Novel Hypotensive Agents, Niazimin A, Niazimin B, Niazicin A and Niazicin B From *Moringa oleifera*: Isolation of First Naturally Occurring Carbamates

Shaheen Faizi,*[a] Bina Shaheen Siddiqui,[a] Rubeena Saleem,[a] Salimuzzaman Siddiqui,[a] Khalid Aftab[a] and Anwar-ul-Hassan Gilani[b]

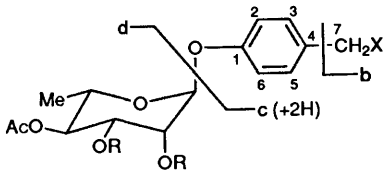
[a] *H.E.J. Research Institute of Chemistry, University of Karachi, Karachi-75270, Pakistan*

[b] *Department of Pharmacology, Faculty of Health Sciences, The Aga Khan University, Karachi-74800, Pakistan*

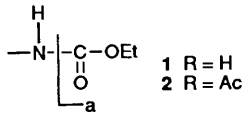
Two novel hypotensive carbamate glycosides designated niazimin A **1** and niazimin B **3** and two new hypotensive mustard oil glycosides possessing the thiocarbamate group, niazicin A **5** and niazicin B **10** along with a benzaldehyde glycoside **12** have been isolated from the fresh leaves extract of *Moringa oleifera* employing bioassay-guided fractionation. The structures of all these glycosides have been elucidated on the basis of spectroscopic evidence (IR, UV, MS, NMR, 2D NMR) and chemical reactions. Compounds **1** and **3** appear to be the first natural products embodying the carbamate moiety. The amide bond in these compounds appears to play an important role in the hypotensive activity, since in both the carbamates and thiocarbamates it is common to those which possess hypotensive activity.

Moringa oleifera Lam., a beautiful tree commonly found in sub-Himalayan range, is widely distributed throughout the hot and hot to temperate regions of the world and has a long history of use at folk level both for medicinal and water purification purposes.[1-3] Its leaves, flowers and fruits are all eaten as vegetables.[1,2] Biological studies carried out on the extracts of its various parts showed that they possess hypotensive,[4] antimicrobial.[5,6] antifertility,[7] antispasmodic, antiinflammatory and diuretic activities.[8]

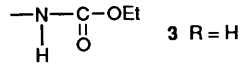
Recent studies showed that the fresh leaves of *M. oleifera* contain hypotensive thiocarbamate glycosides **4**, **7**, **8**, **9** and **11**.[9] Further investigation of the bioactive constituents of the leaves employing bioassay-guided fractionation has led to the isolation of four new hypotensive glycosides, niazimin A **1**, niazimin B **3**, niazicin A **5** and niazicin B **10** along with a new benzaldehyde glycoside **12**. Their structures have been elucidated through mass, NMR and 2D NMR spectroscopy as *O*-ethyl-4-[(4′-*O*-acetyl-α-L-rhamnosyloxy)benzyl] carbamate (*Z*) **1** and (*E*) **3**; *O*-methyl-4-[(4′-*O*-acetyl-α-L-rhamnosyloxy)-benzyl] thiocarbamate (*E*) **5**; (*Z*) **10** and 4-(4′-*O*-acetyl-α-L-rhamnosyloxy)benzaldehyde **12**.



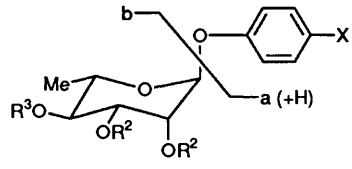
X

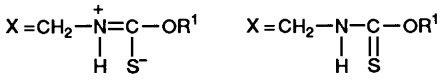


1 R = H
2 R = Ac



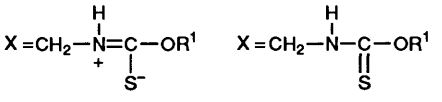
3 R = H





a **b**

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



a **b**

9 R^1 = Me, R^2 = R^3 = H
10 R^1 = Me, R^2 = H, R^3 = Ac
11 R^1 = Et, R^2 = H, R^3 = Ac

12 X = CHO, R^2 = H, R^3 = Ac
13 X = CH_2NCS, R^2 = R^3 = H
14 X = CH_2NCS, R^2 = H, R^3 = Ac

Results and Discussion

On extraction of *M. oleifera* leaves with ethanol and preliminary classical separation of the extract by initial solvent partitioning, a hypotensive factor M − 80 was obtained. Thick layer chromatography of M − 80 afforded three bands M − 2a, M − 2b and M − 4. Both M − 2a and M − 2b appeared as a single spot on TLC, the ^{1}H NMR spectrum of which indicated that it was a mixture of more than two compounds. Reversed-phase HPLC of M − 2a afforded four new glycosides **1**, **3**, **5** and **12** along with **8** and **14**, while HPLC of M − 2b gave pure thiocarbamate **10**.

Niazimin A **1** formed plates (m.p. 168–170 °C) from $CHCl_3$–MeOH (1:1) and had an analysis consistent with the molecular

Table 1 ^{1}H NMR data of compounds **1**–**3** and **5**, **6**, **10** and **12** in $(CD_3)_2SO$

Proton	1	2	3	5	6	10	12
2,6-H	6.97 d (8.7)	7.05 d (8.6)	6.99 d (8.7)	7.01 d (8.7) 7.02 d (8.8)	7.06 d (8.6) 7.04 d (8.6)	7.05 d (8.8) 7.04 d (8.6)	7.25 d (8.8)
3,5-H	7.15 d (8.7)	7.19 d (8.6)	7.17 d (8.7)	7.21 d (8.7) 7.16 d (8.6)	7.21 d (8.6) 7.20 d (8.6)	7.26 d (8.8) 7.27 d (8.6)	7.87 d (8.8)
7-H	4.09 d (5.7)	4.10 d (6.1)	4.01 d (5.8)	4.57 d (5.9) 4.25 d (6.1)	4.60 d (5.5) 4.10 d (6.1)	4.65 d (5.2) 4.10 d (5.3)	9.89 s
1′-H	5.37 d (1.7)	5.62 d (1.6)	5.37 d (1.6)	5.40 d (1.5)	5.63 d (1.6)	5.44 d (1.8)	5.61 d (1.6)
2′-H	3.87 m	5.30 dd (3.5, 1.6)	3.88 m	3.86 m	5.31 m	3.87 m	3.88 m
3′-H	3.81 ddd (9.7, 6.1, 3.3)	5.27 dd (9.8, 3.5)	3.83 ddd (9.6, 6.0, 3.3)	3.84 ddd (9.6, 6.0, 3.3)	5.30 m	3.80 m	3.82 m
4′-H	4.84 t (9.7)	4.95 t (9.8)	4.86 t (9.6)	4.86 t (9.6)	4.96 t (9.8)	4.83 t (9.6)	4.86 t (9.6)
5′-H	3.63 qd (9.7, 6.2)	3.89 qd (9.8, 6.2)	3.65 qd (9.6, 6.2)	3.65 qd (9.6, 6.2)	3.91 qd (9.8, 6.2)	3.44 qd (9.6, 6.2)	3.64 m
6′-H	0.97 d (6.2)	1.07 d (6.2)	0.98 d (6.2)	0.99 d (6.2)	1.07 d (6.2)	0.97 d (6.2)	0.99 d (6.2)
2′-OH	5.28 d (4.3)	—	5.30 d (4.3)	5.31 d (4.3)	—	4.99 d (4.8)	5.41 d (4.5)
3′-OH	4.97 d (6.1)	—	4.98 d (6.0)	4.99 d (6.0)	—	5.09 d (6.2)	5.06 d (6.1)
OCH_2CH_3	4.40 q (7.1)	4.40 q (7.0)	4.40 q (7.1)	—	—	—	—
OCH_2CH_3	1.13 t (7.1)	1.14 t (7.0)	1.15 t (7.1)	—	—	—	—
OCH_3	—	—	—	3.87 s 3.89 s	3.54 s	3.87 s 3.89 s	—
NH	7.52 t (5.7)	7.54 t (6.1)	4.53 t (5.8)	9.57 t (5.9)	4.56 t (5.5)	4.52 t (5.2)	—
$OCOCH_3$	2.03 s	2.12, 2.03, 1.95 s	2.04 s	2.04 s	2.13, 2.05, 1.97 s	2.02 s	2.06 s

formula $C_{18}H_{25}NO_8$ (HRMS). Its IR spectrum displayed important peaks at 3450 (OH), 1735 (ester carbonyl) and 1715 (carbamate carbonyl). Its EIMS showed a distinct molecular ion peak at *m/z* 383 whilst HRMS gave its accurate mass at *m/z* 383.1570 ($C_{18}H_{25}NO_8$); other significant fragments were at *m/z* 196.0950 (base peak, fragment c) and 189.0754 (fragment d) resulting from the elision of sugar and aglycone moieties, respectively, from the molecular ion peak (see structure). The broad-band ^{1}H-decoupled ^{13}C NMR spectrum of **1** in $(CD_3)_2SO$ exhibited 18 signals which were assigned using DEPT (distortionless enhancement by polarization transfer) experiments to three methyls, two methylenes, five methines, four sp^2 CH and four quaternary carbon atoms.

The ^{1}H NMR spectrum (Table 1) revealed the presence of an acetyl sugar moiety by five one-proton signals at δ 5.37 (d, $J_{1',2'}$ 1.7 Hz, 1′-H), 3.87 (m, 2′-H), 3.81 (ddd, $J_{3',4'}$ 9.7, $J_{3',2'}$ 3.3, $J_{3',OH}$ 6.1 Hz, 3′-H), 4.84 (t, $J_{4',5'} = J_{4',3'}$ 9.7 Hz, 4′-H), 3.63 (qd, $J_{5',4'}$ 9.7, $J_{5',6'}$ 6.2 Hz, 5′-H), a three-proton doublet at δ 0.97 ($J_{6',5'}$ 6.2 Hz, 6′-H), and a three-proton singlet at δ 2.03 (OAc). In addition, it showed two, one-proton doublets at δ 5.28 (*J* 4.3 Hz, 2′-OH) and 4.97 (*J* 6.1 Hz, 3′-OH) due to the two hydroxyl protons of the sugar which disappeared on shaking with D_2O and a 2′-H multiplet together with a 3′-H doublet of double doublets each changed to a double doublet (*J* 3.3, 1.7 Hz, 2′-H; *J* 9.7, 3.3 Hz, 3′-H). Exact assignment of these protons was made through their connectivities in COSY-45° (2D homonuclear chemical-shift correlation).

These ^{1}H NMR data (Table 1) and the ^{13}C NMR chemical shifts (Table 3) established that the sugar residue in **1** is a 4-*O*-acetylrhamnoside, whilst the magnitude of the coupling constant of the anomeric proton in the ^{1}H NMR spectrum ($J_{1',2'}$ 1.7 Hz) established the presence of an α-glycosidic linkage with the aglycone. The chemical shifts and multiplicities are also consistent with those of a 4′-*O*-acetyl-α-L-rhamnosyl moiety in compounds reported earlier[9] showing that **1** is 4′-*O*-acetyl-α-L-rhamnoside. That the sugar has a chair conformation[10] and exists in only one anomeric form was established from the ^{13}C NMR spectrum which had only one anomeric carbon signal (δ 98.14) and the ^{1}H NMR spectrum which had only one doublet for the anomeric proton.

The C_7H_6O fragment (*m/z* 107.0499) in the EI mass spectrum indicated the presence of a *p*-benzyloxy unit in the molecule which was also evidenced by the chemical shift values exhibited by aromatic protons [δ_H 6.97 (2 H, d, *J* 8.7 Hz, 2,6-H) and 7.15 (2 H, d, *J* 8.7 Hz, 3,5-H)] and the benzylic methylene protons [δ_H 4.09 (2 H, d, *J* 5.7 Hz)]. The ^{1}H–^{13}C heteronuclear correlation (HETCOR) spectrum had cross peaks showing ^{1}H–^{13}C direct correlation of 2,6-H and 3,5-H with aromatic carbons (CH, DEPT) at δ 116.35 and 128.28, respectively. It also showed correlation of δ_H 4.09 with δ_C 43.12 (CH_2, DEPT). Benzylic and sugar moieties accounted for the five double-bond equivalence in the molecule. The downfield shift of the anomeric proton[6,9,11] as compared to its chemical shift when the sugar is attached with aliphatic carbon[10,11] pointed out that it is linked with the aromatic ring and its site of attachment at the *para* carbon of the benzyl moiety was indicated by the mass fragments c and d and confirmed by the 2D nuclear Overhauser enhancement and

Table 2 ^{1}H NMR data of compounds **1**, **3**, **5**, **10** and **12** in $CDCl_3$

Proton	**1**	**3**	**5**	**10**	**12**
2,6-H	7.01 d (8.6)	7.01 d (8.6)	7.02 d (8.7)	7.02 d (8.7) 7.00 d (8.7)	7.17 d (8.8)
3,5-H	7.21 d (8.6)	7.21 d (8.6)	7.23 d (8.7) 7.17 d (8.7)	7.25 d (8.7) 7.17 d (8.7)	7.84 d (8.8)
7-H	4.29 d (5.4)	4.29 d (5.4)	4.68 d (5.6) 4.37 d (5.6)	4.69 d (5.5) 4.38 d (5.8)	9.91 s
1′-H	5.53 d (1.5)	5.53 d (1.5)	5.53 d (1.4)	5.53 d (1.4)	5.67 d (1.6)
2′-H	4.14 dd (3.5, 1.5)	4.14 dd (3.5, 1.5)	4.13 dd (3.5, 1.4)	4.11 dd (3.7, 1.4)	4.18 m
3′-H	4.09 dd (9.6, 3.5)	4.09 dd (9.6, 3.5)	4.11 dd (9.5, 3.5)	4.08 dd (9.4, 3.7)	4.07 m
4′-H	4.86 t (9.6)	4.86 t (9.6)	4.88 t (9.5)	4.86 t (9.4)	4.87 t (9.6)
5′-H	3.86 qd (9.6, 6.2)	3.86 qd (9.6, 6.2)	3.85 qd (9.5, 6.2)	3.87 qd (9.4, 6.2)	3.83 m
6′-H	1.17 d (6.2)	1.17 d (6.2)	1.16 d (6.2)	1.17 d (6.2)	1.19 d (6.2)
OCH_2CH_3	4.40 m	4.40 m	—	—	—
OCH_2CH_3	1.24 t (7.1)	1.24 t (7.1)	—	—	—
OCH_3	—	—	3.99 s 4.07 s	3.88 s 4.00 s	—
$OCOCH_3$	2.14 s	2.13 s	2.13s	2.17s	2.14 s

Table 3 ^{13}C NMR chemical shifts for **1**, **3**, **5** and **10** in $(CD_3)_2SO$

Carbon	**1**	**3**	**5**	**10**
1	154.66	155.01	154.72	155.70 155.54
2,6	116.35	116.41	116.16 116.24	116.29
3,5	128.28	128.68	128.48 128.36	128.25
4	133.55	131.51	131.40	134.56
7	43.12	45.26	47.21	48.42
8	160.01	160.09	190.75	199.27
1′	98.14	98.47	98.02	98.23
2′	70.10	70.45	67.79	69.40
3′	67.98	69.40	69.89	70.67
4′	73.59	73.65	73.38	73.38
5′	66.88	67.25	66.68	66.35
6′	17.46	17.87	17.18	17.91
OCH_2CH_3	59.67	59.67	—	—
OCH_2CH_3	14.64	14.64	—	—
OCH_3	—	—	56.23 56.96	58.46
$OCOCH_3$	169.89	169.89	169.64	170.01
$OCOCH_3$	20.89	20.89	20.60 20.79	23.15 21.25

exchange (NOESY) experiment which showed the spatial proximity of 1′-H and 2,6-H.

In addition to these structural elements, the NMR spectra revealed the presence of a carbamate group [δ_H 1.13 (3 H, t, J 7.1 Hz) and δ_C 14.64 (CH_3); δ_H 4.40 (2 H, q, J 7.1 Hz) and δ_C 59.67 (OCH_2); δ_C 160.01 ($NHCO_2$); δ_H 7.52 (1 H, t, J 5.7 Hz, NH)]. In keeping with the presence of this group the IR spectrum of compound **1** showed strong absorption at 1715 cm^{-1}. The ^{1}H NMR spectrum recorded after shaking with D_2O showed connectivity of the NH proton of the carbamate with the benzylic CH_2, as the doublet of methylene protons were converted into a singlet while the triplet of NH disappeared. A COSY-45° plot having a cross peak for δ 4.09 and 7.52 and the diagnostic fragments a, b and c at m/z 73.0270, 102.0546 and 196.0950 in the HRMS corresponding to the formulae $C_3H_5O_2$, $C_4H_8NO_2$ and $C_{10}H_{14}NO_3$, respectively, supporting the CH_2NH relationship. Inclusion of carbamate group in the molecule justified the remaining double bond in the structure.

This evidence supported the assignment of structure **1** to niazimin A without specification of the stereochemistry of the NH proton. In agreement with this structural assignment, compound **1** afforded the triacetyl derivative **2** on acetylation with acetic anhydride and pyridine. Compound **2** showed a molecular ion peak at m/z 467.1791 and strong IR absorption at 1745 cm^{-1} for the ester carbonyl. The ^{1}H NMR spectrum (Table 1) displayed three three-proton sharp singlets at δ 2.12, 2.03 and 1.95 (3 × AcO), while carbinylic protons shifted downfield to δ 5.30 (2′-H, dd, J 3.5, 1.6 Hz) and 5.27 (3′-H, dd, J 9.8, 3.5 Hz) as compared to those of **1**.

Niazimin B **3** formed needles from chloroform–methanol (1:1) (m.p. 182–184 °C) and had a molecular ion peak at m/z 383.1613 (HRMS) corresponding to the molecular formula $C_{18}H_{25}NO_8$ identical with niazimin A **1**. Its IR and UV absorption, and mass spectral fragmentation were also similar to those of **1**. It is evident from the ^{1}H (Table 1) and ^{13}C NMR (Table 3) spectral data that all the protons and carbons in niazimin A and B have virtually the same chemical shifts, the only difference being in the resonances of the NH protons [δ 7.52 (t, J 5.7 Hz) in **1** and δ 4.53 (t, J 5.8 Hz in **3**)]. This evidence establishes that compounds **1** and **3** are isomers, only the orientation of the NH proton with respect to the carbonyl group,[12–14] differentiating the two compounds.

It is important to note that the feature distinguishing the two isomers **4** and **9** isolated earlier from *M. oleifera*,[9] was also the chemical-shift values of the NH protons in the two isomers, the downfield shift being assigned to the NH proton *cis* to the thiocarbonyl. Since the magnitude of the carbonyl magnetic anisotropy is opposite to that of the thiocarbonyl,[13] in the present case the downfield value (δ 7.52) was assigned to the NH proton which is *trans* to the carbonyl group (Z isomer, **1**), and the upfield value (δ 4.53) was attributed to the NH proton which is *cis* to the carbonyl function (E isomer, **3**). These attributions are of the reverse order from those of the thiocarbamates **4** and **9**.[9] It is noteworthy that in a recently isolated pair of amides,[15] the NH proton *cis* to the carbonyl group resonated upfield at δ 5.31 while the NH proton *trans* to the carbonyl group appeared at δ 5.40.

Moreover, it was clear from the ^{1}H NMR spectra that the thiocarbamates **4** and **9** exist as two discrete tautomers **4a**, **4b** and **9a**, **9b**.[9] This contrasts with the ^{1}H NMR spectra for compounds **1** and **3** which showed only one signal for the methylene protons (7-H) in each case, indicating rapid interconversion of the tautomers of **1** and **3**. These observations confirm that thioamide sulfur has a stronger tendency to acquire negative charge than amide oxygen.[14]

On acetylation (acetic anhydride–pyridine, room temp. 2 days) isomer **3** afforded a derivative which was identical with **2** (MS, NMR, UV, IR). Formation of a common acetyl derivative **2** from both **1** and **3** showed that, as with thiocarbamates,[9] the E compound had isomerized to the Z *via* a carbinolimine intermediate.

On the basis of the above-described evidence the structures of niazimin A and B have been elucidated as *O*-ethyl-4-[(4′-*O*-acetyl-α-L-rhamnosyloxy)benzyl]carbamate (Z) **1** and (E) **3**, respectively. The ^{13}C NMR spectral data (Table 3) agree well with the assigned structures. It is noteworthy that the ^{1}H NMR spectra of **1** and **3** when run in $CDCl_3$ (Table 2) have almost identical chemical shifts, differentiation between the two isomers being impossible in the absence of an NH signal.

Compound **12** had an analysis consistent with the molecular formula $C_{15}H_{18}O_7$ and in agreement with the molecular ion peak at *m/z* 310.1050 in its high-resolution mass spectrum. The IR spectrum showed absorption at 1690 and 1740 cm^{-1}, characteristic of conjugated keto or aldehyde and ester carbonyl functions, respectively. The aldehydic nature of **12** was consistent with a sharp singlet at δ 9.89 in its ^1H NMR spectrum, which was non-exchangeable with D_2O. The AA'BB' pattern [δ 7.25 (*J* 8.8 Hz, 2,6-H) and δ 7.87 (*J* 8.8 Hz, 3,5-H)] in its ^1H NMR spectrum (Table 1) was reminiscent of two proton doublets (*J* 8.6 Hz) of *p*-hydroxybenzaldehyde {δ_H[300 MHz, $(CD_3)_2SO$], δ 7.75 and 6.91}. The signal for the anomeric proton of the sugar appeared at δ 5.61 (d, 1.6 Hz) and those for 2'-H, 3'-H and 5'-H at δ 3.88, 3.82 and 3.64 (each 1 H, m), respectively. A signal at δ 0.99 (d, *J* 6.2 Hz) was assigned to the methyl group of the sugar and those at δ 5.41 (d, 4.5 Hz) and 5.06 (d, 6.1 Hz) exchangeable with D_2O were attributed to 2'-OH and 3'-OH. Signals at δ 4.86 (t, 9.6 Hz) and 2.06 (s) were suggestive of a 4'-acetoxy group, as indicated by the IR spectrum. These chemical shifts, confirmed by COSY-45 experiments, implied the presence of 4-*O*-acetyl-α-L-rhamnosyl group in the molecule and together with the spectral data discussed above support the assignment of structure **12** to the glycoside. This assignment was also supported by significant mass fragments at *m/z* 189.0765 (fragment b) and 122.0348 (fragment a). This is the first report of the isolation of the rhamnoside of *p*-hydroxybenzaldehyde from a natural source, although the glucosides of 4-hydroxy-3-methoxybenzaldehyde, used both as a flavouring agent and in pharmaceutical work have been isolated from green vanilla.[16] Moreover, 4-hydroxyacetopheno-β-D-glucopyranosides have been obtained from *Penstemon pinifolius*.[17]

The spectral results (IR, UV, MS and NMR) for the thiocarbamates **5** and **10** resembled those for compounds **4** and **9**,[9] respectively, except for the presence of an acetoxy methyl group (IR: 1735 cm^{-1}); ^1H NMR: δ 2.04 in **5** and δ 2.02 in **10**). The increment of 42 a.m.u. in the mass spectrum, and ^1H NMR signals for the acetoxy methyl function in the two compounds confirmed the presence of an acetoxy group in compounds **5** and **10** which was placed at C-4' in the light of evidence discussed in case of **1** and **12**. In analogy with compounds **4** and **9**, **5** and **10** also exist as two discrete tautomers **5a**, **5b** and **10a**, **10b** as revealed by two sets of methylene signals in each case at δ 4.57 (d, *J* 5.9 Hz), 4.25 (d, *J* 6.1 Hz) and 4.65 (d, *J* 5.2 Hz), 4.10 (d, *J* 5.3 Hz), respectively. The structures of **5** and **10** were substantiated by the important ions in the mass spectrum (see Experimental section) at *m/z* 197 ($C_9H_{11}NO_2S$) and 189 ($C_8H_{13}O_5$) corresponding to fragments a and b and a significant fragment at *m/z* 107 (C_7H_7O).

The assignments of all the protons of **5** and **10**, confirmed by heteroCOSY and proton–proton coupling constants are presented in Table 1. Moreover, ^1H NMR spectra (Table 2) recorded in $CDCl_3$ could not distinguish between two isomers as in case of **1** and **3**.

On acetylation with acetic anhydride and pyridine both the isomers afforded the common acetylated product **6**, in which the NH proton appeared at δ 4.56 (t, *J* 5.5 Hz). This showed that during acetylation the *E* isomer was transformed into the *Z* isomer, a phenomenon observed in case of thiocarbamates,[9] and the carbamates **1** and **3**. Compound **6** was found to be identical (TLC, spectral data) with the common derivative of compounds **4** and **9** reaffirming the structures of the isomers **5** and **10**.

The biogenesis of thiocarbamate glycosides, a relatively rare group of mustard oils apparently limited to *M. oleifera* within the family Moringaceae,[9] may involve the addition of ethanol or methanol to isothiocyanates which occur in the plant.[6] Hitherto only the seven glycosides **4**, **5**, **7**, **8**, **9**, **10** and **11** have been obtained from this source as genuine natural products (autolysis).[18] Earlier, two isothiocyanate glycosides **13** and **14** were isolated from *Moringa*, through the action of myrosinase or ascorbic acid.[6,19] Glycosides **1** and **3** are the only examples of natural products incorporating the carbamate group, although synthetic carbamates possessing tranquilizing,[20] anaesthetic,[21] insecticidal,[22] nematocidal and fungicidal activity[23] are known. Since the glycosides **1**, **3** and **5**, **10** and **12** were detected in a fresh ethanolic extract of the leaves, they are considered genuine natural products. Benzaldehyde derivatives are commonly found in Nature and formation of compound **12** it is conjectured may occur through the shikimate pathway.[24]

Biological Results

In pursuance of studies on the isolation of pure hypotensive[9] and antispasmodic[25] principles from the ethanolic extract of *M. oleifera* leaves, further four hypotensive agents **1**, **3**, **5** and **10** have been obtained through a bioassay-guided fractionation of the ethanolic extract showing hypotensive activity. Their effects on the blood pressure of normotensive wistar rats (200–250 g) anaesthetized with pentobarbital sodium (50 mg/kg, i.p.) was studied as described previously.[26]

Intravenous administration of compounds **1**, **3**, **5** and **10** caused a fall in systolic, diastolic and mean arterial blood pressure in a dose-dependent manner. Compound **12** was not examined because of the small quantity available. Hypotensive responses for all four compounds were similar. They produced a 15–20% fall in the control mean blood pressure at a dose level of 1 mg kg^{-1} and a 35–40% reduction was observed at a dose level of 3 mg kg^{-1}. The results revealed that both the carbamates **1** and **3** and the thiocarbamates **5** and **10** are equally potent hypotensive agents, showing that both the amide and thioamide groups in these molecules are responsible for the activity. This observation supports the view that the amide group plays an important role in drug structure, since a number of biologically active compounds bearing this group are known in literature.[27–30] Recently several *O*-carbamoyl taxol analogues have been patented as neoplasm inhibitors,[31] and it has also been shown that the carbonyl group of carbamoyl moiety is essential for platelet inhibition activity.[32]

Experimental

M.p.s were determined using Gallenkamp melting point apparatus and are uncorrected. UV (in MeOH) and IR (in $CHCl_3$) spectra were recorded on Hitachi-U-3200 and JASCO-A-302 spectrophotometers respectively. The EI, FD and HREI mass spectra were obtained on Finnigan MAT-112, MAT-312 and JMS HX-110 spectrometers. The ^1H NMR spectra were run in $CDCl_3$ and $(CD_3)_2SO$ on Bruker Aspect AM-300 and AM-400 spectrometers operating at 300 and 400 MHz, respectively. The ^{13}C NMR spectra (Broad Band and DEPT) were recorded in $(CD_3)_2SO$ on Bruker Aspect AM-400 spectrometer operating at 100 MHz. The chemical shifts are given in ppm (δ) based on the residual solvent peak and coupling constants (*J*) are in Hz. The ^{13}C NMR spectral assignments have been made partly through DEPT and heteroCOSY/HMQC and partly through comparison with reported values of model compounds.[6,9,33] Exact assignments of proton chemical shifts were made through COSY-45, NOESY and heteroCOSY/HMQC. TLC analysis was performed on silica gel GF_{254} coated plates.

The leaves of *Moringa oleifera* were collected from the Karachi region in November 1990 and the plant was authenticated at the Department of Botany, University of Karachi.

Bioassay-directed Isolation of Compounds **1**, **3**, **5**, **10** *and* **12**.—Fresh, undried and uncrushed leaves (8 kg) of *M. oleifera* were repeatedly extracted with EtOH at room temperature. The 3rd–6th* extracts were combined and freed of the solvent under reduced pressure to provide a viscous mass which when subjected to a classical isolation procedure[9] gave a fraction identified as M – 80 (3.8 g). On thick layer chromatography (silica gel, $CHCl_3$–MeOH, 9:1) of M – 80, seven bands M – 1, M – 2a, M – 2b, M – 3, M – 4, M – 5 and M – 6 were obtained. Band M – 2a (194.3 mg) showing a single spot on TLC was resolved into niazimin B **3** (14.3 mg), compound **12** (2.3 mg), niazimin A **1** (17.9 mg), niazicin A **5** (9.8 mg), niaziminin A **8**[9] and **14** in the order of peaks through reverse-phase high-performance liquid chromatography [Shimadzu, C18, techspher 50 DS, 30 cm × 10 mm, mobile phase 70% MeOH–H_2O (v/v), few drops of acetic acid, loop 20 mm^3,† flow rate 4 cm^3 min^{-1}. Niazicin B **10** (8.5 mg) was obtained through HPLC of band M – 2b (100 mg) under the same HPLC conditions.

Niazimin A **1**: λ_{max}(MeOH)/nm 193.4, 201.8, 222.0, 246.0 and 272.2; ν_{max}($CHCl_3$)/cm^{-1} 3450, 2906, 1735, 1715, 1455–1612 (4 peaks), 1365, 1128, 1056 and 1020; *m/z* (FD–MS) 383 (M^+); *m/z* (%) 383.1570 (M^+) (calc. for $C_{18}H_{25}NO_8$, 383.1579) (1), 196.0950 (fragment c, $C_{10}H_{14}NO_3$) (42), 189.0754 (fragment d, $C_8H_{13}O_5$) (100), 178 (6), 171.0657 (fragment d – H_2O) (54), 166 (50), 147.0589 (fragment d – Ac) (6), 129.0529 ($C_6H_9O_3$) (88), 111 (62), 107.0499 (C_7H_7O) (75), 102.0546 (fragment b, $C_4H_8NO_2$) (48), 87 (13), 77.0390 (C_6H_5) (15) and 73.0270 (fragment a, $C_3H_5O_2$) (11); ^{1}H NMR results in Tables 1 and 2 and ^{13}C NMR results in Table 3.

Acetylation of niazimin A **1**. Acetic anhydride (0.5 cm^3) was added to a solution of **1** (5 mg) in pyridine (0.5 cm^3) and the reaction mixture kept at room temperature for 2 days. Work-up of the reaction mixture gave the triacetyl product **2**; λ_{max}(MeOH)/nm 201.6 and 260.6; ν_{max}($CHCl_3$)/cm^{-1} 3445, 2916, 1745, 1721, 1610, 1542, 1365 and 1115; *m/z* (EIMS) (%) 487.1771 (M^+) (calc. for $C_{22}H_{29}NO_{10}$, 467.1791) (4), 273 (70), 213 (35), 171 (28), 153 (85), 111 (100), 107 (15), 102 (8) and 87 (28); ^{1}H NMR results in Table 1.

Acetylation of **3**, **5** and **10** was carried out in a similar manner. The acetyl derivative of **3** is exactly the same as that of **1** while characterization of **6** which is the common acetyl derivative of **5** and **10** and niazimin A **4** and B **9**[9] is reported in the following pages.

Niazimin B **3**: λ_{max}(MeOH)/nm 193.4, 200.6, 221.2 and 270.8; ν_{max}($CHCl_3$)/cm^{-1} 3440, 2920, 1732, 1720, 1460–1605, 1384, 1122 and 1026; *m/z* (FD–MS) 383 (M^+); *m/z* (%) 383.1613 (M^+) (calc. for $C_{18}H_{25}NO_8$, 383.1579) (2); 196.0942 (fragment c, $C_{10}H_{14}NO_3$) (48), 189.0757 (fragment d, $C_8H_{13}O_5$) (100), 178 (6), 171.0664 (fragment d – H_2O) (20), 166 (50), 147.0589 (fragment d – Ac) (6), 129.0546 ($C_6H_9O_3$) (92), 111.0455 ($C_6H_7O_2$) (72), 107.0497 (C_7H_7O) (62), 102.0548 (fragment b, $C_4H_8NO_2$) (42), 87 (18), 77.0387 (C_6H_5) (17) and 73.0273 (fragment a, $C_3H_5O_2$) (10). ^{1}H NMR results in Tables 1 and 2 and ^{13}C NMR results in Table 3.

4-(4′-O-*Acetyl*-α-L-*rhamnosyloxy*)*benzaldehyde* **12**: λ_{max}(MeOH)/nm 201.0, 222.6, 246.6 and 270.2; ν_{max}($CHCl_3$)/cm^{-1} 3440, 3125, 1742, 1692, 1605 and 1120; *m/z* (FD–MS) 310 (M^+); *m/z* (%) 310.1050 (M^+) (calc. for $C_{15}H_{18}O_7$, 310.1052) (0.5), 189.0765 (fragment b, $C_8H_{13}O_5$) (90), 171.0660 (fragment b – 18) (15), 129.0556 ($C_6H_9O_3$) (80), 122.0348 (fragment a, $C_7H_6O_2$) (20), 106 (62) and 77.0388 (C_6H_5) (8); ^{1}H NMR results in Tables 1 and 2.

Niazicin A **5**: λ_{max}(MeOH)/nm 200.8, 223.2 and 245.2; ν_{max}($CHCl_3$)/cm^{-1} 3450, 2905, 1735, 1602, 1442, 1385, 1110 and 1015; *m/z* (FD–MS) 385 (M^+); *m/z* (%) 385.1200 (M^+) (calc. for $C_{17}H_{23}NO_7S$, 385.1194) (6), 384 (10), 230 (5), 197.0489 (fragment a, $C_9H_{11}NO_2S$) (100), 189.0762 (fragment b, $C_8H_{13}O_5$) (75), 181 (46), 171.0676 (fragment b – H_2O) (34), 147 (10), 129 (72), 107.0514 (C_7H_7O) (84) and 77.0383 (C_6H_5) (14); ^{1}H NMR results in Tables 1 and 2 and ^{13}C NMR results in Table 3.

Acetyl derivative **6** *of niazicin A* **5**: λ_{max}(MeOH)/nm 200.4, 221.2 and 246.2; ν_{max}($CHCl_3$)/cm^{-1} 3445, 2910, 1745, 1605, 1556, 1362, 1256 and 1025; *m/z* (%) 469.1446 (M^+) (calc. for $C_{21}H_{27}NO_9S$, 469.1406) (1), 273.0956 (fragment d, $C_{12}H_{17}O_7$) (24), 231.0911 (fragment d – Ac) (5), 171.0629 ($C_8H_{11}O_4$) (20), 129.0541 ($C_6H_9O_3$) (10), 111 (90) and 107.0503 (C_7H_7O) (20); ^{1}H NMR results in Table 1.

Niazicin B **10**: λ_{max}(MeOH)/nm 200.8, 223.0 and 245.2; ν_{max}($CHCl_3$)/cm^{-1} 3455, 2910, 1735, 1605, 1445, 1380, 1115 and 1016; *m/z* (FD–MS) 385 (M^+); *m/z* (%) 385.1208 (M^+) (Calc. for $C_{17}H_{23}NO_7S$, 385.1194) (4), 384 (6), 343 (6), 197.0487 (fragment a, $C_9H_{11}NO_2S$) (100), 189.0722 (fragment b, $C_8H_{13}O_5$) (75), 181 (40), 171.0651 (fragment b – H_2O) (34), 147 (10), 129 (72), 107.0504 (C_7H_7O) (80) and 77.0380 (C_6H_5) (14), ^{1}H NMR results in Tables 1 and 2 and ^{13}C NMR results in Table 3.

* Work on the 1st and 2nd extracts has been published earlier.[9]

† 1 mm^3 = 1 μl.

References

1 B. N. Sastari, *The Wealth of India*, Council of Scientific and Industrial Research, New Delhi, 1962, vol. 1, p. 425.
2 K. M. Nadkarni, revised by A. K. Nadkarni, *The Indian Materia Medica*, Popular Parkashan, Bombay, 1976, p. 810.
3 S. A. A. Jahn, *J. Am. Water Works Assoc.*, 1988, **80**, 43 (*Chem. Abstr.*, 1988, **109**, 61191).
4 S. Siddiqi and M. I. Khan, *Pak. J. Sci. Ind. Res.*, 1968, **11**, 268.
5 A. Caceres, O. Cabrera, O. Morales, P. Mollinedo and P. Mendia, *J. Ethanopharmacol.*, 1991, **33**, 213.
6 U. Eilert, B. Wolters and A. Nahrstedt, *Planta Med.*, 1981, **42**, 55.
7 S. Shukla, R. Mathur and A. O. Prakash, *J. Ethanopharmacol.*, 1988, **22**, 51.
8 A. Caceres, A. Saravia, S. Rizzo, L. Zabala, E. D. Leon and F. Nave, *J. Ethanopharmacol.*, 1992, **36**, 233.
9 S. Faizi, B. S. Siddiqui, R. Saleem, S. Siddiqui, K. Aftab and A. H. Gilani, *J. Chem. Soc., Perkin Trans. 1*, 1992, 3237.
10 D. D. McLantyre, M. W. Germann and H. J. Vogel, *Can. J. Chem.*, 1990, **68**, 1263.
11 R. Kasai, M. Okihara, J. Asakawa, K. Mizutani and O. Tanaka, *Tetrahedron*, 1979, **35**, 1427.
12 J. Sandström, *J. Phys. Chem.*, 1967, **71**, 2318.
13 R. A. Bauman, *J. Org. Chem.*, 1967, **32**, 4129.
14 J. Dale, *Stereochemistry and Conformational Analysis*, Universitets-forlaget, Oslo, 1978, p. 82.
15 H. Greger, F. Hadacek, O. Hofer, G. Wurtz and G. Zechner, *Phytochemistry*, 1993, **32**, 933.
16 V. E. Tyler, L. R. Brady and J. E. Robbers, *Pharmacognosy*, Lea & Febiger, Philadelphia, 1988, 73.
17 P. Junior, *Planta Med.*, 1986, 218.
18 V. Gil and A. J. Macleod, *Phytochemistry*, 1980, **19**, 227.
19 A. Kjaer, O. Malver, B. El-Menshawi and J. Reisch, *Phytochemistry*, 1979, **18**, 1485.
20 J. W. Bastian and G. R. Clement, *Diseases Nervous System*, 22, 1961, 9 (*Chem. Abstr.*, 1963, **58**, 1829).
21 M. Wassef, H. Lal and B. J. Pleuvry, *Br. J. Anaesth.*, 1974, 473 (*Chem. Abstr.*, 1975, **83**, 37629).
22 A. C. Baillie, *Pestic. Sci.*, 1981, **12**, 7 (*Chem. Abstr.*, 1981, **95**, 97315).
23 D. W. Gier and R. W. Pritchard, US Pat. 3, 308, 018, 1967 (*Chem. Abstr.*, 1967, **67**, 2874).
24 E. Haslam, in *Comprehensive Organic Chemistry*, ed. D. Barton, W. D. Ollis and E. Haslam, Pergamon Press, Oxford, 1979, vol. 5, p. 1194.
25 A. H. Gilani, K. Aftab, F. Shaheen, B. S. Siddiqui, S. Siddiqui, R. Saleem and S. Faizi, in *Natural Drugs and The Digestive Tract*, eds. F. Capasso and N. Mascolo, EMSI, Roma, 1992, p. 179.
26 A. H. Gilani, K. Aftab, S. S. Siddiqui, R. Saleem, B. S. Siddiqui and S. Faizi, *Phytotherapy Research*, 1994, **8**, 87.
27 L. Crombie and S. R. M. Jarrett, *J. Chem. Soc., Perkin Trans. 1*, 1992, 3179.

28 J. Zabrocki, J. B. Dunbor, Jr., K. W. Marshall, M. U. Toth and G. R. Marshall, *J. Org. Chem.*, 1992, **57**, 202.
29 C. S. J. Walpole, R. Wrigglesworth, S. Bevan, E. A. Campbell, A. Dray, I. F. James, M. N. Perkins, D. J. Reid and J. Winter, *J. Med. Chem.*, 1993, **36**, 2362, 2373, 2381.
30 H. Okujima, T. Niifuku, H. Betsusho, A. Kyono, J. Hayashi, A. Tobe and M. Kobayashi, Jpn. Kokai Tokkyo Koho JP 05 59, 003 (*Chem. Abstr.*, 1993, **119**, 159902).
31 J. D. Bourzat, A. Commercon, D. Guenord, F. Gueritte-Voegelein and P. Potier, Eur. Pat. Appl. EP 524093 (*Chem. Abstr.*, 1993, **119**, 9005).
32 R. N. Misra, B. R. Brown, P. M. Sher, M. M. Patel, S. E. Hall, W. Han, J. C. Barrish, O. Kocy, D. N. Harris, H. J. Goldenberg, M. Michel, W. A. Schumacher, M. L. Webb, H. Monshizadegan and M. L. Ogletree, *J. Med. Chem.*, 1993, **36**, 1401.
33 W. Voelter and O. Oster, *Org. Mag. Res.*, 1973, **5**, 547.

Paper 4/00675E
Received 3*rd February* 1994
Accepted 10*th June* 1994