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Isomerization of Linolenic Acid by Rumen Micro-organisms

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The main fatty acid in the natural pasture diet of the ruminant is α-linolenic acid (*cis–cis–cis*-octadeca-9,12,15-trienoic acid) esterified in a number of complex lipids. After ingestion the linolenic acid is released in the rumen by the action of bacterial lipases and also hydrogenated with the eventual formation of stearic acid (Garton, 1964). Wilde & Dawson (1966) showed that the first stage of the metabolic sequence was a migration of the double bond in the 12-position to either the 11- or 13-position so that it formed a conjugated system either with the 9- or 15-double bonds. In this communication the isomer formed is identified as *cis–trans–cis*-octadeca-9,11,15-trienoic acid. A preliminary account of these results has been given (Kemp & Dawson, 1967).

The isomeric product was isolated by the following procedure. Rumen fluid was removed from a rumen fistulated sheep, strained to remove large food particles and centrifuged to obtain a pellet containing the micro-organisms (Wilde & Dawson, 1966). The pellet was washed twice with the buffered mineral-salt solution described by Coleman (1958). The micro-organisms were resuspended in the same solution (to the original volume of rumen fluid) and 60 ml. of the suspension was incubated for 30 min. at 39° with [U-^{14}C]- or [1-^{14}C]-linolenic acid (6 μc, 12 mg.) under $N_2 + CO_2$ (95:5). In some isolations the micro-organisms were treated ultrasonically (for 20 min. at 0° under N_2 with the MSE 60 w apparatus) before the incubation, which was prolonged for up to 2 hr. The lipids were extracted from the incubation medium and hydrolysed as described by Wilde & Dawson (1966), and the mixture containing the free fatty acids was methylated with diazomethane. Analytical gas–liquid chromatography at this stage showed that conversion of the added linolenic acid into the isomer ranged between 10 and 70%. The methyl esters of the fatty acids were isolated from the lipid mixture by preparative thin-layer chromatography on silica gel H (E. Merck A.-G., Darmstadt, Germany) with benzene–hexane (1:1, v/v) containing 5 mg. of butylated hydroxytoluene/100 ml. The single band of fatty acid methyl esters (R_F 0·5) was eluted and separated by thin-layer chromatography on 5% $AgNO_3$–silica gel H with benzene–ether (9:1, v/v) containing 5 mg. of butylated hydroxytoluene/100 ml. The plates were scanned for radioactivity and the labelled band (R_F 0·35) moving just ahead of α-linolenic acid was eluted. The product (about 80% pure) was rechromatographed for 4 hr. again on 5% $AgNO_3$–silica gel H plates but with toluene as solvent with a wad of absorbent paper to extend the solvent front. After scanning, the slowest running radioactive band was eluted; it gave a single mass and radioactive peak on gas–liquid chromatography on a polyethylene

Table 1. *Identification of the hydrazine reduction products of the linolenic acid isomer after separation by thin-layer chromatography*

Abbreviations: TLC, thin-layer chromatography; GLC, gas–liquid chromatography.

Band	R_F	Characterization by TLC and GLC (methyl esters)	Dicarboxylic acids formed on oxidation (monomethyl esters)	Identity of band (methyl esters)
1	0·66	Stearate	—	Stearate
2	0·55	trans-$C_{18:1}$	C_{11}	*trans*-Octadec-11-enoate
3	0·43	Conjugated $C_{18:2}$	C_9	*cis–trans*-Octadeca-9,11-dienoate*
4	0·38	cis-$C_{18:1}$	C_9	*cis*-Octadec-9-enoate
			C_{15}	*cis*-Octadec-15-enoate
5	0·1	Non-conjugated $C_{18:2}$	C_9	Octadeca-9,15-dienoate
			C_{11}	Octadeca-11,15-dienoate
		Unchanged linolenate isomer	—	—

* On further reduction of this fatty acid methyl ester with hydrazine *cis*-octadec-9-enoate and *trans*-octadec-11-enoate are formed in equal portions.

glycol adipate column (180°) with a retention volume relative to methyl palmitate of 4·3.

The methyl ester isolated absorbed strongly at 233 mμ, indicative of a conjugated diene system. It was partially reduced by treatment with hydrazine in 95% ethanol (Privett & Nichell, 1966), the progress of the reduction being followed by analytical gas–liquid chromatography. After 4 hr. the reaction products were separated by preparative thin-layer chromatography on 5% $AgNO_3$–silica gel H with benzene–hexane (1:1, v/v) containing 5 mg. of butylated hydroxytoluene/100 ml. Scanning for radioactivity showed five bands, which were eluted and examined by gas–liquid chromatography. This allowed a partial characterization according to the double-bond content (Table 1). The products were oxidized at the double bonds with permanganate–periodate reagent (Rudloff, 1956) and the dicarboxylic acid monomethyl esters were extracted and further methylated with diazomethane. The dicarboxylic acid dimethyl esters produced were identified on a polyethylene glycol adipate column at 180°, the effluent being scanned continuously for mass and radioactivity (James & Piper, 1961). The dicarboxylic acids produced by the oxidation and the identity of the original reduction products are given in Table 1. These reduction products indicate that the isomer is *cis–trans–cis*-octadeca-9,11,15-trienoic acid.

The *cis–trans–cis* structure was confirmed by infrared spectrometry, the isomer giving a doublet absorption at 10·18 and 10·45μ consistent with published values for *cis–trans–cis*-fatty acids from seed oils (10·13 and 10·66μ; Wolff & Mikwa, 1965). The absence of a peak at 10·35μ typical for a *trans*-monoenoic acid was originally wrongly interpreted as meaning that the isomer was all-*cis* (Wilde & Dawson, 1966). The structure of the linolenic acid isomer is the same as that reported by Kepler & Tove (1967) for the primary metabolic product of α-linolenic acid incubated with *Butyrivibrio fibrosolvens*. Although this organism is present in the rumen, recent work in this Laboratory has indicated that a number of other rumen organisms isolated in pure culture are also capable of isomerizing α-linolenic acid in the same way (Kemp & White, 1968).

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