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STUDIES ON THE ANTIMICROBIAL SUBSTANCES OF SPONGES II. STRUCTURE AND SYNTHESIS OF A BROMINE-CONTAINING ANTIBACTERIAL COMPOUND FROM A MARINE SPONGE*

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In a previous paper (1) we described the isolation of four new antibacterial substances from different species of marine sponges. One of these compounds was a broad spectrum bromine-containing antibiotic, m.p. 193-195°C, isolated from the sponge Verongia cauliformis and related species. The elementary analysis and the mass spectrum established the molecular formula $C_8H_7NO_3Br_2$ for this compound. The infrared bands at ν max(nujol) 3445, 3420, 3125, 1700, 1675, 1660, and 1650 cm^{-1} and the uv absorption at λ max(methanol) 257mu (8000) of the compound, and ν max (nujol) 3445, 1749, 1685, 1675 cm^{-1}, λ max(methanol) 266(9000) of its monoacetate suggested the presence of hydroxyl group, amide function and α,β unsaturated ketone. This communication presents chemical evidence for structure and a synthesis which confirms that the antibiotic is 2,6,-dibromo-4-acetamido-4-hydroxy-cyclohexadienone (I, R=H).

The acetyl derivative of the antibiotic** was reduced with excess lithium borohydride in tetrahydrofuran and, after acidification, was worked up to give a phenolic compound $C_8H_7NO_2Br_2$, m.p. 190°C; ν max(nujol) 3410, 3150, 1650, 1615 and 1550 cm^{-1}; λ max(methanol) 290 mu and 283 mu. In a basic medium, the

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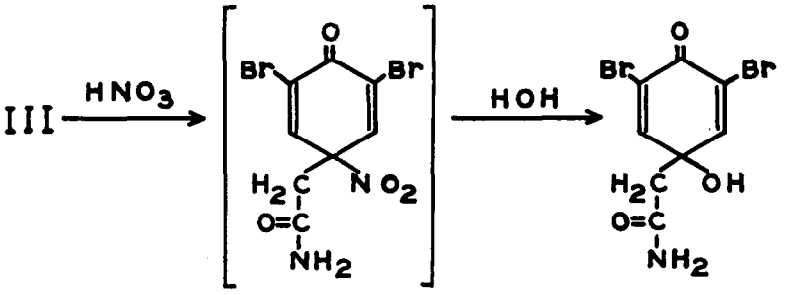
**The method of preparation of the acetyl derivative m.p. 185°C has been reported earlier (1).

To confirm this structure, the dienone (I, R=H) was synthesized by reacting 4-hydroxy-3,5,-dibromophenyl acetamide (III) in acetic acid with concentrated nitric acid according to the procedure developed by Müller, et al. (2) for the synthesis of a similar type of 4-hydroxy dienone. The dienone obtained from our reaction was identical in all respects with the antibiotic isolated from natural sources.

In the n.m.r. spectrum of the antibiotic [singlet bands in deuterated

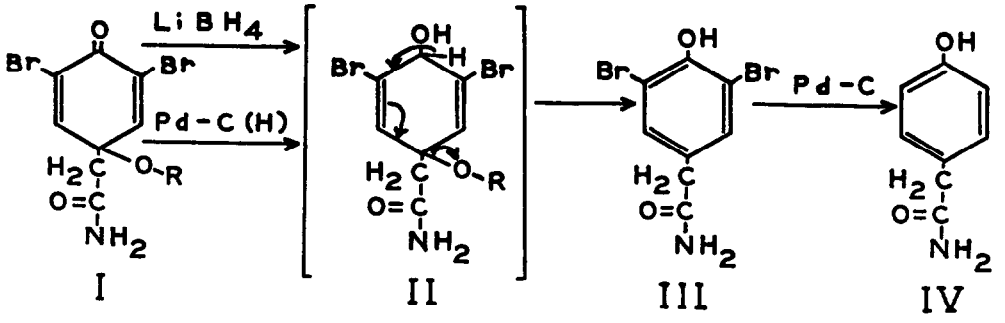


acetone at 2.75 p.p.m. 2H, methylene protons; 2.97 p.p.m. 3H, primary amide and hydroxyl protons; 7.59 p.p.m. 2H, olefinic protons] and of its acetyl derivative (I, R=CH_3CO) [singlet bands at 2.08 p.p.m. 3H, acetyl group; 2.92 p.p.m. 2H, methylene protons; 2.96 p.p.m. 2H, amide protons; 7.75 p.p.m. 2H, olefinic hydrogens] the band due to the protons attached with amide nitrogen is very sharp and appears at an unusually high field. Another characteristic feature of the n.m.r. spectrum of the antibiotic is the shifting of the amide band at 2.97 p.p.m. to 3.72 p.p.m. on addition of a few drops of D_2O without decrease in its relative intensity. However, the amide band completely disappeared (due to exchange) when the spectrum was taken in methanol-d_4 or deuterated water. Further, the singlet character of the olefinic proton absorption band in the n.m.r. spectrum of the antibiotic and of its acetyl derivative is consistant only with the proposed structure (I).

The biosynthesis of the antibiotic is easily envisioned from tyrosine

absorption shifted to λ max 310 mu and 248 mu. This compound was shown, by direct comparison with the synthetic sample*, to be 4-hydroxy-3,5,-dibromo-phenylacetamide(III). By reducing the antibiotic with 0.25 mole of lithium borohydride and neutralizing the reaction mixture with an equivalent amount of hydrochloric acid, a compound which has no uv absorption was isolated. This compound changed into III on crystallization, and also on treatment with excess base or acid. A tentative structure II, R=H was assigned to this intermediate. The reduction of the antibiotic or its monoacetate with sodium borohydride has not been fruitful, as the presence of hydroxide ions, produced by hydrolysis of sodium borohydride, promotes other side reactions** and the isolation of pure product becomes difficult.

On hydrogenation over palladium charcoal the antibiotic absorbed 2.8 moles of hydrogen to give a bromine-free compound with m.p. 168-170°C. This compound was confirmed to be p-hydroxy phenylacetamide (IV) by comparison with an authentic sample. Accepting structure I, R=H for the sponge antibacterial compound, the formation of IV from it can be explained as shown in the sequence II→III→IV. In a separate experiment, III was shown to undergo facile reductive debromination with palladium on charcoal to give IV.



*4-hydroxy-3,5,-dibromo-phenylacetamide is not reported in the literature. It was synthesized from p-hydroxy phenylacetic acid by standard procedures of bromination and amide formation.

**The antibacterial compound, on treatment with one equivalent of .01N sodium hydroxide solution, forms four products which are currently under consideration.

which, after bromination (3), may be transformed into 4-hydroxy-3,5,-dibromo-phenylacetaldehyde through pathways already suggested in the literature (4). The aldehyde can then give rise to 4-hydroxy-3,5,- dibromo-phenylacetamide. Oxidation of the amide to compound I is analogous to the known metabolism of tyrosine itself (5).

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