2-(β-D-Glucopyranosyloxy)-3-(3-methyl-2-butenyl)-1,4-naphthoquinone (4). A suspension of the foregoing tetraacetate (0.0007 mol) in dry methanol (5 ml) was treated with 2 *N* sodium methoxide (0.5 ml), agitated until complete solution occurred, and kept a further 12 hr in the refrigerator. Water was added; the product was extracted with ethyl acetate and chromatographed over silica gel with EtOAc–MeOH, giving 2-(β-D-glucopyranosyloxy)-3-(3-methyl-2-butenyl)-1,4-naphthoquinone (4) (0.0006 mol, 85.71%): ν_{max} (KBr) 3400 (hydroxy), 1630 and 1660 cm^{-1} (quinone); mass spectrum (RMU-7MG Hitachi) *m/e* 242 (17, lapachol fragment), 163 (3.1, glucose fragment). Anal. Calcd for $C_{21}H_{24}O_8 \cdot H_2O$: C, 59.65; H, 6.20. Found: C, 59.22; H, 5.84.

This glycoside gave lapachol and β-lapachone[6] on acid (2 *N* H_2SO_4–MeOH, 1:1) hydrolysis.

β-D-Glucopyranose Pentaacetate (6). This compound was obtained from β-D-glucopyranose by the usual way: crystallized from ethanol; mp 130–131.5°.

Biological Assay. (a) Leukemia P-388. The procedure of the Cancer Chemotherapy National Service Center[8] was used. BDF_1 mice from the cross of female C57BL/6 and male DBA/2 were inoculated with 10^6 viable P-388 lymphocytic leukemia cells. The control groups received physiological saline containing 1.2% Tween 80 and the test groups received the drug under investigation emulsified with 1.2% Tween 80. The increase of survival time of the treated group over controls was determined, a value equal to or exceeding 25% being considered demonstrative of activity. The results are shown in Table I.

(b) Walker 256. The National Cancer Institute procedure[9] was used. Wistar rats were inoculated intramuscularly with Walker 256 tumor cells. Treatment of animals with the drugs began the third day after implantation. Activity was measured as tumor weight. The degree of inhibition was calculated as 100 − 100 T/C, where T and C are the mean tumor weight from treated and control animals, respectively.

A value of 58% or above is statistically significant antitumor activity. The results are summarized in Table II.

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Nonclassical Nicotine Antagonists[1]

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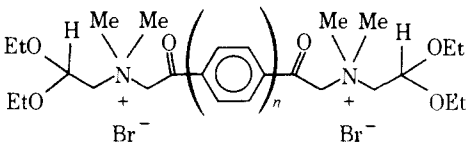
A series of "nonclassical" nicotine antagonists was synthesized and compared to the "classical" nicotine antagonist, hexamethonium, by means of the isolated guinea pig atria preparation. **2** was found to be the most potent, followed by hexamethonium and the other antagonists. With the exception of **5**, the bisquaternary compounds **1–3** and **7–9** were found to be more potent than the monoquaternary compounds **4**, **6**, and **10–12**. Within a series of compounds (**1–6** or **7–12**), those compounds possessing two phenyl rings proved to be more potent than those possessing one or three phenyl rings. These and other aspects of the structure–activity relationship of this class of compounds are discussed.

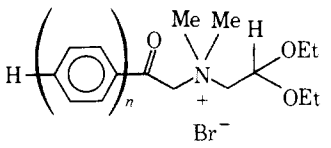
In light of nicotine's role[2] in elevating the blood pressure and increasing the heart rate of smokers, a drug which could block this pressor effect of nicotine may safeguard smokers from cardiovascular damage. Unfortunately, "classical" nicotine antagonists, such as hexamethonium, tetraethylammonium, and triethylcholine, also inhibit normal ganglionic transmission at doses which block nicotine. A "nonclassical" nicotine antagonist was discovered by Wong and Long[3] in 1967 and has been studied extensively. This compound, 4,4′-bis[*N*-(2,2-diethoxyethyl)-*N*,*N*-dimethylammonioacetyl]biphenyl dibromide (**2**), given the trivial name DMAE in the pharmacological literature, was found to have the ability to completely antagonize the pressor effects of nicotine at dose levels too low to affect ganglionic transmission.[4,5] Because **2** did exhibit neuromuscular blockade as well as catecholamine potentiation,[6] its structure was altered to form 4,4′-bis[*N*,*N*-bis(2-ethoxyethyl)-*N*-methylammonioacetyl]biphenyl dibromide (**8**), given the trivial name DEO in the pharmacological literature. **8** was found to be devoid of catecholamine potentiation, but it did exhibit some neuromuscular and ganglionic blockade as well as a drastically reduced duration of action.[7] Because **8** and two other congeners of **2** (dibenzofuran and *p*-terphenyl) exhibited different potencies and spectra of activity from **2**, it was felt that a detailed structure–activity relationship study of this class of compounds was in order.[8]

The structure–activity relationship study has two potential goals: (1) the determination of the molecular parameters needed to maximize the "nonclassical" antagonism of nicotine and (2) the further elucidation of the site of action of nicotine in the adrenergic nervous system.

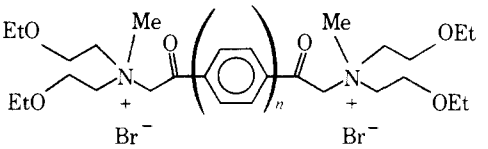
Chemistry. As shown in Schemes I and II, all the compounds **1–12** were prepared by a Hofmann alkylation procedure involving the addition of the desired amine (**19** or



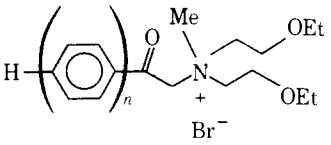
1, $n = 1$ (monophenyl DMAE)
2. $n = 2$ (DMAE)
3. $n = 3$ (terphenyl DMAE)



4. $n = 1$ (half DMAE)
5. $n = 2$ (phenyl half DMAE)
6. $n = 3$ (biphenyl half DMAE)



7. $n = 1$ (monophenyl DEO)
8. $n = 2$ (DEO)
9. $n = 3$ (terphenyl DEO)



10. $n = 1$ (half DEO)
11. $n = 2$ (phenyl half DEO)
12. $n = 3$ (biphenyl half DEO)

20) to the appropriate bromo or dibromo intermediate (**13–18**) in cyclohexanone. *N*-(2,2-Diethoxyethyl)-*N*,*N*-dimethylamine (**19**) is commercially available. *N*,*N*-Bis(2-ethoxyethyl)-*N*-methylamine (**20**) was prepared from *N*,*N*-bis(2-ethoxyethyl)amine by means of the Eschweiler–Clarke procedure[9] involving formaldehyde, formic acid, and hydrochloric acid.

Dibromo intermediate **13** was prepared[10] by bromination of 1,4-diacetylbenzene with bromine in glacial acetic acid. Dibromo intermediates **14** and **15** were prepared by Friedel–Crafts acylation of biphenyl and *p*-terphenyl, respectively, with 2 equiv of bromoacetyl bromide and aluminum chloride in carbon disulfide.

Bromo intermediate **16** is commercially available. Bromo intermediates **17** and **18** were prepared by careful addition of 1 equiv of premixed bromoacetyl bromide and aluminum chloride in methylene chloride to biphenyl or *p*-terphenyl, respectively, in methylene chloride. Even with this procedure, some dibromo impurity was found via thin-layer chromatography during the preparation of **18**; therefore, column chromatography was used to purify **6** and **12** (Table I).

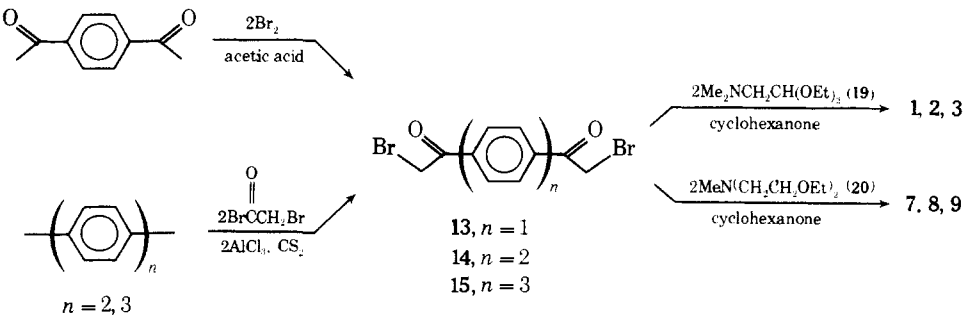
Experimental Section

For the compounds which did not decompose upon reaching their melting points, the melting points were taken on a Mel-Temp hot block apparatus and are corrected. For those compounds which did decompose, the melting points were taken on a Dupont Model 900 differential thermal analyzer and are corrected. Infrared spectra were recorded on a Perkin-Elmer 367 spectrophotometer. Nuclear magnetic resonance spectra were recorded on a Varian Associates T-60 spectrophotometer using tetramethylsilane as an internal standard. Elemental analyses were performed on compounds **1–20** by the Division of Medicinal Chemistry and Natural Products and the Department of Chemistry of the University of Iowa and Midwest Microlab, Ltd. Detailed experimental procedures are given for only a representative number of compounds.

***N*,*N*-Bis(2-ethoxyethyl)-*N*-methylamine (20).** A solution of 20 g (0.25 mol) of 37% formaldehyde in 20 g (0.40 mol) of 90% formic acid was slowly added to 16.1 g (0.10 mol) of *N*,*N*-bis(2-ethoxyethyl)amine in a 200-ml flask. The solution was stirred on the steam bath for 5 hr, then 12 ml (0.15 mol) of concentrated HCl was added through the condenser, and the solution was stirred for an additional 3 hr. The solution was cooled, made strongly basic with 100 ml (0.50 mol) of 5 *M* NaOH, then extracted into three 150-ml portions of ether, and finally dried over $MgSO_4$ overnight. The ether was distilled and the residual oil distilled at 43–47° (0.40 Torr) to yield 12.6 g (73%) of water-white amine. Anal. ($C_9H_{21}NO_2$) C, H, N.

1,4-Bis(bromoacetyl)benzene (13). To a solution of 1.60 g (0.55 ml, 10.0 mmol) of bromine in 5 ml of glacial acetic acid was added a solution of 0.81 g (5.0 mmol) of 1,4-diacetylbenzene in 5

Scheme I



Scheme II

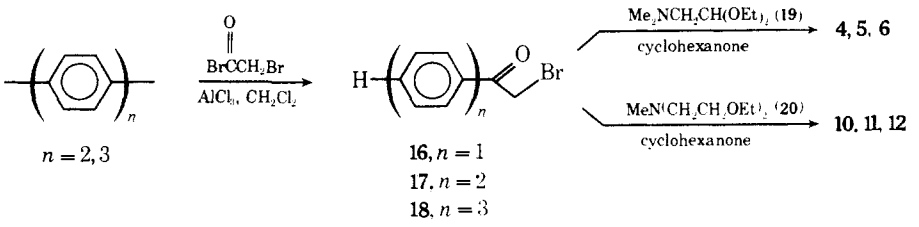
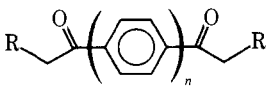


Table I. Nicotine Antagonists and Intermediates



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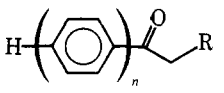

II

Compd	Type	n	R	Mp,[a] °C	Crystn solvent[b]	Yield, %	Formula[c]
1	I	1	$(EtO)_2CHCH_2N^+(CH_3)_2Br^-$	172–179 dec	A	56	$C_{26}H_{46}Br_2N_2O_6$
2	I	2	$(EtO)_2CHCH_2N^+(CH_3)_2Br^-$	181–186 dec[d]	B	63	$C_{32}H_{50}Br_2N_2O_6$
3	I	3	$(EtO)_2CHCH_2N^+(CH_3)_2Br^-$	200–207 dec[e]	C	63	$C_{38}H_{54}Br_2N_2O_6$
4	II	1	$(EtO)_2CHCH_2N^+(CH_3)_2Br^-$	168–170 dec	A	74	$C_{16}H_{26}BrNO_3$
5	II	2	$(EtO)_2CHCH_2N^+(CH_3)_2Br^-$	149–153 dec	B	68	$C_{22}H_{30}BrNO_3$
6	II	3	$(EtO)_2CHCH_2N^+(CH_3)_2Br^-$	167–172 dec	A	50	$C_{28}H_{34}BrNO_3$
7	I	1	$(EtOCH_2CH_2)_2N^+CH_3Br^-$	187–196 dec	C	77	$C_{28}H_{50}Br_2N_2O_6$
8	I	2	$(EtOCH_2CH_2)_2N^+CH_3Br^-$	196–205 dec	C	53	$C_{34}H_{54}Br_2N_2O_6$
9	I	3	$(EtOCH_2CH_2)_2N^+CH_3Br^-$	195–205 dec	C	43	$C_{40}H_{58}Br_2N_2O_6$
10	II	1	$(EtOCH_2CH_2)_2N^+CH_3Br^-$	129–131	A	64	$C_{17}H_{28}BrNO_3$
11	II	2	$(EtOCH_2CH_2)_2N^+CH_3Br^-$	188–191 dec	A	60	$C_{23}H_{32}BrNO_3$
12	II	3	$(EtOCH_2CH_2)_2N^+CH_3Br^-$	172–179 dec	A	65	$C_{29}H_{36}BrNO_3$
13	I	1	Br	173–176[f]	D	60	
14	I	2	Br	225–229 dec[g]	E	46	
15	I	3	Br	208–211 dec[h]	F	52	
17	II	2	Br	121–124[i]	G	71	
18	II	3	Br	182–185	H	63	$C_{20}H_{15}BrO$

[a]See Experimental Section. [b]A, $CHCl_3$–hexane; B, 2-propanol–heptane; C, 2-propanol–H_2O; D, toluene; E, 2-butanone; F, cyclohexanone; G, DMF–H_2O. [c]All compounds analyzed for C, H, and N (18 analyzed for C and H) were within ±0.4% of theory. [d]Lit.[4] mp 164–166°. [e]Lit.[8] mp 197° dec. [f]Lit.[10] mp 177–178°. [g]Lit.[15] mp 226–227°. [h]Lit.[11] mp 220–222°. [i]Lit.[16] mp 124–125°.

ml of glacial acetic acid. The solution was allowed to stand for 8 hr and then filtered, and the resultant crystals were washed with water and dried. Crystallization from toluene afforded 0.96 g (60%) of light yellow plates melting at 173–176° (lit.[10] mp 177–178°).

4,4″-Bis(bromoacetyl)-*p*-terphenyl (15). By means of a solid particle adding device, 11.5 g (0.050 mol) of *p*-terphenyl was added, during 5 min, to a stirred solution of 30.3 g (13.1 ml, 0.150 mol) of bromoacetyl bromide and 26.6 g (0.200 mol) of aluminum chloride in 100 ml of carbon disulfide in a 500-ml flask outfitted with a condenser, drying tube, and stirring motor. The solution was stirred at reflux for 1 hr and then cooled, the carbon disulfide decanted, and the sludgy aluminum chloride complex poured into 1 l. of ice water. The complex was stirred vigorously in the ice water, filtered, washed well with hot water, and allowed to air dry overnight. The brownish powder was crystallized from 200 ml of boiling cyclohexanone to yield 12.3 g (52%) of pale yellow plates melting at 208–211° dec (lit.[11] mp 220–222°).

4-(Bromoacetyl)-*p*-terphenyl (18). A filtered solution of 2.02 g (0.87 ml, 0.010 mol) of bromoacetyl bromide and 1.33 g (0.010 mol) of aluminum chloride in 40 ml of methylene chloride was added, during 3.5 hr, to a stirred, refluxed solution of 2.30 g (0.010 mol) of *p*-terphenyl in 40 ml of methylene chloride in a 200-ml flask outfitted with an addition funnel, condenser, drying tube, and magnetic stirring bar. The solution was refluxed an additional 0.5 hr and then cooled, and ice water was added. The methylene chloride layer was separated from the water layer and washed with water, and the methylene chloride was removed in vacuo. The resultant crude material was crystallized from toluene to yield 2.22 g (63%) of pale yellow plates melting at 182–185°. Anal. ($C_{20}H_{15}BrO$) C, H.

4,4″-Bis[*N*-(2,2-diethoxyethyl)-*N*,*N*-dimethylammonioacetyl]-*p*-terphenyl Dibromide (3). A solution of 1.29 g (8.0 mmol) of 19 and 1.89 g (4.0 mmol) of 15 in 50 ml of cyclohexanone was stirred at 60° for 0.5 hr. The resultant precipitate was filtered, washed with cold cyclohexanone and then ether, and crystallized from 2-propanol–water to yield 2.00 g (63%) of white powder melting at 200–207° dec (lit.[8] mp 197° dec). Anal. ($C_{38}H_{54}Br_2N_2O_6$) C, H, N.

Pharmacological Evaluation. Guinea pigs (300–600 g) were killed by a blow on the head and the hearts were removed rapidly and placed in oxygenated Feigan's solution. Both atria were dissected free from ventricular tissue and mounted in a 20-ml organ bath consisting of Feigan's solution maintained at 32°. The atrial rate was recorded on a Beckman cardiotachometer modified to record 60–300 beats/min. The antagonists, including hexamethonium bromide, was dissolved in distilled water and selected doses were administered to the organ bath. After 2 min had elapsed, 20 μg/ml of nicotine bitartrate was administered and the increase in atrial rate was recorded and compared to the increase caused by nicotine alone. The data were evaluated using a split plot design six-point bioassay by Finney.[12]

Table II. Effect of Nicotine Antagonists on the Chronotropic Responses Induced by Nicotine on Isolated Guinea Pig Atria

Antagonist	No. of atria	I_{50}, μM[a]	Potency ratio[b]
2	6	0.13	1.00
Hexamethonium	11	0.18	0.85 (0.43–1.67)
3	6	0.25	0.82 (0.58–1.10)
5	7	0.25	0.59 (0.41–0.83)
8	6	0.15	0.54 (0.47–0.61)
1	5	0.70	0.27 (0.19–0.38)
9	7	0.30	0.26 (0.19–0.36)
7	6	0.50	0.18 (0.12–0.26)
11	5	1.03	0.16 (0.13–0.20)
4	8	5.50	0.03 (0.02–0.04)
10	5	4.80	0.02 (0.01–0.03)
6	5	25.40	<0.01
12	5	>60	<0.01

[a]Molar equivalents of drug that reduces the nicotine caused increase in atrial rate by 50%. [b]Relative potency with 95% confidence intervals in reference to 2.

Results and Discussion

The results of the guinea pig atria study are shown in Table II. 2 is the most potent, followed by hexamethonium and the other antagonists. The half molecules, with the exception of 5 and 11, show very low potency. 12 had insufficient solubility to measure its I_{50}.

Three points concerning structure–activity relationship of these compounds can be made from these data: (1) the DMAE (**2**) series of compounds is consistently more potent than the DEO (**8**) series; (2) within a series, the bisquaternary compounds are consistently more potent than their monoquaternary counterparts; and (3) compounds possessing two phenyl rings are consistently more potent than those containing either one or three phenyl rings.

The DEO analogs appear to be less potent than the DMAE analogs due to the presence of ethoxyethyl rather than diethylacetal moieties. DEO itself also has a much shorter duration of action than DMAE, apparently the result of decreased affinity for its receptor.[7]

Just as hexamethonium is more potent than tetraethylammonium as a ganglionic blocker due to two-point rather than one-point binding,[13] probably these bisquaternary compounds are more potent than their monoquaternary counterparts for the same reason. The two- vs. one-point binding concept may be true even though the site of action within this atria preparation appears to be the adrenergic terminal rather than the ganglia.[14]

The greater potency of compounds with two phenyl rings over those possessing one or three rings may reflect the fact that this rigidly held intercationic distance of 14 Å is an ideal distance for binding. The 14-Å distance is a significant feature of neuromuscular blocking activity,[13] but its relevance in this study is not yet known. Because **5** and **11** are also fairly potent without the benefit of a second cationic head, the importance of the second phenyl ring itself must not be overlooked. This ring could be involved in binding or in blocking nicotine's approach to its receptor.

If this second ring is important, then one must account for the very low potencies of **6** and **12**. The third phenyl ring of **6** and **12** may be repulsed from the receptor surface by steric or electronic factors. However, **3** and **9** are again active because the second cationic head overcomes this repulsion by binding to the receptor surface. This would, of course, necessitate the presence of binding sites 18 Å apart in addition to those 14 Å apart. Further studies are needed to confirm or deny the presence of these binding sites.

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Methyl 5(6)-Phenylsulfinyl-2-benzimidazolecarbamate, a New, Potent Anthelmintic[1]

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The preparation and anthelmintic properties of methyl 5(6)-phenylsulfinyl-2-benzimidazolecarbamate are described. It is effective at a dose of 10 mg/kg po against gastrointestinal nematodes in horses and at 5 mg/kg po or less against gastrointestinal nematodes and lungworms in cattle, sheep, and swine.

Benzimidazolecarbamates with anthelmintic activity have been reported by several groups of investigators. Alkyl[2] and benzoyl[3] substituents, for example, enhance this activity. During the course of our investigations we have found, as have others,[4,5] that benzimidazole-5(6) ethers and thioethers possess high activity against intestinal nematodes in laboratory animals. In addition, we have found that the 5(6)-phenylsulfinyl substituent confers particular-