THE POSITION OF CYCLOPROPENOID ACIDS IN A GLYCERIDE MOLECULE

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We have previously reported the determination of cyclopropenoid acids in the seed oil of cotton of variety 108F during the ripening of the seeds [1]. In the present paper we give the results of a determination of the location of the cyclopropenoid acids in the triglyceride molecule. From the oil of the ripe seeds by column chromatography on silica gel [2] we isolated the pure triglycerides and we determined the amount of cyclopropenoid acids in them spectrophotometrically [3]. They amounted to 1.83%. The position of the cyclopropenoid acids were found by the hydrolysis of the triglycerides with pancreatic lipase in an alkaline medium. The hydrolyzate was preparatively separated into monoglycerides, diglycerides, fatty acids, and unhydrolyzed triglycerides on plates with a thin layer of silica gel. The fatty-acid composition of the fractions obtained is given below (%)

Acid	Monoglycerides	Diglycerides	Triglycerides
C_{x_1}	0.6	0.3	1.2
C_{x_2}	0.3	0.2	0.5
$C_{14:0}$	0.5	0.6	1.5
$C_{16:0}$	10.4	28.0	40.3
$C_{16:1}$	0.9	1.1	1.5
C_{x_3}	4.2	1.6	3.6
$C_{18:0}$	1.4	1.8	2.3
$C_{18:1}$	29.2	22.5	23.9
$C_{18:2}$	52.5	43.9	25.2

To determine the amounts of cyclopropenoid acids in the mono-, di-, and triglyceride fractions, they were hydrolyzed with alkali, the fatty acids were isolated, and their methyl esters were prepared.

The Halphen reaction was performed for 90 min.

The diglyceride and triglyceride fractions contained a considerable amount of cyclopropenoid acids while the fraction of the fatty acids split off contained traces of them, and no cyclopropenoid acids were found in the monoglyceride fraction. From a calibration curve plotted for methyl esters of fatty acids with known contents of cyclopropenoid acids it was found that the diglyceride fraction contained 0.17% of cyclopropenoid acids. Since the cyclopropenoid acids retard the process of enzymatic hydrolysis [5], the bulk of them is present in the unhydrolyzed triglycerides (1.44%). The results obtained give grounds for considering that in the cottonseed oil triglycerides the cyclopropenoid acids are located predominantly in position 1 or 3.

EXPERIMENTAL

The oil was extracted from the ripe seeds with chloroform–methanol (1:2). The glycerides were isolated from the oil on a column containing silica gel (size 0.16 mm) [2]: 1 g of the oil was dissolved in 7 ml of chloroform and deposited on a column with a diameter of

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19 mm. Then a 1% solution of diethyl ether in petroleum ether was passed through it; the 75 ml of eluate obtained, containing substances accompanying the glycerides, was discarded.

The pure triglycerides were eluted with 300 ml of a 5% solution of diethyl ether in petroleum ether. After the mixture of solvents had been distilled off, the pure triglycerides were checked in a thin layer of silica gel in the petroleum–diethyl ether (7:3) solvent system.

The cyclopropenoid acids were determined by the method of Bailey et al., [3] in sextuplicate. For this purpose, each flask was charged with 0.2 g of oil, and butanol and a solution of sulfur in carbon disulfide were added. After heating at 110°C, the spectra of the reaction mixture were recorded on a Hitachi instrument. The presence of a maximum at λ 495 nm shows the presence of cyclopropenoid acids.

Enzymatic Hydrolysis [4]. A round-bottomed flask fitted with a stirrer was charged with 1 g of triglycerides, 7 ml of a 1 N buffer solution with pH 8.0 (NH_4Cl + NH_4OH), 0.4 g of lipase, and 0.5 ml of emulsifying agent (sodium salts of bile acids), and then it was placed in a thermostat at 37°C for 30 min. Every 10 min, one drop of 4 M NH_4OH was added to maintain the pH constant. After 30 min, the reaction was stopped by the addition of 2 ml of HCl. The hydrolysis products were extracted with ether. The extract obtained was washed with water, dried, and, after the evaporation of the ether, deposited on a plate (18 × 24 cm) with a fixed layer of silica gel in the form of a 15% solution of the hydrolyzate in chloroform. The hydrolyzate was deposited in 150-mg portions on each of a number of plates and separation was performed in the petroleum ether–diethyl ether (7:3) system. The hydrolysis products were revealed by spraying the edges of the plates with a saturated solution of iodine in ethanol.

The corresponding layers were removed from the plates and eluted with diethyl ether, which was then evaporated, and the residue was saponified and methylated, and its composition was determined by GLC.

Gas–liquid chromatography was performed in a UKh-2 instrument with a column containing poly (ethylene succinate) on Celite at 196°C.

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