231° dec. Recrystallization from acetone–ether afforded a mixture of two polymorphic forms, cubes and fine needles, both of which melted at 237–237.5° dec.

Anal. Calcd. for $C_{17}H_{16}N_2O_5S$: C, 56.66; H, 4.48; N, 7.77; mol. wt., 360. Found: C, 56.47; H, 4.52; N, 8.02; mol. wt., 350 ± 10.

On the basis of this elemental analysis and molecular weight, and on the infrared (Fig. 1, curve C) and ultraviolet (Fig. 2) spectra, the compound was assigned structure VIII.

From 8.1 g. (0.0195 mole) of the α-isomer of V and 100 ml. of phosphorus oxychloride in 460 ml. of benzene in a procedure identical to that described above for the β-isomer, there was obtained 2.94 g. (42%) of crude VIII, m.p. 220–228° dec. Recrystallization from acetone–benzene gave fine silky needles, m.p. 236–236.5° dec.

Similarly, this reaction when carried out with 1.5 g. (3.6 millimoles) of Vγ and 19 ml. of phosphorus oxychloride in 85 ml. of benzene afforded 0.77 g. (60%) of VIII, m.p. 236.5–237° after recrystallization from acetone–benzene.

Reaction of Hydrazine with Methyl 6-Phthalimidopenicillanate.—To a solution of 0.18 g. (0.5 millimole) of VI in 10 ml. of dioxane was added 5.0 ml. of a 0.242 *M* solution of hydrazine in dioxane (two equivalents). After storage at room temperature for 16 hours, the mixture was lyophilized, and the phthalhydrazide complex broken up with 5.4 ml. of 0.0992 *N* hydrochloric acid. The phthalhydrazide was removed by filtration, and the filtrate lyophilized. Crystallization of the product from methanol–ether afforded 110 mg. (74%) of X, m.p. 151–152° dec.

Anal. Calcd. for $C_9H_{19}N_4O_3SCl$: C, 36.13; H, 6.42; N, 18.74. Found: C, 36.08; H, 6.37; N, 18.62.

In another experiment, 360 mg. (1 millimole) of VI and 50 mg. (1 millimole) of hydrazine were allowed to react in 5 ml. of dioxane for 6 hours. A crystalline substance separated and was collected by filtration; addition of ether to the mother liquors afforded more of this material. An infrared absorption spectrum showed the disappearance of the bands characteristic of the phthalimido and β-lactam groups, with the appearance of intense peaks in the regions of 6.0 to 6.1 and 6.4 to 6.6 μ. The substance thus appeared to be the phthalhydrazide complex of 4-carbomethoxy-5,5-dimethyl-α-amino-2-thiazolidineacetyl hydrazide. The yield was 210 mg. (91%, based on hydrazine). Treatment of a portion of this material with an equivalent of aqueous hydrochloric acid afforded the hydrochloride X, m.p. 150–152°. From the mother liquors of the reaction with hydrazine there was recovered 165 mg. (92%) of the unreacted VI.

CAMBRIDGE 39, MASSACHUSETTS

[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY, MASSACHUSETTS INSTITUTE OF TECHNOLOGY]

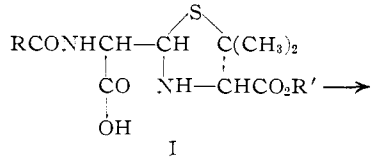
The Synthesis of Substituted Penicillins and Simpler Structural Analogs. XI. Methyl 6-Benzylsulfonamidopenicillanate

BY JOHN C. SHEEHAN AND PHILIP A. CRUICKSHANK

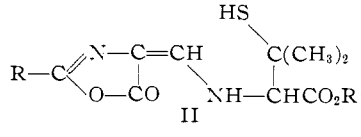
RECEIVED FEBRUARY 15, 1956

By a cyclization procedure, the "sulfonyl analog" of benzylpenicillin has been prepared in which the phenylacetamido side chain has been replaced by the benzylsulfonamido group. Use of the sulfonyl blocking group on the α-amino-2-thiazolidineacetic acid derivative precludes azlactone formation. Two stereoisomeric modifications (two racemates) have been isolated, one of which corresponds in configuration to the natural penicillins. Also obtained was an isomeric material to which has been assigned the 5-keto-2,3,4,5-tetrahydro-1,4-thiazepine ring system.

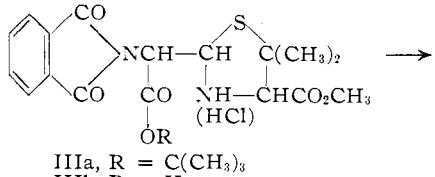
Many attempts directed toward the cyclization of penicilloates of type I have resulted in azlactonization followed by disruption of the thiazolidine ring to give penicillenates (II).[1] Recently, the use of phthalimido derivatives (III), in which the possibility of azlactone formation is precluded, has afforded a fused thiazolidine-β-lactam,[2] the ring system present in penicillin.



I



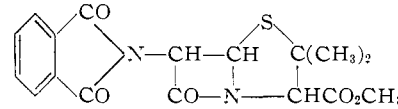
II



IIIa, R = $C(CH_3)_3$
IIIb, R = H

(1) H. T. Clarke, J. R. Johnson and R. Robinson, editors, "The Chemistry of Penicillin," Princeton University Press, Princeton, N. J., 1949, p. 851.

(2) THIS JOURNAL, **78**, 3680 (1956).



IV

The bulkiness of this diacyl blocking group imparts considerable steric hindrance toward cyclization in compounds of type IIIb. This effect apparently has prevented the formation of the methyl 6-phthalimidopenicillanate (IV) having a configuration corresponding to the natural penicillins.[2]

This paper describes the use of the benzylsulfonyl blocking group for penicillanate synthesis. The steric factors of this side chain are very similar to those encountered in the natural penicilloates, while the azlactonization reaction is entirely avoided. Two isomeric benzylsulfonamidopenicillanates (VIII) have been prepared.

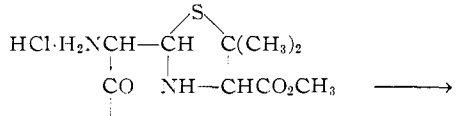
An important consideration in the use of blocking groups for the synthesis of the penicillin structure concerns the ease of their removal after formation of the β-lactam ring. The sulfonamido functions are deficient in this respect for, under conditions of cleavage, hydriodic acid or phosphonium iodide in glacial acetic acid, or sodium in liquid ammonia,[3] the azetidinone ring would be destroyed. Investigations employing the benzylsulfonyl group were

(3) R. A. Boissonnas and G. Preitner, *Helv. Chim. Acta*, **36**, 875 (1953).

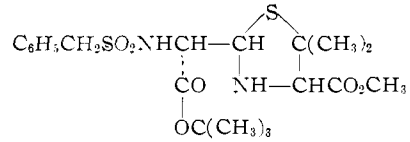
undertaken, however, with two pertinent facts in mind. First, the removal of the benzyl group from certain functions under mild conditions is a well known reaction.[4] Although the cleavage of a benzyl sulfone or benzylsulfonamido type of linkage has not been reported, a reaction of this type might very well be feasible. Second, the penicillanate of natural configuration having this side chain is a sulfonyl analog of benzylpenicillin and, hence, might be expected to display biological activity.

The key intermediates for the synthesis of the β-lactams VIII are the two (of four possible) 4-carbomethoxy-5,5-dimethyl-α-benzylsulfonamido-2-thiazolidineacetic acid hydrochlorides (VII). A straightforward preparation of these substances recently has been made available *via* the corresponding α-aminothiazolidines Vα and Vγ.[5]

The α-isomer of *t*-butyl 4-carbomethoxy-5,5-dimethyl-α-amino-2-thiazolidineacetate hydrochloride (Vα), upon treatment with one equivalent of benzylsulfonyl chloride and a tertiary base, afforded in good yield the benzylsulfonamido derivative VIα. Similarly, the γ-isomer of V gave the isomeric thiazolidine VIγ, although in somewhat poorer yield.

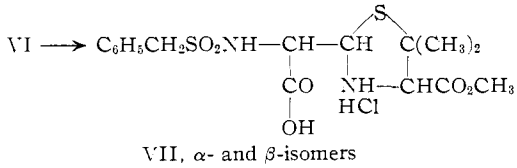


V, α- and γ-isomers



VI, α- and γ-isomers

The unusual lability of the *t*-butoxy group toward anhydrous acid[6] allows the facile cleavage of the carbo-*t*-butoxy group in compounds VI to give the desired β-amino acid hydrochlorides VII. Treatment of nitromethane solutions of VIα and VIγ with anhydrous hydrogen chloride at 0° liberated the acetic acid carboxyl function in very good yield.

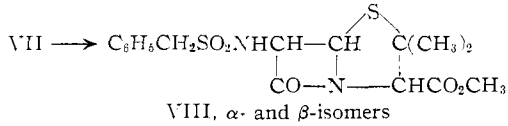


VII, α- and β-isomers

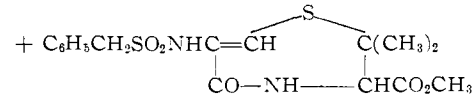
Since a higher temperature during the cleavage step has been shown to lead to a third isomer in the phthaloyl series,[5] samples of VIα and VIγ in nitromethane solution were treated with anhydrous hydrogen chloride at 70°. Material isolated from each of the reactions was found to be identical in all respects to that obtained in the 0° cleavage of VIγ. It would thus appear that the γ-isomer of VI underwent an isomerization during the cleavage, even at 0°. The alternative, isomerization of VIα to the γ-configuration at elevated temperature, does not seem likely. If this were the case, a similar phenomenon should have been observed in the phthaloyl series; actually the α- and γ-isomers of IIIa were found to give the same cleavage product at 70°, but it was different from either 0° cleavage product. The isomer of 4-carbomethoxy-5,5-dimethyl-α-benzylsulfonamido-2-thiazolidineacetic acid hydrochloride derived from VIγ was designated beta to signify that an isomerization had occurred.[5]

The cyclizations of VIIα and VIIβ were carried out employing conditions found to be optimum for the synthesis of methyl 6-phthalimidopenicillanate (IV), *i.e.*, a 25% solution of thionyl chloride in methylene chloride. In preliminary experiments, the crude mixtures from the lactamization reactions were oxidized by means of potassium permanganate in 80% acetic acid, affording the crystalline methyl 6-benzylsulfonamidopenicillanate sulfones (IX). Their infrared spectra are shown in Fig. 1 (curve A, α-isomer; curve B, β-isomer). Note the absorption peak at 5.55 μ characteristic of the azetidinone carbonyl in such environment.

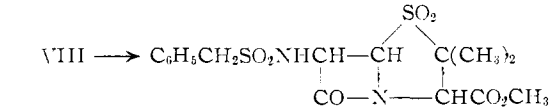
Efforts to bring about the direct crystallization of the α-isomer of methyl 6-benzylsulfonamidopenicillanate (VIIIα) from the products of the reaction of VIIα with thionyl chloride were unsuccessful.



VIII, α- and β-isomers



X (from α-isomer only)



IX, α- and β-isomers

However, its separation in crystalline form was achieved by means of chromatography over alumina. The compound, isolated in 36% yield, displayed the intense absorption at 5.62 μ in the infrared characteristic of a β-lactam carbonyl (Fig. 1, curve C).

During the attempts to crystallize VIIIα, there was obtained an isomeric substance to which structure X has been assigned. Evidence that it is 2,2-dimethyl-3-carbomethoxy-5-keto-6-benzylsulfonamido-2,3,4,5-tetrahydro-1,4-thiazepine was obtained from its ultraviolet (Fig. 2) and infrared (Fig. 1, curve E) spectra. The intense peak at 6.3 μ in the infrared is assignable to a vinyl sulfide chromophore[7] and peaks at 3.1, 6.1 and 6.6 μ are all characteristic of a monosubstituted amide. The absorption maxima present in the ultraviolet are consistent with the chromophoric system present in X. It is of interest to note that the spectra of this compound correspond closely with those of an analogous substance isolated from the reaction of phosphorus oxychloride with IIIb.[2]

(4) R. Adams, *et al.*, Editors, "Organic Reactions," Vol. VII, John Wiley and Sons, Inc., New York, N. Y., 1953, p. 263.

(5) The synthesis of these compounds and an explanation of the isomer designations will be found in paper IX in this series.

(6) J. C. Sheehan and G. D. Laubach, THIS JOURNAL, **73**, 4752 (1951).

(7) C. C. Price and H. Morita, *ibid.*, **75**, 4747 (1953).

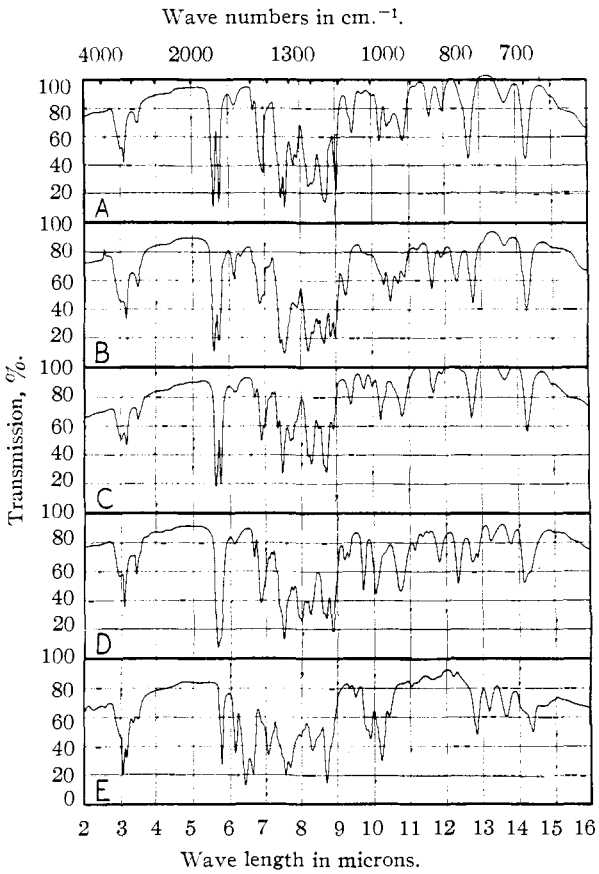


Fig. 1.

The β-isomer of methyl 6-benzylsulfonamidopenicillanate (VIIIβ) crystallized directly from the products of the reaction of VIIβ and thionyl chloride after a preliminary purification. This compound, isolated in 46% yield, also showed intensive absorption at 5.62 μ (Fig. 1, curve D). None of the isomeric by-product X appeared to be formed from this stereoisomer of VII under these conditions.

We are indebted to Bristol Laboratories, Syracuse, N. Y., for generous financial support of this work.

Experimental[8]

***t*-Butyl 4-Carbomethoxy-5,5-dimethyl-α-benzylsulfonamido-2-thiazolidineacetate (VI). A. α-Isomer.**—To a solution of 5.0 g. (0.0146 mole) of *t*-butyl "α"-4-carbomethoxy-5,5-dimethyl-α-amino-2-thiazolidineacetate hydrochloride (Vα) and 3.03 g. (0.03 mole) of triethylamine in 75 ml. of methylene chloride at a temperature of 0°, there was added a solution of 2.95 g. (0.0154 mole) of benzylsulfonyl chloride[9] in 25 ml. of methylene chloride. After 12 hr., the solution was washed thoroughly with water, dried over magnesium sulfate and concentrated under reduced pressure to a colorless resin. From absolute ethanol there was obtained 4.8 g. (71.5% of colorless needles, m.p. 150–151°. Two recrystallizations from ethanol gave an analytical sample, m.p. 153.5–155°.

(8) All melting points are corrected. We are indebted to Dr. S. M. Nagy and his associates for the microanalyses, and to Dr. N. A. Nelson and his associates for the infrared and ultraviolet spectra. The samples for infrared spectra (Fig. 1) were prepared in 1% concentration in potassium bromide pellets. The solvent for the ultraviolet spectrum was alcohol.

(9) C. Ziegler and J. M. Sprague, *J. Org. Chem.*, **16**, 621 (1951).

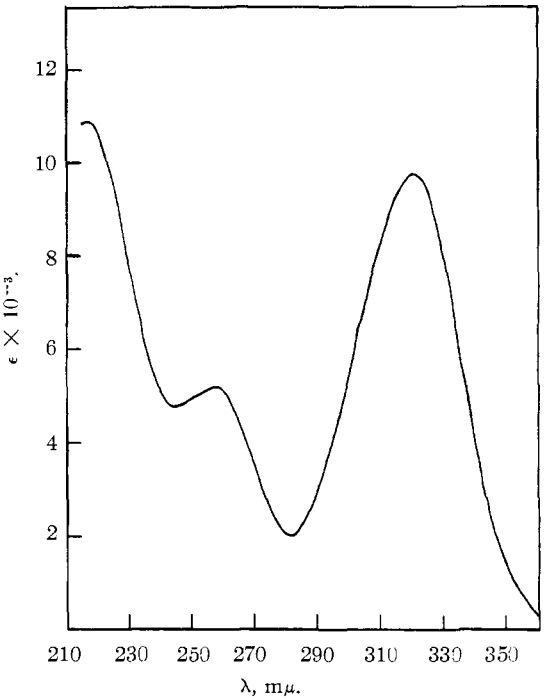


Fig. 2.

Anal. Calcd. for $C_{20}H_{30}N_2O_6S_2$: C, 52.39; H, 6.59; N, 6.11. Found: C, 52.61; H, 6.47; N, 6.09.

B. γ-Isomer.—From the reaction of 6.25 g. (0.0183 mole) of Vγ, 3.70 g. (0.0366 mole) of triethylamine and 3.50 g. (0.0183 mole) of benzylsulfonyl chloride, there was obtained, after crystallization from ether–petroleum ether, 4.43 g. (53%) of VIγ. An analytical sample, recrystallized from ethanol–water, had m.p. 138–139.5°.

Anal. Calcd. for $C_{20}H_{30}N_2O_6S_2$: C, 52.39; H, 6.59; N, 6.11. Found: C, 52.72; H, 6.56; N, 6.19.

4-Carbomethoxy-5,5-dimethyl-α-benzylsulfonamido-2-thiazolidineacetic Acid Hydrochloride (VII). A. α-Isomer.—Through a cold (0°) solution of VIα (4.4 g., 0.0104 mole) in 25 ml. of purified nitromethane[10] was bubbled a stream of anhydrous hydrogen chloride for a period of 5–6 minutes. After storage at 0–5° for 10 hr., 50 ml. of ether was added. The product, 3.67 g. (87%) of feathery needles, m.p. 141.5–142.5° dec., was collected by filtration.

Anal. Calcd. for $C_{16}H_{23}N_2O_6S_2Cl$: C, 43.78; H, 5.28; N, 6.38. Found: C, 43.59; H, 5.46; N, 6.59.

B. β-Isomer.—A solution of 1.0 g. (2.20 mmoles) of VIγ in 10 ml. of purified nitromethane, cooled to 0°, was saturated with hydrogen chloride by passing the anhydrous gas through the solution for 5 minutes. Storage at 0–5° for 14 hr. afforded 0.80 g. (83.5%) of VIIIβ, m.p. 153–154° dec.

Anal. Calcd. for $C_{16}H_{23}N_2O_6S_2Cl$: C, 43.78; H, 5.28; N, 6.38. Found: C, 43.68; H, 5.27; N, 6.47.

Treatment of 0.50 g. (1.10 mmoles) of VIγ in 5.0 ml. of purified nitromethane with anhydrous hydrogen chloride at 72–74° for 5 minutes afforded 0.28 g. (59%) of product, m.p. 151–152° dec. The infrared spectra (1% in KBr) of this material and the substance obtained from the 0° cleavage were identical in every respect in the region 2–16 μ.

Analogous treatment of 0.45 g. (0.99 mmole) of the α-isomer of VI in 3.0 ml. of purified nitromethane at 73–75° gave 0.23 g. (53%) of material, m.p. 149–151° dec. Assignment of the same configuration (β) as in the material obtained from the VIγ was made on the basis that the same β-lactam was formed from each.

Methyl 6-Benzylsulfonamidopenicillanate (VIII). A. α-Isomer.—A suspension of 1.67 g. (0.0038 mole) of VIIα in a mixture of 25 ml. of purified thionyl chloride[11] and 75

(10) J. T. Hays, G. F. Hager, H. M. Engelmann and H. M. Spurlin, THIS JOURNAL, **78**, 5372 (1951).

(11) E. L. Martin and L. F. Fieser, "Organic Syntheses," Coll. Vol. II, John Wiley and Sons, Inc., New York, N. Y., 1943, p. 569.

ml. of methylene chloride was heated under reflux for 105 minutes. The resultant yellow solution was concentrated at 40° under reduced pressure; 50 ml. of benzene was added to flush out the thionyl chloride. The residue was taken up in methylene chloride and extracted with a 5% sodium bicarbonate solution. After concentration, the tan resinous product was dissolved in 10 ml. of benzene; after 1 hr. at room temperature, 0.335 g. (23%) of a crystalline substance, m.p. 212–214° dec., was obtained. An analytical sample, obtained by recrystallization from ethanol, had m.p. 214–215° dec. Assignment of structure X (2,2-dimethyl-3-carbomethoxy-5-keto-6-benzylsulfonamido-2,3,4,5-tetrahydro-1,4-thiazepine) was made from its ultraviolet (Fig. 2) and infrared (curve E, Fig. 1) absorption spectra.

Anal. Calcd. for $C_{16}H_{20}N_2O_5S_2$: C, 49.98; H, 5.24; N, 7.29. Found: C, 49.98; H, 5.06; N, 7.61.

The material in the benzene mother liquors from X was passed through a column containing 6.0 g. of Brockman Grade III ethyl acetate neutralized alumina with benzene as eluent. Crystallization of the material in the first 25 ml. of eluate from benzene–ether–petroleum ether afforded 0.625 g. (43%) of crude VIIIα, m.p. 128–131°. Recrystallization from acetone–petroleum ether gave an analytical sample of this β-lactam,[12] m.p. 130–131.5°.

Anal. Calcd. for $C_{16}H_{20}N_2O_5S_2$: C, 49.98; H, 5.24; N, 7.29. Found: C, 50.10; H, 5.30; N, 7.33.

(12) ADDED IN PROOF.—A preliminary *in vivo* assay of VIIIα in male albino mice against *D. pneumoniae* type II using potassium penicillin G as a standard indicated a potency of approximately 7 units/mg. This assay was carried out under the supervision of Dr. J. Lein, Bristol Laboratories, Syracuse, New York.

From a cyclization run involving 0.250 g. (0.57 mmole) of VIIα and 5.0 ml. of thionyl chloride in 15 ml. of methylene chloride, in which the crude reaction products were oxidized with potassium permanganate in 80% acetic acid, there was isolated 0.120 g. (51%) of the sulfone IXα, m.p. 212–213° dec. Recrystallization from acetone–water gave colorless needles, m.p. 214–215° dec.

Anal. Calcd. for $C_{16}H_{20}N_2O_7S_2$: C, 46.14; H, 4.85; N, 6.72. Found: C, 46.18; H, 4.89; N, 6.63.

B. β-Isomer.—A sample of 0.75 g. (0.0017 mole) of VIIβ was treated with 15 ml. of thionyl chloride in 50 ml. of methylene chloride, as described above for the α-isomer. After the preliminary purification of the crude reaction products by extraction with 5% sodium bicarbonate solution, 0.30 g. (46%) of VIIIβ, m.p. 128–130°, was obtained directly by crystallization from benzene. An analytical sample was obtained by recrystallization from acetone–ether–petroleum ether, m.p. 128.5–130°. The mixed m.p. with VIIIα (m.p. 130–131.5°) was depressed (114–124°).

Anal. Calcd. for $C_{16}H_{20}N_2O_5S_2$: C, 49.98; H, 5.24; N, 7.29. Found: C, 50.12; H, 5.22; N, 7.41.

None of the isomeric substance X appeared to have been formed in this reaction.

The sulfone IXβ was prepared by oxidation of the crude reaction products obtained from 0.16 g. (0.36 mmole) of VIIβ and 5 ml. of thionyl chloride in 15 ml. of methylene chloride. The yield of crude sulfone was 86 mg. (59%), m.p. 158–159°. An analytical sample, prepared by recrystallization from acetone–water, had m.p. 159.5–160°.

Anal. Calcd. for $C_{16}H_{20}N_2O_7S_2$. C, 46.14; H, 4.85; N, 6.72. Found: C, 46.11; H, 4.91; N, 6.59.

CAMBRIDGE 39, MASSACHUSETTS

[CONTRIBUTION FROM THE ENZYME CHEMISTRY BRANCH, CHEMICAL WARFARE LABORATORIES]

Chemical Reactions of Nerve Gases in Neutral Solution. I. Reactions with Hydroxylamine[1]

BY BERNARD J. JANDORF

RECEIVED FEBRUARY 14, 1956

Hydroxylamine and certain of its N-substituted derivatives react with organophosphorus anticholinesterases (DFP, GB) stoichiometrically at room temperature and *p*H 7.5. The over-all reaction is $(R)(R'O)P(:O)X + 3NH_2OH \rightarrow (R)(R'O)P(:O)OH + HX + N_2 + NH_3 + 2H_2O$, and is accompanied by loss of anticholinesterase activity. Evidence for several steps in this over-all reaction is presented. Hydroxylamine in 2000-fold excess does not prevent inhibition of cholinesterase by GB and does not reactivate GB-inhibited cholinesterase. An adaptation of the hydroxamic acid reaction to the colorimetric determination of micromole amounts of NH_2OH is described.

Organophosphorus anticholinesterases ("nerve gases") react with susceptible enzymes in a stoichiometric, irreversible fashion. The reaction consists of several steps, the last of which represents an alkylphosphorylation of a serine moiety.[2]

A search has been in progress in this laboratory for compounds which can react rapidly with nerve gases under physiological conditions of *p*H and temperature with the aim of finding substances which may successfully compete in the animal body with cholinesterase (ChE) for the inhibitor. In the first attempt to find such a competitor, naturally occurring amino acids were screened for reactivity with DFP (diisopropyl phosphorofluoridate) and GB (Sarin, isopropylmethyl phosphonofluoridate) under physiological conditions. These experiments were completely unsuccessful, and similar negative results have been reported by others.[3-5] The search was therefore widened to include other representative chemical structures, not necessarily known to exist in protein molecules but potentially able to react with nerve gases.

There exists in many respects a similarity between the reactions of susceptible esterases with their substrates and with nerve gases (leading to acylation and phosphorylation of the enzymes, respectively). With this in mind, one of the first substances to be tested was hydroxylamine which was known to react rapidly with acyl anhydrides and esters,[6] including acetylcholine,[7] with formation of the corresponding acylhydroxamic acid.

As a working hypothesis it was assumed that an

(1) This paper describes experiments which have been carried out during 1950–1951 and formed the basis of a classified report issued at that time.

(2) B. J. Jandorf, H. O. Michel, N. K. Schaffer, R. Egan and W. H. Summerson, *Faraday Soc. Disc.*, in press (1956).

(3) E. F. Jansen, M.-D. F. Nutting, R. Jang and A. K. Balls, *J. Biol. Chem.*, **185**, 209 (1950).

(4) T. Wagner-Jauregg, J. J. O'Neill and W. H. Summerson, THIS JOURNAL, **73**, 5201 (1951).

(5) W. K. Berry, K. P. Fellowes, P. J. Fraser, J. P. Rutland and A. Todrick, *Biochem. J.*, **59**, 1 (1955).

(6) F. Lipmann and L. C. Tuttle, *J. Biol. Chem.*, **159**, 21 (1945).

(7) S. Hestrin, *ibid.*, **180**, 249 (1949).