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Structure-reactivity relationships in the chemical hydrolysis of prodrug esters of nicotinic acid

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Summary

The rate of chemical hydrolysis of 25 esters of nicotinic acid was measured at pH 7.4 and 37 °C. The most stable esters, which are also the least water-soluble ones, do not undergo any detectable hydrolysis over a period of 5 weeks. In contrast, the most labile esters display half-lives of less than 3 h, but the half-life of most compounds falls in the range 100–1000 h. The rate constants of hydrolysis (as log k values) correlate positively with Taft's polar substituent parameter, as well as with the chemical shift of the carbonyl carbon, in compatibility with a mechanism of general base catalysis demonstrated by the pH profile of the reaction.

Introduction

Nicotinic acid is best known for its vitaminic properties but has also interesting pharmacodynamic effects which have been cogently reviewed by Weiner and Van Eys (1983). First, a major interest of nicotinic acid relates to its effect on patterns of hyperlipidemia associated with atherosclerosis. Thus, the drug in humans lowers the blood levels of cholesterol, triglycerides, LDL and VLDL, while HDL cholesterol is actually increased. Second, the compound also affects vascular tone by acting as a vasodilator and eliciting a flush reaction that is often viewed as an undesirable side-effect. Third, intravenously administered nicotinic acid is a potent but short-acting inducer of fibrinolysis.

A rapid elimination (mainly by biotransformation), a short half-life (20–45 min), the necessity of high doses (2–8 g/day) and the occurrence of side-effects have limited the use of nicotinic acid. In contrast, one bioprecursor (β-pyridylcarbinol) and some esters (e.g. inositol hexanicotinate, pentaerythritol tetranicotinate, sorbitol nicotinate and tocopherol nicotinate) have therapeutic utility and are used on a large or smaller scale (e.g., Grey and Carlson, 1971; Weiner and van Eys, 1983). This suggests that nicotinate esters may have pharmacokinetic advantages as compared to the parent drug, but a systematic examination of the factors influencing their chemical and enzymic hydrolysis has not been published.

To explore structure-hydrolysis relationships, a number of nicotinate esters were prepared with substituents varying markedly in size, polarity, lipophilicity and electronic character. The present

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study reports and rationalizes their chemical hydrolysis under physiological conditions of pH and temperature, while work in progress examines their protein binding and enzymic hydrolysis in various tissues and subcellular preparations.

Materials and Methods

Chemicals

Most of the esters of nicotinic acid used in this study (Table 1) were synthesized in our laboratory according to known methods (see Reymond et al. (1987). The remaining compounds were either obtained from commercial sources (Table 1, footnotes a and b) or were kindly donated by pharmaceutical companies (Table 1, footnotes c–f). The identity and purity of the compounds were checked by ^{1}H-NMR spectroscopy, IR spectroscopy, elementary analysis and HPLC. Methanol, acetonitrile, *n*-decylamine, 3-morpholinopropanesulfonic acid (Mops), and Tris (Fluka, Buchs, Switzerland) were of analytical grade.

Chemical hydrolysis

The hydrolysis of the nicotinates was studied at pH 7.4 ($\pm$0.05) in Mops buffer (0.05 M) whose ionic strength was adjusted to 0.1 with KCl. The temperature was kept constant at 37.0 $\pm$ 0.2°C using a shaking water-bath (Infors WTR-1, Zürich). The pH-rate profile of hydrolysis was determined for the ethyl and benzyl nicotinates at 60 $\pm$ 1°C in 0.05 M Tris buffers (Tris-maleate at pH 5.0 and 6.0; Tris-HCl at pH 7.0 and 8.0).

The water-soluble nicotinates were dissolved in 100 ml buffer to a concentration of 10^{-3} M. In contrast, the slightly water-soluble esters (nos 8, 9, 19 and 20 in Table 1) were dissolved in 500 ml buffer to reach a concentration of 5×10^{-5} M. All kinetic studies were carried out in duplicate.

Sampling and sample preparation

In each experiment, sampling was carried out during at least one half-life, 10 samples or more being taken at intervals depending on the rate of hydrolysis. The sample size was 0.3 ml for the soluble nicotinates and 10 or 20 ml for the slightly soluble ones. The samples of the soluble esters were injected directly into the HPLC system as detailed later and analysed for both nicotinic acid and the ester.

In the case of the poorly water-soluble esters, the concentrations of substrates and product were below the detection limit and direct analysis was excluded. The esters in these samples were therefore concentrated on reversed-phase C_{18} cartridges (Baker, Phillipsburg, NJ, U.S.A.) according to the

TABLE 1

Rate constants of chemical hydrolysis (pH 7.4 and 37°C), relative chemical shifts of the carbonyl carbon (Δδ), and Taft's polar parameter for alkyl groups (σ)*

No. Nicotinates	*k* (min^{-1}) ($\times 10^4$)	Δδ (ppm)	σ* [g]
1. Methyl [a]	0.392	0.000	0.49
2. Ethyl [b]	0.152	0.497	0.00
3. *n*-Propyl	0.157	0.478	−0.10
4. Isopropyl	0.083	1.070	–
5. *n*-Butyl	0.147	0.398	−0.12
6. Isobutyl	0.132	0.491	−0.19
7. tert-Butyl	0.860	1.389	–
8. *n*-Hexyl [c]	– [h]	0.424	–
9. *n*-Octyl	– [h]	0.421	–
10. 2-Chloroethyl	0.628	0.838	0.94
11. 2-Hydroxyethyl	0.379	0.476	–
12. 3-Hydroxypropyl	0.283	0.260	0.21
13. 2-Methoxyethyl	0.353	0.494	0.66
14. 2-Butoxyethyl [d]	0.473	0.531	–
15. Carbamoylmethyl	0.914	2.039	1.68
16. 1-Carbamoylethyl	1.08	1.645	–
17. 2-Dimethylaminoethyl	41.1	0.551	–
18. 3-Aminopropyl	1.23	– [i]	–
19. Cyclohexyl	– [h]	1.123	–
20. 3,3,5-Trimethylcyclohexyl [e]	– [h]	1.120	–
21. Tetrahydrofurfuryl [f]	0.299	0.522	–
22. Benzyl [a]	0.518	0.730	0.75
23. Phenoxyethyl	0.354	0.646	0.85
24. *p*-Chlorophenyl	6.82	2.112	–
25. *p*-Nitrophenyl	39.2	2.693	–

[a] Fluka AG, Buchs, Switzerland.
[b] Aldrich-Europe, Beerse, Belgium.
[c] Ega-Chemie, Steinheim/Albuch, F.R.G.
[d] Knoll AG, Liestal, Switzerland.
[e] Dr Karl Thomae GmbH, Biberach an der Riss, F.R.G.
[f] Ciba-Geigy AG, Basel, Switzerland.
[g] Taken from Hansch and Leo (1983) and Perrin et al. (1981).
[h] No hydrolysis detected (i.e. less than 2%) after 5 weeks.
[i] Uncertain.

following procedure. The stationary phase was conditioned successively with acetonitrile and water (twice 3 ml of each). The sample (10.0 or 20.0 ml) was then passed through the wet column, and after elimination of the remaining water by slight suction the stationary phase was extracted with 3.0 ml acetonitrile. The acetonitrile phase was evaporated to dryness and the residue dissolved in 0.500 ml of mobile phase for injection into the HPLC system. A preliminary investigation had demonstrated that this method of concentration allows a quantitative extraction of the poorly water-soluble esters. However, the nicotinic acid liberated by hydrolysis is lost in this procedure and in such samples only the ester was measured, implying a somewhat decreased precision.

Analysis

Analyses were carried out using an automated HPLC system (Anacomp 220, Kontron, Zürich) composed of an LC 414 T pump, an MSI autosampler and a Uvikon 730 SLC UV detector operating at 262 nm. Lichrosorb RP-18 (10 μm) packed in a 25 cm × 4 mm column was used as stationary phase. The mobile phases were composed of Mops buffer (0.02 M; pH 7.4) and acetonitrile at different ratios; they contained 0.2% (v/v) *n*-decylamine as a masking agent. They were filtered (Millipore 0.45 μm) and deaerated by ultrasonication prior to use. The eluent composition (water/acetonitrile 75 : 25, 60 : 40 or 20 : 80 v/v) and the flow rate (1.0 or 1.5 ml/min) were chosen to optimize retention times.

No internal standard was added, the variations between successive injections of 20 μm by the autosampler being 0.2%. All samples were analysed twice. For the water-soluble nicotinates, both the ester and the acid were analyzed simultaneously and their concentrations used to calculate the rate constants. For the poorly water-soluble compounds, nos 8, 9, 19 and 20, only the ester could be analyzed.

Calibration

Calibration curves were established for each nicotinate using 7 concentrations in the working range 10^{-4}–10^{-3} M. Excellent linearity was obtained for all nicotinates ($r^2 > 0.99$).

Electronic parameters

Various parameters have been proposed to assess the electronic effects of substituents (Van de Waterbeemd and Testa, 1986). Of particular interest for this study is Taft's polar parameter σ^* of the substituent R′ in alcohols of structure $R'CH_2OH$. Table 1 lists available σ^* values for alcohols used to obtain several of the nicotinates.

^{13}C-NMR spectroscopy affords an alternative means of assessing electronic effects. In the case of esters, the chemical shift of the carbonyl carbon is very sensitive to the electronic influence of the alkyl or aryl moiety and was used here as an electronic parameter of experimental origin. The ^{13}C-NMR spectra of the nicotinates (concentrations 10^{-4}–10^{-3} M in $CDCl_3$, TMS as reference, room temperature) were registered using a Varian VXR 200 spectrophotometer. A preliminary investigation showed that the chemical shift (δ in ppm) of the carbonyl carbon is concentration-independent in the range of 5×10^{-5}–5×10^{-3} M. The electronic parameter is expressed here as the difference:

$$\Delta\delta = \delta_i - \delta_{Me} \qquad (1)$$

where δ_i is the chemical shift of the carbonyl carbon of a given nicotinate and δ_{Me} that of the methyl ester (165.514 ppm). The $\Delta\delta$ values given in Table 1 are the mean of two measurements (the mean error being ±0.012 ppm).

Results and Discussion

Chemical hydrolysis

The hydrolysis of all investigated nicotinate esters under physiological conditions (pH 7.4; 37 °C) followed pseudo first-order kinetics. The measured rate constants are listed in Table 1; these are averages of two separate investigations. Some typical first-order plots are shown in Fig. 1. The observed half-lives range from 2.8 h (2-dimethylaminoethyl ester) to 58 days (isopropyl ester).

Compounds 8, 9, 19 and 20 are poorly water-soluble and had to be analyzed differently from the other substrates (see above), since it proved

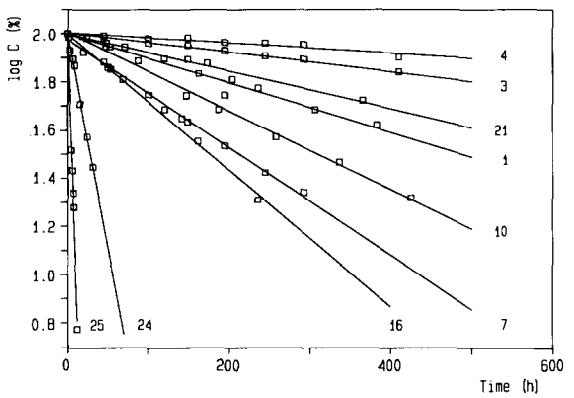

Fig. 1. First-order plots for hydrolysis of some representative nicotinate esters in 0.05 M Mops buffer solution (pH 7.4, 37 °C). The numbers correspond to the compounds in Table 1.

impracticable to monitor nicotinic acid, the product of hydrolysis. Analyzing the ester only resulted in a somewhat decreased precision which could be estimated to ±2%. No hydrolysis of the four compounds was observed after 5 weeks at pH 7.4 and 37 °C, signifying that hydrolysis was below 2%.

Considerable differences are thus evident in the chemical stability of nicotinate esters. Most compounds are in fact poorly susceptible to chemical hydrolysis under physiological conditions of pH and temperature, with k values in the range 0.1×10^{-4}–1×10^{-4} min^{-1}. Four alkyl esters of high lipophilicity and poor hydrosolubility are particularly resistant to hydrolysis. This effect, however, is not caused by steric factors in the molecules, since the *tert*-butyl ester is hydrolysed faster than its *n*-butyl and isobutyl isomers. In contrast to the four esters of high stability, three nicotinates are rapidly hydrolyzed, particularly the *p*-nitrophenyl and dimethylaminoethyl esters. The latter compound being a β-amino ester is likely to experience intramolecular catalysis due to the protonated side chain (Page et al., 1986); in comparable esters, alkaline hydrolysis was increased approx. 1000-fold by protonation of the amino group (Agren et al., 1961).

A preliminary and purely qualitative interpretation of the stability data suggests that the factors influencing the rate of hydrolysis of nicotinic acid esters are not steric but electronic in nature, as electron-withdrawing substituents seem to enhance the rate of reaction.

The kinetics of hydrolysis of two representative compounds, namely the ethyl and benzyl esters, were determined in the pH range 5–8 to ascertain the mechanism of catalysis, i.e. acid or base catalysis, and not to investigate specifically a possible catalytic effect of the buffer. The results are shown in Fig. 2, where the logarithm of the rate constants (log k) is plotted against pH. For both esters and within the investigated pH range, log k values increase linearly with increasing pH, the slopes being 0.63 and 0.49 for the ethyl and benzyl ester, respectively. Such slopes are indicative of a general base catalysis rather than a specific base (hydroxyl) catalysis which implies a slope of 1. Other experiments have confirmed slopes < 1 for other esters and shown that the hydrolysis of nicotinates is also base-catalyzed in phosphate-citrate buffers (data not reported).

Establishing the type of hydrolysis is important in structure-reactivity relationships since Taft has shown that base-catalyzed hydrolysis is sensitive to polar substituent effects whereas acid-catalyzed hydrolysis is not (e.g., Taft, 1956). The fact that the hydrolysis of the nicotinates is base-catalyzed justifies the use of parameters describing substituent polar effects in structure-reactivity relationship studies.

Structure-hydrolysis relationships

Values of Taft's polar parameter σ* were available for 11 nicotinate esters and correlated with

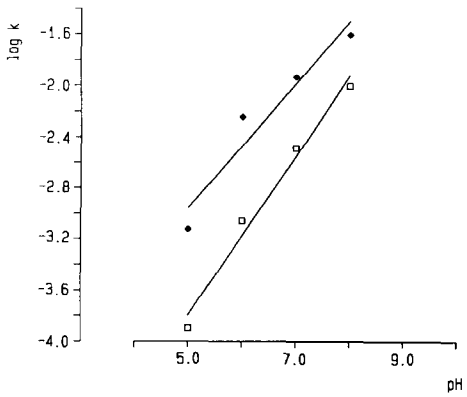

Fig. 2. pH-rate profile for hydrolysis of ethyl (□) and benzyl nicotinate (◆) at 60 °C.

log k values through the following regression equation (95% confidence limits in parentheses):

$$\log k = 0.470(\pm 0.109)\sigma^* - 0.741(\pm 0.079) \quad (2)$$
$$n = 11;\ r = 0.956;\ s = 0.088;\ F = 95.1$$

The correlation is rather good and the positive slope indicates that electron-withdrawing substituents do indeed facilitate hydrolysis. Because such substituents will decrease the electron density on the carbonyl carbon atom and render it more susceptible to nucleophilic attack, Eqn. 2 is compatible with a base-catalyzed reaction. Numerous studies (e.g., Robinson and Matheson, 1969; Nielsen and Bundgaard, 1987) have successfully correlated the σ^* parameter with rates of hydrolysis, which is not surprising when one considers that σ^* values were calculated from hydrolysis rates in the first place (Taft, 1956; Hansch and Leo, 1979). In addition, one shortcoming of Eqn 2 is its narrow predictive power since the σ^* parameter was available for a few substituents only (see Table 1).

Such limitations do not exist for a parameter whose values are readily measurable and obtained by an independent method, as is the case for the chemical shift parameter $\Delta\delta$. The 2-dimethylaminoethyl ester (which is not included in Eqn 2) was excluded from the analysis for the following two reasons. Firstly, it cannot be compared with the other compounds as its hydrolysis at pH 7.4 must be significantly increased by a mechanism of intramolecular catalysis (see above). Secondly, this ester was present in the unprotonated form under the $CDCl_3$ conditions of the ^{13}C-NMR study, and its carbonyl was therefore less electron-deficient than in the protonated state of the compound at pH 7.4.

The relationship between the log k values and the relative chemical shifts of the carbonyl carbon is the following:

$$\log k = 0.759(\pm 0.234)\Delta\delta - 4.97(\pm 0.27) \quad (3)$$
$$n = 19;\ r = 0.856;\ s = 0.34;\ F = 46.8$$

This correlation is not as good as that of Eqn 2, since it explains only 73% of the variance, but it does have the merit of being based on a directly measurable parameter and includes a maximal number of compounds. A closer examination of the log k vs $\Delta\delta$ plot (Fig. 3) reveals that the isopropyl nicotinate deviates somewhat more than the other compounds for unknown reasons, however, there would be no justification on chemical grounds for removing this observation from the regression.

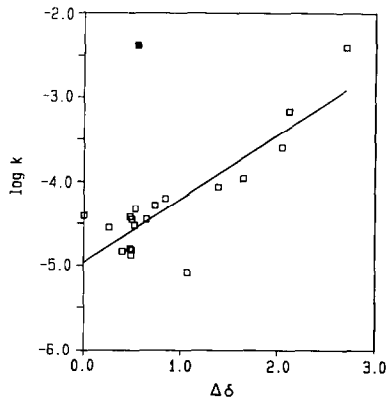

Fig. 3. Plot of the logarithm of hydrolysis rate constants (log k) vs the chemical shift parameter $\Delta\delta$. The solid line represents the regression equation without the 2-dimethylaminoethyl nicotinate (Eqn 3, $n = 19$).

It is interesting to note that the parameters $\Delta\delta$ and σ^* correlated only moderately:

$$\Delta\delta = 0.677(\pm 0.444)\ \sigma^* + 0.305(\pm 0.323) \quad (4)$$
$$n = 11;\ r = 0.754;\ s = 0.360;\ F = 11.9$$

indicating that the structural information they encode overlaps only in part. While Taft's polar parameter (Hansch and Leo, 1979, 1983; Perrin et al., 1981) mainly measures the inductive influence of substituents, the ^{13}C chemical shift results from a complex mixture of electronic and steric effects some of which may not be relevant to the mechanism of hydrolysis.

From the data, it is clear that the rate of hydrolysis of the compounds is not influenced by purely steric features (e.g. steric hindrance) of the substituent, as demonstrated for example by the comparatively rapid hydrolysis of the *tert*-butyl ester. Furthermore, no relationship was found between lipophilicity values (Reymond et al., 1987) and rate constants, despite the fact that the poorly hydrosoluble esters are unreactive.

Conclusion

The 25 esters of nicotinic acid investigated here display extremely large differences in their chemical stability under physiological conditions of pH and temperature. The most stable esters, which are also the least water-soluble ones, did not undergo any detectable hydrolysis during a period of 5 weeks. In contrast, the most labile esters display half-lives shorter than 3 h, but most half-lives reported in Table 1 fall within the range 100–1000 h. This implies that chemical (non-enzymic) hydrolysis should be negligible in biological systems. In contrast, the compounds are good substrates of esterases, as will be reported in forthcoming papers.

Structure-hydrolysis relationships of the nicotinates are described in two regression equations. The correlation of log k values with Taft's polar parameter (Eqn 2) is compatible with a nucleophilic attack on the carbonyl carbon atom, in other words with an independently demonstrated mechanism of general base catalysis. The correlation with ^{13}C chemical shift (Eqn 3) does not lead to mechanistic insights, but has value as an empirical means of estimating the chemical stability of nicotinates.

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References

Agren, A., Hedsten, U. and Jonsson, B., Hydrolysis of prototlytic esters. *Acta Chem. Scand.*, 15 (1961) 1532–1544.

Grey, K.F. and Carlson, L.A., *Metabolic Effects of Nicotinic Acid and its Derivatives*, Huber, Berne, 1971.

Hansch, C. and Leo, A., *Log P and Parameter Database: a Tool for the Quantitative Prediction of Bioactivity*, Pomona College Medicinal Chemistry Project, Comtex Scientific Co., New York, 1983.

Hansch, C. and Leo, A., *Substituent Constants for Correlation Analysis in Chemistry and Biology*, Wiley, New York, 1979.

Nielsen, N.M. and Bundgaard, H., Prodrugs as drug delivery systems. 68. Chemical and plasma-catalyzed hydrolysis of various esters of benzoic acid: a reference system for designing prodrug esters of carboxylic acid agents. *Int. J. Pharm.*, 39 (1987) 75–85.

Page, M.I., Render, D. and Bernath, G., Stereochemical studies. Part 112. Geometrical dependence of intramolecular catalysis in the hydrolysis and aminolysis of aryl esters. *J. Chem. Soc. Perkin Trans. II* (1986) 867–871.

Perrin, D.D., Dempsey, B. and Serjeant, E.P., *pKa Prediction for Organic Acids and Bases*, Chapman and Hall, London, 1981, pp. 109–126.

Reymond, D., Chung, G.N., Mayer, J.M. and Testa, B., Lipophilicity measurement of nicotinates by RP-HPLC. Differences in retention behaviour, but similarities of log k_w values, in methanol-water and acetonitrile-water eluents. *J. Chromatogr.*, 391 (1987) 97–109.

Robinson, J.R. and Matheson, L.E., Linear free-energy relationships between alcohol pK_a and solvolysis rates of esters where substituent variation is in the alkyl portion of the ester. *J. Org. Chem.*, 34 (1969) 3630–3633.

Taft, T.W., *Steric Effects in Organic Chemistry*, Chapman and Hall, London, 1956.

Van de Waterbeemd, H. and Testa, B., The parametrization of lipophilicity and other structural properties in drug design. In Testa, B. (Ed.), *Advances in Drug Research*, vol. 16, Academic Press, London, 1986, pp. 85–225.

Weiner, M. and Van Eys, J., *Nicotinic Acid, Nutrient-Cofactor-Drug*, Dekker, New York, 1983, pp. 227–300.