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Molecular Structure and Adsorption Sequences of Carotenoid Pigments

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The sequence in which carotenoid pigments form bands in Tswett adsorption columns depends upon the adsorbent and the solvent.[1,2,3] This dependence of the relative adsorbability of carotenoid pigments upon the different conditions in the columns indicates that there is a complex relationship between adsorbability and molecular structure.

From the viewpoint of kinetics, separation of mixtures in Tswett columns is contingent upon a reversible distribution of the solutes between the solvent and the adsorbent. Variation of the adsorption sequence with different conditions must result from disproportionate variation of the attractive forces among the adsorbent, the solvent and the solutes. In one instance, the adsorption of fucoxanthin and zeaxanthin in columns of sugar, variation of the attraction between solvent and solutes, as indicated by variation of solubility, has seemed responsible for alteration of the adsorption sequence.[2] Other conditions that might alter the adsorption sequence are variation of the adsorbability of particular structural groups of the carotenoid molecules with different solvents or adsorbents; changes in the structure of the carotenoid molecules induced by adsorbents or solvents; variation in the tendency of the solvents to combine with the adsorbent and thus to displace the adsorbed molecules or particular groups of the adsorbed molecules; etc.

In order to gain further insight into the relationship between the structure and the relative adsorbability of organic molecules, adsorption sequences of several carotenoid pigments have been determined under different conditions. These adsorption sequences have then been compared with the principal structural features of the pigment molecules, with the properties of the adsorbents and solvents and with spectral absorption properties of the pigments. In this way clues to the relative adsorbability of various structural groups have been obtained. Conversely, conditions that determine relative adsorbability and separability of similar organic compounds have been more accurately defined.

Materials and Methods

Natural polyene hydrocarbons, the carotenes, and their hydroxy and oxo derivatives, the xanthophylls, were isolated from common sources by means of partition and chromatographic adsorption methods. These polyene pigments, their sources and the method of preparation, most of which have been described previously,[1,2,4,5] are summarized in Table I.

TABLE I

CAROTENOID PIGMENTS, THEIR SOURCES AND METHOD OF PURIFICATION

Pigments	Sources	Adsorbents	Solvents
	Xanthophylls		
Rhodoxanthin	*Cryptomeria japonica*	Sugar	Pet. ether + 4% acetone
Zeaxanthin	Calyx of *Physalis*	Magnesia	Dichloroethane
Lutein	Leaves of barley	Magnesia	Pet. ether + 25% acetone
Cryptoxanthin	Calyx of *Physalis*	Magnesia	Dichloroethane
	Carotenes		
Lycopene	Tomatoes	Magnesia	Pet. ether + 4% acetone
β-Carotene	Carrots	Magnesia	Pet. ether + 4% acetone
α-Carotene	Carrots	Magnesia	Pet. ether + 4% acetone

Rhodoxanthin, a diketo-dehydro-β-carotene was prepared from the red, winter needles of *Cryptomeria japonica*.[6] It was purified by adsorption from petroleum ether solution in columns of sugar and in columns of Celite 501.

Molecules of each of these carotenoids have many features in common.[7,8] Each pigment contains forty carbon atoms which form similar skeletal structures. The chromophoric group of each pigment consists of eleven or more double bonds at least ten of which occur in conjugation. Oxygen atoms in the four xanthophylls occupy the same relative positions in the molecule; hence, variation of the adsorption sequences due to differences in the location of the oxygen atoms in the molecule was avoided.

Except for lycopene, which has aliphatic instead of cyclic terminal structures, molecules of the several carotenoid pigments differ from one another only in the number and arrangement of the double bonds and in the number and function of the oxygen atoms. A summary of the principal structural features of the pigments is presented in Table II.

TABLE II

PRINCIPAL STRUCTURAL FEATURES OF THE CAROTENOID PIGMENTS

Double bonds are indicated by "═"; conjugated double bonds by "conj. ═"

Pigment	Formula	═	Conj. ═	Conj. C═O	—OH	Rings
Rhodoxanthin	$C_{40}H_{50}O_2$	14	12	2	0	2
Zeaxanthin	$C_{40}H_{56}O_2$	11	11	0	2	2
Lutein	$C_{40}H_{56}O_2$	11	10	0	2	2
Cryptoxanthin	$C_{40}H_{56}O$	11	11	0	1	2
Lycopene	$C_{40}H_{56}$	13	11	0	0	0
β-Carotene	$C_{40}H_{56}$	11	11	0	0	2
α-Carotene	$C_{40}H_{56}$	11	10	0	0	2

Adsorbents employed for determination of the adsorption sequences of the carotenoid pigments were powdered sugar (confectioners powdered sugar with 3% starch to prevent caking, grade XXXXXX[2]), heat-treated siliceous earth (Celite 501) and activated magnesium oxide (Micron

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[1] Strain, "Chromatographic Adsorption Analysis," Interscience Publishers, Inc., New York, N. Y., 1942.

[2] Strain, Manning and Hardin, *Biol. Bull.*, **86**, 169 (1944).

[3] Strain, *Ind. Eng. Chem., Anal. Ed.*, **18**, 605 (1946).

[4] Strain, *J. Biol. Chem.*, **105**, 523 (1934).

[5] Strain, "Leaf Xanthophylls," Carnegie Inst. of Washington, Publ. No. 490, 1938.

[6] Lippmaa, "Das Rhodoxanthin, seine Eigenschaften, Bildungsbedingungen und seine Funktion in der Pflanze," K. Mattiesen'i trükikoda, Tartru, 1925.

[7] Mayer and Cook, "The Chemistry of the Natural Coloring Matters," Reinhold Publishing Corp., New York, N. Y., 1943.

[8] Zechmeister, "Carotinoide," J. Springer, Berlin, 1934.

Brand No. 2641) which was usually mixed with twice its weight of Celite in order to accelerate filtration.[1,3,4]

Adsorption columns were prepared by pressing successive portions of the dry adsorbent into the adsorption tubes (1.3 × 25 cm.). Usually a dilute solution of two or more of the pigments in petroleum ether (about 1 mg. per 100 ml.) was filtered through the adsorption column until the uppermost 3 to 5 mm. of the adsorbent was saturated with the pigments (suction about 0.5 atmosphere). Then fresh solvent or the appropriate solvent mixture was filtered through the column until the bands of adsorbed pigment had migrated from 3 to 15 or 20 cm. Comparison of the chromatograms developed in this way revealed the adsorption sequences of the pigments.

Results

Effect of Different Adsorbents on the Adsorption Sequence.—In columns of different adsorbents, mixtures of carotenes and xanthophylls yielded dissimilar adsorption sequences as is shown in Table III. In all the experiments summarized in this table, the major variable was the adsorbent; hence, the several adsorption sequences must be attributed to selective attraction between the adsorbents and the pigments.

TABLE III

ADSORPTION SEQUENCES OF CAROTENOID PIGMENTS IN COLUMNS OF SUGAR, OF CELITE AND OF MAGNESIA

The solvent was petroleum ether plus 4 to 25% acetone. The most adsorbed pigment is listed at the top of each column and the least adsorbed pigment at the bottom. Brackets indicate pigments that were incompletely separated.

Sugar	Celite	Magnesia
(top of column)	*(top of column)*	*(top of column)*
Zeaxanthin	Zeaxanthin	Rhodoxanthin
Lutein	Lutein	Lycopene
Cryptoxanthin	Rhodoxanthin	Zeaxanthin
Rhodoxanthin	Cryptoxanthin	Lutein
Lycopene	Lycopene	Cryptoxanthin
β-Carotene	β-Carotene	β-Carotene
α-Carotene	α-Carotene	α-Carotene

Inseparability of the carotenes in columns of sugar and in columns of Celite as indicated in Table III is due, in part, to their weak adsorbability relative to their concentration. At extremely low concentration carotenes are very weakly adsorbed on Celite from petroleum ether solution. Comparison of the amount of colorless solvent obtained by adsorption of equally dilute solutions of each pigment in separate columns revealed lycopene to be more adsorbed than β-carotene and α-carotene. In order to observe this separation one must adsorb the pigments from petroleum ether solutions so dilute that the bands in the columns are barely visible.

Effect of Different Solvents on the Adsorption Sequence.—Use of different solvents for development of the chromatograms in columns of a given adsorbent also gave different adsorption sequences. Results obtained in columns of powdered sugar are summarized in Table IV. Except for the variation of the adsorbability of rhodoxanthin, the different solvents had little effect upon the relative adsorbabilities of the pigments.

TABLE IV

ADSORPTION SEQUENCES OF CAROTENOID PIGMENTS IN COLUMNS OF SUGAR WHEN DIFFERENT SOLVENTS WERE EMPLOYED FOR DEVELOPMENT OF THE CHROMATOGRAMS

Petroleum ether	Petroleum ether plus 4% acetone	Petroleum ether plus 0.5% propanol
(top of column)	*(top of column)*	*(top of column)*
Rhodoxanthin	Zeaxanthin	Zeaxanthin
Zeaxanthin	Lutein	Lutein
Lutein	Cryptoxanthin	Cryptoxanthin
Cryptoxanthin	Rhodoxanthin	Rhodoxanthin
Lycopene	Lycopene	Lycopene
β-Carotene	β-Carotene	β-Carotene
α-Carotene	α-Carotene	α-Carotene

Use of different solvents for development of the chromatograms in columns of Celite yielded the results summarized in Table V. With minor exceptions these results are similar to those observed in columns of sugar. In the columns of Celite the presence of propanol in petroleum ether had an unusual effect upon the adsorbability of rhodoxanthin relative to the adsorbability of zeaxanthin and lutein. When these pigments were adsorbed from solution in petroleum ether rhodoxanthin formed a band above the zeaxanthin and lutein. Then as the chromatogram was developed with petroleum ether containing 0.5% propanol the rhodoxanthin appeared below the zeaxanthin and lutein, but upon continued washing of the column with the petroleum ether plus propanol the rhodoxanthin band widened and migrated more slowly so that the zeaxanthin and lutein moved below it.

TABLE V

ADSORPTION SEQUENCES OF CAROTENOID PIGMENTS IN COLUMNS OF CELITE 501 WHEN DIFFERENT SOLVENTS WERE EMPLOYED FOR DEVELOPMENT OF THE CHROMATOGRAMS

Petroleum ether	Petroleum ether plus 4% acetone	Petroleum ether plus 0.5% propanol
(top of column)	*(top of column)*	*(top of column)*
Rhodoxanthin	Zeaxanthin	Rhodoxanthin
Zeaxanthin	Lutein	Zeaxanthin
Lutein	Rhodoxanthin	Lutein
Cryptoxanthin	Cryptoxanthin	Cryptoxanthin
Lycopene	Lycopene	Lycopene
β-Carotene	β-Carotene	β-Carotene
α-Carotene	α-Carotene	α-Carotene

In columns of magnesia polar solvents in petroleum ether did not alter the adsorbability of rhodoxanthin relative to the other carotenoid pigments as is shown by the results presented in Table VI.

Effect of Solvents and Adsorbents on Molecular Structure.—Dissolved in polar solvents, many ketonic carotenoid pigments exhibit spectral absorption maxima that are less distinct than the absorption bands of the same pigments

TABLE VI

ADSORPTION SEQUENCES OF CAROTENOID PIGMENTS IN COLUMNS OF MAGNESIA WHEN DIFFERENT SOLVENTS WERE EMPLOYED FOR DEVELOPMENT OF THE CHROMATOGRAMS

Petroleum ether	Petroleum ether plus 25% acetone	Petroleum ether plus 4% propanol
(*top of column*)	(*top of column*)	(*top of column*)
Rhodoxanthin	Rhodoxanthin	Rhodoxanthin
Lycopene	Lycopene	Zeaxanthin
Zeaxanthin	Zeaxanthin	Lycopene
Lutein	Lutein	Lutein
Cryptoxanthin	Cryptoxanthin	Cryptoxanthin
β-Carotene	β-Carotene	β-Carotene
α-Carotene	α-Carotene	α-Carotene

dissolved in nonpolar solvents. This effect, which is not pronounced with carotenes or with hydroxy carotenes,[5] indicates an alteration of the chromophoric structure of the ketonic pigments by the polar solvents.[2,9,10] Analogous effects of polar solvents upon the spectral absorption properties of rhodoxanthin are shown by the characteristic spectral absorption curves of Fig. 1. Compared to the characteristic absorption curves of other xanthophylls plotted on the same scale[2,5] the absorption curves of rhodoxanthin exhibit remarkably indistinct absorption maxima which are shifted to longer wave lengths by the polar solvent.

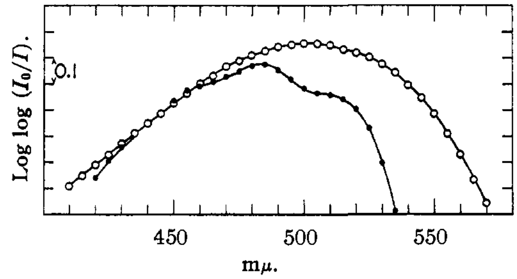

Fig. 1.—Characteristic spectral absorption curves of rhodoxanthin measured at the same concentration in petroleum ether and in 95% ethanol: ●, in petroleum ether; O, in ethanol.

Adsorption of rhodoxanthin either on solids or on liquids also alters the spectral properties of the pigment. Adsorbed on sugar or Celite or magnesia from solution in petroleum ether the keto carotenoid forms a violet-red band; the other carotenoids form orange or orange-red bands. Adsorbed on water droplets by shaking a yellow petroleum ether solution with water, rhodoxanthin causes the emulsion to appear pink. As the emulsion separates, the adsorbed rhodoxanthin liberated at the interface again forms a yellow solution in the petroleum ether.

Discussion

Variation of the adsorbability of the carotenoid pigments in columns of different adsorbents (Table III) can be related to the preferential attraction of the adsorbents for particular structural units in these organic molecules. In columns of sugar adsorbability of the carotenoids is determined to a very limited extent by the conjugated double bonds. Comparison of the structural characteristics of the carotenoids (Table II) with the adsorption sequence observed in columns of sugar (Table III) shows that the single hydroxyl group of cryptoxanthin causes this pigment to be much more adsorbed than the parent hydrocarbon, β-carotene, or the related hydrocarbon lycopene, which contains two additional double bonds. This single hydroxyl group also causes the cryptoxanthin to be much more adsorbed than rhodoxanthin which has three additional unsaturated linkages. Zeaxanthin and lutein, which differ only in the arrangement of one double bond in the molecule, are separable with difficulty in columns of sugar. On the basis of these results sugar is useful as an adsorbent for the separation of carotenoids which differ in the number of hydroxyl or keto groups; it is less useful for separation of pigments which differ only in total unsaturation or in the arrangement of the double bonds.

In columns of magnesia adsorbability of the carotenoids is determined to a large degree by the conjugated double bonds. Comparison of the molecular characteristics of the carotenoids (Table II) with the adsorption sequences (Table III) shows that with magnesia adsorbability of the carotenes increases markedly with the number and with the arrangement of the double bonds, even a small difference in the conjugation of the double bonds being reflected in an appreciable difference in adsorbability as with β-carotene and α-carotene. By contrast with the results obtained in columns of sugar, the two non-conjugated double bonds in lycopene cause this pigment to be more adsorbed than zeaxanthin. These two double bonds in lycopene have, therefore, a slightly greater influence on adsorbability than the two hydroxyl groups in zeaxanthin. Rhodoxanthin with fourteen conjugated, unsaturated linkages is even more adsorbed than lycopene. The small difference between the arrangement of one double bond in zeaxanthin and lutein which has little effect upon the adsorbability of these two pigments in columns of sugar has, by contrast, a great effect upon their adsorbability in columns of magnesia in which they are readily separable. From these experiments, magnesia is much more effective than sugar as an adsorbent for separation of carotenoid pigments which differ principally in the number and arrangement of their double bonds. With respect to adsorption capacity and to the specificity of the adsorption Celite is intermediate between sugar and magnesia (Table III).

Differences between the relative adsorbabilities of carotenoids on sugar and on magnesia must depend upon the nature or quality of the attractive forces of these two adsorbents. Strongly polar, neutral powdered sugar (with eight hydroxyl

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(9) Heilbron and Lythgoe, *J. Chem. Soc.*, 1376 (1936).

(10) Polgar and Zechmeister, THIS JOURNAL, **66**, 186 (1944).

groups and three additional oxygen atoms comprising more than 50% of the molecule) probably attracts the polar hydroxyl groups of the xanthophylls and repels the large, non-polar hydrocarbon portions. Strongly alkaline magnesia, on the other hand, must attract the conjugated polyene system of the xanthophylls as well as the hydroxyl groups.

Polar solvents, which affect the relative adsorbability and the spectral absorption properties of rhodoxanthin, may alter the molecular structure of this pigment, probably through variation of the proportions of the enolic and ketonic modifications. Decrease of the relative adsorbability of rhodoxanthin in columns of sugar by polar solvents (Table IV) corresponds to a decrease in the enolic properties of the keto carotenoid. The slight effect of polar solvents on the relative adsorbability of rhodoxanthin in columns of magnesia (Table VI) indicates that a decrease in enolic properties must be accompanied by an increase in ketonic or polyene properties. This latter conclusion is supported by the effect of polar solvents on the spectral absorption properties of rhodoxanthin as shown already in Fig. 1. Increase in the spectral absorption capacity and shift of the spectral absorption maxima to longer wave lengths by polar solvents are equivalent to an intensification of the ketonic or polyene properties.

In view of the affinity of adsorbents for particular molecular structures, the adsorbability of a complex molecule, as represented by the adsorption isotherm, may be the sum of the adsorbabilities of all the reactive groups. Prediction of the effect of any one group upon the adsorbability of a complex molecule will require some knowledge of the relation between this group and the other reactive centers in the molecule. But it must be remembered that the adsorbability may be influenced by the effect of solvents upon the molecular structure of the adsorbed substance and by the affinity among the solvent, the solute and the adsorbent. Solvents that increase the proportion of those structural groups for which the adsorbent has greatest affinity intensify the adsorbability and *vice versa*. Adsorbability is, as a rule, inversely proportional to solubility. Pronounced affinity between solvent and adsorbent tends to diminish adsorbability of the solute.

These effects of solvents and adsorbents upon the adsorbability of different groups in an organic molecule point the way to new improvements and applications of the chromatographic adsorption method. Information about the adsorbability of particular molecular structures serves as a guide in the selection of solvents and adsorbents for separation of mixtures of various organic compounds and aids prediction of the adsorbability of complex molecules. Solvents and adsorbents may be varied so that adsorption of similar molecules involves the structural features in which the greatest differences reside. Under these conditions, maximum separation of similar substances may be expected.

Summary

Relative adsorbability of seven carotenoid pigments was determined by observation of the adsorption sequence of these polyene compounds in Tswett columns of powdered sugar, of Celite and of magnesia. Petroleum ether, petroleum ether plus acetone and petroleum ether plus propanol were used for development of the chromatograms.

The seven carotenoid pigments, rhodoxanthin, zeaxanthin, lutein, cryptoxanthin, lycopene, β-carotene and α-carotene, are related structurally in such a way that certain molecular features may be regarded as constant while others vary. Comparison of the observed adsorption sequences with the molecular structures of the pigments indicated that the relative adsorbability is determined to a large extent by the selective affinity of the adsorbents for different groups or portions of the pigment molecules. Powdered sugar attracts preferentially the polar hydroxyl groups of the xanthophylls; it has little affinity for the unsaturated hydrocarbon portions of carotenes or xanthophylls. Magnesia, on the other hand, attracts the unsaturated portions of both carotenes and xanthophylls as well as the hydroxyl groups of the xanthophylls. Adsorbents that are most effective for the separation of mixtures of similar carotenoid pigments in Tswett columns attract preferentially those portions of the pigment molecules which contain the greatest structural differences.

Solvents that have a pronounced effect upon the absorption spectrum of the diketonic rhodoxanthin also have a great effect upon the relative adsorbability of this pigment.

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