ple: mp 209–210° dec; $[\alpha]^{25}D$ +245.9°. *Anal.* ($C_{19}H_{25}ClO_3$) C, H, Cl.

17α-Methylandrost-4-ene-3β,17β-diol 3-(Imidazole-1-thionecarboxylate) (23b). A stock solution of *N,N'*-thiocarbonyldiimidazole (100 ml) was added to a solution of 2.00 g (0.0066 mol) of 17α-methylandrost-4-ene-3β,17β-diol (**23a**) in 50 ml of THF, and the reaction was stirred at room temperature for 7 days. H_2O (10 ml) was added and the reaction was diluted with CH_2Cl_2, washed twice with H_2O, dried, and concentrated. The residue was adsorbed onto silica gel from 1:1 CH_2Cl_2–hexane. Elution with 2–15% EtOAc in CH_2Cl_2 gave fractions which were recrystallized from CH_2Cl_2–Et_2O–hexane to give 0.32 g (12%) of the analytical sample of **23b** as colorless crystals: mp 193.5–196.5°; $[\alpha]^{25}D$ −78.64°. *Anal.* ($C_{24}H_{34}N_2O_2S$) C, H, N, S.

17α-Methylandrost-5-ene-3β,17β-diol 3-(Imidazole-1-thionecarboxylate) (24b). 17α-Methylandrost-5-ene-3β,17β-diol (**24a**) (2 g, 0.0066 mol) was dissolved in 50 ml of THF, 150 ml of the stock solution of *N,N'*-thiocarbonyldiimidazole was added, and the solution was stirred for 6 days. The reaction was diluted with CH_2Cl_2, washed with H_2O, dried, and concentrated. The solid residue was recrystallized from CH_2Cl_2–Et_2O to give 0.554 g (20%) of the analytical sample of **24b** as colorless crystals: mp 191.5° dec; $[\alpha]^{25}D$ −70.44°. *Anal.* ($C_{24}H_{34}N_2O_2S$) C, H, N, S.

Acknowledgment. We wish to thank Dr. V. Toome, Mr. S. Traiman, Dr. T. Williams, and Dr. F. Scheidl for the uv, ir, and nmr spectra and microanalyses, respectively.

References

(1) H. A. Staab and A. Mannschreck, *Chem. Ber.*, **95**, 1284 (1962).
(2) F. Sondheimer, W. McCrae, and W. G. Salmond, *J. Amer. Chem. Soc.*, **91**, 1228 (1969).
(3) H. Kuhl and H.-D. Taubert, *Steroids*, **22**, 73 (1973).
(4) H. A. Staab and W. Rohr in "Newer Methods of Preparative Organic Chemistry," Vol. V, W. Foerst, Ed., Academic Press, New York, N. Y., 1968, pp 61–108.
(5) K. von Auwers and W. Schaich, *Chem. Ber.*, **54**, 1738 (1921).
(6) A. Patchornik, A. Berger, and E. Katchalski, *J. Amer. Chem. Soc.*, **79**, 6416 (1957).
(7) W. John, *Chem. Ber.*, **68**, 2283 (1935).
(8) E. Schnabel, H. Herzog, P. Hoffmann, E. Klauke, and I. Ugi, *Justus Liebigs Ann. Chem.*, **716**, 175 (1968).
(9) M. Murakami, K. Takahashi, I. Tamasawa, and K. Murase, Japanese Patent 70 31,174 (1970); *Chem. Abstr.*, **74**, 64248 (1971).
(10) L. Birkofer and A. Ritter in "Newer Methods of Preparative Organic Chemistry," Vol. V, W. Foerst, Ed., Academic Press, New York, N. Y., 1968, pp 211–237.
(11) P. Fournari, P. DeCointet, and E. Laviran, *Bull. Soc. Chim. Fr.*, 2438 (1968).
(12) E. J. Corey and R. L. Dawson, *J. Amer. Chem. Soc.*, **84**, 4899 (1962).
(13) R. Juhrich, German Patent 1,131,216 (1962).
(14) W. Klee and M. Brenner, *Helv. Chim. Acta*, **44**, 2151 (1961).
(15) H. A. Staab, *Justus Liebigs Ann. Chem.*, **609**, 75 (1957).
(16) A. Bowers, H. J. Ringold, and E. Denot, *J. Amer. Chem. Soc.*, **80**, 6115 (1958); R. E. Counsell, *Tetrahedron*, **15**, 202 (1961).
(17) H. A. Staab and G. Walther, *Justus Liebigs Ann. Chem.*, **657**, 98 (1962).
(18) L.-O. Carlsson and J. Sandstrom, *Acta Chem. Scand.*, **24**, 299 (1970).
(19) P. Adams and F. A. Baron, *Chem. Rev.*, **65**, 567 (1965).
(20) R. Hüttenrauch and A. Schubert, *Arch. Pharm. (Weinheim)*, **299**, 1011 (1966).
(21) R. Hüttenrauch, *Pharmazie*, **22**, 179 (1967).
(22) H. Mori, *Chem. Pharm. Bull.*, **10**, 429 (1962).
(23) B. Camerino, R. Modelli, and B. Patelli, *Farmaco, Ed. Sci.*, **13**, 52 (1958); *Chem. Abstr.*, **52**, 13768*i* (1958).
(24) Y. Nomura, B. Takegawa, and I. Chuma, Japanese Patent 8232 (1960).
(25) U. Stache, W. Haede, and W. Fritsch, German Patent 1,250,437 (1967); *Chem. Abstr.*, **67**, 117,099p (1967).
(26) O. Mancera, G. Rosenkranz, and F. Sondheimer, *J. Chem. Soc.*, 2189 (1953).
(27) S. Bernstein, R. H. Lenhard, and J. H. Williams, *J. Org. Chem.*, **18**, 1166 (1953).
(28) P. E. Shaw, F. W. Gubitz, K. F. Jennings, G. O. Potts, A. L. Beyler, and R. L. Clarke, *J. Med. Chem.*, **7**, 555 (1964).
(29) P. D. Klimstra, U. S. Patent 3,176,013 (March 30, 1965).
(30) B. Pelc, *Collect. Czech. Chem. Commun.*, **25**, 309 (1960).
(31) A. Boris and L. DeMartino, *Steroidologia*, **2**, 57 (1971).

CNDO/2 Study of the Antibacterial Activity of Penicillins and Cephalosporins

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The inhibition of bacterial growth by penicillin-like antibiotics is assumed to proceed *via* a two-step mechanism: reversible binding of drug to enzyme followed by irreversible acylation of enzyme *via* thiol attack at the β-lactam carbonyl carbon. The CNDO/2 procedure for electronic structure calculations is used to estimate relative binding strengths and reactivities for penicillins, cephalosporins, and Δ^2-cephalosporins, making simple but reasonable assumptions about the nature of these reactions. The enhanced activity of penicillins over cephalosporins is seen to be due to stronger binding. The virtual inactivity of Δ^2-cephalosporins is a result of the resistance of these molecules to thiol attack. No evidence is found for expulsion of acetate from cephalosporins during the reaction.

The modification of penicillin and cephalosporin antibiotics has been difficult because little is known about the specific enzyme process inhibited by these drugs. It is probably correct that these molecules inhibit the final step in the synthesis of the bacterial cell wall, the cross linking of the peptidoglycan strands using a pentapeptide chain. The hypothesis that the inhibition results from a structural similarity between penicillin and this pentapeptide is an attractive one.[1] However, this cross linking "transpeptidase" has not been isolated and very little is known about the active site or the reaction mechanism.

In this paper we will consider theoretically some of the experimentally unresolved questions regarding the structure–activity relationship of these antibiotics. In order to do this we will make the simplest assumptions about the nature of the reaction, those of eq 1. The first step is the reversible binding of the substrate to the enzyme with binding constant k_1'/k_1. The second step is the reaction with the enzyme to form the covalently bound enzyme–

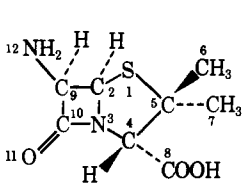
†Supported under NSF Traineeship Grant, Department of Chemistry, Princeton University.

substrate complex ES′. It has been assumed that a similar reaction between penicillins and a D-alanylcarboxypeptidase participating in cell wall synthesis involves thiol attack at the β-lactam carbonyl carbon.[2] Whether or not this enzyme is in fact the unknown transpeptidase, it is reasonable that step 2 proceeds *via* this mechanism. For instance, it is known that penicillins are substantially more sensitive to thiol than to amine attack.[3] Note that we write no reverse reaction for this step; for simplicity we assume that this process is irreversible. With the natural substrate this step is presumably thiol attack at the C-terminal D-Ala D-Ala peptide link and would normally result in the release of D-Ala allowing the reactive complex to complete the cross link.[4] For the antibiotic as sub-

$$E + S \underset{k_1}{\overset{k_1'}{\rightleftharpoons}} ES \xrightarrow{k_2'} ES' \qquad (1)$$

strate this attack is at the β-lactam carbonyl carbon cleaving the β-lactam ring. The molecule remains intact with the ring cleaved and is not free to leave or react further. The enzyme is irreversibly inactivated. We are not concerned with a system which reaches equilibrium. It is known that penicillin will not kill a dormant organism. The cross linking enzyme is only active for short periods in the life cycle of the growing bacteria. It will be the combined effect of steps 1 and 2 which determines the effectiveness of the antibiotic and both must be considered.

Substantial data are available on three groups which are potential inhibitors of this reaction. The first are generically known as penicillins and are derivatives (formed by acylation at the N-12 amine) of 6-aminopenicillanic acid (6-APA, I).[5],‡ (Numbering of atoms in figures is nonstandard and is used for the purpose of clarity in this paper only. Hence, "6-amino" is in accordance with established nomenclature, while in the figure the amine is at C-9.) Second and third are cephalosporins based on 7-aminocephalosporanic acid (7-ACA, II) and Δ^2-cephalosporins based on the Δ^2 ring modification of 7-ACA (Δ^2-7-ACA, III). Generalizations about the relative activities of these compounds are difficult because the pentapeptide employed for cross linking is different in each bacterial system, as is most likely the transpeptidase itself. Broadly speaking the activities of analogous derivatives of these three compounds are approximately in the ratio 6-APA: 7-ACA:Δ^2-7-ACA = 180:15:1.[5b] If we can roughly account for these effects we may have information useful in the development of these drugs.



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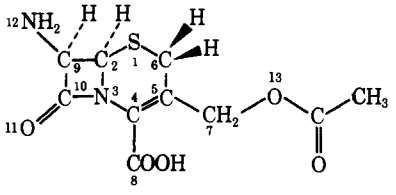

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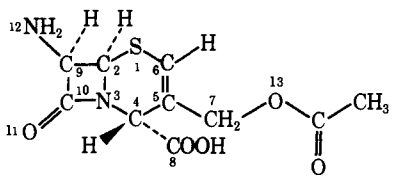


III

In this paper we look for insight into the specific origin of these differences in activity. We assume that acylation at N-12, which is virtually indispensable for antibacterial activity, serves only to increase the number of binding sites to the enzyme and that when considering structures with identical side chains all observable effects are due to properties of the basic bicyclic system. Because these antibiotics will not have the large number of degrees of freedom of the natural substrates, it is possible that geometrical considerations may govern the reversible binding (k_1'/k_1).[6] We calculate the conformation of these rings and with assumptions about the stereochemistry predict relative binding constants for this process. We also calculate the energetics of thiol attack at the β-lactam carbonyl. By considering the differences in energy between the known ground state and an analog of the transition state, relative reactivities toward the formation of the complex ES′ are predicted. The ground state–transition state energy difference is the only one that need be considered because we have assumed the irreversibility of this step. We need not know the nature of the complex ES′.

Calculations

It is well known that the CNDO/2 procedure seriously overestimates bond energies in covalent compounds.[7] Bond angles are much better represented and calculations involving angle and torsional strain can be reliable. To assure reasonable results within the limitations of the theory only questions involving differences between closely analogous compounds should be considered. It is possible to calculate the energetics of small deformations of a molecule or to study the effects of a modification on a series of closely related compounds. It should always be arranged so that large errors are constant and cancel. One should look only at relative effects.

Although the CNDO/2 procedure is well parameterized for first row elements, the inclusion of second row atoms creates problems. D orbitals may play a role in the chemistry of these elements.[8] Orbitals are large and highly polarizable, and the core of electrons is no longer only the small 1s shell but is of considerable extent. Because the d orbitals are difficult to represent within the CNDO/2 framework we have chosen to ignore them.

It is found that different parameterizations of sulfur are needed for the various situations: hydrogen covalently bonded to sulfur, "hydrogen bonding" to sulfur, sulfur–carbon single bonds, and sulfur–carbon multiple bonds. Four parameters are adjustable. In the notation of Pople these are the orbital exponent ζ, which governs the extent of the orbital, and the overlap parameter β, both of which affect the overlap integrals. Also variable are the one-electron s and p energies $\frac{1}{2}(I_s + A_s)$ and $\frac{1}{2}(I_p + A_p)$ determining hybridization and electronegativities.

We have calculated bond lengths and angles for a range of sulfurous compounds including hydrogen sulfide, dimethyl sulfide, 1,4-dithiadiene, carbon disulfide, and carbon monosulfide. Taking as a reference point the values suggested by Santry and Segal,[9] Allen and Demoullin§ found that an increase in the magnitude of both ζ and β of 10% gave a good account of hydrogen bonding. We find that these same parameters adequately describe covalent sulfur–hydrogen bonding. An increase of 10% in ζ and 15% in β gives good results for multiply bonded sulfur. In calculations of sulfur singly bonded to carbon an increase of 2.5% in ζ and 10% in β gave good agreement with observed bond lengths. Bond angles were far too small indicating too much p^2 character. In order to increase hybridization it was necessary to destabilize the s level [$\frac{1}{2}(I_s + A_s)$] 10% and stabilize the p orbitals [$\frac{1}{2}(I_p + A_p)$] 20%.

‡A recent article describing the use of CNDO/2 calculations in the structure–activity relationship in the cephalosporin series has just appeared.[5c]

§L. C. Allen and D. Demoullin, private communication.

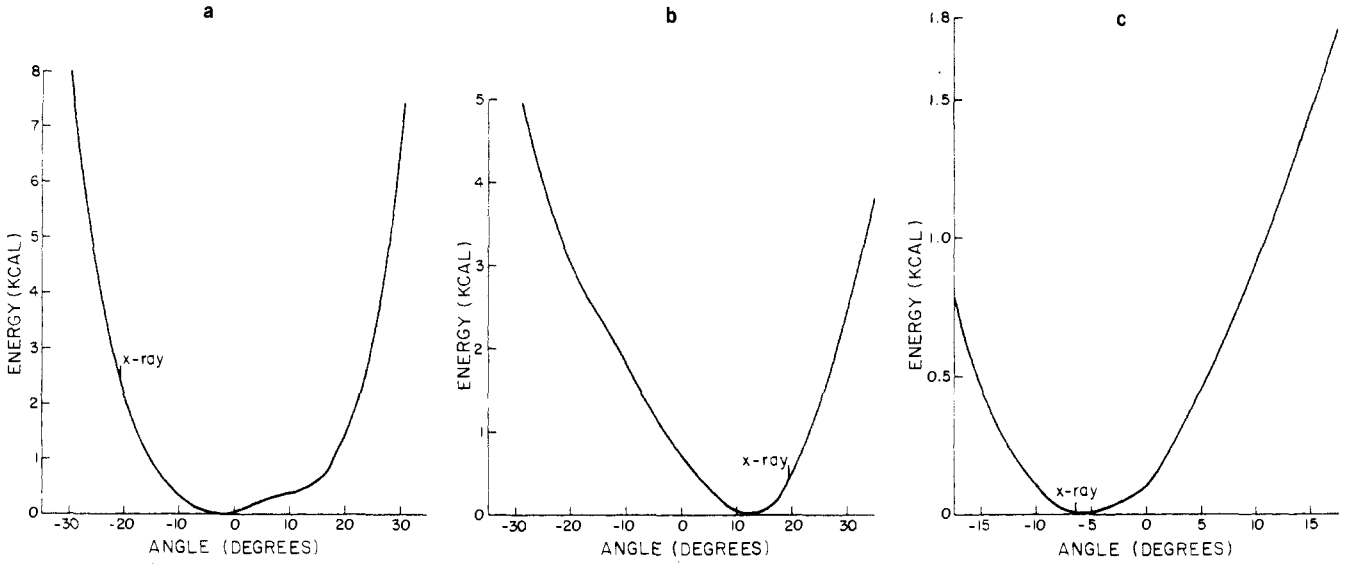


Figure 1. Energy of the fused ring systems as a function of the conformation of the five- and six-membered ring. Energies in kilocalories per mole from the most stable conformation. Conformation parameterized as in calculations section. The conformations as determined by X-ray analysis on molecules having these rings as nuclei are marked: (a) 6-aminopenicillanic acid, (b) 7-aminocephalosporanic acid, (c) Δ^2-7-aminocephalosporanic acid.

These last parameters for singly bonded sulfur-carbon systems were employed in all calculations reported.#

(i) **Conformation of Five- and Six-Membered Rings.** Initial values for atomic coordinates of 6-APA,[10] 7-ACA,[6] and Δ^2-7-ACA[6] were taken from X-ray results on derivatives of these compounds. For 6-APA the position of C-5 (I) was varied, maintaining all bond lengths through distortion of the angle C-4-C-5-S-1. Results are reported as the angle between the planes defined by atoms S-1-N-3-C-4 and N-3-C-4-C-5 which varied from −30 to +30°. Positive angles correspond to a quasi-chair conformation of the fused ring system in which C-5 is bent away from the N-12 amine. Negative angles correspond to a quasi-boat structure. For 7-ACA and Δ^2-7-ACA the positions of C-5 and C-6 were varied maintaining all bond lengths. Results are reported as the angle between S-1-N-3-C-4 and N-3-C-4-C-5, which varied from −18 to +18° for Δ^2-7-ACA and from −28 to +28° for 7-ACA. The angle N-3-C-4-C-5, C-4-C-5-C-6 was varied in proportion to S-1-N-3-C-4, N-3-C-4-C-5 as the ratio of the two in the crystal. Bond angles at C-4 and C-5 were adjusted to maintain bond lengths, the single bond angle in Δ^2-7-ACA being deformed twice as much as the double bond.

(ii) **Strain in β-Lactam Ring.** The energy with coordinates as given by X-ray was calculated. The procedure was repeated with the N-3-C-10 bond cleaved and hydrogen added at N-3 and C-10. Coordinates of five- and six-membered rings were maintained. However, angles and bond lengths for cleaved β-lactam rings were assigned from a table of standard lengths and angles[7] and are those expected for the unstrained reduction product.

(iii) **Reactivity toward Thiol Attack at the β-Lactam Carbonyl Carbon.** Energy was calculated for the molecule with X-ray coordinates and SH^- at infinity. Calculation was repeated with SH^- 1.82 Å below the plane of the β-lactam ring at C-10. These calculations were also repeated assuming a tetrahedral intermediate at the carbonyl carbon and the results were qualitatively similar although the computed energies were lower.** 7-ACA was also calculated with carbonium ion formation by expulsion of acetate at C-7 and with hydrogen replacing the acetate. It was found that absence or orientation (both approximately parallel and perpendicular to the dihydrothiazine ring) of the C-8 carboxylic acid group did not affect the calculation. There was no difference in model activation energy and charge densities on the group remained constant. Thus, the presence of the ionized COO group was used so that the calculation always involved negative SH^- attack on a neutral molecule. The possibility of acetate expulsion during reaction was investigated by calculating the carbonium ion plus free acetate. Cleavage of the S-1-C-2 band was investigated by rotating by 180° about C-5-C-6 in 7-ACA and about C-4-C-5 in 6-APA. Both were carried out with and without SH^- at C-10.

Results

The three fused ring systems are all found to have only one preferred conformation. The presence of a slight inflection in the 6-APA curve suggests a double minimum. Low-temperature nmr work†† on 6-APA and 7-ACA is in progress to check these predictions experimentally. The results are presented graphically in Figure 1a-c where energies are relative to the most stable conformation (x axis as per the previous section). The X-ray conformation is noted on each figure. The minima are broad, and in all cases general agreement with the X-ray structure obtains. The results are also in qualitative agreement with nmr studies.[11,12] For 7-ACA·HCl the crystalline salt is formed between a ring substituent and a chloride ion; for potassium benzylpenicillin the salt is between the ring carboxylic acid and potassium. The X-ray determination of Δ^2-7-ACA is of the electrically neutral molecule. The ring substituents act as a lever on the ring, and any torque exerted on these groups will easily distort the ring. It is interesting that best agreement between theory and the X-ray structure is found for Δ^2-7-ACA, the only crystal in which there is no electrostatic interaction with the ring.

All CNDO/2 calculations reported here were accomplished with a program obtained from the Quantum Chemistry Program Exchange, Department of Chemistry, University of Indiana, Bloomington, Ind. 47401. This is program CNINDO, No. 141, written by Paul A. Dobosh. The program was modified to permit the use of various values for the sulfur parameters and to ignore d orbitals. For a typical molecule (14 nonhydrogen, 10 hydrogen) CPU times were IBM 360/65, 10-12 min; 370/155, 12-15 min; 370/145, 45 min; 360/91, 45 sec.

**These results and the related results of interaction with substituents at C-9 will be reported separately.

††B. Arison, W. C. Topp, and B. G. Christensen, unpublished results.

Table I[a]

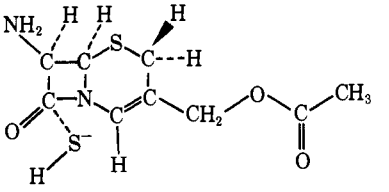
	6-APA	7-ACA	Δ^2-7-ACA	Desacetoxy-7-ACA	Desacetoxy-7-ACA$^+$
Energy	36.4	18.8	30.1	20.0	3.1

[a]The total binding energy of the ground state (molecule + thiol) minus the total binding energy of the transition state in kilocalories per mole. Because bonds with unknown energies are formed and broken in this process, only differences between these values are accurate.

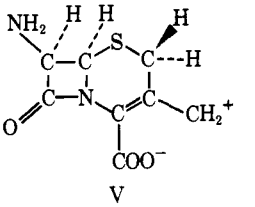
When the energies of the molecular orbitals are plotted against geometry of the five- and six-membered rings, some interesting observations can be made. Many orbitals are relatively conformation invariant although a number show a strong geometrical dependence. For instance, orbitals which are predominantly centered on the five- and six-membered ring atoms are least stable at the planar geometry. Presumably this is the conformation involving the highest angle strain. Orbitals with sizable lone pair nature on sulfur and nitrogen destabilize when the lone pair orbitals eclipse. We conclude that angle strain is a factor, but, more importantly, the presence and nature of the nitrogen and sulfur heteroatoms are a strong feature of the system. Although this analysis gives no insight into hydrogen bonding interactions (Leland C. Allen, private communication) we might expect bonding between sulfur and the amine protons. The behavior of this system strongly reflects the nature of the sulfur orbitals and might be expected to be changed on replacement of S-1 by other heteroatoms with different orbital characteristics or by carbon. We should point out that the correlation of orbital energies with structural effects may be misleading since all molecular orbitals have some density at each atomic center and it is not always possible to isolate a single effect.

An absolute value for the strain present in the four-membered β-lactam ring is not available. The calculation includes not only the energy of ring opening but the energies of formation of C-N bond, an H-H bond, a C-H bond, and an N-H bond. CNDO/2 gives very poor results for bond energies so that these latter quantities cannot be evaluated. However, the bonds involved are nearly identical in 6-APA, 7-ACA, and Δ^2-7-ACA so that differences in energies released in the total process may reasonably be taken as differences in strain energies. The energy released on opening the β-lactam bond in 7-ACA and Δ^2-7-ACA is similar within the context of these calculations, 23 and 19 kcal, but that in 6-APA is larger, 40 kcal. We conclude that both cephalosporin β-lactams are strained by approximately the same amount but that the penicillin β-lactam is significantly more so, by about 20 kcal. Morin, *et al.*,[13] have suggested, on the basis of ir studies of the β-lactam frequencies, that the β-lactam ring in Δ^2-cephalosporins is significantly less strained than those in cephalosporins and penicillins and that this accounts for the decreased Δ^2 activity. Our calculations indicate that ring strain might account for the difference in activity between penicillins and cephalosporins but not between the isomeric cephalosporins.

Reactivity toward thiol attack has been studied by comparing the energy of a transition state analog such as IV (the analog for 7-ACA) with the energies of the reactants (I–III) plus free thiol. We also studied the carbonium ion formed by removal of the acetate V and 7-aminodesacetoxycephalosporanic acid VI. The presence of the carboxylic acid functional group was found to have no influence on the calculation so, for time considerations, this group



IV



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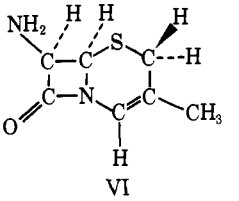
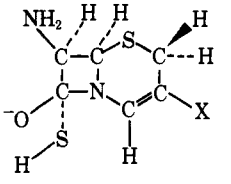


VI

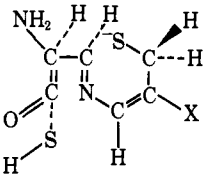
was left off in all calculations. The only exception is the carbonium ion V in which the COO^- was restored to reduce electrostatic effects by neutralizing the positive charge. We present this last calculation but will not discuss it as it is clearly not sufficiently analogous to the others. Results are tabulated in Table I.

The intact molecule (with X-ray coordinates) plus a thiol group at the electrophilic site is a poor analog of the actual transition state. From the various resonance structures which can be written (see below) for this state we might expect a simultaneous change in some bond lengths and an opening of the β-lactam ring to relieve angle strain. Our transition state is arbitrarily higher in energy than the actual.

We may still, however, extract some information from the calculation. We see that the activation energy for thiol attack on Δ^2-7-ACA is about 11 kcal higher than that for 7-ACA. While resonance structures such as VII and VIII can be written for the transition state of both rings, the additional structure IX is possible only for 7-ACA. From



VII



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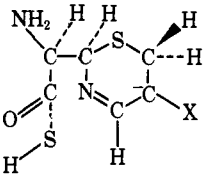


IX

the changes in charge density in going to the transition state (Table II) we see that all structures contribute to the 7-ACA transition state but that only VII and VIII are important for Δ^2-7-ACA. (Interactions of the S-1 and C-5 orbitals also contribute to the Δ^2 structure.) As the strain energies in both rings are similar we would expect that the analog of 7-ACA is worse than that for Δ^2-7-ACA; hence, the actual transition state for 7-ACA will lie lower still with respect to that of Δ^2-7-ACA. We conclude that the calculated 11 kcal difference in the activation energies of the two is the major factor for the difference in reactivity of the isomers. It is possible that if the cephalosporins are strained upon binding to the enzyme such that the fused ring system is forced into a quasi-boat conformation similar to the penicillins, that the increased orthogonality of the double bond to the β-lactam carbonyl will decrease the contribution of IX and narrow this gap. It should by

Table II[a]

Molecule		Atom										
		S-1	C-2	N-3	C-4	C-5	C-6	C-7	C-9	C-10	C-11	C-13
6-APA	Charge	−0.22	+0.16	−0.19	+0.09	+0.12	X	X	−0.08	+0.38	−0.31	X
	Δ	−0.08	+0.01	−0.01	−0.01	0.00	X	X	+0.01	+0.14	−0.20	X
7-ACA	Charge	−0.22	+0.19	−0.17	+0.09	−0.06	+0.11	+0.17	−0.09	+0.37	−0.31	−0.25
	Δ	−0.07	+0.01	−0.02	+0.04	−0.06	+0.02	+0.01	+0.02	+0.13	−0.22	0.00
7-ACA	Charge	−0.22	+0.20	−0.18	+0.11	−0.02	+0.07	+0.17	−0.10	+0.38	−0.33	−0.25
	Δ	−0.07	+0.10	−0.02	+0.01	−0.02	0.00	0.00	+0.03	+0.12	−0.20	0.00
Desacetoxy-7-ACA	Charge	−0.22	+0.19	−0.17	+0.08	−0.03	+0.11	0.00	−0.09	+0.37	−0.32	X
	Δ	−0.08	+0.01	−0.02	+0.04	−0.06	+0.01	+0.01	+0.02	+0.13	−0.21	X
Desacetoxy-7-ACA$^+$	Charge	−0.19	+0.17	−0.11	+0.21	−0.02	+0.09	+0.07	−0.08	+0.38	−0.25	X
	Δ	−0.06	+0.01	+0.05	−0.08	+0.03	+0.01	−0.08	+0.02	+0.13	−0.26	X

[a]Charge densities at nonhydrogen atoms of interest. Numbering of atoms is as in structures I–III. Also, the excess of charge in the transition state over that in the ground state. Negative numbers correspond to excess of negative charge over the conventional valence electrons.

no means disappear, however (see below). This situation is in agreement with the experimentally well-known stability of Δ^2-7-ACA to basic attack.[6] Although the transition state for 6-APA is again 6 kcal higher than that of Δ^2-7-ACA, and the same resonance structures (VII and VIII, see Table II) can be written for each, there is the possibility for the release of 20 kcal more strain energy in 6-APA. The exact position of 6-APA in the order of reactivity cannot be evaluated without a much more detailed study of the actual transition state. This would be impractical for a molecule of this size using this technique. The relative antibacterial activity will be determined by the degree of strain relaxation permitted at the active site of the enzyme.

Discussion

We are now in a position to give some tentative answers to questions concerning the activities of these compounds. These include both geometrical considerations in binding to the transpeptidase (eq 1, k_1'/k_1) and reactivity to irreversible thiol attack (k_2').

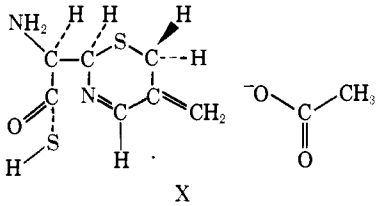
If, as we assume, the two ring carbonyls function as binding sites to the enzyme, it is reasonable that the relative orientation of these groups might determine the strength of binding[6] (if the active site is more rigid than the drug). The distance C-8–O-11 at the theoretical equilibrium in 6-APA is 4.25 Å and in 7-ACA 3.30 Å. Binding is probably to the oxygen of the C-8 carboxylic acid and not to C-8 itself, and there is essentially free rotation about C-4–C-8. Nevertheless, an enzyme to which one may bind easily will not bind the other without distortion. Note that the carboxylic acid acts as a lever on the ring. Substantial movement of this group results in a distortion of the ring. We have seen that the energy curve for ring distortion is broad, so that it is reasonable that the drug will distort in this manner to promote binding. Taking the distance in the most active compound, 6-APA, to be optimal we find that a distortion of 7-ACA from 6° (chair) to 28° (boat) is necessary to produce a similar orientation (3.9 Å apart). This would introduce 4–5 kcal strain. Allowing for some distortion of the active site we would expect Δ^3-cephalosporins to bind much less strongly than analogous penicillins.

On the basis of assumptions about the geometry of binding we would expect Δ^2-7-ACA, with C-8–O-11 = 4.05 Å, to bind approximately as well as 6-APA. The three molecules are quite similar; there are no obvious additional steric factors. We should not neglect the possibility that S-1 is a binding site as well. This atom lies in a substantially different orientation to C-8 and O-11 in Δ^2-7-ACA and in 6-APA. However, this is a site which would not be present in the natural substrate for this enzyme. Such a site would be fortuitous indeed.

The calculation of the energetics of thiol attack on the β-lactam rings of 7-ACA and Δ^2-7-ACA gives insight into this process. We are forming a C–S bond and simultaneously weakening a C–N bond so once again the calculation is inaccurate by this scale factor. Since the process is exactly the same for all three species, we expect this scale factor to remain constant and to cancel in questions involving differences in activation energies. The calculated activation energy for Δ^2-7-ACA lies 11 kcal above that for 7-ACA. We showed in the previous section that this is a minimum difference. An important factor is the presence of the additional resonance structure IX, as seen from Table II. The C-5 carbon develops a substantially greater negative charge in 7-ACA because of this additional structure. The 11-kcal difference suggests that the Δ^2-7-ACA should be virtually inactive.

The reactivity of 6-APA as compared to that of 7-ACA is more difficult to evaluate. The calculated activation energy is here higher by 17 kcal. However, there will be potentially 17 kcal more strain released during the formation of the transition state. In solution where this relaxation is completely free to occur penicillins are more sensitive to basic attack. The relative activities on the enzyme will be determined by the degree of relaxation permitted. It is conceivable that the 7-ACA derivatives will have a greater rate for this step.

It has been proposed that acetate may be expelled during thiol attack and that the presence of this feature (X) accounts for the enhanced activity of cephalosporins over Δ^2-cephalosporins. We find no evidence for this process. At the transition state [VII, X = $COC(=O)CH_3$] the acetate group remains nearly as strongly bound as in the ground state. Although we are ignoring solvation effects these should be similar in the ground and transition states. Further, Table II shows that there is no additional accumulation of negative charge at the acetate oxygen in the transition state, indicating a negligible contribution from X.



X

The presence of a leaving group cannot explain the substantial activity of the 7-aminodesacetoxycephalosporins VI. These compounds have an activity only a factor of 5 to 100 less than the acetoxy compounds, although a hydride ion can in no way be considered a good leaving group. Calculation shows (Table I) that the desacetoxy com-

pound has an activation energy 1.2 kcal higher than 7-ACA. This is very close to the total observed effect. It is more likely that this is due to enhanced ability to stabilize structures such as IX. It is expected that an acetoxy methyl group will better stabilize a negative charge than will a methyl. The acetate may depart at some point as there is a measurable rate for the SN1 displacement of this group in aqueous solutions.[14] However, we find no evidence for a concerted expulsion during the enzymic reaction.

Finally, it is possible that the ring sulfur itself acts as a leaving group during the reaction, corresponding to the formation of VIII as an actual intermediate. The evidence for or against this is much less clear than in the case of acetate expulsion. There is a substantial increase of negative charge at this atom in the transition state, and the calculated bond energy of the C-S bond is nearly halved in going to the transition state. In this case solvation energies could become important. This process remains possible. However, we would expect a larger effect in the Δ^2 system because the sulfur is conjugated with a double bond. We know experimentally that the Δ^2 is stable to basic attack; yet, we find that the expulsion of sulfur is favored in this system. It is perhaps more reasonable that this process does not occur.

Conclusions

We can tentatively draw some conclusions regarding the antibacterial activity of cephalosporins and penicillins. We have considered a two-step mechanism for the inhibiting reaction: first, reversible binding of drug to the enzyme; second, irreversible formation of the covalent drug-enzyme complex. Making simple but reasonable assumptions about the nature of these steps, a clear picture of the activity of these antibiotics emerges which is consistent with experiment.

The 10- to 100-fold difference in the activity of penicillins over cephalosporins can be attributed to differential binding to the enzyme (step 1). We would expect that a cephalosporin derivative with the quasi-boat ring conformation would have considerably higher activity. There is no evidence that the presence of acetate as a leaving group plays any role in the biological activity of cephalosporins. The difference in activity between cephalosporins and desacetoxycephalosporins is well accounted for in the intact molecules. The virtual inactivity of the Δ^2-cephalosporins results from the height of the barrier to reaction with the enzyme; binding to the enzyme should be similar to that of penicillins.

The presence of sulfur as a heteroatom in the five- and six-membered ring plays a major role in the chemistry of these molecules. The size of this atom and the delocalized nature of the lone pair orbitals are important in determining the equilibrium conformation which governs binding to the enzyme. The ability to stabilize negative charge in the transition state in resonance structures such as VIII contributes to reducing the barrier to reaction with the enzyme.

Acknowledgments. We thank Raymond A. Firestone, Fred M. Kahan, Kurt M. Mislow, Jack L. Strominger, John J. Hopfield, and particularly Leland C. Allen for enlightening discussions during the course of this work. One of us (W. C. T.) thanks Merck Sharp & Dohme, and particularly David P. Jacobus, for the opportunity to spend some interesting months at the Merck Institute, where this work was begun.

References

(1) D. J. Tipper and J. L. Strominger, *Amer. J. Med.*, **39**, 708 (1965); *Proc. Nat. Acad. Sci. U. S.*, **54**, 1133 (1965).
(2) P. J. Lawrence and J. L. Strominger, *J. Biol. Chem.*, **245**, 3660 (1970).
(3) E. S. Wagner, W. W. Davis, and M. Gorman, *J. Med. Chem.*, **12**, 483 (1969).
(4) J.-M. Ghuysen, *Bacteriol. Rev.*, **32**, 425 (1968).
(5) (a) K. E. Price, *Advan. Appl. Microbiol.*, 11, 17 (1969); M. L. Sassiver and A. Lewis, *ibid.*, **13**, 163 (1970); (b) M. Gorman and C. W. Ryan in "Cephalosporins and Penicillins," E. H. Flynn, Ed., Academic Press, New York, N. Y., 1972; (c) R. B. Hermann, *J. Antibiot.*, **26**, 223 (1973).
(6) R. M. Sweet and L. F. Dahl, *J. Amer. Chem. Soc.*, **92**, 5489 (1970).
(7) J. A. Pople, "Approximate Molecular Orbital Theory," McGraw-Hill, New York, N. Y., 1970.
(8) C. A. Coulson, *Nature (London)*, **221**, 1106 (1969).
(9) D. P. Santry and G. A. Segal, *J. Chem. Phys.*, **47**, 158 (1967).
(10) A. J. C. Wilson, *Struct. Rep.*, **15**, 553 (1952).
(11) R. D. G. Cooper, P. V. DeMarco, J. C. Cheng, and N. D. Jones, *J. Amer. Chem. Soc.*, **91**, 1408 (1969).
(12) R. D. G. Cooper, P. V. DeMarco, C. F. Murphy, and L. A. Spangle, *J. Chem. Soc. C*, 340 (1970).
(13) R. B. Morin, B. G. Jackson, R. A. Mueller, E. R. Lavagnino, W. B. Scanion, and S. L. Andrews, *J. Amer. Chem. Soc.*, **91**, 1401 (1969).
(14) A. B. Taylor, *J. Chem. Soc.*, 7020 (1965).

Experimental Antileukemic Agents. Coralyne, Analogs, and Related Compounds

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Analogs of the antileukemic alkaloid coralyne (1) and related compounds were synthesized for structure-activity study. It was found that (a) among the 8-alkyl-substituted and the 8-unsubstituted (**7g**) compounds, the 8-ethyl homolog **7d** demonstrates better antileukemic activity against leukemias L1210 and P388 in mice than the parent compound; the 8-propyl derivative **7e** is inactive, suggesting that both lipid solubility and steric effect are significant factors; (b) the planarity and rigidity of molecules of this type are critical to activity; (c) replacement of either or both o-dimethoxy groups of coralyne by methylenedioxy groups causes a slight decrease in the antileukemic activity; interestingly, the two bis(methylenedioxy) analogs **7c** and **7f** displayed activity in the KB cell culture system whereas the corresponding dimethoxy compounds are inactive; (d) elimination of some methoxy groups of coralyne lowers, but does not completely abolish, the original activity; and (e) activities of different salts are comparable but the acetosulfate salt is preferred because of its relatively higher solubility in water. Coralyne forms a stable complex with thymus DNA *in vitro* and exhibits reversible covalent hydration in water. The activity of coralyne against leukemia L1210 was shown to be schedule independent.

Coralyne chloride (**1a**), a hexadehydroberbinium salt, was recently found to exhibit inhibitory activity against both leukemias L1210 and P388 in mice.[1] In connection with our continued study on the structure-activity relationship of certain antileukemic agents containing the N-O-O triangular pharmacophore,[2] some analogs of cora-