EPIRODIN, A POLYENE ANTIBIOTIC FROM THE MOLD *EPICOCCUM NIGRUM*

Sir:

We recently reported the isolation of two amorphous red photosensitive pigments from the pigmented mold *Epicoccum nigrum*, which had antibiotic properties.[1] These pigments represent a new class of compounds to be isolated from this mold for which the names epirodin A and epirodin B have been proposed.[2] Epirodins A and B are interconvertible in solution and the more stable and predominant form appears to be A. The mixture of A and B is referred to simply as epirodin.

A characteristic feature of both A and B is a strong absorption maximum at 429 nm (in ethanol) with a shoulder at 450 nm and the suggestion of a shoulder at 410 nm. The spectrum is almost identical in shape to those of flavomycoin (λ_{max}, 363 nm, in methanol)[3] and dermostatin (λ_{max}, 385 nm, in ethanol)[4], and is a typical blurred or degraded spectrum exhibited by carbonyl-conjugated polyenes. Since flavomycoin is a pentaene and dermostatin a hexaene, the absorption at 429 nm would suggest that the epirodins might be carbonyl-conjugated octaenes. POZSGAY[5] has reported that the acetyl derivatives of lactone-conjugated pentaenes in cyclohexane solution exhibit spectra characteristic of the "classical" hexaenes. Acetylated epirodin gave a prominent peak in cyclohexane at 437 nm and shoulders at 465 and 417 nm as the three longest wavelength absorptions. These would correspond most clearly to a "nonaene" spectrum.[6] C_{25}-β-apo-carotenal, with 7 double bonds in conjugation with a carbonyl function has a λ_{max} at 414 nm in petroleum ether and C_{27}-β-apo-carotenal with 8 double bonds in conjugation, has a λ_{max} at 437 nm.[7a] Since endocyclic double bonds (present in the carotenals) and petroleum ether (when compared with ethanol) both tend to show hypsochromic blue shifts, this comparison would tend to indicate that epirodin is a heptaene. Also, using HIRAYAMA's[8] calculations for an acyclic conjugated carbonyl compound, a heptaene would be expected to absorb at about 425 nm in ethanol and an octaene at about 446 nm. However, if one compares the longest wavelength peak of the non-carbonyl zeta-carotene, which contains 7 acyclic conjugated double bonds, 425 nm in hexane[9] and probably 5~6 nm longer in ethanol[7b], with the longest wavelength peak of the non-carbonyl-conjugated heptaene macrolides, 401~408 nm in ethanol[6], one finds that a significant hypsochromic shift occurs in the macrolides. Therefore direct comparisons with the carotenoids would tend to underestimate the number of double bonds in the macrolides.

A prominent band at 1680 cm^{-1} in the infrared spectrum of epirodin was in the same region as the C=O stretching band of conjugated unsaturated carbonyl compounds and of the lactone-conjugated polyenes flavomycoin (1705 cm^{-1})[8], flavofungin (1680 cm^{-1})[10], mycoticins (1695 cm^{-1})[11], and dermostatin (1700 cm^{-1})[12]. The bulk of the evidence therefore suggests that epirodin is a carbonyl-conjugated octaene. Epirodin was not readily reduced by sodium borohydride but refluxing with sodium borohydride in tetrahydrofuran for 20 hours resulted in an ether-soluble material showing principal longest wavelength peaks at 408, 385, and 365 nm in ethanol, corresponding to a heptaene rather than what would be expected for an octaene. Reduction of the α,β-double bond may have taken place as has been observed in the lithium aluminum hydride reduction of the lactone-conjugated pentaene flavomycoin under drastic conditions to a tetraene[13] and in the reduction, with ease, of Δ^2-cyclopentenones by sodium borohydride.[14]

Exposure of epirodin in ethanol in the presence of iodine to light results in a decrease in absorbance of the main peak and a shift to shorter wavelengths. At the same time there is an increase in absorbance and the appearance of a shoulder at 355 nm. Since, in the case of the carotenoids, a *trans* to *cis* iodine-catalyzed photoisomerization results in a hypsochromic blue shift and a hypochromic effect and in the appearance of a *cis*-peak in the 320~380 nm region[15] the polyene system in epirodin appears to be all *trans*.

In aqueous alkaline solution, epirodin exhibits the same spectrum as in alcohol. However, when the solution is acidified, a drastic decrease in absorbance and a hypsochromic shift to *ca.* 400 nm occurs. Addition of ethanol to the acidic aqueous solution restores the original spectrum. This type of spectral degradation in aqueous solution with acid and its

reversal by ethanol has been observed with the heptaene macrolides[6] and is attributed to the physical condition of the solute—its tendency to form micelles and colloidal dispersions when the pH is lowered and it become increasingly insoluble.

Although the macrolide nature of epirodin has not been established, such a structure might be inferred because, like the known polyene macrolides, a large number of hydroxyl groups appear to be present in the molecule. This is indicated by its insolubility in non-polar solvents, a strong broad O-H stretching band at 3350 cm^{-1} in the infrared, and reaction with periodate. The NMR spectrum of acetylated epirodin in deuterochloroform also showed the presence of vinyl protons, acetyl group protons, and $\mathrm{HC}(-)-\mathrm{O}-$ protons as one would expect from a polyene macrolide. Epirodin also shares some of the biological properties of the antifungal polyene macrolides[16]. It inhibits the growth of *Saccharomyces cerevisiae* and the inhibition can be reversed by sterols. It promotes the hemolysis of erythrocytes, and the hemolysis can be inhibited by sterols, serum, and serum albumin.

The epirodins thus appear to be carbonyl-conjugated octaenes which possess some of the structural features and biological activities of the polyene macrolides of streptomycete origin. The difficulty in isolating epirodin in a pure state and the instability of the pigment have precluded obtaining more detailed information concerning its structure at the present time.

Note: Samples of epirodin were sometimes found to be grossly contaminated with the plasticizer di-(2-ethylhexyl)-phthalate. Although this would not seriously affect the interpretation of results based on visible spectra, the NMR and IR spectra would be seriously affected. The IR spectrum attributed erroneously to epirodin[1] is mainly that of the plasticizer. The IR and NMR results described in this paper are from plasticizer-free preparations.

Acknowledgements

This work was supported by Hatch Project-205. Published with the approval of the Director of the New Hampshire Agricultural Experiment Station as Scientific Contribution No. 886.

MIYOSHI IKAWA[a]
CAROL J. MCGRATTAN[a]
WILLIAM R. BURGE[a]
ROBERT C. IANNITELLI[a]
J. JOHN UEBEL[b]

Departments of Biochemistry[a] and Chemistry[b]
University of New Hampshire
Durham, New Hampshire 03824, U.S.A.

TAMAO NOGUCHI

Laboratory of Marine Biochemistry
Faculty of Agriculture
University of Tokyo
Bunkyo-ku, Tokyo 113, Japan

(Received September 8, 1977)

References

1) BURGE, W. R.; L. J. BUCKLEY, J. D. SULLIVAN, Jr., C. J. MCGRATTAN & M. IKAWA: Isolation and biological activity of the pigments of the mold *Epicoccum nigrum*. J. Agr. Food Chem. 24: 555~559, 1976
2) BURGE, W. R.: Biologically active pigments from *Epicoccum nigrum*. Doctoral Dissertation, Univ. of New Hampshire, Durham, NH, 1972
3) SCHLEGEL, R. & H. THRUM: A new polyene antibiotic, flavomycoin. Structural investigations. I. J. Antibiotics 24: 360~367, 1971
4) NARASIMHACHARI, N. & M. B. SWAMI: Dermostatin: a revised hexaene structure. J. Antibiotics 23: 566, 1970
5) POZSGAY, V.: Ultraviolet absorption spectrum of lactone-conjugated polyene antibiotics. J. Antibiotics 28: 344, 1975
6) OROSHNIK, W. & A. D. MEBANE: The polyene antifungal antibiotics. Fortschr. Chem. Org. Naturst. 21: 17~79, 1963
7) VETTER, W.; G. ENGLERT, N. RIGASSI & U. SCHWIETER: IV. Spectroscopic methods. *in* "Carotenoids" (*edit.* by O. ISLER), (a) p. 193, (b) p. 198, Birkhauser Verlag, Basel, 1971
8) HIRAYAMA, K.: Absorption spectra and chemical structure. IV. Unsaturated aldehydes, ketones, and carboxylic acids. J. Am. Chem. Soc. 77: 383~384, 1955
9) GOODWIN, T. W.: Carotenoids. p. 35, Chemical Publishing Co., New York, 1954
10) BOGNÁR, R.; B. O. BROWN, W. J. S. LOCKLEY, S. MAKLEIT, T. P. TOUBE, B. C. L. WEEDON & K. ZSUPÁN: The structure of flavofungin. Tetrahed. Lett. 1970: 471~474, 1970
11) WASSERMAN, H. H.; J. E. VAN VERTH, D. J. MCCAUSTLAND, I. J. BOROWITZ & B. KAMBER: The mycoticins, polyene macrolides from *Streptomyces ruber*. J. Am. Chem. Soc. 89: 1535~1536, 1967
12) PANDEY, R. C.; K. L. RINEHART, Jr., D. S.

MILLINGTON & M. S. SWAMI: Polyene antibiotics. VI. The structures of dermostatins A and B. J. Antibiotics 26: 475~477, 1973

13) SCHLEGEL, R. & H. THRUM: A new polyene antibiotic, flavomycoin. Structural investigations. II. J. Antibiotics 24: 368~374, 1971

14) BROWN, H. C. & H. M. HESS: Selective reductions. XIII. The reaction of Δ^2-cyclopentenones with representative complex hydrides. Aluminum hydride as a selective reagent for the reduction of the carbonyl group in Δ^2-cyclopentenones. J. Org. Chem. 34: 2206~2209, 1969

15) ZECHMEISTER, L.: *Cis-trans* isomeric carotenoids vitamin A and arylpolyenes. pp. 25~45, Springer-Verlag, Wien, 1962

16) MCGRATTAN, C. J.: The chemistry and biological activity of epirodin, a heptaene antibiotic from *Epicoccum nigrum*. Doctoral Dissertation, Univ. of New Hampshire, Durham, NH, 1976