Short Communications

The Biosynthesis of Ricinoleic Acid by *Claviceps purpurea*

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The major source of ricinoleic acid (D-12-hydroxy-9-octadecenoic acid) is the oil of the castor bean (*Ricinus communis*), where it occurs as a mixture of simple triglycerides. The precursor of ricinoleic acid in this system has been shown to be oleic acid and not linoleic acid (James, 1962, 1963; James, Hadaway & Webb, 1965; Yamada & Stumpf, 1964; Canvin, 1965), though there is still some conflict of evidence as to whether or not oxygen is a cofactor.

Ricinoleic acid also occurs in ergot oil, the oil derived from the sclerotia of the fungus, *Claviceps purpurea* (Matthes & Kürscher, 1931; Bharucha & Gunstone, 1957). Morris & Hall (1966) demonstrated that these glycerides possess a unique structure in that, unlike castor oil, the hydroxyl groups of the ricinoleic acid are not free but are esterified with a variety of long-chain fatty acids. The oil thus contains not only normal triglycerides but also tetra-, penta- and hexa-acid triglycerides. Suitable mycelial cultures of the fungus were also shown to contain ricinoleic acid in similar unusual glycerides. It was therefore decided to investigate the biosynthesis of ricinoleic acid in *Claviceps purpurea*.

Incubation of [2-^{14}C]acetate under aerobic conditions with either cultured mycelial forms of the organism or immature sclerotia isolated from infected rye plants showed incorporation of the label into all fatty acids up to linoleic acid but little or no labelling of ricinoleic acid (Table 1). Under anaerobic conditions only the saturated acids were labelled.

Table 1. *Conversions of labelled precursors by* Claviceps purpurea

In system 1, mycelia were selected from still cultures grown on a medium containing (per l.): $CaNO_3$, 1·0g.; $MgSO_4,7H_2O$, 0·25g.; KH_2PO_4, 0·25g.; KCl, 0·125g.; $ZnSO_4,7H_2O$, 0·027g.; $FeSO_4,7H_2O$, 0·033g.; sucrose, 100g.; asparagine, 10g.; cysteine hydrochloride, 0·01g.; yeast extract, 0·1g.; pH5·0. Three or four fragments of mycelium, each approx. 1cm.^{2} in area, were washed free of medium and added to 3·0ml. of 0·2M-phosphate buffer, pH7·0, containing 2μc of appropriate precursor. In system 2, three to five immature sclerotia (approx. 500mg.), freshly isolated from infected rye inflorescences, were carefully sliced in 4·0ml. of 0·2M-phosphate buffer, pH7·0, and to each preparation was added 1μc of precursor. Results given for acetate incorporations represent the percentage distribution of counts.

System	Substrate	Time (hr.)	Conditions	Percentage conversion into: $C_{16:0}$ acid + $C_{16:1}$ acid	$C_{18:0}$ acid	$C_{18:1}$ acid	$C_{18:2}$ acid	HO–$C_{18:1}$ acid
1	[2-^{14}C]Acetic acid	5	Aerobic	38·0	5·7	39·5	10·8	6·0
1	[1-^{14}C]Oleic acid	5	Aerobic				13·3	Trace
1	[1-^{14}C]Linoleic acid	5	Aerobic					11·8
2	[2-^{14}C]Acetic acid	6	Aerobic	40·5	5·0	45·0	9·5	Trace
2	[2-^{14}C]Acetic acid	6	Aerobic + imidazole	38·2	4·0	35·9	21·9	Trace
2	[2-^{14}C]Acetic acid	6	Anaerobic	49·4	47·8	—	—	—
2	[1-^{14}C]Oleic acid	6	Aerobic				26·0	—
2	[1-^{14}C]Oleic acid	6	Aerobic + imidazole				20·0	—
2	[1-^{14}C]Oleic acid	6	Anaerobic				—	—
2	[1-^{14}C]Linoleic acid	6	Aerobic					20·0
2	[1-^{14}C]Linoleic acid	6	Aerobic + imidazole					20·5
2	[1-^{14}C]Linoleic acid	6	Anaerobic					47·3

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Similarly, incubation with [1-^{14}C]oleic acid never gave rise to any appreciable amount of labelled ricinoleic acid even though, under aerobic conditions, considerable desaturation to linoleic acid occurred (Table 1). Again, as expected, there was no desaturation under anaerobic conditions.

Incubation of mycelia or, more particularly, of immature sclerotia with [1-^{14}C]linoleic acid in phosphate buffer, however, gave rise to labelled ricinoleic acid (Table 1). The conversions into ricinoleate from linoleate listed in Table 1 are probably too high as the system is complicated by the ready enzymic or chemical oxidation of added linoleic acid to products having similar migration characteristics to ricinoleate on both gas–liquid and thin-layer chromatograms. That labelled ricinoleate was indeed formed, however, was proved by careful fractionation of the products on two types of thin-layer plate. Normal adsorption chromatography on a silica-gel layer provided first a hydroxy ester fraction, free from any keto, epoxy or conjugated diene or triene products that might have been formed from linoleate and that would have similar gas–liquid-chromatographic retention times to ricinoleate on an SE–30 stationary phase. Argentation chromatography of the hydroxy ester fraction on a layer impregnated with silver nitrate then ensured the removal of any hydroxy conjugated dienes, which would have been formed from linoleate hydroperoxides, from the ricinoleate that was isolated (Morris & Wharry, 1965).

12-Hydroxy-9-octadecenoic acid

↓ Reduction

12-Hydroxyoctadecanoic acid

↓ Thionyl chloride, then methanolic KOH

12- and 11-Octadecenoic acid

↓ Reduction

Octadecanoic acid

↓ Partial oxidation by $KMnO_4$

Mixture of C_{18}, C_{17}, C_{16}, C_{15} etc. fatty acids, which were analysed by radiochemical gas–liquid chromatography

Scheme 1.

The ricinoleic acid so purified was degraded by the series of reactions (James *et al.* 1965) shown in Scheme 1 and thus shown to be labelled only on the 1-position.

This synthesis of ricinoleic acid from linoleic acid by *Claviceps purpurea* was not only not inhibited by anaerobiosis but was even potentiated by it (Table 1), presumably by decreasing the rapid loss of the precursor by other oxidative reactions. Under aerobic conditions more than half the activity added as linoleic acid was lost from the extractable lipid within 6hr. whereas under anaerobic conditions less than 20% was lost.

The reaction is thus not a formal oxidation but rather a hydration, as is known in the formation of D-10-hydroxystearic acid from oleic acid by a *Pseudomonas* species (Schroepfer, 1965; Niehaus & Schroepfer, 1965). This conclusion is supported by the failure of imidazole to cause any inhibition of the synthesis under conditions that would give inhibition of oxidative hydroxylation (Castelfranco, Stumpf & Contopoulou, 1955; Hitchcock & James, 1966) and that do inhibit the biosynthesis of ricinoleic acid from oleic acid by the castor bean (P. Harris & A. T. James, unpublished work). Morris & Hall (1966) have already advanced some speculations as to the biosynthetic implications of the unusual structures of *Claviceps* glycerides.

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Bharucha, K. E. & Gunstone, F. D. (1957). *J. chem. Soc.* p. 610.
Canvin, D. T. (1965). *Canad. J. Bot.* **43**, 49.
Castelfranco, P., Stumpf, P. K. & Contopoulou, R. (1955). *J. biol. Chem.* **214**, 567.
Hitchcock, C. & James, A. T. (1966). *Biochim. biophys. Acta*, **116**, 413.
James, A. T. (1962). *Bull. Soc. Chim. biol., Paris*, **44**, 951.
James, A. T. (1963). *Symp. biochem. Soc.* **24**, 17.
James, A. T., Hadaway, H. C. & Webb, J. P. W. (1965). *Biochem. J.* **95**, 448.
Matthes, H. & Kürscher, O. H. (1931). *Arch. Pharm.* **269**, 188.
Morris, L. J. & Hall, S. W. (1966). *Lipids*, **1**, 188.
Morris, L. J. & Wharry, D. M. (1965). *J. Chromat.* **20**, 27.
Niehaus, W. G., jun. & Schroepfer, G. J., jun. (1965). *Biochem. biophys. Res. Commun.* **21**, 271.
Schroepfer, G. J., jun. (1965). *J. Amer. chem. Soc.* **87**, 141.
Yamada, M. & Stumpf, P. K. (1964). *Biochem. biophys. Res. Commun.* **14**, 165.