leveled off) but involves the other features of the molecule, then K_I, k_c, and k_{-c} will be independent of X. On the other hand, k_2 will be highly dependent upon X and this dependence will only show up when $k_2 \lesssim k_{-c}$. This scheme also might involve a pentacovalent intermediate.

Although this kind of explanation is *ad hoc*, it should be noted that reversible complexes and substrate-induced conformational changes have played a dominant role in enzyme theory for many years.[10,11]

In a previous study,[12] it was found that the curvature that sets in sharply at $pK_a = 7.0$ did not occur when the leaving group contained a quaternary ammonium function so that these compounds were decidedly more reactive than would be anticipated from the pK_a of their leaving groups. The rationalization offered above would suggest that the conformational change occurs more rapidly when the inducing compound contains a cationic amine function. There is spectral evidence that quaternary amines change the conformation of acetylcholinesterase.[13]

We note that with few exceptions the ring compounds react less rapidly than the open-chain analogs but the distinction is not great enough to account for the poor inhibitory properties of the fluoride and *p*-nitrophenolate in the ring series.

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Synthesis of Some N-Substituted Eleostearic Acid Hydrazides as Potential Monoamine Oxidase Inhibitors†

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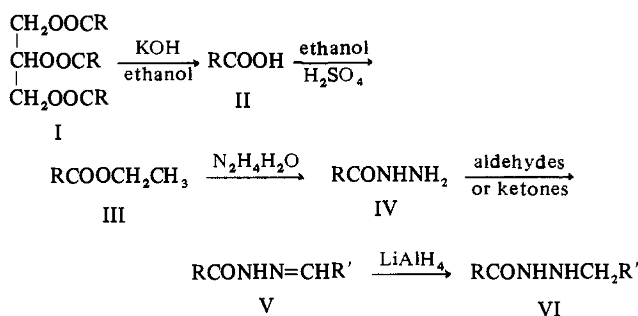
The syntheses of five N-substituted eleostearic acid hydrazides are described: *N*-isopropyl-α-eleostearoyl hydrazide, *N*-benzyl-α-eleostearoyl hydrazide, *N*-phenethyl-β-eleostearoyl hydrazide, *N*-α-methylphenethyl-β-eleostearoyl hydrazide, and *N*-β-methylphenethyl-α-eleostearoyl hydrazide. The compounds were investigated for their ability to inhibit monoamine oxidase *in vitro* and to elevate the brain monoamines in mice. The results showed that these compounds were active monoamine oxidase inhibitors with their slightly lower *in vitro* activity but comparable *in vivo* activity in comparison with the corresponding monosubstituted hydrazines.

The monosubstituted hydrazines such as alkyl- and aralkylhydrazines have provided many potent monoamine oxidase (MAO) inhibitors from which phenelzine has emerged as a clinically used antidepressant.[1] Pheniprazine, benzylhydrazine, and isopropylhydrazine are among the most potent MAO inhibitors but are barred from clinical use because of high toxicity.[2-4] Acylation of benzylhydrazine with 5-methyl-3-isoxazolylcarboxylic acid has produced another currently used hydrazine antidepressant, isocarboxazide, with MAO inhibitory activity but reduced toxicity.[5] Iproniazide, the isonicotinic acid acylated isopropylhydrazine, the first MAO inhibitor antidepressant,[6] also possesses an activity comparable to isopropylhydrazine with less toxicity. The present work was to synthesize some N-substituted hydrazides by acylating some of the most potent monosubstituted hydrazines with α-eleostearic acid (9-*cis*-11-*trans*-13-*trans*-octadecatrienoic acid) and β-eleostearic acid (9-*trans*-11-*trans*-13-*trans*-octadecatrienoic acid) and to investigate their MAO inhibitory activity *in vitro* and *in vivo*.

Scheme I



R, $CH_3(CH_2)_3(CH{=}CH)_3(CH_2)_7$
R', alkyl or aralkyl
I, α- or β-eleostearic acid glyceride in tung oil
II, α- or β-eleostearic acid
III, α- or β-ethyl eleostearate
IV, α- or β-eleostearic acid hydrazides
V, acylhydrazones
VI, N-substituted eleostearoyl hydrazides

Chemistry. The type of reaction series chosen for the synthesis of N-substituted hydrazides is summarized as shown in Scheme I.

α- and β-eleostearic acids II were isolated from the domestic tung oil I from kernels of *Aleurites fordii* by the method

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Table I. Eleostearic Acids and Their Derivatives

Compound	Eleostearic acid isomer	Alkyl and aralkyl	Yield, %	Solvent	Mp, °C[a]	Formula	Analyses[b]
Eleostearic acids							
1	α		56	$EtOH-H_2O$	48	$C_{18}H_{30}O_2$	
2	β		66	$EtOH-H_2O$	70	$C_{18}H_{30}O_2$	
Ethyl eleostearates							
3	α		98		Oil	$C_{20}H_{34}O_2$	C, H
4	β		95		Oil	$C_{20}H_{34}O_2$	
Eleostearic acid hydrazides							
5	α		60	$EtOH-H_2O$	76-77 dec	$C_{18}H_{32}N_2O$	C, H, N
6	β		72	$EtOH-H_2O$	125 dec	$C_{18}H_{32}N_2O$	C, H
Acylhydrazones							
7	α	$=C(CH_3)_2$	85		Semisolid	$C_{21}H_{36}N_2O$	
8	α	$=CHC_6H_5$	92	EtOH	54-55	$C_{25}H_{36}N_2O$	
9	β	$=CHCH_2C_6H_5$	95	EtOH	99-100	$C_{26}H_{38}N_2O$	
10	β	$=C(CH_3)CH_2C_6H_5$	93	$EtOH-H_2O$	55-56	$C_{27}H_{39}N_2O$	
11	α	$=CHCH(CH_3)C_6H_5$	93		Semisolid	$C_{27}H_{39}N_2O$	
N-Substituted eleostearic acid hydrazides							
12	α	$-CH(CH_3)_2$	48		Semisolid	$C_{21}H_{38}N_2O$	C, H, N
13	α	$-CH_2C_6H_5$	40	$Et_2O-EtOH$	63-65[c]	$C_{29}H_{42}N_2O_5$	C, H
14	β	$-CH_2CH_2C_6H_5$	51	$Et_2O-EtOH$	111-113[c]	$C_{30}H_{44}N_2O_5$	C, H
15	β	$-CH(CH_3)CH_2C_6H_5$	23	EtOH	82-84	$C_{27}H_{41}N_2O$	C, H
16	α	$-CH_2CH(CH_3)C_6H_5$	36	$Et_2O-EtOH$	120 dec[d]	$C_{27}H_{42}N_2OCl$	C, H, N

[a]All melting points were taken on a Fisher-Johns apparatus and were corrected. [b]Where analyses are indicated only by symbols of the elements, analytical results obtained for these elements were within ±0.4% of the theoretical values. [c]Maleate. [d]Hydrochloride.

previously described.[7] The ethyl esters III of α- and β-eleostearic acids were prepared by the benzene-alcohol method.[8] Condensation of ethyl eleostearate with a large excess of hydrazine hydrate to yield eleostearic acid hydrazides IV was essentially the method of Szmuszkovicz.[9] The susceptibility of the conjugated triene bonds to the individual reduction by N_2H_4[10] was not evident under the reaction conditions used. Acylhydrazones V were prepared by refluxing the eleostearic acid with the desired aldehydes or ketones by the methods previously described for other acylhydrazones.[11] Hydrogenation of the acylhydrazones with $LiAlH_4$ in dry tetrahydrofuran (THF)[11] afforded the N-substituted eleostearic acid hydrazines VI in desirable quantities. The reduction of the acylhydrazones with sodium borohydride ($NaBH_4$) proceeded very slowly. The use of $NaBH_4$ with $AlCl_3$ was deliberately avoided because of the possibility of the formation of a symmetrically disubstituted hydrazine.[1] Successful reaction was not evident when ethyl eleostearate was allowed to react directly with monosubstituted hydrazines such as benzyl- and phenethylhydrazine. Table I presents all the compounds prepared.

Biological Results. The *in vitro* inhibitory activity of α- and β-eleostearic acids and the N-substituted eleostearoyl hydrazides on the solubilized mitochondrial monoamine oxidase from liver and brain of albino mice was investigated according to the method of Zeller, *et al.*[12] Mitochondria were prepared by the procedure of Mahler, *et al.*[13] Data were analyzed by the method of Wilcoxon and Wilcox.[14] The results of the *in vitro* enzyme study (Table II) showed that the sequence of relative activity for the three established drugs was pheniprazine > phenelzine > isopropylhydrazine ($p < 0.01$). Their eleostearic acid derivatives possessed similar relative potency: **15** > **14** > **12** ($p < 0.01$). Acylated phenelzine and pheniprazine were less active than the unacylated drugs ($p < 0.05$). The two eleostearic acids were equally active. Acylated benzylhydrazine (**13**) was a more powerful inhibitor than acylated isopropylhydrazine (**12**) ($p < 0.01$) as was expected since benzylhydrazine is a stronger inhibitor than isopropylhydrazine. The previous results indicated the superiority of benzylhydrazine to phenethylhydrazine.[1] The same relative potency was not observed for their eleostearic acid derivatives. The concentrations which inhibited only partially the liver enzyme activity led to a total inhibition of the brain enzyme. In general, the inhibitory effects on the brain enzyme were higher than those on the liver enzyme. For the *in vivo* inhibitory activity, the concentrations of brain monoamines (dopamine, norepinephrine, and serotonin) were determined in the control and drug-treated male albino mice by the procedure of Neff, *et al.*[15] Mice were decapitated and brains were removed for analysis 10 hr after the intraperitoneal injection of the test compounds emulsified in 3% Tween 80. Table III summarizes the effects of some N-substituted hydrazides and monosubstituted hydrazines on the brain levels of dopamine (DM), norepinephrine (NE), and 5-hydroxytryptamine (5-HT) in male mice. All the

Table II. *In Vitro* Inhibition of Monoamine Oxidase by Hydrazine Derivatives[a]

Compound	pI_{50} ± S.E.,[b] liver	Concn, *M*	% inhibition[c] Liver	Brain
1	3.9 ± 0.12[d]	1×10^{-5}	27	35
2	3.9 ± 0.08	1×10^{-5}	26	32
12	4.35 ± 0.10	1×10^{-5}	38	48
13	5.15 ± 0.08	1×10^{-5}	62	70
14	5.25 ± 0.13	1×10^{-5}	58	61
15	6.40 ± 0.14	1×10^{-5}	70	86
16	5.80 ± 0.16	1×10^{-5}	43	45
Iproniazide phosphate	5.15 ± 0.10	1×10^{-5}	73	100
Phenelzine sulfate	5.65 ± 0.08	1×10^{-5}	66	100
Pheniprazine hydrochloride	7.10 ± 0.19	1×10^{-5}	100	100

[a]Inhibitors of varying concentrations (10^{-2}–10^{-7} *M*) were incubated with solubilized mitochondrial MAO of mice at 37° for 8 min prior to the addition of *m*-iodobenzylamine (7.2×10^{-4} *M*) in phosphate buffer, pH 7.6. [b]pI_{50} ± standard error. The pI_{50} is the negative logarithm of the concentration required for 50% inhibition of the enzyme activity. [c]Inhibitors (1×10^{-5} *M*) were incubated at 37° for 8 min. Each value represents the mean of duplicate determinations. [d]Comparison of all possible pairs is described in the text.

Table III. Effects of Hydrazine Derivatives on Amine Levels in Brain of Mice (μg/g Wet Tissue)[a]

Compound	DM[b]	NE	5-HT
Control (3% Tween 80)	0.883 ± 0.035	0.466 ± 0.004	0.613 ± 0.012
1	0.820 ± 0.052, N.S.[c]	0.568 ± 0.020, $p < 0.01$,[d] 122%[e]	0.684 ± 0.011, $p < 0.01$, 110%
12	0.903 ± 0.047, N.S.	0.740 ± 0.045, $p < 0.001$, 159%	0.926 ± 0.026, $p < 0.001$, 151%
13	0.886 ± 0.034, N.S.	0.982 ± 0.033, $p < 0.001$, 211%	1.403 ± 0.038, $p < 0.001$, 229%
14	0.921 ± 0.028, N.S.	1.012 ± 0.037, $p < 0.001$, 217%	1.404 ± 0.057, $p < 0.001$, 229%
15	0.950 ± 0.033, N.S.	0.995 ± 0.046, $p < 0.001$, 213%	1.665 ± 0.056, $p < 0.001$, 272%
16	0.867 ± 0.023, N.S.	0.854 ± 0.041, $p < 0.001$, 183%	1.362 ± 0.069, $p < 0.001$, 222%
Iproniazide phosphate	0.887 ± 0.039, N.S.	0.720 ± 0.018, $p < 0.001$, 155%	0.899 ± 0.053, $p < 0.001$, 147%
Phenelzine sulfate	0.907 ± 0.037, N.S.	0.931 ± 0.023, $p < 0.001$, 200%	1.262 ± 0.076, $p < 0.001$, 206%
Pheniprazine hydrochloride	1.014 ± 0.033, N.S.	1.044 ± 0.025, $p < 0.001$, 224%	1.562 ± 0.083, $p < 0.001$, 255%

[a]Each value represents the mean ± standard error for a group of five mice 10 hr after intraperitoneal administration of drug at a dose equimolar to 100 mg/kg iproniazide phosphate (3.6×10^{-4} mol/kg). [b]DM, dopamine; NE, norepinephrine; 5-HT, 5-hydroxytryptamine. [c]No significant difference between treated and control animals ($p > 0.05$). [d]Significance was determined between treated and control animals by t test. [e]Per cent of brain amines compared with control.

treated animals showed increased NE and 5-HT content in brain compared with the control animals injected with vehicle (3% Tween 80) ($p < 0.01$ and $p < 0.001$). The DM levels of all treated mice remained unaffected ($p > 0.05$), a finding consistent with the previous data.[16,17] The eleostearic acid acylated phenelzine and pheniprazine were as powerful as their unacylated hydrazines in elevating both NE and 5-HT. The biological data of the *in vitro* enzyme study and the *in vivo* drug effect on the biogenic amines in the brain of mice indicated that these hydrazides were active MAO inhibitors, with their slightly lower *in vitro* activity but comparable *in vivo* activity in comparison with the corresponding monosubstituted hydrazines.

Experimental Section

Domestic tung oil was obtained from Crosby Forest Products Co. in Picayune, Miss.

α-Eleostearic Acid (1) and β-Eleostearic Acid (2). Tung oil (200 g) was refluxed gently with 60 g of KOH in 50 ml of distilled water and 500 ml of 95% EtOH for 30 min. The soap mixture was acidified with 725 ml of 2 *N* HCl. The oily product was separated and crystallized from 1 l. of 95% EtOH.

Ethyl α-Eleostearate (3) and Ethyl β-Eleostearate (4). To a solution of 278 g (1 mol) of an eleostearic acid in 450 g (8 mol) of dry ethanol was added 375 ml of dry benzene with stirring. Concentrated H_2SO_4 (60 g) was then added dropwise over a period of 30 min. The mixture was refluxed gently for 2.5 hr at 70°. After cooling, 2 l. of cold water was added. The benzene layer was separated and stirred with excess $NaHCO_3$ until effervescence ceased. The mixture was filtered, then washed three times with 100 ml of distilled water, and dried over anhydrous Na_2SO_4. The clear solution was evaporated to dryness *in vacuo* to yield the product.

α- and β-Eleostearic Acid Hydrazides (5 and 6). Compounds **5** and **6** were synthesized by refluxing 3 and 4 (418 g, 1.32 mol) with 85% hydrazine hydrate (330 g, 6.6 mol) at 80° for 18 hr. The products were crystallized from 95% EtOH.

Eleostearic Acid Alkylidene and Aralkylidene Hydrazides (7-11). These acylhydrazones were made by refluxing 5 or 6 (44 g, 0.15 mol) in 80 ml of EtOH with acetone (60 ml), benzaldehyde (21.6 g, 0.2 mol), phenylacetaldehyde (24.5 g, 0.2 mol), phenylacetone (27.3 g, 0.2 mol), and 2-phenylpropionaldehyde (19 g, 0.15 mol) in a water bath for 30 min. The products were crystallized from EtOH or 90% EtOH.

***N*-Alkyl- and *N*-Aralkyleleostearoyl Hydrazides (12-16).** The general method for the preparation of N-substituted hydrazides was as follows. To a suspension of 8 g (0.21 mol) of $LiAlH_4$ in 400 ml of dry THF was added dropwise with stirring a solution of an eleostearic acid alkylidene hydrazide or eleostearic acid aralkylidene hydrazide (0.05 mol) in 150 ml of dry THF. After the exothermic reaction subsided, the stirring was continued for 1 hr. The excess $LiAlH_4$ was decomposed by dropwise addition of 120 ml of ethyl ether saturated with water. After filtration, the filtrate was evaporated to obtain an oily residue. The oily residues derived from 8 and 9 were dissolved in 50 ml of Et_2O and crystallized as a maleate with the addition of maleic acid (6 g, 0.05 mol) in 15 ml of EtOH to yield *N*-benzyl-α-eleostearoyl hydrazide maleate (**13**) and *N*-phenethyl-β-eleostearoyl hydrazide maleate (**14**). The oily residues obtained from 7 and 11 were treated with maleic acid (8.5 g, 0.07 mol) in a similar manner. The maleates were further treated with 0.1 *N* NaOH and the aqueous solutions were extracted with $CHCl_3$. The organic phases were dried over Na_2SO_4, filtered through alumina, and evaporated to afford *N*-isopropyl-α-eleostearoyl hydrazide (**12**) and *N*-β-methylphenethyl-α-eleostearoyl hydrazide (**16**). The oily residue prepared from 10 was crystallized from EtOH to produce *N*-α-methylphenethyl-β-eleostearoyl hydrazide (**15**). The oily residue (1.5 g) prepared from 11 was taken up in 25 ml of Et_2O and treated with 5 ml of 8 *N* ethanolic HCl to yield *N*-β-methylphenethyl-α-eleostearoyl hydrazide hydrochloride as an analytical sample for **16**.

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