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Veratryl Isothiocyanate, a New Mustard Oil, from *Heliophila longifolia* DC. (Cruciferae)*

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Seed of the South African crucifer *Heliophila longifolia* DC. on enzymatic hydrolysis yields a new isothiocyanate, characterized as its crystalline thiourea derivative. By infrared and mass spectroscopic analysis, the latter compound is deduced to be veratrylthiourea (3,4-dimethoxybenzylthiourea), previously underscribed.

Structural confirmation is provided by synthesis of veratryl isothiocyanate (I) and veratrylthiourea, the latter demonstrated to be identical with the naturally derived thiourea.

Unexpected paper-chromatographic properties of the new thiourea are reported.

Heliophila L., now consisting of approximately one hundred species, is the largest genus of crucifers endemic to South Africa.[1] No chemical analysis has, to our knowledge, been previously reported for any member of *Heliophila*. During a survey of natural mustard oils conducted by two of us in the Chemistry Department of Rice University (Houston, Texas, U.S.A.), we examined three seed samples attributed to *H. longifolia* DC. By a standardized procedure,[2] any isothiocyanates or thiooxazolidones that the seed might furnish were liberated enzymatically into ether solution, and the product was tested in three ways: for evolution of thiocyanate ion, as from *p*-hydroxybenzyl isothiocyanate, on extraction with alkali; for thiooxazolidones, by ultraviolet spectrophotometry and with Grote's reagent; and likewise, after treatment with ammonia, for thioureas. The first two combined tests were negative with the seed under discussion, but all three samples gave copious amounts of a crude thiourea, whose chromatographic analysis showed it to consist almost entirely of one component, never before derived from nature. The residues of fatty oil and thiourea, representing 0.2—0.3 g of seed, from the ammonia-

* Part LVI of a series of papers from this laboratory on isothiocyanates. Part LV: *Acta Chem. Scand.* **20** (1966) 698.

treated extracts deposited crystals when kept in the cold. The purified crystals, m.p. 190—191°, were the new thiourea and exhibited bands in the infrared spectrum which were strongly indicative of the presence of a methoxy-substituted benzyl group.

A portion of the minute amount of thiourea available was subjected to mass spectrometry along with 4-methoxybenzylthiourea[3] as a reference compound. In the mass region above *m/e* 100, the two spectra were very similar, with consistent displacement of all peaks in the *Heliophila* thiourea spectrum by 30 mass units towards higher mass numbers. The most conspicuous fragments are listed in Table 1.* Spurious peaks were observed at

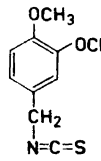
Table 1. Characteristic mass spectral fragments of 4-methoxybenzylthiourea and the thiourea derived from *Heliophila longifolia* DC.

m/e	4-Methoxybenzylthiourea	*Heliophila* thiourea
M^+	196	226 [a]
M−17	179	209
M−34	162	192
M−60	136	166
M−75	121 [b]	151 [b]
M−90	106	136
M−105	91	121
M−120	—	106

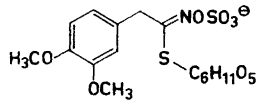
[a] Weak. [b] Base peak.

M + 59 with both thioureas and M + 89 with the *Heliophila* derivative, presumably attributable to thermal decomposition products formed in the heated inlet system. This possibility, however, needs further clarification. The observed fragmentation to a series of ions differing by 15 or 30 mass units is characteristic of aromatic methyl ethers,[4] suggesting that the *Heliophila* thiourea is a dimethoxybenzylthiourea. The 3- and 4-methoxybenzylglucosinolates, as well as the corresponding phenols, occur frequently in Cruciferae and other families containing mustard oil glucosides.[5] Hence, it appeared likely that the *Heliophila* thiourea was 3,4-dimethoxybenzylthiourea. This conclusion was proved to be correct by synthesis. Veratrylamine was converted with thiophosgene to the previously unknown veratryl isothiocyanate (I), m.p. 22—24°, and this in turn to synthetic veratrylthiourea, m.p. 193°. Comparison of the infrared spectra of the thiourea samples of natural and synthetic origin, as well as determination of the melting point of a mixture, established their common identity.

* The observed fragmentation is in accordance with the results of recent studies by Shapiro and Serum[13] of the mass spectra of N-phenylthiourea and a number of its N′-alkyl derivatives.



I



II

Since seed extracts not treated with ammonia did not contain the thiourea, it must have come from the corresponding isothiocyanate. The yield of veratryl isothiocyanate from *Heliophila longifolia* seed, estimated according to the ultraviolet absorption of the thiourea, was strikingly large, namely 3 %. We have not detected more than faint traces of the veratryl derivative in thioureas from any other plant so far examined.

By analogy to the formation of other mustard oils, veratryl isothiocyanate is believed to be an enzymatic cleavage product of a primary constituent of *Heliophila longifolia* seed, the veratrylglucosinolate ion (II),[6] which was characterized by paper chromatography. These are the first known derivatives of a polyphenol in the series of natural mustard oils and their precursors. In view of the established role of phenylalanine [7,8] and possible role of tyrosine [9] in the respective biosyntheses of the benzyl- and *p*-hydroxybenzyl-glucosinolates (anions of glucotropaeolin and sinalbin), it seems reasonable to assume that 3,4-dihydroxyphenylalanine (DOPA) may be involved in the biosynthesis of (II).

Table 2. R_{Ph}-values [10] of benzylthioureas, R-NHCSNH$_2$.

R	Solvent			
	Toluene-butanol [a]	Chloroform [10]	Benzene-ethanol [b]	Toluene-acetic acid [c]
$C_6H_5CH_2$	1.2	0.92	1.1	0.82
3- or 4-$CH_3OC_6H_4CH_2$	1.2—1.25	1.0	1.15—1.2	0.8
3,4-$(CH_3O)_2C_6H_3CH_2$	0.83	0.98	0.6	0.27

[a] Toluene-butanol-water, 10:1:2. [b] Benzene-ethanol-water, 5:1:2. [c] Toluene-acetic acid-water, 5:2:4.

As is apparent from the paper-chromatographic data (Table 2), veratryl-thiourea is remarkably hydrophilic by comparison with its less methoxylated analogues, and from the chromatographic information its structure could hardly have been predicted. Other naturally derived thioureas that we have observed with similarly varying R_{Ph}, some of them associated with mustard oils that yield thiocyanate ion, remain to be identified in the future.

EXPERIMENTAL

Isolation of veratrylthiourea. Seed of *Heliophila longifolia* was obtained from three sources in England: the Royal Botanic Gardens, Kew, and the University of London Botanical Supply Unit, Englefield Green, Surrey, both of whom we thank gratefully; and Thompson and Morgan Ltd. of Ipswich, a commercial seed house. In general, ground, undefatted seed (0.5 g or less) was suspended in 5 ml of 0.1 % aqueous sodium ascorbate with a little glucosinolase,[3] overlaid with 50—75 ml of ether, free of alcohol and peroxides, and allowed to stand 3 h with occasional shaking at room temperature. The ether was separated and washed with a few ml of water. A fifth of the ether was extracted with 0.1 N alkali; in the neutralized extract, no thiocyanate ion was detected with acid ferric nitrate. The remainder of the ether was divided into halves, one of which was treated with 10 ml of ethanol and 2 ml of concentrated aqueous ammonia and let stand 4 h at room temperature or overnight at 5°. Both solutions were evaporated to dryness and the ultraviolet absorption of the residues was measured in ethanol solution at 220, 240, and 260 mμ. For Kew seed, the absorbancies corresponding to 200 mg of seed per aliquot in 625 ml of ethanol were, without ammonia treatment, 0.375, 0.320, 0.096; with ammonia, 0.618, 0.750, 0.206. The untreated material, spotted on paper, sprayed with Grote's reagent,[2,10] and steamed, gave no coloration. Correction of the absorbancy of the crude thiourea at 240 mμ for background [11] reduced the values by 10—15 %, a usual amount. For the example given, the corrected absorbancy is 0.66, equivalent to 2.9 % of veratryl isothiocyanate in the seed. The result for the University of London sample was 3.2 %.

On paper chromatography [10] with the solvents given in Table 2, the *Heliophila longifolia* thiourea, including that from commercial seed, showed one strong spot, with faint indications on a few chromatograms of considerably faster running material. Like benzylthiourea, the major component streaked unless lightly loaded.

The residues of fatty oil and thiourea, stored in open flasks at 5°, formed crystals after two months. Four months thereafter, the dried material was washed with petroleum ether (b.p. 30—60°); the dried, powdery residue was taken up in boiling acetone and freed of insoluble material, and the solution was concentrated to 1 ml. The chilled solution deposited tiny, colorless prisms, sparingly soluble in cold ethanol, m.p. 189—190.5° with prior discoloration, heated from room temperature, or 190—191° with browning, heated from 180°. The ultraviolet absorption of an ethanol solution at 220, 240, and 260 mμ was in the ratio 0.8:1:0.25. Paper chromatography showed that the substance was the *H. longifolia* thiourea. The infrared spectrum of the substance in a potassium bromide disk had bands at 3.0 μ (medium), 3.1 (m), 3.2 (strong), 3.45 (m), 3.6 (weak), 6.15 (s), 6.3 (w), 6.55 and 6.65 (s), 6.8 (w), 6.9 (s, doublet), 7.1 (w), 7.35 (m), 7.5 (w), 7.8 (w), 7.95 and 8.1 (s), 8.25 (w), 8.4 (w), 8.65 and 8.75 (s), 9.0 (m), 9.6 (w), 9.8 (s), 10.55 (m), 10.8 (w), 11.8 (m), 12.1 (w), 12.3 (s), 13.05 (s), 13.45 (m), and 13.9 (w) μ.

Synthesis of veratryl isothiocyanate and its thiourea derivative. A solution of veratrylamine (5.0 g) and triethylamine (6.1 g) in chloroform (150 ml) was added in the course of 45 min to a cooled solution (−10°) of thiocarbonyl chloride (3.8 g) in chloroform (100 ml). The reaction mixture was then washed with water (100 ml), 0.5 N HCl (50 ml), 0.5 N NaOH (50 ml), and finally with three 50-ml portions of water. The organic phase was dried over sodium sulphate, and the solvent was removed through a short column. On distillation at 0.3 mm, veratryl isothiocyanate was obtained as a viscous, yellowish oil (1.80 g), b.p. 135—137°, n_D^{25} 1.5960, crystallizing in fine needles on cooling, m.p. 22—24°. (Found: C 57.36; H 5.32; N 6.60. Calc. for $C_{10}H_{11}NO_2S$: C 57.38; H 5.30; N 6.69). Its infrared absorption was similar to that of the thiourea from 6.5 to 7.1 μ, at 8, 8.7, and 9.75 μ, and between 11.5 and 14 μ.

A solution of the isothiocyanate (300 mg) in chloroform (5 ml), saturated with NH_3 at 0°, was left overnight at room temperature. The crystalline thiourea (350 mg) was recrystallized from ethanol (25 ml), yielding analytically pure veratrylthiourea (225 mg), m.p. 193°. (Found: C 53.31; H 6.32; N 12.23. Calc. for $C_{10}H_{14}N_2O_2S$: C 53.07; H 6.23; N 12.39). On a block under a microscope, the synthetic thiourea and a mixture with the *Heliophila* compound both melted at 199°. The infrared spectrum of the synthetic thiourea was completely identical with the spectrum of the naturally derived compound previously given. The chromatographic behaviour of the two samples in chloroform was the same.

The ultraviolet spectrum of the synthetic 3,4-dimethoxybenzylthiourea in ethanol had maxima at 207 (ε 22 400), 241 (14 800), and 278 (2900; broad) mμ, minima at 225 (10 150) and 267 (2190) mμ. The values of ε at 220, 240, and 260 mμ were 11 300, 14 800, and 3240. Comparison with the spectrum of *m*-methoxybenzylthiourea (ε 14 700 and 12 600 at 220 and 240 mμ)[12] shows the effect of the second methoxyl group in shifting the high intensity aromatic band to longer wave lengths.

Paper chromatography of the parent glucosinolates. A few seeds of each sample were ground in a mortar and extracted with hot 70 % methanol. The concentrated solutions were applied as spots on the starting lines of two paper chromatograms (Schleicher and Schüll 2043 b) one of which was developed with butanol:ethanol:water (4:1:4), and the other with butanol:pyridine:water (6:4:3). On spraying with silver nitrate solution, the characteristic violet-grayish glucosinolate spots appeared with an R_B-value (*i.e.*, R_F-value relative to that of the benzylglucosinolate ion) of 0.79 or 0.93, respectively.

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