Phytochemistry, 1965, Vol. 4, pp. 505 to 509. Pergamon Press Ltd. Printed in England

THE ISOLATION OF XANTHOMEGNIN FROM SEVERAL STRAINS OF THE DERMATOPHYTE, *TRICHOPHYTON RUBRUM**

JOHN C. WIRTH, THOMAS E. BEESLEY and S. R. ANAND
Department of Chemistry, St. John's University, Jamaica 32, N.Y.

(*Received* 27 *August* 1964)

Abstract—One of the three pigments isolated in crystalline form from the mycelium of the dermatophyte, *Trichophyton rubrum*, has been shown to be identical with the pigment, xanthomegnin, previously isolated from *T. megnini*. Several additional derivatives of the compound are reported.

INTRODUCTION

BEFORE 1957, information concerning the pigments of the Trichophyta is almost inconsequential. Simultaneous publications of Mier[1] and Wirth *et al.*[2] reported the isolation in crystalline form of some of the pigments of *T. rubrum*. Mier, by paper chromatography, separated and purified three pigments which were designated as "A", "B", and "C" and succeeded in crystallizing the latter two. Wirth *et al.*, using column adsorption chromatography, presented evidence that the coloration on the undersurface of the mycelium is due to a highly complex mixture of pigments and isolated three of them in crystalline form which were designated as "orange plates", "red needles" and "purple needles". A comparison of the R_f values and absorption spectra (qualitative) in the u.v. and visible region (P. D. Mier, personal communication) established the identity of his pigments "A", "B", and "C" with the "orange plates", "red needles", and, "purple needles", respectively, of Wirth *et al.*

McCabe and Mier[3] investigated three different species of Trichophyta and isolated by fractional precipitation and paper chromatography a total of five different pigment fractions of which only three could be detected in their extracts from *T. rubrum*. While no crystalline material is reported, homogeneity was claimed on the basis of repeated chromatography and constancy of absorption spectra (qualitative). Baichwal and Walker[4] reported the spectral and other properties of their extracts from this organism. However, as these authors were working with non-crystalline material, it is difficult to evaluate their findings. Zussman, Lyon and Vicher[5] confirmed the presence of a nitrogen-free, orange fraction which they resolved into the "orange plates" and "red needles" of Wirth *et al.* On the basis of isotope experiments, they suggested that some of the pigments synthesized by *T. rubrum* may be melanoid in nature.

---

* This investigation was supported by Public Health Research Grant AI-1761 from the National Institute of Allergy and Infectious Diseases.

[1] P. D. MIER, *Nature* 179, 1084 (1957).

[2] J. C. WIRTH, P. J. O'BRIEN, L. F. SCHMITT and A. SOHLER. *J. Invest. Dermatol.* 29, 47 (1957).

[3] M. G. MCCABE and P. D. MIER, *J. Gen. Microbiol.* 23, 1 (1960).

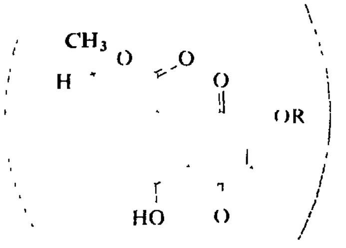
[4] M. R. BAICHWAL and G. C. WALKER, *Can. J. Microbiol.* 6, 383 (1960).

[5] R. A. ZUSSMAN, I. LYON and E. E. VICHER, *J. Bacteriol.* 80, 708 (1960).

While the work on the structure of the "orange plates" was in progress, a paper on the structure of xanthomegnin (I), isolated from a strain of *T. megnini*, appeared.[6] The properties of xanthomegnin pointed either to a close similarity or the identity of the two pigments. Finally, a comparison of the i.r. spectrum of the "orange plates" with that of xanthomegnin, kindly supplied by Dr. George Just, Department of Chemistry, McGill University, conclusively proved the identity of the two pigments.

The preparation of bisnorxanthomegnin (II) and the properties of this compound[7] complement the assignment[6] of the two methoxyl groups to the quinonoid rings. The u.v. spectrum of the leucoacetate is consistent with the 2-2′-naphthoquinone structure.[8,9] The identification of formic acid, on degradation with alkali, is in accord with the high acetyl values found when such analyses were carried out with base hydrolysis.

Whether or not the presence of a compound of the dinaphthoquinone series, very rarely encountered in nature, in two species of the genus, Trichophyton, has any taxonomic significance, remains to be seen.



(I) R = CH_3
(II) R = H

EXPERIMENTAL

All melting points are uncorrected. Microanalyses were run by Weiler and Strauss of Oxford, England, with the exception of the Signer–Barger molecular weight determinations which were run by the Schwarzkopf Microanalytical Laboratory, Woodside 77, N.Y. Ultraviolet spectra were determined with a Beckmann, model DU, spectrophotometer with spectro grade dioxane as solvent. All i.r. spectra were determined with a Perkin–Elmer, No. 21, spectrophotometer, using KBr pellets (0·5% solution). All microanalytical samples were dried at 110° in high vacuum for several hours.

Extraction of the Pigments

The work reported herein was done with strains C-129, M-722, and V-1709. Fortunately, the three pigments isolated in crystalline form seem to be characteristic of the species since they were isolated from each of these strains.

The culturing of the organism and the harvesting of the mycelium was as reported by Wirth *et al.*[2] After removal of the mercuric chloride, the bulk of the water was removed by hand pressure. The moist mycelium was suspended in acetone and macerated for a few minutes in a large Waring blendor. The pulp was filtered on a Buchner funnel and the ground

---

[6] G. JUST, W. C. DAY and F. BLANK, *Can. J. Chem.* **41**, 74 (1963).

[7] R. H. THOMSON, *Naturally Occurring Quinones*, Butterworths Scientific Publications, London (1957)

[8] H. BROCKMANN and G. BUDDE, *Chem. Ber.* **86**, 43 (1953).

[9] R. A. FRIEDEL and M. ORCHIN, *Ultraviolet Spectra of Aromatic Compounds*, (Spectra 303, 306 and 309), John Wiley and Sons, Inc., N.Y. (1951).

mycelium suspended in a mixture of chloroform:methanol (2:1 v/v) under nitrogen. After standing for several days, the mycelium was again removed by filtration; the latter extraction was repeated three times. The extracts from each solvent were taken to dryness separately under reduced pressure. The two residues were combined; extracted with several portions of hexane at room temperature and finally with the same solvent in a Soxhlet apparatus for about 8 hr. This resulted in two fractions: a lipid fraction contaminated with some pigment and a crude, fat free, pigment fraction. The latter was extracted (Soxhlet) for short periods of time (until the extract became light in color) with ethyl and methyl alcohols, successively. The residue was a dark, red–brown powder which was then further separated by chromatography.

Chromatography

Columns, measuring 2·5 × 36 in., were packed with 1·5 kg of a dry mixture (60/40 w/w) of Terra Alba No. 1 (U.S. Gypsum Co.) and Solka-Floc, BW-200 (Brown Co., Berlin, N.H.). The column was prewetted with ethylene dichloride (previously dried by azeotropic distillation) containing 0·1 % (v/v) of acetic acid. The crude pigment (2·0 g), dissolved in about 400 ml of the same mixture, was poured on the column and development was effected with the same solvent mixture. When fully developed (pigment about to come off the bottom of the column), the following bands were discernible: a broad golden yellow band (mainly xanthomegnin); an orange band (mixture of xanthomegnin and red pigment); a red band (mainly red pigment); a diffuse salmon band (discarded); a purple band (mainly purple pigment) and a brown band (discarded). Rechromatography one or more times, on the same adsorbent or on a mixture (70/30 w/w) of Terra Alba No. 1 and Celite, 545 (Johns-Manville Corp.), of the individual fractions eventually yielded homogenous material. All adsorbents with the exception of the Solka-Floc were heated at 175° for 3 hr prior to use.

Particularly in strain M-722, a green pigment contaminated the xanthomegnin and appeared as a brownish-red band in the leading edge of the golden-yellow band. A complete separation of this pigment from xanthomegnin was effected successfully on a heat-activated calcium phosphate ($CaHPO_4.2H_2O$) with ethylene dichloride containing 0·2 % acetic acid (v/v) as the developer.

Xanthomegnin (I)

The pigment crystallized from ethylene dichloride–hexane mixtures in the form of orange plates; from acetic acid–hexane mixtures in the form of needles. With the capillary tube method, it has no discernible, sharp melting or decomposition point but begins to darken at about 200° and appears totally charred at about 300°. Using the preheated metal-block technique, it decomposed instantaneously at a minimum temperature of about 340°. $[\alpha]_D^{22}$ $-156°$ ($c = 9{\cdot}48$ mg/ml in chloroform) and $-155°$ ($c = 10$ mg/ml in chloroform); $\lambda_{max.}$ 228, 288 (inflection), 395 mμ (ϵ 54,090, 17,130, 10,080); ν 1718 (α-β, unsaturated lactone), 1678 (unbonded carbonyl), 1616 cm^{-1} (bonded carbonyl). (Found: C, 62·33; H, 3·87; OCH_3, 9·9; C-Me, 5·3; mol.wt., 593; N, S, Halogen, nil; Ac (base hydrolysis), 3·0, (acid hydrolysis), 2·2. Calc. for $C_{30}H_{22}O_{12}$: C, 62·72; H, 3·86; 2 OCH_3, 10·80; 2 C—CH_3, 5·23 %; mol.wt., 574).

Xanthomegnin Diacetate

50 mg of xanthomegnin in 50 ml of acetic anhydride containing a trace of *p*-toluenesulphonic acid was refluxed 1 hr. After working up in the usual manner, the product was purified

on activated calcium phosphate with anhydrous benzene as developer. The pigment constituting the main yellow band was recrystallized from absolute ethanol or ethyl acetate–hexane mixtures; bright yellow microcrystals; m.p. 234–235 ; $[\alpha]_D^{22}$ −154° ($c = 9{\cdot}75$ mg/ml in chloroform); $\lambda_{max.}$ 262, 348 mμ (ε 44,510, 10,510); ν 1779 (phenylacetate), 1730 (lactone), 1678 cm^{-1} (unbonded carbonyl). (Found: C, 61·46; H, 3·89; Ac (base hydrolysis), 15·4, (acid hydrolysis), 11·1; mol.wt., 616. Calc. for $C_{34}H_{26}O_{14}$: C, 62·01; H, 3·98; 2 Ac, 13·1 %; mol.wt., 658.) When recrystallized from benzene–hexane mixtures a compound with a m.p. of 239–241° was obtained whose analytical properties are consistent with the formula $C_{34}H_{26}O_{14}\cdot\frac{1}{2}C_6H_6$.

Tetrahydroxanthomegnin

A solution of 50 mg of pigment in 50 ml of ethylene dichloride was shaken with 10 ml of a 10 % aqueous solution of sodium dithionite. An instantaneous reaction occurred, the original orange solution turning to bright yellow. The organic layer was washed with deoxygenated water, dried over anhydrous sodium sulphate and concentrated to a small volume. The product was purified by chromatography on unactivated calcium phosphate with ethylene dichloride as developer. The product from the main yellow band crystallized from ethylene dichloride–hexane mixtures as bright yellow plates; decomp. p. 210°; ν 1647 (hydrogen bonded lactone), 1642 cm^{-1} (C = C polycyclic hydrocarbon). (Found: C, 61·88; H, 4·53. Calc. for $C_{30}H_{26}O_{12}$: C, 62·28; H, 4·53 %.)

Tetrahydroxanthomegnin Hexaacetate

A mixture of 100 mg of the diacetate, 200 mg of freshly dried zinc dust, 40 mg of fused sodium acetate, and 10 ml of acetic anhydride (free from acetic acid) was refluxed for 4 hr, according to the procedure of Brockmann and Budde.[8] The crude product was chromatographed on activated calcium phosphate from ethylene dichloride; recrystallized from ethanol–hexane mixtures: very pale yellow microcrystals; m.p. 196–197 ; $\lambda_{max.}$ 262 mμ (ε 87,150); ν 1776 (phenylacetate), 1721 (lactone), 1626 cm^{-1} (C = C polycyclic hydrocarbon). (Found: C, 60·36; H, 4·77; Ac (base hydrolysis), 28·0, (acid hydrolysis), 30·0. Calc. for $C_{42}H_{38}O_{18}$: C, 60·72; H, 4·61; 6 Ac,[5] 31·1 %.)

Bisnorxanthomegnin (II)

100 mg of xanthomegnin, dissolved in 100 ml of N sodium hydroxide, was allowed to stand under nitrogen for 6 days at room temperature. The reaction mixture was strongly acidified with hydrochloric acid and kept in the refrigerator for several hours. Precipitate was filtered off, washed with water, dried and taken up in about 200 ml of hot ethyl acetate. On cooling, a small amount of material precipitated which was filtered off. The solution was chromatographed on Solka-Floc. The first, minor yellow band was discarded. Development was continued until the main, diffuse, brown-yellow band had been eluted. The solution was concentrated and rechromatographed. The ethyl acetate was evaporated and the residue was recrystallized three times from ethylene dichloride–hexane mixtures containing a minute quantity of hydrochloric acid from which the compound precipitated in the form of orange rods; began to darken at about 230°, no further darkening above 245 ; decomp. p. (preheated block) about 245°. The compound is strongly acidic as dilute aqueous solutions give a blue color with congo red paper. If ethyl acetate (above) was washed completely free of acid, the compound migrated into the water layer. ν 1718 (lactone), 1669 (unbonded carbonyl), 1605

cm^{-1} (bonded carbonyl). (Found: C, 59·60; H, 3·70; OCH_3, 0. Calc. for $C_{28}H_{18}O_{12}.H_2O$: C, 59·57; H, 3·57; OCH_3, 0%.)

Tetraacetate of Bisnorxanthomegnin

150 mg of II, dissolved in 30 ml of acetic anhydride containing a trace of *p*-toluenesulphonic acid, was refluxed for 1·5 hr. The reaction mixture was worked up in the usual way and the product dissolved in benzene was chromatographed on activated calcium phosphate. The pigment constituting the main yellow band was recrystallized from ethyl acetate containing a small quantity of hexane; yellow microcrystals; began to darken about 200° and melted with decomposition at 251–253°. (Found: C, 60·25; H, 3·92. Calc. for $C_{36}H_{26}O_{16}$: C, 60·51; H, 3·67%.)

Degradation with Alkali

Xanthomegnin, dissolved in 20 ml of 5 N sodium hydroxide was refluxed 3 hr. The solution was acidified with 85 per cent phosphoric acid and steam distilled. The distillate was made alkaline with ammonium hydroxide and concentrated to a few ml. The ammonium salts were subjected to paper chromatography[10] using *n*-butanol saturated with water as the developer. The unknown, authentic ammonium formate and ammonium acetate, all had the same R_f of 0·11. Two spot tests[11] with mercuric chloride and chromotropic acid were positive; a spot test for acetate with ferric chloride was negative.

Acknowledgements—Thanks are extended to Dr. M. Silva of the Department of Dermatology, College of Physicians and Surgeons, Columbia University, New York, for the cultures of *T. rubrum*.

---

[10] R. L. Reid and M. Lederer, *Biochem. J.* 50, 60 (1951).

[11] F. Feigel, *Spot Tests in Organic Analysis*, 6th Ed., pp. 368, 369 and 371, D. Van Nostrand, N.Y. (1960).