Characterization of 11-Hydroxysaxitoxin Sulphate, a Major Toxin in Scallops Exposed to Blooms of the Poisonous Dinoflagellate *Gonyaulax tamarensis*

By GREGORY L. BOYER, EDWARD J. SCHANTZ, and HEINRICH K. SCHNOES

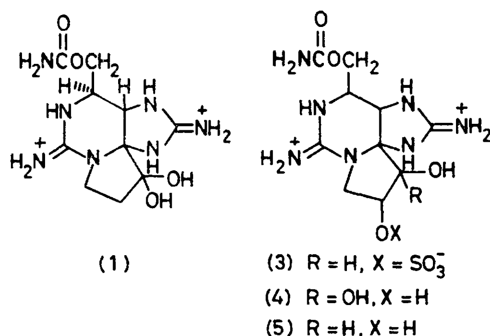
(*Departments of Biochemistry and Food Microbiology and Toxicology, College of Agricultural and Life Sciences, University of Wisconsin-Madison, Madison, Wisconsin* 53706 USA)

Summary The major neurotoxin isolated from scallops collected from the Bay of Fundy during a local bloom of the marine dinoflagellate *Gonyaulax tamarensis* has been identified as the sulphate ester of 11-hydroxysaxitoxin (**2**).

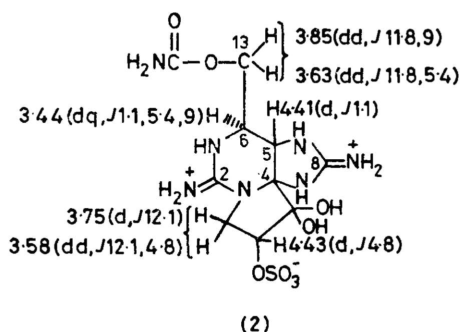
SEVERAL species of marine dinoflagellates produce potent neurotoxins among which saxitoxin (**1**) from *Gonyaulax catenella* is both the best characterized[1,2] and most widely used as a probe of sodium channel structure in excitable membranes.[3,4] The accumulation of these toxins in shellfish during periods of prolific dinoflagellate growth, the so-called 'red tides,' has serious economic and public health repercussions in coastal areas, but also provides convenient sources for toxin isolation. From scallops (*Pecten grandis*) that had become toxic owing to exposure to a bloom of *G. tamarensis* in the Bay of Fundy, we have isolated a new toxin to which, on the basis of chemical and spectroscopic evidence, we can assign structure (**2**), the sulphate ester of 11-hydroxysaxitoxin.

A homogenate produced from a mixture of equal volumes of scallop viscera and Celite covered with acidified 80% aqueous EtOH in a Waring blender (*ca.* 5 kg of scallops and 4 l of EtOH) yielded, after filtration through glass wool and concentration of the filtrate, a viscous toxin extract which was fractionated by the following chromatographic sequence: (i) ion exchange (Amberlite CG-50, H^+ form, elution with 0·5 M HOAc); (ii) gel permeation (4 Bio-gel P-2 columns eluted with either H_2O then 0·5 M HOAc, or 0·1 M HOAc alone); (iii) adsorption (acidic Al_2O_3, elution with MeOH), and (iv) a final clean-up step on Bio-gel P-2. This pro-

cedure gave the toxin (**2**) in *ca.* 17% yield (based on initial toxicity of extract) and afforded a preparation with a specific toxicity of 2500 mouse units per mg.[5]



(1) (3) R = H, X = SO_3^-
(4) R = OH, X = H
(5) R = H, X = H



(2)

The striking similarity between the ^{1}H n.m.r. spectra (270 MHz, in D_2O) of saxitoxin (**1**) and (**2**) (see structure, chemical shifts in p.p.m. downfield from Me_4Si reference, coupling constants in Hz) immediately suggested identity of ring skeleton and basic functionalities. Chief differences, namely the presence of a one-proton doublet at δ 4·43 in the spectrum of (**2**) instead of the two-proton multiplet at δ 2·00 in the case of (**1**), and the downfield shift (by *ca.* 0·35 p.p.m.) of both C(10) protons could be accounted for by an electronegative substituent on C(11), an assignment confirmed by decoupling experiments for the case of (**2**) as well as derivatives (**3**) and (**4**). Similarly the ^{13}C n.m.r. spectrum of (**2**) in D_2O) is easily interpretable [51·1 (C-10), 53·2 (C-6), 57·9 (C-5), 63·3 (C-13), 77·7 (C-11), 81·6 (C-4), 97·6 (C-12), 156·2 and 158·0 (C-2 and 8), and 159·0 (C-14), in p.p.m. downfield from Me_4Si] in terms of an 11-substituted saxitoxin skeleton, since all resonances match those observed for (**1**) very closely with the exception of the marked (44 p.p.m.) and modest (7 p.p.m.) downfield shift of the signals assigned to C(11) and C(10), respectively.

Reduction ($NaBH_4$) of (**2**) led to the alcohol (**3**) [δ 3·99, d, J 5 Hz, (12-H); δ 4·65 t, $J_1 \simeq J_2 \simeq$ 5 Hz, (11-H)] which, like the corresponding borohydride reduction product of saxitoxin [*i.e.* (**1**) with C(12)–OH substituent], lacks biological activity. Hydrolysis of (**2**), under conditions sufficiently mild (2·4 M HCl, 1 h, 90 °C) to leave the carbamate function intact [as determined by model experiments with (**1**)] gave the equally toxic hydroxy-ketone (**4**) exhibiting an ^{1}H n.m.r. pattern (270 MHz, D_2O) differing from that of (**2**) chiefly in the absence of the C(11)–proton (owing to rapid exchange with solvent†) and the appearance of simple doublets for both C(10) protons (δ 3·48 and 3·34, J 11·5 Hz). Borohydride reduction of (**4**) or acid hydrolysis of (**3**) led to the biologically inactive diol (**5**). Periodate treatment, to which saxitoxin, as well as toxin (**2**) and its reduction product (**3**) are insensitive even after 40 h (5M excess, 25 °C), rapidly degraded the hydroxy-ketone (**4**) and diol (**5**) to (uncharacterized) products devoid of toxicity. The observation that saxitoxin (**1**) and products (**4**) and (**5**) exhibit the same electrophoretic mobility which differs markedly from that of toxin (**2**) or its reduction product [relative mobility of 0·55 *vs.* 1·0 for (**1**), (**4**), or (**5**) at pH 2, 4, and 6 and 1400 V] suggested a strongly acidic (but noncarbon) function as the C(11)-substituent. A solution of (**2**) or (**3**) gave negative tests for inorganic phosphate or sulphate, but after brief HCl-treatment of either compound the presence of free sulphate could be demonstrated. Quantitative analysis[6] established the release of 0·92 moles of sulphate per mole of (**2**) upon hydrolysis.

To two toxins, isolated from the softshell clam *Mya arenaria*, and named gonyautoxin II and III (GTX-II and III) the structures of 11α- and 11β-hydroxysaxitoxin, respectively [*i.e.* the two epimers of (**4**)] have been assigned.[7] Published spectral (^{13}C-n.m.r.) data[7] on GTX-II are identical to those we obtained for (**2**), and since the gonyautoxins and (**2**) also exhibit the same chromatographic and electrophoretic behaviour [which differs from that of (**4**)], we believe that GTX-II and III are likely to be the C(11) sulphate ester epimers rather than the hydroxy-compounds as originally proposed.

Preliminary analyses of extracts of *G.tamarensis* indicate the presence of (**2**) as a major toxin of the organism. This observation, and the fact that modification during isolation appears but a remote possibility (no sulphate salts or sulphonate resins were used) suggests that (**2**) is a dinoflagellate toxin rather than a scallop metabolite or isolation artifact.

This work was supported by funds from the Food and Drug Administration. We thank Dr. H. Pivnick (Canadian Department of Health, Education, and Welfare) for arranging, and Mr. Louis Daneault and Captain Elwood Titus for implementing, the collection of toxic scallops, and Professor Frank M. Strong for many discussions and suggestions.

(*Received, 3rd May* 1978; *Com.* 474.)

† Toxin (**2**) also undergoes slow epimerisation in aqueous solution. At equilibrium the two possible C-(11) epimers occur in a *ca.* 4 : 1 ratio, based on t.l.c., ^{1}H n.m.r., and ^{13}C n.m.r. data.

[1] E. J. Schantz, V. E. Ghazarossian, H. K. Schnoes, F. M. Strong, J. P. Springer, J. O. Pezzanite, and J. Clardy, *J. Amer. Chem. Soc.*, 1975, **97**, 1238.
[2] J. Bordner, W. E. Thiessen, H. Bates, and H. Rapoport, *J. Amer. Chem. Soc.*, 1975, **97**, 6008.
[3] M. Evans, *Internat. Qev. Neurobiol.*, 1972, **15**, 83; J. M. Ritchie, *Phil. Trans. Roy. Soc.*, 1975, *B*, **270**, 319.
[4] J. K. Reed and M. A. Raftery, *Biochemistry*, 1976, **15**, 944.
[5] E. J. Schantz, E. F. McFarren, M. L. Schafer, and K. H. Lewis, *J. Assoc. Offic. Analyt. Chem.*, 1958, **41**, 160.
[6] J. Fritz and S. Yamamura, *Analyt. Chem.*, 1955, **27**, 1461.
[7] Y. Shimizu, L. J. Buckley, M. Alam, Y. Oshima, W. E. Fallon, H. Kasai, I. Miura, V. P. Gullo, and K. Nakanishi, *J. Amer. Chem. Soc.*, 1976, **98**, 5414.