The occurrence of the hydrocarbon, squalene, in diverse marine organisms is not uncommon, but quantities of commercial interest are generally limited to the livers of a few members of the shark family. Nova Scotia shallow-water dogfish lack this component in their liver oil. An opportunity to compare several liver oils from deep-water exploratory catches, however, yielded diverse results, confirming its presence in deeper water species. A Pacific eulachon fish body oil rich in squalene was analysed also and the results were compared with the oils from deep sea sharks.

Il n’est pas rare de trouver l’hydrocarbure squalène dans divers organismes marins, mais ce n’est généralement que dans les foies de quelques membres de la famille des requins qu’on le trouve en quantités d’intérêt commercial. Cet hydrocarbure n’est pas présent dans l’huile de foie des aiguillats des eaux peu profondes de la Nouvelle-Écosse. Toutefois, les résultats divers d’une comparaison de plusieurs huiles de foie de poissons provenant de prises exploratoires en eaux profondes confirment la présence de cet hydrocarbure dans des espèces vivant à de plus grandes profondeurs. De l’huile de poisson riche en squalène qui provenait d’un eulakane du Pacifique a également été analysée et comparée aux huiles des requins d’eaux profondes.

INTRODUCTION

A considerable interest in deep-sea shark liver oils has developed in recent years (Summers et al. 1990, Bakes & Nichols 1995, Bordier et al. 1996, Borch-Jensen et al. 1997, Deprez et al. 1970), as well as in shallow water species (Jayasinghe et al. 2003). This type of work is stimulated by a commercial demand for squalene. As the name suggests, squalene was originally isolated from shark liver oil. Our most common Nova Scotian shark species, the shallow-water dogfish *Squalus acanthias* Linnaeus 1758, regrettably, has very little or no squalene in its liver oil. We had an opportunity to examine the liver lipids of a western Atlantic deep-sea shark, also of the family Squalidae, the black dogfish *Centroscyllium fabricii* (Reinhardt) 1825. Livers of the deep-sea cat shark *Apristurus profundorum* (Goode & Bean) 1896 and rough sagre *Etmopterus princeps* Collett 1904 were also provided for research, as was a single liver (274 g) from a deep-sea cartilaginous fish, the longnose chimaera *Harriota raleighana* Goode & Bean 1895, order Chimaeriformes, family Chimaeridae. These were all caught in a commercial exploration using traps set near Nova Scotia in approximate depths of 1000 m or more. For comparison of fish body squalene an earlier study in the west coast euryhaline fish, usually referred to as “eulachon,”

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was repeated on a small scale as new analytical technology (Iatroscan Thin Layer Chromatography/Flame Ionization Detection [TLC/FID]) was available to aid in these analyses, along with fractional crystallization (Nenadis & Tsimidou 2002), a new technique remarkably faster than some proposed liquid handling procedures requiring days.

SAMPLES AND METHODS

The black dogfish (NS-1) were caught about June 11, 1995 in traps and the fish put on ice. Livers were removed after 9 days. The chimaera was caught June 13 and the liver removed after 7 days of being held on ice. The second lot of black dogfish and all other samples were caught in August and were handled similarly. The livers were delivered on ice to the CIFT laboratory from the National Sea Products Ltd. plant in Lunenberg, Nova Scotia in plastic bags with no information other than the species designation. As a first step the livers were sorted and compared, and any that appeared abnormal for colour or consistency were excluded. The appropriate livers were homogenized and extracted by the method of Bligh and Dyer (1959) and the lipid examined for Wijs iodine value and % unsaponifiables by AOCS methods Cd-1-25 and Ca-66-53 respectively.

Lipid composition by Iatroscan TLC/FID was performed with an Iatroscan TH-10 analyzer equipped with a flame ionization detector attached to a SP4200 computing integrator (Spectra Physics) in order to give digital integration of the peaks. The FID hydrogen flow rate was 160 mL min⁻¹. The samples were chromatographed on Chromarods S-III developed in hexane:diethyl ether:formic acid (97:3:1 v/v/v) for 55 min. The solvents were allowed to evaporate from the rods in an oven for 2 min at 110ºC prior to scanning with the FID of the Iatroscan TH-10 analyzer.

The black dogfish liver lipid extract was applied to a TLC plate and separated into two major bands with hexane:diethyl ether:acetic acid (85:15:1 v/v/v). The recovered 1-O-alkyl-diacylglycerol and triacylglycerols were converted to methyl esters with BF₃-MeOH.

For the fatty acid methyl esters (FAME) a modified AOCS Official Method Ce 1b-89 was applied to lipids or lipid fractions by heating 20 mg in methanol containing 4% BF₃ at 100ºC for 1 h. The FAME were recovered in hexane and analyzed by GLC (gas-liquid chromatography). The column was Omegawax-320, 30 m x 0.32 mm id installed in a Perkin Elmer GC model 8420 equipped with a split injection port and a FID. The program used for GLC of FAME was: initially 185ºC for 8 min, a ramp of 3ºC min⁻¹ to 230ºC, and held for 10 min.

RESULTS AND DISCUSSION

Shark Lipids

Sharks are a well-known and prolific fish family, including very large fish with unpopular habits such as attacking shipwrecked sailors. They are
found mainly in a broad band of waters on each side of the equator, but at least some members of the dogfish family are found in the cold waters of the North Atlantic and Pacific oceans. Public interest has stemmed not from shark liver oils, but from a small book rashly promising good health in respect to shark cartilage for treating cancer and degenerative diseases (Lane & Comae 1992). Possible use of these oils in skin creams has also been discussed.

The commercial demand for shark liver oil (Summers et al. 1990) is based on the use of squalene, a C\textsubscript{30}H\textsubscript{50} hydrocarbon with six trans ethylenic bonds (2,6,10,15,19,23-hexamethyl-2,6,10,14,18,22-tetracosahexaene) (Fig 1).

\textbf{Squalene}

\begin{center}
\begin{tikzpicture}
\draw[black, thick] (0,0) -- (1,0) -- (2,0) -- (3,0) -- (4,0) -- (5,0) -- (6,0) -- (7,0) -- (8,0) -- (9,0) -- (10,0) -- (11,0); \\
\draw[black, thick] (1,0) -- (1,1); \\
\draw[black, thick] (2,0) -- (2,1); \\
\draw[black, thick] (3,0) -- (3,1); \\
\draw[black, thick] (4,0) -- (4,1); \\
\draw[black, thick] (5,0) -- (5,1); \\
\draw[black, thick] (6,0) -- (6,1); \\
\draw[black, thick] (7,0) -- (7,1); \\
\draw[black, thick] (8,0) -- (8,1); \\
\draw[black, thick] (9,0) -- (9,1); \\
\draw[black, thick] (10,0) -- (10,1); \\
\draw[black, thick] (11,0) -- (11,1); \\
\draw[black, thick] (0,1) -- (1,1) -- (2,1) -- (3,1) -- (4,1) -- (5,1); \\
\draw[black, thick] (1,1) -- (1,2); \\
\draw[black, thick] (2,1) -- (2,2); \\
\draw[black, thick] (3,1) -- (3,2); \\
\draw[black, thick] (4,1) -- (4,2); \\
\draw[black, thick] (5,1) -- (5,2); \\
\draw[black, thick] (6,1) -- (6,2); \\
\draw[black, thick] (7,1) -- (7,2); \\
\draw[black, thick] (8,1) -- (8,2); \\
\draw[black, thick] (9,1) -- (9,2); \\
\draw[black, thick] (10,1) -- (10,2); \\
\draw[black, thick] (11,1) -- (11,2); \\
\draw[black, thick] (0,2) -- (1,2) -- (2,2) -- (3,2) -- (4,2) -- (5,2); \\
\end{tikzpicture}
\end{center}

\textit{C}_{30}\textit{H}_{50} \quad \textit{M} \ 410.725

\textbf{Fig 1} \quad \text{Squalene}

This hydrocarbon is not rare as it is 0.1-0.7% of olive oil and is in a few other vegetable seed oils such as amaranthus grain (He et al. 2002), as well as in some marine organisms. Squalene itself will oxidize spontaneously, but after hydrogenation to squalane is useful as a stable lubricant. Recovery and refining on an industrial scale is discussed by Summers et al. (1990). It has a low density (0.866 at 5ºC) which sharks themselves may find useful in moving from the surface to considerable depths, as discussed in some detail by Nevenzel (1989) and Morris and Culkin (1989).

\textbf{Shark Lipid Analyses}

The graphic area percentages for major lipid classes (Table 1) for TLC-FID are only an approximation of the neutral lipid classes, but these should have similar relative responses once separated and moved along the Chroma-rod. This quartz rod coated with silica gel is passed through an ionization flame detector. An alternative way to quickly assess the squalene content of a fish liver oil is simply to transesterify the oil and analyse the resulting solution of fatty acid methyl esters and squalene on a polyglycol-based capillary gas-liquid chromatography column. We have used both SUPELCOWAX-10 and Omegawax-320 for this purpose, these being commonly in use for the highly unsaturated and long-chain polyunsaturated fatty acids of marine lipids. Owing to the diverse numbers of ethylenic bonds of FAME (either n-3 or n-6, counting to the first bond from the terminal methyl group\textsuperscript{1})

\textsuperscript{1} There are varied nomenclatures for fatty acids: e.g. 18:2\Delta9, \Delta12, refers to an 18 carbon fatty acid, with 2 cis double bonds between carbons 9 & 10 and 12 & 13, but 18:2n-6 is far simpler.
chain length overlaps often occur. With our program the squalene fell on or just after the 24:1n-9 position, both just after the 22:6n-3 position. Since squalene is chemically different from the methyl esters of fatty acids this position could vary with the temperature program as well as the polarity of the column. The black dogfish oil FAME chromatograms from both samples were remarkable in showing a series of saturated (16%) and monoethylenic (73%) peaks, with almost no significant polyunsaturated fatty acid (PUFA) peaks (Table 2), and then a large squalene peak consisting of ~20% of the total peak area. The monoethylenic fatty acid pattern of the black dogfish liver oil was very conventional and similar to that of herring oil or *S. acanthis* liver oil (Kang et al. 1996). The chimaera liver oil showed no squalene but the FAME gas liquid chromatography analysis showed an unknown peak (8% of FAME area) immediately preceding the 20:0 position. It also had an unusual monoethylenic fatty acid pattern with n-7 fatty acids being extended from 16:1n-7 through 18:1n-7 to 20:1n-7 and similarly 20:1n-9 was extended to 22:1n-9 (Table 2).

The high iodine value in the Russian report (Dolbish et al. 1969) on the liver oil of black dogfish is a firm indicator of squalene as the dominant lipid in their sample. The calculated iodine value (IV) of squalene is 370 and is an example of the effect of squalene inclusion in fish lipids, the addition of 10% squalene to seal oil revised the IV from 152 to 183. It is possible to use the saponification method of Kovacs et al. (1979) with cholestane added to any suitable oil or lipid fraction as an internal standard for GLC determination of squalene. On the polyglycol-based gas-liquid chromatography columns the cholestane emerged immediately after the squalene but with cleanly separated peaks.

The deep-sea cat shark is a small (≤60 cm) shark regarded as a pest when attempts are made to trap other sharks with commercial potential such as the black dogfish. The total liver lipid when transesterified and analysed for FAME included the squalene peak. The fatty acids (Table 2) were not of remarkable interest. A shoulder presumed to be 18:1n-11 is included

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**Table 1** Bligh and Dyer liver extract lipids, properties and proportions from TLC-FID.

<table>
<thead>
<tr>
<th>Lipid content (w/w %)</th>
<th>Black dogfish NS-1</th>
<th>Black dogfish NS-2</th>
<th>USSR* Deep-sea cat shark</th>
<th>Rough sagre Chimaera</th>
</tr>
</thead>
<tbody>
<tr>
<td>Iodine value</td>
<td>90.2</td>
<td>93.7</td>
<td>286</td>
<td>89.0</td>
</tr>
<tr>
<td>Unsaponifiables (w/w %)</td>
<td>61.9</td>
<td>37.0</td>
<td>79</td>
<td>37.7</td>
</tr>
<tr>
<td>Triacylglycerol</td>
<td>9.4</td>
<td>10.7</td>
<td>-</td>
<td>55.9</td>
</tr>
<tr>
<td>Diacylglycerol ether</td>
<td>69.2</td>
<td>73.0</td>
<td>-</td>
<td>3.1</td>
</tr>
<tr>
<td>Hydrocarbon</td>
<td>18.5</td>
<td>13.4</td>
<td>-</td>
<td>36.2</td>
</tr>
<tr>
<td>Polar Lipid</td>
<td>1.0</td>
<td>0.8</td>
<td>-</td>
<td>2.3</td>
</tr>
<tr>
<td>Sterol (wax) Ester</td>
<td>0.9</td>
<td>1.2</td>
<td>-</td>
<td>2.3</td>
</tr>
</tbody>
</table>

*Data from Dolbish et al. 1969*
SQUALENE IN NS DEEP-SEA SHARKS AND IN THE PACIFIC EULACHON

in 18:1n-9, and this correlates with a prominent peak for 20:1n-11. This is suggestive of chain shortening from an original source of 22:1n-11 in the diet (Ackman et al. 1980). Although 18:1n-11 could not be discerned in the GLC analysis of the chimaera liver oil a high proportion of 20:1n-11 relative to 20:1n-9 was also apparent in this species. Thus conversion from 20:1n-11 could also account for the relatively high 22:1n-11 already mentioned. It would seem paradoxical to have n-9 fatty acids going to longer (20:1) chain lengths and then elongation favouring n-11, but 22:1n-11 is common in most fish oils.

The cat shark liver lipid extract showed (Table 1) an even greater proportion of squalene (36%) than did the black dogfish liver oil. The gas liquid chromatography confirmed the hydrocarbon to be squalene. Since the triacylglycerols were just over half of the total lipid the diacylglycerol ethers were only present in trace amounts. The rough sagre is also small, with 75 cm being the maximum length (Scott & Scott 1988). The liver oil of this species was dominated by diacylglycerol ethers but had nearly 30% squalene.

Nova Scotia and other Atlantic provinces could benefit from more deep-sea exploration as there is evidence that deep-sea and northerly teleost fish may also provide squalene in their lipids (Hayashi & Kishimura 2003).

Table 2  Fatty acids of different liver lipid samples

<table>
<thead>
<tr>
<th></th>
<th>Black dogfish</th>
<th>Deep-sea cat shark</th>
<th>Chimaera</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Total Oil</td>
<td>TAG</td>
<td>DAGE</td>
</tr>
<tr>
<td>14:0</td>
<td>1.4</td>
<td>2.5</td>
<td>1.0</td>
</tr>
<tr>
<td>16:0</td>
<td>12.3</td>
<td>13.3</td>
<td>13.8</td>
</tr>
<tr>
<td>18:0</td>
<td>1.0</td>
<td>1.5</td>
<td>1.0</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>16.2</strong></td>
<td><strong>19.0</strong></td>
<td><strong>17.3</strong></td>
</tr>
<tr>
<td>16:1n-7</td>
<td>3.9</td>
<td>6.3</td>
<td>2.8</td>
</tr>
<tr>
<td>18:1n-9</td>
<td>23.7</td>
<td>19.5</td>
<td>27.5</td>
</tr>
<tr>
<td>18:1n-7</td>
<td>3.0</td>
<td>3.2</td>
<td>3.0</td>
</tr>
<tr>
<td>20:1n-11</td>
<td>2.0</td>
<td>1.0</td>
<td>1.1</td>
</tr>
<tr>
<td>20:1n-9</td>
<td>13.1</td>
<td>16.0</td>
<td>13.8</td>
</tr>
<tr>
<td>20:1n-7</td>
<td>0.9</td>
<td>1.0</td>
<td>0.9</td>
</tr>
<tr>
<td>22:1n-11+13</td>
<td>21.4</td>
<td>19.6</td>
<td>22.9</td>
</tr>
<tr>
<td>22:1n-9</td>
<td>3.8</td>
<td>3.4</td>
<td>4.0</td>
</tr>
<tr>
<td>22:1n-7</td>
<td>0.7</td>
<td>0.5</td>
<td>0.4</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>73.2</strong></td>
<td><strong>72.2</strong></td>
<td><strong>78.3</strong></td>
</tr>
<tr>
<td>18:2n-6</td>
<td>0.7</td>
<td>0.7</td>
<td>0.8</td>
</tr>
<tr>
<td>18:3n-3</td>
<td>0.2</td>
<td>0.3</td>
<td>0.2</td>
</tr>
<tr>
<td>18:4n-3</td>
<td>0.4</td>
<td>0.3</td>
<td>0.2</td>
</tr>
<tr>
<td>20:4n-6</td>
<td>0.3</td>
<td>0.5</td>
<td>0.1</td>
</tr>
<tr>
<td>20:5n-3</td>
<td>0.8</td>
<td>0.6</td>
<td>0.2</td>
</tr>
<tr>
<td>22:5n-3</td>
<td>0.6</td>
<td>1.5</td>
<td>0.1</td>
</tr>
<tr>
<td>22:6n-3</td>
<td>2.7</td>
<td>1.8</td>
<td>1.0</td>
</tr>
<tr>
<td><strong>Total PUFA</strong></td>
<td><strong>10.7</strong></td>
<td><strong>9.5</strong></td>
<td><strong>4.7</strong></td>
</tr>
<tr>
<td>Calc. IV</td>
<td>94</td>
<td>86</td>
<td>75</td>
</tr>
</tbody>
</table>

*Confirmed by mixed analysis with canola oil FAME.
The structure of traditional fisheries in this area limits various fisheries to certain times of the year, so deep-sea fisheries could provide additional work periods. In a parallel situation in Tasmanian waters, Deprez et al. (1990) have suggested that these novel resources may be limited.

**Eulachon Lipids**

The shark species described here are not “monsters of the deep,” typically being 100 cm or less (Scott & Scott 1988). Strangely, Canada has a much smaller bony fish that is also rich in squalene. The teleost eulachon *Thaleichthys pacificus* Richardson, 1836, matures at 108 mm and the adults in spawning runs of the Fraser River are only 140-150 mm in length (Hart 1973). It is an anadromous vertebrate of the smelt (Osmeridae) family. The fish are found in western North America from the Russian River of California to the eastern Bering Sea. That it is an oily fish has been long known; the dried fish burned so readily when fitted with a wick that it came to be known as the candlefish. The oil was reputedly solid at room temperature and was collected and traded by the coastal Indians of British Columbia across the Rocky Mountains to the Indians of the Great Plains. In 1968 a few eulachon were collected personally by the author (R.G. Ackman) from a shrimp trawl in Barkley Sound, Vancouver Island. The head and gutted bodies contained 21% lipid of which 84% was triacylglycerols, 3.1% was polar lipid (primarily as phospholipid) and 12% squalene (Ackman et al., 1968). This lipid class analysis was carried out by planar TLC with the squalene confirmed by GLC, and was an unexpected finding. At various times when interest in squalene arose the attempts to develop commercial fisheries were refused by various governmental agencies on the ground that these fish were reserved for Indian use and were needed to support other and more lucrative fisheries.

A more recent capture of eulachon actually taken in the Fraser River, possibly post-spawning, showed only a small amount of squalene (10%) in the lipid, 13.5% of the body and head. The strange feature of the body/head lipids of this lot of eulachon was the fatty acid compositions of the triacylglycerols. Those of the polar lipids were normal for marine fish with respect to the content of long-chain polyunsaturated fatty acids, but the triacylglycerols separated by planar TLC were dominated by very high proportions of 14:0 (9%), 16:0 (20%), 16:1 (7%), 18:0 (5%) and especially high-melting (44°C) vaccenic acid, which is *trans*-18:1n-7, at 4%. Isoprenoid fatty acids (e.g. pristanic) were also present. These observations reinforce the classical description of the oil of the fish taken in freshwater as “solid at ordinary temperatures” (Hart, 1973). These proportions of high-melting fatty acids are in accord with those reported by Kuhnlein *et al.* (1996), using the alternative fish name “ooligan” and basically was a nutritional study of a traditional food fat of the British Columbian indigenous cultures. Neither it nor the similar work of Kuhnlein and Chan (1998) considered squalene, although it would be a normal content of the traditional “grease.” The role of squalene in this species may be to dilute and reduce the viscosity or
melting point of these triacylglycerols in the marine life phase in their cold ocean water life phase.

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