ELECTRONIC JOURNAL OF THEORETICAL CHEMISTRY, VOL. 1, 52–59 (1996)

Experimental and theoretical investigations on the cephalosporin Δ^3–Δ^2 isomerization

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SUMMARY

Cephalosporin esters and amides have been subjected to Δ^3–Δ^2 isomerization in basic medium. Good agreement between the experimentally obtained thermodynamic equilibrium constants and those calculated by molecular mechanics calculations has been found, suggesting the reliability of the MM2 force field as implemented in the program MODEL for calculations on cephalosporins. The Δ^3 isomers generally prove to be more stable than the Δ^2, although the energy difference in the case of esters is small. Reduced accessibility of electrophiles to the Δ^2 double bond is discussed in terms of 3D structure.

KEY WORDS cephalosporin; Δ^3–Δ^2 isomerization; molecular mechanics calculations; free energy calculations.

INTRODUCTION

Orally active Δ^3 cephalosporin esters are potentially useful prodrugs showing better absorption from the intestinal tract than the parent acids[1]. However, the Δ^3 to Δ^2 isomerization of cephalosporin esters is a long-standing problem in their chemistry[2], since the unnatural Δ^2 isomers show enhanced chemical reactivity. Exhibiting reduced hydrolytic stability, the compounds are readily converted to the biologically inactive Δ^2 acids[3]. Although much effort has been devoted to the limitation of this isomerization during synthetic manipulations of cephalosporin derivatives[4], less work has been directed towards the understanding of the associated kinetic and thermodynamic phenomena. Following our previous work in this area[5–7], we became interested in the thermodynamic aspects of this isomerization. We report here a comparison of the experimentally determined equilibrium constants for various substrates with those obtained by molecular mechanics calculations. For the first time, the equilibration of amide derivatives, interesting intermediates for the preparation of enzyme inhibitors[8], has been taken into account.

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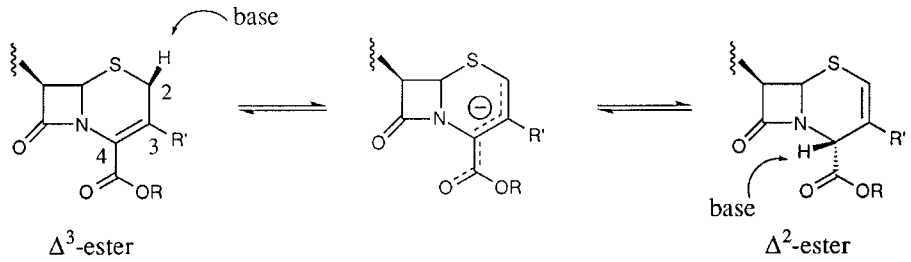
CCC 1082–4928/96/010052–08

Received 1 July 1995
Revised 6 August 1995



Scheme 1.

RESULTS AND DISCUSSION

Chemistry

The conversion of Δ^3 cephalosporin derivatives into the Δ^2 isomers was first described by Green and coworkers[9] and Cocker and coworkers[10] who observed that Δ^3 acids undergo slow isomerization in pyridine, while the corresponding esters and anhydrides are isomerized more rapidly[11,12]. Subsequently Morin *et al.*[13] found that while alkyl 3-acetoxymethyl-7-acylamino-3-cephem-4-carboxylates give a 7:3 (Δ^2:Δ^3) equilibrium mixture, this ratio is completely reversed for the 3-methyl analogues.

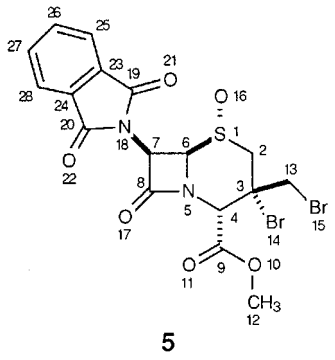
Recently, a systematic study of the kinetic behaviour of pivaloyloxymethyl esters of Δ^3 and Δ^2 3-substituted cephalosporins has shown that the isomerization process to the Δ^2 derivatives, postulated to proceed as depicted in Scheme 1, is the rate-determining step during the degradation of the Δ^3 esters in phosphate buffer solution[1]. In this work the isomerization rate correlates with the steric and electronic effects of the C-3 substituents.

For our study, Δ^3 cephalosporin esters **1a–e** [6] and amides **3a–e** [8] (see Scheme 2) were isomerized in dichloromethane at 25°C at an initial substrate concentration of 0.04 M and in the presence of 0.13 M triethylamine (TEA), followed by HPLC analysis. TEA was chosen as the base to avoid side reactions such as C-7 epimerization [14].

The Δ^3 to Δ^2 isomerization of **1a–e** proved to be an equilibrium reaction. The final position of the equilibrium is independent of the choice of the starting isomer and is unaffected by the addition of more base or more substrate. Conversely, with the exception of **3a** (which after 44 h gave a Δ^3:Δ^2 equilibrium ratio of 88:12), amides **3** do not isomerize under those conditions. From the data reported in Scheme 2 we can assume that in all the cases the Δ^3 isomer is the most stable and the thermodynamically favoured one, even though for the esters the energy gap between the two isomers is considerably smaller than in the case of amides.

Molecular modelling

In 1988 conformational analysis on numerous penicillins was reported by Wolfe and coworkers [15]. A set of parameters, termed MMPEN, was determined for the bicyclic nucleus of penicillin in order to allow a molecular mechanics treatment of penicillins with the MMP2 program. Not knowing whether the program MODEL (generalized MM2 force field) was well parametrized for the molecular mechanics calculations on cephalosporins, we opted, as a first approach, to use it without modification for the treatment of model compound **5**, whose X-ray crystallographic data were available [16]. The reliability of the calculated data was then checked by comparison with those obtained from X-ray analysis of **5** shown in Figure 1.



5

Figure 1.

The input geometry of **5** was generated and initially minimized using the program MODEL (version KS 2.99) [17] and submitted to a conformational search using the batch program BAKMDL [17]. This conformational search (cf. the conformational analysis of **1b**) led to a global minimum energy conformer, whose least-squares fit with the X-ray structure, performed by the MODEL root mean square (rms)-fitting procedure, gave an rms value of 0.081 Å. All the atoms of the bicyclic skeleton were selected and superimposed. The comparison between the calculated and experimentally observed dihedral angles of **5** is reported in Table 1. These results led us to conclude that the MM2 force field as implemented in MODEL is appropriate for the molecular mechanics treatment of cephalosporins.

The conformational analysis of compounds **1–4** was performed following the same general procedure. As an example we describe in detail the procedure used for compound **1b**. The search was carried out stochastically and in two different steps due to the high number of rotatable bonds. In the first step, the bicyclic ring and the ester function at C-4 in **6** were analysed: the S–C_2 bond was broken and the ring treated as a collection of chains, and bonds C_4–C_{10} and C_{10}–O_{12} were rotated according to the default angular values of 180°. All default values offered by the program were accepted with the exception of the energy cutoff (which was lowered to 2 kcal mol^{-1}) and the search was stopped when a new lower energy conformer was not located after 24 CPU hours. While this procedure gives no absolute guarantee that the global minimum has been located, several reasons give us confidence in the reliability of the values obtained. The statistical-search procedure [18] favours the low-energy regions of conformational space. After processing one thousand structures, the calculations identified four low-energy conformations within 2 kcal mol^{-1} of their lowest energy structure.

In the second step of our analysis, the phenoxyacetylamino side chain was added with the correct stereochemistry at the C-7 position of the minimum energy conformer of **6**. The five bonds, C_7–N_{18}, N_{18}–C_{19}, C_{19}–C_{21}, C_{21}–O_{22} and O_{22}–C_{23} were selected and rotated according to the default options: 30, 180, 30, 120 and 60°, respectively. Fourteen conformations were obtained after processing two thousand structures, and their population according to Boltzmann distribution of energy was calculated with MODEL (see Table 2).

The predicted conformational preference of the acylamino side chain is in agreement with the X-ray structure of penicillins [15]: the carbonyl oxygen is directed towards the convex face of the molecule, whereas the aromatic ring is directed towards the concave face of the bicyclic ring, leading to a compact structure. These aspects are of particular interest as the carboxyl group and the amide N–H are supposed to be involved in the binding of these compounds to their receptors [19].

The orientation of both the acylamino and carboxylate groups in the Δ^2 isomers shields, respectively, the concave and the convex faces of the compounds, well explaining the low reactivity of the double

Table 1. Comparison between the crystallographic and calculated dihedral angles of **5**

Dihedral angle	Calc. (°)	Obs. (°)	Dihedral angle	Calc. (°)	Obs. (°)
1-2-3-4	−62	−66	5-6-7-18	−120	−128
1-2-3-13	59	56	5-8-7-6	−1	−7
1-2-3-14	179	177	5-8-7-18	−119	−129
1-6-5-4	62	62	6-5-4-9	−72	−70
1-6-5-8	−118	−109	6-5-8-7	−1	−7
1-6-7-8	108	104	6-5-8-17	−179	−176
1-6-7-18	−11	−18	6-7-8-17	180	177
2-1-6-5	−55	−54	6-7-18-19	−104	−113
2-1-6-7	−151	−150	6-7-18-20	73	77
2-3-4-5	49	49	7-6-1-16	−97	−102
2-3-4-9	−74	−70	7-6-5-8	1	7
2-3-13-15	55	51	7-18-19-21	−2	−5
3-2-1-6	62	63	7-18-19-23	177	174
3-2-1-16	172	171	7-18-20-22	−1	−4
3-4-5-6	−54	−53	8-5-4-9	107	123
3-4-5-8	126	114	8-7-18-19	157	145
3-4-9-10	118	130	8-7-18-20	−25	−25
3-4-9-11	−61	−55	9-4-3-13	−162	−163
4-3-13-15	178	176	9-4-3-14	−45	−47
4-5-6-7	179	178	11-9-10-12	−1	−4
4-5-8-7	178	177	14-3-13-15	−66	−69
4-5-8-17	0	6	17-8-7-18	63	54
4-9-10-12	178	179	18-19-23-24	0	−2
5-4-3-13	75	78	18-19-23-25	−180	−179
5-4-3-14	−168	−167	19-23-24-20	0	0
5-4-9-10	−118	−111	19-23-24-28	180	179
5-4-9-11	62	64	19-23-25-26	180	179
5-6-1-16	167	161	21-19-23-24	180	177
5-6-7-8	−1	−6	21-19-23-25	0	−1

bond towards electrophilic additions [20]. When a phthalimido group, which cannot adopt a folded conformation, is present at C-7, the β-face is more accessible and electrophilic attack at the double bond is allowed. This is also valid for the addition to the exocyclic (C-3/C-3') double bond, whose unusual reactivity towards electrophiles has been reported frequently [21,22].

Free energy calculations

The equilibrium constant for each isomerization reaction (see Table 3) was obtained from the isomeric ratio reported in Scheme 2.

The free-energy difference between isomers was computed at 298 K according to

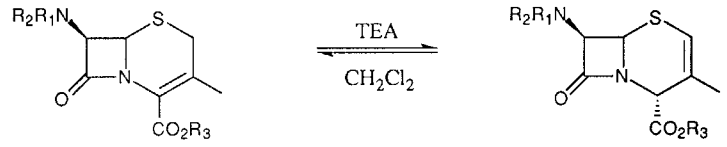
$$\Delta G^{298} = \Delta H^{298} - T\Delta S^{298} \tag{1}$$

The entropic term was calculated by

$$S = -R\sum_i P_i \ln P_i \tag{2}$$

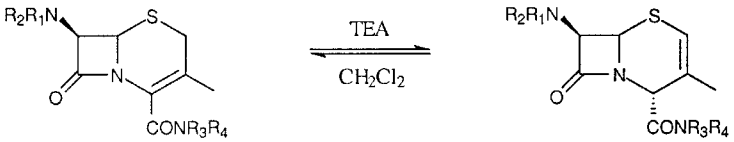
where P_i is the relative population of the i conformer (see Table 3) and the resulting terms $T\Delta S^{298}$ ($\Delta S = S_{\Delta_2} - S_{\Delta_3}$) are reported in Table 3, assuming that solvent ΔS is constant for various structures.

The heats of formation of global minima of compounds **1–4** were obtained by the semiempirical



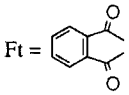
1a-e **2a-e**

a) R_1 = H; R_2 = $PhOCH_2CO$; R_3 = CH_3	Δ_3 = 74%	Δ_2 = 26%	72 h
b) R_1 = H; R_2 = $PhOCH_2CO$; R_3 = $C(CH_3)_3$	Δ_3 = 64%	Δ_2 = 36%	40 h
c) R_1, R_2 = Ft; R_3 = CH_3	Δ_3 = 71%	Δ_2 = 29%	24 h
d) R_1, R_2 = Ft; R_3 = $C(CH_3)_3$	Δ_3 = 49%	Δ_2 = 51%	40 h
e) R_1 = H; R_2 = $PhCH_2CO$; R_3 = CH_3	Δ_3 = 77%	Δ_2 = 23%	20 h



3a-e **4a-e**

a) R_1 = H; R_2 = $PhOCH_2CO$; R_3 = R_4 = H	Δ_3 = 88%	Δ_2 = 12%	44 h
b) R_1 = H; R_2 = $PhOCH_2CO$; R_3 = H; R_4 = CH_3	Δ_3 = 100%		
c) R_1 = H; R_2 = $PhOCH_2CO$; R_3 = R_4 = CH_3	Δ_3 = 100%		
d) R_1, R_2 = Ft; R_3 = R_4 = H	Δ_3 = 100%		
e) R_1, R_2 = Ft; R_3 = R_4 = CH_3	Δ_3 = 100%		

Ft = 

Scheme 2.

molecular orbital method MNDO from the program MOPAC [23] as implemented in SYBYL [24] and used as the enthalpies. ΔH^{298} relative to each couple of Δ^2/Δ^3 isomers is reported in Table 3. The ΔG^{298} values obtained from Equation (1) were used to determine the calculated equilibrium constants.

Comparison between calculated and experimentally derived equilibrium constants shows a good agreement within the limits of experimental approximations, indicating that both MM2, as implemented in MODEL, and MNDO are well parametrized for calculations on cephalosporins.

CONCLUSIONS

We have demonstrated by both calculations and experimentation that the Δ^3 isomers of the considered cephalosporin derivatives are thermodynamically more stable than the corresponding Δ^2 isomers. In the case of esters, the low energy difference between isomers accounts for the usual observation of mixtures of both the isomers during their manipulation, irrespective of the bulkiness of the ester alkyl group. On the other hand, isomerization of Δ^3 cephalosporin amides is not expected on the basis of

Table 2. Relative energies of MM2 (MODEL)-minimized conformations of **1b** and the relative populations

Conformer	Relative energy (kcal mol^{-1})	Relative population (%)	Conformer	Relative energy (kcal mol^{-1})	Relative population (%)
1	0.00	13.81	8	0.81	3.53
2	0.02	13.19	9	0.82	3.52
3	0.06	12.32	10	0.84	3.36
4	0.09	11.96	11	0.89	3.07
5	0.11	11.43	12	0.92	2.92
6	0.14	10.81	13	0.93	2.89
7	0.68	4.39	14	0.95	2.78

the calculated equilibrium constants. This finding is consistent with the experimental results reported in the literature. Although our experimental data are well explained by the theoretical investigations, we must point out that the kinetic aspects of the isomerization have not been considered in our study; they could play an important role, as demonstrated by the possibility of synthesizing Δ^2 cephalosporin *tert*-butyl esters as the sole isomers under particular experimental conditions [25].

EXPERIMENTAL

Chemistry

HPLC was performed using a Waters chromatography system (510 pump, 490 absorbance detector, U6K injector), Perkin Elmer 1020 or Waters 740 integrators, and Merck RP18 5 μm Lichrosorb (4 $\times$ 250 mm) column. Dry dichloromethane and dry triethylamine were distilled over P_2O_5 and KOH, respectively.

General procedure for the equilibration reaction

A solution of 5% triethylamine (TEA) in dry dichloromethane (0.9 ml) was added at 25°C to a solution of the substrate **1a–e** or **3 a–e** (50 mg) in dry dichloromethane (2.5 ml). Aliquots (10 μl) withdrawn at appropriate intervals were diluted with acetonitrile to a final volume of 50 μl, and 20 μl of these solutions were used for HPLC analyses under the following conditions. Mobile phase: H_2O/CH_3CN 70:30 (**1a**,**c**,**e**), H_2O/CH_3CN 50:50 (**1b**,**d**), 0.025 M phosphate buffer (pH 7) /CH_3CN 70:30 (**3a–e**); flow rate: 1.5 ml min^{-1} for **1a–e** and 1.2 ml min^{-1} for **3a–e**; pressure: 2500 psi; AUFS: 2.0; λ: 254 nm. The percentage of ester isomers at the equilibrium and equilibration times are reported in Scheme 1.

Molecular modelling

Molecular mechanics calculations were performed with the use of the program MODEL (version KS 2.99)[17] on a Digital VAXSTATION II/GPX computer and SYBYL program[24] on a Silicon Graphics 4D/35 Personal Iris. Structures were generated by the DRAW option in MODEL program and initially energy minimized by the MM2/M routine of the same program until convergence.

A stochastical conformational search was performed by using the program MODEL selecting the default options, with the exception of the energy cutoff, which was lowered to 2 kcal mol^{-1}. Every search was stopped when a new lower energy conformer was not located after 24 CPU hours of searching from the last located one. Least-squares fittings were performed using the rms-fitting procedure in MODEL.

The final conformationally characterized MM2-minimized structures were transferred to the program SYBYL via the TTY/DATA option of the MODEL program.

Table 3. Comparison between experimental and calculated equilibrium constants

Reaction	ΔH_f	$T\Delta^S$	ΔG	K_{exp}	K_{calc}
1a⇌ **2a**	1.5	0.76	0.7	0.4	0.3
1b⇌ **2b**	1.8	0.70	1.1	0.6	0.2
1c⇌ **2c**	1.9	0.22	1.7	0.4	0.1
1d⇌ **2d**	1.2	0.35	0.9	1	0.2
1e⇌ **2e**	1.9	0.50	1.4	0.3	0.1
3a⇌ **4a**	3.6	0.19	3.4	0.1	0.0
3b⇌ **4b**	2.6	0.35	2.3	0	0.0
3c⇌ **4c**	7.0	0.68	6.3	0	0.0
3d⇌ **4d**	3.3	0.29	3.0	0	0.0
3e⇌ **4e**	5.4	0.41	5.0	0	0.0

Atomic point charges were obtained by the MNDO method of MOPAC[23] through a Mulliken electron population analysis and used for calculating the heats of formation.

ACKNOWLEDGEMENTS

We wish to thank the Italian C.N.R. and the EEC Programme 'Human Capital and Mobility' (contract no. ERBCHRXCT 920027) for supporting this research by grants. Thanks are also due to Dr S. Harper for reading the manuscript.

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