was cooled in an ice-bath and acidified with hydrochloric acid. The oily precipitate crystallized on scratching and was recrystallized from aqueous ethanol, m.p. 125–127° (dec.).

Anal. Calcd. for $C_{15}H_{21}O_5N$: C, 61.18; H, 7.19; N, 4.76. Found: C, 61.05; H, 7.44; N, 4.98.

Attempts to resolve this product with brucine and several other alkaloids were unsuccessful.

Acknowledgment.—The authors are grateful to Dr. O. Wintersteiner for his interest, encouragement and counsel, and to Mr. J. F. Alicino and the Misses Anne Crickenberger and Ruth Karitzky for microanalyses.

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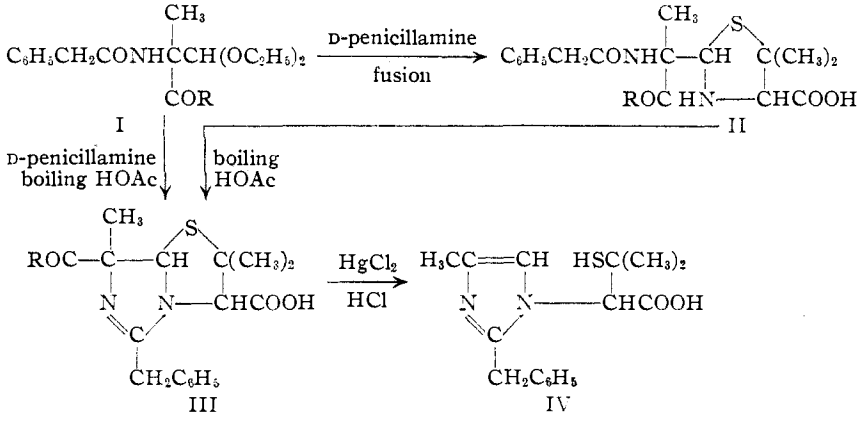
[CONTRIBUTION FROM THE SQUIBB INSTITUTE FOR MEDICAL RESEARCH, DIVISION OF ORGANIC CHEMISTRY]

Homologs of Penicillin Degradation Products. II. 6-Methyl-D-benzylpenillic Acid[1,2]

BY HOMER E. STAVELY[3]

The amides and methyl esters of D- and L-α-methylbenzylpenaldic acid diethyl acetal have been condensed with D-penicillamine hydrochloride in boiling acetic acid. Instead of the expected 6-methyl-D-benzylpenicilloic acid derivatives the products were the α-amides and α-methyl esters of 6-methyl-D-benzylpenillic acid. The two diastereoisomeric α-methyl esters were hydrolyzed to free acids whose chemical and physical properties closely parallel those of D-benzylpenillic acid derived from benzylpenicillin. The two homologous series appear to differ in their mechanism of formation, since an authentic α-methyl-6-methyl-D-benzylpenicilloate can be an intermediate in the formation of 6-methyl-D-benzylpenillic acid.

A number of optically active α-methylbenzylpenaldic acid derivatives (I) have been prepared[1] with the object in view of condensing these compounds with D-penicillamine to form 6-methylbenzylpenicilloic acid derivatives (II). The condensation of



an aldehyde with an α-amino mercaptan to form a thiazolidine is a facile reaction[4] which takes place at room temperature in sodium acetate-buffered aqueous ethanol. Penaldic acid derivatives in which the aldehydic function exists as a hydroxy methylene group, or as an acetal, condense readily with penicillamine to form thiazolidines at room temperature.[5] The acetal group in α-methylpenaldic acid diethyl acetal derivatives, however, has been found to be remarkably stable[6] and the condensation of these derivatives with D-penicillamine could not be accomplished under a variety of conditions at temperatures up to 100°.

In view of these facts the condensation of the L-form of α-methylbenzylpenaldamide diethyl acetal (I, R = NH_2) with D-penicillamine hydrochloride was attempted in boiling acetic acid. The crystalline reaction product was not the expected hydrochloride of α-amido-6-methyl-D-benzylpenicilloic acid (II, R = NH_2), but a substance which according to the analysis contained the elements of one water molecule less. The unexpected loss of an additional water molecule in the condensation was confirmed by the empirical formula of the crystalline methyl ester of the free base, $C_{18}H_{23}O_3N_3S$, prepared by reaction with diazomethane. Treatment of the condensation product with mercuric chloride did not give a water-soluble aldehyde and an immediate precipitate of penicillamine–mercury complex, whereas penicilloic acid derivatives are instantly cleaved by this reagent to these products. Moreover the specific rotation of the condensation product, $[\alpha]D$ +383°, was much higher than was to be expected for a penicilloic acid derivative. A similar condensation of the D-form of the amide with D-penicillamine afforded a crystalline diastereoisomeric product whose methyl ester could not be induced to crystallize.

As was the case with the amides, the methyl esters of D- and L-α-methylbenzylpenaldic acid diethyl acetal (I, R = OCH_3) condensed smoothly with D-penicillamine hydrochloride in boiling acetic acid to form two crystalline diastereoisomeric products. Hydrolysis yielded two highly dextrorotatory dibasic acids having the empirical formula $C_{17}H_{20}N_2O_4S$, (III, R = OH). Reaction of either product with mercuric chloride did not afford an aldehyde, again indicating that they were not D-benzylpenicilloic acids.

The empirical formulas of these condensation products, their high dextrorotation, and their behavior toward mercuric chloride suggested strongly

(1) Paper I of this series, THIS JOURNAL, **73**, 3448 (1951).

(2) Presented in part before the Division of Biological Chemistry, American Chemical Society Meeting, April, 1948.

(3) Pharmaceutical Research Division, Commercial Solvents Corporation, Terre Haute, Indiana.

(4) S. Ratner and H. T. Clarke, THIS JOURNAL, **59**, 200 (1937); M. P. Schubert, *J. Biol. Chem.*, **111**, 671 (1935); **114**, 341 (1936).

(5) H. T. Clarke, J. R. Johnson and R. Robinson, editors, "The Chemistry of Penicillin," Princeton University Press, Princeton, N. J., 1949, p. 545–547.

(6) H. T. Clarke, J. R. Johnson and R. Robinson, *ibid.*, pp. 765, 838. This work was originally reported (in more detail) by J. R. Catch, A. H. Cook and I. M. Heilbron in C.P.S. 105, dated July 4, 1944.

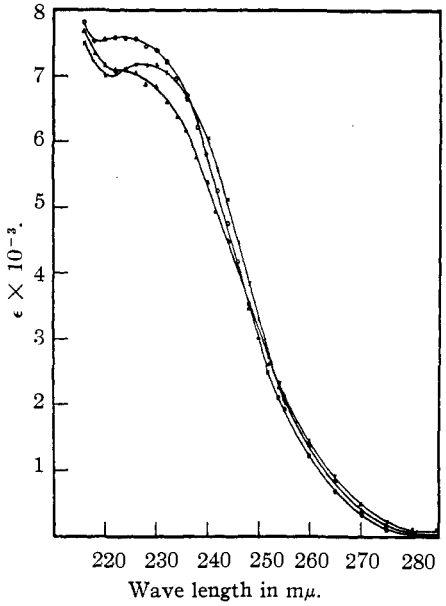


Fig. 1.—Ultraviolet absorption curves in 0.1 *N* hydrochloric acid: x, D-benzylpenillic acid; O, 6-methyl-D-benzylpenillic acid (D_6-isomer); △, 6-methyl-D-benzylpenillic acid (L_6-isomer).

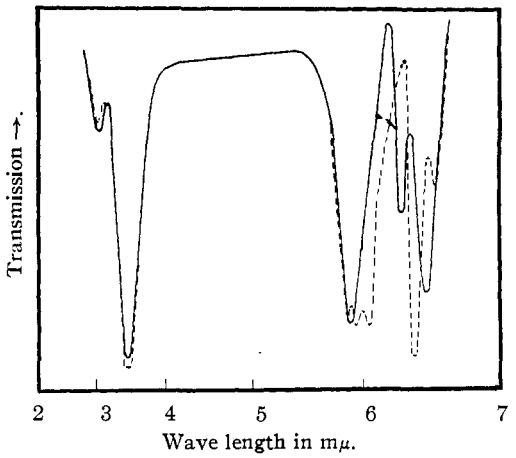


Fig. 2.—Infrared absorption spectra in Nujol suspension of: - - - - - -, 6-methyl-D-benzylpenillic acid (D_6-isomer); ———, D-benzylpenillic acid.

that they were members of the D-benzylpenillic acid series rather than D-benzylpenicilloic acid derivatives. This was confirmed by a comparison of some physical properties of the two synthetic dibasic acids and D-benzylpenillic acid (derived from benzylpenicillin). The ultraviolet absorption spectra of the two synthetic acids were found to be almost identical with that of D-benzylpenillic acid (Fig. 1). The infrared absorption characteristics of one of the synthetic acids (in the hydrogen bond and double bond regions of the spectrum) were compared with those of D-benzylpenillic acid (Fig. 2). Exact correspondence was not to be expected, but the number and approximate positions of the absorption bonds were the same for both compounds. This striking similarity in both chemical and physical properties strongly indicate that the two synthetic acids are diastereoisomeric 6-methyl homologs of D-benzylpenillic acid.

The condensation of α-methylpenaldic acid diethyl acetal derivatives with D-penicillamine hydrochloride to form authentic 6-methylpenicilloic acid derivatives will be described in detail in a subsequent paper. Briefly, however, it has been found that the reaction can be accomplished by the fusion of the two reactants at 110° for a short period. The amorphous hydrochloride of the α-methyl ester of 6-methyl-D-benzylpenicilloate (II, R = OCH_3), prepared in this way, behaved in a manner consistent with this structure rather than as the condensation products described above. After this substance had been refluxed in glacial acetic acid solution for 30 minutes a crystalline hydrochloride was isolated which was hydrolyzed with dilute sodium hydroxide. The crystalline product was identical with that obtained by the direct condensation of the D-form of methyl α-methylbenzylpenaldate diethyl acetal and D-penicillamine in boiling acetic acid. Thus it appears likely that the 6-methylpenicilloates (II) are intermediates in the formation of the penillic acid derivatives III. The dehydration of a 6-methylbenzylpenicilloate to a 6-methylpenillate has no parallel in the D-benzylpenillic acid series. Every attempt to dehydrate a D-benzylpenicilloate to a D-benzylpenillate has failed.[7] Only at elevated temperatures could the imidazole ring be closed, but this was accompanied by the elimination of the sulfur atom from the thiazolidine ring to yield an α-imidazolyl-β,β-dimethylacrylic acid.

The formation of a penillamine (IV) on treatment with mercuric chloride is a characteristic reaction of the penillic acids. Similar treatment of one of the diastereoisomeric 6-methylbenzylpenillic acids yielded an impure hydroxy mercaptan which according to its equivalent weight was partially decarboxylated. Short heating of this material in dilute hydrochloric acid completed the decarboxylation and dehydration and afforded a product which analyzed correctly for 6-methyl-D-penillamine (IV). In this reaction decarboxylation obviously must have preceded dehydration.

Experimental[8]

α-Amido-6-methyl-D-benzylpenillic Acid Hydrochloride (III, R = NH_2).—A solution of 188 mg. (1 millimole) of D-penicillamine hydrochloride and 309 mg. (1 millimole) of the L-form of α-methylbenzylpenaldamide diethyl acetal in 1.0 ml. of glacial acetic acid was refluxed for 30 minutes, cooled and added dropwise to 10 ml. of ether. The precipitate was centrifuged, washed with ether and crystallized from methanol–ether, yield 308 mg.; $[\alpha]^{25}D$ +383° (*c*, 0.61 in methanol).

Anal. Calcd. for $C_{17}H_{21}N_3O_3 \cdot HCl$: C, 53.07; H, 5.78; N, 10.95; S, 8.35; Cl^-, 9.24. Found: C, 52.93; H, 5.76; N, 10.85; S, 8.80; Cl^-, 9.45.

For the preparation of the methyl ester of the free base the hydrochloride (100 mg.) was dissolved in 1.0 ml. of methanol and slightly more than two molar equivalents of diazomethane in ether were added. The product was recrystallized from acetone–hexane, m.p. 212–214°.

Anal. Calcd. for $C_{18}H_{23}N_3O_3S$: C, 59.81; H, 6.41; N, 11.63; OCH_3, 8.59. Found: C, 59.57; H, 6.38; N, 11.31; OCH_3, 8.89.

A similar condensation was carried out with the D-form of α-methylbenzylpenaldamide diethyl acetal and D-penicill-

(7) H. T. Clarke, J. R. Johnson and R. Robinson, ref. 6, p. 121.

(8) The melting points were all taken in open capillary tubes and have been corrected for stem exposure.

amine hydrochloride. A crystalline diastereoisomeric product was obtained, $[\alpha]^{25}D$ +343° (*c*, 0.63 in ethanol).

Anal. Calcd. for $C_{17}H_{21}N_3O_3 \cdot HCl$: C, 53.07; H, 5.78; N, 10.95. Found: C, 53.44; H, 5.56; N, 10.87.

The methyl ester of the free base of this substance, prepared with diazomethane, could not be induced to crystallize.

α-Methyl-6-methyl-D-benzylpenillate Hydrochloride (III, R = OCH_3).—A solution of 93 mg. (0.5 millimole) of D-penicillamine hydrochloride and 162 mg. (0.5 millimole) of the L-form of methyl α-methylbenzylpenaldate diethyl acetal in 2.0 ml. of glacial acetic acid was refluxed for 30 minutes. The solution was lyophilized, the residue was washed with ether and was crystallized from methanol–ether, yield 113 mg.; $[\alpha]^{25}D$ +292° (*c*, 1.02 in ethanol).

Anal. Calcd. for $C_{18}H_{22}N_2O_4S \cdot HCl$: C, 54.20; H, 5.81; N, 7.02; Cl^-, 8.9. Found: C, 54.12: H, 6.16; N, 7.04; Cl^-, 9.6.

By the same method a crystalline diastereoisomeric α-methyl-6-methyl-D-benzylpenillate hydrochloride was prepared by condensing D-penicillamine hydrochloride and the D-form of methyl α-methylbenzylpenaldate diethyl acetal, $[\alpha]^{25}D$ +327° (*c*, 0.44 in 95% ethanol).

Anal. Calcd. for $C_{18}H_{22}N_2O_4S \cdot HCl$: N, 7.02; Cl^-, 8.9. Found: N, 6.56; Cl^-, 9.5.

6-Methyl-D-benzylpenillic Acid (III, R = OH).—The α-methyl ester hydrochloride of 6-methyl-D-benzylpenillic acid prepared from the L-form of the methyl α-methylpenaldate acetal (60 mg.), was dissolved in 1.0 ml. of water and 0.48 ml. of 1.11 *N* sodium hydroxide was added. After standing overnight the alkali was exactly neutralized with 0.99 *N* hydrochloric acid. The resulting crystalline precipitate, 36 mg., was rather insoluble in most solvents. It was purified by dissolving in sodium bicarbonate solution and reprecipitating with dilute hydrochloric acid, m.p. 197–198°; $[\alpha]^{24}D$ +373° (*c*, 0.244 in 95% ethanol).

Anal. Calcd. for $C_{17}H_{20}N_2O_4S$: C, 58.85; H, 5.79; N, 8.04. Found: C, 58.58; H, 5.87; N, 7.79.

By the same procedure the α-methyl ester hydrochloride of 6-methyl-D-benzylpenillic acid prepared from the D-form of methyl α-methylbenzylpenaldate diethyl acetal afforded a diastereoisomeric 6-methyl-D-benzylpenillic acid, m.p. 186–188° (dec.); $[\alpha]^{24}D$ +413° (*c*, 0.210 in 95% ethanol).

Anal. Calcd. for $C_{17}H_{20}N_2O_4S$: C, 58.85; H, 5.79; N, 8.04; S, 9.19. Found: C, 58.73; H, 6.13; N, 7.88; S, 9.43.

6-Methyl-D-benzylpenillamine (IV).—A sample of the 6-methyl-D-benzylpenillic acid prepared from the D-form of methyl α-methylbenzylpenaldate diethyl acetal, 100 mg., was dissolved in 3.0 ml of 5% mercuric chloride solution. After a few minutes a precipitate started to form. After standing for 2 hours the precipitate was centrifuged, washed several times with water and dried *in vacuo* at 60°.

Anal. Found: N, 3.88; S, 4.13; N/S = 2.1.

The nitrogen–sulfur ratio indicated that cleavage of the molecule into D-penicillamine and an aldehyde had not occurred. The substance was suspended in methanol and saturated with hydrogen sulfide. After removal of mercuric sulfide by filtration the filtrate was taken to dryness under reduced pressure. The residue, 75 mg., was dissolved in water and slightly more than one equivalent of 0.55 *N* sodium hydroxide solution was added. The resulting crystalline precipitate had a neutral equivalent weight of 268, indicating partial decarboxylation. It was redissolved in 0.5 ml. of 0.1 *N* hydrochloric acid and heated on the steam-bath for ten minutes, then cooled and exactly neutralized with 0.5 ml. of 0.1 *N* sodium hydroxide. The crystalline precipitate weighed 29 mg. after drying and melted at 148–149°.

Anal. Calcd. for $C_{16}H_{20}O_2N_2S$: C, 63.08; H, 6.62; N, 9.21; neut. equiv., 304. Found: C, 62.56; H, 6.49; N, 9.01; neut. equiv., 310.

The nitroprusside and ferric chloride tests for the sulfhydryl group were strongly positive.

6-Methyl-D-benzylpenillic Acid (III, R = OH) from **α-Methyl-6-methyl-D-benzylpenicilloate** (II, R = OCH_3).—The amorphous α-methyl ester of 6-methyl-D-benzylpenicilloate hydrochloride, 100 mg. (prepared by the direct fusion of equimolar quantities of D-penicillamine hydrochloride and the methyl ester of the D form of α-methylbenzylpenaldate diethyl acetal at 108° for 8 minutes) was dissolved in 2.0 ml. of glacial acetic acid and the solution was refluxed for 30 minutes. After cooling the solution was lyophilized. The residue was dissolved in 0.75 ml. of 1.11 *N* sodium hydroxide. After standing overnight 0.84 ml. of 0.99 *N* hydrochloric acid was added to the solution. The crystalline precipitate (59 mg.) melted at 187–189° and the melting point was not depressed by admixture of 6-methyl-D-benzylpenillic acid of the same melting point.

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