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ISOTHIOCYANATES IN MYROSINASE-TREATED SEED EXTRACTS OF *MORINGA PEREGRINA*

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Key Word Index—*Moringa peregrina*; Moringaceae; seeds; C_3 and C_4 isothiocyanates; 5,5-dimethyl-oxazolidine-2-thione; 4-(α-L-rhamnosyloxy)benzyl isothiocyanate; 4-(4′-*O*-acetyl-α-L-rhamnosyloxy)benzyl isothiocyanate; chemosystematics.

Abstract—Seeds of *Moringa peregrina* (Moringaceae), on treatment with myrosinase, produce 2-propyl, 2-butyl and 2-methylpropyl isothiocyanate in addition to 5,5-dimethyl-oxazolidine-2-thione, all new to the family but known as natural derivatives from other sources. 4-(4′-*O*-Acetyl-α-L-rhamnosyloxy)benzyl isothiocyanate, not previously described, together with substantial quantities of its non-acetylated counterpart, formerly recognized as a component in hydrolysed seeds of *M. oleifera*, constitute the additional mustard oils observed in seeds of *M. peregrina*.

INTRODUCTION

The monogeneric family Moringaceae comprises about 10 xerophytic species distributed from tropical Africa to the East Indies. Two stand out as particularly important, viz. *Moringa oleifera* Lam. and *M. peregrina* (Forssk.) Fiori, both widely cultivated as sources of gums, oils and pungent principles, cherished, because of the latter, as condiments and remedies [1]. Benzyl isothiocyanate (**1a**) is an established product from *M. oleifera* [2, 3], though limited to its roots [3]. A more hydrophilic mustard oil, present also in roots, wood and leaves of the same species, was isolated from defatted seeds in quantities as high as 10% (by weight); it was identified as 4-(α-L-rhamnosyloxy)benzyl isothiocyanate (**2a**) [3], till now the only glycosidic mustard oil of natural derivation. With this background, we have subjected the isothiocyanates produced in seeds of *M. peregrina* to a comparable study.

RESULTS AND DISCUSSION

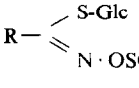
On disintegration, the moist seed meal instantaneously develops pronounced pungency, attributable to isothiocyanate formation, initiated through glucosinolate hydrolysis catalysed by endogenous seed myrosinase. The mixture resulting from myrosinase-catalysed hydrolysis of the glucosinolates (**3**) in defatted seeds of *M. peregrina* was divided into halves.

One was subjected to extractive steam distillation, in a modified Likens and Nickerson apparatus [4], followed by GC–MS analysis of the isopentane extract. 2-Propyl (**1b**), 2-butyl (**1c**) and 2-methylpropyl isothiocyanate (**1d**), in an approximate ratio of 20:1:10, were easily identified upon comparison with authentic specimens; together they constitute virtually the total fraction of volatiles.

The second half was thoroughly extracted with EtOAc. TLC revealed two constituents producing brown-black colours on spraying and heating with $AgNO_3$. After separation by column chromatography, the major component possessed properties (IR, ^1H NMR) comparable to those reported for the *M. oleifera* isolate (**2a**) [3]; additionally, its MS displayed a peak for the molecular ion (*m/e* 311). The second and more lipophilic constituent, amounting to *ca* 20% of **2a**, exhibited a ^1H NMR spectrum very similar to that of **2a**, save for a sharp 3H-singlet at δ 2.13, and an apparent 1H-triplet at a field lower than that of the CH_2-grouping. Decoupling experiments, supplemented with IR data, defined its structure as the monoacetate (**2b**), supported by strong peaks in the MS at *m/e* 43($Me \cdot CO^+$, 100%), 107 ($HO \cdot C_6H_4CH_2^+$, 46%) and 189 ([M − ($O \cdot C_6H_4 \cdot$

R—NCS

1a R = $Ph \cdot CH_2$
1b R = $[Me]_2 \cdot CH$
1c R = Et(Me)CH
1d R = $[Me]_2 \cdot CH \cdot CH_2$



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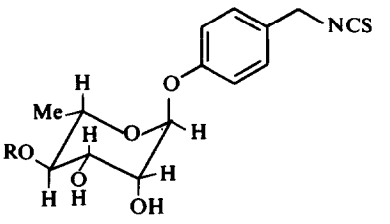
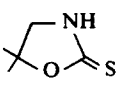


2a R = H
2b R = Ac



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$CH_2NCS)]^+$, 50%). Acetylation of the mixture of **2a** and **2b** gave a chromatographically homogeneous, amorphous triacetate, with the expected ^1H NMR spectrum, whereas treatment of the same mixture with NaOMe in MeOH afforded a homogeneous product, exhibiting a positive Grote reaction, supposedly the methyl thiocarbamate derived from **2a**.

A $CHCl_3$ extract of the aglucones from a large and separately hydrolysed portion of defatted seeds was extracted with NaOH. From the acidified aqueous phase a minute quantity of crystalline material was obtained. It was identified (^1H NMR, IR, MS) as 5,5-dimethyl-oxazolidine-2-thione (**4**) [5, 6], suggesting the presence in *M. peregrina* also of a small quantity of the glucosinolate (**3**, R = $[Me]_2C(OH)\cdot CH_2$), no rarity in Nature [5, 6].

The glucosinolate whence **2a** derives is a major and perhaps genus-characteristic seed constituent of *Moringa*. The acetate (**2b**), when viewed against the background of natural glycoside patterns, appears unexceptional. As previously noted [7, 8], homologized glucosinolates, $R\cdot[CH_2]_n\cdot C(S\cdot Glc)\cdot N\cdot OSO_3^-$ ($n \geq 1$), derived from homologized protein amino acids, $R\cdot[CH_2]_n\cdot CH(NH_3^+)CO_2^-$ ($n \geq 1$), are common in Cruciferae and Resedaceae, rare in Capparaceae, and hardly known elsewhere in glucosinolate-containing taxa. The glucosinolate-derived *Moringa* constituents, **1a–1d**, **2a**, **2b**, and **4**, reflect a biosynthetic derivation from the protein amino acids phenylalanine, valine, isoleucine, leucine and tyrosine, hardly an accidental collection. Assuming the enzymic amino acid homologization, conceivably requiring the operation of several enzymes, to be an advanced character, the pattern of glucosinolate-derived constituents in species of the family Moringaceae places the latter as one of relative antiquity within the order Capparales.

EXPERIMENTAL

Seeds of *M. peregrina* (80 g), collected in the wild near Qosseir in the Red Sea coastal region of Egypt, were disintegrated and defatted in a Waring blendor containing CCl_4 (2 × 200 ml). The seed meal was suspended in a Pi buffer (200 ml, pH 6.8); a cell-free myrosinase soln [9] (2 ml) and a trace of ascorbic acid were added. After 2 hr at room temp. the mixture was divided in half. To one half, H_2O (400 ml) was added before distillation in a modified Likens and Nickerson apparatus [4], with 2-methylbutane (15 ml) as the second solvent. The organic phase was concd by gas entrainment at low temp. [4] to an oily residue (30 mg) which was subjected to GC–MS analysis, performed on a VG MM 70-70 instrument interfaced to a glass column (1.5 m × 2 mm i.d.), packed with 5% OV-101, through a glass jet separator. The operating conditions were: column temp. 50–250° at 8°/min; injector 250°; transfer lines and separator 250°; carrier gas He 15 ml/min; ionization energy 70 eV; ion source temp. 240°. Three significant peaks were observed, in the ratio 20:1:10, arranged in order of increasing retention times. Comparison with authentic specimens and published MS [10] established the identity of these as: 2-propyl (**1b**), 2-butyl (of unproven chirality) (**1c**) and 2-methylpropyl isothiocyanate (**1d**), respectively.

The second half of the enzymic hydrolysis mixture was extracted, first with Et_2O (3 × 200 ml), then with EtOAc (2 × 100 ml). The Et_2O extract, according to TLC (SiO_2, EtOAc–EtOH (9:1), spraying with $AgNO_3/NH_3$ and heating to 100°, as well as observation in UV), contained a minor (R_f 0.6) and a major (R_f 0.4) constituent: the EtOAc extract only the latter. On evapn of the combined extracts, a semi-solid remained (2.5 g). An aliquot of this (450 mg) was subjected to chromatography on a Si gel column (Merck 'Lobar Fertigsäule B, Kieselgel 60') with EtOAc as the mobile phase. Two separated peaks resulted and the corresponding fractions were studied separately; (i) the least mobile fraction (147 mg) was an amorphous solid, displaying IR (in KBr) and ^1H NMR spectra in agreement with those reported for **2a** [3]. A ^{13}C NMR spectrum (in $CDCl_3$) could be completely assigned to structure (**2a**); a measured $^1J[^{13}CH(1)]$ value of 168 Hz for the anomeric C-atom supports the α-configuration [11]. MS: *m/e* (rel. int.): 311 (M^+, <0.2), 165 ($HO\cdot C_6H_4\cdot CH_2NCS$, 12), 147 (rhamnosyl, 41), 146 (30), 129 (27), 107 ($HO\cdot C_6H_4\cdot CH_2$, 100), 85 (59) and 71 (41). (ii) The more lipophilic constituent was obtained as a colourless syrup (37 mg). Its IR spectrum was very similar to that of **2a**, though supplemented with a strong band at 1720 cm^{-1} (C=O). ^1H NMR (90 MHz, $CDCl_3$, TMS as ref.): δ 1.17 (3H, *d*, H-6), 2.13 (3H, *s*, OAc), 3.7–4.0 (2H, *m*, H-3 and H-5), 4.12 (1H, *s(br)*, H-2), 4.62 (2H, *s*, CH_2), 4.89 (1H, *t*, H-4), 5.51 (1H, *s(br)*, H-1), and 7.2 (4H, *dd*, arom.); on irradiation at H-6, the signal of H-5 appeared as a doublet (J = 9.5 Hz): irradiation at H-5 caused conversion of the signal of H-6 into a singlet and collapse of the signal of H-4. MS: *m/e* (rel. int.): 189 (Ac – rhamnosyl, 50), 171 (7), 165 ($HO\cdot C_6H_4CH_2NCS$, 1.5), 129 (189 – HOAc, 21), 111 (16), 107 ($HO\cdot C_6H_4\cdot CH_2$, 44), and 43 (Ac, 100).

Treatment of the mixture of the 2 mustard oils (100 mg) in Py (1 ml) with Ac_2O (0.25 ml) for 1 hr at 20° gave a homogeneous non-crystalline triacetate. ^1H NMR ($CDCl_3$): δ 1.12 (3H, *d*, H-6), 2.00 (6H, *s(br)*, 2OAc), 2.12 (3H, *s*, OAc), 3.9 (1H, *dq*, H-5), 4.61 (2H, *s*, CH_2), 5.09 (1H, *q*, H-4), 5.40 (1H, *dd* (partly hidden), H-3), 5.40 (2H, *s(br)*, H-1 and H-2), and *ca* 7.2 (4H, *dd*, arom.); R_f 0.66 (EtOAc) [0.13 for **2a**, 0.46 for **2b**]. When the same mixture (5 mg) was left standing in MeOH (1 ml) with a trace of NaOMe for 1 hr at 20°, one spot was observed on TLC (R_f 0.32; $CHCl_3$–MeOH, 9:1) in UV, producing a sky-blue colour with Grote's reagent, suggesting formation of the methyl thiocarbamate resulting from MeOH addition to **2a** and **2b**, accompanied by deacetylation.

The $CHCl_3$ extract from a separately hydrolysed seed portion (150 g) was briefly treated with 0.2 M NaOH. The aq. phase was acidified and extracted with $CHCl_3$. Evapn afforded a solid residue (4.1 mg), recrystallized from Et_2O, mp 103°, and identified as 5,5-dimethyl-oxazolidine-2-thione (**4**) on comparison (IR, ^1H NMR, MS) with an authentic specimen [5].

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REFERENCES

1. Pax, F. (1936) in *Die Natürlichen Pflanzenfamilien* (Engler, A. and Prantl, K., eds.) Vol. 17b, p. 693. Duncker & Humblot, Berlin.
2. Kurup, P. A. and Narasimha Rao, P. L. (1954) *Indian J. Med. Res.* **42**, 85.
3. Badgett, B. L. (1964) Ph.D. Thesis, Rice University, Houston, Texas.

4. MacLeod, A. J. and Cave, S. J. (1975) *J. Sci. Food Agric.* **26**, 351.
5. Kjær, A., Gmelin, R. and Boe Jensen, R. (1956) *Acta Chem.* **10**, 432.
6. Schultz, O.-E. and Wagner, W. (1956) *Arch. Pharm.* **289/61**, 597.
7. Ettlinger, M. G. and Kjær, A. (1968) *Recent Advances in Phytochemistry* (Mabry, T. J., Alston, R. E. and Runeckles, V. C., eds.) Vol. 1, p. 126. Appleton-Century-Crofts, New York.
8. Kjær, A., Øgaard Madsen, J. and Maeda, Y. (1978) *Phytochemistry* **17**, 1285.
9. Neuberg, C. and Wagner, J. (1926) *Biochem. Z.* **174**, 457.
10. Kjær, A., Ohashi, M., Wilson, J. M. and Djerassi, C. (1963) *Acta Chem. Scand.* **17**, 2143.
11. Wiebe, L. (1976) Thesis, The Technical University of Denmark.