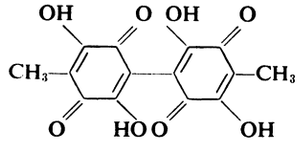
Production of Oosporein and Its Leuco Form by Basidiomycete Species

Abstract. *Oosporein, a dibenzoquinone pigment previously obtained from an Ascomycete and from several species of Fungi Imperfecti, has been isolated from two basidiomycete species of the genus* Phlebia. *The leuco form, not previously reported as a natural product, accompanied the quinone in culture liquids.*

Cultures of *Phlebia mellea* Overholts and *P. albida* Fries (*1*) on malt agar slants produce red pigment which diffuses into the agar. A pigment of similar appearance isolated earlier from the mycelium of *P. strigosozonata* (Schw.) Lloyd was shown to have an unusual terphenyl orthoquinone structure (*2*). The close relation between the organisms producing these substances suggested that the more polar pigment might also be a terphenyl derivative. Analysis (*3*) of the crystalline pigment isolated from culture liquids, as well as of its acetate and leucoacetate, supported this idea. However, the nuclear magnetic resonance (nmr) spectrum (*4*) of the pigment could not be reconciled with any terphenyl structure which fitted the analytical data.

Molecular weight determination was unsatisfactory because of the solubility properties of the pigment. But reconsideration of the analytical results showed that they could be interpreted about equally well for a C_{14} skeleton. Further, a butyrate which was later prepared gave analytical values in agreement with those expected on the basis of a C_{14} but not a C_{15} skeleton. Molecular weight determination on the leucoacetate further supported a C_{14} formula for the parent. Kuhn-Roth determination (*5*) was consistent with the presence of two C-methyl groups, and the nmr spectrum showed a single peak due to methyl protons. The total evidence from analyses, taken in conjunction with the nmr spectra, was completely compatible only with the structure



This structure had already been assigned to oosporein (*6*), a pigment isolated from an ascomycete (*7*) and from several species of Fungi Imperfecti (*6, 8, 9, 10*). Oosporein, like our pigment, formed a tetraacetate and a leucoacetate (*7*). But the melting point (mp) reported for the tetraacetate did not agree with that of the corresponding derivative of the *Phlebia* pigment. To clarify this apparent discrepancy, the *Phlebia* pigment was compared directly with an authentic sample of oosporein obtained from a shake culture of *Verticillium psalliotae* Treschow (*10*). Both pigments showed identical infrared spectra, as did their acetates and leucoacetates. The difference in melting point originally observed for the acetates is probably due to polymorphism; the melting point obtained for the acetate from either source depended on the solvent from which it was crystallized. Acetates with different melting points gave no melting point depression on admixture.

For pigment production, *P. mellea* or *P. albida* was grown in shake culture in 500-ml erlenmeyer flasks at 25°C in a medium consisting of potato dextrose broth (*11*) (130 ml per flask) with inoculum from 2- to 3-week-old malt agar slants. Red color developed in the culture liquid in about 2 weeks; batches of the red liquid were acidified with HCl to a *p*H of 2 to 3, and the resulting yellow solution was extracted exhaustively with ethyl acetate. The extracts were dried with Na_2SO_4 and concentrated under reduced pressure. The pigment crystallized in orange prisms, yielding 50 to 60 mg per liter. After two recrystallizations from 95 percent ethanol, the crystals melted at 308° to 314°C (after subliming between 235° and 260°C). Found: C, 55.14; H, 3.63; O, 41.31. Calculated for $C_{14}H_{10}O_8$: C, 54.91; H, 3.29; O, 41.80.

Kuhn-Roth determination gave 78 percent of the theoretical value for two methyl groups. The ultraviolet absorption spectrum had λ_{max}^{EtOH} 290 mμ (log ε, 4.60), and a broad maximum 425 to 450 mμ (log ε, 2.85). The infrared absorption (IR) spectrum was identical with that of oosporein obtained from *V. psalliotae* Treschow (Fig. 1).

Direct acetylation of the residue from the mother liquors of the crude crystals with acetic anhydride, yielded a product which, after recrystallization from methanol, proved identical (mp, mixed mp, and IR spectra) with the leuco-

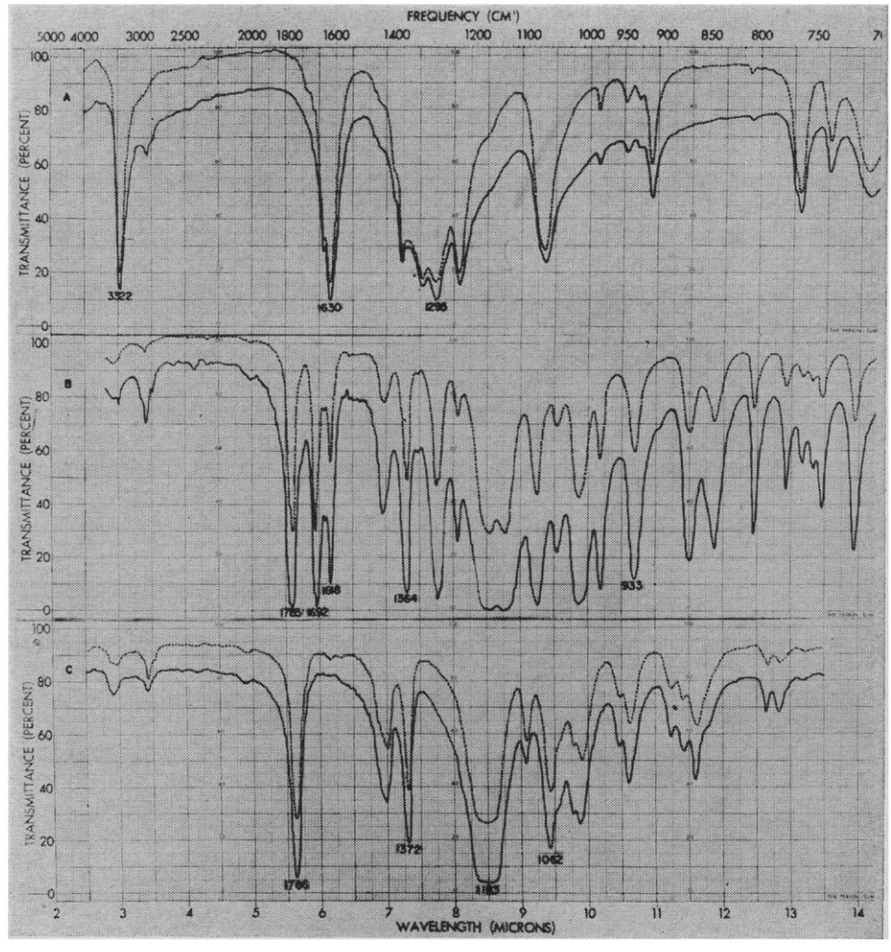


Fig. 1. Comparison of infrared absorption spectra (KBr disc) of oosporein (*A*), acetate (*B*), and leucoacetate (*C*) from *Phlebia* species (—) and from *Verticillium psalliotae* Treschow (- - -).

acetate obtained by reductive acetylation of the pigment.

For preparation of the acetate, a suspension of 8 mg of the *Phlebia* pigment in 2 ml of acetic anhydride was kept at room temperature overnight. The solution was dried under reduced pressure and the residue was recrystallized from methanol, giving a high yield of yellow needles melting at 233° to 234°C. Recrystallization from ethyl acetate or from cyclohexane yielded yellow needles melting at 190° to 191°C. The IR spectra of the two forms in $CHCl_3$ solution of KBr disc were indistinguishable. The ultraviolet absorption spectra had λ_{max}^{ETOH} 262 mμ (log ϵ, 4.39) and a shoulder, 310 to 340 mμ (log ϵ, about 3). The melting point of a mixture of the two forms lay between the respective melting points. For analysis, a sample of the higher-melting form was dried under reduced pressure at about 117°C. Found: C, 56.29; H, 4.04; O, 39.97. Calculated for $C_{22}H_{18}O_{12}$: C, 55.70; H, 3.80; O, 40.48.

Both forms of the acetate could be obtained also from authentic oosporein (from *V. psalliotae*), and these samples did not depress the melting point of samples of the *Phlebia* pigment tetraacetate; IR spectra were identical (Fig. 1).

The leucoacetate was prepared by shaking a suspension of 10 mg of *Phlebia* pigment and 10 mg of PtO_2 catalyst in 10 ml of acetic anhydride in a hydrogen atmosphere; after 30 minutes the mixture became colorless. The catalyst was filtered off and the solvent was removed under reduced pressure. Recrystallization of the residue from methanol yielded 10 mg of colorless prisms melting at 250° to 251°C. These were dried under reduced pressure at about 117°C for analysis. Found: C, 55.97; H, 4.66; O, 38.39; molecular weight, 582. Calculated for $C_{30}H_{30}O_{16}$: C, 55.72; H, 4.68; O, 39.60; molecular weight, 646.

The melting point of the leucoacetate was not depressed by mixture with a sample of the corresponding derivative prepared from authentic oosporein and their IR spectra were identical (Fig. 1). The ultraviolet absorption spectra had λ_{max}^{ETOH} 272 mμ (log ϵ, 3.43). The butyrate was prepared by warming a solution of 15 mg of the pigment in 5 ml of butyric anhydride on the steam bath for 30 minutes. The yellow crytalline residue left by evaporation of the solvent was recrystallized from cyclohexane and yielded 8 mg of yellow prisms melting at 105° to 107°C. Found: C, 61.42; H, 5.84; O, 32.74. Calculated for $C_{30}H_{34}O_{12}$: C, 61.47; H, 5.84; O, 32.83.

Identity of *P. albida* pigment with *P. mellea* pigment was established by comparison of the leucoacetates. The crude pigment obtained from *P. albida* was acetylated directly with acetic anhydride; recrystallized from methanol, the product yielded yellow crystals. The IR spectra of these indicated a mixture of oosporein acetate and leucoacetate. Reductive acetylation of this mixture with acetic anhydride and zinc dust on the steam bath for 30 minutes yielded a crystalline solid, which was purified by adsorption on a silica gel column and elution with a mixture of benzene and ethyl acetate. The IR spectrum of this leucoacetate (KBr pellet) was identical with that of the leucoacetate from *P. mellea*.

The nmr spectrum of the *Phlebia* pigment in deuterodimethylsulfoxide showed only a single peak at τ8.13 (aromatic methyl protons); no aromatic hydrogen was observed. Hydroxyl proton peaks were obscured by background, but the acetate showed two sets of acetoxymethyl protons at τ7.65 and τ7.75, besides the aromatic methylprotons, τ7.97 (relative intensities 6:6:6). The leucoacetate showed three separate methyl peaks: τ7.73 and τ7.95 acetoxymethyl and τ7.93 aromatic methyl (relative intensities 12:12:6). Neither acetate showed a peak in the aromatic proton region.

Vining, Kelleher, and Schwarting (*9*) likewise identified as oosporein a red pigment produced by a culture originally identified as a strain of the basidiomycete *Amanita muscaria;* they were investigating this strain as a possible good producer of the terphenyl quinone pigment, muscarufin. But the culture, like another supposed basidiomycete culture cited, proved to be a *Beauveria* (Fungi Imperfecti).

Since Vining *et al.* made it apparent that mistaking a species of *Beauveria* for a basidiomycete is quite possible, we felt that thorough checking of our *Phlebia* cultures was called for. Rogerson, of this institution, examined our cultures of *P. mellea* and *P. albida* and reported that the mycelia are septate and produce clamp connections; no evidence of the presence of Fungi Imperfecti could be found.

To our knowledge, the only other naturally occurring bibenzoquinone reported is phoenicin (*12*), isolated from a *Penicillium* (*13*). This is therefore the first reported isolation of a compound of this type from a basidiomycete. Although leucophoenicin is reported to occur (*14*) we have found no previous report of the natural occurrence of the leuco form of oosporein. Its presence supports the suggestion (*6*) that oosporein, like phoenicin (*13*), may function as a respiratory pigment.

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References and Notes

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Pyramidal Tract: A Comparison of Two Prosimian Primates

Abstract. *The pyramidal tract of the slow loris* (Nycticebus coucang) *is found in the lateral funiculus of the spinal cord and extends throughout its entire length. Such a course is typical of primates. In the Malayan tree shrew* (Tupaia glis) *the tract occupies a position in the ventral portion of the dorsal funiculus, and in our studies it could not be traced beyond the thoracic cord. In the spinal cord of the slow loris, pyramidal fibers are distributed to the dorsal, intermediate, and ventral gray columns of both sides, while in the tree shrew they are largely restricted to the dorsal horn and do not cross to the opposite side.*

Considerable information may be found in the literature concerning the course of the pyramidal tracts in various animals. At present some data are