m. p. 94–96°. A mixed m. p. with the authentic glycol made from ethyl lactate and phenylmagnesium bromide[9] was not depressed; m. p. 94–96°.

α-Hydroxy-α-phenylacetone Benzoate (VIII).—Phenylacetone (10 g., 0.075 mole) was brominated in 50 ml. of carbon tetrachloride with 12 g. (0.075 mole) of bromine. After the carbon tetrachloride had been evaporated under reduced pressure, a solution of 11.4 g. (0.08 mole) of sodium benzoate in 100 ml. of water and 100 ml. of alcohol was added. The reaction was refluxed for ten hours, after which time the volume of the solvent was reduced to 100 ml. After cooling the reaction mixture, the solid was filtered, washed with dilute hydrochloric acid and dilute sodium hydroxide, and finally recrystallized from 50% alcohol to give 11 g. (58%) of α-hydroxy-α-phenylacetone benzoate, m. p. 57–58°.

Anal. Calcd. for $C_{16}H_{14}O_4$: C, 75.57; H, 5.55. Found: C, 75.31; H, 5.32.

α-Methoxypropiophenone (XI).—The method used recently by Henze, Benz and Sutherland[9] for the preparation of fifteen different α-methoxyketones was used in this synthesis with slight modification. α-Chloroethylmethylether, b. p. 66–68°, prepared in 48% yield from paraldehyde, methyl alcohol, and dry hydrogen chloride, reacted with cuprous cyanide without solvent to give 27% of α-methoxypropionitrile, b. p. 110–113°; $n^{20}D$ 1.3822. Phenylmagnesium bromide converted the nitrile in 50% yield to α-methoxypropiophenone, b. p. 76–77° (0.8 mm.); $n^{25}D$ 1.5220; d^{25}_4 1.095.

Anal. Calcd. for $C_{10}H_{12}O_2$: C, 73.13; H, 7.38. Found: C, 72.79; H, 7.22.

The semicarbazone of α-methoxypropiophenone melted sharply at 161–162°.

Anal. Calcd. for $C_{11}H_{15}O_2N_3$: C, 59.71; H, 6.83; CH_3O, 14.0. Found: C, 59.82; H, 6.89; CH_3O, 14.1.

The methoxyketone was stable to warm methyl alcohol and did not react with benzoic acid at room temperature.

(9) Henze, Benz and Sutherland, THIS JOURNAL, **71**, 2122 (1949).

Summary

A reactive epoxyether (II), isolated from the reaction of α-chloropropiophenone and sodium methoxide, was cleaved rapidly with methyl alcohol and benzoic acid to give an α-hydroxyketal (IV) and an α-ketobenzoate (V), respectively. The structures of these derivatives were proven. The epoxyether gave no evidence of rearrangement to an ester during the formation or isolation.

DETROIT, MICH. RECEIVED APRIL 22, 1950

[CONTRIBUTION FROM THE PHARMACEUTICAL RESEARCH DIVISION, COMMERCIAL SOLVENTS CORPORATION]

Carboxy Derivatives of Benzylpenicillin[1]

BY R. P. HOLYSZ AND HOMER E. STAVELY

Until recently no chemical derivatives of benzylpenicillin had been reported except esters prepared with diazoalkanes. These esters were active parenterally only in species which were able to hydrolyze them to benzylpenicillin.[2] No data have been presented concerning their activity following oral administration.

The relationship of structure to antibiotic activity in derivatives of the free carboxyl group of penicillin has been of interest to a number of investigators. Prompted by the observation that certain penicilloic acid derivatives reacted with isobutyryl chloride in the presence of pyridine to form N-isobutyrylpenicilloic isobutyric anhydride (unpublished experiments) it was decided to investigate the formation of benzylpenicillinic alkanecarboxylic anhydrides and their utilization in the formation of benzylpenicillinic esters and amides. The publication on benzylpenicillinic anhydride[3] afforded an alternative method of approach to these derivatives. Subsequently, the preparation of benzylpenicillinic alkanecarboxylic anhydrides and the preparation of benzylpenicillinamide were reported.[4]

In the present work both procedures for the preparation of some carboxy derivatives of benzylpenicillin were investigated. Under the conditions employed benzylpenicillinic anhydride has proved to be superior to benzylpenicillinic acetic anhydride as an intermediate. The best yields (60–90%) of benzylpenicillinic anhydride, with minimum discoloration of the reaction mixture, were obtained by a modification of the method of Carpenter.[3] The anhydride was prepared by allowing thionyl chloride to react with triethylammonium benzylpenicillinate at −10 to 10° in chloroform solution. For every mole of penicillin salt approximately three-fourths of a mole of thionyl chloride was used. The anhydride was not isolated from such a solution, but the appropriate amine or alcohol was added, either as a solution or suspension in chloroform, and the reaction was allowed to proceed for a few hours at room temperature. The properties of the compounds prepared in this manner are listed in Table I.

$$2RCO_2^-HN^+Et_3 + SOCl_2 \longrightarrow (RCO)_2O + 2Et_3N^+HCl^- + SO_2$$

The product obtained by the addition of anhydrous hydrazine to the benzylpenicillinic anhydride solution was insoluble in most common organic solvents. Nitrogen analysis and its insolubility in acids supported the symmetrical structure, RCONHNHCOR. An attempt was made to prepare the monosubstituted hydrazine by reverse addition. Two products were isolated, one insoluble in ethyl acetate and identical with the disubstituted hydrazine, and the other soluble

(1) Presented in part before the Medicinal Chemistry Division, A. C. S., Atlantic City, New Jersey, September, 1949.

(2) Richardson, *et al.*, *Proc. Soc. Exptl. Biol. Med.*, **60**, 272 (1945); Kirchner, *et al.*, *J. Org. Chem.*, **14**, 388 (1949).

(3) Carpenter, THIS JOURNAL, **70**, 2964 (1948).

(4) Cooper and Binkley, *ibid.*, **70**, 3966 (1948).

TABLE I

PROPERTIES OF SOME CARBOXY DERIVATIVES OF BENZYLPENICILLIN

Compound	Formula	Nitrogen, %[f] Calcd.	Found	M. p., °C.	Specific rotation[g] $[\alpha]^{28}D$	Concn.	Solvent	Yield, %	Iodometric assay[h] (u./mg.)[i]	Bioassay[k] (u./mg.)
Benzylpenicillinamide[a]	$C_{16}H_{19}N_3O_3S$	12.61	12.64	160–161	266	1.93	$CHCl_3$	84	..	219
Benzylpenicillinobutylamide[b]	$C_{20}H_{27}N_3O_3S$	10.79	10.73	145–146	253	2.03	$CHCl_3$	84	1350	1.5
Benzylpenicillinodiethylamide[c]	$C_{20}H_{27}N_3O_3S$	10.79	10.68	151–152	102	2.20	$CHCl_3$	72	1580	0
Benzylpenicillinopiperidide[d]	$C_{21}H_{27}N_3O_3S$	10.47	10.55	176–177	85	1.67	$CHCl_3$	64	1570	0
p-Benzylpenicillinamidobenzoic acid[e]	$C_{23}H_{23}N_3O_5S$	9.27	9.05	130–135, dec.	293	1.37	EtOAc	66	1380	0
p-Benzylpenicillinamidobenzenesulfonamide	$C_{22}H_{24}N_4O_5S_2$	11.47	11.59	195–195.5	272	1.02	EtOAc	56	[j]	1.3
N,N′-Bis-(benzylpenicillinyl)-hydrazine	$C_{32}H_{36}N_6O_6S_2$	12.64	12.43	224–225, dec.	283	1.79	$HCONMe_2$	86	[j]	22
Bis-(benzylpenicillino)-hydroxamic acid	$C_{32}H_{36}N_6O_7S_2$	10.52	10.69	135–140, dec.	134	1.22	$CHCl_3$	74	..	135

[a] Compound was previously reported (see ref. (3)). [b] *Anal.* Calcd.: C, 61.67; H, 6.99. Found: C, 61.72; H, 6.86. [c] *Anal.* Calcd.: C, 61.67; H, 6.99; S, 8.23. Found: C, 61.84; H, 7.05; S, 8.41. [d] *Anal.* Calcd.: C, 62.81; H, 6.78. Found: C, 62.91; H, 7.10. [e] *Anal.* Calcd.: neut. equiv., 453.5. Found: neut. equiv., 456.1, 460.6. [f] Analyses were made by J. F. Alicino, Metuchen, N. J. [g] All rotations were determined in a semimicro 1-dm. polarimeter tube of *ca.* 2 ml. [h] See Experimental Section for details. [i] Sodium benzylpenicillinate assayed 1644 to 1658 u./mg. under these conditions. [j] Compounds were insoluble in 50% ethanol. [k] Activity against *Staph. aureus;* sodium benzylpenicillinate standard, 1667 u./mg.

in ethyl acetate. The latter product appeared to be an impure sample of benzylpenicillinohydrazide, but further purification was not achieved.

Reaction of benzylpenicillinic anhydride with hydroxylammonium chloride in the presence of triethylamine yielded a solid product. It probably was a dihydroxamic acid,[5] as supported by analytical data and by the fact that the color reaction with ferric chloride for the monohydroxamic acid was negative.

Infrared spectra of all of the amides, hydrazine and hydroxylamine derivatives prepared were determined. In each compound the characteristic penicillin absorption band near 5.65 μ (believed to be due to the presence of the β-lactam carbonyl group) was present. Additional evidence that the derivatives were not rearrangement products was obtained from iodometric titration data.[6] All of the compounds (except two which were insoluble in 50% ethanol) were subjected to alkaline hydrolysis followed by treatment with iodine. Consumption of iodine was 80 to 95% of that consumed by the hydrolysis product of sodium benzylpenicillinate under the same conditions.

In the standard penicillin bioassay only benzylpenicillinamide (219 u./mg.), N,N′-bis-(benzylpenicillinyl)-hydrazine (22 u./mg.), and bis-(benzylpenicillino)-hydroxamic acid (135 u./mg.) exhibited appreciable activity. The amides were tested *in vitro* against a representative group of organisms, including gram-positives, gram-negatives and mycobacteria. The derivatives were all considerably less active than sodium benzylpenicillinate. Benzylpenicillinamide, benzylpenicillinopiperidide and bis-(benzylpenicillino)-hydroxamic acid exhibited definite partial inhibition of *M. tuberculosis* at the level of 100 micrograms per milliliter.

(5) The compound was named bis-(benzylpenicillino)-hydroxamic acid by analogy with PhCONHOCOPh, dibenzohydroxamic acid. An alternative name proposed by Prof. Charles D. Hurd was O-(benzylpenicillinyl)-benzylpenicillinohydroxamic acid.

(6) Alicino, *Ind. Eng. Chem., Anal. Ed.*, **18**, 619 (1946).

Benzylpenicillinamide (100,000 u.) was administered orally to two dogs; however, no penicillin levels could be detected in either dog during the ensuing twelve hours, contrary to the findings of Cooper and Binkley.[4] Likewise the N-butyl and N,N-diethyl substituted amides and the piperidide failed to give detectable levels after oral administration, indicating that no enzymatic hydrolysis to benzylpenicillin had occurred, or that these derivatives were inactivated rapidly *in vivo*.

Penicillin levels were not detected at any time during a two-hour period following the intravenous administration of benzylpenicillinamide (4900 u.) to two dogs. In either animal or human serum the amide assayed 3 to 23 units per milligram; while in aqueous buffer at *p*H 6.0 the activity was consistently in the neighborhood of 200 units. In 4% egg albumin and in 0.3% fibrinogen solutions benzylpenicillinamide assayed 134 and 130 units, respectively, indicating that 30–35% apparently was bound with protein. In view of these data and the observation of Cooper and Binkley[4] that penicillinamide was resistant to penicillinase the apparent inactivity of benzylpenicillinamide probably was a result of protein binding.

Reactions of benzylpenicillinic anhydride with methanol, 1-butanol, 1-octanol, glycerol and propylene glycol were also investigated. None of the products, except the methyl ester, was crystalline, and they were not well characterized. When such products were administered orally to dogs no penicillin levels were demonstrated.

Experimental

Benzylpenicillinic Anhydride.—The anhydride was not isolated. A solution consisting of 13.06 g. (30 millimoles) of triethylammonium benzylpenicillinate and 200 ml. of absolute chloroform[7] was cooled to −10°. To this cold solution was added 1.60 ml. (22 millimoles) of purified thionyl chloride[8] and the resulting solution was allowed to

(7) U. S. P. chloroform was refluxed with and then distilled from phosphoric anhydride.

(8) Fieser, "Experiments in Organic Chemistry," D. C. Heath and Company, New York, N. Y., 1935, p. 339.

warm to +10°. Bioassay of such a solution before and after extraction with 2 *M* potassium phosphate buffer at *p*H 7.0–7.5 indicated that substantially all of the penicillin was present in the anhydride form.

Reaction of Benzylpenicillinic Anhydride with Soluble Amines.—The appropriate amine (ammonia, butylamine, diethylamine, piperidine; 50 millimoles) dissolved in 50 ml. of chloroform was added to the chloroform solution of the anhydride at 10°. After standing at room temperature for two hours the reaction mixture was extracted with two 100-ml. portions of 10% phosphoric acid, then with two 100-ml. portions of 2 *M* potassium phosphate buffer at *p*H 7.5, and finally with 100 ml. of water. The chloroform solution was dried over sodium sulfate at 0° and evaporated to dryness *in vacuo*. The resulting solid was taken up in ethyl acetate and recrystallized from ethyl acetate or mixtures of ethyl acetate and ligroin.

Benzylpenicillinamide was prepared from ammonia and the anhydride in chloroform solution. The desired amount of anhydrous ammonia was added in the form of a saturated chloroform solution. The amide was recrystallized from benzene–acetone mixtures.

***p*-Benzylpenicillinamidobenzenesulfonamide.**—Sulfanilamide was not appreciably soluble in the reaction mixture; therefore, it was added to the anhydride solution as a suspension (50 millimoles in 50 ml. of chloroform), and the reaction mixture was agitated constantly for four hours at room temperature. The mixture was processed as indicated above.

***p*-Benzylpenicillinamidobenzoic Acid.**—*p*-Aminobenzoic acid was not appreciably soluble in the reaction medium; therefore, it was added as a suspension in chloroform, and the reaction mixture was agitated for six hours at room temperature. After extraction with dilute phosphoric acid solution, the reaction solution was extracted with five 100-ml. portions of 2 *M* potassium phosphate buffer at *p*H 5.5, then once with water. The resulting product was recrystallized from a mixture of ethyl acetate and petroleum hexane (3:1).

N,N'-Bis-(benzylpenicillinyl)-hydrazine.—Reaction of 15 millimoles of benzylpenicillinic anhydride with 15 millimoles of anhydrous hydrazine under the usual conditions yielded an insoluble crystalline compound. It was not soluble in a wide variety of common solvents, except in N,N-dimethylformamide in which it was freely soluble at room temperature. The compound was recrystallized readily by dissolving it in dimethylformamide and then diluting it with approximately five volumes of boiling toluene.

Benzylpenicillinohydrazide.—A chloroform solution of 15 millimoles of benzylpenicillinic anhydride was added from a dropping funnel surrounded with Dry Ice to a solution of 50 millimoles (1.60 g.) of anhydrous hydrazine in 100 ml. of chloroform. Additions required two hours. On processing 1.14 g. of a crystalline compound, insoluble in ethyl acetate, was isolated. It was identical with N,N'-bis-(benzylpenicillinyl)-hydrazine, since there was no depression of the mixed melting point.

The fraction of the reaction product which was soluble in ethyl acetate was isolated as a powder (2.22 g.) by adding a solution of it to a large volume of cold petroleum pentane or ethyl ether. A suitable simple recrystallizing solvent was not found; m. p. 160–165°, dec.; $[\alpha]^{26}$ +144.7° (*c* 1.726 in chloroform); penicillin activity, 3.2 u./mg.

Anal. Calcd. for $C_{16}H_{20}N_4O_3S$: C, 55.15; H, 5.79; N, 16.08; S, 9.20. Found: C, 55.28; H, 5.87; N, 14.63; S, 9.70.

The infrared spectrum showed strong peaks at 5.70 and 6.03 μ, and it compared favorably with the spectrum of benzylpenicillinamide, indicating that the product was probably a penicillin derivative.

Bis-(benzylpenicillino)-hydroxamic Acid.—Reaction of 15 millimoles of benzylpenicillinic anhydride with 15 millimoles (1.04 g.) of hydroxylammonium chloride in the presence of 30 millimoles (4.20 ml.) of triethylamine under the usual conditions yielded a solid product which was purified by precipitating it from an ethyl acetate solution with ethyl ether. It gave no color reaction with ferric chloride, either in aqueous or aqueous alcoholic solution.

An attempt was made to prepare benzylpenicillinohydroxamic acid by the slow addition of the penicillin anhydride to an excess of hydroxylammonium chloride in the presence of triethylamine; however, only a dark oil was isolated.

Penicillin Esters.—By means of the aforementioned procedures, methyl benzylpenicillinate, m. p. 95–96°, was obtained in approximately 20% yield. The reaction products of the anhydride of penicillin with 1-butanol, 1-octanol, glycerol and propylene glycol were all viscous oils which could be converted to tacky solids by freeze-drying from benzene. None of these products was well characterized.

Benzylpenicillinamide from Benzylpenicillinic Acetic Anhydride.—The mixed anhydride was prepared by adding 0.70 ml. (9.8 millimoles) of acetyl chloride to 3.56 g. (10 millimoles) of sodium benzylpenicillinate in 50 ml. of N,N-dimethylacetamide at 0°. The reaction mixture was kept at 5° for ten minutes, then shaken with 100 ml. of a concentrated solution of ammonium phosphate of *p*H 8.0 at 0°. The product was isolated according to the procedure outlined by Cooper and Binkley[4]; however, only a few mg. of penicillinamide trihydrate, m. p. 61–63°, was obtained. Similar results were obtained when the reaction was carried out in dimethylformamide at lower temperatures (−10°).

Methyl Benzylpenicillinate.—Anhydrous methanol (19.7 millimoles) in 100 ml. of chloroform was added at 5° to a solution of benzylpenicillinic acetic anhydride obtained by the reaction of potassium benzylpenicillinate (20 millimoles) and acetyl chloride (19.7 millimoles) in 75 ml. of dimethylformamide. After one hour at 0° the product was isolated. Only 150 mg. (4.6% yield) of methyl benzylpenicillinate, m. p. 88–90°, was obtained.

Iodometric Assay.—The sample was dissolved in ethanol and the resulting solution was diluted with water to 50% volume. Suitable aliquots were assayed by the method of Alicino.[6] The results are shown in Table I.

Pharmacological Evaluation

Oral Tablets.—Tablets were prepared using aluminum hydroxide base. Each tablet contained a penicillin derivative in amount equivalent to 100,000 u. of benzylpenicillin when the derivative was completely hydrolyzed to penicillin. Benzylpenicillinobutylamide, benzylpenicillinodiethylamide and benzylpenicillinopiperidide each tested in two dogs exhibited no penicillin levels over a 12-hour period. When benzylpenicillinamide (100,000 u., *per se*) was given orally to dogs no penicillin levels could be demonstrated.

Intravenous Benzylpenicillinamide.—Two 10-kg. dogs were given intravenously 29 ml. of a solution of benzylpenicillinamide in physiological saline (169 u./ml.) and blood samples were collected after 5, 15, 30, 60 and 120 minutes. No penicillin activity was detected in any of the blood samples.

Bioassay.—The cup-plate method using *Staph. aureus* was employed. In buffer solution *p*H 6.0 benzylpenicillinamide assayed on the average 219 u./mg. In animal and in human serum the assay ranged from 3.3 to 23 u./mg.; while, in 4% egg albumin and in 0.3% fibrinogen solutions the activities were 134 and 130 u./mg., respectively.

Bacterial Spectra.—The crystalline derivatives were tested *in vitro* against *Staphylococcus*

aureus, Streptococcus faecalis, Streptococcus haemolyticus, Escherichia coli, Pasteurella pseudotuberculosis, Shigella paradysenteriae, Mycobacterium ranae, and *Mycobacterium tuberculosis.* Test levels in phenol red broth base or Long's medium were 0.1, 1.0, 10, 25, 50 and 100 μg./ml.

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Summary

Reactions of benzylpenicillinic anhydride with simple amino compounds such as ammonia, butylamine, diethylamine, piperidine, *p*-aminobenzoic acid and sulfanilamide yielded the corresponding amides of benzylpenicillin in good yields.

Under comparable conditions hydrazine and hydroxylamine yielded disubstituted hydrazine and hydroxylamine derivatives, respectively.

None of the compounds described had appreciable penicillin activity. Apparently these carboxy derivatives were not hydrolyzed to free penicillin in the blood or in the dog's intestinal tract. Some evidence indicated that protein binding was responsible for the apparent inactivity of penicillinamide.

All of the compounds were appreciably less active than sodium benzylpenicillinate against a representative group of organisms. Benzylpenicillinamide, benzylpenicillinopiperidide, and bis-(benzylpenicillino)-hydroxamic acid exhibited partial inhibition of the growth of *M. tuberculosis, in vitro.*

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[CONTRIBUTION FROM THE VENABLE CHEMICAL LABORATORY OF THE UNIVERSITY OF NORTH CAROLINA]

The Spectrophotometric Determination of the Approximate Dissociation Constants of the Monofluoroquinolines

BY WILLIAM K. MILLER WITH SAMUEL B. KNIGHT AND ARTHUR ROE

The optical method of Stenström and Goldsmith[1] for the determination of dissociation constants was applied to the fluoroquinolines. These determinations were carried out not only to test the method when applied to weak bases but also to compare the effects of substituting fluorine in the various positions of quinoline on its ionization. The constants of quinoline and 6-chloroquinoline were measured for comparison. Dissociation constants of the above compounds were also determined by measuring the *p*H of solutions of the hydrochlorides in order to test the spectrophotometric method. Since the quinoline derivatives are insoluble in water, all of the above measurements were made in 10% ethanol by weight.

According to the derivations of Stenström and Goldsmith,[1] the graphical determination of the *p*H at which the extinction coefficient is halfway between the values in alkaline and in neutral solution gives pK_a. Analogous considerations show that $pK_b = p\text{OH}$ when the extinction coefficient is midway between that in neutral and that in acid solution. The method has been applied by Stone and Friedman[2] and Phillips and Merritt[3] to the determination of the acid ionization constant of 8-hydroxyquinoline, and the latter authors also used the method to determine basic dissociation constants of 8-hydroxyquinoline and some of its derivatives. The values obtained by the two authors did not agree, but this discrepancy could probably be traced to the *p*H measurements rather than to the method.

Duplicate determinations at 25° of the dissociation constants of quinoline, the fluoroquinolines, and 6-chloroquinoline were made at each of two wave lengths. The wave lengths of the measurements were predetermined from the spectra of the compounds in neutral and acid solutions[4]; wave lengths at which the ion and molecule absorbed most differently were chosen. Sample plots of *p*H *vs.* extinction coefficient, from which the dissociation constants were determined graphically, are shown in Fig. 1. It was not found necessary to buffer the solutions which were measured since all of the critical values were in the distinctly acid region where absorption of carbon dioxide was not likely.

In order to test the values obtained by the spectrophotometric method, duplicate determinations of the dissociation constants of the bases were made by the approximate method of hydrolysis. The *p*H of solutions of the hydrochlorides of the compounds were measured, and dissociation constants were calculated from the expression

$$[H^+]^2/C - [H^+] = K_w/K_b$$

where C is the concentration of the salt, and $[H^+]$, the hydrogen ion concentration is obtained from the measured *p*H. Since the determinations were made in 10% ethanol, it was necessary to know K_w, the ion product of water, in 10% ethanol. This value was not directly

(1) Stenström and Goldsmith, *J. Phys. Chem.*, **30**, 1683 (1926).
(2) Stone and Friedman, THIS JOURNAL, **69**, 209 (1947).
(3) Phillips and Merritt, *ibid.*, **70**, 410 (1948).
(4) Miller with Knight and Roe, *ibid.*, **72**, 1629 (1950).