# A Note on the Constitutions of Spirilloxanthin and P-481

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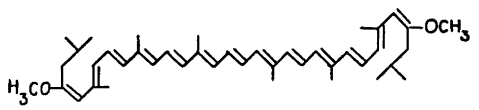
The molecular formula of the carotenoid spirilloxanthin, shown to be identical with that of rhodoviolascin [1], was established by Karrer *et al.*[2,3] and van Niel *et al.*[4,1]. The following constitution (I) was suggested by Karrer *et al.*[5], who isolated from the potassium permanganate oxidation products,




(I)

bixindialdehyde and a methoxyl-free dialdehyde with eleven conjugated double bonds. Karrer *et al.* preferred a symmetrical molecule, and by analogy with the generally occurring 3-substituted carotenoids, they placed the methoxyl groups in the 3,3′-positions. This formula (I) does not contain any *iso*propylidene end groups. Karrer *et al.*[6], however, obtained 0.60–0.73 moles of acetone by ozonolysis. Carotenoids of the lycopene type (two *iso*propylidene end groups) have given 1.24–1.68 moles of acetone by ozonolysis [7,8]; the corresponding values for the γ-carotene type (one *iso*propylidene end group) ranging from 0.6–0.94 [9,10]. The values reported by Karrer *et al.* are unexpectedly high for the structure given (I). By ozonolysis (I) would give two moles of methyl *iso*valerate, from which no measurable quantity of acetone is to be expected. The failure of formula (I) to account for the acetone formed by ozonolysis has already been pointed out by van Niel [11].

Micro determination of *iso*propylidene has been repeated, using the method of Kuhn and Roth [12], on crystalline, chromatographically purified spirilloxantin isolated from *Rhodospirillum rubrum*. The result is presented in Table 1 together with values simultaneously obtained for the carotenoid P-481 [13] from the same source and for chromatographically pure lycopene from tomatoes.

*Table 1.*

| Carotenoid | Found: Moles acetone/mole | Number of *iso*propylidene groups |
|---|---|---|
| Spirilloxanthin | 0.42 | 0 |
| P-481 | 0.82 | 1 |
| Lycopene | 1.70 | 2 |

Although our value for spirilloxanthin is appreciably lower than the values found by Karrer *et al.*, it still exceeds the amount found for carotenoids of the β-carotene type (no *iso*propylidene groups). The reported value for *iso*propylidene determination of β-carotene is 0.2 [9]. We therefore assume that the methoxyl groups of spirilloxanthin belong to a structural element from which some acetone is formed upon ozonolysis.

Assuming a symmetrical formula, Karrer's potassium permanganate oxidation data restrict the location of the methoxyl groups to positions 1, 2 or 3 and 1′, 2′ or 3′. As already mentioned the *iso*propylidene determination does not favour a 3-methoxy-structure. In addition, if the methoxyl groups were placed in the 3,3′-positions the stability of the so-called OH-spirilloxanthin [14] would be difficult to explain. This compound is a bacterial carotenoid with a visible spectrum similar to that of spirilloxanthin, but which according to partition tests and chromatographic behaviour contains one hydroxyl group [15,14]. It has been regarded by Goodwin [15] as a mono-demethylated spirilloxanthin. This assumption seems to agree with the results from kinetic studies of carotenoid synthesis in *Rhodospirillum rubrum* [14]. Mono-demethylated spirilloxanthin, according to formula (I) would be an enol, rearranging in neutral or acidic media to a carotenoid with twelve conjugated double bonds and an isolated keto group. OH-Spirilloxanthin should thus differ remarkably from spirilloxanthin in its visible spectrum. On addition of alkali a pronounced bathochromic shift in the visible spectrum should occur; this is not the case. It may be mentioned that 4,4′-dihydro-rhodoxanthin has a pure β-carotene spectrum [16] with no tendency to prolongation of the chromophore by enolization in neutral solvents.

Carotenoids with allylic methoxyl groups are known to yield products with extended conjugated chains by treatment with

chloroform-hydrochloric acid [17,7]. A series of experiments with chloroform-hydrochloric acid treatment [18] of spirilloxanthin, resulted only in a certain *trans-cis* isomerization of spirilloxanthin and gave no product with an extended conjugated chain. Methoxyl groups are therefore not likely to be present in the 2,2′-positions.

The arguments presented above indicate, by elimination, positions 1,1′ as the most likely sites for the methoxyl groups of spirilloxanthin. Further support for this hypothesis may be obtained from the IR-absorption bands of the methoxyl groups of a series of related compounds (Table 2), although very little data are available.

*Table 2.*

| Compound | C—O Stretching Vibration Frequency $cm^{-1}$ |
|---|---|
| Spirilloxanthin (KBr) | 1 080 |
| 4,4′-dimethoxy-β-carotene (KBr) [18] * | 1 082—1 097 |
| Pigment Y [7] | 1 064 |
| Spheroidenone [7] | 1 066 |
| =C—O—C [19] | near 1 250 |

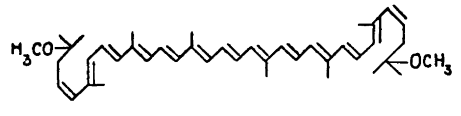
* Private communication from Dr. R. Entschel.

The frequency of the C—O—C stretching vibration of spirilloxanthin is different from that of an enolic methoxyl group.

A relatively intense band around 1 660 $cm^{-1}$ has been shown by Rosenkrantz and Gut [20] to be characteristic for compounds containing the —CH=C—O— grouping. Spirilloxanthin has no absorption band in this region. IR-data are thus not in accordance with formula (I).

As a consequence of the above discussion the constitution (II) is suggested for spirilloxanthin.

As long as the elementary composition of P-481 is unknown, it is premature to indicate a structure for this carotenoid. As seen from Table 1 this carotenoid seems to contain one *iso*propylidene group. A chromophore of twelve conjugated double bonds [14] and the presence of one methoxyl group [21] have already been reported. The IR-absorption band of the methoxyl group [21] at 1 078 $cm^{-1}$ has the same position as that of spirilloxanthin.




(II)

The biological role of P-481 as an intermediate between lycopene and spirilloxanthin in the biosynthesis of carotenoids of *Rhodospirillum rubrum* has been established by Stanier *et al.*[14].

Combining chemical and biological evidence, it is tentatively suggested that the P-481-molecule consists of half a lycopene molecule and half a spirilloxanthin molecule.

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1. Polgàr, A., van Niel, C. B. and Zechmeister, L. *Arch. Biochem.* **5** (1944) 243.
2. Karrer, P. and Solmssen, U. *Helv. Chim. Acta* **18** (1935) 1306.
3. Karrer, P. and Solmssen, U. *Helv. Chim. Acta* **19** (1936) 3.
4. van Niel, C. B. and Smith, J. H. C. *Arch. Mikrobiol.* **6** (1935) 219.
5. Karrer, P. and Koenig, H. *Helv. Chim. Acta* **23** (1940) 460.
6. Karrer, P. and Solmssen, U. *Helv. Chim. Acta* **19** (1936) 1019.
7. Goodwin, T. W., Land, D. G. and Sissins, M. E. *Biochem. J.* **64** (1956) 486.
8. Rabourn, W. J. and Quackenbush, F. J. *Arch. Biochem.* **61** (1956) 111.
9. Haagen-Smit, A. J., Pinchard, J. H. and Zechmeister, L. *Arch. Biochem.* **26** (1950) 358.
10. Kuhn, R. and Grundmann, Chr. *Ber.* **67** (1934) 339.
11. van Niel, C. B. *Bacteriol. Rev.* **8** (1944) 1.
12. Kuhn, R. and Roth, H. *Ber.* **65** (1932) 1285.
13. Goodwin, T. W. and Land, D. E. *Arch. Mikrobiol.* **24** (1956) 305.
14. Liaaen Jensen, S., Cohen-Bazire, G., Nakayama, T. O. M. and Stanier, R. Y. *Biochim. et Biophys. Acta* **29** (1958) 477.
15. Goodwin, T. W. and Osman, H. G. *Biochem. J.* **56** (1954) 222.
16. Kuhn, R. and Brockmann, H. *Ber.* **66** (1933) 828.
17. Wallcave, L. and Zechmeister, L. *J. Am. Chem. Soc.* **75** (1953) 4495.

18. Entschel, R. and Karrer, P. *Helv. Chim. Acta* **41** (1958) 402.
19. Bellamy, L. J. *The Infra-red Spectra of Complex Molecules.* 2nd Ed. Methuen and Co. Ltd., London 1958, p. 114.
20. Rosenkrantz, H. and Gut, M. *Helv. Chim. Acta* **36** (1953) 1000.
21. Liaaen Jensen, S. *Acta Chem. Scand.* **12** (1958) 1698.

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## Synthesis of Certain S-Substituted L-Cysteines

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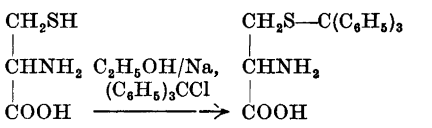
Twenty years ago du Vigneaud [1] introduced the benzylation of the thiol group of cysteine by adding benzyl chloride to the liquid ammonia solution of the sodium salt of cysteine. The S-benzyl group was reversibly split off by reduction with sodium in liquid ammonia. This method has played a fundamental role in the synthesis of various cysteinyl peptides and more recently in the dramatic synthesis of oxytocin [2].

Since the discovery by Weisberger and Levine [3] that leukaemic leukocytes have a high initial cystine-cysteine requirement, several S-cysteine analogues have been synthesized and tested as possible anticancer agents [4]. In most cases du Vigneaud's method has been employed with excellent results. Thus, S-dichlorovinyl-L-cysteine was prepared by McKinney [5] *et al.* in 60—70 % yield.

An alternative procedure for the synthesis of S-alkyl cysteines has been introduced by Zahn and Traumann [6]. Cysteine hydrochloride was treated with the appropriate alkyl bromide in a bicarbonate solution in the presence of nitrogen. Among the products thus produced, S-methyl-L-cysteine and S-ethyl-L-cysteine were obtained in 20 and 10 % yield, respectively. In a similar manner, but in alkaline solution with sodium hydroxide instead of bicarbonate, aminoethylcysteine[7] was synthesized.

In this respect it was found that treatment of cysteine in absolute ethanol with sodium, followed by addition of the appropriate alkyl or phenyl halide, results in the production of the desired S-derivative in excellent yield. Thus, S-benzyl-L-cysteine was synthesized in 70 % yield and its optical value shown to be identical to that reported. Excellent yields were also obtained in the cases of S-butyl-L-cysteine, S-ethyl-L-cysteine and S-methyl-L-cysteine. The thus obtained products were found to be homogeneous according to paper chromatography, as far as the method can detect. This finding suggests that the oxidation of cysteine to cystine is extremely limited or avoided under the experimental conditions described here. On the contrary, the diazonium salt procedure [8,9] for the preparation of S-aryl-L-cysteines gives products always contaminated with cystine, which is difficult to remove.

Treatment of cysteine hydrochloride with trityl chloride in a similar manner, produces S-trityl-L-cysteine in about 20 % yield.




A similar product has been synthesized by selective detritylation of S,N-ditrityl-L-cysteine [10], prepared by direct tritylation [11] of cysteine in the presence of diethylamine, but its reported optical value is entirely different to that described in this parer (see Experimental).

*Experimental. S-Benzyl-L-cysteine.* To a suspension of 1.75 g (0.01 mole) of L-cysteine hydrochloride monohydrate in 30 ml of absolute ethanol, 0.92 g (0.04 mole) of sodium was added within 10 min at room temperature. When the last piece of sodium was about to disappear, 1.26 g (0.01 mole) of benzyl chloride was added with stirring. After 2 min the reaction mixture was poured into 50 ml of water * and the solution acidified with acetic acid to pH 5—5.5. The desired product precipitated immediately. It was cooled in the refrigerator, filtered and washed successively with water and ethylether. Yield 1.5 g (71 %) m. p. 216—218° (decomp.), reported [1] 216—218° (decomp.),

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* Provided that all the sodium had disappeared.