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

Compd	$(CH_2)_n$	R	X	Mp, °C	Yield, %	Formula
12b	CH_2CH_2	CH_3	Cl	190–192	70	$C_{19}H_{19}N_4Cl \cdot HBr$
c	CH_2CH_2	CH_3	Br	218–219	65	$C_{19}H_{19}N_4Br \cdot HBr$
e (HBr)	CH_2CH_2	C_2H_5	Cl	197–199	75	$C_{21}H_{23}N_4Cl \cdot HBr$
f	CH_2CH_2	C_2H_5	Br	187–189	78	$C_{21}H_{23}N_4Br \cdot HBr$
12a	CH_2CH_2	CH_3	H	260–263 dec	65	$C_{19}H_{20}N_4 \cdot 2HCl$
b	CH_2CH_2	CH_3	Cl	271–272 dec	71	$C_{19}H_{19}N_4Cl \cdot 2HCl$
c	CH_2CH_2	CH_3	Br	268–270 dec	62	$C_{19}H_{19}N_4Br \cdot 2HCl$
d	CH_2CH_2	C_2H_5	H	268–269 dec[a]	70	$C_{21}H_{24}N_4 \cdot 2HCl$
e (2HCl)	CH_2CH_2	C_2H_5	Cl	258–259 dec	68	$C_{21}H_{23}N_4Cl \cdot 2HCl$
f	CH_2CH_2	C_2H_5	Br	245–248 dec	65	$C_{21}H_{23}N_4Br \cdot 2HCl$
g	$CH_2CH_2CH_2$	CH_3	H	266–267 dec	92	$C_{20}H_{22}N_4 \cdot 2HCl$
h	$CH_2CH_2CH_2$	CH_3	Cl	261–263 dec	62	$C_{20}H_{21}N_4Cl \cdot 2HCl$
i	$CH_2CH_2CH_2$	CH_3	Br	249–250 dec	56	$C_{20}H_{21}N_4Br \cdot 2HCl$

[a] Reported,[11] mp 205–206°.

line containing 0.5% tragacanth. The ED_{50} and LD_{50} 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 ED_{50} 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 ED_{50} 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 ED_{50} was determined by the ability to abolish the fighting behavior in 50% of the paired mice. Median lethal dose (LD_{50}) 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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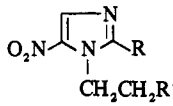
Several mono- and dinitro derivatives of *N*-methyl- and *N,N′*-dimethyl-2,2′-biimidazole have been synthesized. These compounds were tested for *in vitro* and *in vivo* activity against *Trichomonas vaginalis*, *Entamoeba histolytica*, and *Giardia muris*. Most compounds exhibited good *in vitro* activity 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 metronidazole[1] (1), tinidazole[2] (2), flunidazole[3] (3), and nitrimidazine[4] (4). Since it has previously been observed that molecular doubling can lead to enhancement of activity[5] we decided to investigate the 2,2′-biimidazole[6] 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 Me_2SO_4 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 Me_2SO_4 7 was formed exclusively.

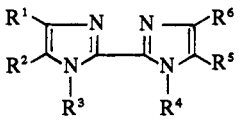
The nitration of 5 was carried out in AcOH–Ac_2O. If only 1 equiv of nitric acid was used the major product was 4-nitro-2,2′-biimidazole (8). Using an excess of HNO_3 the major product is the symmetrical dinitro compound 9. It is of interest that nitration of 5 with an equivalent of a sulfonitric mixture also gave 8. Contrary to Lehmstedt and Zumstein[7]



1, R = CH_3; R' = OH
2, R = CH_3; R' = $SO_2CH_2CH_3$
3, R = 4-F-C_6H_4; R' = OH
4, R = H; R' = morpholino

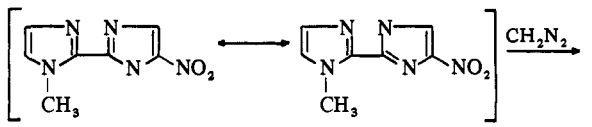
we found no evidence of nitramine formation under these conditions; Novikov, *et al.*,[8] have also recently confirmed our observations.

Nitration of the monomethylated biimidazole **6** with 1 equiv of HNO_3 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 CH_2N_2. 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.

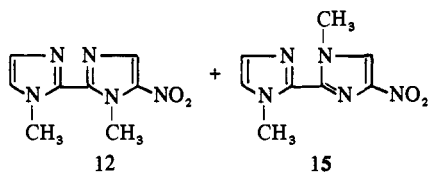


5, R^1 = H; R^2 = H; R^3 = H; R^4 = H; R^5 = H; R^6 = H
6, R^1 = H; R^2 = H; R^3 = CH_3; R^4 = H; R^5 = H; R^6 = H
7, R^1 = H; R^2 = H; R^3 = CH_3; R^4 = CH_3; R^5 = H; R^6 = H
8, R^1 = NO_2; R^2 = H; R^3 = H; R^4 = H; R^5 = H; R^6 = H
9, R^1 = NO_2; R^2 = H; R^3 = H; R^4 = H; R^5 = H; R^6 = NO_2
10, R^1 = H; R^2 = H; R^3 = CH_3; R^4 = H; R^5 = H; R^6 = NO_2
11, R^1 = H; R^2 = NO_2; R^3 = CH_3; R^4 = H; R^5 = H; R^6 = NO_2
12, R^1 = H; R^2 = NO_2; R^3 = CH_3; R^4 = CH_3; R^5 = H; R^6 = H
13, R^1 = H; R^2 = NO_2; R^3 = CH_3; R^4 = CH_3; R^5 = H; R^6 = NO_2
14, R^1 = H; R^2 = NO_2; R^3 = CH_3; R^4 = CH_3; R^5 = NO_2; R^6 = H
15, R^1 = NO_2; R^2 = H; R^3 = CH_3; R^4 = CH_3; R^5 = H; R^6 = H
16, R^1 = NO_2; R^2 = H; R^3 = CH_3; R^4 = CH_3; R^5 = H; R^6 = NO_2
17, R^1 = H; R^2 = NO_2; R^3 CH_3; R^4 = H; R^5 = H; R^6 = H
18, R^1 = NO_2; R^2 = H; R^3 = CH_3; R^4 = H; R^5 = H; R^6 = H
19, R^1 = NO_2; R^2 = H; R^3 = CH_3; R^4 = H; R^5 = H; R^6 = NO_2

Nitration of **6** with excess HNO_3 yielded the expected dinitro biimidazole **11**. Nitration of the dimethylated biimidazole **7** with 1 equiv of HNO_3 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 HNO_3 resulted in nitration at both 5' (**14**) and 4' (**13**) in a ratio of 1:2.

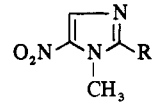


10



12 **15**

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 C_5 and not C_4. 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 $SOCl_2$ 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 = $CNH(OCH_2CH_3)$
24, R = $CNH[NHCH_2CH(OCH_3)_2]$

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 ir 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 CH_3I in DMF. Nitration of **18** with an excess of HNO_3 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, mμ Neutral[b]	Acid[c]	Crystn solvent	Empirical formula	Analyses[a]
6	61	146–147			Petr ether	$C_7H_8N_4$	C, H, N
7	74	113–114			Petr ether	$C_8H_{10}N_4$	C, H, N
8	67	>320	268–329	274	DMF	$C_6H_5H_5O_2$	C, H, N
9	39	>360	225–324	225–324	DMF	$C_6H_4N_6O_4$	C, H, N
10	49	294–295	269–328	274	DMF-H_2O	$C_7H_7N_5O_2$	C, H, N
11	40	206–208	227–344	227–344	MeOH	$C_7H_6N_6O_4$	C, H, N
12	83	179–180	235–238	228–305	MeOH	$C_8H_9N_5O_2$	C, H, N
13	50	173–174	223–323	223–323	EtOH	$C_8H_8N_6O_4$	C, H, N
14	48	174–175	225–336	225–336	MeOH	$C_8H_8N_6O_4$	C, H, N
15	30	176–178	264–316	*d*	MeOH	$C_8H_9N_5O_2$	C, H, N
16	95	292–296	223–316	223–316	DMF-H_2O	$C_8H_8N_6O_4$	C, H, N
17	58	252–254	240–350	234–315	Dioxane-H_2O	$C_7H_7N_5O_2$	C, H, N
18	30	251–253	270–327	274	MeOH	$C_7H_7N_5O_2$	C, H, N
19	50	283–293	225–322	225–322	MeOH	$C_7H_6N_6O_4$	C, H, N

[a] Analytical values were within ±0.4% of the calculated figures. [b] The neutral uv absorptions were determined in MeOH. [c] The acidic solutions were obtained by adding two drops of 0.5 *N* H_2SO_4 to the solutions used for the neutral measurements. [d] Three absorption maxima at: 224, 259, and 294 mμ.

(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 μg/ml of streptomycin. An inoculum of 30,000 protozoa/ml was used. Activity was expressed as the minimal sterilizing concentration in μg/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 Taylor[11] 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 ED_{50} 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 subscutaneously 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 ED_{50} 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.	*In vitro* (MSC) *E. histolytica*, μg/ml	*In vitro* (MSC) *T. vaginalis*, μg/ml	*In vivo* (ED_{50}) *E. histolytica*, 1 × 5 oral dosage, mg/kg	*In vivo* (ED_{50}) *T. vaginalis*, 1 × 3 oral dosage, mg/kg	*In vivo* (ED_{50}) *G. muris*, 1 × 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

[a] This compound proved toxic when given orally at 100 mg/kg twice a day for 3 days. [b] Acute toxicity when given orally in the rat and mouse: LD_{50} 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 Me_2SO_4 was slowly added. The soln was heated under reflux for 7 hr then cooled and filtered. The filtrate was vacuum concd to dryness, and the residue taken up with 50 ml of H_2O and neutralized with 8% HCl. After filtration, 2.72 g of 2,2'-biimidazole was recovered. The filtrate was extracted with $CHCl_3$ and $CHCl_3$ in turn extracted with 8% NaOH. The alkaline soln was neutralized with 8% HCl and extracted with 150 ml of $CHCl_3$. 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 Me_2SO_4. 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 H_2O, and the soln extracted with 150 ml of $CHCl_3$. The $CHCl_3$ 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 Ac_2O was added 1.05 ml (0.025 mole) of 99% HNO_3. The soln was heated at 65° and another 1.05 ml (0.025 mole) of 99% HNO_3 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 H_2O. The soln was neutralized with $NaHCO_3$, 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 Ac_2O at 80° was added dropwise 2.81 ml (0.065 mole) of 99% HNO_3. 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–H_2O.

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 Ac_2O, 0.52 ml (0.0125 mole) of 99% HNO_3 was added. The temp was raised to 75–80° and another 0.52 ml of 99% HNO_3 was carefully added. This temp was maintained for 8 hr. The soln was evaporated to dryness and 20 ml of H_2O was poured into the vessel. After neutralizing with $NaHCO_3$ 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 Ac_2O at 100°, 10 ml (0.24 mole) of 99% HNO_3 was added dropwise. The temp was maintained for 10 hr. After cooling, the soln was evaporated to dryness. Column chromatography on silica gel using Et_2O–petr ether–Et_2NH (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% CH_2N_2 solution in Et_2O was added dropwise. After standing 10 hr at room temp, the excess CH_2N_2 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% CH_2N_2 solution in Et_2O. After standing 10 hr at room temp, the excess CH_2N_2 was decomposed with AcOH. The mixture was filtered and evaporated to dryness, and the residue was treated with 20 ml of boiling Me_2CO. 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 Et_2O–petr ether–Et_2NH (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 CH_3I 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–H_2O 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 CH_2N_2 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 Et_2O was added 22 ml of $SOCl_2$ 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 Et_2O, 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 H_2O. The pH was adjusted to 10 with NH_4OH. The solid which precipitated was collected and crystd from H_2O 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 H_2SO_4. 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 CH_3I. 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 CH_2N_2 in DMF. The mixture of 18 and 17 was separated by column chromatography on silica gel using $CHCl_3$–MeOH–NH_3 (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% HNO_3 was added to a soln of 3.99 g (0.02 mole) of 18 in 100 ml of AcOH and 50 ml of Ac_2O. 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 $NaHCO_3$ 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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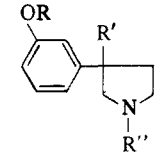
Various *m*-(3-alkyl-1-methyl-3-pyrrolidinyl)phenols and *m*-[3-alkyl-1-(*p*-R-phenethyl)-3-pyrrolidinyl]-phenols were synthesized in order to examine the effects of 3-branched-alkyl substituents on the analgetic activity. In the 1-Me series, going from the earlier 3-*n*-Pr (profadol) to 3-$CHMe_2$, 3-CHEtMe, 3-CH_2CHMe_2, or 3-CH_2CMe_3 increased activity and the activity:toxicity ratio. In the 1-$(CH_2)_2C_6H_4$-*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-$CH_2CH{=}CH_2$, 3-COEt, and 3-CH_2COMe.

In previous papers of this series we have described numerous, variously substituted pyrrolidines of the general formula I, some relationships between structure and analgetic (antimechanoceptive) 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 $(CH_2)_2C_6H_4$-*p*-OH) as determined by the mechanoceptive test in rats employed[1-3] heretofore. A second purpose of the paper is to describe the preparation and analgetic (antimechanoceptive) activities of a limited number of pyrrolidines with unsaturated or oxygenated groups in the 3 position.



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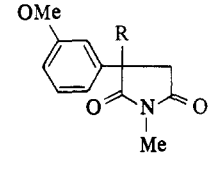

II

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 = $CH_2CH{=}CH_2$). 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 (antimechanoceptive) 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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