The Identity of Leprotene with Isorenieratene

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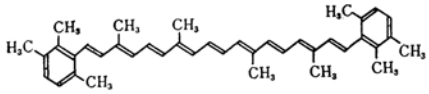
A carotenoid hydrocarbon, leprotene, was first isolated from a strain of the acid-resistant bacteria of a leper in 1937[1]; later it was shown to be a common carotenoid pigment of the *Mycobacterium* genus[2], e. g., *M. phlei*[3] or *M. tuberculosis*[4]. Leprotene has a similar spectral property to that of β-carotene, but it differs in melting point, in chromatographic behavior and in the absence of vitamin A activity.[5] Takeda and Ohta proposed the formula $C_{40}H_{54}$ for leprotene on the basis of their analytical and microhydrogenational results and suggested that leprotene is a dehydrogenated β-carotene.[6] This suggestion has never been established because of a lack of data. Recently, however, one of the present authors, Masaru Yamaguchi, found aromatic carotenoids in a sea sponge[7] and pointed out that one of these aromatic carotenoids, isorenieratene (I), closely resembled leprotene in its properties.[8]



I

In the present study, leprotene was extracted from *M. phlei* and compared with isorenieratene. It was proved that leprotene is completely identical with isorenieratene in mixed melting point determination, ultraviolet- and infrared-spectra, mixed chromatography and CH-analysis.

This result not only clarifies the chemical constitution of leprotene, which has been unknown for a long time, but also establishes the second occurence of an aromatic carotenoid in nature, indicating an even wider distribution of this unique group of carotenoids.

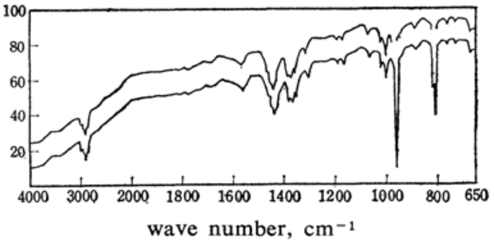


Fig. 1. IR-Spectra of leprotene (upper) and isorenieratene (lower) (KBr disk).

Experimental

A pure strain of typical *M. Phlei* was cultivated at 35°C for thirteen to fifteen days until the deepest coloration developed on Sauton's medium, which contained sodium glutamate (8 g.), citric acid (2 g.), dipotassium hydrogen phosphate (0.5 g.), magnesium sulfate (0.5 g.), ferrous ammonium citrate (0.05 g.), and glycerol (60 g.) in water (1000 g.). The cells were collected by suction, washed with methanol, and extracted with acetone (the dry weight of the residue of the extraction was 800 g.). Pigments were transferred into petroleum benzine by the addition of water, and the benzine layer was washed with water, dried, and evaporated under a vacuum. The deep red oily concentrate separated a minute quantity of a brown precipitate on standing, this was removed by filtration. The concentrate was then

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[1] C. Grundmann and Y. Takeda, *Naturwissenschaften*, **25**, 27 (1937).

[2] T. W. Goodwin, "Carotenoids," Chemical Publishing Co., New York (1954), pp. 120, 124.

[3] Y. Takeda and T. Ohta, *Z. physiol. Chem.*, **262**, 168 (1939); **265**, 233 (1940); T. W. Goodwin and M. Jamikorn, *Biochem. J.*, **62**, 269 (1956).

[4] Y. Takeda and T. Ohta, *J. Pharm. Soc. Japan*, **64**, 67 (1944).

[5] Y. Takeda and T. Ohta, *Z. physiol. Chem.*, **267**, 171 (1941).

[6] Y. Takeda and T. Ohta, ibid., **258**, 6 (1939).

[7] M. Yamaguchi, This Bulletin, **30**, 111, 979 (1957); **31**, 51, 739 (1958).

[8] M. Yamaguchi, ibid., **30**, 111 (1957).

TABLE I. PIGMENTS OF *M. Phlei*

The width of the zones mm.	The color of the zones	UV-Max. (mμ) (Petroleum benzine)
14(upper)	Dark brown (not homogeneous)	
20	Colorless	
22	Reddish orange	481, 451, 425
25	Red	481, 453, 426
41	Colorless	
34	Yellowish orange	480, 451, 425
18(lower)	Yellow	478, 448

saponified with ethanolic potassium hydroxide (10%, 100 cc.) in a benzene solution (200 cc.). The mixture was washed with water, dried, and subjected to chromatography (alumina, 23×4.5 cm.; developer, a benzene-benzine mixture). Table I shows the main zones of a typical chromatogram and their ultraviolet-maxima in a petroleum benzine solution. The third reddish orange zone gave leprotene when the concentrate of the elute was treated with ethanol containing benzine. The pigment was recrystallized several times from benzene-methanol and benzene-ethanol mixtures. Copper red needles (6.5 mg.), m. p. 199°C. Abs. max., 481, 451, 425 mμ (petroleum benzine). Found: C, 90.22; H, 9.07. Calcd. for $C_{40}H_{48}$: C, 90.85; H, 9.15%.

The leprotene thus obtained did not show any depression of melting point when admixed with a sample of the isorenieratene obtained from a sponge ($C_{40}H_{48}$; copper red needles; m. p. 199°C; abs. max. 481, 451, 425 mμ)8) and gave the same absorption curve as that of isorenieratene.7) Infrared-spectra, Fig. 1. Neither of the pigments separated on an alumina column (25×1.2 cm.).

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