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ENT-NORLABDANE TRIOLS FROM *AUSTROEUPATORIUM INULAEFOLIUM*

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Key Word Index—*Austroeupatorium inulaefolium*; Compositae; Eupatorieae; *ent*-norlabdane triols.

Abstract—Two new *ent*-norlabdane triols were isolated from the above-ground parts of *Austroeupatorium inulaefolium*.

INTRODUCTION

Austroeupatorium is a South American genus of about twelve species [1]. Previous phytochemical studies dealt with the roots of the widely distributed type species *A. inulaefolium* [2, 3] and with *A. chaparense* [4]. *Ent*-kauranes and *ent*-labdanes were found in addition to common representatives of other classes of compounds. We now report isolation of two new *ent*-norlabdane triols **1a** and **2a** from the herb of *A. inulaefolium* collected in Córdoba, Argentina.

RESULTS AND DISCUSSION

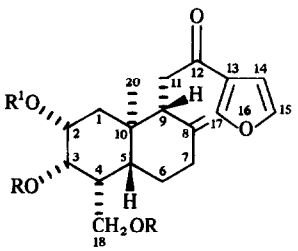
The compounds **1a** and **2a** had the empirical formulas $C_{19}H_{26}O_5$ and $C_{19}H_{26}O_6$, respectively, deduced from the high resolution mass spectra and the ^{13}C NMR spectra (Table 1) which exhibited 19 carbon signals each. The presence of a 3-furoyl group, suspected on the basis of the IR spectra (see Experimental) was confirmed by three characteristic ^1H NMR signals (Table 2) near δ6.75 (H-14), 7.5 (H-15) and 8.1 (H-16), that of H-16 being considerably farther downfield from the more normal position near 7.2 due to the neighboring carbonyl group. It was also made evident by the mass spectral base peak at *m/z* 95 ($C_5H_3O_2$) and the ^{13}C NMR spectra which exhibited the signal of a conjugated carbonyl group near δ196.

Initially it was difficult to reconcile the empirical formulae with the ^1H NMR spectra run in $CDCl_3$–DMSO–H_2O and with the results of small scale acetylation experiments which showed that both **1a** and **2a** gave mixtures of a diacetate (**1b** from **1a**, **2b** from **2a**, major products) and a triacetate (**1c** and **2c**, minor products). In the ^1H NMR spectra of the diacetates two signals, eventually shown to be those of H-3 and H-19b) were shifted downfield and in the spectra of the triacetates three (those of H-2, H-3 and H-19b) were shifted downfield while that eventually shown to be associated with H-19a remained essentially unaffected. However, determination of the ^1H NMR spectra in CD_3OD eventually solved these discrepancies, especially since the ^{13}C NMR spectra clearly showed not only the presence of one CH_2O and two CH–O groups in both compounds, but demonstrated that the extra oxygen of **2a** was a cyclohexanone carbonyl and provided additional evidence for the deduction that **2a** was a norlabdane lacking C-18 in which the C-17 exocyclic methylene group of **1a** had been reduced. The location of the new carbonyl at C-7, also evident from the downfield shifts of H-6a,b, was established by sequential spin decoupling of H-8 through H-11a,b; similar sequential decoupling of H-1 through H-6, and the demonstration that H-4 was coupled to H-19a,b permitted elaboration of the entire carbon skeleton, as the remaining unassigned tertiary methyl group could only be placed on C-10. Chemical shifts of H-2, H-3 and H-19 required that

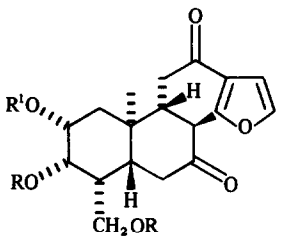
Table 1. ^{13}C NMR spectra of compounds **1a** and **2a** (67.89 MHz)

Carbon	**1a***	**2a**†
1	44.15 *t*§	43.08 *t*§
2	72.32 *d*§	71.35 *d*§
3	75.64 *d*	74.75 *d*
4	} 48.41‡	47.11 *d*‖
5		47.06 *d*‖
6	29.98 *t*	43.87 *t*
7	38.56 *t*§	210.22
8	149.77	48.04‖
9	52.41 *d*	51.03 *d*
10	38.22	36.68
11	37.59§	41.21§
12	196.89	193.79
13	129.34	128.28
14	109.33 *d*	109.19 *d*
15	145.90 *d*	145.08 *d*§
16	149.60 *d*	148.44 *d*
17	108.30 *t*	12.84 *q*§
19	62.15 *t*	60.76 *t*
20	16.75 *q*	15.80 *q*

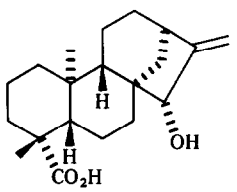
*In CD_3OD.
†In C_5D_5N.
‡Obscured by solvent signal.
§Assignment by selective spin decoupling.
‖Assignments may be interchanged.



1a R, R^1 = H
1b R = Ac, R^1 = H
1c R, R^1 = Ac



2a R, R^1 = H
2b R = Ac, R^1 = H
2c R, R^1 = Ac



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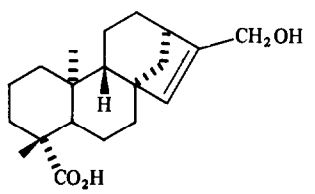

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the three hydroxyl groups be placed on C-2, C-3 and C-19, respectively.

As for stereochemistry, the value of $J_{5,6}$ (14 Hz) showed that H-5 is axial. Comparison of the chemical shifts of H-20 in **1a** and **2a** (and in the corresponding acetates) indicated that the C-10 methyl group is also axial; the usual paramagnetic shift experienced by H-20 of the common labdanes on saturation of the $\delta(17)$-double bond [5] is in this instance exacerbated by presence in **2a** of a 7-keto group which deshields the axially-orientated C-10 methyl (model). Similarly the magnitude of $J_{8,9}$ in **2a** (12 Hz) showed that H-8 and H-9 are axial; hence **1a** and **2a** possess the C-5, C-8, C-9 and C-10 stereochemistry found in other labdanes.

That the C-2 hydroxyl group is axial was made evident by the values of $J_{1\alpha,2}$ and $J_{1\beta,2}$ (both 3 Hz); however, the stereochemistry at C-3 and C-4 remained somewhat questionable although the values of $J_{2,3}$ (3 Hz), $J_{3,4}$ (6 Hz) and $J_{4,5}$ (4.5 Hz) suggested that H-3 and H-4 are equatorial rather than axial. This conclusion and the earlier deductions about the stereochemistries of the other centers were placed on a secure basis by NOE difference spectroscopy. The results for **1a** are shown in Table 3 and are only compatible with the stereochemistry depicted in the formula. The presence of bulky axial substituents at C-4 and C-10 also accounts for the sluggishness with which the axial C-2 hydroxyl of **1b** and **2b** undergoes further acetylation.

The absence of sesquiterpene lactones seems to distinguish the two *Austroeupatorium* species which have been investigated so far from most representatives of *Eupatorium sensu stricto*. How far this generalization can be extended will require further study.

EXPERIMENTAL

Above ground parts (leaves and flowers) of *A. inulaefolium* (HBK) King and Robinson (6.8 kg), collected on the grounds of Ciudad Universitaria de Córdoba, Argentina, in April 1982 were extracted with $CHCl_3$ and worked up in the usual fashion yielding 130 g of crude gum. A 30 g portion of the gum was chromatographed over 400 g of silica gel packed in $CHCl_3$, 50 ml fractions being collected as follows: Fr 1–10 ($CHCl_3$), 11–31 ($CHCl_3$–Me_2CO, 4:1), 32–62 ($CHCl_3$–Me_2CO, 3:2), 53–73 ($CHCl_3$–Me_2CO, 2:3) and 74–94 ($CHCl_3$–Me_2CO, 1:4).

Fractions 14–17 were combined and gave a white solid (15 mg) on recrystallization from C_6H_6–hexane which was a mixture of **3** and **4** (NMR analysis). Combination and recrystallization of fr. 47–51 from C_6H_6–EtOAc gave 0.9 g of solid material contaminated by gum which was rechromatographed over silica gel packed in $CHCl_3$. The fraction eluted with $CHCl_3$–MeOH (24:1) was **1a** (0.188 g), mp 179° (dec), IR ν_{max} cm^{-1}: 3390, 1658, 1554, 1512 and 872 [Calc for $C_{19}H_{26}O_5$: MW, 334.1733. Found: MW(MS), 334.1763] Significant peaks in the low resolution MS were at *m/z* (rel. int.): 334 (5), 316 (1.6), 298 (1.8), 286 (3.4), 268 (1.6), 215 (2.0), 207 (15.8), 197 (2.1), 189 (8.5), 176 (12.3), 170 (5.4), 163 (20.5), 161 (7.6), 158 (17.6), 143 (16.0), 123 (16.8), 121 (18.2), 119 (12.0), 110 (8.9), 107 (14.1), 105 (22.8), 95 (100), 91 (17.8), 81 (14.7), 79 (17.0). In the CI spectrum, the base peak was *m/z* 335 $[M+1]^+$. A 5 mg portion of the substance was acetylated (Ac_2O–pyridine) and worked up in the usual fashion; the NMR spectrum of the resulting 2:1 mixture of **1b** and **1c** is listed in Table 2

Fractions 52–70 were combined and recrystallized from C_6H_6–Me_2CO to give 0.65 g of a mixture of **1a** and **2a** Rechromatography of a 0.45 g portion of the mixture over silica gel and elution with $CHCl_3$–MeOH (24:1) afforded 38 mg of **1a**

Table 2. ^{1}H NMR spectra (270 MHz) of compounds **1** and **2** and their derivatives

Proton	1a*	1a†	1b‡§	1c‡§	2a*	2a†	2b‡§	2c‡§
1α	2.02 *dd* (14, 2 5)	1.94 *dd*			2.03 *dd*	1.95 *dd*		
1β	1.38 *dd* (14, 3)	1.34 *dd*			1.29 *dd*	1.23 *dd*		
2	4.09 *q* (3)	4.03 *q*	4.18 *q*	5.33 *q*	4.09 *q*	4.03 *q*	4.16 *q*	5.33 *q*
3	3.83 *dd* (6, 3.5)	3.78 *dd*	4.84 *dd*	4.96 *dd*	3.82 *dd*	3.75 *dd*	4.84 *dd*	4.96 *dd*
4	2.20 *m*	2.13 *m*			2.17 *m*	2.03 *m*		
5	1.69 *m*	1.71 *m*			1.91 *ddd* (14, 4.5, 3)	1.84 *ddd*		
6α	1 69 *m*	1.71 *m*			2.69 *t* (14)	2.83 *t*		
6β	1.56 *m*	1 50 *m*			2.27 *dd* (14, 3)	2.18 *dd*		
7α	2.40 *ddd* (13, 4, 2)	2.36 *ddd*			—	—	—	—
7β	2 20 *m*	2.13 *m*			—	—	—	—
8	—	—			2.28 *dq* (12, 7)	2.43 *dq*		
9	‖	2.50 *ddbr* (10, 3)	2.62 *ddbr*		2.17 *m*	2.03 *m*		
11a	3.00 *dd* (17, 10)	3 10 *dd*	3 03 *dd*		2.92 *dd* (18, 2.5)	2.88¶ (4.5)	2.87 *dd* (18, 3)	
11b	2.76 *dd* (17, 3)	2.75 *dd*	2.72 *dd*		2.71 *dd* (18, 6)		obsc	
14	6.76 *dd* (1.5, 1)	6.75 *dd* (2, 1)	6 78 *dd* (1.5, 1)		6 78 *dd*	6.80 *dd*	6.78 *dd*	
15	7.45 *t* (1 5)	7.58 *dd* (2, 1)	7.44 *t* (1.5)		7.48 *t*	7.60 *t*	7.47 *t*	
16	8.11 *dd* (1.5, 1)	8.43 *dd* (2, 1.5)	8.09 *dd* (1.5, 1)		8.11 *dd*	8.43 *dd*	8.08 *dd*	8.06 *dd*
17a	4.76 *br*	4.96 *br*	4.80 *br*		0.90 *d*** (7)	0.76 *d***	0.91 *d***	
17b	4.38 *br*	4.36 *br*	4.40 *br*					
19a	4.47 *t* (10)	4.39 *dd* (10.5, 8.5)	4.71 *dd* (11, 7)	4.69 *dd*	4.54 *t* (10)	4.40 *dd* (10.5, 8)	4.72 *dd* (11, 5.5)	4.68 *dd*
19b	3.62 *dd* (10, 2)	3.60 *dd* (10.5, 3 5)	4.36 *dd* (11, 3)	4 33 *dd*	3.66 *dd* (10, 2)	3.75 *dd* (10.5, 2)	4.42 *dd* (11, 5)	4.41 *dd*
20**	0.88	0 85	0.96	0.89	1.24	1 25	1.33	
OAc**	—	—	2.13, 2.01	2.12, 2.05 2 02	—	—	2.13, 2.01	2 22, 2.04 2.02

*In $CDCl_3$–DMSO–D_2O.
†In CD_3OD.
‡In $CDCl_3$.
§Chemical shifts obtained from mixture. Signals are not entered when identical with those of **1a** (for **1b** and **1c**) or **2a** (for **2b** and **2c**)
‖Obscured by solvent.
¶Intensity two protons.
**Intensity three protons.

and 299 mg of **2a**. The latter melted at 207°; IR ν_{max} cm^{-1}: 3465, 1672, 1564, 1511 and 875. [Calc. for $C_{19}H_{26}O_6$: MW, 350.1727. Found: MW(MS), 350.1719]. Significant peaks in the low resolution MS were at *m/z* (rel. int.): 350 $[M]^+$ (6.8), 332 (6.8), 314 (3 9), 296 (2), 241 (10.1), 222 (8.1), 204 (9.0), 192 (6.7), 177 (8.1), 175 (7.6), 159 (10.9), 149 (22.8), 135 (12.0), 123 (18.2), 121 (11.2), 110 (50.0), 107 (11.9), 97 (20.6), 95 (100), 93 (13.9), 82 (20.1), 81 (7.3), 79 (10.9). Acetylation of 5 mg of this substance (Ac_2O–pyridine) and work-up in the usual fashion gave a 2:1 mixture of **2b** and **2c** whose NMR spectrum is listed in Table 2.

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Table 3. NOE difference spectroscopy results for compound **1a**

Saturation (in CD_3OD)	Observed NOE (%)	Saturation (in CD_3OD)	Observed NOE (%)
H-1β	H-2β (9)	H-3β	H-2β (8)
	H-3β (5)		H-1β (4)
	H-5β (5.5)		H-4β (8)
	H-9 (6)		H-5β (6)
	H-1α (20)	H-5β and H-6α	H-3β (9)
H-1α	H-2β (8)		H-19β (9)
	H-11β (11)		H-9β (11)
	H-1β (16)		

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