[CONTRIBUTION FROM THE STERLING-WINTHROP RESEARCH INSTITUTE]

THE USE OF DIAZO COMPOUNDS IN THE PREPARATION OF SOME BENZYLPENICILLIN ESTERS[1]

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In a previous publication (1) interest was indicated in the preparation of benzylpenicillin esters and the results of preliminary tests with the benzyl ester were given. The program was initiated with the hope that some derivative of penicillin would be found which would be more suitable than the salts of benzylpenicillin for oral administration and which would have a depot action on injection. Table I shows the data for the esters reported in this paper. The preparation of crude methyl, ethyl, and *n*-butyl esters has been reported previously (2, 3).

Because of the instability of the penicillin molecule the more common methods of esterification (acid with an alcohol, acid chloride with an alcohol or an alkoxide, etc.) could not be used. Therefore, a milder esterifying agent, the diazoalkanes, was chosen. The esters were prepared by treating free benzylpenicillin in an organic solvent with an excess of the appropriate diazo compound and isolating the pure ester.

The diazo compounds were prepared by an adaptation of Werner's method (4) in which the nitrosourea was decomposed at a low temperature with aqueous potassium hydroxide solution in the presence of an inert immiscible solvent to take up the product.

The necessary ureas were prepared from the corresponding amines by the nitrourea reaction (5) or, when the product was relatively water insoluble, by fusion of the amine with excess urea and isolation of the substituted urea by leaching with water (6).

Nitrosation of the substituted ureas was effected at low temperature using sodium nitrite and acetic acid.

Since the penicillin esters prepared with the usual diazo compounds were water-insoluble, interest was centered in the problem of introducing a group which would make the resulting ester more water-soluble. There was the possibility that such an ester might exhibit variations from the usual benzylpenicillin antibacterial "spectrum." For this purpose there was prepared an ester using 2-(2-pyridyl)diazoethane. The 2-(2-aminoethyl)pyridine used in the preparation of the diazo compound was prepared essentially by Galat's method (7). Further interest in a basic diazo compound stems from the fact that few diazo compounds are known which contain a basic ring-nitrogen. Thus, d'Angelo and

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his co-workers (8, 9) reported some diazoindoles, while Angelico reported diazopyrroles (10). No reference has been found in which diazo compounds containing a pyridine ring have been recorded.

An attempt was made to prepare basic diazoalkanes in which the basic nitrogen was not part of a ring. For this purpose 2-dimethylaminoethyl urea (11) and 3-diethylaminopropyl urea were prepared. However, the usual nitrosation procedure (with nitrous acid) did not yield any of the nitrosourea. In another attempt the preparation of *p*-dimethylaminophenylmethyldiazomethane was undertaken by preparing the ketazine from *p*-dimethylaminoacetophenone and hydrazine sulfate, using the procedure of Curtius and Franzen (12). The ketazine was converted to the hydrazone with anhydrous hydrazine and the hydrazone oxidized with yellow mercuric oxide (13). The preparation of *p*-dimethylaminophenyldiazomethane also was attempted, using anhydrous hydrazine to prepare the hydrazone and then oxidizing it with mercury acetamide (14). However, if any diazo compound was formed it was unusually unstable, for the red ether solution soon began to precipitate a decomposition product.

The use of 2-(2-pyridyl)diazoethane and 2-phenyldiazoethane is reported for the first time. Adamson and Kenner (15) had prepared diazoethane, diazopropane, diazo-*n*-butane, 3-methyldiazobutane, and diazopropene-2 using the corresponding nitrosoalkylaminomesityl oxide as an intermediate. Phenyldiazomethane was reported by Werner (4), who utilized the action of an alkali upon nitrosobenzyl urea, and by Staudinger (13), who oxidized benzalhydrazone with mercuric oxide. The preparation of diazomethane has been reported numerous times (16, 17).

Antibiotic action. Serial dilution tests indicate that the neutral esters are considerably less active *in vitro* than the benzylpenicillin salt (1). Since the solubility of the neutral esters in aqueous media is very low, accurate comparisons are difficult to make. The pyridylethyl ester can be prepared in solution as the hydrochloride (3 mg. per ml. *p*H 3.5) and tested by serial dilution in broth.[3]

The results of *in vivo* mouse-protection tests (1) with *Streptococcus hemolyticus* are summarized in the table.[4] These results are from one hour post-infection subcutaneous administration of the ester in sesame oil. Further tests indicate that considerable species specificity is evidenced in the protection of animals with benzylpenicillin esters. This appears to be related to the ability of the animal serum to hydrolyze the ester with the liberation of free benzylpenicillin.

Mr. W. F. Warner of these laboratories has shown that mouse and rat sera hydrolyze these esters to free benzylpenicillin, whereas rabbit, dog, and human sera do not have this property. These results are in agreement with those recorded by other workers (18, 19, 20).

[3] Tests by Dr. John Hays Bailey of these laboratories indicate comparable activity with this ester and benzylpenicillin salts against *Staphylococcus aureus* 209, and one-thirtieth the activity was displayed against *Bacillus subtilis* and *Streptococcus hemolyticus B.*

[4] The *in vivo* tests were carried out by Mr. H. Grunwald and Miss M. Shibuya.

EXPERIMENTAL[5]

Alkyl ureas. The ethyl, *n*-propyl, *n*-butyl, isobutyl, and allyl ureas were prepared by means of the nitrourea reaction (5).

Aralkyl ureas. The benzyl and 2-phenethyl ureas were prepared following essentially the method of Davis and Blanchard (6).

Nitrosoureas. The monosubstituted ureas, ethyl, *n*-propyl, *n*-butyl, isobutyl, allyl, phenethyl, and benzyl were nitrosated as follows: two-tenths mole of the substituted urea was dissolved in 100 ml. of glacial acetic acid and 15 ml. of water, and the solution cooled to 10° or less. To this cold solution was added from a dropping-funnel a solution of 28 g. (0.4 mole) of sodium nitrite in 60 ml. of water. The rate of addition was such that all of the

TABLE I

BENZYLPENICILLIN ESTERS

ESTER	CARBON, %		HYDROGEN, %		NITROGEN, %		DOSAGE FOR COMPLETE PROTECTION, MG. PER MOUSE[b, c]
	Calc'd	Found	Calc'd	Found	Calc'd	Found	
Methyl[a]	58.60	58.46	5.79	6.17	8.04	8.09	0.05
Ethyl	59.65	59.81	6.12	6.22	7.73	7.92	.05
n-Propyl	60.61	60.26	6.43	6.71	7.44	7.58	.05
n-Butyl	61.51	61.68	6.71	6.87	7.17	7.42	.01
Isobutyl	61.51	61.21	6.71	6.75	7.17	7.10	.02
Allyl	60.94	61.33	5.92	6.16	7.48	7.25	.03
Benzyl	65.07	65.26	5.69	5.57	6.59	6.61	.01
Phenethyl	65.73	65.65	5.98	6.15	6.39	6.50	.05
2-(2-Pyridyl)ethyl	62.85	63.05	5.73	5.36	9.56	9.38	.01

[a] This ester, m.p. 89.5–92.2° (corr.), has been prepared by other workers. This fact will be reported in the monograph, "*The Chemistry of Penicillin*," Princeton University Press, Princeton, N. J.

[b] Quantity of ester required for protection of 20-g. mice inoculated by the intraperitoneal route with 0.3 cc. of a 1:100,000 dilution of an 18 hour broth culture of *Streptococcus hemolyticus* C203.

[c] Sodium benzylpenicillin protective dose by similar test method is approximately 0.1 mg. per mouse.

nitrite solution was added in four hours. The mixture was diluted with 300 ml. of ice-cold water and the nitrosourea filtered off. After washing on the filter with water, the residue was purified by dissolving in 50 ml. of methanol at reflux temperature and then cooling the solution at 0° with stirring. The precipitate was filtered off and washed on the filter with a cold ether-Skellysolve A mixture (1:1). Yields were 35–55%, based on the substituted urea used. Phenethylnitrosourea, m.p. 100–101° (dec.).

Anal. Calc'd for $C_9H_{11}N_3O_2$: N, 21.76. Found: N, 22.14.

Diazo compounds. To 30 ml. of a cold (0–10°) 50% aqueous potassium hydroxide solution layered with 50 ml. of Skellysolve A was added over a period of ten minutes with constant stirring, 0.05 mole of the substituted nitrosourea. The latter usually was dispersed in the alkali layer as a grey or tan powder. Upon continued stirring for fifteen to thirty minutes

[5] In the experimental section physical constants and analyses are reported only for those compounds which have not been reported previously, and for those whose preparation differs from that found in the literature.

The analytical determinations were done under the direction of Mr. M. E. Auerbach in the Analytical Laboratories of this Institute.

the powder underwent spontaneous decomposition to give the diazo compound which was taken up by the Skellysolve A layer. The color of the latter ranged from yellow to red. Occasionally it was found desirable to initiate the decomposition of the nitrosourea salt by adding slowly 10–20 ml. of water. For the preparation of the esters the Skellysolve A layer was decanted and used directly.

2-(2-Aminoethyl)pyridine. The procedure was patterned after Galat's method (7).

In a two-liter, three-neck, round-bottom flask fitted with a thermometer and condenser were placed 380 g. (2.6 moles) of phthalimide and 276 ml. (2.6 moles) of 2-vinylpyridine. After adding 1 ml. of Triton B to the solution the mixture was heated under reflux until only one phase was present and the batch temperature reached 188° without appreciable refluxing (about two hours). The reaction mixture was cooled to 110° and a total of 500 ml. of chloroform was added in small portions through the condenser. The chloroform solution was cooled to 10°. Any solid precipitating at this point was filtered off. To the cooled chloroform solution was added Skellysolve A during mechanical stirring. The resulting precipitate was filtered with suction and washed with a chloroform-Skellysolve A mixture (1:3) until the washings were clear. By concentrating the mother liquor, cooling, and adding Skellysolve A as before, an additional quantity of precipitate was formed. In this manner there was obtained 511 g. (75%) of N-[2-(2-pyridylethyl)]phthalimide as a tan powder of m.p. 95–97°; m.p. of the hydrochloride was 214–215°.

Anal. Calc'd for $C_{15}H_{13}ClN_2O_2$: C, 62.39; H, 4.54; Cl, 12.28.
Found: C, 62.35; H, 4.41; Cl, 12.29.

In a one-liter round-bottom flask fitted with a stirrer were placed 124 g. (0.47 mole) of the substituted phthalimide and 500 ml. of methanol. After stirring for five minutes, 38.3 g. (0.65 mole) of hydrazine hydrate (85%) was added and stirring was continued for 1.5 hours at room temperature. At the end of this period practically all of the alcohol was removed under reduced pressure. The residue was treated with 400 ml. of water, the mixture stirred, and concentrated hydrochloric acid added slowly until the solution was acid to Congo Red paper. The solution was filtered with suction and the residue washed twice with 50-ml. portions of water. The combined filtrate and washings were made alkaline (> *p*H 10) with 40% aqueous sodium hydroxide solution and the basic solution extracted ten times with 50-ml. portions of chloroform. The chloroform solution was concentrated and after removal of the solvent the resulting residue was distilled at reduced pressure. The yield of amine, b.p. 92–93°/12 mm., was 26.5 g. (46%); reported 92–93°/12 mm. (21). M.p. of the dihydrochloride was 189°; reported, 185–186° (22).

Anal. Calc'd for $C_7H_{12}Cl_2N_2$: Cl, 35.68. Found: Cl, 35.89.

2-(2-Pyridylethyl)urea. Forty-one grams (0.34 mole) of the amine and 43 g. (0.41 mole) of nitrourea were added to 200 ml. of methanol contained in a flask fitted with a reflux condenser. Initial heat was applied cautiously; then gradually the contents were heated to reflux, the refluxing being continued for thirty minutes. After cooling to room temperature, ether was added until the solution became cloudy. On cooling in an ice-bath, crystals of the desired urea were precipitated. The yield of the urea, m.p. 143°, was 30 g. (55%).

Anal. Calc'd for $C_8H_{11}N_3O$: C, 58.18; H, 6.73; N, 25.45.
Found: C, 58.11; H, 6.72; N, 25.34.

2-(2-Pyridylethyl)nitrosourea. Thirty-three grams (0.2 mole) of the urea was added to 200 ml. of water and 65 ml. of concentrated hydrochloric acid. The solution was stirred and cooled to 0–10°, and a solution of 40 g. of sodium nitrite in 90 ml. of water was added dropwise over a period of four hours. The clear solution was extracted once with 25 ml. of chloroform, then neutralized at 5° with a saturated aqueous sodium bicarbonate solution. When the aqueous solution was neutral a precipitate formed which was filtered with suction. The residue on the filter paper was washed twice with cold water, then dried at 0° in a drying chamber. The yield of the nitroso compound, m.p. 108° (dec.), was 15.5 g. (40%).

Anal. Calc'd for $C_8H_{10}N_4O_2$: N, 28.86. Found: N, 28.26.

Crude penicillin esters. Two grams of calcium benzylpenicillin (about 800 units per mg.) was dissolved in 500 ml. of distilled water, 100 ml. of ethyl acetate was added, and the

mixture was cooled at 10°. The cooled solution was acidified by slowly adding phosphoric acid with stirring until *p*H 2.5 was reached. The mixture was shaken in a separatory funnel, the layers separated, and the aqueous layer extracted again, once with 75 ml. of ethyl acetate, followed by two 100-ml. portions of ether.

To the combined ethyl acetate-ether solution was added an excess of the desired diazo compound dissolved in Skellysolve A (23). The reaction mixture was allowed to stand for three hours at room temperature. At the end of this period any unused diazo compound was decomposed by washing with three 70-ml. portions of 1% citric acid or 1% acetic acid. The ethyl acetate-ether solution was then extracted with as many 50-ml. portions of 1% sodium bicarbonate solution as was necessary to obtain a colorless bicarbonate layer. In general, only one such washing was necessary. The mixture was washed once more with two 50-ml. portions of water and the organic solvents were removed *in vacuo* to give the crude ester.

With the pyridyl ester a slight modification in the above procedure was necessary. After standing for three hours about 50 ml. of the 1% acid solution was added to the reaction mixture to destroy any excess diazo compound. After separating the aqueous layer, it was made alkaline to *p*H 8 and extracted several times with ethyl acetate. This ethyl acetate extract was added to the original ethyl acetate-ether solution and the latter washed with the bicarbonate solution as outlined above.

Purification of penicillin esters. The crude esters were dissolved in about 15 ml. of ether, warmed slightly on a steam-bath, and Skellysolve B added to the point of turbidity. The flask then was cooled by immersing into a methylene chloride-Dry Ice bath to induce precipitation. During the cooling process the contents of the flask were swirled. The first precipitate obtained was gummy in appearance. The flask was removed from the cooling-bath, allowed to warm slightly, and then after making certain that all of the precipitate adhered to the bottom and sides of the flask, the supernatant liquid was decanted. The residue was redissolved in ether and the above procedure repeated about three more times. In this purification process the precipitate became more flocculent and semi-solid in nature. However, during the warming-up phase of the procedure the semi-solid softened and adhered to the flask so that removal of the supernatant liquid was facilitated. After the final decantation and warming to room temperature there remained a viscous yellow substance. The purified ester was subjected to a high vacuum in the presence of paraffin shavings to help remove the last traces of solvent. With complete removal of the solvent there remained with the exception of the crystalline methyl ester, a highly viscous, and sometimes brittle, substance. When constant weight was obtained the compound was submitted for analysis. The analytical values obtained for carbon and hydrogen were used as a criterion of purity. The preparation of some of these esters using crystalline sodium benzylpenicillin gave similar results.

SUMMARY

1. The following analytically pure benzylpenicillin esters have been prepared: methyl, ethyl, *n*-propyl, *n*-butyl, isobutyl, allyl, benzyl, phenethyl, and 2-(2-pyridylethyl).

2. The use of two diazo compounds, 2-(2-pyridyl)diazoethane and 2-phenyldiazoethane, is considered to be reported for the first time.

3. *In vivo* tests indicate that the penicillin esters are generally more effective than sodium penicillin in protecting mice against *Streptococcus hemolyticus*.

4. The ineffectiveness of the esters in higher animals probably is related to the inability of the sera to hydrolyze the esters.

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