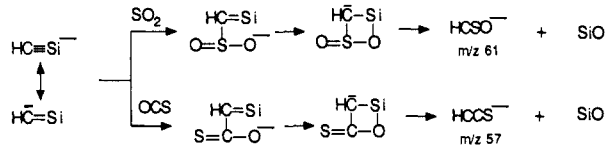
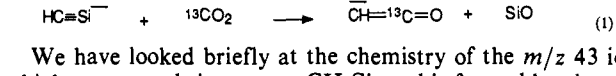
Scheme I



are sampled into a vacuum chamber, the neutral precursor and the helium are removed by pumping, and the ions whose chemistry is to be studied are separated in a quadrupole mass filter from ions of other masses and injected into the second flow tube. There they are allowed to react with neutral reagents, and the resulting anionic products are sampled, separated in a second quadrupole, and detected with an electron multiplier. The results reported in this communication for the silaacetylide ion (m/z 41) were obtained from ions produced from both methylsilane and tetramethylsilane; those for the silene anion (m/z 43) came from methylsilane.

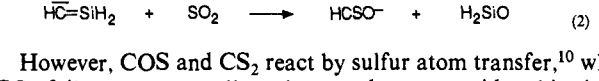
We first measured the basicity of $HCSi^-$. It reacts readily with CH_3SH ($\Delta H°_{(acid)}$ = 357 ± 3 kcal/mol)[5] to form CH_3S^- but not with HF (371 ± 2), acetone (369 ± 3), or acetophenone (362 ± 2). These results suggest that $\Delta H°_{(acid)}$ ($H_2C{=}Si:$) = 360 ± 3 kcal/mol.[6] For comparison, $\Delta H°_{(acid)}$ (acetylene) = 378 ± 2. The silaacetylide ion can have the structure with hydrogen either on silicon or on carbon. Preliminary ab initio computations by Professor M. S. Gordon[7] strongly favor a structure with hydrogen bound to carbon. This same structure also best fits the chemistry of the ion. Its most revealing reactions are cleavages with SO_2 and COS, leading to ions of m/z 61 and 57, respectively. We picture these ions as arising by initial attack by carbon followed by silicon–oxygen bond formation and fragmentation (Scheme I). An analogous cleavage reaction of the allenyl anion has been observed earlier.[8]

The silaacetylide ion does not appear to react with CO_2. However a cleavage reaction like that in Scheme I would lead to the ketene anion, also of m/z 41, so that reaction would be undetectable. Indeed, $^{13}CO_2$ converts the m/z 41 ion to m/z 42 (eq 1).



(1)

We have looked briefly at the chemistry of the m/z 43 ion, which corresponds in mass to CH_3Si^- and is formed by electron impact on methylsilane. There are four possible tautomeric structures for an ion of planned. a mass, with $CH_2{=}SiH^-$ being favored on the basis of preliminary calculations by Gordon.[9] We find that upon reaction with SO_2 (eq 2), the same m/z 61 ion is formed from silaacetylide, a result which seems to favor the alternative tautomeric structure with a single hydrogen on carbon.



(2)

However, COS and CS_2 react by sulfur atom transfer,[10] while CO_2 fails to react at all so that we do not consider this single reaction a definitive proof of structure. Additional studies on these and other unsaturated silicon anions are planned.

Acknowledgment. R.D. and C.H.D. thank the National Science Foundation for support of this work (CHE-8615808 and CHE-8503505, respectively).

(5) Bartmess, J. E., personal communication. All acidity values used come from an unpublished compilation supplied by Professor Bartmess. This is an updated version of the tables published by Bartmess and McIver (Bartmess, J. E.; McIver, R. T., Jr. In *Gas Phase Ion Chemistry*; Bowers, M. T., Ed.; Academic Press: New York, 1979; Vol. 2).

(6) This value assumes that proton abstraction from acetone and acetophenone by the silaacetylide ion is fast enough to be observed if it were exothermic. However, proton transfers to and from carbon and silicon can be slow.[7] Exothermic proton transfers from HF are unlikely to be slow, so that a more conservative value for $\Delta H_{(acid)}$ is 364 ± 7 kcal/mol.

(7) Gordon, M. S., personal communication to R.D. Professor Gordon has initiated a comprehensive computational study of the anions reported in this paper as well as a variety of others for which we have experimental work in progress.

(8) DePuy, C. H. *Org. Mass Spectrom.* **1985**, *20*, 556.

(9) Preliminary computations on the silene anions suggest that $CH_2{=}SiH^-$ is more stable than $SiH_2{=}CH^-$ by some 35 kcal/mol. Though is it unlikely that the energy ordering of these two anions will change with additional computational refinements, computations on related isomeric structures have yet to be carried out and might reveal other stable isomers.

(10) DePuy, C. H.; Bierbaum, V. M *Tetrahedron Lett.* **1981**, *22*, 5129.

(11) We have called this the silaacetylide anion recognizing that the parent is undoubtedly $H_2C{=}Si:$ as has been demonstrated by a number of computational studies (see: Luke, B. T.; Pople, J. A.; Krogh-Jespersen, M.; Apeloig, Y.; Karni, M.; Chandrasekhar, J.; Schleyer, P. von R. *J. Am. Chem. Soc.* **1986**, *108*, 270 and references therein). Both the acidity and reactivity studies in this communication are consistent with the formation of an anion, $[HCSi]^-$, which reacts at a relatively negative carbon to which hydrogen is bound.

Isolation and Structure of the Powerful Cell Growth Inhibitor Cephalostatin 1[1]

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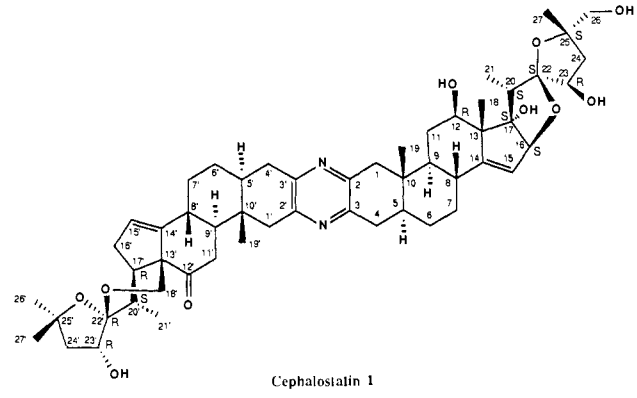
Received July 22, 1987

The invertebrate chordates have some vertebrate characteristics such as a dorsal tubular nervous system and notochord. Among such phyla lacking a vertebral column occurs the Hemichordata. The class Pterobranchia of this phylum has not heretofore been explored in respect to biologically active or other chemical constituents. In late 1972, we collected by SCUBA (ca. -20 m) in the Indian Ocean off Southeast Africa, in areas patrolled by the great white shark *Carcharodon carcharias*, specimens from this class of the marine worm (~5-mm long in tube colonies) *Cephalodiscus gilchristi* (order Cephalodiscida).[2] Two years later, methanol and water extracts of *C. gilchristi* reached the confirmed active level against the U.S. National Cancer Institute's murine P388 lymphocytic leukemia (PS system) with 32–41% life extension at 25–37.5 mg/kg. We now are pleased to report that 15 years of relentless research directed at discovering the active constituent(s) has culminated in the isolation and structural elucidation of a powerful cell growth inhibitory substance designated cephalostatin 1 (**1**) with PS cell line ED_{50} 10^{-7}–10^{-9} μg/mL.



Cephalostatin 1

A number of approaches to the isolation of cephalostatin 1 were made with recollections of *C. gilchristi* until the problem was eventually solved by using a 1981 acquisition (166 kg wet wt, including Coenecium). A methylene chloride–methanol extract was successively partitioned by using the system 9:1 → 4:1 → 3:2 methanol–water against hexane → carbon tetrachloride → methylene chloride, respectively. The active methylene chloride

(1) Contribution 147 of "Antineoplastic Agents". For the prior part, see: Pettit, G. R.; Singh, S. B.; Hamel, E.; Lin, C. M.; Schmidt, J. M.; Alberts, D. S.; Kendall, D. G. *Experientia*, submitted for publication.

(2) Barrington, E. J. W. *The Biology of Hemichordata and Protochordata*; W. H. Freeman and Co.: San Francisco, CA, 1965; pp 1–176, and references therein.

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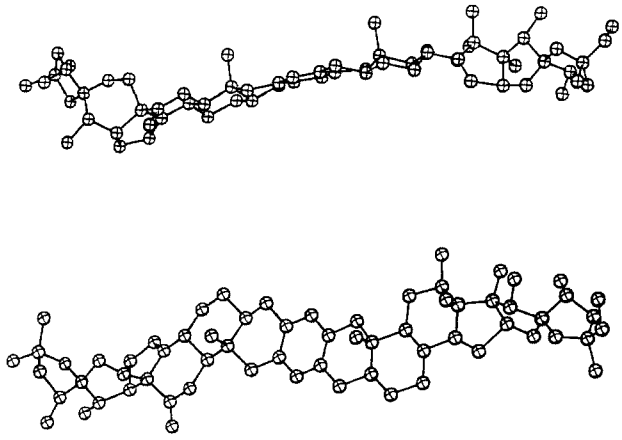

Figure 1. ORTEP-II perspective view of cephalostatin 1.

fraction (28 g) was separated by using a detailed bioassay (PS) guided series of gel permeation, partition (Sephadex LH20 and LH60, silica gel, e.g., with 10:10:1 hexane–ethyl acetate–methanol to 10:10:1 hexane–methylene chloride–methanol) gradient column chromatograms, to HPLC on Partisil M9 employing methanol–water and hexane–ethyl acetate–methanol gradients. Cephalostatin 1 (**1**) was obtained in 8.36×10^{-4}% yield: 138.8 mg as needles from ethyl acetate–methanol; mp 326 °C dec; R_f 0.35 (SiO_2 plate), 90:10:0.8 methylene chloride–methanol–water; $[\alpha]_D$ +102 (*c* 0.04, CH_3OH); SP-HRSIMS[3] 911.5442 ($[M + H]^+$ for $C_{54}H_{74}N_2O_{10}$, calcd 911.5423); UV (CH_3CH_2OH) λ_{max} 289 (ϵ 15233) and 309 (shoulder) nm; IR (KBr) 3430, 3050, 2970, 2930, 2880, 2860, 1708, 1650–1615 (br), 1445, 1400, 1152, 1115, 1090, 1045, 950, and 892 cm^{-1}; see ref 4 and 5 for the ^{13}C and ^1H NMR assignments. The chemical shifts were rigorously assigned by using results of HH-, HC-, and HH-Relayed COSY, COLOC, HHC-RELAY, and NOE experiments.

Cephalostatin 1 gave a tetraacetate derivative: SP-HRSIMS *m/e* 1079.5798 $[M + H]^+$ for $C_{62}H_{82}N_2O_{14}$. But SP-SIMS active hydrogen determination[6] by hydrogen–deuterium exchange indicated five such hydrogen atoms. The yellow-orange reaction to Dragendorf's reagent and positive response to 12-molybdophosphoric acid suggested a steroidal alkaloid-type structure. However, the structural problem proved refractory to complete solution by high field NMR and mass spectral techniques and required crystal structure determination.[7] As cephalostatin persisted in crystallizing in long fibrous needles, preparation of suitable crystals for X-ray proved to be especially difficult and was solved only when this remarkable substance was found to form a useful solvate with pyridine that slowly crystallized (as small plates) at ambient temperature from pyridine–hexane.

Computer-generated perspective views of cephalostatin 1 are shown in Figure 1. On the basis of the absolute configuration of the well established steroid nucleus, the following stereochemical assignments at the remaining eleven asymmetric carbon atoms are as follows: 12*R*, 16*S*, 17*S*, 20*S*, 22*S*, 23*R*, 25*S*, 17′*R*, 20′*S*, 22′*R*, 23′*R*. The structure of cephalostatin 1 is quite unusual, consisting of nine fused rings formed by the coupling of two steroid nucleii at C2 and C3. In addition, two spiroketal rings terminate each end of the fused ring system generating a total of 13 rings. The overall dimensions of cephalostatin 1 are approximately 30-Å long, 9-Å wide and 5-Å thick. The bond angles and distances are within generally accepted limits. The thermal parameters of several atoms were consistently large, i.e., isotropic $U = 0.09$–0.13, for the carbonyl oxygen O_{12}', and the substituents at opposing ends of the molecule—O_{26}, C_{26}', and C_{27}'. A detailed crystallographic analysis has been placed in the Supplementary Material.

Apprently cephalostatin 1 results in part from a biosynthetic condensation of 2-amino-3-oxo steroid units[8] to yield a powerful inhibitor of cell growth that may in turn serve in the chemical defense and/or in other important functions of *C. gilchristi*. Evaluation of the novel marine worm biosynthetic product against various antineoplastic and other biological systems is in progress.

Acknowledgment. For the necessary financial support our appreciation is extended to the Arizona Disease Control Research Commission, the Fannie E. Rippel Foundation, The Robert B. Dalton Endowment, Eleanor W. Libby, the Donald Ware Waddell Foundation, Jack W. Whiteman, Herbert K. and Dianne Cummings (The Nathan Cummings Foundation), Grants CA-16049-05-11 awarded by the NCI, and DHHS and Contract NO1-CM-97262 with the Division of Cancer Treatment, NCI, DHHS. We are pleased to thank for other assistance, Drs. J. D. Douros, J. J. Einck, C. L. Herald, P. Lohavanijaya, M. L. Niven, K. Pandel, J. A. Rideout, D. L. Smith, M. Suffness and L. Lange, G. C. Bryan, and L. D. Vanell. Also, we are grateful for assistance provided by the National Science Foundation for equipment (Grant CHE-8409644) and the NSF Regional Instrumentation Facility in Nebraska (Grant CHE-8620177).

Registry No. 1, 112088-56-9; **1** tetraacetate, 112088-57-0.

Supplementary Material Available: Tables of bond distances and angles and positional parameters for cephalostatin 1 (7 pages). Ordering information is given on any current masthead page.

(3) Pettit, G. R.; Cragg, G. M.; Holzapfel, C. W.; Tuinman, A. A.; Gieschen, D. P. *Anal. Biochem.* **1987**, *162*, 236–241.

(4) 100 MHz ^{13}C NMR (deuteriopyridine): 9.01 (C-21), 11.31 (C-19′), 11.72 (C-19), 12.58 (C-18), 15.49 (C-21′), 26.42 (C-27), 27.94 (C-6), 28.23 (C-6′), 28.69 (C-7), 28.94 (C-11), 29.44 and 29.75 (C-26′ and C-27′), 29.50 (C-7′), 32.36 (C-16′), 32.88 (C-20′), 33.79 (C-8), 35.56 (C-8′), 35.72 (C-4), 35.78 (C-4′), 36.28 (C-10′), 36.32 (C-10), 38.81 (C-11′), 39.51 (C-24), 41.20 (C-5′), 41.78 (C-5), 44.21 (C-17′), 44.50 (C-20), 45.82 (C-1′), 45.98 (C-1), 47.32 (C-24′), 52.20 (C-9′), 53.20 (C-9), 55.39 (C-13), 61.82 (C-13′), 64.19 (C-18′), 69.28 (C-26), 71.51 (C-23), 75.59 (C-12), 81.12 (C-25′), 81.52 (C-23′), 82.81 (C-25), 91.66 (C-17), 93.15 (C-16), 110.96 (C-22′), 117.16 (C-22), 122.28 (C-15), 123.18 (C-15′), 148.38, 148.44, 148.66, 149.01, and 149.46 (C-2, C-3, C-2′, C-3′, and C-14′), 152.71 (C-14), and 211.80 (C-12′) ppm.

(5) 400 MHz ^1H NMR (deuteriopyridine): 0.72 (3 H, s, H-19′), 0.75 (3 H, s, H-19), 0.88 (dt, 4.5, 13.8, H-9), 1.26 (m, H-9′), 1.28 (m, H-6b′), 1.30 (m, H-7b′), 1.33 (3 H, s, H-18), 1.34 (m, H-6b), 1.35 (m, H-7b), 1.35 (3 H, d, 7.0, H-21), 1.39 (3 H, s, H-27′), 1.47 (3 H, d, 7.0, H-21′), 1.47, (3 H, s, H-26′), 1.53 (m, H-6a′), 1.59 (m, H-6a′), 1.60 (m, H-5′), 1.61 (m, H-5), 1.65 (3 H, s, H-27), 1.69 (m, H-7a), 1.77 (dt, 10.0, 14.0, H-11b), 1.95 (dd, 6.2, 12.0, H-24b′), 1.99 (m, H-7a′), 2.04 (m, H-11a), 2.07 (m, H-8), 2.13 (m, H-8′), 2.33 (m, H-16b′), 2.35 (m, H-24a′), 2.36 (m, H-24b), 2.56 (d, 17.0, H-1b′), 2.61 (dd, 14.0, 3.2, H-11b′), 2.64 (d, 17.0, H-1b), 2.65 (2 H, dd, 18.0, 12.5, H-4b and H-4b′), 2.72 (dd, 12.0, 7.0, H-17′), 2.77 (dd, 10.5, 7.9, H-24a), 2.78 (t, 14.0, H-11a′), 2.86 (q, 7.0, H-20), 2.87 (dt, 4.0, 11.8, H-16a′), 2.91 (dd, 17.9, 5.5, H-4a′), 2.93 (dd, 17.9, 5.5, H-4a), 3.04 (d, 17.0, H-1a′), 3.07 (d, 17.0, H-1a), 3.17 (dq, 7.0, 7.0, H-20′), 3.72 (d, 11.2, H-26a), 3.78 (d, 11.2, H-26b), 4.02 (d, 12.2, H-18a′), 4.05 (m, H-12), 4.06 (d, 12.2, H-18b′), 4.70 (s, C-12 OH), 4.80 (2 H, m, H-23 and H-23′), 5.24 (s, H-16), 5.44 (s, H-15′), 5.64 (s, H-15), 6.23 (s, C-17 OH), 6.54 (br s, C-26 OH), 7.19 (br s, C-23′ OH), and 8.06 (br s, C-23 OH) ppm.

(6) Sethi, S. K.; Smith, D. L.; McCloskey, J. A. *Biochem. Biophys. Res. Commun.* **1983**, *112*, 126–131.

(7) Data collection was performed with an Enraf-Nonius CAD-4 diffractometer. The crystal (0.5 × 0.2 × 0.1 mm) was assigned the orthorhombic space group $P2_12_12_1$: $a = 14.863$ (6) Å, $b = 14.992$ (5) Å, $c = 26.354$ Å (6). A density of 1.197 g/cc and mass spectral data indicated one molecule of cephalostatin 1 and two molecules of pyridine per asymmetric unit. All unique reflections with $2\theta < 130°$ were measured at room temperature by using a graphite-monochromated Cu Kα radiation (1.54178 Å) and a ω–2θ scan technique. A complete hemisphere (19570) of reflections was collected. Merging equivalent reflections, removal of systematic absences, and rejection of "unobserved" reflections, $\{[F_o] < 3\sigma[F_o]\}$, yielded a data set consisting of 3380 unique reflections (containing Friedel pairs). Direct methods structure solution was performed with MULTAN-80: Main, P. *A System of Computer Programs for the Automatic Solution of Crystal Structures from X-ray Diffraction Data*; University of York: England, 1980. All refinement calculations were completed by using the "CRYSTALS" computing package: Watkin, D. J.; Carruthers, J. R.; Betteridge, P. W. 1985. A final full-matrix least-squares isotropic refinement of the total asymmetric contents (H atom coordinates and thermal parameters fixed) converged to a residual of $R = 0.088$ and $R_w = 0.056$ (anomalous dispersion corrections made by using 3380 reflections).

(8) A few such reactions have been pursued synthetically with simpler steroids to yield symmetrical pyrazines with UV λ_{max} 290 (ϵ 14000) and 310 (shoulder) nm. Refer to the following: (a) Doorenbos, N. J.; Dorn, C. P. *J. Pharm. Sci.* **1965**, *54*, 1219. (b) Ohta, G.; Koshi, K.; Obata, K. *Chem. Pharm. Bull.* **1968**, *16*, 1487–1497.