THE BIOSYNTHETIC PREPARATION OF 1-^{14}C -TRANS-11-OCTADE--CENOIC ACID

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SUMMARY

1-^{14}C-Trans-11-octadecenoic acid was obtained in 60% overall yield by incubating 1-^{14}C -linoleic acid with sheep rumen micro--organisms. The product, purified in the form of the methyl ester by silver nitrate chromatography, contained small amounts of rela--ted positional isomers as the only impurities.

Introduction

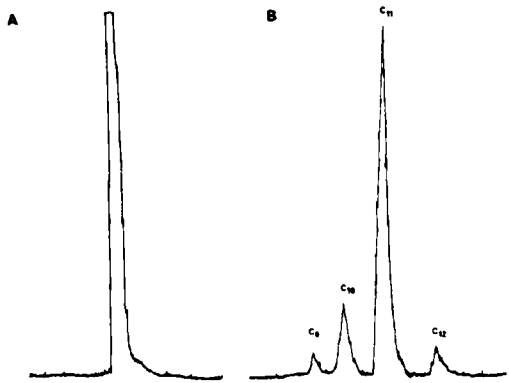
Fatty acids containing double bonds of the trans-configuration, which originate as byproducts or intermediates in the biohydrogenation of polyunsaturated fatty acids in the rumen, are present in significant amounts in the tissues of ruminant animals[1]. Acids of this kind, labelled with ^{14}C, were required for studies of the incorporation of fatty acids into milk and adipose tissue triglycerides in rats and ruminants but were not readily available. A simple in vitro system was therefore devised that utilises rumen microorganisms to convert 1-^{14}C -linoleic acid (cis-9, cis-12-octadecenoic acid) to 1-^{14}C -trans-11-octadecenoic acid in high yield. The preparation and purification of this acid are now described.

Results and discussion

All the isomeric cis-octadecenoic acids have been prepared by total synthesis[2] but a large number of steps are required and the expected yield from radioactive precursors would not be high. Trans-isomers have been prepared in high yield by stereomutation of cis-double bonds[3] but 1-^{14}C -cis-9-octadecenoic acid is the only cis-monoenoic starting material readily available. As the position of the double bond is known to have a marked effect on the rate at which the fatty acid is incorporated into lipids in tissues[4], trans-9-octadecenoic acid would not have been suitable for our purpose. It is well-established[1] that a major pathway in the biohydrogenation of linoleic acid to stearic acid by rumen microorganisms involves intermediate formation of cis-9, trans-11-octadecadienoic acid and trans-11-octadecenoic acid. In numerous studies of this process[5], when high concentrations of free linoleic acid (rather than an esterified form) were incubated anaerobically with sheep rumen contents in vitro, high yields of the trans-11-monoenoic intermediate were obtained with very little stearic acid formation. This was especially pronounced in the absence of added fermentable substrate. In the incubation described here where 1-^{14}C -linoleic acid was the substrate, a typical pattern was obtained and gas-liquid radio-chromatography showed the presence of stearic acid (7%), monoenes (81%), cis-9, trans-11-octadecadienoic acid (4%) and unchanged linoleic acid (8%). The monoene fraction was subsequently shown by analytical silver nitrate thin layer chromatography (TLC) to consist of a trans-isomer (72%) and a cis-isomer (9%).

The trans-monoenoic isomer as the methyl ester derivative was separated from the other products of the incubation by preparative silver nitrate TLC; gas-liquid radio-chromatography (as illustrated in Fig. 1-A) indicated that the

compound was greater than 99% pure. In order to confirm the position of the double bond, a portion of the ester was oxidised with permanganate-periodate reagent[6] and the dibasic acid fragments were methylated for gas-liquid radio-chromatography. The recorder trace that was obtained is illustrated in Fig. 1-B. A C_{11} dibasic acid was by far the major component (80%) although there was also a significant amount of the C_{10} homologue (15%) together with small amounts of C_9 and C_{12} components. (Mass traces concordant with the radioactivity traces were obtained on the recorder). The major product was then trans-11-octadecenoic acid but some of the 9-, 10- and 12-isomers were also present. The radioactivity recovered in the trans-monoenoic components was 60% of that added but the mass recovered was 50% greater than expected on this basis as a result of dilution of the added labelled linoleic acid or of the trans-monoene formed by similar fatty acids endogenous to the system.



Legend to Figure

Gas-liquid radio-chromatography recorder traces. A. the trans-monoene fraction. B.. Dibasic acid fragments obtained by permanganate-periodate oxidation of the trans-monoene fraction.

Although in this instance, the rumen microorganisms were obtained from fistulated animals, rumen contents from freshly slaughtered animals would have been equally suitable for the purpose provided that they were kept anaerobic and at 39°C until ready for use.

Experimental

Materials

1-^{14}C -Linoleic acid was purchased from the Radiochemical Centre, Amersham, England. It was guaranteed 98 per cent pure and this was confirmed by gas-liquid radio-chromatography of the methyl ester derivative before and after oxidation with permanganate-periodate reagent (see below).

Incubation

Rumen contents were obtained from a Suffolk Cross sheep fitted with a rumen fistula and receiving 0.25 Kg hay and 0.25 Kg concentrate mixture at 0900 h and at 1600 h. The rumen contents were taken before the morning feed and were strained through gauze to remove the larger food particles. The strained rumen contents were diluted to 40 per cent by volume with buffer solution[7].

This solution (10 ml) was incubated with the potassium salt of 1-^{14}C -linoleic acid (6 mg; 50 μCi) for 6 hrs at 39°C in an atmosphere of nitrogen to ensure anaerobic conditions. At the end of this time, methanol (33 ml) and chloroform (33 ml) were added and the mixture was refluxed for 1 hour. The pH was adjusted to 2 with dilute hydrochloric acid, the solution was filtered and chloroform (33 ml) and 0.88 per cent aqueous potassium chloride (10 ml) were added. The bottom layer, containing the lipid, was recovered and the fatty acids were methylated with 5 per cent methanolic hydrogen chloride.

Thin layer chromatography with silver nitrate impregnated layers

The methyl esters (in 5 mg portions) were purified by TLC on 20 x 20 cm glass plates coated with a layer 0.5 mm thick of Kieselgel G (E. Merck, Darmstadt) containing 10 per cent (w/w) silver nitrate. Plates were developed twice in the same direction in hexane-diethyl ether (95:5, v/v) at a temperature of 4°C. Bands were located under UV light after spraying with 2', 7'-dichloro-fluorescein in 95 per cent methanol (0.1 per cent, w/v). The required trans-ester migrated between the saturated and cis-monoenoic components and was well separated from both. The band was scraped into a small chromatography column onto a bed of Florisil (BDH Ltd., Poole) about 1 cm deep and the ester was recovered by elution with diethyl ether (100 ml). The radioactivity of the sample was determined by liquid scintillation counting of an aliquot.

Gas-liquid radio-chromatography

Fatty acid methyl esters were separated on glass columns (2 m x 6 mm) packed with 15 per cent EGSS-X on Gas-chrom P (100-120 mesh; Applied Science Laboratories Inc., State College, Pa.) isothermally at 160°C in a Hewlett-Packard 5750 gas chromatograph. The column effluent was divided by a stream splitter so part passed into the flame ionisation detector and the remainder into a Panax gas-flow anticoincident detector equipped with an autoscaler and print-out; in addition, the radioactivity and mass traces were displayed simultaneously on a dual pen recorder.

Permanganate-periodate oxidation

In order to confirm the position of the double bond, unsaturated methyl esters were cleaved at the double bond by permanganate-periodate oxidation using the procedure of von Rudloff[6]. The products were methylated with boron

trifluoride-methanol reagent (BDH Ltd.) and the radioactive dibasic acid fragments were identified and determined by gas-liquid radio-chromatography.

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