

Table VI. 2-Substituted 9-Dialkylaminoalkyl-9H-imidazo[1,2-a]benzimidazoles (12)

Compd	(CH ₂) _n	R	X	Mp, °C	Yield, %	Formula
12b	CH ₂ CH ₂	CH ₃	Cl	190–192	70	C ₁₉ H ₁₉ N ₄ Cl·HBr
c	CH ₂ CH ₂	CH ₃	Br	218–219	65	C ₁₉ H ₁₉ N ₄ Br·HBr
e (HBr)	CH ₂ CH ₂	C ₂ H ₅	Cl	197–199	75	C ₂₁ H ₂₃ N ₄ Cl·HBr
f	CH ₂ CH ₂	C ₂ H ₅	Br	187–189	78	C ₂₁ H ₂₃ N ₄ Br·HBr
12a	CH ₂ CH ₂	CH ₃	H	260–263 dec	65	C ₁₉ H ₂₀ N ₄ ·2HCl
b	CH ₂ CH ₂	CH ₃	Cl	271–272 dec	71	C ₁₉ H ₁₉ N ₄ Cl·2HCl
c	CH ₂ CH ₂	CH ₃	Br	268–270 dec	62	C ₁₉ H ₁₉ N ₄ Br·2HCl
d	CH ₂ CH ₂	C ₂ H ₅	H	268–269 dec ^a	70	C ₂₁ H ₂₄ N ₄ ·2HCl
e (2HCl)	CH ₂ CH ₂	C ₂ H ₅	Cl	258–259 dec	68	C ₂₁ H ₂₃ N ₄ Cl·2HCl
f	CH ₂ CH ₂	C ₂ H ₅	Br	245–248 dec	65	C ₂₁ H ₂₃ N ₄ Br·2HCl
g	CH ₂ CH ₂ CH ₂	CH ₃	H	266–267 dec	92	C ₂₀ H ₂₂ N ₄ ·2HCl
h	CH ₂ CH ₂ CH ₂	CH ₃	Cl	261–263 dec	62	C ₂₀ H ₂₁ N ₄ Cl·2HCl
i	CH ₂ CH ₂ CH ₂	CH ₃	Br	249–250 dec	56	C ₂₀ H ₂₁ N ₄ Br·2HCl

^aReported,¹¹ mp 205–206°.

line containing 0.5% tragacanth. The ED₅₀ and LD₅₀ were calculated by the method of Litchfield and Wilcoxon.¹⁴

The analgetic effects were assayed by the following two methods. The first was antagonism of acetic acid induced writhing method.¹³ Thirty minutes after drug administration, 0.2 ml of 0.6% acetic acid solution was injected ip. Writhing was checked from 5 to 15 min after acetic acid injection. The analgetic ED₅₀ was estimated as the dose which reduced writhing number to 50% of that of control animals over a period of 10 min. The second was a modification of the hot plate method by Eddy, *et al.*¹⁵ The apparatus, which was reported by Takagi, *et al.*,¹⁶ was used, and the bath temperature was kept constantly at 55 ± 1°. Animals showed a reaction time from 5 to 10 sec. The pain responses were determined before and 15, 30, 45, and 60 min after drug administration. The dose which increased the reaction time to 75% longer than that observed before drug administration was considered to be analgetic. The ED₅₀ was calculated as the dose which caused analgesia in 50% of the animals.

The taming effect was measured by the antifighting behavior method. The paired animals were stimulated by an electric current (60 V DC, 1 mA, 3 cps) which was applied through a grid to the feet of the animals, according to the method of Tedeschi, *et al.*¹⁷ The ED₅₀ was determined by the ability to abolish the fighting behavior in 50% of the paired mice. Median lethal dose (LD₅₀) was determined 1 week after the administration.

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Synthesis and Antiprotozoal Activity of Methylnitro Derivatives of 2,2'-Biimidazole

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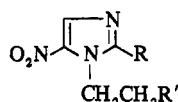
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Several mono- and dinitro derivatives of *N*-methyl- and *N,N'*-dimethyl-2,2'-biimidazole have been synthesized. These compounds were tested for *in vitro* and *in vivo* activity against *Trichomonas vaginalis*, *Entamoeba histolytica*, and *Giardia muris*. Most compounds exhibited good *in vitro* activity against *T. vaginalis*. Only a few were active *in vivo*. The most active compounds were 1,1'-dimethyl-5-nitro-2,2'-biimidazole (12) and 1,1'-dimethyl-5,5'-dinitro-2,2'-biimidazole (14).

Many compounds presently used as drugs for the treatment of protozoal infections contain a nitroimidazole moiety. A few examples are metronidazole¹ (1), tinidazole² (2), flunidazole³ (3), and nitrimidazine⁴ (4). Since it has previously been observed that molecular doubling can lead to enhancement of activity⁵ we decided to investigate the 2,2'-biimidazole⁶ ring structure 5. In this paper we wish to report the synthesis of several nitro derivatives of 5 and their respective antitrichomonas and antiamoebic activities. 2,2'-Biimidazole (5) was selectively monomethylated with

1 equiv of Me₂SO₄ to yield predominantly 6. Some dimethylated biimidazole 7 was always present as a side product regardless of how much excess 2,2'-biimidazole was used. In excess Me₂SO₄ 7 was formed exclusively.

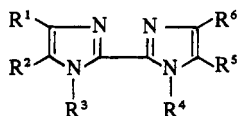
The nitration of 5 was carried out in AcOH-Ac₂O. If only 1 equiv of nitric acid was used the major product was 4-nitro-2,2'-biimidazole (8). Using an excess of HNO₃ the major product is the symmetrical dinitro compound 9. It is of interest that nitration of 5 with an equivalent of a sulfonitric mixture also gave 8. Contrary to Lehmstedt and Zumstein⁷



- 1, R = CH₃; R' = OH
 2, R = CH₃; R' = SO₂CH₂CH₃
 3, R = 4-F-C₆H₄; R' = OH
 4, R = H; R' = morpholino

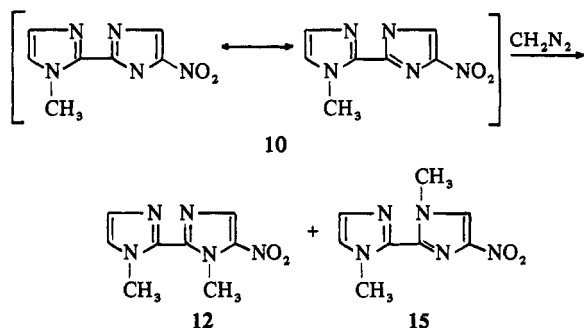
we found no evidence of nitramine formation under these conditions; Novikov, *et al.*,⁸ have also recently confirmed our observations.

Nitration of the monomethylated biimidazole 6 with 1 equiv of HNO₃ unexpectedly yielded 1-methyl-4'-nitro-2,2'-biimidazole (10). It was originally anticipated that nitration of the substituted imidazole ring would predominate since it is more activated for electrophilic substitution. The presence of the nitro group in the unsubstituted imidazole ring was determined by allowing 10 to react with excess CH₂N₂. Two isomeric dimethyl biimidazoles (12 and 15) were obtained. This result necessitates that the nitro group in 10 must be in the unsubstituted imidazole ring. Had the nitro group been in the substituted imidazole ring then only 13 or 15 would have been obtained.



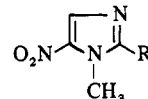
- 5, R¹ = H; R² = H; R³ = H; R⁴ = H; R⁵ = H; R⁶ = H
 6, R¹ = H; R² = H; R³ = CH₃; R⁴ = H; R⁵ = H; R⁶ = H
 7, R¹ = H; R² = H; R³ = CH₃; R⁴ = CH₃; R⁵ = H; R⁶ = H
 8, R¹ = NO₂; R² = H; R³ = H; R⁴ = H; R⁵ = H; R⁶ = H
 9, R¹ = NO₂; R² = H; R³ = H; R⁴ = H; R⁵ = H; R⁶ = NO₂
 10, R¹ = H; R² = H; R³ = CH₃; R⁴ = H; R⁵ = H; R⁶ = NO₂
 11, R¹ = H; R² = NO₂; R³ = CH₃; R⁴ = H; R⁵ = H; R⁶ = NO₂
 12, R¹ = H; R² = NO₂; R³ = CH₃; R⁴ = CH₃; R⁵ = H; R⁶ = H
 13, R¹ = H; R² = NO₂; R³ = CH₃; R⁴ = CH₃; R⁵ = H; R⁶ = NO₂
 14, R¹ = H; R² = NO₂; R³ = CH₃; R⁴ = CH₃; R⁵ = NO₂; R⁶ = H
 15, R¹ = NO₂; R² = H; R³ = CH₃; R⁴ = CH₃; R⁵ = H; R⁶ = H
 16, R¹ = NO₂; R² = H; R³ = CH₃; R⁴ = CH₃; R⁵ = H; R⁶ = NO₂
 17, R¹ = H; R² = NO₂; R³ = CH₃; R⁴ = H; R⁵ = H; R⁶ = H
 18, R¹ = NO₂; R² = H; R³ = CH₃; R⁴ = H; R⁵ = H; R⁶ = H
 19, R¹ = NO₂; R² = H; R³ = CH₃; R⁴ = H; R⁵ = H; R⁶ = NO₂

Nitration of 6 with excess HNO₃ yielded the expected dinitro biimidazole 11. Nitration of the dimethylated biimidazole 7 with 1 equiv of HNO₃ gave 12. The presence of the first nitro group in 12 now apparently acts to deactivate the 5' position of the second biimidazole ring since further reaction of 12 with HNO₃ resulted in nitration at both 5' (14) and 4' (13) in a ratio of 1:2.



Mononitration of the dimethylated biimidazole 7 could conceivably occur at either the 4 or the 5 position of the ring. It was therefore necessary to prove that the nitro group of 12 is attached to C₅ and not C₄. Likewise it was necessary to show that the structural assignments of the isomeric dinitro compounds (13 and 14) were also correct. In order to verify these assignments 13 and 14 were independently synthesized starting with the known compound 1-methyl-2-

formyl-5-nitroimidazole (20). This aldehyde 20 was converted to the oxime 21 which was then refluxed with SOCl₂ in ether to yield the nitrile 22. Compound 22 was then allowed to react at room temperature with ethanol and base to give the imidate 23. The hydrochloride of 23 was condensed with 1 mole of aminoacetaldehyde dimethyl acetal to yield 1-methyl-2-*N*-(β-dimethoxyethyl)amidino-5-nitroimidazole (24) which was then cyclized to the biimidazole 17 by reaction with sulfuric acid.



- 20, R = CHO
 21, R = CH=NOH
 22, R = CN
 23, R = CNH(OCH₂CH₃)
 24, R = CNH[NHCH₂CH(OCH₃)₂]

Nitration of 17 yielded a dinitro compound which must be 1-methyl-5,4'-dinitro-2,2'-biimidazole (11). This compound was identical in all respects with the dinitro compound obtained from exhaustive nitration of 6. The reaction of 11 with diazomethane gave two isomeric compounds which must in turn be 13 and 14. Compounds 13 and 14 could be differentiated by analysis of their respective nmr spectra. The spectrum of 13 showed two 3H peaks at 4.16 and 4.44 (s) for the methyl groups and two 1H peaks at 7.88 and 8.06 (s) for the aromatic protons. The spectrum of 14 indicated a symmetrical molecule with only two narrow peaks at 4.32 and 8.11 (s) of relative intensity 3:1 and consequently it must have the 5,5'-dinitrobiimidazole structure. These data were sufficient to allow unambiguous assignment of structure to all of the nitrobiimidazoles synthesized. For rapid determination of the position of a nitro group in a biimidazole ring it could be used. It was found that a 4-nitro derivative characteristically has an absorption at 750–760 cm⁻¹ whereas this band is shifted to 740–750 cm⁻¹ for the 5-nitro derivatives.

It is interesting to note that methylation of 8 with 1 equiv of diazomethane occurs primarily in the nitrated imidazole ring to yield 17 and 18 in a 2:1 ratio. 18 is better obtained by treating 8 with CH₃I in DMF. Nitration of 18 with an excess of HNO₃ gave in turn 19. Reaction of the dinitrobiimidazole 9 with 1 equiv of diazomethane gave primarily 19. The same reaction with excess diazomethane yielded primarily 13 and 14 and only trace amounts of 16. Compound 16 was obtained in high yield (95%) by allowing 9 to react with excess MeI in DMF.

Biological Methods. The following methods were used to determine *in vitro* and *in vivo* antiprotozoal activity.

(A) *In Vitro* Antiprotozoal Activity on *Entamoeba histolytica* Strain Meah and on *Trichomonas vaginalis* Strain M. The serial dilution method in fluid medium was used. Twenty mg of substance was dissolved with 0.5 ml of 95% EtOH and 1 drop of sterile Tween 80, 4.5 ml of distilled water was then added, and the resulting suspension was diluted serially at a ratio of 1:1.5 in two different culture media for *E. histolytica* and *T. vaginalis*.

(1) *Entamoeba histolytica*. Tests on *E. histolytica* were carried out in 2 ml of Pavlova's monophasic medium, as modified by Jones,⁹ with the addition of 5% sterile horse serum, and a few milligrams of sterile rich starch. An inoculum of 10,000 protozoa/ml was used. Activity was expressed as the minimal sterilizing concentration (MSC) in μg/ml, determined by direct microscopic examination (125X and 500X) of the culture sediment after a 48-hr incubation at 37°.

Table I. Methylnitro-2,2'-biimidazoles

No.	Yield, %	Mp, °C	Uv absorption, λ , m μ		Crystn solvent	Empirical formula	Analyses ^a
			Neutral ^b	Acid ^c			
6	61	146-147			Petr ether	C ₇ H ₈ N ₄	C, H, N
7	74	113-114			Petr ether	C ₈ H ₁₀ N ₄	C, H, N
8	67	>320	268-329	274	DMF	C ₆ H ₅ H ₅ O ₂	C, H, N
9	39	>360	225-324	225-324	DMF	C ₆ H ₅ N ₆ O ₄	C, H, N
10	49	294-295	269-328	274	DMF-H ₂ O	C ₇ H ₇ N ₅ O ₂	C, H, N
11	40	206-208	227-344	227-344	MeOH	C ₇ H ₈ N ₆ O ₄	C, H, N
12	83	179-180	235-238	228-305	MeOH	C ₈ H ₉ N ₅ O ₂	C, H, N
13	50	173-174	223-323	223-323	EtOH	C ₈ H ₉ N ₆ O ₄	C, H, N
14	48	174-175	225-336	225-336	MeOH	C ₈ H ₈ N ₆ O ₄	C, H, N
15	30	176-178	264-316	d	MeOH	C ₈ H ₉ N ₅ O ₂	C, H, N
16	95	292-296	223-316	223-316	DMF-H ₂ O	C ₈ H ₈ N ₆ O ₄	C, H, N
17	58	252-254	240-350	234-315	Dioxane-H ₂ O	C ₇ H ₇ N ₅ O ₂	C, H, N
18	30	251-253	270-327	274	MeOH	C ₇ H ₇ N ₅ O ₂	C, H, N
19	50	283-293	225-322	225-322	MeOH	C ₇ H ₆ N ₆ O ₄	C, H, N

^aAnalytical values were within $\pm 0.4\%$ of the calculated figures. ^bThe neutral uv absorptions were determined in MeOH. ^cThe acidic solutions were obtained by adding two drops of 0.5 N H₂SO₄ to the solutions used for the neutral measurements. ^dThree absorption maxima at: 224, 259, and 294 m μ .

(2) *Trichomonas vaginalis*. Tests on *T. vaginalis* were carried out in 2 ml of Difco fluid thioglycollate medium with the addition of 10% sterile horse serum, 1000 IU of penicillin, and 1000 μ g/ml of streptomycin. An inoculum of 30,000 protozoa/ml was used. Activity was expressed as the minimal sterilizing concentration in μ g/ml, determined by direct microscopic examination (125X and 500X) of 0.2 ml of the culture after a 72-hr incubation at 37°.

(B) *In Vivo Antiprotozoal Activity on E. histolytica, T. vaginalis, and Giardia muris*. (1) *E. histolytica* Meah (rat intestinal amoebiasis). An infection method was used similar to that described by Jones in 1946, and later modified by de Carneri.¹⁰ Young Wistar specific pathogen free albino rats (30-35 g) were used. The rats were fed a water diet with 0.1% vitamin C for 16 hr before the test, and on a Taylor¹¹ diet for 2 days previously. The animals were infected by intracecal injection of a suspension of trophozoites of *E. histolytica* Meah (250,000, 48-hr trophozoites per rat), containing 3% Wilson mucin. The compounds to be tested were suspended in 10% gum arabic and administered by gavage 24, 36, 48, 54, and 72 hr after infection. Each rat was given a total of 0.5 ml per 30 g body wt. Gum arabic alone was given to the control animals. The rats were killed on the fifth day after infection, and the mucosa and fecal content of the cecum were examined. The effect of treatment was

judged by allotting each animal from 0 to 5 points, according to the following scoring system:¹² score 0, no amoebae, no ulceration; score 1, from 1 to 20 amoebae per slide of feces; no amoebae in the scrapings from the cecal wall; no ulceration; score 3, amoebae found in microscopic lesions of the cecal mucosa; score 4, macroscopic amoebic lesions in the cecal wall; score 5, over half the cecum ulcerated by amoebic lesions. The results are expressed as ED₅₀ in mg/kg, calculated on the number of animals totally protected (i.e., with score 0).

(2) *T. vaginalis*. Specific pathogen free CE male albino mice, weighing an average of 20 g, were infected subcutaneously in the upper lateroventral area with 0.5 ml of a 24-hr CPLM culture containing 400,000 protozoa.¹³ The compounds were suspended in 10% gum arabic and administered by gavage. Each animal received 0.5 ml per 20 g body wt, at 6-, 30-, and 54-hr intervals after infection. Therapeutic activity was evaluated by examining for the presence of the abscess which forms at the site of inoculation in treated animals, as well as the presence of live protozoa in the abscess. Results are expressed as ED₅₀ in mg/kg.

(3) *G. muris*. Specific pathogen free CE male albino mice, weighing an average of 20 g, were infected orally by gavage with a suspension of *Giardia* in saline soln (10,000 per mouse) taken from the small intestine of infected mice.

Table II. *In Vitro* and *In Vivo* Activity of Methylnitro-2,2'-biimidazoles

No.	<i>In vitro</i> (MSC)		<i>In vivo</i> (ED ₅₀)		
	<i>E. histolytica</i> , μ g/ml	<i>T. vaginalis</i> , μ g/ml	<i>E. histolytica</i> , 1 \times 5 oral dosage, mg/kg	<i>T. vaginalis</i> , 1 \times 3 oral dosage, mg/kg	<i>G. muris</i> , 1 \times 6 oral dosage, mg/kg
6	100	100			>150
7	100	>100			>150
8	50	50		150	>150
9	25	4		100	>150
10	6.2	3.2		100	150
11 ^a	4	0.25	>25	4 < ED ₅₀ > 20	16
12	0.39	0.10	>45	7	>150
13	1.03	0.07	30	13	150
14 ^b	6.2	0.3	30	2.3	25
15	12.5	0.7	45	100	>150
16	100	0.3		100	>150
17	33	0.26		100	150
18	3.3	1.2	>45	20 < ED ₅₀ < 40	>150
19	16	0.3		100	>150
1	5	0.3	20	2.1	60

^aThis compound proved toxic when given orally at 100 mg/kg twice a day for 3 days. ^bAcute toxicity when given orally in the rat and mouse: LD₅₀ 400 mg/kg.

The compounds to be tested were suspended in 10% gum arabic and administered by gavage, each animal receiving 0.5 ml per 20 g body wt, twice daily for three consecutive days starting from the third day after infection. The median effective dose, determined on the sixth day of infection, was based on the proportion of animals free of protozoa.

Biological Results. Almost all of the compounds tested showed some *in vitro* activity against *E. histolytica* and *T. vaginalis*. When tested *in vivo* the majority of compounds were still effective against *T. vaginalis*. However, against *E. histolytica* only a few were active *in vivo*. Only compound 14 showed *in vivo* activity comparable to metronidazole (1). No compounds were found to be more active than 1 against *E. histolytica* or *T. vaginalis*. However, against *G. muris* two compounds (11 and 14) showed better activity than 1.

From the results listed in Table II it is clear that a nitro group is essential for activity. Compounds 6 and 7 which lack a nitro group show no activity. For a 5-mononitro derivative it appears that activity (both *in vivo* and *in vitro*) is enhanced if both imidazole rings are methylated (13 vs. 17). However for the 4-mononitro derivatives this trend is reversed. The monomethylated compound 18 shows more activity than the corresponding dimethylated compound 15. The observation that 18, which has a 4-nitro group was more active than 17, in which the nitro group is at position 5, is unusual for the imidazoles.^{1,4} However, it should be noted that the 5-nitro compound 12 showed the best *in vivo* activity against *T. vaginalis*.

The introduction of a second nitro group improves activity. With the exception of 16 all dinitro derivatives were more active than their corresponding mononitro analogs. Against *T. vaginalis* at least one of the nitro groups must be in position 5.

Experimental Section

1-Methyl-2,2'-biimidazole (6). A suspension of 4.06 g (0.03 mole) of 2,2'-biimidazole in 60 ml of EtOH and 7 ml of 20% NaOH (0.046 mole) was heated to affect solution, and then 3.2 ml (0.023 mole) of Me₂SO₄ was slowly added. The soln was heated under reflux for 7 hr then cooled and filtered. The filtrate was vacuum condensed to dryness, and the residue taken up with 50 ml of H₂O and neutralized with 8% HCl. After filtration, 2.72 g of 2,2'-biimidazole was recovered. The filtrate was extracted with CHCl₃ and CHCl₃ in turn extracted with 8% NaOH. The alkaline soln was neutralized with 8% HCl and extracted with 150 ml of CHCl₃. The residue obtained after removal of solvent *in vacuo* was crystd from cyclohexane to give 0.9 g of the product (61%), mp 146–147°.

1,1'-Dimethyl-2,2'-biimidazole (7). To a boiling soln of 2.039 g (0.015 mole) of 2,2'-biimidazole in 40 ml of EtOH and 11.5 ml of 20% NaOH (0.07 mole), was added dropwise 6.6 ml (0.068 mole) of Me₂SO₄. The soln was further refluxed for 7 hr then cooled, filtered, and neutralized with 8% HCl. After filtration and evaporation to dryness, the residue was dissolved in 30 ml of H₂O, and the soln extracted with 150 ml of CHCl₃. The CHCl₃ extracts were washed with 30 ml of 8% NaOH soln to remove the monomethyl derivative 6, then dried, and evapd to dryness. The residue was crystd from cyclohexane to give 1.8 g (74%) of the product, mp 113–114°.

4-Nitro-2,2'-biimidazole (8). To a soln of 6.7 g (0.05 mole) of 2,2'-biimidazole in 80 ml of AcOH and 40 ml of Ac₂O was added 1.05 ml (0.025 mole) of 99% HNO₃. The soln was heated at 65° and another 1.05 ml (0.025 mole) of 99% HNO₃ was dropped in. The mixture was allowed to stand at 65° for 8 hr. The solvents were removed *in vacuo*, and the residue was treated with 100 ml of ice-cold H₂O. The soln was neutralized with NaHCO₃, and the solid collected and crystd from DMF to give 6 g (67%) of the product, mp 360°.

4,4'-Dinitro-2,2'-biimidazole (9). To a soln of 3.35 g (0.025 mole) of 2,2'-biimidazole in 40 ml of AcOH and 20 ml of Ac₂O at 80° was added dropwise 2.81 ml (0.065 mole) of 99% HNO₃. The soln was stirred at 90–100° for 8 hr. The reaction mixture was cooled, and the solid filtered and crystd from DMF to give 2.2 g (39%) of the product, mp 360°.

1-Methyl-4'-nitro-2,2'-biimidazole (10). Starting from 6, the method was the same as described for compound 8: yield 49%, mp 294–295° after crystn from DMF-H₂O.

5,4'-Dinitro-1-methyl-2,2'-biimidazole (11). Starting from 6 the method was the same as described for compound 9: yield 40%, mp 206–208° after crystn from MeOH.

1,1'-Dimethyl-5-nitro-2,2'-biimidazole (12). To a stirred solution of 1.62 g (0.01 mole) of 7 in 20 ml of AcOH and 10 ml of Ac₂O, 0.52 ml (0.0125 mole) of 99% HNO₃ was added. The temp was raised to 75–80° and another 0.52 ml of 99% HNO₃ was carefully added. This temp was maintained for 8 hr. The soln was evaporated to dryness and 20 ml of H₂O was poured into the vessel. After neutralizing with NaHCO₃ the solid was collected and crystd from MeOH to give 1.8 g (83%) of the product, mp 179–180°.

1,1'-Dimethyl-4,5'-dinitro-2,2'-biimidazole (13). Method A. To a stirring solution of 5 g (0.024 mole) of 12 in 40 ml of AcOH and 20 ml of Ac₂O at 100°, 10 ml (0.24 mole) of 99% HNO₃ was added dropwise. The temp was maintained for 10 hr. After cooling, the soln was evaporated to dryness. Column chromatography on silica gel using Et₂O-petr ether-Et₂NH (150:50:5) gave 1.5 g (25%) of the product, mp 173–174° and 0.75 g (12.5%) of 14, mp 174–175°.

Method B. To a suspension of 2.24 g (0.01 mole) of 9 in 100 ml of DMF, 0.04 mole of 3% CH₂N₂ solution in Et₂O was added dropwise. After standing 10 hr at room temp, the excess CH₂N₂ was decomposed with AcOH, and the solvent was removed *in vacuo*. Column chromatography of the residue gave 1.26 g (50%) of the product, mp 174–175°, and 0.4 g (16%) of 14.

1,1'-Dimethyl-5,5'-dinitro-2,2'-biimidazole (14). To a suspension of 2.24 g (0.01 mole) of 9 in 100 ml of dioxane, was added 0.04 mole of a 3% CH₂N₂ solution in Et₂O. After standing 10 hr at room temp, the excess CH₂N₂ was decomposed with AcOH. The mixture was filtered and evaporated to dryness, and the residue was treated with 20 ml of boiling Me₂CO. After cooling, the nearly pure product was collected by filtration: 0.82 g (32%), mp 174–175°. The filtrate contained a mixture of 13 and 14 which was separated by column chromatography on silica gel with Et₂O-petr ether-Et₂NH (150:50:5) to give another 0.4 g (16%) of 14 and 0.1 g (4%) of 13.

1,1'-Dimethyl-4-nitro-2,2'-biimidazole (15). Starting from 8 the method was the same as described for compound 13, method B: yield 30%, mp 176–178°. From this reaction a 10% yield of 12 is also obtained.

1,1'-Dimethyl-4,4'-dinitro-2,2'-biimidazole (16). To a stirring solution of 6.7 g (0.03 mole) of 9 in 300 ml of DMF and 100 ml of 1 N NaOH, 6.2 ml (0.1 mole) of CH₃I was added, and the reaction mixture was allowed to stand for 24 hr at room temp. The precipitate which formed was collected and crystd from dioxane or from DMF-H₂O to give 7.2 g (95%) of the product, mp 292–296°.

1-Methyl-5-nitro-2,2'-biimidazole (17). Method A. The method was the same as described for compound 14 except that the starting material was 8 and a stoichiometric quantity of CH₂N₂ was used: yield 58%, mp 252–254°.

Method B. To a solution of 5 g (0.035 mole) of 1-methyl-5-nitroimidazolyl carboxaldehyde in 100 ml of EtOH was added a solution of 3 g (0.09 mole) of hydroxylamine dissolved in EtOH. During the addition the temp was maintained at 5–10°. The reaction mixture was then refluxed 0.5 hr. On cooling, 4 g (84.5%) of the oxime 21 precipitated, mp 234–239°.

To a suspension of 4 g (0.0235 mole) of 21 in 50 ml of Et₂O was added 22 ml of SOCl₂ at such a rate that a gentle reflux was maintained. The mixture was then refluxed for 10 hr, during which time the suspension became a solution. The soln was evaporated to dryness, the solid was extracted with Et₂O, and the ether washings were concentrated to yield a residue which was crystd from petr ether to give 3.2 g (90%) of 1-methyl-2-cyano-5-nitroimidazole (22), mp 78–80°.

To a stirring solution of 1.52 g (0.01 mole) of 22 in 15 ml of EtOH, was added 0.1 g (0.01 mole) of potassium *tert*-butoxide. After 2.5 hr, the precipitate which formed was collected and washed with EtOH to yield 1.2 g (61%) of 23, mp 80–82°.

A solution of 2.3 g (0.01 mole) of the hydrochloride of 23, 1.05 g (0.01 mole) of aminoacetaldehyde dimethyl acetal, and 30 ml of MeOH was refluxed for 10 hr. The solvent was then removed *in vacuo*, and the residue dissolved in H₂O. The pH was adjusted to 10 with NH₄OH. The solid which precipitated was collected and crystd from H₂O to give 1.3 g (51%) of 1-methyl-2-*N*-(β-dimethoxyethyl)amidino-5-nitroimidazole (24), mp 108–111°.

While stirring and cooling, 2.57 g (0.01 mole) of 24 was added to 5.2 g of concd H₂SO₄. A colorless solution was obtained. The soln was then cautiously added to 25 ml of ice-cold water, and the

pH adjusted to 5–6 with 20% NaOH. The precipitate was collected by filtration to yield 1.7 g (89%) of 17, mp 252–254°.

1-Methyl-4-nitro-2,2'-biimidazole (18). Method A. To a solution of 1.79 g (0.01 mole) of 8 in 50 ml of DMF and 5 ml of 2 *N* NaOH was added, dropwise and at room temp, 0.63 ml (0.011 mole) of CH₃I. The soln was kept at 60° for 15 hr, then evaporated to dryness. The residue was crystd from dioxane and recrystd from MeOH to give 0.58 g (30%) of the product, mp 252–253°.

Method B. Compound 18 (20%) together with compound 17 (40%) was obtained by treating 8 with a stoichiometric amount of CH₂N₂ in DMF. The mixture of 18 and 17 was separated by column chromatography on silica gel using CHCl₃–MeOH–NH₃ (190:10:1).

4,4'-Dinitro-1-methyl-2,2'-biimidazole (19). While stirring and heating to 70°, 1.25 ml (0.03 mole) of 99% HNO₃ was added to a soln of 3.99 g (0.02 mole) of 18 in 100 ml of AcOH and 50 ml of Ac₂O. The temp was raised to 85° and maintained for 8 hr. The soln was then evaporated to dryness and the solid crystd from a satd aqueous soln of NaHCO₃ to give 2.6 (50%) of the Na salt of 19. On acidifying 19 was obtained, mp 283–293°.

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Analgetics Based on the Pyrrolidine Ring. 6

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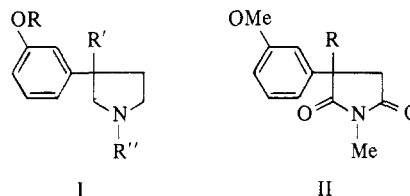
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Various *m*-(3-alkyl-1-methyl-3-pyrrolidinyl)phenols and *m*-[3-alkyl-1-(*p*-R-phenethyl)-3-pyrrolidinyl]-phenols were synthesized in order to examine the effects of 3-branched-alkyl substituents on the analgetic activity. In the 1-Me series, going from the earlier 3-*n*-Pr (profadol) to 3-CHMe₂, 3-CH₂Me, 3-CH₂CHMe₂, or 3-CH₂CMe₃ increased activity and the activity:toxicity ratio. In the 1-(CH₂)₂C₆H₄-*p*-R series superiority of such branched 3-substitutions was not clear. Various *m*-(1-methyl-3-pyrrolidinyl)-phenols and 3-(*m*-methoxyphenyl)-1-methylpyrrolidines were synthesized to study the effects of unsaturated and variously oxygenated groups in place of the earlier 3-*n*-Pr. All were deleterious, albeit clear activity was shown with 3-CH₂CH=CH₂, 3-COEt, and 3-CH₂COMe.

In previous papers of this series we have described numerous, variously substituted pyrrolidines of the general formula I, some relationships between structure and analgetic (antimechanoeptive) activity in rats, and some evidence of varying degrees of separation of narcotic-like, physical-dependence liability from analgetic action.¹⁻³ Of these structures, profadol (I, R = H; R' = Pr; R'' = Me) and its enantiomers have been studied extensively in animals^{4-12,†} and the racemate has been evaluated in man.^{13-18,‡}

One purpose of this paper is to report the effect of substitution of certain branched chains in the 3 position (I, R') on the agonist activity of profadol and related compounds (e.g., I, R'' = Me or (CH₂)₂C₆H₄-*p*-OH) as determined by the mechanoceptive test in rats employed¹⁻³ heretofore. A second purpose of the paper is to describe the preparation and analgetic (antimechanoeptive) activities of a limited number of pyrrolidines with unsaturated or oxygenated groups in the 3 position.



Chemistry. The basic step in the synthesis of several of the compounds with unsaturated or oxygenated groups in the 3 position has been treatment of 2-(*m*-methoxyphenyl)-*N*-methylsuccinimide (II, R = H) with an appropriate halide (e.g., allylbromide) to effect the required 3-substitution (e.g., II, R = CH₂CH=CH₂). Subsequent reduction with lithium aluminum hydride afforded the pyrrolidine. Otherwise the synthetic procedures used are for the most part based on those described in the preceding papers. The physical properties of the compounds prepared are listed in Tables I and II and details of the methods used are given below.

Pharmacology. Acute lethal toxicities and analgetic (antimechanoeptive) potencies were estimated in young male rats by the intraperitoneal route as described earlier.¹⁹ The antinociceptive potencies are based reciprocally on

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