27 acid oxalate: mp 176–177 °C (EtOH); NMR δ 3.21 (dd, 1, J_{BA} = −12.7, J_{BX} = 11.4 Hz, H_B), 3.74 (dd, 1, J_{AB} = − 12.7 Hz, J_{AX} = 2.0 Hz, H_A), 5.24 (dd, 1, J_{XA} = 2.0 Hz, J_{XB} = 11.4 Hz, H_X). Anal. ($C_{15}H_{19}D_2NO_7$) C, H, D, N. 28 acid oxalate: mp 178–180 °C (MeOH); NMR δ 3.10 (dd, 1, J_{BA} = −11.9 Hz, J_{BX} = 11.0 Hz, H_B), 3.58 (dd, 1, J_{AB} = −11.9, J_{AX} = 1.9 Hz, H_A), 5.16 (dd, 1, J_{XA} = 1.9, J_{XB} = 11.0 Hz, H_X). Anal. ($C_{17}H_{23}D_2NO_7$) C, H, D, N.

2-(2,5-Dimethoxyphenyl)-ω-[*N*-benzyl-*N*-(2-hydroxyethyl)amino]acetophenone Hydrochloride (29·HCl). To a solution of *N*-benzylethanolamine (11.5 g, 0.076 mol) in benzene (50 mL) was added a solution of 11 (10.0 g, 0.038 mol) in benzene (100 mL). The reaction mixture was stirred for 3 h at 50 °C, cooled, diluted with benzene (150 mL), and left at room temperature overnight. The solid was filtered off, and the organic solution was extracted with 10% aqueous HCl. Acid extracts were washed with Et_2O and left in a refrigerator overnight. Recrystallization (EtOH) of the formed precipitate gave **26**·HCl (15.7 g, 42%): mp 154–155 °C; IR 1560 (C=O) cm^{-1}. Anal. ($C_{19}H_{24}ClNO_4$) C, H, N.

1-(2,5-Dimethoxyphenyl)-2-[*N*-benzyl-*N*-(2-hydroxyethyl)amino]ethanol (30·HCl). A solution of **29**·HCl (3.6 g, 0.010 mol) in MeOH (60 mL) was reduced with $NaBH_4$, as previously described for the reduction of **12–14** (see Table I).

2-(2,5-Dimethoxyphenyl)-4-benzylmorpholine (31). A stirred suspension of **30**·HCl (8.50 g, 0.023 mol) in 6% aqueous HCl (170 mL) was heated at reflux for 2 h, cooled, made alkaline with solid KOH, and extracted with CH_2Cl_2. Evaporation of the washed (H_2O) and filtered CH_2Cl_2 extracts yielded practically pure **31**, as an oil (7.0 g, 80%). An analytical sample was prepared by distillation, bp 150–155 °C (0.5 mm). Anal. ($C_{19}H_{23}NO_3$) C, H, N.

2-(2,5-Dimethoxyphenyl)morpholine (8). A solution of **31** (5.0 g, 0.016 mol) in EtOH (100 mL) was shaken under hydrogen at 50 °C and atmospheric pressure in the presence of 10% Pd/C (10 g). When the absorption stopped, the catalyst was filtered off, and the solution was evaporated to give a solid residue (2.35 g), which was recrystallized from benzene to give **8** (2.10 g, 60%), mp 147 °C. Anal. ($C_{12}H_{17}NO_3$) C, H, N.

Pharmacological Methods. Isolated Rat Vas Deferens. Vasa deferentia of adult male albino rats (Sprague–Dawley) weighing 250–300 g were isolated and placed in a 10-mL organ bath containing Tyrode's solution aerated with 95% O_2 and 5% CO_2 at a constant temperature of 37 °C. The organs were loaded with 0.5 g and left to stabilize for 30 min. Spontaneous motility and responses to the drugs were recorded isotonically by a force-displacement transducer (Microdinamometer Basile Model 70-50); transmural stimulation was carried out at a frequency of 2, 5, and 10 Hz; the width of rectangular pulses was 1 ms, and the voltage was supramaximal (Grass S 5 stimulator).

Isolated Guinea Pig Atria. The atria, obtained from adult male guinea pigs weighing 300–350 g, were isolated in a 10-mL organ bath and perfused with Tyrode's solution aerated with 95% O_2 and 5% CO_2 at a constant temperature of 34 °C. The atria, loaded with 0.75 g, were left to stabilize for 30 min. Spontaneous activity and responses to the drugs were recorded isometrically by a force-displacement transducer as described for vas deferens. All the drugs were added to the bath at a maximal volume of 0.5 mL. The agonists were allowed to act until the maximal response was achieved, and dose–response curves were obtained. To evaluate the affinity of the agonists for the receptors, we calculated pD_2 values according to Ariëns and Van Rossum.[33] Antagonistic activity of the compounds toward noradrenaline and isoprenaline was evaluated by calculating dose–response curves to the agonists before and after a contact period of 20 min with the amino alcohols. In addition, pA_2 and pA_{10} values were obtained by the method of Arunlakshana and Schild.[34]

The following drugs were used as salts: noradrenaline as bitartrate; phentolamine as mesylate; isoprenaline, amino alcohols **3** (methoxamine), **6**, and **7**, the cyclic analogue of methoxamine (**4**), and practolol as hydrochlorides; amino alcohol **5** and the cyclic derivatives **8–10** as acid oxalates.

Statistical analysis of differences was performed by the Student's *t* test; *n* represents the number of experiments.

Acknowledgment. This work was supported by a grant from the "Programma Finalizzato del Consiglio Nazionale delle Ricerche, Roma, Chimica fine e secondaria". We also thank B. Stacchini for technical pharmacological assistance.

Registry No. **5**, 3600-87-1; **5**·HCl, 60407-53-6; **5** oxalate, 83436-86-6; **6**, 3489-96-1; **6**·HCl, 63991-17-3; **7**, 83436-64-0; **7**·HCl, 83436-85-5; **8**, 83436-71-9; **8** oxalate, 83436-74-2; **9**, 83436-72-0; **9** oxalate, 83447-48-7; **10**, 83436-73-1; **10** oxalate, 83436-75-3; **11**, 1204-21-3; **12**·HCl, 83436-58-2; **13**·HCl, 83436-60-6; **14**·HCl, 83436-59-3; **15**·HCl, 83436-61-7; **16**·HCl, 83436-62-8; **17**·HCl, 83436-63-9; **19**, 83436-65-1; **20**, 60681-99-4; **21**, 83436-66-2; **22**, 83436-07-3; **23**, 83436-68-4; **24**, 83436-69-5; **25**, 83436-70-8; **26**, 83436-76-4; **26** oxalate, 83436-79-7; **27**, 83436-77-5; **27** oxalate, 83436-80-0; **28**, 83436-78-6; **28** oxalate, 83436-81-1; **29**·HCl, 83436-82-2; **30**·HCl, 83436-83-3; **31**, 83436-84-4; $ClCH_2COCl$, 79-04-9; *N*,*N*-dibenzylamine, 103-49-1; benzylisopropylamine, 102-97-6; isopropylamine, 75-31-0; *N*-benzylethanolamine, 104-63-2.

(33) Ariëns, E. J.; Van Rossum, J. M. *Arch. Int. Pharmacodyn.* **1957**, *110*, 275.
(34) Arunlakshana, O.; Shild, H. O. *Br. J. Pharmacol.* **1959**, *14*, 48.

β-Lactam Antibiotics: Geometrical Requirements for Antibacterial Activities

N. Claude Cohen

Department of Medicinal Chemistry, Roussel-UCLAF Research Center, 93230 Romainville, France. *Received June 16, 1982*

Recent observations reveal deficiencies in the accepted theory rationalizing the biological activities of the β-lactam antibiotics, since a study of strained carbapenem β-lactams has shown that the observed antibacterial activities do not correlate either with the pyramidal character of the β-lactam nitrogen atom or with the ease of base hydrolysis of the lactam amide bond. The contradiction can be reconciled by an analysis of the three-dimensional (3-D) features of a set of the representative active and inactive β-lactam structures, which shows that highly specific 3-D recognition sites may exist in the enzymes in their recognition of the antibiotics. The identification of the geometrical requirements for antibacterial activity also reveals how it could be possible to restore antibiotic activities to inactive structures, up to now considered as devoid of any therapeutic interest.

The discovery of the cephalosporin antibiotics, **1**, opened up new perspectives in drug design by showing that structural modifications of the nucleus of the penicillins (**2**) were possible and could lead to different chemical structures still possessing potential antibiotic activities. However, the molecular parameters necessary for good biological activities still remain difficult to define.

Although the working hypothesis correlating the biological activities with the chemical reactivity of the β-lactam ring is widely accepted,[1] recent observations in-

0022-2623/83/1826-0259$01.50/0

dicate serious deficiencies in this model. It is shown in this study how the stereochemical features enable the contradiction to be resolved. Among the molecular structural requirements up to now recognized as necessary for the antibiotic activities, the geometrical aspects have not yet been clearly identified. Analysis of these features reveals that the 3-D aspects may play a key role in the biochemical processes involved in the antibacterial activity and suggests that highly specific 3-D recognition sites may exist in the enzymes in their recognition of the different β-lactams. Likewise, the lack of antibacterial activity is explained, at least for the inactive β-lactams mentioned in this study, on the basis of a nonrecognition rather than as a result of a different chemical reactivity and mechanism.

Previous Studies and Structural Parameters for Antibacterial Activities. In the course of the study of cephalosporins (1), analogous compounds have been prepared in which the double bond is in the Δ^2 position (3), and it was observed that these cephalosporins were devoid of any antibiotic action. This was quite surprising, since the structural differences existing between the two active families of compounds 1 and 2 are much greater than those existing between the isomeric derivatives 1 and 3, differing only by the position of the double bond. When this observation was made 15 years ago, some rational explanations were sought to justify the biological inactivities of the Δ^2-cephalosporins. At that time the α stereochemistry of the carboxylic function in penicillin was already known,[5,6] but in the Δ^2-cephalosporins this stereochemistry was not yet established. For that reason, it was suspected[7] that the biological inactivities of the Δ^2-cephalosporins were possibly due to a "wrong" configuration of this carboxylic function in the β orientation as in 4. Subsequent studies along these lines[7] have shown that the absolute configuration of the carboxy group is, in fact, α as in 5 and, therefore, identical with that in penicillins (2). The biological inactivity was eventually ascribed to a high stability of the β-lactam ring and not to a "wrong" carboxy configuration.

Subsequently, a new attempt was made to analyze the geometrical aspects and was carried out on the basis of X-ray studies.[8] The crystal structures of Δ^2- and Δ^3-cephalosporins were solved, and their molecular structures were compared to that of penicillin G.[6] Surprisingly, 3-D comparison of the cyclic moieties did not reveal obvious similarities between the active compounds, penicillin and Δ^3-cephalosporin. On the contrary, it showed that penicillin more closely resembles the inactive Δ^2-cephalosporin (see Figure 1). This observation was also previously mentioned[7] based on the simple comparison of molecular models. Following on from these observations, it was concluded that the conformational requirements were not very restrictive for the recognition of the antibiotic as a substrate by the enzymes.

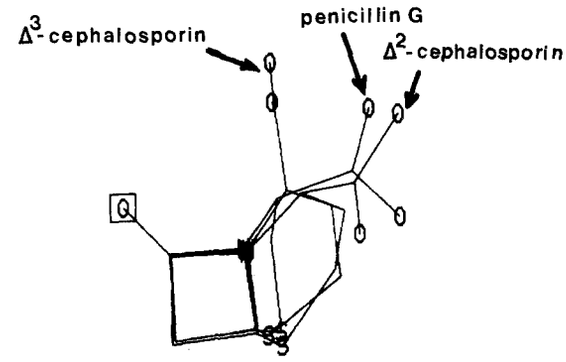


Figure 1. No common 3-D features[8] (atomic coordinates taken from X-rays[6,8]).

Pyramidality of the Nitrogen Atom. In the X-ray studies it was also found that the lactam nitrogen atom of the Δ^2-cephalosporins is nearly planar, in contrast to the pyramidal character existing in penicillin and Δ^3-cephalosporin antibiotics. Using arguments earlier put forward for penicillins by Woodward et al.,[9] the biological activities observed in the three families of compounds (1, 2, and 5) were again ascribed to the chemical reactivity of the β-lactam amide bond: a hindering of the amide resonance in the β-lactam ring of the active compounds being justified by the pronounced pyramidal character of the β-lactam nitrogen atom; the pyramidalization being created either by the strain of the ring fusions in 2 or by electron delocalization through enamine resonance outside the lactam ring in 1.

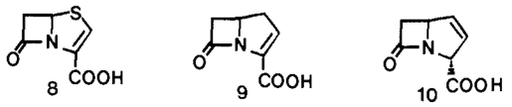
This interpretation, which suggests that the origin of the biological activities is found in the ease of base hydrolysis of the lactam amide bond, sounds reasonable; however, the reduction of the geometrical requirements only to the pyramidal character of the nitrogen is not satisfactory, and the existence of precise molecular 3-D features for the recognition at this level of the antibiotic by the enzymes, although not yet identified, needs to be established.

Deficiencies of the Reactivity Model. Recently, and subsequent to the discovery of the thienamycin[10] antibiotic 6, which possesses high antibacterial properties, Δ^1-thienamycin 7, a double-bond isomer of thienamycin, was found to be devoid of any antibiotic activity.[11] This in-

(1) Subsequent to the molecular interpretations of the biological mode of action initially formulated for penicillins and proposing that the antibiotic acylates the active site of the transpeptidase enzyme involved in the biosynthesis of the peptidoglycan layer of bacterial cell walls.[2-4]
(2) Strominger, J. L.; Tipper, D. J. *Am. J. Med.* **1965**, *39*, 708.
(3) Yocum, R. R.; Waxman, D. J.; Rasmussen, J. R.; Strominger, J. L. *Proc. Natl. Acad. Sci. U.S.A.* **1979**, *76*, 2730.
(4) Ghuysen, J. M.; Frère, J. M.; Leyh-Bouille, M.; Coyette, J.; Dusart, J.; Nguyen-Disteche, M. *Annu. Rev. Biochem.* **1979**, *48*, 73.
(5) Crowfoot, D.; Bunn, C. W.; Rogers-Low, B. W.; Turner-Jones, A. In "The Chemistry of Penicillin"; Princeton University Press: Princeton, NJ, 1949; p 310.
(6) Pitt, G. J. *Acta Crystallogr.* **1952**, *5*, 770.
(7) Van Heyningen, E.; Ahern, L. K. *J. Med. Chem.* **1968**, *11*, 933.
(8) Sweet, R. M.; Dahl, L. F. *J. Am. Chem. Soc.* **1970**, *92*, 5489.
(9) Johnson, J. R.; Woodward, R. B.; Robinson R. In ref 5; p 440.
(10) Albers-Schönberg, G.; Arison, B. H.; Hensens, O. D.; Hirshfield, J.; Hoogsteen, K.; Kaczka, E. A.; Rhodes, R. E.; Kahan, J. S.; Kahan, F. M.; Ratcliffe, R. W.; Walton, E.; Ruswinkle, L. J.; Morin, R. B.; Christensen, B. G. *J. Am. Chem. Soc.* **1978**, *100*, 6491.

activity was likewise ascribed[11] to a chemically less reactive β-lactam amide bond when compared to that found in thienamycin.

At the same time, the antibacterial activities of even simpler but similar highly strained β-lactams were also studied.[12] It was found that compounds **8** and **9** possess potential antibacterial activities, whereas compound **10** was found to be inactive.



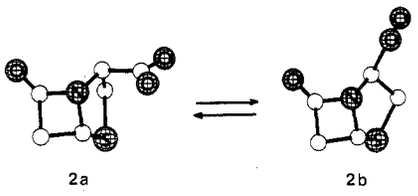
8 **9** **10**

An analysis of the chemical reactivity of the amide bond of these β-lactams and the pyramidal character of the nitrogen atom reveals deficiencies in the admitted model for interpreting the biological activities of the β-lactam antibiotics. It has been observed[12] that the ease of base hydrolysis of the β-lactam ring of the inactive compound **10**, in fact, compares well with that of the active parent compounds **8** and **9** and also with that of the known potent antibiotics. Besides, the X-ray study of the acetonyl esters of the three compounds has shown that the pyramidal character of the lactam nitrogen atom of molecule **10** is more pronounced than in **8** and **9**, and the pyramidality of this atom in the three molecules is in any case much greater than that observed in penicillins.

There are considerable structural similarities between the two inactive compounds **7** and **10**, which have the same carbapen-1-em nucleus. The interpretation on the origin of their biological inactivities is still not clearly established. Shih and Ratcliffe[11] have ascribed this lack of antibacterial activity in **7** to a low chemical reactivity. On the other hand, Woodward initially suggested[13] that the biological inactivity of **10** could be due to an extraordinary chemical reactivity, the compound being immediately destroyed with any nucleophile available in the biological medium. A deeper analysis of this point has lately shown[12] that compound **10** has, in fact, a chemical reactivity, which is perfectly comparable to that of the other active compounds. Although the nature of the substituents may explain the different chemical behavior in the two families of compounds, it still remains that the correlation of these results with the biological observations brings up the question of the nature of the structural parameters rationalizing the antibacterial activities.

The pyramidal character of the nitrogen atom or the reactivity of the β-lactam ring may not represent the only molecular parameters that have to be considered in the interpretation of the biological activities. The 3-D aspects, although not yet clearly understood, may also play an important role in the biochemical processes involved. It is proposed in this study to pay some attention to the geometrical features in order to define with greater precision the 3-D requirements of the molecular structure, which have to be controlled for good design in this area.

Conformational Considerations. Sweet and Dahl's[8] previously mentioned 3-D comparison of compounds **1**, **2**, and **5** has revealed that penicillin resembles the inactive Δ^2-cephalosporins more than the active Δ^3-cephalosporins (see Figure 1). However, this observation, together with the one mentioned earlier on the basis of molecular models,[7] does not give any definitive evidence on the absence of geometrical requirements for the biological activities. A conformational study of the different molecules concerned can reveal that precise geometrical requirements for the recognition of the antibiotics by the enzymes could exist.



2a **2b**

Figure 2. Pseudorotation of the penam nucleus.

One of the keys to this interpretation is based on the conformational possibilities of the penam nucleus, which is endowed with pseudorotating movements. According to this process, the cyclic system can modify the orientation of the substituents and its shape as shown in Figure 2.

In **2a** the carboxy group is in a pseudoaxial orientation (taken from X-ray data[14]), whereas in **2b** it is pseudoequatorial (X-ray study on ampicillin[23]). The two forms have been experimentally observed: in the crystallographic studies of penicillins, the thiazolidine ring is found in the **2a**[14-22] and also in the **2b**[23-30] conformations. Small variations of energies accompany this process, and the carboxy group can easily fluctuate between the two positions. [Molecular modeling studies[31] show that **2a** is more stable by approximately 0.7 kcal/mol. Previous theoretical calculations are either in favor of **2a**[32] or **2b**[33] (in the latter case, the difference of energy between the two forms is 0.5 kcal/mol).] On the contrary, the nucleus of the penem and carbapenem molecules, such as **6–10**, are highly rigid and devoid of any pseudorotating effect: a structure exactly conforming to the precise geometrical requirements of the

(11) Shih, D. H.; Ratcliffe, R. W. *J. Med. Chem.* **1981**, *24*, 639.
(12) Pfaendler, H. R.; Gosteli, J.; Woodward, R. B.; Rihs, G. *J. Am. Chem. Soc.* **1981**, *103*, 4526.
(13) Woodward, R. B. *Philos. Trans. R. Soc. London, Ser. B* **1980**, *289*, 239.
(14) Yoshimoto, M.; Ishihara, S.; Nakayama, E.; Soma, N. *Tetrahedron Lett.* **1972**, 2923.
(15) Csöregh, I.; Palm, T.-B. *Acta Crystallogr., Sect. B* **1977**, *B33*, 2169.
(16) Dexter, D. D.; van der Veen, J. M. *J. Chem. Soc., Perkin Trans. 1* **1978**, 185.
(17) Kukolja, S.; Jones, N. D.; Chaney, M. O.; Elzey, T. K.; Gleissner, M. R.; Paschal, J. W.; Dorman, D. E. *J. Org. Chem.* **1975**, *40*, 2388.
(18) Cox, P. J.; McClure, R. J.; Sim, G. A. *J. Chem. Soc., Perkin Trans. 2* **1974**, 360.
(19) Blanpain, P.; Durant, F. *Cryst. Struct. Commun.* **1976**, *5*, 83.
(20) Abrahamsson, S.; Crowfoot-Hodgkin, D.; Maslen, E. N. *Biochem. J.* **1963**, *86*, 514.
(21) Cameron, A. F.; McElhatton, J.; Campbell, M. M.; Johnson, G. *Acta Crystallogr., Sect. B* **1979**, *B35*, 1263.
(22) Dauter, Z.; Bogucka-Ledochowska, M.; Borowski, E.; Dreissig, W.; Barnickel, G.; Bradaczek, H. *Acta Crystallogr., Sect. B* **1981**, *B37*, 2179.
(23) Boles, M. O.; Girven, R. J. *Acta Crystallogr., Sect. B* **1976**, *B32*, 2279.
(24) Palm, T.-B.; Csöregh, I. *Acta Crystallogr., Sect. B* **1978**, *B34*, 138.
(25) Dexter, D. D.; van der Veen, J. M. *J. Chem. Soc., Perkin Trans. 1* **1978**, 185.
(26) Boles, M. O.; Girven, R. J.; Gane, P. A. C. *Acta Crystallogr., Sect. B* **1978**, *B34*, 461.
(27) Lovell, F. M.; Perkinson, N. A. *Cryst. Struct. Commun.* **1978**, *7*, 7.
(28) Blanpain, P.; Durant, F. *Cryst. Struct. Commun.* **1976**, *5*, 89.
(29) James, M. N. G.; Hall, D.; Hodgkin, D. C. *Nature (London)* **1968**, *220*, 168.
(30) Blanpain, P.; Melebeck, M.; Durant, F. *Acta Crystallogr., Sect. B* **1977**, *B33*, 580.
(31) Cohen, N. C., unpublished results.
(32) Topp, W. C.; Christensen, B. G. *J. Med. Chem.* **1974**, *17*, 342.
(33) Joshi, N. V.; Virudachalam, R.; Rao, V. S. R. *Curr. Sci.* **1978**, *47*, 933.

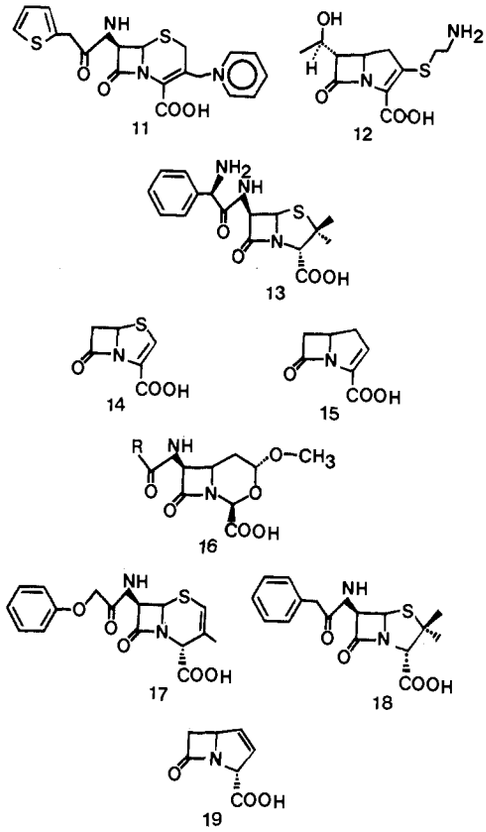


Figure 3. Representative molecules chosen for 3-D comparison.

enzyme will possess the antibiotic activity, whereas a structure not conforming will be unable to modify its geometry to adjust to these requirements and will have no antibacterial activity.

Structures Considered in the Three-Dimensional Comparison. Representative molecules of the active and inactive β-lactam structures mentioned here were selected and are listed in Figure 3. This set of compounds includes the structures of a Δ^3-cephalosporin (**11**), thienamycin (**12**), penicillins (**13** and **18**), a Δ^2-cephalosporin (**17**), and compounds such as **14**, **15**, and **19**. Structure **19** can also be considered as a model of the inactive Δ^1-thienamycin **7**. The crystal structures of all these compounds are available,[5,6,8,10,12,23] except for compound **16**, the molecular geometry of which was obtained with the SCRIPT molecular modeling system.[31,34] Some features of this new family of active antibiotics will be discussed just after the following paragraph.

Two penicillin molecules appear in the set of compounds; their 3-D geometries are representative forms of possible rotamers of the penam nucleus. Compound **13** has the carboxylic function in a pseudoequatorial orientation, whereas in compound **18** it is pseudoaxial. For the other structures only one geometry is sufficient to represent the various analogues. In the model that will be proposed here, the pseudoequatorial form **2b** of the penam nucleus of penicillin will be shown as being the active conformation of these antibiotics, while the pseudoaxial form **2a** is revealed as an inactive form. Six of the first molecules listed in Figure 3 therefore represent active structures, whereas the last three compounds represent inactive geometries.

Concerning molecule **18**, it is worth noting that the compound is an active antibiotic (penicillin G), the crystal structure of which was found to be in the pseudoaxial conformation **2a**. The X-ray data of this molecule are only useful in this study in that it allows us to analyze what we consider the inactive conformation of penicillins, whereas the actual biological action of such a molecule is to be understood on the basis of its conformational possibilities for which the pseudoequatorial active form **2b** can also be envisaged.

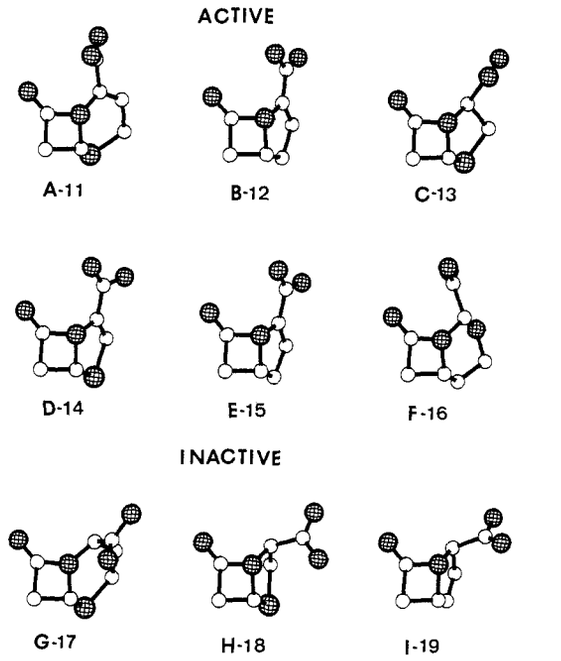


Figure 4. Three-dimensional features of the nine structures listed in Figure 3.

Table I. Distance between the Oxygen Atom of the β-Lactam Amide Group and the Carbon Atom of the Carboxy Group

compd	distance, Å
11	3.198
12	3.568
13	3.899
14	3.607
15	3.613
16	3.024
17	4.111
18	4.263
19	4.276

Comparison of the Nine Compounds. The molecular geometries of the nine compounds are shown on Figure 4. The plane of reference is that of the β-lactam ring, and the molecules are seen from "Woodward's point of view" (with reference to this particular way of drawing the chemical formulas of β-lactams[13]). In order to simplify the comparison, only the heavy atoms of the rings and those of the carboxy group were drawn. It is clear from these views that the inactive structures have very different molecular geometries when compared to the active ones. The active molecules (compounds **11**–**16**) have the carboxy group much closer to the β-lactam amide function than the three inactive geometries **17**–**19**. Table I illustrates, for example, the distance separating the oxygen atom of the amide group and the carbon atom of the carboxylic function for each of the nine structures. These values show that this distance is shorter in the active structures (3.0–3.9 Å) as compared to those observed on the inactive geometries (more than 4.1 Å).

Visualization of the Model. The superposition of the nine molecules is visualized in Figure 5. This picture

(34) Cohen, N. C.; Colin, P.; Lemoine, G. *Tetrahedron* 1981, *37*, 1711.

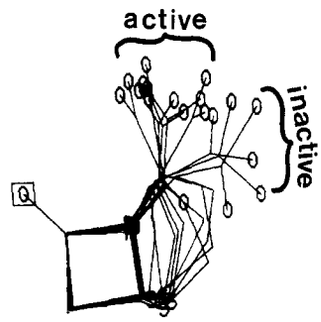


Figure 5. Geometrical separation between active and inactive structures.

clearly reveals the sharp difference existing between the two sets of molecules. The carboxy groups of the active molecules are concentrated in the upper region of the drawing in a cone containing, from right to left, the compounds in the following order: **13**, **14**, **15**, **12**, **11**, and **16**. The carboxy groups of the inactive molecules are located in a different cone, the axis of which is almost perpendicular to that of the active region. One can recognize the molecules in the following order from top to bottom of the view of Figure 5: **19**, **18**, and **17**.

This superposition clearly shows that precise 3-D requirements could exist in the recognition of the antibiotics by the enzymes and, therefore, allows the rationalization of the contradictions mentioned in the interpretation of the biological activities observed; in particular, the biological inactivities of structures such as **5**, **7**, and **10** are justified in the same way.

The difficulty in clarifying the 3-D aspects was, in fact, due to the existence of the stable form of the penicillin molecule **2a**, which appeared with molecular models and also with X-ray crystallography as perfectly capable of mimicking the inactive compounds. In the proposed model, the stable conformation of the penam nucleus of the penicillin molecule at this level is not its active form; only the rotamer having the carboxy group in the pseudoequatorial orientation **2b** is active. Active compounds must, therefore, orient their acidic group in the upper region as revealed in Figure 5, and this requirement should be taken into consideration in the analysis of the structure–activity relationships.

In a study of the broadness of the antibacterial spectrum of β-lactams, Balsamo et al.[35] have proposed the hypothesis that the conformation observed in the crystal structure of a given penicillin molecule (**2a** or **2b**) may be associated with its antibacterial spectrum (narrow or broad) but indicate that the number of structures they have to support this idea is too limited. Although we consider such molecules as possessing some conformational flexibility, the proposed hypothesis partly coincides with the conclusions of our analysis.

New Perspectives in the Design of Antibiotics. This model now opens up new perspectives in the design of new antibiotics in this area. In particular, the possibility of restoring potential activities to the inactive structures can be envisaged. Δ^2-Cephalosporins having their carboxylic function in the "wrong" β configuration (**4**), a configuration which is opposite to that in penicillins, appear capable of presenting the appropriate 3-D requirements (see Figures 4 and 5). One should notice that such a possibility would be impossible to imagine on the basis of the chemical formulas. The validity of this possibility has not yet been

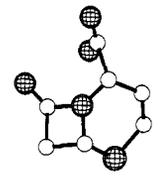


Figure 6. Geometry of the bicyclic moiety of molecule **4**.

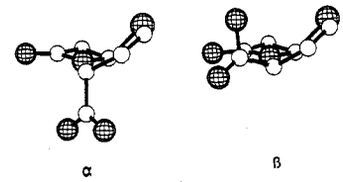


Figure 7. Orientation of the carboxy group in Δ^2-cephalosporins (atomic coordinates taken from X-rays[37]).

proved, as such compounds have never been either isolated or synthesized. Molecule **5** is, in fact, thermodynamically more stable than epimer **4** as estimated[31] with molecular mechanics calculations[34] with a difference in energy of 1 kcal/mol. However, the hypothesis of restoring antibiotic activities to the Δ^2-cephalosporins can find some support in the discovery of a new family of antibiotics: compounds such as **16**[36] with a saturated six-membered ring do have their carboxy group in the β configuration and are endowed with potential antibiotic activities.

The orientation of the carboxy group in this new structure appears on Figure 4 (drawing F16). Likewise, Figure 6 visualizes how good the orientation of the carboxylic function would be in Δ^2-cephalosporins (such as **4**) with this β configuration. In this structure the distance between the oxygen of the amide group and the carbon atom of the carboxylic function is 3.186 Å.

Another comparison of the two epimers of Δ^2-cephalosporins is given in Figure 7. The acid function of the β epimers now fits very well the 3-D requirements discussed in this paper, and this observation allows the consideration that appropriate analogues of this structure may exhibit potential antibiotic activities.

Since the substituents of the β-lactam ring can exert strong electronic interactions with the amide group, this model must also permit the nature of the electronic parameters, which have to be controlled for good antibacterial activities, to be analyzed with greater precision.

Conclusion

It is shown in this work how the molecular aspects for the antibiotic activities of the β-lactam derivatives can have subtle and precise 3-D requirements.

These geometrical features may play a key role in the recognition of the antibiotic by the enzymes in the biological processes. The 3-D aspects allow biological observations to be clarified, which were otherwise difficult to rationalize on the basis of the classical theory correlating the biological activities with the chemical reactivity of the β-lactam ring.

In the course of their important contributions to this area,[38,39] Frère, Ghuysen, and colleagues have recently published[38] an analysis of the chemical reactivity of a wide

(35) Balsamo, A.; Domiano, P.; Macchia, B.; Macchia, F.; Nardelli, M. *Eur. J. Med. Chem.* **1980**, *15*, 559.

(36) Gleason, J. G.; Buckley, T. F.; Holden, K. G.; Bryan, D. B.; Siler, P. *J. Am. Chem. Soc.* **1979**, *101*, 4730.

(37) Kobelt, V. D.; Paulus, E. F. *Acta Crystallogr., Sect. B* **1974**, *B30*, 1608.

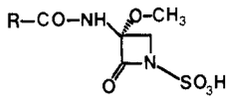
(38) Frère, J. M.; Kelly, J. A.; Klein, D.; Ghuysen, J. M. *Biochem. J.* **1982**, *203*, 223.

(39) De Coen, J. L.; Lamotte-Brasseur, J.; Ghuysen, J. M.; Frère, J. M.; Perkins, H. R. *Eur. J. Biochem.* **1981**, *121*, 221.

range[40] of β-lactam structures and compared it with the kinetic parameters of their interaction with various enzymes (β-lactamases and peptidases), and the intrinsic chemical reactivity is shown not to be correlated with the enzymes' activities. It is proposed that the primary parameter that governs the biological action must be the goodness of fit of the β-lactam to the enzyme cavity, and this is in full agreement with the conclusions of our study.

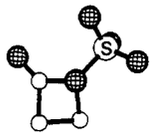
The model proposed in our analysis also enables one to predict the possibility of restoring antibiotic properties to the inactive related structures and must help to arrive at a better understanding of the nature of the structural requirements, both geometric and electronic, that have to be controlled in the design of new therapeutic compounds in this field.

Note Added in Proof: Subsequent to the request of a referee, the analysis of Sulfazecin is presented here. The compound is a monobactam antibiotic (mainly active against Gram-negative bacteria) of the **20** type, the X-ray structure of which has been published.[41] Due to the



20

particular geometrical effects caused by the sulfur atom (bond lengths longer than those with the carbon), the resulting geometrical features of this novel antibiotic appear to be compatible with the 3-D requirements discussed in this paper (see view below). The β-lactam nitrogen is



planar (the distance between the nitrogen and the plane of its three neighbors is 0.13 Å; the sum of the three valency angles around that atom is 357.7°). The distance between the oxygen of the amide group and the sulfur atom is 3.355 Å.

(40) Data on the intrinsic reactivity and some biochemical kinetic parameters of the structures mentioned in the present paper are available in ref 38.

(41) Kamiya, K.; Takamoto, M.; Wada, Y.; Asai, M. *Acta Crystallogr., Sect. B* **1981**, *B37*, 1626.

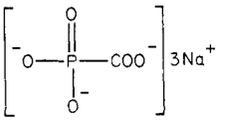
Synthesis of Esters of Phosphonoformic Acid and Their Antiherpes Activity[1,2]

Jan O. Norén,* Erik Helgstrand,[3] Nils G. Johansson, Alfons Misiorny, and Göran Stening

Research and Development Laboratories, Astra Läkemedel AB, S-151 85 Södertälje, Sweden. *Received March 23, 1982*

Aliphatic and aromatic mono-, di-, and triesters of phosphonoformic acid (foscarnet) were synthesized. The triesters were prepared by the Michaelis–Arbuzov reaction and were hydrolyzed to di- and monoesters. The compounds were tested for antiviral activity on isolated herpes simplex virus type 1 (HSV-1) DNA polymerase, in a HSV-1 plaque reduction assay, and on a cutaneous HSV-1 infection in guinea pigs. None of the esters inhibited the activity of isolated HSV-1 polymerases. Monoesters with a free carboxylic group and diesters with an aromatic carboxylic ester function were active against the cutaneous herpes infection. Mono- and diesters with an aromatic phosphonic ester group also showed activity in the plaque-reduction assay. However, mono- and diesters with aliphatic carboxylic ester groups were inactive in all test systems. The results show that all three acidic groups of phosphonoformic acid must be free in order to get antiviral activity at the enzyme level. However, certain esters of this acid may be biotransformed to the acid itself to give antiherpes activity.

Herpes viruses induce a virus-specific DNA polymerase activity in infected cells.[4-6] Therefore, this enzyme is a possible target for selective antiviral drugs. Phosphonoformic acid [(hydroxycarbonyl)phosphonic acid] trisodium salt (PFA) (INN, foscarnet sodium, **1**) and phosphonoacetic acid (PAA) are selective inhibitors of DNA polymerases from several herpes viruses.[7-10] These compounds appear to interfere with the polymerase at a pyrophosphate binding site.[10,11]



1

(1) Part of this work was presented earlier. (a) Helgstrand, E.; Johansson, N. G.; Misiorny, A.; Norēn, J. O.; Stening, G. 1st European Symposium on Organic Chemistry, Cologne, Germany, Aug 1979; abstract, p 368. (b) Norēn, J. O.; Helgstrand, E.; Johansson, N. G.; Misiorny, A.; Stening, G.; Alenius, S.; Stridh, S.; Öberg, B. International Symposium on Medicinal Chemistry, 7th, Torremolinos, Spain, Sept 1980, abstr P 120. (c) Helgstrand, E.; Alenius, S.; Johansson, N. G.; Öberg, B. "Current Chemotherapy and Infectious Disease", Proceedings of the International Congress of Chemotherapy, 11th, and Interscience Conference on Antimicrobial Agents and Chemotherapy, 19th, Boston, Oct 1–5, 1979; Nelson, J. D.; Grossi, C., Eds.; American Society for Microbiology: Washington, DC, 1980; pp 1359–1361.

(2) Helgstrand, E.; Johansson, N. G.; Misiorny, A.; Norēn, J. O.; Stening, G. European Patents 003275 and 003007, 1979.

(3) Present address: AB Draco, Box 1707, S-221 01 Lund, Sweden.

(4) Keir, H. M.; Gold, E. *Biochim. Biophys. Acta* **1963**, *72*, 263–276.

(5) Purifoy, D. J. M.; Lewis, R. B.; Powell, K. L. *Nature (London)* **1977**, *269*, 621–623.

(6) Jofre, J. T.; Schaffer, P. A.; Parris, D. S. *J. Virol.* **1977**, *23*, 833–836.

(7) Helgstrand, E.; Eriksson, B.; Johansson, N. G.; Lannerö, B.; Larsson, A.; Misiorny, A.; Norēn, J. O.; Sjöberg, B.; Stenberg, K.; Stening, G.; Stridh, S.; Öberg, B.; Alenius, S.; Philipson, L. *Science* **1978**, *201*, 819–821.

(8) Reno, J. M.; Lee, L. F.; Boezi, J. A. *Antimicrob. Agents Chemother.* **1978**, *13*, 188–192.

(9) Overby, L. R.; Rabishaw, E. E.; Schleicher, J. B.; Reuter, A.; Schipkowitz, N. L.; Mao, J. C.-H. *Antimicrob. Agents Chemother.* **1974**, *6*, 360–365.

(10) Leinbach, S. S.; Reno, J. M.; Lee, L. F.; Isbell, A. F.; Boezi, J. A. *Biochemistry* **1976**, *15*, 426–430.

0022-2623/83/1826-0264$01.50/0