The alkyl moieties in wax esters and alkyl diacyl glycerols of sharks

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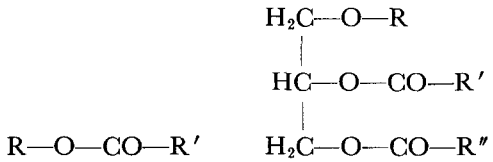
ABSTRACT The alkyl moieties in wax esters and alkyl diacyl glycerols from the liver of the dogfish, soupfin shark, and silky shark are almost exclusively saturated and monounsaturated, the main alkyl moieties being the C_{16} and C_{18} chains in both lipid classes. However, the alkyl moieties in wax esters occur in a wider range of chain lengths. The unsaturated alkyl moieties in the two classes of lipids are mixtures of isomers. The distribution of isomeric octadecenyl moieties in wax esters and alkyl diacyl glycerols is almost the same.

SUPPLEMENTARY KEY WORDS alcohols · alkyl glycerols · isomeric octadecenyl moieties · Elasmobranchii · dogfish liver · soupfin shark liver · silky shark liver · *Squalus acanthias* · *Galeorhinus zyopterus* · *Carcharhinus falciformis*

LONG-CHAIN alcohols occur in nature as such, esterified with fatty acids as wax esters (I), and bound to glycerol as derivatives of alkyl glycerols, such as alkyl diacyl glycerols (II).



(I) (II)

Various tissues of sharks and other cartilaginous fishes (Chondrichthyes) (1) contain large amounts of (I) and (II) and other unusual lipids. The liver of deep-sea sharks is rich in hydrocarbons (2, 3) and wax esters (4), whereas that of species living in more shallow waters contains alkyl diacyl glycerols, alk-1-enyl diacyl glycerols, and triacyl glycerols (5–7).

The long-chain moieties of tissue lipids in cartilaginous fishes have been studied extensively. It has been found that the alkyl moieties in wax esters isolated from tissues of deep-sea sharks are predominantly saturated and monounsaturated, whereas the acyl groups include polyunsaturated structures (4, 8). Similarly, the alkyl moieties in alkyl diacyl glycerols isolated from the liver of sharks and related species are also only saturated and monounsaturated, although among the acyl groups there occur those having four, five, and six double bonds (7, 9).

Analyses of the alkyl moieties in wax esters and alkyl diacyl glycerols of the same tissue of a cartilaginous fish have not been reported. Yet, such comparative analyses would be of particular interest as it has been demonstrated that in a shark, the dogfish, the alkyl moieties in both wax esters (10, 11) and alkyl diacyl glycerols (12, 13) can originate from long-chain alcohols as a common precursor.

The present communication records the results of compositional analyses of the alkyl moieties in wax esters and alkyl diacyl glycerols that were isolated from the liver of three sharks, the dogfish (*Squalus acanthias*) of the suborder Squaloidea, and the soupfin shark (*Galeorhinus zyopterus*) and the silky shark (*Carcharhinus falciformis*), both of the suborder Galeoidea. The relative amounts of the isomeric octadecenyl moieties in wax esters and alkyl diacyl glycerols in the liver of these sharks are also given. These analytical and structural studies permit an evaluation of the metabolic interrelationship between various classes of lipids in shark livers.

MATERIALS AND METHODS

Synthetic Reference Compounds

Isopropylidene derivatives of alkyl glycerols were syn-

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thesized by alkylation of isopropylidene glycerol with long-chain methanesulfonates (14). Alkyl acetates were prepared (15) from alcohols that were purchased from The Hormel Institute Lipids Preparation Laboratory, Austin, Minnesota.

Fish Liver Lipids

The livers of male dogfish (*Squalus acanthias*), soupfin shark (*Galeorhinus zyopterus*), and silky shark (*Carcharhinus falciformis*) were homogenized in chloroform–methanol 2:1. The total lipids were extracted with the same solvent mixture, and they were purified according to established procedures (16).

Analytical Methods

Thin-Layer Chromatography. All fractions isolated, as well as the products of reactions, were analyzed by chromatography on layers of Silica Gel G, using mixtures of hexane and diethyl ether as developing solvents. Fractions were detected under UV light (270 nm) after spraying the plates with a 0.2% solution of 2′,7′-dichlorofluorescein in ethanol, or by charring with chromic sulfuric acid solution.

Gas–Liquid Chromatography. The instrument used was an F & M Scientific Hewlett–Packard gas chromatograph, model 5750, equipped with a flame ionization detector. The column, 150 × 0.4 cm, was filled with 10% diethyleneglycol succinate on Anakrom A, 60/70 mesh. Helium, at an inlet pressure of 30 psi, served as carrier gas. All samples were analyzed isothermally: alkyl acetates at 182°C, and isopropylidene derivatives of alkyl glycerols at 202°C. The relative amounts of the fractions were calculated by triangulation of peak areas on the recording charts. Fractions were identified by comparison of their retention times with those of synthetic reference compounds. In addition, aliquots of the samples, dissolved in ethyl acetate, were hydrogenated with PtO_2 as catalyst, and analyzed for chain length composition. The results of analyses of the original and the hydrogenated samples were in agreement.

Isolation of Wax Esters and Alkyl Diacyl Glycerols

All procedures were carried out under a nitrogen atmosphere as far as was practical. Samples of the oils, 5 g of each, were fractionated (17) on layers of Silica Gel H, 2 mm thick, by developing twice with hexane–diethyl ether 95:5. The fraction of wax esters, which included sterol esters, and the fraction of alkyl diacyl glycerols, which included small amounts of alk-1-enyl diacyl glycerols and triacyl glycerols, were scraped off the plates and eluted from the adsorbent with several portions of water-saturated diethyl ether. The slurries were filtered through sintered glass funnels. The samples yielded 30–60 mg of a wax ester fraction and 80–110 mg of an alkyl diacyl glycerol fraction.

Preparation of Derivatives

The fractions of wax esters and alkyl diacyl glycerols were reacted with methanol–HCl in sealed tubes for 4 hr at 80°C (18). Upon cooling, the reaction mixtures were treated as described below.

Wax Esters. The products of methanolysis, i.e., methyl esters, long-chain alcohols, and sterols, were separated by chromatography on layers of Silica Gel G, 0.5 mm thick, using hexane–diethyl ether 50:50 as developing solvent. The alcohol fraction, R_F 0.6 (sterols R_F 0.5, methyl esters R_F 0.95), was scraped off the plates and eluted from the adsorbent with water-saturated diethyl ether. The alcohols were acetylated and the resulting alkyl acetates were purified by chromatography on layers of Silica Gel G, 0.35 mm thick, using hexane–diethyl ether 90:10 as developing solvent. The purified alkyl acetates were dissolved in hexane and analyzed by gas–liquid chromatography.

Alkyl Diacyl Glycerols. Methanolysis of these fractions under the same conditions as described for wax esters yielded methyl esters and alkyl glycerols. These products were separated and purified by chromatography on layers of Silica Gel G, 0.5 mm thick, using hexane–diethyl ether–glacial acetic acid 50:50:1. The fraction of alkyl glycerols (R_F 0.25) was scraped off the plate, eluted from the adsorbent with water-saturated diethyl ether and, after drying, reacted with absolute acetone in the presence of perchloric acid as catalyst (19). The resulting isopropylidene derivatives of the alkyl glycerols were purified by chromatography on layers of Silica Gel G, 0.35 mm thick, using hexane–diethyl ether 90:10 as the developing solvent.

Isolation of Monounsaturated C_{18} Compounds

Octadecenyl acetates, the isopropylidene derivatives of octadecenyl glycerols, and methyl octadecenoates were isolated by gas–liquid chromatography in a Beckman GC-2A instrument using the experimental conditions described above. Fractions were collected in glass tubing, 4 mm I.D., fitted with ground joints to a heated stainless steel outlet of the gas chromatograph.

Ozonolysis

The monounsaturated C_{18} compounds isolated were ozonized in amounts of 0.2–2 mg in purified pentane (20). The ozonides were reduced with triphenylphosphine (21) and the reaction products were analyzed by gas–liquid chromatography (22, 23). The various aldacetates and aldethers were identified by gas–liquid chromatography using the ozonolysis products of synthetic compounds as standards.

RESULTS

The liver lipids of dogfish, soupfin shark, and silky shark contain less than 1% wax esters. By repeated adsorption chromatography on layers of silicic acid these wax esters can be isolated, together with trace amounts of sterol esters and vitamin A esters, which do not interfere in the analysis of their alkyl moieties. Alkyl diacyl glycerols occur at levels of from 3% in the liver of soupfin shark to 40% in the liver of dogfish, and they can be isolated easily by adsorption chromatography.

The results of gas–liquid chromatographic analysis of the alkyl groups in wax esters and alkyl diacyl glycerols of dogfish and soupfin shark livers are listed in Table 1. In each case, the alkyl chains in the two lipid classes are almost exclusively saturated and monounsaturated. Branched-chain alkyl moieties are present in both types of compounds in small and almost equal proportions. However, the alkyl moieties in wax esters occur in a wider range of chain lengths than those in alkyl diacyl glycerols. The alkyl groups in the alkyl diacyl glycerols consist of about 60% octadecenyl moieties. The results of analyses of the alkyl moieties in the lipids of silky shark were almost identical to those obtained with soupfin shark, a related species, and therefore are not listed.

Determinations of the position of the double bond in octadecenyl moieties reveal a close similarity between these chains in wax esters and alkyl diacyl glycerols (Table 2). Not only the number of isomers but also their percentage distribution is about the same for each pair of octadecenyl moieties. The Δ9 octadecenyl isomer predominates by far in both the wax esters and the alkyl diacyl glycerols isolated from the liver of dogfish and soupfin shark. The distribution of isomeric octadecenyl moieties in the alkyl diacyl glycerols of silky shark was almost identical to that in the alkyl diacyl glycerols of soupfin shark. The isomeric octadecenyl moieties in the wax esters of silky shark could not be determined due to the lack of material.

TABLE 1 COMPOSITION OF THE ALKYL CHAINS IN WAX ESTERS AND IN ALKYL DIACYL GLYCEROLS FROM SHARK LIVERS

Chain Length: Number of Double Bonds	Dogfish		Soupfin Shark	
	Alkyl Moieties		Alkyl Moieties	
	in Wax Esters	in Alkyl Diacyl Glycerols	in Wax Esters	in Alkyl Diacyl Glycerols
	%	%	%	%
13:0	0.2		1.1	
14:0 br		tr.	tr.	
14:0	4.7	2.8	3.5	3.4
14:1		0.6		tr.
14:2				tr.
15:0 br	0.1	0.2	tr.	0.8
15:0	0.9	0.2	1.0	tr.
16:0 br	0.1	0.3	tr.	
16:0	31.7	13.8	18.3	18.5
16:1	6.9	11.8	9.4	13.4
16:2				1.7
17:0 br		0.4		
17:0	1.7	1.1	0.9	tr.
18:0 br	1.4	0.2	tr.	
18:0	6.2	4.5	7.8	5.0
18:1	38.5	59.6	42.7	57.2
18:2 + unkn.	1.1	tr.	1.6 + 0.8	tr.
19:0	tr.		tr.	
20:0	0.5	0.5	1.9	
20:1 + unkn.	9.0 + tr.	1.7	5.6 + 0.5	tr.
20:2	0.2		tr.	
20:4	0.2		0.8	
21:0	tr.		tr.	
22:0	0.1		0.9	
21:1 + unkn.	0.8		2.6 + tr.	
22:3	0.2		tr.	
23:0	0.2		tr.	
24:0	0.1		0.6	

TABLE 2 THE ISOMERIC OCTADECENYL MOIETIES IN WAX ESTERS AND IN ALKYL DIACYL GLYCEROLS FROM SHARK LIVERS

Position of Double Bond in Octadecenyl Moieties	Dogfish		Soupfin Shark	
	Octadecenyl Moiety in Wax Esters	Octadecenyl Moiety in Alkyl Diacyl Glycerols*	Octadecenyl Moiety in Wax Esters	Octadecenyl Moiety in Alkyl Diacyl Glycerols
	%	%	%	%
Δ5	1.2		tr.	0.5
Δ6	tr.	1.5	tr.	0.3
Δ7	4.0	17.7	2.4	2.5
Δ8	1.4	3.6	2.2	0.6
Δ9	77.6	70.6	87.7	86.6
Δ10		2.3		
Δ11	15.1	4.2	7.7	8.3
Δ12	tr.			
Δ13	0.7		tr.	1.2

* See Ref. 22.

DISCUSSION

Several workers have shown that the acyl moieties in the wax esters isolated from various tissues of bony fishes (24–30) and in the alkyl diacyl glycerols from cartilaginous fishes (7–9) occur in a wider range of chain lengths than the alkyl moieties in these two lipid classes. The great similarity in the distribution of isomeric monounsaturated alkyl and acyl moieties in wax esters (27, 28) as well as in alkyl diacyl glycerols (23) indicates that, in fish, alkyl and acyl chains, or long-chain alcohols and fatty acids, can be interconverted. Polyunsaturated moieties occur in rather large proportions among the acyl groups (7, 8, 24–30) of the two classes of lipids, but only traces are detected in the alkyl groups of wax esters (26, 29–31) and alkyl diacyl glycerols (7, 31). These facts can be explained by the finding that in fish, at least in the opaline gourami (*Trichogaster cosby*),

saturated acids having chains of 16 and 18 carbon atoms, and monounsaturated acids, are reduced more easily than fatty acids having longer chain lengths, and polyunsaturated fatty acids (32).

The great similarity in the distribution of isomeric octadecenyl moieties in the two lipid classes lends further support to the assumption that the alkyl groups in both wax esters and alkyl diacyl glycerols of sharks and other fishes are derived from long-chain alcohols.

A comparison of the analyses presented in Table 1 shows that the alkyl moieties in wax esters occur in a wider range of chain lengths than the alkyl moieties in alkyl diacyl glycerols. Thus, it appears that the enzyme system catalyzing the alkylation of the glycerol moiety is more selective than that catalyzing the formation of wax esters.

It is of interest to note that not only in shark livers but also in a mammalian tissue, the preputial gland of the mouse, the distribution of isomeric alkyl moieties in wax esters and alkyl diacyl glycerols has been found to be similar (33). Thus, it has been suggested (33) that the alkyl moieties in the two lipid classes are derived from a common precursor. More recently, metabolic studies have indeed confirmed the role of long-chain alcohols as precursors of the alkyl moieties in alkyl diacyl glycerols of the preputial gland (34).

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