Absolute Configuration of Trisporic Acids and the Stereochemistry of Cyclization in β-Carotene Biosynthesis

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Summary The absolute stereochemistry of trisporic acid C (**1**) is 1*S*, 13*R*; the 1α-methyl, which is equatorial, is selectively labelled by [2-^{14}C]mevalonate, and corresponding chirality at C-1 in [2-^{14}C]mevalonate-labelled β-carotene is thereby indicated with implications for the stereochemistry of cyclization in β-carotene biosynthesis.

THE trisporic acids, *e.g.* trisporic acid C (**1**), which are the principal sexual hormones in Mucorales,[1] are formed from β-carotene by way of retinal.[2] In this process, the prochiral CMe_2 groups of β-carotene afford the chiral centre at at C-1 of (**1**). Now when (**1**) is formed from [2-^{14}C]mevalonate (added to mated *Blakeslea trispora*, total incorporation into trisporic acids 0·65%), degradations show that C-12 [isolated as (−)-*R*-pentane-1,4-diol bis-*p*-nitrobenzoate‡ from ozonolysis followed by $LiAlH_4$ reduction, acylation, and repeated chromatography] contains 26·8 ± 0·5% (*i.e.* 1/4) of the total activity and that the carboxyl-carbon at C-1 is unlabelled [methyl tetrahydrotrisporate-C acetate[3] (**2**) (9·97 × 10^5 d.p.m./mmole)‡ on boiling with KOH–MeOH gave CO_2 (as $BaCO_3$) with 1·4 ± 0·1%, and the hydroxy-ketone (**3**)[3]‡ with 99·3 ± 0·5%, of the original labelling]. The labelling pattern indicated in (**1**) is thus confirmed, and it follows that the cyclization reaction in which the prochiral centres of β-carotene are formed, and the subsequent conversion of these into the chiral centre, must both be fully stereospecific. The stereochemistry of (**1**) is therefore of particular interest.

The carbinol group at C-13 of (**1**) has the *R*-configuration,[2,4] and the unsaturation can be either 7-*trans*-9-*trans* or 7-*trans*-9-*cis*.[4,5] The o.r.d. spectra of *cis*- and *trans*-trisporic acid C and of several of its congeners (with the same chirality) have been published but without any deductions about the chirality at C-1,[4] presumably because of the complex geometry of the interactions between the trienone chromophore and the chiral centre. The situation is somewhat simpler when the side-chain is saturated and accordingly we examined the circular dichroism (c.d.) of the hydrogenation product (**2**).

Observed data (Roussel–Jouan dichrograph model 185, at 20° and a concentration < 1 mg/ml) were, in $CHCl_3$: 342 (+0·37), 290 (−0·39), and 245 nm (−14·49); in EtOH: 334 (+0·40), 245 (−15·13), and 217 nm (+13·9).

In (**2**) the double bond is conjugated with the carbonyl and βγ to the CO_2Me group. The conjugated chromophore should give rise to an $n \rightarrow \pi^*$ band at about 330 nm, a $\pi \rightarrow \pi^*$ band at about 250 nm, and a third band at about 210 nm which is also optically active[6] but cannot be seen in the u.v. spectrum because of its low extinction. The chirality of a non-coplanar enone system can be correlated with the sign of the c.d. at 330 nm,[7,8] and the $\pi \rightarrow \pi^*$ c.d. band usually has the opposite sign;[9] no correlation has hitherto been found for the 210 nm c.d. band.

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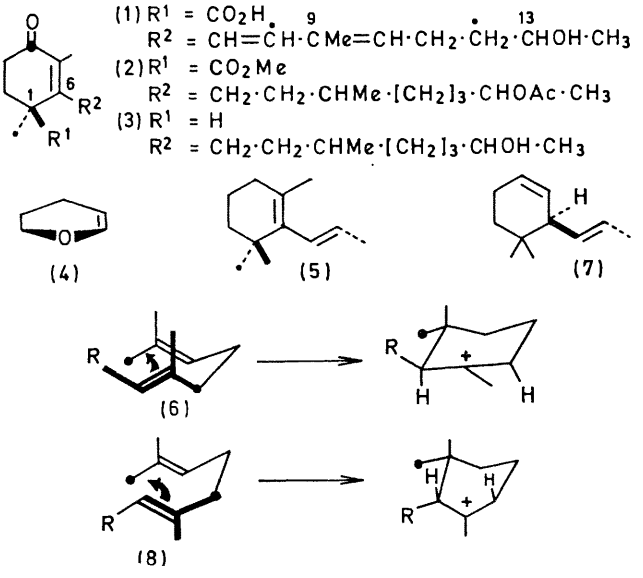
‡Fully satisfactory data for structural assignments and radiochemical purity were obtained.

A $\beta\gamma$-unsaturated acid (or derivative) will give a very enhanced c.d. band at about 220 nm[10-12] if the geometry of the C:C·C:O system is the same as that of analogous ketones;[13,14] such a geometry can be acquired here if the CO_2Me group of (**2**) has the axial conformation. However, if both the conjugated enone and the homoconjugated acid share the same C=C bond, as here, the system forms a new type of chromophore and gives two strong c.d. bands of opposite sign in the region 215—250 nm.[11]

As the exact conformation of the ring in (**2**) is not easily deduced from molecular models, we prefer not to deduce the absolute configuration from the positive sign of the R-band c.d. of the enone. However, the two strong bands of opposite sign at 245 and 217 nm are very similar to those in the reference compounds pregn-4-ene-3,20-dion-19-oic acid and nimbin.[11] This renders the axial conformation of the CO_2Me group very probable and implies the configuration 1*S* which is that shown in the formulae.



Given the configuration, the conformation of the ring can now be deduced from the c.d. at 334 nm. If the torsional angle between the side-chain (R^2) and the equatorial 5-methyl group is increased to about 40° or more in order to lower nonclassical strain, the conformation of the cyclic enone is as given in (**4**), and this must indeed lead to a positive Cotton effect for the R-band c.d.

Alternatively, n.m.r. data can be used to support the conformational picture, and the c.d. at 334 nm then independently verifies the configurational assignment. In going from methyl trisporate-C acetate (*cf.* **1**) to (**2**), the absorption of the equatorial 5-Me group moves from τ 8·15 to 8·30, and the 1-Me absorption similarly moves from τ 8·54 to 8·64 suggesting that it also is approximately equatorial.

We can now conclude that in β-carotene formed from [2-^{14}C]mevalonate and biologically transformable into (**1**), the stereochemistry of the ring is that shown in (**5**). If we then assume that the cyclization leading to β-carotene proceeds through folding in a chair-form—analogously with other terpenoids and irrespective of the actual cyclization mechanism—then the absolute stereochemistry of the cyclization reaction in β-carotene formation must be that indicated in (**6**), being followed by loss of the axial H at C-6. However, if cyclization in this manner is followed by loss of the axial H at C-4, an α-carotene with the stereochemistry shown in (**7**) will be formed,[15] and this is now known to be the enantiomer of natural (+)-α-carotene.[16]

There is good evidence to show that the α- and β-ionone ring-types in cyclic carotenoids are formed by independent pathways from common acyclic precursors, but the more detailed conclusion that the two ring-types arise by selective eliminations of different protons from a common carbonium ion[15] now requires some reconsideration. If chair-form foldings are presumed, the formation of the α- and β-types is now seen to require cyclizations on to opposite faces of the Me_2C=CH group, (**6**) giving the β-ionone type and an enantiomeric folding giving the α-type. Only if the alternative boat-form of folding is involved can cyclization on to the correct face of the Me_2C=CH system [as indicated by the prochirality of (**5**)] also lead to the correct stereochemistry at C-6 in α-carotene. Hence, either the two types represent cyclizations which are different *ab initio*, or selective deprotonations involve a common intermediate which is formed by a boat-folding, as in (**8**). Determination of the prochirality at C-1 of natural α-carotene (or a suitable biological derivative) formed from [2-^{14}C]mevalonate should resolve this problem.

This research was supported at Bonn by the Deutsche Forschungsgemeinschaft and at Manchester by the Science Research Council. We acknowledge helpful discussion with Professor D. Arigoni.

(*Received, December 11th*, 1969; *Com.* 1874.)

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