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 |

1. Scholz, R. G. and Ptak, L. R. *J. Am. Oil Chemists' Soc.* **43** (1966) 596.
2. Buttery, R. G., Hendel, C. E. and Boggs, M. M. *J. Agr. Food Chem.* **9** (1961) 245.
3. Kerr, J. A. and Trotman-Dickenson, A. F. *J. Chem. Soc.* **1960** 1602.
4. Nikkilä, O. E. and Linko, R. R. *Suomen Kemistilehti* **B 24** (1951) 74.

Received November 17, 1967.

# Chemical Studies on Lichens

## 9.* Chlorinated Anthraquinones from *Nephroma laevigatum*

GERD BENDZ, GERD BOHMAN and JOHAN SANTESSON

*The Institute of Chemistry, University of Uppsala, Uppsala, Sweden*

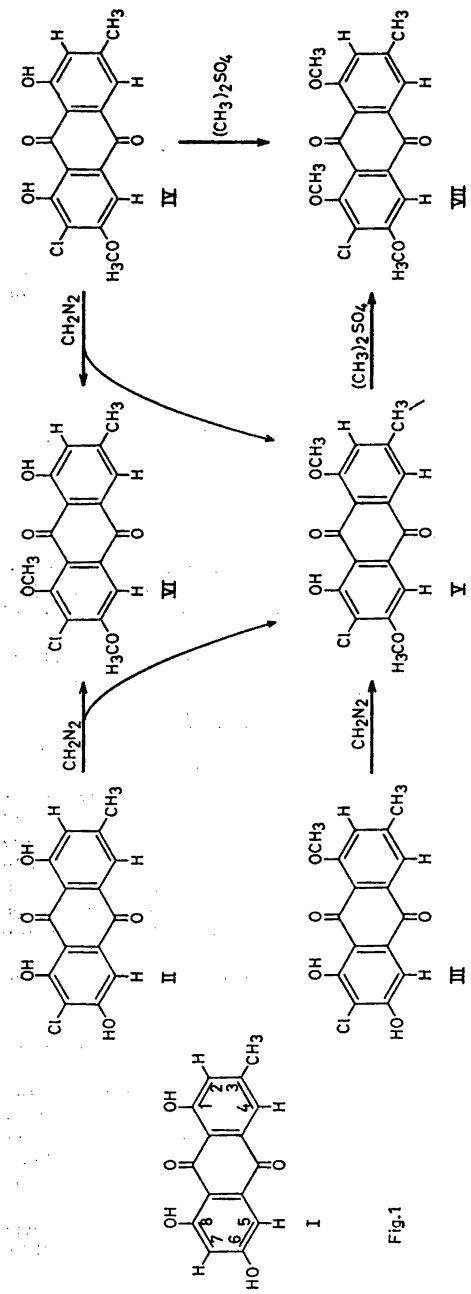
Five different anthraquinones have been isolated in very small amounts from the lichen *Nephroma laevigatum* Ach. non auct. nonn. (syn. *N. lusitanicum* Schaer.). Of these the pigment I has been identified as emodin (3-methyl-1,6,8-trihydroxyanthraquinone) and IV as fragilin [1] (7-chloro-1,8-dihydroxy-6-*O*-methyl-3-methylanthraquinone)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

* Part 8. *Acta Chem. Scand.* **21** (1967) 1111.




treatment with diazomethane. The sole methylation product of III was proved in the same way to be identical with V.

Since the hydroxy group at C-8 should be more reactive towards diazomethane than that at C-1, the main product isolated after methylation of fragilin ought to have structure VI and hence pigment II the assigned structure. Fragilin and pigment V after treatment with dimethyl sulphate yielded the same permethylation product (proved by mass spectroscopy and TLC) (7-chloro-3-methyl-1,6,8-tri-*O*-methylanthraquinone) (VII). Thus the interrelationship between pigments II—V is demonstrated (Fig. 1). Pigment II is 7-chloroemodin, pigment III is 7-chloro-1-*O*-methylemodin, and pigment V is 7-chloro-1,6-di-*O*-methylemodin. A fuller report will be published later.

*Experimental. Nephroma laevigatum*, (18 g) collected 20 km W Uppsala, Sweden, was dried and extracted with acetone. The sodium carbonate soluble part afforded I, II, and III, and the sodium hydroxide soluble part of the remaining extracted material gave IV and V.

I, II, and III were separated by column chromatography on silica gel (chloroform-acetone, 4:1 v/v) and purified by sublimation under vacuum, washed with petroleum ether and recrystallised from glacial acetic acid.

IV and V were separated by column chromatography on silica gel (toluene) and purified by sublimation and washed with petroleum ether.

Fragilin, was obtained from *Sphaerophorus fragilis*,[1] collected in the Varanger peninsula, Norway.

The diazomethane methylation was carried out in acetone-ether for 1 day at room temperature. Fragilin and V were refluxed with dimethyl sulphate and potassium carbonate in acetone for 20 h. The methylation products were purified by preparative TLC on silica gel (toluene or chloroform).

The analytical TLC was carried out on Eastman "Chromagram sheets" type K 301 R 2. NMR-spectra were obtained in hexadeuteroacetone (Varian A 60, equipped with a time averaging computer). Mass spectra were recorded on an LKB 9000.

*Acknowledgements.* Our thanks are due to Professor Arne Fredga for the facilities put at our disposal. We also want to express our gratitude to *Knut and Alice Wallenbergs Stiftelse* for a grant to the university allowing the purchase of the mass spectrometer.

1. Bruun, T., Hollis, D. and Ryhage, R. *Acta Chem. Scand.* **19** (1965) 839.

Received November 23, 1967.