bethoxy-17-(cyclopropylmethyl)morphinan-3,6-diol. It was used in the next reaction without further purification.

CH₃Li (1.84 M, 6.14 mL, 11.3 mmol) was added to a cooled (ice bath) solution of the above diol (792 mg, 2.05 mmol) in 45 mL of THF. The brown reaction mixture was stirred at ambient temperature under N_2 overnight. The reaction was quenched by pouring it into 25 mL of cold 20% NH_4Cl and stirring it in an ice bath for 15 min. The layers were separated, and the aqueous phase was extracted twice with CHCl₃. The organic fractions were combined, washed once with H_2O, dried over Na_2SO_4, and concentrated in vacuo. Purification of the product by column chromatography over Florisil using graded MeOH/CHCl₃ as eluant afforded 295 mg (39%) of **8C**: MS, m/e (relative intensity) 371 (M⁺, 70), 330 (M⁺ – CH₂-c-C₃H₅, 100), 313 (M⁺ – CH₃COCH₃, 23), 272 (330 – CH₃COCH₃, 30); NMR (CDCl₃) δ 0.13–0.53 (br m, 4 H), 1.18 (s, 3 H), 1.28 (s, 3 H), 4.48 (br s, 1 H, exchangeable),

4.75 (br s, 1 H, exchangeable), 6.58–7.03 (m, 3 H); IR (smear) 1610 cm⁻¹. Treatment with ethereal HCl gave the hydrochloride salt as a brown solid, mp foams >200 °C.

17-(Cyclopropylmethyl)-7-(1-hydroxy-1-methylethyl)isomorphinan-3,6-diol (8T). The compound was prepared according to the same procedure as used for **8C**. It was obtained as an off-white solid which begins to decompose at 180 °C (Table I): NMR (CDCl₃/MeOD) δ 0.08–0.58 (m, 4 H), 1.3 (s, 3 H), 1.37 (s, 3 H), 4.1 (br s, 2 H, exchangeable), 4.57 (br s, 1 H, exchangeable), 6.53–7.02 (m, 3 H); IR (CHCl₃) 1610 cm⁻¹.

Aporphines. 39.[1] Synthesis, Dopamine Receptor Binding, and Pharmacological Activity of (R)-(-)- and (S)-(+)-2-Hydroxyapomorphine[2]

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The enantiomers (6aR and 6aS) of 2,10,11-trihydroxyaporphine (THA) were synthesized from thebaine and bulbocapnine and evaluated pharmacologically in vitro in comparison with (-)-apomorphine [(-)-APO] and dopamine by competition with tritiated apomorphine, ADTN, and spiroperidol for binding to a membrane fraction of calf caudate nucleus, as well as for ability to stimulate adenylate cyclase. In all four tests, the rank order of potency was (-)-APO > (-)-THA ≫ (+)-THA. Thus, these results extend the impression that the 6aR configuration for hydroxyaporphines is preferred for interactions with putative dopamine receptors and that 2-hydroxylation reduces potency in comparison with 10,11-dihydroxyaporphines.

Since the discovery of therapeutically useful dopamine (DA) agonist activity in hydroxylated aporphine derivatives, such as apomorphine,[3] considerable interest has developed in delineating the portions of the aporphine molecular structure responsible for dopaminergic properties and the interactions with DA receptors.[4,5] The process of drug design could be considerably improved if receptors and their mode of interaction with active substances were known in precise molecular detail. Such information could then be used to design conformationally defined structures in which pharmacophoric groups are oriented in the appropriate spatial arrangement for optimal receptor interaction. Since DA is an achiral and conformationally flexible molecule, little information concerning interactions with DA receptors can be obtained with this neurotransmitter. Apomorphine [(-)-1, (-)-APO], the enantiomer obtained by the acid-catalyzed rearrangement of morphine, was reported by Saari et al.[6] to be the active enantiomer for dopaminergic and emetic activity. The S(+) enantiomer of apomorphine was shown to be inactive in producing postural asymmetrics in unilaterally caudate-lesioned mice.[6] Despite the complexities of interactions of apomorphine with presumed DA receptors and the status of apomorphine as a possible partial agonist or mixed agonist/antagonist in DA systems, including those in the central nervous system,[7-10] the apomorphine molecule has served as a good starting point for the study of DA receptor interactions. This proposal is supported by
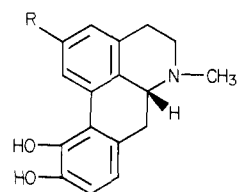
(-)-1 [(-)-apomorphine, APO], R = H
(-)-2 [(-)-2-hydroxyapomorphine, (-)-THA], R = OH
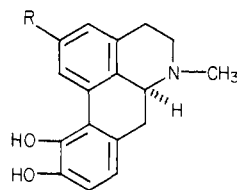
(+)-1 [(+)-apomorphine], R = H
(+)-2 [(+)-2-hydroxy-apomorphine, (+)-THA], R = OH

the facts that the catechol ring and the amino group analogous to those of DA are held in rigid conformation

(1) For Part 38, see R. J. Baldessarini, J. L. Neumeyer, A. Campbell, G. Sperk, V. J. Ram, G. W. Arana, and N. S. Kula, *Eur. J. Pharmacol.*, **77**, 87 (1982).

(2) This paper has been presented in part. See "Abstracts of Papers", 182nd National Meeting of the American Chemical Society, New York, NY, Aug 1981, Chemical Society, Washington, DC, 1981, Abstr MEDI 22.

Table I. Effects of Hydroxyapomorphines on Binding to Dopamine Agonist and Antagonist Sites, and Stimulation of Dopamine-Sensitive Adenylate Cyclase in Brain Tissue[a]

no.	compd	IC$_{50}$,[c] nM [³H]APO	[³H]ADTN	[³H]SPR	% cyclase stimulation (drug concn, μM)[d]
	dopamine (DA)	4.5	3.7	>10 000	58 (@ 50)
1	(−)-APO[b]	1.0	4.9	860	48 (@ 50)
2	(−)-2-OH-APO	11.0	10.0	2 600	59 (@ 250)
2	(+)-2-OH-APO	4000	1500	>50 000	9 (@ 250)

[a] Competition by aporphines for binding to a membrane-rich "subsynaptosomal" tissue fraction of calf caudate nucleus homogenates (see Experimental Section) was evaluated with ³H-labeled apomorphine (APO, 0.5 nM), ADTN (0.5 nM), or spiroperidol (SPR, 0.15 nM). "Blank" assays to define specific or saturable binding were made with unlabeled (±)-ADTN (10 μM), (−)-APO (10 μM) or (+)-butaclamol (1 μM), respectively. [b] APO = apomorphine. [c] Values for IC$_{50}$ were computed by log-probit analysis of data from at least three separate determinations with at least four concentrations of each aporphine tested, in which SD was ≤5% of each value. [d] Cyclase activity was assessed (as picomoles of cAMP formed per 2.5 min assay in excess of basal levels) with at least three concentrations of each test agent between 0 and 250 μM and is reported as percent increase above basal activity with no added agonist (basal activity = 2.70 ± 0.14 pmol of cAMP formed per 2.5 min assay ± SEM) at a representative concentration of test agent (in parentheses). The variance (±SEM) averaged ±12.9% of the mean (N = 4–5). Estimated values for EC$_{50}$ in the stimulation of cyclase activity were 40, 60, 190, and 1300 μM for DA, (−)-1, (−)-2, and (+)-2, respectively.

and that multiple sites for chemical substitution are available in the aporphine system and because APO is optically active. Moreover, the pharmacological characteristics of binding sites defined by [³H]APO or a full agonist, such as [³H]ADTN, are very similar when low ligand concentrations are used.[4,11,12]

The molecular features of DA "receptors" are unknown. Currently, such putative receptors are characterized and differentiated in brain tissue only by behavioral tests or by interactions of compounds with biochemical responses or binding properties proposed as indexes of "receptor" activities.[3,4,7,11] We are using the aporphine ring system as a template for studying DA receptors.[4,12,13] We recently reported that (−)-2,10,11-trihydroxynoraporphines displaced [³H]APO and [³H]spiroperidol ([³H]SPR) from calf caudate membrane fraction[4,5] and stimulated production of adenosine cyclic 3′,4′-monophosphate (cAMP) by adenylate cyclase in rat striatal homogenates in potencies indicative of DA–agonist interactions. One of these compounds, 2,10,11-trihydroxy-N-n-propylnoraporphine, showed in vivo anticonvulsant activity.[14] These findings led to the synthesis and further examination of the properties of (S)-(+)-2,10,11-trihydroxyapophine [(+)-2, (+)-THA] in comparison with (R)-(−)-2,10,11-trihydroxy-aporphine [(−)-2, (−)-THA], (R)-(−)-apomorphine (APO), and dopamine.

Chemistry. The synthesis of (−)-2,10,11-trihydroxy-aporphine [(−)-2, (−)-THA] from thebaine (3) followed the

procedure described for the synthesis of nor-morphothebaine derivatives using concentrated hydrochloric acid in a pressure bottle,[15] followed by O-demethylation with 48% (w/v) HBr (Scheme I). The aporphine alkaloid (S)-bulbocapnine (5), isolated from the roots of *Corydalis cava*, has been converted to **3b** with sodium and liquid ammonia by Ayer and Taylor.[16] These authors isolated the free base (mp 196–197 °C) but did not specify the optical rotation of the product isolated. More recently, Rice and Brossi[17] converted the phosphate ester of bulbocapnine (5) to optically and chemically pure (S)-(+)-2-hydroxy-10-methoxyaporphine using lithium in refluxing liquid ammonia. We chose the latter procedure for the conversion of 5 to (+)-4, which was obtained in 60% yield. O-Demethylation with 48% HBr gave (+)-2 in quantitative yield. An extension of this method has recently been reported[18] for the synthesis of (+)- and (−)-apomorphine.
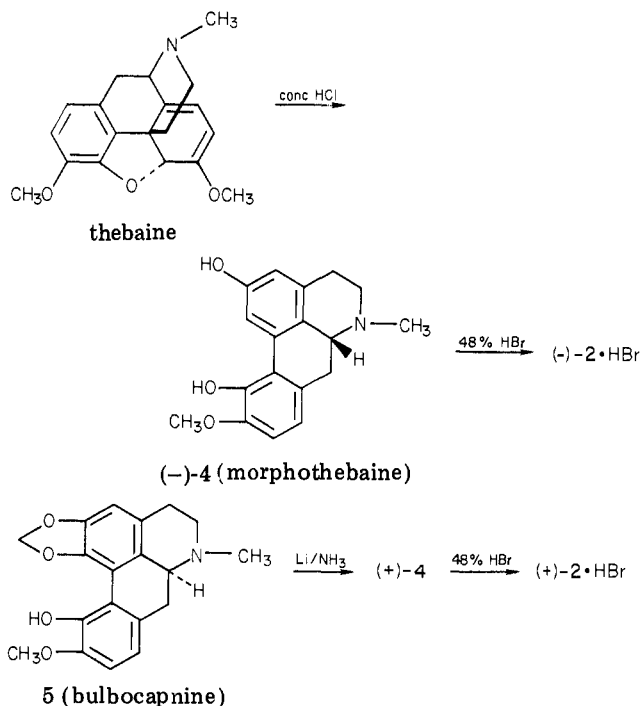
Pharmacology. The ability of aporphines (0 to 250 μM) to stimulate DA-sensitive adenylate cyclase was evaluated with homogenates of rat corpus striatum in the presence of excess ATP and an inhibitor of phosphodiesterase, theophylline; cyclic (cAMP) was then assayed by a protein-binding method.[4] Typical basal activity (without DA agonist present) was 2.70 ± 0.14 pmol of cAMP formed per 2.5 min assay (±SEM). Results are provided in Table I.

Binding assays were carried out with a membrane fraction recovered from an osmotically shocked and Polytron-disrupted synaptosomal preparation of rapidly frozen, protease-free calf caudate nuclei.[4,19] Ligands included (−)-[8,9-³H]apomorphine hydrochloride ([³H]APO; New England Nuclear Corp., 32–36 Ci/mmol), (±)-2-amino-6,7-dihydroxy-1,2,3,4-tetrahydro[5,8-³H]-naphthalene ([³H]ADTN; New England Nuclear Corp., 33 Ci/mmol), and 1-phenyl[4-³H]spiroperidol (SPR; New England Nuclear Corp., 30 Ci/mmol). Blanks were defined by adding excess (10 μM) unlabeled (±)-6,7-ADTN (in the [³H]APO assay), (−)-APO (in the [³H]ADTN assay), or 1 μM (+)-butaclamol (donated by Ayerst Laboratories) in the [³H]SPR assay. Further details concerning the binding assay methods are provided in the legend to Table I and elsewhere.[4,19,20]

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(11) P. Seeman, *Pharmacol. Rev.*, **32**, 229 (1981).

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Scheme I

CH₃

conc HCl

CH₃O   O   OCH₃

thebaine

HO

N—CH₃

H

48% HBr   (-)-2·HBr

HO

CH₃O

(−)-4 (morphothebaine)

O   N—CH₃   Li/NH₃   (+)-4   48% HBr   (+)-2·HBr

H

HO

CH₃O

5 (bulbocapnine)

Results and Discussion

The present results indicate that (−)-2,10,11-tri-hydroxyaporphine [(−)-THA] displaces [³H]APO, [³H]-ADTN, and [³H]SPR from calf caudate membrane fractions and stimulates production of cAMP by adenylate cyclase in rat striatal tissue to indicate DA–receptor interactions (Table I). In all four assays, DA-like activity was less potent with (−)-THA than with apomorphine. Yet the very low activity of (+)-THA in all of the in vitro test systems evaluated indicates stereoselectivity of THA at the putative DA receptor favoring the $R(-)$ enantiomer.

The data presented thus support the suggestion made in previous studies[4,5] that the 6aR configuration and the 10,11-hydroxy groups enable aporphine molecules to fit the presumptive DA receptor, whereas an additional 2-hydroxy moiety interferes with intrinsic agonist actitivity and may even contribute to complex mixed agonist/antagonist properties in vivo.[21] The consequence of other substituents on the 2-position of such aporphines containing hydroxy groups at the 10- or 11-position will be explored further.

Experimental Section

General Methods. All melting points were determined on a Thomas-Hoover apparatus and are uncorrected. Elemental analyses were performed by Galbraith Laboratories, Knoxville, TN. Thin-layer chromatography (TLC) used precoated silica gel

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13179, polyethylene terphthalate sheets (Eastman Kodak, Rochester, NY). Column chromatography was performed on silica gel (Baker, 5-3405, 50–200 mesh). IR spectra were obtained with a Perkin-Elmer Model 700 or Beckman IR-10 spectrophotometer. The NMR spectra were measured in CDCl₃ or CD₃OD on a Varian T-60 spectrometer, and chemical shifts are reported in parts per million (δ) downfield from (CH₃)₄Si as internal standard. Mass spectra were determined on a 12-90-G Nuclide mass spectrometer. Optical rotations were obtained on a Perkin-Elmer polarimeter (Model 141).

(−)-2,11-Dihydroxy-10-methoxyaporphine Hydrochloride [Morphothebaine Hydrochloride, (−)-4·HCl]. This compound was prepared from thebaine with concentrated HCl in a pressure bottle as previously described[15] in 80% yield, mp 260–262 °C (lit.[22] mp 256–260 °C); UV (EtOH) λ_{max} 276 nm (log ϵ 4.13), 268 (4.1), 300 (3.9).

(−)-2,10,11-Trihydroxyaporphine Hydriodide [(−)-2·HI]. A mixture of 1.5 g (5.04 mmol) of (−)-4 and 7.5 mL of 57% HI was stirred while 7.5 mL of acetic anhydride was added carefully dropwise. After the addition, the solution was heated at 140 °C for 1.5 h under N₂. The mixture was cooled and diluted with CH₃CN until no further precipitation occurred. The solid was filtered, washed with CH₃CN, and dried to give 1.4 g (67%) of (−)-2·HI: mp 255–256 °C dec; NMR (Me₂SO-d_6) δ 7.9 (d, 1 H), 6.75 (s, 2 H), 6.65 (d, 1 H), 3.0–4.0 (m, 7 H), 3.2 (s, 3 H); UV (EtOH) λ_{max} 305 nm (log ϵ 3.91), 280 (4.16), 268 (4.13). Anal. (C₁₇H₁₇NO₃·HI) C, H, N, I.

The hydrobromide salt was also prepared by heating (−)-4 with 48% (w/v) HBr at 120–130 °C for 6 h to yield (−)-2·HBr, mp 253–254 °C; $[\alpha]^{20.5}_{546}$ −103.6°. Anal. (C₁₇H₁₇NO₃·HBr) C, H, N.

(+)-2,11-Dihydroxy-10-methoxyaporphine [(+)-Morpho-thebaine Hydrochloride, (+)-4]. Bulbocapnine (1.0 g) was added in small portions to 50 mL of refluxing liquid ammonia under an atmosphere of nitrogen. After the addition was complete, lithium metal (100 mg) was added in small pieces with stirring until the blue color persisted for an hour. The reaction was stirred for an additional 2 h, at which time the ammonia was evaporated and the reaction quenched with a few milliliters of methanol. Water was carefully added, and the pH of the aqueous solution was adjusted to 9.0–9.5 by the addition of 37% (vols) HCl. The aqueous solution was extracted from CHCl₃, dried over Na₂SO₄, and evaporated to dryness to yield 0.85 g. The crude product was purified by column chromatography using silica gel and methanol–chloroform (1:15) as eluant to yield 0.6 g of (+)-4, mp 178–179 °C (lit.[16] mp 196–197 °C).

(+)-2,10,11-Trihydroxyaporphine Hydrobromide [(+)-2-Hydroxyapomorphine Hydrobromide, (+)-2·HBr]. This compound was prepared from (+)-4 as described previously with 48% HBr (w/v) to yield an off-white powder: mp 229–131 °C; $[\alpha]^{20.5}_{546}$ +100.36°. Anal. (C₁₇H₁₇NO₃·HBr·0.5H₂O) C, H, N.

(22) "The Merck Index", 9th ed., Merck & Co., Inc., Rahway, NJ, 1976, p 6120.