Selective Preparation of the Lutein Monomethyl Ethers

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Crystalline 3-hydroxy-3'-methoxy-α-carotene (II) has been prepared by treatment of lutein (I) with methanol and hydrochloric acid. Proton magnetic resonance and infrared evidence support the structure II for this compound.

3-Methoxy-3'-hydroxy-α-carotene (V) was synthesized by nickel peroxide oxidation of lutein (I) to the previously unknown 3-hydroxy-3'-keto-α-carotene (III), followed by methylation with methyl iodide and silver oxide in dimethylformamide to 3-methoxy-3'-keto-α-carotene (IV) and finally borohydride reduction to V.

The two lutein monomethyl ethers II and V could be separated chromatographically; II only was obtained in the pure crystalline state.

Direct comparison between II, V, and the lutein monomethyl ether prepared by others [6] by direct methylation of lutein (I) with methyl iodide and barium oxide in dimethylformamide-dimethyl-sulfoxide, revealed identity of the last named lutein ether with the allylic ether (II).

A lutein monomethyl ether, m.p. 150°C, was first prepared in low yield by Karrer and Jirgensons [1] by methylation of lutein (I) with methyl iodide and potassium t-amyloxylate.

Based on the observation of Petracek and Zechmeister [2] that carotenoids like isozeaxanthin (4,4'-dihydroxy-β-carotene), containing hydroxyl groups in allylic positions to the polyene chain readily underwent methylation by treatment with methanol and hydrochloric acid, Curl [3] prepared the presumed allylic methyl ether of lutein by the same method. Under similar conditions the non-allylic diol zeaxanthin (3,3'-dihydroxy-β-carotene) failed to give analogous products, and it was assumed that specific methylation of the allylic hydroxyl group of lutein (I) had occurred. However, further proof for structure II was not presented.

More recently we have demonstrated that the method of Kuhn, Trischmann and Löw [4] for the methylation of N-acetylglucosamine derivatives with methyl iodide and silver oxide in dimethylformamide, can be employed with advantage for methylation of carotenoids, in particular carotenoids with secondary hydroxyl groups. [5]

Müller and Karrer [6] lately very successfully used the methylation method of Kuhn and co-workers [4,7,8] for the preparation of lutein (xanthophyll) mono- and dimethyl ether. The alternative structures II and V for their lutein monomethyl ether (m.p. 150°C) were not discussed.

On this background it seemed worth-while to attempt a selective synthesis of the two lutein monomethyl ethers II and V.

RESULTS AND DISCUSSION

Crystalline 3-hydroxy-3′-methoxy-α-carotene (II), m.p. 168—169°C, as claimed by Curl,[3] was obtained in good yield by treatment of lutein (I) with methanol and hydrochloric acid according to the procedure of Bush and Zechmeister.[2] Infrared (see Fig. 1) and proton magnetic resonance evidence confirmed structure II for this compound; methoxyl at 1080 cm^{-1} and 6.77 τ (3 protons) and non-allylic secondary hydroxyl in a cyclohexene ring at 1040 cm^{-1}.[9]

For the preparation of 3-methoxy-3′-hydroxy-α-carotene (V) it was desirable to prevent methylation of the hydroxyl group in the 3′-position of lutein. This was achieved by oxidation of this allylic hydroxyl group in lutein to give the ketone III. Recently we have studied in some detail the allylic oxidation of carotenoids by p-chloranil,[10] a reaction first introduced by Warren and Weedon.[11] However, allylic position to only one double bond does not provide sufficient activation for oxidation with this reagent. Hence III could not be obtained on oxidation of lutein with p-chloranil. However, Nakagava, Konaka and Nakata [12] have reported a method for the selective oxidation of allylic hydroxyl groups in terpenoids with nickel peroxide, which proved to be a stronger oxidizing reagent. The previously unknown 3-hydroxy-3′-keto-α-carotene (III), m.p. 168—170°C, was readily obtained by nickel peroxide
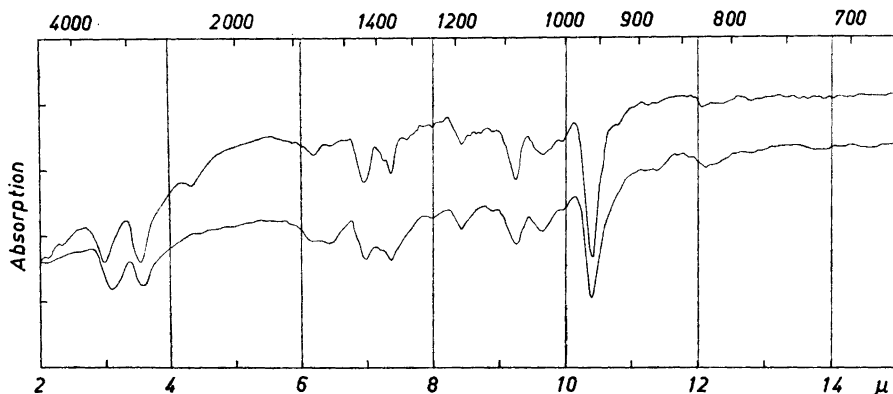

Fig. 1. Infrared spectrum of 3-hydroxy-3′-methoxy-α-carotene (II). Upper curve for compound prepared by hydrochloric acid-methanol treatment of lutein. Lower curve for sample prepared by Müller and Karrer by methylation of lutein with methyl iodide and barium oxide.
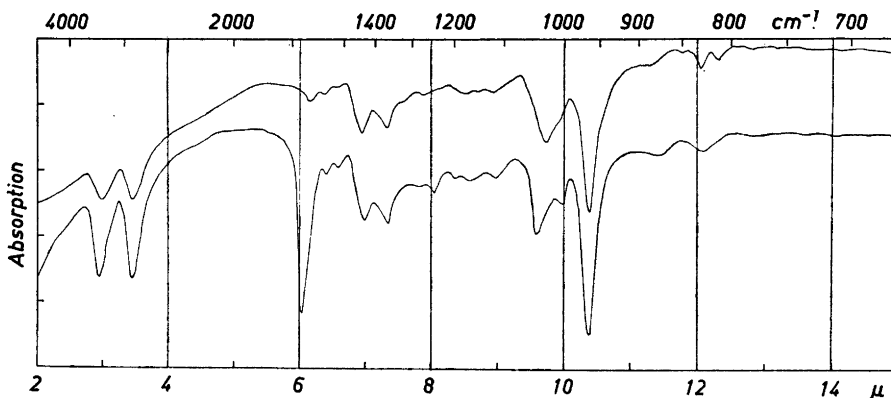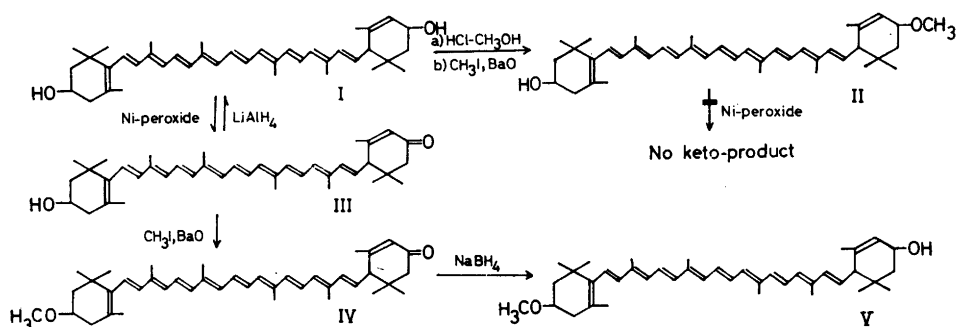
Fig. 2. Infrared spectra of lutein (I) (upper curve) and 3-hydroxy-3'-keto-α-carotene (III) (lower curve).

oxidation of lutein (I). III exhibited the same absorption spectrum in visible light as lutein, and the infrared spectrum further confirmed the presence of a conjugated carbonyl group in 3'-position (see Fig. 2).

Following the method of Kuhn *et al.*,[4,7] III was methylated to 3-methoxy-3'-keto-α-carotene (IV) with methyl iodide and silver oxide or barium oxide in dimethylformamide.

Crude IV was reduced by borohydride to give 3-methoxy-3'-hydroxy-α-carotene (V). Repeated attempts to separate V from non-carotenoid by-products of the methylation reaction unfortunately failed, and V was not obtained in the pure crystalline state.

The two lutein monomethyl ethers II and V showed similar adsorptive properties, but could be separated on kieselguhr or aluminium oxide paper, see Table 2. V was somewhat more strongly retained than II on kieselguhr paper. On aluminium oxide paper the reverse behaviour was observed.

A sample of the lutein monomethyl ether prepared by Müller and Karrer [6] by direct methylation of lutein with methyl iodide and barium oxide, was kindly put to our disposal by Professor P. Karrer, and a direct comparative

study of II and V was carried out. Paper-chromatographically the mono-methyl ether of Müller and Karrer [6] was homogeneous, could not be separated from II, gave no melting point depression with II and exhibited the same infrared spectrum as II (see Fig. 1). On the other hand their ether was chromato-graphically different from V. If the course of direct methylation of lutein (I) should be predicted, the preferential formation of the allylic ether II would be expected, and this indeed is the case.

Hydride reduction of the ketones III and IV should theoretically give a mixture of alcohols with quasi-equatorial and quasi-axial configuration of the hydroxyl groups in the 3'-position. The reduction product of III and IV both comprised two zones on the paper chromatogram. However, the absorption spectra and behaviour on iodine catalysed isomerization of these pigments supported a *trans-cis* double bond relationship rather than steric isomerism of the hydroxyl group. Thus III gave a mixture of *trans* and neo-lutein U, and apparently no separate product of III with opposite configuration of the hydroxyl group at the 3'-position, a compound which would be of interest to compare with diadinoxanthin.[13]

EXPERIMENTAL

Materials and methods have been described in an earlier paper in this journal.[14] This refers to solvents and instruments used, as well as chromatographic methods and determination of partition ratios. Proton magnetic resonance spectra were recorded on an AEI type RS2 spectrometer at 60 Mc/sec. Iodine catalyzed stereoisomerization was carried out as described elsewhere.[5] Melting points were determined in evacuated capillary tubes and are uncorrected. Natural lutein from National Chlorophyll & Chemical Co., Lamar, Colorado, USA, was used. Nickel peroxide was prepared and standardized according to the procedure of Nakagava *et al.*[12] Adsorptive properties for the various lutein derivatives prepared are compiled in Table 1.

Table 1. Adsorptive properties for various lutein derivatives.

Trans carotenoid	Required eluent from deactivated alumina	R_F-value, S. & S. No. 287 kieselguhr paper 5 % acetone *
3-Methoxy-3'-keto-α-carotene (IV)	10 % acetone *	0.70
3-Methoxy-3'-hydroxy-α-carotene (V)	15—20 % acetone	0.67
3-Hydroxy-3'-methoxy-α-carotene (II)	20 % acetone	0.59
3-Hydroxy-3'-keto-α-carotene (III)	25—35 % acetone	0.32
3-Hydroxy-3'-hydroxy-α-carotene (I)	50 % acetone	0.22

* In petroleum ether.

3-Hydroxy-3'-methoxy-α-carotene (II). Lutein (80 mg) was dissolved in chloroform (20 ml) and methanol (100 ml). A freshly prepared saturated solution of dry hydrogen chloride in chloroform (10 ml, *ca.* 0.3 N) was added, and the mixture was left at room temperature. The reaction was complete after 3 h, as revealed by paper-chromatographic examination. The pigments were transferred to ether in the usual manner on admixture with aqueous sodium bicarbonate solution. The washed ether extract was dried over anhydrous sodium sulphate, and the pigments were submitted to column chromato-

graphy on deactivated alumina; pigment recovery 75 %. The reaction mixture contained II only; required eluant 20 % acetone-petroleum ether.

Crystallization was effected from acetone-petroleum ether. After re-crystallization tiny needles, m.p. 168—169°C, were obtained. Crystalline *trans* II had abs. max. in acetone at (428), 449 ($E_{1\,cm}^{1\,\%} = 2590$) and 478 mμ, % III/II [5] = 68, $R_F = 0.67$ on kieselguhr paper (5 % acetone-petroleum ether) and partition ratio 51:49 in petroleum ether/95 % methanol. The IR-spectrum measured in KBr is presented in Fig. 1. The NMR-spectrum, measured in CDCl$_3$, had signals at 6.77 (OCH$_3$, *ca.* 3 protons), 8.03 (in-chain-CH$_3$, *ca.* 12 protons), 8.31 (CH$_3$-groups in 5-position, *ca.* 6 protons) 8.43 (methylene protons), 8.96, 9.0, 9.16, and 9.32 (*gem.* CH$_3$, *ca.* 12 protons). Signals are given in τ-values.

Nickel peroxide oxidation of II was carried out in the same manner as described below. No keto-products were formed.

3-Hydroxy-3′-keto-α-carotene (III). Lutein (60 mg) was dissolved in dry ether (30 ml) and benzene (30 ml). Nickel peroxide (550 mg, corresponding to 30 times molar excess; available oxygen 2.87 × 10⁻³ g atom/g nickel peroxide determined by titration) was added. The mixture was mechanically stirred at room temperature. The course of the reaction was followed paper-chromatographically. After 1/2 h the reaction was interrupted by filtration; spectrophotometrically determined pigment recovery was 45 %. The reaction mixture was submitted to column chromatography on deactivated alumina. III constituted 52 % of the recovered carotenoid mixture and required 25—35 % acetone-petroleum ether for elution, 43 % was recovered lutein (required eluant 50 % acetone-petroleum ether) and 5 % minor yellow decomposition products (required eluant 5—15 % acetone-petroleum ether). Other experiments gave similar results. Prolonged reaction periods resulted in lower pigment recovery.

Crystallization was effected from petroleum ether to give red needles, m.p. 168—170°C, *ca.* 12 mg. Crystalline *trans* III had $R_F = 0.32$ on kieselguhr paper (5 % acetone-petroleum ether) and exhibited abs. max. in acetone at (425), 449 ($E_{1\,cm}^{1\,\%} = 2425$) and 477 mμ, % III/II = 61 in acetone. The IR-spectrum measured in KBr, is presented in Fig. 2 together with that of lutein (I). In petroleum ether/85 % methanol the partition ratio 45:55 was found. The ratio remained unchanged upon addition of alkali.

LiAlH₄-reduction of III. III (4.0 mg) in dry ether (5 ml) was treated with a filtered, fine suspension of LiAlH₄ in dry ether in the usual manner;[15] spectrophotometrically determined pigment recovery was 65 %. Two zones with $R_F = 0.60$ and $R_F = 0.43$ appeared on the circular chromatogram (kieselguhr paper; 10 % acetone-petroleum ether). The former carotenoid (31 % of the total) had abs. max. in acetone at (428), 450 and 478 mμ, % III/II = 57; and the latter (69 % of total) abs. max. at (427), 448 and 476 mμ, % III/II = 52. The interconversion of the two pigments upon iodine catalysis in light was demonstrated by paper chromatography.

In a separate experiment natural lutein (I) in benzene solution was submitted to iodine catalysis in light. Two zones appeared on the circular chromatogram (kieselguhr paper; 10 % acetone-petroleum ether): *trans*, $R_F = 0.60$, abs. max. (428), 449, and 478 mμ, % III/II = 59 in acetone and neo U, $R_F = 0.44$, abs. max. (426), 446, and 474 mμ, % III/II = 48 in acetone.

The two pigments obtained on hydride reduction of III hence presumably represented *trans* and neo-lutein U.

3-Methoxy-3′-keto-α-carotene (IV). III (10 mg) was dissolved in dimethylformamide (6 ml) and silver oxide (1.2 g) and methyl iodide (4 ml) was added. The mixture was mechanically stirred at room temperature. After 18 h a *ca.* 50 % conversion to the corresponding methyl ether (IV) was observed on the paper chromatogram. The reaction mixture was filtered and the pigments transferred to ether in a separatory funnel on admixture with water. The combined ether extracts were washed thoroughly with water and chromatographed on a column of deactivated alumina; pigment recovery was 61 %.

IV required 10 % acetone-petroleum ether for elution and constituted 62 % of the reaction mixture; the rest was unreacted III. *Trans* IV had the same absorption spectrum in visible light as III and $R_F = 0.70$ on kieselguhr paper (5 % acetone-petroleum ether). Attempts at crystallization were unsuccessful due to the presence of non-carotenoid impurities.

Methylation of III (24.6 mg) in dry dimethylformamide (10 ml) with methyl iodide (0.3 ml) and barium oxide (0.3 g) gave a similar result. Transfer of the pigments to chloroform rather than ether resulted in no improved purity of the methylated product.

3-Methoxy-3'-hydroxy-α-carotene (V). Crude IV (4.7 mg, spectrophotometrically determined) in ether (3 ml) and ethyl alcohol (2 ml) was reduced with $NaBH_4$ at room temperature. The reduction, followed paper-chromatographically, was interrupted after 3 h. The pigments were transferred to ether in the usual manner and chromatographed on a column of deactivated alumina; pigment recovery was 70 %. The reaction mixture contained V (89 % of total) and unreacted IV (11 %); for required eluents see Table 1.

V had the same absorption spectrum in visible light as the other prepared lutein derivatives and $R_F = 0.59$ on kieselguhr paper (5 % acetone-petroleum ether).

Repeated attempts to purify the product from non-carotenoid contaminants on columns of deactivated alumina or silica gel according to Müller and Karrer,[6] failed. With the latter column 50 % ether-petroleum ether was used for elution. Pure, crystalline I was not obtained.

Co-chromatographic tests of the two lutein monomethyl ethers II and V were performed on kieselguhr and aluminium oxide papers. The results are presented in Table 2.

Table 2. R_F-values for the lutein monomethyl ethers prepared by various methods.

Trans carotenoid	Schleicher & Schüll No. 287 kieselguhr paper 5 % acetone-petroleum ether	Schleicher & Schüll No. 288 aluminium oxide paper 20 % acetone petroleum ether
Lutein non-allylic ether = 3-Methoxy-3'-hydroxy-α-carotene (V)	0.59	0.68
Lutein allylic ether = 3-Hydroxy-3'-methoxy-α-carotene (II) $HCl-CH_3OH$ procedure	0.67	0.60
Lutein allylic ether (II) CH_3I-BaO procedure, sample from Müller and Karrer [6]	0.67	0.60

Comparison of II and V with the lutein monomethyl ether prepared by direct methylation of lutein with methyl iodide. The latter compound, m.p. 156—158°C, was kindly provided by Professor P. Karrer. Mixed melting point with the allylic ether II (m.p. 168—169°C) gave no depression (m.p. 165—167°C). The results of co-chromatographic tests are included in Table 2. The IR-spectrum (see Fig. 1) further confirmed identity with the allylic ether (II).

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