Bull. Chem. Soc. Ethiop. **2000**, 14(1), 51-55.
Printed in Ethiopia
ISSN 1011-3294

CHEMICAL INVESTIGATION OF THE LEAVES OF *MORINGA STENOPETALA*

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(Received April 17, 2000; revised May 23, 2000)

ABSTARACT. The ethanol extract of the leaves of *Moringa stenopetala* led to the isolation of three glycosides. The compounds were identified as rutin, 4-(4'-O-acetyl-α-L-rhamnosyloxy)-benzylisothiocyanate and 4-(4'-O-acetyl-α-L-rhamnosyloxy)-benzaldehyde. The last two compounds are reported for the first time from *M. stenopetala*. Structure determination was accomplished by means of spectroscopic and chemical studies.

INTRODUCTION

Moringa stenopetala, family Moringaceae, is a 6 to 10 m tall tree characterized by brittle branches, smooth grey bark and softwood. It is one of the five *Moringa* species that grows in Ethiopia and is widely distributed in the southern part of Ethiopia, northern Kenya and eastern Somalia. It is cultivated traditionally as cabbage tree and planted occasionally as an ornamental tree at altitudes between 1,000 and 1,800 m where the temperature range is 20-30 °C [1-3].

M. stenopetala is widely used in Ethiopian folk medicine for the control of diabetes, malaria, hypertension and stomach problems [4]. The seeds are used traditionally for clarifying water and are more effective than *M. oleifera* seeds. Apart from daily use as a vegetable, it is also marketed as a source of cash income in local markets [5-9].

Very few attempts have been made to isolate and characterize the components from the seeds and leaves of *M. stenopetala* of Ethiopia. Studies of the plant's chemical components are limited to the crude extracts of the different parts of the plant [2, 3, 5-7]. In the present work the fresh leaf extracts of the plant were subjected to a systematic phytochemical isolation procedure and the isolated compounds characterized.

RESULTS AND DISCUSSION

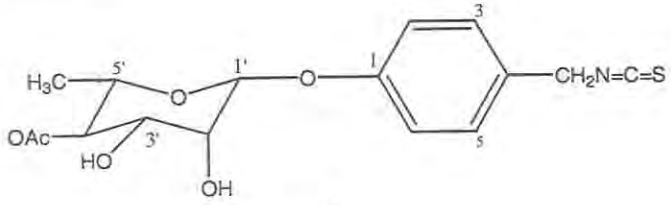
The polar fractions of the EtOH extract of the leaves of *M. stenopetala* were subjected to column chromatography on silica gel and Sephadex LH-20 and prep. TLC to yield compounds **1**, **2** and **3**.

Compound **1** is a light yellow compound whose chromatogram changes to brown with $AgNO_3$ reagent. The IR spectrum showed a strong and broad absorption at 2173 and 2091 cm^{-1} which are unique and characteristic of the N=C=S group. The presence of an acetate and an aromatic group was apparent from the IR signals at 1715, 1611 and 1510 cm^{-1}. The UV (MeOH) absorption maximum at 225 nm is most likely due to the presence of the NCS group. The ^1H NMR spectrum exhibited an anomeric proton signal at 5.50 ppm (*d*, J = 1.6 Hz). The magnitude of the coupling constant of the anomeric proton revealed the α-configuration of the sugar moiety. It also showed a signal due to an acetyl group at 2.1 (*s*) ppm and four methine

protons at 4.05 (*m*), 4.03 (*m*), 5.06 (*t*) and 3.8 (*m*) ppm, while a three proton doublet at 1.10 ppm was due to the C-Me of a rhamnosyl group.

The fragment ion at *m/z* 107 ($C_7H_6O^+$) in the EIMS indicated the presence of a *p*-benzyloxy group in the molecule. The aromatic ring shows a pair of two protons (*d*) at 7.11 and 7.34 ppm which is attributable to the mutually coupled protons, H-2, H-6 and H-3, H-5, respectively. The two proton singlet at 4.7 ppm is attributable to the CH_2 protons. Diagnostic mass fragments were observed at *m/z* 107 ($HOC_6H_5CH_2^+$) and at *m/z* 189 ($M\text{-}OC_6H_5CH_2NCS)^+$ in the EIMS; the latter confirmed the presence of monoacetylated rhamnose in the molecule.

Acetylation of compound **1** gave a compound with R_f value greater than that of compound **1** which confirms the presence of unacetylated groups. Compound **1** displays IR, ^{1}H NMR and EIMS data that are in agreement with those reported for 4-(4'-O-acetyl-α-L-rhamnosyloxy)benzylisothiocyanate [8, 10-14].



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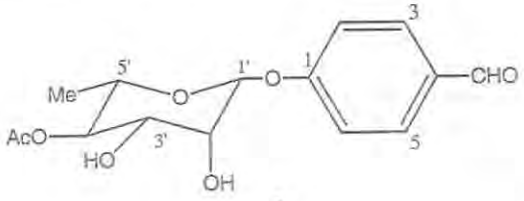
Compound **2** is a light yellow compound. Its chromatogram changed to deep yellow when treated with $AgNO_3$ reagent. The IR spectrum showed absorption at 1617 and 1734 cm^{-1}, characteristic of a conjugated ketone or aldehyde and ester carbonyl functions, respectively. The aldehydic nature of compound **2** was deduced from the appearance of a sharp singlet at 9.95 ppm in its ^{1}H NMR spectrum and the peak at 2852 cm^{-1} in its IR spectrum. Signals at 7.20 (*d*, H-2, H-6) and 7.85 (*d*, H-3, H-5) ppm in the ^{1}H NMR spectrum were characteristic of two proton doublets of *p*-substituted benzaldehyde. The signal for the anomeric proton of the sugar appeared at 5.58 (*d*) ppm and those for H-2', H-3' and H-5' at 4.2, 4.11 and 3.60 ppm (each 1H, *m*), respectively. A signal at 1.20 (*d*) ppm was assigned to the CH_3 group of the rhamnose. Signals at 4.86 (*t*) and 2.16(*s*) ppm were suggestive of a 4'-O-acetyl-α-L-rhamnosyloxy group in the molecule.

The assignment was supported by a significant peak at *m/z* 189 in the EIMS showing that the sugar moiety was monoacetylated rhamnose. The absence of a prominent peak at *m/z* 273 excludes the presence of a fully acetylated sugar. The peak at *m/z* 122 confirms the loss of the sugar moiety from the molecule .The loss of an aldehydic proton from the aglycone gives rise to the fragment at *m/z* 121. On the basis of the above evidence, the structure of compound **2** is 4-(4'-O-acetyl-α-L-rhamnosyloxy)benzaldehyde [11, 15].



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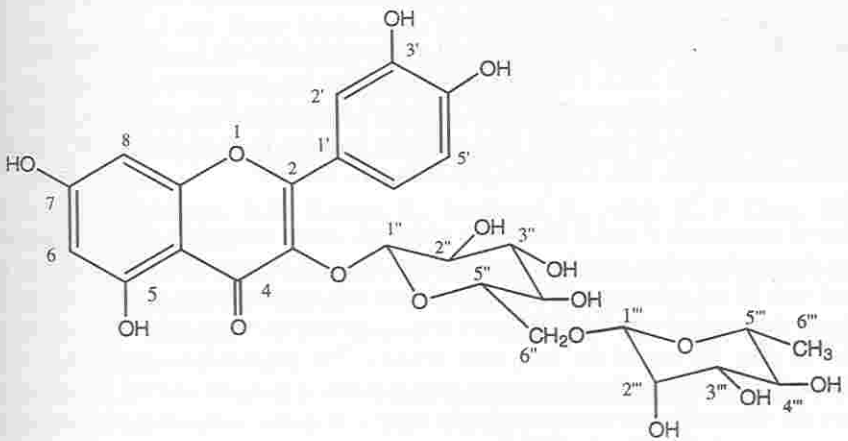
Compound **3** is a pale yellow crystalline solid. Its TLC plate gave blue and deep yellow colors when sprayed with $AgNO_3$ and 5% methanolic KOH solutions, respectively. The UV (MeOH) spectrum displayed absorption maxima at 245, 295, 353 and 390 nm. These indicate the presence of a flavonoid skeleton in the molecule. The IR spectrum showed a broad peak at 3440 cm^{-1}, indicative of the presence of an OH group. The peak at 1630 cm^{-1} was attributed to the presence of a chelated keto group, confirming the presence of 5-OH group. Aromatic peaks were observed at 1560 and 1430 cm^{-1}. A singlet at 12.60 ppm in the ^1H NMR is another characteristic signal of 5-OH grouping. The ^1H NMR spectrum also showed a broad singlet overlapping a doublet at 7.56 ppm; these were assigned to H-2' and H-6'. A one proton doublet at 6.84 ppm corresponds to H-5' while the one proton doublets at 6.18 and 6.38 ppm were assigned to *meta* related H-6 and H-8, respectively.

Many peaks appeared between 3.50-3.06 (*m*) ppm which indicated the presence of a sugar moiety. The presence of L-rhamnose was indicated by 6'''-CH_3 at 0.95 (*d*) ppm and H-1''' at 4.38 (*s*) ppm; it is connected to β-D-glucose, whose H-1'' appears as a doublet at 5.34 ppm. The absence of a singlet in the 6.40-7.20 ppm region strongly suggests that C-3 is substituted. D-glucose and L-rhamnose were identified after hydrolysis by comparison with authentic samples using paper chromatography.

The base peak appeared at *m/z* 302 in the mass spectrum indicating the molecular ion peak of the aglycone, quercetin. Signals at *m/z* 274 and 273 which are due to the loss of CO, CHO moieties, are characteristic fragments of quercetin. The peak at *m/z* 153 ($C_7H_5O_4^+$) is another characteristic fragment of quercetin. The IR, UV, ^1H NMR, mass spectral data and the hydrolysis results are consistent with the structure of rutin [16-20].



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EXPERIMENTAL

General. Melting points were determined on a Block-Monoscop-NR174 apparatus and are uncorrected. UV and IR spectra were recorded using Beckman DU-65 spectrophotometer and Perkin-Elmer FTIR 1600 series, respectively. The EI mass spectra were recorded on a VG ZABSPEC instrument and the ^1H NMR spectra were taken in CD_3OD and DMSO-d_6 on a Varian VXR-500 spectrometer operating at 400 MHz at the Chalmers University of

Technology, Gothenburg, Sweden. The 300 MHz ^{1}H NMR spectra were recorded in CD_3OD on a Brucker 300 MHz instrument at the University of Botswana, Gaborone, Botswana.

Plant material. The leaves of *M. stenopetala* were collected from Arbaminch Water Technology Institute in the months of November, 1998 and April, 1999.

Extraction and isolation. One kg fresh and uncrushed leaves of *M. stenopetala* were successively extracted with 5 L of ethanol at room temperature. The extracts were freed from the solvent under reduced pressure to give a viscous residue. The combined extracts were defatted with petrol and subjected to column chromatography. The column was eluted first with petrol, then with petrol:$CHCl_3$ and $CHCl_3$:MeOH gradients to pure MeOH. These gave different fractions and TLC of each fraction was examined using different solvent systems and those possessing similar R_f values were combined to give four major fractions. The first two fractions contained oily substances and chlorophyll and were discarded. Fractions III (122 mg) and IV (1.2 g) were further purified using column chromatography over Sephadex LH-20 and refractionated using different solvent systems. Purification by prep. TLC was done for each fraction to give compounds **1**, **2** and **3**.

Compound 1. R_f 0.6 ($CHCl_3$:MeOH, 9:1); light yellow; UV (MeOH)λ_{max} (nm) 225, 275, 375; IR (KBr) ν_{max} 3383, 2932, 2173, 2091, 1715, 1611, 1510, 1342, 1235, 1063, 1023, 983, 835, 668, 500 cm^{-1}; ^{1}H NMR (300 MHz, CD_3OD) δ (ppm) 7.34 (*d*, H-3, H-5), 7.11 (*d*, H-2, H-6), 4.71 (*s*, H-7), 5.49 (*d*, H-1'), 4.05 (*m*, H-2'), 4.03 (*m*, H-3'), 5.05 (*t*, H-4'), 3.80 (*m*, H-5'), 1.12 (*d*, H-6'), 2.10 (*s*, OAc): EIMS *m/z* 189, 165, 147, 129, 107, 85, 71.

Compound 2. R_f 0.44 ($CHCl_3$:MeOH, 8:2), light yellow; UV (MeOH) λ_{max} 240, 260, 280 nm; IR (KBr) ν_{max} 3400, 2923, 2852, 1734, 1617, 1489, 1458, 1364, 1094, 860, 776, 450 cm^{-1}; ^{1}H NMR (400 MHz, CD_3OD) δ (ppm) 7.85 (*d*, H-3, H-5), 7.20 (*d*, H-2, H-6), 9.95 (*s*, CHO), 5.58 (*d*, H-1'), 4.2(*m*, H-2'), 4.10 (*m*, H-3'), 4.86 (*t*, H-4'), 3.60 (*m*, H-5'), 1.20 (*d*, H-6'), 2.16 (*s*, OAc); EIMS *m/z* 281, 253, 189, 122 (HOC_6H_4CHO), 121, 105, 93.

Acetylation of compounds 1 and 2. A mixture of 0.5 mL acetic anhydride, 2.0 mg compound **1** and two drops of pyridine was kept for two days at room temperature. The solution was neutralized with 10 mL of saturated $NaHCO_3$ solution and extracted with 50 mL $CHCl_3$. The organic layer was washed with 0.1 N HCl and dried with anhydrous Na_2SO_4. The solvent was evaporated to give 1.3 mg of the acetylated product. The product was analyzed by TLC ($CHCl_3$:MeOH, 9:1) and gave R_f value greater than the starting material and did not change its color when the chromatogram was sprayed with $AgNO_3$ reagent. Acetylation of compound **2** was carried out in a similar manner and gave similar results.

Compound 3 (rutin). R_f 0.55 (n-BuOH:HOAc:H_2O, 4:1:5), pale yellow crystalline solid, mp 187-190°C; UV(MeOH) λ_{max} 245, 295, 353, 395 nm; IR (KBr) ν_{max} 3440, 2918, 1655, 1601, 1505, 1457, 1363, 1295, 1204, 1064, 806 cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm) 12.60 (*s*, 5-OH),7.56 (*d*, H-2', H-6'), 6.84 (*d*, H-5'), 6.38 (*d*, H-8), 6.18 (*d*, H-6), 5.34 (*d*, glucosyl H''-1), 4.38 (*s*, rhamnosyl H'''-1), 3.50 - 3.06 (*m*, 10H sugar), 0.95 (*d*, H-6'''); EIMS *m/z* 302 (M$^+$- rhamnoglucosyl), 274, 273, 187, 153, 121, 73.

Acid hydrolysis of rutin. 10 mg of rutin was dissolved in 10 mL 2 N HCl:MeOH (1:1) and refluxed for 2 h. The solution was cooled and extracted with EtOAc repeatedly. The organic

layer, after drying over Na_2SO_4 and removal of the solvent, gave quercetin, identical to an authentic sample (TLC comparison). Paper chromatographic analysis (n-BuOH:HOAc:H_2O, 4:1:5) of the aqueous phase showed β-D-glucose and α-L-rhamnose by comparison with authentic samples. TLC comparison of compound **3** with an authentic specimen of rutin, gave identical R_f.

ACKNOWLEDGEMENTS

We are thankful to Professor Ermias Dagne of the Department of Chemistry, AAU for the sample of quercetin and to Dr. Kaleab Asres of the School of Pharmacy for the sample of rutin.

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