Confirmation of the Structures of Gonyautoxins I—IV by Correlation with Saxitoxin

By YUZURU SHIMIZU and CHIEN P. HSU

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

Summary The structures of four paralytic shellfish poisons have been unequivocally established by interconversion and ultimate correlation with saxitoxin.

THE gonyautoxins were first isolated as toxic components responsible for the North Atlantic paralytic shellfish poisoning.[1,2] Since then they have been reported in a number of shellfish poisoning samples and the causative dinoflagellates, *Gonyaulax* spp. To date nine toxins, gonyautoxins I—VIII and neosaxitoxin, have been isolated in addition to the previously reported saxitoxin (**1**).[3,4]

In 1976, we proposed that gonyautoxin II and III were epimeric 11-hydroxysaxitoxins.[5] Later they were actually found to exist in the sulphate forms, (**2**) and (**3**).[6,7] Subsequently, neosaxitoxin was assigned the 1-hydroxysaxitoxin structure, (**4**),[8] and gonyautoxins I and IV the epimeric 11-hydroxyneosaxitoxins structures (**5**) and (**6**), respectively.[9]

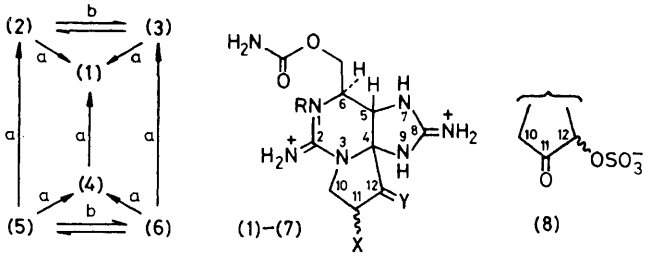
Owing to the scarcity of the toxins, the structural assignments had to be based on spectroscopic data and microchemical degradations. Only the toxin (**4**) could be chemically correlated with (**1**), by the reductive removal of the *N*-hydroxy-group.[8] We now report chemical correlations which interrelate the aforementioned five toxins, and further relate them to (**1**), whose structure has been firmly established by *X*-ray crystallography.[10]

Treatment of (**5**) with zinc powder in 0·01 M HCl at room temperature afforded (**4**) in addition to the initially expected product (**2**). Prolonged treatment resulted in the formation of (**1**) and dihydrosaxitoxin (**7**).† On the other hand, the isomer (**6**) gave (**3**), (**4**), and (**1**). Under the same conditions (**2**) afforded (**1**), and prolonged treatment of (**2**) gave a mixture of (**1**) and (**7**). The reduction of the isomer (**3**) occurred more readily than the reduction of (**2**) and gave the same products, (**1**) and (**7**).

The formation of (**1**) and (**4**) from the gonyautoxins can only be explained by assuming that hydrogenolysis of the *O*-sulphate moiety occurs. This, in turn, supports the 11-*O*-sulphate structures. It is unlikely that the loss of the sulphate group and the hydrogenolysis of the oxygen function at C-11 occurred independently, because the sulphate group is not susceptible to hydrolysis in 0·01 M HCl.

These conversions, which are summarized in the Scheme, confirm the basic skeleton and the stereochemical relationship of the gonyautoxins at C-11. The formation of (**1**) from (**3**) and (**6**) also excludes the possibility that the latter two toxins are regioisomers (**8**) formed by enolization and sulphate-group transfer.‡

Reductive cleavage of free *O*-sulphates may not have many precedents, but the hydrogenolysis of alkane- or arene-sulphonates is certainly a routine practice in organic syntheses. Undissociated sulphates, which exists as such in strongly acidic solution, are expected to be equally good leaving groups. In addition, the neighbouring keto-function present in the gonyautoxins, though in the hydrated form, may further facilitate the hydrogenolysis of the sulphate group.



(**1**) R = H; X = H; Y = $(OH)_2$
(**2**) R = H; X = α-OSO_3^-; Y = $(OH)_2$
(**3**) R = H; X = β-OSO_3^-; Y = $(OH)_2$
(**4**) R = OH; X = H; Y = $(OH)_2$
(**5**) R = OH; X = α-OSO_3^-; Y = $(OH)_2$
(**6**) R = OH; X = β-OSO_3^-; Y = $(OH)_2$
(**7**) R = H; X = H; Y = H,OH

SCHEME. Interconversions of paralytic shellfish toxins. *Reagents and conditions*: a, Zn–HCl; b, pH 8 (AcONa).

The support of a P.H.S. grant is acknowledged.

(*Received, 2nd January* 1981; *Com.* 006.)

† Products were identified by t.l.c. on high performance plates,[2] electrophoresis,[11] and their h.p.l.c. elution pattern.[2] The toxins were eluted from a Bio-Rex 70 column in the order (**6**), (**5**), (**3**), (**2**), (**4**), and (**1**) by a 0·00—0·03 N acetic acid gradient. Their R_F values on Whatman HPK plates were 0·62, 0·81, 0·90, 0·70, 0·90, and 0·81 for (**1**), (**2**), (**3**), (**4**), (**5**), and (**6**), respectively, with the system pyridine:ethyl acetate:water:acetic acid = 75:25:30:15.

‡ Such isomers seemed to be plausible because the two electron-withdrawing guanidinium groups allow the C-12 ketone to exist only as the hydrate, and the exchange of the ketone and sulphate positions would allow the formation of the free keto-group at C-11. Isomerization between (**2**) and (**3**) or (**5**) and (**6**) can occur at just above neutral pH, and is extremely enhanced by a trace amount of base such as sodium acetate. The equilibrium ratio is about 6:4 in both cases.

[1] Y. Shimizu, M. Alan, Y. Oshima, and W. E. Fallon, *Biochem. Biophys. Res. Commun.*, 1975, **66**, 731.
[2] Y. Oshima, L. J. Buckley, M. Alam, and Y. Shimizu, *Comp. Biochem. Physiol.*, 1977, **57**, 31.
[3] Y. Shimizu in 'Recent Advances in Phytochemistry,' eds. T. Swain and G. Waller, Plenum, New York, 1979, Vol. 13, p. 199.
[4] Y. Shimizu in 'Proceedings of the Second International Conference on Toxic Dinoflagellate Blooms,' eds. D. L. Taylor and H. H. Seliger, Elsevier–North Holland, Amsterdam, 1979, p. 321.
[5] Y. Shimizu, L. J. Buckley, M. Alam, Y. Oshima, W. E. Fallon, H. Kasai, I. Miura, V. P. Gullo, and K. Nakanishi, *J. Am. Chem. Soc.*, 1976, **98**, 5414.
[6] G. L. Boyer, F. J. Schantz, and H. K. Schnoes, *J. Chem. Soc., Chem. Commun.*, 1978, 889.
[7] Y. Shimizu, *Kagaku To Seibutsu*, 1980, **18**, 790.
[8] Y. Shimizu, C. P. Hsu, W. E. Fallon, Y. Oshima, I. Miura, and K. Nakanishi, *J. Am. Chem. Soc.*, 1978, **100**, 679.
[9] Y. Shimizu, Symposium Lecture No. 337, The American Chemical Society–Chemical Society of Japan Chemistry Congress, April 1—6, 1979, Honolulu, Hawaii; Y. Shimizu, Proc. 6th FDA Symposium, in the press.
[10] E. J. Schantz, V. E. Ghazarossian, H. K. Schnoes, F. M. Strong, J. P. Springer, J. O. Pezzanite, and J. Clardy, *J. Am. Chem. Soc.*, 1975, **97**, 1238; J. Bordner, W. E. Thiessen, H. A. Bates, and H. Rapoport, *ibid.*, p. 6008.
[11] W. E. Fallon and Y. Shimizu, *J. Environ. Sci.*, 1977, **A12**, 455.