Synthesis and Structure–Toxicity Relationships of Three New Stable Analogues of Acetyl-*seco*-hemicholinium-3

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In order to develop and study inhibitors of neuromuscular function which act presynaptically, three stable analogues of acetyl-*seco*-hemicholinium-3 (AcHC-3, **2**) were prepared. These analogues have 2-ethoxyethyltrimethylammonium, 4-oxopentyltrimethylammonium, and *n*-pentyltrimethylammonium moieties substituted for the 2-acetylethyltrimethylammonium (acetylcholine) moieties of AcHC-3 (**2**) to form the ether **3**, ketone **4**, and alkane **5** analogues of AcHC-3 (**2**). Although AcHC-3 (**2**) has been shown to undergo deesterification rapidly in basic solutions and slowly at pH 7.4, it has been found to be stable in H_2O or D_2O under slightly acidic conditions. All of the analogues are stable for extended time under both slightly acidic conditions and at pH 7.4 in H_2O or D_2O. It has been found that **2** reacts with acetylcholinesterase and butyrylcholinesterase within seconds in H_2O at pH 7.4. However, deesterification of **2** with subsequent cyclization to the hemiacetal form of hemicholinium-3 (HC-3, **1**) is prevented at pH 7.4, possibly by an irreversible binding of **2** to the enzyme. The analogues **3–5**, however, do not react under identical conditions. Mouse toxicity studies (LD_{50}) indicate that **2** is approximately as toxic as HC-3 (**1**), whereas **3**, **4**, and **5** are 14.2, 23.8, and 43.1 times less toxic, respectively. The toxic effects of **3–5**, like **1** and **2**, are antagonized by choline but not by neostigmine in mice. Structure–activity relationships of **1–5** are discussed.

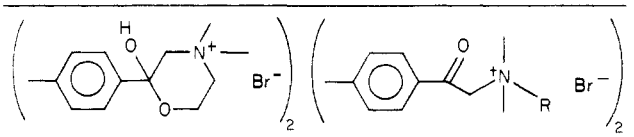
Hemicholinium-3 (HC-3, **1**) is a prototypical agent used to demonstrate prejunctional inhibition in neuromuscular preparations. Although it is initially synthesized as the seco (open ring) form, it rapidly undergoes cyclization on solution in water to form the hemiacetal (closed ring) form.[2] In order to hinder ring closure, the acetate of the seco form of HC-3 (**1**), acetyl-*seco*-hemicholinium-3 (AcHC-3, **2**), was synthesized.[3] Both **1** and **2** produce a slow depression of neuromuscular function and also inhibition of cholinesterase at high concentrations. However, unlike HC-3 (**1**), AcHC-3 (**2**) has been shown to be a potent inhibitor of choline acetylase both in vitro[4] and in vivo.[5] AcHC-3 (**2**) is also a parasympathomimetic and inhibits reuptake of neuronally released catecholamines,[6] a property similar to one exhibited by α,α'-bis(dimethylammonioacetaldehyde diethylacetal) p,p'-diacetylbiphenyl dibromide, DMAE.[7]

Since AcHC-3 (**2**) slowly undergoes hydrolysis with subsequent cyclization to form HC-3 (**1**) on exposure to water at pH 7.4 and rapidly under basic conditions, the probability of an aqueous solution of **2** existing as both the seco derivative **2** and the deacetylated hemiacetal **1** or totally as **1** is quite large. It is thus possible that the actions of a seco derivative of HC-3 (**1**) could be better demonstrated utilizing a stable seco derivative. To investigate this the well-known similarities between acetylcholine and its ether, ketone, and alkane analogues[8] were used as the premise for preparing the corresponding ether **3**, ketone **4**, and alkane **5** analogues of AcHC-3 (**2**). These three compounds were expected a priori to be stable in aqueous solution. The structures of the compounds in question are presented in Table I.

Herein we describe the synthesis, chemistry, and preliminary biological evaluation of **3–5** and compare them to **1** and **2**. Structure–activity relationships of **1–5** are also discussed. Further biological evaluation of all three compounds for cardiovascular function, inhibition of neuromuscular function, and cholinesterase and choline acetylase inhibition is in progress and will be reported subsequently.

Chemistry. The three analogues of AcHC-3 (**2**) were synthesized as the dibromide salts by the reaction of α,α'-dibromo-4,4'-biacetophenone (**6**), prepared by the method of Long and Schueler,[2] with the appropriate amines. The dimethylamino-2-ethoxyethane (**7**) required for the synthesis of the ether analogue **3** was synthesized by a modified method of Grail et al.[9] from sodium ethoxide and 2-dimethylaminoethyl chloride hydrochloride. The amine, 4-oxopentyldimethylamine (**8**), utilized in the preparation of the keto analogue **4** was commercially available from Aldrich Chemical Co., Inc. Reductive alkylation of *n*-pentylamine with formaldehyde and formic acid was utilized to synthesize 1-dimethylaminopentane (**9**), the amine used in the synthesis of the alkyl analogue **5**.

Table I. Structures of Compounds 1-5



1 2-5

Compd no.	Type of substituent	R
2	Acetate	$-CH_2CH_2OCOCH_3$
3	Ether	$-CH_2CH_2OCH_2CH_3$
4	Ketone	$-CH_2CH_2CH_2COCH_3$
5	Alkane	$-CH_2CH_2CH_2CH_2CH_3$

The structures of **3–5** were determined on the basis of the method of synthesis, satisfactory combustion elemental analysis data, and ^1H NMR, ir, and uv spectral data. The ^1H NMR spectrum of each of the analogues as well as the parent compound **2** exhibits a characteristic 8 H aromatic signal centered in the region of δ 7.75–7.79. Similarly, **2–5** have a characteristic 12 H nitrogen methyl singlet located between δ 3.30 and 3.40 depending upon the compound under observation. The spectra of compounds **2–5** also display signals integrating for 12-H between δ 3.20 and 4.20 for the methylenes adjacent to the nitrogen and carbonyl group, adjacent to the nitrogen and insulated from the carbonyl group, and one carbon removed from the nitrogen. The similarity of these portions of the spectra serves to support the conclusion that **2–5** are, in fact, analogues of each other. The ^1H NMR signals for the three terminal chain atoms of **2** and **3**, the terminal chain atom of **4**, and five terminal chain atoms of **5** serve to distinguish among the four compounds under study. A characteristic 6 H singlet at δ 1.60 observed in the spectrum of **2** serves to confirm that **2** is, in fact, the acetyl-*seco* analogue of HC-3 (**1**). The ether analogue **3** exhibits a characteristic ethoxy pattern of a 6 H triplet (J = 7.0 Hz) at δ 0.62 and a 4 H quartet (J = 7.0 Hz) at δ 3.25. The ketone **4** exhibits a characteristic 6 H methyl singlet at δ 2.10 while the aliphatic analogue **5** displays three overlapping multiplets with a total of 22 H between δ 0.65 and 1.91.

In addition, structural support is derived from the ir spectra of **3–5**.[10] The similarities of the three analogues

Table II. Mouse Toxicity Studies

Compd	LD_{50}, mg/kg ip	95% confidence limits	Ratio of LD_{50} doses to that of HC-3 (1)
HC-3 (1)	0.13[a]	0.11-0.17[a]	1.0
AcHC-3 (2)	0.125[a]	*a*	1.0
Ether 3	1.84	1.72-1.98	14.2
Ketone 4	3.1	2.95-3.25	23.8
Alkane 5	5.6	5.54-5.65	43.1

[a] See ref 6.

of AcHC-3 (2) are evident from the presence of signals in the ir spectrum of each for phenyl carbonyl between 1703 and 1694 cm^{-1}, phenyl between 1609 and 1607 cm^{-1}, carbonyl methylene between 1405 and 1403 cm^{-1}, *p*-phenyl between 821 and 813 cm^{-1}, and amine between 594 and 590 cm^{-1}. The presence of a characteristic ether signal at 1120 cm^{-1} with 3 and a characteristic second carbonyl signal at 1717 cm^{-1} with 4, as well as the absence of further distinguishing signals with 5, serves further to distinguish among 3–5. Also, uv data support the assignment of similar central structure to AcHC-3 (2) and the three AcHC-3 (2) analogues. Each of the compounds exhibits a λ_{max} (H_2O) between 304 and 310 nm with an ϵ_{max} between 31000 and 37000.

The ether 3, ketone 4, and alkane 5 analogues of AcHC-3 (2) are stable in H_2O or D_2O solutions under slightly acidic conditions or at pH 7.4 for at least 48 h. They also do not react in vitro in the presence of aqueous pH 7.4 solutions of acetylcholinesterase and butyrylcholinesterase with activities equivalent to those found in blood, i.e., approximately 4 μ/ml. AcHC-3 (2) reacts immediately with these enzymes under identical conditions. Contrary to expectations the hemiacetal form of HC-3 (1) is not produced even after several days. It is possible that 2 undergoes deesterification to the seco form of HC-3 (1) but subsequent cyclization to the hemiacetal form is prevented by binding to the enzyme at pH 7.4. However, it has previously been shown, utilizing a Warburg procedure, that 2 does not produce free acetic acid in the presence of these enzymes.[6] The fact that interaction of 2 with the enzyme does occur is shown by the shift of the λ_{max} (H_2O, pH 7.4) of 2 toward shorter wavelength from 307 to 290 nm. No such interaction is seen with 3–5. Thus, it is quite possible that deesterification of 2 is prevented in some manner by an irreversible binding to the enzyme at pH 7.4 and that the species observed is some type of enzyme–AcHC-3 complex. Similar reactions of 2 in vivo might be expected. Although AcHC-3 (2) was found to undergo slow deesterification with subsequent cyclization to the hemiacetal form of HC-3 (1) at pH 7.4 and 37°,[6] it was found by ^1H NMR spectral studies to be stable in D_2O solvent under slightly acidic conditions at ambient and elevated temperatures for extended periods of time, i.e., <0.5% decomposition in D_2O solvent upon standing for 21 h at ambient temperature, followed by warming to 65 °C for 5.5 h.

Pharmacology and Initial Structure–Activity Relationships. Table II summarizes LD_{50} studies in Charles River CD-1 adult male albino mice (20–30 g). Doses of the respective compounds in normal saline (0.9% NaCl) were injected ip. The animals which died began to show effects within 5 min or less after injection. These effects included exophthalmos, mild to moderate ataxia, respiratory difficulties, loss of righting response, SLUD syndrome,[11] and clonic convulsions. No animals survived after beginning these convulsions. Immediate autopsy revealed that cardiac contractions had not ceased and that peristalsis of the small intestines had increased. There was no excess fluid in the peritoneal cavity or lungs. Thus the apparent cause of death was respiratory failure.

Table III. Antagonism by Choline and Neostigmine of Drug Toxicity in Mice

Compd	LD_{95} of compd, mg/kg ip	Antagonist[a] Choline, mg/kg ip	Antagonist[a] Neostigmine, mg/kg ip	% mortality
HC-3 (1)	0.2	20		15
			0.2	90
AcHC-3 (2)	0.4	20		0
			0.2	80
Ether 3	2.67	20		0
			0.2	80
Ketone 4	4.60	20		0
			0.2	70
Alkane 5	5.80	20		30
			0.2	80
Control		20		0
			0.2	0

[a] Antagonist was administered 1 min prior to compound.

Both HC-3 (1) and AcHC-3 (2) have the same LD_{50} of 0.13 mg/kg.[6] The other compounds, i.e., the ether 3, the ketone 4, and the alkane 5, were 14.2, 23.8, and 43.1 times less toxic. While 1 is a poor choline acetylase inhibitor, 2 is a potent inhibitor of this enzyme.[6] AcHC-3 (2) underwent slow deesterification in H_2O at pH 7.4. However, 2 has also been shown to undergo an immediate reaction with acetylcholinesterase or butyrylcholinesterase in H_2O at pH 7.4. This is apparently an irreversible binding to the esterase without deesterification. Thus it is possible on the basis of these data to eliminate the formation of the hemiacetal form of HC-3 (1) from 2 as the explanation of identical lethality of 1 and 2 (which therefore appears only to be fortuitous). Although at this time we cannot state with certainty whether the ability of 2 to inhibit choline acetylase directly or a similar ability of the esterase–AcHC-3 complex is the cause of the lethality of 2, work is proceeding in this area which may shed light on this subject. At present, the ability of some form of AcHC-3 (2), quite likely the esterase complex, to inhibit choline acetylase is the most appealing explanation of the high toxicity of the compound. Choline acetylase inhibitory studies are currently being conducted on 3–5. Preliminary results indicate that these compounds are less effective choline acetylase inhibitors than 2. The toxicity of 1, which is a very weak choline acetylase inhibitor, is therefore attributed to the cyclic structure of the nonaromatic moieties of the molecule. As stated above, we favor attributing the lethality of 2, a seco compound, to the formation of an esterase–AcHC-3 complex and to its ability to inhibit choline acetylase. Attributing the lethality of 2 to the ability of its esterase–AcHC-3 complex to inhibit choline acetylase is supported by the findings based on preliminary studies that the stable seco analogues of AcHC-3 (2), i.e., the ether 3, the ketone 4, and the alkane 5, which have intermediate toxicities appear to be intermediate in terms of inhibition of choline acetylase.

Studies of antagonism by choline and neostigmine of drug toxicity in mice were conducted and are summarized in Table III. In each case 20 mg/kg of choline or 0.2 mg/kg of neostigmine was administered to the subject 1 min prior to administration of an ip (>LD_{95}) dose of the drug in question. As in the studies with HC-3 (1) and AcHC-3 (2), mice were protected from the ether 3, the ketone 4, and the alkane 5 by choline but not by neo-

stigmine. Although the toxicity of all five compounds may be antagonized by prior treatment with choline, but not neostigmine, other data, as previously discussed, indicate that the mechanism of lethality differs among the five compounds.

Indications of structure–activity relationships in addition to those previously discussed have been noted in further preliminary in vitro and in vivo studies on these compounds. These data will be presented in subsequent papers along with biological evaluations currently in progress for cardiovascular function, inhibition of neuromuscular function, and cholinesterase and choline acetylase inhibition.

Experimental Section

All melting and decomposition points were determined on a Fisher-Jones hot-stage melting point apparatus and are uncorrected. Boiling points were observed during distillation and are likewise uncorrected. Infrared spectra were recorded on Beckman IR-10 and Perkin-Elmer 337 and 257 spectrophotometers and were calibrated against polystyrene. The ultraviolet spectra and extinction coefficients were obtained on a Beckman DB spectrophotometer. Proton magnetic resonance spectra were determined on a Varian A-60 spectrometer using D_2O as the solvent with 1% tetramethylsilane as an external standard unless otherwise indicated. All refractive indices were observed on a Bausch and Lomb refractometer. Elemental analyses were performed by Galbraith Laboratories, Inc., Knoxville, Tenn., are indicated as empirical formulas, and are within ±0.4% of the theoretical values.

α,α'-Dibromo-4,4'-biacetophenone (6). Bisphenacyl bromide (**6**) was prepared by the method of Long and Schueler:[2] mp 223.9–224.4°.

Dimethylamino-2-ethoxyethane (7). The amine **7** was prepared by a modification of the method of Grail et al.[9] To a stirred solution of sodium ethoxide, prepared from sodium (1.76 g, 0.076 mol) and 40 ml of "superdry" EtOH, was added dropwise a similarly dried ethanolic solution of 2-dimethylaminoethyl chloride hydrochloride (5.0 g, 0.035 mol). The reaction mixture was refluxed at 100° for 24 h. Enough H_2O was added to the cooled reaction mixture to dissolve all of the NaCl produced. The resulting solution was made acidic with dilute HCl and excess EtOH was removed under reduced pressure. The liquid residue was made strongly basic with aqueous NaOH and extracted three times with Et_2O. The combined ethereal extracts were dried over anhydrous K_2CO_3 and distilled. A yield of 1.2 g (30%) of the amine **7** was obtained: bp 121° (lit. 116–118°).

[4,4'-Biphenylylenebis(2-oxoethylene)]bis(2-ethoxyethyldimethylammonium bromide) (3). A solution of dimethylamino-2-ethoxyethane (**7**) (1.09 g, 0.0093 mol) in THF was added to a stirred solution of bisphenacyl bromide (**6**, 1.54 g, 0.0039 mol) dissolved in a minimum of THF. The reaction mixture was stirred overnight at ambient temperatures in a stoppered flask. The resulting precipitate was filtered from solution, washed with THF followed by Et_2O, and recrystallized from absolute MeOH to give 1.29 g (53%) of **3**: mp 228–229° dec. Anal. ($C_{28}H_{42}Br_2N_2O_4$) C, H, N.

[4,4'-Biphenylylenebis(2-oxoethylene)]bis(4-oxopentyldimethylammonium bromide) (4). To a stirred solution of bisphenacyl bromide (**6**) (1.7 g, 0.0042 mol) in a minimum of THF was added a solution of 4-oxopentyldimethylamine (**8**, 1.21 g, 0.0094 mol) in THF. The mixture was stirred in a sealed flask at ambient temperature overnight. The precipitate which resulted was removed by vacuum filtration, washed with THF followed by Et_2O, and recrystallized from absolute EtOH to yield 1.84 g (66%) of **4**: mp 214–216° dec. Anal. ($C_{30}H_{42}Br_2N_2O_4$) C, H, N.

1-Dimethylaminopentane (9). The amine **9** was synthesized employing a modified method of Willstatter and Wasser to give 10.1 g (52%) of **9**: bp 60–62° (100 mmHg) [lit. 122–123° (760 mmHg)]; $n^{22}D$ 1.4065 (lit. $n^{20}D$ 1.4083).[12]

[4,4'-Biphenylylenebis(2-oxoethylene)]bis(1-pentyldimethylammonium bromide) (5). A minimum of THF was utilized to dissolve 1.42 g (0.0035 mol) of bisphenacyl bromide (**6**) with stirring at ambient temperature. A THF solution of 1-dimethylaminopentane (**9**, 0.99 g, 0.0085 mol) was added and the resulting solution stirred overnight in a closed flask. The resulting precipitate was isolated, washed with THF followed by Et_2O, and recrystallized from absolute EtOH to give 1.10 g (50%) of **5**: mp 234–235° dec. Anal. ($C_{30}H_{46}Br_2N_2O_2$) C, H, N.

Stability of Analogues 2–5 of HC-3 (1) in D_2O. Procedure A. Deuterium oxide solutions of **3–5** (0.04–0.06 M) were prepared. Each solution as well as a solvent blank was immediately analyzed utilizing a 60-MHz ^1H NMR spectrometer. The solutions were allowed to stand for 20–22 h at ambient temperature after which time each was again analyzed by ^1H NMR methods. The solutions were found to be free of decomposition products within the limits (<0.5%) of our ^1H NMR spectral analysis.

Procedure B. A similar solution of the ester **2**, which would be expected to be the most water labile analogue, was prepared and initially analyzed as in procedure A. The solution was then maintained at ambient temperature and analysis by ^1H NMR methods was conducted at intermittent times up to 21 h. The solution was then heated to 65° for 5.5 h and analyzed by ^1H NMR which indicated that within the limits previously described the solution was free of decomposition products.

Stability of Analogues 2–5 of HC-3 (1) in H_2O at pH 7.4. Water solutions of **2–5** (1.00×10^{-5} M) buffered to pH 7.4 were prepared. The uv absorption spectrum of each was immediately recorded utilizing an identically buffered water sample as a reference. Each sample was maintained at 37° for 48 h. The uv spectrum of each was subsequently recorded at various intervals during this time. No change was observed in the absorption spectra of **3**, **4**, or **5**. A slow steady decrease in the absorbance of **2** at its λ_{max} (H_2O, pH 7.4) of 307 nm was observed. This decrease corresponds to a decrease in the concentration of **2** of approximately 1.5%/h. A similar increase in absorption in the region of the spectrum at which HC-3 (**1**) absorbs maximally (250–260 nm) was also observed. AcHC-3 (**2**) thus slowly undergoes hydrolysis with subsequent cyclization to the hemiacetal form of HC-3 (**1**) in water at pH 7.4 and 37 °C.

Interaction of Analogues 2–5 of HC-3 (1) in H_2O at pH 7.4 with Acetylcholinesterase and Butyrylcholinesterase. Procedure A. Buffered (pH 7.4) aqueous solutions of **1–5** (100×10^{-5} M) were prepared. Employing an identically buffered water sample as a reference, the uv absorption spectrum of a 1-ml aliquot of each was immediately recorded. A 0.1-ml aliquot of a 40 μ/ml buffered (pH 7.4) aqueous solution of acetylcholinesterase from bovine erythrocytes (obtained from Sigma Chemical Co.) was then added to the sample and reference to produce an enzyme concentration similar to that found in blood. The uv absorption spectrum was immediately recorded. An examination of the spectra of HC-3 (**1**), the ether **3**, the ketone **4**, and the alkane **5** recorded before and after addition of enzyme showed no shift in the λ_{max} (H_2O, pH 7.4) of these compounds following enzyme addition. An examination of equivalent spectra for AcHC-3 (**2**) revealed a shift of the λ_{max} (H_2O, pH 7.4) toward shorter wavelength from 307 to 290 nm. Thus no reaction of **1**, **3**, **4**, or **5** with the enzyme was observed. Although reaction of **2** with the enzyme did occur, the expected hemiacetal form of HC-3 (**1**) was not produced. Each solution was then incubated at 37° for 6 h and the uv absorption spectra were again recorded. No significant changes occurred in the spectrum after 6 h indicating that **1**, **3**, **4**, and **5** do not react with the enzyme while **2** reacts apparently irreversibly at pH 7.4 and 37° with acetylcholinesterase.

Procedure B. The experiment described in procedure A above was repeated substituting butyrylcholinesterase from horse serum (obtained from Sigma Chemical Co.) for acetylcholinesterase. Equivalent results were obtained.

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Synthesis and Biological Evaluation of Fragmented Derivatives of Tetrahydroisoquinolines. 3. Trimetoquinol Studies

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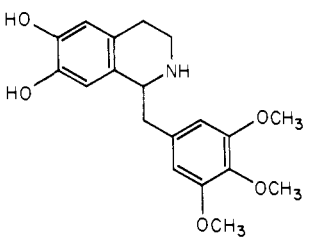
The synthesis of 1-(3,4-dihydroxyphenyl)-2-(3,4,5-trimethoxyphenyl)ethylamine along with the *N*-methyl, *N*-ethyl, and *N*-isopropyl derivatives is presented as fragmented derivatives of trimetoquinol (1). Comparative pharmacological effects of these compounds to 1 are presented and discussed.

In continuing our investigation of tetrahydroisoquinolines as agonists and antagonists in adrenoceptor systems,[1-5] we have initiated a program in determining the relationship of chemical structure to the production of biological actions. One portion of this program involves delineating the importance of an intact tetrahydroisoquinoline ring system for adrenergic activity with a goal toward the development of selective and/or potent β-adrenoceptor stimulants. This report is concerned with the modification of trimetoquinol (1, TMQ) which is known to possess potent β-adrenoceptor activity.[1-8] One of the analogues, **4**, prepared can be considered a fragmented 1 in which the bond between C_4 and the aromatic ring is broken. Other derivatives such as **2**, **3**, and **5** were also prepared and investigated for their profile of β-adrenoceptor activity.



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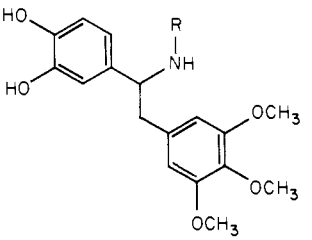
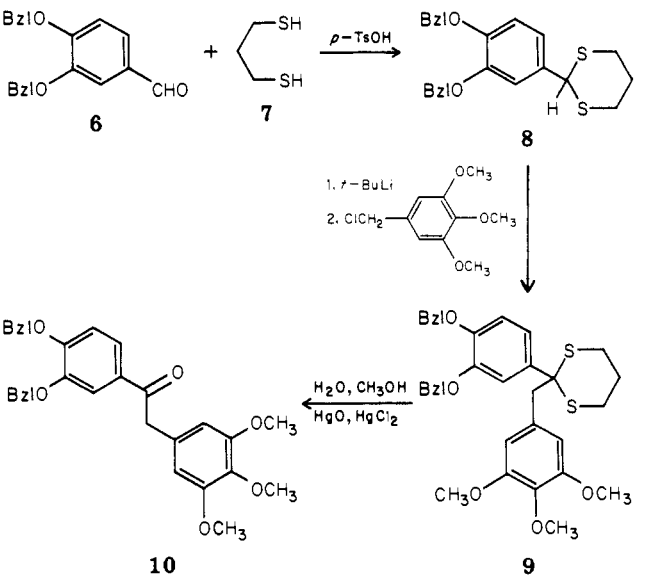


2, R = H
3, R = Me
4, R = Et
5, R = *i*-Pr

The synthesis of the analogues of trimetoquinol was accomplished through the key intermediate ketone, 2-(3',4',5'-trimethoxyphenyl)-1-(3',4'-dibenzyloxyphenyl)-ethanone (**10**, see Scheme I). The ketone was prepared by treating 3,4-dibenzyloxybenzaldehyde with 1,3-propanedithiol in refluxing benzene containing a trace of *p*-TsOH.[9] The resulting dithiane, **8**, was allowed to react with *tert*-butyllithium[10] followed by 3,4,5-trimethoxybenzyl chloride to give **9**. The alkylated dithiane **9** was then hydrolyzed to ketone **10** via the standard method using mercuric oxide and mercuric chloride in aqueous methanol.[11]

The fragmented trimetoquinol derivative **4** along with derivatives **3** and **5** was prepared by the following general reaction procedures (Scheme II). The ketone **10** was converted to the desired imines **11a–c** by allowing the appropriate alkylamine (methylamine, ethylamine, and isopropylamine) to react with **10** in the presence of titanium tetrachloride.[12] The resulting imines were then reduced to the amines **12a–c** using $NaBH_4$ or B_2H_6. The amine was then converted to a hydrochloride salt and the benzyl-protecting groups were removed via hydrogenolysis using 10% palladium on charcoal to give the desired catechols **3**·HCl, **4**·HCl, and **5**·HCl.

Scheme I



6 **7** **8** **10** **9**

The primary amine derivative **2** was prepared by allowing ketone **10** to react with methoxyamine hydrochloride in pyridine and ethanol as shown in Scheme III.[13] The *O*-methyloxime **13** was then reduced using diborane and the resulting amine was converted to hydrochloride salt **14**. Hydrogenolysis of **14** using 10% palladium on charcoal afforded the desired catechol **2**·HCl.

Biological Activity. Pharmacological studies were carried out in the isolated β-adrenoceptor receptor systems of guinea pig trachea, guinea pig atria, and rat adipose tissue. All of the fragmented derivatives possessed an ability to produce tracheal relaxation, chronotropic effects on the heart, and lipolysis.

The relative abilities of each fragmented derivative to stimulate chronotropic response and tracheal relaxation are summarized in Figure 1. Data are presented in terms of the mean pD_2 value ± SEM obtained from cumulative dose–response curves. As indicated, all the analogues possessed pD_2 values which were smaller than the parent