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Haemoventosin

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Haemoventosin, the colouring matter of *Haematomma ventosum*, is given structure as a dihydrofuronaphthoqvinone.

Haematomma ventosum (L.) Mass. is a common, crustose lichen in Norway. The apothecia are prominently coloured, and Culberson states that the colouring matter is acetone soluble.[1] The presence of a colouring substance has been found independently by us and by Dr. C. A. Wachtmeister, Stockholm, Sweden, and by mutual agreement an investigation of its constitution has been undertaken in this Laboratory.

The colouring matter, for which the name haemoventosin is proposed, was obtained in the crystalline state. The IR and UV spectra indicated a quinonoid structure, and this was confirmed by reductive acetylation, which afforded a tri- and a tetra-acetate.

The NMR spectrum in trifluoroacetic acid at 60 or 100 MHz showed the signals given in Fig. 1.

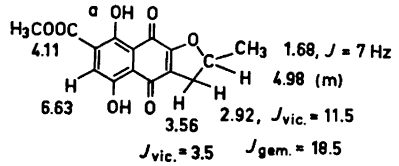
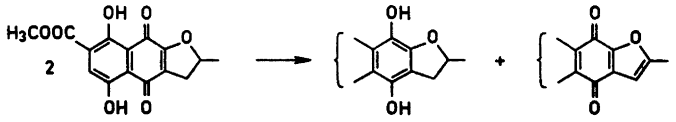


Fig. 1.

The mass spectrum was puzzling, in that it apparently contained three molecular peaks of about equal intensity. The presence of elements other than C, H, and O was ruled out by qualitative elemental analysis. The three peaks were found at *m/e* 302, 304, and 306, by accurate mass measurements shown to correspond to $C_{15}H_{10}O_7$, $C_{15}H_{12}O_7$, and $C_{15}H_{14}O_7$, respectively. Base peak was *m/e* 304. A similar pattern was found in the mass spectra of the acetate and the acid obtained by alkaline hydrolysis, but not in the acetates arising by reductive acetylation. The feature is thought to be due to a hydrogen disproportionation (red-ox reaction) as shown by the following reaction scheme.



The main fragmentation was the loss of C_2H_4O from the molecular ion *m/e* 304 (*m** 222.5, calc. 222.38). It is present also in the acetate and in the acid, but not in the acetates obtained by reductive acetylation. Haemoventosin appeared to give a negative Gibbs test, but observation was made difficult by the intense colour of the substance. However, it unmistakably gave a blue colour as described by Eisenlohr for alkannin in the presence of magnesium ions.[2] This colour reaction is apparently connected with the naphthazarin oxygen pattern.

With acetic anhydride in pyridine at room temperature, haemoventosin afforded an acetate, which by NMR and mass spectral data appeared to be a mono-acetate. The NMR spectrum in deuterochloroform contained, in addition to signals corresponding to those found for haemoventosin in trifluoroacetic acid (above), a three proton singlet at δ 2.40 ($CO-CH_3$). Furthermore, a one proton singlet at 12.4 indicated a strongly hydrogen bonded hydroxyl group.

That the signal at δ 4.11 indicated a methyl ester grouping, was shown by the solvent induced shift in the acetate ($\Delta = \delta_{CDCl_3} - \delta_{benzene} = +0.72$ ppm),[3] and by saponification to the corresponding acid. The acetate gave an unmistakably negative Gibbs test.

The available evidence suggests the constitution given in Fig. 1 for haemoventosin. The only uncertainty concerns the position of the ester group. The one indicated is chosen because haemoventosin may then be thought of as constructed by the condensation of two acetate chains as indicated in Fig. 2,

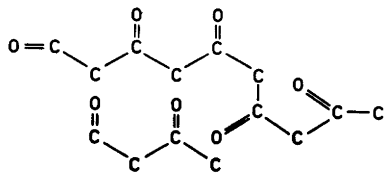


Fig. 2.

whilst an origin from one acetate chain followed by introduction of the carboxyl carbon would require a *meta* dihydroxy pattern. A subsequent oxidation to give the double *para* oxygen pattern appears trivial.

EXPERIMENTAL

IR spectra were obtained in potassium bromide discs with a Perkin-Elmer Model 257 spectrometer. UV spectra were recorded in ethanol solution on a Coleman-Hitachi Model 124 spectrometer. NMR spectra were run on a Varian A-60A or HA 100 instrument. Mass spectra were obtained with an AEI MS 902 instrument. Thin-layer chromatograms were run on Kieselgel G nach Stahl, impregnated with oxalic acid.[4]

Haematomma ventosum (1.8 kg), collected in the Oppdal region, was extracted with ether for one week in a Soxhlet apparatus. The ether coming off the extractor was then nearly colourless. The combined extracts were filtered to leave 60 g of solid material which was extracted with boiling chloroform. The reddish-brown material obtained with chloroform was redissolved in boiling benzene, and on cooling usnic acid precipitated. The filtered benzene solution was concentrated to give more usnic acid. When this procedure had been repeated two or three times, the cold, concentrated solution, together with the precipitated mixture of crystals, was heated to boiling and filtered whilst hot. In this way, dark crystals were obtained on the filter, whilst a precipitate of usnic acid formed on cooling. The process was repeated to give several crops of the dark, reddish-brown crystals, which melted at about 180° with decomposition. On the basis of identical IR spectra, several crops were combined to give a main fraction of 1.87 g and a minor one of 0.4 g. Crystallisation furnished fractions with m.p. ranging from 184° to 196°, all with decomposition. The total amount isolated was about 2 g of a presumed pure *material* shimmering like bronze and of rhombohedral shape. The IR spectrum contained bands at 1725, 1662, 1610, and 1570 (sh) cm^{-1}. The UV spectrum had λ_{max} 5050, 2940, and 2350 Å, ε 8000, 9500, and 27 000, respectively, λ_{min} 3450 and 2650 Å, ε 400 and 5500, respectively. Mass spectrum: 306 (94 %), 304 (100), 302 (52), 260 (88).

Acetylation afforded an acetate, m.p. 193 – 194° (dec.), yellow, felted needles from acetone. The IR spectrum had bands at 1770, 1725, 1650, and 1613 cm^{-1}. The UV spectrum showed λ_{max} 4200 and 2760 Å, ε 5500 and 10 200, respectively, λ_{min} 3255 and 2675 Å, ε 1100 and 9900, respectively. Mass spectrum: 348 (22 %), 346 (13), 344 (4), 306 (100), 304 (96), 302 (82), 260 (30).

Reductive acetylation of haemoventosin. Haemoventosin (58 mg) was treated with acetic anhydride, zinc dust, and anhydrous sodium acetate as directed by Fieser.[5] The substance dissolved gradually, and the colour changed from red *via* green to light yellow. The product was isolated with ether, and the last traces of acetic acid were removed by azeotropic distillation with benzene in a vacuum. The reaction product afforded yellow crystals, m.p. 227 – 228°, with chloroform-ether, and on concentrating the mother liquor, another crop of crystals was obtained, m.p. 232 – 237°. A mixture of the two melted at 214 – 220°. On thin-layer chromatograms the two crystallisates with chloroform-ethyl acetate (9:1) showed two spots of different intensity in the two samples. The spots could be seen under the UV lamp or could be made visible with the anisaldehyde reagent (compare Ref. 4). The lower melting acetate was the faster running and was isolated by preparative thin-layer chromatography followed by crystallisation from chloroform-ether, m.p. 228 – 229°. It showed only one spot on the thin-layer chromatogram. The IR spectrum contained broad bands at 1768, 1667, and 1630 cm^{-1} and a shoulder at 1615 cm^{-1}. The UV spectrum had λ_{max} 3620 and 2670 Å, ε 4800 and 32 000, respectively, λ_{min} 2850 Å, ε 1300. Mass spectrum: M^+ (19 %) 432.1060, calc. 432. 1056 for $C_{21}H_{20}O_{10}$; 390.0958 (14), calc. 390.0951 for $C_{19}H_{18}O_9$; 348.0853 (32), calc. 348.0845 for $C_{17}H_{16}O_8$; 306.0746 (100), calc. 306.0739 for $C_{15}H_{14}O_7$; 287.0569 (8), calc. 287.0556 for $C_{15}H_{11}O_6$. *m** 352.2, 310.5, and 269, calc. 352.17, 310.60, and 269.13, respectively, corresponding to three successive losses of ketene from acetyl groups. The NMR spectrum in deuterochloroform agreed with the presence of three acetyl groups.

The slower moving substance could not be obtained in a pure state. Thin-layer chromatograms, even of samples with the sharp m.p. of 239 – 240°, contained a not negligible, faster running spot, which by thin-layer test was identical with the faster running acetate (above). The acetyl group corresponding to hydroxyl *a* in Fig. 1 is considered to be subject to slow, but facile hydrolysis due to its cramped position. The IR spectrum had peaks at 1765 and 1625 cm^{-1} with a broad, diffuse absorption in the region 1720 – 1680 cm^{-1}. The UV spectrum had λ_{max} 3300 and 2610 Å, ε 640 and 4000, respectively; λ_{min} 2850 Å, ε 290. Mass spectrum: M^+ 474.1159 (3 %), calc. 474.1162 for $C_{23}H_{22}O_{11}$; *m** 394, calc. 393.82 for loss of ketene from the first acetate group. Other peaks: 432 (13), 390 (13), 348 (28), 306 (100), 287 (10).

Alkaline hydrolysis of haemoventosin. Haemoventosin (21 mg) was refluxed for 1/2 h with a 10 % solution of sodium hydroxide in water. The resulting acid was isolated with ether. It gave one spot on thin-layer chromatograms (chloroform-ethyl acetate 9:1). The m.p. was difficult to observe, but appeared to occur at about 200°. The NMR spectrum in trifluoroacetic acid contained no methoxyl signal. The IR spectrum had a broad band centered around 1710 cm^{-1}; UV spectrum: λ_{max} 5050 and 2750 Å, ε 6500 and 21 000,

respectively; λ_{min} 3550 Å, ε 800. Mass spectrum: 292 (8 %), 290.0429 (M^+, 100), calc. 290.0427 for $C_{14}H_{10}O_7$, 288 (10), 273 (11), 261 (10), 246.0165 (79), calc. 246.0164 for $C_{12}H_6O_6$, m^* 209, calc. 208.67 for loss of C_2H_4O from M^+.

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