

R. J. Baker

Department of Chemistry, University of New Orleans, LA 70122

R. J. Majeste

Department of Chemistry, Southern University of New Orleans, LA

*L. M. Trefonas**

Office of Vice-President for Research, University of Central Florida
Orlando, FL 32816

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The crystal and molecular structure of the title compound has been determined by single-crystal x-ray diffraction techniques. The compound is of interest because of its own intrinsic anti-tumor activity and because it is also a common moiety of a series of more active anti-tumor agents, the maytansinoids. The compound crystallizes in the space group $P\bar{1}$ with 2 molecules in a unit-cell of dimensions $a = 6.912 \text{ \AA}$, $b = 7.219 \text{ \AA}$, $c = 15.272 \text{ \AA}$, $\alpha = 119.96^\circ$, $\beta = 104.30^\circ$ and $\gamma = 96.04^\circ$. The structure was solved by direct methods and refined to a final value of $R = 0.063$.

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Introduction.

Alcoholic extracts of plants of the Maytenus family yield minute amounts of a family of compounds called maytansinoids which have proved highly effective in the treatment of selected tumors (1-3). A common moiety of active maytansinoids is the 4-hydro-2-oxo-2,3-dihydro(6*H*)-1,3-oxazine, more commonly referred to as the carbinolamide

ring (Figure 1). If the hydroxyl group of this ring is either absent or substituted (*viz* - methoxide), the biological activity of the entire maytansinoid is destroyed (4a-c). This carbinolamide ring alone shows anti-carcinogenic activity albeit diminished (5). A mechanism explaining the activity of the carbinolamide (and consequently the maytansinoids themselves) has been postulated, involving the formation of an azomethine intermediate under acid conditions and ultimately leading to nucleophilic attack at the hydroxyl group of the carbinolamide (6). The structure determina-

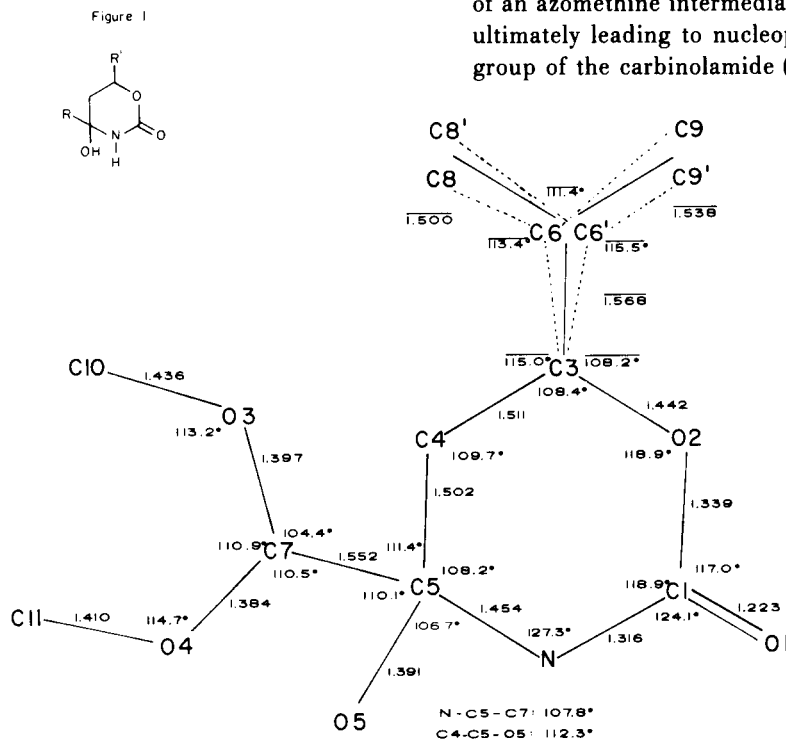


Figure 2. Stick drawing of molecule with distances and angles cited. Average values given for disordered isopropyl group.

tions of a series of carbinolamides (active and inactive) are now under way to see if any correlation of structure, activity, and proposed mechanism exists.

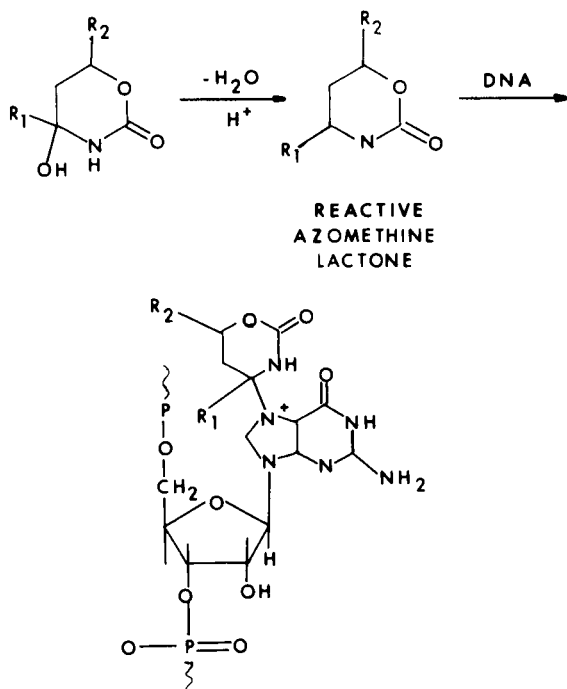


Figure 3. Proposed mechanism for the alkylation of DNA by active carbinolamides.

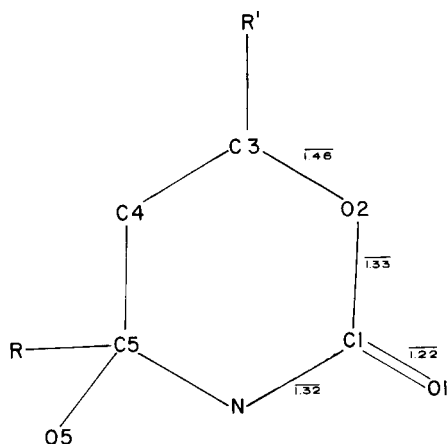


Figure 4. Values in the carbinolamide ring which remain relatively constant regardless of the identity of R, R'.

EXPERIMENTAL

Crystals of **1** with R = CH(OCH₃)₂ and R' = -CH(CH₃)₂ were kindly provided by A. I. Meyers (7) of Colorado State University. A single crystal, cylindrical in shape with a length of 0.20 mm and a diameter of 0.14 mm was selected for the study. The symmetry and cell dimensions

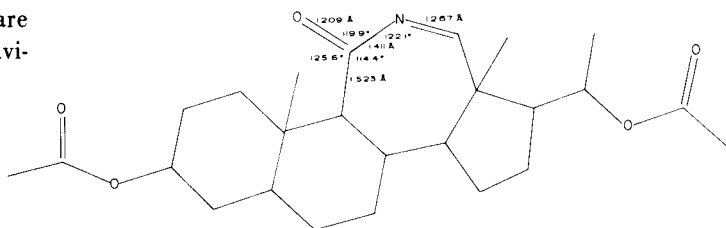


Figure 5. "Limiting value" for a localized CN heterocyclic double bond.

were determined on a General Electric XRD-5 diffractometer using Cu-K α radiation. The lack of any symmetry other than that imposed by Friedl's Law limited the possible space group choices to P1 or P $\bar{1}$. A statistical analysis of the data strongly suggested P $\bar{1}$ and the structure was subsequently solved and refined in that space-group.

Lattice constants were determined by a least-squares fit of thirty reflections at two theta values greater than 65°. A one degree take-off angle and a 0.05° slit were used so that the Cu(K α_1 -K α_2) doublet could be resolved. The resultant lattice constants and their estimated standard deviations are:

$$\begin{aligned} a &= 6.912 \pm .001 \text{ \AA} & \alpha &= 119.96 \pm .02^\circ \\ b &= 7.219 \pm .001 \text{ \AA} & \beta &= 104.30 \pm .01^\circ \\ c &= 15.272 \pm .008 \text{ \AA} & \gamma &= 96.04 \pm .01^\circ \end{aligned}$$

A calculated density of 1.26 g/cc assuming two molecules per unit cell agreed with a measured density of 1.23 \pm 0.3 g/cc using flotation techniques.

Table I
Distances and Angles

Distances (Å)		Angles (°)	
C1-O1	1.223	O1-C1-O2	117.0
C1-O2	1.339	O1-C1-N	124.1
C1-N	1.316	O2-C1-N	118.9
C3-O2	1.442	C1-O2-C3	118.9
C3-C4	1.511	O2-C3-C4	108.4
C3-C6	1.630	O2-C3-C6	98.5
C3-C6'	1.505	O2-C3-C6'	117.9
C4-C5	1.502	C4-C3-C6	110.1
C5-N	1.454	C4-C3-C6'	119.8
C5-O5	1.391	C3-C4-C5	109.7
C5-C7	1.552	C4-C5-N	108.2
C6-C8	1.514	C4-C5-O5	112.3
C6'-C8'	1.485	C4-C5-C7	111.4
C6-C9	1.545	N-C5-C7	107.8
C6'-C9'	1.531	N-C5-O5	106.7
C7-O3	1.397	O5-C5-C7	110.1
C7-O4	1.384	C1-N-C5	127.3
C10-O3	1.436	C3-C6-C8	109.3
C11-O4	1.410	C3-C6'-C8'	117.4
		C3-C6-C9	121.2
		C3-C6'-C9'	109.9
		C8-C6-C9	109.2
		C8'-C6'-C9'	113.7
		C5-C7-O3	104.4
		O5-C7-O4	110.5
		O3-C7-O4	110.9
		C7-O3-C10	113.2
		C7-O4-C11	114.7

Table IIa

Fractional Coordinates and Anisotropic Thermal Parameters (a)
 (Anisotropic thermal parameters $\times 10^4$, estimated standard deviations in Parentheses refer to last decimal place)

Atom	X	Y	Z	β_{11}	β_{22}	β_{33}	β_{12}	β_{13}	β_{23}
O1	0.2976(5)	0.4890(7)	0.4022(3)	177(11)	395(17)	95(4)	146(11)	75(5)	95(7)
O2	0.3353(6)	0.3924(7)	0.2493(3)	223(12)	436(18)	82(3)	198(12)	54(5)	95(7)
O3	0.9244(6)	0.0702(7)	0.2747(3)	282(13)	372(17)	122(4)	248(13)	104(6)	138(7)
O4	0.6466(6)	-0.0117(6)	0.3181(3)	320(14)	281(17)	118(4)	55(13)	79(6)	107(7)
O5	0.8905(5)	0.5013(6)	0.3550(3)	162(11)	291(16)	150(4)	121(11)	87(6)	129(7)
N	0.5934(6)	0.4072(7)	0.3812(3)	128(12)	270(18)	68(4)	94(12)	38(6)	60(7)
C1	0.4089(7)	0.4319(9)	0.3482(4)	134(15)	190(21)	74(5)	42(15)	35(7)	51(8)
C3	0.4741(9)	0.3668(11)	0.1901(5)	332(22)	527(32)	91(6)	297(22)	87(9)	141(12)
C4	0.6023(9)	0.2194(10)	0.2036(4)	274(20)	349(26)	74(5)	189(19)	58(8)	82(10)
C5	0.7273(7)	0.3232(9)	0.3192(4)	132(15)	241(21)	76(5)	74(15)	48(7)	78(8)
C6	0.3023(17)	0.2348(23)	0.0706(9)	229(38)	650(71)	71(10)	214(42)	76(17)	160(23)
C6'	0.3926(17)	0.3419(20)	0.0830(9)	243(39)	264(49)	91(11)	66(36)	9(17)	74(20)
C7	0.8095(8)	0.1533(9)	0.3419(4)	194(17)	256(23)	83(5)	105(16)	58(8)	93(9)
C8	0.1657(20)	0.3795(25)	0.0610(12)	305(48)	562(73)	181(18)	174(49)	51(24)	221(31)
C8'	0.5445(21)	0.3515(24)	0.0306(10)	388(50)	563(71)	84(12)	123(49)	52(20)	124(24)
C9	0.1654(18)	0.0018(25)	0.0222(10)	252(41)	605(72)	100(12)	78(46)	17(19)	149(25)
C9'	0.2496(20)	0.4917(21)	0.0867(9)	392(46)	386(55)	71(11)	214(41)	29(18)	117(20)
C10	1.0591(11)	-0.0453(12)	0.3029(6)	406(27)	465(33)	190(9)	311(25)	128(13)	196(15)
C11	0.6446(12)	-0.0357(12)	0.4043(6)	701(36)	383(32)	186(9)	261(28)	213(16)	201(15)

(a) Anisotropic temperature factors of the form: $\exp[-\beta_{11}h^2 + \beta_{22}k^2 + \beta_{33}l^2 + 2\beta_{12}hk + 2\beta_{13}hl + 2\beta_{23}kl]$.

Table IIb
 Hydrogen Fractional Coordinates

Atom (a)	X	Y	Z	β
HC102	1.2048(0)	0.0168(0)	0.3135(0)	10.0(0)
HC103	1.0108(0)	-0.2074(0)	0.2438(0)	10.0(0)
HC111	0.5033(0)	-0.1282(0)	0.3866(0)	10.0(0)
HC112	0.6688(0)	0.1124(0)	0.4697(0)	10.0(0)
HC113	0.7475(0)	-0.1039(0)	0.4151(0)	10.0(0)
H05	1.0320(0)	0.4780(0)	0.3581(0)	6.0(0)
HN	0.6444(0)	0.4556(0)	0.4626(0)	5.3(0)

(a) Number refers to atom to which hydrogen is attached.

The three dimensional intensity data were collected on a General Electric XRD-490 fully automated diffractometer by the stationary counter, stationary crystal method. Copper $K\alpha$ radiation, balanced Ross filters (Nickel, Cobalt), and a standard counting time were used. Intensity data were taken to a 2θ limit of 100° (1.01 \AA) and of the resultant intensities, 1157 were considered as statistically significant based on the criterion, $[I_{Ni} - 2\sigma(I_{Ni})][I_{Co} + 2\sigma(I_{Co})] > N$ where the sigmas were based solely on counting statistics. N is a constant for a given crystal, based on the intensities read at the location of symmetry extinct reflections, and reflects the minimum number of counts. The intensities were then corrected for α_1 - α_2 splitting as a function of two theta, and for absorption as a function of phi (maximum difference of intensity of $\pm 2\%$ over the entire 360° range of phi at $\chi = 90^\circ$). The usual Lorentz-polarization corrections were made and the intensities were reduced to structure amplitudes.

Structure Determination.

The structure was solved by direct methods. The data was converted to normalized structure factors, scaled by a k-curve (8), and a set of phases determined using the program FAZC (9). The initial map contained thirteen of the sixteen non-hydrogen peaks. Subsequent block-diagonal isotropic least-squares (10) refinement combined with Fourier maps in-

dicated excessive thermal motion of the remaining three carbon atoms (the isopropyl group at C-3). Ultimately, the best fit of the data to the maps resulted from the inclusion of two sets of three half-atoms each for the isopropyl group. At this stage of refinement ($R = .102$), a difference map located hydrogen positions so that hydrogen atoms could be assigned for the nitrogen, hydroxyl and methyl groups. The structure was refined isotropically to $R = .081$ and then the non-hydrogen atoms were allowed to refine anisotropically. Refinements in this fashion with $1/\sigma^2$ weights led to our final value of $R = .063$. At this point, all shifts were less than one-twentieth the estimated standard deviation of the respective parameters and a difference Fourier map showed no region of electron density greater than 0.3 e/\AA^3 and so the structure refinement was concluded.

Results and Discussion.

Bond distances and bond angles for the final refined structure are given in Figure 2, except for the disordered isopropyl group where the average value of each of the parameters is cited. If one momentarily excludes the parameters involving the isopropyl group (C6,C8,C9) attached to the ring at C3, all of the other non-hydrogen bond distances have estimated standard deviations of $\pm .007 \text{ \AA}$ or less, and non-hydrogen bond angle estimated standard deviations of $\pm 0.3^\circ$ or less. The parameters cited for the isopropyl group are the average values of the two "half-atom" models used in the refinement. In such cases the program tends to underestimate the errors and the esd values of such atoms. A more realistic assessment for these particular atoms would be to find the average of the two "half-atom" groups and calculate the deviation of each set from the average. This leads to deviations of $.04 \text{ \AA}$ in bond distances and 4° in bond angles. The more complete set of distances and angles is summarized in Table 1. Table 2 lists the final refined coordinates and temperature factors for the atoms with the esd of each parameter. All methyl hydrogens have fixed isotropic temperature factors whereas the isotropic temperature factors of the non-methyl hydrogens were allowed to refine. All non-hydrogen atoms were refined anisotropically.

The proposed mechanism (6) for the action of carbinolamides, and

analogously for the maytansinoids, is shown in Figure 3. It is postulated that protonation occurs on the hydroxyl at the C-5 position (hence the need for an acid pH) followed by dehydration to the highly electrophilic α -azomethine lactone which is very vulnerable to attack by a nucleophile such as the guanosine of DNA. The facile acid-catalyzed exchange of this -OH groups leads to a particularly tantalizing but still simple explanation. Namely, since tumor cells normally exist at slightly lower pH than normal cells due to their high anaerobic glycolytic rate, the selectivity of these compounds for cancer cells could conceivably depend on such a simple factor as this pH difference.

In an attempt to find structural corroboration for this mechanism, we have looked at the structures of a number of carbinolamides and/or analogous compounds (11-14) relevant to this moiety. In all cases, information as to the biochemical activity was already available. Figure 4 lists average values for those distances in the carbinolamide ring which remain relatively constant among those known structures. These same five atoms whose distances are essentially constant (C3, O2, C1, N and the carbonyl oxygen O1) are very close to planar in all of these structures. At atoms C3 and C4 the geometry becomes tetrahedral with these positions being where the carbinolamide ring is incorporated into the larger ansa macrolide ring in the maytansinoids.

Of the parameters left, that one which is most likely to reflect any of the biochemical activity would be the N-C5 bond since the proposed mechanism assumes an α -azomethine intermediate in which this bond would have some double bond character. In order to define a probable "shortest" double bond distance for this -CN bond, the structure of a steroid containing this functionality was determined (14). Figure 5 shows that resultant structure with only those parameters of possible interest indicated on the drawing. By comparison, for the inactive carbinolamides, C-N = 1.50 Å for the bromopropyl ether of maytansine (11) and 1.47 Å for the cyclic carbamate (12) with *gem*-dimethyl groups on C5. For this structure, which is biochemically active, this C-N distance is discreetly shorter at 1.45 Å. The trend is suggestive of increasing double bond character correlating to biochemical activity although one must recognize that all of these values are far longer than the aforementioned C=N distance (1.267 Å) in the steroid. Hence, one must presently consider this trend as suggestive (in support of the mechanism) although not statistically proven.

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- (10) The conventional reliability index (R), or the weighted reliability index (r) is cited throughout this paper.

$$R = \frac{\sum w | |kF_o| - |Fc| |}{\sum w |kF_o|}$$

$$r = \left\{ \frac{\sum w |kF_o| - |F_c|}{\sum w |kF_o|} \right\}^2$$
 where $w = 1$ or $w = 1/\sigma^2$ and $\sigma = \frac{1}{2}[F(1+B)(1-B)]^{1/2}$ and $B = I_{c\theta}/I_{\theta}$. Scattering factors for phosphorus, carbon, nitrogen and oxygen are taken from the paper by D. Cromer and J. Waber, *Acta Cryst.*, **18**, 104 (1965), while that for hydrogen is from "International Tables for x-ray Crystallography," Vol. III, Kynoch Press, Birmingham, England, 1968.
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