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FULLY ACETYLATED CARBAMATE AND HYPOTENSIVE THIOCARBAMATE GLYCOSIDES FROM *MORINGA OLEIFERA*

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Key Word Index—*Moringa oleifera*; Moringaceae; sahjna; leaves; bioassay-directed isolation; thiocarbamate, carbamate and nitrile glycosides; fully acetylated sugar; 2D NMR; hypotensive activity.

Abstract—Six new and three synthetically known glycosides have been isolated from the leaves of *Moringa oleifera*, employing a bioassay-directed isolation method on the ethanolic extract. Most of these compounds, bearing thiocarbamate, carbamate or nitrile groups, are fully acetylated glycosides, which are very rare in nature. Elucidation of the structures was made using chemical and spectroscopic methods, including 2D NMR techniques. Thiocarbamates showed hypotensive activity.

INTRODUCTION

Moringa oleifera Lam. (syn. *Moringa pterygosperma* Gaertn.), a quick-growing ornamental tree, belongs to the family Moringaceae, which is represented by only one genera [1, 2]. The plant is widely cultivated in Asia, Africa and other tropical parts of the world for food. It is valued for its medicinal properties [1, 2]. Moreover, its seeds are used for the purification of drinking water in rural areas of Asia and Africa. They act as a flocculant and also remove more than 90% of *Schistosoma mansoni* cercariae [3]. Recently, several novel glycosides possessing hypotensive [4–7] and antispasmodic [8] activities have been obtained from the leaves. Prior to these investigations, there was no instance of the isolation of active hypotensive principles, although the antihypertensive and antispasmodic properties have long been attributed to the species [2, 9, 10]. In continuation of these studies, nine new naturally occurring glycosides (**1**–**9**) have been isolated, most possessing fully acetylated sugars. These are rare in nature and the isolation of only a few such glycosides has been reported in the literature [11–14]. Structures of these glycosides were deduced through extensive ^{1}H and ^{13}C NMR studies, including 2D NMR (COSY, NOESY and HMQC). Thiocarbamates **5**–**8**, were examined for hypotensive activity and found to be active; the other compounds were obtained in low yields and could not be tested.

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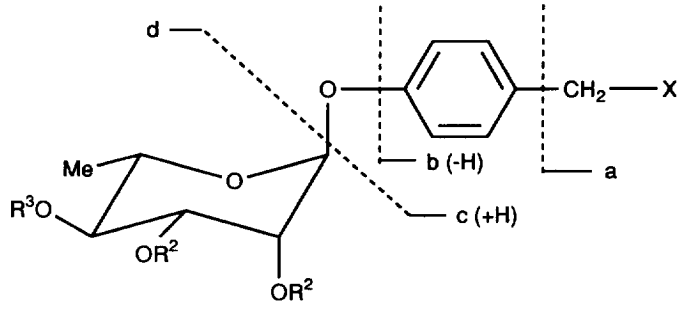
RESULTS AND DISCUSSION

Bioassay-guided fractionation of an ethanolic extract of *M. oleifera* leaves employing classical methods of isolation followed by prep. TLC and HPLC resulted in the isolation of nine new glycosides (**1**–**9**), of which **6**, **7** and **9** have been obtained previously through acetylation of niazinin, niazimicin [4] and niazirin [6], respectively.

The molecular formula of **1** was established as $C_{21}H_{27}NO_{10}$ with the aid of FAB mass spectrometry (negative-ion mode, $[M-1]^+$ *m/z* 452) and important mass ions at *m/z* 273.0958 and 181.0755 (fragment d and c, respectively; *vide* structure). The presence of carbamate, acetate and aromatic rings was apparent in its IR spectrum which lacked hydroxyl absorptions. The ^{1}H NMR spectrum exhibited an anomeric proton signal at δ5.39 which was observed as a doublet of 1.5 Hz. It also showed signals due to three acetoxymethyl groups as singlets at δ2.04, 2.06 and 2.07, and three methine protons geminal to acetoxyl groups at δ5.32 (*dd*, $J_{2',1'}$ = 1.5 Hz, $J_{2',3'}$ = 3.9 Hz, H-2'), 5.14 (*dd*, $J_{3',2'}$ = 3.9 Hz, $J_{3',4'}$ = 9.7 Hz, H-3') and 4.86 (*t*, $J_{4',3'} = J_{4',5'}$ = 9.7 Hz, H-4'). A multiplet at δ3.87 was ascribed to H-5', while a three-proton doublet at δ0.98 ($J_{6',5'}$ = 6.2 Hz) was due to the C-Me group (H-6'); moreover, no hydroxyl proton signal was observed.

The appearance of only one anomeric proton in the spectrum revealed the presence of a sugar in the molecule in a pure anomeric form. The coupling constant of the anomeric proton revealed the α-orientation of the sugar residue. These ^{1}H NMR assignments are comparable to



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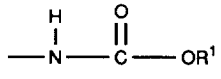
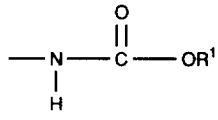


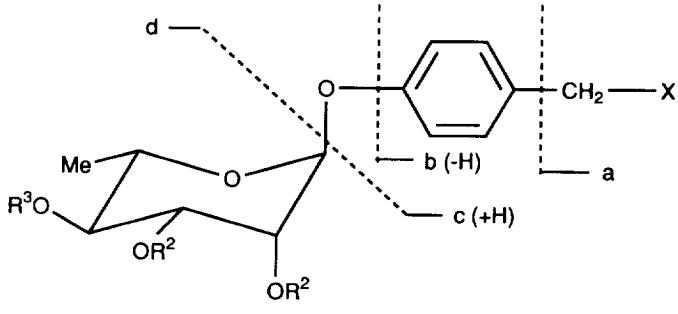
1 R^1 = Me, R^2 = R^3 = Ac

3 R^1 = Et, R^2 = R^3 = Ac

4 R^1 = Me, R^2 = H, R^3 = Ac



2 R^1 = Me, R^2 = R^3 = Ac



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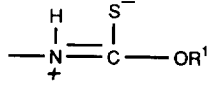
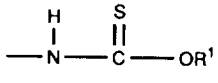
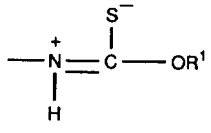


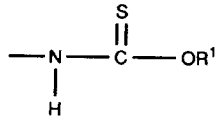
5a R^1 = Me, R^2 = R^3 = Ac



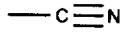
5b R^1 = Me, R^2 = R^3 = Ac



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



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



9 R^2 = R^3 = Ac

those reported for triacetyl rhamnose [4], thus suggesting that **1** is a completely acetylated α-L-rhamnoside. The relatively downfield shift of the anomeric proton, as compared with its chemical shift when it is linked with saturated carbons [15], implied its linkage with the aromatic ring, which was established by the 2D NOESY cross-peak, H-1′/H-2,6. The ^{1}H NMR spectrum is also characteristic for a *p*-oxybenzyl group showing a pair of two-proton doublets (J = 8.8 Hz) at δ7.27 and 7.05 for the mutually-coupled protons, H-3,5 and H-2,6, respectively, of the aromatic ring, and a two-proton doublet at δ4.10 (J = 5.9 Hz, H-7) for the methylene protons. That this methylene is in turn attached with the NH, is evident from the ^{1}H NMR spectrum recorded in DMSO-d_6 + D_2O, in which methylene protons appeared as a singlet, while the NH triplet (J = 5.9 Hz), which resonated at δ4.53 in DMSO-d_6, disappeared. Furthermore, a singlet for the methoxy group appeared at δ3.95. Exact assignment of all these protons, summarized in Table 1, was made through a ^{1}H–^{1}H COSY study.

These ^{1}H NMR spectral data are compatible with those assigned for the previously isolated thiocarbamates [4]. However, diagnostic mass fragment ions at *m/z* 88.0355 (a), 163.0595 (b), 181.0750 (c), 149.0518 (c-MeOH) and 122.0572 (c-COOMe) (see structures) showed the presence of a methyl carbamate group in **1**, instead of methyl thiocarbamate group. Based on these findings, the structure of **1** was established as *O*-methyl, 4-[(2′,3′,4′-tri-*O*-acetyl-α-L-rhamnosyloxy) benzyl] carbamate (*E*), which was corroborated by the significant fragment ions at *m/z* 59.0402 ($C_2H_4O_2$), 60.0140 ($C_2H_3O_2$), 273.0958 (d), 189.0771 ($C_8H_{13}O_5$) and 107.0503 observed in the HREI mass spectrum.

Compound **2** showed an FAB mass spectral (negative ion) peak at *m/z* 452 $[M-1]^+$ in accordance with the molecular formula $C_{21}H_{27}NO_{10}$; this is identical to that of **1**, indicating their isomeric nature. This was supported by the strikingly similar UV and IR spectral data, mass fragmentation and ^{1}H NMR chemical shifts and coupling constant pattern (Table 1) of these compounds. However, in the ^{1}H NMR spectrum of **2**, the exchangeable NH proton triplet (6.1 Hz) resonated downfield (δ7.56) as against that of δ4.53 in **1**. This showed that **2** is the rotational isomer of **1** with the *Z* sterochemistry. The chemical shift assignments for the NH proton in **1** and **2** are the reverse of those of the thiocarbamates niazinin A (*E*) and B (*Z*) [4]. This is based on the fact that the magnitude of magnetic anisotropy of the thiocarbonyl group is opposite to that of the carbonyl [16]. In the recently isolated amides, the penangins, the downfield value (δ5.40) of the NH proton was also assigned to the *Z*-isomer and the upfield chemical shift (δ5.31) ascribed to the NH proton of the *E*-isomer [17]. These facts led to the structure elucidation of **2** as *O*-methyl 4-[(2′,3′,4′-tri-*O*-acetyl-α-L-rhamnosyloxy)benzyl]carbamate (*Z*).

The FAB mass spectrum (negative ion), ^{1}H NMR data and HREI mass spectral fragments c (195.0846) and d (273.0958) of **3** determined its molecular formula as $C_{22}H_{29}NO_{10}$, which indicated that it is the next higher homologue of carbamates **1** and **2**. Its ^{1}H NMR spectrum (Table 1) exhibited signals for sugar and benzylic protons similar to those of **1** and **2**, except that in **3**, the methoxy singlet of the *O*-methyl carbamate function was replaced by ethoxy protons of *O*-ethyl carbamate at δ4.39 (*q*, J = 7.1 Hz, O<u>CH$_2$</u>Me), and 1.24 (*t*, J = 7.1 Hz, OCH$_2$<u>Me</u>). The NH proton triplet (J = 5.8 Hz) appeared at δ4.53, which is identical to that of **1**, revealing the *E*-stereochemistry of the molecule. Thus, the structure of **3** was deduced as *O*-ethyl, 4-[(2′,3′,4′-tri-*O*-acetyl-α-L-rhamnosyloxy)-benzyl]carbamate (*E*), which was substantiated by the diagnostic HREI mass spectral fragment ions (*vide* structure) at *m/z* 102.0550 (a), 148.0450 (c-OEt), 122.0604 (c-COOEt), 231.0877 (d-C_2H_2O), 189.0741 (d-2 × C_2H_2O), 107.0502 (C_7H_7O) and 73.0291 ($C_3H_5O_2$).

The molecular formula $C_{17}H_{23}NO_8$ for niazicinin A (**4**) was based on FD mass spectrometry *m/z* 369 ($[M]^+$), FAB mass spectrometry (negative ion) *m/z* 368, mass fragments at *m/z* 189.0772 (d) and *m/z* 181.0752 (c), and ^{1}H NMR resonances and their integrals. The IR bands at 3415, 1742, 1725 and 1591–1616 cm^{-1} (four peaks) implied the presence of hydroxy, acetate, carbamate and an aromatic ring in the structure. The ^{1}H NMR spectrum of **4** (Table 1) exhibited signals for a sugar moiety at δ5.35 (*d*, $J_{1',2'}$ = 1.6 Hz, H-1′), 3.88 (*m*, H-2′), 3,81 (*m*, H-3′), 4.86 (*t*, $J_{4',5} = J_{4',3'}$ = 9.8 Hz, H-4′), 3.59 (*m*, H-5′) and 0.98 (*d*, $J_{6',5'}$ = 6.2 Hz, H-6′) along with only one singlet for the acetoxymethyl group at δ2.04, suggesting that **4** is a monoacetylated glycoside. These values coincided well with those reported for 4′-*O*-acetylrhamnose [4, 5] and demonstrated that the compound is 4′-*O*-acetyl-α-L-rhamnoside, which was corroborated by the mass fragment d (*vide* structure). Moreover, the ^{1}H NMR spectrum showed characteristic signals for the *O*-methyl benzyloxy carbamate group as described for **1** and **3** (Table 1). On the basis of this evidence, the structure of **4** was elucidated as *O*-methyl 4-[(4′-*O*-acetyl-α-L-rhamnosyloxy) benzyl] carbamate (*E*), which was supported by the significant mass fragment ions at *m/z* 341.1471 $[M-CO]^+$, 163.0595 (b), 107.0508 (C_7H_7O), 88.0355 (a) and 74.0269 ($C_2H_4NO_2$) in the HREI mass spectrum. The structure was finally confirmed by acetylation affording a triacetyl derivative which was found to be identical in every respect to **2** (TLC, UV, IR, MS, ^{1}H NMR). Thus, acetylation was accompanied with the conversion of the *trans* (*E*)-isomer into the *cis* (*Z*)-isomer as **2** has the reverse stereochemistry to that of the parent compound **4** revealed by the resonance of the NH proton in the ^{1}H NMR spectrum (Table 1). This phenomenon was earlier witnessed in the case of both thiocarbamates and carbamates [4, 5]. The importance of naturally occurring carbamates **1** and **3** is thus evident, as they could not be obtained through the normal acetylation of the respective parent compounds.

It is important to note that unlike thiocarbamates which exist in two discrete tautomers [4, 5], carbamates **1**–**4** have only one form as revealed by their ^{1}H NMR spectra which lacked the double signals for the methylene protons. Double signals for the methoxy protons in **1**, **2**

Table 1. ^{1}H NMR chemical shifts (δ) and coupling constants (Hz) for **1**–**9**

H	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**
2,6	7.07 *d* (8.8)	7.07 *d* (8.5)	7.00 *d* (8.7)	7.05 *d* (8.7)	7.07 *d* (8.8)	6.99 *d* (8.5)	7.01 *d* (8.7)	6.97 *d* (8.6)	7.08 *d* (8.5)
					7.00 *d* (8.7)	7.01 *d* (8.6)	6.99 *d* (8.6)	6.96 *d* (8.8)	
3,5	7.27 *d* (8.8)	7.27 *d* (8.5)	7.22 *d* (8.7)	7.27 *d* (8.7)	7.28 *d* (8.8)	7.17 *d* (8.5)	7.21 *d* (8.7)	7.21 *d* (8.6)	7.28 *d* (8.5)
					7.17 *d* (8.7)	7.21 *d* (8.6)	7.27 *d* (8.6)	7.22 *d* (8.8)	
7	4.10 *d* (5.9)	4.09 *d* (6.1)	4.10 *d* (5.8)	4.10 *d* (6.1)	4.57 *d* (5.8)	4.57 *d* (5.4)	4.56 *d* (5.4)	4.57 *d* (5.4)	3.53 *s*
					4.10 *d* (6.1)	4.20 *d* (6.0)	4.23 *d* (5.9)	4.20 *d* (6.0)	
1′	5.39 *d* (1.5)	5.41 *d* (1.8)	5.39 *d* (1.9)	5.35 *d* (1.6)	5.46 *d* (1.7)	5.39 *d* (1.8)	5.39 *d* (1.7)	5.33 *d* (1.9)	5.45 *d* (1.6)
2′	5.32 *dd*	5.06 *dd*	5.06 *dd*	3.88 *m*	5.06 *dd*	5.36 *dd*	5.06 *dd*	3.81 *m*	5.07 *dd*
	(3.9, 1.5)	(3.3, 1.8)	(3.6, 1.9)		(3.3, 1.7)	(4.0, 1.8)	(3.4, 1.6)		(3.6, 1.6)
3′	5.14 *dd*	4.91 *dd*	4.91 *dd*	3.81 *m*	5.40 *m*	5.31 *m*	4.91 *dd*	3.64 *m*	4.91 *dd*
	(9.7, 3.9)	(9.6, 3.3)	(9.6, 3.6)				(9.4, 3.4)		(9.3, 3.3)
4′	4.86 *t* (9.7)	4.86 *t* (9.6)	4.85 *t* (9.6)	4.86 *t* (9.8)	4.86 *t* (9.7)	4.86 *t* (9.6)	4.86 *t* (9.4)	3.27 *dt* (9.1,5.6)	5.33 *t* (9.3)
5′	3.87 *m*	3.70 *m*	3.87 *m*	3.59 *m*	3.99 *m*	3.89 *qd* (9.6,6.2)	3.99 *m*	3.47 *m*	3.97 *m*
6′	0.98 *d* (6.2)	1.09 *d* (6.0)	0.98 *d* (6.2)	0.98 *d* (6.2)	0.89 *d* (6.2)	0.98 *d* (6.2)	1.09 *d* (5.9)	1.09 *d* (6.1)	1.09 *d* (6.2)
2′-OH	—		—	5.41 *d* (4.1)	—		—	4.99 *d* (4.4)	—
3′-OH	—		—	5.14 *d* (5.8)	—	—		4.67 *d* (6.0)	—
4′-OH	—	—		—	—	—		4.82 *d* (5.6)	—
$O\underline{CH_2}Me$	—		4.39 *q* (7.1)	—	—	—	4.39 *q* (7.1)	4.39 *q* (7.1)	—
			4.38 *q* (7.1)	—	—	—	4.38 *q* (7.1)	4.38 *q* (7.1)	
$OCH_2\underline{Me}$	—	—	1.24 *t* (7.1)	—		—	1.24 *t* (7.1)	1.24 *t* (7.1)	
			1.22 *t* (7.1)	—	—	—	1.23 *t* (7.1)	1.22 *t* (7.1)	—
OMe	3.95 *s*	3.96 *s*		3.95 *s*	3.87 *s*	3.85 *s*	—	—	—
	3.88 *s*	3.97 *s*		3.94 *s*	3.88 *s*	3.96 *s*			
NH	4.53 *t* (5.9)	7.56 *t* (6.1)	4.53 *t* (5.8)	4.54 *t* (6.1)	7.55 *t* (5.8)	4.53 *t* (5.4)	4.53 *t* (5.4)	4.53 *t* (5.4)	—
OCOMe	2.04, 2.06	2.04, 2.06	2.03, 2.06	2.04 *s*	2.04, 2.06	2.04, 2.06	2.03, 2.06	—	2.06, 2.07
	2.07 *s*	2.07 *s*	2.07 *s*		2.07 *s*	2.07 *s*	2.07 *s*		2.08 *s*

and **4** and ethoxy protons in **3** (Table 1), however, indicated the phenomenon of *Z*/*E* isomerism along the ester bond [4].

The negative ion FAB mass spectrum ($[M-1]^+$, *m/z* 468) and HREI mass spectral ions at *m/z* 273.0983 (d) and 197.0575 (c) corresponding to the composition $C_{12}H_{17}O_7$ and $C_9H_{11}NO_2S$, respectively, gave the molecular formula of **5** as $C_{21}H_{27}NO_9S$. Its IR spectrum displayed peaks ascribable to acetate and an aromatic ring. The ^{13}C NMR spectrum (Table 2) exhibited signals for 21 carbon atoms in the molecule as five methyls (one each for C-Me and O-Me, and three -OCOMe), one methylene, five methines, four sp^2 CH and six sp^2 quaternary carbons. The structural features were evident from the ^1H NMR spectrum of **5** in DMSO-d_6 (Table 1) which exhibited signals for triacetyl-α-L-rhamnose as observed in the case of carbamates **1**–**3**. This was confirmed by the mass fragment d at *m/z* 273.0983, and ^{13}C NMR chemical shifts at δ95.6, 68.4, 68.8, 70.1, 66.9 and 17.8 which were connected in the HMQC spectrum with protons at δ5.46 (H-1′), 5.06 (H-2′), 5.40 (H-3′), 4.86 (H-4′), 3.99 (H-5′) and 0.98 (H-6′), respectively. In comparison to the nonacetylated rhamnoside [4], the ^{13}C NMR spectrum of **5** showed significant acetylation shifts [18] for C-1′–C-5′ (Table 2). Further, the ^1H NMR chemical shifts and multiplicities for the *p*-substituted benzyl and methyl thiocarbamate functions are in agreement with those of reported constituents [4]. The downfield chemical shift of the NH proton at δ7.55 showed that it is *cis* to the sulphur of the thiocarbonyl group and that **5** has the *trans* (*E*)-stereochemistry. Its structure has thus been elucidated as *O*-methyl, 4-[(2′,3′,4′-tri-*O*-acetyl-α-L-rhamnosyloxy)-benzyl]thiocarbamate (*E*). Important mass fragment peaks at *m/z* 231, 189.0771 ($C_8H_{13}O_5$), 171.0668 ($C_8H_{11}O_4$) and 107.0503 (C_6H_5O) reaffirmed the structure.

Table 2. ^{13}C NMR chemical shifts for **5**–**7**

C	**5**	**6**	**7**
1	155.3	153.1	154.2
	155.0	156.1	151.3
2,6	116.3	116.7	116.7
	116.5	117.9	117.2
3,5	128.7	128.8	128.4
	128.7	128.3	128.7
4	131.7	131.0	131.8
	131.4	132.8	133.9
7	47.4	47.9	47.0
	47.1	44.9	43.1
8	191.0	190.1	190.3
1′	95.6	95.1	95.2
2′	68.4	68.7	68.4
3′	68.8	68.7	68.7
4′	70.1	69.9	69.9
5′	66.9	66.8	66.8
6′	17.8	17.2	17.2
OMe	56.5	58.9	—
	56.0	56.9	
OCH_2Me	—	—	64.2
			64.9
OCH_2Me	—	—	14.6
			14.0
OCOMe	170.2	169.7	169.7
OCOMe	21.6, 21.0, 20.8	20.5, 20.4, 20.6	20.4, 20.5, 20.6

Compound **6** has a molecular formula $C_{21}H_{27}NO_9S$ obtained from the HREI mass spectrum, which showed a $[M]^+$ at *m/z* 469.1441 with important mass fragments at *m/z* 273.0986 (d) and *m/z* 197.0577 (c). These values along with the UV, IR, MS, ^1H NMR and ^{13}C NMR spectral data (Tables 1 and 2) showed that **6** is an isomer of **5**. The upfield value of the NH proton of thiocarbamate group at δ4.53 (*t*, *J* = 5.4 Hz) revealed that in this case the NH is *trans* to the thiocarbonyl group and, thus, the molecule as a whole has *cis* (*Z*)-stereochemistry. On this basis, the structure of **6** has been determined as *O*-methyl, 4-[(2′,3′,4′-tri-*O*-acetyl-α-L-rhamnosyloxy)benzyl]thiocarbamate (*Z*). Mass fragments at *m/z* 189.0763, 171.0681 and 107.0510 corresponding to the formulae $C_8H_{13}O_5$, $C_8H_{11}O_4$ and C_7H_7O, respectively, substantiated the structure. Compound **6** (*Z*) has earlier been obtained through acetylation of both niazinin A (*Z*) and B (*E*) [4]. This showed that, although under normal acetylation conditions it is not possible to obtain the (*E*) acetyl derivative from the parent (*E*) compound, the triacetyl thiocarbamate with *E*-stereochemistry (**5**) does exist in nature.

The HREI mass spectrum of **7** showed a $[M]^+$ at *m/z* 483.1570 consistent with the molecular formula $C_{22}H_{29}NO_9S$. Its UV, IR, ^1H NMR and the mass fragments c (211.0648) and d (273.0980) indicated that it is the thio analogue of compound **3**. This was confirmed by the downfield shift (δ4.56) of the benzylic methylene in the ^1H NMR spectrum and the diagnostic mass fragment at *m/z* 182.0251 resulting from the loss of an ethyl group from fragment c. The same chemical shift δ4.53 for the NH proton in both compounds, however, revealed that **7** has the *cis* (*Z*)-stereochemistry. Thus, its structure has been deduced as *O*-ethyl, 4-[(2′,3′,4′-tri-*O*-acetyl-α-L-rhamnosyloxy)benzyl]thiocarbamate (*Z*), which was supported by the mass fragment ions at *m/z* 399.1318 ($[M]^+ - [2\times42]$), 357.1231 ($[M]^+ - [3\times42]$), 231.0867 ($C_{10}H_{15}O_6$), 189.0756 ($C_8H_{13}O_5$), 122.0624 (C_7H_8NO) and 107.0505 (C_7H_7O). The structure was finally confirmed by the ^{13}C NMR resonances (Table 2) which showed acetylation shifts [18] for the sugar carbons 1′–5′ as compared to those of the non-acetylated compound, niazimicin [4]. Compound **7** was found to be identical (UV, IR, MS, NMR) with the triacetylated derivative of niazimicin and niaziminins A and B [4].

Compound **8** possesses the molecular formula $C_{16}H_{23}NO_6S$, derived from the HREI mass spectrum ($[M]^+$ *m/z* 357.1199), which was identical to that reported for niazimicin [4]. The ^1H NMR spectrum (Table 1) exhibited signals for α-L-rhamnose, a benzylic group and an ethyl thiocarbamate function in the molecule. These spectral data are comparable with those reported for niazimicin [4], except for the NH proton which

appeared upfield (δ4.53, t, J = 5.4 Hz) in **8**, as compared to niazimicin (δ9.52). This observation showed that these compounds are rotational isomers and that **8** is the *cis*-isomer of niazimicin. Further, significant mass fragment ions of **8** at m/z 211.0685 (c), 195.0777 (b), 182.0244 (c-Et), 147.0649 (d) and 107.0510 (C_7H_7O) corroborated its structure, which was defined as *O*-ethyl, 4-[(α-L-rhamnosyloxy)benzyl]thiocarbamate (*Z*). Compound **8** has been named niazimicin B.

In the ^{1}H NMR spectra of all these thiocarbamate glycosides, including the *E*-isomer (**5**) and the *Z*-isomer (**8**), double signals were observed for the benzylic thiocarbamate part of the structure. This may be due to the fact that these isomers exist in two discrete forms a and b in a ratio of 2:1. The assignment of the benzylic signals in the tautomers a and b was rendered possible by comparing the corresponding integrals from the ^{1}H NMR spectra. Thus, the downfield shift (~ 4.57) was attributed to the major form a, while the upfield value (~ 4.20) was assigned to the minor form [4].

The FAB mass spectrum (negative ion) of **9** showed a $[M-1]^+$ at m/z 404 corresponding to the molecular formula $C_{20}H_{23}NO_8$. Its IR spectrum showed absorbance at 2251 cm^{-1} (weak) characteristic for a nitrile group along with strong absorption at 1745 cm^{-1} for ester carbonyl and four peaks at 1505–1601 for an aromatic ring. The ^{1}H NMR spectrum (Table 1) revealed signals for fully acetylated α-L-rhamnose and *p*-substituted benzene as observed in the case of **1–3** and **5–7**. However, in this case, the NH proton resonance of the carbamate or thiocarbamate group was not observed. Further, the protons of the benzylic methylene appeared as a singlet at δ3.53, also observed in other nitrile glycosides of this species [6, 19]. It is thus concluded that **9** is a 4-[(2′,3′,4′-tri-*O*-acetyl-α-L-rhamnosyloxy)benzyl] nitrile. Significant mass fragment ions in the HREI mass spectrum at m/z 273.0986 (d), 133.0508 (c), 117.0553 (b) and 107.0507 (C_7H_7O) confirmed the structure. The compound had earlier been obtained through acetylation of niazirin and niazirinin [6].

Compounds **1–9** have been isolated under natural conditions (autolysis) from an extract of the fresh leaves of the plant without the addition of myrosinase or ascorbic acid [20–22]. Thiocarbamate glycoside may be synthesized in the plant by the addition of methanol or ethanol to isothiocyanate present in the plant [4, 5]. The origin of nitrile glycosides can be conjectured through the degradation of glucosinolates which are versatile progenitors of organic cyanides and isothiocyanates [21, 22]. Biogenesis of carbamates may occur through the hydrolysis of thiocarbamates.

Intravenous administration of glycosides **5–8** caused a fall in systolic, diastolic and mean arterial blood pressure in normotensive anaesthetized rats. Compounds **1–4** and **9** were not tested due to their small quantity. The hypotensive responses of **5–8** were dose dependent and similar in magnitude. At 1 mg kg^{-1}, the fall in mean arterial blood pressure was 10–20% while the next higher dose (3 mg kg^{-1}) produced 30–40% hypotension. These hypotensive responses are similar to those observed for previously reported compounds from this species [4–7].

In conclusion, during the course of our investigation on the hypotensive constituents from *M. oleifera*, several glycosides have been isolated, of which thiocarbamates and carbamates were found to be the hypotensive principles of the plant [4–7]. The isolation of carbamates from nature is very rare and previously only two such compounds have been isolated [5]. All these glycosides contain rhamnose as the sugar residue, which is either nonacetylated, monoacetylated or triacetylated, while no diacetylated rhamnoside was obtained. Hitherto, thiocarbamates and nitriles were isolated as nonacetylated, as well as monoacetylated and triacetylated glycosides, while carbamates were obtained as monoacetylated and triacetylated glycosides, whereas in the case of isothiocyanates, no triacetylated glycoside was found. This is the first instance of the isolation of a large number of triacetylated rhamnosides from a single source. These triacetylated carbamate/thiocarbamate glycosides were obtained both in *E* and *Z* forms. It is important to note that acetylation of the nonacetylated or monoacetylated glycosides of the carbamates and thiocarbamates yielded only the *Z*-isomer of the triacetylated glycoside. Moreover, it is interesting from the point of chemical structure that the thiocarbamates (**5–8**) exist in two discrete forms (a and b), while carbamates **1–4** have only one form [5].

EXPERIMENTAL

General. UV: MeOH. IR: $CHCl_3$. EIMS 80 eV. FABMS: neg. ionization and pos. ionization: 8 kV. HRMS: 70 eV. ^{1}H and ^{13}C NMR 300 and 75 MHz, respectively, with DMSO-d_6 as solvent and spectra referenced to residual solvent signals. ^{13}C NMR spectral assignments have been made partly through DEPT and HMQC and partly through comparison with reported values of similar compounds [4–6, 19]. Unambiguous assignment of proton chemical shifts were made through COSY-45 and NOESY expts. The purity of compounds was checked on silica gel GF_{254} coated plates.

Plant material. Leaves of *Moringa oleifera* were collected from the Karachi region, in November 1990. The species was authenticated by Mr Abrar Hussain, Department of Botany, University of Karachi and the voucher specimen (No. 66250 KUH) is deposited in the same department.

Extraction and isolation. Fresh undried and uncrushed leaves (8 kg) were repeatedly extracted with EtOH at room temp. The 1st and 2nd extracts were combined and the solvent removed under red. pres. to give a residue possessing hypotensive activity. This on subjecting to classical isolation procedures gave a fr. M-80 (5.95 g) [4], which showed very promising hypotensive activity. It was subjected to prep. TLC (silica gel, $CHCl_3$–MeOH, 9:1), yielding 7 bands M-1, M-2a, M-2b, M-3, M-4, M-5 and M-6, of which M-2a, M-2b and M-4 showed single spots and possessed high hypotensive activity. However, spectral studies showed that they were mixts of more than 3 compounds. M-2b (140 mg) was subjected to HPLC [Shimadzu, LC-6A, C-18, techspher 50 DS, 30 cm × 10 mm, mobile phase 70% MeOH–H_2O, few drops of HOAc, loop 20 μl, flow rate 4 ml min^{-1}] to afford **9**

(4.76 mg), **4** (4.87 mg), **1** (3.57 mg), **5** (12.79 mg), **2** (4.27 mg), **6** (10.67 mg), **9** (6.07 mg), **3** (3.26 mg), niazicin B (8.5 mg) [4], **8** (9.86 mg) and niaziminin B (6.2 mg) [4] with R_fs 10 min 15 sec, 10 min 25 sec, 10 min 35 sec, 10 min 40 sec, 10 min 50 sec, 10 min 55 sec, 11 min 5 sec, 11 min 20 sec, 11 min 25 sec, 11 min 40 sec, 11 min 50 sec, respectively.

O-*Methyl*-4-[(2′,3′,4′-*tri*-O-*acetyl*-α-L-*rhamnosyloxy*)-*benzyl*]*carbamate* (E) (**1**). UV λ_{max} (MeOH) nm: 201.2, 221.6, 246.8. IR ν_{max} ($CHCl_3$)cm^{-1}: 1746, 1722, 1520–1615 (four peaks), 1365 and 1120. ^1H NMR in Table 1.

O-*Methyl*-4-[(2′,3′,4′-*tri*-O-*acetyl*-α-L-*rhamnosyloxy*)-*benzyl*]*carbamate* (Z) (**2**). UV λ_{max} (MeOH) nm: 200.4, 221.4, 247.6. IR ν_{max} ($CHCl_3$) cm^{-1}: 1745, 1724, 1620, 1365, 1120. ^1H NMR in Table 1.

O-*Ethyl*-4-[(2′,3′,4′-*tri*-O-*acetyl*-α-L-*rhamnosyloxy*)-*benzyl*]*carbamate* (E) (**3**). UV λ_{max} (MeOH) nm: 202.6, 221.6, 246.2. IR ν_{max} ($CHCl_3$) cm^{-1}: 2950, 1740, 1720, 1625, 1366, 1325, 1115. Negative FAB-MS *m/z*: 466 $[M-1]^+$. ^1H NMR in Table 1.

O-*Methyl*-4-[(4′-O-*acetyl*-α-L-*rhamnosyloxy*)*benzyl*]-*carbamate* (E) (**4**). UV λ_{max} (MeOH) nm: 199.8, 223.0, 270.6. ^1H NMR in Table 1.

O-*Methyl*-4-[2′,3′,4′-*tri*-O-*acetyl*-α-L-*rhamnosyloxy*)-*benzyl*]*thiocarbamate* (*E*) (**5**). UV λ_{max} (MeOH) nm: 201.2, 221.6, 246.8. IR ν_{max} ($CHCl_3$) cm^{-1}: 1742, 1605, 1490, 1366, 1126. ^1H and ^{13}C NMR in Tables 1 and 2.

O-*Methyl*-4-[(2′,3′,4′-*tri*-O-*acetyl*-α-L-*rhamnosyloxy*)*benzyl*]*thiocarbamate* (*Z*) (**6**). UV λ_{max} (MeOH) nm: 200.4, 221.1, 247.6. IR ν_{max} ($CHCl_3$) cm^{-1}: 1742, 1595, 1496, 1364, 1122. Negative FAB-MS *m/z*: 468 $[M-1]^+$. ^1H and ^{13}C NMR in Tables 1 and 2.

O-*Ethyl*-4-[(2′,3′,4′-*tri*-O-*acetyl*-α-L-*rhamnosyloxy*)*benzyl*]*thiocarbamate* (Z) (**7**). UV λ_{max} (MeOH) nm: 202.6, 221.4, 245.6. IR ν_{max} ($CHCl_3$) cm^{-1}: 2915, 1742, 1609, 1360, 1325, 1111. Negative FAB-MS *m/z*: 482 $[M-1]^+$. ^1H and ^{13}C NMR in Tables 1 and 2.

O-*Ethyl*-4-[α-L-*rhamnosyloxy*)*benzyl*]*thiocarbamate* (Z) (**8**). UV λ_{max} (MeOH) nm: 200.1, 223.4, 255.4. IR ν_{max} ($CHCl_3$) cm^{-1}: 3415, 1605, 1475, 1389, 1110. Negative FAB-MS *m/z*: 356 $[M-1]^+$. ^1H NMR in Table 1.

4-[(2′,3′,4′-*Tri*-O-*acetyl*-α-L-*rhamnosyloxy*)*benzyl*]*nitrile* (**9**). UV λ_{max} (MeOH) nm: 199.6, 220.0, 278.0. IR ν_{max} ($CHCl_3$) cm^{-1}: 2915, 2251, 1745, 1505–1601, 1360, 1125. ^1H NMR in Table 1.

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