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Steven M. Kuznicki, Edward M. Eyring*
Department of Chemistry, University of Utah
Salt Lake City, Utah 84112
Received June 26, 1978

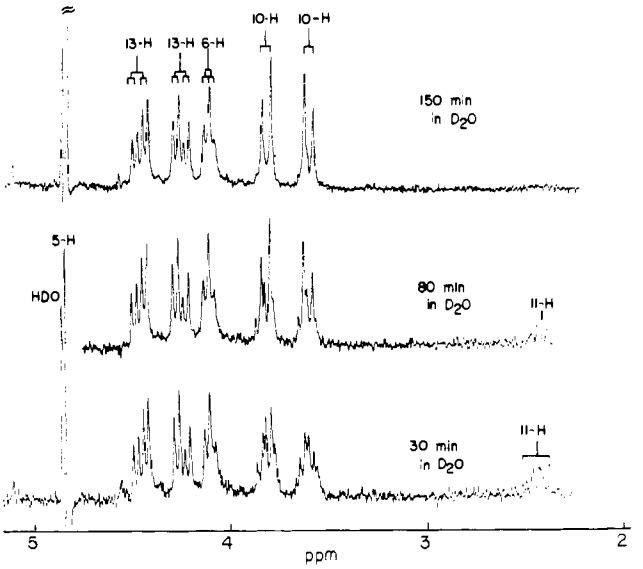


Figure 1. ^1H NMR of neosaxitoxin (**1**) (~1 mg) in D_2O after 30 min, 80 min, and 2.5 h. The strong water band around 4.8 ppm was largely removed by the PRFT technique in order to observe the H-5 signal. All ^1H NMR spectra showed a strong peak at ~2.0 ppm due to the acetate counterions.

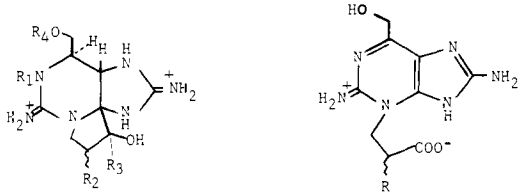
Structure of Neosaxitoxin

Sir:

Neosaxitoxin was first isolated as a minor toxin constituent in the toxic Alaska butter clam, *Saxidomus giganteus*, and later as a major toxin in the cultured dinoflagellate, *Gonyaulax tamarensis*, which causes the North Atlantic paralytic shellfish poisoning (PSP).[1] It has since been isolated from a number of organisms: the toxic mussel, *Mytilus edulis*, from Haines, Alaska;[2] the sea scallop, *Placopecten magellanicus*, from the Bay of Fundy, Canada;[3] and several other unidentified *Gonyaulax* species.[4]

This toxin is of particular interest because, although it is a major toxin in *G. tamarensis*, only trace amounts of it were found in the soft-shell clam, *Mya arenaria*, infested by the dinoflagellate. The major toxin of *Mya arenaria* was found to be saxitoxin, and hence a biotransformation of neosaxitoxin to saxitoxin by the shellfish is suggested.

Neosaxitoxin (**1**) is very similar to saxitoxin (**2**) in its



1 R_1, R_3 = OH; R_2 = H; R_4 = $-CONH_2$
2 R_1, R_2 = H; R_3 = OH; R_4 = $-CONH_2$
3 R_1 = H; R_2 = α-OH; R_3 = OH; R_4 = $-CONH_2$
4 R_1 = H; R_2 = β-OH; R_3 = OH; R_4 = $-CONH_2$
7 R_1, R_2, R_4 = H; R_3 = OH
8 R_1, R_3 = OH; R_2, R_4 = H
11 R_1, R_2, R_3 = H; R_4 = $-CONH_2$

5 R = H
6 R = α-OH

chromatographic behavior and is only separable from the latter by careful gradient ion-exchange chromatography.[1] Its toxicity is comparable with that of saxitoxin (5000 mouse units/mg ± 500); the infrared spectrum is also very similar except for a slightly pronounced shoulder in the carbonyl region (1770 cm^{-1}).[1] The molecular formula could not be obtained directly because of its highly hygroscopic, amorphous, and nonvolatile nature. The following structural studies were carried out on a total of 2 mg of the toxin which was obtained from cultures of *G. tamarensis*.[1]

The structures of saxitoxin (**2**)[5,6] and two other PSP toxins, gonyautoxins II (**3**) and III (**4**),[7] have recently been established. Saxitoxin (**2**) and gonyautoxin II (**3**) gave the aromatized aminopurinylpropionic acid derivatives **5** and **6**, respectively, by oxidation with H_2O_2–NaOH.[7-9] The oxidation products **5** and **6** are highly fluorescent and have characteristic UV absorptions with λ_{max} of 335 and 337 nm, respectively. When neosaxitoxin (**1**) was oxidized under the same condition, however, no UV-absorbing product was obtained; it was only upon H_2O_2 oxidation in neutral aqueous medium that **1** gave a minute amount of a fluorescent product with UV absorption (λ_{max} 335 nm) corresponding to that of **5**. This result suggests the presence of the same nucleus in neosaxitoxin (**1**) as in saxitoxin (**2**) and gonyautoxin II (**3**).

The 220-MHz partially relaxed Fourier transform (PRFT) ^1H NMR of **1** in D_2O (Figure 1)[10] showed again a close resemblance to that of saxitoxin (**2**). It was noted that the ~2.44-ppm 11-H multiplet was rapidly deuterated and disappeared in about an hour, and that two multiplets centered at 3.80 and 3.58 ppm (10-H's) were converted into a well-defined AB-type quartet. This phenomenon corresponds to the known deuterium exchange of 11-H_2 of saxitoxin (**2**).[11] However, in **2** this exchange through enolization takes place at the much slower rate of over a period of 2 weeks. The rapid deuterium exchange in **1** is also noticeable in the ^{13}C NMR spectrum where the C-11 appeared only as a weak signal owing to the two deuterium attachment.[12] The ^{13}C NMR spectrum in D_2O showed only two signals in the sp^2 carbon region which first led us to suspect that **1** might be a decarbamoyl derivative of saxitoxin. However, direct comparison of **1** with decarbamoylsaxitoxin (**7**)[13] clearly ruled out this possibility. Namely, when **1** was treated with 7.5 N HCl, a condition to effect hydrolysis of the carbamoyl group,[13] a new product, **8**, which maintains ~70% of the original toxicity, was isolated.[1] This observation closely parallels that of saxitoxin suggesting presence of the carbamoyl moiety in **1**. This problem was finally resolved with the detection of three peaks at 157.4, 158.5, and 159.0 ppm in C_5D_5N containing a trace of D_2O.

The proton and carbon chemical shifts of neosaxitoxin (**1**) and saxitoxin (**2**) are summarized in structures **9** and **10** (Chart I). The closeness of ^1H NMR coupling patterns of **1** and **2**

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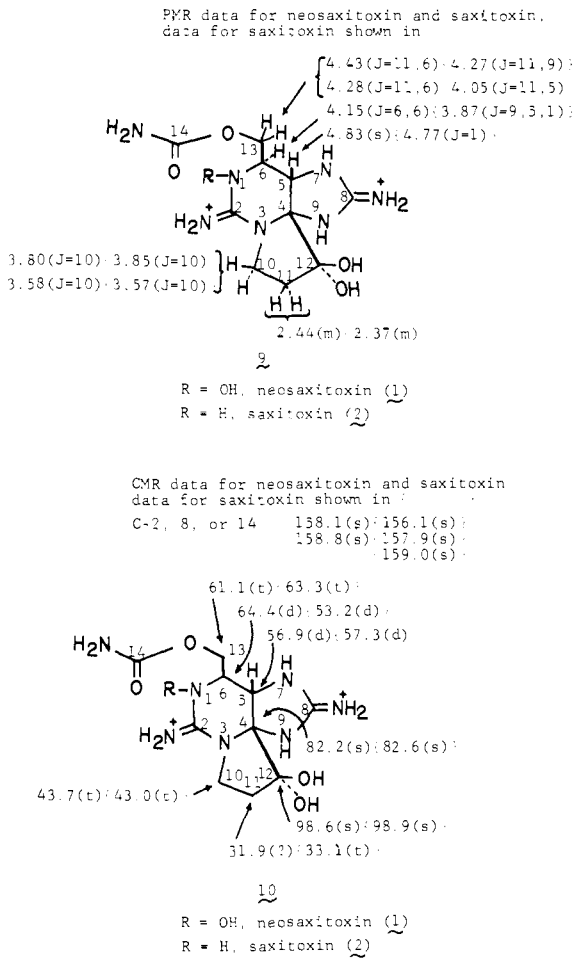
Chart I



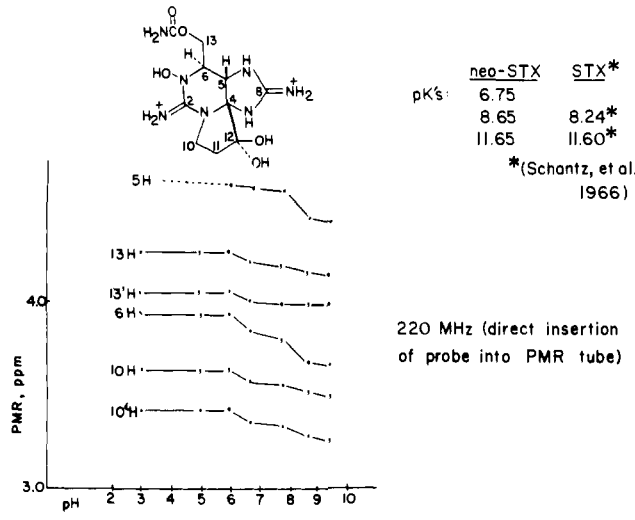
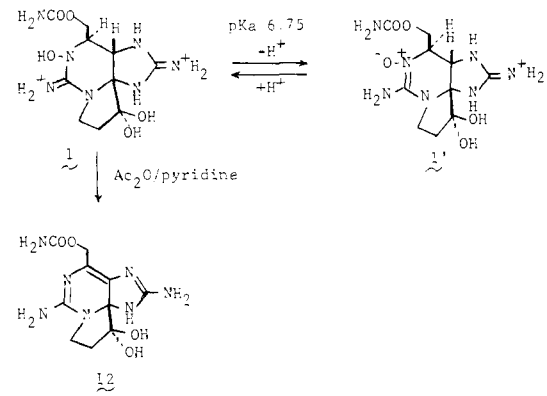


Figure 2. The dependence of ^{1}H NMR signals on pH, 220 MHz, in D_2O.

excluded any major structural deviations of **1** and **2**. The only conspicuous differences in chemical shifts were those relating to C-5, C-6, C-13 and the protons attached to these carbons. This indicated that either N-1 or N-7 in **1** carried an extra substituent. In order to differentiate the two possibilities, the C-5 and C-6 ^{13}C NMR peaks had to be rigidly assigned. This was accomplished by selective irradiation of the 4,83-ppm 5-H signal upon which the 56.9-ppm ^{13}C NMR doublet was converted into a singlet. Hence the 56.9- and 64,4-ppm methine signals are assignable to C-5 and C-6, respectively. Similarly, the previously unassigned carbon signals of **2** at 53.2 and 57.3 ppm[6] were assigned to C-6 and C-5, respectively.

The ^{13}C NMR data for **1** shown in structure **10** indicate an upfield shift of 2.2 ppm for C-13 (γ effect) and a downfield shift of 11.2 ppm (β effect) for C-6, whereas the C-5 signal is hardly shifted. These shifts clearly show that N-1 carries the extra substituent. This conclusion is corroborated by the chemical-shift difference seen in the ^{1}H NMR peaks of 6-H (4.15 vs. 3.87 ppm). The N-1 substituent was found to be readily removable by reduction. Thus treatment of **1** with Zn–AcOH or $NaBH_4$ afforded a mixture of saxitoxin (**2**) and dihydrosaxitoxin (**11**) (TLC and electrophoresis). Microtitration of **1** gave three pK_a values of 6.75, 8.65, and 11.65. The latter two pK_a values correspond to those of the two guanidinium groups in saxitoxin (pK_a of 8.24 and 11.60)[14] but the pK_a of 6.75 is due to the additional substituent which is dissociable. The effect of this lowest pK_a is clearly reflected in the pH dependence of the ^{1}H NMR of **1** (Figure 2) which were measured by inserting a microelectrode directly into the ^{1}H NMR probe. As seen in Figure 2, the 6-H undergoes the largest shift in signal position with pH range relevent to the pK_a of 6,75 indicating the closest proximity of the proton to the changing charge. On the other hand, the 5-H seems to be the most affected[15] with respect to the pK_a of 8.65 suggesting its association with the imidazole guanidine.

From the above data we conclude that neosaxitoxin is 1-hydroxysaxitoxin (**1**).[16] The dissociation of the hydroxylamine



to the *N*-oxide form (**1'**)[17] is assisted by the positively charged guanidinium group. The *N*-hydroxy group also accounts for its easy removability by reduction.[18]

A further support of the *N*-hydroxy structure was obtained when treatment of **1** with acetic anhydride and pyridine at room temperature yielded a product with λ_{max} 295 nm which is tentatively assigned structure **12**.[19] The corresponding product could not be obtained from saxitoxin (**2**) under the same treatment.

In *Gonyaulax tamarensis* cells, saxitoxin (**2**) is only a minor component, whereas gonyautoxins II (**3**) and III (**4**) and neosaxitoxin (**1**) are the major toxins. On the other hand, in the toxic clam saxitoxin is the major toxic constituent and only a trace of neosaxitoxin is detectable. It is plausible that neosaxitoxin, with its easily reducible *N*-hydroxy group, is converted to saxitoxin in the clam bodies. In one experiment, incubation of neosaxitoxin with clam crystalline stylus homogenate afforded saxitoxin.

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(11) J. L. Wong, R. Oesterlin, and H. Rapoport, *J. Am. Chem. Soc.*, **93**, 7344 (1971).
(12) The ^{13}C NMR was measured on a JEOL PS-100 FT instrument equipped with a microprobe; the sample (1 mg) was dissolved in ~30 μL of D_2O containing a trace of dioxane (as reference) and placed in a melting point tube, 50392 scans. The 11-H's had been completely deuterated during the course of the long accumulation time.
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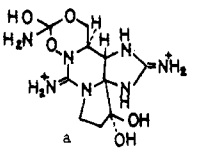
(15) Owing to the DOH signal overlap, some of the chemical shifts were not evaluated exactly.
(16) The presence of a substituent on the purine nucleus also explains the difficulty of the aromatization of 1 by oxidation. The keto function of saxitoxin is known to exist mostly in the hydrate form owing to the two strong electron-withdrawing guanidinium groups. Substitution on the guanidinium nitrogen will change this character, which explains the faster deuterium exchange and the slightly pronounced IR carbonyl absorption of 1 in comparison with saxitoxin. Also, the fairly large chemical-shift differences of the 13-H's in 1 and **2** are attributable to the anisotropic effect on the N–O function.
(17) The pK_a values of 3-hydroxyguanine derivatives range from 5.70 to 8.24: J. C. Parham, T. G. Winn, and G. B. Brown, *J. Am. Chem. Soc.*, **36**, 2639 (1971).
(18) The possibility of the tautomeric cyclitol structure (a) can be discarded under the present condition of NMR measurements because the sp^3 carbon with



a

fully heteroatomic substitution would be expected to show a signal at ~100 ppm[6] which is not the case.
(19) The amount of **12** was too minute for further characterization.

Yuzuru Shimizu,* Chien-ping Hsu
William E. Fallon, Yasukatsu Oshima
Department of Pharmacognosy
College of Pharmacy, University of Rhode Island
Kingston, Rhode Island 02881

Iwao Miura, Koji Nakanishi*
Department of Chemistry, Columbia University
New York, New York 10027

Received June 22, 1978

Book Reviews

Porphyrins and Metalloporphyrins. Edited by KEVIN M. SMITH (University of Liverpool). Elsevier Scientific Publishing Co., Amsterdam. 1975. xxiii + 910 pp. $114.50.

This ambitious volume is a timely and much enlarged revision of Falk's 1964 work of the same name. There are 19 well-written review articles organized around eight general themes surveying the literature through 1974. The first section written by the editor is a succinct outline of the structure and synthesis of porphyrin compounds and serves well as an introduction to the book and the topic. An important biological perspective is supplied by chapters on the biosynthesis (A. R. Battersby and E. McDonald) and oxidative cleavage of heme (P. O'Carra). The coordination chemistry of porphyrins is dealt with in considerable detail (J. W. Buchler; P. Hambright). The chapter on metalloporphyrins of unusual structure (M. Tsutsui and G. A. Taylor) has many large and strikingly informative molecular structures. The section on molecular structure has chapters reviewing X-ray crystallographic results (J. L. Hoard), mass spectroscopy (K. M. Smith), NMR (H. Scheer and J. J. Katz), and vibrational spectra (H. Bürger) related to porphyrins and metalloporphyrins. The section on electronic structure contains chapters on Mössbauer spectroscopy (P. Hambright and A. J. Bearden) and ESR spectroscopy. Taken together the two sections on porphyrin and metalloporphyrin spectroscopy are a valuable reference. Several chapters on the chemical reactivity of porphyrins deal with reversible and electrochemical reactions, reactions of the molecular periphery (J.-H. Fuhrhop), photochemistry (F. R. Hopf and D. G. Whitten), and photosynthesis (D. Mauzerall and F. T. Hong). The short chapter on structural analogs (A. W. Johnson) is very informative.

Anyone actively pursuing porphyrin chemistry will find the most useful chapter in the book to be the last, "Laboratory Methods" (J.-H. Fuhrhop and K. M. Smith), for here are over 100 pages of preparative methodology pertinent to these compounds with many hints and comments which are likely to be found nowhere else.

The only difficulty with the book is the lack of an author index and a rather modest subject index. Although these shortcomings are compensated in part by a highly detailed Table of Contents, it is difficult to find a particular result quickly.

This is a very valuable and readable book for the general reader as well as the specialist. This reviewer found "Porphyrins and Metalloporphyrins" to be highly popular with his colleagues and students. Indeed, it has often been difficult to locate as a result. When retrieved for the purposes of this review, its dust jacket was tattered, its spine was well creased, and it fell open to several particularly topical sections.

John T. Groves, *The University of Michigan*

Terpenoids and Steroids. Volume 7. J. R. HANSON, Senior Reporter. The Chemical Society, London. 1977. x + 349 pp. 25.00 ($50.00).

This volume, the latest Specialist Periodical Report on terpenoids and steroids, covers the literature from September 1975 to August 1976. The subject matter is divided into chapters on monoterpenoids, sesquiterpenoids, diterpenoids, triterpenoids, carotenoids, and polyterpenoids, biosynthesis, steroid properties and reactions with a new section on steroid partial synthesis, and steroid total synthesis, each chapter being the province of a separate reporter or reporter team. The arrangement makes it reasonably simple to locate references to a particular topic. Nevertheless, the absence of a subject index is somewhat of a handicap as, for example, when this reviewer wished to locate references to sesterpenoids, a topic apparently not covered in this volume. Chemists interested in terpene and steroid alkaloids must look elsewhere for full coverage of the literature.

Some of the reporters have approached their task more critically than others; this reviewer particularly enjoyed R. B. Yeats' refreshingly discriminating review of the current monoterpenoid literature. Volume 7 seems relatively free of mistakes, unlike Volume 6 which suffered from an abundance of typographical errors. Its acquisition is, of course, a must for every group engaged in terpenoid or steroid chemistry.

Werner Herz, *Florida State University*

Physical Methods of Chemistry. Part VI: Supplement and Cumulative Index. Edited by ARNOLD WEISSBERGER and BRYANT W. ROSSITER (Eastman Kodak Company). John Wiley & Sons, Inc., New York, N.Y. 1977. xi + 323 pp. $22.95.