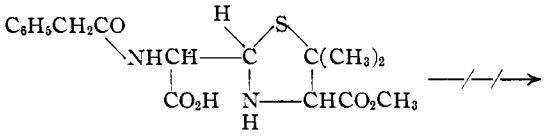
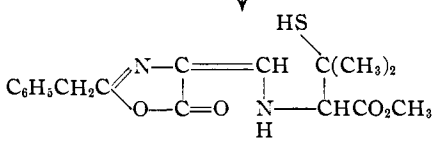
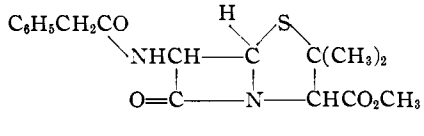


β-Methyl benzylpenicilloate (I)

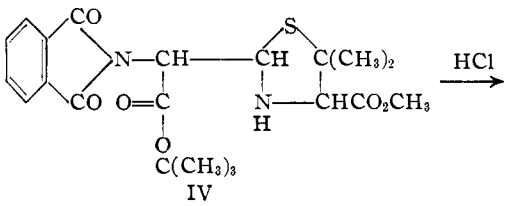


Methyl benzylpenicillenate (III)

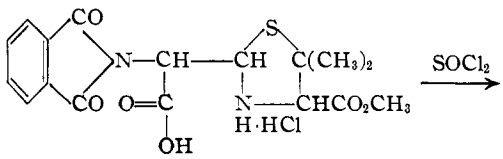


Methyl benzylpenicillinate (II)

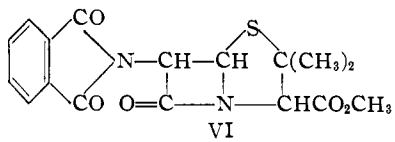
imidopenicillanate.[2] The key intermediate is V, a penicilloic acid derivative structurally incapable of azlactonization.



IV



V



VI

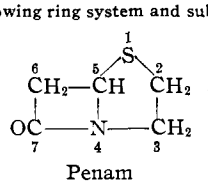
Condensation of *t*-butyl phthalimidoacetate with *t*-butyl formate in the presence of sodium hydride led to 31% of *t*-butyl α-phthalimidomalonaldehydrate, m.p. 155–156° (dec.).[3] *Anal.* Calcd. for $C_{15}H_{15}NO_5$: C, 62.27; H, 5.23; N, 4.84. Found: C, 62.48; H, 5.29; N, 4.70. A crystalline, stereoisomeric mixture of *t*-butyl 4-carboxy-5,5-dimethyl-α-phthalimido-2-thiazolidineacetates was obtained in 84% yield by condensation of this aldehyde-ester with DL-penicillamine. Several recrystallizations from acetone-water afforded a homogeneous sample, m.p. 179.5–180.5° (dec.). *Anal.* Calcd. for $C_{20}H_{24}N_2O_6S$: C, 57.13; H, 5.75; N, 6.66. Found: C, 57.20; H, 5.79; N, 6.35. Treatment with diazomethane generated the corresponding methyl ester IV (90% yield), m.p. 121–122°. *Anal.* Calcd. for $C_{21}H_{26}N_2O_6S$: C, 58.05; H, 6.03; N, 6.45. Found: C, 58.02; H, 6.09; N, 6.52.

By cleavage of the *t*-butyl ester with dry hydrogen chloride, an 85% yield of 4-carbomethoxy-5,5-dimethyl-α-phthalimido-2-thiozalidineacetic acid hydrochloride (V) was formed, m.p. 160–161° (dec.). *Anal.* Calcd. for $C_{17}H_{19}N_2O_6SCl$: C, 49.21; H, 4.62; N, 6.75. Found: C, 48.99; H, 4.86; N, 7.07. Treatment of V with thionyl chloride, followed by oxidation with potassium permanganate in acetic acid solution gave the sulfone of VI in 13% yield; m.p. 200–201° (dec.). *Anal.* Calcd. for $C_{17}H_{16}N_2O_7S$: C, 52.03; H, 4.11; N, 7.14. Found: C, 52.18; H, 4.05; N, 7.27. From a similar reaction mixture before oxidation there was isolated by chromatography over alumina the pure β-lactam-thiazolidine VI,[4] m.p. 171–172° (dec.). *Anal.* Calcd. for $C_{17}H_{16}O_5N_2S$: C, 56.67; H, 4.47; N, 7.78. Found: C, 56.64; H, 4.56; N, 8.04.

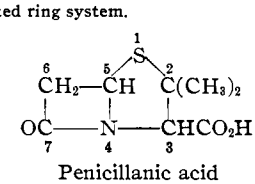
The infrared spectrum of methyl phthalimidopencillanate (VI) has the intense band at 5.62 μ which is associated with the β-lactam carbonyl in natural pencillins[5] and in synthetic β-lactam-thiazolidines. Conversion to the sulfone causes the expected shift[6] of this band to about 5.57 μ; in the spectrum of both VI and VI sulfone the characteristic phthalimido bands at 5.65 μ and 5.82 μ are observed.

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

(2) As a convenience in naming VI and similar analogs of the penicillins we suggest the terms "penam" and "penicillanic acid" for the following ring system and substituted ring system.



Penam



Penicillanic acid

As in the case of the penicilloic acids, these terms carry no stereochemical implications. Thus methyl benzylpenicillinate (penicillin G methyl ester) is one of the stereoisomers of methyl phenylacetamidopenicillanate. The numbering is that generally accepted for the penicillins, and the point of attachment of the side chain is understood to be 6 unless otherwise stated.

(3) All melting points are corrected.

(4) This lactam is inactive when tested by routine penicillin assay procedures (Bristol Laboratories, Syracuse, N. Y.).

(5) Ref. 1, p. 404.

(6) J. C. Sheehan, H. W. Hill, Jr., and E. L. Buhle, THIS JOURNAL, **73**, 4374 (1951); Ref. 1, p. 411.

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FORMATION OF THE ISONICOTINIC ACID HYDRAZIDE ANALOG OF DPN[1]

Sirs:

In a previous paper from this laboratory[2], diphosphopyridine nucleotidase (DPNase) of beef spleen was shown to catalyse the exchange of added C^{14}-labeled nicotinamide with the nicotinamide moiety of DPN resulting in the isolation of C^{14}-labeled DPN. The speculation that structural analogs of nicotinamide might take part in a similar

(1) Contribution No. 53 of the McCollum-Pratt Institute. Aided by grants from the American Tuberculosis Association, the American Cancer Society as recommended by the Committee on Growth of the National Research Council, the Williams-Waterman Fund and the Rockefeller Foundation.

(2) L. J. Zatman, N. O. Kaplan and S. P. Colowick, *J. Biol. Chem.*, **200**, 197 (1953).

exchange reaction to yield a DPN analog,[2,3] has now been tested experimentally with the recently described antituberculous drug isonicotinic acid hydrazide (INH). The over-all reaction can be summarized as $ARPPRN^{+} + INH \rightarrow ARPPR(INH)^{+} + N$, where the symbol $ARPPRN^{+}$ represents the DPN structure adenine-ribose-phosphate-phosphate-ribose-nicotinamide, and the symbol $ARPPR(INH)^{+}$ similarly represents the DPN analog in which the nicotinamide moiety (N) has been replaced by isonicotinic acid hydrazide (INH).

The early studies with INH indicated a surprising species specificity as regards its inhibitory action on the tissue DPNases[3] and recent experiments have shown that the enzyme of human spleen and prostate is remarkably insensitive; thus even at 4×10^{-2} *M* INH the rate of DPN cleavage is unaffected. Upon incubation of DPN with INH in the presence of these "INH-insensitive" DPNases, the development of a yellow color was observed on making the incubation mixture alkaline (*p*H 9.5) prior to addition of the alcohol dehydrogenase system for assay of the residual DPN. Production of the yellow color in alkali after the incubation was subsequently found to depend on (1) the active DPNase, (2) added DPN, (3) INH and (4) the disappearance of DPN during the incubation. For example, the presence of nicotinamide during the incubation prevented DPN breakdown, and, to the same extent, prevented appearance of the yellow compound. Figure 1 illustrates the direct relationship which appears to exist between the disappearance of DPN and the appearance of the yellow compound during an incubation with human prostate homogenate. The yellow compound has an absorption maximum in alkali at 385 mμ, the height of this peak being *p*H dependent; thus while it is maximal in 0.1 *N* NaOH it disappears (reversibly) in acid. That the yellow color is attributable to an N-substituted INH moiety in the DPN analog is strongly suggested by the fact that N'-methyl INH shows practically identical changes in absorption spectrum on treatment with alkali and acid.

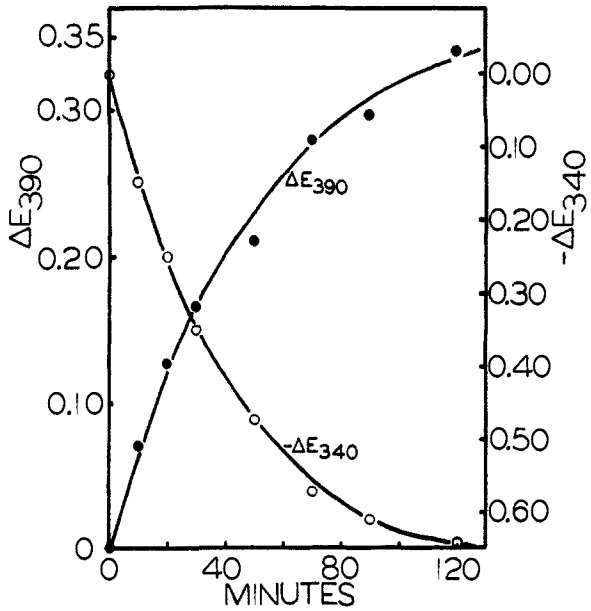

Fig. 1.—Time relationship between disappearance of DPN and formation of analog. Concentrations in 3.6 ml. reaction mixture: 0.02 *M* phosphate buffer *p*H 7.2; 6×10^{-4} *M* DPN; 2×10^{-2} *M* INH; 1.5 ml. 10% human prostate homogenate; temperature, 38°. Aliquots analyzed for (a) DPN, by addition of crystalline yeast alcohol dehydrogenase and ethanol to the aliquot in glycine/NaOH buffer *p*H 9.5 and determining the increase in optical density at 340 mμ, expressed as E_{340}, and (b) the analog, by determining the optical density at 390 mμ in the glycine/NaOH buffer *p*H 9.5, expressed as E_{390}. Changes in these values during the incubation are expressed as $-\Delta E_{340}$ and ΔE_{390}, respectively.

It has been found that the yellow compound is formed by all "INH-insensitive" DPNases tested with the exception of the *Neurospora* enzyme (which is unaffected by 1.3×10^{-1} *M* INH). This latter observation is of particular significance because the *Neurospora* enzyme does not promote the exchange reaction between free nicotinamide and the bound nicotinamide of DPN.[2]

A more detailed study of the reaction involving the production of the yellow compound has been carried out using pig brain as the enzyme source. These experiments have resulted in the isolation, from a reaction mixture originally containing 1000 mg. of DPN (Sigma "90"), of 774 mg. of a yellow product which appears to be the proposed analog of DPN, *i.e.*, the compound in which the nicotinamide moiety of DPN has been replaced by INH. An analysis of the compound has yielded the data shown in Table I—data which conform to the expected values which would obtain for an INH analog of DPN. Paper chromatographic techniques have shown that while the analog spot (R_f = 0.40 in EtOH: 0.1 *N* acetic acid, 1:1) gives a positive picryl chloride test for the hydrazide grouping,[4] prior hydrolysis in acid or alkali abolishes the analog spot and simultaneously releases free INH (R_f = 0.74).

TABLE I

	Moles per mole of analog					
	Ribose	P	INH	5-adenylic acid	Nicotinamide	DPN
Calcd. for $ARPPR(INH)^{+}$	2.0	2.0	1.0	1.0	0	0
Observed	2.2	2.1	0.88	0.84	0.11	0.14

Preliminary investigations of the properties of the analog in enzyme systems have shown it to be a potent inhibitor of the INH-sensitive beef spleen DPNase—at least twice as potent as free INH. Further studies are now being carried out dealing in particular with the possible role of the analog in the antituberculous action of INH and more generally with the significance of such analogs of essential coenzymes in the mechanism of drug action.

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(3) L. J. Zatman, S. P. Colowick, N. O. Kaplan and M. M. Ciotti, *Bull. Johns Hopkins Hosp.*, **91**, 211 (1952).

(4) W. F. J. Cuthbertson and D. M. Ireland, *Biochem. J.*, **52**, xxxiv (1952).