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Preparation and Isomerization of 5-Epibenzylpenicillins

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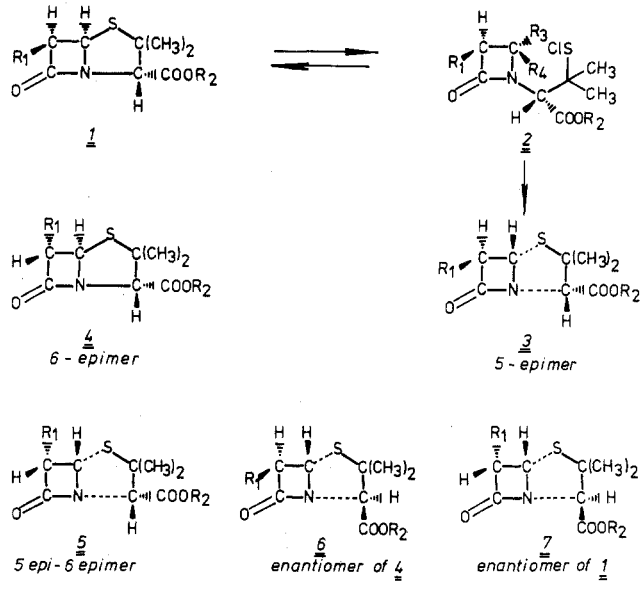
5-Epibenzylpenicillin methyl and benzyl ester were obtained by replacement of the phthalimido side chain of the corresponding 6-phthalimido-5-epipenicillanates, which were prepared from the natural isomer by the method of Kukolja. Base-catalyzed isomerization of silylated 5-epibenzylpenicillin methyl ester in the presence of triethylamine and DBN was investigated. With triethylamine, no epimerization was observed, whereas a mixture of 5 epimer and of the enantiomers of the 6 epimer and natural isomer was obtained when DBN was used as catalyst. These observations, which indicate an epimerization at position 3, are compared with the results of isomerizations of penicillanates with a different configuration and with other side chains. The general mechanism of epimerization of penicillanates is discussed. The antibiotic activity of the sodium salt of 5-epibenzylpenicillin, prepared by hydrogenolysis of the benzyl ester, is less than 0.1% of that observed for natural benzylpenicillin.

In the course of the study of the configuration of the four D-benzylpenicilloates,[1] 5-epibenzylpenicillin was needed. At that moment, only methyl and benzhydryl 6-phthalimido-5-epipenicillanate had been described.[2] Compound **3bx** was prepared by transformation of **1bx** into **2bxv** and **2bxw** by chlorinolysis of the S_1–C_5 bond, and by recyclization of **2bxw** with $SnCl_2$. This method has been applied for the preparation of several 5-epipenicillins having a cyclic or an acyclic imido side chain.[3]

The replacement of the phthalimido side chain by a phenylacetamido group seemed to be impossible in the penicillin series, because treatment with hydrazine causes cleavage of the β-lactam ring.[4] For this reason the procedure of Kukolja[2] was applied to the methyl ester of benzylpenicillin (**1ax**). Treatment of **1ax** with an equivalent amount of chlorine yielded a mixture of 25% of **2axv** and 75% of **2axw** (as determined by NMR). Attempts to cyclize this mixture with $SnCl_2$ gave several compounds of unknown structure but no 5 epimer could be detected. It should be noted that chlorinolysis of the S_1–C_5 bond in benzylpenicillin in the presence of an excess of chlorine has led to an olefinic azetidinone, which has been cyclized to a thiazabicycloheptenone.[5]

This negative result prompted an attempt to remove the phthaloyl group with hydrazine in dimethylformamide, a method which has been used successfully in the cephalosporin series.[6] Application of this procedure to **3bx** gave methyl 6-amino-5-epipenicillanate (**3cx**) in 75% yield. The success of this reaction is probably due to the greater stability of the β-lactam in the 5 epimer, because treatment of **3bx** with hydrazine in dioxane also gave **3cx**, albeit in lower yield, whereas reaction of **1bx** with hydrazine in dimethylformamide still caused cleavage of the β-lactam ring. By reaction of **3cx** with phenylacetyl chloride, 5-epibenzylpenicillin methyl ester (**3ax**) was obtained.

In order to prepare a salt of 5-epibenzylpenicillin, we applied the same scheme to the benzyl ester. Benzyl 6-phthalimidopenicillanate (**1by**), obtained by esterification of 6-phthalimidopenicillanic acid[7] with benzyl bromide in dimethylformamide, was treated with chlorine as described for the methyl ester.[2] The resulting 2-chloro-α-(1-chlorothio-1-methylethyl)-4-oxo-3-phthalimido-1-azetidineacetate was obtained in good yield but it could not be crystallized although it contained practically only the trans isomer (**2byw**). Treatment of this material with $SnCl_2$ afforded **3by** in 85% total yield. Since the presence of water greatly affects the stereoselectivity of the reductive cyclization,[2] it is necessary to perform this step with anhydrous $SnCl_2$ under strictly anhydrous conditions, in order to obtain a high yield of **3by**, because **1by** cannot be separated from the desired isomer **3by** by column chromatography. The presence of appreciable



a. $R_1 = C_6H_5CH_2CONH$ -
b. $R_1 = C_6H_4(CO)_2N$ -
c. $R_1 = Cl^{\ominus}H_3N^{\oplus}$ -
d. $R_1 = C_6H_5OCH_2CONH$ -

x. $R_2 = CH_3$ -
y. $R_2 = C_6H_5CH_2$ -
z. $R_2 = Na$

v. $R_3 = H$, $R_4 = Cl$
w. $R_3 = Cl$, $R_4 = H$

Table I. Optical Rotations and NMR Data[a] for Epimeric Penicillanates

Configuration	Product	^{1}H NMR values 3-H	5-H (J, Hz)	6-H (J, Hz)	$[\alpha]_D$ in acetone	Ref
3*S*,4*S*,5*R*,6*R*	**1ax**	4.37	5.48 (4)	5.64 (4)	+246	10
	1ay	4.40	5.50 (4)	5.61 (4)	+218	*b*
	1bx	4.68	5.60 (4.5)[c]	5.68 (4.5)[c]	+275 +279[d]	11, 12
3*S*,4*S*,5*R*,6*S*	**4ax**	4.44	5.12 (1.8)	5.01 (1.8)	+191	10
	4ay	4.49	5.13 (1.5)	5.03 (1.5)	+149	13
	4bx	4.63	5.57 (2.1)[c]	5.39 (2.1)[c]	+207 +207[d]	11, 12
3*S*,4*R*,5*S*,6*S*	**5ax**	3.78	5.21 (4)	5.51 (4)	−236	1
3*S*,4*R*,5*S*,6*R*	**3ax**	3.69	5.03 (2)	4.78 (2)	−164	*e*
	3ay	3.70	5.00 (2)	4.80 (2)	−119	*e*
	3bx	3.90	5.56 (2)[f]	5.42 (2)[f]	−192[d]	2

[a] In parts per million in $CDCl_3$. [b] E. Roets, unpublished results. [c] Assignment based on deuterium exchange.[12] [d] In chloroform. [e] This publication. [f] Assignment based on the inversion of values of chemical shifts for C-5–C-6 cis relative to C-5–C-6 trans, as observed for the pairs **1ax–4ax, 1ay–4ay, 1bx–4bx,** and **5ax–3ax.**

(more than 5%) quantities of **1by** causes difficulties in the next step. The phthaloyl group of **3by** was removed with hydrazine as described for the methyl ester. Benzyl 6-amino-5-epipenicillanate hydrochloride (**3cy**) could not be isolated but it was immediately phenylacetylated. 5-Epibenzylpenicillin benzyl ester (**3ay**) was obtained in a somewhat lower yield (35%) than the methyl ester (60%). Hydrogenation of **3ay** in the presence of Pd/C in methanol–water containing $NaHCO_3$ gave the sodium salt of 5-epibenzylpenicillin (**3az**). Its activity against *Staphylococcus aureus* ATCC 6538P is less than 0.1% of that observed for natural benzylpenicillin. It is not hydrolyzed by penicillinase of *Bacillus cereus*.

Chemical shift values and optical rotations of four epimeric penicillins are shown in Table I. The trans orientation of the protons on C-5 and C-6 in compounds **3** and **4** is indicated by small J values and by a large shielding of the C-6 proton, causing this proton to resonate at higher field than the C-5 proton, while the converse is true for the cis isomers (**1** and **5**). For the two isomers with 5*S* configuration (**3** and **5**) a strong diamagnetic shift of the C-3 proton was observed, which is indicative of a cis relationship between 5-H and 3-H. This effect is also observed for carboxythiazolidine derivatives[8] and for phthalimidopenicilloates.[1]

An attempt was made to calculate the contribution of the different chiral centers to the optical rotation, using a computation method analogous to that used for the isorotation rules of Hudson in the carbohydrate series.[9] The contribution of the centers N-4 and C-5 can be estimated as ±209 by the following calculation:

$$[\alpha]_D \text{ of } \mathbf{lax} - [\alpha]_D \text{ of } \mathbf{3ax} = 2\,[\alpha]_D \text{ of } 4S,5R = +410$$

$$[\alpha]_D \text{ of } \mathbf{5ax} - [\alpha]_D \text{ of } \mathbf{4ax} = 2\,[\alpha]_D \text{ of } 4R,5S = -427$$

Similar calculations give ±32 for C-6 and ±9 for C-3. In order to check these values, it would be necessary to perform the same calculations for the benzyl esters and for the methyl phthalimidopenicillanates. Only a partial check is possible because only three isomers are available, which gives one pair of epimers. Nevertheless, rather similar values were obtained for the different chiral centers. At any rate, this calculation indicates that the chiral centers N-4 and C-5 provide a predominant contribution to the optical rotation.

In order to extend these calculations, an attempt was made to prepare the fourth isomer of the methyl phthalimidopenicillanates, i.e., **5bx.** It has been shown that penicillins with this side chain can be readily isomerized with base, and in particular, that **1bx** is transformed into **4bx.**[11,14]

Treatment of **3bx** with triethylamine gave only starting product. When **3bx** was treated with a stronger base, 1,5-diazabicyclo[4.3.0]non-5-ene (DBN), a large amount of starting material was recovered after a short reaction time, whereas a more prolonged reaction resulted in extensive decomposition. At any rate, compound **5bx** was not obtained from this isomerization, but surprisingly, **6bx,** which is the enantiomer of **4bx,** was isolated from the reaction mixture in a yield of 5–20% depending on the reaction conditions. The preparation of **5bx** was also attempted by chlorinolysis of **4bx,** and ring closure with $SnCl_2$. Again only **4bx** was recovered. All these experiments indicate that phthalimidopenicillanates with hydrogen on C-5 and C-6 in trans configuration, i.e., **3bx, 4bx,** and **6bx,** are the most favored ones, and that an exo stereochemistry of the C-3 substituent (**6bx**) is preferred to an endo position (**3bx**).

It has also been observed that penicillins with a phenylacetamido side chain can be isomerized with base, provided that the amino group is trimethylsilylated.[10] When **3ax** was silylated with *N,O*-bis(trimethylsilyl)acetamide (BSA) and then treated with DBN for 20 min at room temperature, a mixture of **3ax, 6ax,** and **7ax** was obtained. Prolonged reaction times (1–5 h) resulted in the disappearance of **3ax,** but also a lower yield was obtained for **6ax** and **7ax,** though they were still present in the same ratio as after a short reaction time. Using triethylamine as base, and a reaction time of up to 50 h, only starting product (**3ax**) was recovered. It should be noted that in no case formation of **5ax** or of 1,4-thiazepine (IV) was observed.

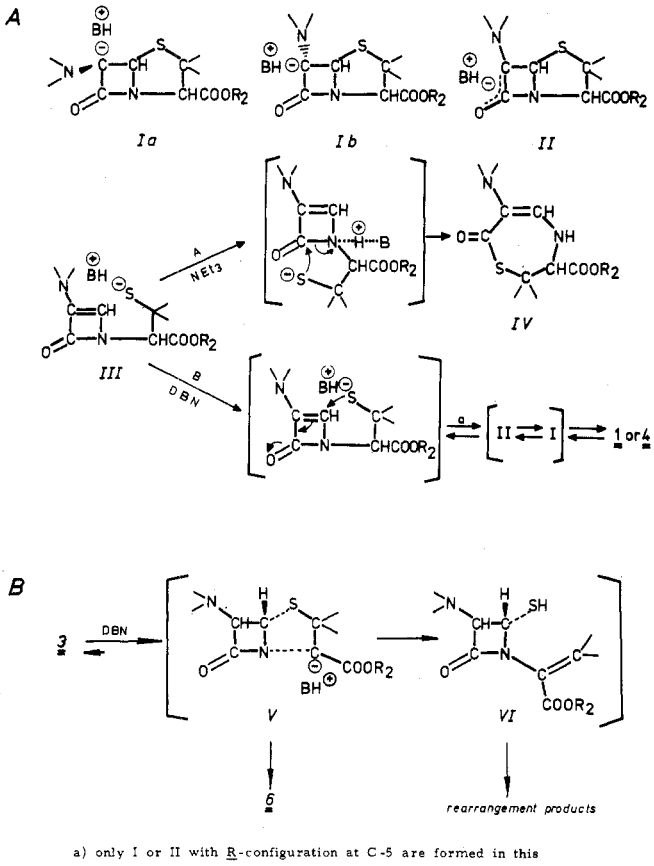
As can be seen in Table II, in which the results of our experiments together with those of other closely related isomerizations are summarized, the behavior of 5-epipenicillin upon base-catalyzed epimerization is clearly different from that of penicillanates having the natural configuration at C-5 (**1** or **4**). Using a strong base like DBN, a penicillin with a phthalimido side chain like **1bx** is transformed almost completely into the 6 epimer **4bx,** whereas a 1:3 ratio of normal to 6 epimer is obtained from a penicillin with a phenyl- (**1ax**) or a phenoxyacetamido (**1dy**) side chain. The strong base causes a fast isomerization, and almost the same equilibrium mixture can be isolated in good yield from either **1** or **4.** Prolonged treatment with DBN results, however, in substantial degradation, and in all cases lower yields of penicillanates are obtained. For these isomerizations, ion pairs Ia and Ib or the enolate II have been proposed as intermediates,[10,14,15] and the free-energy difference between **1** and **4,** which is largely dependent on the nature of the side chain, was considered as the driving force for the predominant formation of 6 epimer or trans isomer. With triethylamine as base, the epimerization

Table II. Base-Catalyzed Isomerization of Penicillanates

Product	Reagent	Reaction conditions[a]	Results	Ref
1bx	NEt_3, 3 equiv	12 h	40% **4bx**, <1% **1bx**, 28% IV	11
		12h	38% **4bx**, ±1% **1bx**, 25% IV	*b*
	DBN, 1 equiv	10 min[c]	82% **4bx**[d,e]	*b*
		1.5 h[c]	50% **4bx**[d,e]	
4bx	NEt_3, 3 equiv	72 h	Large amount **4bx**, 4% IV	11
		240 h	68% **4bx**, 12% IV	11
3bx	NEt_3, 3 equiv	50 h	**3bx** unchanged	*b*
	DBN, 1 equiv	2 min[c]	73% **3bx**, 5% **6bx**[e,f]	*b*
		10 min	14% **3bx**, 18% **6bx**[e,f]	*b*
		1 h	3% **3bx**, 16% **6bx**[e,f]	*b*
1dy	BSA; NEt_3, 5 equiv	24 h	58% (**4dy** + **1dy**) 2:1, 19% IV	10
	BSA; DBN, 1 equiv	10 min	>80% (**4dy** + **1dy**) 3:1	10
4dy	BSA; NEt_3, 5 equiv	24 h	64% (**4dy** + **1dy**) 7:3, 18% IV	10
4dx	BSA; DBN, 1 equiv	10 min	>80% (**4dx** + **1dx**) 3:1	10
1ax	BSA; NEt_3, 5 equiv	24 h	76% (**4ax** + **1ax**) 5:95, 8% IV	*b*
		48 h	60% (**4ax** + **1ax**) 1:5, 25% IV	*b*
	BSA; DBN, 1 equiv	15 min	75% (**4ax** + **1ax**) 3:1[d,e]	*b*
		5 h	20% (**4ax** + **1ax**) 4:1[d,e]	*b*
4ax	BSA; DBN, 1 equiv	10 min	89% (**4ax** + **1ax**) 3:1	10
3ax	BSA; NEt_3, 5 equiv	50 h	>90% **3ax**[e]	*b*
	BSA; DBN, 1 equiv	2 min	>90% **3ax**, ±3% **6ax**[f]	*b*
		20 min	50% **3ax**, 22% **6ax**, 5% **7ax**[e,f]	*b*
		45 min	18% **3ax**, 16% **6ax**, 4% **7ax**[e,f]	*b*
		5 h	<5% **3ax**, 8% **6ax**, 2% **7ax**[e,f]	*b*

[a] In CH_2Cl_2 solution and at room temperature. [b] Results of the present study. [c] Experiment at 0 °C. [d] No compound corresponding to **3** was detected by NMR. [e] No thiazepine IV was detected by TLC. [f] No compound corresponding to **5** was detected by NMR.

is much slower, and an additional compound, 1,4-thiazepine (IV), is formed in an amount increasing with the reaction time. Its formation has been explained by assuming an enethiolate III as a secondary intermediate, produced in a rate-determining step from the initial intermediate I or II by a β-elimination mechanism.



a) only I or II with R-configuration at C-5 are formed in this stereoselective step.

In our opinion this intermediate is probably also formed during the isomerization of **1** or **4** with DBN, although I or II are the predominant intermediate species. We suggest that III, which rearranges by nucleophilic attack on the β-lactam amide to 1,4-thiazepine IV in the presence of triethylamine (pathway A), is not transformed into IV during reaction with a strong base, but leads to degradation products or goes back to penicillanates (pathway B). In this case, the β-lactam amide is not opened by $^-SC(CH_3)_2$–, because the strong base prevents protonation of the bridgehead nitrogen, which is considered to be an essential step for the substitution reaction due to the poor leaving character of an uncharged secondary amine group. Since in no case 5 epimer (**3**) could be detected during the isomerization **1** ⇄ **4**, we also suggest that in pathway B, only penicillanates are produced with natural configuration at C-5, like **1** or **4**. The high stereoselectivity of this addition step may be due to the electrostatic repulsion between S^- and the C-3 carbomethoxy group, forcing the two groups into an anti position before the addition takes place.

In these isomerization reactions, removal of the C-6 hydrogen is considered as the first step, in accordance with the ElcB mechanism proposed by Ramsay and Stoodley.[15] Consequently, since a larger activation energy is expected for the deprotonation at the endo side of the molecule, the epimerization should occur at a slower rate for penicillins with trans-oriented azetidinone protons like **3** and **4**. It can be seen in Table II that this effect is, as anticipated, more pronounced with a weak base like triethylamine than with DBN.

Notable differences of rate of isomerization for **1bx** ⇄ **4bx**, **1dy** ⇄ **4dy**, and **1ax** ⇄ **4ax** under the influence of triethylamine are observed (Table II). They probably are related to the acidity of the C-6 hydrogen, which depends on the structure of the side chain.

We consider that the results obtained for the epimerization of **3ax** or **3bx** are not consistent with the mechanistic scheme as outlined above. In particular, the formation of compounds **6** and **7** upon treatment of **3** with DBN requires the inversion of the configuration of C-3, which, in principle, may be in-

duced by removal of the 3-H atom (V). However, epimerization at position 6 (Ia) which may result in the formation of the 5-epi-6 epimer (**5**), cannot be excluded on the basis of our results, since the absence of compound **5** in the reaction mixture can be explained either by the fact that it is unstable under the reaction conditions used, or that it is not formed as a result of a still larger free-energy difference between **3** and **5** than between **4** and **1**. Since in 5-epipenicillins 3-H has the exo stereochemistry, whereas 6-H as well as the 3 substituent are both at the endo side of the molecule, removal of the 3 hydrogen may become competitive if not preferred to the deprotonation at C-6, although this latter hydrogen is intrinsically more acidic. The negative free-energy difference between **6** and **3**, which is ascribed to the relief of compressional interaction between the C-3 substituent and the azetidinone ring in the process **3** → **6**, is considered as the driving force for the preferential formation of compound **6** upon reprotonation. This isomer, which has the 3*R*,5*S*,6*R* configuration, is the enantiomer of the 6 epimer (**4**) having the 3*S*,5*R*,6*S* stereochemistry, and it is thus expected to undergo base-catalyzed epimerization at position 6, resulting in the formation of compound **7** which is the enantiomer of **1**. In the epimerization of **3ax** with DBN, the isomers **6ax** and **7ax** are obtained in a ratio of 4:1, whereas in the epimerization of methyl 6-phthalimido-5-epipenicillanate (**3bx**) no **7bx** was formed. A similar trend was already mentioned in our discussion of the isomerization **1** ⇄ **4**, where a reduction in the bulkiness of the C-6 substituent also was accompanied with an increase of the 6*β* isomer in the equilibrium mixture.

Base-induced removal of the 3-H atom of penicillanoyl derivatives has been discussed recently by Stoodley.[18] The deprotonation was considered as the slow step in the formation of a series of rearrangement products involving the resulting secoceph-3-em derivative (VI). In accord with the observation[18] that there was little evidence for the secoceph-3-em → penicillanoyl transformation, we suggest that a deprotonation–reprotonation mechanism via intermediate V is operative in the epimerization of 5-epipenicillins at position 3, and that intermediate VI may account for the substantial loss of material observed during these isomerizations.

It should be noted that the formation of about 20% of the enantiomer of 5*R*,6*S*-D-benzylpenicilloate during basic hydrolysis of 5-epibenzylpenicillin methyl ester[1] may be rationalized in a similar way, by assuming partial epimerization of the 5-epipenicillanate at position 3 before the hydrolysis took place.

Experimental Section

General experimental details are given in the preceding paper.[1] For TLC, the following mobile phases were used: I, C_6H_6–Me_2CO, 80:20; II, *n*-BuOAc–*n*-BuOH–H_2O–MeOH–HOAc, 80:15:24:5:40; III, Me_2CO–HOAc, 95:5.

Benzyl 6-Phthalimidopenicillanate (1by). To a solution of 25 g (72 mmol) of 6-phthalimidopenicillanic acid[7] in 130 ml of dimethylformamide was added 10.1 ml (72 mmol) of freshly distilled triethylamine and 11 ml (90 mmol) of freshly distilled benzyl bromide. The mixture was stirred at room temperature for 4 h and then poured into 700 ml of ice–water under vigorous stirring. The aqueous suspension was extracted with two 250-ml portions of chloroform and the combined organic layer was successively washed with 200 ml of $NaHCO_3$ (1%) and H_2O, dried (Na_2SO_4), and evaporated. The yellow oil was crystallized from 200 ml of ether, yielding 34 g (77%) of **1by**, mp 133–136 °C. Recrystallization from acetone–ether raised the melting point to 138–140 °C; TLC (system I) R_f 0.84; $[\alpha]^{25}D$ +253° (*c* 1, $CHCl_3$); *m/e* 436; ir (KBr) 1780 (*β*-lactam), 1795, 1730 (phthalimide), 1750, 1210 cm^{-1} (ester); NMR ($CDCl_3$) *δ* 1.40 (s, CH_3), 1.74 (s, CH_3), 4.62 (s, 3-H), 5.10 (s, $CH_2C_6H_5$), 5.46 (d, *J* = 4 Hz, 5-H), 5.54 (d, *J* = 4 Hz, 6-H), 7.23 (s, C_6H_5), 7.63 (m, C_6H_4).

Benzyl 6-Phthalimido-5-epipenicillanate (3by). Benzyl 6-phthalimidopenicillanate (26.16 g, 60 mmol), dissolved in dry methylene chloride (400 ml) and carbon tetrachloride (200 ml), was treated at room temperature with an equimolecular amount of chlorine (60 mmol, titrated), dissolved in carbon tetrachloride, for 30 min. The solvent was evaporated, and the residual oil was dried over P_2O_5 in a vacuum desiccator for 2 h. From the NMR spectrum it could be deduced that it contained mainly **2byw** with only a small amount of **2byv**. To the dried product, dissolved in anhydrous tetrahydrofuran (200 ml), 12.02 g (62 mmol) of anhydrous[16] $SnCl_2$ was added, and the mixture was stirred at room temperature for 2 h. The solvent was evaporated and the residue was dissolved in ethyl acetate, washed with three volumes of ice–water, and dried (Na_2SO_4). The product was purified on silica gel using benzene–acetone (95:5) as eluent, and the fractions containing the desired compound were dissolved in benzene and freeze dried, yielding 23.0 g (85.1%) of **3by**: mp 66–68 °C; TLC (system I) R_f 0.82; $[\alpha]^{25}D$ −104° (*c* 1, $CHCL_3$); *m/e* 436; ir (KBr) 1780 (broad peak, *β*-lactam and phthalimide), 1725 (broad peak, phthalimide and ester), 1200 cm^{-1} (ester); NMR ($CDCl_3$) *δ* 1.35 (s, CH_3), 1.60 (s, CH_3), 3.85 (s, 3-H), 5.16 (s, $CH_2C_6H_5$), 5.35 (d, *J* = 2 Hz, 6-H), 5.48 (d, *J* = 2 Hz, 5-H), 7.30 (s, C_6H_5), 7.75 (m, C_6H_4).

Methyl 6-Phthalimido-5-epipenicillanate (3bx). To a suspension of 18 g (50 mmol) of **1bx**[7] in 400 ml of carbon tetrachloride was added 70 ml of a solution of Cl_2 (50 mmol, titrated), in carbon tetrachloride, and the mixture was stirred for 30 min at room temperature. The resulting solution was evaporated to a yellow oil, and upon addition of anhydrous ether 12.47 g (58%) of a crystalline product was recovered. From the NMR spectrum [($CDCl_3$) *δ* 1.74 (s, two CH_3), 3.88 (s, OCH_3), 4.59 (s, 3-H), 5.55 (d, *J* = 2 Hz, 5-H or 6-H), 6.04 (d, *J* = 2 Hz, 6-H or 5-H), 7.85 (m, C_6H_4)], which is entirely in agreement with the published values,[2] it was established that the crystalline product was the trans isomer **2bxw**. No more crystalline product could be obtained from the residue (36%) obtained by evaporation of the filtrate and which consisted of a mixture of the trans and the cis isomer. The azetidinone **2bxw** (12.47 g, 29 mmol) was treated with anhydrous $SnCl_2$ (6.06 g, 31 mmol) in 200 ml of anhydrous tetrahydrofuran at room temperature for 2 h. Upon concentration of the mixture, a white precipitate formed, which was removed by filtration. The filtrate was evaporated to dryness and the residue crystallized from acetone–ether, yielding 7.81 g (21.7 mmol, 75%) of **3bx**. The residue, obtained before by evaporation of the filtrate, was also treated with $SnCl_2$ and the resulting mixture of **1bx** (R_f 0.79, system I) and **3bx** (R_f 0.72) was separated on silica gel using benzene–acetone (98:2) as eluent. From the fractions containing the desired compound, another 2.47 g of **3bx** was recovered. The total yield for the transformation of **1bx** into **3bx** amounted to 10.28 g (57%). Recrystallization from acetone afforded a pure sample: mp 174–176 °C; TLC (system I) R_f 0.75; $[\alpha]^{25}D$ −182° (*c* 1, $CHCl_3$); ir (KBr) 1780 (*β*-lactam), 1780, 1715 (phthalimide), 1745 and 1210 cm^{-1} (ester); NMR ($CDCl_3$) *δ* 1.50 (s, CH_3), 1.70 (s, CH_3), 3.82 (s, OCH_3), 3.90 (s, 3-H), 5.45 (d, *J* = 2 Hz, 6-H), 5.56 (d, *J* = 2 Hz, 5-H), 7.85 (m, C_6H_4). Kukolja[2,3] gives mp 174–175 °C and $[\alpha]D$ −192° ($CHCl_3$).

5-Epibenzylpenicillin Methyl Ester (3ax). To a solution of methyl 6-phthalimido-5-epipenicillanate (2.880 g, 8 mmol) in freshly distilled dimethylformamide (30 ml), cooled to −10 °C, was added 8 ml of a 1 M solution of hydrazine hydrate (0.48 ml in 10 ml) in dimethylformamide. After the mixture was stirred at room temperature for 30 min, the phthalhydrazide complex was decomposed by addition of 9.5 ml of 1 NHCl, and the solvent was evaporated under reduced pressure at 40 °C. The resulting oil was taken up in 30 ml of water under vigorous stirring, and the insoluble phthalhydrazide was removed by filtration, washed, and discarded. The combined aqueous phase was evaporated to an oil and dried over P_2O_5 under vacuum for 2 h. Crystallization from absolute methanol and ether afforded 1.600 g (75%) of the hydrochloride of methyl 6-amino-5-epipenicillanate (**3cx**): mp 157 °C dec; TLC (system II) R_f 0.38; $[\alpha]^{25}D$ −210° (*c* 0.5, MeOH); ir (KBr) 2940 (NH_3^+), 1780 (*β*-lactam), 1730, 1195 cm^{-1} (ester); NMR (D_2O–DSSA) *δ* 1.40 (s, CH_3), 1.63 (s, CH_3), 3.76 (s, OCH_3), 4.03 (s, 3-H), 4.70 (d, *J* = 2 Hz, 6-H or 5-H), 5.37 (d, *J* = 2 Hz, 5-H or 6-H). This salt was then N-phenylacetylated by treatment of an ice-cooled suspension of 1.600 g (6 mmol) of **3cx** in 45 ml of anhydrous methylene chloride with 6 ml of a solution containing 2.1 ml of triethylamine in 13 ml of methylene chloride, followed by simultaneous and dropwise addition of 7 ml of the triethylamine solution and of a phenylacetyl chloride solution (1.3 ml in 8.7 ml of methylene chloride). After the addition was complete, stirring was continued for 1 h, and the resulting methylene chloride solution was successively washed with 1 N HCl (50 ml), $NaHCO_3$ 5% (2 × 50 ml), and H_2O (3 × 60 ml). The organic layer was dried (Na_2SO_4) and purified by column chromatography on silica gel using benzene–acetone (97:3) as eluent, yielding after crystallization from ether 1.650 g (79%) of **3ax**: mp 143–145 °C; TLC (system I) R_f 0.52; $[\alpha]^{25}D$ −164° (*c* 0.5, acetone); *m/e* 348 (M^+), 274 [$M - (CH_3)_2CS]^+$;[17] ir (KBr) 3260, 1645, 1560

(amide), 1790 (β-lactam), 1740, 1205 cm^{-1} (ester); NMR ($CDCl_3$) δ 1.38 (s, CH_3), 1.55 (s, CH_3), 3.50 (s, CH_2), 3.69 (s, 3-H), 3.71 (s, OCH_3), 4.78 (dd, J = 2 and 7 Hz, 6-H), 5.03 (d, J = 2 Hz, 5-H), 7.20 (br, -CONH- and C_6H_5).

5-Epibenzylpenicillin Benzyl Ester (3ay). Benzyl 6-phthalimido-5-epipenicillanate (4.36 g, 10 mmol) in 10 ml of dimethylformamide was treated with an equimolecular amount of hydrazine hydrate as described for the methyl ester. Decomposition of the resulting phthalhydrazide complex with 1 N HCl (11 ml) could not yield crystalline **3cy.** The product was immediately transformed to the *N*-phenylacetyl derivative by reaction with phenylacetyl chloride in the presence of triethylamine. The reaction product was purified by column chromatography on silica gel using benzene–acetone (97:3) as eluent, and crystallized from CCl_4 or from benzene–ether, yielding 1.3 g (30.6%) of **3ay:** mp 90–91 °C; TLC (system I) R_f 0.68; $[\alpha]^{25}D$ −119° (*c* 1, acetone); *m/e* 424 (M^+), 350 [M − $(CH_3)_2CS]^+$;[17] ir (KBr) 3320, 1650, 1520 (amide), 1770 (β-lactam), 1745 and 1200 cm^{-1} (ester); NMR ($CDCl_3$) δ 1.30 (s, CH_3), 1.52 (s, CH_3), 3.50 (s, CH_2CO), 3.70 (s, 3-H), 4.80 (dd, J = 2 and 7 Hz, 6-H), 5.00 (d, J = 2 Hz, 5-H), 5.13 (s, $CH_2C_6H_5$), 6.60 (d, J = 7 Hz, NHCO), 7.20 (s, $C_6H_5CH_2CO$–), 7.25 (s, $C_6H_5CH_2O$–).

5-Epibenzylpenicillin Sodium Salt (3az). A solution of **3ay** (200 mg, 0.47 mmol) in 5 ml of distilled water and 20 ml of methanol, containing 1 equiv of $NaHCO_3$ (39.5 mg), was hydrogenated over 10% Pd/C (200 mg) for 3 h at room temperature and at a pressure of 3.5 kg/cm^2. The catalyst was filtered off, and the methanol was evaporated. After addition of water (10 ml) and ethyl acetate (20 ml), the mixture was acidified to pH 3 with 0.1 *N* HCl. The layers were separated, and the water was extracted twice with ethyl acetate. The combined organic layer was washed with H_2O (20 ml), and after addition of 40 ml of H_2O adjusted to pH 6.8 with 0.1 *N* NaOH. The aqueous layer was freeze dried, yielding 0.100 g (60%) of the sodium salt of 5-epibenzylpenicillin: mp 136–138 °C; TLC (system III) R_f 0.56; $[\alpha]^{25}D$ −108° (*c* 1, H_2O); ir (KBr) 3280, 1660, 1540 (amide), 1750 (β-lactam), 1390 cm^{-1} (carboxylate); NMR (D_2O–DSSA) δ 1.44 (s, CH_3), 1.57 (s, CH_3), 3.63 (s, 3 protons, 3-H and CH_2–), 4.82 (d, J = 2 Hz, 6-H or 5-H), 5.10 (d, J = 2 Hz, 5-H or 6-H), 7.31 (s, C_6H_5).

Anal. Calcd for $C_{16}H_{17}N_2O_4SNa$: C, 53.91; H, 4.80; N, 7.86. Found: C, 53.64; H, 4.75; N, 7.61.

As in the case of 6-epibenzylpenicillin,[10] no hydrolysis was observed when sodium 5-epibenzylpenicillin was treated with an aqueous penicillinase solution (Penase Leo Lot 80096) at pH 7.

Epimerization of 5-Epibenzylpenicillin Methyl Ester (3ax). A solution of **3ax** (0.696 g, 2 mmol) in anhydrous methylene chloride (5 ml) was treated with BSA (1.2 ml, 5 mmol), and stirred for 100 min at room temperature under an atmosphere of nitrogen. The reaction mixture was cooled to 0 °C, DBN (0.24 ml, 2 mmol) was added, and stirring was continued for 20 min at room temperature. The solution was then poured into a mixture of ice–water (10 ml) and 1 N HOAc (2 ml), and shaken for 5 min. The layers were separated, the aqueous phase was extracted with two volumes of methylene chloride, and the combined organic layer was washed with H_2O and dried (Na_2SO_4). The solvent was evaporated, and the residue was chromatographed on silica gel (15 g) using benzene–acetone (97:3) as eluent. A first fraction of the eluate consisted of a mixture (50 mg) of two isomers, which were found by TLC and NMR to be identical with natural (R_f 0.64) and 6-epibenzylpenicillin methyl ester (R_f 0.62) in a ratio 7:3 (NMR). The [α]D of this mixture was −194°. From the second fraction of the eluate 0.140 g (20%) of 6-epibenzylpenicillin methyl ester was obtained as a crystalline product after treatment with ether. The $[\alpha]^{25}D$ −184° (*c* 1, acetone) indicated that this product was the enantiomer of 6-epibenzylpenicillin methyl ester with structure **6ax.** As the [α]D of the mixture from the first eluate is more levorotatory, it must be concluded that natural penicillin methyl ester (with [α]D +246°) is not present, but also must be the enantiomer with structure **7ax.** From the third fraction of the eluate 0.350 g (50%) of the starting product **3ax** (R_f 0.52) was recovered. When the reaction with DBN was carried out for 45 min, only 18% of starting material **3ax,** 16% of **6ax,** and 4% of **7ax** were recovered, and after a reaction time of 5 h, the yields of **7ax** and **6ax** were only 2 and 8%, respectively. More than 90% of **3ax** was recovered unchanged after a reaction time of 2 min with DBN, or after 50 h with 5 equiv of triethylamine as base.

Epimerization of Benzylpenicillin Methyl Ester (1ax). Solutions of **1ax**[10] (0.696 g, 2 mmol) in anhydrous methylene chloride (5 ml) were treated with BSA (1.2 ml, 5 mmol) for 2 h at room temperature. The silylated penicillanate was then isomerized with DBN (1 equiv) for 15 min and for 5 h, or with triethylamine (5 equiv) for 24 and 48 h. The reaction mixtures were worked up as described for the 5 epimer, and were analyzed by NMR after purification by column chromatography on silica gel using benzene–acetone (98:2) as eluent. The results are collected in Table II.

Epimerization of Methyl 6-Phthalimidopenicillanate (1bx) and Its 5 Epimer (3bx). Methyl 6-phthalimidopenicillanate (**1bx,** 0.360 g, 1 mmol) was dissolved in anhydrous methylene chloride (5 ml) and treated with DBN (0.12 ml, 1 mmol) at 0 °C for 10 min and for 90 min. After the mixtures were worked up, **4bx** was obtained in a crystalline state from methanol in a yield of 82 and of 50%, respectively. Similarly, methyl 6-phthalimido-5-epipenicillanate[2] (**3bx**) was isomerized with DBN and with triethylamine. The reaction mixtures were worked up and separated by column chromatography on silica gel. The results, which are collected in Table II, were based on optical rotation, TLC, and NMR spectroscopy. Compound **6bx,** which was isolated from the epimerization mixture with DBN, was identified as the enantiomeric form of the 6 epimer **4bx,** since all physical constants (TLC, NMR, ir, *m/e*) except the optical rotation, which was negative [$[\alpha]^{25}D$ −199 (*c* 1, acetone)], were identical with those of **4bx.**

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Registry No.—**1ax,** 653-89-4; **1bx,** 19788-65-9; **1by,** 59034-26-3; **2bxw,** 34734-72-0; **3ax,** 59034-27-4; **3ay,** 59034-28-5; **3az,** 59034-29-6; **3bx,** 34716-53-5; **3by,** 59034-30-9; **3cx,** 59034-31-0; 6-phthalimidopenicillanic acid, 20425-27-8; benzyl bromide, 100-39-0.

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