

### Experimental

**Methyl and Ethyl Carbazates.**—These esters were prepared in about 90% yields from 85% hydrazine hydrate and dimethyl and diethyl carbonates, respectively, by the procedure of Diels.<sup>3</sup>

**Preparation of the Carbomethoxy- and Carboethoxyhydrazones.**—Approximately 1 g. of the aldehyde or ketone was dissolved in 3–4 ml. of alcohol and sufficient water was added to cause a faint turbidity. This was removed by means of a few drops of alcohol, and then 3 drops of acetic acid and 1 g. of the carbazate were added. The mixture was shaken and allowed to stand for a few minutes. If crystallization did not result, the reaction mixture was heated to reflux for one hour and cooled. The precipitate was removed by filtration, weighed and crystallized. In those cases where a solid was not obtained readily, the reaction was carried out without the addition of water, and after refluxing for one hour, the solvent was evaporated and the residue recrystallized.

In practically all of the condensations, the yields of the derivatives were high. The products were purified by recrystallization from dilute alcohol or a mixture of benzene and petroleum ether.

(3) O. Diels, *Ber.*, **47**, 2183 (1914).

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### Streptohydrazid

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Previous investigators<sup>1</sup> have demonstrated the condensation of streptomycin with a wide variety of amino compounds. Since isoniazid and streptomycin are both used in the chemotherapy of tuberculosis and have been proposed for combined therapy,<sup>2</sup> it was of interest to prepare and characterize an analogous condensation product, Streptohydrazid.<sup>3</sup>

The condensation of streptomycin with isoniazid was found to occur readily, and it was possible to isolate Streptohydrazid sulfate and hydrochloride as white crystalline products. Whereas isoniazid exhibits an absorption maximum in water at 262  $m\mu$  ( $\epsilon$  4,360), Streptohydrazid absorbs at 260  $m\mu$  ( $\epsilon$  14,700). The latter value was obtained in the presence of a large molar excess of streptomycin to prevent hydrolysis. It was found that by utilizing the difference in absorption at 260  $m\mu$  the extent of reaction could be estimated. It was also observed that in concentrated aqueous solutions Streptohydrazid hydrolyzes very little, but in very dilute solution it dissociates into its component parts.

In tuberculosis protection studies in animals Streptohydrazid has been found at least as effective as combined therapy utilizing streptomycin and isoniazid.<sup>2</sup>

**Streptomycylideneisonicotinylhydrazine Trihydrochloride.**—A mixture of 30 g. of streptomycin hydrochloride and 6 g. of isoniazid in 300 ml. of absolute methanol was boiled under reflux for 15 minutes. The solution was allowed to stand

(1) W. A. Winsten, C. I. Jarowski, F. X. Murphy and W. A. Lazier, *THIS JOURNAL*, **72**, 3969 (1950).

(2) G. L. Hobby and T. F. Lenert, "The Action of Isoniazid and Streptomycin Alone and in Combination," Annual Meeting Public Health Association, Cleveland, Ohio, October 24, 1952.

(3) Chas. Pfizer and Co., Inc., trade name for streptomycylideneisonicotinylhydrazine.

several days in a refrigerator, and large crystals slowly formed. The supernatant solution was decanted and the product filtered, washed with cold methanol and dried; yield 19.0 g. (54%), decomposes *ca.* 200°.

The infrared spectrum exhibits broad strong absorption at 1650  $cm^{-1}$  and at longer wave lengths is very similar to isoniazid. Weak absorption is evident near 1350, 1300, 1210, 1140, 1110, 1060 and 1010  $cm^{-1}$ .

*Anal.* Calcd. for  $C_{27}H_{47}N_{10}O_{12}Cl_3$ : C, 40.03; H, 5.85; N, 17.29; Cl, 13.13. Found: C, 39.94; H, 5.90; N, 17.23; Cl, 13.18.

**Streptomycylideneisonicotinylhydrazine Sesquisulfate.**—Streptomycin trihydrochloride calcium chloride double salt (50 g.) assaying about 660 mcg. per mg., was dissolved in 160 ml. of water containing 8.5 g. of isoniazid. Over a period of 0.5 hour, 85 ml. of methanolic triethylamine sulfate solution (1.98 *M* in  $SO_4^{2-}$ , pH 5) was introduced with stirring, followed by the addition of 255 ml. of methanol during the succeeding hour. The precipitated  $CaSO_4$  was collected and washed with a mixture of one part methanolic triethylamine sulfate solution, two parts of water, and three parts of methanol.

Methanol (about 100 ml.) was added until a haze persisted and the mixture was then allowed to crystallize. Methanol (2000 ml.) was added dropwise with stirring and the white crystalline product was collected, washed with methanol, and dried *in vacuo*; yield 36.0 g. (75%), decomposes *ca.* 230°.

*Anal.* Calcd. for  $C_{27}H_{44}N_{10}O_{12} \cdot 3/2H_2SO_4$ : C, 38.25; H, 5.59; N, 16.52; S, 5.67. Found: C, 38.04; H, 5.74; N, 16.22; S, 5.63.

Streptohydrazid trihydrochloride and sesquisulfate are extremely soluble in water. The former is partially soluble in methanol, whereas the latter is insoluble. Both salts are insoluble in less polar solvents.

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### Hypotensive Agents. III.<sup>1</sup> Dialkylaminoalkyl Pyrrolidine Derivatives<sup>2a,b</sup>

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In the course of a continuing study of potential hypotensive compounds we investigated the reduction of various N-dialkylaminoalkyl succinimides. The various substituted succinimides were obtained in good yields by the reaction of equimolecular amounts of the appropriate dialkylaminoalkylamine with succinic anhydride. After the exothermic reaction had subsided, the resulting mixture was heated at 160–170° for two hours to complete the reaction. Ohki<sup>4</sup> had prepared this type of imide and studied its electrolytic reduction. He isolated the corresponding pyrrolidone by this means.

The reduction of N-phenylsuccinimide to yield N-phenylpyrrolidine with lithium aluminum hydride has been reported by Spitzmueller.<sup>5</sup> Wojcik and Atkins<sup>6</sup> obtained N-amylpyrrolidine by reduction of amylsuccinimide by means of copper chromite catalyst in excellent yields. In our past experi-

(1) For the first paper in this series see L. M. Rice, A. Popovici, M. Rubin, C. F. Geschickter and E. E. Reid, *THIS JOURNAL*, **74**, 3025 (1952).

(2) (a) Supported (in part) by a research grant from the Geschickter Fund for Medical Research, Inc. (b) Presented at the Meeting of the American Chemical Society at Atlantic City, N. J., Sept., 1952.

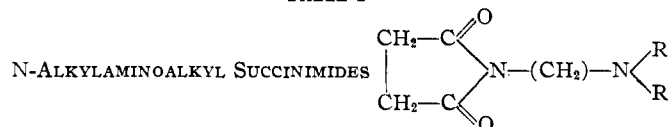
(3) Professor Emeritus, Johns Hopkins University, Baltimore, Md.

(4) Sadao Ohki, *J. Pharm. Soc. Japan*, **70**, 92 (1950).

(5) Weldon G. Brown, "Organic Reactions," Vol. VI, John Wiley and Sons, Inc., New York, N. Y., 1951, p. 492.

(6) B. Wojcik and H. Atkins, *THIS JOURNAL*, **56**, 2419 (1934).

TABLE I



R	n	Formula	B.p.,		Carbon, %		Hydrogen, %		Nitrogen, %		Hydrochloride M.p., °C.
			°C.	Mm.	Calcd.	Found	Calcd.	Found	Calcd.	Found	
CH <sub>3</sub>	2	C <sub>8</sub> H <sub>14</sub> N <sub>2</sub> O <sub>2</sub> <sup>b</sup>	93-97	2	56.45	56.51	8.29	8.03	16.46	16.53	198-199
C <sub>2</sub> H <sub>5</sub>	2	C <sub>10</sub> H <sub>18</sub> N <sub>2</sub> O <sub>2</sub>	122-124	3	60.58	60.44	9.15	8.97	14.13	13.94	208-209
CH <sub>3</sub>	3	C <sub>9</sub> H <sub>16</sub> N <sub>2</sub> O <sub>2</sub>	105-107	2	58.67	58.41	8.75	8.59	15.21	14.91	196-197
C <sub>4</sub> H <sub>9</sub> O <sup>a</sup>	2	C <sub>10</sub> H <sub>16</sub> N <sub>2</sub> O <sub>3</sub>	140-144	2	56.59	56.85	7.60	7.53	13.20	13.03	217-218

<sup>a</sup> Morpholino. <sup>b</sup> M.p. 40-41°.

TABLE II



R	n	Formula	B.p.,		Carbon, %		Hydrogen, %		Nitrogen, %		HCl M.p., °C.	Dimethiodide analysis		M.p., °C.
			°C.	Mm.	Calcd.	Found	Calcd.	Found	Calcd.	Found		Calcd.	Found	
CH <sub>3</sub>	2	C <sub>8</sub> H <sub>18</sub> N <sub>2</sub>	92-94	48	67.55	67.52	12.76	12.64	19.69	19.58	294-295	6.57	6.60	261-262
C <sub>2</sub> H <sub>5</sub>	2	C <sub>10</sub> H <sub>22</sub> N <sub>2</sub>	56-58	3	70.53	70.68	13.02	12.75	16.45	16.89	245-246	6.16	6.37	228-229
CH <sub>3</sub>	3	C <sub>9</sub> H <sub>20</sub> N <sub>2</sub>	50-55	3	69.17	69.06	12.90	12.72	17.93	17.65	264-265	6.36	6.20	295-297
C <sub>4</sub> H <sub>9</sub> O <sup>a</sup>	2	C <sub>10</sub> H <sub>20</sub> N <sub>2</sub> O	78-80	2	65.17	65.00	10.94	10.73	15.20	15.39	295-296	5.98	6.02	245-246

<sup>a</sup> Morpholino.

ence the reduction of imides with lithium aluminum hydride had proved to give excellent yields in other series. When applied to this series of imides the yields were 90-95%. Phillips<sup>7</sup> had reported that the reaction of ethanolamine with succinic anhydride yielded no imide but only the bis-amide under any of the conditions that he employed. We have prepared diethylaminoethylpyrrolidine by the reaction of  $\beta$ -diethylaminoethyl chloride and pyrrolidine and found that the product obtained was identical with that produced by the reduction of diethylaminoethylsuccinimide.

The various imides prepared and the corresponding pyrrolidine reduction products are listed in Tables I and II, respectively. When tested on dogs for hypotensive activity the dimethiodides of these dialkylaminoalkyl pyrrolidines were found to possess a moderate hypotensive effect.

#### Experimental

**General Procedure for the Preparation and Reduction of Dialkylaminoalkyl Succinimides.**—To 0.3 mole of succinic anhydride was slowly added with cooling 0.3 mole of the appropriate dialkylaminoalkyl amine. When the reaction had subsided the mixture was heated in an oil-bath at 175° for two hours. After the product had cooled to room temperature it was fractionated in vacuum to yield the imide as a colorless oil or low melting solid.

The imide (50 g.) was dissolved in twice its volume of anhydrous ether and slowly added with stirring to a solution of 19 g. of lithium aluminum hydride in 1 liter of anhydrous ether. The rate of addition was such as to cause gentle refluxing. After all the imide had been added the reaction mixture was allowed to stand overnight. Decomposition was effected by the slow addition of water and the mixture stirred an additional hour. The suspension was filtered, dried over sodium sulfate, and the ether stripped off. The products were obtained by vacuum distillation as colorless liquids.

The hydrochlorides were prepared in the usual way by means of alcoholic hydrogen chloride.

The dimethiodides were made in the usual manner by adding methyl iodide to an alcohol solution of the amine.

Diethylaminoethylpyrrolidine was obtained in the usual way by reaction of diethylaminoethyl chloride (0.2 mole)

with pyrrolidine (0.4 mole) in benzene solution. The boiling point of the base and melting point of the hydrochloride corresponded to those of the product obtained by reduction. A mixed melting point of the hydrochloride gave no depression.

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### Isolation of Fructose from Beef Serum<sup>1</sup>

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The isolation of additional amounts of the recently reported TPS (*Treponema pallidum* survival) factor,<sup>2</sup> for the purpose of chemical investigations necessitated the processing of considerable quantities (approximately 100 liters) of beef serum. In order to isolate the TPS factor, protein is removed from the serum by means of tungstic acid, the factor is adsorbed from the protein-free filtrate on charcoal (Darco G-60) and eluted with acetone; the acetone-soluble material after having been extracted with ether, is condensed with acetone in the presence of sulfuric acid. The isopropylidene derivative of the TPS factor which is ether-soluble is now precipitated from ether with petroleum ether (b.p. 30-60°) and recrystallized from ethyl acetate.<sup>2</sup> The material soluble in ether-petroleum ether from individual lots of serum (in all approximately 100 liters) was pooled and concentrated to dryness. The thick brown sirup which resulted gave a strong positive test for carbohydrate by the conventional Molisch and Anthrone<sup>3</sup> tests. It was thought to be of interest particularly in view of the major role played by carbohydrates in metabolism, to attempt to separate, and identify

(1) Supported by a grant from the Whitehall Foundation, New York, N. Y.

(2) F. A. H. Rice and Robert A. Nelson, Jr., *J. Biol. Chem.*, **191**, 35 (1951).

(3) R. Dreywood, *Ind. Eng. Chem., Anal. Ed.*, **18**, 499 (1946); L. Sattler and F. W. Zerban, *THIS JOURNAL*, **72**, 3814 (1950).

(7) A. P. Phillips, *THIS JOURNAL*, **73**, 5557 (1951).