Synthesis and Antibacterial Activity of 1-β-Methylcarbapenem Having a 1,3-Diazabicyclo[3.3.0]octan-4-one Moiety

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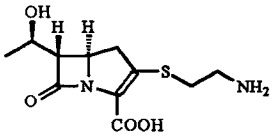
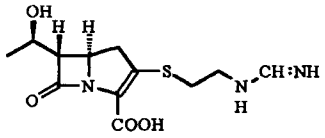
Key Words: *1-β-methylcarbapenems; antibacterial activity; MIC; substituent effect*

Summary

The synthesis of new series of 1-β-methylcarbapenems having a 1,3-diazabicyclo[3.3.0]octan-4-one moiety is described. Their *in vitro* antibacterial activities against both Gram-positive and Gram negative bacteria are reported and the effect of the substituent on the bicyclic ring was investigated and was in agreement with findings from our previous studies.



Thienamycin



Imipenem

Introduction

The isolation of thienamycin, which is one of the most potent naturally occurring broad spectrum β-lactam antibiotics, was the first step in the discovery of the carbapenem family. This new class of β-lactams, carbapenems, exhibited excellent antimicrobial activity against a wide range of Gram-positive and Gram-negative bacteria [1,2]. Thus the traditional and well accepted Structure-Activity Relationship (SAR) in β-lactam antibiotics had to be reexamined.

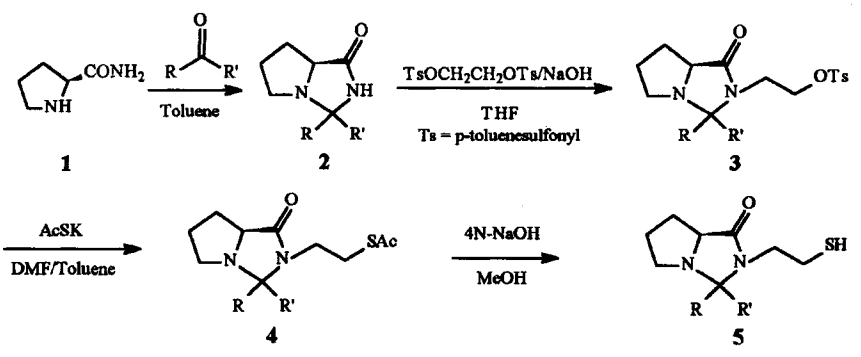
However, naturally obtained carbapenems are in general chemically unstable and easily metabolized (hydrolysis) by renal dehyropeptidase-I (DHP-1) [3,4]. Therefore, extensive efforts have been directed toward the synthesis of new chemically stable carbapenem compounds to overcome these disadvantages and allow their development as clinical drug candidates.

The first chemically stable product, imipenem, was developed through the structural modification of thienamycin by the Merck group. However, in spite of its potent antibacterial activity, it still also lacked resistance against renal dehydropeptidase-I (DHP-I) [4].

Recently, Shih *et al.* reported that the introduction of a methyl substituent at C-1 position of the carbapenem nucleus resulted in much improved stability toward dehydropeptidase-I with high antibacterial potency [5]. The present investigation was undertaken to synthesize the chemically and metabolically stable carbapenem derivatives retaining activity against a wide range of bacteria.

In preceding papers [6–9], we reported the synthesis and biological properties of carbapenems having various substituent groups at C-2. In this study a series of 1-β-methyl carbapenems having a 1,3-diazabiyclo[3.3.0]octan-4-one moiety at the C-2 position were synthesized and their *in vitro* antibacterial activities were tested.

The cyclic amide derivatives (**2**) were synthesized by the known method from prolinamide (**1**) [10]. *N*-Alkylation of the cyclized amide (**2**) was carried out with ethylene glycol di-*p*-tosylate in the presence of sodium hydride. Treatment of **3** with potassium thioacetate in dimethyl formamide and toluene gave 3-(2-acetylthioethyl)-2,2-dialkyl-1,3-diazabicyclo[3.3.0]octan-4-one (**4**). Finally, the acetylthio group of **4** was readily hydrolyzed with 4N NaOH in methanol to give the compound 2,2-dialkyl-3-(2-mercaptoethyl)-1,3-diazabicyclo[3.3.0]octan-4-one (**5**) (Scheme 1).



Scheme 1

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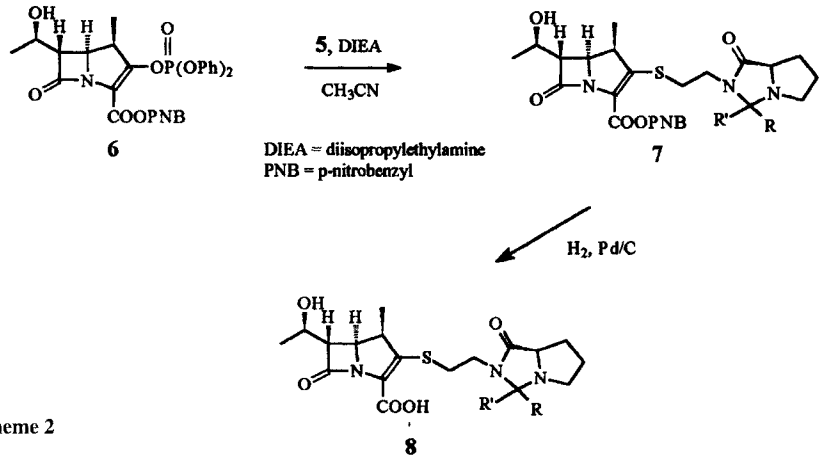


Scheme 2

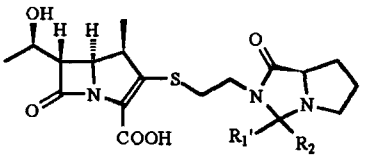
Preparation of the 2-(diphenylphosphoryloxy)carbapenem compound **6** has been reported [11]. Reaction of **6** with **5** in the presence of diisopropylethylamine provided the 2-substituted carbapenem (**7**). The synthesis of final product **8** was completed by catalytic hydrogenolysis over 10% Pd/C in phosphate buffer (pH = 7). Compounds **8a–8h** were prepared analogously (Scheme 2).

Antibacterial Activity

The minimum inhibitory concentrations (MICS) of the new carbapenem compounds **8a~8h** were determined by an agar dilution method using *Mueller-Hinton* agar (Table 1). The effect of the substituent on the bicyclic ring was investigated: the larger the size of substituent, the lower the activities against both Gram-positive and Gram-negative bacteria. Compound **8a** without substituent was more active than compound **8f** having a benzyl and methyl group. Analogous substituent effects were already demonstrated by us [6–9].

Most of the carbapenem products showed well balanced antibacterial spectra except the activity against *Pseudomonas aeruginosa.*

Table 1. Antibacterial activities of the carbapenem derivatives.



Compound	R^1	R^2	MIC(μg/MI)[a]					
			S.p[b]	S.a	E.c	P.a	K.o	En.c
8a	H	H	0.01	0.03	0.10	1.56	0.20	0.10
8b	CH_3	H	0.01	0.05	0.10	6.25	0.40	0.10
8c	CH_3	CH_3	0.01	0.05	0.10	6.25	0.40	0.10
8d	CH_3	C_2H_5	0.03	0.10	0.20	25	0.40	0.20
8e	CH_3	C_3H_7	0.03	0.05	0.20	25	0.40	0.10
8f	CH_3	C_6H_5	0.05	0.05	0.20	100	0.80	0.20
8g	cyclopropyl	H	0.05	0.20	0.80	100	0.40	1.56
8h	cyclopropyl	CH_3	0.10	0.20	0.80	100	0.80	1.56

[a] Agar dilution method.

[b] S.p.: *Streptococcus pyogenes* 77A, S.a.: *Staphylococcus aureus* 503; E.c.: *Escherichia coli* 055, P.a.: *Pseudomonas aeruginosa* 9027, K.o.: *Klebsiella oxytoca* 1082E, En.c.: *Enterobacter cloacae* 1321E.

Experimental Part

Elemental analysis: Fisons instrument EA 1108 CHNS-O.– Melting point (mp): Tomas Hover apparatus, uncorrected.– UV spectra: Hewlett-Packard 8451A UV-VIS spectrophotometer.– ^{1}H-NMR spectra: Varian Gemini 300 spectrometer, tetramethylsilane (TMS), as internal standard.– MS: Hewlett-Packard 5890 GC and Hewlett-Packard 5972 mass selective detector.

Procedures are given only for compounds **2e–8c,** which exemplify typical preparations for the rest of the target molecule.

*(5S)-2,2-Dimethyl-1,3-diazabicyclo[3.3.0]octan-4-one (**2c**)*

Water was azeotropically removed from a solution of (*S*)-prolinamide (10.0 g, 87.6 mmol), toluene (300 ml), and acetone (48 ml, 0.8 mol). Distillation was continued for 6 h, until no more water was separated in a Dean-Stark receiver. The reaction mixture was then cooled to room temperature, evaporated to dryness, and recrystallized (toluene) to give **2c** as color-

less crystals, yield 11.5 g (85.0%).– Anal. $C_8H_{14}N_2O$.– M.p.: 148–150 °C.– ^{1}H-NMR (DMSO-d6): δ (ppm) = 1.29 (S, 3H, CH_3), 1.30 (s, 3H, CH_3), 1.84 (m, 4H, H-6,7), 2.55 (m, 2H, H-8), 3.71 (m, 1H, H-5), 8.0 (S, 1H, NH).– MS (70 eV): *m/z* = 154 (M^+).

(5S)-2,2-Dimethyl-3-(2-p-toluenesulfonylethyl)-1,3-diazabicyclo-[3.3.0]octan-4-one (**3c**)

To a solution of NaH (3.7 g, 0.15 mol) in dry THF (100 ml) at 0 °C was added slowly a solution of **2c** (8.0 g, 51.9 mmol) in THF under N_2 gas. After 2 h the reaction mixture was slowly added ethylene glycol di-*p*-tosylate (38.0 g, 0.10 mol) in THF and stirred for 48 h. The resulting mixture was diluted with water and ethyl acetate. The organic layer was washed with brine and dried over Na_2SO_4. Removal of the solvent gave a crude residue, which was chromatographed on silica gel using ethyl acetate to give a yellowish gum, yield 7.21 g (85.1 %).– Anal. $C_{17}H_{24}N_2O_{17}S$.– ^{1}H-NMR ($CDCl_3$): δ (ppm) = 1.23 (s, 3H, CH_3), 1.30 (s, 3H, CH_3), 1.74–2.17 (m, 4H, H6,7), 2.32 (s, 3H, CH_3), 2.85 (m, 2H, H-8), 3.41–3.74 (m, 4H, NCH_2CH_2S), 3.91 (q, 1H, H-5), 7.20–7.61 (2d,4H).– MS (70 eV): *m/z* = 352 (M^+).

(5S)-3-(2-Acetylthioethyl)-2,2-dimethyl-1,3-diazabicyclo[3.3.0]octan-4-one (**4c**)

A mixture of **3c** (7.0 g, 19.8 mmol), potassium thioacetate (13.6 g, 0.10 mol) in DMF (70 ml), and toluene (70 ml) was stirred at 60 °C for 3 h under N_2. After cooling, the mixture was re-extracted with ethyl acetate (50 ml). The combined organic layer was washed with brine and dried over Na_2SO_4. Removal of the solvent gave a crude residue, which was chromatographed on silica gel using ethyl acetate to give a yellowish gum, yield 4.4 g (86.8 %).– Anal. $C_{12}H_{20}N_2O_2S$.– ^{1}H-NMR ($CDCl_3$): δ (ppm) = 1.33 (s, 3H, CH_3), 1.40 (*s*, 3H, CH_3), 1.84–2.11 (m, 4H, H6,7), 2.30 (s, 3H, $COCH_3$), 2.45 (m, 2H, H-8), 3.01–3.24 (m, 4H, NCH_2CH_2S), 3.81 (m, 1H, H-5).– MS (70 eV): *m/z* = 256 (M^+).

5(S)-2,2-Dimethyl-3-(2-mercaptoethyl)-1,3-diazabicyclo[3.3.0]octan-4-one (**5c**)

To a solution of **4c** (1.2 g, 4.7 mmol) in CH_3OH (10 ml) was added 1.1 ml of 4N NaOH in an ice bath. After stirring for 20 min 1.1 ml of 4N HCl was added and the reaction mixture was diluted with ethyl acetate, washed with water and dried over Na_2SO_4. Removal of the solvent gave **5c**, yield 0.90 g (90.0 %).– Anal. $C_{10}H_{18}N_2O_2S$.– ^{1}H-NMR ($CDCl_3$): δ (ppm) = 1.31 (8, 3H, CH_3), 1.33 (s 3H, CH_3), 1.71–2.13 (m, 4H, H-6,7), 2.55 (m, 2H, H-8), 2.72–3.04 (m, 4H, NCH_2CH_2S), 3.71 (m, 1H, H-5).– MS (70 eV): *m/z* = 214 (M^+).

p-Nitrobenzyl-(1R,5S,6S)-6-[(1R)-1-hydroxyethyl]-2-[(5S)(2,2-dimethyl-4-oxo-1,3-diazabicyclo[3.3.0]octan-3-yl]ethylthio-1-methylcarbapen-2-em-3-carboxylate (**7c**)

A solution of *p*-nitrobenzyl-(1*R*,5*S*,6*S*)-3-(diphenylphosphoryloxy)-6-[(*R*)-1-hydroxyethyl]-1-methylcarbapen-2-em-3-carboxylate (**6**, 7.40 g,12.4 mmol) in CH_3CN (50 ml) was cooled at 0 °C under N_2. To this solution was added diisopropyl ethylamine (2.5 ml, 12.4 mmol) and a solution of the mercapto compound **5c** (2.40 g, 12.4 mmol) in CH_3CN (10 ml). After stirring for 2 h, the mixture was diluted with ethyl acetate, washed with 10% $NaHCO_3$, brine, and dried over $MgSO_4$. Evaporation *in vacuo* gave a foam which was purified by silica gel CC to give **7c** as a yellow foam solid, yield 4.10 g (65.5%).– Anal. $C_{27}H_{34}N_4O_7S$.– ^{1}H-NMR ($CDCl_3$): δ (ppm) = 1.15 (d, 3H, 1-CH_3, *J* = 7.2 Hz), 1.20 (d, 6H, 2′-CH_3×2, *J* = 14.0 Hz), 1.29 (d, 3H, CH_3CHOH, *J* = 6.2 Hz), 1.65–1.99 (m, 4H, H′6 and H7′), 2.52 (t, 2H, H8′), 2.75 (m, 1H, H1), 2.80–2.99 (m, 4H, CH_2CH_2S), 3.35 (m, 1H, H6), 3.60–3.68 (m, 1H, H5′), 4.05 (m, 1H, H5), 4.25 (m, 1H, CH_3CHOH), 5.25 (d, 1H, *J* = 11 Hz), 5.40 (d, 1H, J = 11 Hz), 7.63 and 8.11 (d, 4H, *J* = 7.8 Hz).– MS (70 eV): *m/z* = 558 (M^+).

(1R,5S,6S)-6-[(1R)-1-Hydroxyethyl]-2-[(5S)-(2,2-dimethyl-4-oxo-1,3-diazabicyclo[3.3.0]octan-3-yl]ethylthio-1-methylcarbapen-2-em-3-carboxylic Acid (**8c**)

Compound **7c** (2.01 g, 3.60 mmol) and 1.0 g of Pd/C (10%) were dissolved in THF/phosphate buffer (pH = 7) (1:1, 20 ml each). The mixture was hydrogenated at 50 psi for 1 h. The solution was filtered through celite and washed with water (2 × 10 ml). The combined filtrate was washed with ether (2 × 20 ml) and lyophilized to give a yellow powder which was purified on a Diaion HP-20 column, eluting with 2 % THF in water. Fractions having a UV absorption at 298 nm were collected and lyophilized again to give the title compound **8c** as a white powder, yield 0.21g (12.3%).– Anal. $C_{20}H_{29}N_3O_5S$.– Mp: 98–107 °C (dec).– ^{1}H-NMR (D_2O): δ (ppm) = 1.21 (d, 3H, 1-CH_3, *J* = 7.2 Hz), 1.25 (d, 6H, 2′-CH_3×2, *J* = 14.0 Hz), 1.33 (d, 3H, CH_3CHOH, *J* = 6.2 Hz), 1.70–2.05 (m, 4H, H′6 and H7′, 2.56 (t, 2H, H8′), 2.80–2.95 (m, 4H, CH_2CH_2S and H1), 3.35 (m, 1H, H6), 3.64–3.69 (m, 1H, H5′), 4.02 (m, 1H, H5), 4.30 (m, 1H, CH_3CH-OH).– MS (70 eV): *m/z* = 408 ($M^+ - CH_3$).

(1R,5S,6S)-6-[(1R)-1-Hydroxyethyl]-2-[(5S)-(4-oxo-1,3-diazabicyclo-[3.3.0]octan-3-yl]ethylthio-1-methylcarbapen-2-em-3-carboxylic Acid (**8a**)

Anal. $C_{18}H_{25}N_3O_5S$.– Mp: 108–119 °C (dec).– ^{1}H-NMR (D_2O): δ (ppm) = 1.21 (d, 3H, 1-CH_3, *J* = 7.2 Hz), 1.25 (d, 6H, 2′-CH_3×2, *J* = 14.0 Hz), 1.33 (d, 3H, CH_3CHOH, *J* = 6.2 Hz), 1.70–2.05 (m, 4H, H′6 and H7′), 2.56 (t, 2H, H8′), 2.80–2.95 (m, 4H, CH_2CH_2S and H1), 3.35 (m, 1H, H6), 3.64–3.69 (m, 1H, H5′), 4.02 (m, 1H, H5), 4.30 (M, 1H, CH_3CHOH).– MS (70 eV): *m/z* = 377 ($M^+ - H_2O$).

(1R,5S,6S)-6-[(1R)-1-Hydroxyethyl]-2-[(5S)-(2-methyl-4-oxo-1,3-diaza-bicyclo[3.3.0]octan-3-yl]ethylthio-1-methylcarbapen-2-em-3-carboxylic Acid (**8b**)

Anal. $C_{19}H_{27}N_3O_5S$.– Mp: 95–111 °C (dec).– ^{1}H-NMR (D_2O) δ (ppm) = 0.92 (d, 3H, 2′-CH_3), 1.21 (d, 3H, 1-CH_3, *J* = 7.0 Hz), 1.30 (d, 3H, CH_3CHOH, *J* = 6.2 Hz), 1.680–2.02 (m, 4H, H′6 and H7′), 2.59 (t, 2H, H8′, 2.80–2.95 (m, 4H, CH_2CH_2S and H1), 3.33 (m, 1H, H6), 3.63–3.66 (m, 1H, H5′), 4.06 (m, 1H, H5), 4.30–4.48 (m, 2H, CH_3CHOH and H2′).– MS (70 eV): *m/z* = 394 ($M^+ - CH_3$).

(1R,5S,6S)- 6-[(1R)-1-Hydroxyethyl]-2-[(5S)-(2-ethyl-2-methyl-4-oxo-1,3-diazabicyclo[3.3.0]octan-3-yl]ethylthio-l-methylcarbapen-2-em-3-carboxylic Acid (**8d**)

Anal. $C_{21}H_{31}N_3O_5S$.– Mp: 89–102 °C(dec).– ^{1}H-NMR (D_2O): δ (ppm) = 0.92 (t, 3H, 2′-CH_2CH_3), 1.23 (d, 3H, 1-CH_3, *J* = 7.2 Hz), 1.33 (d 3H, CH_3CHOH, *J* = 6.2 Hz), 1.40 (s, 3H, 2′-CH_3), 1.63 (q, 2H, 2′-CH_2CH_3), 1.70–2.02 (m, 4H, H′6 and H7′), 2.66 (t, 2H, H8′, 2.76–2.92 (m, 4H, CH_2CH_2S and H1), 3.39 (m, 1H, H6), 3.64 (m, 1H, H5′), 4.11 (m, 1H, H5), 4.48 (m, 1H, CH_3CHOH).– MS (70 eV): *m/z* = 437 (M^+).

(1R,5S,6S)-6-[(1R)-1-Hydroxyethyl]-2-[(5S)-(2-methyl-2-propyl-4-oxo-1,3-diazabicyclo[3.3.0]octan-3-yl]ethylthio-l-methylcarbapen-2-em-3-carboxylic Acid (**8e**)

Anal. $C_{22}H_{33}N_3O_5S$.– Mp: 87–99 °C (dec).– ^{1}H-NMR (D_2O): δ (ppm) = 0.91 (t, 3H, 2′-CH_2CH_3), 1.19 (d, 3H, 1-CH_3, *J* = 7.2 Hz), 1.30 (d, 3H, CH_3CHOH, *J* = 6.2 Hz), 1.36 (s, 3H, 2′-CH_3),1.53 (m, 2H, 2′-CH_2CH_2), 1.63 (m, 2H, 2′-CH_2CH_3), 1.70–2.01 (m, 4H, H′6 and H7′), 2.62 (t, 2H, H8′), 2.76–2.90 (m, 4H, CH_2CH_2S and H1), 3.27 (m, 1H, H6), 3.68 (m, 1H, H5′), 4.10 (m, 1H, H5), 4.31 (m, 1H, CH_3CHOH).– MS (70 eV): *m/z* = 451 (M^+).

(1R,5S,6S)-6-[(1R)-1-Hydroxyethyl]-2-[(5S)-(2-methyl-2-phenyl-4-oxo-1,3-dibicyclo[3.3.0]octan-3-yl]ethylthio-1-methylcarbapen-2-em-3-carboxylic Acid (**8f**)

Anal. $C_{25}H_{31}N_3O_5S$.– Mp: 85–98 °C (dec).– ^{1}H-NMR (D_2O): δ (ppm) = 1.21 (d, 3H, 1-CH_3, *J* = 7.2 Hz), 1.33 (d, 3H, CH_3CHOH, *J* = 6.2 Hz), 1.38 (s, 3H, 2′-CH_3), 1.58–1.97 (m, 4H, H′6 and H7′), 2.59 (t, 2H, H8′), 2.80–2.95 (m, 4H, CH_2CH_2S, and H1), 3.35 (m, 1H, H6), 3.61–3.67 (m, 1H, H5′), 4.13

(m, 1H, H5), 4.34 (m, 1H, CH_3CHOH), 7.56 (bs, 5H, 2′-phenyl).– MS (70 eV): $m/z = 485$ (M^+).

*(1R,5S,6S)-6-[(1R)-1-Hydroxyethyl]-2-[(5S)-(2-cyclopropyl-4-oxo-1,3-diazabicyclo[3.3.0]octan-3-yl]ethylthio-1-methylcarbapen-2-em-3-carboxylic Acid (**8g**)*

Anal. $C_{21}H_{29}N_3O_5S$.– Mp: 134–146 °C (dec).– ^{1}H-NMR (D_2O): δ (ppm) = 0.55–0.74 (m, 5H, 2′-cyclopropyl), 1.21 (d, 3H, 1-CH_3, $J = 7.2$ Hz), 1.33 (d, 3H, CH_3CHOH, $J = 6.2$ Hz), 1.70–2.05 (m, 4H, H′6 and H7′), 2.56 (t, 2H, H8′), 2.80–2.95 (m, 4H, CH_2CH_2S and H1), 3.35 (m, 1H, H6), 3.64–3.69 (m, 1H, H5′), 4.02 (m, 1H, H5), 4.30 (m, 1H, CH_3CHOH), 4.33 (m, 1H, H2′).– MS (70 eV): $m/z = 417$ ($M^+ - H_2O$).

*(1R,5S,6S)-6-[(1R)-1-Hydroxyethyl]-2-[(5S)-(2-cyclopropyl-2-methyl-4-oxo-1,3-diazabicyclo[3.3.0]octan-3-yl]ethylthio-1-methylcarbapen-2-em-3-carboxylic Acid (**8h**)*

Anal. $C_{22}H_{31}N_3O_5S$.– Mp: 116–124 °C (dec).– ^{1}H-NMR (D_2O): δ (ppm) = 0.62–0.94 (m, 5H, 2′-cyclopropyl), 1.23 (d, 3H, 1-CH_3, $J = 6.8$ Hz), 1.30 (d, 3H, CH_3CHOH, $J = 6.1$ Hz), 1.36 (s, 3H, 2′-CH_3), 1.70–2.02 (m, 4H, H′6 and H7′), 2.56 (t, 2H, H8′), 2.80–2.95 (m, 4H, CH_2CH_2S and H1), 3.35 (m, 1H, H6), 4.02 (m, 1H, H5), 4.30 (m, 1H, CH_3CHOH).– MS (70 eV): $m/z = 449$ (M^+).

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Received: April 3, 1996 [FP109]