to 7.5 with hydrochloric acid, 2 ml of 1 % ferric chloride added, and air was bubbled through *via* a sintered glass filter. After 27 h, the solution became clear yellow, and it was acidified to pH 3. An unidentified yellow oil was first removed by extraction with five 100 ml portions of benzene, and then the product (III) was isolated by extraction with three 100 ml portions of ether. The crude yield of 1,2-dithiane-4,4-dicarboxylic acid was 5 g (52 %). Recrystallization from alcohol-water produced white crystals which melted with decarboxylation at 152°. Equiv. wt. calc. 104.1; found 107.0.

1,2-Dithiane-4-carboxylic acid (IV). 4 g (0.0192 mole) of crude 1,2-dithiane-4,4-dicarboxylic acid (III) was heated at 150—170° for half an hour. Recrystallization from alcohol-water and subsequently from petroleum ether-benzene produced 1.85 g (59 %) of pure 1,2-dithiane-4-carboxylic acid melting at 115.2—116.2°. (Found: C 37.10; H 4.90; S 38.78; equiv. wt. 165.0. Calc. for $C_5H_8S_2O_2$ (164.2); C 36.81; H 4.91; S 39.05; equiv. wt. 164.2.)

Preliminary resolution of 1,2-dithiane-4-carboxylic acid. Quinine and cinchonidine salts were found to be suitable for the resolution of racemic IV. Brucine, α-phenyl-ethylthiuronium chloride and α-(2-naphthyl)-ethylamine proved to be ineffective as resolving agents. The impure dextro enantiomorph, $[\alpha]_D^{25} = +71.5°$ in ethanol solution; m. p. 109—113°, was obtained after four crystallizations of the cinchonidine salt from abs. ethanol. The impure levo antipode, $[\alpha]_D^{25} = -123.5°$; m. p. 106.5—108°, was obtained after four crystallizations of the quinine salt from abs. ethanol. The UV spectra of the two enantiomorphs were essentially identical, with absorption maxima at 211 and 298 mμ (in ethanol).

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A Note on the Constitution of Rhodopin

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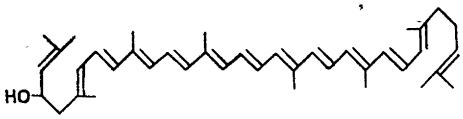
The carotenoid rhodopin was first isolated in 1936 by Karrer and Solmssen [1] from mixed cultures containing *Rhodovibrio* species and was also shown to be present in *Thiocystis* [2]. The elementary analysis was in good agreement with a molecular formula $C_{40}H_{56}O$, although Karrer *et al*[3]. did not exclude the possibility of $C_{40}H_{56}O$. The oxygen function was shown to be a hydroxyl group by quantitative determination of active hydrogen [2,3]. Because of its resistance to acetylation rhodopin was assumed to have a tertiary hydroxyl group [3]. Upon catalytic hydrogenation an uptake of 11.93 and 11.71 moles of hydrogen per mole rhodopin was recorded, and Karrer and Solmssen [3] assumed that twelve carbon-carbon double bonds were present in rhodopin. Rhodopin occurred in complex mixture with other carotenoids including a carotene which these workers named rhodopurpurin[1], and they suggested that rhodopin might be a derivative of rhodopurpurin [1]. They also pointed out the possible identity of lycopene and rhodopurpurin [3] *, but no structural formula for rhodopin was suggested.

More recently Goodwin *et al.*[4,5] have isolated from a number of photosynthetic bacteria *(Athiorhodaceae* and *Thiorhodaceae)* a carotenoid which has the lycopene chromophore and which according to partition tests and chromatographic behaviour was assumed to be a mono-OH-lycopene. Taking into account the source of isolation and the visible spectra and chromatographic behaviour Goodwin and Land concluded that this pigment was identical with rhodopin.

A mono hydroxy derivative of lycopene, lycoxanthin, had previously been isolated by Zechmeister and Cholnoky [6] from the berries of *Solanum dulcamara*. Lycoxan-

* This suggestion has since been verified by Goodwin and Land [5] by mixed chromatography and comparison of spectroscopic properties. It should, however, be kept in mind that lycopene from photosynthetic bacteria has so far not been isolated in crystalline state.



(I)

thin was easily acetylated, and Zechmeister and Cholnoky suggested the formula (I). The hydroxyl group was allocated to the 3-position in accordance with the normal, smooth formation of acetates from secondary alcohols, and the widespread occurrence of 3-OH-substituted carotenoids.

Goodwin and Land [5] reisolated lycoxanthin from *Solanum dulcamara*, and compared it with the rhodopin isolated from a photosynthetic sulphur-bacterium, *Chromatium spp.* It was concluded that rhodopin was identical with lycoxanthin on the basis of their similar chromatographic behaviour, and because of the fact that the two pigments exhibited the same visible absorption spectrum in different solvents before and after iodine isomerization. Whilst the similarity of the visible absorption spectra and chromatographic behaviour indicate that lycoxanthin and rhodopin have the same chromophore and the same number of hydroxyl groups, it is by no means certain that the positions of the latter are the same in the two compounds. Goodwin and Land did, however, exclude hydroxyl in the 4-position on the grounds of a negative $HCl-CHCl_3$ test. It is surprising that these authors made no comment on the different behaviour of rhodopin and lycoxanthin [3,6] towards acetylating agents. In later work the rhodopin of the photosynthetic bacteria has been referred to mainly as lycoxanthin in the literature [7], although Stanier *et al.*[8] preferred the designation OH-lycopene until this structural problem was satisfactorily settled.

It has been established by Stanier *et al.*[8] that OH-lycopene is an intermediate between lycopene and spirilloxanthin in the biosynthesis of carotenoids in *Rhodospirillum rubrum*. Since the structure of OH-lycopene is of importance in this connection, the properties of this carotenoid have been investigated in more detail.

OH-Lycopene was isolated from *Rhodospirillum rubrum* in the exponential growth phase and from *Rhodopseudomonas palustris*. According to van Niel [9] the genetic name *Rhodopseudomonas* is synonymous with *Rhodovibrio* and *Rhodobacillus*, from which Karrer and Solmssen isolated rhodopin.

As already reported by Goodwin and Land [5] no difference in structure for lycoxanthin and rhodopin can be predicted from comparison of visible spectra in different solvents, nor from partition tests and chromatographic behaviour. Also the melting points recorded are so close that they yield no useful information. The melting point reported for lycoxanthin is 168°C, whilst that for rhodopin varies from 159—160°C [1] to 171°C [3] with increasing purity. The OH-lycopene used in the present studies melted at 166—168°C.

The outstanding difference between lycoxanthin and rhodopin was in their behaviour towards acetylating agents, and this aspect was therefore studied first. Numerous attempts were made using different methods to acetylate OH-lycopene from *Rhodospirillum rubrum* and *Rhodopseudomonas palustris*. After standing with acetic anhydride in pyridine at room temperature for periods of up to two days the OH-lycopene could be completely recovered. A parallel experiment with lutein (3,3′-di-OH-α-carotene) resulted in the formation of lutein diacetate in quantitative yield. Treatment with freshly distilled acetyl chloride in pyridine at room temperature for periods up to four days gave a product with properties corresponding to an acetate. However, the yield did not exceed 8 % of the OH-lycopene used for the test. Upon saponification of this acetate with 15 % methanolic KOH about 30 % was converted to OH-lycopene. This behaviour of OH-lycopene towards acetylating agents is similar to that reported by Karrer *et al.*[3] for rhodopin, but not for lycoxanthin [6].

Further support for the tertiary character of the hydroxyl group in OH-lycopene from photosynthetic bacteria is obtained from the IR absorption data presented in Table 1.

Carotenoids with the hydroxyl groups in 3- or 4-positions show strong absorption in the 1 030 cm^{-1} region. Chloroxanthin and OH-lycopene, however, have no absorption band in this region. Chloroxanthin is a mono- or di-OH-neurosporene which was isolated by Nakayama [12] from a green mutant of *Rhodopseudomonas spheroides*. Less than 5 % of chloroxanthin is acetylated on treatment with acetic anhydride in pyridine, and this led Nakayama to the conclusion that the hydroxyl group(s) of chloroxanthin must be tertiary. Both chloroxanthin and OH-lycopene exhibit a band of somewhat weaker intensity around 1 140 cm^{-1} which might be due to tertiary hydroxyl groups [13]. It might be argued that this region is also typical for *iso*propyl groups (1 170—1 140 cm^{-1}) [13], but an IR-spectrum of lycopene isolated from tomatoes showed no band of similar intensity in this region. The

Table 1.

Carotenoid	IR-absorption connected with the hydroxyl group Frequency cm^{-1}
Cryptoxanthin (3-OH-β-carotene) $CHCl_3$[10]	*ca.* 1 035
Isozeaxanthin (4,4′-di-OH-β-carotene) CCl_4[11]	*ca.* 1 035
Zeaxanthin (3,3′-di-OH-β-carotene) KBr	1 038
Violaxanthin (3,3′-di-OH-β-carotene-5,6,5′,6′-di-epoxide) $CHCl_3$	1 025
Chloroxanthin KBr	1 145
OH-Lycopene KBr	1 140

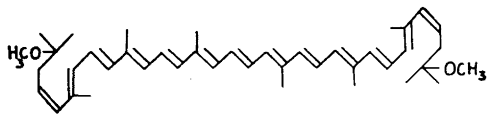
IR-spectrum of lycopene is in fact surprisingly simple, and corresponds closely to that of OH-lycopene apart from a band at 3 450 cm^{-1} (OH), a relatively weak band at 1 260 cm^{-1} and the band at 1 140 cm^{-1} already discussed.

Microdetermination of *iso*propylidene was carried out, using the method of Kuhn and Roth [14]. The result is presented in Table 2 together with the value obtained simultaneously for lycopene isolated from tomatoes.

Table 2.

Carotenoid	Found: Moles of acetone/mole	Number of *iso*-propylidene groups
Lycopene	1.70	2
OH-Lycopene	1.12	1

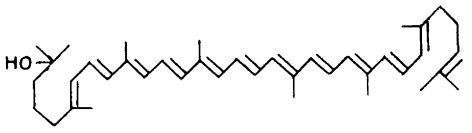
It is seen that the value obtained for OH-lycopene is much lower than that for lycopene. It has been shown that spirilloxanthin to which the formula (II) is now ascribed [15],



(II)

and which contains no *iso*propylidene groups, yields 0.42—0.73 [15,2] moles of acetone upon ozonolysis. The *iso*propylidene value obtained for OH-lycopene is therefore interpreted as being due to the presence of one *iso*propylidene end group together with a second end group which yields a small amount of acetone on ozonolysis.

Combining the chemical evidence obtained by Karrer *et al.*[1-3] for rhodopin, and the evidence presented here, the following structure (III) is suggested for the OH-lycopene from photosynthetic bacteria.



(III)

In accordance with the generally accepted nomenclature, this should now be referred to as rhodopin. The name lycoxanthin should be reserved for the hydroxy derivative of lycopene which was first isolated from *Solanum dulcamara* and for which the structure (I) has been suggested by Zechmeister and Cholnoky [6].

In view of the findings described above, it is clear that as far as the biosynthetic reactions leading from lycopene to spirilloxanthin are concerned, no definite conclusions should be drawn until the chemical structure of all the intermediates which can be isolated in reasonable amounts is firmly established.

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The Mass Spectra of Methyl Oleate, Methyl Linoleate, and Methyl Linolenate

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In connexion with studies on the mass spectra of esters of long-chain fatty acids we have examined a number of unsaturated esters. In general, unsaturated esters give more complex spectra than esters of saturated, normal-chain acids [1,2]. In order to interpret the spectrum of methyl oleate we have examined the isomeric 2-, 6-, 8-, 10-, 13- and 17-octadecenoates, but it has proved desirable to study also the ester of an acid with the double bond at position 4:5 or 5:6. None of these acids has been described in the literature. Synthetic work will therefore have to be undertaken and may also be required for the interpretation of the spectra of the di- and triethenoid esters. In view of the common occurrence of oleic, linoleic, and linolenic acids, however, and the analytical possibilities afforded by the mass spectrometer [3], a brief description of the mass spectra of their esters will be made.

The mass spectrum of methyl oleate (Fig. 1a) shows that the introduction of a double bond in the hydrocarbon chain leads to a fragmentation pattern considerably different from that of saturated methyl esters. Compared with methyl stearate [2], the peaks in the low-mass range due to fragments containing an intact methoxycarbonyl group are much less prominent than those due to hydrocarbon fragments. The peak at $m/e = 55$ ($C_4H_7^+$) is of the same order of magnitude as the rearrangement peak at $m/e = 74$. In the sequence of the higher-methoxycarbonyl-type fragments, the peak at $m/e = 87$ is the most prominent one (45 % of the base peak). The higher members of this series of peaks do not show the marked rhythmic variation in height which is characteristic of methyl esters of normal-chain saturated acids. In the high-mass range, marked peaks are found at $m/e = 296$, 264, 222, and 180. The peak due to the molecule-ion at $m/e = 296$ is relatively small, and the base peak at $m/e = 264$ corresponds to a fragment formed with the loss of 32 mass units. A comparison with the ethyl ester shows that the molecule-ion loses a methoxyl group and one hydrogen atom (formally, this corresponds to the loss of one molecule of methanol). The peak at $m/e = 265$ is higher than expected for the isotope peak corresponding to the peak at $m/e = 264$, which shows that ions are also formed with the loss of the methoxyl group only. The peak at $m/e = 222$ ($= M-74$) is due to a fragment formed by the loss of methylene-methoxycarbonyl, $-CH_2COOCH_3$, of mass 73, together with one hydrogen atom. The appearance of fairly marked peaks at $m/e = 180$, 222, 264, and 265 in both the methyl and the ethyl esters shows that the corresponding ions do not contain the ester alkoxy group.

The mass spectrum of methyl elaidate is practically indistinguishable from that of methyl oleate. Methyl esters of *cis-* and *trans-*petroselinic acids were both found to be indistinguishable from that of methyl oleate. Even a shift of the double bond to the 17:18