lated guinea pig ileum. The ileum was suspended in Krebs–Henseleit solution, aerated with 95% O_2 and 5% CO_2, and maintained at 37°. AcCh and unknown drug were tested at minimal, medium, and submaximal concentrations as determined prior to the actual assay. The compounds were run on separate days using different guinea pigs for each experiment. The data were subjected to variance analysis which showed that none of the compounds altered the sensitivity of the tissue to AcCh.

Enzymology.—Enzyme-catalyzed hydrolysis of the compounds and inhibition of ACh hydrolysis were determined at pH 7.5 by titration of the liberated AcOH with NaOH solution (0.0065 N) using a Sargent pH-Stat. Concentrations of substrate varying from 1.0 to 5.0 $\times 10^{-4}$ M were used in a medium consisting of 0.01 M $MgCl_2$, 0.1 M Na Cl, and 0.01 mg/ml of AChE for the K_m determinations. Inhibitor concentrations were 3.0 $\times 10^{-4}$ M. The reaction rates were measured at 25° and were linear. A graphic plot of S/V *vs.* S provided K_m and K_i values. Comparisons of hydrolysis rates were carried out at 5.0 $\times 10^{-4}$ M substrate concentration.

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Phosphorus–Nitrogen Compounds. XI. Phosphamidase Studies. I. Unsubstituted Amides[1,2]

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That enzymes capable of catalyzing reactions involving P–N bonds occur in higher concentration in cancer than in normal cells has been well established.[3] These may be the same as, or closely related to, phosphamidase which has been isolated from *Escherichia coli*,[4] hog eye,[5] and beef spleen.[6] Neither a natural substrate nor a substrate-like inhibitor has been determined for this enzyme. In the design of the latter a common preliminary step is the selection of compounds with high affinity for the enzyme receptor site for alteration, if necessary, to produce increased binding capacity.

There have been only 12 different P–N compounds, excluding a few qualitative histochemicals,[3] previously used to study phosphamidase preparations, including 2 phosphorodiamides and 5 substrates with unsubstituted amido groups. This initial paper reports the examination of 25 P–N substrates of type $=P(O)NH_2$, $P(O)(OH$ or $OEt)NH_2$, and $P(O)(NH_2)_2$ with 60 to 90 SAS beef spleen fraction. Twenty-one of these have not been investigated previously with phosphamidase and 7 are new chemical entities.

The only substrates subject to P–NH_2 hydrolysis by the beef spleen preparation are certain triamides having two unsubstituted amido groups and two phosphonic diamides (XIII, XIV). As shown in Table I, the order of degree of hydrolysis is XVI > XIII > XVIII > XIX > XIV, XX > XXIII, XXV. With the triamides XVI–XXIII, where the alkyl substituents were N,N-Me_2, N,N-Et_2, N-Bu, N-hex, and N-cyclohexyl, hydrolytic activity decreased with increasing number of carbons. Loss of activity resulted when the alkyl chain was lengthened to eight carbons (XXI), when a double bond was introduced (XVII), and when the β carbon atoms of XVIII were linked to give a pyrrolidine derivative (XXIV). Hydrolysis of the P–NH_2 bond is, however, restored in the piperidyl homolog, XXV. The high activity of XIII is especially interesting since it contains a $ClCH_2P(O)$ moiety, an analog of the halomethylcarbonyl group which has been used as a covalent bonder in the design of inhibitors.[7]

Since the ultimate goal of this continuing study is the design of phosphamidase inhibitors, several substrates were screened for this property. Representative inactive compounds IV, V, X, XII, and XXIV did not affect phosphoramidate cleavage at 12 mM whereas XI and XV gave evidence of inhibition at this concentration, reducing NH_3 liberation by 50%.

Although the P–NH_2 bond in XI was shown not to be cleaved enzymatically in this study it is used as a histochemical substrate for the detection of phosphamidase.[8] This compound is probably cleaved preferentially at the aryl N–P bond to phosphoramidic acid which, in turn, is converted into H_3PO_4 and NH_3. Under conditions herein reported this acid, being formed in low concentration, is not appreciably acted upon. Each of the triamides, as well as IX and XII, also contains substituted amido groups subject to phosphamidase activity, and the short series of five N-substituted phosphoramidic acids previously studied with beef spleen preparation[6] does not permit the relating of the effect of this substituent on enzymatic activity. A future study in this series is expected to yield more definite information concerning the effect of N-substitution of phosphoramidic acid on enzyme interaction. It may then be possible to devise a substrate, *i.e.*, R(R or H)P(O)$N(CH_3)_2$ where R is an optimal substituent(s), possessing high enzyme affinity.

Experimental Section

Chemistry.—Previously unreported XIII, XVII, and XX–XXIV were prepared from the appropriate phosphorodichloridates according to the procedure of Goehring and Niedenzu.[9] Their elemental analyses (C, H, N) were obtained with Coleman analyzers with the results being within ±0.4% of the theoretical values and spectra using a Beckman IR-8 were as expected. Melting points were determined on a Fisher–Johns apparatus and are uncorrected. NH_3 (2.0-ml sample) was determined using Conway microdiffusion dishes[10] with Obrink modification.

Enzyme Studies.—Substrates I–XXV (0.012 M), 0.1 M acetate buffer (pH 6.0), enzyme preparation 60 to 90 SAS 1.0 $(EU)^{PA}$/ml, and 0.02 M β-mercaptoethanol were incubated 10 min at 37° according to a previously described procedure.[6] Although this

(1) This investigation was supported by Grant E-297 from the Robert A. Welch Foundation, Houston, Texas, and Grant CA-08711 from the National Cancer Institute, U. S. Public Health Service, Bethesda, Md.

(2) For the previous paper in this series see L. A. Cates, *J. Med. Chem.*, **11**, 1075 (1968).

(3) M. S. Burstone, "Enzyme Histochemistry," Academic Press, New York, N. Y., 1962, p 231.

(4) R. A. Smith and D. J. Burrow, *Biochim. Biophys. Acta*, **34**, 274 (1959).

(5) T. Wong, *Comp. Biochem. Physiol.*, **17**, 139 (1966).

(6) M. F. Singer and J. S. Fruton, *J. Biol. Chem.*, **229**, 111 (1957).

(7) B. R. Baker, *J. Pharm. Sci.*, **53**, 347 (1964).

(8) G. Gomori *Methods Enzymol.*, **4**, 387 (1957).

(9) See footnote *o*, Table I.

(10) R. B. Johnston, M. J. Mycek, and J. S. Fruton, *J. Biol. Chem.*, **185**, 629 (1950).

TABLE I
ENZYMATIC HYDROLYSIS OF UNSUBSTITUTED P–N AMIDES

Compd	Formula	Reference, °C	μmol of NH_3/(ml) per 10 min
I[a]	$(HO)_2P(O)NH_2$	*b*	1.0
II	$(HO)P(O)(NH_2)_2$	*c*	0.8
III	$(EtO)_2P(O)NH_2$	*d*	0.0
IV	$[i\text{-PrO}]P(O)NH_2$	*d*	0.0
V	$(C_6H_5O)_2P(O)NH_2$	*e*	0.0
VI[f]	$(C_6H_5CH_2O)_2P(O)NH_2$	*d*	
VII[f]	$(C_6H_5NH)_2P(O)NH_2$	*g*	
VIII[h]	$C_6H_5OP(OH)NH_2$	*i*	0.0
IX	$C_6H_5NHP(O)(OH)NH_2$	*j*	0.0
X[i]	$C_6H_5CH_2OP(O)(OH)NH_2$	*k*	0.0
XI	$4\text{-}ClC_6H_4NHP(O)(OH)NH_2$	*l*	0.0
XII	$C_6H_5NHP(O)(OEt)NH_2$	*j*	0.0
XIII	$ClCH_2P(O)(NH_2)_2$	120–122[m]	3.1
XIV	$C_6H_5P(O)(NH_2)_2$	*n*	0.8
XV	$EtOP(NH_2)_2$	*o*	0.0
XVI	$(CH_3)_2NP(O)(NH_2)_2$	*o*	5.5
XVII	$CH_2{=}CHCH_2NHP(O)(NH_2)_2$	59–62 (85%)[m]	0.0
XVIII	$Et_2NP(O)(NH_2)_2$	*o*	1.9
XIX	$n\text{-}BuNHP(O)(NH_2)_2$	*o*	1.1
XX	$CH_3(CH_2)_5NHP(O)(NH_2)_2$	104–108 (95%)[m]	0.8
XXI	$CH_3(CH_2)_7NHP(O)(NH_2)_2$	106–110 (87%)[m]	0.0
XXII[f]	$CH_3(CH_2)_9NHP(O)(NH_2)_2$	110–113 (87%)[m]	
XXIII	$C_6H_{11}NHP(O)(NH_2)_2$	113–117 (96%)[m]	0.5
XXIV	$(CH_2)_4NP(O)(NH_2)_2$	150–152 (86%)[m]	0.0
XXV	$(CH_2)_5NP(O)(NH_2)_2$	*p*	0.5

[a] Na salt. [b] H. N. Stokes, *Amer. Chem. J.*, **15**, 198 (1893). [c] H. N. Stokes, *ibid.*, **16**, 124 (1894). [d] F. R. Atherton, H. T. Openshaw, and A. R. Todd, *J. Chem. Soc.*, 660 (1945). [e] F. Ephraim, *Chem. Ber.*, **44**, 631 (1911). [f] Precipitates upon addition to incubation mixture. [g] A. V. Kirsanov and L. P. Zhuravlova, *Zh. Obshch. Khim.*, **31**, 598 (1961); *Chem. Abstr.*, **55**, 25751 (1961). E. S. Levchenko and I. E. Sheinkman, *ibid.*, **34**, 1145 (1964); *Chem. Abstr.*, **61**, 1787 (1965). [h] Ba salt. [i] R. M. Caven, *J. Chem. Soc.*, **81**, 1362 (1902). [j] Li salt. [k] V. M. Clark and A. R. Todd, *ibid.*, 2030 (1950). [l] K. Rorig, *J. Amer. Chem. Soc.*, **71**, 3561 (1949). [m] See Experimental Section. [n] A. Michaelis, *Ann. Chem.*, **293**, 193 (1896). W. C. Smith and L. F. Audrieth, *J. Org. Chem.*, **22**, 265 (1957). [o] M. Goehring and K. Niedenzu, *Chem. Ber.*, **89**, 1768 (1956). [p] Farbenfabriken Bayer-A.-G., British Patent 830,800 (1961); *Chem. Abstr.*, **56**, 3329 (1962).

concentration was shown not to inhibit the enzyme, each incubation vessel also contained 20% propylene glycol as a solvent for all substrates except I, II, VIII, and X (H_2O). All determinations were made in duplicate. Only two substrates were investigated at one time with standardization against I each determination. Controls and samples were treated identically except that the enzyme preparation was added to the former after incubation and cooling. Enzyme activity was stopped by addition of an equal volume of cold 10% Cl_3CCO_2H. Vessels were maintained in an ice bath prior to, and after, incubation. VIII was treated with an equimolar amount of Na_2SO_4 prior to incubation. As a true control I was treated with sufficient $BaCl_2$ and Na_2SO_4 to yield a corresponding 0.012 *M* concentration of $BaSO_4$. Liberated NH_3 attributable to both chemical and enzymatic hydrolysis did not exceed 5% of theoretical with any substrate.

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The Synthesis of Pentazocine

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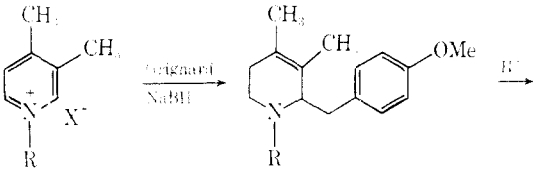
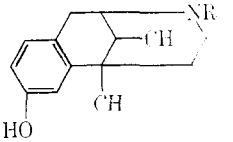
Although pentazocine (III, R = $CH_2CH{=}CMe_2$) is now commerically available, the route for its synthesis has not been described in the chemical literature[1] except for the brief statement that "our synthetic procedure does not give the *N*-methylbenzomorphan."[2]

(1) Belgium Patent 719,408 covering the synthesis has been issued Feb 13, 1969.

(2) B. F. Tullar, *et al.*, *J. Med. Chem.*, **10**, 383 (1967).

The accompanying formulas where RX is MeI show May's original synthesis of 6,7-benzomorphans.[3] Demethylation of III, R = CH_3, to produce III, R = H, requires three steps. Our modification utilizes benzyl chloride for the quaternization of 3,4-lutidine. This affords a more favorable *cis:trans* isomer ratio in III,





IIIa, R = CH_3
b, R = $C_6H_5CH_2$

and the *N*-benzyl group is removed in one operation. Alkylation of the resulting base completes the synthesis of pentazocine.

Kametani and coworkers recently described[4] essentially the same synthesis *via* the benzyl route, but

(3) E. L. May and E. M. Fry, *J. Org. Chem.*, **22**, 1366 (1957); N. B. Eddy, J. G. Murphy, and E. L. May, *ibid.*, **22**, 1370 (1957).

(4) T. Kametani, K. Kigasawa, M. Hiiragi, T. Hayasaka, N. Wagatsuma, and K. Wakisaka, *J. Heterocycl. Chem.*, **6**, 43 (1969).