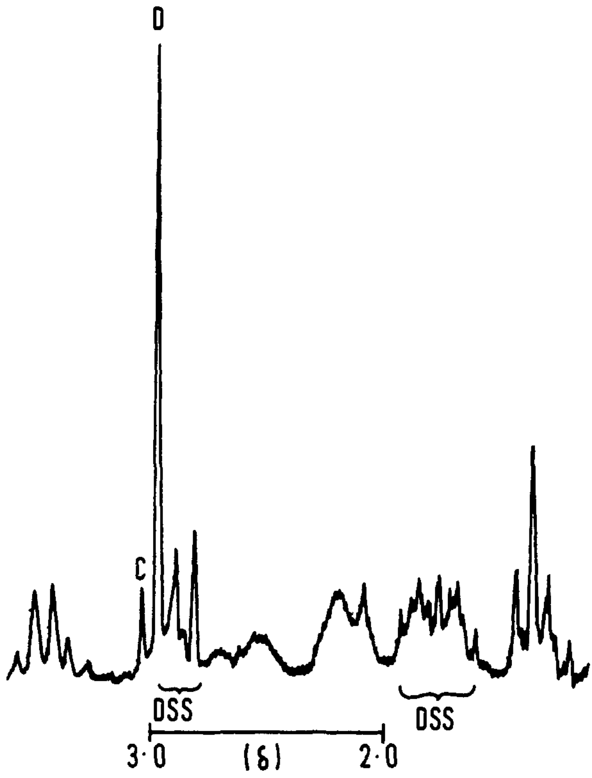

Figure 3. Part of the nmr spectra of the more soluble form of atropine ethiodide, obtained from mother liquors. Peaks C and D are *N*-methyl signals from axial and equatorial *N*-methyl groups, respectively, and indicate that about 85% of the ethyl group is axial (*cf.* Figure 1).

Table II. Values of Log *K* for Postganglionic Acetylcholine Receptors of the Guinea-Pig Ileum[a]

	N_{ax}-Ethyl	N_{eq}-Ethyl
Atropinium iodide	*9.29*	8.22
(*R*)-Hyoscyaminium iodide		7.06
(*S*)-Hyoscyaminium iodide	*9.59*	8.79*
		8.40

[a]See Table IE of the previous paper.[1] Calculated values are shown in italics; the asterisk indicates that this experimental value is made with material containing 10% of the axial epimer.

ethylated epimer (though this did not produce any significant difference in optical rotation, see Table I of the previous paper[1]). If, as with the racemic mixture, N_{ax}-ethyl-(*S*)-hyoscyaminium iodide is appreciably more active than the N_{eq}-ethyl epimer, the calculated value for the racemate will be higher than it should be. From the value of log *K* for N_{ax}-ethylatropinium iodide, 9.29, the value for N_{ax}-ethyl-(*S*)-hyoscyaminium iodide should be about 9.59 and the value for N_{eq}-ethyl-(*S*)-hyoscyaminium iodide, corrected for the 10% N_{ax}-ethyl epimer present in the sample tested, should be 8.40. The value for N_{eq}-ethylatropinium iodide should then be 8.12, which is in reasonable agreement with the experimental value (Table II).

It seems likely that the samples studied by Green, *et al.*,[2] contained appreciable amounts of axially alkylated material.

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Nitrones. 6. α-(5-Nitroimidazol-2-yl)-N-substituted Nitrones[1,†]

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The disclosure[2,3] of the antibacterial and antiprotozoal properties of derivatives of 1-alkyl-5-nitroimidazole-2-carboxaldehydes led us to extend our work in the nitrone area to include nitrone derivatives of these aldehydes (a portion of this work has been described[4]).

The desired nitrones were readily prepared by condensation of the appropriate 5-nitroimidazole-2-carboxaldehyde with an N-substituted hydroxylamine (eq 1). The aldehydes employed in the synthesis had $R_1 = CH_3$, CH_2CH_2OH, and $CH_2CH_2OCOCH_3$ and were prepared by modifications of the methods described by Henry and Hoff.[2] The hydroxylamines utilized were either known compounds or have been described in our previous publications on nitrones.[1] The nitrones prepared in this investigation are listed in Table I.

$$\text{5-nitroimidazole-2-CHO }(R_1) + R_2NHOH \longrightarrow \text{5-nitroimidazol-2-yl-}CH{=}N(\rightarrow O)R_2 \ (R_1) \quad (1)$$

Biological Activity. From the data presented in Table II it is apparent that the nitroimidazolyl nitrones as a group are more active against the gram-negative *Salmonella schottmuelleri* than the two representative gram-positive organisms. Variation of the nitrone side chain (R_2) from lower alkyl (**1**, **2**) to higher alkyl (**3**, **4**) or aryl (**11**) led to a decrease in activity. Side chains having hydroxyl groups (**5**, **12**, **13**, **15**, **18**) were introduced; however, this modification did not lead to an increase in activity as had been found with the nitrofurylnitrones.[5] Introduction of other functional groups into the side chain (**8**, **10**, **20**), with the exception of $-OC_2H_5$ (**9**), also decreased activity. The observation that **9** was one of the most active nitroimidazole nitrones was not too surprising as the corresponding compound in the nitrofuran series was also highly active.[5]

Metronidazole [1-(2-hydroxyethyl)-2-methyl-5-nitroimidazole], the first orally active drug for the treatment of trichomoniasis,[6] has a hydroxyethyl group on the imidazole ring. We felt that the use of this group as R_1 in our nitrones might enhance their activity. However, neither this group nor its acetate (**14–20**) enhanced antibacterial activity, although **14**, **17**, and **18** were approximately as active as **1**, **2**, and **9** against *S. schottmuelleri in vivo.*

†Part of this work was carried out at the Hess & Clark Division of Richardson-Merrell, Inc., Ashland, Ohio, now a division of Rhodia, Inc.

Table I. α-(5-Nitroimidazol-2-yl)-N-substituted Nitrones

O_2N–[imidazole, N, R_1]–$CH{=}NR_2$ (↓ O)

Compd no.	R_1	R_2	Time, hr	Mp, °C	Recrystn solvent	Yield,[a] %	Formula[b]
1	CH_3	CH_3	0.5	184-185	EtOH	66	$C_6H_8N_4O_3$
2	CH_3	C_2H_5	0.5	125-128	EtOH	78	$C_7H_{10}N_4O_3$
3	CH_3	n-$C_{10}H_{21}$	0.5	66-68	EtOH	61	$C_{15}H_{26}N_4O_3$
4	CH_3	C_6H_{11}[c]	1	155-156	*i*-PrOH	71	$C_{11}H_{16}N_4O_3$
5	CH_3	CH_2CH_2OH	0.5	159-162	EtOH	73	$C_7H_{10}N_4O_4$
6	CH_3	$CH(C_2H_5)CH_2OH$	0.5	128-129	EtOH	48	$C_9H_{14}N_4O_4$
7	CH_3	$C(CH_2OH)_3$	0.5	174-175	EtOH	33	$C_9H_{14}N_4O_6$
8	CH_3	CH_2CH_2OAc	0.5	120-123	C_6H_6	37	$C_9H_{12}N_4O_5$
9	CH_3	CH_2CH_2OEt	0.5	103-104	EtOH	49	$C_9H_{14}N_4O_4$
10	CH_3	$CH(CH_3)CF_3$	24	185-187	EtOH	28	$C_8H_9F_3N_4O_3$
11	CH_3	C_6H_5	4	158-160	EtOAc	59	$C_{11}H_{10}N_4O_3$
12	CH_3	$CH_2CH(OH)C_6H_4$-p-OCH_3	0.25	154-156	MeOH	77	$C_{14}H_{16}N_4O_5$
13	CH_3	$CH_2CH(OH)$–[benzene ring with O–CH_2–O]	0.5	203 dec	DMF-H_2O	92	$C_{14}H_{14}N_4O_6$
14	CH_2CH_2OH	CH_3	3.5	167-169	EtOH	46	$C_7H_{10}N_4O_4$
15	CH_2CH_2OH	CH_2CH_2OH	2.5	151-152	*i*-PrOH	76	$C_8H_{12}N_4O_5$
16	CH_2CH_2OH	C_6H_{11}[c]	3	203-205	$MeNO_2$	22	$C_{12}H_{18}N_4O_4$
17	CH_2CH_2OAc	CH_3	1.25	124-126	EtOH	59	$C_9H_{12}N_4O_5$
18	CH_2CH_2OAc	CH_2CH_2OH	1.25	138-140	MeOH	73	$C_{10}H_{14}N_4O_6$
19	CH_2CH_2OAc	C_6H_{11}[c]	1.5	115-118	EtOH	58	$C_{14}H_{20}N_4O_5$
20[d]	CH_2CH_2OAc	CH_2CH_2OAc		93-96	C_6H_6	59	$C_{12}H_{16}N_4O_7$

[a]Yield of purified product. [b]All new compounds were analyzed for C, H, N, and where applicable F; analytical results obtained varied within ±0.4% of the calculated values. [c]Cyclohexyl. [d]Prepared by acetylation of 18.

Table II. Antibacterial Activity[a]

Compd no.	*In vitro*[b] MIC, μg/ml			*In vivo*[c] ED_{50}, mg/kg	
	S.a.	S.ag.	S.s.	S.a.	S.s.
1	I	1000	100	I	22
2	1000	1000	1000	I	10
3	1000	I	I	I	I
4	I	I	1000	I	I
5	I	1000	100	I*	I
6	I	I	100		
7	I	I	1000		
8	I	I	100	I*	50
9	1000	I	100	10	10
10	I	I	1000	I	50
11	1000	I	1000	I*	87
12	I	I	I	I	I
13	I	I	I	I	I
14	I	1000	100	I	22
15	I	I	1000	I	I
16	I	I	I	78	I
17	1000	I	100	I	25
18	1000	I	1000	I	15
19	I	I	I	I	95
20	I	I	1000	I	I
Nifuratrone[d]	100	I	10	23	6.2
2-Amino-5-(1-methyl-5-nitro-2-imidazolyl)-1,3,4-thiadiazole[e]	1000	I	100	50	17

[a]S.a. = *Staphylococcus aureus*; S.s. = *Salmonella schottmuelleri*; S.ag = *Streptococcus agalactiae*. [b]I = No activity at 1000 μg/ml or less. [c]I = Inactive at 200 mg/kg or less. The bacterial challenge was approximately 10-100 LD_{50}'s. Route of drug administration was subcutaneous except where noted by an asterisk, in which case the oral route was used. [d]USAN name for *N*-(2-hydroxyethyl)-α-(5-nitro-2-furyl)nitrone (see ref 5). [e]Reference 3.

Only compound **9** showed *in vivo* activity comparable to the recently disclosed[3] 2-amino-5-(1-methyl-5-nitro-2-imidazolyl)-1,3,4-thiadiazole in our test systems. Thus, only the ethyl ether nitrone function is comparable to the aminothiadiazole group for enhancing the antibacterial activity of the 5-nitroimidazole moiety.

Preliminary antiprotozoal evaluation of compound **1** against *Histomonas meleagridis* in turkeys and *Trypanosoma cruzi* in mice has indicated interesting activity against these organisms.

Experimental Section

Melting points were taken in open capillary tubes with a calibrated thermometer using a Thomas-Hoover melting point apparatus. Elemental analyses were performed by Spang Microanalytical Laboratory, Ann Arbor, Mich., and by the Analytical Department of Merrell-National Laboratories. The ir and nmr spectra of each compound were consistent with assigned structures. Solvents were removed under vacuum on a rotary evaporator.

1-(2-Acetoxyethyl)-5-nitroimidazole-2-carboxyaldehyde (21). A mixture of 1-(2-acetoxyethyl)-2-hydroxymethyl-5-nitroimidazole[2] (124.7 g, 0.55 mol), active MnO_2 (375 g), and C_6H_6 (4.5 l.) was refluxed for 1 hr and filtered hot. The MnO_2 was washed with C_6H_6; the organic phases were combined and evaporated to an oil (99.8 g). Crystallization from C_6H_6–cyclohexane gave the aldehyde (90.9 g, 73%), mp 45–46.5°. The preparation of this aldehyde by another route has been reported by Asato and English.[7]

5,6-Dihydro-8-hydroxy-3-nitro-8*H*-imidazo[2,1-*c*][1,4]oxazine. A mixture of **21** (69.6 g, 0.3 mol) and 10% H_2SO_4 (200 ml) was refluxed 1 hr and cooled giving the cyclic hemiacetal (52.4 g, 92%). Recrystallization from $MeNO_2$ gave the pure hemiacetal, mp 197–200° dec (lit.[7] 206.5–207° dec). This hemiacetal reacted readily with hydroxylamines to give the expected nitrones.

Preparation of α-(5-Nitroimidazol-2-yl)-N-substituted Nitrones. The following description of the preparation of compound **1** illustrates the general method used to prepare the nitrones described in this report.

***N*-Methyl-α-(1-methyl-5-nitroimidazol-2-yl)nitrone (1).** To 51.2 g (0.33 mol) of 1-methyl-5-nitroimidazole-2-carboxaldehyde[2] dissolved in 1500 ml of hot absolute EtOH was added 32 g (0.38 mol) of *N*-methylhydroxylamine hydrochloride and 40 g (0.48 mol) of $NaHCO_3$. The mixture was heated, with stirring, at reflux for 30 min and filtered hot. The filtrate was cooled and the solid filtered off

giving 1 (40.3 g, 66%), mp 180–182°. Recrystallization from EtOH gave pure 1, mp 184–185°.

***N*-(2-Acetoxyethyl)-2-[1-(2-acetoxyethyl)-5-nitroimidazol-2-yl]-nitrone (20).** A solution of 18 (20 g, 0.07 mol), Ac_2O (12 ml), pyridine (11 ml), and THF (200 ml) was refluxed for 4 hr and evaporated. The residue was recrystallized from C_6H_6 (150 ml) to give 20 (13.8 g, 59%), mp 93–96°.

Biological Test Procedures. The *in vitro* testing of the compounds was carried out using the paper disk method. In this method a standard 12.7-mm diameter sterile filter paper disk impregnated with 0.1 ml of the indicated concentration of the compound was placed on a trypticase soy agar plate seeded with the test organism. The seeded plates were incubated for 24 hr at 37° and then observed for zones of inhibition of bacterial growth around the disks.

In vivo testing was carried out in CF_1, ♂, 18–20-g mice. The compounds were administered subcutaneously or orally. Two doses of compound were given on both day 1 and 2, with the bacterial challenge given intraperitoneally between doses on day 1. Doses protecting 50% of the animals were calculated by the method of Reed and Muench[8] as milligrams per kilogram based on individual doses and not total compound given.

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7-Acetylthioetiojervanes, New Antialdosterone Agents

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Several 12α-etiojervane derivatives have been found to be potent antialdosterone agents when administered parenterally.† In an attempt to increase the oral efficacy of these compounds, structural modifications were made paralleling those successfully employed for a similar purpose in the steroidal spirolactones, a class of well-known antialdosterone agents.[2,3] The particular molecular changes reported in this communication involve the introduction of the acetylthio group at the 7α position of the etiojervane molecule.

Direct chloranil oxidation of the unsaturated ketone **1d** led to mixtures containing moderate amounts of the desired dienone 2d as evidenced by the uv spectrum of the crude product. Purification of this material was hampered by partial decomposition of the dienone 2d on chromatography. The partially purified material was saponified at room temperature to yield the desired alcohol 2a. The structure of this dienone was unambiguously shown by its spectral profile.

The lack of stability of the unsaturated ketone **1** was also seen when acid-catalyzed ketal[1a] or enol ether formation was attempted. One example of this instability was the intractable mixture produced by room temperature treatment of the unsaturated ketone **1a** with trimethyl orthoformate and acid. This instability presumably reflects the steric strain imposed by the cis-fused C/D hydrindan system. However, enol ether formation was successfully achieved by use of dimethoxypropane. Subsequent mild oxidation with dichlorodicyanoquinone (DCDCQ) gave the desired dienone in an overall yield of 40% from the starting unsaturated ketone 1a.

The addition of thiolacetic acid was found to proceed readily at room temperature in contrast to the standard procedure which employs prolonged heating.[2] The product was a stable, crystalline product. Assignment of the 7α configuration to the acetylthio group was done in analogy to the spirolactone work, since no obvious steric factors would favor addition of the thiolacetic acid to the β face of the molecule. Corroboration of this configurational assignment was obtained from the nmr spectrum of this molecule and its comparison to the spectrum of the corresponding spirolactone.[4]

Derivatives with a 17α substituent were also prepared. The starting 4,6-diene 2e could be obtained in 15% yield by direct crystallization of the product resulting from dehydrobromination of the 17α-acetoxy-2,4-dibromo derivative **6** (X = Br). This type of elimination has been previously noted with steroidal 2,4-dibromo 3-ketones.[5] The dienone 2e could also be obtained by direct oxidation of the 3-keto-Δ^4 compound 1e with DCDCQ but again this procedure gave only a low yield of product. Saponification of the 17α-acetate 2e gave the dienone **2b**. Alternatively, the 17α-acetate **2b** was synthesized by room temperature DCDCQ oxidation of the enol ether **4b**. Thiolacetic addition proceeded smoothly to give the desired thiolacetate **5b** (or 5e) from the 17α-hydroxy derivative **2b** (or its acetate 2e) (Scheme I).

The 17-keto dienone 2c was prepared from the 17α-hydroxy derivative **2b** by oxidation of the corresponding alcohol with chromic acid. To circumvent possible isomerization of the labile 13-methyl group,[6] the 7α-thiol acetate was prepared by oxidation of either the 17β- or the 17α-hydroxy derivative 5a or **5b**, reactions which proceeded without complication to give the desired 17-keto derivative **5c**.

Sequential treatment of the 1,4-diene 3d with *N*-bromosuccinimide and magnesium oxide or treatment of the 4,6-dienone 2a with DCDCQ failed to give discernible amounts of the 1,4,6-trienone corresponding to **2**.

Biological Activity. The compounds were assayed in a 4-hr test in adrenalectomized rats treated with desoxycorticosterone acetate (DCA) and isotonic saline solution prior to the administration of the test compound.[7] The enhancement of potencies produced in the spirolactones by these chemical modifications were not observed in the etiojervanes (see Table I). In the present case the potent activity of the parent compound 1a (450%) was decreased sharply by either introduction of the 6,7 double bond (50%, 2a) or the 7-thioacetate (52%, 5a). Of the compounds possessing parenteral activity, only **5a** was active orally but at a reduced (13%) potency.

Experimental Section‡

3-Methoxy-12α-etiojerva-3,5-dien-17β-ol (4a) (Procedure A). *p*-Toluenesulfonic acid (50 mg) was added to a solution of 1.53 g of

†See ref 1a for the chemical synthesis of these compounds. The biological activities will be described in a forthcoming paper by L. M. Hofmann and W. F. Johns. See also ref 1b.

‡Optical rotations were determined in chloroform, infrared spectra in chloroform, ultraviolet spectra in methanol, and nmr spectra in deuteriochloroform. Nmr's are reported with tetramethylsilane as an internal standard ($\Delta\nu$ = 0) as recorded on a Varian A-60 spectrometer. These data were supplied by Dr. J. W. Ahlberg and staff for which we thank them.