Phytochemistry, Vol. 25, No. 3, pp. 723–727, 1986.
Printed in Great Britain.

0031–9422/86 $3.00 + 0.00

DITERPENOIDS AND FLAVONOIDS FROM *GRINDELIA DISCOIDEA*

BARBARA N. TIMMERMANN, JOSEPH J. HOFFMANN, SHIVANAND D. JOLAD*, ROBERT B. BATES† and TERUNA J. SIAHAAN†

University of Arizona, Office of Arid Lands Studies, Bioresources Research Facility, 250 E. Valencia Road, Tucson, AZ 85706, U.S.A. and *University of Arizona, College of Pharmacy; †Department of Chemistry, University of Arizona, Tucson, AZ 85721, U.S.A.

(*Received* 28 *June* 85)

Key Word Index—*Grindelia discoidea*; Asteraceae; diterpenoid acids; labdanes; discoidic acid; flavonoids; mass spectrometry.

Abstract—A new labdane-type diterpenoid acid, discoidic acid, has been isolated from the aerial parts of *Grindelia discoidea*. Its structure has been deduced as 4β-hydroxymethyllabd-7-en-15-oic acid on the basis of the spectral data of its methyl ester derivative and comparison of these data with those of the methyl ester derivative of its isomer, 3β-hydroxylabd-7-en-15-oic acid, also isolated from the same extract. In addition, a dihydroflavonol and two flavonols have been isolated.

INTRODUCTION

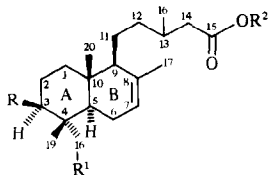
In the course of our investigations of the New World genus *Grindelia* (Astereae, Soladiginae) we have examined the aerial parts of one of its South American species, *Grindelia discoidea* Hook & Arn. Quantitative GC analysis of the methyl ester mixture of the acid fraction of *G. discoidea* gave a chromatogram which showed none of the typical grindelane-type diterpenoid acids previously observed in the chromatograms of methyl ester mixtures of the acid fractions of two North American *Grindelia* species [1, 2] and several others currently under investigation. This prompted us to extend our investigation to the acid fraction of *G. discoidea* which led to the isolation of two labdane-type diterpenoid acid isomers (**1** and **2**) and three flavonoids (**3**–**5**).

One of the labdanoids, which is new and designated as discoidic acid, was identified as 4β-hydroxymethyllabd-7-en-15-oic acid (or 1,4,4a,5,6,7,8,8a-octahydro-5-hydroxymethyl-β,2,5,8a-tetramethyl-1-naphthalenepentanoic acid, **1**) and the other, isomeric with **1**, was characterized as 3β-hydroxylabd-7-en-15-oic acid (or 1,4,4a,5,6,7,8,8a-octahydro-6-hydroxy-β,2,5,5,8a-pentamethyl-1-naphthalenepentanoic acid, **2**), previously found in the South American *Chrysothamnus nauseous* by Bohlmann *et al.* [3]. However, as their identification of **2** was based solely on ^{1}H NMR data obtained on a mixture of the methyl ester of **2** and its dehydro (7,13*E*-dien) derivative, we decided to fully characterize our compound **2**. The dihydroflavonol was identified as (2*R*,3*R*)-dihydroquercetin 7,3′-dimethyl ether (**3**) from its ^{1}H NMR, mass and UV spectral properties. Since the substitution pattern in ring B was inconclusive despite comparison of its physical and spectral properties with those reported for **3** [4, 5] and its structural isomer (**3b**) [6], additional evidence was obtained to define the B-ring substitution pattern. The two flavonols were identified as quercetin 3,3′-dimethyl ether (**4**) and quercetin 3,7,3′-trimethyl ether (pachypodol, **5**) by comparison of their spectral data with those of authentic samples.

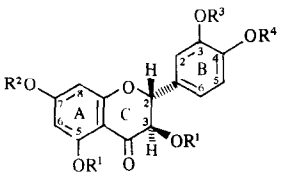
RESULTS AND DISCUSSION

Compounds **1**–**5** were isolated from the aerial parts of *G. discoidea*. Compound **1** was separated as its methyl ester (**1a**) by treatment with ethereal diazomethane and was then saponified to regenerate pure **1**. The purity of **1a** and **2a** (obtained by treatment of **2** with methyl iodide–acetone), which we used to elucidate the structures of corresponding naturally occurring acids **1** and **2**, was checked by GC using our standard techniques [1].

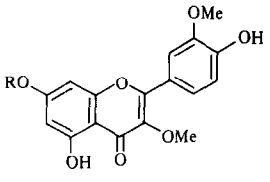
Compound **1a**, $C_{21}H_{36}O_3$ (high resolution MS), had



	R	R^1	R^2
1	H	OH	H
1a	H	OH	Me
2	OH	H	H
2a	OH	H	Me
2b	H	H	Me(13–en)



	R^1	R^2	R^3	R^4
3	H	Me	Me	H
3a	Ac	Me	Me	Ac
3b	H	Me	H	Me
3c	H	H	Me	Me



	R
4	H
5	Me

IR absorptions at $\nu_{max}^{CCl_4}$ cm^{-1}: 3640 (OH), 1738 (COOMe), 3020 and 1665 (C=C) and 1375 (Me), but lacked gem-dimethyl group absorptions. These assignments were supported by the NMR spectra of **1a**. The ^{1}H NMR spectrum of **1a**, which showed an olefinic hydrogen at δ5.35, a methyl ester at 3.66, CH_2OH at 3.47 and 3.84 (ab quartet, J = 10.8 Hz) and four methyl groups, one secondary at δ0.96 (*d*, J = 6.6 Hz, 16-Me), two tertiary at 0.73 (20-Me) and 0.96 (18-Me), and an olefinic methyl at δ1.65 (*s*, *br*, 17-Me), was reminiscent of ^{1}H NMR spectra of compounds containing a labdane skeleton. The presence of a β-methylpentanoic acid methyl ester side chain was clearly indicated by a strong mass spectral ion peak at m/z 207 $[M-C_7H_{13}O_2]^+$ in **1a** which did not shift in **1**. The absence of gem-dimethyl group absorptions in the IR and ^{1}H NMR spectra of **1a** and the presence of a third oxygen atom in ring A of the octahydronapthalene ring system, established from the elemental composition of m/z 196 $[M-C_9H_{16}O]^+$ peak by high resolution MS, suggested that the hydroxymethyl group must be attached to C-4 replacing the C-19 methyl of the gem-dimethyl group present in many labdanes. The chemical shift assigned to the quaternary carbon C-4 (δ35.3) and oxygenated carbon C-9 (δ64.8) in the ^{13}C NMR spectrum of **1a** clearly confirmed this supposition.

The stereochemistry of **1a** was deduced by correlation with **2a** whose configuration has been shown to be as depicted [3]. Both **1a** and **2a** had similar NMR parameters except for the differences expected for the change in the hydroxyl group location: C-3 and the attached proton were shifted downfield in **2a**, C-18 and its protons were shifted downfield in **1a**, and C-2 in **2a** was much farther downfield due to a β-effect. The same NMR absorptions for C-19 and its attached protons were displayed for **1a** and **2a**. This indicates that C-18 in **1a** is oxidized. The configurations at all other centres in **1a** are assumed to be the same as in **2a**.

Except for stereochemistry, the structure of **1a** was elucidated solely on the basis of the mass spectral fragmentation pattern of **1a** in the EI mode. The mechanistic proposals outlined for certain fragment ions in Fig. 1, all verified by exact mass measurements (HR-MS), are consistent with structure **1a**. The transitions shown in Fig. 1 were substantiated by metastable peaks. The appearance of an abundant peak at m/z 207 $[C_{14}H_{23}O]^+$ derived from $[M]^+$ indicated that the hydroxymethyl function was a part of either ring-A or ring-B of the octahydronaphthalene system. It was confined to ring-A because another prominent peak at m/z 196 $[C_{12}H_{20}O_2]^+$ was derived from $[M^+]$ by retro-Diels–Alder fragmentation.

The EI mass spectrum of **2a** was very similar to that of **1a**, differing only with respect to the mass of satellite peaks derived from $[M]^+$ and to the abundance of certain peaks below m/z 210.

Flavonoid **3** was identified as (2*R*,3*R*)-dihydroquercetin-7,3′-dimethyl ether. The molecular ion peak at m/z 332 in the EI mass spectrum of **3** and its elemental analysis (HR-MS) indicated a molecular formula $C_{17}H_{16}O_7$. The IR spectrum suggested the presence of hydroxyl (3440 cm^{-1}), methoxyl (3000, 2950 and 2900 cm^{-1}) and conjugated carbonyl (1630 cm^{-1}) functions. The ^{1}H NMR data indicated a dihydroquercetin skeleton having *trans*-diaxial (2*R*,3*R*) stereochemistry at C-2 and C-3 [doublets at δ5.02 (H-2) and 4.57 (H-3), J = 11.9 Hz], two methoxyl groups (δ3.83 and 3.95), five aromatic protons [δ7.05, 7.00 and 7.08 (H-2′, H-5′ and H-6′); doublets at δ6.07 (H-6, J = 2.2 Hz) and δ6.13 (H-8, J = 2.2 Hz)], two free hydroxyls at C-3 (doublet sharpened on exchange with D_2O) and C-5 and a third hydroxyl either in ring-A or ring-B (Table 2). This substitution pattern suggested three possible structures, **3**, **3b** or **3c**, for the flavonoid. Structure **3c** was ruled out by the high resolution exact mass measurements of major fragment

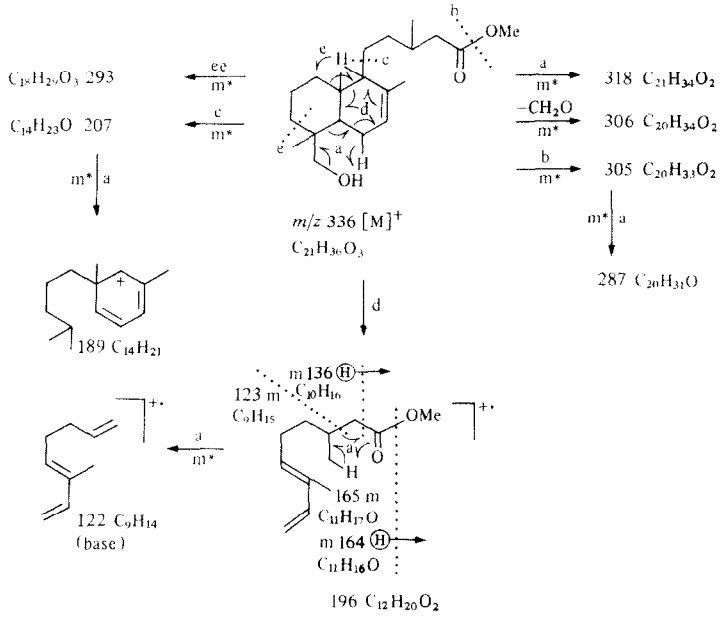


Fig. 1. Fragmentation pathways of some characteristic ions (m/z ratios), established by exact mass measurements and metastable peaks (m*), in the mass spectrum of discoidic acid methyl ester (**1a**).

Table 1. ^{1}H NMR (for **1a**, **2a** and **2**) and ^{13}C (for **1a**, **2a** and **2b**) NMR chemical shifts (δ, $CDCl_3$, TMS as internal standard, J, in Hz, in parentheses)

	^{1}H			^{13}C		
Atom	**1a**	**2a**	**2**	**1a**	**2a**	**2b** [7]
1	1.40–1.55 *m*			39.3	39.4	39.2
2				18.4	27.4	18.8
3		3.24 *dd* (10.3, 5.3)	3.24 *dd* (9.8, 5.5)	39.1	79.2	43.2
4				35.3	38.7	32.9
5	1.34 *dd* (12.2, 4.8)			51.1	49.7	50.1
6a	1.90 *m*			24.6	24.5	23.8
6b	1.85 *m*					
7	5.35 *s* (*br*)	5.38 *s* (*br*)	5.38 *s* (*br*)	122.0	122.1	122.6
8				135.3	135.2	134.4
9	1.97 *m* (*br*)			55.4	55.2	54.4
10				36.8	36.5	36.8
11	1.00–2.00			23.3	23.5	22.0
12				37.9	37.3	43.3
13	1.59 *m* (2.6)			31.2	31.3	160.2
14a	2.34 *dd* (14.6, 6.0)	2.34 *dd* (14.6, 6.0)	2.39 *dd* (15.0, 5.8)	41.3	41.4	115.1
14b	2.12 *dd* (14.6, 8.0)	2.12 *dd* (14.6, 8.0)	2.16 *dd* (15.0, 8.1)			
15				173.6	173.7	(166.9)
16	0.96 *d* (6.6)	0.96 *d* (6.3)	1.00 *d* (6.6)	19.8	19.9	(18.8)
17	1.65 *s* (*br*)	1.66 *s* (*br*)	1.66 *s* (*br*)	26.6	27.9	25.3
18a	3.84 *d* (10.8)	0.85 *s*	0.85 *s*	64.8	15.1	32.9
18b	3.47 *d* (10.8)					
19	0.96 *s*	0.97 *s*	0.97 *s*	21.9	21.9	21.8
20	0.73 *s*	0.75 *s*	0.75 *s*	14.5	13.6	13.6
OMe	3.66 *s*	3.66 *s*		51.3	51.4	(50.6)

Table 2. ^{1}H NMR data for compounds **3** and **3a** (J, in Hz, in parentheses)

	3			**3a**
H	$CDCl_3$ (TMS)	Me_2CO-d_6 (TMS)	NaOD (DSS)	$CDCl_3$ (TMS)
2	5.02 *d* (11.9)	5.11 *d* (11.6)	obscured by HOD signal	5.38 *d* (12.1)
3	4.57 *dd* (11.9, 1.5)	4.72 *d* (11.6)	4.57 *d* (11.4)	5.72 *d* (12.1)
6	6.07 *d* (2.2)	6.06 *d* (2.3)	5.64 *d* (2.5)	6.42 *d* (2.5)
8	6.13 *d* (2.2)	6.09 *d* (2.3)	5.81 *d* (2.5)	6.34 *d* (2.5)
2′	7.05 *dd* (1.8, 0.5)	7.22 *d* (1.9)	7.04 *d* (2.2)	7.08 *d* (1.7)
5′	7.00 *dd* (7.8, 0.5)	6.88 *d* (8.1)	6.64 *d* (8.1)	7.07 *d* (8.1)
6′	7.08 *dd* (7.8, 1.8)	7.04 *dd* (8.1, 1.9)	6.92 *dd* (8.1, 2.2)	7.02 *dd* (8.1, 1.7)
3-OH	3.46 *d* (1.5)			2.02 *s* (MeCOO)
5-OH	11.20 *s*	2.94 *s* (*br*)		2.33 *s** (MeCOO)
4′-OH	5.73 *s*			2.38 *s** (MeCOO)
3′-OMe	3.83 *s**	3.87 *s*	3.75 *s*	3.84 *s*
7-OMe	3.95 *s**	3.88 *s*	3.78 *s*	3.86 *s*

*Values interchangeable.

ions and metastable peaks (Fig. 2). Comparison of the aromatic region of the ^{1}H NMR and mass spectra of our flavonoid (**3**) with those reported in ref. [6] for **3b** did not permit a clear cut distinction between **3** and **3b** although the mp and specific rotation of **3** were noticeably different from those of **3b**. In 1972 Herz *et al.* [5] reported the isolation of the dihydroflavonol (mp 188–191°) from a *Eupatorium* hybrid having its structure as **3** or **3b**. In the same year Rodriguez *et al.* [4] reported the isolation of a flavonoid from *Artemisia pygmaea* having **3** as its structure. The mp reported for this isolate (182–184°) was in good agreement with that of ours (180°) but their UV data did not quite match with our data although a bathochromic shift (29 nm) and an increase in intensity of band I upon the addition of sodium methoxide clearly indicated that the third hydroxyl group in our flavonoid was indeed in ring-B at C-4′. This was confirmed independently from the ^{1}H NMR of our flavonoid in sodium deuteroxide in

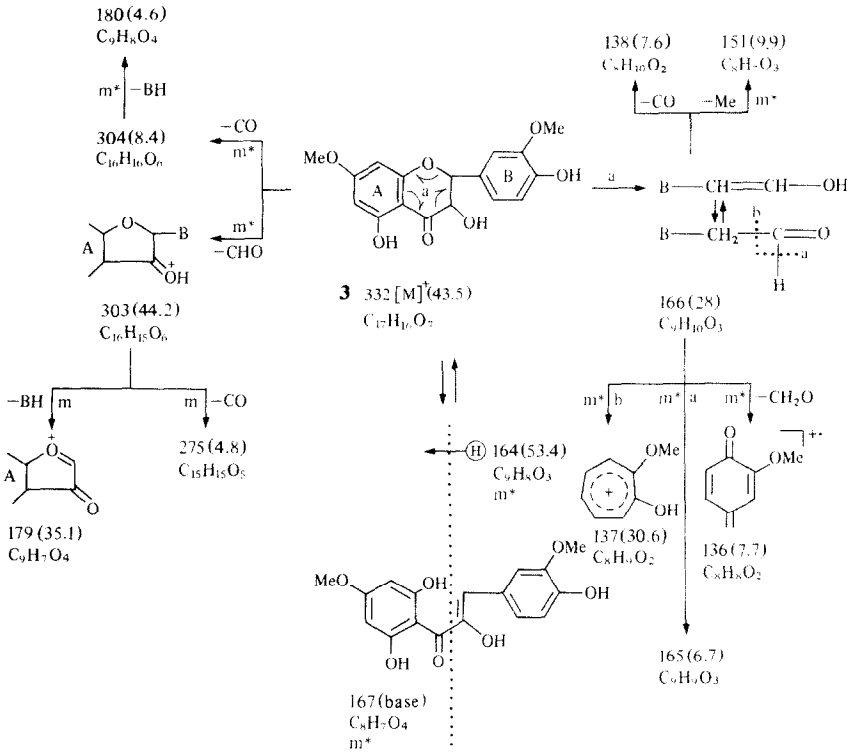


Fig. 2. Fragmentation pathways of major fragment ions (*m/z* ratios, relative intensities in parentheses), established by exact mass measurements and metastable peaks (m*), in the mass spectrum of dihydroquercetin 7,3′-dimethyl ether (**3**).

which C-5′ proton shifted from δ7.00 to 6.64. Based on this observation together with evidence deduced from other spectral arguments, our flavonoid and the flavonoid reported in ref. [4] are both **3**.

EXPERIMENTAL

General. Spectroscopic procedures were the same as in previous work [8].

Plant material. This was obtained from plants grown in the greenhouse in Tucson, Arizona, from seeds collected in the city of Cordoba, Argentina, in Jan 1982. A herbarium specimen has been deposited at the University of Arizona. All plant material was airdried, ground to 3 mm particle size and stored at −10° prior to extraction.

Extraction and fractionation. The milled aerial parts of *G. discoidea* (985 g) were extracted exhaustively with CH_2Cl_2 in a Soxhlet extractor. The solvent free extract (59 g) was extracted by stirring with Et_2O (3 l.) at room temp. (4 hr), left in the freezer overnight and filtered. The filtrate, after reducing the vol. to 1 l. under vacuum, was separated into neutral (12.3 g) and acidic (46.5 g) fractions using 5% aq. Na_2CO_3 followed by neutralization of the alkaline soln with 25% aq. HCl.

Chromatography of acid fraction. The acid fraction (46.5 g), when treated with Et_2O, gave a residue (1.08 g) which was filtered off, crystallized from CH_2Cl_2 and identified as compound **5**. The Et_2O-soluble extract (44 g), obtained after evaporation of the solvent *in vacuo*, was subjected to silica gel 60 (1350 g) CC. Elution of the column with *n*-hexane containing increasing concns of EtOAc (initial concn 2%) and finally with 100% EtOAc gave 60 fractions of 1 l. each. TLC analyses (toluene–EtOAc, 2:1; hexane–EtOAc, 3:2, 1:1; CH_2Cl_2–MeOH, 49:1) of these fractions showed that fraction 30 contained essentially **3**, as did fraction 48 in which **4** was the major component with traces of **2**. Fraction 32, which was complex, contained **1** and **3** as the major components and in fraction 53, compound **2** was the major component with traces of **4** and **5**. The analyses of the remaining chromatographic fractions is underway and will be reported later.

(2R,3R)-Dihydroquercetin-7,3′-dimethyl ether (**3**). This was crystallized out when fraction 30 was treated with Et_2O. Recrystallization (petrol) gave **3** (30 mg) as white powder, mp 180°, $[\alpha]_D^{25}$ + 54.33°(C_5H_5N; *c* 1.2); UV λ_{max}^{MeOH} nm: 239 (sh), 287, 325 (sh), 375 (sh); λ_{max}^{NaOMe} nm: 255 (sh), 288, 355 (sh), 404 (sh); $\lambda_{max}^{AlCl_3}$ nm: 242 (sh), 315, 388 (sh); $\lambda_{max}^{AlCl_3-HCl}$ nm: 239 (sh), 293, 354 (sh), 384; λ_{max}^{NaOAc} nm: 287, 325 (sh), 405 (sh); IR ν_{max}^{KBr} cm^{-1}: see text; MS *m/z* (rel. int.): see Fig. 2; ^{1}H NMR (see text): spectra were in accord with structure **3**. The ^{1}H NMR data (Table 2) of the acetate of **3** (**3a**, prepared by treatment with Ac_2O–C_5H_5N at room temp., 24 hr) was in accord with structure **3a**.

4β-Hydroxymethyllabd-7-en-15-oic acid methyl ester (**1a**). An additional amount of **3** (12 mg) was separated out when fraction 32 was decolourized followed by treatment with Et_2O. The Et_2O-soluble residue (405 mg) was esterified with CH_2N_2 in Et_2O and the reaction product, when subjected to prep. TLC (silica gel 60 PF-254; toluene–EtOAc, 2:1), gave **1a** (226 mg) as transparent oil, homogeneous by TLC and GC. IR $\nu_{max}^{CCl_4}$ cm^{-1}: see text; MS *m/z* (rel. int.): 336.2614 ($[M]^+$, calc. for $C_{21}H_{36}O_3$: 336.2619) (3.9), 318 (3), 306 (13), 305 (34.7), 303 (2), 293 (3.8), 291 (6.2), 287 (2.3), 275 (1.7), 235 (3.2), 223 (4.8), 207 (59.6), 196 (25), 189 (11.5), 175 (10), 165 (7), 164 (11.7), 153 (15.7), 149 (18.5), 147 (12.3), 136

(16.8), 135 (17.6), 133 (15.5), 123 (51.2), 122 (100), 121 (32.3), 119 (34.5), 109 (93.3), 107 (44.4), 105 (28.2), 95 (62.6), 93 (38.6), 91 (31.2), 81 (53.5), 79 (33), 77 (17.1), 69 (31.3), 67 (33.7), 59 (19.8) and 55 (56.8); ^{1}H and ^{13}C NMR (Table 1) spectra were in accord with structure **1a**.

3β-Hydroxylabd-7-en-15-oic acid (**2**). This was isolated from fraction 53 by prep. TLC (silica gel 60 PF-254) (*n*-hexane–Et_2O–AcOH, 40:10:1). Crystallization from CH_2Cl_2 gave **2** (250 mg) as transparent needles, homogeneous by TLC and GC, mp 172°, $[\alpha]_D$ −39.1° (C_5H_5N; *c* 0.046). IR ν_{max}^{KBr} cm^{-1}: see text; *m/z* (rel. int.): 322 $[M]^+$ (12.8), 307 (3.71), 304 (16.4), 289 (9.1), 271 (3.7), 261 (3), 207 (29.5), 190 (15.4), 189 (30.9), 182 (78.6), 175 (10.8), 153 (12.1), 147 (10.8), 135 (12.7), 133 (11.5), 125 (14), 123 (65), 122 (100), 121 (23), 119 (21.9), 109 (13.8), 107 (38.6), 105 (1.6), 96 (31.5), 95 (54.5), 93 (20.7), 91 (18.1), 81 (37.5), 79 (19.7), 69 (18.6), 68 (18.6), 67 (20.5) and 55 (31.6); ^{1}H NMR (Table 1): spectra were in accord with structure **2**.

3β-Hydroxylabd-7-en-15-oic acid methyl ester (**2a**). Methylation of **2** (50 mg) with MeI (1.5 ml) in dry Me_2CO (4 ml) and dry K_2CO_3 (94 mg) at 56–60° for 4 hr gave **2a** (49 mg, oil) homogeneous by TLC and GC. IR $\nu_{max}^{CHCl_3}$ cm^{-1}; 3620, 1730, 1380–1363 (doublet); MS *m/z* (rel. int.) *m/z* 336 $[M]^+$ (18.3), 321 (5.3), 318 (20.8), 303 (14.3), 196 (30.2) all shifted by 14 mass units, the rest of the spectrum with the base peak at *m/z* 122 was very similar to that of **2**; ^{1}H NMR (Table 1) spectra were in accord with structure **2a**.

Acknowledgements—We thank Mr. Peter Baker for mass spectral data, Mr. Pramuk Shivanonda for technical assistance and Dr. Carlos Oberti, Universidad Nacional de Cordoba, Argentina, for seed collection. This investigation was supported by NSF (Grant PCM-8304771) whom we gratefully acknowledge.

REFERENCES

1. Timmermann, B. N., Luzbetak, D. J., Hoffmann, J. J., Jolad, S. D., Schram, K. H., Bates, R. B. and Klenck, R. E. (1983) *Phytochemistry* **22**, 523.
2. Timmermann, B. N., Hoffmann, J. J., Jolad, S. D. and Schram, K. H. (1985) *Phytochemistry* **24**, 1031.
3. Bohlmann, F., Dutta, L., Robinson, H. and King, R. M. (1979) *Phytochemistry* **18**, 1889.
4. Rodriguez, E., Carman, N. J., Van der Velde, G., McReynolds, J. H., Mabry, T. J., Irwin, M. A. and Geissman, T. A. (1972) *Phytochemistry* **11**, 3509.
5. Herz, W., Gibaja, S., Bhat, S. V. and Srinivasan, A. (1972) *Phytochemistry* **11**, 2859.
6. Ruangrungsi, N., Tappayuthpijarn, P., Tantivatana, P., Borris, R. P. and Cordell, G. A. (1981) *J. Nat. Prod.* **44**, 541.
7. Imamura, P. M., Marsaioli, A. J., Barata, L. E. S. and Ruveda, E. A. (1977) *Phytochemistry* **16**, 1842.
8. Jolad, S. D., Hoffmann, J. J., Schram, K. H., Cole, J., Tempesta, M. and Bates, R. B. (1981) *J. Org. Chem.* **46**, 4267.