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Metabolism *in vitro* of potential apomorphine prodrugs

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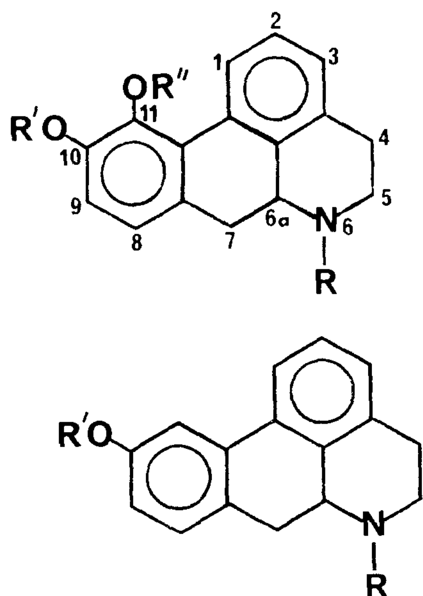
Numerous analogs of apomorphine* (I) have been tested for dopaminergic activity. Lal *et al.* [1] and Saari *et al.* [2] reported that apocodeine (II) exerted weak apomorphine-like effects in rats and mice respectively. II elicits a mild emetic response in pigeons [3] which is not observed for isoapocodeine (III) [4].

N-*n*-propylnorapomorphine (IV) is reported [5] to be thirty-five times more potent than I in provoking stereotyped behavior in rats. The monohydroxy analog of IV, 10-hydroxy-N-*n*-propylnoraporphine (V), is a considerably less potent dopaminergic agent, yet is at least three times as active as 10-hydroxyaporphine (VI) [5, 6]. By analogy, it is expected that the apomorphine-like activity of 10-methoxyaporphine (VII) would be poor.



(I) R = CH_3, R′R″ = H
(II) R, R′ = CH_3, R″ = H
(III) R, R″ = CH_3, R′ = H
(IV) R = $CH_2CH_2CH_3$,R′, R″ = H
(V) R = $CH_2CH_2CH_3$, R′ = H
(VI) R = CH_3, R′ = H
(VII) R, R′ = CH_3

Since a catechol moiety is generally considered to be required for dopaminergic activity, it has been suggested that the actions of II, III, V and VI are related to their metabolic conversions to apomorphine or N-*n*-propylnorapomorphine via appropriate *O*-dealkylation or aromatic hydroxylation reactions [1, 6–8]. These possible biotransformations have been investigated with a rat liver microsomal preparation and are subjects of this report.

Apomorphine hydrochloride hemihydrate was obtained commercially (Merck, Rahway, N.J.). Literature methods were used in the synthesis of II [9], III [4], IV [(±)-hydroiodide] [10], V [(±)-hydroiodide] [11], VI (hydrobromide) [12], and VII (hydrochloride) [12]; homogeneity of these compounds was established by thin-layer chromatography (t.l.c.) [13] prior to use. All solvents and reagents were analytical reagent grade.

Male Sprague–Dawley rats (250–300 g) (ARS Sprague–Dawley, Madison, Wis.) were decapitated, exsanguinated, and hepatectomized following induction with sodium phenobarbital [14]. Livers were blotted dry and homogenized (glass tube–teflon pestle) at 0° in 2 vol. of 0.25 M sucrose. The homogenate was centrifuged at 10,000 *g* (av) at 0° for 15 min and the supernatant fraction retained. Incubations contained 1 ml of 0.2 M Tris buffer (pH 7.4, 37°); 1 ml of 10,000 *g* supernatant (= 0.5 g liver); 1 ml Tris-buffered co-factor solution containing 2 μmoles NADP (Sigma, St. Louis, Mo.), 10 μmoles glucose 6-phosphate (Sigma) and 25 μmoles $MgCl_2$; and 2 μmoles substrate contained in 0.5 ml of 0.01 N HCl. Blanks devoid of substrate and prepared with boiled enzyme were similarly treated. Duplicate experiments were also performed in the presence of 0.05% sodium bisulfite to completely prevent air-oxidation of I and IV [13]. Incubations were performed in the presence of air for 15 min at 37° in a Dubnoff metabolic shaker. Protein precipitation was effected by adding 0.5 ml of 1 N HCl; after centrifugation at 2000 *g* for 5 min, the resulting supernatants were neutralized with 1.0 M Tris (pH 7.2, 22°) and extracted five times with 6-ml portions of nitrogenated ethyl acetate. The extracts were reduced to dryness *in vacuo* and reconstituted with 1-ml aliquots of nitrogenated chloroform–methanol (1:1) for t.l.c. using the solvent systems described previously [13]. For analyses by gas chromatography (g.c.), residues of the combined ethyl acetate extracts were spiked with 4.0 μmoles boldine (2,9-dihydroxy-1,10-dimethoxyaporphine; Nutritional Biochemicals, Cleveland, Ohio), reacted with 0.3 ml of *N,O*-bis-(trimethylsilyl) acetamide (BSA) by shaking at 5-min intervals for 30 min, and 4-μl aliquots chromatographed under conditions reported earlier [15]; t_R (solvent front) = 6.00 min (apomorphine), 6.89 min (10-hydroxyaporphine), 7.24 min (apocodeine and isoapocodeine), 7.48 min (10-methoxyaporphine) and 17.2 min (boldine). Gas chromatographic determinations of 4-μl portions of BSA solutions containing 6.7, 3.3, 1.7, 0.8, 0.4 and 0.2 μmoles/ml of apomorphine plus 13.3 μmoles/ml of boldine gave a standard curve (peak height ratio, apomorphine/boldine vs concn of apomorphine) with an $r \geq 0.999$.

Compounds II, III, V, VI and VII were incubated with rat liver microsomal fractions (from phenobarbital-induced rats) fortified with an NADPH-generating system. Metabolites of these compounds are listed in Table 1. The metabolic products indicated were characterized by identical R_f values and color reactions using diazotized sulfanilic acid and Gibb's reagent in at least three t.l.c. systems [13] and by g.c. retention times (trimethylsilyl derivatives) when compared to authentic reference compounds.

In cases where apomorphine was detected as a metabolite, difficulty was initially encountered in determining per cent conversions because of rapid air-oxidation of I. Such decomposition can be totally prevented by the use of 0.05% sodium bisulfite in the incubation medium [13]. This level of bisulfite was earlier shown to be compatible with microsomal *N*-dealkylation, *O*-dealkylation and aromatic hydroxylation reactions as tested with model substrates [16]. When apomorphine *per se* was incubated (in the presence of bisulfite) with rat liver microsomes, no metabolic prod-

* All aporphines referred to in this paper possess the R-configuration at position 6a and are levorotatory unless otherwise indicated.

Table 1. Metabolites *in vitro* of aporphine derivatives

Substrate	Metabolite identified* (Average per cent conversion)
Apocodeine (II)	Apomorphine (15)
Isoapocodeine (III)	Apomorphine (2)
10-Hydroxy-N-*n*-propylnoraporphine (V)	None†
10-Hydroxyaporphine (VI)	‡
10-Methoxyaporphine (VII)	10-Hydroxyaporphine (75)

* Results of three experiments performed in duplicate; quantitative estimations by g.c.
† Examined by t.l.c. only.
‡ See text.

ucts were detected by t.l.c. or g.c. and greater than 95 per cent of the substrate was recovered as determined by g.c.

The *O*-methylated aporphines, II and III, were both converted to apomorphine *in vitro*; however, there was an approximate 10-fold difference in the extent to which this transformation occurred. The greater *O*-dealkylation of II may be due to the less sterically hindered methoxyl group in this compound compared to III. Daly [17] noted similar findings with a series of substituted anisole derivatives. The extensive *O*-dealkylation of VII leading to 10-hydroxyaporphine (see Table 1) reinforces the notion that *O*-dealkylation in aporphines may be markedly influenced by steric effects. This phenomenon has also been described in studies of the microbial *O*-dealkylation of 10,11-dimethoxyaporphine [18].

The reported dopaminergic activity of II [1–3] may be due to its conversion to apomorphine. This is supported as well by work *in vivo* reported earlier [7]. The fact that isoapocodeine (III) is less active than apocodeine (II) as a dopaminergic agent [4] is interesting in light of the poorer conversion *in vitro* of III to apomorphine.

No evidence was found for the microsomal hydroxylation of VI and V to apomorphine and N-*n*-propylnoraporphine respectively*. Thus, the demonstrable dopaminergic action of V may support the contention that this compound can behave as a direct (albeit weak) agonist [6]. This suggestion is very interesting in terms of the current thoughts on structure–activity relationships of dopaminergic agonists [1, 6–8, 11], and the selective dopaminergic (antinociceptive) activity recently described for V [11].

With compounds II, III and VI (but not V), more polar metabolites (other than those indicated in Table 1) were detected by t.l.c. The identity of these substances and complete metabolic studies of compounds I through VI will be described in future reports.

* Hydroxylation of V and/or VI to their corresponding 9,10-dihydroxylated anologs was not specifically investigated; however, the t.l.c. systems [13] utilized are capable of distinguishing monophenols from catecholic substances, and there was no evidence that the latter materials were formed.

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Drug Dynamics Institute, College of Pharmacy, The University of Texas at Austin, Austin, Tex. 78712 — ROBERT V. SMITH

Department of Medicinal Chemistry, College of Pharmacy and Allied Health Professions, Northeastern University, Boston, Mass. 02115 — PAUL W. ERHARDT, JOHN L. NEUMEYER

Arnar-Stone Laboratories, Mount Prospect, Ill. 60056, *U.S.A.* — ROBERT J. BORGMAN

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