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ISOLATION OF (−)-5-ALLYL-2-THIOOXAZOLIDONE FROM *BRASSICA NAPUS* L.

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Abstract—A new compound, (−)-5-allyl-2-thiooxazolidone (napoleiferin) has been isolated from rape (*Brassica napus* L. var. *oleifera*) and its presence demonstrated by chromatographic methods in turnip (*B. campestrus* L.). The structure was established with the aid of NMR, u.v. and i.r. spectral data. ORD data indicates a similar absolute configuration to that of goitrin isolated from the same tissue. Evidence suggests a new glucoside, 2-hydroxy-4-pentenyl-glucosinolate, is present in fresh tissue.

DURING a preliminary investigation of methods suitable for analysis of the amounts of various glucosinolate[1] aglycones obtained from seed, leaf and stem tissue of rape (*Brassica napus* L. var. *oleifera*) a hitherto unidentified thiooxazolidone was noted. The name napoleiferin is proposed for this compound, which has been shown to be (−)-5-allyl-2-thiooxazolidone (Fig. 1). Ettlinger and Thompson[2] have listed the glucosinolates found in this and related species, while a comprehensive review by Kjær[3] outlines much of the chemistry and biochemistry of most known glucosinolates. Thiooxazolidones and isothiocyanates are formed by the action of myrosinase on the parent glucosinolates. That napoleiferin appeared only after preparations had been treated with myrosinase, strongly suggests that the fresh tissue contains a corresponding 2-hydroxy-4-pentenyl-glucosinolate.

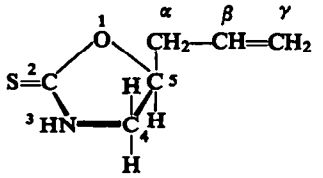

FIG. 1. ABSOLUTE CONFIGURATION OF NAPOLEIFERIN.

In the preliminary work, paper chromatography was used to separate goitrin ((−)-5-vinyl-2-thiooxazolidone) and the thioureas formed by ammonia treatment of the volatile isothiocyanates. Napoleiferin was observed as a small spot causing decolourization of iodine-azide[4] spray reagent. With all solvent systems used napoleiferin migrated further than goitrin (Table 1) and occurred in lesser amounts. Napoleiferin reacted with Grote's reagent[5] in a manner similar to goitrin giving a pale green spot which faded to a pale pink after a few

[1] M. G. ETTLINGER and G. P. DATEO, *Studies of Mustard Oil Glucosides—I* Final Report, Contract DA19-129-QM-1059, P12, Rice University, Houston, Texas (1961).
[2] M. G. ETTLINGER and C. P. THOMPSON, *Studies of Mustard Oil Glucosides—II* Final Report, Contract DA19-129-QM-1689, Rice University, Houston, Texas (1961).
[3] A. KJÆR, *Fortschr. Chem. Org. Naturstoff.* **18**, 122 (1960).
[4] F. FEIGL, *Spot Tests in Organic Analysis*, 5th ed., p. 88. Elsevier, Amsterdam (1956).
[5] I. W. GROTE, *J. Biol. Chem.* **93**, 25 (1931).

days in darkness. In contrast, the thioureas of the naturally occurring isothiocyanates gave dark blue spots which changed to bright pink when in high concentration. Phenyl thiourea used as a marker gave a blue-green spot. An u.v. absorption spectrum of napoleiferin in water gave a curve with a peak at 239 nm which in alkali was reduced in intensity and displaced to 230 nm. This effect is typical of the thiooxazolidone structure.[6]

TABLE 1. R_f VALUES OF THIOOXAZOLIDONES

	Solvent systems*		
	1	2	3
Goitrin	0·27	0·54	0·57
Barbarin	—	0·90	—
Cleomin	0·63	—	0·71
Napoleiferin	0·50	0·67	0·71

* 1, Carbon tetrachloride: 30% acetic acid, 1:1 v/v; 2, Benzene: methanol: water, 2:1:1 v/v; 3, Benzene: hexane: water, 9:2:9 v/v. To obtain R_f measurements all solvents were used with Whatman No. 1 paper by the descending technique.

The isolation of napoleiferin from the enzymically treated sap of steamed leaf and stem tissue is described in the Experimental Section. Results of microanalysis were consistent with the napoleiferin being a thiooxazolidone with an unsaturated three-carbon atom side-chain. Confirmation of the 5-allyl-2-thiooxazolidone structure was obtained by analysis of the NMR spectrum (60 Mc) (Fig. 2) and comparison with the NMR spectra of goitrin and

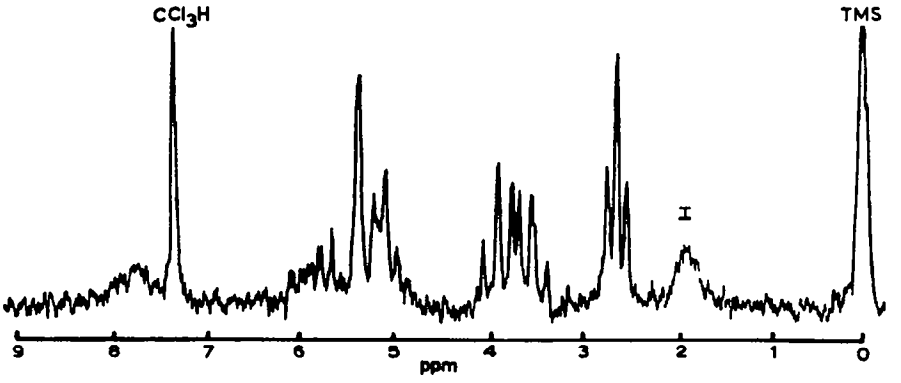

FIG. 2. NMR SPECTRUM (60 Mc) OF NAPOLEIFERIN.
Tetramethylsilane (TMS) and chloroform were used to calibrate the spectrum. The small peak (0·8 H) at 2·0 ppm is attributed to an unidentified impurity (I).

barbarin ((−)-5-phenyl-2-thiooxazolidone). All three compounds showed similar six peak patterns (2H) in the range 3·3–4·3 ppm, the AB part of an ABX system. This group must be due to the two non-equivalent (4)-CH_2 protons. The additional splitting is due to the (5)-CH proton which appears as a complex multiplet at about 5·1 ppm. Kjær and Thomsen[7] have

[6] E. B. ASTWOOD, M. A. GREER and M. G. ETTLINGER, *J. Biol. Chem.* **181**, 121 (1949).
[7] A. KJÆR and H. THOMSEN, *Acta Chem. Scand.* **16**, 591 (1962).

shown that (4)-CH_2—N protons occur at about 3·6 ppm in cleomin ((−)-5-ethyl-5-methyl-thiooxazolidone) and conringiin (5,5-dimethyl-2-thiooxazolidone) while the (5)-CH_2—O protons in 4-methyl-2-thiooxazolidone occur in the range 4·0–5·0 ppm. The triplet (2H) at 2·5–2·8 ppm can be assigned to the (α)-CH_2 protons which are coupled to both (5)-CH and to (β)-CH protons with coupling constants ($J_{5\alpha}$, $J_{\alpha\beta}$) of about 7 c/s. The protons of the vinyl group appear as a complex multiplet at 4·8–6·1 ppm. The NH proton appears as a broad peak at 7·8 ppm. The i.r. spectrum (thin film) was typical of thiooxazolidones. A small sharp peak at 6·1 μ and other peaks at 10·1 μ and 10·8 μ were consistent with the presence of a vinyl group.

The optical rotatory dispersion curves of napoleiferin and goitrin (Fig. 3) showed similar Cotton effects, which suggests that napoleiferin has the same absolute configuration (Fig.1) as goitrin.[8] It is notable that with napoleiferin the optical rotation at the sodium D wavelength is negative but small. No attempt has been made to measure its magnitude accurately.

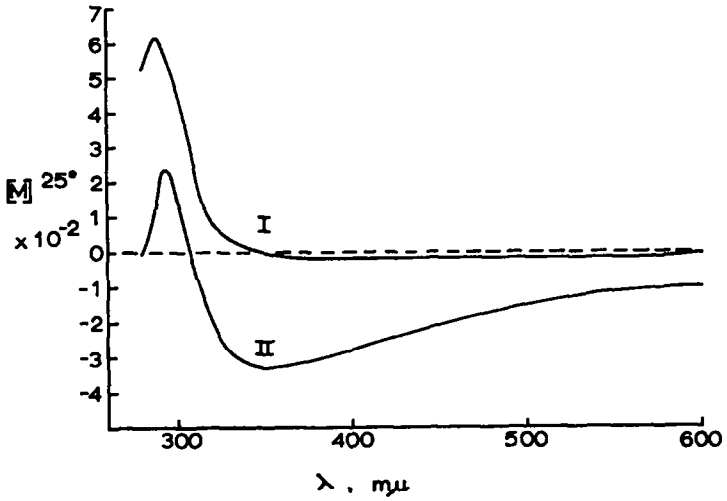

FIG. 3. OPTICAL ROTATORY DISPERSION CURVES OF NAPOLEIFERIN (I) AND GOITRIN (II). Measured as 0·25 per cent solution in methanol.

Ettlinger and Thompson[2] have reported a "hydrophobic thiooxazolidone" from Early Purple Top Strapleaved Turnip seeds (*B. campestris* L.) which may be the same compound as napoleiferin.

Although napoleiferin has been isolated in pure form only from rape leaf and stem tissue, paper chromatography has shown it to be present in turnip (*B. campestris* L.) and swede (*B. napus* L. var. *napobrassica*). Estimations of the amounts of napoleiferin and goitrin in these plants based on the relative size of chromatogram spots are shown in Table 2. The ratio of amounts of napoleiferin to goitrin tended to reflect the ratio of 4-pentenyl isothiocyanate to 3-butenyl isothiocyanate in the same plant. This is perhaps not surprising as a biogenetic relationship between these compounds is indicated.

There are a number of reports indicating that thiooxazolidones, isothiocyanates and the parent glucosides are important as either goitrogenic substances[9] or as factors controlling

[8] A. KJÆR, B. W. CHRISTENSEN and S. E. HANSEN, *Acta Chem. Scand.* **13**, 144 (1959).
[9] C. A. LAMP, *J. Australian Inst. Agri. Sci.* **29**, 8 (1963).

parasitic insects of crops.[10] The significance of napoleiferin or 2-hydroxy-4-pentenyl-glucosinolate in these fields remains to be demonstrated.

TABLE 2. AMOUNTS OF THIOOXAZOLIDONES IN SOME *Brassica* CROPS

	Napoleiferin*	Goitrin
B. napus L. var. *oleifera*		
Rangi rape, tops	5	18
Broad leaf Essex II rape, tops	4	12
B. napus L. var. *napobrassica*		
Sensation swede, tops	Trace	15
Sensation swede, roots	Not detected	10
B. campestris L.		
Kapai 66 turnip, tops	2	36
Kapai 66 turnip, roots	1	20
EPTS turnip, seed†	400	2000
B. oleracea		
Medium stem choumollier, tops	Not detected	0·5
Golden Acre cabbage, tops	Not detected	5

* As μg per g fresh weight.
† Early Purple Top strap-leaved turnip seed kindly supplied by Dr. M. G. Ettlinger.

EXPERIMENTAL

Thiooxazolidones and Isothiocyanates from Rape

Fresh rape leaf and stem tissue was steamed at atmospheric pressure for 20 min to inactivate enzymes and precipitate proteins. The sap was pressed from the cooked residue and about 80 l. stored at −10°. Portions of about 10 l. were thawed, adjusted to pH 6·5 with $NaHCO_3$, and incubated overnight with cell-free myrosinase.[11] The liberated thiooxazolidones and isothiocyanates were extracted into an equal volume of $CHCl_3$. Centrifugation gave a partial separation of the emulsion formed during the extraction. The clear aqueous layer was removed and the $CHCl_3$ layer treated with sufficient Celite 545 to allow the $CHCl_3$ extract to be filtered free of the emulsified water. No attempt was made to obtain quantitative yields.

Separation of Thiooxazolidones from Isothiocyanates

Thiooxazolidones were separated from isothiocyanates by reducing the volume of the $CHCl_3$ extract to about one-tenth, and extracting twice with equal volumes of 0·1 N NaOH. The alkaline extracts containing the thiooxazolidones were combined, acidified to pH 3 with HCl and shaken with an equal volume of $CHCl_3$. A crude oily mixture of the thiooxazolidones was obtained upon evaporation.

Chromatography Solvents

Carbon tetrachloride: 30% acetic acid, 1:1 v/v, was used with the ascending technique for preparative separations on Whatman No. 17 paper as well as for qualitative work using Whatman No. 1 or No. 4 papers. Benzene: methanol: water, 2:1:1 v/v, and benzene: hexane: water, 9:2:9 v/v, were used descending.

Preparative Chromatography of Thiooxazolidones

The thiooxazolidones were dissolved in a small volume of ethanol and filtered before application as streaks on to Whatman No. 17 paper for ascending chromatography. Thiooxazolidones equivalent to about 2 l. of the pressed sap were applied to each 25 cm square sheet. The chromatograms were developed using the lower phase of carbon tetrachloride: 30% acetic acid, 1:1 v/v, in a tank saturated with the upper phase.

[10] S. D. BECK, *Ann. Rev. Entomol.* **10**, 207 (1965).
[11] M. SANDBERG and Q. M. HOLLY, *J. Biol. Chem.* **96**, 443 (1932).

Narrow strips cut from the centre of dried chromatograms were sprayed with iodine-azide[4] solution. Two bands of decolourization could be seen on the sprayed strips. The slower-moving and larger band was finally shown to contain goitrin while the faster-moving and lesser band contained napoleiferin.

Isolation of Napoleiferin

The faster-moving band of the preparative chromatograms was eluted with ethanol. Much of the coloured impurity was removed by reducing the volume of ethanol and filtering through B.D.H. activated charcoal. Upon drying a pale yellow oil was obtained. Crystallization was induced only after distillation of a portion under high vacuum at 150° on to a cold finger cooled with liquid nitrogen. Once seed crystals had been obtained napoleiferin could be crystallized from cooled ether and hexane mixtures. Colourless prisms were obtained, m.p. 60–61°. (Found: C, 50·40; H, 6·64%. C_6H_9NOS required: C, 50·32; H, 6·33%.)

Isolation of Goitrin

The slower-moving band of the preparative chromatograms was treated in a similar manner to the lower one and finally yielded goitrin, m.p. 48·7–50·0°. Its identity was established by an i.r. spectrum and by paper chromatography using as a standard a sample of authentic goitrin kindly supplied by Professor A. Kjær.

Barbarin[12]

About 100 g of fresh leaves and inflorescences of *Reseda luteola* L. were blended with 400 ml of hot 70% aqueous ethanol to give a crude preparation of glucobarbarin. Ethanol was removed under reduced pressure and the water-soluble portion of the residue was extracted with $CHCl_3$ to remove pigments. The aqueous phase was freed of dissolved $CHCl_3$ under reduced pressure and incubated overnight with a preparation of cell-free myrosinase. Crystals of barbarin formed spontaneously and were filtered off. This material was recrystallized from hot water to give colourless needles, m.p. 123–124°. The identity was confirmed by an i.r. spectrum.

Cleomin[7]

One gramme of seeds of *Cleome spinosa* Jacq. was ground with sand and extracted with two 25 ml portions of boiling methanol for 10 min. The methanol was removed under reduced pressure and the water-soluble portion treated with cell-free myrosinase. A $CHCl_3$ extract of this enzymically treated preparation was used as a source of cleomin for paper chromatography.

Estimation of Thiooxazolidones and Isothiocyanates

Suitable portions of leaf tissue or finely ground seeds were extracted with boiling 70% aqueous ethanol. Ethanol was removed from the extracts under reduced pressure and the water-soluble residues incubated with cell-free myrosinase. Thiooxazolidones and isothiocyanates were extracted with $CHCl_3$ then treated with ethanol and ammonia to convert isothiocyanates to thioureas. After concentration, small portions of the extracts were chromatographed on Whatman No. 1 paper together with a range of standards for each compound. Iodine-azide spray reagent was used to detect the thiooxazolidones and thioureas, and the amounts were estimated by visual comparison of the spot sizes with those of the standards.

Infrared spectra were obtained with a Perkin-Elmer "Infra-red 137" by either the KBr pressed-disc method or by depositing a thin film on NaCl plates with solvent evaporation. A Varian D.P. 60 spectrometer was used for the NMR spectra and a Jasco O.R.D./u.v.—5 was used to record the optical rotatory dispersion curves. Microanalyses were carried out by Dr. A. D. Campbell and associates, Otago University, New Zealand.

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[12] A. KJÆR, *Acta Chem. Scand.* **12**, 1693 (1958).