The Stereochemistry of α -Oxidation of Fatty Acids in Plants: the Configuration of Biosynthetic Long-Chain 2-Hydroxy Acids

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(Received 7 October 1971)

All natural long-chain 2-hydroxy acids that have been isolated and tested by polarimetry are optically active and have the D-configuration at C-2; in particular, lipids of pea (*Pisum sativum*) leaves contain a small proportion of D-2-hydroxypalmitate (Hitchcock, Morris & James, 1968b). This evidently accumulates after biosynthesis by α -hydroxylation of palmitate (Hitchcock & James, 1966; Morris & Hitchcock, 1968), though α -oxidation of palmitate apparently involves preferential formation and oxidation of L-2-hydroxypalmitate (Hitchcock, Morris & James, 1968a). When [1- 14 C]palmitic acid is incubated with pea leaves or solutions of their acetone-dried powders, 2-hydroxy[1- 14 C]palmitate accumulates; the configuration of this metabolite cannot be determined directly, but appears to be the D-hydroxy acid by crystallization of the isolated hydroxy esters with authentic enantiomers (Hitchcock *et al.* 1968b). This finding has now been confirmed by converting the hydroxy esters into their L-acetylmandelates and resolving the derivatives by t.l.c. (Morris & Crouchman, 1971).

Methods. (a) Preparation of L-acetylmandelyl chloride. L-Mandelic acid (1g) and acetyl chloride (1.5g) were allowed to react at room temperature until a clear mixture was obtained (15 min). The excess of acetyl chloride was removed *in vacuo* at 60°C for 30 min, and the residue was taken up in 25 ml of benzene. Oxalyl chloride (2g) was added; after 2 h at room temperature followed by refluxing for 1 h, the solvent was removed *in vacuo*. The crude yellow oil was used without purification.

(b) Preparation of methyl DL-2-(L-acetylmande-

lyloxy)palmitate. Methyl DL-2-hydroxypalmitate (20 mg) was dissolved in 2 ml of pyridine, and 40 mg of crude L-acetylmandelyl chloride was slowly added. After 16 h at room temperature, the reaction mixture was diluted with diethyl ether, and washed successively with aqueous hydrochloric acid until acid, aqueous sodium hydrogen carbonate until alkaline and water. The ethereal solution was dried (over anhydrous sodium sulphate) and evaporated; the required mandelates were isolated from the residue, and their subsequent purity was checked, by t.l.c. They were separated on t.l.c. plates of silica gel G by two developments with 3% (v/v) diethyl ether in benzene; under these conditions the R_F values of the methyl esters are approximately as follows: 2-hydroxypalmitate, 0.42; L-2-(L-acetylmandelyloxy)palmitate, 0.56; D-2-(L-acetylmandelyloxy)palmitate, 0.72; palmitate, 0.85.

(c) Biosynthesis of 2-hydroxy[1- 14 C]palmitate. [1- 14 C]Palmitic acid was incubated with either freshly sliced pea leaves or acetone-dried powders therefrom, and the biosynthesized 2-hydroxy-[1- 14 C]palmitate was isolated as the methyl ester as described by Hitchcock *et al.* (1968b). After dilution with unlabelled DL-2-hydroxypalmitate, the mandelates were prepared and separated as described above with excess of L-acetylmandelyl chloride. They were located by comparing with external markers and by scanning for radioactivity; the radioactivity of appropriate areas was determined by scraping off the adsorbent, eluting the esters with ether and measuring the radioactivity

Table 1. Configuration of 2-hydroxy[1- 14 C]palmitate biosynthesized by pea leaf preparations

Experimental details are given in the text.

System	Radioactivity of accumulated 2-hydroxy[1- 14 C]palmitates (d.p.m.)		D-Enantiomer/L-enantiomer radioactivity ratio
	D-Enantiomer	L-Enantiomer	
Freshly chopped pea leaves	32 300	700	45:1
Pea leaf acetone-dried powders	138 300	4300	32:1

of samples in a Philips liquid-scintillation counter.

Results. When biosynthetic radioactive 2-hydroxypalmitate is resolved almost all the radioactivity is associated with the D-hydroxy acid, with less than 3% with the L-enantiomer. When t.l.c. plates with the separated mandelyl derivatives are scanned for radioactivity, no peak corresponding to the L-hydroxy acid can be observed; quantitative determination (Table 1) reveals a small amount of radioactivity that may be due to this enantiomer. This confirms a previous report (Hitchcock *et al.* 1968b), based on resolution by recrystallization, that D-2-hydroxy[1-¹⁴C]palmitate (and not the L-enantiomer) accumulates during the α -oxidation of [1-¹⁴C]palmitate in pea leaves and in solutions of their acetone-dried powders. It illustrates a novel chromatographic method developed by Morris &

Crouchman (1971) for the resolution of small quantities of long-chain hydroxy acids, which has proved useful in the preparation of microgram quantities of radioactive enantiomorphs of high specific radioactivity.

We are grateful to Dr L. J. Morris for valuable discussions.

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