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*.

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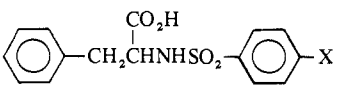
§J. H. Brown and R. F. Borne, unpublished results.

#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.

Table I. Physical Properties of 

No.	X	Configuration	% yield	Mp, °C	Recrystn solvent	$[\alpha]^{22}D$, deg	Formula	Analyses[a]
1	H	DL	58	115-117[b]	C_6H_6		$C_{15}H_{15}NO_4S$	C, H, N
2	H	D	58	130-132[c]	C_6H_6	+6.7 ($CHCl_3$)[c]	$C_{15}H_{15}NO_4S$	
3	H	L	68	131-133[d]	C_6H_6	−7.2 ($CHCl_3$)[d]	$C_{15}H_{15}NO_4S$	
4	F	DL	60	107-109	C_6H_6		$C_{15}H_{14}FNO_4S$	C, H, N, F
5	Cl	DL	77	138-140	EtOH-H_2O		$C_{15}H_{14}ClNO_4S$	C, H, N, Cl
6	Br	DL	64	165-166	EtOH-H_2O		$C_{15}H_{14}BrNO_4S$	C, H, N
7	Br	D	50	136-138	EtOH-H_2O	+18.3 (EtOH)	$C_{15}H_{14}BrNO_4S$	C, H, N
8	Br	L	60	137-139	EtOH-H_2O	−18.9 (EtOH)	$C_{15}H_{14}BrNO_4S$	C, H, N, Br[e]
9	OCH_3	DL	55	149-151	EtOH-H_2O		$C_{16}H_{17}NO_5S$	C, H, N
10	$NHCOCH_3$	DL	45	224-227[f]	EtOH-H_2O		$C_{17}H_{18}N_2O_5S$	

[a] Where analyses are indicated by symbols of the elements, analytical results obtained for those elements were within ±0.4% of the theoretical values. [b] Lit.[12] mp 127-128°. [c] Lit.[13] mp 133°, $[\alpha]^{25}D$ +6.7° ($CHCl_3$). [d] Lit.[13] mp 133°, $[\alpha]^{25}D$ −7.0° ($CHCl_3$). [e] C: calcd, 46.89; found: 47.32; Br: calcd, 20.79; found: 21.29. [f] Lit.[14] mp 218-219°.

Table II

No.	% inhibition of heat-induced hemolysis (n)[a] Concn, M 10^{-3}	10^{-4}	10^{-5}	% inhibition of edema (n)[a] Dose, mg/kg 60	120	LD_{50}, mg/kg
1	34 (12)	31 (9)	18 (9)	18 (32)	19 (32)	820
2	35 (12)	29 (9)	27 (9)	32 (16)	35 (16)	850
3	17 (9)	0 (9)	0 (9)	0 (16)	0 (16)	
4	0 (9)	0 (9)	0 (9)	50 (16)	19 (16)	
5	65 (9)	0 (9)	0 (9)	37 (32)	31 (32)	
6	80 (15)	32 (15)	5 (9)	43 (32)	74 (32)	385
7	79 (18)	23 (18)	0 (9)	26 (16)	32 (16)	350
8	67 (9)	11 (9)	11 (9)	31 (16)	60 (16)	
9	29 (9)	48 (9)	0 (9)	10 (16)	35 (16)	
10	24 (9)	26 (9)	27 (9)	34 (16)	28 (16)	
Phenylbutazone	75 (15)	50 (15)	11 (15)	34 (32)	52 (32)	336[b]

[a] n = No. of determinations per concn or dose level. [b] Ref 15.

to a pH of 2-3 and vigorously stirred. The resulting solid was collected, dried, and recrystallized from the appropriate solvent system.

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Quaternary 4- (and 5-) Azolylpyridazinium Salts. A New Class of Oral Hypoglycemic Agents

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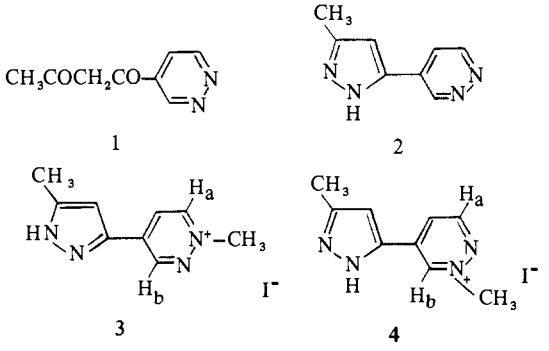
An extensive series of quaternary azolylpyridinium salts including pyrazolyl,[1] isoxazolyl,[2-5] 1,2,4-oxadiazolyl,[6] thiazolyl,[7] oxazolyl,[8] thienyl, furyl, and pyrrolyl[9] derivatives has been found to display oral hypoglycemic activity in laboratory animals. We have also described[10] a number of azolylpyridinium salts which do not induce hypoglycemia. In order to delineate further the effect of structural changes on hypoglycemic activity, we have synthesized a series of quaternary salts in which the pyridinium group is replaced by pyridazinium and the five-membered heterocycle is chosen from those included in the active families listed above.

The pyrazolylpyridazinium salts **3** and **4** were prepared in a manner similar to that used for the pyrazolylpyridinium salts.[1] Thus, ethyl pyridazine-4-carboxylate[11] was condensed with Me_2CO to give the β diketone **1**, which was then allowed to react with N_2H_4 to provide the pyrazolylpyridazine **2**. Quaternization of **2** with MeI gave a separable mixture of the 4- and 5-pyrazolylpyridazinium salts



3 and **4**. The structural assignments are based upon pH-dependent uv spectra. In earlier work,[1] it was observed that