Isolation and Structure Elucidation of New Nitrile and Mustard Oil Glycosides from Moringa oleifera and Their Effect on Blood Pressure

Shaheen Faizi, Bina Shaheen Siddiqui, Rubeena Saleem, Salimuzzaman Siddiqui, Khalid Aftab, and Anwar-ul Hassan Gilani

J. Nat. Prod., **1994**, 57 (9), 1256-1261• DOI: 10.1021/np50111a011 • Publication Date (Web): 01 July 2004

Journal of Natural Products
Vol. 57, No. 9, pp. 1256-1261, September 1994

ISOLATION AND STRUCTURE ELUCIDATION OF NEW NITRILE AND MUSTARD OIL GLYCOSIDES FROM *MORINGA OLEIFERA* AND THEIR EFFECT ON BLOOD PRESSURE

SHAHEEN FAIZI,* BINA SHAHEEN SIDDIQUI, RUBEENA SALEEM, SALIMUZZAMAN SIDDIQUI, KHALID AFTAB,

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

and ANWAR-UL HASSAN GILANI

Department of Pharmacology, Faculty of Health Science, The Aga Khan University, Karachi 74800, Pakistan

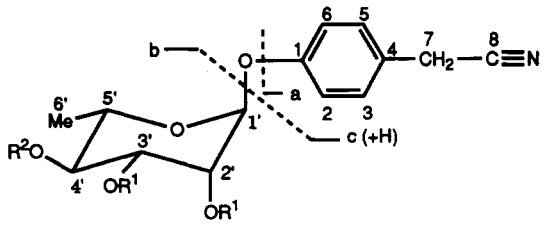
ABSTRACT.—Bioassay-guided analysis of an EtOH extract of *Moringa oleifera* leaves showing hypotensive activity led to the isolation of two nitrile glycosides, niazirin [**1**] and niazirinin [**2**], and three mustard oil glycosides, 4-[(4′-*O*-acetyl-α-L-rhamnosyloxy)benzyl]isothiocyanate [**4**], niaziminin A, and niaziminin B. Glycoside **2** is a new compound. Niaziminins A and B have previously been obtained from the leaf extract as a mixture, while compound **4** is new from this source. Structural determination was accomplished by means of spectroscopic methods including appropriate 2D nmr experiments and chemical reactions. This is the first report of the isolation of nitriles, an isothiocyanate, and thiocarbamates from the same plant species. Isothiocyanate **4** and the thiocarbamate glycosides niaziminin A and B showed hypotensive activity while nitrile glycosides **1** and **2** were found to be inactive in this regard.

Moringa oleifera Lam. (syn. *Moringa pterygosperma* Gaertn.) (Moringaceae) commonly known as "sahjna," is a fast-growing ornamental tree, which is widely distributed in tropical areas (1,2). Its medicinal value has long been recognized in the indigenous system of medicine (1–4). The plant is a cardiac and circulatory tonic and antiseptic (4) and has a folkloric reputation as a hypotensive (4,5) and antidiarrheal agent (6,7). Recent pharmacological studies on several parts of the plant showed that they possess antimicrobial activity (6), while the seeds reportedly have antispasmodic, anti-inflammatory, and diuretic properties (7). *Moringa oleifera* is also known as "clarifier tree" due to flocculating properties of the seeds and is employed in the treatment of drinking water in rural areas of Asia and Africa (2). Moreover, almost every part of the plant is used as a vegetable (4).

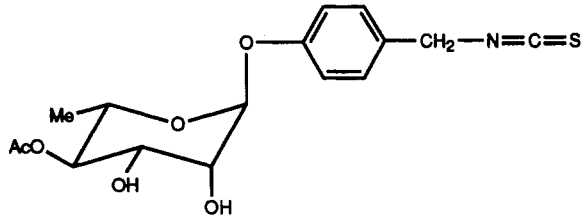
As a result of an investigation on the hypotensive constituents of *M. oleifera* leaves, the novel thiocarbamates, niazinin A, niazinin B, niazimicin, and a mixture of niaziminins A and B were previously reported to possess hypotensive activity (8). In a continuation of this study, two nitrile glycosides, niazirin [**1**] (9,10) and niazirinin [**2**], which is the 4′-*O*-acetyl derivative of **1**, have been isolated along with the mustard oil glycoside, 4-[(4′-*O*-acetyl-α-L-rhamnosyloxy)benzyl]isothiocyanate [**4**], which was obtained previously from a myrosinase-treated seed extract of *M. peregrina* (11). The isolation of niaziminins A and B as pure entities has also been achieved. Studies on the hypotensive activity of these constituents showed that the isothiocyanate **4** and the thiocarbamates, niaziminin A and B, are active, while the nitriles, **1** and **2**, are inactive.

Bioactivity-directed fractionation of an EtOH extract of *M. oleifera* leaves possessing hypotensive activity afforded an active fraction, M-80, which on prep. tlc followed by reversed-phase hplc gave two nitrile glycosides [**1**,**2**], and three mustard oil glycosides, 4-[(4′-*O*-acetyl-α-L-rhamnosyloxy)benzyl]isothiocyanate [**4**], and niaziminins A and B.

The molecular formula for niazirinin [**2**] was established as $C_{16}H_{19}NO_6$ through hreims (*m/z* 321.1227) and field-desorption ms (*m/z* 321). Its ir spectrum exhibited absorbance maxima at 3400 (OH),



1 $R^1=R^2=H$
2 $R^1=H$, $R^2=Ac$
3 $R^1=R^2=Ac$



4

2550 (C-N), and 1730 (OAc) cm^{-1}. The ^{13}C-nmr spectra (broad band and DEPT) indicated that the 16 carbons of the molecule were present as two methyls, one methylene, five methines, four sp^2 CH carbons, and three sp^2 and one sp quaternary carbons. Other structural features were evident from the 300 MHz ^1H-nmr spectrum of **2** in $CDCl_3$ (Table 1), which showed the presence of a mono-acetylated sugar moiety from a one-proton doublet at δ 5.55 ($J_{1',2'}$=1.6 Hz, H-1′), a pair of one-proton doublet of doublets at δ 4.15 ($J_{2',3'}$=3.5 Hz, $J_{2',1'}$=1.6 Hz, H-2′) and δ 4.09 ($J_{3',4'}$=9.6 Hz, $J_{3',2'}$=3.5 Hz, H-3′), a one-proton triplet at δ 4.87 ($J_{4',5'}$=9.6 Hz, $J_{4',3'}$=9.6 Hz, H-4′), a one-proton quartet of doublets at δ 3.58 ($J_{5',4'}$=9.6 Hz, $J_{5',6'}$=6.3 Hz, H-5′), a three-proton doublet at δ 1.19 ($J_{6',5'}$=6.3 Hz, H-6′), and a three-proton singlet at δ 2.13 ($OCOCH_3$). The chemical shift and coupling constant of the anomeric proton (H-1′) showed that the sugar was linked to the aglycone molecule by an α-glycosidic linkage (8, 11–13). These values are comparable with those of 4-*O*-acetyl-α-L-rhamnose and suggested that the compound is a 4-*O*-acetyl-α-L-rhamnoside (8). The ^1H-nmr data (Table 1) also showed that the rhamnose ring has the chair conformation (14). Exact assignment of these protons was made through COSY-45 and NOESY nmr experiments. The attribution of ^{13}C-nmr shifts (Table 2) is based on ^1H-^{13}C HETCOR which also confirmed the sugar moiety as 4-*O*-acetyl-α-L-rhamnose (8). Moreover, a base peak at *m/z* 189.0772 ($C_8H_{13}O_5$, fragment b) in the hreims confirmed the presence of monoacetylated rhamnose in the molecule. Two mutually coupled protons at δ 7.24 (J=8.7 Hz) and 7.05 (J=8.7 Hz) showed the presence of a *p*-substituted benzene ring in the molecule, which was supported by the correlation of these protons with CH carbons at δ 129.25 and 116.87, respectively, in the HETCOR spectrum. An ion at *m/z* 77.0382 (C_6H_5) in the hreims also indicated the presence of a phenyl ring in the structure. Thus six of the eight unsaturations implied by the molecular composition were accounted for by the sugar and phenyl rings. The site of glycosidic linkage with the aromatic ring was inferred from the 2D-nOe (NOESY) spectrum in which H-2, -6 had through-space interactions with H-1′ and H-3, -5; H-3, -5 with H-7, and H-1′ had an

TABLE 1. ^{1}H-Nmr Spectral Data for Compounds **1–4**.

Proton	Compound					
	1[a]	**1**[b]	**2**[a]	**3**[a]	**4**[a]	**4**[b]
H-2, -6	7.05 d (8.9)	7.04 d (8.8)	7.05 d (8.7)	7.07 d (8.7)	7.07 d (8.8)	7.09 d (8.7)
H-3, -5	7.25 d (8.9)	7.26 d (8.8)	7.24 d (8.7)	7.25 d (8.7)	7.25 d (8.8)	7.32 d (8.7)
H-7	3.69 s	3.94 s	3.68 s	3.68 s	4.64 s	4.86 s
H-1′	5.51 d (1.9)	5.37 d (1.8)	5.55 d (1.6)	5.44 d (1.8)	5.55 d (1.6)	5.45 d (1.6)
H-2′	4.14 dd (3.4, 1.9)	3.81 m	4.15 dd (3.5, 1.6)	5.40 dd (3.5, 1.8)	4.14 dd (3.5, 1.6)	3.88 m
H-3′	3.97 dd (9.1, 3.4)	3.62 ddd (9.2, 5.9, 3.2)	4.09 dd (9.6, 3.5)	5.49 dd (9.9, 3.5)	4.09 dd (9.4, 3.5)	3.81 m
H-4′	3.54 t (9.1)	3.26 m	4.87 t (9.6)	5.14 t (9.9)	4.85 t (9.4)	4.89 t (9.8)
H-5′	3.75 m	3.48 m	3.58 qd (9.6, 6.3)	3.94 qd (9.9, 6.2)	3.87 qd (9.4, 6.2)	3.65 qd (9.8, 6.2)
H-6′	1.27 d (6.2)	1.09 d (6.2)	1.19 d (6.3)	1.19 d (6.2)	1.19 d (6.2)	1.00 d (6.2)
2′-OH	—	5.01 d (4.3)	—	—	—	5.14 d (4.7)
3′-OH	—	4.69 d (5.9)	—	—	—	5.07 (5.8)
4′-OH	—	4.84 d (5.6)	—	—	—	—
OCOC*H*$_3$	—	—	2.13 s	2.18, 2.08 2.02 s	2.13 s	2.04 s

[a]Solvent $CDCl_3$.
[b]Solvent DMSO-d_6.

interaction with H-2′. Furthermore, a two-proton singlet at δ 3.68 in the ^{1}H-nmr spectrum correlated with the carbon resonance at δ 22.91 in the HETCOR nmr spectrum and indicated the presence of a benzylic methylene in the molecule. The ^{13}C-nmr spectrum (broad band) showed a peak at δ 123.70 attributable to the carbon of the nitrile group, the presence of which was confirmed by the weak absorption at 2250 cm^{-1} in the ir spectrum and a significant mass fragment at *m/z* 116.0477 (C_8H_6N, fragment a) in the hreims. This accounted for the remaining two double bonds in the molecule. In light of the above discussion the structure of niazirinin was elucidated as 4-[(4′-*O*-acetyl-α-L-rhamnosyloxy)benzyl] nitrile [**2**], which was corroborated by the important mass fragments b and c and the fragment at *m/z* 107.0494 (C_7H_7O) in the hreims (see Experimental). Moreover, the ^{13}C-nmr data were also in good agreement with structure **2**. The structure was finally confirmed through formation of its di-*O*-acetyl derivative **3**, the hreims of which displayed a prominent peak at *m/z* 273.0980 ($C_{12}H_{17}O_7$, fragment b) corresponding to the mass of a fully acetylated sugar residue. However, the molecular ion peak was not observed. The ^{1}H-nmr spectrum (Table 1) of **3** showed, apart from the benzylic signals, a downfield shift of the H-2′ and H-3′ resonances as compared to those of **2**. Also present in the ^{1}H-nmr spectrum were three, three-proton singlets at δ 2.18, δ 2.08, and δ 2.02 assignable to the three acetoxy methyl protons. Additionally, **3** exhibited an ester carbonyl absorbance at 1742 cm^{-1} in the ir spectrum.

TABLE 2. ^{13}C-Nmr Spectral Data for Compounds **1**, **2**, and **4** in $CDCl_3$.

Carbon	Compound		
	1	**2**	**4**
C-1	156.03	156.00	155.65
C-2, -6	116.65	116.87	116.80
C-3, -5	129.23	129.25	128.81
C-4	132.64	131.70	131.68
C-7	22.90	22.91	47.50
C-8	123.72	123.70	128.60
C-1′	97.96	97.19	98.00
C-2′	70.87	70.65	67.94
C-3′	71.70	70.13	70.01
C-4′	73.48	75.49	73.48
C-5′	68.83	66.36	66.99
C-6′	17.49	17.46	17.42
$OC\underline{O}CH_3$	—	172.01	169.98
$OCOC\underline{H}_3$	—	21.05	20.87

The ^{1}H- and ^{13}C-nmr chemical shifts and the uv, ir, and mass spectral data of niazirin [**1**] revealed that it is the 4′-deacetylated derivative of niazirinin [**2**]. It has been isolated earlier from the roasted and raw seeds of *M. oleifera* (9,10) and was also found to exhibit mutagenic activity (9). The spectral data of **1** compared well with the reported values (10). However, in the previous report, the nmr data of **1** were recorded in deuterated MeOH, while in the present studies the nmr spectra were recorded in $CDCl_3$ and DMSO-d_6 solution and are presented in Tables 1 and 2, which provide a ready comparison with data for niazirinin [**2**]. On acetylation with Ac_2O and pyridine at room temperature it afforded the triacetyl derivative, which was identical with the diacetyl derivative **3** of **2** (tlc, ir, mass, and ^{1}H-nmr spectra).

Compound **4** was identified as 4-[(4′-*O*-acetyl-α-L-rhamnosyloxy)benzyl] isothiocyanate on the basis of interpretation and comparison of its spectral data with those reported in the literature (11). The ^{1}H-nmr spectral data of the isolate are comparable with those published earlier (11). This mustard oil glycoside had previously been isolated from the myrosinase-treated seed extract of *M. peregrina* (11). Since the ^{13}C-nmr data have not been reported previously, they are presented in Table 2.

The identities of niaziminin A and its rotational isomer, niaziminin B, previously obtained as a mixture and existing in two tautomeric forms, were determined by comparison of their spectral data (uv, ir, ms, nmr) with literature values (8).

The origin of nitrile glycosides **1** and **2** can be conjectured through the degradation of a glucosinolate molecule (15–19). It has already been reported that glucosinolates which possess a unique molecular framework are versatile progenitors of organic isothiocyanate and cyanides (15–19). The nitrile glycosides **1** and **2**, the isothiocyanate **4**, and the thiocarbamates, niaziminin A and niaziminin B, were obtained without the addition of external enzyme thioglucoside glucohydrolase (myrosinase) or ascorbic acid.

The bioassay-guided isolation steps and subsequent evaluation of the hypotensive effects of the isolates were conducted using normotensive anesthetized rats. The thiocarbamates, niaziminins A and B and the isothiocyanate **4** were found to lower the arterial blood pressure, whereas the nitriles **1** and **2** were devoid of any activity at dose levels up to 5 mg/kg. At a dose of 3 mg/kg, the percentage decreases (mean±SEM; $n=3$) in mean blood pressure exhibited by niaziminin A (37.5±8.3) and niaziminin B (40.3±5.4) were similar ($p>0.05$) to the order of activity (48.6±6.3) obtained from their mixture (8). At the same dose level, isothiocyanate **4** produced a similar fall (34.9±8.6%) in mean arterial blood pressure (Table 3).

The similarity in the hypotensive potency of isothiocyanate [**4**] and the thiocarbamates, niaziminin A and B, coupled with the absence of activity in the nitriles [**1**] and [**2**] suggested that the amide or -N=C- group and/or sulfur atom may be essential for the hypotensive activity.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Mp

TABLE 3. Effects of the EtOH Extract, Fractions, and Pure Compounds of *M. oleifera* Leaves on Mean Arterial Blood Pressure (MABP) in Normotensive Anesthetized Rats.

Extract/Fraction/ Pure Compound	% Fall in MABP (mm Hg)[a]
ML	56.90±01.10
MAQ	32.11±01.57
ME	53.91±02.56
MEA	51.83±02.62
MMB	18.23±01.11
MPES	23.33±03.33
MPEI	39.00±02.96
MBPE	27.06±03.58
M-80	69.66±03.71
M-1	29.00±03.50
M-2a	45.50±05.10
M-2b	40.15±05.50
M-3	10.55±03.15
M-4	50.33±04.22
M-5	12.21±05.33
M-6	15.55±03.51
Niaziminin A	37.51±08.31
Niaziminin B	40.33±05.41
Compound **4**	34.91±08.63

[a]Values shown represent the mean ± SEM of three determinations at the dose of 10 mg/kg for extracts and fractions and at the dose of 3 mg/kg for bands M-1–M-6 and pure compounds.

was determined on a Gallenkamp melting-point apparatus and are uncorrected. Uv and ir spectra were recorded on a Hitachi-U-3200 and a Jasco A-302 spectrophotometer, respectively. The ei, fd, fab (positive) and hrms were recorded on Finnigan MAT-112, MAT-312, and JMS HX-110 spectrometers. The ^{1}H-nmr spectra were taken in $CDCl_3$ and DMSO-d_6 on a Bruker Aspect AM-300 spectrometer operating at 300 MHz, while the ^{13}C-nmr (broad band and DEPT) spectra were obtained in $CDCl_3$ on the same instrument operating at 75 MHz. The spectra were referenced to residual solvent signals and the chemical shifts are in ppm (δ) and coupling constants (J) are in Hz. The ^{13}C-nmr spectral assignments were made partly through DEPT and HETCOR and partly through comparison with the reported values of reference compounds (8, 10–12, 20, 21). Assignments of protons were based on COSY-45 and NOESY experiments. The purity of compounds was monitored on Si gel GF_{254} tlc plates.

PLANT MATERIAL.—The leaves of *M. oleifera* were collected from the Karachi region in November 1990. The plant was authenticated by Prof. Dr. Syed Irtifaq Ali of the Department of Botany, University of Karachi, and a voucher specimen was deposited in the same department.

EXTRACTION AND ISOLATION.—Fresh, undried, and uncrushed leaves (8 kg) of *M. oleifera* were repeatedly extracted with EtOH at room temperature. The first two extractions (8) and the third through the sixth extractions were combined separately and the solvent removed under reduced pressure to give two residues (A and B) possessing hypotensive activity. Work on the first combined residue has been reported elsewhere (8). Residue B from the latter extractions (ML) was partitioned between H_2O and EtOAc. Hypotensive activity (Table 3) was most evident in the EtOAc phase (ME), while the aqueous phase (MAQ) was found to be less active. Fraction ME was washed with H_2O, dried with anhydrous Na_2SO_4, clarified with charcoal, and filtered. The filtrate was freed of the solvent to give a residue (MEA). The charcoal bed was treated with a MeOH-C_6H_6 (1:1) solution to give the eluate (MMB). Fraction MEA possessing the greater activity, was divided into petroleum ether-soluble (MPES, less active) and insoluble (MPEI, more active) portions. Fraction MPEI was partitioned between 80% aqueous MeOH and C_6H_6-petroleum ether (1:1) solution (MBPE) and the aqueous MeOH phase was extracted with EtOAc after saturation with NaCl. The residue M-80 (3.80 g) obtained on workup of this EtOAc phase was found to be most active, while fraction MBPE was found to be less active. M-80 was subjected to repeated prep. tlc (Si gel, $CHCl_3$-MeOH, 9:1) yielding seven bands, M-1, M-2a, M-2b, M-3, M-4, M-5, and M-6 with R_f values of 0.97, 0.28, 0.25, 0.19, 0.12, 0.06, and 0.03, respectively, with bands M-2a, M-2b, and M-4 showing the highest levels of hypotensive activity. Band M-2a (194.3 mg) was resolved into six components, of which three were identified as niazirinin [**2**] (22 mg), niaziminin A (23 mg), and isothiocyanate **4** (10 mg). Niaziminin B (6.2 mg) was obtained by reversed-phase hplc of band M-2b (single spot, 100 mg). Hplc of band M-4 (117.3 mg) afforded niazirin [**1**] (36 mg) along with niazinin B (21 mg), niazimicin (20 mg), niazinin A (19 mg), and 4-[(α-L-rhamnosyloxy)benzyl]isothiocyanate (5 mg) (12).

Niazirin [**1**] was identified as 4-[(α-L-rhamnosyloxy) benzyl] nitrile through comparison of its spectral data with the reported values (10). ^{1}H- and ^{13}C-nmr data are shown in Tables 1 and 2, respectively.

Niazirinin [**2**].—Needles, ($CHCl_3$/MeOH), mp 183–184°; uv (MeOH) λ max 200.2 and 221.8 nm; ir ($CHCl_3$) ν max 3400, 2900, 2250, 1730 (br), 1580–1620, 1370, 1120, and 1020 cm^{-1}; hreims *m/z* 321.1227 (M^+) (calcd for $C_{16}H_{19}NO_6$, 321.1212) (1), 189.0772 (fragment c, $C_8H_{13}O_5$) (100), 190.0810 ($C_8H_{14}O_5$) (20), 171.0661 ($C_8H_{11}O_4$)(12), 147.0654 ($C_6H_{11}O_4$)(3), 133.0488 (fragment d, C_8H_7NO) (32), 129.0477 ($C_6H_9O_3$) (72), 116.0477 (fragment b, C_8H_6N)(8), 111.0438

($C_6H_7O_2$) (48), 107.0494 (C_7H_7O) (8), 77.0382 (C_6H_5) (6), and 43.0177 (C_2H_3O) (70); ^{1}H- and ^{13}C-nmr data are shown in Tables 1 and 2, respectively.

Acetylation of Niazirinin [**2**].—Ac_2O (0.5 ml) was added to a solution of **2** (8 mg) in pyridine (0.7 ml) and the reaction mixture was kept for two days at room temperature. The usual workup of the reaction mixture afforded the diacetyl derivative **3**: uv (MeOH) λ max 191.2 and 203.7 nm; ir ($CHCl_3$) ν max 3412, 2910, 2210, 1742, 1605, 1361, and 1125 cm^{-1}; hreims *m/z* 273.0980 (fragment c, $C_{12}H_{17}O_7$) (40), 231 (2), 213 (18), 171 (22), 153 (70), 129 (20), 111 (100), and 107 (40); ^{1}H-nmr data are shown in Table 1.

4-{(4'-O-Acetyl-α-L-rhamnosyloxy)benzyl} isothiocyanate [**4**].—Identification was confirmed by comparison with reported spectral data (11), and ^{13}C-nmr data (Table 2).

BIOASSAYS (HYPOTENSIVE EVALUATION).—The effect of fractions and isolates on blood pressure was studied in normotensive anesthetized Wistar rats (200–250 g) as described in a previous study (22). Animals were anesthetized with Sodium Pentothal® (60–80 mg/kg; i.p.). The arterial blood pressure was recorded from the carotid artery via the arterial cannula connected to a pressure transducer (Statham P_{23}) coupled with Grass model 79 polygraph. Drugs were injected via the cannula inserted in the jugular vein. Mean blood pressure was calculated as the diastolic blood pressure plus one-third of pulse width. Acetylcholine (1 μg/kg) was used as a control which produced 59.5±8.3% fall (mean±SEM; n=4) in the mean blood pressure.

LITERATURE CITED

1. B.N. Sastari, "The Wealth of India," Council of Scientific and Industrial Research, New Delhi, 1962, p. 425.
2. S.A.A. Jahn, *J. Am. Water Works Assoc.*, **80**, 43 (1988); *Chem. Abstr.*, **109**, 61191, 1988.
3. W. Dymock, C.J.H. Warden, and D. Hooper, "Pharmacographia Indica," The Institute of Health and Tibbi Research, republished under the auspices of Humdard National Foundation, Pakistan, 1890, p. 396.
4. K.M. Nadkarni (revised by A.K. Nadkarni) "The Indian Materia Medica;" Popular Parkashan, Bombay, 1976, p. 810.
5. S. Siddiqi and M.I. Khan, *Pak. J. Sci. Ind. Res.*, **11**, 268 (1968).
6. A. Càceres, O. Cabrera, O. Morales, P. Mollinedo, and P. Mendia, *J. Ethnopharmacol.*, **33**, 213 (1991).
7. A. Càceres, A. Saravia, S. Rizzo, L. Zabala, E.D. Leon, and F. Nave, *J. Ethnopharmacol.*, **36**, 233 (1992).
8. S. Faizi, B.S. Siddiqui, R. Saleem, S. Siddiqui, K. Aftab, and A.H. Gilani, *J. Chem. Soc., Perkin Trans. I*, 3237 (1992).
9. I.M. Villasenor, C.Y. Lim-Sylianco, and F. Dayrit, *Mutation Res.*, 209 (1989).
10. F.M. Dayrit, A.D. Alcantar, and I.M. Villasenor, *Philip. J. Sci.*, **119**, 23 (1990).
11. A. Kjaer, O. Malver, B. El-Menshawi, and J. Reisch, *Phytochemistry*, **18**, 1485 (1979).
12. U. Eilert, B. Wolters, and A. Nahrstedt, *Planta Med.*, **42**, 55 (1981).
13. L.A.P. Anderson, P.S. Steyn, and F.R.V. Heerden, *J. Chem. Soc., Perkin Trans. I*, 1573 (1984).
14. D.D. McIntyre, M.W. Germann, and H.J. Vogel, *Can. J. Chem.*, **68**, 1263 (1990).
15. V. Gil and A.J. Macleod, *Phytochemistry*, **19**, 227 (1980).
16. A. Kjaer, in: "Progress in The Chemistry of Organic Natural Products," Ed. by L. Zechmeister, Springer-Verlag, Vienna, 1960, p. 122.
17. M.G. Ettlinger and A. Kjaer, in: "Recent Advances in Phytochemistry," Ed. by T.J. Mabry, Appleton-Century-Crofts, New York, 1968, p. 59.
18. M.E. Daxenbichler, G.F. Spencer, D.G. Carlson, G.B. Rose, A.M. Brinker, and R.G. Powell, *Phytochemistry*, **30**, 2623 (1991).
19. A.I. Virtanen, *Phytochemistry*, **4**, 207 (1965).
20. E. Breitmaier, G. Haas, and W. Voelter, "Atlas of Carbon-13 Nmr Data," Heyden and Son, Ltd., London, 1979, Vol. 2, p. 1392.
21. H.O. Kalinowski and H. Kessler, *Org. Mag. Res.*, **6**, 305 (1974).
22. A.H. Gilani, *Drug Dev. Res.*, **24**, 127 (1991).

Received 20 September 1993