Bacterial Carotenoids XXII*

The Carotenoids of Thiorhodaceae 5. ** Structural Elucidation of Okenone

SYNNØVE LIAAEN JENSEN

Organic Chemistry Laboratories, Norway Institute of Technology, Trondheim, Norway

Further chemical investigations on the structure of okenone, the main carotenoid of some purple sulphur bacteria, have been carried out.

Okenone is a conjugated keto carotenoid with one trimethylphenyl end-group and one methoxylated end-group of the spirilloxanthin type. By mass spectrometry its molecular formula was established as $C_{41}H_{54}O_2$. From chemical and physical properties, including IR- and NMR-data of okenone and the derivatives okenol (III), okenol acetate (IV) and anhydro-okenol (V), the structure II for okenone is suggested.

A CCORDING to the investigation by Schmidt, Pfennig and Liaaen Jensen,[1] the occurrence of okenone appears to be restricted to certain purple sulphur bacteria, more specifically to various strains of *Chromatium okenii* and *Chromatium weissei*. In these organisms okenone is present in rather high quantity and as the single carotenoid component.[1]

Okenone was first characterized by Schmidt, Liaaen Jensen and Schlegel,[2] who described it as a new keto carotenoid containing a methoxy group.

RESULTS AND DISCUSSION

In the present study okenone was isolated from *Chromatium okenii*, strain Ostrau. Okenone constituted about one per cent of the dry cell material, and could be obtained in relatively large amounts. Moreover, okenone itself and its derivatives crystallized readily.

The presence of a conjugated carbonyl group in okenone was inferred from the solvent effects on the absorption spectrum in visible light and from IR-absorption at 1652 cm^{-1}.[2] Hydride reduction to okenol, which has a shorter

---

* Part XXI. *Acta Chem. Scand.* **21** (1967) 371.
** No. 4, *Arch. Mikrobiol.* **52** (1965) 132.

Table 1. Adsorptive properties and partition behaviour of various okenone derivatives.

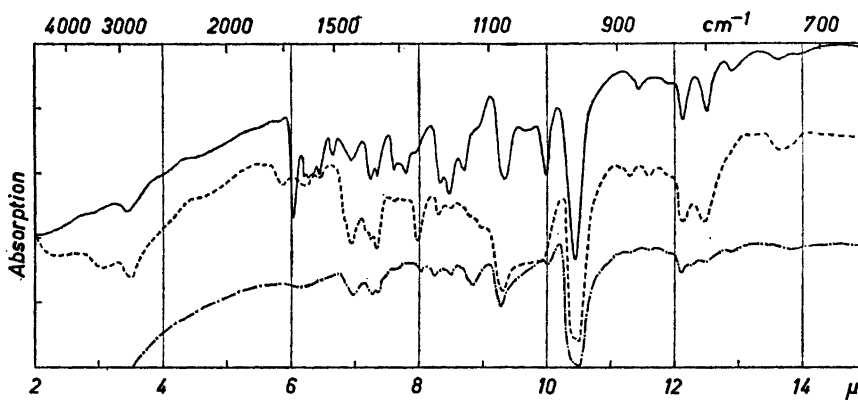| Carotenoid	Required eluant from deactivated alumina	R_F-value, kieselguhr paper		Partition ratio petroleum ether/ 95 % methanol
		5 % acetone *	10 % acetone *	
Okenol acetate	5 % acetone *	0.60		92 : 8
Anhydro-okenol	5 % acetone	0.49		99 : 1
Okenone	8 % acetone	0.33	0.52	87 : 13
Okenol	15 — 20 % acetone	0.18	0.40	56 : 44

* In petroleum ether.

Fig. 1. IR-spectra of KBr of —— okenone, - - - - okenol, and — · — anhydro-okenol.

chromophoric system, gave further support. Okenol exhibited adsorptive properties and partition behaviour (see Table 1), as well as IR-absorption (Fig. 1) consistent with its being a mono-ol. Upon acetylation okenol gave an acetate. Partition behaviour and adsorptive properties of the acetate (see Table 1) supported the absence of free hydroxyl groups. This evidence suggested the presence of one conjugated carbonyl group and the absence of free hydroxyl groups in okenone. The ketonic nature of the conjugated carbonyl group was indicated by the observed carbonyl stretching frequency and lack of aldehydic CH-stretching absorption in the IR-spectrum. The NMR-spectrum also revealed that aldehydic protons were not present in okenone (Fig. 2).

The IR-spectrum of okenone exhibited a medium intensity absorption band at 1072 cm^{-1}, suggesting the presence of a methoxy group. This was confirmed by a signal at 6.84 τ (3 protons) in the NMR-spectrum. Judging from a sharp singlet at 8.80 τ (6 protons) the methoxyl group appeared to be part of a spirilloxanthin type end-group (I, *cf.* Refs. 3, 4).

$$
\begin{array}{c}
CH_3 \\
| \\
R-C-OCH_3 \qquad\qquad (I) \\
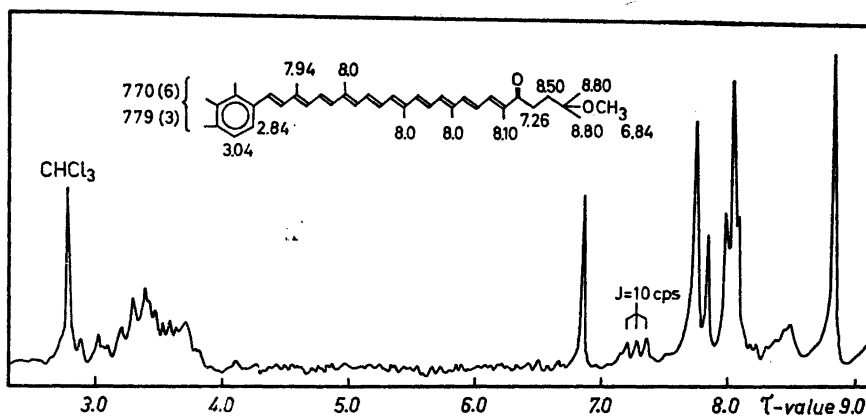| \\
CH_3
\end{array}
$$
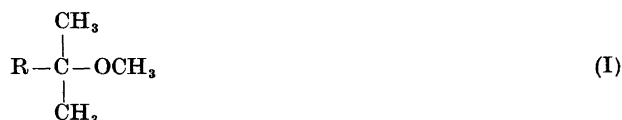
Fig. 2. NMR-spectrum, recorded at 100 Mc/sec of okenone in deuterochloroform.

Furthermore, NMR-evidence revealed the presence of five methyl groups attached to the polyene chain and a tetrasubstituted phenyl ring; *ca.* two aromatic protons and three aromatic methyl groups with signals at 7.70 τ (6 protons) and 7.79 τ (3 protons).[5-7] IR-absorption at 800 cm^{-1} was consistent with the presence of a phenyl substituent with two free adjacent hydrogens.[8] Okenone also exhibited signals at 7.26 (triplet, 2 protons, $J = 10$ cps) and 8.50 τ, thus demonstrating the presence of methylene groups in different surroundings.

We shall now consider the chromophoric system of okenone. Okenol, obtained by hydride reduction of okenone, displayed absorption maxima in
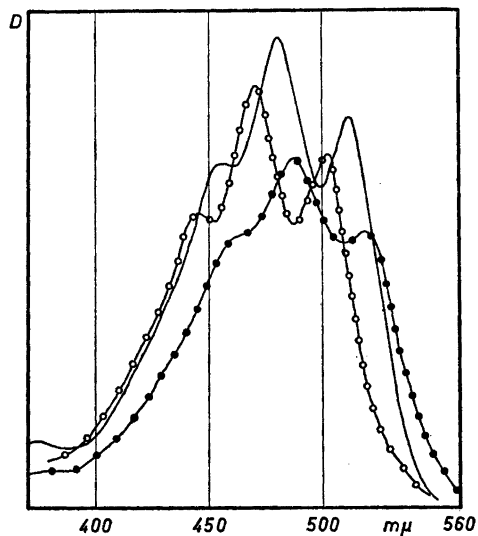

Fig. 3. Absorption spectra in petroleum ether of ——— okenone, ——— O ——— okenol, and — ● — anhydro-okenol.

Table 2. Absorption characteristics in visible light of some carotenoids.

Carotenoid	Petroleum ether Abs. max. in mμ					% III/II	Acetone Abs. max. in mμ				
Okenone	(360)	375	(460)	484	516	13	(360) 381	(465)	487		518
Okenol			444	470	500	51		445	471.5		501
Lycopene			446	472	503	83	(365)	448	474		506
Anhydro-okenol		372	454	479	511	48	378	(460)	483		514
3,4-Dehydro-rhodopin	358	374	454	483	516	81	377	459	487.5		522

visible light (see Fig. 3 and Table 2) in rather similar positions to those of lycopene (with 11 conjugated double bonds in an aliphatic polyene chain) and at considerably longer wavelengths than those of γ-carotene (with 10.3 spectroscopically efficient double bonds [9]). Since okenol was reversibly oxidized to okenone by air under the catalytic influence of iodine and light,[10] no hydrogenolysis or rearrangement could be involved in the hydride reduction of okenone.

Treatment of okenol with acid chloroform according to the method of Karrer and Leumann,[11] gave a dehydration product, anhydro-okenol, in quantitative yield. Adsorptive properties and partition behaviour of anhydro-okenol (see Table 1), indicated the absence of hydroxyl groups. Anhydro-okenol exhibited absorption maxima at somewhat shorter wavelengths than 3,4-dehydro-rhodopin [12] (with 12 conjugated double bonds in an aliphatic polyene chain) and at considerably longer wavelengths than lycopene (see Table 2 and Fig. 3). IR-Evidence (Fig. 1) demonstrated the presence of a non-enolic methoxyl group in anhydro-okenol; the methoxyl frequency being essentially unchanged through the series okenone → okenol → anhydro-okenol. Resistance towards hydride reduction and IR-data (Fig. 1) excluded the presence of carbonyl groups in anhydro-okenol.

Additional information was obtained from a mass-spectrometric determination of the molecular weight of okenone, establishing its molecular formula as $C_{41}H_{54}O_2$.

The above evidence is in accord with structure II for okenone; okenol being III; okenol acetate IV and anhydro-okenol V.
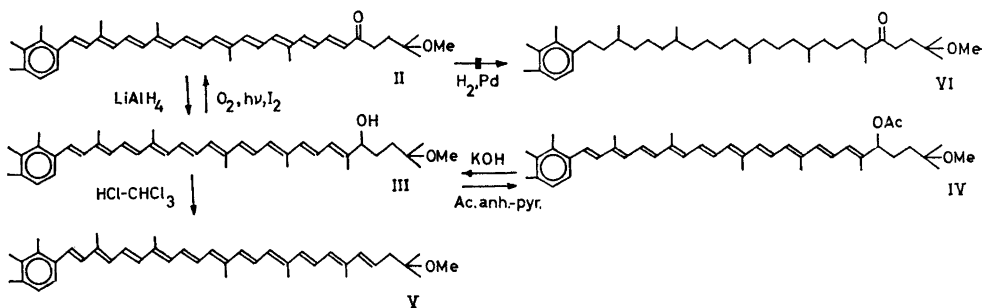
Table 3. NMR-signals in τ-value for some trimethylphenyl-substituted carotenoids.

Carotenoid	Aromatic protons	Aromatic methyl groups				Reference
Chlorobactene (VII)	3.05	7.75	7.77		(2:1) *	6
β-Isorenieratene (VIII)		7.73	7.78		(2:1)	5
β-Renierapurpurin (X)		7.72		7.81	(2:1)	5
Okenone (II)	ca. 3.04, 2.84	7.70		7.79	(2:1)	
Okenol (III)		7.71		7.80	(2:1)	

* Approximate signal ratio.

To meet the requirements of a chromophore corresponding to *ca.* 11 spectroscopically efficient conjugated carbon-carbon double bonds in okenol (III) and the presence of two methylene groups and two adjacent aromatic protons in okenone (II), it was necessary to postulate a 1,2,3-trimethylphenyl end-group. Because of steric hindrance between a possible methyl substituent in the 5-position and the hydrogen atom in the 8-position, the aryl ring, in the case of 1,2,5-trimethyl substitution, would be twisted out of the plane of the polyene chain. In the latter case a shorter chromophoric system, as in chlorobactene (VII), which displays a γ-carotene-type spectrum (10.3 spectroscopically efficient double bonds) is obtained.[5,6] The assignments of the NMR-signals of okenone (II) are given in Fig. 2. In Table 3 a correlation has been attempted of the signal positions for the aromatic protons and aromatic methyl groups of various carotenoids with 1,2,5 and 1,2,3-trimethylphenyl substituents; β-isorenieratene being VIII[5] and β-renierapurpurin X.[5] Data for okenol (III) are also included. It is seen that the aromatic protons of okenone

VII

X

VIII

XI

IX

XII
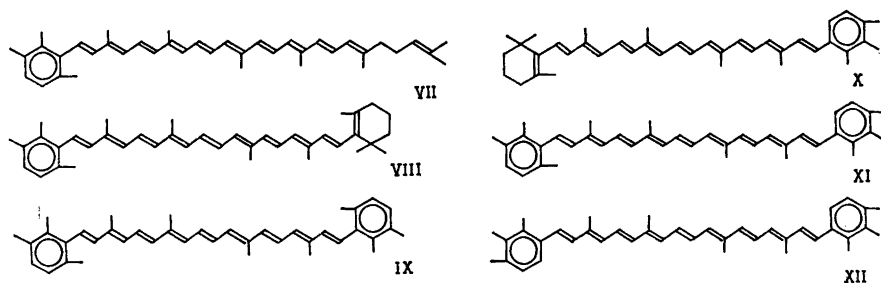
(II) come to resonance at lower fields than those of the hydrocarbon VII. This originally led to the assumption that okenone was an aryl ketone with the keto group in the 7-position. However, all attempts at anionotrophic rearrangement of okenol were negative, thus disfavouring a bi-allylic arrangement for its hydroxy group. To exclude an aryl ketone structure also by NMR-data, attempts were made to synthesize VI by catalytic hydrogenation of okenone with palladium catalyst at conditions known to retain the phenyl residue intact.[13] However, IR- and NMR-data for the product revealed that the keto group had suffered hydrogenation under the conditions employed.

According to Cooper *et al.*[5] the 1,2,5-trimethylphenyl end-group exhibits methyl signals at 7.73 and 7.78 τ (relative intensities 2:1), whereas the 1,2,3-trimethylphenyl end-group has bands at 7.72 and 7.81 τ (2:1). Although the effects appear to be small, the signal positions of the aromatic methyl groups of okenone and okenol (see Table 3) seem to support a 1,2,3-trimethyl substitution pattern.

Some other spectral points deserve comment. As seen from Fig. 3 and Table 1 the hypsochromic shift on reduction of okenone (II) to okenol (III) is 15 mμ in petroleum ether. Spectral shifts on selective reduction of conjugated keto groups in other carotenoids are of the order 6—10 mμ if the keto group is located in a ring and about 26 mμ if the keto group is part of an aliphatic chromophore.[14] Moreover, the carbonyl stretching absorption of okenone (1652 cm^{-1}) is exceptionally low.[14]

In order to establish beyond doubt the structure II for okenone and to answer some of the questions just raised, total syntheses of okenone and related carotenoids were carried out. These results are treated in a subsequent communication.[15]

A direct comparison of okenone with renieraxanthin, isolated by Yamaguchi from a sea sponge *Reniera japonica*,[16] would be interesting. Renieraxanthin, $C_{40}H_{56}O_2$ (estimated from combustion analysis), m.p. 161°C, showed rather similar absorption to okenone in visible light and occurred together with the aryl carotenoids renieratene (XI), isorenieratene (IX), and renierapurpurin (XII), the structures of which have since been well established (*cf.* Ref. 14 for review).

Beside renierapurpurin (XII) and renieratene (XI), okenone (II) is the third naturally occurring aryl carotenoid with a 1,2,3-trimethylphenyl end-group. Although it has never been experimentally proved, aryl carotenoids are thought to be derived from the common carotenoids with 2,2,6-trimethylcyclohexenyl rings by dehydrogenation and methyl migration.[5,6] However, the biosynthetic pathway leading to the 1,2,3-trimethyl substitution pattern of the phenyl ring is intriguing,[5,17] as is the introduction of the conjugated carbonyl function under strictly anaerobic growth conditions.[18] Conditions allowing the isolation of biosynthetic precursors of okenone have not yet been defined.

EXPERIMENTAL

Materials and methods were similar to those described in previous papers of this series; for a summary see Ref. 19. Melting points were measured on a Berl block with abbreviated (50°C) thermometers and are uncorrected. The terms in which spectral fine-structure are expressed have been defined elsewhere.[20]

Biological material. Chromatium okenii. Perty strain Ostrau, obtained from Professor H. G. Schlegel, Institut für Mikrobiologie der Universität, Göttingen, was used. The cultivation was carried out by Docent K. E. Eimhjellen according to the procedure previously described.[2] Mauve cells from *ca.* 50 l of culture in the stationary phase of growth were harvested by centrifugation and were deep-frozen.

Pigment extraction. The deep-frozen cell suspension was thawed and extracted with successive portions of acetone-methanol (7:3) at room temperature. Centrifugation at 4000 g for 20 min was employed between each extraction in order to separate the clear, coloured supernatant from the cell residue. This residue was finally treated with chloroform. The dry cell residue weighed 5.1 g; average yield 0.4 g wet cells/l culture.

Saponification. Preliminary experiments revealed that alkali treatment did not alter the carotenoids, and saponification was carried out in 6 % methanolic KOH-solution for 1 h at room temperature. The unsaponifiable matter was transferred to ether in the usual manner and the pigments submitted to chromatographic purification.

Column chromatography was performed on deactivated alumina. A total of 49 mg okenone (37 % *cis* and 63 % *trans*) was eluted from the chromatogram; for adsorptive properties see Table 1. Other carotenoids were not detected. The yield of okenone was 0.98 % of the dry, extracted cell residue or in average 1.0 mg okenone/l culture.

O k e n o n e (II)

Crystallization. After repeated recrystallizations from dry acetone-carbon disulphide-petroleum ether tiny violet needles with a metallic sheen, forming clusters in solution, were obtained; yield 16 mg. Further amounts of less pure okenone were obtained from the mother liquors. The purest sample melted at 161 — 163°C. Paper-chromatographic examination revealed the presence of a single zone of *trans* okenone; for R_F-value see Table 1.

Solubility. Crystalline okenone was readily soluble in carbon disulphide, moderately soluble in ether and acetone and hardly soluble in petroleum ether.

Absorption spectrum in visible light. Absorption maxima in petroleum ether and acetone solution are given in Table 2, $E \, ^{1 \, \%}_{1 \, cm} = 2320$ at 484 mμ in petroleum ether. The spectrum in petroleum ether is reproduced in Fig. 3.

IR-spectrum (KBr pellet). This is shown in Fig. 1; abs. max. at 2900 (CH), 1652 (conj. ketone), 1600—1550 complex, 1502, 1442, 1380—1360 (methyl, *gem.* methyl), 1315, 1285, 1205, 1185, 1150, (1118), 1072 (methoxyl), 1000, 955 (disubstituted *trans* double bonds), 875, 822 (trisubstituted *trans* double bonds), and 800 cm^{-1} (tetrasubstituted benzene with two free adjacent hydrogens).

NMR-spectrum of okenone in CDCl$_3$ at 100 Mc/sec is presented in Fig. 2.

Mass-spectrometric molecular weight determination gave a molecular peak corresponding to C$_{41}$H$_{54}$O$_2$.

Partition ratio is given in Table 1.

Stereoisomerization studies. The composition of the iodine catalyzed equilibrium is given in the succeeding communication.[15] The true nature of the neo A and neo B isomers as members of the okenone stereoisomeric set was established by reversible isomerization in light.

O k e n o l (III)

Preparation. Okenone (7.1 mg) was reduced with LiAlH$_4$ in dry ether for 1 min in the usual manner; pigment recovery 96 %. The reaction mixture, examined by paper chromatography, contained *cis* and *trans* okenol only. Other experiments gave a similar result. The product was purified by column chromatography, *cf.* Table 1.

Crystallization. Trans okenol was obtained as red needles from acetone-petroleum ether; yield 2.8 mg, m.p. 176 — 177°C.

Solubility. Crystalline okenol was hardly soluble in petroleum ether and fairly readily soluble in ether, acetone, and carbon disulphide.

Adsorptive properties are given in Table 1.

Absorption spectrum in visible light. Absorption characteristics in petroleum ether and acetone are given in Table 2; $E \, ^{1 \, \%}_{1 \, cm} = 2560$ at 471.5 mμ in acetone. The spectrum in petroleum ether is given in Fig. 3.

IR-spectrum (KBr pellet). This is presented in Fig. 1; abs. max. at 3380 (OH), 2860 (CH), 1600, 1440, 1380—1360 (methyl, *gem.* methyl), 1250, 1072 (methoxyl), 1045 (*sec.* hydroxyl), 1000, 955 (disubstituted *trans* double bonds), 823 (trisubstituted *trans* double bonds), and 800 cm^{-1} (tetrasubstituted phenyl with two adjacent hydrogens).

NMR-spectrum of okenol in CDCl$_3$ at 100 Mc/sec. This had methyl signals at 6.72 (*ca.* 3 protons, methoxyl), 7.71 (*ca.* 6 protons, aromatic methyl), 7.79 (*ca.* 3 protons, aromatic methyl), 7.95 (*ca.* 3 protons, methyl in 9-position), 8.03 (*ca.* 9 protons, in-chain

methyls), 8.20 (*ca.* 3 protons, end-of-chain methyl), and 8.87 τ (*ca.* 6 protons, *gem.* methyl in 1′-position).

Partition ratio is given in Table 1.

Stereoisomerization and oxidation.[10] To okenol (85 μg) in benzene (1 ml) and petroleum ether (50 ml) was added a pale violet solution of iodine in hexane (10 capillary drops). The mixture was flushed with nitrogen and kept in indirect daylight for 3 h. The mixture was chromatographed on a) a deactivated alumina column and b) kieselguhr paper. The mixture consisted of 45 % *cis* and *trans* okenone and 55 % *cis* and *trans* okenol. Other experiments gave a similar result.

The *trans*, neo A and neo B okenone produced exhibited absorption spectra in visible light and adsorptive properties identical with those of the corresponding stereoisomers of natural okenone.

The ratio of *cis* and *trans* okenol was determined by paper chromatography and subsequent spectrophotometry in the usual manner, (see the following paper). On standing for 1 h in diffuse daylight the neo-okenol A was reversibly isomerized to *trans* okenol.

Attempts at allylic rearrangement. Treatment with various concentrations of sulphuric or oxalic acid did not result in a product with properties expected for an allylic rearrangement product.

Okenol acetate (IV)

Okenol (0.14 mg) was acetylated with acetic anhydride in dry pyridine by the standard procedure.

Okenol acetate exhibited absorption in visible light identical with that of okenol, see Table 2. Adsorptive properties and partition behaviour are given in Table 1.

On saponification under standard conditions okenol acetate (0.1 mg) was quantitatively converted to okenol.

Anhydro-okenol (V)

Preparation. To okenol (1.8 mg) in chloroform (5 ml) was added 0.3 N HCl−CHCl₃-solution (0.3 ml). After 17 min in daylight the products were isolated in the usual manner; pigment recovery 94 %. Paper-chromatographic examination revealed the presence of *cis* and *trans* V only. Other experiments gave a similar result.

Adsorptive properties are listed in Table 1.

Crystallization. Violet crystals were obtained from chloroform; yield 0.3 mg, m.p. 169−170°C.

Solubility. Crystalline anhydro-okenol was slightly soluble in petroleum ether and methanol, fairly soluble in acetone and readily soluble in carbon disulphide.

Absorption spectrum in visible light. Spectral characteristics in petroleum ether and acetone are listed in Table 2. The spectrum in petroleum ether is given in Fig. 3.

IR-spectrum (KBr pellet). This is presented in Fig. 1; abs. max. at 1630, 1440, 1380−1360 (methyl, *gem.* methyl), 1250, 1175, 1120, 1078 (methoxyl), 1000, 955 (disubstituted *trans* double bonds), 825 (trisubstituted *trans* double bonds), and 800 cm⁻¹ (tetrasubstituted phenyl with two adjacent hydrogens).

Partition ratio is given in Table 1.

Stereochemical studies. The main *cis* isomer, neo A, was reversibly isomerized to *trans* anhydro-okenol in light; for other properties of the stereosiomeric set see the following paper.

Hydride reduction. Treatment of anhydro-okenol (0.2 mg) with LiAlH₄ in dry tetrahydrofurane for 2 min resulted in 87 % pigment recovery. No reduction products were formed.

Hydrogenated okenone

Preparation. Okenone (9.74 mg) in ethyl acetate (7 ml) was hydrogenated at room temperature using 10 % palladium on calcium carbonate (20.7 mg) as catalyst. A hydrogen uptake of *ca.* 9.5 moles per mole of okenone was estimated. The reaction mixture was filtered, the products transferred to ether in a separatory funnel and chromatographed

on a column of deactivated alumina. The waxy hydrogenation product required 15 % ether in petroleum ether for elution.

UV-spectrum. In ether there were no maxima but a slight inflexion at 265 mμ; $E \frac{1 \%}{1 \text{ cm}} = ca. 22$.

IR-spectrum (in chloroform). This exhibited abs. max. at 2950 (strong; CH), 1760 (weak), 1680 (medium), 1460 (strong; CH$_2$), 1375–1360 (medium; methyl, *gem*.methyl), 1280 (weak), 1075 (strong; methoxyl), and 805 cm^{-1} (medium; two adjacent hydrogens in tetrasubstituted phenyl).

NMR-spectrum in CDCl$_3$, recorded at 60 Mc/sec. This exhibited signals at 3.13 (*ca.* 2 protons; aromatic), 6.92 (*ca.* 3 protons; methoxyl), 7.81 and 7.89 (*ca.* 9 protons; aromatic methyls), 8.83–8.91 (*ca.* 40 protons; methylene and *gem.* methyl 1'-position) 9.05–9.19 (*ca.* 15 protons; methyl). Approximate relative integrals are given together with tentative assignments.

REFERENCES

1. Schmidt, K., Pfennig, N. and Liaaen Jensen, S. *Arch. Mikrobiol.* **52** (1965) 132.
2. Schmidt, K., Liaaen Jensen, S. and Schlegel, H. G. *Arch. Mikrobiol.* **46** (1963) 117.
3. Jackman, L. M. *Application of Nuclear Magnetic Resonance Spectroscopy in Organic Chemistry*, Pergamon, London 1959.
4. Jackman, L. M. and Liaaen Jensen, S. *Acta Chem. Scand.* **18** (1964) 1403.
5. Cooper, R. D. G., Davis, J. B. and Weedon, B. C. L. *Proc. Chem. Soc.* **1963** 5637.
6. Liaaen Jensen, S., Hegge, E. and Jackman, L. M. *Acta Chem. Scand.* **18** (1964) 1703.
7. Bonnett, R., Spark, A. A. and Weedon, B. C. L. *Acta Chem. Scand.* **18** (1964) 1739.
8. Bellamy, L. J. *Infra-red Spectra of Complex Molecules*, 2nd Ed., Methuen, London 1958.
9. Liaaen Jensen, S. *Pure Appl. Chem. In press.*
10. Liaaen Jensen, S. *Acta Chem. Scand.* **19** (1965) 1166.
11. Karrer, P. and Leumann, E. *Helv. Chim. Acta* **34** (1951) 445.
12. Jackman, L. M. and Liaaen Jensen, S. *Acta Chem. Scand.* **15** (1961) 2058.
13. Schiller, G. In Houben-Weyl *Methoden der organischen Chemie*, 4th Ed., Thieme, Stuttgart 1955, Vol. 4, Part 2, p. 241.
14. Liaaen Jensen, S. and Jensen, A. In Holman, R. T. *Progress in the Chemistry of Fats and other Lipids*, 1965, Vol. 8, Part 2.
15. Aasen, A. J. and Liaaen Jensen, S. *Acta Chem. Scand.* **21** (1967) 371.
16. Yamaguchi, M. *Bull. Chem. Soc. Japan* **30** (1957) 111.
17. Yamaguchi, M. *Bull. Chem. Soc. Japan* **31** (1958) 739.
18. Liaaen Jensen, S. In Gest, H., San Pietro, A. and Vernon, L. P. *Bacterial Photosynthesis*, Antioch Press, Yellow Springs, Ohio 1963, p. 19.
19. Aasen, A. J. and Liaaen Jensen, S. *Acta Chem. Scand.* **20** (1966) 1970.
20. Liaaen Jensen, S. *Kgl. Norske Videnskab. Selskabs, Skrifter* **1962** No. 8.