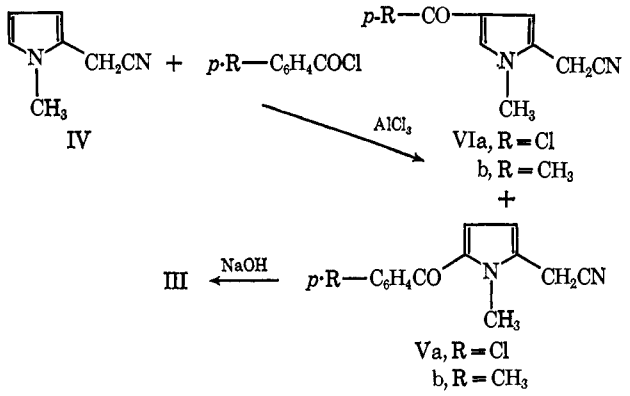
induced arthritis assay. Compound IIIb was estimated to be 5.79 (2.43–10.08) times as potent as phenylbutazone and 0.14 (0.08–0.25) times as potent as indomethacin in the cotton pellet granuloma test.

In addition, significant analgetic activity, as measured by the acetylcholine bromide induced writhing test,[3] and antipyretic activity[4] were observed.

Chemistry.—Compounds of type III can be prepared *via* Friedel–Crafts aroylation of 1-methylpyrrole-2-acetonitrile (IV)[5] with the appropriate aroyl halide in the presence of $AlCl_3$. Both the 5-aroyl (V) and the 4-aroyl (VI) derivatives are formed in this reaction. The desired 5-aroyl derivatives (V) can be isolated by fractional crystn and/or adsorption chromatography on Al_2O_3. The structures of V and VI can be demonstrated from their nmr spectra. The spectra of the 1,2,5-substituted compounds V show a 3.8–4.0 Hz coupling of the pyrrole protons indicative of coupling across the 3 and 4 positions. The 1,2,4-substituted compounds show a 1.5–1.8 Hz 3,5 coupling.

Saponification of the nitriles V affords the corresponding acids III.



Experimental Section

All melting points were detd using a Thomas-Hoover capillary mp apparatus and are uncorrected. The nmr spectra were obtained using a Varian A60 instrument (Me_4Si). Elemental analyses were performed by the Scandinavian Microanalytical Laboratories, Herlev, Denmark. Where analyses are indicated by the symbols of the elements, the anal. results obtained for those elements are within ±0.4% of the theoretical values.

Chemistry. Friedel–Crafts Reactions.—A soln of 13.3 g (0.1 mole) of anhyd $AlCl_3$ and 0.1 mole of the appropriate acid chloride (*p*-ClBzCl or *p*-CH_3BzCl) in 50 ml of $ClCH_2CH_2Cl$ was added over 30 min to a soln of 1-methylpyrrole-2-acetonitrile in 50 ml of $ClCH_2CH_2Cl$. The mixt was stirred for 15 min and heated under reflux for 5 min. The soln was poured into ice-HCl. The residue remaining in the reaction flask was triturated with $CHCl_3$–HCl. The combined org soln was washed with H_2O, *N*,*N*-dimethyl-1,3-propanediamine soln,[6] HCl, and brine. It was dried ($MgSO_4$) and the solvent was evapd *in vacuo*. The residue was chromatographed on acid-washed Al_2O_3. Elution with C_6H_6, evapn of the eluate, and recrystn of the residue from MeOH afforded the 5-aroyl-1-methylpyrrole-2-acetonitrile: Va, 5.4 g (21%), mp 128–130°, *anal.* ($C_{14}H_{11}ClN_2O$) C, H, N; Vb, 6.0 g (25%), mp 103–105°, *anal.* ($C_{15}H_{14}N_2O$) C, H, N.

Further elution of the above chromatographic column with 10% $CHCl_3$ in C_6H_6, evapn of the eluate, and recrystn of the residue from MeOH gave the 4-aroyl-1-methylpyrrole-2-acetonitrile: VIa, 1.9 g (7.4%), mp 93–95°, *anal.* ($C_{14}H_{11}ClN_2O$) C, H, N; VIb, 1.59 g (6.7%), mp 117–119°, *anal.* ($C_{15}H_{14}N_2O$) C, H, N, O.

Saponifications.—A soln of 1 equiv of the appropriate 5-aroyl-1-methylpyrrole-2-acetonitrile (Va or Vb) in 2 equiv of 1 *N* NaOH and twice that vol of EtOH was heated under reflux overnight. The EtOH was evapd *in vacuo* and the residue was acidified with HCl. The pptd solid was extd into Et_2O and the Et_2O was evapd to give the 5-aroyl-1-methylpyrrole-2-acetic acid: IIIa, 81% yield (from Et_2O–hexane), mp 188–191° dec, *anal.* ($C_{14}H_{12}ClNO_3$) C, H, N; IIIb, 69% yield, (from CH_3CN), mp 155–157° dec, *anal.* ($C_{15}H_{15}NO_3$) C, H, N.

Pharmacology.—Compounds of type III and phenylbutazone were administered as solns of their sodium salts. EtOH was added to facilitate the dissolution of phenylbutazone. Indomethacin was administered as an aq suspension contg 0.1% Tween.

Antiinflammatory activity was assessed by measuring the inhibition of edema induced by 0.1-ml intraplantar injections (1% carrageenin or 10% kaolin) in the hind paw of male Holtzman rats (170–180 body weight). Paw vol were detd by the mercury displacement method of Van Arman as described by Winter, *et al.*,[7] with modifications[8] to provide digital readout on paper tape. All statistical analyses of data were processed by computer.

A min of 3 doses for each test or ref compd was administered orally to groups of 10 rats per dose, 1.0 hr prior to the intraplantar injections. Edema was measured 3 hr later in the carrageenin test and 6 hr later in the kaolin test. Analysis of variance was performed and group means were compared by Dunnett's procedure at the 5% protection level. Any treatment mean significantly less than the control mean was indicative of significant antiinflammatory activity.

Rat paw edema vol of treated animals was compared to that of animals receiving saline, indomethacin, or phenylbutazone by computer estn of relative potency (95% confidence limits) using Finney's parallel line assay.[9] Results are presented in Table I.

Inhibition of cotton pellet granuloma was studied using the procedure of Meier, *et al.*,[10] with minor modifications. Only the granuloma formed in the cotton pellets was retained and dried for analysis of response. The granuloma forming a capsule was not included in the studies.

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(3) H. O. J. Collier, L. C. Deneen, C. A. Johnson, and C. Schneider, *Brit. J. Pharmacol. Chemother.*, **38**, 295 (1968).

(4) U. M. Teotino, L. P. Friz, A. Gandini, and D. Della Bella, *J. Med. Chem.*, **6**, 248 (1963).

(5) W. Herz and J. L. Rogers, *J. Amer. Chem. Soc.*, **73**, 492 (1951).

(6) L. F. Fieser and M. Fieser, "Reagents for Organic Synthesis," Wiley, New York, N. Y., 1967, p 274.

(7) C. A. Winter, E. A. Risley, and G. W. Nuss, *Proc. Soc. Exp. Biol. Med.*, **111**, 544 (1962).

(8) Modifications of the method are to be described in detail elsewhere.

(9) D. J. Finney, "Statistical Method in Biological Assay," Hafner Publishing Co., New York, N. Y., 1952, pp 99–117.

(10) R. Meier, W. Schuler, and P. Desaulles, *Experientia*, **6**, 469 (1950).

Phosphorus–Nitrogen Compounds. 12. Phosphamidase Studies. 2. *N*-Alkylphosphoramidic Acids[1,2]

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Although a few *N*-arylphosphoramidic acids, most notably *N*-(4-chlorophenyl)phosphoramidic acid (CPA), have been studied with phosphamidase prepara-

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(2) For paper 11 see L. A. Cates, *J. Med. Chem.*, **13**, 301 (1970).

tions,[3a-f] there has been no comparable investigation involving *N*-alkyl derivatives. A lack of literature reference to these latter compounds indicates difficulty in their synthesis. Preparative problems leading to controversy over exactness of composition are encountered even with the more stable, less hygroscopic aromatic acids.[4,5]

Chemical hydrolysis studies of both types of phosphoramidic acids have been reported, investigations of *N*-alkyl substituted compounds being confined to the esters[6] and dichlorides.[7] The P–N bond in these latter derivatives is extremely stable to alkali and the possibility of preparing compounds of the nature $RRNPO(OH)_2$ *in situ* is suggested by Lapidot and Halmann[8] who showed that the dichloride of *N*-phosphourethane was rapidly and completely converted to the dibasic acid by hydrolysis with NaOH solution.

The *N*-alkylphosphoramidic acid substrates used in this study were prepared by alkaline hydrolysis of the corresponding dichlorides with conversion to dibasic acids being determined by the quantitative measurement of liberated Cl^- (Table I). The basic solutions

TABLE I
ENZYMATIC HYDROLYSIS OF *N*-ALKYLPHOSPHORAMIDIC ACIDS, $RPO(OH)_2$

Compd	R	Reference	Chloride, %	μmoles of P/ml per 10 min
I	CH_3NH	*a*	99.6	0.00
II	$(CH_3)_2N$	*a*	98.7	0.00
III	C_2H_5NH	*a*	100.3	0.00
IV	$(CH_3)_2CHNH$	*b*	98.5	0.00
V	$(C_2H_5)_2N$	*a*	99.7	0.00
VI	Pyrrolidyl	*c*	99.2	0.00
VII	Piperidyl	*a*	98.3	0.00
VIII	$(C_3H_7)_2N$	*a*	99.2	0.00
IX	$C_6H_{13}NH$	*d*	98.2	0.30
X	*c*-$C_6H_{11}NH$	*e*	94.1	0.29
XI	Morpholinyl	*e*	99.7	0.16
XII	$(C_4H_9)_2N$	*b*		
XIII	NH_2	*f*		1.00[g]

[a] A. Michaelis, *Justus Liebigs Ann. Chem.*, **326**, 129 (1903). [b] See Experimental Section. [c] C. Christol and H. Christol, *J. Chim. Phys.*, **62**, 246 (1965). [d] T. Mizuma, Y. Minaki, and S. Toyoshima, *Yakagaku Zasshi*, **8**, 48 (1961). [e] R. V. Artemkina and V. M. Berezovskii, *Zh. Obshch. Khim.*, **36**, 823 (1966); *Chem. Abstr.*, **65**, 12776*f* (1966). [f] H. N. Stokes, *Amer. Chem. J.*, **15**, 198 (1893). [g] μmoles of NH_3/ml per 10 min.

were adjusted to physiological pH prior to chloridometric analysis and addition to the incubation mixtures. The limitation of this method is shown by the failure of *N*,*N*-dibutylphosphoramidic dichloride, where the alkyl chains total 8 C atoms, to dissolve when subjected to high alkalinity and heat. Although the P–N bond is readily hydrolyzed by acid with the half-life of *N*,*N*-dimethylphosphoramidic acid, prepared *in situ*, being a few min at pH 2.3,[7] the substrates were found to be sufficiently stable for study under the incubation conditions (pH 6.0 and 37° for 10 min) and during the assay procedure (pH 4.0).[9] The total concentration of phosphate resulting from alkaline treatment, enzymatic activity, and the assay procedure varied between 5.7 and 7.4×10^{-4} *M* and, therefore, did not exceed the 10^{-3} *M* concentration which has been reported to inhibit bovine spleen phosphamidase.[3a] Since several of the inactive substrates gave total concentrations in the lower range, lack of hydrolytic activity was not attributable to enzyme inhibition by phosphate. The Lowry–Lopez method,[9] originally devised for the determination of phosphate in the presence of labile phosphate esters, was selected as the assay procedure after attempting other methods[10-12] which require highly acidic conditions.

The effect of bovine phosphamidase preparation on *N*-alkylphosphoramidic acids indicates that these substrates possess a relatively low order of reactivity. Hydrolysis of the P–N bond occurred with the morpholino derivative XI and with the (di)alkyl compounds only when substitution reached C_6 (IX, X). Each substrate herein reported was also investigated in combination with phosphoramidate for the purposes of determining if inhibitory substances other than the phosphoramidic acids were present and of estimating possible affinity without intrinsic activity. If such materials as mono- or dichlorides were present in the incubation mixture as a result of incomplete conversion to the corresponding dibasic acids they had no discernible effect on the enzyme preparation. Only the three hydrolyzable substrates (IX–XI) showed slight inhibitions of 16, 17, and 9%, respectively. Conditions for alkaline hydrolysis of the dichlorides were kept as mild as possible to achieve quantitative, or nearly quantitative, conversions to the acids while restricting P–N cleavage to a minimum.

The most active substrate for a partially purified phosphamidase preparation yet to be reported is *N*,*N*-dimethylphosphoric triamide.[2] Since II is inactive it is likely that the only hydrolysis occurring in the triamide is at the $P-NH_2$ bond(s) and that the effect of the $(CH_3)_2N$ group is limited to increasing enzyme affinity. The structural similarity between the dimethylamino moiety and the aziridinyl group, a common substituent in many oncolytics, suggests that this latter group contributes to enzymatic affinity as well as alkylating ability.

Evidence thus far obtained indicates that the enzyme present in bovine spleen is a phosphodiamidase, as distinguished from the phosphomonoamidase reported to be present in normal and malignant tissue,[13a,b] and is, therefore, at least of the same nature as the enzyme thought to be responsible for activation of latent phosphoramide mustards. The majority of such agents displaying good antineoplastic properties are diam-

(3) (a) M. F. Singer and J. S. Fruton, *J. Biol. Chem.*, **229**, 111 (1957); (b) M. Ichihara, *J. Biochem. (Tokyo)*, **18**, 87 (1933); (c) H. Holter and Si-Oh Li, *C. R. Trav. Lab. Carlsberg, Ser. Chim.*, **27**, 393 (1951); (d) Si-Oh Li, *Acta Chem. Scand.*, **4**, 610 (1950); (e) G. Gomori, *Proc. Soc. Exp. Biol. Med.*, **69**, 407 (1948); (f) Si-Oh Li and Chi-Ping Chang, *Sheng Li Hsueh Pao*, **21**, 142 (1957); *Chem. Abstr.*, **53**, 13220*g* (1959).
(4) K. Rorig, *J. Amer. Chem. Soc.*, **71**, 3561 (1949).
(5) J. D. Chanley and E. Feageson, *ibid.*, **80**, 2686 (1958).
(6) M. Selim and T. N. Thanh, *C. R. Acad. Sci.*, **250**, 2377, 2724 (1960).
(7) D. F. Heath and P. Casapieri, *Trans. Faraday Soc.*, **47**, 1093 (1951).
(8) A. Lapidot and M. Halmann, *J. Chem. Soc.*, 1713 (1958).
(9) O. H. Lowry and J. A. Lopez, *J. Biol. Chem.*, **162**, 421 (1946).
(10) I. Berenblum and E. Chain, *Biochem. J.*, **32**, 295 (1938).
(11) C. H. Fiske and Y. Subbarow, *J. Biol. Chem.*, **66**, 375 (1925).
(12) R. Gibbs, P. M. Roddy, and K. Titus, *ibid.*, **240**, 2181 (1965).
(13) (a) O. M. Friedman and E. Boger, 139th National Meeting of the American Chemical Society, St. Louis, Mo., March 1961, 26-C; (b) O. M. Friedman, S. Schichor, and E. Boger, *Proc. Amer. Soc. Cancer Res.*, **3**, 320 (1954).

ides.[14-17] The information gained during the study of 33 substrates of 4 chemical types with a phosphamidase preparation reveals that the most active substrates are phosphorodi- or triamides and that the central grouping most suitable for modification in the design of substrate-like inhibitors is RR'NPON(N or O) in which R and/or R' are H or small alkyls.

Experimental Section

Chemistry.—Known phosphoramidic dichlorides used for the *in situ* preparation of the corresponding phosphoramidic acids (I–III, V–XI) were synthesized by the procedures indicated in Table I. Ir spectra of all dichlorides, recorded on a Beckman IR-8, were compatible with structures. Elemental analyses, where indicated only by symbols of the elements, are within ±0.4% of the theoretical values.

***N*-Isopropylphosphoramidic Dichloride.**—Reaction of *i*-$PrNH_2$ and $POCl_3$ (2:1) according to the method of Michaelis[18] yielded the white cryst product (Et_2O), mp 51–55° (Fisher–Johns apparatus, uncorr). *Anal.* ($C_3H_8Cl_2NOP$) N (Coleman N analyzer), Cl.[19]

***N,N*-Dibutylphosphoramidic Dichloride.**—Similarly prepd this compd was a colorless liquid, bp 122–124° (1.5 mm). *Anal.* ($C_6H_{18}Cl_2NOP$) C, H, Cl.[20]

Portions of 1 *N* NaOH were added to the phosphoramidic chlorides with magnetic stirring and maintenance of pH at 10–12 (Corning Model 12 pH meter) until solns were obtd. Dil HNO_3 (to pH 7.2–7.4) and then H_2O were added to make 0.03 *M* solns. A soln of the standard reference, phosphormidate, was similarly prepd from the monosodium salt.[21] Triplicate Cl^- analyses[19] were run on samples of these solns and the results are shown in Table I. NH_3 (2.0-ml sample) was determined using Conway microdiffusion dishes[22] with Obrink modification. Inorganic phosphate was detd according to a modified method of Lowry and Lopez[9] with color readings taken after exactly 4 min.[23]

Biology.—Substrates I–XI (0.006 *M*), 0.1 *M* acetate buffer (pH 6.0), and enzyme prepn 60–90 SAS (1.0 EU of phosphoramidate/ml)[3a] were incubated 10 min at 37°. These mixts are the same as those previously described[2] except 2-mercaptoethanol was deleted since it completely interfered with the method used for inorganic phosphorus analysis. Phosphoramidate (0.006 *M*) in lieu of a portion of the buffer soln was included in the mixts for the estimation of inhibitory activity. All detns were made in duplicate with standardization against the reference for each detn. The enzyme prepn was added to controls after incubation and cooling. Enzyme activity was stopped by the addition of equal vols of cold Cl_3CCOOH and, to separate incubation flasks, a NaOH soln of sufficient concn to adjust the pH to 4.0. Duplicate NH_3 detn were run on samples of the former solns and triplicate P analyses were conducted on the alkaline adjusted solns.

(14) H. Arnold, F. Bourseaux, and N. Brock, *Arzneim.-Forsch.*, **11**, 143 (1961).
(15) O. M. Friedman, *Cancer Chemother. Rep.*, **51**, 327 (1967).
(16) Z. F. Chmielewicz, T. J. Bardos, A. Munson, H. B. Babbitt, and J. L. Ambrus, *J. Pharm. Sci.*, **56**, 1179 (1967).
(17) O. M. Friedman, E. Boger, V. Grubliauskas, and H. Sommer, *J. Med. Chem.*, **6**, 50 (1963).
(18) See footnote *a*, Table **1**.
(19) Buchler–Cotlove chloridometer.
(20) Schwarzkopf Microanalytical Laboratory, Woodside, N. Y.
(21) See footnote *f*, Table I.
(22) R. B. Johnston, M. J. Mycek, and J. S. Fruton, *J. Biol. Chem.*, **185**, 629 (1950).
(23) T. Winnick, *Arch. Biochem.*, **12**, 209 (1947).

New Compounds

Potential Antidiabetics. 9. Biological Activity of Some Pyrazoles[1]

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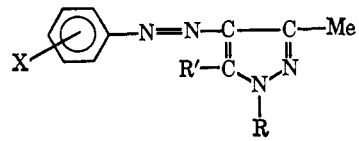
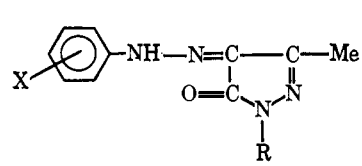
This paper describes the synthesis and evaluation of 4-arylazo-1-(guanyl nitrate)-3-methyl-5-phenylpyrazoles against diabetes mellitus and also includes the evaluation of earlier reported 4-arylazo-1-(2,4-dinitrophenyl)- 3,5- dimethylpyrazoles[2] and 4-arylhydrazono-1-carbamoyl-3-methyl-2-pyrazolin-5-ones[3] against viral infections, as well as 4-arylazo-1-(2,4-dinitrophenyl)-3-methyl-5-phenylpyrazoles[3] and 4-arylhydrazono-1-(2,4-dinitrophenyl)-3-methyl-2-pyrazolin-5-ones[4] against *Trichinella spiralis*.

Pharmacology. Antidiabetic Activity.—Compounds **2, 4, 5, 6, 11, 12, 17,** and **18** have been evaluated for their hypoglycemic activity in CF-1.5 mice (25–30 g) with the aid of a Technician Auto-Analyzer using the modified method of Hoffman.[5] No activity has been shown by these compounds.



Ia, R = —C(=NH)—$NH_2 \cdot HNO_3$
R' = Ph
b, R = DNP
R' = Me or Ph



IIa, R = DNP
b, R = $CONH_2$
X = Substituted phenyl

(1) Part VIII: H. G. Garg and C. Prakash, *J. Pharm. Sci.*, in press.
(2) H. G. Garg and P. P. Singh, *J. Med. Chem.*, **11**, 1103 (1968).
(3) H. G. Garg and P. P. Singh, *J. Chem. Soc. C*, 1141 (1969).
(4) H. G. Garg and P. P. Singh, *J. Med. Chem.*, **11**, 1104 (1968).
(5) W. S. Hoffman, *J. Biol. Chem.*, **120**, 51 (1937).