PHILIP MORRIS U. S. A.

INTER-OFFICE CORRESPONDENCE

RICHMOND, VIRGINIA

To: Dr. T. S. Osdene

Date: October 20, 1980

From: J. Seeman

Subject: Nicotine Program Memos

The attached memos are in response to your request for information regarding the last year's efforts in nicotine analogue synthesis and chemistry and in our external pharmacological testing program being conducted with Professor Leo Abood. Also attached is a memo convering future work we are gearing up to do in the field of nicotine receptors.

I should like to emphasize that the concepts discussed in those memos and the results enumerated are due to a continued effort by many individuals in Charge #2500 and Charge #1600. They include Chuck Chavdarian, Ted Sanders and Henry Secor in our division and Lisa Carron, Vic DeNoble and Carolyn Levy in the Behavioral Group. The collaboration of numerous other individuals from the Analytical Research Division and the Physical Chemical Group is also to be noted.

Questions regarding these memos can be directed to me or to any of those individuals involved in this program.

Jeffrey Seeman

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Enclosures

PHILIP MORRIS U. S. A.

INTER-OFFICE CORRESPONDENCE

RICHMOND, VIRGINIA

To: Dr. T. S. Osdene Date: October 20, 1980

From: J. Seeman

Subject: External Pharmacological Testing at University of Rochester/ Professor Leo Abood

This memo is in response to your request for a summary of the past year's efforts with Professor Leo Abood.

Professor Abood has rendered considerable support to the nicotine program in a variety of ways.

1. Dr. Abood, one of the leaders in the field of neurochemistry, has consulted with us on many aspects of the nicotine program in general, including the contract peripheral pharmacological testing program in progress.

2. Dr. Abood's development of the nicotine "prostration syndrome" was a major advance in nicotine central nervous system (CNS) activity studies. He has subsequently aided PM's efforts along these lines and has retested some of the analogues examined here for cross-laboratory comparisons. The PM Behavioral Group has subsequently made significant advancements using in-house developed modifications and refinements of the "prostration syndrome" test.

3. We have chosen and supplied samples of nicotine analogues prepared at PM for Professor Abood to test using receptor binding techniques (c.f. item #4 below), binding to glass fiber filters (c.f. item #5 below), rat blood pressure procedures (c.f. item #6 below) and prostration syndrome (c.f. item #7 below).

4. Binding constants for nicotine analogues have been determined by competition of the analogue with ^{3}H-acetylcholine to torpedo membranes. Nicotine and nicotine analogue binding was also determined on solubilized rat brain tissue. Binding constants are a reflection of the degree of association of a compound to a receptor preparation. Inasmuch as binding is the first stage in drug action, binding constants are of fundamental interest in the nicotine program. The ultimate goal is the correlation of binding data with <u>in vivo</u> activity.

Also, for a compound such as nicotine which has a wide range of activities, e.g., peripheral and CNS, binding data for different receptor preparations will be a clue to the separation of activity profiles.

5. Binding to glass fiber filters is a technique that Dr. Abood has developed. One advantage of such a procedure is the ability to obtain a binding constant without the need for a biochemical preparation, e.g., rat brain solubilized receptors or torpedo receptors. Unfortunately, but not unexpectedly, the correlation between glass binding and receptor binding data is rather poor. We have concluded that one cannot use glass fiber filters to derive receptor binding data via a pairwise correlation relationship.

6. One of the most classical pharmacological tests for nicotine activity is blood pressure. The effect of nicotine on blood pressure is often considered one of its undesirable side effects, and as cited in other reports by us, a separation of nicotine effects by an analogue would be of considerable value. Toward this end, we must have blood pressure evaluations of the analogues. While rat blood pressure determinations have been made for us under contract, results have been delayed for some time. Dr. Abood volunteered to do these tests for us. Most interestingly, a number of the nicotine analogues at low doses acted as hypotensive agents, i.e., they caused a decrease in rat blood pressure. A number of the more active analogues, including 6-methylnicotine, were hypertensive as is nicotine at the doses tested. A small number showed the most interesting result: hypotensive upon jugular vein infusion and hypertensive upon iliac vein administration. Work is continuing in this area.

7. Early in the year, Dr. Abood evaluated a large number of the compounds using the prostration syndrome test. This work, coupled with results from PM's Behavioral Group, is giving us an excellent bioassay for nicotinic CNS activity.

Jeffrey Seeman

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PHILIP MORRIS U. S. A.

INTER-OFFICE CORRESPONDENCE

RICHMOND, VIRGINIA

To: Dr. T. S. Osdene Date: October 20, 1980

From: Jeff Seeman

Subject: Future Work in the Nicotine Receptor Field

We have made major advances in our efforts to quantitate nicotinic activity at various receptors (e.g., rat brain receptors and torpedo membrances via binding data). We intend to continue this work with a goal of correlating binding data with in vivo tests, such as prostration syndrome and discrimination paradigm.

In addition, we are beginning a series of studies to be done in collaboration with Professor Leo Abood aimed at isolating the nicotine receptor from a variety of preparations. This work can be divided into two distinct approaches:

1. A nicotine analogue or group of analogues having a reactive site, in particular, an eletrophilic site, will be prepared. A receptor preparation, or possibly a whole animal, will be treated with the analogue. It is hoped that, in addition to nonspecific binding, specific binding and covalent bonding will occur at the nicotine receptor. At this stage, experiments to determine decreased sensitization to nicotinic stimulation may be examined. If the analogue were labeled, then one in theory could isolate the receptor by isolating the radioactive portions of the preparation.

2. Similarly, the nicotine analogue(s) could be activated in such a fashion to react with a column chromatography support. Subsequently, a solubilized receptor preparation could then be passed through the column. The nicotine moiety, hanging off, or more descriptively dangling off the column support backbone, would then bind non-covalently to the receptor. This affinity chromatography procedure would allow the receptor(s) to be purified from other proteinaceous materials.

It is to be noted that the ultimum in activity studies involves the isolation, identification and characterization of receptors. This type of research is clearly at the forefront of SAR research and is among the most challenging but potentially most valuable type of experiment in the field.

3. Continued emphasis will be placed in binding studies. This may lead to studies aimed at determining analogue distribution using labeled analogues and examining brain slices, etc.

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Jeffrey Seeman

PHILIP MORRIS U. S. A.

INTER-OFFICE CORRESPONDENCE

RICHMOND, VIRGINIA

To: Dr. T. S. Osdene Date: October 20, 1980

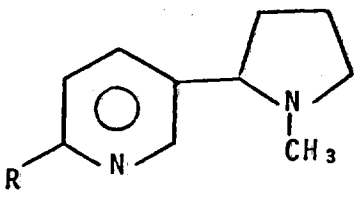
From: J. Seeman

Subject: Synthesis and Chemistry of Nicotine Analogues (Oct 1, 1979 - September 30, 1980)

This summary of the above topic is in response to your recent request. The nicotine program is aimed at (1) understanding and defining more precisely the many facets of nicotine pharmacology, (2) determining structure-activity relationships, and (3) preparing a compound (or group of compounds) which have specific activity profiles.

During the past year, series of nicotine analogues have been synthesized as a central feature in the program.

1. A series of 6-alkylnicotines (1) were prepared. Included in this series are 6-methyl, 6-ethyl, 6-isopropyl, 6-*t*-butyl, 6-cyclopropyl, and 6-vinyl. In some cases, the corresponding nornicotines and myosmines were prepared. (It is of importance to note that the procedures often used in this work generate the myosmine first, from which are sequentially prepared the corresponding nornicotine and then nicotine derivative.) These compounds were synthesized because of the observation that 6-methylnicotine was found to be as active and in some cases more active than nicotine itself.



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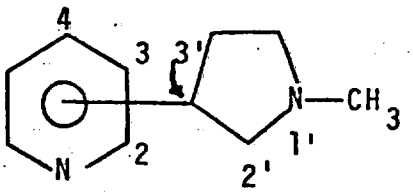
2. A series of x,3'-isonicotines (2) were prepared. These have interest because the point of attachment to the pyridine ring is the same as in the x,2'-nicotine series prepared previously except that the pyrrolidine ring attachment is one atom removed. Of consequence is the increased pyrrolidine accessibility and the increased N---N' distance.



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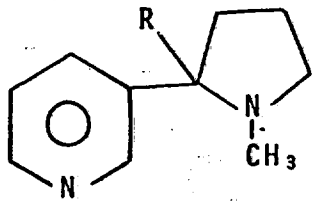
3. A series of 2'-alkylnicotines (3) were prepared. These are of interest because the basicity of the pyrrolidine ring is increased while its accessibility is decreased; because the conformational properties of the molecule are significantly altered; and because biological studies of 2'-methylnicotine were extraordinarily promising. Included in this series are 2'-methyl, 2'-ethyl and 2'isopropyl. Efforts to prepare 2'-*t*-butyl have been unsuccessful to date..



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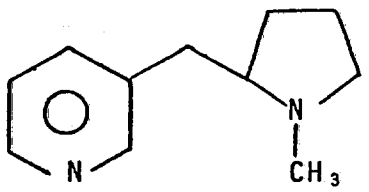
4. The novel homo and bishomo nicotines, 4 and 5 and the related nornicotines and myosmines were prepared. These are of interest because the bonding positions remain the same at C-3 on the pyridine ring and at C-2' on the pyrrolidine ring, but the rings are separated from each other by one and two carbons respectively. Steric, electronic and conformational features change in a novel fashion in this series.



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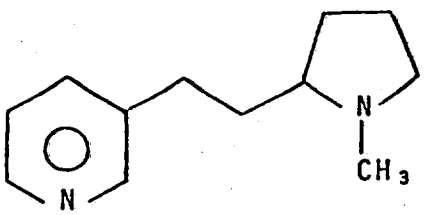

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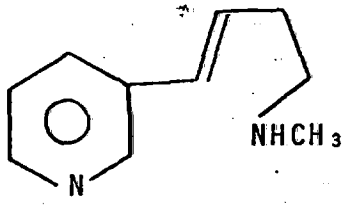
5. Metanicotine (6), dihydrometanicotine (7), and dihydromethylmetanicotine (8) were prepared. These compounds are part of a series of analogues in which an acyclic moiety replaces nicotine's pyrrolidine ring. Involved in the structural variability are unsaturation, degree of nitrogen substitution, chain flexibility and N---N' distance.



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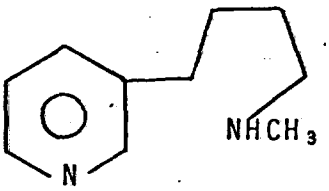

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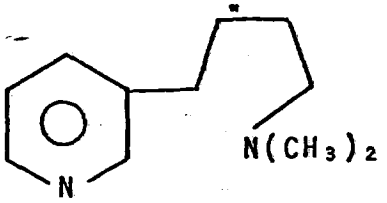

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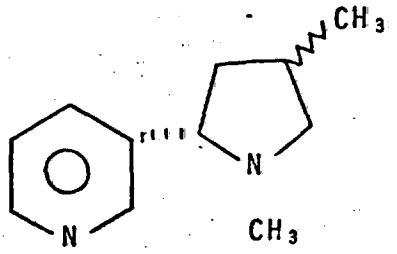
6. The preparation of optically active 4'-alkylnicotinoids has been accomplished, e.g., *cis* and *trans*-4'-methylnicotines (9). These compounds and the related 3'-alkyl and 5'-alkylnicotinoids will give information regarding substitution and spatial tolerances at the nicotinic receptor.



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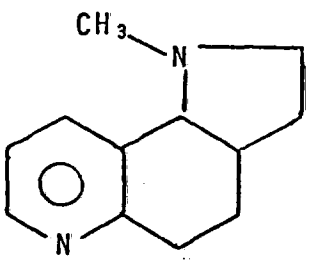
7. A novel bridged nicotinoid, 2,3'-bismethylenenicotine (10), and its myosmine and nornicotine analogues have now been prepared. Bridged nicotines may allow us to assay the importance of ring-ring orientation and molecular rigidity at the nicotinic receptor.



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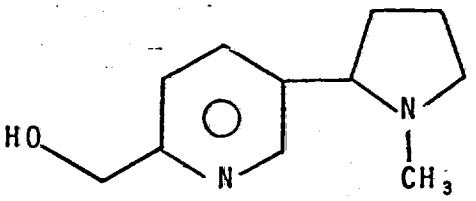
8. Oxygenated 6-substituted nicotines have been prepared for the first time. These include 6-hydroxymethyl- and 6-(2-hydroxyethyl)nicotine (11 and 12). These compounds are of significant value for two different reasons. Firstly, they are additional 6-substituted nicotinoids which may well have interesting pharmacological properties. (See item 1 above.) Secondly, they will be used in experiments aimed at isolation of the nicotinic receptor -- both by covalent binding at the receptor (as the 6-haloalkylnicotines) and by affinity chromotography during which a solubilized receptor preparation will be purified.



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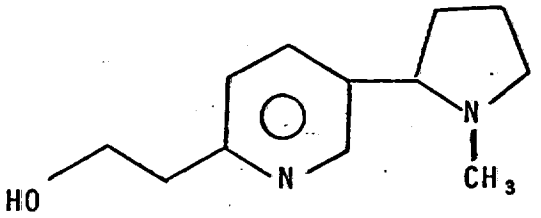

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B. We have also attacked the problem of preparing the enantiomers of the interesting nicotine analogues.

9. A procedure is being developed in which a *d,l*-nornicotine analogue can be separated into its enantiomers: first, by converting it to a diastereomeric mixture; second, by separating the diasteromers by HPLC; and third, by chemically removing the inducing chiral centers. Excellent results have been obtained and we anticipate that this procedure will meet fruition shortly.

10. We have prepared nicotine and 5-methylnicotine in optically active form by asymmetric synthesis. This is the first time any nicotine compound has been synthesized asymmetrically.

C. In order to better evaluate the pharmacological results we are obtaining in this work and to assist us in designing new compounds for study, we are examining the physical and chemical properties of nicotine and many of its analogues. It has been standard practice in pharmacological programs to determine such properties as lipophilicity, basicity, etc. This aspect of our program is designed to push chemical concepts to their limits in order to allow a better understanding of the biochemical questions involved.

11. Studies have been carried out to determine the conformational properties and motional processes of nicotine and some of its analogues in solution. We have examined the effect of substitution on these physical parameters, using nicotine as the standard by which the analogues are compared.

12. We have determined that the nucleophilicity of the two nitrogens of nicotine are critical to its activity. We have begun to monitor this parameter by determining the reactivity of nicotine and its analogues with a standard alkylating agent, iodomethane. The basic hypothesis of this work is that the factors which stabilize (or destabilize) the alkylation transition states, as judged by kinetic reactivity measurements, similarly effect the noncovalent bonding interactions of nicotine with its receptor(s). Correlations have now been made between alkylation rates and biological activity for nicotine and its methyl-substituted derivatives.

13. A wide range of 1-methyl-2-phenylpyrrolidines and the quaternary salts of these and many of the other nicotine analogues have been prepared for the kinetic analyses discussed below.

14. We shall soon begin determining the pK_a's of the analogues and their partition coefficients, parameters which quantitate basicity and lipophilicity.

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Jeffrey Seeman