Transformation of 13,14- and 11,12-Monoepoxycanthaxanthin on Magnesia

Matthew J. Cyronak,[1] Diona Oşianu, George Britton, and Kenneth L. Simpson*

13,14- and 11,12-monoepoxycanthaxanthin underwent cleavage to form the apo-14'- and apo-12'-canthaxanthal, respectively, when brought into contact with adsorptive magnesia. It was also found that these two apocanthaxanthals readily undergo aldol condensation with acetone to form methyl ketones in the presence of magnesia. These reactions may be attributed to the basicity of the magnesia and/or possible contaminants. Therefore, care should be taken when magnesia is used as an adsorbent in carotenoid research.

Carotenoid research had depended on the use of chromatographic methods for the isolation and identification of these pigments. These procedures utilize numerous types of chromatographic adsorbents and the adsorbent being employed should be free of any reactions between it and the pigments involved. Adsorbents once thought to be inert, however, have been found to cause alterations of carotenoid pigments. β-Carotene, for example, has recently been found to undergo significant hydroxylation and oxidation in the presence of Micro-Cel C to form isocryptoxanthin and other products (Rodriguez et al., 1976). An earlier report had linked Micro-Cel C and other siliceous adsorbents with the isomerization of carotenoid epoxides (Strain et al., 1967), and isomerization of carotenoids has also been attributed to adsorbtive alumina (Zechmeister, 1948; Zechmesiter and Sandoval, 1945; Tsukida and Zechmeister, 1958). It was also noted that fucoxanthin was quickly isomerized to isofucoxanthin when exposed to basic alumina (Liaaen-Jensen, 1971).

Magnesia, on the contrary, has only been reported to alter chlorophyll pigments (Strain, 1958; Strain et al., 1965) and has been widely used in carotenoid research. However, we wish to report that the in-chain epoxides of canthaxanthin undergo degradation in the presence of magnesia. This represents a new reaction for this class of carotenoid pigments and illustrates the extreme precautions necessary in the selection of adsorbents to be used in carotenoid research.

EXPERIMENTAL SECTION

Preparation of 11,12- and 13,14-Monoepoxycanthaxanthin. Canthaxanthin was treated with perbenzoic or *m*-chloroperbenzoic acid in a 1:2 molar ratio, and the products were isolated under the conditions previously reported (Nicoară et al., 1970; Oşianu et al., 1977).

Exposure to Magnesia. Two methods were used to expose the in-chain epoxides of canthaxanthin to fresh magnesia (adsorbtive, Fisher Scientific, N.J.). First, the reaction mixture containing the unpurified epoxides was directly chromatographed on a column of magnesia-Hyflo Super Cel (1:2, w/w). The upper layer of the column turned green and this color quickly disappeared with the addition of 25% ethyl ether in petroleum ether. Development continued until all the diffuse pigments (fraction A) adsorbed below canthaxanthin were collected (elution time, approximately 1 h). Fraction A was then rechromatographed on a column of magnesia–Hyflo Super Cel developed with 5% ethyl ether in petroleum ether (on occasion acetone was substituted for ethyl ether). Further purification of each pigment was accomplished by thin-layer chromatography (TLC) on silica gel G plates developed with 3% MeOH in benzene.

Alternatively, magnesia was added to the individual purified in-chain epoxides dissolved in petroleum ether until all of the pigment was adsorbed. This was allowed to stand for 30 min at room temperature in the dark. After the allotted time, the pigments were extracted from the adsorbent with ethyl ether and concentrated under vacuum. The pigments were then separated by TLC on silica gel G plates developed with 3% MeOH in benzene. Each epoxide was found to give a main product and the starting material.

Identification of Conversion Products. Identification of the products was based on the absorption spectra, mass spectra, and group chemical reactions.

The epoxide test was carried out by adding a few drops of concentrated HCl to the pigment dissolved in ethyl ether and the color of the acid layer was noted. A blue color indicates the presence of the epoxy group and absence of color constitutes a negative result (Davies, 1976).

Reduction of the carbonyl groups was accomplished by adding a few crystals of $NaBH_4$ to a solution of the pigment in 95% EtOH (Krinsky, 1963). The reaction mixture was allowed to stand for 3 h under refrigeration. The reduced pigment was transferred to petroleum ether by the addition of water.

Since carotenoid aldehydes react with hippuric acid to form azlactones, this was used as a method of identification (Tămaş et al., 1973; Tămaş and Bodea, 1974). To the crystalline pigment in a test tube equal amounts of hippuric acid and anhydrous sodium acetate were added. This was stirred well with a glass rod and a few drops of acetic anhydride were added to the mixture and kept for 1.5 h at 85 °C. After cooling to room temperature, the residue was dissolved in chloroform, filtered, and concentrated under vacuum. Purification of the pigment was accomplished by TLC on silica gel G plates developed in the solvent system described above. The red-purple pigment was eluted from the adsorbent with chloroform and crystallized by the addition of petroleum ether.

All analytical operations were carried out under subdued light. Reagents used were all analytical grade. All solvents except for MeOH and EtOH were distilled prior to use. Precoated silica gel G sheets (20 × 20 cm., 0.25 mm; E. M. Labs, Elmsford, N.Y.) were used for TLC.

Visible absorption spectra were determined using a Cary 15 recording spectrophotometer. Mass spectra were determined on an MS12 instrument in the Department of Biochemistry of the University of Liverpool, England.

Department of Food Science and Technology, Nutrition and Dietetics, University of Rhode Island, Kingston, Rhode Island 02881 (M.J.C., K.L.S.), Department of Biochemistry, Institute of Agronomy, Cluj-Napoca, Romania (D.O.), and the Department of Biochemistry, University of Liverpool, Liverpool, England (G.B.).

[1]Present address: General Foods Corp., Technical Center, Tarrytown, New York 10591.

0021-8561/78/1426-0712$01.00/0

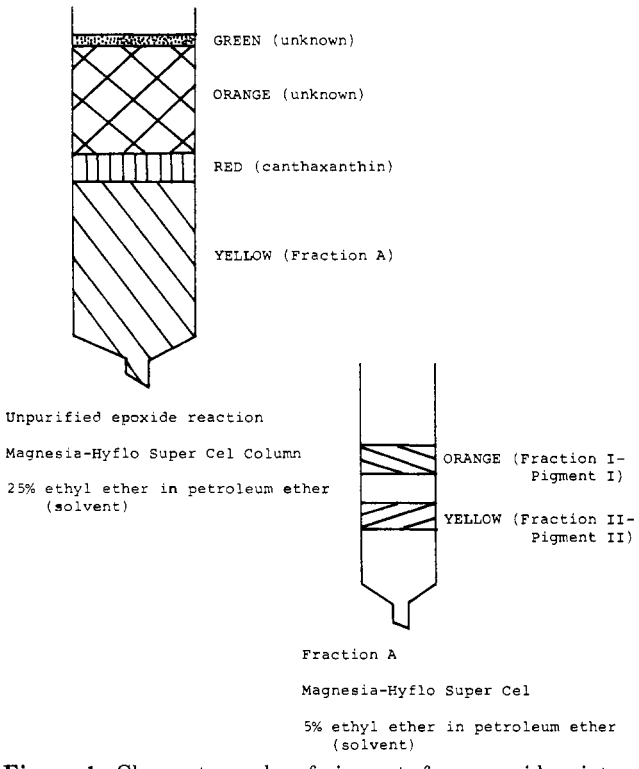

Figure 1. Chromatography of pigments from epoxide mixture exposed to magnesia.

RESULTS AND DISCUSSION

Two main fractions were observed when fraction A was rechromatographed on magnesia-Hyflo Super Cel developed with 5% ethyl ether in petroleum ether (Figure 1). Fractions I and II isolated from fraction A were identical, respectively, to the pigments isolated when pure 13,14- and 11,12-monoepoxycanthaxanthin were individually exposed to magnesia.

Pigment I (14′-Apocanthaxanthal). The pigment from fraction I and from the exposure of 13,14-monoepoxycanthaxanthin (VII) to magnesia was light yellow and gave a negative epoxide reaction. It also exhibited a degenerated absorption spectrum (λ_{max} at 392 nm in petroleum ether, 402 nm in benzene, and 406 nm in 95% EtOH) characteristic of ketocarotenoids (Reichenbach and Kleinig, 1971; Davies, 1976). The pigment was easily reduced by $NaBH_4$ and gave a product with a decrease in R_f value on silica gel G thin-layer plates (from 0.44 to 0.03). The absorption spectrum of the reduced compound (λ_{max} at 342 and 355 nm) showed a hypsochromic shift of 50 nm in 95% EtOH (Figure 2) with some increase in fine structure. This indicated the presence of two carbonyl groups in conjugation with the polyene chromophore in the parent compound (Reichenbach and Kleinig, 1971). Also, the position of the absorption maxima of the reduced compound suggested six conjugated carbon–carbon double bonds with one being in the β position of the ring (Davies, 1976; Vetter et al., 1971; Moss and Weedon, 1976).

The mass spectrum of I showed a parent ion, M^+ at m/e 324 (100), which is equivalent to a molecular formula of $C_{22}H_{28}O_2$. The fragment ions at m/e 309 (27), 295 (25), 255 (22), 232 (5), 69 (78), 29 (54) provide evidence for a terpenoid structure and that one of the carbonyls is an aldehyde (Vetter et al., 1971; Moss and Weedon, 1976). The condensation of the pigment with hippuric acid confirmed the presence of the aldehyde group with the resulting azlactone exhibiting a bathochromic shift of 81 nm in benzene (λ_{max} at 483 and 505 nm in benzene and 492 nm in $CHCl_3$) and an increase in R_f value (0.79) on silica gel G thin-layer plates. The mass spectrum of the azlactone gave a parent ion, M^+ at m/e 467 (12, $C_{31}H_{33}NO_3$) and fragment ions at m/e 465 (11), 450 (4), 439 (1), 362 (1), 334 (3), 318 (2), 203 (62), 105 (100), and 77 (99), which were found to be characteristic of azlactones formed from apocarotenals (Tămaş, 1976). From the above data it was concluded that pigment I is 14′-apocanthaxanthal (4-keto-14′-apo-β-caroten-14′-al, I) and that the reduction product and the corresponding azlactone have structures III and IV, respectively (Figure 3).

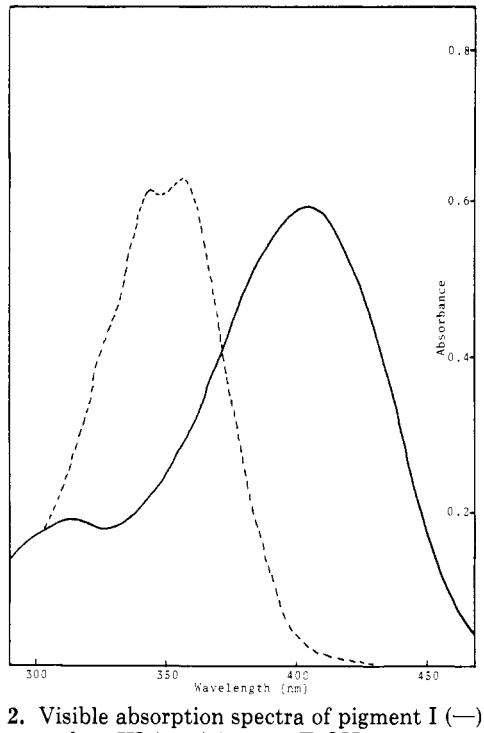

Figure 2. Visible absorption spectra of pigment I (—) and its reduction product III (– – –) in 95% EtOH.

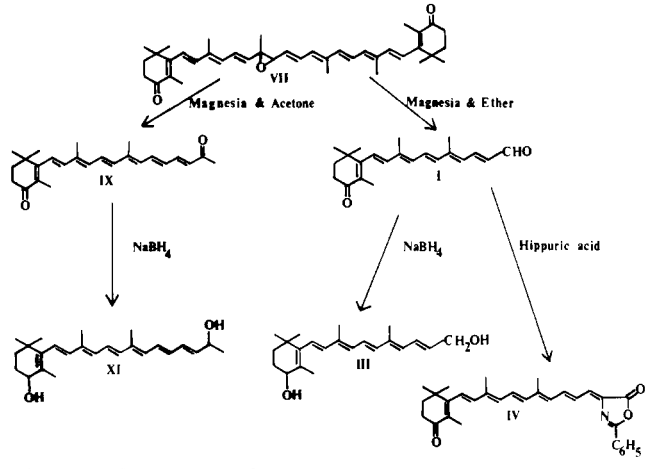

Figure 3. Reaction products of 13,14-monoepoxycanthaxanthin (VII).

Pigment II (12′-Apocanthaxanthal). The pigment isolated from fraction II and from the exposure of 11,12-monoepoxycanthaxanthin (VIII) to magnesia was orange and, also, gave a negative epoxide reaction. Again, the pigment exhibited a degenerated absorption spectrum (λ_{max} at 409 and 418 nm in petroleum ether, 424 nm in benzene, and 430 nm in 95% EtOH) and gave, upon $NaBH_4$ reduction, a product with a decreased R_f value (0.53 to 0.02) and a hypsochromic shift of 53 nm in 95% EtOH (λ_{max} at 360, 377, and 394 nm; Figure 4). This indicated, along with the position of the absorption maxima, the presence of two carbonyls in conjugation with

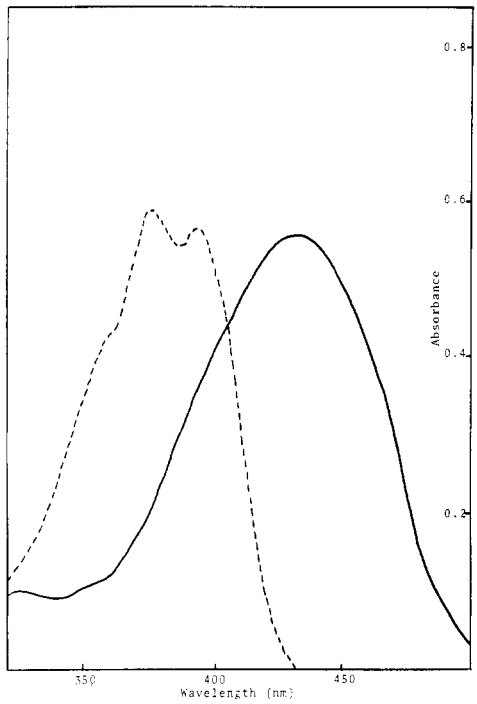

Figure 4. Visible absorption spectra of pigment II (—) and its reduction product (---) in 95% EtOH.

a polyene chromophore of seven carbon–carbon double bonds in the parent compound. The mass spectrum gave the parent ion M^+ at m/e 364 (100, $C_{25}H_{32}O_2$) and fragment ions at m/e 349 (14), 335 (2), 295 (12), 272 (3), 69 (50), and 29 (30). Again it was evident that one of the carbonyls was an aldehyde. The azlactone of pigment II had a bathochromic shift of 82 nm in benzene (λ_{max} at 506 and 535 nm in benzene and 510 nm in $CHCl_3$) with an increase R_f value (0.84). The mass spectrum was characteristic of azlactone with M^+ at m/e 507 (18, $C_{34}H_{37}NO_3$). Fragment ions were at m/e 492 (1), 479 (3), 415 (1), 402 (2), 374 (3), 358 (2), 303 (3), 105 (100), and 77 (98). It was concluded that pigment II is 12′-apocanthaxanthal (4-keto-12′-apo-β-caroten-12′-al, II) with a reduction product and the azlactone having the respective structures V and VI (Figure 5).

Besides the above two compounds, other pigments have been isolated in small quantities from the epoxide reaction after adsorption on the magnesia–Hyflo Super Cel column. These compounds have been tentatively identified as the 8′-, 10′-, and 15-apocanthaxanthal arising possibly from the cleavage of the corresponding in-chain epoxides. However, the low quantity of the pigments isolated has prevented confirmation of their structures.

It should be noted that the primary reason for this investigation was to synthesize in-chain epoxides of canthaxanthin to be used in other studies. Thus the procedure of Nicoară et al. (1970) was followed in which acetone was used in the developing solvent. However, two pigments were constantly isolated from the magnesia–Hyflo Super Cel column, the properties of which were not characteristic of in-chain epoxides; the double absorption spectrum, indicative of in-chain structure was not observed (Ogata et al., 1973) and they both gave negative epoxide reactions. When these two compounds were subjected to the identification procedures described above, it was concluded that they were the methyl ketones, IX (4-keto-11′,12′-dihydro-11′-apo-20′-nor-β-caroten-12′-one) and X (4-keto-9′,10′-dihydro-9′-apo-19′-nor-β-caroten-10′-one), which were formed from the aldol condensation of acetone with the breakdown products of the in-chain epoxides,

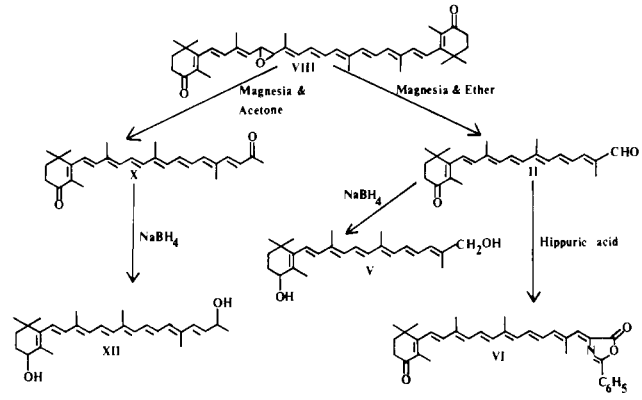

Figure 5. Reaction products of 11,12-monoepoxycanthaxanthin (VIII).

apocanthaxanthals I and II, respectively, on the magnesia column. The structures of IX and X are given in Figures 3 and 5. An important feature of the mass spectra of IX [M^+ at m/e 364 ($C_{25}H_{32}O_2$) with fragment ions at m/e 321 (M – 43) and 43 ($COCH_3$); R_f value, 0.75] and X [M^+ at m/e 404 ($C_{28}H_{36}O_2$) with fragment ions at m/e 361 (M – 43) and 43 ($COCH_3$); R_f value, 0.43], which led us to the conclusion that they were methyl ketones, was a strong peak at m/e 43 (base peak) and a reasonably strong M – 43 peak. This was shown to be due to the loss of a terminal $COCH_3$ group which is characteristic of a methyl ketone (Schmidt et al., 1971; Vetter et al., 1971). Also the mass spectra of the alcohols XI [M^+ at m/e 368 ($C_{25}H_{36}O_2$) with fragment ions at m/e 350 (M – H_2O), 332 (M – $2H_2O$), and 323 (M – 45); R_f value, 0.20] and XII [M^+ at m/e 408 ($C_{28}H_{40}O_2$) with fragment ions at m/e 390 (M – H_2O), 372 (M – $2H_2O$), and 363 (M – 45); R_f value, 0.03] from the $NaBH_4$ reduction of IX and X had a prominent fragment ion at m/e M – 45 which was due to the loss of a $CHOHCH_3$ terminal group, again providing conclusive evidence for the methyl ketone structures of the parent compounds. The type of chromophore present was determined from the absorption spectra of IX (λ_{max} at 410 nm in petroleum ether and 430 nm in 95% EtOH) and X (λ_{max} at 427 nm in petroleum ether and 440 nm in 95% EtOH) and their reduction products, XI (λ_{max} at 352, 370, and 388 nm in 95% EtOH) and XII (λ_{max} at 386, 403, and 426 nm in 95% EtOH). When ethyl ether was substituted for acetone in the developing solvent, the two methyl ketones, as expected, disappeared and the corresponding apocarotenals were then isolated.

It is known that an aldol condensation reaction is catalyzed by either a basic or an acidic medium (Morrison and Boyd, 1973). Other apocarotenals have been reported to undergo this reaction with acetone during isolation procedures where a basic medium was involved (Schmidt et al., 1971; Stewart and Wheaton, 1973; Britton et al., 1976). Accordingly, it is no surprise that the basic nature of magnesia could catalyze the aldol condensation between apocanthaxanthal I and II and acetone to yield the isolated methyl ketones, IX and X, respectively. This increases the precautions needed when apocarotenals are chromatographed on magnesia.

In order to further investigate the cleavage reaction caused by magnesia, this reaction was performed in Cluj-Napoca, Romania. The magnesia utilized (Riedel de Haen A. G. Seelze-Hanover, licht chem. pure D. A. B. 6) was the same as that originally used in the isolation of the in-chain epoxides of canthaxanthin (Nicoară et al., 1970). No cleavage products were observed with only the in-chain epoxides being isolated when fresh and reused magnesia were employed (however, it was noted that the aldol

condensation between apocanthaxanthals I and II and acetone did occur using this magnesia). It was also found that the magnesia used in Cluj-Napoca is less alkaline than that in Kingston (pH <9 vs. pH <11) and that the regeneration and reuse of the magnesia in Cluj-Napoca does not alter its alkalinity. Thus, the cleavage mechanism seems to be a very complex reaction with the pH of the magnesia and possible trace contaminates playing an important role. Also, the fact that the in-chain epoxides of canthaxanthin are found to be labile in dilute solutions of alkali (Oşianu et al., 1977) may provide a clue. It is, therefore, conceivable that a base-catalyzed ring opening could occur, characteristic of epoxides (Rosowsky, 1964), with rearrangement leading to bond cleavage and aldehyde formation. But to propose a viable mechanism, it is necessary to isolate and identify the other fragments of the cleaved in-chain epoxides and to investigate the properties and composition of the two magnesias involved.

Although the above reactions do occur on magnesia, its use as an adsorbent for the separations of carotenoids should not be discontinued. However, care must be exercised with regard to the pH of the magnesia employed, the class of carotenoids being separated, and the type of solvent system used.

Biochemically, the cleavage mechanism may provide some insight into the metabolism of carotenoids. It is known that various types of apocarotenals occur naturally in plants and animals which represent breakdown products of the parent carotenoids (Goodwin, 1976; Weedon, 1971). An oxidative mechanism has been postulated hypothesizing that the polyene chain degrades in a stepwise manner at one end with the formation of the corresponding carotenals or carotenic acids (Glover and Redfearn, 1954; Glover, 1960). The major support of this view is the widespread distribution of the apocarotenoids. However, to this date, no known intermediates between the parent carotenoid and the apocarotenals have been isolated, but it has been proposed that the intermediate may be an in-chain epoxide (Tămaş, 1972; Bodea, 1969). Thus, the cleavage reaction of these in-chain epoxides with magnesia may mimic a biological mechanism and could provide a possible model for study.

ACKNOWLEDGMENT

We acknowledge with thanks the gift of synthetic canthaxanthin from Hoffman–LaRoche Inc., Nutley, N.J.

LITERATURE CITED

Bodea, C., *Pure Appl. Chem.* **20**, 517 (1969).
Britton, G., Malhotra, H. C., Singh, R. K., Goodwin, T. W., Ben-Aziz, A., *Phytochemistry* **15**, 1749 (1976).
Davies, B. H., "Chemistry and Biochemistry of Plant Pigments", Vol. 2, 2nd ed, Goodwin, T. W., Ed., Academic Press, New York, N.Y., 1976, p 38.
Glover, J., Redfearn, E. R., *Biochem. J.* **58**, 15 (1954).
Glover, J., *Vitam. Horm. (N.Y.)* **18**, 371 (1960).
Goodwin, T. W., "Chemistry and Biochemistry of Plant Pigments", Vol. 1, 2nd ed, Goodwin, T. W., Ed., Academic Press, New York, N.Y., 1976, p 225.
Krinsky, N. I., *Anal. Biochem.* **6**, 293 (1963).
Liaaen-Jensen, S., "Carotenoids", Isler, O., Ed., Birkhäuser-Verlag, Basel, 1971, p 61.
Morrison, R. T., Boyd, R. N., "Organic Chemistry", 3rd ed, Allyn and Bacon, Boston, 1973, p 567.
Moss, G. P., Weedon, B. C. L., "Chemistry and Biochemistry of Plant Pigments", Vol. 1, 2nd ed, Goodwin, T. W., Ed., Academic Press, New York, N.Y., 1976, p 149.
Nicoară, E., Oşianu, D., Bodea, C., *Rev. Roum. Chim.* **15**, 1965 (1970).
Ogata, Y., Tomizawa, K., Takagi, K., *Tetrahedron* **29**, 47 (1973).
Oşianu, D., Nicoară, E., Bodea, C., *Rev. Roum. Chim.*, in press (1977).
Reichenbach, H., Kleinig, H., *Arch. Mikrobiol.* **76**, 364 (1971).
Rodriguez, D. B., Tanaka, Y., Katayama, T., Simpson, K. L., Lee, T-C., Chichester, C. O., *J. Agric. Food Chem.* **24**, 819 (1976).
Rosowsky, A., "Heterocyclic Compounds with Three and Four-Member Rings", Part I, Weissberger, A., Ed., Interscience, New York, N.Y., 1964, p 1.
Schmidt, K., Francis, G. W., Liaaen-Jensen, S., *Acta Chem. Scand.* **25**, 2476 (1971).
Stewart, I., Wheaton, T. A., *Phytochemistry* **12**, 2947 (1973).
Strain, H. H., *Annu. Priestly Lect.*, 32 (1958).
Strain, H. H., Sherma, J., Benton, F. C., and Katz, J. J., *Biochim. Biophys. Acta* **109**, 1, 16, 23 (1965).
Strain, H. H., Sherma, J., Grandolfo, M., *Anal. Chem.* **39**, 926 (1967).
Tămaş, V., *St. Cerc. Biochim.* **15**, 315 (1972).
Tămaş, V., Ciurdaru, V., Bodea, C., *Rev. Roum. Chim.* **18**, 1409 (1973).
Tămaş, V., Bodea, C., *Rev. Roum. Chim.* **19**, 701 (1974).
Tămaş, V., azlactone characteristics, private communication, Institute of Agronomy, Cluj-Napoca, Romania, 1976.
Tsukida, K., Zechmeister, L., *Arch. Biochem. Biophys.* **74**, 408 (1958).
Vetter, W., Englert, G., Rigassi, N., Schwieter, U., "Carotenoids", Isler, O., Ed., Birkhäuser-Verlag, Basel, 1971, p 189.
Weedon, B. C. L., "Carotenoids", Isler, O., Ed., Birkhäuser-Verlag, Basel, 1971, p 29.
Zechmeister, L., Sandoval, A., *Science* **101**, 585 (1945).
Zechmeister, L., *Ann. N.Y. Acad. Sci.* **49**, 145 (1948).

Received for review June 9, 1977. Accepted December 15, 1977. This work was supported in part by grants from National Science Foundation (NSF-OIP/FRR04/253) and NCST-Romania. Contribution No. 1742 of the Agricultural Experiment Station of the University of Rhode Island, Kingston, R.I.