New Pterocarpanoids of *Crotalaria pallida* and *Crotalaria assamica*

by **Jing-Ru Weng**, **Ming-Hong Yen**, and **Chun-Nan Lin***

School of Pharmacy, Kaohsiung Medical University, Kaohsiung, Taiwan 807, Republic of China

Four new pterocarpanoids crotafurans A (**1**), B (**2**), C (**3**), and D (**4**) were isolated from the bark of *Crotalaria pallida* and the seeds of the *C. assamica*, respectively. The structures, including relative configurations were elucidated by spectroscopic data.

1. Introduction. – Various pyrrolizidine alkaloids isolated from *Crotalaria pallida* AIT. and *C. assamica* BENTH. have been reported [1]. In a continued search for bioactive constituents from this plant, three new pterocarpanoids, crotafurans A (**1**), B (**2**), and C (**3**) were isolated from the bark of *C. pallida*, and a new pterocarpanoid, crotafuran D (**4**), was isolated from the seeds of *C. assamica*. In the present paper, the structure elucidations of the four new pterocarpanoids are reported.

2. Results and Discussion. – The molecular formula of crotafuran A (**1**) was determined to be $C_{20}H_{16}O_4$ by HR-EI-MS (*m/z* 320.1048 (M^+)), which was consistent with the ^{1}H- and ^{13}C-NMR data. The IR absorption of **1** implied the presence of OH (3422 cm^{-1}) and aromatic ring (1604 cm^{-1}) moieties.The ^{1}H- and ^{13}C-NMR spectra of **1** (*Table 1*) were assigned by COSY90, HMQC, HMBC, and NOESY experiments and comparison with corresponding reported data [2–4]. Consequently, the structure of crotafuran A (**1**) was established as 5′-(1-methylethenyl)furo[2′,3′:9,10]pterocarpan-3-ol (see *Fig. 1*).

In the ^{1}H-NMR spectrum of **1**, signals at δ 3.66 (*dd*, J = 10.4, 10.4 Hz, 1 H), 3.73 (*ddd*, J = 11.0, 7.2, 4.8 Hz, 1 H), 4.33 (*dd*, J = 10.4, 4.8 Hz, 1 H), and 5.69 (*d*, J = 7.2 Hz, 1 H) were assigned to H_β–C(6), H_α–C(6a), H_α–C(6), and H_α–C(11a) protons of the pterocarpan (= 6a,11a-dihydro-6*H*-benzofuro[3,2-*c*]benzopyran) moiety, suggesting a *cis* arrangement of H_α–C(6a) and H_α–C(11a) [2]. The ^{13}C-NMR signals at δ 40.8, 67.3, and 80.4 were in agreement with the signals assigned to C(6a), C(6), and C(11a) of the pterocarpan moiety [2]. The ^{1}H- and ^{13}C-NMR spectra revealed signals due to a trisubstituted and a tetrasubstituted benzene moiety, a trisubstituted furan moiety [3], as well as an exocyclic methylene, tertiary Me, and phenolic OH group. The position of the trisubstituted benzene moiety (ring A) of **1**, *i.e.*, its fusion at C(1a)–C(4a), was established by COSY90, HMQC, and the HMBC correlations H–C(2)/C(1a), H–C(4)/C(4a), H–C(4)/C(1a), H_β–C(6)/C(4a), H–C(11a)/C(4a), H–C(11a)/C(1) (*Table 1*). Similarly, the fusion of the tetrasubstituted benzene moiety (ring D) at C(7a)–C(10a) was suggested by COSY90, HMQC, and the HMBC correlations H–C(7)/C(10a) and H–C(8)/C(7a). The attachment of the methyl and exocyclic methylene groups at C(14) was confirmed by the HMBC correlations Me(16)/C(14), Me(16)/C(15), H–C(15)/C(16), and H–C(15)/C(14). The HMBC correlations H–C(15)/C(13), H–C(12)/C(13), H–C(12)/C(10), H–C(8)/C(10), and H–C(7)/C(9) established the connectivity of the 1-methylethenyl moiety at the trisubstituted furan (ring E) moiety through the C(13)–C(14) bond and the fusion site of the trisubstituted furan (ring E) moiety at C(9)–C(10) of ring D.

The presence of characteristic peaks at *m/z* 303 ([320 – OH]$^+$), 277 ([M – a – 2 H]$^+$), and 186 ([M – b + 2 H]$^+$) in the EI-MS of **1** (*Fig. 2*) supported the proposed structure. The relative configurations at C(6a) and

Table 1. *^{1}H- and ^{13}C-NMR Data of* **1** *and* **3** *in $(CD_3)_2CO$.* Arbitrary numbering, see *Fig. 1*; δ in ppm, J in Hz

	1			**3**		
	δ(H)	δ(C)	HMBC (^{1}H)[a])	δ(H)	δ(C)	HMBC (^{1}H)[a])
H–C(1)	7.39 (*d*, *J* = 8.4)	133.1	5.69 (H–C(11a))	7.38 (*d*, *J* = 8.4)	133.9	5.49 (H–C(11a))
C(1a)		113.3	6.38 (H–C(4)), 6.58 (H–C(2))		113.8	6.58 (H–C(2))
H–C(2)	6.58 (*dd*, *J* = 8.4, 2.4)	110.5	6.38 (H–C(4)), 8.61 (OH–C(3))	6.58 (*dd*, *J* = 8.8, 2.4)	111.6	6.32 (H–C(4)), 8.69 (OH–C(3))
HO–C(3)	8.61 (*s*)	159.8	6.58 (H–C(2)), 7.39 (H–C(1)), 8.61 (OH–C(3))	8.69 (*s*)	160.5	6.58 (H–C(2)), 7.38 (H–C(1)), 8.69 (OH–C(3))
H–C(4)	6.38 (*d*, *J* = 2.4)	104.0	6.58 (H–C(2)), 8.61 (OH–C(3))	6.32 (*d*, *J* = 2.4)	105.1	6.58 (H–C(2)), 8.69 (OH–C(3))
C(4a)		157.8	3.66 (H_β–C(6)), 5.69 (H–C(11a)), 6.38 (H–C(4))		158.1	4.20 (H_β–C(6)), 5.49 (H–C(11a)), 6.32 (H–C(4))
H_α–C(6)	4.33 (*dd*, *J* = 10.4, 4.8)	67.3	5.69 (H–C(11a))	4.16 (*d*, *J* = 11.6)	71.4	
H_β–C(6)	3.66 (*dd*, *J* = 10.4, 10.4)			4.20 (*d*, *J* = 11.6)		
H_α–C(6a)	3.73 (*ddd*, *J* = 11.0, 7.2, 4.8)	40.8	3.66 (H_β–C(6))		78.1	4.20 (H_β–C(6))
HO–C(6a)				5.19 (*s*)		
C(7a)		121.2	7.03 (H–C(8))		124.1	7.10 (H–C(8))
H–C(7)	7.29 (*d*, *J* = 8.4)	121.7	7.03 (H–C(8))	7.35 (*d*, *J* = 8.4)	121.5	7.10 (H–C(8))
H–C(8)	7.03 (*d*, *J* = 8.4)	103.8	7.29 (H–C(7))	7.10 (*d*, *J* = 8.4)	104.6	7.35 (H–C(7))
C(9)		153.3	7.29 (H–C(7))		154.4	7.35 (H–C(7))
C(10)		114.9	6.74 (H–C(12)), 7.03 (H–C(8))		115.8	6.74 (H–C(12)), 7.10 (H–C(8))
C(10a)		157.6	7.29 (H–C(7))		159.0	
H–C(11a)	5.69 (*d*, *J* = 7.2)	80.4	3.66 (H_β–C(6)), 4.33 (H_α–C(6))	5.49 (*s*)	87.8	4.20 (H_β–C(6)), 4.20 (H_α–C(6))
H–C(12)	6.74 (*s*)	100.3		6.74 (*s*)	101.0	
C(13)		157.2	5.19 (H–C(15)), 5.69 (H–C(11a)), 6.74 (H–C(12))		157.9	2.11 (Me(16)), 5.18 (H–C(15)), 5.71(H–C(15)), 6.74 (H–C(12))
C(14)		133.8	2.11 (Me(16)), 5.69 (H–C(11a))		134.5	2.11 (Me(16)), 5.71 (H–C(15))
H–C(15)	5.19 (*s*)	112.6	2.11 (Me(16))	5.18 (*s*)	114.1	2.11 (Me(16))
H–C(15)	5.71 (*s*)			5.71 (*s*)		
Me(16)	2.11 (*s*)	19.3	5.19 (H–C(15)), 5.69 (H–C(11a))	2.11 (*s*)	19.9	5.18 (H–C(15)), 5.71 (H–C(15))

[a]) Only key interactions.

C(11a) were established by the NOESY cross-peaks (*Fig. 2*) H_α–C(6)/H–C(6a) and H–C(6a)/H–C(11a), while H–C(6a) and H–C(11a) adopted the relative α-configuration. Further experiments are required to elucidate the absolute configuration of **1**.

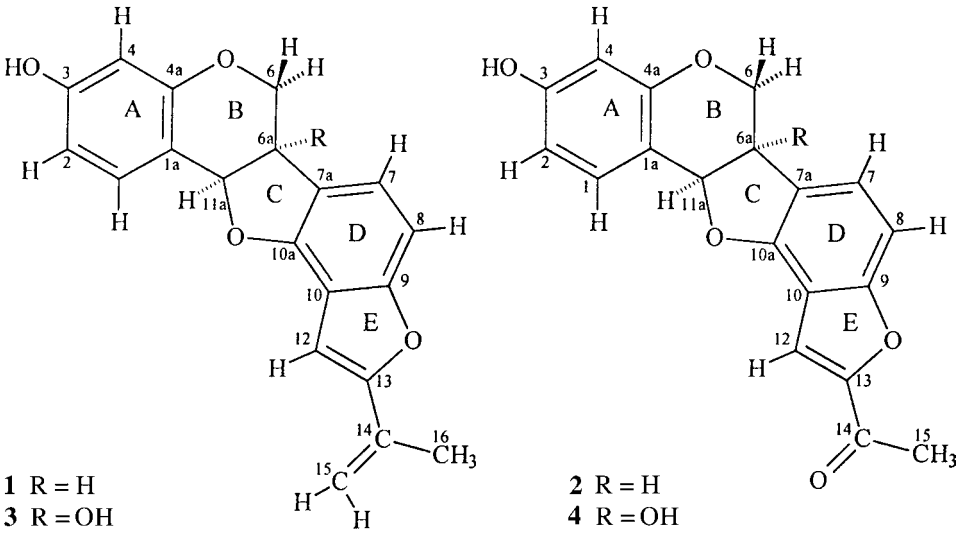

Fig. 1. *Structures of* **1**–**4**. Numbering arbitrary.

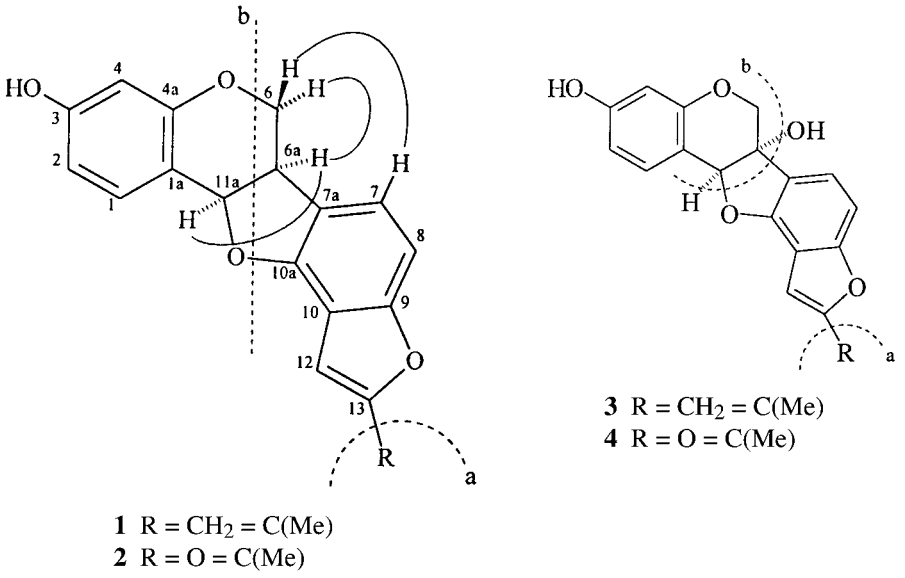

Fig. 2. *Key NOESY interactions of* **1** *and* **2** *and EI-MS fragmentation patterns of* **1**–**4**

The molecular formula of crotafuran B (**2**) was determined to be $C_{19}H_{14}O_5$ by HR-EI-MS (*m/z* 322.0848 (M^+)), which was consistent with the ^{1}H- and ^{13}C-NMR data. The IR absorptions of **2** were indicative of OH (3262 cm^{-1}), conjugated CO (1669 cm^{-1}), and aromatic-ring (1626 cm^{-1}) moieties. The UV spectrum and the EI-MS of **2** (*Fig. 2*) resembled that of crotafuran A (**1**). The ^{1}H-NMR data of **2** were very similar to those of **1**, except for the absence of signals due to the 1-methylethenyl group and the

Table 2. *^{13}C-NMR Data of* **2** *and* **4**[a]); Arbitary numbering, see *Fig. 1*; δ in ppm.

	C(1)	(C(1a)	C(2)	C(3)	C(4)	C(4a)	C(6)	C(6a)	C(7)	C(7a)	C(8)	C(9)	C(10)	C(10a)	C(11a)	C(12)	C(13)	C(14)	C(15)
2[b])	133.2	112.4	110.6	159.9	104.0	157.9	67.2	40.8	125.3	121.9	104.6	155.0	113.7	158.2	81.1	110.6	153.4	187.9	26.5
4	134.0	114.7	111.7	160.6	106.0	158.0	71.3	78.1	125.2	124.6	104.6	156.0	113.7	159.7	88.6	111.3	154.3	188.5	27.2

[a]) The number of protons directly attached to each C-atom was verified by DEPT experiments. [b]) Signals obtained by ^{1}H,^{1}H-COSY, HMQC, HMBC, and NOESY techniques and comparison with the corresponding reported data [4][5].

appearance of signals due to an acetyl group. In the ^{13}C-NMR spectra of **2** (*Table 2*), the chemical-shift values of C(1) to C(15) were almost identical to corresponding data of **1** (*Table 1*) except for C(7), C(9), C(10), and C(12) to C(15). Based on these results, the acetyl group was located at C(13). The ^{1}H- and ^{13}C-NMR, COSY90, HMQC, HMBC, and NOESY data allowed to assign to crotafuran B (**2**) the structure of 1-(3-hydroxyfuro[2′,3′:9,10]pterocarpan-5′-yl)ethanone.

The molecular formula of crotafuran C (**3**) was determined to be $C_{20}H_{16}O_5$ by HR-EI-MS (*m/z* 336.1016 (M^+)), which was consistent with the ^{1}H- and ^{13}C-NMR data. The IR absorptions of **3** were indicative of OH (3416 cm^{-1}), conjugated CO (1625 cm^{-1}), and aromatic ring (1602 cm^{-1}) moieties, and the UV spectrum resembled that of **1**, suggesting a pterocarpanoid structure. The ^{1}H- and ^{13}C-NMR (*Table 1*), COSY90, HMQC, HMBC, and NOESY data confirmed the structure of 5′-(1-methylethenyl)-furo[2′,3′:9,10]pterocarpan-3,6a-diol for crotafuran C (**3**).

The ^{1}H-NMR spectrum of **3** was similar to that of **1**, except for the lack of signals due to H_β–C(6), H_α–C(6a), H_α–C(6), and H_α–C(11a) of the pterocarpan moiety and the appearance of signals due to methylene protons at δ 4.16 (*d*, *J* = 11.6 Hz, 1 H) and 4.20 (*d*, *J* = 11.6 Hz, 1 H) and a methine proton at δ 5.49 (*s*). The ^{13}C-NMR data were almost identical to those of **1**, except for the signals of C(6) to C(7a) and C(10a). The presence of a quaternary C-atom at δ 78.1 and four deshielded C-signals at δ 71.4, 124.1, 159.0, and 87.7 compared to those of corresponding signals of **1** suggested that **3** was a pterocarpan-3,6-diol. The presence of characteristic EI-MS peaks at *m/z* 318 ($[M-H_2O]^+$), 277 ($[318-a]^+$), and 163 ($[318-b-H_2O-H]^+$) (*Fig. 2*) supported the structure proposed for **3**. In $(CD_3)_2CO$, the chemical shifts of H_α–C(6), H_β–C(6), and H_α–C(11a) experienced a low-field shift of 0.22, 0.06, and 0.28 ppm, respectively, compared with those measured in $CDCl_3$ [5]. This clearly indicated that the ring fusion of rings B and C is *cis* [6].

The molecular formula of crotafuran D (**4**) was determined to be $C_{19}H_{14}O_6$ by HR-EI-MS (*m/z* 338.0791 (M^+)), which was consistent with the ^{1}H- and ^{13}C-NMR data. The IR absorptions of **4** were indicative of OH (3417 cm^{-1}), conjugated CO (1660 cm^{-1}), and aromatic ring (1625 cm^{-1}) moieties, and the ^{1}H-NMR data were very similar to those of crotafuran C (**3**), except for the absence of signals of the 1-methylethenyl group and the appearance of signals due to an acetyl group. In the ^{13}C-NMR spectra of **4** (*Table 2*), the chemical-shift values of C(1) to C(15) were almost identical to corresponding data for **3** (*Table 1*), except for C(7), C(9), C(10), and C(12) to C(15). Based on these results, the acetyl group was located at C(13). The ^{1}H- and ^{13}C-NMR spectra and comparison with those of **3** and with reported data [4] allowed us to assign to crotafuran D (**4**) the structure of 1-(3,6a-dihydroxyfuro[2′,3′:9,10]pterocarpan-5′-yl)ethanone (**4**).

Crotafurans A, B, C, and D are the first natural products containing a furan ring fused at the C(9)–C(10) bond of the pterocarpan skeleton.

This work was partly supported by a grant from the *National Science Council of R. O. C.* (NSC 89-2320-B-037-077).

Experimental Part

General. M.p.: uncorrected. Optical rotations: *Jasco* model *DIP-370* digital polarimeter. UV Spectra: *Jasco UV-VIS* spectrophotometer; λ_{max} (log ε) in nm. IR Spectra: *Hitachi 260-30* spectrophotometer; $\tilde{\nu}$ in cm^{-1}. ^1H- and ^{13}C-NMR Spectra: *Varian Unity-400* spectrometer; 400 and 100 MHz, resp.; δ in ppm, J in Hz. MS: *JMS HX100* mass spectrometer; *m/z* (rel. %).

Plant Material. Whole plants of *C. pallida* and the seeds of *C. assamica* were collected at Ping Tung Hsieng, Taiwan, in July 2000. A voucher specimen (2003) has been deposited at the Department of Medicinal Chemistry, School of Pharmacy, Kaohsiung Medical University.

Extraction and Isolation. Pieces of the bark (8 kg) of *C. pallida* were chipped and extracted with MeOH at r.t. The extract (85 g) was subjected to column chromatography (silica gel, C_6H_6/acetone 2:1): **1** (30 mg), **2** (15 mg), and **3** (10 mg). The seeds (125 g) of *C. assamica* were pressed and extracted with MeOH at r.t. The extract (20 g) was subjected to column chromatography (silica gel, CH_2Cl_2/MeOH 9:1): **4** (5 mg).

Crotafuran A (= *5′-(1-Methylethenyl)furo[2′,3′:9,10]pterocarpan-3-ol* = rel-*(5b*R,*11b*R*)-5b,11b-Dihydro-2-(1-methylethenyl)-6*H-*furo[2′,3′:6,7]benzofuro[3,2-*c*][1]benzopyran-9-ol*; **1**): Yellow needles (C_6H_6/acetone). $[\alpha]_D^{25} = -18$ ($c = 0.16$, acetone). UV (MeOH): 245 (4.14), 279 (4.25), 288 (sh, 4.80). IR (KBr): 3432, 2952, 2360, 1604. ^1H-NMR ((D_6)acetone, 400 MHz; for numbering, see *Fig. 1*): *Table 1*. ^{13}C-NMR ((D_6)acetone, 100 MHz): *Table 1*. EI-MS (70 eV): 320 (67, M^+), 303 (13), 256 (2), 211 (6), 198 (15), 186 (23), 147 (18), 55 (79). HR-EI-MS: 320.1048 ($C_{20}H_{16}O_4^+$; calc. 320.1049).

Crotafuran B (= *1-(3-Hydroxyfuro[2′,3′:9,10]pterocarpan-5′-yl)ethanone* = rel-*1-[(5b*R,*11b*R*)-5b,11b-Dihydro-9-hydroxy-6*H-*furo[2′,3′:6,7]benzofuro[3,2-*c*][1]benzopyran-2-yl]ethanone*; **2**): Yellow needles (C_6H_6/acetone). $[\alpha]_D^{25} = -16$ ($c = 0.14$, acetone). UV (MeOH): 237 (3.93), 288 (3.94), 338 (sh, 3.48). IR (KBr): 3262, 2922, 2858, 1669, 1626. ^1H-NMR ((D_6)acetone, 400 MHz; for numbering, see *Fig. 1*): 2.54 (*s*, Me(16)); 3.73 (*dd*, $J = 10.4$, 10.4, H_β–C(6); 3.83 (*ddd*, $J = 11.0$, 7.2, 4.8, H_α–C(6a)); 4.36 (*dd*, $J = 10.4$, 4.8, H_α–C(6)); 5.80 (*d*, $J = 7.2$, H–C(11a)); 6.37 (*d*, $J = 2.4$, H–C(4)); 6.59 (*dd*, $J = 8.4$, 2.4, H–C(2)); 7.15 (*d*, $J = 8.4$, H–C(8)); 7.40 (*d*, $J = 8.4$, H–C(1)); 7.53 (*d*, $J = 8.4$, H–C(7)); 7.63 (*s*, H–C(12)); 8.64 (*s*, OH–C(3)). ^{13}C-NMR ((D_6)acetone, 100 MHz): *Table 2*. EI-MS (70 eV): 322 (58, M^+), 305 (9), 251 (5), 147 (18), 134 (37), 55 (27). HR-EI-MS: 322.0848 ($C_{19}H_{14}O_5^+$; calc. 322.0841).

Crotafuran C (= *5′-(1-Methylethenyl)furo[2′,3′:9,10]pterocarpan-3,6a-diol* = rel-*(5b*R,*11b*R*)-5b,11b-Dihydro-2-(1-methylethenyl)-6*H-*furo[2′,3′:6,7]benzofuro[3,2-*c*][1]benzopyran-5b,9-diol*; **3**): Yellow needles (C_6H_6/acetone). $[\alpha]_D^{25} = -20$ ($c = 0.11$, acetone). UV (MeOH): 275 (4.32), 285 (4.36), 306 (sh, 3.92). IR (KBr): 3146, 1625, 1602. ^1H-NMR ((D_6)acetone, 400 MHz; for numbering, see *Fig. 1*): *Table 1*. ^{13}C-NMR ((D_6)acetone, 100 MHz): *Table 1*. EI-MS (70 eV): 336 (17, M^+), 318 (5), 317 (8), 291 (8), 277 (7), 163 (10), 115 (12), 69 (22). HR-EI-MS: 336.1016 ($C_{20}H_{16}O_5^+$; calc. 336.0998).

Crotafuran D (= *1-(3,6a-Dihydroxyfuro[2′,3′:9,10]pterocarpan-5′-yl)ethanone* = rel-*1-[(5b*R,*11b*R*)-5b,11b-Dihydro-5b,9-dihydroxy-6*H-*furo[2′,3′:6,7]benzofuro[3,2-*c*][1]benzopyran-2-yl]ethanone*; **4**): White powder (CH_2Cl_2/MeOH). $[\alpha]_D^{25} = -23$ ($c = 0.16$, MeOH). UV (MeOH): 237 (4.24), 287 (4.23), 330 (sh, 3.81). IR (KBr): 3417, 1660, 1625. ^1H-NMR ((D_6)acetone, 400 MHz; for numbering, see *Fig. 1*): 2.55 (*s*, Me(15)); 4.20 (*d*, $J = 11.6$, H_α–C(6)); 4.25 (*d*, $J = 11.6$, H_β–C(6)); 5.27 (*s*, OH–C(6a)); 5.58 (*s*, H–C(11a)); 6.32 (*d*, $J = 2.4$, H–C(4)); 6.60 (*dd*, $J = 8.4$, 2.4, H–C(2)); 7.22 (*d*, $J = 8.4$, H–C(8)); 7.59 (*d*, $J = 8.4$, H–C(7)); 7.64 (*s*, H–C(12)); 8.65 (*s*, OH–C(3)). ^{13}C-NMR ((D_6)acetone, 100 MHz): *Table 2*. EI-MS (70 eV): 338 (9, M^+), 320 (1), 319 (2), 293 (5), 257 (5), 236 (5), 97 (45), 69 (66), 57 (83). HR-EI-MS: 338.0791 ($C_{19}H_{14}O_6^+$; calc. 338.0790).

REFERENCES

[1] L. W. Smith, C. C. Culvenor, *J. Nat. Prod.* **1981**, *44*, 129.

[2] L. A. Mitscher, S. R. Gollapudi, D. C. Gerlach, S. D. Drake, E. A. Véliz, J. A. Ward, *Phytochemistry* **1988**, *27*, 381.

[3] K. Biemann, 'Spectral Data for Structure Determination of Organic Compounds', Springer-Verlag, Cambridge, MA 02139/USA, 1989, H265.

[4] A. V. K. Prasad, R. S. Kapil, S. P. Popli, *Indian J. Chem., Sect. B* **1985**, *24*, 236.

[5] W. Lwande, M. D. Bentley, C. Macfoy, F. N. Lugemwa, A. Hassanali, E. Nyandat, *Phytochemistry* **1987**, *26*, 2425.

[6] P. V. Demacro, E. Farkas, D. Doddrell, B. L. Mylari, E. Wenkert, *J. Am. Chem. Soc.* **1968**, *90*, 5480.

Received October 1, 2001