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IDENTIFICATION OF THE VOLATILE PHAGOSTIMULANTS IN *SOLANUM CAMPYLACANTHUM* FOR *EPILACHNA FULVOSIGNATA*

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Abstract—The oil obtained by steam distillation of fresh leaves of *Solanum campylacanthum*, which is known to have a phagostimulatory effect on *Epilachna fulvosignata* larvae, has been fractionated. The constituents of the biologically active fraction have been isolated and identified as hexanol, *cis*-hex-2-en-1-ol, and *cis*-hex-3-en-1-ol.

INSECTS of the coccinellid genus *Epilachna* are known from host plant records to feed predominantly on either solanaceous or cucurbitaceous plants although some species are associated with other plant families. Chemical factors are undoubtedly important in this host plant selection, with one or more of the plant constituents having an attractant or inhibitory effect on the insect. From studies of the behaviour of *Epilachna fulvosignata* larvae which feed on *Solanum campylacanthum* (Solanaceae) one of us (STRIDE, 1965) has concluded that the leaves of this plant must contain both volatile and non-volatile phagostimulants. By steam distillation of fresh leaves a volatile, pleasant smelling oil was isolated which, when incorporated in appropriate agar gels, stimulated the *Epilachna* larvae to feed. The isolation and structural elucidation of the phagostimulatory constituents, referred to by Stride as VPS, of the steam volatile oil from *S. campylacanthum*, are the subject of the present investigation.

MATERIALS AND METHODS

Analytical GLC separations were made on a Perkin–Elmer F11 gas chromatograph. GC–MS data were recorded on an LKB 9000 gas chromatograph–mass spectrometer. Kieselgel HF_{254} (Merck) was employed for analytical (0·25 mm) and preparative (0·5 mm) TLC. Ether refers to anhydrous AnalaR diethyl ether and light petroleum to AnalaR light petroleum, b.p. 40 to 60°C, which was redistilled and the fraction b.p. 40 to 55°C collected.

Our studies have been hampered by the pronounced volatility of the components of the oil, which even made staining on TLC difficult. For satisfactory results, analytical TLC plates had to be developed with iodine vapour, then before the

spots faded, the plate sprayed with a 1% solution of ceric ammonium sulphate in 4 N sulphuric acid followed by rapid heating to 130°C, then held at that temperature for 4 to 5 min.

When the crude *Solanum* oil was allowed to stand in an unstoppered flask for 24 hr at 0°C, considerable diminution in the intensities of the peaks with retention times less than that of peak 1 (Fig. 1) was observed, indicating the pronounced volatility of these components. The most convenient method for storing the oil, or the separated fractions, was absorbed on silica from which it could be recovered by ether extraction when required.

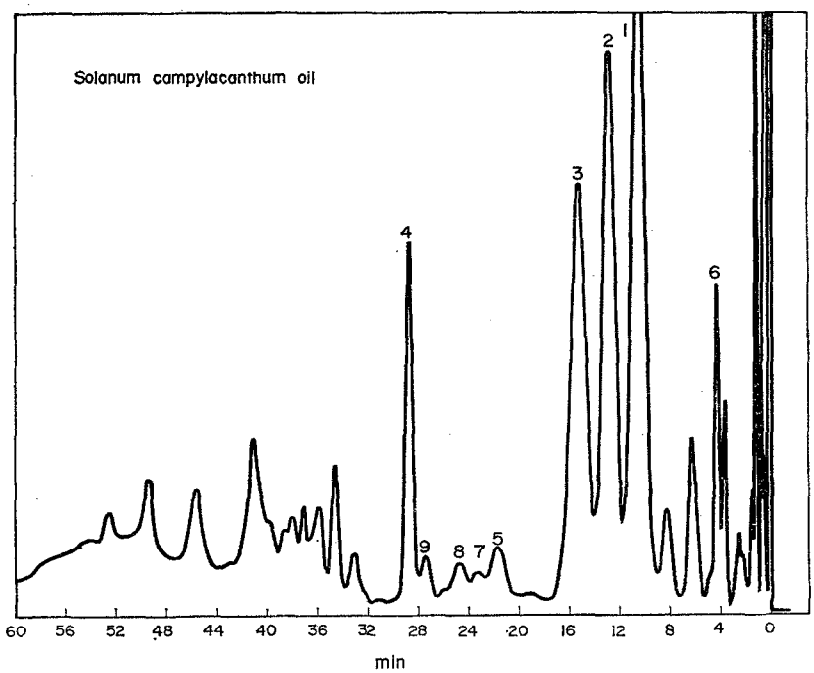

FIG. 1. Separation on 8% Carbowax

Fractionation of the Solanum *oil by preparative TLC*

A sample (~75 mg) of the *S. campylacanthum* steam distillate in light petroleum was absorbed on to a preparative chromatoplate. After elution with ether–light petroleum (1 : 3), bands of material were detected by their appearance on irradiation at 254 nm. Band A (R_f ~ 0·5–0·9) contained at least three bands the least polar of which (diffuse blue) was separated from the most polar (intense blue) by a narrow visible yellow band. Band B (R_f ~ 0·2–0·3) appeared as two closely spaced bands (blue), while band C (R_f ~ 0·1–0·2) was a visible yellow band having at its head an intense blue u.v. active band.

Mixed GLC analysis of the extract of band B with the crude oil identified its constituents as peaks 4 and 5 (Fig. 1). Careful rechromatography of this fraction

in ether–light petroleum (1 : 4) (three elutions) resulted in the isolation of the less polar component which was shown to be linalool, identical by NMR and GLC (8% Carbowax at 95°C and 20% Cyano P programmed at 5°C/min from 65 to 125°C) with an authentic sample.

Mixed GLC analysis of the extract of band C with the crude oil identified its constituents as peaks 1, 2, and 3 (Fig. 1). Rechromatography of this fraction on silver nitrate-impregnated silica and elution with ether–light petroleum (3 : 7) resulted in the isolation of the components of peaks 1 and 3 (Fig. 1) from the less polar band and the compound which gave peak 2 from the more polar band. Structural assignments were based on GC–MS data while additional support for the structures of *n*-hexanol (peak 1), *cis*-hex-3-en-1-ol (peak 2) and *cis*-hex-2-en-1-ol (peak 3) respectively came from mixed GLC with authentic samples (8% Carbowax at 70°C and 20% Cyano P at 65°C).

RESULTS AND DISCUSSION

Preliminary GLC studies on a 1% SE 30 column at 50°C indicated that the oil consisted of a complex mixture of volatile compounds, 70 per cent of the material being eluted within 5 min. A much greater degree of separation was achieved on the more polar 8% Carbowax column (20 min at 65°C, then programmed at 4°C/min to 160°C), and evidence for the presence of more than 30 components in the mixture was obtained (Fig. 1). A major step towards the isolation of the phagostimulatory components was made with the discovery that the crude oil could be conveniently separated into three fractions by TLC (see Materials and Methods section). By incorporation of appropriate TLC bands into agar prior to biological testing, it was found that the least polar band, A, showed negligible activity; fraction B showed variable activity depending on the batch of steam distillate used; whereas *fraction C*, the most polar band on TLC, exhibited consistent biological activity, always of a phagostimulatory nature. Recovery of separated organic material from chromatographic silica gel presented a problem because the volatility of the compounds were such that on concentration of ether extracts of the TLC bands, losses occurred through co-distillation. These difficulties were overcome by use of a micro-Soxhlet apparatus such that the volume of ether was minimized ($\sim$0·8 ml), concentration being effected by very gentle warming. From i.r. evidence, fractions B and C were found to contain hydroxylic compounds, while their u.v. spectra revealed only end absorption characteristic of non-conjugated alkenes.

GLC analysis of the biologically most active fraction, C, showed it to contain the three most abundant components of the oil, peaks 1, 2, and 3 (Fig. 1). Additional separation of the components of fraction C could be effected by preparative TLC on silver nitrate-impregnated silica. The more mobile of the two bands thus obtained contained two compounds (peaks 1 and 3, Fig. 1) while the more polar band contained only one compound (peak 2, Fig. 1). The two fractions now constituting C were then analysed by combined GC–MS (5% Carbowax, isothermal, 85°C; 70 eV). Peaks 1 and 3 were found from their mass spectral

fragmentation patterns (Table 1) to be *n*-hexanol and *cis*-hex-2-en-1-ol respectively, whereas peak 2 proved to be the isomer, *cis*-hex-3-en-1-ol. All three assignments were supported by mixed GLC analyses with authentic samples on two columns.

TABLE 1—CONSTITUENTS OF *S. Campylacanthum* OIL

GLC peak	TLC band	Structure	Retention index*	Molecular formula	Mass spectral peaks†
1	C	*n*-Hexanol‡	1312	$C_6H_{14}O$	84, 69, *56*, 55, 43, 42, 41
2	C	*cis*-Hex-3-en-1-ol§	1343	$C_6H_{12}O$	100(M), 82, 69, 67, 55, 42, *41*, 39
3	C	*cis*-Hex-2-en-1-ol§	1360	$C_6H_{12}O$	100(M), 82, 67, *57*, 44, 43, 41, 39
4	B	Linalool‖	1500	$C_{10}H_{18}O$	154(M), 136, 121, 93, 80, *71*, 55, 43, 41
5	B	Unknown	1400		99, 85, 72, *57*, 55, 43, 41
6	A	Hex-2-en-1-al	1200	$C_6H_{10}O$	98(M), 83, 69, 57, 55, 43, 42, *41*, 39, 29
7	A	Monoterpene alcohol‖	1440	$C_{10}H_{20}O$	*138*, 123, 109, 96, 82, 55, 43, 41
8	A	Monoterpene alcohol‖	1455	$C_{10}H_{20}O$	*138*, 123, 109, 96, 82, 55, 43, 41
9	A	Monoterpene alcohol‖	1490	$C_{10}H_{16}O$	152(M), *137*, 123, 109, 81, 67, 55, 43, 41, 39, 29

* Retention indices were obtained (ETTRE, 1964) from GLC data using standard *n*-C_{11} to C_{15} alkanes.
† Base peak italicized.
‡ FRIEDEL *et al.* (1956).
§ HONKANEN *et al.* (1963).
‖ SYDOW (1963); Willhalm *et al.* (1964).

Fraction B, which exhibited variable biological activity, was shown by TLC and GLC to contain two compounds (peaks 4 and 5, Fig. 1). The more abundant of these was isolated by careful chromatography and identified as linalool, but the nature of peak 5 has not yet been established. Although it accounts for approximately half of the steam distillate by weight, fraction A has been examined in only a cursory manner since it was found to be biologically inactive. GLC has revealed the presence of at least 23 compounds whereas GC–MS data (Table 1) suggest that peak 6 is hex-2-en-1-al and peaks 7 to 9 are probably monoterpene alcohols.

GLC examination revealed slight variations in the composition of different batches of oil, but whether this implies seasonal fluctuations in the leaf constituents or is a function of loss of material during the isolation procedure is not known. In every sample of oil, however, the four major peaks were always present (peaks 1 to 4, Fig. 1) though often peak 2 predominated. The composition of the most important constituents of the oil, estimated by GLC, was *n*-hexanol (14–22 per cent),

cis-hex-3-en-1-ol (22–16 per cent), *cis*-hex-2-en-1-ol (14–13 per cent), and linalool (2·5–6 per cent).

From our results, it is clear that the fraction of *S. campylacanthum* leaf oil which has a phagostimulatory effect on *E. fulvosignata* larvae is composed entirely of three C_6 alcohols, two of which, hexanol and *cis*-hex-3-en-1-ol, are present with *trans*-hex-2-enal and linalool in a number of plant aromas. It is significant, however, from the phagostimulation viewpoint, that WATANABE (1958) has concluded that the compound present in mulberry leaves which most strongly attracts the silkworm larvae is our third alcohol, hex-2-en-1-ol, with, in his case, hexanol and hex-2-enal showing some attractivity.

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