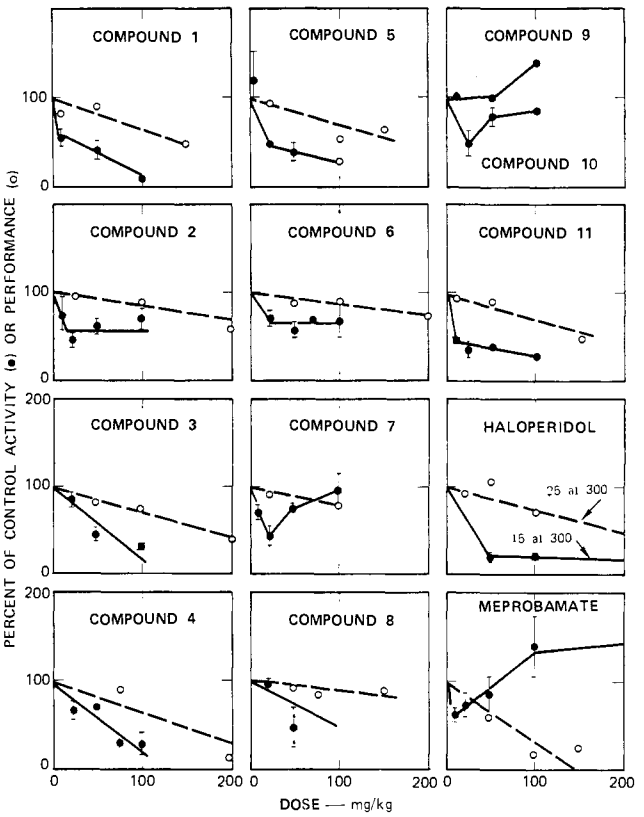

Figure 1.—Effects of trimethoxybenzamides on motor activity of mice as measured by photocell activity cage and rotarod methods. Dose–response data are expressed as per cent of control activity (photocell). Bars represent ± standard errors for means of three to four determinations; all other points are means of two determinations (groups of five mice). Open circles (rotarod) are means of ten animals. Note that doses of haloperidol are scale values $\times 10^{-2}$, *i.e.*, 0.5, 1.0, and 3.0 mg/kg. Rotarod data were not obtained for compounds **9** and **10**.

Experimental Section

Melting points were taken on a Fisher-Johns apparatus and are corrected. Elemental analyses were within ±0.4% of theoretical values.

Pharmacological Methods.—A photocell–activity cage–rotarod screening procedure similar to that described by Kinnard and Carr[7] was used. Male, albino mice (17–20 g) of a Swiss–Webster strain were utilized, and were used only once. All drugs were administered intraperitoneally as suspensions in 10% Tween 80 in 0.9% saline (0.2 ml). Photocell activity was determined as cumulative counts over a 1-hr period beginning 0.5 hr after administration of drug or vehicle to groups of five mice. Activity was determined for one control group with each two treatment groups. Values for the latter were calculated as percentages of the former. Rotarod performance times were determined in groups of five trained mice 0.5 hr after administration of drug or vehicle. Mean performance time for control groups was 113.9 sec. Rod rotation was at the rate of 15 rpm.

Synthesis.—The amines in benzene were refluxed for 2–5 hr with 3,4,5-trimethoxybenzoyl chloride in a 2.2:1 ratio. The precipitated product was either isolated directly or the solution was washed with 3 *N* HCl and a saturated $NaHCO_3$ solution. The C_6H_6 layer was dried ($MgSO_4$) and evaporated *in vacuo*. The products were recrystallized using the solvents listed in Table I.

Acknowledgment.—The authors are grateful to Mrs. A. W. Burfine for technical assistance in the pharmacological evaluations. This work was supported by Stanford Research Institute Research and Development Program.

(7) W. J. Kinnard, Jr., and C. J. Carr, *J. Pharmacol. Exptl. Therap.*, **121**, 354 (1957).

Purine N-Oxides. XXIX. The Synthesis of 6-Hydroxylaminopurine 3-Oxide and Related Derivatives[1]

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6-Hydroxylaminopurine,[2] an analog of adenine and hypoxanthine, was found to possess a growth-inhibitory activity against Sarcoma 180 in tissue culture,[3] and inhibited the growth of Ehrlich ascites carcinoma in mice.[4] It was suggested that 6-hydroxylaminopurine may act as an antagonist of adenine and hypoxanthine metabolism and as an inhibitor of purine nucleotide biosynthesis.[4] Studies of the inhibitory effects of 6-hydroxylaminopurine on Ehrlich ascites cells[5] showed that the drug blocked the conversion of inosinate to adenylate and guanylate, thus leading to a decrease in the biosynthesis of nucleic acids.

6-Hydroxylaminopurine was toxic[2b,c] when administered to animals mainly because it was metabolized to the sparingly soluble 2,8-dihydroxyadenine which deposited in the kidney tubules in crystalline form.[6]

The toxicity of biologically active purines may be decreased by their conversion to an N-oxide.[7] It was therefore desirable to synthesize 6-hydroxylaminopurine N-oxide in order to learn whether the toxicity of 6-hydroxylaminopurine[2b,c] could be decreased with retention of its growth-inhibitory activity.

It will also be of interest to see whether 6-hydroxylaminopurine 3-oxide would exhibit a cancerogenic activity, a property shown by a variety of purine N-oxides (especially the 3-oxides)[8] and by the potent oncogenic structural analog 4-hydroxylaminoquinoline 1-oxide.[9]

The parent compound, 6-hydroxylaminopurine, is not significantly oncogenic when administered for prolonged periods to rats,[10] but it has shown mutagenic

(1) This investigation was supported by funds from the National Cancer Institute (Grant No. CA 08748), The Atomic Energy Commission (Contract No. AT[30-1], 910), and aided by the Grant No. T-128F from the American Cancer Society.

(2) (a) A. Giner-Sorolla and A. Bendich, *J. Am. Chem. Soc.*, **80**, 392 (1958); (b) A. Giner-Sorolla, Ph.D. Thesis, Cornell University; *Dissertation Abstr.*, **20**, 1148 (1959); (c) A. Giner-Sorolla, *Galenica Acta* (Madrid), **19**, 97 (1966); *Chem. Abstr.*, **68**, 11303 (1968).

(3) J. J. Biesele, Proceedings of the 3rd National Cancer Conference, Detroit, Mich., 1956, p 405; *cf.* ref 2a.

(4) (a) A. C. Sartorelli and H. F. Upchurch, *Cancer Res.*, **23**, 1077 (1963); (b) A. C. Sartorelli, A. L. Bieber, P. K. Chang, and G. A. Fischer, *Biochem. Pharmacol.*, **13**, 567 (1964).

(5) P. F. Pecora and M. Earl Balis, *ibid.*, **13**, 1071 (1964).

(6) (a) A. Bendich, G. B. Brown, F. S. Philips, and J. B. Thiersch, *J. Biol. Chem.*, **183**, 267 (1950); (b) F. S. Philips, J. B. Thiersch, and A. Bendich, *J. Pharmacol. Exp. Ther.*, **104**, 20 (1952).

(7) (a) M. A. Stevens, A. Giner-Sorolla, H. W. Smith, and G. B. Brown, *J. Org. Chem.*, **27**, 567 (1962); (b) G. B. Brown, G. Levin, S. Murphy, A. Sele, H. C. Reilly, G. S. Tarnowski, F. A. Schmidt, and C. S. Stock, *J. Med. Chem.*, **8**, 190 (1965).

(8) G. B. Brown, *Progr. Nucleic Acid Res. Mol. Biol.*, **8**, 209 (1968).

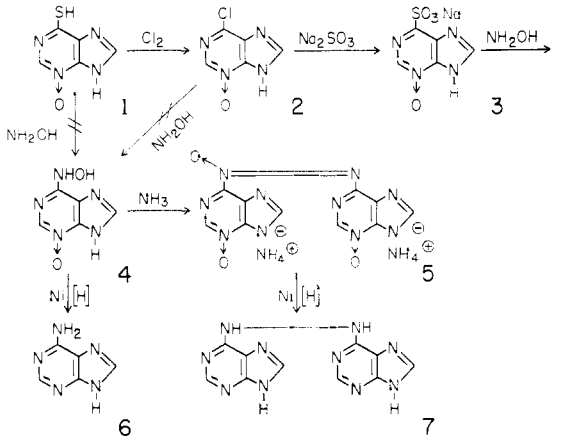
(9) (a) Y. Shiasu and A. Ohta, *Gann*, **54**, 221 (1963); (b) C. E. Searle and D. L. Woodhouse, *Acta Unio Intern. Contra Cancrum*, **19**, 519 (1963); (c) H. Endo and F. Kume, *Gann*, **54**, 443 (1963); (d) Y. Shirasu, *Proc. Soc. Exptl. Biol. Med.*, **118**, 812 (1965); (e) M. Hozumi, S. Inuzuka, and T. Sugimura, *Cancer Res.*, **27**, 1378 (1967).

(10) K. Sugiura and G. B. Brown, in preparation.

action in bacterial viruses[11] and a teratogenic effect in rats.[12]

Synthetic Studies.—The synthesis of 6-hydroxylaminopurine 3-oxide (**4**) was achieved when a suspension of purine-6-sulfonate 3-oxide (**3**)[13] was stirred for 15 days at 25°, in excess 0.6 *M* hydroxylamine in 90% aqueous EtOH (Scheme I). Treatment of 6-hydroxylaminopurine 3-oxide (**4**) with Raney nickel or dilute hydrazine gave adenine (**6**). 6-Hydroxylaminopurine 3-oxide (**4**) reacted in concentrated aqueous NH_3 to yield a red crystalline precipitate of 6,6′-azoxypurine 3,3′-dioxide (**5**). In contrast with 6-hydroxylaminopurine,[2c] the reaction of the N-oxide with NH_3 takes place in only a few seconds, instead of several days. The azoxy derivative **5** was transformed to 6,6′-bisadenine (**7**)[14] upon boiling with Raney nickel.

SCHEME I



Several attempts failed to yield 6-hydroxylaminopurine 3-oxide (**4**). Treatment of 6-chloro- (**2**), bromo-, and iodopurine 3-oxides[13b] with ethanolic hydroxylamine gave 6-hydroxylaminopurine.[2a] Similar reaction with 6-methylmercaptopurine 3-oxide[7b] resulted in the formation of hypoxanthine 3-oxide.[13b] 6-Mercaptopurine 3-oxide (**1**)[7b] failed to yield **4** upon reaction with hydroxylamine. 6-Chloropurine-9-cyanamido 3-oxide[13b] also gave no reaction with hydroxylamine. Treatment of 6-hydroxylaminopurine or its triacetyl derivative (**8**) in a variety of oxidizing agents (peracetic, trifluoroperacetic, and *m*-chloroperbenzoic acids[15]) resulted in no reaction, or the formation of hypoxanthine.

Experimental Section[16]

Sodium Purine-6-sulfonate 3-Oxide Hydrate (3).[13b,17]—A solution of sodium sulfite (2.52 g, 20 mmoles) in H_2O (40 ml) was added to 6-chloropurine 3-oxide[13b] (**2**, 3.41 g, 20 mmoles) in a H_2O (60 ml) suspension and the resulting mixture was heated at 80° for 2 hr. After cooling, the solution was treated with charcoal, filtered, and poured into EtOH (175 ml). The resulting precipitate was filtered and dissolved in H_2O (75 ml), and the pH was adjusted to 5 with 20% AcOH. After standing at 5° for 18 hr, the precipitate which formed was collected, washed with a little cold EtOH, and dried to give 2.70 g of a white crystalline product, mp >350°. Upon concentration of the mother liquor *in vacuo*, an additional crop of 1.0 g of a white crystalline product, mp >350°, was obtained; total yield 78%. An analytical sample was prepared by repeated H_2O–EtOH treatment, as described above. *Anal.* ($C_5H_3N_4NaO_3S \cdot H_2O$) C, H, N, S, Na.

6-Hydroxylaminopurine 3-Oxide (4).—A solution of **3** (4.6 g, 19 mmoles) in H_2O (250 ml) was added slowly to a 0.6 *M* solution of ethanolic NH_2OH[2a] (2.7 l.) containing 5 ml of 30% aqueous $NH_2OH \cdot HCl$.[18] The mixture was stirred in the dark at 25° for 15 days. The resulting flocculent precipitate was collected, washed with a little cold EtOH, and suspended in H_2O (40 ml). The pH of the suspension was adjusted to pH 3 by dropwise addition of glacial AcOH. The suspension was stirred 15 min, and the solid was collected and washed with a little cold H_2O and EtOH to yield 1.78 g (59%) of light yellow microneedles, mp 215° (exploded when inserted at 210°).

An analytical sample was prepared by repeated washing with H_2O and with 90% aqueous EtOH; light yellow needles, mp 220° (exploded when inserted at 215°). *Anal.* ($C_5H_5N_5O_2$) C, H, N.

6-Hydroxylaminopurine 3-oxide (**4**) darkened on exposure to light. Aqueous solutions of **4** turned yellow when boiled for a few minutes. Compound **4** gave strong positive $FeCl_3$ (intense blue color) and azoxy test (deep red brick color, which later yields a crystalline precipitate of the same color) when dissolved in a small amount of concentrated NH_3.

6-Hydroxylaminopurine 3-oxide (**4**) showed at pH 2 λ_{max} 225 mμ (ϵ 10.1 × 10^3) and 288 (14.7 × 10^3) and at pH 6.47 (0.01 *M* phosphate buffer) λ_{max} 232 mμ (ϵ 14.4 × 10^3) and 302 (13.2 × 10^3). Instability in alkaline solution did not allow the determination of ϵ. The solubility of **4** was 0.29 g/l. of H_2O at 25° (±1°).

Treatment of 6-Hydroxylaminopurine 3-Oxide (4) with Raney Ni.—6-Hydroxylaminopurine 3-oxide (**4**, 5 mg) was suspended in H_2O (5 ml), and Raney Ni (100 mg) was added. After boiling for 30 min the suspension was filtered while hot and the Ni was washed with 5 ml of boiling H_2O. The combined filtrates were concentrated *in vacuo* yielding a product which no longer gave $FeCl_3$ and azoxy tests. Paper chromatograms in three solvent systems and uv spectra at pH 1, 6.8, and 12 were identical with those of an authentic sample of adenine. Similar results were obtained when **4** (5 mg) was refluxed for 2 hr with 20% ethanolic $(H_2N)_2$ (5 ml).

6,6′-Azoxypurine 3,3′-Dioxide Diammonium Salt (5).—Compound **4** (43 mg, 0.4 mmole) was dissolved in concentrated aqueous NH_3 (1 ml) at 25°. The yellow solution rapidly turned orange and then brick red and a copious crystalline precipitate appeared with evolution of gas, presumably O_2.[19] The suspension was filtered after 1 hr and the precipitate was washed thoroughly with cold EtOH to yield 36 mg (75%) of brick red needles, mp >300°. *Anal.* ($C_{10}H_{12}N_{12}O_3$) C, H, N.

Compound **5** showed at pH 6.7 (phosphate buffer), λ_{max} 217, 260, and 456 mμ; at pH 1, λ_{max} 233 and 456 mμ; at pH 13, λ_{max} 466 mμ. The instability of the aqueous solutions at all pHs did not allow determination of ϵ.

Treatment of 6,6′-Azoxypurine 3,3′-Dioxide Diammonium Salt (5) with Raney Ni.—A solution of (**5**) (5 mg) in H_2O (5 ml) was boiled with Raney Ni (100 mg) for 30 min. The resulting colorless solution showed uv spectra and R_f values identical with those of 6,6′-bisadenine **7**.[14]

6-Hydroxylaminopurine Triacetate (8).—A solution of 6-hydroxylaminopurine (3 g, 20 mmoles) in Ac_2O (60 ml) was refluxed for 1 hr. The solution was evaporated *in vacuo* and the residue was washed with EtOH to yield 4.3 g (78%) of needles, mp 158°. A sample, after recrystallization from EtOH gave colorless

(11) E. B. Freese, *Mutation Res.*, **5**, 299 (1968).

(12) S. Chauhe, *Biochem. Pharmacol.*, in press.

(13) (a) I. Scheinfeld, J. C. Parham, S. Murphy, and G. B. Brown, *J. Org. Chem.*, in press; (b) A. Giner-Sorolla, C. Gryte, A. Bendich, and G. B. Brown, *ibid.*, in press.

(14) A. Giner-Sorolla, in preparation.

(15) T. J. Delia, M. J. Olsen, and G. B. Brown, *J. Org. Chem.*, **30**, 2766 (1965).

(16) Uv spectra were determined with a Cary recording spectrophotometer Model 11. Ascending paper chromatography was run on Whatman No. 1 paper in the following solvent systems: concentrated NH_4OH–H_2O–*i*-PrOH (10:20:70), *n*-BuOH–H_2O–AcOH (50:25:25), and 1 *M* NH_4OAc–EtOH (30:70). The determination of melting points was carried out with a Hoover–Thomas melting point apparatus and the temperatures were corrected. Analyses were performed by Spang Microanalytical Laboratory, Ann Arbor, Mich. Where analyses are indicated only by symbols of the elements or functions, analytical results obtained for those elements or functions were within ±0.4% of the theoretical values.

(17) This modified procedure, based on the previously reported synthesis,[13b] gave a better quality product and greater yield.

(18) A. Giner-Sorolla, S. O. Bryant, J. H. Burchenal, and A. Bendich, *Biochemistry*, **5**, 3057 (1966).

(19) *Cf.* (a) E. Bamberger and F. Tschirner, *Ber.*, **32**, 342 (1899); (b) E. Bamberger, *ibid.*, **33**, 113 (1900).

needles, mp 160°. *Anal.* ($C_{11}H_{11}N_5O_4$) C, H, N. Compound **8** decomposed with evolution of AcOH when kept at 25°.

Treatment of 6-Hydroxylaminopurine Triacetate (8) with AcO_2H.—To a solution of **8** (0.27 g) in Ac_2O (3 ml) 30% H_2O_2 (0.4 ml) dissolved in Ac_2O (4 ml) was added and the mixture was kept at 25° for 3 weeks. Evaporation to dryness *in vacuo* resulted in the recovery of the starting material **8**, mp 158°.

Similar treatment of 6-hydroxylaminopurine (0.3 g) in Ac_2O (10 ml) and 30% H_2O_2 (0.4 ml) gave no oxidation product, and the starting material was recovered. Reaction with trifluoroacetic acid and H_2O_2 in Ac_2O gave hypoxanthine. Chloroperbenzoic acid[15] in ether and 6-hydroxylaminopurine after 3 weeks gave no reaction and the purine was recovered unchanged.

6-Mercaptopurine 3-Oxide (1) and NH_2OH.—6-Mercaptopurine 3-oxide[7b] (**1**, 0.20 g, 1.2 mmoles) was suspended in a solution of 0.6 *M* ethanolic NH_2OH (200 ml) and $NH_2OH \cdot HCl$ (20 mg). The mixture was refluxed for 6 hr. Upon evaporation to dryness *in vacuo*, **1** was recovered unchanged.

Biological Activity.—6-Hydroxylaminopurine 3-oxide (**4**) has been tested in the screening program of the Divisions of Applied Therapy and Experimental Chemotherapy. It showed no toxicity when administered to mice at 200 mg/kg, but was found toxic at 300 mg/kg (no survivors in control mice after 7 days). The kidneys of these animals presented a normal aspect, and no crystalline deposit was detected on microscopic observation.

In cell suspension culture, **4** caused very slight inhibition (10%) at 30 μg/ml of leukemia L5178Y/Ca55. At the dosage level of 150 and 100 mg/kg/day for 10 days, **4** prolonged slightly (increased life span: +23 and +24%, respectively) the survival time of mice with leukemia LE1210S. At 100 mg/kg for 7 days, **4** failed to inhibit the growth of Sarcoma 180 and Ridgway osteogenic sarcoma in mice.

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Nitrones. I. α-(5-Nitro-2-furyl)-N-arylnitrones

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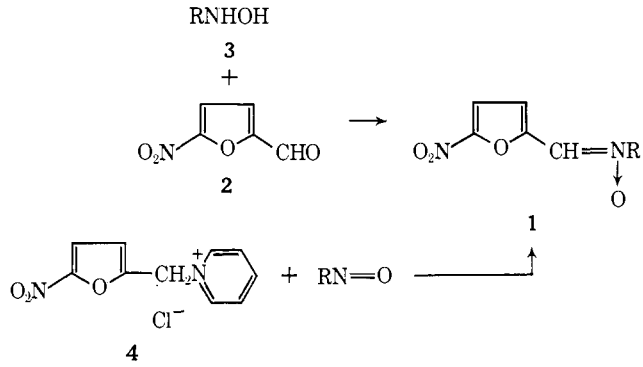
In the course of studies for new antibacterial agents based on nitrofuran,[1] we synthesized a number of the title compounds.[2] At the outset of this work only one nitrofurylnitrone was reported;[3a] subsequently, however, several reports have appeared.[3b-d]

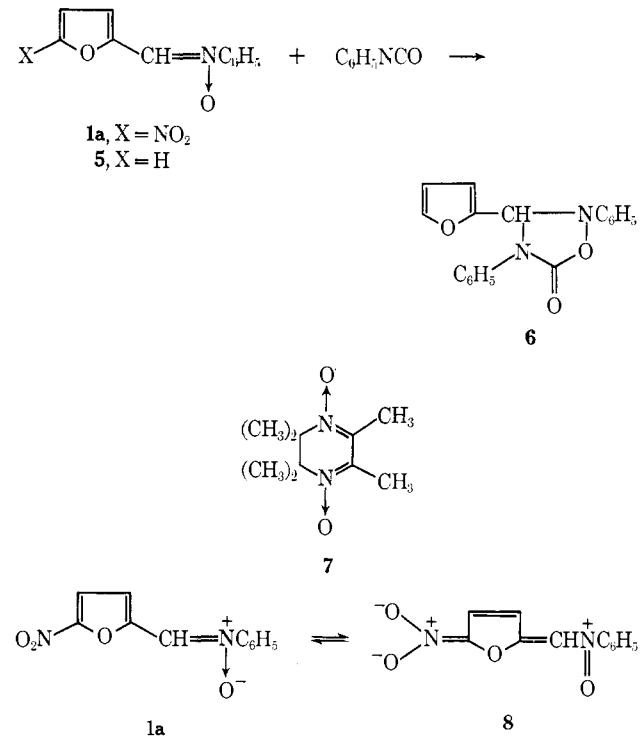
We report here several additional nitrofurylnitrones (**1**) and evidence for the back polarization of the nitrone group.

Nitrones **1a–c**, **f**, and **g** were obtained in 41–64%



yield by reaction of 5-nitrofurfural (**2**) and the corresponding N-arylhydroxylamine (**3**).[4] Nitrones **1a**, **d**, and **e** were prepared in 35–83% yield by the Kröhnke reaction[5] (Table I).

An attempt to cyclize **1a** with phenyl isocyanate in a 1,3 cycloaddition[6] was unsuccessful; the starting material was recovered completely. Yet when α-furyl-N-phenylnitrone (**5**) was treated similarly, the expected cycloaddition product **6** was obtained in good



yield. We could find only one other example of thermal inertness in a 1,3 cycloaddition reaction of this type and this involved the cyclic α-dinitrone **7**.[7] We believe that since nitrophenylnitrones undergo the 1,3 cycloaddition[8] the inability of **1a** to do so is good evidence for "back polarization" as depicted in **1a** ⇌ 8.

Screening Results.—The compounds described above were tested against *Salmonella choleraesuis* (mice)

(1) R. E. Bambury, H. K. Yaktin, and K. K. Wyckoff, *J. Heterocyclic Chem.*, **5**, 95 (1968).

(2) For a general review on nitrones see J. Hamer and A. Macaluso, *Chem. Rev.*, **64**, 473 (1964); G. R. Delpierre and M. Lamchen, *Quart. Rev.* (London), **19**, 329 (1965).

(3) (a) α-(5-Nitro-2-furoyl)-N-(*p*-dimethylaminophenyl)nitrone was reported by N. Saldabols and S. Hillers, *Latvijas PSR Zinatnu Akad. Vestis, Kim. Ser.*, 585 (1963); *Chem. Abstr.*, **61**, 4297 (1964); (b) for structures related to **1** see Dainippon Pharmaceutical Co., Ltd., British Patent 1,105,007 (1968); *Chem. Abstr.*, **69**, 86809 (1968); (c) R. Paul and S. Tchelitcheff, *Bull. Soc. Chim. France*, 4179 (1967); (d) α-(5-Nitro-2-furyl)-N-benzhydrylnitrone was reported by E. Bellasio, F. Parravicini, T. La Noce, and E. Testa, *Farmaco* (Pavia), *Ed. Sci.*, **23**, 372 (1968); *Chem. Abstr.*, **69**, 10143 (1968).

(4) The N-arylhydroxylamines mentioned in this report were prepared by the procedure of H. E. Baumgarten, A. Staklis, and E. M. Miller [*J. Org. Chem.*, **30**, 1203 (1965)], of E. C. Taylor and P. K. Loeffler [*ibid.*, **24**, 2035 (1959)], and of P. K. Chang [*J. Med. Chem.*, **8**, 884 (1965)].

(5) F. Kröhnke, *Ber.*, **71**, 2583 (1938).

(6) R. Huisgen, *Angew. Chem. Intern. Ed. Engl.*, **2**, 565 (1963).

(7) M. Lamchen and T. W. Mittag, *J. Chem. Soc., C*, 1917 (1968).

(8) G. Cum, M. C. Aversa, and N. Uccella, *Gazz. Chim. Ital.*, **98**, 782 (1968); *Chem. Abstr.*, **69**, 77144 (1968).