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SHORT COMMUNICATION

CHROMATOGRAPHY OF CAROTENOIDS USING PAPERS FILLED WITH SILICA GEL AND WITH ALUMINA

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Abstract—the R_f values of twenty-two carotenoids separated on Whatman paper Chromedia AH 81 and SG81 are given The R_f values are readily reproducible and separation of a large number of carotenoids can easily be carried out in about half-an-hour

CIRCULAR paper chromatography has been used successfully to separate carotenoids by a number of workers.[1,2] Arpin and Liaaen-Jensen[3] used a silica-impregnated paper (Whatman SG 81) in an attempt to separate the *cis*- and *trans*-compounds gazaniaxanthin and rubixanthin. Jensen and Liaaen-Jensen[2] obtained good separation of carotenoids with a polarity greater or equal to that of β-carotene on papers impregnated with kieselguhr They also found that papers impregnated with alumina were very good for those carotenes with a polarity equal to or less than β-carotene There is little other information on the use of two commercially available impregnated papers (Whatman's Chromedia AH 81 and SG 81) and it was decided to investigate the suitability of these papers for separating carotenoids. AH 81 is loaded with aluminium hydroxide equivalent to 7 5% Al_2O_3 and SG 81 with 22% SiO_2.

Circular chromatography was carried out using Rutter's[4] method with minor modifications with a circle 16 cm in diameter, the carotenoids being applied to the centre of the paper as a very thin arc The appropriate solvent was then carefully added to the 14 cm Petri dish so that the folded centre strip was dipping in the solvent The lid was placed in position, development took place for 20–30 min and R_f values determined The different bands obtained were immediately cut out, packed in small capillary tubes with fine elongated ends and the pigment eluted with acetone or methanol The R_f values of 22 carotenoids separated on the two papers are given in Table 1 The solvents used were *n*-hexane and increasing concentrations of acetone in *n*-hexane

Very few of the less polar carotenoids move in pure *n*-hexane but there is good separation between α-, β-, γ-carotenes and lycopene The R_f values, however, indicate that there is different adsorption by the two papers, e g phytofluene moves behind α-carotene on SG 81 and in front of α-carotene on AH 81 A number of compounds move in 1% acetone in *n*-hexane and there is very good separation especially with AH 81, whereas monoepoxy-α-carotene, diepoxy-β-carotene and cryptoxanthin have R_f values of between 0·25 and 0·29

[1] A JENSEN, *Acta Chem Scand* **14**, 2051 (1960)

[1a] A JENSEN, in Carotine und Carotinoide (edited by K LANG), Dietrich Steinkopff, Darmstadt (1963), N ARPIN, *Les Caroténoïdes des Discomycètes* Thèse de Doctorat *Service de Phytochimie et Phytophysiologie, Lyon* (1968), J -L FIASSON, *Les Caroténoïdes des Basidiomycètes* Thèse de Doctorat *Service de Phytochimie et Phytophysiologie, Lyon* (1968)

[2] A JENSEN and S LIAAEN-JENSEN, *Acta Chem Scand* **13**, 1863 (1959).

[3] N ARPIN and S LIAAEN-JENSEN, *Phytochem* **8**, 185 (1969)

[4] L RUTTER, *Nature, Lond* **161**, 435 (1948)

TABLE 1 R_f VALUES OF CAROTENOIDS ON AH 81 AND SG 81 PAPERS

Carotenoid	R_f value Concentrations of acetone in *n*-hexane 0%		1%		5%	
	SG81	AH81	SG81	AH81	SG81	AH81
Phytofluene*	0 40	0 78	—	—		
α-Carotene†	0 45	0 73	—	—		
β-Carotene†	0 35	0 50	1 00	1 00		
γ-Carotene†	0 20	0 08	0 47	0 50		
Lycopene†	0 10	0 11	0 42	0 37		
ζ-Carotene*			1 00	1 00		
5,6-Monoepoxy-α-carotene*			0 25	0 31		
5,6-Monoepoxy-β-carotene*			0 38	0 46		
5,6 5′,6′-Diepoxy-β-carotene*			0 27	0 60		
Cryptoxanthin†			0 29	0 64		
Rubixanthin‡	0 00	0 08	0 22	0 41		
Mutatochrome*			0 00	0 00	0 61	0 61
Flavochrome*			0 00	0 00	0 65	0 64
Torulene¶			0 32	0 26	—	—
Canthaxanthin†					0 20	0 21

	5%		15%		20%	
Canthaxanthin†	0 20	0 21	0 60	1 00	1 00	1 00
Violaxanthin‡			—	—	0 35	0 32
Rhodoxanthin§			—	—	0 60	0 62
Lutein*			0 28	0 25	0 53	0 60
Taraxanthin*			0 71	0 66	0 80	0 81
Zeaxanthin*			—	—	0 85	0 64
Flavoxanthin*			0 62	0 50	—	—
Neoxanthin‖			—	—	0 07	0 00

* From various Compositae flowers (L R G VALADON and R S MUMMERY, *Phytochem* **10**, (1971))
† Synthetic product from Hoffman–La Roche & Co Ltd , Switzerland
‡ From rose hips (L R G VALADON and R S MUMMERY, *Ann Bot* **33**, 671 (1969))
§ From *Taxus baccata* (P KARRER and E JUCKER, *Carotenoids*, Elsevier, Amsterdam (1950))
‖ From *Dimorphotheca aurantiaca* (L R G VALADON and R S MUMMERY, *Phytochem* **6**, 983 (1967))
¶ From *Rhodotorula rubra* (P KARRER and E JUCKER, *Carotenoids*, Elsevier, Amsterdam (1950))

in SG 81, they are 0·31, 0 60 and 0 64 in AH 81 The compounds that are separated in this solvent are carotenes, 5,6 and 5,6,5′,6′ epoxycarotenes and monohydroxyxanthophylls but not 5,8 and 5,8,5′,8′ furanoid epoxides of carotenes which are stationary at the starting line

As the solvent is increased to 5% acetone in *n*-hexane, the furanoid oxides mutatochrome and flavochrome are well separated and also the diketocompound canthaxanthin The R_f values of the carotenoids on the two papers in this solvent are very similar, and similarly for the other xanthophylls when 15 and 20% acetone in *n*-hexane are used The two papers are therefore equally good for the separation of the more polar xanthophylls The R_f values are readily reproducible and the rapidity of the technique and the ready availability of these two papers make them commendable for the separation of a whole range of carotenoids

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Key Word Index—Carotenoids, chromatography, silica filled paper, alumina filled paper