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OXIDATIONS OF PENICILLIN ESTERS

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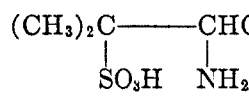
With few exceptions the preparation of derivatives of penicillins leads to products having no biological activity. One of the functional derivatives of penicillins which is claimed to have biological activity is the sulfone (1). Although the sulfone of methyl benzylpenicillin reputedly shows activity after hydrolysis of the ester group, or by *in vivo* assay, the corresponding sulfoxide is declared to be inert (2). In an investigation of oxidative degradations of penicillin esters with organic peracids, we have found that neither the mono- nor di-oxide[2] (sulfoxide and sulfone?) is active biologically by our testing procedures. The published activity of the sulfone (one-tenth that of the ester) (1) may have resulted from the presence of unoxidized ester with the sulfone. The stepwise preparation of the sulfone from ester through sulfoxide has not been described. Stepwise oxidations are described here for both the methyl and benzyl esters of benzylpenicillin.

In agreement with previous work, it has not been possible to isolate crystalline oxidation products of free penicillins. The methyl ester is known to yield a monoxide upon treatment with metaperiodate (2) and a dioxide upon oxidation with permanganate (3). The present work was initiated with the observation that treatment of the benzyl ester of benzylpenicillin with essentially anhydrous peracetic acid in acetonitrile yielded a crystalline product which was shown to be benzyl penicillaminate (I) which on catalytic hydrogenolysis yielded penicillaminic acid. When the oxidation was interrupted prior to separation of the penicillaminic ester, a crystalline compound was isolated from the reaction mixture which gave an analysis for a benzyl benzylpenicillin monoxide. Treatment of the isolated monoxide with peracetic acid led to formation of I. Oxidation of methyl benzylpenicillin with peracetic acid also yielded a monoxide which appeared to be identical with the monoxide prepared by oxidation with metaperiodate.[3] Perbenzoic acid could also be used for the oxidation.



I

An intermediate dioxide could not be isolated upon peracetic acid oxidation of monoxide to I. When the monoxide was oxidized with permanganate, the

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[2] The terms "monoxide" and "dioxide" are used rather than "sulfoxide" and "sulfone" since rigorous proof is not available that the latter terms represent the true structures.

[3] The sulfoxide described in reference (2) was stated to be a hemihydrate and melted at 123°. Our preparations were anhydrous; m.p. 127° (corr.).

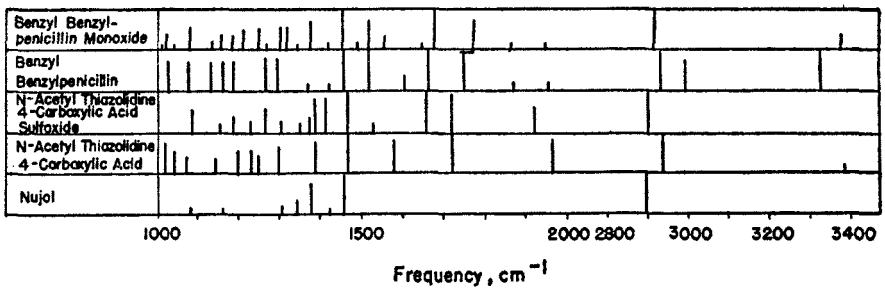
dioxide was obtained which upon treatment with peracetic acid also yielded I. Hydrogen peroxide had no effect on either the penicillin ester or its monoxide. Acetic acid had no effect on the penicillin esters in the time required for the oxidations described.

The various esters were tested for biological activity by suspension in rat blood serum, which hydrolyzed the ester group, followed by serial-dilution assay.[4] Both methyl and benzyl benzylpenicillin showed activity, while none of the oxidation products was active (less than $\frac{1}{10}$ unit per milligram). The same results were obtained by assay after mild alkaline hydrolysis of these compounds according to published procedures (1). Duplication of the described *in vivo* assay in mice was not attempted.

The monoxide obtained from benzyl benzylpenicillin was an easily crystallizable, sharply-melting product in contrast to the glassy starting ester. Ultraviolet absorption spectra show no evidence of penicillenate formation. The

TABLE I

Line Chart of Infrared Absorptions



oxides are neutral and show no basic groups by perchloric acid titration. In contrast, penicillin esters, at room temperatures, show the presence of one basic nitrogen in this titration. The basic nitrogen appears simultaneously with inactivation of the penicillin during titration. Mild alkaline hydrolysis of the monoxide utilizes from one to nearly two equivalents of alkali depending upon length of time of reaction. The monoxide did not react with iodine but one equivalent of iodine was reduced in the titration of a mild alkaline hydrolysis solution of the monoxide. The benzyl ester monoxide was unchanged after 16 hours refluxing in ethanol containing a trace of mercuric chloride.

The model compound N-acetylthiazolidine-4-carboxylic acid yielded the sulfoxide when treated with one mole of peracetic acid and the sulfone upon treatment with an excess. There was no evidence of cysteic acid formation.

Results of infrared absorption measurements of benzyl benzylpenicillin, its

[4] This method has proven to be quite reliable with penicillin esters. It appears to show *in vitro* activity wherever the activity appears *in vivo* since the latter activity of the esters depends on prior hydrolysis of the ester group by blood serum of the test animal. The *in in vitro* method also eliminates the factor of differences in rates of absorption of various esters which affect *in vivo* assays.

monoxide, and N-acetylthiazolidine-4-carboxylic acid and its sulfoxide are described as supplementary data in Table I. Until very recently (4) infrared absorption characteristics for the >SO bond were not available. The characteristic bond stretching vibration frequency for the sulfoxide group is given by Barnard, *et al.* (4) as 1055 cm^{-1}. The data in Table I show absorption bands at about 1080 cm^{-1} for both acetylthiazolidine-4-carboxylic acid sulfoxide and benzyl benzylpenicillin monoxide. However, benzyl benzylpenicillin absorbs in this region prior to its oxidation.

Attempts to isolate the products other than I obtained in oxidations with excess peracid have yielded glassy products difficult to characterize.

EXPERIMENTAL

Benzyl benzylpenicillinate monoxide. Method A. To 3 g. (7 millimoles) of benzyl benzylpenicillinate in 30 cc. of acetonitrile was added a solution of 14 millimoles of 70% peracetic acid in 10 cc. of acetonitrile; the mixture was kept at 5–10° for 30 minutes and then at 25° for 30 minutes. The solution was concentrated *in vacuo* to about 15 cc., then poured into aqueous sodium bicarbonate solution to yield a gummy precipitate which crystallized. The product was recrystallized from a dioxane-Skellysolve B solution as white plates, m.p. 146–147° (corr.), yield 2.1 g. or 68%.

Method B. The oxide was also obtained when a chloroform solution of perbenzoic acid was used in place of the peracetic acid. After the usual reaction period, the solution was shaken with aqueous sodium bicarbonate to remove acids and the chloroform evaporated to yield the oxide.

Anal. Calc'd for $C_{23}H_{24}N_2O_5S$; C, 62.71; H, 5.49; N, 6.36.
Found: C, 62.90; H, 5.38; N, 6.30.

A solution of 0.75 millimoles of the oxide and 3.8 millimoles of 90% hydrogen peroxide in 10 cc. of acetonitrile was allowed to stand at 25° for six hours. Upon dilution with water, the unchanged oxide was recovered.

Benzyl penicillaminate. To 482 mg. (1.1 millimoles) of the benzyl benzylpenicillinate monoxide in 25 cc. of acetonitrile was added 5 millimoles of peracetic acid in 5 cc. of acetonitrile. The solution was allowed to stand at 25°; crystals began to separate after two hours and separation was complete after about 24 hours with a yield of 375 mg. (60%) of benzyl penicillaminate, m.p. 245° dec.

Anal. Calc'd for $C_{12}H_{17}NO_5S$; C, 50.16; H, 5.96; N, 4.87; S, 11.16.
Found: C, 50.45; H, 6.14; N, 5.06; S, 11.01.

Potentiometric titration with 0.1 *N* alkali showed an equivalent weight of 209 between the points of inflection in the titration curve at *p*H 4.9 and 9.0; Calc'd mol. wt. is 197.

Catalytic hydrogenolysis of the compound in water with a palladium catalyst and evaporation of the solvent led to isolation of a crystalline acid, m.p. dec. slowly >270°. This product showed an identical potentiometric titration curve with a synthetic sample of penicillaminic acid.

Anal. Calc'd for $C_5H_{11}NO_5S$; C, 30.96; H, 5.52.
Found: C, 30.90; H, 5.62.

Methyl benzylpenicillinate monoxide. To one gram (3 millimoles) of methyl benzylpenicillinate (m.p. 97°) in 5 cc. of acetonitrile at 5–10° was added one cc. of 40% peracetic acid (5 millimoles) in 10 cc. of acetonitrile. The solution was kept at this temperature for 30 minutes and then allowed to warm to room temperature for 30 minutes. The mixture was poured into aqueous sodium bicarbonate solution and the oil which separated was extracted with chloroform. The chloroform extract was evaporated and the crystalline residue was recrystallized from either dioxane-Skellysolve B (or equivalent) or ethanol-water solutions and dried at 80°. Yield of white crystalline monoxide, 80%; m.p. 127° (corr.).

A preparation of methyl benzylpenicillinate sulfoxide from the ester by metaperiodate oxidation (2) yielded a compound with m.p. 127° and showed no depression of melting point when mixed with the peracetic acid oxidation product.

Anal. Calc'd for $C_{17}H_{20}N_2O_5S$; C, 56.03; H, 5.53.

Found: C, 56.07; H, 5.67.

Oxidation of benzyl benzylpenicillinate monoxide to a dioxide with permanganate. A solution of 17 cc. of 5% potassium permanganate in 80% acetic acid was shaken with one gram of benzyl benzylpenicillinate monoxide until solution was complete and the excess permanganate decolorized with 30% hydrogen peroxide [procedure used in the preparation of sulfone of methyl benzylpenicillinate (3)]. The mixture was poured into water, the precipitate was extracted with benzene, and the extract dried over sodium sulfate. Addition of Skellysolve B (or equivalent hydrocarbon) to the benzene solution yielded a precipitate which was dissolved in benzene and again precipitated. The product was dried *in vacuo* over paraffin to yield 350 mg. of white powder, m.p. 58–62°.

Anal. Calc'd for $C_{23}H_{24}N_2O_6S$; C, 60.51; H, 5.30.

Found: C, 60.73; H, 5.16.

A portion of the dioxide treated with excess peracetic acid in acetonitrile yielded crystalline benzyl penicillaminate.

Peracetic acid oxidation of 3-acetylthiazolidine-4-carboxylic acid. To a solution of 3.5 g. (0.02) mole) of 3-acetylthiazolidine-4-carboxylic acid (5) in 100 cc. of acetonitrile at 40° was added 0.02 mole of peracetic acid in 15 cc. of acetonitrile. After one hour the solution was concentrated under reduced pressure, diluted with water, and extracted with ethyl acetate. The extract was dried over sodium sulfate, evaporated to dryness, and the residue recrystallized from hot ethanol. The sulfoxide (about 50% yield) melted at 195–196°.

Anal. Calc'd for $C_6H_9NO_4S$; C, 37.68; H, 4.74.

Found: C, 37.73; H, 4.97.

When 0.1 mole of peracetic acid was used in the above preparation, the sulfone was obtained, m.p. 191–192°.[5]

Anal. Calc'd for $C_6H_9NO_5S$; C, 34.44; H, 4.33.

Found: C, 34.25; H, 4.20.

Reactions of benzyl benzylpenicillinate monoxide. A solution of 0.5 g. of oxide in 50 cc. of absolute ethanol containing 5 mg. of mercuric chloride was refluxed for 18 hours. A slight precipitate was filtered off and the unchanged oxide was recovered from solution.

To a solution of 215 mg. of the oxide in 10 cc. of dioxane and 5 cc. of water was added 10 cc. of 0.1 *N* sodium hydroxide in dropwise fashion while keeping record of the changes in *p*H. The solution was kept at *p*H 12 for one minute and then back-titrated with 0.1 *N* hydrochloric acid. The oxide utilized 1.5 equivalents of alkali during the hydrolysis; partial hydrolysis of the benzyl ester group apparently occurred. The neutralized hydrolysis solution was titrated with 0.1 *N* iodine solution. One equivalent of iodine was utilized. The original oxide did not react with iodine prior to hydrolysis.

Basicity of nitrogen in penicillins. Perchloric acid titration of sodium penicillins in acetic acid is known to demonstrate the presence of two basic groups (Na and N); esters show only one basic group. The titration of sodium penicillin with sulfuric acid in acetic anhydride with a Crystal Violet indicator also demonstrated two basic groups; the penicillin was inactivated by this treatment.

A solution of 100 mg. of potassium benzylpenicillin in 10 cc. of acetonitrile and 1 cc. of acetic acid was cooled to −20° to −10° and titrated with 0.1 *N* perchloric acid in acetic acid with Methyl Violet indicator. Rapid reaction occurred with one equivalent of acid and very slow reaction thereafter with a second equivalent. An aliquot of the titration mixture, diluted with *p*H 7 buffer and assayed, showed no inactivation of the penicillin after addition

[5] Ratner and Clarke (ref. 5) give m.p. 188–190° for the sulfoxide and m.p. 190° for the sulfone.

of the first equivalent of acid but nearly complete inactivation after the second. The first equivalent apparently reacted with the potassium.

Solutions of 100 mg. of methyl benzylpenicillin, benzyl benzylpenicillin, and the monoxide and dioxide of each in 10 cc. of acetic acid were titrated with perchloric acid in the usual manner. Only the first two reacted with one equivalent of perchloric acid; neither the monoxide nor dioxide showed the presence of basic nitrogen under these conditions.

Infrared absorption measurements. All samples were run in 0.025-mm. sections on a Perkin-Elmer Model 12B recording spectrophotometer. Solids were mulled in Nujol; benzyl benzylpenicillin was run without Nujol. In the high frequency region, a quartz prism was used to obtain greater dispersion than possible with the standard rock-salt prism. Only the data from the frequency sections from 1000 to 2000 and 2800 to 3400 cm^{-1} are given. The intensity of absorption at various wave numbers is denoted in the table by line charts.

Penicillin activity assays. The compounds were dissolved in a small volume of acetone, diluted with water and incubated with rat serum prior to assay by serial dilution techniques (6).

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