chlorides in Et_2O was usually followed by recrystallization from cyclohexane and/or C_6H_6. The tlc's were done on silica gel. They were developed in 99% C_6H_6–1% MeOH or *i*-PrOH and visualized with uv light. The R_f values were essentially the same with either of these two developing media.

4-(2-*n*-Dibutylamino-1-hydroxyethyl)phenanthrene Hydrochloride.—A solution of 5.0 g (0.227 mol) of 4-phenanthrylethylene oxide in 35 ml of *n*-Bu_2NH was refluxed at 160° for 16 hr. The excess amine was removed at reduced pressure. The residue was distilled at 199–212° (0.05 mm) with a molecular still to yield 1.8 g (23%) of 4-(2-*n*-dibutylamino-1-hydroxyethyl)-phenanthrene. This was dissolved in 250 ml of C_6H_6 and saturated with HCl. The solution was refluxed for 2 hr with a Dean–Stark trap. The C_6H_6 was removed under reduced pressure and 250 ml of Et_2O was added to the oily residue. The solution was refluxed overnight and the solid collected by filtration to yield 1.8 g (90%) of product, mp 131–134° (softens 125°). *Anal.* ($C_{24}H_{36}ClNO$) C, H, N.

The nmr spectrum[5] of the product was as expected and typical of these compounds, *e.g.*, δ ($CDCl_3$) 0.80 (CH_3), 1.24 (CH_2), 1.62 (NCH_2CH_2), 3.06 (NCH_2), 6.56 (CHOH), 7.35–8.07 (phenanthryl protons), and 8.60–8.70 (phenanthryl 4 and 5 protons) ppm. Formation of the free base by washing the $CDCl_3$ solution with aqueous $NaHCO_3$ resulted in a shift in CH_2 peaks centered at δ 3.50–2.70 ppm (peak at δ 3.06 ppm) to 3.30–2.50 ppm as well as a concentration-dependent shift in the CH proton peaks to a doublet of doublets at δ 6.26 ppm ($CHOHCH_2NR_2$) and to a triplet at 4.62 ppm ($CHCH_2OHNR_2$). The integration ratios of these two groups permitted an analysis of the isomer content of the sample when the undesirable isomer was present. This nmr analysis showed the product to be 88% isomer A and 12% of the undesired isomer B (see Table X).

All of the amino alcohols showed some antimalarial activity in mice. Only 1-(2-*n*-dihexylamino-1-hydroxyethyl)-9-bromophenanthrene gave cures (2 out of 5) at 640 mg/kg. This series is being extended to include additional halogenated phenanthrenes.

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Nitrones. II.[1] α-(5-Nitro-2-furyl)-*N*-cycloalkyl- and -*N*-alkylnitrones

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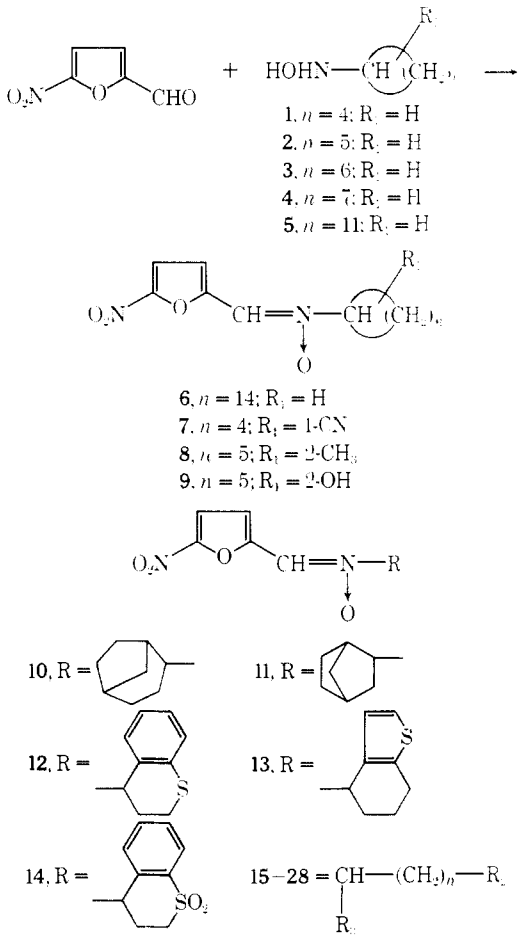
A series of α-(5-nitro-2-furyl)-*N*-cycloalkylnitrones, *N*-bicycloalkyl and *N*-heterocycloalkylnitrones, and *N*-alkylnitrones were synthesized and evaluated as antibacterial, antifungal, and anticoccidial agents. Saturation of the phenyl ring of α-(5-nitro-2-furyl)-*N*-phenylnitrone[1] enhanced its antibacterial activity. Replacement of the cyclohexyl moiety by Me (**15**) further enhanced the antibacterial activity. Structure–activity relationships are discussed.

In a previous paper,[1] the preparation and biological activities of some α-(5-nitro-2-furyl)-*N*-arylnitrones were reported. This paper describes an extension of this series to include analogs in which the *N*-aryl group was replaced by cycloalkyl, bicycloalkyl, heterocycloalkyl, and alkyl groups. Compounds **1–28** were obtained in 6–93% yield by the reaction of 5-nitrofurfural and the corresponding *N*-substituted hydroxylamines either directly or by liberating them *in situ* from their HCl salts as illustrated in eq 1. Physical and analytical data for the nitrones are listed in Tables I and II. Compounds **15–17** and **22** were reported[3] subsequent to our work.

Direct interaction of free lower *N*-alkylhydroxylamines, *e.g.*, *N*-propylhydroxylamine, with 5-nitrofurfural caused rapid decomposition of the aldehyde, whereas treatment with cycloalkyl-, heterocycloalkyl-, *e.g.*, **30–32**, and higher alkylhydroxylamines, *e.g.*, **33–39**, resulted in the formation of the desired nitrones without difficulty. In the case of **28**, the reaction was carried out in an aqueous medium containing base to give the product as its Na salt.

The N-substituted hydroxylamines (Table III) were prepared by diborane reduction of the corresponding oximes according to Feuer, *et al.*,[4] or by the cyanide–



(1) For paper I, see H. K. Kim and R. E. Bambury, *J. Med. Chem.*, **12**, 719 (1969).

(2) Deceased May 21, 1968.

(3) Dainippon Pharmaceutical Co., Ltd., British Patent 1,105,007; *Chem. Abstr.*, **69**, 86809 (1968).

(4) H. Feuer, B. F. Vincent, Jr., and R. S. Bartlett, *J. Org. Chem.*, **30**, 2877 (1965).

TABLE I

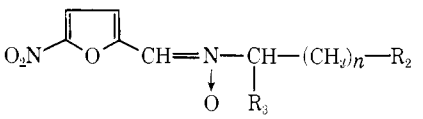
α-(5-NITRO-2-FURYL)-N-CYCLOALKYLNITRONES

Compd	Prepn method	Mp, °C	Recrystn solvent	Yield,[a] %	Formula[b]	*In vivo* antibacterial act. rel to 15[c]
1	A	105–106	Et_2O	69	$C_{10}H_{12}N_2O_4$	0.50
2	A	150–151	Et_2O–C_6H_6	76	$C_{11}H_{14}N_2O_4$	0.83
3	A	133–135	Et_2O	74	$C_{12}H_{16}N_2O_4$	0.63
4	A	128–130	Et_2O	79	$C_{13}H_{18}N_2O_4$	<0.50
5	A	154–155	Et_2O–C_6H_6	87	$C_{17}H_{26}N_2O_4$	<0.30
6	A	128–129	Et_2O	71	$C_{20}H_{32}N_2O_4$	<0.30
7	C	152–153	EtOH	76	$C_{11}H_{11}N_3O_4$	<0.30
8	B	101–103	Et_2O–hexane	20	$C_{12}H_{16}N_2O_4$	<0.40
9	B	147–148	MeOH	6	$C_{11}H_{14}N_2O_5$	<0.40
10	B	154	Et_2O	61	$C_{13}H_{16}N_2O_4$	<0.40
11	B	136–138	MeOH	48	$C_{12}H_{14}N_2O_4$	0.42
12	A	163–164	MeOH–CH_3NO_2	79	$C_{14}H_{12}N_2O_4S$	I[d]
13	A	177–178	Et_2O	38	$C_{13}H_{12}N_2O_4S$	I
14	C	212–213 dec	CH_3NO_2	89	$C_{14}H_{12}N_2O_6S$	I

[a] Yield is of purified product. [b] All compounds were analyzed for C, H, N, and where applicable S; analytical results obtained were within ±0.4% of the calculated values. [c] Activity, 1.00. [d] I = inactive.

TABLE II

α-(5-NITRO-2-FURYL)-N-ALKYLNITRONES



Compd	*n*	R_2	R_3	Prepn method	Mp, °C	Recrystn solvent	Yield,[a] %	Formula[b]	*In vivo* antibacterial act. rel to 15
15	0	H	H	C	163–164[d]	CH_3NO_2	78	$C_6H_6N_2O_4$	1.00
16	1	H	H	C	173–174[e]	CH_3NO_2	71	$C_7H_8N_2O_4$	0.50
17	2	H	H	C	83–84[f]	Et_2O	36	$C_8H_{10}N_2O_4$	0.67
18	3	H	H	B	62–63	Et_2O	57	$C_9H_{12}N_2O_4$	<0.30
19	6	H	H	B	76–77	Et_2O	39	$C_{12}H_{18}N_2O_4$	<0.30
20	9	H	H	C	86	Et_2O	71	$C_{15}H_{24}N_2O_4$	<0.30
21	13	H	H	C	95–96	C_6H_6	68	$C_{19}H_{32}N_2O_4$	<0.30
22	1	H	CH_3	C	106–107[g]	Et_2O	93	$C_8H_{10}N_2O_4$	0.38
23	2	H	CH_3	B	68–69	MeOH	90	$C_9H_{12}N_2O_4$	0.50
24	1	C_6H_{11}[h]	CH_3	A	95–97	Cyclohexane	57	$C_{14}H_{20}N_2O_4$	<0.30
25	1	H	CF_3	B	144–147	C_6H_6–hexane	65	$C_8H_7F_3N_2O_4$	<0.40
26	1	Cl	CH_2Cl	B	114–116	C_6H_5–cyclohexane	34	$C_8H_8Cl_2N_2O_4$	<0.40
27	3	H	CN	C	102–104	C_6H_6–petr ether (bp 60–70°)	76	$C_{10}H_{11}N_3O_4$	<0.40
28	3	H	CO_2Na	D	153[i]	EtOH–C_6H_6	48	$C_{10}H_{11}NaN_2O_6$	<0.30

[a] Yield is of purified product. [b] All compounds were analyzed for C, H, N, and where applicable halogen; analytical results obtained were within ±0.4% of the calculated values. [c] See ref 3. [d] Lit. mp 165–166°.[3] [e] Lit. mp 175–177°.[3] [f] Lit. mp 82–83°.[3] [g] Lit. mp 103–104°.[3] [h] Cyclohexyl. [i] It detonated at this temperature.

oxime reduction of Neelakantan, *et al.*,[5] *e.g.*, 1-hydroxylaminocyclopentanecarbonitrile (**37**), 2-(hydroxylamino)pentanonitrile (**38**), and 2-(hydroxylamino)pentanoic acid (**39**). *N*-(2-Methylcyclohexyl)-, *N*-(2-hydroxycyclohexyl)-, *N*-(2-bicyclo[3.2.1]octyl)-, *N*-(2-norbornyl)-, and *N*-2-(1,3-dichloropropyl)hydroxylamines obtained by the former method were not isolated,[6] but were converted into their HCl salts and used without purification. The structures of these new N-substituted hydroxylamines were confirmed by their ir and nmr spectra. The compounds also gave a positive Tollens test. Diborane reduction of 1,1,1-trifluoroacetone oxime and 1,3-dichloroacetone oxime to the corresponding *N*-alkylhydroxylamines illustrates the selectivity[7] shown by this reagent when both oxime and halogen groups are present in the same molecule.

The ir spectra of all the nitrones showed nitrone (CH=N→O), nitro, and furan ether group bands, and the nmr spectra were consistent with the nitrone structure.

Structure–Activity Relationships.—These nitrones showed slight to moderate *in vitro* antibacterial and antifungal activity against representative bacteria and fungi, as shown in Table IV.

It was interesting to find that saturation of the phenyl moiety in α-(5-nitro-2-furyl)-*N*-phenylnitrone[1] enhanced antibacterial activity.

The antibacterial activity of the nitrones against a *Salmonella choleraesuis* infection in mice relative to **15** (assigned activity of 1.00) is shown in Tables I and

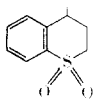
(5) L. Neelakantan and W. H. Hartung, *J. Org. Chem.*, **23**, 964 (1958).

(6) Attempts to purify them by sublimation under reduced pressure were unsuccessful.

(7) H. C. Brown, "Hydroboration," W. A. Benjamin, Inc., New York, N. Y., 1962, p 249.

TABLE III

N-SUBSTITUTED HYDROXYLAMINES, RNHOH

Compd	R	Mp, °C	Recryst solvent	Yield,[a] %	Formula[b]
29	Cyclopentadecyl	100–101	Cyclohexane	20	$C_{15}H_{31}NO$
30		130–131	EtOH	29	$C_9H_{11}NOS$
31		79–80	C_6H_6	32	$C_9H_{11}NOS$
32		171–173	EtOH	32	$C_9H_{11}NO_3S$
33	$CH(CF_3)CH_3$	86–88	Hexane	50	$C_3H_6F_3NO$[c]
34	$CH(CH_3)CH_2C_6H_{11}$[d]	95–96	Cyclohexane	32	$C_9H_{19}NO$
35	n-$C_{10}H_{21}$	83	EtOH	39	$C_{10}H_{23}NO$
36	n-$C_{14}H_{29}$	61	EtOH	61	$C_{14}H_{31}NO$

[a] Yield is of purified product. [b] All compounds were analyzed for C, H, N, and where applicable S; analytical results were within ±0.4% of the calculated values. [c] F: calcd, 44.16; found, 40.66. [d] C_6H_{11} = cyclohexyl.

TABLE IV

In Vitro ANTIBACTERIAL AND ANTIFUNGAL ACTIVITY (MINIMUM INHIBITORY CONCENTRATION, μG/ML)

Compd	SG[a]	ST	SA	SAG	PsA	PrM	EI	BS	EC	PM	AF	CA
1	100	100	>100	100	>100	>100		100	<100	10	1	10
2	100	100	100	10	>100	>100		10	100	100	100	1
3	>100	>100	100	100	>100	>100		10	>100	10	>100	>100
4	>100	>100	>100	100	>100	>100		>100	>100	10		100
5	>100	>100	>100	>100	>100	>100		>100	>100	>100	>100	>100
6	>100	>100	>100	>100	>100	>100	>100	>100	>100		>100	>100
7	>100	100	10	100	>100	>100		10	>100	100	>100	>100
8	100	>100	100	100	>100	>100		10	>100	10	100	100
9	100	100	10	1	>100	>100		1	100	10	100	100
10	>100	>100	>100	>100	>100	>100	>100	>100	>100		>100	>100
11	>100	>100	>100	>100	>100	>100	>100	>100	>100		>100	>100
12	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100
13	>100	>100	>100	>100		>100	>100	>100	>100	>100	>100	>100
14	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100
15	10	100	100	10	>100	100	10	10	100	1		
16	10	10	100	10	>100	100	100	10	1	10	1	10
17	10	100	100	10	>100	100	100	10	100	1	100	100
18	100	100	100	10	>100	>100	100	10	100	100	10	10
19	100	>100	100	10	>100	>100	100	10	>100	10	10	>100
20	>100	>100	100	100	>100	>100	>100	100	>100	>100	>100	>100
21	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100
22	10	100	100	100	>100	100	100	10	10	1	10	10
23	100	>100	>100	100	>100	100	>100	>100	100	10	100	100
24	>100	>100	100	>100	>100	>100	100	1	>100	100	100	>100
25	100	>100	100	100	>100	>100	>100	10	>100	10	100	10
27	100	100	100	100	>100	100	100	100	100	10	100	100
28	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100

[a] SG = *Salmonella gallinarum*, ST = *Salmonella typhimurium*, SA = *Staphylococcus aureus*, SAG = *Streptococcus agalactiae*, PsA = *Pseudomonas aeruginosa*, PrM = *Proteus mirabilis*, EI = *Erysipelothrix insidiosa*, BS = *Bacillus subtilis*, EC = *Escherichia coli*, PM = *Pasteurella multocida*, AF = *Aspergillus fumigatus*, CA = *Candida albicans*.

II. Cyclohexyl was found to be the most active ring substituent with activity decreasing with changing ring size either larger or smaller. The activity decreased in the order: cyclohexyl > cycloheptyl > cyclopentyl. The ED_{50} of **2** is 54.0 mg/kg as compared to 12.0 mg/kg for furazolidone.[8] Thus, the potency of **2** relative to furazolidone[8] is 0.22,[9] and the potencies of **1** and **3** are 0.16 and 0.15, respectively. Substitution of functional groups, such as cyano, hydroxyl, or methyl, into the cycloalkyl ring caused a significant decrease in activity. Replacement of the cycloalkyl group with bicycloalkyl groups, *e.g.*, bicyclo[3.2.1]-octyl (**10**) and norbornyl (**11**), and heterocycloalkyl groups, *e.g.*, 2,3-dihydro-4*H*-1-benzothiopyran-4-yl (**12**), tetrahydrobenzothiophene (**13**), and 1,1-dioxido-2,3-dihydro-4*H*-1-benzothiopyran-4-yl (**14**), also decreased biological activity.

α-(5-Nitro-2-furyl)-*N*-methylnitrone (**15**) was found to be the most active antibacterial agent in this series. In this same test, the potency, 0.27, of **15** (ED_{50} 44.6 mg/kg) relative to furazolidone was slightly in-

[8] Furazolidone, 3-(5-nitrofurfurylideneamino)-2-oxazolidinone.

[9] Potency was determined by the following calculation described by M. T. Litchfield, Jr., and F. Wilcoxon [*J. Pharmacol. Exp. Ther.*, **96**, 99 (1949)]. Potency = ED_{50} of furazolidone/ED_{50} of **2**.

creased. In general, the antibacterial activity decreased with increasing chain length or substitution.

Compound **15** also demonstrated anticoccidial activity against *Eimeria tenella* in chickens at a dose of 63 mg/kg, but was considerably less active than nitrofurazone.[10] However, none of the cycloalkylnitrones displayed anticoccidial activity.

Experimental Section[11]

Starting Materials.—The 1 *M* borane in tetrahydrofuran (THF) solution was employed as received from Metal Hydrides Division, Ventron Corporation, Beverly, Mass. THF was purified by known methods.[12] All oximes were prepared by methods described in the literature. Cyclohexylacetone oxime (**40**) was obtained in typical fashion and distilled through a 15.2 cm Vigreaux column to yield a viscous oil; yield, 87%, bp 82–84° (0.03 mm), ν_{max} 3175 (=N—OH) and 1667 cm^{-1} (C=N). *Anal.* ($C_9H_{17}NO$) C, H.

Diborane Reduction of Oximes to the Corresponding N-Substituted Hydroxylamines (29–36) (Table III).—A 1 *M* solution of borane in THF was introduced, dropwise, to a cooled solution of the corresponding oxime in anhydrous THF (200–800 ml), at such a rate that the temperature did not exceed 5°. The reaction mixture was stirred overnight at room temperature, the temperature was lowered to 0°, and 50% NaOH (35 ml) was added at such a rate that the temperature did not exceed 5°. After refluxing for 1 hr, the reaction mixture was dried ($MgSO_4$), and the solvent was removed to give an oily residue, which was triturated with petroleum ether (bp 60–70°) to give crude products which were purified by recrystallization. Results are shown in Table III.

Similarly *N*-(2-methylcyclohexyl)-, *N*-(2-hydroxycyclohexyl)-, *N*-(2-bicyclo[3.2.1]octyl)-, and *N*-(2-norbornyl)hydroxylamine·HCl were obtained by reducing the corresponding oximes followed by treatment with HCl. In the case of *N*-(2-(1,3-dichloropropyl)hydroxylamine, HCl was used for hydrolysis.

The N-substituted hydroxylamines not listed in Table III were described previously in the literature.

Preparation of α-(5-Nitro-2-furyl)-*N*-cycloalkyl- and -*N*-alkylnitrones. Method A.—A mixture of 5-nitrofurfural (0.01 mol) and the corresponding *N*-cycloalkylhydroxylamine[13] or *N*-alkylhydroxylamine (0.01 mol) in dry C_6H_6 (45–50 ml) was refluxed 45 min, using a Dean–Stark water separator. The solvent was removed and the residue triturated with petroleum ether (bp 60–70°) and recrystallized to yield the corresponding nitrone. Results are shown in Tables I and II.

Method B.—The corresponding *N*-cycloalkyl- or *N*-alkylhydroxylamine·HCl (0.01 mol) in absolute EtOH (10 ml) was stirred with 5-nitrofurfural (0.01 mol) in absolute EtOH (10 ml) containing $NaHCO_3$ (1.26 g, 0.015 mol). Stirring was continued for 3–16 hr and the mixture was filtered. The filter cake was thoroughly washed with Et_2O in the case of **8, 10, 15–19, 22–23,** and **25–26,** warm MeOH in the case of **9,** and $CHCl_3$ in the case of **11** until no yellow color remained. Evaporation of the combined filtrate and washings gave a residue which was recrystallized from the appropriate solvents. Results are shown in Tables I and II.

Method C.—A mixture of 5-nitrofurfural (0.01 mol) and **37**[5] or the corresponding *N*-heterocycloalkyl- or -*N*-alkylhydroxylamine (0.01 mol) in warm EtOH (30–50 ml) was stirred for 0.2–2 hr at room temperature. After cooling at *ca.* 0°, filtration gave the corresponding nitrone which was purified by recrystallization. Results are shown in Tables I and II.

Method D.—A mixture of 5-nitrofurfural (5.30 g, 0.0376 mol), **39**[5] (5.01 g, 0.0376 mol), and $NaHCO_3$ (3.60 g, 0.0376 mol) in H_2O (50 ml) was heated on the steam bath for 10 min. The mixture was stirred overnight at room temperature and then evaporated to dryness. The residue was recrystallized from EtOH–C_6H_6 to obtain the sodium salt of α-(5-nitro-2-furyl)-*N*-(1-carboxybutyl)nitrone (**28**).

In vitro **Antibacterial and Antifungal Test Procedure.**—Each compound to be tested (10 mg) was placed in 10 ml of 0.1% Trypticase Soy Agar (TSA). This solution contained 10^3 μg/ml of compound. Five test tubes containing 0.9 ml of Trypticase Soy Broth (TSB) were arranged to make tenfold dilutions of each compound tested against each organism in the test spectrum. A 0.1-ml sample was removed from the solution containing 10^3 μg/ml and placed into the first tube in each series. Tenfold dilutions were made to give final concentrations ranging from 100 to 0.01 μg/ml and differing by factors of 10. The test solutions were inoculated with 0.1 ml of a 1:1000 dilution of a 24-hr TSB culture of the respective organism. All tubes were incubated for 24 hr at 37° and observed visually for turbidity.

In vivo **Antibacterial Screening Procedure.**—Random bred, male albino mice weighing 19–21 g were placed in cages (5 mice/cage) and were allowed free access to a preweighed quantity of feed containing the test drug at 0.1 to 0.0016% levels. In each test there were three control groups, noninfected control, infected control, and infected control, receiving 0.0125% furazolidone[8] in feed. The feed remaining after 48 hr was weighed to determine the amount of feed consumed. All mice designated to be infected were then injected intraperitoneally with 0.2 ml of a 1:100,000 dilution of a 5-hr *Salmonella choleraesuis* variety Kunzendorf (ATCC #12011) brain heart infusion broth culture. Mortality records were maintained for 14 days postinfection with the mice receiving their designated test feeds throughout this period. At the completion of an evaluation, per cent survival and milligrams per kilogram dose corresponding to each level were plotted on logarithmic probability paper in order to determine ED_{50} values. The methods described by Litchfield and Wilcoxon[9] were used to fit the curve.

Anticoccidial Screening Procedure.—Anticoccidial screening in chickens against a strain of *Eimeria tenella* was carried out as described by Johnson and O'Connor.[14]

Acknowledgments.—The authors are indebted to Dr. W. E. Meredith and D. R. Filson, formerly of the Microbiology Department, for antibacterial and antifungal testing, and C. A. Johnson for anticoccidial testing.

(10) P. D. Harwood and D. I. Stunz, *J. Parasitol.*, **35**, 175 (1949).

(11) Melting points were taken in open capillary tubes using a Thomas–Hoover melting point apparatus, and are uncorrected. Elemental analyses were performed by Spang Microanalytical Laboratory, Ann Arbor, Mich. Ir spectra were obtained with a Beckman IR-5 spectrophotometer (KBr). Nmr spectra were obtained with a Varian A-60 spectrometer (Me_4Si). Evaporation of solvents was done under reduced pressure using a rotary evaporator.

(12) L. F. Fieser and M. Fieser, "Reagents for Organic Synthesis," John Wiley & Sons, Inc., New York, N. Y., 1967, p 1140.

(13) (a) *N*-Cyclopentylhydroxylamine: E. Perotti, M. Lanzoni, G. Daniel, and M. DeMalde, *Ann. Chim.* (Rome), **55** (5), 485 (1965); *Chem. Abstr.*, **64**, 9607 (1966); (b) *N*-cyclohexylhydroxylamine, see ref 4; (c) *N*-cycloheptyl- and *N*-cyclooctylhydroxylamine: E. Müller, D. Fries, and H. Metzger, *Chem. Ber.*, **88**, 1891 (1955); (d) *N*-cyclododecylhydroxylamine: H. Meister, *Ann. Chem.*, **679**, 83 (1964).

(14) C. A. Johnson and J. O'Connor, *Poultry Sci.*, **44**, 578 (1965).