

ally to the hot, dark reaction product in order to decompose the excess acetic anhydride. After cooling, the mixture was extracted with chloroform. Distillation gave, after a forerun of several drops, 10.5 g. (46%) of diester, b.p. 143–146° (16 mm.),  $n_D^{25}$  1.4936. The infrared spectrum was identical with that of XIII prepared by acetylation<sup>14</sup> of *trans*-2-hydroxycyclohexanethiol obtained by the ring-opening of cyclohexene oxide with sodium hydrogen sulfide.<sup>16</sup>

The latter material possessed the following physical constants: b.p. 147–150° (19 mm.),  $n_D^{25}$  1.4940.

**Acknowledgment.**—The author wishes to express his gratitude to Mr. Donald Johnson for the infrared spectra used in this investigation.

MADISON, WIS.

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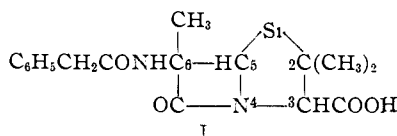
[CONTRIBUTION FROM THE SQUIBB INSTITUTE FOR MEDICAL RESEARCH, DIVISION OF ORGANIC CHEMISTRY]

## Homologs of Penicillin Degradation Products. I. $\alpha$ -Methylpenaldic Acid Derivatives

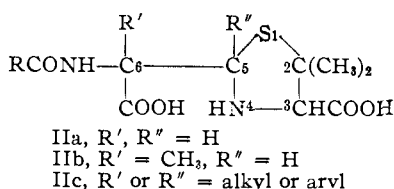
BY HOMER E. STAVELY<sup>1</sup> AND MARY BERESTECKI

Some alkyl substituted penaldic acid derivatives were prepared as intermediates for attempted syntheses of penicillin homologs. Racemic  $\alpha$ -methylbenzylpenaldic acid diethyl acetal was resolved *via* the brucine salts and the *D*- and *L*-forms were converted into the corresponding oxazolones, methyl esters and amides. The preparation of  $\alpha$ -methylbenzylpenaldic acid diethyl dithioacetal and  $\alpha$ -methylphenylpenaldic acid diethyl acetal is described.

This series of papers will describe the preparation and reactions of some compounds which theoretically could be derived by degradation from the unknown substance 6-methylbenzylpenicillin (I). The eventual goal of this work was the synthesis of I.

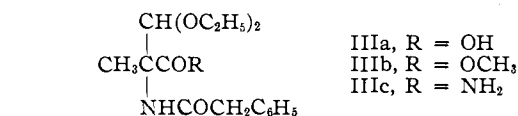


The final step of many unsuccessful attempts to synthesize a penicillin has been closure of the  $\beta$ -lactam ring, a penicilloic acid derivative (IIa) being the penultimate compound in the synthesis.<sup>2</sup>



Since most of the monocyclic and bicyclic  $\beta$ -lactams known are heavily substituted with alkyl or aryl groups<sup>3</sup> it might be expected that a penicilloic acid derivative having alkyl or aryl radicals on carbon atoms 5 and/or 6 (IIc) could be  $\beta$ -lactamized more readily than the unsubstituted derivative.<sup>4</sup>

As the first step in the synthesis of penicillin homologs with substituents on carbon atom 6, the preparation of certain  $\alpha$ -methylpenaldic acid derivatives was undertaken. Heilbron and his associates<sup>5</sup> were the first to report the preparation of *DL*- $\alpha$ -phenylacetyl-amido- $\alpha$ -diethoxymethylpropionic acid (IIIa) ( $\alpha$ -methylbenzylpenaldic acid diethyl acetal) by reaction of *DL*-phenylacetylala-



nine<sup>6</sup> with ethyl orthoformate in hot acetic anhydride. The British investigators found that the acetal group of IIIa possessed an unusual degree of stability, since it was unaffected by dilute acid and failed to form a 3,5-dinitrophenylhydrazone or a semicarbazone under mild conditions. Treatment with a warm solution of dinitrophenylhydrazine in dilute sulfuric acid degraded the acid but the product was the dinitrophenylosazone of methylglyoxal. In the reaction with phenylacetylalanine, ethyl orthoformate might conceivably have reacted with the active methylene of the phenylacetyl residue, but the isolation of this osazone proved that the reaction product had structure IIIa. This conclusion has been further strengthened by the successful synthesis of *DL*- $\alpha$ -benzamido- $\alpha$ -diethoxymethylpropionic acid ( $\alpha$ -methylphenylpenaldic acid diethyl acetal) from benzoylalanine by the same procedure in this Laboratory.

In the present work a number of alkaloids were utilized in unsuccessful attempts to resolve the racemic acid IIIa, but the resolution was finally accomplished with the aid of brucine in dilute aqueous solution. The separation of the two diastereoisomeric brucine salts was aided by the fact that the more soluble salt apparently has a negative differential solubility, since heating the cold mother liquor caused an oily precipitation which redissolved on cooling. Decomposition of the insoluble crystalline salt by the usual methods afforded an acid with an  $[\alpha]_D$  of  $-10.5 \pm 1^\circ$  (chloroform) and  $[\alpha]_D +9.6 \pm 1^\circ$  in normal sodium hydroxide. Decomposition of the first mother liquor gave the slightly optically impure optical antipode,  $[\alpha]_D +8.8^\circ$  in chloroform,  $[\alpha]_D -9.2^\circ$  in normal sodium hydroxide, but the rotation could be raised somewhat by crystallization from aqueous ethanol. The *L* configuration has been tentatively assigned to the acid forming the insoluble brucine salt, since this form has a dextro rotation in alkaline solution, as is the case with *L*(+)-benzoylalanine and other *L*(+)-acylamino acids.

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

(2) H. T. Clarke, J. R. Johnson and R. Robinson, editors, "The Chemistry of Penicillin," Princeton University Press, Princeton, N. J., 1949, pp. 849–891.

(3) See Chapter XXVI, "The Chemistry of Penicillin," for an excellent review of  $\beta$ -lactam chemistry.

(4) This has been borne out by the recent synthesis of a 5-phenyl penicillin derivative by J. C. Sheehan, *et al.*, *THIS JOURNAL*, **72**, 3828 (1950).

(5) Ref. 2, pp. 502, 765, 838. The work mentioned was reported in detail in C. P. S. 105 by J. R. Catch, A. H. Cook and I. M. Heilbron, dated July 4, 1944.

(6) G. J. Shiple and C. P. Sherman, *J. Biol. Chem.*, **53**, 463 (1922).

The methyl esters of the DL-, D- and L-forms were all prepared by reaction with diazomethane. When the esters were treated with ethanolic ammonia they were recovered unchanged. In order to prepare the amides it was necessary to dehydrate the free acids with acetic anhydride to the corresponding oxazolones, which were then dissolved in dry ether and saturated with dry ammonia. By this procedure a good yield of  $\alpha$ -methylbenzylpenaldamide diethyl acetal was obtained. The oxazolones prepared from the D- and L-acids had specific rotation of  $-74$  and  $+77^\circ$ , respectively. This optical activity was expected because no hydrogen is attached to the carbon atom  $\alpha$  to the carbonyl group and racemization is prevented.

The marked stability of the acetal group in these compounds renders impractical the preparation of derivatives containing a free aldehyde group, substances which would be desirable from the standpoint of ease of condensation with D-penicillamine to form benzylpenicilloic acid derivatives. Such compounds probably could be prepared, however, from the corresponding mercaptal by reaction with mercuric chloride.<sup>7</sup> The diethyl mercaptal of  $\alpha$ -methylpenaldic acid was prepared by treating the diethyl acetal with ethyl mercaptan and dry hydrogen chloride.

### Experimental<sup>8</sup>

**DL- $\alpha$ -Methylbenzylpenaldic Acid Diethyl Acetal (IIIa).**<sup>9</sup>—DL-Phenylacetylalanine, 18 g., 25 ml. of acetic anhydride and 17 ml. of ethyl orthoformate were refluxed for 30 minutes. The solvents were removed under reduced pressure and the oily residue was shaken with 100 ml. of *N* sodium hydroxide for 4 hours. During the shaking most of the product dissolved. After extraction of the alkaline solution with ether it was cooled in an ice-bath and acidified to pH 2 with cold concentrated hydrochloric acid. The resulting precipitate solidified on scratching. After standing in the refrigerator overnight the crystalline acid was filtered, dried, washed with ether and recrystallized from aqueous ethanol, m.p. 136–137° (dec.); yield 8.2 g. (31%).

*Anal.* Calcd. for  $C_{16}H_{23}O_5N$ : C, 62.12; H, 7.49; N, 4.53. Found: C, 62.48; H, 7.56; N, 4.63.

**Methyl DL- $\alpha$ -Methylbenzylpenaldate (IIIb).**—To a solution of the acid IIIa (3.2 g.) in methanol (10 ml.) was added an ethereal solution of diazomethane until the yellow color persisted. After concentration of the solution to dryness the residue was recrystallized several times from ether-pentane, m.p. 105–106°.

*Anal.* Calcd. for  $C_{17}H_{25}O_5N$ : N, 4.34. Found: N, 4.19.

**DL- $\alpha$ -Methylbenzylpenaldamide Diethyl Acetal (IIIc).**—Methyl DL- $\alpha$ -methylbenzylpenaldate diethyl acetal (1.0 g.) was dissolved in 10 ml. of ethanol and an equal volume of concentrated aqueous ammonia was added. After 24 hours the crystalline deposit was filtered off and was shown to be the starting material. The experiment was repeated in a pressure bottle at 45° for ten days. Again the starting methyl ester was recovered.

The oxazolone was prepared by dissolving the acid IIIa (3.2 g.) in acetic anhydride, heating on the steam-bath for 10 minutes, and removing the solvent under reduced pressure. After drying overnight in a vacuum desiccator the oxazolone was dissolved in 50 ml. of dry ether and the solution was saturated with dry ammonia, stoppered and allowed to stand overnight. On concentration of the solution and the addition of hexane, crystals separated, which were recrystallized from ether-hexane, m.p. 123–124°.

*Anal.* Calcd. for  $C_{16}H_{21}O_4N_2$ : C, 62.31; H, 7.85; N, 9.09. Found: C, 62.67; H, 7.84; N, 9.19.

(7) M. L. Wolfrom, *THIS JOURNAL*, **51**, 2188 (1929).

(8) All m.p.'s were taken in open capillary tubes and are corrected for stem exposure.

(9) This preparation is essentially that of Catch, Cook and Heilbron (ref. 5) but has been modified considerably to obtain better yields.

**DL- $\alpha$ -Phenylacetyl-amido- $\alpha$ -diethylmercaptomethylpropionic Acid.**—DL- $\alpha$ -Methylbenzylpenaldic acid diethyl acetal (200 mg.) was covered with 5 ml. of ethyl mercaptan, placed in an ice-bath, and dry hydrogen chloride passed into the suspension until the solid went into solution. The flask was tightly stoppered and left in the refrigerator overnight. The solvent was blown off with nitrogen, the crystalline residue washed with ether and recrystallized from aqueous ethanol, m.p. 116°.

*Anal.* Calcd. for  $C_{16}H_{23}O_3S_2N$ : S, 18.8; N, 4.12. Found: S, 18.4; N, 4.16.

**Resolution of DL- $\alpha$ -Methylbenzylpenaldic Acid Diethyl Acetal.**—The racemic acid (9.3 g.) and 12.1 g. of anhydrous brucine were suspended in 300 ml. of water and heated to boiling. When the solid material had all dissolved the solution was filtered and left in the refrigerator for two days. The brucine salt which had crystallized out, 10.8 g., was filtered. Concentration of the mother liquor to about half-volume afforded a further small crop. The combined salts were recrystallized from water containing a little ethanol, until the specific rotation became constant ( $-33^\circ$ ).

The salt was suspended in 50 ml. of *N* sodium hydroxide, and stirred for 30 minutes. After removal of the brucine by filtration the filtrate was cooled in an ice-bath and acidified to pH 2 with concd. hydrochloric acid. The precipitated acid was filtered and recrystallized from aqueous alcohol, 2.2 g., m.p. 148–150° (dec.);  $[\alpha]^{25}_D -10.5 \pm 1.0^\circ$  (*c*, 1.08 in chloroform);  $[\alpha]^{25}_D +9.6 \pm 1.0^\circ$  (*c*, 1.06 in *N* sodium hydroxide).

*Anal.* Calcd. for  $C_{16}H_{23}O_5N$ : C, 62.12; H, 7.49; N, 4.53. Found: C, 62.39; H, 7.44; N, 4.49.

To the mother liquor from the first crystallization of the brucine salts was added 40 ml. of *N* sodium hydroxide, with stirring. The brucine was filtered, the filtrate cooled in an ice-bath, and acidified to pH 2 with concentrated hydrochloric acid. The precipitated acid was removed by filtration and was twice recrystallized from aqueous ethanol, 2.4 g., m.p. 148–149.5°;  $[\alpha]^{25}_D +8.8 \pm 1.0^\circ$  (*c*, 1.26 in chloroform);  $[\alpha]^{25}_D -8.3 \pm 1.0^\circ$  (*c*, 1.17 in *N* sodium hydroxide).

*Anal.* Calcd. for  $C_{16}H_{23}O_5N$ : N, 4.53. Found: N, 4.44.

**Methyl Esters of the D- and L-Acids.**—The optically active acid (1.0 g.) obtained from the insoluble brucine salt (which has tentatively been assigned the L configuration) was dissolved in 2.0 ml. of dry methanol, and a solution of diazomethane in ether was added until the yellow color persisted. The reaction product was crystallized from ether-pentane m.p. 91.5–93°;  $[\alpha]^{25}_D -20^\circ$  (*c*, 0.9 in chloroform).

*Anal.* Calcd. for  $C_{17}H_{25}O_5N$ : C, 63.14; H, 7.79; N, 4.34. Found: C, 62.92; H, 7.92; N, 4.54.

The methyl ester of the D-acid (obtained from the more soluble brucine salt) was similarly prepared, m.p. 91–93°;  $[\alpha]^{25}_D +18^\circ$  (*c*, 1.02 in chloroform).

*Anal.* Found: N, 4.48.

**Oxazolones and Amides of the L- and D-Acids.**—A sample of the L-acid (10 mg.) was dissolved in 1 ml. of acetic anhydride and the rotation measured at intervals until it became constant. After two hours the specific rotation attained a constant value of  $+77^\circ$  (*c*, 1.0 in acetic anhydride). In a similar experiment the D-acid was likewise dehydrated to an optically active oxazolone,  $[\alpha]^{25}_D -74^\circ$  (*c*, 0.98 in acetic anhydride). The L-acid (2.0 g.) was heated on the steam-bath for 30 minutes with 15 ml. of acetic anhydride. The solvent was removed under reduced pressure and the resulting oxazolone was left in an evacuated desiccator overnight. It was dissolved in 20 ml. of dry ether and saturated with dry ammonia. After standing overnight the ether was removed and the residue crystallized several times from ether-pentane, m.p. 103–104.5°;  $[\alpha]^{25}_D -33^\circ$  (*c*, 1.04 in chloroform);  $[\alpha]^{25}_D -23^\circ$  (*c*, 1.11 in ethanol).

*Anal.* Calcd. for  $C_{16}H_{21}O_4N_2$ : C, 62.31; H, 7.85; N, 9.09. Found: C, 62.68; H, 7.79; N, 8.98.

The amide of the D-acid was similarly prepared, m.p. 102–104°;  $[\alpha]^{25}_D +32^\circ$  (*c*, 1.0 in chloroform);  $[\alpha]^{25}_D +23^\circ$  (*c*, 1.11 in ethanol).

**DL- $\alpha$ -Methylphenylpenaldic Acid Diethyl Acetal.**—A mixture of 12 g. of DL-benzoylalanine, 17 ml. of acetic anhydride and 11 ml. of ethyl orthoformate was refluxed for 90 minutes. The solvents were removed under reduced pressure and the residual oil was shaken with 50 ml. of *N* sodium hydroxide for 3 hours. After extraction with ether the basic solution

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  $C_{13}H_{21}O_5N$ : 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.

NEW BRUNSWICK, N. J.

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

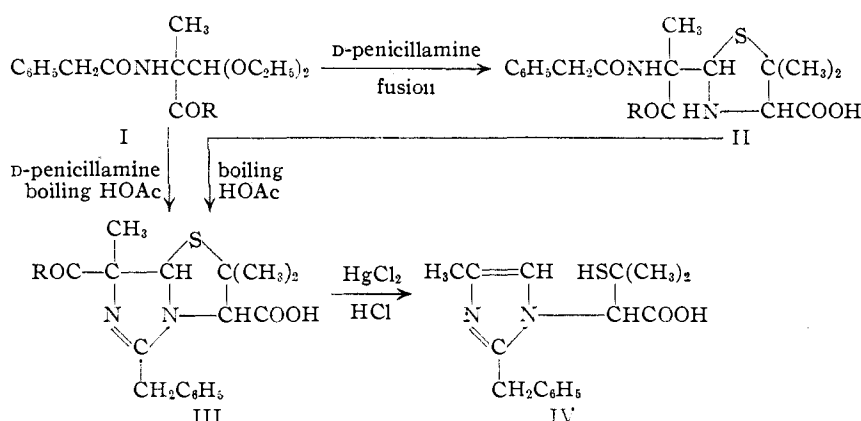
## Homologs of Penicillin Degradation Products. II. 6-Methyl-D-benzylpenillic Acid<sup>1,2</sup>

BY HOMER E. STAVELY<sup>3</sup>

The amides and methyl esters of D- and L- $\alpha$ -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  $\alpha$ -amides and  $\alpha$ -methyl esters of 6-methyl-D-benzylpenillic acid. The two diastereoisomeric  $\alpha$ -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  $\alpha$ -methyl-6-methyl-D-benzylpenicilloate can be an intermediate in the formation of 6-methyl-D-benzylpenillic acid.

A number of optically active  $\alpha$ -methylbenzylpenaldic acid derivatives (I) have been prepared<sup>1</sup> with the object in view of condensing these compounds with D-penicillamine to form 6-methylbenzylpenicilloic acid derivatives (II). The condensation of

In view of these facts the condensation of the L-form of  $\alpha$ -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  $\alpha$ -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,  $C_{13}H_{23}O_3N_3S$ , 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,  $[\alpha]_D +383^\circ$ , 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.



an aldehyde with an  $\alpha$ -amino mercaptan to form a thiazolidine is a facile reaction<sup>4</sup> 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.<sup>5</sup> The acetal group in  $\alpha$ -methylpenaldic acid diethyl acetal derivatives, however, has been found to be remarkably stable<sup>6</sup> and the condensation of these derivatives with D-penicillamine could not be accomplished under a variety of conditions at temperatures up to 100°.

As was the case with the amides, the methyl esters of D- and L- $\alpha$ -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.