continued for 24 hr at 30°. The reaction mixture was poured on H_2O (400 ml) and the product isolated by filtration.

Method H. **2-[β-(4-Pyridyl)ethyl]- and 2-[β-(2-Quinolyl)ethyl]-1,2,3,4,6,7,12,12a-octahydropyrazino[2',1':6,1]pyrido[3,4-*b*]indole (14 and 15).** A solution of 4-vinylpyridine or 2-vinylquinoline (11 mmol), glacial AcOH (10 mmol), and **6** (10 mmol) in 95% EtOH (150 ml) was refluxed for 20 hr. The reaction mixture was evaporated to dryness. Residue was taken in H_2O (20 ml) and made alkaline with 2 *N* NaOH to give **14** or **15**.

Method I. **2-(*p*-Fluorophenacyl)-1,2,3,4,6,7,12,12a-octahydropyrazino[2',1':6,1]pyrido[3,4-*b*]indole (20).** *p*-Fluorophenacyl bromide (5 mmol) in dry THF was added slowly to a stirred solution of **6** (10 mmol) in dry THF. Stirring was continued for 24 hr at 30°. The I·HBr which separated was filtered and the filtrate on concentration gave **20**.

Method J. **1,2,3,4,6,7,12,12a-Octahydropyrazino[2',1':6,1]pyrido[3,4-*b*]indole-2-carboxamide (40).** A mixture of **6** (5 mmol), KCNO (7.5 mmol), concentrated HCl (1 ml), and absolute EtOH (15 ml) was refluxed for 30 hr. The reaction mixture was evaporated to dryness and triturated with H_2O to yield **40**.

Method K. **β-[2-(1,2,3,4,6,7,12,12a-Octahydropyrazino-[2',1':6,1]pyrido[3,4-*b*]indolyl)]propionic Acid (34).** A mixture of **33** (3 mmol), aqueous NaOH (6 ml of 1 *N*), and EtOH (15 ml) was boiled for 45 min. The reaction mixture was evaporated to dryness. The residue was taken in H_2O (8 ml) and just neutralized with 5 *N* HCl to give **34**.

Method L. **2-(γ-Hydroxypropyl)-1,2,3,4,6,7,12,12a-octahydropyrazino[2',1':6,1]pyrido[3,4-*b*]indole (39).** A solution of **33** (3 mmol) in dry THF (60 ml) was added to a stirred suspension of $LiAlH_4$ (12 mmol) in dry Et_2O (150 ml). The reaction mixture was heated at 50–55° for 4 hr and worked up as usual to give **39**.

Method M. **2-(1,2,3,4,6,7,12,12a-Octahydropyrazino[2',1':6,1]pyrido[3,4-*b*]indolyl)amidine (41).** A mixture of **6** (5 mmol), *S*-methylthiourea sulfate (5 mmol), and EtOH (95%, 25 ml)–H_2O (4 ml) was refluxed for 20 hr. The reaction mixture was evaporated to dryness; residue was taken in H_2O (20 ml), basified with aqueous NH_4OH, and extracted with EtOAc. The EtOAc extracts were dried over anhydrous Na_2SO_4 and concentrated to give **41**.

Method N. A mixture of the appropriate hydroxy compound (2 mmol), Ac_2O (4 mmol), and dry C_5H_5N (10 ml) was stirred for 14 hr at 30°. The reaction mixture was dried *in vacuo*, residue washed with H_2O, and the compound isolated by extraction with EtOAc.

Method O. A solution of **29** (3.3 mmol) in dry THF (40 ml) was added slowly to the appropriate Grignard reagent (10 mmol) in dry Et_2O (150 ml). The reaction mixture was stirred and heated at 50–55° for 4 hr. The complex was decomposed with saturated NH_4Cl, the organic layer separated, and the aqueous layer extracted with EtOAc. The EtOAc extracts were dried on anhydrous Na_2SO_4 and evaporated to give the products.

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Epimeric Forms of Quaternary Derivatives of Atropine

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The values of log *K* for the *R* and *S* forms of *N*-ethyl-, *N*-*n*-propyl-, and *N*-*n*-butylhyoscyaminium iodides, described in the previous paper,[1] gave calculated values for the racemate (Table I) which differed appreciably from values obtained experimentally for the racemates by others.[2] With our own samples of *N*-ethyl- and *N*-*n*-propylatropinium iodides, we obtained estimates of log *K* which were closer to the values calculated from our results with the separate enantiomers, but the values for *N*-ethylatropinium iodide were still not as close to the calculated values as would be expected simply from our experience of the errors attached to the biological tests.

With these compounds there is the possibility, with substituents other than methyl, of obtaining two epimeric forms, one with the substituent axial and the other with the substituent equatorial. Although the alkylation of tropine, pseudotropine, and some related compounds has been found to give products with the substituent mainly equatorial,[3,4] the axial products are formed as well to some extent. The ratio of equatorially substituted to axially substituted products varied from 9:1 to 7:3. It seemed possible, therefore, that the various samples of these alkylated tropine derivatives differed in epimeric composition. This might account for some of the discrepancies in Table I. Nador[5] did not observe any special differences between the pharmacological properties of some epimers of this type (though he did with aralkyl derivatives), but it was remarkable that the results in Table I show reasonable agreement for atropine and atropine methiodide, where there are no complications due to the existence of epimers.

We have, therefore, examined the nmr spectra of some of these quaternary salts of atropine in order to assess the relative proportions of epimers present and we have also investigated the effects of recrystallization on epimeric composition and on biological activity.

Experimental Section

Spectra were obtained with a Varian HA 100 instrument with the samples dissolved in D_2O. The substances examined were (recrystallized) specimens of *N*-methyl-, *N*-ethyl-, and *N*-*n*-propylatropinium iodides and of the *R* and *S* enantiomers of *N*-ethyl- and *N*-*n*-propylhyoscyaminium iodides. A crude preparation of

Table I. Affinities of Quaternary Derivatives of Atropine for Postganglionic Acetylcholine Receptors of the Guinea-Pig Ileum[a]

	Values of log *K*		
	Previous paper	Green, *et al.*[2]	Calcd
Atropine sulfate	9.007		9.080
Atropine methiodide	9.454	9.53	9.370
Atropine ethiodide	8.239 8.198	8.82	8.494
Atropine *n*-propyl iodide	7.244	7.88	7.224
Atropine *n*-butyl iodide		7.45	6.813

[a] Values of log *K* from Table IE of the previous paper[1] are compared with values obtained by Green, *et al.*,[2] and values calculated from the results for the separate enantiomers.

N-ethylatropinium iodide, obtained by removing all the solvent from the reaction mixture after atropine had been treated with excess ethyl iodide, was also examined and a similar crude preparation obtained by treating atropine with CD_3I.

Results and Discussion

The *N*-methyl signals, relative to internal DSS, were sharp singlets and the chemical shifts were 312 and 304 Hz (δ 3.12 and 3.04; the peaks were of equal height) for the methiodide, 302 Hz (δ 3.02) for the ethyl iodide, and 304 Hz (δ 3.04) for the *n*-propyl iodide. With the recrystallized specimens of *N*-ethyl- and *N*-*n*-propylatropinium iodides, there was only one signal in this region so it appears that these samples contain only one epimeric form. With the crude product from the ethylation of atropine, however, there were two *N*-methyl signals at 300 and 294 Hz (δ 3.00 and 2.94; the latter superimposed on a signal due to DSS; Figure 1) and the peak heights were approximately in the ratio of 6:1, respectively. It has been shown[4] that with tropines the higher field *N*-methyl signals are associated with equatorial *N*-methyl groups, so the crude ethiodide obtained from the ethylation of atropine appears to contain about one-seventh of the epimer with the ethyl group in the axial position. This component is removed by recrystallization.

The crude product obtained by treating atropine with CD_3I showed *N*-methyl signals at 312 and 304 Hz (δ 3.12 and 3.04; Figure 2) whose peak heights were approximately

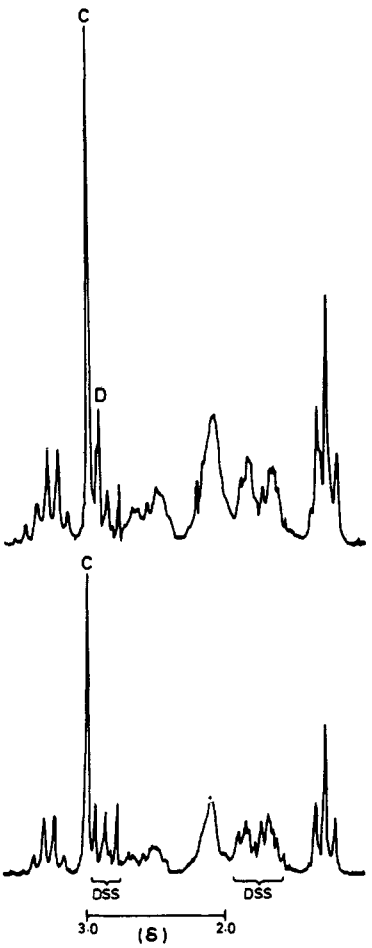

Figure 1. Part of the nmr spectra of crude atropine ethiodide (above) and recrystallized material (below). Peaks C and D correspond to *N*-methyl signals from the axial and equatorial *N*-methyl groups, respectively.

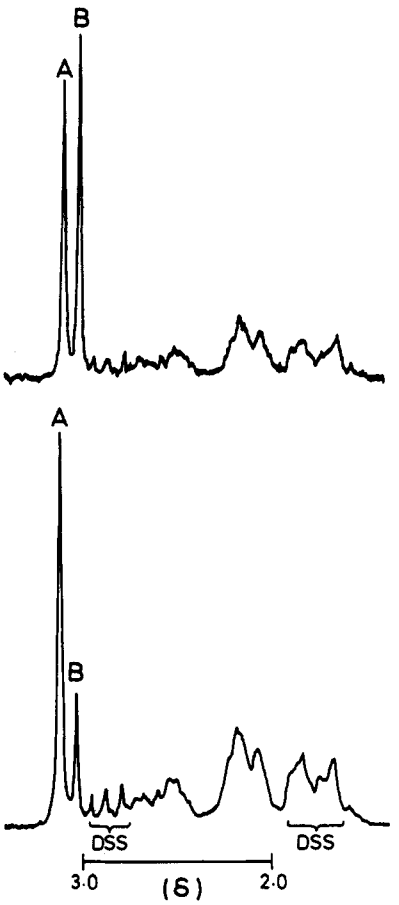

Figure 2. Part of the nmr spectra of atropine methiodide (above) and crude atropine CD_3 iodide (below). Peaks A and B correspond to *N*-methyl signals from the axial and equatorial *N*-methyl groups, respectively. The signal B is approximately one-third of A and indicates the proportion of methyl groups in the equatorial position, *i.e.*, the proportion of axial attack by CD_3I.

in the ratio 3:1, which indicates that there is a lower proportion of equatorial attack in this reaction compared with ethylation.

The discrepancies in Table I might, therefore, be accounted for by supposing that the epimers with the alkyl groups in the axial position had higher affinity than those in which it is equatorial. The crude *N*-ethylatropinium iodide, estimated to contain one-seventh of the axially ethylated epimer, was tested on the guinea-pig ileum and the estimate of log *K* was 8.627 ± 0.030 (six estimates), compared with values of 8.239 and 8.198 for samples which should be purely equatorially ethylated. The axially ethylated epimer appeared therefore to be more soluble and much more active. From the results with the pure equatorially ethylated epimer and with the mixture containing one-seventh of the axial ethylated epimer, the value of log *K* for the pure axially ethylated epimer would be expected to be about 9.29. By concentrating the mother liquors and discarding the less soluble equatorial epimer it was possible to obtain material, mp 177–180°, which from the nmr spectra appeared to be about 85% pure axially ethylated epimer (Figure 3) and this had log *K* 9.265 ± 0.019 (six estimates).

The nmr spectra of the samples of the *R* and *S* forms of *N*-*n*-propylhyoscyaminium iodide showed that in these the *n*-propyl group was purely equatorially substituted. The sample of *N*-ethyl-(*R*)-hyoscyaminium iodide was likewise purely equatorially ethylated but the sample of the *S* enantiomer was found to contain about 10% of the axially

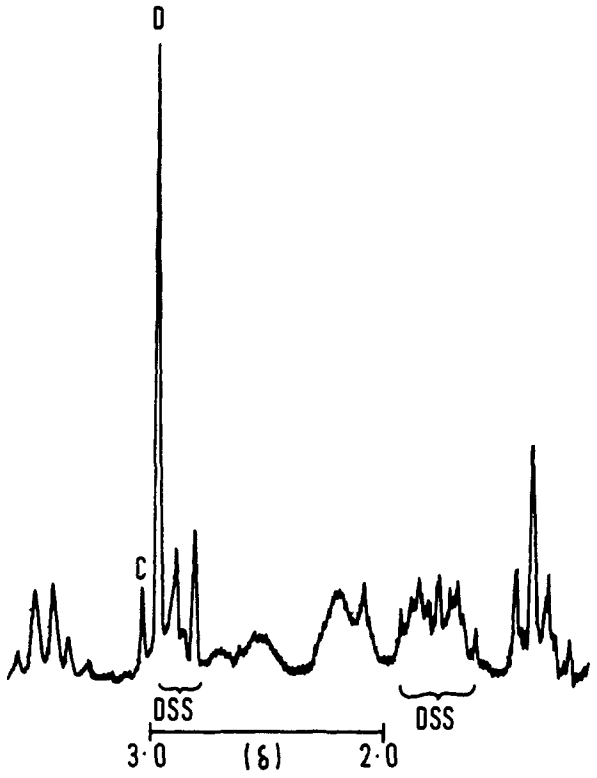

Figure 3. Part of the nmr spectra of the more soluble form of atropine ethiodide, obtained from mother liquors. Peaks C and D are *N*-methyl signals from axial and equatorial *N*-methyl groups, respectively, and indicate that about 85% of the ethyl group is axial (*cf.* Figure 1).

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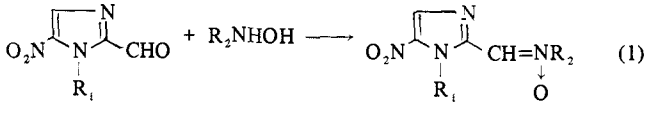
The disclosure[2,3] of the antibacterial and antiprotozoal properties of derivatives of 1-alkyl-5-nitroimidazole-2-carboxaldehydes led us to extend our work in the nitrone area to include nitrone derivatives of these aldehydes (a portion of this work has been described[4]).

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.



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.