product was isolated in essentially 100% yield and, recrystallized from 50% ethanol, melted at 216–217°.

*Anal.* Calcd. for $C_9H_{11}BrN_2O$: N, 11.5. Found: N, 11.6.

The picrate was prepared, m.p. 166–167°.

*Anal.* Calcd. for $C_{15}H_{14}BrN_5O_8$: N, 14.8. Found: N, 14.7.

**2-Amino-5-bromo-4,6-dimethylpyridine (IX).**—A solution of 1.5 g. of VIII in 35 ml. of 15% sodium hydroxide was refluxed for two hours. The free base separated out on cooling as fluffy white crystals, m.p. 145–146°. A mixture of this with II melted at 90–95°.

*Anal.* Calcd. for $C_7H_9BrN_2$: N, 13.9. Found: N, 14.1.

The monopicrate was prepared, m.p. 226–228°.

*Anal.* Calcd. for $C_{13}H_{12}BrN_5O_7$: C, 36.29; H, 2.81; N, 16.3. Found: C, 36.73; H, 2.76; N, 16.3.

**5-Bromo-4,6-dimethyl-2-pyridol (X) (a) From IX.**—A solution of 0.5 g. of IX in 30 ml. water and 10 ml. of concd. hydrochloric acid was heated to boiling and filtered. To the clear hot filtrate was added with swirling a solution of 0.5 g. of sodium nitrite in 10 ml. of water. Fumes of oxides of nitrogen were evolved. On cooling, a white precipitate X was isolated in 78% yield. This was recrystallized from 95% ethanol, m.p. 240–241°. A mixture of this with III melted at 200–205°. It gave a weak ferric chloride color test and was not affected by boiling water nor boiling alcoholic silver nitrate.

*Anal.* Calcd. for $C_7H_8BrNO$: N, 6.9. Found: N, 6.7.

The picrate was prepared, m.p. 195–197°.

*Anal.* Calcd. for $C_{13}H_{11}BrN_4O_8$: N, 13.0. Found: N, 13.5.

The addition of an excess of bromine to an acetic acid solution of X in glacial acetic acid gave III, m.p. 235–236°. This sample of III did not depress the melting point of III prepared by the above methods.

**(b) From 3-Cyano-4,6-dimethyl-2(1)-pyridone.**—The bromination of 3-cyano-4,6-dimethyl-2(1)-pyridone in glacial acetic acid gave 5-bromo-3-cyano-4,6-dimethyl-2(1)-pyridone in 80% yield even though neither the starting material nor the product are appreciably soluble in this solvent. Recrystallized from 70% ethanol, it melted at 259–260°. A solution of 1 g. of this compound in 22 ml. of sulfuric acid (50% by volume) was refluxed for five hours. The reaction mixture was poured into 100 ml. of a slurry of ice and water containing 17 g. of sodium hydroxide. The neutralization was completed with sodium bicarbonate. The insoluble product was isolated as fluffy white crystals (85% yield). Recrystallized from 95% ethanol it melted at 241–242° and did not depress the melting point of X prepared by method (a).

**3-Bromo-4,6-dimethyl-2-pyridol (XI).**—The silver salt of 3-carboxy-4,6-dimethyl-2-pyridol[3b] was prepared according to the method of Barnes and Prochaska.[17] To a suspension of 0.25 g. of the dry salt in 25 ml. of dry carbon tetrachloride was added an equivalent amount of dry bromine. The reaction mixture was refluxed on a steam-bath for five hours, cooled, and filtered. The filtrate was distilled to dryness under reduced pressure. The residue recrystallized from 95% ethanol gave white crystals, m.p. 228–229°. A mixture of this with X melted at 180–185°. It gave a weak ferric chloride color test.

*Anal.* Calcd. for $C_7H_8BrNO$: N, 6.9. Found: N, 7.2.

(17) R. A. Barnes and R. J. Prochaska, THIS JOURNAL, **72**, 3188 (1950).

EVANSTON, ILLINOIS RECEIVED NOVEMBER 5, 1951

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[CONTRIBUTION FROM THE STERLING-WINTHROP RESEARCH INSTITUTE]

# Antispasmodics. III. N-Methyl-2(4)-(ω-R-ω-phenylalkyl)-piperidines and 2-R-2-Phenyl-4(5)-(N-methyl-4-piperidyl)-alkanenitriles

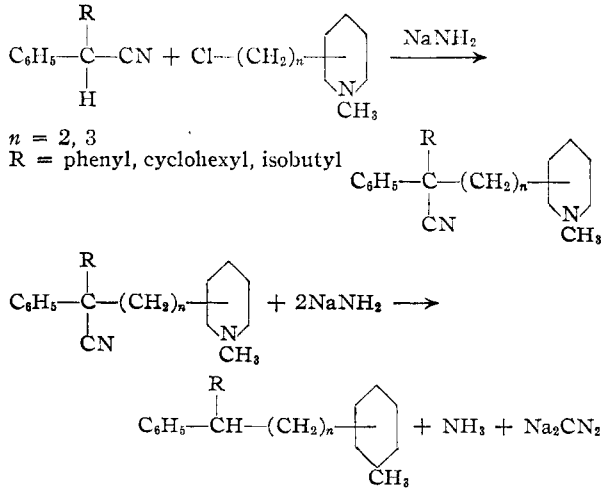
BY A. WAYNE RUDDY[1] AND HOWARD W. BISHOP

A series of amines has been synthesized to determine the effect on spasmolytic activity of moving various ω-substituted phenylalkyl groups from the nitrogen into the 2- and 4-positions of the piperidine ring. 2-R-2-Phenyl-4(5)-(N-methyl-2(4)-piperidyl)-alkanenitriles have been prepared where R is phenyl, cyclohexyl and isobutyl. The corresponding N-methyl-2(4)-(ω-R-ω-phenylalkyl)-piperidines were obtained from these nitriles by treatment with excess sodamide. The most active compound in the series was 2-(3,3-diphenylpropyl)-N-methylpiperidine methiodide.

In continuing work in these laboratories on spasmolytics it was believed that the availability of the 2- and 4-pyridylethanols and propanols offered an excellent opportunity to study the effect on spasmolytic activity of moving various ω-substituted phenylalkyl groups from the 1-position[2] into the 2- and 4-positions of the piperidine ring.

Although a number of phenylalkylpiperidines have been reported, a literature search revealed only one substituted phenylalkylpiperidine, N-methyl-3-benzhydrylpiperidine[3] and no N-methyl-2(4)-(ω-R-ω-phenylalkyl)-piperidines. The only piperidylphenylacetonitriles found were α-phenyl-α-(N-methyl-3-piperidyl)-acetonitrile[4] and α,α-diphenyl-(N-methyl-3-piperidyl)-acetonitrile[5] prepared by alkylating the nitriles with N-methyl-3-chloropiperidine.

From the 2- and 4-pyridylalkanols were prepared the corresponding N-methylpiperidylalkanols which were then chlorinated with thionyl chloride to give the N-methyl-2- and 4-piperidylethyl chloride and propyl chloride hydrochlorides. These basic side chains were used to alkylate diphenylacetonitrile,




(1) Chilcott Laboratories, Morris Plains, N. J.

(2) A. W. Ruddy, THIS JOURNAL, **73**, 4096 (1951).

(3) M. Bockmühl, E. Ehrhart and L. Stein, U. S. Patent 2,446,522, Aug. 10, 1948.

(4) Society of Chemical Industry of Basle, British Patent 589,625.

(5) I. G. Farbenindustrie A. G., German patent 731,560, Jan. 14, 1943.

TABLE I

N-METHYL-2(4)-(ω-R-ω-PHENYLALKYL)-PIPERIDINES AND CORRESPONDING NITRILES $C_6H_5-\underset{\displaystyle R}{\overset{\displaystyle R'}{\overset{|}{\underset{|}{C}}}}-(CH_2)_n-$ (N-$CH_3$)

| R | R' | n | Yield, % | B.p., °C. | Base Mm. | $n^{25}D$ | M.p., °C. (cor.) | Formula | Hydrochlorides and methiodides: Carbon, % Calcd. | Carbon, % Found | Hydrogen, % Calcd. | Hydrogen, % Found | Halogen, % Calcd. | Halogen, % Found |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **2-Piperidyl derivatives** | | | | | | | | | | | | | | |
| Phenyl | H | 2 | 74 | 173–178 | 1.5 | 1.5567 | 187.3–188.5 | $C_{21}H_{27}N\cdot HCl$ | 76.45 | 76.49 | 8.56 | 8.53 | 10.75 | 10.71 |
| | | | | | | | 203.2–205 | $C_{22}H_{30}IN$ | 60.69 | 60.67 | 6.95 | 6.98 | 29.15 | 29.40 |
| Cyclohexyl | H | 2 | 71 | 140–145 | 0.15 | 1.5280[a] | 177.6–178.4 | $C_{21}H_{33}N\cdot HCl$ | 75.07 | 75.14 | 10.20 | 10.30 | 10.55 | 10.41 |
| Cyclohexyl | CN | 2 | 69 | 170–178 | 0.2 | 1.5327 | [b] | $C_{22}H_{32}N_2$ | [c] | | | | | |
| **4-Piperidyl derivatives** | | | | | | | | | | | | | | |
| Phenyl | H | 2 | 86 | 144–147 | 0.1 | 1.5566 | 160–162 | $C_{21}H_{27}N\cdot HCl$ | 76.45 | 76.20 | 8.56 | 8.32 | 10.75 | 10.90 |
| | | | | | | | 210.8–211.8 | $C_{22}H_{30}IN$ | 60.69 | 60.63 | 6.95 | 6.74 | 29.15 | 28.92 |
| Phenyl | H | 3 | 76 | 163–176 | 0.3 | 1.5527 | 191.8–194 | $C_{22}H_{29}N\cdot HCl$ | 76.83 | 76.90 | 8.97 | 9.05 | 10.31 | 10.15 |
| | | | | | | | 226–227.5 | $C_{23}H_{32}IN$ | 61.47 | 61.45 | 7.18 | 7.18 | 28.24 | 28.40 |
| Cyclohexyl | CN | 3 | 92 | 179–186 | 0.05 | 1.5275 | 181–182.5 | $C_{23}H_{34}N_2\cdot HCl$ | [d] | | | | 9.46 | 9.44 |
| | | | | | | | 244–245.1 | $C_{24}H_{37}IN_2$ | [e] | | | | 26.42 | 26.54 |
| Cyclohexyl | H | 3 | 93 | 158–163 | 0.1 | 1.5210 | 219–220.8 | $C_{22}H_{33}N\cdot HCl$ | 75.50 | 75.35 | 10.37 | 10.18 | 10.13 | 10.27 |
| | | | | | | | 198.1–199.2 | $C_{23}H_{36}IN$ | 60.65 | 60.65 | 8.41 | 8.21 | 27.87 | 27.93 |
| Isobutyl | CN | 3 | 92 | 135–140 | 0.05 | 1.5091 | 165.6–167.6 | $C_{21}H_{32}N_2\cdot HCl$ | 72.28 | 72.57 | 9.53 | 9.80 | 10.16 | 9.94 |
| | | | | | | | 232.5–233.8 | $C_{22}H_{35}IN_2$ | 58.14 | 58.20 | 7.76 | 7.67 | 27.93 | 28.20 |
| Isobutyl | H | 3 | 90 | 130–135 | 0.5 | 1.5007 | 149–149.8 | $C_{20}H_{33}N\cdot HCl$ | 74.15 | 74.34 | 10.58 | 10.46 | 10.95 | 10.68 |
| | | | | | | | 205–206.1 | $C_{21}H_{36}IN$ | 58.73 | 58.84 | 8.45 | 8.24 | 29.56 | 29.46 |

[a] Eventually crystallized m.p. 46–47.5°. [b] Hydrochloride would not crystallize. [c] *Anal.* Calcd.: neut. equiv., 324.49. Found: neut. equiv., 321.5. [d] *Anal.* Calcd.: N, 7.47. Found: N, 7.40. [e] *Anal.* Calcd.: N, 5.83. Found: N, 5.59.

cyclohexylphenylacetonitrile and isobutylphenylacetonitrile with the aid of sodium amide. The basic nitriles were then treated with excess sodium amide to replace the cyano group by hydrogen.[2]

The N-methyl-2- and 4-piperidylethyl chlorides and N-methyl-4-piperidylpropyl chloride condensed with the nitriles in good yield, but no basic nitriles were obtained from a reaction with N-methyl-2-piperidylpropyl chloride. Apparently the only product formed from the last side chain was N-methyloctahydropyrrocolinium chloride produced by ring closure within the molecule. None of the 2-(4)-piperidylalkyldiphenylacetonitriles were isolated in pure form. The nitrile group was so labile that the product isolated was always a mixture of the basic nitrile and the corresponding N-methyl-2(4)-(diphenylalkyl)-piperidine. In contrast the basic cyclohexyl- and isobutylphenylacetonitriles, since they required the temperature of refluxing xylene to remove the nitrile group, were purified and characterized before further treatment with sodium amide.

Among the compounds containing two asymmetric centers only one racemate of 2-(3-cyclohexyl-3-phenylpropyl)-N-methylpiperidine hydrochloride was obtained, and no solid derivatives could be obtained of 2-cyclohexyl-2-phenyl-4-(N-methyl-2-piperidyl)-butanenitrile, 2-isobutyl-2-phenyl-4-(N-methyl-2-piperidyl)-butanenitrile or N-methyl-2-(5-methyl-3-phenylhexyl)-piperidine.

The preliminary data published by Dr. A. M. Lands of our Pharmacology Department[6] indicate that the most active antispasmodic in this series is 2-(3,3-diphenylpropyl)-N-methylpiperidine methiodide. It appears to have about one-half of the activity of atropine sulfate in relaxing the spasms induced by acetylcholine in isolated rabbit intestine. It is about ten times as active as N-(3,3-diphenylpropyl)-piperidine under comparable conditions.

(6) A. M. Lands, *J. Pharmacol. Exptl. Therap.*, **101**, 313 (1951).

**Acknowledgment.**—The authors are indebted to Mr. M. E. Auerbach, Mr. K. D. Fleischer and staff for the analytical data.

## Experimental

**2(4)-(Chloroalkyl)-N-methylpiperidines.**—Chlorination of the N-methyl-2(4)-piperidylethanols and N-methyl-2(4)-piperidylpropanols[7] was accomplished with thionyl chloride in chloroform. The chloroform was removed under reduced pressure and the products obtained in 90 to 92% yields by recrystallization from ethyl acetate–ethanol mixtures. 2-(2-Chloroethyl)-N-methylpiperidine hydrochloride, m.p. 137.5–139°; reported m.p. 132–133.[8]

*Anal.* Calcd. for $C_8H_{16}ClN\cdot HCl$: N, 7.07; Cl, 35.79. Found: N, 7.08; Cl, 35.70.

4-(2-Chloroethyl)-N-methylpiperidine hydrochloride melted at 121–121.5°.

*Anal.* Calcd. for $C_8H_{16}ClN\cdot HCl$: C, 48.50; H, 8.60; Cl, 35.79. Found: C, 48.55; H, 8.86; Cl, 35.45.

2-(3-Chloropropyl)-N-methylpiperidine hydrochloride melted at 120.5–121.2°.

*Anal.* Calcd. for $C_9H_{18}ClN\cdot HCl$: C, 50.95; H, 9.03; $Cl^-$, 16.71. Found: C, 50.84; H, 9.03; $Cl^-$, 16.88.

4-(3-Chloropropyl)-N-methylpiperidine hydrochloride melted at 134.8–135.2°.

*Anal.* Calcd. for $C_9H_{18}ClN\cdot HCl$: N, 6.60; Cl, 33.43. Found: N, 6.88; Cl, 33.02.

**2-Cyclohexyl-2-phenyl-5-(N-methyl-4-piperidyl)-pentanenitrile.**—The preparation of this nitrile illustrates the procedure used in preparing all of the basic nitriles.

A mixture of 40 g. (0.2 mole) of cyclohexylphenylacetonitrile, 42.5 g. (0.2 mole) of 4-(3-chloropropyl)-N-methylpiperidine hydrochloride and 17.5 g. (0.45 mole) of sodium amide was vigorously stirred and gradually heated to 65°. The exothermic reaction was controlled by occasional cooling with an ice-bath. The reaction was heated at 70° for two more hours, cooled and the excess sodamide decomposed by the dropwise addition of water. The benzene layer was separated, washed with water and extracted with 10% hydrochloric acid. The combined acid extracts were made strongly alkaline with 35% sodium hydroxide and the base extracted with ether. After drying over sodium hydroxide pellets the ether was removed and the product distilled under reduced pressure, b.p. 179–186° (0.05 mm.).

The basic nitriles prepared in this manner are described in Table I.

(7) R. R. Burtner and J. M. Brown, THIS JOURNAL, **69**, 630 (1947).

(8) T. R. Norton, R. A. Seibert, A. A. Benson and F. W. Bergstrom, *ibid.*, **68**, 1572 (1946).

**4-(4-Cyclohexyl-4-phenylbutyl)-N-methylpiperidine.**—A solution of 33.9 g. (0.1 mole) of the nitrile just described in 100 ml. of xylene was added gradually to a vigorously stirred mixture of 12 g. (0.3 mole) of sodium amide in 100 ml. of refluxing xylene. The mixture was refluxed and stirred for ten hours, then cooled and decanted from the excess sodium amide. The benzene solution was washed with water and then extracted with 10% hydrochloric acid. The acid extracts were made alkaline and the base taken up in ether and dried over sodium hydroxide pellets. The ether was removed and the amine distilled under reduced pressure, b.p. 158–163° (0.1 mm.).

The amines prepared in this way are described in Table I.

**Hydrochlorides.**—Samples of the basic nitriles and amines were converted to their hydrochlorides with hydrogen chloride in alcohol and precipitated with ether. The hydrochlorides were recrystallized from acetone–ether or alcohol–ether combinations and are described in Table I.

Only one racemate of 2-(3-cyclohexyl-3-phenylpropyl)-N-methylpiperidine hydrochloride was obtained in crystalline form.

**Methiodides.**—The basic nitriles and amines were converted to their methiodides by warming the bases in benzene or ethyl acetate with excess methyl iodide. The methiodides were recrystallized from ethyl acetate–methanol and are described in Table I.

MORRIS PLAINS, N. J. RECEIVED OCTOBER 2, 1951

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[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY, UNIVERSITY OF MARYLAND]

# The Action of Rat Liver Tissue on Mixtures of Methionine and Ethanolamine

BY F. P. VEITCH AND GUNTER ZWEIG

This paper describes experimental work which shows that when methionine and ethanolamine are incubated with rat liver slice or homogenate, the presence of D-amino acid oxidase in this tissue causes an apparent disappearance of methionine as judged by the McCarthy–Sullivan method for methionine. In the light of this finding, reports of *in vitro* transmethylation from methionine to ethanolamine to form choline based on the disappearance of methionine from the reaction mixture are subject to grave doubt. The validity of the reported isolation of choline as the reineckate from such reaction mixtures is also shown to be questionable.

The reported[1–5] *in vitro* synthesis of choline from methionine and ethanolamine using rat or guinea pig kidney or liver, slices or homogenates as a source of a transmethylase enzyme or enzyme system led us to attempt to isolate this enzyme or enzyme system. Consistent failure to isolate such a system or even duplicate the cited reports has caused us grave doubt as to the validity of these reports.

Steensholt[2–5] has used the disappearance of methionine from the reaction mixture, as measured by the McCarthy–Sullivan method,[6] as proof that transmethylation has occurred. Both Steensholt and Barrenscheen[1] report the formation of choline as evidenced by precipitation of a supposed choline reineckate from the reaction mixture. No attempt was made to identify this precipitate by physical properties or analysis. Steensholt recognized that this latter was poor evidence, as ethanolamine also gave a reineckate under the conditions employed for the formation of choline reineckate. We concur in this observation of Steensholt and in addition have shown that the choline reineckate obtained by Barrenscheen in his experiments was in all probability the reineckate of ethanolamine.

We have confirmed the observation of Krebs[7] that rat and guinea pig liver or kidney is rich in D-amino acid oxidase, and further observed that the formation of α-keto-γ-methiolbutyric acid by the action of this enzyme on methionine accounts for the apparent disappearance of methionine, from reaction mixtures containing these tissues, as measured by the McCarthy–Sullivan method. When rabbit liver homogenates, which have been shown to contain little if any D-amino acid oxidase,[8] were substituted for rat liver no methionine disappearance was noted.

Analysis of a sample of pure sodium salt of α-keto-γ-methiolbutyric acid by the McCarthy–Sullivan method revealed that while this keto analog of methionine gives a color in this reaction the color is much less intense than for an equivalent amount of methionine. We have described a method for determining the amount of keto analog present in mixtures of methionine and α-keto-γ-methiolbutyric acid.

## Experimental

The experiments herein described were conducted in Warburg flasks where possible and desirable, and any gaseous exchange recorded. Suitable blanks were included in all runs. Samples of D-methionine and L-methionine were obtained through the courtesy of Dr. J. P. Greenstein of the Cancer Institute, National Institutes of Health, Bethesda, Maryland. The sodium salt of α-keto-γ-methiolbutyric acid was furnished by Dr. Alton Meister of the same address and had the analysis: Calcd. for $C_5H_7O_3SNa$: C, 35.3; H, 4.1; S, 18.8; Na, 13.5. Found: C, 35.6; H, 4.1; S, 18.7; Na, 13.5.

A number of experiments were run under conditions which duplicated those of Barrenscheen and Steensholt. That is, methionine and ethanolamine neutralized with either phosphoric or hydrochloric acid, were dissolved in McIlvaine's phosphate–citrate buffer (*p*H 7.1) and either 1.0 ml. of a 10% tissue homogenate in Krebs-phosphate Ringer, or approximately 100 mg. of tissue slice added as the source of enzyme. All reactions were conducted at 37°.

At the end of a run the contents of the Warburg flasks were transferred to volumetric flasks, or pooled in cases where isolation procedures were to be followed. In either case the solutions were deproteinized with trichloroacetic acid after which determinations for methionine, choline or isolation procedures were carried out. The results of these experiments are best presented in the form of Table I.

**Analysis of Methionine in the Presence of α-Keto γ-Methiolbutyric Acid.**—The colored compound formed from methionine in the McCarthy–Sullivan method demonstrated

(1) H. E. Barrenscheen, *et al.*, *Z. physiol. Chem.*, **284**, 228 (1949).
(2) G. Steensholt, *Acta Physiol. Scand.*, **10**, 333 (1945).
(3) G. Steensholt, *ibid.*, **11**, 294 (1946).
(4) G. Steensholt, *ibid.*, **14**, 340 (1947).
(5) G. Steensholt, *ibid.*, **17**, 276 (1949).
(6) T. E. McCarthy and M. X. Sullivan, *J. Biol. Chem.*, **141**, 871 (1941).
(7) H. A. Krebs, *Biochem. J.*, **29**, 1620 (1935).
(8) F. Berheim and M. L. C. Berheim, *J. Biol. Chem.*, **109**, 131 (1935).