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**Note**

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# Separation of β-apocarotenals and related compounds by reversed-phase paper and thin-layer chromatography

The separation and characterization of vitamins $A_1$ and $A_2$ and related compounds by reversed-phase paper chromatography as well as by thin-layer chromatography have been reported earlier[1,2]. Thin-layer chromatography has also been used for the separation and characterization of carotenoids from natural sources[3,4]. However, BOLLIGER[5] observed that carotenoid mixtures cannot be separated on a single adsorbent with a single solvent. The separation and determination of carotenoid aldehydes from plants, microorganisms and animal tissues have been carried out by means of thin-layer chromatography[6]. Apocarotenals and apocarotenoic acid have been detected in oranges by the same technique[7,8].

Various apocarotenals and their epoxides were prepared and characterized in this laboratory[9,10]. Different apocarotenoic acids were prepared from their corresponding apocarotenals according to the procedure of BARUA AND NAYAR[11] and

TABLE I

REVERSED-PHASE CIRCULAR PAPER CHROMATOGRAPHIC RESOLUTION OF APOCAROTENALS AND RELATED COMPOUNDS

| *Compound* | *λmax. in light petroleum (nm)* | *$SbCl_3$ colour test λmax. (nm)* | *$R_F$ value*[a] |
|---|---|---|---|
| β-Apo-8′-carotenal | 455 | 825 (light blue) | 0.26 |
| β-Apo-10′-carotenal | 437 | 770 (bluish green) | 0.38 |
| β-Apo-12′-carotenal | 410 | 710 (bluish green) | 0.43 |
| 5,6-Epoxy-β-apo-8′-carotenal | 430, $\overline{450}$[b], 477 | 600 (blue) | 0.49 |
| 5,8-Epoxy-β-apo-8′-carotenal | 405, $\overline{430}$, 455 | 645 (blue) | 0.51 |
| 5,6-Epoxy-β-apo-12′-carotenal | $\overline{403}$, 423 | 555 (violet) | 0.69 |
| 5,8-Epoxy-β-apo-12′-carotenal | $\overline{380}$, 400 | 555 (violet) | 0.81 |
| β-Apo-8′-carotenyl acetate | $\overline{425}$, 450 | 785 (light blue) | 0.19 |
| 5,6-Epoxy-β-apo-8′-carotenyl acetate | 395, $\overline{418}$, 440 | 695 (deep blue) | 0.26 |
| 5,8-Epoxy-β-apo-8′-carotenyl acetate | 378, $\overline{395}$, 420 | 695 (deep blue) | 0.41 |
| β-Apo-8′-carotenoic acid | 450 | 820 (light blue) | 0.36 |
| β-Apo-10′-carotenoic acid | 425 | 755 (light blue) | 0.60 |
| β-Apo-12′-carotenoic acid | 400 | 685 (deep blue) | 0.58 |
| 5,8-Epoxy-β-apo-8′-carotenoic acid | 420 | 645 (deep blue) | 0.72 |
| 5,8-Epoxy-β-apo-12′-carotenoic acid | 370 | 555 (violet) | 0.95 |
| Retinoic acid | 350 | 560 (violet) | 0.88 |
| 3-Dehydroretinoic acid | 295, $\overline{370}$ | 628 (blue) | 0.94 |
| Retinal | 370 | 640 (blue) | 0.76 |
| 3-Dehydroretinal | 390 | 680 (blue) | 0.84 |
| β-Carotene | 430, $\overline{450}$, 470 | 960 (light blue) | 0.00 |

[a] Mean of six observations.
[b] (—) denotes the main band of spectral absorption.

characterized spectroscopically. In the present communication, a method for the resolution and identification of various $\beta$-apocarotenals and related compounds is described.

*Reversed-phase circular paper chromatography*

Circular paper chromatography on filter-paper (Whatman No. 1) impregnated with 3 % of vaseline using 90 % methanol as the solvent system, was carried out[12]. The chromatogram was run for 8–9 h. A definite separation of a number of $\beta$-apocarotenals and allied compounds was achieved (Table I, Fig. 1). Various bands were eluted with light petroleum–diethyl ether (1:1) and the absorption spectra were recorded on a Beckman DU spectrophotometer. The absorption spectra of the chromogens of the compounds formed with antimony trichloride reagent were also checked for identification purposes.

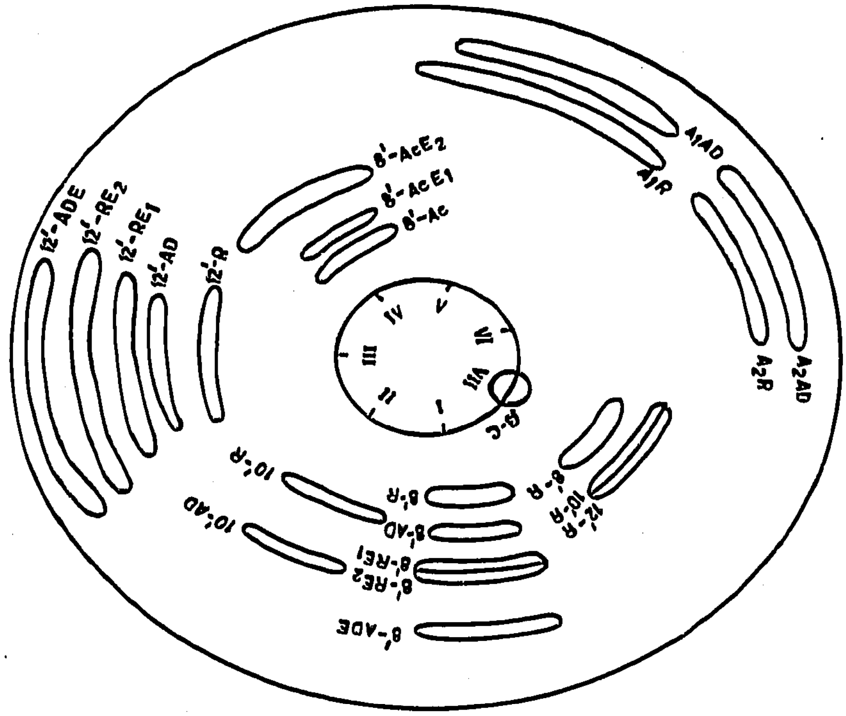


Fig. 1. Schematic representation of the separation of $\beta$-apocarotenals and related compounds by reversed-phase circular paper chromatography. Mixture I: 8'-R = 8'-apocarotenal; 8'-AD = 8'-apocarotenoic acid; 8'-$RE_1$ = 5,6-epoxy-8'-apocarotenal; 8'-$RE_2$ = 5,8-epoxy-8'-apocarotenal; 8'-ADE = 5,8-epoxy-8'-carotenoic acid. Mixture II: 10'-R = 10'-apocarotenal; 10'-AD = 10'-apocarotenoic acid. Mixture III: 12'-R = 12'-apocarotenal; 12'-AD = 12'-apocarotenoic acid; 12'-$RE_1$ = 5,6-epoxy-12'-apocarotenal; 12'-$RE_2$ = 5,8-epoxy-12'-apocarotenal; 12'-ADE = 5,8-epoxy-12'apocarotenoic acid. Mixture IV: 8'-Ac = 8'-apocarotenyl acetate; 8'-$AcE_1$ = 5,6-epoxy-8'-apocarotenyl acetate; 8'-$AcE_2$ = 5,8-epoxy-8'-apocarotenyl acetate. Mixture V: $A_1R$ = retinal; $A_1AD$ = retinoic acid. Mixture VI: $A_2R$ = 3-dehydroretinal; $A_2AD$ = 3-dehydroretinoic acid. Mixture VII: $\beta$-C = $\beta$-carotene; 8'-R = 8'-apocarotenal; 10'-R = 10'apocarotenal; 12'-R = 12'-apocarotenal.

*Thin-layer chromatography*

The procedure for preparing thin-layer plates was essentially similar to that of STAHL[13, 14], which is briefly as follows. Silica gel (40.0 g, National Chemical Laboratory, Poona) was mixed with plaster of Paris (5.0 g, 300 mesh) in the ratio 8:1 (w/w) and stirred with 80 ml of glass-distilled water. The slurry was then applied on to five plates (20 cm × 20 cm) with the aid of a special applicator to give a fine thin layer of the adsorbent with a uniform thickness of 0.5 mm. The plates were dried in air and activated at 120° for 1 h before use.

The compounds under examination were applied as described by JOHN *et al.*[2]. The chromatogram was developed by the ascending method for about 1 h in a closed rectangular glass chamber using 200 ml of the solvent. The solvent systems chosen were 10 % acetone in light petroleum, 25 % diethyl ether in light petroleum and 6 % acetone in cyclohexane. A good separation of apocarotenals and allied compounds was achieved with all three solvent systems. A suitable system, 25 % acetone in light petroleum, was also developed for the separation of various apocarotenoic acids, epoxycarotenoic acids, vitamin $A_1$ acid and vitamin $A_2$ acid (Table II). The exact location of the individual compounds was ascertained by observing the plate in daylight as well as under ultraviolet light. The conclusive characterization of the spots was carried out by spraying the chromatograms with antimony trichloride reagent, when the different compounds gave coloured spots. Alternatively, for identification the various spots located under ultraviolet light were scrapped out and eluted with light petroleum–diethyl ether (1:1), for scanning the spectrum directly or after reaction with antimony trichloride.

TABLE II

THIN-LAYER CHROMATOGRAPHIC SEPARATION OF APOCAROTENALS AND RELATED COMPOUNDS

| *Compound* | *$R_F$ value*[a] | | | |
|---|---|---|---|---|
| | *10% (v/v) acetone in light petroleum* | *25% (v/v) diethyl ether in light petroleum* | *6% (v/v) acetone in cyclohexane* | *25% (v/v) acetone in light petroleum* |
| β-Apo-8′-carotenal | 0.67 | 0.67 | 0.48 | — |
| β-Apo-10′-carotenal | 0.58 | 0.53 | 0.36 | — |
| β-Apo-12′-carotenal | 0.71 | 0.63 | 0.50 | — |
| 5,8-Epoxy-β-apo-8′-carotenal | 0.50 | 0.39 | 0.29 | — |
| 5,8-Epoxy-β-apo-12′-carotenal | 0.55 | 0.42 | 0.32 | — |
| β-Apo-8′-carotenyl acetate | 0.77 | 0.78 | 0.60 | — |
| 5,8-Epoxy-β-apo-8′-carotenyl acetate | 0.61 | 0.56 | 0.38 | — |
| β-Apo-8′-carotenoic acid | 0.00 | 0.00 | 0.00 | 0.22 |
| β-Apo-10′-carotenoic acid | 0.00 | 0.00 | 0.00 | 0.12 |
| β-Apo-12′-carotenoic acid | 0.03 | 0.03 | 0.00 | 0.28 |
| 5,8-Epoxy-β-apo-8′-carotenoic acid | 0.04 | 0.05 | 0.00 | 0.33 |
| 5,8-Epoxy-β-apo-12′-carotenoic acid | 0.05 | 0.07 | 0.03 | 0.42 |
| Retinoic acid | 0.08 | — | — | 0.42 |
| 3-Dehydroretinoic acid | 0.08 | — | — | 0.37 |
| Retinal | 0.65 | — | — | — |
| 3-Dehydroretinal | 0.61 | — | — | — |
| β-Carotene | 1.00 | 1.00 | 1.00 | — |

[a] Mean values of eight observations.

The $R_F$ values of the various compounds analysed by thin-layer chromatography using three solvent systems are given in Table II. Fig. 2 shows a typical schematic representation of the separation of the compounds in 10 % acetone in light petroleum. The thin-layer chromatography as applied to apocarotenals and related compounds has the advantage of giving clear and complete resolution of individual components in micro-amounts in a short period. Attempts to apply this method to the 5,6-epoxides of apocarotenals were unsuccessful owing to their rapid furanization on silica gel.

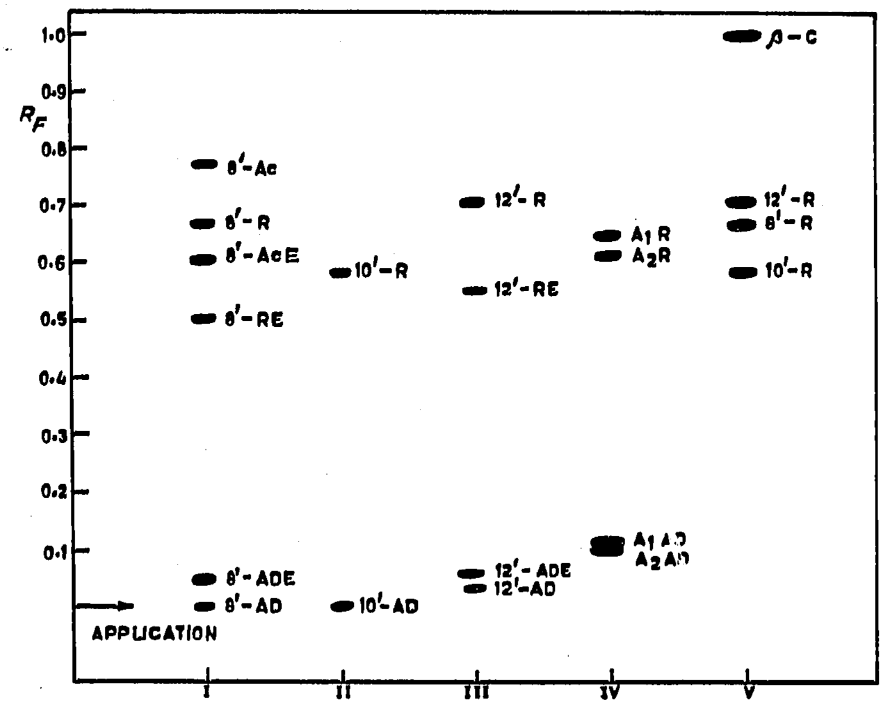


Fig. 2. Schematic representation of the separation of $\beta$-apocarotenals and related compounds by thin-layer chromatography. Mixture I: 8'-AD = 8'-apocarotenoic acid; 8'-ADE = 5,8-epoxy-8'-apocarotenoic acid; 8'-RE = 5,8-epoxy-8'-apocarotenal; 8'-AcE = 5,8-epoxy-8'-apocarotenyl acetate; 8'-R = 8'-apocarotenal; 8'-Ac = 8'-apocarotenyl acetate. Mixture II: 10'-AD = 10'-apocarotenoic acid; 10'-R = 10'-apocarotenal. Mixture III: 12'-AD = 12'-apocarotenoic acid; 12'-ADE = 5,8-epoxy-12'-apocarotenoic acid; 12'-RE = 5,8-epoxy-12'-apocarotenal; 12'-R = 12'-apocarotenal. Mixture IV: $A_2AD$ = 3-dehydroretinoic acid; $A_1AD$ = retinoic acid; $A_2R$ = 3-dehydroretinal; $A_1R$ = retinal. Mixture V: 10'-R = 10'-apocarotenal; 8'-R = 8'-apocarotenal; 12'-R = 12'-apocarotenal; $\beta$-C = $\beta$-carotene.

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