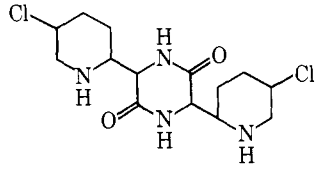
Structure of an Antineoplastic Agent from *Streptomyces griseoluteus*[1]

Sir:

A South African specimen of the soil microorganism *Streptomyces griseoluteus* has been found to produce an antineoplastic and antibiotic component designated 593A (NSC-135758).[2a] The new substance was shown to inhibit growth of the L1210 lymphocytic leukemia (T/C > 200), Ridgway osteogenic sarcoma and Krebs 2 murine tumor systems, the rat Walker 256 carcinosarcoma, and several neoplastic cell lines.[2b] Recently the National Cancer Institute brought 593A to clinical trial[2c] and preliminary results with certain human lymphomas have been very encouraging.[3] In 1973 the piperazinedione structure **1** was proposed for 593A on the basis of spectral measurements, principally ^{1}H NMR and electron impact fragmentation.[4] However, the results of chlorine and oxygen elemental analyses for 593A were unsatisfactory, and mass spectral measurement of a molecular ion was not obtained. These uncertainties, combined with the promising clinical results, led to an urgent need for an unequivocal structure determination.



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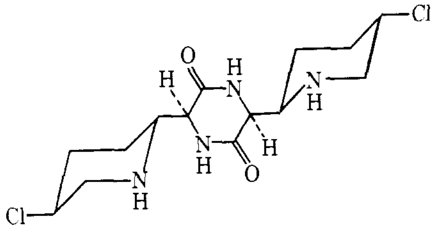

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We are pleased to report that conversion of 593A hydrochloride to the sulfate salt, dp 235–240 °C, provided a suitable derivative for structural elucidation by x-ray crystallographic methods and that the provisionally assigned structure (**1**) is indeed correct. In addition, the conformational and chiral assignments resulting from the present study now greatly enhance the prospect of obtaining antineoplastic agent 593A by total synthesis.

Single crystals of the sulfate salt pentahydrate of sufficient size for data collection were obtained from methanol–water. The observed Laue symmetry and extinctions correspond uniquely to the space group $P2_12_12_1$ with $a = 26.570$ (14), $b = 14.913$ (8), $c = 6.006$ (3) Å; $Z = 4$; $\rho_{\text{calcd}} = 1.500$ g/cm^3 for $C_{14}H_{24}O_2N_4Cl_2{}^{2+}SO_4{}^{2-}\cdot 5H_2O$, and $\rho_{\text{obsd}} = 1.450$ g/cm^3. Diffraction intensities were measured in the θ–2θ scan mode using graphite monochromated Cu Kα radiation on a Syntex P$\bar{1}$ autodiffractometer. Of the 2580 reflections examined within the limits of the diffractometer ($2\theta \leq 140°$), a total of 2241 reflections were retained as objectively observed ($|F_o| \geq 1.0\sigma_{F_o}$). No corrections were applied for either absorption or extinction.

The structure was readily solved by direct methods using the MULTAN-74 system of programs.[5] Large block least-squares refinements[6] of the two enantiomeric structural configurations with anisotropic thermal parameters and fixed hydrogen positions using the anomalous scattering factors for S, Cl, O, N and C[7] (289 independent variables in two blocks) yielded the standard residuals $R = 0.0945$ and 0.0993 for all observed data, respectively. The weighted residuals $R_w = (\Sigma_w(|F_o| - |F_c|)^2/\Sigma_w|F_o|^2)^{1/2}$ of 0.0948 and 0.0998, respectively, were obtained with $w = 1/\sigma_F{}^2$. The hydrogen atoms on carbon and nitrogen had been placed at idealized positions and all O–H atoms were placed as found in a difference synthesis. After the hydrogen positions had been refined a subsequent final refinement of the configuration with the lowest set of residuals gave $R = 0.0889$ and $R_w = 0.0872$, as represented by structure **2**.

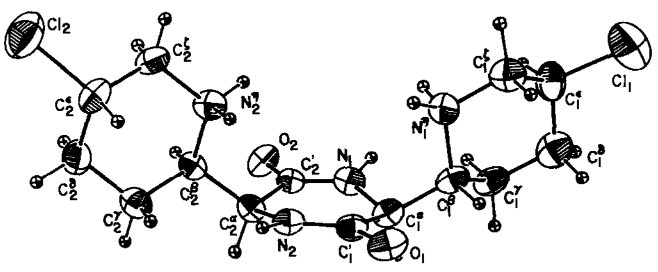


Figure 1.

The perspective view shown in Figure 1 displays the essential configurational and conformational features of the molecular cation. The six chiral centers are (S)-C$_1{}^\alpha$, (R)-C$_1{}^\beta$, (S)-C$_1{}^\epsilon$, (S)-C$_2{}^\alpha$, (R)-C$_2{}^\beta$, and (S)-C$_2{}^\epsilon$; thus the molecule is a diketopiperazine composed of two identical (and hitherto unknown[8]) amino acids.

Interestingly, 2-chloroethylamine **2** appears to represent the first naturally occurring prototype (to be isolated) of the synthetic nitrogen mustards.[9] Details of the molecular geometry and the extensive hydrogen bonding scheme will be discussed in a complete presentation of the structure.

Acknowledgment. We are grateful for support of this investigation by the National Cancer Institute (performed pursuant to Contract No. N01-CM-12308 with the Division of Cancer Treatment, NCI, National Institutes of Health, Department of Health, Education and Welfare), Public Health Service Research Grant CA-16049-03 from the National Cancer Institute, the Fannie E. Rippel Foundation, Talley Industries, the Phoenix Coca-Cola Bottling Co., The J. W. Kieckhefer Foundation, Mr. E. Romley, and the Ladies Auxiliary of the Arizona VFW. The calculations for the structure analysis were performed with computer time provided by Arizona State University. We also wish to thank Dr. Cherry L. Herald for performing several experiments.

References and Notes

(1) Contribution 47 in the series Antineoplastic Agents. For part 46 see G. R. Pettit, R. H. Ode, C. L. Herald, R. B. Von Dreele, and C. Michel, *J. Am. Chem. Soc.*, **98**, 4677 (1976).

(2) (a) C. O. Gitterman, E. L. Rickes, D. E. Wolf, J. Madas, S. B. Zimmerman, T. H. Stoudt, and T. C. Demny, *J. Antibiot.* **23**, 305 (1970); (b) G. S. Tarnowski, F. A. Schmid, D. J. Hutchison, and C. C. Stock, *Cancer Chemother. Rep.*, **57**, 21 (1973); (c) R. M. Folk, A. C. Peters, K. L. Pavkov, and J. A. Swenberg, *ibid.*, **5**, 37 (1974).

(3) Private communication from Dr. S. E. Jones, Department of Internal Medicine, University of Arizona Medical School, Tucson, Ariz.

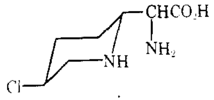
(4) B. H. Arison and J. L. Beck, *Tetrahedron*, **29**, 2743 (1973).

(5) M. H. J. Koch, *Acta Crystallogr., Sect. A*, **30**, 67 (1974); J. P. Declercq, G. Germain, P. Main, and M. M. Woolfson, *ibid.*, **29**, 231 (1973); G. Germain, P. Main, and M. M. Woolfson, *ibid.*, **27**, 368 (1971).

(6) All calculations other than data reduction and direct methods were performed with the "CRYSTALS" computing package adapted to the Univac 1110: R. S. Rollett and J. R. Carruthers, personal communication.

(7) J. A. Ibers and W. C. Hamilton, Ed., "International Tables for X-Ray Crystallography", Vol. IV, The Kynoch Press, Birmingham, England, 1974, p 149.

(8) Apparently piperazinedione **2** is derived from the still to be isolated amino acid i, and we suggest that this substance be designated streptolutine and abbreviated Slt. The very convenient systematic name *cyclo*-(streptolutyl-streptolutyl) or *cyclo*-(Slt-Slt) then follows for piperazinedione **2**.



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(9) The well-known and clinically useful nitrogen mustard *N*-methylbis(2-chloroethyl)amine was synthesized over 40 years ago: V. Prelog and Z. Blazek, *Collect. Czech. Chem. Commun.*, **6**, 211-224 (1934). The utility of such nitrogen mustards in cancer chemotherapy began to be evaluated in earnest about 30 years ago but this lead still needs further development.

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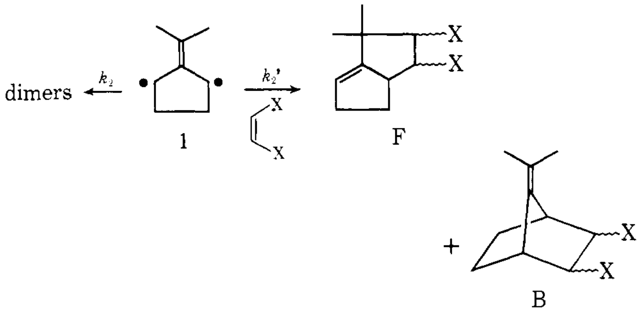
Absolute Rates of Triplet-Triplet Dimerization and Cycloaddition of Trimethylenemethane Biradicals[1]

Sir:

Previous studies[2-4] have shown that the triplet molecules which constitute the ground state[4,5] of the trimethylenemethane (TMM), **1**, dimerize or, in the presence of olefins, are intercepted as fused (F) or bridged (B) cycloadducts. Competition experiments[3] establish an order of *relative* reactivities for a series of olefins, but absolute rates of dimerization or cycloaddition reactions of TMM species heretofore have not been reported. The present description of such measurements is among the very few absolute rates available for biradical reactions.[6,7]

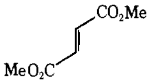
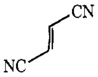
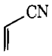
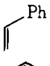


The dimerization and entrapment can be followed by monitoring the intensity of the electron spin resonance (ESR) signal of the $\Delta m_s = 1$ transition in the spectrum[4,5] of triplet **1**. The rates themselves can be made to fall into a convenient range by the choice of the moderately viscous solvent system three parts *i*-PrOH:two parts PrOH, which has $\eta = 2 \times 10^3$ poise[8] at the temperature of our observations, 143.5 K.

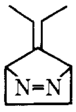
One might imagine that, in fluid media, the quality of the triplet spectrum could be degraded by tumbling of the molecule, which if fast enough, could either collapse the six-line $\Delta m_s = 1$ transitions to a single line by averaging of the direct magnetic dipolar coupling between the two electrons, or produce broad, difficultly observable lines through spin-lattice relaxation. However, for most organic triplet molecules, the rotational correlation time, τ_c, would have to be in the range 10^{-11} to 10^{-12} s in order for these effects to become important.[9] From the Debye theory,[10] we can estimate $\tau_c \sim 8.5 \times 10^{-7}$ s under our conditions, so that a safety factor of 5-6 orders of magnitude protects the experiment.

Irradiation at 360 nm of a 1-2 M solution of the diazene **2**, a precursor of **1**,[4] under these conditions in the cavity of a Varian E-line ESR spectrometer balances the formation and decay of **1** and generates a spectrum which is essentially the same as that previously reported for triplet **1** in rigid glasses.[4,5] The steady-state concentration (±25%) of triplet **1**, usually 10^{-5} to 10^{-4} M, is established by calibration of the spectrometer response against known concentrations of diphenylpicrylhydrazyl (DPPH) and di-*tert*-butylnitroxyl (DTBN).[11,12]

Table I. Second-Order Rate Constants for Cycloaddition of 1 to Olefins at 143.5 K[d]

Olefin	k'_2, l. M^{-1} s^{-1} [b,c]
	3.2
	1.0
	0.20[a]
	0.12
	0.16

[a] At initial olefin concentration = 1.07 M. The values at 1.78 and 1.42 M are 0.21 and 0.20, respectively. [b] The values are probably accurate to ±20%. The major uncertainty is contributed by the solvent contraction, which is assumed to be the same throughout (20% volume decrease relative to the volume at room temperature). [c] Calculated from the equations ln $(I/I_0) = k_1 t$ and $k'_2 = k_1$ [olefin]$^{-1}$. [d] Material balance of dimer plus cycloadducts in each case is >90% based on azo compound 1. No other products are observed.



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When the irradiation is switched off, the ESR signal intensity (I) declines with a rate that is cleanly second order, $-\mathrm{d}I/\mathrm{d}t = k_2[\text{triplet } \mathbf{1}]^2$. The products are the previously reported[4] dimers of **1**. The rate-constant, $k_2 = (2 \pm 0.8) \times 10^3$ l. M^{-1} s^{-1}, is about 0.13 times the bimolecular encounter rate constant k_d calculated from the Stokes-Einstein diffusion theory.

The rates of the diyl + olefin cycloaddition are followed by the same experimental technique used in the dimerization studies, except that the reaction mixtures initially contain a 10-20-fold molar excess of olefin (0.1-1 M) over diazene (0.01-0.1 M). At low light intensity, the reaction in each case is first-order in triplet **1**.[13] The order in olefin is established for the case of acrylonitrile by the observation that the pseudo-first-order rate constant is directly proportional to the acrylonitrile concentration. Table I summarizes the data.

If we assume that a lower Arrhenius activation energy is responsible for the entire factor of 15.2 ± 3 favoring cycloaddition of dimethyl fumarate over acrylonitrile at 143.5 K, we may extrapolate to a corresponding ratio of 3.3 ± 0.7 at 333 K. Direct competition experiments[2,3] at 333 K in fluid medium give a ratio of 1.7 ± 0.4, which in view of the uncertainties in the extrapolation, may be considered to be in satisfactory agreement with the ratio of absolute values.

The rate constants k'_2 at seven temperatures between 120.8 and 143.5 K for the reaction of **1** with acrylonitrile give Arrhenius parameters (least-squares rms = 0.0788) $E_a = 6.3$ kcal/mol, log $A = 8.9$ (A in s). These are very similar to those observed[14] for free radical-olefin additions.