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Thorpe–Ingold effects in cyclizations to five-membered and six-membered rings containing planar segments. The rearrangement of N(1)-alkyl-substituted dihydroorotic acids† to hydantoinacetic acids in base

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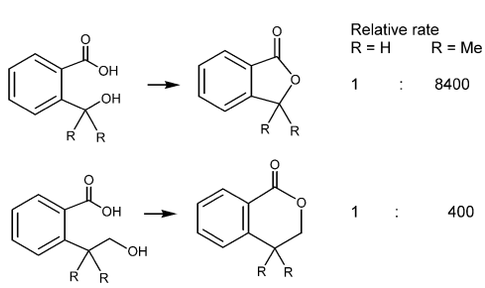
While the *gem*-dimethyl effect (GDME) is quantitatively similar for cyclizations to cyclopentane and cyclohexane rings and their homomorphs, in systems containing planar segments the GDME is stronger for the formation of five-membered rings. Planar pentagons have smaller angles than planar hexagons and their formation is helped by the decrease in the potential internal bond angle caused by substituents, as suggested by Thorpe and Ingold for small rings. The phenomenon is illustrated with crystal structure data on five-membered hydantoins and six-membered dihydrouracils containing four-atom planar segments. Such a Thorpe-Ingold effect explains the rearrangement in base of *N*-alkyl substituted dihydroorotic acids **1** to hydantoinacetic acids **3**. The reaction involves initial hydrolysis to *N*-(*N*-alkylcarbamoyl)aspartic acids **2** and their subsequent cyclization. The unsubstituted *N*-carbamoylaspartic acid **2a** is stable in 1 M KOH, the N(1)-methyl and ethyl compounds **2b** and **2c** are in equilibrium with the hydantoinacetic acids **3**, while the cyclization of the N(1)-isopropyl and cyclohexyl derivatives **2d** and **2e** is irreversible. Experimental data on equilibria and pK_as for ionization of the carboxy and NH groups allow equilibria and rates involving the *N*-unsubstituted compounds to be estimated and compared with those for the *N*-alkyl derivatives. The strongest effect is observed on the equilibrium [$\mathbf{3}^{2-}$]/[$\mathbf{2}^{2-}$], where substitution of H by methyl increases *K* 600-fold. *In vitro* the kinetic regioselectivity for acid catalyzed cyclization of *N*-carbamoylaspartic to hydantoinacetic acid against dihydroorotic acid is only 10 : 1. This, together with the weaker acidity of the remote carboxyl group, favours cyclization to dihydroorotic acid under biological conditions.

Geometry of normal rings and the Thorpe–Ingold effect

The Thorpe–Ingold or *gem*-dimethyl (more generally dialkyl) effect is defined by the increase in both rate and equilibrium constants of cyclization reactions resulting from the introduction of substituents in the linking chain. The original concept of Thorpe and Ingold referred to the reduction of the angle between the relevant ring atoms in the open-chain reactant resulting from compression by the substituent(s).[1] However, alkyl substitution also favours the formation of normal (five to seven-membered) rings, where this reduction in bond angle is no longer obviously beneficial because the original tetrahedral angles are preserved in the ring. The broader term *gem*-dimethyl[2] or *gem*-dialkyl effect[3] was coined,‡ and the effect explained in terms of increased strain and a reduced entropy of rotation in the open-chain compound.[4] Quantitatively the effect on the formation of normal rings varies considerably, and depends on the type of reaction as well as on the position of the substituent(s) in the chain.[5]

These variations can often be understood in terms of tightness or looseness of the transition state and of specific interactions in the transition state involving the substituent.[5b,6] Data for the *gem*-dialkyl effect in the formation of five-membered *vs.* six-membered rings are available for many systems but seem not to have been analyzed in detail. A convenient point of reference is the parent cycloalkane. Equilibrium constants for the formal cyclizations of alkanes and *gem*-dimethylalkanes calculated from thermodynamic data show very similar increases in stability for cyclopentane and cyclohexane (around 70§ for substitution at C(3)).[7] Similarly Warren and coworkers[8] have shown that in competitive cyclizations to tetrahydrofurans and pyrans the GDME is of equal importance. However, some systems show a significantly stronger GDME for the formation of five membered rings. The lactonizations of *o*-hydroxyalkylbenzoic acids (Scheme 1) present such an example.[5b]



Scheme 1

A second example is provided by the relative rates of acid catalyzed cyclization of hydantoic[9] and 2-ureidopropionic acids[5a] (Scheme 2).

This difference in behaviour, of compounds resembling the cyclic alkanes on the one hand and the lactones of benzoic acid and cyclic ureides on the other, can be rationalized by the inter-

† The numbering of dihydroorotic acid differs from that of dihydrouracils due to priority of the carboxy group. For easier comparison with the parent ring systems we use 1-alkyldihydroorotic acid to denote 3-alkyl-2,6-dioxohexahydropyrimidine-4-carboxylic acid and 1-alkylhydantoinacetic acid to denote (3-methyl-2,5-dioxo-imidazolidin-4-yl)-acetic acid.

‡ The effect is not limited to *gem*-substitution.

§ The number given in Table 14 of ref. 7 is incorrect.

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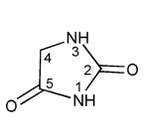
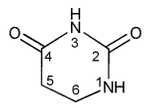
Table 1 Ring geometries in hydantoins and dihydrouracils[a]



Hydantoin



Dihydrouracil

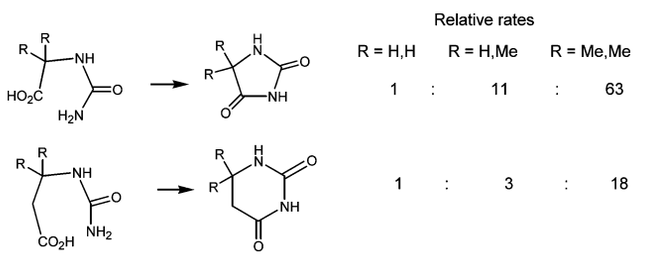
(a) Mean internal angles (°) at apex atoms

Ring atom	N(1)	C(2)	N(3)	C(4)	C(5)	C(6)
Hydantoins[b]	112.3 ± 1.2	107.6 ± 0.9	111.5 ± 1.0	106.9 ± 0.6	101.2 ± 1.1	
Dihydrouracil[c]	122.0 ± 1.7	116.7 ± 0.9	125.2 ± 1.6	115.3 ± 1.3	109.9 ± 1. 9	109.7 ± 1.6

(b) Average torsion angles (°)

Hydantoins	*1234*	*2345*	*3451*	*4512*	*5123*	
	2.8 ± 2.2	3.3 ± 2.8	3.5 ± 3.4	3.4 ± 3.4	3.3 ± 2.7	
Dihydrouracils	*1234*	*2345*	*3456*	*4561*	*5612*	*6123*
	11.7 ± 4.7	5.7 ± 4.6	34.8 ± 6.6	50.6 ± 6.0	39.2 ± 7.5	9.3 ± 4.0

[a] Averages are for compounds containing the hydantoin or dihydrouracil ring without spiro or fused linkages to other rings. Data from the Cambridge Crystal Structure Data Base. [b] From 37 structures. [c] From 32 structures.



Scheme 2

vention of the original Thorpe–Ingold effect in the second case. This arises from the geometrical requirements imposed by the presence of planar segments of conjugated sp^2-hybridized atoms. von Baeyer suggested many years ago that cyclopentane is strainless because the tetrahedral bond angles at carbon (~109°) match those of a regular pentagon (108°). In an irregular pentagon, as long as it is planar, the sum of the angles remains fixed at 540°. When the intrinsic bond angles of some of the atoms are 120°—there are four such atoms in the cases cited above—the sum of bond angles exceeds 540°. With conjugation enforcing local regions of planarity the conflict can be resolved by a combination of ring puckering and decreases in some or all bond angles. The latter course is helped by the Thorpe–Ingold effect, which thus favours cyclization more strongly.

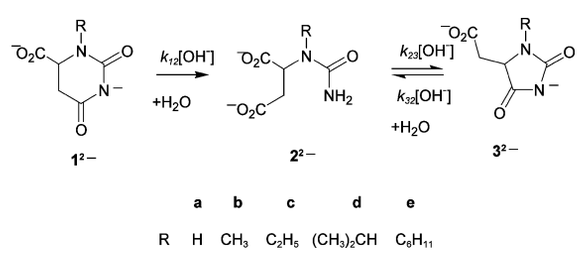
The opposite situation prevails in six-membered rings. A tendency to planarity demands ultimately the 120° bond angles of a regular hexagon, and thus atypically large angles at tetrahedral centres. This is borne out by the X-ray structural data on hydantoins and dihydrouracils shown in Table 1.

The averaged internal bond angles of hydantoins are all considerably reduced, that at C(5) by 8° compared with the standard tetrahedral angle, while the torsion angles (Table 1(b)) indicate that the ring is essentially planar. Although the average values for the angles at C(5) and C(6) in dihydrouracils are close to tetrahedral these derive mainly from compounds with one or two substituents at these atoms. In the parent dihydrouracil the angles are 112.6° and 110.3°, respectively.[10] The torsion angles for the six-membered rings show that there is considerable puckering involving the tetrahedral carbon atoms, and even the two amide groups are slightly twisted with respect to each other (torsion angle *1234*).

A measure of the Thorpe–Ingold effect on the formation of five-membered rings with five planar atoms is founded in the intramolecularly catalyzed hydrolysis of maleamic acids (which involves cyclization to the anhydrides). Introduction of one and two methyl groups increases the rate by factors of 30 and 1.5×10^4 respectively, reflecting substantial decreases in the prospective internal bond angles at the alkene carbons.[11]

The rearrangement of 1-alkyl-substituted dihydroorotic acids to hydantoinacetic acids in base

We report a strong Thorpe–Ingold effect on the formation of five-membered rings from the rearrangement of dihydroorotic to hydantoinacetic acids in KOH solutions: which occurs on alkylation of N(1) of the dihydrouracil ring (Scheme 3).



Scheme 3

The change in behaviour in aqueous alkali is quite spectacular. Dihydroorotic acid **1a** is readily hydrolyzed to *N*-carbamoylaspartate dianion **2a**, which is stable under the conditions. The hydrolysis products **2b** and **2c** of the methyl and ethyl derivatives slowly cyclize, to form equilibrium amounts of the dianion of hydantoinacetate **3**. And the formation of hydantoinacetate **3** is so fast for the secondary alkyl derivatives **2d** and **2e** that the process followed by UV appears superficially as a direct conversion of **1d (e)** into **3d (e)**.

The rate increase in the cyclization of the dianion of *N*-carbamoylaspartic acid (k_{23} of Scheme 3) was found to be *ca*. 40 when R = H was substituted for Me—a value comparable to the maleamic acid case quoted above.

Results

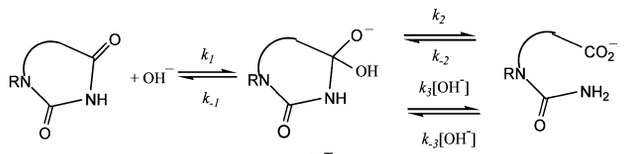
The alkaline hydrolysis of 1-alkyldihydroorotic acids **1** has been studied previously,[12] to assess the effect on rates of an axial carboxylate group. (This conformational preference is the result of allylic strain: in the parent acid the COOH group is more or

less equally axial and equatorial.) When the study was extended to include R = isopropyl and R = cyclohexyl the reaction studied by UV appeared to show a direct conversion into the corresponding hydantoic acid **3**. This could in principle have resulted from intramolecular attack of carboxylate anion on the C=O at position 4, perhaps helped by buttressing by the neighbouring secondary alkyl group.

However, this pathway is not consistent with the observed kinetics. In 0.1–1 M KOH dihydroorotic acids **1** exist as the dianions (Scheme 3). The rate of formation of **3** shows a first order dependence on [OH^-]: an intramolecular reaction of the dianion of **1d** would be independent of hydroxide concentration. Furthermore, a ^{1}H NMR study of the course of the reaction with **1d** revealed transient formation of the intermediate *N*-(1′-isopropylcarbamoyl)aspartic acid **2d**. Finally, when solutions of the hydrolyzed products **2** of methyl and ethyl derivatives **1b** and **1c** were left to stand they slowly cyclized, forming equilibrium mixtures of **2** and **3**.

The course of the rearrangement is thus that outlined in Scheme 3, involving base catalyzed nucleophilic attack of the urea NH_2 on the carboxylate group. In the case of **1b** and **1c** the two consecutive reactions are well separated in time and separate pseudo first order rate constants could be obtained from the decrease in absorbance of $\mathbf{1}^{2-}$ at λ_{max} = 240 nm and the subsequent increase in absorbance at 232 nm (λ_{max} for **3b** and **3c**). The isopropyl and cyclohexyl derivatives **2b** and **3b**, on the other hand, cyclized much faster so that this simple treatment was no longer possible: the rate constants were obtained by curve fitting of the variation of absorbance with time to the integrated first order rate equation for two consecutive reactions.

The preferred mechanism[13] of alkaline hydrolysis of dihydrouracils and hydantoins (shown in Scheme 4) is characterized by two parallel modes of decomposition of the tetrahedral intermediate $\mathbf{T}^-$, catalyzed by water and OH^- respectively.



$\mathbf{T}^-$

Scheme 4

The steady state approximation affords the following rate equation for the forward reaction:

$$k_{cor} = \frac{k_1[OH^-]\{k_2/k_{-1} + k_3/k_{-1}[OH^-]\}}{1 + k_2/k_{-1} + k_3/k_{-1}[OH^-]} \qquad (1)$$

where k_{cor} is the observed first-order rate constant corrected for unproductive ionization at 3-NH:

$$k_{cor} = k_{obs}\frac{[OH^-] + K_w/K_{NH}}{K_w/K_{NH}} \qquad (2)$$

In the region of pH 10–14 **1a** and **3a** exhibit pH-rate profiles for k_{cor} in which the slope changes from 1 to 2, then back to 1, due to rate determining k_1k_2/k_{-1}, k_1k_3/k_{-1} and k_1 respectively. Upon increasing the substituent size in 1,6-disubstituted dihydrouracils the term second order in [OH^-], k_1k_3/k_{-1}, tends to become dominant throughout the pH region.[12] This is the case for compounds like 1,6-dimethyldihydrouracil and 1-ethyl-dihydroorotic acid **1c**. Similarly the formation of 1,3,5,5-tetramethylhydantoin (stable in base up to 1 M KOH) from the corresponding hydantoic acid is second order in [OH^-] (based on neutral substrate), consistent with a transition state carrying two negative charges.[14]

As expected, in 0.1–1 M KOH the hydrolyses of the *N*-isopropyl and *N*-cyclohexyl-dihydroorotic acids **1d** and **1e**, and the formation of the hydantoinacetic acids **3d** and **3e** are apparently first order in [OH^-]:

$$V = k_{12}[\mathbf{1}^{2-}][OH^-] \text{ and } V = k_{23}[\mathbf{2}^{2-}][OH^-].$$

The kinetics are revealed as being of the second order when the unproductive ionizations are accounted for,

$$k_{cor} = k_1k_3/k_{-1}.$$

The cyclizations of the *N*-methyl and *N*-ethyl-carbamoylacetic acids **2b** and **2c** are too slow for a pH–rate profile to be measured, so rates were measured in 1 M KOH. To check the kinetic order a pH–rate profile for the cyclization of the ethyl derivative **2c** was obtained at 50 °C, and reaction found to be first order in [OH^-] (see Fig. 1).

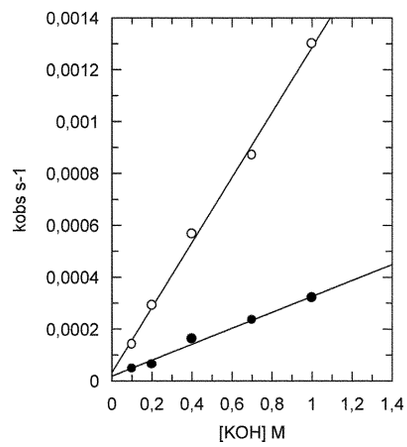


Fig. 1 Plots of the observed pseudofirst order rates, s^{-1}, against [KOH] at 50.0 °C: open circles hydrolysis of **1c**, $k_1 = 1.25 \times 10^{-3}$ dm^3 mol^{-1} s^{-1}; filled circles cyclization of **2c**, $k_2 = 3.07 \times 10^{-4}$ dm^3 mol^{-1} s^{-1}.

Measuring the equilibria for cyclization of the carbamoylaspartic acids **2b** and **2c** also provided the rate constants (k_{32}) for hydrolysis of hydantoinacetic acids **3b** and **3c**, because $k_{obs\,23} = k_{23} + k_{32}$.

The full set of kinetic data is shown in Table 2. Two sets of rate constants are presented for comparison. For each compound the first column presents the apparent second or first order rate constant determined in 1 M KOH. However, our earlier work[12] indicates that the observed hydrolysis rates, k_{12}, of the parent dihydroorotic acid **1a** and its *N*-methyl derivative **1b** in 1 M KOH are determined mainly by k_1, the rate constant for formation of the tetrahedral intermediate. For the remaining compounds the OH^- catalyzed breakdown of the tetrahedral intermediate (k_1k_3/k_{-1}) is rate determining at 1 M KOH. The latter mechanism is the one for which rate constants could be obtained in all cases, and these are given in parentheses in Table 2. These rate constants are readily obtained from the experimental second order rate constants and pK_{NH}: from eqns. (1) and (2), under conditions where [OH^-] >> K_w/K_{NH}:

$$k_1k_3/k_{-1} = (k_{12} \text{ or } k_{32})K_{NH}/K_w \qquad (3)$$

The pK_{NH}-values for **1** and **3** measured for this purpose appear in Table 3.

The data obtained for the equilibrium:

$$K^{2-} = [\mathbf{3}^{2-}]/[\mathbf{2}^{2-}] \qquad (4)$$

in the case of the *N*-methyl and *N*-ethyl derivatives, **b** and **c**, make an estimate of equilibrium with the *N*-H compounds **a** of particular interest. This is possible because constants for two of

Table 2 Rate constants for the hydrolysis of dihydroorotic acid and hydantoinacetic acid and the cyclization of *N*-carbamoylsuccinic acid in 0.1–1 M KOH (I = 1.0 M, KCl) at 25.0 °C, and k_1k_3/k_{-1}-values according to eqn. (1) (in parentheses)

R	k_{12} dm^3 mol^{-1} s^{-1} (dm^6 mol^{-2} s^{-1})	$k_{rel} = k_o/k_x$	k_{23} dm^{3}mol^{-1} s^{-1}	$k_{rel} = k_x/k_o$	k_{32} dm^3 mol^{-1} s^{-1} (dm^6 mol^{-2} s^{-1})	$k_{rel} = k_o/k_x$
H	2.21×10^{-3} [a] (9.78 [d])	1 (1)	2.7×10^{-7} [b]	1	2.88×10^{-5} [c] (0.91)	1
CH_3	5.09×10^{-4} [a] (0.0783) [d]	4.3 (125)	1.15×10^{-5}	43	2.00×10^{-6} (0.047)	14 (19)
C_2H_5	3.32×10^{-4} [a] (0.0200) [d]	6.7 (490)	3.78×10^{-5}	140	2.52×10^{-6} [e] (0.048)	
$(CH_3)_2CH$	2.50×10^{-5} (0.0022)	88 (4400)	1.62×10^{-4}	600		
C_6H_{11}	2.58×10^{-5}		2.22×10^{-4}			

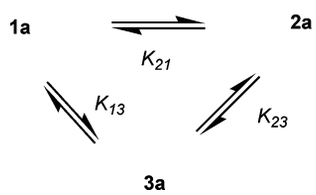
[a] Apparent first order rate constant in 1 M KOH s^{-1}. [b] Calculated value, see text. [c] Interpolated from a temperature dependence study from ref. 15. The pH–rate profile in the same paper indicates that this value, measured as the apparent bimolecular k_{32} in 0.1 M KOH, applies to the third order k_1k_3/k_{-1} reaction. [d] Ref. 12. [e] Value inaccurate because of the large equilibrium constant.

Table 3 Acidity constants for dissociation at NH of hydantoinacetic acids and dihydroorotic acids at 25.0 °C and I = 1.0 M (KCl)

Compound	pK_{NH}	Compound	pK_{NH}
3a	9.50	**1a** [a]	11.60
3b	9.63	**1b** [b]	12.22
3c	9.72	**1c** [b]	12.25
3d	9.97	**1d**	12.06

[a] Ref. 16. [b] Ref. 12

the equilibria of the thermodynamic cycle shown in Scheme 5 are available.



Scheme 5

K_{13} between dihydroorotic acid and hydantoinacetic acid was determined [17] in HCl at 70 °C, while the equilibrium K_{23} between carbamoylaspartic acid and dihydroorotic acid could be set up enzymatically [18] around pH 7 at 36 °C. As all the necessary pK_a-values involved are known, the equilibrium constants between the various ionization states can be calculated. The equilibrium data at 70 °C were corrected to 25 °C, as described in the footnote of Table 4. (pK_a-values for the carboxy groups of *N*-carbamoylaspartic acid, and its equilibrium constant, were determined at 36 °C, while the remaining pK_a-values were measured at 25 °C: this difference is ignored.) The equilibrium data—experimental and estimated—are summarized in Table 4.

Discussion

Intrinsic regioselectivity of cyclization of *N*-carbamoylaspartic acids

The cyclization of *N*-carbamoylaspartic **2a** to dihydroorotic acid is a step in the "*de novo*" biosynthesis of pyrimidine bases.[19] An intriguing point, recently reexamined,[20] is that in mineral acids the opposite regioselectivity is observed: cyclization proceeds by attack of the ureido NH_2-group on the 1-CO_2H to give hydantoinacetic acid, the only product isolated by Nyc and Mitchell.[21] The new interpretation [20] is that in the chemical reaction, attack on the distant carboxyl is hindered by a strong hydrogen bond between the two carboxy groups, which reduces the flexibility of the chain. Attack on the 1-CO_2H takes place in acid because the *geminal* ureido is favourably disposed. It was also postulated that this hydrogen bond is broken in the enzyme active site. From ^{1}H vicinal coupling constants assigned from specifically deuterium-labeled **2a** we have estimated [22] that the conformations with the carboxy groups gauche account for less than 60% of the neutral form in D_2O—not consistent with stabilization by a strong hydrogen bond. In contrast the population of conformations with the ureido and 4-CO_2H gauche is 90%, so that proximity in forming the six-membered ring should not be problem. Further, we showed previously [17] that in HCl solutions the cyclization of *N*-carbamoylaspartic acid actually gives both products, with a kinetic regioselectivity [**3a**] : [**1a**] of no more that 10 : 1, much less than the thermodynamic preference discussed above.

At biological pH, near 7, both carboxy groups are ionized. Because carboxylate anions are poor electrophiles reaction must involve the neutral CO_2H group. Since the more distant carboxy group is a 30-times weaker acid more of it will be present in the neutral CO_2H form near pH 7, thus over-riding the kinetic selectivity. The conclusion from chemical reactivity data is thus that dihydroorotic acid is the preferred product at neutral pH, so that enzyme catalysis is not in fact working against strong intrinsic regioselectivities upon cyclization of *N*-carbamoylaspartic acid.

The effect of *N*-substituents

Cyclizations of ureido acids are observed even in alkaline solution in fixed molecules or highly encumbered systems like *o*-ureidobenzoic acid [23] or 2,2,3,5-tetramethylhydantoic acid.[14] Remarkably, just two substituents of the size of carboxymethyl and isopropyl in the 1,5-position of hydantoin are enough to make the ring stable in 1 M KOH. There is abundant evidence, for example in the acid catalyzed cyclization of ureido acids,

Table 4 Equilibrium constants for the cycle on Scheme 5

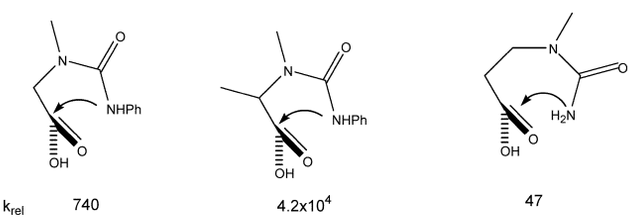
Equilibrium (compound)	K^o	$K^{1-/2-}$ mol^{-1}	K^{2-}	k_{rel}
[**1a**]/[**2a**]	135 [a]	1.51×10^6 [a]	3.8×10^{-6} [b]	
[**3a**]/[**1a**]	80 [c]	$K^{1-} = 20$ [d]	2500	
[**3a**]/[**2a**]	1.08×10^4 [b]	3.02×10^7	9.5×10^{-3} [e]	1
[**3b**]/[**2b**]		2.13×10^{10}	5.7	600
[**3c**]/[**2c**]		8.05×10^{10}	15	1600

[a] Ref. 18; from equilibrium achieved enzymatically at pH around 7 (dilute solutions) and pK_{1a} = 3.10, pK_{2a} = 2.83, pK_{2a^-} = 4.32 obtained at 37 °C and I = 0.5 M. [b] pK_{1a^-} = 11.60. [c] Determined[17] at 70 °C and corrected for a temperature of 25 °C by assuming $\Delta F = \Delta H$ and Cp constant. [d] pK_{COOH3a} = 3.70.[18] [e] pK_{NH3a^-} from Table 3.

that substitution at N(1) in the product causes the greatest accelerations (k_{rel}-values obtained by comparison with the corresponding unsubstituted ureido acids):[24,25,5a]

Agami and Couty[26] have identified strong Thorpe–Ingold effects due to *N*-alkyl substitution facilitating the formation of 1,3-oxazolidin-2-ones or oxazolidin-2,5-diones compared to the *N*-unsubstituted compounds. AM1 calculations showed decreases in the C–NR–C angle of the open-chain reactant when R = Me, leading Agami *et al.*[27] to the conclusion that a classical Thorpe–Ingold effect operates to favour ring-closure upon *N*-methyl substitution.

A very strong such effect is observed on the equilibrium hydantoinacetic acid $\rightleftharpoons$ *N*-carbamoylaspartic acid [$\mathbf{3}^{2-}$]/[$\mathbf{2}^{2-}$] (Table 4), where an *N*-methyl shifts the equilibrium by a factor of 600. This seems too large to be simply a bond-angle effect: the relative rates of similar acid-catalyzed cyclizations (Schemes 2 and 5) show that rate increases for substitution at the nitrogen atom are much larger than that at C(5), even though significant reductions of bond angles occur at both centres (data of Table 1); and the substantial increase caused by *N*-substitution in the rate of formation of the 6-membered ring shown in Scheme 6 involves no bond-angle reduction. Studies of rotational barriers around partial double bonds indicate[28] that in ureas considerable strain accumulates in the ground state upon *N*-substitution. This is released upon cyclization as one pair of the eclipsing groups is incorporated in the ring, as explained by Allinger[4] for the case of substituted hexanes.



Scheme 6

In the absence of special effects on the transition state the rate increases upon cyclization or decreases upon ring-opening should be smaller than those for equilibrium: this is borne out by the data in the final 4 columns of Table 2 (which compare N–Me with N–H). The rate increase on cyclization is larger than that on ring opening, which suggests a transition state closer to the cyclic form. The hydrolyses of compounds **a** and **b** allow a comparison of six *versus* five-membered ring behaviour. When the apparent second order rate constants for hydantoinacetic acids **3** are compared to the first order constants for dihydroorotic acids **1** in 1 M KOH the decrease is larger for the five-membered ring. As explained above, however, the data for formation and hydrolysis of the hydantoinacetic acids refer to the doubly negatively charged transition state,¶ so that it is more appropriate to compare them with the third order rate constants k_1k_3/k_{-1} for hydrolysis of **1**. The latter exhibit a very large decrease upon substitution of H for Me, which has been interpreted previously[12] as an "inverse" GDME on ring opening. The same trend is observed when the size of the *N*-substituent is increased further (Table 2).

Increasing the size of the substituent from methyl to isopropyl or cyclohexyl exhibits substantial effects on the rates of cyclization of the *N*-alkylcarbamoylaspartic acids to hydantoinacetic acids (see columns 4 and 5 of Table 2)

Experimental

Unless otherwise stated IR-spectra are for Nujol mulls, using a Bruker IFS 113v instrument, and frequencies in cm^{-1}. ^1H NMR spectra were recorded on a Bruker Spectrospin WM 250 instrument (chemical shifts in ppm against TMS, couplings in Hz), and UV measurements made using Unicam SP 800 UV and Carl Zeiss Jena UV VIS spectrophotometer provided with a thermostatted cell compartment, wavelengths in nm. Melting points were taken on a Kofler block.

Materials

Inorganic reagents and buffer components were of analytical grade and were used without further purification. Potassium hydroxide and buffer solutions were prepared with CO_2-free distilled water. The preparation of 3-alkyl-2,6-dioxo-hexahydropyrimidine-4-carboxylic acids **1b**,**c**, and **d** has been described previously.[29]

3-Cyclohexyl-2,6-dioxohexahydropyrimidine-4-carboxylic acid 1e. The parent 2-cyclohexylaminosuccinic acid monoamide was prepared as previously described.[30] This was converted into the *N*-ethoxycarbonyl derivative by dissolving the mono-amide (0.5 g, 2.23 mmol), followed by 1 ml of ethyl chloroformate, in 10 ml of dry triethylamine. After standing for 2 hours at 50 °C the triethylamine was removed and the residue dried *in vacuo* at 50 °C. The residual 1 g was treated for 4 h under reflux with sodium ethoxide (7 mmol) in 5 ml of dry ethanol. The precipitate formed under cooling was filtered rapidly to avoid moisture and washed with dry ethanol. The solid was dissolved in a 3 ml of cold water and acidified with HCl (Congo Red), then the precipitate filtered and recrystallized from ethanol yielding **1e** (180 mg, 34%), mp 234–235 °C. (Found: C, 54.97; H, 6.70; N, 11.73. Calc. for $C_{11}H_{16}N_2O_4$: C, 54.99; H, 6.71; N 11.66%); δ_H (DMSO-d_6) 1.0–1.8 (10 H, m, $(CH_2)_5$), 2.566 (1 H, d, J_{44} 16.6, $J_{4e5} < 1.5$, 4e-H), 2.851 (1 H, dd, J_{44} 16.6, J_{4a5} 7.2, 4a-H), 4.052 (1 H, s, broad unresolved, 1′-H), 4.220 (1 H, d, J_{4a5} 7.2, $J_{4e5} < 1.5$, 5-H), 10.055 (1 H, s, NH).

(3-Alkyl-2,5-dioxo-imidazolidin-4-yl)-acetic acids 3. The 3-alkyl-2,6-dioxohexahydropyrimidine-4-carboxylic acid **1** (0.44 mmol) was dissolved in 20 ml of hydrochloric acid diluted 1 : 1 with water and refluxed for two hours. The residue was evaporated to dryness and the residue recrystallized from ethanol. Data for these compounds are summarized in Table 5.

^1H NMR in DMSO-d_6. The CH_2 protons and the 4-H proton formed an ABX system. The data for these protons were obtained from the analysis of this system. **1b**. 2.741 (3 H, s, Me), 2.760 (2 H, octet, $\Delta\nu_{AB}$ 11.1 Hz, J_{AB} 17.1, J_{AX} 4.7, J_{BX} 4.3, CH_2), 4.160 (1 H, t, *J* 4.5, 4-H), 10.73 (1 H, s, NH), 12.76 br (CO_2H). **1c**. 1.022 (3 H, t, *J* 7.1, Me), 2.767 (2 H, octet, $\Delta\nu_{AB}$ 10.7 Hz, J_{AB} 17.2, $J_{AX} = J_{BX} = 4.5$ CH_2), 3.040 (1 H, sextet, *J* 14.1, *J* 7.1, CH*H*N), 3.415 (1 H sextet, *J* 14.1, *J* 7.1, C*H*HN), 4.251 (1 H, t, *J* 4.5, 4-H), 10,74 (1 H, s, NH), 12.58 (1 H, s, CO_2H). **1d**.‖ 1.123 (3 H, d, *J* 6.9, Me), 1.169 (3 H, d, *J* 6.9, Me), 2.765 (2 H, octet, $\Delta\nu_{AB}$ 7.2 Hz, J_{AB} 17.0, J_{AX} 4.6, J_{BX} 4.0, CH_2), 3.869 (1 H, septet, *J* 6.9), 4.199 (1 H, t, *J* 4.3, 4-H). **1e**. 1.0–1.8 (10 H, m, $(CH_2)_5$), 2.822 (2 H d (*J* 3.4) CH_2), 3.494 (1 H, t unresolved, 4-H), 4.225 (1 H, br, 1′-H), 10.73 (1 H, s, NH), 12.55 (1 H, br, CO_2H).

Determination of pK_as for dissociation at NH

pK_a-values were determined spectrophotometrically in a series of buffers at 25.0 °C using the absorptions of the anions: $\lambda_{max} \cong 234$ nm for *N*-alkylhydantoinacetic acids and 238 nm for *N*-alkyldihydroorotic acids. pH-values were measured using a Radiometer pH M 84 Research pH-meter, equipped with a GK 2401 C electrode standardized at pH 6.87, 4.01 and 9.18.

¶ Actually triply charged if the charge of the carboxylate group not involved in the reaction is also taken into account.

‖ Spectrum taken on a 400 MHz instrument.

Table 5 Data for (3-alkyl-2,5-dioxo-imidazolidin-4-yl)-acetic acids **3**

Compound (formula)	Yield (%)[a]	Mp/°C	Found (%) (required)		
			C	H	N
3b $C_6H_8N_2O_4$	92	184–185	41.79 (41.86)	4.75 (4.68)	16.24 (16.28)
3c $C_7H_{10}N_2O_4$	91	190–191	45.21 (45.16)	5.38 (5.41)	15.02 (15.05)
3d $C_8H_{12}N_2O_4$	92	196–197	48.11 (47.99)	6.01 (6.04)	13.89 (13.99)
3e $C_{11}H_{16}N_2O_4$	90	199–200	55.01 (54.99)	6.90 (6.71)	11.72 (11.66)

[a] Of recrystallized product.

Kinetic measurements

Reactions were followed using stoppered cells, in the temperature-controlled cell holder of the spectrophotometer or in sealed vials at 25.0 °C. The reaction was initiated by adding 20 μl of a 0.05 M solution of the substrate in UV-grade methanol to 2.75 ml of the KOH solution. The ionic strength was maintained at 1 M with KCl.

(a) *N*-Methyl and *N*-ethyl derivatives. The rates of hydrolysis of dihydroorotic acids **1a**, **1b** and **1c**, reported before,[12] were followed by monitoring the decrease of the absorbance of the dianion at 238 nm. In the case of **1b** and **1c**, on standing the subsequent increase of absorbance with a λ_{max} at 234 nm showed that the formation of the respective hydantoinacetic acids took place at a considerably slower rate. The two processes are well separated in time and with some approximation their rates could be measured separately, justifying the previous treatment of the hydrolysis of dihydroorotic acids **1b** and **1c** as single reactions. The rates of cyclization of *N*-carbamoylaspartic acids **2b** and **2c** were monitored by following the increase in the absorbance at 234 nm after opening of the dihydrouracil ring. Rate constants were obtained by curve fitting to pseudo first order rate equations.

The final absorbances after cyclization were smaller than those of hydantoins $\mathbf{3b^{2-}}$ or $\mathbf{3c^{2-}}$ at the same concentration. This could be because the reaction had reached an equilibrium, or a result of hydrolysis of the ureido function in the carbamoyl aspartic acid. The latter possibility was excluded by treating the end product with HCl. Because the absorbances of the hydantoins are more readily measured for the dianions, the resulting solution was made alkaline: this increased the absorbance to that expected at the same concentration. Repeated experiments were carried out as follows. 0.363 ml of a 5×10^{-2} M solution of **1b** were added to 25 ml of 0.5 M KOH, $I = 1.0$ M (KCl). The solution was allowed to stand at room temperature for 8 days and the spectrum taken after 1 : 3 dilution with 0.5 M KOH. 5 ml of the equilibrated solution were then acidified by mixing with 7.5 ml 1 M HCl and heated for 3 hours at 50 °C. 7.5 ml of 1 M KOH were added to this solution and the spectrum recorded.

(b) The *N*-isopropyl and *N*-cyclohexyl derivatives. Following the UV-spectral changes of compounds **1d** and **1e** in potassium hydroxide solutions indicated complete and all but direct conversion into the hydantoinacetic acids **3d** and **3e**. To exclude the possibility of a direct conversion of **1d** to **3d** the reaction course was examined by means of ^{1}H NMR in 1 M KOD in D_2O. This revealed the transient formation of up to 10–15% of *N*-isopropyl-carbamoylaspartic acids **2**, confirming that the reaction proceeds according to Scheme 3. The rate constants were then obtained by curve fitting of the absorption data at five wavelengths to the integrated rate equation for two first order consecutive reactions:

$$\mathbf{A} \xrightarrow{k_1} \mathbf{B} \xrightarrow{k_2} \mathbf{C}$$

where k_1 and k_2 are k_{12} and k_{23} of Scheme 3. Product analysis and NMR and UV spectra showed that the cyclization of **2d** and **2c** is practically irreversible.

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