satisfactory since it can be treated with sulphuric acid and exposed to high charring temperatures without the danger of breakage experienced by the use of glass plates.

Vapor phase chromatography on column 1 provided an excellent method for separation of the alkoxyaminocyclohexanols and aminocyclohexanols. Most noteworthy in this connection is the capability of the procedure to separate the three methoxyaminocyclohexanols on a preparative scale, thereby providing a suitable means for the isolation of the difficultly accessible 1 α-methoxy-2 α-amino-3 β-hydroxycyclohexane[1] in a pure condition. This column was not satisfactory for separation of the acetylated derivatives but the latter compounds were resolved when column 2 was used. The relatively short retention times achieved by the use of this column made for rapid analysis and the method was convenient for checking the products from the degradation of the di-N-acetylamino compounds. This column was not satisfactory, however, for the separation of the alkoxyacetylaminocyclohexanols from their O-acetyl derivatives (*e.g.* 13 and 15) but these compounds are readily separable by the thin-layer chromatographic method mentioned earlier.

It is evident from Table I that by selecting one of the three chromatographic methods examined, any individual member of any of these groups of isomers can be separated. These results underline the importance of concurrent examination of several chromatographic techniques when difficulty is encountered in the separation of isomers by more conventional means.

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Stabilization of xanthophyll and carotene by ethoxyquin during thin-layer chromatography

The use of thin-layer adsorption chromatography (TLC) for the quantitative and qualitative determination of xanthophyll and carotene is limited due to the rapid oxidation and isomerization of these compounds during TLC. Employment of impregnated and reverse-phase TLC[1] as well as saturated solvent chambers[2] has eliminated some of these losses, however, these procedures are more complex and require more time and equipment than the more common technique of adsorption chromatography. Recently, a procedure was described for retarding autoxidation of lipids

during TLC by the addition of antioxidants to the developing solvent[3]. Previously, ethoxyquin (6-ethoxy-2,2,4-trimethyl-1,2-dihydroquinoline) has been shown to be an effective antioxidant for carotene[4] and xanthophyll[5] in dehydrated alfalfa meal. This present paper describes the stabilization of these carotenoids during adsorption TLC by the addition of ethoxyquin.

Materials and methods

A slurry of Silica Gel G*–calcium hydroxide (1:6 by wt) in 50 ml of water, was applied with a mobile applicator as a 250 μ layer to glass plates, 20 cm × 20 cm, and activated at 105° for 30 min. After preparation the plates were left in the laboratory atmosphere with a relative humidity of approximately 50 % until used.

Extracts of dehydrated alfalfa meal were prepared according to the procedure of KOHLER *et al*[6] and concentrated under reduced pressure to a final concentration equivalent to 0.5 g meal/ml of extract. Aliquots of the extracts were applied to the chromatoplates while under dim light, and the chromatograms developed in the dark at room temperature with benzene–1-butanol (100:2, v/v). Five levels of redistilled ethoxyquin were added to the developing solvent (Table I).

TABLE I

% OF CAROTENOIDS RECOVERED FROM THIN-LAYER CHROMATOGRAMS

Carotenoid	*Spectral adsorption maxima*, mμ*	R_F	*mg of ethoxyquin per 200 ml developing solvent*				
			0	*150*	*450*	*900*	*1500*
Neoxanthin	466, 438, 418	0.07	35	97	99	99	97
Xanthophyll No. 1	470, 443, 426	0.23	< 1	79	91	100	100
Violaxanthin	469, 441, 417	0.31	< 1	73	81	80	82
Xanthophyll No. 2	471, 445, 424	0.48	< 1	58	87	97	95
Lutein	474, 446, 421	0.60	59	96	100	100	100
β-Carotene	477, 449, 421	0.87	84	88	94	97	100

* In hexane–acetone (7:3).

Six carotenoid spots were separated as shown in Fig. 1. Following drying of the developed plate, initial carotenoid content was determined by immediately scraping the spots from the chromatoplate and eluting the carotenoids with hexane–acetone (7:3). Related developed and dried plates were stored in the dark with exposure to the air for 2 h, prior to the scraping and elution of the carotenoid spots. The absorbance of the filtered eluates was measured in a spectrophotometer at 475 mμ for the xanthophyll (neoxanthin at 445 mμ) and 436 mμ for the β-carotene, employing the absorption coefficients of 236 and 196, respectively.

Results and discussion

The use of ethoxyquin as an antioxidant during thin layer chromatography has been useful in the quantitative determination of carotenoids. At the higher levels of ethoxyquin almost complete stability was obtained. Lutein was the most stable of the hydroxylated carotenoids, however, carotene was more stable without antioxidant treatment than any of the xanthophylls. The least stable of the xanthophylls was violaxanthin. This is apparently due to violaxanthin being a dihydroxy diepoxide

* Reference to a company or product name does not imply approval or recommendation of the product by the U.S. Department of Agriculture to the exclusion of others that may be suitable.

and hence more labile. Neoxanthin, a trihydroxy, monoepoxide[7], suffered only a 3 % loss at the high level of ethoxyquin, However, due to isomerization the main spectral peak was shifted from 465 mμ to 440 mμ. The absorbance measured at 445 mμ did not show the effect of this shift.

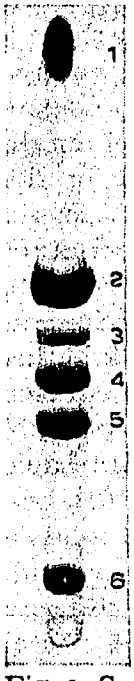

Fig. 1. Separation of dehydrated alfalfa xanthophylls and carotene by TLC. 1 = β-Carotene; 2 = lutein; 3 = xanthophyll No. 2; 4 = violaxanthin; 5 = xanthophyll No. 1; 6 = neoxanthin.

In the solvent system employed, only a very slight separation of zeaxanthin from lutein, and cryptoxanthin from β-carotene was achieved. Since zeaxanthin and cryptoxanthin account for less than 10 % of the total xanthophylls in dehydrated alfalfa[8], they were not isolated in the present study, but were included in the lutein and β-carotene fractions, respectively.

In addition to acting as an antioxidant the added ethoxyquin may also act as an antacid to counteract any acidity of the Silica Gel G particles. This, of course, would prove very advantageous during chromatography of the acid labile epoxide containing xanthophylls.

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