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Report

The Solid-State Decarboxylation of the Diammonium Salt of Moxalactam

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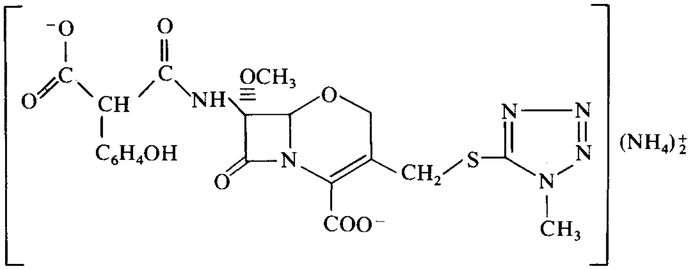
This paper reports studies of the solid-state chemistry of the diammonium salt of moxalactam. The methods employed include X-ray crystallography, molecular mechanics calculations, thermogravimetric analysis, and high-pressure liquid chromatography. The crystal structure shows that the malonic acid amide functionality in crystals of the diammonium salt is not planar. If the common decarboxylation mechanism is operating, then considerable rotation would be required for this functionality to attain coplanarity. Simultaneous HPLC and thermogravimetric analysis studies indicate that the decarboxylation of the diammonium salt of moxalactam is preceded by desolvation. Molecular mechanics calculations indicate that the barrier to rotation of the malonic acid amide functionality is relatively small in the dehydrated crystals, perhaps explaining the facile decarboxylation of this antibiotic. Alternatively, the amorphous desolvated crystals may allow enough molecular freedom for the malonic acid amide functionality to attain coplanarity and decarboxylate.

KEY WORDS: decarboxylation; moxalactam; solid state; thermogravimetric analysis; molecular mechanics calculations.

INTRODUCTION

Solid-state decarboxylation reactions are of pharmaceutical importance. For example, the antituberculosis agent *p*-aminosalicylic acid decarboxylates in both the solid state and solution to give *m*-aminophenol (1). In addition, 5-tetradecyloxy-2-furoic acid also decarboxylates in the solid state, probably in a liquid layer formed on the surface of the crystal, as reported by Carstensen and Kothari (2). The decarboxylation both of *p*-aminosalicyclic acid and of 5-tetradecyloxy-2-furoic acid involves the loss of CO_2 attached to an aromatic nucleus.

Malonic acids and antibiotics containing the malonamide functionality also decarboxylate. This type of decarboxylation has been extensively studied in solution (3–6) and the solid-state decarboxylation of malonic acid itself has been investigated (7). However, to our knowledge, there are no detailed studies of the solid-state decarboxylation of drugs containing this functionality. Thus, this paper reports a detailed study of the solid-state decarboxylation of the diammonium salt of moxalactam (I) (5-oxa-1-azabicyclo[4,2,0]oct-2-ene-2-carboxylic acid, 7-[[carboxy(4-hydroxyphenyl)acetyl]amino]-7-methoxy-3[[(1-methyl-1H-tetrazol-5-yl)thio]methyl]-8-oxo, diammonium salt).



EXPERIMENTAL

A high-pressure liquid chromatography (HPLC) (Waters Associates) equipped with a 6000A pump, a 401 detector, a U6K injector, and a Partisil ODS-2-10-μm, 25-cm column (Whatman) was used. The thermogravimetric analyses were performed on a Perkin–Elmer TGS-2. The drawings of the crystal packing were made using the ORTEP program. The molecular mechanics calculations were done using the CAMSEQ program (3).

Thermogravimetric Analysis (TGA). The weight loss of various samples of moxalactam and its salts was analyzed using a heating rate of 6°/min and a starting temperature of 27.5°C.

High-Pressure Liquid Chromatography. A mobile phase of 3% methanol in 97% NaH_2PO_4 (adjusted to pH 6.8

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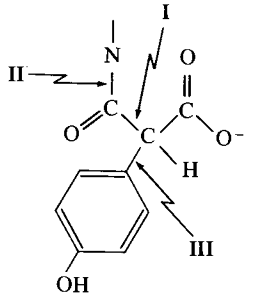
Table I. Steric Energies from the CAMSEQ Program for Various Dihedral Angles About Bonds I, II, and III of the Malonic Acid Functionality in Crystals of Moxalactam $2(NH_4) \cdot 4.5H_2O$ with the NH_4 and H_2O molecules removed[a]



Bond I	Bond II	Bond III	Relative energy (kcal/mol)
0	0	0	−40
0	0	20	−38
0	0	120	−35
0	0	140	−40
0	0	160	−39
0	−20	0	−45
0	−20	20	−44
0	−20	80	−37
0	−20	100	−45
0	−20	120	−44
0	−20	140	−42
0	−20	160	−45
0	20	0	−30
0	40	140	−30
20	20	140	−30
40	20	120	−30
60	0	0	−36
60	0	40	−35
60	0	60	−34
60	0	80	−32
60	0	100	−33
60	0	120	−34
60	20	0	−38
60	20	20	−39
60	20	40	−39
60	20	60	−40
60	20	80	−34
60	20	100	−39
60	40	0	−30
60	40	40	−30
80	−20	100	−46
80	−20	120	−46
80	20	20	−41
80	20	40	−42
80	20	60	−42
80	20	80	−35
80	40	0	−41
80	40	20	−41
80	40	40	−41
80	40	60	−37
80	40	160	−34
100	40	0	−39
100	40	160	−34

[a] All energies < -30 kcal/mol are shown (20° increments in the angles were used).

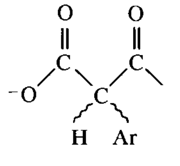
prior to mixing) was used (8,9). Decarboxylated moxalactam was used as a control and was a gift from Eli Lilly and Co.

Molecular Mechanics Calculations. The ORTEP drawings available from the X-ray analysis (10) of the diammonium salt of moxalactam were used to determine the atoms which lined the water tunnels running through the crystal. These atoms were used to construct a hypothetical molecule representing the cavity and the malonamide functionality. Then a CAMSEQ calculation was done by rotating the bonds shown in Table I using 20° increments. Molecular mechanics calculations involved only the use of Lennard–Jones steric potential functions, which give large energies when atoms "run into" each other. Coulombic terms and torsional terms were not included because of the way the "molecule" was constructed. These calculations were made assuming that the water and at least one of the ammonia molecules had exited the crystal.

RESULTS

Crystallographic Studies of the Diammonium Salt of Moxalactam. The studies in this paper were done using atomic coordinates obtained from crystal data collected in our laboratory on a prism of the diammonium salt of moxalactam, $C_{20}H_{35}N_8O_{13.5}S$. The coordinates are within three standard deviations of the coordinates reported by Shiro *et al.* (10) except for atoms C32, C21′, and C24′, which differ by five standard deviations. These differences are not expected to affect either the drawings shown in this paper or the molecular mechanics calculations. An ORTEP drawing of the contents of the unit cell is shown in Fig. 1. A crystal packing drawing is shown in Fig. 2.

Molecular Mechanics Calculations. The approach used can be viewed as a computerized examination of a CPK molecular model of the structure of moxalactam without ammonium or water in the crystal. Table I reports the values of the lowest energies for 20° increments in the dihedral angles about the CO–CH (Bond I) bond in diammonium moxalactam. These lowest energies were obtained by rotating the amide bone (Bond II) and the minimum energy value at each C–Ar bond (Bond III) to their minimum energy value at each increment of rotation of bond I. This approach approximates the motion necessary to make the functionality coplanar and the last value in the table Bond I = 100°. Bond II = 40° represents the conformation where the



group is coplanar.

Thermal Degradation Studies. The degradation of the diammonium salt of moxalactam was studied using TGA, HPLC, and elemental analysis. Figure 3 shows a typical TGA trace obtained with a 6°/min heating rate and a 10 ml/min N_2 flow rate. Separate runs were interrupted at various temperatures, sealed in containers under nitrogen, and sub-

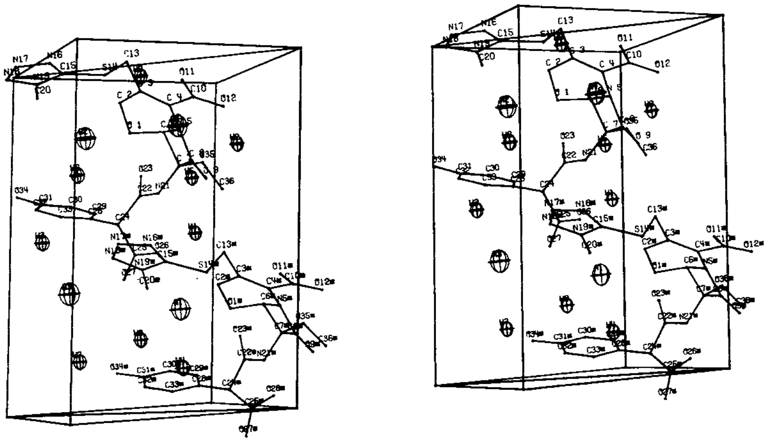


Fig. 1. An ORTEP drawing of the contents of one unit cell of the diammonium salt of moxalactam. The large circles with crosses are the NH_4 groups. The small circles are the water molecules. The direction of the axes are (*a*) across, (*b*), vertical, and (*c*) out of the plane of the paper.

jected to HPLC and elemental analysis. Table II shows the result of these studies.

DISCUSSION

Figures 1 and 2 show that the moxalactam molecule is U-shaped in the solid state. The tetrazole and aryl groups form the sides of the "U" and the β-lactam group forms the base. The aryl group is not coplanar with the carbonyl groups because of steric interactions that prevent coplanarity. The conformation of the malonic acid functionality in diammonium moxalactam is essentially the same as that in the other malonic acids, despite the fact that the acid is in the salt form.

The crystal packing of diammonium moxalactam shows that there is a plane containing both water molecules and ammonia molecules that runs through the crystal parallel to the *c* axis, approximately bisecting the γ angle (see Fig. 2). The two independent moxalactam molecules are held together by nonbonding attractions as well as by a N_{21}–N_{16}* hydrogen bond of 2.96 Å, three O . . . NH_4 . . . O* hydrogen bonds and one O . . . H_2O* hydrogen bond. All of these contacts are well within those expected of such hydrogen bonds except for the NH_4 . . . O_{27}* distance of 2.64 Å, which is significantly shorter than the average NH . . . O distance of 2.88 Å (11). This distance is also shorter than the N . . . O distances in ammonium hydrogen bis-chloroacetate (12) (2.771 to 3.369), ammonium oxalate monohydrate (2.850 to 3.172) (13), ammonium hydrogen terephthalate (2.78 Å) (14), and ammonium hydrogen oxalate hemihydrate (2.841) (15).

An understanding of the decarboxylation reaction in the solid state under anhydrous conditions requires consideration of a solid-state reaction as a four step process as first proposed by Paul and Curtin (16). The first step is nucleation and molecular loosening of the reacting functionalities in the solid state. The second step is the chemical reaction which corresponds to the same reaction in solution. The third step is solid solution formation and the fourth step is crystallization of the reaction product. These last two steps are usually

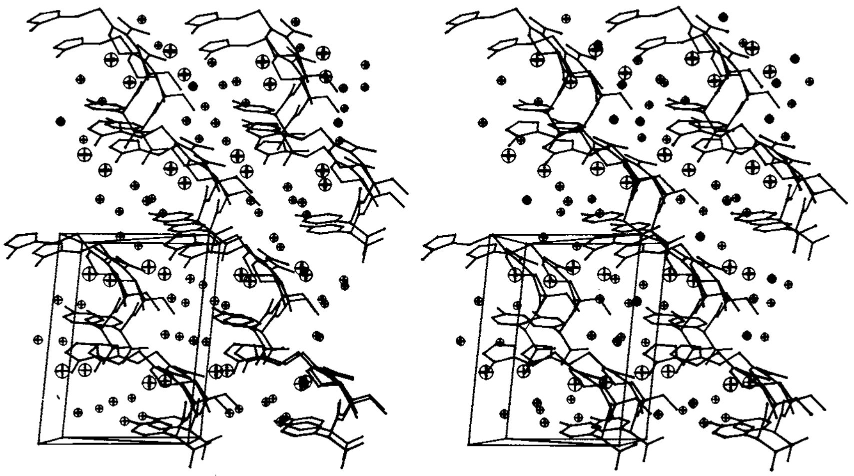


Fig. 2. An ORTEP drawing of the crystal packing of the diammonium salt of moxalactam. The unit cell is in the same orientation as in Fig. 1.

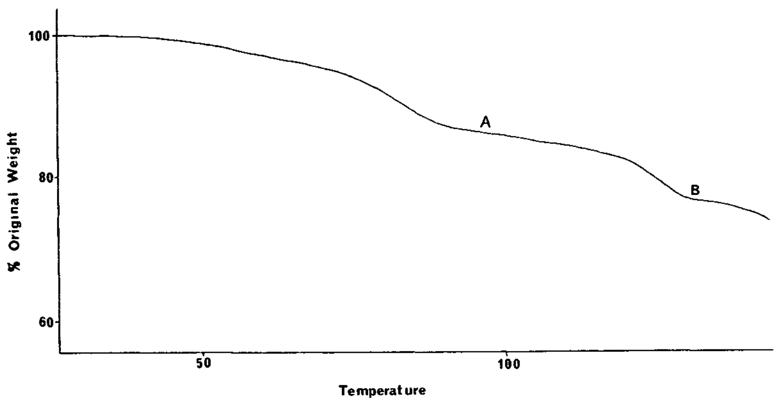


Fig. 3. Typical thermal gravimetric analysis trace of diammonium moxalactam. $C_{20}H_{18}N_6O_9S_{4.5}H_2O$. Elemental analyses were taken after stopping the reaction at points A and B. A heating of 6°/min and a N_2 flow rate of 10 ml/min were used.

not rate determining. For antibiotics of the β-lactam type, where an amorphous product may be formed, these steps may be absent. In addition, the formation of an amorphous phase could further accelerate the reaction rate.

The decarboxylation of diammonium moxalactam can be investigated by simultaneous thermal gravimetric analysis and HPLC. We have used this technique to determine whether solvent loss occurs before reaction. We have also investigated the loss of solvent of crystallization and ammonia using thermogravimetric analysis and elemental analysis. These data are shown in Fig. 3 and Table II. Analysis of these data indicates that the first step in the decarboxylation of diammonium moxalactam (which occurs up to 90°) involves the loss of 4.5 water molecules. The first step is hypothesized to be the molecular loosening step.

The second step—the chemical reaction—involves the loss of one NH_3 molecule and CO_2 and occurs between 90 and 130°C. It was not possible to determine the sequence of events in this reaction, but we have attempted to model this second step using molecular mechanics calculations. The calculations were based on the Fraenkel model (6) for decarboxylation, which assumes a planar transition state for COOH . . . O = C. The molecular mechanics calculations show that the barrier to rotating the carboxylic acid group in the dehydrated diammonium moxalactam crystal is not extremely large, and thus, such a reaction can occur in the 90–130°C temperature range.

In summary, this study indicates that the solid-state decarboxylation of the diammonium salt of moxalactam under anhydrous conditions occurs by (i) molecular loosening via loss of the water molecules, (ii) loss of NH_3, (iii) rotation of the carboxylic acid group into coplanarity with the carbonyl group, and (iv) decarboxylation. Unfortunately, the exact order of the last three events could not be established.

ACKNOWLEDGMENTS

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Table II. Elemental and HPLC Analysis of Diammonium Moxalactam After Heating to Various Temperatures in a Thermogravimetric Analyzer[a]

Temperature and position on TGA trace (Fig. 3)	Formula	Results of HPLC analysis	Calculated		Observed		Conclusions
			C	H	C	H	
At start	$C_{20}H_{18}N_6O_9S$ $4.5H_2O \cdot 2NH_4$	Moxalactam	37.80	5.55	—	—	Pure diammonium moxalactam · $5H_2O$
95°C, point A	$C_{20}H_{18}N_6O_9S \cdot 2NH_4$	Moxalactam	43.32	4.72	43.17	4.95	Diammonium moxalactam (dehydrated)
120°C	$C_{20}H_{18}N_6O_9S \cdot NH_4$	Mixture of moxalactam and decarboxylated moxalactam	44.69	4.31	—	—	Ammonium moxalactum (dehydrated and $1NH_3$ lost)
135°C, point B	$C_{19}H_{19}N_6O_7S \cdot NH_4$	>85% decarboxylated moxalactam	46.24	4.70	46.05	4.92	Decarboxylated ammonium moxalactum (dehydrated and $1NH_3$ and CO_2 lost)

[a] A heating rate of 6°/min and a N_2 flow rate of 10 ml/min were used.

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