[CONTRIBUTION FROM THE RESEARCH DIVISION, BRISTOL LABORATORIES, INC.]

Carboxy Derivatives of Benzylpenicillin

BY DAVID A. JOHNSON

RECEIVED MARCH 18, 1953

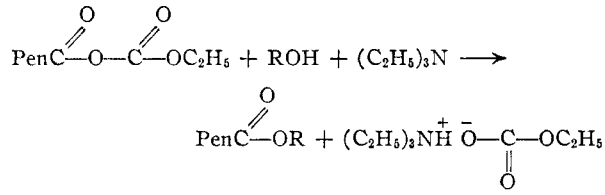
The reaction of triethylammonium benzylpenicillinate with ethyl chloroformate has provided a mixed anhydride which has served as the reactive intermediate for the preparation of a variety of carboxy derivatives of benzylpenicillin *via* a convenient and efficient procedure.

With the exception of salts, relatively few carboxy derivatives of benzylpenicillin have been reported. This apparent lack of interest can be attributed partly to the comparatively low order of antibacterial activity exhibited by the derivatives which are already known. The deficiency of suitable methods for the preparation of other derivatives in good yield has also been a factor.

Esters of benzylpenicillin have been prepared by treatment of the free acid with diazoalkanes,[1,2] by the interaction of salts of benzylpenicillin with activated alkyl halides,[3,4] and by treatment of benzylpenicillin anhydride with alcohols.[5]

Benzylpenicillinamide and its N-substituted derivatives have been obtained from both the mixed[6,7] and symmetrical[8] anhydrides of benzylpenicillin. Several of the mixed anhydrides have been described as stable crystalline solids.[9]

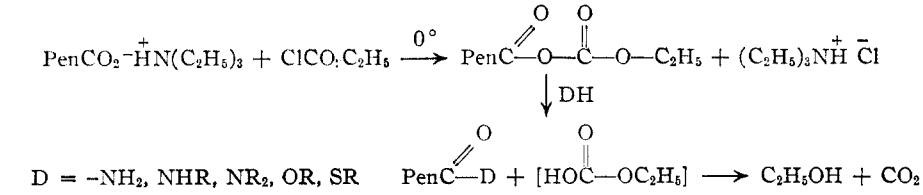
In this communication is described a simple and economical procedure which is applicable to the preparation of carboxy derivatives of benzylpenicillin in general. The reaction sequence can be represented by the equation



In general, the intermediate mixed anhydride was not isolated. However, by operating at low temperatures, it was obtained in a nearly pure condition as a colorless gum which decomposed slowly at 5°.

Table I lists examples of various types of derivatives prepared. The reported yields were obtained with a minimum of developmental work and do not necessarily represent optimum conditions. The simplicity and versatility of the method makes readily available the carboxy derivatives of benzylpenicillin.

The application of ethyl chloroformate to this problem was suggested by the recent disclosures of its use in peptide syntheses.[10-12]



Thus, a solution of triethylammonium benzylpenicillinate in methylene chloride was treated at 0° with ethyl chloroformate. After 30 minutes, the appropriate reagent (amine, alcohol, etc.) was added and the solution was allowed to warm to room temperature to complete the reaction. For the preparation of esters, it was found necessary to add an equivalent of triethylamine with the alcohol. The triethylamine acts as a catalyst and also prevents the liberation of ethanol, which could complicate the reaction.

Experimental[13]

Benzylpenicillin Ethyl Carbonic Mixed Anhydride.—A solution of triethylammonium benzylpenicillinate (4.36 g., 0.0100 mole) in 50 ml. of methylene chloride was treated at 0° with ethyl chloroformate (1.05 ml., 0.0110 mole). After storage at 0° for 30 minutes, the solution was washed with 25 ml. of cold *M* potassium phosphate buffer of *p*H 7.3 and with two 25-ml. portions of cold water. The methylene chloride solution was dried over magnesium sulfate and concentrated under reduced pressure at 0° to a colorless oil. Traces of solvent were removed at a pressure of 1–2 mm. to yield a colorless glass. The yield was 4.00 g. (98.5%), $[\alpha]^{25}D$ in acetone +207° (*c* 1.02).

Anal. Calcd. for $C_{19}H_{22}N_2O_6S$: C, 56.14; H, 5.46. Found: C, 55.4; H, 5.35.

The product was insoluble in water and the saturated hydrocarbons, but it dissolved readily in the other common organic solvents. A solution of 100 mg. of the mixed anhydride in 10 ml. of acetone was added to 75 ml. of *M* potassium phosphate buffer of *p*H 7.3. The milky solution was diluted with water to a volume of 100 ml. and stored at room temperature for 4 hours, after which time only a faint turbidity remained. Biological assay of the solution

(1) H. T. Clarke, J. R. Johnson and R. Robinson, "The Chemistry of Penicillin," Princeton University Press, Princeton, N. J., 1949, O. Wintersteiner, *et al.*, Chapter V, p. 92.

(2) F. K. Kirchner, J. R. McCormick, C. J. Cavallito and L. C. Miller, *J. Org. Chem.*, **14**, 388 (1949).

(3) K. A. Jensen, P. J. Dragstedt, I. Kioer, E. J. Nielsen and E. Fredericksen, *Acta Path. Microbiol. Scand.*, **28**, 407 (1951).

(4) H. F. McDuffie, Jr., and D. E. Cooper (to Bristol Laboratories, Inc.) U. S. Patent 2,578,570 (Dec. 11, 1951) [*C. A.*, **46**, 7127 (1952)].

(5) F. H. Carpenter, THIS JOURNAL, **70**, 2964 (1948).

(6) D. E. Cooper and S. B. Binkley, *ibid.*, **70**, 3966 (1948).

(7) D. E. Cooper (to Bristol Laboratories, Inc.) U. S. Patent 2,593,852 (April 22, 1952).

(8) R. P. Holysz and H. E. Stavely, THIS JOURNAL, **72**, 4760 (1950).

(9) D. E. Cooper (to Bristol Laboratories, Inc.), U. S. Patent 2,577,699, (Dec. 4, 1951) [*C. A.*, **46**, 7127 (1952)].

(10) R. A. Boissonnas, *Helv. Chim. Acta*, **34**, 874 (1951).

(11) T. Wieland and H. Bernhard, *Ann.*, **572**, 190 (1951).

(12) J. R. Vaughan, Jr., THIS JOURNAL, **73**, 3547 (1951); **74**, 676 (1952).

(13) All melting points are uncorrected. The microanalyses were performed by Mr. Richard M. Downing.

TABLE I

	Compound name	Yield,[a] %	Solvent[b]	M.p., °C.	$[\alpha]^{25}D$	In acetone[c]	Empirical formula	Carbon % Calcd.	Carbon % Found	Hydrogen, % Calcd.	Hydrogen, % Found	Nitrogen, % Calcd.	Nitrogen, % Found
1	Benzylpenicillinamide	82	A–W	66.5–67.5	+256°	1.07	$C_{16}H_{19}N_3O_3S\cdot 3H_2O$	(Reported m.p. 65°)[6]					
2	N-*n*-Butylbenzylpenicillinamide	81.5	EA–SB	157–158[e]	+276	1.14	$C_{20}H_{27}N_3O_3S$	61.67	61.7	6.99	7.04	10.79	10.9
3	N,N-Diethylbenzylpenicillinamide	68	EA–SB	151–152	+165	0.84	$C_{20}H_{27}N_3O_3S$	(Reported m.p. 151–152°)[8]					
4	Benzylpenicillin-*p*-anisidide	84	A–W	209–210 (dec.)	+299	1.04	$C_{23}H_{25}N_3O_4S$	62.85	62.6	5.73	5.64	9.56	9.28
5	N-2-Diethylaminoethylbenzylpenicillinamide	72.5	EA–SA	144.5–145.5	+256	1.15	$C_{22}H_{31}N_4O_3S$	61.23	61.0	7.24	7.53	12.98	12.7
6	N-2-Hydroxyethylbenzylpenicillinamide	83.5	MC–CT	115–116	+276	0.62	$C_{18}H_{23}N_3O_4S$	57.28	57.5	6.14	6.43	11.13	11.2
7	N-(2-Pyridyl)-benzylpenicillinamide	76.5	A–SB	140–141	+259	.83	$C_{21}H_{22}N_4O_3S\cdot C_3H_6O$	61.52	61.6	6.02	5.92	11.96	12.0
8	1-Benzylpenicillinyl-2-isonicotinylhydrazine	71.5	A–W	109–110 (dec.)	+214	.73	$C_{22}H_{23}N_5O_4S\cdot 2H_2O$	53.97	53.8	5.56	5.53	14.31	14.2
9	Ethyl benzylpenicillinylglycinate sulfone[d]	72.5	A–W	202–203 (dec.)	+156	.81	$C_{20}H_{25}N_3O_7S$	53.20	53.2	5.58	5.53	9.31	9.23
10	Methyl benzylpenicillinate	80	EA–SB	96–97	+234	.95	$C_{17}H_{20}N_2O_4S$	(Reported m.p. 97–98°)[1]					
11	Phenyl benzylpenicillinate	74.5	EA–SB	153.5–155	+210	1.10	$C_{22}H_{22}N_2O_4S$	64.37	64.6	5.40	5.51	6.83	6.74
12	Phenyl benzylthiolpenicillinate	72	EA–SA	147–148.5	+350	0.79	$C_{22}H_{22}N_2O_3S_2$	61.95	62.0	5.20	5.37	6.57	6.93

[a] After one recrystallization. [b] Solvent for recrystallization: A, acetone; CT, carbon tetrachloride; EA, ethyl acetate; MC, methylene chloride; SA, Skellysolve A (b.p. 28–38°); SB, Skellysolve B (b.p. 60–71°); W, water. [c] Reported m.p. 145–146°.[8] [d] Obtained by oxidation of the non-crystalline sulfide with potassium permanganate in 80% acetic acid.

indicated an activity of 1170 u./mg. which corresponded to an 80% regeneration of benzylpenicillin. After storage for 48 hours at 5°, another sample of the anhydride was subjected to similar conditions with a 64% regeneration of penicillin activity.

Benzylpenicillinamide.—A solution of triethylammonium benzylpenicillinate (4.36 g., 0.0100 mole) in 50 ml. of methylene chloride was treated at 0° with ethyl chloroformate (1.05 ml., 0.0110 mole). After storage at 0° for 30 minutes, 100 ml. of 15% diammonium phosphate solution was added, the ice-bath was removed, and the mixture was stirred vigorously for 3 hours. The layers were separated, 25 ml. of fresh 15% diammonium phosphate solution was added, and the two-phase system was chilled as 3 volumes of cyclohexane were added slowly. The benzylpenicillinamide trihydrate separated as colorless needles. The yield was 3.18 g. (82%), m.p. 63–64°. Recrystallization from acetone–water afforded colorless needles, m.p. 66.5–67.5° (reported m.p. 65°),[6,8] $[\alpha]^{25}D$ in acetone +256° (*c* 1.07). Alternatively, the mixed anhydride was formed from the sodium or potassium salts of benzylpenicillin by employing dimethylformamide or dimethylacetamide as solvents. Concentrated ammonium hydroxide was used as the ammonia source, and subsequent dilution with water provided the amide in yields of 64–76%.

N-Substituted Amides of Benzylpenicillin.—A methylene chloride solution of the mixed anhydride (0.0100 mole), prepared in the usual manner, was treated with a solution of the amine (0.0110 mole) in 20 ml. of methylene chloride and the ice-bath was removed. The solution was stored for 3–4 hours and washed with 25 ml. portions of 5% phosphoric acid, *M* potassium phosphate buffer of *p*H 7.3, and water. (For basic amides, the acid wash was replaced by water.) After drying over magnesium sulfate, the solution was concentrated under reduced pressure and the product was recrystallized from appropriate solvents.

Esters of Benzylpenicillin.—To the methylene chloride solution of the mixed anhydride (0.0100 mole) at 0°, was added a solution of the alcohol (0.0200 mole) and triethylamine (0.0100 mole) in 20 ml. of methylene chloride. The ice-bath was removed, and after storage for 3–4 hours, the product was worked up as for the amides. This same procedure was used for esters of phenols, thiophenols or mercaptans.

Acknowledgment.—The author is grateful to Dr. Lee C. Cheney and Professor John C. Sheehan for their invaluable advice and criticism.

SYRACUSE 1, NEW YORK