compound had been formed, possibly of the amino sulfonate type.

Failure to isolate an amino sulfonate from *N*-phenylisopropyl - D - mannosylamine and amphetamine bisulfite would suggest that the mannosylamine had reacted with bisulfite to form an unstable amino sulfonate. The amino sulfonate would then undergo expulsion of the amine, as in the hydrolytic reactions of glycosylamines (16), to form the more stable aldose bisulfite addition product. The reaction was undoubtedly driven in this direction as a result of the insolubility of the aldose bisulfite addition product which was observed to crystallize out of the reaction solution.

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Reactions of Amine Drugs with Sugars II

Synthesis and *In Vivo* Evaluation of Mannose Amphetamine Sulfonate and *N*-Phenylisopropyl-D-mannamine

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Mannose amphetamine sulfonate was synthesized from the reaction of amphetamine bisulfite with D-mannose. *N*-Phenylisopropyl-D-mannamine was synthesized by catalytic hydrogenation of *N*-phenylisopropyl-D-mannosylamine. The products were evaluated *in vivo* by the Williamson activity cage method. Mannose amphetamine sulfonate was found to have a significantly longer duration of activity and produced a higher level of mean activity, from 3.5 hr. to 4.5 hr. after administration, than dextroamphetamine sulfate. *N*-Phenylisopropyl-D-mannamine was found to be orally inactive at dosage levels of 10 and 50 mg./Kg. of body weight.

ADAMS *et al.* (1, 2) have extensively investigated the reaction of various amine bisulfites with aldehydes and ketones. The products isolated, using aldehydes such as benzaldehyde, were of the α-hydroxy sulfonate type. They were, however, unsuccessful in obtaining products with D-glucose. The formation of alkylamine bisulfite addition products with aldoses has been reported by Ingles (3).

The preparation of glycamines by catalytic hydrogenation of *N*-glucosides of alkylamines has been reported by a number of workers (4, 5). Mitts and Hixon (6) have reported on the properties of a number of alkylglucamines obtained by catalytic hydrogenation of glucosylamines.

The purpose of this work was the synthesis and *in vivo* evaluation of an aldose bisulfite addition product and a glycamine derived from an alkylamine drug.

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EXPERIMENTAL

Synthesis of Mannose Amphetamine Sulfonate—The synthesis of this product was carried out by reacting dextroamphetamine bisulfite with D-mannose as described in the first paper of this series (7).

Synthesis of N-Phenylisopropyl-D-mannamine—*N*-Phenylisopropyl-D-mannosylamine was prepared by reacting dextroamphetamine,[1] 6.80 Gm. (0.05 mole), with D-mannose,[2] 9.00 Gm. (0.05 mole), in methanol, 5 ml., at 60–65° for 30 min. The resulting light amber colored solution of the mannosylamine was used without further purification for the catalytic hydrogenation.

Hydrogenation of the mannosylamine was carried

[1] Prepared from dextroamphetamine sulfate, Sigma Chemical Co., St. Louis, Mo.
[2] Nutritional Biochemicals Corp., Cleveland, Ohio.

out in a Parr type bomb using activated Raney nickel as the catalyst. The mannosylamine was mixed with 250 ml. of ethanol and 10 Gm. of the catalyst, and hydrogenation was carried out below 100° at 1,000 p.s.i. hydrogen pressure. The reaction was terminated after 24 hr. and the solvent reduced to a small volume under vacuum. The resulting crystalline product was filtered off and dried in a vacuum desiccator over calcium sulfate.

Amphetamine Assay—A qualitative test for amphetamine was considered necessary because of the possibility that the unsaturated benzene ring of the amphetamine moiety had been reduced to cyclohexane during the catalytic hydrogenation of the mannosylamine. Amphetamine exhibits a characteristic ultraviolet absorption spectrum which would be destroyed by hydrogenation of the benzene ring. A qualitative test, based on the ultraviolet absorption spectrum of amphetamine was, therefore, selected. Solutions of the mannamine and of dextroamphetamine sulfate in pH 10 buffer were prepared and the ultraviolet absorption spectra of the solutions were compared and found to be identical. The amphetamine content of the mannamine as determined spectrophotometrically was 46.3%; the theoretical amphetamine content is 45.3%.

In Vitro Acid-Base Stability—A quantitative test for amphetamine content, based on the extraction of amphetamine base from alkaline solutions by an organic solvent, was used to determine the acid-base stability of the mannamine. An aqueous solution containing 10.13 mg. of the mannamine per ml. was prepared and 1 ml. of this solution was added to each of three extraction tubes. One milliliter of 1 *N* sodium hydroxide solution was added to each tube and the time recorded. Ten milliliters of chloroform was added to one tube immediately after the addition of the sodium hydroxide solution. Extraction was carried out and the amphetamine content of the chloroform determined spectrophotometrically. The second sample was extracted after 1 hr. and the third sample after 9 hr. Results indicated that less than 50 mcg. of amphetamine was being extracted at each extraction, and that the value did not change during the course of the experiment.

Since the mannamine was apparently stable in the presence of 1 *N* sodium hydroxide, the procedure for the determination of its acid stability characteristics was considerably simplified. If the mannamine were unstable in acid solution, it would liberate the free base which would immediately convert to the salt form; subsequent addition of base would convert the salt form to the free base which could be extracted by chloroform.

The mannamine was found to be stable over a 9-hr. period in artificial gastric fluid.

In an experiment designed to study the partitioning of the mannamine between a pH 8 aqueous solution and chloroform, it was found that when 1 ml. of the aqueous solution containing the equivalent of 3.24 mg. of amphetamine per milliliter was shaken with 10 ml. of chloroform for 24 hr., the concentration of amphetamine present in the chloroform was 10 mcg./ml., thus indicating partitioning strongly in favor of the buffer. The product was found to have a solubility in chloroform equivalent to 50 mcg. of amphetamine per milliliter.

In Vivo Evaluation—For the *in vivo* evaluation by the Williamson[3] activity cage method, male Holtzman rats were selected as the test animal. Their initial weight was between 150 and 175 Gm. In all test runs, the rats were fasted for 10 to 12 hr. in an isolation cage prior to dosing and were preconditioned 30 min. to acquaint the animal with the cage. In each experiment three animals were run simultaneously. Each cage was used an equal number of times for each drug system and six observations were conducted for each of the drugs evaluated. All doses were administered as a solution. The control animals received 3.75 to 5.0 ml. of distilled water by stomach tube, corresponding to the volume of solution required to dose an equivalent weight test animal. All animals were maintained on a diet of Wayne Lab Blox and water *ad libitum* and were housed in an air-conditioned animal room at 23–25°. Animal colony size was 30 rats, maintained in two cages of 15 rats each.

Dosage was calculated on the basis of 10 mg. and 50 mg. of amphetamine per kilogram of body weight. Solutions of the test drugs were prepared in a concentration representing 1 or 5 mg. of amphetamine per 2.5 ml. of solution. All tests were conducted so that the starting time of the test period was between 7:30 and 8:00 p.m.

This procedure was used to evaluate dextroamphetamine sulfate, mannose amphetamine sulfonate, and *N*-phenylisopropyl-D-mannamine at a dosage level of 10 mg./Kg. and to establish control activity levels. *N*-Phenylisopropyl-D-mannamine was also evaluated at a dosage level of 50 mg./Kg.

At the end of the test period, the average number of responses per 0.5-hr. interval was calculated. A statistical analysis of the data was accomplished by first applying an over-all *F* test to determine if a significant difference existed in any of the possible combinations. If a significant F ($\alpha = 0.05$) was found, the Student Newman-Kuels test was used to evaluate each of the possible combinations (8). Table I presents the average activity, at each 0.5-hr.

TABLE I—MEAN ACTIVITY COUNTS PER 0.5-hr. PERIOD FOR DEXTROAMPHETAMINE COMPOUNDS[a] AND FOR THE CONTROL

Time, hr.	1 Control $\bar{X}$	2 H2 $\bar{X}$	3 5H2 $\bar{X}$	4 AMS $\bar{X}$	5 Amp. SO_4 $\bar{X}$
0.5	5.34	5.66	5.88	16.22	14.11
1.0	2.78	2.11	4.00	15.44	15.22
1.5	3.00	4.00	4.56	15.67	14.00
2.0	4.56	2.78	4.22	14.77	13.00
2.5	4.00	3.89	3.78	14.78	13.34
3.0	4.34	4.78	5.34	13.89	12.45
3.5	5.45	3.67	4.56	15.67	11.22
4.0	4.89	4.34	4.11	14.77	10.00
4.5	3.45	4.45	5.34	11.55	7.89
5.0	4.78	4.00	4.11	8.67	6.45
5.5	4.45	2.78	3.78	4.34	6.22
6.0	3.22	2.89	4.22	7.22	5.22

[a] H2, *N*-phenylisopropyl-D-mannamine; dosage level equivalent to 10 mg. dextroamphetamine per Kg. of body weight; $5XH_2$, *N*-phenylisopropyl-D-mannamine, dosage level equivalent to 50 mg. dextroamphetamine per Kg. of body weight; AMS, mannose amphetamine sulfonate, dosage level equivalent to 10 mg. of dextroamphetamine per Kg. of body weight; Amp. SO_4, dextroamphetamine sulfate, dosage equivalent to 10 mg. dextroamphetamine per Kg. of body weight.

[3] Williamson Development Co., Inc., West Concord Mass.

TABLE II—STATISTICAL ANALYSIS OF THE *In Vivo* DATA FOR SIGNIFICANCE OF DIFFERENCE BETWEEN THE VARIOUS DEXTROAMPHETAMINE COMPOUNDS AND BETWEEN THE COMPOUNDS AND THE CONTROL

Time, hr.	Significance at 0.05 Level[a]									
	1 × 2[b]	1 × 3	1 × 4	1 × 5	2 × 3	2 × 4	2 × 5	3 × 4	3 × 5	4 × 5
0.5	...	...	*	*	...	*	*	*	*	...
1.0	...	...	*	*	...	*	*	*	*	...
1.5	...	...	*	*	...	*	*	*	*	...
2.0	...	...	*	*	...	*	*	*	*	...
2.5	...	...	*	*	...	*	*	*	*	...
3.0	...	...	*	*	...	*	*	*	*	...
3.5	...	...	*	*	...	*	*	*	*	*
4.0	...	...	*	*	...	*	*	*	*	*
4.5	...	...	*	*	...	*	...	*	...	*
5.0	...	...	*	...	...	*	...	*	...	...
5.5	...	...	...	...	...	...	*	...	...	...
6.0	...	...	*	...	...	*	...	*	...	*

[a] An asterisk indicates a significant difference exists between the mean activity counts for the two drug systems being compared or between the drug system and the control at that 0.5-hr. period. [b] The numbers refer to the systems described in Table I and compare differences in mean activity between these systems at each 0.5-hr. period given.

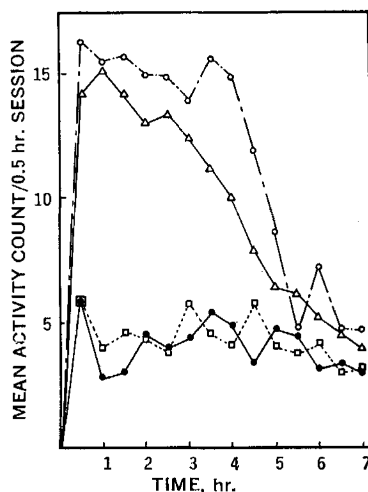

Fig. 1—In vivo *evaluation of amphetamine compounds. Key:* ○, *AMS;* △, *amphetamine SO_4;* □, *5H2;* ●, *control. Equivalent dextroamphetamine dosage level and compound identification are the same as reported in Table I.*

interval, for all test groups. Results of the statistical analysis are given in Table II. Figure 1 is a plot of the mean activity exhibited by the animals used in the study at 0.5-hr. intervals.

DISCUSSION

Mannose amphetamine sulfonate and dextroamphetamine sulfate were found to have an immediate onset of pharmacologic action. The dextroamphetamine sulfate had a duration of activity of 4.5-hr. while the mannose amphetamine sulfonate had a 5-hr. duration of activity. Furthermore, the mannose amphetamine sulfonate produced a significantly higher CNS response than dextroamphetamine sulfate, from 3.5 hr. after administration, to 4.5 hr. after administration. No significant difference in the activity of dextroamphetamine sulfate and mannose amphetamine sulfonate was demonstrated during the first 3 hr. Further investigations of this and similar compounds would seem to be necessary for an elucidation of the mechanism responsible for the observed increased duration and level of activity produced by mannose amphetamine sulfonate.

N-Phenylisopropyl-D-mannamine was found to be inactive when administered orally to rats at a dosage level equivalent to 10 and 50 mg./Kg. of dextroamphetamine. This observation would, therefore, indicate that the compound was not degraded in the gastrointestinal tract to release the amphetamine moiety. It is highly improbable that the mannamine itself was absorbed, since the *in vitro* tests indicated a very low lipid solubility and almost no partitioning from an aqueous (pH 8) phase to a chloroform phase. From the results of this study, it could be assumed that the conversion of this class of amine drugs to glycamines would probably result in the formation of orally inactive compounds.

SUMMARY

Mannose amphetamine sulfonate was synthesized and evaluated *in vivo*. The compound was found to have a significantly longer duration of activity and produced a higher level of mean activity from 3.5 hr. to 4.5 hr. after administration than dextroamphetamine sulfate.

N-Phenylisopropyl-D-mannamine was synthesized and evaluated *in vitro* and *in vivo*. The compound was found *in vitro* to be stable over a 9-hr. period in both acidic and basic solutions. Solubility determinations and partitioning experiments indicated the product was relatively insoluble in chloroform and its partitioning between pH 8 buffer and chloroform was strongly in favor of the buffer. The mannamine was found to be inactive when administered orally to rats.

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