SEPARATION OF VITAMINS A_1 AND A_2 AND ALLIED COMPOUNDS BY THIN-LAYER CHROMATOGRAPHY

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Within the last few years, thin-layer chromatography has made very rapid strides in the separation of fat-soluble vitamins and particularly of a few derivatives of vitamin A, kieselgel and alumina being employed as adsorbents[1–7]. A recent report[8] also indicates distinct separation of the isomers of vitamins A_1 and A_2 on kieselgel plates. The present communication describes a simple, rapid and reproducible method for the resolution of vitamin A_1 and A_2 compounds, including the epoxy-derivatives of vitamin A with established structures[9].

EXPERIMENTAL

The modified procedure for preparing thin-layer plates described below is essentially similar to that of STAHL[10,11]. Kieselgel (25.5 g, E. Merck) mixed with plaster of Paris (4.5 g, 300 mesh) in the ratio 85:15 (w/w) is well slurried with 60.0 ml of distilled water. The slurry is then applied on five glass plates (20 × 20 cm) with the help of a special applicator to give a fine thin layer of the adsorbent with a uniform thickness of 0.25 mm. The plates are dried at 120° for one h and stored in big desiccators until used.

Vitamins A_1 and A_2 and the allied compounds under examination are applied with the aid of a 0.1 ml micropipette, either individually or in mixtures, each spot containing about 5–30 μg of the substance. The substances are applied along a straight line on the plate, about 2.5 cm from the bottom, and the spots are spaced at a distance of 3 cm from one another. The chromatogram is developed by the ascending method for about 2 h in a special closed rectangular all-glass chamber, with 200 ml of any one of the following three solvent systems: (a) 6% (v/v) acetone in light petroleum (40–60°), (b) 15 % (v/v) diethyl ether in light petroleum and (c) 3 % (v/v) acetone in iso-octane.

When the solvent front reaches a distance of about 16–18 cm from the point of application of the compounds, the plate is removed from the chamber and allowed to dry at room temperature for a few seconds. Immediate examination of the plate, under ultraviolet light (wavelength 366 mμ) reveals the distinct separation of the various compounds which either fluoresce or absorb, depending on their characteristic property. The boundary of each concentric spot can be very easily marked with the help of a pin and further conclusive characterisation of the various spots is carried out by spraying the chromatogram with $SbCl_3$ reagent (25 % w/v) when the various compounds of the vitamin A_1 and A_2 group give characteristic blue, violet, pink and yellow spots. Alternatively, the various spots located under ultraviolet light can also be

TABLE I

THIN-LAYER CHROMATOGRAPHIC SEPARATION OF VITAMIN A_1 AND A_2 COMPOUNDS

Substance	*Band as observed in U.V. light**	*R_F values***			*$\lambda_{max.}$ in light petrol (mμ)*	*$SbCl_3$ colour-test $\lambda_{max.}$ (mμ)*
		6% (v/v) acetone in light petrol	*15% (v/v) diethyl ether in light petrol*	*3% acetone in iso-octane*		
Anhydrovitamin A_1	Bright yellow fluor.	0.93 ± 0.01	0.82 ± 0.01	0.82 ± 0.01	350, 370, 390	620 (blue)
Vitamin A_1 palmitate	Blue fluor.	0.91 ± 0.01	0.76 ± 0.02	0.79 ± 0.01	325	620 (blue)
Vitamin A_1 acetate	Blue fluor.	0.70 ± 0.02	0.47 ± 0.01	0.49 ± 0.01	325	620 (blue)
Vitamin A_1 aldehyde	Dull black absorb.	0.53 ± 0.01	0.26 ± 0.01	0.21 ± 0.02	370	664 (blue)
Vitamin A_1 alcohol	Blue fluor.	0.20 ± 0.01	0.08 ± 0.02	0.08 ± 0.01	325	620 (blue)
Vitamin A_1 acid	Dull black absorb.	0.00	0.00	0.00	350	573 (red)
Anhydrovitamin A_2	Bright yellow fluor.	0.87 ± 0.02	0.69 ± 0.02	0.61 ± 0.01	350, 370, 390	693 (blue)
Vitamin A_2 acetate	Green fluor.	0.69 ± 0.01	0.45 ± 0.01	0.47 ± 0.01	350	693 (blue)
Vitamin A_2 aldehyde	Dull black absorb.	0.50 ± 0.01	0.33 ± 0.01	0.20 ± 0.01	385	735 (greenish blue)
Vitamin A_2 alcohol	Green fluor.	0.17 ± 0.02	0.08 ± 0.02	0.08 ± 0.01	350	693 (blue)
Vitamin A_2 acid	Dull black fluor.	0.00	0.00	0.00	370	—
5,6-Monoepoxy-vitamin A palmitate	Faint blue fluor.	0.84 ± 0.01	—	—	310, 325	460 (yellow)
5,6-Monoepoxy-vitamin A acetate	Faint blue fluor.	0.68 ± 0.01	—	—	310, 325	460 (yellow)
5,6-Monoepoxy-vitamin A aldehyde	Dull black absorb.	0.44 ± 0.02	—	—	352	440 (yellow)
5,6-Monoepoxy-vitamin A alcohol	Faint blue fluor.	0.07 ± 0.01	—	—	310, 325	465 (yellow)
5,8-Monoepoxy-vitamin A palmitate	Faint blue fluor.	0.81 ± 0.01	—	—	280	460 (yellow)
5,8-Monoepoxy-vitamin A acetate	Faint blue fluor.	0.65 ± 0.01	—	—	280	460 (yellow)
5,8-Monoepoxy-vitamin A aldehyde	Dull black absorb.	0.42 ± 0.01	—	—	317	440 (yellow)
5,8-Monoepoxy-vitamin A alcohol	Faint blue fluor.	0.07 ± 0.01	—	—	280	465 (yellow)
β-Carotene	Orange spot	1.00	1.00	1.00	430, 450, 470	555 (blue)

* Fluor. = fluorescence; absorb. = absorbance.

** Mean values of eight observations.

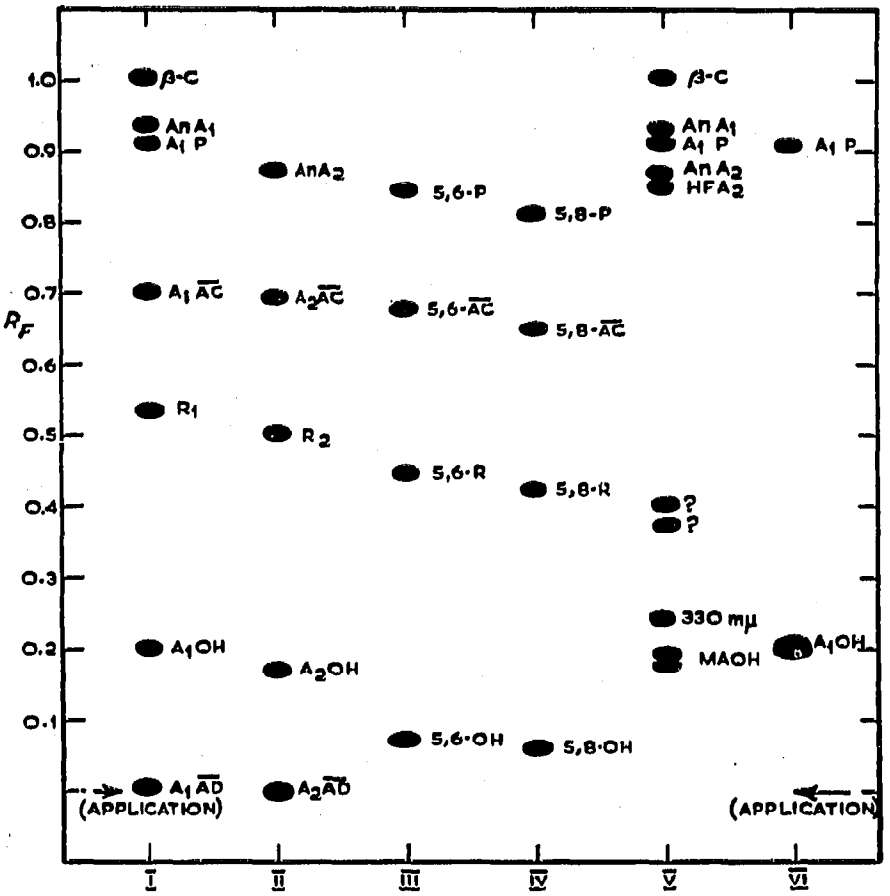

Fig. 1. Schematic representation of the separation of vitamins A_1 and A_2 and allied compounds by thin-layer chromatography, using kieselgel.

Mixture I: β-C = β-carotene; AnA_1 = anhydrovitamin A_1; A_1P = vitamin A_1 palmitate; $A_1\overline{AC}$ = vitamin A_1 acetate; R_1 = vitamin A_1 aldehyde; A_1OH = vitamin A_1 alcohol; $A_1\overline{AD}$ = vitamin A_1 acid.

Mixture II: AnA_2 = anhydrovitamin A_2; $A_2\overline{AC}$ = vitamin A_2 acetate; R_2 = vitamin A_2 aldehyde; A_2OH = vitamin A_2 alcohol; $A_2\overline{AD}$ = vitamin A_2 acid.

Mixture III: 5,6-P = 5,6-monoepoxy-vitamin A palmitate; 5,6-$\overline{AC}$ = 5,6-monoepoxy-vitamin A acetate; 5,6-R = 5,6-monoepoxy-vitamin A aldehyde; 5,6-OH = 5,6-monoepoxy-vitamin A alcohol.

Mixture IV: 5,8-P = 5,8-monoepoxy-vitamin A palmitate; 5,8-$\overline{AC}$ = 5,8-monoepoxy-vitamin A acetate; 5,8-R = 5,8-monoepoxy-vitamin A aldehyde; 5,8-OH = 5,8-monoepoxy-vitamin A alcohol.

V. Wallago Attu liver oil: β-C = β-carotene; AnA_1 = anhydrovitamin A_1; A_1P = vitamin A_1 palmitate; AnA_2 = anhydrovitamin A_2; HFA_2 = higher fatty acid esters of vitamin A_2; 330 mμ = the 330 mμ compound; MAOH = mixture of vitamins A_1 and A_2 alcohols.

VI. Rat-liver oil: A_1P = vitamin A_1 palmitate; A_1OH = vitamin A_1 alcohol.

scraped out and eluted with a diethyl ether–light petroleum mixture (1:1 v/v).

After removal of the solvent under reduced pressure, the absorption spectra of the various compounds in light petroleum were recorded in a Beckman DU spectrophotometer. In addition, to confirm the authenticity of the respective compounds, the $SbCl_3$ colour-test absorption maxima were also recorded in the spectrophotometer as described by CAMA, COLLINS AND MORTON[12].

RESULTS AND DISCUSSION

The R_F values of the various compounds analysed as well as some of their properties are given in Table I. Fig. 1 shows a typical schematic representation of the separation of compounds in a 6 % (v/v) acetone in light petroleum system.

This technique has been successfully applied to the separation of constituents of fish-liver oils and rat-liver extracts. Confirming the separation achieved by column chromatography[13] and reverse phase paper chromatography[14], freshwater fish-liver oil of *Wallago attu* gave nine distinct bands corresponding to β-carotene, anhydrovitamins A_1 and A_2, vitamin A_1 palmitate, higher fatty acid esters of vitamin A_2, two unidentified substances, the uncharacterised compound with $\lambda_{max.}$ at 330 mμ and a mixture of vitamins A_1 and A_2 alcohols. The rat-liver unsaponifiable fraction gave a faint band of vitamin A_1 palmitate and a prominent band of vitamin A_1 alcohol. The presence of trace amounts of vitamin A palmitate in the rat-liver unsaponifiable fraction may well be due to incomplete saponification. Furthermore, the 5,6- and 5,8-monoepoxy-vitamin A group of compounds also gave distinct separations agreeing very well with respect to the palmitate, acetate, aldehyde and alcohol forms.

Thus, thin-layer chromatography as applied to the vitamin A group of compounds has the advantage of clear and complete resolution of individual components from a mixture in micro and macro quantities without any trailing whatsoever. Further, as it is much less time-consuming, it has the added advantage that the decomposition of the generally extremely unstable and labile vitamin A_1 and A_2 group of compounds is prevented. Attempts to apply the above method quantitatively were, however, unsuccessful.

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SUMMARY

A simple and rapid method for the separation of vitamins A_1 and A_2 and allied compounds by thin-layer chromatography using kieselgel has been described. The method, however, cannot be applied for quantitative estimation.

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