Chemical Studies of Potential Relevance to Penicillin Hypersensitivity. The Synthesis of DL-2-Phenoxymethylpenicillenic Acid and of DL-2-(2,6-Dimethoxyphenyl)penicillenic Acid (1)

Kenneth H. Dudley, Daniel L. Bias, and Dorothy Johnson

Center for Research in Pharmacology and Toxicology,
University of North Carolina School of Medicine, Chapel Hill, North Carolina 27514

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Crystalline samples of DL-2-phenoxymethylpenicillenic acid (**14**) and of DL-2-(2,6-dimethoxyphenyl)penicillenic acid (**21**) have been synthesized by the reaction of DL-penicillamine with 2-phenoxymethyl-4-(methoxymethylene)oxazol-5-one and 2-(2,6-dimethoxyphenyl)-4-(ethoxymethylene)oxazol-5-one, respectively. These key oxazol-5-one intermediates were synthesized by azlactonization of the appropriately substituted penaldic acid dialkyl acetals. The reaction of mercury(II) chloride with D-phenoxymethylpenicillin was studied and found to be inapplicable to the synthesis of D-**14**. A comparison of crystalline DL- and of amorphous D-2-(2,6-dimethoxyphenyl)penicillenic acid (**21**), which were prepared by the "oxazolone" and "mercury(II) chloride" methods, respectively, suggests a marked improvement in the purity of the DL-material.

The spontaneous rearrangement of benzylpenicillin to benzylpenicillenic acid in aqueous solutions was first recognized during the penicillin investigations of 1940-1945 (2,3). In 1960, when it was recognized that benzylpenicillenic acid can form conjugates with protein, a role of the penicillenic acid in penicillin allergy was proposed (4,5). The Bern group (6) is at present of the opinion that the direct reaction of the β-lactam of penicillin with protein to form the penicilloyl determinant is the more important pathway by which antigen is formed. There is, however, still sufficient evidence to point to the involvement of a penicillenic acid in production of specific determinant groups, including the penicilloyl determinant (7,8,9).

The more widely employed method for synthesis of a penicillenic acid is the one-step rearrangement reaction by mercury(II) chloride of a "natural" D-penicillin to its *amorphous* D-penicillenic acid (10). A second method requires the synthesis of a 2-substituted-4-alkoxymethyleneoxazol-5-one, which in the final step of the sequence may be condensed under anaerobic conditions with DL-penicillamine (11). In the example of benzylpenicillenic acid the "oxazolone" method (8,11) is far more laborious than the "mercury(II) chloride" method (10), but it offers the versatility of permitting the preparation of a pure *crystalline* DL-benzylpenicillenic acid. In the previous investigation (8) a marked difference was observed in the kinetic behaviors in dilute phosphate buffers between penicillenic acids prepared by the "oxazolone" and "mercury(II) chloride" methods. Because "mercury(II) chloride" preparations of D-benzylpenicillenic acid appeared to contain an impurity which affected kinetic behavior in dilute phosphate buffers (8), we believe the "oxazolone" method is the more reliable means for securing pure penicillenic acids.

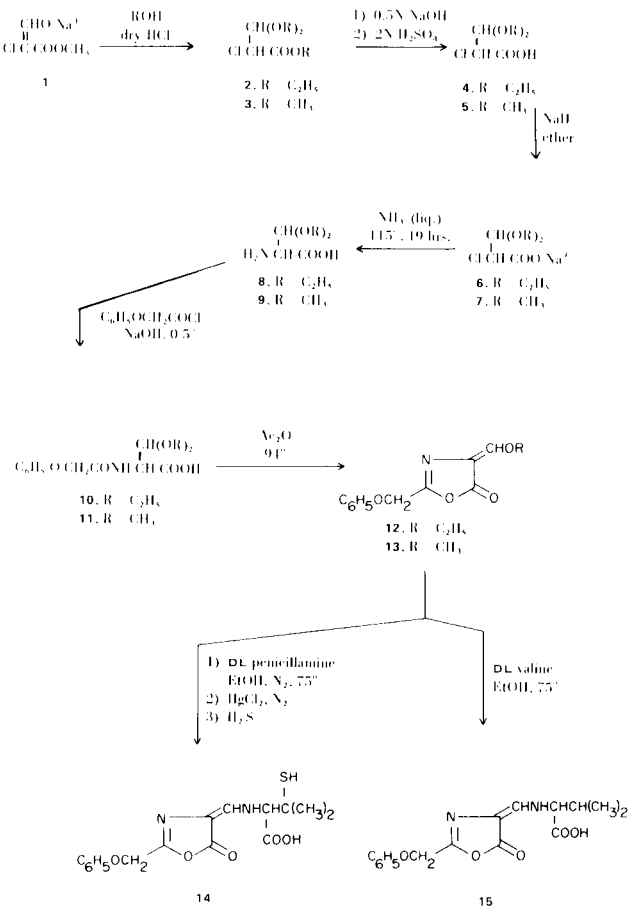
In the present study we report the synthesis of crystalline samples of DL-2-phenoxymethylpenicillenic acid (**14**) and of DL-2-(2,6-dimethoxyphenyl)penicillenic acid (**21**). These penicillenic acids are of interest in relation to the hypersensitivity reactions of phenoxypenicillin and methicillin, respectively. As was the case for benzylpenicillin and its penicillenic acid (8,9), kinetic studies have been made of these crystalline DL-penicillenic acids and of the appearance and disappearance of these penicillenic acids in aqueous buffered solutions of their respective penicillins. The results of these kinetic studies will be reported elsewhere.

Synthesis of DL-2-Phenoxymethylpenicillenic Acid (14).

Our early attempts to synthesize the key 4-alkoxymethyleneoxazol-5-one, **12**, by azlactonization of *N*-(phenoxyacetyl)glycine with a mixture of acetic anhydride and ethyl orthoformate were unsuccessful. This "orthoformate" modification of the azlactone synthesis is also inapplicable to the synthesis of 2-benzyl-4-alkoxymethyleneoxazol-5-one (12), a compound closely related to **12** and the intermediate required for synthesis of DL-benzylpenicillenic acid. In light of this limitation of the "orthoformate" modification, variations of the "penaldate"

CHART I

The Synthesis of DL-2-Phenoxymethylpenicillenic Acid and of DL-2-Phenoxymethyl*desthiol*penicillenic Acid



method (12) were considered for the synthesis of the key oxazolone, **12**. The efforts to construct a penaldic acid by *C*-formylation (12) of *N*-(phenoxyacetyl)glycine methyl ester in a manner successful in the benzyl series (8) were unsuccessful, only heterogeneous mixtures being formed in repeated trials. In another variation of the "penaldate" method (12), there was a choice of elaborating the *sodio* derivative of methyl chloromalonaldehydate (**1**) as its diethyl acetal, **2**, or as its dimethyl acetal, **3**. An advantage of the use of the diethyl acetal, **2**, rested on the fact that the isolation of 2-amino-3,3-diethoxypropionic acid (β,β-diethoxyalanine), **8**, had already been reported (13).

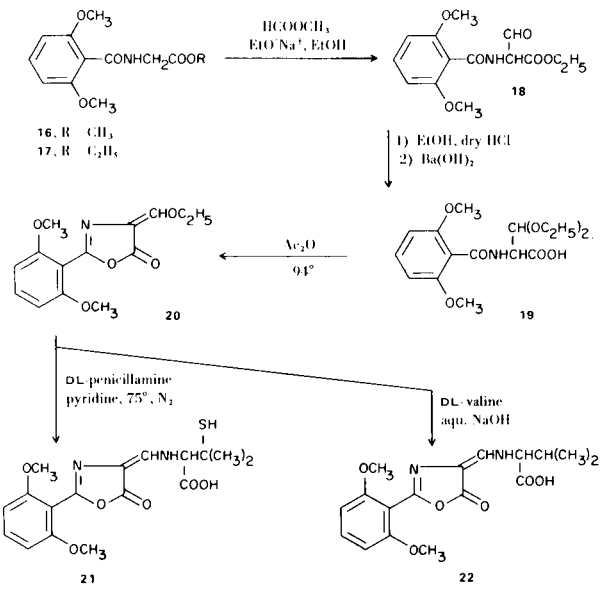
The endeavor to synthesize the oxazolone, **12**, by the latter variation of the "penaldate" method commenced with the diethyl acetal, **2**. Several synthetic steps were accomplished without difficulty to prepare 2-(phenoxyacetamido)-3,3-diethoxypropionic acid (**10**), this compound being the requisite intermediate for preparation of the ethoxymethyleneoxazol-5-one, **12**. Numerous trials were made at azlactonization of **10** with acetic anhydride, and this route involving the diethyl acetal derivatives (*i.e.* **4** → **6** → **8** → **10** → *etc.*) was abandoned when it was recognized that the ethoxymethyleneoxazol-5-one, **12**, could not be obtained in any form of a reasonable degree of purity. The best obtained samples of **12** were sharply odoriferous gums, the ultraviolet spectra indicating purities of about 50% for these preparations.

A successful sequence of reactions to give the key oxazolone intermediate, **13**, and 2-phenoxymethylpenicillenic acid (**14**) was achieved by use of the dimethyl acetal derivative, **3**, of Skokina and Knunyants (14). This compound was converted to its sodium salt, **7**, and the sodium salt **7**, when heated with anhydrous ammonia in a bomb for 19 hours at 110-115°, afforded 2-amino-3,3-dimethoxypropionic acid (β,β-dimethoxyalanine), **9**. The 3,3-dimethoxy acid, **9**, could not be isolated in crystalline form as was the case (13) with the 3,3-diethoxy acid, **8**. The workup procedure, however, was designed so that the amount of 3,3-dimethoxy acid, **9**, produced in the reaction could be quantitated by titration. In this way the conditions of temperature and length of heating period were optimized for the high pressure ammonolysis reaction of **7**. With the knowledge of the quantity of the dimethoxy acid, **9**, present, the solution was made weakly alkaline and was stirred vigorously at 0-5° while a somewhat less than equimolar quantity of phenoxyacetylchloride was added. It was advantageous to add slightly less than the theoretical amount of phenoxyacetylchloride since formation of appreciable amounts of phenoxyacetic acid hindered the isolation of pure crystalline 2-phenoxyacetamido-3,3-dimethoxypropionic acid (**11**). Azlactonization of **11** with acetic anhydride provided in moderate yield the key intermediate, pure crystalline 2-phenoxymethyl-4-(methoxymethylene)oxazol-5-one (**13**).

Special conditions had to be devised for the condensation reaction of 2-phenoxymethyl-4-(methoxymethylene)oxazol-5-one (**13**) and DL-penicillamine. It was evident that the solvent medium of pyridine employed in the synthesis of DL-benzylpenicillenic acid (8) was unsuitable for use in the synthesis of 2-phenoxymethylpenicillenic acid (**14**). Oxygen-free condensation reactions of **13** and DL-penicillamine in pyridine (75°) developed deep yellow-orange colors, and only very inferior penicillenic acid samples with low extinction coefficients at 322-324 nm were obtained. This problem was circumvented by use of absolute ethanol as solvent for the condensation reaction. It was shown in a parallel study that the spectrophotometric yield of 2-phenoxymethylpenicillenic acid (**14**) was optimal when the condensation reaction of **13** and DL-penicillamine was carried out in the absence of oxygen in ethanol for 15 minutes at 75°. Isolation of the penicillenic acid was accomplished by precipitating the phenoxymethylpenicillenic acid as its

CHART II

The Synthesis of DL-2-(2,6-Dimethoxyphenyl)penicillenic Acid and of DL-2-(2,6-Dimethoxyphenyl)*desthiol*penicillenic Acid



mercury(II) salt. The penicillenic acid mercury(II) salt was decomposed by treating its cold suspension in benzene with hydrogen sulfide. The amorphous DL-2-phenoxymethylpenicillenic acid [ϵ (324 nm, ethanol) 20,800] obtained by lyophilization of the benzene was obtained in crystalline form [ϵ (324 nm, ethanol) 26,800-28,500] by recrystallization from methyl acetate-hexane.

A crystalline sample of DL-2-phenoxymethyl*desthiol*-penicillenic acid (**15**) was also prepared by the condensation reaction of **13** and DL-valine in absolute ethanol. The *desthiol* compound had ϵ (322 nm, ethanol) 28,000.

Synthesis of DL-2-(2,6-Dimethoxyphenyl)penicillenic Acid (**21**).

Although the "orthoformate" modification of the azlactone synthesis is limited in scope, the method has had noted success in cases where a substrate is a *N*-(*p*-substituted-benzoyl)glycine (12). In the present investigation the "orthoformate" method proved unsuitable for effecting azlactonization of *N*-(2,6-dimethoxybenzoyl)-glycine to form the corresponding 2-(2,6-dimethoxyphenyl-4-(ethoxymethylene)oxazol-5-one (**20**). This key intermediate was obtained by the "penaldate" method.

Methyl *N*-(2,6-dimethoxybenzoyl)glycinate (**16**) was prepared and its ester function was exchanged for an ethyl group. The glycinate **17** was formylated with methyl formate and sodium ethoxide by the method of Erlenmeyer and Stoop (15,13) to yield a crystalline penaldate, m.p. 123.0-132.5°. The crystallinity of the penaldate compound, **18**, was surprising in view of the fact that penaldates are usually obtained as unstable, polymerizable oils or gums (13). The penaldate **18** was converted to its diethyl acetal and the ethyl ester group was hydrolyzed, the penaldic acid diethyl acetal **19** being isolated as a crystalline product. Azlactonization of **19** with acetic anhydride provided the key intermediate, 2-(2,6-dimethoxyphenyl)-4-(ethoxymethylene)oxazol-5-one (**20**), from which both 2-(2,6-dimethoxyphenyl)-penicillenic acid (**21**) and 2-(2,6-dimethoxyphenyl)*desthiol*-penicillenic acid (**22**) were prepared by the "oxazolone" method. Each of these penicillenic acid derivatives occluded solvent of crystallization. This phenomenon was suggested by the analytical data and, in the case of the penicillenic acid **21**, the presence of solvent of crystallization (methyl acetate) in the crystal lattice was confirmed by mass spectrographic evidence.

Discussion.

When account is taken of the benzyl ($C_6H_5CH_2$-) and the phenoxymethyl ($C_6H_5OCH_2$-) groups as they exist in benzylpenicillin and in phenoxymethylpenicillin, respectively, it is interesting that such slight variation in molecular design can alter so strikingly the apparent chemical properties of these two compounds. Phenoxymethylpenicillin is more stable in acid solution than benzylpenicillin (18) and the rates of formation of the penicillenic acids of these two penicillins are noticeably different in phosphate buffers of *p*H 7.4 (19). Methicillin has an acid stability and rate of penicillenic acid formation more comparable to benzylpenicillin (18,19).

In addition to those differences already noted for the benzyl and phenoxymethyl intermediates in the present investigation, a marked difference was observed in the behaviors of benzylpenicillin and of phenoxymethylpenicillin in their rearrangement reactions to their respective penicillenic acids in the presence of mercury(II) chloride. We find that the "mercury(II) chloride" method does not elicit the rearrangement of phenoxymethylpenicillin and its stabilization as its penicillenic acid as was the case with benzylpenicillin (10) and methicillin (20).

In absolute alcohol the ultraviolet absorbancy of a penicillenic acid is stable (5), and spectrophotometric measurements of solutions of various preparations of benzylpenicillenic acid in this solvent have been used to assess purities (8). It may be seen that a small but definite difference does exist in extinction coefficients when a comparison is made of the extinction coefficients at 322 nm of ethanolic solutions of crystalline DL-2-phenoxymethylpenicillenic acid (ϵ, 28,600) of the present study and of crystalline DL-benzylpenicillenic acid (ϵ, 29,500) of the previous study (8). An explanation for this small discrepancy in the extinction coefficients is not presently at hand. A comparison of the extinction coefficient a 330 nm of crystalline DL-2-

(2,6-dimethoxyphenyl)penicillenic acid (ϵ, 28,400) of the present study and the reported (20) value at 333 nm of amorphous D-2-(2,6-dimethoxyphenyl)penicillenic acid (ϵ, 23,400), which was prepared by the "mercury-(II) chloride" method, indicates a substantial improvement in the purity of this penicillenic acid when prepared by the "oxazolone" method.

EXPERIMENTAL

Thin layer chromatograms were prepared by coating microscope slides with Silica Gel H. Suitable solvent systems were benzene:ethyl acetate:acetic acid in proportions of 7:3:1 and of 90:10:1. Zones were visualized by spraying the eluted chromatograms with 5% phosphomolybdic acid in ethanol (PMA) and, then, baking the slide on the surface of a hot plate (zones were developed within minutes).

Micromelting points were taken on a Kofler hot stage microscope and are uncorrected.

Infrared spectra were measured with a Perkin-Elmer Model 257 instrument; samples were prepared in the form of pressed KBr disks.

Mass spectra (70 eV) were measured on a AEI-MS-902 spectrometer at the Research Triangle Institute Center for Mass Spectrometry. High resolution mass spectrographic measurements (peak matchings) were conducted by Mr. F. Williams (RTI Laboratory).

Microanalyses were carried out by Micro-Tech Laboratories, Skokie, Illinois.

Sodio Methyl Chloromalonaldehydate (1) and Methyl Chloromalonaldehydate.

The sodio derivative, **1**, was prepared in 84% yield by the method of Skokina and Knunyants (14). Methyl chloromalonaldehydate was obtained by addition of **1** (244 g.) to 1400 ml. of cracked ice containing 130 ml. of 12 *N* hydrochloric acid. This mixture was extracted immediately with 6 x 300 ml. of ether. The combined ether extract was washed with saturated sodium sulfate (200 ml.), dried (anhydrous sodium sulfate), and evaporated to an oil. The vacuum dried oil was further dried by allowing its chloroform solution to stand over anhydrous magnesium sulfate. Evaporation gave a solid (112 g., 55%), m.p. 64-89° with sublimation. An analytical sample, m.p. 70-90°, was prepared by recrystallization from ethyl acetate-hexane. Mass spectrum: Calcd. for $C_4H_5ClO_3$, 135.9927; Found, 135.9930.

Anal. Calcd. for $C_4H_5ClO_3$: C, 35.18; H, 3.69. Found: C, 35.20; H, 3.63.

Ethyl 2-Chloro-3,3-diethoxypropionate (2).

A solution of methyl chloromalonaldehydate (31 g.) in absolute ethanol (350 ml.) saturated with dry hydrogen chloride at 0-5° was allowed to stand at 25° for 2 days. Volatiles were removed under reduced pressure (aspiration) with the aid of benzene, and the residual oil was distilled through an annular spinning band column to give 28 g. of **2**, b.p. 104-105°/10 mm. Lit. (16) b.p. 108-109°/11 mm.

2-Chloro-3,3-diethoxypropionic Acid (4) and its Sodium Salt (6).

The free acid was prepared by alkaline hydrolysis of its ethyl ester **2** in nearly quantitative yield (17). The dry acid was converted to the sodium salt **6** as described for the case of the dimethoxy acid sodium salt of **7**.

2-Amino-3,3-diethoxypropionic Acid (β,β-Diethoxyalanine) (8).

This compound was prepared in the present study in 22% yield by the reaction of 2-chloro-3,3-diethoxypropionic acid sodium salt, **6**, (5.0 g.) and dry, distilled ammonia (50 ml.) in a stainless steel bomb (200 ml.) at 110° for 23 hours. The ethanol-soluble material of the bomb residue was recrystallized from ethanol containing a small amount of water, the amino-acid **8** being isolated as white needles (0.781 g., m.p. 186-188° dec.). The compound gave a neutralization equivalent of 196.5 (Calcd. for $C_7H_{15}NO_4 \cdot H_2O$: 195.3) and an apparent pK_2 of 8.70. Lit. (13,17), m.p. 189-190°, m.p. 170-200°.

2-Phenoxyacetamido-3,3-diethoxypropionic Acid (10).

β,β-Diethoxyalanine, (**8**) (532 mg., 3.0 mmoles) in 17 ml. of water containing 6.0 meq of sodium hydroxide was *N*-acylated at 0-5° with phenoxyacetylchloride (513 mg., 3.0 mmoles) in the manner described below for 2-phenoxyacetamido-3,3-dimethoxypropionic acid (**11**). The usual workup gave a nearly chromatographically uniform oil (900 mg.) which was recrystallized from ethyl acetate-hexane to give **10** (645 mg.), m.p. 61-64° and 71-73.5° (dimorphic).

Anal. Calcd. for $C_{15}H_{21}NO_6$: C, 57.78; H, 6.89; N, 4.32. Found: C, 57.87; H, 6.80; N, 4.49.

Attempts to prepare 2-phenoxymethyl-4-ethoxymethylene-oxazol-5-one (**12**), by cyclization of the above penaldic acid diethyl acetal with acetic anhydride in the usual manner (12) resulted only in isolation of gums [ϵ (284 nm, ethanol) 8,300-9,200] which resisted all attempts at crystallization.

Methyl 2-Chloro-3,3-dimethoxypropionate (3).

The sodium salt, **1**, was converted to **3** by the method of Skokina and Knunyants (14), a yield of 120 g. of analytically pure **3**, b.p. 96°/18 mm, being obtained by distillation of the crude product (173 g.) through an annular spinning band column. Lit. (14) b.p. 95-97°/18 mm. The nmr spectrum was in accordance with **3** and elemental analyses of C, H, and Cl were satisfactory (± 0.20).

2-Chloro-3,3-dimethoxypropionic Acid (5) and its Sodium Salt (7).

The alkaline hydrolysis of **3** to **5** was conducted as described previously for conversion of the ethyl ester **2** to its acid **4** (17). The acid was dried repeatedly by allowing its methylene chloride solution to stand over fresh portions of anhydrous sodium sulfate. A small sample was distilled for analysis at b.p. 101° and an initial pressure of 3.0 mm. Distillation of a large sample (45 g.), was accompanied by considerable decomposition of the sample, only 20% yield of distillate being obtained; nmr (3% deuteriochloroform): δ 3.46 (s, 6p, *gem*-OC*H*$_3$), δ 4.33 (d, J = 7 cps, 1p, >C*H* CH<), and δ 4.73 (d, J = 7 cps, 1p, >CH–C*H*<).

Anal. Calcd. for $C_5H_9ClO_4$: C, 35.62; H, 5.38; Cl, 21.03. Found: C, 35.88; H, 5.44; Cl, 21.20.

To prepare the sodium salt **7**, the dry acid **5** (18.9 g., 0.11 mole) was added dropwise during 30 minutes to a vigorously (mechanically) stirred suspension of sodium hydride dispersion (50% in oil, 5.2 g., 0.11 mole) in 1.2 l. of dry ether (freshly distilled from sodium hydride). The mixture was stirred for two days. In runs where hydride still persisted, excess hydride was decomposed with methanol prior to filtration, washing (ether), and drying *in vacuo* (40°) of the sodium salt **7**. The yield is nearly quantitative.

2-(Phenoxyacetamido)-3,3-dimethoxypropionic Acid (11).

A mixture of 10.0 g. of 2-chloro-3,3-dimethoxypropionic acid sodium salt (**7**) and 100 ml. of dry distilled ammonia in a stainless steel bomb (470 ml. capacity) was allowed to stand for 19 hours

in an oven maintained at 115°. The ammonia was allowed to evaporate, and the bomb was warmed gently (60-75°) for 1-2 minutes. The vacuum dried residue was dissolved in 100 ml. of methanol, this solution was filtered and evaporated under reduced pressure (45°), and the dark brown residual gum was dried in high vacuum for 18 hours. Titration of a solution of the gum in 80 ml. of water with 1 *N* sodium hydroxide indicated the presence of 17.9 meq of 2-amino-3,3-dimethoxypropionic acid, the solution being titrated with base until a total of 36.0 meq of base had been added. The alkaline solution was transferred to a Morton flask and, after chilling to 5-10° and with vigorous mechanical stirring, 2.90 g. (17.0 mmoles) of phenoxyacetylchloride was added dropwise. After the reaction had been stirred for 2 hours at 5-10° and for 0.5 hour at 25°, the mixture was treated with charcoal and filtered, and the alkaline filtrate was extracted with 2 x 50 ml. of ether. The ether extracts were discarded.

The alkaline solution was chilled by addition of crushed ice (so that it persisted), an equal volume of ice-cold chloroform was added, and the mixture was acidified with 6 ml. of 6 *N* hydrochloric acid and shaken. The organic layer was drawn off and the aqueous layer was further extracted with 3 x 50 ml. of chloroform. The combined chloroform extract was dried (anhydrous sodium sulfate) and evaporated to a light brown oil (3.4 g.). Tlc showed two zones, the more mobile zone (which with 5% PMA developed as an orange-brown zone) being the contaminant, phenoxyacetic acid. Chromatographically pure *2-phenoxyacetamido-3,3-dimethoxypropionic acid* (1.68 g., m.p. 106-112°) was obtained by dissolving the sample (3.4 g.) in the minimum volume of hot ethyl acetate, chilling (-12°), seeding, and after the recrystallization appeared complete at -12°, by addition of small volumes of hexane to the mixture at 25°. The analytical sample, m.p. 111.5-114.5°, was prepared by further recrystallization from ethyl acetate-hexane; ir: 3430, 3350, 2940, 2840, 1741, 1720, and 1625 cm^{-1}.

Anal. Calcd. for $C_{13}H_{17}NO_6$: C, 55.12; H, 6.05; N, 4.95. Found: C, 54.86; H, 6.11; N, 4.95.

2-Phenoxymethyl-4-(methoxymethylene)oxazol-5-one (**13**).

A solution of 2.0 g. of **11** in 10 ml. of acetic anhydride was heated in a water bath (94°) for 30-35 minutes and then was stripped of volatiles (40°/40 mm). The residue, a gum, crystallized upon standing at -12° (12 hours). The solid was triturated under small portions of ether until the vacuum dried product contained a weak, if any, odor of acetic anhydride. The yield of chromatographically uniform (tlc) **13** was 990 mg., m.p. 81-86°; [ϵ (283 nm, absolute ethanol) 17,300]. A sample recrystallized from *dry* ether for analysis had m.p. 86.3-87.0°; [ϵ (283 nm, ethanol) 17,300]; ir: 1800 (shoulder), 1775, and 1682 cm^{-1}.

Anal. Calcd. for $C_{12}H_{11}NO_4$: C, 61.80; H, 4.75; N, 6.01. Found: C, 61.86; H, 4.82; N, 5.86.

DL-Phenoxymethylpenicillenic Acid (**14**).

A four-neck flask was equipped with a mechanical stirrer, two pressure equalizing funnels fitted with nitrogen inlets, and a condenser fitted with a drying tube. A capillary tubing (1 mm inner diameter) was inserted through one of the nitrogen inlet lines, the tubing extending to the base of the addition funnel. The exterior end of the tubing contained an inserted 18-ga. syringe needle with a Luer-Lok fitting. This tubing aided the anaerobic addition of a cold aqueous mercury(II) chloride solution to the addition funnel after completion of the "oxazolone-condensation" reaction.

The conditions of temperature and of length of heating period for the oxazolone-condensation reaction were determined in separate experiments by study of the course of development of 324 nm absorbancy in anaerobic ethanolic solutions of DL-penicillamine and 2-phenoxymethyl-4-(methoxymethylene)oxazol-5-one (**13**) under varying conditions of temperature and time.

The reaction vessel was charged with the dry powders of 2-phenoxymethyl-4-(methoxymethylene)oxazol-5-one (**13**) (250 mg. 1.08 mmoles) and DL-penicillamine (188 mg., 1.25 mmoles), and the addition funnel not having the inserted capillary tubing was charged with absolute ethanol (30 ml.). The system was flushed for 5 minutes with a strong stream of nitrogen saturated with absolute ethanol. The nitrogen stream was reduced, the absolute ethanol was added, vigorous stirring was commenced, and a bath preheated to 75° was raised around the vessel. Vigorous stirring and heating were maintained for exactly 15 minutes, whereafter the heating bath was removed and the reaction mixture was quickly chilled to 5-10°. A cold solution of 406 mg. (1.50 mmoles) of mercury(II) chloride in 15 ml. of water was added anaerobically to the addition funnel *via* the capillary tubing. This solution was added to the condensation reaction and was followed by the dropwise addition under oxygen free conditions (7-9 minutes) of 75 ml. of cold water. Stirring was continued (5-10°) for 5 minutes after the addition was complete and, then, the white precipitate containing the mercury(II) salt of the penicillenic acid was collected by centrifugation and filtration. The salt was washed thoroughly with cold water and vacuum dried. While partially damp, the sample was triturated under 4 x 5 ml. portions of ether and was then vacuum dried to constant weight. The yield of salt varied in the range of 314-334 mg. In ethanol the salt had an absorption maximum that varied in different preparations from 334-339 nm (ϵ, 14,200-15,600). The ethanol-insoluble portion of the salt is probably a mercury(II) salt of unreacted DL-penicillamine.

The mercury(II) salt (316 mg.) was wetted with 5 ml. of cold water, 30 ml. of cold benzene was added, and hydrogen sulfide was bubbled through the cold mixture (8°) for 2-5 minutes until the precipitation of mercury(II) sulfide was complete. The cold benzene layer was isolated, dried (anhydrous sodium sulfate), filtered, and lyophilized to yield an amorphous white powder (40 mg.), [ϵ (324 nm, ethanol) 20,800]. The powder (40 mg.) was covered with 0.20 ml. of methyl acetate and the crystalline material which formed shortly was collected and washed successively with 0.10 and 0.20 ml. of methyl acetate. The yield of the vacuum dried material was 30 mg. This solid was dissolved in the minimum volume of methyl acetate (~4 ml.) at ambient temperature and recrystallized by the gradual addition of hexane. The yield of crystalline DL-phenoxymethylpenicillenic acid was 14 mg., m.p. 137.0-138.5° dec. The uv spectrum of this material showed [ϵ (324 nm, ethanol) 26,800]. The highest extinction coefficient obtained for a sample of this penicillenic acid was 28,600; ir: 2550, 1740, 1655, and 1600 cm^{-1}. Mass spectrum (70 ev) m/e (relative intensity): M^+ not observed, 332 (13), 209 (23), 191 (11), 179 (12), 168 (15), 163 (27), 135 (12), 115 (11), 114 (20), 108 (27), 107 (67), 100 (33), 94 (47), 87 (19), 82 (12), 79 (40), 78 (20), and 77 (100). Calcd. for $C_{16}H_{16}N_2O_4S$ (M^+ -18): 332.0831, Found: 332.0831.

Anal. Calcd. for $C_{16}H_{18}N_2O_5S$: C, 54.84; H, 5.18; N, 7.99. Found: C, 54.67; H, 5.09; N, 7.75.

Mercury(II) Chloride Rearrangement of Potassium Phenoxymethylpenicillin.

The mercury(II) chloride reaction of 4.0 g. of the potassium salt of phenoxymethylpenicillin (Pen Vee K, Wyeth) and 3.26 g. of mercury(II) chloride, as described by Levine (10), afforded

2.6 g. of a white insoluble mercury salt. This material did not have an ultraviolet absorption peak (320-340 nm) characteristic of a penicillenic acid mercury(II) salt (8). Decomposition of the salt with hydrogen sulfide and the usual workup (8,10) gave 50 mg. of white powder, [ϵ (322 nm, ethanol) 7,300].

DL-Phenoxymethyl*desthiol*penicillenic Acid (**15**).

A mixture of 112 mg. (0.48 mmole) of **13**, 65 mg. (0.55 mmole) of DL-valine, and 50 ml. of absolute ethanol was stirred mechanically and heated at 75° for 75 minutes. Samples were withdrawn and the changes in absorbancy in the range of 260-350 nm were observed during the course of the reaction. The absorbancy at 322 nm remained constant after 70 minutes, the final value indicating a spectrophotometric yield of *desthiol*-penicillenic acid of 73%. The reaction mixture was evaporated to dryness under reduced pressure and the residual gum was partitioned between ethyl acetate-water. The organic layer was dried (anhydrous sodium sulfate) and impinged with nitrogen to a crystalline residue, which was triturated under a small volume of cold ethyl acetate and filtered off. The product (64 mg.) was recrystallized from ethyl acetate to give 37 mg. of chromatographically uniform (tlc) needles of **15**, m.p. 168-173° [ϵ (322 nm, ethanol) 28,500]. The analytical sample which was prepared by further recrystallization from ethyl acetate, had m.p. 168-172°; [ϵ (322 nm, ethanol) 28,800].

Anal. Calcd. for $C_{16}H_{18}N_2O_5$: C, 60.37; H, 5.70; N, 8.80. Found: C, 60.21; H, 5.62; N, 8.74.

Methyl *N*-(2,6-Dimethoxybenzoyl)glycinate (**16**).

A solution of 150 g. (0.75 mole) of 2,6-dimethoxybenzoyl-chloride in 200 ml. of methylene chloride was added dropwise to a cold (0-5°), mechanically stirred mixture of 104 g. (0.83 mole) of methyl glycinate hydrochloride and 168 g. (1.65 mole) of triethylamine in 1.5 l. of methylene chloride. The addition required 1 hour and the final mixture was stirred at 0-5° for 2 hours. The reaction mixture, which contained insoluble triethylamine hydrochloride, was extracted successively with 2 x 1 l. of 0.5 *N* hydrochloric acid, 1 l. of water, and 2 x 500 ml. of 0.1 *M* sodium bicarbonate solution. The organic layer was dried (anhydrous sodium sulfate) and evaporated to a chromatographically pure crystalline residue of **16** (157 g.), m.p. 108-109°, which was finely pulverized, washed with ether, and vacuum dried. An analytical sample of m.p. 110-111° was prepared by recrystallization from ethyl acetate.

Anal. Calcd. for $C_{12}H_{15}NO_5$: C, 56.91; H, 5.97; N, 5.53. Found: C, 57.12; H, 6.03; N, 5.63.

N-(2,6-Dimethoxyphenyl)glycine.

A solution of 50 g. (0.198 mole) of **16** in 500 ml. of absolute methanol was treated with 50 ml. of 4 *N* sodium hydroxide solution and allowed to stand at 25° for 4 hours. The solution was concentrated at the aspirator (40°) to a volume of 75 ml., and the concentrate was diluted with an equal volume of water and acidified with 20 ml. of 12 *N* hydrochloric acid. The white precipitate was filtered off, washed thoroughly with water, and vacuum dried. The chromatographically pure product (46 g.) had m.p. 209-219°. After two recrystallizations from absolute methanol, the compound had m.p. 207-221°; uv: [ϵ (280 nm, absolute ethanol) 1,560].

Anal. Calcd. for $C_{11}H_{13}NO_5$: C, 55.23; H, 5.48; N, 5.86. Found: C, 55.22; H, 5.55; N, 5.90.

Ethyl *N*-(2,6-Dimethoxybenzoyl)glycinate (**17**).

A solution of 25 g. of *N*-(2,6-dimethoxybenzoyl)glycine in 475 ml. of absolute ethanol containing 0.6 ml. of concentrated sulfuric acid (sp. gr. 1.84) was stirred overnight at 25°, after which time 250 ml. of benzene was added and the ternary was distilled off over a period of 6 hours. The residual liquid was evaporated *in vacuo* to a solid which was suspended in water and filtered off. The vacuum dried product (22 g.) was recrystallized from ethyl acetate-hexane to give soft white needles (21 g.), m.p. 110.0-110.5°.

Anal. Calcd. for $C_{13}H_{17}NO_5$: C, 58.39; H, 6.42; N, 5.24. Found: C, 58.69; H, 6.36; N, 5.06.

Ethyl 2,6-Dimethoxyphenylpenaldate (**18**).

To a solution of sodium ethoxide prepared by dissolving 8.28 g. (0.36 mole) of cleanly cut sodium in 160 ml. of absolute ethanol was added 21.6 g. (0.36 mole) of methyl formate. After 1 hour, 20.0 g. (0.075 mole) of **17** was added and the resulting mixture was stirred at ambient temperature for 24 hours. The solvent was removed *in vacuo* with the aid of benzene (bath 40-50°) and the solid residue was added to a cold (0-5°) mixture composed of 600 ml. of water and ice, 400 ml. of chilled chloroform and 40 ml. of chilled 12 *N* hydrochloric acid. The mixture was shaken, the organic layer was isolated, and the aqueous acid layer was washed with fresh portions of chloroform. The combined chloroform extract was washed with a small portion of water, dried (anhydrous sodium sulfate), and evaporated to a white crystalline residue (14.2 g.). The product was recrystallized from ethyl acetate-hexane to give 12.2 g. of **18**, m.p. 123.0-132.5° which was chromatographically pure by tlc; uv: [ϵ (274 nm, absolute ethanol) 5,700].

Anal. Calcd. for $C_{14}H_{17}NO_6$: C, 56.94; H, 5.80; N, 4.74. Found: C, 56.77; H, 5.78; N, 4.66.

A dinitrophenylhydrazone derivative, m.p. 197-199°, was prepared in the usual manner and was recrystallized from absolute ethanol for analysis.

Anal. Calcd. for $C_{20}H_{21}N_5O_9$: C, 50.50; H, 4.46; N, 14.73. Found: C, 50.44; H, 4.49; N, 14.55.

2-(2,6-Dimethoxyphenyl)-3,3-diethoxypropionic Acid (**19**).

A suspension of 22.0 g. (0.0746 mole) of **18** in 350 ml. of absolute ethanol was treated with 70 ml. of cold absolute ethanol which had been saturated at 5° with dry hydrogen chloride. The mixture was allowed to stand at 5-7° with occasional swirling, and, after 22 hours, the pure yellow solution was diluted with 200 ml. of benzene and was evaporated under reduced pressure (aspirator, 45°). The residue was dissolved in 250 ml. of ether and this solution was washed with 500 ml. of iced-water. The aqueous layer was back-washed with ether, and the combined ether solution was dried (anhydrous sodium sulfate) and evaporated to a pale yellow oil (19.2 g.) assumed to be the diethyl acetal of ethyl (2,6-dimethoxyphenyl)penaldate. Tlc indicated the material was essentially pure, a minor contaminant being the starting penaldate.

The mixture of the oily diethyl acetal (19.2 g., 0.052 mole) in 250 ml. of water containing 8.91 g. (0.052 mole) of barium hydroxide (anhydrous) was stirred at 25° until complete solution formed (~ 2 hours). The solution was filtered, and enough ice was added to the filtrate so that ice still persisted after an equal volume of chloroform had been added and chilled by shaking. Cold 12 *N* hydrochloric acid (10 ml., 0.120 Equiv.) was added, the mixture was shaken, and, after drawing off the chloroform, the mixture was further extracted with 2 x 100 ml. of cold chloroform. The combined chloroform extract was dried (anhydrous magnesium sulfate) and evaporated to a chromatographi-

cally uniform (tlc) oil which was recrystallized from ethyl acetate-hexane with the aid of seed crystals to give 12.1 g., m.p. 110-112°, of 2-(2,6-dimethoxyphenyl)-3,3-diethoxypropionic acid (**19**). The analytical sample, which was prepared by further recrystallization from ethyl acetate-hexane had m.p. 115.5-117.5°; uv: [ϵ (280 nm, absolute ethanol) 2,160].

Anal. Calcd. for $C_{16}H_{23}NO_7$: C, 56.29; H, 6.79; N, 4.10. Found: C, 56.44; H, 6.79; N, 4.15.

2-(2,6-Dimethoxyphenyl)-4-(ethoxymethylene)oxazol-5-one (**20**).

A mixture of 5.0 g. of **19** in 15 ml. of acetic anhydride was heated on the water bath (94°) for 10 minutes, the volatiles were removed under reduced pressure (45°/10 mm), and the crystalline residue was recrystallized from ethyl acetate-hexane. The white crystalline product (2.9 g.) was chromatographically uniform and had m.p. 189-194°. The analytical sample had m.p. 187.5-193.5°; ir: 1780 and 1673 cm^{-1}; uv: [ϵ (292 nm, absolute ethanol) 19,400].

Anal. Calcd. for $C_{14}H_{15}NO_5$: C, 60.64; H, 5.45; N, 5.05. Found: C, 60.68; H, 5.49; N, 4.97.

DL-2-(2,6-Dimethoxyphenyl)penicillenic Acid (**21**).

A solution of 600 mg. (2.17 mmoles) of **20** and 360 mg. (2.42 mmoles) of DL-penicillamine in 100 ml. of dry pyridine containing 2.6 ml. of triethylamine was stirred and heated at 75° (oil bath) under nitrogen for 30 minutes. The reaction was worked up as previously described in the "oxazolone method" for DL-benzylpenicillenic acid (8) except that chloroform was used in place of benzene as a solvent for partitioning the crude product against buffered phosphate solutions (8,11). The chloroform was dried (anhydrous sodium sulfate) and evaporated under reduced pressure (nitrogen atmosphere) to give a residue which after vacuum drying amounted to 506 mg. (orange powder). This powder was dissolved completely in 1.2-1.5 ml. of chloroform (34-37°) and crystalline material was induced to separate by scratching. After chilling the mixture, the snow-white microcrystalline powder (309 mg.) was collected and was recrystallized from its solution in methyl acetate (25 ml.) by addition of hexane (25 ml.). The yield was 184 mg., m.p. 131-134°. The analytical data suggested the presence of ½ mole of methyl acetate per mole of compound. The occluded solvent could not be removed by vacuum drying at 45°. The presence of methyl acetate in the dried product was confirmed by mass spectrographic data. Based upon a molecular formula of $C_{17}H_{20}N_2O_6S \cdot \frac{1}{2}C_3H_6O_2$ (417.4), [ϵ (330 nm, ethanol) 28,400]; Lit. (20) [ϵ (333 nm, methanol) 23,400]. The analytical sample m.p. 131-134° and [ϵ (330 nm, ethanol) 28,700] was prepared by further recrystallization from methyl acetate-hexane and was dried in high vacuum at 45°.

Anal. Calcd. for $C_{17}H_{20}N_2O_6S \cdot \frac{1}{2}C_3H_6O_2$: C, 53.18; H, 5.51; N, 6.70; S, 7.68. Found: C, 53.21; H, 5.51; N, 6.69; S, 7.62.

The mass spectral evidence for the presence of methyl acetate in the crystal lattice of the penicillenic acid **21** was obtained by Dr. D. Rosenthal and Mr. F. Williams of the Research Triangle Institute Center for Mass Spectrometry. In the first experiment, the mass spectrographs were recorded at 70 ev of samples of **21** at inlet temperatures of 90, 100, 110, 120, and 130°, each of these spectra showing the characteristic predominant peaks of methyl acetate at m/e 74, 59, and 43. In a second experiment, the appearance and disappearance of m/e 74 was observed as a function of time and as the inlet temperature of a sample of **21** was raised from 90 to 150°.

DL-2-(2,6-Dimethoxyphenyl)*desthiol*penicillenic Acid (**22**).

To a magnetically stirred solution of 264 mg. (2.25 mmoles) of DL-valine in 10 ml. of water containing 2.30 meq of sodium hydroxide was added a suspension of 600 mg. (2.17 mmoles) of **20** in 25 ml. of absolute ethanol. The mixture was stirred at 25° for 75 minutes, a yellow solution forming. The ethanol was removed under reduced pressure and the syrupy residue was acidified to *p*H 2 with 6 *N* hydrochloric acid. The orange solid which separated was triturated under 25 ml. of water, filtered, washed well with water, and vacuum dried. The crude product (667 mg.) was recrystallized from ethyl acetate (4 ml., -12°) to give 138 mg., m.p. 110-117° dec., of chromatographically pure crystalline material. The analytical sample was prepared by an additional recrystallization from ethyl acetate and by drying the compound at 75° in high vacuum. The analytical data indicated the presence of ½ mole of ethyl acetate and the extinction coefficient at 330 nm, when based upon a molecular weight of 392.5, was [ϵ (330 nm, ethanol) 28,400].

Anal. Calcd. for $C_{17}H_{20}N_2O_6 \cdot \frac{1}{2}C_3H_8O_2$: C, 58.13; H, 6.16; N, 7.13. Found: C, 57.70; H, 6.16; N, 7.00.

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