

GUAIANOLIDES FROM THE LEAVES OF *CENTAUREA INCANA**

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(Revised received 28 June 1985)

Key Word Index—*Centaurea incana*; Compositae; sesquiterpene lactones; guaianolides.

Abstract—Six guaianolides have been isolated from the aerial parts of *Centaurea incana* from Algeria. They were identified as desoxyrepin, repin or subluteolide, repin monochlorhydrin, acroptilin, janerin and repidiolide triol.

INTRODUCTION

Centaurea species have been the subjects of many phytochemical investigations [1–7]. They have been reported to contain a wide variety of sesquiterpene lactones, many of which are biologically active [8]. As part of a systematic examination of plants native to the Aures area (Algeria), we set out to study the content of the aerial parts of *Centaurea incana* Desf., a plant allegedly used in folk medicine [9].

RESULTS AND DISCUSSION

Six compounds were isolated. They were identified by their physical properties and comparison with literature data. In order of increasing polarity, they were: desoxyrepin (1), repin or subluteolide (2), 'repin' [10, 11] monochlorhydrin, acroptilin (3) and its 4,15-regioisomer (4), janerin (5) and repidiolide triol (6). To the best of our knowledge compounds 3 and 6 are novel.

The mass spectral ($[M]^+$, 300–400 a.m.u.), IR (double bands at 1770 and 1660 cm^{-1}) and high field ^1H NMR data of compounds 1–6 indicated that they were sesquiterpene lactones of the guaiane type. This was not totally unexpected on chemotaxonomic grounds. Compounds 1, 4 and 5 were identified as 17,18-desoxyrepin (18-desoxyjanerin) [12], acroptiline (chlorohyssopifolin C) [13, 14] and janerin [15] (UV, IR, NMR, MS).

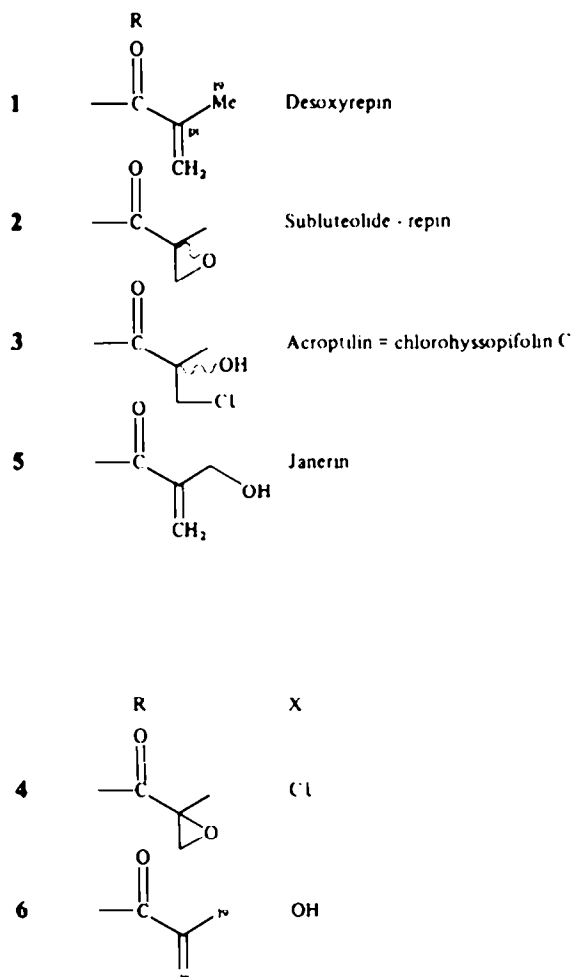
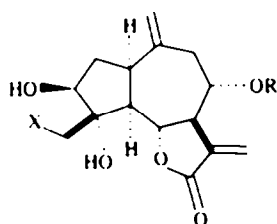
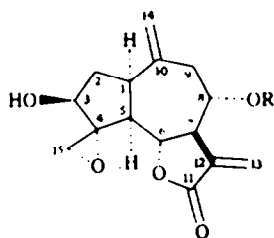
The MS of compound 2 showed a molecular ion at m/z 362 with fragments at m/z 57 and 260. Its ^1H NMR spectrum showed signals for an α -methylene lactone, an isolated $\text{CH}_2=\text{C}$ and two epoxy rings. All these data, as well as the $[\alpha]_D$, IR and UV data, suggested that 1 was either repin or subluteolide. The somewhat tenuous differences between those two products have been summarized by Stevens [16]. The ^1H NMR spectrum of 2, obtained at 400 MHz was not fundamentally different from the spectrum of subluteolide [7, 8] obtained at

240 MHz; minor differences between them may be due to solvent effects or to traces of impurities. The spectrum of repin was recorded at 90 MHz at which field, overlap or second order effects may arise. An important difference between the two spectra may be eliminated by inversion of the assignments of H-15 and H-18 in repin (glycidic esters invariably have a larger geminal coupling constant than ordinary epoxides [19]). It remains a possibility, however, that repin and subluteolide could differ in the configuration of the epoxy isobutyric acid moiety. In the absence of any other evidence it is not possible to make a distinction between 2, repin and subluteolide.

Compound 3 was a chlorinated substance as shown by the presence in its mass spectrum of a double molecular ion at m/z 398–400 ($\text{C}_{19}\text{H}_{23}\text{O}_7\text{Cl}$). The presence of ions at m/z 297–299 ($\text{C}_{15}\text{H}_{19}\text{O}_4\text{Cl}$), 260 and 57 indicated the presence of a $\text{C}_4\text{H}_3\text{O}_3$ side chain and of a chlorine atom on the sesquiterpene nucleus. ^1H NMR spectroscopy established that the side chain took the form of an epoxy isobutyric acid residue (Me at δ 1.58, CH_2 at δ 2.82 and 3.18, $J = 6$ Hz; these data are in good agreement with those found for the side chain of 2). Decoupling experiments allowed determination of the protons attached to C-1, C-2, C-3, C-5, C-6, C-7, C-8, C-9, C-13 and C-14. An AB system (δ 4.32, 3.94, $J = 11.5$ Hz) was attributed to CH_2 -15; the geminal coupling constant and chemical shifts were best accommodated into a chlorhydrin system with the chlorine on the primary carbon atom. The deshielding of H-3 (+0.2 ppm) in 3 was a consequence of the disappearance of the shielding epoxide ring. We thus propose for 3 the structure of 15,4-chlorohydrin of repin-subluteolide.

Compound 6 was a methylacrylic acid derivative as shown by a base peak at m/z 69 in the MS. Other fragments were found at m/z 260 and 278, indicating a supplementary hydroxyl on the sesquiterpene nucleus. The peak at highest mass appeared at m/z 333. Confirmation of the presence of an α -methylacrylic ester chain was provided by the ^1H NMR data (two broad quintuplets at δ 6.20 and 5.67; Me at δ 1.98). No epoxide signal was detected; instead an AB system appeared at δ 3.96 and 4.03 with a 12 Hz coupling constant. This was attributed to a CH_2OH unit and formula 6, a triol, was

*This article is dedicated to the memory of the late Dr R. Toubiana.



proposed for this compound. The m/z 333 ion has now been assigned to $[M - CH_2OH]^+$. As expected, 6 yielded a triacetate on acetylation with acetic anhydride and DMAP.

Further characterization of the guaianolides was obtained by ^{13}C NMR spectroscopy of acroptilin (3) and janerin (5). The data were interpreted on the basis of previous assignments [10] (Table 1). Although some resonances were not assigned without ambiguity (C-6 and C-8), it is clear that 3 and 5 possess the same relative stereochemistry of their carbons 1, 3-8.

Inspection of the high field 1H NMR data for 1-6 also revealed that all these compounds belong to the same series. The coupling constants observed between H-1, -2, -3, -5 and -6 were identical within experimental error. Curiously, opening of the 4,15-epoxide had its strongest effect on the H-8, H-9 α coupling constant indicating a deformation of the seven membered ring. We thus conclude that 1-6 have the configuration of subluteolide, which was established by Mompon and Toubiana, on the basis of lanthanide induced shifts and nuclear Overhauser effects [18].

Note. After this manuscript was submitted for publication, we found that compound 3 had been isolated from *Centaurea aegyptica* by Dr F. Bohlmann [11].

EXPERIMENTAL

General. Plant material was collected in June, 1984, at Djerna Pass, 20 km West of Batna (elevation 1200 m) and was identified

Table 1. ^{13}C NMR data for compounds 3 and 5 (15 MHz, EtOAc- d_6 , TMS as int. standard)

C	3	5	C	3	5
1	46.7*	46.3*	11	139.0	139.1
2	36.1†	36.8†	12	169.3	169.3
3	76.2‡	76.0	13	121.9	121.5
4	69.0	69.0	14	118.5	118.1
5	54.0	53.5	15	51.4	48.4
6	76.3‡	77.6	16	173.3	165.9
7	48.1*	48.2	17	75.8	141.8
8	76.2‡	74.7	18	48.5	61.3
9	39.2†	38.9	19	24.0	125.0
10	143.3	143.5			

*†‡Assignments within the same column may be interchanged.

Table 2. ¹H NMR data for compounds 1-6

	H-1	H-2	H-2'	H-3	H-5	H-6	H-7	H-8	H-9	H-9'	H-13	H-13'	H-14	H-14'	H-15	H-15'	H-18	H-18'	H-19
1°	3.33	2.43	1.85	4.0	4.64	5.13	2.76	2.4	6.2	5.59	5.17	4.95	3.08	3.33	6.19	5.67	1.99	s	
q	ddd	ddd	ddd	ddd	ddd	ddd	ddd	ddd	ddd	ddd	ddd	ddd	ddd	ddd	ddd	ddd	ddd	ddd	
q	3.33	2.43	1.78	3.96	2.02	4.58	3.03	5.0	2.74	2.34	5.72	4.92	3.04	3.28	3.14	2.8	1.57	s	
q				ddd	ddd	ddd	ddd	ddd	ddd	ddd	ddd	ddd	ddd	ddd	ddd	ddd	ddd	ddd	
3°	3.58	2.5	1.56	4.16	2.3	4.7	3.1	5.0	2.64	2.4	5.74	4.8	4.32	3.94	3.18	2.82	1.58	s	
ddd	ddd	ddd	ddd	ddd	ddd	ddd	ddd	ddd	ddd	ddd	ddd	ddd	ddd	ddd	ddd	ddd	ddd	ddd	
4°	3.35	2.5	1.8	3.95	1.98	4.68	3.03	5.15	2.75	2.45	5.8	5.15	4.9	3.05	3.3	3.84	3.63	1.53	s
ddd	ddd	ddd	ddd	ddd	ddd	ddd	ddd	ddd	ddd	ddd	ddd	ddd	ddd	ddd	ddd	ddd	ddd	ddd	
5°	3.36	2.45	1.82	3.98	2.06	4.65	3.08	5.15	2.78	2.4	5.6	4.95	3.08	3.33	6.32	5.96	4.38	d	
q	ddd	ddd	ddd	ddd	ddd	ddd	ddd	ddd	ddd	ddd	ddd	ddd	ddd	ddd	ddd	ddd	ddd	ddd	
6°	3.33	2.35	1.87	4.16	2.2	4.56	3.15	5.05	2.78	2.4	6.2	5.64	4.94	4.03	3.96	6.2	5.67	1.98	s

by Drs K. Absdessem (Batna Forests Institute) and J. Alyafi. $[\alpha]_{20}^{20}$: CHCl_3 ; IR: CHCl_3 , films; ^1H NMR: 400 MHz; ^{13}C NMR: 15 MHz.

Extraction procedure. Air dried material (2.15 kg) was extracted by percolation with 40 l. CH_2Cl_2 . Evaporation of the solvent gave 109 g of a thick oil, which was diluted with EtOH (1 l.) and hot H_2O (500 ml) and stirred overnight with 4% $\text{Pb}(\text{OAc})_2$ and charcoal. After filtration, the filtrate was partitioned between CH_2Cl_2 and H_2O . The organic phase yielded 42 g (20 g/kg) of a waxy residue, 15 g of which was chromatographed on silica gel. Elution of the column with CHCl_3 and a gradient of MeOH in CHCl_3 (up to 5%) yielded sesquiterpenes 1 (2%), 2 (1%), 3 (6%), 4 (10%), 5 (12%) and 6 (15%).

Repin (subluteolide)-15,4-chlorhydrine (3). IR $\nu_{\text{max}}^{\text{CHCl}_3}$, cm^{-1} : 3500, 1765, 1740, 1660, 1640, 1170, 1140, 755, 730; MS m/z (rel. int.): 400 (0.1), 398 (0.2), 362 (0.2), 348 (0.4), 278 (5), 260 (10), 57 (100).

$$[\alpha]_{20}^{20} = \frac{589}{+58} \frac{578}{+59} \frac{546}{+68} \frac{436}{+110} \frac{365}{+163} \quad (c = 0.44; \text{CHCl}_3).$$

Repdiolide triol (6). IR $\nu_{\text{max}}^{\text{CHCl}_3}$, cm^{-1} : 3420, 1765, 1715, 1670, 1645, 1160, 810, 750; MS m/z (rel. int.): 333 (5), 316 (2), 278, 260 (2), 247 (5), 69 (100).

$$[\alpha]_{20}^{20} = \frac{589}{+42} \frac{578}{+44} \frac{546}{+51} \quad (c = 0.65; \text{CHCl}_3).$$

Acknowledgements— Support of the program 'Plantes des Aurès' by O.N.R.S. (Algeria), C.N.R.S. (France) and the French Embassy in El-Djazair is gratefully acknowledged. We thank Dr Mompon (LERS, Synthelabo) for fruitful discussion and exchange of information and Dr S. K. Kan for access to his NMR spectrometer.

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