Isolation and Structure Elucidation of Novel Hypotensive Agents, Niazinin A, Niazinin B, Niazimicin and Niaziminin A + B from *Moringa oleifera*: The First naturally occurring Thiocarbamates

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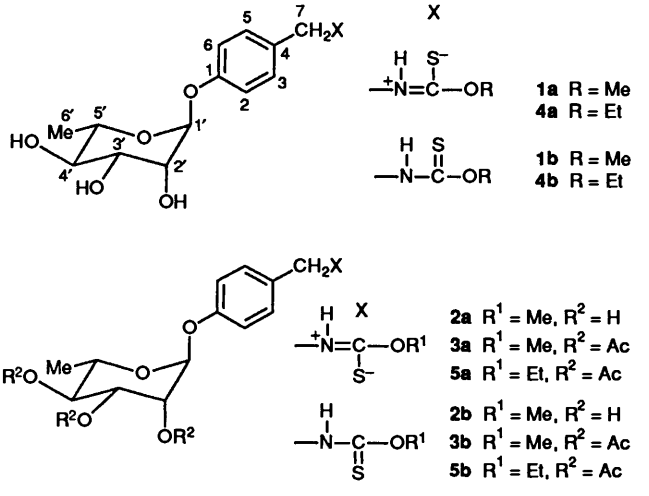
Novel hypotensive principles, niazinin A **1**, niazinin B **2**, niazimicin **4** and niaziminin A and B **6** + **7**, have been obtained from the ethanolic extract of the fresh leaves of *Moringa oleifera*, employing a bioassay-guided isolation procedure. Their structures have been elucidated through spectroscopic (including 2D NMR techniques) and chemical methods. These compounds are mustard oil glycosides which are very rare in Nature and are also the first examples of naturally occurring thiocarbamates.

Moringa oleifera Lam. (syn. *Moringa pterygosperma* Gaertn.) known in the vernacular as 'Sahjna', belongs to the single-genus family Moringaceae, and is widely distributed in Asia, Africa, and other tropical parts of the world.[1,2] It is a small-to-medium-sized tree with multiple uses, and its different parts are reputed to be used in folk medicine for the treatment of a variety of human ailments such as rheumatism, paralysis, epilepsy and ascites; in addition, almost every part of the tree is used as a vegetable.[1-5] Moreover, seeds of the plant are traditionally utilized for water purification because of their strong coagulating properties for sedimentation of suspended mud and turbidity, and their disinfectant effect against pathogens.[2,6,7] Recently, different parts of the plant have been subjected to biological evaluation, which showed that they possessed antibacterial properties.[8] Moreover, crude ethanolic and aqueous extracts of the leaves have been found to have hypotensive properties;[9] on the other hand there is also a report of the isolation of bases from the plant possessing hypertensive activity.[4,10] Phytochemical studies on its different parts resulted in the isolation of various chemical constituents such as flavonoids,[11] steroids,[12] mustard oils,[7] and proteins;[13] the last are active flocculating agents in the seed.

In view of the facts that, so far, no phytochemical work has been reported on the leaves, and that studies of the plant's hypotensive activity are limited to the crude extracts, in the present work the fresh leaves extract of *M. oleifera* was subjected to a systematic bioassay-guided isolation procedure. These studies have led to the isolation of mustard oil glycosides as active hypotensive principles. The stuctures of these compounds, namely niazinin A, niazinin B, niazimicin and niaziminin A + B, have been elucidated as *O*-methyl 4-[(α-L-rhamnosyloxy)benzyl]thiocarbamate (*E*)-**1** and (*Z*)-**2**, *O*-ethyl 4-[(α-L-rhamnosyloxy)benzyl]thiocarbamate (*E*)-**4** *O*-ethyl 4-[(4′-*O*-acetyl-α-L-rhamnosyloxy)benzyl]thiocarbamate (*E* + *Z*)-(**6** + **7**), respectively, through detailed ^1H and ^{13}C NMR spectroscopy, including 2D NMR (COSY, NOESY, *J*-resolved, heteroCOSY or HMQC),† NOE‡ difference and ^1H–^1H homo-decoupling experiments, and chemical transformations. Rotamers **6** and **7** were not separated and were characterized as their mixture.



Results and Discussion

Bioactivity-directed fractionation of the ethanolic extract of *M. oleifera* leaves, employing classical methods of isolation, afforded an active hypotensive factor M-80. This, on thick-layer chromatography and reversed-phase HPLC (see Experimental section), furnished novel hypotensive agents, namely niazinin A (**1**), niazinin B (**2**), niazimicin (**4**), and niaziminin A and B (**6** + **7**).

The molecular formula of niazinin A **1** was established as $C_{15}H_{21}NO_6S$ by high-resolution EI–MS (*M*, 343.1095), field desorption (343), and fast-atom-bombardment mass spectrometry (positive) (MH^+, 344). ^{13}C NMR spectra (broad band and DEPT)§ indicated that the 15 carbons of the molecule are present as two methyls (one CMe and one OMe), one methylene, five methines, four sp^2 CH and three sp^2 quaternary carbons. The structural features were evident from the 300 MHz ^1H NMR spectrum of compound **1** in $(CD_3)_2SO$ (Table 1) which showed the presence of a sugar moiety by a one-proton doublet at δ 5.33 ($J_{1',2'}$ 1.8, 1′-H), a pair of one-proton doublets of double doublets at δ 3.81 ($J_{2',2'\text{-OH}}$ 3.9, $J_{2',3'}$ 2.9, $J_{2',1'}$ 1.8, 2′-H) and δ 3.63 ($J_{3',4'}$ 9.2, $J_{3',3'\text{-OH}}$ 5.9, $J_{3',2'}$ 2.9, 3′-H), and a one-proton doublet of triplets at δ 3.26 ($J_{4',3'}$ 9.2, $J_{4',5'}$ 9.2,

† COSY: 2D homonuclear chemical-shift correlation; NOESY: 2D nuclear Overhauser enhancement and exchange; HMQC: 2D heteronuclear multiple quantum coherence.

‡ NOE: nuclear Overhauser enhancement.

§ DEPT: distortionless enhancement by polarization transfer.

Table 1 ^{1}H NMR data for compounds **1**–**7**; coupling constants (*J*/Hz) are in parentheses

Proton	1	2	3	4	5	6	7
2-, 6-H	6.97 d (8.6)	6.98 d (8.7)	7.07 d (8.7)	6.97 d (8.6)	7.03 d (8.6)	7.00 d (8.7)	7.07 d (8.7)
	6.96 d (8.6)	6.97 d (8.7)	7.06 d (8.7)	6.98 d (8.6)	7.01 d (8.6)	6.99 d (8.7)	7.01 d (8.7)
3-, 5-H	7.21 d (8.6)	7.16 d (8.7)	7.21 d (8.7)	7.21 d (8.6)	7.24 d (8.6)	7.21 d (8.7)	7.27 d (8.7)
	7.18 d (8.6)	7.20 d (8.7)	7.19 d (8.7)	7.16 d (8.6)	7.26 d (8.6)	7.17 d (8.7)	7.00 d (8.7)
7-H	4.56 d (6.1)	4.54 d (6.1)	4.58 d (5.7)	4.55 d (6.0)	4.30 d (5.5)	4.56 d (6.0)	4.57 d (6.0)
	4.17 d (5.7)	4.24 d (6.3)	4.12 d (6.0)	4.22 d (6.0)	4.11 d (5.5)	4.10 d (6.1)	4.21 d (6.2)
1′-H	5.33 d (1.8)	5.33 d (1.8)	5.63 d (1.6)	5.33 d (1.9)	5.64 d (1.6)	5.43 d (1.7)	5.40 d (1.9)
2′-H	3.81 ddd (3.9, 2.9, 1.8)	3.80 ddd (4.4, 3.6, 1.8)	5.31 dd (3.5, 1.6)	3.80 ddd (4.3, 3.4, 1.9)	5.31 dd (3.3, 1.6)	3.90 m	3.90 m
3′-H	3.63 ddd (9.2, 5.9, 2.9)	3.62 ddd (9.3, 6.1, 3.6)	5.27 dd (9.8, 3.5)	3.63 ddd (9.2, 5.9, 3.4)	5.26 dd (9.1, 3.3)	3.83 m	3.83 m
4′-H	3.26 dt (9.2, 5.6)	3.26 dt (9.3, 5.0)	4.96 t (9.8)	3.27 dt (9.2, 5.6)	4.96 t (9.8)	4.87 t (9.6)	4.86 t (9.6)
5′-H	3.46 qd (9.2, 6.1)	3.42 qd (9.3, 6.2)	3.91 qd (9.8, 6.4)	3.48 qd (9.2, 6.1)	3.92 qd (9.8, 6.3)	3.66 qd (9.6, 6.2)	3.66 qd (9.6, 6.2)
6′-H_3	1.09 d (6.1)	1.08 d (6.2)	1.07 d (6.4)	1.09 d (6.1)	1.07 d (6.3)	0.98 d (6.2)	0.98 d (6.2)
2′-OH	4.99 d (3.9)	5.00 d (4.4)		5.01 d (4.3)		5.33 d (4.3)	5.35 d (4.4)
3′-OH	4.66 d (5.9)	4.69 d (6.1)		4.69 d (5.9)		5.01 d (5.3)	5.02 d (5.7)
4′-OH	4.82 d (5.6)	4.84 d (5.0)		4.84 d (5.6)			
OCH_2Me				4.38 q (7.0)	4.39 q (7.0)	4.39 q (7.1)	4.38 q (7.1)
				4.37 q (7.0)	4.38 q (7.0)		
OCH$_2$*Me*				1.24 t (7.0)	1.27 t (7.0)	1.24 t (7.1)	1.20 t (7.1)
				1.22 t (7.0)	1.30 t (7.0)	1.22 t (7.1)	1.23 t (7.1)
OMe	3.87 s, 3.89 s	3.87 s, 3.89 s	3.54 s				
NH	9.55 t (6.1)	4.56 t (5.8)	4.56 t (5.8)	9.52 t (6.0)	4.54 t (5.5)	9.53 t (6.0)	7.55 t (5.6)
OAc			2.13, 2.05, 1.97 s		2.13, 2.04, 1.97 s	2.03 s	2.03 s

Sharp multiplicities were observed in the spectra of **1**, **2**, **4** and **6** + **7** after shaking with D_2O.

$J_{4',4'\text{-OH}}$ 5.6, 4′-H), a one-proton doublet of quartets at δ 3.46 ($J_{5',4'}$ 9.2, $J_{5',6'}$ 6.1, 5′-H) and a three-proton doublet at δ 1.09 ($J_{6',5'}$ 6.1, 6′-H_3). The chemical shift and coupling constants of the anomeric proton (1′-H) showed that the sugar is linked with the aglycone molecule by an α-glycosidic linkage.[7,14,15] In addition the presence of three hydroxy groups was shown by three one-proton doublets at δ 4.99 (*J* 3.9, 2′-OH), 4.66 (*J* 5.9, 3′-OH) and 4.82 (*J* 5.6, 4′-OH). On shaking with D_2O these signals disappeared, while the signals for 2′-H and 3′-H each collapsed into a double doublet, and that for 4′-H changed into a triplet. These values are comparable with those of α-L-rhamnose and suggested that the compound is an α-L-rhamnoside.[7,14,15] An exact assignment of these protons was made through ^{1}H–^{1}H-COSY-45 measurements and double-resonance experiments.

The assignment of ^{13}C NMR shifts (Table 2) is based on ^{1}H–^{13}C heteroCOSY, which also evaluates the sugar moiety as α-L-rhamnose.[15,16] In the ^{1}H NMR spectrum, the two two-proton mutually coupled doublets at δ 7.21 (*J* 8.6) and 6.97 (*J* 8.6) showed the presence of a *para*-substituted benzene ring in the molecule, which was supported by the correlation of these protons at δ 7.21 (3-, 5-H) and 6.97 (2-, 6-H) with the CH carbons at δ_C 128.50 and 116.29, respectively, in the heteroCOSY spectrum. Thus, five of the six unsaturation degrees implied by the molecular formula were accounted for by the sugar and the phenyl rings.

The site of the glycosidic linkage with the aromatic ring was inferred from the relatively downfield shift of the anomeric proton[7,14,16] as compared with its chemical shift when it is linked with saturated carbons.[15,16] This was supported by the 2D NOESY spectrum in which 1′-H has connectivity with 2′-H and aromatic protons 2-, 6-H. This was also confirmed by NOE difference experiments. Hence, when 1′-H was irradiated, the signals of 2-, 6-H (20% NOE) and 2′-H (16% NOE) were enhanced, while irradiation of 2-, 6-H altered the signals of 3-, 5-H (18% NOE) and 1′-H (19% NOE).

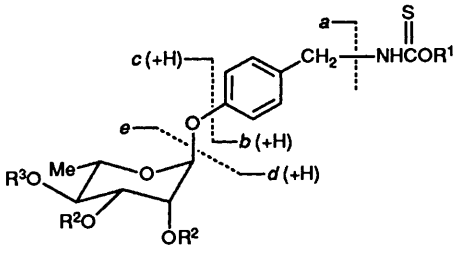
Furthermore, a doublet at δ 4.56 (*J* 6.1, 7-H) in the ^{1}H NMR spectrum correlated with the carbon resonance at δ_C 47.40 in the heteroCOSY, indicating the presence of a benzylic methylene in the structure. These data are comparable with the values reported for 4-(α-L-rhamnosyloxy)benzyl isothiocyanate.[7] However, signals for the isothiocyanate moiety were missing in the IR as well as in the ^{1}H and ^{13}C NMR spectra of compound **1** and instead a methyl thiocarbamate function was indicated by the presence of a triplet for NH at δ 9.55 (*J* 6.1) and a singlet for the methoxy group at δ 3.87, which was also

Table 2 ^{13}C NMR chemical shifts[a] for compounds **1**, **2**, **4**, **6** and **7** in $(CD_3)_2SO$

Carbon	1	2	4	6	7
1	154.88, 155.01	155.26	155.00	154.92, 154.71	155.20, 155.25
2,6	116.29	116.33	116.08, 116.13	116.38, 116.82	117.05, 116.99
3,5	128.50, 128.64	128.75, 128.32	128.46	128.79, 128.36	129.40, 128.86
4	132.29, 132.71	131.36	131.18, 131.36	130.51, 131.92	131.75, 132.61
7	47.40, 41.52	47.44, 41.80	47.96, 44.88	47.41, 45.09	47.14, 43.12
8	190.72	191.00	189.84, 187.81	190.13, 191.55	190.13, 190.49
1′	98.49	98.47	98.31	98.05	98.13
2′	70.14	69.44	69.96	69.97	69.97
3′	70.42	70.21	70.25	70.04	70.10
4′	71.78	70.45	71.60	73.56	73.56
5′	69.34	71.81	69.17	66.92	66.98
6′	17.78	17.87	17.59	17.44	17.44
OMe	56.43, 57.10	56.53			
OCH_2Me			65.06, 65.86	65.39	65.39
$OCH_2\mathit{Me}$			13.97, 13.77	14.24, 14.63	14.24, 14.03
OCOMe				170.04, 170.30	170.04, 170.30
$OCO\mathit{Me}$				20.89, 21.57	20.89, 21.08

[a] In a pair of chemical shifts the upper value corresponds to the major isomer.

supported by the ions at *m/z* 311.0767, 165.0276, and 182.0259 resulting from the loss of MeOH from the parent ion, and loss of MeOH and the Me group, respectively, from fragment *d* (see Fig. 1 and Experimental section). That the NH is linked with the benzylic methylene carbon was evident from the diagnostic peak at *m/z* 253 (fragment *a*) and from the multiplicities and coupling constants of these protons. On shaking with D_2O, the signal of NH vanished while the doublet of the methylene group changed to a singlet. In the ^{13}C NMR spectrum the OMe carbon was noted at δ_C 56.43 and the thiocarbonyl carbon was observed at δ_C 190.72 which also justified the sixth double bond in the molecule. In the light of the above discussion, the structure of niazinin A has been elucidated as *O*-methyl 4-[(α-L-rhamnosyloxy)benzyl]thiocarbamate (*E*)-**1**, which was corroborated by the fragments *a*–*e* and the base peak at *m/z* 107.0499 (C_7H_7O) in the EI–HR mass spectrum (see Fig. 1 and Experimental section). In conformity to structure **1**, it yielded the tri-*O*-acetyl derivative **3**, which has the molecular ion peak



1, **2** R^1 = Me, R^2 = R^3 = H
3 R^1 = Me, R^2 = R^3 = Ac
4 R^1 = Et, R^2 = R^3 = H
5 R^1 = Et, R^2 = R^3 = Ac
6 + **7** R^1 = Et, R^2 = H, R^3 = Ac

Fig. 1

at *m/z* 469.1440 ($C_{21}H_{27}NO_9S$) in the HR mass spectrum, and a strong absorption at 1742 cm^{-1} in the IR spectrum for the acetate carbonyls. The ^1H NMR spectrum (Table 1) has three three-proton, sharp singlets at δ 2.13, 2.05, and 1.97 instead of the doublets of the hydroxy groups, and showed the downfield shifts for the signals of 2′-, 3′- and 4′-H.

It is important to note that compound **1** exists in two forms, **1a** and its resonance hybrid **1b**, in the ratio 2:1, which was inferred by the presence of double signals for the benzyl and methoxy nuclei in the NMR spectra (Table 1 and 2). In both the hybrids, the NH proton appeared at the same chemical shift (δ 9.55). This was manifested by its connectivity (COSY-45) with 7-H in each case (δ 4.56, 4.17). The downfield shift, δ 4.56, has been attributed to the 7-H of the hybrid **1a** and its larger integration implied that it is the major form, which has also been observed earlier in the case of thioamides.[17,18] The protons on C-7 of tautomers **1a** and **1b** were related by δ_C 47.40 and 41.52, respectively, in the heteroCOSY spectrum.

Niazinin B **2** gave a molecular ion (*M*, 343.1117) in the high-resolution EI–MS and FAB–MS (positive) spectra (MH^+, 344) consistent with the same molecular formula, $C_{15}H_{21}NO_6S$, as that of compound **1**. Its IR, UV absorption and mass fragments were strikingly similar to those of niazinin A **1**, and the NMR (^1H and ^{13}C) spectra have the same chemical-shift pattern for the sugar moiety, benzyl ring and the methoxy group. The only difference between the two compounds was in the chemical shift of NH protons which now resonated upfield at δ 4.56 (t, *J* 5.8). In this case also, double signals were observed in a 2:1 ratio for the benzyl and methoxy nuclei (Table 1 and 2), showing the existence of niazinin B **2** in two forms, **2a** and **2b**, **2a** being the major form. These observations indicated that compound **2** is a rotational isomer[19,20] of niazinin A **1**, and that the two rotamers **1** and **2** differ in the orientation of the NH group with respect to sulfur. Thus, in both tautomers **1a** and **1b**, NH is *Z* to sulfur and appears downfield (δ 9.55) whereas in both tautomers **2a** and **2b** it is *E* to sulfur and resonates upfield (δ 4.56).[20]

In each pair discussed above (**1a**, **1b** and **2a**, **2b**) the phenomenon of *Z*–*E* isomerism also exists along the ester bond,[20,21] which causes two more methoxy singlets to appear in each case, although as minor contributors. Thus in the case of niazinin A **1** two minor singlets were present at δ 3.54 and 3.56 and two minor singlets were noted at δ 3.49 and 3.53 in the ^1H NMR spectrum of niazinin B **2**.

On acetylation compound **2** afforded a tri-*O*-acetyl derivative, which is identical in every respect (molecular formula, IR, UV absorptions and ^1H NMR spectral data) with compound **3**, in which NH and methoxy protons appeared upfield. The ^1H NMR data (Table 1) showed that compound **3** also exists in two forms, **3a** and **3b**, in the ratio 2:1. The upfield shift of the NH signal from δ 9.55 in **1** to δ 4.56 in **3** indicated that the *E* isomer had been changed to the *Z* isomer during acetylation. This could be envisaged as being due to the presence of trace amounts of acetic acid in the acetylation mixture. In order to verify this, compound **1** was kept in 3% acetic acid–chloroform for three days and its ^1H NMR spectrum was recorded. It also showed an upfield shift of NH from δ 9.55 to δ 4.90, thus confirming that the trace of acid transforms the *E* thiocarbamylamine **1** to *Z* thiocarbamylamine **2** through its thiolimine tautomer.

The high-resolution EI–MS (M, 357.1197) and FAB positive mass spectrum (MH^+, 358) of niazimicin showed that it is 14 atomic mass units heavier than compounds **1** and **2**. Its mass spectral fragmentation is similar to those of compounds **1** and **2** except that the aglycone portion has the composition $C_{10}H_{13}NO_2S$ (*M*, 211.0686, base peak, fragment *d*), *i.e.* it has an additional methylene group which could be rationalized as a

methylene of an ethoxy group from the ^{1}H NMR spectrum which further indicated that niazimicin **4** also exists in resonating hybrids **4a** and **4b** in the ratio 3:1 (Table 1). It showed two triplets (δ 1.24, J 7.0; δ 1.22, J 7.0) and two quartets (δ 4.38, J 7.0; δ 4.37, J 7.0) for the ethoxy function which was also corroborated by a fragment at m/z 312 resulting from the loss of the ethoxy group from the molecular ion. In the light of these observations the structure of niazimicin has been evaluated as *O*-ethyl 4-[(α-L-rhamnosyloxy)benzyl]thiocarbamate (*E*)-**4**. The fragments *a*–*e* in the mass spectrum of niazimicin **4** and ^{13}C NMR spectral data are also compatible with the structure. The NH of both tautomers **4a** and **4b** appeared at the same chemical shift, *i.e.* δ 9.52 (t, J 6.0) as shown by a COSY-45 plot. The ^{1}H NMR chemical shifts of 7-H and NH of tautomers **4a** and **4b** are comparable to those of tautomers **1a** and **1b**. Weak quartets at δ 4.00 (J 7.1) and 4.09 (J 7.1) in the ^{1}H NMR spectrum of niazimicin **4** indicated the presence of minor conformers of tautomers **4a** and **4b** due to isomerism along the ester bond, as observed in the case of compounds **1** and **2**. The complementary pair of triplets for the ethoxy methyl protons was hidden under the methyl signals of the major contributors. Acetylation of niazimicin **4** (**4a** + **4b**) gave the tri-*O*-acetyl product **5** (M^+, 483.1575), consisting of tautomers **5a** and **5b** in the ratio 3:1. As in the case of the acetyl derivative of compound **1**, the NH signal of compound **5** also shifted upfield (δ 4.54, t, J 5.5) in the ^{1}H NMR spectrum.

The high-resolution EI–MS (M, 399.1355) and fast-atom-bombardment (positive) mass spectrum (MH^+, 400) evaluated the molecular formula of niaziminin as $C_{18}H_{25}NO_7S$. Its ^{1}H NMR spectral data (Table 1) were very similar to those of compound **4** except that it has a singlet at δ 2.03 and the signal of 4′-H was shifted downfield to δ 4.87 (t, J 9.6, compound **6**) and 4.86 (t, J 9.6, compound **7**). These observations suggested that it has an acetoxy substituent at C-4′, the presence of which was indicated by the strong peak at 1745 cm^{-1} in the IR spectrum. This was further supported by the downfield shift of C-4′ due to the α-effect of the acetyl group, and the upfield shifts of C-3′ and C-5′ due to the β-effect of this group[22] in the ^{13}C NMR spectrum (Table 2) as compared with the values for compounds **1**, **2** and **4**. Thus niaziminin is the 4′-*O*-acetyl derivative of niazimicin **4**. Important fragments at m/z 211.0667 (fragment *d*), 189.0763 (fragment *e*) and 171.0667 (fragment *e* − 18) supported the structure, which was confirmed by the formation of the di-*O*-acetyl derivative $C_{22}H_{29}NO_9S$ (M, 483.1575) on reaction with acetic anhydride and pyridine. Multiple signals observed in the NMR spectra of niaziminin (Tables 1 and 2) indicated that it is a 1:1 mixture of *E*–*Z* rotamers **6** and **7**, and each of these consists of resonance hybrids **6a**, **6b** and **7a**, **7b**.[17–19] On acetylation both the *E* (**6a** + **6b**) and *Z* (**7a** + **7b**) isomers afforded the common acetylation product, due to the transformation of the *E* to the *Z* rotamer as discussed above. These were identical with the *Z* pair **5a** and **5b**.

Thiocarbamates **1**, **2**, **4** and **6** + **7** are mustard oil glycosides, which are very rare in nature,[23–25] and previously only two such glycosides have been reported from the seed and root of *M. oleifera*[7] and seeds of *M. peregrina*.[14] There is also no report of the isolation of naturally occurring thiocarbamates and the existence of all four contributing structures, although dithiocarbamates have been isolated as phytoalexins from natural sources and their isomerism due to restricted rotation about the C–N bond has been observed.[26]

It is noteworthy that these thiocarbamates were obtained in a genuine, natural situation (autolysis)[27] from the ethanol extract of the leaves of *M. oleifera* without treatment with myrosinase or ascorbic acid.[7,14,23–25,27] These compounds may be present in the leaves as their glucosinolates,[7,23–25,27] and the myrosinase activity in leaves is sufficient for liberation of the thiocarbamates from the corresponding parent compounds. Compounds **4** and **6** + **7** were detected in the fresh leaves extract along with compounds **1** and **2**, showing that they are naturally occurring constituents and not artefacts.

Biological Results.—Effects of the ethanolic extract, fractions, and pure compounds from *M. oleifera* leaves were studied in Wistar rats anaesthetized with pentobarbital sodium (50 mg/kg i.p.). The extract caused a fall in blood pressure; its bioassay-directed separation has led to the isolation of compounds **1**, **2**, **4** and **6** + **7**. All these compounds caused a fall in arterial blood pressure in a dose-dependent manner.

The hypotensive effects of all compounds were similar. At a dose level of 1 mg/kg, they produced a 14–22% fall in control mean blood pressure, and a fall of 40–65% was observed at a dose of 3 mg/kg. These results indicated that all these compounds are potent hypotensive agents, and the *Z*–*E* isomerism observed in these compounds might play a role in their biological activity as reported in the case of numerous peptides with important biological activity.[28] Detailed pharmacological evaluation of these compounds has been presented elsewhere.[29]

Experimental

UV (in MeOH) and IR (in $CHCl_3$) spectra were recorded on Hitachi-U-3200 and JASCO-A-302 spectrophotometers, respectively. The EI, FD, FAB positive and HR mass spectra were recorded on Finnigan MAT-112, MAT-312 and JMS HX-110 spectrometers. The ^{1}H and ^{13}C NMR (broad band and DEPT) spectra were run in $(CD_3)_2SO$ on a Bruker Aspect AM-300 spectrometer operating at 300 and 75 MHz, respectively, with spectra referenced to residual protio–deuterio solvent signals. The chemical shifts are in ppm (δ) and coupling constants (J) are in Hz. The ^{13}C NMR spectral assignments have been made partly through DEPT and heteroCOSY (or HMQC) and partly through comparison with the reported values of similar compounds.[7] Assignments of protons are based on homo-decoupling experiments, COSY-45, NOESY, J-resolved and NOE difference spectroscopy. The purity of compounds was checked on silica gel GF_{254} coated plates. Light petroleum refers to the fraction boiling in the range 66–70 °C.

Leaves of *M. oleifera* were collected from the Karachi region, in November 1990; the plant was authenticated at the Department of Botany, University of Karachi, and a voucher specimen was deposited in the same department.

Bioassay-directed Isolation of Niazinin A **1**, *B* **2**, *Niazimicin* **4** *and Niaziminin A and B* **6** + **7**.—Fresh, undried and uncrushed leaves (8 kg) of *M. oleifera*, were repeatedly extracted with EtOH at room temperature. The first and second ethanolic extracts were combined and freed from solvent under reduced pressure to give a thickish mass, which was partitioned between water and ethyl acetate. The ethyl acetate phase, after usual work-up, was freed from solvent to give a residue, which was divided into light petroleum-soluble and -insoluble portions. The latter fraction was partitioned between 80% aq. methanol and benzene–light petroleum (1:1 v/v). The 80% methanolic phase was extracted with ethyl acetate after saturation with sodium chloride. The residue (M-80) (5.95 g, 0.1008% dry wt.) obtained on usual work-up of the ethyl acetate phase was subjected to preparative thick-layer chromatography [silica gel; $CHCl_3$–MeOH (9:1)] as a result of which seven bands, M-1, -2a, -2b, -3, -4, -5 and -6, were obtained. Band M-2a (167 mg, 0.0028%) was identified as niaziminin A + B **6** + **7**. Band M-4 was resolved into three compounds, niazinin B **2** (116 mg, 0.0019%), niazimicin **4** (146 mg, 0.0024%), and niazinin A **1** (110 mg, 0.0018%) in the order of peak elution through reversed-

phase high-performance liquid chromatography [Shimadzu; C_{18}, techspher 50 DS, 30 cm × 10 mm; mobile phase 70% MeOH–water (v/v) containing a few drops of acetic acid; loop 20 mm^3, flow rate 4 cm^3/min]. Compounds **1**, **2**, **4**, and **6** + **7**, and the acetyl derivatives **3** and **5**, were obtained as amorphous substances.

Niazinin A **1**.—λ_{max}/nm 200.3, 223.6 and 245.1; ν_{max}/cm^{-1} 3400, 1606, 1426 and 1050; FAB–MS (pos.) *m/z* 344 (MH^+, 100%), 436 (MH^+ + 92, 25) and 528 (MH^+ + 92 + 92, 3); *m/z* 343.1095 (M^+, 1%) ($C_{15}H_{21}NO_6S$ requires *M*, 343.1089), 311.0767 (M^+ − MeOH, 1), 253 (fragment *a*, 1), 197.0504 (fragment *d*, $C_9H_{11}NO_2S$, 30), 182.0259 (fragment *d* − Me, 12), 181 (fragment *b*, 6), 165.0276 (fragment *d* − MeOH, 16), 164 (fragment *c*, 4), 147.0653 (fragment *e*, $C_6H_{11}O_4$, 44), 129.0549 ($C_6H_9O_3$, 26), 107.0499 (C_7H_7O, 100), 89.0330 (C_7H_5, 7) and 77.0396 (C_6H_5, 26). ^1H and ^{13}C NMR data in Tables 1 and 2.

Acetylation of Niazinin A **1**.—Acetic anhydride (1 cm^3) was added to a solution of niazinin A **1** (15 mg) in pyridine (1 cm^3) and the reaction mixture was kept at room temperature for three days. Usual work-up of the reaction mixture gave the acetyl product **3**, λ_{max}/nm 202.4, 221.2, and 245.8; ν_{max}/cm^{-1} 3400, 2900, 1742, 1600, 1500, 1360, 1240, 1250 and 1020; *m/z* 469.1440 (M^+, 1%) ($C_{21}H_{27}NO_9S$ requires *M*, 469.1406), 273.0958 (fragment *e*, $C_{12}H_{17}O_7$, 24), 231.0906 ($C_{10}H_{15}O_6$, 5), 223 (10), 171.0631 ($C_8H_{11}O_4$, 20), 153.0540 ($C_8H_9O_3$, 76), 129.0540 ($C_6H_9O_3$, 10), 111.0445 ($C_6H_7O_2$, 100), 107.0503 (C_7H_7O, 15) and 43.0188 (C_2H_3O, 60). ^1H NMR data in Table 1.

Acetylation of compounds **2**, **4** and **6** + **7** was also carried out in the manner described above. Acetyl derivative of compound **2** is same as that of compound **1**, while characterization of acetate **5**, which is the common acetyl derivative of compounds **4** and **6** + **7**, is reported below.

Niazinin B **2**.—λ_{max}/nm 200.2, 223.6 and 245.0; ν_{max}/cm^{-1} 3400, 1600, 1420 and 1040; FD–MS 343 (M^+); FAB–MS (pos.) 344 (MH^+, 100%), 436 (MH^+ + 92, 25) and 528 (MH^+ + 92 + 92, 14); *m/z* 343.1117 (M^+, 14%) ($C_{15}H_{21}NO_6S$ requires *M*, 343.1089), 311.0766 (M^+ − MeOH, 1), 253 (1), 239 (2), 197.0503 (fragment *d*, $C_9H_{11}NO_2S$, 100), 182.0307 (fragment *d* − Me, 40), 181 (fragment *b*, 6), 165.0509 (fragment *d* − MeOH, 6), 164 (4), 147.0639 (fragment *e*, $C_6H_{11}O_4$, 12), 129.0554 ($C_6H_9O_3$, 20), 121 (10), 107.0511 (C_7H_7O, 70), 89.0331 (C_7H_5, 12) and 77.0391 (C_6H_5, 16). ^1H and ^{13}C NMR in data Tables 1 and 2.

Niazimicin **4**.—λ_{max}/nm 200.4, 223.2 and 245.8; ν_{max}/cm^{-1} 3600, 3400, 1600, 1480, 1380, 1310, 1110, 9090 and 830; FAB–MS (pos.) 358 (MH^+, 100%), 450 (MH^+ + 92, 30) and 542 (MH^+ + 92 + 92, 2); *m/z* 357.1197 (M^+, 24%) ($C_{16}H_{23}NO_6S$ requires *M*, 357.1245), 312 (2), 253 (fragment *a*, 1), 211.0686 (fragment *d*, $C_{10}H_{13}NO_2S$, 100), 195.0775 (fragment *b*, $C_{10}H_{13}NOS$, 2), 182.0247 (fragment *d* − C_2H_5, $C_8H_8NO_2S$, 68), 164 (fragment *c*, 2), 147.0649 (fragment *e*, $C_6H_{11}O_4$, 10), 129.0589 (fragment *e* − H_2O, $C_6H_9O_3$, 13), 111.0444 (fragment *e* − $2H_2O$, $C_6H_7O_2$, 8), 107.0512 (C_7H_7O, 53), 106.0390 (C_7H_6O, 8), 95.0432 (9), 77.0393 (C_6H_5, 12), 59.0512 (C_3H_7O, 14) and 57.0358 (C_3H_5O, 24). ^1H and ^{13}C NMR data in Tables 1 and 2.

Acetyl Derivative of Compounds **4** *and* **6** + **7**.—λ_{max}/nm 201.4, 220.4 and 250.2; λ_{max}/cm^{-1} 3400, 2997, 1740, 1600, 1500, 1360, 1212 and 1020; *m/z* 483.1575 (M^+, 0.5%) ($C_{22}H_{29}NO_9S$ requires *M*, 483.1562), 273.0984 (fragment *e*, $C_{12}H_{17}O_7$, 46), 213 (14), 211.0620 (fragment *d*, $C_{10}H_{13}NO_2S$, 2), 195.0768 (fragment *b*, $C_{10}H_{13}NOS$, 2), 171.0624 ($C_8H_{11}O_4$, 22), 154.0564 ($C_8H_{10}O_3$, 8), 153 (78), 111.0444 ($C_6H_7O_2$, 100), 107.0512 (C_7H_7O, 17) and 43.0174 (C_2H_3O, 42). ^1H NMR data in Table 1.

Niaziminin A and B **6** + **7**.—λ_{max}/nm 195.0, 200.6, 222.0 and 246.0; ν_{max}/cm^{-1} 3600, 3400, 1745, 1600, 1490, 1360, 1110 and 1010; *m/z* 399.1355 (M^+, 8%) ($C_{18}H_{25}NO_7S$ requires *M*, 399.1351), 384 (1), 211.0667 (fragment *d*, $C_{10}H_{13}NO_7S$, 42), 195 (fragment *b*, 10), 189.0763 (fragment *e*, $C_8H_{13}O_5$, 100), 182 (32), 171.0667 ($C_8H_{11}O_4$, 20), 129.0552 ($C_6H_9O_3$, 50), 107.0494 (C_7H_7O, 52) and 59 (20). ^1H and ^{13}C NMR data in Tables 1 and 2.

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