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STUDIES ON THE ANTIMICROBIAL SUBSTANCES OF SPONGES.

VI. STRUCTURES OF TWO ANTIBACTERIAL SUBSTANCES ISOLATED FROM THE MARINE SPONGE DYSIDEA HERBACEA.*

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While screening marine sponges for antimicrobial activity, the sponge *Dysidea herbacea*, obtained from the western Caroline Islands, was found to inhibit the growth of both Gram-negative and Gram-positive organisms (1,2). Chromatography of the benzene extract of this sponge over a florisil column gave an active fraction which was repeatedly crystallised from benzene to give an antibacterial substance A: mp 185-186°. Steam distillation of the residue obtained after evaporation of the solvent from the combined mother liquors gave another closely related antimicrobial agent B: mp 95-98°. In this communication we wish to report the structures of these two compounds.

Microanalysis and mass spectrometric measurements established molecular composition of $C_{12}H_5O_2Br_5$ (M^+ at m/e 580, 582, 584, 586, 588, and 590) for the antibacterial substance A. The ir spectrum (ν_{max}^{KBr} 3500, 3410, 1580, 1555, 1470, 1440, 890, 875, 862, 841, and 820 cm^{-1}) revealed the presence of a hydroxyl group and phenyl rings. The uv spectrum was similar to that of phenolic compounds with a broad band at λ_{max}^{MeOH} 288 nm (ε4000) which shifted to 303 nm in a basic medium. Further indication of the presence of a phenolic ring was obtained by reacting A with diazomethane to form the monomethyl ether 3, mp 155° in quantitative yield, which no longer showed the typical uv bathochromic shift in base.

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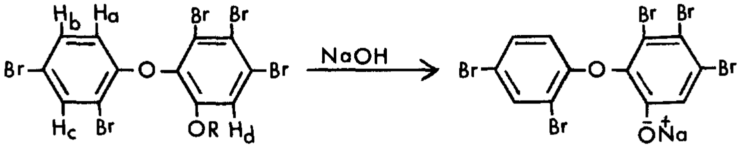

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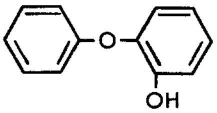

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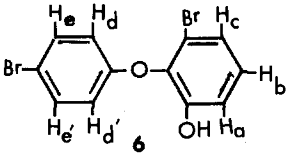
1 $R = H$

2 $R = CH_3-C=O$

3 $R = OCH_3$



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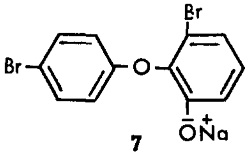

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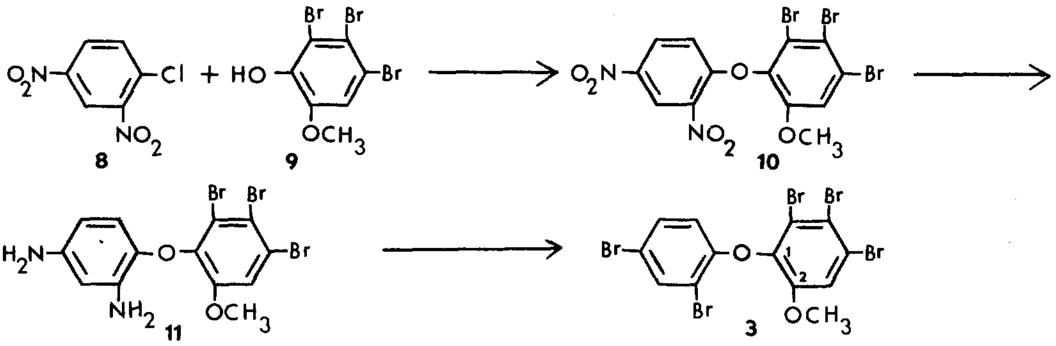
The presence of a single hydroxyl group was confirmed by monoacetylation ($1 \rightarrow 2$) as evidenced by an increase of 42 mass units for its molecular ion and a new methyl singlet at δ2.27 in its nmr spectrum. Since the ir spectrum of A indicated the absence of carbonyl function, the second oxygen atom of this antibacterial substance should be an ether linkage.

Catalytic hydrogenation of A gave the known compound (3) 2-hydroxy-diphenyl ether 5, mp 105°C. The antibacterial substance is, therefore, a pentabromo derivative of 5.

The nmr spectrum of A had the following characteristic features: a hydroxyl proton at δ3.1 (demonstrated by exchange with deuterium oxide); a sharp one proton singlet at δ7.5, which was assigned to H_d, the proton ortho to the phenolic hydroxyl because of its upfield shift to δ6.67 in the spectrum of the phenolate ion 4 (4,5); and finally three multiplets at δ6.56 (d, 1H, J_{ab}= 9Hz, H_a), 7.38 (d of d, 1H, J_{ba} = 9 and J_{bc}=3Hz, H_b) and 7.8 (d, 1H, J_{cb}=3Hz, H_c) due to three aromatic protons arranged in a 1,2,4 pattern on a benzene ring. This spectral data is consistent with the proposed structure 1 for the antibacterial substance A.

The structure 1 assigned to the antibacterial substance A was confirmed by synthesis. The 3,4,5-tribromoguaiacol 9 was prepared as follows (6): bromination of guaiacol with 2 or 3 mols of bromine in carbon disulphide gave 4,5-dibromoguaiacol (mp 88-90°). Bromination of dibromoguaiacol with 1 mol of bromine in acetic acid gave tribromoguaiacol 9 (mp 115°). The condensation of 2,4-dinitrochlorobenzene 8 with 9 in the presence of 1 mol of sodium hydroxide in ethanol afforded 10 (mp 195°) which was reduced with sodium dithionite (7) to give 1-(2',4'-diaminophenoxy)-2 methoxy-4,5,6-tribromobenzene 11 (mp 198-200°) in 50% yield. The compound 11 was tetraazotised and treated with cuprous bromide (Sandmeyer reaction) essentially according to a procedure given in literature (8). The reaction mixture was extracted with ethyl acetate. Processing the organic phase in the usual way afforded a solid which was chromatographed over silica gel. Elution with hexane gave 1-(2',4'-dibromophenoxy)-2-methoxy-4,5,6-tribromobenzene 3 (mp 153-154°) which was identical in all respects with the methyl ether obtained by reacting A with diazomethane.



The second antibacterial substance B ($C_{12}H_8O_2Br_2$; M^+ at m/e 342, 344 and 346, ν_{max}^{KBr} 3500-3400, 1587, 1570, 1480, 1455, 1242, 920, 830, 745, 700 cm^{-1}; λ_{max}^{MeOH} 278 and 285 nm) was shown to be a dibromo derivative of 5 by catalytic hydrogenation. Structure 6 was assigned to this compound on the basis of its 220 MHz nmr spectrum which showed bands in DMSO-d_6 at δ6.84 (d, 2H, J_{ortho}=9Hz, H_d and H_d'), 7.09 (d of d, 1H, J_{ab}=8 and J_{ac}=1.9Hz, H_a), 7.16 (t,1H, J_{ba}=8 and J_{bc}=8Hz, H_b), 7.25 (d of d, 1H, J_{cb}=8 and J_{ca}=1.9Hz, H_c) and 7.56 (d, 2H; J_{ortho}=9Hz, H_e and H_e'). The resonances at δ7.09 and 7.25 were assigned to the protons ortho and para to the phenolic hydroxyl, because of their upfield shift to δ6.70 and δ6.40, respectively, in the nmr spectrum of the phenolate ion 7.

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NOTES & REFERENCES

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