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A Semi-empirical Study of some Clavulanic Acid Derivatives in Relation to their Activity as β-Lactamase Inhibitors

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Abstract—Clavulanic acid and some of its derivatives are inhibitors of β-lactamases and hence enhance the activity of antibiotics such as cephaloridine, amoxycillin and ampicillin against β-lactamase-producing bacteria. Several empirical structure-activity relationships have been proposed for these compounds; bearing these findings in mind, we have carried out a semi-empirical structural study on clavulanic acid and twelve of its derivatives lacking the carboxyl group. We relate our results to the available activity data, focusing on features such as planarity at the *N* atom, charge distribution and the orientation of the substituents, all of which are related to the activity of these systems.

Clavulanic acid has been the object of much research due to its activity as a potent, competitive and irreversible β-lactamase inhibitor and its consequent enhancement of the efficacy of antibiotics such as amoxycillin, ampicillin or cephaloridine, which are inactivated by β-lactamases produced by some target bacteria (Cole 1984; Labia et al 1985; Allan 1987). Clavulanic acid derivatives include both more active and less active β-lactamase inhibitors and compounds with no such activity; those that have been most intensively studied lack the carboxyl group at C2 or the methoxy group at C9 or have additional substituents at C6 or C2 (Fig. 1). Several empirical structure-activity relationships have been established (Roberts & Price 1985). Following the lead of these studies, we have carried out a structural and charge distribution study of clavulanic acid and twelve of its derivatives that lack the C2 carboxyl group (Fig. 1), because clavulanic acid analogues lacking this group have also been shown to be potent β-lactamase inhibitors (Mak et al 1983; Vasudevan & Rao 1981).

Mak et al (1983) reported that of several compounds studied, compounds *9*, *10* and *11* were the most active against β-lactamases; they also found that methyl-substitution at C2 (compound *7*) had no effect on β-lactamase inhibition, that substitution at C6 (compound *3*) removed all biological activity, and that activity was enhanced by the presence of a carboxyl group at C9 (compound *11*).

In this study, we used molecular orbital (MO) calculations, in particular the AM1 method (with the Parasok option) was implemented by the MOPAC program (Department of Chemistry, Indiana University). Quantum mechanics gives the total energy (E) of a molecule as the sum of an electronic and a nuclear contribution (ε_N):

$$E = \varepsilon_N + 1/2 \, \Sigma_{\mu\tau} \, P_{\tau\mu} \, (H(\text{core})_{\mu\tau} + F_{\mu\tau})$$

where ε_N is the nuclear-nuclear repulsion energy, $\Sigma_{\mu\tau}$ sum over the atomic orbitals, P the density matrix, H(core) the core Hamiltonian matrix, and F the Fock matrix. The energy depends on the molecular geometry. In this way, solving the Schrödinger equation for different geometries, the potential energy surface of the system can be calculated; and, once the minima (conformers) have been located, their charge distributions are obtained from the wave function (Szabo & Ostlund 1982).

Semi-empirical methods are based on this methodology, but contrary to the ab-initio techniques, they use approximations for solving the problem, such as intermediate or complete neglect of some integrals (Hirst 1990). To account for these approximations they are parameterized to give results in agreement with experiment. The AM1 method is a semi-empirical technique, very useful when dealing with large molecules, for which ab-initio methods are still prohibitive. As an example of the application of these semi-empirical techniques to the study of β-lactam systems, Boyd et al (1988) recently carried out MNDO, AM1 and MINDO/3 calculations on the potential energy curve for rotation around a torsional angle of a monocyclic β-lactam.

The two-ring system of clavulanic acid and its analogues is known to have only one stable conformation, as against the two puckered conformations that are possible for the five-membered ring of penicillins (Joshi et al 1978; Vasudevan & Rao 1981). In this work, we started from the stable two-ring arrangement and studied all the possible conformations of the substituents, searching for the most stable ones. The torsional angles involving the two-ring system substituents were assigned all possible combinations of the values 60°, −60° and 180°; the torsional angle of the phenyl group with respect to the rest of the system was taken as 90° and 180°, and for each orientation of the phenyl ring its substituents were oriented both in the plane of the ring and perpendicular to this plane. Once the most stable conformation of each molecule had been obtained, we studied geometrical features including non-planarity at the *N* atom (taken as the distance between the *N* atom and the plane of its three substituents) and the C7-N bond lengths (both these features are known to be related to the reactivity of β-lactam systems (Boyd 1977, 1982)), and the orientation of the substituents—which affects the fit of the molecule to the active site of the target enzymes (Vasudevan & Rao 1981). We also calculated

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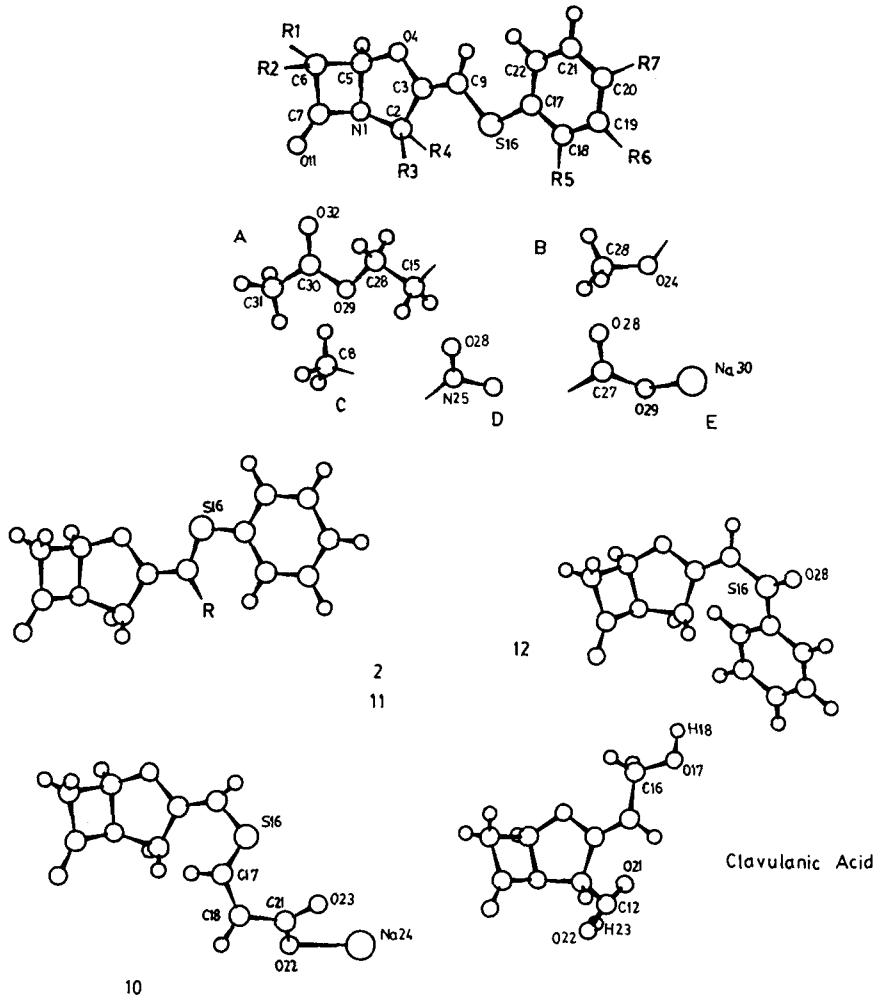

Compound	R1	R2	R3	R4	R5	R6	R7
1	H	H	H	H	H	H	H
3	A	H	H	H	H	H	H
4	H	A	H	H	H	H	H
5	H	H	H	H	H	B	H
6	H	H	H	C	H	H	H
7	H	H	C	H	H	H	H
8	H	H	H	H	H	H	D
9	H	H	H	H	E	H	H

FIG. 1. Compounds studied, with their atom numbering.

charge distributions to determine the centres of nucleophilic and electrophilic attack.

Results

Our results for the most stable conformations of each compound studied (Fig. 1) are displayed in Tables 1–4 (the complete final geometries are available from the authors on request). Table 1 lists their absolute energies, dipole moments and ionization potentials, Table 2 compares the non-planarity at the *N* atoms and C7-*N* distances with the reported experimental activities for several enzymes (Mak et al 1983). Table 3 lists the main torsional angles, and Table 4 the charges on the skeletal atoms.

Table 1 shows that compounds *9*, *10* and *11* bear much larger dipole moments and lower ionization potentials than

Table 1. Energies, dipole moments and ionization potentials of the compounds under study.

Compound	Energy (eV)	Dipole moment (Db)	Ionization potential (eV)
1	−15924·99398	0·839	8·89890
2	−15851·92514	1·537	8·74569
3	−26853·09631	2·969	8·75531
4	−26322·85607	2·767	8·72665
5	−19684·80150	1·379	9·09154
6	−17887·28073	1·014	8·73516
7	−17543·57156	1·649	8·86072
8	−20912·31654	6·211	9·34015
9	−21606·03246	16·844	7·69603
10	−15015·99552	14·152	7·66322
11	−21262·79193	14·801	7·48378
12	−17978·72217	5·223	9·98429
Clavulanic acid	−15021·59024	0·321	9·82981

Table 2. Non-planarities at the *N* atom, defined as the distance (h_N) from *N* to the plane of its three substituents, C7-*N* bond lengths and experimental IC50 values (concentration of inhibitor that inhibits 50% of the enzyme activity).

Compound	h_N (Å)	C7-*N* (Å)	IC50[a] (μM) *E. cloacae* P99	IC50[a] (μM) *E. coli* TEM 1
1	0·574	1·457	27	0·2
2	0·577	1·458		
3	0·573	1·457		
4	0·576	1·457		
5	0·576	1·457	19	0·3
6	0·573	1·469		
7	0·569	1·469		
8	0·575	1·457	20	0·4
9	0·577	1·460	⩽5	<0·05
10	0·580	1·455	2·5	0·03
11	0·575	1·460	⩽0·5	⩽0·05
12	0·579	1·457	50	0·2
Clavulanic acid	0·574	1·463	>50	0·05

[a] Values reported by Mak et al (1983).

the other molecules. From Table 2 it is seen that compound *10* has a short C7-*N* bond, but the greatest non-planarity at the *N* atom. Compounds *3*, *6* and *7* have the most planar *N* atoms, and compound *3* also a short C7-*N* bond.

The configuration of the 5-membered ring is the same in compounds *1*, *2*, *4*, *5*, *8* and *12*, and in *6*, *10* and *11*: in both these groups the best 4-atom plane is defined by atoms 2, 3, 4 and 5. In *7* the best 4-atom plane is defined, as in the most stable clavulanic conformer, by atoms 1, 2, 3 and 4, though the global configuration of the 5-membered ring differs from that of clavulanic acid. Comparison of compounds *3* and *4*, and of compounds *6* and *7*, shows that changing the stereochemistry at C6 and C2 changes the configuration of the 5-membered ring. In compound *9* the best 4-atom plane is defined by atoms 3, 2, 1 and 5.

In all the compounds studied, the 4-membered ring has the same configuration as in clavulanic acid. In compound *4*, the ring is much less planar, and in compound *9* it is much more planar, than in the other systems. The relative orientation of the two adjacent rings is the same in all molecules; the sum of the angles between them is larger in compound *3*.

The S-Ph groups are ±gauche in all the compounds, the maximum deviations of the torsional angle 17-16-9-3 from ±60° being 19·1° and 43·0° in *1* and *7*, respectively. The large deviation in compound *7* is due to steric repulsion between the phenyl group and the methyl group at C2. The orientation of the phenyl group is, in all cases, such as to minimize this type of interaction; in *11* it is almost coplanar with the S-C9 bond. In compounds *5* and *8* the phenyl substituents are almost in the plane of the phenyl ring, but in *9* the substituent is almost perpendicular to the ring.

In all the molecules studied, the most positively charged atom, the centre of nucleophilic attack, is C7 (Table 4). In the clavulanic acid derivatives the most negative atom, the one most exposed to electrophilic attack is C9, followed by atoms C11 and C1, whereas in clavulanic acid itself the target of this type of attack is atom 1.

Discussion

In all the conformations, the configuration of the β-lactam

Table 3. Main torsional angles (degrees) of the most stable conformations obtained.

Description	Compound *1*	*2*	*3*	*4*	*5*	*6*	*7*	*8*	*9*	*10*	*11*	*12*	Clavulanic acid
5-4-3-2	−1·3	−1·4	0·4	−1·9	−1·8	2·5	−6·7	−1·8	−6·1	1·1	1·6	−0·2	−3·4
4-3-2-1	−3·6	−3·6	−4·9	−4·0	−2·8	−7·2	1·7	−3·6	3·1	−5·5	−6·1	−3·9	−1·6
3-2-1-5	6·7	6·9	7·1	7·9	6·0	8·7	3·8	7·2	1·1	7·4	7·8	6·2	5·6
4-5-1-2	−7·9	−8·1	−7·2	−9·4	−7·3	−7·8	−8·0	−8·6	−4·8	−7·0	−7·2	−6·5	−7·9
1-5-4-3	5·7	5·9	4·2	7·0	5·6	3·2	9·2	6·4	6·8	3·6	3·4	4·1	6·9
6-5-1-7	−3·4	−3·6	−4·0	−5·2	−3·2	−3·3	−2·5	−3·8	−1·3	−3·0	−3·0	−2·9	−3·3
6-7-1-5	3·4	3·6	4·0	5·3	3·3	3·4	2·5	3·9	1·3	3·1	3·0	2·9	3·3
1-7-6-5	−3·3	−3·5	−3·9	−5·1	−3·2	−3·3	−2·5	−3·8	−1·3	−3·0	−2·9	−2·9	−3·2
7-6-5-1	3·2	3·4	3·8	4·9	3·1	3·2	2·4	3·7	1·2	2·9	2·8	2·8	3·1
6-5-1-2	−123·6	−123·4	−124·2	−125·2	−123·1	−123·5	−123·5	−124·1	−121·2	−122·9	−123·2	−122·5	−123·4
4-5-1-7	112·4	111·7	113·0	110·6	112·5	112·4	113·0	111·6	115·1	112·9	113·1	113·0	112·2
11-7-1-2	−69·7	−69·0	−68·6	−66·8	−69·6	−69·0	−71·3	−69·0	−73·9	−70·9	−70·9	−70·6	−68·4
9-3-2-1	176·3	175·3	174·1	175·2	177·2	170·6	−176·4	178·7	179·2	174·9	173·1	176·3	176·3
17-16-9-3	−40·9	51·3	−65·2	−56·8	−56·8	−61·6	−103·0	−55·1	−60·9	−46·7	65·0	22·5	11·1
18-17-16-9	106·1	53·3	166·1	160·0	94·2	166·4	155·9	169·2	153·2	160·4	−175·7	74·0	−57·9
15-6-5-1			120·4	−110·9									
28-15-6-5			179·4	166·1									
29-28-15-6			−108·3	−177·6									
30-29-28-15			83·7	82·7									
31-30-29-28			178·7	177·4									
28-24-19-20					175·6								
8-2-1-5						−111·9	127·9						
28-25-20-19								179·0					
28-27-18-17									98·0				
21-18-17-16										−3·4			
22-21-18-17										−1·0			
29-27-9-3											−157·3		
28-16-9-3												−85·0	
23-22-12-2													178·5

Table 4. Charges on skeletal atoms.

	Compound												
Atom	*1*	*2*	*3*	*4*	*5*	*6*	*7*	*8*	*9*	*10*	*11*	*12*	Clavulanic acid
1	−0·25	−0·25	−0·25	−0·24	−0·25	−0·25	−0·25	−0·25	−0·25	−0·24	−0·25	−0·25	−0·25
2	−0·07	−0·07	−0·08	−0·08	−0·07	−0·01	−0·01	−0·08	−0·07	−0·07	−0·06	−0·08	0·00
3	0·00	0·02	0·03	0·02	0·02	0·03	0·06	0·03	0·05	−0·01	0·04	0·09	−0·01
4	−0·21	−0·20	−0·21	−0·21	−0·21	−0·21	−0·21	−0·21	−0·22	−0·22	−0·21	−0·21	−0·21
5	0·06	0·06	0·06	0·06	0·06	0·06	0·06	0·06	0·06	0·06	0·06	0·06	0·07
6	−0·23	−0·23	−0·18	−0·17	−0·23	−0·23	−0·23	−0·23	−0·24	−0·24	−0·24	−0·23	−0·23
7	0·27	0·27	0·27	0·27	0·27	0·27	0·27	0·26	0·29	0·27	0·27	0·27	0·27
9	−0·35	−0·36	−0·37	−0·36	−0·35	−0·37	−0·37	−0·38	−0·35	−0·35	−0·32	−0·52	−0·20
11	−0·25	−0·25	−0·25	−0·24	−0·24	−0·25	−0·25	−0·24	−0·25	−0·25	−0·27	−0·24	−0·23

ring and the relative orientation of the two rings are the same. The arrangement of the substituents can be explained in terms of minimizing steric repulsions. Knowledge of the relative activities of stereoisomers gives insight into the way in which these compounds fit into the enzyme: if the clavulanic acid derivative binds to the enzyme with its convex face towards the latter, then compounds *3* and *6* should be less active than their counterparts *4* and *7* because of possible steric repulsions, whereas the reverse may be expected if the clavulanic acid derivative binds concave side first.

Studying the activity of these systems, both goodness of the fit and chemical reactivity have to be taken into account. Regarding the latter, it is well known that in β-lactam compounds reactivity requires a labile C7-N bond. From the data of Table 2 it follows that to explain the anti-β-lactamase activity of these compounds, not only the *N*-planarity data have to be taken into account, but also geometrical features like the C7-*N* bond lengths. The most active compounds have *N*-non-planarities in the top half of the range of this parameter and a C7-*N* distance of at least 1·460 Å. In compound *10* the short C7-*N* bond is compensated for by its having the greatest non-planarity at the *N* atom. In compound *3* the planar *N* atom environment and the short C7-*N* bond are in keeping with its lower activity. Since compounds *6* and *7* have the same C7-*N* distance but the former has more *N*-non-planarity (close to that of clavulanic acid), compound *6* should be the more active of the two.

The charge distribution is closely related to the reactivity of β-lactam compounds. Boyd (1983, 1984) studied this relationship in cephalosporins and penicillins, concluding that the more positive the charge on the carbonyl oxygen, the more reactive the compound will be. This is in keeping with the mechanism they proposed for the opening of the bicyclic system by a nucleophile. From our results it is seen that the centre of nucleophilic attack is also the C7, but in the most active compound (*11*) the carbonyl oxygen bears the most negative charge. This result supports the fact that clavulanic acid analogues tend to react following mechanisms like those proposed by Charnas et al (1978), Reading & Hepburn (1979) and Brenner & Knowles (1984), where there is no intermediate with a negatively charged O11 atom.

From the results it can be concluded that the most active systems bear the larger dipole moments, a relationship that was also found in the study of other lactams (Bowden 1990); however, it has to be borne in mind that the use of dipole moments in quantitative structure-activity relationships has not been sufficiently tested.

In this work the effect of the solvent was not taken into account, because many enzymatic amide bond breakages are known to occur without an active role of the solvent (Petrongolo & Ranghino 1980).

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