microbiological assays. This work was supported by grants CA 06516, CA 17718, and CA 10914, awarded by the National Cancer Institute, Department of Health, Education and Welfare.

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Antifilarial Agents. 1,2-Cyclobutanediamines as Analogues of Diethylcarbamazine. Status of Structure–Activity Relationships among Diethylcarbamazine Analogues

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cis- and *trans*-1,2-cyclobutanediamines bearing appropriate *N*-methyl and *N*-acyl substituents were prepared as analogues of diethylcarbamazine (DEC). None displayed activity against *Litomosoides carinii* in the gerbil despite substantial structural and stereochemical similarities to the parent drug. The inactivity of these drugs is rationalized in terms of eclipsed pharmacophore configurations and the increased population of unfavorable rotational conformations made possible by the exocyclic position of both pharmacophores. To provide perspective for these conclusions, the literature on DEC analogues is briefly summarized and structure–activity data are discussed in terms of critical structural factors associated with microfilaricidal activity. Generalizations on structural principles governing activity are advanced which encompass test results for the large majority of DEC analogues.

In the preceding papers of this series[1,2] we presented several groups of active diethylcarbamazine (DEC, 1) analogues derived from 3-aminopyrrolidine and various bridged piperazines. These compounds were characterized by relatively rigid skeletons that held the pharmacophoric groups in spatial orientations that closely resembled those of DEC. Our rationalization for their biological activity was based on this similarity. We now report the synthesis of a group of *cis*- and *trans*-1,2-diaminocyclobutane derivatives in which the pharmacophores are again held by the ring system in orientations generally comparable to those of DEC but among which no antifilarial activity has been found. In this paper we attempt to rationalize these results in terms of conformational characteristics of the cyclobutanediamine derivatives. We also attempt to summarize the essential characteristics of active DEC analogues and to assess the validity of the resulting description by comparison with the accumulated results of structure–activity studies in this area.[1-20]

Results and Discussion

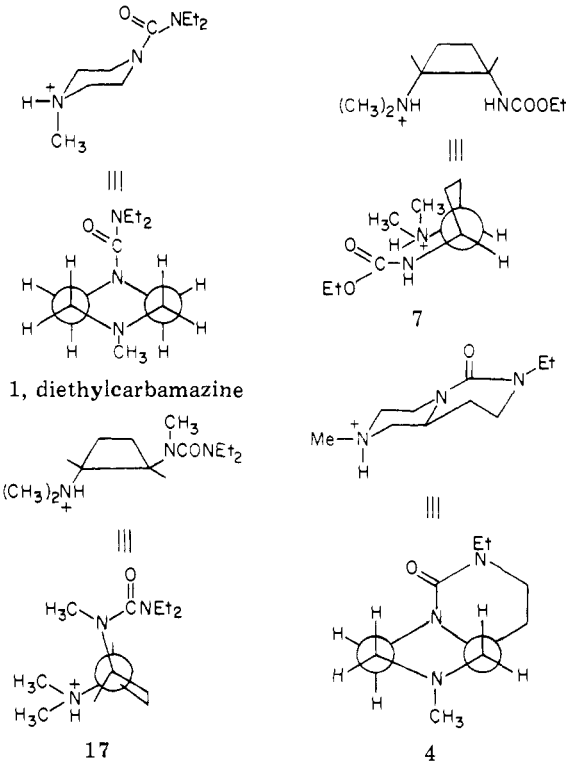
Table I presents the antifilarial assay results obtained from DEC and the substituted *cis*- and *trans*-1,2-diaminocyclobutanes (**7**, **10–17**). The procedure used to evaluate the compounds against *Litomosoides carinii* in the gerbil was described in our first publication.[1] None of the new compounds caused a reduction of microfilaremia or had an effect on the number of adult worms in this test system. The cyclobutane derivatives of this series are similar to previously described active DEC analogues in several significant respects. The spacing of the pharmacophores in the cis derivative **7** (2.7 Å between N atoms) is very similar to that of DEC (2.9 Å) according to Dreiding models. Although the spacing in the trans series is larger (3.5 Å) compounds with greater internitrogen separations but similar functional groups have provided reduced but readily demonstrable antifilarial activity according to prior work.[8] Both acyl groups used in this series have previously provided highly active derivatives,[1,7] and the lipid–water partition properties of the members of this series should not differ substantially from those of known active groups, according to estimates derived from partition additivity concepts.[21] A rigid orientation of the pharmacophores has proven favorable previously.[1-3,9] The lack of activity in the 1,2-diaminocyclobutane series must therefore be attributed to more subtle stereochemical differences.

Table I. Chemical and Antifilarial Properties of 1,2-Cyclobutane Analogues of DEC

Compd	Formula[b]	Mp, °C	Microfilaria count, day 0 (range)	Antifilarial act.,[a] % of day 0 count (std dev)				
				Day 1	Day 3	Day 7	Day 9	Day 14
1	DEC		132 (55-194)	6 (±0.2)	0.1 (±0.1)		29 (±11)	43 (±12)[c]
7	$C_9H_{18}N_2O_2 \cdot C_4H_4O_4$	102-106	214 (134-276)	127 (±21)	59 (±68)	169 (±69)	154 (±57)	199 (±51)
10	$C_7H_{14}N_2O_2 \cdot 1.5HCl$	150-153	95 (11-207)	107 (±14)	163 (±52)	148 (±47)	182 (±68)[d]	153 (±63)
11	$C_{10}H_{18}N_2O_4$	127-129	192 (18-497)	189 (±82)	236 (±64)	201 (±90)	240 (±122)	208 (±112)
12	$C_8H_{16}N_2O_2 \cdot HCl$	134-137	164 (81-222)	101 (±6)	113 (±14)	122 (±22)	99 (±9)	151 (±21)
14	$C_9H_{18}N_2O_2 \cdot C_4H_4O_4$	93-96	99 (67-116)	150 (±42)	160 (±61)	150 (±47)	95 (±33)	165 (±46)
15	$C_9H_{18}N_2O_2 \cdot C_4H_4O_4$	107.5-111.5	312 (13-752)	96 (±32)	190 (±53)	158 (±66)	133 (±33)	213 (±148)
16	$C_{11}H_{23}N_3O \cdot C_6H_8O_7$	133-135	236 (70-403)	106 (±26)	113 (±26)	111 (±37)	137 (±65)	161 (±72)
17	$C_{12}H_{25}N_3O \cdot C_4H_4O_4$	128-129	353 (3-1184)	185 (±24)	99 (±52)	174 (±99)	159 (±135)	100 (±56)

[a] Drugs administered at dosages of 25, 50, 100, and 200 mg/kg on days 0, 1, 2, and 3, respectively. See ref 1 for further details. [b] Elemental analyses for carbon, hydrogen, and nitrogen were within ±0.4% of the theoretical value for these compounds. [c] This figure refers to day 11 rather than day 14. [d] This figure refers to day 10 rather than day 9.

Chart I



Two conformational characteristics distinguish the cyclobutane derivatives from earlier active analogues. First, the substituents on the carbon–carbon bond connecting the pharmacophores are eclipsed, whereas all prior analogues with two-carbon links between pharmacophores have staggered (or near-staggered[2]), gauche relationships. To illustrate this point, Chart I presents perspective views and Newman projections of DEC (1), cis derivative 7, and trans derivative 17. In the case of 7, the pharmacophores eclipse each other; in 17, each pharmacophore is eclipsed by a hydrogen. DEC illustrates the usual gauche conformation. Second, both carbon–nitrogen bonds connecting the pharmacophores to the supporting carbon skeleton are exocyclic to the cyclobutane ring. This permits free rotation of both pharmacophores about these bonds. The rotational freedom allows the two groups to interact freely, and CPK molecular models indicate that hydrophobic or polar contacts are easily achieved. Most rotameric conformations, in which the two pharmacophores interact, differ sharply from those associated with active analogues. We therefore attribute the inactivity of 7 and 10–17 to high populations of unfavorable conformers made possible by the eclipsed relationships of the pharmacophores and their increased rotational freedom.

The highly active DEC analogue of Anand and coworkers[3,9] (4, Chart I), whose bicyclic structure permits very few rotameric isomers, further demonstrates the importance of these conformational factors. The pharmacophores are fixed in a gauche relationship. The urea carbonyl and associated atoms are essentially immobilized relative to the basic nitrogen of the piperazine ring, and neither pharmacophore can interact with the other. Compound 4 was reported to be substantially more active than DEC by one group[3,9] and to be equally active by another.[10]

The DEC pharmacophores have been incorporated into a variety of structures by various investigators, and it is instructive to try to interpret the bioassay results from these studies in terms of a unified hypothesis. Consideration of published structure–activity studies on DEC analogues[1-20] discloses the following characteristics.

1. Two pharmacophores are necessary—a tertiary aliphatic amine and an oxygen dipole usually provided as part of an amide moiety. Additional functional groups in the molecule are inactivating.

2. Significant activity falls within a narrow range of lipid–water partition properties.

3. The interpharmacophore separation found with DEC (2.9 Å) has provided the best compounds, but much larger separations are permissible.

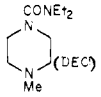
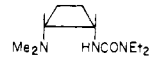
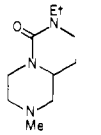
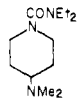
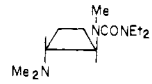
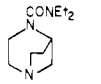
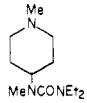
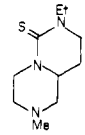
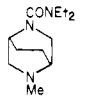
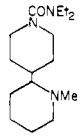
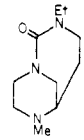
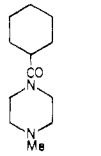
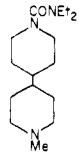
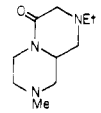
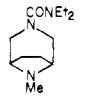
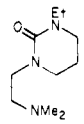
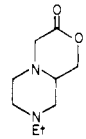
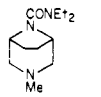
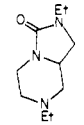
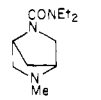
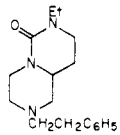
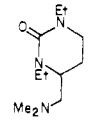
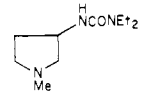
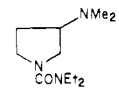
4. Optimum activity is achieved with conformationally restricted structures; an important, but not fully defined, angular relationship between the pharmacophores exists.

5. Considerable variety in the carbon structure supporting the two pharmacophores is permissible and either one may be exocyclic to a ring.

Table II lists examples of the various types of DEC analogues. No effort has been made to include all active analogues, but each skeletal class is shown. Specific compounds were selected because they had pharmacophores most similar to those of DEC; in most studies, these compounds were also the most active. The activity ranking is somewhat arbitrary as three host animal systems with variable end points were used by different investigators and not all reports present quantitative assay data. However, all investigations used DEC as the positive standard and some basis for intercomparison was therefore available. Compounds from our previous work[1,2] were assigned a +++ rating if they reduced microfilaremia levels to 1% or less of pretreatment levels and a ++ for reduction to the 1–10% range; the dose schedule was the same as that used for DEC.

In the first reports of Hewitt et al.[7] on DEC and its piperazine-derived congeners, they established that the two pharmacophores were necessary for significant activity. High activity clearly required that the amine be tertiary and *N*-methyl was optimum, but some activity was re-

Table II. Summary of DEC Analogues and Their Activities against *L. carinii* Microfilaria

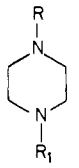
Active analogues				Active analogues				Inactive analogues		
No.	Structure	Act.[a]	Ref	No.	Structure	Act.[a]	Ref	No.	Structure	Ref
1		+++	7	31		++	8, 11, 14	7		*b*
4		+++	3, 9	32		+	8	17		*b*
23		+++	2	33		+	8	40		3
24		+++	1	34		+	8	41		5
25		+++	13	35		+	8	42		6
26		++	1	36		+	4	43		6
27		++	1					44		6
28		++	1	37		+	3	45		4
29		++	2	38		+	12			
30		++	2	39		+	8			

[a] Ability to reduce blood levels of *L. carinii* microfilaria in the gerbil or cotton rat. +++ = activity comparable or superior to that of DEC; ++ = high activity but clearly less than that of DEC; + = detectable activity but not comparable to that of DEC. [b] This paper.

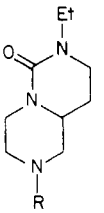
tained if the basic nitrogen was substituted with amidino (**3**). However, in bicyclic ring system **10**, replacement of *N*-methyl with amidino destroyed activity,[3] and amidino was associated only with inactivity in another series.[18] Other functional groups associated with the basic nitrogen (e.g., a second amine,[3,19] double bond,[7] hydroxyl,[3,7] carbethoxy,[3] phenyl[7]) destroyed activity. Although the ureido moiety, as provided by the *N,N*-diethylcarbamyl group of DEC, gave the optimum nonbasic pharmacophore, a carbamate group formed by *N*-carbethoxylation could replace it (e.g., **2**) with only a small loss of activity. These results tended to guide the majority of subsequent studies, but it has been demonstrated that the amide group, as in **25**, can provide potent DEC-like activity and that a sulfuramide, as in **39**, is compatible with low but significant activity. This latter result is important because it demonstrates that the necessary component of the nonbasic pharmacophore is an oxygen dipole (supplied in **39** by an S–O bond) rather than the carbon–oxygen double bond, as provided by all other N-substituents. That an oxygen dipole is the key feature is supported by the inactivity of **40**, the sulfur analogue of **4**.

The inactivity of bicyclic analogues **42** and **43** can be attributed to the presence of two basic centers in each. There are no published examples of definitive activity being associated with molecules with more functional groups than the two required pharmacophores.

Discussion of the lipophilic character of the compounds in Table II must be limited to generalizations because no comparative experimental partition data are available. However, most of the derivatives in Table II can be considered roughly isolipophilic (exceptions discussed below) because they contain the same polar functional groups and between 9 and 11 lipophilic methyl, methylene, or methine groups. The original work leading to DEC[7] clearly established that adding or subtracting as little as one or two methylene groups to the substituents on either nitrogen of the piperazine ring caused loss or precipitous decreases in activity. This sensitivity to small changes in alkyl substituents was even more pronounced in the structure–activity studies evolving from bicyclic analogue **4**.[3] Replacing the ethyl of **4** by methyl, or exchanging the methyl of **4** with H or ethyl, caused a 30-fold decrease in potency. Thus we conclude that for optimum activity,

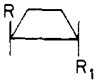


1, R = $CONEt_2$; $R_1 = CH_3$
2, R = CO_2Et; $R_1 = CH_3$
3, R = CO_2Et; $R_1 = C(=NH)NH_2$

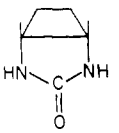


4, R = CH_3
5, R = $CH_2CHOHC_6H_5$
6, R = CH_2CO_2Et

7, R = $N(CH_3)_2$; $R_1 = NHCO_2Et$
8, R = $R_1 = NH_2$
9, R = NH_2; $R_1 = NHCO_2Et$



10, R = NH_2; $R_1 = NHCO_2Et$
11, R = R_1 = $NHCO_2Et$
12, R = $NHCH_3$; $R_1 = NHCO_2Et$
14, R = $NHCH_3$; $R_1 = N(CH_3)CO_2Et$
15, R = $N(CH_3)_2$; $R_1 = NHCO_2Et$
16, R = $N(CH_3)_2$; $R_1 = NHCONEt_2$
17, R = $N(CH_3)_2$; $R_1 = NCH_3CONEt_2$
18, R = $R_1 = NH_2$
19, R = $R_1 = NHCH_3$
20, R = $N(CH_3)_2$; $R_1 = NH_2$
21, R = $N(CH_3)_2$; $R_1 = NHCH_3$



13

DEC analogues must fall within a quite narrow lipophilicity range.

Compounds **34** and **35** each possess 15 lipophilic carbons with associated hydrogens and would be expected to have partition coefficients about 2–3 log units higher than those of DEC.[21] This places them at the fringe of the lipophilicity region found compatible with activity by Hewitt et al.[7] Compound **37**, in which phenethyl replaces methyl in the highly active analogue **4**, also displayed low activity. This result is anomalous in terms of partitioning properties because compounds from the same series with lower, equal, or higher lipophilicity (e.g., phenethyl replaced by benzyl, *n*-pentyl, *n*-hexyl, and *n*-octyl) were inactive. However, the calculated increase in partition coefficient upon changing **4** to **37** is about 2.5 log units, and **37** should fall within the lipophilicity limits provided by the work of Hewitt et al.[7] The possibility remains that phenethyl has special properties (e.g., receptor binding) that compensate for its lipophilicity. The insertion of hydroxyl group into the phenethyl side chain (e.g., **5**), which should largely compensate for its lipophilic character, provided an inactive compound, thus suggesting that bulk effects of substituents on the basic nitrogen are also important. This suggestion is supported by the inactivity[3] of the analogue of **4** where methyl is replaced by carbethoxymethyl (**6**); the latter substituent has a calculated π value only 0.04 log units lower than that of methyl.[21]

The rationale behind the work reported in our first publication on DEC analogues[1] was based in part on the correlation of decreased microfiliarial activity with the increase in distance between pharmacophores. Thus DEC and analogue **4** may be compared with analogues **31–35** of Brookes et al.[8] where there are stepwise increases in interpharmacophore separation. However, in view of the above discussion of partition properties, the less activity of **31–35** may be interpreted as due to increased lipophilicity rather than to increased interpharmacophore separation. With the exception of a series of acyclic DEC analogues based on α,ω-diaminoalkanes[15] whose inactivity may be easily rationalized in conformational terms (see below) only compounds **31–35** can be considered in terms of the effect of pharmacophore separation. It is especially unfortunate that numerical test data on these analogues were not published, but it is nevertheless significant that **34** and **35**, those compounds with the maximum separation of pharmacophores, are described as "markedly active in reducing the numbers of circulating microfiliaria". Assuming that **34** and **35** maintain chair–chair conformations, the internitrogen separations are approximately 5.2 and 7.1 Å, respectively. This compares with 2.9 Å for DEC and **4**. Thus, it cannot be concluded that any interpharmacophore separation is proven optimal. The dimensions of DEC and **4** are obviously satisfactory in a variety of ring systems, but the possibility that considerably greater separations could be associated with high activity in molecules with appropriate lipophilicities has not been tested.

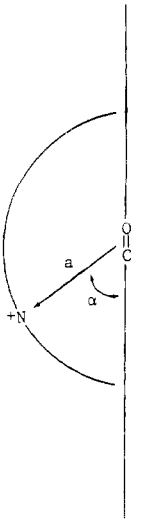


Figure 1. Hypothesized relationship of DEC pharmacophores. The line through the carbonyl represents the oxygen dipole, arrow *a* is the distance between the dipole and tertiary amine, and α is the angle between the dipole and the amine.

Conformational factors are clearly important to the activity of DEC analogues, but they cannot yet be fully defined. Our working hypothesis assumes that one conformational orientation of the basic pharmacophore to the oxygen dipole is optimal. This orientation is best represented by compound **4**, where the fused bicyclic ring system almost completely inhibits conformational flexibility. Given this assumption, and assuming other factors are equal, analogues should be active according to their ability to achieve the right conformation and according to the population of the right conformation among all other possible conformations. Molecular models indicate that all of the active analogues with two-carbon links between pharmacophores can achieve the same orientation of pharmacophores as in **4**. Unfortunately, conformer populations are very difficult to estimate in any but the simplest molecules. However, it is clear that as the pharmacophores in DEC analogues are released from the constraints of being in rings (e.g., become exocyclic as in **29**, **30**, **32–36**, and **45** or acyclic as in **38**), the number of possible conformers increases greatly. To include compounds with linkages having more than two carbons between pharmacophores in this generalization requires the assumption that the angular relationship between the oxygen dipole and the basic center is fixed but that the

distance between the dipole and amine may be variable.

As noted earlier, we attribute the inactivity of our 1,2-diaminocyclobutane analogues (e.g., **7** and **17**) to a high population of suboptimum conformers made possible by the extra degrees of freedom associated with exocyclic substituents and to their eclipsed relationship. Compounds in which only one substituent is exocyclic (e.g., **29**, **30**, **32–35**) and in which the pharmacophores cannot sterically interact may show significant activity. The low activity of **36** and **38** must be assigned to an adequate population of correct conformers, which is permitted by the absence of the eclipsed interactions found in the cyclobutanes.

Compound **41** is a good example of the probable inability of a molecule to achieve the correct orientation of carbonyl dipole to basic center. This analogue is isomeric with **4**. It is bicyclic and therefore rigid. However, the two rings are bridged and the carbonyl dipole is twisted relative to the piperazine ring. As shown by models, this twisting substantially alters the angular relationship between the pharmacophores, apparently enough to destroy activity entirely. The angular relationship concept is represented schematically in Figure 1. The data suggest that α must be relatively constant for activity but that a can be variable.

Compound **44** is another very close relative of compound **4** and rationalization of its inactivity is difficult. The orientation of the carbonyl and amine moieties is very similar to that of **4** according to molecular models and to more accurate computer conformational analysis.[22,23] Based on present information, the loss of activity must be attributed to the very small difference in α, to an exceptional sensitivity to replacement of *N*-methyl by *N*-ethyl, or possibly to a combination of both.

The conformation of the methyl group in DEC and most of its analogues can theoretically be either axial or equatorial. The high activity of analogue **23** establishes that the axial orientation is acceptable, although the preferred orientation cannot be specified because no fixed equatorial N-substituent has yet been incorporated into an analogue.

Activity among DEC analogues is not highly sensitive to the nature of the carbon structure bearing the pharmacophores, as can be seen by the diversity of ring systems in Table II. Although the piperazine ring has served as the structural unit on which a great deal of analogue work has been based, the activity of pyrrolidine derivatives **29** and **30** and piperidine derivatives **32–35** is notable. Little work on C-substituted piperazine derivatives has been done except for the very early work of American Cyanamid investigators in which some 2-methyl- and 2,5-dimethylpiperazine compounds were prepared.[16] No activity was seen in this group, and it must be tentatively assumed that the high activity found with bridged piperazines **23** and **24**, which may be regarded as 2-substituted and 2,5-disubstituted piperazines, respectively, is due to the more compact structural characteristics of the bridged systems. A tentative generalization concerning carbon structure of DEC analogues is that it must provide support and spacing for the pharmacophores that permit them to achieve optimum conformations but does not impede access of the pharmacophores to their respective receptor sites. Further exploration of this view with respect to the elongated DEC analogues **34** and **35** would be especially interesting.

Although the above analysis of structure–activity characteristics of DEC analogues is based on comparisons of rather uncertain data in many cases, useful principles are beginning to emerge and a new generation of improved analogues may be possible through optimization of the several critical characteristics.

Chemistry. Buchman et al.[24] reported the synthesis of the *cis*- and *trans*-1,2-diaminocyclobutanes (**8** and **18**) via both Schmidt and Curtius rearrangements from the corresponding diacids. We were unable to isolate the *trans*-diamine **18** from the Schmidt degradation and therefore employed the Curtius rearrangement for its preparation. Yields were poor (4–5%) for **8** and could be undependable (19–40%) for **18**.

Asymmetry was introduced into the diamines by monoacylating at controlled pH.[7] The reaction conditions used for monoacylation of 2,5-diazabicyclo[2.2.2]octane[1] at pH 3 were successful for conversion of **8** to **9** and also provided by-product **13**. In the trans series, successful monoacylation of **18** was poor at pH 3 but proceeded well at pH 4, as had been found by Moore et al. for monoacylation of ethylenediamine.[25]

The Borch[26] and Eschweiler–Clark (HCOOH–HCHO) reductive methylations gave equal results for the preparation of **7** from **9** when using **9** in 0.5-g quantities. The latter method gave an unidentified compound when used on a larger scale.

Monomethylation of compound **10** to give **12** was achieved by sequential trifluoroacetylation, treatment with methyl iodide and base, and hydrolysis, according to a procedure of Johnstone et al.[27] *N,N'*-Dicarbethoxy derivative **11**[24] was reduced with $LiAlH_4$ to give **19** and monoacylated to give **14**.

The *N*-carbethoxy analogue **10** was dimethylated with HCOOH–HCHO to give **15**, hydrolyzed to **20**, and treated with diethylcarbamyl chloride to give **16**. Compound **15** was reduced with $LiAlH_4$ to give trimethyldiamine **21** and then acylated to give target **17**.

Experimental Section

All boiling and melting points are uncorrected. Melting points were determined using a Fisher block or a Mel-Temp apparatus. Infrared spectra were obtained on a Perkin-Elmer Infracord. Where analyses are indicated only by symbols of the elements, analytical results obtained for those elements were within ±0.4% of the theoretical values.

***cis*-*N*-Carbethoxy-1,2-diaminocyclobutane (9).** A solution of **8**[24] (500 mg, 3.14 mmol) in 50% aqueous Me_2CO (10 mL), stirred at room temperature and monitored with a pH meter, was adjusted to pH 3 (2 N HCl) and then treated dropwise alternately with ethyl chloroformate (0.4 mL, 4.19 mmol) and a buffer of $Na_3PO_4 \cdot 12H_2O$ (25 g/100 ml H_2O), keeping the pH between 2 and 3. After the slow addition was completed, the reaction was stirred at room temperature for 0.5 h, treated with 40% KOH, and saturated with K_2CO_3. Extraction with Et_2O, drying (Na_2SO_4), and evaporation of Et_2O in vacuo afforded 407 mg of oil. The oil was acidified and partitioned between H_2O and Et_2O. The free base was regenerated (320 mg) and purified by preparative TLC (alumina–dioxane). Elution of the appropriate band with aqueous dioxane yielded 154 mg (31%) of oily free base. Treatment of an ether solution with ethanolic HCl gave a crystalline HCl salt, mp 143–153 °C. Anal. ($C_7H_{14}N_2O_2 \cdot HCl$) C, H, N.

During the large-scale preparation, a solution of the crude reaction mixture in Et_2O was treated with ethanolic HCl to yield a solid. Recrystallization ($EtOH \cdot Et_2O$) gave 5.3 g of **9** (38%). The mother liquors were combined and evaporated in vacuo, dissolved in H_2O, and extracted with Et_2O. The H_2O solution was then treated with NaOH to pH 11, saturated with K_2CO_3, and extracted with Et_2O. The Et_2O residue yielded 60 mg of crystals, mp 138–142 °C, identified by elemental analysis and NMR as **13**. Anal. ($C_5H_8N_2O$) C, H, N.

Alumina dry column chromatography (elution with dioxane) of the mother liquors from the large-scale extraction gave an additional 2.4 g (18%) of **9** for a total yield of 56%.

***cis*-*N*-Carbethoxy-*N'*,*N'*-dimethyl-1,2-diaminocyclobutane (7).** This compound was synthesied by two different methods.

Method A. Compound **9** (96 mg, 0.61 mmol) was combined with $NaBH_3CN$ (39 mg, 0.62 mmol), paraformaldehyde (182 mg, 6.1 mmol), and a few Linde 3A molecular sieves in anhydrous MeOH (2 mL). Ethanolic dry HCl (5.9 M) was added to adjust the pH to 6. This was stirred at room temperature for 48 h. A second portion of paraformaldehyde (182 mg) was gradually vaporized by heating and the gaseous HCHO was bubbled into the reaction mixture. The reaction was stirred another 18 h, treated with ethanolic HCl, and evaporated in vacuo. H_2O was added and this solution was extracted with Et_2O; the H_2O was made alkaline (pH 12, NaOH) and extracted with Et_2O to yield, after evaporation, a colorless oil (39 mg, 34.5%). The oil yielded a picrate salt (43 mg), mp 192.5–195.5 °C, when recrystallized (absolute EtOH). Anal. ($C_9H_{18}N_2O_2 \cdot C_6H_3N_3O_7$) C, H, N.

Method B. To compound **9** (400 mg, 2.53 mmol), chilled at 0 °C, was added 88% HCOOH (0.56 mL, 12.8 mmol) followed by 36% HCHO (0.44 mL, 5.7 mmol). This was heated at 70 °C until gas evolution ceased; thereafter it was heated at 110 °C for 4 h. The reaction was evaporated in vacuo, treated with 40% KOH, and extracted with Et_2O. The Et_2O extract was evaporated to leave an oil. A maleic acid salt (490 mg, 64%), mp 102–106 °C, formed readily. Anal. ($C_9H_{18}N_2O_2 \cdot C_4H_4O_4$) C, H, N. The NMR spectrum was compatible with structure **7**.

***trans*-*N*-Carbethoxy-1,2-diaminocyclobutane (10).** To a vigorously stirring solution of **18** (15.0 g, 0.0944 mol) in 147 mL of Me_2CO and 147 mL of H_2O at pH 4 and room temperature was added ethyl chloroformate (12.0 mL, 0.126 mol) alternately with $Na_3PO_4 \cdot 12H_2O$ (25 g/100 mL of H_2O) at such a rate that the pH was controlled between 3 and 4. After all the ethyl chloroformate was added (9 h), the reaction was stirred an additional 0.5 h. A solid began crystallizing from the solution and stirring was continued at 0 °C for 1.5 h. The reaction was filtered to yield 0.82 g of a brownish solid, the diacylated product. The chilled filtrate was then brought to pH 13 with 50% aqueous KOH. The solution was then saturated with K_2CO_3, which caused two layers to separate. Extraction with Et_2O afforded 13.06 g of orange oil.

A solution of the oil in anhydrous Et_2O (400 mL) was treated with 6.37 M dry HCl in EtOH (18.6 mL) to yield a crystalline HCl salt, 12.79 g (63.5%), mp 150–153 °C. A previous reaction yielded an analytical sample, mp 153–155 °C.

***trans*-*N*-Carbethoxy-*N'*-methyl-1,2-diaminocyclobutane (12).** To a cold stirred mixture of **10** (3.41 g, 0.0215 mol) and $NaHCO_3$ (13.0 g, 0.155 mol) in Et_2O (65 mL) was added trifluoroacetic anhydride (3.37 mL, 0.0239 mol). The mixture was stirred 2 h at room temperature. $CHCl_3$ (65 mL) was added; the reaction was filtered, washed (H_2O), dried (Na_2SO_4), filtered, and evaporated in vacuo to leave a white solid: 4.47 g (81%); homogeneous on TLC (alumina–$CHCl_3$). This solid was combined with CH_3I (3.66 mL, 0.0588 mol) and anhydrous K_2CO_3 (5.9 g, 0.0427 mol) in dry Me_2CO (30 mL). The mixture was stirred 48 h at room temperature and refluxed for 3 h. The reaction was filtered and the filtrate was washed (H_2O), dried (Na_2SO_4), filtered, and evaporated to give 4.6 g of oil. The oil was then stirred with 20% aqueous NaOH (20 mL) in a steam bath for 20 min. The hydrolysis mixture was cooled and extracted (Et_2O and $CHCl_3$). The extracts were dried (Na_2SO_4), filtered, and evaporated in vacuo to give a white crystalline solid, which was recrystallized (Et_2O) to give 1.1 g of **12** as a free base, mp 87–91 °C. Further purification was obtained by partitioning this solid between aqueous HCl and $CHCl_3$. The aqueous phase was evaporated to a crystalline solid, which was triturated in Me_2CO and filtered to afford 1.212 g (31%) of white solid (**12**), mp 134–137 °C. Anal. ($C_8H_{16}N_2O_2 \cdot HCl$) C, H, N.

***trans*-*N*,*N'*-Dimethyl-1,2-diaminocyclobutane (19).** A solution of **11**[24] (20.0 g, 0.087 mol) in dry THF (500 mL) was added dropwise to a chilled, stirring suspension of $LiAlH_4$ (13.2 g, 0.348 mol) in dry THF (200 mL). The reaction was refluxed for 20 h and cooled; excess $LiAlH_4$ was destroyed by the cautious addition of H_2O. The white salts were filtered through sintered glass. The filtrate was distilled, collecting first the THF and then a colorless liquid, bp 160–169 °C (6.44 g). A solution of the liquid in Et_2O (200 mL) was treated with 5.7 M dry HCl in EtOH until no further turbidity was produced. After prolonged chilling, a crystalline solid formed. The mother liquor was decanted and the residual solid was triturated with Me_2CO. The solid was then dried in vacuo to yield a very hygroscopic pink solid (8.6 g, 51%), mp 137–150 °C. An aqueous solution of a small portion of the hygroscopic HCl salt was treated with a saturated aqueous solution of picric acid to yield a dipicrate salt, mp 184–187 °C after recrystallization (absolute EtOH). Anal. ($C_6H_{14}N_2 \cdot 2C_6H_3N_3O_7$) C, H, N.

***trans*-*N*-Carbethoxy-*N*,*N'*-dimethyl-1,2-diaminocyclobutane (14).** This compound was prepared by the procedure described for **10**. The maleic acid salt was prepared and recrystallized from Me_2CO–Et_2O: mp 93–96 °C; yield 58%. Anal. ($C_9H_{18}N_2O_2 \cdot C_4H_4O_4$) C, H, N.

***trans*-*N*-Carbethoxy-*N'*,*N'*-dimethyl-1,2-diaminocyclobutane (15).** This compound was prepared from **10** using method B as described for **7**. The product was isolated as the free base by crystallizing from cyclohexane (dry ice) and by distillation [bp 92–94 °C (0.7 mm), mp 56–58 °C] in 52% yield. Anal. ($C_9H_{18}N_2O_2$) C, H, N. The maleic acid salt had mp 108–111 °C. Anal. ($C_9H_{18}N_2O_2 \cdot C_4H_4O_4$) C, H, N.

***trans*-*N*,*N*-Dimethyl-1,2-diaminocyclobutane (20).** Compound **15** (2.28 g, 0.0123 mol) was combined with KOH (4.6 g, 0.082 mol) in H_2O (25 mL) and MeOH (10 mL) and refluxed for 5 h. Steam distillation gave 375 mL of distillate, which was treated with concentrated HCl and evaporated to dryness to yield 1.25 g of white solid, mp 245 °C dec. Anal. ($C_6H_{14}N_2 \cdot 2HCl$) C, H, N.

***trans*-*N*-Diethylcarbamyl-*N'*,*N'*-dimethyl-1,2-diaminocyclobutane (16).** A mixture of **20**·2HCl (1.25 g, 6.7 mmol), diethylcarbamyl chloride (0.92 g, 6.8 mmol), and triethylamine (3.0 mL, 0.0218 mol) in dioxane (4 mL) was stirred at room temperature for 18 h. Et_2O was added and the solid (triethylamine hydrochloride) was collected. The filtrate was partitioned between H_2O and Et_2O. The Et_2O phase yielded 170 mg of oil. The H_2O layer was treated with concentrated HCl and evaporated in vacuo to give 1.4 g of an oily hydrochloride. The free base was regenerated with 20% aqueous NaOH and isolated by extraction with Et_2O. A crystalline citric acid salt, 0.90 g (33%), was obtained from Et_2O: mp 133–136 °C. Anal. ($C_{11}H_{23}N_3O \cdot C_6H_8O_7$) C, H, N.

***trans*-*N*,*N*,*N'*-Trimethyl-1,2-diaminocyclobutane (21).** This compound was prepared in 54% yield from **15** by the method described for **19**. The dihydrochloride salt was obtained from Et_2O–EtOH and triturated with EtOH: mp 213–220 °C. Anal. ($C_7H_{16}N_2 \cdot 2HCl$) C, H, N.

***trans*-*N*-Diethylcarbamyl-*N*,*N'*,*N'*-trimethyl-1,2-diaminocyclobutane (17).** A solution of diethylcarbamyl chloride (1.52 g, 0.016 mol) in $CHCl_3$ (2 mL) was added to a chilled, stirring two-phase system of **21** (1.48 g, 0.016 mol) in $CHCl_3$ (15 mL) and 10% aqueous NaOH (10 mL, 0.025 mol). After 2 h of stirring, **21** was still readily detectable on TLC (alumina–Me_2CO). Another 0.5 g (0.005 mol) of diethylcarbamyl chloride was added and the reaction was stirred for another 2 h. The two phases were separated and the water layer was extracted with $CHCl_3$; the combined extracts were dried (Na_2SO_4), filtered, and evaporated in vacuo to afford a light yellow oil (1.99 g, 76%). An Me_2CO solution of the oil was poured through a 20-g pad of acid-washed alumina in a sintered glass funnel and eluted with three column lengths of Me_2CO to remove an impurity appearing at the origin on TLC. An ethereal solution of the recovered oil (1.6 g) was treated with an ethanolic solution of maleic acid (0.87 g) to yield a white solid (1.71 g, 43%), mp 128–129 °C. Anal. ($C_{12}H_{25}N_3O \cdot C_4H_4O_4$) C, H, N.

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Antifilarial Agents. 3-Aminopyrrolidine and 1,4-Diazabicyclo[3.2.1]octane Derivatives as Analogues of Diethylcarbamazine

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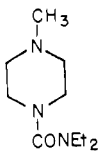
3-Aminopyrrolidines bearing acyl substituents on either nitrogen and N-acylated 1,4-diazabicyclo[3.2.1]octanes are potent microfilaricides in the *Litomosoides carinii* gerbil test system but have no effect on adult worms. The high activity of the pyrrolidine derivatives establishes that diethylcarbamazine (DEC) like antifilarial activity does not require that both pharmacophores be incorporated into one ring. Results with the 1,4-diazabicyclo[3.2.1]octanes establish that an axial conformation of the alkyl substituent corresponding to the equatorial *N*-methyl group of diethylcarbamazine is fully consistent with high activity. Other conformational considerations pertinent to DEC analogues are discussed.

In the preceding paper of this series, we discussed the reasoning behind our current work on diethylcarbamazine (DEC, 1) analogues.[2] That study was concerned with defining the nature of the carbon skeleton connecting the two pharmacophoric groups of 1. We concluded from the first study that there was a surprisingly low specificity for carbon skeleton structure among active DEC analogues if the approximate separation between the essential tertiary amine and the amide group seen in the parent compound was maintained.

In general, ring systems with more rigidity than 1 but with similar functional groups on the ring nitrogen displayed activity parallel to 1 against *Litomosoides carinii* in the gerbil. This paper reports the results of continued investigation in this area.

One stereochemical point not addressed in the first report was the preferred orientation of the *N*-methyl group of DEC and its active analogues. To evaluate the significance of orientation of the *N*-methyl group, we synthesized and evaluated the antifilarial activity of three 1,4-diazabicyclo[3.2.1]octanes (2–4) in which, because of the bridgehead nitrogen atom, the substituents on the tertiary amino group of the substituted piperazine were



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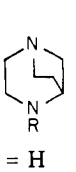
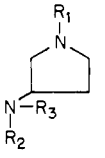


2, R = H
3, R = CO_2Et
4, R = $CONEt_2$



5, $R_1 = CH_3$; $R_2 = CONEt_2$; $R_3 = H$
6, $R_1 = CH_3$; $R_2 = CO_2Et$; $R_3 = CH_3$
7, $R_1 = CONEt_2$; $R_2 = CH_3$; $R_3 = H$
8, $R_1 = CONEt_2$; $R_2 = R_3 = CH_3$

locked into a rigid conformation. In addition, we continued our investigation of DEC analogues with the synthesis and antifilarial evaluation of 3-aminopyrrolidine analogues of DEC (**5–8**). These compounds possess the functional groups shown to be consistent with significant activity in the piperazine[3,4] and bridged piperazine[2] congeners of DEC. Most importantly, however, compounds **5–8** maintain nearly the same interatomic distance between the pharmacophoric groups as that in the piperazine