first, and to the best of our knowledge only, *dimeric* aminoacid–sesquiterpene adduct reported in a natural source. It is not known whether **7** arises via dimerization of a monomeric valine–lactone adduct or through addition of valine dimer **10** (a diketopiperazine) to two molecules of onopordopicrin **1f** (Scheme 2). As a matter of fact, our attempts to generate **7** by means of addition of the dianion of **10**–**1f** only led to decomposition.

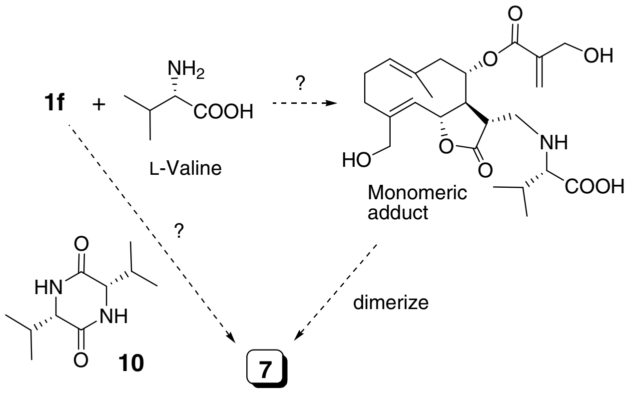
With the exception of **7**, the compounds now isolated from both subspecies of *C. aspera* are well in line with the known chemical behavior of both the genus *Centaurea* and the tribe Cynareae (Zdero and Bohlmann, 1990; Buděšínský et al., 1994). It is worth mentioning here that the elemanolides melitensin and 11β,13-dihydromelitensin, both reported in the previous investigations on *C. aspera* subsp. *stenophylla* (Picher et al., 1984), have not been found in the course of our work. Indeed, it has been shown that many elemanolides isolated from natural sources are actually artifacts originated through Cope rearrangements of germacranolides, which took place during prolonged Soxhlet extractions with boiling solvents (Barrero et al., 1989). However, it is also noteworthy that, while the Cope rearrangement products of germacranolides **1a**–**h** have not been detected in our extracts, we have isolated two elemane esters, **6d** and **6f**, but not their putative germacrane precursors.

Tables 2 and 3 contain the ^{13}C NMR data of most of the sesquiterpenes isolated in this work. The signals were assigned with the aid of 2D C,H correlations.

As described in Section 3, the secondary metabolites isolated from the different plant materials are very similar. Both subspecies contain germacranolides of the same type and only show minor differences in the side chains. Since the materials were collected in different locations and years, the observed small differences in the chemical composition (chemotypes) may be attributed to seasonal variations or changes in the nature of the soil. In our opinion therefore, the chemical data do not lend support to the taxonomic subdivision of *C. aspera* into distinct subspecies.



Scheme 1. Chemical conversion of **2** into **3**. *Reaction conditions*: (a) TBDPSCl, py, RT; (b) PDC, CH_2Cl_2, RT; (c) tetra-*n*-butylammonium fluoride, THF, RT.



Scheme 2. Possible biosynthetic ways towards **7**.

3. Experimental

3.1. General experimental procedures

NMR spectra were measured on Bruker NMR spectrometers WM-400 and AC-200 operating at the ^{1}H frequencies indicated in the tables. Mass spectra were run on a Varian MAT 711 spectrometer. IR spectra were determined as oily films on NaCl plates. Optical rotations were measured at 22 °C. HPLC separations were performed in the reverse phase mode (LiChrosorb RP-8, 250 × 8 mm, flow, 3 ml/min, elution with MeOH/H_2O mixtures). Medium pressure column chromatography (MPCC) was made on silica gel Merck (40–63 μm).

3.2. Plant material

Specimens of *C. aspera* subsp. *aspera* were collected in Buñol, in July 1989. Those of *C. aspera* subsp. *stenophylla* were collected in two locations: El Saler, May 1990, and Sollana, June 1991. All locations are in the province of Valencia, Spain. Voucher specimens are deposited in the herbarium of the Department of Botany at the Faculty of Biology in Valencia (Prof. M. Guara).

3.3. Extraction and isolation

The plant material (1000–1200 g in each case) was air-dried at room temperature, finely ground and macerated 4 days at room temp. in MeOH. The obtained extract was concentrated in vacuo, suspended in boiling MeOH (10 ml/g of extract), stirred for 5 min and cooled in a freezer to −15 °C. After standing for 4 h at this temperature, the waxy precipitate was eliminated by filtration. After evaporation of the supernatant solution in vacuo, the dark-green, dense oil was prefractionated by means of column chromatography on silica gel: fraction A, hexane–Et_2O 1:3; fraction B, Et_2O, fraction C, Et_2O–MeOH, 9:1. The three fractions were subjected to further chromatographic separations as described below.

3.3.1. C. aspera subsp. aspera

Chromatographic treatment of fractions A and B yielded only waxes and common triterpenes and sterols. Fraction C was fractionated by MPCC (gradient elution from $CHCl_3$–MeOH 25:1 to 5:1). Purification of the intermediate fractions by means of preparative TLC afforded **1d** (82 mg), **1f** (107 mg), **6d** (23 mg), **6f** (61 mg), **2** (19 mg), **3** (7 mg), **4** (67 mg) and **5** (13 mg). Compound **7** precipitated from one of the fractions as a sparingly soluble, colorless solid.

3.3.2. C. aspera subsp. stenophylla (material from El Saler)

Fraction A was subjected to MPCC (elution with $CHCl_3$–MeOH 25:1). This provided lactones **1b** (22 mg) and **1e** (23 mg). When processed in the same way, fraction B gave **1g** (32 mg) and **1h** (328 mg). Fraction C was also subjected to MPCC (elution with $CHCl_3$–MeOH 25:1) to yield several fractions, which were further purified by means of HPLC (elution with MeOH–H_2O 60:40 or 55:45). This afforded **1a** (9 mg), **1c** (63 mg), **1d** (18 mg), **1f** (15 mg), **1g** (212 mg), **1h** (10 mg), **4** (142 mg) and **5** (515 mg).

3.3.3. C. aspera subsp. stenophylla (material from Sollana)

Fraction A yielded only waxes and sterols. Fraction B gave **1e** (24 mg). Fraction C contained considerable amounts of **1g**, which precipitated in crystalline form (ca. 2 g) from the fractions. MPCC of the mother liquors (elution with $CHCl_3$–MeOH 25.1), followed where necessary by HPLC, allowed the isolation of **1a** (50 mg), **1d** (900 mg), **1e** (26 mg), **1g** (32 mg), **1h** (44 mg), loliolide (15 mg) and matairesinol (10 mg).

3.3.4. 8α-(Z-2-Methyl-4-acetoxybut-2-enoyloxy)-15-hydroxygermacra-1(10),E,4Z,11(13)-trien-12,6α-olide (1e)

Colorless oil; $[\alpha]_D + 54$ (c, 3.26; $CHCl_3$). IR ν_{max} 3450, 1765, 1745, 1720, 1655, 1520, 1450, 1380, 1265, 1230, 1145, 1030, 820, 740 cm^{-1}. EIMS *m/z* (% rel. int.): 246 ($M^+ - C_7H_{10}O_4$, 3), 228 (9), 199 (5), 149 (16), 43 (100). Approx. R_f value on silica gel: 0.3 ($CHCl_3$–EtOAc 9:1). For NMR data, see Tables 1 and 2.

3.3.5. 8-Oxo-15-hydroxygermacra-1(10),E,4Z-dien-11βH-12,6α-olide (3)

Colorless oil; $[\alpha]_D - 393$ (c, 2.76; $CHCl_3$). IR ν_{max} 3450, 1760, 1690, 1684, 1427, 1370, 1350, 1310, 1200, 1155, 980, 940, 890 cm^{-1}. EIMS *m/z* (% rel. int.): 246 ($M^+ - H_2O$, 8), 218 (10), 199 (12), 145 (15), 107 (16), 69 (100). Approx. R_f value on silica gel: 0.35 ($CHCl_3$–Et_2O 5:1). For NMR data, see Tables 1 and 3.

3.3.6. Methyl 8α-(Z-2-hydroxymethyl-4-acetoxybut-2-enoyloxy)-6α,15-dihydroxyelema-1,3,11(13)-trien-12-oate (6d)

Colorless oil; $[\alpha]_D + 11.5$ (c, 1.2; $CHCl_3$). IR ν_{max} 3400, 1745, 1710 (*br*), 1650, 1625, 1440, 1370, 1325, 1225, 1155, 1050, 910, 815 cm^{-1}. EIMS *m/z* (% rel. int.): 278 ($M^+ - C_7H_{10}O_5$, 18), 260 (24), 246 (15), 229 (14), 119 (69), 115 (100). Approx. R_f value on silica gel: 0.3 ($CHCl_3$–EtOAc 1:5). For NMR data, see Tables 1 and 3.

3.3.7. Silylation of 2–8

A solution of lactone **2** (85 mg, 0.32 mmol) in dry pyridine (1.5 ml) was treated at 0 °C under Ar with *t*-butyldiphenylsilyl chloride (0.1 ml, 0.38 mmol). The solution was then stirred for 1 h at the same temperature and for another hour at room temperature. The reaction mixture was poured onto 5% aqueous HCl and extracted with CH_2Cl_2. The organic layer was washed with satd. aq $NaHCO_3$, then with brine and subsequently dried over anhydrous Na_2SO_4. Filtration, evaporation of the solution in vacuo and column chromatography on silica gel (hexane–EtOAc 1:1) afforded **8** (56 mg, 36%) as an oil: ^{1}H NMR (200 MHz) δ 7.70–7.30 (*br m*, 10H), 4.93 (*br dd*, $J = 10$, 5 Hz, 1H), 4.81 (*br t*, $J = 9.5$ Hz, 1H), 4.66 (*br d*, $J = 9.5$ Hz, 1H), 4.17 (*br s*, 2H), 3.83 (*br ddd*, $J = 10$, 10, 2.5 Hz, 1H), 2.68 (*dq*, $J = 11.5$, 7 Hz, 1H), 2.53 (*br dd*, $J = 12$, 2.5 Hz, 1H), 2.38 (*dd*, $J = 12$, 10 Hz, 1H), 2.25–1.80 (*br m*, 4H), 2.00 (overlapped *m*, 1H), 1.42 (*d*, $J = 7$ Hz, 3H), 1.31 (*br s*, 3H), 1.07 (*s*, 9H).

3.3.8. Oxidation of 8–9

Lactone **8** (56 mg, 0.11 mmol) was dissolved in dry CH_2Cl_2 (0.1 ml) and added under Ar at room temperature to a suspension of pyridinium dichromate (70 mg, 0.18 mmol) in dry CH_2Cl_2 (1 ml). After stirring for 4 h at room temp., the reaction mixture was diluted with Et_2O (5 ml) and filtered through a pad of silica gel. The filtrate was then concentrated in vacuo and chromatographed on silica gel (hexane–EtOAc 7:3) to yield **9** (30 mg, 54%) as an oil: ^{1}H NMR (200 MHz) δ 7.70–7.30 (*br m*, 10H), 5.16 (*br dd*, $J = 9$, 7 Hz, 1H), 5.00 (*m*, 2H), 4.13 (*d*, $J = 14$ Hz, 1H), 3.88 (*d*, $J = 14$ Hz, 1H), 3.29 (*br d*, $J = 10$ Hz, 1H), 3.05 (*m*, 2H), 2.93 (*br d*, $J = 10$ Hz, 1H), 2.55 (*br dd*, $J = 11$, 2.5 Hz, 1H), 2.20 (*br m*, 3H), 1.19 (*d*, $J = 7$ Hz, 3H), 1.31 (*br s*, 3H), 1.06 (*s*, 9H).

3.3.9. Desilylation of 9–3

A solution of keto lactone **9** (30 mg, 0.06 mmol) in dry THF (2 ml) was treated under Ar at room temperature with an 1 M solution of tetra-*n*-butylammonium fluoride in the same solvent (0.25 ml, 0.25 mmol) in dry CH_2Cl_2 (1 ml). After stirring for 1 h at room temp., the reaction mixture was poured onto brine and extracted with CH_2Cl_2. The organic layer was then dried over anhydrous Na_2SO_4, filtered and concentrated in vacuo. Column chromatography of the residue on silica gel (hexane–EtOAc 1:1) furnished an oil (10 mg, 61%), identical in all its chromatographic and spectral properties with natural lactone **3**.

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