

THIN-LAYER CHROMATOGRAPHY OF β -SITOSTERYL ESTERS

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(Received October 9th, 1963)

Sterols are often present in plant tissues not only as the free sterols, but also as their esters. In connection with their studies on corn oil constituents, KUKSIS AND BEVERIDGE^{1,2,2a} prepared a number of steryl esters of high purity which could be used as reference compounds for the sterol derivatives present in plants, and studied their separation by reversed-phase chromatography on impregnated paper with three solvent systems.

In studies on the sterol fraction of mulberry (*Morus alba*) leaves, which are believed to contain a factor, or factors, essential for the feeding of the silkworm *Bombyx mori*^{3,4} NAYAR AND FRAENKEL⁵ have obtained strong evidence that the active material is very similar to β -sitosterol and occurs in the plant in the form of esters which, however, have not been isolated in a crystalline form. Like these authors, we have used the technique of thin-layer chromatography and have studied the R_F values, separation and identification of fifteen sitosteryl esters in six solvent systems. With this method, we have been able to isolate from mulberry leaves a major sterol which, indeed, was identical or at least very similar to β -sitosterol, as well as sitosteryl caprate; it appears that at least one more ester is present which is either sitosteryl palmitate or stearate.

EXPERIMENTAL

Preparation of esters

Most of the esters had been described before^{1,2}; the new compounds have been prepared by the following method: a mixture of 0.2 mole each of β -sitosterol and the acid with 0.015 mole of *p*-toluenesulphonic acid in 150 ml of dry benzene was refluxed for 4 h in an oil bath. The filtered solution was concentrated *in vacuo* and the solid residue triturated with acetone and recrystallized from methanol.

β -Sitosteryl pelargonate, m.p. 82–83°. Calculated for $C_{38}H_{66}O_2$: C, 82.3; H, 11.9. Found: C, 82.3; H, 12.0 %.

β -Sitosteryl 10-undecenoate, m.p. 72–73°. Calculated for $C_{40}H_{68}O_2$: C, 82.8; H, 11.7. Found: C, 83.0; H, 11.9 %.

β -Sitosteryl arachidonate, m.p. 70–71°. Calculated for $C_{40}H_{80}O_2$: C, 84.0; H, 11.4. Found: C, 83.4; H, 11.8 %.

Preparation of plates

The chromatography was carried out on glass plates (20 × 20 cm), coated with a layer (250 μ thick) of silica gel G (E. Merck).

The slurry for five plates was prepared by shaking 30 g of silica gel and 60 ml of water in a stoppered flask for 30 sec; it was then transferred to a thin-layer applicator

(Desaga, Heidelberg) which was drawn across the plates. The plates were allowed to dry for 15 min at room temperature and then activated in an oven at 120–130° for 30 min. After cooling they were kept in a vacuum desiccator.

Development

To ensure equilibrium conditions inside the chromatography chamber, the walls were lined with a strip of filter paper dipped into the solvent system (150 ml).

The following solvent systems were used as mobile phases (v/v):

- (1) Cyclohexane–benzene (1:1),
- (2) Cyclohexane–benzene (2:1),
- (3) Cyclohexane–benzene (4:1),
- (4) Carbon tetrachloride–chloroform (19:1),
- (5) *n*-Heptane–ethyl acetate (19:1),
- (6) Chloroform–acetone (19:1).

The starting line was drawn at a distance of 2 cm from the base line. The esters were dissolved in chloroform (0.5 mg/ml chloroform), and 1 μ l of each solution was applied with a micropipette.

The glass plates were placed inside a Desaga rectangular glass chamber (21 × 22 × 10 cm) and developed by the ascending technique. The experiments were performed at room temperature (27–30°); 30–90 min. were usually required for the solvent front to reach a distance of 13–15 cm from the starting line.

The plates were then taken out of the chamber, and after marking the solvent front, dried in air for a few minutes and at 120° for 3 min.

Detection

The spots of the esters were detected by spraying the plates in a horizontal position with two reagents, (a) a saturated solution of antimony trichloride in chloroform^{6,7} and (b) phosphomolybdic acid (10% in ethanol)⁸ and heating at 120° for 5 min.

TABLE I

No.	Ester of β -sitosterol	Empirical formula	R_F values in solvent system					
			1	2	3	4	5	6
1.	Acetate	C ₃₁ H ₅₂ O ₂	0.40	0.22	0.19	0.18	0.48	0.83
2.	Propionate	C ₃₂ H ₅₄ O ₂	0.53	0.33	0.26	0.25	0.53	0.84
3.	Butyrate	C ₃₃ H ₅₆ O ₂	0.55	0.36	0.29	0.26	0.56	0.85
4.	Caproate	C ₃₅ H ₆₀ O ₂	0.70	0.41	0.33	0.31	0.59	0.86
5.	Caprylate	C ₃₇ H ₆₄ O ₂	0.75	0.46	0.39	0.37	0.60	0.88
6.	Pelargonate	C ₃₈ H ₆₆ O ₂	0.74	0.48	0.40	0.36	0.59	0.87
7.	Caprate	C ₃₉ H ₆₈ O ₂	0.76	0.50	0.42	0.38	0.62	0.88
8.	10-Undecenoate	C ₄₀ H ₆₈ O ₂	0.70	0.46	0.35	0.34	0.61	0.87
9.	Laurate	C ₄₁ H ₇₂ O ₂	0.80	0.53	0.43	0.40	0.63	0.90
10.	Myristate	C ₄₃ H ₇₆ O ₂	0.82	0.54	0.44	0.41	0.64	0.90
11.	Palmitate	C ₄₅ H ₈₀ O ₂	0.85	0.54	0.47	0.43	0.65	0.91
12.	Stearate	C ₄₇ H ₈₄ O ₂	0.88	0.55	0.50	0.44	0.66	0.92
13.	Oleate	C ₄₇ H ₈₂ O ₂	0.86	0.53	0.45	0.42	0.65	0.91
14.	Linolate	C ₄₇ H ₈₀ O ₂	0.89	0.56	0.52	0.45	0.67	0.92
15.	Arachidonate	C ₄₉ H ₈₀ O ₂	0.92	0.58	0.54	0.49	0.69	0.93

Reagent (a) gave violet spots, except for β -sitosteryl pelargonate which gave an orange spot. Reagent (b) gave blue spots against a yellow background.

The R_F values of the β -sitosteryl esters are summarized in Table I.

In order to separate mixtures of β -sitosteryl esters, two-dimensional chromatography was employed, using the solvent systems No. 2 and 4. The starting line was drawn at a distance of 3 cm from the base line and the mixture was developed by ascending chromatography. After the solvent had traveled 14 cm, the plate was removed, dried and developed in a second direction.

The following three mixtures of esters have been studied (Table II):

A: 1, 2, 7, 13, 14, 15

B: 1, 6, 8, 9, 11, 12

C: 1, 2, 3, 4, 5, 7, 10, 14, 15.

TABLE II

No.	Ester of β -sitosterol	R_F in solvent system			
		2		4	
		A	B	B	C
1.	Acetate	0.09	0.10	0.10	0.10
2.	Propionate	0.25			0.13
3.	Butyrate				0.15
4.	Caproate				0.20
5.	Caprylate				0.22
6.	Pelargonate		0.27	0.24	
7.	Caprate	0.28			0.24
8.	10-Undecenoate		0.30	0.28	
9.	Laurate		0.37	0.34	
10.	Myristate				0.26
11.	Palmitate		0.38	0.35	
12.	Stearate		0.34	0.32	
13.	Oleate	0.32			
14.	Linoleate	0.34			0.28
15.	Arachidonate	0.35			0.30

SUMMARY

Fifteen esters of β -sitosterol have been separated by thin-layer chromatography on silica gel G plates, using six solvent systems.

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