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(21) International Application Number: PCT/US99/11785 (22) International Filing Date: 4 June 1999 (04.06.99) (30) Priority Data: 60/088,117 5 June 1998 (05.06.98) US (71) Applicant: REGENT COURT TECHNOLOGIES [US/US]; 709 The Hamptons Lane, Town and Country, MO 63017 (US). (72) Inventors: WILLIAMS, Jonnie, R.; 1 Starwood Lane, Manakin-Sabot, VA 23103 (US). DELORENZO, Robert, J.; 221 Dryden Lane, Richmond, VA 23229 (US). BURTON, Harold, R.; 3129 Chelsea Drive, Lexington, KT 40503 (US). (74) Agents: BOLAND, Mark et al.; Sughrue, Mion, Zinn, MacPeak & Seas, PLLC, Suite 800, 2100 Pennsylvania Avenue, N.W., Washington, DC 20037-3213 (US).	(81) Designated States: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, UZ, VN, YU, ZA, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SL, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG). Published <i>With international search report. Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.</i>	
(54) Title: MONOAMINE OXIDASE (MAO) INHIBITORS AND USES THEREOF		
(57) Abstract		
<p>The present invention provides a group of tobacco alkaloids, tobacco extract, Yerbamaté extract, and an extract of chewing gum and lozenges which are modulators of monoamine oxidase (MAO) activity (i.e., compounds and substances which inhibit MAO enzyme and prevent its biological activity). The MAO inhibitors of the present invention can cause an increase in the level of norepinephrine, dopamine, and serotonin in the brain and other tissues, and thus can cause a wide variety of pharmacological effects mediated by their effects on these compounds. The MAO inhibitors of the present invention are useful for a variety of therapeutic applications, such as the treatment of depression, disorders of attention and focus, mood and emotional disorders, Parkinson's disease, extrapyramidal disorders, hypertension, substance abuse, smoking substitution, antidepressant therapy, eating disorders, withdrawal syndromes, and the cessation of smoking.</p>		

MONOAMINE OXIDASE (MAO) INHIBITORS AND USES THEREOF

FIELD OF THE INVENTION

The present invention relates to the novel use of compounds and substances
5 which are capable of modulating monoamine oxidase (MAO) activity by inhibiting
the MAO enzyme. The present invention also relates to MAO inhibitors and their
therapeutic use as a drug or dietary supplement in the treatment of various conditions
or disorders, including psychiatric and neurological illnesses. More particularly, the
10 present invention relates to the therapeutic use of tobacco alkaloids, Yerbamaté (*Ilex
paraguariensis*) extract, or tobacco extracts to inhibit MAO activity to provide a
treatment for various disorders or conditions.

BACKGROUND OF THE INVENTION

By inhibiting MAO activity, MAO inhibitors can regulate the level of
monoamines and their neurotransmitter release in different brain regions and in the
body (including dopamine, norepinephrine, and serotonin). Thus, MAO inhibitors
20 can affect the modulation of neuroendocrine function, respiration, mood, motor
control and function, focus and attention, concentration, memory and cognition, and
the mechanisms of substance abuse. Inhibitors of MAO have been demonstrated to
have effects on attention, cognition, appetite, substance abuse, memory,
25 cardiovascular function, extrapyramidal function, pain and gastrointestinal motility
and function. The distribution of MAO in the brain is widespread and includes the
basal ganglia, cerebral cortex, limbic system, and mid and hind-brain nuclei. In the
peripheral tissue, the distribution includes muscle, the gastrointestinal tract, the
30 cardiovascular system, autonomic ganglia, the liver, and the endocrinic system.

MAO inhibition by other inhibitors have been shown to increase monoamine content in the brain and body. Regulation of monoamine levels in the body have been shown to be effective in numerous disease states including depression, anxiety, stress disorders, diseases associated with memory function, neuroendocrine problems, cardiac dysfunction, gastrointestinal disturbances, eating disorders, hypertension, Parkinson's disease, memory disturbances, and withdrawal symptoms.

It has been suggested that cigarette smoke may have irreversible inhibitory effect towards monoamine oxidase (MAO). A.A. Boulton, P.H. Yu and K.F. Tipton, "Biogenic Amine Adducts, Monoamine Oxidase Inhibitors, and Smoking," Lancet , 1(8577): 114-155 (January 16, 1988), reported that the MAO-inhibiting properties of cigarette smoke may help to explain the protective action of smoking against Parkinson's disease and also observed that patients with mental disorders who smoke heavily do not experience unusual rates of smoking-induced disorders. It was suggested that smoking, as an MAO inhibitor, may protect against dopaminergic neurotoxicity that leads to Parkinson's disease and that the MAO-inhibiting properties of smoking may result in an antidepressive effect in mental patients.

L.A. Carr and J. K. Basham, "Effects of Tobacco Smoke Constituents on MPTP-Induced Toxicity and Monoamine Oxidase Activity in the Mouse Brain," Life Sciences, 48:1173-1177 (January 16, 1991), found that nicotine, 4-phenylpyridine and hydrazine prevented the decrease in dopamine metabolite levels induced by 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) in mice, but there was no significant effect on dopamine levels. Because tobacco smoke particulate matter caused a marked inhibition of MAO A and MAO B activity when added *in vitro*, it was suggested that one or more unidentified substances in tobacco smoke are capable of inhibiting brain MAO and perhaps altering the formation of the active metabolite of

MPTP.

J.S. Fowler, N.D. Volkow, G.J. Wang, N. Pappas, and J. Logan, "Inhibition of Monoamine Oxidase B in the Brain of Smokers," Nature (Lond), 379(6567):733-736 (February 22, 1996), found that the brains of living smokers showed a 40% decrease
5 in the level of MAO B relative to non-smokers or former smokers. MAO inhibition was also reported as being associated with decreased production of hydrogen peroxide.

It has also been suggested that nicotine may not be the only constituent of tobacco responsible for tobacco addiction. J. Stephenson, "Clues Found to Tobacco
10 Addiction," Journal of the American Medical Association, 275(16): 1217-1218 (April 24, 1996), discussing the work of Fowler, *et al.*, pointed out that the brains of living smokers had less MAO B compared with the brains of nonsmokers or former smokers. MAO B is an enzyme involved in the breakdown of dopamine, which is a pleasure-enhancing neurotransmitter. The results suggested that the inhibition of
15 MAO B in the brains of smokers may make nicotine more addictive by slowing down the breakdown of dopamine, thereby boosting its levels. The findings provided an explanation as to why cigarette smokers were less susceptible to developing Parkinson's disease. Further, the findings suggested that MAO inhibitors could be used for smoking cessation.

20 K.R.R. Krishnan, "Monoamine Oxidase Inhibitors," The American Psychiatric Press Textbook of Pharmacology, American Psychiatric Press, Inc., Washington, DC 1995, pp. 183-193, suggest various uses for monoamine oxidase inhibitors. The uses include atypical depression, major depression, dysthymia, melancholia, panic disorder, bulimia, atypical facial pain, anergic depression, treatment-resistant
25 depression, Parkinson's disease, obsessive-compulsive disorder, narcolepsy, headache,

chronic pain syndrome, and generalized anxiety disorder.

D. Nutt and S.A. Montgomery, "Moclobemide in the Treatment of Social Phobia," Int. Clin. Psychopharmacol, 11 Suppl. 3: 77-82 (June 11, 1996), reported that moclobemide, a reversible MAO inhibitor, may be effective in the treatment of social phobia.

I. Berlin, et al., "A Reversible Monoamine Oxidase A Inhibitor (Moclobemide) Facilitates Smoking Cessation and Abstinence in Heavy, Dependent Smokers," Clin. Pharmacol. Ther., 58(4): 444-452 (October 1995), suggested that a reversible MAO A inhibitor can be used to facilitate smoking cessation.

U.S. Patent No. 3,870,794 discloses the administering of small quantities of nicotine and nicotine derivatives to mammals, including humans, to reduce anger and aggression and to improve task performance.

U.S. Patent No. 5,276,043 discloses the administering of an effective amount of certain anabasine compounds, certain unsaturated anabasine compounds, or unsaturated nicotine compounds to treat neurodegenerative diseases.

U.S. Patent No. 5,516,785 disclose a method of using anabasine; and DMAB-anabasine for stimulating brain cholinergic transmission and a method for making anabasine.

U.S. Patent Nos. 5,594,011, 5,703,100, 5,705,512, and 5,723,477 disclose modulators of acetylcholine receptors.

Known irreversible MAO inhibitors also inhibit MAO in the stomach and liver as well as the brain. As a result, their use has been limited because hypertensive crisis may occur when certain types of food (for example, fermented foods) are ingested, thereby creating an adverse drug-food interaction. Tyramine, which has a pressor action and which is normally broken down by the MAO enzymes, can be present in

certain foods.

Thus, it would be desirable to provide MAO inhibitors which are effective, but less potent (i.e., those which provide an asymptotic effect on MAO inhibition) than known MAO inhibitors, for the treatment of various conditions and disorders. It would also be desirable to provide MAO inhibitors which are easily synthesized and which could be provided to patients as an "over the counter" medication or dietary supplement.

SUMMARY OF THE INVENTION

10 The present invention relates to the discovery that certain tobacco alkaloids or extracts, a certain tea plant extract, and a certain extract of tobacco extract-containing chewing gum and lozenges provide MAO-inhibiting effects. The present invention also relates to the use of these compounds or substances in the treatment of certain conditions and disorders in mammals, including humans.

15 The compounds and substances of the present invention are capable of inhibiting MAO activity in mammalian brain and peripheral tissue. These compounds and substances act by increasing the concentration of monoamine compounds (norepinephrine, dopamine, and serotonin) in the body and brain.

The present invention provides a method of treating certain medical, psychiatric and/or neurological conditions or disorders. In a first embodiment of the invention, the method comprises administering a MAO-inhibiting effective amount of anabasine, anatabine or nornicotine to a mammal, particularly a human, for the treatment of medical, psychiatric and/or neurological conditions and disorders such as, but not limited to, Alzheimer's disease, Parkinson's disease, major depression, minor depression, atypical depression, dysthymia, attention deficit disorder, hyperactivity,

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conduct disorder, narcolepsy, social phobia, obsessive-compulsive disorder, atypical facial pain, eating disorders, drug withdrawal syndromes and drug dependence disorders, including dependence from alcohol, opioids, amphetamines, cocaine, tobacco, and cannabis (marijuana), melancholia, panic disorder, bulimia, anergic depression, treatment-resistant depression, headache, chronic pain syndrome, generalized anxiety disorder, and other conditions in which alteration of MAO activity could be of therapeutic value.

In a second embodiment of the invention, the method comprises administering a MAO-inhibiting effective amount of an extract of Yerbamaté (*Ilex paraguariensis*) tea plant to a mammal, particularly a human, for the treatment of medical, psychiatric and/or neurological conditions and disorders such as, but not limited to, Alzheimer's disease, Parkinson's disease, major depression, minor depression, atypical depression, dysthymia, attention deficit disorder, hyperactivity, conduct disorder, narcolepsy, social phobia, obsessive-compulsive disorder, atypical facial pain, eating disorders, drug withdrawal syndromes and drug dependence disorders, including dependence from alcohol, opioids, amphetamines, cocaine, tobacco, and cannabis (marijuana), melancholia, panic disorder, bulimia, anergic depression, treatment-resistant depression, headache, chronic pain syndrome, generalized anxiety disorder, and other conditions in which alteration of MAO activity could be of therapeutic value.

In a third embodiment of the invention, the method comprises administering a MAO-inhibiting effective amount of a tobacco extract to a mammal, particularly a human, for the treatment of medical, psychiatric and/or neurological conditions and disorders such as, but not limited to, Alzheimer's disease, Parkinson's disease, major depression, minor depression, atypical depression, dysthymia, attention deficit disorder, hyperactivity, conduct disorder, narcolepsy, social phobia, obsessive-

compulsive disorder, atypical facial pain, eating disorders, drug withdrawal syndromes and drug dependence disorders, including dependence from alcohol, opioids, amphetamines, cocaine, tobacco, and cannabis (marijuana), melancholia, panic disorder, bulimia, anergic depression, treatment-resistant depression, headache, chronic pain syndrome, generalized anxiety disorder, and other conditions in which alteration of MAO activity could be of therapeutic value.

In a fourth embodiment of the invention, the method comprises administering a MAO-inhibiting effective amount of an extract of gum and lozenges formulated with tobacco extract to a mammal, particularly a human, for the treatment of medical, psychiatric and/or neurological conditions and disorders such as, but not limited to, Alzheimer's disease, Parkinson's disease, major depression, minor depression, atypical depression, dysthymia, attention deficit disorder, hyperactivity, conduct disorder, narcolepsy, social phobia, obsessive-compulsive disorder, atypical facial pain, eating disorders, drug withdrawal syndromes and drug dependence disorders, including dependence from alcohol, opioids, amphetamines, cocaine, tobacco, and cannabis (marijuana), melancholia, panic disorder, bulimia, anergic depression, treatment-resistant depression, headache, chronic pain syndrome, generalized anxiety disorder, and other conditions in which alteration of MAO activity could be of therapeutic value.

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BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 shows a plot of MAO inhibition versus time for anabasine.

Figure 2 shows the inhibition of MAO A and MAO B for anabasine.

Figure 3 shows a plot of MAO inhibition versus time for anatabine.

25 Figure 4 shows the inhibition of MAO A and MAO B for anatabine.

Figure 5 shows a plot of MAO inhibition versus time for nornicotine.

Figure 6 shows the inhibition of MAO A and MAO B for nornicotine.

Figure 7 shows a plot of MAO inhibition versus time for Yerbamaté.

Figure 8 shows the inhibition of MAO A and MAO B for Yerbamaté.

5 Figure 9 shows a plot of MAO inhibition versus time for tobacco extract.

Figure 10 shows the inhibition of MAO A and MAO B for tobacco extract.

Figure 11 shows a plot of MAO inhibition versus time for GUMSMOKE.

Figure 12 shows a plot of MAO inhibition versus time for a lozenge extract.

10 DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS

MAO is an important enzyme that plays a major role in the metabolic transformation of catecholamines and serotonin. Neurotransmitters from this group are metabolized by MAO, and thus their effect is decreased at their receptor sites. MAO is important for the regulation of the levels of dopamine, norepinephrine and
15 serotonin. Accordingly, inhibition of this major enzyme system will have major effects on the functions regulated by these compounds.

In a first embodiment of the invention, the method comprises administering a MAO-inhibiting effective amount of anabasine, anatabine or nornicotine to a mammal, particularly a human, for the treatment of medical, psychiatric and/or
20 neurological conditions and disorders such as, but not limited to, Alzheimer's disease, Parkinson's disease, major depression, minor depression, atypical depression, dysthymia, attention deficit disorder, hyperactivity, conduct disorder, narcolepsy, social phobia, obsessive-compulsive disorder, atypical facial pain, eating disorders, drug withdrawal syndromes and drug dependence disorders, including dependence
25 from alcohol, opioids, amphetamines, cocaine, tobacco, and cannabis (marijuana),

melancholia, panic disorder, bulimia, anergic depression, treatment-resistant depression, headache, chronic pain syndrome, generalized anxiety disorder, and other conditions in which alteration of MAO activity could be of therapeutic value.

Anabasine, anatabine and nornicotine are minor tobacco alkaloids. These
5 compounds are commercially available. However, they may be synthesized according to known techniques or extracted directly from tobacco itself.

Preferably, anatabine is synthesized according to the method disclosed by N.M. Deo and P.A. Crooks, "Regioselective Alkylation of N-(diphenylmethyldine)-3-(aminomethylpyridine: A Simple Route to Minor Tobacco Alkaloids and Related
10 Compounds," 1137-1141 (11 December 1995), which is incorporated herein by reference.

In addition, nornicotine is preferably synthesized according to the method disclosed by S. Brandange and L. Lindblom, "N-Vinyl as N-H Protecting Group: A Convenient Synthesis of Myosmine," Acta Chem. Scand., B30, No. 1, p. 93 (1976),
15 which is also incorporated herein by reference.

In a second embodiment of the invention, the method comprises administering a MAO-inhibiting effective amount of an extract of Yerbamaté (*Ilex paraguariensis*) tea plant to a mammal, particularly a human, for the treatment of medical, psychiatric and/or neurological conditions and disorders such as, but not limited to, Alzheimer's
20 disease, Parkinson's disease, major depression, minor depression, atypical depression, dysthymia, attention deficit disorder, hyperactivity, conduct disorder, narcolepsy, social phobia, obsessive-compulsive disorder, atypical facial pain, eating disorders, drug withdrawal syndromes and drug dependence disorders, including dependence from alcohol, opioids, amphetamines, cocaine, tobacco, and cannabis (marijuana),
25 melancholia, panic disorder, bulimia, anergic depression, treatment-resistant

depression, headache, chronic pain syndrome, generalized anxiety disorder, and other conditions in which alteration of MAO activity could be of therapeutic value.

The Yerbamaté extract may be prepared by shredding the Yerbamaté materials, mixing the shredded materials with a water/ethanol (for example, 1/1 by
5 volume) solution in a mixture of about four leaves per 10 ml of the water/ethanol mixture, extracting with continuous stirring, and then removing the solution from the Yerbamaté residue. The residue can then be further extracted two more times with the same volume of water/ethanol mixture, and then the extracts may be combined and filtered to remove the particulate Yerbamaté materials. The combined extracts may
10 then be subject to vacuum evaporation to yield the Yerbamaté extract.

In a third embodiment of the invention, the method comprises administering a MAO-inhibiting effective amount of a tobacco extract to a mammal, particularly a human, for the treatment of medical, psychiatric and/or neurological conditions and disorders such as, but not limited to, Alzheimer's disease, Parkinson's disease, major
15 depression, minor depression, atypical depression, dysthymia, attention deficit disorder, hyperactivity, conduct disorder, narcolepsy, social phobia, obsessive-compulsive disorder, atypical facial pain, eating disorders, drug withdrawal syndromes and drug dependence disorders, including dependence from alcohol, opioids, amphetamines, cocaine, tobacco, and cannabis (marijuana), melancholia,
20 panic disorder, bulimia, anergic depression, treatment-resistant depression, headache, chronic pain syndrome, generalized anxiety disorder, and other conditions in which alteration of MAO activity could be of therapeutic value.

The tobacco extract may be prepared by shredding tobacco leaves (for example, processed tobacco obtained from STAR TOBACCO AND
25 PHARMACEUTICALS, INC.), mixing the shredded leaves with a water/ethanol (for

example, 1/1 by volume) solution in a mixture of about four leaves per 10 ml of the water/ethanol mixture, extracting with continuous stirring, and then removing the solution from the tobacco residue. The residue can then be further extracted two more times with the same volume of water/ethanol mixture, and then the extracts may be
5 combined and filtered to remove the particulate tobacco leaf material. The combined extracts may then be subject to vacuum evaporation to yield the tobacco extract.

In a fourth embodiment of the invention, the method comprises administering a MAO-inhibiting effective amount of an extract of chewing gum and lozenges formulated with tobacco extract to a mammal, particularly a human, for the treatment
10 of medical, psychiatric and/or neurological conditions and disorders such as, but not limited to, Alzheimer's disease, Parkinson's disease, major depression, minor depression, atypical depression, dysthymia, attention deficit disorder, hyperactivity, conduct disorder, narcolepsy, social phobia, obsessive-compulsive disorder, atypical facial pain, eating disorders, drug withdrawal syndromes and drug dependence
15 disorders, including dependence from alcohol, opioids, amphetamines, cocaine, tobacco, and cannabis (marijuana), melancholia, panic disorder, bulimia, anergic depression, treatment-resistant depression, headache, chronic pain syndrome, generalized anxiety disorder, and other conditions in which alteration of MAO activity could be of therapeutic value.

20 The chewing gum and lozenges extract may be prepared by extracting five slices of GUMSMOKE chewing gum and NICOMINT lozenges (obtained from STAR TOBACCO AND PHARMACEUTICALS, INC.), which are formulated with tobacco extract, with distilled water (50 ml) at room temperature for 12 hours, and then removing the undissolved gum substance by filtration.

25 The above compounds and substances were evaluated for their MAO

inhibiting activity. Test results surprisingly showed that the compounds and substances of the present invention all provided MAO inhibition. It was also discovered that the MAO inhibiting effects had a different character than for known MAO inhibitors in that they reached an asymptotic or ceiling effect, so that further increases in the dose beyond maximal inhibition did not produce any further increase in the MAO inhibition. This asymptotic effect would provide many benefits. For example, the problems associated with previously known, irreversible MAO inhibitors, such as hypertensive effects, can be avoided. Furthermore, the inventive MAO inhibitors may be provided as an "over the counter" drug or dietary supplement in view of its safety and efficacy.

The MAO inhibitors of the present invention may be provided in forms well known to one skilled in the art. They may be formulated in a pharmaceutically acceptable carrier, diluent or vehicle and administered in effective amounts. They may be provided in the form of a capsule, pill, tablets, lozenge, gum, troches, suppositories, powder packets or the like.

The determination of the effective amounts for a given treatment can be accomplished by routine experimentation and is also well within the ordinary skill in the art.

20 EXAMPLES

To determine the effectiveness of compounds and substances of the present invention, experiments were conducted as follows:

MAO Reaction

The MAO activities of the compounds and substances were determined using standard reaction conditions as described in Halt, A., et al., Analytical Biochemistry,

244:384-392 (1997).

Tissue Preparation:

Liver samples from cow or rat were obtained immediately after sacrifice.

Liver was homogenized in a Polytron mechanical homogenizer in a ratio of 1 gram of
5 liver to 1 ml of potassium phosphate buffer (0.2 M at pH of 7.6). Large membranes
were removed by low speed centrifugation at 1000xg for 15 minutes. The supernatant
was removed from the pellet and used immediately for MAO activity assays or stored
at 0 degrees Centigrade. Protein levels were determined in the liver homogenate by
the Bradford protein reaction.

10 **Reaction Conditions:**

The standard reaction conditions were developed as a modification of the
spectrophotometric assay using standard conditions (Halt, A., et al., Analytical
Biochemistry, 244:384-392 (1997)). Total MAO activity was determined by
incubating the liver preparations for 30 minutes at 37 degrees Centigrade with a 1/1
15 dilution of a test fraction (compound or substance to be tested dissolved in distilled
water) or control condition (water alone). This incubation allowed the test compound
or substance to interact with the enzyme under physiological conditions. The final
tissue concentration in the reaction mixture was 3.5 mg per 100 ml.

Following the incubation with test compounds/substances or control, the MAO
20 reactions were initiated and the reactions were incubated at 37 degrees Centigrade.
The reaction was initiated by mixing 150 µl of preincubated tissue with 150 µl of
chromogenic solution (containing 10 mM vanillic acid, 5 mM 4-amino antipyrine, 20
units/ml of peroxidase in 0.2 M potassium phosphate buffer final concentration pH
7.6), 600 µl of amine substrate (tyramine 500 micromolar), and 100 µl of distilled
25 water (1 ml reaction volume). The standard reaction time was for 1 hour, but reaction

times varied from 1 minute to 3 hours to evaluate the time course of the reaction in the presence or absence of test substance or control. The reactions were terminated by the addition of 30 μ l of a stop solution of phenelzine (10 mM). The stopped reactions were stored on ice and placed at room temperature for reading in a spectrophotometer at a wavelength of 498 nm. The resulting values were analyzed to determine the amount of reaction product produced by MAO activity. This assay was reliable and simple to perform. A standard curve using hydrogen peroxide for enzyme activity was prepared for each experiment to determine the activity of the enzyme.

Selective assays of MAO A and MAO B isoforms were determined by using selective inhibitors of each of these enzymes. During the preincubation of the enzyme with the test solutions, either pargyline or chlorgyline (final drug concentrations in the reaction mixture of 500 nM) was added to the reaction mixture. This technique allowed for the assay of MAO A or MAO B activity in the absence of the activity of the other isoform of the enzyme. All other reaction conditions were conducted as for total MAO activity studies.

Each of the compounds and substances of the present invention were evaluated by initially determining a concentration curve at a reaction time of one hour. After determining the concentration curves of each compound or substance on MAO activity, a reaction time course in the presence or absence of test compound or substance was determined and time course curves were generated. Following these experiments, the effect of each test compound or substance was evaluated on MAO A and MAO B activity by the same reaction studies as described above for the total enzyme activity.

EXAMPLE 1

Anabesine, in its purified form, was dissolved in distilled water in a maximal inhibition concentration of 0.2 mg/ml, and tested according to the procedure described above. At maximal or saturating inhibition concentrations, anabesine was effective at inhibiting MAO activity by approximately 10-13%, and was effective at inhibiting the enzyme at all time points in the reaction.

Figure 1 presents the means (plus or minus the standard errors of the means) for the percent inhibition of MAO activity produced by saturating concentrations of anabesine over 60 minutes of MAO activity measured as described above. Each data point represented the mean of 5 determinations. All the data points shown in Figure 1 were statistically, significantly different from the sham control at each time point tested (student t test, $p < 0.01$), and were representative of multiple experiments.

Since anabesine was an inhibitor of MAO activity, further studies were conducted to evaluate if this agent was inhibiting MAO A or B activity using the methods described above. Anabesine was found to inhibit both MAO A and MAO B activity as shown in Figure 2. Figure 2 presents the means (plus or minus the standard errors of the means) for 5 determinations for the percent inhibition of MAO A and MAO B activity. The effects of anabesine on both forms of MAO activity were statistically, significantly different from control enzyme conditions (student t test, $p < 0.05$). The results demonstrate that anabesine inhibits both MAO A and B forms of the enzyme.

EXAMPLE 2

Anatabine in its purified form, was dissolved in distilled water in a maximal inhibition concentration of 0.1 mg/ml, and tested according to the procedure described

above. At maximal or saturating inhibition concentrations, anatabine was effective at inhibiting MAO activity by approximately 60%. This result shows that anatabine may be much safer as a medication than standard MAO enzyme inhibitors. Anatabine was effective at inhibiting the enzyme at all time points in the reaction, and was equally effective in inhibiting both MAO A and MAO B activities.

Figure 3 presents the means (plus or minus the standard errors of the means) for the percent inhibition of MAO activity produced by saturating concentrations of anatabine over 60 minutes of MAO activity measured as described above. Each data point represented the mean of 6 determinations. Anatabine was an effective MAO inhibitor at maximal concentrations, inhibiting the enzyme by approximately 60 %, as discussed above. All the data points shown in Figure 3 were statistically, significantly different from the sham control at each time point tested (student t test, $p < 0.005$) and were representative of multiple experiments.

Since anatabine was an inhibitor of MAO activity, further studies were conducted to evaluate if this agent was inhibiting MAO A or B activity using the methods described above. Anatabine was found to inhibit both MAO A and MAO B activity as shown in Figure 4. Figure 4 presents the means (plus or minus the standard errors of the means) for 6 determinations for the percent inhibition of MAO A and MAO B activity. The effects of anatabine on both forms of MAO activity were statistically, significantly different from control enzyme conditions (student t test, $p < 0.01$). The results demonstrate that anatabine inhibits both MAO A and B forms of the enzyme.

EXAMPLE 3

Normicotine in its purified form, was dissolved in distilled water in a maximal

inhibition concentration of 0.08 mg/ml, and tested according to the procedure described above. At maximal or saturating inhibition concentrations, nornicotine was effective at inhibiting MAO activity by approximately 80 to 95%, and was effective at inhibiting the enzyme at all time points in the reaction. Nornicotine was also equally effective in inhibiting both MAO A and MAO B activities.

Figure 5 presents the means (plus or minus the standard errors of the means) for the percent inhibition of MAO activity produced by saturating concentrations of nornicotine over 60 minutes of MAO activity measured as described above. Each data point represented the mean of 6 determinations. Nornicotine was an effective MAO inhibitor at maximal concentrations, inhibiting the enzyme by approximately 80-95%, as discussed above. All the data points shown in Figure 5 were statistically, significantly different from the sham control at each time point tested (student t test, $p < 0.01$) and were representative of multiple experiments.

Since nornicotine was an inhibitor of MAO activity, further studies were conducted to evaluate if this agent was inhibiting MAO A or B activity using the methods described above. Nornicotine was found to inhibit both MAO A and MAO B activity as shown in Figure 6. Figure 6 presents the means (plus or minus the standard errors of the means) for 6 determinations for the percent inhibition of MAO A and MAO B activity. The effects of nornicotine on both forms of MAO activity were statistically, significantly different from control enzyme conditions (student t test, $p < 0.01$). The results demonstrate that nornicotine inhibits both MAO A and B forms of the enzyme.

EXAMPLE 4

The Yerbamaté extract was prepared as follows: Yerbamaté materials

(obtained from STAR TOBACCO AND PHARMACEUTICALS, INC.) were shredded and mixed with a water/ethanol (1/1 by volume) solution in a mixture of about four leaves per 10 ml of the water/ethanol mixture; the materials were then extracted overnight with continuous stirring; the solution was then removed from the Yerbamaté residue and stored; the residue was then further extracted overnight two more times with the same volume of water/ethanol mixture, and the three extracts were combined and filtered to remove the particulate Yerbamaté material; and the combined extracts were subjected to removal of the water/ethanol by vacuum evaporation. The resultant extract was then weighed and solubilized in distilled water.

When tested, Yerbamaté extract was effective in inhibiting MAO activity. The maximal inhibition concentration was 10 mg/ml. At maximal or saturating inhibition concentrations, the Yerbamaté extract inhibited MAO activity by approximately 40 to 50%. The results suggest that Yerbamaté may be much safer as a medication than standard MAO enzyme inhibitors. The extract was effective in inhibiting MAO at all time points in the reaction, and was equally effective in inhibiting both MAO A and MAO B activities.

Figure 7 presents the means (plus or minus the standard errors of the means) for the percent inhibition of MAO activity produced by saturating concentrations of Yerbamate over 60 minutes of MAO activity measured as described above. Each data point represented the mean of 5 determinations. Yerbamate was an effective MAO inhibitor at maximal concentrations, inhibiting the enzyme by approximately 40-50%, as discussed above. All the data points shown in Figure 7 were statistically, significantly different from the sham control at each time point tested (student t test, $p < 0.005$) and were representative of multiple experiments.

Since Yerbamate was an inhibitor of MAO activity, further studies were

conducted to evaluate if this agent was inhibiting MAO A or B activity using the methods described above. Yerbamate was found to inhibit both MAO A and MAO B activity as shown in Figure 8. Figure 8 presents the means (plus or minus the standard errors of the means) for 5 determinations for the percent inhibition of MAO A and MAO B activity. The effects of Yerbamate on both forms of MAO activity were statistically, significantly different from control enzyme conditions (student t test, $p < 0.01$). The results demonstrate that Yerbamate inhibits both MAO A and B forms of the enzyme.

10 EXAMPLE 5

The tobacco extract was prepared in the same manner as in Example 4, except that processed tobacco leaves (obtained from STAR TOBACCO AND PHARMACEUTICALS, INC.) were substituted for the Yerbamaté materials.

When tested, the tobacco extract was effective in inhibiting MAO activity. At maximal or saturating inhibition concentrations, the tobacco extract was able to inhibit MAO activity by approximately 60%. The results suggest that the extract may be much safer as a medication than standard MAO enzyme inhibitors. The tobacco extract was effective at inhibiting MAO at all time points in the reaction, and was equally effective in inhibiting both MAO A and MAO B activities.

Figure 9 presents the means (plus or minus the standard errors of the means) for the percent inhibition of MAO activity produced by saturating concentrations of tobacco extract over 60 minutes of MAO activity measured as described above. Each data point represented the mean of 8 determinations. Tobacco extract was an effective MAO inhibitor at maximal concentrations, inhibiting the enzyme by approximately 60%, as described above. All the data points shown in Figure 9 were statistically,

significantly different from the sham control at each time point tested (student t test, $p < 0.001$) and were representative of multiple experiments.

Since tobacco extract was an inhibitor of MAO activity, further studies were conducted to evaluate if this agent was inhibiting MAO A or B activity using the methods described above. Tobacco extract was found to inhibit both MAO A and MAO B activity as shown in Figure 10. Figure 10 presents the means (plus or minus the standard errors of the means) for 8 determinations for the percent inhibition of MAO A and MAO B activity. The effects of tobacco extract on both forms of MAO activity were statistically, significantly different from control enzyme conditions (student t test, $p < 0.005$). The results demonstrate that tobacco extract inhibits both MAO A and B forms of the enzyme.

EXAMPLES 6 and 7

The extract of GUMSMOKE chewing gum or lozenges was prepared as follows: five slices each of gum or lozenges, formulated with tobacco extract, were extracted with 50 ml of distilled water at room temperature for 12 hours. The undissolved gum substance was removed by filtration. (The lozenges dissolved completely.) Dilutions of these extracts were prepared for evaluation.

The gum and lozenges extracts were effective in inhibiting MAO activity. At maximal or saturating concentrations, the extracts were able to inhibit MAO activity by approximately 50 to 60%.

Figure 11 presents the means (plus or minus the standard errors of the means) for the percent inhibition of MAO activity produced by saturating concentrations of an extract of GUMSMOKE chewing gum prepared as described above over 60 minutes of MAO activity measured as described above. Each data point represented the mean

of 4 determinations. GUMSMOKE extract was an effective MAO inhibitor at maximal concentrations, inhibiting the enzyme by approximately 50-60%. All the data points shown in Figure 11 were statistically, significantly different from the sham control at each time point tested (student t test, $p < 0.05$) and were representative of multiple experiments.

Figure 12 presents the means (plus or minus the standard errors of the means) for the percent inhibition of MAO activity produced by saturating concentrations of an extract of the lozenge prepared as described above over 60 minutes of MAO activity measured as described above. Each data point represented the mean of 4 determinations. The lozenge extract was an effective MAO inhibitor at maximal concentrations, inhibiting the enzyme by approximately 50-60%. All the data points shown in Figure 12 were statistically, significantly different from the sham control at each time point tested (student t test, $p < 0.05$) and were representative of multiple experiments.

Both MAO A and MAO B were also inhibited by these extracts.

Throughout this specification and the claims which follow, unless the context requires otherwise, the word "comprise", and variations such as "comprises" and "comprising", will be understood to imply the inclusion of a stated integer or step or group of integers or steps but not the exclusion of any other integer or step or group of integers or steps.

The reference to any prior art in this specification is not, and should not be taken as, an acknowledgment or any form or suggestion that that prior art forms part of the common general knowledge in Australia.

THE CLAIMS DEFINING THE INVENTION ARE AS FOLLOWS:

1. A method of treating major depression, minor depression, atypical depression, dysthymia, attention deficit disorder, hyperactivity, conduct disorder, narcolepsy, obsessive-compulsive disorder, atypical facial pain, eating disorders, drug withdrawal syndromes and drug dependence disorders, melancholia, pain disorder, bulimia, anergic depression, treatment-resistant depression, headache, or chronic pain syndrome, comprising administering to a mammal a MAO-inhibiting effective amount of an active agent selected from the group consisting of anabasine, anatabine, nornicotine, and tobacco extract.
5
10
2. The method according to claim 1, wherein the mammal is human.
3. The method according to claim 2, wherein the active agent is anabasine.
15
4. The method according to claim 2, wherein the active agent is anatabine.
5. The method according to claim 2, wherein the active agent is nornicotine.
- 20 6. The method according to claim 2, wherein the active agent is a tobacco extract.
7. The method according to claim 2, wherein the disorder treated is major depression.
8. The method according to claim 2, wherein the active agent is provided in a pharmaceutically acceptable carrier, diluent, or carrier.
25
9. A method of treating Alzheimer's disease, Parkinson's disease, major depression, minor depression, atypical depression, dysthymia, attention deficit disorder, hyperactivity, conduct disorder, narcolepsy, social phobia, obsessive-compulsive disorder, atypical facial pain, eating disorders, drug withdrawal syndromes and drug
30

dependence disorders, melancholia, pain disorder, bulimia, anergic depression, treatment-resistant depression, or chronic pain syndrome, comprising administering to a mammal a MAO-inhibiting effective amount of Yerbamaté (*Ilex paraguariensis*) extract.

5 10. The method according to claim 9, wherein the mammal is human.

 11. The method according to claim 10, wherein the active agent is Yerbamaté (*Ilex paraguariensis*) extract.

 12. The method according to claim 11, wherein the disorder treated is major depression.

10 13. The method according to claim 11, wherein the active agent is provided in a pharmaceutically acceptable carrier, diluent or carrier.



15 DATED this 4th day of August, 2003

REGENT COURT TECHNOLOGIES

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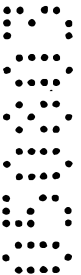


FIG. 1

ANABASINE TIME COURSE

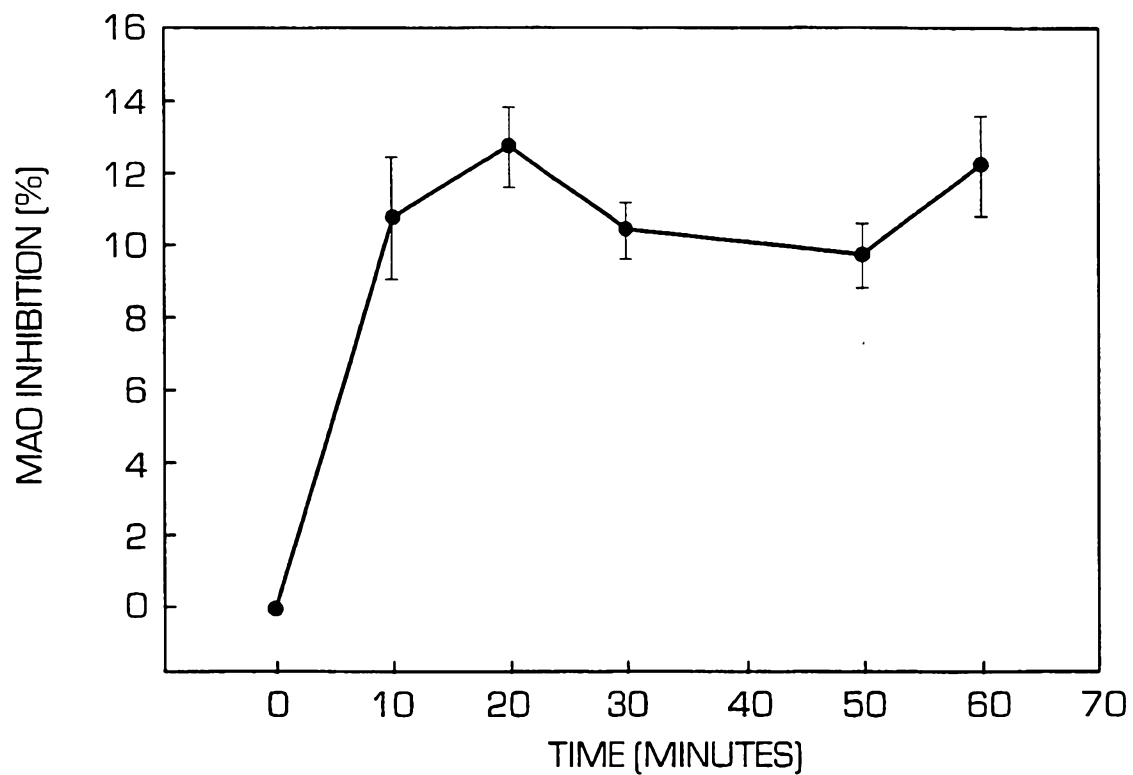


FIG. 2

ANABASINE INHIBITION OF MAO A & B

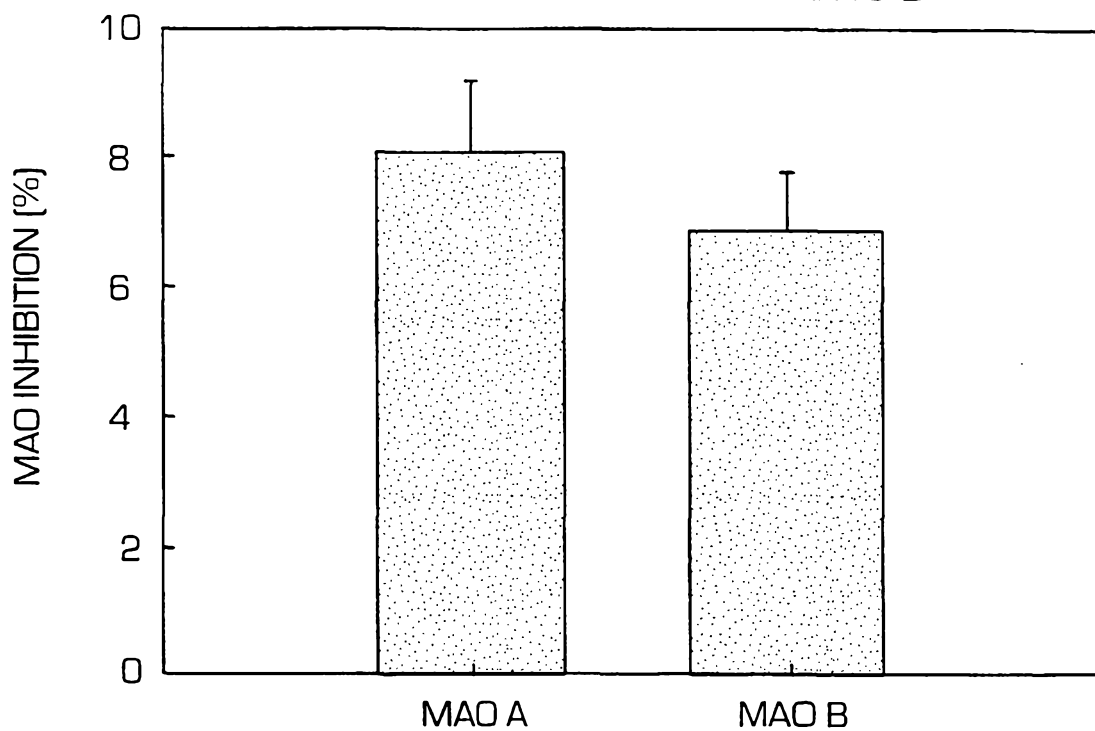
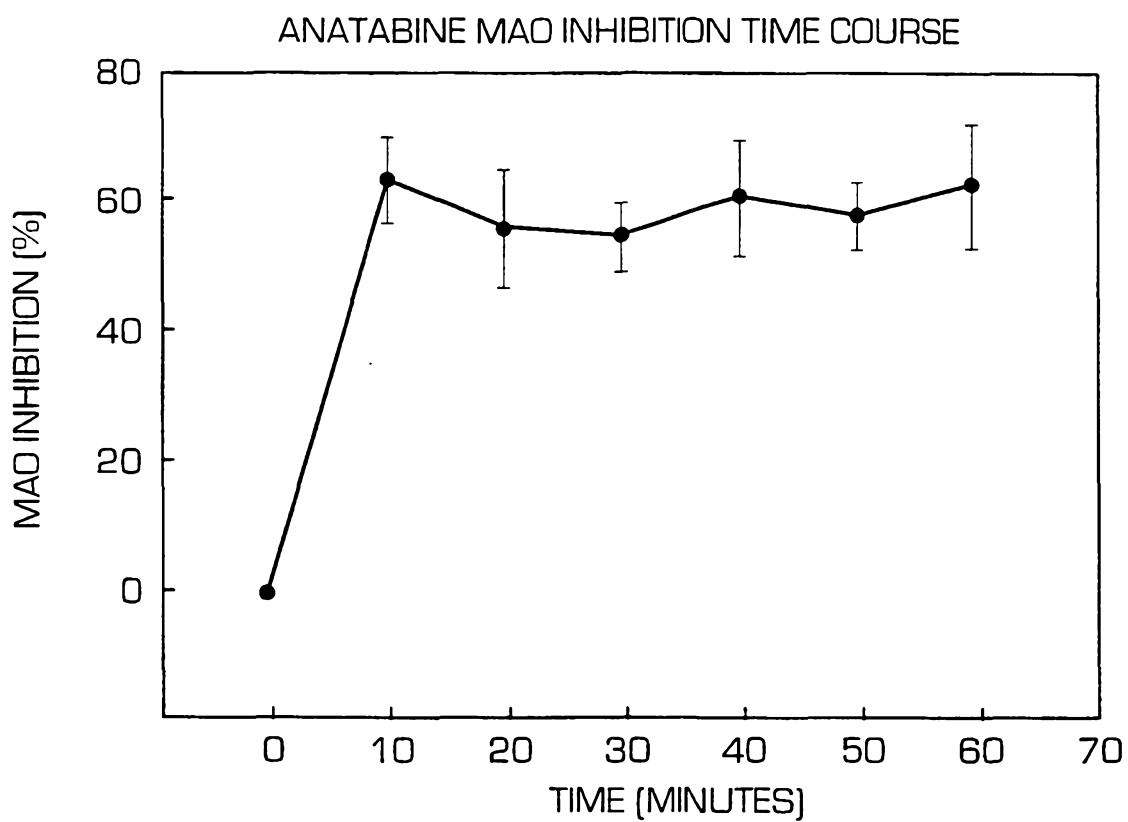
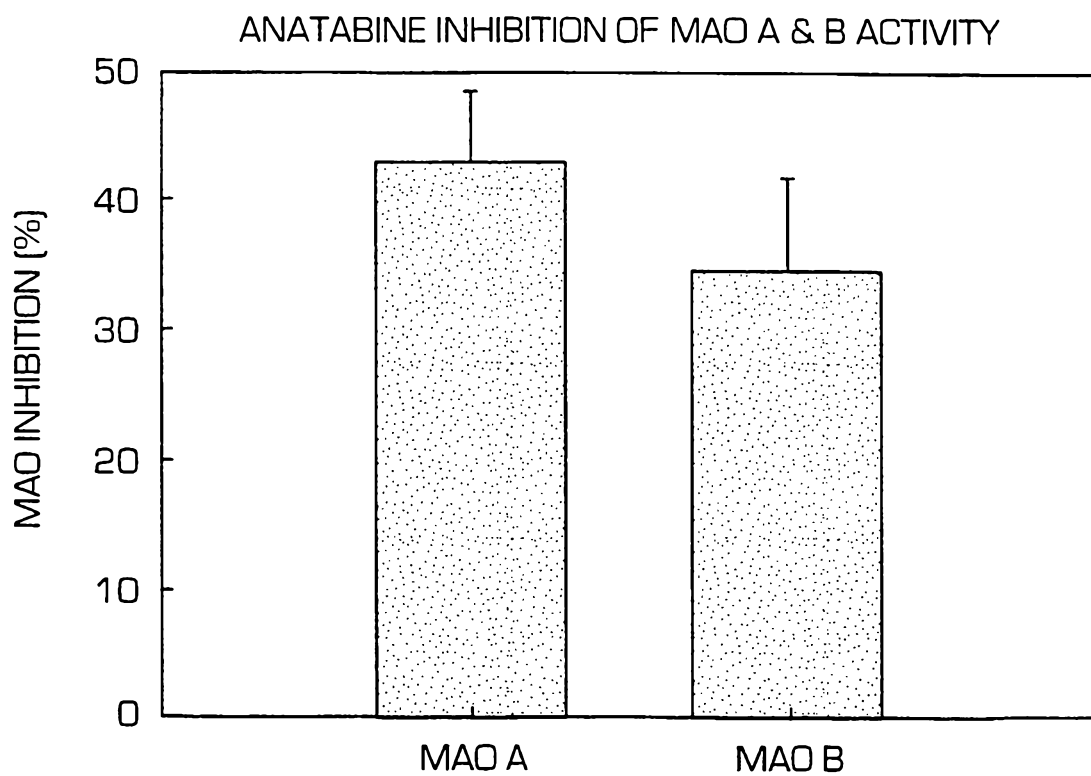


FIG. 3



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FIG. 4



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FIG. 5

NORNICOTINE MAO INHIBITION TIME COURSE

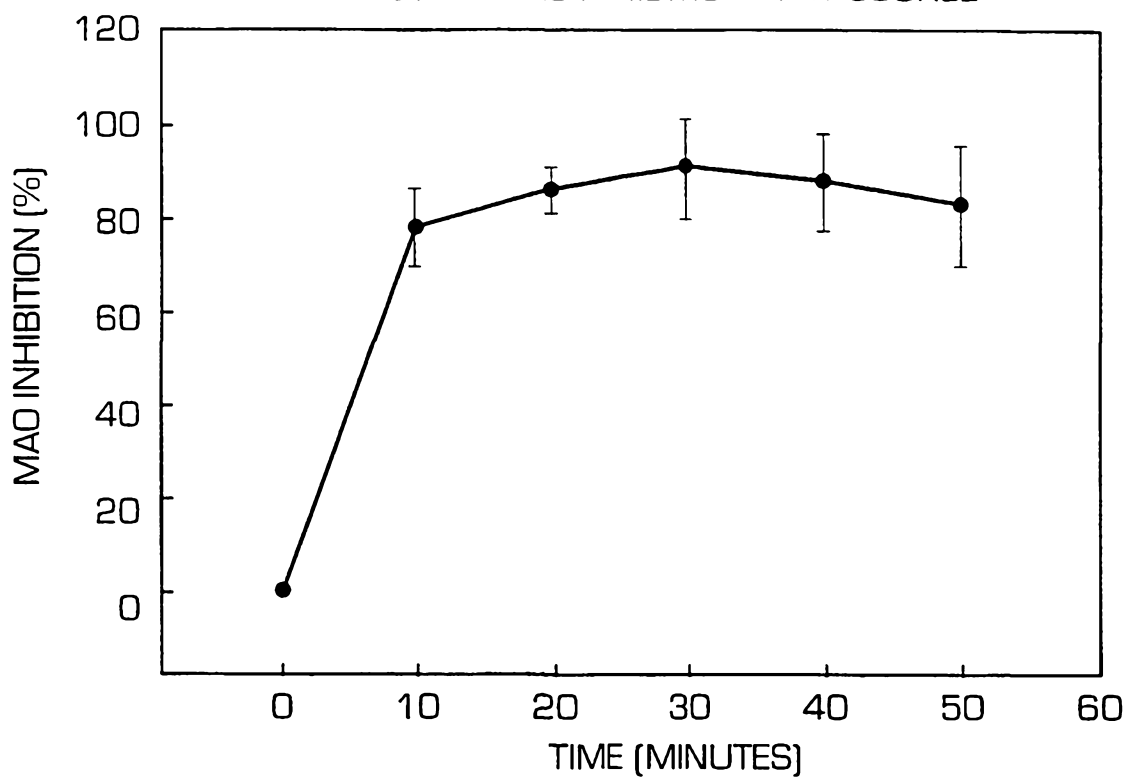
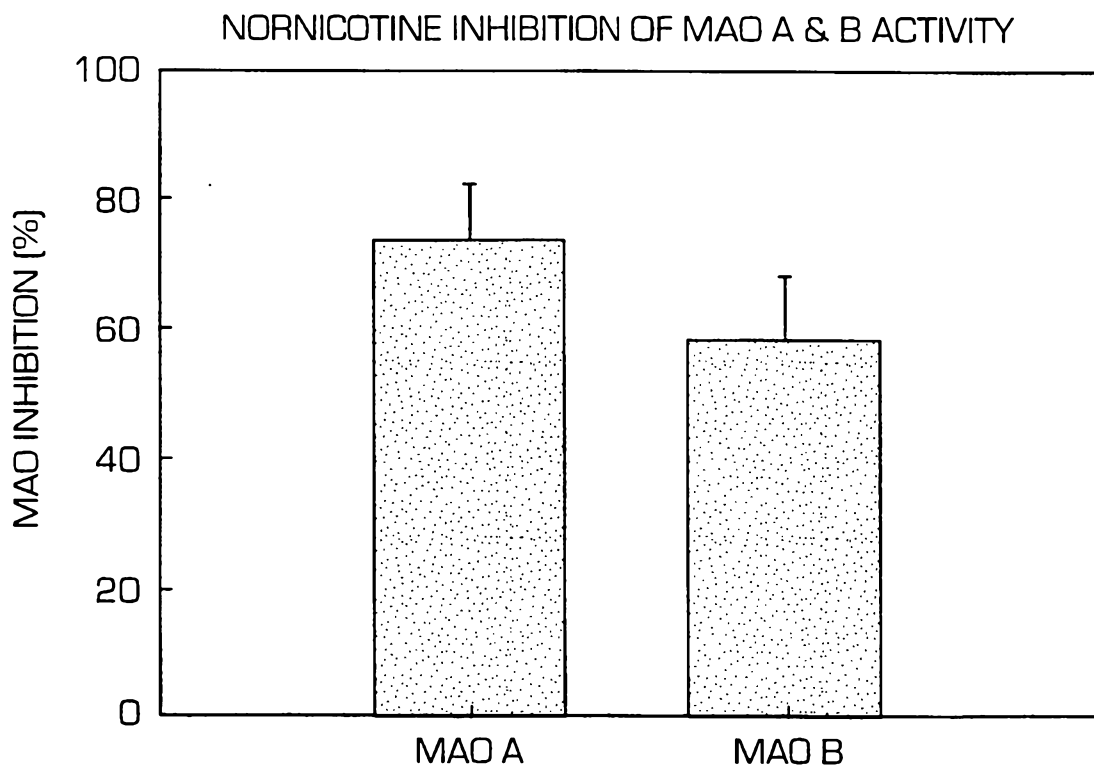


FIG. 6



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FIG. 7

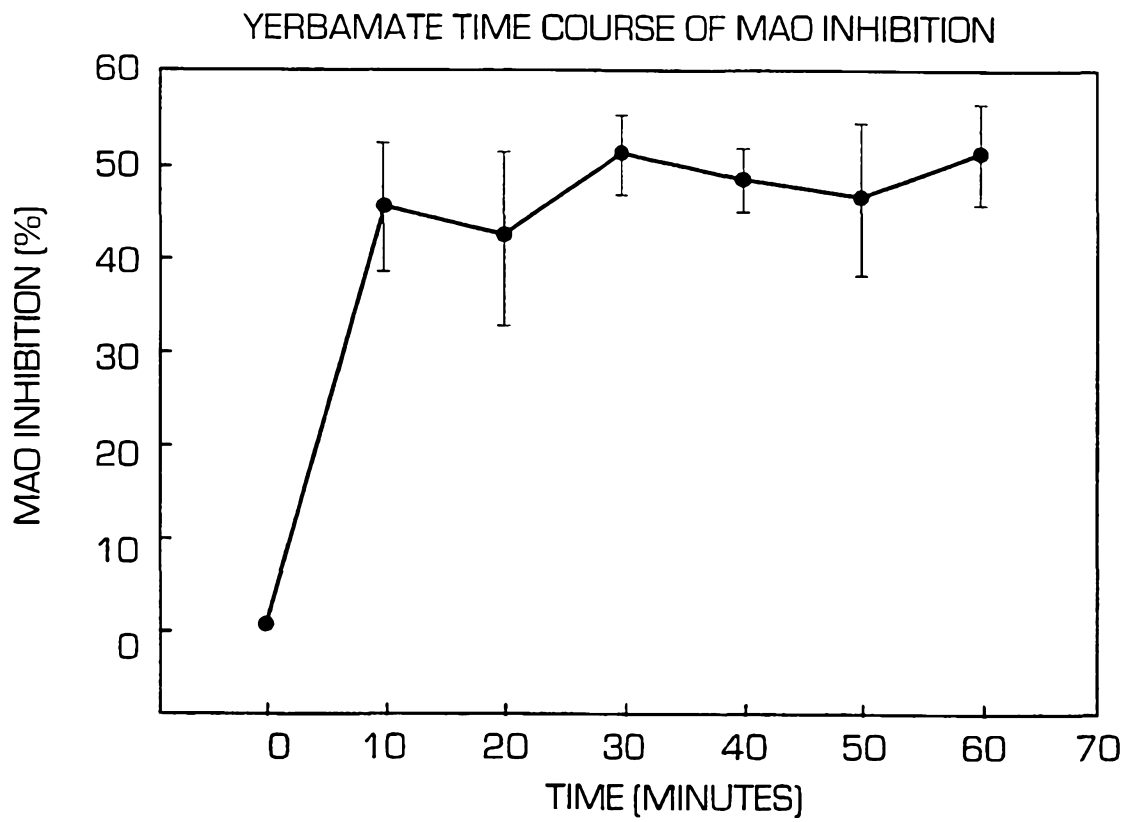
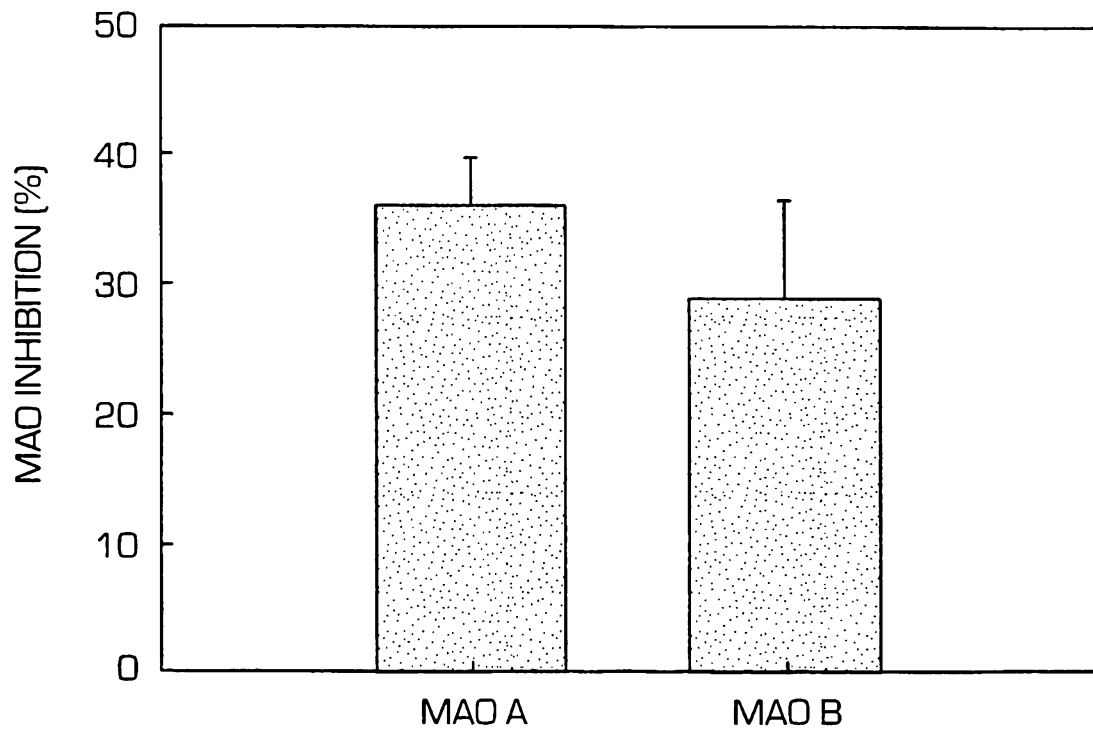


FIG. 8

YERBAMATE INHIBITION OF MAO A & B ACTIVITY



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FIG. 9

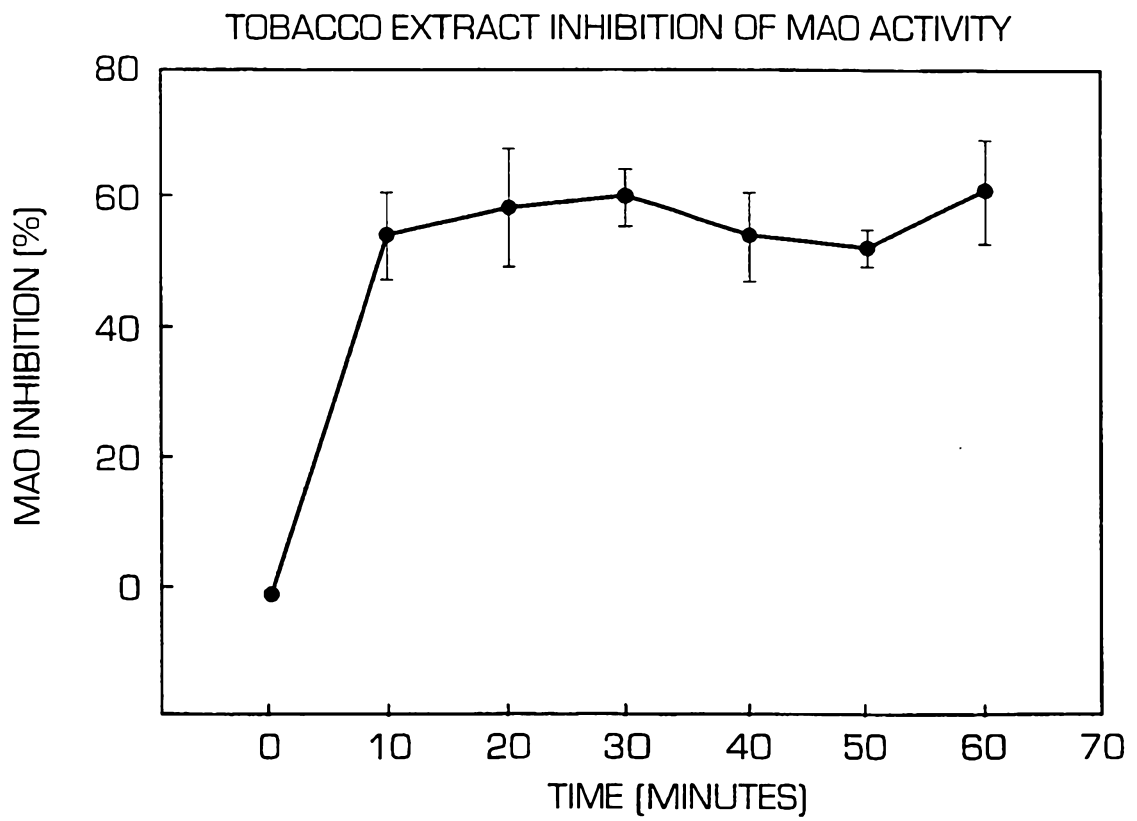
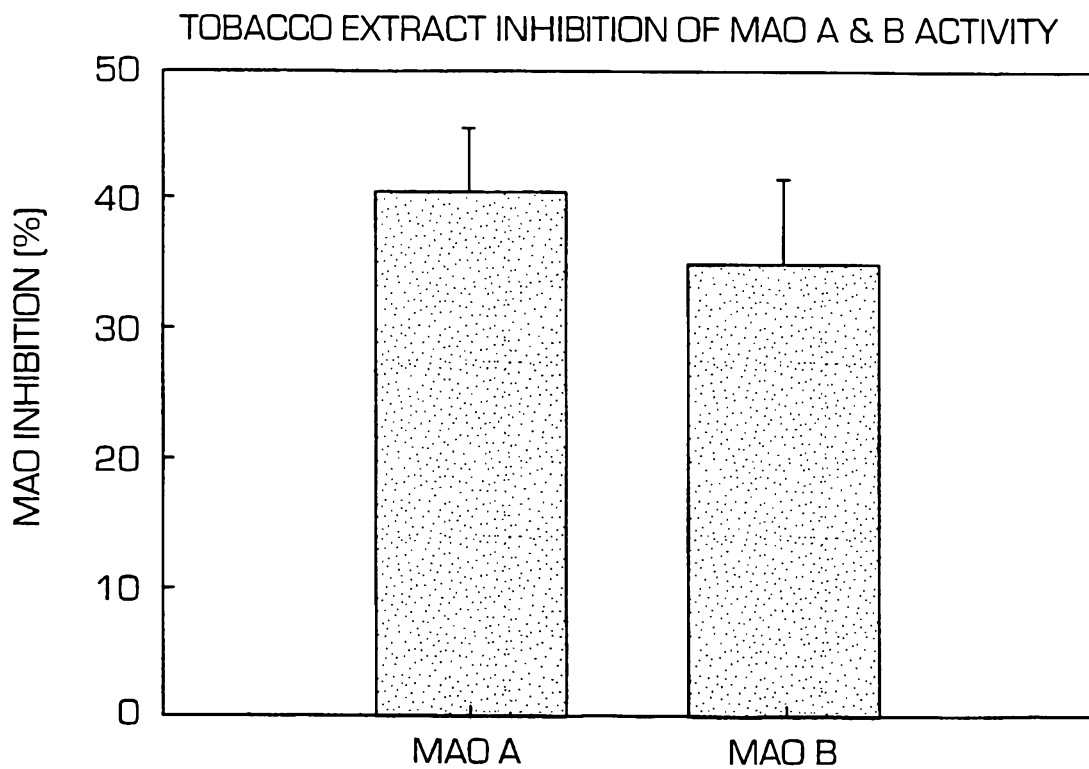


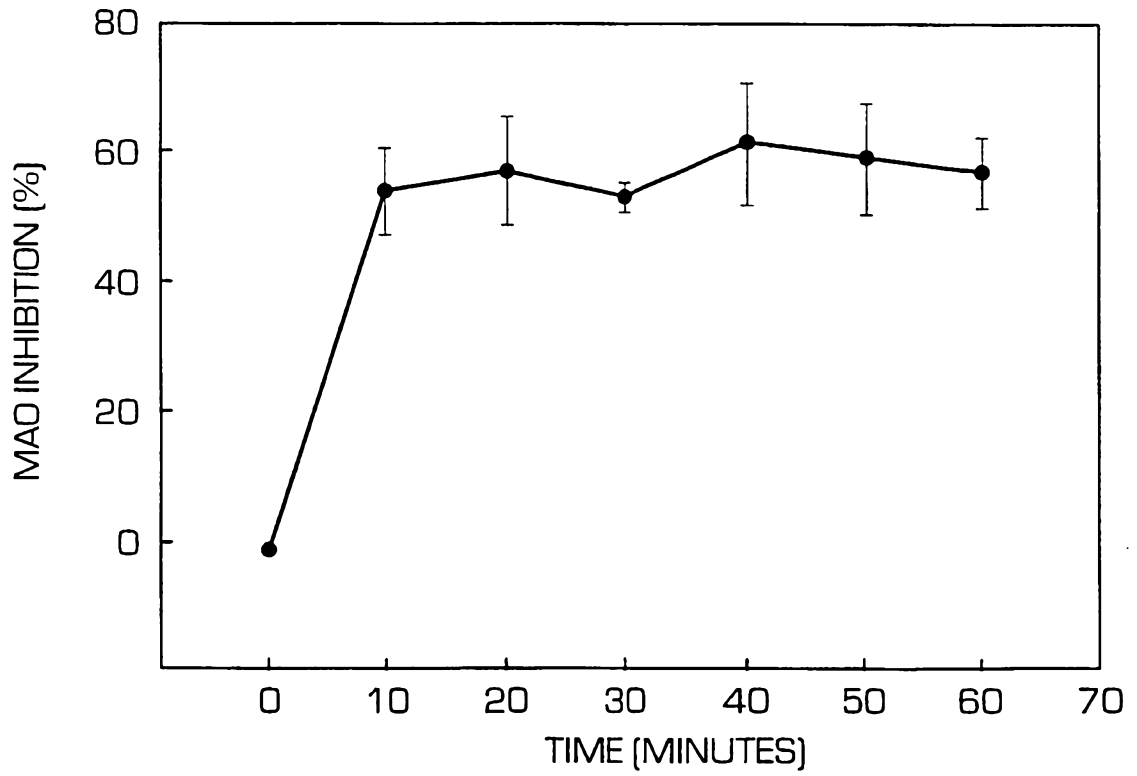
FIG. 10



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FIG. 11

GUMSMOKE INHIBITION OF MAO ACTIVITY



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FIG. 12

