Hydrolytic Cleavage of *p*-Nitrophenyl Alkanoates in Aqueous Solutions of Cyclodextrins

Gian Maria Bonora
Centro C.N.R. 'Studi sui Biopolimeri,' Istituto di Chimica Organica, Università di Padova, 35131 Padova, Italy
Roberto Fornasier,* Paolo Scrimin, and Umberto Tonellato*
Centro C. N. R. 'Meccanismi di Reazioni Organiche,' Istituto di Chimica Organica, Università di Padova, 35131 Padova, Italy

The hydrolytic cleavage of *p*-nitrophenyl alkanoates has been investigated in moderately alkaline aqueous solutions of α- and β-cyclodextrins. The catalytic effects of cyclodextrins (the k_c/k_{un} ratios) first decrease and then increase with increasing chain length. Such a trend is apparently related to the mode of insertion of the alkanoates into the cyclodextrin. Induced c.d. measurements indicate that only in the case of short-chain alkanoates (up to butyrate or hexanoate) the *p*-nitrophenyl moiety is included in the cyclodextrin cavity whereas for long-chain esters the hydrocarbon chain is inserted into it. The kinetic consequences of the dual mode of complexation and differences between the two types of cyclodextrins investigated are briefly discussed.

The hydrolytic cleavage of phenyl esters promoted by cyclodextrins has been extensively investigated[1] and the mechanism of the catalysed reaction clearly established.[2] This involves nucleophilic attack by a secondary hydroxy group (in the anionic form, pK_a *ca.* 12) of the cyclodextrins on the carbonyl carbon atom of the bound substrate. The proper inclusion of the ester so as to allow for smooth formation of the tetrahedral intermediate, rather than strong binding, is a critical feature of high reactivity of these host–guest complexes.[2,3]

In the case of the reactive phenyl ester complexes so far investigated it has been assumed that the aromatic portion of the molecule (the phenolic or other aromatic moieties) is buried in the cyclodextrin cavity. In the cleavage of *p*-carboxyphenyl 2-methylpropionate and 3,3-dimethylbutyrate, where the alkyl portion and not the more hydrophilic aryl moiety is assumed to be included in the cyclodextrin cavity, 'non-productive' complexes were formed and retardation was observed.[2a,4] We have recently reported,[5] however, kinetic evidence that in the cleavage of 1-methylheptyl *p*-nitrophenyl carbonate with α- and β-cyclodextrins the most productive complex is the one with the alkyl chain bound into the cavity.

We have investigated and here report the kinetic consequences of alkyl *versus* aryl insertion in the hydrolytic cleavage of the *p*-nitrophenyl alkanoates: acetate, PNPA, butyrate, PNPB, hexanoate, PNPH, octanoate, PNPO, and dodecanoate, PNPD. Molecular models show that, as the length of the hydrocarbon chain increases, the alkyl portion may better fill the cyclodextrin (CDX) cavity than the *p*-nitrophenyl portion and this prediction has been substantially confirmed by induced c.d. measurements.

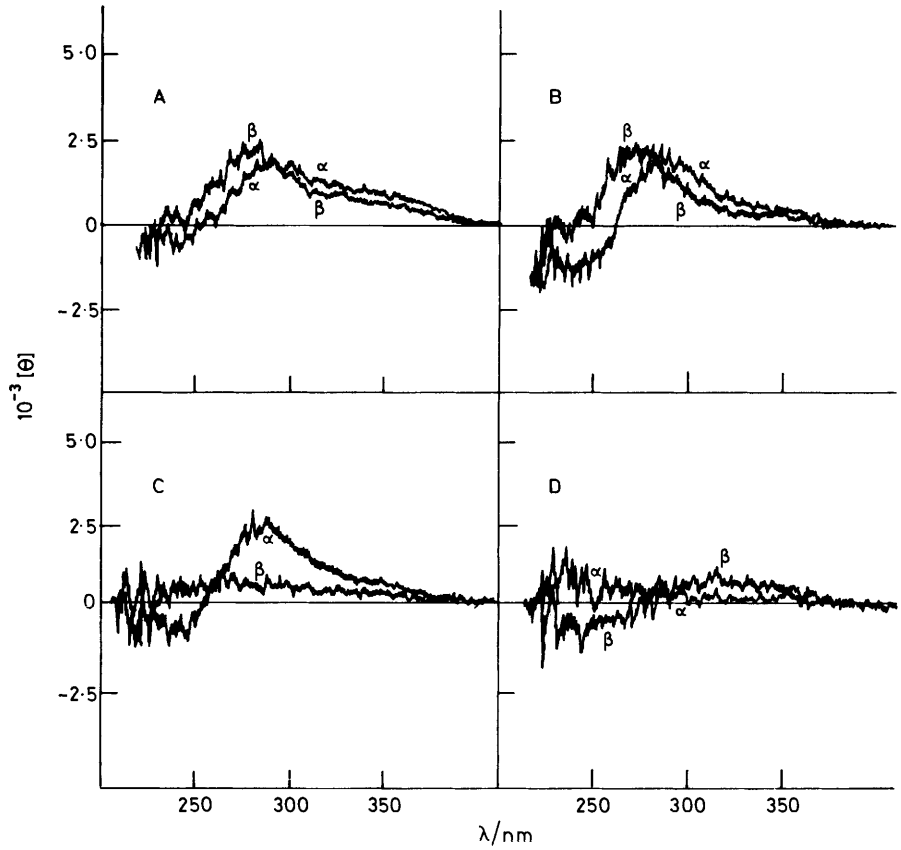
Results

Induced C.d. Measurements.—The induced c.d. spectra of α- and β-CDXs complexes with the *p*-nitrophenyl alkanoates shown in the Figure were recorded for aqueous 5% v/v MeOH solutions of CDX (0.95×10^{-2}M) and substrate (4.75×10^{-4}M). In the case of short-chain alkanoates a weak and positive dichroism is clearly observed in the 300—250 nm region, arising from the asymmetric interaction between the *p*-nitrophenyl ester chromophore and the α- or β-CDX cavities.[6,7] In particular it appears that: (a) the PNPA and PNPB complexes with both CDXs and that of PNPH with α-CDX give rise to c.d. bands, similar in shape and intensity with a maximum at *ca.* 280 (α-CDX) or 270 nm (β-CDX). This clearly indicates that, in the above cases, the aromatic moiety is included in the CDX's cavity; (b) no significant c.d. band is observed in the case of PNPH with β-CDX and in that of PNPO with both CDXs, thus ruling out aromatic insertion as a major mode of complexation for these esters. Turbidity prevented reproducible c.d. measurements in the case of PNPD although the recorded spectra did not show, as with PNPO, any band in the 250—300 nm region.

Thus it may be argued that, as the hydrocarbon chain of the alkanoates increases, alkyl insertion is preferred to aryl insertion in the CDX's complexes, PNPH being the turning point as a substrate in the series here investigated.

Kinetic Measurements.—Rate measurements were made for sodium carbonate buffers, pH 10.4, with added 1% v/v CH_3CN at 25 °C. In the absence of CDXs rate measurements for long-chain alkanoates are complicated by the low solubility or micellization of these substrates. Guthrie[8] and, more recently, Murakami and his co-workers[9] reported that, whereas at very low ester concentrations the rate of alkaline hydrolysis is independent of [ester], beyond a critical substrate concentration, the rate decreases, log k_ψ being linearly related to log [ester]. Following Murakami and his co-workers, the critical concentration is the c.m.c. for self-micellization of the substrate; according to Guthrie,[8b] it is rather the solubility limit of the alkanoate. The critical concentrations for PNPH, PNPO, and PNPD, according to Guthrie (aqueous buffers 2% v/v CH_3CN, 25 °C) are 1×10^{-4}, 1×10^{-5}, and (estimated) 1.2×10^{-7}M. The last value is higher (5.6×10^{-7}M) under Murakami's conditions (aqueous 1% v/v dioxane, 40 °C).

We carried out our kinetic measurements using the following ester concentrations: 1×10^{-5}M (PNPA, PNPB, and PNPH), 1×10^{-6}M (PNPO), and 5—15 $\times 10^{-7}$M (PNPD). In the last case we also noticed a decrease in the apparent pseudo-first-order rate constants on increasing the substrate concentrations and also distinct deviations from first-order kinetics for [PNPD] > 1×10^{-6}M. By taking the rates measured for [PNPD] in the range 5—10 $\times 10^{-7}$M, we estimated the rate of alkaline hydrolysis of the substrate from the plot of log k_ψ *versus* log [PNPD] at [PNPD] = 1×10^{-7}M, a conservative estimate of the critical concentration of this ester. The pseudo-first-order rate constants are shown in the Table as k_{un}. The relative rates for PNPA, PNPH, PNPO, and PNPD are 1.0, 0.60, 0.54, and 0.17, in reasonably good agreement with those observed by Guthrie, 1.0, 0.61, 0.50, and 0.10, under comparable conditions.[8b]



C.d. spectra of α- and β-CDX complexes with: A, PNPA; B PNPB; C, PNPH; D, PNPO. Molar ellipticity: [θ]/(deg cm^2 dmol^{-1})

Table. Kinetic parameters[a] for the α- and β-CDX accelerated cleavage of *p*-nitrophenyl alkanoates

		α-CDX			β-CDX		
Ester	k_{un}	k_c	k_c/k_{un}	K_d	k_c	k_c/k_{un}	K_d
PNPA	1.75	5.65 ± 0.8	3.2	10.5 ± 2	21.3 ± 3.5	12.2	6.5 ± 1.1
PNPB	1.05	1.67 ± 0.2	1.6	4.8 ± 0.9	8.6 ± 0.6	8.2	3.9 ± 0.4
PNPH	1.05	2.65 ± 0.3	2.5	2.0 ± 0.5	6.1 ± 0.3	5.8	2.3 ± 0.5
PNPO	0.95	3.3 ± 0.6	3.6	0.98 ± 0.3	9.3 ± 0.5	9.8	1.9 ± 0.4
PNPD	0.3	3.2 ± 0.5	10.6	0.37 ± 0.15	20.2 ± 2	67	0.75 ± 0.3

[a] $10^3k_{un}/s^{-1}$, $10^3k_c/s^{-1}$, and K_d/mM.

In the presence of cyclodextrins rate measurements were made for solutions of the ester at the concentrations indicated above (5—8 × 10^{-7}M in the case of PNPD without apparent kinetic effects) using 6—10 different CDX concentrations from 1 × 10^{-4} to 5 × 10^{-3}M. From the k_ψ values, by the use of Lineweaver–Burk-type plots, $1/(k_\psi - k_{un})$ *versus* 1/[CDX], as described,[1,10] the following parameters were estimated: K_d, the dissociation constant of the CDX–substrate complex, and k_c, the first-order rate constants for the cleavage of fully bound substrates. The Table also shows the k_c/k_{un} ratios, the way CDX accelerations are normally shown.[2,3,11]

Discussion

From the data in the Table, the following trends are apparent on going from PNPA to PNPD: (a) the K_d constants decrease regularly; (b) the k_c constants and the k_c/k_{un} ratios first decrease and then increase, PNPH, in the case of β-CDX, and PNPB, in that of α-CDX, being the substrates with the lowest catalytic ratio. The rate enhancements are not related to the strength of binding but rather to the mode of substrate complexation with CDX. The largest rate enhancements were observed in the extreme cases, PNPA and PNPD, where i.c.d. measurements indicate that either aromatic (PNPA) or alkyl (PNPD) insertion is largely predominant.

At least in the case of β-CDX, the rate parameters observed on going from PNPH to PNPD indicate that the kinetic benefits of alkyl insertion increase with increasing chain length. It seems reasonable to assume, as molecular models indicate, that as the folded hydrocarbon chain increasingly occupies the cavity, the carbonyl group of the ester is allowed to surface the better to approach the nucleophilic hydroxy group of the rim. Short-chain alkanoates would therefore yield alkyl insertion complexes poorly productive or non-productive as reported by Bender and his co-workers.[2a] In the case of PNPB and perhaps PNPH, a productive aryl insertion and a non-productive alkyl

insertion are likely to be competitive modes of complexation: as a result, for these substrates low catalytic ratios are observed.

In the case of α-CDX, the same rationale seems to apply. It is however, evident that the k_c values (although not the k_c/k_{un} ratios) level off instead of increasing on going from PNPO to PNPD. The kinetic benefits of alkyl insertion are reached earlier in the case of α- than in that of β-CDX and this could be accounted for by the smaller volume of the α-CDX cavity to be filled by the alkyl moiety.

The rate enhancements here observed are rather modest; in any case, neither optimal geometry nor sufficient freezing of rotational degrees of freedom in the transition state of the reaction, which have been indicated [3] as the factors for high rate enhancements, are achieved in the *p*-nitrophenyl alkanoate–CDX complexes.

Experimental

Materials.—*p*-Nitrophenyl alkanoates were reagent-grade commercial products used without further purification.

Circular Dichroism Measurements.—Measurements were made for solutions prepared by mixing 1.9 ml of a 1×10^{-2}M CDX solution in bidistilled water and 0.1 ml of a freshly prepared solution of ester (1×10^{-2}M) in MeOH. C.d. spectra were recorded on a JASCO J-500A spectropolarimeter equipped with DP-501 N data processor using cylindrical fused quartz cells of 0.2 cm pathlength.

Kinetic Measurements.—Each run was initiated by adding 20 μl of a stock solution of ester in CH_3CN to 2 ml of 0.02M-sodium carbonate buffer, pH 10.4, containing the proper amount of CDX, pre-equilibrated at 25 °C in the thermostatted cell holder of a Varian–Cary 219 spectrophotometer. The liberation of *p*-nitrophenol was followed at 400 nm; conditions in the case of PNPD: 0.01 OD/full scale and 2 s response.

Acknowledgements

We thank Professor C. Toniolo for discussions, E. Castiglione and V. Moretto for technical assistance, and the Ministry of Public Education (Italy) for financial support.

References

1 Reviews: M. L. Bender and M. Komiyama, 'Cyclodextrin Chemistry,' Springer Verlag, Weinheim, 1978; J. Szejtli, 'Cyclodextrins and their Inclusion Complexes,' Akademiai Kiado, Budapest, 1982.
2 (*a*) R. L. Van Etten, G. A. Glowes, J. F. Sebastian, and M. L. Bender, *J. Am. Chem. Soc.*, 1967, **89**, 3242; (*b*) R. L. Van Etten, J. F. Sebastian, G. A. Glowes, and M. L. Bender, *ibid.*, p. 3253
3 (*a*) R. Breslow, M. F. Czarniecki, J. Emert, and H. Hamaguchi, *J. Am. Chem. Soc.*, 1980, **102**, 762; (*b*) G. L. Trainor and R. Breslow, *ibid.*, 1981, **103**, 154; (*c*) R. Breslow, G. Trainor, and A. Ueno, *ibid.*, 1983, **105**, 2739.
4 See also M. Komiyama and S. Inoue, *Bull. Chem. Soc. Jpn.*, 1980, **53**, 2330, 3266.
5 R. Fornasier, P. Scrimin, and U. Tonellato, *Tetrahedron Lett.*, 1983, **24**, 5541.
6 C. Toniolo, M. L. Falxa, and M. Goodman, *Biopolymers*, 1968, **6**, 1579.
7 M. Bodaroszky, M. L. Fuuk, K. W. Fuuk, M. Kondo, C. Yanglin, and A. Bodanzky, *J. Am. Chem. Soc.*, 1974, **96**, 2234.
8 J. P. Guthrie, (*a*) *J. Chem. Soc., Chem. Commun.*, 1972, 897; (*b*) *Can. J. Chem.*, 1973, **51**, 3494.
9 Y. Murakami, Y. Aoyama, and M. Kida, *J. Chem. Soc., Perkin Trans. 2*, 1977, 1947.
10 H. J. Brass and M. L. Bender, *J. Am. Chem. Soc.*, 1973, **95**, 5391.
11 See, however: R. Breslow and D. J. Cram, *Chem. Eng. News*, 1983, April 11, p. 4.

Received 2nd April 1984; *Paper* 4/533