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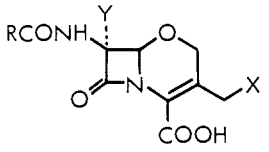
Communications to the Editor

Synthetic Studies on β-Lactam Antibiotics. Part 10.[1] Synthesis of 7β-[2-Carboxy-2-(4-hydroxyphenyl)acetamido]-7α-methoxy-3-[[(1-methyl-1*H*-tetrazol-5-yl)thio]-methyl]-1-oxa-1-dethia-3-cephem-4-carboxylic Acid Disodium Salt (6059-S) and Its Related 1-Oxacephems[2]

Sir:

Recently, syntheses and antibacterial activity[3-5] of 1-oxacephems have increasingly attracted notice. Christensen and his co-workers have published the synthesis of *dl*-1-oxacephalothin (*dl*-**1**)[4a] and *dl*-1-oxacefamandole (*dl*-**2**),[4b] and the available data suggested that the former was somewhat less potent than cephalothin but the latter compound was approximately twice as superior to cefamandole with respect to antibacterial activity. In a preceding communication[6] from our laboratories, the synthesis of several optically active 3-methyl-1-oxacephems **3** was reported, as well as the finding of a four- to eightfold superiority of these compounds in activity as compared with their 1-thia congeners. These findings stimulated subsequent studies on the synthesis and antibacterial activity of optically active 3-substituted methyl-1-oxacephems and 7α-methoxy compounds, including 1-oxacephalothin (**1**), 1-oxacefamandole (**2**), 7α-methoxy-1-



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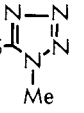
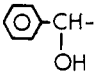
2, Y = -H; X =  ; R = [D]- 

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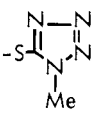
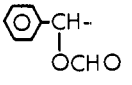
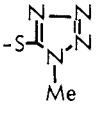
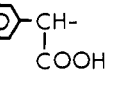
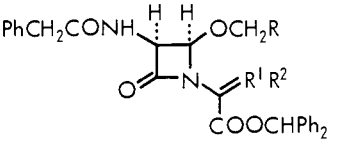
4, Y = -OMe; X =  ; R = [D]- 

5, Y = -OMe; X =  ; R = HO- 

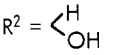
oxacefamandole *O*-formate (**4**), and the title compound (the disodium salt of **5**).[7]

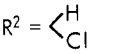
Azetidinone **6**, prepared[6] from 6-aminopenicillanic acid,



R¹, R² = =CMe₂ R = -COCH₂X

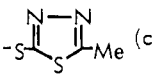
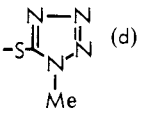
6, R = -C≡CH **11**, R¹, R² = =O

7, R = -CH=CH₂ **12**, R¹, R² = 

8, R =  **13**, R¹, R² = 

9, R = -CH(OH)CH₂X **14**, R¹, R² = =PPh₃

10, R = -COCH₂X

X = -OAc (a), -OMe (b),  (c),  (d)

was selectively hydrogenated (5% Pd–$CaCO_3$, MeOH) to **7**, which was then epoxidized (1.3 equiv of *m*-chloroperbenzoic acid, CH_2Cl_2, 15 h) to an epimeric mixture of **8** (67% from **6**). Cleavage of the epoxide ring of **8** either with X^- or with HX proceeded regiospecifically, producing the corresponding secondary alcohols. Thus, acetolysis (7% NaOAc in HOAc, 55–60 °C, 5 h) and acid-catalyzed methanolysis (99:1 MeOH–concentrated H_2SO_4, 25 °C, 1 h) of **8** yielded **9a** and **9b**, respectively. Cleavage of **8** with lithium 2-methyl-1,3,4-thiadiazole-5-thiolate (1 equiv of lithium 2-methyl-1,3,4-thiadiazole-5-thiolate, THF, 25 °C, 1 h) and lithium 1-methyl-1*H*-tetrazole-5-thiolate (1 equiv of 1-methyl-1*H*-tetrazole-5-thiol and 0.2 equiv of lithium 1-methyl-1*H*-tetrazole-5-thiolate, THF, 25 °C, 5 h) proceeded smoothly, producing **9c** and **9d**, respectively. Each of the alcohols **9a–d** was oxidized (CrO_3–H_2SO_4, acetone, 1.5–3 h), and the product was purified by silica gel chromatography to give the corresponding ketone **10** (yield from **8**: **a**, 74%; **b**, 62%; **c**, 63%; **d**, 89%).

Conversion of the isopropylideneacetate side chain of **10** into triphenylphosphonylideneacetate was carried out by a procedure established in our laboratories[8] involving ozonolysis of **10** (ozone, CH_2Cl_2, −78 °C then Me_2S), which gave **11**; selective reduction with zinc and acetic acid (0 °C, 1.5 h or 25 °C, 0.5 h), which produced a 1:1 mixture of epimeric alcohols **12**; chlorination (1.2 equiv of $SOCl_2$, pyridine, CH_2Cl_2, 0 °C, 0.5–2 h), which yielded a mixture of unstable chlorides **13**; and a final treatment with triphenylphosphine (CH_2Cl_2, reflux, 2.5–4 h), which formed ylide **14**, which was purified by silica gel chromatography. Heating **14** in refluxing dioxane under nitrogen (**a**, 20 h;

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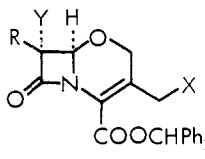
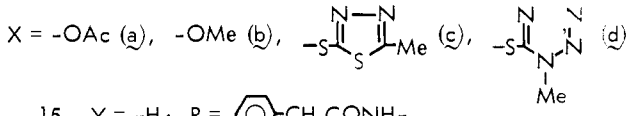
Table I. Minimum Inhibitory Concentrations (MIC), μg/mL

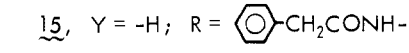
compd	*S.a*[a]	*S.a*[b,l]	*S.p.*[c]	*E.c.*[d]	*K.p.*[e]	*K.p.*[f,l]	*P.m.*[g]	*P.v.*[h]	*E.c.*[i]	*S.m.*[j]	*P.a.*[k]
1	0.013	0.1	0.025	0.8	0.2	>100	0.4	50	100	>100	>100
cephalothin	0.05	0.2	0.1	6.3	0.8	>100	3.1	50	>100	>100	>100
2	0.025	0.4	0.025	0.05	0.05	100	0.1	0.1	0.4	6.3	>100
cefamandole	0.1	0.4	0.1	0.4	0.4	>100	0.8	0.8	3.1	50	>100
4	0.4	0.4	0.2	0.05	0.1	0.1	0.2	0.2	3.1	3.1	>100
7α-methoxycefamandole *O*-formate	0.8	3.1	1.6	1.6	0.8	0.8	1.6	0.8	12.5	12.5	>100
disodium salt of **5**	6.3	6.3	3.1	0.1	0.1	0.05	0.1	0.1	0.2	0.4	25

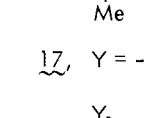
[a] *S.a.*, *Staphylococcus aureus* 209P JC-1. [b] *S.a.*, *Staphylococcus aureus* C-14. [c] *S.p.*, *Streptococcus pneumoniae* I. [d] *E.c.*, *Escherichia coli* NIHJ JC-2. [e] *K.p.*, *Klebsiella pneumoniae*. [f] *K.p.*, *Klebsiella pneumoniae* 363. [g] *P.m.*, *Proteus mirabilis* PR-4. [h] *P.v.*, *Proteus vulgaris* CN-329. [i] *E.c.*, *Enterobacter cloacae* 233. [j] *S.m.*, *Serratia marcescens* ATCC 13880. [k] *P.a.*, *Pseudomonas aeruginosa* 24. [l] β-Lactamase-producing strain.

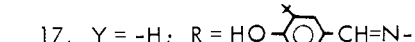
b, 40 h; **c**, 18 h; **d**, 15.5 h) yielded the desired compound **15**, the 1-oxa-1-dethia-3-cephem structure being confirmed

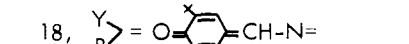


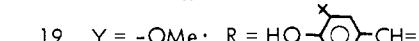












on the basis of its spectral data (yield from **10**: **a**, 20%; **b**, 46%; **c**, 14%; **d**,[9] 48%). Deacylation of **15** was smoothly carried out by the conventional method (PCl_5–pyridine, 25 °C, 0.5 h/MeOH, 25 °C, 0.5 h/H_2O, 25 °C, 0.5 h) to give **16** (a,[10] 75%; **b**,[11] 77%; **c**,[12] 89%; **d**,[13] 90%).

Introduction of the 7α-methoxy group into **16d** was successfully carried out by a sequence of reactions[14] involving condensation of **16d** with 3,5-di-*tert*-butyl-4-hydroxybenzaldehyde, which gave **17**; dehydrogenation with nickel peroxide[15] (1.8 equiv of nickel peroxide, $MgSO_4$, CH_2Cl_2–benzene, −12–25 °C, 1 h), which yielded **18**; addition of methanol at the 7α position, which gave **19**; and a final exchange reaction with the Girard reagent T, which produced the desired methoxyamine **20**[16] (74% from **16d**).

Acylation of **16a** with 2-thienylacetyl chloride and pyridine and **16d** with D-mandelic *O*-carboxylanhydride and sodium bisulfite, followed by deprotection (CF_3COOH–anisole, CH_2Cl_2, 0 °C, 1 h), yielded optically active 1-oxacephalothin (**1**) ($[\alpha]^{22}_D$ −10°, MeOH) and 1-oxacefamandole (**2**) ($[\alpha]^{22}_D$ −153°, MeOH), respectively. Methoxyamine **20** was acylated with D-2-(formyloxy)-2-phenylacetyl chloride and pyridine, and the product was similarly deprotected, producing 7α-methyl-1-oxacefamandole *O*-formate (**4**) ($[\alpha]^{22}_D$ −79°, MeOH). Finally, **20** was treated with 2-[4-[(4-methoxybenzyl)oxy]phenyl]-2-[[(4-methoxybenzyl)oxy]carbonyl]acetyl chloride, prepared in situ from the corresponding acid[17] (oxalyl chloride, Et_3N, CH_2Cl_2, 0 °C, 1 h), in the presence of pyridine (CH_2Cl_2, 0 °C, 0.5 h), and the product was deprotected (CF_3COOH–anisole, CH_2Cl_2, 0 °C, 1 h, or $AlCl_3$–anisole,[18] 5–0 °C, 0.5 h) producing **5**, which on treatment with sodium 2-ethylhexanoate gave the title compound[19] (disodium salt of **5**; $[\alpha]^{22}_D$ −45°, H_2O) as a 1:1 mixture of epimers at the C-α position.

Table I shows the MIC values of these optically active 1-oxacephems in comparison with those of the corresponding cephalosporins against various organisms. These 1-oxacephems clearly showed four- to eightfold superior potency to the 1-thia congeners against most of the susceptible bacteria. The title compound, which has the *p*-hydroxyphenylmalonylamido function at carbon 7, exhibited excellent activity and a widely expanded spectrum against Gram-negative bacteria, including *Pseudomonas aeruginosa* and a β-lactamase-producing strain of *Klebsiella pneumoniae*.

References and Notes

(1) Part 9: S. Uyeo, T. Aoki, H. Itani, T. Tsuji, and W. Nagata, *Heterocycles*, **10**, 99 (1978).

(2) The trivial name of 1-oxacephems, first described by Wolfe et al.,[3] was used for synthetic cephalosporin analogues possessing the 1-oxa-1-dethia-3-cephem or 5-oxa-1-azabicyclo[4.2.0]oct-2-en-8-one skeleton.

(3) S. Wolfe, J. B. Ducep, K. C. Tin, and S. L. Lee, *Can. J. Chem.*, **52**, 3996 (1974).

(4) (a) L. D. Cama and B. G. Christensen, *J. Am. Chem. Soc.*, **96**, 7582 (1974); (b) R. A. Firestone, J. L. Fahey, N. C. Maciejewicz, G. S. Patel, and B. G. Christensen, *J. Med. Chem.*, **20**, 551 (1977).

(5) E. G. Brain, C. L. Branch, A. J. Eglington, J. H. C. Nayler, N. F. Osborne, M. J. Pearson, J. C. Smale, R. Southgate, and P. Tolliday, "Recent Advances in the Chemistry of β-Lactam Antibiotics", J. Elks, Ed., The Chemical Society, Burlington House, London, 1977, p 204.

(6) M. Narisada, H. Onoue, and W. Nagata, *Heterocycles*, **7**, 839 (1977).

(7) The interesting antibacterial activity and the pharmacological properties of this compound were reported from our laboratories. (a) T. Yoshida, M. Narisada, S. Matsuura, W. Nagata, and S. Kuwahara, 18th Interscience Conference on Antibacterial Agents and Chemotherapy, Oct 1978, Atlanta, G., abstract no. 151; (b) S. Matsuura, T. Yoshida, K. Sugeno, Y. Harada, M. Harada, and S. Kuwahara, *ibid.*, abstract no. 152.

(8) S. Yamamoto, N. Haga, T. Aoki, S. Hayashi, H. Tanida, and W. Nagata, *Heterocycles*, **8**, 283 (1977).

(9) **15d**: mp 197–200 °C; $[\alpha]^{22}_D$ −193.2 ± 8.7° ($CHCl_3$, *c* 0.263); UV (CH_2Cl_2) λ_{max} 282 nm (ε 9150); IR ($CHCl_3$) ν 1799 (β-lactam), 1718 (ester), 1680 (amide) cm^{-1}; NMR ($CDCl_3$) δ 3.60 (s, $PhCH_2CO$), 3.78 (s, N-Me), 4.20 (s, C-2 H), 4.53 (br s, CH_2S), 4.95 (d, *J* = 4 Hz, C-6 H), 5.65 (dd, *J* = 4 and 9 Hz, C-7 H), 6.23 (d, *J* = 9 Hz, NH), 6.83 (s, $OCHPh_2$).

(10) **16a**: NMR ($CDCl_3$) δ 1.70 (br s, NH_2), 2.03 (s, OCOMe), 4.49 (br s, C-2 and C-7 H), 4.96 (d, *J* = 4 Hz, C-6 H), 5.00, 5.07 (AB q, *J* = 14 Hz, CH_2OAc), 6.91 (s, $OCHPh_2$).

(11) **16b**: colorless crystals; IR ($CHCl_3$) ν 1785 (β-lactam), 1722 (ester) cm^{-1}; NMR ($CDCl_3$) δ 1.8 (br s, NH_2), 3.25 (s, OMe), 4.47–4.60 (C-2 and C-7 H, CH_2OMe), 4.97 (d, J = 4 Hz, C-6 H), 6.98 (s, $OCHPh_2$).

(12) **16c**: IR ($CHCl_3$) ν 1794 (β-lactam), 1723 (ester) cm^{-1}; NMR ($CDCl_3$) δ 1.88 (s, NH_2), 2.67 (s, C-Me), 4.25, 4.55 (AB q, J = 14 Hz, C-2 H), 4.52 (d, J = 4 Hz, C-7 H), 4.68 (s, CH_2-S), 5.00 (d, J = 4 Hz, C-6 H), 7.07 (s, $OCHPh_2$).

(13) **16d**: $[\alpha]^{22}_D$ −232.8 ± 7.6° (Me_2SO, c 0.360); UV (Me_2SO) λ_{max} 286 nm (ϵ 8700); IR ($CHCl_3$) ν 1790 (β-lactam), 1718 (ester) cm^{-1}; NMR ($CDCl_3$) δ 1.75 (br s, NH_2), 3.81 (s, N-Me), 4.28 (br s, C-2 H), 4.50 (d, J = 4 Hz, C-7 H), 4.64 (br s, CH_2-S), 4.98 (d, J = 4 Hz, C-6 H), 6.90 (s, $OCHPh_2$).

(14) An excellent procedure for 7α-methoxylation of cephalosporins was applied; see H. Yanagisawa, M. Fukushima, A. Ando, and H. Nakao, *Heterocycles*, **3**, 1130 (1975); *Tetrahedron Lett.*, 259 (1976).

(15) Oxidation with nickel peroxide in place of originally reported lead dioxide proceeded under milder conditions.

(16) **20**: mp 160–162 °C (dec); IR ($CHCl_3$) ν 1792 (β-lactam), 1724 (ester) cm^{-1}; NMR ($CDCl_3$) δ 2.00 (br s, NH_2), 3.38 (s, OMe), 3.87 (s, N-Me), 4.32 (s, C-2 H), 4.73 (s, CH_2-S), 4.92 (s, C-6 H), 7.00 (s, $OCHPh_2$).

(17) Prepared by alkylation of *p*-hydroxyphenylacetic acid (2.4 equiv of *p*-anisyl chloride, 2.4 equiv of NaI, and 3 equiv of K_2CO_3, acetone, 50–60 °C, 48 h) giving 4-methoxybenzyl 4-[(4-methoxybenzyl)oxy]phenylacetate and subsequent carboxylation of the corresponding anion produced by deprotonation with lithium diisopropylamide [1.2 equiv of $LiN(i\text{-}Pr)_2$, THF, −78 °C, 30 min].

(18) A novel method for deprotection of esters, developed in our laboratories, was applied; see T. Tsuji, M. Yoshioka, T. Kataoka, Y. Sendo, S. Hirai, T. Maeda, and W. Nagata, Belgium Patent 856444 (1977); *Chem. Abstr.*, **89**, 6100*s* (1978).

(19) Disodium salt of **5**: UV (H_2O) λ_{max} 270 nm (ϵ 12000); NMR (D_2O–external Me_4Si) δ 3.91, 3.98 (two s, OMe), 4.44, 4.46 (two s, N-Me), 4.55, 4.60, 4.63 (C-2 H), 4.91, 4.95 (two br s, CH_2-S, benzyl proton), 5.57, 5.58 (two s, C-6 H).

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Cyclopropylamines as Suicide Substrates for Cytochromes P-450

Sir:

There is considerable current interest in enzyme inhibitors of the k_{cat} or suicide substrate variety.[1] Because the action of such inhibitors is intimately related to the enzymatic mechanism, knowledge of the latter often provides an excellent starting point for the rational design of highly specific and effective inhibitors. Conversely, the discovery of such inhibitors for an enzyme whose mechanism and active site are not well characterized should, in principle, provde an equally specific and effective probe of catalytic mechanism and active-site structure for that enzyme. The cytochrome P-450 mixed-function oxidases constitute an important family of enzymes whose mechanism and active sites remain incompletely characterized despite more than a decade of intense effort. A recent report[2] that allylisopropylacetamide (AIA), long known for its ability to deplete cytochrome P-450 in vivo, undergoes metabolic activation in vivo, leading to its covalent attachment to the heme group of P-450, now prompts us to report our own work with cyclopropylamines as suicide substrates for cytochrome P-450.

Table I. Inhibition of in Vitro Aminopyrine Demethylation by Para-Substituted *N*-Cyclopropyl- and *N*-Isopropylbenzylamines (p-$XC_6H_4CH_2NHR$)[a]

X	inhibitor, % inhibn	
	R = c-Pr	R = *i*-Pr
CH_3O	1a, 54 ± 1	1b, 0 ± 2
CH_3	2a, 77 ± 2	2b, 2 ± 4
H	3a, 61 ± 1	3b, 9 ± 6
Cl	4a, 79 ± 4	4b, 25 ± 3
Br	5a, 79 ± 1	5b, 27 ± 4

[a] The inhibitor (1 mM) and aminopyrine (3 mM) were each added at the start of the assay. Incubations were carried out in triplicate for a total of 12 min at 33 °C under air, followed by quenching with $ZnSO_4$ and $Ba(OH)_2$; formaldehyde was determined colorimetrically with Nash reagent.[4]

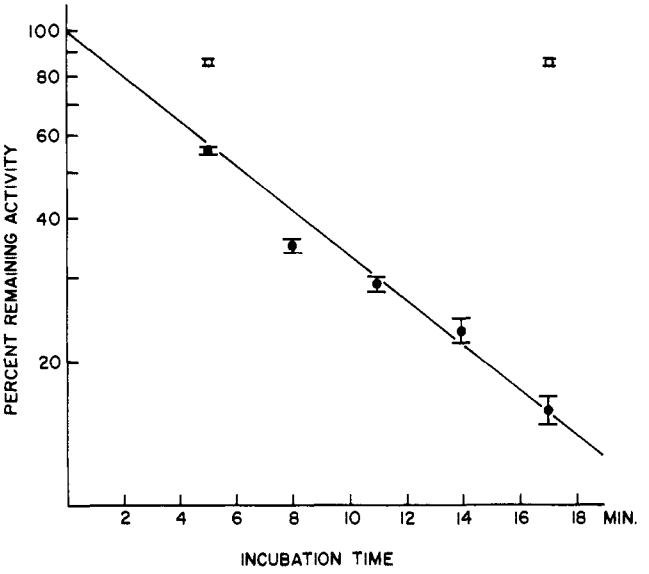

Figure 1. Kinetics of aminopyrine demethylase inhibition by **2a** (closed circles) and **2b** (open circles). Assays were carried out under conditions described in Tables I and II. The points represent the mean ± SE of four experiments.

In the course of some mechanistic studies aimed at developing effective inhibitors of first-pass N-dealkylation reactions, a series of para-substituted *N*-cyclopropyl- and *N*-isopropylbenzylamines was prepared[3] and evaluated in vitro[4] for inhibition of aminopyrine demethylation using rat liver microsomes. The cyclopropylamines were consistently found to be significantly more inhibitory than the corresponding isopropylamines (Table I).[5] During attempts to determine the kinetics of inhibition of aminopyrine metabolism by the *N*-cyclopropylbenzylamines, it was noticed that the degree of inhibition appeared to be both time and concentration dependent. In addition, among numerous individual batches of microsomes, those with the highest overall cytochromes P-450 activity were the most susceptible to inhibition. When the time dependence was specifically investigated, it was found that loss of enzyme activity was kinetically a first-order process with a rather short half-life (e.g., 6 min with **2a** in Figure 1). In contrast, inhibition due to **2b**, a very weak inhibitor with a high pK_a, and *N*-benzylmorpholine (**8**), a good inhibitor with a low pK_a, was time independent (Figure 1 and Table II).[6] Similar results (not shown) were obtained with **3a** and **3b**. Since control experiments indicated that the cyclopropylamines did not inhibit NADPH–cytochrome P-450 reductase nor interfere with cofactor regeneration, it appeared that the cyclopropylamines might be undergoing metabolic activation to a

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