by reacting *N*-chloroacetylbenzamide with various amines.

EXPERIMENTAL

Chloroacetylbenzamide.—This intermediate was prepared according to the method of Cadwallader and LaRocca (8).

Preparation of Substituted Aminoacetylbenzamides.—*Aminoacetylbenzamide.*—*N*-Chloroacetylbenzamide (0.05 mole) was dissolved in 8 ml. of anhydrous benzene with the aid of heat. Dry ammonia was passed through the hot solution. On cooling, unreacted chloroacetylbenzamide precipitated and was filtered. The filtrate was evaporated until aminoacetylbenzamide precipitated. The precipitate was recrystallized three times from petroleum ether.

N-Diethylaminoacetylbenzamide Hydrochloride.—*N*-Chloroacetylbenzamide (0.05 mole) was refluxed for 5 hr. with 0.05 mole of diethylamine in 8 ml. of anhydrous benzene. Diethylaminoacetylbenzamide hydrochloride precipitated and was removed by filtration while hot. The precipitate was washed with hot benzene. The filtrate and washings were combined and distilled to remove benzene and any unreacted amine. The residue was found to be benzamide. Its presence probably was due to the hydrolysis of chloroacetylbenzamide because of the acidic nature of imino hydrogen. In an attempt to prepare the free amine, the experiment was repeated using pyridine in a molar ratio to take up the hydrogen chloride gas produced during the reaction. However, instead of free amine, the hydrochloride salt of diethylaminoacetylbenzamide precipitated. The precipitate was removed by hot filtration and washed repeatedly with hot anhydrous benzene. The yield obtained was 75%, in contrast to 20% without the pyridine, indicating that the pyridine had acted as a catalyst.

All subsequent experiments were carried out in a similar manner, with and without pyridine as a catalyst. The effects of pyridine on yield, melting points, and analytical data are summarized in Table I.

A rather extensive survey of the pharmacological properties of these compounds has been completed.[1] Although the compounds are relatively nontoxic (oral LD_{50} ranging from a minimum of 750–1000 mg./Kg. to a maximum of over 2000 mg./Kg.), they also are devoid of significant response to the tests employed. In addition to anticonvulsant activity, representative compounds were tested for systemic antibacterial, antifungal, antiparasitic, and psychotropic actions.

SUMMARY

The synthesis of 13 new substituted aminoacetylbenzamides is described, and the catalytic effect of pyridine on the condensation of secondary amines and chloroacetylbenzamide is reported. These compounds have been screened for pharmacological activity.

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[1] The authors are indebted to Dr. U. R. Schock, Abbott Laboratories, North Chicago, Ill., for the pharmacological screening data.

Modification of the Spectrophotofluorometric Determination of Griseofulvin

By M. KRAML, J. DUBUC, and D. DVORNIK

The spectrophotofluorometric method of Bedford *et al.* for the determination of griseofulvin is rendered more reliable and sensitive by measuring the fluorescence in anhydrous methanol.

LEVELS OF griseofulvin in the blood of laboratory animals or humans are usually determined by the fluorometric method of Bedford *et al.* (1). The fluorescence of griseofulvin in 1% ethanol at 450 mμ (uncorrected) is measured after activation at 295 mμ (uncorrected).

Following the procedure of Bedford *et al.* (1) in the past, the authors were often unable to dissolve the residue of extracted griseofulvin in 1% ethanol, and the resulting faintly turbid suspensions gave erratic fluorescence spectra. To overcome this difficulty at first, ethanol was used (2, 3). Since ethanol is often contaminated with traces of highly fluorescent impurities, it had to be redistilled before use. Later, the procedure was modified to use reagent grade methanol instead of 1% ethanol. This offered two advantages: (*a*) the intensity of fluorescence of griseofulvin is increased nearly twofold (Table I), and (*b*) commercial reagent grade methanol does not contain fluorescent impurities. As with ethanol (2), the fluorescence maximum in methanol was shifted to 420 mμ (uncorrected) (Fig. 1). The activation maxima remained unchanged at 295 and 335 mμ (uncorrected).

The procedure of Bedford *et al.* (1) using 1%

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TABLE I.—FLUORESCENCE OF GRISEOFULVIN STANDARDS

Concn., mcg./10 ml.	Arbitrary Fluorescent Units			
	1% Ethanol		Methanol	
1.0	8.0	(8.0)[a]	16.0	(16.0)
2.0	18.0	(9.0)	32.0	(16.0)
3.0	27.0	(9.0)	49.0	(16.3)
4.0	36.0	(9.0)	64.0	(16.0)
5.0	44.0	(8.8)	79.0	(15.8)
Av./1.0 mcg.		8.8		16.0
Ratio[b]	1.00		1.82	

[a] Values in parentheses are given as fluorescent units per 1.0 mcg. of griseofulvin. [b] Value for 1% ethanol taken as 1.00.

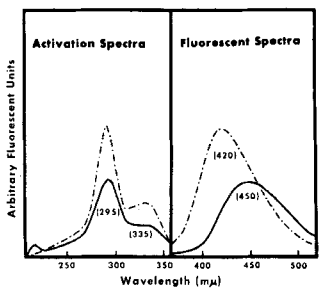

Fig. 1.—Activation and fluorescent spectra of griseofulvin. Key: ———, spectra in 1% aqueous ethanol; ---------, spectra in 100% methanol. Wavelength of peaks are given in parentheses in millimicrons (uncorrected).

ethanol and the authors' modification using anhydrous methanol were compared by determining the levels of griseofulvin[1] in the serum of 16 adult male volunteers who received one single oral dose of 500 mg. of micronized griseofulvin.[2] Blood samples were taken at 0, 4, 8, 12, and 24 hr. Within the limits of the experimental errors, both procedures gave identical values (Table II).

The authors have applied the modified procedure in analyzing about 1500 samples of human or animal serums. Only a few spectra were lost due to turbid extracts causing light scattering or because of interference from high background fluorescence. The increased fluorescence of griseofulvin in methanol enhances the sensitivity of the method and is thus particularly useful in determining submicrogram quantities of griseofulvin regularly encountered in human serums.

The method also is readily applicable to the analysis of griseofulvin in whole blood samples. Whole blood blanks were no higher than those obtained with control serums and, calculated as griseofulvin, gave background values in the 0.20–0.30 mcg./ml. range. Using tritiated griseofulvin and liquid scintillation counting, McNall reported (4, 5) that in mice levels of griseofulvin in whole blood were two to three times higher than in the serum. With the modified spectrophotofluorometric technique, no significant differences were found between levels of griseofulvin in whole blood and plasma of rats, 2, 4, or 6 hr. after oral administration of 100 mg./Kg. of griseofulvin (Table III).[3]

TABLE II.—LEVELS OF GRISEOFULVIN (mcg./ml.) IN THE SERUM OF MALE VOLUNTEERS AFTER A SINGLE ORAL DOSE OF 500 mg. OF MICRONIZED GRISEOFULVIN

Patient	Hr. After Dose							
	4		8		12		24	
	A[a]	B	A	B	A	B	A	B
1	3.67	4.02	3.37	3.42	2.37	2.68	1.05	0.89
2	2.97	3.12	3.13	3.12	2.18	2.53	1.29	1.64
3	1.07	0.89	1.35	1.19	1.03	1.04	1.53	1.79
4	1.27	1.34	1.03	1.19	0.89	1.04	0.88	0.89
5	0.96	1.04	0.88	0.89	0.74	0.74	0.41	0.45
6	1.09	1.04	1.12	1.19	0.74	0.89	0.39	0.60
7	0.88	0.89	0.83	0.89	1.04	0.89	0.85	0.89
8	2.79	2.68	1.91	2.53	2.29	2.23	1.32	1.49
9	1.61	1.64	1.37	1.34	0.91	0.89	0.62	0.74
10	1.54	1.49	1.61	1.64	1.09	1.49	0.72	0.74
11	1.34	1.04	0.81	0.60	0.90	1.19	0.80	0.60
13	2.05	1.64	1.86	2.08	1.78	1.79	1.05	1.04
14	1.59	1.19	0.87	0.74	1.01	0.74	0.83	0.74
15	1.83	1.49	1.61	1.19	1.25	1.04	0.73	0.60
16	1.83	1.93	1.87	1.49	1.36	1.19	0.57	0.45
Av.	1.77	1.70	1.57	1.57	1.31	1.36	0.87	0.90
± S.E.[b]	0.21	0.23	0.20	0.22	0.15	0.20	0.08	0.11

[a] Fluorescence measured in anhydrous methanol (A) and 1% aqueous ethanol (B). [b] Standard error.

TABLE III.—LEVELS OF GRISEOFULVIN IN THE WHOLE BLOOD AND PLASMA OF RATS AFTER ORAL ADMINISTRATION OF 100 mg./Kg. OF MICRONIZED GRISEOFULVIN

Hr. After Administration	Griseofulvin, mcg./ml.	
	Whole Blood	Plasma
2 (16)[a]	2.22 ± 0.28[b]	2.09 ± 0.19
4 (20)	2.60 ± 0.23	2.70 ± 0.27
6 (20)	1.93 ± 0.22	2.12 ± 0.28

[a] Values in parentheses are the number of animals employed in each test. [b] Standard error.

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[1] An Aminco-Bowman spectrophotofluorometer coupled to an Electro Instruments X-Y recorder was used.
[2] Marketed as Grisactin by Ayerst Laboratories.
[3] Animal work was performed under the supervision of Dr. A. Marton.