Low-dose radiation processing can be accomplished at reasonable costs. The processing cost for destruction of trichina in pork carcasses at a radiation dose as low as 15,000 rads would be as low as 0.05 cent per pound, according to Murray's data (*11*). Foods treated at sterilization doses of 3 to 5 megarads, however, would entail a higher processing cost above 2 cents per pound. Radiation sterilization, therefore, at the moment does not present the economic potentialities of those currently recognized in radiation processing with low dose treatment.

Acknowledgment

The authors acknowledge the assistance and contribution of Frederick G. Moote, radiation physicist, Nuclear Radiation Department, Curtiss-Wright Corp., in calculation of some of the data relating to economics of radiation processing.

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Received for review February 9, 1960. Accepted June 27, 1960. Radiation Processing Section, Nuclear Congress, New York, N. Y., April 1960.

FRACTIONATION OF CAROTENOIDS

Two New Solvent Systems for the Countercurrent Distribution of Carotenoids

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Two new solvent systems for the countercurrent distribution of carotenoids have been discovered. A clean separation of the carotenoid diol, monoepoxide diol, diepoxide diol, and polyol fractions can be obtained in 100 transfers with the system petroleum ether and 73.5% methanol. A good system for the further fractionation of the carotenoid polyols consists of petroleum ether, acetone, methanol, and water (1.25 to 1.00 to 0.10 to 0.65 by volume, respectively). Determination of N_{100} values of individual polyol carotenoids with the latter system has considerable value in showing nonidentity, probable identity, or close relationship, as of stereoisomers.

Earlier work (*2*) shows that carotenoids can be fractionated by means of countercurrent distribution in a Craig apparatus. Two different solvent systems were used: I, petroleum ether and 99% methanol and, II, benzene, petroleum ether, and 87% methanol, 1 to 1 to 1.15 by volume.

More recent investigation has led to the discovery of two other useful solvent systems for the countercurrent distribution of carotenoids. By means of system IV (petroleum ether and 73.5% methanol) the diol-polyol fraction can be much more completely resolved into four fractions in 100 transfers than in 200 transfers with system II. System III (see below) can be used to fractionate further the very complex polyol fraction of fruits such as oranges (*4*) or cling peaches (*1*). Determination of N_{100} values with system III was especially useful in showing nonidentity or possible identity of various carotenoid polyols; with systems I, II, or IV, the N_{100} values are too close to zero.

Experimental

Solvent system IV consisted of petroleum ether, methanol, and water, 10 to 7.35 to 2.65, by volume. System III consisted of petroleum ether, acetone, methanol, and water, 1.25 to 1.00 to 0.10 to 0.65 by volume.

Countercurrent distribution runs were carried out in a 100-tube Craig apparatus in which the volume of the lower layer was 10 ml. The volume of the upper layer added in all cases was also 10 ml. The procedure used was essentially that previously described (*2*). At the end of the run, the contents of the various tubes were transferred to numbered test tubes by means of a glass syringe, and diluted with sufficient acetone to make them homogeneous and to a definite volume (50 ml. with systems III and IV). The depth of color was then measured in an Evelyn photoelectric colorimeter using filter 440. The results obtained were calculated as β-carotene by the use of a conversion table. (The resulting values are roughly approximate for most common carotenoids, and are used here mainly to show the positions of the various fractions.) In the case of some individual constituents, with absorption maxima at somewhat shorter wave lengths than 440 mμ, a 420 mμ filter was

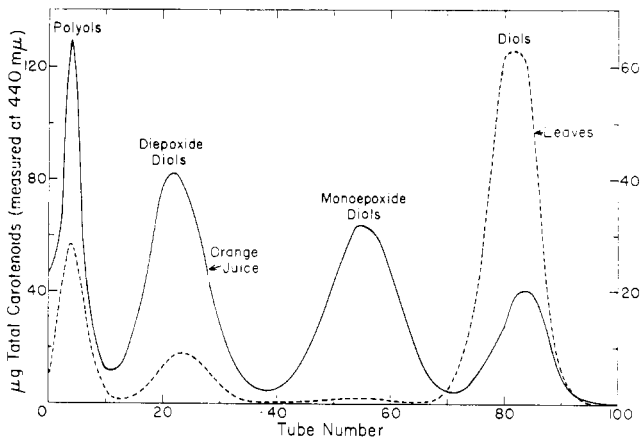

Figure 1. Countercurrent distribution of carotenoids in system IV, petroleum ether–73.5% methanol

Orange juice (scale on left) —— Pyracantha leaves (scale on right) - - - -

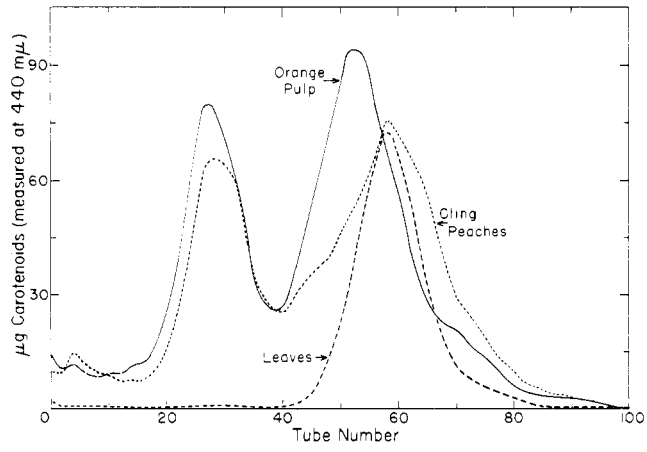

Figure 2. Countercurrent distribution of carotenoid polyols in system III, petroleum ether–acetone–methanol-water, 1.25 to 1.00 to 0.10 to 0.65, by volume, respectively.

Orange pulp —— Cling peaches - - - - Pyracantha leaves – – –

used in order to determine the position of the maximum in the Craig apparatus. Where the absorption maxima were below 420 mμ, the absorbance was measured in a Cary recording spectrophotometer at the wave length of the principal absorption maximum.

The carotenoid mixtures and fractions were obtained from orange juice or pulp (*4*), cling peaches (*7*), red bell peppers, or leaves (*3*). Much of this work was done during the winter months, at which time Pyracantha leaves were a convenient source; Strain (*9*) showed that the pigment mixture of leaves from various angiosperms is quite uniform. The diol-polyol fractions were obtained by countercurrent distribution with system I (or by distribution between petroleum ether and 95% methanol), the polyol fractions by means of system II. In some cases, further resolution of the polyol fraction was achieved by chromatography on magnesia, either Westvaco 2642 with an equal volume of filter aid, or Sea Sorb 43. The latter absorbant was used in columns 7 to 10 cm. in height and 14 mm. in diameter without a diluent. The bands were considerably sharper on Sea Sorb 43; the eluents used (*4*) were the same as on magnesia 2642.

Results and Discussion

Of the considerable number of solvent systems which have been investigated, many systems, some otherwise promising, were unsuitable because of the formation of persistent emulsions. Chlorinated hydrocarbons in combination with aqueous methanol usually formed an appreciable amount of acid, which is very undesirable because of the resulting isomerization of the carotenoid 5,6-epoxides to 5,8-epoxides. Cis-trans isomerization is also catalyzed by acids.

Table I. N_{100} Values of Various Carotenoids in System III—Petroleum Ether 1.25, Acetone 1, Methanol 0.1, Water 0.65, by Volume

No.	Constituent	Type of Epoxide	Source[a]	Spectral Absorption Maxima in Benzene, Mμ	N_{100}[b]
1	Diols	Non	O	...	97
2	Monoepoxide diols	Both	O	...	96
3	Diepoxide diols	Both	O	...	93
4	Capsorubin	Non	BP	521, 486, 459	78
5	Trollein *a*	Non	O	485, 456, 431	73
6	Trollein *b*	Non	O	484, 455, (431)	55
7	Trans-neoxanthin	5, 6	P	482, 450, 425	62
8	Neoxanthin	5, 6	L	476, 446, 421	61
9	Taraxanthin-like	5, 6	P	480, 452, 428	49
10	Trolliflor-like	5, 6	P	481, 450, 425	30
11	Unknown	Non?	P	480, 449, 422	4
12	Neochrome *a*	5, 8	L	459, 431, 406	72
13	Neochrome *b*	5, 8	L	458, 430, 405	70
14	Tarachrome-like	5, 8	O	458, 430, 406	58
15	Trolliflavin-like	5, 8	O	458, 430, 406	38
16	Sinensiaxanthin	5, 6	O	429, 404, 383	46
17	Sinensiachrome	5, 8	O	403, 380, 361	ca. 52
18	Valenciaxanthin	5, 6	O	402, 379, 361	50
19	Persicaxanthin	5, 6	P	401, 378, 360	39–40
20	Unknown	?	P	400, 377, 357	ca. 4
21	Valenciachromes	5, 8	O	375, 353, 336	ca. 54
22	Persicachromes	5, 8	P	373, 353, 336	48

[a] O: Valencia oranges; BP: bell peppers; L: leaves; P: cling peaches.
[b] N_{100}: tube number of maximum per 100 transfers.

System IV. The combined diol-polyol fraction can be separated into diols, monoepoxide diols, diepoxide diols, and polyols within 100 transfers on countercurrent distribution with systems containing petroleum ether, methanol, and water (70 to 85% methanol). The optimum separation was achieved at about 73.5% methanol (Figure 1). The N_{100} values of the polyol, diepoxide diol, monoepoxide diol, and diol fractions in this system were 2, 22, 55, and 83, respectively; the corresponding values with system II were 9, 39, 56, and 70 (*2*). With system IV a much better separation is obtained in 100 transfers than with 200 transfers with system II; considerably larger quantities of material can be used with the latter system.

A convenient method of determining the fractional composition of a carotenoid mixture is to make a 100-transfer run with system I. The diol-polyol fraction (or an aliquot thereof equivalent to 1 or 2 mg., as β-carotene) is then used in a 100-transfer run with system IV. With system I, the sample may be conveniently dissolved in 50 ml. of the upper layer, and five 10-ml. aliquots are added successively to tube 0, or initially to tubes 0 to 4. For system IV, it may be necessary to dissolve the sample in 50 ml. each of the upper and lower layers, and 10-ml. aliquots of each layer are added initially to tubes 0 to 4.

The curve for pyracantha leaf carotenoids (Figure 1) shows the preponderance of the diol fraction, mainly lutein, the

considerably smaller amounts of diepoxide diols and polyols, mainly violaxanthin and neoxanthin, respectively, and the very low content of monoepoxide diols, in good agreement with earlier work (*3*).

System III. A system containing petroleum ether, acetone, methanol, and water in the ratios 0.87 to 1 to 0.14 to 0.25, by volume, respectively, gave separations similar to those with system II. The water content of the former can be increased threefold or even more without excessive emulsion formation. A system in which the partition coefficient of the carotenoid polyol fraction of cling peaches was about 1 (system III) was petroleum ether 1.25, acetone 1.00, methanol 0.10, and water 0.65, by volume. Countercurrent distributions with this system of the carotenoid polyol fractions from Valencia orange pulp, cling peaches, and pyracantha leaves are shown in Figure 2.

The leaf polyols had only one maximum at N_{100} of 59. This is close to the N_{100} value of neoxanthin (Table I), the principal constituent of the polyol fraction of leaves. Neoxanthin was discovered by Strain (*9*) and is apparently a triol 5,6-epoxide (*3*). The curve shows the presence of very little carotenoids elsewhere, except for a minor bump at about tube number 73. The peach polyols had two major maxima with N_{100} values of 60 and 30, which are attributable to the presence of triols (such as neoxanthin) and tetraols, respectively, a minor maximum with N_{100} of 4 (pentaols or hexaols ?), and several minor inflections. The orange polyols also had a complex curve, with major maxima at 54 and 28, and several minor maxima and inflections. By the use of countercurrent distribution with system III, it is thus possible to separate the complex polyol fractions of fruits such as peaches and oranges into two major fractions, and perhaps two minor ones (by combining tubes 0 to about 17, and tubes 70 to 90). These subfractions are more readily resolvable by chromatography than is the entire polyol fraction. The curves in Figure 2 confirm the much greater complexity of the polyol carotenoids of cling peaches and oranges than of leaves (*1*, *3*).

Distribution of Individual Constituents in System III. Another means of fractionating the carotenoid polyols is to chromatograph the entire fraction, and then subject each of the separated bands to a countercurrent distribution with system III. Where the band is a single substance, only one maximum will appear with an N_{100} value characteristic of that substance, which will aid in its identification. In other cases, the distribution may show separation into two or more subfractions having different N_{100} values. The tubes representing these subfractions can then be combined, the samples recovered and dissolved in benzene, and spectral absorption curves run.

By this means, the polyol fractions of Valencia oranges, cling peaches, and leaves were separated into a considerable number of constituents. Eighteen of the more important ones are listed in Table I. For purposes of comparison, data are also included for the diol, monoepoxide diol (mainly antheraxanthin), and diepoxide diol (mainly violaxanthin) fractions of orange juice, and also for capsorubin, a dihydroxydiketocarotenoid obtained from bell peppers. The structure of none of the polyols listed in Table I is completely known.

A number of carotenoids have been found in nature which appear to contain three or more hydroxyl groups, including neoxanthin (*9*), taraxanthin (*5*), trollixanthin (*6*), trolliflor (*7*), fucoxanthin, myxoxanthophyll, and sulfatoxanthin; the complete structure is known only for trollixanthin. With the exception of neoxanthin, a universal constituent of green leaves, none of the above appears to occur as a major constituent in a readily available source. Hence, no authentic preparations of any of the above polyol carotenoids except neoxanthin were available. Trollein *a*, trollein *b*, valenciaxanthin, valenciachromes, sinensiaxanthin, sinensiachrome, persicaxanthin, and persicachromes were found by the present author in oranges or peaches.

In several instances, fractions which appeared to be cis-trans isomers were found to give closely agreeing N_{100} values in system III, such as neoxanthin and transneoxanthin. However, neochromes *a* and *b*, the 5,8-epoxide (furanoid oxide) isomers of neoxanthin, had N_{100} values higher by about 10 than neoxanthin. This behavior appears to be general, as in No. 9 and 14; 10 and 15; 16 and 17; 18 and 21; and 19 and 22 (Table I). This difference in the N_{100} values of 5,6- and the isomeric 5,8-epoxides was not observed in either systems I or II. The N_{100} value of 5,8-epoxide pairs showed no significant difference in the only instance observed, neochromes *a* and *b*.

There are a number of naturally occurring substances which have a spectral absorption curve and maxima which resemble closely those of violaxanthin (main part of No. 3). In Table I are Nos. 7, 9, 10, and 11, also lutein 5,6-epoxide and cryptoxanthin 5,6, 5′,6′-diepoxide (found in Meyer lemon peel). All of these substances are readily distinguished by their N_{100} values in either system II or III. Valenciaxanthin and persicaxanthin were shown to be distinct substances by their significant difference in N_{100} values (*1*).

Determination of the N_{100} value of carotenoid polyols with system III is thus of value in showing nonidentity in some cases, as in valenciaxanthin and persicaxanthin; in other cases identity of two substances may be indicated, or at least close relationship such as in cis-trans or other stereoisomers. The N_{100} values in solvent systems I, II, or IV are likewise useful in the identification of naturally occurring carotenoids containing fewer than three hydroxyl groups. Two substances having similar chromatographic behavior, closely agreeing spectral absorption curves, and N_{100} values (preferably not near either zero or 100) in a given solvent system are very probably either identical or closely related substances such as stereoisomers. Carotenoids differing in the position of functional groups, such as zeaxanthin and isozeaxanthin (3, 3′- and 4, 4′dihydroxy-β-carotenes, respectively), have distinctly different N_{100} values (in system II, 69 and 78, respectively), while isomers such as zeaxanthin and lutein (3, 3′-dihydroxy-α-carotene), which differ in the position of one double bond, have very similar N_{100} values.

N_{100} values are, of course, dependent on partition coefficients of the solvent system employed. If the distribution coefficient is 1.00, the N_{100} value should be 50. Petracek and Zechmeister (*8*) have recently published the partition coefficients of numerous carotenoids in hexane–95% methanol and hexane–85% methanol; their data show how the partition is affected by the presence of certain functional groups.

Acknowledgment

Spectrophotometer curves of carotenoid fractions were run by Glen F. Bailey and Edith Gong. The sample of isozeaxanthin was supplied by the late G. F. Siemers of Hoffmann–LaRoche, Inc., Nutley, N. J.

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Received for review January 18, 1960. Accepted May 8, 1960.