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Aliphatic Amines II. The *n*-Heptylamines, Preparation and Toxicity*

By Melvin F. W. Dunker†, Walter H. Hartung and C. W. Chapman

With the exception of the primary heptylamine, no studies on toxicity have been made on the C_7 amines. The work on heptylamine is limited to pressor studies (1), studies on the oxidation of heptylamine by tissues (2) and by amine oxidase (3), studies on the antibacterial efficiency (4) of the compound, the production of specific precipitating and complement-fixing antibodies when injected in rabbits (5), and its toxicity for paramecia (6). As a preliminary experiment, it was thought interesting to prepare the amines in pure form and determine whether the position of the amino group in the chain had any effect on the toxicity of the compounds.

The amines were administered intraperitoneally in neutral solutions to white mice. According to the results obtained in comparative experiments, the 1- and 4-aminoheptanes seemed to be the least toxic, the 2- and 3-aminoheptanes being approximately equal but more toxic than the 1-aminoheptane.

EXPERIMENTAL

The amines were prepared free of secondary or tertiary amines by the reduction of the oximes. The necessary aldehyde and ketones were obtained as follows. Eastman technical heptaldehyde was fractionated twice and the cut boiling 152–156° used. Methyl *n*-amylketone and dipropylketone were generously supplied by Carbide and Carbon Company. The samples were fractionated before use. Methyl *n*-amyl ketone boiling at 150–151° and dipropylketone boiling at 142–144° were used. The heptanone-3 was prepared by the dry distillation of an intimate mixture of calcium propionate and calcium valerate. The product was fractionated and a fraction boiling 147–153° used for the preparation of oximes. The oximes were all prepared according to the method given in the literature (7) and illustrated below.

Preparation of Oximes.—To 87 Gm. (1.25 mol) of hydroxylamine hydrochloride in 125 cc. water in a 1-L. 3-neck flask equipped with a reflux condenser, mechanical stirrer, thermometer and dropping funnel were added 114 Gm. (1 mol) heptaldehyde and the mixture was stirred vigorously. A solution of 66 Gm. (0.63 mol) sodium carbonate in 175 cc. water was added slowly so that the temperature did not rise above 45°. The stirring was continued for an hour after the complete addition of the solution. On standing over night, the oxime solidified in large lumps which were filtered out and dried in a desiccator. The yield was quantitative. The crystals melted at 53–55° and after crystallization from 60% alcohol melted at 53.5–54.5°. The preparation of the three ketoximes was carried out in like manner and the properties are given in Table I.

Reduction of Oximes.—Test experiments carried out on the reduction of heptaldoxime in either glacial acetic acid or alcoholic hydrochloric acid in the presence of palladium on charcoal at atmospheric pressure or pressures up to an initial pressure of 750 lb. were not successful in the production of primary amine.

Since the catalytic reduction did not work successfully under the conditions investigated, the reductions were carried out with sodium and absolute alcohol (8). Nine-tenths mol methyl *n*-amyl ketoxime was dissolved in 1.8 L. absolute alcohol and the solution heated to boiling in a water bath under a reflux condenser. The sodium, cut in thin strips (9.8 mols or 225 Gm.), was added slowly through the condenser. Near the end, the sodium melted to a ball which settled to the bottom and it was necessary to continue the heat to retard the formation of a crystalline mass of sodium ethoxide which trapped globules of sodium. When the sodium appeared to be gone, the cautious addition of water was begun and finally 1.5 L. of water were added. The flask was arranged for distillation and the distillate collected in a mixture of 135 cc. concentrated hydrochloric acid and 135 cc. water. The alcohol and most of the water were then distilled from a water bath under reduced pressure. To the amine hydrochloride crystals were added 500 cc. of 40% solution of sodium hydroxide while cooling. The aqueous layer was withdrawn and the amine dried

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over solid potassium hydroxide. The dry 2-aminoheptane was then fractionated and 60 Gm. boiling 141.8–142.3° at 758 mm. were obtained. The other amines were similarly prepared, and their properties are summarized in Table I.

sexes, weighing 18 to 25 Gm. The animals were observed for three days. Usually death occurred within 15 to 30 minutes after injection. Animals surviving 12 hours did not subsequently die while under observation. From preliminary experiments

Table I.—Properties of Aminoheptanes

Oxime	Boiling Point, °C.	Amine	Per Cent Yield	Boiling Point, °C.	M. P., °C. Benzamide	M. P., °C. Picrate	LD_{50}, Mg./Gm. Mouse
Heptaldoxime	53.5–54.5[a]	1-Aminoheptane	65	154.5–155.5 (765 mm.)[b]	34–35	120–121[c]	0.1[d]
Methyl *n*-amyl ketoxime	88–90 (5–6 mm.)[e]	2-Aminoheptane[j]	58	141.8–142.5 (758 mm.)[f]	68–69	97–98	0.06
Ethyl *n*-butyl ketoxime	85–105 (5 mm.)[g]	3-Aminoheptane	71	140–144 (761 mm.)	89.5–90	120–121.5	0.07
Dipropyl ketoxime	85–86 (6–7 mm.)[h]	4-Aminoheptane	65	140–141 (761 mm.)[i]	108.5–109.5	165.5–166.5	0.11

[a] 57–58°, Ponzio, *J. prakt. Chem.* [2], 53 (1896), 432.
[b] $b._{760}$ 154.5–155.5°, Ralston, A. W., *Oil and Soap*, 17 (1940), 89; 153°, Paul, R., *Bull. soc. chim.* [5], 4 (1937), 1121.
[c] 118.5–119.5°, Adamson, D. W., and Kenner, J., *J. Chem. Soc.*, (1934), 838; 120–121°, Takaki, S., and Ueda, T., *J. Pharm. Soc. Japan*, 58 (1938), 276.
[d] The sample supplied by Sharples Solvents Corp. gave the same value.
[e] $b._{13}$ 99.65°, Simon, I., *Bull. soc. chim. Belg.*, 38 (1929), 47.
[f] 142–144°, Clarke, *J. Am. Chem. Soc.*, 21 (1899), 1027.
[g] $b._{15}$ 97.5°, Timmermans, J., *Bull. soc. chim. Belg.*, 36 (1927), 502.
[h] $b._{772}$ 196°, Trapesongjanz, *Ber.*, 26 (1893), 1433; $b._{760}$ 195°, Mailhe, *Bull. Soc. Chim.*, [4], 15 (1914), 328.
[i] 139–140°, Noyes, W. A., *J. Am. Chem. Soc.*, 15 (1893), 542; 140–141°, Kishner, *J. Russ. Phys.-Chem. Ges.*, 31, 874.
[j] Chloroaurate melted 77–78.5°; Clarke gave 63–64°, *J. Am. Chem. Soc.*, 21 (1899), 1027.

A sample *n*-heptylamine was generously supplied by Sharples Solvents Corporation. It was fractionated and a sample boiling 154.5–155.0° at 765 mm. taken. The derivatives prepared from it agreed in properties with those of the synthetic sample and when tested on mice, no difference in toxicity could be detected.

The picrates were prepared by adding a saturated solution of picric acid in benzene to a benzene solution of the amine. The product was crystallized from benzene or dilute alcohol. The benzamides were prepared by treating the amine with an equivalent of benzoyl chloride in the presence of a slight excess of sodium hydroxide solution. All except the benzamides of 1-aminoheptane crystallized easily from dilute alcohol. The 1-aminoheptane benzamide persisted in remaining as an oil for some time. However, when an ether solution was chilled in a carbon dioxide ice bath, very fine, small needles formed which after three crystallizations from ether and ether-petroleum ether mixtures melted 34–35°. The various properties are summarized in Table I.

Acute Toxicity Determinations.—Toxicity studies were made for the four aminoheptanes synthesized and for a purified sample of commercial 1-aminoheptane.[1] Aqueous solutions of each amine were prepared of such a concentration that the appropriate dose in mg. per Gm. of mouse was contained in 0.7 to 1.0 cc. These solutions were made neutral to litmus with *N*/1 hydrochloric acid. Injections were made intraperitoneally into white mice of both

[1] Obtained from Sharples Solvents Corporation.

on three different samples of 1-aminoheptane (Sharples) the LD_{50} was found to be between 0.09 and 0.10 mg. per Gm. body weight. The integrated distribution curve (*b* curve) was extremely steep.

A series of determinations was made on all of the isomers between June 21 and July 12, 1940. Three to five mice were injected with each of a series of five doses (equal logarithmic interval) for each compound. Not all compounds were injected on the same day. The LD_{50} for 1-aminoheptane did not vary more than 10% during the experiments, and it is therefore assumed that no appreciable change in sensitivity occurred during the experiments. From the data obtained, the LD_{50}'s were calculated by Karber's method (9). The synthesized 1-aminoheptane had exactly the same acute toxicity as the Sharples sample. Apparently the 1-amino- and the 4-aminoheptanes had about the same toxicity, while the 2-amino- and 3-aminoheptanes were slightly more toxic. A sufficient number of animals was not employed to determine significant differences.

SUMMARY

1. The four primary 1 amines from *n*-heptane have been prepared in pure form and characterized.

2. The acute toxicity for white mice by intraperitoneal injection has been determined. The 1- and 4-aminoheptanes are of about equal toxicity whereas the 2- and 3-aminoheptanes are more toxic.

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Determination of Iodine in Desiccated Thyroid*

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In the course of another investigation, the authors have had occasion to determine iodine in various samples of desiccated thyroid and various extracted fractions of thyroid and iodinated protein. For extremely accurate work, and especially when very small amounts of iodine are encountered, a recent modification (1) of Leipert's wet digestion procedure (2) is preferred, but for ordinary purposes we have found that ignition in a simple alkaline fusion mixture free from oxidizing agents will give recoveries of iodine comparable to those obtained by the U. S. P. XI thyroid assay and within three per cent of the calculated theoretical values in the case of known mixtures of low iodine content. Interference by nitrates and nitrites is eliminated and the reagents are simplified.

Using four samples of desiccated thyroid and two standardized mixtures of diiodotyrosine and casein, the proposed procedure was compared with three published procedures: the U. S. P. XI assay, the Burnett and Warkow modification (3) and the Manganese Dioxide Method (4). The latter method employs a manganese dioxide ignition mixture and is particularly applicable to the determination of iodine in the presence of oxidizing metallic catalysts. In a study involving 106 analyses by four individuals, the proposed procedure when applied to thyroid gave recoveries essentially identical with those obtained by the U. S. P. XI assay. Applied to the standard diiodotyrosine-casein mixtures, recoveries were slightly higher than by the U. S. P. method and varied between 97.2 per cent and 98.9 per cent of theoretical.

In a previous paper (4), the degree of acidity at which iodine should be liberated by iodic acid was reported. At pH 2.6 or above, the recovery was depressed slightly and the proper reagent blank was not obtained. At pH values below 2.0, the rate of return of color after titrating was increased. Burnett and Warkow (3), in their modification of the U. S. P. XI assay, state that the uncertain end-point and the blank are eliminated and accurate results obtained when the pH is adjusted to 2.5-2.7 and the temperature to about 33° before the addition of potassium iodide. In view of this criticism, the recovery of iodine from standard quantities of potassium iodate was again investigated.

Effect of pH on the Liberation of Iodine.—The iodine liberated by standard quantities of potassium iodate at different pH values was titrated with sodium thiosulfate. Recovery was depressed when the liberation of iodine was conducted at pH values above 2.55.

A series of standard potassium iodide samples (2.00 mg.) were treated exactly according to the U. S. P. XI thyroid assay, the iodine being liberated at different pH values. At values above pH 2.5, the solution was adjusted to 33° C. before the addition of potassium iodide. The results in Table I indicate that the recovery and the reagent blank are depressed above pH 2.5.

Table I.—Analysis of Potassium Iodide

pH	Blank as Cc.	Per Cent Recovery	Determinations
2.52-2.69	0.0	95.1- 96.9	8
1.92-1.98	0.25	100.2-100.8	5
1.52	0.30	99.74	2

Using 0.40-cc. portions of 0.005*N* potassium iodate as representing a normal blank, it is illustrated in Table II that iodine recovery from the blank is low at pH 2.1-2.2 (the range usually encountered in the U. S. P. XI assay) and that no recovery is obtained

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