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COMMUNICATION

Synthesis and Properties of Disodium Tetraethyleneglycol-bis-(α-carboxybenzylpenicillin)

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ABSTRACT

Disodium tetraethyleneglycol-bis-(α-carboxybenzylpenicillin) (TEG-carbenicillin), a tetraethyleneglycol (TEG) diester of carbenicillin, was synthesized to develop a carbenicillin prodrug with enhanced acid stability for oral administration. Antimicrobial activities of TEG-carbenicillin tested against gram-negative Escherichia coli *(TG-1) and gram-positive* Staphylococcus aureus *(ATCC-12228) and* Bacillus subtilis *(NA-1) were comparable to that of carbenicillin. Stability of the β-lactam ring of TEG-carbenicillin was determined by iodometry at pH 6.8, pH 4.5, and pH 2.0 at varied time intervals and was compared to that of carbenicillin. In 26 hr, both of the compounds were stable at pH 6. 8. At pH 4.5, about 41% of the carbenicillin was decomposed, while TEG-carbenicillin was not appreciably decomposed. At pH 2.0, carbenicillin was decomposed about 61% after 6 hr, while TEG-carbenicillin was decomposed about 21% during the same period.*

Key Words: *Carbenicillin; Disodium tetraethyleneglycol-bis-(α-carboxybenzylpenicillin); Tetraethyleneglycol.*

INTRODUCTION

Drug derivatization has long been recognized as an important means to produce more efficacious pharmaceutical products and to reduce undesirable properties inherent in a drug molecule that may limit the use of the pharmaceuticals in clinical practice.

Carbenicillin, α-carboxybenzylpenicillin, is a broad-spectrum antimicrobial agent that shows antibacterial activity on both gram-positive and gram-negative bacteria.

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Even though its in vitro activity against the gram-positive bacteria is substantially lower than that of ampicillin, it is active against *Pseudomonas aeruginosa* and certain *Proteus* strains that are usually resistant to ampicillin (1,2). Carbenicillin is usually administered parenterally due to its poor gastrointestinal absorption characteristics. In addition, it is rendered inactive in the gastric contents due to its acid liability since the presence of intact β-lactam rings is required for the β-lactam antibiotics to show antimicrobial activities.

Indanyl and the phenyl ester of carbenicillin are the representative orally administered prodrugs introduced to overcome the shortcomings of carbenicillin (3,4). The degradation of these prodrugs is reported to proceed through a total cleavage of the β-lactam ring and of the ester bond. In acidic media below pH 3, the β-lactam degradation pathway predominates, and at pH above 7, degradation is superseded in importance by ester hydrolysis that is independent of the electronic nature of the side chain (5).

Polyethylene glycols, including tetraethyleneglycol (TEG), are known to be nontoxic, are soluble in both water and organic solvent, and are available in various well-defined molecular weights and have been utilized as a promoiety in a variety of drugs. The synthesis and pharmacological evaluation of polyoxyethyleneglycol derivatives of 4-isobutylphenyl-2-propionic acid (ibuprofen) have been reported; all exhibited a prolonged anti-inflammatory activity and a higher plasma half-life compared to free drug after oral administration of the equivalent dose to rats (6–9). Among the polyoxyethyleneglycol derivatives tested, the TEG derivative showed a remarkably increased initial bioavailability.

In order for the ester prodrug of carbenicillin to be orally active, it should be dissolved sufficiently and readily absorbable, the ester bond should be chemically stable, and the β-lactam ring should be intact at the upper gastric pH.

In the present study, the TEG derivative of carbenicillin was synthesized as prodrug of carbenicillin able to be orally administered in the expectation that it might be acid stable and lipophilic enough for gastric absorption.

MATERIALS AND METHODS

Materials

The TEG was purchased from Tokyo Kasei (Tokyo, Japan), and carbenicillin disodium, 6-aminopenicillanic acid (6-APA), and phenylmalonic acid (PMA) were purchased from Sigma Chemical Company (St. Louis, MO) and were used as received. The ingredients of the medium used for the antimicrobial test were received from DIFCO (Detroit, MI). All other chemicals used were reagent grade and were used without further purification. Ultraviolet (UV) and infrared (IR) spectra were recorded with a Bomem MB 100 FT-IR spectrophotometer (Quebec, Canada) and Shimadzu UV 2101-PC (Tokyo, Japan), respectively. The ^{1}H-NMR (nuclear magnetic resonance) spectra were taken on a Brucker AC-200 spectrometer (Tübingen, Germany), and the chemical shifts are in parts per million downfield from tetramethylsilane. Thin-layer chromatography (TLC) was performed on a Merck Kieselgel 60 F_{254} (Frankfurt, Germany), eluting with butylacetate/acetic acid, glacial/methanol/*n*-butanol/phosphate buffer at pH 7.3 (80:4:5:15:20); the spots were treated with 10% $FeCl_3$/2% $K_3Fe(CN)_6$/HCl (1:2:6), and an Orion 320 pH meter (Beverly, MA) was used for the pH measurements.

Preparation of Tetraethyleneglycol-bis-(α-carboxy-α-phenylacetate)

In a 100-ml three-neck flask equipped with a mechanical stirrer, a condenser with drying tube ($CaCl_2$), and a dropping funnel, 2.7 g (15.0 mM) of PMA and 16.5 ml of tetrahydrofuran (THF) were added. The mixture was stirred and heated to reflux, and 1.2 ml (16.5 mM) of thionyl chloride was added dropwise over 10 min and stirred for 1.5 hr under reflux to form the monoacid chloride. This was then cooled to 30°C–40°C, and 1.2 ml (6.9 mM) of TEG was added dropwise. After reacting at 66°C for 1 hr and cooling to room temperature, 10 ml of chloroform and 10 ml of water were slowly added. The aqueous layer was removed, and the organic layer was washed with additional water and dried with anhydrous sodium sulfate. After removing the organic solvents under reduced pressure, 5.1 g of oily residue (yield 65.4%) was obtained, which was used for the preparation of tetraethyleneglycol-bis-(α-carboxybenzylpenicillin) (TEG-carbenicillin) without further purification.

Preparation of Tetraethyleneglycol-bis-(α-carboxybenzylpenicillin)

Tetraethyleneglycol-bis-(α-carboxy-α-phenylacetate), 2.53 g (4.88 mM) in 18 ml of methylene chloride, was heated to reflux and 0.8 ml (11.02 mM) of thionyl chloride was added dropwise. After the addition was complete, the mixture was refluxed for 2 hr. Residual thionyl chloride was removed under reduced pressure to yield a viscous oil of the acid chloride. 6-APA, 2.109 g (9.75

mM) in 15 ml of water, was adjusted to pH 7.5 with 1 N NaOH, 15 ml of acetone was added, and the pH was readjusted to 8 at 18°C. To this solution, the acid chloride prepared above was added, maintaining the pH at 5.7 to 5.9 with 2 N Na_2CO_3. Then, 30 ml of methyl isobutyl ketone was added, and the pH was adjusted to 2.0 with 6 N HCl. After filtering the reaction mixture, the organic layer was collected, and the aqueous layer was extracted with 3 ml of the ketone. The combined organic layer was dried with anhydrous sodium sulfate, the pH was adjusted to 7.8 with sodium 2-ethylhexanoate, and the mixture was left to stand for a day. The resulting oily residue was dissolved in a minimum amount of methanol and 30 ml of acetone was added to precipitate TEG-carbenicillin disodium (yield 25.8%). The precipitates were collected by suction filtration and recrystallized with several portions of dry acetone. The compound was a pale yellow solid that decomposed (160°C–183°C). Values as calculated for $C_{42}H_{50}N_4S_2O_{15}$ were C, 55.14; H, 5.47; N, 6.12; S, 7.00, and as found, were C, 55.0; H, 5.5; N, 6.1; S, 6.9.

Effect of pH on the Stability of β-Lactam Ring of Carbenicillin and Tetraethyleneglycol-bis-(α-carboxybenzylpenicillin)

The stabilities of the β-lactam ring of carbenicillin and TEG-carbenicillin were determined at pH 6.8, 4.5, and 2.0 at 37°C. The amount of intact β-lactam ring was determined at varied time intervals by the iodometric method described by Alicino (10). A calibration curve using carbenicillin disodium as a standard was constructed by measuring the volumes of standard iodine solution consumed by the intact β-lactam of carbenicillin at varied concentrations. The amount of intact β-lactam ring in TEG-carbenicillin was determined from the calibration curve by measuring the volume of standard iodine solution consumed by the sample.

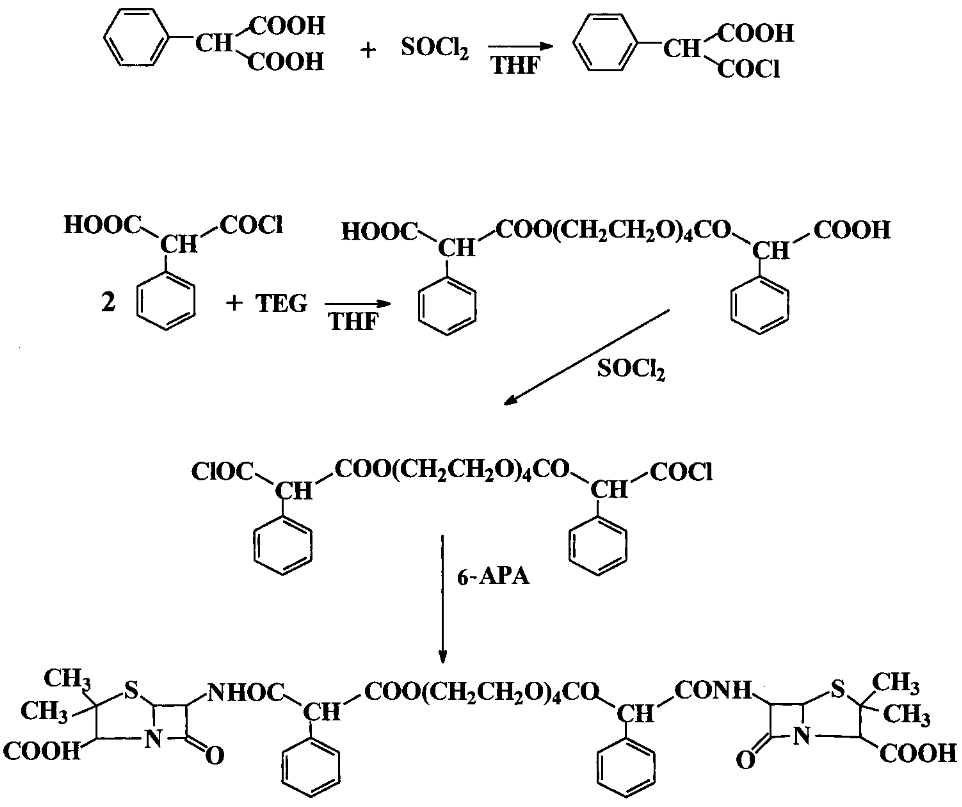
Antimicrobial Activities of Tetraethyleneglycol–Carbenicillin Disodium

A loopful of *Bacillus subtilis* (NA-1) obtained from a recently grown slant culture was used to inoculate the culture tube, which contained 10 ml of nutrient broth. This was incubated at 37°C for 24 hr. The inoculum was prepared by diluting the inoculated nutrient broth with 200 ml of medium I on the day of the test. Inoculum for *Staphylococcus aureus* (ATCC 12228) and *Escherichia coli* (TG-1) were prepared according to the same procedure. The antimicrobial activities of TEG-carbenicillin were evaluated by measuring the diameters of the inhibition zone according to the cup-plate method (11) at two different concentration levels. Medium I, 21 ml, was placed in a culture plate (diameter 90 mm and height 15 mm) and allowed to harden to give a smooth base layer with a uniform 3–4-mm depth. To this plate, 6.0 ml of inoculum was added, and the plate was tilted back and forth to spread the inoculum evenly and entirely over the surface; this was allowed to harden. Four stainless steel cylinders (o.d. 11 mm, i.d. 9 mm, and height 10 mm) were dropped from 24 mm above the inoculated surface for each culture plate using a mechanical guide or other device and covered the plate to avoid contamination. After filling these cups with a test solution of TEG-carbenicillin, the culture plate was incubated at 37°C for 16 hr, the cups were removed, and the diameter of the inhibition zone was measured.

RESULTS AND DISCUSSION

Synthesis of TEG-carbenicillin was achieved by two consecutive reaction processes (12,13) as shown in Scheme 1.

Phenylmalonic acid monochloride, which was obtained by treating PMA with thionyl chloride, was condensed with TEG to produce tetraethyleneglycol-bis (α-carboxy-α-phenylacetate). This product was treated again with thionyl chloride to convert the free carboxylic acid to acid chloride, which, in turn, reacted with 6-APA to produce TEG-carbenicillin. Isolation of the products was achieved by converting TEG-carbenicillin to its disodium salts by adjusting the pH with sodium ethylhexanoate so that the compound could be precipitated as disodium salts, leaving most other organic by-products and impurities dissolved in the organic solvent system. The TLC was performed on a Merck Kisselgel $60F_{254}$ plate, eluting with butylacetate/acetic acid, glacial/methanol/*n*-butanol/pH 7.3 phosphate buffer (80 : 4 : 5 : 15 : 20); the spots were treated with 10% $FeCl_3$/2% $K_3Fe(CN)_6$/HCl (1 : 2 : 6) (14). The TEG-carbenicillin appeared as a spot at the original position, which was different from the carbenicillin disodium (R_f = 0.54). From the IR spectra of tetraethyleneglycol-bis-(α-carboxy-α-phenylacetate), typical hydroxyl group absorption of carboxylic acid at 3200–3600 cm^{-1}, strong absorption of ester carbonyl at 1720 cm^{-1}, and C—O single-bond absorption of the ether group around 1000–1200 cm^{-1} were observed. The TEG-carbenicillin displayed IR absorption bands at 1760 cm^{-1} (β-lactam C=O), 1680 cm^{-1} (amide C=O), 1610 cm^{-1} (β-lactam carboxylate), and the ^{1}H-NMR spectrum



Scheme 1. Synthesis of TEG-carbenicillin.

(DMSO-d$_6$) showed signals at (ppm) 1.5 (12H; dimethyl group in β-lactam ring), 3.5 (16H; methylene group in TEG), 4.1–4.3 (6H; H in position 3 of the β-lactam ring, –NH, benzylic H), 5.3 (4H; H in positions 5 and 6 of the β-lactam ring), 7.3 (10H; aromatic).

Since the presence of an intact β-lactam ring is required for the β-lactam antibiotics to show antimicrobial activities, the stability of the β-lactam ring of TEG-carbenicillin was investigated by iodometric assay, for which the penicilloate ion that takes up iodine is determined. The time course of the degradation of the β-lactam ring was followed by iodometry for carbenicillin and TEG-carbenicillin at 37°C at pH 2.0, 4.5, and 6.8, which represents the pH of stomach, upper duodenum, and small intestine, respectively. The results are shown in Figs. 1 and 2. Within 26 hr, both of the compounds were stable at pH 6.8. At pH 4.5, about 41% of the carbenicillin was decomposed, while TEG-carbenicillin was not appreciably decomposed. At pH 2.0, about 61% of the carbenicillin was decomposed in 6 hr, while TEG-carbenicillin was decomposed about 21% during the same period. The acid stability of the β-lactam ring was greatly enhanced in TEG-carbenicillin and was stable at a wider

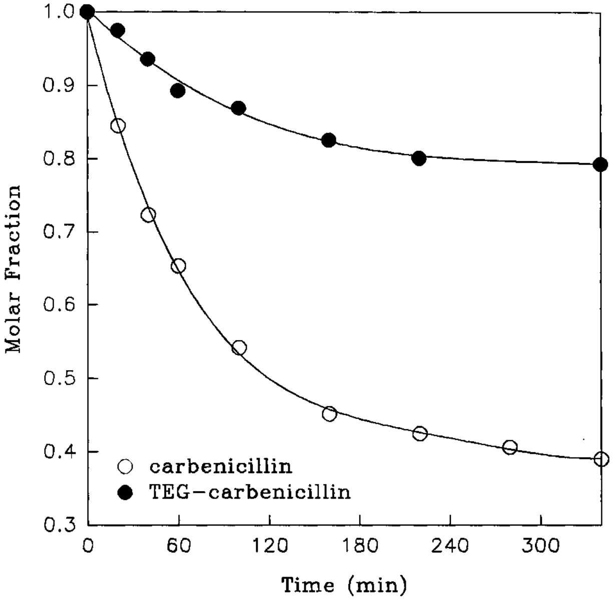


Figure 1. Degradation profile of TEG-carbenicillin and carbenicillin at pH 2.0 and 37°C.

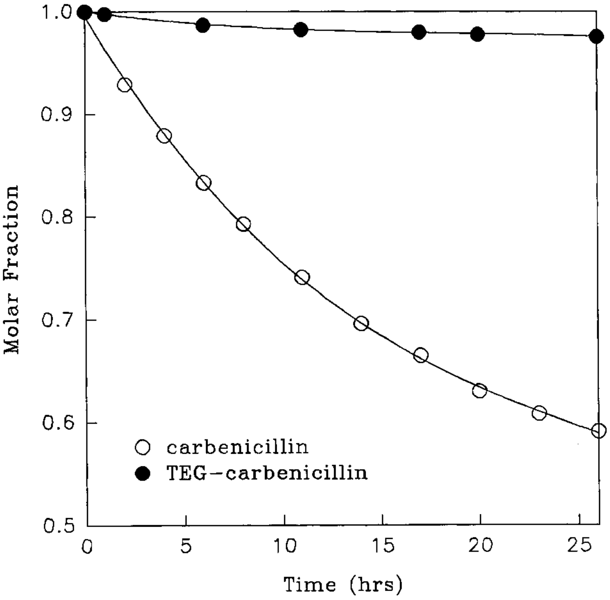


Figure 2. Degradation profile of TEG-carbenicillin and carbenicillin at pH 4.5 and 37°C.

pH range compared to that of carbenicillin. The susceptibility of β-lactam of penicillins to acid-catalyzed degradation is reported to be attributed not only to the strained ring, but also the intramolecular attack of the side-chain amide on the β-lactam carbonyl, which is accelerated by electron-donating substituents, while electron-withdrawing substituents reduce the β-lactam cleavage rate (15). Enhanced stability of TEG-carbenicillin compared to carbenicillin may be explained in terms of the increased inductive effect of ester compared to the carboxylate anion.

Antimicrobial activities of carbenicillin disodium and TEG-carbenicillin disodium were carried out by the cup-plate method at two different concentration levels. As shown in Table 1, the antimicrobial activities of TEG-carbenicillin were comparable to those of carbenicillin.

In conclusion, by the formation of TEG ester, the stability of the β-lactam ring of carbenicillin in acidic media was greatly enhanced. Antimicrobial activities of TEG-carbenicillin were comparable to free carbenicillin.

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Table 1

Antimicrobial Activities of Carbenicillin and TEG-Carbenicillin

	Diameter of Inhibition Zone (mm)			
	Carbenicillin		TEG-Carbenicillin	
Microorganism	30 μg/ml	60 μg/ml	30 μg/ml	60 μg/ml
S. aureus	24.8	25.8	23.8	25.2
B. subtilis	17.2	18.0	17.1	17.8
E. coli	23.9	25.7	23.2	24.8

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