Bacterial Carotenoids

XVI. A Comparative Study of Leprotene and Isorenieratene

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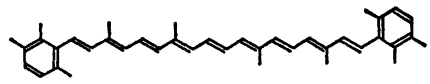
In 1937 Grundmann and Takeda [1] described the isolation of a new carotenoid hydrocarbon, leprotene, from a strain of acid-resisting bacteria obtained from a leper. The same pigment was later found in a strain of *Mycobacterium phlei*.[2] Leprotene agreed well with β-carotene in light absorption properties, but differed markedly from the latter compound (m.p. 183°C) in chromatographic behaviour and in melting point (198–200°C). Takeda and Ohta [3] assigned the molecular formula $C_{40}H_{54}$ to leprotene, but the suggestion that it was a dehydro-β-carotene was later withdrawn.[4] It has since been claimed that leprotene is present in various *Mycobacterium* species.[5-10]

In 1954–1960 Yamaguchi [11-17] isolated a number of carotenoids from the marine sponge *Reniera japonica* and demonstrated that three of them, *viz.* renieratene, isorenieratene and renierapurpurin (which had the elementary composition $C_{40}H_{48}$) possessed trimethylphenyl end groups instead of the more usual 2,2,6-trimethylcyclohexene rings. Identity of leprotene and isorenieratene was considered by Yamaguchi,[13,14] but discounted.

Liaaen Jensen and Weedon [18] have recently claimed the identical nature of leprotene and synthetic isorenieratene (I) based on a direct comparison of the two compounds.



(I)

In the present paper experimental details of the comparative study of leprotene and synthetic isorenieratene are reported.

A sample of 0.2 mg leprotene, left from the investigations of Grundmann and Takeda,[1] was used for the following studies.

The specimen, sealed under vacuum for some 25 years, consisted of cerise red clusters of tiny needles, which were slightly soluble in pet.ether and fairly readily soluble in acetone, benzene, and CS_2. A paper-chromatographic purity test on aluminiumoxide-containing paper (Schleicher and Schüll No. 288)[19] gave one yellow zone (R_F = 0.66; 1 % acetone-pet. ether) and a reddish spot of decomposition products (R_F = 0). The latter constituted less than 20 % of the former. Under similar conditions β-carotene exhibited a higher R_F-value (R_F = 0.84). The chromatographic system mentioned above was used throughout this study.

The carotenoids having been dissolved and filtered, absorption spectra in visible light were determined in various solvents (see Table 1). Similar measurements were recorded for synthetic isorenieratene, the preparation of which has been described by Cooper, Davis and Weedon.[20] The spectra of the two compounds showed close agreement.

Iodine-catalyzed stereoisomerization was carried out in the usual manner.[21,22] To 0.149 mg leprotene in 50 ml 2 % benzene-petroleum ether was added 10 capillary drops of a pale violet solution of iodine in pet.ether. The equilibrium was reached after 3 h in indirect daylight. The spectral changes observed are given in Table 2, and the composition of the iodine-catalyzed equilibrium mixture, spectro-

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Table 1. Absorption maxima in visible light of leprotene and isorenieratene in various solvents.

Carotenoid	Pet. ether b.p. 40–60°C	Acetone	Benzene	CS_2
Leprotene	(428) 452 480	(435) 458 485	(440) 465 494	(460) 484 515
Isorenieratene	(430) 452 480	(435) 456 484	(440) 465 495	(460) 483 514

Table 2. Spectral changes on separate iodine catalysis of *trans* leprotene and *trans* isorenieratene in petroleum ether.

Carotenoid	Stereoisomer	In pet. ether *			Drop in $E^{1\%}_{1\,cm}$ at λ_{max} in % of initial
		Abs. max. in mμ	% D_B/D_{II}[22]	% III/II[22]	
Leprotene	*Trans*	(428) 452 480	7	42	
	I_2-cat. equil. mixture	335 (425) 445 472	19	11	12
Isorenieratene	*Trans*	(430) 452 480	6	40	
	I_2-cat. equil. mixture	335 (425) 445 470	17	8	12

* containing 2 % benzene

Table 3. Composition of the iodine-catalyzed equilibrium mixtures of leprotene and isorenieratene.

Carotenoid	Member of the stereo-isomeric set	R_F-value*	In acetone			% of total	
			Abs. max. in mμ	% D_B/D_{II}[22]	% III/II[22]		
Leprotene	Neo B	0.75	342 (425) 445 471	*ca.* 35	0	37	61
	Neo A	0.72	(340) (425) 448 475	*ca.* 20	*ca.* 30	22	
	Trans	0.66	(435) 458 485	8	35	41	
Isorenieratene	Neo B	0.76	344 (425) 444 470	*ca.* 35	0	*ca.* 40	59
	Neo A	0.72	344 (425) 448 477	*ca.* 20	*ca.* 25	*ca.* 20	
	Trans	0.66	(435) 456 484	9	36	93	

* S. & S. No. 288 paper, 1 % acetone-pet. ether.

photometrically determined after paper-chromatographic separation according to the techniques previously described[22,23] is presented in Table 3. The neo A and neo B isomers were reversibly isomerized to *trans* leprotene during 2½ h in acetone solution in daylight, as was shown by subsequent paper-chromatographic examination. A similar study was carried out with synthetic isorenieratene (0.22 mg). The results are summarized in Tables 2 and 3.

On co-chromatography of *trans* leprotene and *trans* isorenieratene, using the 3-divided-paper technique,[24] a single zone (R_F = 0.66) was obtained.

Similarly, co-chromatography tests of the iodine-catalyzed equilibrium mixtures of the two carotenoids revealed identity of the corresponding isomers (see Table 3).

Mixed-melting-point determination in evacuated capillary tubes of leprotene and isorenieratene showed no evidence of depression; leprotene m.p. 185–189°C, mixed m.p. 189–192°C, and isorenieratene m.p. 192°C. Abridged thermometers were used. Melting points are uncorrected.

The corresponding spectral shifts of leprotene and isorenieratene on iodine catalysis, and in particular the qualitative (absorption spectra and R_F-values) and quantitative (percentage of each stereoisomer) agreement in the composition of the iodine-catalyzed equilibrium mixtures, are considered as conclusive evidence for the identity of leprotene and isorenieratene (I).

In selecting the molecular formula for leprotene Takeda and Ohta [2] emphasized the carbon content. Their H-value (9.12 %) was very close to that required for $C_{40}H_{48}$ (9.16 %), although the C-value was about 1 % too low. For aryl carotenoids the hydrogen uptake may vary considerably with the hydrogenation conditions.

The name leprotene has priority over isorenieratene. However, the nomenclature of aromatic carotenoids will be discussed elsewhere.

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Circular Dichroism and Chelation: Complexes of N,N′-Bis (2-butan-1-ol)-ethylenediamine

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Larsen and Olsen [1] have recently reported that optically active monodentate ligands co-ordinating through nitrogen (*e.g. d*-2-aminobutane) or oxygen (*e.g. d*-α-methylbutyrate) do not impose an observable optical activity onto the $d-d$ transitions of metal ions. An optically active chelate, however, causes its complexes (*e.g.* mono (*l*-propylenediamine)copper(II)) to exhibit circular dichroism. A vicinal effect of the asymmetric carbon in the monodentate was not sufficient to cause a measurable activity but the puckering of the chelate ring lowered the effective symmetry and the Cotton effect was observed. Therefore, circular dichroism should prove to be a useful tool for determining whether an optically active ligand behaves as a chelate.

In this paper, the above observations will be applied to some metal complexes formed in solution by N,N′-bis(2-butan-1-ol)-ethylenediamine ('ethambutol'; structure below with X = OH). These complexes are important because they are thought to be responsible for the compound's considerable antituberculous activity.[2] From a comparison of the stability of these com-