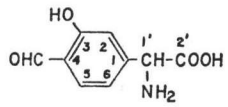
Communications to the editor

THE STRUCTURE OF FORPHENICINE

Sir:

The biological properties, isolation and characterization of forphenicine, a new alkaline phosphatase inhibitor, produced by actinomycetes were reported in the preceding paper[1]. Here we report the chemical structure of forphenicine. Forphenicine is crystallized from water as pale yellow crystals; m.p. >300°C; $[\alpha]_D^{20}$ +140° (*c* 1.0, 1 N HCl); pKa′ <2, 7.3, 9.1 (titration equivalent 195). The molecular formula of forphenicine was established as $C_9H_9NO_4$ by elemental analysis and mass spectrometry (M^+, *m/e* 195).

Forphenicine gave positive ninhydrin, ferric chloride, FOLIN, 2,4-dinitrophenylhydrazine, FEHLING and RYDON-SMITH, but negative SAKAGUCHI and anthrone reactions. On paper electrophoresis (600 V, 30 minutes) using formic acid - acetic acid - water (25: 75: 900), forphenicine moved toward the cathode; Rm 0.34 (Ala=1). Strong absorptions in the infrared spectrum were shown at ν_{max} (KBr) 3150 (intramolecular hydrogen-bonded hydroxyl), 2850~3050 (ammonium), 1660 (intramolecular hydrogen-bonded aldehyde), 1600 and 1400 (carboxylate), 1500 cm^{-1} (phenyl). The NMR spectrum of forphenicine in deuterotrifluoroacetic acid (TMS as the internal reference) showed three aromatic protons at δ 7.91 (1H, doublet, J=8.0 Hz) and δ 7.2~7.5 (2H), a methine proton at δ 5.51 (singlet) and an aldehyde proton at δ 10.02 (singlet). The ultraviolet spectrum of forphenicine in 0.1 M phosphate buffer (pH 7.0) shows maxima at 214 (shoulder), 258 (ε 15210), 324 (ε 3900) and 375 nm (shoulder), in 0.1 N hydrochloric acid solution, maxima at 212 (ε 23790), 255 (ε 15600) and 322 nm (ε 4100), and in 0.1 N sodium hydroxide solution, maxima at 230 (shoulder), 271 (ε 11120) and 377 nm (ε 7610). This characteristic pattern indicates



the presence of salicylaldehyde chromophore in the molecule. Treatment of forphenicine in acetic acid with acetic anhydride at 45°C for 2.5 hours gave colorless needles of N-acetylforphenicine, m.p. 167~169°C (dec.). Anal. Calcd. for $C_{11}H_{11}NO_5$: C, 55.69; H, 4.67; N, 5.91. Found: C, 55.58; H, 4.60; N, 5.80, M^+, *m/e* 237. NMR ($CD_3COOD+D_2O$, TMS): δ 2.36 (3H, singlet, N-acetyl), δ 5.78 (1H, singlet, 1′-H), δ 7.32 (1H, doublet, 2-H, $J_{2,6}$=1.8 Hz), δ 7.39 (1H, double doublet, 6-H, $J_{5,6}$=7.8 Hz), δ 7.99 (1H, doublet, 5-H), δ 10.17 (1H, singlet, aldehyde). Coupling constants of three aromatic protons of N-acetylforphenicine indicated the presence of the 1,3,4-trisubstituted benzene ring shown in the formula described above. As forphenicine contained a salicylaldehyde group, the hydroxyl and aldehyde groups should be on C-3 and C-4, and the methine (C-1′) group, which is substituted with amino and carboxyl groups, should be attached to C-1. Compared with the other two aromatic protons, the chemical shift (δ 7.99) of the proton on C-5 was at the lowest field, and thus the aldehyde group was shown to be attached to C-4, indicating the hydroxyl group to be at C-3. Thus, the structure of forphenicine was elucidated to be 4-formyl-3-hydroxy-phenylglycine. The presence of the salicylaldehyde structure in forphenicine was confirmed by formation of a coumarin derivative. The reaction[2] of N-acetylforphenicine and diethyl malonate in ethanol containing piperidine and glacial acetic acid gave a new coumarin derivative of N-acetylforphenicine. m.p. 196.5~197.5°C (dec.). Anal. Calcd. for $C_{15}H_{15}NO_5$: C, 62.28; H, 5.23; N, 4.84. Found: C, 62.15; H, 5.23; N, 4.75, M^+,

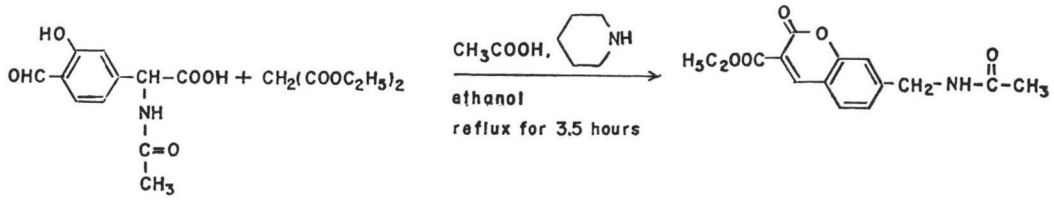
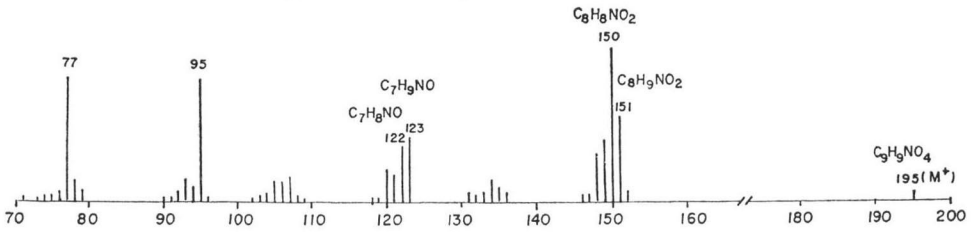


Fig. 1. The mass spectrum of forphenicine



m/e 289. NMR (CD_3COOD, TMS): δ 1.36 (3H, triplet), δ 2.14 (3H, singlet), δ 4.36 (2H, quartet), δ 4.56 (2H, singlet), δ 7.2~7.4 (2H), δ 7.68 (1H, doublet, J=8.0 Hz), δ 8.61 (1H, singlet).

The structure of forphenicine was further confirmed by high resolution mass spectrometry as shown in Fig. 1. This structure has also been proved by its chemical synthesis which will be reported in another paper. The configuration of the asymmetric carbon (C-1') is now under study.

Acknowledgement

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References

1) Aoyagi, T.; T. Yamamoto, K. Kojiri, F. Kojima, M. Hamada, T. Takeuchi & H. Umezawa: Forphenicine, an inhibitor of alkaline phosphatase produced by actinomycetes. J. Antibiotics 31: 244~246, 1978
2) Horning, E. C.; M. G. Horning & D. A. Dimmig: Organic Syntheses. Coll. Vol. III, pp. 165~167, John Wiley & Sons Inc., New York, N.Y., 1955