Separation of Pristane from Herring

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In earlier papers we have reported a method including gas liquid chromatography for the separation of glyceryl ethers in the unsaponifiable matter of shark oils [1,2]. We have later on been interested in that from herring oil. When separating the unsaponifiable material from herring on silicic acid, elution with light petroleum gave a clear oily fraction. This fraction was submitted to gas liquid chromatography and showed only one component. Separation on different stationary phases, gave shorter retention times relative to hexadecane on polyesters and polyglycols than on silicones. The shortest retention time was found on Carbowax and the longest one on a fluorosilicone fluid. On the non-polar silicone elastomer (SE-30) the retention time was something between these two and almost the same as normal heptadecane.

The mass spectrometric analysis * showed that the substance was a branched hydrocarbon. The relative intensity of the C_8 and C_{13} fragments in the mass spectrum, see Fig. 1, indicates two branches in the chain. The molecular weight was 268 thus giving the formula $C_{19}H_{40}$.

Because of its short retention time in gas chromatography the substance isolated must be a multi-branched hydrocarbon. Tsujimoto [3] reported a saturated hydrocarbon later named pristane [4] as a minor component in shark livers. Sörensen *et al.*[5,6] showed that this hydrocarbon had at least nineteen carbon atoms. Later Sörensen and Sörensen [7] were able to prove the identity between a synthesized hydrocarbon norphytane (2,6,10,14-tetramethyl pentadecane) and pristane. The physical constants showed good agreement. In a review of the composition of fish oils given by Swain [8] pristane was only reported to be a component in shark liver oils, unfortunately quoted as $C_{18}H_{38}$.

* The mass spectrometric analysis was kindly performed by Dr. R. Ryhage, Laboratory for Mass Spectrometry, Karolinska Institutet, Stockholm, Sweden.

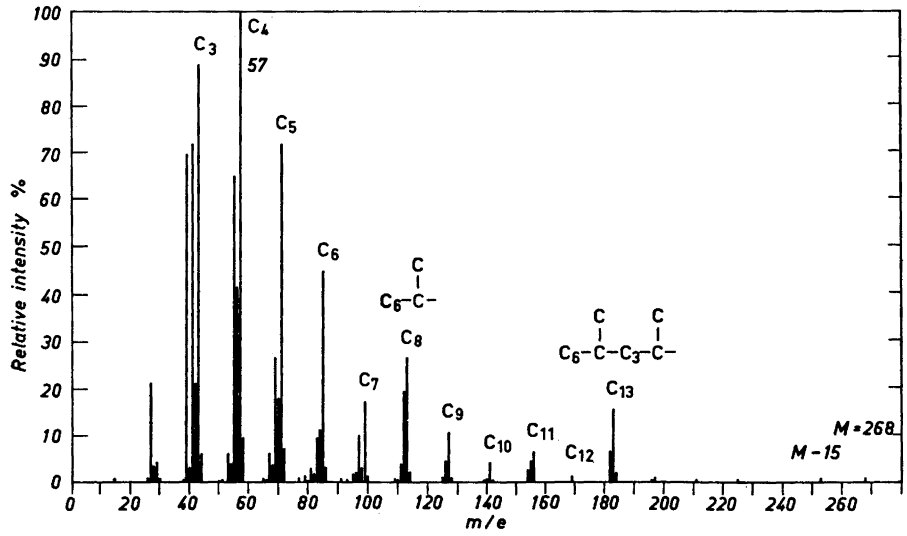

Fig. 1. Mass spectrum of hydrocarbon isolated from *Clupeus harengus*. M = 268

Table 1. Retention times relative to hexadecane.

	Fluoro-silicone	Carbowax 4000
Hexadecane	1.00	1.00
Hydrocarbon from *Clupeus*	1.57	1.24
Pristane	1.57	1.24
Octadecane	2.00	2.16

A comparison between pristane (L Light & Co. Ltd., Colnbrook, England) and the C_{19} hydrocarbon found in herring gave the same gas chromatographical data on two different phases, *viz.* fluorosilicone FS 1265 and Carbowax 4000. The retention times including those of hexadecane and octadecane are summarized in Table 1. Different stationary phases retarded pristane in the following order: Carbowax < polyester < methylsilicone < fluorosilicone. The commercial pristane and the hydrocarbon from herring gave identical mass spectra. The infrared spectrum of the pristane obtained from Light & Co. and that of the hydrocarbon from herring were identical and very similar to that of squalane.

Experimental. Material. Small specimens of herring *Clupeus harengus* were frozen at sea in solid carbon dioxide immediately after the capture. The fish was caught outside the coast of Halland. It was then taken still frozen to the laboratory and the whole fish was minced in a mincing-machine, an "Electrolux Assistent". The material was then lyophilized. An amount of 846 g lyophilized herring was extracted with 7000 ml of a mixture of chloroform-metanol 1:1 (v/v). After refluxing on a steam-bath for half an hour the mixture was filtered. The solvent was then evaporated on a water-bath not exceeding 60°. The yield was 251 g (29.7 %).

Saponification. 251 g of the lipid was boiled in 1250 ml 95 % ethanol and 84 g KOH (*pro analysi* 86/88 %) under reflux for 2 h. The mixture was then diluted with 625 ml distilled water and the unsaponifiable matter was extracted with 5 × 300 ml of light petroleum (b.p. 60–80°). The combined extract was first washed with 125 ml diluted KOH (0.5 N), then twice with 150 ml distilled water. The solvent was evaporated, the residue was resaponified in 100 ml ethanol and 5 g KOH under reflux for 2 h. The unsaponifiable material was extracted as above. The petroleum extract was dried over $MgSO_4$ and then filtered. The yield amounted to 1.79 g (0.7 %).

Chromatography on silicic acid. Silicic acid (Baker AR, Lot. No. 23043) with particles passing a 325 mesh sieve was used. The powder was washed with methanol and diethyl ether. The finest particles were removed by decanting the solvent after 5 min of settling. This procedure was repeated once with methanol and twice with ether. After drying in air, the silicic acid was finally activated at 120° for 24 h.[9] A 60 g column was packed in diethyl ether and then rinsed with 500 ml of light petroleum. About half of the unsaponifiable material, 880 mg, was applied to the column. The first eluant was light petroleum (b.p. 60–80°) in an amount of 700 ml. It contained 141 mg of a clear oily substance (hydrocarbon). Some higher fatty alcohols *viz.* eicosenol and docosenol were eluted with 8 % diethyl ether in light petroleum. The alcohols were separated by means of gas chromatography and identified by mass spectrometry. The main part of the unsaponifiable matter was eluted with 25 % diethyl ether in light petroleum and consisted of sterols mainly cholesterol.

Conditions for gas liquid chromatography I. Chromatograph: Perkin-Elmer Model 116 E equipped with flame ionization detector. *Column dimensions:* 300 × 0.6 cm o.d. aluminium tubing. *Solid support:* Johns-Manville Chromosorb W (60–80 U.S. mesh) washed with concentrated HCl and 5 % KOH in water and washed until neutrality[10]. Dried at 200° and treated with dimethyldichlorosilane. *Stationary phase:* Fluorosilicone fluid FS 1265, identical to QF-1-0065 pilot plant lot., Dow Corning (25 % by weight of the solid support). *Temperatures:* Injection: 275°. Detector and column: 175°. (All temperatures measured with Mercury thermometer.) *Carrier gas:* He at 75 ml/min Inlet pressure: 1.5 kp/cm². Outlet pressure: atmospheric. *Recorder:* 2 mV; 1/4 sec.; 0.5 cm/min. *Detector:* Flame ionization detector, one flame hydrogen detector, model Bodenseewerk. *Sample size:* 0.10 µl of a diluted solution in hexane. *Analysis time:* About 10 minutes to octadecane.

Conditions for gas liquid chromatography II. Chromatograph: See I. *Column dimensions:* 200 × 0.6 cm o.d. aluminium tubing. *Solid support:* See I, but not silane treated. *Stationary phase:* Carbowax 4000, a polyethylene glycol (25 % by weight of the solid support). *Temperatures:* Injection: 272°. Detector and column: 170°. *Carrier gas:* He at 55 ml/min. Inlet pressure: 1.0 kp/cm². Outlet pressure: atmospheric. *Recorder:* See I. *Detector:* See I. *Sample size:* 0.10 µl of a diluted solution in

hexane. *Analysis time:* About 7 minutes to octadecane.

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Alkali Stability of 2-*O*-(4-*O*-Methyl-α-D-Glucopyranosyluronic Acid)-D-Xylopyranose

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The alkaline degradation of 1,4-linked polysaccharides proceeds by a peeling process starting from the reducing end group. A substituent at the C-2 hydroxyl group will prevent the peeling process "because saccharides substituted in the C-2 hydroxyl group are unable to form the necessary carbonyl group in the position *beta* to the substituted hydroxyl group" [1]. If the substituent is split off the peeling can proceed and it will continue until a stable metasaccharinate ion, terminating the chain, is formed [2]. The average number of units peeled off in a cellulose chain has recently been estimated to be of the order of 70—90 [3].

The behaviour of a substituent at the C-2 hydroxyl is of special importance to the xylans in kraft pulping of hardwoods. Hardwood xylan consists of straight 1,4 β-linked xylose chains, substituted in every tenth to fifteenth unit by a 4-*O*-methyl-D-glucuronic acid residue in the C-2 hydroxyl group [4]. The substituent will prevent the peeling to proceed, when the reaction reaches it. On the basis of this fact, it has been postulated that as long as the xylan chain contains glucuronic acid groups, these groups will protect the xylan against peeling, disregarding the initial peeling reaction [5,6]. During the kraft cook most of the glucuronic acid residues are split off, but xylan isolated from birch kraft pulps still contains 3—5 % glucuronic acid [6]. If the glucuronic acid residues were randomly distributed and if they had the postulated preventing effect on the peeling, this type of stopping of the peeling reaction would be of considerable importance. The major part of the xylan chains would then be terminated by reducing xylose units substituted at the C-2 hydroxyl by glucuronic acid residues and the minor part would be terminated by xylometasaccharinic acid units.

The above reasoning presumes, however, that the rate of hydrolysis of a glucuronic acid residue attached to a reducing xylose end-group is the same as that of a glucuronic acid residue attached to a nonreducing xylose unit. Experimental evidence indicates that this is not the case. If xylan is exposed to alkali at 100°C for some hours, its percentage of glucuronic acid is not influenced, but the degree of polymerization is lowered and only 65—70 % of

Table 1. The alkali stability of 2-*O*-(4-*O*-methyl-α-D-glucurono)-D-xylose

Treatment, 4 % NaOH		Presence of aldobiouronic acid
Temperature °C	Time, h	
20	0.5	+++
40	64	++
70	8	+
100	1	0