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AROMATASE INHIBITORS IN CIGARETTE SMOKE, TOBACCO LEAVES AND OTHER PLANTS

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A chance observation that cigarette smoke interferes with the aromatase assay led us to investigate tobacco leaf and smoke extracts for the presence of aromatase inhibitors. The highest inhibitory activity was found in the basic fraction of cigarette smoke. Further purification of this fraction led to the identification of N-n-octanoylnornicotine. Synthesis and testing of a series of acylated nornicotines and anabasines for their ability to inhibit aromatase showed an interesting correlation of activity with the length of the acyl carbon chain, with maximum activity at C-11. The acylated derivatives showed activity which was significantly greater than that of nicotine and anabasine. *In vivo* studies in rats indicated that administration of this inhibitor delayed the onset of NMU-induced breast carcinoma and altered the estrus cycle. These *in vivo* studies suggest that tobacco alkaloid derivatives exert their effects by suppression of the aromatase enzyme system. Toxicity studies indicated relatively low toxicity with LD_{50} for N-n-octanoylnornicotine = 367 mg/kg body weight. When extracts from thirty five varieties of vegetables, plant leaves, and fruits were analyzed, seventeen showed quantitatively significant aromatase inhibition which was comparable to that of green tobacco leaf, suggesting that naturally occurring substances may affect endocrine function through aromatase inhibition.

KEY WORDS: Aromatase inhibitor, cigarette smoke, tobacco, acylnornicotine, acylanabasine, N-n-octanoylnornicotine.

INTRODUCTION

Aromatase, the enzyme responsible for the production of estrogens from androgens, has been a topic of interest in the field of steroid biochemistry for decades. The ability to regulate aromatase activity would have many implications in the physiology involved in puberty, reproduction, aging, fetal sex differentiation, the growth of sex hormone-dependent cancers, and other biological processes both normal and abnormal. Many compounds which block the production of estrogens through inhibition or inactivation of aromatase are currently under investigation and a few are in the treatment of estrogen-dependent breast carcinoma.

In the process of developing a simple and sensitive method for routine assays of aromatase activity,[1] we realized that a puff of cigarette smoke during pipetting greatly influenced the outcome of the experiment. By eliminating smoking in the laboratory

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area, we were able to carry the original project to completion. However, the nagging question of why tobacco smoke affected aromatase activity eventually led to the investigation not only of cigarette smoke, but also tobacco leaves and several purified components of tobacco extracts, and extracts from other plants.

We devised an apparatus to collect cigarette smoke by passing it through aqueous and organic (methylene chloride) traps under slightly reduced pressure. Extracts of the unburned tobacco were made by soaking the material in aqueous ethanol. Preliminary tests indicated that these extracts did, indeed, inhibit aromatase. The smoke and leaf extracts were then separated by solvent partition into five crude fractions which were subsequently tested for their ability to inhibit aromatase activity. The basic fraction of tobacco smoke extract and the basic and acid fractions of tobacco leaf extract showed relatively strong inhibitory effects.

Since the known components in tobacco and smoke number over 1,200 molecular species,[2] we realized that isolating the active compound(s) would be a cumbersome task. However, after our unsuccessful testing of many commercially available components, we decided to pursue purification of the active fractions from cigarette smoke. GC/MS analyses of an active component of the basic extracts showed it to be N-n-octanoylnornicotine; a minor component was identified as N-n-hexanoylnornicotine. We sought therefore, to prepare synthetically pure material of a series of N-acyl-nornicotines and anabasines in order to assess their inhibitory activity and attempt to determine a structure-activity relationship. We were able to obtain 19 acylnornicotines and 15 structurally related anabasines. We also evaluated the biological activity of octanoylnornicotine by monitoring acute toxicity and determining the effect on the estrus cycle and on the incidence of N-nitroso-N-methylurea (NMU)-induced breast tumors in Buffalo/N rats.

The presence of inhibitory activity in green tobacco leaves led us to check for its presence in other plants, fruits, and vegetables. When the ethanolic extracts of 35 different plants were assayed for aromatase inhibition, approximately half showed no significant effect. However, several extracts clearly exhibited enough inhibitory activity to warrant more detailed investigation.

MATERIALS AND METHODS

Cotinine, NADPH, benzoic acid, trans-cinnamic acid, nicotine and anabasine were purchased from Sigma Chemical Co. (St. Louis, MO). Azelaic acid, 4-hydroxybenzoic acid, 3,4-dihydrocinnamic acid, 3,4-dihydroxybenzoic acid, *p*-hydroxycinnamic acid and phenylacetic acid were purchased from Aldrich Chemical Co. (Milwaukee, WI). Benzo[a]pyrene was obtained from Eastman Kodak Co. (Rochester, NY). N-Nitroso-N-methylurea (NMU) was purchased from ICN (Cleveland, OH). [1β,2β-$^3H_2$]Androstenedione (45 Ci/mmol) was obtained from DuPont Co., NEN (Boston, MA). [^{14}C]Acetic anhydride (100,000 dpm/μmol) and [4-^{14}C]androstenedione (58 Ci/mmol) were from Amersham Co. (Arlington Heights, IL). The substrate for the aromatase assay, [1β-^{3}H, 4-^{14}C]androstenedione was prepared by drastic alkali treatment of the [1β,2β-^{3}H] labeled compound as described previously.[3] Several different brands of cigarettes and the other fresh vegetable material were purchased locally. ($\pm$)Acylnornicotines were generous gifts from the Central Research Institute of Japan Tobacco, Inc. Research cigarettes (2-A-1) were purchased from the Tobacco and Health Re-

search Institute (Lexington, KY). Rats and mice were purchased from Harlan Sprague Dawley Inc. (Indianapolis, IN). All reagents were of analytical grade.

Isolation of Aromatase Inhibitors

Cigarette smoke was drawn by water vacuum (-15 cm H_2O) through aqueous (0.1 M phosphate buffer, pH 7.5) and organic (methylene chloride) traps. Methylene chloride in the organic extract was evaporated under N_2 at 37°C in the presence of propylene glycol and the residue was dissolved in 0.1 M phosphate buffer (pH 7.5) for the inhibition assay. The extracts were separated into several fractions by solvent partition according to the procedures illustrated in Figure 1, (diagram at the top). The aqueous and organic extract portions were combined, adjusted to pH 1–2 with 6 N HCl, and extracted with ether. The ether layer was partitioned with 1 N NaOH, washed with water, dried over Na_2SO_4 and evaporated yielding the ether-soluble

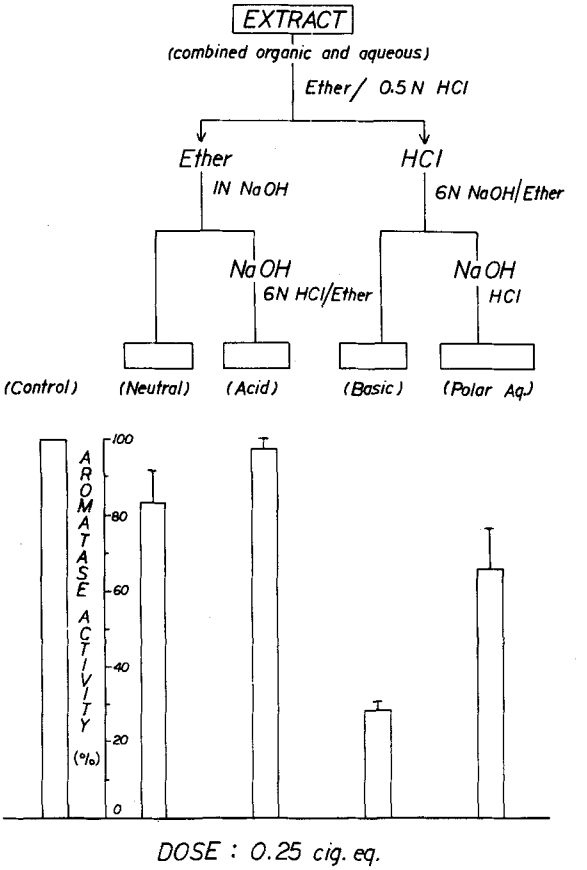


FIGURE 1 Flow chart of the fractionation of the alcoholic extract from cigarette smoke. The bar graph represents the effect of 0.25 cig. equiv. on aromatase activity ($n = 5$).

neutral fraction. The NaOH fraction was acidified with 6 N HCl and extracted with ether to give the ether-soluble acid fraction. This was partitioned with 6% $NaHCO_3$ to separate the acid fraction from the phenolic fraction. The original acid layer was made basic with 2 N NaOH and extracted with ether to separate the ether-soluble basic fraction and the polar aqueous fraction. The polar aqueous fraction was neutralized with HCl, lyophilized, and dissolved in 0.1 M phosphate buffer before being tested for inhibitory activity. Crude extracts of cigarettes, cured tobacco leaf, and green tobacco leaf were made by soaking the material in 70% ethanol for 24 h. The crude residues obtained after removal of the solvent were taken up in ether and partitioned in the same manner as for the cigarette smoke extracts. Other plant materials were homogenized in 70% ethanol (600 ml/100 gm raw material) in a similar fashion, but only their crude extracts were tested for aromatase inhibitory activity.

Enzyme Preparation

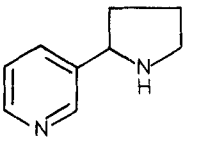
Lyophilized powder of 900 × g precipitates from human term placenta, prepared as described previously,[1] was suspended in 0.1 M phosphate buffer (pH 7.5) containing 1 mM DTT, and centrifuged at 12,000 × g for 20 min. The precipitates were re-suspended in the same buffer and stored at −20°C. The protein concentration was determined by the method of Bensadoun and Weinstein[4] using bovine serum albumin as a standard.

Aromatase Assay

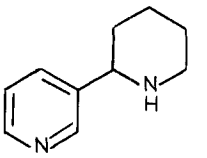
A suspension or solution of the material to be tested for inhibitory activity in 0.1 M phosphate buffer (0.5 ml, pH 7.2–7.5) containing NADPH (1 mg/ml) was added to a suspension of the enzyme preparation in 0.1 M phosphate buffer (34–39 pmol/min/mg, 2.5 mg protein, 0.5 ml, pH 7.5) and mixed at room temperature for a specific time (0–60 min for the time-dependent study, 20 min under standard conditions). The mixture was added to the incubation flask containing [1β-^{3}H, 4-^{14}C]androstenedione (1.4×10^5 dpm ^{3}H, 2.8×10^3 dpm ^{14}C, 6 nmol) and NADPH (1.2 μmol) in 1 ml of phosphate buffer (pH 7.5) and incubated at 37°C for 10 min. The incubation was terminated with addition of 0.25 ml of 20% trichloroacetic acid followed by the addition of 1 ml of 5% activated charcoal. After continued shaking for 20 min, the mixture was centrifuged and the supernatant filtered through a cotton plugged disposable pipette. A 2 ml aliquot of the effluent was combined with 1 ml distilled water and 10 ml Liquiscint (National Diagnostics, Sommerville, NJ) for counting in a Packard Instruments liquid scintillation spectrophotometer. From the determination of the ^{3}H released from the 1β-^{3}H of the substrate (75% at the 1β position) upon aromatization,[3,5] the aromatase specific activity was calculated as the number of picomoles of estrogen produced per minute per mg protein.

Synthesis of Acylanabasines

The acylations of anabasine were carried out in the usual manner. For example, N-n-nonanoylanabasine was made in the following manner. To a solution of (±)anabasine (400 mg, 2.47 mmol) in anhydrous ethyl ether (50 ml) was added triethylamine (314 mg, 3.14 mmol) at 0°C under N_2. A solution of n-nonanoyl chloride (550 mg, 3.11 mmol) in ether (10 ml) was added dropwise to the mixture and stirred at 0°C for



Nornicotine



Anabasine

90 min under N_2. Water (10 ml) was added and the organic layer was removed. The aqueous layer was extracted two times with ether, and the combined ether extract was washed with brine and dried over anhydrous Na_2SO_4. Following flash-evaporation of the solvent, the residue was purified by alumina column chromatography (Brockman Activity I, neutral, 80–200 mesh). Elution with hexane/ethyl acetate (9:1) gave 476 mg (64%) of N-n-nonanoylanabasine as a light yellow oil. These synthetic compounds were examined by gas chromatography and determined to be 94–99.8% pure. Their structures were confirmed by IR, NMR, and elemental analysis or MS. Acylnornicotines were identified by NMR and GC/MS and chemical purity were found to be 95–99.9%.

In Vivo *Studies of Inhibitors*

a. Toxicity The preliminary acute toxicity of the synthetic tobacco alkaloid aromatase inhibitors was assessed in female mice by i.p. injection of different doses of the inhibitor in sesame oil, and compared with the LD_{50} of nicotine and nornicotine. For N-n-octanoylnornicotine, the method of Behrens[6] was followed using 45 male Harlan Sprague Dawley mice and the LD_{50} was calculated accordingly.

b. Effect of N-n-octanoylnornicotine on the estrus cycles of BUF/N rats The procedures are as previously published.[7] The proestrus day of normal cycling BUF/N rats was determined by means of the daily vaginal smear test observed for at least three normal cycles, after which the rats were injected i.p. with 50 mg/kg body weight of N-n-octanoylnornicotine in sesame oil. The control group received only sesame oil. Daily vaginal smears were taken until a normal four-day cycle returned for three continuous cycles.

c. Effect of N-n-octanoylnornicotine on NMU-induced breast tumors The procedures are as previously published as Protocol I.[7] N-Nitroso-N-methylurea (NMU), wet with 3% acetic acid was dissolved in distilled water (10 mg/ml) and given in three intravenous injections four weeks apart at a dose of 50 mg/kg body weight to BUF/N inbred female rats which were 55 days old at the first injection. One group of animals

(n = 10) was ovariectomized at the 4th week. The test group (n = 9) was given N-n-octanoylnornicotine (50 mg/day/kg body weight for 1 week) at the 4th week, and the control group (n = 9) received only sesame oil. The rats were monitored for tumors by visual inspection and by palpation of the mammary areas three times a week.

RESULTS

The aqueous and organic extracts of cigarette smoke demonstrated dose-dependent inhibition of placental aromatase with 50% inhibition at 0.25 and 0.07 cigarette equivalent (cig. equiv.), as shown in Figure 2. The inhibition of the tobacco leaf extact was also dose-dependent, 50% at 0.025 cig. equiv.[8] Nicotine demonstrated moderate activity (50% at 4 cig. equiv.), whereas cotinine, menthol, and benzo[a]pyrene were not inhibitory. When the more common tobacco acids previously identified by Chuman[9] (benzoic, trans-cinnamic, 4-hydroxy and 3,4-dihydroxylated benzoic and cinnamic, azelaic and phenylacetic acid) were assayed, they produced no inhibition (data not shown). Fractionation of the leaf extract and the combined aqueous and organic smoke extracts yielded five and four fractions, respectively (Figures 3 and 1). The results of aromatase inhibition assays on the fractions are shown in Figures 1 and 3. The acid leaf fraction and the basic smoke fraction contained the highest inhibitory activity. As the results summarized in Table I indicate, the acid fraction of tobacco leaf showed a dependency on premixing time which suggests an irreversible inactivation of aromatase. On the other hand, change of premixing time had no effect on aromatase activity in either basic fraction, suggestive of a competitive type of inhibition (data not shown).

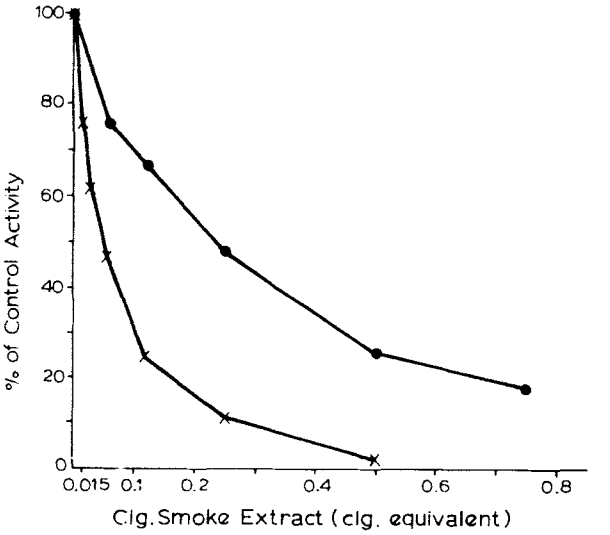


FIGURE 2 Inhibition of aromatase by cigarette smoke extract. The aqueous extract (o) and organic extract (x) are compared against the aromatase activity of the control. They demonstrate dose-dependent inhibiton.

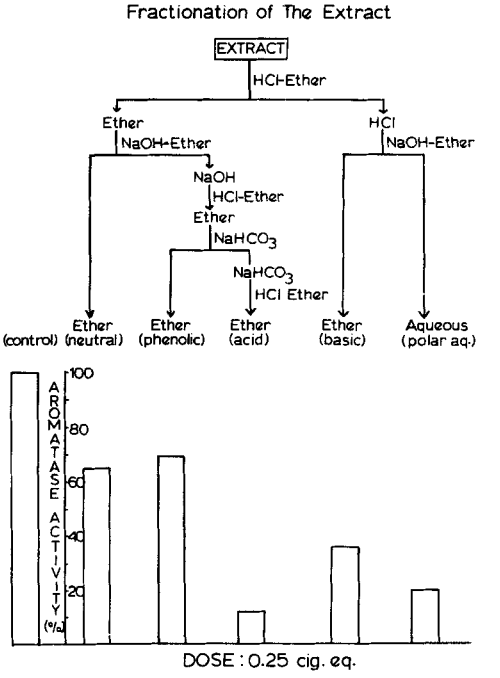


FIGURE 3 Flow chart of the fractionation of the extract of cigarette tobacco leaf. The bar graph represents the effect of an 0.25 cig. equiv. on aromatase activity, this is the average of triplicate determination.

TABLE I
Aromatase activity of the acid fraction with respect to premixing time.

	Aromatase Activity – % of Control			
Premixing Time (min)	0	10	30	60
Control	100	104	107	102
0.062 cig. equiv.	100	83	56	31
0.125 cig. equiv.	100	68	29	11
0.25 cig. equiv.	92	43	15	5

TLC of the basic smoke fraction in cyclohexane/ethyl acetate (8:1) and $CHCl_3$/MeOH (99:1) resulted in two active peaks (MT6, R_f0.6 and MT7, R_f0.7). In GC/MS analysis, the lower R_f area was composed of a mixture of more than 20 different compounds, while the area at R_f0.7 produced one main GC peak (Figure 4). The MS of this major peak gave a characteristic spectrum with probable molecular ion (M^+) at m/e 274, the base peak at m/e 70, and fragment ions at m/e 203, 190, 189, 175, 147, 130, 120, 106, and 98 (Figure 5). Acetylation with [^{14}C]acetic anhydride and pyridine and subsequent GC/MS analysis indicated the compound to be non-acetylatable, with a molecular formula $C_{17}H_{26}N_{20}$. The total mass spectra agreed with those cited in the literature[10] and with synthetic N-n-octanoylnornicotine.

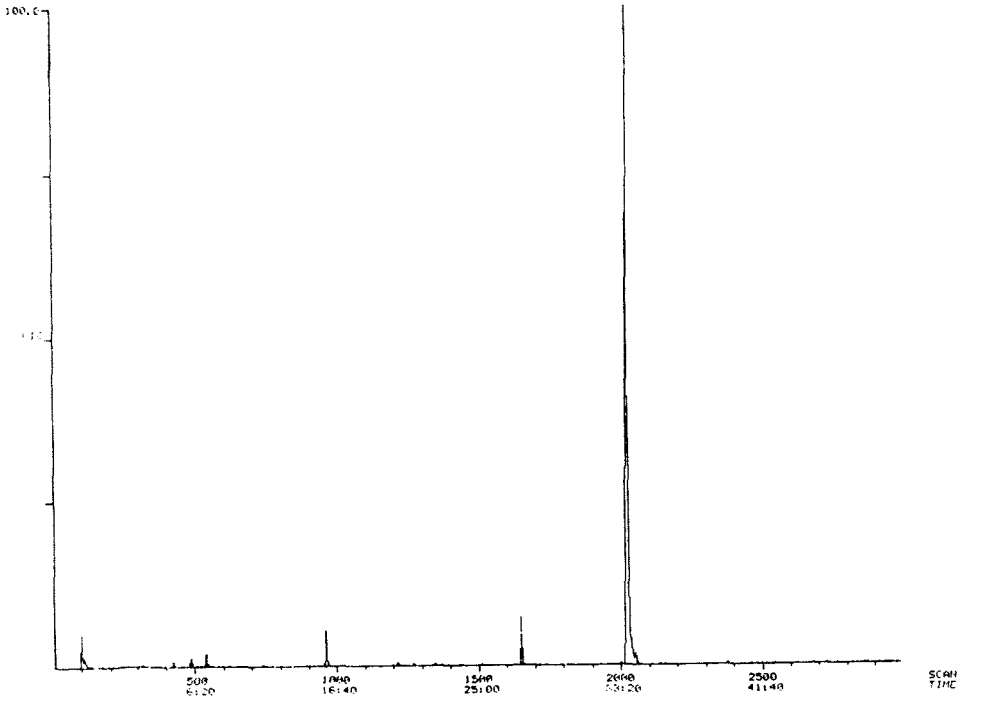


FIGURE 4 The Gas chromatographic spectrum of the active inhibitor from the cigarette smoke basic fraction (MT-7).

Results of the screening of synthetic acylated nornicotines and anabasines are presented in Table II and III, respectively. The acyl derivatization increased the activity considerably from that of the parent compounds. We observed correlation of inhibitory activity with the size of the acyl carbon chain, with a maximum activity at C-11 as shown in Figure 6 and 7 for the nornicotine and anabasine series. Over seventy-fold enhancement of the inhibitory activity of nicotine was observed in derivatives such as N-n-octanoyl (C-8), N-n-decanoyl (C-10), N-(4-hydroxy-n-undecanoly) (C-11), and N-(5-hydroxy-n-nonanoyl) (C-9) nornicotine derivatives. Over fifty-fold enhancement of the activity of anabasine was found in N-n-undecanoyl (C-11) and N-(4-hydroxyundecanoyl) anabasine (C-11) derivatives. When the size of the acyl group reached C-14, the activity decreased markedly.

The tobacco extracts were relatively highly toxic even after fractionation, and *in vivo* assessment of aromatase inhibition was not feasible. With the synthetic tobacco alkaloids, the acyl derivatives were found to be much less toxic than the parent alkaloid. The reported values for LD_{50} are 9.5 mg/kg (in mice) for nicotine[11] and 10 and 25 mg/kg (in rats) for anabasine[12] and nornicotine respectively.[13] Our preliminary tests showed lethal doses between 200–320, 400–600, 400–900, 600—800 and > 1300 mg/kg body weight for N-(4-hydroxy-n-undecanoyl)nornicotine, N-n-decanoylnornicotine, N-formylnornicotine, N-n-nonanoylanabasine, and N-(4-hydroxy-n-butyryl)nornicotine, respectively. We further assessed acute toxicity of

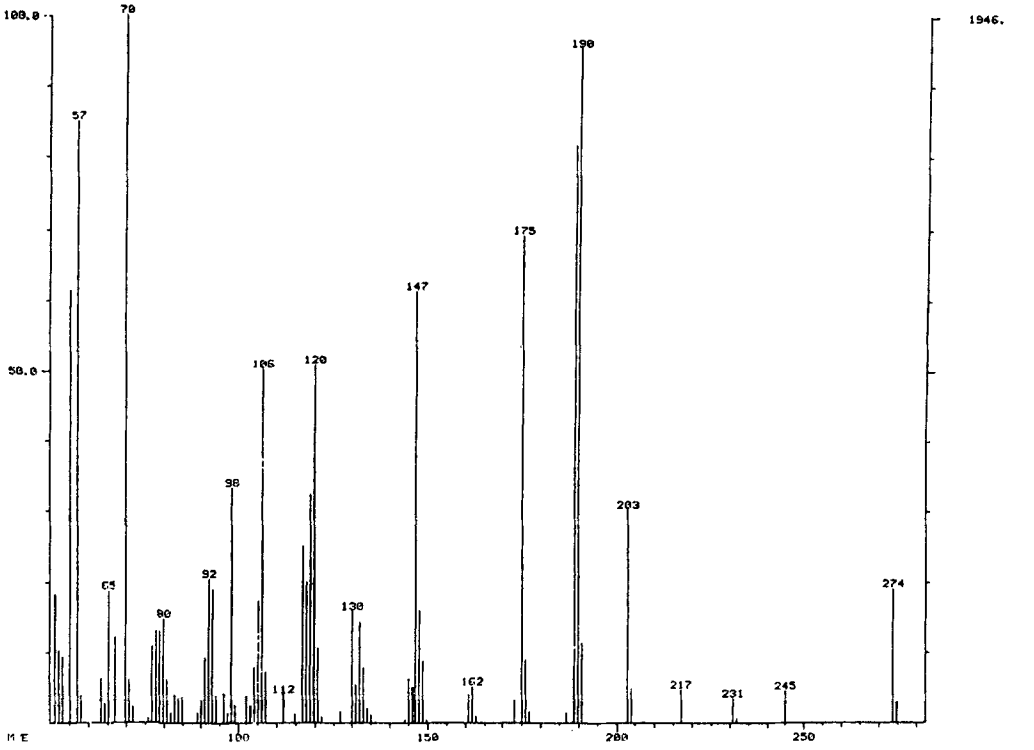


FIGURE 5 Mass spectrometry of the major component of the active inhibitor from cigarette smoke basic fraction (MT-7). Spectrum shows the characteristic M^+ ion at m/e 274.

N-n-octanoylnornicotine by Behrens' method[6] using 45 mice and 5 different dose levels and determined the LD_{50} to be 367 mg/kg body weight.

Administration of N-n-octanoylnornicotine to BUF/N rats with normal estrus cycles delayed the cycles by 3 to 4 days and the delay was repeated for at least three cycles (Figure 8). Treatment of BUF/N rats for one week at 50 mg/kg/day with N-n-octanoylnornicotine delayed occurrence of NMU-induced breast tumors by an average of two weeks. On the 70th day after the initial NMU injection, tumor incidence for the control group was 30% (3/10), 11% (1/9) for the ovariectomized group and 0% (0/9) for the N-n-octanoylnornicotine treated group. On the 90th day, tumor incidence was 60% for the control, 11% for the ovariectomized, and 33% for the inhibitor treated group.

An investigation of other common plant leaves was undertaken to check for similar inhibitory activity. Various vegetables, fruits and plant leaves were homogenized in 70% ethanol and soaked overnight. A second extraction was then carried out in 70% ethanol. An aliquot of the combined alcohol extracts was taken, evaporated under N_2 and assayed for aromatase inhibition. The results are given in Table IV. The inhibitory activity was expressed as units per 100 gm of raw material. A unit is defined as the dose required to achieve 50% inhibition of aromatase activity under the standard conditions. Many of the extracts which demonstrated high inhibitory activity were from green vegetables.

TABLE II
IC_{50} and Relative Potency of Nornicotine Derivatives as Aromatase Inhibitors.

Compound	$IC_{50}(\mu M)$	Relative Potency
nicotine	26000	1
nornicotine	no activity	–
anabasine	6600	4
N-formylnornicotine	980	27
N-n-butyrylnornicotine	480	54
N-n-octanoylnornicotine	360	72
N-(4-hydroxy-n-butyryl)nornicotine	650	40
N-n-decanoylnornicotine	260	100
N-myristoylnornicotine	> 5000	–
N-palmitoylnornicotine	> 5000	–
N-(4-oxo-valeryl)nornicotine	900	29
N-(3-hydroxy-n-butyryl)nornicotine	> 2000	< 13
N-(5-hydroxy-n-decanoyl)nornicotine	450	58
N-(5-hydroxy-n-nonanoyl)nornicotine	290	90
N-(5-hydroxyvaleryl)nornicotine	1100	24
N-(4-hydroxy-n-nonanoyl)nornicotine	600	43
N-(6-hydroxy-n-hexanoyl)nornicotine	2100	12
N-(4-hydroxy-n-undecanoyl)nornicotine	130	200
N-linoleylnornicotine	920	28
N-linolenylnornicotine	2100	12
N-(4-hydroxyvaleryl)nornicotine	580	45
N-(5-hydroxylauroyl)nornicotine	530	49

TABLE III
IC_{50} and Relative Potency of Anabasine Derivatives as Aromatase Inhibitors

Compound	$IC_{50}(\mu M)$	Relative Potency
anabasine	6600	4
N-valerylanabasine	240	28
N-octanoylanabasine	50	132
N-nonanoylanabasine	40	165
N-undecanoylanabasine	25	264
N-lauroylanabasine	50	132
N-(4-cyanobenzoyl)anabasine	100	66
N-(4-cyanocinnamoyl)anabasine	240	28
N-(4-hydroxyundecanoyl)anabasine	30	220
N-(4-acetoxyundecanoyl)anabasine	60	110
N-myristoylanabasine	4500	2
N-acetylanabasine	2000	3
N-(4-oxo-undecanoyl)anabasine	70	94
N-(bromoacetoxyundecanoyl)anabasine	105	63
Unknown anabasine derivative	30	220

DISCUSSION

Our study of tobacco inhibitors of aromatase began with the serendipitous discovery that cigarette smoke interfered with aromatase assays in our laboratory. This led to

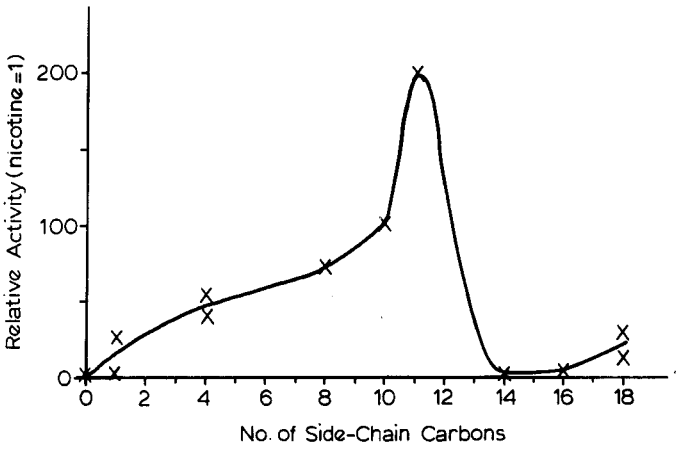


FIGURE 6 Structure–activity relationship of acylnornicotine derivatives. The relative activity of the increasing carbon chain derivatives is compared to that of nicotine.

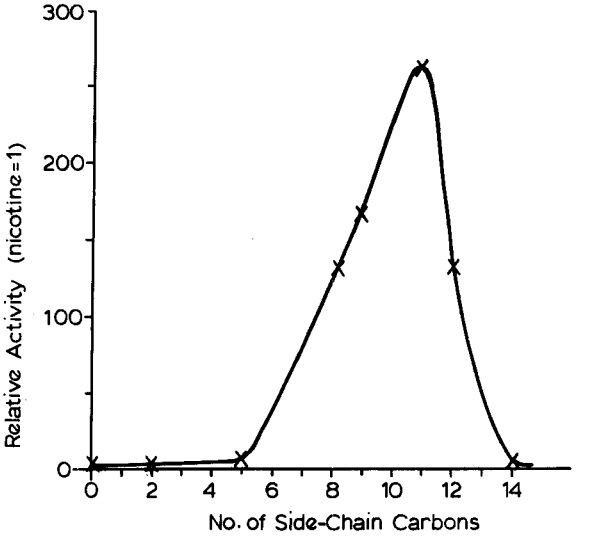


FIGURE 7 Structure-activity relationship of acylanabasine analogues. The relative activity of the increasing carbon chain derivatives is compared to that of nicotine.

the study of cigarette smoke and tobacco leaf extracts. The crude organic extract of cigarette smoke showed higher aromatase inhibition than the crude aqueous smoke extract suggesting the presence of a hydrophobic inhibitor. Nicotine and anabasine[14] had inhibitory activity which was weaker than that of our extract and therefore were eliminated from consideration as the active component. Cotinine, menthol, and benzo[a]pyrene produced no inhibition and were also eliminated from consideration.

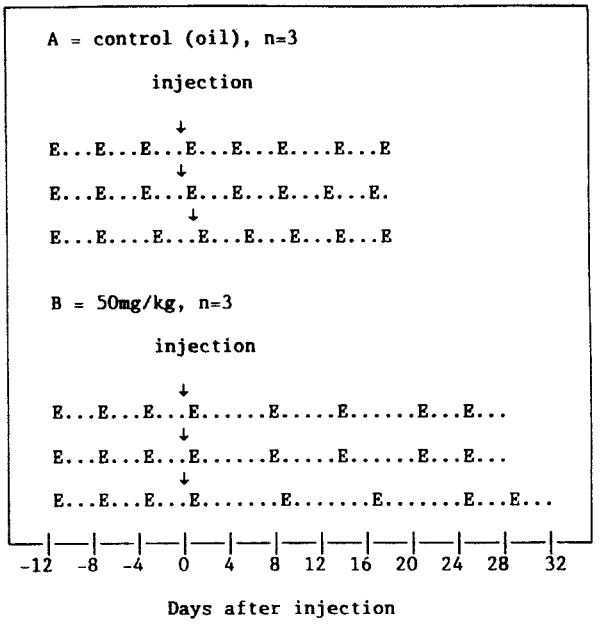


FIGURE 8 Effect of N-n-octanoylnornicotine on the estrus cycle of normal cycling BUF/N rats. *E* denotes estrus. A single injection of sesame oil (control) or N-n-octanoylnornicotine was given on day *O* (proestrus).

On fractionation, the basic fraction of the cigarette smoke extract demonstrated strong dose-dependent competitive inhibition. Purification of this fraction by TLC and GC/MS analysis led to the identification of the active component as N-n-octanoylnornicotine.

BUF/N rats with NMU-induced breast tumors were selected as a suitable animal model for human breast carcinoma.[15,16] Estrogen, progesterone, and prolactin receptors are present in the majority of these tumors so the majority of the NMU-induced rat tumors are hormone-dependent. This tumor model appears to be estradiol-dependent and thus, resembles human breast cancer more closely than DMBA (7,12-dimethylbenz[a]anthracene)-induced mammary tumors which are prolactin dependent. When rats, with the NMU-induction, were given N-n-octanoylnornicotine daily for one week, tumor incidence was delayed for an average of two weeks. In the ovariectomized rats, long term absence of tumors was observed. Since tobacco alkaloid aromatase inhibitors are reversible, competitive inhibitors, we did not expect the effect to last so long. We can assume that continuous administration of the inhibitor would produce long term results. A single dose of the compound altered the four day estrus cycle to a 7–8 day cycle continuing for a 20 to 24 day period, after which it returned to normal. These results suggest that tobacco alkaloid derivatives affect endocrine function *in vivo* through the suppression of the aromatase enzyme system. We found similar inhibitory activity in other plant leaves such as eggplant, potato, and tomato, and in some popular green vegetables such as kale, romaine lettuce, spinach, and asparagus. It is quite possible that such naturally-occurring aromatase inhibitors affect animal and human endocrine function through their effect

TABLE IV
Aromatase Inhibitory Activity in Plant Extracts.

Extract[a]	Units/ 100 gm[b]	Extract[a]	Units/ 100 gm[b]
Tea	27,000	Apples	< 80[c]
Coffee	13,000	Banana	0
Cocoa	9,000	Potato	0
Collards	8,500	Cauliflower	0
Tomato Leaves	6,000	Orange	0
Kale	4,700	Ginger root	0
Potato leaves	4,500	Carrot	0
Broccoli leaves	3,600	Iceberg lettuce	0
Dandelion greens	2,900	Pea	0
Pepper leaves	2,800	Cabbage	0
Mustard greens	2,700	Celery	0
Spinach	2,400	Tomato	0
Asparagus	1,300	Cucumber	0
Parsley	1,200	White onions	< 0
Endive	850	Green onions	0
Escarole	830	Cantaloupe	0
Eggplant leaves	800	Plums	0
Lemons	660	Turnips	0
Tobacco Leaves	590	Seedless grapes	0
Romaine lettuce	560	Beet greens	0
Peppers	330	Broccoli	0
Spanish onions	310	Avocado meat	0
Eggplant	190	Chocolate	0

[a] Plant leaves and vegetables were homogenized in approximately 70% ethanol in a Waring blender and kept overnight at room temperature. The extract was filtered and the insoluble part was re-extracted with 70% ethanol. Several aliquots of the combined extract were taken for the inhibition assay with 30 min premixing in the presence of NADPH.

[b] One unit is defined as the dose required for 50% inhibition (ID_{50}) of aromatase under our standard conditions. The inhibitory activity is expressed as Units per 100 gm wet weight of green leaf or vegetable. The ID_{50} was taken directly from the observed dose responsive curve. When the maximum inhibition was less than 20% it was classified to be inactive (0).

[c] The inhibitory activity was not strong enough to suppress to the 50% level within the measured range, though dose responsive inhibition was observed. The dose required for 50% inhibition was extrapolated on the graph and the total activity was estimated.

on regulation of estrogen production. A study of aromatase in Oriental herb medicines may also show an interesting correlation.

Lower incidence of endometrial cancer has been reported in women who smoke.[17] Since this cancer is estrogen-dependent, smoking may affect its development by aromatase inhibition. It is known that menopause occurs an average of a year and a half earlier in smokers than in non-smokers.[18] This phenomenon could be due to the effect of aromatase inhibitors in the cigarette smoke.

Neither acylnornicotines nor acylanabasines have any apparent structural resemblance to natural androgen substrates. However, they seem to compete with each other at the active site of aromatase, and therefore more detailed kinetic studies are desirable. Some of the hydroxyacyl and bromoacetoxyacyl derivatives reported in this paper have potent inhibitory activities. This offers an opportunity to alkylate the aromatase inhibition site with a radioisotope-labeled group and to elucidate the topography of inhibition and catalytic sites and determine the correlation between them.

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