Optically Pure (+)-Nicotine from (±)-Nicotine and Biological Comparisons with (-)-Nicotine

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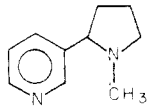
Optically pure (+)-nicotine has been obtained from (±)-nicotine using a combination of *d*-tartaric acid and di-*p*-toluoyl-*l*-tartaric acid. As the di-*d*-tartrate salt, (+)-nicotine is *less* potent than (-)-nicotine di-*l*-tartrate in producing lethality in mice, on blood pressure in anesthetized rats, and in the isolated guinea-pig ileum, indicating substantial stereospecificity for nicotine receptors. Potency ratios are 0.14, 0.06, and 0.019, respectively.

Nicotine has long played an important role in furthering



nicotine

our understanding of the cholinergic nervous system. As early as 1905, Langley[1] reported that low doses of nicotine stimulated and high doses paralyzed autonomic ganglia. The differentiation of cholinergic receptors by Dale[2] was provided by the alkaloids nicotine and muscarine. Although much is known about nicotinic receptors in the peripheral nervous system, more remains to be learned regarding the role of these receptors, especially in the central nervous system.

It has been shown that cholinergic receptors in the central nervous system may be either nicotinic or muscarinic.[3,4] Many studies have been performed[5] to determine the central site(s) and mechanism(s) of action of nicotine. These studies have attempted to correlate brain nicotine levels with pharmacologic effects such as changes in spontaneous activity, EEG patterns, tremors, convulsions, and antidiuretic effects.

Other approaches have been used in an attempt to relate brain-nicotine distribution with behavior and/or amine turnover.[5] Although these researches have added to our knowledge of the action of nicotine, as yet, the mechanism(s) of action remains obscure. There is, however, a growing body of evidence which suggests that the central effects of nicotine correlate better with its levels at nicotine receptors than with its total concentration in different brain regions.[6-8] Some work has been reported regarding nicotine binding with receptors,[9-11] but little work has been reported with optically pure (+)-nicotine, the enantiomer of naturally occurring (-)-nicotine. Barlow and Hamilton using (+)-nicotine which they estimated to be 97.8% optically pure demonstrated pharmacological stereospecificity with the isomers.[12] Hicks and Sinclair using (+)-nicotine obtained from optically pure (+)-nornicotine simply reported that the acute toxicities of (-)- and (+)-nicotine are similar.[13] Because of the growing implications regarding nicotine receptors, we decided to demonstrate an important criterion, namely, pharmacological stereospecificity of these optically pure isomers of nicotine.

Chemistry. Heretofore, (-)-nicotine has been characterized as the *d*-tartrate and (+)-nicotine as the *l*-tartrate salts,[14] and these salts were used as the basis for resolutions of (±)-nicotine. According to the literature,[12,15] repeated recrystallizations of (+)-nicotine *l*-tartrate obtained from (±)-nicotine have never given optically pure (+)-nicotine. We have found that (+)-nicotine di-*d*-tartrate (1) is a better characterized (nonhygroscopic) salt than the *l*-tartrate and that *d*-tartaric acid combined with the use of di-*p*-toluoyl-*l*-tartaric acid will consistently give optically pure (+)-nicotine from (±)-nicotine.

MeOH–Me_2CO is the solvent system used with 2 equiv of *d*-tartaric acid for the initial separation. 1 so obtained is 90–95% pure after one recrystallization from MeOH. Treatment of the free base of this material in Me_2CO with 1 equiv of di-*p*-toluoyl-*l*-tartaric acid gives essentially pure (+)-nicotine di-*p*-toluoyl-*l*-tartrate which can be converted directly to optically pure 1 with a slight excess of *d*-tartaric acid in 95% EtOH or MeOH. The di-*d*-tartrate residues from these operations, now enriched with respect to the (-)-isomer, can be converted to free base which with *l*-tartaric acid and a repetition of the above procedure gives optically pure (-)-nicotine di-*l*-tartrate.

The two operations that ensure the success of this six-step resolution procedure and, thus, that optically pure 1 is, indeed, obtained are (1) the conversion of the (+)-nicotine containing 5–10% of the (-)-isomer to the di-*p*-toluoyl-*l*-tartrate salt, **5**, and (2) transformation of the resultant **5** directly to 1 with *d*-tartaric acid in 95% EtOH or MeOH. In operation 1, the volume of Me_2CO used is such as to preclude crystallization of the (-)-nicotine di-*p*-toluoyl-*l*-tartrate, **4** (whose solubility in Me_2CO was predetermined by us to be 1%), present in the mixture to the extent of at most 0.6 g/100 mL but more probably 0.3–0.4 g/100 mL. Furthermore, because of the high dilutions and crystallization of **5** in small prisms, occlusion should not be a factor. Regarding operation 2, attempts to convert *pure* **4** directly to (-)-nicotine di-*d*-tartrate by the same conditions that gave 1 from **5** in good yield after 24 h yielded no (-)-nicotine *d*-tartrate during 72 h.

A final crystallization of 1 prepared directly from **5** was for elimination of possible (minor) contaminants other than (-)-nicotine salts and delivery of a refined product. The 1 so obtained (whose melting point and optical rotation did not change on further recrystallization) gave (+)-nicotine (base) with specific rotations identical (within experimental precision) to those of authentic (-)-nicotine. "Recycling" of this (+)-nicotine through the above-described two key steps did not change physical constants.

It should be noted that nicotine (base) is hygroscopic and subject to autoxidation and absorption of CO_2. Samples stored in closed vials at 0 °C gradually became discolored, and after 3 months of such storage rotation values were −159 to −161° ($CHCl_3$) compared with the initial −170°. Thus, particularly when handling small amounts, extraordinary precautions are necessary to obtain reproducible results in the measurement of optical rotations of the bases.

(+)-Nicotine di-*d*-tartrate and (-)-nicotine di-*l*-tartrate, **2**, equal amounts of which gave 0.00° rotation in H_2O, are anhydrous, stable salts suitable for biological studies.

Biological Results. (a) Acute Lethality in Mice. The intravenous LD_{50} values were calculated to be 0.38 (0.32–0.45) mg/kg for the (-)-isomer and 2.75 (1.87–4.04) mg/kg for the (+)-isomer by the method of Litchfield and Wilcoxon.[28] The overt behavioral effects noted were

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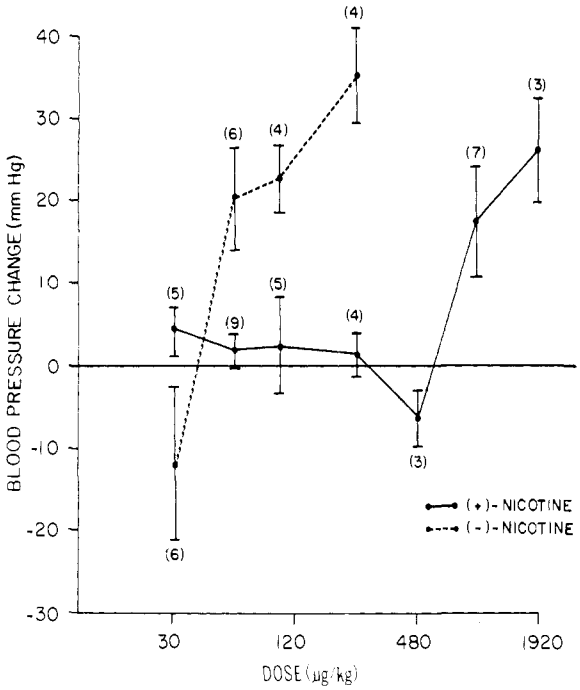

Figure 1. Effects of (+)- and (-)-nicotine on the blood pressure of the anesthetized rat. The number of animals tested is indicated in parentheses at each point. Each point represents the mean, with vertical lines indicating SE. Saline controls produced changes of 2.7 (±1.2 SE) mmHg. The blood pressures just prior to injection of (+)- and (-)-nicotine were 89 ± 2.9 and 85 ± 3.4, respectively.

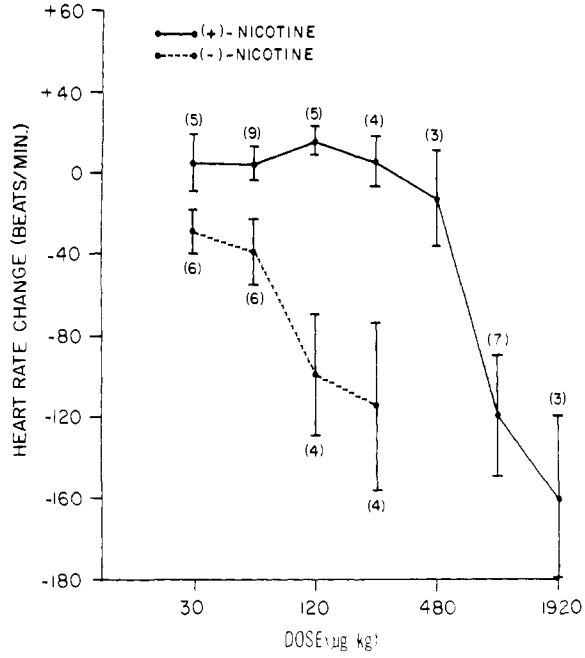

Figure 2. Effects of (+)- and (-)-nicotine on the heart rate of the anesthetized rat. The number of animals is indicated in parentheses at each point. Each point represents the mean, with vertical lines indicating SE. Saline controls produced changes of 10.7 ± 2.8 beats/min. The heart rates just prior to injecting (+)- and (-)-nicotine were 323 ± 12 and 335 ± 14, respectively.

similar, and all animals that died did so within 3 min of tonic extensor convulsions. Survivors lived for at least 24 h.

(b) Rat Blood Pressure and Heart Rate. The acute effects of (-)- and (+)-nicotine on blood pressure are illustrated in Figure 1. (-)-Nicotine was tested at 15–420 μg/kg. Initially it produced a fall in pressure at 30 μg/kg; higher doses caused increases in blood pressure. (-)-Nicotine was lethal to one animal receiving the highest dose and produced apnea in the other animals. (+)-Nicotine, tested at 15–1920 μg/kg, did not produce remarkable results until a dose of 480 μg/kg was reached. At this dose, a drop in pressure was noted reminiscent of that seen with 30 μg/kg of the (-)-isomer. Rises in blood pressure were seen at all of the higher doses. At the highest dose some apnea was noted. In general, the isomers behaved similarly; the (+)-isomer was approximately 0.06 times as potent as its antipode. Saline controls produced changes of 2.7 ± 1.2 mmHg (mean ± SE).

Effects on heart rate are shown in Figure 2. The isomers behaved essentially in the same way; i.e., both produced dose-related decreases in heart rate. The (-)-isomer was active beginning at 240 μg/kg; the (+)-isomer again appears to be 0.06 as potent as its enantiomer. Saline (controls) increased the heart rate by 10.7 ± 2.8 beats/min.

(c) Isolated Guinea Pig Ileum. The potency of the (+)-isomer relative to (-)-nicotine was calculated to be 0.019 (0.013–0.026) in the first assay and 0.013 (0.010–0.020) in the second.

Discussion

Probably because (+)-nicotine has been difficult to obtain optically pure, there have been conflicting reports about its pharmacological properties. Macht and Davis[16] reported that (-)-nicotine was more toxic than (±)-nicotine in a variety of species, while Hicks and Sinclair[13] found little difference in the toxicities of these two isomers in a study in rats and guinea pigs. We found that (-)-nicotine was much more potent. The calculated potency ratio was 7.24. Furthermore, our LD_{50} for (-)-nicotine is the lowest yet reported.

Regarding pharmacological comparisons of the activities of the isomers, Hicks and Sinclair[13] reported that (-)-nicotine was much more active than (+)-nicotine in raising the blood pressure of cats and rabbits and for its effects on the superior cervical ganglion of the cat. However, no quantitative values were given. Domino[17] also found that (-)-nicotine was more potent in raising the blood pressure of anesthetized dogs, but the sample of (+)-nicotine was judged to be only 87% optically pure. Barlow and Hamilton[12] using (+)-nicotine, estimated to be 97.8% optically pure, reported a number of equipotent molar ratios for the two isomers. These authors reported that the difference between the activities varied greatly on the different preparations and appeared to be greatest at receptors in ganglia, particularly in the guinea-pig ileum. There also were great differences between the isomers on the cat superior cervical ganglia and on blood pressure. Our results in the rat blood-pressure experiments indicate that the (-)-isomer is approximately 16 times more potent than the (+)-isomer. The greatest difference in potency was shown by us to be in the isolated guinea-pig ileum preparation. Thus, the stereospecificity indicated by our results confirms and quantitates with optically pure (+)-nicotine that noted by Barlow and Hamilton.[12]

Experimental Section

Melting points (uncorrected) were taken in a Thomas-Hoover (capillary) apparatus and optical rotations with a Perkin-Elmer 141 polarimeter. Elemental analyses (C, H, N), all values within ±0.3% of theory, are courtesy of Paula Parisius and Alice Wong of the Section on Instrumentation and Analytical Services, National Institutes of Health.

(-)-Nicotine Di-*l*-tartrate (2). (-)-Nicotine[18] (1.8 g, 0.011 mol), *l*-tartaric acid[19] (4.0 g, 0.026 mol), and 20 mL of hot 95% EtOH left at 20–23 °C[20] for 3–5 h gave, after washing with 1:1 MeOH–MeCO, 4.7 g (92%) of **2**, mp 138–139.5 °C. After a re-

crystallization from MeOH, it melted at 138.5–139.5 °C; $[\alpha]_D^{21-23}$ −9.6° (*c* 1.8, H_2O). Anal. ($C_{18}H_{26}N_2O_{12}$) C, H, N.

(−)-Nicotine Di-*p*-toluoyl-*d*-tartrate (3). (−)-Nicotine[18] (100 mg, 0.6 mmol), 250 mg (0.64 mmol) of di-*p*-toluoyl-*d*-tartaric acid,[19] and 4 mL of Me_2CO were warmed to solution to give, after 3–6 h at 20–23 °C,[20] 300 mg of **4**, mp 144–145 °C (dec). It was dissolved in ca. 40 mL of boiling Me_2CO. Concentration (hot plate) of the solution to 3–4 mL and cooling at 20–23 °C[20] gave 250 mg: mp 143–144 °C (dec); $[\alpha]_D^{24}$ −92.0° (*c* 0.70, MeOH). Anal. ($C_{30}H_{32}N_2O_8$) C, H, N.

(−)-Nicotine Di-*p*-toluoyl-*l*-tartrate (4). As described for **3**, this salt was prepared from (−)-nicotine and di-*p*-toluoyl-*l*-tartaric acid·H_2O[19] and gave the following: mp 148–149 °C (dec); $[\alpha]_D^{24}$ +103.9° (*c* 0.688, MeOH). Anal. ($C_{30}H_{32}N_2O_8$) C, H, N.

Resolution of (±)-Nicotine. To 1.05 g (7.0 mmol) of *d*-tartaric acid[19] in 5 mL of warm MeOH was added 0.5 g (3.1 mmol) of (±)-nicotine[21] followed by 2 mL of Me_2CO. The mixture was warmed to solution, treated with a few crystals of **1**,[22] and left for 2–5 days[23] at 20–23 °C. The resultant heavy crystals were filtered and washed with 3:2 MeOH–Me_2CO, giving 0.55–0.65 g of mainly (+)-nicotine di-*d*-tartrate (**1**): mp 127–133 °C, $[\alpha]_D^{22}$ +11.5–12.5° (H_2O). Three such fractions were combined and recrystallized[24] from 6–8 mL of MeOH (cooling at 20–23 °C[20]) to give 1.5 g of **1**: mp 134–137 °C, $[\alpha]_D^{22}$ +10.5° (H_2O). This gave 0.53 g (3.3 mmol) of (+)-nicotine, $[\alpha]_D^{23}$ +126° ($CHCl_3$) [contaminated with about 10% of (±)-nicotine], by use of excess 6 M NH_4OH and 10–15 mL of Et_2O[20] (three to four extractions dried over Na_2SO_4). This (+)-nicotine was added to a warm solution of 1.3 g (0.32 mmol) of di-*p*-toluoyl-*l*-tartaric acid·H_2O[19] in 30 mL of Me_2CO. After 2–3 h at 20–23 °C,[20] the yield of heavy crystals of (+)-nicotine di-*p*-toluoyl-*l*-tartrate (**5**), mp 143–144 °C (dec); $[\alpha]_D^{24}$ +92.4° (*c* 0.708, MeOH), was 1.5 g. A recrystallization from Me_2CO (see **3** and **4** above) did not essentially change the melting point or optical rotation. Anal. ($C_{30}H_{32}O_8$) C, H, N.

5 (1.5 g, 2.7 mmol) was added to a warm solution of *d*-tartaric acid (1.25 g, 8.2 mmol) in 12–13 mL of 95% EtOH.[25] The mixture was left at 20–23 °C[20] for 24 h to give 1.1 g of **1**, mp 137–138.5 °C. Recrystallization from MeOH (ca. 5 mL) or 95% EtOH (6–8 mL)[24] gave, after cooling at 20–23 °C[20] for 24–48 h, 0.8 g of **1**,[26] mp 138.5–139.5 °C; $[\alpha]_D^{22}$ +9.6° (*c* 1.15, H_2O), unchanged on further recrystallization from 95% EtOH. Anal. ($C_{18}H_{26}N_2O_{12}$) C, H, N.

When 10.5 mg each of **1** and authentic **2** were mixed in 1.00 mL of H_2O, the observed rotation was 0.00°.

Furthermore, (+)-nicotine prepared from **1** as described above (Et_2O–NH_4OH) gave, after molecular distillation at 0.1–0.5 mm (bath temperature 45–60 °C), $[\alpha]_D^{22}$ +171.2° (*c* 1.100, $CHCl_3$) and +130.8° (*c* 1.118, MeOH). Similarly, authentic (−)-nicotine (see note 18) purified through the di-*p*-toluoyl-*l*- or di-*p*-toluoyl-*d*-tartrate and di-*l*-tartrate salts had $[\alpha]_D^{22}$ −171.4° (*c* 0.878, $CHCl_3$) and −130.6° (*c* 1.174, MeOH). Standard-error limits are about ±1°.

The filtrate and washings from the 0.55–0.65 g of **1** above were evaporated to dryness at the H_2O pump, and the residue was converted to 0.26 g of free base, $[\alpha]_D^{22}$ −55–70° ($CHCl_3$), as described above for **1**. This was added to 0.6 g of *l*-tartaric acid[19] in 3 mL of warm MeOH, followed by 2 mL of Me_2CO and warming to homogeneity. Addition of a few crystals of authentic **2** and keeping at 20–23 °C[20] for 24–60 h gave 0.36–0.4 g of **2**, mp 128–133 °C. As described above for **1**, this crude **2** (with or without recrystallization from MeOH) was converted to base, $[\alpha]_D^{22}$ −110 to −130° ($CHCl_3$), in turn purified through either **3** or **4** (see also **5**). Either one, with a slight excess of *l*-tartaric acid, gave pure **2** (identical with authentic **2**), mp 138.5–139.5 °C; $[\alpha]_D^{22}$ −9.8° (*c* 1.38, H_2O), after one recrystallization from MeOH or 95% EtOH.[26]

(+)-Nicotine Di-*p*-toluoyl-*d*-tartrate (6). This salt was obtained from either pure or 90% optically pure (+)-nicotine with di-*p*-toluoyl-*d*-tartaric acid[19] as described above for **5**: mp 148–149 °C (dec); $[\alpha]_D^{22}$ −102.4° (*c* 1.11, MeOH). Anal. ($C_{30}H_{32}N_2O_8$) C, H, N.

An equal mixture of **3** and **5** and of **4** and **6** gave $[\alpha]_D^{22}$ essentially 0° within the limits of mechanical error.

Solubility of 4 and 5 in Me_2CO. Compound **4** (158 mg) was dissolved in 20–25 mL of boiling Me_2CO. The solution was concentrated on the hot plate to 3.5 mL and allowed to crystallize at 20–23 °C[20] for 1–24 h to give 123 mg of **4** (small prisms), leaving 35 mg in the filtrate. This corresponds to 1000 mg in 100 mL or a solubility of 1.0%.

Similarly, **5** was obtained in a yield of 129 mg. Thus, the solubility of **5** in Me_2CO is 0.8%.

Attempted Conversion of 4 Directly to (−)-Nicotine *d*-Tartrate. Compound **4** (0.64 g, 1.2 mmol), 0.54 g (3.2 mmol) of *d*-tartaric acid, and 5–6 mL of 95% EtOH were warmed to solution and left at 20–23 °C. Nothing had crystallized from the clear solution after 3 days. On cooling to 0 °C, a little oily material separated, but this quickly became redissolved on returning the mixture to 20–23 °C (see procedure for conversion of **5** to **1**).

Biological Methods. (A) Nicotinic Extensor Convulsions and Lethality in Mice. Male ICR mice (10–15/dose) weighing 20–25 g were injected intravenously. All doses were calculated as free base and geometrically spaced (2×) and given a total volume of 0.1 mL/10 g of body weight. All solutions were injected for 3–5 s into the tail vein. If an animal underwent a tonic-extensor convulsion, it invariably died within 3 min. Otherwise it survived for at least 24 h. The LD_{50} values were calculated by the method of Litchfield and Wilcoxon.[28]

(B) Blood Pressure and Heart Rate in Anesthetized Rats. Sprague-Dawley male rats weighing 275–350 g were anesthetized by an intraperitoneal injection of 1.2 g/kg of urethane in aqueous solution and secured to an animal operating board. The left femoral artery and vein were isolated and cannulated using polyethylene tubing (PE-50). Both cannulas were filled with saline, and 100 units/mL of heparin was added to the arterial cannula to prevent clotting. A Statham pressure transducer was connected to the arterial cannula and blood pressure and heart rate were recorded using a Grass Polygraph (Model 7) fitted with appropriate channels. After surgery, the blood pressure was allowed to stabilize for approximately 30 min before initiating injections. Each rat always received an injection of saline, which was followed 15 min later by an injection of either (+)- or (−)-nicotine. All solutions were given in a volume of 0.1 mL/100 g. Because tachyphylaxis was obvious when additional doses either of (+)- or (−)-nicotine were given, only the results of the first injection of either isomer are included in this study. The responses (mean ± SE) were calculated for each dose. The maximum change in arterial pressure and heart rate was calculated for each dose. Average blood pressure was calculated as the diastolic pressure plus one-third the difference between the systolic and diastolic pressures in mm/Hg.

(C) Isolated Guinea Pig Ileum. Male guinea pigs weighing 450–500 g were killed by a blow on the head. The terminal portion of the ileum less the 10-cm portion adjacent to the ileocecal junction of the ileum was used. A 3-cm segment was suspended in a 10-mL organ bath maintained at 37 °C. Krebs solution was used and was aerated using 95% oxygen and 5% CO_2. Contractions of the ileum were recorded by means of a force transducer connected to a Grass Polygraph Model 5D.

The initial tension on the ileum was 1 g. A stabilization period of 60 min was allowed before addition of the drugs.

Three different doses of each drug were added to the bath three times in a randomized fashion (3 × 3 assay). Drugs were allowed to act for 45 s, and then the ileum was washed; time between doses was 10–15 min. Two assays were completed and the potency ratios were calculated according to the method of Bliss.[29]

Acknowledgment. We are grateful to Dr. T. S. Osdene and his associates at the Philip Morris Research Center, Richmond, Va., for a generous supply of (±)-nicotine, to Dr. Arthur Jacobson (NIH) for arranging for C, H, N analyses, to Dr. Kenner Rice (NIH) for suggestions and supply of some of the resolving agents used, and to F. T. Grove for his excellent technical assistance. Supported by grants from the USPHS (DA-00490, 07027, and 00326) and the Council for Tobacco Research, U.S.A. (1157).

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(18) From Eastman Kodak. This material and other freshly distilled samples of (−)-nicotine gave $[\alpha]_D^{20-23}$ −170° (*c* 0.20–1.00, $CHCl_3$) and $[\alpha]_D^{25}$ −131° (*c* 0.98, MeOH); lit., K. D. Jackson, *Chem. Rev.* **24**, 162 (1941), $[\alpha]_D^{20}$ −157 and −118° in $CHCl_3$ and MeOH, respectively.
(19) From Aldrich.
(20) Room temperature.
(21) Generously supplied by Dr. T. S. Osdene, Philip Morris Research Center, Richmond, Va.
(22) Obtained initially by repeated recrystallization of a crude specimen of **1** which crystallized on cooling to 0 °C from a solution of (±)-nicotine and *d*-tartaric acid in MeOH.
(23) Occasional warming at first appeared to facilitate crystallization.
(24) With addition of 100 mg of *d*-tartaric acid.
(25) MeOH (4–6 mL) may be used instead of 95% EtOH, but crystallization is slower in MeOH and cooling to 0 °C is essential for good yields.
(26) A 3:2 or 2:1 mixture of MeOH–Me_2CO was always used to wash the crystals of **1**.
(27) A sixfold scale-up in this resolution procedure increases the overall yield of **1** and **2** to 30–35%. Furthermore, evaporation to dryness of all filtrates from the nicotine salts and conversion of the latter to free base gives 20–25% more of the antipodes on resolution again.
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Synthesis of Pyridinium Analogues of Acetylcholine and Their Interactions with Intestinal Muscarinic Receptors

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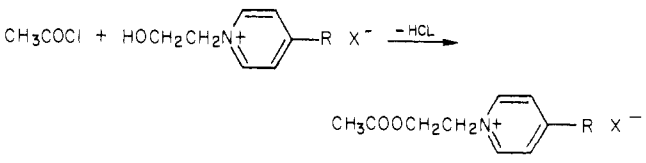
Received April 12, 1978

N-(β-Acetoxyethyl)pyridinium salts were synthesized and tested for muscarinic receptor interactions by the guinea pig ileum assay. Agonist activity indicates that receptor binding is substantially retained when the ammonium group of acetylcholine is formally replaced by a pyridinium ring. Introduction of alkyl groups into the ring yields antagonists. The 4-*tert*-butylpyridinium derivative is proved to have an activity superior to that of the 4-methylpyridinium salt. Competitive antagonism is favored by the more hydrophobic property of the *tert*-butyl group. A nonpolar area is suggested to be situated in the direct vicinity of the anionic binding sites of muscarinic receptors. The interaction of hydrophobic substituents with this area determines the antimuscarinic properties of pyridinium salts.

A large series of pyridinium salts was synthesized in a systematical search for drugs with antidotal properties against organophosphorus poisoning.[1-4] The most potent drugs against soman, *O*-(1,2,2-trimethylpropyl) methylfluorophosphonate, in vivo[5,6] and in vitro[6] are those which bear a hydrophobic group at the pyridinium ring. Their effect can be attributed to a protection of active sites of acetylcholinesterase (AChE, EC 3.1.1.7)[6] and to antimuscarinic properties.[7-10] Tentatively, it was assumed that hydrophobic surroundings of the anionic centers of AChE[11,12] and muscarinic receptors[13,14] are involved in the binding mechanisms of hydrophobic substituted pyridinium salts. The hypothesis was propounded that the pyridinium ring binds to the same site where the onium group of acetylcholine (ACH) can be attached.

To answer the question of whether the pyridinium ring can bind to the muscarinic receptor, ACh analogues were synthesized in which the quaternary ammonium group was formally replaced by a pyridinium ring. In order to test the significance of hydrophobic areas near the active site of the receptor, derivatives were substituted with alkyl groups in the ring. To our knowledge, these compounds were unknown until now. Their muscarinic or antimuscarinic activities were tested with the isolated longitudinal muscles of the guinea pig ileum. The interaction with other systems than cholinergic receptors cannot be excluded. This problem, however, was beyond the scope of this paper.

Scheme I



R = H, $-CH_3$, $-C(CH_3)_3$
X = Cl^-, Br^-, I^-

Chemistry. The synthesis of the ACh analogues succeeded according to the reaction in Scheme I. The *N*-(hydroxyethyl)pyridinium salts **1–3** (Table I) could be prepared by the reaction of the respective pyridine derivative with chloro- or bromoethanol.[15] In order to synthesize **4** (HB 14, Table I), it is suitable to prepare

0022-2623/79/1822-0177$01.00/0