

A New Diterpene and Flavonoids from *Pulicaria somalensis*

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Abstract. A new diterpene of clerodane type and three known methylated flavones (chryso splenol D, chryso splenetin and casticin) have been isolated from the aerial parts of *Pulicaria somalensis* and characterized by spectroscopic methods. Chryso splenetin and casticin have been isolated from the *Pulicaria* genus for the first time.

Introduction

In the course of our research on natural products from Saudi plants and in view of the absence of any information about the chemistry of *Pulicaria somalensis*, we have undertaken the investigation of this plant. Recently, we reviewed the chemistry of various members of the *Pulicaria* genus [1]. Characteristic constituents of this genus are sesquiterpenoids and flavonoids. Diterpenoids and triterpenoids are however less frequent. Only a total of six diterpenes of the clerodane series have been isolated from three *Pulicaria* species [2-5]. In the present article we describe the isolation of previously unknown clerodane lactone (1) as well as three flavonoids from the aerial parts of *P. somalensis*.

Experimental

Melting points are uncorrected and were taken on a Kofler hot stage. UV spectra were recorded on uv/vs PU 8800 Pye Unicam spectrophotometer. NMR spectra were obtained with a Jeol-100 MHz instrument in CDCl₃ using TMS as an internal reference. Known compounds were identified by comparison of their spectral data with those reported in the literature and in some cases with authentic samples available in our laboratory.

Plant material

Aerial parts of *Pulicaria somalensis* were collected in the vicinity of Al-Hariq (200 Km from Riyadh, Saudi Arabia) during August 1991 and identified by Dr. Al-Farhan, Botany Department, College of Science, King Saud University, Saudi Arabia.

Extraction and Isolation

The air dried plant material (1.6 Kg) was extracted at room temperature with MeOH-Et₂O-Petrol (1:1:1)(9 L, 10 days) [6]. After filtration and removal of solvents under vacuum, the residue (40 gm) was chromatographed on silica gel, to give the collected fractions A-E. Fraction A (petrol-CHCl₃; 1:1) consisted mainly of waxes and volatile terpenes including α -cadinene (65 mg). Fraction B (petrol-CHCl₃; 3: 7) was fractionated by CC on silica gel (petrol-CHCl₃ mixtures) and those intermediate fractions which gave positive tests for flavones were further purified by TLC to give flavone (3) (70 mg). Fraction C (CHCl₃) gave after purification by TLC (CHCl₃-Et₂O; 8:2), the diterpene (1) (35 mg). Fraction D (MeOH-CHCl₃; 2:8) contained a high percentage of flavonoids as revealed from qualitative tests. This fraction was chromatographed on silica gel and the individual flavonoidal containing fractions were purified by TLC (CHCl₃-Et₂O; 1:1) to give compounds (2) (110 mg) and (4) (40 mg); the first is the less polar. Fraction E (MeOH) yielded an amorphous solid which showed a very low solubility in most solvents. This solid was acid hydrolysed to give β -sitosterol and glucose as judged from co-chromatography with authentic samples.

Diterpene lactone (1) (Oil) IR: ν (cm⁻¹)(neat): 3250-2500 (COOH), 1797 (carbonyl lactone), 1719 (COOH) 1627, 909, 854; MS (relative intensity): 330, [M]⁺(8%), 286[M-CO₂]⁺(78), 271[286-CH₃]⁺(67), 195[M-C₈H₇O₂]⁺(72), 175[286-C₆H₇O₂]⁺(96), 111[C₆H₇O₂]⁺(100); ¹H NMR (CDCl₃): δ 9.1 (br s, 1H), 7.38 (br d, 1H, H-3), 6.0(dd, 1H, H-2), 5.86(dt, 1H, H-16), 5.24(d, 1H, H-3), 4.87 and 5.1(each 1H, br s, H-19), 4.77(br d, 2H, H-14), 1.01(s, 3H), 0.82(d, J=7 Hz, 3H); ¹³C NMR (see Table 2).

Identification of compounds (2-4): These compounds were identified from their UV spectral data and ¹H NMR spectra. Casticin (4), identified by direct comparison of its melting point and various spectroscopic data with those of authentic sample.

Results and Discussion

Chromatographic separation of *P. somalensis* extractives gave compounds (1-4) in addition to the known terpenoids, α -cadinene and β -sitosterol-3-glucose. The diterpene lactone (1) was obtained as an oily product and the mass spectrum was consistent with the molecular formula. IR spectrum of (1) exhibited a broad absorption

band in the region 3250-2500 cm^{-1} , together with a strong absorption (1719 cm^{-1}) indicative of a conjugated carboxylic acid function. This spectrum also showed the presence of γ -lactone moiety (1779 cm^{-1}). ^1H NMR spectrum of (1) showed broadened signals when recorded at room temperature, but at elevated temperatures, signals were easily assigned. This spectrum also showed typical signals of β -substituted butenolide function; a doublet of a triplet at δ 5.86 (1H, $J = 1$ and 1.5 Hz) and a broad doublet at δ 4.77 (2H, $J = 1.5$ Hz). The mass spectrum of (1) confirms the presence of β -substituted butenolide ring. The main three fragments are at m/e 286, 175 and 111. The one at m/e 286 comes from the loss of carbon dioxide from the molecular ion. When the latter fragment loses the lactone side chain as a radical, the peak at m/e 175 is produced. On the other hand, when the lactone side chain is lost as a cation, the base peak at m/e 111 is produced. Comparison of the ^1H -NMR spectrum of (1) with those reported in the literature for compounds (5a) [5], (5b) [7] and (7) [8] allowed the assignment of the remaining signals. The stereochemistry of the methyl groups at C-8 and C-9 in (1) was based upon the comparison of their chemical shifts with those reported for the methyl groups in the previously identified compounds (5a), (5b) and (7). The chemical shift values for the methyl groups in the new diterpenoid (1) were the same as those in (7) and different from those in (5a) and (5b) (Table 1). As a result the stereochemistry in (1) was chosen to be the same as in (7).

Table 1. ^1H -NMR chemical shifts (δ) for the methyl groups at C₈ and C₉ in (1), (5a), (5b) and (7)

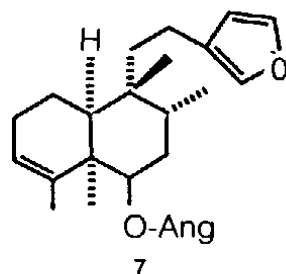
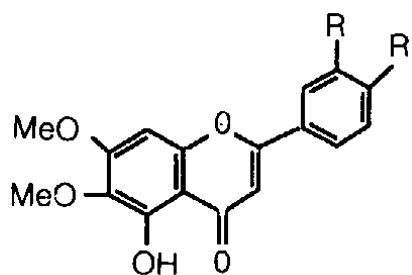
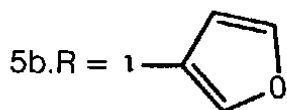
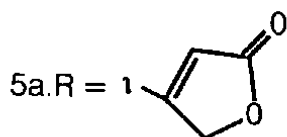
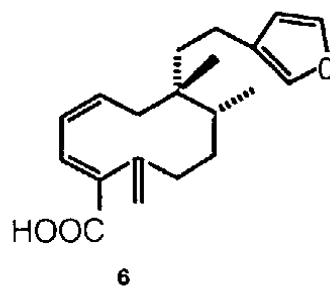
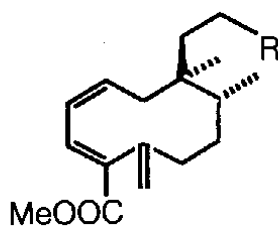
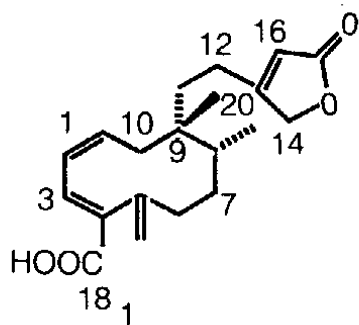
	Chemical shift			
	(1)	(5a)	(5b)	(7)
C ₈ - CH ₃	0.82	0.75	0.76	0.87
C ₉ - CH ₃	1.01	0.68	0.71	1.07

The ^{13}C -NMR spectrum of (1) (Table 2) supported the suggested structure. The assignment of all ^{13}C signals in (1) was made by their comparison with those reported for (6) [9]; (no ^{13}C -NMR was given for 5a); and other structurally related clerodanes [8], and by the aid of the single frequency off-resonance decoupling technique (SFORD).

Mehtylated flavonoids chryso splenol D, (2), chryso splenetin, (3), and casticin (4) were obtained from the flavonoid fraction of *P. somalensis* extracts (see experimental). These flavonoids were characterized by their spectroscopic data (UV, NMR) and by comparison with literature data and by direct comparison in the case of compound (4). Oxygenation patterns of flavonoids (2-4) were determined from their ^1H NMR spectra. Each of these spectra had a single aromatic A-ring proton absorption (H-8) and ABX system characteristic of 1, 2, 4-trisubstituted aromatic

Table 2. ^{13}C NMR spectral data of diterpene (1)

C. No.		C. No.	
1	128.21	11	29.19
2	126.74	12	19.26
3	142.89	13	170.28
4	136.49	14	73.26
5	144.5	15	171.89
6	33.71	16	115.23
7	37.38	17	15.30
8	35.76	18	170.68
9	37.38	19	118.84
10	35.59	20	18.55



2. Chrysosplenol D

3. Chrysosplenetin

4. Casticin

R	R ¹
OH	OH
OMe	OH
OH	OMe

ring (B-ring). ^1H NMR spectra of (2-4) also showed signals due to three methoxy groups for (2) and four methoxyls for (3) and (4). The UV spectra of (2-4) and their changes in the presence of diagnostic shift reagents pointed clearly to the presence of a free hydroxyl group at C-5 in the three flavones and free hydroxyl at C-4 in flavones (2) and (3) [10].

In conclusion, this first investigation of the chemical constituents of *Pulicaria somalensis* resulted in the identification of a new diterpenoid (1), of a clerodane type, and three flavonoids (chrysosplenol D (2), chrysosplenetin (3) and casticin (4)). The latter two flavonoids are observed in the genus for the first time. It is worth mentioning that the chemistry of *P. somalensis* is somewhat similar in the terpenoid content with that reported for *P. angustifolia*. Further investigation of the terpenoid contents of this plant is still in progress in our laboratory.

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مركب تريين ثنائي جديد وفلافونويدات من نبات بوليكاريا سومالينسيس

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(سُلِّمَ في ١٥ محرم ١٤١٣هـ، وقُبِلَ للنشر في ٣ رمضان ١٤١٣هـ).

ملخص البحث. لقد استخلص تريين ثنائي جديد له هيكل كليرودان وكذلك ثلاثة مركبات فلافونويدية معروفة (كرايسوسبيلينول D وكرايسوسبيلتين وكاستيسين) من سوق نبات بوليكاريا سومالينسيس وأوراقه. وقد تمّ التعرف على التركيب البنائي لهذه المركبات بواسطة الطرق الطيفية. وتجدر الإشارة إلى أن الفلافونويدات كرايسوسبيلتين وكاستيسين تم عزلها لأول وهلة من نباتات جنس بوليكاريا.