Tetrahedron Letters No.17, pp. 1589-1595, 1967. Pergamon Press Ltd. Printed in Great Britain.

ACTINOBOLIN. I. BASIC DEGRADATION STUDIES AND THE STRUCTURE OF A MAJOR DEGRADATION PRODUCT (1)

Robert F. Struck, Martha C. Thorpe, W. C. Coburn, Jr., and Y. Fulmer Shealy

Kettering-Meyer Laboratory, Southern Research Institute
Birmingham, Alabama 35205

(Received 11 February 1967)

Actinobolin, an antibiotic obtained from a Streptomyces culture (2), has been shown to possess broad-spectrum antibacterial activity (3) as well as some antitumor (4, 5) and antileukemic (6) activity. Haskell and Bartz (2) reported an empirical formula of $C_{13}H_{20-22}N_2O_6$ for actinobolin free base.

By mass spectral studies we have shown that the molecular formula of the antibiotic is $C_{13}H_{20}N_2O_6$. A high-resolution mass measurement of the molecular ion gave m/e = 300.1325. The calculated m/e value for $C_{13}H_{20}N_2O_6$ is 300.1321.

Basic degradation of the antibiotic liberates ammonia, and carbon dioxide is formed upon acidification of the basic solution. Extraction of the basic solution with ether gave no product, but ether extraction after acidification yielded a crystalline degradation product (A). From the aqueous, acidic solution after ether extraction was obtained an amino acid, which was identified as L-(+)-alanine by a comparison of its infrared spectrum, thin-layer chromatographic behavior, and optical rotation with

comparable data for authentic L-(+)-alanine.

A was obtained by degradation of actinobolin in basic solutions ranging from 20% aqueous potassium hydroxide at reflux for one hour to 1 N potassium hydroxide at reflux for 30 minutes. Refluxing a solution of the antibiotic in 0.1 N potassium hydroxide for five minutes did not generate A. The antibiotic was degraded routinely in 1 N potassium hydroxide at reflux for one hour and gave A in crude yield (crystallized on standing) of 38-50%. A was purified by crystallization from benzene-ethanol, giving white needles with a melting point of 115°.

A shows ultraviolet absorption maxima at 225 mμ ($\epsilon = 4.4 \times 10^3$) and 293 mμ ($\epsilon = 3.6 \times 10^3$) in ethanol and at 289 ($\epsilon = 3.2 \times 10^3$), 289 ($\epsilon = 3.1 \times 10^3$), and 273 mμ ($\epsilon = 5.2 \times 10^3$), respectively, at pH 1, 7, and 13. The infrared spectrum is shown in Figure 1. Aromatic and aliphatic C-H, OH, and C=C absorption is apparent. The optical rotation of A ($[\alpha]_D^{25}$) is $-10.6° \pm 0.8$ (C = 1.0 g. in 100 ml. ethanol).

Elemental analysis of A indicated an empirical formula of C_3H_4O (Found: C, 64.98; H, 7.43), and an ebullioscopic molecular-weight determination suggested a molecular formula of $C_9H_{12}O_3$. Mass spectral analysis verified the suggested molecular formula; a high-resolution mass measurement of the molecular ion gave m/e = 168.0787. The calculated m/e value for $C_9H_{12}O_3$ is 168.0786.

The proton magnetic resonance spectrum of A in DMSO-d_6 showed peaks attributed to a methyl group [doublet at 1.07 p.p.m. (7)], a methylene group (doublet at 2.56 p.p.m.), a methine proton (complex multiplet centered at about 3.9 p.p.m.), a multiplet for three aromatic protons centered at about 6.5 p.p.m., and two types of hydroxyl absorption, the one at 8.54 p.p.m. equivalent to two protons and the other (doublet at 4.74 p.p.m.) equivalent to one proton.

The p.m.r. spectrum of A in D_2O (Figure 2) was consistent with a trisubstituted cycloheptatriene structure (I), since in D_2O the methine proton appeared to be

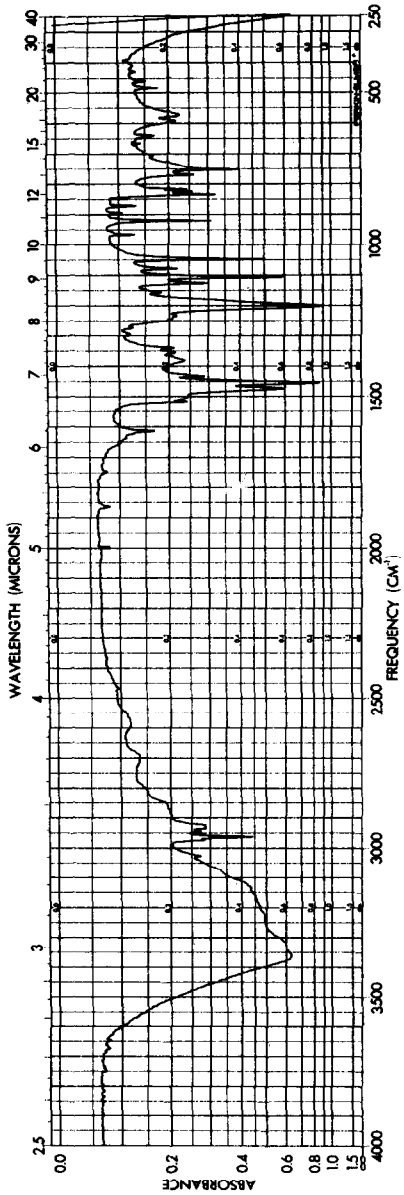


FIG. 1

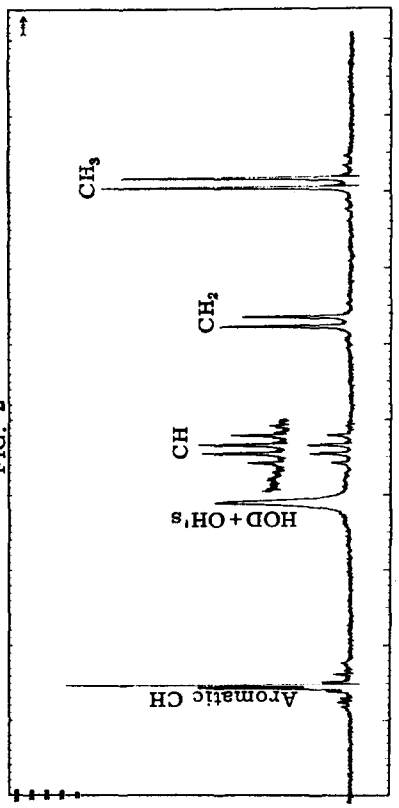
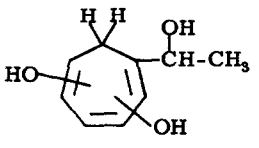


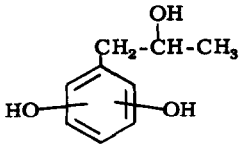
FIG. 2

SOLVENT: D_2O; NOT REFERENCED

a simple quartet, but IR and UV data suggested a 1, 2, 4-trisubstituted phenolic structure such as II (phenyl absorption bands at 1863, 1745, 1612, and 1520 cm^{-1} and the strong -C-O- stretching band at 1200 cm^{-1}). High-resolution mass spectral analysis of A supported the postulation of the presence of an α-hydroxyethyl group but did not distinguish between a cycloheptatriene nucleus and a benzyl nucleus since both would be expected to yield comparable mass fragmentation patterns. Further p. m. r. studies on A with the aid of a homonuclear spin decoupler (Varian V-6058A) allowed a choice to be made between I and II. Spin decoupling experiments proved the methine proton to be equally coupled to the methyl and methylene protons. This results in three quartets so superimposed that an equally-spaced sextet results. In our first spectra the small outer peaks were obscured by the noise, but with improved instrumentation we were later able to observe them. This equal coupling of the methine proton to the methyl and methylene protons supports structure II.



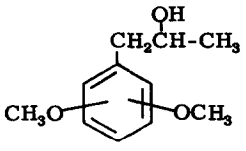
I



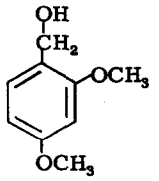
II

Treatment of A with diazomethane in ether-methanol gave a product whose elemental analysis was acceptable for III (Found: C, 67.01; H, 8.42; infrared: $\bar{\nu}_{max}$ (cm^{-1}) 3400, 3060, 3030, 2990, 2965, 2930, 2830, 1600, 1580, 1490, 1460, 1420, 1365, 1300, 1275, 1215, 1170, 1150, 1110, 1040, 1020, 940, 925, 870, 825, 795, 720, 705, 700 (sh), 630, 575, 550, 460) and whose ultraviolet maximum appeared at 287 mμ at pH 1, 7, and 13. The absence of a shift at pH 13 is good evidence for a dimethylated phenol such as III; as mentioned earlier, at pH 13 the ultraviolet maximum of A had shifted to 273 mμ. Ultraviolet spectral comparison was sought for dimethylated A by

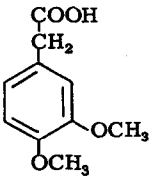
examining maxima of samples of the model compounds IV, V, and VI. Maxima appeared at 276 mμ for IV, at 277-8 mμ for V, and at 287 mμ for VI, at pH 1, 7, and 13. Dimethylated A, then, appeared to be 1-(2, 5-dimethoxy)phenyl-2-propanol (VII).



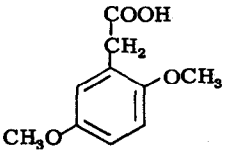
III



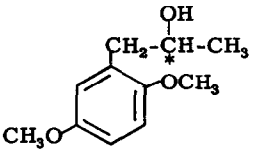
IV



V



VI



VII

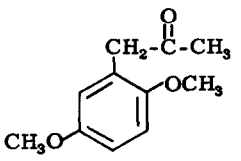
The ketone VIII (8), 1-(2, 5-dimethoxy)phenyl-2-propanone, was synthesized from 2, 5-dimethoxyphenylacetyl chloride and diethyl ethoxymagnesiummalonate (9) (Found: C, 67.84; H, 7.07; b.p. 84-93° at 0.05 mm [reported (8) b.p. 95° at 0.25 mm]; $\bar{\nu}_{max}$ (cm^{-1}) 3050(sh), 2990, 2940, 2900, 2830, 1710, 1600, 1585, 1495, 1460, 1420, 1350, 1315, 1275, 1220, 1175, 1150, 1120, 1040, 1020(sh), 925, 870, 800, 720, 705, 700(sh), 640, 520, 450; λ_{max} (mμ) 288 at pH 1, 7, and 13 [ϵ = 2.7, 2.7, 2.9×10^3, respectively]) and converted into the corresponding secondary alcohol IX, D, L-1-(2, 5-dimethoxy)phenyl-2-propanol (Found: C, 67.42; H, 8.08), by reduction with sodium borohydride in methanol (10). The infrared (liquid, capillary film) and ultraviolet spectra of IX were identical in every respect to the spectra of VII. In addition, VII was oxidized to the corresponding ketone by means of an Oppenauer oxidation and produced VIII (8), which was identified as its 2, 4-dinitrophenylhydrazone. Chemical and spectral data show that A is (-)-1-(2, 5-dihydroxyphenyl-2-propanol (X).

It was interesting to note that a summation of the identified fragments is equivalent to actinobolin plus a molecule of water:

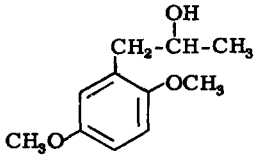
$$C_{13}H_{20}N_2O_6 + H_2O \rightarrow C_9H_{12}O_3 + C_3H_7NO_2 + NH_3 + CO_2$$

$$C_{13}H_{22}N_2O_7 \equiv C_{13}H_{22}N_2O_7$$

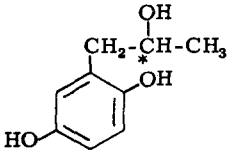
Infrared, ultraviolet, p. m. r., and mass spectral studies on actinobolin itself have not provided sufficient data to permit us to depict a complete structure. The mass fragmentation pattern of the free base was such that no high mass fragments were obtained that could be investigated by high-resolution methods. A consideration of spectral data and the identified degradation products indicates that the degradation of the antibiotic does not proceed by a simple route. Further studies on the structure of actinobolin are in progress.



VIII



IX (D,L-mixture)



X

Acknowledgment. The authors are indebted to Dr. W. J. Barrett and the members of the Analytical Service Section of Southern Research Institute for determinations of optical rotations, elemental analyses, and infrared and ultraviolet spectra. Mass spectral measurements were performed on an A. E. I. MS-9 spectrometer by Dr. R. E. Rhodes of Mellon Institute and on a Hitachi Perkin-Elmer RMU-6D spectrometer by Drs. Morgan and Schaffer of Morgan-Schaffer Corporation; the authors are indebted to these men and to Prof. Fred W. McLafferty of Purdue University and Dr. Roger Foltz of Battelle Memorial Institute for helpful discussions on the interpretation of mass spectra.

REFERENCES

1. This investigation was supported by the Cancer Chemotherapy National Service Center, National Cancer Institute, National Institutes of Health, Contract No. SA-43-ph-1740, and by the C. F. Kettering Foundation.
2. T. H. Haskell and Q. R. Bartz, Antibiotics Annual 1958-1959, Medical Encyclopedia, Inc., New York, 1959, p. 505.
3. R. F. Pittillo, M. W. Fisher, R. J. McAlpine, P. E. Thompson, and J. Ehrlich, ibid., p. 497.
4. M. N. Teller, P. C. Merker, J. E. Palm, and G. W. Woolley, ibid., p. 518.
5. K. Sugiura and H. C. Reilly, ibid., p. 522.
6. J. H. Burchenal, E. A. D. Holmberg, H. C. Reilly, S. Hemphill, and J. A. Reppert, ibid., p. 528.
7. Chemical shifts are in parts per million downfield from tetramethylsilane as internal reference.
8. F. W. Bollinger and M. Sletzinger, Belg. Patent 614,410, August 27, 1962; Chem. Abstr., 59, 1753 f (1963).
9. Organic Syntheses, Collective Volume IV., edited by N. Rabjohn, John Wiley and Sons, Inc., New York, 1963, p. 708.
10(a) N. G. Gaylord, Reduction With Complex Metal Hydrides, Interscience Publishers, Inc., New York, 1956, p. 100. (b) S. W. Chaikin and W. G. Brown, J. Am. Chem. Soc., 71, 122 (1949).