(9) "Pharmacological Experiments on Isolated Preparations," 2nd ed, Livingstone, Edinburgh, 1971, p 112.
(10) J. M. Van Rossum, *Arch. Int. Pharmacodyn.*, 143, 299 (1963).
(11) L. Villa, V. Ferri, E. Grana, O. C. Mastelli, and D. Sosci, *Farmaco Ed. Sci.*, 24, 329 (1969).
(12) H. L. Friedman, *Pharmacologist*, 13, 199 (1971).
(13) A. R. Laddu and P. Somani, *Eur. J. Pharmacol.*, 8, 167 (1969); A. M. Barrett and J. L. Wale, *ibid.*, 12, 372 (1970).
(14) *Beilstein*, 4th ed, 4, 153 (1922).

p-Dimethylaminobenzonitrile. A Chemically Simple Coccidiostat

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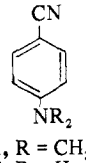
p-Dimethylaminobenzonitrile (**1**) possesses an unexpected degree of anticoccidial activity for so simple a chemical. For example, **1** at 0.025% in the diet prevented mortality and nearly controlled blood loss in feces of chicks infected with a virulent strain of *Eimeria tenella*. Per cent fecal score was 86.4 and weight gain was 82.5% that of noninfected, nonmedicated controls. The same infection caused 70% mortality in infected, nonmedicated controls.

p-Dimethylaminobenzonitrile (**1**) resembles *p*-aminobenzonic acid (PABA) chemically. In protozoa, PABA is a precursor for the biosynthesis of folates. The inhibition of this synthesis by PABA antagonist sulfonamides leads to cellular damage since these organisms are almost completely unable to utilize exogenous folates. In the organism the utilization of folic acid is inhibited by dihydrofolate reductase inhibitors such as certain diaminopyrimidines and related compounds. Synergistic combinations of a dihydrofolate reductase inhibitor and a PABA antagonist have provided reliable therapy for several protozoal diseases and are used in the prophylaxis of coccidiosis.[1-8] The chemical resemblance of **1** to PABA prompted us to attempt to potentiate the activity of **1** by combining with it a folic acid antagonist.



1, R = CH_3
8, R = H
9, R = C_2H_5

Two folic acid antagonists, both of which are known to potentiate coccidiostatic sulfonamides, were found to be effective: 2,4-diamino-5-(4,5-dimethoxy-2-methylbenzyl)-pyrimidine (**2**)[4,8] and 2,4-diamino-5-(4-chlorophenyl)-6-ethylpyrimidine (**3**).[2,5-7] Chicks fed prophylactically a combination of **1** (0.025%) and **2** (0.0075%) and infected with *Eimeria tenella* performed as well as noninfected, nonmedicated controls; there was no mortality, weight gain was equivalent and no blood was detected in feces. The combination of **1** (0.025%) and **3** (0.004%) protected the chicks nearly as well; there was no mortality, weight gain was 84.6% that of controls and only a trace of blood was detected in the feces. Three other folic acid antagonists did not do as well although optimum doses may not have been found for these combinations: 4,6-diamino-1-(*p*-chlorophenyl)-1,2-dihydro-2,2-dimethyl-*s*-triazine·HCl (**4**), 4,6-diamino-1-(3,4-dichlorophenyl)-1,2-dihydro-2,2-dimethyl-*s*-triazine·HCl (**5**),[7] and 2,4-diamino-5-(3,4,5-trimethoxybenzyl)pyrimidine (**6**).[9] Although no mortality was observed with these combinations, weight gain was poorer or blood loss was more severe. Compound **5** is reported to have coccidiostatic activity[7] but did not control mortality in this test. Compound **4** (cycloguanil) and **6** (trimethoprim) are best known for their antimalarial[10] and antibacterial[9] activity, respectively, and do not have significant coccidiostatic activity at these levels. In combination with **1**, compounds **4**, **5**, and **6** did not significantly improve the activity of **1** alone.

Table I. Activity of *p*-Aminobenzonitriles and Combinations with Folate Antagonists against *Eimeria tenella* in Chicks

Compd	% diet	Compd	% diet	% gain	% FS	% survival
1	0.05			44.7	82.5	100.0
1	0.025			82.5	86.4	100.0
1	0.0125			42.3	35.3	100.0
1	0.0125	2	0.0075	102.4	100.0	100.0
1	0.0125	3	0.004	84.6	98.4	100.0
1	0.025	3 7	0.004 0.025	89.0	95.4	100.0
1	0.025	4	0.005	69.7	54.4	100.0
1	0.025	5	0.0025	85.1	85.4	100.0
1	0.025	6	0.0075	60.3	47.7	100.0
8	0.0125			35.8	33.2	90.0
8	0.0125	3	0.004	80.8	90.7	100.0
9	0.025			49.0	48.7	100.0
9	0.025	2	0.0075	80.2	77.4	100.0
9	0.025	3	0.004	71.6	56.9	100.0
9	0.025	6	0.0075	73.2	48.3	100.0
2	0.0075			60.2	20.8	90.0
3	0.004			25.3	24.7	66.7
5	0.0025			39.7	37.4	90.0
Infected, nonmedicated				46.6	15.4	30.0
Noninfected, nonmedicated				100.0	100.0	100.0

A combination of **1** and **2** was also tested against a strain of *E. tenella* which was resistant[11] to the coccidiostat amprolium [1-(2-propyl-4-amino-5-pyrimidylmethyl)-2-methylpyridinium chloride hydrochloride]. Using the same experimental method (14 birds per group), a mixture of **1** (0.025%) and **2** (0.0075%) prevented mortality. Birds receiving amprolium (Amprol Plus, 0.0125%) suffered 29% mortality. Infected, nonmedicated controls suffered 50% mortality.

p-Aminobenzonitrile (**8**) also demonstrated anticoccidial activity and a combination of **8** (0.0125%) and **3** (0.004%) appeared synergistic. Compound **9** at 0.025% in the diet also controlled mortality and may be potentiated by diaminopyridines **2**, **3**, and **6**.

Experimental Section

Cockerel broiler chicks, 16 days old, were weighed individually and separated into weight-balanced groups. Replicate groups of 10 birds were used for each experiment. Data reported are mean values for the 2 groups. Medicated diets were fed 48 hr prior to infection with 200,000 oocysts and continued for 7 days. Individual weights were recorded and mean weight gains relative to noninfected, nonmedicated controls were calculated.

Percentage fecal score (% FS, the relative area of the pan under the cage free of hemorrhagic fecal droppings) was calcd using a grid system and proportionately adjusted for mortality.

Benzonitriles **1**, **8**, and **9** are well known.[12-14] They are commercially available and were purified before use. We are indebted to Hoffmann-LaRoche for a sample of **2** and to Burroughs-Wellcome & Company for samples of **3** and **6**. Compd **4** is a well-known antimalarial.[10] Compd **5** was prepared according to a literature procedure,[15] mp 226–227°. *Anal.* ($C_{11}H_{13}Cl_2N_5$·HCl) C, H, Cl, N.

We are indebted to Dr. R. R. Baron of Salsbury Laboratories for providing the virulent strain of *E. tenella* used in this study and to Dr. W. C. Campbell of the Merck Institute for Therapeutic Research for providing the amprolium-resistant *E. tenella*.

References

(1) L. P. Joyner and S. B. Kendall, *Nature (London)*, **176**, 975 (1955).
(2) L. P. Joyner and S. B. Kendall, *Brit. J. Pharmacol.*, 11, 454 (1956).
(3) C. Horton-Smith, P. L. Long, and H. O. Collier, *ibid.*, **15**, 298 (1956).
(4) M. Mitrovic, E. G. Schildknecht, and G. Fusiek, *Poultry Sci.*, **48**, 210 (1969).
(5) M. L. Clarke, *Vet. Rec.*, **74**, 845 (1962).
(6) M. L. Clarke, *ibid.*, **76**, 818 (1964).
(7) R. E. Lux, *Antibiot. Chemother.*, **4**, 971 (1954).
(8) W. L. Marusich, E. G. Ogrinz, B. Hecht, and M. Mitrovic, *Poultry Sci.*, **50**, 512 (1972).
(9) S. R. M. Bushby and G. H. Hitchings, *Brit. J. Pharmacol. Chemother.*, **33**, 72 (1968).
(10) H. C. Carrington, A. F. Crowther, and G. J. Stacey, *J. Chem. Soc.*, 1017 (1954).
(11) D. K. McLoughlin and M. B. Chute, *J. Parasitol.*, **54**, 696 (1968).
(12) T. van Es, *J. Chem. Soc.*, 1564 (1965).
(13) N. J. Ashley, H. J. Barber, A. J. Ewins, G. Newbery, and A. D. H. Self, *ibid.*, 103 (1942).
(14) G. Lohaus, *Chem. Ber.*, **100**, 2719 (1957).
(15) E. J. Modest, *J. Org. Chem.*, **21**, 1 (1956).

Antiinflammatory Activity of Para-Substituted *N*-Benzenesulfonyl Derivatives of Amino Acids†

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An examination of the literature in the areas of pharmacology and medicinal chemistry reveals great interest in the search for more effective nonsteroidal antiinflammatory agents and the understanding of the inflammatory processes.[1,2] A wide variety of compounds possess clinically useful antiinflammatory properties, most notably pyrazolidinediones, arylalkanoic acids, carboxylic acid amides, salicylates, and anthranilic acids. Certain amino acids have been shown to possess significant activity.[3,4] In view of the above facts, and recent reports that various sulfonamides possess antiinflammatory activity,[5-8] the *N*-benzenesulfonyl derivatives of glycine, sarcosine, DL-alanine, β-alanine, L-histidine, DL-tryptophan, L-proline, L-asparagine, and DL-phenylalanine were prepared and tested for their ability to protect erythrocytes from heat-induced hemolysis.[9] Of all the above derivatives the *N*-benzenesulfonyl derivatives of DL-phenylalanine possessed significant stabilization activity.§ We wish to report here the effects of various substituents on the benzenesulfonyl ring and the effects of stereochemistry in the phenylalanine moiety on antiinflammatory activity.

Chemistry. Compounds **1-10** were prepared in a straightforward manner from either DL-, D-, or L-phenylalanine and the appropriate benzenesulfonyl chloride in aqueous NaOH. Optimum yields were obtained when the pH of the basic solution of phenylalanine was maintained between 9.5 and 10.5 during the course of addition of the benzenesulfonyl chloride. Appropriate physical data are summarized in Table I.

Pharmacology. The ability of a compound to inhibit heat-induced hemolysis of red blood cells has been suggested as a rapid, *in vitro* technique for screening potential antiinflammatory agents.[9] This procedure was followed with the exception that fresh human blood from fasted Type O+ donors was used rather than blood from anesthetized mongrel dogs. We found that fresh human blood gave more consistent results, perhaps due to a lack of stabilizing action of the anesthetic, sodium pentobarbital. The compounds were tested at three dose levels and compared to the standard phenylbutazone. Each value is the average of 9-18 separate values determined as triplicates on the blood of three to give separate subjects. The results are summarized in Table II.

Antiinflammatory activity was measured as inhibition of carrageenin-induced edema in the hind paw of the rat (Sprague-Dawley, 150-200 g) according to the procedure of Winter, *et al.*[10] Edema formation was measured 3 hr after an intraperitoneal injection of test drug suspended in saline and Tween 60 and 2.5 hr after carrageenin. The edema inhibition of each compound (8 rats/group) was compared with animals receiving only the vehicle and animals receiving phenylbutazone. Each value is the average per cent inhibition of paw edema measured on 16-32 rats. The LD_{50} of the more active compounds was determined by the method of Litchfield and Wilcoxon[11] using four dose levels for each compound.

Discussion

In general, compounds which were active in the EMS assay were also active in inhibiting edema formation. The obvious exception is **4** which failed to stabilize erythrocyte membranes but did show activity in the edema assay. The halogenated derivatives (**4-8**) were generally more active in the edema assay than the unsubstituted parent compound (**1**). Stereochemical factors were particularly interesting in the edema assay. Where X = H (**1-3**) the order of activity was D > DL > L, whereas when X = Br (**6-8**) DL > L > D. The effect of halogen substitution is apparent since Br > Cl > F in both assays. *p*-Methoxy or *p*-acetamido substitution was less effective in both assays. Of particular interest is the fact that **6** was more potent than phenylbutazone in both assays although both compounds show similar toxicity as indicated by LD_{50} determinations.

Experimental Section#

General Procedure. A solution of the appropriate benzenesulfonyl chloride (1.2 moles) in dioxane was added to a solution of phenylalanine (in a sufficient amount of 2 *N* NaOH to effect solution) at such a rate as to maintain the pH between 9.5 and 10.5. After addition, the resulting solution was stirred until no further pH decrease was observed. The solution was acidified with concd HCl

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§J. H. Brown and R. F. Borne, unpublished results.

#Melting points were determined on a Thomas-Hoover Uni-Melt apparatus and are uncorrected. Optical rotations were taken on a Perkin-Elmer Model 141 automatic recording polarimeter at *c* 0.56. Elemental analyses were performed by Alfred Berhnardt Mikroanalytisches Laboratorium, Bonn, West Germany, or Chemalytics, Inc., Tempe, Arizona. The ir spectra (KBr, Perkin-Elmer Model 257) and nmr spectra (DMSO-d_6, Jeolco Model C-60-HL) were consistent with all structures.