# Isolation and Structure Elucidation of a Novel Glycoside Niazidin from the Pods of *Moringa oleifera*[1]

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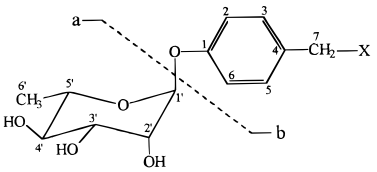
Studies on the EtOH extract of fresh pods of *Moringa oleifera* have resulted in the isolation of a novel glycoside niazidin (**1**) possessing an *O*-nitrile thiocarbamate group, along with thiocarbamate, carbamate, and isothiocyanate glycosides. Their structures have been determined through spectral studies, including appropriate 2D NMR experiments and chemical reaction. Fatty acid esters, long-chain hydrocarbons, carbamic acid, isocyanates, isothiocyanates, phenolic esters, nitriles, nitrile ester (**3**), polysulfide sulfinate (**4**), and a benzyl thiocarbamate (**5**), along with elemental sulfur ($S_8$), have also been identified through GC−MS.

*Moringa oleifera* Lam. (Syn. *Moringa pterygosperma* Gaertn.) belongs to the monogeneric family Moringaceae. It is widely cultivated in the tropics for its edible fruits.[2] Its fried seeds taste like peanuts and are also used externally for the treatment of rheumatism and gout.[2−4] Seeds are also traditionally used in Asia and Africa for water purification because of their strong coagulation and antibacterial properties.[5,6] All parts of the plant have medicinal importance and are used in folk medicine for the treatment of various ailments,[2−6] particularly for lowering blood pressure.[2,7] Based on these facts, systematic work was initiated in 1989 on its pods and leaves, which resulted in the isolation of thiocarbamate,[8−11] carbamate,[9,11] isothiocyanate,[10] and nitrile glycosides[10] from the fresh leaves. Except for 4-(α-L-rhamnosyloxy)benzenecarbonitrile and its 4′-*O*-acetyl derivative, these compounds are the hypotensive principles of the leaves.[8−11]

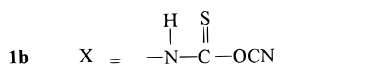
In a continuation of these studies, work on the pods has afforded a novel glycoside niazidin along with thiocarbamate[8,11] and isothiocyanate glycosides.[6,10] The structure of niazidin has been elucidated as (*E*)-*O*-cyano 4-(α-L-rhamnosyloxy)benzenethiocarbamate (**1**) through extensive spectroscopic studies including 2D NMR experiments and chemical reaction. Moreover, various long-chain esters, nitriles, isothiocyanate, isocyanates, carbamic acid, hydrocarbon, nitrile ester (**3**), phenolic esters, thiocarbamate (**5**), and polysulfide (**4**), along with elemental sulfur ($S_8$), have also been identified for the first time from this plant through GC−MS. Most of these compounds are new natural products.

The EtOH extract of *M. oleifera* pods was subjected to classical method of fractionation proceeded through solvent separation, which afforded the petroleum ether insoluble neutral (NPEI) fraction (see Experimental Section). This, on subjecting to vacuum liquid chromatography (VLC) followed by TLC, gave a novel glycoside, niazidin (**1**).

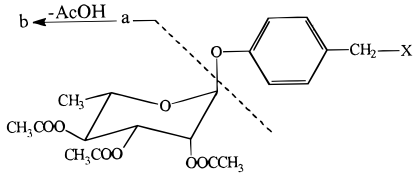
The EIMS of niazidin (**1**) showed a very weak molecular ion peak at *m*/*z* 354, while its FABMS (positive) showed a peak at *m*/*z* 355 ($MH^+$). The molecular formula $C_{15}H_{18}N_2O_6S$ was determined through integration of $^1$H-NMR spectrum (Table 1), $^{13}$C-NMR data (broad band and DEPT) (Table 1), and HREIMS, which showed diagnostic ions at *m*/*z* 147.0649 and *m*/*z* 207.0247



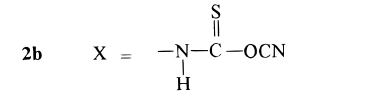
**1a** X = −N⁺=C(H)−S⁻−OCN



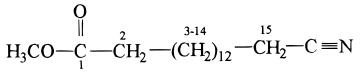
**1b** X = −N(H)−C(=S)−OCN



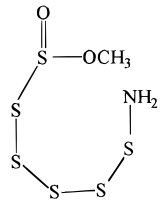
**2a** X = −N⁺(H)=C(S⁻)−OCN



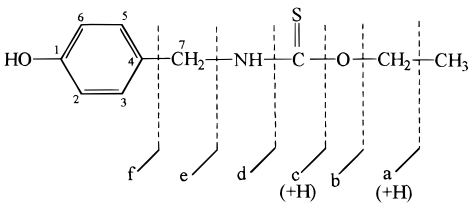
**2b** X = −N(H)−C(=S)−OCN



**3**



**4**



**5**

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**Table 1.** $^1$H- and $^{13}$C-NMR Chemical Shifts ($\delta$) for Compound **1** in $(CD_3)_2SO$

| assignment | $\delta^1$H | $\delta^{13}$C |
|---|---|---|
| 1 | | 155.14 |
| | | 154.94 |
| 2, 6 | 6.95 d (8.5) | 116.36 |
| 3, 5 | 7.19 d (8.5) | 128.61 |
| | | 128.31 |
| 4 | | 132.66 |
| 7 | 4.58 d (5.0) | 46.48 |
| | 4.16 d (5.2) | 42.28 |
| 8 | | 182.88 |
| | | 182.90 |
| 9 | | 110.05 |
| | | 110.06 |
| 1′ | 5.31 d (1.6) | 98.53 |
| 2′ | 3.80 dd (3.3, 1.6) | 70.22 |
| 3′ | 3.62 dd (9.2, 3.3) | 70.50 |
| 4′ | 3.26 t (9.2) | 71.88 |
| 5′ | 3.46 qd (9.2, 6.2) | 69.42 |
| 6′ | 1.08 d (6.2) | 17.89 |
| OH | 4.84, 5.01 br m | |
| NH | 7.95 t (5.0) | |
| | 6.40 t (5.2) | |

corresponding to the fragments a ($C_6H_{11}O_4$) and b ($C_9H_7N_2O_2S$), respectively.

The structural features of **1** were evident from the 300 MHz $^1$H-NMR spectrum in $(CD_3)_2SO$ (Table 1), which showed the presence of a sugar moiety by a one-proton doublet at $\delta$ 5.31 ($J_{1',2'}$ 1.6 Hz, H-1′), a pair of one-proton doublet of doublets at $\delta$ 3.80 ($J_{2',3'}$ 3.3 Hz; $J_{2',1'}$ 1.6 Hz, H-2′), 3.62 ($J_{3',4'}$ 9.2 Hz; $J_{3',2'}$ 3.3 Hz, H-3′), a one-proton triplet at $\delta$ 3.26 ($J_{4',3'}$ 9.2 Hz; $J_{4',5'}$ 9.2 Hz, H-4′), a one-proton doublet of quartet at $\delta$ 3.46 ($J_{5',6'}$ 6.2 Hz; $J_{5',4'}$ 9.2 Hz, H-5′), and a three-proton doublet at $\delta$ 1.08 ($J_{6',5'}$ 6.2 Hz, H-6′). The chemical shifts and coupling constants of the anomeric proton (H-1′) showed that the sugar is linked with the aglycon molecule by an α-glycosidic linkage. These values are comparable with those of α-L-rhamnose and suggested that the compound is an α-L-rhamonoside.[6,8] An exact assignment of these protons was made through $^1$H−$^1$H COSY-45 measurements. The hydroxyl groups of rhamnose were observed at $\delta$ 5.01 and 4.84 as broad signals in the $^1$H-NMR spectrum, which disappeared on shaking with $D_2O$. The $^{13}$C-NMR spectrum (Table 1) showed 15 signals of carbons that were identified as one methyl (C-Me), one methylene, five methines, four sp$^2$ CH, three sp$^2$ quaternary, and one sp quaternary carbon(s). The assignment of $^{13}$C-NMR chemical shifts is based on a $^1$H−$^{13}$C hetero COSY experiment, which also evaluates the sugar moiety as α-L-rhamnose.[8] In the $^1$H-NMR spectrum, two one-proton mutually coupled doublets at $\delta$ 7.19 ($J$ = 8.5 Hz) and 6.95 ($J$ = 8.5 Hz) showed the presence of a para-substituted benzene ring in the molecule, which was supported by the correlation of these protons with CH carbons at $\delta_C$ 128.61 and 116.36, respectively, in the hetero COSY plot. Thus, five of the eight unsaturation degrees implied by the molecular formula were accounted for by the sugar and the phenyl rings. The site of glycosidic linkage with the aromatic ring was inferred from the relatively downfield shift of the anomeric proton[8−11] as compared to its chemical shift when it is linked with saturated carbons.[12] This was supported by the 2D NOESY spectrum, in which H-1′ has through-space connectivity with H-2′ and aromatic protons H-2, 6.

Furthermore, a doublet at $\delta$ 4.58 ($J$ = 5.0 Hz, H-7) in the $^1$H-NMR spectrum correlated with the carbon resonance at $\delta$ 46.48 in the hetero COSY, indicating the presence of a benzylic methylene in the structure. This methylene is in turn attached with NH as indicated by the triplet of NH protons at $\delta$ 7.95 ($J$ = 5.0 Hz). On shaking with $D_2O$, this triplet disappeared, while doublet of methylene protons changed to a singlet. A cross peak in the COSY-45 plot, showing through-bond connectivity of $\delta$ 4.58 with 7.95, confirmed the relationship. That the NH is linked with the benzylic methylene was also supported by the diagnostic peaks at $m/z$ 253 [($M^+$ + 1) − $C_2H_2N_2OS$] and $m/z$ 270 [($M^+$ + 1) − $C_2HN_2S$] in the chemical ionization mass spectrum (CIMS) and $m/z$ 269 in the EIMS. The NOESY plot showed spacial proximity of H-7 with H-3, H-5. An ion at $m/z$ 311 ($M^+$ − HOCN) in the EIMS and a peak at $\delta$ 182.88 for the quaternary carbon in the $^{13}$C-NMR spectrum implied the presence of thiocarbonyl group in the molecule. These data are comparable with the values reported for the thiocarbamates, isolated earlier from *M. oleifera* leaves.[8,9,11] However, signals for the methoxy or ethoxy group of the thiocarbamate moiety were missing in the NMR spectra of niazidin, and, instead, a cyanato (OCN) function was indicated by the mass fragments at $m/z$ 311 in the EIMS and $m/z$ 312 [($M^+$ + 1) − HOCN] in the FABMS (positive). A peak at $\delta$ 110.05 in the broad band $^{13}$C-NMR spectrum of **1** strengthened this argument. The chemical shift ($\delta$ 110.05) is comparable to the reported values for cyanato compounds.[13] The presence of cyanoxythiocarbamate group justified the remaining three double bonds in the molecule. Moreover, the chemical shift of the NH proton ($\delta$ 7.95, t, $J$ = 5.0 Hz) revealed that this is cis to sulfur, and the molecule as a whole has a trans (*E*) stereochemistry as observed earlier in case of thiocarbamates.[8,9,11] In light of the above discussion, the structure of niazidin has been elucidated as (*E*)-*O*-cyano 4-(α-L-rhamnosyloxy)-benzenethiocarbamate (**1**), which was corroborated by various mass fragments (see Experimental Section) and the base peak at $m/z$ 107.0499 ($C_7H_7O$) in the HREIMS.

Prolonged refluxing with EtOH produced no effect on **1**, further confirming the presence of a cyanate function instead of an isocyanato group, which is more reactive to addition than cyanate.[14] In conformity to structure **1**, niazidin yielded the tri-*O*-acetyl derivative **2** ($Ac_2O$−pyridine, room temperature). Compound **2** showed no molecular ion peak in the EIMS; however, it has a [($M^+$ + 1) − 15] peak in the CIMS at $m/z$ 466 and a strong absorption at 1740 cm$^{-1}$ in the IR spectrum for the acetate carbonyls. The $^1$H-NMR spectrum recorded in $CDCl_3$ (Table 2) has three three-proton sharp singlets at $\delta$ 2.01, 2.03, and 2.17 for the acetoxy methyls instead of the signals of the hydroxyl groups and showed the downfield shifts for the signals of H-2′, H-3′ and H-4′. The $^{13}$C-NMR spectrum (Table 2) along with $^1$H−$^1$H COSY, 2D NOESY, and hetero COSY experiments supported the structure, which was corroborated by important fragments in the HREIMS at $m/z$ 273.0980 (fragment a) and 213 (fragment b), showing the presence of a triacetylated rhamnose in the molecule.

It is important to note that niazidin (**1**) in DMSO solution exists in two discrete tautomeric forms **1a** and **1b** in a ratio of 9:1, which was inferred by the presence of double signals for the benzyl group in the $^1$H- and $^{13}$C-NMR spectra and double signals for the carbon of

**Table 2.** $^1$H- and $^{13}$C-NMR Chemical Shifts ($\delta$) for Compound **2** in $CDCl_3$

| assignment | $\delta^1$H | $\delta^{13}$C |
|---|---|---|
| 1 | | 155.37 |
| | | 155.61 |
| 2, 6 | 7.03 (8.7) | 116.96 |
| | | 116.83 |
| 3, 5 | 7.21 d (8.7) | 129.40 |
| | | 129.17 |
| 4 | | 131.31 |
| | | 130.81 |
| 7 | 4.58 d (4.6) | 44.18 |
| | 4.82 d (4.8) | 42.20 |
| 8 | | 184.62 |
| 9 | | 112.00 |
| 1′ | 5.42 d (1.8) | 95.94 |
| 2′ | 5.42 m | 69.82 |
| 3′ | 5.48 dd (3.5, 9.9) | 69.01 |
| 4′ | 5.13 t (9.9) | 71.11 |
| 5′ | 3.96 qd (6.2, 9.9) | 67.29 |
| 6′ | 1.18 d (6.2) | 17.44 |
| NH | 5.96 m | |
| OCO*CH*$_3$ | 2.17, 2.03 | 20.79 |
| | 2.01 s | 20.67 |
| | | 20.64 |
| O*CO*CH$_3$ | | 169.91 |

the nitrile group in the $^{13}$C-NMR spectrum (Table 1). The downfield shift $\delta$ 4.58 has been attributed to the H-7 of the tautomer **1a**, and its larger integration implied that it is the major form that has also been observed earlier in the case of thioamides[15] and the thiocarbamates.[8,9,11] The protons at C-7 of **1a** ($\delta$ 4.58 d) and **1b** ($\delta$ 4.16 d) were related to $\delta_C$ 46.48 and 42.28, respectively, in the hetero COSY spectrum, and to $\delta$ 7.95 (t, 5.0 Hz, NH) and 6.40 (t, 5.2 Hz, NH) in the $^1$H−$^1$H COSY plot. It is important to note in this regard that in the case of niazidin (**1**) the NH protons of tautomer **1a** and **1b** resonate at different chemical shifts ($\delta$ 7.95 and 6.40) in contrast to the NH of thiocarbamates, which has the identical resonance for both the tautomers **a** and **b**.

The $^1$H-NMR data showed that the acetyl derivative **2** also exists in two forms, **2a** and **2b**, in a ratio of 1:2, and the chemical shift ($\delta$ 4.58) for the form **2b** has larger integration than the downfield shift ($\delta$ 4.82) for the form **2a**. Moreover, the tautomeric forms of niazidin (**1**) and its acetyl derivative **2** are observed in DMSO solution on the NMR time scale and may or may not be the forms in solid. It is worth mentioning that in the $^1$H-NMR spectrum of **2** recorded in $(CD_3)_2SO$ (300 MHz), the NH proton appeared upfield at $\delta$ 4.64 (t, 5.5 Hz) as compared to that of the parent compound (**1**) in which it resonated at $\delta$ 7.95 and 6.40. Thus in **2**, the NH and sulfur are trans and the molecule as a whole has a cis (*Z*) stereochemistry. This showed that during acetylation the *E* isomer, has changed into *Z* isomer, as observed earlier in the case of thiocarbamate[8,9,11] and carbamate glycosides.[9,11]

The formation of **1** may be envisaged through the addition of the elements of cyanic acid (HOCN)[16] to an isothiocyanate glycoside present in the plant.[6,10] This is the first report of the isolation of a natural product with a cyanato group.

The initial nonpolar VLC fractions N-1 and N-5 (see Experimental Section) possessed a strong "sulfur" odor reminiscent of the sulfur-containing volatile compounds of *Allium* species.[17] Indeed, the GC−MS and HRMS of N-1 showed that elemental sulfur is preponderant among its constituents and exists in the molecular form $S_8$ ($M^+$ *m/z* 256). The $M^+ + 2$ peak confirmed the presence of eight sulfur atoms in the molecule, and each ion in the mass spectrum has the +2 peak, the percentage of which varied depending on the number of sulfur atoms present in the fragment.[18] The mass spectrum is identical to that of $S_8$ in the mass spectral data base.[19] There are several reports of isolation of sulfur from vegetables[20] and grapes;[21] however, its isolation in the molecular form, $S_8$, which is relatively more stable than other forms of molecular sulfur,[22] rarely occurs in the plant kingdom.[23] Moreover, detection of sulfur in various cruciferous plants has been utilized as a method for the identification of glucosinolates in various parts of the plants.[24−26]

Other components of the fraction N-1 were tentatively identified as heptadecane isocyanate[27] ($M^+$ *m/z* 281; 239), octadecane isocyanate[28] ($M^+$ *m/z* 295; 253), tricosane isothiocyanate ($M^+$ *m/z* 381; 323), and henicosane carbamic acid ($M^+$ *m/z* 355, 337, 295). The uniform loss of 14 amu between a number of ion peaks in their mass spectra showed the presence of a long aliphatic chain in these compounds, which are new natural products. The preparation of isocyanates is reported in literature.[26,27]

GC−MS, HREIMS, and $^1$H-NMR spectra of fraction N-5 revealed that its main components are phenolic esters provisionally identified as methyl *p*-hydroxy benzoate[29] ($M^+$ *m/z* 152; base peak *m/z* 121) and propyl *p*-hydroxy benzoate[30] ($M^+$ *m/z* 180, base peak *m/z* 121). The methyl ester has been isolated from a number of plants, while there is only one report for detection of the propyl ester.[30] Other compounds of the fraction are *O*-ethyl henicosenoate ($M^+$ *m/z* 352; base peak *m/z* 88) in which the location of double bond is not certain and 4,5-dimethyl decane ($M^+$ *m/z* 170; 127, 99), which is a new natural product. GC−MS, HRMS, and $^1$H-NMR spectra of the acetylated product ($Ac_2O$−pyridine, room temperature, overnight) of N-5 showed the presence of methyl *p*-acetoxy benzoate ($M^+$ *m/z* 194), propyl *p*-acetoxy benzoate ($M^+$ *m/z* 222), and *p*-acetoxy phenyl acetonitrile ($M^+$ *m/z* 175; base peak *m/z* 133) confirming the structures of the ester of N-5 and supported the presence of *p*-hydroxy phenyl acetonitrile in pods, which has earlier been isolated from the roasted and raw seeds of *M. oleifera*.[4]

The components of N-16, as characterized by its HREIMS, $^1$H-NMR, $^{13}$C-NMR, and 2D NMR spectra, are niazinin A, niazinin B, niazimicin A, and 4-(α-L-rhamnosyloxy)benzeneisothiocyanate[6] isolated earlier from *M. oleifera*.[6,8,10] These compounds exist in a ratio of 1:1:1:3 as revealed by the proton integrals. It is important to note that N-16 showed a single spot on TLC that exactly corresponds to M-4 band of *Moringa* leaves. In addition to these compounds, however, M-4 also contains 4-(α-L-rhamnosyloxy)benzenenitrile.[10]

On subjecting it to TLC, N-9 resolved into four bands, two minor ones (9A, 9B) and two major ones (9C and 9D). The latter two exactly match with the M-2a and M-2b bands of *Moringa* leaves.[8,9,11] However, in the present case 9C is mainly a mixture of niaziminin A and niaziminin B,[8] as revealed by the spectral data, while HPLC, MS and 2D NMR data of 9D showed that it is pure (*E*)-*O*-methyl 4-(2′,3′,4′-tri-*O*-acetyl-α-L-rhamnosyloxy)benzenethiocarbamate, isolated from the leaves

along with several other glycosides.[11] GC−MS of band 9A showed the presence of a hydrocarbon, tricosane ($M^+$ *m/z* 324) isolated from a number of plants, for example, *Brassica campestris*,[31] a cyanoester, methyl 15-cyanopentadecanoate (**3**), and a major compound, methyl 1-aminopentasulfide 5-sulfinate (**4**). Review of literature revealed that natural products **3** and **4** are new compounds.

Compound **3** showed a molecular ion peak at *m/z* 281 in GC−MS, indicating the presence of a nitrogen atom in the molecule. The $M^+-26$ ion at *m/z* 255 suggested the presence of a cyano group in the molecule, and its location at one end of the hydrocarbon chain was supported by the appearance of a number of ion peaks at the regular intervals of 14 amu. A base peak at *m/z* 74, characteristic of methyl esters, and a peak at *m/z* 224 $\{M^+-(26 + 31)\}$ established the other end of hydrocarbon chain. These mass fragments were comparable to those of straight chain nitriles and methyl esters of saturated fatty acids.[19] The $^1$H-NMR spectrum of 9A in $CDCl_3$ at 300 MHz displayed signals for this minor compound. Two triplets resonating at $\delta$ 2.34 ($J$ = 7.6 Hz) and 2.27 ($J$ = 7.5 Hz) were attributed to two methylene protons (H-15 and H-2) adjacent to cyano and ester groups. A singlet at $\delta$ 3.47 was ascribed to the methyl protons of the ester and two multiplets at $\delta$ 1.58 and 1.28 were due to the methylene protons (C-3−C-14) of straight-chain hydrocarbons. Hence, the structure of compound **3** has been derived as methyl 15-cyanopentadecanoate.

The MS of compound **4** showed the molecular ion peak at *m/z* 255, which is also the base peak and has 36% $M^+ + 2$ peak at *m/z* 257, indicating the presence of six sulfur atoms,[18] while the odd number of molecular ion peak revealed the presence of a nitrogen atom in the molecule. It also displayed diagnostic ion peaks at *m/z* 224, 208, and 207 corresponding to the loss of methoxy; methoxy + amino, and methoxy + hydroxy groups, respectively, from the molecular ion peak. Strong peaks at *m/z* 192 ($S_6$), 160 ($S_5$), 128 ($S_4$), 96 ($S_3$), and 64 ($S_2$) each accompanied with the ion + 2 peak confirmed the structure **4**. The abundance of ion at *m/z* 194 is approximately 22% of ion peak at *m/z* 192, indicating the presence of six sulfur atoms in the fragment. Polysulfides[17,32] and sulfinates[17] are not very common in Nature and are mainly found in *Allium* species.[17]

The major compounds of fraction 9B identified through GC−MS, HREIMS, and $^1$H-NMR spectral data are methyl *p*-hydroxybenzoate and propyl *p*-hydroxybenzoate, while the minor ones are methyl hexadecanoate ($M^+$ *m/z* 270, base peak *m/z* 74), isolated from various plants,[31,33] and a new compound *O*-ethyl *p*-hydroxy benzyl thiocarbamate (**5**).

The HREIMS of **5** showed $M^+$ peak at *m/z* 211.0712, corresponding to molecular formula $C_{10}H_{13}NO_2S$, with significant mass fragments (see structure) at *m/z* 197.1539 (fragment a, $C_9H_{11}NO_2S$), 182.0280 (fragment b, $C_8H_8NO_2S$), and 107.0702 (fragment e, $C_7H_7O$). The mass fragment at *m/z* 197 and chemical shift pattern in the $^1$H-NMR spectrum ($CDCl_3$, 400 MHz) showed that it is a benzyl thiocarbamate. The $^1$H-NMR spectrum showed a pair of doublets ($J$ = 8.4 Hz) at $\delta$ 6.99 (H-2, 6) and 7.22 (H-3, 5) for *p*-substituted benzene ring, while a doublet at $\delta$ 4.64 ($J$ = 5.7 Hz, H-7) was attributed to the methylene protons of benzyl moiety. A quartet at $\delta$ 4.51 ($J$ = 7.1 Hz, $OCH_2CH_3$) and a multiplet at $\delta$ 1.24 ($OCH_2CH_3$) were assigned to the ethyl group of the thiocarbamate moiety, while the NH proton was not observed. On shaking with $D_2O$, the doublet of benzylic methylene at $\delta$ 4.64 was converted into a singlet, indicating that both benzylic methylene and NH are adjacent to each other. All these values are similar to those observed earlier in the case of aglycon part of niaziminin A and B and niazimicin A and B,[8,9,11] isolated earlier from leaves of this plant. In light of the foregoing data, structure of **5** has been elucidated as *O*-ethyl *p*-hydroxy benzyl thiocarbamate without specification of stereochemistry, as resonance for NH was not observed in the $^1$H-NMR spectrum.

## Experimental Section

**General Experimental Procedures.** $^1$H- and $^{13}$C-NMR spectra were obtained using a Bruker Aspect AM-300 spectrometer operating at 300 and 75 MHz, respectively. For $^{13}$C-NMR spectra, broad band and DEPT experiments were employed to distinguish multiplicity. All chemical shifts are reported with respect to residual solvent signals. The EIMS, FDMS, FABMS (positive) and HRMS were recorded on Finnigan MAT-112, MAT-312, and JMS HX-110 analyzers. UV (in MeOH) and IR (in $CHCl_3$) spectra were obtained from Hitachi-U-3200 and JASCO-A 302 spectrophotometers, respectively.

The GC analyses were performed on a gas chromatograph GC-9A (Shimadzu) equipped with FID detector. Temperature programing was from 150−250 °C at 5 °C/min with 5-min initial and final hold time. Nitrogen was used as a carrier gas, with 30 mL/min flow rate. Temperature of detector and injector was 300 °C.

For the GC−MS analysis a JMS HX-110 JEOL MS instrument, connected to HP-5890 gas chromatograph filled with a 30-m SPB-1 (fused silica) column was used. Temperature programing was from 70−250 °C at 5 °C/min. EI energy emission current and ion temperature of the mass spectrometer were 70 eV, 300 mA, and 250°, respectively. Helium was used as a carrier gas at a flow of 48 mL/min. The purity of compounds was checked on Si gel $GF_{254}$ coated plates. Light petroleum refers to the fraction boiling in the range 66−70 °C.

**Plant Material.** Pods of *M. oleifera* were collected from the Karachi region, in December 1989. The plant was authenticated at the Department of Botany, University of Karachi, and a voucher specimen (no. 66250 KUH) is on file with the herbarium of the department.

**Extraction and Isolation.** Fresh and undried pods (36 kg) of *M. oleifera* were cut into 2-inch pieces and were extracted six times with EtOH at room temperature. The extracts were combined together and their solvent removed under reduced pressure to give a thickish mass possessing hypotensive activity. It was partitioned between $H_2O$ and EtOAc, and, as the activity accumulated in the EtOAc phase, it was treated with 4% $Na_2CO_3$ to separate the neutral from the acidic fraction. On usual work up and charcoaling of the neutral EtOAc phase, a residue (71.1 g, 1.05%) was obtained possessing the hypotensive activity. It was divided into light petroleum ether-soluble (34.5 g, 0.51%) and -insoluble (NPEI, 36.1 g, 0.53%) portions. The latter fraction, showing numerous spots on TLC, was subjected to VLC[34] (light petroleum ether, petroleum

ether−EtOAc, EtOAc−MeOH, MeOH), which afforded a number of fractions. On the basis of TLC, these fractions were combined together to give five main fractions, N-1 (0.97 g, 0.014%), N-5 (1.21 g, 0.018%), N-9 (1.67 g, 0.024%), N-16 (5.67 g, 0.084%), and N-71 (1.25 g, 0.019%). All percentages are with respect to dry weight of pods.

Preparative TLC (Si gel, $CHCl_3$−MeOH, 9:1) of N-9 (67 mg) afforded two major bands 9C (15.71 mg) and 9D (17.21 mg) and two minor bands 9A (4.23 mg) and 9B (5.57 mg). Band 9C was found to be the mixture of niaziminins A and B, while 9D was pure (*E*)-*O*-methyl 4-(2′,3′,4′-tri-*O*-acetyl-α-L-rhamnosyloxy)benzenethiocarbamate. 9A band was composed of tricosane, methyl 15-cyanopentadecanoate (**3**), and methyl 1-aminopentasulfide-5-sulfinate (**4**), while methyl *p*-hydroxybenzoate, propyl *p*-hydroxybenzoate, methyl hexadecanoate, and a new compound, *O*-ethyl *p*-hydroxy benzyl thiocarbamate (**5**), were the constituents of band 9B.

Fraction N-16 (single spot) consists of niazinin A, niazinin B, niazimicin A,[8] and 4-(α-L-rhamnosyloxy)-benzeneisothiocyanate[6,10] in a ratio of 1:1:1:3. On subjecting it to preparative TLC (Si gel, $CHCl_3$−MeOH, 8:2), fraction N-71 (296 mg) gave pure niazidin (**1**) in amorphous form (205 mg).

**Niazidin (1):** UV (MeOH) $\lambda_{max}$ 200.8, 226.8, 242.4 nm; IR ($CHCl_3$) $\nu_{max}$ 3400−3420, 1508−1602, 1120 $cm^{-1}$; FABMS (pos) *m/z* 355 ($MH^+$, 5), 329 (8); CIMS *m/z* 311 $[MH - 42 - 2H]^+$, 296 $[MH - 42 - 17]^+$, 253 $[MH - 102]^+$; EIMS *m/z* (%) 354 $[M]^+$ (1), 336 $[M - 18]^+$ (1), 311 (1), 207.0247 [fragment b, $C_9H_7N_2O_2S$] (6), 205 (3), 165 (6), 147.0649 [fragment a, $C_6H_{11}O_4$] (18), 146 (23), 123 (43), 107.0499 [$C_7H_7O$] (100); $^1$H- and $^{13}$C-NMR data, see Table 1.

**Acetylation of niazidin (1):** $Ac_2O$ (5 mL) added to a solution of **1** (75 mg) in pyridine (5 mL); reaction mixture kept at room temperature for three days; work up of the reaction mixture yielded triacetyl derivative **2** (47.32 mg); UV (MeOH) $\lambda_{max}$ 201.3, 222.6, 243.6 nm; IR (MeOH) $\nu_{max}$ 3415, 2920, 1740, 1503−1600, 1365, 1235, 1010 $cm^{-1}$; FABMS (pos) *m/z* 481 $[MH]^+$ (3), 455 (4), 439 (5), 421 (6), 395 (4); CIMS *m/z* 466 (4), 438 (12), 396 (2), 378 (4), 273 (100), 231 (6), 213 (12), 171 (4), 153 (10), 111 (4); EIMS *m/z* (%) 394 $[M - 86]^+$ (1), 273.0980 [fragment a, $C_{12}H_{17}O_7$] (41), 231 (4), 213 [fragment b, $C_{10}H_{15}O_5$] (12), 189 (3), 171 (20), 153 (68), 122 (8), 111 (100), 107 (19).

**Elemental sulfur ($S_8$):** GC−MS *m/z* (rel int) 258 $[M + 2]^+$ (8), 255.7228 $[M]^+$ (23), 226 (1), 224 [$S_7$] (3), 194 (4), 192 [$S_6$] (13), 162 (5), 160 [$S_5$] (20), 130 (4), 128 [$S_4$] (28), 98 (3), 96 [$S_3$] (15), 66 (9), 64 [$S_2$] (100).

**Methyl-15-cyanopentadecanoate (3):** GC−MS *m/z* (rel int) 281 $[M]^+$ (1), 255 (1), 227 (3), 224 (2), 185 (2), 171 (2), 143 (10), 129 (4), 87 (55), 74 (100), 59 (5).

**Methyl 1-aminopentasulfide 5-sulfinate (4):** GC−MS *m/z* (rel int) 257 $[M + 2]^+$ (36), 255 $[M]^+$ (100), 226 (3), 224 $[M - OCH_3]^+$ (5), 209 (1), 207 $[M - OCH_3 - OH]^+$ (4), 208 $[M - OCH_3 - NH_2]^+$ (1), 194 (7), 192 [$S_6$] (33), 162 (13), 160 [$S_5$] (60), 130 (8), 128 [$S_4$] (55), 98 (4), 96 [$S_3$] (24), 66 (8), 64 [$S_2$] (76).

***O*-Ethyl *p*-hydroxybenzenethiocarbamate (5):** GC−MS *m/z* (rel int) 211.0712 [M, $C_{10}H_{13}NO_2S]^+$ (5), 197.1539 [$C_9H_{11}NO_2S$] (45), 182.0280 [$C_8H_8NO_2S$] (30) 166 (21), 122.0586 [$C_7H_8NO$] (91), 107.0702 [$C_7H_7O$] (98), 93 (40).

## References and Notes


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