C(2)-CARBOXY AND CARBOXYMETHYL CEPHEMS

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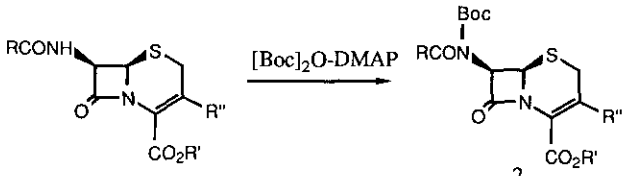
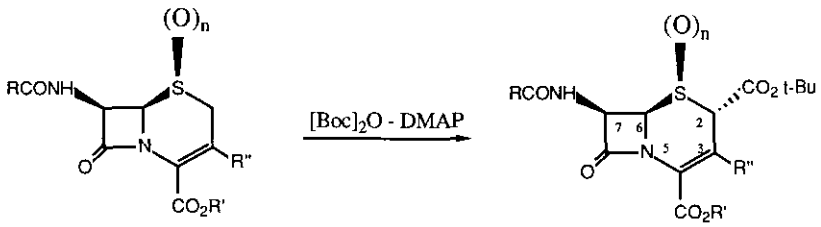
Abstract - A series of C(2)-substituted [methyl, carboxy and carboxymethyl] cephems have been prepared. Test data show a significant reduction in antibiotic activity. Penicillin binding protein (PBP) studies show a decreased affinity to essential PBP-3 in S. aureus X1.1 and E. coli K12. The effect is believed to be steric in nature.

We recently reported the synthesis of N-Boc-cephems using Boc anhydride, DMAP [4-dimethylamino-pyridine] on the cephem amides.[1] [1 to 2]



1 2

Although this reaction also works on the α or R sulfoxides, the corresponding β or S sulfoxides failed to give the N-Boc derivatives, resulting instead in C-carboxylation to give the C(2)-t-Bu ester 4.



3 4

	R	n	R'	R"	%
a	U	1	PNB	Me	50-65
b	U	1	PNB	Cl	57
c	Me	1	TCE	Me	54-65
d	U	1	PNB	OMe	44-48
e	U	1	BH	CH_2OAc	49-58
f	U	1	Me	H	0
g	U	1	PNB	H	11
h	U	1	BH	H	14
i	U	1	PNB	$OCHPh_2$	16
j	U	2	allyl	Me	67

PNB = p-nitrobenzyl; TCE = trichloroethyl;
BH = diphenylmethyl; U = phenoxyacetyl

Cephem sulfones also gave C(2)-carboxylation, using $[Boc]_2O$-DMAP, but required somewhat longer reaction times [4 h vs 30 min to 2 h].

Recycling 4 [R,R"=Me,R'=TCE][pKa=6.4] under the reaction conditions failed to give any new products, ie the N-Boc derivatives or dicarboxylation, while attempts to alkylate 4 [R,R"=Me, R'=TCE] or the corresponding C(2)-methyl ester [vida infra] with alkyl halides, for example methyl iodide, also failed, either at C(2) or C(4).

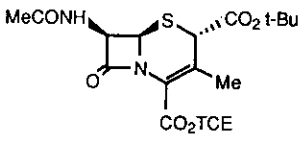
The β-sulfoxide is known to activate the C(2)-position of the cephem molecule sufficiently for it to undergo the Mannich[2] reaction, chlorination[3], alkylation[4,5], thiolation[6] and diazo exchange[7]. The α-sulfoxides, which are not intramolecularly hydrogen bonded to the amide proton, are known, however, to behave differently from the β-sulfoxides.[7,8,10]

Miller has shown that simple sulfoxides can be alpha-carboxylated with $[Boc]_2O$ under somewhat more drastic conditions [LDA].[11]

Campbell has reported that attempts to directly carboxylate the cephem sulfoxide using alkyl chloroformates-triethylamine resulted in C(2)-alkyl carbonates via a Pummerer mechanism.[7]

Acid (TFA=trifluoroacetic) cleavage of the t-butyl ester 4 gave the intermediate sulfoxide C(2)-carboxylic acid which readily underwent decarboxylation, analogous to a β-keto carboxylic acid.

Sulfoxide reduction of 4 [C(3)-Me] using PBr_3 (80-90%) or acetyl bromide/amylene (80%) went cleanly to give the sulfide-C(2)-t-Bu-ester whose stereochemistry was shown to be α by nmr studies. Thus 5 and its β-sulfoxide showed an NOE between the C(3)-Me and the C(2)-methine, indicating that the C(2)-proton is in the β-configuration. One would also predict carboxylation from the less hindered α-face of the molecule.



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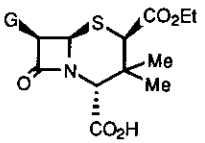
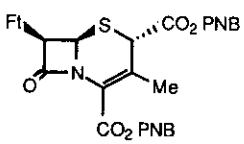


6

G=phenylacetamido

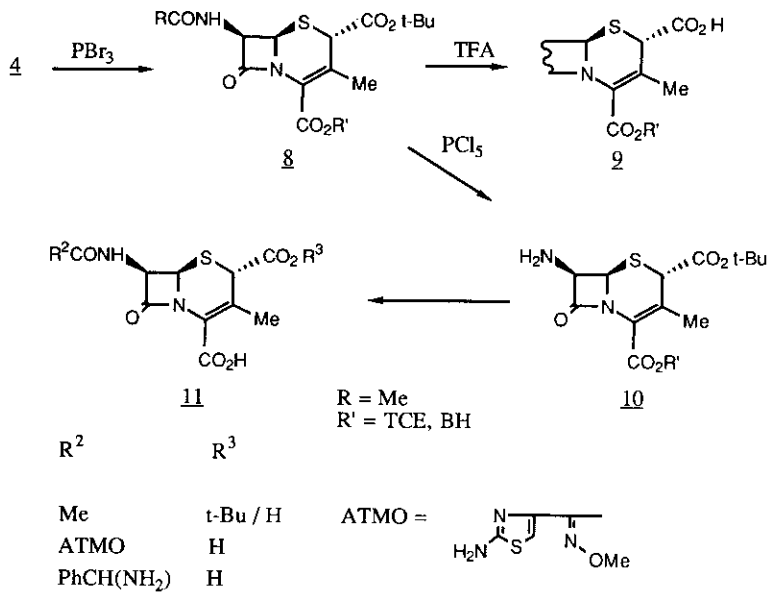


7

Ft=phthalimido

Yoshimoto et al. have prepared 6 from penicillin via a carbenoid reaction followed by a Michael reaction [35%] and ester deblocking. Its stereochemistry was shown to be C(2)-β-carboxy via W coupling between H^2-H^6.[12]

Kametani et al., via an analogous route of Yoshimoto's, have prepared 7 [14% overall], and their stereochemical assignment is C(2)-α-carboxy based on long range H^2-H^7 coupling.[13] We observe no H^2-H^7 coupling, however, it is known that the phthalimido side chain accentuates this type of coupling.[3]

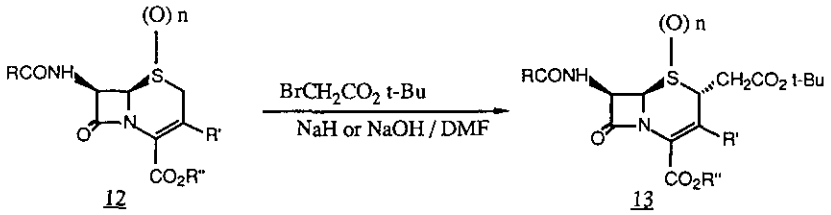


Acid cleavage of the t-Bu ester sulfide gave a stable sulfide-C(2)-acid 9 which on treatment with diazomethane gave 61% chromatographed C(2)-methyl ester. Attempts to prepare the C(2)-diazomethyl ketone via oxalyl chloride/diazomethane or ethyl chloroformate/base/diazomethane failed. We desired the diazomethyl ketone in order to study the intramolecular sulfur-ylide type rearrangement[14] and the Arndt Eistert reaction.

Application of Yamada's modified Curtius reaction also proved fruitless.[15,16]

Side chain cleavage [PCl_5] of 8 went smoothly [80%], and 10 was acylated and deblocked to give the various acids 11, all of which showed poor antibiotic activity [MICs of 64-128].

The C(2)-carboxymethylcephems 13 were then prepared by a slight modification of Kim's procedure[5].



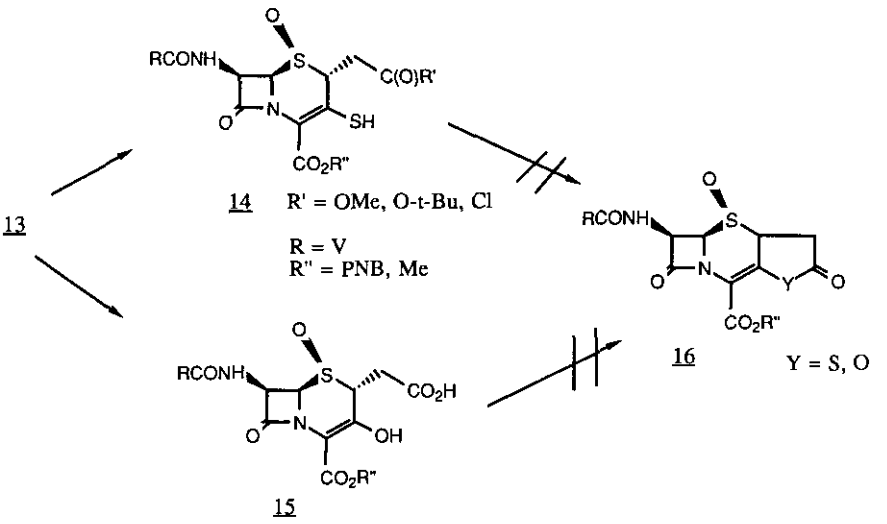
12 13

	R	n	R'	R"	%
a	U	2	Me	Allyl	62
b	Me	1	Me	TCE	83
c	U	1	CH_2OAc	PNB	40
d	U	1	OME	PNB	43 + 28%C(4)
e	U	1	SMe	PNB	50
f	U	1	Cl	PNB	70-80
g	U	1	H	BH	58
h	U	1	Cl	Me	55

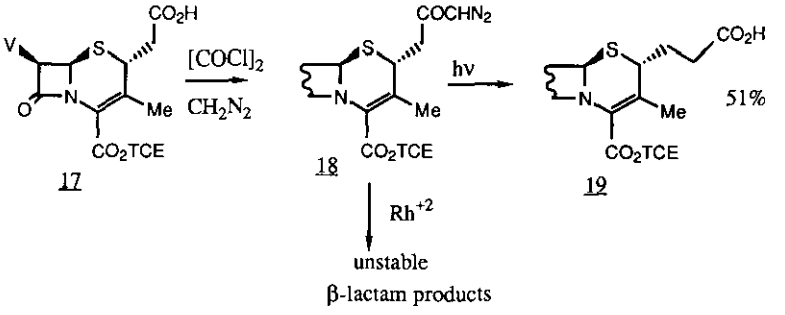
n=1 is β-sulfoxide only.

Only in the case of C(3)-OMe did we observe any significant amounts of C(4)-alkylation [28%]. The stereochemical assignment at C(2), when R' is methyl, was confirmed to be α-carboxymethyl by observing an NOE between the C(3)-Me and the C(2)-methine. The C(2)- stereochemistry of the major isomer of the other C(3)- derivatives is assumed to be α-carboxymethyl. There was, however, nmr evidence for the presence of the other C(2)-isomer.

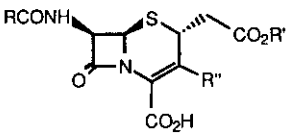
Attempts to cyclize the various sulfoxide carboxylmethyl derivatives 14 and 15 to the tricyclic lactones or thiolactones were not successful.



Synthesis of the diazomethyl ketone 18 was successful and it underwent the Arndt-Eistert reaction in 51%. The rhodium catalyzed ylide reaction yielded two products whose irs showed the presence of β-lactam and whose mass spectra showed loss of nitrogen and the addition of hydrogen. The products, however, were unstable.



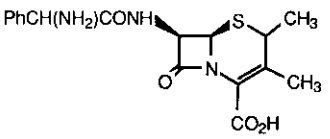
Sulfoxide reduction of 13 followed by side chain cleavage, acylation and deblocking gave the various acids 20, all of which showed very poor antibacterial activity.



20

R	R'	R"
$PhCH(NH_2)$	H	Cl
$PhCH(NH_2)$	Me	Cl
ATMO	H	Me

Since the minimal inhibitory concentrations (MICs) of the C(2)-carboxy and carboxymethyl cephems were so poor we prepared the C(2)-α and β-methyl derivatives 21 and 22 of cephalexin via Wright's[2] chemistry.

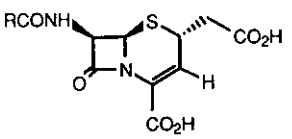


21 α-Me

22 β-Me

Both showed the same level of activity, which was significantly less than that of cephalexin.

Kamiya[17] and Long[18] have shown that C(2)-methyl-C(3)-hydrogen derivatives are in most cases equal or slightly better in activity than the corresponding C(3)-Me-C(2)-hydrogen compounds. We then prepared 23 and 24. 23 showed poor MICs while 24 showed reduced activity- *E. coli* [EC14] value of 1 vs. .015 for the corresponding descarboxymethyl derivative.



	R
23	$PhCH(NH_2)$
24	ATMO

Expression of antibacterial activity by β-lactam antibiotics can be generalized to require coincidence of at least three factors: physical access to the bacterial target enzyme (e.g., outer membrane permeability), avoidance of destruction by bacterial enzymes (e.g., β-lactamases), and the ability to bind to and inactivate the target enzymes (penicillin binding proteins). In an attempt to determine the reason for the lack of activity expressed by some of the compounds in this series, we studied their binding affinity for the PBPs in β-lactamase free inner membrane preparations of Staphylococcus aureus and Escherichia coli K12.[19]

In β-lactamase negative strains of S. aureus and E. coli such as the ones used in this study, cephalosporins usually bind to one of the essential PBPs at concentrations similar to the observed MICs in whole cell experiments.[20] Deviations from these generalizations must be attributed to other factors. We found general agreement between the affinity for PBP3 and the MIC for most of the compounds tested. Thus, we conclude that in most cases, the high MICs for these compounds can be attributed to low affinity for the essential PBPs in the test organisms.

EXPERIMENTAL

The following instruments were used for obtaining the spectral data: ^{1}H nmr: Varian T-60, G.E. QE-300; ir spectra: Perkin Elmer 281; mass spectral data: Varian-m.a.t.-731. NOE data were collected on a Bruker WM-270 spectrometer, using the Aspect 3000 data system. The "difference method" was used to determine the NOEs.[21] All chromatographic separations were done using Merck silica gel (Kieselgel 60).

Trichloroethyl (7β)-Acetamido-(2α)-t-butoxycarbonyl-3-methyl-3-cephem-4-carboxylate-1-β-oxide (4c).

The trichloroethyl (7β)-acetamido-3-methyl-3-cephem-4-carboxylate-1-β-oxide (3c) [12.43 g, 30.8 mmol] was combined with 13.44 g (61.6 mmol, 2 eq) of $[Boc]_2O$ and 3.951 g (32.3 mmol, 1.05 eq) of DMAP in 425 ml of CH_2Cl_2. After stirring 30 min at room temperature the mixture was washed with cold 1N HCl and brine, dried (Na_2SO_4), evaporated to dryness and chromatographed on silica gel using 20% ethyl acetate-toluene vs 20% acetone-ethyl acetate gradient to give 8.3 g [54%] product as a froth; m/z 504; ir ν ($CHCl_3$) 1795 cm^{-1} (β-lactam); uv λ (EtOH) 265 nm, ε=8,490, 373 nm, ε=17,500; nmr ($CDCl_3$) δ 1.52 (s, 9, t-bu), 2.07 (s, 3, Ac), 3.97 (s, 3, vinyl Me), 4.53 (s, 1, H^2), 4.80 (d, J=4 Hz, 1, H^6), 5.00 (s, 2, TCE), 6.12 (dd, J=4,10 Hz, 1, H^7), 6.88 (d, J=10 Hz, 1, NH).

Trichloroethyl (7β)-Acetamido-(2α)-t-butoxycarbonyl-3-methyl-3-cephem-4-carboxylate (5).

The corresponding sulfoxide (4c) (237 mg, 0.47 mmol) was dissolved in 8 ml of acetonitrile and 2 ml of DMF and treated at 0°C with 0.07 ml (0.706 mmol, 1.5 eq) of PBr_3 for 10 min, followed by 10 min at room temperature. The mixture was combined with ethyl acetate and washed with water, brine, dried (Na_2SO_4), evaporated and chromatographed on silica gel using a toluene-ethyl acetate gradient to give 194 mg (85%) product as a froth; m/z 487, 397; ir ν ($CHCl_3$) 1780 cm^{-1} (β-lactam); uv λ (EtOH) 264 nm, ε=6,350; nmr ($CDCl_3$) δ 1.52 (s, 9, t-bu), 2.05 (s, 3, Ac), 2.20 (s, 3, vinyl Me), 4.00 (s, 1, H^2), 4.97 (s, 2, TCE), 5.37 (d, J=5 Hz, 1, H^6), 5.93 (dd, J=5, 8 Hz, 1, H^7), 6.90 (d, J=8 Hz, 1, NH).

p-Nitrobenzyl (7β)-Phenoxyacetamido-(2α)-t-butoxycarbonyl-3-chloro-3-cephem-4-carboxylate-1-β-oxide (4b).

The p-nitrobenzyl (7β)-phenoxyacetamido-3-chloro-3-cephem-4-carboxylate-1-β-oxide (3b) (0.520 g, 1.0 mmol) was combined with 0.437 g (2.0 mmol, 2.0 eq) of $[Boc]_2O$ and 0.128 g (1.05 mmol, 1.0 eq) of DMAP in 25 ml of CH_2Cl_2 and allowed to stir at room temperature 1h. The mixture was diluted with ethyl acetate and then washed with 1N HCl and brine, dried (Na_2SO_4), evaporated and chromatographed on silica gel using a toluene-ethyl acetate gradient to give 353 mg (57%) product as a froth; m/z 520 (loss of Boc); ir ν ($CHCl_3$) 1800 cm^{-1} (β-lactam); nmr ($CDCl_3$) δ 1.52 (s, 9, t-bu), 4.57 (s, 2, phenoxyacetyl), 4.72 (s, 1, H^2), 4.88 (d, J=5 Hz, 1, H^6), 5.48 (s, 2, PNB), 6.24 (dd, J=5, 10 Hz, 1, H^7).

Trichloroethyl (7β)-Acetamido-(2α)-t-butoxycarbonylmethylene-3-methyl-3-cephem-4-carboxylate-1-β-oxide (13b).

To a stirred solution of the trichloroethyl (7β)-acetamido-3-methyl-3-cephem-4-carboxylate-1-β-oxide (12b) (6.456 g, 16.0 mmol) in 400 ml of DMF was added 24 ml (24 mmol, 1.5 eq) of 1N NaOH followed by 9.36 g (48 mmol, 32 eq) of t-butyl bromoacetate. The reaction mixture was allowed to stir for 45 min at room temperature and then diluted with cold ethyl acetate and acidified with excess 1N HCl. The aqueous was separated and reextracted with ethyl acetate. The combined ethyl acetate extract was washed with water and brine, dried (Na_2SO_4), evaporated and chromatographed on silica gel using 10% ethyl acetate-toluene vs 10% acetone-ethyl acetate gradient to give 6.90 g (83%) product as a froth; m/z 517; ir ν ($CHCl_3$) 1790 cm^{-1} (β-lactam); uv λ (EtOH) 265 nm, ε=8,171,372 nm, ε=14,661; nmr ($CDCl_3$) δ 1.22 (s, 9, t-bu), 1.82 (s, 3, Ac), 1.90, 1.96 (ABX, J=9,

17 Hz, 1, CH_2CO_2t-bu), 1.99 (s, 3, vinyl Me), 2.38, 2.44 (ABX, J=4, 17 Hz, 1, CH_2CO_2t-bu), 3.81 (m, J=4, 9 Hz, 1, H^2), 4.28 (d, J=5 Hz, 1, H^6), 4.60, 4.75 (AB, J=12 Hz, 2, TCE), 5.90 (dd, J=5, 10 Hz, 1, H^7), 9.94 (d, J=10 Hz, 1, NH).

Methyl (7β)-Phenoxyacetamido-(2α)-t-butoxycarbonylmethyl-3-chloro-3-cephem-4-carboxylate-1-β-oxide (13h).

To a cooled (0°C), stirred solution of the methyl (7β)-phenoxyacetamido-3-chloro-3-cephem-4-carboxylate-1-β-oxide (12h) (0.399 g. 1.0 mmol) in 15 ml of DMF was added 0.144 g (3.0 mmol, 3.0 eq) of 50% NaH. The reaction mixture was allowed to stir at 0°C for 2 min, t-butyl bromoacetate (0.48 ml, 3.0 mmol, 3.0 eq) was added and the reaction was allowed to stir at room temperature for 1h. Excess 1N HCl was then added along with ethyl acetate and the mixture was washed with water and brine, dried (Na_2SO_4), evaporated and chromatographed on silica gel using a toluene-ethyl acetate gradient to give 280 mg (55%) product as a yellow solid which was crystallized from CH_2Cl_2-hexane to give a white solid; mp 163-165°C; m/z 513; ir ν (KBr) 1805 cm^{-1} (β-lactam); nmr (DMSOd^6) δ 1.38 (s, 9, t-bu), 2.62, 2.65 (ABX, J=9, 18 Hz, 1, CH_2CO_2t-bu), 2.79, 2.81 (ABX, J=4, 18 Hz, 1, CH_2CO_2t-bu), 3.80 (s, 3, CO_2Me), 4.22 (m, J=4, 9 Hz, 1, H^2), 4.64 (s, 2, phenoxyacetyl), 5.17 (d, J=5 Hz, 1, H^6), 6.07 (dd, J=4, 9 Hz, 1, H^7); Anal. Calcd for $C_{22}H_{25}N_2O_8SCl$: C, 51.53; H, 4.91; N, 5.46. Found: C, 51.33; H, 4.78; N, 5.31.

Trichoroethyl (7β)-Acetamido-(2α)-carboxyethylene-3-methyl-3-cephem-4-carboxylate (19).

Trichoroethyl (7β)-acetamido-(2α)-carboxymethyl-3-methyl-3-cephem-4-carboxylate (17) (1.312 g, 2.44 mmol) in 25 ml of methylene chloride was treated with 2.6 eq of oxalyl chloride (0.55 ml, 6.35 mmol) and 5 drops of DMF and allowed to stir at 5°C for 15 min and then at room temperature for 30 min. The reaction mixture was evaporated to dryness at room temperature in vacuo, methylene chloride (15 ml) was added and the solution was again evaporated to dryness at room temperature. The crude acid chloride, dissolved in 15 ml of methylene chloride, was then added dropwise to a stirred, cooled (5°C) solution of 2 equiv of CH_2N_2 in 10 ml of methylene chloride plus 20 ml of Et_2O. The reaction mixture was then stirred at 5°C for 30 min and evaporated to dryness at room temperature. The mixture was then chromatographed on silica gel using a 10% ethyl acetate-toluene vs 10% acetone-ethyl acetate gradient to give 673 mg (49%) 18; ir ν ($CHCl_3$) 2100 cm^{-1} ($COCHN_2$), 1778 cm^{-1} (β-lactam); nmr ($CDCl_3$) δ 2.18 (s, 3, vinyl Me), 2.6 (m, 1, $CH_2CO_2CHN_2$), 2.85, 2.91 (ABX, J=2, 16 Hz, 1, $CH_2CO_2CHN_2$), 4.06 (dd, J=2, 10 Hz, 1, H^2), 4.52 (s, 2,

phenoxyacetyl), 4.89, 4.92 (AB, J=12 Hz, 2, TCE), 5.03 (d, J=5 Hz, 1, H^6), 5.30 (s, 1, $C\underline{H}N_2$), 5.98 (dd, J=5, 9 Hz, 1, H^7).

The diazomethyl ketone 18 (629 mg, 1.12 mmol) was dissolved in 487 ml of dioxane and 163 ml of water was added and the mixture was photolyzed for 1 h in an immersion vessel using a Hanovia 450 watt type L lamp with a pyrex insert. The mixture was evaporated in vacuo to a low volume, combined with ethyl acetate and extracted with aq $NaHCO_3$. The neutral ethyl acetate layer was dried (Na_2SO_4), evaporated to dryness and chromatographed on silica gel using a 20% ethyl acetate-toluene vs 20% acetone-ethyl acetate gradient to give 97 mg of starting material. The bicarbonate extract was layered with ethyl acetate and acidified with cold 1N HCl. The ethyl acetate solution was then washed with brine, dried (Na_2SO_4) and evaporated to give 317 mg (51%) 19; m/z 552; ir ν ($CHCl_3$) 1781 cm^{-1} (β-lactam); nmr ($CDCl_3$) δ 2.20 (s, 3, vinyl Me), 2.3 (m, 2, $C\underline{H}_2CH_2CO_2H$), 2.6 (m, 2, $CH_2C\underline{H}_2CO_2H$), 3.35 (m, 1, H^2), 4.53 (s, 2, phenoxyacetyl), 4.80, 4.96 (AB, J=12 Hz, 2, TCE), 5.00 (d, J=5 Hz, 1, H^6), 5.92 (dd, J=5, 9 Hz, 1, H^7).

The methyl ester of 19 was prepared using CH_2N_2; m/z 566, 564; nmr ($CDCl_3$) δ 2.24 (s, 3, vinyl Me), 2.3 (m, 2, $C\underline{H}_2CH_2CO_2Me$), 2.59 (m, 2, $CH_2C\underline{H}_2CO_2Me$), 3.37 (m, 1, H^2), 3.69 (s, 3, CO_2Me), 4.54 (s, 2, phenoxyacetyl), 4.81, 4.92 (AB, J=12 Hz, 2, TCE), 5.02 (d, J=5 Hz, 1, H^6), 5.95 (dd, J=5, 9 Hz, 1, H^7).

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REFERENCES

1. D. O. Spry, N. J. Snyder, A. R. Bhala, C. E. Pasini, and J. M. Indelicato, Heterocycles, 1987, 26, 2911.
2. I. G. Wright, C. W. Ashbrook, T. Goodson, G. V. Kaiser, and E. M. Van Heyningen, J. Med. Chem., 1971, 14, 420.
3. D. O. Spry, Tetrahedron Lett., 1972, 3717.
4. D. O. Spry, Tetrahedron Lett., 1973, 165.
5. C. U. Kim, P. F. Misco, U. J. Haynes, and D. N. McGregor, J. Med. Chem., 1984, 27, 1225.
6. A. Yoshida, S. Oida, and E. Ohki, Chem. Pharm. Bull., 1975, 23, 2507.
7. D. H. Bremner and M. C. Campbell, J. Chem. Soc., 1977, 2298.
8. J. Cs. Jaszberenyi and T. E. Grunda, Progress in Medicinal Chemistry, 1975, 12, 395, 1975.[9]
9. α-Sulfoxides undergo the Mannich reaction with Eschenmoser's reagent. Unpublished data of D. O. Spry.
10. J. J. Koning, A. F. Marx, M. M. Poot, P. M. Smid, and J. Verweij, "Recent Advances in the Chemistry of β-Lactam Antibiotics," ed. J. Elks, Chemical Society, Special Publication, No. 28, London, 1977, 161.
11. R. D. Miller and R. Hassig, Tetrahedron Lett., 1985, 26, 2395.
12. M. Yoshimoto, S. Ishihara, E. Nakayama, E. Shoji, H. Kuwano, and N. Soma, Tetrahedron Lett., 1972, 4387.
13. T. Kametani, N. Kanaya, T. Mochizuki, and T. Honda, Heterocycles, 1983, 20, 435.
14. I. Ernest, Tetrahedron, 1977, 33, 547.
15. T. Shioiri, K. Ninomiya, and S. Yamada, J. Am. Chem. Soc., 1972, 94, 6203.
16. D. O. Spry, Chem. Comm., 1974, 1012.
17. T. Kamiya, T. Teraji, M. Hashimoto, O. Nakaguchi, and T. Oku, J. Am. Chem. Soc., 1976, 98, 2342.
18. A. G. Long, "Recent Advances in the Chemistry of β-Lactam Antibiotics," ed. J. Elks, Chemical Society, Special Publications, No. 28, London, 1977, 214.
19. B. G. Spratt, Eur. J. Biochem., 1977, 341.
20. N. A. C. Curtis, D. Orr, G. W. Ross, and M. G. Boulton, Antimicrob. Agents Chemother., 1979, 533.
21. M. P. Williamson and D. H. Williams, J. Am. Chem. Soc., 1981, 103, 6580.

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