Phytochemistry, Vol. 26, No. 8, pp. 2315–2320, 1987.
Printed in Great Britain.

0031–9422/87 $3.00 + 0.00

MELAMPOLIDES FROM *MIKANIA CORDIFOLIA*

ALICIA B. GUTIÉRREZ, JUAN CARLOS OBERTI, VIRGINIA E. SOSA* and WERNER HERZ*

Instituto Multidisciplinario de Biologia Vegetal, CONICET and Facultad de Ciências Quimicas, Universidad Nacional de Córdoba, 5016 Córdoba, Argentina; *Department of Chemistry, The Florida State University, Tallahassee, Florida 32306-3006, U.S.A.

(*Received* 4 *December* 1986)

Key Word Index—*Mikania cordifolia*; Eupatorieae; Compositae; Melampolides; sesquiterpene lactones.

Abstract—Aerial parts of two collections of *Mikania cordifolia* from northern Argentina afforded eight new melampolides whose structures were established by chemical and spectroscopic means. Two of these appear to be mixtures of ester side chain epimers.

INTRODUCTION

Mikania cordifolia (L.f.) Willd. (Eupatorieae, Compositae) is a widespread species ranging from Gulf coastal U.S.A. (Louisiana, Mississippi and Florida) through wet-tropical America into northern Argentina. In previous work on *M. cordifolia*, a collection from Puerto Rico furnished the interesting polyfunctionalized elemanolide micordilin (**1**) [1]. Work on the isolation of micordilin [1] dates back to the late 1960s and it is possible that other, noncrystalline lactone constituents present in the extract of Puerto Rican *M. cordifolia* were overlooked. A collection of the plant from Ecuador gave no micordilin, but several germacradienolides of type **2** [2]. As part of our continuing study of *Mikania* species [3] we now report isolation from two Argentine collections of *M. cordifolia* of a series of closely related melampolides **3a**–**3h** where **3d** and **3g** are apparently mixtures of C-2′ epimers. Again no micordilin was found. Both collections also contained a small amount of acetophenetidine. As this substance has not been reported previously as a natural product, it may have been a contaminant.

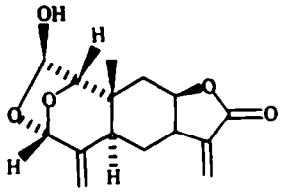
RESULTS AND DISCUSSION

The two collections of *M. cordifolia* from the provinces of Salta and Catamarca gave similar mixtures of the melampolide esters **3a**–**3g** which also contained the parent diol **3h**. In the Catamarca material ester **3f** predominated. The constituents were very difficult to purify, with **3a** and **3b**, and **3g** and **3h**, being obtained only in the form of binary mixtures, and they tended to polymerize. Characterization was therefore carried out primarily by spectroscopic methods. ^{1}H NMR spectra of the purified lactones in $CDCl_3$ or C_6D_6 at room temperature showed, except for the narrowly split low field doublets of H-13a, b and the signals which allowed identification of the various ester residues, only broad partially superimposed signals. Significant improvement in the spectra was achieved at elevated temperature, especially in C_6D_6.

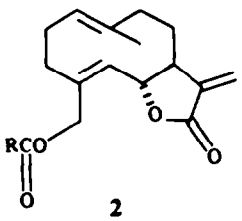
The main features of the very similar ^{1}H NMR spectra of **3a**–**3h** (Table 1) were the H-13 doublets near δ6.20 and 5.50 (J = 3 Hz) and the two vinylic protons (H-1 and H-5) partially superimposed on the H-13b signal. An AB system centered at about δ4.50 (J_{AB} = 12 Hz) could be ascribed to allylic $-CH_2OCOR$, either H-14 or H-15, and a broad two proton singlet at δ4.12 to allylic $-CH_2OH$ (H-15 or H-14). The proton under the lactone oxygen was seen at approx. δ3.85 as a broad multiplet which sharpened to a *dt* (J = 8, 3, 3 Hz) on raising the temperature. Irradiation at this frequency, which because of its multiplicity was tentatively assigned to H-8, collapsed a pair of *dd* at δ2.72 and 2.44, presumably due to H-9a, b, to an AB system (J_{AB} = 14 Hz) and simplified a two proton multiplet near δ2.8 which represented H-7 and one of the other four methylene protons, either H-2a or H-6a. Further irradiation at the frequency of the δ2.8 multiplet converted not only the H-13a, b doublets into singlets, but also collapsed the *dd* of H-5 (or H-1) under the H-13b signal into a doublet.

Comparison of the ^{1}H NMR spectra of **3a**–**3h** with the spectra of the acetates **4d**, **4i**–**4l** (Table 2) did not resolve the question of whether the acyl groups of the various lactones were located on C-14 or C-15 and did not remove the slight uncertainty about the orientation of the lactone ring. Therefore **3a**–**3c** and **3e** were oxidized with manganese dioxide to the corresponding aldehydes **5a**–**5c** and **5e** in whose ^{1}H NMR spectra (Table 3) the relevant signals were now nicely separated, with the broadened triplet near δ6.6 being clearly assignable to hydrogen on the β-carbon of an α,β-unsaturated aldehyde. Irradiation at the frequency of the second vinylic ring hydrogen, now shifted somewhat upfield to near δ5.3, located the two protons of the neighboring methylene group at δ2.74 (superimposed on H-7) and δ2.15, the latter a sharp *dt* (J = 13, 11, 11 Hz). Irradiation at δ2.15 in turn simplified *both* components of the two proton signal at δ2.74, thus identifying the signals at δ2.74, 2.15 and 5.30 as arising from H-6a together with H-7, H-6b and H-5, respectively and locating the aldehyde as C-14. Hence, the parent compounds **3a**–**3g** carried the acyl functions on C-15 and the lactone rings were closed to C-8.

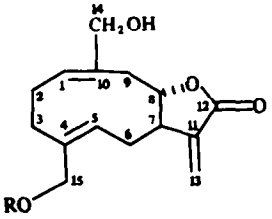
The remaining problem was the stereochemistry of the two ring double bonds. The chemical shift of the aldehyde protons of **5a**–**5c** and **5e** which appeared near δ9.5 indicated clearly that the 1(10)-double bond was *E* [4]; additional evidence for this and for *Z*-stereochemistry of



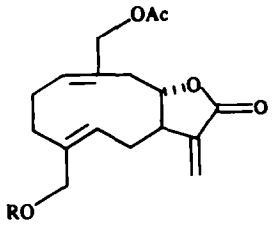
1



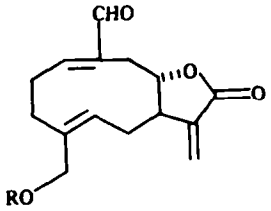
2



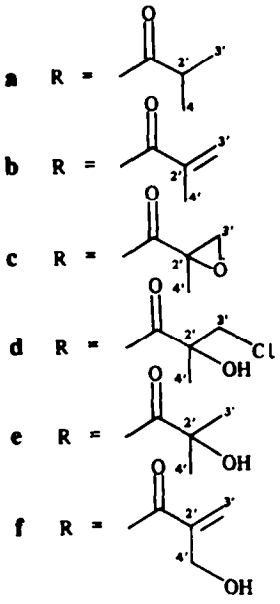
3



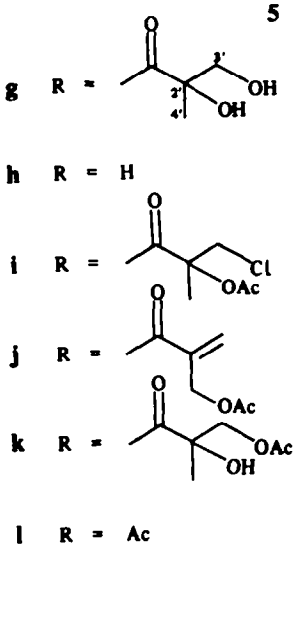
4



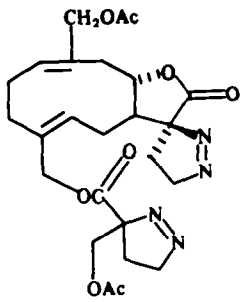
5



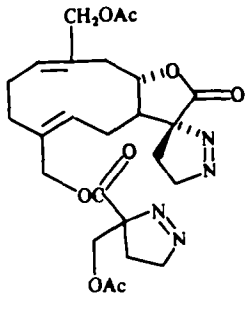
a R =
b R =
c R =
d R =
e R =
f R =



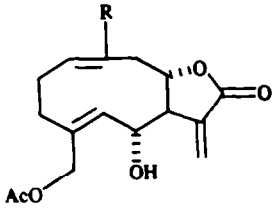
g R =
h R = H
i R =
j R =
k R =
l R = Ac



6



7



8a R = CH_2OAc
8b R = CHO

the 4,5-double bond was provided by NOE difference spectrometry using a freshly prepared mixture of **5a** and **5b**, the other aldehydes having decomposed in the interval. It seems necessary to stress that while the conformational equilibrium evident from the NMR spectra of many 1(10),4,5-germacradien 8β,12-olides interferes with use of NOE measurements for deducing conformations and orientations of ring substituents, it does not negate use of the technique for determining *E* and *Z* double bond stereochemistry. Irradiation at the frequency of H-14

Table 1. ^{1}H NMR spectra of compounds **3a**–**3h** ($CDCl_3$, 270 MHz, 60°)

H	3a*	3b*	3c	3d§	3d†	3e	3f	3f†	3g‡¶	3h‡
1	5.38 *br t* (8)	5.36 *br t*	5.62 *br t*	5.62 *br t* (7)	5.36 *br t* (8)	5.60 *br t*	5.55	5.34 *br t*	5.61 *br t* (7)	5.65 *br t* (8)
5	5.04 *dd* (11, 5)	5.04 *dd*	5.50 *br dd* (10, 6)	5.60 *br dd* (11, 5)	5.04 *dd*	5.55	5.59 *br dd* (10, 6)	5.01 *br dd* (11, 5.5)	5.58 *br dd*	5.45 *dd* (12, 5)
6a			2.80 *m*	2.80 *m*		2.80 *m*			2.80 *m*	2.80 *m*
7	~2.2 *m*	~2.2 *m*	2.82 *m*	2.82 *m*	~2.2 *m*	2.82 *m*	2.82 *m*	~2.2 *m*	2.80 *m*	2.80 *m*
8	3.55 *dt* (8, 3.5)	3.55 *dt*	3.80 *dt* (8, 3)	3.82 *dt* (9.4)	3.50 *dt* (8, 3.5)	3.87 *dt* (9, 3.5)	3.87 *dt*	3.52 *dt* (8.5, 3.5)	3.80 *dt* (8, 3)	3.90 *dt* (8, 3.5)
9a	2.29 *dd* (14, 3.5)	2.29 *dd*	2.89 *dd* (14, 3)	2.72 *dd* (14, 4)	2.22 *dd* (14, 3.5)	2.72 *dd* (15, 3.5)	2.72 *dd*	2.25 *dd* (14, 3.5)	2.71 *dd* (14, 3)	2.71 *dd*
9b	2.17 *dd* (14, 3.5)	2.17 *dd*	2.47 *dd* (14, 3)	2.45 *dd* (14, 4)	2.10 *dd* (14, 3.5)	2.44 *dd* (15, 3.5)	2.46 *br dd*		2.45 *dd*	2.45 *dd*
13a	6.08 *d*(3)	6.08 *d*	6.25 *d*	6.22 *d*	6.08 *d*	6.22 *d*(3.5)	6.22 *d* (3)	6.09 *d*	6.22 *d*	6.22 *d*
13b	4.98 *d* (3)	4.98 *d*	5.55 *d*	5.54 *d*	4.99 *d*	5.55 *d*	5.50 *d*	4.97 *d* (2.5)	5.50 *d*	5.50 *d*
14a, b	3.92 *br*	3.92 *br*	4.12 *br*	4.13 *br*	3.91 *br*	4.12 *br*	4.12 *br*	3.89 *br*	4.12 *br*	4.12 *br*
15a	4.51 *d* (12)	4.54 *d*	4.64 *d*	4.73 *d*	4.50 *d*	4.66 *d* (12.5)	4.69 *br d*	4.49 *br d*	4.68 *d* (12)	4.02 *br*
15b	4.25 *d* (12)	4.32 *d*	4.48 *d*	4.50 *d*	4.27 *d*	4.50 *d* (12.5)	4.58 *d*	4.24 *d*	4.55 *d* (12)	4.02 *br*
2'	2.40 *m*	—	—	—	—	—	—	—	—	—
3'a	1.06 *d* (7)‖	5.26 *br*	3.08 *d* (6)	3.77 *d* (11.5)	3.57 *d*	1.41 *s*‖	6.32 *br*	6.15 *br q* (1.5)	3.80 *d* (11.5)	—
b	—	4.98 *br*	2.86 *d* (6)	3.56 *d*	3.33 *d*		5.86 *br q*	5.58 *br q*	3.58 *d* (11.5)	—
4'	1.06 *d* (7)‖	1.82 *br*‖	1.58 *s*‖	(1.475)‖	1.30 *s*‖	1.43 *s*‖	(1.35) *br*	4.18 *br*	1.38 *s*‖	—

*Run in C_6D_6 at 70° on a 3:2 mixture.
†Run in C_6D_6 at 70°.
‡Chemical shifts from mixture of **3g** and **3h**.
§Second acyl epimer—3.78 *dd* (11.5) (H-3'a), 3.57 *d* (11.5) (H-3'b), 1.49 *s* (H-4').
¶Second acyl epimer—3.80 *d* (11.5) (H-3'a), 3.60 *d* (11.5) (H-3'b), 1.41 *s* (H-4').
‖Intensity three protons.

produced a 21% enhancement in the H-1 signal (in the converse experiment irradiation at the frequency of H-1 produced a 23% enhancement of H-14) while irradiation at the frequency of H-5 caused no significant change in the intensity of H-14a or H-14b. Hence **3a**–**3h** were melampolides. Chemical shifts and coupling constants of **3a**–**3h** and **5a**, **5c**–**5e** conform reasonably closely to those of 'acetoxydicomanolide' and '14-oxodicomanolide' from *Dicoma anomala* ssp. *cirsioides* [5] whose originally assigned 1(10)-*Z*,4,5-*Z* stereochemistries were subsequently [6] changed to **8a** and **8b** for reasons that were not clearly specified but must depend on the chemical shift of H-14 of **8b**.

Whether the acyl function esterifying the C-15 hydroxyl group of **3d** and **4d** was derived from α-chloro-β-hydroxyisobutyric or from β-chloro-α-hydroxyisobutyric acid was not immediately clear from the NMR spectra. However, when the spectra of **4d** and **4i** are compared the relatively small paramagnetic shift of H-3'a, b indicates the latter. This conclusion is supported by comparing the spectra of **3d** and **4i** with those of **3g** and **4k** where acetylation of the primary hydroxyl group of the ester side chain has resulted in considerably larger paramagnetic shifts of H-3'a, b than in the case of the conversion of **3d** to **4i**.

In the ^{1}H NMR spectra of **3d** and **3g** ($CDCl_3$, 60°), the signals of the ester side chain were duplicated. As this phenomenon was not observed in the spectra of **3a**–**3c**, **3e**, **3f** we think that the duplicate signals may be due to the presence of C-2' epimers in the approximate ratio 3:1. It is likely that **3d** and **3g** are artifacts resulting from acid-catalysed opening of the ester side chain of **3c**.

The ^{13}C NMR spectra of **3a**–**3f** given in Table 4 resembled each other closely except for the signals of the acyl moieties whose shifts and multiplicities confirmed the inferences about their structure deduced from the proton spectra and which were very sharp in contrast to the signals of the carbons of the 10-membered ring. The latter were somewhat broadened as a result of conformational interconversion, a phenomenon especially marked in the case of the C-9 signal. Assignments were easily made except for those of the triplets of C-2, C-3 and C-6 which remain tentative. As regards the pairs C-1, C-5 and C-4, C-10, their components could be distinguished by comparing the spectra of **3f** and **4j**. Acetylation of the 14-hydroxyl should result in shielding of C-10α and deshielding of C-1β to C-14, whereas the resonances of C-4 and C-5 should not be affected significantly.

Because of the conformational flexibility of the lactones described in this report, the CD curve of **3e** given in the Experimental section provides no clue to the absolute conformation which we assume is that shown in the formulas. Reaction of **4c** with diazomethane furnished two pyrazolines **6** and **7** to which structures were assigned on the basis of the chemical shift differences exhibited by the H-7 and H-8 signals (Table 5).

Table 2. ^{1}H NMR spectra of compounds **4d**, **4i**–**4l** ($CDCl_3$, 270 MHz, 60°)

H	4d	4i	4j	4j*	4k†	4l†
1	5.61 *br t* (7)	5.60 *br t*	5.60 *br t* (8)	5.32 *br t*	5.60 *br t*	5.60 *br t*
2a, b } 3a, b }		2–2.4		2.18–2.30	2.15–2.28	2.15–2.28
5	5.55 *br dd* (11, 5.5)	5.54 *br dd*	5.53 *dd* (11, 5)	5.50 *dd* (11, 5.5)	5.51 *dd*	5.51 *br dd*
6a	2.80 *m*	2.80 *m*	2.80 *m*	2.79 *m*	2.80 *m*	2.80 *m*
6b				2.18–2.3	2.15–2.28	2.15–2.28
7	2.80 *m*	2.80 *m*	2.80 *m*	2.80 *m*	2.80 *m*	2.80 *m*
8	3.81 *dt* (8, 3)	3.82 *dt*	3.88 *br dt* (10, 4)	3.52 *dt*	3.86 *dt* (8, 3)	3.86 *dt*
9a	2.71 *dd* (14, 3)	2.69 *dd*	2.70 *dd* (15, 4)		2.68 *dd* (14, 3)	2.68 *dd*
9b	2.42 *dd* (14, 3)	2.48 *dd*	2.49 *dd* (15, 4)		2.50 *dd* (14, 3)	2.50 *dd*
13a	6.23 *d* (3)	6.22 *d*	6.22 *d*	6.08 *d*	6.22 *d*	6.22 *d*
13b	5.56 *d* (2.9)	5.55 *d* (2.5)	5.55 *d* (3)	4.98 *d* (2.5)	5.53 *d* (3)	5.53 *d*
14a	4.61 *d* (13)	4.60 *d* (13)	4.60 *d* (12)	4.56 *d* (13)	4.60 *d* (12)	4.60 *d*
14b	4.53 *d* (13)	4.52 *d* (13)	4.52 *d* (12)	4.48 *d* (13)	4.53 *d* (12)	4.53 *d*
15a	4.72 *d* (12)	4.74 *d*	4.74 *br d*	4.53 *d*	4.72 *d*	4.52 *d*
15b	4.40 *d* (12)	4.40 *d*	4.52 *br d*	4.32 *d*	4.39 *d*	4.42 *d*
3'a	3.79 *d* (11.5)	4.06 *d*	6.32 *br q* (1.5)	6.22 *br q*	4.49 *d* (11.5)	—
3'b	3.57 *d* (11.5)	3.78 *d*	5.74 *br q* (1.5)	5.55 *br q*	4.32 *d* (11.5)	—
4'	1.42 *s*‡	1.62 *s*‡	4.80 *br*§	4.81 *br*§	1.58 *s*‡	—
Ac‡	2.06 *s*	2.06 *s*	2.06 *s*	1.81 *s*	2.07 *s*	2.07 *s*
		2.05 *s*	2.04 *s*	1.70 *s*	2.04 *s*	2.04 *s*

*Run in C_6D_6 at 70°.

†Chemical shifts taken from mixture of **4h** and **4k**.

‡Intensity three protons.

§Intensity two protons.

EXPERIMENTAL

Extraction of M. cordifolia. Collection A of *Mikania cordifolia* (L.f) Willd. came from Salta Province, Argentina, collection B from Dique el Jumeal, Catamarca Province, Argentina. The collections were identified by Dr Luis Ariza Espinar. Voucher specimens are on deposit in the Museo Bótanica, Universidad Nacional de Córdoba.

Aerial parts of collection A (600 g) were exhaustively extracted with $CHCl_3$. The usual work-up [7] gave 12 g of gum which was chromatographed on 500 g of silica gel packed in $CHCl_3$ and eluted with $CHCl_3$ and increasing amounts of MeOH in four major fractions IA, IIA, IIIA and IVA. Fraction IA (2.3 g) was rechromatographed on 150 g of silica gel with C_6H_6–Me_2CO as eluent. Me_2CO–C_6H_6 (1:19) afforded 96 mg of a 3:2 mixture of **3a** and **3b** containing traces of acetophenetidine, 50 mg of a mixture of **3a**, **3b** and **3c**, 36 mg of relatively pure **3c** and 40 mg of a mixture of **3c** and **3d**. Attempts to separate **3a** and **3b** were unsuccessful. The mixture was used to record the NMR measurements recorded in Tables 1 and 4. Compound **3c** was a gum; IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3480, 1760, 1735; EIMS: **3c** failed to give the molecular ion, significant peaks were observed at *m/z* (rel. int.): 246 $[M-C_4H_6O_3]^+$ (6.4), 228 (9.2), 215 (17.2). Oxidation of the mixture of **3a**–**3c** in 25 ml of spectrograde $CHCl_3$ with 0.5 g of activated MnO_2, filtration through silica gel after 1.5 hr at which time TLC revealed disappearance of starting material, evaporation of the solvent and purification of the residue by radial chromatography (1 mm silica gel plate, CH_2Cl_2–DEE gradient, flow rate 3 ml/min) afforded 10 mg of a 3:2 mixture of **5a** and **5b** which was used for the NMR measurements recorded in Table 3; PCIMS *m/z* (rel. int.): 333 $[M+1]^+$ of **5a** (8.4), 331 $[M+1]^+$ of **5b** (40.7), 305 (1.5), 289 (7.1), 247 (51.3), 229 (100), 89 (1), 87 (1), and 35 mg of **5c**, IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 1765, 1730, 1690 (*br*); this substance decomposed by the time MS could be run.

Compound **3d** was also a gum; IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3500 (*br*), 1760; 1730; PCIMCS *m/z* (rel. int.): 385 and 383 $[M+1]^+$ (11), 247 (33.1), 229 (17.7), 141 and 139 $[C_4H_8O_3Cl]^+$ (24.9 and 100), 105 (12.8), 108 (33.5). Acetylation of 50 mg of **3d** (pyridine, Ac_2O) in the usual way and purification of the crude product by radial chromatography in the manner described above afforded 5 mg of **4d** (gum); PCIMS *m/z* (rel. int.): 427 $[M+1]^+$ (9), 409 (12.6), 399 (29.4), 386 (11.5), 384 (15.5), 369 (29.6), 367 (25.4), 229 (47.6), 141 (23.4), 139 (81.4), and 30 mg of **4i** (gum); EIMS *m/z* (rel. int.): 289 $[M-C_6H_8O_4Cl]^+$ (2.2), 246 (3.5), 228 (14.8), 180 (21), 167 (9), 165 (18), 137 (29.3); PCIMS *m/z* (rel. int.): 471 and 469 $[M+1]^+$ (9.3 and 14.0), 441 (23.7), 409 (48.9), 289 (39.9), 229 (68.6), 183 (35.3), 181 (100), 165 (22.5), 163 (88.3).

Fraction IIA (0.40 g) on rechromatography over silica gel (eluent Me_2CO–C_6H_6) gave 73 mg of **3d** and 35 mg of a mixture of **3d** and **3e** while rechromatography of fraction IIIA yielded 91 mg of **3e** as a gum; IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3480, 1760, 1735; EIMS:

Table 3. ^{1}H NMR spectra of compounds **5a–3c**, **5e** ($CDCl_3$, 270 MHz)

H	5a*	5b*	5c	5e
1	6.62 *br t* (7.5)	6.60 *br t* (8)	6.65 *br t* (8.5)	6.64 *br t*
2a, b	2.4–	2.4–	2.4–	
3a, b	2.8	2.8	2.8	2.52 *m*
5	5.32 *br dd* (10, 5.5)	5.30 *br dd*	5.37 *br dd*	5.36 *br dd*
6a	2.70 *m*			
6b	2.16 *dt* (13, 11, 11)	2.16 *dt*	2.17 *dt*	2.15 *dt*
7	2.70 *m*			2.68 *m*
8	3.99 *m*	3.99 *m*	3.96 *m*	3.97 *m*
9a	2.89 *br d* (15)	2.89 *br d*	2.89 *br d*	2.88 *br d* (14)
9b	2.50–2.60 *c*	2.50–2.60 *c*	2.50–2.60 *c*	2.52 *m*
13a	6.23 *d* (3.5)	6.23 *d*	6.23 *d* (3)	6.25 *d* (3.4)
13b	5.52 *d* (2.9)	5.52 *d*	5.54 *d* (3)	5.58 *d* (2.9)
14	9.51 *s*	9.49 *s*	9.51 *s*	9.52 *s*
15a	4.62 *d* (12)	4.70 *d*	4.62 *m*	4.71 *d* (12.5)
15b	4.53 *d* (12)	4.63 *d*	4.62 *m*	4.60 *d* (12.5)
2′	2.20 *sept* (6.5)	—	—	—
3′a	1.19 *d* (6.5)†	6.11 *br*	3.12 *d* (6)	1.44 *s*†
3′b		5.629 *q* (1.5)	2.80 *d* (6)	
4′†	1.19 *d* (6.5)	1.97 *br*	1.59 *s*	1.44 *s*

*Taken from a 3:2 mixture of **5a** and **5b**.
†Intensity three protons.

3e failed to give the molecular ion; significant peaks were observed at *m/z* (rel. int.): 246 $[M-C_4H_8O_3]^+$ (3.5), 228 (9.2), 215 (17.2), 105 (53.4), 59 (100); CD curve (MeOH) $[\theta]_{246}$ −2700 (sh), $[\theta]_{228}$ −3700, $[\theta]_{225}$ −2950, $[\theta]_{210}$ −13500 (last reading). Oxidation of 50 mg of **3e** with activated MnO_2 as described above and purification of the crude product by radial chromatography gave 15 mg of **5e** as a gum; IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 1765, 1730, 1690; PCIMS *m/z* (rel. int.). 349 $[M+1]^+$ (68.5), 245 (57.2), 105 (100).

Rechromatography of fraction IVA over silica gel with $CHCl_3$–Me_2CO (3:2) as eluent gave 13 mg of a mixture of **3f** and **3g** and 49 mg of a mixture of **3g** and **3h**. Compound **3f** which was obtained pure from the Catamarca collection (*vide infra*) was a gum which decomposed rapidly; IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3370 *br*, 1755, 1710, 1690; PCIMS *m/z* (rel. int.): 349 $[M+1]^+$ (7.1), 263 (2.3), 247 (100), 229 (76.6), 103 (47.9), 85 (8.9). MnO_2 oxidation of **3f** gave a complex mixture which was not studied further. Acetylation of 0.2 g of **3f** followed by the usual work-up and chromatography (10 g silica gel, C_6D_6–EtOAc, 9:1 as eluent) furnished 0.17 g of **4j** as a gum; IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 1760, 1740, 1690; PCIMS *m/z* (rel. int.): 433 $[M+1]^+$ (34.5), 373 (64.5), 289 (100), 247 (21.6), 229 (73.7), 193 (19.6), 145 (41.7). Attempts to separate **3g** and **3h** were unsuccessful. The mixture was used to record the NMR spectra in Table 1 and to prepare, after purification by radical chromatography, the mixture of diacetates **4k** and **4l** whose NMR spectra are recorded in Table 2.

Collection B (400 g) gave 9 g of crude extract whose main lactone constituent was **3f**. CC (silica gel, 250 g) in the manner described above furnished four main fractions IB, IIB, IIIB and IVB. On further purification, fraction IB afforded 0.18 g of a mixture of **3a**, **3b** and acetophenetidine, 40 mg of a mixture of **3c** and **3d**, 10 mg of **3d** and 20 mg of **3f**, fraction IIB afforded 1.07 g

Table 4. ^{13}C NMR spectra of compounds **3a–3f**, **4j** and **6** ($CDCl_3$)

C	3a†,§	3b†,§	3c†	3d†	3e†	3f‡	4j‡	6‡
1	125.6 *d*	125.6 *d*	125.1 *d*	125.2 *d*	125.0 *d*	125.03 *d*	128.53 *d*	129.00 *d*
2	28.4 *t**	28.4 *t**	28.4 *t**	28.4 *t**	28.3 *t**	29.20 *t**	28.63 *t**	28.08 *t**
3	29.5 *t**	29.5 *t**	29.7 *t**	29.8 *t**	29.6 *t**	29.86 *t**	29.64 *t**	not seen
4	139.6 *s*	139.6 *s*	139.0 *s*	139.0 *s*	139.1 *s*	139.43 *s*	139.08 *s*	not seen
5	127.4 *d*	127.4 *d*	128.1 *d*	128.6 *d*	128.1 *d*	127.95 *d*	128.28 *d*	129.00 *d*
6	31.1 *t**	31.1 *t**	30.9 *t**	30.8 *t**	30.6 *t**	30.91 *t**	31.00 *t**	30.90 *t**
7	46.5 *d*	46.5 *d*	46.5 *d*	46.5 *d*	46.5 *d*	46.63 *d*	46.86 *d*	52.42 *d*
8	84.1 *d*	84.1 *d*	84.1 *d*	84.2 *d*	84.1 *d*	84.28 *d*	83.55 *d*	83.64 *d*
9	34.7 *t*¶	34.7 *t*¶	34.8 *t*¶	35.0 *t*¶	35.2 *t*¶	35.09 *t*¶	35.61 *t*¶	not seen
10	138.4 *s*	138.4 *s*	138.1 *s*	138.1 *s*	138.1 *s*	138.44 *s*	133.98 *s*	133.41 *s*
11	135.8 *s*‖	136.1 *s*‖	134.9 *s*	134.9 *s*	135.5 *s*	135.54 *s*	135.34 *s*	99.36 *s***
12	169.7 *s*	169.7 *s*	169.6 *s*	169.6 *s*	169.6 *s*	169.80 *s*	169.52 *s*	171.82 *s*
13	120.7 *t*	120.7 *t*	120.8 *t*	120.8 *t*	120.8 *t*	120.91 *t*	120.81 *t*	23.21 *t****
14	66.2 *t*	66.2 *t*	66.1 *t*	66.1 *t*	66.0 *t*	66.11 *t*	67.67 *t*	67.67 *t*
15	63.1 *t*‖	63.3 *t*‖	64.2 *t*	64.7 *t*	64.0 *t*	63.37 *t*	63.52 *t*	64.31 *t*
16	—	—	—	—	—	—	—	78.97 *t*
1′	176.5 *s*	165.9 *s*		173.6 *s*	176.9 *s*	166.10 *s*	164.96 *s*	167.84 *s*
2′	33.9 *d*	135.75 *s*	61.9 *s*	50.9 *s*	72.1 *s*	139.19 *s*	135.14 *s*	97.53 *t***
3′	18.8 *q*	125.8 *t*	63.2 *t*	75.3 *t*	27.1 *q*	126.22 *t*	127.92 *t*	23.47 *t****
4′	18.8 *q*	18.2 *q*	17.3 *q*	23.5 *q*	26.9 *q*	61.99 *t*	63.39 *t*	64.60 *t*
Ac							170.27 *s*	170.08 *s*
							170.64 *s*	170.66 *s*
							20.90 *q*	20.91 *q*
							20.68 *q*	20.59 *q*
								78.52 *t*

*,**,***Assignment may be interchangeable within same column.
†Run at 20.15 MHz. §From 3:2 mixture of **3a** and **3b**.
‡Run at 67.89 MHz. ¶Very broad.
‖Assignments may be interchangeable within adjacent columns.

Table 5. ^{1}H NMR spectra of compounds **6** and **7** (C_6D_6, 270 MHz, 70°)

H	6	7
1	5.63 *br t* (8)	5.35 *br dd* (8, 7)
2a, b 3a, b	2.1–2.4 *c*	1.75–2.0 *c*
5	5.45 *dd* (12, 5.5)	4.99 *dd* (11, 5.5)
6a, b	2.1–2.4 *c*	
7	2.5 *m*	3.05 *ddd* (12, 10, 4)
8	4.99 *m*	3.86 *dt* (10, 4)
9a	2.1–2.4 *c*	2.48 *dd* (15, 4)
9b	2.62 *br d* (14)	2.38 *br dd* (15, 4)
13a, b	1.65 *m*	1.3–1.5 *c*
14a, b 14a, b 16a, b	4.48–4.8 *m*	4.1–4.7 *m*
H-3'a, b	1.52 *m*	
H-4'a, b	2.1–2.4 *m*	1.3–1.5 *m*
H-5'a, b	4.48–4.8 *m*	4.1–4.7 *m*
Ac*	2.10 *s*	1.85 *s*
	2.00 *s*	1.50 *s*

*Intensity three protons.

of impure **3f**, fraction IIIB gave 0.21 g of a mixture of **3f** and **3g** and 0.93 g of **3f**, fraction IVB gave 13 mg of the **3f**, **3g** mixture and 49 mg of a mixture of **3g** and **3h**.

Pyrazoline **6** *and* **7**. Reaction of 0.15 g of **4j** with excess CH_2N_2 in $CHCl_3$ followed by removal of solvent and radial chromatography of the crude product gave 95 mg of **6** and 26 mg of **7**, both gums. Pyrazoline **6** had IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 1780, 1740 *br*, 1690, 1540; PCIMS *m/z* (rel. int.): 489 $[M+1-N_2]^+$ (1), 461 $[M+1-2N_2]^+$ (7.9), 303 (100), 243 (82.5), 159 (32.5), 99 (3.9). Pyrazoline **7** had IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 1780, 1740 *br*, 1690, 1540; EIMS *m/z* (rel. int.): 460 $[M-2N_2]^+$ (0.1), 401 (6.3), 331 (0.1), 303 (7.8), 243 (42.6), 141 (96.3), 81 (100).

Acknowledgements—Work in Argentina was supported by a grant from CONICOR, Córdoba. V. E. S. thanks CONICET for a fellowship. We also express our thanks to Dr Manuel Gonzalez Sierra for some ^{13}C NMR spectra.

REFERENCES

1. Herz, W. Subramaniam, P. S., Murari, R., Dennis, N. and Blount, J. F. (1977) *J. Org. Chem.* **42**, 1720.
2. Bohlmann, F., Natu, A. A. and Mahanta, P. K. (1978) *Phytochemistry* **17**, 483.
3. Gutiérrez, A. B., Oberti, J. C., Kulanthaivel, P. and Herz, W. (1985) *Phytochemistry* **24**, 2967.
4. Herz, W. and Kalyanaraman, P. S. (1975) *J. Org. Chem.* **40**, 3486.
5. Bohlmann, F. and LeVan, N. (1978) *Phytochemistry* **17**, 570.
6. Bohlmann, F., Singh, P. and Jakupovic, J. (1982) *Phytochemistry* **21**, 2029.
7. Herz, W. and Högenauer, J. (1962) *J. Org. Chem.* **27**, 905.