Journal of Natural Products
Vol. 53, No. 3, pp. 559-564, May-Jun 1990

NEW POLYHYDROXYLATED TRITERPENES FROM *UNCARIA TOMENTOSA*

R. AQUINO, F. DE SIMONE, F.F. VINCIERI, C. PIZZA,*

Dipartimento di Chimica delle Sostanze Naturali, Università degli Studi di Napoli, Via D. Montesano, 49, I-80131 Napoli, Italy

and E. GAĆS-BAITZ

Central Research Institute of Chemistry, Hungarian Academy of Sciences, Pusztaszeri út 59-57, H-1525 Budapest, Hungary

ABSTRACT.—Three novel polyhydroxylated triterpenes have been isolated from *Uncaria tomentosa*. Their structures were established as **1**, **2**, and **3** by detailed spectral studies including ^{1}H-^{13}C correlations via long range couplings using the INAPT pulse sequence, nOeds, and 2D ^{1}H-^{13}C direct chemical shift correlation (HETCOR) nmr techniques.

Recently we described the occurrence of a number of quinovic acid glycosides with moderate antiviral activity in two plants of the Rubiaceae that are used in South American traditional medicine (1–4). Such compounds were isolated from the Brazilian plant *Guettarda platypoda* DC. (2,3) and from the Peruvian plant *Uncaria tomentosa* (Willd.) DC. (1,4).

During the course of our search for other biologically active metabolites from these plants, we have isolated three novel polyhydroxylated triterpenes, **1**, **2**, and **3**, from the $CHCl_3$ extract of *U. tomentosa*, and in the present paper we describe the structures of these three compounds.

Displaying a number of structural features frequently encountered with terpenic natural products, the molecules studied here may be of some general phytochemical interest. Therefore, we report here a detailed spectral analysis of **1**, **2**, and **3**, including 2D-nmr techniques (HETCOR) and 1D-nmr techniques (INAPT or insensitive nuclei assigned by polarization transfer, and nOeds or nuclear Overhauser enhancement difference spectra).

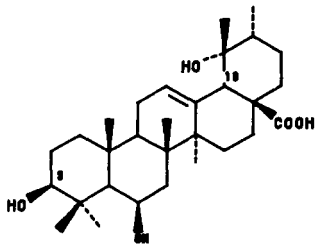
RESULTS AND DISCUSSION

The dried bark of *U. tomentosa* was extracted with petroleum ether followed by $CHCl_3$. From the $CHCl_3$ extract **1**, **2**, and **3** were isolated by a SiO_2 column and a reversed-phase hplc separation.

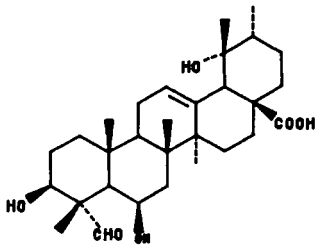
Hreims analysis showed a molecular ion at *m/z* 488.3508 $[M]^+$ (488.3502 calcd for $C_{30}H_{48}O_5$), which gave the molecular formula $C_{30}H_{48}O_5$ for **1**; this formula was also derived by the ^{13}C- and DEPT ^{13}C-nmr analysis and indicated its triterpenic nature.

The ^{13}C-nmr spectrum of **1** revealed thirty carbon signals due to a pentacyclic triterpenic skeleton; these were sorted by ^{13}C and DEPT ^{13}C nmr into -Me × 7, $-CH_2-$ × 8, >CH × 5, >C< × 6, >CH-O- × 2, $-\overset{|}{C}-O-$ × 1, and -COOH × 1 (Table 1). The $\Delta^{12,13}$ structure was derived from the resonance of the sp^2 carbons at C-12 (tertiary carbon, deduced by DEPT pulse sequence) at 129.4 ppm and at C-13 (quaternary carbon) at 137.6 ppm. The chemical shifts of the olefinic carbons were useful to distinguish between an urs-12-ene and an olean-12-ene analogue (5). The signal at 181.1 ppm (quaternary carbon) appeared to be diagnostic for the presence of a 28 carboxyl group on the urs-12-ene skeleton (1,6).

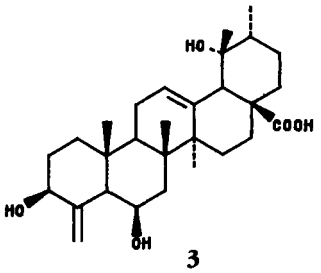
The ^{1}H-nmr spectrum of **1** in CD_3OD at 250 MHz (see Experimental) showed signals for one olefinic proton at δ 5.25 (1H, t, J = 3.5 Hz, H-12) and for two -C*H*-OH at



1



2



3

δ 3.10 and δ 4.51. There were also six partially overlapped singlets due to Me groups at quaternary carbons in the region δ 1.08–1.33 and one doublet at δ 0.96 (3H, d, $J = 6$ Hz, H-30) due to an Me group at a tertiary carbon.

These spectral evidences together with the appearance of characteristic signals at δ 2.59 (1H, s, H-18) in the ^{1}H-nmr spectrum and at 73.3 ppm (C-19, quaternary carbon) in the ^{13}C-nmr spectrum suggested that **1** was an ursolic acid derivative containing an Me group and an OH group at C-19 (7,8). We also noted that the OH group at the 19 position induced a downfield shift of the resonance of the axial proton at C-16. This signal in ursolic acid derivatives has not been reported in the literature because it is overlapped with other signals below δ 2.0 whereas in **1** it resonated at δ 2.62 and was observed as a ddd with $J = 13.5$, 13.5, 4.5 Hz, thus supporting the 19α-OH stereochemistry and being compatible only with a cis stereochemistry of the ring-D/E junction.

In addition the eims spectrum (see Experimental) showed prominent fragment ions at *m/z* 264 and 246 ([264 − H_2O], base peak), usually resulting from the typical retro-Diels-Alder cleavage of urs-12-enes that possess a C-17 carboxyl group and a hydroxy group on ring D or E (8,9). The ready loss of the carboxyl group from the fragment at *m/z* 246 to afford the ion at *m/z* 201 was also in agreement with its positioning at C-17 (8,9). Further, the appearance of a fragment at *m/z* 224 indicated that two hydroxyl groups were present on ring A and/or B (10).

The β-OH substitution at C-3 was evident from the chemical shift and the J value of the axial proton at C-3 (δ 3.10, dd, $J = 11.5$ and 4 Hz, H-3). The location of the other secondary -OH group at C-6 was deduced by direct 2D-^{1}H, ^{13}C chemical shift cross correlations (Table 1) and by spin decoupling experiments, which showed a proton sequence H-5 (δ 0.78), H-6 (δ 4.51), H-7 (δ 1.50 and 1.72) starting from the H-5 signal (δ 0.78, 1H, d, $J = 1.5$ Hz). The β configuration of this OH group was evident from the unresolved signal of the equatorial H-6 proton (δ 4.51, 1H, m, $W½ = 6$ Hz) and from the resonances of Me-24 (δ 1.19), Me-25 (δ 1.33), and Me-26 (δ 1.11), which were significantly shifted downfield by 1,3 diaxial interactions with respect to unreported ursolic acid spectrum run in CD_3OD (+0.31, +0.34, +0.30 ppm, respectively) and 6α-OH triterpenic derivatives (11,12).

TABLE 1. Nmr Data for Compounds **1**, **2**, and **3** in $CD_3OD/CDCl_3$.[a]

Position	Compound								
	1[b]			**2**[b]			**3**[c]		
	δ ^{13}C	DEPT[d]	δ ^1H, (J_{HH}, Hz)	δ ^{13}C	DEPT[d]	δ ^1H, (J_{HH}, Hz)	δ ^{13}C	DEPT[d]	δ ^1H, (J_{HH}, Hz)
1	40.9	CH_2	0.96,1.55[e]	40.5	CH_2	1.07,1.62[e]	39.5	CH_2	
2	27.0	CH_2	1.62	26.2	CH_2	1.27,1.77[e]	31.7	CH_2	
3	79.2	CH	3.14 dd (11.3,4.0)	72.7	CH	3.76 dd (10.5,4.5)	72.9	CH	3.98 dd (11.5,5.5)
4	39.7	C	—	56.4	C	—	150.9	C	—
5	55.9	CH	0.76 d (1.5)	49.6	CH	1.39 d (2)	52.1	CH	1.72 d (2)
6	68.6	CH	4.54 m ($W_{1/2}$ = 6)	70.8	CH	3.88 m ($W_{1/2}$ = 6)	69.8	CH	4.44 m ($W_{1/2}$ = 6)
7	40.6	CH_2	1.53,1.72 each dd (11.5,3.0)	40.5	CH_2	1.46,1.76 each dd (11.5,3.0)	40.6	CH_2	—
8	39.1	C	—	39.7	C	—	38.9	C	—
9	47.7	CH	1.68	47.7	CH	1.80	44.9	CH	—
10	36.6	C	—	35.9	C	—	37.8	C	—
11	23.7	CH_2	2.05 m	23.7	CH_2	2.07 m	24.5	CH_2	—
12	129.4	CH	5.39 br t (3.5)	128.6	CH	5.38 br t (3.5)	129.6	CH	5.41 br t (3.5)
13	137.6	C	—	137.8	C	—	137.8	C	—
14	41.9	C	—	42.0	C	—	42.3	C	—
15	28.3	CH_2	0.98,1.76[e]	28.4	CH_2	1.01,1.78[e]	28.1	CH_2	—
16	25.6	CH_2	1.56,2.48 ddd (13.5,4.5,2.0) ddd (13.5,13.5,4.5)	25.6	CH_2	1.56,2.47 ddd (13.5,4.5,2.0) ddd (13.5,13.5,4.5)	25.6	CH_2	—
17	47.8	C	—	47.9	C	—	47.7	C	—
18	53.5	CH	2.58 s	53.6	CH	2.56 s	53.5	CH	2.58 s
19	73.3	C	—	73.2	C	—	73.1	C	—
20	41.4	CH	1.41	41.5	CH	1.40	41.2	CH	—
21	26.2	CH_2	1.25,1.62[e]	26.2	CH_2	1.24,1.60[e]	26.1	CH_2	—
22	37.7	CH_2	1.60,1.75[e]	37.7	CH_2	1.65,1.76[e]	37.5	CH_2	—
23	17.1	Me	1.06 s	209.3	CH	9.52 s	—	—	—
24	27.8	Me	1.16 s	10.1	Me	1.42 s	—	—	—
25	16.8	Me	1.30 s	17.0	Me	1.32 s	16.5	Me	1.16 s
26	17.7	Me	1.06 s	17.7	Me	1.06 s	18.4	Me	1.09 s
27	24.5	Me	1.26 s	24.5	Me	1.28 s	24.6	Me	1.29 s
28	181.1	C	—	181.2	C	—	181.1	C	—
29	27.2	Me	1.23 s	27.1	Me	1.23 s	27.5	Me	1.23 s
30	16.2	Me	0.95 d (6)	16.1	Me	0.95 d (6)	16.1	Me	0.95 d (6)
$=CH_2$ at C-4							104.5	CH_2	4.72 t (3.5) 5.27 br s

[a]s = singlet, d = doublet, t = triplet, m = multiplet, br = broad.

[b]Assignments confirmed by 2D-^{13}C, ^1H direct cross correlation spectroscopy (HETCOR) recorded on a Varian XL-400 spectrometer.

[c]One-dimensional ^1H-nmr spectrum obtained at 400 MHz, one-dimensional ^{13}C-nmr spectrum obtained at 101 MHz.

[d]DEPT were obtained on a Bruker MW-250 instrument.

[e]Partially obscured by adjacent signals or overlapped signals.

Because in the literature regarding triterpenoids the ^1H-nmr Me resonances are normally reported unassigned (8,9,13), and to assign unambigously all ^{13}C-nmr signals, particularly signals arising from C-1 and C-7, C-16 and C-21, and C-5 and C-18, which resonate very close to each other, we initiated a detailed nmr study of compounds **1–3**. Methyl proton signals that are partially overlapped in CD_3OD at 250 MHz became visible at 400 MHz in a mixture of $CD_3OD/CDCl_3$. In these conditions, spectra with isolated signals were obtained, all eight methyls were observed distinctly, and 2D-^1H, ^{13}C nmr direct chemical shift correlation experiments (Table 1) allowed all signals to be interrelated.

In addition, the unambigous assignments of all Me signals were made by some INAPT experiments (14), which delineated the correlation of each methyl proton with carbons linked via long-range couplings. The INAPT experiments showed that the Me signal at δ 1.06 (Me-26) was correlated with the carbon resonances at 39.1 ppm (C-8), 47.7 ppm (C-9), 41.9 ppm (C-14), and 40.6 ppm (C-7); furthermore the Me signal at δ 1.30 (Me-25) was observed to correlate with the resonances of C-10, C-1, C-9, and C-5 (Table 1).

These experiments also established connectivities between Me-27 (δ 1.26) and the resonances of C-14, C-8, and C-15 and between the Me singlet at δ 1.23 (Me-29) and the resonances of C-19, C-18, and C-20. The methyl doublet signal at δ 0.95 (Me-30) was observed to correlate with the C-20, C-19, and C-21 resonances. This provided an unequivocal assignment of all the methyl group signals. From all these data it was concluded that **1** is 3β,6β,19α-trihydroxyurs-12-en-28-oic acid.

Compound **2** had mol wt 502 and a molecular formula $C_{30}H_{46}O_6$, and compound **3** had mol wt 472 and a molecular formula $C_{29}H_{44}O_5$ derived from ms and ^{13}C- and DEPT ^{13}C-nmr analyses.

Comparisons of the nmr spectral data of **2** and **3** with those of **1** (Table 1) indicated that **2** and **3** are 3β,6β,19α-trihydroxyurs-12-en-28-oic acid derivatives. In particular, hydrogens and carbons due to the C, D, and E rings of **2** and **3** resonated at almost the same frequencies as the corresponding signals in **1**, while the A- and B-ring hydrogen and carbon signals were observed at somewhat different positions.

The nmr spectra of **2** contained one less methyl and one more signal [^1H nmr 9.52 (1H, s); ^{13}C nmr 209.3 ppm (CH)] than those of **1**, suggesting that in **2** one of the Me group was replaced by a formyl group. The eims spectrum of **2** showed an intense peak at *m/z* 472, which could be explained as elimination of an aldhedic group from the molecular ion (*m/z* 502). Other prominent fragments were observed at *m/z* 354, 264, and 208 for the retro-Diels-Alder cleavage of the 472 ion. The ^1H-nmr spectrum of **2** showed that H-3 (δ 3.76) and H-5 (δ 1.39) signals were shifted downfield by 0.62 and 0.63 ppm, respectively, while H-6 (δ 3.88) signal was shifted upfield by 0.66 ppm in comparison with the spectrum of **1**.

In addition, one of the methyl signals was affected by the presence of the formyl group; its resonance was shifted downfield to δ 1.42 in **2** from δ 1.16 in **1**. Therefore the -CHO group should be restricted to the C-23 or C-24 positions.

The most significant features of the ^{13}C-nmr spectrum of **2**, which suggested the location of the formyl group at C-23, were the downfield shifts exhibited by C-4 (+16.7 ppm) and C-6 (+2.2 ppm) and the upfield shifts experienced by C-3 (−6.5 ppm), C-5 (−6.3 ppm), and Me-24 (−17.6 ppm). Similar shifts were reported for gypsogenin, which has a 23 equatorial -CHO group, when compared with oleanolic acid (13,15).

Furthermore, ^1H-nOeds experiments were applied for substantiation of the above relative stereochemistry at C-23 and C-24: by irradiation of the signal at δ 9.52 (-*CHO*) we observed an nOe with the signal at δ 1.39 (H-5α), 1.42 (Me-24 axial), 3.88 (H-6α), and 3.76 (H-3α). NOe's were also observed between H-5α, H-6α, H-3α, and the aldehydic signal at δ 9.52. These nOe's led us to confirm the equatorial orientation of the formyl group. Therefore the structure of **2** was elucidated as 3β,6β,19α-trihydroxy-23-oxo-urs-12-en-28-oic acid.

The eims spectrum of **3** showed a fragmentation pattern identical to that of **2**, starting from the molecular ion at *m/z* 472. The ^1H-nmr spectrum, by comparison with that of **1**, showed the lack of two Me signals (Me-23 and Me-24) and the presence of complex signals at δ 4.72 (1H, t, $J = 3.5$ Hz) and 5.27 (1H, br s), which could be assigned to the CH_2=C group. The major differences between the two spectra involved the H-3 and H-5 signals which were shifted downfield by 0.84 ppm (from δ 3.14 in **1** to δ 3.98 in **3**) and 0.96 ppm (from δ 0.76 in **1** to δ 1.72 in **3**), respectively, and the H-6 signal shifted upfield by 0.1 ppm to δ 4.44 in **3** from δ 4.54 in **1**.

Further evidence for the presence of a CH_2=C group was provided by the ^{13}C-nmr resonances of the sp^2 carbons C-4 (quaternary) at 150.9 ppm and CH_2= (secondary) at 104.5 ppm. The location of the =CH_2 group at C-4 was also justified by the disappearances of the signals arising from Me-23 and Me-24 and by the shift values of the carbons

neighboring the C-4 position; in fact C-3 and C-5 were shielded by -6.3 and -3.8 ppm, respectively, while C-6 was deshielded by +1.2 ppm with respect to the corresponding signals in **1**. It follows that **3** can be formulated as 23-nor-24-esomethylene-3β,6β,19α-trihydroxyurs-12-en-28-oic acid.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—The following instruments were used: for nmr, Bruker MW-250 Spectrospin and Varian XL-400 spectrometers; for eims, AEI MS-30; for hplc, Waters Model 6000 A pump equipped with a U6K injector and a differential refractometer, model 401; for optical rotation, Perkin-Elmer Model 141 polarimeter.

Monodimensional spectra were measured both in CD_3OD and in CD_3OD-$CDCl_3$ (1:1); the 2D and the INAPT experiments were measured in CD_3OD-$CDCl_3$ (1:1). Chemical shifts are reported in ppm (δ) downfield from internal TMS, and coupling constants (J) are given in Hz.

The nOe, DEPT, direct HETCOR, and INAPT experiments were carried out using Varian or Bruker commercial microprograms. The nOe experiments were performed using the spectral subtraction technique (nOeds). The samples for nOe measurements were previously degassed by bubbling argon through the solution for 40 min.

The DEPT (Distortionless Enhancement by Polarization Transfer) experiments were performed using polarization transfer pulses of 90° to obtain only CH groups and 135° to obtain positive signals for CH and Me and negative ones for the CH_2. Polarization transfer delays were adjusted to an average CH coupling of 135 Hz. These delays were also applied for 2D direct ^{13}C-^1H shift correlations (HETCOR) on a 512 × 2048 data matrix (Varian XL-400).

INAPT (selective polarization transfer spectroscopy via long-range ^1H-^{13}C- couplings) spectra were obtained using the pulse sequence described by Bax and co-workers (14,16) and Radics and Sàndor (17). The delays used in the pulse sequence were set to 60 msec (Δ_1) and 25 msec (Δ_2) for Me groups.

EXTRACTION AND ISOLATION.—The plant material and the extraction procedure have been described earlier (1,4). The $CHCl_3$ extract of the bark of *U. tomentosa* was evaporated to dryness to give 1.63 g of residue which was then chromatographed on a SiO_2 column by using $CHCl_3$ and increasing MeOH content to 10%. After the monitoring [tlc on SiO_2 plates, $CHCl_3$-MeOH (95:5)], the fractions were combined to give three main fractions, A (50.3 mg), B (39.6 mg), and C (89.3 mg). Purification of each fraction was achieved by hplc on a C_{18} μ-Bondapak column (30 cm × 8 mm, flow rate 2 ml/min) using MeOH-H_2O (9:1) as the eluent to afford pure compound **1** (32.5 mg, elution time 3.5 min) from fraction A, compound **2** (28.7 mg, elution time 5.2 min) from fraction B, and compound **3** (16.7 mg, elution time 5.5 min) from fraction C.

Compound **1**.—$[\alpha]^{25}D$ +52.7° (c = 1.0, MeOH); ms *m/z* $[M]^+$ 488, $[M-H_2O]^+$ 470, $[M-2H_2O]^+$ 452, $[M-HCOOH]^+$ 442, $[M-HCOOH-H_2O]^+$ 424, $[452-15]^+$ 437 $[424-15]^+$ 409, $[409-H_2O]^+$ 391, 370, 264, $[264-18]^+$ 246 base peak, $[246-15]^+$ 251, 224, $[264-COOH]^+$ 219, $[264-HCOOH]^+$ 218, $[224-18]^+$ 206, $[246-COOH]^+$ 201, $[201-14]^+$ 187; ^1H nmr (CD_3OD at 250 MHz) δ 0.78 (1H, d, J = 1.5 Hz, H-5), 0.96 (3H, d, J = 6 Hz, Me-30), 1.08 (3H, s, Me-23), 1.11 (3H, s, Me-26), 1.19 (3H, s, Me-24), 1.23 (3H, s, Me-27), 1.33 (6H, br s, Me-29 and Me-25), 1.50 (1H, br, H-7eq), 1.72 (1H, dd, J = 11.6 and 3 Hz, H-7ax), 2.59 (1H, s, H-18), 2.62 (1H, ddd, J = 13.5, 13.5, 4.5 Hz, H-16ax), 3.10 (1H, dd, J = 11.5, 4.0 Hz, H-3), 4.51 (1H, m, H-6), 5.25 (1H, t, J = 3.5 Hz, H-12).

Compound **2**.—$[\alpha]^{25}D$ −15.2° (c = 1.0, MeOH); ms *m/z* $[M]^+$ 502, $[M-H_2O]^+$ 484, $[M-HCHO]^+$ 472, $[M-HCOOH]^+$ 456, $[472-HCOOH]^+$ 426, $[426-H_2O]^+$ 408, 384, 354, 264, 246 (base peak), 231, 219, 218, 208, 201, 190, 187; ^1H nmr (CD_3OD at 250 MHz) δ 0.96 (3H, d, J = 6 Hz, Me-30), 1.12 (3H, s, Me-26), 1.24 (3H, s, Me-27), 1.37 (6H, br s, Me-29, Me-25), 1.44 (3H, s, Me-24), 2.60 (1H, s, H-18), 2.62 (1H, ddd, J = 13.5, 13.5, 4.5 Hz, H-16ax), 3.70 (1H, dd, J = 11.5, 3 Hz, H-3), 3.91 (1H, m, H-6), 5.26 (1H, m, H-12).

Compound **3**.—$[\alpha]^{25}D$ +50° (c = 1.0, MeOH); ms *m/z* $[M]^+$ 472, $[M-H_2O]^+$ 454, $[M-HCOOH]^+$ 426, $[426-H_2O]^+$ 408, 354, 264, 246 (base peak), 231, 219, 218, 208, 201, 190, 187; ^1H nmr (CD_3OD at 250 MHz) δ 0.96 (3H, d, J = 6 Hz, Me-30), 1.11 (3H, s, Me-26), 1.21 (3H, s, Me-25), 1.24 (3H, s, Me-27), 1.39 (3H, s, Me-29), 2.60 (1H, s, H-18), 2.62 (1H, ddd, J = 13.5, 13.5, 4.5, H-16ax), 3.98 (1H, br m, H-3), 4.40 (1H, m, H-6), 5.25 (1H, m, H-12), under H_2O signal, 5.38 (each 1H, br s, $=CH_2$).

ACKNOWLEDGMENTS

The authors are grateful to Dr. F.P. Iaccarino for the supply of the plant. The ms spectra were pro-

vided by the "Servizio di Spettrometria di massa del CNR e dell'Università di Napoli." We also thank Prof. M. Nógrádi, Institute of Organic Chemistry, Technical University of Budapest, and Dr. L. Radics, Central Research Institute of Chemistry, Budapest, Hungary, for the hospitality during the use of the Varian XL-400 instrument for the present study.

LITERATURE CITED

1. R. Cerri, R. Aquino, F. De Simone, and C. Pizza, *J. Nat. Prod.*, **51**, 257 (1988).
2. R. Aquino, F. De Simone, C. Pizza, R. Cerri, and J.F. De Mello, *Phytochemistry*, **27**, 2927 (1988).
3. R. Aquino, F. De Simone, C. Pizza, and J.F. De Mello, *Phytochemistry*, **28**, 199 (1989).
4. R. Aquino, F. De Simone, C. Pizza, M.L. Stein, and C. Conti, *J. Nat. Prod.*, **52**, 679 (1989).
5. D.M. Doddrell, P.W. Khong, and K.G. Lewis, *Tetrahedron Lett.*, **27**, 2381 (1974).
6. S. Seo, Y. Tomita, and K. Tori, *Tetrahedron Lett.*, **1**, 7 (1975).
7. M. Takani, K. Kubota, M. Nozawa, T. Ushiki, and K. Takahashi, *Chem. Pharm. Bull.*, **25**, 981 (1977).
8. K. Hidaka, M. Ito, Y. Matsuda, H. Kohda, K. Yamasaki, and J. Yamahara, *Phytochemistry*, **26**, 2023 (1987).
9. H. Budzikiewicz, C. Djerassi, and D.H. Williams, "Structure Elucidation of Natural Products by Mass Spectroscopy," Holden-Day, San Francisco, 1964, Vol. II.
10. F.N. Ngounou, D. Lontsi, and B.L. Sondengam, *Phytochemistry*, **27**, 301 (1988).
11. Z.Z. Liang, R. Aquino, V. De Feo, F. De Simone, and C. Pizza, *Planta Med.*, in press (1989).
12. M.A. Khan and A.U. Rahman, *Phytochemistry*, **14**, 789 (1975).
13. M. Iwamoto, H. Okabe, T. Yamauchi, M. Tanaka, Y. Rokutani, S. Hara, K. Mihashi, and R. Higuchi, *Chem. Pharm. Bull.*, **33**, 464 (1985).
14. A.J. Bax, *J. Magn. Reson.*, **57**, 314 (1984).
15. K. Tori, S. Seo, A. Shimaoka, and Y. Tomita, *Tetrahedron Lett.*, **48**, 4227 (1974).
16. A. Bax, J.A. Ferretti, N. Nashed, and D.M. Jerina, *J. Org. Chem.*, **50**, 3029 (1985).
17. L. Radics and P. Sàndor, *Magnetic Moments*, **3**, 2 (1988).

Received 25 July 1989