Determination of the Glyceride Structure of Fats. Glyceride Structure of Fats with Unusual Fatty Acid Compositions[1]

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Abstract

Five fats containing less common fatty acids, nutmeg butter (myristic), rapeseed oil (erucic, eicosenoic), peanut oil (arachidic, behenic, lignoceric), tung oil (eleostearic), and coriander seed oil (petroselinic) were oxidized, and the oxidized esterified glycerides were analyzed by gas-liquid chromatography (GLC). The values obtained are compared with those calculated from lipase hydrolysis data. Although there was a general over-all agreement between the compositions calculated from lipase hydrolysis data and that obtained by GLC analysis of the oxidized glycerides, there were some discrepancies that exceeded the range of experimental error.

Introduction

In a previous publication results of the analysis of 14 animal and vegetable fats were described (1). These fats contain the common fatty acids, palmitic, stearic, oleic, linoleic, and linolenic acids. For such fats the elution of the oxidized, esterified glycerides in the GLC unit starts from triazelain, which has an effective carbon number of 36 on a silicone column, and continues up to 54 with the elution of tristearin. In this paper the scope of the method has been extended to cover fats containing some less common fatty acids in fairly large amounts, such as petroselinic, eicosenoic, erucic, eleostearic, myristic, and arachidic acids. The fats analyzed were nutmeg butter, rapeseed, peanut, tung, and coriander seed oils. Pancreatic lipase hydrolysis of the oils was carried out to determine the composition of fatty acids esterified in the 1,3 positions of the glycerol molecule. The glyceride compositions calculated from pancreatic lipase hydrolysis are compared with those obtained by GLC of oxidized glycerides.

Experimental Section

The fats analyzed were extracted from the seed except the peanut oil and nutmeg butter, which were commercial samples. About 20 mg of each fat were oxidized with permanganate-periodate as previously described (2). The resulting azelaoglyceride mixture was esterified with diazomethane and analyzed by GLC on an F&M Model 1609 temperature-programmed unit with a flame ionization detector. The column was a 4-ft × $\frac{3}{16}$-in. stainless steel tube, packed with 2% S.E. 30 on 60–70 mesh Anakrom A.B.S. Temperature programming was from 250–325C at 3°/min, and the helium flow rate was 100 ml/min. The significance of carbon numbers used to denote emergence times of the esterified azelaoglycerides has been discussed earlier (2). Glycerides containing erucic acid are converted by oxidation into those with tridecanedioic acid. A carbon number of 16 was assigned to this acid by analogy with methyl azelate, which emerges with methyl laurate. Similarly oxidized products of eicosenoic, eleostearic, and petroselinic acids were assigned carbon numbers of 14, 12, and 9 respectively. As before (2), the quantitative results were based on the molar response of the detector as proportional to the number of carbon atoms remaining after as many CO_2 groups as possible have been split off. Thus trimethyl-triadipin (formed from tripetroselinin) with 24 actual carbon atoms and six ester groups would have an effective carbon number of 18. The GLC peak areas were divided by the appropriate effective carbon numbers to give mole percentages.

Fatty acid compositions of the oils before and after pancreatic lipase hydrolysis were determined by GLC of their methyl esters by using an 8-ft × $\frac{3}{16}$-in. copper column, packed with 15% o-phthalic ethylene glycol polyester on 60–60 mesh Chromosorb W.

GLC analysis of methyl esters of coriander seed oil gave the proportion of palmitic, palmitoleic, stearic, oleic + petroselinic, and linoleic esters. Another portion of the methyl esters was oxidized with permanganate-periodate to convert oleate and linoleate into azelaic acid and petroselinate into adipic acid. The oxidized material was esterified and analyzed by GLC to get relative molar amounts of azelate (from oleate + linoleate) and adipate (from petroselinate). The method used to determine the eleostearic acid content of *Momordica charantia* (3) was utilized to obtain the eleostearic acid content of tung oil. All results are reported as mole percentages.

Results and Discussion

Fatty acid analyses for the original fats and for the 1,3 positions are given in Table I. Glyceride compositions, as calculated from the lipase hydrolysis data by the method of Vander Wal (4) and Coleman and Fulton (5), are given in Tables II to VI along with the analysis as obtained by GLC of the oxidized esterified glycerides.

TABLE I

Fatty Acid Composition of Fats (Mole %)[a]

Nutmeg butter	L	M	P	O			
Original	3.3	87.2	4.5	5.0			
1,3 positions	2.3	83.5	6.6	7.6			
Rapeseed oil	P	S	O	L	Li	E	Er
Original	3.5	1.2	34.8	19.4	7.5	11.7	21.9
1,3 positions	5.2	1.8	31.4	11.6	4.5	16.5	29.0
Peanut oil	P	S	A	B	Lg	O	L
Original	14.0	3.5	2.0	2.0	1.0	47.8	29.7
1,3 positions	20.0	4.0	3.0	3.0	2.0	48.6	19.4
Tung oil	P	S	O	L	El		
Original	2.5	2.9	6.4	6.7	81.5		
1,3 positions	3.7	4.3	7.4	3.4	81.2		
Coriander seed oil	P	Po	S	O	Pe	L	
Original	3.4	0.6	1.2	10.0	71.8	13.0	
1,3 positions	5.0	1.0	1.6	8.0	80.0	4.4	

[a] L—lauric, M—myristic, P—palmitic, S—stearic, A—arachidic, B—behenic, Lg—lignoceric, Po—palmitoleic, O—oleic, Pe—petroselinic, E—eicosenoic, Er—erucic, L—linoleic, El—eleostearic acid.

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TABLE II

Glyceride Composition of Nutmeg Butter (Mole %)

Carbon No.	Glyceride	From lipase data	GLC detn.
38	LMO	0.32	
	OMO	0.61	
	LLM	0.10	
	MLO	0.70	
		1.73	.....
40	LMM	3.16	
	MMO	12.61	
	MLM	3.60	
		19.37	12.90
42	LMP	0.28	
	MMM	65.45	
	PMO	1.06	
	MLP	0.60	
		67.39	78.50
44	MMP	11.04	8.60
46	PMP	0.47	.....

Nutmeg Butter

The glyceride composition of this fat is given in Table II. The principal glycerides were trimyristin (65%), oleodimyristin (12%), and palmitodimyristin (11%). Collin and Hilditch (6) found 55% of trimyristin in material with a saturated acid content of 88.2%. From pancreatic lipase data 13 glycerides were calculated for nutmeg butter. The GLC analysis showed more of the mono- and trimyristo glycerides and less of the dimyristo glycerides than were predicted from the lipase data.

Rapeseed Oil

Oil was extracted with petroleum ether from a sample of commercial rapeseed. The erucic acid content of the sample used in this investigation was 22%, which is somewhat lower than the usual 45–50% reported earlier (7,8). The proportions of eicosenoic, linolenic, and linoleic acids were accordingly somewhat higher than is usual for erucic-rich oils of the Cruciferae. The amounts of 114 glycerides were obtained from pancreatic lipase hydrolysis data (Table III). Hilditch and Paul (7) found in rapeseed oil 6% of mixed palmito-oleo (or linoleo) erucins, 50% of di-C_{18}-erucin; the C_{18} acid was oleic, linoleic, or linolenic. Probably α and β eruco-di-oleins were present. Hilditch, Laurent, and Meara (8) crystallized rapeseed oil from acetone into three fractions at −20C and −30C. The chief classes of glycerides

TABLE III

Glyceride Composition of Rapeseed Oil (Mole %)

Carbon No.	Glyceride	From lipase data	GLC detn.	Carbon No.	Glyceride	From lipase data	GLC detn.
36	OOO	3.93		42	POE	0.72	
	OOL	3.21			SOO	0.52	
	OOLi	1.04			SOL	0.21	
	LOL	0.60			SOLi	0.08	
	LOLi	0.40			EOEr	4.25	
	LiOLi	0.06			PLE	0.58	
	OLO	3.36			SLO	0.44	
	OLL	2.60			SLL	0.18	
	OLLi	0.85			SLLi	0.08	
	LLL	0.50			ELEr	3.50	
	LLLi	0.34			PLiE	0.24	
	LiLLi	0.06			SLiO	0.20	
	OLiO	1.30			SLiL	0.08	
	OLiL	1.10			ELiEr	1.40	
	OLiLi	0.34			PEO	0.06	
	LLiL	0.20			OEEr	0.45	
	LLiLi	0.14			LEEr	0.14	
		20.03	8.50		EEE	0.06	
38	OOE	4.50			OErE	0.74	
	LOE	1.71			LErE	0.30	
	LiOE	0.56			LiErE	0.10	
	OLE	3.60				14.33	18.20
	LLE	1.42		44	POP	0.11	
	LiLE	0.48			POEr	1.22	
	OLiE	1.48			SOE	0.30	
	LLiE	0.56			ErOEr	3.48	
	LiLiE	0.20			PLP	0.09	
	OEO	0.27			PLEr	1.00	
	OEL	0.18			SLE	0.24	
	LEL	0.03			ErLEr	2.90	
		14.99	12.60		PLiP	0.04	
40	POO	1.30			PLiEr	0.42	
	POL	0.50			SLiE	0.10	
	POLi	0.16			ErLiEr	1.16	
	OOEr	7.39			EEEr	0.20	
	LOEr	2.99			PErO	0.22	
	LiOEr	0.98			PErL	0.08	
	EOE	1.22			OErEr	1.22	
	PLO	1.08			LErEr	0.52	
	PLL	0.42			LiErEr	0.18	
	PLLi	0.14			EErE	0.20	
	OLEr	6.30				13.68	13.20
	LLEr	2.44		46	POS	0.08	
	LiLEr	0.80			SOEr	0.48	
	ELE	1.02			PLS	0.06	
	PLiO	0.44			SLEr	0.52	
	PLiL	0.16			PLiS	0.02	
	PLiLi	0.04			SLiEr	0.16	
	OLiEr	2.52			PEEr	0.06	
	LLiEr	0.98			ErEEr	0.20	
	LiLiEr	0.32			PErE	0.12	
	ELiE	0.40			SErO	0.10	
	OEE	0.23			SErL	0.04	
	LEE	0.08			EErEr	0.70	
	LiEE	0.04				2.54	2.80
	OErO	0.65					
	OErL	0.60					
	OErLi	0.16		48	PErP	0.02	
	LErL	0.11			PErEr	0.22	
		33.47	43.90		SErE	0.06	
					ErErEr	0.58	
						0.88	0.80
				50	SErEr	0.08	.....

were stated to be monosaturated-mono-C_{18}-unsaturated-mono-C_{22}-unsaturated (18%), mono-C_{18}-unsaturated-di-C_{22}-unsaturated (54%), and di-C_{18}-unsaturated-mono-C_{22}-unsaturated (28%).

The principal glyceride types, as determined in the present work, are as follows: tri-C_{18}-unsaturated (20%), monoeicoseno-di-C_{18}-unsaturated (15%), and monoeruco-di-C_{18}-unsaturated (35%) glycerides. Glyceride composition by GLC showed a lower percentage of the tri-C_{18}-unsaturated glycerides and a higher percentage of monoeruco-di-C_{18}-unsaturated glycerides than those calculated from pancreatic lipase hydrolysis data. In order to investigate thoroughly the glyceride structure of rapeseed oil, it would be preferable to use a combination of silicic acid silver nitrate column and GLC separations as outlined earlier (9).

Peanut Oil

The sample used in the present investigation contained 2% arachidic, 2% behenic, and 1% lignoceric acids. Crawford and Hilditch (10) deduced the presence of monosaturated dioleins in large amounts (65% of total glycerides) with minor amounts of saturated oleolinoleins, oleodilinolein, and triolein. Vander Wal (4) analyzed a sample of peanut oil with a saturated acid content of 21% and found the glyceride structure to be (S saturated, U unsaturated) SSS 0.1, SSU 0.6, SUS 9.3, SUU 41.8, USU 0.7, and UUU 47.5.

In the present analysis 64 glycerides were obtained from pancreatic lipase hydrolysis data (Table IV). Values determined by GLC analysis agreed well with calculated values. The principal glycerides were triolein 11%, dioleolinolein 21%, saturated oleo linoleins 22%, dilinoleo-olein 12%, saturated diolein 15%, and minor amounts of saturated dilinoleins (6%).

TABLE V

Glyceride Composition of Tung Oil (Mole %)

Carbon No.	Glyceride	From lipase data	GLC detn.
36	OOEl	0.61	
	LOEl	0.26	
	ElOEl	3.00	
	OLEl	1.82	
	LLEl	0.69	
	ElLEl	9.00	
	OElO	0.44	
	OElL	0.43	
	OElEl	9.86	
	LElL	0.10	
	LElEl	4.52	
	ElElEl	54.08	
		84.81	85.0
40	POEl	0.27	
	PLEl	0.91	
	PElO	0.44	
	PElL	0.21	
	PElEl	4.92	
		6.75	11.00
42	SOEl	0.36	
	SLEl	1.08	
	SElO	0.52	
	SElL	0.24	
	SElEl	5.72	
		7.92	4.00
44	PElP	0.11	.....
46	PElS	0.26	.....
48	SElS	0.15	.....

Tung Oil

The anomalous glyceride structure of *Momordica charantia,* which contains high amounts of eleostearic

TABLE IV

Glyceride Composition of Peanut Oil (Mole %)

Carbon No.	Glyceride	From lipase data	GLC detn.	Carbon No.	Glyceride	From lipase data	GLC detn.
36	OLO	11.88		48	PLA	0.64	
	OLL	10.14			SLS	0.09	
	LLL	1.92			LgLO	0.98	
	OOO	11.02			LgLL	0.38	
	OOL	8.70			POA	0.56	
	LOL	1.60			SOS	0.07	
		45.26	50.00		LgOO	0.88	
40	PLO	10.18			LgOL	0.36	
	PLL	3.94			PPP	0.10	
	POO	8.82			OPA	0.06	
	POL	3.60				4.12	1.80
	OPO	0.50		50	PLB	0.64	
	OPL	0.50			SLA	0.14	
	LPL	0.09			POB	0.56	
		27.63	28.20		SOA	0.10	
42	SLO	2.14			OPB	0.04	
	SLL	0.80				1.48	1.00
	SOO	1.80		52	PLLg	0.40	
	SOL	0.72			ALA	0.50	
		5.46	7.50		POLg	0.36	
44	PLP	2.08			SOB	0.10	
	ALO	1.52			AOA	0.04	
	ALL	0.60			SLB	0.14	
	POP	1.84				1.09	.....
	PPO	0.40		54	SLLg	0.08	
	PPL	0.20			SOLg	0.08	
	AOL	0.54			ALB	0.10	
	AOO	1.36			AOB	0.08	
		8.54	7.00			0.34	.....
46	PLS	0.84		56	ALLg	0.06	
	POS	0.72			AOLg	0.06	
	BOO	1.36			BOB	0.05	
	BOL	0.54			BLB	0.04	
	BLO	1.54				0.21	.....
	BLL	0.60		58	BLLg	0.06	
	SPO	0.11			BOLg	0.06	
		5.71	4.50			0.12	.....
				60	LgLLg	0.02	
					LgOLg	0.02	
						0.04	.....

TABLE VI

Glyceride Composition of Coriander Seed Oil (Mole %)

Carbon No.	Glyceride	From lipase data	GLC detn.	Carbon No.	Glyceride	From lipase data	GLC detn.
27	PePePe	35.80	41.80	38	?	.....	1.10
30	OPePe	8.23		39	SPeP	0.17	
	PePeL	3.86			SPeL	0.08	
	PeOPe	8.95			SOPe	0.35	
	PeLPe	19.22			SLPe	0.75	
		40.26	42.50			1.35	0.80
33	OPeO	0.46		40	POO	0.12	
	OPeL	0.44			POL	0.06	
	LPeL	0.12			PLO	0.27	
	OOPe	2.06			PLL	0.13	
	PeOL	0.96				0.58	.....
	OLPe	4.34					
	PeLL	2.10		41	PPeP	0.14	.....
		10.48	3.40	42	SOO	0.14	
34	PPePe	4.44	2.10		SOL	0.02	
					SLO	0.09	
35	?	.....	0.30		SLL	0.04	
36	SPePe	1.42				0.19	.....
	OOO	0.12					
	OOL	0.11		43	PPeS	0.10	.....
	LOL	0.03		44	POP	0.04	
	OLO	0.25			PLP	0.07	
	OLL	0.24				0.11	.....
	LLL	0.05					
		2.22	1.00	46	POS	0.03	
37	PPeO	0.50			PLS	0.05	
	PPeL	0.24				0.08	.....
	POPe	1.11					
	PLPe	2.40					
		4.25	7.00				

acid, has been reported earlier (3). In marked contrast, tung oil, which contained 81.5% of eleostearic acid, follows the glyceride pattern obtained for other vegetable oils (Table V). A total of 25 glycerides was calculated from pancreatic lipase hydrolysis data. Hilditch and Mendelowitz (11) reported the presence, in tung oil, of 4% of monoeleostearo-di others (saturated, oleate, and linoleate), 40% of dieleostearo-mono others, and 56% of trieleostearin. The present results both from pancreatic lipase data and GLC determination show that, in tung oil, the following glycerides are present: 54% of trieleostearin, 27% mono-C_{18}-unsaturated dieleostearin, 11% of saturated dieleostearins, with minor amounts of eleolinoleo-eleostearin (4%) and saturated oleo(linoleo)eleo-stearins (4%).

Coriander Seed Oil

This oil was selected for study because of the presence of petroselinic acid, a C_{18} monoenoic acid with the double bond in the 6,7 position. On oxidation of the oil the petroselinyl groups are converted into adipyl groups. Christian and Hilditch (12) found 53% of petroselinic acid in coriander seed oil fatty acids besides oleic (32%), linoleic (7%), and palmitic (8%) acids. The present analysis shows a higher proportion of petroselinic (72%) acid and a lower oleic acid content (10%).

The glyceride structure of this oil does not seem to have been investigated so far. Triadipin (the free carboxyl group of the dicarboxylic acid was esterified with diazomethane), for purposes of elution, has a carbon number of 27 and was found to the extent of 42% in the present glyceride analysis (Table VI). Dipetroselinomono-oleo (or linoleo) glycerides amounted to 43%. The rest was made up mostly by dipetroselino-monosaturated glycerides. Agreement between the glyceride composition, obtained by pancreatic lipase data (a total of 42 glycerides), and that by GLC determination was fairly good excepting for dioleo (or dilinoleo) monopetroselinin, where the calculated value was higher by 7% than that determined by GLC whereas tripetroselinin was 6% lower than that determined by GLC.

The present results show the usefulness of the GLC technique for determining glyceride compositions of various natural fats and demonstrates the applicability of the method to fats containing some less common fatty acids. Although there is a general over-all agreement between the compositions calculated from lipase hydrolysis data and that obtained by GLC analysis of oxidized glycerides, there are some discrepancies that exceed the range of experimental error. Thus the assumption of a 1,3 random, 2 random distribution appears to hold for a number of fats (1) but cannot be applied as a general rule to all fats.

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