Penicillin Dipeptides

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A series of dipeptide acids and esters of benzylpenicillin, methicillin, oxacillin, and cloxacillin with 6-aminopenicillanic acid were prepared and their *in vitro* activities were determined against nonpenicillinase- and penicillinase-producing staphylococci. These studies indicated that none of the new semisynthetic penicillin derivatives was more active than the parent penicillin.

The successful acylation of 6-aminopenicillanic acid to give the first semisynthetic preparation of benzylpenicillin was described in a classical paper in 1959.[1] This work led the way to the preparation of numerous new amide derivatives of penicillin.[1,2] In a modification of this work we report the preparation of peptide analogs of types **4** and **5** in which the amine function of 6-aminopenicillanic acid is acylated by the carboxylic acid moiety of a penicillin. We anticipated that the penicillin moiety would act as a sterically hindered side chain and, therefore, increase the stability of these new penicillins to penicillinase inactivation.[3]

The preparation of these new compounds was achieved by using known synthetic methods in peptide chemistry,[4] methods for the protection of carboxylic acid and amine functions, together with coupling and deblocking reactions. Our approach to the synthesis of the dipeptide of benzylpenicillin with 6-aminopenicillanic acid is summarized in Scheme I.

The benzyl ester was chosen for the protection of the carboxyl group due to its facile cleavage under mild conditions. Protection of the amino moiety was dictated by the penicillin analog desired. We were able to prepare good yields of pure dipeptides through a coupling by the mixed anhydride method.[5] The synthesis of the dipeptide of the benzylpenicillin **4a** was therefore carried out by converting the triethylammonium salt of potassium penicillin G to the mixed anhydride **1a** and treating this *in situ* at 0° with the *p*-toluenesulfonate salt of benzyl 6-aminopenicillanate (**2**). Acidic and basic extraction of the dipeptide esters were carried out to remove any uncoupling materials. The resultant dipeptide (**4a**) was obtained analytically pure as a noncrystalline solid in 96% yield. The procedure was successfully employed to include other penicillin derivatives. Thus, the dipeptide benzyl esters of methicillin (**4b**), oxacillin (**4c**), and cloxacillin (**4d**) were synthesized. The debenzylation of **4a** was carried out by hydrogenation (method A) at atmospheric pressure in a glacial acetic acid–dioxane mixture using 25% Pd–C as the catalyst and afforded a good yield of the analytically pure dipeptide acid **5a** as a noncrystalline solid.

The dipeptide acid **5a** was also obtained directly (method B) by coupling the triethylammonium salt of 6-aminopenicillanic acid (**3**) with the mixed anhydride **1a**. This dipeptide acid which was identical with the product obtained by method A was converted to the potassium salt **6** and the methyl ester **7** by standard methods.

This direct procedure (method B) was successfully employed to include other penicillin derivatives. Thus, the dipeptide acids of methicillin (**5b**), oxacillin (**5c**), and cloxacillin (**5d**) were also synthesized by this method. Since the penicillin dipeptides resisted all attempts at crystallization partial purification was effected by lyophilization. Nevertheless products of high purity were obtained (see Experimental Section).

Table I summarizes the analytical data for all the penicillin dipeptides.

Biological Results.—Four strains of *Staphylococcus aureus* were used in this work.[6] Two of these strains are penicillin resistant (258γW and 147βW) and two strains are penicillin sensitive (258N and 147N). The activities of the penicillins were recorded as the minimal inhibitory concentration from an overnight growth of a culture in tryptone–yeast broth at 35°. The activities of all the compounds were observed in a final concentration of 10% DMSO[7] in broth. The semisynthetic penicillins were tested for antistaphylococcal activity by a serial threefold broth dilution test.

The activities of the semisynthetic penicillins as compared to their respective parent penicillins are summarized in Table II. In the methicillin, oxacillin, and cloxacillin series, the dipeptide acids **5b**, **5c**, and **5d**, respectively, exhibited appreciably more activity than the respective dipeptide benzyl esters **4b**, **4c**, and **4d** to both resistant and sensitive organisms. In the cases of **5b** and **5c** activity against three strains was comparable to that of methicillin and oxacillin, respectively.

None of the compounds in the penicillin G series showed activity comparable to penicillin G against sensitive organisms. It should be noted that in the penicillin G series, the dipeptide benzyl ester (**4a**) was slightly more active than the dipeptide acid (**5a**). We have no explanation for this observed difference with the methicillin, oxacillin, and cloxacillin series where the dipeptide acids (**5b**, **5c**, **5d**) were more active than the dipeptide benzyl esters (**4b**, **4c**, **4d**).

(1) F. R. Batchelor, F. P. Doyle, J. H. C. Naylor, and G. N. Rolinson, *Nature*, **183**, 257 (1959).

(2) (a) F. R. Batchelor, D. Butterworth, J. Cameron-Wood, M. Cole, G. C. Eustace, M. V. Mart, M. Richards, and E. B. Chain, *ibid.*, **187**, 236 (1960); (b) F. R. Batchelor, E. B. Chain, and G. N. Rolinson, *Proc. Roy. Soc.* (London), **B154**, 478 (1961); (c) F. R. Batchelor, E. B. Chain, T. L. Hardy, K. R. L. Mansford, and G. N. Rolinson, *ibid.*, **B154**, 498 (1961).

(3) For a general review see (a) N. J. Harper and A. B. Simmonds, *Advan. Drug Res.*, **1**, 1 (1964); and (b) G. T. Stewart, "The Penicillin Group of Drugs," Elsevier Publishing Co., Amsterdam, 1965, Chapter 3.

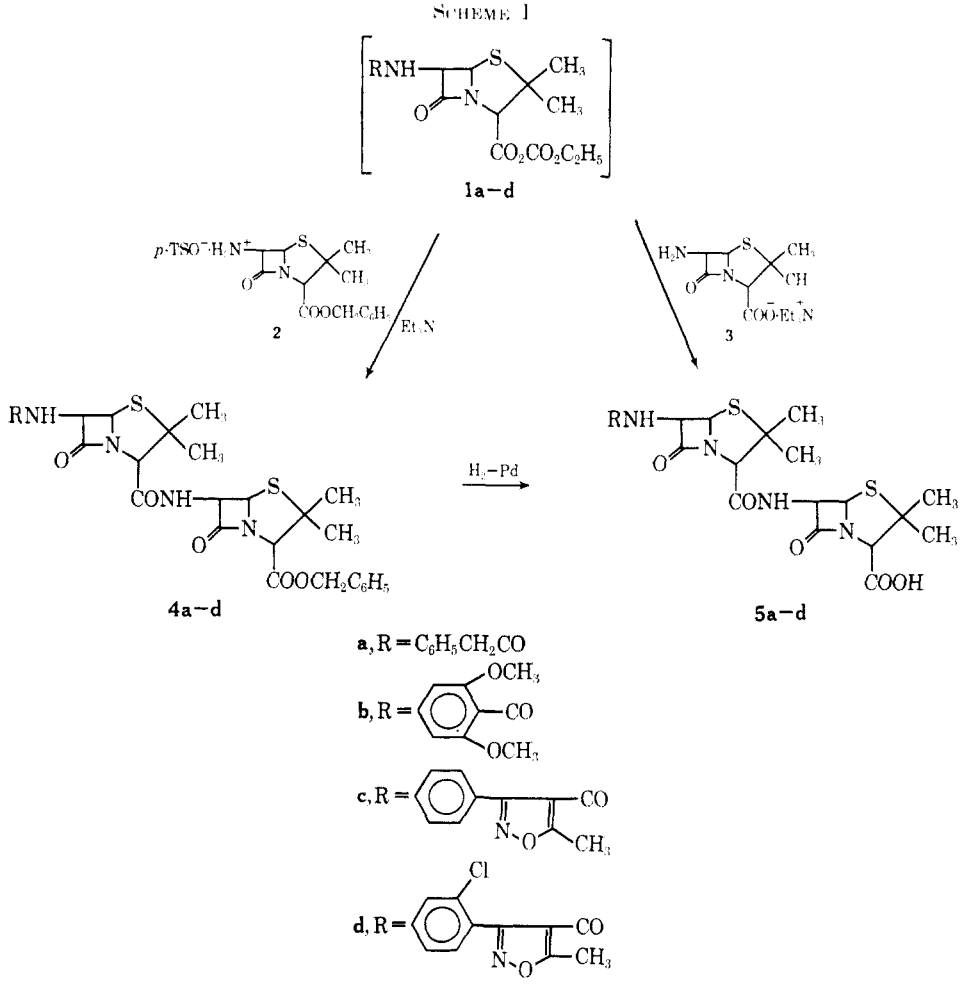
(4) For a general review see E. Schroder and K. Lubke, "The Peptides," Academic Press Inc., New York, N. Y., 1965.

(5) (a) T. Wieland and H. Bernhard, *Ann. Chem.*, **572**, 190 (1951); (b) R. A. Boissonnas, *Helv. Chim. Acta*, **34**, 874 (1951); (c) J. R. Vaugh, Jr., *J. Am. Chem. Soc.*, **73**, 3547 (1951); (d) R. L. Barnden, *et al.*, *J. Chem. Soc.*, 3733 (1953); (e) D. A. Johnson, *J. Am. Chem. Soc.*, **75**, 3636 (1953).

(6) (a) Samples were obtained from Dr. R. P. Novick; (b) R. P. Novick and M. H. Richmond, *J. Bacteriol.*, **90**, 467 (1965).

(7) This concentration of dimethyl sulfoxide has no effect on the growth of these organisms.

SCHEME I



In general, it can be said that if the parent compound was active against resistant and sensitive strains of staphylococci, then the dipeptide acid derivatives showed the same relative activity (*e.g.*, **5c** showed the same activity as sodium oxacillin against strains 147βW and 147N). If the parent compound was less active against resistant strains of staphylococci, then the dipeptide derivatives also showed the same relative activity (*e.g.*, **4a** showed the same activity as penicillin G against strains 258γW and 147βW; both were inactive).

Experimental Section

All melting points were determined microscopically on a hot stage and are corrected. Ir spectra were determined on a Beckman IR-9 spectrophotometer and nmr spectra were determined with a Varian A-60 instrument. In all cases where penicillin dipeptides were prepared ir spectra confirmed the structure from an examination of the carbonyl region. The relative intensity of the ester band at 1755 cm^{-1} for the dipeptides (**4a, 7, 4b, 4c, 4d**) is considerably less (about one-half) than the intensity of both the amide band at 1685 cm^{-1} and the β-lactam band at 1790 cm^{-1}. In the case of a model benzylpenicillanic acid benzyl ester the three regions had bands of equal intensities. The same observations were made for the relative intensity of the acid band at 1745 cm^{-1} for the dipeptides (**5a, 5b, 5c, 5d**) with respect to the amide band at 1685 cm^{-1} and β-lactam band at 1790 cm^{-1}. These observations are in accord with the expectation that the ratio of ester (acid):amide:lactam for the dipeptides is 1:2:2 compared with 1:1:1 for noncoupling starting material. Nmr analysis confirmed the ratio of 1:1 for each substituted penicillanic acid nucleus in the dipeptide, and each penicillin dipeptide was shown to be free of starting materials by a visual estimation of thin layer chromatograms (silica gel G).

Triethylammonium penicillins were prepared by a modification of the procedure of Goldman.[8] A solution of 10.0 g (0.027 mole) of potassium penicillin G in 2 l. of H_2O was treated with 300 g of $(NH_4)_2SO_4$, 9.2 ml of 85% H_3PO_4, and 61.6 ml of $(C_2H_5)_3N$. The resulting solution was extracted (cold $CHCl_3$, three 250-ml portions). The organic layers were combined, dried ($MgSO_4$), filtered, and concentrated to a volume of 40 ml. EtOAc (30 ml) was added and the mixture was again concentrated to remove $CHCl_3$. Acetone (10 ml) was added and the reaction mixture was cooled overnight at 0°. The product was obtained by filtration and was washed (Me_2CO) to afford 7.45 g (64%) of product, mp 145.5–147.5° dec (lit.[8] mp 145–147° dec).

The triethylammonium salts of methicillin, oxacillin, and cloxacillin were prepared by analogy in yields of 80, 68, and 75%, respectively.

Benzyl 6-Aminopenicillinate *p*-Toluenesulfonate (2).—Benzyl 6-aminopenicillinate *p*-toluenesulfonate (**2**) was prepared by two methods. Method A involved the detritylation of benzyl 6-tritylaminopenicillinate.[9] Alternatively, the benzyl 6-aminopenicillinate salt was prepared directly more conveniently from 6-aminopenicillanic acid by method B.

Method A.—Detritylation was carried out by a modification of the procedure of Koe.[10] A solution of 2.74 g (0.005 mole) of benzyl 6-tritylaminopenicillinate[9] in 15 ml of acetone was treated with a solution of 0.95 g (0.005 mole) of *p*-toluenesulfonic acid monohydrate in 15 ml of acetone and the resulting solution was stirred at 25° for 2 hr. CH_2Cl_2 (10 ml) was added and the mixture was treated with excess acetone. Filtration afforded 2.05 g (83%) of the product as white prisms, mp 153.5–154° dec.

Method B is a modification of an earlier method.[11] A solution of 3.3 g (0.015 mole) of 6-aminopenicillanic acid in 200 ml of

(8) L. Goldman, U. S. Patent 2,547,640 (April 3, 1951).

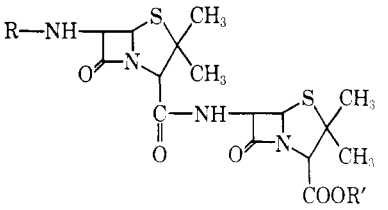
(9) J. C. Sheehan and K. R. Henery-Logan, *J. Am. Chem. Soc.*, **84**, 2983, (1962).

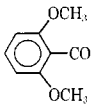
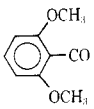
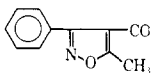
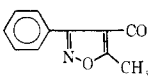
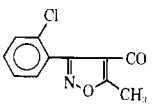
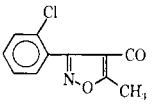
(10) B. K. Koe, *Nature*, **195**, 1200 (1962).

(11) A. A. Patchett, E. F. Rogers, and W. J. Leanza, Belgian Patent 634,-374 (Jan 2, 1964); *Chem. Abstr.*, **61**, 1869c (1964).

TABLE I

PENICILLIN DIPEPTIDES



Compd	R	R′	Coupling yield, %	$[\alpha]^{25}D$, deg (c, $CHCl_3$)[a]	Formula	Analyses[b]
4a	$C_6H_5CH_2CO$	$CH_2C_6H_5$	92	+232.7 (1.012)	$C_{31}H_{34}N_4O_6S_2$	C, H, N
5a	$C_6H_5CH_2CO$	H	82	+267.5 (1.060)	$C_{24}H_{28}N_4O_6S_2$	C, H, S; N[d]
6	$C_6H_5CH_2CO$	K	...	+198.7 (1.020)[c]	$C_{24}H_{27}KN_4O_6S_2 \cdot 2H_2O$	C, H, N
7	$C_6H_5CH_2CO$	CH_3	...	+251.2 (0.740)	$C_{25}H_{30}N_4O_6S_2$	C, H
4b		$CH_2C_6H_5$	65	+261.7 (1.130)	$C_{32}H_{36}N_4O_8S_2 \cdot H_2O$	C, H, N
5b		H	47	+225.4 (1.170)	$C_{25}H_{30}N_4O_8S_2$	C, H
4c		$CH_2C_6H_5$	84	+198.2 (0.950)	$C_{34}H_{35}N_5O_7S_2$	C, H, N
5c		H	55	+152.3 (1.225)	$C_{27}H_{29}N_5O_7S_2 \cdot 0.5H_2O$	C, H
4d		$CH_2C_6H_5$	34	+166.5 (0.940)	$C_{34}H_{34}ClN_5O_7S_2 \cdot H_2O$	H; C,[e] N[f]
5d		H	62	+190.5 (0.960)	$C_{27}H_{28}ClN_5O_7S_2 \cdot H_2O$	C, H, N

[a] All optical rotations were taken on a Perkin-Elmer Model 141 polarimeter using a 10-cm path length. [b] Satisfactory analyses were obtained for the elements indicated. [c] In MeOH. [d] N: calcd, 10.52; found, 9.89. [e] C: calcd, 55.01; found, 55.49. [f] N: calcd, 9.44; found, 8.91.

TABLE II

COMPARATIVE ANTIBACTERIAL ACTIVITY OF SEMISYNTHETIC PENICILLINS

	% act. of deriv against *S. aureus* compared to parent compd[a]			
Test Compd	(resistant)[b] 258γW	(sensitive)[c] 258N	(resistant)[b] 147βW	(sensitive)[c] 147N
Penicillin G, Na Salt	100 (37)	100 (0.14)	100 (37)	100 (<1.0)
7	33	<1	33	<3
5a	11	4	100	<25
4a	33	10	100	<25
Sodium methicillin	100 (4)	100 (4)	100 (4)	100 (4)
5b	33	100	100	100
4b	4	4	4	4
Sodium oxacillin	100 (1.3)	100 (1.3)	100 (1.3)	100 (1.3)
5c	33	100	100	100
4c	3	3	3	3
Sodium cloxacillin	100 (1.3)	100 (1.3)	100 (1.3)	100 (1.3)
5d	11	33	33	33
4d	<1	<1	1	1

[a] The minimal inhibitory concentration in μg/ml of the parent compound is in parentheses. [b] The organism is resistant to penicillin G by the production of penicillinase. [c] The organism is sensitive to penicillin G.

absolute MeOH was treated by the dropwise addition of an ethereal solution of excess phenyldiazomethane.[12] The resulting mixture was stirred for 2 hr at 25°. Ether (150 ml) was added and the mixture was filtered to remove 2.2 g of unreacted 6-aminopenicillanic acid. The filtrate was evaporated to dryness. The resultant oil was taken up in acetone–ether (1:1), filtered, and then treated with a solution of 1.0 g of *p*-toluenesulfonic acid monohydrate in 10 ml of acetone. Filtration gave 1.8 g (74%) of **2**, mp 152–153°, which was identical in all respects with the product obtained by method A.

6-(6-Phenylacetamidopenicillamido)penicillanic Acid Benzyl Ester (4a).—A solution of 3.1 g (7.05 mmoles) of triethylammonium benzylpenicillin in 50 ml of dry CH_2Cl_2 was stirred under

(12) G. F. Fasman and P. S. Sarin, *Biochim. Biophys. Acta*, **82**, 175 (1964).

N_2 and treated with 0.765 g (7.05 mmoles) of ethyl chloroformate. The resulting solution was stirred at 0° for 20 min and was then treated at 0° by the dropwise addition of a solution of a mixture of 3.5 g (7.05 mmoles) of benzyl 6-aminopenicillinate *p*-toluenesulfonate, 50 ml of dry CH_2Cl_2, and 0.714 g (7.05 mmoles) of $(C_2H_5)_3N$. The reaction mixture was stirred at 0° for 0.5 hr and at 25° for 0.5 hr, transferred to a separatory funnel, and washed with 0.1 *M* citric acid, then with 0.1 *M* Na_2HPO_4, and finally with H_2O.[13] The solution was dried (Na_2SO_4), filtered through Norit, and evaporated to dryness. The residue was lyophilized from C_6H_6 to give 4.2 g (96%) of **4a** as a white amorphous powder (one spot on tlc, R_f 0.36, 3.2% MeOH in $CHCl_3$) which melted with slow decomposition from 55°.

6-(2,6-Dimethoxybenzamidopenicillamido)penicillanic acid benzyl ester monohydrate (4b) was prepared from 1.444 g (3.00 mmoles) of triethylammonium methicillin and from 1.435 g (3.00 mmoles) of benzyl 6-aminopenicillanate *p*-toluenesulfonate exactly as described above for the synthesis of **4a**. Work-up gave 1.3 g (65%) of **4b** as a white powder which melted with decomposition from 97° (tlc, one spot, R_f 0.65, 4.8% MeOH in $CHCl_3$).

6-(5-Methyl-3-phenyl-4-isoxazolylcarboxamidopenicillamido)-penicillanic acid benzyl ester (4c) was prepared as described above for the synthesis of **4a** from 2.00 g (4.00 mmoles) of triethylammonium oxacillin and from 1.914 g (4.00 mmoles) of benzyl 6-aminopenicillanate *p*-toluenesulfonate. Work-up afforded 2.3 g (84%) of **4c** as a white amorphous powder, which melted with decomposition from 78° (tlc, one spot, R_f 0.74, 5% MeOH in $CHCl_3$)

6-[5-Methyl-3-(2-chlorophenyl)-4-isoxazolylcarboxamidopenicillamido]penicillanic Acid Benzyl Ester Monohydrate (4d).—This reaction was carried out as described above for the preparation of **4a** from 2.148 g (4.00 mmoles) of triethylammonium cloxacillin and from 1.914 g (4.00 mmoles) of benzyl 6-aminopenicillanate *p*-toluenesulfonate. After the usual work-up, the product was purified by column chromatography using Florisil (Floridin Co., Hancock, W. Va.) with $CHCl_3$ as the eluent. Removal of solvent afforded 1.0 g (34%) of **4d** as a white powder, which melted with decomposition from 58° (tlc, one spot, R_f 0.78, 6.6% MeOH in $CHCl_3$).

6-(6-Phenylacetamidopenicillamido)penicillanic Acid (5a). Method A.—The hydrogenation of **4a** was carried out by a modification of the procedure outlined by Sheehan[14] for the penicillin system. A mixture of 8.4 g of 25% Pd–C, 200 ml of dry dioxane, and 12 ml of AcOH was prereduced with H_2 at atmospheric pressure and then treated with 3.11 g (5.00 mmoles) of **4a**. Hydrogenation was resumed at atmospheric pressure until the theoretic amount of H_2 had been absorbed (122 ml). Catalyst was removed by filtration and the filtrate was lyophilized to afford 2.5 g of a white amorphous solid. Purification was effected by dissolving the crude product in 2 *N* $KHCO_3$, filtration through Norit, and reacidification of the cooled (0°) filtrate with 2 *N* HCl. The resultant solid was taken up in CH_2Cl_2, dried ($MgSO_4$), filtered, and evaporated to give 1.7 g (64%) of **5a** as a white amorphous powder which melted with decomposition from 113°.

Method B.—A solution of 8.71 g (20.0 mmoles) of triethylammonium benzylpenicillin in 100 ml of dry CH_2Cl_2 at $-20 \pm 5°$ was stirred under N_2 and treated with 2.25 g (20.8 mmoles) of ethyl chloroformate. The resulting solution was stirred at $-15°$ for 20 min and then treated with a solution of 6.35 g (20.0 mmoles) of triethylammonium 6-aminopenicillanic acid (**3**)[15] in 40 ml of dry CH_2Cl_2. The addition was carried out dropwise with stirring at $-15°$ over a period of 15 min. The resulting mixture was then stirred at $-15 \pm 5°$ for 40 min and allowed to reach room temperature while stirring was continued for an additional 2 hr. The mixture was washed with 2 *N* HCl, dried ($MgSO_4$), filtered through Norit, and evaporated to dryness to give 8.9 g (77%) of **5a** as a white amorphous powder which melted with decomposition from 110°, $[\alpha]^{25}D$ + 263.0° (*c* 1.09, $CHCl_3$). The ir and nmr spectra were identical with those observed for the product prepared above by method A.

6-(2,6-Dimethoxybenzamidopenicillamido)penicillanic acid (5b) was prepared as described above for **5a** (method B) from 4.82 g (10.0 mmoles) of triethylammonium methicillin and with 3.17 g (10.0 mmoles) of **3**. Work-up afforded 2.7 g (47%) of **5b** as a white amorphous powder which melted with decomposition from 122°.

6-(5-Methyl-3-phenyl-4-isoxazolylcarboxamidopenicillamido)-penicillanic acid hemihydrate (5c) was prepared as described above for **5a** (method B) from 5.026 g (10.0 mmoles) of triethylammonium oxacillin and 3.17 g (10.0 mmoles) of **3**. Work-up as before yielded 3.3 g (55%) of **5c** as a white amorphous solid which melted with decomposition from 102°.

6-[5-Methyl-3-(2-chlorophenyl)-4-isoxazolylcarboxamidopenicillamido]penicillanic acid monohydrate (5d) was prepared in the usual manner as described above for **5a** (method B) from 2.148 g (4.00 mmoles) of triethylammonium cloxacillin and 1.27 g (4.00 mmoles) of **3**. Work up afforded 1.6 g (62%) of **5c** as a white amorphous solid which melted with decomposition from 120°.

Potassium 6-(6-Phenylacetamidopenicillamido)penicillinate Dihydrate (6).—A mixture of 1.066 g (2.0 mmoles) of **5a**, 0.202 g (2.0 mmoles) of $KHCO_3$, and 40 ml of distilled H_2O was stirred, filtered through Norit and Celite to remove a trace of insoluble material, and lyophilized. The resultant off-white powder was purified by dissolving in absolute EtOH and precipitation with hexane to give 0.9 g (79%) of **6** as a white powder, mp 187° dec.

6-(6-Phenylacetamidopenicillamido)penicillanic Acid Methyl Ester (7).—A mixture of 0.533 g (1.0 mmole) of **5a**, 5 ml of MeOH, and a solution of CH_2N_2 in ether[16] (10 ml of an ethereal solution obtained from 20 g of nitrosomethylurea) was stirred at 25° for 30 min. Solvents were removed and the residue was taken up in C_6H_6. The solution was extracted with 2 *N* $KHCO_3$, dried ($MgSO_4$), filtered, and evaporated to give 0.40 g (73%) of **7** as a white amorphous powder which melted with decomposition from 84° (tlc, one spot, R_f 0.49, 1:25 MeOH–$CHCl_3$).

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(13) Extraction with 1 *M* HCl, 1 *M* sodium bicarbonate, and water, respectively, gave equally satisfactory results.

(14) J. C. Sheehan and D. R. Hoff, *J. Am. Chem. Soc.*, **79**, 237 (1957).

(15) (a) H. B. Koenig, Belgian Patent 612,733 (July 17, 1962); *Chem. Abstr.*, **58**, 1465*a* (1963); (b) Y. G. Perron and W. F. Minor, French Patent 1,332,557 (1963); *Chem. Abstr.*, **60**, 1761 (1964).

(16) F. Arndt, "Organic Syntheses," Coll. Vol. II, John Wiley and Sons, Inc., New York, N. Y., 1943, p 165.