Some Derivatives of Phenethanolamine as MAO Inhibitors

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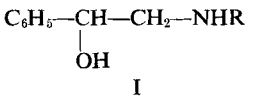
Abstract □ A series of *N*-methyl and *o*-alkyl phenethanolamine derivatives has been prepared and tested against monoamine oxidase *in vitro*. A substantial increase in MAO inhibition over that of the parent phenethanolamine derivatives was noted in the *o*-benzyl analogs.

Keyphrases □ Monoamine oxidase inhibition *in vitro*—phenethanolamine derivatives □ *N*-Methyl, *o*-alkyl derivatives—monoamine oxidase inhibition *in vitro* □ Inhibition analysis—radioactive content method

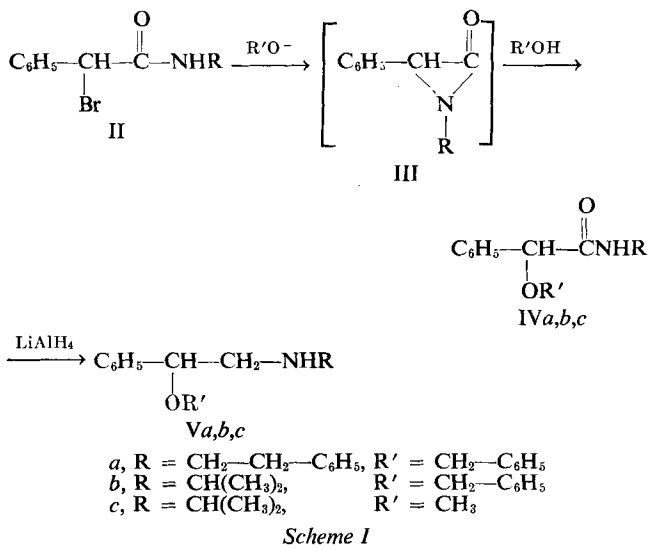
A previous report (1) from these laboratories indicated that 2-phenethylamino-1-phenylethanol (I*a*) and 2-isopropylamino-1-phenylethanol (I*b*) were good monoamine oxidase (MAO) inhibitors *in vitro*. A more recent investigation has shown that the *in vivo* duration of action of I*a* is very short and characterized by potent CNS stimulant properties (2).



I

a, R = CH_2—CH_2—C_6H_5
b, R = $CH(CH_3)_2$

The purpose of the studies reported here was to improve the inherent MAO-inhibiting properties of I*a* and I*b* by structural modification. Based on the report (3) that etherification of the hydroxyl group in a hydrazinoalkanol series increased the *in vivo* potency, the authors have prepared a few ether and *N*-alkyl analogs of I*a* and I*b* and tested their effectiveness against MAO *in vitro*.



Scheme I

CHEMISTRY

Preparation of *N*-methyl derivatives of I*a* and I*b* was accomplished by a standard reductive amination procedure (4).

Synthesis of the desired amino-ethers (V*a,b,c*) was accomplished *via* the corresponding α-bromophenylacetamide (II) (Scheme I). α-Bromoamides have been shown to form α-lactams when treated with an alkoxide (5). It was assumed that the α-lactam was formed as an intermediate in this sequence since product ratios and yields under various conditions correspond to those predicted from studies of α-lactam reactivity (6). For example, α-lactam formation could have occurred when the appropriate α-bromoamide (II) was reacted with an equimolar amount of either sodium methoxide or benzoxide suspended in the respective alcohol or benzene. However, yields of the ether-amides decreased when benzene was the solvent. This would be expected from proposed ring opening mechanisms attributing formation of the amino-ester (VI) to alkoxide attack on the carbonyl carbon of α-lactams and ether-amide (VII) formation to solvolysis of the α-carbon by an alcohol molecule (Scheme II) (5–8).

One experiment was conducted in an attempt to isolate both the products expected if an α-lactam was involved. When *N*-phenethyl α-bromophenylacetamide (II*a*) was reacted with an equimolar amount of sodium benzoxide in ether at room temperature, both the amino-ester (VI, R′ = CH_2—C_6H_5, R = CH_2—CH_2—C_6H_5) and ether-amide (IV*a*) were isolated. In no other case was an attempt made to recover the amino-ester or to quantify the yield. It is interesting to note that in the reaction of sodium methoxide with II*a*, only the amino-ester (methyl α-phenethylaminophenylacetate) was isolated.

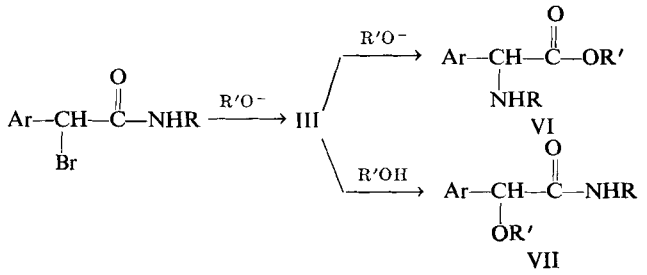
The desired amino-ether compounds (V*a,b,c*) were readily obtained by lithium aluminum hydride reduction of the corresponding ether-amides (IV*a,b,c*).

***In Vitro* Monoamine Oxidase Inhibition**—Inhibition of monoamine oxidase *in vitro* was assessed by the method of Wurtman and Axelrod (9). The potential MAO inhibitor and the substrate (2-^{14}C-tryptamine) were incubated with rat liver homogenate. After a suitable length of time the deaminated product (^{14}C-indolacetic acid) was extracted and counted for its radioactive content. The compounds were screened at 5×10^{-4} *M* concentration as the hydrochloride salt by using: 1 ml. of a 10^{-3} *M* solution of the potential inhibitor, 0.625 ml. of phosphate buffer, 0.10 ml. (0.02 μc.) of ^{14}C-tryptamine substrate, and 0.25 ml. of rat liver homogenate to give a final volume of 2 ml. The degree of inhibition was compared to 5×10^{-4} *M* iproniazid standard. The results are given in Table I.

EXPERIMENTAL

Melting points were determined on a Buchi apparatus with open capillary tubes and are uncorrected. NMR spectra were obtained with a Varian Associates A-60-A spectrophotometer in $CDCl_3$ with tetramethylsilane as an internal standard. Combustion analyses were conducted by Galbraith Laboratories, Inc., Knoxville, Tenn. IR spectra were determined on a Perkin-Elmer model 21 spectrophotometer and were consistent with structures given.

***N*-Methyl-2-phenethylamino-1-phenylethanol Hydrochloride**—Formic acid (25.6 g. of 90%, 0.50 mole), formaldehyde (9.0 g. of 40%, 0.30 mole), and 2-phenethylamino-1-phenylethanol (24.1 g., 0.10 mole) were reacted by standard procedures (4). The product was isolated as the hydrochloride salt and recrystallized from hexane–acetone to give a white solid (8.6 g., 30%), m.p. 153–154°; NMR



Scheme II

(D_2O) δ7.25 (m, 10, Ar), 5.25 (m, 1, C<u>H</u>), 3.22 (b m, 6, CH_2), 2.95 (s, 3, CH_3).

Anal.—Calcd. for $C_{17}H_{22}ClNO$: C, 69.97; H, 7.60. Found: C, 69.93; H, 7.76.

***N*-Methyl-2-isopropylamino-1-phenylethanol Hydrochloride**—Formic acid (51.2 g. of 90%, 1.0 mole), formaldehyde (18.0 g. of 40%, 0.60 mole), and 2-isopropylamino-1-phenylethanol (35.8 g., 0.20 mole) were reacted as above. Recrystallization of the hydrochloride salt from hexane–acetone gave 16.5 g. (36%) of white solid, m.p. 134–135°; NMR (D_2O) δ7.4 (s, 5, Ar), 5.15 (t, 1, C<u>H</u>), 3.7 (m, 1, CH), 3.25 (d, 2, CH_2), 1.27 (m, 6, CH_3).

Anal.—Calcd. for $C_{12}H_{20}ClNO$: C, 62.73; H, 8.77. Found: C, 62.86; H, 8.97.

***N*-Phenethyl-α-benzoxyphenylacetamide (IV*a*)**—*Method A*—*N*-Phenethyl α-bromophenylacetamide (11) (20.0 g., 0.06 mole) in 150 ml. of benzyl alcohol was added over 1 hr. to a heated solution of sodium benzoxide (7.8 g., 0.06 mole) in 50 ml. of benzyl alcohol. The mixture was heated under reflux for 10 hr. and then filtered. The filtrate was added to water and extracted with ether. The dried organic extract was evaporated *in vacuo* and the residue distilled under reduced pressure to remove benzyl alcohol. The thick residue solidified after 2 days. The solid was recrystallized from hexane to give 5.0 g. (23%) of white crystalline solid, m.p. 86–88°; NMR ($CDCl_3$) δ7.25 (m, 9, Ar), 6.8 (b m, 1, NH), 4.75 (s, 1, CH), 4.40 (d, 2, C<u>H</u>$_2$), 3.5 (q, 2, NHC<u>H</u>$_2$), 2.75 (t, 2, CH_2).

Anal.—Calcd. for $C_{23}H_{23}NO_2$: C, 79.97; H, 6.71. Found: C, 79.75; H, 6.33.

Method B—*N*-Phenethyl-α-bromophenylacetamide (25.0 g., 0.08 mole) in 100 ml. of benzene was added dropwise to a suspension of 10.4 g. (0.08 mole) of sodium benzoxide in 100 ml. of benzene. After 24 hr. at room temperature the precipitate which had formed was removed and the filtrate was evaporated *in vacuo*. The solid residue was recrystallized from hexane to give 2.5 g. (9%) of white solid identical to that obtained by *Method A*.

***N*-Isopropyl-α-methoxyphenylacetamide (IV*c*)**—Utilizing *Method A* above, *N*-isopropyl-α-bromophenylacetamide (40.0 g., 0.16 mole) was reacted to give 5 g. (15%) of recrystallized material, m.p. 51–56°; NMR ($CDCl_3$); δ7.3 (m, 5, Ar), 6.43 (m, 1, NH), 4.51 (s, 1, CH), 4.0 (t, 1, CH), 3.3 (s, 3, OCH_3), 1.15 (m, 6, CH_3). This material was used without further purification.

***N*-Phenethyl-α-benzoxyphenylacetamide (IV*a*) and Benzyl-α-phenethylaminophenylacetate**—To a solution of sodium benzoxide (13.0 g., 0.10 mole) in ether was added in one portion an ether solution of *N*-phenethyl-α-bromophenylacetamide (32.0 g., 0.1 mole). After 1 hr. the solid was collected and discarded. Ethereal HCl was added dropwise to the filtrate. The precipitate was recrystallized from acetone–ethanol (4:1) to give 2.3 g. (6.2%) of VI·HCl, m.p. 224–225°.

The ether filtrate was evaporated *in vacuo*. The resulting residue was treated with water and extracted with ether. The ether extracts were dried and evaporated to give an oil which could not be purified. The aqueous phase was chilled and a precipitate formed. The solid was recrystallized from hexane to give 1.0 g. (2.1%) of IV*a*.

Attempted Synthesis of *N*-Phenethyl-β-methoxyphenethylamine Hydrochloride—Following *Method B*,*N*-phenethyl-α-bromophenylacetamide (32.0 g., 0.10 mole) was reacted with sodium methoxide (5.4 g., 0.10 mole) to give a crude solid, m.p. 222–225°. Without further purification (7.5 g., 0.028 mole) was reacted with lithium aluminum hydride according to the general reduction procedure. The product obtained was found to be 2-phenyl-2-phenethylaminoethanol hydrochloride, 1.7 g. (19%), m.p. 131–132°; NMR ($CDCl_3$), δ6.40 (m, 10, Ar), 5.6 (m, 1, CH), 4.35 (m, 3, C<u>H</u>$_2$—OH), 3.15 (m, 4, C<u>H</u>$_2$—Ar, C<u>H</u>$_2$—N).

Table I—Results of *In Vitro* Screening for MAO Inhibition Using Rat Homogenate

Compound	% Inhibition
Iproniazid	100
I*a*	36 ± 7[a,b]
I*b*	36[c]
V*a*	93 ± 2[a]
V*b*	70 ± 5[a]
V*c*	15 ± 4[a]
N-Methyl I*a*	51 ± 4[a]
N-Methyl I*b*	24 ± 6[d]

[a] Triplicate determinations reported as the mean and average deviation from the mean value. [b] Found to be less active than previously reported (1). Dioxane was used in the earlier testing and if not properly purified would contain ethanol which has been reported to inhibit MAO *in vitro* (10). [c] Single determination. [d] Duplicate determinations reported as the mean and average deviation of an individual result from this mean value.

Anal.—Calcd. for $C_{16}H_{20}ClNO$: C, 69.18; H, 7.27. Found: C, 69.47; H, 7.24.

***N*-Phenethyl-β-benzoxyphenethylamine Hydrochloride (V*a*)**—*General Reduction Procedure*—*N*-Phenethyl-α-benzoxyphenylacetamide (8.0 g., 0.023 mole) suspended in 200 ml. of dry ether was added in 10-ml. portions to a suspension of lithium aluminum hydride (1.74 g., 0.046 mole) in 100 ml. of ether. The mixture was heated under reflux for 12 hr. and then 9.6 ml. of water was added. The resulting precipitate was removed by filtration and the ether filtrate dried. The ether was placed in a dry-ice acetone bath and dry ethereal HCl was added dropwise. The solid that precipitated was recrystallized from acetone–methanol (4:1) by cooling the solution to 0° to give 1.6 g. (19%) of pure V*a*·HCl, m.p. 136–137.5°; NMR ($CDCl_3$) δ7.37 (m, 15, Ar), 5.15 (q, 1, CH), 4.5 (dd, 2, O—C<u>H</u>$_2$—Ar), 3.3 (m, 6, C<u>H</u>$_2$—N—C<u>H</u>$_2$—C<u>H</u>$_2$—Ar).

Anal.—Calcd. for $C_{23}H_{26}ClNO$: C, 75.08; H, 7.12. Found: C, 75.26; H, 7.21.

***N*-Isopropyl-β-benzoxyphenethylamine Hydrochloride (V*b*)**—*N*-Isopropyl-α-benzoxyphenylacetamide (6.0 g., 0.021 mole) was reacted according to the general reduction procedure to give 1.3 g. (22%) of V*b*, m.p. 188–189°; NMR ($CDCl_3$) δ7.4 (m, 10, Ar), 5.17 (q, 1, CH), 4.5 (dd, 2, O—CH_2), 3.33 (m, 3, CH, N—CH_2), 1.5 (d, 6, CH_3).

Anal.—Calcd. for $C_{18}H_{24}ClNO$: C, 70.69; H, 7.91. Found: C, 70.39; H, 7.90.

***N*-Isopropyl-β-methoxyphenethylamine Hydrochloride (V*b*)**—*N*-Isopropyl-α-methoxyphenylacetamide (5.0 g, 0.024 mole) was reacted according to the general reduction procedure to give 1.1 g. (20%) of V*c*·HCl, m.p. 225–226°; NMR (D_2O) δ7.3 (m, 5, Ar), 4.65 (m, 1, CH), 3.3 (m, 6, CH, N—C<u>H</u>$_2$, OCH_3), 1.50 (d, 6, CH_3).

Anal.—Calcd. for $C_{12}H_{20}ClNO$: C, 62.73; H, 8.77. Found: C, 62.61, H. 8.69.

DISCUSSION

From Table I it can be observed that *in vitro* inhibition of MAO by the benzyl ether compounds V*a* and V*b* was substantially increased over that of the parent compounds (I*a* and I*b*). It is interesting to note that the methyl ether of 2-isopropylamino-1-phenylethanol causes a decrease in *in vitro* MAO inhibition from the parent compound which corresponds to observations in the hydrazinoalkanol series of Schuler and Wyss (3). Methylation of the nitrogen of the parent compounds increased MAO inhibition in one case (II*a*) over the parent but decreased inhibition in relation to the parent with compound II*b*. *In vivo* testing of these compounds will be carried out at a later date.

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Some Cyclic Ketals and Acetals of Digitoxin, Digoxin, and Ouabain

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Abstract □ A new and milder method has been utilized to reinvestigate the preparation, properties, and biological activities of the mono-*o*-isopropylidene (acetonides) derivatives of digitoxin, digoxin, and ouabain. The preparation, properties, and biological activities of the mono-*o*-ethylidene derivatives of digitoxin and digoxin also are described. The latter derivatives show greater activity than the former.

Keyphrases □ Digitoxin, digoxin cyclic ketals, acetals—synthesis □ Ouabain cyclic ketals, acetals—synthesis □ Bioanalysis—digitoxin, digoxin, ouabain derivatives □ TLC, paper chromatography—separation, identification □ UV spectrophotometry—identity

In a previous publication the preparation and activities of the mono-*o*-isopropylidene derivatives (glycoside acetonides) of digitoxin, digoxin, and ouabain have been reported (1). Their biological activities were much less than had been expected on the basis of their physical properties and, in the cases of digitoxin and of digoxin, the position of the isopropylidene group. Because these activities were so unexpectedly low, a reinvestigation of the preparation of these derivatives by much milder methods was sought and one such was found. Acetonation can be effected in a very short time at room temperature *via* the use of 2,2-dimethoxypropane, acetone, a trace of acid, and room temperature conditions. The yields are almost quantitative and derivatives readily can be crystallized. Bioassay of these derivatives prepared by this new, very mild method gave comparable results in the case of digoxin but not in the case of digitoxin or ouabain. The ease with which the isopropylidene derivatives (dioxolanes) of digitoxin and digoxin could be prepared *via* the use of ketal-interchange techniques suggested the preparation of other cyclic ketals and cyclic acetals (dioxolanes) to test further the contribution to activity such derivatives might exert. Because the isopropylidene group, even though it was the simplest type of a cyclic ketal and on the terminal digitoxose residue, reduced activity so markedly that the preparation of a cyclic acetal as a second type of a dioxolane derivative was investigated. The ethylidene derivative readily could be prepared *via* 1,1-dimethoxyethane, acetaldehyde, a trace of acid, and room temperature conditions. Bioassays of these ethylidene derivatives *via* LD_{50} intravenous assays in cats showed that mono-*o*-ethylidene digoxin had the same activity as that of acetyldigoxin whereas acetyldigitoxin was 2.35 times as active as mono-*o*-ethylidene digitoxin. Thus the activity (toxicity) of digitoxin was reduced to a greater extent by both types of derivatives than was the case with digoxin. Also it is of interest to note that the mono-*o*-ethylidene digoxin activity was the same as that of acetyldigoxin.

In the case of digoxin, its usefulness is improved in its acetyl derivatives.[1] Acetyldigoxin is considered by some to be the drug of choice because it is well absorbed orally, is less toxic to the CNS, and has a shorter duration of action than digitoxin or acetyldigitoxin. Acetyldigoxin was isolated directly from *Digitalis lanata* by Hopponen and Gisvold (2) in 1952, and its good oral absorption properties in cats were first reported by White and Gisvold (3).

It now appears that the physical properties of the monoethylidene derivative of digoxin, *i.e.*, R_f value measuring liposolubility, should enhance its degree of absorption upon oral administration. Its toxicity and duration of action would depend in part upon its distribution and metabolism *in vivo*. This derivative is sensitive to hydrolysis at condition below pH 7 but is quite stable above pH 7. Dioxolanes are usually stable *in vivo* and thus such derivatives of the cardiac glycosides might exert some interesting effects upon their metabolism *in vivo*. Increased stability without a significant increase in stereochemical bulk would be effected by the substitution of F for H in the ethylidene residue. Such investigations are now in progress.

EXPERIMENTAL

The details of some of the paper chromatographic techniques used in these studies have been previously described (4). Thin-layer

[1] Marketed in Europe as Novadigal and Lanatilin.