Polyene Antibiotics

William Oroshnik, Leo C. Vining, Alexander D. Mebane, Willard A. Taber

Ortho Research Foundation, Raritan, New Jersey, and
Institute of Microbiology, Rutgers, University, New Brunswick, New Jersey

DURING the past few years a considerable number of antibiotics of a highly unsaturated nature have been isolated. Of these, mycomycin (I), agrocybin (II), and diatretyne I (III), have had their structures elucidated, and the highly unsaturated constituent of fumagillin (IV), has been shown to be 2,4,6,8-decatetraene-1,10-dioic acid.

$$HC{\equiv}C{-}C{\equiv}C{-}CH{=}C{=}CH{-}CH{=}CH{-}CH_2COOH \quad (I)$$

$$CH_3{-}C{\equiv}C{-}C{\equiv}C{-}C{\equiv}C{-}CONH_2 \quad (II)$$

$$HOOC{-}CH{=}CH{-}C{\equiv}C{-}C{\equiv}C{-}CONH_2 \quad (III)$$

$$HOOC{-}CH{=}CH{-}CH{=}CH{-}CH{=}CH{-}CH{=}CH{-}COOH \quad (IV)$$

These materials have aroused widespread interest since they represent the first examples of highly conjugated nonisoprenoid chromophores to be found among the products of microorganisms. The presence of conjugated polyenyne chromophores, such as those of (II) and (III), has also been demonstrated in the higher plants as far back as 1935 by Russian workers (*1*) and very recently by Sorensen and his coworkers (*2*).

Since 1950 a number of new antibiotic substances have been reported whose ultraviolet absorption spectra, while characteristic of highly conjugated systems, are nevertheless fundamentally different from those of polyenyne compounds. This is illustrated in Fig. 1. Despite this, workers in the field have continued to group all of these unsaturated antibiotics together.

It is our purpose here to point out that the new antibiotics can definitely be characterized as conjugated polyenes rather than polyenynes by their ultraviolet absorption spectra. Figures 2, 3, and 4 illustrate the very close relationship between the spectra of nystatin, flavacid, and candidin and those of known tetraene, hexaene, and heptaene (*3*) examples, respectively. In addition, the position of the wavelengths of the absorption maxima in these spectra indicates with fair certainty that the responsible chromophores are at least disubstituted and at most tetrasubstituted.

Table 1 gives the wavelengths of the absorption maxima of nine recently isolated antibiotics. These show wave number separations of 140–152 mm^{-1} and 139–155 mm^{-1} characteristic of conjugated polyene systems. They fall into three distinct groups, each of which appears to contain the same chromophore. In the first, this is a tetraene (Fig. 2). Although possessing an identical chromophoric group, rimocidin and actimycoin are known to be different from nystatin and from each other. Flavacid, the only member of the second group so far reported, contains a hexaene (Fig. 3). In the third group, the chromophore is a heptaene (Fig. 4). Of these four antibiotics, candidin and candicidin have been proved different; ascosin and trichomycin are very similar to candicidin but cannot as yet be proved identical or nonidentical with it. A number of other antifungal agents with the same absorption spectrum have been isolated but not as yet reported.

Although fumagillin is also a polyene antibiotic (*3*), it nevertheless differs significantly in several respects from the polyene antibiotics just discussed. Its absorption curve (Fig. 5) shows considerably less fine structure than is seen in the curves of the others, which would indicate that the carboxyl groups in the polyene acid (IV) are conjugated with the chromophore. The effect of a conjugated carbonyl on the fine structure of the absorption spectrum is clearly illustrated in Figs. 4 and 5. Preliminary chemical evi-

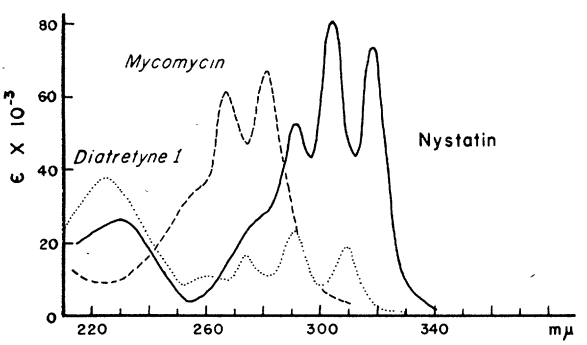

Fig. 1. Ultraviolet absorption spectra of polyunsaturated antibiotics, in ethanol or methanol. [Redrawn from references cited in text.]

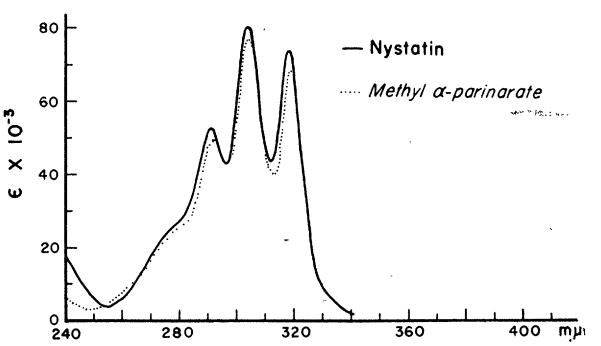

Fig. 2. Ultraviolet absorption spectra, in ethanol, of nystatin and methyl α-parinarate, C_2H_5 $(CH{:}CH)_4(CH_2)_7$ COOMe [J. P. Riley, *J. Chem. Soc.* 2579 (1951)].

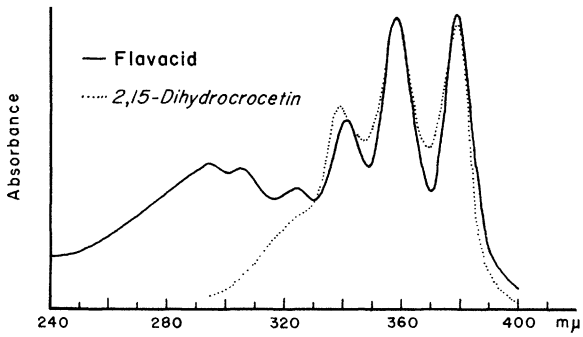

Fig. 3. Ultraviolet absorption spectra, in ethanol, of flavacid and 2,15-dihydrocrocetin, HOOC—CH(Me)—CH: CH—CH: C(Me)—CH: CH—CH: CH—C(Me): CH—CH: CH—CH(Me)COOH [A. Smakula, *Angew. Chem.* **47**, 657 (1934)]. The absorbance of the antibiotic is arbitrarily shown as equal to that of the reference compound.

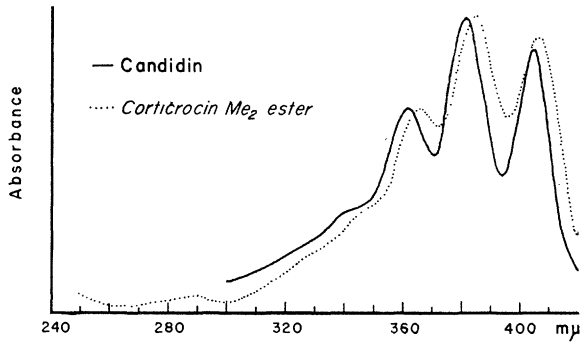

Fig. 4. Ultraviolet absorption spectra, in ethanol, of candidin and corticrocin dimethyl ester, $MeOOC(CH:CH)_6COOMe$ [H. Erdtman, *Acta Chem. Scand.* **2**, 209 (1948)]; curve corrected for bathochromic effect of chloroform solvent used. The absorbance of the antibiotic is arbitrarily shown as equal to that of the reference compound.

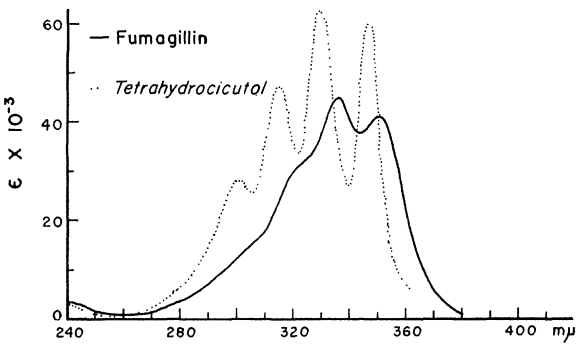

Fig. 5. Ultraviolet absorption spectra, in ethanol, of fumagillin and tetrahydrocicutol, $C_4H_9(CH:CH)_5CH_2CH_2CH_2OH$ [E. F. L. J. Anet, *et al., J. Chem. Soc.* 309 (1953)].

dence obtained with candicidin corroborates the spectral evidence that its acidity is not due to a conjugated carboxyl group.

In addition fumagillin shows a totally different antibiotic spectrum from those of the other substances listed in Table 1. The latter are characterized by high activity against the yeasts and fungi and a very low order of activity against most other microorganisms. It is also noteworthy that fumagillin is a product of the mold *Aspergillus fumigatus,* and the remaining substances have all been isolated from streptomycetes and represent a high proportion of the antifungal antibiotics so far isolated from this genus. It appears therefore, that at least two distinct types of antibiotics containing a conjugated polyene system exist in nature.

References and Notes

1. V. V. Williams, V. S. Smirnov, and V. P. Gol'mov, *J. Gen. Chem. (U.S.S.R.)* **5**, 1195 (1935).
2. N. A. Sorensen *et al., Acta Chem. Scand.* **8**, 26, 34, 280 (1954).
3. Although corticrocin contains eight conjugated double bonds, two of these being derived from carboxyl groups, its spectrum indicates, in line with A. Smakula's generalization [*Angew. Chem.* **47**, 657 (1934)] that only one of the carbonyl groups shows the spectral effects of conjugation. Hence, corticrocin exhibits the spectrum of a conjugated heptaene rather than an octaene and is used here for want of a better example. Fumagillin, for the same reason, shows the spectrum of a conjugated pentaene rather than a hexaene.
4. F. Raubitscheck, R. F. Acker, and S. A. Waksman, *Antibiotics & Chemotherapy* **2**, 179 (1952).
5. E. L. Hazen and R. Brown, *Proc. Soc. Exptl. Biol. Med.* **76**, 93 (1951).

Table 1. Absorption spectra of polyene antibiotics.

	λ_1(mμ)	λ_2(mμ)	λ_3(mμ)	$\bar{\nu}_1-\bar{\nu}_2$(mm^{-1})	$\bar{\nu}_2-\bar{\nu}_3$(mm^{-1})	Solvent
Antimycoin (*4*)	291	304–5	318	152	139	EtOH
Fungicidin (Nystatin) (*5, 6*)	292	304.5	318	141	139	EtOH
Rimocidin (*7*)	291	304	318	147	144	80% MeOH
Chromin (*8*)	292.5	305	320	140	154	EtOH
Flavacid (*9*)	341	358	379	141	155	EtOH
Candidin (*10*)	363	383	406	144	148	EtOH
Candicidin (*11*)	359.5	379.5	401.5	147	146	EtOH
Trichomycin (*12*)	364	384	406	143	141	EtOH
Ascosin (*13*)	358	377	399	141	146	MeOH
Fumagillin (*14*)	$322_{infl.}$*	336	351	130	120	EtOH

* Inflection point.

6. J. D. Dutcher, G. Boyack, and S. Fox, *Antibiotics Annual, 1953–1954* (Medical Encyclopaedia, New York), p. 191.
7. J. W. Davisson *et al.*, *Antibiotics & Chemotherapy* **1**, 289 (1951).
8. S. Wakaki *et al.*, *J. Antibiotics (Japan)* **6B**, 247 (1953).
9. Spectra taken on a sample kindly supplied by J. Takahashi, Tohoku University, Japan.
10. W. A. Taber, L. C. Vining, and S. A. Waksman, *Antibiotics & Chemotherapy* **4**, 455 (1954).
11. H. Lechevalier *et al.*, *Mycologia* **45**, 155 (1953). The wavelengths of the absorption maxima of different batches of candicidin vary somewhat. Those given refer to a specimen prepared by the Heyden Chemical Co.
12. S. Hosoya, *J. Antibiotics (Japan)* **5**, 564 (1952).
13. R. J. Hickey *et al.*, *Antibiotics & Chemotherapy* **2**, 472 (1952).
14. T. E. Eble and E. R. Hanson, *Antibiotics & Chemotherapy* **1**, 54 (1951).

University of Pennsylvania Radiocarbon Dates I

Elizabeth K. Ralph

University Museum and *Department of Physics, University of Pennsylvania, Philadelphia*

CONSTRUCTION of a carbon-14 dating laboratory at the University of Pennsylvania was started in October 1951 (*1*). The equipment is essentially the same as that developed by Anderson, Arnold, and Libby (*2*). Numerous sieges of contamination and other minor difficulties have delayed the completion of the first series of results until this time.

Initial and subsequent control measurements have been made with "dead" anthracite and modern wood samples. With the exception of periods of contamination, the anthracite samples have shown no net counts greater than two σ different from the background rates which have varied between 4.0 and 7.0 counts/min. The modern wood determinations were lower than expected (*3*). Therefore, archeological samples of known age were measured as shown in Fig. 1. The line through these points is drawn with the C^{14} half-life slope [5568 ± 30 yr (*4*)]. Eight determinations of 10- to 50-yr old modern wood from New Jersey and Pennsylvania have given an average value of 6.42 ± 0.04 counts/min; two runs of young poplar from Afghanistan, 6.37 ± 0.12 counts/min. The graph of known-age samples indicates that the basis for the time scale should be higher. Since the unknown samples are comparable in physical and chemical characteristics to those of known age rather than to modern wood, it was decided to base the age calculations on the figure obtained from the archeological measurements. (This corresponds to the assumption of a value of 6.70 ± 0.10 counts/min for modern wood). When more data are available from this and other laboratories, it is hoped that this discrepancy will be clarified.

The error listed with each age includes the standard error σ and the ± 0.10 uncertainty for the time scale. Counting times have been limited to 48 hr. The prefix "P-" is used to designate the sample numbers for this laboratory. The letters *a*, *b*, or *c* following a number represent the 2nd, 3rd, or 4th portion of a particular sample. A separate counting run has been made on each portion.

The caves that have been dated were excavated by Carleton S. Coon. Belt Cave was first excavated in 1949 (*5*). Samples of charred bone then taken were dated by Libby (*6*). In 1951 Belt Cave was excavated further, and work at Hotu Cave was begun. The samples dated in this laboratory consisted of charcoal collected from many levels in both caves. In the few cases where these dates correspond with levels dated by Libby, it must be remembered that his samples were charred bone, collected in 1949, whereas the ones in the present list were charcoal, obtained in 1951.

Belt (*Ghar-i-kamarband*) and Hotu are two neighboring caves eroded out of Jurassic limestone by wave action during some early high-water period of the Caspian Sea. They are situated in the face of a limestone crop just south of the railroad and motor roads on the southern Caspian shore of Iran, 4½ mi west of Behshahr and an equal distance south of the present shoreline, at about latitude 36°20′ N, longitude 53°35′ E, just east of the modern village of Turujan. The unexcavated floor of Belt Cave lay 15.42 m above the 1951 Caspian shoreline; that of Hotu, 18.37 m. The rock-bottom of Belt, which was reached by excavation, lies 9.7 m above this line, and the lowest point reached in excavating Hotu was 6.0 m above this line.

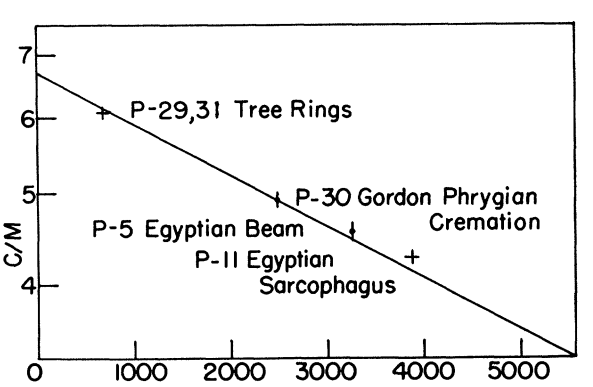

Fig. 1. Counting rates for samples of known age.