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 paper1. Here we report the chemical structure of forphenicine. Forphenicine is crystallized from water as pale yellow crystals; m.p. > 300°C; [α]D +140° (c 1.0, 1 N HCl); pKα' < 2, 7.3, 9.1 (titration equivalent 195). The molecular formula of forphenicine was established as C9H9NO4 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 (25:75:90), forphenicine moved toward the cathode; Rm 0.34 (Ala = 1).

Strong absorptions in the infrared spectrum were shown at \( \nu_{\text{max}} \) (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 deuter trifluoroacetic acid (TMS as the internal reference) showed three aromatic protons at \( \delta 7.91 \) (1H, doublet, \( J= 8.0 \) Hz) and \( \delta 7.2 \sim 7.5 \) (2H), a methine proton at \( \delta 5.51 \) (singlet) and an aldehyde proton at \( \delta 10.02 \) (singlet). The ultraviolet spectrum of forphenicine in 0.1 N phosphate buffer (pH 7.0) shows maxima at 214 (shoulder), 258 (\( \varepsilon 15210 \)), 324 (\( \varepsilon 3900 \)) and 375 nm (shoulder), in 0.1 N hydrochloric acid solution, maxima at 212 (\( \varepsilon 23790 \)), 255 (\( \varepsilon 15600 \)) and 322 nm (\( \varepsilon 4100 \)), and in 0.1 N sodium hydroxide solution, maxima at 230 (shoulder), 271 (\( \varepsilon 11120 \)) and 377 nm (\( \varepsilon 7610 \)). 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\(_3\)COOD+D\(_2\)O, TMS): \( \delta 2.36 \) (3H, singlet, N-acetyl), \( \delta 5.78 \) (1H, singlet, 'H), \( \delta 7.32 \) (1H, doublet, 2-H, \( J_{2,3} = 1.8 \) Hz), \( \delta 7.39 \) (1H, double doublet, 6-H, \( J_{3,4} = 7.8 \) Hz), \( \delta 7.99 \) (1H, doublet, 5-H, \( J_{4,5} = 10.17 \) (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 (\( \delta 7.99 \)) 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$_3$COOD, TMS): $\delta$ 1.36 (3H, triplet), $\delta$ 2.14 (3H, singlet), $\delta$ 4.36 (2H, quartet), $\delta$ 4.56 (2H, singlet), $\delta$ 7.2~7.4 (2H), $\delta$ 7.68 (1H, doublet, $J=8.0$ Hz), $\delta$ 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.

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References
