THEORETICAL STUDIES ON β-LACTAM ANTIBIOTICS

III. Conformational Flexibility of the Five Membered Ring in Clavulanic Acid and Penicillin and the Binding Specificity of β-Lactamases

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ABSTRACT

The possible conformations of clavulanic acid and penicillin were analysed by semiempirical potential energy calculations. The oxazolidine ring of clavulanic acid favours only one type of puckered conformation. On the other hand, the thiazolidine ring of penicillin favours two types of puckered conformations similar to those observed in ampicillin and penicillin G or V in the solid state. The present study predicts that both these conformations can coexist in solution in agreement with the NMR results. Comparison of the possible conformations of penicillin with that of clavulanic acid indicates that the C_3 puckered conformation of the thiazolidine ring (as in penicillins G or V in the solid state) is important for the binding of penicillin with β-lactamases.

INTRODUCTION

PENICILLIN is one of the widely used antibiotics to combat many bacterial diseases. However, in recent years its use has been limited by the emergence of resistant bacteria. These strains achieve their resistance mainly by producing β-lactamases which inactivate the antibiotic by hydrolysing the lactam peptide bond before the antibiotic reaches the target enzyme (transpeptidase).

Recently we have studied the binding specificity of transpeptidase by theoretical methods and have shown that for optimal binding with the enzyme, the aminoacyl and the carboxyl groups should assume specific conformations[1-3]. For the binding specificity of β-lactamases, such an information is not available. Recent observation that clavulanic acid is a potent inhibitor of various β-lactamases indicates that to bind with β-lactamases the aminoacyl group of the antibiotic is not as essential as the lactam ring and the carboxyl group. Since the carboxyl group of penicillin can assume different orientations due to different modes of puckering of the thiazolidine ring[4] it is not known which of these forms is essential for binding with β-lactamases. In order to understand this, a detailed analysis of the conformational flexibility of the five membered ring in clavulanic acid and penicillin was made by semiempirical potential energy calculations, and, the results are presented in this paper. These studies are important for the design of a suitable β-lactam compound active against resistant bacteria.

METHODS

Nomenclature

The numbering of the atoms and the dihedral angles in clavulanic acid and penicillin are shown in Fig. 1. R_1 and R_2 (Fig. 1) were treated as methyl groups for calculational simplicity. The torsional angles α_1, α_2 and α_3 (in clavulanic acid) specifies the relative orienta-
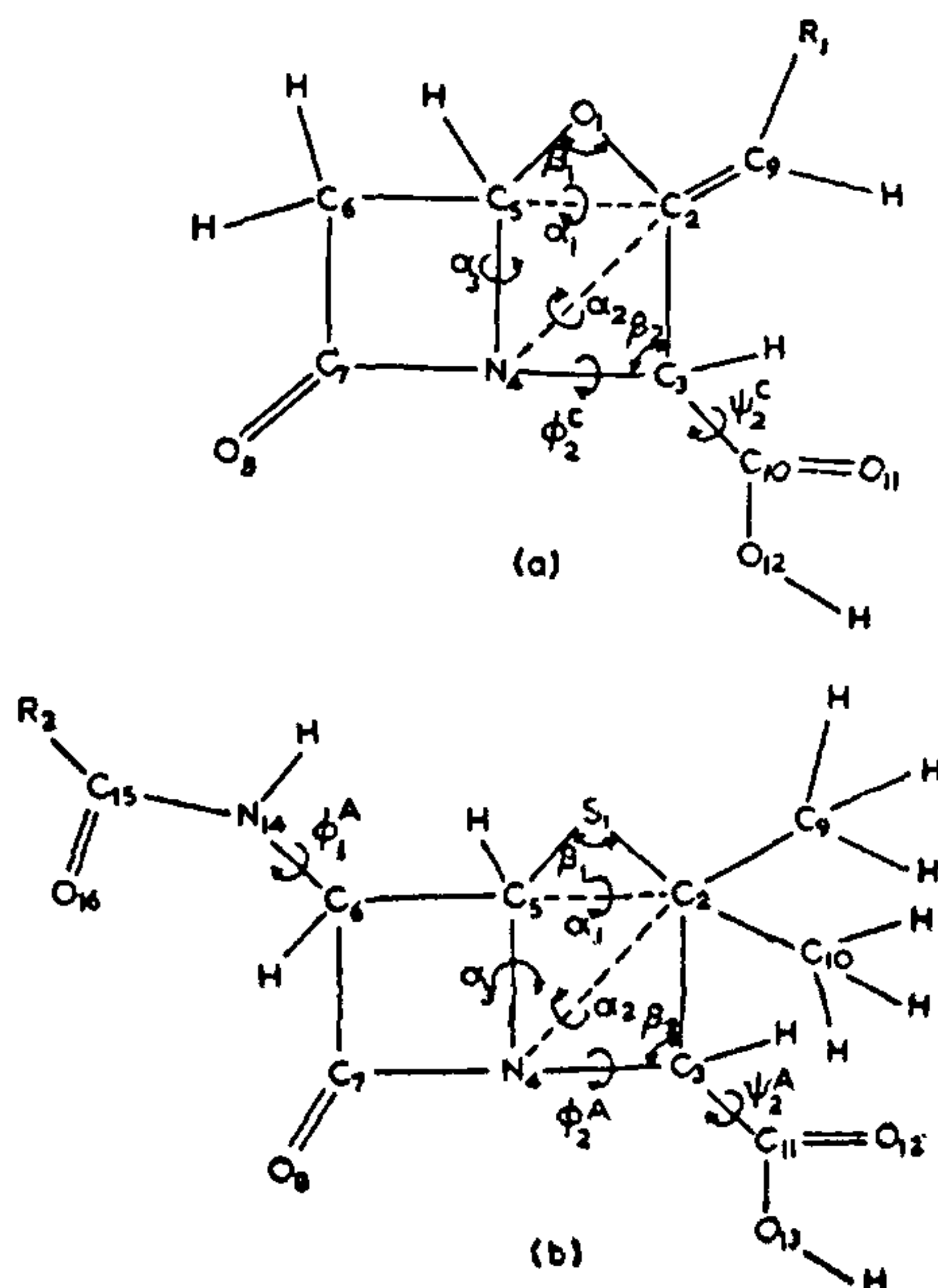

FIG. 1. Numbering of atoms and dihedral angles in (a) clavulanic acid and (b) penicillin.

tion of the planes (C_5, O_1, C_2), (C_2, C_3, N_4) and (N_4, C_5, C_6, C_7) respectively with respect to the reference plane (C_2, N_4, C_5). Hence in this notation α_1 and α_2 define the conformation of the five membered ring and α_3 defines its orientation with respect to the lactam ring. In the initial conformation ($\alpha_1 = \alpha_2 = \alpha_3 = 0$) all the ring atoms ($O_1$, C_2, C_3, N_4, C_5, C_6 and C_7) are in one plane as in Fig. 1 a. Clockwise rotation about the virtual or real bonds (indicated by arrows) looking

along C_5-C_2, C_2-N_4 and N_4-C_6 is considered as positive for the dihedral angles a_1, a_2 and a_3. The definition of the torsional angles (q_2^c, ψ_2^c) which define the orientation of the carboxyl group is according to the IUPAC-IUB nomenclature. Similar definition holds good for penicillin also. The superscript ' c ' in (q_1^c, ψ_1^c) refers to clavulanic acid. Similarly ' A ' in (a_1^A, ψ_1^A) refers to the antibiotic penicillin.

Energy Calculations

The total conformational energy (V_{TOT}) of a molecule can be expressed as

$$V_{TOT} = V_{NB} + V_{ES} + V_\theta + V_{TOR}$$

where

V_{NB} is the non-bonded interaction energy,

V_{ES} is the electrostatic interaction energy,

V_θ is the energy due to bond angle strain and

V_{TOR} is the torsional potential energy.

The form of the functions and the parameters used to calculate V_{NB}, V_{ES}, V_θ and V_{TOR} are described elsewhere[5-7].

Since our main aim is to study the flexibility of the five membered ring, the lactam ring was fixed in a planar conformation[4,8-12] and the energy of the molecule was calculated as a function of (a_1, a_2). These dihedral angles were varied in the range $-60°$ to $+60°$ *at 10° intervals and at each grid point, the energy* was minimized with respect to (a_3, β, β_2) in clavulanic acid and (a_3, q_1^A, β_1, β_2) in penicillin. In both these compounds ψ_2^c or ψ_2^A was fixed at 30° which is the preferred value in simple peptides[2,3]. Iso-energy contours of these molecules on the (a_1, a_2) plane are shown in Figs. 2 and 3. The projection diagrams
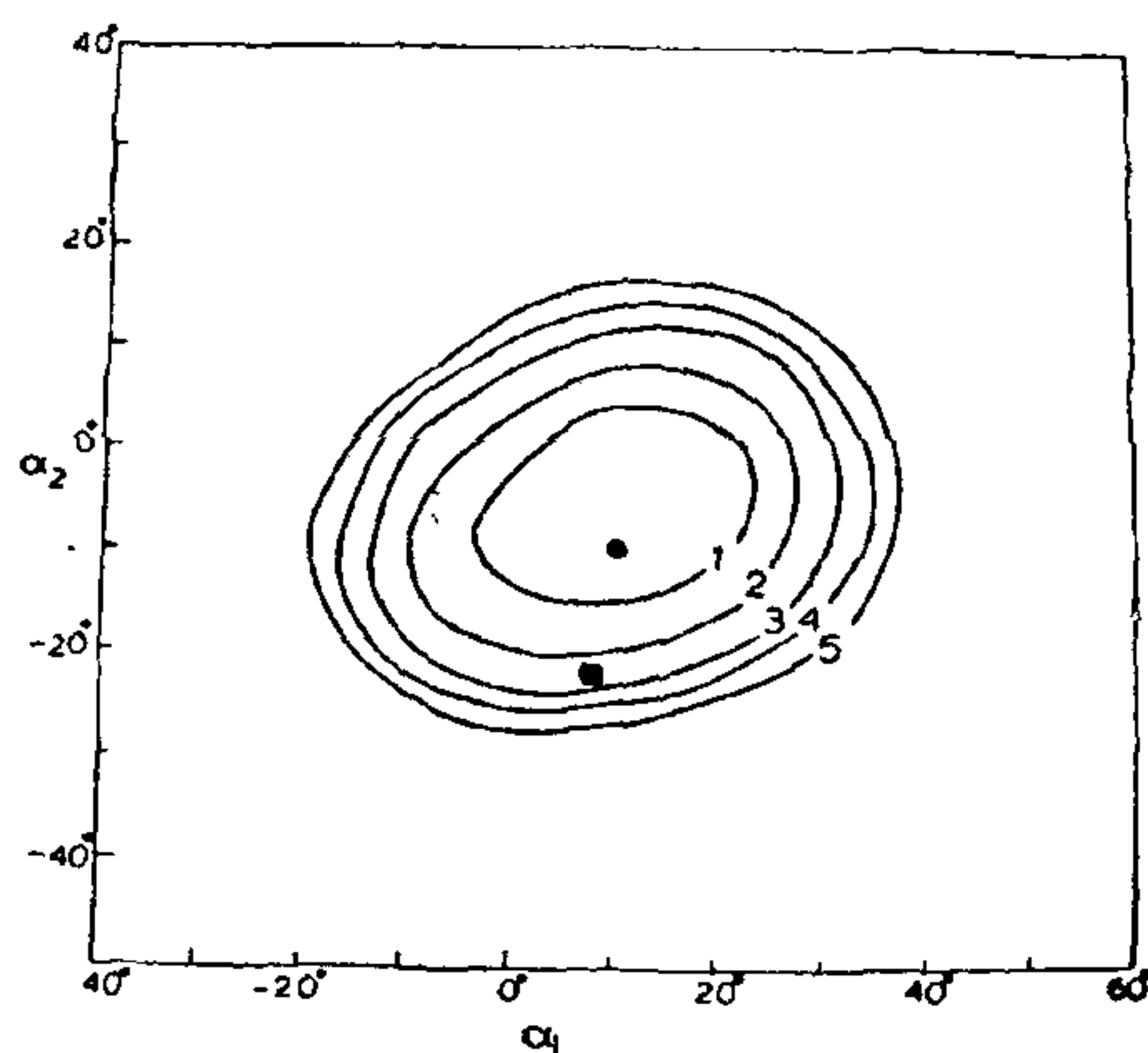
FIG. 2. Iso-energy contours for clavulanic acid in kcal. mole⁻¹. ●—Global minimum; ■—solid state conformation.
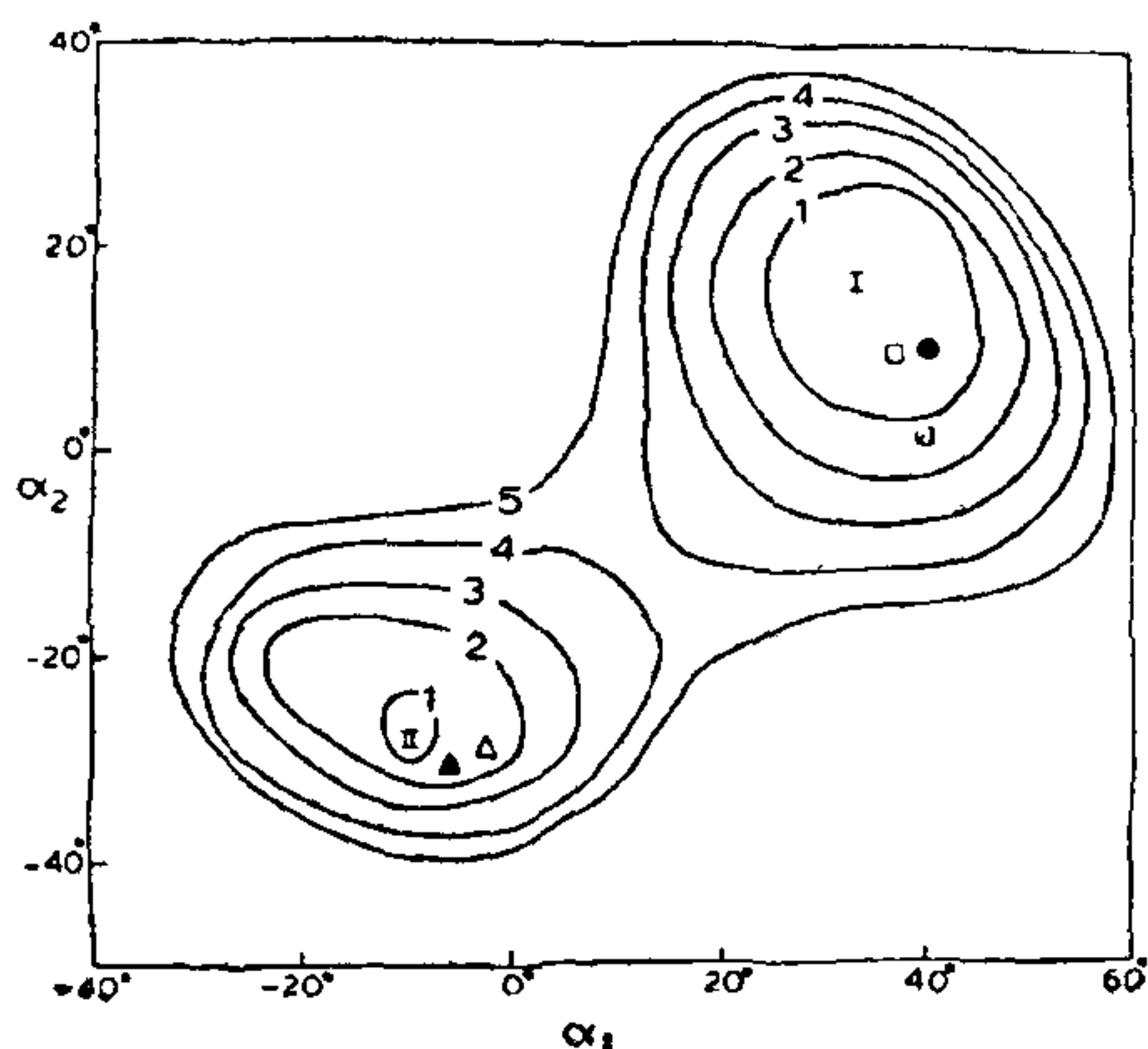
FIG. 3. Iso-energy contours for penicillin in kcal. mole⁻¹. In region I the thiazolidine ring is in the C_2 puckered conformation and in region II it is in the C_3 puckered conformation. ●—Global minimum. Solid state conformations of the thiazolidine ring in ampicillin trihydrate (■); ampicillin anhydrate (□); penicillin V (▲) and penicillin G (△).

of the minimum energy conformers are shown in Fig. 4 a, b, c. The values of a_3, q_2^c or q_2^A corresponding to the minimum energy and solid state conformations are given in Table I.

TABLE I

Calculated and crystal structure values of a_3 and q_2^c or q_2^A

Molecule	Relative orientation of the bicyclic ring system (180-a_3)		Orientation of the carboxyl group (q_2^c or ϕ_2^A)	
	Calcu-lated[a]	Experi-mental[b]	Calcu-lated[a]	Experi-mental[b]
Clavulanic acid	122°	120°	148°	161°
Penicillin (C₃ puckered)	143°	147°	111°	113°
Penicillin (C₃ puckered)	113°	119°	161°	158°

a—Corresponding to the minimum energy conformations.

b—Data taken from References 4, 9 and 12.

RESULTS AND DISCUSSION

Figure 2 shows a single minimum for the oxazolidine ring of clavulanic acid indicating that the ring is highly rigid and favours only one type of conformation. The energy minimum occurs at (a_1, a_2) = (10°, $-10°$).
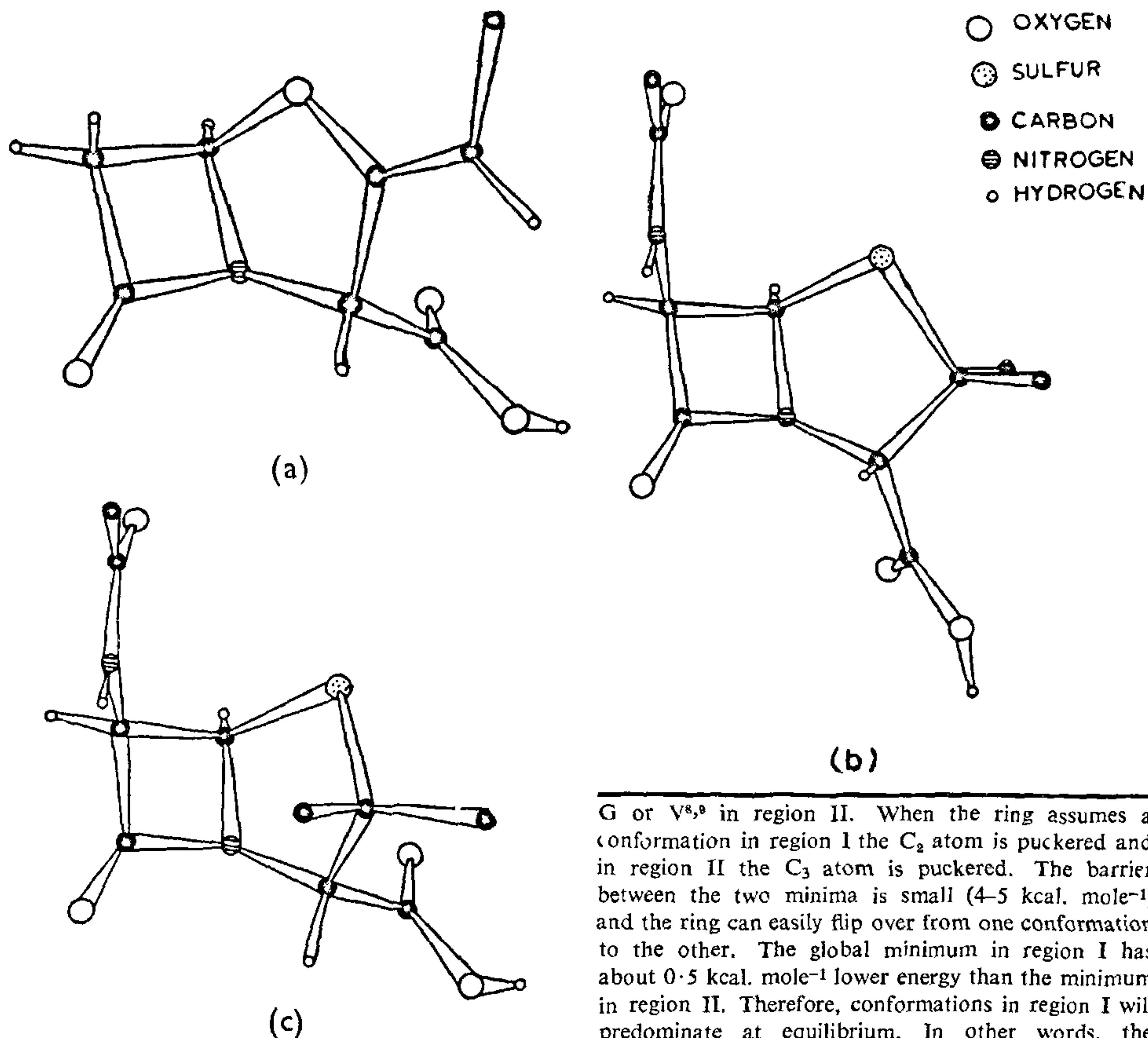
O OXYGEN
⊚ SULFUR
● CARBON
⊖ NITROGEN
o HYDROGEN

(a)

(b)

(c)

FIG. 4. Projection diagrams of clavulanic acid (a), and penicillin in the C_2 (b) and C_3 (c) puckered conformations.

In the solid state the ring assumes a conformation around ($10°$, $-20°$) and is very close to the energy minimum. However, the solid state conformation has about 2 kcal. mole^{-1} higher energy than the energy minimum which may be compensated by lattice energy in the crystal. The relative orientations of the two rings (a_3) and the orientation of the carboxyl group (ϕ_2^c) as predicted from theory are in agreement with the crystal structure results (Table I).

Figure 3 shows two minima for the thiazolidine ring of penicillin. The global minimum occurs at (a_1, a_2) = ($40°$, $10°$) in region I and the local minimum at ($-10°$, $-25°$) in region II. The solid state conformations of the thiazolidine ring as observed in ampicillins[4,10] fell in region I and that of penicillin

G or V[8,9] in region II. When the ring assumes a conformation in region I the C_2 atom is puckered and in region II the C_3 atom is puckered. The barrier between the two minima is small (4–5 kcal. mole^{-1}) and the ring can easily flip over from one conformation to the other. The global minimum in region I has about 0.5 kcal. mole^{-1} lower energy than the minimum in region II. Therefore, conformations in region I will predominate at equilibrium. In other words, the population of the C_2 puckered conformation will be higher than that of the C_3 puckered conformation in solution. This agrees with the recent NMR studies[13]. Depending upon the mode of puckering the relative orientations of the lactam and the thiazolidine rings (a_3) and the orientation of the carboxyl group (ϕ_2^A) vary. The values of (a_3. ϕ_2^A) at the minimum energy conformations are very close to the experimental values (Table I). The influence of the thiazolidine ring puckering on the preferred orientation of the aminoacyl group i not significant and the latter favours a conformation around $\phi_1^A = 170°$. Interestingly, this is the conformation required for optimal binding with transpeptidase[1,2].

It is interesting to note that clavulanic acid which exists in only one type of conformation is a potent inhibitor of β-lactamases. This suggests that this molecule has the right conformation to interact with

these enzymes. Comparison of the favoured conformations of penicillin with that of clavulanic acid indicates that the relative orientations of the bicyclic ring system and the orientation of the carboxyl group of the former are very similar to the latter when the thiazolidine ring is in the C_3 puckered conformation (Table I and Fig. 4). This suggests that for optimal binding with β-lactamases the thiazolidine ring of penicillin should assume a conformation in region II (C_3 puckered form). As pointed out in our earlier papers[2,3], to bind with transpeptidase also the thiazolidine ring should assume a similar conformation. As mentioned already, in solution, the population of this conformation will be small for penicillin compared to clavulanic acid since the latter favours only one type of conformation. This may explain the competitive inhibitory property of clavulanic acid.

Experimental studies indicate that the rate of hydrolysis of the lactam peptide bond by β-lactamases is very slow in clavulanic acid compared to that in penicillin. It is not clear at this stage whether the presence of the aminoacyl group or the flexibility of the five membered ring is required for this reaction to take place.

ACKNOWLEDGEMENTS

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OBSERVATION OF HIGHER SEISMICITY ASSOCIATED WITH LOWER PREMONSOON RAINFALL IN NORTH-EAST INDIA

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ABSTRACT

A comparison of the temporal variation of the premonsoon rainfall with that of seismicity for about fifty-five years in North-Eastern Indian region shows that both premonsoon rainfall and seismicity in the same year are inversely related to each other. However, heavy premonsoon rain seems to occur a year or so before every earthquake of magnitude seven or greater. Influence of premonsoon rainfall on earthquake occurrence in this region is discussed.

INTRODUCTION

ACCORDING to plate tectonic theory, the N.E. Indian region is marked by underthrusting of the Indian plate to the north and Burmese plate to the east and south-east. The region has the highest seismicity compared to the rest of the country. The region is also characterised by high rainfall especially during monsoon (June to September). Since more than 50%

of the earthquakes of this region having magnitudes greater than seven (M > 7) occurred during the first half of the monsoon season, it is suspected that premonsoon rainfall may have some influence on the occurrence of earthquake in this region. If some relation between the two phenomena can be found, it will be a very convenient method for prediction of earthquakes in the North-East India by observing only the variation of premonsoon rainfall.