Effect of Ascorbic Acid and Related Compounds on the Tobacco Hornworm, *Manduca sexta* Johannson (Lepidoptera: Sphingidae)

Karl J. Kramer,* Leon H. Hendricks, Yun T. Liang, and Paul A. Seib

The effect of L-ascorbic acid (1) and related compounds on the growth of the tobacco hornworm, *Manduca sexta* Johannson, was determined. The dietary level of 1 necessary for normal development was 0.5 mM. Magnesium 2-*O*-phosphono-L-ascorbate, sodium 6-*O*-myristoyl-L-ascorbate, and L-dehydroascorbic acid were equally potent as 1. In contrast, analogues such as D-ascorbic acid, 6-bromo-6-deoxy-L-ascorbic acid, and D-isoascorbic acid were approximately one-half, one-fifth, and one-tenth as effective. Potassium 2-*O*-sulfato-L-ascorbate, L-isoascorbic acid, reductic acid, L-gluconic acid γ-lactone, piperidinohexose reductone, dimethylaminohexose reductone, α-tocopherol acetate, *p*-hydroquinone, pyrocatechol, 2,3-dihydroxy-1,4-dithiobutane, sodium dithionite, and ferrous sulfate were at most only one-twentieth as active as 1.

Many insects, especially green plant feeders, require exogenous L-ascorbic acid (1, vitamin C) to grow and develop fully (Dadd, 1973; House, 1974a; Chatterjee et al., 1975). Others, it has been suggested, may not require the vitamin, or they may synthesize it from glucose or rely on symbiotic organisms to produce it (Day, 1949; Gamo and Seki, 1954; Pierre, 1962; Briggs, 1962; Raychaudhuri and Banerjee, 1968). In the present paper we report the effects of ascorbic acid on the development of the plant feeding lepidopteran species, *Manduca sexta* Johannson. A bioassay for vitamin C utilizing this insect has been developed and used to measure the growth-promoting activity of several other related compounds.

EXPERIMENTAL SECTION

Insects. *Manduca sexta* eggs were obtained from Dr. J. P. Reinecke (Agricultural Research Service, U.S. Department of Agriculture, Fargo, N. Dak.), and larvae were reared using a standard agar-based diet (Bell and Joachim, 1976) at 28 °C and 60% relative humidity with a 16-h photophase. After the diet had cooled to 60 °C, L-ascorbic acid or a derivative thereof was added and thoroughly blended. Compound 1 is stable at these conditions (Vanderzant, 1975), and the 2-sulfate, 2-phosphate, and 6-*O*-myristoyl esters are approximately an order of magnitude more stable than 1 (Seib et al., 1974; Lee, 1976; Hoseney et al., 1977). The only relatively labile derivative, L-dehydroascorbic acid (**9**, Velisek et al., 1972), was added to the diet either as described above or was applied to the surface of the gelled diet at room temperature. The diet (13 g) was poured into 110 cm^3 plastic bottles and allowed to harden. Neonate larvae were used in all tests. The rates at which larvae grew on the control diet were compared with those on an ascorbic acid-substituted diet. At 1–4-day intervals up to 40 days, the mean weight of 10–20 animals was determined. Fecal matter was removed at each observation.

Chemicals. L-Ascorbic acid, D-isoascorbic acid, 2,3-dihydroxy-1,4-dithiobutane, *p*-hydroquinone, pyrocatechol, sodium dithionite, and ferrous sulfate were purchased from J. T. Baker, Sigma, Mallinckrodt or Matheson, Coleman and Bell. L-Gulonic acid γ-lactone was obtained from K and K Laboratories and α-tocopherol acetate was from Applied Sciences. Potassium 2-*O*-sulfato-L-ascorbate (mp 87–89 °C) was synthesized as described by Seib et al. (1974). Magnesium 2-*O*-phosphono-L-ascorbate was prepared by cation-exchange chromatography of the tricyclohexylammonium salt of the phosphate ester (mp 178–182 °C), which was synthesized in 70% yield by a modification (Lee, 1976) of the method of Cutolo and Larizza (1961). Magnesium 2-*O*-phosphono-L-ascorbate is an amorphous hydrate with approximately 5 mol of water, so the amount present in solution was determined by UV spectroscopy [$\epsilon_m = 1.62 \times 10^4$ at 264 nm and pH 10 (Lee, 1976)]. Sodium 6-*O*-myristoyl-L-ascorbate was prepared by evaporation to dryness of a biphasic liquid system containing 6-*O*-myristoyl-L-ascorbic acid in chloroform mixed with water containing 1 equiv of sodium bicarbonate (Klaui, 1963). 6-*O*-Myristoyl-L-ascorbic acid (mp 109–111 °C) was synthesized using the procedure of Swern et al. (1943) as modified by Cousins et al. (1977). The following compounds were obtained as follows: reductic acid (mp 211–212 °C) from Professor M. S. Feather, University of Missouri, Columbia, Mo; morpholino- and dimethylhexose reductones (mp 216–217 and 209–210 °C, respectively) from Dr. J. E. Hodge, Northern Regional Research Center, ARS, USDA, Peoria, Ill.; and 6-bromo-6-deoxy-L-ascorbic acid (mp 168–170 °C) from Dr. K. Bock and Dr. C. Peterson at the Technical University of Denmark, Lyngby. L-*erythro*-Hex-2-enonic acid γ-lactone (L-isoascorbic acid, **8**, mp 168–171 °C) was prepared by alkali-catalyzed racemization of L-ascorbic acid (Brenner et al., 1964), whereas the D-threo isomer (D-ascorbic acid, mp 190–194 °C) was produced in the same manner from D-*erythro*-hex-2-enonic acid γ-lactone (D-isoascorbic acid). L-Dehydroascorbic acid was prepared in aqueous solution by oxidation of 1 with freshly sublimed benzoquinone (Schultz et al., 1939). After removal of the aromatic materials with ether, the aqueous phase was held under vacuum to remove the residual ether and then the aqueous phase was admixed with or applied directly to the surface of the diet. Thin-layer chromatography on plates coated with silica gel using acetonitrile–propiononitrile–water (66:33:2) was used to examine the purity of L-dehydroascorbic acid. After developing and charring on a hot plate, L-dehydroascorbic acid gave a single spot of R_f 0.7, whereas L-ascorbic acid gave R_f 0.1. The purity of all crystalline compounds was >99%.

RESULTS AND DISCUSSION

The effect of L-ascorbic acid (1) on the development of *M. sexta* is shown in Figure 1. All neonate larvae de-

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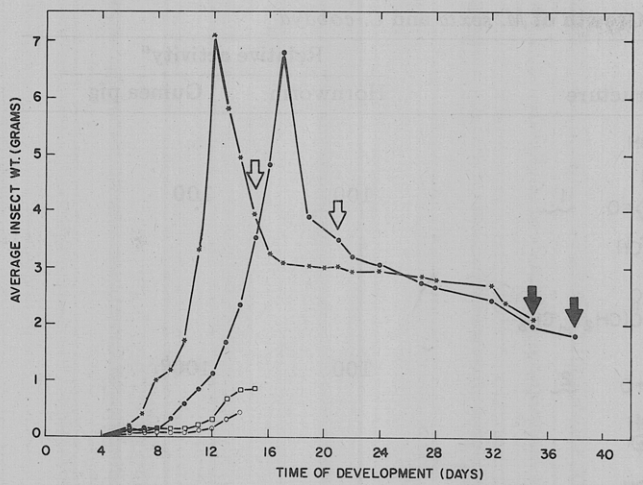


Figure 1. Growth curves of *Manduca sexta* fed diet containing 0.50 mM (-*-), 0.25 mM (-●-), 0.05 mM (-□-) or an ascorbic acid free diet (-O-). Open arrow and closed arrow denote the time of pupal and adult ecdysis, respectively.

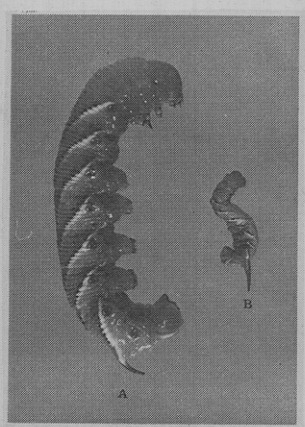


Figure 2. Comparison of the sizes of 8-day-old larvae of *M. sexta* with and without L-ascorbic acid (0.50 mM) in the diet.

veloped to adults on the artificial diet that contained 0.5 mM 1; also, the insects raised on this diet were robust and had the normal bright blue-green coloration. As the amount of 1 in the diet was decreased, pathological effects appeared after a period of feeding that depended on the concentration of 1. Figure 2 shows 8-day-old larvae of *M. sexta* reared on diets without and with 1 (0.5 mM). Those animals reared on a 1-deficient diet were much reduced in size and were colored a dull yellowish green. In all tests, larvae appeared normal through development to the second instar, probably because of the presence of an endogenous level of 1 passed from the parent insects to the neonate larvae. On diet completely lacking 1, physical breakdown occurred during the third instar: at the beginning of the third molting period, the insects began to shrivel, and 1 day later became moribund. Larvae fed medium containing 0.05 mM 1 were similarly affected, but at one stadium later. Fifty percent of the insects fed diet supplemented with 0.25 mM vitamin died in the prepupal stage; the other half underwent pupal and adult eclosion 3 to 6 days later than the control group.

The efficacy of several other compounds in promoting hornworm growth is presented in Table I. The activity of those chemicals in the guinea pig is also listed for comparison. Several organic esters of 1 have been postulated as natural sources of vitamin C and have been found to counteract scorbutic effects in fish, monkey, and guinea pig (Tolbert et al., 1975). Both the 6-myristate (2) and 2-phosphate esters (3) were effective vitamin replacements for the tobacco hornworm at 0.5 mM concentration (Table I) although the average time of adult eclosion of the insects was delayed by several days. Thus, the insect may be metabolically like the guinea pig because both can utilize those ester derivatives of L-ascorbic acid. 6-Fatty acid esters of 1 have full vitamin C potency in guinea pigs (Inagaki et al., 1968). When L-ascorbate-2-phosphate (3) was fed or injected, Cutolo and Larizza (1961) demonstrated that guinea pigs excrete L-ascorbate in their urine; furthermore, the quantity of 1 excreted equalled the amount excreted by animals given an equivalent amount of 1. Carboxyesterases and phosphatases in the digestive tract or other tissues of the hornworm probably convert the respective esters to the free vitamin (House, 1974b) The widespread occurrence of these enzymes in Insecta indicates that fatty acid and phosphate esters of L-ascorbic acid should be active sources of L-ascorbate for all species.

The 2-sulfate ester (7) of L-ascorbic acid was one of the least effective growth factors for the hornworm. To develop normally, the insect required a 20 times greater concentration of this conjugate (10 mM). The hornworm, therefore, does not easily metabolize the sulfate ester to an ascorbate-active compound, perhaps because of the absence of a sulfohydrolase enzyme in insect tissue. L-Ascorbate 2-sulfohydrolyase has been demonstrated in rainbow trout (Tolbert, 1977), mammalian liver (Roy, 1975; Tolbert et al., 1975), a marine mollusc (Hatanaka et al., 1976), and human urine (Fluharty et al., 1976). Although 7 is effective in preventing scurvy in rainbow trout and Coho salmon (Halver et al., 1975), it is devoid of antiscorbutic activity in the guinea pig (Tolbert et al., 1975). Apparently, the growth promoting activity of the 7 as well as of all ascorbic acid type derivatives is species dependent. In fact, preliminary results of Chippendale (1977) suggest that 7 possesses about half the activity of free ascorbic acid for the southwestern corn borer, *Diatraea grandiosella* Dyar. These larvae require a dietary supplement of about 21 mM of 1 for optimal growth, development, and adult fertility (Chippendale, 1975), approximately 40 times more than the level required by the tobacco hornworm. These differences, no doubt, reflect the metabolic needs of individual species.

Several optical isomers of 1 were also bioassayed using the tobacco hornworm (Table I). In the guinea pig, D-isoascorbic acid (6) and D-ascorbic acid (4) have 5 and 0% antiscorbutic activity, respectively (Hay et al., 1967). This indicates that configurational changes at the C-4 and C-5 carbons of 1 reduce or diminish the compound's vitamin potency. In the hornworm, these configurational changes also affect activity, but not as drastically. For example, the enantiomer (4) has ~40% activity, while the C-5 epimer (6) has 10% activity. Ito and Arai (1965), Mittler et al. (1970) and Chippendale (1975) have reported similar results in other insects with compound 6. It should be noted that the relative potency of the two isomers 4 and 6 are reversed in the vertebrate and invertebrate animals. L-Isoascorbic acid (8) has no vitamin potency in either *Manduca* or the guinea pig.

In order to determine whether *Manduca* itself has the capacity to synthesize 1, we tested a well-known precursor of 1 for growth activity. L-Gulonic acid lactone (11) was not active at 0.5 mM concentration. This compound is directly converted to 1 by L-gulonolactone oxidase in some animals (Touster, 1969).

Another derivative with very potent vitamin activity in vertebrates, L-dehydroascorbic acid (9), was inactive in our bioassay when it was admixed with hot, pregelled diet. We

Table I. Effect of L-Ascorbic Acid and Assorted Compounds on Growth of *M. sexta* and *C. cobaya*

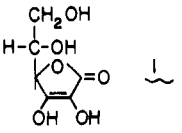
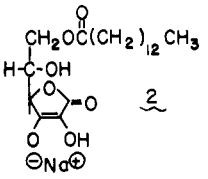
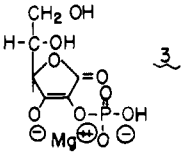
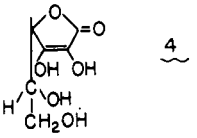
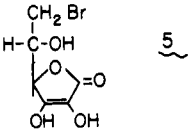
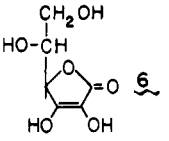
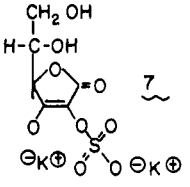
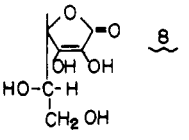
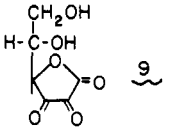
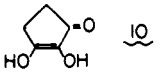
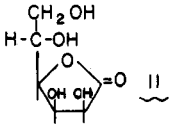
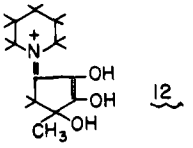
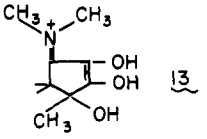
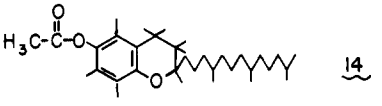
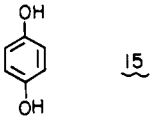
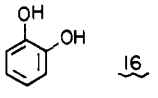
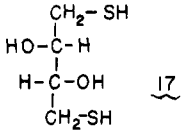
Compound	Structure	Relative activity[a]	
		Hornworm	Guinea pig
L-*threo*-Hex-2-enonic acid γ-lactone (L-ascorbic acid)	 1	100	100
Sodium 6-*O*-myristoyl-L-ascorbate	 2	100	100[b]
Magnesium 2-*O*-phosphono-L-ascorbate	 3	100	100[c]
D-*threo*-Hex-2-enonic acid γ-lactone (D-ascorbic acid)	 4	40 ± 10	0[d,e]
6-Bromo-6-deoxy-L-ascorbic acid	 5	20 ± 10	Not available
D-*erythro*-Hex-2-enonic acid γ-lactone (D-isoascorbic acid)	 6	10 ± 10	5[d,e]
Potassium 2-*O*-sulfato-L-ascorbate	 7	5	0[f]
L-*erythro*-Hex-2-enonic acid γ-lactone (L-isoascorbic acid)	 8	0	0[d,e]
L-*threo*-Hex-2,3-diulosic acid γ-lactone (L-dehydroascorbic acid)	 9	100[g]	100[d]
2,3-Dihydroxycyclopent-2-en-1-one (reductic acid)	 10	0	0[d,h]
L-Gulonic acid γ-lactone	 11	0	0[i]

Table I (*Continued*)

Compound	Structure	Relative activity[a] Hornworm	Guinea pig
N-[1-Methyl-1,2,3-trihydroxy-2-cyclopenten-4-ylidene]-piperidinium betaine (*N*→2 or →3)(piperidinohexose reductone)	 12	0	Not available
Dimethylaminohexose reductone	 13	0	Not available
α-Tocopherol acetate	 14	0	0
p-Hydroquinone	 15	0	0
Pyrocatechol	 16	0	0
2,3-Dihydroxy-1,4-dithiobutane	 17	0	0

[a] Compound tested at 0.5 mM concentration using 10–30 insects in each experiment, except for compounds 4 and 7 where 0.25–10.0 mM was fed. [b] Inagaki et al., 1968. [c] Cutolo and Larizza, 1961. [d] Hay et al., 1967; Hornig, 1975. [e] Demole, 1934. In this original reference, Demole gave an erroneous structure for D-ascorbic acid; instead he gave the structure of D-*erythro*-hex-2-enonic acid γ-lactone. Since the compounds he tested were gifts from Professor Richstein, who in 1933 and 1934 synthesized all four isomeric six-carbon ascorbic acids, we assume that D-ascorbic acid was indeed tested. [f] Kuenzig et al., 1974. [g] Because of instability, L-dehydroascorbic acid was applied to the diet surface, all other compounds were admixed with diet before gelling. [h] This compound was called reductinic acid in ref *d*. [i] Burns and Ashwell, 1969.

thought that this result could be misleading because **9** is rather unstable and may have decomposed under the conditions required to prepare the diet (Velisek et al., 1972). We therefore repeated the bioassay using a surface treatment of **9** and, under this condition, **9** proved to be as effective as **1** in promoting hormone growth.

We also tested the possibility that the vitamin C active compounds were exerting their effects on the hornworm as nonessential, albeit effective reducing agents instead of as a vitamin. The carbon ring analogue, reductic acid (**10**) was inactive. Compounds **12** through **17** are also all organic reducing agents, and none of those showed any growth promoting activity. Ferrous sulfate and sodium dithionite (0.05 M) were two inorganic salts that did not support *Manduca* development. It thus appears that L-ascorbate is an essential nutrient for *Manduca* and does have specific vitamin activity, the precise mechanism for which is not known. In vertebrates, it has been proposed that **1** serves as a nonspecific antioxidant and also functions specifically in protein and fat metabolism and in enzymatic hydroxylation and sulfation reactions (Chatterjee et al., 1975; Hatanaka and Egami, 1976). Because the earliest pathological effects in *Manduca* were observed during a larval molting period, this vitamin may have some role in the production or degradation of insect cuticle. In any event, the structural requirements for vitamin C activity in *M. sexta* are symbatic with those observed in higher animals and, as first noted by Dadd (1963), it is even more apparent that the place of ascorbic acid in insect nutrition may be compared to that of vitamin C for vertebrates.

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LITERATURE CITED

Bell, R. A., Joachim, F. G., *Ann. Entomol. Soc. Am.* **69**, 365–373 (1976).

Brenner, G. S., Hinkley, D. F., Perkins, L. M., Weber, S., *J. Org. Chem.* **29**, 2389–2392 (1964).

Briggs, M. H., *Comp. Biochem. Physiol.* **5**, 241–252 (1962).

Burns, J. J., Ashwell, G., in "The Enzyme", 2nd ed, Vol. 3, Part B, Boyer, P. D., Lardy, H. A., Myrback, K., Ed., Academic Press, New York, N.Y., 1969, pp 397–398.

Chatterjee, I. G., Majumder, A. K., Nandi, B. K., Subramanian, N., *Ann. N.Y. Acad. Sci.* **258**, 24–47 (1975).

Chippendale, G. M., *J. Nutr.* **105**, 499–507 (1975).

Chippendale, G. M., private communication, University of Missouri, 1977.

Cousins, R. C., Seib, P. A., Hoseney, R. C., Deyoe, C. W., Liang, Y. T., Lillard, D. C., *J. Am. Oil Chem. Soc.* **54**, 308–312 (1977).
Cutolo, F., Larizza, A., *Gazz. Chim. Ital.* **91**, 964–972 (1961).
Dadd, R. H., *Adv. Insect Physiol.* **1**, 47–109 (1963).
Dadd, R. H., *Ann. Rev. Ent.* **18**, 381–420 (1973).
Day, M. F., *Aust. J. Sci. Res.* **B2**, 19-30 (1949).
Demole, V., *Biochem. J.* **28**, 770–703 (1934).
Fluharty, A. L., Stevens, R. L., Miller, R. T., Sharpiro, S. S., Kihara, H., *Biochim. Biophys. Acta* **429**, 508–16 (1976).
Gamo, T., and Seki, H., *Shinshu Daigaku Sen'igakubu Kenkyu Hokoku* **4**, 29–38 (1954).
Halver, J. E., Smith, R. R., Tolbert, B. M., Baker, E. M., *Ann. N.Y. Acad. Sci.* **258**, 81–102 (1975).
Hatanaka, H., Egami, F., *J. Biochem. (Tokyo)* **80**, 1215–1221 (1976).
Hatanaka, H., Ogawa, Y., Egami, F., *Biochem. J.* **159**, 445–448 (1976).
Hay, G. W., Lewis, B. A., Smith, F., in "The Vitamins", Sebrell, W. H., Jr., Harris, R. S., Ed., Academic Press, New York, N.Y., Vol I, 1967, p 331; see original references therein.
Hornig, D., *World Rev. Nutr. Diet* **23**, 225–258 (1975).
Hoseney, R. C., Seib, P. A., Deyoe, C. W., *Cereal Chem.* **54**, 1062–1069 (1977).
House, H. L., "Physiology of Insecta", 2nd ed, Vol. 5, Rockstein, M., Ed., Academic Press, New York, N.Y., 1974a, pp 1–62.
House, H. L., "Physiology of Insecta", 2nd ed, Vol. 5, Rockstein, M., Ed., Academic Press, New York, N.Y., 1974 b, pp 63–117.
Inagaki, C., Arakawa, N., Suzuki, N., Sago, Y., Nogami, K., *Vitamins* **37**, 152–158 (1968); *Chem. Abstr.* **68** 66890a (1968).
Ito, T., Arai, N., *Bull. Sinc. Exp. Sta., Tokyo* **20**, 1–19 (1965).
Klaui, H., *Wiss. Veroeff. Dtsch. Ges. Ernahr.* **9**, 390–400 (1963).
Kuenzig, W., Avenia, R., Kamm, J. J., *J. Nutr.* **104**, 952–956 (1974).
Lee, C. H., Ph.D. Dissertation, Kansas State University, Manhattan, Kans., 1976.
Mittler, T. E., Tsitsipis, J. A., Kleinjan, J. E., *J. Insect Physiol.* **16**, 2315–2326 (1970).
Pierre, L. L., *Nature (London)* **193**, 904–905 (1962).
Raychaudhuri, D. N., Banerjee, M., *Sci. Cult.* **34**, 461–463 (1968).
Roy, A. B., *Biochim. Biophys. Acta* **377**, 356–363 (1975).
Schultz, M. O., Harrer, C. J., King, C. G., *J. Biol. Chem.* **131**, 5–12 (1939).
Seib, P. A., Liang, Y. T., Lee, C. H., Hoseney, R. C., Deyoe, C. W., *J. Chem. Soc., Perkin Trans. 1*, 1220–1224 (1974).
Swern, D., Stirton, A. J., Turer, J., Wells, P. A., *Oil Soap* **20**, 224 (1943).
Tolbert, B. M., Downing, M., Carlson, R. W., Knight, M. K., Baker, E. M., *Ann. N.Y. Acad. Sci.* **258**, 48–69 (1975).
Tolbert, B. M., private communication, University of Colorado, 1977.
Touster, O., in "Comprehensive Biochemistry", Vol. 17, Florkin, M., Stotz, E. H., Ed., Elsevier, Amsterdam, 1969, pp 219–240.
Vanderzant, E. S., *J. Econ. Entomol.* **68**, 375–376 (1975).
Velisek, J., Davidek, Janicek, G., *Coll. Czech. Chem. Commun.* **37**, 1465–1470 (1972).

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Analysis of Toxic Mussels (*Mytilus* sp.) from the Alaskan Inside Passage

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An analysis of the toxins present in two samples of PSP infested mussels from Haines and Elfin Cove, Alaska, is described. The toxins are extracted and fractionated by gel filtration and liquid chromatography (ion exchange). Both samples are found to contain gonyautoxin-I, gonyautoxin-II, gonyautoxin-III, gonyautoxin-IV, gonyautoxin-V, and a new PSP poison. In addition, the Haines sample contains minor amounts of neosaxitoxin and the Elfin Cove sample contains minor amounts of saxitoxin. In both samples, gonyautoxin-I is the predominant toxin. Some implications of these finding vis-a-vis PSP in the Alaskan butter clam are considered.

Paralytic shellfish poisoning (PSP), a severe and occasionally fatal form of food poisoning caused by the ingestion of certain shellfish which have been exposed to blooms of toxic dinoflagellates, is a recurring health hazard in certain areas of the world.

The potent neurotoxin, saxitoxin (**1**), has been isolated and identified as the responsible PSP toxin from: California mussels, *Mytilus californianus*; Alaska butter clams, *Saxidomus giganteus*; and the Pacific dinoflagellate, *Gonyaulax catenella* (Schantz et al., 1957, 1966).

In addition to saxitoxin, New England soft shell clams, *Mya arenaria*, and cultures of the Atlantic dinoflagellate *Gonyaulax tamarensis* were found to contain three related toxins: gonyautoxin-I (GTX_1), gonyautoxin-II (GTX_2), and gonyautoxin-III (GTX_3) (Shimizu et al., 1975).

The heterogeneity of the PSP toxins was confirmed and extended by the subsequent isolation of three additional toxins: gonyautoxin-IV (GTX_4), gonyautoxin-V (GTX_5), and neosaxitoxin (neoSTX) from *G. tamarensis* cells. Soft shell clams *Mya arenaria* exposed to a bloom of *G. tamarensis* were also found to contain GTX_4 and neosaxi-

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