Synthesis and Anti-Inflammatory Activity of Fluorinated Benzamides

WILLIAM S. DORSEY and WILLIAM D. EASTERLY, Jr.

Abstract □ Six fluorine-containing *N*-substituted benzamides were synthesized, and their physical constants were determined. Their acute toxicity was determined, and LD_{50} values are given. The compounds were screened for anti-inflammatory activity using a modification of the rat-paw edema method. None of the compounds showed activity comparable to hydrocortisone acetate or indomethacin. However, two of the compounds showed sufficient activity to warrant further study.

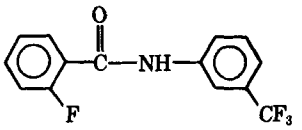
Keyphrases □ Benzamides, fluorinated—synthesized as potential anti-inflammatory agents, physical constants determined, screened using rat-paw edema test □ Anti-inflammatory agents, potential—synthesis and pharmacological evaluation of six fluorine-containing *N*-substituted benzamides

The increased recognition of the widespread and diversified nature of the inflammatory diseases, together with the empirical manner of treatment, recently has stimulated more research in this area. Some research has resulted in the development of new, nonsteroidal drugs, several of which have shown significant therapeutic activity. Indomethacin (1, 2), mefenamic acid (3), and flufenamic acid (4) are typical examples.

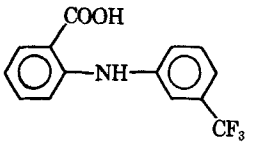
The compounds synthesized in this investigation were screened for anti-inflammatory activity because of their structural similarity to flufenamic acid and because they all contain the trifluoromethyl group which has been associated with anti-inflammatory activity, as shown by Rorig and Wagner (5). The similarity in structure may be seen when one of the prepared compounds is compared with flufenamic acid. The work of Rorig and Wagner (5) indicated that certain 4-trifluoromethyl pyrimidines are quite effective in alleviating the heat, swelling, and rubor characteristics of the inflammatory response to tissue injury.

EXPERIMENTAL

Synthesis—All of the compounds were prepared by a modification of the procedure used by Chase and Weller (6). Equimolar quantities of the appropriate amine[1] and acid chloride[2] were reacted together at room temperature in a mixture of chloroform and pyridine. The amine and an equimolar portion of pyridine were placed in the chloroform, and the acid chloride was added dropwise with stirring. When the initial exothermic reaction subsided, the mixture was refluxed for 30 min. The resulting amide was separated from the reaction mixture by filtration and then recrystallized from $CHCl_3$, ethanol, or aqueous ethanol, depending upon its solubility. The product was then dried in an oven at 40–50° and assayed.



N-(3-trifluoromethylphenyl)-2-fluorobenzamide



flufenamic acid

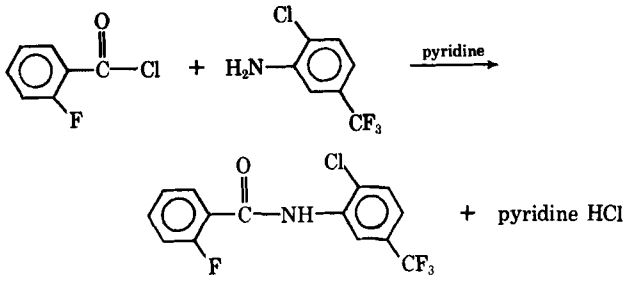
The preparation of *N*-(2-chloro-5-trifluoromethylphenyl)-2-fluorobenzamide represents the general reaction (Scheme I) involved in the preparation of the compounds reported in this paper.

The analytical results for the compounds are given in Table I[3].

Acute Toxicity Studies—The approximate LD_{50} of each compound was determined, using basically the method of Deichman and LeBlanc (7). This method consisted of injecting intraperitoneally four albino rats with the test compound suspended in 1% methylcellulose solution. The dosage to be administered to each of the four animals was based on data from Jordan and Easterly (8), in which *N*-(3-trifluoromethylphenyl)-2,4-dichlorobenzamide, an analog of the compounds prepared in this study, showed an LD_{50} greater than 7.0 g./kg. body weight. Two animals were given doses above 7.0 g./kg., and two animals were given doses below 7.0 g./kg. After 24 hr., the animals were observed and any fatalities were recorded. Two additional doses were subsequently given at dosage levels in between the closest fatal and nonfatal doses. This procedure allowed for a more exact determination of the approximate LD_{50} values.

Results are shown in Table II.

Anti-Inflammatory Studies—The method used to test the compounds for anti-inflammatory activity was a modification of that described by Winter *et al.* (9). Each compound tested was suspended



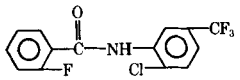
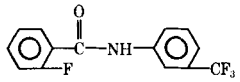
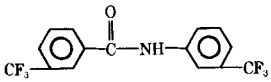
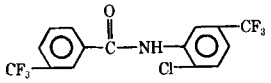
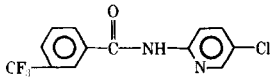
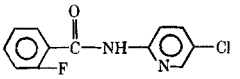
Scheme I

[1] Obtained from Aldrich Chemical Co., Inc., Milwaukee, Wis.

[2] Obtained from Matheson Chemical Co., Inc., Norwood, Ohio; Pierce Chemical Co., Rockwood, Ill.; and K & K Laboratories, Inc., Plainview, N. Y.

[3] Nitrogen assays were by the Kjeldahl method and halogen determinations were performed by Galbraith Laboratories, Inc., Knoxville, Tenn.

Table I—*N*-Substituted Benzamides

Compound Substituent Group	Structure of Compound	Melting Point[a]	Analysis, %—Calc.	Found
N-(2-Chloro-5-trifluoromethylphenyl)-2-fluoro-		98–100°	Cl 11.18 F 23.94 N 4.41	11.10 24.08 4.52
N-(3-Trifluoromethylphenyl)-2-fluoro-		96–98°	Cl — F 26.95 N 4.94	— 26.66 4.99
N-(3-Trifluoromethylphenyl)-3-trifluoromethyl-		122–124°	Cl — F 34.23 N 4.20	— 33.04 4.12
N-(2-Chloro-5-trifluoromethylphenyl)-3-trifluoromethyl-		123–125°	Cl 9.67 F 31.02 N 3.81	9.81 31.35 3.81
N-(4-Chloro-2-pyridyl)-3-trifluoromethyl-		115–117°	Cl 11.81 F 18.96 N 9.32	11.90 19.37 9.25
N-(4-Chloro-2-pyridyl)-2-fluoro-		105–108°	Cl 14.17 F 7.58 N 11.18	13.92 7.75 11.05

[a] All melting points were taken on a Thomas-Hoover capillary melting-point apparatus.

in a 1% methylcellulose solution and administered orally by stomach tube to each of six albino male Holzman rats, weighing approximately 250–300 g. each. To ensure equal hydration in all animals, the suspensions were prepared to contain the desired dosage in a 5.0-ml. volume.

One hour after feeding of the compound, 0.05 ml. of a 1% suspension of carrageenin in sterile saline was injected into the plantar region of the hind paw of each animal. The volume of the paw was determined immediately and again after 3 hr.

Foot volume was measured by immersing the paw into mercury contained in a Pyrex U-tube of approximately 30 mm. diameter. The paw was immersed to a previously marked ink line at the level of the lateral malleolus. The mercury in the left column of the U-tube was in contact with isopropyl alcohol containing a small amount of the dye, eosin Y. The interface of the two immiscible liquids was at the midpoint of a bulb, of approximately 85 mm. diameter, connected at the top with a capillary tube graduated in millimeters. The mercury displaced by the immersed paw displaced a proportionate amount of the lighter alcohol, the level of which extended into the calibrated capillary tube. Volume was measured by reading the difference in millimeters of the colored liquid in the capillary tube before and after immersion and converting this millimeter reading into milliliters. Immersion of an object of 1 ml. in volume caused a displacement of 28.4 mm. in the level of the liquid in the capillary tube.

The paws appeared to reach a condition of peak edema in about 3 hr.; therefore, another volume determination was performed at that time. The increase in volume was calculated by comparing the paw volume measurements at injection time and 3 hr. later. Control animals received only tap water, and another group of animals received only 1% methylcellulose. Results are given in Table III.

Table II—Acute Toxicity

Compound	LD_{50}, g./kg.
Benzamide, *N*-(2-chloro-5-trifluoromethylphenyl)-2-fluoro-	>15.7
Benzamide, *N*-(3-trifluoromethylphenyl)-2-fluoro-	4.50
Benzamide, *N*-(3-trifluoromethylphenyl)-3-trifluoromethyl-	>12.5
Benzamide, *N*-(2-chloro-5-trifluoromethylphenyl)-3-trifluoromethyl-	>15.1
Benzamide, *N*-(4-chloro-2-pyridyl)-3-trifluoromethyl-	13.70
Benzamide, *N*-(4-chloro-2-pyridyl)-2-fluoro-	4.80

RESULTS AND DISCUSSION

None of the compounds tested showed edema-inhibiting activity comparable to hydrocortisone acetate or indomethacin. Two of the compounds, the *N*-(4-chloro-2-pyridyl)-2-fluorobenzamide and the *N*-(2-chloro-5-trifluoromethylphenyl)-2-fluorobenzamide, were significantly more active than the others and may warrant further testing.

It would appear that the fluorine atom in the *ortho*-position of the benzamide ring may be a necessary structural component for the anti-inflammatory activity. Furthermore, it would appear that the *N*-chloropyridylbenzamides have slightly more anti-inflammatory (antiedema) activity than those without this chemical group. More compounds in this series would have to be prepared and tested, however, before definitive structure–activity relationships could be established.

Results were calculated as a percentage increase rather than as a total increase in the volume of the paw before and 3 hr. after in-

Table III—Anti-Inflammatory Studies

Compound	Dose, mg./kg.	Mean Percent Increase in Paw Volume (±*SEM*)	Percent Inhibition of Edema (Mean)[a]
Controls			
Tap water	5.0 ml.	35.50 ± 4.29	0
Methylcellulose, 1%	5.0 ml.	37.94 ± 1.28	−7.34[b]
Hydrocortisone[c] acetate	20	2.08 ± 0.65	93.40
Indomethacin[d]	10	14.35 ± 2.71	59.60
Benzamide, *N*-(2-chloro-5-trifluoromethylphenyl)-2-fluoro-	500	27.65 ± 8.49	21.59
Benzamide, *N*-(3-trifluoromethylphenyl)-2-fluoro-	500	33.28 ± 3.77	5.85
Benzamide, *N*-(3-trifluoromethylphenyl)-3-trifluoromethyl-	500	37.39 ± 7.18	−5.78
Benzamide, *N*-(2-chloro-5-trifluoromethylphenyl)-3-trifluoromethyl-	500	35.58 ± 5.95	−0.65
Benzamide, *N*-(4-chloro-2-pyridyl)-3-trifluoromethyl-	500	32.09 ± 5.94	9.64
Benzamide, *N*-(4-chloro-2-pyridyl)-2-fluoro-	500	23.35 ± 3.31	34.00

[a] Data were calculated on the basis that the controls showed no inhibition. [b] Negative values indicate a decrease in inhibition (*i.e.*, increased edema). [c] Produced by Roussel Corp., New York, N. Y. [d] Supplied by Merck Sharp & Dohme Research Lab., Rahway, N. J.

jection of the phlogistic agent. This was done to offset any possible error caused by the difference in the size of the animals used. The standard error of the mean (*SEM*) was calculated according to the formula given by Burn *et al.* (10):

$$s\bar{y} = \sqrt{\frac{S(y - \bar{y})^2}{N(N-1)}} \qquad \text{(Eq. 1)}$$

where $s\bar{y}$ = *SEM*, $\bar{y}$ = mean value, y = any single value, N = total number of animals used in the assay, and S = sum.

The method and techniques developed for the anti-inflammatory screening carried out in this investigation proved to be quite satisfactory. The method was simple and quick, and the apparatus was sufficiently sensitive for the determination of therapeutic levels of antiedema activity. Results with the hydrocortisone acetate and indomethacin, the control compounds, compared favorably with the results of others using antiedema assay procedures (2). Carrageenin seemed to possess a distinct advantage as a phlogistic agent because it produced an edema effectively controlled by the single, oral, nontoxic doses of the known anti-inflammatory agents used in the studies.

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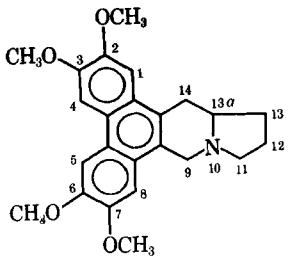
Alkaloids of Tylophora III: New Alkaloids of *Tylophora indica* (Burm) Merrill and *Tylophora dalzellii* Hook. f.

KOPPAKA V. RAO*, RICHARD A. WILSON, and BERNICE CUMMINGS

Abstract □ During a reexamination of the alkaloidal constituents of *Tylophora indica*, three new alkaloids (designated A, B, and C) were isolated. Analytical and spectral data indicate that these alkaloids are related to tylophorine and tylophorinine, which are also present in the plant. Alkaloid B is shown to be a desmethyltylophorine, and Alkaloid C is shown to be a desmethyltylophorinine. *T. dalzellii* has a relatively low alkaloid content, and desmethyltylophorinine is the major constituent. A brief description of the antileukemic activity of desmethyltylophorinine is presented.

Keyphrases □ *Tylophora indica*—isolation of three new alkaloids □ *Tylophora dalzellii*—desmethyltylophorinine determined as major constituent □ Desmethyltylophorinine—isolated and identified from *Tylophora indica* and *Tylophora dalzellii* □ Desmethyltylophorine—isolated and identified from *Tylophora indica*

In a recent publication, isolation of six new alkaloids from *Tylophora crebriflora*, together with the known members tylocrebrine, tylophorine, and septicine, was described (1). Structures for the new members were proposed based on the dibenzo[*f,h*]pyrrolo[1,2*b*]isoquinoline skeleton with four or five oxygen-bearing substituents (2). Interest in these alkaloids arose because of the antitumor activity shown by some members of this group. In this connection, it seemed worthwhile to reexamine the alkaloidal constituents of the related species *T. indica* (Burm) Merrill. A related species, *T. dalzellii* Hook. f., had not been examined, so a brief study of it also was undertaken.



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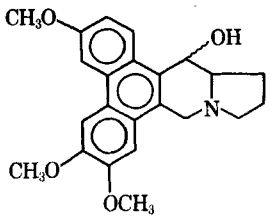

II

DISCUSSION

From *T. indica*, Govindachari *et al.* (3) isolated two alkaloids tylophorine and tylophorinine. They assigned Structures I and II for these compounds, respectively, and later recorded their syntheses (4–7).

The crude alkaloid from *T. indica*[1] was isolated by the following steps: extraction with 0.5% methanolic acetic acid, concentration,

[1] The plant material used was received from India. It was identified and a voucher specimen was preserved at Chas. Pfizer & Co., Inc., Maywood, N. J.