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Note

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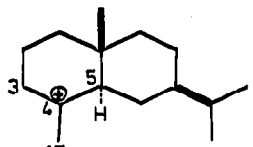
Separation and characterisation of Δ^{3}- and $\Delta^{4(15)}$-cyclopyrethrosin acetates via chromatography on a lipophilic dextran gel

A common source of double-bond isomerism in terpenoid compounds arises from deprotonation of a tertiary carbonium ion centre. In the eudesmane system, Δ^{3}-, $\Delta^{4(15)}$-, and $\Delta^{4(5)}$-isomers are formed in this manner from the cationic intermediate (I). Many analogous compounds have been obtained by cyclisation of germacranolides and related ten-membered sesquiterpenoids.



I

Methods previously employed to effect separation of such mixtures of olefins have involved the techniques of gas–liquid chromatography (GLC) on selective phases[1,2] and/or adsorption chromatography on silica gel, with[3,4] or without[5] the addition of complexing silver salts. To these procedures can now be added the comparatively new technique of liquid–gel partition chromatography on lipophilic Sephadex gels of the type introduced by ELLINGBOE *et al.*[6] and also studied by BROOKS AND KEATES[7].

Experimental

Liquid–gel chromatography

Following the procedures of ELLINGBOE *et al.*[6], Sephadex G-25 (superfine) (Pharmacia, Uppsala, Sweden) was hydroxypropylated and subsequently alkylated with NEDOX 1518 (Ashland Oil and Refining Co., Minneapolis, Minn., U.S.A.), a mixture of C_{15}–C_{18} *n*-alkene epoxides. The observed increase in weight for the gel corresponds to 58% (w/w) substitution of alkyl residues. The modified gel was washed exhaustively with benzene before use. All glassware in contact with the gel was previously "silanised" by treatment with dichlorodimethylsilane in toluene (5% w/v).

Analytical-scale gel chromatography. This was effected using 1 m × 2 mm I.D. glass tubing fitted with PTFE end-pieces. Columns were operated at normal temperatures and atmospheric pressure. Flow-rates so obtained were in the range 0.1–0.2 ml/h. Eluates were monitored on a moving-wire flame ionisation detector (FID) (type HAAHTI–SJÖVALL)[8].

Samples (approx. 10 μg of each component) were applied in methylene chloride solution (0.1 *M*) to the surface of the gel bed prior to elution with freshly distilled anhydrous methanol.

Preparative-scale gel chromatography. This was effected using an 80 × 1 cm I.D. WHATMAN glass column fitted with adjustable end-pieces. Sephadex G-25, refined

previously by sedimentation to a 30–40-μ particle size range, was used as the basis for the lipophilic gel described above. Flow-rates at atmospheric pressure were adjusted to 10 ml/h by means of a screw clamp fitted to the exit tubing.

In a typical procedure, the cyclopyrethrosin acetates (100 mg), dissolved in a small quantity (*ca.* 0.5 ml) of methylene chloride, were applied to the surface of the gel (which had been swollen in "AnalaR" grade methanol) and allowed to penetrate the bed before elution with methanol. Samples were collected in 0.5-ml aliquots for analysis by GLC. Sample recovery was consistently in excess of 95% by weight for a number of runs.

Analytical GLC

Apparatus. The gas chromatograph used was an Aerograph 204 fitted with an FID. The column used was a glass helix, 2 m × 3 mm I.D.

Operating conditions. GLC was carried out under the following conditions: stationary phase, OV-1, 1% on Gas-Chrom Q, 100–120 mesh; carrier gas: nitrogen, 30 ml/min; temperatures: oven, 225°; injector, 260°; detector oven, 200°. Samples containing 2 μg of each component were sufficient to produce full-scale deflection.

Preparative GLC

Apparatus. The instrument used was a Pye Model 105, fitted with a helical glass column, 2 m × 3 mm I.D.

Operating conditions. Preparative GLC was carried out under the following conditions: stationary phase, OV-25, 5% on Gas-Chrom Q, 100–120 mesh; oven temperature, 225°; carrier gas: nitrogen, 60 ml/min; sample loading, 100 μg per injection.

Mass spectrometry

Spectra of the cyclopyrethrosin acetates were obtained from an LKB 9000 gas chromatograph–mass spectrometer unit fitted with a 10-ft. glass column: stationary phase, OV-1, 1% on Gas-Chrom Q, 100–120 mesh; carrier gas; helium, 25 ml/min; oven temperature 220°. Spectra were recorded at 15-eV nominal ionisation potential.

Samples

Cyclopyrethrosin acetates. Preparation by the method of BARTON AND DE MAYO[9].

Cyclopyrethrosins. Preparation by the reaction of pyrethrosin (300 mg) in acetone (5 ml) with *p*-toluenesulphonic acid (30 mg) at 40° during 30 min. After neutralisation, the product was recovered by evaporation to dryness and purified by preparative thin-layer chromatography.

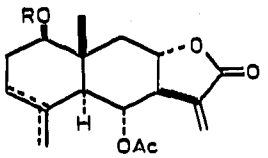
NMR spectra were recorded on a Varian HA 100 instrument, with deutero-chloroform as solvent.

Melting points were recorded on a Kofler block and are uncorrected.

Results and discussion

Our interest in the particular separations effected in this note stemmed from the work of IRIUCHIJIMA AND TAMURA[10] whose assignment of stereochemistry to the

cyclopyrethrosin acetates was based upon studies of mixtures of the double-bond isomers containing different proportions of each isomer, obtained by repetitive fractional crystallisation. Disadvantages associated with techniques previously used for separations of double-bond isomers are well illustrated by the example of the cyclopyrethrosin acetates (II).



II R = Ac

III R = H

By use of a selective stationary phase (OV-25), the two isomers are completely separable by preparative GLC. Degradation of the sample at the required operating temperature (225°), however, contributes to a poor overall yield (*ca.* 12%). Thin-layer chromatography on silica gel impregnated with up to 20% by weight of silver nitrate failed to produce any discernible difference in the relative mobilities of each isomer.

Analytical-scale liquid–gel chromatography of the cyclopyrethrosin acetates and the corresponding cyclopyrethrosins (III) on a C_{15}–C_{18} *n*-alkylated derivative of hydroxypropyl Sephadex G-25 achieved complete separation in both cases (Fig. 1)

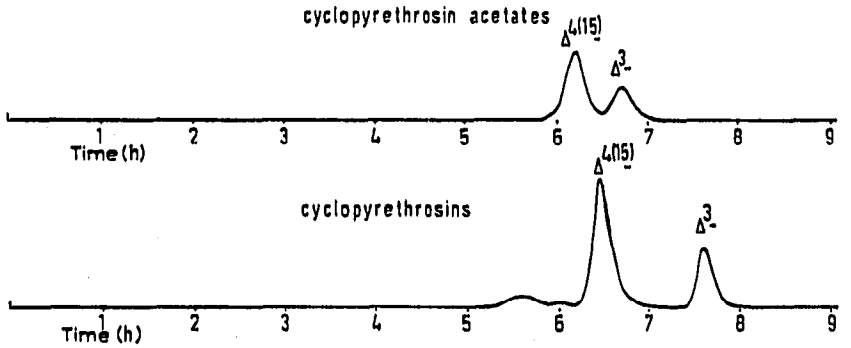


Fig. 1. Liquid–gel chromatogram of the cyclopyrethrosins and cyclopyrethrosin acetates. Solvent, methanol; flow-rate, 0.15 ml/h.

when operated in a strongly reversed-phase mode[7], with methanol as the liquid phase. Upon extension of the method to achieve preparative-scale separation of the double-bond isomers, it was found necessary to resort to the recycling of column fractions resulting from a partial separation in the first stage of chromatography (Fig. 2). Complete separation of the Δ^3- and $\Delta^{4(15)}$-isomers was achieved after liquid–gel chromatography of the fractions obtained at 52–54 S.E.V.[7] and 46–50 S.E.V., respectively.

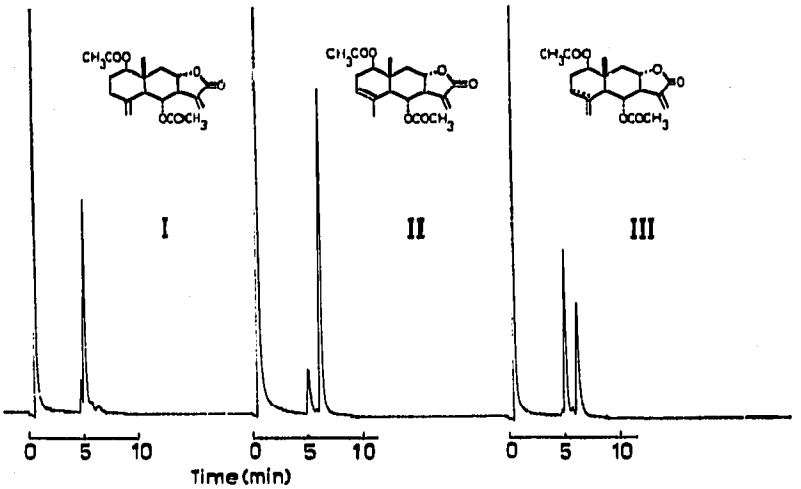


Fig. 2. GLC of column fractions obtained after one cycle of preparative liquid–gel chromatography of the cyclopyrethrosin acetates showing: I, 46–50 S.E.V.; II, 52–54 S.E.V.; III, original mixture.

TABLE I

PHYSICAL AND CHROMATOGRAPHIC DATA FOR Δ^{3}- AND $\Delta^{4(15)}$-CYCLOPYRETHROSIN ACETATES

	Δ^{3}-isomer	*$\Delta^{4(15)}$-isomer*
m.p. (°C)	186–186.5 (prisms)	172–173 (needles)
f (1% OV-1) (225°)	2480	2420
f (3% OV-25) (200°)	3075	3005
S.E.V.[a]	52	49

[a] 58% C_{16}–C_{18} hydroxyalkoxypropyl Sephadex G-25, 10–40 μ (methanol).

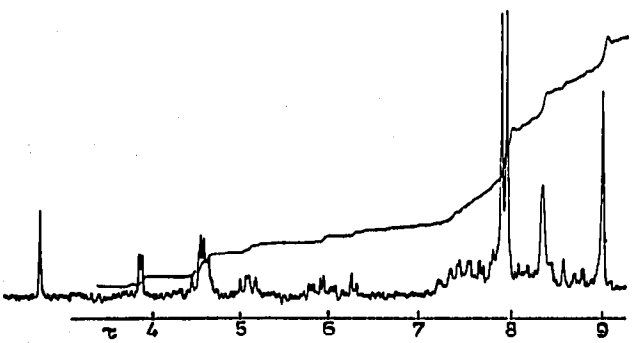


Fig. 3. 100-MHz NMR spectrum (deuterochloroform) of Δ^{3}-cyclopyrethrosin acetate.

Physical and chromatographic data for the double-bond isomers are given in Table I. NMR spectra of the isolated components (Figs. 3 and 4) were fully compatible with the assignment of Δ^{3}- and $\Delta^{4(15)}$-structures. Supporting evidence for the assignment is available from a comparative study of the major fragment ions produced

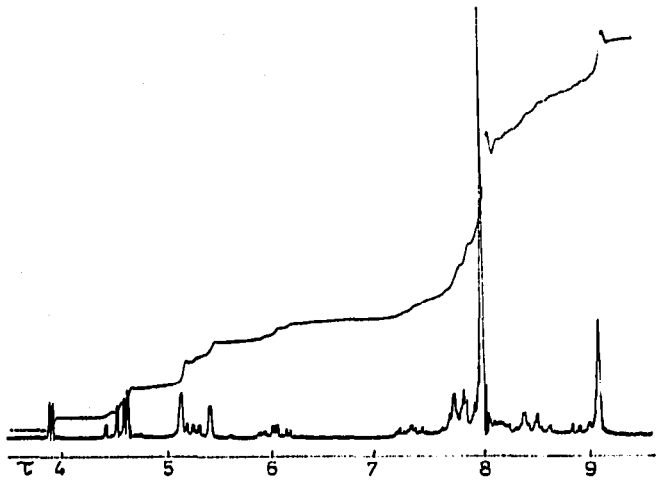


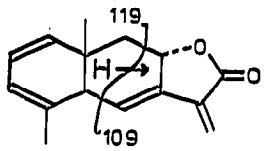
Fig. 4. 100-MHz NMR spectrum (deuterochloroform) of $\Delta^{4(15)}$-cyclopyrethrosin acetate.

TABLE II

COMPARATIVE MASS SPECTRA OF THE Δ^{3}- AND $\Delta^{4(15)}$-CYCLOPYRETHROSIN ACETATES
LKB 9000 spectrometer operating at 15 eV nominal ionisation potential.

m/e	*Relative abundance (% of base peak)*	
	Δ^{3}	$\Delta^{4(15)}$
348	0.1	0.1
288	2	3
246	2	8
229	10	28
228	61	100
213	12	16
200	6	19
199	10	6
119	100	7
43	14	24

during GLC–MS of the cyclopyrethrosin acetates (Table II). The major difference between the two sets of spectra lies in the presence of an ion at *m/e* 119 accounting for a large proportion (26%) of the total ion current from the Δ^{3}-isomer. This species (of type IV) can be attributed to the formation of an *endo*cyclic conjugated π-system, favouring fragmentation of ring A intact.



IV

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