Nonenzymatic Conversion of Penicillins to 6-Aminopenicillanic Acid

D. A. JOHNSON,[1] C. A. PANETTA, AND R. R. SMITH

Chemical Development Division, Bristol Laboratories, Division of Bristol-Myers Company, Syracuse, New York

Received March 7, 1966

The nonenzymatic conversion of several *o*-nitrophenyl-substituted penicillins (**3**) to 6-aminopenicillanic acid (**8**) is reported. Spontaneous cleavage of the side chain occurs at room temperature after reduction to the substituted amine (**4**) with hydrogen over palladium catalyst or, preferably, to the hydroxylamine (**5**) with alkali borohydride in the presence of palladium on carbon.

The availability of the key intermediate 6-aminopenicillanic acid (6-APA) has led to the preparation of thousands of new semisynthetic penicillins. At least nine of these, all of which have been tailor made to provide certain desirable properties, are now being marketed. 6-APA can be produced in commercial quantities either by direct fermentation of precursor-free penicillin broth[2] or by enzymatic cleavage of natural penicillins.[3,4] It has also been prepared by total synthesis[5] and by acid cleavage of the N-trityl derivative.[6] The only reported nonenzymatic preparations of 6-APA from a penicillin involved catalytic hydrogenolysis of an N-carbobenzyloxy 6-APA derivative using massive amounts of catalyst.[7]

Conversion of a penicillin to 6-APA clearly requires some highly specific reagent since, of the two amide linkages, the β-lactam is by far the more reactive and is actually more similar to an acid chloride or anhydride in its reactivity.

Highly selective cleavages of peptide bonds are known to result from reactions involving participation of neighboring groups.[8] In a recent important example of this type, Morin and co-workers[9] reported the successful conversion of cephalosporin C (**1**) to 7-aminocephalosporanic acid (**2**) *via* reaction of nitrosyl chloride on the aminoadipyl side chain (Scheme I).

Successful application of this reaction to the corresponding penicillin (penicillin N) has not been reported, but this is not surprising in view of the greater lability of the penicillin nucleus toward acids.

In 1952, Holley and Holley[10] reported removal of the N-*o*-nitrophenoxyacetyl group from peptides *via* thermal cyclization of the corresponding amino compounds which were produced by catalytic hydrogenation. The utility of this approach in cleaving the side chain from a penicillin would be limited by the need to use a large amount of catalyst to overcome the poisoning affect of the sulfur atom and by the high temperature generally required to promote cyclization of the amine.

We wish to report, however, the successful conversion of several *o*-nitrophenyl-substituted penicillins (**3**) to 6-APA (**8**) and the expected benzoxazines (**6**) *via* catalytic reduction using a palladium catalyst (Scheme II).

The substituted anilines (**4**) were probably formed as unstable intermediates. Although none were isolated and characterized, their presence was indicated by the appearance of a new zone on thin layer chromatograms immediately after reduction and by gradual disappearance of this zone after acidification as zones for 6-APA and the benzoxazine (**6**) appeared. In the more successful cases, the crystalline 6-APA separated spontaneously from a methyl isobutyl ketone extract of filtered and acidified reduction mixture. At the extraction pH of 2, the weakly basic amine (**4**) transferred into the methyl isobutyl ketone layer and, within a few minutes at room temperature, began to degrade into 6-APA (**8**) and the corresponding benzoxazine (**6**). If the extraction was not performed rapidly, a large amount of the 6-APA (which is insoluble in methyl isobutyl ketone and soluble in water) would transfer into the aqueous phase before the latter could be separated. In this case, the 6-APA was isolated from the aqueous phase by acylation with α-phenoxypropionyl chloride to form the easily isolable penicillin, phenethicillin. In the least successful cases, the formation of 6-APA was shown only by thin layer chromatography (tlc). Results are tabulated in Table I.

TABLE I

YIELDS OF 6-APA AND PHENETHICILLIN WHICH WERE OBTAINED BY THE REDUCTION OF *o*-NITROPHENYL-SUBSTITUTED PENICILLINS

	Reduction with potassium borohydride			Reduction with hydrogen and 30% Pd–C		
	% 6-APA as			% 6-APA as		
Penicillin	6-APA	Phenethicillin	Total	6-APA	Phenethicillin	Total
3a	46		46		2.6	2.6
3b				Tlc evidence		
3c	12		12	Tlc evidence		
3d	43	26	69	43	13	56
3e	4.7		4.7			
3f	53		53		38	38
3g	17	41	58	31	34	65

In 1963, Coutts and co-workers[11] described the facile reductive cyclization of *o*-nitrophenyl-substituted esters using sodium borohydride and palladium on carbon to

(1) To whom inquiries should be addressed.

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

(3) (a) G. N. Rolinson, *et al.*, *ibid.*, **187**, 236 (1960); (b) C. A. Claridge, A. Gourevitch, and J. Lein, *ibid.*, **187**, 237 (1960).

(4) (a) H. T. Huang, A. R. English, T. A. Seto, G. M. Shull, and B. A. Sobin, *J. Am. Chem. Soc.*, **82**, 3790 (1960); (b) W. Kaufmann and K. Bauer, *Naturwissenschaften*, **47**, 474 (1960).

(5) J. C. Sheehan and K. R. Henery-Logan, *J. Am. Chem. Soc.*, **81**, 5838 (1959).

(6) J. C. Sheehan and K. R. Henery-Logan, *ibid.*, **84**, 2983 (1962).

(7) (a) British Patent 894,368 to Chas. Pfizer and Co., Inc. (April 18 1962); *Chem. Abstr.*, **57**, 2346*h* (1962); (b) I. R. Hoover, U. S. Patent 3,107,250 (1963); *Chem. Abstr.*, **60**, 2940*g* (1964).

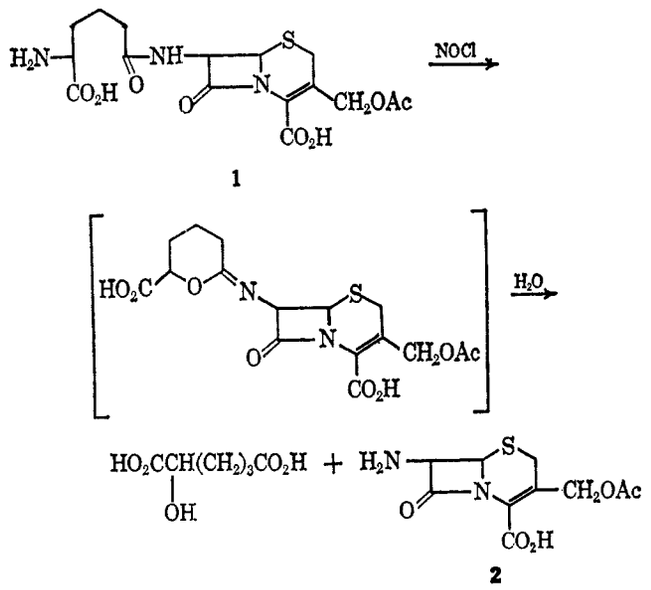
(8) For a review, see B. Witkop, *Advan. Protein Chem.*, **16**, 221 (1961); L. A. Cohen and B. Witkop, *Angew. Chem.*, **73**, 253 (1961)

(9) R. B. Morin, B. G. Jackson, E. H. Flynn, and R. W. Roeske, *J. Am. Chem. Soc.*, **84**, 3400 (1962).

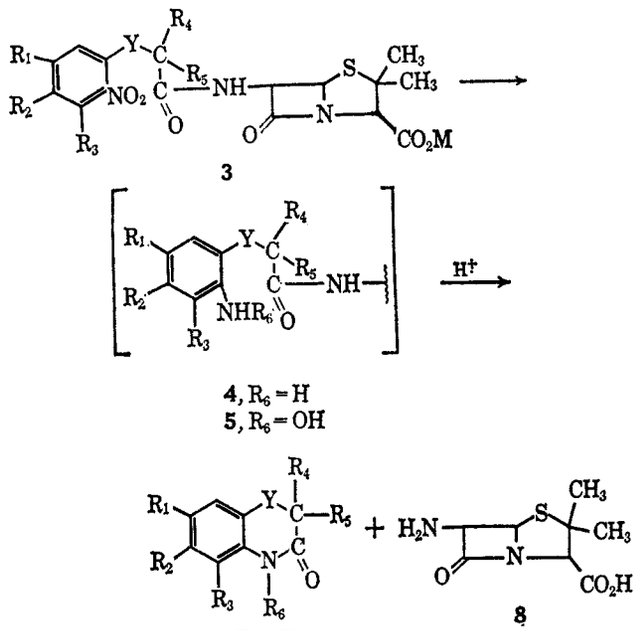
(10) R. W. Holley and A. D. Holley, *ibid.*, **74**, 3069 (1952).

(11) (a) R. T. Coutts and D. G. Wibberley, *J. Chem. Soc.*, 4610 (1963); (b) R. T. Coutts, D. Noble, and D. G. Wibberley, *J. Pharm. Pharmacol.*, **16**, 773 (1964).

SCHEME I



SCHEME II



	R_1	R_2	R_3	R_4	R_5	Y	R_6	M
3a	H	H	H	H	H	O		K
3b	H	H	H	H	H	S		K
3c	H	H	H	H	H			Na
3d	H	H	H	CH_3	CH_3	O		K
3e	CH_3	CH_3	CH_3	H	H	O		K
3f	H	$(CH_3)_3C$	H	H	H	O		Na
3g	H	$(CH_3)_3C$	H	CH_3	CH_3	O		K
4a, 6a	H	H	H	H	H	O	H	
5a, 7a	H	H	H	H	H	O	OH	
4b, 6b	H	H	H	CH_3	CH_3	O	H	
5b, 7b	H	H	H	CH_3	CH_3	O	OH	

produce cyclic hydroxamic acids; *i.e.*, ethyl *o*-nitrophenoxyacetate was converted to the benzoxazine in 84% yield. Despite the mild reaction conditions required and the good yields obtained, no application of this concept to cleavage of an amide linkage has appeared.

We now wish to report on application of the borohydride reductive cyclization to the selective cleavage of a peptide bond and specifically on the use of this technique to prepare 6-APA from *o*-nitrophenyl-substituted penicillins. Reduction of an aqueous solution of the *o*-nitrophenyl-substituted penicillins by 2 moles of alkali borohydride in the presence of a catalytic amount of 5% palladium on carbon was accomplished in 15 min at pH 8.5–9.0 and 0–5°. The intermediate products were probably the substituted hydroxylamines (**5**). As in the case of the anilines however, none were isolated but their presence was indicated by tlc and by subsequent generation of 6-APA and the hydroxybenzoxazines (**7**). Here again efficiencies were monitored by the amount of crystalline 6-APA and/or phenethicillin isolated. Results are tabulated in Table I.

The formation of 6-APA from *o*-nitrophenyl-substituted penicillins was greatly dependent on a number of variables. The most important of these was the structure of the penicillin side chain. The optimum number of atoms between the benzene ring and the amide linkage was two as in *o*-nitro Pen V (**3a**). Shortening the chain by one atom as in 6-(*o*-nitrophenylacetamido)penicillanic acid (**3c**) lowered the yield of 6-APA considerably and lengthening it by the same number as in 6-(*o*-nitrophenoxypropionamido)penicillanic acid gave no 6-APA at all. The replacement of the two α hydrogens in *o*-nitro Pen V with methyl groups (penicillin **3d**) greatly increased the amount of 6-APA obtained by either reductive method as shown in Table I. The facilitating effect of alkyl substituents on the ease of ring closure is well known and the underlying theory has been discussed in a recent review.[12] Addition of a *p*-*t*-butyl group to *o*-nitro Pen V (penicillin **3f**) was also advantageous and the combination of the *p*-*t*-butyl and α,α-dimethyl groups on the *o*-nitro Pen V molecule (penicillin **3g**) gave a good yield of 6-APA, but their effects were not additive.

A pH in the range of 1.5–3.0 appears to be necessary for the optimum conversion of the reactive intermediate to 6-APA. In one case, the yield of 6-APA was cut almost in half when a higher pH (4.0) was tried and it was assumed that the product is too labile at any pH below 1.5. The best temperature for the cleavage to 6-APA was in the range 0–30°. Lower yields were obtained using temperatures of 40 or 100°.

6-(*o*-Nitrophenylsulfonylacetamido, *o*-nitrophenylsulfonamidoacetamido, *o*-nitrophenoxypropionamido, *o*-nitrophenylbutyramido, and *o*,*p*-dinitrophenoxyacetamido)penicillanic acids gave no 6-APA by either method of reduction.

The by-product benzoxazines (**6** and **7**) were isolated from the methyl isobutyl ketone filtrates after 6-APA was removed. They were characterized by the usual analyses or directly compared with authentic samples.

Crystalline 6-APA was also obtained by the borohydride reduction technique in which nickel chloride replaced Pd–C (14.7% yield) or by electrolytic reduction of the *o*-nitrophenyl-substituted penicillin (14.0% yield).[13]

The *o*-nitrophenyl-substituted penicillins used in this study were conveniently prepared by reaction of the appropriate acid chloride with 6-APA. The prepara-

(12) B. Capon, *Quart. Rev.* (London), **18**, 109 (1964).

(13) Performed by J. Bomstein and P. Montileone of the Chemical Control Department of Bristol Laboratories.

tion of penicillins of this type by direct fermentation has also been reported in the patent literature.[14]

Experimental Section[15]

The thin layer chromatograms were run on dip-coated glass microscope slides (25 × 75 mm).[16] Spotting was performed using 0.5–1.0 μl of a 1% solution in pH 7.0 phosphate buffer. The solvent system used for the penicillins, nitrophenoxyacetic acids, and benzoxazines was 60% benzene, 35% acetone, and 5% acetic acid and that used for 6-APA was 95% acetone and 5% acetic acid. The zones were detected as yellow areas on a purple background after spraying with a 0.5% aqueous potassium permanganate solution.

2-Methyl-2-(*o*-nitrophenoxy)propionic Acid.—*o*-Nitrophenol, 114.1 g (0.82 mole), 111.5 g (0.91 mole) of α-chloroisobutyric acid,[17] 1250 ml of dry acetone, and 251.8 g (1.82 moles) of anhydrous potassium carbonate were heated to reflux for 2 days. The solvent was removed by distillation under reduced pressure and the residual solid was taken up in 1 l. of water and 1 l. of benzene. The pH of the mixture was adjusted to 8.2 and the aqueous layer was washed with a fresh portion (400 ml) of benzene. Another portion (1 l.) of benzene was added to the water solution and the pH was adjusted to 2.0 with 6 *N* hydrochloric acid. The layers were separated and a second extraction of the aqueous phase was made with 400 ml of benzene. The two acidic benzene extracts were combined and dried over anhydrous magnesium sulfate. The benzene filtrate was concentrated under reduced pressure to about one-fifth the original volume. Crystals began to separate and the mixture was diluted with 2 l. of Skellysolve B (bp 60–70°) and cooled. The product was collected on a filter funnel, washed with Skellysolve B (petroleum ether, bp 60–70°), and dried: weight, 83.5 g (45.2%); mp 111.0–112.0°; ν_{max}^{KBr} 1717 (C=O), 1532 and 1358 (NO_2), and 1281 cm^{-1} (Ph–O–C). The nmr spectrum was consistent with the kinds and number of protons present and tlc showed the product to be homogeneous.

The analytical sample of 2-methyl-2-(*o*-nitrophenoxy)propionic acid was recrystallized from a mixture (1:4) of benzene and Skellysolve B (bp 60–70°). It melted at 111.2–113.1°.

Anal. Calcd for $C_{10}H_{11}NO_5$: C, 53.33; H, 4.92; N, 6.22. Found: C, 53.42; H, 4.82; N, 6.28.

Potassium 6-[2-methyl-2-(*o*-nitrophenoxy)propionamido]penicillanate (3d).—The following procedure was generally applicable to the preparation of all of the nitrophenyl-substituted penicillins (**3a–g**). A solution of 88.1 g (0.391 mole) of 2-methyl-2-(*o*-nitrophenoxy)propionic acid and 31.1 ml (51.1 g, 0.43 mole) of thionyl chloride in 700 ml of methylene chloride and 5.0 ml of N,N-dimethylformamide was heated to reflux for 4²/₃ hr. The methylene chloride and the excess thionyl chloride were removed by distillation under reduced pressure and the residual oil was taken up in 200 ml of methyl isobutyl ketone. This solution was added dropwise during a 15-min period to a cold (5°) mixture of 84.6 g (0.391 mole) of 6-APA, 4.0 l. of water, and 2.0 l. of methyl isobutyl ketone which was adjusted to a pH of 3.0 with 2.4 *N* hydrochloric acid. The pH was held at this level during the addition and for 30 min thereafter by the dropwise addition of 10% aqueous sodium hydroxide solution. The cooling bath was removed after all of the acid chloride was added and the reaction mixture was allowed to warm to the ambient temperature during the next 30 min. The pH was then adjusted to 2.0 with 6 *N* hydrochloric acid and the organic layer was washed with 500 ml of water and dried over magnesium sulfate. The filtrate was treated with 271 ml of a 30.4% solution of potassium 2-ethylhexanoate (0.391 mole) in methyl isobutyl ketone. The product crystallized immediately and the resultant mixture was stirred cold for 20 min. The crystals were collected by filtration, washed successively with methyl isobutyl ketone and Skellysolve B (bp 60–70°), and dried in a 40° vacuum oven: weight, 162.1 g (89.9%); ν_{max}^{KBr} 3410 (NH), 1780 (β-lactam C=O), 1688 (amide C=O), 1610 (carboxylate), 1530 and 1360 (NO_2), and 1249 cm^{-1} (Ph–O–C). Tlc indicated a homogeneous product.

Anal. Calcd for $C_{15}H_{20}KN_3O_7S$: C, 46.90; H, 4.36; N, 9.11. Found: C, 46.59; H, 4.31; N, 8.85.

Similarly these were prepared by this procedure: potassium 6-(*o*-nitrophenoxyacetamido)penicillanate (**3a**, 88.5%); potassium 6-(*o*-nitrophenylthioacetamido)penicillanate (**3b**, 69.0%); sodium 6-(*o*-nitrophenylacetamido)penicillanate (**3c**, 31.8%); potassium 6-(2-nitro-3,4,5-trimethylphenoxyacetamido)penicillanate (**3e**, 80.9%); sodium 6-(*p*-*t*-butyl-*o*-nitrophenoxyacetamido)penicillanate (**3f**, 94.5%); and potassium 6-[2-(*p*-*t*-butyl-*o*-nitrophenoxy)-2-methylpropionamido]penicillanate (**3g**, 89.6%). Structural assignments were supported by infrared and nuclear magnetic resonance spectral data.

***p*-*t*-Butylphenoxyacetic Acid.**[18]—A two-phase mixture of 100 g (0.665 mole) of *p*-*t*-butylphenol, 30 g (0.75 mole) of sodium hydroxide, 233 g (2.0 moles) of sodium chloroacetate, and 500 ml of water was heated at reflux for 16 hr. Concentrated hydrochloric acid (300 ml) was added and the mixture was cooled to room temperature. The crude product separated and was collected by filtration. It was washed with water and dried: weight, 118 g.

The above crude material (94.0 g) was dissolved in about 900 ml of warm water using 25 g of sodium hydroxide. Crystals of sodium *p*-*t*-butylphenoxyacetate separated on cooling to 5°. These were collected on a filter funnel and washed with 200 ml of cold (5°) water. The wet solid was added to 400 ml of water and the resultant mixture was heated to about 50°. The pH was adjusted to 1.5 with concentrated hydrochloric acid. An oil separated which soon crystallized. The solid was isolated, washed with water, and dried: weight, 47.5 g. This was recrystallized from 300 ml of hot Skellysolve B (bp 60–70°) to afford pure *p*-*t*-butylphenoxyacetic acid: weight, 46.5 g (42.2%); mp 96.0–97.0° (lit.[19] mp 88–89°).

***p*-*t*-Butyl-*o*-nitrophenoxyacetic Acid.**[18]—Concentrated nitric acid (sp gr, 1.44), 1.27 ml (0.02 mole), was added dropwise during a 5-min period to a cold (15°) solution of 4.17 g (0.02 mole) of *p*-*t*-butylphenoxyacetic acid in 50 ml of acetic anhydride. The resultant solution was stirred at 25–30° for 1.5 hr after which 75 ml of acetone and 605 ml of water were added. An oil separated which quickly crystallized. The mixture was cooled for 30 min and filtered. The crystalline product was washed with water and dried in a 90° oven: weight, 4.25 g (84.0%); mp 156.0–158.0°; tlc showed one zone; ν_{max}^{KBr} 1760 (C=O), 1240 (Ph–O–C), and 1530 and 1340 cm^{-1} (NO_2).

Anal. Calcd for $C_{12}H_{15}NO_5$: C, 56.91; H, 5.97; N, 5.53. Found: C, 57.28; H, 6.13; N, 5.52.

This product was used in the preparation of sodium 6-(*p*-*t*-butyl-*o*-nitrophenoxyacetamido)penicillanate (**3f**).[18]

***p*-*t*-Butyl-*o*-nitrophenol.**—Dilute nitric acid (8 *N*), 86.0 ml (0.69 mole), was added dropwise to a solution of 50.0 g (0.33 mole) of *p*-*t*-butylphenol in 216 ml of benzene, which was maintained at 20–22° by means of a cooling bath. The mixture was stirred at 20° for 2 hr after which time the benzene was removed by distillation. The aqueous mixture was extracted with methylene chloride. The organic layer was dried and the solvent was removed by concentration under reduced pressure. The oily residue weighed 59.0 g (91.4%) and was used in the next step without further purification.

2-(*p*-*t*-Butyl-*o*-nitrophenoxy)-2-methylpropionic Acid.—*p*-*t*-Butyl-*o*-nitrophenol (crude oil), 19.51 g (0.10 mole), 13.47 g (0.11 mole) of α-chloroisobutyric acid,[17] 195 ml of dry acetone, and 30.5 g (0.22 mole) of anhydrous potassium carbonate were combined and subjected to the same procedure as that which was used for the preparation of 2-methyl-2-(*o*-nitrophenoxy)propionic acid (see above). The final benzene solution was concentrated under reduced pressure to a brown syrup which crystallized after the addition of 300 ml of Skellysolve B (bp 60–70°). The crystalline product was collected by filtration, washed with Skellysolve B, and dried: weight, 9.54 g (33.9%); mp 116.0–118.0°; ν_{max}^{KBr} 2975 (CH), 1727 (C=O), 1536 and 1368 (NO_2), and 1291 cm^{-1} (Ph–O–C). It was homogeneous according to tlc.

Anal. Calcd for $C_{14}H_{19}NO_5$: C, 59.75; H, 6.82. Found: C, 60.13; H, 6.94.

(14) O. K. Behrens, J. W. Corse, R. G. Jones, and Q. F. Soper, U. S. Patents 2,479,295 (1949), 2,479,296 (1949), 2,562,410 (1951), and 2,623,876 (1952); *Chem. Abstr.*, **44**, 6441*bf* (1950); *ibid.*, **46**, 9616*a* (1952); *ibid.*, **47**, 2944*h* (1953).

(15) All melting points are corrected. Microanalyses are by Mr. R. M. Downing and the spectroscopic measurements by Mr. D. F. Whitehead.

(16) K. Randerath, "Thin-Layer Chromatography," Verlag Chemie, GmbH Weinheim/Bergstr, translated by D. D. Libman, Academic Press Inc., New York, N. Y., 1963, p 29.

(17) D. G. Kundiger, E. A. Ikenberry, E. B. W. Ovist, J. G. Peterson, and C. R. Dick, *J. Am. Chem. Soc.*, **82**, 2953 (1960).

(18) Prepared by H. H. Silvestri.

(19) C. M. Hill, M. E. Hill, A. O. Williams, and E. M. Shelton, *J. Am. Chem. Soc.*, **75**, 1084 (1953).

This product was used to prepare potassium 6-[2-(*p*-*t*-butyl-*o*-nitrophenoxy)-2-methylpropionamido]penicillanate (**3g**).

3,4,5-Trimethylphenoxyacetic Acid.—A solution of 22.1 g (0.162 mole) of 3,4,5-trimethylphenol and 6.48 g (0.162 mole) of sodium hydroxide in 81.0 ml of water was added to a solution of 18.9 g (0.162 mole) of sodium chloroacetate in 122.0 ml of water. The resulting mixture was heated to reflux for 24 hr. It was acidified to pH 1.8 with concentrated hydrochloric acid and the crystals that separated were collected by filtration, washed with cold water, and dried. This crude product weighed 27.0 g. It was partially dissolved in 600 ml of 5% aqueous sodium bicarbonate solution and the resultant mixture was filtered. The filtrate was washed with 100 ml of ether and the aqueous layer was adjusted to pH 1.8 with dilute sulfuric acid. The white product was collected, washed with water, and dried: weight, 15.7 g (50.0%); mp 150.0–151.0° (lit.[20] mp 149°); ν_{max}^{KBr} 1750 (C=O) and 1255 cm^{-1} (Ph–O–C).

2-Nitro-3,4,5-trimethylphenoxyacetic Acid.—Concentrated nitric acid (sp gr 1.44), 1.27 ml (0.02 mole), was added dropwise to a cold (15°) mixture of 3.88 g (0.02 mole) of 3,4,5-trimethylphenoxyacetic acid in 85.0 ml of acetic anhydride. After about 2 min, a solution resulted which was followed almost immediately by precipitation of a crystalline material. The mixture was stirred at 20–30° for 1 hr and then added to 254 ml of 50% aqueous acetone solution. The resultant solution was then diluted with 890 ml of water and the product crystallized. It was collected, washed with water, and dried: weight, 2.84 g (59.4%); mp 223.0–225.0°; ν_{max}^{KBr} 1748 (C=O), 1530 and 1330 (NO_2), and 1258 cm^{-1} (Ph–O–C).

Anal. Calcd for $C_{11}H_{13}NO_5$: C, 55.20; H, 5.47; N, 5.86. Found: C, 55.25; H, 5.68; N, 5.95.

This product was used to prepare potassium 6-(2-nitro-3,4,5-trimethylphenoxyacetamido)penicillanate (**3e**).

***o*-Nitrophenylthioacetic Acid.**—A mixture of 15.8 g (0.10 mole) of *o*-chloronitrobenzene, 8.0 g (0.20 mole) of sodium hydroxide, and 6.95 ml (9.2 g, 0.10 mole) of mercaptoacetic acid in 300 ml of water and 30 ml of 2-propanol was heated to reflux for 3 hr. The 2-propanol was distilled under reduced pressure and the red aqueous solution was extracted with two portions of benzene. The pH of the aqueous solution was adjusted to 2.0 with 6 *N* hydrochloric acid and yellow needles separated. The mixture was cooled for 45 min and filtered. The crystalline product was washed with water and dried: weight, 9.7 g (45.5%); mp 152.5–161.0° (lit.[21] mp 162–164°). This product was used in the preparation of potassium 6-(*o*-nitrophenylthioacetamido)penicillanate (**3b**).

Reduction of Potassium 6-[2-Methyl-2-(*o*-nitrophenoxy)-propionamido]penicillanate (3d) with Potassium Borohydride and Subsequent Conversion of the Reduced Intermediate 5b to 6-APA and 3,4-Dihydro-2,2-dimethyl-4-hydroxy-2H-1,4-benzoxazin-3-one (7b). General Procedure.—Potassium borohydride, 1.50 g (0.028 mole) was dissolved in 140 ml of cold (5°) water and this solution was stirred and cooled in an ice bath while 0.10 g of 5% palladium on charcoal was added. Hydrochloric acid (6 *N*), 0.4 ml, was added in order to adjust the pH from 11.5 to 9.0–8.5 and a cold (5°) solution of 6.36 g (0.0138 mole) of potassium 6-[2-methyl-2-(*o*-nitrophenoxy)propionamido]penicillanate in 60 ml of water was immediatedly added in portions to the chilled borohydride mixture. The pH drifted up and alternate dropwise addition of 6 *N* hydrochloric acid was necessary in order to keep it in the range of 8.5–9.0. Efficient stirring during the additions was essential to the success of the procedure and the hydrochloric acid should be added very carefully in order to avoid partial precipitation of the dissolved penicillin. The mixture was stirred at 0–5° for 15 min. Methyl isobutyl ketone, 200 ml, was added and the pH was adjusted to 2.0 with 6 *N* hydrochloric acid. The mixture was immediately filtered through a layer of Dicalite and the aqueous portion of the filtrate was extracted with two 80-ml portions of methyl isobutyl ketone. It was especially important to perform the preceding extractions as rapidly as possible in order to transfer as much as possible of the reduced penicillin (**5**) into the organic phase before it degraded to 6-APA. The wet methyl isobutyl ketone extracts were combined and crystals of 6-APA separated after 3–5 min. This mixture was stored for 90 min at room temperature and an equal period of time at 5°. The 6-APA was collected on a filter funnel, washed with methyl isobutyl ketone, and dried in a 40° vacuum oven: weight, 1.30 g (43.4%). This white crystalline product was identical with authentic 6-APA with respect to the R_f value on a silica gel coated glass plate, melting point, and infrared spectrum. It was 100% pure by chemical (hydroxylamine) assay.

The pH 2.0, methyl isobutyl ketone extracted aqueous filtrate was combined with 100 ml of fresh methyl isobutyl ketone and the resultant mixture was cooled to 0–5°. The pH was adjusted to 2.5–3.0 with 10% aqueous sodium hydroxide solution and 1.48 ml (1.73 g, 0.0094 mole) of α-phenoxypropionyl chloride was added in one portion. The mixture was stirred at 0–5° and the pH was maintained at the above level for 1 hr after the addition of the acid chloride. The pH was adjusted to 2.0 with 6 *N* hydrochloric acid and the aqueous layer was extracted with two portions (100 and 50 ml) of methyl isobutyl ketone. The three organic extracts were combined and passed through a Dicalite-coated filter funnel. The clear filtrate was then treated with 15 ml of a 54% aqueous solution of potassium acetate (sp gr 1.30) and the resulting mixture was stirred at room temperature for 4 hr. During this time, crystalline potassium phenethicillin separated. It was collected and dried in a 40° vacuum oven: weight, 1.42 g (25.5%). This sample was identical with authentic phenethicillin with respect to the R_f value on a silica gel coated glass plate and infrared spectrum. It was 91% pure by chemical (hydroxylamine) assay.

After the 6-APA was separated, the methyl isobutyl ketone filtrate was dried and almost all of the solvent was removed by distillation under reduced pressure. The oily residue quickly crystallized and the crystals were suspended in Skellysolve B (bp 60–70°) before being collected and dried: weight, 1.45 g; mp 122.1–129.6°. This solid was recrystallized from 31 ml of boiling water and simultaneously decolorized with 0.1 g of Darco KB. Cooling the filtered mixture for 3 hr afforded off-white needles. These were collected and dried: weight, 0.48 g (18.0%); mp 132.7–134.0°; ν_{max}^{KBr} 3100 (OH), 2980 (CH), 1677 and 1646 (C=O), 1262 (Ph–O–C), and 754 cm^{-1} (aromatic hydrogens). A dilute aqueous solution of the product changed to a purple color when treated with a 5% aqueous solution of ferric chloride. This observation would indicate the presence of a hydroxamic acid moiety.[22] This sample of 3,4-dihydro-2,2-dimethyl-4-hydroxy-2H-1,4-benzoxazin-3-one (**7b**) was homogeneous according to tlc.

Anal. Calcd for $C_{10}H_{11}NO_3$: C, 62.16; H, 5.74; N, 7.25. Found: C, 62.63, 62.45; H, 5.85, 5.91; N, 7.20.

Similarly, there was obtained, after the borohydride reduction of potassium 6-(*o*-nitrophenoxyacetamido)penicillanate (**3a**) and separation of 6-APA, 3,4-dihydro-4-hydroxy-2H-1,4-benzoxazin-3-one (**7a**): mp 163.2–164.1° (lit.[11b] mp 156°); ν_{max}^{KBr} 2800–3200 (OH, CH), 1685 and 1648 (C=O), 1280 (Ph–O–C), and 747 cm^{-1} (aromatic hydrogens). A dilute aqueous solution of this compound turned deep purple when treated with 5% aqueous ferric chloride solution.

Anal. Calcd for $C_8H_7NO_3$: C, 58.18; H, 4.27; N, 8.48. Found: C, 58.00; H, 4.37; N, 8.83.

Catalytic Hydrogenation of Potassium 6-[2-Methyl-2-(*o*-nitrophenoxy)propionamido]penicillanate (3d) under Pressure and Subsequent Conversion of the Reduced Intermediate 4b to 6-APA and 3,4-Dihydro-2,2-dimethyl-2H-1,4-benzoxazin-3-one (6b). General Procedure.—A solution of 3.18 g (0.0069 mole) of potassium 6-[2-methyl-2-(*o*-nitrophenoxy)propionamido]penicillanate in 50 ml of water was added in one portion to a suspension of 3.18 g of prehydrogenated (for 30 min under 50 psig of hydrogen) 30% palladium on diatomaceous earth in 50 ml of water. The resultant mixture was shaken under 50 psig of hydrogen for 1 hr at room temperature. Methyl isobutyl ketone, 100 ml, was added and this mixture was adjusted to pH 2.0 with 6 *N* hydrochloric acid. In rapid succession, the mixture was filtered through Dicalite and the aqueous phase was extracted with two 40-ml portions of methyl isobutyl ketone. The three wet organic solutions were combined and stored for 1 hr at room temperature and 2 hr at 5°. Crystalline 6-APA separated during this time. It was collected by filtration, washed with methyl isobutyl ketone, and dried in a 40° vacuum oven: weight, 0.65 g (43.4%). This sample was identical with authentic 6-APA with respect to tlc R_f value and infrared spectrum. It was 86% pure by chemical (hydroxylamine) assay.

(20) O. Kruber and A. Marx, *Chem. Ber.*, **73**, 1175 (1940).

(21) G. M. Badger, D. J. Clark, W. Davies, K. T. H. Farrer, and N. P. Kefford, *J. Chem. Soc.*, 2624 (1957).

(22) R. L. Shriner, R. C. Fuson, and D. Y. Curtin, "The Systematic Identification of Organic Compounds," 4th ed, John Wiley and Sons, Inc., New York, N. Y., 1956, p 122.

The acidic, methyl isobutyl ketone extracted aqueous filtrate was treated as in the preceding procedure to obtain potassium phenethicillin in 12.7% yield.

After the 6-APA was removed, the methyl isobutyl ketone filtrate was concentrated *in vacuo* to a brown solid: weight, 1.05 g; mp 164.3–166.5°. This was recrystallized twice from a boiling mixture of 2-propanol and water (3:1). Darco KB was used in the first recrystallization. A white, crystalline sample of 3,4-dihydro-2,2-dimethyl-2H-1,4-benzoxazin-3-one (**6b**) was obtained: weight, 0.45 g (36.8%); mp 165.6–166.1° (lit.[23] mp 161.5°); ν_{max}^{KBr} 2900–3200 (NH, CH), 1690 (C=O), 1251 (Ph-O-C), and 760 cm^{-1} (aromatic hydrogens). An aqueous solution of this compound gave no color change when treated with 5% aqueous ferric chloride solution.

Anal. Calcd for $C_{10}H_{11}NO_2$: C, 67.78; H, 6.26; N, 7.91. Found: C, 68.00; H, 6.41; N, 8.21.

In a similar manner, 3,4-dihydro-2H-1,4-benzoxazin-3-one (**6a**) was obtained after the catalytic reduction of potassium 6-(*o*-nitrophenoxyacetamido)penicillanate under hydrogen pressure: mp 171.0–174.8° (lit.[24] mp 173.5°); ν_{max}^{KBr} 2900–3200 (NH, CH), 1700 (C=O), 1220 (Ph-O-C), and 748 cm^{-1} (aromatic hydrogens). An aqueous solution of this compound did not change color when treated with 5% aqueous ferric chloride solution.

(23) C. A. Bischoff, *Chem. Ber.*, **33**, 931 (1900).

(24) W. A. Jacobs and M. Heidelberger, *J. Am. Chem. Soc.*, **39**, 2188 (1917).

Photochemistry of 2-Alkylaminophenoxaz-3-ones.[1a] II

M. C. WANI AND SAMUEL G. LEVINE[1b]

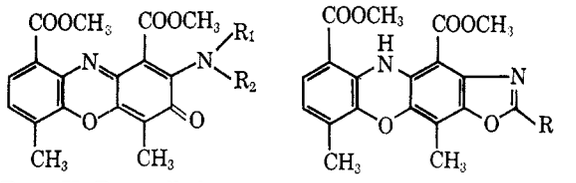
Natural Products Laboratory, Research Triangle Institute, P. O. Box 490, Durham, North Carolina

Received March 9, 1966

As a continuation of our previous study[2] the photochemistry of 2-dialkylaminophenoxaz-3-ones has been investigated. In general these compounds have been found to be more photoreactive than the corresponding monoalkylaminophenoxaz-3-ones. The 2-dimethylaminophenoxaz-3-one **4** underwent photochemical demethylation. Irradiation of the 2-polymethyleniminophenoxaz-3-ones gave various products depending upon the size of the polymethylenimine ring. The phenoxazones **9**, **11**, and **12** gave novel pentacyclic compounds **23**, **25**, and **26**, respectively. Stable dihydrophenoxaz-3-ones have been obtained by the reduction of compounds **10**, **11**, and **12**.

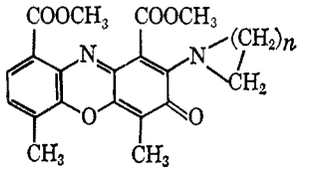
In an earlier paper,[2] we described the preparation and photochemistry of 2-monoalkylaminophenoxaz-3-ones **1**. Depending upon the nature of the 2-alkylamino substituent, an oxazolophenoxazine **2** or an oxazolinophenoxazine **3** was observed to be the photoproduct (Scheme I). We have now extended our studies to the photochemistry of 2-dialkylamino- and 2-polymethyleniminophnoxaz-3-ones.

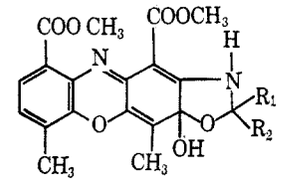
SCHEME I



1, R_1 = H, R_2 = alkyl
4, R_1 = R_2 = CH_3
6, R_1 = H, R_2 = $CH_2CH(OCH_3)_2$
7, R_1 = H, R_2 = $CH_2CH_2OCH_3$
13, R_1 = H, R_2 = CH_3

2, R = H
16, R = $CH(OCH_3)_2$
17, R = CH_2OCH_3





3, R_1 = R_2 = alkyl
5, n = 1
8, n = 2
9, n = 3
10, n = 4
11, n = 5
12, n = 6

The phenoxaz-3-ones[3] **4** to **12** were prepared from the corresponding 2-chlorophenoxaz-3-one by treatment with an appropriate amine as described in the preceding paper.[3] With the exception of the ethyleniminophenoxaz-3-one **5**, all of these products were found to be much more light sensitive than the corresponding monosubstituted compounds. Purification of these compounds had to be carried out by crystallization in the absence of light and all except **5** and **8** decomposed during thin layer chromatography (tlc). The nmr spectra of all the phenoxaz-3-ones were consistent with assigned structures (*cf.* Experimental Section).

Photolysis of 2-Dialkylaminophenoxaz-3-ones.—Irradiation of 2-dimethylaminophenoxaz-3-one **4** gave an unstable intermediate (Experimental Section) which on work-up formed 2-methylaminophenoxaz-3-one[3] **13**. The first step in this demethylation process appears to be the formation of a zwitterionic compound **14** and the latter then gives **13** by hydrolysis and oxidation. An alternative cyclization of **14** to the oxazoline **15** did not take place probably because of the ease of hydrolysis of the former (Scheme II). The intermediates derived from the irradiation of 2-polymethyleniminophenoxaz-3-ones may be less susceptible to hydrolysis than **14**, thus providing an opportunity for cyclization. It was, therefore, decided to study the photochemistry of a series of 2-polymethyleniminophenoxaz-3-ones.

The ethyleniminophenoxaz-3-one **5**, the first member of this series, did not give any identifiable product upon photolysis in benzene; most of the starting

(1) (a) This work was supported by Grant No. DA-ARO(D)-31-124-G250 and Contract No. DA-31-124-ARO(D)-169 with the U. S. Army Research Office; (b) Department of Chemistry, North Carolina State University, Raleigh, N. C.

(2) S. G. Levine and M. C. Wani, *J. Org. Chem.*, **30**, 3185 (1965).

(3) In connection with the preparation of 2-azetidinophenoxaz-3-one **8**, it is interesting to note that, although the nmr spectrum of the sample of azetidine prepared according to the method of I. M. Roberts and D. Horvitz [*Chem. Abstr.*, **58**, 10172 (1963)] indicated the presence of allylamine, the product was pure **8** as shown by its tlc and nmr spectrum. Since a large excess of the amine was used, a possible explanation of this observation may lie in the greater nucleophilicity of azetidine over allylamine.