Acyloxymethyl as a Drug Protecting Group. Part 3. Tertiary *O*-Amidomethyl Esters of Penicillin G: Chemical Hydrolysis and Anti-Bacterial Activity

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Purpose. *O*-(*N*-alkylamido)methyl esters of penicillin G were studied as a new class of prodrugs.

Methods. Their hydrolysis in aqueous buffers containing 20 % (v/v) of acetonitrile was investigated by HPLC.

Results. A U-shaped pH-rate profile was seen with a pH-independent process extending from pH *ca.* 2 to pH *ca.* 10. This pathway is characterised by kinetic data that are consistent with a unimolecular mechanism involving rate-limiting iminium ion formation and penicillinoate expulsion. Penicillin G and the corresponding amide are the ultimate products detected and isolated, indicating that β-lactam ring opening is much slower than ester hydrolysis. The *O*-(*N*-alkylamido)methyl esters of penicillin G displayed similar *in vitro* antibacterial activity to penicillin G itself.

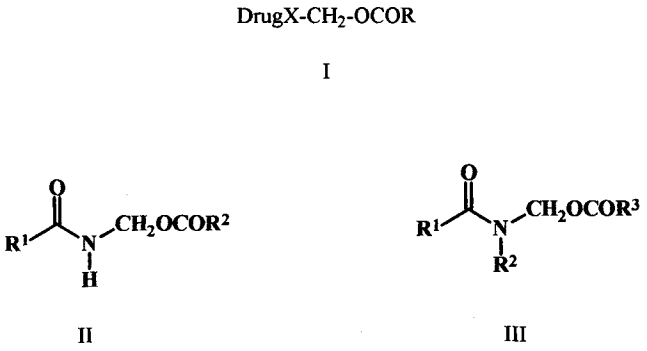
Conclusions. Compared to the penicillin G derivatives, the much higher stability of the *O*-(*N*-methylbenzamido)methyl benzoate, acetate and valproate esters (which gave rise to a Bronsted β_{lg} value of *ca.* -1) suggests that tertiary *N*-acyloxymethylamides may be useful prodrugs for carboxylic acid drugs with $pK_a > 4$.

KEY WORDS: *O*-amidomethylation; penicillin G; carboxylic acids; prodrugs; hydrolysis kinetics; mechanism of hydrolysis; antibacterial activity.

INTRODUCTION

The double prodrug concept has been widely used for improving the physicochemical properties of a large number of drugs containing carboxylic acid and NH-acidic functional groups (1). A common strategy is to prepare acyloxymethyl derivatives of the type I. Drugs such as triazenes (2), amides (3), peptides (4), hydantoins (5) and uracils (6) have been well studied, though only a few examples are described in which the amide is used as the pro-moiety (7). For these, (eg. II) decomposition is rapid at pH 7.4 and proceeds through a base-catalysed elimination mechanism (8).

Recently, we reported that the corresponding *tertiary N*-acyloxymethylamides III are significantly more stable (8). Therefore, *O*-(*N*-alkyl)amidomethylation is a potential approach for prodrug development of drugs that contain free carboxylic acid groups, especially those that have their therapeutic effectiveness greatly reduced after oral administration. This is the case for several β-lactam antibiotics, which are poorly absorbed from the gastrointestinal tract and unsuitable for oral administration. Some α-(acyloxy)alkyl esters of neutral β-lactam antibiotics are also poorly active orally because of their low aqueous solubility. The introduction of a basic α-amino group into the acyl pro-moiety increases the water-solubility but generally reduces *in vitro* stability (9). Unfortunately, esterification at the C-2 carboxylic acid group of penicillins generally increases the rate of alkaline β-lactam hydrolysis (10), therefore contributing to the reduction of *in vitro* stability.



Following our development of a new general synthetic method that allows the direct coupling of the $R^1CONR^2CH_2$ moiety to an R^3CO_2H drug (11), here we report upon the hydrolysis and *in vitro* antimicrobial activity of *O*-(*N*-alkyl)amidomethyl esters of penicillin G IV-VII. This study is directed towards: 1) uncovering the mechanism of chemical hydrolysis of *O*-(*N*-alkyl)amidomethyl penicilloates; 2) assessing the extent of competitive β-lactam ring opening, and 3) evaluating the suitability of *O*-(*N*-alkyl)amidomethyl esters as prodrugs for β-lactam antibiotics. The data are compared with the *O*-(*N*-methylbenzamido)-methyl esters of benzoic, IX, acetic, X, and valproic acids, XI. The structures of the compounds used in this study are contained in Figure 1.

MATERIALS AND METHODS

Apparatus

Melting points were recorded on a Buchi 510 capillary melting-point apparatus and are uncorrected. The ^{1}H-NMR spectra were recorded on a Bruker MSX-300 spectrometer. Readings of pH were made on a Crison micropH 2002 pH-meter at the temperature of study. High-performance liquid chromatography (HPLC) was carried out using a system consisting of a Shimadzu LC-9A pump, a Shimadzu SPD-6AV variable-wavelength UV detector or a Shimadzu SPD-M6A diode-array detector, a 20 μL Rheodyne loop injection valve and a Merck LiChrosorb RP-8 column with 5 μm particles.

Chemicals

Penicillin G was purchased from Sigma. All other chem-

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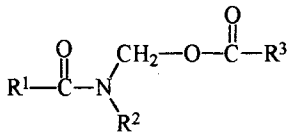
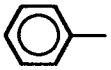
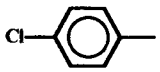
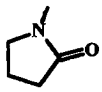
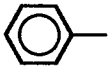
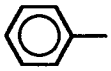
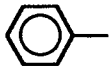
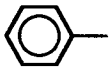
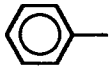


Compound	R^1	R^2	R^3
IV	CH_3	CH_3	A
V		CH_3	A
VI		CH_3	A
VII			A
VIII		$CH_2CO_2C_2H_5$	A
IX		CH_3	
X		CH_3	CH_3
XI		CH_3	$(n\text{-}C_3H_7)_2CH$

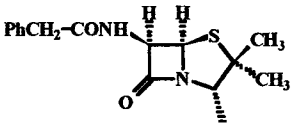
A: 

Fig. 1. Structures of the *O*-amidomethyl esters used in the current study.

icals and solvents were of reagent grade, except buffer substances and HPLC solvents which were analytical grade and LiChrosolve (Merck) grade, respectively. Column chromatography was performed using silica gel 60 mesh 70-230 (Merck). The substrates IV-XI were synthesized as described previously (11). The following are new compounds:

2'-[(N-Ethoxycarbonylmethylbenzamido)methyl]-(2S,5R,6R)-3,3-Dimethyl-7-oxo-6-phenylacetamido-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylate (VIII): cream gum; ^{1}H-NMR, δ_H: 1.33 (3H, t, J = 7 Hz, CH_3), 1.43 (3H, s, C_3-CH_3), 1.46 (3H, s, C_3-CH_3), 3.65 (2H, s, $PhCH_2$), 4.25 (2H, q, J = 7 Hz, CH_2), 4.29 (2H, s, NCH_2CO), 4.38 (1H, s, C_2-H), 5.40 (1H, dd, J = 11 Hz, NCH_2O), 5.43 (1H, dd, J = 11 Hz, NCH_2O), 5.46 (1H, d, J = 4 Hz, C_5-H), 5.76 (1H, dd, J = 4 and 9 Hz, C_6-H), 6.01(1H, d, J = 9 Hz), 7.20-7.67 (5H, m). Found: C, 60.0; H, 5.58; N, 7.49. Calculated for $C_{28}H_{31}N_3O_7S$: C, 60.8; H, 5.61; N, 7.59.

2'-[(N-Methylbenzamido)methyl ethanoate. (X): oil; ^{1}H-NMR, δ_H: 2.03 (3H, s, CH_3CO), 3.06 (3H, s, N-CH_3), 5.24 (2H, s, NCH_2O), 7.30-7.36 (5H, m, Ph). Found: C, 63.5; H, 6.25; N, 6.68. Calculated for $C_{11}H_{13}NO_3$: C, 63.8; H, 6.28; N, 6.76.

2'-[(N-Methylbenzamido)methyl-2-propylpentanoate. (XI): oil; ^{1}H-NMR, δ_H: 0.97 (6H, t, J = 6 Hz, CH_3), 1.36 (4H, sext., J = 6 Hz, CH_3CH_2), 1.42-1.73 (4H, m, CH_2CH), 2.43-2.52 (1H, m, CH), 3.20 (3H, s, N-CH_3), 5.33 (2H, broad s, NCH_2O), 7.35-7.83 (5H, m, Ph). Found: C, 69.9; H, 8.50;

N, 4.75. Calculated for $C_{17}H_{25}NO_3$: C, 70.1; H, 8.59, N, 4.81.

Kinetic Studies

All kinetic experiments were carried out in aqueous buffers (sodium hydroxide, borate, phosphate, acetate, chloroacetate and hydrochloric acid) containing 20%(v/v) of acetonitrile, with an ionic strength maintained at 0.5 M ($NaClO_4$). A 20 µl aliquot of a 10^{-2} M stock solution of substrate in acetonitrile, was added to 10 ml of the appropriate thermostatted buffer solution. At regular intervals, samples of the reaction mixture were analysed by HPLC using the following conditions: detector wavelength, 230 nm; mobile phase, methanol-water containing 0.04 M tetrabutylammonium phosphate (55:45 to 60:40%) for compounds IV-VIII, or acetonitrile-water containing 0.2 M sodium acetate buffer (55:45 to 70:30%) for compounds IX-XI.

Antimicrobial Susceptibility Tests

The minimum inhibitory concentration (MICs) of penicillin G and of compounds IV and VI-VIII were determined by the Agar Dilution Method (Mueller-Hinton) using a multipoint inoculater. The plates were incubated in aerobic atmospheres at 37 °C for 48 hours. MICs were recorded as the lowest concentration completely inhibiting visible bacterial growth. The range of concentrations used for all the compounds was $2.24x10^{-10}$ - 0.5 $x10^{-6}$ mol ml^{-1}. The following reference bacterial organisms were used: *Pseudomonas aeruginosa* ATCC 27853, *Klebsiella pneumoniae* ATCC 10031, *Escherichia coli* ATCC 25992, *Salmonella typhimurium* ATCC 43971, *Shigella dysenteriae* ATCC 13313, *Serratia marcescens* NCTC 1377, *Staphylococcus aureus* ATCC 25923 and *Streptococcus faecalis* ATCC 10541. The following bacterial organisms belong to the collection of the Faculty of Pharmacy: β-lactamase producing *Klebsiella pneumoniae*, β-lactamase producing *Escherichia coli, Salmonella enteritidis, Shigella flexneri,* and *Proteus mirabilis*.

RESULTS AND DISCUSSION

Kinetics of Hydrolysis and pH-Rate Profiles

The pH-rate profiles for the hydrolysis of the esters of penicillin G IV-VIII exhibit a broad U-shape (Figure 2), indicative of the presence of acid-catalysed, base-catalysed and pH-independent processes (Eq.(1)).

$$k_{obs} = k_o + k_H[H^+] + k_{HO}[OH^-] \quad (1)$$

The apparent first-order rate constant, k_o, for the pH-independent process was determined from the pH-rate profile, while the catalytic second-order rate constants, k_{H^+} and k_{HO^-}, were obtained from the plots of k_{obs} *versus* $[H^+]$ and $[OH^-]$, respectively. The values of these rate constants for the *O*-amidomethyl esters of penicillin G IV-VIII, and for the benzoate, IX, acetate, X, and valproate, XI, esters are listed in Table I.

Apart from the relative positions of the plateau regions, these profiles are analogous to those of simple acyloxymethylamides, *eg.* compound IX (8). Thus, amidomethyl derivatives of penicillin G behave as typical acyloxymethylamides, albeit with enhanced reactivity. The source of this enhanced reactivity is discussed below.

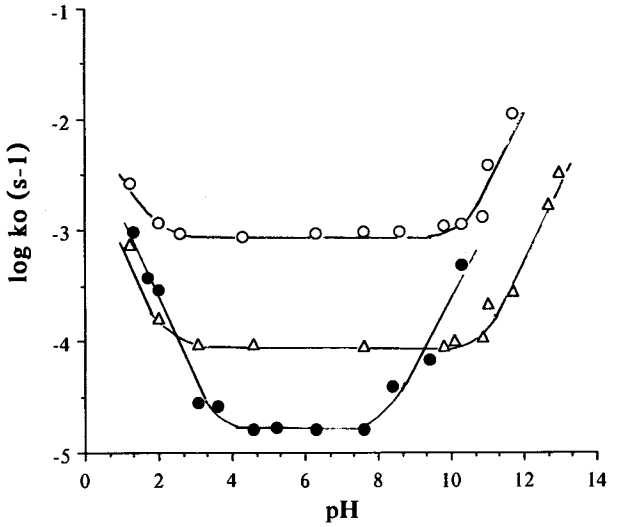
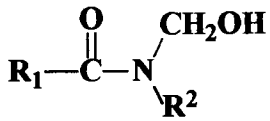


Fig. 2. pH-rate profiles for the hydrolysis of compounds VII (○), VIII (●) and IX (△) in aqueous buffers containing 20% of acetonitrile (at 20 °C).

A striking feature of these pH-rate profiles is the unusually broad plateau extending from pH *ca.* 2 to pH *ca.* 10. This contrasts markedly with simple ester hydrolysis, which usually exhibits specific base catalysis at *ca.* pH 6-7. Moreover, the pH-rate profiles of the penicilloates observed here differ significantly from those of the hydrolysis of penicillins, which either have no significant spontaneous reaction (eg. penicillin G) or have only small plateaus around neutral pH (eg. cyclacillin) (10). The apparent first-order rate constants for the pH-independent reactions for these latter penicillins are *ca.* 10^{-7} s^{-1} at 30 °C, several orders of magnitude smaller than the values *ca.* 10^{-3} s^{-1} obtained for the derivatives IV-VIII at 20 °C.

pH-Independent Region. The hydrolysis of the esters IV-VIII in the pH-independent region liberates penicillin G and the corresponding tertiary *N*-hydroxymethylamide XII in quantitative yield.



XII

Figure 3a depicts a reaction profile for the decomposition of compound VI at pH 7.4. Over the timescale of the hydrolysis reactions, neither the decomposition of penicillin G to penicilloic acid (through β-lactam ring opening) nor decomposition of the hydroxymethylamide to the corresponding secondary amide, were detected. The rate of formation of the *N*-hydroxymethyl derivative, XII, follows first-order kinetics and is identical to the rate of decompo-

Table I. Second-Order Rate Constants, k_{H^+} and k_{HO^-}, for Acid and Base-Catalysed Pathways and Pseudo First-Order Rate Constants, k_o, for the pH-Independent Hydrolysis at 20°C, Half-Lives at 37°C in pH 7.4 Phosphate Buffer and Lipophilicity Parameters of Derivatives IV–XI

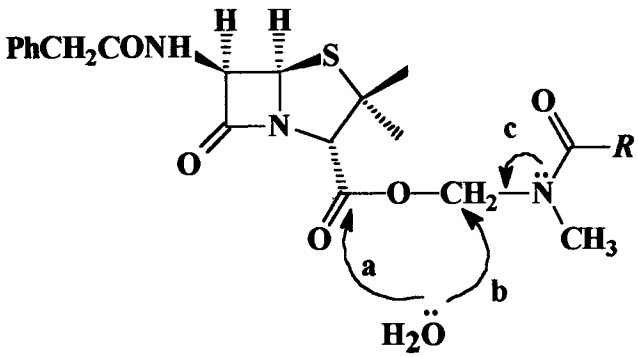
Compound	$10^2\ k_{H^+}/M^{-1}s^{-1}$	$10^2\ k_{HO^-}/M^{-1}s^{-1}$	$10^5\ k_o/s^{-1}$	$t_{1/2}$/min (pH 7.4)	log *P*[b]
IV	—	—	518	1[a]	1.00
V	—	—	176	1[a]	1.77
VI	—	—	89.8; 89.0[c]; 21.5[d]; 50.5[e]; 141[f]; 281[g]	2[a]	2.48
VII	2.78	238	91.1; 136[h]; 65.5[i]; 38.8[j]; 13.4[k]; 6.82[l]	2[a]	1.11
VIII	1.92	1460	1.66	23	1.60
IX	1.18	3.25	7.72	7	0.79
X	12.7	19.6	3.85	10	−0.22
XI	0.127	2.60	0.361	57	3.00

[a]From reference 11.
[b]*P*, octanol-water partition coefficient (calculated).
[c]in D_2O.
[d]10°C.
[e]15°C.
[f]25°C.
[g]30°C.
[h]20% dioxane.
[i]30% dioxane.
[j]40% dioxane.
[k]50% dioxane.
[l]60% dioxane.

sition of the substrate. In contrast, due to the slower rate of hydrolysis of the *O*-benzamidomethyl acetate X (Figure 3b), small amounts of *N*-methylbenzamide can also be detected alongisde the *N*-hydroxymethylamide.

The pH-rate profiles with broad plateaus, the high values of the apparent first-order rate constants for the pH-independent process and the quantitative formation of penicillin G clearly indicate that the source of reactivity of the *O*-amidomethyl derivatives IV-VIII is neither β-lactam ring opening nor conventional ester hydrolysis. What then is the mechanism of the hydrolysis?

The three potential mechanisms that could be operating in the solvolysis of any tertiary *N*-acyloxymethylamide are shown in XIII. Of these, path *a* would be expected to be independent of, or slightly retarded by, electron-donating R substituents, path *b* should be largely retarded by electron-donating R groups while path *c* should be enhanced by the electron-donating R substituents.



XIII

The k_o values for the compounds IV-VI clearly increase with the increased electron donation of the R group (as reflected by Taft σ* values), which is consistent with path *c*, unimolecular ionisation of the substrate to generate both the penicilloate anion and an acyliminium ion (Figure 4). Further evidence in favour of path *c* comes from the following observations:

1. The absence of buffer catalysis (data not shown) contrasting with the decomposition of penicillin G itself, which is catalysed by buffers and is characterised by a Bronsted β value of 0.39 consistent with general-base catalysis (10).

2. The temperature dependence for the reaction of compound VI reveals an entropy of activation, $\Delta S^{\neq}$, of -9.6 J K^{-1} mol^{-1} and an enthalpy of activation, $\Delta H^{\neq}$, of 86.5 kJ mol^{-1} (Table I). The value of $\Delta S^{\neq}$ is small and well within the range observed for unimolecular ionisation reactions (12).

3. The rate constants for compound VII are solvent dependent (Table I), and log k_o values correlate (Eq.(2)) with the corresponding Winstein-Grunwald *Y* parameter (a measure of the ionizing power of the solvent).

$$\log k_o = 0.60Y - 4.63 \quad (n = 5,\ r^2 = 0.99,\ s = 0.055) \qquad (2)$$

The value of the slope, 0.6, is slightly lower than the range normally observed for unimolecular ionization processes (0.7-1.0), but this could be attributable to the good leaving group ability of the penicillinoate anion (pK_a = 2.7) which is better able to disperse the developing negative charge. A similar effect has been observed elsewhere (13).

4. The solvolysis of the ester VI proceeds slightly faster in H_2O than in D_2O, giving a solvent kinetic isotope K_{H_2O}/K_{D_2O} effect of 1.01. This value is consistent with an S_N1 ionization process being the rate-limiting step, and contrasts sharply with that for the hydrolysis of the β-lactam ring in penicillins, 4.5 (10).

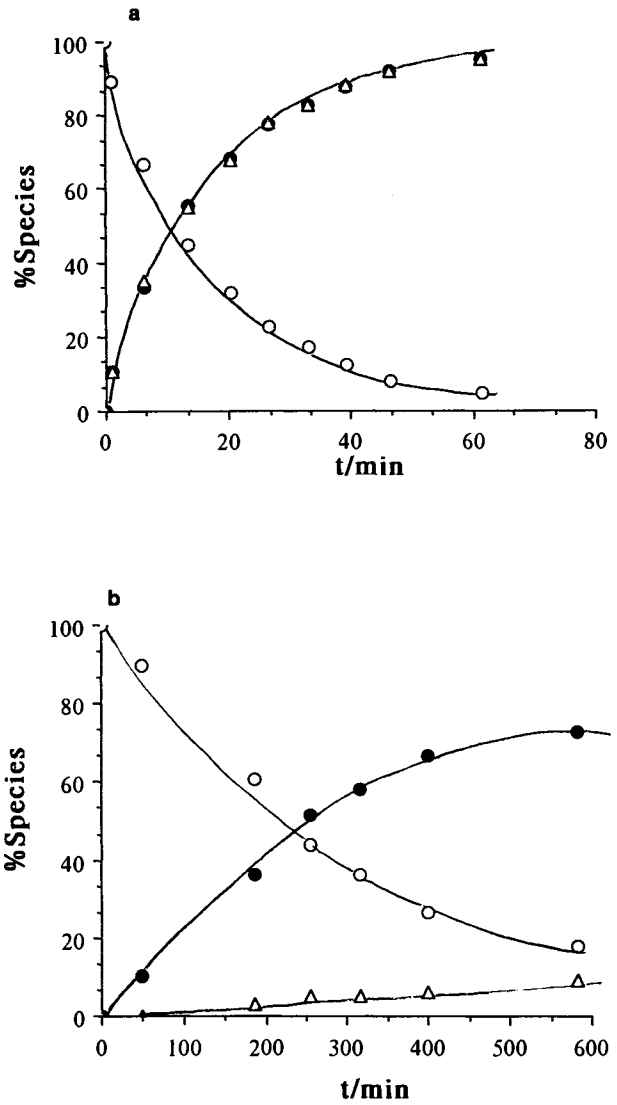


Fig. 3. a) HPLC product analysis for the hydrolysis of the *O*-amidomethyl penicilloate VI at pH 7.4 phosphate buffer containing 20% of acetonitrile at 20°C; (○), ester; (●), penicillin G; (△), *N*-hydroxymethylamide; b) HPLC product analysis for the hydrolysis of the *O*-amidomethyl acetate X at pH 7.4 phosphate buffer containing 20% of acetonitrile at 20°C; (○), ester; (●), *N*-hydroxymethylamide; (△), amide.

The greater reactivity of the *O*-(*N*-methylbenzamido)-methyl esters of penicillin G IV-VIII over those of simple carboxylic acids IX-XI can also be accomodated by the mechanism depicted in Figure 4. The observed rate constants for compounds V, IX, X and XI follow a Bronsted relationship with the pK_a of the carboxylate leaving group (Eq.(3)).

$$\log k_o = -1.06\, pK_a + 0.29 \quad (n = 4,\ r^2 = 0.91,\ s = 0.37) \qquad (3)$$

The Bronsted β_{lg} value of *ca.* -1.0 indicates that the reactivity of these esters is highly dependent on the nucleofugacity of the carboxylate, a trend expected for the S_N1 mechanism of solvolysis of tertiary *N*-acyloxymethylamides *via* iminium ion formation. Furthermore, the fact that penicillin G is included in this correlation clearly shows that β-lactam ring opening is not a major pathway is the hydrolysis of compounds IV-VIII.

Acid and Base-Catalysed Regions. Compounds IV-VII are too reactive to study in the acid and base-catalysed regions of the pH-rate profiles. Nevertheless, product analysis reveals that in both regions the major product is penicillin G. This is also true for the less reactive compound VIII. Under acidic conditions (*eg.* pH 3) the co-product is the corresponding *N*-hydroxymethylamide while in basic media (*eg.* pH 11) rapid formation of the secondary amide from the hydroxymethylamide is observed. For compounds IX-XI, the rate data in Table I reveal that, as the steric crowding in the carboxylate moiety is increased, the value of k_{OH^-} diminishes, as expected for nucleophilic attack of OH- at the ester carbonyl group.

In vitro *Antibiotic Activity.* The *in vitro* antibiotic activities of compounds IV and VI-VIII against a range of Gram positive and Gram negative bacteria (Table II) show that, in general, the esters have a similar activity profile to penicillin G. This is particularly true of compounds IV, VI and VII. Compound VIII, however, appears to be at least 4 times less active than penicillin G and the other derivatives, which can be ascribed to the much greater chemical stability of this prodrug at pH 7. The *in vitro* activity data thus appear to correlate with the reactivity in the pH-independent region of the pH-rate profile, but there is no obvious correlation between either antibiotic activity or chemical reactivity with the log *P* values of these compounds (cf Tables I and II).

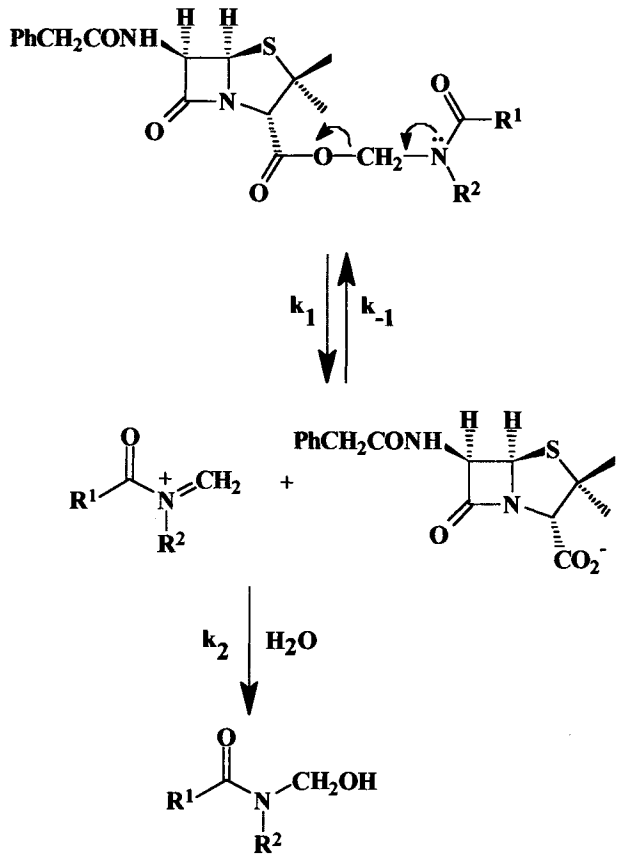


Fig. 4. Mechanism for the hydrolysis of *O*-amidomethyl esters of penicillin G.

Table II. *In vitro* Activity of *O*-Amidomethyl Penicillinoates IV, VI–VIII and of Penicillin G Against Several Bacteria

Bacteria	MIC × 10^{-3}/μmol l^{-1}				
	IV	VI	VII	VIII	Penicillin G
P. aeruginosa ATCC 27853	>500	>500	>500	>500	>500
K. pneumonia[a]	>500	>500	>500	>500	>500
K. pneumonia ATCC 10031	125	250	125	250	62
E. coli[a]	>500	>500	>500	>500	>500
E. coli ATCC 25922	62	125	125	250	62
S. typhimurium ATCC 43971	2	4	2	16	4
S. enteritidis	16	31	16	62	8
S. flexneri	62	62	62	125	31
S. dysenteriae ATCC 13313	31	16	31	62	16
S. marcescens NCTC 1377	>500	>500	>500	>500	>500
P. mirabilis	8	16	8	31	8
S. aureus ATCC 25923	<0.2	<0.2	<0.2	4	<0.2
S. faecalis ATCC 10541	16	31	31	31	16

[a] β-lactamase producing bacteria.

Relevance to Prodrug Design

O-(*N*-alkylamido)methyl esters of penicillin G hydrolyse *via* cleavage of the ester group. This is a clear advantage over the majority of α-acyloxyalkyl double prodrugs of β-lactam antibiotics, which can suffer β-lactam ring opening depending on the substrate and the pH. For example, at 25 °C and pH 7.5 hydrolysis of bacampicillin involves 22 % β-lactam ring opening and 78 % ester hydrolysis, whereas at pH 3.5, β-lactam hydrolysis accounts for 74% of the total hydrolysis reaction (14). Even so, simple *O*-(*N*-alkylamido)methyl esters of penicillin G IV-VIII are clearly too unstable to be of general use as prodrugs. However, the compounds containing electron-withdrawing acylamido substituents have reduced rates of hydrolysis, especially the prodrug VIII derived from penicillin G and ethyl hippurate which is *ca.* 100-fold more stable than its *N*-methylbenzamide counterpart V. This is a trend observed elsewhere for similar derivatives of naproxen and ibuprofen (11). Such a difference in reactivity can be ascribed to the strong electron-withdrawing effect of the *N*-CH_2CO_2Et group compared to *N*-CH_3, which depresses the rate of *N*-acyliminium ion formation. Studies are in progress to evaluate more thoroughly the influence of the *N*- substituents in the amide promoiety on the chemical and enzymatic reactivity of *O*-amidomethyl esters in general.

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REFERENCES

1. H. Bundgaard. Design of Bioreversible Drug Derivatives and the Utility of the Double Prodrug Concept. In E.B. Roche (Ed.), *Bioreversible Carriers in Drug Design: Theory and Application;* Pergamon Press, New York, 1987; pp. 13-94.
2. J.N. Iley, R. Moreira, and E. Rosa. Triazene drug metabolites. 4. Kinetics and mechanism of the decomposition of 1-aryl-3-benzoyloxymethyl-3-methyltriazenes in mixed aqueous-organic solvents. *J. Chem. Soc. Perkin Trans. 2:* 1503-1508 (1987).
3. M. Johansen and H. Bundgaard. Pro-drugs as drug delivery systems. XVI. Novel water-soluble pro-drugs types for chlorzoxazone by esterification of the *N*-hydroxymethyl derivative. *Arch. Pharm. Chemi, Sci. Ed.* 9: 43-54 (1981).
4. H. Bundgaard and G.J. Rasmussen. Prodrugs of peptides. 11. Chemical and enzymatic hydrolysis kinetics of N-acyloxymethyl derivatives of a peptide-like bond. *Pharm. Res.* 8: 1238-1242 (1991).
5. S.A. Varia; S. Schuller; K.B. Sloan, and V.J., Stella. Phenytoin prodrugs. III. Water-soluble prodrugs for oral and parenteral use. *J. Pharm. Sci.* 73: 1068-1073 (1984).
6. A. Buur, H. Bundgaard, and E. Falch. Prodrugs of 5-fluorouracil. IV. Hydrolysis kinetics, bioactivation and physicochemical properties of various N-acyloxymethyl derivatives of 5-fluorouracil. *Int. J. Pharm.* 24: 43-60 (1985).
7. H. Bundgaard and N.M. Nielsen. Prodrugs as drug delivery systems. 74. Facile hydrolysis of *N*-(acyloxymethyl)amide derivatives and implications for the design of prodrugs of NH-acidic compounds and of carboxylic acids. *Acta Pharm. Suec.* 24: 233-246 (1987).
8. J. Iley, R. Moreira, and E. Rosa. Acyloxymethyl as a drug protecting group. Kinetics and mechanism of the hydrolysis of *N*-acyloxymethylbenzamides. *J. Chem. Soc. Perkin Trans. 2:* 563-570 (1991).
9. W.J. Wheeler, P.A. Preston, W.E. Wright, G.W. Huffman, H.E. Osborn, and D.P. Howard. Orally active esters of cephalosporin antibiotics. 3. Synthesis and biological properties of aminoacyloxymethyl esters of [D-(-)-mandelamido]-3-[[(1-methyl-1*H*-tetrazol-5-yl)thio]methyl]-3-cephem-4-carboxylic acid. *J. Med. Chem.* 22: 657-661 (1979).
10. M.I. Page. The mechanisms of reactions of β-lactam antibiotics. *Adv. Phys. Org. Chem.* 23: 165-270 (1987).
11. R. Moreira, E. Mendes, T. Calheiros, M.J. Bacelo, and J. Iley. A new direct synthesis of tertiary *N*-acyloxymethylamide prodrugs of carboxylic acid drugs. *Tetrahedron Lett.* 35: 7107-7110 (1994).
12. S. Winstein and A.H. Fainberg. Correlation of solvolysis rates. IV. Solvent effects on enthalpy and entropy of activation for solvolysis of *t*-butyl chloride. *J. Am. Chem. Soc.* 79: 5937-5950 (1957).
13. J.S. Lomas and J.-E. Dubois. Solvent effects in the solvolysis of aryl di-*tert*-butylcarbinyl *p*-nitrobenzoates in aqueous acetic acid. Substituent effects on transition state charge separation. *J. Org. Chem.* 40: 3303-3305 (1975)
14. N.-A.T. Nguyen, L.M. Mortada, and R.E. Notari. Potential improvement in the shelf life of parenterals using the prodrug approach: bacampicillin and talampicillin hydrolysis kinetics and utilization time. *Pharm. Res.* 5: 288-296 (1988).