THE PIGMENTS OF *TRICHOPHYTON* SPECIES*

YUKI ITO, M.D., TATSUZO FUJII AND YOSHINORI NOZAWA

Pigments of dermatophytes have been studied by a number of workers, but only recently have purified preparations been used for study of their chemical properties and structures. Wirth *et al.* (1), employing column chromatography, demonstrated that the coloring matter of *T. rubrum* is not a single substance but a mixture of several different compounds, and they isolated three pigments in crystalline form. Mier (2) similarly succeeded in isolating three pigment fractions from *T. rubrum* by means of paper chromatography, which he presumed to be anthraquinone derivatives. His work was subsequently extended by McCabe and Mier (3) to include *T. mentagrophytes* and *T. violaceum*. More recently, Koehne *et al.* (4) obtained three kinds of pigments from *Microsporum cookei* which proved to be identical with those from *T. rubrum*, and they further concluded, mainly based on the infrared spectral analysis, that two of these pigments are in fact polyhydroxy-methyl-anthraquinones. A contradictory view was expressed by Zussman *et al.* (5) on their pigment preparations isolated from *T. rubrum* according to modified Wirth's method that they were melanoid in nature.

Attempts were made by the present authors first to attempt to answer the above-mentioned discrepancy with reference to the chemical nature of these pigments, and then to compare the characteristics of the pigments produced by four different species of dermatophytes in the hope that the result would help in the identification of these dermatophyte species.

Materials and Methods: The cultures studied, *T. rubrum*, *T. tonsurans*, *T. mentagrophytes* and *T. megnini*, were obtained from the Institute for Fermentation, Osaka, Japan. They were grown in 500 ml conical flasks, containing 120 ml of Sabouraud's dextrose broth, at 28° C for 4 weeks.

Crude pigment preparation, obtained by either Zussman's extraction method (5) or by Mier's method (2, 3), was subjected to column chromatography on kaolin-Celite 545 (30:70 w/w) with ethylene dichloride containing 1% hydrochloric acid as developing solvent. After development, each colored band was cut off and was divided into two portions. The one half was eluted with glacial acetic acid and the other half with 5% sodium hydroxide. Ultraviolet and visible absorption was measured on these eluates with a Hitachi model EPS-2U automatic-recording spectrophotometer.

Results and Discussion: After column chromatography of the crude pigment preparation obtained from *T. rubrum* by one of the two extraction methods, three bands were similarly detected, which were in the descending order, brown (Fraction 1), red to reddish violet (Fr. 2) and yellow (Fr. 3) in color. Absorption spectrum measurement on the acid and alkaline eluate from the individual band indicated the similar characteristics between the corresponding pigments isolated from the two separate preparations as mentioned above. Color changes produced by treating them with acid, alkali or magnesium acetate, etc., also revealed no difference between the pigment preparations obtained by Zussman's method and those by Mier's method. Thus, it is concluded that the pigments isolated by Zussman's method are practically identical with those obtained by Mier's method which were previously believed to be anthraquinone derivatives (2, 3, 4).

Column chromatography of the crude pigment fractions from *T. tonsurans*, *T. mentagrophytes* and *T. megnini*, obtained according to Zussman's procedures, consistently gave three bands similar in color and in the order of development to those from *T. rubrum*, though naturally with slight differences in the color tone or in the width of the band. The absorption maxima of these pigments are shown in Table I. In general, they showed, in acetic acid solution, an absorption peak at around 260 to 270 mμ and also one at around 380 mμ, with slight but definite diversity in the exact position of these peaks on respective species. In alkaline solution, the maximum in visible range shifted to longer wave-length side, while the peak in ultraviolet range appeared indistinctly and therefore were not cited in the Table.

For the sake of comparison of their absorption characteristics, the pigments of *T. rubrum* were tentatively named A, B and C, as shown in Table I. The pigment A′ and A″ with absorption spectra similar to but slightly different from A, were detected in *T. tonsurans* and in *T. megnini*, respectively, while such a type pigment was lacking in the corresponding fraction from *T. mentagrophytes*. The pigment B and C of *T. rubrum* were common to *T. tonsurans* and *T. mentagrophytes*. In the corresponding fractions of *T. megnini*, the pigment B′ and C′ which were analogous to but distinct from B and C, respectively, were detected.

It is clear from these facts that all four of the species of the genus *Trichophyton* which were examined are distinguishable with reference to the characteristic absorption spectrum of the pigment of type A which varies from species to species. Furthermore, *T. megnini* differs from the other three in that it contains even two more specific pigments, different from the two kinds of pigments common to the latter, and also *T. mentagrophytes* differs from the others in that it contains no pigment of type A.

Received for publication July 25, 1963.

* From the Biochemical Institute, Gifu Medical School, Gifu, Japan.

TABLE I

Absorption maxima in ultra-violet and visible range of the pigments isolated from dermatophytes

Species	Fraction No.	Absorption Maxima (mu) in			Type (Tentatively Named)
		Glacial acetic acid		5% NaOH	
T. rubrum	1	260–270	384	490	A
	2	268	380	530–540	B
	3	264	380	520	C
T. tonsurans	1	268	384	480	A′
	2	268	380	530–540	B
	3	266	380	520	C
T. mentagrophytes	1	—	—	—	—
	2	268	376	530–540	B
	3	264	380	520	C
T. megnini	1	275	380	495	A″
	2	275	380	520–530	B′
	3	272	384	500–510	C′

The absorption characteristics of these pigments, as well as the results of their color reactions characteristic to anthraquinone derivatives, strongly suggest that all the pigments under examination are closely related to each other, and are anthraquinoid rather than melanoid compounds. Besides these pigments, there exists at least one kind of water-soluble pigment in respective culture, not presently clarified.

Considering the significant difference in the pattern of the pigments found among species of the dermatophytes examined, the use of such an analytical technic may aid in the identification of certain dermatophytes on a chemical basis.

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