Synthesis and Biological Evaluation of Substituted α-Phenylcinnamonitriles

J. T. STEWART

Abstract □ The synthesis of some new α-phenylcinnamonitriles is reported along with biological results in the areas of chemotherapy, gastrointestinal activity, fertility control, parasitology, and agricultural screening. The compounds showed negative or marginal biological activity in the areas tested.

Keyphrases □ α-Phenylcinnamonitriles, substituted—synthesis, biological evaluation □ Pharmacological, biological screening—substituted α-phenylcinnamonitriles

Interest in this laboratory in the use of α-phenylcinnamonitriles as potential analytical reagents resulted in the synthesis of some α-phenylcinnamonitrile derivatives (Table I). A literature search revealed that derivatives of this type have been shown to possess some antibacterial, antifungal, hypocholesterolemic, and estrogenic activity (1–4).

In this paper, the synthesis of some new α-phenylcinnamonitriles is reported, along with biological results for these compounds in the areas of chemotherapy, gastrointestinal activity, fertility control, parasitology, and agricultural screening.

The α-phenylcinnamonitriles were synthesized *via* base-catalyzed condensations of substituted phenylacetonitriles and benzaldehydes (5, 6). Starting materials required for the synthesis either were available commercially or were readily obtained in a few synthetic steps from commercial materials.

The nitro, isothiocyanate, amine, and quaternary amino compounds which were synthesized are shown, together with their melting points and elemental analysis data, in Table I. Compounds 3, 4, and 6–13 are new compounds. Compounds 1, 2, and 5 are known in the literature, but no biological data have been reported for them.

BIOLOGICAL SCREENING[1]

Compounds 1, 2, 4–10, and 13 were evaluated using the assay method of Conkey and Carlson (7) in a chemotherapy screen, involving a variety of Gram-positive and Gram-negative bacteria and fungi of industrial importance. The bacteria and fungi used were *Aerobacter aerogenes* (IPC 500)[2], *Bacillus mycoides* (IPC 509), *Staphylococcus aureus* (ATCC 6538), *Pseudomonas aeruginosa* (ATCC 10145), *Saccharomyces cerevisiae*[3], *Rhizoctonia solani* (ATCC 16115), and *Pullularia pullulans* (ATCC 9348). None of the substances tested showed any activity in this preliminary screen.

The gastrointestinal assay on Compounds 1 and 5 evaluated the ability of these substances to depress the volume of gastric secretions to one-half or less of the control value and/or raise the pH of the secretions above 2.5. Male rats, starved for 24 hr., were given a suspension of the test compound in 1% methylcellulose solution by stomach tube. After 30 min., the stomach was exposed under light anesthesia and the pyloric end tied off. The incision was closed, and the animals were allowed to recover from the anesthesia. Two hours later, the animals were killed and the volume and pH of the stomach secretions were measured. The compounds were tested in this assay at a standard dose level of 16 mg./kg., and both were inactive.

Two milligrams each of Compounds 1, 2, 4, 7, and 12 was tested in a fertility control assay. Each compound was administered orally once a day for 12 days to adult female mice. The suspending medium was carboxymethylcellulose. On the 4th treatment day, a fertile male was placed with the female, where he remained for 8 days. On the 5th day after cessation of treatment, the females were sacrificed and the uteri examined for implants. A positive response was obtained when none of the six treated animals showed implants. Estrone was used as a reference compound, and an untreated control group of six animals was used to establish fertility of the group of animals. In this screen, Compounds 1, 2, 4, 7, and 12 showed negative or marginal activity.

Compounds 1–13 were evaluated in *in vitro* and *in vivo* parasitology screens (8, 9). The *in vitro* screen consisted of an anthelmintic test in which larvae or eggs of trichostrongyle nematodes were exposed to the test drug at a concentration of 100 mcg./ml. Efficacy was determined by direct microscopic inspection of the condition of the parasites. Any active compounds were then retested in 10-fold serial dilutions to determine the end-point. None of the compounds, 1–13, was active in this test.

The *in vivo* parasitology screen tested the compounds in a coccidiosis screen at a dose level of 0.05% of the diet. For measurement of anticoccidial effects of drugs, 2-week-old-sex and weight-balanced "white-cross" chicks in groups of 10 were fed a standard laboratory ration to which drugs were added just prior to use. The uninfected and infected control birds were fed the basal ration. On the 2nd day of the test, the chicks were inoculated orally with 50,000 sporulated oocysts of *Eimeria tenella*. The chicks were held for 6 days following inoculation. Several criteria were employed for evaluation of drug efficacy. These included observations and records on the mortality rate, growth, severity of pathological lesions, and number of oocysts produced. All of the compounds, 1–13, were inactive in this test.

Several of the compounds were also tested in a liver fluke assay at a dosage level of 200 mg./kg. *in vivo*. In this assay, immature rodents were infected with 5–8 *Fasciola hepatica* metacercaria by the oral route. After 3 weeks, the test drug was administered orally (two doses spaced 2 days apart). The animals were sacrificed 1 week after initial dosing, the results being estimated from the presence or absence of living or dead flukes in the liver.

The agricultural screen consisted of testing Compounds 2, 7, and 10 in a herbicide test at a dose level of 15 lb./acre and against mosquito larvae at 10-p.p.m. concentration. In both assays, Compounds 2 and 7 were inactive. Compound 10 was inactive in the herbicide test but produced an 80% kill of larvae at 10 p.p.m. and a 20% kill at 1 p.p.m. This level of activity was, however, not considered promising enough to pursue.

EXPERIMENTAL[4]

Reduction of Nitro to Amino Compounds (Procedure A)—A suspension or solution of a nitro compound and 100 mg. of Adams' catalyst/10 g. of compound in absolute ethanol was allowed to consume a calculated amount of hydrogen in a Parr hydrogenator. The

[1] Tests for the various biological activities were carried out by the Merck Sharp and Dohme Research Laboratories, Rahway, N. J.

[2] IPC = Institute of Paper Chemistry, Appleton, Wis.

[3] Isolate obtained from Carolina Biological Supply Co., Burlington, N. C.

[4] Catalytic hydrogenation reactions were carried out at room temperature and 60 p.s.i. by means of a Parr hydrogenator. Melting points were taken in open capillary tubes with a Thomas-Hoover apparatus; they are corrected. IR spectra were obtained with a Perkin-Elmer model 237B grating spectrometer and were as expected. Microanalyses were performed by Atlantic Microlab, Atlanta, Ga.

Table I—α-Phenylcinnamonitrile Derivatives

Compound	R_1	R_2	Melting Point	Method of Synthesis[a]	Yield, %	Analysis, % Calc.	Analysis, % Found
1	4-Methoxyphenyl	3-Aminophenyl	107–108° (lit.)	—[b]	—	—	—
2	4-Methoxyphenyl	4-Dimethylaminophenyl	148–150° (lit.)	—[c]	—	—	—
3	Phenyl	2-Methoxy-5-nitrophenyl	158–160°	—[c,d]	92	C, 68.56 H, 4.31 N, 9.99	C, 68.69 H, 4.46 N, 9.92
4	Phenyl	2-Methoxy-5-aminophenyl	101–103°	A	85	C, 76.77 H, 5.63 N, 11.19	C, 76.88 H, 5.67 N, 11.06
5	4-Aminophenyl	2-Methoxyphenyl	105° (lit.)	—[e]	—	—	—
6	Phenyl	4-Trimethylammonium-phenyl iodide	189–190°	B	80	C, 55.38 H, 4.90 N, 7.17	C, 55.51 H, 5.05 N, 7.39
7	4-Isothiocyanatophenyl	2-Methoxyphenyl	193–194°	C	86	C, 69.84 H, 4.13 N, 9.58	C, 69.44 H, 4.31 N, 9.36
8	3,4-Dimethoxyphenyl	3-Nitro-4-methoxyphenyl	195–197°	—[f]	93	C, 63.52 H, 4.73 N, 8.23	C, 63.58 H, 4.89 N, 8.17
9	3,4-Dimethoxyphenyl	3-Amino-4-methoxyphenyl	184–186°	A	88	C, 69.66 H, 5.84 N, 9.02	C, 69.75 H, 5.97 N, 9.11
10	3,4-Dimethoxyphenyl	3-Trimethylammonium-4-methoxyphenyl iodide	158–162°	B	75	C, 52.49 H, 5.24 N, 5.83	C, 52.31 H, 5.37 N, 5.78
11	3,4-Dimethoxyphenyl	2-Methoxy-5-nitrophenyl	205–207°	—[f]	92	C, 63.52 H, 4.73 N, 8.23	C, 63.67 H, 4.85 N, 8.29
12	3,4-Dimethoxyphenyl	2-Methoxy-5-aminophenyl	85–88°	A	88	C, 69.66 H, 5.84 N, 9.02	C, 69.62 H, 5.95 N, 9.09
13	3,4-Dimethoxyphenyl	2-Methoxy-5-trimethylammoniumphenyl iodide	164–168°	B	80	C, 52.49 H, 5.24 N, 5.83	C, 52.63 H, 5.48 N, 5.88

[a] Superscripts are for general literature procedures and key intermediates. Letters refer to the author's experimental procedures. [b] See *Reference 10*. [c] Synthesized according to general procedure described in *Reference 5*. [d] See *Reference 11*. [e] See *Reference 12*. [f] Synthesized according to general procedures described in *References 5* and *6*.

catalyst was removed by filtration through Celite 545, and the ethanol was evaporated, The residue was recrystallized from aqueous ethanol or another appropriate solvent.

Quaternary Salt Formation (Procedure B)—A suspension of 0.01 mole of amino compound, 0.03 mole of dimethyl sulfate, 5 ml. of water, and 5 g. of potassium carbonate in 60 ml. of acetone was heated on a steam bath for 2 hr. Filtration of the hot suspension followed by air evaporation of the filtrate (hood) gave the crude methosulfate salt. Addition of potassium iodide to an aqueous solution of the methosulfate formed the iodide, which was collected by filtration and dried. The iodide salts were recrystallized from absolute ethanol and/or water.

Formation of Isothiocyanate from Amino Compound (Procedure C)—An acetone solution of the amino compound and an excess of thiophosgene (hood) was refluxed on a steam bath for 2 hr. Removal under reduced pressure at room temperature of the unreacted thiophosgene (hood) and acetone left a material, which was recrystallized from glacial or aqueous acetic acid.

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