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Effect of temperature on the acidolysis of *N*-acyl-*N*,α,α-trialkyl glycine amides as related to the nature of substituents

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Abstract: The *C*-terminal amide bond of *N*-acyl-*N*,α,α-trialkyl glycine amides is labile to acid and this has been currently assigned to steric crowding within the amino acid residue. However, our previous work has shown that in the acidolysis of some of these compounds steric hindrance seems to play a less important role than what one would expect. Thus, the cleavage of two sets of such compounds bearing different degrees of crowding was investigated at five different temperatures in order to clarify the effect of structure on reactivity in terms of enthalpy and entropy of activation. The compounds exhibited an Arrhenius-type behaviour, and both enthalpies and entropies of activation were calculated by taking advantage of the transition state theory. In addition, the kinetic data were analysed in terms of isokinetic relationships in order to find evidence to support that the compounds react under the same mechanism. The changes in the reaction rate are governed by the changes in both the enthalpy and the entropy of activation, which are related to bond energy and steric hindrance, respectively. In general, the entropies of activation are very negative for all compounds investigated, which reflects large steric constrictions associated with the formation of the transition state. In addition, they are very sensitive to the structure of the substrates.

Keywords: acidolysis; α,α-dialkyl glycine; rate constant; enthalpy of activation; entropy of activation; isokinetic relationship; structure-reactivity relationships

INTRODUCTION

Amino acids having two chains bonded to their α-carbon atom (α,α-dialkyl glycines) are useful for incorporation into the peptide chain of peptidomimetics [1,2]. This has led various authors to investigate the conformational behaviour of these compounds, in order to allow rationalising their structural features that may be incorporated into the structures of peptidomimetics, which makes them useful in practical pharmacological application [3,4]. Most of these amino acids are not commercially available and are not easy to synthesise owing to steric crowding, which reflects in their reactivity by causing serious problems concerning manipulation [5–7]. Nevertheless, we developed a methodology to synthesise these compounds starting with the preparation of *N*-acyl-*N*,α,α-trialkyl glycine amides by a Ugi–Passerini reaction, followed by full acidolytic cleavage of the product to yield the required amino acid or by selective acidolysis with trifluoroacetic acid (TFA) to give the corresponding *N*-acyl [8] or the *N*-acyl-*N*-alkyl derivative [9]. In principle, these amino acid derivatives may be elongated towards their *C*-terminus, thus offering a useful and direct route for their incorporation into peptides. As for the synthesis of the above derivatives, we took advantage of the lability of the amide bond at the *C*-terminus of the Ugi–Passerini adducts towards acid [10], their availability depending on the efficiency of the selective acidolysis. Bearing this in mind, we have been concerned with the investigation of the effect of the various substituents at these substrates on reactivity and selectivity. A mechanistic investigation of the cleavage of the *C*-terminal amide bond of *N*,α,α-trimethyl glycine derivatives with TFA carried out by Goodman and his co-workers [11] led the authors to postulate the acidolytic formation of a short-lived intermediate oxazolonium species, which would then undergo hydrolytic ring opening with trace water in the reaction solvent. In our investigation of the kinetic behaviour at 25 °C with four *N*-acyl-*N*-alkyl amides in each of two sets of derivatives of α,α-dimethyl glycine and α,α-dibenzyl glycine, we found evidence in support of such oxazolonium salt [12] and also that this species undergoes decomposition by either hydrolysis or acid-promoted elimination, or both [13]. The kinetic data obtained correlated well with the bulkiness of the substituents both at C^{α} and at the nitrogen atom of the *C*-terminal amide. However, when five analogous compounds differing from each other only in size and branching within the substituents at C^{α} were submitted to a similar kinetic investigation, polar effects were shown to override steric ones. A good correlation with structure could be obtained only when both effects were taken into account and evaluated

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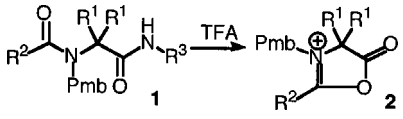
by means of a Taft multi-parametric treatment of the kinetic data [14]. This outcome prompted us to further investigate the acidolysis of the above two sets of compounds (Scheme 1) by collecting kinetic data at five different temperatures in order to obtain information on their thermodynamics of activation with the aim to clarify the effect of structure on reactivity in terms of enthalpy and entropy of activation. We now present the results of this investigation.

EXPERIMENTAL

Substrates **1a**–**h** were prepared according to the procedures published elsewhere [9]. All solvents and reagents used were obtained from commercial sources. Tri-distilled and deionised water was used in HPLC experiments. HPLC measurements were carried out with a Jasco PU-980 intelligent HPLC Pump, a Shimadzu SPD-6AV UV-Vis Spectrophotometric Detector and a Shimadzu C-R6A Chromatopac Printer. A reverse-phase LiChrospher® 100 RP-18 (5 μm) column was used throughout the work. Temperature stability (±0.01 °C) was maintained in the thermostatic bath throughout the kinetic work with the aid of a HAAKE Circulator DL30 and Precision® thermometers. Peak areas were measured for each substrate **1a**–**h** at five to seven different concentrations in neat acetonitrile in the interval 0.005–0.035 mol dm^{-3} and at least three results were obtained for each concentration. The analysis of these data revealed an excellent linear relationship between concentrations and peak areas, which allowed calculation of reaction rate constants directly from peak areas. For the kinetic measurements, each substrate [9] was dissolved in dry acetonitrile followed by addition of the amount of TFA necessary to give a 0.02 M solution of substrate containing the acid at a concentration of 5%. These solutions were allowed to react until all the reagents had been consumed. Samples were collected for HPLC monitoring at regular intervals and injected as quickly as possible to minimise errors due to temperature fluctuations. In general, the peak areas were highly reproducible. The detection wavelength was 260 nm, and eluents of acetonitrile/water mixtures with compositions of 1 : 1, 2 : 1 and 3 : 1 (v/v) were used. For each substrate, the dependence of the reaction rate on the TFA concentration was investigated for several values within the interval 1–20%; in all cases a first-order behaviour was found.



Pmb = 4-CH_3O–C_6H_4–CH_2

1a: R^1 = Me, R^2 = $PhCH_2$, R^3 = Pmb
1b: R^1 = Me, R^2 = Ph, R^3 = Pmb
1c: R^1 = Me, R^2 = $PhCH_2$, R^3 = C_6H_{11}
1d: R^1 = Me, R^2 = Ph, R^3 = C_6H_{11}
1e: R^1 = $PhCH_2$, R^2 = $PhCH_2$, R^3 = Pmb
1f: R^1 = $PhCH_2$, R^2 = Ph, R^3 = Pmb
1g: R^1 = $PhCH_2$, R^2 = $PhCH_2$, R^3 = C_6H_{11}
1h: R^1 = $PhCH_2$, R^2 = Ph, R^3 = C_6H_{11}

Scheme 1

RESULTS AND DISCUSSION

All reactions exhibited pseudo-first-order behaviour with respect to the amino acid derivative, which is shown by the excellent linear variation of ln A, where A is an HPLC peak area, as a function of time. As an example, ln A vs t plots for one experiment involving each substrate at 30 °C are presented in Figure 1. The observed rate constants, k, were calculated by the linear least squares methodology for a straight line. The standard deviation of the fits never exceeded 1% of the corresponding k value. Table 1 presents the mean values of at least three independent kinetic experiments for each compound and temperature, and the values of the corresponding mean deviations, dk. It was not possible to investigate all compounds under the same range of temperatures owing to the large differences in their reactivity. However, the same number of points (five) equally spaced was used for all compounds, the reference temperature of 25 °C being always within the interval of temperatures, as is statistically required. Moreover, the acidolysis of all compounds has been done at three common temperatures, 20, 25 and 30 °C, to further support the soundness of comparisons. It was found that the rate of substrate cleavage increases appreciably with temperature. The effect of temperature on the observed rate constants can be statistically best described by an Arrhenius-type equation, $\ln k = a_0 + a_1/T$, where a_0 and a_1 are estimated parameters (Figure 2). The least squares procedure was applied to the variation of ln k with T^{-1} using all the reliable data points. The values of the Arrhenius activation energy, E_A, are presented in Table 2. Taking advantage of the transition state theory, the enthalpies of activation, $\Delta^{\ddagger}H$, and the entropies of activation, $\Delta^{\ddagger}S$, were calculated. These values at 25 °C are also presented in Table 2 together with the mean standard deviations. For each compound, the values found for the enthalpy of activation at different temperatures did not differ much from temperature to temperature; the same applies to the entropies of activation.

When a series of structurally related substrates undergo the same general reaction, the enthalpies and the entropies of activation sometimes satisfy the so-called isokinetic relationship (IKR) represented by the equation $\partial\Delta^{\ddagger}H = \beta\partial\Delta^{\ddagger}S$, where β is the isokinetic temperature [15–17]. In order to obtain statistically correct calculations, Berthelot and co-workers [18,19] proposed a computer programme to test the kinetic data according to the new equation $\log k_{ij} = y^o + b_i(T_{ij}^{-1} - \beta^{-1})$, which is equivalent to the above. An analysis of the kinetic data obtained for the eight amides does not allow verifying the occurrence of an IKR. Nevertheless, for the plot log k vs $1/T$ concerning the dimethyl derivatives (compounds **1a**–**d**), an IKR with $s_0 = 0.0383$ and $s_{00} = 0.0371$ was found and accepted as an approximate relationship ($F = 1.47$; $\psi = 0.097$); the isokinetic temperature $\beta = 784$ K was determined although with

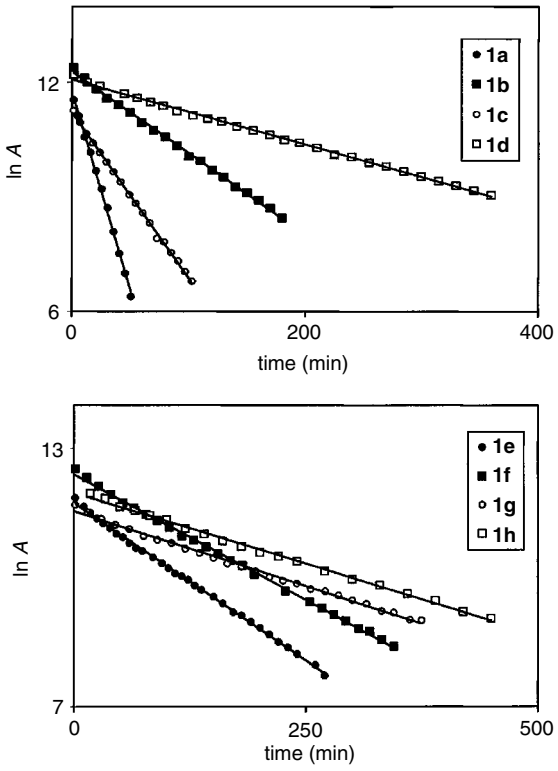


Figure 1 Plots of values of ln A vs t for acidolysis of α,α-dialkyl glycine derivatives **1a–d** and **1e–h** ($T = 30\,^{\circ}C$).

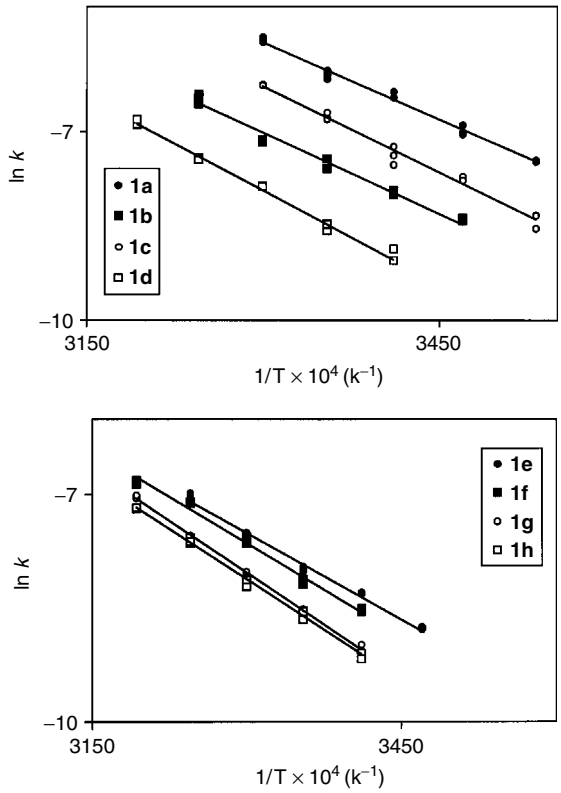


Figure 2 Temperature dependence of rate constants (k) for acidolysis of α,α-dialkyl glycine derivatives **1a–d** and **1e–h**.

great uncertainty. However, the plot for the dibenzyl analogues (compounds **1e–h**) confirms a valid IKR within experimental uncertainty, $s_0 = 0.0259$ and $s_{00} = 0.0271$ ($F = 0.40$, accepted; $\psi = 0.085$, accepted); the optimum value of β is 351 K. Other combinations were tested, namely, the sets of compounds with identical substituent at R^2 and R^3, but no valid IKR was found.

CONCLUSIONS

For the reaction under investigation, the reactivities are greatly influenced by the nature of the substituents. The changes in the reaction rates are not only governed by changes in the enthalpy of activation but also, as one would expect, by changes in the entropy of activation, which are related to bond energy and steric hindrance, respectively. As the compounds having benzyl at R^1 react under the same reaction mechanism, as revealed by a valid IKR, and this is most probably also the case of their dimethyl analogues, one may compare the results obtained with them and conclude that (i) the reactivities are higher and the values of the activation functions are lower for Pmb amides than in the case of the corresponding cyclohexyl amides and (ii) replacement of $PhCH_2$ by Ph at R^2 decreases reactivity and increases

Table 1 Rate constants and mean deviations ($k \pm dk$) $\times 10^4$ (s^{-1}) for the acidolysis of α,α-dialkyl glycine derivatives at different temperatures

Compound	10 °C	15 °C	20 °C	25 °C[a]	30 °C	35 °C	40 °C
1a	5.74 ± 0.07	9.35 ± 0.57	16.41 ± 0.71	23.14 ± 0.99	40.44 ± 1.18	—	—
1b	—	2.27 ± 0.05	3.46 ± 0.12	5.07 ± 0.35	7.84 ± 0.15	15.89 ± 0.83	—
1c	2.26 ± 0.20	4.34 ± 0.12	6.29 ± 0.62	11.56 ± 0.44	19.40 ± 0.12	—	—
1d	—	—	1.30 ± 0.12	1.97 ± 0.10	3.83 ± 0.02	5.92 ± 0.09	10.64 ± 0.44
1e	—	1.57 ± 0.03	2.49 ± 0.03	3.38 ± 0.08	5.32 ± 0.13	9.13 ± 0.17	—
1f	—	—	1.99 ± 0.04	2.91 ± 0.06	5.02 ± 0.16	8.16 ± 0.06	10.70 ± 0.28
1g	—	—	1.21 ± 0.05	1.96 ± 0.04	3.27 ± 0.02	5.25 ± 0.07	8.84 ± 0.18
1h	—	—	1.08 ± 0.04	1.95 ± 0.03	2.83 ± 0.12	4.95 ± 0.15	7.61 ± 0.05

[a] Values from Refs. [13,14].

Table 2 Arrhenius activation energy (E_A) and enthalpy of activation ($\Delta\ddagger H$) and entropy of activation ($\Delta\ddagger S$) at 25 °C[a]

Compound	**1a**	**1b**	**1c**	**1d**	**1e**	**1f**	**1g**	**1h**
E_A (kJ mol^{-1})	68.1	71.3	75.6	82.4	62.8	68.0	75.9	73.8
$\Delta\ddagger H$ (kJ mol^{-1})	65.6	68.8	73.4	79.9	60.4	65.5	73.4	71.3
$\Delta\ddagger S$ (J mol^{-1}K^{-1})	−75	−76	−55	−47	−108	−92	−69	−77

[a] Mean standard deviations: σ (E_A) = 1.0 kJ mol^{-1}; σ ($\Delta\ddagger H$) = 1.0 kJ mol^{-1}; σ ($\Delta\ddagger S$) = 3 J mol^{-1} K^{-1}.

the values of the activation functions except for the case of **1g** and **1h**. Each compound having $PhCH_2$ at R^1 exhibits a lower reactivity (smaller rate constants) and almost always lower values for the activation functions than the corresponding Me analogue. In general, the entropies of activation are very negative for all the eight compounds investigated, which reflects large steric constrictions associated with the formation of the transition state. They are very discriminative, since their values change a lot from compound to compound according to the structure. Thus, it is not unexpected that some of the compounds in the α,α-dibenzyl glycine series present really very negative values. The difference between the α,α-dimethyl and the α,α-dibenzyl glycine derivatives is in full agreement with the difficulties met while manipulating derivatives of α,α-dialkyl glycines having side chains bulkier than methyl.

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