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ACKNOWLEDGMENTS

The authors thank Richard Privette and Dr. David Rosenthal of Research Triangle Institute for technical assistance and computer programming work, respectively. They also thank Dr. Madison J. Cawein and Terry D. Dusebout of the Clinical Pharmacology Department at the Merrell Research Center for conducting the clinical portion of the study.

Prodrugs of Etilefrine: Synthesis and Evaluation of 3′-(*O*-Acyl) Derivatives

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Received March 10, 1980, from the **Department of Chemical Research, Klinge Pharma, Munich, West Germany,* and the ‡*Institute of Pharmacology and Toxicology, University of Würzburg, Würzburg, West Germany.* Accepted for publication June 27, 1980.

Abstract □ A series of 3′-(*O*-acyl) derivatives of etilefrine {α-[(ethylamino)methyl]-3′-hydroxybenzyl alcohol} was synthesized. Correlations between structure and solubility, pKa value, lipophilicity, and esterase-catalyzed hydrolysis were demonstrated. Of special interest is the 3′-(*O*-pivaloyl) derivative, which shows, in addition to favorable solubility and improved lipophilicity, marked stability against enzymatic cleavage in blood along with a high rate of hydrolysis in the liver.

Keyphrases □ Etilefrine—synthesis and evaluation of 3′-(*O*-acyl) derivatives as prodrugs □ Prodrugs—etilefrine, synthesis and evaluation of 3′-(*O*-acyl) derivatives as prodrugs

The uncertain activity of orally administered phenolethanolamines is partly assumed to result from metabolic processes during absorption. Phenolethanolamines with a free hydroxyl group in the *meta*-position are primarily subject to this gut wall first-pass metabolism. As they pass the intestinal wall, they are conjugated to inactive compounds. For example, in the intestinal wall, 80% of orally administered isoproterenol is converted to sulfate (1, 2) in the *meta*-position. During the gut wall passage, a similar conjugation was found for orciprenaline (metaproterenol) (3), terbutaline (4), norfenefrine (5), and etilefrine (6). Due to this early metabolic reaction, the bioavailability of compounds, even those with a high rate of absorption, is frequently reduced (7).

To avoid the attack of conjugating enzymes at the 3′- or 5′-hydroxy group, it was masked by acylation, thus converting the phenolethanolamines into prodrugs. *O*-Acyl derivatives of this type are known for phenylephrine (8, 9), etilefrine (8, 9), norepinephrine (10), *meta*-norepinephrine (10), epinephrine (8, 9), *meta*-epinephrine (8, 9), isoproterenol (10), orciprenaline (10), *N*-*tert*-butylnorepinephrine (11), and terbutaline (12). These *O*-acyl derivatives differ from their parent drug significantly in their solubility, dissociation constants, and lipophilicity. In addition to these properties that affect the absorption rate, enzymatic cleavage is influenced by the structure of the acyl radicals (8, 9, 13).

Since enzymatic hydrolysis also is dependent on the number and position of the acyl radicals, it was of interest to test *O*-acyl derivatives of one phenolethanolamine that only differed by the acyl radical. By taking into account all properties essential for bioavailability, a search has been centered on a prodrug applicable for therapeutic use. Therefore, the model compound selected was etilefrine {α-[(ethylamino)methyl]-3′-hydroxybenzyl alcohol}, which is used in the treatment of circulatory diseases. Its systemic bioavailability as unmetabolized etilefrine is ~50% (6, 7), although it is completely absorbed.

A series of 3′-(*O*-acyl) derivatives of etilefrine was synthesized according to various methods (Table I). Their physical properties and hydrolysis rates are listed in Table II.

RESULTS AND DISCUSSION

Starting from 3′-hydroxy-2-(benzylethylamino)acetophenone (I), which was prepared according to known procedures for the synthesis of hydroxy-2-(benzylalkylamino)acetophenones (14, 15), acylation was carried out according to Methods A–E (Scheme I). The esters (II) obtained were debenzylated with hydrogen in the presence of palladium-on-carbon to give intermediates (III), which were reduced to the corresponding alcohols (IV), usually without interrupting the reaction (Method F). In cases where the substituents at the nucleus were sensitive to hydrogenolysis (IV*r*), the keto group was reduced with sodium borohydride (Method G). In one case (IV*f*), isolation of the levorotatory form (IV*g*) was achieved by reaction with (+)-dibenzoyltartaric acid (Method H).

The compounds thus obtained had characteristic IR, PMR, and mass spectra, consistent with the structures indicated. All IR spectra exhibited strong, sometimes broad absorptions at 3340–3160 cm^{-1} (OH), bands between 2500 and 2360 cm^{-1} with several splits (NH_2^+), and intensive absorptions at ~1760–1730 cm^{-1} (C=O) (Table III).

Common to all PMR spectra were CH_3 signals from NCH_2CH_3 as a triplet at ~1.3–1.4 ppm, both CH_2 absorptions due to CH_2NHCH_2 as a multiplet at 2.9–3.1 ppm, a multiplet between 5.0 and 5.5 ppm due to the CH proton from CHOH, and broad absorptions centered in the regions of 5.7–5.8 ppm (OH) and 9.0–9.2 ppm (NH). The latter absorptions disappeared on treatment with deuterium oxide. The absorptions of the *m*-disubstituted phenyl-ring protons and those due to the R group are summarized in Table III.

The mass spectra of IV*a*–IV*s* contained the molecular ions of the free amines and peaks at M − 18 (H_2O) with low intensity. In addition, diagnostic fragments appeared in all cases at *m/e* 162[1] (M − H_2O − RCO) and 134[2] (M − H_2O − RCO − CO). The dominant ions at *m/e* 58

[1] High-resolution mass spectrum: calc. for $C_{10}H_{12}NO$, 162.0919; found, *m/e* 162.0922.
[2] High-resolution mass spectrum: calc. for $C_9H_{12}N$, 134.0970; found, *m/e* 134.0967.

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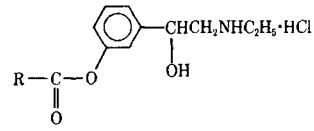


Table I—Etilefrine 3′-(*O*-Acyl) Derivatives

Compound (as Hydrochloride)	R	Melting Point	Yield (Pure), %	Method	Formula
IV*a*	CH_3	114°[a]	49	B/F	$C_{12}H_{18}ClNO_3$
IV*b*	CH_3CH_2	116.1°[b]	37	A/F	$C_{13}H_{20}ClNO_3$
IV*c*	$CH_3(CH_2)_2$	138.1°[b]	55	D/F	$C_{14}H_{22}ClNO_3$
IV*d*	$(CH_3)_2CH$	159.8°[b]	57	B/F	$C_{14}H_{22}ClNO_3$
IV*e*	$CH_3(CH_2)_3$	134.2°[b]	45	D/F	$C_{15}H_{24}ClNO_3$
IV*f*	$(CH_3)_3C$	209.4°[b]	65	A/F	$C_{15}H_{24}ClNO_3$
IV*g*[c]	$(CH_3)_3C$	225°[a]	6[d]	H[e]	$C_{15}H_{24}ClNO_3$
IV*h*	$CH_3(CH_2)_{16}$	117.4°[a]	60	E/F	$C_{28}H_{50}ClNO_3$
IV*i*	1-$CH_3C_6H_{10}$	204.3°[a]	51	D/F	$C_{18}H_{28}ClNO_3$
IV*j*	$C_6H_5CH_2$	127.6°[b]	46	B/F	$C_{18}H_{22}ClNO_3$
IV*k*	$C_6H_5CH(CH_3)$	169°[b]	51	D/F	$C_{19}H_{24}ClNO_3$
IV*l*	$C_6H_5CH(C_2H_5)$	138.4°[b]	63	D/F	$C_{20}H_{26}ClNO_3$
IV*m*	$C_{10}H_7$-1-CH_2	144°[a]	17	E/F	$C_{22}H_{24}ClNO_3$
IV*n*	4-$CH_3C_6H_4$	190.1°[a]	41	D/F	$C_{18}H_{22}ClNO_3$
IV*o*	2-$CH_3C_6H_4$	161°[a]	69	A/F	$C_{18}H_{22}ClNO_3$
IV*p*	2,4,6-$(CH_3)_3C_6H_2$	180.1°[a]	77	C/F	$C_{20}H_{26}ClNO_3$
IV*q*	2,6-$(CH_3O)_2C_6H_3$	207.1°[a]	52	C/F	$C_{19}H_{24}ClNO_5$
IV*r*	2,6-$Cl_2C_6H_3$	190.2°[a]	27	D/F/G	$C_{17}H_{18}Cl_3NO_3$
IV*s*	2-$C_6H_5C_6H_4$	186.2°[b]	55	D/F	$C_{23}H_{24}ClNO_3$

[a] Recrystallized from methanol–ether. [b] Recrystallized from isopropanol. [c] (−)-Enantiomer of IV*f*. [d] Referenced to racemate. [e] For resolution of racemic salt, see *Experimental*.

($CH_2NHC_2H_5$, mostly base peak) and *m/e* 30 (CH_2NH_2) were due to characteristic amine fragmentations. Furthermore, all mass spectra, with the exception of IV*h* and IV*q*, showed typical fragment ions (R, RCO, or RCOH) from ester cleavage (Table III).

Table II illustrates that the high water solubility of the etilefrine hydrochloride was hardly influenced by introducing short aliphatic acyl radicals (IV*a*–IV*e*). Surprisingly, water solubility was reduced markedly whenever the acyl radical was highly branched (IV*f* and IV*g*). While araliphatic acyl radicals still led to sufficient solubility in water (IV*j*, IV*l*, and IV*m*), this was no longer the case either with very long aliphatic acyl radicals (IV*h*) or with aromatic acyl radicals that carry some substituents (IV*p*–IV*r*) at the nucleus.

Phenolethanolamines in solution represent a mixture of the uncharged form and ionic species (cation, anion, and zwitterion) (16). At half-neutralization, these components are in a specific equilibrium, which is represented by an apparent average pKa value. The pKa value is dependent on the substituent at the amino nitrogen and is increased from 8.67 (norfenefrine) (17) to 8.89 (phenylephrine) (17) or 9.0 (etilefrine) (18) for the 3′-hydroxyphenylethanolamines when introducing a methyl or ethyl radical into the amino group. The same value also was obtained for etilefrine in 1.5×10^{-3} *M* aqueous solution (Table II).

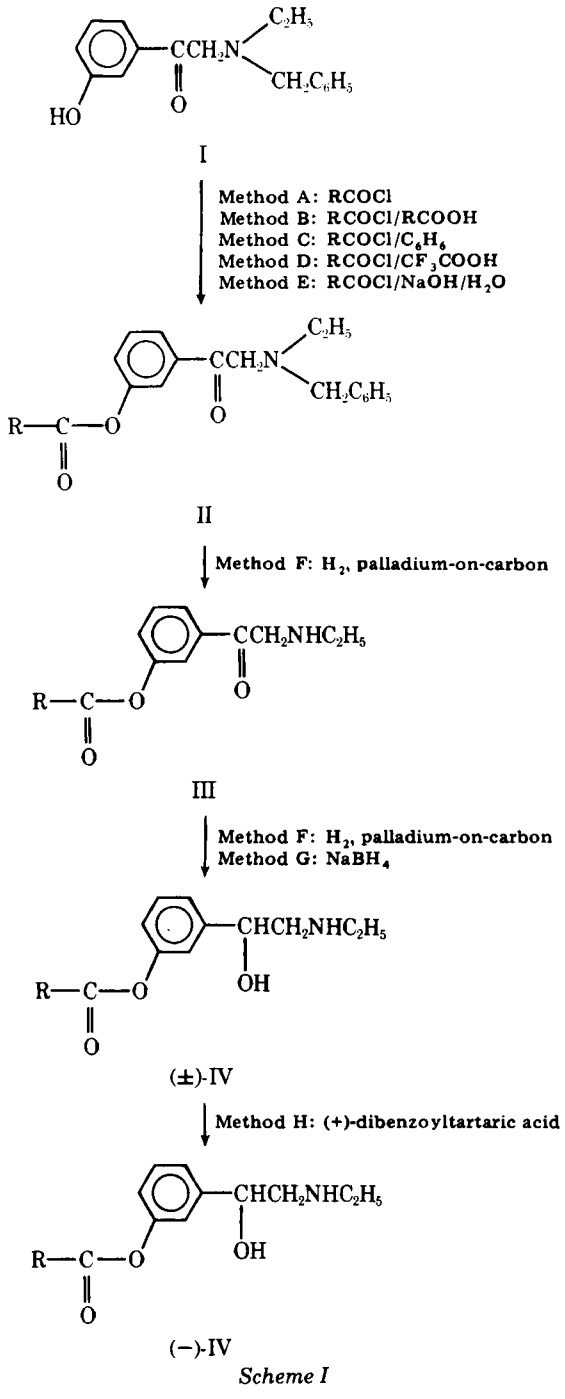
When the phenolic hydroxyl group is lost, the participating components of the dissociation equilibrium are reduced to the uncharged form and the ammonium compound. This results in a change of the pKa value (17). As shown by the pKa values of the acyl derivatives (IV*a*–IV*f*) in aqueous solution, the same effect can be observed when the phenolic hydroxyl group is masked with an acyl radical. However, in methanolic solution, the pKa values decreased from 9.4 (water) to 8.7 (90.7% methanol). The pKa values of IV*g*–IV*s* could not be ascertained because of insufficient solubility, although some of these compounds, being hydrochlorides, would be easily water soluble (IV*j*, IV*l*, and IV*m*). However, considering that all acyl derivatives in water–methanol (9.3:90.7) showed identical pKa values, it is assumed that the pKa value of 9.4 found for IV*a*–IV*f* in water would be the same for IV*g*–IV*s*.

As suggested by the pKa values, 2.45% of the etilefrine and 0.99% of the acyl derivatives (IV*a*–IV*s*) are present in aqueous solution of pH 7.4 in the uncharged form. Judging by the partition coefficient, 2.1% of etilefrine was present in the lipophilic phase. This value closely corresponds

Table II—Physical Properties and Hydrolysis Rates of Etilefrine 3′-(*O*-Acyl) Derivatives

Compound (as Hydrochloride)	Solubility[a]		pKa[b]		log *P* in *n*-Octanol–Buffer[c]	Hydrolysis Rates[d], μl of CO_2		
	Percent	*M*	Water[e]	Water–Methanol[f] (9.3:90.7)		Human Blood[g]	Rat Blood[g]	Rat Liver[h]
Etilefrine	75	3.4	9.0	8.9	−1.66			
IV*a*	60	2.3	9.4	8.7	−1.57	57.5	44	182
IV*b*	65	2.4	9.4	8.7	−0.85	47.5	46.5	226
IV*c*	60	2.1	9.4	8.7	−0.39	32	25.4	204
IV*d*	60	2.1	9.4	8.7	−0.16	34.5	32	220
IV*e*	65	2.1	9.4	8.7	0.23	30.7	34	194
IV*f*	12	4.0×10^{-1}	9.4	8.7	0.20	5.6	4.6	196
IV*g*	2	6.6×10^{-2}	—[i]	8.7	0.20	5.6	4.6	196
IV*h*	<0.05	$<1.0 \times 10^{-3}$	—[i]	8.7	>2	—[i]	—[i]	—[i]
IV*i*	9	2.6×10^{-1}	—[i]	8.7	1.5	0.4	0	45
IV*j*	55	1.6	—[i]	8.7	0.23	6.9	80.5	130
IV*k*	5	1.4×10^{-1}	—[i]	8.7	0.91	3.0	2.4	95
IV*l*	25	6.9×10^{-1}	—[i]	8.7	1.36	1.0	1.6	25
IV*m*	25	6.5×10^{-1}	—[i]	8.7	1.41	1.4	4.2	75
IV*n*	8	2.4×10^{-1}	—[i]	8.7	1.20	9.0	0.2	60
IV*o*	9	2.7×10^{-1}	—[i]	8.7	1.20	0.8	0.3	21
IV*p*	2	5.5×10^{-2}	—[i]	8.7	1.89	—[i]	—[i]	—[i]
IV*q*	1	2.6×10^{-2}	—[i]	8.7	0.38	0.3	0.1	0.1
IV*r*	0.4	1.0×10^{-2}	—[i]	8.7	1.72	—[i]	—[i]	—[i]
IV*s*	0.3	7.0×10^{-3}	—[i]	8.7	1.82	—[i]	—[i]	—[i]

[a] In water; accuracy of ±5%. [b] Accuracy of ±0.05 pK unit. [c] Phosphate buffer, pH 7.4, 0.15 *M*; initial concentrations = 1.0×10^{3}–1.8×10^{3} *M*. [d] Substrate concentration of 10^{-2} *M*. [e] $c = 1.5 \times 10^{-2}$ *M*. [f] $c = 2 \times 10^{-2}$ *M*. [g] Blood (30 μl) and substrate (30 μmoles) in 2.5×10^{-2} *M* $NaHCO_3$ (2970 μl). [h] A suspension of a liver homogenate (60 μl) and substrate (30 μmoles) in 2.5×10^{-2} *M* $NaHCO_3$ (2940 μl). [i] Not determinable because of poor solubility of the base.



Scheme I

to the existing amount of the uncharged form of etilefrine. A similar lipophilicity was shown by IV*a*, although the addition of an acetyl radical in the molecule would suggest an increase. However, since IV*a* has a higher pKa value than etilefrine itself, its normally higher lipophilicity is reduced because of the higher dissociation at pH 7.4. With increasing lipophilicity of the acyl moiety (IV*b*–IV*s*), the amount of ionized acyl etilefrine in the lipophilic phase is augmented.

The experimentally determined Δ log P values of homologs are in most cases in reasonable agreement with the reported π values (19–23). However, in some cases (IV*a*/IV*b*, IV*c*/IV*e*, and IV*j*/IV*k*), considerable discrepancies were observed. This finding is attributed to the fact that the Hansch π value system will falter whenever aliphatic groups are near a very polar moiety such as the carbonyl function.

On the basis of log P values found through experimentation, it is evident that the lipophilicity increases with the chain length (IV*b* < IV*c* < IV*e* < IV*h*). However, the lipophilicity of the *n*-acyl derivatives is not necessarily higher than that of the *iso*-acyl compounds (IV*c* < IV*d*). A similar inconsistency was observed for the 5′-(*O*-acyl) derivatives of 9-β-D-arabinofuranosyladenine (24). The levorotatory form of the pivaloyl derivative (IV*g*) showed no difference compared to the racemate (IV*f*). Even the change of position of a methyl substituent in the benzoyl radical (IV*n* and IV*o*) did not alter the lipophilicity.

After absorption through the gut wall, orally administered substances are transported by the blood into the liver by way of mesentery veins and the vena portae. Through this passage, the 3′-(*O*-acyl) derivatives of etilefrine are protected from attacking conjugating enzymes, provided they do not become subject to esterase-catalyzed hydrolysis. To obtain better insight into the cleavage, tests were conducted with these esters in diluted rat and human blood and in rat liver homogenate.

The rate of enzymatic hydrolysis of the aliphatic acyl radicals decreased in human blood with increasing alkyl chain length (IV*a* > IV*b* > IV*c* > IV*e*) or when the radical was highly branched (IV*c* > IV*f* = IV*g*) (Table II). In most cases, rat blood was slightly less active in hydrolyzing acyl radicals than was human blood, but the deviations in general were rather low. However, there was one exception. The phenyl acetyl derivative (IV*j*) was hydrolyzed extremely rapidly by rat blood, probably due to a specific esterase in this species. The hydrolysis carried out by the enzymes of the rat liver was much faster and was nearly the same with the aliphatic acyl radicals, whereas compounds with araliphatic or aromatic acyl radicals were split up according to their steric hindrance in the blood as well as in the liver. Thus, the hydrolysis rate was decreased as the size of the aromatic system of the araliphatic acyl derivatives was enlarged (IV*j* > IV*m*) or the length of the alkyl radical grew (IV*j* > IV*k* > IV*l*). For the aromatic acyl radicals, the splitting rate was reduced considerably if substituents were introduced at position 2 or positions 2 and 6 (IV*n* > IV*o* > IV*q*). Due to insufficient solubility in 2.5×10^{-2} *M* $NaHCO_3$, hydrolysis rates of IV*h*, IV*p*, IV*r*, and IV*s* could not be ascertained enzymatically.

As the data in Table II illustrate, the 3′-(*O*-acyl) derivatives of etilefrine can differ by over 100-fold with respect to solubility, lipophilicity, and enzymatic cleavage. In selecting a prodrug with improved bioavailability after oral administration, the following criteria are decisive: (*a*) sufficient solubility and enhanced lipophilicity for accelerated absorption; (*b*) very reduced enzymatic hydrolysis during absorption, thus giving improved protection against conjugation; and (*c*) extensive enzymatic hydrolysis while passing through the liver, resulting in the fast release of drug activity.

Judging by the cleavage through hepatic enzymes, the aliphatic acyl derivatives (IV*a*–IV*g*) are superior to IV*h*[3]–IV*s*. Of these compounds, IV*e*–IV*g* promise more accelerated absorption than etilefrine due to their 70 times greater lipophilicity. Since the pivaloyl derivative (IV*f*) exhibits a hydrolysis rate in the blood of less than one-fifth of that of IV*e* and possesses a solubility six times higher than that of IV*g*, IV*f* was preferred. Comparative absorption studies with dogs[4] and humans[5] confirmed the superiority of this compound to etilefrine. As shown with tritium-labeled material, the absorption rate was increased with dogs[4] and humans[5] when a pivaloyl radical was introduced, and bioavailability was increased by 46%[5].

Investigations of the pharmacological properties of etilefrine pivalate will be discussed elsewhere.

EXPERIMENTAL[6]

Chemical Methods—*Method A/F: Etilefrine 3′-Pivalate (IVf) Hydrochloride*—A mixture of I·HCl (30.6 g, 0.1 mole) in pivaloyl chloride (60.3 g, 0.5 mole) was stirred and heated slowly to 106° with an increasing rate of hydrogen chloride evolution. The suspension was maintained at

[3] After completion of these studies, results of comparative pharmacokinetic investigations on etilefrine and its stearoyl derivative (IV*h*) were published (25). It was shown that introduction of the stearoyl radical reduced the bioavailability of etilefrine by 49%.

[4] D. Henschler, unpublished results.

[5] J. H. Hengstmann, Department of Medicine, University of Bonn, Bonn, West Germany, personal communication.

[6] All melting points were obtained using a Mettler FP 1 melting-point apparatus and are uncorrected. Microanalyses were carried out at the Analytical Laboratories, 5279 Gummersbach, West Germany. Purity of intermediates not subjected to elemental analysis was ascertained by TLC performed on Merck silica gel 60 F_{254}. Spots were located under UV light or with iodine vapor. Solvent mixtures used for TLC were chloroform–methanol–water (59:33:8) (Solvent A), chloroform–methanol–water (90:9:1) (Solvent B), and chloroform–methanol (98:2) (Solvent C).

UV absorption curves were recorded on a Beckman ACT A III UV–visible spectrophotometer. IR spectra were run on a Perkin-Elmer model 257 spectrophotometer, and PMR spectra were determined on a Varian EM 360 spectrometer. Mass spectra were obtained from a Varian MAT 112 S spectrometer using a direct-sample insertion system and ionization by electron impact (70 ev). Optical rotations were measured with a Schmidt-Haensch (model Polartronic I) electronic polarimeter. The pH values were obtained using a Metrohm A 603 pH meter.

Table III—Spectral Data for Etilefrine 3′-(*O*-Acyl) Derivatives

Compound (as Hydrochloride)	IR[a], $\nu_{C=O}$	PMR[b] Solvent	PMR[b] δ for $RCOOC_6H_4$—	Mass Fragmentations, *m/e* (relative intensity, %)
IV*a*	1760	A	1.38 (t, 3H, CH_3) and 6.73–7.53 (m, 4H, aromatic H)	223 (M^+, 0.1) and 43 (8)
IV*b*	1760	A	1.22 (t, 3H, CH_3), 2.55 (q, 2H, CH_2), and 6.73–7.47 (m, 4H, aromatic H)	237 (M^+, 0.1), 29 (16), and 57 (9)
IV*c*	1760	A	1.02 (t, 3H, CH_3), 1.73 (m, 2H, CH_2CH_3), 2.50 (t, 2H, $COCH_2$), and 6.80–7.50 (m, 4H, aromatic H)	251 (M^+, 0.2), 43 (15), and 71 (9)
IV*d*	1755	A	1.27 [d, 6H, $CH(CH_3)_2$], 2.90 (m, 1H, CH), and 6.73–7.50 (m, 4H, aromatic H)	251 (M^+, 0.5), 43 (28), and 71 (11)
IV*e*	1760	A	0.95 (t, 3H, CH_3), 1.20–1.97 [m, 4H, $(CH_2)_2CH_3$], 2.52 (t, 2H, $COCH_2$), and 6.75–7.46 (m, 4H, aromatic H)	265 (M^+, 0.3), 57 (8), and 85 (3)
IV*f*	1745	B	1.33 [s, 9H, $C(CH_3)_3$] and 6.83–7.60 (m, 4H, aromatic H)	265 (M^+, 0.2), 57 (20), and 85 (2)
IV*h*	1750	A	0.90 (t, 3H, CH_3), 1.05–1.93 (m, 30H, $(CH_2)_{15}CH_3$), 2.50 (t, 2H, $COCH_2$), and 6.80–7.37 (m, 4H, aromatic H)	447 (M^+, 0.1) and 267 (0.7)
IV*i*	1745	A	1.07–2.45 (m, 13H, 1-$CH_3C_6H_{10}$) and 6.81–7.50 (m, 4H, aromatic H)	305 (M^+, 0.3), 97 (11), and 125 (0.7)
IV*j*	1740	A	3.80 (s, 2H, CH_2) and 6.70–7.50 (m, 9H, aromatic H)	299 (M^+, 0.1), 91 (18), and 118 (6)
IV*k*	1755	A	1.58 (d, 3H, CH_3), 3.93 (q, 1H, CH), and 6.7–7.5 (m, 9H, aromatic H)	313 (M^+, 0.4), 105 (33), and 132 (10)
IV*l*	1750	A	0.95 (t, 3H, CH_3), 1.95 (m, 2H, CH_2), 3.65 (t, 1H, CH), and 6.67–7.50 (m, 9H, aromatic H)	327 (M^+, 0.6), 119 (14), and 146 (12)
IV*m*	1750	A	4.21 (s, 2H, CH_2) and 6.68–8.10 (m, 11H, aromatic H)	349 (M^+, 1), 141 (39), and 168 (21)
IV*n*	1730	B	2.45 (s, 3H, CH_3)and 6.96–8.15 (m, 8H, aromatic H)	299 (M^+, 1), 91 (43), and 119 (100)
IV*o*	1730	A	2.61 (s, 3H, CH_3) and 6.88–8.25 (m, 8H, aromatic H)	299 (M^+, 2), 91 (52), and 119 (95)
IV*p*	1730	A	2.30 (s, 3H, 4-CH_3), 2.40 (s, 6H, 2- and 6-CH_3), and 6.76–7.43 (m, 6H, aromatic H)	327 (M^+, 1), 119 (25), and 147 (100)
IV*q*	1740	B	3.86 (s, 6H, 2O-CH_3) and 6.58–7.63 (m, 7H, aromatic H)	345 (M^+, 1) and 165 (100)
IV*r*	1750	C	7.06–7.82 (m, 7H, aromatic H)	353 (M^+, 0.1), 145 (5), and 173 (16)
IV*s*	1740	B	6.62–8.08 (m, 13H, aromatic H)	361 (M^+, 2), 153 (25), and 181 (75)

[a] IR spectra were run in potassium bromide, and absorption values are in reciprocal centimeters. [b] PMR spectra were run at 60 MHz in deuterochloroform (A), methanol-d_4 (B), or dimethyl sulfoxide-d_6 (C), and chemical shifts (δ) are given in parts per million relative to tetramethylsilane as the internal standard. Multiplicities of the peaks are expressed as singlet (s), doublet (d), triplet (t), quartet (q), and multiplet (m).

this temperature until the starting material went into solution; the temperature then was raised to 110° and maintained there until cessation of gas evolution (~2 hr). After evaporation of the remaining pivaloyl chloride, the residue was dissolved in methanol (30 ml) and poured into petroleum ether (700 ml). The precipitate, R_f 0.8 on TLC (Solvent B), was filtered, dissolved in isopropanol–water (330 ml), and hydrogenated in the presence of 10% palladium-on-carbon (3.3 g) at room temperature and normal pressure until the calculated amount of hydrogen had reacted (about 3 hr). After removal of the catalyst and then the solvent by vacuum evaporation, the residue was crystallized from isopropanol to give 19.6 g (65%) of IV*f*·HCl, mp 209.4°, R_f 0.4 (Solvent A).

Anal.—Calc. for $C_{15}H_{23}NO_3$·HCl: C, 59.69; H, 8.01; N, 4.64. Found: C, 59.69; H, 7.91; N, 4.39.

*Method B/F: Etilefrine 3′-(2-Methylpropionate) (IV*d*) Hydrochloride*—A suspension of I·HCl (30.6 g, 0.1 mole) in 2-methylpropionyl chloride (85.2 g, 0.8 mole) and 2-methylpropionic acid (85 ml) was stirred and heated slowly until the starting material went into solution; then the mixture was refluxed for 0.5 hr. After evaporation to dryness, the residue, R_f 0.8 on TLC (Solvent B), was reprecipitated and hydrogenated under the same conditions as described for the preparation of IV*f*·HCl to give 16.4 g (57%) of IV*d*·HCl after crystallization from isopropanol, mp 159.8°, R_f 0.4 (Solvent A).

Anal.—Calc. for $C_{14}H_{21}NO_3$·HCl: C, 58.43; H, 7.71; N, 4.87. Found: C, 58.48; H, 7.59; N, 4.85.

*Method C/F: Etilefrine 3′-(2,4,6-Trimethylbenzoate) (IV*p*) Hydrochloride*—A mixture of I·HCl (30.6 g, 0.1 mole) in 2,4,6-trimethylbenzoyl chloride (36.5 g, 0.2 mole) and benzene (20 ml) was refluxed for 1.5 hr. The mixture was extracted at room temperature with petroleum ether. The bottom layer was separated and evaporated under reduced pressure. The residue, R_f 0.8 on TLC (Solvent B), was dissolved in isopropanol–water (450 ml) and hydrogenated in the presence of 10% palladium-on-carbon (11.0 g) in the same manner as described for the preparation of IV*f*·HCl to give 28.0 g (77%) of IV*p*·HCl after recrystallization from methanol–ether, mp 180.1°, R_f 0.4 (Solvent A).

Anal.—Calc. for $C_{20}H_{25}NO_3$·HCl: C, 66.01; H, 7.20; N, 3.85. Found: C, 65.96; H, 7.10; N, 3.81.

*Method D/F: Etilefrine 3′-(4-Methylbenzoate) (IV*n*) Hydrochloride*—To a suspension of I·HCl (30.6 g, 0.1 mole) in trifluoroacetic acid (39.9 g, 0.35 mole) was added 4-methylbenzoyl chloride (30.9 g, 0.2 mole) over 0.5 hr with vigorous evolution of hydrogen chloride. The mixture was refluxed for 1.5 hr. The volatile portion was distilled off under vacuum, and the residue was stirred in ether. The precipitate was poured into a mixture of ether (500 ml) and water (100 ml). After cooling, the two-phase system was adjusted to pH 9 with cold ammonium hydroxide. The organic layer was separated and washed twice with cold 1 *N* NaOH (400 ml) and water (400 ml). After removal of the solvent under vacuum, the residue, R_f 0.8 on TLC (Solvent B), was dissolved in isopropanol–water (300 ml), acidified to pH 2 with hydrochloric acid, and hydrogenated in the presence of 10% palladium-on-carbon (6.5 g) in the same manner as described for the preparation of IV*f*·HCl to give 13.8 g (41%) of IV*n*·HCl after recrystallization from methanol–ether, mp 190.1°, R_f 0.4 (Solvent A).

Anal.—Calc. for $C_{18}H_{21}NO_3$·HCl: C, 64.38; H, 6.60; N, 4.17. Found: C, 64.43; H, 6.56; N, 4.17.

*Method D/F/G: Etilefrine 3′-(2,6-Dichlorobenzoate) (IV*r*) Hydrochloride*—A mixture of I·HCl (30.6 g, 0.1 mole) and 2,6-dichlorobenzoyl chloride (42.0 g, 0.2 mole) in trifluoroacetic acid (114.0 g, 1 mole) was stirred and heated slowly to 80° with an increasing rate of hydrogen chloride evolution. Then the mixture was refluxed for 1.5 hr. After evaporation of the volatile portion, the oily residue was poured into a mixture of ice and ammonium hydroxide and extracted twice with ether (500 ml). The organic layer was separated and washed with cold 1 *N* NaOH and water. The solvent was evaporated to give 23.0 g (52%) of 3′-hydroxy-2-(benzylethylamino)acetophenone 3′-(2,6-dichlorobenzoate) (II*r*) as an oil, R_f 0.9 (Solvent B).

A solution of II *r* (23.0 g, 0.052 mole) in methanol–water (9:1, 300 ml) was adjusted to pH 2 with hydrochloric acid and then hydrogenated in the presence of 10% palladium-on-carbon (2.3 g) at room temperature and atmospheric pressure until 1 equivalent of hydrogen had reacted. After the catalyst and then the solvent were removed by vacuum evaporation, the residue was crystallized from methanol–isopropanol to give 12.0 g (60%) (31% referenced to I·HCl) of 3′-hydroxy-2-(ethylamino)-acetophenone 3′-(2,6-dichlorobenzoate) (III*r*) hydrochloride, mp 170° dec., R_f 0.85 (Solvent A). The PMR and IR spectral data supported the assigned structure.

Sodium borohydride (4.0 g) was added in portions to a mixture of III*r*·HCl (12.0 g, 0.031 mole) in dry methanol (200 ml) over 1 hr while the temperature of the reaction was kept below 5°. Excess sodium borohydride was destroyed by the addition of acetic acid. After evaporation to dryness, the residue was suspended in diluted ammonium hydroxide (200 ml) and extracted with ether. The organic layer was separated, washed with water, and evaporated to dryness. The residue was dissolved in isopropanol–water (9:1, 400 ml), acidified to pH 2 with hydrochloric acid, and concentrated. The solid residue was crystallized with methanol–ether to give 10.5 g (87%) (27% referenced to I·HCl) of IV*r*·HCl, mp 190.2°, R_f 0.4 (Solvent A).

Anal.—Calc. for $C_{17}H_{17}Cl_2NO_3$·HCl: C, 52.26; H, 4.64; N, 3.59. Found: C, 52.35; H, 4.63; N, 3.58.

Method E/F: Etilefrine 3′-Stearate (IVh) Hydrochloride—A mixture of I·HCl (30.6 g, 0.1 mole) and stearoyl chloride (33.3 g, 0.11 mole) in 1 *N* NaOH (250 ml) was stirred at ambient temperature for 1 hr. The mixture was extracted with ether, and the organic layer was washed with 1 *N* NaOH and water.

After removal of the solvent under reduced pressure, the waxy residue, R_f 0.8 on TLC (Solvent C), was taken up in methanol (1 liter) and adjusted to pH 2 with hydrochloric acid. The mixture was hydrogenated in the presence of 10% palladium-on-carbon (10.0 g) at 40° and atmospheric pressure until the calculated amount of hydrogen had reacted. After the catalyst and solvent were removed by vacuum evaporation, the residue was crystallized from methanol–ether to give 29.1 g (60%) of IV*h*·HCl, mp 117.4°, R_f 0.5 (Solvent A).

Anal.—Calc. for $C_{28}H_{49}NO_3$·HCl: C, 69.46; H, 10.41; N, 2.89. Found: C, 69.82; H, 10.27; N, 2.77.

Method H: (−)-Etilefrine 3′-Pivalate (IVg) Hydrochloride—To a mixture of IV*f*·HCl (301.8 g, 1 mole) in water (2.7 liters) and (+)-*O,O*-dibenzoyltartaric acid monohydrate (188.0 g, 0.5 mole) in methanol (2.7 liters) was added sodium bicarbonate (42.0 g, 0.5 mole) at room temperature. The solution was cooled slowly to 2° and maintained at this temperature for 5 days. The white crystalline precipitate was filtered off and washed with cold methanol–water (1:1). This product was recrystallized (13 times) from methanol–water (55:45) until the optical rotation remained constant. The yield was 39.1 g of (−)-etilefrine 3′-pivalate (+)-*O,O*-dibenzoylhydrogentartrate, mp 163.5° dec., $[\alpha]_D^{20}$ +55.3° (*c*, 1 in methanol).

This product was dissolved in methanol–water (9:1, 200 ml), acidified to pH 2 with hydrochloric acid, and concentrated. The residue was crystallized with methanol–ether to give 18.1 g of IV*g*·HCl (6%) referenced to IV*f*·HCl, mp 225°, R_f 0.4 (Solvent A), $[\alpha]_D^{20}$ −36.7° (*c*, 1 in methanol).

Anal.—Calc. for $C_{15}H_{23}NO_3$·HCl: C, 59.69; H, 8.01; N, 4.64. Found: C, 59.72; H, 7.98; N, 4.58.

Determination of Solubility—An excess of each compound (100–1000 mg weighed accurately) was stirred in a portion of water at room temperature for 1 hr. Additional portions of water were added at 1-hr intervals until the entire sample was dissolved under stirring. The percentage solubility, *S*, was calculated according to:

$$S(\%) = \frac{\text{weight}_{\text{sample}}}{\text{weight}_{\text{solution}}} \times 100 \qquad \text{(Eq. 1)}$$

Measurement of pKa Values—The pKa values listed in Table II were determined by measuring the pH of a solution containing equivalent concentrations of the amine and its salt. These solutions were obtained by adding to a solution of the salt the calculated amount of aqueous 0.1 *N* NaOH for half-neutralization. The concentrations of the compound were 1.5×10^{-3} *M* in water and 2×10^{-2} *M* in water–methanol. For IV*g*–IV*s*, which were insufficiently soluble in water at the point of half-neutralization, aqueous solutions were made up to contain 90.7% methanol at half-neutralization. The pH values were measured using a pH meter with a digital scale at room temperature. The results were estimated to be accurate to ±0.05 pK unit.

The percentage of the protonated form, $[BH]^+$, at pH 7.4 is expressed by:

$$[BH]^+(\%) = \frac{100}{1 + \text{antilog}\,(7.4 - \text{pKa})} \qquad \text{(Eq. 2)}$$

Measurement of Partition Coefficients—For the partitioning, pure *n*-octanol saturated with 0.15 *M* phosphate buffer (pH 7.4) and phosphate buffer saturated with *n*-octanol were used. Normally, 20–40-mg samples were dissolved in the buffer solution (50 ml) and shaken with the *n*-octanol solution (50–600 ml) at room temperature for 0.5 hr. After centrifugation, an aliquot of the bottom layer was removed and diluted to give an absorbance range of 0.2–0.7, measured at the absorption maximum of the compounds (206–223 nm) using a 1-cm cell. A standard curve of at least four points was used for each sample to determine the concentration in the bottom layer. Multiple analyses showed the data in Table II to be reproducible. The partition coefficient, *P*, was calculated from:

$$P = \frac{V_{\text{buffer}}}{V_{n\text{-octanol}}} \left(\frac{C_{\text{buffer},\,i}}{C_{\text{buffer},\,f}} - 1 \right) \qquad \text{(Eq. 3)}$$

The first factor in Eq. 3 is the ratio of the volumes of the buffer and *n*-octanol used for partitioning of the sample. The second factor contains the ratio of the initial to the final concentration in the buffer layer, as determined by readings of the absorbance in the UV region.

Determination of Enzymatic Ester Cleavage—The Warburg manometric technique was used as described by Ammon (26). Blood was obtained from one healthy female volunteer and from rats (female Wistar, 180 g). One part of rat liver (female Wistar, 180 g) was homogenized in one part of buffer (2.5×10^{-2} *M* $NaHCO_3$).

Heparinized blood (30 μl) or rat liver homogenate (60 μl) diluted with buffer to 300 μl was pipetted into the side arm, and the solution of the respective ester substrate in the buffer (30 μmoles/2700 μl) was put into the main trough of the calibrated reaction vessels. After equilibration for 10 min with a stream of nitrogen and carbon dioxide (95:5), the vessels were closed, and the reaction was started by mixing the enzyme preparation with the substrate–buffer solution. Readings were taken at 5-min intervals for up to 1 hr, corrected for volume and actual atmospheric pressure, transformed into volume units, and plotted as microliters of carbon dioxide against time. The plotted curves were idealized graphically and adjusted for spontaneous hydrolysis as determined in the control vessels without the addition of enzymes. With some sparingly water-soluble substrates, stock solutions were obtained with pure ethanol. With final ethanol concentrations not exceeding 5%, no inhibition of esterase activities was recorded.

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