A New Triterpene Glycoside from the Roots of Symphytum officinale

Viqar Uddin Ahmad, Mushtaq Noorwala, Faryal Vali Mohammad, and Bilge Sener

J. Nat. Prod., **1993**, 56 (3), 329-334• DOI: 10.1021/np50093a003 • Publication Date (Web): 01 July 2004

Journal of Natural Products
Vol. 56, No. 3, pp. 329-334, March 1993

A NEW TRITERPENE GLYCOSIDE FROM THE ROOTS OF *SYMPHYTUM OFFICINALE*

VIQAR UDDIN AHMAD,* MUSHTAQ NOORWALA, FARYAL VALI MOHAMMAD,

H.E.J. Research Institute of Chemistry, University of Karachi, Karachi 75270, Pakistan

and BILGE SENER

Department of Pharmacognosy, Faculty of Pharmacy, Gazi University, Ankara, Turkey

ABSTRACT.—Chemical investigation of the EtOH-soluble portion of *Symphytum officinale* roots has resulted in the isolation of a new pentacyclic triterpene glycoside of oleanolic acid. Its structure was elucidated by spectroscopic studies, including 2D nmr, and chemical evidences as 3-*O*-[β-D-glucopyranosyl-(1→4)-β-D-glucopyranosyl-(1→4)-α-L-arabinopyranosyl]-oleanolic acid [**1**].

Symphytum officinale L. (Boraginaceae), commonly known as "comfrey," is widely distributed in North Asia, England, and Europe, and is found abundantly in Turkey. The EtOH extract of the roots of the plant are used extensively in the indigenous medicine for the treatment of various ailments (1,2). No work has apparently been carried out on the saponin constituents of the roots of *S. officinale*. The present study describes the isolation and structure determination of a new pentacyclic triterpenoid saponin, 3-*O*-[β-D-glucopyranosyl-(1→4)-β-D-glucopyranosyl-(1→4)-α-L-arabinopyranosyl]-oleanolic acid [**1**] from the roots of *S. officinale*.

RESULTS AND DISCUSSION

Compound **1** was purified by repeated cc on Si gel followed by hplc on a reversed-phase column (RP-8) with MeOH-H_2O (70:30) as the mobile phase: $[\alpha]^{24}D$ +22.86 (c = 0.14, MeOH). The ir spectrum showed carboxylic (1700 cm^{-1}) together with strong hydroxyl (3400 cm^{-1}) absorption bands. The uv spectrum showed only end absorption. The ^{13}C nmr (Table 1) was assigned on the basis of BB, DEPT, 2D direct ^1H/

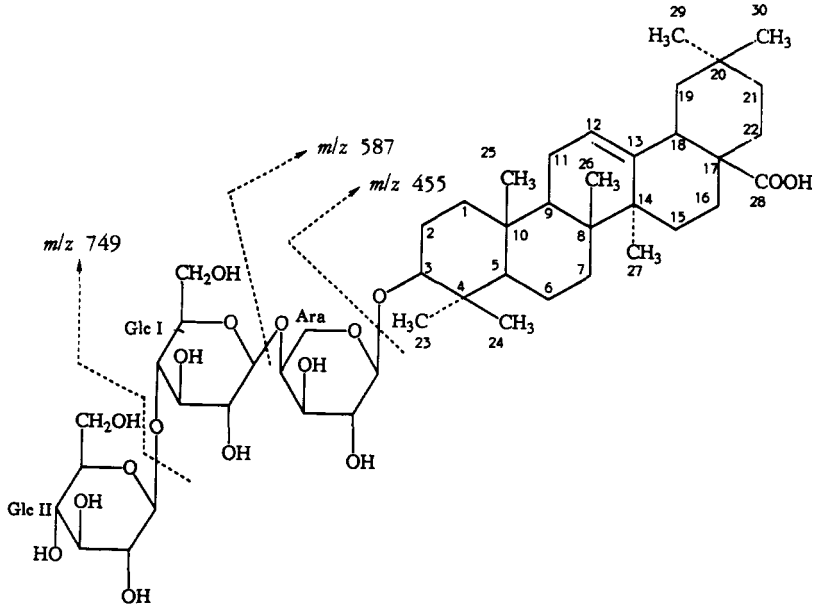

TABLE 1. Nmr Assignments and $^{1}H/^{13}C$ Direct Correlation (HMQC) of Compound **1**.

Position	^{1}H nmr (CD_3OD, 300.13 MHz)			^{13}C nmr (CD_3OD, 75.43 MHz)		
	δ	Multiplicity	*J* (Hz)	δ	GASPE	HMQC
1	[a]	—	—	39.79	CH_2	—
2	[a]	—	—	26.95	CH_2	—
3	3.12	br t	9.0	91.47	CH	3.20 (H-3)
4	—	—	—	40.37	Quaternary	No coupling
5	[a]	—	—	57.02	CH	—
6	[a]	—	—	19.39	CH_2	—
7	[a]	—	—	34.12	CH_2	—
8	—	—	—	40.57	Quaternary	No coupling
9	[a]	—	—	48.14	CH	—
10	—	—	—	37.94	Quaternary	No coupling
11	1.88	m	—	24.54	CH_2	1.88 (H-11)
12	5.21	distorted t	—	122.78	CH	5.21 (H-12)
13	—	—	—	146.40	Quaternary	No coupling
14	—	—	—	43.03	Quaternary	No coupling
15	1.24	m	—	29.13	CH_2	1.24 (H-15)
16	[a]	—	—	24.54	CH_2	—
17	—	—	—	47.94	Quaternary	No coupling
18	[a]	—	—	43.23	CH	—
19	[a]	—	—	47.94	CH_2	—
20	—	—	—	31.72	Quaternary	No coupling
21	[a]	—	—	35.35	CH_2	—
22	[a]	—	—	34.31	CH_2	—
23	1.05	s	—	28.58	Me	1.05 (H-23)
24	0.85	s	—	16.90	Me	0.85 (H-24)
25	0.94	s	—	15.90	Me	0.94 (H-25)
26	0.86	s	—	18.17	Me	0.86 (H-26)
27	1.14	s	—	26.40	Me	1.14 (H-27)
28	—	—	—	—	Quaternary	No coupling
29	0.88	s	—	33.79	Me	0.88 (H-29)
30	0.95	s	—	24.21	Me	0.95 (H-30)
Sugar moiety						
Ara						
1′	4.51	d	5.30	105.16	CH	4.51 (H-1′)
2′	3.86	m	—	73.34	CH	3.86 (H-2′)
3′	3.26	m	—	71.92	CH	3.26 (H-3′)
4′	3.98	m	—	78.29	CH	3.98 (H-4′)
5′	3.55	m	—	64.54	CH_2	3.55 (H-5′)
Glc I						
1″	4.47	d	7.63	105.66	CH	4.47 (H-1″)
2″	3.22	m	—	75.79	CH	3.22 (H-2″)
3″	3.36	m	—	78.07	CH	3.36 (H-3″)
4″	3.24	m	—	78.18	CH	3.24 (H-4″)
5″	3.32	m	—	77.93	CH	3.32 (H-5″)
6″	3.80	m	—	63.12	CH_2	3.80 (H-6″)
Glc II						
1‴	4.60	d	7.66	104.49	CH	4.60 (H-1‴)
2‴	3.20	m	—	75.45	CH	3.20 (H-2‴)
3‴	3.36	m	—	78.07	CH	3.36 (H-3‴)
4‴	3.28	m	—	71.51	CH	3.28 (H-4‴)
5‴	3.88	m	—	78.65	CH	3.88 (H-5‴)
6‴	3.64	m	—	62.76	CH_2	3.64 (H-6‴)

[a]Peaks are not discernible.

^{13}C chemical shift correlation (HMQC), and HMBC experiments. Anomeric carbon resonances at δ 104.49, 105.16, and 105.66 ppm suggested the presence of three monosaccharide moieties. The ^{13}C-nmr spectrum of **1** (Table 1) showed the presence of 47 carbon atoms in the molecule. Sixteen carbon signals were seen for the sugar moieties, and the signal at δ 78.07, assigned to C-3 signals of both glc I and glc II, confirmed the presence of three monosaccharide units, two hexoses and one pentose. The remaining 30 carbon signals were due to the triterpenoid aglycone. The broad band ^{13}C-nmr spectrum of **1** in conjunction with analysis of the gated spin-echo (GASPE) spectrum showed the presence of seven methyl, 13 methylene, 19 methine, and eight quaternary carbon atoms in agreement with structure **1**. The downfield ^{13}C chemical shift at δ 91.47 indicated that the trisaccharide moiety was attached to C-3 of the aglycone (3). Olefinic carbon signals at δ 122.78 and 146.40, methine and quaternary, suggested the presence of an endocyclic double bond at the 12 position in an oleanane skeleton. Moreover, the ^{13}C-nmr spectrum showed the presence of seven tertiary methyl groups at δ 15.90, 16.90, 18.17, 24.21, 26.40, 28.58, and 33.79. The ^{13}C-nmr shielding data were compared with reported data of oleanane triterpenoids and led to identification of the aglycone as oleanolic acid (4).

The structure of compound **1** was further supported by ^{1}H-nmr and negative ion fab mass spectra. The ^{1}H-nmr spectrum of **1** (CD_3OD, 300.13 MHz) indicated the presence of seven tertiary methyl singlets for C-23, C-24, C-25, C-26, C-27, C-29, and C-30 at δ 1.05, 0.85, 0.94, 0.86, 1.14, 0.88, and 0.95, respectively. The attachment of the trisaccharide moiety shifted the carbinylic proton resonance of H-3 at δ 3.12. The ^{1}H-nmr spectrum also showed the presence of an olefinic proton resonance as a distorted triplet at δ 5.21, characteristic of the Δ^{12} proton in pentacyclic triterpenes. The ^{1}H-nmr spectrum also indicated the presence of three sugar moieties. The anomeric proton resonance as a doublet at δ 4.51 (d, $J = 5.30$ Hz) showed the α configuration for an L-arabinose moiety, while the anomeric signals appearing at δ 4.47 (d, $J = 7.63$ Hz) and 4.60 (d, $J = 7.66$ Hz) implied the β configuration for two D-glucose moieties. The ^{1}H-nmr assignments were confirmed with the help of 2D J-resolved, COSY 45°, long range COSY, NOESY, and HOHAHA experiments. A molecular weight of 912 daltons was confirmed by an intense peak at *m/z* 911 in the negative ion fabms of compound **1**, which is due to an $[M-H]^-$ ion, corresponding to the molecular formula $C_{47}H_{76}O_{17}$, indicating ten double bond equivalents. Furthermore, negative fragment ions at *m/z* 749 and 587 may be as ascribed to the loss of terminal glucose and glucose-glucose disaccharide units, respectively. A third fragment ion at *m/z* 455 may be attributed to the loss, from the $[M-H]^-$ ion, of a glucose-glucose-arabinose trisaccharide unit. This sequence indicated that the terminal glucose moiety was linked to inner glucose which was linked to arabinose. This, in turn, was attached to the C-3 of oleanolic acid. This fragmentation also showed that all the sugars of compound **1** were linked to each other by interglycosidic linkages as determined by ^{13}C-nmr data (Table 1).

The acid hydrolysis of compound **1** yielded the aglycone that was identified as oleanolic acid by comparing with spectral and physical data reported in the literature (4,5). The sugars obtained from the hydrolysate were identified as glucose and arabinose by comparison with authentic samples of galactose, glucose, and arabinose through pc and Si gel tlc. The ^{13}C-nmr spectral data (Table 1) indicated the β-D-pyranosyl configuration for glucose and the α-L-pyranosyl configuration for arabinose (6).

The points of attachment of sugar units were determined through ^{13}C chemical shifts in which the upfield shifts of β carbons and the downfield shifts of α carbons were characteristic for the establishment of interglycosidic linkages (7). Comparison of the ^{13}C-nmr spectrum of compound **1** showed that the C-3 and C-5 signals of α-L-

arabinose appeared upfield and the C-4 signal of α-L-arabinose appeared downfield, which allowed us to place a (1↦4) linkage between the arabinose and inner glucose moieties (8). The downfield ^{13}C chemical shift of C-4 of inner glucose at δ 78.18 and the small upfield shift of C-5 of inner glucose at δ 77.93 showed that C-4 of inner glucose is substituted and showed (1↦4) linkage between two glucose units (9). The almost identical ^{13}C-nmr chemical shifts of one β-D-glucose with corresponding methyl glycosides (6) suggested that it was a terminal sugar. The possibility of (1↦2) linkage between two glucose units was eliminated by comparing the ^{13}C-nmr data of saponins reported by Xu *et al.* (10). It was demonstrated by these authors that when terminal glucose was attached to C-2 of inner glucose, the ^{13}C chemical shift was observed at about 83–84 ppm.

One-bond correlations between ^{1}H and ^{13}C nuclei were established by HMQC experiments (11), which provided conclusive evidence to establish the structure of compound **1**. The signals of C-3, C-11, C-12, and C-15 in the ^{13}C-nmr spectrum at δ 91.47, 24.54, 122.78, and 29.13 correlated with the corresponding protons in the ^{1}H-nmr spectrum at δ 3.12, 1.88, 5.21, and 1.24. The methyl carbons resonated at δ 28.58 (C-23), 16.90 (C-24), 15.90 (C-25), 18.17 (C-26), 26.40 (C-27), 33.79 (C-29), and 24.21 (C-30). These carbons were coupled with protons at δ 1.05, 0.85, 0.94, 0.86, 1.14, 0.88, and 0.95, respectively, in the HMQC spectrum. Anomeric carbons C-1′, C-1″, and C-1‴ were coupled to the protons resonating at δ 4.51, 4.47, and 4.60, respectively.

The 2D homonuclear ^{1}H-^{1}H chemical shift correlation (COSY-45°) (11) provided further insights into the structure of **1**. The double bond at C-12 was confirmed by COSY-45°, which showed the connectivity of H-11 (δ 1.88) to vinylic H-12 (δ 5.21). Similarly, COSY interaction was also observed between anomeric H-1′ (δ 4.51) and the vicinal methine H-2′ (δ 3.86). The possibility of (1↦2) linkage between the inner glucose and arabinose was eliminated, because in the HMQC spectrum the methine proton at δ 3.86 was coupled with the carbon at δ 73.35. It is reported that when glucose is attached to C-2 of arabinose, the ^{13}C chemical shift was observed at about 80–84 ppm (9,12). The (1↦4) linkage between glucose and arabinose was finally confirmed by COSY 45°, which showed that H-4′ methine (δ 3.98) exhibited cross peaks with the H-5′ methine (δ 3.55). Again the possibility of (1↦2) linkage between two glucose units was eliminated, as the COSY-45° spectrum showed cross peaks between anomeric methine H-1″ (δ 4.47) and H-2″ (δ 3.22). COSY interactions between H-1‴ (δ 4.60) and H-2‴ (δ 3.20) methines were also observed. Finally H-5‴ (δ 3.88) showed cross peaks with the hydroxyl-bearing methylene H-6‴ (δ 3.64).

The HOHAHA experiments (11) proved valuable in assigning protons of the three monosaccharide units in **1**. The experiment was recorded with mixing times of 20, 60, 80, and 100 msec (13). The spectrum obtained with a mixing time of 20 msec closely resembled the COSY-45° spectrum showing the direct connectivities, whereas with longer mixing intervals the magnetization was seen to spread to more distant protons (11,13). With mixing time of 100 msec, H-1′ (δ 4.51) showed cross peaks in the HOHAHA spectrum with H-2′ (δ 3.86), H-4′ (δ 3.98), and H-5′ (δ 3.55), thus confirming assignments of the protons in arabinose. This result also confirmed that C-4′ of arabinose was linked to C-1″ of glucose.

Similarly H-1″ (δ 4.47) showed cross peaks with H-2″ (δ 3.22), H-3″ (δ 3.36), H-4″ (δ 3.24), H-5″ (δ 3.32), and H-6″ (δ 3.80) in its HOHAHA spectrum. The above data confirmed that C-4″ of the inner glucose must be linked to C-1‴ of the terminal glucose. Other important cross peaks appeared between the protons at δ 4.60 (H-1‴) with H-2‴ (δ 3.20), H-3‴ (δ 3.36), H-4‴ (δ 3.28), H-5‴ (δ 3.88), and H-6‴ (δ 3.64), which confirmed the assignments of carbons C-1‴, C-2‴, C-3‴, C-4‴, C-5‴, and C-6‴.

Long range connectivities were differentiated from vicinal and geminal connectivities by comparison of the HOHAHA spectrum with the COSY-45° spectrum.

Thus, the structure of **1** was established as 3-*O*-[β-D-glucopyranosyl-(1↦4)-β-D-glucopyranosyl-(1↦4)-α-L-arabinopyranosyl]-oleanolic acid.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Melting points were determined in an H_2SO_4 bath (capillary) and are uncorrected. Optical rotations were measured in MeOH with a JASCO DIP-360 digital polarimeter. Uv (MeOH) and ir (KBr) spectra were measured on Hitachi-U-3200 and JASCO A-302 spectrophotometers, respectively. Eims was recorded on a Finningan MAT-112 spectrometer coupled with a PDP 11/34 computer system. The negative ion fabms was recorded on a Jeol JMS HX-110 spectrometer coupled with a PDP 11/73 computer systems operating at an accelerating voltage of −10 kV, using MeOH as a solvent and glycerol as a matrix. Samples were ionized by bombardment with xenon (gas) atoms. The ^{1}H-nmr spectra were recorded in CD_3OD on a Bruker Aspect AM-300 spectrometer operating at 300 MHz. ^{13}C-nmr spectra (BB and GASPE) were recorded in CD_3OD on an Aspect AM-300 spectrometer operating at 75 MHz. The chemical shifts are expressed as ppm (δ), and coupling constants (J) are in Hz with TMS as an internal standard. The ^{13}C-nmr spectral assignments were made partly through a comparison of the chemical shifts with the published data for a similar compound (8) and partly through the appearance of signals in GASPE and HMQC spectra (Table 1). All the above proton signals and multiplicities were determined through 2DJ- resolved spectra, and coupling interactions were established by COSY-45 spectra. Kieselgel 60 (70–230 mesh) was used for cc. Precoated Kieselgel 60, F_{254} cards (thickness 0.25 mm, Riedel de Haëns, Art No. 37360) were used for tlc. The purity of the sample was checked on RP-8 F_{254}S precoated tlc plates (thickness 0.25 mm, E. Merck, Art No. 15684). The hplc consisted of a Shimadzu model LC-6A pump as a solvent delivery system, a Rheodyne sample injector with a 100 μl loop, a Hibar RP-8 column (24.4 cm × 10 mm i.d.), and a Shimadzu model RID-6A refractive index detector connected with a Kipp & Zonen BD-41 recorder.

The 2D COSY-45° spectra were acquired at 300 MHz with sweep widths of 2016 Hz (1K data points in ω_2) and 1008 Hz (256 t_1 values zero-filled to 1K) in ω_1. A 1.5 sec relaxation delay was used, and 16 transients were accumulated for each t_1 value. The 2D ^{1}H-^{13}C chemical shift correlation (HMQC) experiment was carried out at 400 MHz with a sweep width of 2325 Hz (2K data points in ω_2) and 8064 Hz (128 t_1 values zero-filled to 1K) in ω_1. A 1.0 sec relaxation delay was used, and 32 transients were performed for each t_1 value.

PLANT MATERIAL.—The roots of *S. officinale* (dry wt 3 kg) were collected from the Mudanya-Bursa, Ankara, Turkey during the flowering stage in 1989. The plant was identified by Prof. Dr. Bilge Sener, Department of Pharmacognosy, Gazi University, and a voucher specimen (GUE 1018) is preserved in the herbarium of the Faculty of Pharmacy, Gazi University, Ankara.

EXTRACTION AND ISOLATION OF COMPOUND **1**.—The dried and powdered roots (3 kg) of *S. officinale* were repeatedly extracted with EtOH at room temperature. The residue obtained on removal of solvent from the combined extracts under vacuo was dissolved in a minimum amount of MeOH and diluted with cold Et_2O to precipitate the crude glycoside mixture. This procedure was repeated several times, and the yellow precipitate (55 g) was collected by filtration. The crude saponin mixture was subjected to cc on Si gel. Elution was carried out with mixtures of solvents of increasing polarity starting with *n*-hexane, $CHCl_3$, and MeOH. The fractions eluted with $CHCl_3$-MeOH (80:20) were combined on the basis of similar tlc profile and further purified by hplc using MeOH-H_2O (7:3) on a semipreparative reversed-phase (RP-8) column at a flow rate 3.0 ml/min. This procedure led to isolation of saponin **1** (40 mg).

Compound **1**.—$[\alpha]^{24}$D +22.86 (c = 0.14, MeOH); ir (KBr) 3400 cm^{-1} (OH), 1700 cm^{-1} (C=O of COOH group); uv λ (MeOH) max 203.6 nm (end absorption); ^{1}H nmr (CD_3OD, 300 MHz) δ 5.21 (1H, distorted t, H-12), 1.05 (3H, s, H-23), 0.85 (3H, s, H-24), 0.94 (3H, s, H-25), 0.86 (3H, s, H-26), 1.14 (3H, s, H-27), 0.88 (3H, s, H-29), 0.95 (3H, s, H-30), 4.51 (1H, d, J = 5.30 Hz, H-1′), 4.47 (1H, d, J = 7.63 Hz, H-1″), 4.60 (1H, d, J = 7.66 Hz, H-1‴); ^{13}C nmr (CD_3OD, 75.43 MHz) see Table 1; negative ion fabms *m/z* [M − H]$^-$ 911, [M − H − glucose]$^-$ 749, [M − H − 2 × glucose]$^-$ 587, [M − H − 2 × glucose − arabinose]$^-$ 455.

ACID HYDROLYSIS OF COMPOUND **1** AND IDENTIFICATION OF RESULTING MONOSACCHARIDES.—Compound **1** (25 mg) was hydrolyzed with 2 M HCl in aqueous MeOH (10 ml) on a boiling H_2O bath for 3 h. The MeOH was evaporated under reduced pressure, the mixture was diluted with H_2O, and the hydrolysate was then extracted with EtOAc. The aqueous layer thus separated was evaporated under reduced pressure with repeated addition of H_2O to remove HCl. The residue obtained was compared with standard sugars on Si gel tlc [EtOAc-HOAc-H_2O-MeOH (6:1:1:2)], which showed that the sugars

were arabinose and glucose in compound **1**. The identity of the monosaccharides was further confirmed by comparison with standard sugars on pc (Whatman Filter paper No. 1, serrated edges along the lower descending end) using the solvent system *n*-BuOH–pyridine–H_2O (10:3:3) and developing time 48 h. Spots were detected by spraying with freshly prepared aniline phthalate sugar reagent followed by heating. Two spots were present, whose R_f's were identical to the R_f of L-arabinose and D-glucose.

IDENTIFICATION OF THE AGLYCONE.—The EtOAc layer was evaporated under reduced pressure, and the aglycone (8 mg) was compared with the original saponin **1** on tlc using the solvent system *n*-BuOH–HOAC–H_2O (12:3:5). The tlc showed that compound **1** was fully hydrolyzed. The aglycone was identified by eims, ^{1}H nmr, and ^{13}C nmr as oleanolic acid: mp 295–298°, $[\alpha]^{23}D$ +75.0° (c = 0.025, $CHCl_3$), [lit. (14) mp 306–308°, $[\alpha]^{12}D$ +79.5°]; eims *m/z* $[M]^+$ 456 ($C_{30}H_{48}O_3$) [RDA fragment a] 248 ($C_{16}H_{24}O_2$), [RDA fragment b] 207 ($C_{14}H_{23}O$), [RDA fragment a–COOH group] 203; ^{1}H nmr ($CDCl_3$), δ 3.22 (m, H-3α), 5.26 (t, H-12), 0.97 (s, H-23), 0.76 (s, H-24), 0.94 (s, H-25), 0.74 (s, H-26), 1.12 (s, H-27), 0.89 (s, H-29), 0.92 (s, H-30); ^{13}C-nmr ($CDCl_3$) see Tori *et al.* (4).

LITERATURE CITED

1. L. Jarrett, "Herbal Medicine," Dian Dincin Buchman, New York, 1979, pp. 1–8.
2. H.A. Hoppe, "Drogen Kunde, Vol. 1, Angiosperms," 8th ed., Walter de Gruyter, Berlin, 1975, p. 1028.
3. V.U. Ahmad, N. Bano, S. Bano, A. Fatima, and L. Kenne, *J. Nat. Prod.*, **49**, 784 (1986).
4. K. Tori, S. Seo, A. Shimaoka, and Y. Tomita, *Tetrahedron Lett.*, 4227 (1974).
5. K. Tori, S. Seo, and Y. Tomita, *Chem. Ind. (London)*, 434 (1975).
6. P.A.J. Gorin and M. Mazurek, *Can. J. Chem.*, **53**, 1212 (1975).
7. R. Lanzetta, G. Laonigro, and M. Parrini, *Can. J. Chem.*, **62**, 2874 (1984).
8. X-C. Li, D-Z. Wang, S-G. Wu, and C-R. Yang, *Phytochemistry*, **29**, 595 (1990).
9. S.B. Mahato, N.P. Sahu, S.K. Roy, and S. Sen, *Tetrahedron*, **47**, 5215 (1991).
10. J-P. Xu, R-S. Xu, and X.Y. Li, *Phytochemistry*, **31**, 233 (1992).
11. Atta-ur-Rahman, "One and Two Dimensional Nuclear Magnetic Resonance Spectroscopy," Elsevier, Amsterdam, 1989.
12. M.G.L. Brandao, M-A.L. Dubois, M.A. Teixera, and H. Wagner, *Phytochemistry*, **31**, 352 (1992).
13. D.G. Davis and A.C. Bax, *J. Am. Chem. Soc.*, **107**, 2820 (1985).
14. J. Buckingham, "Dictionary of Organic Compounds," 5th ed., 3rd suppl., Chapman and Hall, New York, 1985, p. 332.

Received 26 June 1992