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9-(METHYLSULPHINYL)NONANENITRILE, A STRESS METABOLITE OF *RORIPPA SYLVESTRIS*

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Key Word Index—*Rorippa sylvestris*; Cruciferae; 9-(methylsulphinyl)nonanenitrile; 8-(methylsulphinyl)octyl isothiocyanate.

Abstract—A stress metabolite, 9-(methylsulphinyl)nonanenitrile, was isolated from all aerial parts of *Rorippa sylvestris* after induction by $CuCl_2$ and its structure proved by the high resolution NMR and mass spectra.

INTRODUCTION

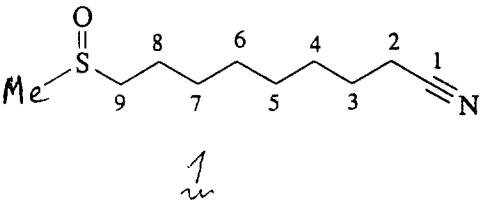
Rorippa sylvestris is a Eurasian subatlantic species, secondarily circumpolarly distributed. 8-(Methylsulphinyl)octyl glucosinolate, glucohirsutin, has been found in the roots and aerial parts of this species and some other cruciferous plants [1–5]. The hydrolytic product, 8-(methylsulphinyl)octyl isothiocyanate released into the rhizosphera was identified as the allelopathin [6, 7].

We were interested in accumulation of phytoalexins in cruciferous plants after induction by abiotic stress. This paper reports the presence of 9-(methylsulphinyl)nonanenitrile (**1**) in all aerial parts of *Rorippa sylvestris* as a stress metabolite. Only the low resolution mass spectrum of this compound has been published previously [5]. We focused our attention on the ^1H and ^{13}C NMR and high resolution mass spectra to corroborate the structure of 9-(methylsulphinyl)nonanenitrile (**1**).

RESULTS AND DISCUSSION

By HPLC analysis the peak corresponding to compound **1** caused by abiotic stress induced by $CuCl_2$ was detected in the aerial organs (leaf, stem and flower) of *R. sylvestris*. The compound was present only in very small amounts in the leaf, but was not in the stem or flower of control plants.

The ^1H NMR spectrum shows nineteen protons in the region δ 1.30–2.80 including the well resolved end protons of an eight-carbon aliphatic chain. Two of them, H-9a and H-9b at δ 2.65 and 2.73, respectively, are distinctly nonequivalent due to the vicinal chiral methylsulphinyl group. Nonequivalency is observed also for H-8a and H-8b at δ 1.77, while the protons of more distant methylene groups are apparently equivalent. Other deshielded proton signals were seen for CH_3SO (δ 2.57), H_2-2 (δ 2.34) and H_2-3 (δ 1.66) group. Two overlapped multiplets at δ 1.46 and 1.36 resolvable in the DQF COSY spectrum, were observed for the eight remaining internal methylene protons (H_2-4 to H_2-7).



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In the ^{13}C NMR spectrum signals of ten carbons, eight of methylene type, one methyl and one quarternary carbon were present according to the DEPT-135 measurement. The last signal at δ 119.68 belonged to the carbon of the nitrile group which possessed a strong magnetic anisotropy. As seen from the HSQC spectrum, it strongly shields C-2 (δ 17.12) compared to the other carbons. This observation contrasts the situation with deshielded H-2 protons. Such a difference is an unambiguous proof of the end nitrile group. On the contrary, both the protons and carbon at C-9 are well deshielded due to a withdrawing effect of the methylsulphinyl substituent. Other carbons with slightly different chemical shifts were assigned on the basis of the HSQC spectrum.

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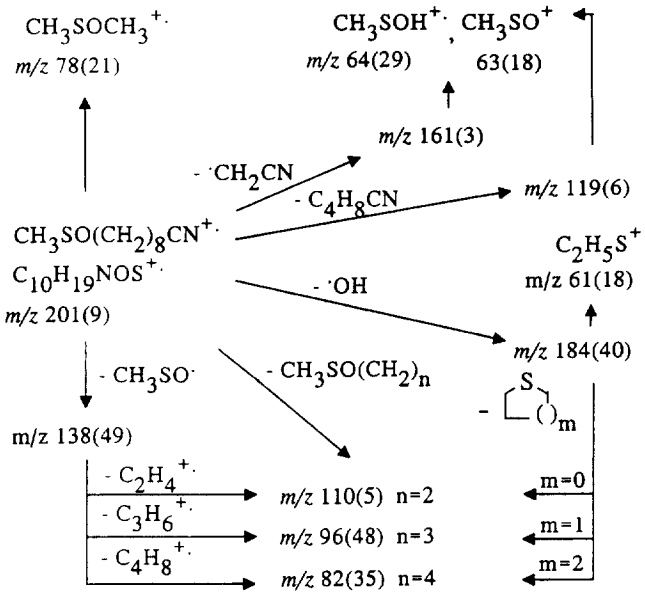

Scheme 1.

The high-resolution electron ionization mass spectrum revealed an elemental composition of $C_{10}H_{19}NOS^{+\cdot}$ in accordance with the structure concluded from the NMR spectra. The accurate masses of the primary and most abundant secondary fragment ions together with the fragmentation pathways solved by MS-MS daughter ion studies on the ZABspec-oaTOF confirmed the 9-(methylsulphinyl)nonanenitrile (**1**) structure (Scheme 1). Further support for the straight aliphatic chain is given by the abundant $C_xH_y^+$ ions where y equals $x+1$, x, $x-1$, $x-2$ or $x-3$, e.g. *m/z* (rel. int.) $C_5H_9^+$ (40), $C_4H_9^+$ (18), $C_4H_7^+$ (100), $C_4H_6^{+\cdot}$ (16), $C_3H_7^+$ (43), $C_3H_6^{+\cdot}$ (31), $C_3H_5^+$ (96), C_3H_3 (24) as examples of most abundant species of each type.

EXPERIMENTAL

Plants of *Rorippa sylvestris* (L.) Bess. grown in the Botanical Garden of P. J. Šafárik University, (Košice, Slovakia) were used. A voucher specimen has been deposited in the Herbarium KO. To elicit the stress metabolite accumulation, the plants were sprayed with a 2% aq. soln of $CuCl_2$ [8]. In the experiment and the control 10 plants were used. After 48 hr the plants were collected and dried at room temp.

Extraction. Powdered leaf, stem and flower (380 g) were extracted (EtOH) on a warm water bath. The extract was partitioned with hexane—MeOH—H_2O (50:25:25), then the H_2O—MeOH layer was partitioned with CH_2Cl_2—MeOH—H_2O (50:25:25). The CH_2Cl_2 layer was dried, dissolved in EtOH and analyzed by HPLC.

HPLC. Column (3 × 150 mm, SGX C18, 7 μm) and mobile phases A (H_2O—MeCN—H_3PO_4, 80:19:1) and B (MeCN—MeOH—H_3PO_4, 59:40:1), elution profile 0–20 min, 0–100% B and UV detection at 218 nm were used.

Isolation. Purified extract (0.401 g) was separated by CC on silica gel (35 to 60 μm, deactivated by 11% of water) eluted with $CHCl_3$. Pure compound (60 mg) was prepared after rechromatography.

NMR spectra were taken on Bruker AMX-600 NMR spectrometer in $CDCl_3$ at laboratory temp. ^1H and ^{13}C spectra were calibrated to tetramethylsilane (0 ppm). Following spectra were measured: ^1H (600.13 MHz) spectrum, broadband decoupled ^{13}C (150.903 MHz) spectrum, DEPT-135, double quantum filtered homonuclear correlation (H,H DQF-COSY) and H,C-heteronuclear single-quantum coherence spectrum (HSQC).

^1H NMR (δ, ppm): 1.36 (2H, *m*, H-6), 1.37 (2H, *m*, H-5), 1.46 (2H, *m*, H-4), 1.47 (2H, *m*, H-7), 1.66 (2H, *m*, H-3), 1.77 (2H, *m*, H-8a, H-8b), 2.34 (2H, *t*, J = 7.1 Hz, H-2), 2.57 (3H, *s*, CH_3SO), 2.65 (1H, *ddd*, J = 12.9, 8.5, 7.6 Hz, H-9a), 2.73 (1H, *ddd*, J = 12.9, 8.5, 6.3 Hz, H-9b).

^{13}C NMR spectrum (δ, ppm): 17.12 (C-2), 22.50 (C-8), 25.28 (C-3), 28.47 (C-6), 28.51 (C-4), 28.61 (C-7), 28.89 (C-5), 38.60 (CH_3), 54.66 (C-9), 119.68 (CN).

The electron impact mass spectra were recorded on a VG 7070E double focusing mass spectrometer equipped with an OPUS software (Altrincham Manchester, U.K.). A water cooled direct insertion probe was used for introduction of the samples at the ambient temp. The ionization energy of 70 eV and the trap current of 100 μA were used for creating positive ions. The resolution of 1000 was used for obtaining the low resolution spectra. The daughter ions were obtained by linked scan method (B/E constant) from all major parent ions or as the TOF spectra on a ZABspec-oaTOF. The accurate mass measurements of all

important peaks were done by computerized peak matching method using perfluorokerosine (PFK, Merck Art. 10145) as the reference compound at the resolution of 5000. HR-MS *m/z*, (rel. int.): 201.11929 ($CH_3SO(CH_2)_8CN$, $C_{10}H_{19}NOS^{+\cdot}$), $[M]^{+\cdot}$ (9), 184.11618 ($C_{10}H_{18}NS^+$), $[M—OH]^+$ (40), 161.10010 ($C_8H_{17}OS^+$), $[M—CH_2CN]^+$ (3), 138.12856 ($C_9H_{16}N^+$), $[M—CH_3SO]^+$, (49), 119.05313 ($C_5H_{11}OS^+$), $[M—(CH_2)_4CN]^+$ (6), 96.08134 ($C_6H_{10}N^+$), $[M—CH_3SO(CH_2)_3]^+$ (48), 82.06539 ($C_5H_8N^+$), $[M—CH_3SO(CH_2)_4]^+$ (35), 78.01408 ($C_2H_6OS^{+\cdot}$), $[HOCH_2CH_2SH]^{+\cdot}$ (21), 69.07069 $[C_5H_9]^+$ (40), 63.99842 $[CH_3SOH]^{+\cdot}$ (29).

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