An Approach to the Synthesis of 6-Methylpenicillin G (1)

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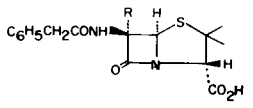
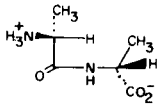
The evidence that penicillin G (**1**) may block bacterial cell wall synthesis as a result of a resemblance to D-alanyl-D-alaine (**3**) led Strominger (2) to suggest that 6-methylpenicillin (**2**) might correspond more closely to the peptide substrate and, as a result, possess an enhanced antibacterial effectiveness. We have not succeeded in synthesizing **2** but the results of our efforts seemed to be of sufficient interest to report at this time. The introduction of a methyl group at C-6 of the penicillin nucleus has been achieved by others (3) by the methylation of the C-6 anion of a derivative of 6-aminopenicillanic acid.

Our approach is based on the report of Robinson (4) who observed that the condensation of oxazolone **4** and thiazoline **5** in pyridine yielded the penicillenic acid analog **7** (Scheme I). A possible intermediate is the adduct **6** which underwent a base-catalyzed elimination to give **7**. If the benzyloxazolone **8** (5) (Scheme II) were substituted for the phenyloxazolone **4** in this transformation, the reaction with **5** might then yield adduct **9**, a potential precursor to the desired **2**. The anticipated result was realized and adduct **9** was isolated in 32% yield.



1 R = H
2 R = CH_3



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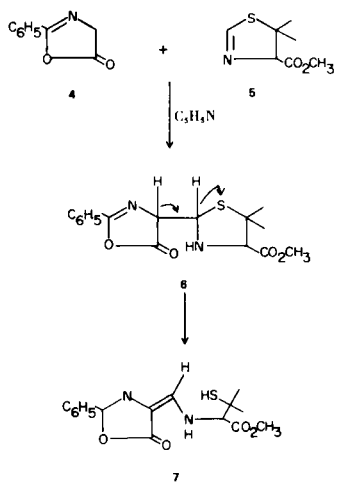
Our efforts to convert **9** to a penicillin under a variety of conditions were unsuccessful. For example, the penillic acid derivative **10** formed upon heating **9** in benzene, a result of attack of the thiazolidine ring nitrogen at the iminoether rather than the carbonyl group of the oxazolone ring to form a five- rather than a four-membered ring. Lithium azide in hot DMF converted **9** into the acyl enamine **13**. We had hoped to generate an equilibrium mixture of **9** and acid azide **15** which would include the desired penicillin ester. Although it is tempting to assume that a penicillin is an intermediate in the formation of **13**, we believe that this is an unlikely possibility.

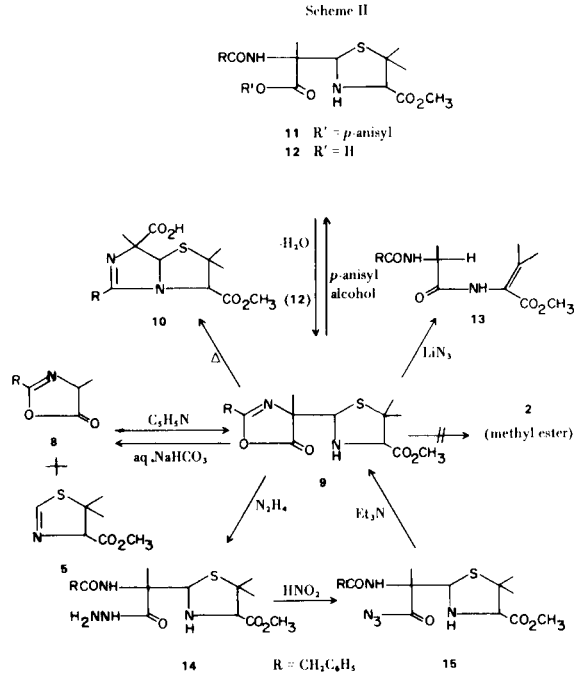
The lack of success in finding conditions for the direct conversion of **9** to a penicillin led us to adopt a more conventional approach *via* the carboxylic acid **12**. Reaction of **9** with aqueous sodium bicarbonate gave the thiazoline and *N*-phenylacetylalanine, resulting from dissociation of the adduct and hydrolysis of **8**. Alcoholysis of **9** with *p*-anisyl alcohol in the presence of triethylamine, however, gave ester **11** cleavage of which with trifluoroacetic acid yielded the carboxylic acid **12**. Oxazolone rather than β-lactam ring formation occurred when **12** was treated with a carbodiimide reagent. A penicillin could not be detected in the reaction product. This result was not wholly unexpected in view of the very low yield of penicillin G reported by Sheehan in the cyclization of benzylpenicilloic acid (6). Acid azide **15** was prepared by hydrazinolysis of **9** and subsequent nitrosation of the hydrazide **14**. It also returned adduct **9** on exposure to triethylamine in methylene chloride.

Scheme I



The stereochemistry of **9** is unknown. If it is assumed that this compound is formed by addition of the anion of oxazolone **8** to the thiazoline, it is possible that the oxazolone ring and the carbomethoxy group are *trans*, a relationship which would correspond to the relative configuration at C-3 and C-5 of the natural penicillins, but there appears to be no firm basis for assignment of configuration at the remaining assymmetric center of **9**.

Scheme II



EXPERIMENTAL

Melting points are uncorrected. The melting points correspond to compounds in the DL series. Most of the transformations were also carried out in the D series starting with the more readily available D-thiazoline methyl ester prepared by trifluoroacetic acid degradation of penicillin G methyl ester (7).

Methyl 2-(2-benzyl-4-methyl-5-oxo-2-oxazolin-4-yl)-5,5-dimethyl-4-thiazolidinecarboxylate (**9**).

A solution of 6.4 g. (0.037 mole) of DL-**5** (8) and 7.0 g. (0.037 mole) of **4** (5) in 70 ml. of dry pyridine was left at room temperature for 24 hours. The pyridine was evaporated *in vacuo* at 35° and the residue was extracted with 300 ml. of boiling hexane. When the hexane solution was cooled a colorless gum separated which solidified. The crude solid was recrystallized from ether-hexane (1:1) to give 4.25 g. (32%) of **9** as colorless needles, m.p. 92-95°. Repeated crystallization from the same solvent gave product, m.p. 95-97°; ir (chloroform): 2.98 μ (NH), 5.48, 5.52 (C=O, oxazolone), 5.75 (C=O, ester), 5.95 (C=N); nmr (deuteriochloroform): δ 1.19, 1.35, 1.53 (s each, 3 each, 3 C-CH_3's), 3.68 (app. s, 4, OCH_3, CH), 3.79 (s, 2, $C_6H_5CH_2$), 4.85 (s, 1, -CHS), 7.24 (s, 5, aromatic); (perdeuteriopyridine): δ 4.05 (d, 1, J = 13 Hz, CH), 4.4-5.1 (m, 1, NH), 5.25 (d, 1, J = 8 Hz, CHS); deuterium oxide exchange δ 4.05 (s, 1, CH), 5.25 (s, 1, CHS).

Anal. Calcd. for $C_{18}H_{22}N_2O_4S$: C, 59.64; H, 6.12; N, 7.73. Found: C, 59.73; H, 6.20; N, 7.67.

5-Benzyl-3-(methyoxycarbonyl)-2,3,7,7a-tetrahydro-2,2,7-trimethylimidazo[5,1-*b*]thiazole-7-carboxylic Acid (**10**).

A solution of 1.0 g. of **9** in 10 ml. of dry benzene was refluxed for 24 hours. The solution was cooled and the colorless crystals collected: wt. 450 mg. (45%), m.p. 197-199°. An analytical sample was prepared by recrystallization from acetonitrile without change in m.p.; ir (Nujol mull): 3.2-4.2 μ ($=\overset{+}{N}H$), 5.70, 5.75 (C=O, ester), 6.1-6.25 (CO_2^-, C=N); nmr (trifluoroacetic acid): δ 1.49 (app. s, 6, 2 C-CH_3's), 1.94 (s, 3, N-C-CH_3), 3.80 (s, 3, OCH_3), 4.10 (s, 2, CH_2), 4.59 (s, 1, $CHCO_2CH_3$), 5.97 (s, 1, NCHS), 7.0-7.5 (m, 5, aromatic), 9.1-9.5 (s, broad, 1, NH).

Anal. Calcd. for $C_{18}H_{22}N_2O_4S$: C, 59.64; H, 6.12; N, 7.73. Found: C, 59.80; H, 6.00; N, 7.82.

Methyl 2-[1-(*p*-Methoxybenzyloxycarbonyl)-1-(phenylacetamido)-ethyl]-5,5-dimethyl-4-thiazolidinecarboxylate (**11**).

A solution of 9 g. (0.025 mole) of **9** in 18 ml. of *p*-anisyl alchohol and 2.52 g. (0.025 mole) of triethylamine was allowed to stand overnight and diluted with 50 ml. of dry ether. Colorless crystals formed which were collected, washed (ether) and dried, yielding 3.6 g. (29%) of **11**, m.p. 149-150°; ir (chloroform): 2.94, 2.98 μ (NH), 5.75 (C=O, ester), 5.98 (C=O, amide); nmr (deuteriochloroform): δ 1.10, 1.34, 1.54 (s, each, 3 each, 3 C-CH_3's), 3.0-3.5 (m, 2, NH-CH), 3.49 (s, 2, $-CH_2C_6H_5$), 4.67, 4.73 (s each, 3 each, 2 OCH_3's), 5.01 (s, 2, $CH_2C_6H_5$), 5.10 (s, 1, NCHS), 6.30 (s, 1, NH), 6.6-7.4 (m, 4, A_2B_2 aromatic), 7.19 (s, 5, aromatic).

Anal. Calcd. for $C_{26}H_{32}N_2O_6S$: C, 62.39; H, 6.44; N, 5.60. Found: C, 62.35; H, 6.54; N, 5.51.

1-[5,5-Dimethyl-4-(methoxycarbonyl)-2-thiazolidinyl]-1-phenylacetamidopropanoic Acid (**12**).

To 15 ml. of cold trifluoroacetic acid was added 1.5 g. of **11**. Within a few minutes a purple solution resulted. After 5 minutes the excess trifluoroacetic acid was evaporated *in vacuo* at room temperature. The residue was shaken with 25 ml. of cold saturated sodium bicarbonate and 30 ml. of ethyl acetate. The aqueous layer was separated, washed with ethyl acetate and ether, and acidified with concentrated hydrochloric acid. The aqueous solution was extracted with 50 ml. of methylene chloride and the dried (sodium sulfate) solution was evaporated *in vacuo*. The residue crystallized when triturated with ether-cyclohexane to yield 0.98 g. (85%) of the **trifluoroacetic acid salt**, m.p. 117-118.5° dec.; ir (Nujol mull): 3.0-3.7 μ (bonded OH), 5.73 (C=O, ester), 5.95 (C=O, amide); nmr (perdeuteriopyridine): δ 1.28, 1.54, 1.95 (s each, 3 each, 3 C-CH_3's), 3.63 (s, 3, OCH_3), 3.70 (s, 2, $-CH_2-$) 3.94 (s, 1, $CHCO_2CH_3$), 5.95 (s, 1, NCHS), 7.0-7.7 (m, 5, aromatic), 8.30 (s, 1, NH amide). 9.25 (s, 3 exchangeable H's: $2CO_2H$'s, NH).

Anal. Calcd. for $C_{20}H_{25}F_3N_2O_7S$: C, 48.58; H, 5.09; N, 5.66. Found: C, 48.86; H, 5.22; N, 5.72.

Methyl *N*-(Phenylacetylalanyl)-α-amino-β,β-dimethylacrylate (**13**).

A solution of 362 mg. (0.001 mole) of **9** and 48 mg. (0.001 mole) of lithium azide in 5 ml. of dry DMF was stored at 80° for 18 hours. The reaction mixture was evaporated *in vacuo* at 30° and the residue was taken up in methylene chloride. The methylene chloride solution was washed with water, dried (sodium sulfate) and evaporated *in vacuo*. The partially crystalline residue was tirturated with ether and the colorless crystals were collected. The crude product, 150 mg. (47%), was recrystallized from ethyl acetate to yield **13** as white crystals, m.p. 175-177°, ir (chloroform): 2.92, 3.02 μ (NH), 5.80 (C=O, ester), 5.92 (C=O, amide), 6.02 (C=O, amide); nmr (deuteriochloroform): δ 1.33 (d, 3, J = 7.5 Hz, $CHCH_3$), 1.70, 2.08 (s each, 3 each, $C=C(CH_3)_2$) 3.51 (s, 2, $-CH_2-$) 3.66 (s, 3, OCH_3), 4.4-4.9 (dd, 1, J = 7.5, 10 Hz, CH), 6.75 (d, 1, J = 10 Hz, NH), 7.21 (s, 5, aromatic), 8.05 (s, 1, NH).

Anal. Calcd. for $C_{17}H_{22}N_2O_4$: C, 64.13; H, 6.97 N, 8.80. Found: C, 64.16; H, 7.10; N, 8.78.

Methyl 2-[1-(Hydrazinocarbonyl)-1-(phenylacetamido)ethyl]-5,5-dimethyl-4-thiazolidinecarboxylate (**14**).

A solution of 362 mg. (0.001 mole) of **9** and 50 mg. (0.001 mole) of 100% hydrazine hydrate in 10 ml. of anhydrous methanol was stirred at room temperature for 2 hours. The precipitated

solid was filtered and washed (MeOH) to afford 260 mg. (66%) of colorless crystals, m.p. 183-186°. The analytical sample was recrystallized from methanol, m.p. 182-184°; ir (Nujol mull): 3.0, 3.03 μ (NH), 5.74 (C=O, ester), 5.99, 6.08 (C=O, amide, hydrazide); nmr (DMF-D_7): δ 1.18, 1.43, 1.55 (s each, 3 each, 3 C-CH_3's), 3.57 (s, 2, CH_2), 3.63 (s, 1, C_3H), 3.72 (s, 3, OCH_3), 5.39 (s, 1, C_5H), 5.4 (m, 3, $NHNH_2$), 7.25 (s, 5, aromatic), 7.53 (s, 1, C_6NH).

Anal. Calcd. for $C_{18}H_{26}N_4O_4S$: N, 14.20; S, 8.13. Found: N, 14.15; S, 8.31.

Methyl 2-[1-(Azidocarbonyl)-1-(phenylacetamido)ethyl]-5,5-dimethyl-4-thiazolidinecarboxylate (**15**).

To an ice-cooled, stirred solution of 1.18 g. (0.003 mole) of **14** in 20 ml. of glacial acetic acid and 0.2 ml. of 1 *N* hydrochloric acid was added dropwise over 20 minutes a solution of 228 mg. (0.0033 mole) of sodium nitrite in 5 ml. of water. The mixture was poured into 40 ml. of ice water. The gum was extracted (chloroform) and the organic fractions washed in the cold (water, saturated sodium bicarbonate). The dried (sodium sulfate) extract was evaporated at room temperature to afford 900 mg. of a gum. Crystallization from absolute ether gave 250 mg. (21%) of white needles, m.p. 110-111° dec.; ir (potassium bromide) 3.00, 3.08 μ (NH), 4.68 (azide), 5.71 (C=O, azide), 5.87 (C=O, ester), 6.02 (C=O, amide); nmr (deuteriochloroform): δ 1.15, 1.37, 1.40 (s, 3 each, 3 C-CH_3's), 3.19 (s, 1, NH), 3.33 (s, 1, C_3H), 3.56 (s, 2, CH_2), 3.69 (s, 3, OCH_3), 4.97 (s, 1, C_5H), 6.25 (s, 1, NH), 7.24 (s, 5, aromatic).

Anal. Calcd. for $C_{18}H_{23}N_5O_4S$: C, 53.32; H, 5.72; N, 17.27. Found: C, 53.49; H, 5.75; N, 16.95.

The compound is not stable at room temperature. Recrystallization (benzene-hexane) lowered the m.p. to 103.104.5° dec.

Reaction of **12** with a Carbodiimide.

To a solution of 472 mg. (0.96 mmole) of D-**12** trifluoroacetate and 85 mg. (1.0 mmole) of sodium bicarbonate dissolved in 5 ml. water and 45 ml. of *p*-dioxane was added 435 mg. (1.05 mmoles) 1-cyclohexyl-3-(2-morpholinoethyl)carbodiimide metho-*p*-toluenesulfonate. After 2 hours at room temperature the solution was diluted with 600 ml. water and extracted with four 150-ml. portions of methylene dichloride. After washing with water and saturated sodium chloride solution the methylene dichloride was dried, filtered, and evaporated *in vacuo* affording 339 mg. (97%) of crystalline material. Infrared and tlc analysis established the product as D-**9**.

Reaction of **15** with Triethylamine.

To a stirred suspension of 150 mg. of the azide **15** in 3 ml. of methylene chloride was added 0.1 ml. of triethylamine. After 40 minutes at room temperature the reaction mixture was poured into water and the organic layer separated. It was washed with water, cold 10% aqueous phosphoric acid, water, and saturated brine. The dried (sodium sulfate) extract was evaporated to afford impure **9** (ir and nmr) as a viscous yellow gum. The crude product was readily converted to pure **14** (*vide supra*).

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