Hybromet: A Ligand for Purifying Opioid Receptors

Sydney Archer,*† Josephine Michael,† Peter Osei-Gyimah,† Ahmad Seyed-Mozaffari,† R. Suzanne Zukin,‡ Rhoda Maneckjee,‡ Eric J. Simon,§ and Theresa L. Gioannini§

Cogswell Laboratory, Department of Chemistry, Rensselaer Polytechnic Institute, Troy, New York 12180-3590, Departments of Biochemistry and Neuroscience, Albert Einstein College of Medicine, Bronx, New York 10461, and Departments of Psychiatry and Pharmacology, New York University School of Medicine, New York, New York 10016.
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Condensation of the Grignard reagent derive from 2-[4-(allyloxy)phenyl]ethyl bromide (**4b**) with 7α-acetyl-6,14-*endo*-ethenotetrahydrothebaine (**5**) furnished the (*R*) tertiary carbinol, **7**, which upon methoxymercuration followed by treatment with the KBr gave the bromomercurio compound **10** (Hybromet). The corresponding *N*-cyclopropylmethyl analogue, **11**, was prepared also. The bromomercurio compound, **1**, and the mercaptobenzothiazole derivative, **3**, gave allyl phenyl ether when treated with BAL at room temperature. Similar treatment of **10** with BAL gave **7** in high yield. Binding studies using rat brain homogenates indicated that **7**, **13**, and **14** have moderately high affinities for μ rather than δ binding sites. Although much weaker, **10** showed preferential μ binding also. These results along with the fact that **10** reacted smoothly with sulfhydryl groups suggest that Hybromet would be a suitable ligand for use in affinity chromatography.

For some time we have been interested in the isolation and purification of opioid receptors using affinity chromatography.[1-3] To this end we wanted to prepare an opioid ligand that not only could bind covalently to a suitable matrix but also could bind covalently and chemically reversibly to an opioid receptor. Since sulfhydryl-containing matrices such as Affi-Gel 401 are commercially available and opioid receptors are known to be inactivated by reagents that react with sulfhydryl groups,[4] it occurred to us that a properly designed opioid mercurial could accomplish both objectives.

As a preliminary test of this concept, the model reactions shown in Scheme I were carried out. The mercury derivative **1** served as a surrogate for the opioid ligand, and 2-mercaptobenzothiazole (**2**) played a similar role for the opioid receptor and a thiol-containing insoluble matrix.

Allyl phenyl ether was treated with mercuric acetate, methanol, and then KBr to give the oily bromomercurio compound **1**. The regiochemistry of the methoxymercuration reaction was established by NMR spectroscopy. Treatment of **1** with the anion derived from **2** gave **3** as a crystalline, stable substance. When **3** was allowed to react with 1,2-dithioglycerol (BAL) overnight at room temperature, allyl phenyl ether and 2-mercaptobenzothiazole were isolated in 68% and 89% yields, respectively. Similarly, treatment of **1** with BAL gave allyl phenyl ether in 82.5% yield.

In order to incorporate all the features of **1** in an opioid ligand, we turned to the 6,14-*endo*-ethenotetrahydrothebaines. Bentley, Hardy, and Meek[5] have shown that 7α-acetyl-6,14-*endo*-ethenotetrahydrothebaine (**5**) reacts with (2-phenylethyl)magnesium bromide to give the (*R*) tertiary carbinol, **6**, which was about 500 times as potent as morphine in a rodent analgesic assay. The preparation of Hybromet (**10**) and some related compounds is shown in Scheme II. 2-[4-(Allyloxy)phenyl]ethanol was converted to the corresponding *p*-toluenesulfonate which was treated with NaI to give the iodide **4a**. The latter was converted to the Grignard reagent which was allowed to react with **5** to afford the (*R*) carbinol **7**. This reaction was erratic and even when successful never proceeded in higher than 10% yield. The bromide, **4b**, gave **7** more consistently and in higher yield. Methoxymercuration of **7**, followed by treatment with KBr, gave Hybromet (**10**) in 34% yield. The NMR spectrum showed that only the allylic double bond had reacted. There was a signal for a new methoxyl group[6] and two new methylene protons. When **10** was treated with BAL, **7** was obtained in 88% yield.

Scheme I

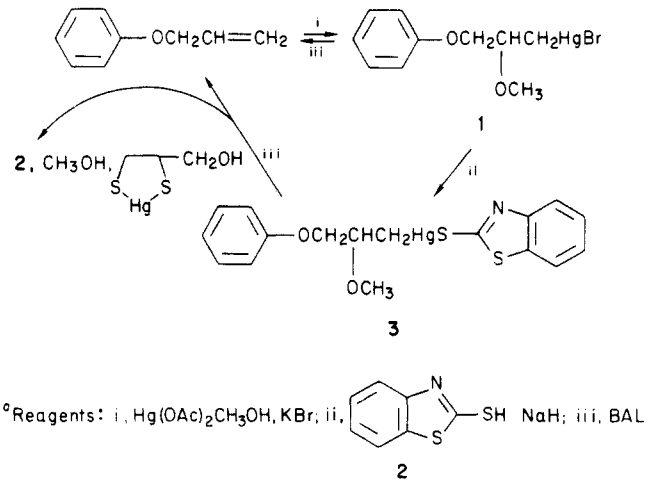


Table I. Affinities of Hybromet and Some Congeners in Rat Brain Homogenate Preparation

compd	IC_{50},[a] nM	
	[^{3}H]naltrexone	[^{3}H]DADLE
10	95	250
11	100	100
7	10	84
13	2.5	38
14	6.4	50

[a] The values reported are the mean of three determinations. The IC_{50} for naltrexone against itself is 1.5 nM, and the IC_{50} for DADLE against itself is 2 nM.

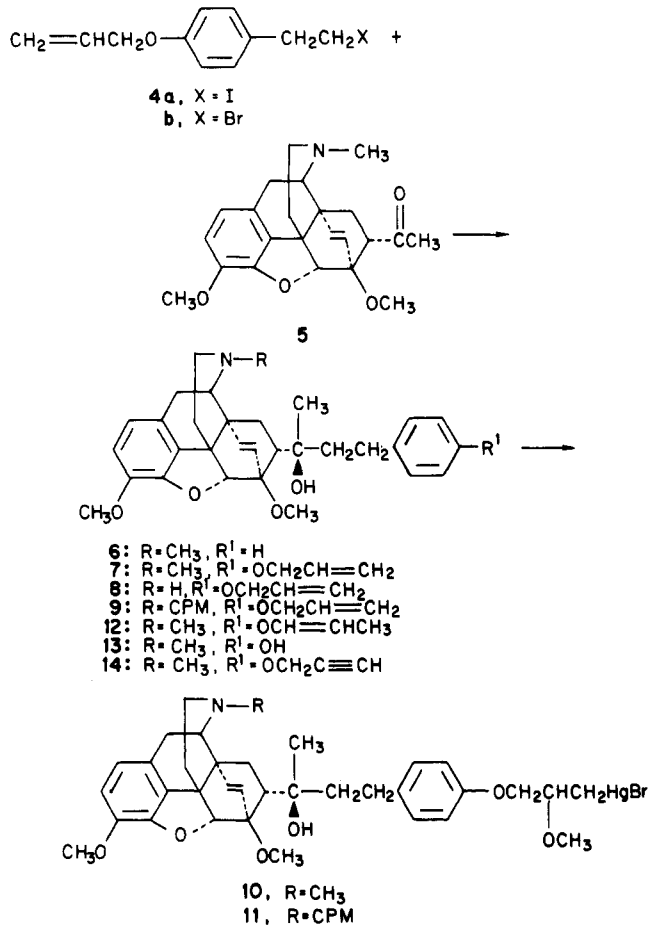
The cyclopropylmethyl (CPM) analogue **11** was prepared by demethylation of **7** to give **8** followed by treatment with cyclopropylmethyl bromide to furnish **9** which on methoxymercuration followed by KBr treatment gave

† Rensselaer Polytechnic Institute.
‡ Albert Einstein College of Medicine.
§ New York University College of Medicine.

(1) Bidlack, J. M.; Abood, L. G.; Osei-Gyimah, P.; Archer, S. *Proc. Natl. Acad. Sci. U.S.A.* **1981**, *78*, 626.
(2) Gioannini, J. L.; Howard, A.; Hiller, J. M.; Simon, E. J. *Biochem. Biophys. Res. Commun.* **1984**, *119*, 624.
(3) Maneckjee, R.; Zukin, R. S.; Archer S.; Michael, J.; Osei-Gyimah, P. *Proc. Natl. Acad. Sci. U.S.A.* **1985**, *82*, 594.
(4) Simon, E. J.; Groth, J. *Proc. Natl. Acad. Sci. U.S.A.* **1975**, *72*, 2402.
(5) Bentley, K. W.; Hardy, D. A.; Meek, B. *J. Am. Chem. Soc.* **1967**, *89*, 3273.
(6) The chirality of the carbon containing the new methoxyl group is not known.

0022-2623/85/1828-1950$01.50/0

Scheme II



11, which will be used as a ligand for an affinity column.

The acetylene **14** was prepared because it could serve as an intermediate to prepare tritium-labeled **7**. The deallylation of **7** proceeded smoothly according to the method of Gigg.[7] Isomerization of **7** with strong base afforded the propenyl ether **12** which was hydrolyzed to the phenol **13** with the aid of mercury salts. Alkylation of **13** with propargyl bromide gave **14**. Alkylation of **8** with methyl iodide gave **7** in >80% yield. This reaction can also be used to prepare tritium-labeled **7**.

Biological Results. In order for a ligand to be functional in affinity chromatography, it must be recognized by the target receptor. IC_{50} values for the displacement of [^{3}H]naltrexone (a μ-selective ligand) and [^{3}H]DADLE (a δ-selective ligand) in rat brain homogenates[8] were determined for Hybromet (**10**), **11**, **7**, **13**, and **14**. The results are summarized in Table I.

Compounds **7**, **13**, and **14** show preferential affinity for μ-binding sites. Although much weaker than its mercury-free congeners Hybromet (**10**) showed a modest preference for μ-binding sites also. The *N*-CPM analogue, **11**, is weaker than the mercury-free ligands but shows no preference for μ-over δ-binding sites.

Hybromet and naltrexone were added simultaneously to a rat brain homogenate preparation followed by two washings with Tris buffer. The naltrexone afforded less then 20% protection of the μ receptors. A similar experiment with **11** gave essentially identical results. These experiments suggest but do not prove that covalent binding of **10** and **11** to the μ receptors may have occurred.

As described in greater detail elsewhere[3] opioid receptors from rat neural membranes were solubilized with CHAPS, a zwitterionic detergent derived from cholic acid. The affinity column was prepared by condensing **10** with Affi-Gel 401, a sulfhydryl-containing derivative of agarose. The solubilized crude receptor preparation was applied to the column. The selectivity of the column was enhanced by the use of the highly selective μ ligand, normorphine, as the eluant. A 500-fold purification relative to the crude extract was achieved.[9] Binding studies on the partially purified receptor using ligands such as etorphine (μ selective), ethylketocyclazocine (κ selective), and DADLE (δ selective) indicated that the partially purified receptor was μ selective.

Experimental Section

Melting points were taken on a laboratory device Mel-Temp apparatus and are corrected. The ^{1}H NMR spectra were run on a 60-MHz Hitachi Perkin-Elmer R600 and a Varian XL200 spectrometer (as indicated below) using $CDCl_3$ as the solvent and $(CH_3)_4$Si as the internal standard. Microanalyses were performed by the Spang Microanalytical Laboratory, Eagle Harbor, MI, and Galbraith Laboratories. All analytical results but one were within ±0.4% of the theoretical values.

(2-Methoxy-3-phenoxypropyl)mercuric Bromide (1). A solution of 100 mg (0.75 mmol) of allyl phenyl ether in 10 mL of MeOH was added to a suspension of 1.20 g (3.76 mmol) of $Hg(OAc)_2$ in 10 mL of MeOH while the mixture was being stirred with the exclusion of moisture. After 16 h, 448 mg (3.76 mmol) of KBr in 10 mL of H_2O was added and stirring was continued for 45 min under reflux. The cooled suspension was extracted with 3 × 40 mL portions of $CHCl_3$. The combined extracts were washed with H_2O, filtered, and evaporated to dryness to leave a heavy colorless oil, wt 300 mg (90%). Purification was achieved by chromatography on silica gel using ethyl acetate–hexane (1:1). The resulting clear colorless oil was dried at 80 °C (0.02 torr). NMR (XL-200): δ 2.16 (d, fine splittings, 2 H, CH_2Hg), 3.46 (s, 3 H, OCH_3), 4.00 (d, 2 H, OCH_2), 4.10 (m, 1 H, CH), 6.96–7.40 (m, 5 H, aromatic H). Irradiation of the region δ 4.06–4.16 caused the doublets at δ 2.16 and 4.00 to become singlets. Anal. ($C_{10}H_{13}BrHgO_2$), C, H.

(2-Methoxy-3-phenoxypropyl)mercuric (2-Benzothiazolyl)mercaptide (3). To a stirred solution of 1.00 g (5.98 mmol) of 2-mercaptobenzothiazole (**2**) in 30 mL of dry THF kept under N_2 at 0 °C was added 287 mg (5.98 mmol) of a 50% suspension of NaH in mineral oil. After the addition was complete, stirring was continued for another 15 min before a solution of 2.66 g (5.98 mmol) of **1** in 10 mL of dry THF was added dropwise. The suspension was allowed to warm to room temperature, and stirring was continued for another 2 h. The cloudy suspension was treated with H_2O and extracted with $CHCl_3$. The extracts were thoroughly washed with H_2O and concentrated. The residual brown oil crystallized when triturated with EtOH. The almost white crystals were collected, wt 3.10 g (97%). After crystallization from EtOH, the mecaptide melted at 89–90 °C. NMR (XL-200): δ 2.16 (d, fine splittings, 2 H, CH_2Hg), 3.50 (s, 3 H, OCH_3), 3.92–4.10 (m, 2 H, OCH_2), 4.10–4.26 (m, 1 H, CH), 6.88–7.74 (3 m, 9 H, aromatic H). Irradiation of the region δ 3.92–4.26 caused the doublet at δ 2.16 to become a singlet. Anal. ($C_{17}H_{17}HgNO_2S_2$) C, H, N.

Reaction of (2-Methoxy-3-phenoxypropyl)mercuric (2-Benzothiazolyl)mercaptide (3) with 1,2-Dithioglycerol (BAL). A solution of 300 mg (0.564 mmol) of the mercaptide, **3**, and 70 mg (0.564 mmol) of BAL in 15 mL of $CHCl_3$ was stirred overnight. The filtered suspension was evaporated to dryness and chromatographed on silica gel. Elution with CH_2Cl_2 gave 51 mg (68%) of an allyl phenyl ether, whose IR and NMR spectra were identical with those of an authentic sample. Elution with $CHCl_3$–MeOH (9:1) gave crude 2-mercaptobenzothiazole (**2**). The crude **2** was dissolved in 1 N NaOH. The cloudy suspension was

(7) Gigg, J.; Gigg, R. *J. Chem. Soc.* **1966**, 82. Gigg, R.; Warren, C. D. *Tetrahedron Lett.* **1967**, 1683.

(8) Simon, E. J.; Hiller, J.; Groth, J.; Edelman, I. *J. Pharmacol. Exp. Ther.* **1975**, *192*, 531.

(9) **Note Added in Proof**: Further work has resulted in a >4000-fold purification of the μ receptor (Maneckjee, R.; Zukin, R. S.; Archer, S., unpublished data).

clarified by filtration, and the filtrate was washed with chloroform, acidified with dilute HCl, and finally extracted with $CHCl_3$. The washed $CHCl_3$ extract was dried and evaporated to leave 84 mg (89%) of 2-mercaptobenzothiazole, mp 180–182 °C, undepressed when admixed with an authentic specimen. The IR spectra of the isolated and authentic **2** were identical.

Reaction of (2-Methoxy-3-phenoxypropyl)mercuric Bromide (1) with BAL. A solution of 6.3 g (14.1 mmol) of **1** and 1.76 g (14.1 mmol) of BAL in 100 mL of $CHCl_3$ was stirred at room temperature for 2 h. The resulting suspension was filtered, and the filtrate was evaporated to leave an oil that was chromatographed on silica gel using $CHCl_3$ as the eluant. The appropriate fractions were combined and evaporated to furnish 1.56 g (82.5%) of allyl phenyl ether, which was identified by means of IR and NMR spectroscopy.

2-[4-(Allyloxy)phenyl]ethyl Iodide (4a). To a stirred solution of 6.2 g (0.035 mol) 2-[4-(allyloxy)phenyl]ethyl alcohol in 35 mL of dry pyridine kept below 5 °C was added portionwise 7.24 g (0.038 mol) of *p*-toluenesulfonyl chloride. The mixture was stirred for 30 min and allowed to warm to room temperature, and it was stirred for an additional 30 min. The mixture was poured onto crushed ice and acidified with 3 N HCl solution. This aqueous suspension was extracted with ether. The ethereal layer was washed with H_2O, dried, and concentrated to give 10.2 g (87%) of the crude tosylate. This was dissolved in 60 mL of dry acetone, and 7.5 g (0.05 mol) of NaI was added in one portion. The stirred solution was heated under reflux for 24 h, cooled, and filtered. The filtrate was diluted with H_2O and extracted with ether. The organic layer was washed with saturated Na_2SO_3 solution and then with brine. The dried extract was distilled to give the desired phenethyl iodide: bp 96–100 °C (0.1 torr); wt 10.0 g (96%); NMR (R-600) δ 3.00–3.50 (m, 4 H, CH_2CH_2), 4.40–4.60 (m, 2 H, CH_2O), 5.20–6.60 (m, 3 H, vinyl H), 6.9 (4 H aromatic H, AB system). Anal. ($C_{11}H_{13}IO$) C, H.

2-[4-(Allyloxy)phenyl]ethyl Bromide (4b). A solution of 12.0 g (0.1 mol) of allyl bromide in 60 mL of dry acetone was added over a period of 15 min to a stirred suspension of 13.0 g (0.12 mol) of K_2CO_3 and 13.0 g (0.09 mol) of 2-(*p*-hydroxyphenylethyl) alcohol in 250 mL of dry acetone heated in a water bath kept at 70 °C. After the addition was complete, the suspension was heated under reflux for 24 h. The cooled mixture was poured into H_2O and extracted with $CHCl_3$, and the organic layer was washed with H_2O. The dried extract (K_2CO_3) was evaporated to dryness to give crude 2-[4-(allyloxy)phenyl]ethyl alcohol: wt 16.0 g (96%); NMR (R-600) δ 2.10 (br s, 1 H, OH), 2.80 (t, 2 H, CH_2), 3.80 (t, 2 H, CH_2OH), 4.50 (d, 2 H, OCH_2), 5.20–5.50 (m, 2 H, vinyl H), 5.80–6.40 (m, 1 H, vinyl H), 6.90 (4 H, aromatic H, AB system).

To a cooled solution of 22.4 g (0.085 mol) of triphenylphosphine in 70 mL of dry CH_3CN was added over a period of 15 min with stirring 13.5 g (0.084 mol) of Br_2. To this stirred, cooled solution of triphenylphosphine dibromide was added a solution of 15.0 g (0.084 mol) of the above crude alcohol in 15 mL of CH_3CN. The CH_3CN was removed by distillation and the desired bromide was dissolved in dry ether and filtered. The ether was dried and then evaporated to leave an oil that was purified by chromatography on a column of silica gel using hexane as the eluent. There was obtained 13.0 g (62%) of the desired bromide as an oil. NMR (R-600): δ 3.10 (t, 2 H, CH_2), 3.50 (t, 2 H, CH_2Br), 4.50 (d, 2 H, OCH_2), 5.20–5.50 (m, 2 H, vinyl H), 5.80–6.40 (m, 1 H, vinyl H), 6.9 (4 H, aromatic H, AB system). Anal. ($C_{11}H_{13}BrO$) C, H.

7α-[(1*R*)-1-Hydroxy-1-methyl-3-[4-(allyloxy)phenyl]propyl]-6,14-*endo*-ethenotetrahydrothebaine (7). (A) A suspension of 1.0 g (0.041 mol) of Mg chips in 5.0 mL of dry THF was heated under reflux. A solution of 5.9 g (0.02 mol) of 2-[4-allyloxy)phenyl]ethyl bromide and 1.8 mL (0.02 mol) of 1,2-dibromoethane in 25 mL of dry THF was prepared. Two milliliters of this solution was added to the Mg suspension to initiate a vigorous reaction. After the Grignard reaction had started, the remainder of the bromide mixture was added dropwise under reflux. Where the addition was complete, the whole was refluxed for an additional 2 h and then cooled to room temperature. A solution of 3.2 g (0.008 mol) of 7α-acetyl-6,14-endo-ethenotetrahydrothebaine in 10 mL of dry THF was added dropwise over a period of 15 min. The mixture was stirred under reflux for 6 h, cooled, and treated with cold aqueous NH_4Cl solution. The mixture was extracted thoroughly with $CHCl_3$. The extracts were washed with H_2O, dried, and evaporated. Trituration of the residue with ether furnished a crystalline solid, wt 2.50 g (55%) that melted at 160–162 °C after crystallization from ethanol. NMR (XL-200): δ 0.80 (dd, 1 H, H-7?), 1.03 (s, 3 H, CH_3), 1.44–2.08 (m, 4 H, H-8, H-15), 2.36 (s, 3 H, NCH_3), 2.24–2.56 (m, 4 H, H-16, [$(CH_3)COHCH_2$]), 2.66–2.79 (t, 2 H, $CH_2C_6H_4O$), 2.79–3.30 (m, 3 H, H-9α, H-10α, H-10β), 3.80 (s, 3 H, C-6 OCH_3), 3.84 (s, 3 H), C-3 OCH_3), 4.54 (d, 2 H, OCH_2), 4.59 (s, 1H, H-5β), 5.04 (s, 1 H, OH), 5.75 (dd, 2 H, vinyl H, AB system, J = 8Hz), 5.24–6.20 (m, 3 H, allylic H), 6.60 (dd, 2 H, aromatic H, AB system, J = 8Hz), 7.03 (dd, 4 H aromatic H, AB system, J = 8 Hz). IR (KBr): 3460 cm^{-1} (OH). Anal. ($C_{34}H_{41}NO_5$) C, H, N.

(B) A Grignard reagent was prepared from 16.6 g (0.050 mol) of 2-[(allyloxy)phenyl]ethyl iodide and 1.4 g of Mg turnings in 60 mL of dry ether. A solution of 11.0 g (0.03 mol) of 7α-acetyl-6,14-*endo*-ethenotetrahydrothebaine was added dropwise, and the mixture was heated under reflux for 24 h. The cooled mixture was worked up in the usual manner to give an oil that was chromatographed on silica gel to give 2.8 g (10%) of the desired product, mp 156–157 °C, after crystallization from ethanol. Except for the slight difference in melting point, the sample was identical (NMR, IR, mixed melting point) with the material prepared from the corresponding bromide.

(C) A mixture of 15 mg (0.023 mmol) of the fumarate salt of the nor compound **8**, 45 mg (0.425 mmol) of Na_2CO_3, and 5 mg (0.035 mmol) of methyl iodide in 3 mL of 10% aqueous acetone was heated under reflux with stirring for 1 h. The mixture was poured onto water, and the whole was extracted with CH_2Cl_2. The extract was washed with H_2O and evaporated to leave an oil, which was chromatographed on silica gel using ethyl acetate–methanol (19:1) as the eluant. There was obtained 10.5 mg (83%) of the desired product **7**, identical (NMR, IR) with an authentic sample.

7α-[(1*R*)-1-Hydroxy-1-methyl-3-[4-[3-(bromomercurio)-2-methoxypropoxy]phenyl]propyl]-6,14-*endo*-ethenotetrahydrothebaine (10). A solution of 2.8 g (5.1 mmol) of the above allyl ether (**7**) in 10 mL of $CHCl_3$ was added with stirring to a solution of 1.64 g (5.1 mmol) of $Hg(OOCCH_3)_2$ in 30 mL of CH_3OH. The solution was stirred at room temperature for 72 h before being treated with 600 mg (5.1 mmol) of KBr dissolved in 10 mL of H_2O. The resulting suspension was heated at 50 °C for 30 min, and then the warm solution was filtered. The filtrate was evaporated to dryness in vacuo, and the residue was dissolved in $CHCl_3$. The solution was dried and evaporated to leave a residue that was chromatographed on silica gel with $CHCl_3$ as the developing solvent. There was obtained 1.5 g (34%) of the desired bromomercurio compound **10**, mp 117–118 °C, after crystallization from CH_2Cl_2/CH_3OH. NMR (XL-200): δ 0.82 (dd, 1 H, H-7?), 1.04 (s, 3 H, CH_3), 1.46–2.14 (m, 4 H, H-8, H-15), 2.16 (d, fine splittings, 2 H, CH_2Hg), 2.38 (s, 3 H, NCH_3), 2.28–2.60 (m, 4 H, H-16, [$(CH_3COHCH_2]$]), 2.68–2.83 (t, 2 H, $CH_2C_6H_4O$), 2.82–3.32 (m, 3 H, H-9α, H-10α, H-10β), 3.46 (s, 3 H, OCH_3), 3.82 (s, 3 H, C-6 OCH_3), 3.85 (s, 3 H, C-3 OCH_3), 4.00 (d, fine splitting, 2 H, OCH_2), 4.04–4.18 (m, 1 H [$CH_2(OCH_3)CHCH_2$]), 4.60 (s, 1 H, H-5β), 5.06 (s, 1 H, OH), 5.77 (dd, 2 H, vinyl H, AB system, J = 8 Hz), 6.63 (dd, 2 H, aromatic H, AB system, J = 8 Hz), 7.10 (dd, 4 H, aromatic H, AB system, J = 8 Hz). Irradiation of the region δ 3.94–4.10 caused the doublet at δ 2.16 to become a singlet. IR (KBr): 3470 cm^{-1} (OH). Anal. ($C_{35}H_{44}O_6BrHgN$) C, H, N.

7α-[(1*R*)-1-Hydroxy-1-methyl-3-[4-(propenyloxy)-phenyl]propyl]-6,14-*endo*-ethenotetrahydrothebaine (12). A solution of 2.0 g (3.6 mmol) of the allyl ether (**7**) and 410 mg (3.6 mmol) of potassium *tert*-butoxide in 4 mL of Me_2SO was heated at 100 °C for 30 min. The mixture was cooled and poured into H_2O. The solid that separated was collected, washed with H_2O, and dried; wt. 190 g (95%). After crystallization from C_2H_5OH the white crystals melted at 166–167 °C. NMR (R-600): δ 1.00 (s, 3 H, CH_3), 1.70 (d, 3 H, CH_3), 2.36 (s, 3 H, NCH_3), 3.76, 3.83 (2 s, 6 H, 2 OCH_3), 4.50 (s, 1 H, H-5β), 4.93 (s, 1 H, OH), 4.70–6.36(m, 4 H, vinyl H), 6.50–7.20 (m, 6 H, aromatic H). IR (KBr): 3470 cm^{-1} (OH). Anal. ($C_{34}H_{41}NO_5$) C, H, N.

7α-[(1*R*)-1-Hydroxy-1-methyl-3-(4-hydroxyphenyl)-propyl]-6,14-*endo*-ethenotetrahydrothebaine (13). To a stirred suspension of 1.0 g of the above propenyl derivative and 1.0 g of HgO in 21 mL of acetone–H_2O (4:1) was added a solution of 1.0 g of $HgCl_2$ in 21 mL of the same solvent mixture. After 1 h the mixture was filtered and 2.5 g of $NaHCO_3$ was added to the

filtrate. Soluble mercury salts were removed by passing H_2S into the solution. The black suspension was filtered, and the filtrate was evaporated. The residue was dissolved in $CHCl_3$, washed with H_2O, and dried. Evaporation of the solvent left a residue that was chromatographed on silica gel with $CHCl_3$–10% CH_3OH as the eluant. There was obtained 500 mg (55%) of phenol **13**, mp 229–230 °C, after crystallization from ethanol. NMR (R-600): δ 1.00 (s, 3 H, CH_3), 2.33 (s, 3 H, NCH_3), 3.78, 3.85 (2 s, 6 H, 2 OCH_3), 4.50 (s, 1 H, H-5β), 5.13 (s, 1 H, OH), 5.20, 6.10 (dd, 2 H, vinyl H), 6.40–7.16 (m, 7 H, aromatic 6 H, 1 OH). Anal. ($C_{31}H_{27}NO_5 \cdot 0.5H_2O$) C, H, N.

7α-[(1*R*)-1-Hydroxy-1-methyl-3-[4-(propargyloxy)phenyl]propyl]-6,14-*endo*-ethenotetrahydrothebaine (14). To a solution of 400 mg (0.78 mmol) of phenol **14** and 35 mg (0.87 mmol) of NaOH in 25 mL of C_2H_5OH was added 100 mg (0.84 mmol) of propargyl bromide. The solution was refluxed for 48 h and evaporated to dryness, and the residue was chromatographed on a silica gel column with $CHCl_3$–1% CH_3OH as the eluant. There was obtained 200 mg (47%) of the propargyl ether, mp 162–163 °C, after recrystallization from heptane. NMR (R-600): δ 1.05 (s, 3 H, CH_3), 2.33 (s, 3 H, NCH_3), 2.36 (m, 1 H, C≡CH), 3.73–3.83 (2 s, 6 H, 2 OCH_3), 4.50–4.70 (m, 3 H, OCH_2, H-5β), 4.96 (s, 1 H, OH), 5.30–6.10 (dd, 2 H, vinyl H), 6.33–7.23 (m, 6 H aromatic H). IR (KBr): 3450 cm^{-1} (OH). Anal. ($C_{34}H_{39}NO_5$) C, H, N.

7α-[(1*R*)-1-Hydroxy-1-methyl-3-[4-(allyloxy)phenyl]propyl]-6,14-*endo*-ethenotetrahydronorthebaine (8). A solution of 2.0 g (3.7 mmol) of allyl ether **7** and 1.06 g (6 mmol) of ethyl azodicarboxylate in 60 mL of dry benzene was refluxed for 5 h. The benzene was removed in vacuo, and the residue was treated with 8.0 mL of H_2O, 8.0 mL of C_2H_5OH, and 6.0 mL of saturated NH_4Cl. After being heated for 7 h, the mixture was evaporated to dryness in vacuo, neutralized with $NaHCO_3$ solution, and extracted with $CHCl_3$. The $CHCl_3$ extract was washed with H_2O, dried, and evaporated to leave a residue that was chromatographed on silica gel with ethyl acetate as the developing solvent to furnish a crude solid, wt 1.2 g (60%). It formed a crystalline fumarate salt, mp 248–250 °C. NMR (R-600) (free base): δ 1.00 (s, 3 H, CH_3), 3.76–3.80 (2 s, 6 H, 2 OCH_3), 4.40–4.66 (d, 3 H, OCH_2, H-5β), 5.10–6.33 (m, 5 H, vinyl H and allylic H), 6.30–7.30 (m, 6 H, aromatic). Anal. ($C_{33}H_{39}NO_5 \cdot C_4H_4O_4$) C, H, N.

7α-[(1*R*)-1-Hydroxy-1-methyl-3-[4-(allyloxy)phenyl]propyl]-6,14-*endo*-ethenotetrahydro-*N*-(cyclopropylmethyl)northebaine (9). A suspension of 970 mg (1.8 mmol) of the nor compound (**8**), 1.5 g (10 mmol) of NaI, 1.06 (0.01 m) of Na_2CO_3, and 400 mg (2.96 mmol) of cyclopropylmethyl bromide in 30 mL of 10% aqueous acetone was heated under reflux with stirring for 3 h. The mixture was poured onto H_2O, and the whole was extracted with CH_2Cl_2. The extract was washed with H_2O, dried (Na_2SO_4), and evaporated to leave an oil that was chromatographed on silica gel with ethyl acetate–hexane (1:1) as the eluant. There was obtained 770 mg (77%) of the desired product, **9**, mp 111–112 °C. NMR (R-600): δ 1.00 (s, 3 H, CH_3), 3.76–3.83 (2 s, 6 H, 2 OCH_3), 4.40–4.63 (d, 3 H, OCH_2, H-5β), 4.93 (s, 1 H, OH), 5.10–6.33 (m, 5 H, vinyl H and allylic H), 6.30–7.30 (m, 6 H, aromatic H). IR (KBr): 3480 cm^{-1} (OH). Anal. ($C_{37}H_{45}NO_5$) C, H, N.

7α-[(1*R*)-1-Hydroxy-1-methyl-3-[4-[3-(bromomercurio)-2-methoxypropoxy]phenyl]propyl]-6,14-*endo*-ethenotetrahydro-*N*-(cyclopropylmethyl)northebaine (11). A solution of 790 mg (1.35 mmol) of allyl ether **9** in 10 mL of CH_3OH was added to a solution of 430 mg (1.35 mmol) of $Hg(OOCCH_3)_2$ in 10 mL of CH_3OH. The mixture was stirred for 3 days at room temperature and was then treated with a solution of 160 mg (1.35 mmol) of KBr in 10 mL of H_2O. The mixture was heated for 30 min, poured into H_2O, and extracted with $CHCl_3$. Chromatography (silica gel with $CHCl_3$ as the eluant) furnished the desired product: wt 350 mg (29%); mp 78–80 °C. NMR (R-600): δ 1.00 (s, 3 H, CH_3), 3.33 (s, 3 H, OCH_3), 3.73, 3.80 (2 s, 6 H, 2 OCH_3), 3.90 (2 H, OCH_2), 4.46 (s, 1 H, H-5β), 4.87 (s, 1 H, OH), 5.26–6.00 (m, 2 H, vinyl H), 6.43–7.16 (m, 6 H aromatic). IR (KBr): 3460 cm^{-1} (OH). Anal. ($C_{38}H_{48}O_6BrHgN$) C, H, N.

Reaction of 10 with 1,2-Dithioglycerol (BAL). A solution of 50 mg (0.0585 mmol) of **10** and 7.3 mg (0.0587 mmol) of 1,2-dithioglycerol in 2 mL of $CHCl_3$ was stirred overnight at room temperature. The suspension was filtered and the filtrate was evaporated to dryness, leaving an oily residue that was chromatographed on silica gel with ethyl acetate–MeOH (19:1) as the eluant. There was obtained 28 mg (88%) of the allyl ether, **7**, mp 159–161 °C, identical in all respects (mixed melting point IR, NMR) with an authentic sample.

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1-Benzylcyclopropylamine and 1-(Phenylcyclopropyl)methylamine: An Inactivator and a Substrate of Monoamine Oxidase

Richard B. Silverman*† and Paul A. Zieske

Departments of Chemistry and of Biochemistry, Molecular Biology, and Cell Biology, Northwestern University, Evanston, Illinois 60201. *Received April 1, 1985*

1-Benzylcyclopropylamine (1) and 1-(phenylcyclopropyl)methylamine (2), cyclopropane analogues of phenethylamine, were tested as inactivators for monoamine oxidase (MAO). Compound 1 is a potent competitive reversible inhibitor of the oxidation of benzylamine and also is a mechanism-based inactivator. It requires 2.3 equiv of 1 to inactivate 1 equiv of MAO. The excess equivalents of 1 are converted into benzyl vinyl ketone. A one-electron mechanism of inactivation is proposed. Compound 2 is a substrate for MAO and is converted into 1-phenylcyclopropanecarboxaldehyde without inactivation of the enzyme. Mechanistic consequences are discussed as a result of this observation.

Mitochondrial monoamine oxidase (MAO; E.C. 1.4.3.4) is one of the enzymes responsible for the catabolism of the biogenic amines. Compounds that inhibit MAO exhibit antidepressant activity;[1-3] many substituted cyclopropyl-

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