mination using polyfluorokerosine as the standard gave M^+ = 442.3745 (calc. M^+ = 442.3811).

The difference in melting points [lit. (10) betulin mp 252–253°] was due to traces of betulinic acid. The IR spectra of the isolated betulin (KBr pellet) and that of an authentic sample of betulin plus traces of betulinic acid were in complete agreement.

Preparation of Betulin Diacetate—Betulin (12 mg), pyridine (0.5 ml), and acetic anhydride (0.5 ml) were refluxed for 1 hr. After cooling, the reaction mixture was poured over crushed ice. The precipitated compound was collected and washed thoroughly with water until free of acetic acid and pyridine. After drying under vacuum, the compound was recrystallized from absolute ethanol. Approximately 10 mg of solid needles of betulin diacetate was obtained, mp 218–221° [lit. (10) mp 223–224°]. The IR spectra of the prepared betulin diacetate and that of an authentic sample were in complete agreement.

REFERENCES

(1) F. E. Lloyd, "The Carnivorous Plants," Chronica Botanica Co., Waltham, Mass., 1942, pp. 17–39.

(2) G. L. Plummer and J. B. Kethley, *Bot. Gaz.*, **125**, 245(1964).

(3) R. M. Harper, *J. Elisha Mitchell Sci. Soc.*, **34**, 110(1918).

(4) C. R. Bell, *ibid.*, **65**, 137(1949).

(5) S. McDaniel, Ph.D. dissertation, Florida State University, Tallahassee, Fla., 1966.

(6) M. E. Wall, M. C. Wani, C. E. Cook, K. H. Palmer, A. T. McPhail, and G. A. Sim, *J. Amer. Chem. Soc.*, **88**, 3888(1966).

(7) K. Sheth, E. Bianchi, R. Wiedhopf, and J. R. Cole, *J. Pharm. Sci.*, **62**, 139(1973).

(8) J. Simonsen and W. C. J. Ross, "The Terpenes," vol. IV, Cambridge University Press, London, England, 1957, p. 287.

(9) "Dictionary of Organic Compounds," vol. I, Oxford University Press, New York, N.Y., 1965, p. 391.

(10) "The Merck Index," 8th ed., Merck & Co., Inc., Rahway, N.J., 1968, p. 146.

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Anti-Inflammatory Activity of *para*-Substituted *N*-Benzenesulfonyl Derivatives of Anthranilic Acid

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Abstract □ Several *para*-substituted *N*-benzenesulfonyl derivatives of anthranilic acid were prepared and evaluated for anti-inflammatory activity using erythrocyte membrane stabilization and carrageenin-induced rat paw edema assays. *N*-*p*-Bromobenzenesulfonylanthranilic acid possessed greater activity than phenylbutazone in both assays. Quantitative structure–activity correlations employing the Hansch method were unsuccessful in the erythrocyte membrane stabilization assay. However, correlations were obtained in the rat paw edema assay, and it appears that the activity of these derivatives depends solely on the lipophilic character of the substituents on the benzenesulfonyl ring.

Keyphrases □ Anthranilic acid, *para*-substituted *N*-benzenesulfonyl derivatives—synthesized and screened for anti-inflammatory activity □ Anti-inflammatory activity—*para*-substituted *N*-benzenesulfonyl derivatives of anthranilic acid, synthesis and screening □ Structure–activity relationships—*para*-substituted *N*-benzenesulfonyl derivatives of anthranilic acid

Considerable effort continues to be expended in the search for effective nonsteroidal anti-inflammatory agents. Various chemical classes have been demonstrated to possess therapeutically useful properties—*viz.*, salicylates, carboxylic acid amides, arylalkanoic acids, pyrazolidinediones, and anthranilic acids. Recently, significant anti-inflammatory activity was reported for members of a series of *para*-substituted *N*-benzenesulfonyl derivatives of amino acids, most notably phenylalanine (1). While phenylalanine itself possesses anti-inflammatory activity (2), the *N*-benzenesulfonyl moiety was found to enhance this activity significantly. In view of the pronounced anti-inflammatory activity of the anthranilic acid derivatives mefenamic acid[1] and flufenamic acid[2], earlier studies were extended to the synthesis of various *N*-*para*-substituted benzenesulfonyl derivatives of anthranilic acid (I–VI) and the examination of the influence of the *para*-substituent through quantitative structure–activity correlations.

EXPERIMENTAL[3]

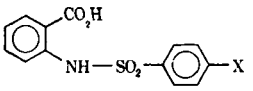
General Synthetic Procedure—A solution of the appropriate benzenesulfonyl chloride (1.2 moles) in dioxane was added to a solution of anthranilic acid (1.0 mole dissolved in sufficient 2 *N* NaOH) at such a rate as to maintain the pH of the mixture between 9.5 and 10.5. Additional 2 *N* NaOH was added when the

[1] Ponstel, Parke, Davis and Co.

[2] Arlef, Parke, Davis and Co.

[3] Melting points were determined on a Thomas-Hoover Unimelt apparatus and are uncorrected. Elemental analyses were performed by Chemalytics, Inc., Tempe, Ariz. The IR spectra (KBr, Perkin-Elmer model 256) and NMR spectra (dimethyl sulfoxide-d_6, Jeolco model C-60-HL) were consistent with the proposed structures.

Table I—Properties of Anthranilic Acid Derivatives



Compound	X	Yield, %	Melting Point	Formula	Analysis, % Calc.	Found
I	H	80	211–214°[a]	—	—	—
II	CH_3	78	229–231°	$C_{14}H_{13}NO_4S$	C 57.72	57.77
					H 4.50	4.22
					N 4.81	4.53
III	NO_2	80	215–218°	$C_{13}H_{10}N_2O_6S$	C 48.46	48.64
					H 3.10	3.59
					N 8.69	8.56
IV	F	65	171–173°	$C_{13}H_{10}FNO_4S$	C 52.89	52.87
					H 3.42	3.19
					N 4.74	4.45
V	Cl	72	201–203°	$C_{13}H_{10}ClNO_4S$	C 50.09	50.16
					H 3.24	3.17
					N 4.49	4.19
VI	Br	81	221–223°	$C_{13}H_{10}BrNO_4S$	C 43.84	43.83
					H 2.84	2.78
					N 3.93	3.79

[a] Lit. (12) mp 211–212°.

Table II—Pharmacological Results

Compound	Percent Inhibition of Heat-Induced Hemolysis Concentration, M 10^{-5}	10^{-4}	10^{-3}	Percent Inhibition of Edema Dose, mg/kg[a] 30	60	120	Estimated I_{50}[b]	LD_{50}, mg/kg[a]
I	0	0	55.9	26.3	29.2	41.0	168	540
II	18.7	37.4	31.7	23.2	−25.6	−0.1	—	890
III	22.8	26.0	73.2	16.8	14.6	47.4	125	—
IV	17.9	37.4	87.8	21.4	20.8	64.0	100	395
V	0	62.6	66.7	22.6	35.0	58.5	99	275
VI	17.0	30.2	85.7	22.6	71.5	83.8	47	385
Phenylbutazone	17.2	35.0	83.7	—	34	52	—	336[c]

[a] Administered intraperitoneally. [b] Graphically estimated dose that produces 50% inhibition of edema. [c] Ref. 13.

pH fell below 9.5. After addition of the benzenesulfonyl chloride, the resulting solution was stirred until no further pH decrease was observed. The solution was then acidified with concentrated hydrochloric acid to pH 2–3 and vigorously stirred. The resulting solid was collected and recrystallized from aqueous ethanol. Appropriate physical data are summarized in Table I.

Erythrocyte Membrane Stabilization Assay—The procedure of Brown *et al.* (3) was followed using fresh human blood from fasted Type O$^+$ donors. The compounds were tested at three dose levels and compared to phenylbutazone as a standard. Each value (Table II) is the average of 18 separate values determined as triplicates on the blood of three to five subjects.

Carrageenin Rat Paw Edema Assay—Anti-inflammatory activity was measured as inhibition of carrageenin-induced edema in the hindpaw of a Sprague–Dawley rat (150–200 g) according to the procedure of Winter *et al.* (4). Edema formation was measured 3 hr after an intraperitoneal injection of test drug suspended in saline and polysorbate 60 and 2.5 hr after carrageenin. The edema inhibition of each compound (eight rats per group) was compared with animals receiving only the vehicle and animals receiving phenylbutazone. No irritant effects were observed upon administration of any compound tested. Each value (Table II) is the average percent inhibition of paw edema measured on 32 rats.

Toxicity Data—The LD_{50} values were determined and calculated by the method of Litchfield and Wilcoxon (5) following intraperitioneal injections of four dose levels for each compound (Table II).

Quantitative Structure–Activity Correlations—The concentration of *para*-substituted *N*-benzenesulfonylanthranilic acid required for 50% inhibition of edema (I_{50}) was estimated graphically from the data reported herein. In accordance with the procedure developed by Hansch (6), the I_{50} values are measures of the concentration of anthranilic acid derivative with substitutent X required to exert a standard biological response. The structural contributions to this response are then factored in the form of the Hammett-type free energy relationship as follows:

$$\log BR_x = k\pi x + k'\sigma x + k'' Es_x + k''' \qquad \text{(Eq. 1)}$$

where BR_x is the biological response, $1/I_{50}$, for a molecule with substituent X; and the constants k, k', k'', and k''' are fixed for a given system and are determined by computerized multiparameter regression analysis. The π values for substituents were determined from the apparent log P values for the molecules where $\pi = \log P_x - \log P_H$, where P_x is the octanol-water partition coefficient for *N*-benzenesulfonylanthranilic acid with substituent X and P_H is the partition coefficient for unsubstituted *N*-benzenesulfonylanthranilic acid. The partition coefficients were measured in octanol *versus* 0.13 M phosphate buffer, pH 8.0, and are the average of five or more determinations for each substituent. The π values are used here instead of log P since the partition coefficients measured were apparent values and not true partition coefficients. Also, the substituent constant, π, is more useful in emphasizing the effect of substituents changes at a single position. The Hammett σ values (7) and the Taft E_s values (8) were taken from the sources indicated. Table III contains the data on substituent changes and biological activities. The correlations were derived using a computer[4] with a multiparameter regression analysis program.

RESULTS AND DISCUSSION

The syntheses of the target compounds were achieved in a straightforward manner (Table I). Optimum yields were obtained when pH of the basic solution of anthranilic acid was maintained

[4] IBM 360/65.

Table III—Edema Inhibition by Anthranilic Acid Derivatives

Compound	π^a	E_s^b	σp^c	Obs. log $1/I_{50}^d$	Calc. log $1/I_{50}^e$
I	0	1.24	0.0	0.22	0.22
II	0.51	0.0	−0.17	—	—
III	0.43	−1.28	0.78	0.41	0.40
IV	0.49	0.78	0.06	0.47	0.43
V	1.00	0.27	0.23	0.50	0.65
VI	1.29	0.08	0.23	0.88	0.78

[a] Calculated from measured log P values; apparent log P for Compound I is −0.46. [b] From Ref. 14. [c] From Ref. 15. [d] Estimated graphically from data in Table II; I_{50} value for Compound II could not be derived; I_{50} is in millimoles. [e] Calculated *via* Eq. 2.

between 9.5 and 10.5 during the addition of the benzenesulfonyl chloride.

Pharmacological results are presented in Table II. The carrageenin rat paw edema model of acute inflammation has been widely used as an accurate model for the detection of potential anti-inflammatory agents (9). 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 anti-inflammatory agents (3), and this procedure was followed. The reasonable correlation observed between the carrageenin rat paw edema assay and the erythrocyte membrane stabilization assay in Table II substantiates the proposal (3) that the erythrocyte membrane stabilization assay can serve as a rapid *in vitro* screening procedure for this class of pharmacological agents. The halogenated derivatives (IV, V, and VI) possessed significant activity in both assays and were more active than the unsubstituted parent compound (I). The nitro derivative (III) possessed greater activity in the erythrocyte membrane stabilization assay than in the carrageenin rat paw edema assay. The methyl derivative (II) gave interesting results. While weak activity was observed in the erythrocyte membrane stabilization assay, II induced edema formation at higher doses in the carrageenin rat paw edema assay. The *p*-bromo derivative possessed the greatest activity in the carrageenin rat paw edema assay. In previous studies of *N-para*-substituted benzenesulfonyl derivatives of phenylalanine (1), the *N-p*-bromobenzenesulfonyl derivative was also the most potent member of the series and was more active than phenylbutazone (74% *versus* 52% inhibition of carrageenin rat paw edema at 120 mg/kg, respectively). Unfortunately, while VI possesses significant activity in the carrageenin rat paw edema assay, it is only slightly less toxic than phenylbutazone based upon LD_{50} data.

In an attempt to correlate edema I_{50} values with the substituent constants in Table III, all possible combinations of the parameters were investigated including potential parabolic relationships. The best relationship was Eq. 2 involving π alone:

$$\log 1/I_{50} = 0.44\,(\pm 0.34)\,\pi + 0.22\,(\pm 0.27)$$
$$n = 5 \quad s = 0.11 \quad r = 0.92 \qquad \text{(Eq. 2)}$$

where the values in parentheses are the 95% confidence intervals for the coefficients, n is the number of data points, s is the standard deviation, and r is the correlation coefficient. An equation involving a combination of steric and electronic parameters (r = 0.96) must be discounted because of the extremely high degree of covariance between E_s and σ, as indicated by a squared correlation coefficient of 0.97 for E_s *versus* σ. Neither term alone can account for the variation in biological activity, nor does addition of either parameter to π significantly improve Eq. 2.

The positive coefficient associated with π in Eq. 2 suggests that increases in lipophilic character will result in increases in biological activity. The relative size of this coefficient places it in the intermediate range of lipophilic sensitivity, 0.40–0.85 (10). While no similar biological test system was correlated, Eq. 2 is similar to that derived for the ability of aromatic acids to induce fibrinolysis of hanging blood clots (10, 11). This may suggest that the biological effect of the anthranilic acid derivatives results from an analogous interaction with some protein at the site of action.

Since the biological test employed here is an *in vivo* system, one might anticipate a parabolic relationship to arise between lipophilic character and biological action as a result of the "random-walk" process (6). It is highly probable that the substituents evaluated all lie on a linear portion of the parabola since the substituent variations examined were primarily designed to delineate electronic effects and do not cover a wide range in lipophilicity.

Attempts to correlate the effects of I–VI on heat-induced hemolysis were unsuccessful.

These preliminary studies provide some valuable information. The biological activity of these derivatives, at least against edema, solely depends on lipophilic character with respect to variations on the benzenesulfonyl ring. More lipophilic substituents could provide valuable information about the limits of this dependency.

REFERENCES

(1) R. F. Borne, R. L. Peden, I. W. Waters, M. Weiner, and M. Walz, *J. Med. Chem.*, **15**, 1325(1972).

(2) R. H. Davis, J. S. Fisher, and L. McGowan, *J. Endocrinol.*, **41**, 603(1968).

(3) J. H. Brown, H. K. Mackey, and D. A. Riggilo, *Proc. Soc. Exp. Biol. Med.*, **125**, 837(1967).

(4) C. A. Winter, E. A. Risley, and G. W. Nuss, *J. Pharmacol. Exp. Ther.*, **141**, 369(1963).

(5) J. T. Litchfield and F. Wilcoxon, *ibid.*, **96**, 99(1949).

(6) C. Hansch, in "Drug Design," vol. I, E. J. Ariëns, Ed., Academic, New York, N.Y., 1971, pp. 271–342.

(7) L. P. Hammett, "Physical Organic Chemistry," 2nd ed., McGraw-Hill, New York, N.Y., 1970, pp. 347–390.

(8) R. W. Taft, Jr., in "Steric Effects in Organic Chemistry," M. S. Newman, Ed., Wiley, New York, N.Y., 1956, pp. 556–675.

(9) M. DiRosa, *J. Pharm. Pharmacol.*, **24**, 89(1972).

(10) C. Hansch and W. J. Dunn, III, *J. Pharm. Sci.*, **61**, 1(1972).

(11) C. Hansch and K. N. Von Kaulla, *Biochem. Pharmacol.*, **19**, 2193(1970).

(12) L. Bauer and S. V. Miarka, *J. Amer. Chem. Soc.*, **79**, 1983(1957).

(13) J. Ben-Bassat, E. Peretz, and F. G. Sulman, *Arch. Int. Pharmacodyn. Ther.*, **122**, 439(1959).

(14) E. Kutter and C. Hansch, *J. Med. Chem.*, **12**, 647(1969).

(15) D. H. McDaniel and H. C. Brown, *J. Org. Chem.*, **23**, 420(1958).

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