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Conformational analysis of β and γ-lactam antibiotics

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Summary — Geometry optimization, superimposition searches and conformational analysis carried on several lactam antibiotics show that reactivity with the active-site serine penicillin-binding proteins is related to a particular spatial disposition of 2 flanking functional groups — namely a C=O or C-OH on 1 side and a carboxylate on the other — with respect to the central scissile amide bond. Such a binding entity is found in one of the most stable conformers of the tripeptide diacetyl-L-Lys-D-Ala-D-Ala conferring substrate activity, and in benzylpenicillin, cephapyrin, thienamycin, γ-lactam, the 6-spiro-epoxypenicillin S and in one epimer of lactivicin, conferring inactivating potency. This binding entity generates a particular electronic distribution and the fact that it is conserved in compounds belonging to very different chemical families strongly suggests that it is an important feature required for enzyme recognition.

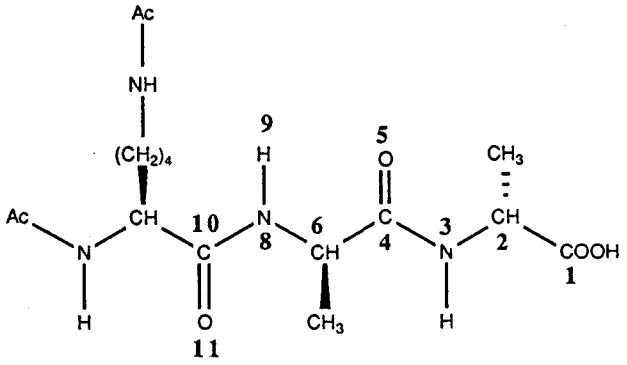
Résumé — Analyse conformationnelle d'antibiotiques β et γ-lactamiques. *L'étude par optimisation de géométrie, superposition et analyse conformationnelle réalisée sur plusieurs antibiotiques à noyau lactame montre que leur réactivité vis-à-vis des protéines liant la pénicilline et possédant une sérine active dépend de la disposition spatiale particulière des groupements fonctionnels C=O ou C-OH et carboxylate de part et d'autre de la liaison amide sensible. Ce motif existe dans l'un des conformères les plus stables du tripeptide substrat diacétyl-L-Lys-D-Ala-D-Ala et dans les inhibiteurs benzylpénicilline, céphapyrine, thiénamycine, γ-lactame, ainsi que dans l'un des épimères de la 6-spiroépoxypénicilline et de la lactivicine. Ce motif génère une distribution électronique très particulière et le fait qu'il soit conservé dans des composés appartenant à des familles chimiques très différentes suggère fortement qu'il constitue une caractéristique nécessaire à la reconnaissance enzymatique.*

antibiotics / β-lactam / γ-lactam / penicillin / cephalosporin / thienamycin / conformational analysis

The targets of the β-lactam antibiotics in susceptible bacteria are membrane-bound proteins, collectively designated as penicillin-binding proteins. The bacteria also produce defensive enzymes, the β-lactamases, which hydrolyse the antibiotics into biologically metabolites. The penicillin-binding proteins and the majority of the β-lactamases react with the β-lactam antibiotics *via* formation of an acyl enzyme in which the carbonyl of the amide bond of the β-lactam ring becomes ester-linked to a serine residue. The acyl enzyme formed by reaction with the penicillin-binding proteins is essentially hydrolytically inert while that formed by reaction with the β-lactamases is very short-lived [1, 2].

The penicillin-binding proteins have specific functions in cell division, cell elongation and determination of cell shape [1, 2]. Some of them catalyse key reactions in wall peptidoglycan metabolism. In these reactions, the *R-L*-Xαα-D-alanyl moiety of *R-L*-Xαα-D-alanyl-D-alanine-terminated peptidoglycan precursors is sequentially transferred to the essential serine residue (with formation of a serine ester-linked acyl enzyme) and from this, either to water (DD-carboxypeptidation reaction) or a suitable amino acceptor (DD-transpeptidation reaction). Though the D-alanyl-D-alanine peptide bond in non-cyclic carbonyl donors and the endocyclic amide bond in the β-lactam antibiotics are thus functionally equivalent, there has been, for many years, a widely held view according to which the non-planarity of the β-lactam nitrogen atom would play a role in biological reactivity as a transition state analogue. This assumption might explain why the reactivity of the endocyclic CON unit is important but not essential [3]. Moreover, antibacterial monocyclic β-lactams and bicyclic and monocyclic γ-lactams are known, suggesting that the goodness of fit of the molecules in the enzymes active sites is the essential feature for biological activity.

The molecules selected for this study are shown in figures 1 and 2. The tripeptide diacetyl-L-Lys-D-Ala-D-Ala (fig 1) is a substrate analogue while the lactams (fig 2) are suicide substrates of various penicillin-



Ac_2 L Lys D Ala D Ala

Fig 1. Structure of the tripeptide substrate analogue.

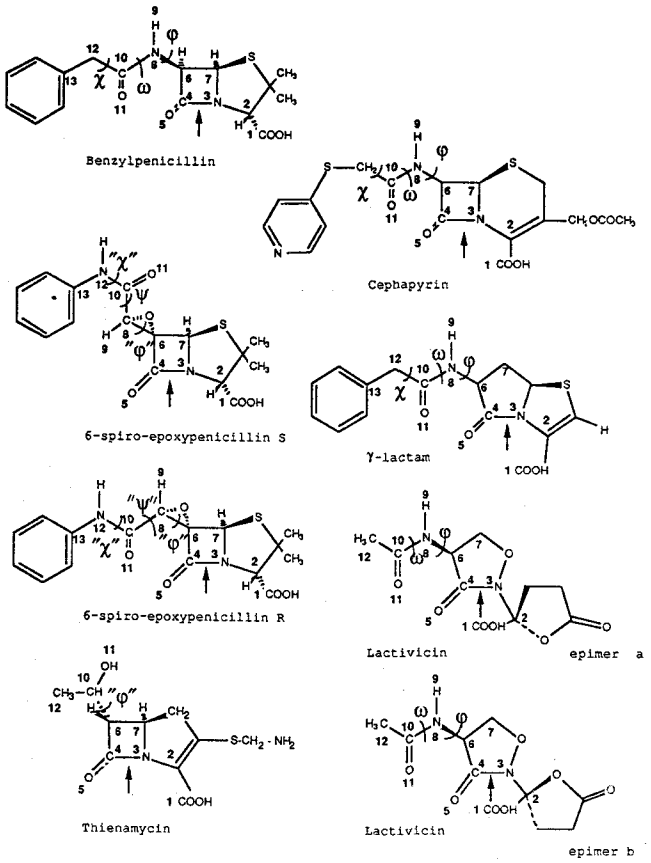


Fig 2. Structures of the selected lactams with atom numbering and dihedral angle labelling. The black arrow shows the scissile amide bond.

binding proteins. These lactams are also substrates and/or inactivators of some β-lactamases. All these molecules possess on both sides of the scissile amide C4=O5 bond, C10=O11 (or C10-O11H) and Cl OOH functional groups whose spatial disposition has been explored by methods of theoretical chemistry, superimposition searches and conformational analysis. Since the compounds studied belong to distinct chemical families, the presented atom numbering is arbitrary; it has only been used here for the sake of uniformity.

Materials and Methods

Selected lactams (fig 2)

Benzylpenicillin from the penams family was used as a reference. From its crystal structure, it is known that the thiazolidine ring can adopt 3 different puckerings depending on which the sulfur or 1 of the carbon atoms of the cycle comes out of the mean plane [4]. The 6-amino acyl side chain is extended in the complex formed with procaine and folded in the potassium salt [5]. Penicillin has also been the subject of theoretical studies. In particular, Rao [6] attempted to determine the minimum energy conformation of several antibiotics, Cohen [7] emphasized the pseudoaxial-pseudoequatorial relationships in the conformational variations of the penam nucleus, and Wolfe *et al* [8] investigated penicillin conformations in solution using molecular mechanics. Existence of a global minimum where the penicillin adopts a compact structure was proposed (in which, on the convex face of the molecule, the N8H9C10O11 amide group is exposed to interactions with proteins).

Cephapyrin was selected as a member of the 3-cephems family. Its crystal structure is known [9].

Like benzylpenicillin, the 6-spiro-epoxypenicillins [10] and thienamycin [11] are bicyclic β-lactams. In 6-spiro-epoxypenicillins, where the conformation of the C6 side chain is frozen by the configuration of the epoxy cycle, the *S* isomer is biologically active while the *R* isomer is not. In turn, the carbapenem thienamycin bears an isopropylalcohol on the β-face of the molecule.

The γ-lactam in which a benzyl group replaces the phenoxy group of the original compound studied by Baldwin [12] is also bicyclic but, in this compound, the scissile amide bond is in a 5-membered ring which itself is fused to a thiazoline moiety (the double bond of which was thought to be important to enhance the reactivity of the γ-lactam ring).

Lactivicin [13] possesses 2 cycles which are not fused to each other. The scissile amide bond-containing isoxazolidinone is substituted by a lactone on N3. In solution, the 2 epimers a and b occur in a ≈ 1 to 1 molar ratio. The crystal structure of aminolactivicinic acid is known [14]. This molecule whose side chain is limited to an amino group, adopts a zwitterionic form and the crystal structure is stabilized by intermolecular hydrogen bonds.

Computational methods

All the degrees of freedom of the compounds were fully optimized within the MNDO quantum chemistry framework [15] using the link 402 of the GAUSSIAN 86 program [16], except for lactivicin to which the MONSTERGAUSS program [17]

was applied at the *ab initio* STO-3G level (the reason for this is that a study of the chemical reactivity of isoxazolidinone derivatives is in progress in the laboratory). Moreover, the consistency between semiempirical MNDO and minimal basis set STO-3G results was checked as described in the literature [18]. The guess geometry used as a starting point for the optimization procedure rested upon the available X-ray data and/or standard parameters of smaller compounds. From the quantum chemistry optimized geometry, conformational analyses around torsional angles were performed using the molecular mechanics program MM2 [19] keeping all the other degrees of freedom unchanged. Geometrical adjustment and dynamic fit were included in the graphic ULYSSE package from this laboratory (improved version of the PAKGGRAPH program) [20]. It was run on a Data General MV7800 computer with an attached graphic processor GDC2400.

Results

Optimized geometries and conformational analysis

Figure 3 shows the optimized geometries of the lactams studied. Optimization of lactivicin gives rise to a cisoid configuration of the 2 substituents of the isoxazolidinone, which configuration is more stable by 2.77 kcal/mol than transoid configuration. In the cisoid diastereoisomers a and b, the 6-acetylamino side chain has the same orientation but the carboxylate at position 2 points to different regions of space, compound b being more stable by 1.44 kcal/mol than compound a.

The conformation of side chains on C6 depends on the values of several dihedral angles (fig 2). In benzylpenicillin, the γ-lactam and lactivicin, the amide bond is in the transplanar conformation ($\omega = 180°$). Geometry optimization yields a ϕ value of 175° for the 3 compounds and a χ value of 75° for benzylpenicillin and the γ-lactam.

The optimized ϕ and χ values correspond to the minima of the conformational maps. As illustrated with benzylpenicillin, plots of energy as a function of ϕ and χ gives maximum stability for ϕ values ranging between 140° and 300° (with $\chi = 75°$) and for 3 discrete χ values (with $\phi = 175°$) (figs 4a, b). Two-D map contouring at levels of 0.5, 3, 5 and 10 kcal/mol above the minimum energy (fig 4c) allows identification of 3 main stable conformers having a common ϕ value of $\approx 175°$ and χ values of 70° (a value which is very similar to that found by MNDO optimization), 180° and 285°, respectively. The $\phi 175°$–$\chi 70°$ conformer is more stable than the 2 others by 1.1 kcal/mol and the energy barrier between the 3 conformers does not exceed 3 kcal/mol. The γ-lactam behaves in the same way (not shown). The 3 most stable conformers have a common ϕ value of 170° and a χ value of 75°, 190° and 270°, respectively. In lactivicin, the permitted values of ϕ range from 170° to 300° (fig 4d).

The orientation of C10-O11 in the 6-spiro-epoxy-penicillins depends on dihedral angles referred to as 'ϕ' and 'ψ'. 'ϕ' is frozen to 140° in epimer *S* and − 2.9° in epimer *R*. Rotation around ψ generates minima at − 100° and − 122° for the optimized epimers *S* and *R*, respectively.

The orientation of the OH group at position 11 in thienamycin is governed by 'ϕ'. Optimized geometry and conformational analysis yields a 'ϕ' value of 75°, which is also that found in the crystal structure.

Rigid fit

Pair-wise superimposition of the optimized structures, using benzylpenicillin as a reference, were carried in order to obtain the best possible fit between either atoms N3C4O5C6C7 (referred to as the 5 atom fit) or atoms C2N3C4O5C6C7 (referred to as the 6 atom fit). For the pairs benzylpenicillin-γ-lactam and benzylpenicillin-lactivicin (epimers a and b), the sum of the squared distances between corresponding atoms was minimized (table I) in order to achieve a common orientation of the skeletons.

Whether the 5 atom fit or the 6 atom fit is used (fig 5), the carbonyl of the scissile amide groups superimpose almost exactly. Moreover, the distances between fitted pairs of atoms are of the same order of magnitude and never exceed 0.5 Å (table I). Depending on the kind of fit, however, the central nuclei are rotated by about 10° (fig 5). Consequently, the 5 atom fit yields a better match between the carbon atoms C6 and, conversely, the 6 atom fit gives a better match between the sulfur atoms (of benzylpenicillin and γ-lactam) and, of course, the carbon atoms C2 and the carboxylates.

A view of benzylpenicillin (fig 6a) and cephapyrin (fig 6b) (chosen as model of the 3-cephems) along the plane of the β-lactam ring shows that the carboxylates do occur on the same face of the molecules but are distant from each other by more than 1.5 Å. This difference is a direct consequence of the hybridization character of C2 which is sp3 in the penams and sp2 in the 3-cephems. Similarly, the unsaturated bond in the thiazoline ring of the γ-lactam compels the carboxylate to adopt a position which resembles that found in cephapyrin (not shown). Finally, lactivicin epimer b (fig 6d) – but not epimer a (fig 6c) – has its carboxylate oriented in a way that also resembles that found in benzylpenicillin and, much better, in cephapyrin. On this basis, epimer b is assumed to be the biologically active form of lactivicin.

Dynamic fit

As a result of the 5 atom fit, the distances between each of the atoms C10 and O11 in benzylpenicillin and in the other compounds are respectively equal to: 0.26 and 0.56 Å for lactivicin epimer b, 0.59 and

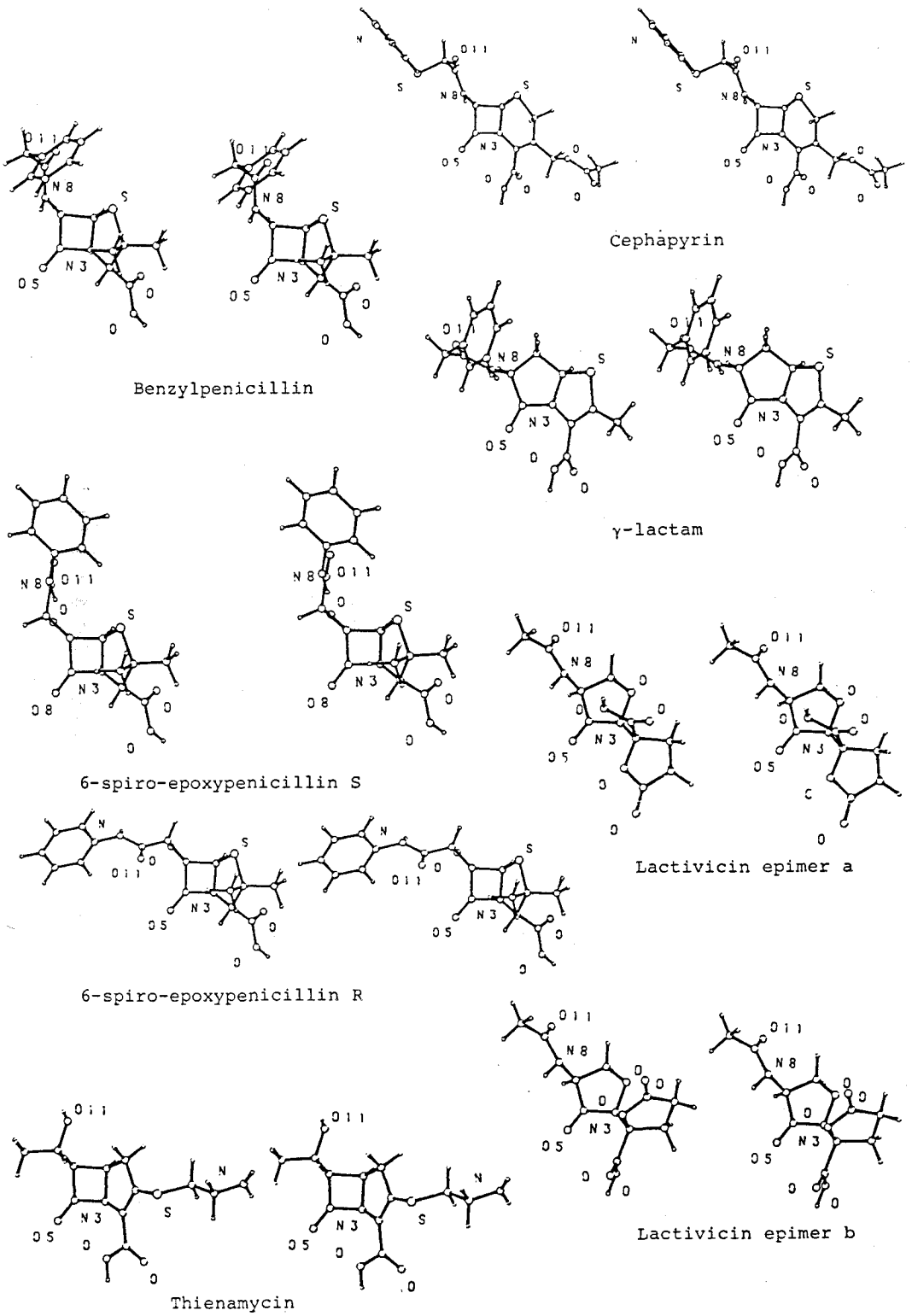


Fig 3. Stereoview of the optimized geometries of the selected lactams.

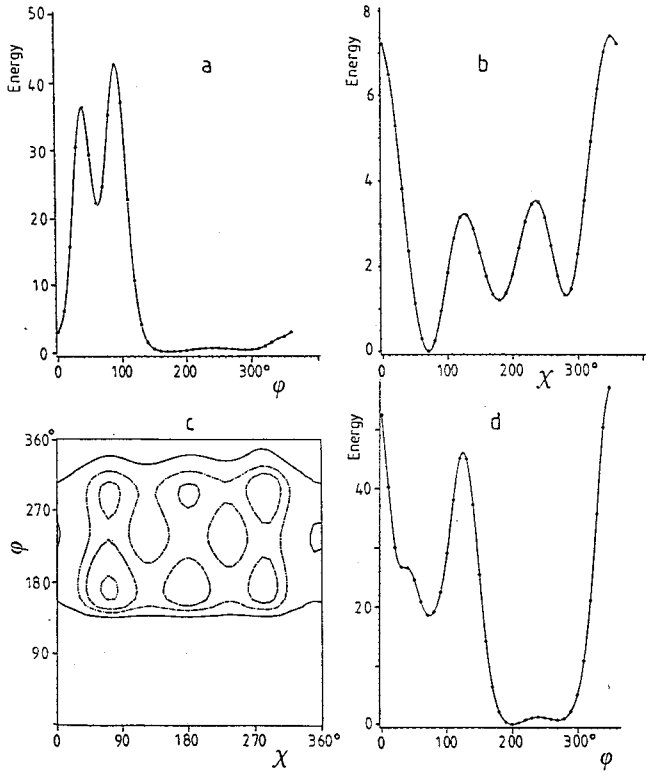


Fig 4. Energy (kcal/mole) as a function of the dihedral angles that govern the conformation of the side chain at C6 of benzylpenicillin (a, b, c) and lactivicin (d). In figure c, the energy contour levels are 10 (—), 5 (–•–•–), 3 (– – –) and 0.5 kcal/mol (•••)

0.89 Å for the 6-spiro-epoxypenicillin *S*, 0.42 and 1.30 Å for thienamycin, 0.76 and 1.16 Å for the γ-lactam and 1.73 and 3.65 Å for the 6-spiro-epoxypenicillin *R*.

In those cases where the distances exceed 1 Å, a dynamic fit was performed using the program ULYSSE, by rotating the C6 side chains to minimize the distance between each pair of the carbonyl oxygens O11 and by calculating for the adjusted geometry, the loss of MNDO energy. As a result of this fit, the distances are equal to 0.42 and 0.85 Å for the pair benzylpenicillin-thienamycin (at the expense of 1.54 kcal/mol), and 0.37 and 0.37 Å for the pair benzylpenicillin-γ-lactam (at the expense of 0.56 kcal/mol). In contrast, the side chain of the biologically inactive 6-spiro-epoxypenicillin *R* can never adopt an orientation similar to that found in benzylpenicillin, C10 and O11 distances remaining larger than 2.8 Å. In a similar way, using X-ray data of other spiro-

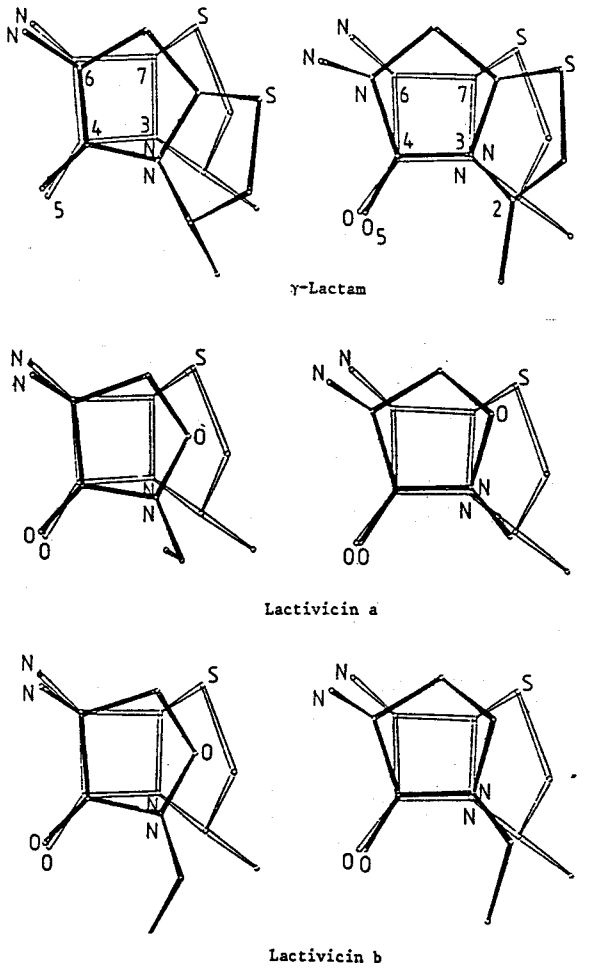


Fig 5. Pair-wise superimposition of the nucleus of benzylpenicillin and those of γ-lactam and lactivicin epimers a and b (unfilled bonds). Superimposition was carried using the 5 atom fit (left) and the 6 atom fit (right). For details, see text.

Table I. Distances (Å) between pairs of atoms by reference to benzylpenicillin.

Atoms	*5 atom fit*			*6 atom fit*		
	γ-lactam	*lactivicin a*	*lactivicin b*	*γ-lactam*	*lactivicin a*	*lactivicin b*
C2	–	–	–	0.11	0.14	0.19
N3	0.45	0.38	0.38	0.08	0.14	0.15
C4	0.09	0.09	0.08	0.07	0.06	0.07
O5	0.11	0.13	0.11	0.11	0.16	0.19
C6	0.09	0.08	0.07	0.45	0.45	0.46
C7	0.50	0.42	0.41	0.45	0.41	0.41
$\Sigma\Delta^2$(Å)^2	0.49	0.35	0.34	0.44	0.44	0.48
C1	2.57	2.97	2.68	1.82	2.91	1.91

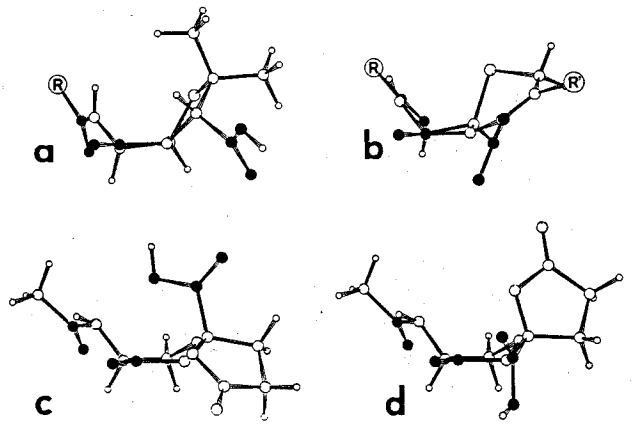


Fig 6. Orientation of the C10-O11, C4-O5 and Cl-OO bonds (in black) in benzylpenicillin (a), cephapyrin (b), lactivicin epimer a (c) and epimer b (d). All the molecules are viewed along the N3-C5-C7 plane. R = acyl side chain on C10; R' = alkyloxy substituent of cephapyrin.

epoxy derivatives, Shute [21] showed that the available conformational space for the side chains of the 3*R*- and 3*S*-anilides is mutually exclusive and that the conformational space for the more flexible benzylpenicillin can accomodate the side chains of both the constrained spiro derivatives.

Figure 7 shows the final results of the superimposition. In benzylpenicillin, the 6-spiro-epoxypenicillin *S*, thienamycin, the γ-lactam and lactivin epimer b, the C4=O5 carbonyl of the scissile amide groups on the one hand, and the C10=O11 (or C10-OH11) groups on the other superimpose remarkably well, while at the other end of the molecules, the carboxylates are at least similarly oriented. The same type of fitting was carried with the non-cyclic tripeptide diacetyl-L-Lys-D-Ala-D-Ala, a substrate analogue of the low-M_r DD-peptidases/penicillin-binding proteins. Figure 7 also shows that in one of the most stable conformers of this tripeptide, previously designated BB*B* [22], the carbonyl of the L-Lys-D-Ala bond, that of the scissile D-Ala-D-Ala bond and the carboxylate, superimpose almost perfectly with the equivalent atom groupings of benzylpenicillin.

Structure–activity relationships

As shown by the data of table II, the value of the dihedral angle between the plane O11C4O5 and the plane C4O5C1 exhibits minor variations, from 175 to 195°, in all the compounds studied except in the 2 biologically inactive compounds lactivicin epimer a (230.9°) and 6-epoxy-spiropenicillin *R* (217.6°). The atomic distance O5-O11 vary from 4.70 to 5.85 Å, in all the compounds studied except in 6-epoxy-spiropenicillin *R* (3.74 Å). Finally, depending on the type of structure in which N3 is involved, the distance O5-C1 varies from 2.75 to 4.25 Å. These O5-C1 distances and those reported by Cohen [7] emphasize the incidence of the thiazoline ring conformation. In the 3 penam optimized structures studied here, the 'C3 conformation' of the fused ring [4] generates a distance O5-C1 of about 4.2 Å which characterizes the pseudo-axial conformation described by Cohen. However, the pseudoequatorial conformation which is assumed to be the energetically accessible 'active conformation' (by about 0.7 kcal) is induced during formation of the acyl enzyme (as shown in a model of methanolysis which is under current study in this laboratory).

From the foregoing, it thus follows that biological activity, whether as inactivator or as substrate, essentially rests upon an almost coplanar disposition of atoms O11, O5, C4 and C1 and is compatible with O5-O11 distances ranging from 4.70 to 5.85 Å, O5-C1 distances ranging from 2.75 to 4.25 Å and C1-O5-O11 angle values of 95–133°.

Discussion

The lactam antibiotics and D-alanyl-D-alanine-terminated substrates have a common backbone R-CONH-C-CON-C-COOH where CON is the electrophilic center involved in the nucleophilic attack by the active-site serine of the DD-peptidases/penicillin binding proteins. Geometry optimization, superimposition searches and conformational analysis carried on

Table II. Spatial disposition of atoms O11, O5 and C1 in the lactam inactivators and the tripeptide Ac_2-L-Lys-D-Ala-D-Ala substrate.

Compound	*Distance O5-O11 (Å)*	*Distance O5-C1 (Å)*	*Dihedral angle O11-C4-O5-C1 (°)*	*Angle O11-O5-C1 (°)*
Benzylpenicillin	5.16	4.16	177.4	100.8
6-spiro-epoxypenicillin *S*	5.85	4.20	184.6	95.3
6-spiro-epoxypenicillin *R*	3.74	4.21	217.6	120.0
Thienamycin	4.70	3.57	175.4	106.7
Cephapyrin	5.12	3.35	174.8	118.0
γ-lactam	4.88	3.06	195.3	112.7
Lactivicin epimer a	4.98	3.49	230.9	96.7
Lactivicin epimer b	5.01	2.75	188.6	133.4
Ac_2-L-Lys-D-Ala-D-Ala conformer BB*B* [19]	4.88	4.25	187.1	108.2

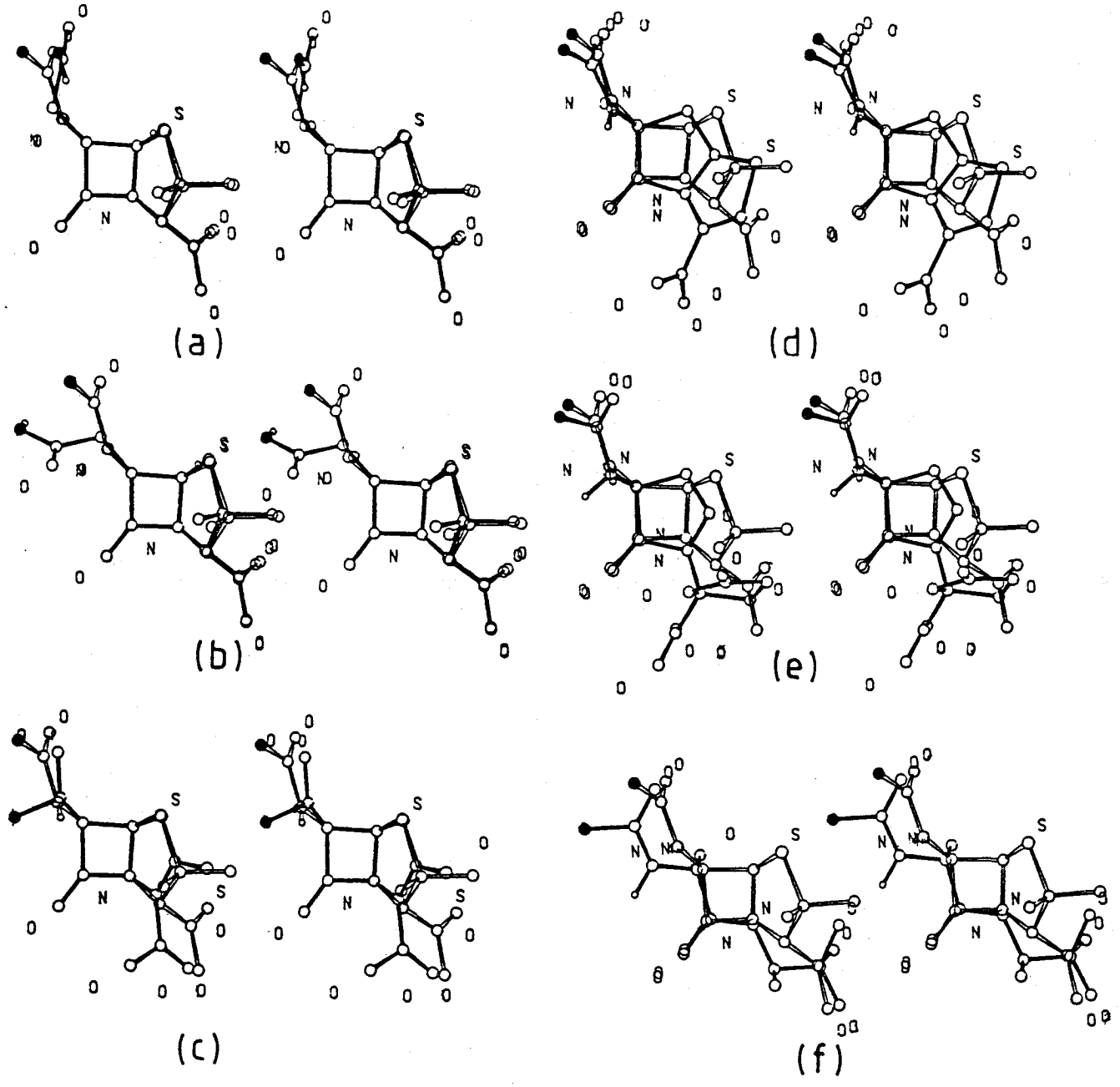


Fig 7. Superimposed stereoviews of benzylpenicillin (unfilled bonds) and 6-spiro-epoxypenicillin *S* (a), 6-spiro-epoxypenicillin *R* (b), thienamycin (c), γ-lactam (d), lactivicin b (e) and Ac_2-L-Lys-D-Ala-D-Ala conformer BB*B* (f). The R substituent on C10 is represented by a black ball.

antibiotics belonging to different chemical families (penicillins, 6-epoxy-spiropenicillin *S*, cephalosporins, thienamycin, γ-lactams and lactivicin) lead to the suggestion that carbonyl donor activity requires a particular spatial disposition of the R-CO-NH moiety and the COOH function with respect to the central CON scissile amide group. The same preferred spatial disposition is found in all the lactams studied and in

one of the most stable conformers of the tripeptide Ac_2-L-Lys-D-Ala-D-Ala substrate analogue. Given the particular electronic distribution that these functional groups generate, which itself gives rise to a negative electrostatic potential of defined shape and volume [23], one can speculate that this common feature is an essential component of the binding entity involved in enzyme recognition.

It is well-known in β-lactam medicinal chemistry that enzyme recognition depends very much on the nature of the *R* side chain, which *R* side chain is differently oriented in the lactams and the D-alanyl-D-alanine-terminated peptides (fig 7). The occurrence of distinct enzyme subsites may explain the development of resistance in important bacterial pathogens such as *Staphylococcus aureus, Streptococcus pneumoniae, Neisseria gonorrhoeae* and *Streptococcus faecium* [24–30], by the evolution of altered penicillin-binding proteins that have greatly reduced their affinity for the lactam antibiotics without impairing their ability to process their structurally-analogous normal substrate. Conversely, it may also explain the fact that penicillin-binding proteins exist which have no function in peptidoglycan metabolism and act only as penicillin-signal transducer for the synthesis of intracellular proteins (B Joris, P Ledent, T Kobayashi, JO Lampen, JM Ghuysen, submitted).

Acknowledgments

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References

1 Ghuysen JM (1988) *Rev Infect Dis* 10, 726
2 Ghuysen JM, Frère JM, Joris B, Dusart J, Duez C, Leyh-Bouille M, Nguyen-Distèche M, Coyette J, Dideberg O, Charlier P, Dive G, Lamotte-Brasseur J (1989) *In: Design of Enzyme Inhibitors as Drugs* (Sandler M, Smith HJ, eds) Oxford University Press, 523–573
3 Frère JM, Kelly JA, Klein D, Ghuysen JM, Claes P, Vanderhaeghe H (1982) *Biochem J* 203, 223
4 Domiano P, Nardelli M, Balsamo A, Macchia B, Macchia F (1979) *Acta Cryst* B35, 1363
5 Dexter DD, van der Veen JM (1978) *J Chem Soc (Lond)* Perkin I, 185
6 Rao VSR (1983) *In: The Target of Penicillin* (Hakenbeck R, Höltje JV, Labischinski H, eds) Walter de Gruyter, Berlin, 359–367
7 Cohen NC (1983) *J Med Chem* 26, 259
8 Wolfe S, Yang K, Khalil M (1988) *Can J Chem* 66, 2733
9 Declercq JP, Germain G, Moreaux C, Van Meersche M (1977) *Acta Cryst* B33, 3868
10 Bycroft BW, Shute RE, Begley MJ (1988) *J Chem Soc Chem Comm* 274
11 Albers-Schönberg G, Arison BH, Hensens OD, Hirshfield J, Hoogsteen K, Kaczka EA, Rhodes RE, Kahan JS, Kahan FM, Ratcliffe RW, Walton E, Ruswinkle LJ, Morin RB, Christensen BG (1978) *J Am Chem Soc* 100 (20), 6491
12 Baldwin JE, Lowe C, Schofield CJ (1986) *Tetrahedron Lett* 27, 3461
13 Nozaki Y, Katayama N, Ono H, Tsubotani S, Harada S, Okazaki H, Nakao Y (1987) *Nature* 325, 179
14 Wada Y, Takamoto M, Tsubotani S, Kamiya K (1987) *Acta Cryst* C43, 1786
15 Dewar MJS, Thiel W (1977) *J Am Chem Soc* 99, 4899
16 Frisch MJ, Binkley JS, Schlegel HB, Raghavachari K, Melius CF, Martin RL, Stewart JJP, Bobrowicz FW, Rohfing CM, Kahn LR, Defrees DJ, Seeger R, Whiteside RA, Fox DJ, Fleuder EM, Pople JA (1984) *In: Gaussian 86*, Carnegie-Mellon Quantum Chemistry Publishing Unit, Pittsburgh, PA
17 Peterson MR, Poirier RA, Csizmadia IG (1980) Program Monstergauss, Dept Chem, University of Toronto
18 Dewar MJS, Thiel W (1977) *J Am Chem Soc* 99, 4907
19 Allinger NL (1977) MM2-(77), Quantum Chemistry Program Exchange, Bloomington, Indiana
20 Honig B, Kabat EA, Katz L, Levinthal C, Wu TT (1973) *J Mol Biol* 80, 277
21 Shute RE, Jackson DE, Bycroft BW (1989) *J Comput Aided Mol Des* 3, 149
22 De Coen JL, Lamotte-Brasseur J, Ghuysen JM, Frère JM, Perkins HR (1981) *Eur J Biochem* 121, 221
23 Lamotte-Brasseur J, Dive G, Ghuysen JM (1984) *Eur J Med Chem Chim Ther* 19, 319
24 Song MD, Maesaki S, Wachi M, Takahashi T, Doi M, Ishino F, Maeda Y, Okonogi K, Imada A, Matsuhashi M (1988) *In: Antibiotic Inhibition of Bacterial Cell Surface Assembly and Function* (Actor P, Daneo-Moore L, Higgins ML, Salton MRJ, Shockman GD, eds) American Society for Microbiology, Washington DC, 352–359
25 Spratt BG (1989) *In: Microbial Resistance to Drugs* (Bryan LE, ed) Handbook of Experimental Pharmacology, Springer Verlag, Berlin, 29, 77–100
26 Laible G, Hakenbeck R, Sicard MA, Joris B, Ghuysen JM (1989) *Molecular Microbiology* (in press)
27 Dowson CG, Jephcott AE, Cough KR, Spratt BG (1989) *Molecular Microbiology* 3, 35
28 Spratt BG (1988) *Nature* 332, 173
29 Canepari P, Del Mar Lleo M, Cornaglia G, Fontana R, Satta G (1986) *J Gen Microbiol* 132, 625
30 Hedge P, Spratt BG (1985) *Eur J Biochem* 151, 111