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Fungus Pigments

II. * Cortisalin, a New Polyethenoid Pigment

JARL GRIPENBERG

Chemistry Department, Finland Institute of Technology, Helsingfors, Finland

A few years ago Erdtman [1] reported the isolation of a new pigment, named corticrocin, from the "yellow mycorrhiza" the fungal component of which is considered to be the mycelium of *Corticium croceum* Bres. (= *C. sulphureum* (Fr.) Fr.). Erdtman also showed that corticrocin is dodecahexaene-(1,3,5,7,9, 11)-dicarboxylic acid-(1,12)(VII) [1].

In his paper, Erdtman pointed out that it would be of considerable interest to see if other *Corticium* species, several of which are coloured, also contain corticrocin or related polyethenoid pigments, since this would strengthen the opinion that the fungal component of the "yellow mycorrhiza" is, indeed, *Corticium croceum*. He has recently been able to isolate corticrocin from a sporophore which according to Professor E. Melin, Uppsala, was an immature sporophore of *Corticium croceum* [2]. The mycelium from this sporophore was obviously connected with the "yellow mycorrhiza" the other component of which in this case was *Vaccinium vitis ideae*.

An investigation of the pigment of *Corticium salicinum* Fries has now been carried out. This fungus grows on dead branches of *Salix* species, particularly in wet places such as marshes and the shores of lakes. The red sporophore is rather small, being seldom more than a few centimeters wide and 1—2 millimeters thick. Although the fungus is rather common, much work is required to collect appreciable quantities.

From this fungus we have isolated a crystalline pigment for which we propose the name cortisalin. It is thus in this case the sporophore, not the mycelium as in the case of *Corticium croceum*, which has given the pigment. The reddish mycelium is less readily accessible and has not been investigated, but undoubtedly it contains the same pigment.

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Cortisalin can be obtained from the fungus by extraction with acetone. The extraction of the dried fungus proceeds very slowly, but the pigment is more rapidly extracted from the fresh material. It was therefore found advantageous to add water to the powdered fungus from time to time during the extraction. The addition of water causes a swelling of the fungus material and this appears to facilitate in some way the removal of the pigment. That this is not due to a higher solubility of the pigment in aqueous acetone follows from the fact that the pure pigment is extremely difficultly soluble in acetone and quite insoluble in water.

The pigment precipitates from the acetone extract as a brown powder and after purification, as described in the experimental section, it is obtained as beautiful violet-red needles. The yield has been somewhat varying in different batches of the fungus, the best being 0.6 per cent. A total of about 2.5 g of crystalline cortisalin has been available for this investigation.

Cortisalin does not melt but decomposes gradually above 290°. It is extremely insoluble in all common organic solvents except pyridine, in which it is rather easily soluble particularly on warming. This low solubility has made the work with cortisalin very difficult and time consuming. Cortisalin dissolves in conc. sulphuric acid, giving a very intense indigo-blue solution. When crystals of cortisalin are suspended in dilute sodium hydroxide they immediately turn black, but do not dissolve.

Diazomethane converts cortisalin very slowly into a red methyl derivative, m. p. 255—256° (dec.), which is somewhat more soluble in chloroform but less soluble in pyridine than is cortisalin. This methylcortisalin can be sublimed in a high-vacuum, but with great loss of material. With acetic anhydride cortisalin is converted into an acetate, m. p. 280—282°(dec.).

It has been very difficult to obtain consistent analyses for cortisalin and its derivatives and it appears doubtful if the pigment has ever been obtained in a state of complete purity. Similar difficulties were encountered in the case of corticrocin. What appeared to be the most reliable of these analyses indicated a formula $C_{24}H_{24}O_4$ for cortisalin, and $C_{26}H_{28}O_4$ with two methoxyl groups for the methyl derivative [3]. Cortisalin itself contains no methoxyl groups. As will be shown below, this formulation of cortisalin can no longer be upheld.

When dimethylcortisalin is hydrolysed with alcoholic potassium hydroxide it is slowly converted into an insoluble, dark-yellow, substance. This is evidently a potassium salt, and upon trituration with dilute sulphuric acid it is converted into a dark-red compound, m. p. 280—282°(dec.). This has only about half of the methoxyl content of the original dimethylcortisalin, and this fact points to the presence of a carboxyl group in cortisalin.

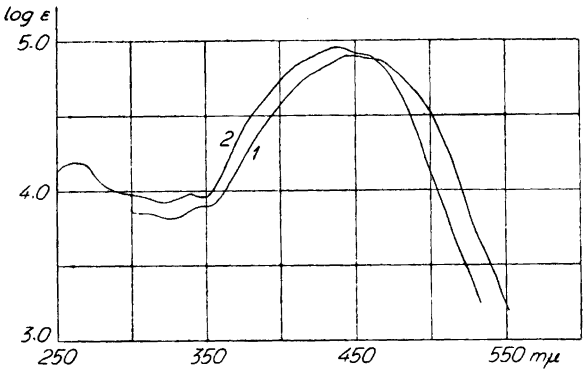
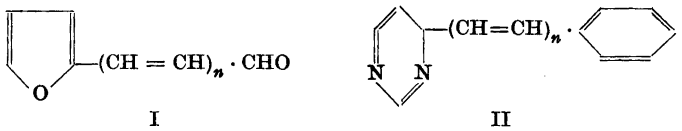


Fig. 1. Absorption spectra of: 1. cortisalin (in pyridine) and 2. dimethylcortisalin (in chloroform).

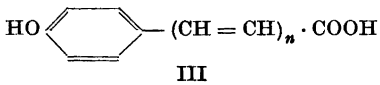
The nature of the second methoxyl group was shown by oxidation of dimethylcortisalin with potassium permanganate in acetone solution, to anisic acid. This shows the presence in cortisalin of a hydroxylated benzene ring connected in the *p*-position to the rest of the molecule.

The absorption spectra of cortisalin and dimethylcortisalin (Fig. 1) bear a striking resemblance to the spectra of polyethenoid compounds. Although the well developed three-peaked maximum of, for example, corticrocin [1] is lacking in these spectra, they resemble the spectra of unsymmetrically substituted polyenes such as (I) [4] and (II) [5].



I

II



III

A similar unsymmetrically substituted polyene structure appears therefore very probable for cortisalin. Taking into account the presence of a carboxyl group and a *p*-hydroxyphenyl group cortisalin might well correspond to the general formula (III). From the position of the maximum it can be judged that n is either 6 or 7 but no definite decision can be made between these two alternatives.

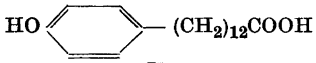
If this view is correct, cortisalin should correspond to either $C_{19}H_{18}O_3$ or $C_{21}H_{20}O_3$, and dimethylcortisalin to $C_{21}H_{22}O_3$ or $C_{23}H_{24}O_3$. The analytical

data are however in poor agreement with either of these formulations. In particular, the methoxyl values of dimethylcortisalin are much too low to correspond with either $C_{21}H_{22}O_3$ or $C_{23}H_{24}O_3$.

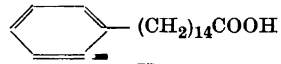
Upon catalytic hydrogenation in ethanol suspension cortisalin is converted into a colourless crystalline compound. This hydrogenation is extremely slow, requiring several days for the hydrogenation of 0.2 g of cortisalin, due to the extremely low solubility of cortisalin. It has therefore not been possible to determine the exact amount of hydrogen consumed in the hydrogenation. The hydrogenation product which is obtained only in moderate yield has m. p. 98—100° and its U. V. absorption spectrum shows maxima at 225 mμ (log $\varepsilon = 3.6$) and 279 mμ (log $\varepsilon = 3.1$). In alkaline solution, these maxima are displaced to 242 mμ (log $\varepsilon = 3.75$) and 297 mμ (log $\varepsilon = 3.2$). This behaviour is almost identical with that of *p*-cresol with maxima at 224 mμ (log $\varepsilon = 3.8$) and 280 mμ (log $\varepsilon = 3.3$) [6]. The displacement of the maxima in alkaline solution is also typical of phenols [7]. The presence of a phenolic group in cortisalin is thus confirmed.

Upon treatment with diazomethane, hydrocortisalin is converted into a methyl ester. That the phenolic hydroxyl group has not entered into reaction is shown by the fact that alkaline hydrolysis regenerates hydrocortisalin, and by the identity of the U. V. absorption spectrum with that of hydrocortisalin in both neutral and alkaline solution. The formation of an ester thus confirms the presence of a carboxyl group in cortisalin. Hydrocortisalin gives a sodium salt that is very sparingly soluble in cold water, and dissolves in warm water giving a soap-like solution. It is thus reminiscent of the long chain fatty acids.

If the above mentioned assumption regarding the structure of cortisalin is correct, hydrocortisalin would be either (IV) or (V)



IV



V

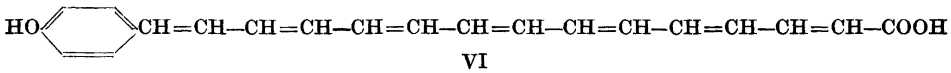
The analytical figures do not however allow any definite decision between these two possibilities.

In view of the indecisive analytical data on all the above-mentioned derivatives of cortisalin, it was decided to synthesise the two hitherto unreported acids (IV) and (V) and compare them with hydrocortisalin.

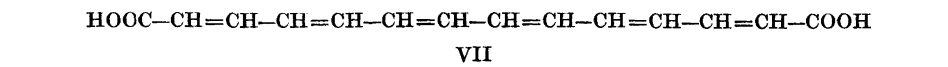
The syntheses of these acids are described in the following paper [8]. 13-*p*-hydroxyphenyltridecanoic acid (IV) has m. p. 94—96° and its methyl ester m. p. 71—72°. Both give definite depressions of m. p. when mixed with hydrocortisalin and its methyl ester respectively. On the other hand 15-*p*-hydroxyphenylpentadecanoic acid (V) has m. p. 99—100° undepressed in admixture

with hydrocortisalin. The identity was further confirmed by comparison of the x-ray diffraction patterns *.

Hydrocortisalin has therefore the structure (V) and there is little doubt that cortisalin must be represented by the structure (VI) in spite of the poor agreement of the analytical data with the theoretical.



VI



VII

Regarding the configuration of the double bonds, nothing definitive can be said, but it seems not unreasonable to expect an all-*trans* arrangement (compare corticrocin [1]).

Cortisalin (VI) thus differs from corticrocin (VII) in having two more carbon atoms in the aliphatic chain, and a *p*-hydroxyphenyl group in the place of the second carboxyl group. The great similarity in structure between corticrocin and cortisalin can be taken as further evidence that the mycelium from which corticrocin was isolated really belonged to a fungus of the genus *Corticium*, and may further indicate that both compounds are derived from a common progenitor in much the same way as crocetin and azafrin may be derived from a C_{40}-carotenoid.

It should be noted that the *p*-hydroxyphenyl group occurs also in atromentin [9], another fungus pigment.

EXPERIMENTAL

Isolation of cortisalin. Of various methods tried for the isolation of the piment, the following was found to be the most satisfactory. The finely ground fruit body of *Corticium salicinum* (222 g) was extracted with acetone in a Soxhlet-apparatus. Initially the extract was dark brown, but later the colour changed to yellow. When no more coloured material was being removed, water was added, after which the extract became yellow again. The extraction was continued until no change in colour occurred on the addition of water. This requires a time of about two weeks. The cortisalin precipitates as a brown powder in the flask of the extraction apparatus. It is advisable to filter off the precipitate from time to time to avoid unnecessary exposure to heat. The precipitate was washed with water and extracted with ether in a Soxhlet apparatus to remove wax-like impurities. The cortisalin remains undissolved as a dark brown powder. This was extracted with hot pyridine which easily dissolves cortisalin leaving a small amount of insoluble impurities. Crude cortisalin (2.6 g) crystallised from the pyridine solution on cooling. This material contains an appreciable amount of brown amorphous impurities and was extracted again with ether and recrystallised from pyridine. In this way 1.25 g of essen-

* The author wishes to express his thanks to Docent E. Stenhagen, Uppsala, for this information.

tially pure cortisalin was obtained as violet-red needles. It has no melting point but decomposes above 290°. From another batch of 270 g of fungus 1.05 g of cortisalin was obtained by a similar procedure. Samples for analysis were purified by repeated recrystallisation from pyridine. (Found: C, 77.0, 77.0, 77.8; H, 6.3, 6.2, 6.4. $C_{21}H_{20}O_3$ requires C, 78.7; H, 6.3 %.)

Dimethylcortisalin. Cortisalin (200 mg) was suspended in ether (200 ml) and an ethereal solution of diazomethane (ca. 2 g) was added. Only a very slight evolution of nitrogen could be observed. The mixture was allowed to stand, with occasional shaking, for two weeks, during which time the red-violet crystals had been transformed into a bright red powder. This was collected and extracted with chloroform in a continuous extraction apparatus. Dimethylcortisalin precipitated from the chloroform extract during the extraction, and was recrystallised from diethyl succinate giving 100 mg of bright red glistening leaflets, m. p. 255—257° (dec.). Samples for analysis were sublimed in vacuum (0.5 mm) and then recrystallised from diethyl succinate. (Found: C, 78.4, 77.8, 78.7; H, 7.0, 6.9, 6.8; OCH_3, 14.2, 14.8, 15.1. $C_{23}H_{24}O_3$ requires C, 79.3; H, 7.0; OCH_3, 17.8 %).

Cortisalin methyl ether. Dimethylcortisalin (15 mg) was suspended in 2 per cent alcoholic potassium hydroxide solution (200 ml), and the mixture was heated on a water-bath for 10 hours, during which time the crystals of dimethylcortisalin changed to a brown microcrystalline powder. The alcohol was removed and water was added. The insoluble brown precipitate was filtered off, extracted with boiling chloroform in order to remove unchanged dimethylcortisalin, then suspended in 2-N sulphuric acid and set aside overnight. The precipitate which was then dark red was filtered off, washed with water and extracted with pyridine. From the pyridine extract, cortisalin methyl ether crystallised, on cooling, as dark red micro needles (4 mg), m. p. 280—282° (dec.). (Found: OCH_3, 8.4; $C_{22}H_{22}O_3$ requires OCH_3, 9.3 %).

Acetylcortisalin. Cortisalin (50 mg) was dissolved in pyridine and acetic anhydride (1 ml) was added. The solution darkened appreciably, and was set aside overnight then poured into water. The precipitate was collected, dried, and extracted with chlorobenzene. The acetylcortisalin separated as yellow-brown needles which were purified by recrystallisation from chlorobenzene-dimethyl succinate. M. p. 280—282° (dec.). (Found: C, 76.2, H, 6.1; $C_{23}H_{22}O_4$ requires C, 76.2, H, 6.1 %).

Oxidation of dimethylcortisalin. Dimethylcortisalin (50 mg) was placed in a folded filter paper suspended in one neck, of a two-necked flask. The same neck was equipped with a reflux condenser. Acetone was placed in the flask which was heated on a water-bath. The refluxing acetone slowly dissolved the dimethylcortisalin. Finely powdered potassium permanganate was added in small portions through the other neck. The rate of addition of the potassium permanganate must be adjusted so that the solution does not contain too large an excess of potassium permanganate or so much dimethylcortisalin that it precipitates. The oxidation was interrupted after two days although part of the dimethylcortisalin still remained in the filter paper. This amounted to 20 mg, 30 mg thus having been dissolved. Water was added and the acetone was distilled off on a water-bath. The solution was then acidified with dilute sulphuric acid and sodium hydrogen sulphite was added to dissolve the manganese dioxide. 2 Mg of unreacted dimethylcortisalin was removed by filtration, and the solution was extracted with ether. The ether solution was extracted with sodium carbonate solution, which was then acidified and again extracted with ether. Evaporation of the ether yielded a white crystalline mass (10 mg). After sublimation and recrystallisation from ether-light petroleum this had m. p. 182—184°, undepressed in admixture with anisic acid.

Hydrogenation of cortisalin. Cortisalin (200 mg) was suspended in absolute alcohol and hydrogenated at 2 atm. pressure, in the presence of a Pt-catalyst. After about one week the cortisalin had dissolved and the solution was colourless. The alcohol was removed in vacuum, and the white solid mass obtained was dissolved by warming with a few ml of 2 *N* sodium hydroxide and the solution was filtered. On cooling, the sodium salt of hydrocortisalin separates as a white amorphous powder. When attempts were made to extract an ether solution of hydrocortisalin with sodium hydroxide the formation of this difficultly soluble sodium salt caused the formation of a very troublesome emulsion. Hydrocortisalin was obtained from the sodium salt by acidification, as a slightly yellow precipitate (140 mg). The yellow colour was removed by filtering an ether solution through a small amount of acid-washed aluminium oxide, and the product was recrystallised from dilute alcohol or ether-light petroleum, m. p. 98—100°. Mixed with synthetic 15-*p*-hydroxyphenylpentadecanoic acid of m. p. 99—100° it melted at 99—100°. (Found: C, 74.7, H, 10.0; $C_{21}H_{34}O_3$ requires C, 75.4, H, 10.3 %).

Methylation of hydrocortisalin. Hydrocortisalin (100 mg) was dissolved in ether and an excess of diazomethane in ether was added. After three hours the evolution of nitrogen had ceased, and the excess of diazomethane was distilled off with part of the ether. The remaining colourless solution was filtered through a small amount of aluminium oxide and evaporated. The crystalline residue was recrystallised from light petroleum, m. p. 76—76.5°. (Found: C, 76.2, H, 10.4, OCH_3, 8.4; $C_{22}H_{36}O_3$ requires C, 75.8, H, 10.4, OCH_3, 8.9 %).

On alkaline hydrolysis, hydrocortisalin m. p. 99—100°, identified by a mixed m. p. determination, was regenerated.

SUMMARY

From the sporophore of *Corticium salicinum* Fries a redviolet pigment has been isolated. This has been termed cortisalin and has been shown to be 15-*p*-hydroxyphenylpentadeca-2,4,6,8,10,12,14-heptenoic acid.

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