[Chem. Pharm. Bull. 24(1) 102—107 (1976)]

UDC 615.31.011.5 : 615.076.7

Lactol Esters of Ampicillin

Ichiro Isaka, Kohzi Nakano, Teruya Kashiwagi, Akio Koda, Hiroshi Horiguchi, Hidefumi Matsui, Kozo Takahashi, and Masuo Murakami

Central Research Laboratories, Yamanouchi Pharmaceutical Co., Ltd.[1]

(Received May 17, 1975)

Several lactol esters of D-α-aminobenzylpenicillin hydrochloride (Va—e) were synthesized from the corresponding lactol esters of benzylpenicillin (IIa—e) or from D-α-aminobenzylpenicillin (ampicillin). The lactol esters IIa—e were prepared with potassium benzylpenicillinate (I) and certain halides of lactols. Among them, two isomers of 2(5H)-furanone-5-yl benzylpenicillinate (IIb) could be easily separated from the mixture of the stereoisomers due to C_5' position of the lactol moiety. We found that all of these lactol esters of ampicillin hydrochloride (Va—e) showed higher blood concentrations of ampicillin after oral administration to rats than that of ampicillin itself. Particularly Va and Ve were much superior.

A broad spectrum antibiotic, D-α-aminobenzylpenicillin (ampicillin) (VI), has been used successfully in the therapeutics of wide variety of bacterial infections. In general, however, absorption of VI is not sufficient after oral administration. On the other hand, it has been shown recently by Daehne, *et al.*[2] that orally administered acyloxymethyl esters of VI were absorbed readily, and high blood levels as VI were achieved by hydrolysis of the esters with an esterase in blood and tissues.

Among these acyloxymethyl esters of VI already reported, pivaloyloxymethyl ester of VI (pivampicillin generally called) is the best. But it has been reported[3] that the ester might have side-effects, because it would release harmful formaldehyde when hydrolyzed.

We studied to develop new esters of VI that they are easily absorbed, hydrolyzed in organism, no release of harmful materials, highly safe, and clinically useful. In order to discover easily absorbable derivatives of VI, we have synthesized various ester derivatives of VI and examined the absorption of them after oral administration to rats.[4]

γ-Aldehydic acids containing both formyl and carboxyl groups in a molecule generally produce a 5-membered lactol by intramolecular ring closure.

We were interested in such lactol esters, since it is expected that the lactol ester, in which hydroxy group of lactol is acylated, is easily hydrolyzed. Thus several new lactol esters of VI (Va—e)[5] were synthesized. We found that these lactol esters of VI were stable in pharmaceutical preparations contrary to expectation, and rapidly hydrolyzed with enzyme *in vitro* experiment[6] in spite of its high stability.

We also found that all their lactol esters of VI were easily absorbed and rapidly hydrolyzed by an esterase of rat to give high plasma concentrations of ampicillin.

1) Location: *Azusawa, Itabashi-ku, Tokyo, Japan.*
2) W.V. Daehne, E. Frederiksen, E. Gundersen, F. Lund, P. Mønch, H.J. Petersen, K. Roholt, L. Tybring, and O. Godtfredsen, *J. Med. Chem.*, **13**, 607 (1970).
3) W.V. Daehne, W.O. Gotfredsen, K. Roholt, and L. Tybring, *Antimicr. Agents & Chemoth.*, **1970**, 431 (1971); M.C. Jordan, J.B. deMaine, and W.M.M. Kirby, *ibid.*, **1970**, 438 (1971).
4) I. Isaka, K. Nakano, N. Kawahara, T. Kashiwagi, T. Ozasa, H. Horiguchi, K. Takahashi, and M. Murakami, *Rep. Yamanouchi Cent. Res. Lab.*, **2**, 95 (1974); H. Matsui, N. Sakamoto, H. Sasaki, F. Kumagai, S. Kawahara, and M. Murakami, *ibid.*, **2**, 109 (1974).
5) We later found that Beecham Group Ltd. had applied 6 days earlier than us for a patent on the invention of another process of Ve among these new lactol-esters and applications were laid open as Japan Patent Publication number (unexamined) 48—5793 and 48—5794.
6) Y. Shiobara, A. Tachibana, H. Sasaki, T. Watanabe, and T. Sado, *J. Antibiot.*, **27**, 665 (1974).

A pathway for the synthesis of the lactol esters Va—e is outlined in Chart 1.[7] Corresponding benzylpenicillin esters (IIa—e) were obtained in good yield by the reaction of potassium benzylpenicillinate (I) with some halides, 5-chloro-dihydro-2(3H)-furanone,[8] 5-bromo-2(5H)-furanone,[9] 5-chloro-3,4-dimethyl-2(5H)-furanone, 5-bromo-4-ethyl-3-methyl-2(5H)-furanone, and 3-bromo-(3H)-isobenzofuranone (phthalidylbromide)[10] in N,N-dimethylformamide (DMF) or dimethylsulfoxide (DMSO) in the presence of potassium hydrogen carbonate. All these lactol esters IIa—e are new compounds and are the equimolar mixture of stereoisomers due to the asymmetric carbon on the individual lactol ester moieties (the C_5, on the furanone moieties or the C_3, on the phthalidyl moiety respectively). Among those lactol esters (IIa—e), dihydro-2(3H)-furanone-5-yl benzylpenicillinate (IIa) and phthalidyl benzylpenicillinate (IIe) were obtained as crystals, and 3,4-dimethyl-2(5H)-furanone-5-yl benzylpenicillinate (IIc) and 4-ethyl-3-methyl-2(5H)-furanone-5-yl benzylpenicillinate (IId) as noncrystalline powders.

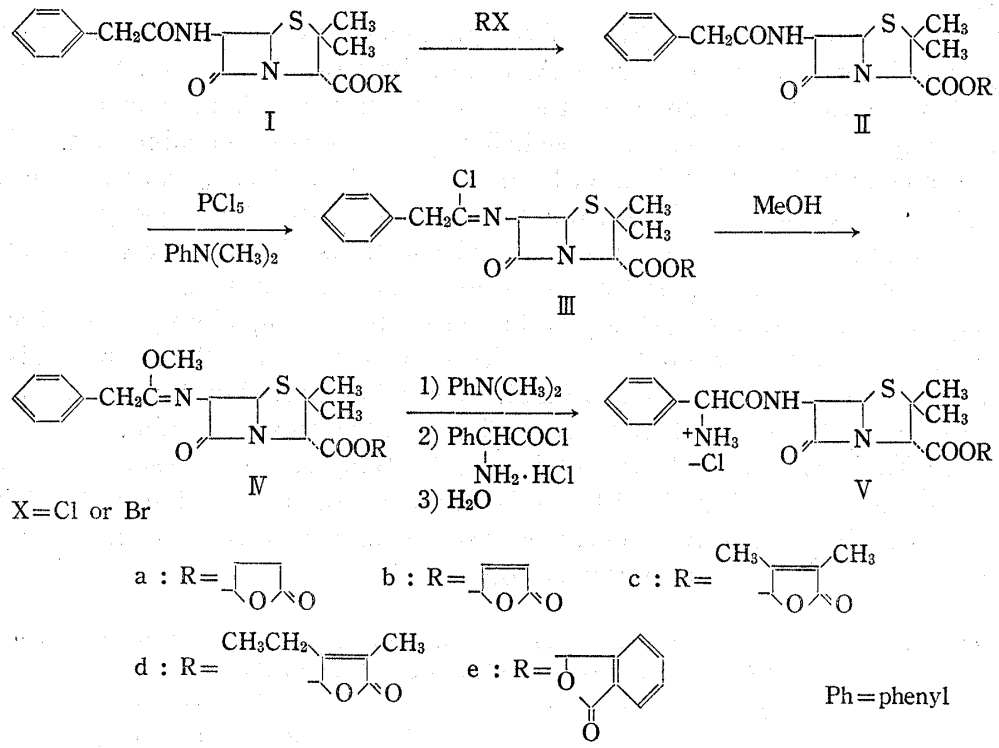

Chart 1

Two isomers of 2(5H)-furanone-5-yl benzylpenicillinate (IIb), could be easily separated by fractional recrystallization technique. Recrystallization of IIb from ethyl acetate gave one stereoisomer IIb-1, $[\alpha]_D^{20}$ +118°, as crystal. After removal of the crystals, the concentrated filtrate was chromatographed on silica gel to afford the other stereoisomer IIb-2, $[\alpha]_D^{20}$ +185°, as powder.

The intended derivatives of ampicillin were synthesized by the reaction[7] in which IIa—e were treated with phosphorous pentachloride, methanol, D-(—)-α-phenylglycylchloride

7) We already reported the acyl group exchange of natural penicillin and its mechanism. I. Isaka, T. Kashiwagi, K. Nakano, N. Kawahara, A. Koda, Y. Numasaki, S. Kawahara, and M. Murakami, *Yakugaku Zasshi*, **92**, 454 (1972); A. Koda, K. Takanobu, I. Isaka, T. Kashiwagi, K. Takahashi, S. Kawahara, and M. Murakami, *ibid.*, **92**, 459 (1972).

8) S. Motoki, S. Satsumabayashi, and I. Tajima, *Bull. Chem. Soc. Japan*, **37**, 646 (1964).

9) N. Elming and N. Clauson-Kaas, *Acta Chem. Scand.*, **6**, 565 (1952).

10) R.L. Shriner and F.J. Wolf, "Organic Syntheses," Coll. Vol. II, ed. by A.H. Blatt, John Wiley, and Sons, Inc., New York 1955, p. 737.

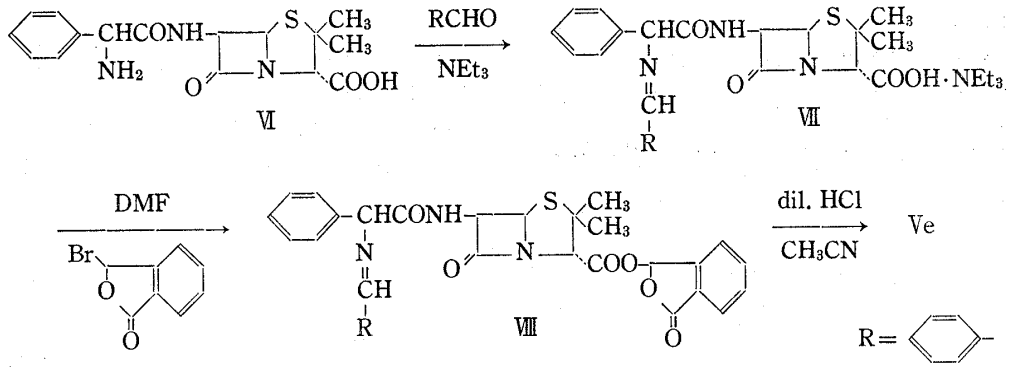

Chart 2

hydrochloride, and water successively. Thus obtained Va—e were all easy to dissolve in water expectedly.

Alternative syntheses of 1(3H)-isobenzofuranone-3-yl D-α-aminobenzylpenicillinate (Phthalidyl ampicillinate) hydrochloride (Ve) were illustrated in Chart 2. For example, treatment of VI with benzaldehyde in the presence of triethylamine in dichloromethane afforded N-benzylidene-D-α-aminobenzylpenicillin (VII) as triethylamine salt. The Schiff base (VII) was esterified with phthalidyl bromide at 0—5° in DMF, and the resulted Schiff base ester (VIII) was hydrolyzed with diluted aqueous hydrochloric acid in acetonitrile at the same temperature to give Ve. The physical constants obtained here were completely consistent with those of Ve prepared by the route shown in Chart 1.

In order to estimate the absorbability of the lactol esters of ampicillin (Va—e), their plasma concentrations as VI were measured in rats. To four or five male Wistar rats (about 170 g of a body weight) in each group fasted overnight were administered orally an aqueous solution of VI or Va—e in a dose equivalent to 20 mg/kg of anhydrous ampicillin. The results are summarized in Table I. Thus all of the six lactol esters, Va to Ve, especially Va and Ve have been found to have higher blood levels of ampicillin than ampicillin itself. This seems to indicate that the six lactol derivatives can be absorbed more readily than ampicillin. Detailed biological properties of Ve have been published.[6]

Table I. 30 min Plasma Concentrations of Ampicillin Following Oral Administration of Ampicillin or Its Esters Hydrochloride to Rats

Ester No.	Number of rats	Plasma concentrations (mcg/ml)	
		Mean	SE
Ampicillin	5	1.04	0.06
Va	5	4.58	0.57
Vb-1	5	2.50	0.27
Vb-2	5	1.24	0.26
Vc	5	2.00	0.14
Vd	5	1.74	0.24
Ve	4	5.58	0.73

Experimental[11]

Dihydro-2(3H)-furanone-5-yl Benzylpenicillinate (IIa)——To a stirred suspension of potassium benzylpenicillinate (I) (35.4 g) and $KHCO_3$ (2.2 g) in DMF (60 ml) was added 5-chloro-dihydro-2(3H)-8-furanone[8]

11) All melting points were uncorrected. Infrared (IR) and nuclear magnetic resonance (NMR) spectra were measured with a Hitachi 215 grating infrared spectrometer and with a Nippondenshi NH-100 or Hitachi R-20 spectrometer using tetramethylsilane (TMS) as an internal standard.

(11.4 g) and the mixture was stirred at room temperature for 6 hr. The reaction mixture was poured into ice water and extracted with AcOEt. The AcOEt layer was washed with 5% aq. solution of $NaHCO_3$ and H_2O successively and dried over anhyd. $MgSO_4$. The crystalline solid of IIa was precipitated from concentrated AcOEt layer, and ether was added to make complete precipitation. 28.4 g (71.2%) of IIa were obtained as a colorless crystalline solid. mp 170—171° IR ν_{max}^{KBr} cm^{-1}: 3280 (NH) 1800—1770 broad (lactone, lactam and ester) 1665 (amide). NMR ($CDCl_3$) δ: 1.48 (12H, s, $C(CH_3)_2\times 2$), 2.35—2.72 (8H, m, C_3' and C_4'-H$\times 2$), 3.64 (4H, s, C_6H_5-$C\underline{H}_2$-$\times 2$), 4.37, 4.39 (2H, s, C_3-H), 5.43—5.78 (4H, m, lactam-H$\times 2$), 6.19 (2H, m, NH$\times 2$), 6.08—6.22 (2H, m, C_5'-H$\times 2$), 7.34 (10H, s, $C_6H_5\times 2$).

2(5H)-Furanone-5-yl Benzylpenicillinate (IIb-1 and IIb-2)——To a stirred suspension of I (37.2 g) and $KHCO_3$ (5.0 g) in DMSO (200 ml) was added dropwise 5-bromo-2(5H)-furanone[9] (16.3 g) over a period of 30 min and the mixture was stirred at room temperature for 2 hr. The reaction mixture was poured into ice water (*ca.* 1 liter) and the resulting precipitate was collected, washed with H_2O. The precipitate was dissolved in dichloromethane (300 ml). The solution was washed with 5% aq. NaCl, then treated with charcoal and filtered. The filtrate was dried over anhyd. $MgSO_4$ and evaporated. The resulting solid was triturated with ether–petr. ether to give a mixture of stereoisomers, II-b 35 g as a pale yellow powder (84%), $[\alpha]_D^{20}$ +158° (*c*=1, $CHCl_3$).

Recrystallization from AcOEt gave 15.4 g of colorless prisms of IIb-1, $[\alpha]_D^{20}$ +118° (*c*=1, $CHCl_3$). IR ν_{max}^{KBr} cm^{-1}: 3320 (NH), 1800—1765 broad (lactone, lactam, and ester) 1680 (amide). NMR ($CDCl_3$) δ: 1.50, 1.61 (6H, >$C(CH_3)_2$), 3.58 (2H, s, C_6H_5-$C\underline{H}_2$-), 4.42 (1H, s, C_3-H), 5.48 (1H, d, *J*=4 Hz C_5-H), 5.57 (1H, dd, *J*=8, 4 Hz, C_6-H), 6.41 (1H, d, *J*=6 Hz, C_3'-H), 7.09 (1H, s, C_5'-H), 7.38 (5H, s, C_6H_5-), 7.54 (1H, d, *J*=6 Hz, C_4'-H), 8.51 (1H, d, *J*=8 Hz, 2 NH).

The AcOEt mother liquor was condensed and residual oil was chromatographed on silica gel. Elution with AcOEt–benzene (1: 1) gave a stereoisomer, IIb-2 as a pale yellow powder, yield 11.0 g $[\alpha]_D^{20}$ +185° (*c*=1, $CHCl_3$). The same IR as IIb-1 was shown. NMR ($CDCl_3$) δ: 1.50, 1.60 (6H, >$C(CH_3)_2$), 3.58 (2H, s, C_6H_5-$C\underline{H}_2$-), 4.44 (1H, s, C_3-H), 5.40—5.62 (2H, m, lactam-H), 6.38 (1H, d, *J*=6 Hz, C_3'-H), 7.01 (1H, s, C_5'-H), 7.20—7.30 (5H, m, C_6H_5-), 7.48 (1H, d, *J*=6 Hz, C_4'-H), 8.35 (1H, d, *J*=8 Hz, NH).

5-Chloro-3,4-dimethyl-2(5H)-furanone——A mixture of 5-hydroxy-3,4-dimethyl-2(5H)-furanone[12] (6.6 g) and $SOCl_2$ (10 ml) was stirred at room temperature for 4 hr. Evaporation of excess $SOCl_2$ under reduced pressure gave 5-chloro-3,4-dimethyl-2(5H)-furanone as a pale yellow oil. Yield 7.5 g. IR ν_{max}^{neat} cm^{-1}: 1760—1790 broad (lactone) NMR (CD_3OD) δ: 1.77 (3H, s, C_3-CH_3), 1.98 (3H, s, C_4-CH_3), 5.87 (1H, s, C_5-H).

3,4-Dimethyl-2(5H)-furanone-5-yl Benzylpenicillinate (IIc)——To a stirred suspension of I (19.0 g) and $KHCO_3$ (3.0 g) in DMSO (100 ml) was added dropwise 5-chloro-3,4-dimethyl-2(5H)-furanone (7.5 g) over a period of 30 min and the mixture was stirred at room temperature for 2 hr. The reaction mixture was poured into ice water (*ca.* 500 ml) and the precipitate was collected, washed with H_2O. The precipitate was dissolved in dichloromethane (100 ml). The solution was washed with H_2O (50 ml$\times$3), dried over anhyd. $MgSO_4$ and evaporated. The residue was crystallized from benzene–ether to give a mixture of stereoisomers, IIc as a colorless powder. Yield 17.7 g (78%). IR ν_{max}^{KBr} cm^{-1}: 3350 (NH), 1770 (lactone, lactam, and ester), 1680 (amide). NMR ($CDCl_3$) δ: 1.43—1.51 (12H, m, >$C(CH_3)_2\times 2$), 1.89 (6H, s, C_3'-$CH_3\times 2$), 1.98 (6H, s, C_4'-$CH_3\times 2$), 3.63 (4H, s, C_6H_5-$C\underline{H}_2$-$\times 2$), 4.41 (2H, s, C_3-H$\times 2$), 5.39—5.77 (4H, m, lactam-H$\times 2$), 6.1 (2H, m, NH$\times 2$), 6.74 (2H, m, C_5'-H$\times 2$), 7.32 (10H, s, C_6H_5-$\times 2$).

5-Bromo-3-methyl-4-ethyl-2(5H)-furanone——Phosphorus tribromide (8.13 g) was added to 5-hydroxy-3-methyl-4-ethyl-2(5H)-furanone[12] (12.6 g) below 0° with stirring. After stirring at 0° for 1 hr, stirring was continued at room temperature for 30 min. The reaction mixture was evaporated and residual oil was distilled under reduced pressure to give a colorless oil of 5-bromo-3-methyl-4-ethyl-2(5H)-furanone. Yield 12.1 g (67%), bp_3 105—106°.

3-Methyl-4-ethyl-2(5H)-furanone-5-yl Benzylpenicillinate (IId)——To a stirred suspension of I (30.0 g) and $KHCO_3$ (5.0 g) in DMSO (60 ml) was added dropwise a solution of 5-bromo-3-methyl-4-ethyl-2(5H)-furanone (10.6 g) in DMSO (20 ml) over a period of 30 min. and the mixture was stirred at room temperature for 5 hr. The reaction mixture was poured into ice water (*ca.* 500 ml) and the precipitate was extracted with AcOEt (100 ml$\times$3). Combined AcOEt layer was washed with 5% aq. NaCl, then treated with charcoal and filtered. The filtrate was dried over anhyd. $MgSO_4$ and evaporated. The residue was solidified from *n*-hexane to give a mixture of stereoisomers IId as a colorless powder. Yield 34.7 g (94%). IR ν_{max}^{KBr} cm^{-1}: 3310 (NH), 1765 (lactone, lactam, and ester), 1660 (amide) NMR ($CDCl_3$) δ: 1.14 (6H t, *J*=8 Hz, C_4'-C-$CH_3\times 2$), 1.45—1.51 (12H, m, -$C(CH_3)_2\times 2$), 1.91 (6H, s, C_3'-$CH_3\times 2$), 2.40 (4H, m, C_4'-CH_2-$\times 2$), 3.16 (4H, s, $C_6H_5C\underline{H}_2$-$\times 2$), 4.42 (2H, s, C_3-H$\times 2$), 5.41—5.78 (4H, m, lactam-H$\times 2$), 6.28 (2H, m, NH$\times 2$), 6.87 (2H, m, C_5'-H$\times 2$), 7.36 (10H, s, C_6H_5-$\times 2$).

1(3H)-Isobenzofuranone-3-yl Benzylpenicillinate (IIe)——To a stirred suspension of I (19.0 g) and $KHCO_3$ (4.0 g) in DMF (70 ml) was added 3-bromo-1(3H)-isobenzofuranone[10] (12.5 g) at 5°, and the mixture was stirred at room temperature for 5 hr. The reaction mixture was poured into ice-water (400 ml), and the precipitate was collected, washed with H_2O. The precipitate was dissolved in dichloromethane (70 ml), and the organic layer was washed with H_2O, dried over anhyd. Na_2SO_4 and evaporated under reduced pressure.

12) J. Shreiber and C.G. Wermuth, *Bull. Soc. Chim. France*, **1965**, 2242.

The residue was crystallized from iso-PrOH. The resulting crystal was recrystallized from dichloromethane–iso-PrOH to give a mixture of stereoisomers, IIe as colorless fine crystals. Yield 17.5 g (77.7%). IR ν_{max}^{KBr} cm^{-1}: 3390 (NH), 1770—1785 broad (lactone, lactam, and ester), 1685 (amide) NMR (d_6-DMSO) δ: 1.50—1.58 (12H, m, $>C(CH_3)_2\times2$), 3.54 (4H, s, C_6H_5–C<u>H</u>$_2$–$\times2$), 4.54 (2H, s, C_3–H$\times2$), 5.48—5.65 (4H, m, lactam-H$\times2$), 7.27 (10H, s, $C_6H_5\times2$) 7,63 (2H, s, COO–CH–$\times2$), 7.30—8.03 (8H, m, aromatic-H of phthalide$\times2$).

Dihydro-2(3H)-furanone-5-yl D-α-Aminobenzylpenicillinate·HCl (Va)——To a solution containing IIa (4.9 g) and N,N-dimethylaniline (4.81 ml) in dichloromethane (49 ml), was added PCl_5 (2.69 g) at −25°, and the mixture was stirred at the same temperature for 1.5 hr. MeOH (47 ml) was added dropwise to the reaction mixture at −25±5°, and stirring was continued at the same temperature for 3 hr to give iminoether solution. To this solution N,N-dimethylaniline (8.02 ml) and then D-(−)-α-phenylglycylchloride·HCl (2.82 g) were added at −25±5° with stirring. After stirring at the same temperature for 2 hr, the reaction mixture was allowed to stand overnight at −20——25°. Water (23 ml) was added to the reaction mixture, and after stirring for 30 min, at 0°, the mixture was filtered and water layer was separated. The organic layer was extracted with H_2O (10 ml×2). Combined H_2O layer was washed with dichloromethane and after the pH value was adjusted to 7 with $NaHCO_3$, the deposited oil was extracted with dichloromethane. The dichloromethane solution was washed with H_2O, dried over anhyd. Na_2SO_4, evaporated under reduced pressure. Precipitation by adding AcOEt (5 ml) and ether (20 ml) to the residual oil gave dihydro-2(3H)-furanone-5-yl D-α-aminobenzylpenicillinate as a colorless powder. mp 181—182° (decomp.) The compound (2.1 g) was dissolved in dichloromethane. To the resulting solution was added iso-PrOH saturated with HCl gas (*ca.* 1.5 ml) to make pH 3. After stirring for 15 min ether (30 ml) was added. Deposited precipitate was collected to give a mixture of stereoisomers Va as a colorless powder. Yield 2.2 g *Anal.*calcd. for $C_{20}H_{23}O_6N_3S\cdot HCl$: C, 51.12; H, 5.15; N, 8.94; Cl, 7.54. Found: C, 50.87; H, 5.62; N, 8.40; Cl, 7.29. IR ν_{max}^{KBr} cm^{-1}: 1790—1760 broad (lactone, lactam, and ester), 1685 (amide) NMR (D_2O) δ: 1.36 (12H, s, $>C(CH_3)_2\times2$), 2.2—2.85 (2H, m, C_4'–H$\times2$) 4.56, 4.59 (2H, s, C_3–H), 5.27(2H, s, C_6H_5C<u>H</u>–$\times2$), 5.52 (4H, s, lactam-H$\times2$), 6.68 (2H, s, C_5'–H$\times2$), 7.53 (10H, s, C_6H_5–$\times2$).

2(5H)-Furanone-5-yl D-α-Aminobenzylpenicillinate-HCl (Vb-1 and Vb-2)——To a solution containing IIb-1 (8.32 g) and N,N-dimethylaniline (8.25 ml) in dichloromethane (80 ml), was added PCl_5 (4.6 g) at −25°, and the mixture was stirred at the same temperature for 1.5 hr. MeOH (50 ml) was added dropwise to the reaction mixture at −25°, and stirring was continued at the same temperature for 3 hr. To the resulting iminoether solution, was added N,N-dimethylaniline (13.8 ml) and the D-(−)-α-phenylglycylchloride·HCl (5.0 g) portionwise over a period of 1 hr at −25±5° with stirring. After 2 hr stirring at the same temperature, the reaction mixture was allowed to stand overnight at −20——25°. The reaction mixture was diluted with dichloromethane (80 ml), washed with saturated aq. NaCl (20 ml×2), dried over anhyd. $MgSO_4$ and evaporated under reduced pressure. The residual oil was washed with ether, and resulting solid material was dissolved in H_2O (100 ml). The H_2O solution was washed with AcOEt (50 ml×3) and saturated with NaCl to deposit a colorless precipitate. The precipitate was collected, and dissolved in dichloromethane containing MeOH. The resulted solution was dried over anhyd. $MgSO_4$ and evaporation gave a crystalline solid. Recrystallization from dichloromethane–iso-PrOH afforded colorless fine prisms of optically active Vb-1. Yield: 6.25 g (67.0%) mp. 192—193° (dec.) $[\alpha]_D^{20}$ +175° (c=1 H_2O) *Anal.*. calcd. for $C_{20}H_{21}O_6N_3S\cdot HCl$: C, 51.33; H, 4.74; N, 8.98; Cl, 7.58; S, 6.85. Found: C, 50.58; H, 4.93; N, 8.61; Cl, 7.45; S, 6.75. IR ν_{max}^{KBr} cm^{-1}: 1780—1760 broad (lactone, lactam, and ester), 1685 (amide). NMR ($CDCl_3$–CD_3OD) δ: 1.49 (6H, s, $>C(CH_3)_2$), 4.43 (1H, s, C_3–H), 5.22 (1H, s, C_6H_5–C<u>H</u>–), 5.53 (2H, q, J=4 Hz, lactam H), 6.42 (1H, dd, J=6, 1.5 Hz, C_3'–H), 7.09 (1H, d, J=1.5 Hz, C_5'–H), *ca.* 7.5 (6H, m, C_6H_5– and C_4'–H).

The other optical isomer, Vb-2 was obtained as a colorless powder from the optical isomer, IIb-2 by the same procedure with Vb-1. Yield. 6.0 (56%) mp 154—160° (decomp.) *Anal.* Calcd. for $C_{20}H_{21}O_6N_3S\cdot HCl$: C, 51.33; H, 4.74; N, 8.98; Cl, 7.58; S, 6.85. Found: C, 50.74; H, 4.98; N, 8.59; Cl, 7.51; S, 6.54. $[\alpha]_D^{20}$ +184° (c=1, H_2O). IR ν_{max}^{KBr} cm^{-1} 1780—1760 broad (lactone, lactam, and ester), 1685 (amide) NMR ($CDCl_3$–CD_3OD) δ: 1.49 (6H, s, $>C(CH_3)_2$) 4.45 (1H, s, C_3–H), 5.18 (1H, s, C_6H_5–C<u>H</u>–), 5.51 (2H, q, J=4 Hz, lactam-H), 6.42 (1H d—d, J=6, 1.5 Hz, C_3'–H), 7.05 (1H, d, J=1.5 Hz, C_5'–H), *ca.* 7.5 (6H, m, C_6H_5– and C_4'–H).

3,4-Dimethyl-2(5H)-furanone-5-yl D-α-aminobenzylpenicillinate·HCl (Vc) and 3-methyl-4-ethyl-2(5H)-furanone-5-yl D-α-aminobenzylpenicillinate·HCl (Vd) were also obtained as a mixture of stereoisomers by the same procedure as Vb-1. IR and NMR data as follows. Vc: IR ν_{max}^{KBr} cm^{-1}: 1770 (lactone, lactam, and ester), 1680 (amide), NMR ($CDCl_3$–CD_3OD) δ: 1.48 (12H, s, $>C(CH_3)_2\times2$), 1.90 (6H, s, C_4'–$CH_3\times2$), 2.04 (6H. s, C_3'–$CH_3\times2$), 4.43, 4.47 (2H, s, C_3–H$\times2$), 5.18 (2H, m, C_6H_5–C<u>H</u>–$\times2$), 5.42—5.60 (4H, m, lactam-H$\times2$), 6.70 (2H, m, C_5'–H$\times2$), 7.51 (10H, s, C_6H_5–$\times2$) Vd: IR ν_{max}^{KBr} cm^{-1}: 1770 (lactone, lactam, and ester), 1680 (amide) NMR [D_2O] 1.09 (6H, t, J=8 Hz, C_4'–CH_2C<u>H</u>$_3\times2$), 1.37 (12H, s, $>C(CH_3)_2\times2$), 1.80 (6H, s, C_3'–$CH_3\times2$), 2.20—2.70 (4H, m, C_4'–C<u>H</u>$_2CH_3\times2$), 4.23 (2H, s, C_3–H$\times2$). 5.39 (2H, s, C_6H_5–C<u>H</u>–$\times2$), 5.58 (4H, s, lactam-H$\times2$), 6.95 (2H, s, C_5'–H$\times2$), 7.66 (10H, s, C_6H_5–$\times2$).

Phthalidyl Ampicillinate·HCl (Ve)——a) From 1(3H)-Isobenzofuranone-3-yl Benzylpenicillinate (IIe): To a stirred solution containing IIe (50 g) and N,N-dimethylaniline (44.2 ml) in dichloromethane (250 ml), was added PCl_5 (25 g) at −25°, and the mixture was stirred at −20°——25° for 1.5 hr. MeOH (160 ml) was added dropwise to the reaction mixture at −25±5° and stirring was continued at −20——25° for 2.5 hr. To the resulting iminoether solution, was added N,N-dimethylaniline (74 ml) and then D-(−)-α-

phenylglycylchloride·HCl (27 g) dividing in three portions below −25°, and then the resulting pale yellow clear solution was kept overnight at −20——25°. The reaction mixture was washed with 15% aq. NaCl (250 ml × 2), dried over anhyd. Na_2SO_4 and then evaporated under reduced pressure. The residual oil was dissolved in H_2O (800 ml) and the aq. solution was washed successively with AcOEt until yellow color had not been shown in AcOEt layer. To the aq. solution was added NaCl to deposit an oil, and this was extracted two times with dichloromethane (200 ml, 50 ml). Combined dichloromethane solution was washed with 15% aq. NaCl, dried over anhyd. Na_2SO_4 and filtered. Iso-PrOH (240 ml) was added to the filtrate, which then was concentrated under reduced pressure. After removal of the greater part of dichloromethane, deposition of a colorless precipitate began. Concentration was continued until the volume of the mixture had become to about 200 ml. After cooling in a refrigerator overnight, the precipitate was filtered off to give a mixture of stereoisomers, Ve as a colorless amorphous powder. Yield 39.6 g (71.2%) $[\alpha]_D^{20}$ +164.5 (c=1, EtOH) *Anal.* calcd. for $C_{24}H_{23}O_6N_3S$·HCl: C, 55.65; H, 4.67; N, 8.11; S, 6.19; Cl, 6.84. Found C, 55.27; H, 4.95; N, 7.84; S, 6.21; Cl, 6.35. IR ν_{max}^{KBr} cm^{-1}: 1780 (lactone, lactam and ester), 1685 (amide) NMR [CD_3OD] δ: 1.42—1.50 (12H, m, >C-$(CH_3)_2$ × 2), 4.51, 4.53 (2H, s, C_3-H × 2), 5.19 (2H, s, C_6H_5-CH- × 2), 5.42—5.63 (4H, m, lactam-H × 2), 7.37—7.61 (10H, m, C_6H_5 × 2), 7.66—8.05 (10H, m, aromatic-H of phthalide and -COOCH- × 2).

b) From D-α-Aminobenzylpenicillin (VI): To a stirred suspension of VI (20 g) and anhyd. $MgSO_4$ (12 g) in dichloromethane (200 ml), was added dropwise triethylamine (6.9 ml), and benzaldehyde (8.0 g). After stirring for 30 min, the reaction mixture was filtered, and the filtrate was condensed under reduced pressure at a low temperature to give a yellow residual oil. Washing the oil with ether and petr. ether gave D-α-benzylideneaminobenzylpenicillin (VII) triethylamine salt as a yellow gum. Yield 23.5 g (88%) IR ν_{max}^{KBr} cm^{-1}: 1770 (lactam), 1680 (amide), 1640 (C=N), 1600 (carboxylate). To a solution of triethylamine salt of VII (23.0 g) in DMF (60 ml) was added $KHCO_3$ (5.0 g) and portionwise 3-bromo-1(3H)-isobenzofuranone[10] (9.1 g) at 0—5° with stirring. After stirring for 2 hr with ice cooling, the reaction mixture was poured into ice water (300 ml). The precipitate was extracted with AcOEt (100 ml × 2) and the combined AcOEt layer was washed with H_2O (100 ml × 2), dried over anhyd. $MgSO_4$ and evaporated under reduced pressure. The pale yellowish residual oil was chromatographed on silica gel (Wakogel C 200; Wako Pure Chemical Industries, Ltd. 500 g). Elution with AcOEt–benzene (1: 1) gave phthalidyl D-α-benzylideneaminobenzylpenicillinate (VIII) as a yellowish white amorphous powder. Yield 15.0 g (61.5%) mp 104—109° (decomp.) IR ν_{max}^{KBr} cm^{-1}: 1780 broad (lactone, lactam, and ester), 1680 (amide), 1640 (C=N) VIII (10.0 g) was dissolved in CH_3CN (20 ml). To this solution was added dropwise 10% HCl (5 ml) with stirring at room temperature and stirred for 10 min. The reaction mixture was concentrated under reduced pressure below room temperature. After removal of the greater part of CH_3CN, H_2O (100 ml) was added to the condensed solution, which was extracted with AcOEt (500 ml × 3) to remove water insoluble oily materials. The water layer was saturated with NaCl, and the deposited colorless oil was extracted with dichloromethane (50 ml × 2). The combined dichloromethane solution was washed with 15% aq. NaCl, dried over anhyd. Na_2SO_4 and filtered. Iso-PrOH was added to the filtrate, concentrated and then precipitate was collected to give Ve, which was identical in every respect with that obtained by above described method of a). Yield. 6.4 g (70.4%).