Electrochemical Determination of the pK_a of Weak Acids in *N*,*N*-Dimethylformamide

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Abstract: The electroreduction of NH-protic α-bromo amides in DMF generates an enolate-type base which undergoes a fast proton transfer from the parent compound (self-protonation), affording the corresponding reduced amide together with the conjugate base of the bromo amide. When an acid weaker than the bromo amide is added to the solution, a current increase in a potential region more negative than the main voltammetric reduction peak is observed under suitable conditions. The voltammetric pattern is in agreement with an unfavored protonation of the conjugate base of the starting compound by the added proton donor with regeneration of the electroactive bromo amide. The theoretical analysis of this reduction sequence has been carried out, and the voltammetric profiles have been simulated. Comparison of the experimental and simulated voltammetries led to the determination of the acidity difference, ΔpK_a, between the α-bromo amide and the added acid. For each α-bromo amide it was possible to obtain ΔpK_a data ranging from 1.4 to 4.2. The use of α-bromo amides of different acidity with the same exogenous acids provided the link between the different sets of relative acidities. In this way, using six α-bromo amides, a relative acidity scale encompassing an overall pK_a variation in DMF of about 10 units could be established. The relative scale was then anchored to the low pK_a scale in DMF through both the determination of the acidity of selected acids and using a correlation between literature pK_a data obtained in both DMF and DMSO. The application of this original electrochemical mechanism provided absolute pK_a data in DMF ranging from about 16 to 26, i.e., a pK_a region that is practically unexplored in this solvent.

Introduction

Potentiometric and conductometric methods have been widely employed to determine the equilibrium acidity of strong or relatively strong organic acids, such as phenols and carboxylic acids, in dipolar nonprotogenic solvents.[1] Although the bulk of literature data comes from measurements by the above techniques and spectrophotometric methods, less traditional electrochemical approaches have been reported for particular cases.

A polarographic acidity scale of CH-acids in DMF was developed by Reutov, Beletskaya, and Butin.[2a] The method is based on the use of a few selected pK_a data and the irreversible reduction potentials of organomercuric compounds, R_2Hg, corrected by taking into account the transfer coefficient α. However, owing to the use of a nonthermodynamic electrochemical quantity and of the pK_a data utilized, the equilibrium acidities thus determined are only rough estimates.[2b] Sawyer and co-workers presented a voltammetric method in which the half-peak potentials for proton reduction are utilized to determine effective acidities of Brønsted acids.[3] Breslow and co-workers combined the potentials for the stepwise reduction of carbocations in organic solvents with the pK_{R^+} values determined in aqueous acid to calculate pK_a's.[4] A different approach, making use of gas-phase bond dissociation energies and the redox potential of the radical/carbanion couples, was later proposed.[5] Kern, Sauer, and Federlin reported a correlation between the oxidation potential of carbanions in DMSO and the pertinent pK_a values. By using this correlation, some pK_a values of weak CH-acids were evaluated.[6] Very recently, Wayner, Griller, and co-workers reported the reduction potentials of radicals generated photolytically in acetonitrile. These data were combined with gas-phase bond dissociation energies to calculate the pK_a's of the corresponding, very weak CH-acids.[7] Thermodynamic cycles making use of electrochemical potentials and different thermodynamic data have been applied to the determination of the pK_a's of reactive species such as radical cations,[8a-c] dications,[8c] carbocations,[8d] carbanions, and neutral radicals.[8e]

In general, most of the pK_a values determined by electrochemical methods other than potentiometry and conductometry suffer from the use of irreversible redox potentials (sluggishness of the heterogeneous electron transfer or fast chemical reaction following the reversible or quasi-reversible electron transfer) and/or approximate evaluations of the solution bond dissociation energies. On the other hand, the potentiometric approach cannot be confidently extended to the determination of the acidity in the high pK_a region, as pointed out by Ritchie[9] and Bordwell and co-workers.[10] In this paper, some peculiar features of a self-protonation electroreduction mechanism will be shown to provide a new way to obtain acidity data for weak acids in DMF.

The electrochemical reduction of organic compounds is usually associated with the formation of basic species, such as carbanions, radical anions, and dianions. In weakly protogenic media, an interesting mode of reaction of electrogenerated bases is the self-protonation reaction, a proton-transfer reaction in which the actual proton source for the electrogenerated base is the starting compound itself.[11] Since in the following extensive use will be

(1) (a) *Treatise on Analytical Chemistry*; Kolthoff, I. M., Elving, P. J., Eds.; Wiley: New York, 1979; Part I, Vol. 2. (b) Ritchie, C. D. In *Solute-Solvent Interactions*; Coetzee, J. F., Ritchie, C. D., Eds.; Marcel Dekker: New York, 1969; Vol. 1, Chapter 4.

(2) (a) Reutov, O. A.; Beletskaya, I. P.; Butin, K. P. *CH-Acids*; Pergamon: Oxford, 1978. (b) See for instance: Streitwieser, A., Jr., Juaristi, E.; Nebenzahl, L. L. *Stud. Org. Chem. (Amsterdam)* **1980**, *5*, 323-381.

(3) Barrette, W. C., Jr.; Johnson, H. W., Jr.; Sawyer, D. T. *Anal. Chem.* **1984**, *56*, 1890-1898.

(4) Breslow, R.; Chu, W. *J. Am. Chem. Soc.* **1973**, *95*, 411-418.

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(6) Kern, J. M.; Sauer, J. D.; Federlin, P. *Tetrahedron* **1982**, *38*, 3023-3033.

(7) (a) Wayner, D. D. M.; McPhee, D. J.; Griller, D. *J. Am. Chem. Soc.* **1988**, *110*, 132-137. (b) Sim, B. A.; Griller, D.; Wayner, D. D. M. *J. Am. Chem. Soc.* **1989**, *111*, 754-755.

(8) (a) Nicholas, A. M. P.; Arnold, D. R. *Can. J. Chem.* **1982**, *60*, 2165-2179. (b) Bordwell, F. G.; Cheng, J.-P. *J. Am. Chem. Soc.* **1991**, *113*, 1736-1743 and references therein. (c) Parker, V. D.; Tilset, M. *J. Am. Chem. Soc.* **1988**, *110*, 1649-1650. (d) Nagaoka, T.; Berinstain, A. B.; Griller, D.; Wayner, D. D. M. *J. Org. Chem.* **1990**, *55*, 3707-3708. (e) Parker, V. D.; Tilset, M.; Hammerich, O. *J. Am. Chem. Soc.* **1987**, *109*, 7905-7906.

(9) Ritchie, C. D. In *Solute-Solvent Interactions*; Coetzee, J. F., Ritchie, C. D., Eds.; Marcel Dekker: New York, 1976; Vol. 2, Chapter 12.

(10) Matthews, W. S.; Bares, J. E.; Bartmess, J. E.; Bordwell, F. G.; Cornforth, F. J.; Drucker, G. E.; Margolin, Z.; McCallum, R. J.; McCollum, G. J.; Vanier, N. R. *J. Am. Chem. Soc.* **1975**, *97*, 7006-7014.

(11) Although the term "self-protonation" is, stricto sensu, incorrect since it does not refer to a proton-transfer reaction between two identical molecules, it is commonly employed by electrochemists to describe the proton-transfer reaction between an electrogenerated base and the parent compound.[11a] Although the occurrence of such a mechanism has been often reported or can be suspected on the basis of the electrochemical results, detailed kinetic studies have been reported only recently[11c] on the basis of pertinent theoretical treatments.[11b,d-f] (a) See, for instance, ref 11b and references therein. (b) Amatore, C.; Capobianco, G.; Farnia, G.; Sandonà, G.; Savéant, J. M.; Severin, M. G.; Vianello, E. *J. Am. Chem. Soc.* **1985**, *107*, 1815-1824. (c) For leading references, see: Roffia, S.; Concialini, V.; Paradisi, C.; Maran, F.; Vianello, E. *J. Electroanal. Chem.* **1991**, *302*, 115-129. (d) Triebe, F. M.; Borhani, K. J.; Hawley, M. D. *J. Am. Chem. Soc.* **1979**, *101*, 4637-4645. (e) Amatore, C.; Anne, A.; Florent, J. C.; Moiroux, J. *J. Electroanal. Chem.* **1986**, *207*, 151-160. (f) Arevalo, M. C.; Farnia, G.; Severin, M. G.; Vianello, E. *J. Electroanal. Chem.* **1987**, *220*, 201-211.

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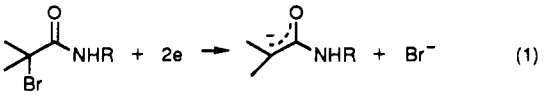
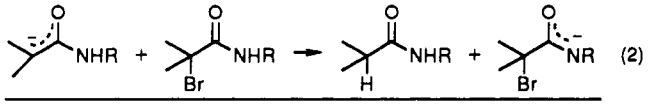
made of the acquired knowledge on the reduction mechanism of self-protonating α-bromo amides, the main results we obtained in this field will be shortly reviewed.[12] The electroreduction of NH-protic α-bromo amides in dipolar nonprotogenic solvents, such as DMF, affords two irreversible voltammetric peaks. A schematic picture of the electrode reaction mechanism is given for α-bromoisobutyramides in eqs 1–3.


(1)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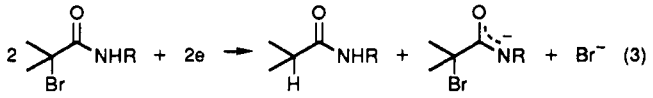
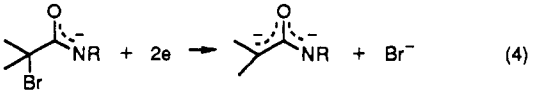

(3)

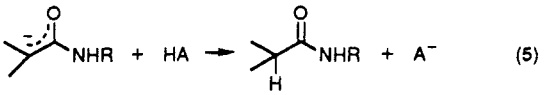
The reduction at the potentials of the first peak causes the breaking of the C–Br bond and leads to the formation of the corresponding carbanion (eq 1).[13] Although the latter species is a two-electron reduction product, one electron per molecule is apparently consumed in the process. This is a consequence of the fast and irreversible protonation of the electrogenerated carbanion by a molecule of the parent compound (typical rate constants span in the 10^7–10^8 M^{-1} s^{-1} range),[12c] affording the reduced amide and the conjugate base of the bromo amide (step 2). Since half of the α-bromo amide is transformed into an anionic species which is not reducible at the potentials of the first peak, the electron consumption is cut to one electron per molecule (overall stoichiometry 3).

The bromo amide anion undergoes dissociative electron transfer at more negative potentials (second peak) according to the overall two-electron stoichiometry 4, but, owing to the conversion of one-half of the starting compound into the bromo amide anion (eq 3), an apparent one-electron process should also be observed at the second peak. However, since the bromo amide anion is


(4)

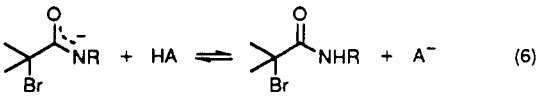
a labile species releasing bromide ion in an intramolecular nucleophilic substitution,[14] an apparent one-electron second peak can be detected only when the bromo amide anion is stable in the time scale of cyclic voltammetry. Some results of a current investigation on the detection of electroactive labile intermediates have been used to obtain kinetic information on the decay of α-bromo amide anions.[14] From the results of such analyses and the determination of the self-protonation rate constant, we know the scan rate range and thus the time window is which the bromo amide anion is quantitatively formed (total efficiency of the self-protonation reaction) and is stable (insufficient time for the chemical decay to take place). In this time window, we have the opportunity to provoke and study other reactions involving the bromo amide anion. In this paper, we will report the effect of adding an acidic species to the solution, the way by which the voltammetric curves are affected, and how to obtain information on proton-transfer reactions other than self-protonation.

On addition of an acid, HA, considerably stronger than the bromo amide, the self-protonation step 2 is suppressed owing to the quantitative protonation of the electrogenerated carbanion by the exogenous acid:


(5)

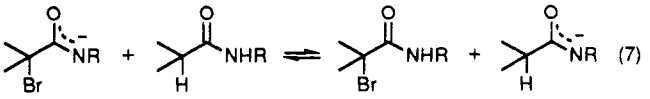
In this way, the first peak becomes a two-electron peak and the second one disappears, since the formation of the bromo amide anion is prevented.[12]

On the other hand, we observed that if the acid HA is weaker than the bromo amide, the voltammetric pattern is modified in quite a different way: the first peak remains unaffected, the second one decreases, and a broad current increase is observed in between the two main peaks.[15] The following equilibrium was hypothesized[15] and later confirmed[16] to play a significant role in the overall mechanism:


(6)

In this reaction, it is the bromo amide anion formed upon self-protonation, instead of the electrogenerated carbanion, which undergoes the proton transfer from the acid HA. In the unfavored reaction 6, the electroactive starting compound is regenerated, thus causing the current increase.

It is worth noting that in the self-protonation step 2 we have the formation of the reduced amide which is also an NH-acid, although weaker than the α-bromo amide. Therefore, in principle, the acid HA could be the endogenous acid represented by the reduced amide, as shown in the following equation:


(7)

In any case, the equilibrium constant K of either the proton-transfer reaction 6 or 7 is linked to the difference in thermodynamic acidity, ΔpK_a, between the bromo amide and the exogenous or endogenous acid, respectively, and is defined as $-\log K = pK_a(\text{acid}) - pK_a(\text{bromo amide}) = \Delta pK_a$. The analysis of the above proton-transfer equilibria 6 and 7, referred to as Exogenous Regeneration (ExR) or Endogenous Regeneration (EnR) depending on the nature of the proton donor, may then provide a method to calculate relative acidities, as shown in a preliminary note on the ExR mechanism.[16]

In this paper, the voltammetric theoretical analysis will be presented for both cases. Six bromo amides were used to obtain acidity data for several weak acids through comparison of the simulated voltammetries with the experimental curves. The sets of data collected with the various bromo amides, once linked to one another and anchored to the low pK_a scale in DMF, will be shown to provide equilibrium acidities covering a pK_a region from ca. 16 to 26, i.e., a pK_a region which is practically unexplored in this solvent.

Results and Discussion

EnR Mechanism. In order to evaluate the interference caused by the formation of the reduced amide on the voltammetric pattern of the corresponding α-bromo amide, we resorted to comparisons of the experimental voltammograms with those simulated on the basis of the reduction scheme eq 1 + eq 2.[11f] Close comparison with the experimental curves revealed that, in the region between the first and second peak, the experimental curves at low potential

(12) (a) Maran, F.; Vianello, E.; D'Angeli, F.; Cavicchioni, G.; Vecchiati, G. *J. Chem. Soc., Perkin Trans. 2* **1987**, 33–38. (b) Maran, F.; Fabrizio, M.; D'Angeli, F.; Vianello, E. *Tetrahedron* **1988**, *44*, 2351–2358. (c) Maran, F.; Roffia, S.; Severin, M. G.; Vianello, E. *Electrochim. Acta* **1990**, *35*, 81–88.

(13) In line with the general electroreduction behavior of organic halides,[13a,b] the overall reduction (1) is actually a multistep process, the rate being determined by the uptake of the first electron. The reductive bond cleavage reaction affords a radical which is reduced at the electrode as soon as it is produced, with formation of the carbanion. (a) Hawley, M. D. In *Encyclopedia of Electrochemistry of the Elements*; Bard, A. J., Lund, H., Eds.; Dekker: New York, 1980; Vol. XIV. (b) Becker, J. Y. In *Chemistry of Functional Groups, Supplement D*; Patai, S., Rappoport, Z., Eds.; Wiley: New York, 1983; Chapter 6, pp 203–285.

(14) Maran, F.; Vianello, E.; Severin, M. G.; D'Angeli, F. Work in progress.

(15) Maran, F.; Vianello, E. *Stud. Org. Chem. (Amsterdam)* **1987**, *30*, 231–234.

(16) Maran, F.; Severin, M. G.; Vianello, E. *Tetrahedron Lett.* **1990**, *31*, 7523–7526.

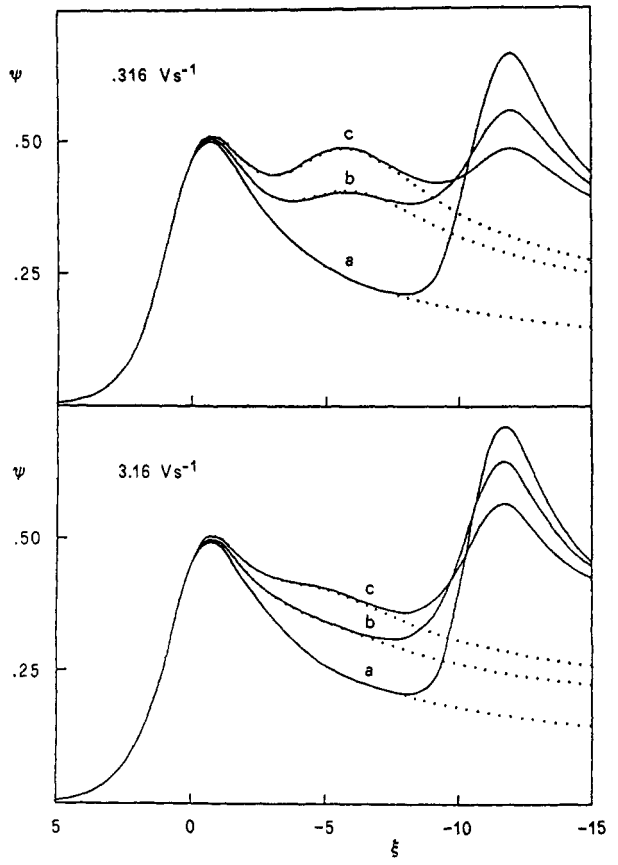


Figure 1. Comparison of experimental and simulated voltammetries for the EnR mechanism. Solid lines pertain to experimental voltammograms obtained in DMF–0.1 M TEAP at 25 °C with 1.34 mM $BrCMe_2CONHPh$ at 0.316 V/s (upper figure) and 3.16 V/s (lower figure) before (curves a, $\gamma = 0$) or after the addition of $HCMe_2CONHPh$ (curves b, $\gamma = 10$; curves c, $\gamma = 20$). The dotted lines are the corresponding simulated voltammetries (second peak not included in the calculations). The abscissa and ordinate are the dimensionless potential ξ and current ψ, respectively.

scan rates, v, were not exactly reproduced in that the simulated current was slightly lower than the experimental one. Such a difference vanished on increasing v. On the basis of the hypothesis that the excess current observed experimentally was caused by a regeneration of the starting material through equilibrium 7, the effect of adding the reduced amide to a solution of the corresponding α-bromo amide was investigated. Qualitatively, it was observed that an increase of the current in the potential region between the first and second peaks and a decrease of the reduction peak of the bromo amide anion (second peak) occurred. The first peak was left untouched in shape, position, and current. The current increase between the two main peaks took the shape of a broad peak (in the following referred to as the third peak) when the addition of the reduced amide was large enough. As before, the above effects tended to vanish on increasing v.

Since the above observations are general for all of the protic α-bromo amides investigated, the following α-bromoisobutyramides were selected as representative examples: 2-bromo-2-methyl-*N*-(*p*-cyanophenyl)propanamide, 2-bromo-2-methyl-*N*-(*p*-chlorophenyl)propanamide, 2-bromo-2-methyl-*N*-phenylpropanamide, and 2-bromo-2-methyl-*N*-benzylpropanamide. The selection took into account that the substrates differ significantly in acidity[12c,17] and that they afford relatively stable bromo amide anions (stability >95% for v values higher than 0.2, 0.2, 0.316, and 2 V/s, respectively).[14]

All experiments were carried out in DMF–0.1 M Et_4NClO_4 at 25 °C by using a mercury microelectrode and typically a 1 × 10^{-3} M substrate concentration. Linear sweep voltammetries were recorded at selected scan rates in the range 0.2–50 V/s. After the addition of the corresponding isobutyramide, a new set of voltammetries was recorded under the same conditions. The molar ratio γ between the added acid and the bromo amide was varied from 10 to 20. The sets of experimental curves were then compared with the voltammetries simulated for the EnR mechanism (see the Experimental Section). The second peak was not included in the simulation procedure as it is unimportant from a quantitative point of view in the present study. For each γ value and for all scan rates employed, good overlapping of the experimental curves with the simulated voltammetries was obtained (see the example reported in Figure 1) by proper choice of the equilibrium constant K of reaction 7 and the dimensionless parameter λ, which describes the kinetics of the process

$$\lambda = k_b RTC^*/\alpha Fv \qquad (8)$$

where k_b is the backward rate constant of the proton-transfer 7, C^* the analytical concentration of the bromo amide, and α the transfer coefficient. The forward rate constant k_f of proton-transfer 7 is readily obtained from λ, and $K = k_f/k_b$. Optimization of the simulated voltammetric curves (see the Experimental Section) afforded the results reported in Table I.

Table I. Equilibrium and Kinetic Acidity Data for $HCMe_2CONHR$ Relative to $BrCMe_2CONHR$ Obtained in DMF at 25 °C According to the EnR Mechanism

R	K	ΔpK_a	k_f (M^{-1} s^{-1})	k_b (M^{-1} s^{-1})
p-C_6H_4CN	4.2×10^{-4}	3.38	1.8×10^5	4.4×10^8
p-C_6H_4Cl	3.5×10^{-4}	3.46	1.2×10^5	3.3×10^8
C_6H_5	3.1×10^{-4}	3.51	7.1×10^4	2.3×10^8
$CH_2C_6H_5$	2.5×10^{-4}	3.60	1.8×10^5	7.3×10^8

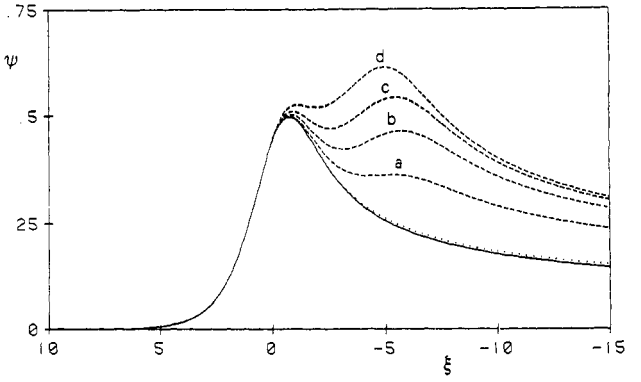


Figure 2. Simulated voltammetries for the ExR mechanism: effect of increasing the concentration ratio γ. Dashed lines pertain to the following γ values: a, 2; b, 5; c, 10; d, 20. $K = 10^{-3}$, $\lambda = 10^5$. The solid line represents the uncomplicated one-electron voltammetry and the dotted line the corresponding endogenous contribution ($\gamma = 0$, $\lambda = 10^5$) for the typical value of the equilibrium constant of the proton transfer 7, $K = 3 \times 10^{-4}$. The abscissa and ordinate are the dimensionless potential ξ and current ψ, respectively.

The ΔpK_a values reported in Table I indicate that a noticeable acidifying effect is brought about by replacing the α-hydrogen with bromine and further support a recent finding that α-substitution in amides causes a variation in acidity that is scarcely dependent on the substituent at nitrogen.[18] From a quantitative point of view and taking into account the different halogens considered, our results are in agreement with those concerning the ΔpK_a values between α-fluoro amides and corresponding amides.[18]

ExR Mechanism. The analysis performed in the preceding section points to a good agreement between the experimental and simulated voltammetries, thus supporting the overall mechanism

(17) Maran, F.; Goldblum, A.; D'Angeli, F.; Vianello, E. *Electrochim. Acta* **1988**, *33*, 227–229.

(18) Bordwell, F. G.; Fried, H. E.; Hughes, D. L.; Lynch, T.-Y.; Satish, A. V.; Whang, Y. E. *J. Org. Chem.* **1990**, *55*, 3330–3336.

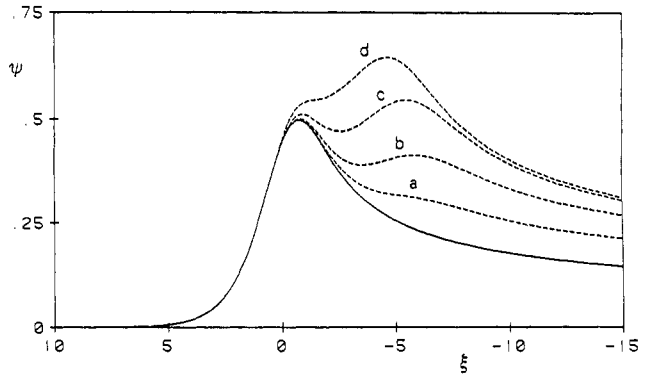


Figure 3. Simulated voltammetries for the ExR mechanism: effect of increasing the equilibrium constant K of the proton transfer 6. Dashed lines pertain to the following K values: a, 10^{-4}; b, 3×10^{-4}; c, 10^{-3}; d, 3×10^{-3}. $\lambda = 10^5$, $\gamma = 10$. The solid line represents the uncomplicated one-electron voltammetry. The abscissa and ordinate are the dimensionless potential ξ and current ψ, respectively.

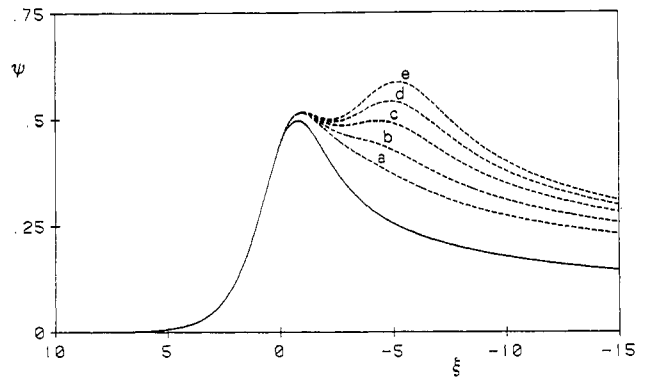


Figure 4. Simulated voltammetries for the ExR mechanism: effect of increasing the kinetic parameter λ. Dashed lines pertain to the following λ values: a, 10^3; b, 3×10^3; c, 10^4; d, 3×10^4; e, 10^5. $K = 10^{-3}$, $\gamma = 15$. The solid line represents the uncomplicated one-electron voltammetry. The abscissa and ordinate are the dimensionless potential ξ and current ψ, respectively.

and providing a basis for the extension of the voltammetric analysis to the effect of exogenous acids weaker than the bromo amide itself. In the simulated ExR mechanism (see the Experimental Section), the contribution from the third peak due to the reduced amide, formed together with the bromo amide anion in a 1:1 ratio, was neglected. A posteriori it was verified that, owing to the large third peak effects that we are going to describe, the error introduced by such a simplification is lower than the actual experimental error (see for instance the dotted line in Figure 2).

A fundamental requirement is that the added exogenous acid is not in competition with the acidic bromo amide in the protonation of the electrogenerated carbanion. In other words, the self-protonation reaction 2 must be always quantitative. On the other hand, the acidity of the exogenous proton donor should not be much lower than that of the bromo amide, otherwise too high γ values would be required.

The modifications on the voltammetric pattern brought about by properly selected exogenous acids are of the same type as described in the preceding section, i.e., practically no effect on the first peak, growth of the third peak, and decrease of the second peak. At this point, it is useful to analyze separately the effects caused by the variation of the three parameters, γ, K, and λ, on the simulated curves. The effect on the simulated voltammetric curves of increasing γ for fixed values of λ and K is depicted in Figure 2. The main features are a current increase and a positive shift of the third peak potential. A similar effect is brought about by an increase of K, as can be seen in Figure 3, for fixed values of γ and λ. Finally, when K and γ are kept constant, the increase of the kinetic parameter λ causes a current increase and a negative shift of the third peak potential (Figure 4).

Table II. Equilibrium Acidities of a Series of NH-Acids Relative to $BrCMe_2CONHPh$ Obtained in DMF at 25 °C According to the ExR Mechanism

HA	pK_a[a]	γ[b]	K	ΔpK_a[c]
formanilide	19.4^d	0.6;0.9	3.6×10^{-2}	1.44
N,N'-diphenylurea	19.5_5	0.6;0.9	$1.8_5 \times 10^{-2}$	1.73
carbazole	19.9	1;2	1.7×10^{-2}	1.77
phenylacetanilide	20.6^e	2;5	2.1×10^{-3}	2.68
indole	20.9_5	2;5	1.7×10^{-3}	2.77
acetanilide	21.4_5	10;20	3.9×10^{-4}	3.41
isonicotinamide	21.5	10;20	5.9×10^{-4}	3.23
phenoxazine	21.6_5	10;20	3.5×10^{-4}	3.46
nicotinamide	22.0	20;30	2.3×10^{-4}	3.64
2-thiophenecarboxamide	22.3	30;50	8.0×10^{-5}	4.10

[a] In DMSO at 25 °C.[19] [b] Ratios between the concentration of the exogenous acid and of the bromo amide. [c] The maximum error is ±0.03. [d] Reference 20. [e] Reference 18.

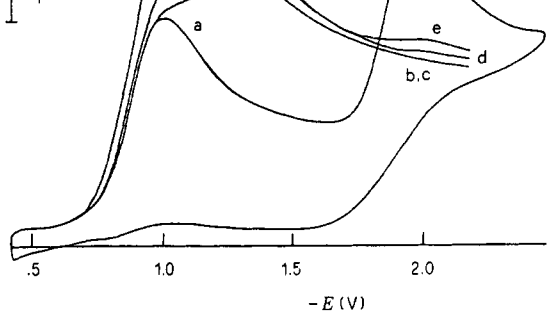


Figure 5. Cyclic voltammetry of 1.0 mM $BrCMe_2CONHPh$ (curve a) in DMF–0.1 M TEAP compared with the linear scan voltammetries obtained in the presence of phenol (curve b, $\gamma = 2$; curve c, $\gamma = 1$), benzoylhydrazine (curve d, $\gamma = 1$), or formanilide (curve e, $\gamma = 1$). $T = 25$ °C, $v = 0.2$ V/s.

At this point, one might wonder why the addition of an acid weaker than the α-bromo amide results in the development of a new peak, which is also due, as the first one, to the reduction of the α-bromo amide. This effect can be accounted for, at least qualitatively, by remembering that the weak acid is unable to compete with the bromo amide in the protonation of the electrogenerated carbanion. The only possible proton transfer involving the weak acid is then toward the conjugate base of the substrate (eq 6). Although such a reaction is thermodynamically unfavored, reduction of the resulting bromo amide continuously displaces the equilibrium. However, the amount of bromo amide regenerated depends on both the rate and equilibrium constants of reaction 6. Since a finite amount of time is needed for this process to occur, the current increase due to the reduction of the bromo amide thus produced is displaced along the time/potential axis.

The third peak effect brought about on the voltammetric pattern of 2-bromoisobutyranilide by a series of exogenous NH-acids of known pK_a in DMSO[18-20] was studied in DMF–0.1 M Et_4NClO_4 at 25 °C. Good fitting of the experimental voltammograms was obtained with the simulated curves for all scan rates and γ values investigated, of the same quality as that depicted in Figure 1. In

(19) Bordwell, F. G. *Acc. Chem. Res.* **1988**, *21*, 456–463.

(20) The pK_a of formanilide in DMSO originally reported[20a] to be 19.7 has been corrected to Bordwell's scale according to Petrov.[20b] (a) Kravtsov, D. N.; Peregudov, A. S.; Petrov, E. S.; Terekhova, M. I.; Shatenshtein, A. I. *Izv. Akad. Nauk SSSR, Ser. Khim.* **1981**, 1259. (b) Petrov, E. S. *Russ. Chem. Rev.* **1983**, *52*, 1144–1155.

Table II are reported experimental conditions and equilibrium results obtained for the NH-exogenous proton donors meeting the above requirements of the method.[21]

As stressed above, if the acidity of the added acid is not substantially lower than that of the bromo amide, both proton donors compete in the protonation of the electrogenerated carbanion, thus making the results unreliable. The transition from protonation of the carbanion by the exogenous acid (eq 5) up to conditions fulfilling the above requirement is illustrated in Figure 5. It was reported earlier[12c,15] that phenol (pK_a = 18.0 in DMSO)[19] is a good proton donor for the carbanion electrogenerated from α-bromoisobutyranilide, as its effect at $\gamma = 2$ is to double the current of the first peak and to cause the total disappearance of the second one. A broader peak is obtained in the presence of imidazole (pK_a = 18.6 in DMSO),[19] and this indicates that the protonation of the carbanion by the exogenous acid is not as effective as in the case of phenol and that some third peak contribution starts affecting the voltammetric profile. The third peak effect is more evident with benzoylhydrazine (pK_a = 18.9 in DMSO),[19] but the experimental curves indicate that some protonation of the carbanion by the exogenous acid is still taking place. We found that reasonable data can be obtained when $\Delta pK_a \approx 1.4$ (cf. Table II, formanilide), i.e. when the contribution of the exogenous acid to the carbanion protonation (eq 5) can be neglected relative to the self-protonation step 2. On lowering the acidity too much, typically for acids hypothetically exceeding $\Delta pK_a = 4.2$, third peak effects are hardly detectable and in any case too high γ values would be required.

Acidity Scale in DMF. The method just described provides relative pK_a values for a series of exogenous acids. Although the covered range can be extended to ca. 2.8 pK_a units at maximum, the use of bromo amides having different acidities allows one to investigate the acidities of a wide range of exogenous acids. Moreover, since the acidity of the same exogenous proton donor can be calculated relative to bromo amides of not too different acidity, a link between the various sets of data can be established, and thus all acidity determinations can be referenced to the most acidic bromo amide used. This procedure was applied to protic α-bromoisobutyramides carrying the following substituents at nitrogen: 4-CNC_6H_4 (**1**), 4-ClC_6H_4 (**2**), Ph (**3**), 4-$MeOC_6H_4$ (**4**), $CHPh_2$ (**5**), and CH_2Ph (**6**).

In order to establish the relative acidities of the selected α-bromo amides, we used an overlapping approach similar to the overlapping indicator method described by Bordwell and co-workers for acidity measurements in DMSO[10] and *N*-methylpyrrolidinone (NMP).[22] For each couple of bromo amides of proximate acidity, such as **1** and **2**, **2** and **3**, etc., the acidity of one or two acids was measured. Selected acids were then used, whenever possible, to obtain cross-links, such as between **1** and **3**, **2** and **4**, etc. These relative acidity determinations were processed, taking into account any possible independent route, to calculate the average pK_a values for all bromo amides with respect to the reference compound **1**. The ladder depicting the accessible pK_a range for each bromo amide,[23] the exogenous acids employed, and the pertinent ΔpK_a's is illustrated in Figure 6. The whole set of acidity data for all bromo amides and exogenous and endogenous acids investigated, along with the corresponding maximum error, are reported in Table III in the first and second columns, respectively.[24]

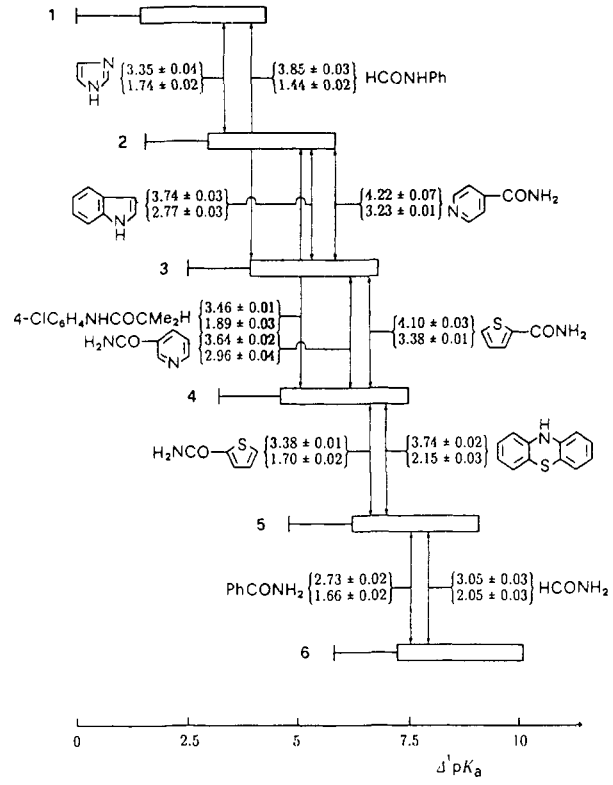


Figure 6. Ladder illustrating the acidities of α-bromo amides **1–6** in DMF with respect to compound **1**. The rectangles depict the ΔpK_a range in which ExR measurements are possible (see text). The acids used for the linkages are indicated as well as the experimental ΔpK_a's obtained with respect to the couple of bromo amides connected.

Table III. Relative pK_a Values in DMF at 25 °C and Corresponding Absolute Values in DMF and DMSO

substrate	Δ^1pK_a	error	pK_a^{DMF} [a]	pK_a^{DMSO} [b]
2-bromo-*p*-cyanoisobutyranilide	0.00	0.04	16.4	15.4[c]
2-bromo-*p*-chloroisobutyranilide	1.54	0.04	17.9	17.0[c]
phenol	2.00	0.08	18.4	18.0
2-bromoisobutyranilide	2.49	0.04	18.9	18.0[c]
2-bromo-*p*-methoxyisobutyranilide	3.18	0.04	19.6	18.8[c]
imidazole	3.32	0.08	19.7	18.6
p-cyanoisobutyranilide	3.38	0.06	19.8	19.0[c]
formanilide	3.89	0.08	20.3	19.4[d]
N,N'-diphenylurea	4.22	0.05	20.6	19.5_5
carbazole	4.26	0.06	20.6	19.9
2-bromo-*N*-benzhydryliso-butyramide	4.83	0.04	21.2	20.5[c]
p-chloroisobutyranilide	5.04	0.08	21.4	20.7[c]
phenylacetanilide	5.17	0.06	21.5_5	20.6[e]
indole	5.27	0.07	21.6_5	20.9_5
isonicotinamide	5.74	0.06	22.1	21.5
2-bromo-*N*-benzylisobutyramide	5.86	0.04	22.2	21.5_5[c]
acetanilide	5.90	0.05	22.3	21.4_5
phenoxazine	5.95	0.06	22.3	21.6_5
isobutyranilide	6.00	0.05	22.4	21.7[c]
nicotinamide	6.13	0.06	22.5	22.0
2-thiophenecarboxamide	6.56	0.07	22.9	22.3
phenothiazine	6.95	0.07	23.3	22.7
benzamide	7.54	0.06	23.9	23.3_5
formamide	7.90	0.07	24.3	23.4_5
2-pyrrolidinone	8.60	0.07	25.0	24.2
urethane	8.71	0.07	25.1	24.6
diphenylamine	9.16	0.07	25.5	24.9_5
N-benzylisobutyramide	9.46	0.06	25.8	25.3[c]

[a] See text. [b] Reference 19. [c] Values estimated through correlation 9. [d] Reference 20. [e] Reference 18.

(21) The proton transfer 6 between the bromo amide anion and the NH-acids of Table II proceeds in the thermodynamically favorable direction at almost diffusional rates so that the forward rate constants are in the 10^4–10^7 M^{-1} s^{-1} range, depending on the ΔpK_a. On the other hand, when both rate constants are significantly smaller, as we found for instance with CH-acids such as 2-bromofluorene and phenylacetonitrile, the overlapping of the experimental and simulated curves becomes inaccurate. In fact, with acids of this type, high λ values cannot be attained and thus high γ values are required in order to observe significant third peak effects. Under these experimental conditions, the resulting voltammograms are rather featureless because the third peak effect is more spread out along the voltammogram.

(22) Bordwell, F. G.; Branca, C. J.; Hughes, D. L.; Olmstead, W. N. *J. Org. Chem.* **1980**, *45*, 3305–3313.

(23) The accessible pK_a ranges for compounds **4**, **5**, and **6** are indeed slightly reduced on the right hand side with respect to the range $1.4 \leq \Delta pK_a \leq 4.2$ depicted in Figure 6. This is due to the fact that the bromo amide anions of **4**, **5**, and **6** are stable only for scan rates higher than ca. 1, 1, and 2 V/s, respectively, and thus measurements with exogenous acids providing ΔpK_a values higher than ca. 3.8 are precluded.

Table IV. Equilibrium Acidities of Benzoic Acids in DMSO and DMF

benzoic acid	$pK_{a\,av}^{DMSO}$ [a]	pK_a^{DMSO}	$pK_{a\,av}^{DMF}$ [a]	pK_a^{DMF}
4-NH_2	12.8	12.8[b]	14.0	13.96[h]
3,4-$(Me)_2$	11.4	11.41[c]	13.0	12.98[c]
4-Me	11.2	11.2[d]	12.6	12.58[h]
3-OH	11.1	11.1[e]	12.5	12.50[h]
3-Me	11.0	11.0,[d] 11.0[e]	12.4	12.38[h]
unsubstituted	11.0	11.0,[b] 11.0,[c] 10.9,[d] 11.0,[e] 11.1[f]	12.3	12.39,[c] 12.27,[i] 12.20,[j] 12.28,[k] 12.27[l]
4-Br	10.5	10.5[b]	11.6	11.57[h]
4-Cl	10.1	10.1[e]	11.5	11.54[h]
3-Br	9.7	9.68[c]	11.2	11.29,[c] 11.16[h]
2-Cl	9.3	9.29[c]	11.1	11.18,[k] 11.0[l]
3,4-$(Cl)_2$	9.2	9.20[c]	10.9_5	10.95[c]
3-NO_2	9.2	9.2,[b] 9.17[c]	10.7	10.82,[c] 10.51[h]
4-NO_2	9.0	9.0,[b] 9.04,[c] 8.9[e]	10.6	10.58,[h] 10.6,[i] 10.63,[k] 10.62[l]
3,5-$(Cl)_2$	8.8	8.81[c]	10.4	10.43[c]
4-Cl-3-NO_2	8.6	8.62[c]	10.0	10.04[c]
2-NO_2	8.2	8.18[c]	9.9	9.90,[c] 9.60,[j] 10.06[k]
3,5-$(NO_2)_2$	7.4	7.40,[c] 7.4[f]	8.8	8.87,[c] 8.95,[i] 8.49,[k] 8.48[l]
2-OH	6.8	6.6,[d] 6.8,[f] 6.9[g]	8.2	8.24,[c] 8.24,[i] 7.85,[j] 8.24,[k] 8.2[l]
2,4-$(NO_2)_2$	6.5	6.52[c]	8.2	8.16[c]
2,6-$(OH)_2$	3.1	3.1[f]	3.6	3.56[i]

[a] Average pK_a values. [b] Jasiński, T.; Stefaniuk, K. *Chem. Anal. (Warsaw)* **1965**, *10*, 211–216. The pK_a values have been corrected according to notes c and f. [c] Kolthoff, I. M.; Chantooni, M. K., Jr. *J. Am. Chem. Soc.* **1971**, *93*, 3843–3849. [d] Courtot-Coupez, J.; Le Dêmêzet, M. *Bull. Soc. Chim. Fr.* **1969**, 1033–1040. [e] Ritchie, C. D.; Uschold, R. E. *J. Am. Chem. Soc.* **1968**, *90*, 2821–2824. [f] Kolthoff, I. M.; Chantooni, M. K., Jr.; Bhowmik, S. *J. Am. Chem. Soc.* **1968**, *90*, 23–28. [g] Kolthoff, I. M.; Reddy, T. *Inorg. Chem.* **1962**, *1*, 189–194. [h] Ludwig, M.; Baron, V.; Kalfus, K.; Pytela, O.; Večeřa, M. *Collect. Czech. Chem. Commun.* **1986**, *51*, 2135–2142. [i] Kolthoff, I. M.; Chantooni, M. K., Jr.; Smagowski, H. *Anal. Chem.* **1970**, *42*, 1622–1628. [j] Petrov, S. M.; Umanskii, Yu. I. *Russ. J. Phys. Chem.* **1968**, *42*, 1627–1628. [k] Juillard, J. *J. Chim. Phys. Physicochim. Biol.* **1970**, *67*, 691–700. [l] Demange-Guerin, G. *Talanta* **1970**, *17*, 1075–1084 and 1099–1107.

In recent years, a variety of acidity scales in dipolar nonprotogenic solvents have been constructed.[1,2,19,22] Whereas the most frequently employed solvent system in DMSO,[19,20b] measurements of the pK_a's of weak acids in amide solvents are more rarely found in the literature. This is true in particular in DMF for acids having $pK_a > 15$. Owing to the extensive use of DMF in both organic and electroorganic chemistry, it seemed to us that an effort in this direction might be useful.

Since the conjugate bases of the bromo amides are labile species, at least on a time scale longer than in cyclic voltammetry, conventional methods for determining the pK_a of the bromo amides could not be employed. Therefore, we had to measure the relative acidity of compounds of known pK_a in DMF, possibly in the low pK_a region where acidity data are less dependent on the experimental approach employed and where the results from different laboratories best agree. This is not trivial. For instance, in their work on the determination of an absolute pK_a scale in DMSO, Bordwell and co-workers compared pK_a data obtained by potentiometric, conductometric, and spectrophotometric methods and found poor agreement between the different analytical methods for acids having $pK_a > 14$.[10] As reported by Ritchie[25] and later discussed,[9,10] it seems that potentiometric measurements, which have been extended in the mentioned solvents to pK_a values up to 20, suffer from sluggishness of the electrode system at high pH values. In DMSO, the acidity scale encompasses reliable pK_a values ranging from 0 to 32 and includes OH-, SH-, NH-, and CH-acids,[19] so that DMSO is certainly the reference solvent of choice. In DMF, however, only pK_a values up to ca. 20 are reported, although a method able to provide a rough estimate of the pK_a's of weaker acids has been also presented.[2a]

Since our experimental data provide relative pK_a values, the absolute pK_a in DMSO of the exogenous acids that we studied was taken as a guideline for the selection of the acids to be used in the anchoring procedure. The difference between absolute acidities in DMF and DMSO was indeed expected to be relatively small on the basis of a comparative study on the acidities in DMSO and NMP,[22] considering that NMP and DMF are amide solvents with similar properties. Ritchie and Megerle potentiometrically measured the pK_a's of a family of substituted phenols in DMF but encountered troubles with electrodes for substrates having $pK_a > 17$. The highest reliable value was 16.78 for *p*-chlorophenol, and the pK_a of phenol was only estimated to be > 18.[25a] As a first attempt to anchor our relative pK_a data to literature values coming from well-established procedures, we used α-bromo amide **1**, i.e., the most acidic compound on our hands, and *p*-chlorophenol as the exogenous acid. *p*-Chlorophenol caused a broad increase of the first peak. Also, using the best conditions for observing the third peak, i.e., low γ and high bromo amide concentration values, the voltammetric pattern was not significantly improved in the desired direction. Clearly, this proton donor enters into competition with bromo amide **1** in the protonation of the electrogenerated carbanion, and thus the main requirement of the ExR method is violated. On the other hand, phenol provided a good third peak effect on the voltammetric pattern of bromo amide **1** and led to the determination of a relative acidity of 2.00 ± 0.04 pK_a units. Taking into account that phenol should have a $pK_a > 18$,[25a] a rough determination of the pK_a region in which possible reference acids should be found has been assessed.

A good experimental outcome has been obtained with cytosine and bromo amide **1**, providing a $\Delta pK_a = 2.62 \pm 0.02$. Since the potentiometric pK_a of cytosine in DMF was reported to be 18.9[26] in comparison with the reference system given by benzoic acid, the acidity of 2-bromo-*p*-cyanoisobutyranilide is 16.3. This result is of interest since benzoic acid appears to be one of the best reference acids in both DMF and DMSO (cf. the Experimental Section, Table IV), especially considering the fairly large and

(24) The fact that the reduction of α-bromo amides is affected to some degree by the mercury electrode was taken into consideration. Accordingly, we performed some explorative third peak measurements with bromo amide **3** using an inert electrode surface such as that provided by the glassy carbon electrode. With this electrode, the difference between the potential of the first and second peak is significantly reduced with respect to the behavior at mercury, in particular owing to a negative shift of the first peak. Nevertheless, the results obtained with mercury were reproduced within the experimental error. However, the use of glassy carbon is unpractical because the potential difference between the two main peaks must be as large as possible in order to have a third peak current unaffected by the foot of the second peak. We also performed some measurements with bromo amides **2** and **3** using tetrabutylammonium perchlorate in place of TEAP. Although the nature of the supporting electrolyte affects the positions of both peaks, the outcome of the measurements reported in Table III was not altered, at least within the overall error on pK_a.

(25) (a) Ritchie, C. D.; Megerle, G. H. *J. Am. Chem. Soc.* **1967**, *89*, 1447–1451. (b) Ritchie, C. D.; Uschold, R. E. *J. Am. Chem. Soc.* **1967**, *89*, 1721–1725. (c) Reference 9.

(26) Mikstais, U. Ya.; Smolova, N. T.; Veveris, A. Ya. *Zh. Anal. Khim.* **1977**, *32*, 362–366.

Table V. Equilibrium Acidities of Phenols and Phenolic-Type Compounds in DMSO and DMF

phenol	$pK_{a\,av}^{DMSO}$ [a]	pK_a^{DMSO}	$pK_{a\,av}^{DMF}$ [a]	pK_a^{DMF}
unsubstituted	18.0[c]	18.03,[d] 16.4[e] 16.9[f]		>18,[o] 15.4[p]
4-Cl	16.4	16.74,[d] 16.1[f]	16.8	16.78[o]
3-Cl	15.8	15.83[d]	16.3	16.29[o]
3-CF_3	14.9_5	14.30,[g] 15.6[h]	15.7	15.70[o]
3-NO_2	14.1	14.39,[d] 13.75[g]	14.6	15.43,[o] 13.85[q]
2-NO_2	11.0	11.0[e]	12.2	12.14,[q] 12.20[r]
4-NO_2	10.8	11.0,[e] 10.93,[g] 10.8,[h] 10.4[i]	12.3	12.34,[o] 11.84,[q] 12.19,[r] 12.64[s]
3,5-$(NO_2)_2$	10.6	10.6[e]	11.3	11.42,[g] 11.25[s]
3-CF_3-4-NO_2	9.3	9.33[g]	10.4	10.38[g]
2,6-Bu^t-4-NO_2	7.6	7.6[e]	8.3	8.27[g]
2,4-$(NO_2)_2$	5.2	5.4,[g] 5.2,[j] 5.12[k]	6.3	6.43,[g] 6.0,[j] 6.34,[p] 6.36,[q] 6.34,[r] 6.33[s]
2,6-$(NO_2)_2$	4.9	4.9[e]	6.1	6.4,[p] 6.07,[q] 6.18,[r] 5.77[s]
4-Cl-2,6-$(NO_2)_2$	3.6	3.54,[e] 3.56,[k] 3.65[l]	4.7	4.70[s]
thiophenol	10.0	9.8,[f] 10.28[m]	10.7	10.7[j]
4-NO_2-C_6H_4SH	5.5_5	5.6,[j] 5.5[m]	6.3	6.3[j]
TAN[b]	10.2	10.24[n]	11.9	11.91[n]

[a] Average pK_a values. [b] 1-(2-Thiazolylazo)-2-naphthol. [c] Selected value. [d] Bordwell, F. G.; McCallum, R. J.; Olmstead, W. N. *J. Org. Chem.* **1984**, *49*, 1424–1427. [e] Kolthoff, I. M.; Chantooni, M. K., Jr.; Bhowmik, S. *J. Am. Chem. Soc.* **1968**, *90*, 23–28. [f] Courtot-Coupez, J.; Le Dêmêzet, M. *Bull. Soc. Chim. Fr.* **1969**, 1033–1040. [g] Chantooni, M. K., Jr.; Kolthoff, I. M. *J. Phys. Chem.* **1976**, *80*, 1306–1310. [h] Reference 8b. [i] Reference 25b. [j] Clare, B. W.; Cook, D.; Ko, E. C. F.; Mac, Y. C.; Parker, A. J. *J. Am. Chem. Soc.* **1966**, *88*, 1911–1916. [k] Reference 22. [l] Kolthoff, I. M.; Reddy, T. B. *Inorg. Chem.* **1962**, *1*, 189–194. [m] Bordwell, F. G.; Hughes, D. L. *J. Org. Chem.* **1982**, *47*, 3224–3232. [n] Šimuničová, E.; Rûriková, D. *Chem. Zvesti* **1979**, *33*, 57–63. [o] Reference 25a. [p] Demange-Guerin, G. *Talanta* **1970**, *17*, 1075–1084 and 1099–1107. [q] Petrov, S. M.; Umanskii, Yu. I. *Russ. J. Phys. Chem.* **1967**, *41*, 728–730. [r] Juillard, J. *J. Chim. Phys. Physicochim. Biol.* **1970**, *67*, 691–700. [s] Kolthoff, I. M.; Chantooni, M. K., Jr.; Smagowski, H. *Anal. Chem.* **1970**, *42*, 1622–1628.

well-shaped potential jumps reported for cytosine and similar derivatives.[26] Moreover, this result is in line with both the failure to use *p*-chlorophenol (the theoretical ΔpK_a would be 16.8 − 16.3 = 0.5, i.e., lower than the minimum ΔpK_a that can be measured with the ExR method) and the determination of the acidity of phenol.

A survey of literature allows one to see that a relatively large amount of acidity data determined in both DMF and DMSO can be found for OH-acids such as benzoic acids, phenols, aliphatic carboxylic acids, and dicarboxylic acids. Tables IV–VI report information pertaining to the acidity of 51 selected compounds, whose pK_a values have been reported in both of the above solvents. The correlation of pK_a^{DMF} vs pK_a^{DMSO}, based on values ranging from ca. 3 to 18 in DMSO, is described by the equation

$$pK_a^{DMF} = 1.56 + 0.96pK_a^{DMSO} \quad (9)$$

Despite the fact that different types of OH-acids are included in the correlation, the quality of the result appeared promising (r = 0.991). In principle, one should not expect to find a correlation when the properties of the two solvents considered are not very similar. This is true in particular when other types of acids, such as for instance NH- and CH-acids, are taken into consideration. However, if one compares the slope of the above correlation with the value, 0.97 ± 0.02, that can be obtained using our relative acidity data in DMF vs the pertinent absolute values in DMSO (Table III), the coincidence of the two results, within the experimental error, supports the fact that DMF and DMSO display similar properties, at least for OH- and NH-acids and their conjugate bases.

A further estimate of the absolute pK_a of bromo amide **1** in DMF has been made comparing the $\Delta^1pK_a^{DMF}$ values with the expected pK_a^{DMF}'s calculated through correlation 9 and using the experimental pK_a^{DMSO}'s. The average of these differences, 16.38 ± 0.04, is in good agreement with the estimate of 16.3 coming from the determination of cytosine acidity. The absolute acidity of bromo amide **1** in DMF was taken to be 16.4 and, also taking into account the error of the $\Delta^1pK_a^{DMF}$ values, the absolute error was estimated to be ca. ±0.1 pK_a unit. The whole set of pK_a^{DMF} values is reported in Table III third column. It is worth noting that the present method provides a measure of the equilibrium acidity of α-bromo amides, a family of acids whose conjugate bases are unstable in the time scale of conventional pK_a measurements.

In Figure 7 are reported the absolute data of Table III, except for bromo amides and endogenous acids together with benzoic acids (see Table IV), phenols (see Table V), aliphatic mono- and dicarboxylic acids (see Table VI), and benzenesulfonamides.[27]

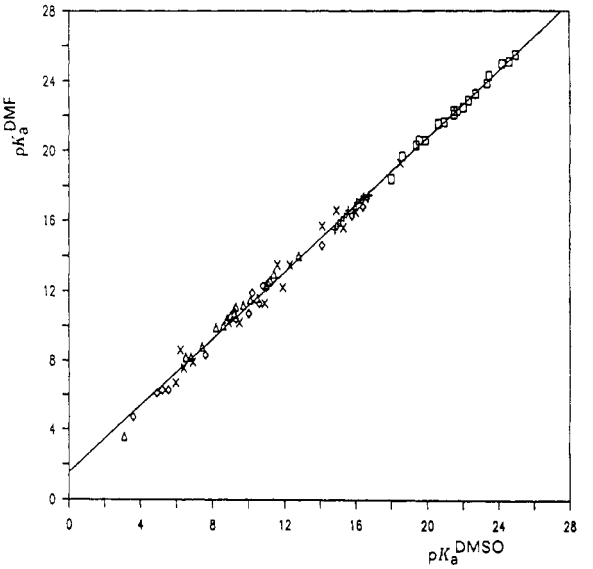


Figure 7. Correlation between the equilibrium acidities in DMF vs those determined in DMSO: Δ, benzoic acids (cf. Table IV); ◇, phenols (cf. Table V); ×, mono- and dicarboxylic acids (cf. Table VI); +, sulfonamides (ref 27); □, amides and NH-heterocycles (this work). The solid line results from the linear regression analysis (see text).

Since our data fit the correlation well and substantially extend the set of data reported in the literature, the whole series of pK_a couples (84 total), except for those amides of unknown pK_a in DMSO, was used to obtain a new equation correlating the acidities in DMF with those in DMSO. The correlation is again described by eq 9, but the correlation coefficient is improved (r = 0.997) and thus the uncertainties on the intercept (± 0.11) and slope (± 0.01) are improved also.

This result can be compared with the correlation between the acidities in NMP against those in DMSO that can be constructed using 47 data points reported by Bordwell et al. and pertaining to OH-, NH-, and CH-acids,[22] leading to 1.35, 0.99, and 0.998 for the intercept, slope, and correlation coefficient, respectively. As a consequence, it can be observed that the absolute acidities in DMF and NMP are indeed very similar as previously sug-

(27) The originally reported pK_a values in DMSO[27a] have been corrected to Bordwell's scale[19] by adding 0.9 pK_a unit. (a) Ludwig, M.; Pytela, O.; Večeřa, M. *Collect. Czech. Chem. Commun.* **1984**, *49*, 2593–2601.

Table VI. Equilibrium Acidities of Mono- and Dicarboxylic Acids in DMSO and DMF

acid	$pK_{a\,av}^{DMSO}$ [a]	pK_a^{DMSO}	$pK_{a\,av}^{DMF}$ [a]	pK_a^{DMF}
CH_3COOH	12.3	12.6,[d] 12.6,[e] 11.6[f]	13.5	13.5,[j] 13.95,[k] 13.25,[l] 13.3[m]
$CH_2ClCOOH$	8.9	8.9[d]	10.2	11.5,[k] 10.10[l] 9.0[n]
$CHCl_2COOH$	6.4	6.36[g]	7.5_5	7.9,[k] 7.2[n]
$PhCH_2COOH$	11.6	11.6[d]	13.5	13.5[k]
oxalic	6.2[b]	6.2[d]	8.6[b]	8.57[o]
	14.9[c]	14.9[d]	16.6[c]	16.62[o]
malonic	6.9[b]	7.2,[h] 6.6[i]	7.9[b]	7.8,[p] 8.2,[q] 7.80[r]
	18.5[c]	18.55,[h] 18.4[i]	19.3[c]	18.03,[o] 20.8[p]
succinic	9.5[b]	9.50,[h] 9.5[i]	10.2[b]	10.05,[j] 10.34,[o] 10.4,[p] 10.20,[r]
	16.5_5[c]	16.7,[h] 16.4[i]	17.3[c]	17.21,[j] 15.49,[o] 19.9,[p] 16.8,[s]
glutaric	10.9[b]	10.9[h]	11.3[b]	11.28[s]
	15.3[c]	15.3[h]	15.6[c]	15.6[s]
adipic	11.9[b]	11.9[h]	12.2[b]	12.28,[o] 12.13[s]
	14.1[c]	14.1[h]	15.7[c]	15.32,[o] 16.0[s]
o-phthalic	5.9_5[b]	6.2,[d] 5.7[i]	6.7[b]	6.73[s]
	16.0[c]	16.0,[b] 16.0[i]	16.5[c]	16.5[s]

[a] Average pK_a values. [b] pK_a(I). [c] pK_a(II). [d] Courtot-Coupez, J.; Le Démézet, M. *Bull. Soc. Chim. Fr.* **1969**, 1033–1040. [e] Kolthoff, I. M.; Chantooni, M. K., Jr.; Bhowmik, S. *J. Am. Chem. Soc.* **1968**, *90*, 23–28. [f] Reference 25b. [g] Kolthoff, I. M.; Chantooni, M. K., Jr. *J. Am. Chem. Soc.* **1976**, *98*, 5063–5068. [h] Chantooni, M. K., Jr.; Kolthoff, I. M. *J. Phys. Chem.* **1975**, *79*, 1176–1182. [i] Martin, J. A.; Duperis, J. *Bull. Soc. Chim. Fr.* **1968**, 138–145. Values estimated by Kolthoff and Chantooni Jr. in note g and *J. Am. Chem. Soc.* **1976**, *98*, 7465–7470. [j] Kolthoff, I. M.; Chantooni, M. K., Jr.; Smagowski, H. *Anal. Chem.* **1970**, *42*, 1622–1628. [k] Demange-Guerin, G. *Talanta* **1970**, *17*, 1075–1084 and 1099–1107. [l] Petrov, S. M.; Umanskii, Yu. I. *Russ. J. Phys. Chem.* **1968**, *42*, 1627–1628. [m] Tézé, M.; Schaal, R. *Bull. Soc. Chim. Fr.* **1962**, 1372–1379. [n] Clare, B. W.; Cook, D.; Ko, E. C. F.; Mac, Y. C.; Parker, A. J. *J. Am. Chem. Soc.* **1966**, *88*, 1911–1916. [o] Gavrilov, A. V.; Kurmaeva, A. I.; Tret'yakova, A. Ya.; Barabanov, V. P.; Kitsya, V. P. *Zh. Anal. Khim.* **1979**, *34*, 771–774. [p] Roletto, E.; Juillard, J. *J. Solution Chem.* **1974**, *3*, 127–138. [q] Boulanger, E. Thesis, Université Paris VI, Paris, France, 1971. [r] Roletto, E.; Vanni, A. *Talanta* **1977**, *24*, 73–75. [s] Roletto, E.; Vanni, A.; Zelano, V. *Ann. Chim. (Rome)* **1980**, 147–159.

gested.[22] The higher absolute pK_a's in DMF or NMP relative to DMSO are likely to be linked to a lower basicity of the carboxamide solvents. On the other hand, Bagno and Scorrano reviewed the acid–base properties of organic solvents and concluded that DMSO can be considered to be less basic than amide solvents in aqueous medium.[28] However, an inversion of basicity can occur on changing the medium to which the equilibrium acidities of the protonated solvent molecules are referenced. On the basis of the data collected in DMF, NMP, and DMSO, it seems that such an inversion is indeed likely to take place. In their work on acidities in NMP, Bordwell et al. concluded that acidities in DMSO can be used to predict relative acidities in NMP and other carboxamide solvents.[22] We can add that the use of the improved eq 9 for DMF can satisfactorily provide absolute acidities in DMF from DMSO data with a standard deviation of 0.4 pK_a unit.

The method presented here is rather different from many nontraditional electrochemical approaches for acidity determinations[2-8] in the sense that approximate electrochemical data such as irreversible potentials are not used, as well as other thermodynamical data. With respect to nonelectrochemical methods, possible problems arising from ion pair interactions are greatly reduced as alkali metal cations are avoided. As a final consideration, we can add that, apart from the present application to the determination of the acidities of weak acids, third peak type effects caused by either exogenous or endogenous acids must be taken into account in the study of electroreduction processes in which the substrate under investigation bears an acidic function.

Experimental Section

Chemicals. The syntheses of 2-bromo-2-methyl-*N*-(*p*-cyanophenyl)-propanamide, 2-bromo-2-methyl-*N*-(*p*-chlorophenyl)propanamide, 2-bromo-2-methyl-*N*-phenylpropanamide, 2-bromo-2-methyl-*N*-(*p*-methoxyphenyl)propanamide, 2-bromo-2-methyl-*N*-benzylpropanamide, 2-methyl-*N*-(*p*-cyanophenyl)propanamide, 2-methyl-*N*-(*p*-chlorophenyl)-propanamide, 2-methyl-*N*-phenylpropanamide, and 2-methyl-*N*-benzyl-propanamide have been previously described.[12a,b]

Pure samples (≥99%) of phenol, imidazole, formanilide, nicotinamide, isonicotinamide, acetanilide, phenothiazine, benzamide, diphenylamine, *p*-chlorophenol, and 2-pyrrolidinone (Janssen reagents); indole, carbazole, and formamide (Erba reagents); 2-thiophenecarboxamide (Aldrich); urethane (Fluka); and cytosine (Sigma) were used as received. *N,N'*-Diphenylurea (Fluka), phenoxazine (Janssen), and benzoylhydrazine (Janssen) were recrystallized from ethanol–water.

2-Bromo-2-methyl-*N*-benzhydrylpropanamide. 2-Bromopropanoyl bromide (3.7 mL, 0.03 mol) in toluene (10 mL) was added while stirring over 30 min at 0 °C to a toluene solution (80 mL) containing aminodiphenylmethane (5.2 mL, 0.03 mol) and triethylamine (4.2 mL, 0.03 mol). Stirring was continued for 1 h, and then the resulting mixture was treated with water (200 mL) at room temperature. The organic phase was washed with 0.05 N HCl and water and then dried. The solvent was removed to give 5.75 g (58%) of crude product melting at 132–133 °C, which was recrystallized (ethanol–water) to afford 2-bromo-2-methyl-*N*-benzhydrylpropanamide[29] as white needles: mp 135–136 °C; ^1H NMR (DMSO-d_6, TMS) δ 1.93 (s, 6 H, $2CH_3$), 6.18 (d, 1 H, J = 8.6 Hz, CH), 7.31 (m, 10 H, 2Ph), 8.87 (d, 1 H, J = 8.6 Hz, NH).

Phenylacetanilide. Phenylacetanilide was prepared from phenylacetic acid (Erba) by reaction with $SOCl_2$ (Erba) in $CHCl_3$ and then with two equivalent amounts of aniline (Erba). It was recrystallized from ethanol to yield white needles, mp 117 °C (lit.[30] mp 118–119 °C).

Solvents and Electrolytes. *N,N*-Dimethylformamide (Aldrich, 99+%) was treated over some days with anhydrous Na_2CO_3 with stirring and then twice distilled at reduced pressure (17 mmHg) under nitrogen atmosphere. Some explorative runs were carried out in dimethyl sulfoxide (Janssen), purified, and handled along similar lines as reported.[10] In order to keep the water content at a minimum, some experiments were carried out after cycling the solvent–electrolyte system through a column of alumina (Merck, activity grade 1) previously activated overnight at 350 °C under vacuum, a method well-established for electrochemistry in organic solvents.[31] However, it was verified that such a procedure was not necessary for most experiments.

Tetraethylammonium perchlorate (Erba) was recrystallized from ethanol. Tetrabutylammonium perchlorate was prepared by reacting tetrabutylammonium hydroxide (Fluka) with perchloric acid (Erba) and recrystallizing from 2:1 water–ethanol. Both salts were carefully dried under vacuum at 60 °C and then stored over P_2O_5.

Electrochemical Apparatus. Electrochemical measurements were conducted in an all-glass cell thermostated at 25 ± 0.2 °C. The solution was deoxygenated with argon (SIAD, 99.9995%), and then a blanket of gas was maintained over the liquid.

The working electrode was homemade by slow galvanostatic electrodeposition of mercury onto a small platinum bead. In between different experiments, the Hg electrode was dipped into a clean Hg pool. In this way the surface proved to be clean, reproducible, and of constant area. After introduction into the cell, the electrode was subjected to electrochemical activation in the blank solution by means of several cycles at

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0.5 V/s between −0.3 and −2.8 V vs the KCl saturated calomel electrode (SCE). The reference electrode was a homemade Ag/AgCl one.[32] Its potential was always calibrated against SCE after each experiment. A large-area platinum ring was the counter electrode.

A conventional electrochemical instrumentation was employed: PAR 173/179 potentiostat digital coulometer, PAR 175 universal programmer, Nicolet 3091 digital oscilloscope, and Amel 863 X/Y pen recorder.

Digital Simulation of the Voltammetric Curves and Data Treatment. The endogenous regeneration (EnR) mechanism can be described by the reaction sequence 10 + 11:

$$2A + 2e \rightarrow B + C \quad (10)$$

$$B + C \underset{k_b}{\overset{k_f}{\rightleftharpoons}} A + D \quad (11)$$

where reaction 11 is characterized by an equilibrium constant, $K = k_f/k_b \ll 1$. Species B, C, and D are not reducible in the potential range of interest.

Reaction 10 corresponds to the irreversible reduction of A in a formal slow transfer of one electron per molecule with the formation of species B and C in half amounts. Equation 10 may be taken as a satisfactory representation of the electrode reaction 1 + 2 in view of the fast self-protonation reaction. Under these conditions, eq 10 simply represents the direct formation of the bromo amide anion (B) and the reduced amide (C) from two molecules of the bromo amide (A). The formation of bromide ion is neglected since it does not enter in any further step. D represents the conjugate base of C formed in the regeneration step 11, which corresponds to eq 7.

The voltammetric response (i vs E) for this process can be obtained by solving a system of partial derivative equations for the relevant concentrations as a function of space x and time t, describing the diffusion to a plane electrode and the homogeneous kinetics, together with the initial ($t = 0$) and boundary ($x = 0$ and $x \rightarrow \infty$) conditions.

The equations are conveniently expressed in dimensionless form by means of the quantities defined as follows: a, b, c, and d are the concentrations of species A, B, C, and D, respectively, divided by the analytical concentration C^* of the substrate A; $\tau = t\alpha Fv/RT$ is the time variable; $y = x(\alpha Fv/DRT)^{1/2}$ is the variable linked to the distance x from the electrode surface (D is the common value of the diffusion coefficient for all species); $\xi = -\alpha F(E - E_{in})/RT + \ln [2k(E_{in})(RT/\alpha FvD)^{1/2}]$ is the potential variable, $k(E_{in})$ being the heterogeneous electron transfer rate constant at the initial potential E_{in} of the voltammetric scan; $\psi = i/[FSC^*(\alpha FvD/RT)^{1/2}]$ is the current function (S = electrode area); and $\lambda = k_bRTC^*/\alpha Fv$ is the kinetic parameter (eq 8).

Since the concentrations of species C and D are linked to those of species A and B by mass balances

$$c = b + \gamma, \qquad d = 1 - a - 2b$$

the only equations needed for a complete description of the process are those pertaining to species A and B

$$\partial a/\partial \tau - \partial^2 a/\partial y^2 = -\partial b/\partial \tau + \partial^2 b/\partial y^2$$

$$\partial b/\partial \tau = \partial^2 b/\partial y^2 + \lambda[a(1 - a - 2b) - Kb(\gamma + b)]$$

with the initial and boundary conditions:

$$\tau = 0, y \geq 0 \text{ and } \tau > 0, y \rightarrow \infty: \; a = 1, b = 0$$

$$\tau > 0, y = 0: \; \partial a/\partial y = -2\partial b/\partial y = a \exp(\xi) = \psi$$

where the electrode boundary condition for A is a dimensionless formulation of the Butler–Volmer rate law for its electrode reduction, also taking into account the presence of the fast self-protonation reaction.[11f,12c]

The system was solved numerically by using an implicit finite difference method[33] in order to obtain the variation of the dimensionless current, ψ, as a function of the dimensionless potential, ξ, for different values of the three parameters γ, λ, and K.

The exogenous regeneration (ExR) mechanism can be represented by the sequence 12 + 13:

$$2A + 2e \rightarrow B \quad (12)$$

$$B + E \underset{k_b}{\overset{k_f}{\rightleftharpoons}} A + F \quad (13)$$

where species E and F, representing the exogenous acid and its conjugate base, respectively, are not reducible in the potential region of interest, as well as the bromo amide anion B. In the ExR mechanism, the formation of the reduced amide (species C in the EnR case) can be neglected. As already discussed, it is considered an unimportant proton source with respect to the exogenous acid in the very regeneration step. The formation of bromide ion is also neglected, as before.

The dimensionless concentrations e and f of species E and F, respectively, are related to a and b by the mass balances

$$e = \gamma - f, f = 1 - a - 2b$$

and therefore the set of partial derivative equations to be solved numerically is

$$\partial a/\partial \tau - \partial^2 a/\partial y^2 = -\partial b/\partial \tau + \partial^2 b/\partial y^2$$

$$\partial b/\partial \tau = \partial^2 b/\partial y^2 + \lambda[a(1 - a - 2b) - Kb(\gamma - 1 + a + 2b)]$$

with initial and boundary conditions as for the EnR case.

The experimental curves were recorded in the form of dimensionless current and potential in order to compare them with the simulated voltammetric curves. Since in the potential region of interest only a capacitive component was observed in the background current, a simple vertical shift was needed for background correction.

For each α-bromo amide, the voltammetric behavior of the first peak, in the absence of added proton donors, was taken as the basis for calibration of the vertical and horizontal scales of the X–Y recorder. For all compounds, the ratio $i_p/v^{1/2} = 0.496FSC^*(\alpha FD/RT)^{1/2}$ under the self-protonation regime is constant within 2–3%, and the values of the α coefficient (**1**, 0.27; **2**, 0.29; **3**, 0.30; **4**, 0.29; **5**, 0.25; **6**, 0.28), determined through the dependence of the peak potential on log v and the peak width, agree[12c] and are quite reproducible (±0.005). The above α coefficients were used in the form $\alpha F/RT$ to scale the potential axis. The vertical axis was scaled by taking into account the average value of the ratio between the current of the first peak and $v^{1/2}$.

The two-parameter fit of the experimental and simulated curves was carried out by a trial-and-error procedure using a very large set of simulated voltammograms. All of the features of the voltammetric curve (first to third peak separation, current of both peaks, third peak width, and position and height of the current minimum between the two peaks), together with the dependence of the voltammetric pattern on v and γ, were needed for the best optimization of the parameters K and λ. We stress that only the best combination of K with λ led to good simulation of the experimental curves over all the scan rate and γ ranges investigated. If erroneous values of K and λ, but similar to the correct ones, are chosen, it is possible to have a somewhat acceptable fit but only within less than 1 order of magnitude in scan rate variation and only for similar γ's. Therefore, since for each γ value more than 1 order of magnitude in the kinetic parameter λ can be varied through the experimental parameter v and C^* (eq 8), the error on the K value is usually rather small.

Literature pK_a Data in DMSO and DMF. During the last 30 years a number of equilibrium acidity determinations of organic acids have been carried out in nonaqueous solvents by electrochemical and spectrophotometric methods. Tables IV–VI report literature pK_a values in DMSO and DMF from different laboratories for three classes of OH-acids, benzoic acids, phenols, and mono- and dicarboxylic acids, along with the average values. Provided that at least three similar values in the same solvent were reported, a single value differing of more than ca. ±1 pK_a unit was discarded. In those cases in which no selection could be made, all reported pK_a values have been averaged.

Acknowledgment. Financial support by the European Economic Community, within the Research Project SCI 0183-C (EDB), is gratefully acknowledged.

Registry No. 1, 116275-13-9; **2**, 1970-52-1; **3**, 2322-45-4; **4**, 1970-56-5; **5**, 61071-68-9; **6**, 60110-37-4; TAN, 1147-56-4; *p*-HC(CH$_3$)$_2$CONHC$_6$H$_4$CN, 113715-23-4; *p*-HC(CH$_3$)$_2$CONHC$_6$H$_4$Cl, 7160-05-6; HC(CH$_3$)$_2$CONHPh, 4406-41-1; HC(CH$_3$)$_2$CONHCH$_2$Ph, 4774-58-7; *p*-NH$_2$C$_6$H$_4$CO$_2$H, 150-13-0; *p*-CH$_3$C$_6$H$_4$CO$_2$H, 99-94-5; *m*-HOC$_6$H$_4$CO$_2$H, 99-06-9; *m*-CH$_3$C$_6$H$_4$CO$_2$H, 99-04-7; C$_6$H$_5$CO$_2$H, 65-85-0; *p*-BrC$_6$H$_4$CO$_2$H, 586-76-5; *p*-ClC$_6$H$_4$CO$_2$H, 74-11-3; *m*-BrC$_6$H$_4$CO$_2$H, 585-76-2; *o*-ClC$_6$H$_4$CO$_2$H, 118-91-2; *m*-NO$_2$C$_6$H$_4$CO$_2$H, 121-92-6; *p*-NO$_2$C$_6$H$_4$CO$_2$H, 62-23-7; *o*-NO$_2$C$_6$H$_4$CO$_2$H, 552-16-9; *o*-HOC$_6$H$_4$CO$_2$H, 69-72-7; *p*-ClC$_6$H$_4$OH, 106-48-9; *m*-ClC$_6$H$_4$OH, 108-43-0; *m*-F$_3$CC$_6$H$_4$OH, 98-17-9; *m*-NO$_2$C$_6$H$_4$OH, 554-84-7; *o*-NO$_2$C$_6$H$_4$OH, 88-75-5; *p*-NO$_2$C$_6$H$_4$OH, 100-02-7; *p*-NO$_2$C$_6$H$_4$SH, 1849-36-1; CH$_3$CO$_2$H, 64-19-7; CH$_2$ClCO$_2$H, 79-11-8; CHCl$_2$CO$_2$H, 79-43-6; PhCH$_2$CO$_2$H, 103-82-2; formanilide, 103-70-8; *N,N'*-diphenylurea, 102-07-8; carbazole, 86-74-8; phenylacetanilide, 621-06-7; indole, 120-72-9; acetanilide, 103-84-4; isonicotinamide, 1453-82-3; phenoxazine, 135-67-1; nicotinamide, 98-92-0; 2-thiophenecarboxamide, 5813-89-8; phenol, 108-95-2; imidazole, 288-32-4; phenothiazine, 92-

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84-2; benzamide, 55-21-0; formamide, 75-12-7; 2-pyrrolidinone, 616-45-5; urethane, 51-79-6; diphenylamine, 122-39-4; *N*-benzylisobutyramide, 4774-58-7; 3,4-dimethylbenzoic acid, 619-04-5; 3,4-dichlorobenzoic acid, 51-44-5; 3,5-dichlorobenzoic acid, 51-36-5; 4-chloro-3-nitrobenzoic acid, 96-99-1; 3,5-dinitrobenzoic acid, 99-34-3; 2,4-dinitrobenzoic acid, 610-30-0; 2,6-dihydroxybenzoic acid, 303-07-1; 3,5-dinitrophenol, 586-11-8; 3-(trifluoromethyl)-4-nitrophenol, 88-30-2; 2,6-*tert*-butyl-4-nitrophenol, 728-40-5; 2,4-dinitrophenol, 51-28-5; 2,6-dinitrophenol, 573-56-8; 4-chloro-2,6-dinitrophenol, 88-87-9; thiophenol, 108-98-5; oxalic acid, 144-62-7; malonic acid, 141-82-2; succinic acid, 110-15-6; glutaric acid, 110-94-1; adipic acid, 124-04-9; *o*-phthalic acid, 88-99-3.

Mechanism of Inactivation of γ-Aminobutyric Acid Aminotransferase by 4-Amino-5-hexynoic Acid (γ-Ethynyl GABA)†

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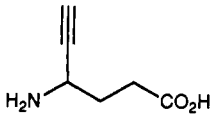
Abstract: γ-Aminobutyric acid (GABA) aminotransferase is a pyridoxal phosphate (PLP) dependent enzyme that catalyzes the degradation of γ-aminobutyric acid. The inactivation of GABA aminotransferase has been shown to be an important treatment for epilepsy. The mechanism of inactivation of GABA aminotransferase by γ-ethynyl GABA, a mechanism-based inactivator of GABA aminotransferase that shows anticonvulsant activity in animal models, is investigated in this paper. Although it appears that azaallylic isomerization (the normal catalytic pathway for substrates) of the PLP-bound inactivator occurs (pathway a, Scheme VII), little or no inactivation of the enzyme results from that isomerization. Essentially all of the inactivation is derived from a propargylic isomerization (pathway b) to the allenamine bound PLP adduct **10**, which undergoes nucleophilic attack at two different sites. It appears that an active site lysine residue reacts at the Schiff base to give the free enamine **18** (pathway c) or reacts at the allene to give the enzyme and cofactor bound enamine **12** (pathway d); possible attack by water (pathway e) would lead to metabolite **26**. The enamine **18** does not become attached to the PLP (Scheme III, pathway a), but a small amount (5–10%) may become attached to the enzyme at a site other than at lysine (**9**, Scheme III, pathway b). Adduct **9** also could be derived from azaallylic isomerization of the inactivator-PLP Schiff base followed by conjugate addition to the acetylene by an active site nucleophile other than a lysine residue (Scheme I). Mostly **18** is released into solution to give **27** (Scheme VII). Adduct **12** is believed to be a transient intermediate that partitions between conversion to metabolite **26** (Scheme VII, pathway f) and conversion to a more stable isomer (**13**, pathway g). Upon denaturation, adduct **13** partitions equally (Scheme VIII) between release of metabolite **26** and the formation of another covalent adduct (**17**). Isolation and identification of the amine and nonamine metabolites produced during processing of γ-ethynyl GABA showed that, on average, for every 13 molecules of γ-ethynyl GABA that are turned over, 1.2 undergoes transamination (pathway a, Scheme VII), 2.6 are metabolized to **27** (pathways b and c), 8.2 are converted to **26** (pathways b, d, and f and/or pathways b and e), and 1.0 becomes attached to the enzyme, almost all, as **13** (pathways b, d, and g), but possibly 5–10% as **9** (X ≠ Lys) as discussed above.

Epilepsy is a disease characterized by convulsive seizures that result from repeated and excessive electrical discharges in the neurons. In its many forms, epilepsy may affect as much as one percent of the world population.[1] Although the etiological mechanism leading to the electrical discharges in convulsive epilepsy is not yet understood, it is known that γ-aminobutyric acid (GABA) is a major neurochemical component in seizure inhibition. Indeed, convulsions occur when GABA levels fall below a certain threshold level in the brain,[2-6] and direct injection of GABA into the brain causes the convulsions to cease.[7]

The catabolism of GABA is catalyzed by the pyridoxal 5′-phosphate (PLP)-containing and α-ketoglutarate-dependent enzyme, GABA aminotransferase (EC 2.6.1.19), to yield succinic semialdehyde and L-glutamate. An important treatment of epileptic convulsions has focused on raising brain GABA levels by the irreversible inactivation of GABA aminotransferase. So far, this has been the most effective way to increase the presynaptic levels of GABA.[8]

γ-Ethynyl GABA (4-amino-5-hexynoic acid, **1**), one of the first rationally designed mechanism-based inactivators,[9] was shown to



1

be a potent inactivator of GABA aminotransferase.[10-12] In vivo, γ-ethynyl GABA causes a long-lasting decrease in mouse brain

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† Dedicated to Professor Robert H. Abeles, with admiration for his creative contributions to enzymology, on the occasion of his 65th birthday.

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0002-7863/91/1513-9329$02.50/0