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Studies on Prodrugs. I. Preparation and Characterization of Acyloxyallylester 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

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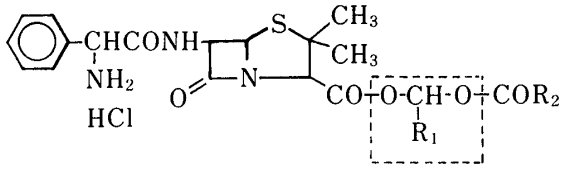
In order to investigate a new type of promoiety for ampicillin prodrugs, acyloxyallyl esters were prepared and their oral absorbabilities, stabilities and hydrolyzabilities to parent ampicillin were studied. As starting materials for (3-phthalidylidene)ethyl esters, ethylidenephthalide and its derivatives were regiospecifically prepared by a new method using the Wittig reaction. (3-Phthalidylidene)ethyl esters (**14a—c**) given orally were well absorbed, but (thiophthalidylidene)ethyl ester (**14d**) and (isocoumarin-4-yl)methyl ester (**14e**) were poorly absorbed.

Keywords——ampicillin ester; prodrug; promoiety; ethylidenephthalide; oral absorption; Wittig reaction; (3-phthalidylidene)ethyl ester

The use of prodrugs as a method to improve oral absorption has been carried out widely in the case of penicillins.[1] In particular, esterification of dipolar D-α-aminobenzylpenicillin (ampicillin) has been studied, and some of the esters are used clinically as prodrugs of ampicillin.[2] These effective prodrugs have acyloxymethyl ester as a structurally common feature, namely a double ester of a geminal diol (see Chart 1).



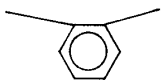
	R_1	R_2
pivampicillin	$-H$	$-C(CH_3)_3$
talampicillin		
bacampicillin	$-CH_3$	$-OC_2H_5$

Chart 1

It is considered that they are hydrolyzed by initial cleavage of the unhindered terminal ester bond, followed by spontaneous degradation of the unstable hydroxymethyl ester to give ampicillin and aldehyde.[3]

When the unstable hydroxymethyl ester degrades, an electron pair is transferred, and an additional carbon-carbon double bond would probably transfer the electron pair in the same manner. Acyloxyallyl ester, in which a carbon-carbon double bond is inserted into the terminal ester bond of the acyloxymethyl ester, is thus expected to function as a prodrug (see

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Chart 2). In order to investigate whether the acyloxyallyl ester moiety can act as a promoiety or not, (3-phthalidylidene)ethyl ester and (isocoumarin-4-yl)methyl ester were selected from the point of view of easy preparation. R_3 and R_4 cyclize and form *exo*-olefin in ampicillin (3-phthalidylidene)ethyl ester, and R_2 and R_4 cyclize and form *endo*-olefin in ampicillin (isocoumarin-4-yl)methyl ester, as shown in Chart 2.

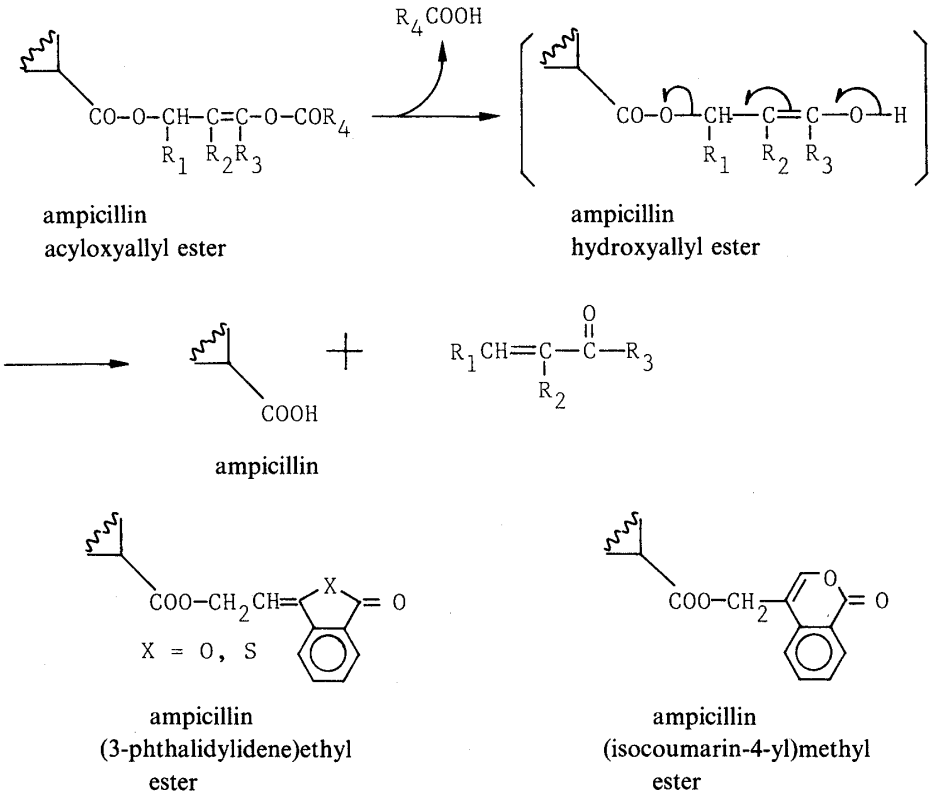

Chart 2

Since such a type of ampicillin ester, to our knowledge, has not been described previously, we decided to investigate in detail the possibility of improving the oral absorption of ampicillin, and the results of our studies are presented here.

Chemistry

For the preparation of ethylidenephthalide, the Perkin reaction is known.[4] Although the yield of this thermal condensation reaction is good, it is not a general method for synthesizing ethylidenephthalide derivatives. For instance, when an attempt was made to prepare a fluorine-substituted derivative from 4-fluorophthalic anhydride, the result was an inseparable mixture of 5- and 6-fluorinated derivatives.

Thus, for regiospecific synthesis, we modified the method of Howe, who had prepared 3-benzylidenephthalide by Wittig reaction of triphenyl (3-phthalidyl)phosphonium bromide and benzaldehyde.[5] Ethylidenephthalides were prepared regiospecifically from acetaldehyde and corresponding phosphonium bromides. The known phthalides[6] (**1**) were brominated with *N*-bromosuccinimide in carbon tetrachloride, and reacted with triphenylphosphine in benzene to give phosphonium bromides (**3**). Ethylidenephthalides (**4**, **5**) were obtained from the phosphonium bromides and acetaldehyde in the presence of triethylamine (see Chart 3).

It is known that alkylidenephthalides have *E*- and *Z*-isomers.[5] In the case of ethylidenephthalide, only the thermodynamically stable *Z*-isomer (**4**) is the isolated from the Perkin reaction, but in this Wittig reaction, the *E*-isomer (**5**) is mainly obtained kinetically. These isomers were isolated by chromatography and the *E*-isomer (**5**) was found to be easily

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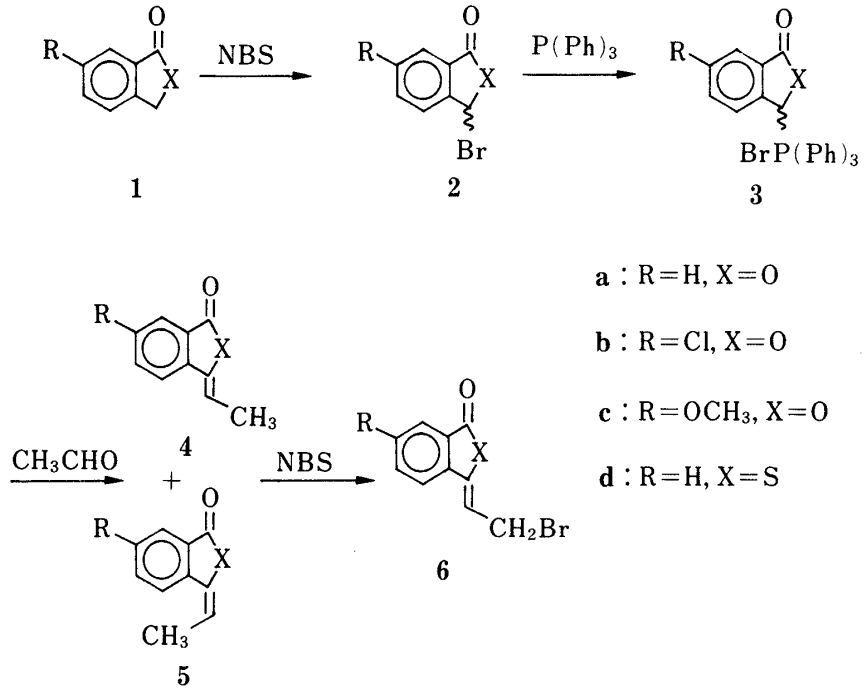

Chart 3

transformed to the more stable *Z*-isomer (**4**) by iodine-catalyzed isomerization.

Bromination of ethylidenephthalides was carried out under radical conditions, and (*Z*)-3-(2-bromoethylidene)phthalides (**6**) were obtained from the *Z*-isomer and also from the *E*-isomer of ethylidenephthalides.

There are several methods for the preparation of 4-methylisocoumarin. It is known that the ring closure reaction of acetonyl benzoate proceeds only when the compound has electron-donating groups at *meta*-positions of the benzoate.[7] Thus, 3,5-dihydroxybenzoic acid (**7**) was selected as a starting material, and 5,7-dihydroxy-4-methylisocoumarin (**9**) was obtained from acetonyl 3,5-dihydroxybenzoate (**8**) by the ring closure reaction with conc. sulfuric acid; however, bromination did not proceed. Thus it was converted to the diacetate and dimethyl ether, and it was found that the dimethyl ether (**10**) could be brominated by *N*-bromosuccinimide (Chart 4). The obtained bromides are summarized in Table I.

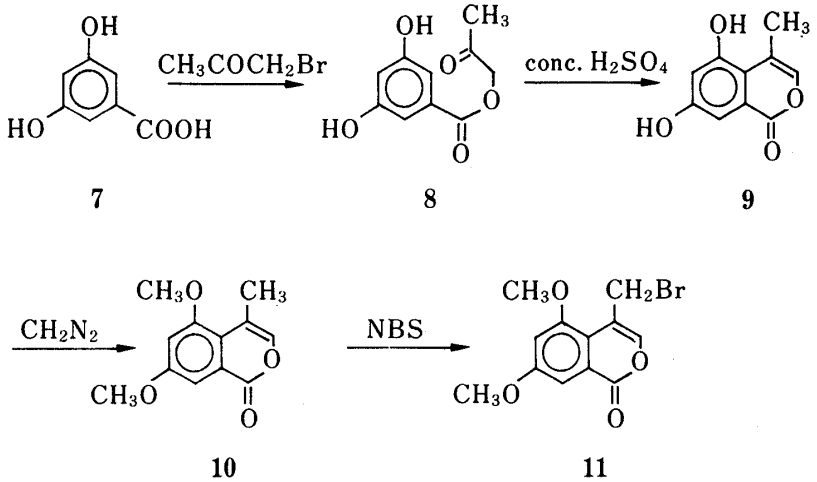

Chart 4

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TABLE I. Yields, Melting Points and Analysis Data of Bromides

Compd. No.	Yield (%)	mp (°C)	Analysis (%)					
			Calcd			Found		
			C	H	Br	C	H	Br
6a	68	106—108	50.24	2.95	33.42	50.23	3.09	33.13
6b	40	106—108	43.91	2.22	29.21	44.06	2.31	29.38
6c	62	91—93	49.10	3.37	29.70	49.28	3.34	29.80
6d	32	109—109.5	47.08	2.77	31.32	47.07	2.71	31.62
11	28	158—160	48.19	3.71	26.71	48.27	3.74	26.81

Ampicillin was esterified with these bromides as shown in Chart 5, by a modification of Isaka's method.[8]

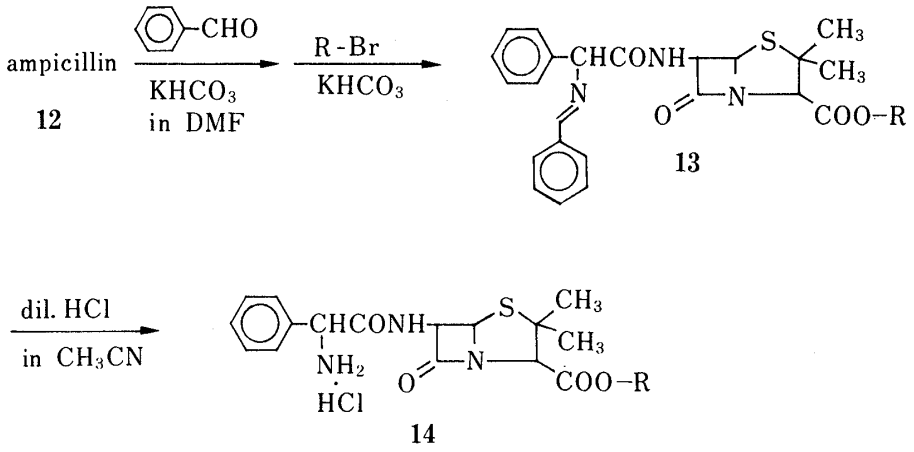

Chart 5

Treatment of ampicillin (**12**) with benzaldehyde in the presence of potassium bicarbonate in *N*,*N*-dimethylformamide, followed by esterification with the bromide (**6** or **11**) below 10 °C, afforded the corresponding *N*-benzylidene-D-α-aminobenzylpenicillanic acid ester (**13**).

The Schiff base was hydrolyzed with aqueous hydrochloric acid in acetonitrile at 0 °C to give the ampicillin ester hydrochloride. The results are shown in Table II; the preparation and characterization of some of the compounds were described previously.[9]

Absorption Test in Mice

In order to estimate the absorbability of ampicillin ester hydrochlorides (**14**), serum concentrations of ampicillin were measured in mice after administration of **14**.

Five fasted male ddY mice (about 20 g body weight) in each group received orally an aqueous suspension or solution of ampicillin trihydrate or ampicillin ester hydrochloride at a dose equivalent to 50 mg/kg of anhydrous ampicillin. Mice were killed at 15, 30, 60 and 120 min after dosing, and blood was taken from the cut axilla region and 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, inhibition zone diameters were measured and the ampicillin concentrations of the test specimens were derived from standard lines constructed by the use of standard solutions. The

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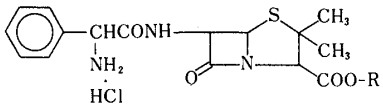
TABLE II. Structures, Yields, Melting Points and Analysis Data of Ampicillin Ester Hydrochlorides



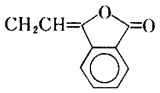
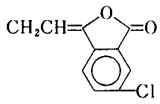
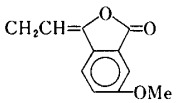
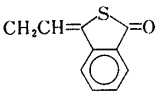
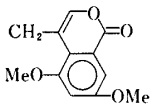
Compd. No.	R	Yield (%)	mp (°C)	Analysis (%)	Calcd	Found
14a		48	145—155	C, H, N, S,	55.56 5.02 7.48 5.70	55.21 4.86 7.31 5.93
14b		34	141—147	C, H, N, S,	52.35 4.56 7.04 5.38	52.01 4.76 6.77 5.02
14c		36	143—150	C, H, N, S,	54.68 5.27 7.09 5.41	54.32 5.45 6.88 5.26
14d		42	140—145	C, H, N, S,	54.02 4.88 7.27 11.09	53.78 4.92 7.03 11.31
14e		38	155—159	C, H, N, S,	54.06 5.19 6.75 5.15	53.89 5.31 6.52 5.38

TABLE III. Serum Concentrations of Ampicillin (mcg/ml)

Compd. No.	15	30	60	120 (min)
14a	26.5	14.5	6.4	3.1
14b	21.0	13.3	3.5	3.0
14c	13.5	12.3	4.9	3.4
14d	3.3	2.8	1.0	0.3
14e	4.2	2.3	1.2	1.1
Ampicillin trihydrate	7.0	8.6	4.8	1.7

results are summarized in Table III.

Stability Test

To estimate the stability of the ampicillin esters in the digestive tract, their half-lives in artificial gastric and intestinal juices were measured by high performance liquid chromatography (HPLC) method. Each of the esters were dissolved to a predetermined concentration in simulated gastric juice having a pH of 1.2 and in simulated intestinal juice having a pH of 7.5[10] and the solutions were shaken at 37 °C. The concentrations of remaining esters were measured periodically by HPLC using a reversed phase partition column, and the half-lives

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TABLE IV. Hydrolysis of Ampicillin Esters in SGJ, SIJ and Mouse Blood

Compd. No.	Half-life		Time (min) taken for complete hydrolysis in mouse blood
	In SGJ[a] (h)	In SIJ[b] (min)	
14a	12	85	<2
14b	10	45	<5
14c	7	120	5
14d	>20	210	50
14e	>20	>300	>60

a) Simulated gastric juice.
b) Simulated intestinal juice.

were determined. A Waters Assoc. HPLC machine equipped with a Model 3000A 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 0.033 M citrate buffer (pH 2.6)–acetonitrile (55 : 45, v/v) and the flow rate was 1.5 ml/min. The results are summarized in Table IV.

Recovery of Ampicillin

To confirm the effective hydrolysis of the ester to ampicillin *in vivo*, the ester was incubated in 40% mouse blood (heparinized and diluted with saline) at 37 °C and the period required for the ester to be completely hydrolyzed was measured by using bioautography. Test samples were taken 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 plates were sprayed with 10% aq. mouse serum and incubated at 37 °C for 30 min to hydrolyze the unchanged ester. The bioautography was carried out by using nutrient agar plates with *B. subtilis* ATCC 6633 as a test organism. Some of the esters were observed to release ampicillin rapidly in the blood, as shown in Table IV.

In order to identify the metabolites of the ester, the hydrolyzed products of ampicillin (3-phthalidylidene)ethyl ester (**14a**) in weak alkali were investigated by HPLC under the same conditions as for the stability test. The main alkaline hydrolysis products in 2.5% aq. sodium bicarbonate coincided with authentic 2-carboxyphenyl vinyl ketone (**15**) and ampicillin.

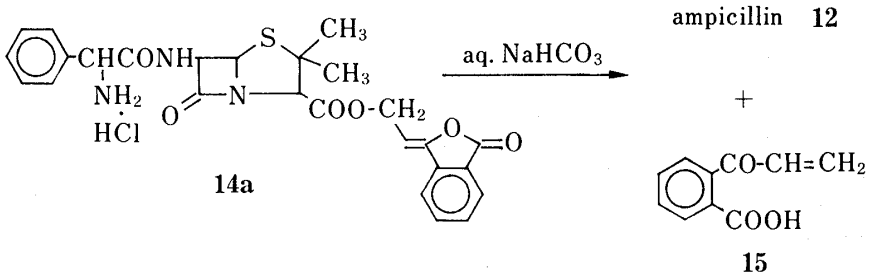

Chart 6

The mechanism of hydrolysis was assumed to involve initial hydrolysis of the terminal lactonyl ester, followed by electron transfer (enol–keto isomerization) and cleavage of the penicillin ester bond to give ampicillin and the vinylketone (**15**). As esterase-catalyzed hydrolysis is considered to be similar to alkaline hydrolysis,[3] an analogous reaction should

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occur *in vivo*.

Discussion

The resistance to hydrolysis of thiophthalidylidenethyl ester (**14d**) may be due to reduction of the electrophilicity of the thiolactonyl carbonyl carbon through back-donation of the sulfur atom. In the case of (isocoumarin-4-yl)methyl ester (**14e**), the high stability to hydrolysis may be a result of the longer conjugated system and reduced ring strain energy of the six-membered ring.

Hydrolyzabilities of the esters in blood were parallel to those in the case of non-enzymatic hydrolysis in simulated intestinal juice (pH 7.5). This supports the view that the ester-hydrolysis mechanism *in vivo* is analogous to that of the alkali-catalyzed reaction. Since the product of the weak alkali-catalyzed hydrolysis of **14a** is the vinyl ketone (3-(2-carboxyphenyl)-1-propen-3-one) (**15**), it is assumed that the terminal lactonyl ester is hydrolyzed first and then the resulting hydroxyallyl ester is cleaved spontaneously by electron transfer, as expected initially.

It was confirmed that some of the acyloxyallyl esters functioned as promoieties for ampicillin prodrugs, like the conventional acyloxymethyl esters. (3-Phthalidylidene)ethyl esters of ampicillin were well absorbed from the digestive tract, and showed 2.5 fold or more higher blood levels of parent drug as compared with ampicillin trihydrate itself. These well-absorbed esters were hydrolyzed rapidly (within five minutes) in mouse blood *in vitro*, whereas the acyloxyallyl esters such as **14d** and **14e**, which gave low levels of ampicillin in blood, were slowly hydrolyzed under the same conditions. These results suggest that the blood levels of parent penicillins *in vivo* can be estimated from the hydrolyzabilities of the esters *in vitro* to some extent. Further studies on other new promoieties are in progress.

Experimental

Melting points were determined on a Yamato capillary melting point apparatus, Model MR-21. 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. Infrared (IR) spectra were recorded with Shimadzu IR-440 machine. Main intermediates and ester hydrochloride products were analyzed for C, H, S, *etc.* and values were within 0.4% of the calculated theoretical values. No attempts were made to maximize the yields.

Triphenyl(3-phthalidyl)phosphonium Bromide (3a)——Phthalide (20 g), *N*-bromosuccinimide (26.6 g) and α,α'-azobisisobutyronitrile (0.2 g) were refluxed for 1 h in carbon tetrachloride (300 ml). The solution was cooled, succinimide was filtered off, and the solvent was removed *in vacuo* to leave a solid, which was recrystallized from benzene–cyclohexane to give 3-bromophthalide (**2a**), (27 g, yield 85%). A mixture of **2a** (18.7 g), triphenylphosphine (23 g) and dry benzene (100 ml) was stirred at 90—95 °C under N_2 for 24 h. The reaction mixture was allowed to cool, then filtered. The resultant solid was washed with hot acetonitrile (300 ml) and filtered to give a colorless solid (30.5 g, yield 73%). mp 250—254 °C. IR ν_{max}^{KBr} cm^{-1}: 1780 (C=O). ^{1}H-NMR ($CDCl_3$) δ: 7.0—8.0 (19H, m, arom. H), 9.80 (1H, s, 3-H). *Anal.* Calcd for $C_{26}H_{20}BrO_2P$: C, 65.70; H, 4.24; P, 6.52. Found: C, 65.96; H, 4.23; P, 6.41.

Triphenyl(3-phthalidyl)phosphonium bromides (**3b—d**) were obtained by the same procedure and the physical properties were as follows.

Triphenyl(6-chloro-3-phthalidyl)phosphonium Bromide (3b)——(overall yield 54%). mp 229.5—232 °C. IR ν_{max}^{KBr} cm^{-1}: 1780 (C=O). ^{1}H-NMR ($CDCl_3$) δ: 7.3—8.0 (18H, m, arom. H), 9.8 (1H, s, 3-H).

Triphenyl(6-methoxy-3-phthalidyl)phosphonium Bromide (3c)——(overall yield 41%). mp 233.5—234.5 °C. IR ν_{max}^{KBr} cm^{-1}: 1780 (C=O). ^{1}H-NMR ($CDCl_3$) δ: 3.82 (3H, s, OCH_3), 7.1—8.0 (18H, m, arom. H), 9.65 (1H, s, 3-H).

Triphenyl3-(2-thiophthalidyl)phosphonium Bromide (3d)——(overall yield 47%). mp 228—233 °C. IR ν_{max}^{KBr} cm^{-1}: 1710 (C=O). ^{1}H-NMR ($CDCl_3$) δ: 7.3—8.2 (19H, m, arom. H), 9.8 (1H, d, J=8.0 Hz, 3-H).

(*Z*)- and (*E*)-3-Ethylidenephthalide (4a and 5a)——Triethylamine (4 g) was added dropwise to a solution of triphenyl(3-phthalidyl)phosphonium bromide (20 g) and acetaldehyde (2 g) in dichloromethane (300 ml) at 0—5 °C with stirring. After being stirred for 2 h at room temperature, the mixture was washed with water, dried over anhydrous sodium sulfate and concentrated *in vacuo* to give a solid. The solid was chromatographed on silica gel

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(Merck Silica gel 60 No. 7734). Elution with benzene–ethyl acetate (30 : 1, v/v) gave (*Z*)-3-ethylidenephthalide and (*E*)-3-ethylidenephthalide, which were recrystallized from benzene–hexane.

(*Z*)-3-Ethylidenephthalide (4a)——(2.4 g, yield 36%). mp 64—65 °C. IR ν_{max}^{KBr} cm^{-1}: 1770 (C=O). ^{1}H-NMR ($CDCl_3$) δ: 1.98 (3H, d, J=8.0 Hz, CH_3), 5.50 (1H, q, J=8.0 Hz, CH_3–CH=C), 7.25—7.9 (4H, m, arom. H). *Anal.* Calcd for $C_{10}H_8O_2$: C, 74.99; H, 5.03. Found: C, 74.73; H, 5.17.

(*E*)-3-Ethylidenephthalide (5a)——(3.6 g, yield 54%). mp 59—60 °C. IR ν_{max}^{KBr} cm^{-1}: 1765 (C=O). ^{1}H-NMR ($CDCl_3$) δ: 2.11 (3H, d, J=8.0 Hz, CH_3), 5.82 (1H, q, J=8.0 Hz, CH_3–CH=C), 7.2—8.0 (4H, m, arom. H). *Anal.* Calcd for $C_{10}H_8O_2$: C, 74.99; H, 5.03. Found: C, 74.81; H, 5.11.

By the same procedure, (*E*)- and (*Z*)-3-ethylidenephthalides (**4b—d** and **5b—d**) were obtained. Their physical properties were as follows.

(*Z*)-6-Chloro-3-ethylidenephthalide (4b)——(yield 24.4%). mp 109—110 °C. IR ν_{max}^{KBr} cm^{-1}: 1780 (C=O). ^{1}H-NMR ($CDCl_3$) δ: 1.99 (3H, d, J=8.0 Hz, CH_3), 5.59 (1H, q, J=8.0 Hz, CH_3CH=C), 7.45—7.7 (3H, m, arom. H). *Anal.* Calcd for $C_{10}H_7ClO_2$: C, 61.71; H, 3.63; Cl, 16.44. Found: C, 61.81; H, 3.52; Cl, 16.61.

(*E*)-6-Chloro-3-ethylidenephthalide (5a)——(yield 36.6%). mp 113—114 °C. IR ν_{max}^{KBr} cm^{-1}: 1775 (C=O). ^{1}H-NMR ($CDCl_3$) δ: 2.10 (3H, d, J=8.0 Hz, CH_3), 5.84 (1H, q, J=8.0 Hz, CH_3CH=C), 7.48—7.88 (3H, m, arom. H). *Anal.* Calcd for $C_{10}H_7ClO_2$: C, 61.71; H, 3.63; Cl, 16.44. Found: C, 61.86; H, 3.54; Cl, 16.51.

(*Z*)-6-Methoxy-3-ethylidenephthalide (4c)——(yield 14.4%). mp 93—94 °C. IR ν_{max}^{KBr} cm^{-1}: 1770 (C=O). ^{1}H-NMR ($CDCl_3$) δ: 1.95 (3H, d, J=8.0 Hz, CH_3), 3.82 (3H, s, OCH_3), 5.43 (1H, q, J=8.0 Hz, CH_3CH=C), 7.1—7.5 (3H, m, arom. H). *Anal.* Calcd for $C_{11}H_{10}O_3$: C, 69.46; H, 5.30. Found: C, 69.37; H, 5.25.

(*E*)-6-Methoxy-3-ethylidenephthalide (5c)——(yield 31.2%). mp 95—96 °C. IR ν_{max}^{KBr} cm^{-1}: 1770 (C=O). ^{1}H-NMR ($CDCl_3$) δ: 2.05 (3H, d, J=8.0 Hz, CH_3), 3.82 (3H, s, OCH_3), 5.66 (1H, q, J=8.0 Hz, CH_3CH=C), 7.09—7.79 (3H, m, arom. H). *Anal.* Calcd for $C_{11}H_{10}O_3$: C, 69.46; H, 5.30. Found: C, 69.58; H, 5.33.

(*Z*)- and (*E*)-3-Ethylidene-2-thiophthalide (4d and 5d)——(yield 49%). A mixture of (*Z*)- and (*E*)-isomers was obtained as a pale yellow liquid and subjected to bromination without separation. IR ν_{max}^{neat} cm^{-1}: 1710 (C=O). ^{1}H-NMR ($CDCl_3$) δ: 1.95 (d, J=7.0 Hz, CH_3), 2.22 (d, J=8.0 Hz, CH_3), 6.11 (q, J=8.0 Hz, CH_3CH=C), 6.59 (q, J=7.0 Hz, CH_3CH=C), 7.2—7.9 (m, arom. H). *Anal.* Calcd for $C_{10}H_8OS$: C, 68.15; H, 4.58; S, 18.19. Found: C, 68.03; H, 4.63; S, 18.21.

(*Z*)-3-(2-Bromoethylidene)phthalide (6a)——(*Z*)- or/and (*E*)-3-Ethylidenephthalide (**4a** or **5a**) (52.5 g), *N*-bromosuccinimide (67 g) and α,α′-azobisisobutyronitrile (0.5 g) were refluxed in carbon tetrachloride (1500 ml) for 20 h. The solution was cooled, succinimide was filtered off and the solvent was removed *in vacuo*. The resultant solid was recrystallized from benzene to give pale yellow needles (53 g, yield 68%). mp 106—108 °C. IR ν_{max}^{KBr} cm^{-1}: 1780 (C=O), 1685 (*exo*-olefin). ^{1}H-NMR ($CDCl_3$) δ: 4.3 (2H, d, J=8.0 Hz, CH_2Br), 5.7 (1H, t, J=8.0 Hz, $BrCH_2CH$), 7.3—7.6 (4H, m, arom. H). *Anal.* Calcd for $C_{10}H_7BrO_2$: C, 50.24; H, 2.95; Br, 33.42. Found: C, 50.23; H, 3.09; Br, 33.13.

(*Z*)-3-(2-Bromoethylidene)phthalides (**6b—d**) were obtained by the same procedure, and their physical properties were as follows.

(*Z*)-6-Chloro-3-(2-bromoethylidene)phthalide (6b)——(yield 40%). mp 106—108 °C. IR ν_{max}^{KBr} cm^{-1}: 1790 (C=O), 1680 (*exo*-olefin). ^{1}H-NMR ($CDCl_3$) δ: 4.32 (2H, d, J=8.0 Hz, CH_2Br), 5.83 (1H, t, J=8.0 Hz, $BrCH_2CH$), 7.6—8.0 (3H, m, arom. H). *Anal.* Calcd for $C_{10}H_6BrClO_2$: C, 43.91; H, 2.22; Br, 29.21; Cl, 12.96. Found: C, 44.06; H, 2.31; Br, 29.38; Cl, 13.11.

(*Z*)-6-Methoxy-3-(2-bromoethylidene)phthalide (6c)——(yield 62%). mp 91—93 °C. IR ν_{max}^{KBr} cm^{-1}: 1780 (C=O), 1680 (*exo*-olefin). ^{1}H-NMR ($CDCl_3$) δ: 3.87 (3H, s, OCH_3), 4.32 (2H, d, J=8.0 Hz, CH_2Br), 5.68 (1H, t, J=8.0 Hz, $BrCH_2CH$), 7.1—7.6 (3H, m, arom. H). *Anal.* Calcd for $C_{11}H_9BrO_3$: C, 49.10; H, 3.37; Br, 29.70. Found: C, 49.28; H, 3.34; Br, 29.80.

(*Z*)-3-(2-Bromoethylidene)-2-thiophthalide (6d)——(yield 32%). mp 109—109.5 °C. IR ν_{max}^{KBr} cm^{-1}: 1710—1680 (C=O, *exo*-olefin). ^{1}H-NMR ($CDCl_3$) δ: 4.26 (2H, d, J=8.0 Hz, CH_2Br), 6.85 (1H, t, J=8.0 Hz, $BrCH_2CH$), 7.4—8.0 (4H, m, arom. H). *Anal.* Calcd for $C_{10}H_7BrOS$: C, 47.08; H, 2.77; Br, 31.32; S, 12.57. Found: C, 47.07; H, 2.71; Br, 31.62; S, 12.41.

5,7-Dimethoxy-4-methylisocoumarin (10)——Diazomethane in ether was added to a stirred suspension of 5,7-dihydroxy-4-methylisocoumarin (**9**) (1.6 g) in ether–ethyl acetate (1 : 1, v/v, 100 ml), and the mixture was stirred at room temperature for 24 h. Dilute aq. acetic acid was added, and the mixture was washed with water, sat. aq. sodium bicarbonate and water, then dried over anhydrous sodium sulfate and concentrated *in vacuo*. The resultant solid was recrystallized from ethanol to give pale yellow needles (1.0 g, yield 54%). mp 169—170 °C. IR ν_{max}^{KBr} cm^{-1}: 1710 (C=O). ^{1}H-NMR ($CDCl_3$) δ: 2.4 (3H, d, J=1.0 Hz, CH_3), 3.95 (3H, s, OCH_3), 4.00 (3H, s, OCH_3), 6.84 (1H, d, J=2.0 Hz, 6-H), 6.96 (1H, d, J=1.0 Hz, 3-H), 7.45 (1H, d, J=2.0 Hz, 8-H). *Anal.* Calcd for $C_{12}H_{12}O_4$: C, 65.45; H, 5.49. Found: C, 65.59; H, 5.37.

4-Bromomethyl-5,7-dimethoxyisocoumarin (11)——5,7-Dimethoxy-4-methylisocoumarin (**10**) (5 g), *N*-bromosuccinimide (4 g) and α,α′-azobisisobutyronitrile (0.2 g) in carbon tetrachloride (150 ml) were refluxed for 2 h. The solution was cooled, succinimide was filtered off and the solvent was removed *in vacuo* to leave a solid, which was recrystallized from benzene to give 4-bromomethyl-5,7-dimethoxyisocoumarin as pale yellow needles (1.9 g, yield

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28%). mp 158—160 °C. IR ν_{max}^{KBr} cm^{-1}: 1720 (C=O). ^{1}H-NMR ($CDCl_3$) δ: 3.93 (3H, s, OCH_3), 3.95 (3H, s, OCH_3), 4.70 (2H, s, CH_2Br), 6.75 (1H, d, J=3.0 Hz, 6-H), 7.23 (1H, s, 3-H), 7.43 (1H, d, J=3.0 Hz, 8-H). *Anal.* Calcd for $C_{12}H_{11}BrO_4$: C, 48.19; H, 3.71; Br, 26.71. Found: C, 48.27; H, 3.74; Br, 26.81.

Ampicillin (3-Phthalidylidene)ethyl Ester Hydrochloride (14a)——Ampicillin trihydrate (1 g) was suspended in *N*,*N*-dimethylformamide (10 ml) and potassium bicarbonate (0.25 g) and benzaldehyde (0.5 ml) were added at 0 °C. The mixture was stirred at 0—5 °C for 3 h. Then the bromide (**6a**) (0.7 g) and potassium bicarbonate (0.29 g) were added, and the mixture was further stirred at 0—5 °C for 3 h. The reaction mixture was poured into ice-water and extracted with ethyl acetate (30 ml). The extract was washed with 10% aq. sodium chloride (20 ml × 3), dried over anhydrous magnesium sulfate and concentrated *in vacuo* to give a yellow syrup. The syrup was dissolved in acetonitrile (6 ml) and the pH of the solution was adjusted to 2.0 with 1 N HCl. The solution was stirred at 0—5 °C for 30 min, then water (20 ml) was added, and the mixture was concentrated under reduced pressure below 20 °C to remove acetonitrile. The aqueous layer was washed with ethyl acetate (10 ml × 3), then saturated with sodium chloride, and the separated oil was extracted with dichloromethane (30 ml × 2). The dichloromethane solution was washed with sat. sodium chloride and dried over anhydrous sodium sulfate. The dried organic layer was concentrated under reduced pressure until half the dichloromethane had been removed. Isopropyl alcohol (20 ml) was added and the solution was again concentrated under reduced pressure to give a colorless solid. The solid was collected by filtration and washed with cold isopropyl alcohol and ether (0.6 g, yield 48%). mp 145—155 °C (dec.). IR ν_{max}^{KBr} cm^{-1}: 1790—1740 (β-lactam, ester), 1690 (amide). ^{1}H-NMR (DMSO-d_6) δ: 1.36 (3H, s, 2-CH_3), 1.49 (3H, s, 2-CH_3), 4.40 (1H, s, 3-H), 5.07 (2H, d, J=8.0 Hz, $\underline{CH_2}CH=C$), 5.12 (1H, s, Ph–$\underline{CH}$–NH_2), 5.4—5.6 (2H, m, 5- and 6-H), 6.16 (1H, t, J=8.0 Hz, $CH_2\underline{CH}=C$), 7.3—8.1 (9H, m, arom. H), 8.60 (2H, NH_2), 9.30 (1H, d, NHCO). *Anal.* Calcd for $C_{26}H_{25}N_3O_6S$–HCl–H_2O: C, 55.56; H, 5.02; N, 7.48; S, 5.70. Found: C, 55.21; H, 4.86; N, 7.31; S, 5.93.

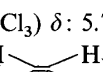
Ampicillin ester hydrochlorides were obtained by the same procedure, and their physical properties were as follows.

Ampicillin (3-(6-Chlorophthalidylidene))ethyl Ester Hydrochloride (14b)——(yield 34%). mp 141—147 °C (dec.). IR ν_{max}^{KBr} cm^{-1}: 1790 (β-lactam), 1725 (ester), 1690 (amide). ^{1}H-NMR (DMSO-d_6) δ: 1.37 (3H, s, 2-CH_3), 1.50 (3H, s, 2-CH_3), 4.40 (1H, s, 3-H), 5.04 (2H, d, J=8.0 Hz, $\underline{CH_2}CH=C$), 5.12 (1H, s, Ph–$\underline{CH}$–NH_2), 5.4—5.6 (2H, m, 5- and 6-H), 6.24 (1H, t, J=8.0 Hz, CH_2–$\underline{CH}=C$), 7.3—8.2 (8H, m, arom. H), 8.6 (2H, NH_2), 9.3 (1H, d, NHCO). *Anal.* Calcd for $C_{26}H_{24}ClN_3O_6S$–HCl–H_2O: C, 52.35; H, 4.56; N, 7.04; S, 5.38. Found: C, 52.01; H, 4.76; N, 6.77; S, 5.02.

Ampicillin (3-(6-Methoxyphthalidylidene))ethyl Ester Hydrochloride (14c)——(yield 36%). mp 143—150 °C (dec.). IR ν_{max}^{KBr} cm^{-1}: 1780 (β-lactam), 1745 (ester). 1690 (amide). ^{1}H-NMR (DMSO-d_6) δ: 1.36 (3H, s, 2-CH_3), 1.49 (3H, s, 2-CH_3), 3.91 (3H, s, OCH_3), 4.39 (1H, s, 3-H), 4.88 (2H, d, J=9.0 Hz, $\underline{CH_2}CH=C$), 5.12 (1H, s, Ph–$\underline{CH}$–NH_2), 5.4—5.6 (2H, m, 5- and 6-H), 6.01 (1H, t, J=9.0 Hz, CH_2–$\underline{CH}=C$), 7.3—8.0 (8H, m, arom. H), 8.8 (2H, NH_2), 9.3 (1H, NHCO). *Anal.* Calcd for $C_{27}H_{28}N_3O_7S$–HCl–H_2O: C, 54.68; H, 5.27; N, 7.09; S, 5.41. Found: C, 54.32; H, 5.45; N, 6.88; S, 5.26.

Ampicillin (3-(2-Thiophthalidylidene))ethyl Ester Hydrochloride (14d)——(yield 42%). mp 140—145 °C (dec.). IR ν_{max}^{KBr} cm^{-1}: 1780 (β-lactam), 1740 (ester), 1700—1680 (amide and thiolactone). ^{1}H-NMR (DMSO-d_6) δ: 1.35 (3H, s, 2-CH_3), 1.48 (3H, s, 2-CH_3), 4.40 (1H, s, 3-H), 4.97 (2H, d, J=7.0 Hz, $\underline{CH_2}CH=C$), 5.12 (1H, s, Ph–$\underline{CH}$–NH_2), 5.4—5.6 (2H, m, 5- and 6-H), 7.12 (1H, t, J=7.0 Hz, CH_2–$\underline{CH}=C$), 7.3—8.3 (9H, m, arom. H), 8.4—9.0 (2H, NH_2), 9.3 (1H, d, NHCO). *Anal.* Calcd for $C_{26}H_{25}N_3O_5S_2$–HCl–H_2O: C, 54.02; H, 4.88; N, 7.27; S, 11.09. Found: C, 53.78; H, 4.92; N, 7.03; S, 11.31.

Ampicillin (5,7-Dimethoxyisocoumarin-4-yl)methyl Ester Hydrochloride (14e)——(yield 38%). mp 155—159 °C (dec.). IR ν_{max}^{KBr} cm^{-1}: 1780 (β-lactam), 1740—1680 (lactone, ester and amide). ^{1}H-NMR (DMSO-d_6) δ: 1.34 (3H, s, 2-CH_3), 1.42 (3H, s, 2-CH_3), 3.83 (3H, s, OCH_3), 3.90 (3H, s, OCH_3), 4.35 (1H, s, 3-H), 5.00—5.30 (3H, m, $COO\underline{CH_2}$ and Ph–$\underline{CH}$–NH_2), 5.36—5.60 (2H, m, 5- and 6-H), 7.02, 7.15 (2H, d, J=2.0 Hz, arom. H), 7.35—7.40 (5H, m, arom. H), 7.55 (1H, s, $CH_2C=\underline{CH}$), 8.9 (2H, NH_2), 9.35 (1H, d, NHCO). *Anal.* Calcd for $C_{28}H_{29}N_3O_8S$–HCl–H_2O: C, 54.06; H, 5.19; N, 6.75; S, 5.15. Found: C, 53.89; H, 5.31; N, 6.52; S, 5.38.

3-(2-Carboxyphenyl)-1-propen-3-one (Vinyl Ketone) (15)——(*Z*)-3-(2-Bromoethylidene)phthalide (**6a**) (1.4 g) in acetone (5 ml) was added to sat. aq. sodium bicarbonate (50 ml), and the suspension was stirred at room temperature for 30 min. The pH was adjusted to 2.0 with 2 N HCl, then ethyl acetate (80 ml) was added and the organic layer was taken and washed with sat. sodium chloride. The solution was dried over anhydrous magnesium sulfate and concentrated *in vacuo*. The resultant syrup was recrystallized from benzene to give colorless columns (0.5 g, yield 50%). mp 94.5—95 °C. IR ν_{max}^{KBr} cm^{-1}: 1695, 1675 (C=O). ^{1}H-NMR ($CDCl_3$) δ: 5.78 (1H, d, J=17 Hz, ), 5.94 (1H, d, J=10 Hz, ), 6.62 (1H, dd, J=17 Hz and 10 Hz, ), 7.3—8.1 (4H, m, arom. H), 10.3 (1H, COOH). *Anal.* Calcd for $C_{10}H_8O_3$: C, 68.18; H, 4.58. Found: C, 68.27; H, 4.48.

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