BENZYLPCNILLNRC ACID AS AN INTERMEDIATE IN THE SYNTHESIS OF BENZYLPCNICILLN (PCNICILLN G)*

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During the war several English and American laboratories participated in a study of the structure and synthesis of penicillin (1, 2). In this work it was noted that when the methyl ester of benzylpenicillin (I) (see Fig. 1) was treated with mercuric chloride in ether solution and the resulting mercury derivative (II) was decomposed with hydrogen sulfide, a neutral, amorphous product was obtained. This crude product possessed an absorption peak in the ultraviolet at 320 mp ($E_M = 13,700$) and was degraded by sodium hydroxide to the sodium salt of 2-benzyl-4-hydroxymethylene-5(4)-oxazolone. For these and other reasons the product was assigned structure (III) and was given the trivial name methyl $\alpha$-benzylpenicillenate (3).

After the close of the war, studies were continued in this Laboratory on the synthesis and the mechanism of synthesis of benzylpenicillin from $\alpha$-penicillamine hydrochloride (V) and 2-benzyl-4-methoxymethylene-5(4)-oxazolone (IV) (4, 5). It was found that when the two compounds were allowed to react in pyridine containing triethylamine, a biologically inactive, amorphous product was obtained. However, when this product was heated in pyridine containing pyridinium chloride, benzylpenicillin was formed in small yield. The intermediate product possessed an ultraviolet absorption spectrum similar to that described for natural methyl $\alpha$-benzylpenicillenate (III). So far attempts to isolate the intermediate compound in crystalline form have not been successful. During the course of fractionation studies on this product, the formation of $\alpha$-benzylpenillic acid (VI) was encountered (6). This $\alpha$-benzylpenillic acid was identical with that formed by rearrangement of benzylpenicillin.

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1 Throughout this paper $E_M$ is the molar absorption coefficient and is equal to $D/cd$, where $D$ is $\log I_0/I$, $c$ is concentration in moles per liter, and $d$ is cell thickness in cm.

2 In order to simplify phraseology in this article, $\alpha$-benzylpenicillenic acid which has been prepared by rearrangement of benzylpenicillin is called "natural" $\alpha$-benzylpenicillenic acid in contrast to that which has been prepared by total synthesis.
When DL-penicillamine hydrochloride was used instead of D-penicillamine hydrochloride in the condensation with the oxazolone (IV) in pyridine containing triethylamine, it was possible to isolate a crystalline compound from the reaction mixture. Since this compound had an absorption peak at 322.5 nm ($E_M = 26,000$ to $28,000$) and other properties in agreement with those of natural methyl D-benzylpenicillenate, it was called DL-benzylpenicillenic acid (IIIa) (7).

The crystalline DL-benzylpenicillenic acid (IIIa) rearranged in alcoholic solution to give a racemic benzylpenillic acid (VI). When the DL-benzylpenicillenic acid was heated in pyridine containing pyridinium chloride, antibiotic activity was produced, and furthermore in an amount proportional to the amount of D-benzylpenicillenic acid present. This ability to produce antibiotic activity was retained unchanged through repeated recrystallizations of the DL-benzylpenicillenic acid (7).

Since these results indicated that benzylpenicillenic acid (IIIa) was an intermediate in the synthesis of penicillin, it was desirable to demonstrate that the synthetic D-benzylpenicillenic acid was identical with natural D-benzylpenicillenic acid formed by rearrangement of benzylpenicillin.

Previous attempts in this and other Laboratories to obtain either natural or synthetic D-benzylpenicillenic acid in crystalline form had been unsuccessful (3, 5). Thus we were faced with the problem of trying to establish identity between two amorphous compounds. Since DL-benzylpenicillenic acid had been obtained in crystalline form, it occurred to us that amorphous D-benzylpenicillenic acid, either natural or synthetic, might be converted to a crystalline product by mixing it with an equivalent amount of amorphous L-benzylpenicillenic acid. Thus one might expect to obtain, on the one hand, a crystalline DL-benzylpenicillenic acid in which the D moiety arose by rearrangement of benzylpenicillin and, on the other hand, a crystalline DL-benzylpenicillenic acid in which the D moiety arose by synthesis from D-penicillamine (V) and the oxazolone (IV). In both cases, the L moiety would consist of synthetic L-benzylpenicillenic acid. If these two crystalline DL-benzylpenicillenic acids could be shown to be identical, then it would necessarily follow that the natural D-benzylpenicillenic acid was identical with the synthetic D-benzylpenicillenic acid.

Methyl benzylpenicillenate (III) had been prepared by the action of mercuric chloride on the methyl ester of benzylpenicillin (3). Since saponification of a compound as unstable as methyl benzylpenicillenate did not seem feasible, it was decided to investigate the action of mercuric chloride on sodium benzylpenicillin. If the reaction with sodium benzylpenicillin proceeded in a fashion analogous to that with the ester, one would expect to obtain D-benzylpenicillenic acid directly. Investigators at the Abbott Laboratories (3) had reported that treatment of sodium benzyl-
penicillin in aqueous solution with mercuric chloride resulted in the precipitation of a mercury derivative. This product possessed an absorption peak in dioxane at 320 μm ($E_M = 15,400$) (3). The material was probably the mercury derivative (IIa) of 3-benzylpenicillenic acid, and therefore a study of this reaction was initiated. Conditions were developed by which a mercury derivative (IIa) with an absorption peak in dioxane at 320 μm ($E_M = 18,000$ to 19,000) and in ethanol at 342 μm ($E_M = 21,000$ to 23,000) could be obtained from benzylpenicillin. This mercury derivative (IIa) was successfully converted to crude 3-benzylpenicillenic acid (IIIa) by treating a suspension of the compound in a water-ethyl acetate mixture with hydrogen sulfide. The amorphous 3-benzylpenicillenic acid which was isolated from the ethyl acetate layer possessed an absorption peak at 322.5 μm ($E_M = 17,600$). When this crude 3-benzylpenicillenic acid was allowed to stand in methanol, it rearranged to 3-benzylpenillic acid (VI). In this respect it behaved similarly to the 3-benzylpenicillenic acid prepared by synthesis from 3-penicillamine (V) and the oxazolone (IV) (6).

Preliminary experiments were undertaken to determine whether crystalline D,3-benzylpenicillenic acid could be obtained from a mixture of amorphous, synthetic D- and L-benzylpenicillenic acids. At first some difficulty was encountered in obtaining crystalline material upon admixture of the D and L compounds in solution. Presumably this was due to impurities present in these preparations. This difficulty was eliminated to a large degree when a method of partial purification of the crude penicillenic acids, based upon solvent extraction, was devised. It was noted that when a chloroform solution of the crude benzylpenicillenic acid was shaken with an equal volume of 2 M phosphate buffer solution at pH 5.4, a portion of the impurity went into the buffer layer. Although some of the benzylpenicillenic acid was either extracted or destroyed by this procedure, the benzylpenicillenic acid remaining in the chloroform layer was considerably purer than the starting material. The use of this information made it possible to isolate crystalline D,L-benzylpenicillenic acid from a mixture of the synthetic D and L compounds.

Natural 3-benzylpenicillenic acid was mixed with an equivalent amount of synthetic L-benzylpenicillenic acid. After a chloroform solution of this mixture had been purified by the extraction procedure, D,L-benzylpenicillenic acid was isolated from the solution in crystalline form. This D,L-benzylpenicillenic acid was identical in melting point, mixed melting point, and in infra-red and ultraviolet absorption spectra with the D,L-benzylpenicillenic acid synthesized from D,L-penicillamine (7) and also with material prepared from a mixture of synthetic D- and L-benzylpenicillenic acids. In addition, the D,L-benzylpenicillenic acid (IIIa), in which the D moiety was natural, rearranged in methanolic solution to racemic benzylpenillic acid (VI).
When the DL-benzylpenicillenic acid containing natural D-benzylpenicillenic acid was heated in pyridine and pyridinium chloride, antibiotic activity was produced. The amount of antibiotic activity was equal, within experimental error, to that produced under similar conditions from synthetic DL-benzylpenicillenic acid. Synthetic L-benzylpenicillenic acid did not give rise to antibiotic activity under these conditions. Therefore, in the case of the DL-benzylpenicillenic acid, the activity must have arisen entirely from the D moiety of the compound.

These results prove that synthetic D-benzylpenicillenic acid is identical in all respects with natural D-benzylpenicillenic acid. Moreover the production of antibiotic activity, previously shown (2) to be due to benzylpenicillin, in identical amounts from two samples of crystalline DL-benzylpenicillenic acid in which the D moiety was prepared in two altogether different ways indicates beyond a reasonable doubt that benzylpenicillin, and not a small impurity present in the preparation, is an intermediate in the synthesis of benzylpenicillin from penicillamine (V) and the oxazolone (IV). In any event, these data, in connection with those of other experiments cited above (2, 4, 5), demonstrate that benzylpenicillin may be rearranged to an antibiotically inactive product which under certain conditions can be converted in small part back to benzylpenicillin. The relationships discussed here are illustrated on the basis of the β-lactam formula for penicillin in Fig. 1.

The present communication also contains evidence as to the nature of the racemic benzylpenillic acid obtained by rearrangement of DL-benzylpenicillenic acid (7). Since there are three asymmetric carbon atoms in benzylpenillic acid, there are four possible racemic forms of this compound. Admixture of equal quantities of synthetic D- and L-benzylpenillic acids gave rise to crystalline DL-benzylpenillic acid that was identical with the material prepared by rearrangement of DL-benzylpenicillenic acid. These results demonstrate that the racemic benzylpenillic acid formed by rearrangement of DL-benzylpenicillenic acid contains a D moiety which is identical with the D-benzylpenillic acid produced by rearrangement of D-benzylpenicillin.

It should be pointed out that, since benzylpenicillenic acid (IIIa) can rearrange to benzylpenillic acid (VI), it is possible that the rearrangement of D-benzylpenicillin (Ia) to D-benzylpenillic acid (VI) in aqueous solution at pH 2 (8) takes place through the intermediate formation of D-benzylpenicillenic acid (IIIa).

**EXPERIMENTAL**

**D- and L-Benzylpenicillenic Acids**—To a mixture of 3.04 gm. (0.015 mole) of L-penicillamine hydrochloride hydrate and 3.04 gm. (0.014 mole) of L-penicillamine hydrochloride hydrate and 3.04 gm. (0.014 mole) of...
2-benzyl-4-methoxymethylene-5(4)-oxazolone were added 225 cc. of pyridine, and solution was effected by swirling the mixture. After addition of 24 cc. of triethylamine, the mixture was heated at 65-70° for 20 minutes. The yellow solution was distilled in vacuo in a stream of nitrogen at a bath temperature of 50° until the solvents were removed. A solution of the residue in 300 cc. of chloroform was shaken with 150 cc. of 2 M phosphate buffer solution at pH 1.6 (prepared by admixture of equal volumes of

\[
\text{H}_3\text{PO}_4 \text{ and } \text{NaH}_2\text{PO}_4). \]  

Then the chloroform layer was shaken for 1 minute with two 150 cc. portions of 2 M phosphate buffer solution at pH 5.4.\(^4\) The separated chloroform layer was dried over anhydrous MgSO\(_4\) for 20 minutes, filtered by suction, and distilled in vacuo almost to dryness. The residual gum was dissolved in 30 cc. of chloroform and added gradually

\[^4\text{This buffer solution was prepared by admixture of 81 volumes of 2 M NaH}_2\text{PO}_4 \text{ with 19 volumes of 2 M K}_2\text{HPO}_4. \]  

The resulting solution gave a pH of 5.4 when measured without dilution with the glass electrode.

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**Fig. 1. Some reactions of benzylpenicillenic acid**
BENZYL-PENICILLENIC ACID

to 600 cc. of agitated hexane. The precipitate which formed was immediately filtered and dried in vacuo at room temperature. The weight of amorphous L-benzylpenicillenic acid ranged from 3.10 to 3.65 gm. (66 to 78 per cent); $E_M = 15,900$ to 17,000 at 320 mp in 95 per cent ethanol. The maximum in the ultraviolet absorption peak occurred at 322.5 mp.

The specific rotation of the product varied slightly with different preparations, having an average value of about $[\alpha]_{22}^2 = -82^\circ$ (1.3 per cent solution in 95 per cent ethanol). Since the rotation of ethanolic solutions of either of the enantiomorphs of benzylpenicillenic acid gradually increased in value with time, the rotations were determined as soon as possible (within 15 minutes) after the solutions had been prepared.

D-Benzylpenicillenic acid was prepared from D-penicillamine hydrochloride hydrate in the manner described for the L acid. The specific rotation was approximately equal in amount but opposite in sign to that found for L-benzylpenicillenic acid.

**Purification of DL-Benzylpenicillenic Acid by Extraction**—Synthetic D- and L-benzylpenicillenic acids having approximately the same absorption at 320 mp ($E_M = 16,500$) were selected for this experiment. A mixture of 100 mg. of each compound was dissolved in 50 cc. of distilled chloroform. The chloroform solution was shaken for 1 minute with 50 cc. of 2 M phosphate buffer solution at pH 5.4.

Aliquots for determination of ultraviolet absorption were removed from the chloroform solution before and after the extraction. The results of the absorption measurements indicated that 21 per cent of the benzylpenicillenic acid was removed or destroyed by the extraction. After the chloroform layer had been dried over anhydrous MgSO$_4$, it was filtered and concentrated to dryness in vacuo. The residue weighed 98 mg. (49 per cent) and possessed a molar absorption at 320 mp of $E_M = 20,100$. Thus the extraction removed from the chloroform 51 per cent of the material on a weight basis but only 21 per cent of the benzylpenicillenic acid on an absorption basis. An additional extraction with the pH 5.4 buffer solution did not effect appreciable further purification.

**Preparation of DL-Benzylpenicillenic Acid by Admixture of Synthetic Enantiomorphs**—To 300 cc. of ice-cold chloroform were added 0.73 gm. of amorphous L-benzylpenicillenic acid ($E_M = 15,900$ at 320 mp, $[\alpha]_{22}^2 = -82^\circ$) and 0.70 gm. of amorphous D-benzylpenicillenic acid ($E_M = 16,600$ at 320 mp; $[\alpha]_{22}^2 = +80^\circ$). These quantities were equivalent on the basis of molar absorption coefficients. The solution was shaken for 1 minute with 100 cc. of ice-cold 10 per cent $H_2PO_4$, and then was shaken with 300 cc. of ice-cold 2 M phosphate buffer solution at pH 5.4. After 10 minutes (to allow complete separation of phases) the lower layer was drawn off, placed in an ice bath, and dried with anhydrous MgSO$_4$ for 20 minutes. The desiccant was then filtered by suction, and the clear, yellow solution was
evaporated in vacuo in the absence of air of ebullition in a bath at 40–60°. Before half of the solvent was evaporated, the solution became cloudy and crystallization commenced. The mixture was concentrated to a volume of about 10 cc., allowed to stand in an ice bath for 40 minutes, and filtered. The white crystals were washed three times with a total of 5 cc. of distilled chloroform and dried in vacuo at room temperature; weight, 328 mg. (23 per cent); m.p. 131–133° (with decomposition); $E_M = 24,600$ at 320 mμ in 95 per cent ethanol.

When 298 mg. of this material were warmed for 5 minutes in 15 cc. of dry ethyl acetate and finally boiled for a minute, all but a trace of substance dissolved. The solution was filtered with the aid of gentle suction into a tared centrifuge tube and left in the cold. After 18 hours the white crystals were collected by centrifugation and dried in vacuo; weight, 178 mg. (60 per cent); m.p. 136–137° (with decomposition); $E_M = 26,600$ at 320 mμ in 95 per cent ethanol; absorption peak located at 322.5 mμ; $[α]_{25}^0 = 0^\circ$ (0.35 per cent solution in 95 per cent ethanol). The melting point of this

### Table I

**Reaction of Mercuric Chloride with Sodium Benzylpenicillin**

<table>
<thead>
<tr>
<th>Concentration of reactants</th>
<th>Yield</th>
<th>$E_M$ at 320 mμ</th>
</tr>
</thead>
<tbody>
<tr>
<td>mole per l.</td>
<td>per cent</td>
<td></td>
</tr>
<tr>
<td>0.0833</td>
<td>92</td>
<td>14,500</td>
</tr>
<tr>
<td>0.0417</td>
<td>89</td>
<td>16,000</td>
</tr>
<tr>
<td>0.0208</td>
<td>91</td>
<td>17,600</td>
</tr>
<tr>
<td>0.0104</td>
<td>85</td>
<td>18,600</td>
</tr>
</tbody>
</table>

DL-benzylpenicillenic acid was not depressed upon admixture with DL-benzylpenicillenic acid prepared from DL-penicillamine (7).

**Effect of Concentration on Reaction of Mercuric Chloride with Sodium Benzylpenicillin**—Samples of sodium benzylpenicillin (0.25 mμ) were dissolved in various amounts of water. An aqueous solution of mercuric chloride containing 0.25 mμ was added to each penicillin solution and the final volume was noted. The solutions were allowed to stand at room temperature (25°) for 2 hours and then refrigerated at 5° for 16 hours. The precipitates of the mercury derivative (IIa) were collected by filtration and dried in vacuo over phosphoric anhydride at room temperature. The yield and molar absorption coefficient of each material at 320 mμ in dioxane are shown in Table I.

**Preparation of Mercury Derivative (IIa) from Sodium Benzylpenicillin**—To 1.78 gm. (0.005 mole) of sodium benzylpenicillin dissolved in 100 cc. of water were added 1.49 gm. (0.0055 mole) of mercuric chloride dissolved in 100 cc. of water. The clear solution gradually developed turbidity while
standing at room temperature for 3 hours. After the mixture had been allowed to stand at 5° for 16 hours, a precipitate had formed which was collected on a sintered glass filter. The precipitate was washed thoroughly with water and then dried in vacuo over phosphoric anhydride at room temperature. The light yellow powder weighed 2.67 gm. (94 per cent). The mercury derivative (IIa) was slightly soluble in dioxane, ethanol, and acetone, very slightly soluble in ethyl acetate, and insoluble in water.

C₁₆H₁₇N₃O₇SClHg. Calculated. N 4.92, S 5.63
569.5 Found. N 4.86, S 5.40

![Graph of molar absorption spectra of mercury derivative (IIa) in 95 per cent ethanol (Curve 1) and in dioxane (Curve 2).]

**Fig. 2.** Molar absorption spectra of the mercury derivative (IIa) in 95 per cent ethanol (Curve 1) and in dioxane (Curve 2).

The molar absorption spectra of the mercury derivative (IIa) in 95 per cent ethanol (Curve 1) and in dioxane (Curve 2) are shown in Fig. 2.

**Conversion of Mercury Derivative (IIa) to Amorphous d-Benzylpenicillenic Acid**—A suspension of 2.40 gm. of the mercury derivative (IIa) in 30 cc. of water and 75 cc. of ethyl acetate was treated with hydrogen sulfide. The mixture was centrifuged and the ethyl acetate layer was filtered through diatomaceous earth (Filter-Cel). The filtrate was placed at -70° for 30 minutes to freeze out most of the water. The ice crystals were separated by rapid filtration and washed with 50 cc. of ethyl acetate at -70°. The combined ethyl acetate solutions were allowed to stand for 30 minutes over anhydrous MgSO₄. After the desiccant had been separated by gravity filtration, the ethyl acetate solution was concentrated in vacuo in the absence of air of ebullition to a volume of 30 cc. The concentrated ethyl
acetate solution was added dropwise to 600 cc. of agitated hexane. The amorphous precipitate which formed turned to a gum when the mixture was allowed to stand for 2 hours at 5°. The supernatant liquid was decanted and the gum was dissolved in 30 cc. of chloroform. Upon dropwise addition of the chloroform solution to 500 cc. of hexane, a white, amorphous precipitate formed. The precipitate was filtered, washed well with hexane, and, while still moist with hexane, placed in a vacuum desiccator to dry under suction. The amorphous D-benzylpenicillenic acid weighed 0.85 gm. (60 per cent). This material possessed an absorption peak in 95 per cent ethanol at 322.5 μ (Ε₉₅ = 17,600). The specific rotation determined within 10 minutes after preparing the solution was [α]₀⁻²⁴ = +86° (0.47 per cent solution in 95 per cent ethanol).

**DL-Benzylpenicillenic Acid by Admixture of Natural D- with Synthetic L-Benzylpenicillenic Acid**—Natural D-benzylpenicillenic acid (0.70 gm.) (Ε₉₅ = 17,600 at 322.5 μ, [α]₀⁻²⁴ = +86°) was admixed with 0.70 gm. of synthetic L-benzylpenicillenic acid (Ε₉₅ = 17,800 at 322.5 μ; [α]₀⁻²⁴ = −89°). The mixture was treated by a procedure similar to that described above for the isolation of crystalline D₁L-benzylpenicillenic acid by admixture of the synthetic enantiomorphs. The crystalline D₁L-benzylpenicillenic acid isolated by this procedure weighed 0.283 gm. (21 per cent), m.p. 133–134° (with decomposition).

A 100 mg. sample of this product was recrystallized from 5 cc. of ethyl acetate. 50 mg. of D₁L-benzylpenicillenic acid were recovered; m.p. 137–139° (with decomposition); [α]₀⁻³ = 0° (0.26 per cent solution in 95 per cent ethanol). There was no depression in the melting point upon admixture with D₁L-benzylpenicillenic acid prepared from D₁L-penicillamine (V) and the oxazolone (IV) (7). The molar absorption spectrum in 95 per cent ethanol is shown in Fig. 3.

C₉H₉N₄O₅S. Calculated. C 57.45, H 5.43, N 8.38
334.4 Found. C 57.31, H 5.82, N 8.33

**Infra-Red Absorption Measurements**—The infra-red absorption spectra from 690 cm⁻¹ to 3600 cm⁻¹ of D₁L-benzylpenicillenic acid made by admixture of natural D- with synthetic L-benzylpenicillenic acid (upper curve) and of D₁L-benzylpenicillenic acid made by synthesis from D₁L-penicillamine (lower curve) are shown in Fig. 4. The absorption spectra were determined on samples of the crystalline compounds mulled in mineral oil between two sodium chloride plates. The measurements were made on a Perkin-Elmer infra-red spectrometer, model 12A, with a gain control to compensate for the energy distribution of the Globar source. It should be pointed out that the curves include absorption peaks due to mineral oil and to air as well as those due to benzylpenicillenic acid.

**Rearrangement of Benzylpenicillenic Acid to Benzylpenicillin**—Crystalline
FIG. 3. Molar absorption spectrum\(^1\) of crystalline \textit{dL}-benzylpenicillenic acid in 95 per cent ethanol.

FIG. 4. Infra-red absorption spectra of crystalline \textit{dL}-benzylpenicillenic acids: upper curve, \textit{dL}-benzylpenicillenic acid prepared by admixture of natural \textit{d}- with synthetic \textit{l}-benzylpenicillenic acid; lower curve, \textit{dL}-benzylpenicillenic acid synthesized from \textit{dL}-penicillamine.
DL-benzylpenicillenic acid (30 mg.) prepared by admixture of natural D- with synthetic L-benzylpenicillenic acid was dissolved in 10 cc. of pyridine containing 6.5 mg. of pyridinium chloride per cc. Aliquots (1 cc.) of this solution were placed in a series of test-tubes. To one of these tubes about 0.05 cc. of triethylamine was added and the tube was placed in an ice bath. The rest of the tubes were placed in an oil bath at 120°. At noted time intervals a tube was removed from the oil bath, triethylamine was added to it, and the tube was cooled in the ice bath. The solvents were removed from each tube in vacuo at a bath temperature of 50°. The residues were moistened with 0.2 cc. of acetone and then dissolved in various amounts of 1 per cent phosphate buffer solution at pH 6. These buffer solutions were assayed against Bacillus subtilis ATCC 6051 by a modification of the method of Vincent and Vincent (9) with crystalline sodium benzylpenicillin as a standard. The results are shown in Curve 1, Fig. 5.

Synthetic n-benzylpenicillenic acid was heated in pyridine and pyridinium chloride, and the products were prepared for assay under conditions similar to those described above. The results are shown in Curve 2, Fig. 5. It should be noted that this crude L-benzylpenicillenic acid possessed a slight...
amount of antibiotic activity (about 0.05 unit per mg.) when assayed at high concentration. However, since this antibiotic activity increased only very slightly, if at all, during the heating period, it was probably not due to the presence of a penicillin-like compound.

In another experiment, crystalline DL-benzylpenicillenic acid made by admixture of natural D with synthetic L acid was dissolved at a concentration of 3 mg. per cc. in pyridine containing 0.5 mg. of pyridinium chloride per cc. An identical solution was prepared from DL-benzylpenicillenic acid made by synthesis from DL-penicillamine (7). The two solutions were placed in an oil bath at 110° for 12 minutes, and then removed and prepared for assay as described above. The DL-benzylpenicillenic acid in which the D moiety arose from penicillin yielded 1.14 units of penicillin per mg. of starting DL-benzylpenicillenic acid, while the entirely synthetic DL-benzylpenicillenic acid gave rise to 1.19 units. Aliquots which had not been heated were also assayed. These showed no detectable activity when assayed at a concentration of 3 mg. per cc.

Rearrangement of Natural D-Benzylpenicillenic Acid to D-Benzylpenillic Acid—A solution of 75 mg. of amorphous, natural D-benzylpenicillenic acid in 1 cc. of methanol was seeded with a trace of D-benzylpenillic acid. The solution was allowed to stand at room temperature for 18 hours and then at 5° for 24 hours. Long, needle-like crystals of D-benzylpenillic acid separated; weight, 10.5 mg. (14 per cent); \([\alpha]_D^{24} = +490°\) (0.1 per cent solution in methanol) (6); \(E_M = 6300\) at 237.5 mp in 95 per cent ethanol (6). The melting point was determined on a sample which had been recrystallized by dissolving it in an equivalent amount of 0.1 N NaOH and then adding an equivalent amount of 0.1 N HCl. This sample of D-benzylpenillic acid melted at 180-185° (with decomposition). The melting point was not lowered upon admixture with D-benzylpenillic acid prepared by rearrangement of benzylpenicillin in water at pH 2 (8).

Rearrangement of DL-Benzylpenicillenic Acid to DL-Benzylpenillic Acid—A methanolic solution (2 cc.) of 100 mg. of crystalline DL-benzylpenicillenic acid in which the D moiety arose from penicillin was seeded with a trace of DL-benzylpenillic acid. After the solution had been allowed to stand for 17 hours at room temperature and 24 hours at 5°, 18.2 mg. (18 per cent) of long, needle-like crystals separated. The DL-benzylpenillic acid had a specific rotation of \([\alpha]_D^{24} = 0°\) (0.1 per cent solution in methanol); \(E_M = 5700\) at 240 m\(\mu\) in 95 per cent ethanol; and m.p. 179-180° (with decomposition). The melting point was not lowered upon admixture with DL-benzylpenillic acid prepared by synthesis from DL-penicillamine through the intermediate DL-benzylpenicillenic acid (7).

In the determination of the melting points of the benzylpenillic acids reported in this paper, the compounds were placed in the bath at 170° and heated at a rate of 1.5° per minute at the melting point.
DL-Benzylpenillic Acid by Admixture of Enantiomorphs—D-Benzylpenillic acid and its enantiomorph were prepared from the corresponding D- and L-benzylpenicillenic acids by rearrangement in methanol (6). Each of the penillic acids was dissolved in 0.1 N NaOH solution so that the concentration was 30 mg. per cc. Equal volumes of the two solutions were mixed, and the resulting solution was made acid to Congo red paper with 0.1 N HCl. After the solution had stood at 5° overnight, it yielded white crystals, m.p. 177-178° (with decomposition). The melting point of this DL-benzylpenillic acid was not lowered upon admixture with racemic benzylpenillic acid (m.p. 178.5-179.5°) formed by rearrangement of DL-benzylpenicillenic acid (7).

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SUMMARY

D-Benzylpenicillenic acid synthesized from D-penicillamine hydrochloride and 2-benzyl-4-methoxymethylene-5(4)-oxazolone was shown to be identical with D-benzylpenicillenic acid prepared by rearrangement of D-benzylpenicillin (penicillin G).

Benzylpenicillin was converted to an antibiotically inactive product which was in turn reconverted in small yield to benzylpenicillin. Evidence was presented to show that this inactive compound was identical with D-benzylpenicillenic acid and that D-benzylpenicillenic acid was an intermediate in the synthesis of benzylpenicillin from D-penicillamine and 2-benzyl-4-methoxymethylene-5(4)-oxazolone.

Racemic benzylpenillic acid prepared by rearrangement of DL-benzylpenicillenic acid was shown to contain a D moiety identical with D-benzylpenillic acid prepared by rearrangement of D-benzylpenicillin.

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