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THE STRUCTURE OF PB-1, AN UNUSUAL TOXIN ISOLATED FROM THE RED TIDE DINOFLAGELLATE PTYCHODISCUS BREVIS

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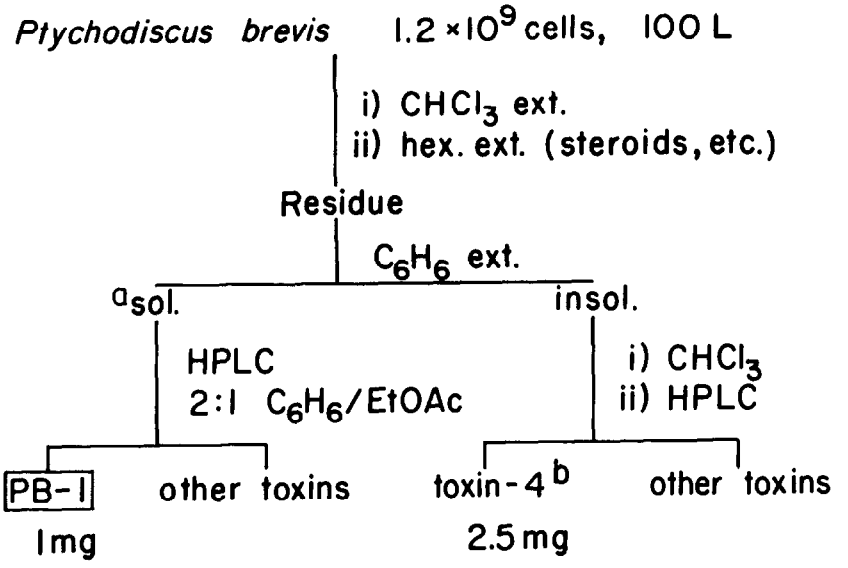
Abstract: The structure of PB-1, 1, an ichthyotoxin isolated from Ptychodiscus brevis was determined to be 0,0-diphenyl-N-cyclooctyl phosphoramidate.

The toxins associated with "blooms", or explosive growths of certain dinoflagellates, have caused massive fish kills off the coasts of the United States, Canada, and Japan, and have been the subject of intense studies over the last thirty years.[1,2] Although most of the 400 species of dinoflagellates are non-toxic, there are notable exceptions, i.e., the Gonyaulax spp. which give rise to saxitoxins/gonyautoxins[1,2] and Ptychodiscus brevis which give rise to brevetoxins.[3,4]

Unlike the Gonyaulax species which accumulate in the hepatopancreas of shellfish and thus facilitate the collection of toxins, P. brevis must be grown in the laboratory to obtain the lipophilic toxins. One hundred liters of artificial sea water was inoculated with P. brevis cultures and grown under fluorescent lights at 25° for three weeks. The cells were extracted with $CHCl_3$ and then hexane to remove steroids and other non-polar materials. The residue was dissolved in benzene and submitted to HPLC, C_6H_6/EtOAc 2:1, to give 1 mg of PB-1; ichthyotoxicity was traced with the common guppy, Lebestes reticulatus, the toxicity of PB-1 being LD_{100} (1hr), 1 ppm.

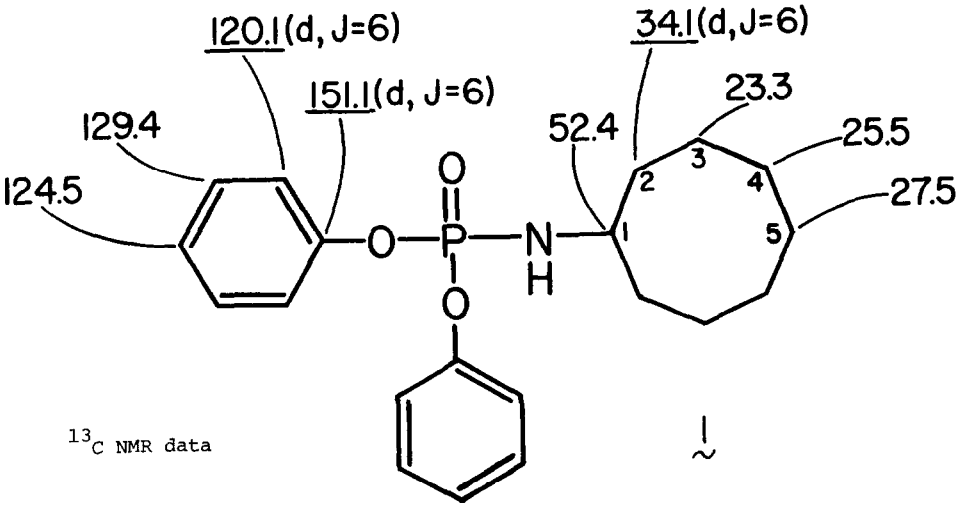
Because of the unexpected presence of phosphorous, derivation of structure 1 was not straightforward. It was finally achieved as follows. The UV (MeOH),



Scheme 1 Isolation of PB-1

a: Brevetoxin B[3] has been detected by TLC from this fraction.

b: The structure has recently been elucidated by X-ray to be (E)-2-(1-methyl-2 oxopropylidene)phosphorohydrazidothioate (E)-oxime: Alam, M.; Sanduja, R.; Hossain, M.B.; van der Helm, D. J. Am. Chem. Soc. 1982, 104, 5232.



^{13}C NMR data

λ_{max} 262nm (ε 475) with fine structures at 259 and 268 nm, was characteristic for phenyl groups. The ^{1}H-NMR spectrum ($CDCl_3$) showed a 10-H (m) signal at 7.0-7.4 ppm (aromatic), 1-H (dd, 11, 11) at 3.72 ppm (exchangeable N-H), 1-H (m) at 3.45 ppm (1-H), 4-H (m) at 1.7-1.9 ppm (m) (2-H), and a 10-H (m) signal around 1.3-1.6 ppm (CH_2 groups). The 3.72 ppm N-H dd signal still retained one of the 11 Hz couplings when the 3.45 ppm 1-H signal was irradiated and this suggested the linkage to a heteroatom of non-integral spin, i.e., I=1/2.

The scarcity of sample led to difficulties in ^{13}C-NMR measurements. Pulsing of the sample for 2 days with a long relaxation delay (RD = 5sec) and a shorter pulse width (30°) than usual revealed all carbons including the weak 151.1 ppm signal; however, this signal and the 120.1 and 34.1 ppm signals all appeared as closely (J = ca. 6 Hz) spaced twin peaks. That these were three doublets (rather than twin peaks) originating from a quaternary C (151.1 ppm), a C-H (120.1 ppm) and a CH_2 (34.1 ppm) was finally clarified, together with the nature of the other carbons, by the INEPT technique[5] which differentiates quaternary, methyl, methylene and methine carbons. The doublet nature of the three ^{13}C peaks, as well as the doublet nature of the 3.72 ppm signal (after irradiation of the 3.45 ppm signal), suggested the extra multiplicities to be due to long range coupling with phosphorous. In support of this, the FTIR showed a strong band at 930 cm^{-1}, characteristic of P-O-C_{aryl} bonds.[6] Inclusion of P in the molecular formula gave $C_{20}H_{26}NO_3P$ (found 359.16472,[7] calc'd 359.16503) which then led to structure 1.

The structure was confirmed by synthesis. Two equivalents of cyclooctylamine were reacted with 0,0-diphenylphosphochloridate in THF at room temperature for 30 min. upon which PB-1 was produced in quantitative yield.

Is PB-1 a natural product? Compounds of this structure type act as acetylcholinesterase inhibitors and are commonly found as commercial insecticides (or nerve gases). However, as far as we are aware, compound 1 is not on the market. The following experiments were hence repeatedly carried out for over a year in an attempt to trace the origin of this unnatural natural product structure. Repeated attempts to biosynthesize [^{32}P]PB-1 from ^{32}P-conaining medium gave ambiguous results, the radioactivity level of isolated PB-1 being merely twice that of the background. Therefore, despite its unnatural structure, PB-1 does

appear to be a toxic metabolite of P. brevis, although the possibility remains that it may be derived from an unsuspected contaminant in the artificial culture medium.[8]

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4. James, J.C.; Golik, J.; Nakanishi, K. Tetrahedron Lett. 1982, 2535.

5. We are grateful to Dr. H.C. Charles, JEOL, for this measurement.

6. Nakanishi, K.; Solomon, P. in "Infrared Absorption Spectroscopy", 2nd Ed., Holden-Day, San Francisco, 1977. p 53.

7. We are grateful to Prof. K. Biemann for this measurement.

8. The common NH-15 medium was employed which uses $NaNO_3$ as the nitrogen source: see refs. 7 and 8 in Alam, M.; Sanduja, R.; Hossain, M.B.; van der Helm, D. J Am. Chem. Soc. 1982, 104, 5232.

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