NOTES

Cremeomycin, a Novel Cytotoxic Antibiotic from *Streptomyces cremeus*

Structure Elucidation and Biological Activity

JAMES N. MCGUIRE, SCOTT R. WILSON and KENNETH L. RINEHART*

School of Chemical Sciences,
University of Illinois at Urbana-Champaign,
600 S. Mathews Ave., Urbana, IL 61801, U.S.A.

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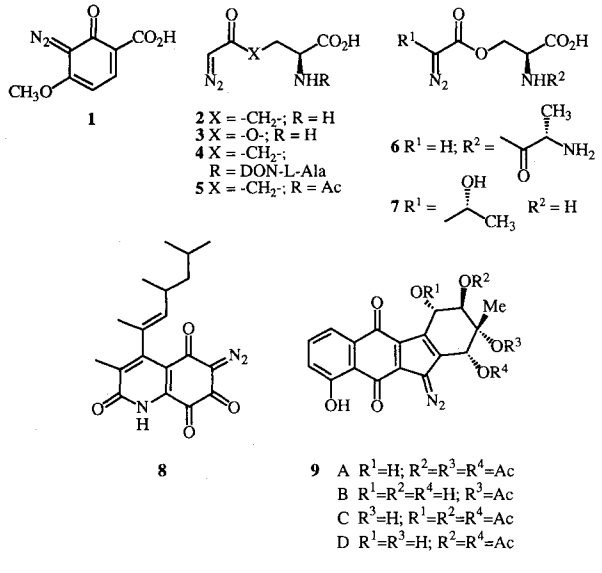
Although several antitumor diazo ketones have been isolated from natural sources, they have all been modified α-amino acids: 6-diazo-5-oxo-L-norleucine (**2**, DON),[1] azaserine (**3**),[2] alazopeptin (**4**, L-Ala-DON-DON),[3] duazomycin A (**5**, *N*-acetyl DON),[4] *N*-(L-Alanyl)azaserine (**6**),[5] and *O*-[(3*R*)-2-diazo-3-hydroxybutyryl]-L-serine (**7**, thrazarine).[6,7] Other diazo compounds recently reported include lagunamycin (**8**), a 5-lipoxygenase inhibitor from *Streptomyces* sp. AA0310,[8] and the kinamycins A ~ D (**9**), antibiotics originally isolated from *Streptomyces murayamaensis*.[9,10] We report here the structure of cremeomycin (**1**), which shares the diazoketone functionality and cytotoxicity, but represents a completely novel structure in this class of compounds.

Fermentation and Isolation

Cremeomycin was originally reported by BERGY and PYKE at the Upjohn Company in 1967 from *Streptomyces cremeus* NRRL 3241.[11] Neither a structural formula nor functional group information was reported.

Physico-chemical and Biological Properties

Cremeomycin is a bright yellow solid that is soluble in solvents from dichloromethane to water but insoluble in hexane. Solutions of the compound slowly decompose when exposed to light at room temperature, but fairly concentrated solutions are stable for weeks at 0°C when protected from light. Cremeomycin was shown in the present study to inhibit the growth of murine lymphocytic leukemia L-1210 cells, with IC_{50} 1.5 μg/ml, and earlier displayed broad antibacterial properties.[11]



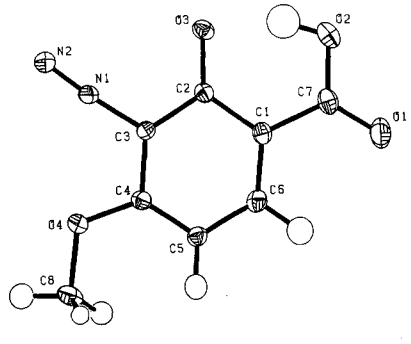
Results and Discussion

The structure of cremeomycin was solved primarily by X-ray crystallography. An ORTEP drawing is shown in Fig. 1. Large, yellow, prismatic crystals were grown at −20°C in dichloromethane solution with a trace of hexane. Preliminary photographs and systematic conditions suggested the monoclinic space group $P2_1$ or $P2_1/m$. Cell parameters determined from automatically centered settings of 25 reflections ($24.0 < 2\theta < 30.1°$) on an Enraf-Nonius CAD4 diffractometer at −75°C were a = 7.746(2) Å, b = 5.919(2) Å, c = 9.111(2) Å, $\beta = 98.98(2)°$, V = 412.6(13) Å^3, Z = 2 and $\rho_{calc} = 1.563$ g/cm^3 for the molecular formula $C_8H_6N_2O_4$. The acentric choice was confirmed by refinement.

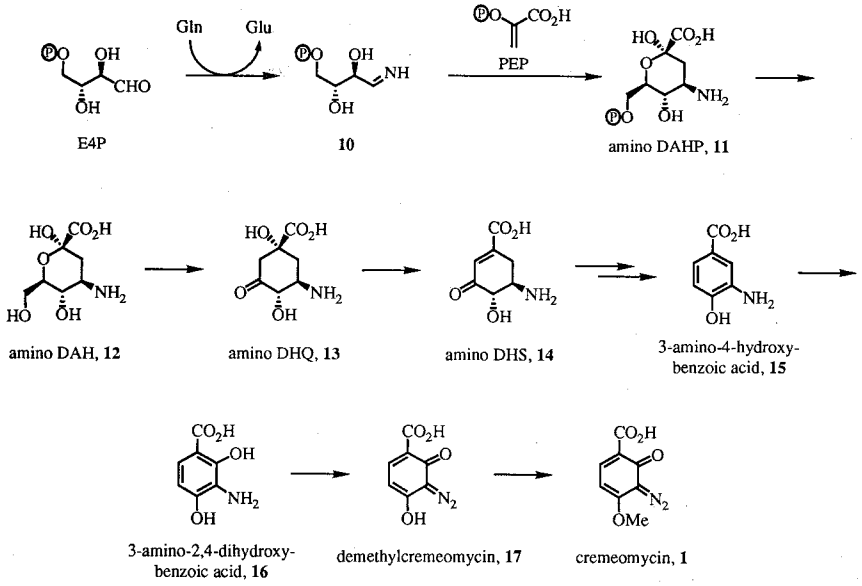
A total of 2161 $\omega/2\theta$ scans ($2\theta < 70°$ for −h, +k, ±l) were collected at −75°C using a variable scan speed (1.5 to 8°ω/minute) and width ($\omega = 1.50[1.00 + 0.35\tan(\theta)]°$) on a κ-axis diffractometer equipped with a graphite monochromator (λ(MoKα) = 0.71073 Å). These data were corrected for anomalous dispersion, absorption (transmission factor range 0.967 to 0.945), Lorentz and polarization effects. Of 1952 unique intensities measured ($R_i = 0.013$), only the 1338 "observed" ($I > 2.58\sigma(I)$) data were used for refinement of the proposed model.

The structure was solved by direct methods (SHELXS-86)[12]; correct positions for all non-H atoms were deduced from an E-map in the acentric space group. All positional parameters were refined independently, anisotropic thermal coefficients were refined for non-H atoms, isotropic coefficients were refined for H atoms, and an empirical extinction parameter converged

Fig. 1. ORTEP drawing of cremeomycin.



Scheme 1.



$(1.0(2) \times 10^{-6})$. Successful convergence was indicated by the maximum shift/error for the last cycle. Refinement in the centric space group $P2_1/m$ converged with conventional residuals R=0.040 and wR=0.057 for 102 variables against 1338 data. Differences between the centric and acentric models were consistently less than 2σ. Refinement in the acentric space group $P2_1$ converged with R=0.038 and wR=0.053 for 151 variables against 1338. The polarity of the acentric model could not be distinguished. The highest peaks in both final difference Fourier maps were located between C atoms in the aromatic ring. A final analysis of variance between observed and calculated structure factors showed no systematic errors.

The diazo ketone was apparent in both IR and ^{13}C NMR spectra. A strong infrared band at 2180 cm^{-1} was consistent with the asymmetric stretch for a highly conjugated diazo group.[13] Initial carbon NMR spectra showed only seven resonances but the addition of a paramagnetic agent, $Cr(acac)_3$, reduced the relaxation time and allowed observation of all eight carbons,[14] with the added signal at 84.0 ppm. This is upfield for a carbon that is formally sp^2-hybridized, but the value is not surprising when compared to the shift of the diazo carbon (~60 ppm) in the azaserine group of compounds.[5]

Cremeomycin displays a strong molecular ion as well as a characteristic loss of N_2 in both low resolution EI and FAB mass spectra. FAB-MS/CID/MS performed on *m/z* 195 (M+H) showed the molecule to lose water followed by N_2. The molecular ion by EI-MS/CID/MS (*m/z* 194) gave *m/z* 150 ($M-CO_2$) and *m/z* 122 ($M-CO_2-N_2$). Neither FAB-MS/CID/MS nor EI-MS/CID/MS showed direct loss of N_2 from the molecular ion.

The unique structure of cremeomycin warrants further study, especially with respect to its biosynthesis. We presume the antibiotic arises from glucose through a variant of the shikimic acid pathway related to the one in *Nocardia mediterranei* S 699 that produces 3-amino-5-hydroxybenzoic acid[15] or the one in *Streptomyces pactum* which produces 3-aminobenzoic acid.[16,17]

Experimental

X-Ray

A sample of cremeomycin was dissolved in CH_2Cl_2 with a drop of hexane added and left at −20°C for one week. The mother liquor was decanted from the resulting crystals, which were then dissolved in a minimum amount of CH_2Cl_2. A small drop of hexane was added and the sample was allowed to sit for one additional week at −20°C. Large, yellow, prismatic crystals were obtained. The crystals became opaque when exposed to light for more than a few minutes and readily dissolved in mother liquor at room temperature. The data crystal was excised under oil (Paratone-N, Exxon) from a parent crystal which had non-uniform transluscent domains. The data crystal uniformly extinguished plane-polarized light; however, there were several internal flaws suggesting the possibility of more than one domain. The sample was mounted using oil to a thin glass fiber then cooled to −75°C with the (−3 1 2) scattering planes roughly normal to the spindle axis. The data crystal was approximately bound by the {0 0 1}, {1 1 0} and {1 −1 −1} inversion forms. Distances from the crystal center to these facial boundaries were 0.14, 0.23 and 0.29 mm, respectively. The X-ray interpretation is described under Results and Discussion.

NMR

^1H NMR spectra were acquired on a General Electric QE-300 instrument operating at 300 MHz with deuterio-

chloroform as solvent and reference. Chemical shifts listed are relative to TMS. The listed ^{13}C NMR data were obtained on a Varian Unity 400 spectrometer operating at 100 MHz. Chromium(III) *tris*-acetylacetonate [$Cr(acac)_3$] was added to help the diazo carbon relax at a faster rate. A concentration of 0.06 M $Cr(acac)_3$ in a saturated solution of cremeomycin (0.7 M) was sufficient to see all eight carbons with a 22° pulse width after 200 transients with no delay time. ^1H NMR (300 MHz, $CDCl_3$), δ 4.06 (3H, s, OCH_3), δ 5.98 (1H, d, J=8.6 Hz), δ 8.38 (1H, d, J=8.6 Hz), δ 13.73 (1H, br s, CO_2H). ^{13}C NMR (100 MHz, $CDCl_3$), δ 176.3, δ 165.3, δ 161.8, δ 145.7, δ 113.4, δ 94.8, δ 84.0, δ 57.4.

Mass Spectrometry

Low resolution FAB spectra were acquired on a VG ZAB-SE spectrometer with a matrix of dithiothreitol-dithioerythritol.[18] HRFAB-MS, FAB-MS/CID/MS and EI-MS/CID/MS spectra were obtained on a VG 70-SE-4F four-sector instrument. LREI and HREI spectra were obtained on Finnigan-MAT CH5 and 731 spectrometers, respectively. HRFAB, m/z 195.0409 ($M+H$, $C_8H_7N_2O_4$, Δ 0.3 mDa); FAB-MS/CID/MS, m/z 195→177, 149, 138, 134, 107, 106, 53. HREI, m/z 194.0336 (M, $C_8H_6N_2O_4$, Δ 1.0 mDa); EI-MS/CID/MS, m/z 194→150, 122, 107, 95, 79, 53, 39.

Ultraviolet

A Hewlett-Packard 8451A diode array spectrophotometer was used to collect the UV data: λ_{max}^{MeOH} 214 nm (ε 20,900), 252 (7,800), 292 (3,900), 414 (3,800).

Infrared

Infrared spectra were taken on an IBM IR/30S FTIR instrument as a film on an NaCl disk: ν_{max} cm^{-1} 2180, 1725, 1590, 1550, 1490, 1465, 1425, 1340, 1210, 1100, 965, 935, 780, 585, 515, 485, 465, 450, 425, 410.

L-1210 Assays

Initial L-1210 assays were performed according to a procedure modified from that originally developed by HUGHES.[19] IC_{50} values were determined by Dr. ROBERT G. HUGHES, Jr. at the Roswell Park Cancer Institute in Buffalo, NY (U.S.A.).

Acknowledgements

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