

## The Constitution of Fragilin

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Based on the results of high resolution nuclear magnetic resonance and mass spectroscopy measurements fragilin has been given the constitution (I).

During his systematic investigations on lichen constituents Zopf also dealt with the lichens *Sphaerophorus fragilis* (L.) Ach. (= *S. fragilis* Pers.) Ref. 1, p. 340 and Ref. 2, p. 276, and *S. corraloides* Pers. (= *S. globosus* Vain.) Ref. 1, p. 344. He reported the presence of a very minor coloured constituent, which he named fragilin. On the basis of colour reactions, Ref. 1, p. 343, Zopf considered fragilin to be an anthracene derivative, but reported no m.p. or analysis of the substance or any crystallographic data.

Asahina and Hashimoto<sup>3</sup> extracted *S. melanocarpus* DC (= *S. compressus* Ach.) in order to obtain sphaerophorin, which is the main constituent also of the two lichens mentioned above. They encountered minute amounts of a coloured substance sparingly soluble in benzene, which they thought might be fragilin, but could not isolate it in a pure form for lack of material.

For other purposes one of us (T. B.) during the course of some years collected large amounts of *S. globosus* and *S. fragilis*. Thanks to the fact that fragilin could be extracted from an ether solution with sodium hydroxide but not with sodium carbonate, and to its very low solubility in most of the

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common organic solvents, it was possible to obtain sufficient material for an investigation of the substance by taking advantage of the possibilities for micro scale work that mass spectroscopy and high resolution nuclear magnetic resonance spectroscopy now offer.

Our pure samples of fragilin were found to have m.p. 267–268°. Thin layer chromatography on silica gel impregnated with oxalic acid,<sup>4</sup> with benzene-chloroform 1:1 or benzene or benzene-petroleum, b.r. 60–70°, as solvents showed only one spot with sulphuric acid or potassium hydroxide as spraying reagent. Gas chromatographic check could not be carried out owing to the low solubility of fragilin in organic solvents. However, chlorine analysis gave a result too low for the formula finally arrived at (compare below) and the mass spectrum indicated the presence of a small, but indeterminable amount of impurities of molecular weight higher than that of fragilin. The ultraviolet spectrum in chloroform exhibited maxima at 2715, 3125, and 4345 Å, and the infrared spectrum in potassium bromide had bands in the carbonyl region at 1680 and 1630  $\text{cm}^{-1}$ , whilst the fully methoxylated compound showed only one band, at 1680  $\text{cm}^{-1}$ . The merging of the two bands indicates OH groups only in the 1 and/or 8 positions of the anthraquinone nucleus according to the findings of Bloom *et al.*<sup>5</sup> for hydroxyanthraquinones, and supports the contention of Zopf that fragilin would be an anthracene (*i.e.* anthraquinone) derivative.

The NMR spectrum of fragilin is shown in Fig. 1.

The peaks at 11.86 and 12.70  $\delta$  which correspond to one proton each, are readily caused to disappear by addition of  $\text{D}_2\text{O}$ . They can therefore be assigned to two non-equivalent OH groups, which, judging from their chemical shifts, are quite strongly hydrogen bonded. Both OH groups must therefore be in positions 1 and 8, *peri* to a carbonyl group in agreement with the IR data. The two strong, sharp peaks at 2.42 and 4.08  $\delta$ , which correspond to three protons

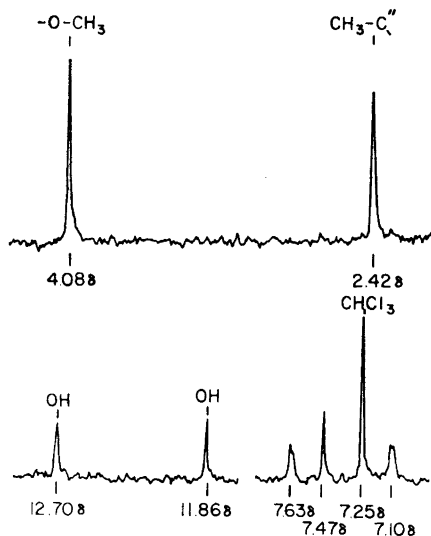


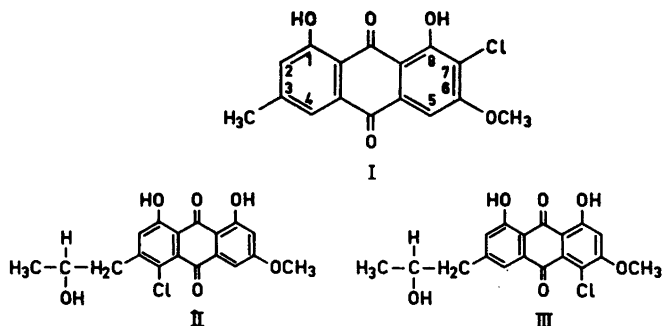
Fig. 1. 100 Mc/sec NMR spectrum of fragilin in  $\text{CDCl}_3$  solution. Temperature: 80°C. Chemical shifts are in ppm ( $\delta$ ) from internal tetramethylsilane.

each, are assigned to a methyl group and a methoxyl group, respectively, on an aromatic ring. The peaks at 7.63, 7.47, and 7.10  $\delta$  account for one proton each, so that only seven of the eight positions on the anthraquinone ring system are accounted for by the NMR spectrum. As will be shown below, the remaining substituent is chlorine. The resonances at 7.10 and 7.63  $\delta$  show a small splitting ( $\sim 1$  cps) which is consistent with a *meta* arrangement of the two protons as in the spectrum of 1,3-dimethyl-9,10-anthraquinone.<sup>6</sup> The remaining sharp peak at 7.47  $\delta$  represents the aromatic proton on the second ring.

The methyl group is placed at position 3 since its chemical shift is very close to that of the 3-methyl in 1,3-dimethylanthraquinone.<sup>6</sup> The methyl is probably not in the 2-position, *ortho* to the OH, since this would be expected to cause an upfield shift of at least 0.1 ppm (compare spectra 157 and 340, Ref. 6). The chemical shift of the methyl group also constitutes evidence that neither Cl nor OCH<sub>3</sub> groups occupy positions 2 or 4, since these would be expected to alter the CH<sub>3</sub> shift. Further evidence that protons occupy positions 2 and 4 is the fact that the observed chemical shifts of the two coupled aromatic protons can be calculated using equations and additive constants given by Martin and Dailey<sup>7</sup> for the *meta* and *para* disubstituted benzenes. The calculated values are 7.08 and 7.64  $\delta$  for the 2 and 4 positions respectively, again using 1,3-dimethyl-9,10-anthraquinone as the model compound. The observed values are 7.10 and 7.63  $\delta$ . Although the work of Martin and Dailey concerned only substituted benzenes, it is reasonable to expect it to apply also to the anthraquinones. This expectation was tested for the case of 1,3-dimethyl-9,10-antraquinone where the calculated values for the 2 and 4 protons are 7.37 and 7.89  $\delta$ , as compared with observed values of 7.33 and 7.99  $\delta$ .

There remains the problem of arranging the Cl, OCH<sub>3</sub>, and a proton in the 5, 6, and 7 positions. If it is assumed on the biogenetic grounds referred to below that the OCH<sub>3</sub> group is in position 6, then the proton can be assigned to position 5, because the combined effects of the substituents is expected to produce an approximate 1 ppm upfield shift, relative to the corresponding protons in 1,3-dimethyl-9,10-anthraquinone, whether the proton is in the 5 or 7 position. This would lead to a shift of about 6.7  $\delta$  in position 7 and about 7.2  $\delta$  in position 5, the latter being much closer to the observed value of 7.47  $\delta$ . Also consistent with this assignment is the fact that the OH resonance appearing at lower field (12.70  $\delta$ ) is considerably broader than the one at 11.86  $\delta$ . This could be due to slight coupling of the OH proton to the Cl to which it would be hydrogen bonded or to an exchange of the proton between environments in which it is alternately hydrogen bonded to oxygen and the chlorine. The lower field shift of this proton would then be due to its greater opportunity for hydrogen bonding.

The three strongest peaks of the mass spectrum of fragilin are the base peak at *m/e* 284 and the peaks at *m/e* 318 and 320. The intensity of *m/e* 320 relative to *m/e* 318 is 37 %. In the mass spectrum of fragilin there are two other pairs of mass peaks with a similar relationship, namely *m/e* 277 which is 32 % of 275, and 249 which is 34 % of 247. These pairs may be taken to indicate that fragilin contains chlorine, which occurs naturally as <sup>35</sup>Cl and <sup>37</sup>Cl,



the latter in an amount about 32 % of the former. The presence of chlorine in the sample was confirmed by a positive Beilstein test and by analysis.

The data given above together with biogenetic considerations for the formation of anthraquinone derivatives as set out by Birch<sup>8,9</sup> combine to establish the structure of fragilin as (I).

It will be seen that the suggested formula for fragilin is that of a chlorinated physcion. Physcion has a molecular weight of 284, which is the base peak of fragilin. It occurred to us that a confirmation of the suggested formula in a general way could be obtained by a comparison with the mass spectrum of physcion. As will be seen from Figs. 2 and 3 the mass spectrum of fragilin below  $m/e$  285 is very similar to that of physcion.

The pairs  $m/e$  277—275 and 249—247 deserve some comment. The former corresponds to loss of mass 43, the latter of mass 71. Beynon has studied the mass spectra of anthraquinone and of phenols and has found that CO and COH appear to be readily lost from phenols, whilst the loss of CO appears to be characteristic of anthraquinones.<sup>10</sup> The observed loss of masses 43 and 71 is analogously interpreted as loss of  $\text{COCH}_3$  and of  $\text{CO} + \text{COCH}_3$ , respectively.

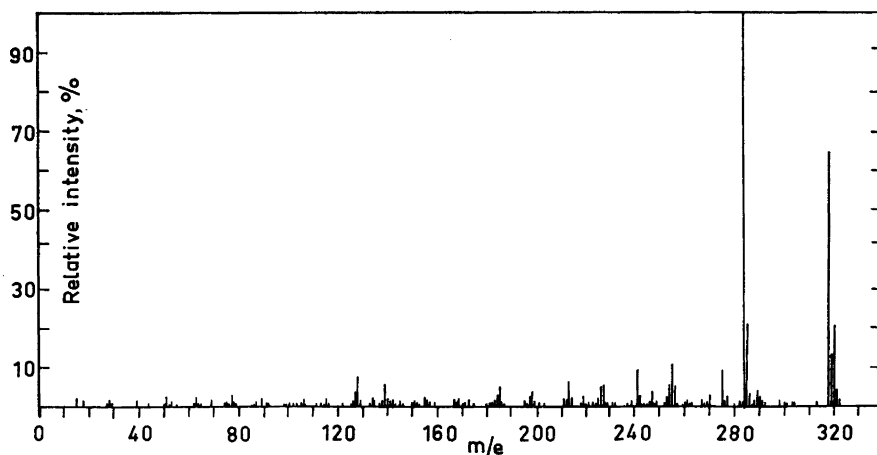


Fig. 2. Mass spectrum of fragilin.

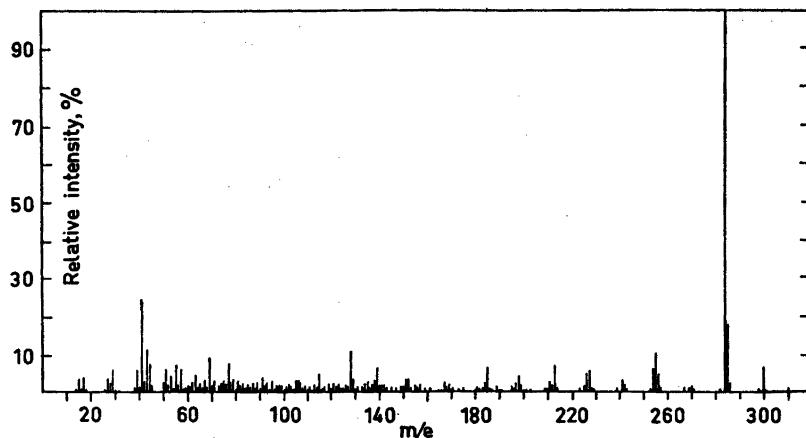


Fig. 3. Mass spectrum of physcion.

We believe this interpretation to be supported by the findings of Barnes and Occolowitz.<sup>11</sup> These authors studied the mass spectra of several methyl phenyl ethers. They did not discuss the loss of mass 43 from their compounds, but from their published data can be seen that it occurs frequently. A study of the mass spectrum of physcion reveals that loss of CO or COH or COCH<sub>3</sub> or a combination of them does occur.

As far as is known to us, no anthraquinone of established formula with halogen in the nucleus has been found previously in nature. For the chlorine containing anthraquinone derivative nalgiolaxin (isolated from *Penicillium nalgiovensise* Laxa) only alternative structures have been given, (II) and (III).<sup>12</sup>

#### EXPERIMENTAL

Infrared spectra were measured with a Perkin-Elmer Model 21 spectrophotometer in KBr pellets. Ultraviolet spectra were measured with a Perkin-Elmer Model 13 spectrophotometer. M.p.'s are not corrected. Nuclear magnetic resonance spectra were obtained using the Varian A-60 and the HR 100 spectrometers with associated variable temperature probes. A 180° mass spectrometer was used and 0.5 mg of material was introduced into an all glass heated inlet system. The temperature of the inlet system was kept at 180°C and the energy of the bombarding electrons at 70 eV.

The lichen material consisted of *Sphaerophorus globosus* Vain. collected in Trondheim Bymark or at Kongsmoen, some 200 km north of Trondheim, and of *S. fragilis* Pers. collected in the Oppdal region, 120 km south of Trondheim. The various collections were extracted with ether for 24 h. The ether extracts were concentrated and extracted successively with sodium hydrogen carbonate, sodium carbonate and sodium hydroxide. The organic material in the sodium hydroxide extract was liberated by addition of dilute sulphuric acid and extracted with ether. Removal of the ether left an oil which gradually solidified, when coloured crystals could be seen in an otherwise colourless paste. The coloured crystals could be isolated by treatment with ether or (less successfully) with chloroform, and purified by fractionated sublimation in a vacuum and/or crystallisation from chloroform, m.p. 267–268°. (Found: C 60.0, 60.1; H 4.3, 3.5; Cl 9.2; OCH<sub>3</sub> 9.7; C—CH<sub>3</sub> 10.5. C<sub>16</sub>H<sub>11</sub>ClO<sub>5</sub> (318.72) requires: C 60.3; H 3.5; Cl 11.1; OCH<sub>3</sub> 9.7; C—CH<sub>3</sub> 4.7).

Light absorption maxima in chloroform at 2715, 3125, and 4345 Å,  $\epsilon$  36 500, 14 000, and 15 000, respectively, minima at 2415, 3000, and 3350 Å,  $\epsilon$  12 500, 12 500, and 1800, respectively.

Methylation of fragilin with dimethyl sulphate and potassium carbonate in acetone furnished the *methoxy derivative*, m.p. 208–209°, fine yellow needles from chloroform-methanol; light absorption maxima in ethanol at 2730 and 3845 Å,  $\epsilon$  41 500 and 5500, respectively, minima at 2390 and 3155 Å,  $\epsilon$  9000 and 2100, respectively. (Found: C 63.3; H 4.6; OCH<sub>3</sub> 26.8. C<sub>18</sub>H<sub>15</sub>ClO<sub>5</sub> (346.77) requires: C 62.4; H 4.4; OCH<sub>3</sub> 26.9).

Acetylation with acetic anhydride in pyridine with standing overnight at room temperature afforded the *acetate*, m.p. 234–235°, yellow needles from acetone; light absorption maxima in ethanol at 2750 and 3430 Å,  $\epsilon$  29 500 and 3700, respectively, minima at 2335 and 3110 Å,  $\epsilon$  7000 and 2400, respectively. (Found: C 60.0, 59.6; H 2.5, 2.8; OCH<sub>3</sub> 6.8. C<sub>20</sub>H<sub>15</sub>ClO<sub>7</sub> (402.79) requires: C 59.6; H 3.8; OCH<sub>3</sub> 7.7).

*Acknowledgements.* T.B. likes to thank Professor D. H. R. Barton for the opportunity to have the majority of the analyses carried out at the University of Glasgow, Glasgow, Scotland, and at Imperial College of Science and Technology, London, England, Professor P. de May, University of Western Ontario, London, Ontario, Canada, for the chlorine analysis, Professor N. A. Sørensen for his interest in the work and *Norges Almenvitenskapelige Forskningsråd* for a fellowship held during the initial stages of the work.

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Received February 23, 1965.