Synthetic Biologically Active Polymers. 9. Comparison of Antimalarial Activity in Copolymers Containing Common Sulfonamide Monomers but Different Comonomers

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Previous publications in this series have described the preparation, characterization, and certain biological activities of a number of polymers and copolymers[3] with a view to observing the effect of polymerization or copolymerization upon drugs, hoping to elucidate from the data obtained some generalities concerning what can be expected in the way of biological activity when a drug is polymerized or copolymerized. In other studies,[3h] various observations indicate that the biological activity of a polymerized or copolymerized drug may be influenced by the nature of the comonomer present in the polymer other than the drug, and this paper attempts to display data relevant to this phenomenon. The sulfonamide copolymers employed in this study were prepared and characterized by methods reported previously.[3c-h]

Biological Activity.—Table I displays comparative data concerning the antimalarial activity of various sulfonamide–dimethylolurea copolymers (D) relative to sulfonamide–formaldehyde copolymers (F). Each copolymer system being compared has the same sulfonamide incorporated into the copolymer.

TABLE I
COMPARISON OF SULFONAMIDE–DIMETHYLOLUREA COPOLYMER (D) AND SULFONAMIDE–FORMALDEHYDE COPOLYMER (F) ANTIMALARIAL ACTIVITY[a,b]

Sulfonamide system	D act.	F act.
Sulfanilamide	Active[c]	Curative[d]
Sulfapyridine	Active	Curative
Sulfacetamide	Inactive	Curative
Sulfabenzamide	Inactive (nontoxic)	Curative (toxic[e])
4,4′-Diaminodiphenylsulfone	Curative	Curative

[a] The prepn, characterization, and antimalarial activity compared to the antimalarial activity of the pertinent sulfonamide monomers of the copolymers have been reported previously.[3c-f] Other activity testing also has been reported.[3g,h] [b] Antimalarial testing was carried out with white ICR/Ha Swiss mice infected with *Plasmodium berghei*. Five mice were employed for each compd at each dose level. Drugs were administered ip by injection in oil. [c] Active = when mice in a test group survive 14 days. [d] Curative = when mice in a test group survive 30 days. [e] Control animals do not die before day 6. Deaths through day 5 are attributable to drug action (toxic).

As can be seen from Table I, in general, the antimalarial activity of the F copolymers tends to be better than that of the D copolymers, except in the case of the 4,4-diaminodiphenylsulfone systems where the activity is the same. An interesting observation concerning toxicity can be seen in the case of the sulfabenzamide system. Thus, as in the case of similar comparative data derived from related work,[3h] it would appear that the biological activity obtained under identical test conditions may be dependent, at least in part, on factors other than the sulfonamide content of the copolymers. For if only the sulfonamide content was involved, it would be expected that in all cases the observed activity and/or toxicity of the F copolymers would be greater than that of the D copolymers since the same dose levels were used in each comparative case and thus the sulfonamide content of an equal weight of D copolymer would be less than that present in the same weight of F copolymer.

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(2) Taken in part from the thesis to be submitted by Mr. John Razzano in partial fulfillment of the requirements for the Ph.D. degree.

(3) (a) R. J. Cornell and L. G. Donaruma, *J. Polymer Sci., Part A*, **3**, 827 (1965); (b) R. J. Cornell and L. G. Donaruma, *J. Med. Chem.*, **8**, 388 (1965); (c) L. G. Donaruma and J. Razzano, *ibid.*, **9**, 258 (1966); (d) J. R. Dombroski, L. G. Donaruma, and J. Razzano, *ibid.*, **10**, 963 (1967); (e) J. R. Dombroski, L. G. Donaruma, and J. Razzano, *ibid.*, **10**, 964 (1967); (f) J. R. Dombroski, M.S. Thesis, Clarkson College of Technology, Oct 9, 1967; J. R. Dombroski and L. G. Donaruma, *J. Appl. Polymer Sci.*, **15**, 1219 (1971). (g) L. G. Donaruma and J. Razzano, *J. Med. Chem.*, **14**, 244 (1971); (h) J. R. Dombroski, L. G. Donaruma, and J. Razzano, *ibid.*, **14**, 460 (1971).

Antihistomonal Activity of Ipronidazole and Closely Related Nitroimidazoles

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The 5-nitroimidazoles dimetridazole (**1**)[1] and ipronidazole (**2**)[2] are potent histomonastats.[3] Comparison with their 4-nitro isomers **4**[1,4] and **5** demonstrates that both 5-nitro analogs are much superior histomonastats.

In order to collect additional information regarding the structure–activity relationship of isomeric isopropyl nitroimidazoles, we have included in our comparison study the two new 1-isopropyl-substituted nitroimidazoles **6** and **7** and the known 5-isopropyl-2-nitroimidazole **8**.[5] Compds **6** and **7** were obtained by alkylating 2-methyl-4(5)-nitroimidazole under different reaction conditions.[6]

Their structures could easily be assigned by uv spectroscopy and pK measurements.[7] A pure sample of the

(1) V. K. Bhagwat and F. L. Pyman, *J. Chem. Soc.*, **127**, 1832 (1925).

(2) K. Butler, H. L. Howes, J. E. Lynch, and D. K. Pirie, *J. Med. Chem.*, **10**, 891 (1967). Ipronidazole **2** was recognized by this group to be a systemically highly active trichomacide in mice.

(3) M. Mitrovic, M. Hoffer, and E. Schildknecht, *Antimicrob. Ag. Chemother.*, 445 (1968).

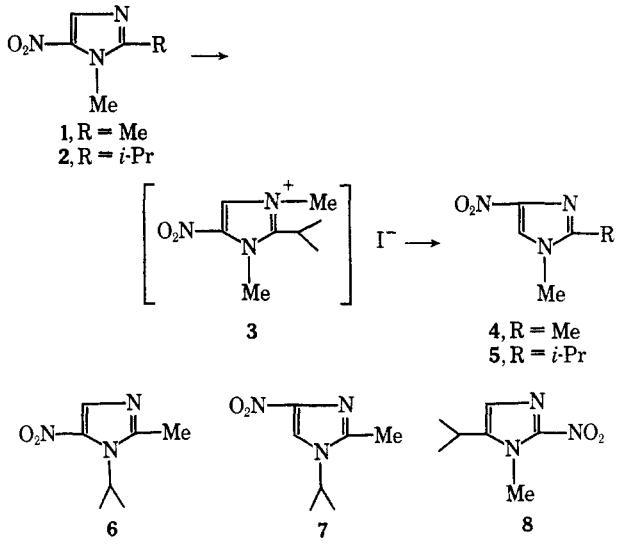
(4) C. Cosar, C. Crisan, R. Horclois, R. M. Jacob, J. Robert, S. Tchelitcheff, and R. Vaupre, *Arzneim-Forsch.*, **16**, 27 (1966).

(5) G. Lancini and E. Lazzari, U. S. Patent 3,499,967, (March 10 1970); also see the discussion regarding that compd in I. de Carneri, *Arzneim-Forsch.*, **19**, 383 (1969).

(6) F. Kadjfež, V. Šunjić, D. Kolbah, T. Fajdiga, and M. Oklobdžija, *J. Med. Chem.*, **11**, 167 (1968).

(7) M. Hoffer, V. Toome, and A. Brossi, *J. Heterocycl. Chem.*, **3**, 454 (1966).

4-nitro derivative **5** was obtained by thermal decomposition[8] of the quaternary iodide **3** prepd from **2** and MeI.



Biological Activity.—Two-week-old Broad Breasted White (BBW) turkey poults were used throughout the experiments. Sixteen birds (4 × 4), selected according to wt and sex (50% female and 50% male), were used in each group. Turkey prestarter mash, free of other feed additives, was used as the basal ration. The chem compds cited were added to the basal ration in various amts to obtain the desired concns, which varied from as low as 0.00156% to as high as 0.0125% of active drug in feed. The medicated feed was given 3 days in advance of infection and for 21 consecutive days postinfection (total medication period, 24 days). The infection was standardized by administering to each turkey 500 *Heterakis gallinarum* embryonated eggs contg *Histomonas meleagridis*. For each experiment, parallel sets of uninfected, unmedicated controls and infected, unmedicated controls were used.

Parameters of Activity.—The parameters of each compound's activity were expressed as the minimal effective dose in feed, an active compd being one which, at the lowest concn tested, met the following criteria: (i) prevented mortality (0%), (ii) prevented or reduced pathology (average degree of infection, 0 to 1.0), and (iii) maintained the weight gain of the medicated birds within 10% of that of the control birds on the basal ration. The rather strict criteria for the evaluation of efficacy were imperative in assessing the chemotherapeutic potency of the closely related compds tested.

Turkey Poults, LD_{50}.—These studies were carried out in BBW turkey poults, 1 week old. The poults, properly assembled into groups of 6 each (50% female and 50% male), based on body wt, were treated individually by a single oral dose of compd *via* gelatin capsule. Following a 14-day observation period, the LD_{50} values were extrapolated by the method of Miller and Tainter.[9]

(8) C. E. Hazeldine, F. L. Pyman, and J. Winchester, *J. Chem. Soc.*, **126**, 1436 (1924).

(9) L. C. Miller and M. L. Tainter, *Proc. Soc. Exp. Biol. Med.*, **57**, 261 (1944).

Results

The structure–activity relationship data pertinent to the antihistomonal activity of isomeric isopropylnitroimidazoles are shown in Table I. It can be seen that 1-methyl-2-isopropyl-5-nitroimidazole[10] and the known 5-isopropyl-2-nitroimidazole were equally active, MED 0.00312% active drug in feed; as far as the LD_{50} parameter is concerned, the former compd appeared at least 3 times less toxic. Neither of the two 1-isopropyl-substituted nitroimidazoles appeared effective at the highest dosages tested (0.0125%). Dimetridazole, even though it was least toxic, appeared 4 times less effective than 1-methyl-2-isopropyl-5-nitroimidazole (ipronidazole) and 5-isopropyl-2-nitroimidazole.

TABLE I
ANTIHISTOMONAL ACTIVITY OF ISOMERIC ISOPROPYLNITROIMIDAZOLES

Compound	Med,[a] % in feed	LD_{50}, mg/kg
2	0.00312	640 ± 25
6	>0.0125	700 ± 40
7	>0.0125	400 ± 32
8	0.00312	200 ± 22
1	0.0125	>1000

[a] Minimal effective dose.

Experimental Section[11]

1,3-Dimethyl-2-isopropyl-4(5)-nitroimidazolium Iodide (3).—A mixt of 5 g of the nitroimidazole **2** and 50 ml of MeI was refluxed and stirred for 72 hr. The yellow powder was filtered, washed with MeI, and dried to yield 3.08 g of **3**. A sample was recrystd from MeOH to give yellow crystals, mp 184–185° dec. *Anal.* ($C_8H_{14}IN_3O_2$) C, H, N.

1-Methyl-2-isopropyl-4-nitroimidazole (5).—The quaternary iodide **3** (3g) was heated *in vacuo* (0.1 mm) with a free flame. The decompn proceeded smoothly and the distillate (1.26 g) soon solidified. Recrystn from Et_2O gave colorless crystals: mp 76–76.5°; uv λ_{max} (H_2O) infl 230 mμ (ε 3400), 316 (7650). *Anal.* ($C_7H_{11}N_3O_2$) C, H, N. The material, mp 76–76.5°, can be converted into a higher melting form, mp 86–86.5°. The ir spectra of the two modifications in $CHCl_3$ soln are identical.

1-Isopropyl-2-methyl-5-nitroimidazole (6).—A mixt of 2.6 g of 2-methyl-4(5)-nitroimidazole and 6.0 g of 2-propyl tosylate[12] was refluxed in 10 ml of $C_6H_5CH_3$ for 8 hr. The $C_6H_5CH_3$ layer, which sepd upon chilling, was decanted and the residue was dissolved in 40 ml of CH_2Cl_2. The CH_2Cl_2 ext was washed with 1 *N* NaOH until the exts were colorless. Evapn of the CH_2Cl_2 soln yielded **6** as an oil. Treatment with HCl in MeOH yielded 0.8 g of a cryst hydrochloride. Recrystn from *i*-PrOH gave **6**·HCl: mp 169–170°; uv λ_{max} (H_2O) 230 mμ (ε 3330), 321 (8300); pK_a = 2.95. *Anal.* ($C_7H_{11}N_3O_2$.HCl) C, H, N, Cl.

1-Isopropyl-2-methyl-4-nitroimidazole (7).—Na (2.3 g) was dissolved in 100 ml of MeOH and 13.1 g of 2-methyl-4(5)-nitroimidazole was added. After evapn to dryness the resulting Na salt was refluxed for 2 hr in a mixt of 50 ml of DMF and 20 g of *i*-PrI. The soln was evapd, the residue was dissolved in 200 ml of CH_2Cl_2 and washed thoroughly with 3 *N* NaOH. Distn of the CH_2Cl_2 gave a residue, which on crystn from H_2O, gave 12 g of **7**: mp 90–91°; uv λ_{max} (H_2O) infl 225 mμ (ε 3680), 315 (8480); pK_a = 0.80. *Anal.* ($C_7H_{11}N_3O_2$) C, H, N.

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(10) Generic name ipronidazol, trademark Ipropran.

(11) All new compds described gave acceptable analytical data.

(12) H. Gilman and N. J. Beaber, *J. Amer. Chem. Soc.*, **47**, 521 (1925). Prepd as described in this reference for 2-butyl tosylate.