Epimeric 3,3′-Dihydroxy-ε,ε-carotenes from the Skin of the Yellow Costa Rican Frog, *Atelopus chiriquiensis*

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Summary Two epimeric 3,3′-dihydroxy-ε,ε-carotenes have been identified from the skins of the yellow Costa Rican frog, *Atelopus chiriquiensis;* both compounds have the (6*R*,6′*R*,3′*S*) stereochemistry but differ in absolute configuration at C-3.

In connection with research on toxins of Central American frogs,[1] we have isolated from the skins of the yellow Costa Rican frog, *Atelopus chiriquiensis*, several carotenoids, the two most abundant of which have been shown by chemical reactions, and i.r., u.v., n.m.r., and mass spectral data to be the diastereomers of 3,3′-dihydroxy-ε,ε-carotene. This constitution has been suggested previously for a carotenoid (or mixture of carotenoids) called tunaxanthin,§ isolated from several species of fish.[2] As no information has been published on the stereochemistry of tunaxanthin we are

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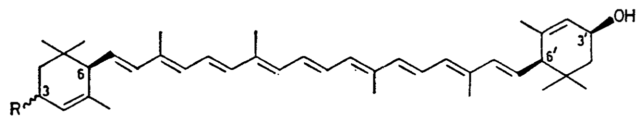
§ *Added in Proof*: A referee has pointed out a recent review wherein the 3,6-*trans*-3′,6′-*trans* geometry is suggested for tunaxanthine: B. C. L. Weedon, *Pure Appl. Chem.*, 1976, **47**, 161.

unable to ascertain whether it is identical with either of these frog skin carotenoids. Therefore we will refer to these as chiriquixanthin A and B.

Fresh homogenized skins were extracted with acetone, the solvent was removed, and the residue was saponified with 5% KOH in 50% methanol–benzene at room temperature. T.l.c. of the saponified product on silica gel (25% acetone–cyclohexane) gave one major (R_f 0·20) and two minor bands (R_f 0·48 and 0·74). The major band separated into two approximately equal components upon rechromatographing with 35% ethyl acetate–benzene to give chiriquixanthin A, R_f 0·32 and chiriquixanthin B, R_f 0·38.

Both compounds exhibited identical visible absorption spectra, λ_{max} 417, 441, and 470 nm (acetone), indicative of a chromophore with nine conjugated *trans* double bonds. The mass spectra of chiriquixanthin A and B were almost identical with M^+ at *m/e* 568, consistent with the molecular formula of $C_{40}H_{56}O_2$.



Chiriquixanthin A: R = α-OH, (3*R*, 6*R*, 3′*S*,6′*R*)
Chiriquixanthin B: R = β-OH, (3*S*, 6*R*, 3′*S*,6′*R*)

Although only minor differences between chiriquixanthin A and chiriquixanthin B were observed in the i.r. spectra, the ^{1}H n.m.r. spectra in the methyl region were very characteristic and most useful in assigning the relative configurations at positions C-3, C-6 and C-3′, C-6′. Examination of the ^{1}H n.m.r. spectra for chiriquixanthin A and B showed the C-9,9′, C-13,13′, and C-5,5′ methyl signals to have identical chemical shifts (δ 1·90, 1·95, and 1·63, respectively) for both compounds, consistent with the 3-hydroxy-ε end-group formulation. The C-1 *gem*-dimethyl group on a *trans*-ε end-group (where the C-3 substituent and polyene chain are on opposite sides of the cyclohexene ring as in lutein[3]) gives rise to two singlets at δ 0·99 and 0·84.† The signals for the C-1 methyls in chiriquixanthin A appeared as three sharp singlets at δ 0·99 (3H), 0·94 (3H), and 0·84 (6H). Based on symmetry considerations, we therefore assign one end group as 3,6-*trans* and the other as 3′,6′-*cis* in chiriquixanthin A. Chiriquixanthin B showed the C-1 methyl signals at δ 0·94 (6H) and 0·84 (6H) and it is therefore assigned the *cis–cis* stereochemistry at C-3, C-6 and C-3′, C-6′.

Both chiriquixanthin A and B show qualitatively identical c.d. curves from 225 to 400 nm, with a strong positive Cotton effect at 268 nm. The position and sign of the c.d. curves of the allylic methylation products of these two pigments, epimerized at C-3 and C-3′, are essentially unchanged from those of the parent compounds. It has been shown that –OR substituents at C-3 or alkyl substituents at C-2 have no appreciable effect on the nature of the c.d. curve of these types of compound.[4] Therefore, comparison with the c.d. curve of decaprenoxanthin,[4] with a negative Cotton effect at 268 nm and whose absolute configuration is known to be *S* at both C-6 and C-6′, establishes the C-6 and C-6′ centres of both chiriquixanthin A and B as having the *R* configuration. Based on this assignment and the relative stereochemistry of C-3,C-6 and C-3′,C-6′ as shown by ^{1}H n.m.r. spectroscopy we therefore assign the absolute stereochemistry for chiriquixanthin A and B as (3*R*, 6*R*, 3′*S*,6′*R*) and (3*S*,6*R*,3′*S*,6′R), respectively.

Previous work on animal pigments has been reviewed by Fox.[5] The two carotenoids reported here are unusual relative to plant carotenoids in several respects. The first striking difference is the (3*S*) hydroxy-configuration in the ε-cyclohexene end-group;‡ secondly, the two compounds, isolated from the same source, are epimeric at C-3. Further, chiriquixanthin A is unique in that both the end-groups are constitutionally identical but stereochemically different.

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† XL-100 n.m.r. spectrum run on authentic sample of lutein in our laboratory.

‡ The *S* configuration has been previously suggested for the carotenoid calthaxanthin, (isomeric with lutein) which was shown upon treatment with acid to yield products identical to those obtained from lutein. No n.m.r. data were presented. A. G. Dabbagh and K. Egger, *Z. Pflanzenphysiol.*, 1974, **72**, 177.

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