CHLOROBIUM CHLOROPHYLL-660. THE ESTERIFYING ALCOHOL.

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The chlorophyllous pigments produced by the photosynthetic bacteria Chlorobium thiosulfatophilum have been recognized as unique among the various chlorophylls. In particular, they do not contain a methoxyl group and do not give the Molisch phase test (Stanier and Smith, 1960; Holt and Morley, 1960; Holt and Hughes, 1961). Also, Holt, et al, (1960, 1961), have reported that the alcohol portion of the remaining ester appears to differ from phytol, which is found in other chlorophyll pigments (Fischer and Stern, 1940).

In this report, we present evidence which identifies the esterifying alcohol of chlorobium chlorophyll-660, produced by Chlorobium thiosulfatophilum (strain PM).[2]

Purified chlorobium chlorophyll-660 (Stanier and Smith, 1960; Holt and Morley, 1960), obtained in about 5% yield from dried cells, was converted to the corresponding pheophytin derivative by treatment with acid (Golden, Linstead, and Whitham, 1958). Base catalyzed hydrolysis of this pheophytin gave a complex mixture of alcohols. However, direct alkaline hydrolysis of

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chlorobium chlorophyll-660 gave an 85% yield (assuming mol. wt. 825) of an alcohol which was over 95% a single component as determined by gas phase chromatography (g.p.c.). Thus, it was evident that the mixture of alcohols obtained from the pheophytin was due to the acid used in converting chlorophyll-660 to pheophytin. When the acid treatment was omitted, the esterifying alcohol was obtained essentially as a single component.

The alcohol, purified by g.p.c. on a poly m-phenyl ether column, had the molecular formula $C_{15}H_{26}O$ (Anal. Calcd.: C, 81.0; H, 11.8. Found: C, 81.2; H, 11.6.). There were three C-methyl groups (calcd., 20.2; found, 13.6.) and three double bonds (absorption of 300 mole % of hydrogen to yield the saturated alcohol $C_{15}H_{32}O$). The double bonds were not conjugate, since there was no ultraviolet absorption, and they were located by the following procedures:

Ozonolysis (Knowles and Thompson, 1960) gave levulinaldehyde, isolated in 56% yield as its bis-2,4-dinitrophenylhydrazone, m.p. 234-236° [reported m.p. 235.5-236.5° (Strain, 1935). Anal. Calcd. for $C_{17}H_{16}N_8O_8$: C, 44.4; H, 3.5; N, 24.3. Found: C, 44.2; H, 3.6; N, 24.0.].

A periodate-permanganate oxidation of the alcohol (Lemieux and Rudloff, 1955) in aqueous t-butyl alcohol was carried out for six hours at 25°. Sodium bisulfite was added, and the resulting solution was treated with sodium carbonate and semicarbazide hydrochloride. Removal of the t-butyl alcohol in vacuo and continuous extraction of the aqueous solution with ether gave, on evaporation of the ether and sublimation, acetone semicarbazone (85% yield), identical with an authentic sample by mixed m.p. (184-187°) and infrared absorption.

The nuclear magnetic resonance spectrum of the alcohol (in carbon tetrachloride with tetramethylsilane as internal standard,

Varian model A-60 spectrometer) showed a peak at τ = 6.04 (doublet, J = 6 c.p.s.), characteristic of methylene hydrogens of an allylic alcohol, $=CH—CH_2OH$. This was confirmed in the hydrogenated alcohol which exhibited a triplet at τ = 6.40 (J = 12.5 c.p.s.), assigned to the methylene hydrogens of a primary alcohol, $—CH_2—CH_2OH$.

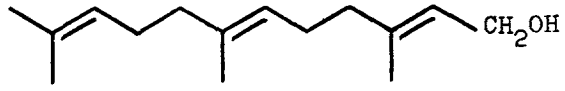
Also, the alcohol showed two sharp singlets at τ = 8.34 and τ = 8.41, characteristic of olefinic methyl hydrogens, $=C<^{C}_{CH_3}$. The absence of any absorption at higher fields eliminated the presence of a methyl group on a saturated carbon.

The foregoing data unambiguously locate the double bonds and establish the alcohol as a farnesol. This was confirmed by comparison with a sample of synthetic farnesol, the major component[3] of which was identical by g.p.c., i.r., and n.m.r. with the alcohol from chlorobium chlorophyll-660.

The only remaining question was the stereochemistry at the Δ^2 and Δ^6 double bonds. Comparison in the infrared with nerolidol (Ofner, et al, 1959) indicated the configuration at the Δ^6 double bond was trans. cis-Nerolidol exhibited a single, medium intensity band, ν_{max} 833 cm^{-1}; trans-nerolidol exhibited a weak band, 835 cm^{-1}, and a shoulder, 809-817 cm^{-1}. The chlorobium alcohol showed weak absorption at 835 cm^{-1} and a shoulder at 815 cm^{-1} Confirmation for this assignment and an assignment for the Δ^2 double bond is based on the work of Bates and Gale (1960). They have reported n.m.r. data for the various farnesol isomers, and the trans, trans isomer shows two sharp peaks, at τ = 8.34 and 8.41. This is exactly what is found for the alcohol from

[3] This sample contained a small amount (ca. 10%) of a closely related substance, probably a stereoisomer.

chlorobium chlorophyll-660, and therefore we conclude its structure is trans, trans-farnesol.



trans, trans-farnesol

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