Synthesis and Substituent Effects on Antibacterial Activity, Alkaline Hydrolysis Rates, and Infrared Absorption Frequencies of Some Cephem Analogues Related to Latamoxef (Moxalactam)

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Relationships between intrinsic antibacterial activity and β -lactam reactivity of 7β -[(4-hydroxyphenyl)acetyl]aminoand 7β -[(4-hydroxyphenyl)malonyl]amino derivatives of 1-oxa- and 1-thiacephems, with or without the 7α -methoxy group (1-8), were investigated in order to clarify the enhanced antibacterial activity of latamoxef disodium (1). Substituent effects of a carbon atom at the 1- and 7α -positions were also investigated by using racemic 1-carbacephem 9 and 7α -methyl-1-oxacephem 10. Syntheses of 2-8 and 10 are also described. Acid chlorides derived from the O-benzyloxycarbonyl derivative of (4-hydroxyphenyl)acetic acid and the p-methoxybenzyl derivative of (4hydroxyphenyl)malonic acid smoothly effected the introduction of these side chains. Conjugate addition of lithium dimethylcuprate to the quinoid system in 16 proceeded stereospecifically, furnishing the 7α -methyl group for the synthesis of 10. Values of log (1/C) averaged for the sensitive Gram-negative strains (Escherichia coli NIHJ JC-2 and Klebsiella pneumoniae SRL-1) were taken as an estimation of the intrinsic antibacterial activity. The chemical reactivity of the β -lactam ring was estimated either by pseudo-first-order rate constants (k) of alkaline hydrolysis measured at pH 9.20 and 35.0 $^{\circ}$ C or by infrared stretching frequencies of the β -lactam carbonyl measured in dimethyl sulfoxide. Substitution of an oxygen atom at the 1-position increases both the hydrolysis rates and the antibacterial activity by a factor of \sim 6.3, while substitution of a 7 α -methoxy group increases the antibacterial activity by a factor of ~ 3.2 without significant change in the hydrolysis rates. The effect of the 7 α -methoxy group on the transition state in alkaline hydrolysis is discussed. Substitutions at the 1-position with a methylene group and, especially, at the 7α -position with a methyl group greatly diminished the antibacterial activity, whereas the hydrolysis rate remained high with the substitution of a methylene group. Substitution of an oxygen atom for the sulfur atom at the 1-position of 1-thiacephems increased the β -lactam carbonyl frequencies by ~ 6 cm⁻¹, whereas introduction of a 7α -methoxy group in 1-thia- and 1-oxacephems reduced the frequencies by ~ 5 cm⁻¹.

Penicillin molecules inhibit the activity of membranebound enzymes that catalyze the final step of peptide cross-linking between nascent and preexisting peptidoglycans in the cell wall synthesis in bacteria;¹ inhibition of bacterial growth was suggested as the primary physiological response to this disturbance in cell wall synthesis.^{1c} These enzymes are believed to correspond to some of penicillin-binding proteins, of which inhibition also related to the various morphological changes observed during treatment of bacteria with particular β -lactam antibiotics. As the main secondary aspects, penicillin may also interfere with peptidoglycan biosynthesis and the control system for murein hydrolase activity, resulting in a triggering of the hydrolase activity, which leads to the lysis of the bacterial cell wall.^{1c} β -Lactam antibiotics structurally similar to the C-terminal D-Ala-D-Ala moiety of the peptidoglycans can compete for the enzymes with the natural substrates² and inhibit the enzymes by forming fairly stable acyl enzymes,³ which was recently confirmed by X-ray crystallography.⁴

The interest of medicinal chemists has been focused on the relationships between antibacterial activity of β -lactam

- (a) Frére, J.-M.; Duez, C.; Ghuysen, J.-M.; Vanderkerckhove, (3) J. FEBS Lett. 1976, 70, 257. (b) Yocum, R. R.; Waxman, D. J.; Rasmussen, J. R.; Strominger, J. L. Proc. Natl. Acad. Sci. U.S.A. 1979, 76, 2730. (c) Georgopapadakou, N. H.; Liu, F. Y.; Ryono, D. E.; Neubeck, R.; Ondetti, M. Eur. J. Biochem. 1981, 115, 53.
- (4) Kelly, J. A.; Moews, P. C.; Knox, J. R.; Frére, J.-M.; Ghuysen, J.-M. Science 1982, 218, 479.

antibiotics and their ability to acylate the active center of the enzymes,⁵ which was recently found to be the serine hydroxy group.³ Relationships between antibacterial activity and reactivity of penicillins and cephalosporins have been investigated by using alkaline hydrolysis rates of the β -lactam ring⁶ and molecular orbital treatments⁷ to estimate the reactivity. The antibacterial activity or chemical reactivity of the β -lactam antibiotics has been correlated with bond characteristics around the β -lactam moiety, which are inferred from infrared stretching frequencies of the β -lactam carbonyl,⁸ kinetic parameters,⁹ geometrical parameters obtained by X-ray crystallographic analyses,¹⁰ and ¹³C NMR chemical shifts.¹¹

In connection with the enhanced antibacterial activity¹² and the high stability to β -lactamases¹³ demonstrated by latamoxef disodium (1),¹⁴ we investigated the effects of its

- Yamana, T.; Tsuji, A. J. Pharm. Sci. 1976, 65, 1563.
 (a) Hermann, R. B. J. Antibiot. 1973, 26, 223. (b) Topp, W. (7)C.; Christensen, B. G. J. Med. Chem. 1974, 17, 342. (c) Boyd, D. B.; Hermann, R. B.; Presti, D. E.; Marsh, M. M. Ibid. 1975, 18, 408. (d) Boyd, D. B.; Lunn, W. H. W. Ibid. 1979, 22, 778. (e) Boyd, D. B.; Lunn, W. H. W. J. Antibiot. 1979, 32, 855. (f) Boyd, D. B.; Herron, D. K.; Lunn; W. H. W.; Spitzer, W. A. J. Am. Chem. Soc. 1980, 102, 1812. (g) Boyd, D. B. Ann. N.Y. Acad. Sci. 1981, 367, 531.
- (8) Morin, R. B.; Jackson, B. G.; Mueller, R. A., Lavagnino, E. R.; Scanlon, W. B.; Andrews, S. L. J. Am. Chem. Soc. 1969, 91, 1401.
- Indelicato, J. M.; Norvilas, T. T.; Pfeiffer, R. R.; Wheeler, W. J.; Wilham, W. L. J. Med. Chem. 1974, 17, 523.
- (10) Sweet, R. M. In ref 5, p 280.
- (11) (a) Paschel, J. W.; Dorman, D. E. J. Org. Chem. 1978, 43, 2013. (b) Modelli, R.; Ventura, P. J. Chem. Soc., Perkin Trans. 2 1977, 1749.
- Yoshida, T.; Matsuura, S.; Mayama, M.; Kameda, Y.; Kuwa-(12)hara, S. Antimicrob. Agents Chemother. 1980, 17, 302.
- (13)Murakami, K.; Yoshida, T. Antimicrob. Agents Chemother. 1981, 19, 1.

^{(1) (}a) Blumberg, P. M.; Strominger, J. L. Bacteriol. Rev. 1974, 38, 291. (b) See also: Charlier, P.; Coyette, J.; Dideberg, O.; Duez, C.; Dusart, J.; Frére, J.-M.; Ghuysen, J.-M.; Joris, B.; Leyh-Bouille, M.; Nguyen-Distésche, M. In "Recent Advances in the Chemistry of β -Lactam Antibiotics"; Gregory, G. I., Ed.; The Royal Society of Chemistry, Special Publication No. 38; Burlington House: London, 1981; p 184. (c) Tomasz, A. Annu. Rev. Microbiol. 1979, 33, 113.

^{(2) (}a) Tipper, D. J.; Strominger, J. L. Proc. Natl. Acad. Sci. U.S.A. 1965, 54, 1133. (b) Lee, B. J. Mol. Biol. 1971, 61, 463.

⁽⁵⁾ Gorman, M.; Ryan, C. W. In "Cephalosporins and Penicillins: Chemistry and Biology"; Glynn, E. H., Ed.; Academic Press: New York, 1972; p 532.

Scheme I



substituents on the antibacterial activity and the reactivity of the β -lactam ring. Here we report the effects of the atoms at the 1-position and the substituents at the 7α position, as well as the carboxyl group of the side chain in cephem analogues 1-10, upon their antibacterial activity and chemical reactivity, which is expressed by the pseudo-first-order rate constants of alkaline hydrolvsis and correlated with the infrared stretching frequencies of the β -lactam carbonyl.

HO (
	х	Y	R	
Τ	0	OCH3	COONo	
2	S	H	н	
3	0	н	н	
4	s	OCH ₃	н	
5	0	OCH3	н	
6	S	н	COONa	
7	0	н	COONa	
8	s	OCH3	COONa	
9	CH ₂	OCH3	COONa (<u>racemic</u>)	
10	0	CH₃	COONa	

Results

Synthesis of Cephem Analogues. Amine 11a-d^{14a} was prepared as reported previously, and side chains were introduced by two methods. The (p-hydroxyphenyl)acetyl group was introduced by using [p-[(benzyloxycarbonyl)-

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Table I.	Antibacterial	Activity	(MIC _a	and MIC _b) of 1-10
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MIC, µg/mL	compd	<i>E. coli</i> NIHJ JC-2	Kleb. pneumoniae SRL-1
MIC _a ^a	2 3 4 5 6 7 8	$1.2 \\ 0.34 \\ 0.5 \\ 0.08 \\ 1.5 \\ 0.29 \\ 0.55$	1.4 0.31 1.3 0.1 2.3 0.3 0.37
MIC _b ^b	1 8 1 (<i>dl</i>)-9 10	$\begin{array}{c} 0.03\\ 0.09\\ 0.78\\ 0.1\\ 6.25\\ 12.5\end{array}$	0.06 0.39 0.1 3.13 12.5

^a Obtained by the gradient plate method. ^b Obtained by the agar dilution method.

oxy]phenyl]acetic acid (12), which was prepared as described in the Experimental Section. The corresponding acid chloride 13, obtained by treatment with oxalyl chloride and dimethylformamide, smoothly acylated amine 11a-d to yield 14a-d, respectively (Scheme I). Treatment of 14a-d with aluminum trichloride and anisole¹⁵ cleanly effected the deprotection of both benzyloxycarbonyl and benzhydryl groups to produce acids 15a-d, respectively, which were converted into the corresponding sodium salts 2-5 with sodium 2-ethylhexanoate.

The [(p-hydroxyphenyl)malonyl]amino group was introduced by using the method reported for preparing 1 and **21b.**^{14a,b} The 7α -methyl- 7β -amino derivative 11e was successfully prepared by conjugate addition of lithium dimethylcuprate to 16, an intermediate reported^{14a} in the synthesis of 11d, followed by treatment of the resulting 17 with Girard reagent T. The addition appeared to be stereospecific and was considered to proceed from the less-hindered α -side.¹⁶ Activation of the protected halfester 18 reported previously^{14a,b} by treatment with oxalyl chloride in the presence of triethylamine afforded a solution of the corresponding acid chloride 19, which was used in situ for the acylation of 11a,c,e. Removal of both etherand ester-protecting groups by aluminum trichloride treatment yielded acids 21a,c,e, respectively. Neutralization of acid 21a,c,e, including 21b,14b gave the corresponding disodium salts 6-8 and 10, respectively.

Total synthesis of 9 in a racemic form has already been reported from our laboratories.¹⁷

Antibacterial Activity. Table I shows minimal inhibitory concentrations, MIC_a and MIC_b (in micrograms per milliliter), determined by the gradient plate technique and the agar dilution method, respectively, representing the in vitro antibacterial activity of 1-10. The MIC_a values agreed well with the MIC_b for 1 and 8. The geometric means of MIC_a and MIC_b were calculated for two different β -lactamase-nonproducing organisms (E. coli NIHJ JC-2 and Kleb. pneumoniae SRL-1) and designated as Ca and C_b (in moles per liter), respectively (Table II). Values of $\log (1/C_{\rm s})$ were used as an estimation of the intrinsic activity of 1-8. Values of log $(1/C_b)$ were used when com-

^{(14) (}a) Narisada, M.; Yoshida, T.; Onoue, H.; Ohtani, M.; Okada, T.; Tsuji, T.; Kikkawa, I.; Haga, N.; Satoh, H.; Itani, H.; Nagata, W. J. Med. Chem. 1979, 22, 757. (b) Narisada, M.; Yoshida, T.; Onoue, H.; Ohtani, M.; Okada, T.; Nagata, W. J. Antibiot. 1982, 35, 463. (c) Nagata, W. Philos. Trans. R. Soc. London 1980, B289, 225.

Tsuji, T.; Kataoka, T.; Yoshioka, M.; Sendo, Y.; Nishitani, Y.; (15)Hirai, S.; Maeda, T.; Nagata, W. Tetrahedron Lett. 1979, 2793.

⁽¹⁶⁾ The corresponding 1-thia compound was prepared as a major product by a similar conjugate addition of methylmagnesium bromide to the corresponding quinoid system. See: Yanagisawa, H.; Nakao, H. Tetrahedron Lett. 1976, 1815. Uyeo, S.; Ona, H. Chem. Pharm. Bull. 1980, 28, 1563.

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Prepared by methoxylation of 11a by the method reported by (18)Yanagisawa et al.: Yanagisawa, H.; Fukushima, M.; Ando, A.; Nakao, H. Tetrahedron Lett. 1975, 2705.

 	$C_{a}^{a}, \frac{a}{mol}$	C_{b}, b	lor	log			v_{\max} , d cm ⁻¹		capacity ^e =
compd	$L \times 10^7$	$L \times 10^7$	$(1/C_a)$	$(1/C_b)$	$k, c h^{-1} \times 10^2$	$\log k$	β-lactam	amide I	factor (k')
 2	26.8		5.57		2.27 ± 0.10	-1.64	1773.1	1676.3	
3	6.93		6.16		13.92 ± 0.32	-0.86	1778.2	1674.5	
4	15.70		5.80		3.51 ± 0.04	-1.45	1768.1	1690.6	
5	1.79		6.75		22.82 ± 0.10	-0.64	1772.9	1694.9	
6	33.7		5.47		2.39 ± 0.14	-1.62	1770.0	1669.0	11.4
7	5.02		6.30		12.74 ± 0.12	-0.89	1779.2	1665.4	8.2
8	7.77	9.5	6.11	6.0	2.39 ± 0.08	-1.62	1767.8	1682.3	10.6
1	1.30	1.8	6.89	6.7	17.78 ± 0.10	-0.75	1771.8	1686.6	7.7
					(14.09 ± 0.22)	(-0.85)			
(dl)-9		39^{f}		5.4	6.94 ± 0.05	-1.16	1757.0	1684.5	10.3
10		228		4.6	1.48 ± 0.05	-1.83	1774.5	1668.2	8.3

Table II.	Antibacterial	Activity,	Hydrolysis	Rates,	and IR Frequencies
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^a Geometrical mean of MIC_a calculated from MIC_a values for *E. coli* NIHJ JC-2 and *Kleb. pneumoniae* SRL-1 in Table I. ^b Geometrical mean of MIC_b calculated from MIC_b values for *E. coli* NIHJ JC-2 and *Kleb. pneumoniae* SRL-1 in Table I. ^c Pseudo-first-order rate of β -lactam cleavage at pH 9.2 and 35 °C. ^d Measured in dimethyl sulfoxide. ^e See Experimental Section. ^f Corrected for the biologically active enantiomer.

parisons were made among 1 and 8–10, since $\log (1/C_a)$ values were not available for 9 and 10.

Alkaline Hydrolysis, Infrared Spectra, and HPLC Capacity Factors. Hydrolysis of 1 under the standard conditions of pH 10.0 and 35 °C⁶ was difficult due to the transient appearance of a new band at 252 nm, probably because of decomposition of the ionized phenolic moiety in the side chain. Accordingly, the hydrolysis rate constants of 1–10 were determined by measuring the loss of absorbance near 260 nm at pH 9.2 and 35.0 °C. Hydrolysis of 1, 3–5, and 7–10 followed pseudo-first-rate kinetics, whereas that of 2 and 6 was accompanied by successive gradual decomposition. The resulting pseudo-first-order rate constants (k) of 1-10 and that of 1 measured by the HPLC method, value in parentheses, are shown in Table II.

Infrared stretching frequencies of β -lactam carbonyl and amide I bands measured in dry dimethyl sulfoxide solution and HPLC capacity factors (k) representing lipophilicity of 1 and 6–10 are also given in Table II.

Experimental Section

Synthesis. All reactions were carried out under anhydrous conditions in a nitrogen atmosphere with anhydrous solvents that had been dried over type 4Å molecular sieves. Melting points were determined on a Yanagimoto apparatus and were not corrected. Infrared (IR) spectra were recorded on a Hitachi 215 or JASCO DS-403G spectrometer. Proton nuclear magnetic resonance (¹H NMR) spectra were obtained on a Varian A-60 or T-60A spectrometer with tetramethylsilane or sodium 2,2-dimethyl-2-silapentane-5-sulfonate (in D_2O) as an internal reference. Ultraviolet (UV) spectra were recorded on a Hitachi 323 spectrometer. Rotations were determined on a Perkin-Elmer 141 spectrometer. For column chromatography, silica gel (Merck silica gel 60) deactivated by the addition of 10% water was used.

Preparation of [4-[(Benzyloxycarbonyl)oxy]phenyl]acetic Acid (12). An ethereal solution of (4-hydroxyphenyl)acetic acid (10.6 g) was treated with an excess of diphenyldiazomethane. The product was purified by silica gel chromatography using benzene-ethyl acetate (4:1) as the eluant and crystallized from methanol to give **diphenylmethyl (4-hydroxyphenyl)acetate** (17.0 g, 76.3%).

To a solution of the ester (17.0 g, 53.34 mmol) in methylene chloride (100 mL) were added triethylamine (8.90 mL, 1.2×53.34 mmol) and benzyl chloroformate (10.9 g, 1.2×53.34 mmol), and the resulting solution was stirred at 0 °C for 30 min. The reaction solution was concentrated in vacuo and diluted with ethyl acetate. The diluted solution was washed successively with H₂O, 2 N HCl, and H₂O and dried (Na₂SO₄). Removal of the solvent, followed by crystallization of the residue from methanol, gave diphenylmethyl [4-[(henzyloxycarbonyl)oxy]phenyl]acetate (20.3 g, 84.1%): mp 70–72 °C; IR (CHCl₃) 1758, 1732 cm⁻¹; NMR (CDCl₃) δ 3.67 (s, 2 H), 5.23 (s, 2 H), 6.83 (s, 1 H).

The carbonate (2.0 g) dissolved in methylene chloride (40 mL) and anisole (4.0 mL) was treated with trifluoroacetic acid (4.0 mL) at 0 °C for 45 min. The reaction mixture was concentrated in vacuo and the residue was crystallized from petroleum ether to give 12 (1.24 g, 98.0%): mp 113–115 °C; IR (CHCl₃) 1755, 1708 cm⁻¹; NMR (CDCl₃) δ 3.63 (s, 2 H), 5.27 (s, 2 H).

Preparation of Compound 14d (General Procedure for Compounds 14a-c). A suspension of 12 (8.0 g, 2.2×14 mmol) in benzene (70 mL), mixed with oxalyl chloride (2.39 mL, 2.0 \times 14 mmol) and DMF (70 μ L), was stirred at room temperature for 1 h. Crude acid chloride 13, obtained as a crystalline mass on removal of the solvent in vacuo, was used for the next reaction. To a solution of 11d^{14a} (7.12 g, 14 mmol) in ice-cold methylene chloride (210 mL) were added pyridine (1.69 mL, 1.5×14 mmol) and a solution of the crude acid chloride 13 in methylene chloride (30 mL), and the resulting mixture was stirred at 0 °C for 30 min. The reaction solution was washed successively with 2 N HCl, H₂O, a 5% solution of NaHCO₃, and H_2O . The organic solution was dried (Na_2SO_4) and concentrated in vacuo. The residue was chromatographed on silica gel (200 g) using benzene-ethyl acetate (4:1) as the eluant to give 14d (10.7 g, 98.5%) as a colorless foam: IR (CHCl₃) 3420, 1788, 1762, 1715 cm⁻¹; NMR (CDCl₃) δ 3.48 (s, 3 H), 3.63 (br s, 2 H), 3.77 (s, 3 H), 4.27 (br s, 2 H), 4.60 (br s, 2 H), 5.05 (s, 1 H), 5.27 (s, 2 H), 6.67 (br s, 1 H), 6.90 (s, 1 H).

In a similar way, 14a-c were prepared. Compound 14a, foam $(\sim 100\%)$: IR (CHCl₃) 1792, 1767, 1725, 1690 cm⁻¹; NMR (CDCl₃) δ 3.52 (br s, 4 H), 3.67 (s, 3 H), 4.23 (br s, 2 H), 4.87 (d, J = 4 Hz, 1 H), 5.22 (s, 2 H), 5.80 (dd, J = 8 and 4 Hz, 1 H), 6.92 (s, 1 H), 7.00–7.67 (m, 20 H). Compound 14b, foam (79.5%): IR (CHCl₃) 1799, 1764, 1722, 1687 cm⁻¹; NMR (CDCl₃) δ 3.60 (s, 2 H), 3.73 (s, 3 H), 4.23 (s, 2 H), 4.57 (s, 2 H), 4.98 (d, J = 4 Hz, 1 H), 5.27 (s, 2 H), 5.72 (dd, J = 9 and 4 Hz, 1 H), 6.68 (d, J = 9 Hz, 1 H), 6.93 (s, 1 H), 7.13–7.67 (m, 19 H). Compound 14c, foam (92.8%): IR (CHCl₃) 3.43 (s, 3 H), 3.50, (s, 2 H), 3.57 (s, 2 H), 3.75 (s, 3 H), 4.20, 4.44 (AB q, J = 14 Hz, 2 H), 5.00 (s, 1 H), 5.23 (s, 2 H), 6.88 (s, 1 H).

Preparation of Compound 15d (General Procedure for Compounds 15a-c). An ice-cold, stirred solution of 14d (8.4 g, 10.8 mmol) dissolved in anisole (50 mL) was treated with AlCl₃ (14.4 g, 10 × 10.8 mmol), and the resulting mixture was stirred for 30 min at 0 °C. The reaction mixture was partitioned between ethyl acetate and NaHCO₃ solution. The precipitates were filtered off. The aqueous filtrate was separated, washed with ethyl acetate, acidified with concentrated HCl to pH 1.5, and extracted with ethyl acetate after saturation with sodium chloride. The organic solution was dried (Na₂SO₄) and concentrated in vacuo to give crude crystals, which were recrystallized from methanol-ethyl acetate to give 15d (4.25 g, 82.7%): mp 165-168 °C dec; IR (KBr) 3300, 1780, 1710, 1678 cm⁻¹; UV (MeOH) λ_{max} 274 nm (log ϵ 4.05); NMR (acetone- d_6) δ 3.42 (s, 3 H), 3.57 (s, 2 H), 4.00 (s, 3 H), 4.33 (s, 2 H), 4.65 (s, 2 H), 5.10 (s, 1 H), 6.70-7.27 (m, 4 H).

In a similar way, 15a-c were prepared. Compound 15a (99.3%): mp 131-135 °C (from ether); characterized as sodium salt 2. Compound 15b (97.9%): mp 182-185 °C dec; characterized as sodium salt 3. Compound 15c, colorless foam (91.6%): IR (KBr) 3290, 1772, 1710, 1675 cm⁻¹; UV (MeOH) λ_{max} 276 nm (log ϵ 3.98); NMR (CD₃OD) δ 3.43 (s, 3 H), 3.53 (s, 4 H), 3.97 (s, 3 H), 4.25, 4.38 (AB q, J = 14 Hz, 2 H), 5.00 (s, 1 H), 6.63–7.22 (m, 4 H).

Preparation of Compound 5 (General Procedure for Compounds 2-4). A solution of 15d (3.50 g, 7.35 mmol) in MeOH (20 mL) mixed with a 2.0 mol/L methanolic solution of sodium 2-ethylhexanoate (9.2 mL, 2.5 × 7.35 mmol) was stirred at room temperature for 10 min. Precipitates generated on dilution of the reaction solution with ethyl acetate (300 mL) and ether (100 mL) were obtained by filtration and washed well with ether to give 5 (3.53 g, 96.4%) as an amorphous powder: $[\alpha]^{25}_{D} + 5.5 \pm$ 0.9° (MeOH, c 0.531); UV (MeOH) λ_{max} 273 nm (log ϵ 4.03); NMR (D₂O, DSS internal reference) δ 3.50 (s, 3 H), 3.65 (s, 2 H), 4.03 (s, 3 H), 4.17 (br s, 2 H), 4.50 (br s, 2 H), 5.17 (s, 1 H), 6.82-7.32 (m, 4 H).

In a similar way, 2-4 were prepared. Compound 2, powder (81.7%): IR (KBr) 3400, 1760, 1660, 1610 cm⁻¹; UV (MeOH) λ_{max} 274 nm (log ϵ 4.00). Compound 3, powder (~100%): IR (KBr) 3410, 1770, 1613 cm⁻¹; UV (MeOH) λ_{max} 267 nm (log ϵ 3.80). Compound 4, powder (88.2%): IR (Nujol) 1760, 1676, 1610 cm⁻¹; $[\alpha]^{25}_{D}$ +94.5 ± 2.5° (MeOH, c 0.544); UV (MeOH) λ_{max} 275 nm (log ϵ 4.02).

Preparation of Compound 17. A suspension of cuprous iodide (1.48 g, 2×3.88 mmol) in ether (20 mL) mixed with an ethereal solution of methyllithium (1.7 mol/L, 9.50 mL, 4×3.88 mmol) was stirred at -10 °C for 5 min. To the resulting mixture cooled at -78 °C was added a solution of crude 16^{14a} (prepared from 3.88 mmol of amine 11b) in THF (40 mL), and stirring was continued at -78 °C for 2 h. The reaction solution was poured into ice-water and extracted with ethyl acetate. The organic solution was washed with H₂O, dried (Na₂SO₄), and concentrated in vacuo to give crude 17, which appeared to be homogeneous on the grounds of its NMR spectrum: NMR (CDCl₃) δ 1.43 (s, 18 H), 1.73 (s, 3 H), 3.78 (s, 3 H), 4.28 (s, 2 H), 4.58 (s, 2 H), 4.97 (s, 1 H), 5.53 (s, 1 H), 6.93 (s, 1 H), 7.23-7.57 (m, 12 H).

Preparation of Compound 11e. The crude 17, dissolved in THF (20 mL) and MeOH (50 mL), was mixed successively with Girard reagent T (1.00 g, 1.5×3.88 mmol), H₂O (0.10 mL), and HOAc (0.10 mL). The resulting solution was stirred at room temperature for 30 min. The reaction solution was concentrated in vacuo, and the residue was dissolved in methylene chloride. The organic solution was washed with H₂O, dried (MgSO₄), and concentrated in vacuo. The residue was chromatographed on silica gel (60 g) using methylene chloride-ethyl acetate as the eluant. The main fractions were triturated with ethyl acetate-ether to give 11e (769 mg, 40%) as crystals: mp 190–192 °C; IR (CHCl₃) 1785, 1718, 1628, 1600 cm⁻¹; NMR (CDCl₃) δ 1.57 (s, 3 H), 1.75 (br s, 2 H), 3.83 (s, 3 H), 4.30 (s, 2 H), 4.67 (s, 2 H), 4.73 (s, 1 H), 6.92 (s, 1 H), 7.25–7.63 (m, 10 H).

Preparation of Compounds 20a,c,e. Acylation^{14a,b} of amines 11a, 11c¹⁸ and 11e by using a solution of acid chloride 19 prepared from 18 yielded 20a,c,e, respectively. Compound 20a (66.4%): mp 170–175 °C; NMR (CDCl₃) δ 3.72 (br s, 2 H), 3.77 (s, 3 H), 3.78 (s, 3 H), 3.80 (s, 3 H), 4.33 (br s, 2 H), 4.88 + 4.53 (s, 1 H, two epimers), 4.95 (d, J = 6 Hz, 1 H), 4.97 (s, 2 H), 5.10 (s, 2 H), 5.88 (dd, J = 10 and 6 Hz, 1 H). Compound 20c, foam (75.0%): IR (CHCl₃) 1787, 1732, 1700 cm⁻¹; NMR (CDCl₃) δ 3.38 (br s, 2 H), 3.40 (s, 3 H), 3.50 (s, 3 H), 3.52 (s, 3 H), 3.57 (s, 3 H), 4.17, 4.50 (AB q, J = 12 Hz, 2 H), 4.67 (br s, 1 H), 4.92 (s, 2 H), 4.97 (s, 1 H), 5.10 (s, 2 H). Compound 20e, not analyzed.

Preparation of Compounds 21a,c,e. Deprotection of **20a,c,e** with AlCl₃, in a similar way to that described for the preparation of **15d**, yielded **21a,c,e**, respectively. Compound **21a**, foam (92.0%): $[\alpha]^{25}_{D} + 23.1 \pm 1.5^{\circ}$ (CHCl₃, c 0.416); IR (KBr) 3378, 3318, 2585, 1770, 1727, 1675 cm⁻¹; UV (MeOH) $\lambda_{max} 271$ nm (log ϵ 3.90). Compound **21c**, foam (~100%): $[\alpha]^{23}_{D} + 70.6 \pm 1.1^{\circ}$ (MeOH, c 1.021); IR (KBr) 1775, 1725 cm⁻¹; UV (MeOH) $\lambda_{max} 276$ nm (log ϵ 3.99); NMR (acetone-d₆) δ 3.32 + 3.50 (s, 3 H, two epimers), 3.67 (m, 2 H), 3.97 + 4.00 (s, 3 H, two epimers), 4.40 (m, 2 H), 4.53 (br s, 3 H), 4.78 (s, 1 H), 5.07 (s, 1 H), 6.87, 7.25 (A₂B₂ q, J = 8 Hz, 4 H). Compound **21e**: NMR (acetone-d₆) δ 1.97 (s, 3 H), 3.98 (s, 3 H), 4.03 (m, 3 H), 4.32 (s, 2 H), 4.56 (m, 3 H), 4.88 + 4.90 (s, 1 H, two epimers), 6.81, 7.22 (A₂B₂ q, J = 8 Hz, 4 H).

Preparation of Compounds 6-8 and 10. Treatment of diacids 21a, 21b,^{14b} 21c, and 21e with sodium 2-ethylhexanoate in a similar

way to that described for the preparation of 5 yielded 6, 7, 8, and 10, respectively. Compound 6, colorless powder (60.8%): UV (H₂O) λ_{max} 272 nm (log ϵ 4.02); NMR (D₂O) δ 3.30, 3.66 (AB q, J = 18 Hz, 2 H), 3.97 (s, 3 H), 4.05, 4.25 (ÅB q, J = 14 Hz, 2 H), 4.47 (s, 1 H), 4.99 (d, J = 5 Hz, 1 H), 5.57 (d, J = 5 Hz, 1 H), 6.83, 7.20 (A₂B₂ q, J = 8 Hz, 4 H). Compound 7: UV (H₂O) λ_{max} 267.5 nm (log e 4.06); IR (KBr) 3410, 1765, 1650, 1600 cm⁻¹; NMR (D₂O, external Me₄Si) δ 4.50 (s, 3 H), 4.55–5.06 (m, 4 H), 5.43 (s, 1 H), 5.62 (d, J = 4 Hz, 1 H), 5.93 (d, J = 4 Hz, 1 H), 7.36, 7.73 (A₂B₂ q, J = 9 Hz, 4 H). Anal. Calcd for $C_{19}H_{16}N_6O_8SNa_2\cdot 1.5H_2O$. C, 40.64; H, 3.41; N, 14.96. Found: C, 40.90; H, 3.62; N, 14.94. Compound 8, colorless powder (70.8%): UV (H₂O) λ_{max} 273.5 nm $(\log \epsilon 4.05)$; NMR $(D_2O) \delta 3.20 + 3.25$, 3.60 + 3.65 (AB q, J = 18Hz, 2 H, two epimers), 3.43 + 3.53 (s, 3 H, two epimers), 3.98 (s, 3 H), 3.91, 4.32 (AB q, J = 14 Hz, 2 H), 4.47 + 4.53 (s, 1 H, two epimers), 5.05 (s, 1 H), 6.84, 7.26 (A_2B_2 q, J = 8 Hz, 4 H). Compound 10: $[\alpha]^{23}_{D} -50.4 \pm 0.9^{\circ} (H_2O, c \ 0.997); UV (H_2O) \lambda_{max}$ 267 nm (log e 4.05); IR (KBr) 3430, 1762, 1658, 1602, 1515 cm

Determination of Antibacterial Activity. (a) Method A. $MIC_{a}s$ were determined by the gradient plate technique, which is essentially same as that described by Bryson and Szybalski.¹⁹ The compound was incorporated into the agar medium used as the bottom layer with the plate tilted. The plate was then returned to the horizontal position, and agar medium without the compound was laid over the first layer. Each plate was incoulated by streaking a bacterial suspension of 10⁶ cells/mL across the gradient of compound. After incubation at 37 °C for 18–20 h, the MIC was determined by measuring the distance of growth of the test organism and calculating from the original concentration of the compound used in the bottom layer, since bacterial growth was visible along the streak to the point of inhibition.

(b) Method B. MIC_bs were determined by the agar dilution method using sensitivity test agar (Eiken, Japan). An overnight culture of bacteria in tryptosoy broth (Eiken, Japan) was diluted to about 10^6 cells/mL with the same broth and inoculated with an inoculating device onto agar containing serial twofold dilutions of an antibiotic. Organisms were incubated at 37 °C for 18–20 h. The MIC_b of an antibiotic was defined as the lowest concentration that inhibited visible growth. Determination of Rate Constants. (a) Spectroscopic

Determination of Rate Constants. (a) Spectroscopic Measurements of Rate Constants of Compounds 1-10. The method reported by Tsuji and Yamana⁶ was modified by using a slightly lower pH value, because during an attempted hydrolysis of 1 in a pH 9.94 buffer solution (NaHCO₃ + Na₂CO₃ + NaCl, $\mu = 0.5$) at 35 °C, a new band at 252 nm increased gradually in intensity to attain the maximal value after 25 min, while the maximum at 270 nm, characteristic of the cephem β -lactam system, almost disappeared after 10 min. Hydrolysis of 1-10 in a pH 9.20 buffer solution (0.084 mol/L glycine + 0.016 mol/L NaOH + NaCl, $\mu = 0.5$) at 35.0 ± 0.5 °C under nitrogen atmosphere proceeded without the appearance of the new band while showing isosbestic points near 248 nm in most cases.

The mode of hydrolysis of 1-10 fits the pseudo-first-order kinetics expressed by:

$$\ln\left[\left(A_t - A_{\infty}\right) / \left(A_0 - A_{\infty}\right)\right] = -kt$$

where A_t is the optical density at 270 nm after t hours; A and A_{∞} are that at t = 0 h and infinity, respectively. Values of A_0 were estimated by extrapolation to time zero of the A_t/t curves. Values of A_{∞} were assumed to be based on the A_t values at t = 24-30 h. Even though the estimated A_{∞} values might be slightly incorrect, good linear correlations between t and $\ln [(A_t - A_{\infty})/(A_0 - A_{\infty})]$ were obtained up to 7 h. In the cases of compounds 2 and 6, the reaction rates were so slow that competitive decomposition became significant enough to result in overestimation of the A_{∞} values to give small k values. Table II shows the k values obtained by using the least-squares method in the linear regions with 95% confidence levels.

(b) Chromatographic Measurements of Rate Constants of 1. The hydrolysis rate of 1 under the above conditions was measured by the HPLC method using Nucleosil 10 C18 (30 cm \times 4.0 mm i.d.) as the stationary phase, a 73:27 mixture of a 5 mmol/L *n*-Bu₄NOH-phosphate buffer solution (pH 6.0) and

⁽¹⁹⁾ Bryson, V.; Szybalski, W. Science 1952, 116, 45.

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Figure 1. Correlation between $\log (1/C)$ and $\log k$.

methanol as the mobile phase, 1.0 mL/min flow rate, and optical density at 230 nm for detection. The integrated area over the peaks at 9.7 and 10.2 min corresponding to the R and S epimers of 1, respectively, was calculated. Plots of ln (I_t/I_0) against time up to 24 h exhibited a very good linear correlation, expressed as:

 $\ln\left(I_t/I_0\right) = -kt$

where I_t and I_0 are integrated areas at time t and 0 hours, respectively. The k value with the 95% confidence level is also given in Table II.

Measurements of Infrared Spectra. Infrared stretching frequencies of the β -lactam carbonyl and amide I bands were measured with an accuracy of ± 0.5 cm⁻¹ in a ~ 0.02 mol/L solution in dry dimethyl sulfoxide using a 0.25-mm cell on a JASCO DS-403G spectrometer. The frequencies were calibrated for the rotational bands of the vapor.

Measurements of HPLC Capacity Factors.²⁰ The retention time (t_R) was measured using Nucleosil 5 C18 (15 cm × 4 mm i.d.) as the stationary phase, a 75:25 mixture of a 5 mmol/L *n*-Bu₄NOH-phosphate buffer solution (pH 6.5) and methanol as the mobile phase, and a flow rate of 1.0 mL/min. The capacity factor, k', was calculated as $(t_R - t_0)/t_0$, where t_R is the mean value of the retention time of the corresponding R and S epimers, and t_0 is the retention time of the unretained component (water).

Discussion

Our previous reports^{13,21,22} described the significant effects of the substituents upon the antibacterial spectrum of activity of 1–8 and related compounds. Figure 1 shows the relationships between antibacterial activity against sensitive Gram-negative bacteria [log $(1/C_a)$] and hydrolysis rates (log k).

Substitution of the oxygen atom (compare 3 with 2; 5 with 4; 7 with 6; and 1 with 8) increased both the values of log $(1/C_{\rm s})$ and log k by ~0.80 and ~0.79, respectively, which indicate that the enhanced antibacterial activity is associated with the increased chemical reactivity of the β -lactam ring. Since antibacterial activity has previously been correlated with the increased rates in hydrolysis by β -lactamases, as well as the high frequency shifts of the β -lactam carbonyl band,²³ the enhanced antibacterial activity and the increased enzymatic hydrolysis rates are concluded to be associated with the improved ability of

- (20) The experiment was carried out by Mr. K. Kuruma of our laboratories.
- (21) Yoshida, T. Philos. Trans. R. Soc. London 1980, B289, 231.
 (22) Hamashima, Y.; Matsumura, H.; Matsuura, S.; Nagata, W.;
- (22) Hamashima, Y.; Matsumura, H.; Matsuura, S.; Nagata, W.; Narisada, M. In ref 1b, p 57.
 (23) Murakami, K.: Takasuka, M.: Motokawa, K.: Yoshida, T. J.
- (23) Murakami, K.; Takasuka, M.; Motokawa, K.; Yoshida, T. J. Med. Chem. 1981, 24, 88.

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the β -lactam ring in 1-oxacephems to acylate the target enzymes and β -lactamases, respectively. The increase in the chemical reactivity of the β -lactam ring in 1-oxacephems has been correlated with ¹³C NMR chemical shifts and interpreted mainly by the decrease in amide resonance caused by increment mainly by the decrease in amide resonance caused by increment of the pyramidal structure of the lactam nitrogen atom,²⁴ which has been observed by X-ray crystallography²⁵ in both 7α -hydrogen and 7α methoxy 1-oxacephems. The increase in permeability through cell membranes of Gram-negative bacteria is an additional factor.²⁶ Actually, shortened HPLC capacity factors (k') were observed for 1-oxacephems (7 and 1) as previously reported,²⁷ indicating lowered lipophilicity,²⁸ which is probably necessary for better permeability. As reported previously for the 7α -hydrogen series,²⁶ the higher frequency shifts (ca. 5.8 cm⁻¹) of the β -lactam carbonyl caused by substitution of the oxygen atom in both the 7α -hydrogen and 7α -methoxy series might also support the correlation described above.

The effects of the introduction of the 7 α -methoxy group (compare 4 with 2; 5 with 3; 8 with 6; and 1 with 7) are rather puzzling. The values of log $(1/C_a)$ substantially increased (ca. 0.51), whereas log k remained almost constant (ca. 0.14). A lower shift of the β -lactam carbonyl frequencies (ca. -5.0 cm⁻¹), as well as an interestingly higher frequency shift of the amide I bands (ca. 17.3), was observed. Although the lower frequency shift of the β lactam carbonyl can be interpreted^{24,29} as due to the increased amide resonance caused by restored planarity in the β -lactam nitrogen atom, which was indicated by X-ray crystallography,²⁵ the small change in the hydrolysis rates is rather surprising. Indeed, the β -lactam ring in the



ground state was deactivated, whereas the probable transition state shown above in the alkaline hydrolysis might have been stabilized greatly by forming a strong hydrogen bond between the anion-charged oxygen atom and the cis-oriented amide hydrogen, resulting in accelerating the hydrolysis rates. Introduction of the 7α -methoxy group probably makes the hydrogen more acidic and closer to the carbonyl oxygen, resulting in a stronger hydrogen bond. The acidic character of the amide hydrogen might be associated with the higher frequency shift of the amide band I caused by the inductive effect of the methoxy group. Thus, the enhanced antibacterial activity generated by

- (24) Nishikawa, J.; Tori, K.; Takasuka, M.; Onoue, H.; Narisada, M. J. Antibiot. 1982, 35, 1723.
- (25) (a) For 7α-hydro-1-oxacephem, see: Shiro, M.; Nakai, H.; Onoue, H.; Narisada, M., to be published. (b) For 7α-methoxy-1-oxacephem: see Shiro, M.; Nakai, H.; Onoue, M.; Narisada, M. Acta Crystallogr., Sect. B 1980, B36, 3137.
- (26) Murakami, K.; Yoshida, T. Antimicrob. Agents Chemother. 1982, 21, 254.
- (27) Miller, R. D.; Affolder, C.; Neuss, N. Experientia 1981, 37, 928.
- (28) The linear correlation log P = B log k'+ constant, where B = 1, has been found for a wide range of aromatic compounds. See (a) McCall, M. J. Med. Chem. 1975, 18, 549. (b) Carlson, R. M.; Carlson, R. E.; Kopperman, H. L. J. Chromatogr. 1975, 107, 219. (c) Mirrlees, M. S.; Moulton, S. J.; Murphy, C. T.; Taylor, P. J. J. Med. Chem. 1976, 19, 615. (d) Hammers, W. E.; Meurs, G. J.; DeLigny, C. L. J. Chromatogr. 1982, 247, 1.
- (29) Takasuka, M.; Nishikawa, J.; Tori, K. J. Antibiot. 1982, 12, 1729.

introduction of the 7α -methoxy group may be attributed to the increased ability of the β -lactam ring to acylate RO⁻ located on the α -side.³⁰

The effects of introducing the carboxyl group to the side chain (compare 6 with 2; 7 with 3; 8 with 4; and 1 with 5) were not significant. Only a slight increase in the values of log $(1/C_{\rm s})$ for antibacterial activity was observed. Hydrolysis rates (log k) and the β -lactam carbonyl frequencies remained almost constant.

Substitution of a methylene group for the sulfur atom in cephalosporins (compare 9 to 8) greatly diminished log $(1/C_{\rm b})$ (-0.6), whereas log k significantly increased (0.56). The increase in the alkaline hydrolysis rate for 7α hydro-1-carbacephem at pH 10 and 35 °C was larger²⁴ than that caused by substitution of the oxygen atom. The increase was interpreted by a probable similarity in the geometry of 1-carbacephem to that of 1-oxacephems, which might force the lactam nitrogen atom into a more pyramidal structure. The diminished antibacterial activity might be ascribed to some disturbance in the complex formation to the target enzymes at the right position, probably caused by conformational changes in the amide side chain generated by the bulkier methylene group at the 1-position. Actually, antibacterial activity of 7α -hydro-1-carbacephem (24) against Gram-negative bacteria is of a similar level



to that for 22 and 23,²² in which the side chain probably contributes to efficient complex formation with enzymes.

Introduction of the 7α -methyl group resulted in substantial decreases in log k and log $(1/C_b)$ (-0.21 and -1.48, respectively). Steric hindrance against RO⁻ caused by the bulky methyl group³¹ and conformational changes in the side chain³² might be the reason for the decreases, respectively. The indifferently high frequency of the β -lactam carbonyl probably indicates little contribution of the inductive effect of the 7α -methyl group.

We came to the following four conclusions. First, the enhancement of antibacterial activity, against sensitive Gram-negative bacteria, caused by substitution of an oxygen atom for the sulfur atom in cephalosporins can be interpreted as an increase in the chemical reactivity of the β -lactam ring. Second, introduction of the 7α -methoxy group results in some enhancement of the antibacterial activity mainly caused by the increased reactivity of the β -lactam ring which may be associated with the presumed transition state stabilized by a stronger hydrogen bond between the amide hydrogen and the charge-generating carbonyl oxygen atom. Third, substitution of a methylene group for the sulfur atom in 7β -[[(4-hydroxyphenyl)malonyl]amino]-7 α -methoxycephalosporin lowers the antibacterial activity. Fourth, introduction of the 7α -methyl group greatly diminishes the antibacterial activity.

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Registry No. 1, 76858-80-5; 2, 77016-90-1; 3, 77059-22-4; 4, 77016-91-2; 5, 77059-23-5; 6, 86940-51-4; 7, 86862-79-5; 8 (isomer 1), 74157-37-2; 8 (isomer 2), 86862-93-3; 9, 86862-80-8; 10, 86862-81-9; 11a, 53090-86-1; 11b, 66429-65-0; 11c, 56610-72-1; 11d, 66510-99-4; 11e, 86862-82-0; 12, 86862-83-1; 13, 86862-84-2; 14a, 86862-85-3; 14b, 86862-86-4; 14c, 86862-87-5; 14d, 86862-88-6; 15a, 81362-32-5; 15b, 75007-69-1; 15c, 86862-89-7; 15d, 75007-70-4; 16, 70371-42-5; 17, 86862-90-0; 18, 70175-90-5; 19, 64952-86-9; 20a (isomer 1), 86862-91-1; 20a (isomer 2), 86862-94-4; 20c, 66216-32-8; 20e, 86884-68-6; 21a, 86940-52-5; 21c (isomer 1), 66216-37-3; 21c (isomer 2), 86862-95-5; 21e (isomer 1), 86940-96-7; 4-hydroxyphenylacetic acid, 156-38-7; diphenylmethyl (4-hydroxyphenylacetate, 78984-21-1; diphenylmethyl [4-[(benzyloxycarbonyl)oxy]phenyl]acetate, 86862-92-2.

Synthesis and Antihypertensive Activity of Substituted trans-4-Amino-3,4-dihydro-2,2-dimethyl-2H-1-benzopyran-3-ols

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A series of novel substituted *trans*-4-amino-3,4-dihydro-2,2-dimethyl-2*H*-1-benzopyran-3-ols was prepared and tested for antihypertensive activity in the conscious deoxycorticosterone acetate (DOCA)/saline treated hypertensive rat. Optimum blood pressure lowering activity requires 6-substitution by a strong electron-withdrawing group, together with a pyrrolidino or piperidino group at the 4 position. Exceptions to this were the 7-nitro-4-pyrrolidine analogue and the 6-nitro-3-chloropropylamine, which retained marked antihypertensive activity. All of these compounds were direct vasodilators and had comparable antihypertensive activity to hydralazine and to the calcium antagonist, nifedipine. The synthetic route to these compounds involves cyclization of propargyl ethers to 2H-1-benzopyrans, followed by conversion via bromohydrins to 3,4-epoxides, which were ring opened with the appropriate amines. Meta-substituted propargyl ethers gave both 5- and 7-substituted benzopyrans on thermal cyclization, the former predominating. A new route to 2,2-dimethyl-7-nitrobenzopyran is described.

During the evaluation of a series of benzopyrans developed in these laboratories, we discovered that 3,4-dihydro-2,2-dimethyl-*trans*-4-(isopropylamino)-6-nitro-2*H*-1-benzopyran-3-ol (1) possessed antihypertensive activity

⁽³⁰⁾ The effects of the 7α-methoxy group in cephalosporins on the reactivity of the β-lactam ring and antibacterial activity have been discussed in a different way. See: (a) Ho, P. P. K.; Towner, R. D.; Indelicato, J. M.; Wilham, W. J.; Spitzer, W. A.; Koppel, G. A. J. Antibiot. 1973, 26, 313. (b) Indelicato, J. M.; Wilham, W. L. J. Med. Chem. 1974, 5, 528.

⁽³¹⁾ The inductive effect of the 6α-methyl group on the decrease in the hydrolysis rate of methyl 6β-[(phenylacetyl)amino]-6αpenicillinate has been described. see: Bohme, H. W.; Applegate, H. E.; Ewing, J. B.; Funke, R.; Puar, M. S.; Dolfini, J. E. J. Org. Chem. 1973, 38, 231.

⁽³²⁾ Virudachalam, R.; Rao, V. S. R. Int. J. Pept. Protein Res. 1977, 10, 51.