Derivatives of Apomorphine and of Other *N*-Substituted Norapomorphines

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Abstract □ Derivatives of apomorphine and of *N*-*n*-propylnorapomorphine were prepared to obtain modified pharmacological activity and enhanced chemical stability. Mouse profile and dog emesis screens were performed, and the activity of various *N*-substituted derivatives and their esters was evaluated and compared to the parent compounds. The *N*-*n*-propyl diacetate derivative and *N*-methyl and *N*-*n*-propyl ascorbate salts were remarkably stable to air; apomorphine etherate was no more stable than the free base. The dimers, the major products formed during the acid-catalyzed rearrangement of morphines to apomorphines, were all potent emetics. Additionally, two showed a significant antagonism to morphine in mice and dogs.

Keyphrases □ Apomorphine—*N*-substituted derivatives synthesized, screened for emetic activity, stabiliy □ Structure–activity relationships—*N*-substituted derivatives of apomorphine screened for emetic activity □ Emetic activity—*N*-substituted derivatives of apomorphine screened

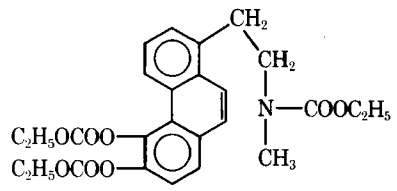
Following the conclusion that *N*-*n*-propylnorapomorphine was the most potent member of the apomorphine series (1–3), derivatives were prepared (I–IX, Table I) that might possess appropriately modified pharmacological activity or greater stability. In this work, apomorphine was used as a model; the *n*-propyl compound was used only for the synthesis of the more promising derivatives. The work was restricted to compounds preparable from morphine and belonging to the (6aR) (−) series. Some parallel work with apomorphines prepared by total synthesis was reported previously (4).

The pharmacological activity of four "dimers" (X–XIII), the major products formed during the acid-catalyzed rearrangement of normorphine and its *N*-substituted derivatives to the corresponding apomorphines, is also reported.

DISCUSSION

Chemistry—Conventional procedures for the synthesis of I–IX and workup procedures for X–XIII are described under *Experimental*. Little is known about the structure and chemistry of the dimers (X–XIII). Their behavior in gel chromatography indicates that they have molecular weights considerably higher than the 535 required for a true dimer[1], and a parent peak in their mass spectra has not been identified. They are readily soluble in dilute acid but insoluble in all other solvents. Although it was possible to free them from the corresponding apomorphine, homogeneous samples were not obtained; the analytical values given in Table I are for the specific sample used for pharmacological evaluation. They are formed during the rearrangement reaction (even at room temperature) and are not formed from the apomorphine itself under the conditions of rearrangement (3).

Compound III was prepared by the reaction of apomorphine with ethyl chloroformate in pyridine. When aqueous Schotten–Bauman conditions were used, ring opening occurred to give the phenanthrene



XIV

(XIV). Analogous reactions were reported for the conditions of the Hoffmann and Emde degradation reactions (5).

Compound XIV was evaluated because of the possibility that hydrolysis *in vivo* might give an aminoalkylphenanthrenediol known (5) to have apomorphine-like activity.

Pharmacology[2]—The mouse profile and dog emesis screens were those used previously (3). Quaternary derivatives of apomorphine and of morphine are known to differ markedly from the parent compounds because of their inability to enter the central nervous system (CNS) (6); the unusally low activity of VII in comparison to its parent tertiary amine is a striking example of this effect. The quaternary compound was also much more toxic.

Alkyl ethers derived from apomorphine compounds do not cause the apomorphine response or syndrome (4, 7, 8). Compound V was prepared in the hope that, as a formal, it might undergo cleavage to apomorphine *in vivo*. Its low activity suggests that this cleavage does not occur readily.

The quantitative pharmacological equivalence of apomorphine and its ester derivatives was reported previously (9, 10). In the treatment of parkinsonism, the esters have a prolonged effect and a lower toxicity than apomorphine itself (10). The esters of catecholamines enter the CNS more rapidly and provide for a sustained release of the catecholamine within the CNS (11).

The equivalence of diacetates to the parent phenols (3) is confirmed by the activity of I and II. TLC showed that the hydrolysis of II *in vitro* was slow. The onset of emesis in dogs following intravenous administration of the carbonate ester (III) was slightly delayed. At a dose of 0.05 mg/kg, 2 min was required. At 0.1 mg/kg, emesis occurred at 90 sec and again at 2 hr. At 0.2 mg/kg, emesis occurred at 90 sec and again at 30 min. These observations are in accord with the hypothesis that hydrolysis of III to apomorphine is slower than with the acetates (I and II).

The carbamate (IV) and the methoxyacetate (VI) were relatively inactive in mice, suggesting that hydrolysis *in vivo* is slow. The LD_{50} and MED values in mice for XIV were both greater than 20 mg/kg; had hydrolysis occurred, greater activity would have been expected for the resulting aminoalkylphenanthrenediol (5).

The dimers (X–XIII) were all quite active in the mouse screen, although less potent than the related apomorphines (3). Their emetic potency in dogs was only slightly less than that of the apomorphines. During a routine study of other pharmacological properties, it was observed that all four dimers showed insignificant analgesia in a modified mouse tail-flick test (12) at doses up to 100 mg/kg iv but that only XII and XIII showed marked antagonism to morphine in that test. For example, when 18 mice were treated with 10 mg/kg of morphine (which produced 79% analgesia), XII had a morphine antagonist value (AD_{50}) of 9.0 mg/kg; the values ranged between 82% analgesia at 3 mg/kg of XII and 8% analgesia at 30 mg/kg. The AD_{50} for XIII was 21 mg/kg.

[1] J. G. Cannon, University of Iowa, Iowa City, Iowa, personal communication.

[2] In conducting the described research, the investigators adhered to the "Guide for the Care and Use of Laboratory Animals," as promulgated by the Committee on Revision of the "Guide for Laboratory Animals, Facilities and Care" of the Institute of Laboratory Animal Resources, National Research Council.

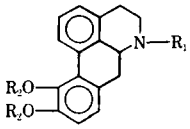


Table I—Derivatives of Apomorphine and of Other *N*-Substituted Norapomorphines

Compound	R_1	R_2	Melting Point	Yield, %	Formula	Analysis, % Calc.	Analysis, % Found	MED[a], mg/kg iv, Mouse (Fiducial Limits)	LD_{50}, mg/kg iv, Mouse (Fiducial Limits)	Dog Emesis, Minimum Effective Dose, mg/kg iv
I	CH_3	CH_3CO	125–128°[b]	50	$C_{21}H_{21}NO_4$	—	—	0.042 (0.024–0.075)	40.0 (18.0–89.0)	0.006
II	n-C_3H_7	CH_3CO	138.5–139.5°	50	$C_{23}H_{25}NO_4$	C 72.73 H 6.64 N 3.68	72.18 6.71 3.60	0.0042 (0.0024–0.0075)	50.0 (39.0–63.0)	—
III	CH_3	C_2H_5OCO	111–113°	55	$C_{23}H_{24}NO_6$	C 67.14 H 6.12 N 3.40	67.09 6.04 3.31	0.056 (0.034–0.094)	36.0 (28.0–45.0)	<0.05
IV	CH_3	$(CH_3)_2NCO$	75–125°	50	$C_{23}H_{27}N_3O_4$	C 67.46 H 6.65 N 10.26	67.48 6.47 10.27	1.8 (0.56–5.6)	18.0 (5.6–56.0)	>0.1
V[c]	CH_3	CH_3OCH_2	164–166° dec.	58	$C_{21}H_{25}NO_4 \cdot C_4H_6O_6$	C 59.39 H 6.18 N 2.77	58.86 6.12 2.64	1.8 (0.56–5.6)	>100	>0.1
VI[c]	CH_3	CH_3OCH_2CO	162–163°	67	$C_{23}H_{25}NO_6 \cdot C_4H_6O_6$	C 57.75 H 5.56 N 2.49	57.56 5.63 2.45	0.13 (0.075–0.24)	63 (50.0–79.0)	
VII[d]	n-C_3H_7, CH_3	H	—[e]	40	$C_{20}H_{24}INO_2$	C 54.92 H 5.53 I 29.02 N 3.30	54.26 5.30 29.58 3.30	1.8 (0.56–5.6)	5.6 (1.8–18.0)	>0.1
VIII[f]	CH_3	H	100° dec.	95	$C_{17}H_{17}NO_2 \cdot C_6H_8O_6 \cdot H_2O$	C 59.86 H 5.90 N 3.04	59.48 5.74 2.69	—	—	—
IX[f]	n-C_3H_7	H	85° dec.	80	$C_{19}H_{21}NO_2 \cdot C_6H_8O_6 \cdot 2H_2O$	C 59.16 H 6.55 N 2.76	58.90 6.07 2.37	—	—	—
X[g]	CH_3	H	290–300° dec.	60	—	C H Cl N Ash	61.6 6.04 11.21 4.22 1.44	0.13 (0.075–0.24)	32.0 (25.0–40.0)	0.01
XI[g]	C_2H_5	H	285° dec.	70	—	C H Cl N Ash	62.8 5.99 10.60 4.14 1.83	0.042 (0.024–0.075)	32.0 (20.0–50.0)	0.005
XII[g]	n-C_3H_7	H	270–280° dec.	70	—	C H Cl N Ash	65.4 6.59 8.46 4.48 0.9	0.01 (0.005–0.019)	36.0 (28.0–45.0)	0.002
XIII[g]	CH_2=$CHCH_2$	H	270–290° dec.	40	—	C H Cl N Ash	66.9 6.51 10.1 3.75 1.93	0.56 (0.18–1.8)	56.0 (18.0–180.0)	0.1

[a] Minimum dose at which reactive signs were observed in half of mice tested (3). [b] Recrystallized from ethyl acetate–petroleum ether; lit. (21) mp 128–129°. [c] Tartrate salt. [d] Quaternary methiodide. [e] Shrinks at 220°, decomposes at much higher temperature. [f] L-Ascorbate salt hydrate. [g] Dimer hydrochloride.

The morphine antagonism of XII and XIII was also observed in dogs, where these compounds reversed morphine-induced respiratory depression as measured by both respiration rate and pulmonary ventilation rate. Thus, 5 mg/kg of morphine was administered intravenously to anesthetized beagle dogs of either sex, and the well-known respiratory depression was observed. After 5 min, 0.1 mg/kg of XII was administered intravenously and caused an immediate reversal of depression. The antagonism persisted for at least 1 hr, because two subsequent 5-mg/kg doses of morphine given at 15 and 65 min caused no further respiratory depression. A dose of 0.01 mg/kg of XII did not prevent respiratory depression caused by 5 mg/kg of morphine, but it did prevent additional depression by a second 5-mg/kg dose of morphine. Similar results were observed with doses of 0.03 and 0.1 mg/kg of XIII. An alternative explanation of the apparent morphine antagonism of XII and XIII is that the dogs rapidly developed a tolerance to morphine.

Stability Studies—All apomorphines are easily oxidized by air to form a green oxidation product, which has been identified (13) and used as the basis of an analytical method for apomorphine (14, 15). Dilute solutions of apomorphine hydrochloride at pH 3 develop no color when protected by sulfite and an inert gas. When the solutions contain sulfite and the sodium salt of ethylenediaminetetraacetic acid, they can still be exposed intermittently to air and yet suffer only a 7% deterioration during 7 months (16). The standard procedure for liberating apomorphine bases from their hydrochloride salts by the use of aqueous sulfite solutions was undoubtedly developed to take advantage of the protective effect of sulfite. Thiourea also protects apomorphine (17).

Ascorbic acid protects apomorphine hydrochloride solutions from oxidation by air (18). The ascorbate salts (VIII and IX) were characterized and found to be nonhygroscopic and to develop no color after prolonged exposure to air. The protective effect of the ascorbate ion is so great that these salts do not react in the standard iodine oxidation color test for apomorphines (19). No attempt was made to establish the pharmacological potency of VIII and IX; it is likely that these salts are equivalent to the hydrochloride salts *in vivo*.

It was expected that the diacetate ester (II) would also resist oxidation. It did not become discolored in air and was very stable even in the molten state. Samples were repeatedly melted (pronounced supercooling was observed) on a microscope stage, and the melting point fell only to 131–136°. Solutions of II in dimethyl sulfoxide remained colorless for 1 week; when allowed to evaporate in air, long colorless needles of II separated.

A 24% solution of II in dimethylacetamide at 22° had a very pale-green color. During 8 weeks of storage in contact with air, the absorption coefficient at 266 nm did not change within experimental error; the color darkened somewhat. It has been the authors' experience [as with others (16)] that marked discoloration of apomorphine compounds in contact with air can occur before significant decreases in actual apomorphine content. A 1% aqueous stock solution of *N*-*n*-propylnorapomorphine (pH 4–5) became dark green, but the MED value in the mouse screen was unchanged after 24 hr.

When apomorphine base is liberated from its hydrochloride salt by aqueous sulfite and then extracted into ether, concentration by boiling of the ether solution gives a remarkably stable etherate [$C_{17}H_{17}NO_2 \cdot (C_2H_5)_2O$], whose stability was noted previously (20). The compound loses ether at 145° to form the apomorphine base, mp 208°. Evaporation of an acetone solution of the etherate does not decompose it, but evaporation of a chloroform solution causes complete loss of ether. An analogous etherate could not be prepared from *N*-*n*-propylnorapomorphine. Apomorphine etherate developed a faint green color after contact with air in the dark during 3 months; but in parallel experiments, the discoloration of the apomorphine base and of the free base of *N*-*n*-propylnorapomorphine (mp 195–197°) was about the same. Thus, etherates do not possess greatly enhanced stability to air.

While the stability studies are qualitative in nature, the results confirm the observations of other investigators who found that significant stabilization of apomorphine can be achieved by use of appropriate salts. They also show that the diacetate esters (I and II) are very stable derivatives with high pharmacological potency.

EXPERIMENTAL[3]

***N*-*n*-Propylnorapomorphine Diacetate (II)**—The procedure was similar to that used for the preparation of apomorphine diacetate (21). Following acetylation by acetic anhydride in pyridine, the crude product hydrochloride was dissolved in water and the base was liberated by addition of potassium bicarbonate. The base was extracted into chloroform, recovered by evaporation, and recrystallized from ethyl acetate–hexane.

Apomorphine Bis(ethyl Carbonate) (III)—Ethyl chloroformate (1.6 g, 0.0148 mole) was added rapidly to a stirred, cold (0°) solution of 1.5 g (0.00454 mole) of apomorphine hydrochloride[4] NF in 60 ml of pyridine. The mixture was stirred in the dark for 36 hr, during which time a transient pale-yellow precipitate was observed. Pyridine was removed by vacuum evaporation to 20 ml, 20 ml of toluene was added, and the solution was evaporated to dryness. The gelatinous residue was taken up in 100 ml of water, and the base was liberated by addition of excess sodium sulfite solution. It was extracted into ether, recovered by evaporation, and recrystallized from acetone–heptane.

Ethyl *N*-[2-[1-[5,6-Bis(ethoxycarbonyloxy)]phenanthryl]-ethyl]-*N*-methylcarbamate (XIV)—To a stirred mixture of 5 g (0.0158 mole) of apomorphine hydrochloride, 50 ml of water, and 30 g (0.28 mole) of ethyl chloroformate under nitrogen at 0–3° was added 10% aqueous sodium hydroxide (containing a little sodium dithionite) until the mixture became permanently basic to litmus. The mixture was stirred in the dark at room temperature for 3 days. The solid that separated was washed with water, dried in air, and recrystallized from ether–hexane to give almost colorless XIV in 60% yield, mp 84–87°.

Anal.—Calc. for $C_{26}H_{29}NO_8$: C, 64.61; H, 6.05; N, 2.88. Found: C, 65.19; H, 6.06; N, 2.91.

Because of its insolubility in standard solvents for intravenous administration, XIV was administered as a solution in 85% lactic acid.

Apomorphine Bis(dimethylcarbamate) (IV)—To a solution of apomorphine etherate (21) (1.7 g, 0.005 mole) in 15 ml of dry pyridine was added 1.08 g (0.01 mole) of freshly distilled dimethylcarbamyl chloride[5]. The yellow solution was warmed under nitrogen on the steam bath until TLC (silica, methyl alcohol–chloroform) of an aliquot (free base liberated by sodium sulfite and extracted into ether) showed little remaining apomorphine. Pyridine was removed under vacuum and by evaporation of its azeotrope with toluene.

The residual hydrochloride salt was treated with sodium sulfite solution, and the free base was recovered after extraction into ether. TLC showed a trace of residual apomorphine, which was removed by passing a chloroform solution of the product through a column of neutral alumina. The product recovered by evaporation of chloroform was a pale-yellow glass, homogeneous by TLC. It developed a pronounced yellow color when exposed to light and air. It was examined in the animal screens without delay.

10,11-Di(methoxymethoxy)aporphine Tartrate (V)—Apomorphine etherate (20) was prepared from 1.5 g (0.00492 mole) of apomorphine hydrochloride. A solution of naphthalene sodium was prepared by stirring a mixture of 2 g of naphthalene, 226 mg (0.00984 mole) of sodium, and 60 ml of dry 1,2-dimethoxyethane for 3.5 hr. A solution of the etherate in 30 ml of the same solvent was added dropwise under nitrogen to the stirred naphthalene sodium solution, and a clear pale-green solution formed.

A solution of 800 mg (0.00994 mole) of chloromethyl methyl ether in 10 ml of the solvent was added, and the mixture was refluxed for 2 hr, during which time sodium chloride precipitated and an orange color developed. When the product could not be characterized as the free base, it was converted to a tartrate salt in boiling isopropyl alcohol solution and then recrystallized from ethyl alcohol–ether.

Apomorphine Bis(methoxyacetate) Tartrate (VI)—Acylation in pyridine, as for III, was used with methoxyacetyl chloride (22). The product was characterized as the tartrate salt from boiling isopropyl alcohol.

***N*-*n*-Propylnorapomorphine Methiodide (VII)**—Excess methyl iodide was added to a dry (magnesium sulfate) ether solution of *N*-*n*-propylnorapomorphine under nitrogen, and a heavy colorless

[3] Melting points were obtained in capillaries in a bath heated from room temperature at 5°/min and are uncorrected. Elemental analyses were performed by Galbraith Laboratories, Knoxville, Tenn., and by the late Dr. S. M. Nagy (Belmont, Mass.). Satisfactory IR, UV, and NMR spectra were recorded for all synthesized compounds. All workup procedures were designed to eliminate unreacted starting material that might have significantly affected the pharmacological evaluation.

[4] Penick.

[5] Aldrich.

crystalline solid separated during 24 hr. The quaternary salt was recrystallized twice from methyl alcohol–acetone and additionally leached with ether until free (TLC) from unreacted base. The salt formed a 1.8% solution in water at room temperature; both the salt and its solution discolored rapidly in air.

Ascorbate Salts (VIII and IX)—Apomorphine hydrochloride (2 g, 0.0066 mole) was dissolved in 150 ml of water, and the colorless base was precipitated by addition of excess sodium sulfite solution. The base was extracted into ether, and a solution of 1.16 g (0.0066 mole) of ascorbic acid[6] USP in 10 ml of methyl alcohol was added to the dry (magnesium sulfate) ether solution. The colorless ascorbate salt (VIII) that precipitated was washed with ether and dried in air at 65°.

Ascorbate salt IX was prepared similarly from *N*-*n*-propylnorapomorphine. The hydrate character was assigned on the basis of analytical data and melting-point behavior.

Dimers (X–XIII)—The starting materials for these preparations were the tan-colored pastes formed as the major product during the rearrangement of morphine and its *N*-alkyl derivatives to the corresponding apomorphines, from which the apomorphines had been extracted by ether (3). No distinction between the dimers formed in the hot phosphoric acid process and those formed in methanesulfonic acid at room temperature was made, except that the latter were less discolored. The yields given in Table I are based on quantities used in specific earlier preparations (3); no allowance was made for manipulative losses experienced during the following procedure.

Crude dimer pastes were obtained as free bases. The bases became green on standing in air, particularly when exposed to light. Dried samples often contained up to 10% ash on analysis. The ash is believed to be derived from occluded or adsorbed sodium sulfite because sulfur dioxide evolved when the bases were dissolved in dilute hydrochloric acid.

The pastes were boiled with acetone to remove any remaining apomorphine and then were suspended in water at 60°. Then, 6 *N* HCl was added to the stirred suspension until the solid dissolved. The brown solution was stirred at 60° until no odor of the sulfur dioxide remained and then was filtered by gravity unless clear. The solution was then stirred at 40°, and an equal volume of concentrated hydrochloric acid was added slowly to precipitate the dimer hydrochloride. The precipitate was washed by stirring with 6 *N* HCl and then was allowed to dry in air at 40° until no odor of hydrogen chloride remained; forced drying at 65° caused severe discoloration. Final drying was at 65°/1 mm to give an olive-colored powder.

Typical analyses are given in Table I. It was apparent that these polymeric materials held metal ions tenaciously; ash-free samples were never obtained. In a standard color test for apomorphine (20), the dimer hydrochloride salts gave very weak colors. Their UV spectra in ethyl alcohol all showed λ_{max} 275 nm and a shoulder at 310 nm. Their IR spectra were not well resolved but showed absorption at 1700 cm^{-1} and a double band at 1600 cm^{-1}.

[6] Pfizer.

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