Journal of Natural Products
Vol. 53, No. 3, pp. 703-705, May-Jun 1990

2,3,4-TRIBROMOPYRROLE FROM THE MARINE POLYCHAETE *POLYPHYSIA CRASSA*[1]

R. EMRICH,* H. WEYLAND, and K. WEBER

Alfred-Wegener-Institute for Polar and Marine Research, 2850 Bremerhaven, Federal Republic of Germany

ABSTRACT.—Tribromopyrrole was isolated from the marine worm *Polyphysia crassa*. The compound was found to be the major component responsible for the antibacterial activity of the animal extracts.

Brominated pyrrole derivatives have been reported from sponges (1) and bacteria (2). Halogenated metabolites isolated from marine worms were mostly identified as tyrosine-derived phenolic bromocompounds (3,4).

We now report the isolation of 2,3,4-tribromopyrrole, previously unknown as a natural product, from *Polyphysia crassa* Oersted (Scalibregmindae), a scalibregmid polychaete, which occurs in the North Atlantic and lives in shallow sediment burrows.

Individual animals were extracted with Me_2CO/*n*-hexane. Crude extracts as well as acetylated and methylated samples were subjected to gc analysis. Two halogenated compounds were found by electron capture detection and tentatively identified by combined gc and ms analysis as tribromopyrrole (major component) and dibromopyrrole (trace amounts). Dilute solutions of tribromopyrrole decomposed slowly when stored above $-18°$, forming di- and tetrabromopyrrole due to a disproportionation reaction. Thus, the dibromopyrrole detected in the animal extracts might result from decomposition of the metabolite.

For further structure elucidation, authentic material was synthesized by bromination of pyrrole. The complex reaction mixture was separated by silica chromatography to yield a small amount of pure 2,3,4-tribromopyrrole, which was distinguished from the 2,3,5-tribromo isomer by comparison of ^{1}H-nmr and gc data with those reported in the literature (5,6). Gc retention and mass spectra of authentic 2,3,4-tribromopyrrole were in all respects identical with those of the natural product. Concentrations, determined by gc analysis using authentic material as reference, were found to be 55–80 μg/g wet wt, varying for different individuals.

The compound exhibited marked antibacterial activity. Growth inhibition of 9 different Gram-positive and Gram-negative marine bacteria, tentatively identified as members of the genera *Vibrio, Alteromonas, Corynebacterium, Bacillus,* and *Flavobacterium* and isolated from surface sediments from the Northeast Atlantic, was tested using the disk assay method. At 50 μg/disk strong inhibition was observed for all test organisms. At 10 μg/disk tests were negative for one of two strains of either *Vibrio* and *Alteromonas* and moderately positive for the other strains. Obviously a broad activity is exerted by the compound, even though the Gram-positive strains, *Bacillus* and *Corynebacterium*, are somewhat more inhibited than most of the Gram-negative bacteria. Antibacterial activity of crude animal extracts was evaluated with respect to their content of metabolite. Correlation with the results obtained from authentic material indicated that the predominant antibacterial component of the extracts was 2,3,4-tribromopyrrole.

[1]Contribution No. 237 of the Alfred-Wegener-Institute for Polar and Marine Research.

Because *P. crassa* is usually predated by bottom-dwelling fish, the brominated metabolite was also investigated for its ichthyotoxic properties. No effect was observed when exposing goldfish (*Carassius auratus*) to either pure samples of 2,3,4-tribromopyrrole (max, 500 μg/200 ml H_2O) or crude extracts of *P. crassa* (max. 1 g animal/200 ml H_2O). Experimental procedures have previously been described in detail (7,8). For evaluation of animal behavior, criteria as described by Green (7) were applied. The ecological significance of the antimicrobial activity exhibited by the compound has yet to be proven.

EXPERIMENTAL

ANIMAL COLLECTION AND EXTRACTION.—Samples of *P. crassa* were collected from −85 m in Gulmarfjord (Sweden) and frozen immediately. A voucher specimen is stored at the Alfred-Wegener-Institute, Bremerhaven.

Frozen animals (2–4 g wet wt) were ground with 15 g Na_2SO_4 and 5 g quartz sand in a mortar mill. Column extraction using 100 ml of Me_2CO–*n*-hexane (1:1) was applied for extraction of metabolites. Crude extracts were stored at −18°, protected from light.

IDENTIFICATION.—Quantitative gc was conducted with a Packard Model 428 equipped with a ^{63}Ni-electron capture detector and a 30 m fused-silica capillary column, i.d. 0.25 mm, coated with 25 μm SE-54, on column injection, temperature program 100° (1 min), 10°/min to 300°. Gc-ms was performed using a Finnigan Mat 1050 mass spectrometer directly coupled to a Perkin-Elmer Sigma 3B gas chromatograph. Gc conditions as described above; gc-ms interface 300°; eims (+) 70 eV. All retention data were derived by linear interpolation from gc-ms investigations (9).

2,3,4-Tribromopyrrole.—^{1}H nmr (Et_2O-d_8) δ 6.58 (s, CH); ms *m/z* (%) $[M]^+$ 307 (24), $[M]^+$ 305 (92), $[M]^+$ 303 (100), $[M]^+$ 301 (30), 226 (25), 224 (42), 222 (24), 199 (15), 197 (30), 195 (15), 118 (17), 116 (18), 81 (12), 79 (13); gc retention index 1652.

N-*Acetyl-2,3,4-tribromopyrrole.*—Ms *m/z* (%) $[M]^+$ 349 (3), $[M]^+$ 347 (8), $[M]^+$ 345 (8), $[M]^+$ 343 (3), 307 (18), 305 (49), 303 (55), 301 (19), 226 (8), 224 (14), 222 (8), 43 (100); gc retention index 1758.

N-*Methyl-2,3,4-tribromopyrrole.*—Ms *m/z* (%) $[M]^+$ 321 (35), $[M]^+$ 319 (100), $[M]^+$ 317 (91), $[M]^+$ 315 (23), 199 (17), 197 (31), 195 (17).

SYNTHESIS AND DERIVATIZATION.—*2,3,4-Tribromopyrrole.*—Bromine (800 mg) was added dropwise to 350 mg pyrrole and 400 mg Et_3N in 15 ml CCl_4 at −10°. Et_2O was added, and the solution was washed with aqueous Na_2SO_3. The organic layer was concentrated at 0° and subjected to liquid chromatography immediately [Kieselgel Si 60 Merck, eluent *n*-hexane–Et_2O (1:1)] to yield 20 mg of the desired product (mono-, di-, and tetrabromo derivatives were also obtained). Compounds were dissolved in Et_2O with a few drops of Et_3N added and stored at −18°.

Acetylation.—Pyridine (40 μl) and Ac_2O (20 μl) were added to either 50 μl of crude animal extract or 20 μg of synthetic material. After the reaction mixture was kept at room temperature for 1.5 h, 3 ml of hexane was added, and the solution was washed with 0.1 N HCl and 0.1 N NaOH to remove excess reagent.

Methylation.—Excess ethereal CH_2N_2 was added to either 50 μl of crude animal extract or 20 μg of synthetic material. After 1 h at room temperature, the solvent was evaporated and the residue dissolved in hexane.

ANTIMICROBIAL ACTIVITY.—Tests were conducted with 100 μg, 50 μg, and 10 μg of authentic material and aliquots of crude extract containing 10 μg of metabolite, according to gc analysis. Ethereal solutions were applied on a sterilized disk (10 mm diameter) and the solvent evaporated. The disk was placed on a marine agar plate freshly inoculated with the test organisms. Tests were evaluated after 48 h by measuring the bacteria-free zone around the disk (>5 mm, strong inhibition; 2–5 mm, moderate inhibition; <1 mm, no inhibition).

ACKNOWLEDGMENTS

We thank Dr. H. Goerke, AWI Bremerhaven, for collection and identification of animals and Prof. Dr. D. Leibfritz, University of Bremen, for aquisition of nmr spectra.

LITERATURE CITED

1. L. Chevolot, S. Padua, B.N. Ravi, P.C. Blyth, and P.J. Scheuer, *Heterocycles*, **7**, 891 (1977).
2. R.J. Andersen, M.S. Wolfe, and D.J. Faulkner, *Mar. Biol.*, **27**, 281 (1974).
3. T. Higa, T. Fujiyama, and P.J. Scheuer, *Comp. Biochem. Physiol.*, **65B**, 525 (1980).
4. T. Higa and P.J. Scheuer, *Tetrahedron*, **31**, 2379 (1975).
5. H.M. Gilow and D.E. Burton, *J. Org. Chem.*, **46**, 2221 (1981).

6. G.A. Cordell, *J. Org. Chem.*, **40**, 3161 (1975).
7. G. Green, *Mar. Biol.*, **40**, 207 (1977).
8. J.E. Thompson, R.P. Walker, and D.J. Faulkner, *Mar. Biol.*, **88**, 11 (1985).
9. "The Sadtler Standard Gas Chromatography Retention Index Library," Sadtler Research Laboratories, Philadelphia, Pennsylvania, 1985.

Received 20 September 1989