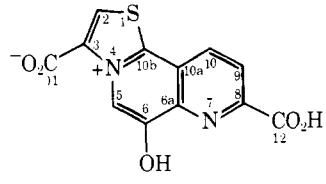
Berninamycin. I. The Structure of Berninamycinic Acid[1]

Sir:

The antibiotic berninamycin[2] has been shown to be a potent inhibitor of bacterial protein synthesis[3] and, hence, a potentially useful chemotherapeutic agent. An earlier report[2] also described the isolation of a sulfur-containing degradation product which was given the name berninamycinic acid. In the present report we describe the separation of berninamycin into two components and the preparation of a crystalline hydrate of berninamycinic acid, and assign this zwitterionic acid (which accounts for one-fourth of the molecular formula of berninamycin) the structure **1**, containing the hitherto unreported pyridothiazolopyridinium chromophore shown.



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Purification of crude berninamycin line product by counter-current distribution (chloroform:cyclohexane:0.1 N aqueous sodium chloride:methanol 27:13:10:30; 800 transfers) afforded a major component, berninamycin A (K 0.33), and a minor component, berninamycin B (K 0.21), with approximate formulas $C_{51}H_{50}N_{14}O_{16}S$ and $C_{59}H_{74}N_{14}O_{22}S$, respectively, based on microanalyses, NMR spectra, and degradation products.[1b] Berninamycin A appears to be identical with the purified berninamycin which was used in the earlier studies.[2,3] In spite of the antibiotics' high degree of unsaturation proton magnetic resonance spectra (C_5D_5N) of berninamycins A and B contain only four aromatic protons—doublets at 8.04 and 8.34 ppm (J = 8 Hz), singlets at 8.57 and 8.84 ppm for berninamycin A; doublets at 8.07 and 8.34 ppm (J = 8 Hz), singlets at 8.59 and 8.89 ppm for berninamycin B. These aromatic protons and the sulfur atom are found in berninamycinic acid, as we shall see.

Purified berninamycin A was hydrolyzed for 18 h at 100 °C in 6 N aqueous hydrochloric acid. The insoluble residue from the hydrolysis (crude berninamycinic acid) was dissolved in a minimal volume of 0.1 N aqueous sodium hydroxide and diluted to approximately 3 mg/ml. Careful acidification with concentrated hydrochloric acid to pH 4–5 afforded golden needles of berninamycinic acid, $C_{12}H_6N_2O_5S$:[4] mp 210° dec; pK_a = 5.8; λ_{max} (0.01 N HCl) 228 (ϵ 13 500) and 272 nm (ϵ 13 500); λ_{max} (0.01 N NaOH) 232 (ϵ 9500) and 294 nm (ϵ 13 000). The ultraviolet spectra of berninamycins A and B, which contain other chromophores as well as that of **1**, show intense broad absorption (ϵ >15 000) covering the region from 210 to 280 nm. Similar hydrolysis of berninamycin B also yielded berninamycinic acid.

Since berninamycinic acid is insoluble in water, magnetic resonance spectra were obtained on the sodium salt, mp 300 °C dec, prepared by dissolving crude berninamycinic acid in 1 N sodium hydroxide and allowing the solution to stand overnight at 5 °C, and the ammonium salt, mp 270 °C dec, prepared by lyophilizing a sample of berninamycinic acid dissolved in 1 N ammonium hydroxide. The ^{1}H NMR spectrum (D_2O, pH 9) of sodium berninamycinate, like spectra of berninamycins A and B, shows four aromatic protons at low field—doublets at 8.00 and 8.41 ppm (J = 8 Hz), singlets at 8.39 and 9.21 ppm. The ^{13}C NMR spectrum of ammonium berninamycinate has 12 peaks in the region 118–172 ppm, also in accord with a highly aromatic compound.

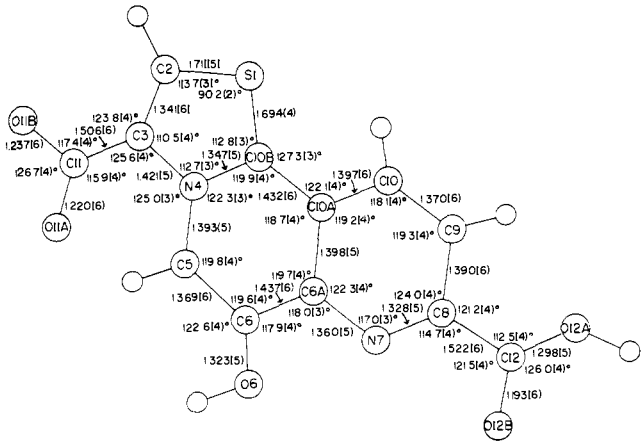


Figure 1. Bond lengths (Å) and angles (deg) for the molecule of berninamycinic acid.

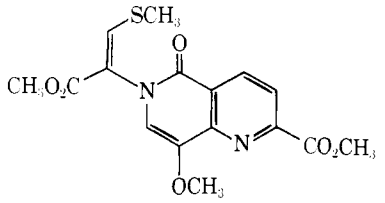
Ammonium berninamycinate inhibits bacterial protein synthesis under the previously described conditions[3] to the extent of 34% at a concentration of 0.2 mM, whereas berninamycins A and B inhibit synthesis to the extent of 35 and 6%, respectively, at 0.01 mM. It does not, however, inhibit bacterial growth in vitro, while both antibiotic components are active.

The structure of the dihydrate of berninamycinic acid (**1**) was determined by x-ray crystallographic direct methods[6] and the structure of the molecule is shown in Figure 1. A small (~0.05 × 0.1 × 0.5 mm), amber-colored crystal was used to collect 2153 unique reflections on a Picker FACS-1 diffractometer using Cu K_α radiation. The needle shaped crystal is monoclinic (a = 8.499 (3) Å, b = 7.116 (4) Å, c = 22.158 (14) Å, β = 108.08(4)°), and the space group is $P2_1/c$ with four $C_{12}H_6N_2O_5S\cdot 2H_2O$ groups per unit cell. The positions of all atoms were refined by full-matrix least-squares methods (all atoms were allowed to vibrate anisotropically except the hydrogens) to a weighted R factor (R_2) of 0.052 and an unweighted R factor (R_1) of 0.059 on all observed reflections. The molecule is quite planar. The deviations from the best plane of the atoms in the three rings range from −0.037 to 0.019 Å. There are two water molecules of crystallization per asymmetric unit which are strongly hydrogen bonded to each other and fall between the planar berninamycinic acid molecules in the z direction. The berninamycinic acid molecules are stacked over each other in the y direction with an interplanar distance of 3.5 Å.[7]

The novel structure assigned berninamycinic acid (**1**, 8-carboxy-6-hydroxypyrido[3,2-*c*]thiazolo[3,2-*a*]pyridinium-3-carboxylate) correlates well with the elemental analyses and mass spectrometric results,[4] and its zwitterionic character accounts for its intrinsic high polarity and the difficulties encountered during attempted derivatization.

During the course of the structural studies attempts were made to form less polar derivatives of berninamycinic acid for mass spectral analysis. The most successful of these involved exhaustive permethylation with methyl iodide–silver oxide, which afforded a pure product, tetramethyl berninamycinate hydrate (**2**, $C_{16}H_{16}N_2O_6S$, mol wt 364.0734 by HRMS), in very low yield. The ^{1}H NMR spectrum ($CDCl_3$) of **2** exhibits three *O*-methyl singlets at 3.80, 3.94, and 4.07 ppm, one *S*-methyl singlet at 2.53 ppm, an AB quartet (8.25 ppm, 1 H, J = 8 Hz; 8.86 ppm, 1 H, J = 8 Hz), and two singlets (6.61 ppm, 1 H; 7.92 ppm, 1 H). The ultraviolet spectrum of **2** (λ_{max} 275 nm, ϵ 15 000) is similar to that of berninamycinic acid in acidic solution.

The only way that an *S*-methyl group could arise in tet-



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ramethyl berninamycinate hydrate is by cleavage of the thiazole ring system. In view of the pyridine ring the likely site of hydrolytic cleavage of the thiazole ring system in **1** is the S–C(10b) bond. Thus, structure **2** is assigned for tetramethyl berninamycinate hydrate.

Acknowledgment. This study was supported in part by grants from the National Institute of Allergy and Infectious Diseases (AI 01278) and the National Institute of General Medical Sciences (GM 19336). High resolution and field desorption mass spectra were obtained on a mass spectrometer provided by grants from the National Cancer Institute (CA 11,388) and National Institute of General Medical Sciences (GM 16864). The berninamycin used was provided by The Upjohn Company.

Supplementary Material Available: structure factors and atomic coordinates (15 pages). Ordering information is given on any current masthead page.

References and Notes

(1) (a) Presented in part at the 170th National Meeting of the American Chemical Society, Chicago, Ill., Aug 1975; *cf.* Abstract No. ORGN 20. (b) Taken in part from the Ph.D. Thesis of J. M. Liesch, University of Illinois, Urbana, 1975.
(2) M. E. Bergy, J. H. Coats, and F. Reusser, U.S. Patent 3 689 639 (23 Jan 1969); *Chem. Abstr.*, **77**, 150582 (1972).
(3) F. Reusser, *Biochemistry*, **8**, 3303 (1969).
(4) Even after drying at 0.02 Torr for 24 h at 110 °C the anhydrous acid could not be obtained. Calcd for $C_{12}H_6N_2O_5S \cdot 0.2H_2O$: C, 49.04; H, 2.19; N, 9.53; S, 10.91. Found: C, 48.93; H, 2.30; N, 9.46; S, 10.93. The molecular weight corresponding to $C_{12}H_6N_2O_5S$ is 290. Electron impact mass spectra of berninamycinic acid vary due to decomposition. The field desorption mass spectrum[5] of ammonium berninamycinate showed only fragmentation peaks at *m/e* 246 (M − NH_3 − CO_2) and 202 (246 − CO_2).
(5) K. L. Rinehart, Jr., J. C. Cook, Jr., K. H. Maurer, and U. Rapp, *J. Antibiot.*, **27**, 1 (1974).
(6) A program called LSAM by P. Main and M. M. Woolfson, Department of Physics, University of York, York, England, 1972, was used.
(7) A listing of the final values for the atomic coordinates and for the x-ray structure factors will appear following these pages in the microfilm edition of this volume of the journal. See paragraph at end of paper regarding supplementary material.
(8) University of Illinois Fellow, 1971–1973; Mobil Foundation Fellow, 1973–1974; Uniroyal Fellow, 1974–1975.
(9) NIH Postdoctoral Fellow, 1974–1976.

Jerrold M. Liesch,[8] Joyce A. McMillan,[9] Ramesh C. Pandey
Iain C. Paul,* Kenneth L. Rinehart, Jr.*
School of Chemical Sciences, University of Illinois
Urbana, Illinois 61801

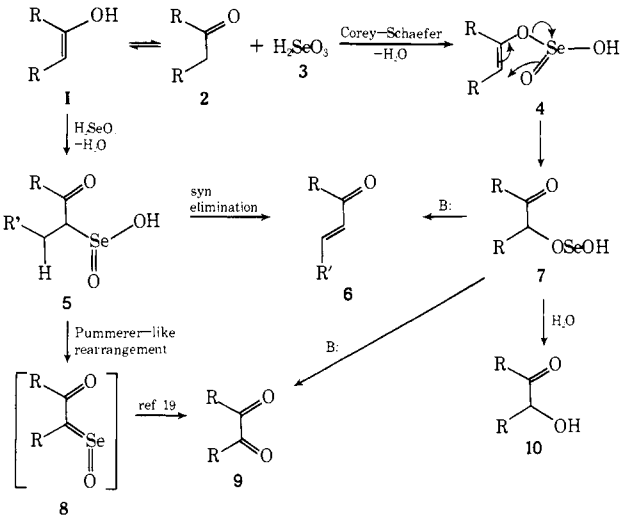
Fritz Reusser
The Upjohn Company
Kalamazoo, Michigan 49001
Received September 8, 1975

Selenium Dioxide Oxidation of Ketones and Aldehydes. Evidence for the Intermediacy of β-Ketoseleninic Acids

Sir:

Olefins, ketones, and aldehydes are the three organic functional groups most often subjected to oxidation by selenium dioxide. We previously established that olefin oxidations proceed via allylseleninic acids,[1] and we now present evidence that the selenium dioxide oxidation of ketones and aldehydes to α-diketones and glyoxals also involves an organoselenium species.

Scheme I



Our mechanism (Scheme I) proposes that the key intermediate in this sequence is the β-ketoseleninic acid **5** formed by electrophilic attack of selenous acid, **3**, on the enol **1**;[2] Pummerer-like[3] decomposition yields the α-diketone **9**. For comparison, Scheme I also includes the mechanism proposed by Corey and Schaefer[4] which is widely accepted at this time, but does not involve an organoselenium intermediate. Ample precedent exists for the formation of carbon–selenium bonds during selenium dioxide oxidations,[5] and, in several cases, the putative β-ketoseleninic acid intermediate appears to have been trapped by a second molecule of the substrate.[5d-f]

Our principal objection to the mechanism of Corey and Schaefer arises from our observation[1a] that selenium II esters such as **7** hydrolyze very rapidly to alcohols (**10**). Furthermore, in their kinetic study of the oxidation of deoxybenzoin, Corey and Schaefer explicitly excluded the intermediacy of such a ketol on the basis that oxidation of benzoin to benzil proceeds at only one-twentieth the oxidation rate of deoxybenzoin. We therefore do not consider **7** to be a likely intermediate.[6]

To date, we have been unable to isolate a β-ketoseleninic acid (**5**). We have sought instead to generate this species in situ by the oxidation of α,α′-diketodiselenides[7a] and observe the resulting organic products. To eliminate the possibility that β-ketoseleninic acids thus formed might revert to selenous acid and ketone which could conceivably afford diketone by some alternate mechanism, we synthesized the two isomeric α,α′-diketodiselenides **16** and **17**.[8] As indicated in Table I, ozonolysis at 25° of each isomer afforded the corresponding α-diketone only (**16** → **11** and **17** → **12**);[9] careful GLPC analysis[10] revealed no crossover products. We submit this as evidence that the carbon–selenium bond remains intact until the Pummerer rearrangement effects oxidation of the α-carbon, and we propose the selenine **8** as a likely intermediate. Selenines have not yet been characterized, but Barton[11] has demonstrated that oxidation of di-*tert*-butyl selenoketone gives the corresponding ketone, presumably via a selenine intermediate, and Strating[12] has reported that the analogous sulfine groups readily hydrolyze to ketones.

Although the yields of α-diketones are low (Table I) for the ozonolysis of **16** and **17**, selenium dioxide oxidation of the parent ketone, 3-octanone, in hot 70% acetic acid gave