GONIODOMIN, A NEW ANTIBIOTIC FROM A DINOFLAGELLATE*

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(Received for publication October 12, 1968)

A new antifungal substance, goniodomin, has been isolated from a marine dinoflagellate, *Goniodoma* sp. collected in Puerto Rico. The molecular formula, $C_{43}H_{58}O_{11}$, has been established for this compound. Physical and chemical data indicate the presence of five hydroxyl groups, a lactone ring, four ether linkages and a dihydrogeranyl side chain in this molecule. Goniodomin strongly inhibits *Cryptococcus*, *Trichophyton*, and other fungi, but shows little or no activity against bacteria.

Marine dinoflagellates, numbering altogether about 1,100 species, possess considerable biomedical significance because they form red tides (BRONGERSMA-SANDERS, 1957) that sometimes result in mass mortality of marine animals, produce antibiotic substances (BURKHOLDER, BURKHOLDER and ALMODÓVAR, 1960), synthesize toxic materials lethal for many kinds of organisms (ABBOTT and BALLANTINE, 1957) and cause paralytic shellfish poisoning in man. Extensive investigations of toxins in various dinoflagellates, including several species of *Gonyaulax* and *Gymnodinium*, have been reviewed by HALSTEAD (1965) and by RUSSELL (1965, 1967). Certain other chemical properties of dinoflagellates, especially their carotenoid pigments and chlorophyll *c*, provide interesting examples of biochemical diversity (BOGOROD, 1962). Mass mortality of fish has been associated with blooms of dinoflagellates all too frequently along the coasts of Florida, California, Africa and other regions (CONNELL and CROSS, 1950; HOWELL, 1953; and GRINDLEY and TAYLOR, 1964). Many species require for their growth exogenous sources of B vitamins, especially cobalamine, thiamine and biotin (PROVASOLI, 1958). Endogenous growth rhythms and flashing luminescence are curious properties of some genera (SWEENEY and HASTINGS, 1962). The biochemistry of these phenomena deserves further investigation for both theoretical and practical reasons.

Antimicrobial activity of dinoflagellates has been demonstrated in red tides caused by *Goniodoma* sp. and *Cochlodinium* sp. in Puerto Rico, and in *Gyrodinium cohnii* cultivated in the Lamont laboratories. This paper reports the isolation and properties of an antibiotic substance obtained from a bloom of *Goniodoma* collected in Puerto Rico. Generic identification of the organism was made by Dr. ESTELA DE

* Contribution No. 1272 from Lamont Geological Observatory. This investigation was supported by NSF grant GB 4914 and by a contribution from the G. Unger Vetlesen Foundation. Grateful acknowledgement is made to Dr. LUIS ALMODÓVAR for assistance in collecting material at the University of Puerto Rico marine laboratory and to Dr. JOHN WEBB for the high resolution mass spectrum determined at Lederle Laboratories.

SOUSA E SILVA, who is continuing research on the cytology and taxonomy of the flagellate.

Field Observations

Goniodoma sp. grows sporadically and sometimes abundantly in protected bays near La Parguera, Peurto Rico, where the waters may become golden brown in color because of tremendous numbers of cells accumulating near the sea surface (BURKHOLDER, BURKHOLDER and ALMODÓVAR, 1967). This flagellate was observed to inhibit *Staphylococcus aureus*, *Candida albicans* and other microorganisms (BURKHOLDER, BURKHOLDER and ALMODÓVAR, 1960). The motile cells of *Goniodoma* commonly make their appearance each morning after sunrise and remain to form patches of red or yellow blooms throughout most of the day. Late in the afternoon, the flagellates show phototactic response to changing conditions of daylight, and swim toward the bottom where they disappear from sight. During the morning of a sunny day, they leave the bottom and swarm again to the surface.

It was possible to gather large quantities (several kilograms) of *Goniodoma* cells by taking advantage of their phototactic responses. Plastic tanks were filled with sea water containing the suspended golden brown plankton. Then blue cellophane covers, selected to simulate blue skylight of late afternoon, were placed on the tops of the tanks, with the result that the dinoflagellates promptly swam to the bottom of the containers. By decanting the water, a slurry of the organisms was obtained, and used for filtering on a large Buchner funnel. Tests of the sea water filtrates indicated toxicity for the small fish, *Jenkinsia lamprotaenia*. The brown residue on the filter showed strong antimicrobial activity when small amounts of the material were applied directly to test plates of yeasts and bacteria. The masses of flagellates filtered easily to form a dry filter cake, which was frozen and maintained at low temperature until chemical studies could be performed in the laboratory at Lamont Geological Observatory.

Isolation Procedure

One kilogram of wet cells of *Goniodoma* was extracted eight times with methanol until the residue, obtained after filtering the solvent, was inactive against the indicator organism, *Candida albicans*. The combined methanol extract (8×1.5 liters) was freed of solvent under reduced pressure, and the residue was then suspended in water and extracted exhaustively with ethyl acetate. The ethyl acetate extract was dried over anhydrous Na_2SO_4 and the solvent completely distilled off under reduced pressure, leaving behind about 30 g of a dark green residue. This residue was dissolved in a minimum amount of benzene and chromatographed on a silica gel column (750 g; 48″×2.5″ (121.92 cm×6.35 cm)). By eluting the column with four liters of benzene, 19 g of inactive material were removed. The column was then washed with ether and fractions active against *Candida* were collected as recorded in Table 1. Fraction 6 (1.7 g) gave the largest zone of inhibition against *Candida albicans*. To this fraction 10 ml of cold ether were added and the insoluble white compound

was collected by suction and crystallized from an ethyl acetate-hexane mixture. The yield was about 250 mg, having a m.p. 199°C. The analytical sample was prepared by crystallizing three times from dilute methanol. The name Goniodomin is suggested for this compound, after *Goniodoma*, the organisms from which it was isolated.

Table 1. Fractions obtained from chromatography of *Goniodoma* extract on a silica gel column.

Fraction Number	Solvent	Vol. (liters)	Color of fractions	Wt. eluted (g)	Activity against *C. albicans*
1	Benzene	2	Orange	8.0	not active
2	Benzene	2	Brown yellow	16.0	not active
3	Benzene	2	Pale yellow	1.0	
4	Benzene	1	Colorless	—	
5	Ether	2	Green	7.5	active
6	Ether	2	Green	1.7	active
7	Ether	2	Orange	0.9	active
8	Ether	2	Orange	0.7	active
9	Ether	2	Lemon yellow	50 mg	not active
10	Ether	2	Lemon yellow	traces	not active

Cells of *Goniodoma* emitted a strong odor, reminiscent of acrylic acid. Since acrylic acid is an effective inhibitor of Gram-positive bacteria, the possibility was considered that the activity of crude *Goniodoma* material against *Staphylococcus aureus* might be due to this acid. However, experiments designed to isolate acrylic acid from the organism, indicated the absence of this compound.

Physical and Chemical Properties

Goniodomin is freely soluble in chloroform, benzene and methanol, but sparingly soluble in ether and hexane. When a few crystals of this compound are applied to a plate of *Candida albicans*, an exceedingly small zone of inhibition results. If a paper disc is dipped into a methanolic solution of this compound, and then dried and applied to the test organisms in an agar plate, the resulting inhibition zone is very large (Table 2). The reason for this phenomenon is not clear. Goniodomin

Table 2. Antifungal activity of goniodomin in agar plate tests*.

Concentration (microgm/ml)	Radius of inhibition (in mm)				
	Candida albicans	*Saccharomyces cerevisiae*	*Cryptococcus neoformans*	*Trichophyton mentagrophytes*	*Penicillium* sp.
500	8	8	13	12	15
50	7	7	12	9	14
5	5.5	5.5	7	3.5	10
0.5	2.5	1.5	6	3	7

* Paper discs 0.25 inch diameter were dipped in methanol solutions, dried in air, and applied to the surface of seeded agar plates. Only slight traces of activity were observed against numerous kinds of bacteria.

crystallized with one molecule of water, and analyzed for $C_{43}H_{58}O_{11}\cdot H_2O$ (found: C 66.58%, H 7.71%, required: C 67.18%, H 7.81%). Elemental and functional group analysis were provided by ALFRED BERNHARDT, Mulheim, Germany. This molecular formula was confirmed by high resolution mass spectrum (observed: $M^+=750.3938$; calculated for $C_{43}H_{58}O_{11}=750.3975$).

Functional group analysis indicated the presence of five hydroxyl groups (ZEREWITINOFF) and one C-CH_3 (found: C-CH_3 3.09%, active H 0.7%, required:

Fig. 1. Infrared spectrum of goniodomin (in KBr pellet).

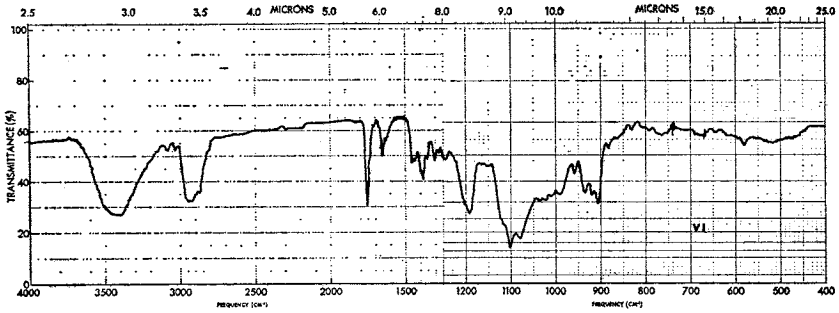


Fig. 2. Infrared spectrum of diacetyl goniodomin (in KBr pellet).

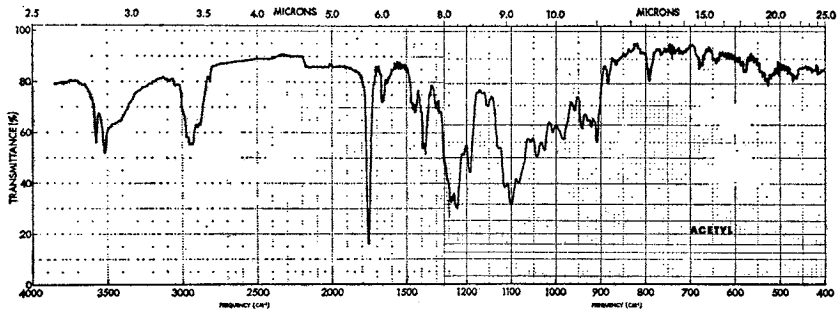
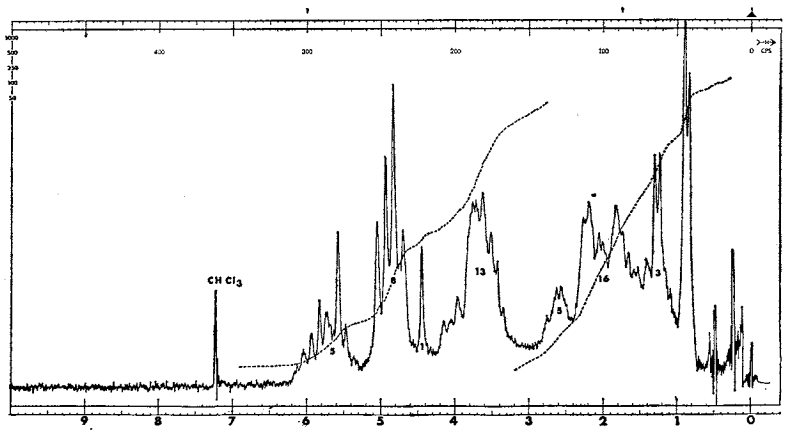


Fig. 3. Nuclear magnetic resonance spectrum (100 Mc) of goniodomin.



C-CH_3 3.49 %, active H 0.66 %). The compound is dextrorotatory $[\alpha]_D^{24}$ +56.5°, and does not absorb in the ultraviolet. The I. R. spectrum of this compound (Fig. 1) has characteristic bands at 3450 cm^{-1} (broad, OH groups); 1750 cm^{-1} (-$\overset{O}{\overset{\|}{C}}$- in a lactone or ester); 1190 cm^{-1} (C-O stretching); 1100 cm^{-1} (OH groups); 905 cm^{-1} ($HC{=}CH_2$ group).

On reaction with excess acetic anhydride and pyridine the antimicrobial substance gave the diacetyl derivative, m. p. 202~205°C (found: C 66.23 %, H 7.89 %, $CH_3C{=}O$ 10.43 %, required for $C_{47}H_{64}O_{14}$: C 66.73 %, H 7.43 %, $CH_3C{=}O$ 10.44 %). The I. R. spectrum of the acetate (KBr pellet) showed two bands, at 3510 cm^{-1} and 3575 cm^{-1}

(Fig. 2), suggesting that some of the hydroxyls were not acetylated and, therefore, are tertiary in nature.

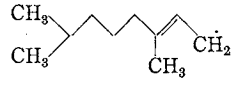
Attempted reduction of the compound with sodium borohydride in ethanol at room temperature resulted in the formation of a complex mixture from which no component could be isolated in the pure form. However, the band at 1750 cm^{-1} in the I. R. spectrum of the mixture indicated the ketonic function was not reduced. The antimicrobial substance was recovered unchanged, on reacting with hydroxyl amine hydrochloride under varied experimental conditions. The above experiments indicate absence of a ketone group, and support the concept that an ester or lactone ring may be present in the molecule.

On hydrogenation over palladium charcoal (10 % catalyst) the compound absorbed 4.25 moles of hydrogen. Thin layer chromatography indicated the presence of four major components in the reduced product.

Hydrolysis of the natural product with dilute hydrochloric acid or methanolysis with methanolic HCl produced extensive degradation of the molecule. The above chemical transformations were carried out on a micro-scale because of the scarcity of material. Full investigation of these reactions will be undertaken when more material has been prepared.

In the 100 Mc N. M. R. spectrum (Fig. 3) certain structural features of the molecule are discernable. Two sharp signals at 0.92 ppm and 0.95 ppm (6 protons) are assigned to two tertiary methyl groups or $(CH_3)_2CH$. A doublet at 1.25 ppm (3 protons) is due to a CH_3CH group. The groups of bands between 1.35 and 2.4 ppm (16 protons) are due to methylene and methine protons. The envelope centered around 3.7 (13 protons) is assigned to protons on carbon atoms bearing the oxygen function (H-C-O-). The signal at 4.1 ppm (one proton) and 4.45 ppm (one proton) are assigned to hydroxyl groups (which disappear on addition of (D_2O). The four line pattern between 4.6 ppm and 5.2 ppm appears to be two closely spaced doublets, due to two $\diagdown C{=}C \diagup$ groups (J cis = 12 cps). The group of lines between 5.25 ppm and 6.2 ppm integrate for another set of 5 olefinic protons.

The mass spectrum of goniodomin exhibits prominent ions at m/e 750 (M^+); 732 ($M-H_2O$); 714 ($732-H_2O$); 706 (M-44; or 732-26); 688 ($706-H_2O$); 670 ($688-H_2O$); 611 (M-139); 593 ($611-H_2O$); 575 ($593-H_2O$); 563, 557 ($575-H_2O$); 539 ($557-H_2O$); 431 (M-319); 413 (431-18); 401, 387; 383 (401-18); 369 (383-18); 319; 301 (319-18; metastable ion at 284); 283 ($301-H_2O$) and 139 ($C_{10}H_{19}$, dihydrogeranyl [I]).



(I)

The ion at m/e 611 appears to have been formed by a one step loss of a $C_{10}H_{19}$ fragment from the molecular ion. This fragment may be a dihydrogeranyl radicle, representing one of the side chains of the antimicrobial substance. The ion at m/e 431 also appears to have been produced directly from the molecular ion by loss of a fragment of mass 319. The presence of peaks at m/e 139 and m/e 319 in the mass

spectrum supports the idea of loss of these fragments from M^+ to produce ions m/e 611 and 431.

From the above physical and chemical data it may be concluded that the antimicrobial substance has two primary and three tertiary hydroxyl groups. Two of the oxygen atoms are part of an ester group or lactone ring. The remaining four oxygen atoms appear to be involved in ether linkages. The spectral data excludes the presence of any aromatic ring.

When more material can be collected, further chemical work will be done toward elucidation of the complete structure of goniodomin and the nature of the unknown antibacterial substance produced by the flagellate.

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