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THE STRUCTURE OF SOME ANTIBIOTICS FROM THE SPONGE <u>IRCINIA</u> <u>STROBILINA</u>

Irvin Rothberg* and Peter Shubiak

Department of Chemistry, Rutgers University, Newark, New Jersey 07102

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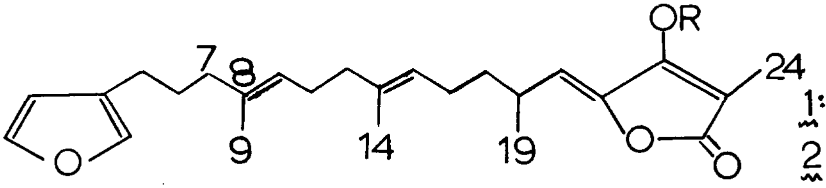
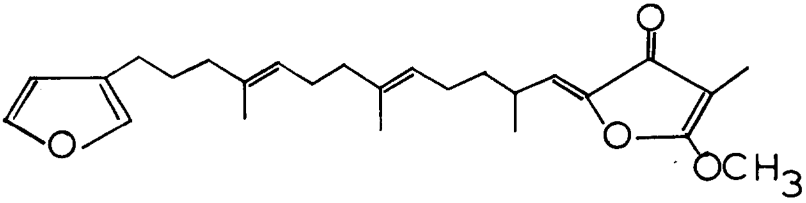
Four related sesterterpenes, which contain one or more furan rings and a tetronic acid moiety, have been isolated from sponges of the genus *Ircinia*.[1,2] We wish to report the isolation and structure elucidation[3] of a new sesterterpene, strobilinin 1 which coexists with variabilin 4 in the sponge *Ircinia strobilina*.



1:R=H
2:R=CH$_3$



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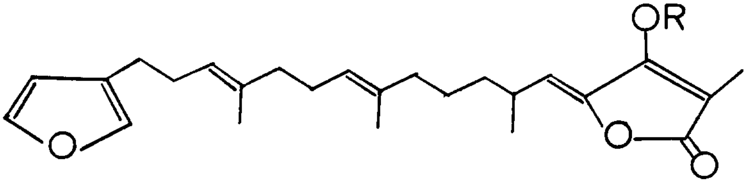
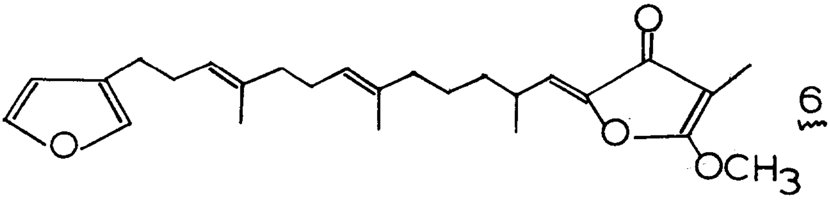


4:R=H
5:R=CH$_3$



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TLC on silica gel (65:35 hexane/dioxane) of an ether extract gave a 0.05% yield of a mixture of 1 and 4 active against *Staphylococcus aureus* and *Bacillus subtilis* at a minimum inhibitory concentration of 3-6 ppm. The uv and ir spectrum were similar to those reported for variabilin 4.[2] The nmr spectrum (100 MHz, $CDCl_3$) was also similar to that reported for variabilin except in the vinylic methyl region. Singlet signals at δ 1.55, 1.59 and 1.65 indicated the presence of vinylic methyl groups at C-9 and C-14. The signal at 1.65 represented about 25% of the other methyl groups and strongly suggested the presence of a second isomer. High resolution mass spectrometry of the mixture of 1 and 4 showed the following fragments: 398.2515 ($M, C_{25}H_{34}O_4$), 317.2162 ($M-C_4H_3OCH_2$), 303.2000 ($M-C_4H_3OCH_2CH_2$), and 289.1822 ($M-C_4H_3OCH_2CH_2CH_2$). The last fragment which represents cleavage of the C7-C8 bond, would not be expected from variabilin, but should be present in strobilinin.[4]

Ozonolysis of the mixture of 1 and 4 followed by oxidative workup and methylation with diazomethane gave two sets of products. Quantitized GLC and GLC-MS using authentic esters as standards showed methyl 5-oxohexanoate and dimethyl 2-methylglutarate from strobilinin 1 and dimethyl succinate and methyl 2-methyl-6-oxoheptanoate from variabilin 4. The two sets of products were in the ratio of 27:73. Methyl levulinate was also found and is common to both isomers.

GLC (3% OV-1) of acetates and trimethylsilyl ether derivatives of the mixture of 1 and 4 both showed two components in the ratio of 25:75. Small samples of the acetates and trimethylsilyl ethers were able to be purified by preparative GLC and each material, from the mixture, was collected in greater than 98% purity. Similarities in the ir, uv and mass spectrum of the collected materials were consistent with the proposal that the two compounds present in the 25:75 mixtures were isomers.

Preparation of methyl ethers was carried out using diazomethane. This leads to a mixture of four compounds (2, 3, 5, and 6) due to the introduction of isomerization within the lactone moiety.[1] Compounds 2 and 5 which represented about 80% of the mixture were separated from 3 and 6 by means of TLC on silica gel (90:10 benzene/ethyl ether). Compounds 2 and 5, which were not separated from each other, had an R_f of 0.72 while 3 and 6 had an R_f of 0.28. This very closely resembles the behaviour reported previously for the reaction of diazomethane with tetronic acid derivatives.[1] GLC of the major zone (10% UCW-98) showed a 25:75 ratio of 2 : 5.

The ir, uv, and mass spectrum of both zones were consistent with two isomeric O-methyl derivatives. The ir spectrum of the major zone (R_f 0.72) corresponding to a mixture of 2 and 5 showed bands at 1760 (5 membered α,β-unsaturated γ- lactone), 1640 (olefin), 1500, 1025, 875, and 775 (furan ring)[1,2,3,5] cm^{-1}.

The nmr spectrum of the mixture of 2 and 5 displayed signals at δ 7.37, 7.20, and 6.32 (broad singlets, 1 H each) showing the presence of a β-substituted furan ring. Signals centered at 5.20 (complex multiplet, 3 H) showed the vinyl protons in the polyisoprenoid chain. A band at 4.12 (singlet, 3 H) showed the presence of the O-methyl group in the tetronic acid moiety. Singlet signals at 1.65 and 1.57 were indicative of vinylic methyl groups. These, as was found for the mixture of 1 and 4 , were present in the ratio of 25:75. The C-19 methyl appeared at δ 1.07 (d,J = 7Hz,3 H) and the C-24 methyl at 2.05 (singlet, 3H).

Preparative TLC on silica gel impregnated with silver nitrate (90:10 benzene/ethyl ether) enabled compound 2 (R_f0.68) to be separated from compound 5 (R_f 0.53). Visualization was carried out by spraying a portion of the plate with 10% sulfuric acid in methanol and heating. Each compound appeared as a pinkish-brown spot. GLC showed that each compound after purification had less than 5% of its isomer present. The ir spectra of the pure compounds 2 and 5 were essentially identical with each other and unchanged from the spectrum of the mixture present before TLC separation. The nmr spectra of 2 and 5 were essentially identical to each other and to the spectrum of the mixture with the following exception. The spectrum of 2 showed equal intensity C-9 and C-14 vinylic methyl signals at δ 1.65 and 1.57 (3 protons each). The spectrum of 5 showed absorption for the C-9 and C-14 vinylic methyl protons only at 1.57 (singlet, 6H).

The location of the double bonds in the polyisoprenoid chain was confirmed by carrying out ozonolysis reactions on separated samples of 2 and 5 followed by oxidative workup, and methylation with diazomethane. The only ozonolysis products obtained from 2 were methyl levulinate, methyl 5-oxohexanoate, and dimethyl 2-methylglutarate. The ozonolysis products from 5 were methyl levulinate, dimethyl succinate, and methyl 2-methyl-6-oxoheptanoate.

All available evidence shows the presence of two isomeric sesterterpenes 1 and 4 in the ratio of 1:3 in the sponge *Ircinia strobilina*.[6] This serves as another example of a geographically separated species of *Ircinia* having a tetronic acid moiety and furan ring contained in a sesterterpene.

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References

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2. D. J. Faulkner, *Tetrahedron Lett.*, 3821 (1973).
3. The stereochemistry of the double bonds in the polyisoprenoid chain is unknown.
4. G. Cimino, S. De Stefano, and L. Minale, *Tetrahedron*, **28**, 5983 (1972).
5. T. Kubota, *Tetrahedron*, **4**, 68 (1958); A. Quilico, F. Piozzi, and M. Pavan, *ibid*, **1**, 177 (1957).
6. *Ircinia strobilina* was captured in the Florida Keys. Identification was made by Professor Lowell Thomas of the University of Miami School of Marine and Atmospheric Science.