part, to the removal of small aliquots after each chain lengthening for ninhydrin reaction and for amino acid analyses. From this point on 5.0 g of peptidyl-resin was used for the continuation of the synthesis. The 23-peptidyl resin weighed only 5.78 g. There are obvious losses in the deprotection steps: trifluoroacetic acid removes a not negligible portion of the peptide from the resin.

15-Lysine-secretin-(5–27). A sample of the tricosapeptide resin (400 mg) was treated with 20% trifluoromethanesulfonic acid in trifluoroacetic acid (4 mL) for 2 h. After filtration, the resin was washed with F_3Ac (5 mL). The acids were removed in vacuo and the residue was dried in vacuo over NaOH. After 1 h the crude material was treated with ether and the ether solubles were removed by centrifugation. After overnight storage in a desiccator in vacuo over NaOH, the residue was dissolved in methanol (1 mL) and applied to a column of Sephadex LH-20 poured in methanol (30 g, 2.4 × 45 cm) and eluted with methanol; 1-mL fractions were collected. Fractions 55–70 contained the major component. After removal of the solvents the residue was dissolved on 50% aqueous methanol (1 mL) and passed through a small column (0.7 × 7.5 cm) of Dowex 1-acetate. The column was washed with the same solvent mixture (9 mL). After evaporation the residue was dissolved in 0.005 N HCl (1 mL) and applied to a Sephadex LH-20 column (30 × 2.4 cm) prepared with 0.005 N HCl. Elution was carried out with the same solvent. After 40 mL of eluate 2.5-mL fractions were collected. They were monitored by UV absorption and also examined by TLC (silica gel, fluorescamine, and charring, solvent system A). Fractions 21–34 contained the desired tricosapeptide amide (56 mg). According to the recovery in amino acid analysis, this material contained only 28 mg of peptide corresponding to an overall yield of 13%: R_f (cellulose) 0.58; R_f (silica gel) 0.33. Amino acid analysis after acid hydrolysis gave Lys, 1.1; Arg, 3.6; Thr, 1.6; Ser, 2.6; Glu, 3.1; Gly, 1.0; Ala, 1.1; Val, 1.0; Leu, 6.1; Phe, 0.8. After hydrolysis with aminopeptidase M, amino acid analysis gave Lys, 1.1; Arg, 3.7; Thr, 1.7; Glu, 0.6; Gly, 1.0; Ala, 1.1; Val, 1.0; Leu, 5.9; Phe, 0.8. (Ser and Gln were not determined since their peaks were not separated.[25])

Biological Tests. The experiments with smooth muscle preparations were carried out as described for VIP.[21] The flow of pancreatic juice was determined in rats.[5] The affinity of VIP and secretin receptors in acinar cells from guinea pig pancreas (Table I) was measured according to the procedure described in ref 23.

Attempted Synthesis of 15-Lysine-Secretin. Continuation of the chain lengthening until the 27-peptide was completed, cleavage from the resin with CF_3SO_3H, and purification as described for the 23-peptide gave a product which was homogeneous by TLC and had quite satisfactory amino acid analysis. After digestion with aminopeptidase M, however, the values for Asp and Gly were very low (about 0.2 for Asp and 1.2 for Gly). We had to assume that ring closure took place at residues 3 and 4. The enzymic hydrolysis seems to skip aminosuccinylglycine and to go on with the hydrolysis in the rest of the chain. A similar situation can be discerned in the studies of Ondetti and his associates,[24] who used also aminopeptidase M in the digestion of aminosuccinyl peptides.

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Synthesis and β-Adrenoceptor Activity of the Erythro and Threo Isomers of Substituted α-Hydroxytrimetoquinol

Peter Osei-Gyimah, Michael T. Piascik, John W. Fowble, Dennis R. Feller, and Duane D. Miller*

Divisions of Medicinal Chemistry and Pharmacology, College of Pharmacy, The Ohio State University, Columbus, Ohio 43210.
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The synthesis and pharmacological activity of erythro and threo isomers of 1-(3′,4′,5′-trimethoxy-α-hydroxybenzyl)-6,7-dihydroxy-1,2,3,4-tetrahydroisoquinoline, **2** and **3**, are reported. The structural assignments of **2** and **3** are based upon NMR spectra of the 6,7-dibenzyl precursors, **6** and **10**, and of the synthetic derivatives of 13α- and 13β-hydroxy-2,3-(dibenzyloxy)-9,10,11-trimethoxytetrahydroprotoberberine, **8** and **12**, respectively. The erythro isomer **2** was a more potent β-adrenoceptor stimulant than the threo isomer **3** in guinea pig atrial, guinea pig tracheal, and rat adipocyte preparations. The differential activity of these compounds on lipolysis was favorably correlated to changes in the stimulation of adenylate cyclase activity and cAMP accumulation in rat adipocytes.

Although 1-benzyltetrahydroisoquinolines have been studied intensively, both chemically and pharmacologically,[1–3] the corresponding α-oxo- and α-hydroxybenzyltetrahydroisoquinolines are less frequently encountered

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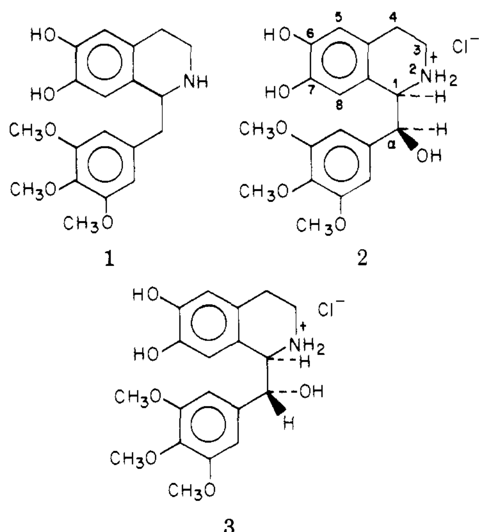
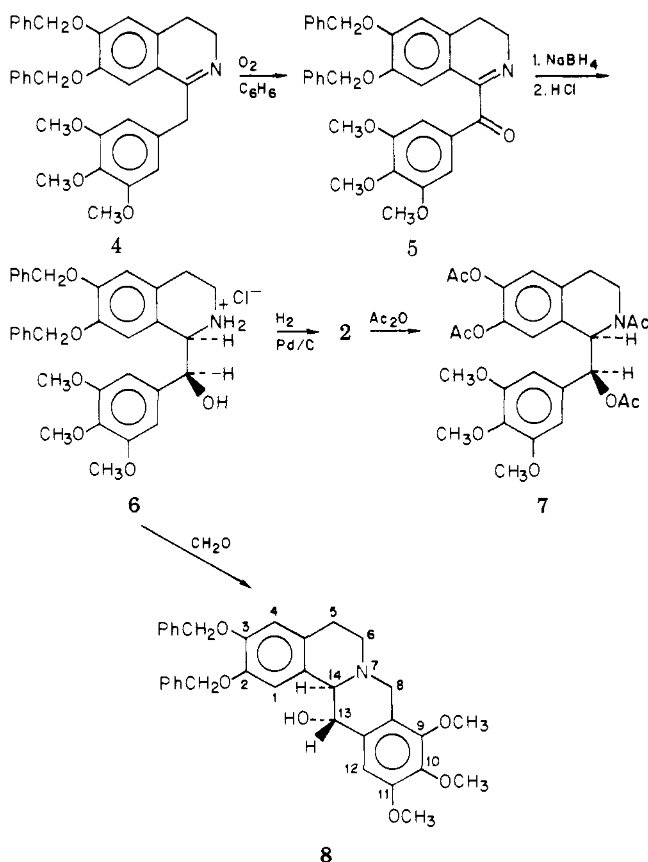
in the literature.[4-6] We wish to report the synthesis and the pharmacological profile and biochemical mechanism of β-adrenergic activity possessed by the *erythro*- and *threo*-α-hydroxy analogues **2** and **3** of the potent bronchodilator, trimetoquinol (1). These compounds, **2** and **3**, were prepared in a program attempting to find more selective adrenergic drugs since trimetoquinol possesses β_1- and β_2-adrenergic stimulant actions.

Chemistry. In our report of the synthesis of trimetoquinol (1),[2] an intermediate, 1-(3′,4′,5′-trimethoxy-

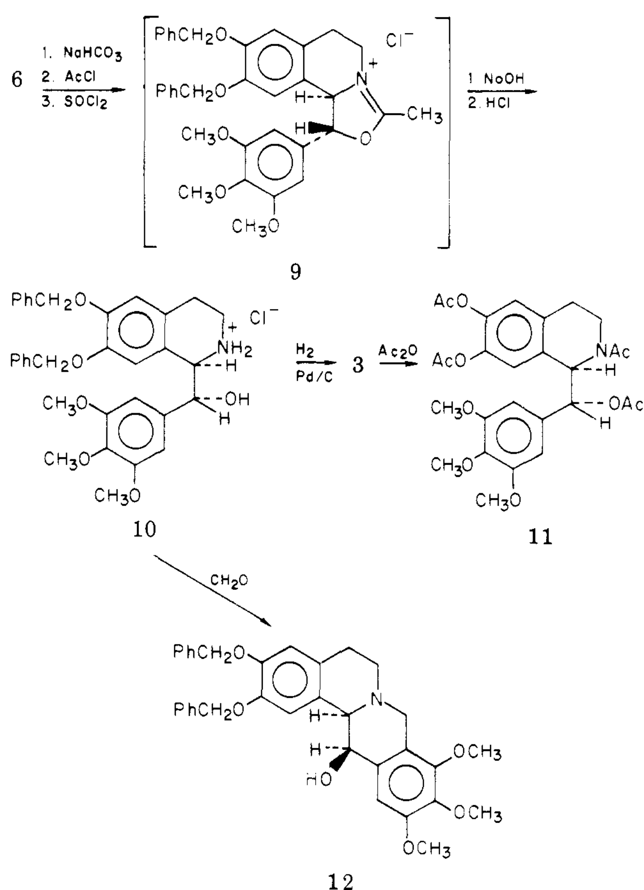


benzyl)-6,7-(dibenzyloxy)-3,4-dihydroisoquinoline (4),[2,7] was reported to undergo oxidation rapidly in benzene solution[8] to yield the α-oxo derivative **5** (Scheme I). Treatment of **5** with sodium borohydride in methanol solution afforded stereoselectively 1-(3′,4′,5-trimethoxy-

Scheme I



Scheme II



α-hydroxybenzyl)-6,7-(dibenzyloxy)-1,2,3,4-tetrahydroisoquinoline (**6**), which served as a precursor to both the desired erythro and threo isomers of 1-(3′,4′,5′-trimethoxy-α-hydroxybenzyl)-6,7-dihydroxy-1,2,3,4-tetrahydroisoquinolines **2** and **3**.

In the NMR spectrum of the free base of **6**, the C-1 proton appeared as a doublet at δ 4.21 and the C-α proton at δ 4.84 ($J_{1,\alpha}$ = 4.4 Hz). Compound **6** was tentatively assigned as the erythro isomer based on the small vicinal coupling constant, which indicated that on the average the dihedral angle between the C-1 and C-α protons would be small.[9-13] Removal of the benzyl-protecting groups by catalytic hydrogenation afforded the desired catechol **2** which was further characterized as the tetraacetyl derivative **7**.

Prompted by an earlier report[4] that the stereochemistry between the C-13 and C-14 protons of C-13 hydroxytetrahydroprotoberberines was determined by NMR studies, we treated *erythro*-**6** with formalin solution under Mannich conditions to yield the tetrahydroprotoberberine **8**. In the NMR spectrum of **8** the C-13 proton doublet appeared at δ 4.45 ($J_{13,14}$ = 8.7 Hz). The C-14 proton doublet collapsed into a singlet at δ 3.26 when the C-13 proton was irradiated (decoupled) at δ 4.45. The coupling constant of 8.7 Hz was consistent with the approximate 180° dihedral angle between the C-13 and C-14 protons. Bohlman bands in the infrared spectrum at 2750, 2790, 2810, and 2820 cm^{-1} indicated a trans B/C ring juncture; therefore, **8** has the stereochemistry indicated in Scheme I.

The conversion of the protected amino alcohol **6** to **3** is outlined in Scheme II. Using a method similar to that reported by Mardle et al.,[9] sequential treatment of the free base **6** with acetyl chloride in the presence of triethylamine and then with thionyl chloride provided an intermediate

Table I. Intrinsic Activity (I) and Affinity Constants (pD_2) for (±)-Trimetoquinol (1) and the Erythro and Threo Isomers of α-Hydroxytrimetoquinol (2 and 3, Respectively) in β-Adrenoceptor Systems[a]

pharmacological system	analogue						
	(±)-1		(±)-2		(±)-3		
	I	pD_2	I	pD_2	I	pD_2	IAR[b]
A. guinea pig trachea (relaxation)	0.88	9.0 ± 0.2	0.88	6.8 ± 0.2	0.76	6.0 ± 0.1	0.8
B. guinea pig atria (positive chronotropy)	0.9	9.1 ± 0.1	0.8	6.5 ± 0.1	0.78	6.1 ± 0.1	0.4
C. rat adipocytes							
1. lipolysis (glycerol release)	0.86	7.3 ± 0.1	0.75	7.0 ± 0.1	0.44	6.8 ± 0.1	0.2
2. cAMP accumulation	0.8	7.7 ± 0.5	0.86	6.7 ± 0.3	0.86	6.4 ± 0.2	0.3
3. adenylate cyclase activation	0.75	8.5 ± 0.1	0.55	6.8 ± 0.2	0.49	6.2 ± 0.1	0.6

[a] Within each test system, the intrinsic activity (I) was calculated as the maximal response obtained with each drug to the maximum response obtained with (−)-isoproterenol. Drug concentrations used were in the 10^{-10}–10^{-4} M range. Each point on the dose–response curves for the test systems represented the mean of 4–12 observations. pD_2 values are expressed as the mean ± SEM. [b] Negative logarithm of the molar ED_{50} of (±)-2 minus negative logarithm of the molar ED_{50} of (±)-3.

that was assumed to be the *trans*-oxazoline derivative. Alkaline hydrolysis of **9** gave **10** which was hydrogenated as the hydrochloride salt over Pd/C catalyst to afford the desired catechol **3**, which was characterized further as the tetraacetyl derivative **11**. In contrast to the low coupling constant ($J_{1,\alpha}$ = 4.4 Hz) of the vicinal protons of **6**, a coupling constant $J_{1,\alpha}$ = 7.3 Hz was obtained from the C-α proton at δ 4.52. The C-1 proton was obscured by the methoxy signals. NMR vicinal coupling constants have been a powerful tool in the classification of diastereomeric β-amino alcohols into their erythro and threo configurations.[9–13] In such cases it has been noted that the vicinal coupling constants of erythro isomers are smaller than threo isomers, and this is consistent with our structural assignments of **6** and **10**. Unique to the threo compound **10** was the observation that one set of benzyloxymethylene protons showed nonequivalency with the methylene protons appearing as a set of doublets at δ 4.62 and 4.81 (J = −12.1 Hz). To confirm the stereochemistry of **10** it was treated with formalin solution to give the tetrahydroprotoberberine **12**. The spectrum of **12** showed a broad doublet at δ 4.67 due to coupling with both the hydroxyl and C-14 proton. After deuterium exchange the C-13 proton appeared as a partially resolved doublet at δ 4.62 ($J_{13,14}$ = 1.0 Hz). This small coupling constant was in accord with an approximate 60° dihedral angle between the C-13 and C-14 protons. Bohlman bands in the infrared spectrum at 2760, 2810, and 2820 cm^{-1} indicate a trans B/C ring juncture; thus, **12** has the stereochemistry indicated in Scheme II.

Biological Results. A summary of the dose-dependent β-adrenoceptive stimulatory properties of trimetoquinol (**1**) and the α-hydroxy derivatives (**2** and **3**) in guinea pig tracheal and atrial and rat adipocyte preparations is presented in Table I. It is clear that the α-hydroxy isomers of trimetoquinol possess less activity than **1** in these β-adrenoceptor tissue systems. The rank order of potency for these THI's is **1** > **2** > **3**. On the basis of the isomeric activity ratio (IAR) calculated for the α-hydroxy derivatives, analogue **2** was 1.6-, 2.5-, and 6.3-fold more potent than **3** on lipolysis, atrial heart rate, and tracheal relaxation, respectively. As can be seen similar intrinsic activities were obtained for these analogues in guinea pig atria and trachea. The only notable exception is that **3** possessed a much lower maximum drug effect than either **1** or **2** on lipolysis. The observed reduction in intrinsic activity for **3** is related to the fact that the highest drug concentration studied in rat adipocytes was 10^{-5} M; therefore, the maximal lipolytic effect and affinity (pD_2 value) for **3** in this preparation could not be accurately estimated in the molar concentration range used.

These tetrahydroisoquinolines were also assessed for their ability to produce changes in intracellular cAMP levels and in the activity of adenylate cyclase in rat adipocyte preparations (see Table I, part C, 2 and 3). Again the parent drug (**1**) was a more potent stimulant than the corresponding α-hydroxy isomers. Of the latter compounds, *erythro*-α-hydroxytrimetoquinol (**2**) was two and four times more potent than the threo isomer in cAMP accumulation and adenylate cyclase activation in rat adipocytes, respectively. The quantitative differences in cAMP accumulation and formation induced by these tetrahydroisoquinolines in rat adipocytes are in good agreement with their differential pharmacological effects in these β-adrenoceptor systems.

Discussion

The data reported herein demonstrate that the substitution of trimetoquinol on the α carbon attached at the 1 position gave analogues which retain a stereoselective effect in β-adrenoceptor tissues. Both of the diasteromeric α-hydroxytrimetoquinol analogues showed a reduced potency as compared to trimetoquinol (**1**). Additionally, the isomeric activity ratio for the diastereoisomers of α-hydroxytrimetoquinol remained relatively constant in the β-adrenoceptor tissues examined (see Table I). These latter findings are suggestive of a common site of interaction in the various β-adrenoceptor systems.[14]

The initial interaction of catecholamines with the β-receptor-coupled membrane-bound adenylate cyclase system has been correlated for the pharmacological and physiological response of β-adrenoceptor tissues, including rat adipocytes.[15,16] The quantitation of drug effects on the activity of adenylate cyclase and on the level of intracellular cyclic nucleotide (cAMP) has served as a valuable biochemical model for the delineation of the β-adrenoceptor mechanism for catecholamines.[17] Previously, Grunfeld et al.[18] found that **1** was a stimulant of frog erythrocyte adenylate cyclase (a β_2-adrenoceptor system). In the present study, **1** and the α-hydroxytrimetoquinol analogues produced a dose-dependent elevation in intracellular cAMP and activation of adenylate cyclase which were in good quantitative agreement with their differential pharmacological effects in rat adipocytes (a β_1-adrenoceptor system). In this regard we have reported that **1–3** are unable to inhibit cAMP phosphodiesterase activity in rat adipose tissue.[19] Inamasu et al.[20] have demonstrated in tracheal muscle that the intracellular cAMP accumu-

lation induced by 1 is also unrelated to an inhibition of cAMP phosphodiesterase activity. These workers demonstrated a dose-dependent and temporal elevation in cAMP levels by trimetoquinol in tracheal muscle which coincided with the pharmacological effects. Taken together, the positive correlation between biochemical and pharmacological effects for tetrahydroisoquinolines clearly supports the notion that the β-adrenoceptor actions of catecholamines and trimetoquinol analogues are mediated by an identical biochemical mechanism. However, quantitative radioligand binding displacement analysis will be required to fully establish the similarity or dissimilarity in β-receptor occupation for these analogues.

The close structural resemblance between catecholamines and 1 has been proposed as the basis for the β-adrenoceptor properties possessed by 1 and closely related tetrahydroisoquinolines.[21] In our laboratories, modification of 1 by fragmentation has revealed analogues with less activities,[2,22,23] while the addition of certain functionalities to the basic nucleus of trimetoquinol has led to a class of selective β-adrenoceptor blocking agents.[24] In the present study, substitution at the α-carbon atom of the 1-(3′,4′,-5′-trimethoxybenzyl) group has resulted in the retention of significant potency as compared to the β-adrenoceptor activity of 1, albeit the erythro isomer was about 0.3–2.6 log units less active than the parent drug (1). Since our studies have shown that the α-OH-TMQ isomers possess significant biological activity, we feel that further structural modification at the α-carbon position of trimetoquinol is warranted.

Experimental Section

All melting points were taken on a Thomas-Hoover melting point apparatus and were corrected. Infrared spectra were obtained on Perkin-Elmer Model 257 and Beckman Model 4230 spectrophotometers. The NMR spectra were recorded using Varian A-60A and Bruker HX-90E NMR spectrometers with Me_4Si as internal standard. The mass spectra were determined with a Du Pont Model 491 (direct inlet mode) at an ionizing voltage of 70 eV. Chemical analyses were determined by Galbraith Laboratory, Knoxville, Tenn. Analytical results for elements indicated were within ±0.4% of the theoretical values.

1-(3′,4′,5′-Trimethoxybenzoyl)-6,7-(dibenzyloxy)-3,4-dihydroisoquinoline (5). A solution of 4[2,7] (2.0 g, 0.004 mol) in 20 mL of C_6H_6 was refluxed on a steam bath for 0.5 h. On cooling 40 mL of hexane was added to the mixture and allowed to crystallize in the refrigerator. The solid was recrystallized twice from C_6H_6–hexane to give 5 as a light yellow solid: 2.1 g (88%); mp 149–150 °C; IR (KBr) 1660 cm^{-1} (C=O); NMR ($CDCl_3$) δ 2.74 (t, 2 H, J = 7.5 Hz, CH_2), 3.85 (s, 6 H, 2OCH_3), 3.93 (s, 3 H, OCH_3), 3.75–4.05 (2 H, CH_2), 5.08 (s, 2 H, $ArCH_2O$–), 5.20 (s, 2 H, $ArCH_2O$), 6.82 (s, 1 H, aromatic), 7.05 (s, 1 H, aromatic), 7.15–7.50 (m, 12 H, aromatic). Anal. ($C_{33}H_{33}NO_6$) C, H, N.

***erythro*-1-(3′,4′,5′-Trimethoxy-α-hydroxybenzyl)-6,7-(dibenzyloxy)-1,2,3,4-tetrahydroisoquinoline Hydrochloride (6).** To a stirred suspension of 5 (3.0 g, 5.6 mmol) in 60 mL of MeOH, $NaBH_4$ (4.0 g, 0.1 mol) was added in small portions while maintaining the temperature at 20 °C. The mixture was allowed to stir at room temperature for an additional 3 h and then the solvent was removed in vacuo. The residue was suspended in H_2O and extracted with $CHCl_3$. The $CHCl_3$ extract was washed with H_2O, dried over anhydrous $MgSO_4$, and concentrated in vacuo to give an oil, 2.6 g (86%). The hydrochloride salt was prepared and recrystallized from MeOH–Et_2O to give a white solid: mp 192–194 °C; IR (neat, free base) 3400 cm^{-1} (OH); NMR (90 MHz) ($CDCl_3$, free base) δ 2.3–2.91 (m, 6 H, $ArCH_2CH_2NH$ and OH), 3.64 (s, 6 H, 2OCH_3), 3.78 (s, 3 H, OCH_3), 4.21 (d, 1 H, J = 4.4 Hz, Ar*CH*OH–), 4.84 (d, 1 H, J = 4.4 Hz, ArCHOHC*H*N–), 4.99 (s, 2 H, $ArCH_2O$), 5.08 (s, 2 H, $ArCH_2O$), 6.35 (s, 2 H, aromatic), 6.61 (s, 1 H, aromatic), 6.70 (s, 1 H, aromatic), 7.19–7.5 (m, 10 H, aromatic). Anal. ($C_{33}H_{36}NO_6Cl$) C, H, N.

***erythro*-1-(3′,4′,5′-Trimethoxy-α-hydroxybenzyl)-6,7-dihydroxy-1,2,3,4-tetrahydroisoquinoline Hydrochloride (2).** To a solution of 6 (1.0 g, 1.7 mmol) in 60 mL of absolute EtOH, 10% Pd/C (300 mg) was added. The mixture was hydrogenated on a Parr apparatus at room temperature at 40 psi for 6 h. The mixture was filtered with the aid of Celite and the solvent volume was reduced to about 4 mL under reduced pressure. Et_2O (2 mL) was added and the mixture was allowed to crystallize while sitting in an Et_2O chamber. The solid collected by filtration gave 0.46 g (68%) of 2 as a light grey solid: mp 153–156 °C; IR (KBr) 3390 cm^{-1} (OH, NH). Anal. ($C_{19}H_{24}NO_6Cl$) C, H, N.

***erythro*-1-(3′,4′,5′-Trimethoxy-α-acetoxybenzyl)-2-acetyl-6,7-diacetoxy-1,2,3,4-tetrahydroisoquinoline (7).** To a suspension of 2 (0.31 g, 0.8 mmol) in 3 mL of Ac_2O was added 3.5 mL of pyridine. The mixture was stirred at room temperature for 24 h and excess reagent was removed under reduced pressure. The resulting oil was stirred in 10% aqueous hydrochloric acid for 0.5 h and extracted with $CHCl_3$. The $CHCl_3$ solution was washed with water, dried ($MgSO_4$), and concentrated in vacuo to give an oil which crystallized under Et_2O. The solid recrystallized from the C_6H_6–Et_2O mixture to afford 7: 0.31 g (73.5%); mp 169–171 °C; IR (KBr) 1770, 1740 (ester), 1650 cm^{-1} (amide); NMR ($CDCl_3$) δ 2.10 (s, 3 H, $NCOCH_3$), 2.12, 2.26, 2.29 (3 s, 9 H, 3$OCOCH_3$), 2.3–3.7 (m, 3 H, Ar$CH_2CH_aH_b$N), 3.76 (s, 6 H, 2OCH_3), 3.8 (s, 3 H, OCH_3), 4.3–5.1 (m, 1 H, CH_aH_bN), 5.85–6.21 (m, 2 H, methine), 6.4–7.35 (m, 4 H, aromatic). Anal. ($C_{27}H_{31}NO_{10}$) C, H, N.

13α-Hydroxy-2,3-(dibenzyloxy)-9,10,11-trimethoxytetrahydroprotoberberine (8). To a solution of 6 (0.4 g, 0.69 mmol) in a hot mixture of 6 mL of EtOH and 12 mL of H_2O, 37% CH_2O solution (1.5 mL) was added. The mixture was refluxed for 2 h, cooled, and basified with 10% aqueous ammonium hydroxide. The mixture was extracted with chloroform. The chloroform solution was washed with water, dried ($MgSO_4$), and concentrated in vacuo to give an oil which solidified under methanol. The solid was recrystallized from MeOH to afford 8, 0.33 g (85.5%), as a white solid: mp 155–156 °C; IR (KBr) 3450 cm^{-1} (OH); NMR (90 MHz) ($CDCl_3$) δ 2.4–3.13 (m, 5 H, $ArCH_2CH_2N$ and OH), 3.26 (d, 1 H, J = 8.7 Hz, C_{14}-methine), 3.42 (d, 1 H, J = 12.5 Hz, C_8-H_aH_b), 3.73 (s, 3 H, OCH_3), 3.77 (s, 3 H, OCH_3), 3.81 (s, 3 H, OCH_3), 3.9 (d, 1 H, J = 12.5 Hz, C_8-CH_aH_b), 4.45 (d, 1 H, J = 8.7 Hz, C_{13}-methine), 5.1 (s, 4 H, 2$ArCH_2O$), 6.65, 6.8 (2 s, 2 H, aromatic), 7.21–7.57 (m, 11 H, aromatic). Anal. ($C_{34}H_{35}NO_6$) C, H, N.

***threo*-1-(3′,4′,5′-Trimethoxy-α-hydroxybenzyl)-6,7-(dibenzyloxy)-1,2,3,4-tetrahydroisoquinoline Hydrochloride (10).** Acetyl chloride (0.25 g, 0.003 mol) was added in one portion to a stirred solution of the free base of 6 (1.7 g, 0.003 mol) and triethylamine (0.32 g, 0.003 mol) in benzene. After stirring for an additional 1 h, triethylamine hydrochloride was removed by filtration and the filtrate evaporated in vacuo to give an oil. The oil was dissolved in $CHCl_3$, washed with H_2O, and dried ($MgSO_4$), and the solvent was removed under reduced pressure. The resulting oil was purified by column chromatography on silica gel ($CHCl_3$–MeOH, 19:1) to give the amide as an oil: 1.5 g (82%); IR (neat) 1620 cm^{-1} (amide); NMR ($CDCl_3$) δ 1.80 (s, 1 H, OH), 2.1 (s, 3 H, $NCOCH_3$), 2.15–3.4 (m, 4 H, $ArCH_2CH_2N$), 3.6 (s, 6 H, 2OCH_3), 3.75 (s, 3 H, OCH_3), 5.0 (s, 2 H, $ArCH_2O$), 5.08 (br d, s, 3 H, $ArCH_2O$ and ArCHOHC*H*N–), 5.65 (br d, s, 1 H, ArC*H*OHCHN–), 6.24, 6.58 (2 s, 4 H, aromatic), 7.15–7.50 (m, 10 H, aromatic).

Thionyl chloride (0.93 g, 0.8 mmol) was added to 1.5 g (0.3 mmol) of the oil and stirred to dissolve at 0 °C. The yellow solution was stirred at room temperature for 10 min and excess thionyl chloride was removed in vacuo. The oily residue solidified under EtOH at 0 °C and was collected by filtration and washed with Et_2O. Without further purification, a solution of the solid in 60 mL of MeOH and 10 mL of 15% aqueous NaOH was refluxed for 2.5 h. On cooling, the white precipitate was collected by filtration. The solid was dissolved in chloroform, washed with water, dried ($MgSO_4$), and concentrated in vacuo to give 10, 1.1 g (68%). The hydrochloride salt was prepared and recrystallized from MeOH–Et_2O: mp 121–123 °C; IR (neat, free base) 3320 cm^{-1} (br, NH, OH); NMR (90 MHz) ($CDCl_3$, free base) δ 2.63 (t, 2 H, J = 6.0 Hz, CH_2), 2.95–3.30 (m, 4 H, Ar$CH_2$$CH_2$N*H* and OH), 3.73 (d, 1 H, J = 7.3 Hz, ArCHOHC*H*NH–), 3.80 (s, 6 H, 2OCH_3), 3.86 (s, 3 H, OCH_3), 4.52 (d, 1 H, J = 7.3 Hz, ArC*H*OHCHNH–), 4.62 (d, 1 H, J = 12.1 Hz, ArCH_aH_bO), 4.81 (d, 1 H, J = 12.1 Hz.

$ArCH_aH_bO$), 5.09 (s, 2 H, $ArCH_2O$), 5.87, 6.55, 6.63 (3 s, 4 H, aromatic), 7.2–7.5 (m, 10 H, aromatic). Anal. ($C_{33}H_{36}NO_5Cl$) C, H, N.

***threo*-1-(3′,4′,5′-Trimethoxy-α-hydroxybenzyl)-6,7-dihydroxy-1,2,3,4-tetrahydroisoquinoline Hydrochloride (3).** To a solution of **10** (1.0 g, 1.7 mmol) in 60 mL of absolute EtOH, 10% Pd/C (300 mg) was added. The mixture was hydrogenated on a Parr apparatus at room temperature at 40 psi for 6 h. The mixture was filtered with the aid of Celite and the solvent volume reduced to about 4 mL. Et_2O (2 mL) was added and the mixture was allowed to crystallize in an Et_2O chamber. The solid was collected by filtration to give 0.43 (65%) of **3**: mp 153–158 °C; IR (KBr) 3410 cm^{-1} (br, OH, NH). Anal. ($C_{19}H_{24}NO_6Cl$) C, H, N.

***threo*-1-(3′,4′,5′-Trimethoxy-α-acetoxybenzyl)-2-acetyl-6,7-diacetoxy-1,2,3,4-tetrahydroisoquinoline (11).** A mixture of **3** (0.31 g, 0.8 mmol), Ac_2O (3 mL), and 3.5 mL of pyridine was stirred at room temperature for 24 h and the excess reagent was removed under reduced pressure. The resulting oil was stirred in 10% aqueous HCl for 0.5 h and extracted with $CHCl_3$. The $CHCl_3$ extract was washed with H_2O, dried ($MgSO_4$), and concentrated in vacuo to give an oil which crystallized under Et_2O. The solid was recrystallized from C_6H_6–Et_2O to give **11**: 0.34 g (75%); mp 184–185.5 °C; IR (KBr) 1765, 1745 (ester), 1665 cm^{-1} (amide); NMR ($CDCl_3$) δ 2.1 (s, 3 H, $NCOCH_3$), 2.2 (s, 6 H, $2OCOCH_3$), 2.31 (s, 3 H, $OCOCH_3$), 2.6–3.9 (m, 3 H, $ArCH_2CH_aH_bN$), 3.83, 3.86, 3.89 (3 s, 9 H, $3OCH_3$), 4.4–5.1 (m, 1 H, $ArCH_2CH_aH_bN$), 5.9–7.4 (m, 6 H, aromatic and methine). Anal. ($C_{27}H_{31}NO_{10}$) C, H, N.

13β-Hydroxy-2,3-(dibenzyloxy)-9,10,11-trimethoxytetrahydroprotoberberine (12). A 37% formalin (1.6 mL) solution was added to a hot solution of **10** (0.45 g, 0.75 mmol) in 6 mL of EtOH and 12 mL of H_2O. After refluxing for 2 h, the mixture was cooled, basified with aqueous 10% ammonium hydroxide solution, and extracted with $CHCl_3$. The $CHCl_3$ extract was washed with H_2O, dried ($MgSO_4$), and concentrated in vacuo. The oily residue was purified by silica gel column chromatography (Et_2O). The resulting oil crystallized under ether to give **12** as yellow microcrystals: 0.31 g (72%); mp 152–154 °C; IR (KBr) 3320 cm^{-1} (OH); NMR (90 MHz) ($CDCl_3$ + D_2O at 325 K) δ 1.7 (s, 1 H, OH), 2.4–3.3 (m, 4 H, $ArCH_2CH_2N$), 3.45 (d, 1 H, J = 10 Hz, C_8-H_aH_b), 3.65 (br s, 1 H, C_{14}-methine), 3.8, 3.83, 3.88 (3 s, 9 H, $3OCH_3$), 4.1 (d, 1 H, J = 10 Hz, C_8-H_aH_b), 4.62 (d, 1 H, $J \sim$ 1.0 Hz, C_{13}-methine), 5.1 (s, 4 H, $2ArCH_2O$), 6.7, 6.72, 6.88 (3 s, 3 H, aromatic), 7.21–7.55 (m, 10 H, aromatic). Anal. ($C_{34}H_{35}NO_6$), C, H, N.

Pharmacological Testing. Guinea pigs of either sex (weighing 300–500 g) and male Sprague–Dawley rats (weighing 160–220 g) were employed in all experiments. The isolation and procedures for the testing of each compound in rat adipocytes and isolated guinea pig atria and trachea were identical with those described previously.[22] In all biological experiments, dose–response curves (DRC) were obtained with each drug (10^{-10}–10^{-4} M range) and the data expressed in terms of the percent of the maximal effect obtained with 10^{-5} M (–)-isoproterenol. pD_2 values (negative logarithm of molar ED_{50}) and I (intrinsic activities) were determined from the DRC of each test compound. In these experiments, each point on the DRC represented the mean of four to eight observations. All drug solutions were freshly prepared in normal saline containing 0.05% sodium metabisulfite.

Biochemical Studies. A. Adenosine Cyclic 3′,5′-Monophosphate (cAMP) Accumulation in Isolated Fat Cells. The procedures for the isolation of rat adipocytes and measurement of cAMP were essentially identical with those described previously.[25,26] Final reaction mixtures contained 0.3 mL of the adipocyte suspension, 0.1 mL of theophylline (10^{-6} M), 0.05 mL of test drug solution, and 1.95 mL of Krebs–Ringer bicarbonate buffer, pH 7.4, containing 2% albumin (bovine fraction V; Sigma). All flasks were preincubated for 15 min prior to drug addition and then incubated for an additional 10-min period. A 2-mL aliquot of TCA (10% w/v) was then added to terminate the reaction. In each experiment, a maximal cAMP accumulation was obtained with 10^{-6} M (–)-isoproterenol and this value was used to calculate the percent of the maximal cAMP response for each test drug concentration. The maximal rate of cAMP accumulation produced by (–)-isoproterenol in rat adipocytes was 950 ± 49 pmol/0.3-mL cells/10 min (mean ± SEM of N = 27). Theophylline-induced cAMP formation rates were subtracted from drug-induced cAMP accumulation rates prior to the construction of the DRC for each drug.

B. Adenylate Cyclase Activity in Plasmalemma of Rat Adipocytes. Rat adipocytes (from 10–15 animals per experiment) were placed in a lysing medium [1:10 dilution of Krebs–Ringer bicarbonate buffer (pH 7.4) containing 2% albumin–water][27] and adipocyte plasmalemma was isolated by centrifugation and resuspension as described previously.[28] Reaction mixtures contained 4 μmol of Tris buffer (pH 7.6), 3 μmol of $MgCl_2$, 0.1 μmol of cAMP, 0.7 μmol of theophylline, 14 enzyme units of creatine phosphokinase, 0.51 mg of phosphocreatine, 1.0 mM [α-^{32}P]-ATP (0.5 μCi/assay), and drug in a final volume of 0.1 mL. Reactions were initiated by the addition of plasmalemma (30–100 μg of protein) and incubated for 10 min, after which time 0.1 mL of EDTA (100 mM) was added to terminate the reaction. Demineralized, doubly distilled H_2O and 15 000–20 000 dpm of [8-^{3}H]-cAMP were added to each flask in a 0.3-mL volume to monitor the extent of product recovery during the isolation procedure. Recovery of cAMP varied from 25 to 45%. The double column chromatographic method outlined by Salomon et al.[29] was used to isolate [^{3}H]- and [α-^{32}P]-cAMP from the reaction mixture. The ^{3}H and ^{32}P present in the final column eluates were determined simultaneously on a Beckman LS-355 scintillation counter using external standardization as a quench-monitoring method. Efficiencies of ^{3}H and ^{32}P detection were 30 and 98%, respectively.

The basal rate of cAMP formation was subtracted from the drug-induced rate to obtain the net picomoles of cAMP formed per milligram of protein per minute. In each experiment, a maximal cAMP formation rate was generated in the presence of 10^{-8} M (–)-isoproterenol and this value was used to calculate the percent of maximal cAMP response for each drug concentration required for the construction of the individual DRC. The maximal stimulation of adenylate cyclase activity by 10^{-8} M (–)-isoproterenol was (65 ± 12 net pmol/mg)/min (mean ± SEM of N = 10). Basal adenylate cyclase activity was calculated as (28 ± 15 pmol/mg)/min (mean ± SEM of N = 10).

Assays. Protein content was determined by the method of Lowry et al.[30] using bovine serum albumin as a standard.

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Synthesis of Substituted 1-Hydroxy-2-naphthanilides as Potential Cestodicidal Agents[1]

S. K. Dubey, A. K. Singh, H. Singh, S. Sharma, R. N. Iyer,*

Division of Medicinal Chemistry

J. C. Katiyar, P. Goel, and A. B. Sen

Division of Parasitology, Central Drug Research Institute, Lucknow-226001, India.

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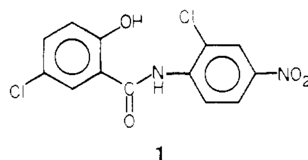
A series of substituted 1-hydroxy-2-naphthanilides 4–14 has been synthesized by treating 1-hydroxy-2-naphthoic acids 2 with substituted anilines 3. The nitronaphthanilides, on reduction and subsequent treatment with thiophosgene, gave the corresponding substituted 2-naphthanilide isothiocyanates 30–33. Substitution of the chlorine of 8 by various cyclic amines gave 3′-nitro-4′-substituted 1-hydroxy-2-naphthanilides 15–21. Various 3-aryl-4-oxo-2,3-dihydro-1,3-naphthoxazine-2-thiones 34–43 and 3-aryl-2,4-dioxo-2,3-dihydro-1,3-naphthoxazines 44–51 have been prepared by reacting the corresponding naphthanilides with thiophosgene and ethyl chloroformate, respectively. All the compounds were tested for their cestodicidal activity against *Hymenolepis nana* infection in rats; 30 was found to be the most active compound of the series, showing 100% clearance of infection at a single oral dose of 7.5 mg/kg.

During the course of structure–activity relationship studies carried out in the analogues of 2′,5-dichloro-4′-nitrosalicylanilide (niclosamide, 1),[2] we have reported the



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synthesis and biological activity of a large number of substituted salicylanilides of which many showed powerful cestodicidal activity.[3–5] In a further probe in this direction, the synthesis of various substituted 1-hydroxy-2-naphthanilides **4–33** and some of their cyclic analogues, viz., 3-aryl-4-oxo-2,3-dihydro-1,3-naphthoxazine-2-thiones **34–43** and 3-aryl-2,4-dioxo-2,3-dihydro-1,3-naphthoxazines **44–51**, has been undertaken. All these compounds have been screened for their cestodicidal activity against *Hymenolepis nana* in rats, and the results are reported in this communication.

Chemistry. Condensation of substituted 1-hydroxy-2-naphthoic acids **2** with different mono- and disubstituted anilines **3** in the presence of PCl_3 in refluxing xylene or toluene gave substituted 1-hydroxy-2-naphthanilides **4–14**.[6,7] The nitronaphthanilides, thus obtained, were reduced to the corresponding aminonaphthanilides **22–29** which were smoothly converted into the respective naphthanilide isothiocyanates **30–33** by treatment with thiophosgene. Various 3′-nitro-4′-substituted 1-hydroxy-2-naphthanilides **15–21** were prepared by treating 4′-chloro-3′-nitro-1-hydroxy-2-naphthanilide (**8**) with different cyclic amines. The synthesis of 3-aryl-4-oxo-2,3-dihydro-1,3-naphthoxazine-2-thiones **34–43** and 3-aryl-2,4-dioxo-2,3-dihydro-1,3-naphthoxazines **44–51** was accomplished by treating the corresponding naphthanilides with thiophosgene and ethyl chloroformate, respectively, in the presence of triethylamine[8] (see Scheme I).

Biological Activity. All the compounds were tested for their in vivo cestodicidal activity against *H. nana* infection in rats by the technique of Steward,[9] and the results are summarized in Tables I and II. The compounds were given orally at dosages of 250, 100, 50, 13, and 7.5 mg/kg, using three rats per experimental group. For all the control experiments, 1 was used as the standard drug. The active compounds of this series were **14** and **30**, showing 100% clearance of worm load at a single oral dose of 13 and 7.5 mg/kg, respectively, while **10** was equipotent to 1. Compounds **12**, **31**, and **32** were active at a dose of 100 mg/kg, and **11**, **13**, and **33** showed activity at 250 mg/kg. The other compounds were inactive at 250 mg/kg.

In view of the marked cestodicidal activity exhibited by **30**, its efficacy was assessed against a related cestode species *Hymenolepis diminuta* in rats and also against *Taenia* sp. in naturally infected dogs. A single oral dose of 10 mg/kg of the compound caused 100% reduction of worm load in both of the above-mentioned hosts. The standard compound **1** produced similar results when given orally at a dose of 50 mg/kg.[10] The detailed toxicity studies of **30** in rats and mice have also been carried out. At a dose of 5 g/kg given orally or intraperitoneally to uninfected rats, **30** was found to be tolerated well without any mortality. The young rats infected with *H. nana* also tolerated 1 g/kg of **30**. The acute toxicity experiments carried out on *Mastomys natalensis* and dogs showed **30** to be safe when given orally or intraperitoneally. Further chronic toxicity studies in rats, dogs, and monkeys are in progress.

The cestodicidal testing results of **4–51** establish a definite structure–activity relationship. Among various

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