Pyrrolidine-Substituted Nicotine Analogs: Synthesis and Pharmacology

WARREN HANKINS* and ALFRED BURGER

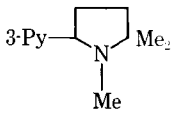
Abstract □ (—)5′,5′-Dimethylnicotine (I) was synthesized in one step from (—) cotinine; it had approximately 0.03 times the mouse toxicity of nicotine but did not cause the same qualitative toxic signs as nicotine; it stimulated and then depressed autonomic ganglia (anesthetized dog) and directly affected the respiratory center and the cardiovascular system. The total synthesis of 5,5-dimethyl-2-(3-pyridyl)-Δ^1-pyrroline (V) and its 1-oxide (VI) was performed. Compound V was hydrogenated to 2,2-dimethyl-5-(3-pyridyl)pyrrolidine (II), and this was converted to its 1-propyl homolog. Compound VI was reduced to 2,2-dimethyl-1-hydroxy-5-(3-pyridyl)pyrrolidine (VIII).

Keyphrases □ Nicotine analogs, pyrrolidine substituted—synthesis □ Pharmacology—pyrrolidine-substituted nicotine analogs □ NMR spectroscopy—structure □ Mass spectroscopy—identity □ Nitrone—synthesis, reactions

Because of the blocking action of neighboring methyl groups upon the piperidine-nitrogen in pempidine (1), the authors undertook a study of derivatives with sterically crowding methyl groups in the pyrrolidine ring of nicotine.[1] Of compounds which have been studied pharmacologically, 5′-methylnornicotine (2), and nicotine analogs with alkyl groups in the pyridine ring, including 4- and 6-methylnicotine (3, 4), and 2-(or 6-) alkyl-1,2-(or 1,6-)dihydro-1-methylnicotine compounds (5) may be mentioned; the latter paralyzed the respiratory center but lowered the dog's blood pressure.

THEORETICAL

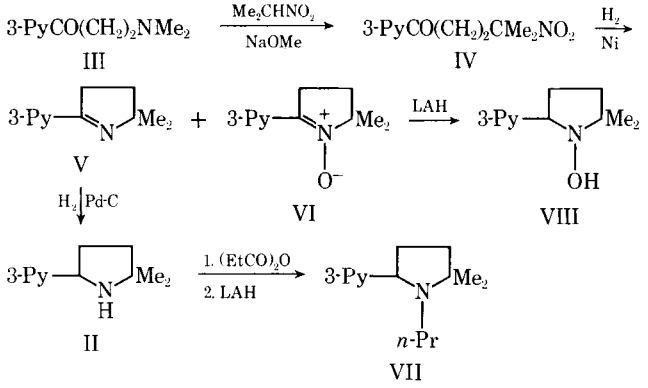
In this work, (—) cotinine was treated with excess MeMgI to give (—) 5-(3-pyridyl)-1,2,2-trimethylpyrrolidine [(—)5′,5′-dimethylnicotine (I)].



I

The corresponding 1-*nor* compound [(±)5,5-dimethyl-2-(3-pyridyl)pyrrolidine] (II) was synthesized as follows. Methoxide-induced condensation of 3-dimethylamino-1-(3-pyridyl)-1-propanone (III) with 2-nitropropane, and hydrogenation of the resulting 4-methyl-4-nitro-1-(3-pyridyl)-1-pentanone (IV) gave 5,5-dimethyl-2-(3-pyridyl)-Δ^1-pyrroline (V) together with 5,5-dimethyl-2-(3-pyridyl)-Δ^1-pyrroline-1-oxide (VI) (6). The pyrroline V was then hydrogenated to II.

Confirmation of structures V and VI rested on chemical reactions and spectra (see *Experimental*). Propionylation of II followed by reduction of the resulting amide gave 2,2-dimethyl-1-*n*-propyl-5-(3-pyridyl)pyrrolidine (VII), the propyl homolog of I. Furthermore,



the nitrone VI could be reduced to 2,2-dimethyl-1-hydroxy-5-(3-pyridyl)pyrrolidine (VIII).

The nicotine homolog I (as the dihydrochloride) was tested (Dr. Marvin J. Bleiberg of Woodard Research Corp.) and compared to nicotine (dihydrochloride and sulfate) under identical test conditions which are described in the *Experimental* section. The LD_{50} ratios in the mouse for I (I·2HCl, LD_{50} 11.0 mg./kg., 95% confidence limits) and nicotine were approximately 30.6, *i.e.*, I was about 0.03 times as toxic as nicotine. The confidence intervals of the LD_{50}'s for each compound show that the dose-mortality curves are virtually parallel. However, toxic signs such as Straub tail, tremors, eyelid ptosis, and mydriasis, which were seen at lethal doses of nicotine, were not observed with I.

The MED_{50} to obtain a pharmacodynamic effect in the mouse are (95% confidence limits) 0.018 mg./kg. for nicotine·2HCl, and 1.8 mg./kg. for I·2HCl, respectively; thus the potency ratio of nicotine/I is 100.0. There was evidence of ganglionic stimulation expressed by a greater rise in blood pressure caused by dimethylphenylpiperazinium iodide (DMPP) for I; in a dog receiving atropine before all but the initial dose of I, 0.200 mg. of I caused a marked rise in blood pressure equivalent to 0.016 mg. of nicotine sulfate. This is consistent with the potency ratio in the mouse. A ganglionic blocking action after large doses of I was suggested by the progressive depression of the blood pressure rise after AcCh following increasing doses of I. A direct effect on the respiratory center and on the cardiovascular system was indicated by the increased depth of respiration and the large blood pressure rise following injection of I.

The odor of I (free base) was very similar to, if not indistinguishable from, that of nicotine.

EXPERIMENTAL

Melting points were determined with a Thomas-Hoover apparatus and are uncorrected. IR spectra were obtained on a Perkin-Elmer 337 spectrophotometer, NMR spectra on a Perkin-Elmer R-20 instrument (60 M c.p.s.), mass spectra with a Hitachi-Perkin-Elmer RMU-6E instrument (see Table I). IR and NMR spectra were taken on all compounds and were consistent with the proposed structures; they are listed only if not essentially identical with those tabulated by Castagnoli *et al.* (7). NMR spectra (p.p.m.) were measured in $CDCl_3$ (TMS as standard) or in D_2O (DSS as standard), respectively. Microanalyses were performed by Galbraith Laboratories, Knoxville, Tennessee.

Pharmacological Methods—Mice of the HA/ICR strain, random breed (2 per dose level), received I at one-half log-dosage intervals i.v. Observations included the standard pharmacologic profile in

[1] After completion of both the synthetic and pharmacologic work (*vide infra*) the authors learned of an article by Castagnoli *et al.* (7) who had synthesized several of the compounds described in this paper [(±)I, II, V, VI, VIII] by a completely different route. For this reason, the authors' experimental report lists only those data that differ significantly from those of the California authors (7).

Table I—Mass Spectra (70 ev.) *m/e*

Compound	M+	Fragmentation M minus ( )
I	190	175 (CH_3)
II	176	161 (CH_3)
V	174	159 (CH_3)
		146 (CH_2=CH_2)
VI	190	175 (CH_3)
		173 (OH)
VII	218	203 (CH_3)
		189 (C_2H_5)
VIII	192	177 (CH_3)
		174 (H_2O)

dose range studies, number of mice reacting, time of onset of the pharmacologic signs, their degree and severity, and the time for recovery. LD_{50} and MED_{50} (both at 95% confidence limits) were estimated.

For tests in the dog, two mongrel dogs which were housed in temperature-controlled quarters, conditioned to the laboratory, and previously given canine distemper vaccine, were used. Food was withheld for 18–24 hr. prior to anesthesia (phenobarbital sodium, 140 mg./kg., i.v. or i.p.). The trachea was exposed and cannulated in order to maintain the respiratory airway. The right common carotid artery was exposed and cannulated to record blood pressure by means of an E and M pressure transducer, connected to an E and M physiograph. The anticoagulant was benzo fast pink (1.66 g./l. of 9% saline). Bilateral electrodes, placed on the skin next to the lower ribs, recorded the respiratory excursions by means of an impedence pneumograph; the EKG was recorded on a separate channel of the physiograph. Injections of the test material (and of nicotine dihydrochloride as a standard) were administered through a cannula inserted into a femoral vein; each injection was washed with Krebs-Ringer solution. In addition to graded increasing doses of I (dihydrochloride) and of the standard, Dog No. 1 also received dimethylphenylpiperazinium iodide, acetylcholine chloride, and atropine sulfate before a final dose of nicotine sulfate. Dog No. 2 also received epinephrine, ACh, and atropine.

Chemistry—(−)*1,2,2-Trimethyl-5-(3-pyridyl)pyrrolidine* (*I*)—To a solution of MeMgI [from 106.5 g. (0.75 mole) of MeI and 18.2 g. (0.75 mole) of Mg in dry ether (300 ml.) under N_2] was added (−) cotinine (8) (26.4 g., 0.15 mole) in dry benzene (40 ml.). Ether was removed by distillation and replaced by benzene, and the solution was refluxed at 65–75° under N_2 for 24 hr. (9). The reaction mixture was poured into ice water, the basic mixture was steam distilled, and the distillate (3–4 l.) was acidified and evaporated under vacuum. The residual oil was made basic, extracted (ether), and dried well (Na_2SO_4). Evaporation of the ether yielded 5 g. (17%) of a brown liquid. The colorless dihydrochloride, prepared in ether, was recrystallized from ethanol–ether, m.p. 258–260°.

Anal.—Calcd. for $C_{12}H_{20}Cl_2N_2$: C, 54.8; H, 7.6; N, 10.6. Found: C, 55.0; H, 7.8; N, 10.4.

The yellow dipicrate was obtained in, and recrystallized from, ethanol, m.p. 210–212°: $[\alpha]_{Hg}^{25}$ − 76.9° (c 0.13, ethanol).

Anal.—Calcd. for $C_{24}H_{24}N_8O_{14}$: C, 44.4; H, 3.7; N, 17.3. Found: C, 44.4; H, 3.8; N, 17.1.

4-Methyl-4-nitro-1-(3-pyridyl)-1-pentanone (*IV*)—A solution of sodium methoxide (3 g., 0.054 mole) in methanol (40 ml.) was added gradually to a stirred solution of 3-dimethylamino-1-(3-pyridyl)-1-propanone (III) (10.7 g., 0.05 mole) (10) and 2-nitropropane (31 g., 0.35 mole) in methanol (60 ml.) (11). After slight heating for 10 min. the stirred solution was heated to boiling, and dimethylamine was removed by distilling off about 10 ml. of methanol over a period of 20 min. Solvent and excess nitropropane were then removed under reduced pressure, the residue was taken up in water (100 ml.) containing a few drops of 10% NaOH solution, and IV crystallized on cooling. It was recrystallized from ethanol. The colorless crystals weighed 7.6 g. (68%), m.p. 73–75°.

Anal.—Calcd. for $C_{11}H_{14}N_2O_3$: C, 59.5; H, 6.3; N, 12.6. Found: C, 59.7; H, 6.4; N, 12.5.

Reduction of IV—A solution of IV (8.4 g., 38 mmoles) in absolute ethanol (40 ml.) and 2 g. of W-7 Raney nickel (12) was hydrogenated at an initial pressure of 3.6 kg./cm.[2] for 24 hr. Standard workup yielded 6.5 g. of crude reduction product which was chromatographed on magnesium silicate.[2] Elution with ether gave 5,5-dimethyl-2-(3-pyridyl)-Δ^1-pyrroline (V) (4.6 g., 69%); elution with ether–methanol (9:1) gave 5,5-dimethyl-2-(3-pyridyl)-Δ^1-pyrroline-1-oxide (VI) (0.9 g., 12%).

Compound V separated from ether as colorless crystals, m.p. 43–44°; it had previously been described as an oil (7).

Anal.—Calcd. for $C_{11}H_{14}N_2$: C, 75.8; H, 8.1; N, 16.1. Found: C, 75.8; H, 8.1; N, 16.2.

The nitrone VI had m.p. 75–76° after recrystallization from ether or hexane, and vacuum drying.

Anal.—Calcd. for $C_{11}H_{14}N_2O$: C, 69.5; H, 7.4; N, 14.7. Found: C, 69.3; H, 7.4; N, 14.6.

IR (KBr) 3400 cm.$^{-1}$ [the band at 3660 cm.$^{-1}$ reported by Castagnoli *et al.* (7) was not observed].

1-Hydroxy-2,2-dimethyl-5-(3-pyridyl)pyrrolidine (*VIII*)—A suspension of LAH (200 mg.) in dry ether (50 ml.) was added gradually over a period of 15 min. to a warm stirred solution of VI (0.5 g., 2.6 mmoles) in dry ether (150 ml.) and the mixture was refluxed for 18 hr. After dropwise decomposition with water, the ether layer was dried ($MgSO_4$) and evaporated in vacuum. VIII (0.3 g., 62%) crystallized from ether, m.p. 127–128°. IR (KBr) 3170 cm.$^{-1}$; the band at 3580 cm.$^{-1}$ (7) was not observed.

Anal.—Calcd. for $C_{11}H_{16}N_2O$: C, 68.7; H, 8.4; N, 14.6. Found: C, 68.6; H, 8.5; N, 14.3.

2,2-Dimethyl-5-(3-pyridyl)pyrrolidine (*II*)—A solution of V (3 g.) in absolute ethanol (30 ml.) was hydrogenated at 3.82 kg./cm.[2] with 1.5 g. of 10% Pd-C for 7 hr. Standard workup gave a residual oil which was converted to its dihydrochloride in ether. The salt was recrystallized from ethanol–ether, m.p. 135–137°. Drying over P_2O_5 raised the m.p. to 210–212°.

Anal.—Calcd. for $C_{11}H_{16}N_2 \cdot 2HCl \cdot H_2O$: C, 49.4; H, 7.5; N, 10.5. Found: C, 49.6; H, 7.3; N, 10.4.

Reconversion of the dihydrochloride to the base with sodium carbonate solution and ether extraction gave a clear liquid. The yellow dipicrate, formed in and recrystallized from ethanol, had m.p. 185–186°.

Anal.—Calcd. for $C_{23}H_{22}N_8O_{14}$: C, 43.5; H, 3.5; N, 17.7. Found: C, 43.7; H, 3.3; N, 17.5.

2,2-Dimethyl-1-n-propyl-5-(3-pyridyl)pyrrolidine (*VII*)—A mixture of II (1.5 g.), propionic acid (2.5 ml.), and propionic anhydride (2.5 ml.) was refluxed for 1 hr. and then cooled and poured into ice water. The solution was made strongly basic with 50% KOH solution, extracted with ether, and the extract was dried ($MgSO_4$) and evaporated. The crude 2,2-dimethyl-1-propionyl-5-(3-pyridyl)-pyrrolidine (1.4 g.), dissolved in dry ether (30 ml.), was added to LAH (0.5 g.) in dry ether (25 ml.) with stirring. After refluxing for 4 hr. the mixture was worked up [water (1.5 ml.), 10% NaOH (1.5 ml.), water (5 ml.), ether extraction, drying ($MgSO_4$), evaporation]. Yield of clear oil VII was 1.2 g. The dihydrochloride, prepared in dry ether, was recrystallized from ethanol–ether, m.p. 245–247°. NMR (D_2O) δ 0.78 (t, 3, CH_3 of propyl), 1.3 (m, 2, CH_2 of propyl), 1.52 (s, 3, CH_3), 1.66 (s, 3, CH_3), 2.6 (m, 4, protons at C-3 and C-4 of pyrrolidine), 3.2 (m, 2, N—CH_2—), 5.07 (q, 1, proton at C-2), 8.1–9.15 (4, pyridinium salt pattern).

Anal.—Calcd. for $C_{14}H_{22}N_2 \cdot 2HCl$: C, 57.7; H, 8.3; N, 9.6. Found: C, 57.4; H, 8.1; N, 9.4.

The yellow dipicrate was obtained in and recrystallized from ethanol, m.p. 205–206°.

Anal.—Calcd. for $C_{26}H_{28}N_8O_{14}$: C, 46.2; H, 4.1; N, 16.6. Found: C, 46.3; H, 4.2; N, 16.4.

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Synthesis of Sugar Moiety Substituted Nucleosides I: 9-[3-*O*-(*n*-Hexyl)-α,β-D-xylofuranosyl]adenine and 9-[3-*O*-(*n*-Hexyl)-5-deoxy-α,β-D-xylofuranosyl]adenine

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Abstract □ Isopropylidene-D-xylose was coverted *via* the 5-trityl compound to a 3-*O*-(*n*-hexyl) derivative. Following detritylation, benzoylation, and acetolysis, condensation with chloromercuri-6-benzamidopurine in the presence of titanium tetrachloride gave a crude nucleoside mixture. After deacylation, the mixture of anomeric nucleosides was resolved on ion-exchange resin (Bio-Rad AGl) to give 9-[3-*O*-(*n*-hexyl)-α-D-xylofuranosyl]adenine and 9-[3-*O*-(*n*-hexyl)-β-D-xylofuranosyl]adenine. Similarly, isopropylidene-5-deoxy-D-xylose was converted to a 3-*O*-(*n*-hexyl) derivative. Acetolysis and condensation with chloromercuri-6-benzamidopurine followed by deacylation and resolution on ion-exchange resin led to isolation of 9-[3-*O*-(*n*-hexyl)-5-deoxy-α-D-xylofuranosyl]adenine and 9-[3-*O*-(*n*-hexyl)-5-deoxy-β-D-xylofuranosyl]adenine.

Keyphrases □ Nucleosides, sugar moiety substituted—synthesis, isolation, separation □ 3-*O*-(*n*-Hexyl)adenine derivatives—synthesis, isolation □ Column chromatography—separation □ IR—identification □ UV spectrophotometry—identification □ Polarimetry—identification

In a continuing series of investigations, the authors have been exploring the structural features of the sugar moiety of adenine nucleosides required for interaction with the enzyme adenosine deaminase and/or inhibition of whole cells (1, 2). Other groups as well have devoted considerable attention to this area of study, particularly the laboratories of LePage (3), Schaeffer (4), and Bloch (5), among others. Compositely, the results of many studies such as those cited suggest that the 3′-hydroxyl group is usually not an important participant in an interaction with enzymes by which an adenine nucleoside may function as an *inhibitor* rather than as a *substrate*.

Recently, Baker (6) has collated many examples of the application of a principle he enunciated earlier (7): that a group which is found not to be important in interaction with an enzyme may be an ideal candidate for further modification with even quite bulky groups, including those which may react covalently with an enzyme to yield an active site directed, irreversible inhibitor. Both Baker (8) and Schaeffer (9) have now prepared a number of such irreversible inhibitors.

To date there seems not to have been any attempt to apply the implications of the Baker principle to "unimportant" groups on the sugar moiety of nucleosides. As noted, the 3′-hydroxyl would appear to be such a group. The present and following reports describe the syntheses of a number of 3′-*O*-substituted nucleosides whose availability will allow a beginning to be made in assessing the practicality of designing an active site directed, irreversible inhibitor of a sugar moiety substituted type.

Initially, the *n*-hexyl substituent was selected since not only could it serve as the carrier of an alkylating function, but of itself might enhance binding of the nucleoside through interaction with potentially accessible hydrophobic regions on susceptible enzymes. That such a hydrophobic region exists on adenosine deaminase has been demonstrated by Schaeffer and Vogel with a series of 9-alkyl substituted adenines (10). Xylofuranosyladenine (3*c*) and 5′-deoxyxylofuranosyladenine (11), both of which show affinity toward adenosine deaminase, were slected as candidates for 3′-*O*-substitution. The present paper, therefore, reports the syntheses of the 3′-*O*-(*n*-hexyl) derivatives of these two nucleosides.

PROCEDURES

Etherification of 1,2-*O*-isopropylidene-5-*O*-triphenylmethyl-D-xylofuranose (12) (I, Scheme I) with 1-chlorohexane in the presence of potassium hydroxide gave the 3-*O*-(*n*-hexyl) derivative (II) as a noncrystallizing syrup in quantitative yield. Attempts to remove the trityl group by hydrogenolysis over palladium black or palladium-on-charcoal were unsuccessful. This group, however, was readily removed in good yield when II was refluxed in an aqueous ethanolic solution of acetic acid. The resulting distillable syrup (III) was contaminated with 8% of triphenylcarbinol which could be removed by