observed during 39 days of the experiment appearence were carefully recorded. The results obtained are summarized in Table 1, indicating the average body weight changes per day and the content of free and bound ascorbic acids in the whole liver homogenates of the guinea pigs.

Table 1. Average body weight changes per day and content of free and bound ascorbic acids in the liver of guinea pigs fed on a scorbutic diet (group 1), ascorbic acid (group 2) and ascorbigen (groups 3 and 4).

Group	Average body weight change per day (g)	Ascorbic acid in liver (mg)	
		Free	Bound
1	−5.4	0.25	0.024
2	+3.0	1.60	0.110
3	+0.16	0.31	0.027
4	+3.0	0.71	0.076

As is shown in Table 1, the weight of the animals in the control group decreased continuously at an average rate of 5.4 g per day after the 12th day of the experiment. Typical symptoms of scurvy were clearly seen between the 14th and 16th days: lack of appetite, apathy, and sore joints. Four animals died on the 23rd day after feeding on the scorbutic diet. In group 3, the weight of the animals increased at an average rate of 0.16 g per day, but two of them showed clear symptoms of scurvy and died on the 24th and 29th days. Meanwhile in groups 2 and 4 no symptoms of scurvy could be observed in the animals, whose weight increased at the same rate of 3.0 g per day on an average. In parallel with the increase of the body weight, the contents of the free and bound forms of ascorbic acid in the whole liver homogenates increased, but more free and bound ascorbic acid accumulated in the animals fed on 2 mg ascorbic acid per day than in those fed on 21 mg of ascorbigen, as is shown in Table 1.

According to these results, guinea pigs fed on ascorbigen were able to utilize only 15 to 20 % of the ascorbic acid bound in ascorbigen as a source of vitamin C. This is in agreement with the findings of Kiesvaara and Virtanen.[3]

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A Gas Chromatographic Method for Determination of the Rancidity of Herring Oil

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As with other oils and fats, the rancidity of the oil of semi-sterile herring preserves is generally measured by determination of the peroxide number, carbonyl number, thiobarbituric acid (TBA) number, and so on. In the main, these numbers increase at the beginning of rancidity, but diminish again after some time. Consequently their application for evaluation of the quality of a mature product is limited.

A method has been published by Scholz and Ptak[1] for gas-chromatographic measurement of the rancidity of vegetable oils. They detected pentane in cottonseed oil, peanut oil, corn oil, and soybean oil, and further demonstrated the correlation of organoleptic quality and the content of pentane. It has been known for many years that pentane and other hydrocarbons are present in rancid oils. Buttery *et al.*[2] have suggested that hexanal is the source of saturated hydrocarbons C_1—C_5, and that their production is catalysed by fat peroxides.

Scholz and Ptak have found that pentane is always present in hexanal purified by distillation and preparative gas chromatography. The concentration of pentane increases on standing. They assumed that pentane was produced from hexanal by a photo-initiated reaction, described by Kerr and Trottman-Dickenson.[3] As heptanal is the aldehyde present in the highest amounts in rancid herring oil,[4] it was to be expected that hexane would prove to be one of the final products in rancid fish oil.

In the present experiment, use was made of herring from Northern Iceland. It was packed in glass jars under light vacuum and stored at +30°C. Analyses were made after 1, 3, 5, 7, and 10 days. The oil was separated by centrifugation, and the hydrocarbons were enriched in the apparatus illustrated in Fig. 1. 20 ml

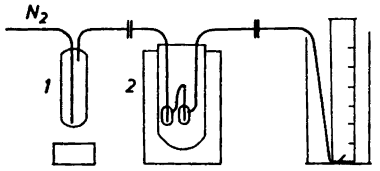

Fig. 1. Apparatus for the enrichment of volatiles in herring oil.

oil, *i.e.* about 17 g, was weighed in bottle 1. Trap 2 was cooled with liquid oxygen. The oil was flushed with two litres of nitrogen (for about 16 h), and after this procedure the trap was connected to a gas-chromatograph. For the removal of traces of air, helium carrier gas was passed through the trap for about 30 sec while it was still immersed in liquid oxygen. It was observed that the laboratory air caused disturbing peaks.

Analysis was effected with a gas chromatograph Fraktometer Model 116 Perkin Elmer, flame ionization detector and with helium as the carrier gas; the flow rate was 120 ml/min. The column was, like that of Scholz and Ptak, Carbowax 20 M, 15 % on Chromosorb P, 10 ft. The temperature of the column was 20°C. The trap was warmed to +30°C, and the sample was blown to the column with the carrier gas.

The chromatograms after storage for one day and for ten days are illustrated in Figs. 2 and 3. In Fig. 4 is a chromatogram of pure pentane, hexane, and acetone. Acetone was chosen as the internal standard owing to its short retention time at this temperature, and as it was eluted at a position which

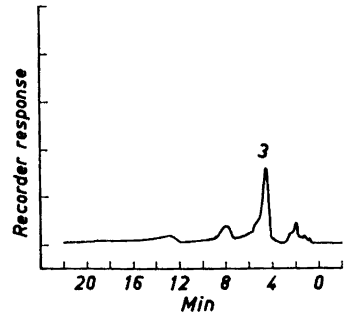

Fig. 2. Gas chromatogram of herring oil after storage for one day at +30°C; acetone as the internal standard (peak 3). During the first 3 min the attenuation is 16, thereafter 64.

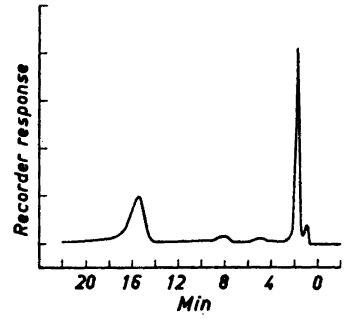

Fig. 3. Gas chromatogram of herring oil after storage for ten days at +30°C, without internal standard. Attenuation 64.

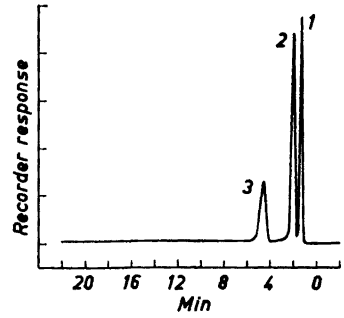

Fig. 4. Gas chromatogram of pure pentane, hexane, and acetone (peaks 1, 2, and 3, respectively).

ensured that no other components interfered (Figs. 1 and 2). One microlitre of acetone was added to 20 ml of oil. Comparable results were obtained by dividing the ratio of the areas of the assumed hexane peak and acetone peak by the weight of oil sample (kg).

In herring oil, a 7-day lag phase was observed before an appreciable increase was apparent in the value derived (Table 1). In ten days, the content of the component assumed to be hexane had increased 52 fold. The peroxide number returned to its initial level in 5 days. The assumed hexane peak greatly exceeds that of pentane. There was a slight indication of rancid odour in the oil after ten days.

Table 1. Peroxide number and ratio of areas of assumed hexane peak and internal standard (acetone) in herring oil during storage at +30°C.

Storage days	Peroxide number	Peak area ratio /kg of oil
1	8	2.5
3	2	4.4
5	0	2.1
7	0	3.8
10	0	130.0

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Chemical Studies on Lichens

9.* Chlorinated Anthraquinones from *Nephroma laevigatum*

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Five different anthraquinones have been isolated in very small amounts from the lichen *Nephroma laevigatum* Ach. non auct. nonn. (syn. *N. lusitanicum* Schaer.). Of these the pigment I has been identified as emodin (3-methyl-1,6,8-trihydroxyanthraquinone) and IV as fragilin [1] (7-chloro-1,8-dihydroxy-6-*O*-methyl-3-methylanthraquinone) by comparing their mass spectra and TLC with authentic specimens.

The remaining anthraquinones have tentatively been assigned the structures II, III, and V. The pigments I—III are soluble in sodium carbonate in contrast to IV and V, suggesting that not only I but also II and III have at least one hydroxyl in the β-position.

According to mass spectra and NMR spectra the pigments II and III might be derivatives of I containing chlorine at C-7. They have molecule peaks at $m/e = 304$ and $m/e = 318$, the relative intensities of M + 2 to M being 36 % and 46 %, respectively. They have the same base peak $m/e = 270$ which corresponds to M − 34 for II and M − 34 − 14 for III. Below these base peaks their spectra are very similar to that of emodin. The NMR spectra of II and III show the same signals as fragilin [1] due to aromatic protons indicating the presence of protons at C-2, C-4, and C-5. Consequently the chlorine in II and III ought to be in the same position as in fragilin.

When treated with diazomethane fragilin gave two isomers (M = 332) separable by TLC. One of these was obtained only in trace amount. The same isomers, in the same proportions, were also obtained from II after methylation. Their identities were proved by mass spectroscopy and TLC. The isomer obtained in trace amounts from fragilin and II was identified by mass spectroscopy and TLC as V which remained unchanged after

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