Gonyautoxin VIII, a Cryptic Precursor of Paralytic Shellfish Poisons

By MASARU KOBAYASHI and YUZURU SHIMIZU*

(*Department of Pharmacognosy and Environmental Health Sciences, College of Pharmacy, University of Rhode Island, Kingston*, RI 02881)

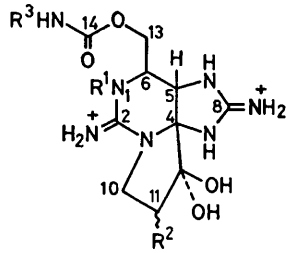
Summary A new dinoflagellate toxin, gonyautoxin VIII, which exhibits enhanced toxicity upon mild acid treatment, seems to possess a novel sulphonatocarbamoyl function.

PARALYTIC shellfish poisons are primarily derived from dinoflagellates, *Gonyaulax spp.*,[1] and toxin levels in affected shellfish are monitored by a mouse assay.[2] In the assay process, which involves brief acid treatment, unexplained enhancement of toxicity has sometimes been observed.[3,4] Conceivably a cryptic form of a toxin, which can be activated by acid treatment, may be present.

The Ipswich strain of *Gonyaulax tamarensis* is a very virulent organism that prevails in the northeastern United States. Its toxin profile is that of a mixture of gonyautoxins I—IV (**1**)—(**4**),[5-8] gonyautoxin V (unknown structure), neosaxitoxin (**5**),[9] and saxitoxin (**6**),[10] which accounted for over 80% of the total toxicity of the organism and paralleled the toxin profile of shellfish.[11-13] During our biosynthetic study of the toxins, we noted in the early eluates of Bio-Gel P-2 column chromatography a peak of weak toxicity but representing substantial radioactivity;† work-up led to the isolation and characterization of the new toxin.

A total of 30 l of a *G. tamarensis* culture, fed with 15 μCi of [*guanido*-^{14}C]arginine, was harvested and processed as previously reported.[13] The toxin fraction [21,900 mouse units (m.u.)] was adjusted to pH 5·8 and chromatographed on a Bio-Gel P-2 column. Elution with water yielded the new toxin, gonyautoxin VIII (**7**) (1,200 m.u.), while elution with 0·03N-acetic acid produced the other known toxins which were further separated into individual toxins on a Bio-Rex 70 column. The radioactivity of (**7**) (11,500 c.p.m.) represented 28% of the total activity (41,500 c.p.m.). Comparison of the toxicity:radioactivity ratio with those of other toxins having known specific toxicity[7] resulted in a specific toxicity of 280 m.u. μmol^{-1} for (**7**), or 14% of the toxicity of saxitoxin (**6**) (2,045 m.u. μmol^{-1}).[14]

Compound (**7**) showed the following n.m.r. spectral data: ^{1}H, (270 MHz, D_2O), δ 3·54 (q, J 10·6, 8 Hz, 10α-H), 3·81 (q, J 9·5, 5 Hz, 6-H), 4·11 (q, J 12, 5 Hz, 13α-H), 4·12 (q, J 10·6, 8 Hz, 10β-H), 4·32 (q, J 12, 9·5 Hz, 13β-H), 4·78 (s, 5-H), and 4·91 (t, J 8 Hz, 11-H); ^{13}C, (D_2O), δ (p.p.m.) 47·7 (C-10), 53·0 (C-6), 57·5 (C-5), 64·0 (C-13), 76·0 (C-11), 81·9 (C-4), 97·5 (C-12), and 154·3, 156·1, and 158·3 (C-2, -8, -14). These chemical shifts were essentially the same as those of the major toxin gonyautoxin III (**3**),[7] except for the slight difference in the proton and carbon chemical shifts for C-13: $\Delta\delta$ + 0·04 for 13α-H, +0·09 for 13β-H and +0·7 p.p.m. for C-13. Toxin (**7**) gradually changes to a *ca.* 1:2 mixture of (**7**) and its epimer (**8**) at room temperature. A similar equilibrium has been also noted between (**3**) and its epimer (**2**).[5] On electrophoresis using tris buffer [tris = 2-amino-2-(hydroxymethyl)propane-1,3-diol] at pH 8·7, (**7**) migrated to the anode in contrast with the other toxins which migrated to the cathode.[15] This indicated that (**7**) has a net negative charge and an additional acidic function in the molecule. Upon treatment with 0·1N-HCl at 37 °C for 7 days or with 0·05N-HCl at 85 °C for 30 min (**7**) was found to be converted nearly quantitatively into (**3**). Similar treatment of a mixture of (**7**) and (**8**) resulted in the formation of (**2**) and (**3**). Both reaction mixtures gave a positive test for free sulphate ($BaCl_2$ and benzidine–thymol); upon heating 1·4 μmol of (**7**) in 0·05N-HCl, 1·23 μmol of (**3**) and 1·3 μmol of free



(**1**); R^1 = OH, R^2 = α-OSO_3^-, R^3 = H
(**2**); R^1 = R^3 = H, R^2 = α-OSO_3^-
(**3**); R^1 = R^3 = H, R^2 = β-OSO_3^-
(**4**); R^1 = OH, R^2 = β-OSO_3^-, R^3 = H
(**5**); R^1 = OH, R^2 = R^3 = H
(**6**); R^1 = H, R^2 = R^3 = H
(**7**); R^1 = H, R^2 = β-OSO_3^-, R^3 = OSO_3^-
(**8**); R^1 = H, R^2 = α-OSO_3^-, R^3 = OSO_3^-

† Guanido-labelled arginine was incorporated indiscriminately into all toxins. The details of the feeding experiment will be published elsewhere.

sulphate [determined colorimetrically (benzidine–thymol)] were generated.[16] Under the same condition, toxins bearing only *O*-sulphate esters did not liberate free sulphate.

These observations point to the structure of (**7**) as a derivative of (**3**) with an additional sulphate moiety.‡ Among several possible conjugation sites, the carbamate amino-group seems to be the reasonable location in view of the n.m.r. data. Among the other possible conjugation sites, the ketone hydrate hydroxy-groups were ruled out because the formation of the isomer (**8**) was considered to take place *via* enolization of the keto-form. Thus the remaining possible conjugation sites are the carbamato- or one of the guanidino-nitrogens. However, complete agreement of the chemical shifts of the protons and carbons in (**3**) and (**7**) except for C-13 and its attached protons seems to support the sulphonatocarbamate structure. Sulphamato-groups and sulphated amide groups were reported in heparin and the recently found β-lactam antibiotics, sulfazecin and isosulfazecin.[17] Although a sulphated carbamoyl group is unprecedented in nature, it is expected to undergo more facile acid hydrolysis than ordinary sulphamates.

Already the reductive transformation of some paralytic shellfish toxins has been demonstrated in scallop homogenates.[18] The discovery of this new, virtually cryptic toxin, which can be activated to a toxin of several times its original potency, could signify an additional problem to the current safety standards for shellfish toxicity. Furthermore, the marked difference in toxicity observed between (**3**) and (**7**) may be due to important differences in the binding mechanism between the toxin molecules and sodium channels.[19]

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‡ The two 'new toxins' described by Oshima and Yasumoto (Y. Oshima and T. Yasumoto in 'Proceedings of the Second International Conference on Toxic Dinoflagellate Blooms,' eds. D. L. Taylor and H. H. Seliger, Elsevier-North Holland, Amsterdam 1979, pp. 377—380) seem to correspond to compounds (**7**) and (**8**). Also, Hall and his co-workers reported four new toxins which they denoted B1, B2, C1, and C2 from an Alaskan isolate of *Protogonyaulax sp.*, which on brief treatment with 0·1N-HCl at 100 °C afforded (**6**), (**5**), (**2**), and (**3**), respectively (ref. 4). Direct comparison of the 270 MHz ^{1}H n.m.r. spectra of toxin C2 and (**7**) proved their identity.

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