Screening of Five New Synthetic Compounds for Anticonvulsant Activity

By CHUNG AE PARK and JOHN F. BESTER

Five new synthetic compounds were tested for ability to control the convulsions caused by diphenhydramine in overdose in rats. Test animals were given sublethal but convulsive doses of diphenhydramine intraperitoneally. The drugs tested were administered intraperitoneally in varying dosage, either with diphenhydramine or upon onset of convulsions. Of the five, four were found to be ineffective, and one of these was found to be a convulsant. A fifth, isopropyl-4-amino-2-sulfamoylbenzoate, showed ability to protect against convulsive seizures, particularly in doses of 100 to 150 mg./Kg. of rat. Doses below 100 mg./Kg. were ineffective; those above 200 mg./Kg. were toxic.

IN AN EARLIER paper, Mael and Bester (1) reported upon attempts to control the convulsive seizures induced by excessive doses of antihistaminic drugs. That antihistaminic overdosage results in convulsive seizures of varying degrees of severity has been demonstrated amply (2–9).

Hamor has for a number of years been engaged in the synthesis of compounds intended to show muscle relaxant and/or anticonvulsant capabilities. Both the synthesis and preliminary screening of several of these have been reported (10). Five of the most recently developed were made available for testing, and it was decided that one of the tests might well be against convulsions precipitated by antihistaminic excess. Structures, chemical names, and code designations of the compounds appear in Table I.

EXPERIMENTAL

Diphenhydramine was chosen as the challenging drug because its actions and side effects are typically characteristic of the antihistaminic group of drugs (1, 11).

Adult albino rats of both sexes, weighing between 190 and 300 Gm., were used. They were housed at normal room temperature and allowed free access to food and water at all times, except during the periods of test.

All drugs were administered intraperitoneally. Of the five compounds under test, only GHH-SC5 was soluble and was given as an aqueous solution; the other four were administered as aqueous suspensions. Dosages of the test compounds ranged between 25 and 500 mg./Kg.

The challenging dose of diphenhydramine was in all instances 50 mg./Kg. of rat. This dose, administered intraperitoneally, was consistently convulsant but usually not lethal.

Anticonvulsant activity was tested in two ways. First, diphenhydramine and the test drug were administered simultaneously. Second, the test drug was given at the onset of convulsions. In all series control animals received intraperitoneally equivalent doses of the test drug alone.

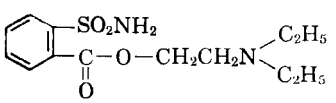
Anticonvulsant capability was based upon reduction in severity or duration of antihistaminic induced convulsions. The stated dose of diphenhydramine induces convulsions in 3 to 5 min., and convulsions persist for up to 2 hr. if not terminated by death. Convulsions are preceded by excitation, erratic movement, and muscle tremors. Convulsions are clonic and violent.

Three of the compounds tested, GHH-SC1, GHH-SC3, and GHH-SC4, were found to have no apparent effect upon onset, severity, or duration of convulsions; nor did any of the three show any apparent effect upon control animals. GHH-SC5 not only failed to ameliorate convulsive attacks, but, on the contrary, was itself a convulsant. Control rats receiving 50 mg./Kg. or more of the compound exhibited mild seizures, and the attacks in test animals were worsened by this agent. No further testing was done on these four compounds.

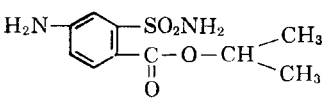
Only GHH-SC2 showed a degree of protective ability against diphenhydramine intoxication; these data appear in Table II. At a dose of 100 mg./Kg. or more with the challenging dose of diphenhydramine or at a dose of 125 mg./Kg. or more at the

TABLE I.—STRUCTURES OF THE COMPOUNDS TESTED

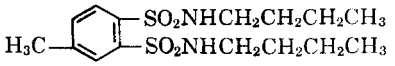
1, GHH-SC1; 2-Diethylaminoethyl-*o*-sulfamoylbenzoate



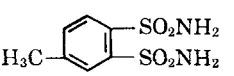
2, GHH-SC2; Isopropyl-4-amino-2-sulfamoylbenzoate



3, GHH-SC3; Toluene-*N*,*N*′-di-*n*-butyl-3,4-disulfonamide



4, GHH-SC4; Toluene-3,4-disulfonamide



5, GHH-SC5; 2-(2-Dimethylaminoethyl)-saccharin HCl

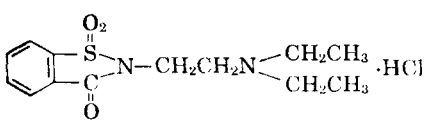


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TABLE II.—ANTICONVULSANT ACTIVITY OF GHH-SC2

Dose, mg./Kg.	Animals, No.	Intensity of Convulsions[a] ++++	+++	++	+	0	−	Deaths, No.
Controls	6	0	0	0	0	2	4	0
Simultaneous Injection with Diphenhydramine								
50	4	0	2	1	1			0
100	4			1	3			0
150	4			1	2	1		1
Injection at Onset of Convulsions								
50	4	1	3					0
125	4		1	1	2			0
150	4		1		2	1		0

[a] ++++, Violent, continuing, clonic convulsions; +++, convulsions, less violent, followed by depression; ++, mild convulsions, followed by depression; +, muscle tremors only; 0, absence of symptoms; −, depression only.

TABLE III.—EFFECTS OF GHH-SC2 ON RATS

Dose, mg./Kg.	Animals, No.	Mean Time to Onset, min.	Mean Duration, hr.	Effects
Controls[a]	10	...	...	None
25	3	...	...	None
50	3	...	...	None
75	3	...	...	None
100	3	30	2	Sedation
125	3	28	2–3	Sedation
150	3	15	3	Sedation
200	3	13	4–5	Depression and paralysis
250	3	10	6	Depression and paralysis
300	3	5	8	Depression and paralysis; one death
350	3	3	...	Three deaths within 2 hr.

[a] Controls injected with distilled water only.

onset of convulsions, a definite reduction in the severity and duration of convulsions was observed. However, only rarely were seizures completely eliminated. Control animals receiving 125 mg./Kg. or more of GHH-SC2 showed significant depression in from 5 to 15 min. and remained in this state for varying periods of time, depending upon dose given. Because of this, GHH-SC2 was then given alone intraperitoneally to rats in doses ranging from 25 to 300 mg./Kg. Controls in this series were injected similarly with distilled water. Results of this study are shown in Table III. As will be observed, doses of less than 100 mg./Kg. were without apparent effect on the animals. Doses above 200 mg./Kg. caused prolonged depression with loss of skeletal muscle function; still higher doses led to death. In the dosage range between 100 and 200 mg./Kg., a state of sedation was produced. During this phase, both heart and respiration remained normal. The righting reflex was not lost but impaired. The rats had a tendency to lie quietly but did not sleep.

Comparable effects were observed when rats, similarly treated, were subjected to the inclined screen test; the angle of incline was 45° and the screen 0.25-in. gauge. There was no detectable loss of skeletal muscle function at doses below 100 mg./Kg., and loss of function resulted from doses above 200 mg./Kg. Between these two extremes there was some loss because animals would slide free, but, if they were replaced on the screen, they could cling to it for periods of several minutes, then slide free again. Muscle function in this intermediate dosage range was not totally lost at any time.

DISCUSSION

Due to the fact that only GHH-SC2 or isopropyl-4-amino-2-sulfamoylbenzoate, of the five compounds tested, showed any ability to produce sedation, muscle relaxation, and some inhibition of convulsions, it alone warrants consideration. At best, its anticonvulsant ability appears limited because seizures are decreased but are not usually eliminated, and the dosage required is not small. As a muscle relaxant, a possible correlation may be drawn between GHH-SC2 and mephenesin since the muscle relaxant and indeed the paralyzing action of the latter has been shown to be free of excitation before the loss of the righting reflex and following its recovery as well as from hyperexcitability during the paralytic stage (12). These characteristics are shared by GHH-SC2.

Loev and Kormendy (13), reporting on the abilities of compounds similar to those reported here, tested against strychnine convulsions and against maximal electric shock in mice and stated that their isopropyl derivatives were most active. GHH-SC2 contains the isopropyl moiety; but its significance, if any, cannot be stated yet. Similarly, future studies on additional compounds of this type now being synthesized might be expected to indicate whether the amino substitution on the ring of GHH-SC2 contributes to its biological activity.

Finally, GHH-SC2 again illustrates that ability to produce sedation, muscle relaxation, and some anticonvulsant effect can occur in a single molecule. Hence, the synthesis of related molecules should be continued in anticipation of their finding useful application.

CONCLUSIONS

Five compounds—2-diethylaminoethyl-*o*-sulfamoylbenzoate, isopropyl-4-amino-2-sulfamoylbenzoate, toluene-*N,N'*-di-*n*-butyl-3,4-disulfonamide, toluene-3,4-disulfonamide, and 2-(2-diethylaminoethyl)-saccharin hydrochloride—were tested against diphenhydramine convulsions.

Only isopropyl - 4 - amino - 2 - sulfamoylbenzoate showed any anticonvulsant ability against diphenhydramine. In addition, it exhibited sedation and skeletal muscle relaxation.

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Acid Stability of Lincomycin

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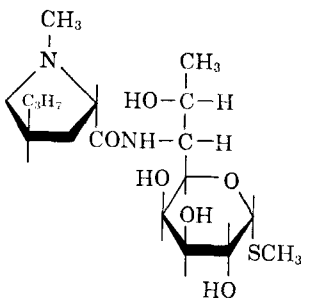
Lincomycin degrades slowly in 0.1 *N* HCl at 70° (half-life, 39 hr.), and the decrease in optical rotation shows a direct correlation with microbiological assays. At 37°, lincomycin shows no degradation for at least 48 hr. in 0.1 *N* HCl. Under these conditions, penicillin V degrades with a half-life of 29 min.

THE ISOLATION, characterization, microbiological assay, and biological evaluation of the new Gram-positive specific antibiotic, lincomycin,[1] have been reported recently together with absorption and excretion studies in man and animals (1–9). Structure I has been assigned to lincomycin (10). Crys-



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talline lincomycin hydrochloride was found to be stable for at least 6 months at 70° (2). To predict the stability of this antibiotic in the stomach following oral administration, studies have been conducted in acid solution at 37° and 70°. A comparative examination of the acid stability of penicillin V at 37° was also made.

EXPERIMENTAL

Lincomycin Degradation at 70°.—A 0.4% solution of lincomycin hydrochloride in 0.1 *N* HCl was incubated at 70°. At intervals over a period of 389 hr., 10-ml. aliquots were removed for potentiometric titrations. At the same time, 5-ml. aliquots were removed and frozen for subsequent optical rotation and spectral measurements and 100-μl. aliquots were diluted to 25 ml. with 0.1 *M* phosphate buffer, pH 7.0, and frozen for bioassay by agar diffusion *versus Sarcina lutea* (3).

Titrations were run immediately after sampling. Following addition of 9 ml. of 0.1 *N* NaOH to each sample, titrations were completed potentiometrically with 1 *N* NaOH. Twenty milliliters of ethanol then was added to each sample and the solutions back-titrated with 1 *N* HCl. Consumption of acid or base and apparent pKa's were calculated for each sample (Table I).

Lincomycin Degradation at 37°.—A 0.4% solution of lincomycin hydrochloride in 0.1 *N* HCl was incubated at 37°. At intervals over a period of 48 hr., 10-ml. aliquots were removed. From each aliquot, a 100-μl. portion was removed and diluted to 25 ml. with 0.1 *M* phosphate buffer, pH 7.0. Three-milliliter aliquots of the resulting buffered solutions were diluted further with equal volumes of the phosphate buffer. All the buffered solutions were frozen for subsequent microbiological assay. The remainder of each sample aliquot was frozen for optical rotation determinations.

Penicillin V Degradation at 37°.—A 10-mg. sample of penicillin V was dissolved in 45 ml. of water in a 50-ml. volumetric flask and brought to 37° in a water bath. A 5-ml. portion of 1 *N* HCl was added, the solution was mixed, and a 2-ml. aliquot was removed. The aliquot was quickly added to 8 ml. of 0.1 *M* phosphate buffer, pH 7.0,

TABLE I.—POTENTIOMETRIC TITRATION STUDIES OF LINCOMYCIN AS A FUNCTION OF DEGRADATION TIME AT 70°C. IN 0.1 *N* HCl

Time, hr.	Meq. of Amine/ml. H_2O	Meq. of Amine/ml. 51% EtOH	pKa' H_2O	pKa' 51% EtOH
0	0.0095	0.0092	7.92	7.00
1.8	0.0101	0.0092	8.00	...
5.5	0.0097	0.0093	7.94	...
22.5	0.0104	0.0093	7.95	...
46.5	0.0096	0.0094	7.92	6.84
77.5	0.0096	0.0095	7.92	...
150	0.0101	0.0093	7.95	...
249	0.0094	0.0092	7.87	...
389	0.0094	0.0093	7.80	7.12
Mean	0.0098	0.0093	7.92	6.99
S.D.	0.0004	0.0001	0.06	0.14

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[1] Lincomycin hydrochloride. Marketed as Lincocin by The Upjohn Co., Kalamazoo, Mich.