[Chem. Pharm. Bull. 32(6)2241—2248(1984)]

Studies on Prodrugs. II. Preparation and Characterization of (5-Substituted 2-Oxo-1,3-dioxolen-4-yl)methyl Esters of Ampicillin

FUMIO SAKAMOTO,* SHOJI IKEDA and GORO TSUKAMOTO

Pharmaceuticals Research Center, Kanebo Co., Ltd., 5–90, Tomobuchi-cho 1-chome, Miyakojima-ku, Osaka 534, Japan

(Received August 19, 1983)

(5-Substituted 2-oxo-1,3-dioxolen-4-yl)methyl esters were designed as a new type of ampicillin prodrug. These esters were prepared and confirmed to produce higher blood levels of ampicillin than ampicillin trihydrate itself after oral administration to mice. The compounds which produced particularly high blood levels of ampicillin were found to be hydrolyzed readily in blood *in vitro*. Ampicillin (5-methyl-2-oxo-1,3-dioxolen-4-yl)methyl ester hydrochloride (KBT-1585) showed the best oral absorbability in mice.

Keywords——ampicillin ester; prodrug; acyloxyallyl ester; (5-substituted 2-oxo-1,3-dioxolen-4-yl)methyl ester; oral absorption; KBT-1585

Some pharmaceuticals, despite their high pharmacological activities, are not sufficiently effective in practice because of their poor bioavailabilities. The prodrug approach is a fruitful method to overcome such difficulties; for example, some ampicillin esters are used clinically as prodrugs.[1] These ampicillin esters (such as pivampicillin, talampicillin and bacampicillin) have the acyloxymethyl ester moiety as a structurally common feature, that is, a double ester of a geminal diol. They appear to be hydrolyzed by initial cleavage of the sterically unhindered terminal ester bond, followed by spontaneous degradation of the unstable hydroxymethyl ester to give ampicillin and aldehyde as shown in Chart 1.[2]

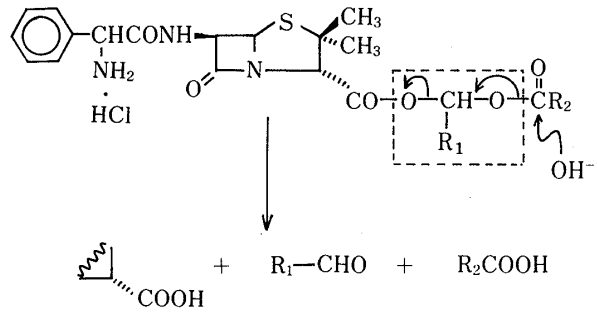
Recently we reported that some acyloxyallyl esters of ampicillin also act as a new type of prodrug.[3] These (3-phthalidylidene)ethyl esters of ampicillin are shown as the type A structure in Chart 2, and are well absorbed by the digestive tract, readily hydrolyzed, and produce high blood levels of the parent ampicillin. The mechanism of hydrolysis was assumed to involve initial attack at the terminal lactonyl ester, followed by electron transfer and cleavage of the penicillin ester bond to give ampicillin and a vinylketone derivative as shown in Chart 2.

We designed another acyloxyallyl ester structure, (5-substituted 2-oxo-1,3-dioxolen-4-yl)methyl ester, which is shown as the type B structure in Chart 2, in the expectation that it would be easy to prepare and would be readily hydrolyzed to the parent ampicillin. This type of ester should be hydrolyzed in the same manner as the type A ester, to give ampicillin, an α-dicarbonyl compound and carbon dioxide.

The present paper describes the preparation and characterization of ampicillin (5-substituted 2-oxo-1,3-dioxolen-4-yl)methyl ester hydrochlorides. This type of ampicillin ester has not been reported previously.

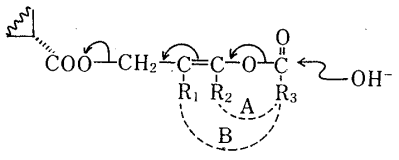
Chemistry

For the preparation of 5-substituted 4-methyl-2-oxo-1,3-dioxolenes (**3**), several methods have been reported.[4] Cyclization of α-hydroxyketone (**1**) with carbonyldichloride is a general



	R_1	R_2
pivampicillin	$-H$	$-C(CH_3)_3$
talampicillin		
bacampicillin	$-CH_3$	$-OC_2H_5$

Chart 1



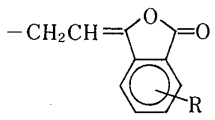
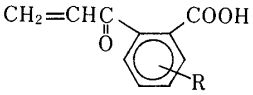
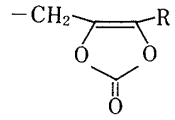
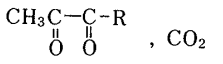
structure	hydrolysis products
A-type  (3-phthalidylidene)ethyl ester	ampicillin,  vinylketone
B-type  (5-substituted 2-oxo-1,3-dioxolen-4-yl)methyl ester	ampicillin,  α-dicarbonyl compound

Chart 2

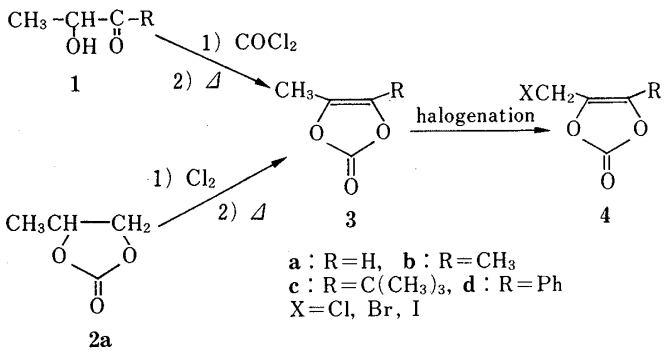


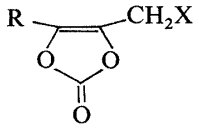
Chart 3

method, but some α-hydroxyketones are so unstable that they cannot be used as starting compounds. Thus, the method of chlorination and then dehydrochlorination of 2-oxo-1,3-dioxolane (**2a**) as shown in Chart 3 was also employed. The 5-methyl, 5-*tert*-butyl and 5-phenyl derivatives were prepared by the former method, and unsubstituted 4-methyl-2-oxo-1,3-dioxolene by the latter one.

Bromination of **3** was carried out easily with *N*-bromosuccinimide or bromine under radical reaction conditions. Because radical substitution with bromine generally accompanies the olefinic addition reaction in an allylic system, bromine was added slowly and an inorganic base was used to scavenge the generated hydrobromide to avoid side reactions. Chlorination with *N*-chlorosuccinimide or chlorine proceeded very slowly under the same conditions as used for bromination. The iodide was obtained quantitatively from the bromide or the chloride by a halogen-exchange reaction using potassium or sodium iodide in acetone. The physical properties of the obtained halides are summarized in Table I.

Ampicillin was esterified with these halides by the method shown in Chart 4. Protection of the amino group of ampicillin (**5**) with benzaldehyde in the presence of potassium bicarbonate in *N*,*N*-dimethylformamide, followed by esterification with the bromide or iodide (**4**, X=Br or I), afforded the *N*-protected ampicillin ester (**6**). Then the Schiff base (**6**) was hydrolyzed with 1 N hydrochloric acid in acetonitrile to give the ampicillin ester hydrochloride (**7**) as shown in Table II.

TABLE I. 4-Halogenomethyl-2-oxo-1,3-dioxolenes (**4**)



Compd. No.	R	X	Yield (%)	mp (°C) or bp (°C/Torr)
4a	H	Br	34	93/3
4b	CH_3	Cl	9	91—93/2
4b	CH_3	Br	81	92/2
4b	CH_3	I	92	—[a]
4c	$C(CH_3)_3$	Br	66	112—115/4
4d	Ph	Br	66	90.5—91.5
4d	Ph	I	88	88—89

a) Gradually became colored at room temperature.

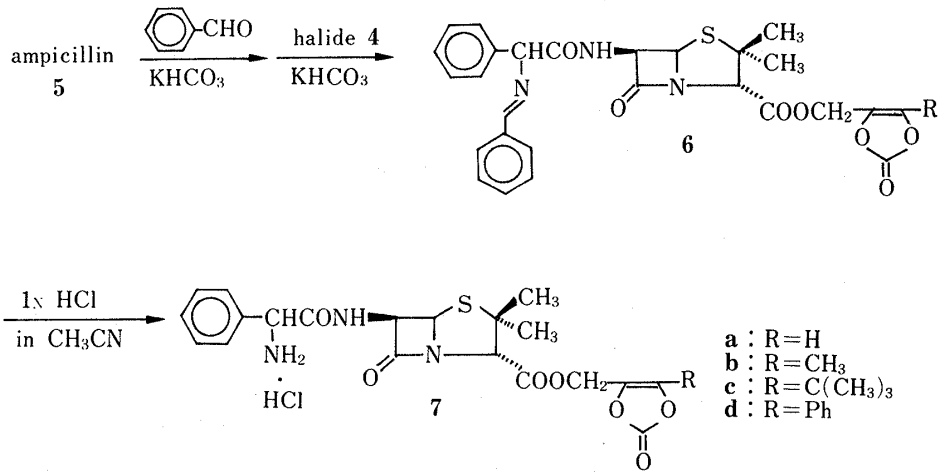
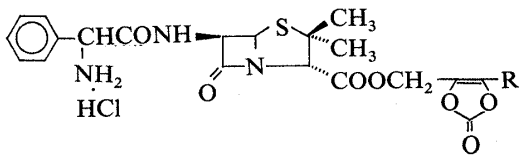


Chart 4

TABLE II. Ampicillin (5-Substituted 2-oxo-1,3-dioxolen-4-yl)methyl Esters (**7**)



Compd. No.	R	Yield (%)	mp (°C)
7a	H	26	130
7b	CH_3	60	145
7c	$C(CH_3)_3$	60	133
7d	Ph	46	140

Oral Absorption Test in Mice

In order to estimate the absorbability of ampicillin ester hydrochloride (**7**), serum concentrations of ampicillin were measured in mice after oral administration of **7**.

Groups of five fasted male ddY mice (about 22 g body weight) were given an aqueous

Table III. Serum Concentrations of Ampicillin after Oral Administration of Compounds to Mice

Compd. No.	Concentration of ampicillin (mcg/ml)			
	15	30	60	120 (min)
7a	31.0	22.5	11.9	5.6
7b	43.1	33.1	21.3	3.1
7c	18.5	10.5	8.2	3.3
7d	35.2	26.3	19.4	5.0
Ampicillin trihydrate	11.3	14.4	10.6	3.1

Table IV. Hydrolyses of Ampicillin Esters in Artificial Gastric (G) and Intestinal (I) Juices, and Mouse Blood

Compd. No.	Half-life (h)		Time (min) taken for complete hydrolysis in 40% mouse blood
	In G	In I	
7a	6.0	1.6	<2
7b	12.6	2.7	<2
7c	>20	>20	20
7d	9.0	2.5	<2

suspension or solution of ampicillin trihydrate or **7** orally at a dose equivalent to 100 mg/kg of anhydrous ampicillin. The mice were killed at 15, 30, 60 and 120 min after dosing, and the blood taken from the cut axilla region was centrifuged to obtain serum samples.

Serum specimens obtained at the same time were combined and assayed on the day of sampling. Concentrations of ampicillin were measured by microbioassay using *B. subtilis* ATCC 6633 as a test organism. Specimens were assayed against standard solutions of ampicillin prepared in mouse serum. The assay plates were incubated overnight at 35 °C, the diameters of the inhibition zones were measured and the ampicillin concentrations of the test specimens were derived from standard plots constructed by the use of standard solutions. The results are summarized in Table III.

Stability Test

To estimate the stabilities of the ampicillin esters in the digestive tract, their half-lives in artificial gastric juice (pH 1.2) and intestinal juice (pH 6.8)[5] were determined by the high performance liquid chromatography (HPLC) method. Each of the ampicillin ester solutions was shaken at 37 °C, the concentration of remaining ester was measured periodically by HPLC, and the half-life of the degradation was determined. A Waters Assoc. HPLC machine equipped with a model 6000A pump, a model U6K universal injector, a model 440 absorbance detector (at 254 nm) and a μ-Bondapak C_{18} column (30 cm × 4 mm i.d.) was used. The mobile phase consisted of 0.1 M acetate buffer (pH 4.0)–methanol (56 : 44—40 : 60, v/v) and the flow rate was 1.0 ml/min. The results are summarized in Table IV.

Recovery of Ampicillin

In order to confirm the effective hydrolysis of the ester to ampicillin *in vivo*, the period required for the complete hydrolysis of the ester was determined by bioautography. Each of the esters was incubated at 37 °C in 40% mouse blood which had been heparinized and diluted with 1/15 M phosphate buffer. A test sample was taken out periodically, spotted on a silica gel

thin-layer chromatography (TLC) plate (Merck silica gel plate No. 5715), and developed with chloroform–methanol (10:1, v/v). The dried TLC plate was sprayed with 30% aq. mouse blood and incubated at 37 °C for 30 min to convert the unchanged ester to ampicillin. The bioautography was carried out by using nutrient agar plate with *B. subtilis* ATCC 6633 as a test organism. Three esters were observed to release ampicillin completely within two min in the blood, but the *tert*-butyl derivative (**7c**) was stable and required 20 min for complete hydrolysis as shown in Table IV.

Alkaline Hydrolysis of Ampicillin Ester

If these esters are hydrolyzed by electron-transfer after the cleavage of the cyclic carbonate, the hydrolysis products should be ampicillin, α-dicarbonyl compound and carbon dioxide as shown in Chart 2. In order to confirm this, the degradation products of ampicillin (2-oxo-5-phenyl-1,3-dioxolen-4-yl)methyl ester (**7d**) in weak alkali were investigated by HPLC under the same conditions as used for the stability test. The main alkaline hydrolysis products in 2.5% aq. sodium bicarbonate coincided with authentic methyl phenyl diketone (**8d**) and ampicillin. By the same procedure, diacetyl (**8b**) and ampicillin were detected as the main alkaline hydrolysis products of **7b**. The mechanism of the hydrolysis was assumed to be the same as in the case of (3-phthalidylidene)ethyl ester[3] as shown in Chart 5. As esterase-catalyzed hydrolysis is similar to alkaline hydrolysis, an analogous reaction is expected to occur *in vivo*. However, no diketone was detected in a study on metabolites of **7b**. Thus, the *in vivo* hydrolysis mechanism seems to be somewhat different from the *in vitro* one.[6]

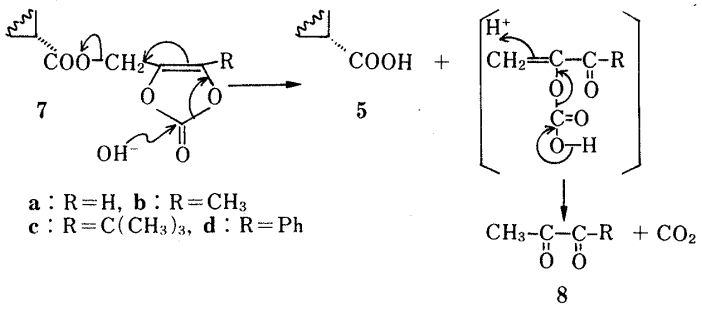


Chart 5

Discussion

These ampicillin (5-substituted 2-oxo-1,3-dioxolen-4-yl)methyl esters were confirmed to function effectively as ampicillin prodrugs, like the conventional acyloxymethyl esters such as pivampicillin, talampicillin or bacampicillin.

The 5-methyl (**7b**) and 5-phenyl (**7d**) derivatives were well absorbed by the digestive tract, and showed 2.5-fold or higher blood levels of ampicillin as compared with ampicillin trihydrate itself.

These ampicillin esters were found to be hydrolyzed readily in mouse blood *in vitro*, but showed moderate stability in artificial digestive juices. Among them, the unsubstituted derivative (**7a**) seemed to be too labile in the digestive tract to produce high blood levels. On the other hand, the 5-*tert*-butyl derivative (**7c**) was about 10-fold more stable than the effective prodrugs **7b** and **7d** in mouse blood, and hence the blood level of ampicillin may be low *in vivo*. The substituent effect on the lability to the esterase-catalyzed hydrolysis is under detailed investigation.

The (5-substituted 2-oxo-1,3-dioxolen-4-yl)methyl ester moiety was confirmed to be as effective as the (3-phthalidylidene)ethyl ester moiety which was reported recently.[3] Both compounds can be regarded as acyloxyallyl esters, and are presumed to be hydrolyzed by

similar mechanisms, that is, through electron-transfer after the fission of the terminal carbonate ester bond as shown in Chart 5. These new acyloxyallyl esters should widen the choice of available promoieties, so that optimum prodrugs can be selected from the viewpoints of safety, side effects, pharmacokinetics and so on.

In order to investigate the safety and efficacy of ampicillin (5-methyl-2-oxo-1,3-dioxolen-4-yl)methyl ester hydrochloride (**7b**), clinical trials are in progress with this compound under the code name of KBT-1585.

Experimental

Melting points were determined on a Yamato capillary melting point apparatus, model MR-21, and all melting points are uncorrected. ^{1}H Nuclear magnetic resonance (^{1}H-NMR) spectra were determined on a Nihon Denshi PS-100 NMR spectrometer and a Hitachi R-24A NMR spectrometer using tetramethylsilane (TMS) as an internal standard. IR spectra were recorded with a Shimadzu IR-440 machine. Main intermediates and the ester hydrochlorides (which were freeze-dried to eliminate trace solvents) were analyzed for C, H, N, S, *etc.* and the values were within 0.4% of the calculated theoretical ones. No attempts were made to maximize the yields.

4-Bromomethyl-2-oxo-1,3-dioxolene (4a, X=Br)——*N*-Bromosuccinimide (17.8 g) and a catalytic amount of α,α′-azobisisobutyronitrile were added to a solution of 8.6 g of 4-methyl-2-oxo-1,3-dioxolene [**3a**, synthesized from 4-methyl-2-oxo-1,3-dioxolane (**2a**) by chlorination with chlorine, followed by thermal dehydrochlorination][4a] in 200 ml of carbon tetrachloride, and the mixture was refluxed for 90 min. The reaction mixture was concentrated to one-half of the initial volume, and the insoluble material was removed by filtration. The filtrate was concentrated and the syrupy residue was distilled under reduced pressure to give 5.2 g (33.6%) of a colorless liquid. bp 93 °C (3 Torr). IR ν_{max}^{neat} cm^{-1}: 1830 (C=O). ^{1}H-NMR (CCl_4) δ: 4.1 (2H, s, CH_2Br), 7.0 (1H, s, C=CH). *Anal.* Calcd for $C_4H_3BrO_3$: C, 26.84; H, 1.69. Found: C, 26.94; H, 1.66.

4-Bromomethyl-5-methyl-2-oxo-1,3-dioxolene (4b, X=Br)——*N*-Bromosuccinimide Method: *N*-Bromosuccinimide (5.34 g) and a catalytic amount of α,α′-azobisisobutyronitrile were added to a solution of 3.42 g of 4,5-dimethyl-2-oxo-1,3-dioxolene [**3b**, synthesized from acetoin with carbonyldichloride][4b] in 150 ml of carbon tetrachloride, and the mixture was refluxed for 15 min.

The reaction mixture was concentrated to one-half of the initial volume, and the insoluble material was removed by filtration. The filtrate was concentrated, and the syrupy residue was distilled under reduced pressure to give 4.2 g (73%) of a colorless liquid.

Bromine Method: **3b** (5.7 g) was dissolved in 200 ml of carbon tetrachloride, and 4.7 g of sodium bicarbonate and a catalytic amount of α,α′-azobisisobutyronitrile were added. Under a nitrogen gas atmosphere, 8.8 g of bromine in 40 ml of carbon tetrachloride was added dropwise over the course of 50 min with vigorous stirring at 70 °C. The reaction mixture was stirred for 15 min at the same temperature, then worked up by the same procedure as described above to give 7.8 g (80.8%) of a colorless liquid. bp 92 °C (2 Torr). IR ν_{max}^{neat} cm^{-1}: 1825 (C=O). ^{1}H-NMR ($CDCl_3$) δ: 2.16 (3H, s, CH_3), 4.18 (2H, s, CH_2Br). *Anal.* Calcd for $C_5H_5BrO_3$: C, 31.12; H, 2.61; Br, 41.40. Found: C, 31.30; H, 2.49; Br, 41.31.

4-Bromomethyl-5-*tert*-butyl-2-oxo-1,3-dioxolene (4c, X=Br)——2-Hydroxy-4,4-dimethyl-3-pentanone[7] (25 g) was dissolved in 200 ml of benzene and 455 ml of a toluene solution of carbonyldichloride (0.126 g/ml) was added at 0 °C. The solution was stirred at 0 °C, then 350 ml of pyridine was added, and the whole was stirred overnight. The resultant salt of pyridine was removed by filtration and the filtrate was washed with water and dil. aq. hydrochloric acid, and dried over anhydrous magnesium sulfate. The organic layer was concentrated, and the residue was dissolved in 200 ml of xylene. Then 5 g of *p*-toluenesulfonic acid was added and the mixture was refluxed for 8 h to isomerize the produced olefin.[4d] The solution was washed with water and 5% aq. sodium bicarbonate, then dried over anhydrous magnesium sulfate and concentrated to a syrupy residue, which was distilled to give 16.6 g of 4-*tert*-butyl-5-methyl-2-oxo-1,3-dioxolene (**3c**, 55%). bp 88—91 °C (5 Torr). IR ν_{max}^{neat} cm^{-1}: 1820 (C=O). ^{1}H-NMR ($CDCl_3$) δ: 1.26 (9H, s, $C(CH_3)_3$), 2.12 (3H, s, CH_3). *Anal.* Calcd for $C_8H_{12}O_3$: C, 61.52; H, 7.75. Found: C, 61.63; H, 7.80.

N-Bromosuccinimide (3.6 g) and a catalytic amount of α,α′-azobisisobutyronitrile were added to a solution of 3.0 g of **3c** in 150 ml of carbon tetrachloride, and the mixture was refluxed for 60 min, then cooled to 5 °C. Insoluble materials were separated by filtration. The filtrate was concentrated, and the residue was distilled under reduced pressure to give 3.0 g of the bromide (**4c**, 66.4%) as a colorless liquid. bp 112—115 °C (4 Torr). IR ν_{max}^{neat} cm^{-1}: 1820 (C=O). ^{1}H-NMR ($CDCl_3$) δ: 1.35 (9H, s, $C(CH_3)_3$), 4.27 (2H, s, CH_2Br). *Anal.* Calcd for $C_8H_{11}BrO_3$: C, 40.87; H, 4.72; Br, 33.99. Found: C, 40.92; H, 4.81; Br, 34.05.

4-Bromomethyl-2-oxo-5-phenyl-1,3-dioxolene (4d, X=Br)——*N*-Bromosuccinimide (2.9 g) and a catalytic amount of α,α′-azobisisobutyronitrile were added to a solution of 2.4 g of 4-methyl-2-oxo-5-phenyl-1,3-dioxolene (**3d**)[4c] in 150 ml of carbon tetrachloride, and the reaction mixture was refluxed for 90 min, then cooled to 5 °C.

Insoluble materials were separated by filtration. The filtrate was concentrated, and the residue was recrystallized from a mixture of benzene and cyclohexane to give 2.3 g (66%) of colorless needles. mp 90.5—91.5 °C. IR ν_{max}^{KBr} cm^{-1}: 1825 (C=O). ^{1}H-NMR (CCl_4) δ: 4.35 (2H, s, CH_2Br), 7.40 (5H, s, arom. H). *Anal.* Calcd for $C_{10}H_7BrO_3$: C, 47.09; H, 2.77; Br, 31.33. Found: C, 47.22; H, 2.64; Br, 31.29.

4-Chloromethyl-5-methyl-2-oxo-1,3-dioxolene (4b, X=Cl)——A suspension of 11.4 g of **3b** and 32 g of *N*-chlorosuccinimide in 400 ml of carbon tetrachloride was stirred at room temperature for 80 h under irradiation with a 400 W high-pressure mercury lamp (Fuji model HL-400). Insoluble materials were separated by filtration, the filtrate was concentrated and the residue was distilled under reduced pressure to give 1.36 g (9.2%) of colorless liquid. bp 91—93 °C (2 Torr). IR ν_{max}^{neat} cm^{-1}: 1820 (C=O). ^{1}H-NMR ($CDCl_3$) δ: 2.18 (3H, s, CH_3), 4.31 (2H, s, CH_2Cl). *Anal.* Calcd for $C_5H_5ClO_3$: C, 40.43; H, 3.39. Found: C, 40.46; H, 3.25.

4-Iodomethyl-2-oxo-5-phenyl-1,3-dioxolene (4d, X=I)——A suspension of 1.3 g of 4-bromomethyl-2-oxo-5-phenyl-1,3-dioxolene (**4d**, X=Br) and 1.7 g of powdered potassium iodide in 10 ml of acetone was stirred at room temperature for 3 h. Insoluble materials were filtered off and the solvent was removed *in vacuo*. Next, 50 ml of ethyl ether was added to the residue and the insoluble solids were filtered off. The filtrate was washed with 5% aq. sodium chloride (50 ml) and dried over anhydrous sodium sulfate. The solution was concentrated and the residue was recrystallized from benzene to give 1.32 g (88%) of yellow needles. mp 88—89 °C. IR ν_{max}^{KBr} cm^{-1}: 1810 (C=O). ^{1}H-NMR ($CDCl_3$) δ: 4.33 (2H, s, CH_2I), 7.45 (5H, s, arom. H). *Anal.* Calcd for $C_{10}H_7IO_3$: C, 39.76; H, 2.34. Found: C, 40.02; H, 2.46.

4-Iodomethyl-5-methyl-2-oxo-1,3-dioxolene (4b, X=I) was obtained in 92% yield by the same procedure as described above; its physical properties were as follows. IR ν_{max}^{neat} cm^{-1}: 1820 (C=O). ^{1}H-NMR ($CDCl_3$) δ: 2.08 (3H, s, CH_3), 4.09 (2H, s, CH_2I). *Anal.* Calcd for $C_5H_5IO_3$: C, 25.02; H, 2.10. Found: C, 25.40; H, 2.25.

Ampicillin (5-Methyl-2-oxo-1,3-dioxolen-4-yl)methyl Ester Hydrochloride (7b, KBT-1585)——Ampicillin trihydrate (5 g) was suspended in 50 ml of *N*,*N*-dimethylformamide, and 1.25 g of potassium bicarbonate and 2.5 ml of benzaldehyde were added at 0 °C. The mixture was stirred at 0 °C for 3 h, then 1.25 g of potassium bicarbonate and 2.5 g of the bromide (**4b**, X=Br) were added, and the mixture was stirred at 0 °C for 3 h. The reaction mixture was poured into ice water and extracted with ethyl acetate (200 ml). The organic layer was washed with 10% aq. sodium chloride (100 ml × 3), dried over anhydrous magnesium sulfate and concentrated *in vacuo* to give a yellow syrup. The resultant residue was dissolved in 40 ml of acetonitrile and the pH of the solution was adjusted to 2.0 with 1 N hydrochloric acid. The solution was stirred at 0—5 °C for 30 min, and then water (100 ml) was added, and the mixture was concentrated under reduced pressure at below 20 °C to remove acetonitrile. The aqueous layer was washed with ethyl acetate (50 ml × 2), and then saturated with sodium chloride, and the separated oil was extracted with dichloromethane (50 ml × 2). The dichloromethane solution was washed with sat. aq. sodium chloride and dried over anhydrous sodium sulfate, and the solution was concentrated under reduced pressure to one-half of the initial volume. Iso-propanol (20 ml) and ethyl acetate (40 ml) were added, and the mixture was again concentrated under reduced pressure to a volume of about 20 ml. Then the solution was allowed to stand overnight at 5 °C to give colorless crystals, which were collected by filtration and washed with cold ethyl acetate to give 3.7 g of **7b** (60%). mp 145 °C (dec.). IR ν_{max}^{KBr} cm^{-1}: 1825, 1785, 1750, 1690 (C=O). ^{1}H-NMR (DMSO-d_6) δ: 1.33 (3H, s, 2-CH_3), 1.49 (3H, s, 2-CH_3), 2.18 (3H, s, C=C–CH_3), 4.44 (1H, s, 3-H), 5.12 (2H, s, C=C–CH_2), 5.18 (1H, s, Ph–CH–NH_2), 5.44—5.66 (2H, m, 5- and 6-H), 7.3—7.6 (5H, m, arom. H), 8.97 (2H, NH_2), 9.4 (1H, d, NHCO). *Anal.* Calcd for $C_{21}H_{23}N_3O_7S$–HCl: C, 50.65; H, 4.86; N, 8.44; S, 6.44. Found: C, 50.77; H, 4.92; N, 8.29; S, 6.41.

Other ampicillin ester hydrochlorides were obtained by the same procedure as described above and their physical properties were as follows.

Ampicillin (2-Oxo-1,3-dioxolen-4-yl)methyl Ester Hydrochloride (7a)——(Yield 26%). mp 130 °C (dec.). IR ν_{max}^{KBr} cm^{-1}: 1835, 1790, 1750, 1690 (C=O). ^{1}H-NMR (D_2O) δ: 1.36 (6H, s, 2-CH_3), 4.58 (1H, s, 3-H), 5.11 (2H, s, C=C–CH_2), 5.23 (1H, s, Ph–CH–NH_2), 5.49 (1H, d, J=2 Hz, 5-H), 5.58 (1H, d, J=2 Hz, 6-H), 7.5 (6H, m, arom. H and C=CH). *Anal.* Calcd for $C_{20}H_{21}N_3O_7S$–HCl–H_2O: C, 47.86; H, 4.82; N, 8.37; S, 6.39. Found: C, 48.21; H, 5.03; N, 8.09; S, 6.69.

Ampicillin (5-*tert*-Butyl-2-oxo-1,3-dioxolen-4-yl)methyl Ester Hydrochloride (7c)——(Yield 60%). mp 133 °C (dec.). IR ν_{max}^{KBr} cm^{-1}: 1830, 1785, 1755, 1690 (C=O). ^{1}H-NMR (DMSO-d_6) δ: 1.26 (9H, s, C(CH_3)$_3$), 1.32 (3H, s, 2-CH_3), 1.46 (3H, s, 2-CH_3), 4.41 (1H, s, 3-H), 5.08 (1H, s, Ph–CH–NH_2), 5.11 (2H, S, C=CCH_2), 5.4—5.6 (2H, m, 5- and 6-H), 7.3—7.6 (5H, m, arom. H), 8.55—8.8 (2H, NH_2), 9.3 (1H, d, NHCO). *Anal.* Calcd for $C_{24}H_{29}N_3O_7S$–HCl–H_2O: C, 51.66; H, 5.78; N, 7.53; S, 5.75. Found: C, 51.29; H, 5.65; N, 7.73; S, 5.88.

Ampicillin (2-Oxo-5-phenyl-1,3-dioxolen-4-yl)methyl Ester Hydrochloride (7d)——(Yield 46.4%), mp 140 °C (dec.). IR ν_{max}^{KBr} cm^{-1}: 1830, 1785, 1760, 1690 (C=O). ^{1}H-NMR (DMSO-d_6) δ: 1.32 (3H, s, 2-CH_3), 1.45 (3H, s, 2-CH_3), 4.44 (1H, s, 3-H), 5.12 (1H, s, PH–CH–NH_2), 5.31 (2H, s, C=C–CH_2), 5.4—5.6 (2H, m, 5- and 6-H), 7.3—7.6 (10H, m, arom. H), 8.8 (2H, NH_2), 9.3 (1H, d, NHCO). *Anal.* Calcd for $C_{26}H_{25}N_3O_7S$–HCl–$2H_2O$: C, 52.39; H, 5.07; N, 7.05; S, 5.38. Found: C, 52.17; H, 4.83; N, 7.31; S. 5.64.

Acknowledgement We are grateful to the late Dr. I. Utsumi, former director of this research center, for his encouragement. We also wish to thank Dr. M. Moriyama for valuable advice regarding the manuscript.

References and Notes

1) W. V. Daehne, E. Frederiksen, E. Gundersen, F. Lund, P. Morch, H. J. Peterson, K. Roholt, L. Tybring and W. O. Godtfredson, *J. Med. Chem.*, **13**, 607 (1970); J. P. Clayton, M. Cole, S. W. Elson, H. Ferres, J. C. Hanson, L. W. Mizen and R. Sutherland, *ibid.*, **19**, 1385 (1976); N. O. Bodin, B. Ekstrom and U. Forsgren, *Antimicrob. Agents Chemother.*, **8**, 518 (1975).
2) W. Morozowich, M. J. Cho and F. J. Kezdy, "Design of Biopharmaceutical Properties through Prodrugs and Analogs," ed. by E. B. Roche, American Pharmaceutical Association, Washington, D.C., 1977, pp. 344—391.
3) F. Sakamoto, S. Ikeda and G. Tsukamoto, *Chem. Pharm. Bull.*, **31**, 2698 (1983).
4) *a*) P. H. Moss, U. S. Patent 3020290 (1962); *b*) H. M. Fischer, H. G. Heine and W. Hartmann, *Tetrahedron Lett.*, 1701 (1972); *c*) K. R. Stahlke, H. G. Heine and W. Hartmann, *Liebigs Ann. Chem.*, **764**, 116 (1972); *d*) T. Hiyama, S. Fujita and H. Nozaki, *Bull. Chem. Soc. Jpn.*, **45**, 2797 (1972); *e*) F. W. Beitbeil, L. Wright, T. H. Foss and R. C. LaRose, *Trans. Ill. State Acad. Sci.*, **67**, 139 (1974).
5) The artificial gastric and intestinal juices correspond to the 1st and 2nd fluids, respectively, for the J. P. disintegration test. The Pharmacopoeia of Japan, 10th edition (1981).
6) Details will be reported at a later date.
7) S. B. Bowlus and J. A. Katzenellenbogen, *J. Org. Chem.*, **39**, 3309 (1974).