ACTA CHEMICA SCANDINAVICA 10 (1956) 1111—1115

# Fungus Pigments

## IV. * Aurantiacin, the Pigment of *Hydnum aurantiacum* Batsch

JARL GRIPENBERG

*Department of Chemistry, Institute of Technology, Helsingfors, Finland*

The fungus, *Hydnum aurantiacum* Batsch has yielded a red pigment, termed aurantiacin. It has been shown that aurantiacin is 2,5-bis(benzoyloxy)-3,6-bis(*p*-hydroxyphenyl)-1,4-benzoquinone, that is, a dibenzoate of the previously known fungus pigment atromentin.

*Hydnum aurantiacum* Batsch is a fungus growing on the ground, particularily in shady places in spruce forests. The foot of the sporophore is dark red-brown, whereas the cap is lighter in colour with darker streaks. The pigment can very readily be extracted with ether from the fresh material, and somewhat more slowly from the dried fungus. When the extraction is carried out in a Soxhlet apparatus, a red crystalline precipitate is formed in the extraction flask. This precipitate gives after purification, as described in the experimental part, aurantiacin as dark red, small needles, m.p. 285—295°. Aurantiacin gives a yellow acetate, m.p. 235—238° and a colourless leuco-acetate, m.p. 246—253°.

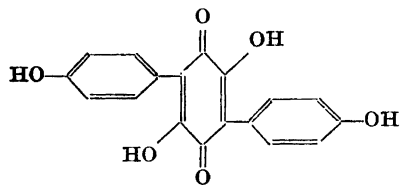
Analytical data indicated a composition $C_{32}H_{20}O_8$ for aurantiacin, $C_{36}H_{24}O_{10}$ for the acetate and $C_{40}H_{30}O_{12}$ for the leuco-acetate. The two derivatives are thus a diacetate and a dihydrotetra-acetate, respectively. Molecular weight determination gave somewhat high values, but this is not considered too important, because the low solubility of the substances necessitated working with relatively low concentrations, making the values obtained rather uncertain.

A clue to the structure of aurantiacin was obtained in an attempt to recover more of the pigment from the highly coloured ether extract. This was shaken first with sodium hydrogen carbonate and then with sodium carbonate solutions, which, however, extracted only minute amounts of material. With 2 N sodium hydroxide, on the other hand, all of the pigment could be removed from the ether solution giving a dark red-brown alkaline solution. When this was acidified and the colouring matter taken up in ether most of it could now

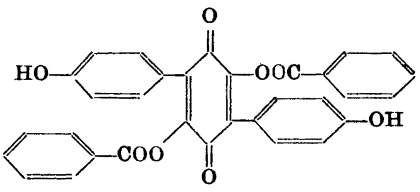
* Part III. *Acta Chem. Scand.* **6** (1952) 587.

be extracted with sodium hydrogen carbonate. Reprecipitation with acid gave a mixture which upon treatment with benzene was separated into two components. The benzene soluble component was identified as benzoic acid. The insoluble component formed upon recrystallisation dark brown leaflets, decomposing without melting at about 300°. This substance corresponds to the formula $C_{18}H_{12}O_6$ and gave a yellow tetra-acetate, $C_{26}H_{20}O_{10}$. This melts at 242—245°, but resolidifies and then melts at 290—294°. A colourless leuco-acetate, $C_{30}H_{26}O_{12}$, m.p. 235—240° was obtained upon reductive acetylation. The formation of these derivatives shows the presence of four hydroxyl groups and a quinone group in the compound $C_{18}H_{12}O_6$. The parent hydrocarbon is thus $C_{18}H_{14}$, which is the composition of terphenyl. Because several diphenylbenzoquinones have been found in fungi [1,2] the formulation of the compound $C_{18}H_{12}O_6$ as a tetrahydroxydiphenylbenzoquinone suggested itself. In fact the properties of this compound and its derivatives are in close agreement with those given by Kögl and Postowsky [3] for atromentin isolated from *Paxillus atrotomentosus* Batsch. The only serious difference is that Kögl and Postowsky report only a single m.p. 240—241° for atromentin tetra-acetate. Through the kindness of Professor Kögl, who sent a very generous sample of authentic atromentin, it could be shown that the compound obtained in the present work was identical with atromentin. When the m.p. of atromentin tetra-acetate was determined in the Kofler hot-stage microscope it showed the same characteristic double m.p. as observed with the acetate of our compound.

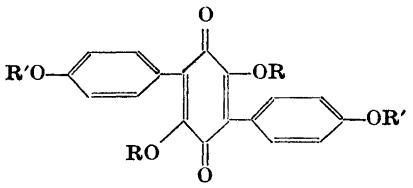
It is obvious that both the benzoic acid and the atromentin have been formed upon treatment of the extract with alkali. This suggests that they might be combined with another in aurantiacin. This was borne out by a separate experiment, where aurantiacin was dissolved in sodium hydroxide, giving atromentin and benzoic acid.



I



II



III ; R = OC—⟨benzene⟩ ; R′ = $CH_3$

IV ; R = $CH_3$ ; R′ = OC—⟨benzene⟩

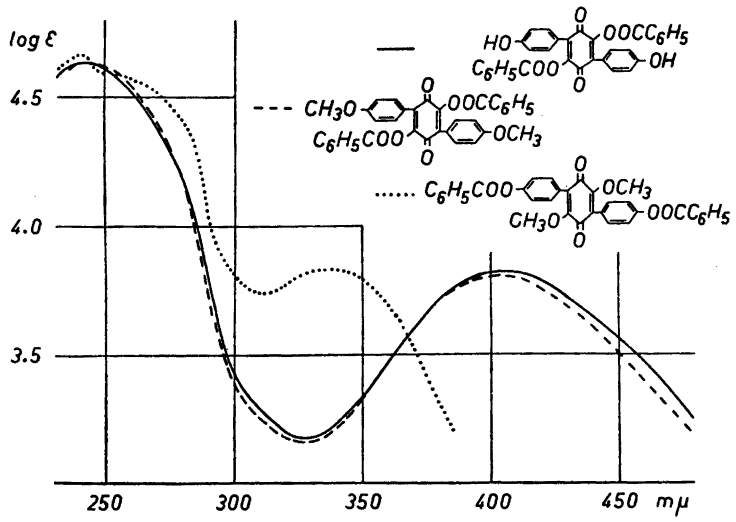



*Fig. 1.*

Aurantiacin is thus most probably a benzoate of atromentin, and the analyses reported above show that it is a dibenzoate. This view is further supported by benzoylation of aurantiacin to a dibenzoate. which is identical with the tetrabenzoate obtained by benzoylation of atromentin.

The structure of atromentin (I) has been established by Kögl and coworkers, both by degradation [4] and by synthesis [5]. It remains to show which of the four hydroxyl groups are benzoylated in aurantiacin. Three dibenzoates of atromentin are possible and, as will be shown below, aurantiacin must be (II)

This conclusion rests on the following observations. Aurantiacin is only a very weak acid, being insoluble in 2 N sodium carbonate, whereas atromentin is soluble even in sodium hydrogen carbonate. This shows that aurantiacin cannot have any free hydroxyl groups in the quinone ring. The almost instantaneous hydrolysis of aurantiacin, when treated with alkali also indicates that it is the hydroxyl groups in the quinone ring that are benzoylated, giving what can be considered as a vinylogous acid anhydride. Thirdly a comparison of the UV-absorption curve of aurantiacin with the absorption curves of the compounds (III) and (IV) prepared by unambiguous routes from atromentin, shows, as can be seen from Fig. 1, that the spectrum of aurantiacin is very close to that of (III), but quite different from that of (IV). (All spectra in dioxan).

Final proof of the structure (II) for aurantiacin was obtained by methylating it to its dimethyl ether, which was identical with the synthetically prepared (III). This methylation was unexpectedly difficult to achieve. Only a rather low yield (20 %) of the desired compound could be obtained. At least three other compounds were formed in the methylation but they have not been studied further.

## EXPERIMENTAL

(All m.p:s have been determined on a Kofler hot-stage microscope, unless otherwise stated. The analyses have been carried out by Dr. A. Bernhardt, Mülheim.)

*Isolation of aurantiacin.* The finely ground sporophores of the fungus were extracted with ether in a Soxhlet apparatus and the red crystalline precipitate was filtered off. The yield was varying, one batch of 140 g giving 2.7 g, another of 170 g giving 1.7 g of crude pigment. This (100 mg) was dissolved in acetone and chromatographed on acid washed alumina (10 g). In the upper part of the column a narrow green band was formed and immediately below this a broad dark brown zone. Development with acetone caused a slow broadening of the brown zone but no clear separation from the green band could be achieved. The green band was removed from the column and the brown zone was eluated with acetone containing a few drops of acetic acid (3 drops per 40 ml). A dark red eluate was obtained. The pigment could be recovered either by evaporation of the solvent in vacuum, or by addition of water. In either case dark red crystals (85 mg) were obtained. Rechromatography of this gave only the brown zone. The pigment was recrystallised from dioxan giving small orange red needles. Upon standing they slowly turned dark red. They evidently contain dioxan of crystallisation. M.p. 285–295°. (Found: C 72.25; H 3.72; O 23.89; Mol.wt. (Rast) 610; $C_{32}H_{30}O_8$ requires C 72.18; H 3.79; O 24.04; Mol.wt. 532.5).

*Aurantiacin diacetate.* Aurantiacin (200 mg) was suspended in acetic anhydride (10 ml) containing two drops of pyridine. After standing overnight all the red crystals of aurantiacin had disappeared and the solution contained the yellow crystals of aurantiacin diacetate. These were filtered off (180 mg) and recrystallised from dioxan. M.p. 235–238°. (Found: C 69.61; H 3.88; O 26.85; Mol.wt. 745, 760; $C_{36}H_{24}O_{10}$ requires C 70.13; H 3.92; O 25.95; Mol.wt. 616.5).

*Aurantiacin leuco-acetate.* Aurantiacin diacetate (100 mg) was suspended in acetic anhydride (2 ml) to which had been added a few drops of pyridine. Zinc powder was then added and the mixture was allowed to stand. The yellow acetate disappeared rapidly and the almost colourless solution was filtered from the zinc, and poured into cold water. A white crystalline precipitate was formed (100 mg) This was recrystallised from acetic acid. M.p. 246–253°. (Found: C 68.35; H 4.50; O 27.30; $C_{40}H_{30}O_{12}$ requires C 68.46; H 4.29; O 27.25).

*Aurantiacin dibenzoate.* Aurantiacin (100 mg) was dissolved in pyridine (5 ml) and benzoyl chloride (0.25 ml) was added. The mixture turned warm and after some time a mixture of colourless and yellow crystals was formed. The crystals were filtered off and washed with water to remove the colourless pyridine hydrochloride. The remaining yellow aurantiacin dibenzoate was recrystallised from dioxan. M.p. 310–315°. (Found: C 74.22; H 3.93; O 22.02; $C_{46}H_{28}O_{10}$ requires C 74.59; H 3.81; O 21.60).

*Treatment of the ether extract.* The ether extract obtained in the extraction of one batch of fungus (170 g) was shaken successively with portions of saturated sodium hydrogen carbonate, 2 N sodium carbonate and 2 N sodium hydroxide, each time until the aqueous phase was almost colourless. The two first extracts gave upon acidification only minute amounts of precipitate, which were not further investigated, while the sodium hydroxide extract gave a large precipitate. This was taken up in ether and the extraction repeated as above. The precipitate obtained by acidification of the sodium hydrogen carbonate extract was filtered off and washed with benzene, whereby a part of it dissolved. The mother liquor from the filtration was extracted with ether, the ether was removed and the residue was divided into a benzene soluble and benzene insoluble part, which were combined with the corresponding fractions from the precipitate. 3.3 g of a brown benzene insoluble powder was thus obtained. This was extracted with hot methanol. Upon cooling 1.9 g of dark brown crystals of atromentin separated. By adding dioxan to the mother liquor a further amount (0.35 g) was obtained. Recrystallisation from methanol-dioxan mixture gave orange red crystals which rapidly darken in the air. It decomposes without melting at about 300°. (Found: C 66.68; H 3.96; O 29.54; $C_{18}H_{12}O_6$ requires C 66.67; H 3.73; O 29.60).

The benzene solution obtained above was evaporated leaving a slightly red crystalline mass. Sublimation gave colourless needles, m.p. 120–121°, identified as benzoic acid by mixed m.p.

When pure aurantiacin was dissolved in 2 N sodium hydroxide and the solution acidified a precipitate was obtained that in the same way could be separated into atromentin and benzoic acid.

*Atromentin tetra-acetate* was prepared from atromentin and acetic anhydride in the presence of pyridine in the same way as aurantiacin diacetate. Recrystallisation from acetic acid and then from dioxan gave yellow needles. When the m.p. was determined in a conventional m.p. apparatus a m.p. of 242—245°, not depressed by admixture of authentic atromentin tetra-acetate was observed. In a m.p. microscope the same initial m.p. was observed, but the melt recrystallised to very characteristic clusters of needles which then melted at 290—294°. The same behaviour was observed also with authentic atromentin tetra-acetate. (Found: C 63.09; H 4.06; O 32.53; $C_{26}H_{20}O_{10}$ requires C 63.41; H 4.09, O 32.49).

*Atromentin leuco-acetate.* Reductive acetylation in the same manner as described above for aurantiacin gave the colourless leuco-acetate, m.p. 235—240°. Kögl and Postowsky [3] report 235—236°. (Found: C 61.83; H 4.82; O 33.13; $C_{30}H_{26}O_{12}$ requires C 62.28; H 4.53; O 33.19).

*Atromentin tetrabenzoate.* Atromentin (100 mg) was suspended in pyridine (3 ml) and benzoyl chloride (0.5 ml) was added. The atromentin dissolved and after a short time precipitation began. After standing overnight the crystals were filtered off and washed thoroughly with water. The yellow crystals (140 mg) were recrystallised from anisole, m.p. 308—315°. Gives no depression of m.p. when mixed with aurantiacin dibenzoate. (Found: O 22.19; $C_{46}H_{28}O_{10}$ requires O 21.60).

*Aurantiacin dimethylether* (III). *a*) From atromentin. Atromentin was converted to its *p,p'*-dimethylether according to Kögl and Becker [4]. This (50 mg) was suspended in pyridine (2 ml) and benzoyl chloride (0.1 ml) was added. After a few hours the same amount of benzoyl chloride was added. After 24 hours the crystalline material was filtered off and washed with water. The residue, after recrystallisation from dioxan-water was obtained as orange red plates, m.p. 243—247°. (Found: C 73.09; H 4.45; $C_{34}H_{28}O_8$ requires C 72.85; H 4.32).

*b*) From aurantiacin. Aurantiacin (300 mg) was dissolved in dry acetone (50 ml) and dimethyl sulphate (2 ml) and potassium carbonate (3 g) were added. The mixture was boiled one day on a water bath, filtered and the acetone evaporated in vacuum. The residue was dissolved in benzene and the solution chromatographed on alumina. It formed a broad yellow, rapidly moving band and above that several narrow red to brown coloured bands. The eluate of the yellow band was evaporated under vacuum, whereby a partly crystalline residue was obtained. The crystals were filtered off (50 mg) and recrystallised from dioxan-water, giving orange red plates, m.p. 243—246°, no depression when mixed with a specimen prepared according to *a*).

*Atromentin-2,5-dimethylether*-p,p'-*dibenzoate* (IV). Atromentin-2,5-dimethylether was prepared from atromentin with diazomethane [3]. This (120 mg) was dissolved in pyridine (5 ml) and benzoyl chloride (0.3 ml) was added. After some time crystallisation set in and the mixture was allowed to stand for 2 days. The crystals were removed by filtration and were washed with water and benzene. The residue was recrystallised from pyridine, giving light brown needles, m.p. 278—290°. (Found: C 72.45; H 4.79; $C_{34}H_{24}O_8$ requires C 72.85; H 4.32).

The author wishes to express his indebtedness to Prof. F. Kögl, Utrecht, for a sample of atromentin. A grant from *Statens Naturvetenskapliga Kommission* is gratefully acknowledged.

## REFERENCES

1. Mayer, F. and Cook, A. H *The Chemistry of Natural Coloring Matters*, Reinhold Publishing Co., New York 1943, p. 97 ff.
2. Akagi, M. *J. Pharm. Soc. Japan* **62** (1942) 129; *Chem. Abstracts* **45** (1951) 2898.
3. Kögl, F. and Postowsky, J. J. *Ann.* **440** (1924) 19.
4. Kögl, F. and Becker, H. *Ann.* **465** (1928) 211.
5. Kögl, F. *Ann.* **465** (1928) 243.

Received May 8, 1956.