Studies in Potential Filaricides. 5. 3-Ethyl-8-methyl-1,3,8-triazabicyclo[4.4.0]decan-2-one, a New Antifilarial Agent[1]

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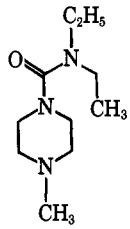
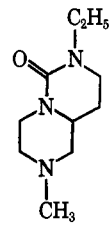
The synthesis of 3-ethyl-8-methyl-1,3,8-triazabicyclo[4,4,0]decan-2-one possessing high microfilaricidal activity is reported.

In earlier studies of this series, the syntheses of certain open-chain[2,3] and cyclic[4-8] analogs of diethylcarbamazine (I) were described, but none of these compds possessed significant antifilarial activity. This communication describes the synthesis and antifilarial activity of 3-ethyl-8-methyl-1,3,8-triazabicyclo[4.4.0]-decan-2-one (II), where the diethylcarbamazine molecule has been incorporated into a rigid framework.



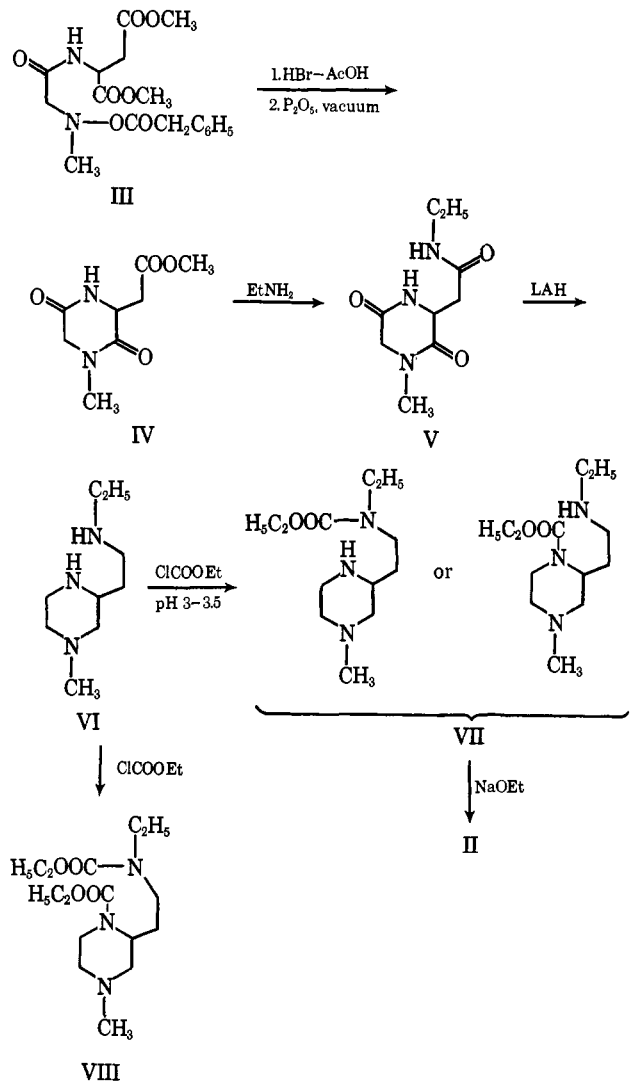
I



II

Chemistry.—Condensation of dimethyl aspartate[9] with benzyloxycarbonylsarcosine[10] using DCI gave dimethyl benzyloxycarbonylsarcosylaspartate (III). Removal of the benzyloxycarbonyl group from III by catalytic hydrogenation or by treatment with HBr–AcOH followed by cyclization, effected either by heating in abs EtOH or by storage over P_2O_5 *in vacuo* for 48 hr, gave methyl 2-(1-methyl-2,5-dioxopiperazin-3-yl)acetate (IV). When heated with $EtNH_2$ in a sealed tube for 36 hr, IV yielded *N*-ethyl-2-(1-methyl-2,5-dioxopiperazin-3-yl)acetamide (V), which on reduction with LAH gave 1-methyl-3-(2-ethylamino)-ethylpiperazine (VI). When VI was condensed with ClCOOEt at pH 3–3.5, the monocarbamate VII was obtained. The same reaction gave a dicarbamate (VIII) when treatment with ClCOOEt was carried out in the presence of NaOEt or Et_3N. Cyclization of the monocarbamate VII to 3-ethyl-8-methyl-1,3,8-triazabicyclo[4.4.0]decan-2-one (II) could not be achieved by heating. This was eventually effected by treatment of VII with NaOEt (Scheme I).

SCHEME I



Biological Activity.—II, when tested for its antifilarial activity in cotton rats infected with *Litomosoides carinii*, showed a high microfilaricidal activity. In this test II is about 5 times more effective than diethylcarbamazine. It is equally effective by both ip and oral routes of administration. The MED and LD_{50} results have clearly shown that the safety margin of II is higher than diethylcarbamazine. Its activity persists much longer than that of this drug.[11]

When tested against *Hymenolepis nana* in mice and *Nippostrongylus brasiliences* in rats by the technique of Steward[12,13] and *Chandlerella hawkingi* in crows by the

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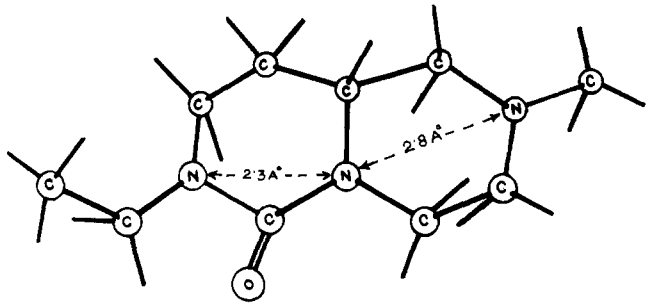

Figure 1.—Interatomic distances in 3-ethyl-8-methyl-1,3,8-triazabicyclo[4.4.0]decan-2-one.

method of Chatterjee and Sen,[14] II showed no activity but was effective in clearing about 40% of worms in fowls experimentally infected with *Ascaridia galli* at an oral dose of 250 mg/kg.

II, like diethylcarbamazine, inhibited the passive cutaneous anaphylactic response, and also showed significant antiinflammatory activity when tested in rats according to Winter, *et al.*[15] At a dose of 28 mg/kg po it caused 30% inhibition of carrageenin-induced edema as compared to 31% inhibition by 25 mg/kg of phenylbutazone.

None of the open-chain analogs of diethylcarbamazine described earlier showed antifilarial activity. This and the fact that the corresponding homopiperazine analog was about 0.5 as active would suggest that, in addition to functionality of the three N, the geometry of the molecule and the resulting N–N distance are of great importance in determining the activity of diethylcarbamazine and its analogs. The diethylcarbamoyl side chain of diethylcarbamazine can assume different conformations. The fact that analog II in which one of the Et groups forms part of a ring has the full profile of biological activity of diethylcarbamazine would suggest that the preferred conformation of diethylcarbamazine in the biophase is such in which bond distances are similar to those described for II in Figure 1.

Experimental Section

Mps and bps are uncor. The various compds were routinely checked by ir and nmr on a Perkin-Elmer infracord and Varian A-60D instrument and only such data as are relevant to the discussion are given. The nmr values are expressed in τ units (TMS). The mass spectra were detd with a Hitachi RMU-6E single focusing spectrometer. Analyses are indicated only by symbols of the elements or functions analyzed and were within ±0.4% of the calcd values.

Dimethyl Benzyloxycarbonylsarcosylaspartate (III).—A soln of DCI (12.0 g, 0.058 mole) in dry EtOAc (20 ml) was added dropwise at 0–5° to a stirred soln of benzyloxycarbonylsarcosine (12.0 g, 0.05 mole) and dimethyl aspartate (8.60 g, 0.05 mole) in dry EtOAc (30 ml). The mixt was stirred for an addnl 2 hr at 0° and then left overnight. The soln was treated with AcOH (1 ml) to decomp excess of DCI and stirred again at 0° for 1 hr, the pptd dicyclohexylurea was removed by filtration, the ppt was washed with dry EtOAc (10 ml), and the combined filtrates were washed successively with H_2O, 1 *N* HCl, 1 *N* NaOH soln, and satd aq NaCl (200 ml each) and dried (Na_2SO_4). The solvent was removed *in vacuo* to give the peptide III as a viscous oil, yield 16.20 g (83%). *Anal.* ($C_{17}H_{22}N_2O_7$) C, H, N.

Methyl 2-(1-Methyl-2,5-dioxopiperazin-3-yl)acetate (IV). **Method A.**—The dipeptide III (1.5 g, 0.004 mole) and 10% Pd/C (0.20 g) in MeOH (15 ml) were hydrogenated at 3.52 kg/cm² and 70° for 48 hr. The catalyst was removed by filtration and washed with MeOH (15 ml) and the combined filtrates were evapd *in vacuo* to give an oil, which when refluxed with abs EtOH (20 ml) for 24 hr gave IV as a solid product which crystd (C_6H_6) in colorless needles: yield 0.20 g (26%); mp 98°, ir, $\nu_{max}^{KBr,\ 1742\ cm^{-1}}$ ($COOCH_3$), 1656 cm^{-1} (CONH); nmr ($CDCl_3$) 5.70 (t, 1, CH), 5.98 (d, 2, $COCH_2N$, J = 1 cps), 6.25 (s, 3, OCH_3), 7.0 (s, 3, NCH_3), 7.10 (m, 2, CH_2); IV gives the characteristic red color of dioxopiperazines[16] with a soln of Na_2CO_3 and picric acid. *Anal.* ($C_8H_{12}N_2O_4$) C, H, N.

Method B.—A soln of III (16.0 g, 0.04 mole) in AcOH (40 ml) was treated with 4 *N* HBr–AcOH (40 ml) and kept at 20° for 1 hr. The soln was dild with excess of dry Et_2O (100 ml), and dried *in vacuo*. The solid was dissolved in dry $CHCl_3$ (150 ml) and satd with dry NH_3, NH_4Br filtered, and washed with dry $CHCl_3$ (25 ml), the solvent was removed, and the residual oil was kept over P_2O_5 in a vacuum desiccator for 48 hr when it gradually solidified: yield 4.0 g (60%), mp 98°, identical with the product obtained by method A.

***N*-Ethyl-2-(1-methyl-2,5-dioxopiperazin-3-yl)acetamide** (V).—A soln of IV (1.0 g, 0.005 mole) and $EtNH_2$ (0.7 ml, 0.01 mole) in abs EtOH (25 ml) was heated in a sealed tube at 135–140° for 36 hr. The solvent was removed under reduced pressure, the residual oil was triturated with dry Et_2O, and the product was crystd (MeOH–Et_2O): yield 0.70 g (70%); mp 104°; ir, $\nu_{max}^{KBr,\ 1680\ cm^{-1}}$ (CONH); nmr ($CDCl_3$), 5.70 (t, 1, CH), 6.0 (d, 2, $COCH_2N$, J = 1 cps), 6.7 (q, 2, CH_2CH_3), 7.0 (s, 3, NCH_3), 8.9 (t, 3, CH_2CH_3). *Anal.* ($C_9H_{15}N_3O_3$) C, H, N.

1-Methyl-3-(2-ethylamino)ethylpiperazine (VI).—A soln of V (2.6 g, 0.009 mole) in dry THF (20 ml) was added to a stirred suspension of LAH (0.7 g, 0.018 mole) in dry THF (40 ml), the mixt was refluxed for 100 hr, and the product was worked up in the usual manner. The residual oil was distd under reduced pressure: yield 0.83 g (55%); bp 110–112° (8 mm); ir ν_{max}^{neat} 3268 cm^{-1} (NH); nmr ($CDCl_3$), 7.75 (s, 3, NCH_3), 8.82 (t, 3, CH_2CH_3).

Its hydrochloride crystd from abs EtOH and was very hygroscopic: mp 213–216° (sealed tube); picrate, crystd (EtOH) as yellow pellets, mp 240° dec. *Anal.* ($C_9H_{21}N_3 \cdot 3C_6H_2OH(NO_2)_3$) C, H, N.

***N*-Ethoxycarbonyl-1-methyl-3-(2-ethylamino)ethylpiperazine** (VIII).—To a stirred soln of VI (6.0 g, 0.035 mole) in H_2O (25 ml), cooled to 0°, HCl was added to bring the pH of the soln to 3–3.5. ClCOOEt (4.0 ml, 0.04 mole) was added dropwise maintaining the pH between 3 and 3.5 by the gradual dropwise addn of NaOAc soln. Stirring was contd for an addnl 1 hr and then the reaction mixt was kept in a refrigerator overnight. The soln was washed with Et_2O (50 ml), and the aq layer was satd with K_2CO_3; the oil, which seps, was extd with Et_2O (3 × 75 ml), the combined exts were dried (Na_2SO_4), and solvent was removed. A C_6H_6 soln of the residual oil was chromatogd on a basic alumina column when the product was obtd as an oil from the C_6H_6 eluates: yield 7.0 g (82%); ir ν_{max}^{neat} 3268 (NH) and 1706 cm^{-1} (NCOOEt); nmr ($CDCl_3$), 5.85 (q, 2, OCH_2CH_3), 6.72 (complex m, 2, NCH_2CH_3), 7.75 (s, 3, NCH_3), 8.78 (t, 3, OCH_2CH_3), 8.89 (t, 3, NCH_2CH_3). *Anal.* ($C_{12}H_{25}N_3O_2$) C, H, N.

1-Ethoxycarbonyl-2[β-(*N*-ethoxycarbonyl-*N*-ethyl)amino-ethyl]-4-methylpiperazine (VIII).—ClCOOEt (0.2 ml, 0.001 mole) was added dropwise to a stirred soln of VI (0.3 g, 0.001 mole) contg Et_3N (0.5 ml, 0.003 mole) in dry Et_2O (25 ml) at 0°. Stirring was contd for an addnl 4 hr at the same temp followed by 1 hr at room temp. Pptd $Et_3N \cdot HCl$ was removed by filtration. Concn of the filtrate gave an oil which was chromatogd on neutral alumina using C_6H_6 as eluant: yield 0.3 g (60%); ν_{max}^{neat} 1692 cm^{-1} (NCOOEt). *Anal.* ($C_{15}H_{29}N_3O_4$) C, H, N.

3-Ethyl-8-methyl-1,3,8-triazabicyclo[4.4.0]decan-2-one (II).—A soln of VII (50.0 g, 0.205 mole) and NaOEt (from 5.75 g, 0.25 mole, of Na and 200 ml of abs EtOH) in abs EtOH (50 ml) was refluxed for 36 hr. The solvent was removed under reduced pressure, H_2O (75 ml) was added to the residue, and the mixt was extd with CH_2Cl_2 (3 × 150 ml). The combined ext was dried (Na_2SO_4), solvent was removed, and the oil obtained was distd: yield 35.0 g (86%); bp 130–132° (5 × 10^{-2} mm); ir ν_{max}^{neat} 1668 cm^{-1} (NCON); nmr ($CDCl_3$) 5.7 (m, 1, CH_2CHCH_2), 6.7 (q, 2, NCH_2CH_3), 7.70 (s, 3, NCH_3), 8.90 (t, 3, NCH_2CH_3); mass

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spectrum, major signals at m/e 197, 182, 155, 140, 127, 125, 113, 111, 98, and 97. *Anal.* ($C_{10}H_{19}N_3O$) C, H, N.

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Deuterium Isotope Effect in Chloramphenicol Action

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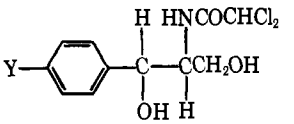
α-Deuteriochloramphenicol was synthesized and its activity against *Escherichia coli* was tested in comparison with chloramphenicol itself. α-Deuteriochloramphenicol had only approximately 80% of the chloramphenicol activity. This finding supports the hypothesis that the benzylic C-H bond is broken in the kinetically determining step of chloramphenicol action. This is consistent with the idea that the antibiotic blocks an enzyme covalently.

Several hypotheses have been developed to explain the mode of action of chloramphenicol in bacterial cells.[1,2] Evidence is increasing that chloramphenicol interferes with the synthesis of proteins essential in cell division.[3] However, the biochemical studies on the mechanism of action of chloramphenicol and the structure–activity studies in the chloramphenicol series of compounds essentially remained unrelated.[2] An interesting exception is the work of Jardetzky and Julian.[4] These authors suggest that the antibiotic acts *via* a competitive inhibition mechanism because of some structural similarities between chloramphenicol and pyrimidine nucleotides. However, Richmond recently pointed out,[5] that antibacterial substances which act solely by competitive inhibition of a single enzyme or receptor have proved to be relatively ineffective as chemotherapeutic agents. Even in cases where antibacterial compounds affect the operation of regulatory processes in the cell in a competitive manner the evolutionary flexibility of bacterial populations ensures that a process of mutation followed by selection allows the bacterial population to survive. Therefore, the most effective bacterial inhibitors are compounds that bind irreversibly to an enzyme active center or are bound covalently to an enzyme product that is used for a subsequent biosynthetic step. Azaserine, puromycin, and penicillin, for example, act in this manner. From a chemical point of view it is not difficult to detect the chemically reactive site in azaserine, puromycin, or penicillin. Chloramphenicol is a very potent antibiotic, too. However, compared to other antibiotics chloramphenicol appears to be quite unreactive (Figure 1).

Recently, Hansch, *et al.*,[6] reported a good quantitative correlation with a set of chloramphenicols of structure I obtained using regression analysis. In eq 1

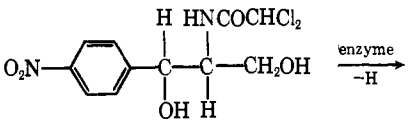


I

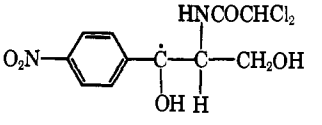
$$\log A = [3.069\ (\pm 1.2)]E_R + [0.227\ (\pm 0.16)]\pi + 0.769\ (\pm 0.25) \quad (1)$$

$$n = 8 \qquad s = 0.140 \qquad r = 0.954$$

log A represents variations in chloramphenicol activity on the growth of *Escherichia coli* due to changes of the substituent Y.[7] E_R is a radical parameter[8] and π a hydrophobic constant,[9] both dependent on the properties of Y. Although substituent effect analysis *per se* cannot elucidate a particular mechanism of action it still can provide valuable information about the nature of drug–receptor interactions. From eq 1 it was concluded that changes in activity are proportional to the ability of the substituents to stabilize a free radical provided the lipophilic character of the substituents is kept constant. This led Hansch, *et al.*, to postulate that chloramphenicol may act *via* a hydrogen radical transfer mechanism.[6] Therefore the benzylic CH bond



II



III

is proposed to be the point of chemical attack at the site (Figure 1). The enzyme involved could be in-

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