THE DETECTION OF OPTICAL ACTIVITY IN NATURAL ASYMMETRIC TRIGLYCERIDES

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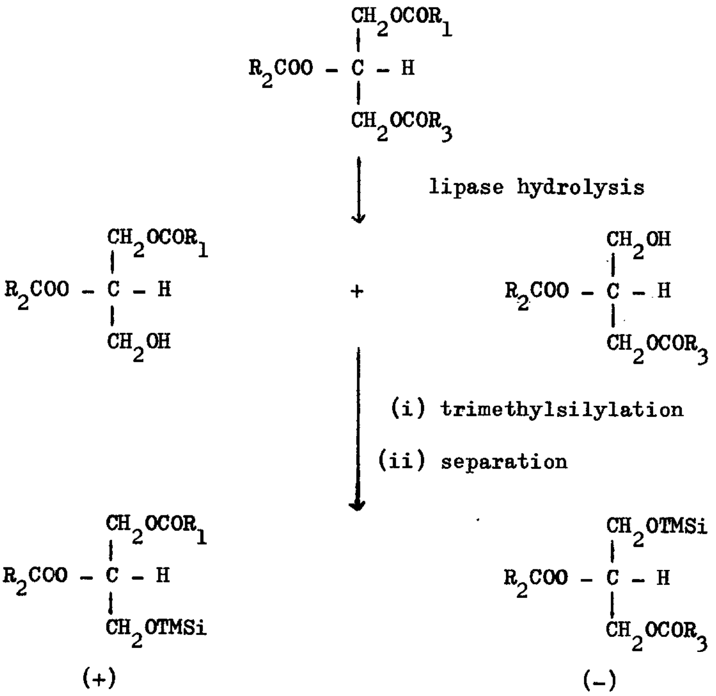
Many asymmetric triglycerides occur in nature but it has never been demonstrated that any of these is optically active. Indeed, currently favoured theories of fatty acid distribution in natural triglyceride mixtures (Vander Wal, 1960; Coleman & Fulton, 1961; Gunstone, 1962) consider, as a basic assumption, that the 1- and 3-positions of glycerol are exactly equal and equivalent, i.e. that asymmetric triglycerides must be racemic. The difficulty in demonstrating optical activity has been the fact that even optically pure enantiomeric triglycerides of the common long-chain fatty acids have no measurable optical rotation (Sowden & Fischer, 1941).

Recently, Schlenk (1962) has described how optical asymmetry in a pure triglyceride can be demonstrated directly by several physical procedures, notably X-ray crystallography and measurement of piezoelectric excitability. The only natural triglyceride he examined by these procedures, the 1-palmityl, 2-oleyl, 3-stearyl glyceride from cocoa butter, was found to be racemic.

Brockerhoff (1965) has devised a scheme, based entirely on lipolytic enzyme specificities, for the analysis of the fatty acids esterified with each distinct position of glycerol. Any difference in composition of the acids of the 1- and 3-positions indicates indirectly that optically active triglycerides are present in the mixture but, when applied to whole fats, does not indicate what are the optically asymmetric triglyceride species present. Brockerhoff et al. (1965) state that in mammalian depot fats there is a preference of saturated acids for the 1-position and of unsaturated acids for the 3-position, suggesting that the mono- and di-unsaturated triglycerides (SSU and SUU) in these fats are predominantly in one enantiomeric form.

Almost simultaneously with Brockerhoff's publication, this author (Morris, 1965) proposed a scheme for detection of optical asymmetry of individual asymmetric triglycerides by polarimetry of the diglycerides separated after lipase hydrolysis or of suitable derivatives of these diglycerides. The detection, for the first time, of optical activity in several natural triglycerides by this procedure is the subject of this communication. The procedure used, slightly modified from the original proposal, is summarised in Figure 1. Glycerides are drawn in Fischer projection in the L-configuration and are then numbered from the top, in accordance with the convention proposed by Hirschmann (1960).

Figure 1



EXPERIMENTAL

Standard L-1,2-dipalmitin ([α] = 2.3°, 5.6% in $CHCl_3$) was synthesised by conventional methods (Sowden & Fischer, 1941). Of the various derivatives we tried to prepare from this 1,2-dipalmitin, including the sorbic, p-nitrobenzoic and trifluoroacetic esters and the benzyl and trimethylsilyl ethers, the trimethylsilyl ether was chosen as being the derivative with reasonable optical rotation ([α] = +4.7°, 3.4% in $CHCl_3$) which was most convenient to prepare and whose preparation was least likely to cause isomerisation and racemisation of the αβ-diglycerides.

Relatively pure samples of natural asymmetric triglycerides were isolated from various fats by complementary use of low temperature crystallisation, argentation-crystallisation (Gunstone et al., 1965) and preparative thin-layer chromatography (TLC) on silica gel layers impregnated with silver nitrate (Morris, 1964).

Lipase hydrolyses were carried out on 500 mg samples with 50 mg amounts of purified pig pancreatic lipase by a scaled-up modification of the procedure of Luddy et al. (1964). Under our conditions a reaction time of 3 minutes gave nearly maximal yields of diglycerides with a relatively small proportion of monoglyceride being formed. The whole hydrolysate from a 500 mg sample was applied as a streak to one 200 x 200 x 1 mm thin-layer plate and the diglyceride fraction isolated quantitatively in a pure state and with generally less than 10% isomerisation to the αα'-form. The recovered diglyceride mixture, amounting normally to about 100 mg, was dissolved in 1.0 ml of dry pyridine, treated with 0.2 ml of hexamethyldisilazane and 0.1 ml of trimethylchlorosilane and the trimethylsilyl (TMSi) ethers recovered in hexane solution.

Each pair of diglyceride-TMSi ethers was separated by preparative TLC on silica gel impregnated with silver nitrate, by virtue of the difference in unsaturation of the acids on positions 1 and 3 respectively. The optical rotations of the two fractions were then determined at 546.1 mμ in chloroform solution with an ETL/NPL automatic polarimeter. The results obtained from asymmetric triglycerides isolated from one animal fat and three vegetable fats are summarised in Table 1. In each case, the two diglyceride-TMSi ethers were optically active, the rotations of the individuals of each pair being of opposite sign. The absolute configuration of the sole or predominating enantiomer of the original triglyceride is shown in the Table both as a diagrammatic Fischer projection, according to the Hirschmann convention, and as (R) or (S), according to system of Cahn, Ingold and Prelog (1956).

Table 1*

Fat - Triglyceride		Diglyceride-TMSi ethers ([α])		Absolute configuration	
		1,2-	2,3-	Hirschmann	Cahn et al.
lard	SSO	SS-TMSi(+1.6°)	SO-TMSi(-1.3°)	S S — O	(R)
palm oil	SOO	OO-TMSi(+2.5°)	SO-TMSi(-2.3°)	O O — S	(S)
malabar tallow	SOO	OO-TMSi(+0.5°)	SO-TMSi(-3.9°)	O O — S	(S)
cocoa butter	SOO	OO-TMSi(+1.0)	(SO-TMSi(-3.2°)	O O — S	(S)

* S = palmitic and/or stearic acid;
O = oleic acid

DISCUSSION

All of the natural asymmetric triglycerides that we have so far examined by this procedure have been found to be optically active. In no case, however, did the specific rotation, either positive or negative, of a diglyceride-TMSi ether derived from a natural triglyceride attain the value of 4.7° of the synthetic standard derivative. Whereas this may mean that the natural triglycerides studied are only predominantly and not exclusively of one enantiomeric form, this cannot be assumed from the present work. There are a number of factors which would give rise to reduced rotations. Thus, the presence in the di-unsaturated triglyceride (SOO) of the positional isomer (OSO) or of the linoleyl-disaturated triglyceride (SLS), which would migrate with it on argentation-TLC, would result in the lowering of the rotations of the mono- and di-unsaturated diglyceride derivatives respectively and such αβ- to αα'-isomerisation of the diglycerides as occurred during their isolation would diminish the rotations of both derivatives equally. Also, incomplete separation of the pair of TMSi ethers would reduce the rotation of one or both of them as would any decomposition of the ethers to diglycerides after their separation. All of these deleterious factors could be demonstrated to be affecting some or all of the experiments reported and the low positive rotations of the di-unsaturated ether derivatives from cocoa butter and malabar tallow were shown to be largely due to a combination of the presence of linoleate-containing glycerides and of their incomplete separation from the mono-unsaturated derivatives.

The positive result obtained for the lard triglyceride is in accord with the difference in fatty acid compositions of the 1- and 3-positions of this fat reported by Brockerhoff et al. (1965). Of considerable interest is the finding that the di-unsaturated triglyceride from cocoa butter is optically active whereas the 1-palmityl, 2-oleyl, 3-stearyl glyceride from the same fat was racemic (Schlenk, 1962). This suggests that the 1,3-positional specificity operating during biosynthesis of this fat only differentiates between saturated and unsaturated acids and that a distinction between two saturated acids, palmitic and stearic, is not made and it was to test this supposition that this particular triglyceride was chosen for study. From the few results here reported and from the work of Brockerhoff et al. (1965), it would also appear that in animal depot fats saturated acids prefer the 1-position and unsaturated acids the 3-position whereas in vegetable fats the converse is true.

For these fats whose triglycerides we have examined the 1- and 3- positions of glycerol have been shown not to be equal and equivalent so that, for these fats at least, the 1,2-random, 3-random hypothesis of triglyceride distribution (Vander Wal, 1960; Coleman and Fulton, 1961; Gunstone, 1962) is wrong as stated.

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