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N-(2,4,5-Trihydroxyphenethyl)normetazocine, a Potential Irreversible Inhibitor of the Narcotic Receptor

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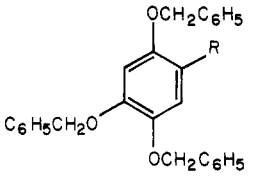
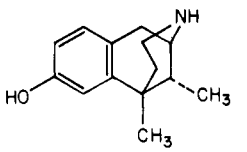
The reaction of *N*-(2,4,5-tribenzyloxyphenyl)ethyl methanesulfonate, prepared in a seven-step sequence, with normetazocine followed by hydrogenolysis of the benzyloxy-protecting groups, gave *N*-(2,4,5-trihydroxyphenethyl)normetazocine. This compound was prepared to study the effect of a narcotic analgesic containing a functional group which could be activated in situ to a moiety potentially capable of reacting irreversibly with the narcotic receptor. This 6-hydroxydopamine derivative of normetazocine did not prove to be a useful affinity label. Its low toxicity could indicate the necessity for the formation of an aminochrome system for the expression of toxicity by 6-hydroxydopamine.

Much effort is currently being expended in search of a useful affinity label for the opiate receptor.[2,3] The ideal reagent for this purpose would interact specifically with the opiate receptor with high affinity so that it could be employed at very low concentrations. A reactive group is needed in the reagent, capable of forming a covalent bond with one or more types of functional groups on the receptor. It is probably also important, to ensure the greatest specificity, that the reactive group be neither too readily reactive nor too weakly so. Alternatively, groups which can be activated after binding to receptors can be employed. One such approach has been the use of photoaffinity probes.[2] We chose to make use of a functional group which may require oxidation by molecular oxygen prior to reaction. The 2,4,5-trihydroxyphenethylamine moiety may be such a group and its potential usefulness is explored in this communication.

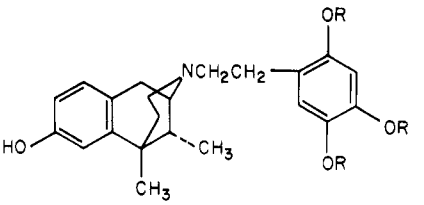
2,4,5-Trihydroxyphenethylamine (6-hydroxydopamine) is known to effect the release of norepinephrine and cause long-term reduction of cardiac uptake of norepinephrine and selective destruction of peripheral noradrenergic nerve terminals.[4] The central administration of 6-hydroxydopamine results in a profound depletion of both dopamine and norepinephrine as a consequence of the selective destruction of catecholamine-containing nerve terminals.[4] A possible mechanism for the cytotoxic action of 6-hydroxydopamine results from the ability of the initial oxidation product, the *p*-quinone of 6-hydroxydopamine, to bind covalently to sulfhydryl groups on macromolecules leading to the polymerization of proteins at the plasma membrane and within the neuronal terminal.[5-7] We hoped to deliver a 6-hydroxydopamine-like molecule to the vicinity of the narcotic receptor in the brain by combining it with a compound from which potent analgesics have been prepared, namely, 5,9α-dimethyl-2′-hydroxy-6,7-benzomorphan (normetazocine, **6**).[8] Thus, *N*-(2,4,5-trihydroxyphenethyl)normetazocine (**8**) was synthesized and the biological activity of the hydroiodide salt of **8** was explored.



1, R = CH_2Br
2, R = CH_2CN
3, R = CH_2COOH
4, R = CH_2CH_2OH
5, R = $CH_2CH_2OSO_2CH_3$



6



7, R = $CH_2C_6H_5$
8, R = H

N-Phenethyl-substituted benzomorphans and morphinans, whether or not the aromatic ring has a para substituent, are very potent analgesics and have high affinity for the narcotic receptor.[9] Thus, *N*-phenethylnormetazocine is about ten times more potent than morphine in vivo (in mice, hot-plate assay, subcutaneous injection). *N*-*p*-Hydroxyphenethylnormetazocine has been

Table I. Inhibition of Norepinephrine Uptake and Its Release

Compound	ED_{50}[a]	Release[b]
N-(2,4,5-Trihydroxyphenethyl)-normetazocine	160	0[c]
α-Methyltyramine	0.34	13
N,N-Dimethyltyramine	14.1	>400
2,4,5-Trihydroxyphenethylamine	0.73	6.8
α-Methyl-2,4,5-trihydroxy-phenethylamine	0.60	4.2
Cocaine	1.8	0
Morphine	>400	

[a] Dose of drug in μmol/kg giving 50% inhibition of norepinephrine uptake by intravenous administration (ref 19). Dose-response curves for uptake and release were constructed over a range of five doses with five male N.I.H. General Purpose mice per dose. An average of two or three dose-response curves was obtained for the new compounds. [b] Dose of drug administered subcutaneously in μmol/kg giving 50% release of tritiated norepinephrine (administered intravenously 60 min prior to drug administration; ref 20). [c] > 150 μmol/kg gave no release.

noted to retain about half of the activity (in rats)[10] of the potent phenazocine (*N*-phenethylnormetazocine). *N*-*p*-Aminophenethylnormetazocine has about 20 times the potency of morphine and *N*-*p*-methoxyphenethylnormetazocine is about eight times as potent. Similar potencies were noted for the comparably N-substituted *levo*-3-hydroxymorphinans.[11] Further, *N*-phenethylnormetazocine is comparable to morphine in its affinity for the narcotic receptor (in vitro binding assay procedure) and *levo*-3-hydroxy-*p*-aminophenethylmorphinan has more than ten times the affinity of morphine for the narcotic receptor.

Results

A. Receptor Binding Studies. *N*-(2,4,5-Trihydroxyphenethyl)normetazocine interacts only relatively weakly with the rat brain opiate receptor. Binding affinity is ~700 nM in standard assay conditions,[12] i.e., 0.32 M sucrose–0.01 M Tris pH 8.0 at 37 °C. The binding affinity of morphine, under comparable conditions, is 3 nM. Although the salt of **8** is somewhat unstable in solution, the measured binding affinity is the same no matter whether the incubation with receptors at 37 °C is for 10 or 1 min. The solution slowly darkens on standing over several hours. Therefore, its low affinity for receptor is not likely to be due primarily to instability.

N-(2,4,5-Trihydroxyphenethyl)normetazocine, at concentrations as high as 2×10^{-4} M, has no effect on the opiate-sensitive adenylate cyclase of neuroblastoma X glioma hybrid cells.[13] At even higher concentrations, however (10^{-3} M), there is an inhibiting effect of the substance on adenylate cyclase activity. Morphine inhibits the adenylate cyclase of these cells at 10^{-7} M. Thus, in binding to the opiate receptor, as well as in inhibition of adenylate cyclase activity, the compound is less than $^1/_{100}$th as active as morphine.

All experiments were performed with freshly prepared solutions. Binding to opiate receptors was carried out as described previously,[12] except that the time of incubation was sometimes varied as described above. Adenylate cyclase activity was measured with homogenates of neuroblastoma X glioma (NG108-15) cells.[13] Incubation times were 3 min.

B. Inhibition and Release of Norepinephrine. *N*-(2,4,5-Trihydroxyphenethyl)normetazocine was 230 times less effective than 6-hydroxydopamine alone but more effective than, for example, morphine alone, in inhibiting norepinephrine uptake (Table I). It had no effect on norepinephrine release in the in vitro system.[7] Comparisons between related compounds are shown in Table I. Normetazocine alone was toxic at 5 mg/kg (LD_{100} = 10 mg/kg), by intravenous injection, and could not be utilized for the assay. In contrast, **8** showed no signs of toxicity at doses as high as 80 mg/kg.

C. Antinociceptive Activity. In the hot-plate assay[14,15] *N*-(2,4,5-trihydroxyphenethyl)normetazocine appeared to be considerably less potent than codeine (in mice, subcutaneous injection); only seven (out of ten) mice were affected at 50 mg/kg.

Discussion

The inactivity of *N*-(2,4,5-trihydroxyphenethyl)normetazocine in the various assay procedures could be rationalized as follows.

The hydrophilic character of the polyhydroxy-substituted substrate could, conceivable, hinder access to the brain, in vivo. The hydroxyl groups could also serve as sites for rapid metabolic inactivation in vivo, thus causing the observed reduced antinociceptive activity.

The large number of hydroxyl groups in **8** might, also, promote, by hydrogen bonding to solvent molecules, the formation of intermolecular complexes of sufficient size to hinder its interaction with the opiate receptor in vitro.

The low affinity of the compound for the norepinephrine uptake mechanism coupled with its relatively low toxicity may be an indication of the necessity for the formation of an aminochrome system for the expression of toxicity by 6-hydroxydopamine. The aminochrome formed from 6-hydroxydopamine appears to be responsible for inactivation of some enzymes with catechol-binding sites, according to Borchardt.[16] The inability of *N*-(2,4,5-trihydroxyphenethyl)normetazocine to form an aminochrome system would exclude this type of interaction. The likely ability of this derivative of normetazocine to form a *p*-quinone casts some doubt upon the critical role of the *p*-quinone of 6-hydroxydopamine in the manifestation of the cytotoxicity of 6-hydroxydopamine.

The very weak affinity of *N*-(2,4,5-trihydroxyphenethyl)normetazocine for the opiate receptor, determined both by binding measurements and with receptor-linked adenylate cyclase, shows that this compound will not be a useful affinity label. Nevertheless, the general approach used here, namely, construction of a narcotic with a functional group which can be activated in situ, seems to us to offer the best chance of providing the high degree of specificity required. We are currently investigating alternate means of achieving this goal.

Experimental Section

Melting points were determined in open capillary tubes using a Thomas-Hoover apparatus and are corrected. Microanalyses were performed by the Laboratory of Chemistry's Section on Microanalytical Services and Instrumentation. IR (Perkin-Elmer 257) and NMR spectra (Varian A-60) were consistent with the assigned structures. Mass spectra of the free bases were obtained using a Hitachi Perkin-Elmer RMU6E spectrometer (70 eV); each spectrum was consistent with the assigned structure and showed the expected molecular ion.

2,4,5-Tribenzyloxyphenylacetonitrile (2). To a slurry of NaCN (2.0 g, 40.8 mmol) in dry dimethyl sulfoxide (70 mL) was added crude 2,4,5-tribenzyloxybenzyl bromide (**1**) prepared as previously described[17] from the corresponding alcohol (7.7 g, 18.1 mmol). After stirring 20 h at 25–30 °C, the reaction mixture was poured into a mixture of ice and H_2O (500 mL). The oil that separated was extracted into benzene (150 mL) and the solution was washed successively with H_2O, 5% $NaHCO_3$ solution, and brine. After drying ($MgSO_4$) the solution was evaporated to give tan crystalline material which was recrystallized from EtOH to give essentially pure **2** (6.1 g, 78%). Recrystallization from

benzene–hexane gave pure material, mp 103–104 °C. Anal. ($C_{29}H_{25}NO_3$) C, H, N.

2,4,5-Tribenzyloxyphenylacetic Acid (3). The nitrile **2** (22.0 g, 50.5 mmol) was added to a solution of 85% KOH (22.0 g, 333.3 mmol) in 2-methoxyethanol (250 mL). The mixture was refluxed 48 h, cooled, diluted with H_2O (2.0 L), and extracted with Et_2O (3 × 200 mL). The aqueous phase was then acidified with 37% HCl and extracted with $CHCl_3$ (2 × 200 mL). The combined extracts were dried ($MgSO_4$), filtered, and evaporated to a dark syrup which was dissolved in CH_3CN and treated with dicyclohexylamine (11.0 g, 60.6 mmol). The salt that separated almost immediately was filtered, washed with cold CH_3CN, dissolved in EtOH (100 mL), acidified with 15% HCl, and poured into H_2O (1.5 L) which was extracted with $CHCl_3$ (2 × 200 mL). The combined extracts were backwashed with H_2O (2 × 200 mL), dried ($MgSO_4$), and evaporated to give purified crystalline **3** which was recrystallized from methyl isobutyl ketone to give essentially pure **3** (13.7 g, 60%), mp 160–162 °C. Recrystallization from MeOH gave pure material, mp 162–164 °C (lit.[18] mp 152–154 °C). Anal. ($C_{29}H_{26}O_5$) C, H.

2-(2,4,5-Tribenzyloxyphenyl)ethanol (4). A solution of the acid **3** (10.0 g, 22.0 mmol) in dry tetrahydrofuran (100 mL) was added dropwise to a stirred, refluxing solution of $LiAlH_4$ (1.1 g, 29.0 mmol) in dry tetrahydrofuran (350 mL). After the addition was complete, the mixture was refluxed 3 h and cooled, and a solution of H_2O (1.1 mL) in tetrahydrofuran (20 mL) was added dropwise to the well-stirred solution. Aqueous 15% NaOH (1.1 mL) and a solution of H_2O (3.3 mL) in tetrahydrofuran (20 mL) were then added successively to the mixture while stirring. Stirring was continued 0.5 h and the solids were filtered, washed well with tetrahydrofuran, and discarded. The filtrate was evaporated to give essentially pure **4** (7.8 g, 80%). Two recrystallizations from isopropyl ether gave pure **4**, mp 84.5–85.5 °C. Anal. ($C_{29}H_{28}O_4$) C, H.

2-(2,4,5-Tribenzyloxyphenyl)ethyl Methanesulfonate (5). Methanesulfonyl chloride (1.09 g, 9.5 mmol) was added dropwise to a stirred solution of the alcohol **4** (3.70 g, 8.4 mmol) in dry pyridine at 23–27 °C. After stirring 2.0 h, H_2O (1 mL) was added to the reaction mixture and stirring was continued 0.25 h. The mixture was then poured into Et_2O (200 mL) and the pyridine was removed by extraction with excess 3 N HCl. The Et_2O solution was washed with 8% $NaHCO_3$ solution (75 mL) and then brine, dried ($MgSO_4$), and evaporated to give crude **5** (4.18 g, 96%), mp 89–91 °C. Two recrystallizations from 100% ethanol gave pure **5**, mp 93–95 °C. Anal. ($C_{30}H_{30}SO_6$) C, H.

***N*-(2,4,5-Tribenzyloxyphenethyl)normetazocine (7).** A mixture of the mesylate **5** (3.48 g, 6.7 mmol), normetazocine **6** (1.52 g, 7.0 mmol), anhydrous K_2CO_3 (6.0 g, 43.5 mmol), and dry dimethylformamide (25 mL) was heated at 60–65 °C for 20 h while stirring. The reaction mixture was then cooled and filtered and the solvent was removed in vacuo. The residue was dissolved in Et_2O (200 mL) and the solution washed with H_2O (50 mL), dried (Na_2SO_4), filtered, and rendered slightly acidic with ethereal HCl. After standing 0.25 h, the ether was decanted from the gummy hydrochloride salt which adhered to the side of the flask. The salt was rinsed with Et_2O (~100 mL), dissolved in MeOH (20 mL), and treated with excess 28% NH_4OH. Et_2O (200 mL) was added and the solution was washed with H_2O (2 × 30 mL) and brine, dried (Na_2SO_4), and evaporated to give the crude basic fraction (2.94 g). Crystallization from Et_2O (20 mL) then gave essentially pure **7** (1.99 g, 46%). The analytical sample was prepared by recrystallization from isopropyl ether (mp 134–135.5 °C). Anal. ($C_{43}H_{45}NO_4$) C, H, N.

***N*-(2,4,5-Trihydroxyphenethyl)normetazocine Hydroiodide Sesquihydrate (8).** A stirred solution of **7** (319 mg, 0.5 mmol) in MeOH (25 mL) containing AcOH (5 drops) was purged well with N_2. After addition of 10% Pd/C (80 mg), H_2 was passed through the stirred solution for 3 h. At the end of this time, 57% HI (5 drops) was added and the solution was filtered under N_2 and evaporated (<30 °C), and the syrupy residue was dissolved in 96% AcOH (4 mL) without heating. After seeding, crystalline material rapidly separated from the solution. The solid was filtered, washed with 96% AcOH, and dried (25 °C) to give **8**·HI·1.5H_2O (156 mg, 60%), mp 144.5–146.5 °C dec. Considerable difficulty was experienced, initially, in obtaining a crystalline salt of **8**. However, once seeded, crystallization of **8**·HI·1.5H_2O proceeded readily from 96% AcOH (but not glacial AcOH). A number of other salts and crystallization solvents were examined; satisfactory results could only be obtained as described above. Anal. ($C_{22}H_{27}NO_4$·HI·1.5H_2O) C, H, N.

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