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Studies on Prodrugs. VI. Preparation and Characterization of (5-Substituted 2-Oxo-1,3-dioxol-4-yl)methyl Esters of Mecillinam

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As a new type of mecillinam prodrug, mecillinam (5-substituted 2-oxo-1,3-dioxol-4-yl)methyl esters were prepared. These esters were found to produce at least 4-fold higher mecillinam levels in blood than mecillinam itself after oral administration to mice. Mecillinam (5-methyl-2-oxo-1,3-dioxol-4-yl)methyl ester hydrochloride was hygroscopic, but its L-tartrate was not.

Keywords—(5-methyl-2-oxo-1,3-dioxol-4-yl)methyl ester; mecillinam prodrug; oral absorbability; pro-moiety; mecillinam ester

Mecillinam is a unique penicillin derivative which exhibits strong antimicrobial activities against *Escherichia coli* and other gram-negative bacilli.¹⁾ As it is not absorbed orally,²⁾ its sodium salt is used parenterally and two of its esters, namely pivmecillinam³⁾ and bacmecillinam⁴⁾ are clinically used as oral prodrugs mainly for urinary tract infections.

We have been developing the (5-substituted 2-oxo-1,3-dioxol-4-yl)methyl group as a new, useful pro-moiety, and the effectiveness of the prodrugs of ampicillin⁵⁾ and norfloxacin⁶⁾ has been reported recently. As an extension of this prodrug study we prepared some new mecillinam (5-substituted 2-oxo-1,3-dioxol-4-yl)methyl esters and examined their oral absorbabilities. The present paper describes the preparation and characterization of these mecillinam esters.

Chemistry

6-Aminopenicillanic acid triethylammonium salt (**1**) was reacted with activated *N*-formylhexamethyleneimine (**2**)⁷⁾ to give crude mecillinam (**3**). This product was esterified with the halides (**4**) without further purification to give mecillinam ester hydrochlorides (**5**) as shown in Chart 1. The method for preparing the halides, (5-substituted 4-halomethyl-2-oxo-1,3-dioxoles (**4**)), was reported previously.^{5,8)} We have already reported a convenient and practical method for preparing (5-substituted 2-oxo-1,3-dioxol-4-yl)methyl 6-aminopenicillanate,⁹⁾ and the mecillinam esters can also be synthesized by the reaction of these esters and activated *N*-formylhexamethyleneimine (**2**).

Crystals of ester hydrochlorides (**5a**·HCl and **5b**·HCl) were found to have equimolar alcohol of crystallization (isopropanol or ethanol), and **5a**·HCl was hygroscopic. In an attempt to obtain a non-hygroscopic derivative, the hydrochloride was converted to other acid salts. Compound **5a** was found to form crystalline salts with L-tartaric acid, and L- and D-dibenzoyltartaric acid, but not with D-tartaric acid, malonic acid, maleic acid, *p*-toluenesulfonic acid or sulfuric acid. Among the crystalline salts, the L-tartrate did not have solvent of crystallization and was not hygroscopic.

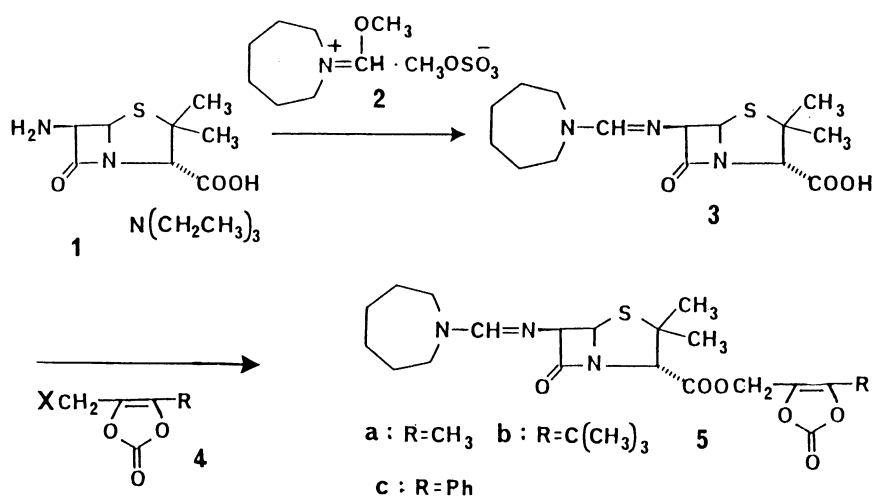


Chart 1. Synthesis of Mecillinam Esters

TABLE I. Serum Concentrations of Mecillinam ($\mu\text{g/ml}$)

Compd.	15	30	60	90	(min)
5a · HCl · EtOH	24.0	19.0	7.3	3.3	
5a · HCl · iso-PrOH	23.5	17.8	9.6	4.5	
5a · L-tartrate	25.5	22.8	11.9	3.2	
5b · HCl · iso-PrOH	10.1	7.0	3.2	0.8	
5c · HCl	11.2	5.8	2.1	0.6	
Mecillinam	2.3	2.4	2.0	0.7	

Oral Absorption in Mice

In order to estimate the oral absorbability of the mecillinam esters (**5**), serum concentrations of mecillinam were measured in mice after oral administration of these esters, and compared with serum levels after administration of the parent drug (mecillinam). The results are summarized in Table I.

The mecillinam (5-substituted 2-oxo-1,3-dioxol-4-yl)methyl esters were found to be effective as mecillinam prodrugs, showing at least 4-fold higher serum concentrations of mecillinam as compared with mecillinam itself. In particular, (5-methyl-2-oxo-1,3-dioxol-4-yl)methyl ester (**5a**) showed the highest C_{max} and a normal absorption pattern. There was no significant difference between the oral absorbabilities of the hydrochloride and L-tartrate of **5a**.

Stability Test

To estimate the stability of the mecillinam esters in the digestive tract, hydrolysis tests in artificial gastric juice (pH 1.2) and intestinal juice (pH 6.8)¹⁰ were performed by a bioautography method, as described in the experimental section. The predetermined R_f values of mecillinam and its esters (**5**) separately obtained on bioautograms were 0.5 and about 0.9, respectively. All of the test solutions sampled after 10, 30 and 50 min showed the inhibitory zone only at R_f about 0.9, and not at R_f 0.5. Therefore, it is concluded that these mecillinam esters are stable for a period of more than 50 min in the artificial digestive juices.

Recovery of Mecillinam

In order to confirm the effective hydrolysis of the mecillinam esters to the parent drug *in vivo*, the hydrolysis test in 40% mouse blood was carried out in the same way as the above-mentioned stability tests. Each of the mecillinam esters was dissolved in 40% mouse blood which had been heparinized and diluted with 1/15 M phosphate buffer (pH 7.4). Each solution was shaken at 37 °C and bioautographed after 5 and 10 min.

In the cases of **5a** and **5c**, the inhibitory zones were observed only at *R_f* 0.5 and not at *R_f* about 0.9. On the other hand, the inhibitory zones from **5b** were observed at both *R_f* 0.5 and *R_f* about 0.9, though the latter zone was small at 10 min. From these results, **5a** and **5c** were concluded to be hydrolyzed readily within 5 min at 37 °C in mouse blood *in vitro*, while **5b** was more stable.

Discussion

(5-Substituted 2-oxo-1,3-dioxol-4-yl)methyl groups, which have been developed as pro-moieties of ampicillin prodrugs were applied to mecillinam. The mecillinam esters obtained were well absorbed orally and showed at least 4-fold higher serum concentrations of mecillinam as compared with mecillinam itself.

The 5-methyl derivative (**5a**) was the best compound among these esters and showed a 10-fold higher C_{\max} than that of mecillinam, and a normal absorption pattern. Some of the mecillinam ester hydrochlorides were found to contain alcohol of crystallization and were hygroscopic. These properties are different from those of the ampicillin ester hydrochlorides with the same pro-moiety, as well as those of other conventional mecillinam ester hydrochlorides, such as pivmecillinam hydrochloride and bacmecillinam hydrochloride. Formulation of this prodrug was expected to be difficult because of the hygroscopicity. Among the other acid salts of **5a** prepared in an attempt to eliminate this hygroscopicity, the L-tartrate was found to have no solvent of crystallization and was not hygroscopic. Further, it showed good oral absorbability. L-Tartaric acid is widely used as a acidulant for soft drinks and confectionary products,¹¹⁾ and is considered to be safe.

The 5-*tert*-butyl derivative (**5b**) exhibited poorer oral absorbability than the 5-methyl derivative (**5a**), analogously with the ampicillin ester.⁵⁾ This may be partially attributable to the lack of lability in mouse blood, as noted before. On the other hand, the 5-phenyl derivative (**5c**) was labile in mouse blood and stable in artificial digestive juices, but it was not absorbed as well as **5a**. The reason for this poor absorbability is not clear.

The degradation products of mecillinam esters *in vivo* have not yet been examined, but it can be predicted that the mecillinam ester **5a** would be hydrolyzed in the same way as the corresponding ampicillin ester, lenampicillin.¹²⁾ Thus, the metabolites of **5a** should be mecillinam, acetoin and 2,3-butanediol.

Experimental

Melting points were determined on a Yamato capillary melting point apparatus, model MR-21, and all melting points are uncorrected. Proton nuclear magnetic resonance (¹H-NMR) spectra were determined on a Nihon Denshi PS-100 NMR spectrometer using tetramethylsilane (TMS) as an internal standard. Infrared (IR) spectra were recorded with a Shimadzu IR-440 machine. The esters were analyzed for C, H, N, and the values were within 0.4% of the calculated theoretical ones. No attempts were made to maximize the yields.

Mecillinam (5-Methyl-2-oxo-1,3-dioxol-4-yl)methyl Ester Hydrochloride Isopropanoate (5a · HCl · iso-PrOH)—A mixture of 6-aminopenicillanic acid (6.5 g) and triethylamine (12.6 ml) in 60 ml of dichloromethane was stirred at 35 °C for 30 min, then cooled to 0–5 °C. *N*-Formylhexamethylene dimethylsulfate (9.1 g) was added, and the whole was stirred for 1 h at the same temperature. The solvent was evaporated off to give a pale yellow syrup, which was dissolved in a mixture of ethyl acetate (70 ml) and *N,N*-dimethylformamide (30 ml). Then, sodium iodide (0.5 g) and 4-chloromethyl-5-methyl-2-oxo-1,3-dioxole (**4a**, X = Cl, 6 g) were added and the mixture was stirred for 20 h at room

temperature. Ethyl acetate (70 ml) and ice water (100 ml) were added, and after vigorous stirring, the organic layer was separated, washed with ice water, mixed with ice water (150 ml) and adjusted to pH 2.5 in the aqueous layer with 2N hydrochloric acid. The aqueous layer was separated, and sodium chloride was added to saturation. The resulting oily product was extracted with dichloromethane (100 and 50 ml), and the combined dichloromethane extracts were washed with saturated aqueous sodium chloride, dried with anhydrous magnesium sulfate, and concentrated under reduced pressure. The resulting syrup was dissolved in isopropanol (50 ml) and the solution was allowed to stand overnight at 0–5 °C. The solid was filtered off and washed with cold isopropanol to give 8 g of the title compound as colorless crystals (50%). mp 70–71 °C. IR ν_{\max}^{KBr} cm^{-1} : 1820, 1790, 1755, 1690. $^1\text{H-NMR}$ (DMSO- d_6) δ : 1.4–1.9 (14H, m, 2-CH₃ and (CH₂)₄), 2.20 (3H, s, C=C-CH₃), 3.5–4.6 (4H, m, (CH₂)₂N), 4.6 (1H, s, 3-H), 5.14 (2H, s, C=C-CH₂), 5.5–5.65 (2H, m, 5- and 6-H), 8.22 (1H, s, CH=N). *Anal.* Calcd for C₂₀H₂₇N₃O₆S·HCl·C₃H₇OH: C, 51.72; H, 6.79; N, 7.86. Found: C, 51.46; H, 6.75; N, 7.91.

Mecillinam (5-*tert*-Butyl-2-oxo-1,3-dioxol-4-yl)methyl Ester Hydrochloride Isopropanoate (5b·HCl·iso-PrOH)

—According to the above procedure, crude mecillinam was treated with 4-bromomethyl-5-*tert*-butyl-2-oxo-1,3-dioxole (4b, X=Br), and the obtained syrup was crystallized from a mixture of isopropanol and ethyl ether to give the title compound as colorless crystals (35%). mp 75–80 °C. IR ν_{\max}^{KBr} cm^{-1} : 1835, 1775, 1755, 1690. $^1\text{H-NMR}$ (DMSO- d_6) δ : 1.28 (9H, s, C(CH₃)₃), 1.4–1.9 (14H, m, 2-CH₃ and (CH₂)₄), 3.5–3.8 (4H, m, (CH₂)₂N), 4.62 (1H, s, 3-H), 5.17 (2H, s, C=C-CH₂), 5.58 (2H, s, 5- and 6-H), 8.22 (1H, s, CH=N). *Anal.* Calcd for C₂₃H₃₃N₃O₆S·HCl·C₃H₇OH: C, 54.20; H, 7.34; N, 7.29. Found: C, 54.01; H, 7.13; N, 7.33.

Mecillinam (2-Oxo-5-phenyl-1,3-dioxol-4-yl)methyl Ester Hydrochloride (5c·HCl)—According to the above procedure, crude mecillinam was treated with 4-bromomethyl-2-oxo-5-phenyl-1,3-dioxole (4c, X=Br), and the obtained residue was crystallized from a mixture of acetone and ethyl ether to give the title compound as colorless crystals (30%). mp 85–90 °C. IR ν_{\max}^{KBr} cm^{-1} : 1835, 1795, 1760, 1690. $^1\text{H-NMR}$ (DMSO- d_6) δ : 1.5–2.1 (14H, m, 2-CH₃ and (CH₂)₄), 3.6–4.1 (4H, m, (CH₂)₂N), 4.53 (1H, s, 3-H), 5.20 (2H, s, C=C-CH₂), 5.52–5.68 (2H, m, 5- and 6-H), 7.4–7.6 (5H, m, arom. H), 7.74 (1H, s, CH=N). *Anal.* Calcd for C₂₅H₂₉N₃O₆S·HCl·H₂O: C, 54.20; H, 5.82; N, 7.58. Found: C, 54.10; H, 6.00; N, 7.32.

Mecillinam (5-Methyl-2-oxo-1,3-dioxol-4-yl)methyl Ester Hydrochloride Ethanolate (5a·HCl·EtOH)—A solution of 10 g of 5a·HCl·iso-PrOH in ice water (50 ml) was treated with active carbon under ice cooling and filtered. The aqueous solution was saturated with sodium chloride, and the separated oily product was extracted with dichloromethane (60 and 20 ml). The combined dichloromethane extracts were washed with saturated aqueous sodium chloride, dried and concentrated under reduced pressure to give a colorless syrup. The syrup was dissolved in ethanol (10 ml) and mixed with diisopropyl ether (25 ml), and the mixture was allowed to stand overnight at 0–5 °C. The resulting solid was filtered off and washed with a cold mixed solvent (diisopropyl ether : ethanol, 2 : 1) to give 8 g of the title compound as colorless crystals. mp 89–91 °C (dec.). IR ν_{\max}^{KBr} cm^{-1} : 1820, 1780, 1750, 1680. $^1\text{H-NMR}$ (DMSO- d_6) δ : 1.4–1.9 (14H, m, 2-CH₃ and (CH₂)₄), 2.22 (3H, s, C=C-CH₃), 3.3–4.2 (4H, m, (CH₂)₂N), 4.64 (1H, s, 3-H), 5.18 (2H, s, C=C-CH₂), 5.63 (2H, brs, 5- and 6-H), 8.35 (1H, s, CH=N). *Anal.* Calcd for C₂₀H₂₇N₃O₆S·HCl·C₂H₅OH: C, 50.75; H, 6.29; N, 8.45. Found: C, 50.54; H, 6.07; N, 8.49.

Mecillinam (5-Methyl-2-oxo-1,3-dioxol-4-yl)methyl Ester L-Tartrate (5a·L-Tartrate)—A solution of 10 g of 5a·HCl·iso-PrOH in ice water (50 ml) was treated with active carbon under ice cooling and filtered. Ethyl acetate (60 ml) was added to the filtrate with stirring under ice cooling, and the pH of the aqueous layer was adjusted to 7.3 with 5% aqueous sodium bicarbonate. The organic layer was separated, washed with cold saturated aqueous sodium chloride, and dried with anhydrous magnesium sulfate. A methanol solution (15 ml) of L-tartaric acid (2.8 g) was added to the organic layer, and the mixture was allowed to stand overnight at –10 °C. The separated solid was filtered off, washed with cold ethyl acetate, suspended in diisopropyl ether (100 ml) and refluxed for 5 min. After cooling, the solid was filtered off and washed with diisopropyl ether to give 8 g of the title compound as colorless crystals. mp 75–79 °C. IR ν_{\max}^{KBr} cm^{-1} : 1820, 1780, 1740, 1680. $^1\text{H-NMR}$ (DMSO- d_6) δ : 1.3–1.9 (14H, m, 2-CH₃ and (CH₂)₄), 2.21 (3H, s, C=C-CH₃), 3.3–3.7 (4H, m, (CH₂)₂N), 4.23 (2H, s, HOCH-CHOH), 4.4 (1H, s, 3-H), 5.15 (2H, s, C=C-CH₂), 5.22 (1H, d, $J=4.0$ Hz, 5-H), 5.52 (1H, d, $J=4.0$ Hz, 6-H), 7.6 (4H, br, OH and COOH), 7.80 (1H, s, CH=N). *Anal.* Calcd for C₂₀H₂₇N₃O₆S·C₄H₆O₆: C, 49.05; H, 5.66; N, 7.15. Found: C, 49.18; H, 5.72; N, 7.01.

Mecillinam (5-Methyl-2-oxo-1,3-dioxol-4-yl)methyl Ester Dibenzoyl-L-tartrate (5a·Dibenzoyl-L-tartrate)—By the same procedure as described above, 5a·HCl·iso-PrOH was treated with dibenzoyl-L-tartaric acid and the title compound was solidified with ethyl acetate as colorless crystals (35%). mp 163–165 °C (dec.). IR ν_{\max}^{KBr} cm^{-1} : 1825, 1790, 1765, 1740, 1725, 1690. $^1\text{H-NMR}$ (DMSO- d_6) δ : 1.3–1.9 (14H, m, 2-CH₃ and (CH₂)₄), 2.19 (3H, s, C=C-CH₃), 3.3–3.7 (4H, m, (CH₂)₂N), 4.40 (1H, s, 3-H), 5.10 (2H, s, C=C-CH₂), 5.30 (1H, d, $J=4.0$ Hz, 5-H), 5.50 (1H, d, $J=4.0$ Hz, 6-H), 5.70 (2H, s, PhCOOCH-CHOCOPh), 7.3–8.1 (11H, m, arom. H and CH=N), 8.5–10.5 (2H, br, COOH). *Anal.* Calcd for C₂₀H₂₇N₃O₆S·C₁₈H₁₄O₈: C, 57.35; H, 5.19; N, 5.28. Found: C, 57.41; H, 5.04; N, 5.06.

Oral Absorption Test—An aqueous solution of a mecillinam ester (5) or mecillinam was given to groups of five fasted male ddY mice (about 21 g body weight) at a dose of 50 mg equivalent of mecillinam per kg body weight. Blood was taken from the cut axilla region at 15, 30, 60 and 90 min after dosing, and allowed to stand for 30 min at 0 °C. The serum was obtained by centrifugation. Serum specimens obtained at the same time were combined and assayed on the

day of sampling. Concentrations of mecillinam were measured by bioassay using *E. coli* NIHJ as a test organism.¹³⁾

Stability Test—Each of the mecillinam esters was dissolved in artificial digestive juices and the solutions were shaken at 37°C, and bioautographed after 10, 30 and 50 min, in the following way. The test solution was spotted on a cellulose plate (Art 5718 supplied by Merck & Co.) for thin layer chromatography, and the plate was developed with the upper layer of a mixture of *n*-butanol, ethanol and water in a ratio of 4:1:5. The plate was dried in air, sprayed with a 30% aqueous mouse serum solution and left to stand at 37°C for 30 min to convert the unchanged ester to mecillinam, which was detectable on the bioautogram. The thin layer plate was kept in intimate contact with a nutrient agar plate inoculated with *E. coli* NIHJ for 30 min, then the agar plate was incubated for 18 h at 37°C. The *R_f* value of the inhibitory zone on the plate against *E. coli* NIHJ was determined for mecillinam and mecillinam esters.

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