EUR. J. MED. CHEM. — CHIM. THER., 1984-19, N° 4, pp. 319-330

On the structural analogy between D-alanyl-D-alanine terminated peptides and β-lactam antibiotics

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(Received July 4, 1983, accepted January 5, 1984)

Summary. — Structural analogy between D-alanyl-D-alanine terminated peptides (and analogues) of varying substrate activity toward D-alanyl-D-alanine-cleaving peptidases, and bicyclic fused ring azetidinone structures of varying inactivating potency toward the same enzymes has been examined by comparing the relative spatial disposition of the carboxylate function at the C-terminal position and the amide function at the N-terminal position with respect to the scissile amide bond at the central position. The observed variations in the geometric parameters and the molecular electrostatic potential maps generated by these functional groups suggest multiple modes of binding. In the monobactam sulfazecin, the relative disposition of at least the scissile amide bond and the terminal sulphamate group is comparable to that of the corresponding functions in the bicyclic β-lactams.

Résumé. — Le degré d'analogie structurale entre, d'une part, peptides se terminant par la séquence D-alanyl-D-alanine (et analogues) et doués d'activité de substrat variable vis-à-vis des DD-peptidases et, d'autre part, β-lactamines bicycliques douées de pouvoir inactivateur variable vis-à-vis de ces mêmes enzymes a été examiné en comparant la disposition spatiale relative des fonctions carboxylate et amide qui se situent, respectivement, en position C-terminale et en position N-terminale par rapport à la liaison peptidique (amidique) sensible. Les paramètres géométriques et les cartes de potentiel électrostatique générées par ces trois fonctions suggèrent des modes multiples de fixation aux centres actifs de ces enzymes. La disposition relative du groupement sulfamate terminal et de la liaison amidique sensible dans la β-lactamine monocyclique, sulfazecine, est comparable à celle des fonctions correspondantes dans les β-lactamines bicycliques.

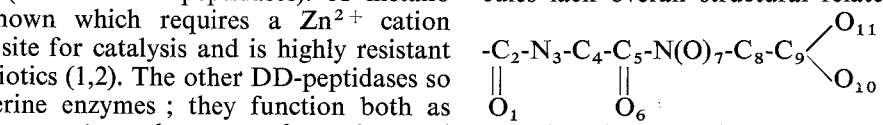
Zusammenfassung. — Die strukturelle Analogie zwischen D-Alanyl-D-alanin-terminalen Peptiden (und Analoga) mit verschiedener Substrataktivität gegenüber D-Alanyl-D-analin-spaltenden Peptidasen und bicycschen kondensierten Azetidinonen mit unterschiedlicher Hemmwirkung auf die gleichen Enzyme wurde untersucht. Dazu wurden die relative räumliche Anordnung der C-terminalen Carboxyl-Gruppe und der N-terminalen Amid-Gruppe in Hinblick auf die zu spaltende Amid-Bindung verglichen. Die beobachteten Variationen der geometrischen Parameter und die Verteilung der molekularen elektrostatischen Potentiale, die durch diese funktionellen Gruppen erzeugt werden, lassen auf verschiedene Bindungspartner schließen. In dem Monolactam Sulfazecin ist zumindest die relative Anordnung der zu spaltenden Amid-Bindung und der terminalen Sulfamat-Gruppe vergleichbar jener der entsprechenden Funktionen in den bicyclischen β-Lactamen.

Key-words : β-Lactams. — D-alanyl-D-alanine terminated peptides. — DD-peptidases. — Structural analogy.

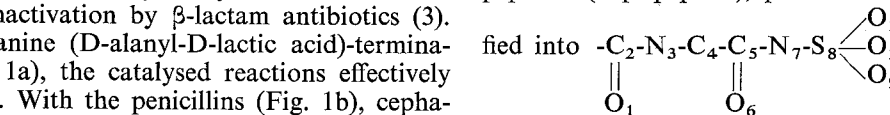
INTRODUCTION

The transpeptidation and carboxypeptidation reactions involved in the last stages of wall peptidoglycan synthesis in bacteria are catalysed by multiple D-alanyl-D-alanine-cleaving peptidases (in short DD-peptidases). A metallo DD-peptidase is known which requires a Zn^{2+} cation bound to the active site for catalysis and is highly resistant to all β-lactam antibiotics (1,2). The other DD-peptidases so far identified are serine enzymes ; they function both as peptidases and esterases *via* acyl enzyme formation and are susceptible to inactivation by β-lactam antibiotics (3). With D-alanyl-D-alanine (D-alanyl-D-lactic acid)-terminated substrates (Fig. 1a), the catalysed reactions effectively proceed to products. With the penicillins (Fig. 1b), cephalosporins (Fig. 1c) and monobactams (Fig. 1d), the reaction flux stops at the level of the acyl enzyme intermediate, immobilizing the enzymes in an inactive form.

The scissile bond in the peptide (depsipeptide) substrates and β-lactam inactivators are functionally equivalent (see arrows in Fig. 1). However, i) these two types of molecules lack overall structural relatedness ; ii) the backbone



which is common to the peptides (depsipeptide), penams and cephems can be modified into



in the monobactams (4,5) ; and iii) the scissile bonds are far from being isosteric with each other. Thus, the nitrogen atom is pyramidal in the β-lactams and planar in the peptides (depsipeptide) ;

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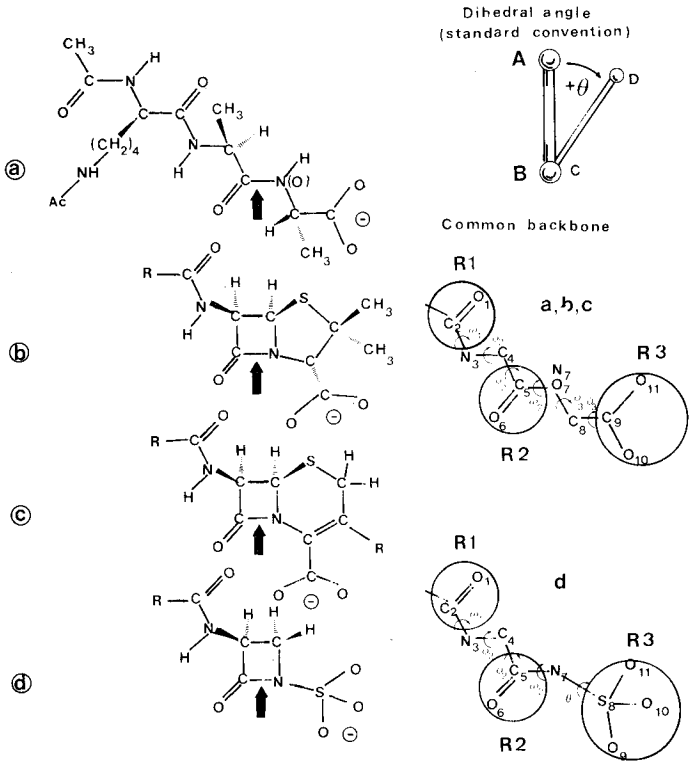


FIG. 1. — *Structure and common backbone (showing the atoms and positions numbering used in this present report) of substrate analogues [a) Ac_2-L-Lys-D-Ala-D-Ala and Ac_2-L-Lys-D-Ala-D-Lac] and β-lactam inactivators [b) penams ; c) Δ^3-cephems ; d) monobactams] of DD-peptidases. Standard convention for defining dihedral angles.*

The arrows show the amide or ester bond whose attak within the active site of the serine DD-peptidases leads to the formation of the serine ester-linked acyl enzyme.

Standard convention for defining dihedral angles, using four atoms A, B, C, D in sequence order, is that looking along the bond between the central two atoms B and C (in either direction), the end atom in front is used as the 0° angle reference. The dihedral angle is then measured by the relative position of the end atom in back (positive is clockwise, negative is conterclockwise) with respect to the reference atom position.

In the peptides (depsipeptide) the backbone angles are variable except ω_2 which is fixed to 180°. In any of the bicyclic β-lactams, ω_2, ψ_2 and φ_3 are fixed to specific values while the variable angles φ_2 and ψ_3 determine the orientation of the amino acyl substituent and the carbonyl grouping, respectively. In the monobactams (sulfazecin), the angle θ combines φ_3 and ψ_3 of the bicyclic β-lactams.

the $O_6{=}C_5{\leftrightarrow}\begin{matrix}C_4\\ \diagup\\ \diagdown\\ N(O)_7\end{matrix}$ bond angle is 90° in the β-lactams and 117° in the peptides (depsipeptide) ; the dihedral angle ω_2 is 135° in the penicillins, 155° in the cephalosporins, 172.5° in sulfazecin and 180° in the peptides (depsipeptide).

In spite of these variations, bioactivation of the C_5-$N(O)_7$ amide (ester) bond at position R_2 [both in peptide (depsipeptide) substrates and azetidinone inactivators] can be achieved providing that a proper substituent occurs on the C_2-N_3 amide bond at position R_1 and that an anionic charge is present at position R_3. Therefore, instead of comparing a same sequence of atoms (6), the work presented here has been based on a search of possible analogy in the relative spatial disposition of these three functional groups.

The atomic coordinates of the β-lactam compounds are from crystal structures (Cambridge Data Bank ; level 1982, update 1). None of the peptides (depsipeptide) studied has been crystallized and, consequently, the atomic coordinates are those of the most probable conformers as revealed by empirical conformational analysis (7) and CNDO calculations carried out on these selected conformers. Altogether, these conformers cover almost or more than 90 % of the whole conformational space of each peptide (depsipeptide) concerned.

The present study, which extends the excellent discussion of Cohen (8), concerns only the static properties of the peptides (depsipeptide) of varying substrate activity and β-lactam compounds of varying inactivating potency before any interaction with the enzyme active sites. It does not take into account the numerous forces (hydrophobic, van der Waals, hydrogen bonding and electrostatic interactions) which govern the molecular enzyme-ligand association.

MATERIALS AND METHODS

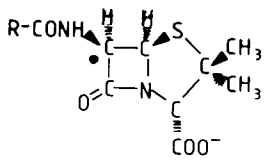
Peptides

The tripeptides Ac_2-L-Lys[1]-D-Ala[2]-D-Ala[3] (**1**), Ac-L-Ala[1]-D-Ala[2]-D-Ala[3] (**2**), Ac_2-L-Lys[1]-D-Leu[2]-D-Ala[3] (**3**), Ac_2-L-Lys[1]-D-Ala[2]-D-Leu[3] (**4**), Ac_2-L-Lys[1]-L-Ala[2]-D-Ala[3] (**6**) and Ac_2-L-Lys[1]-D-Ala[2]-L-Ala[3] (**7**), and the depsipeptide Ac_2-L-Lys[1]-D-Ala[2]-D-lactic acid[3] (**5**) have been used (Table 1). With the R61 serine DD-peptidase, the enzyme catalytic efficiency ($M^{-1}s^{-1}$) for the hydrolysis of (**5**) and (**1**) is 27 600 (J. M. Frère ; unpublished data from this laboratory) and 4 600, respectively. The D-Leu[3] analogue (**4**) has a much decreased substrate activity (310 $M^{-1}s^{-1}$). The L-Ala[1], D-Leu[2], L-Ala[2] and L-Ala[3] analogues have very little or no substrate activity at all. The L-Ala[2] and L-Ala[3] analogues do not bind to the enzyme active site (for more details, see [3]). As previously suggested (7), the four most probable blackbone conformers of each peptide (depsipeptide) are designated by a three-letter system where each letter qualifies that portion of the $(\varphi_i\psi_i)$ space of minimum energy for each residue. BB*B*, EB*B*, BE*B* and EE*B* refer to the LDD peptides (depsipeptide) (**1-5**), BBB*, EBB*, BEB* and EEB* to the LLD peptide (**6**) and BB*B, EB*B, BE*B and EE*B to the LDL peptide (**7**). The peptide backbone is an extended structure in conformers (B or E) B*B*, (B or E) BB* and (B or E) B*B and has a tendancy to fold back on itself in conformers (B or E) E*B*, (B or E) EB* and (B or E) E*B. For more information, see footnote of table 1 and (7).

β-Lactams

The β-lactam structures (**8**) to (**42**) shown in Fig. 2 fall into three main groups depending on wether the β-lactam ring is fused to a five-membered ring (Fig. 2A and 2B), a six-membered ring (Fig. 2C-F) or is not fused to any other ring (Fig. 2G). The penams (**8**) to (**21**) and the Δ^3-cephems (**24**) to (**35**) are effective antibiotics (when possessing a free carboxyl group at the C-terminal position). In the order of decreasing acylating efficacy ($M^{-1}s^{-1}$) toward the R61 serine DD-peptidase, the following β-lactams stand as follows : benzylpenicillin : 14 000 ; cephalothin : 3 000 ; phenoxymethylpenicillin : 1 500 ; cepha-

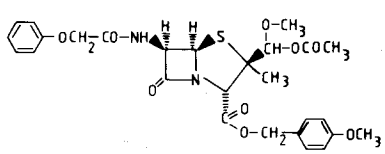
A] Classical penams



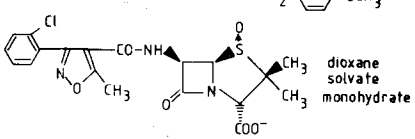
	R		
Benzylpenicillin	CH_2	potassium salt sodium salt procaine salt 1' diethylcarbonate esther	(8) (9) (10) (11)
Phenoxymethyl-penicillin	OCH_2	 methyl ester	(12) (13)
Ampicillin	CH(NH$_2$)		(14)
Amoxycillin	HO-C$_6$H$_4$-CH(NH$_2$)		(15)
Methicillin	2,6-(OCH$_3$)$_2$-C$_6$H$_3$	methyl ester	(16)
Oxacillin	3-(2-CH$_3$-5-CH$_3$-oxazolyl)-C$_6$H$_4$		(17)
Cloxacillin	3-(2-Cl-5-CH$_3$-oxazolyl)-C$_6$H$_4$	methyl ester	(18)
Dicloxacillin	3-(2-Cl-5-CH$_3$-oxazolyl)-2,6-Cl$_2$-C$_6$H$_3$	methyl ester a b	(19) (20)
Piperacillin	CH(NH-C(=O)-N(C$_2$H$_5$)-C(=O)-N(=O)-C$_6$H$_4$-O)		(21)

B] Non classical penams

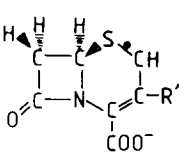
MBAPEN (22)



Cloxacillin sulfoxide — dioxane solvate monohydrate (23)



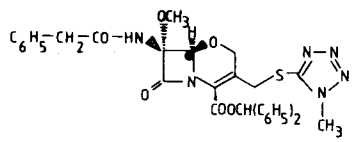
C] Classical Δ^3-cephems



	R	R'		
Cephalosporin C	HOOC-CH(NH$_2$)-(CH$_2$)$_2$-CH$_2$-	$-CH_2-O-COCH_3$	sodium salt	a (24) b (25)
Cephaloglycine	CH(NH$_2$)	$-CH_2-O-COCH_3$		(26)
Cephalothin	2-thienyl-CH_2	$-CH_2-O-COCH_3$		a (27) b (28)
Cephapyrine	N-pyridyl-S-CH_2	$-CH_2-O-COCH_3$ + HSO_4^-		(29)
Cephaloridine	2-thienyl-CH_2	$-CH_2-N^+$-pyridyl		(30)
TZACOL	2-thienyl-CH_2	$-S$-(1,2,4-triazolyl-CH$_3$)		a (31) b (32)
Cefmenoxime	2-(2-NH$_2$-thiazolyl)-C(=N-O-CH$_3$)	$-CH_2-S$-(1,2,4-triazolyl-CH$_3$)		a (33) b (34)
Cephazolin	1,2,3,4-tetrazolyl-CH_2	$-CH_2-S$-(1,3,4-thiadiazolyl-CH$_3$)	sodium salt	(35)

D] 7-α-methoxy-oxa-Δ^3-cephem

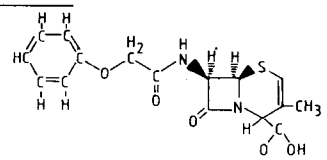
MOACPM



diphenylmethylester (36)

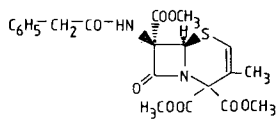
E] Δ^2-cephems

PODACE



(37)

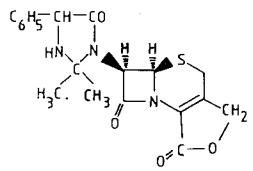
PAMXCP



(38)

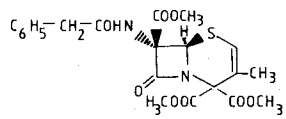
F] Bicyclic β-lactams : miscellaneous

IZCEPL (Δ^3-cephem)



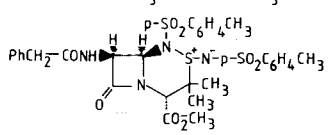
(39)

PACXCM (Δ^2-cephem)



(40)

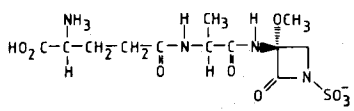
Pentos (β-lactam fused Ylide derivative)



(41)

G] Monobactam

Sulfazecin



(42)

FIG. 2. — *Selected β-lactam crystal structures.*

TABLE I. — *Relative energies (Kcal/mol) of conformers of the standard tripeptide. Ac_2-L-Lys^1-D-Ala^2-D-Ala^3 and analogues.*
A : Empirical method ; B : CNDO method.

Peptide / Conformer	Ac_2-L-Lys^1-D-Ala^2-D-Ala^3 (1)		L-Ala^1 analogue (2)		D-Leu^2 analogue (3)		D-Leu^3 analogue (4)		D-Lac^3 analogue (depsipeptide) (5)	
	A	B	A	B	A	B	A	B	A	B
a) BB*B*	0.0	2.61	0.43	0.0	0.0	17.22	2.52	14.59	0.28	0.0
b) EB*B*	1.01	0.0	0.92	1.65	1.70	2.51	3.375	0.0	1.83	3.50
c) BE*B*	0.37	3.60	0.56	2.32	0.93	0.0	1.35	16.44	0.0	4.33
d) EE*B*	0.11	6.49	0.0	3.99	1.11	2.88	0.0	19.27	0.15	6.95

Peptide / Conformer	L-Ala^2 analogue (6)	
	A	B
a) BBB*	0.33	0.0
b) EBB*	1.02	6.50
c) BEB*	0.0	3.60
d) EEB*	0.70	5.30

Peptide / Conformer	L-Ala^3 analogue (7) (*)	
	A	B
a) BB*B	0.0	0.0
b) EB*B	1.02	4.18
c) BE*B	0.45	2.65
d) EE*B	0.26	5.52

(*) Conformer EA*B (not mentionned in the table) has a relative energy of 0.53 (empirical method) or 3.57 (CNDO method).

The capital letters A, B and E correspond to the following $\varphi_i\psi_i$ conformational space ; Space A : φ from - 90° to 0° ; ψ : from - 90° to 0° ; Space B : φ from - 180° to - 90° ; ψ : from 90° to 180° ; Space E : φ from - 90° to 0° ; ψ : from 0° to 90°.

losporin C : 1 150 ; oxacillin : 130 ; ampicillin : 110 ; sulfazecin : 40 ; methicillin, cloxacillin and cephaloglycine : 15-30 (2,3).

Energy estimation

Using empirical conformational analyses (7), the energy values obtained for the tripeptides (depsipeptide) essentially reflect steric interactions. In this method, several highly probable conformers for each residue at positions 1, 2 and 3, considered as independent units, are first characterized and then these sets of conformers are recombined to select the peptide (depsipeptide) conformers of minimal energy both with regard to the backbone and the lateral chains. The energy of these most probable conformers has been also estimated by M.O. (molecular orbitals) calculation at the CNDO level (Complete Neglect of Differential Overlap : see [9]) and the same procedure has been applied to benzylpenicillin (**10**), cephapyrine (**29**), cephaloridine (**30**) and sulfazecin (**42**).

Dihedral angles

The dihedral angles used in the present study (Table 2 and Fig. 3) differ from angles φ_i, ψ_i and ω_i of Fig. 1 in that they involve groups of atoms not directly linked to each other.

In this context, the term « bond » may thus relate to unlinked atoms. Angles α, β, γ and δ define the relative orientation of the terminal anionic grouping at position R_1 with respect to atom O_6 at position R_2 (angles α and β) and with respect to atom O_1 at position R_1 (angles γ and δ) when looking along the scissile amide (ester) bond C_5-N(O)$_7$. In all cases, clockwise rotation is positive and atom O_{10} (O_9 in sulfazecin) is that oxygen atom at the R_3 position which has the smallest angle β absolute value.

Interplanar angles

The interplanar angles, Δ_1, Δ_2 and Δ_3, formed between the reference plane O_6-C_5-N(O)$_7$ and the planes C_5-N(O)$_7$-C_9, C_5-N(O)$_7$-O_{10} and C_5-N(O)$_7$-O_{11}, respectively, are equivalent to the dihedral angles α, $\beta(O_{10})$ and $\beta(O_{11})$. In turn, the interplanar angle Δ_4 formed between the same reference plane O_6-C_5-N(O)$_7$ and plane O_1-C_5-N(O)$_7$ is equivalent to the dihedral angle O_1-C_5-N(O)$_7$-O_6 (Fig. 3). It can be calculated by (α-γ) — 180°, or [$\beta(O_{10})$ — $\delta(O_{10})$] — 180°, or [$\beta(O_{11})$ — $\delta(O_{11})$] — 180° (examples : α = 28°, γ = — 169°, α-γ = 197°, Δ_4 = 17° ; α = 28°, γ = 170°, α-γ = — 142° or 218°, Δ_4 = 38° ; α = 12°, γ = — 163°, α-γ = 175°, Δ_4 = — 5°). Positive values of Δ_1 (or α), Δ_2 [or $\beta(O_{10})$] and Δ_3 [or $\beta(O_{11})$] indicate that atoms C_9, O_{10} and O_{11} are below the plane O_6-C_5-N(O)$_7$. Positive values of Δ_4 indicate that atom O_1 is above the same plane O_6-C_5-N(O)$_7$.

TABLE 2. — *Dihedral angles in peptides (**1** to **4** ; **6-7**), depsipeptide (**5**), bicyclic β-lactams (**8-41**) and monobactam sulfazecin (**42**).*

(Numbering of atoms is that shown in Fig. 1 ; see also Fig. 3).

Angles	Pairs of atoms considered in		
	Peptides and bicyclic β-lactams	Depsipeptide	Sulfazecin
α	O_6-C_5-N_7-C_9	O_6-C_5-O_7-C_9	O_6-C_5-N_7-S_8
$\beta(O_9)$			O_6-C_5-N_7-O_9
$\beta(O_{10})$	O_6-C_5-N_7-O_{10}	O_6-C_5-O_7-O_{10}	O_6-C_5-N_7-O_{10}
$\beta(O_{11})$	O_6-C_5-N_7-O_{11}	O_6-C_5—O_7-O_{11}	O_6-C_5-N_7-O_{11}
γ	O_1-C_5-N_7-C_9	O_1-C_5-O_7-C_9	O_1-C_5-N_7-S_8
$\delta(O_9)$			O_1-C_5-N_7-O_9
$\delta(O_{10})$	O_1-C_5-N_7-O_{10}	O_1-C_5-O_7-O_{10}	O_1-C_5-N_7-O_{10}
$\delta(O_{11})$	O_1-C_5-N_7-O_{11}	O_1-C_5-O_7-O_{11}	O_1-C_5-N_7-O_{11}

Molecular electrostatic potential maps

The electrostatic potential generated by the electron density (as estimated in the CNDO approximation) has been calculated according to approximation I (10). The maps show contours of equipotential values and localized electrostatic potential wells. The electrostatic potential at any calculated point of the maps represents the value, at the first order of perturbation, of the interaction energy of the molecule with a unitary point charge (i.e. a proton) (11).

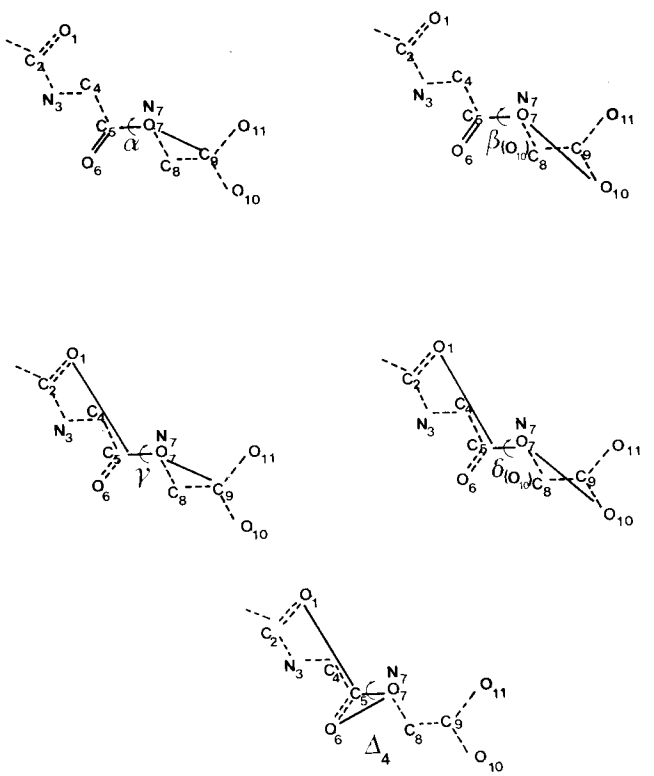


FIG. 3. — *Angles* α, $\beta(O_{10})$, γ, $\delta(O_{10})$ *and* Δ_4 *in peptides (depsipeptide) and bicyclic* β*-lactams.*

The above angles apply to sulfazecin, taking into account that the sequence N_7-C_8-C_9 (O_{11}, O_{10}) in the bicyclic β-lactams is N_7-S_8 (O_{11}, O_{10}, O_9) in sulfazecin.

Spatial disposition

An orthogonal system of coordinates has been used. Plane x, y corresponds to a section made through the plane O_6-C_5-$N(O)_7$ of the selected molecule and is qualified as level zero. Origin is C_5 ; axis x coincides with bond C_5-$N(O)_7$; axis y is close to bond C_5-C_4 ; and the coordinates of atom O_6 are negative values of x and y. The x and y coordinates have not been recorded as such since the positions of the functional groups within the reference plane are better expressed by the molecular electrostatic potential maps which take into account any steric effect that may influence the electronic environment of the molecules. Finally, axis z is perpendicular to plane O_6-C_5-$N(O)_7$ and refers to sections parallel to level zero. Both the values of the interplanar angles Δ_1, Δ_2, Δ_3 and Δ_4, and the direct spanning distances C_5-C_9, C_5-O_{10}, C_5-O_{11} and O_1-C_5, define the z coordinate of atoms C_9, O_{10}, O_{11} and O_1, respectively.

RESULTS

Relative energy of peptides (depsipeptide)

The relative energies of the most probable conformers, as calculated (Table 1) by the empirical method (Column A) and the CNDO procedure (column B), are expressed relative to that of the most stable conformer (or leader conformer) for which the energy is set to 0.0 kcal × mol^{-1}. The following observations can be made.

1) With peptides (depsipeptide) (**1**, **2**, **5**, **6** and **7**), the energy differences between the four conformers considered are small irrespective of the method used, but the leader conformer is not the same depending on the method.

2) As revealed by the CNDO method, the electronic perturbations caused by the bulky isopropyl side chain considerably destabilizes conformer BB*B* in the case of the D-Leu[2] analogue (**3**) and the three conformers BB*B*, BE*B* and EE*B* in the case of the D-Leu[3] analogue (**4**).

3) Replacement of an amide bond by an ester bond influences the conformational distribution patterns. The leader conformer selected by the CNDO method is conformer EB*B* for the tripeptide (**1**) and conformer BB*B* for the depsipeptide (**5**). Note that (**1**) and (**5**) have identical backbone conformations except that ψ_3 is 20° in (**1**) and 60° in (**5**) (for the other dihedral angles, see [7]).

Dihedral and interplanar angles

The values of the dihedral and interplanar angles are given in Table 3. The variation ranges observed with angles α, $\beta(O_{10})$, γ, $\delta(O_{10})$ and Δ_4 are also shown in the form of Newman projections (Fig. 4).

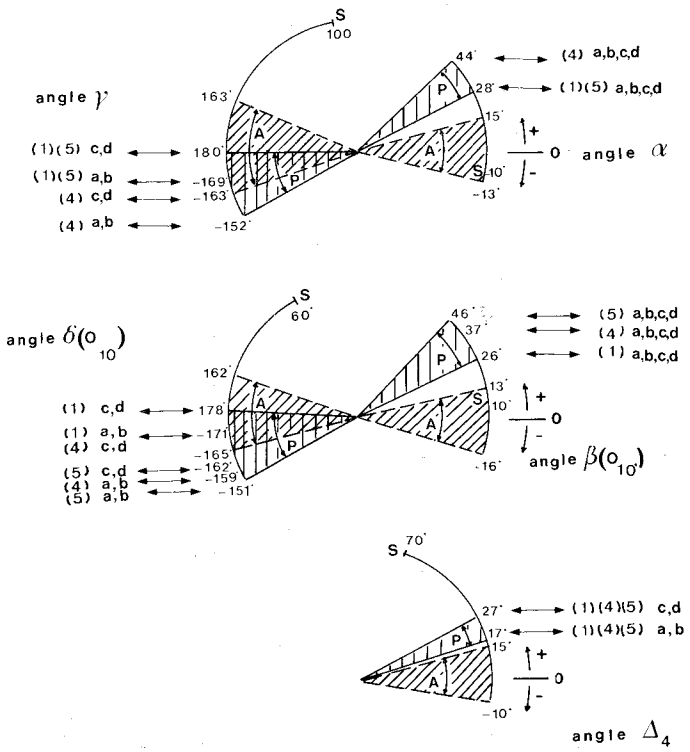


FIG. 4. — *Variations range (Newman projections) of the interplanar angle* Δ_4 *and angles* α, γ, $\beta(O_{10})$ *and* $\delta(O_{10})$ *[or* $\beta(O_9)$ *and* $\delta(O_9)$ *in sulfazecin].*

P = peptides (**1**), (**4**) and depsipeptide (**5**) ; A = bicyclic β-lactams ; S = sulfazecin. In sulfazecin, angles $\beta(O_9)$ and $\delta(O_3)$ (see Table 3) are equivalent to angles $\beta(O_{10})$ and $\delta(O_{10})$ in bicyclic β-lactams, respectively.

TABLE 3. — *Dihedral [α, β(O_{10}), β (O_{11}), γ, δ (O_{10}) and δ (O_{11})] and interplanar (Δ_1, Δ_2, Δ_3 and Δ_4) angles looking along bond C_5-$N(O)_7$; distances [$z(O_1)$, $z(C_9)$, $z(O_{10})$ and $z(O_{11})$] of atoms O_1, C_6, O_{10} and O_{11} to plane O_6-C_5-$N(O)_7$.*

Compound	α (Δ_1) (°)	β(O_{10}) (Δ_2) (°)	β(O_{11}) (Δ_3) (°)	γ (°)	δ(O_{10}) (°)	δ(O_{11}) (°)	Δ_4 (°)	$z(O_1)$ pm	$z(C_9)$ pm	$z(O_{10})$ pm	$z(O_{11})$ pm
Peptide (1) (a,b)	28	26	130	- 169	- 171	- 67	17	104	- 49	- 43	- 95
(c,d)	28	26	130	180	178	- 78	27	134	- 49	- 43	- 95
Peptide (2) (a,b)	28	26	130	- 169	- 171	- 67	17	104	- 49	- 43	- 95
(c,d)	28	26	130	180	178	- 78	27	134	- 49	- 43	- 95
Peptide (3) (a,b)	28	26	130	170	168	- 88	37	230	- 49	- 43	- 95
(c,d)	28	26	130	180	178	- 78	27	134	- 49	- 43	- 95
Peptide (4) (a,b)	44	37	86	- 152	- 159	- 110	17	104	- 123	- 163	- 187
(c,d)	44	37	86	- 163	- 170	- 121	27	134	- 123	- 163	- 187
Peptide (5) (a,b)	28	46	- 82	- 169	- 151	81	17	104	- 49	- 160	22
(c,d)	28	46	- 82	180	- 162	70	27	134	- 49	- 160	22
Peptide (6) (a)	28	26	130	- 136	- 138	- 34	- 17	- 104	- 49	- 43	- 95
(b)	28	26	130	- 169	- 171	- 67	17	104	- 49	- 43	- 95
(c)	28	26	130	180	178	- 78	27	134	- 49	- 43	- 95
(d)	28	26	130	- 125	- 127	- 23	- 27	- 134	- 49	- 43	- 95
Peptide (7) (a,b)	- 28	- 26	- 130	136	138	34	17	104	49	43	49
(c,d)	- 28	- 26	- 130	125	127	23	27	134	49	43	49
(8) BZPENK	- 1	- 1	66	178	178	- 115	1	4	15	6	- 53
(9) NABZPE	- 13	- 5	92	163	171	- 92	5	18	23	19	- 32
(10) PRPENG	12	0	56	- 163	- 176	- 120	- 4	- 28	- 34	0	- 123
(11) BENPEN	- 6	- 11	91	177	171	- 86	- 3	- 16	9	39	- 58
(12) PMEPEN	- 11	- 16	78	167	162	- 104	2	9	17	58	- 62
(13) MEPPAP	3	0	77	169	165	- 118	15	108	- 7	1	- 81
(14) AMCILL	- 1	- 14	31	- 177	170	- 145	- 4	- 23	3	73	- 116
(15) AMOXCT	8	- 6	42	- 171	174	- 137	- 1	- 5	- 26	30	- 147
(16) METHIC	2	- 7	43	175	166	- 144	7	50	- 5	37	- 125
(17) MPIXPS	4	8	109	180	- 176	- 75	- 4	27	- 7	- 29	- 43
(18) CMIPEN	- 7	- 9	88	180	178	- 84	- 7	- 44	11	35	- 47
(19) DCLOXL	3	5	61	- 175	- 174	- 117	- 2	- 10	- 7	- 20	- 49
(20) DCLOXL	- 8	- 7	105	- 178	- 177	- 64	- 10	- 63	13	27	- 33
(21) PIPCIL	0	- 15	33	180	165	- 147	0	0	- 1	75	- 118
(22) MBAPEN	- 1	- 13	64	- 179	169	- 114	- 2	- 14	2	49	- 91
(23) IPENSX	5	- 10	42	- 170	175	- 132	- 5	- 39	- 14	49	- 127
(24) CEPHNA	15	5	38	- 171	180	- 148	6	40	- 61	- 35	- 177
(25) CEPHNA	7	- 9	31	- 168	174	- 145	- 4	- 29	- 28	52	- 138
(26) CEPGLY	- 13	13	- 27	169	- 165	155	- 2	- 10	54	- 65	151
(27) CETHNA	5	- 10	28	- 174	171	- 152	0	- 3	- 22	57	- 133
(28) CETHNA	8	- 7	30	- 175	169	- 153	4	26	- 35	40	- 145
(29) CEPHAP	7	- 7	31	- 177	168	- 154	5	37	- 30	42	- 138
(30) CEPHHM	10	- 6	34	- 167	177	- 143	- 3	- 19	- 40	34	- 155
(31) TZACOL	9	- 1	35	- 173	177	- 147	2	15	- 37	7	- 153
(32) TZACOL	6	- 8	31	- 172	174	- 148	- 1	- 8	- 25	45	- 140
(33) CEFMEN	11	- 3	34	- 176	170	- 153	8	55	- 45	20	- 155
(34) CEFMEN	8	- 6	31	- 175	162	- 161	12	92	- 33	33	- 33
(35) CEPHNB	11	- 4	33	178	163	- 160	13	95	- 46	22	150
(36) MOACPM	1	- 5	19	168	162	- 174	13	93	- 4	28	84
(37) PODACE	50	34	83	- 127	- 144	- 94	- 2	- 16	- 129	- 142	- 214
(38) PAMCXP	- 10	5	- 37	157	172	130	13	94	42	- 29	171
(39) IZCEPL	- 8	1	- 17	102	111	93	69	252	34	- 3	83
(40) PACXCM	- 15	- 3	- 38	- 124	- 111	- 147	- 71	- 260	63	19	169
(41) PENTOS	15	0	31	125	110	142	69	270	- 62	- 1	- 149
	α	β(O_9)	β(O_{10}) (O_{11})	γ	δ(O_9)	δ(O_{10}) (O_{11})	Δ_4	$z(O_1)$	$z(S_8)$	$z(O_9)$	$z(O_{10})$ (O_{11})
(42) SULFAZ	- 10	10	30 - 50	100	120	140 60	70	261	21	- 44	163 - 41

The same geometric parameters are given for the monobactam sulfacezin **42**, taking into account that atoms S_8 , O_9 and O_{10} are equivalent to atoms C_9 , O_{10} and O_{11} in the bicyclic β-lactams. Angles are expressed in (°) and distances in pm (1 pm = 1×10^{-4} Å).

Angles β(O_{10}) and δ(O_{10}) have rather remarkably coherent values in the LDD peptides, on the one hand, and the bicyclic β-lactams, on the other. Angles β(O_{11}) and δ(O_{11}) (projections not shown but see Table 3) show much wider dispersions. The reason for this apparent discrimination between atoms O_{10} and O_{11} is that these oxygen atoms are not directly linked to bond C_5-$N(O)_7$ but are connected to it through the intervening C_8-C_9

sequence. This effect may be even more pronounced in the penams and cephems where the hybridization of atom C_8 is sp^3 and sp^2, respectively.

The β-lactams were crystallized either as such, in the form of various salts or as ester derivatives. Moreover, cases exist where two distinct conformers (qualified a and b in Fig. 2) occur in the same crystal unit. The question therefore arises which relates to the biological significance of the data thus obtained. Examination of Table 3 shows that : i) compounds (**8**, **9**, **10**) (benzylpenicillin salts) and (**11**) (an ester derivative of benzylpenicillin) ; ii) compounds (**12**) (phenoxymethylpenicillin) and (**13**) (an ester derivative of it) ; and iii) the two conformers of dicloxacillin (**19**, **20**), cephalosporin C (**24** and **25**), cephalothin (**27** and **28**), TZACOL (**31** and **32**) and cefmenoxime (**33** and **34**), respectively, are similar to each other with respect to the α, γ, $\beta(O_{10})$ and $\delta(O_{10})$ angle values. These similarities strongly suggest that the various coordinates, as they were calculated, are true features of the free β-lactam molecules.

In benzylpenicillin, conformer (**8**), angles α, $\beta(O_{10})$ and Δ_4 are — 1°, 1° and 1°, respectively. Hence, atom O_1, the triad O_6-C_5-N_7 and both atoms C_9 and O_{10} are almost coplanar (Fig. 5). Note that variations in the dihedral angles among the penams are not related to the puckering of the bicyclic fused ring system. However, the distances O_6-C_9 are longer in the C_3 conformers [0.435 nm in (**8**)] than in the S_1 conformers [0.390 nm in ampicillin (**14**)].

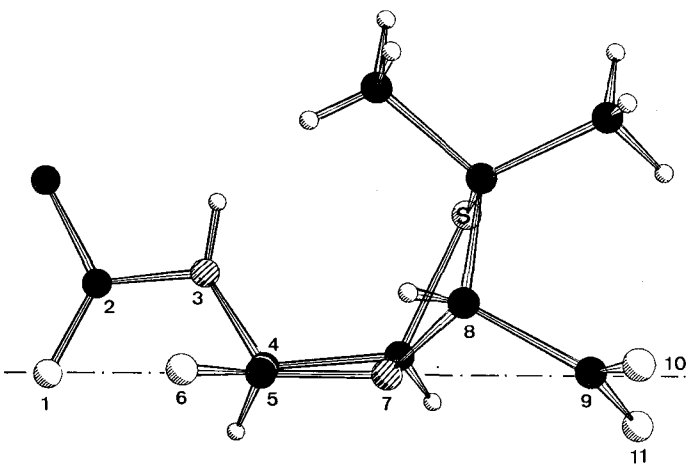


FIG. 5. — *Model of benzylpenicillin, C_3 puckered conformer* **8**.

In this molecule, angles α, $\beta(O_{10})$ and Δ_4 are — 1°, 1° and 1°, respectively. Hence, atoms O_1, O_6, C_5, N_7 (the other two carbon atoms of the β-lactam ring), C_9 and O_{10} are virtually coplanar. The spanning distances are : O_1-O_6 : 0.37 nm ; O_6-C_9 : 0.43 nm ; O_6-O_{10} : 0.50 nm.

Molecular electrostatic potential maps

A selection of the molecular electrostatic potential maps (at level zero ; see the Materials and Methods section) are shown in Figs 6 to 9. These maps relate to neutral molecules and therefore, one of the oxygen atoms, either O_{10} or O_{11}, at the R_3 position in the peptides (depsipeptide) has been substituted by a proton. Since this OH group may be *trans* or *cis* with respect to atom O_6, each of the conformers listed in Table 1 occurs in two forms. Fig. 6A and C, for example, show the maps generated by peptide (**1**), conformer BB*B*, in the *trans* and *cis* forms, respectively (note the effect on potential well n° 3).

Protonation of either O_{10} or O_{11} at the C-terminal position mimics the two limit cases of charge pairing which is thought to be part of the process of enzyme-peptide association. This process probably involves formation of a salt linkage between the carboxylate ion at position R_3 and the guanidinium cation of an Arg residue of the enzyme cavity (1,12). (Note that locating the proton at any intermediate position between the two carbonyl functions is equally arbitrary).

In benzylpenicillin and cephapyrin (Fig. 9A, B), protonation of the C-terminal carboxylate has been made on the basis of the crystallographic data, the longest C-O bond supposedly being protonated. With sulfazecin, the tautomeric form found in the crystal ($COOH$; NH_3^+ ; SO_3^-) is the most polarized one ; the SCF procedure does not converge in the CNDO approximation but convergence occurs with the two other forms : COO^- ; NH_3^+ ; SO_3H and $COOH$; NH_2 ; SO_3H (which is the form shown in Fig. 9D).

Cephaloridine (Fig. 9C) is unprotonated since the C-terminal carboxylate forms a zwitterion with the pyridinium substituent of the dihydrothiazine ring.

Spatial disposition of the functional groups at positions R_1, R_2 and R_3 in the peptides (depsipeptide) and β-lactams

For each of the parameters studied, variations are observed among the biologically active peptides (depsipeptide) (**1**, **4**, **5**) and bicyclic β-lactams (**8-36**). Inactive compounds may fall within these limits. Those which fall outside are specifically mentioned.

A) Peptides (depsipeptide)

The average spanning distances O_6-C_9, O_6-O_{10} and O_6-O_{11} are given in Table 4. On the basis of the α and $\beta(O_{10})$ values (Table 3 and Fig. 4), bonds $N(O)_7$-C_9 and $N(O)_7$-O_{10} extend below the reference plane O_6-C_5-$N(O)_7$. Atoms C_9 and O_{10} have z values ranging between — 0.049 and — 0.123 nm, and between — 0.043 nm and — 0.163 nm, respectively (Table 3). All the L-Ala[3] analogue conformers (**7**) fall outside these limits ; bonds N_7-C_9 and N_7-O_{10} extend above the reference plane [$\alpha \simeq \beta(O_{10}) \simeq -27°$].

The average spanning distances O_1-O_6, O_1-O_{10} and O_1-O_{11} in the extended (a,b) and folded (c,d) conformers are given in Table 4. On the basis of the Δ_4 values (Table 3 and Fig. 4), bond O_1-C_5 extends above the reference plane O_6-C_5-$N(O)_7$. Atom O_1 has a z value of + 0.104 nm in the extended conformers a, b and + 0.134 nm in the folded conformers c, d (Table 3). Conformers a, b of peptide (**3**) fall outside these limits (z = + 0.230 nm). In conformers a, d of peptide (**6**), bond O_1-C_5 extends below the reference plane.

Unless bulky side chains exert some masking effect (see below), the potential wells generated at positions

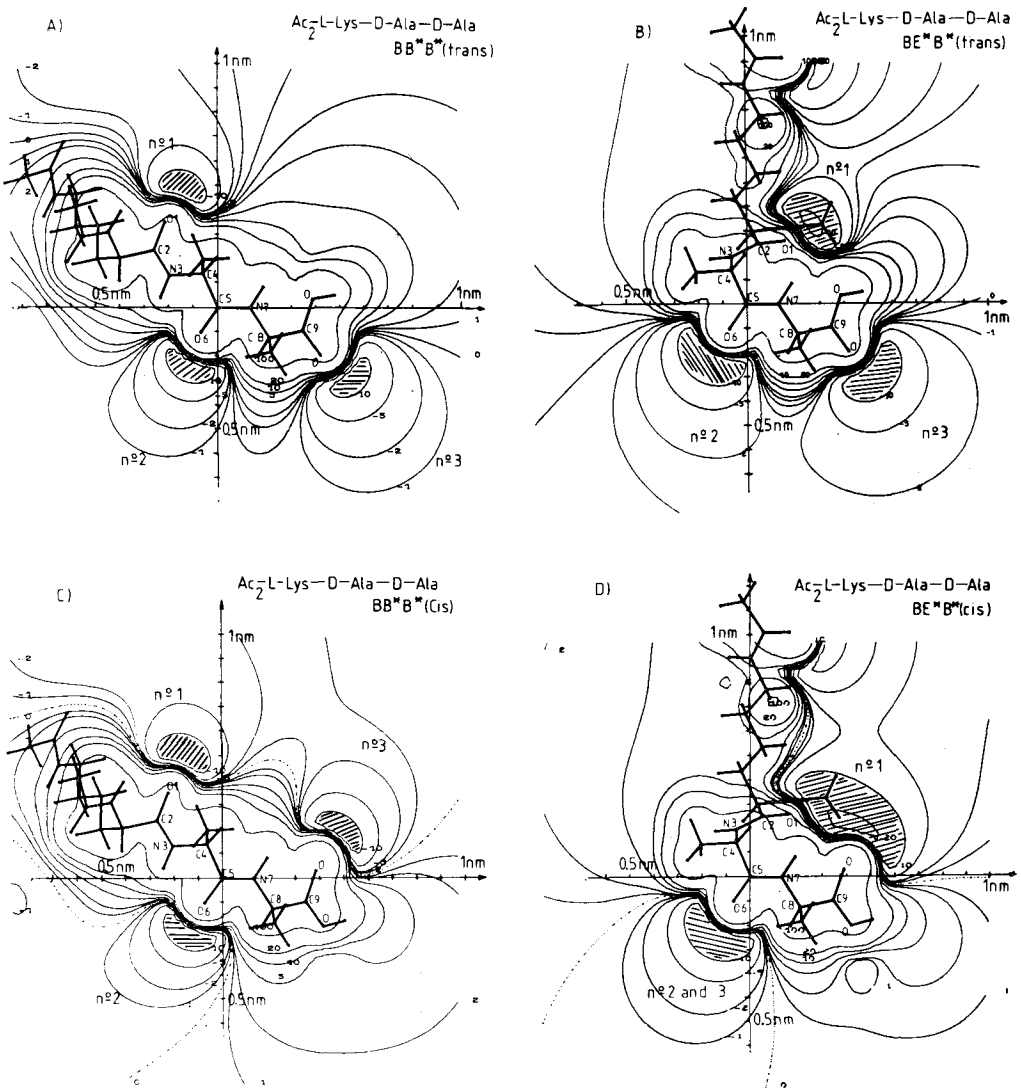


FIG. 6. — *Potential electrostatic maps at level zero of Ac_2-L-Lys^1-D-Ala^2-D-Ala^3 inconformations BB*B* and BE*B*.*
(cis) and *(trans)* refer to the orientation of the OH group at position R_3 . Shadowed areas show negative potential holes of - 10 Kcal/mol or less.

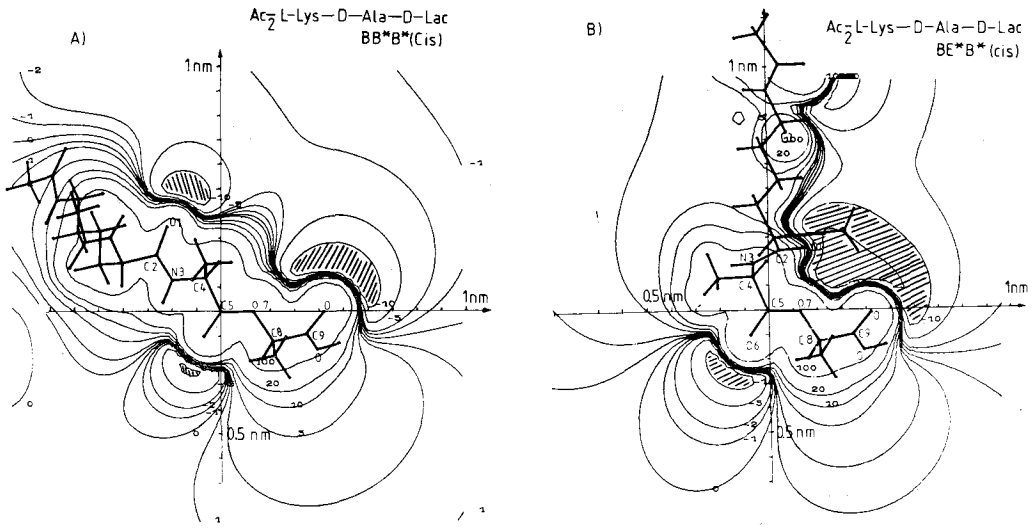


FIG. 7. — *Potential electrostatic maps at level zero of Ac_2-L-Lys^1-D-Ala^2-D-Lac- in conformations BB*B* and BE*B*.*
(cis) refers to the orientation of the OH group at position R_3.

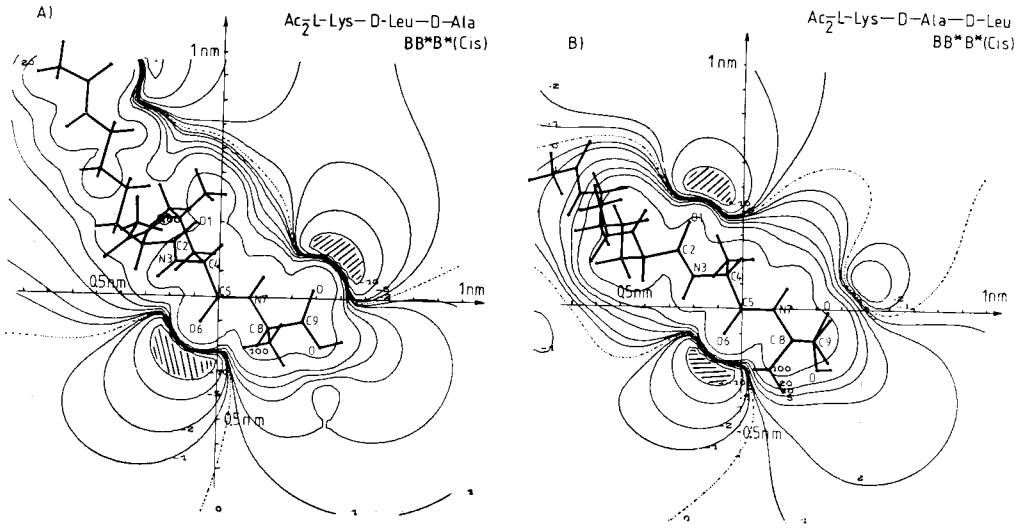


FIG. 8. — *Potential electrostatic maps at level zero of Ac_2-L-Lys^1-D-Leu^2-D-Ala^3 in conformation BB*B*. and Ac_2-L-Lys^1-D-Ala^2-D-Leu^3 in the same conformation BB*B*.*

(cis) refers to the orientation of the OH group at position R_3 .

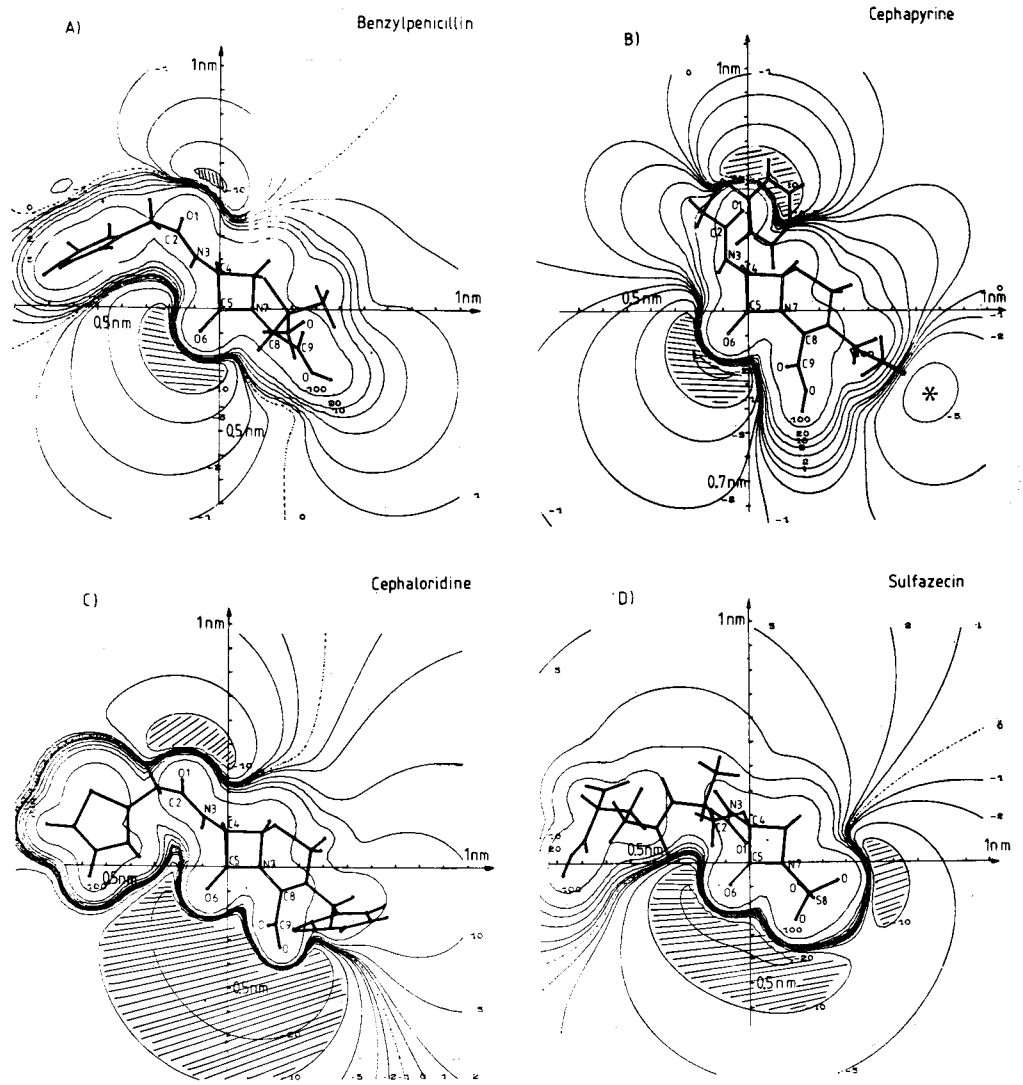


FIG. 9. — *Potential electrostatic maps at level zero of benzylpenicillin, cephapyrine, cephaloridine and sulfazecin (form COOH ; NH_2 ; SO_3H).*

TABLE 4. — *Average spanning distances (nm) between pairs of atoms.* (Standard deviation values are given into parentheses).

	Pairs of atoms (Fig. 2e)	Peptides (depsipeptide) (1)-(7) (Table 2)	β-Lactams (8)-(23) (Fig. 5)	β-Lactams (24)-(41) (Fig. 5)	Sulfazecin (42) (Fig. 5)
R_1-R_2	C_5-C_9	All conformers 0.364 (0.007)	0.370 (0.0095)	0.319 (0.013)	
	C_5-S_8				0.2795
	O_6-C_9	All conformers 0.420 (0.013)	0.426 (0.022)	0.331 (0.024)	
	O_6-S_8				0.335
	O_6-O_{10}	0.507 (0.017)	0.503 (0.016)	0.407 (0.030)	
	O_6-O_{11}	All conformers 0.485 (0.014)	0.473 (0.038)	0.314 (0.011)*	
	O_6-O_9				0.454
	O_6-O_{10}				0.388
	O_6-O_{11}				0.315
R_1-R_2	O_1-C_5	conformers a,b 0.422 (0.013) conformers c,d 0.332 (0.0)	0.401 (0.022)	0.417 (0.010)**	0.2785
	O_1-O_6	conformers a,b 0.484 (0.008) conformers c,d 0.448 (0.0)	0.458 (0.044)	0.501 (0.017)**	0.326
R_1-R_3	O_1-C_9	conformers a,b 0.725 (0.029) conformers c,d 0.442 (0.0225)	0.7095 (0.030)	0.702 (0.020)**	
	O_1-S_8				0.420
	O_1-O_{10}	conformers a,b 0.848 (0.025) conformers c,d 0.571	0.823 (0.033)	0.802 (0.020)**	
	O_1-O_{11}	conformers a,b 0.701 (0.036)	0.701 (0.042)	0.697 (0.036)**	
	O_1-O_9				0.517
	O_1-O_{10}				0.496
	O_1-O_{11}				0.378

* For compound **37**, O_6-O_{10} is 0.502 and O_6-O_{11} is 0.436. These values are similar to those found in the penams **8-23**.

** For compounds **39**, **40** and **41**, the distances are :

	(39)	(40)	(41)
O_1-C_5	0.2775	0.274	0.289
O_1-O_6	0.307	0.323	0.331
O_1-C_9	0.502	0.492	0.521
O_1-O_{10}	0.594	0.560	0.586
O_1-O_{11}	0.5305	0.543	0.562

Note that normal distances occur in the diastereomer PAMXCP **(38)** where in contrast to PACXCM **(40)**, the β-lactam substituents have the same orientation as that found in the active cephamycins.

R_1 (well n° 1), R_2 (well n° 2) and R_3 (well n° 3) of protonated peptides are clearly visible in the x, y sections made at level zero. Maps of the type shown in Fig. 6A and C are generated by peptides (**1**, **2**, **6** and **7**) in the extended conformation (B or E) B*B*, (B or E) BB* or (B or E) B*B. Well n° 3, however, is located differently depending on whether the OH group at position R_3 is *trans* (Fig. 6A) or *cis* (Fig. 6C). With conformers (B or E) E*B*, (B or E) EB* and (B or E) E*B whose peptide backbone has a tendency to fold back on itself, well n° 1 is generated on the right side of axis y. Depending on whether the OH group at position R_3 is *trans* or *cis*, it appears as a distinct entity (Fig. 6B) or is fused with well n° 2 (Fig. 6D).

The maps shown in Fig. 6 A-D are generated by conformers BB*B* or BE*B* of Ac_2-L-Lys-D-Ala-D-Ala ; residue at position 1 is in the B conformation. A shift to the E conformation for this residue has little effect on the electronic environment (not shown). Similarly, replacement of the scissile amide bond in the tripeptide Ac_2-L-Lys^1-D-Ala^2-D-Ala^3 by an ester bond in the depsipeptide Ac_2-L-Lys^1-D-Ala^2-D-Lac^3 has also little effect on the electronic environmemt of the corresponding molecules (in the same conformations) (compare Fig. 7A, B with Fig. 6C, D). However, well n° 2 is shallower and well n° 3 is deeper in the depsipeptide than in the tripeptide.

Bulky side chains, such a the leucine isopropyl group, may modify the electrostatic potential maps. The effect however, depends on the backbone conformation of the peptide. Thus, with the extended (B or E) B*B* backbone conformation, well n° 1 completely disappears in the D-Leu^2 analogue (compare Fig. 8A with Fig. 6C) and well n° 3 almost completely disappears in the D-Leu^3 analogue (compare Fig. 8B and Fig. 6C). On the contrary, with a folded (B or E) E*B* backbone conformation, replacement of D-Ala by D-Leu at position 2 or 3 of the tripeptide (**1**) has little effect on the electrostatic maps (not shown) which, essentially, remain very similar to that of Fig. 6B and D.

B) Bicyclic β-lactams (**8-38**)

The unusual compounds (**39-41**) are excluded from the present analysis.

The average spanning distances O_6-C_9, O_6-O_{10} and O_6-O_{11} in the penams and penam-like β-lactams (**8-23**) are similar to those found in the peptides (Table 4). They are significantly shorter in the cephems and cephem-like β-lactams (**24-38**) (Table 4). On the basis of the α and β(O_{10}) values (Table 3 and Fig. 4) (excluding the Δ^2-cephem **37**), bonds N_7-C_9 and N_7-C_{10} extend below, above or within the reference plane O_6-C_5-N_7. Atoms C_9 and O_{10} have z values ranging between + 0.054 and — 0.061 nm, and between + 0.075 and — 0.065 nm, respectively (Table 3).

The average spanning distances O_1-O_6, O_1-O_{10} and O_1-O_{11}, respectively, are very similar in all bicyclic β-lactams but longer than in the peptides (Table 4). On the basis of the Δ_4 values (Table 3 and Fig. 4), bond O_1-C_5 extends either below, above or within the reference plane O_6-C_5-N_7. Atom O_1 has z values ranging between + 0.108 and — 0.063 nm (Table 3).

The molecular electrostatic maps of the three bicyclic β-lactams examined lack well n° 3.

With benzylpenicillin (Fig. 9A), this situation is due to the masking effect exerted by the thiazolidine ring and the two methyl substituents on C_2. However, a well of about — 8 Kcal/mol is generated at level — 0.1 nm (x = 0.45 nm ; y = 0.06 nm). With the two cephalosporins (Fig. 9B and C), the value of the backbone dihedral angle φ_3 (44° instead of 139° in the penams and 155° in the peptides) forces well n° 3 to become fused with well n° 2, thus possibly creating a very large zone of electrostatic potential which is enhanced in the zwitterion cephaloridine. Note that the acetoxy group on C_3 of the dihydrothiazine ring in cephapyrine generates an additional potential well which is hardly significant at level zero (and indicated

by a star in Fig. 9B) but is — 15 Kcal/mol at level + 0.1 nm.

C) The monocyclic β-lactam sulfazecin

The spanning distance O_6-S_8 is the same as the corresponding distance O_6-C_9 in the cephems and the spanning distances O_6-O_9 and O_6-O_{10} are intermediate to the corresponding distances O_6-O_{10} and O_6-O_{11} found in the penams and Δ^3-cephems, respectively (Table 4). Angles α (— 10°) and β(O_9) (10°) fall within the limits of angles α and β(O_{10}) observed with the bicyclic β-lactams (Table 3 ; Fig. 4).

The spanning distances O_1-O_6 (0.33 nm), O_1-O_9 (0.52 nm) and O_1-O_{10} (0.50 nm) are shorter than in any other compound studied (Table 4). In parallel to this, angle Δ_4 (70°) is unusual (Table 3 ; Fig. 4). Bond O_1-C_5 extends above the reference plane O_6-C_5-N_7 and atom O_1 has a z value of + 0.26 nm (Table 3).

Sulfazecin has no additional ring that could mask the sulphamate group. But it has a complex side chain (see **42**) where, in addition to the COOH-CH-NH_2 terminal grouping, two amide functions occur with their carbonyl groups oriented toward position R_2. As a consequence, a very large zone of electrostatic potential surrounds the β-lactam ring by more than 180° along the negative branch of axis y. In the molecular form shown in Fig. 9D, this zone contains two minima ; in the other form, COO^- ; NH_3^+ ; SO_3H, these two minima are fused to each other (not shown). No potential well n° 1 is seen in Fig. 9D.

DISCUSSION

Looking at models of peptides (depsipeptide) and bicyclic β-lactams, one sees, well exposed on the α-face of these molecules, the scissile amide (ester) bond C_5-$\overset{\overset{O_6}{\|}}{N(O)_7}$ at the central position R_2, flanked by two important functional groups. The carboxylate function $C_9\begin{matrix}\diagup O_{11}\\ \diagdown O_{10}\end{matrix}$ at the C-terminal position R_3 is probably involved in charge pairing with a cationic side chain of the enzyme cavity (1,12). The amide C_2-$\overset{\overset{O_1}{\|}}{N_3}$ function at position R_1 bears a substituent whose structure, orientation, electrical charge, etc. is crucial (in most cases) for substrate activity or inactivating potency (3). The present study has therefore been focused on these three functional groups.

The average spanning distances between homologous pairs of atoms, in particular the distances O_1-O_6 , O_1-C_9 (O_{10}) and O_6-C_9(O_{10}), are very similar in the extended peptide conformers and in the penicillins and cephalosporins. In the folded peptide conformers, at least the distance O_6-C_9(O_{10}) is the same as in the other compounds studied.

With respect to the angles that the « bonds » $N(O)_7$-C_9 , $N(O)_7$-O_{10} and O_1-C_5 form with the reference plane C_5-$\overset{\overset{O_6}{\|}}{N(O)_7}$, the active peptides (depsipeptide) and the bicyclic β-lactams fall into two groups that do not overlap [see angles α, β(O_{10}) and Δ_4 , respectively, in Fig. 4]. In the β-lactams, coplanarity is within a ± 15° variation range whereas in the peptides (depsipeptide) bonds $N(O)_7$-C_9 (see angle α) and $N(O)_7$-O_{10} [see angle β(O_{10})] definitely extend below and bond O_1-C_5 (see angle Δ_4) extends above the reference plane. As a result of the combining effect of the spanning distances and interplanar angles, the z values for atom O_1 vary from + 0.230 to + 0.104 nm in the biologically active peptides (depsipeptide) and from + 0.108 to — 0.063 nm in the biologically active bicyclic β-lactams. The corresponding z values for atom O_{10} range between — 0.043 to — 0.163 nm, and from + 0.075 to — 0.065 nm.

The molecular electrostatic potential maps well reflect the x, y coordinates of the carbonyl groups at position R_1 , R_2 and R_3. The wells 1, 2 and 3 thus generated create large zones of electrostatic potential around the common sequence O_1-C_2-N_3-C_4-C_5-$N(O)_7$-C_8-$C_9\begin{matrix}\diagup O\\ \diagdown OH\end{matrix}$ and can be inscribed in a 1.2 nm^2 square that intersects axis x at — 0.4 and + 0.8 nm and axis y at + 0.6 and — 0.6 nm.

In the monobactam sulfazecin (the only monocyclic β-lactam antibiotic for which the crystallographic data are available at this time), bonds O_6-S_8 and O_6-O_9 are equivalent to bonds O_6-C_9 and O_6-O_{10} in the bicyclic β-lactams both in terms of spanning distances and relative orientations with respect to plane C_5-$\overset{\overset{O_6}{\|}}{N_7}$. Moreover, a large electrostatic potential surrounds the β-lactam ring and its sulphamate substituent by more than 180° (along the negative branch of axis y). However, the presence of a very unusual 3-β-amido substituent results in an « abnormal » geometry in the R_1-R_2 part of the molecule and prevents any potential well from being expressed along the positive branch of the y axis (in the level zero section).

The selected set of peptide and β-lactam conformers have common reactive features around the carbonyl of the scissile bond (well n° 2). But, depending on which of the oxygen atoms has been protonated, the conformation of the peptide backbone, the presence of bulky side chains, the type of bicyclic (or monocyclic) framework in the β-lactams and the presence of ionized or electron-withdrawing substituents, potential wells n° 1 and/or n° 3 may be modified, displaced along the x or y axes, fused together or with well n° 2, eliminated or better expressed in another section of the molecule along axis z.

The relative spatial disposition and strength of the positive and negative potentials form a pattern which is characteristic of each molecule in a certain geometric conformation. This pattern is an important feature (among others ; see the Introduction) that governs the orientation of the whole molecule within the enzyme's active site and its reactivity towards the catalytically important side chains. The widely varying patterns exhibited by the $L^1D^2D^3$

peptides and β-lactams strongly suggest multiple modes of binding leading to enzyme-ligand associations of highly varying productiveness. This conclusion is in agreement with the well-documented observations that : i) the catalytic efficiency of the DD-peptidases on the peptides largely depends on the structure of the side chain at position R_1 (3) ; ii) the inactivating potency of the β-lactams largely depend on the structure of the amide substituent at the same position R_1 (3) ; and iii) a substituent at position R_1 that confers high substrate activity to a peptide does not confer inactivating potency when substituting a bicyclic fused ring azetidinone structure, and vice versa (13). The R61 serine DD-peptidase has been crystallized. Study of the high-resolution structure of this enzyme is in progress (14) and should lead to a detailed understanding of the various parameters involved in the enzyme-substrate and enzyme-inactivator interactions.

Acknowledgements. — The work was supported by an *Action Concertée* from the Belgian Government (convention 79/84-I1) and the *Fonds de la Recherche Scientifique Médicale*, Brussels, Belgium (contract n° 3.4507.83). G.D. is *Chercheur qualifié* of the *Fonds de la Recherche Scientifique*, Brussels.

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