

CHROM. 6033

Magnesium hydroxide as a thin-layer chromatographic adsorbent

III. Application to separations of vitamin A and related carotenoids

Magnesium hydroxide is a thin-layer chromatographic adsorbent of considerable potential utility in the resolution of organic chemical mixtures^{1,2}. Among its many advantages is its remarkable, perhaps unique, sensitivity to the nature of the π -electron systems of aromatic adsorbates, a factor which can lead to separations not possible with more commonly used chromatographic media.

In order to determine this adsorbent's applicability to non-aromatic systems, we have investigated the chromatographic behavior of several carotenoids and vitamin A derivatives. The data for these polyolefinic compounds, like those of the polynuclear aromatic¹ and azaaromatic² compounds studied previously, have confirmed the importance of π -electrons as determinants of adsorbability on magnesium hydroxide. In addition, the influence of various oxygenated functional groups on chromatographic mobility has been studied. Structure-adsorbability relationships have been derived and compared with those previously deduced for the column chromatography of carotenoids on magnesia, and the observed similarities have been exploited in devising a superior system for the preparative thin-layer chromatographic separation of α - and β -carotene mixtures.

Experimental

Thin-layer chromatographic data were collected essentially as previously described¹.

Retinol, retinal, retinyl acetate, and β -carotene were obtained from Fisher Chemical Company (St. Louis, Mo. and Silver Spring, Md.) and bixin, crocin, and crocetin were purchased from Fluka, AG (Buchs, Switzerland).

Isolation of α -carotene. A commercially available carotene mixture (Mann Research Laboratories, New York, N.Y.) containing 15% of the α isomer was dissolved in chloroform and applied in streaks to twenty (20 \times 20 cm) 1-mm magnesium hydroxide plates at a sample load of 4 mg of the mixture per plate. After development in carbon disulfide for 30 min, the band corresponding to α -carotene was scraped off, placed in a small column and eluted with ethanol-chloroform (75:25). The eluate was evaporated at reduced pressure, and the residue was crystallized twice from benzene-methanol, m.p. 178.5–179.5° (ref. 3 m.p. 184°).

Isolation of lycopene. A sample of tomato paste weighing 170 g was mixed with 250 ml of methanol and filtered after standing overnight in the cold. The filter cake was twice shaken for periods of 1 h with a mixture of 100 ml of isooctane and 100 ml of methanol, then with a mixture of 100 ml of isooctane with 50 ml of methanol, and finally with 100 ml of cyclohexane. The isooctane and cyclohexane layers were combined and extracted three times with 50-ml portions of water, dried over sodium sulfate, and evaporated under reduced pressure. The residue was crystallized from hot benzene by dropwise addition of methanol, m.p. 164° (ref. 4, m.p. 174°).

Results

The chromatographic data for a series of carotenoids (Table I) show that

TABLE I

 R_F VALUES FOR CAROTENOID DERIVATIVES ON MAGNESIUM HYDROXIDE

Compound	Carbon disulfide	Pyridine	Tetrahydrofuran	Ethanol
α -Carotene	0.43	0.74	0.70	0.82
β -Carotene	0.29	0.74	0.70	0.82
Lycopene	0.02	0.75	0.05	0.08
Crocin	a	a	a	a
Crocetin	a	a	a	a
Bixin	a	a	a	a

^a Compound retained at the origin.

magnesium hydroxide is quite sensitive to π -electronic structure in these non-aromatic molecules. The R_F value of α -carotene is 50 % greater than that of the isomeric β derivative in carbon disulfide, suggesting that there is an increase in adsorption energy upon bringing the isolated double bond into conjugation with a polyene system located elsewhere in the molecule.

The very small R_F values of lycopene relative to those of the carotenes in most solvents are probably determined by a combination of factors. In the first place, the π -electrons of all olefinic linkages should contribute significantly to the adsorption energy, even when unconjugated; thus lycopene, with eleven conjugated and two isolated double bonds, should be more strongly adsorbed than the isomeric β -carotene, which has eleven conjugated and no non-conjugated double bonds. In addition, steric interactions such as those attributable to the quasi-axial methyl groups of the cyclohexenyl rings of the carotenes undoubtedly tend to prevent these molecules from approaching the adsorption sites normally. Isomerization to the ring-opened lycopene can be expected to relieve at least part of this steric hindrance, and, as a consequence, to lead to stronger adsorption. Finally, a "flatness effect" may be operative here. This interesting concept has been described by NICOLAIDES⁵, who invoked it to explain the small R_F values of saturated steroidal esters relative to those of saturated straight-chain esters of similar molecular weight.

Introduction of polar substituents into these hydrocarbons also decreases their mobility. In the extreme case, crocetin and bixin were retained at the origin even with the most polar and basic developing agents. These compounds possess free carboxylic acid functions, and are quite possibly ionized by the basic adsorbent. Crocin, the di-gentobiose ester of crocetin, also shows the marked effects of exagger-

TABLE II

 R_F VALUES OF SOME VITAMIN A DERIVATIVES ON MAGNESIUM HYDROXIDE

Compound	Benzene	Carbon tetrachloride	Carbon disulfide
Retinol (vitamin A)	0.29	0.05	0.03
Retinal	0.61	0.22	0.15
Retinyl acetate	0.75	0.48	0.39

ated polar substitution; with fourteen free hydroxyl groups in the sugar residues, this compound is also retained at the point of application.

The relative contributions of various oxygenated substituents to total adsorption energy are better illustrated with the data of Table II. Vitamin A contains a single, free hydroxyl group in addition to a 10-carbon conjugated olefinic system, but it is more strongly adsorbed than β -carotene, which has a 22-carbon polyene chain and no hydroxyl groups. Esterification of the hydroxyl function, as in retinyl acetate, greatly masks its retarding influence, the R_F value of vitamin A being consistently smaller than that of the corresponding acetate. The chromatographic polarity of the aldehyde function (*cf.* the R_F value of retinal) appears to be intermediate between those of the free and esterified hydroxyl groups.

Discussion

The structural factors which determine adsorbability on thin layers of magnesium hydroxide appear to parallel those which govern separability in column chromatography on magnesia. Thus, STRAIN⁶ has noted the marked influence of the number and location of double bonds (as well as hydroxyl groups) on the course of carotenoid separations on magnesia columns. SABACKY *et al.*⁷ have pointed out that benzene is a more powerful eluent than acetone or ether on magnesia; this is the opposite of the eluotropic order observed with all other adsorbents (except magnesium hydroxide); they have interpreted this fact in terms of the great importance of double bonds relative to polar groups as determinants of adsorbability on magnesia. Finally, STRAIN⁸ has observed that a relatively small quantity of magnesia is sufficient for the chromatography of large amounts of substrate (*cf.* earlier reports of the high capacity^{1,2} of magnesium hydroxide).

On the other hand, KARTNIG AND MIKULA⁸ have achieved migration of various carboxylic acids on thin layers of magnesium oxide. This result stands in contrast to our own observation that bixin and crocetin fail to migrate even with pyridine or ethanol as developer, and we must emphasize that magnesium hydroxide and magnesium oxide are quite probably different adsorbents, even after suspension in water during plate preparation*. Nevertheless, there are clear similarities between them, as demonstrated above, and we suggest that the wealth of published information^{10,11} on the magnesia column chromatography of carotenoids should be most helpful in attempting to devise conditions for a given thin-layer chromatographic separation**. The use of this approach to the solution of a specific separation problem is illustrated in the following section.

Application

Pure α -carotene did not appear to be commercially available at the time this study was undertaken, and we were faced with the problem of isolating a reference sample of this material from a commercially supplied mixture of the α and β isomers, which contained only 15% of the former. The most promising literature method

* Some data on the chemical hydration of magnesium oxide have been presented by CHOWN AND DEACON⁹.

** Results with alumina columns might also furnish relevant data; SNYDER¹² has observed that alumina and magnesia resemble each other quite closely, except for the stronger relative adsorption of the carbon-carbon double bond on the latter adsorbent.

for the thin-layer chromatographic separation of this mixture* seemed to be that of STAHL *et al.*, who completely separated these isomeric compounds in one pass on calcium hydroxide using a multicomponent** developer¹⁴. Our attempts to effect this separation on a preparative scale consistently led to failure, however. After noting STRAIN's¹⁵ observation that "of the numerous adsorbents tested for the separation of carotenes, magnesium oxide possesses the greatest number of desirable properties", we attempted the separation using magnesium hydroxide thin-layer chromatography. Preliminary experiments showed that the isomers could be separated completely using carbon disulfide as developer (Table I), and a total of 80 mg of the mixture was resolved on twenty plates (20 × 20 cm; layer thickness, 1 mm). Crystalline α -carotene was obtained by elution of the faster moving band.

This easy separability, together with the additional advantages of high capacity, excellent layer durability, and experimental convenience, have led us to recommend magnesium hydroxide/carbon disulfide as the thin-layer chromatographic system of choice in the separation of α - and β -carotene.

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* BOLLIGER *et al.*¹³ have reported separating six carotene isomers at once on thin layers of magnesium oxide, but this adsorbent has not yielded suitable plates in our hands.

** Pentane-hexane-heptane-octane-undecane-methylene chloride (570:190:95:57:38:50).

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