

Short Communications

Silylation as a Method for Establishment of Tertiary Hydroxyl Groups in Carotenoids

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The assignment of tertiary hydroxyl groups in carotenoids is often based on the failure of such groupings to form acetates under standard conditions.¹ Infrared absorption in potassium bromide pellets at 1140 cm^{-1} (medium strength band) and 905 cm^{-1} (weak band) is also indicative of such groupings,¹ as are nuclear magnetic resonance signals at *ca.* 8.72τ (in deuteriochloroform), provided adjacent methyl groups are present.^{2,3} Dehydration with phosphorus oxychloride may furthermore give useful information.⁴⁻⁷

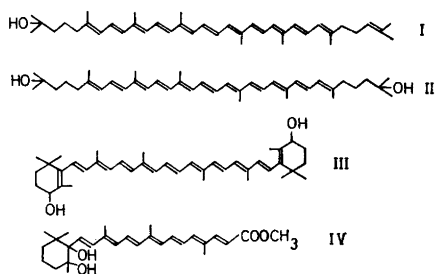
We now report on another positive criterion for the presence of tertiary hydroxyl groups in carotenoids. The recent paper by Friedman and Kaufman⁸ prompts us to publish our results.

Whereas carotenoid acetates are only readily furnished from primary and secondary hydroxy compounds, silyl ethers may equally easily be formed from tertiary hydroxy compounds. Thus rhodopin (I) was quantitatively converted to its trimethylsilyl ether, and 1,2,1',2'-tetrahydro-1,1'-dihydroxy-lycopene (II) to its di-trimethylsilyl ether, by treatment with trimethylchlorosilane and hexamethyldisilazane in dry pyridine, according to the procedures of Sweeley, Bentley, Makita and Wells⁹ and Golding and Rickards,¹⁰ under conditions where isozeaxanthin (III) gave the corresponding di-silyl ether. The silyla-

tion may conveniently be carried out on a micro scale; the silyl ether formation being characterized by a large increase in R_F -value.

Whereas workers in other fields generally have employed acid or neutral conditions for hydrolysis of the silyl ether to the parent alcohol,^{8,9,11} alkaline hydrolysis was shown to be preferable in the present investigation. Treatment with 5% methanolic KOH-solution at room temperature promoted complete hydrolysis. However, the rate of hydrolysis was distinctly lower for tertiary than secondary trimethylsilyl ethers, exemplified by alkaline hydrolysis of rhodopin (I) trimethylsilyl ether and isozeaxanthin (III)-di-trimethylsilyl ether. The latter secondary ether was quantitatively hydrolyzed during 1 h under conditions where the tertiary ether was only partly affected. However, the tertiary ether was quantitatively hydrolyzed during 22 h.

In recent years mass spectrometry of various silyl ethers has been employed with great success.^{10,12} For pertrimethylsilyl ethers the fragmentation pattern at the high mass end of the spectrum is characterised by successive eliminations of neutral trimethylsilanol (90 mass units). This method can also be extended to carotenoids. The mass spectra of the trimethylsilyl ethers of azafrin methyl ester (IV), 1,2,1',2'-tetrahydro-1,1'-dihydroxy-lycopene (II), and rhodopin (I) are reproduced in part in Fig. 1(a), (b), and (c), respectively. All three compounds give an abundant molecular ion and a peak 90 mass units less than this. A further loss of 90 mass units occurs in the spectrum of II (m/e 536). I and II also show eliminations of 92 and 106 mass units from the molecular ion. The free alcohols also exhibited this fragmentation. According to Schwieter *et al.*¹³ carotenoids are known to lose toluene (92 mass units) and xylene (106 mass units) in the mass spectrometer by a *cis*-oid rearrangement of the polyene chain.



Only carotenoids with sterically very hindered hydroxyl groups do not form trimethylsilyl ethers. Thus azafrin methyl ester (IV) gave a mono-trimethylsilyl ether only, the hydroxyl group in the 6-position presumably not being accessible for silylation. As seen from Fig. 1 (a) the spectrum also has a peak at $M-72$. That this is due in part to a fragmentation process is evident from the presence of a metastable peak at m/e 378 (calculated for $512 \rightarrow 440 = 378.1$). Some unreacted IV (M.wt. 440) may also have been present as the sample was not purified prior to introduction into the mass spectrometer.

Mass spectrometry of the parent carotenoid, the fully acetylated product thereof

and the silyl ether of the final acetate, thus in most cases offers a useful method for establishment of the total number of hydroxyl functions in a carotenoid, and for the determination of the number of tertiary hydroxyl groups present.

Materials and methods. I, II, and III were synthetic compounds, IV was prepared by esterification of azafrin with dimethylsulphate and alkali.¹⁴ Solvents and reagents used were of analytical grade. All reactions were performed under nitrogen at room temperature. Concentrations were carried out under vacuum. The paper-chromatographic method previously described,¹⁵ was used. Visible light absorption spectra were recorded on a Beckman DB recording spectrophotometer and mass spectra on an AEI Type MS9 mass spectrometer with a direct inlet system.

Rhodopin (I) trimethylsilyl ether. To rhodopin (1.16 mg) in dry pyridine (0.5 ml) was added hexamethyldisilazane (0.2 ml) and trimethylchlorosilane (0.1 ml). After 1 h the reaction was interrupted by repeated addition of portions of carbontetrachloride, followed by concentrations to dryness *in vacuo*. Finally the trimethylsilyl ether was extracted from the residue with petroleum ether; spectrophotometrically determined pigment recovery was 95 %.

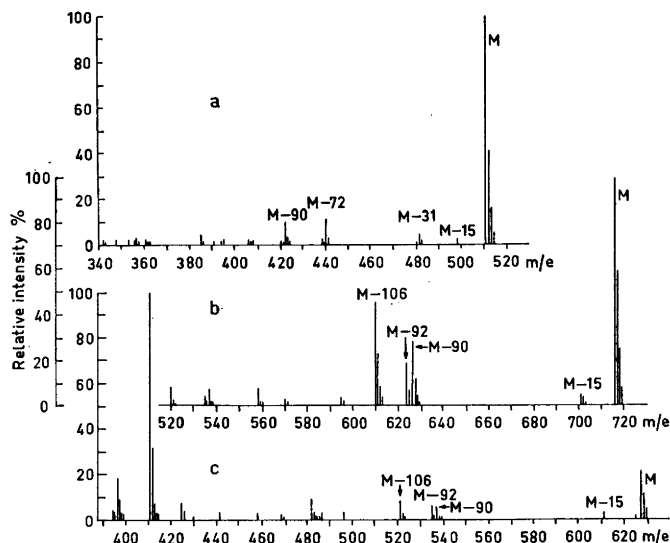


Fig. 1. High mass end of mass spectra of rhodopin(I) trimethylsilyl ether (c), 1,2,1',2'-tetrahydro-1,1'-dihydroxylycopene (II) di-tri-methylsilyl ether (b) and azafrin methyl ester (IV) trimethylsilyl ether (a).

Rhodopin (I) trimethylsilyl ether had the same absorption spectrum in visible light as I¹ and R_F -value = 0.75 (2 % acetone in petroleum ether) on circular kieselguhr paper,¹⁵ compared with R_F = 0.18 for I in the same system.

In a parallel experiment isozeaxanthin (III, 1.21 mg) was treated in like manner. The di-trimethylsilyl ether was obtained in quantitative yield, exhibited the same absorption spectrum in visible light as II and had R_F = 0.11 (petroleum ether) on kieselguhr paper, compared with R_F = 0.60 (10 % acetone in petroleum ether) for II.

Hydrolysis of rhodopin (I) trimethylsilyl ether to I. Treatment of I-trimethylsilyl ether in petroleum ether with excess of water by repeated shaking in a separatory funnel, resulted in a 3 % conversion to I as judged by spectrophotometric and paper-chromatographic examination.

Mechanical shaking for 1 h of the same mixture resulted in a 5 % hydrolysis to I. Shaking overnight in 30 % aqueous methanol resulted in no further hydrolysis.

Treatment of the trimethylsilyl ether with 10 % glacial acetic acid in ether overnight gave no significant hydrolysis, whereas alkaline hydrolysis in 5 % methanolic KOH-solution (followed paper-chromatographically) was completed after 22 h.

In parallel experiments hydrolysis of III-di-trimethylsilyl ether was tested. Treatment with water or methanol resulted in no significant hydrolysis. Only partial hydrolysis was achieved in 10 % glacial acetic acid in ether for 19 h. Alkaline hydrolysis in 5 % methanolic KOH-solution was completed in 1 h.

Acknowledgement. Rhodopin and 1,2,1',2'-tetrahydro-1,1'-dihydroxy-lycopen were kindly provided by Dr. J. D. Surmatis and isozeaxanthin by Dr. O. Isler, Hoffmann-La Roche & Co. Azafirin was a gift from Professor R. Kuhn, Heidelberg. One of us (SLJ) is grateful to *Norges tekniske hogskoles Fond* for a grant.

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Received July 8, 1966.

Spectroscopic Studies on Metal Carbonyls

V. Mean Amplitudes of Vibration and Shrinkage Effects for Nickel Tetracarbonyl

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The previous papers of this series^{1,2} are concerned with hexacarbonyls of octahedral symmetry. In one of them² the calculated mean amplitudes of vibration and Bastiansen-Morino shrinkage effects for chromium and molybdenum hexacarbonyls are reported. The studies on

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