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REGIOSELECTIVE *O*-METHYLATION OF TETRAHYDROPAPAVEROLINE AND TETRAHYDROXYBERBINE *IN VIVO* IN RAT BRAIN

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Abstract—Enzymatic *O*-methylation is a primary pathway for the metabolism of catecholamines in mammals and of isoquinoline alkaloids in plants. This report describes the differential *O*-methylation patterns of the racemates and enantiomers of two catecholamine-derived alkaloids, tetrahydropapaveroline (THP) and 2,3,10,11-tetrahydroxyberbine (THB), in the brain of the rat. One hour after intracerebroventricular administration of a specific isomeric form of each alkaloid, the *O*-methylated metabolites were isolated from the rat brain and subsequently quantified using high performance liquid chromatography. The isomeric form of THP or THB which was administered markedly influenced the pattern of O-methylation. The racemate and *R*-(+)-enantiomer of THP were mono-*O*-methylated predominantly at the 7 and 3′ positions, while the *S*-(−)-enantiomer of THP was mono-*O*-methylated to an essentially equal degree at the 6, 7 and 3′ positions. Minimal mono-*O*-methylation at the 4′ position was detectable only with the racemate and (−)-enantiomer of THP. The racemate and enantiomers of THB were mono-*O*-methylated predominantly at the 2 and 11 positions and to a lesser extent at the 3 and 10 positions. Although minimal with the *R*-(+)-enantiomer, the 3 and the 10-*O*-methylation pathways were enhanced significantly with the *S*-(−)-enantiomer of THB. These results demonstrate that both enantiomers of THP and THB are *O*-methylated *in vivo* in rat brain and that the chiral centers of these alkaloids influence the position of *O*-methylation, thereby dictating the relative amounts of specific products formed.

Enzymatic *O*-methylation is a primary pathway for the metabolism of 1-benzyltetrahydroisoquinoline alkaloids in plants, and the specific *O*-methylated metabolites derived markedly influence the mode of phenolic oxidative coupling, a further metabolic process, leading to different classes of alkaloids [1]. Evidence that mammalian systems can affect the formation and further metabolism of tetrahydroisoquinoline alkaloids via *O*-methylation has emerged from several laboratories. Tetrahydropapaveroline (THP), a 1-benzyltetrahydroisoquinoline, has been detected in rat brain after the administration of L-dopa or L-dopa in combination with ethanol [2] to the intact animal. Furthermore, the biotransformation of THP [3] and reticuline [4] to tetrahydroprotoberberines *in vivo* in rats has been reported. Four tetrahydroprotoberberine alkaloids, including two *O*-methylation products, coreximine and a 2- or 3-mono-methylated derivative of tetrahydroxyberbine, were identified in the urine of rats after intraperitoneal administration of THP [3]. Additionally, the two positional isomeric alkaloids, 2,3,9,10- and 2,3,10,11-tetrahydroxyberbine (THB), which are formed through the intermediacy of THP, were also identified as urinary excretion products in Parkinsonian patients receiving L-dopa therapy [3]. Another indication that THP is metabolized *in vivo* in rat brain possibly via *O*-methylation derives from the work of Melchior *et al.* [5] who reported that the half-life ($T_{1/2}$) of intracerebroventricularly-injected THP in rats is approximately 17 min and is increased 3-fold in pyrogallol-treated animals.

Further evidence for the *O*-methylation and biotransformation of THP-related alkaloids has been obtained from *in vitro* experiments [6, 7]. Kametani *et al.* [6] reported that reticuline is transformed into the protoberberine alkaloids, coreximine and scoulerine, by rat liver preparations in the presence of cofactors. Collins *et al.* [7], using rat liver catechol-*O*-methyltransferase (COMT) preparations, demonstrated that THP is a substrate for COMT with apparent K_m and maximal velocity for THP one-tenth and five times, respectively, those of dopamine. Additionally, the stereoselective *O*-methylation of the racemates and enantiomers of THP and THB by partially purified rat liver COMT preparations has been described [8].

This paper elaborates on the *O*-methylation of the racemates and enantiomers of THP and THB *in vivo* in the brain of the rat and demonstrates that *O*-methylation of enantiomers of THP and THB in the intact animal is regioselective.

MATERIALS AND METHODS

Materials. Racemic (±)-, *R*-(+)- and *S*-(−)-1-(3′,4′ - dihydroxybenzyl) - 6,7 - dihydroxy - 1,2,3,4 -

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tetrahydroisoquinoline hydrobromide (tetrahydropapaveroline, THP); (±)-, *R*-(+)- and *S*-(−)-2,3,10,11-tetrahydroxyberbine hydrobromide (THB); (±)-1-(3′,4′-dihydroxybenzyl)-6-methoxy-7-hydroxy-1,2,3,4-tetrahydroisoquinoline hydrochloride (6-methoxytetrahydropapaveroline, 6-OMe-THP); (±)-1-(3′,4′-dihydroxybenzyl)-6-hydroxy-7-methoxy-1,2,3,4-tetrahydroisoquinoline hydrochloride (7-methoxytetrahydropapaveroline, 7-OMe-THP); (±)-1-(3′-methoxy-4′-hydroxybenzyl)-6,7-dihydroxy-1,2,3,4-tetrahydroisoquinoline hydrochloride (3′-methoxytetrahydropapaveroline, 3′-OMe-THP); (±)-1-(3′-hydroxy-4′-methoxybenzyl)-6,7-dihydroxy-1,2,3,4-tetrahydroisoquinoline hydrochloride (4′-methoxytetrahydropapaveroline, 4′-OMe-THP); (±)-2-methoxy-3,10,11-trihydroxyberbine hydrochloride (2-OMe-THB); (±)-3-methoxy-2,10,11-trihydroxyberbine hydrochloride (3-OMe-THB); (±)-10-methoxy-2,3,11-trihydroxyberbine hydrochloride (10-OMe-THB); and (±)-11-methoxy-2,3,10-trihydroxyberbine hydrochloride (11-OMe-THB) were synthesized in our laboratory using established synthetic routes [1, 8]. All other reagents and chemicals were the highest quality commercially available. Alkaloid solutions were prepared immediately before use in 0.001 N HCl containing 0.1 mM dithiothreitol (DTT), and all other solutions were prepared in triple distilled water. All glassware was siliconized with dilute AquaSil (Pierce, Rockford, IL) before use.

Intracerebroventricular administration of alkaloids. Male rats of the Sprague–Dawley strain weighing 200–230 g were used. Each rat received an intracerebroventricular injection of 10 μl (50 nmoles) of a 5.0 mM solution of the selected alkaloid in 0.001 N HCl–0.1 mM DTT or the vehicle alone according to a technique described by Noble *et al.* [9]. For this procedure, the animals were lightly anesthetized with CO_2. The skull of each rat was rapidly exposed by a midsagittal incision, and a small burr hole was drilled in the skull 1.5 mm lateral to the crossing of the sagittal and coronal sutures. After intracerebroventricular administration of the solution, the needle was allowed to remain in place 5 sec, then withdrawn, and the incision closed with stainless steel skin clips. The alkaloids administered to the animals were (±)-THP, *R*-(+)-THP, *S*-(−)-THP, (±)-THB, *R*-(+)-THB or *S*-(−)-THB.

Isolation and quantification of O-methylated metabolites. The animals were decapitated 1 hr after the intracerebroventricular injection, and the entire brain was rapidly removed, rinsed with saline, and weighed. Each brain was homogenized in 19 vol. of a cold (4°) solution of 0.5 M HCl, 0.1 M $HClO_4$, and 0.5% sodium metabisulfite [5] with a glass homogenizer. The homogenate was centrifuged at 20,000 *g* for 20 min at 4°. The supernatant fraction was transferred to a 50 ml graduated cylinder and placed in an ice bath. The precipitate was resuspended in 10 ml of homogenizing fluid, and the mixture was centrifuged at 20,000 *g* for 5 min at 4°. This supernatant fraction was combined with the first supernatant and diluted to a final volume of 50 ml with distilled water. A differential liquid chromatographic method was used for isolation and concentration of the alkaloids from the supernatant fractions (Cashaw *et al.*, to be published). The eluate (total volume, 3.0 ml) obtained was assayed by high performance liquid chromatography (HPLC) with an electrochemical detector. To detect trace amounts of metabolites, 1.0 ml aliquots of the eluates were brought to dryness under vacuo and the residue obtained was dissolved in 100 μl of 0.01 N HCl–0.1 mM DTT. For recovery determinations, either a standard mixture of THP, 6-OMe-THP, 7-OMe-THP, 3′-OMe-THP and 4′-OMe-THP or a standard mixture of 2,3,10,11-THB, 2-OMe-THB, 3-OMe-THB, 10-OMe-THB and 11-OMe-THB was added to each control rat brain homogenate. The homogenates containing the standard compounds were carried through the same procedure described for test animals.

High performance liquid chromatography. The analyses were performed with a modular chromatograph consisting of an LC-3 electrometer/electrochemical detector (Bioanalytical Systems, West Lafayette, IN), an OmniScribe recorder (Houston Instruments, Austin, TX), a model 110 A metering pump, and a model A type injection valve (Altex, Berkeley, CA). Additionally, a model 308 Computing Integrator (Laboratory Data Control, Riviera Beach, FL) which is capable of quantifying all recognized peaks (fused peaks), precluding the need for complete resolution of peaks in a chromatogram, was employed for retention time measurements and quantification. The HPLC conditions were: column, Supelcosil/C18 (5 μm particle size), 25 cm × 4.6 mm i.d.; mobile phase, 0.1 M $NH_4H_2PO_4$ containing 6% dioxane; temperature, ambient; flow rate, 1.0 ml/min; sample size injected, 40 μl of reference mixture of authentic alkaloids or 40 μl of rat brain extract (eluate or concentrate), corresponding to ≈12 mg rat brain for the eluate and ≈120 mg for the concentrate. All values reported are corrected for recovery using the specified standard compound.

RESULTS

O-methylation of (±)-THP, R-(+)-THP and S-(−)-THP. The structures of THP and the four positional isomers of mono-*O*-methyl-THP are shown in Fig. 1. A representative HPLC profile illustrating the separation of a reference mixture of THP and the positional isomers of mono-*O*-methyl-THP is illustrated in Fig. 2A. The intracerebroventricular (i.c.v.) administration of (±)-THP, *R*-(+)-THP or *S*-(−)-THP resulted in the formation of readily detectable amounts of three of the four possible mono-*O*-methylated products of the parent alkaloid. Representative chromatographic profiles of rat brain extracts (equivalent to 12 mg wet weight of tissue), obtained 1 hr after i.c.v. administration of *R*-(+)-THP and *S*-(−)-THP, are shown in panels B and C, respectively, of Fig. 2. Each of these chromatograms contains four peaks which have retention times identical to those of the corresponding numbered peaks in Fig. 2A representing (1) THP, (2) 3′-OMe-THP, (3) 7-OMe-THP and (4) 6-OMe-THP. The differences in peak areas in Fig. 2B (peaks 2–4) as compared to Fig. 2C (peaks 2–4) clearly indicate that the *O*-methylating enzymes in

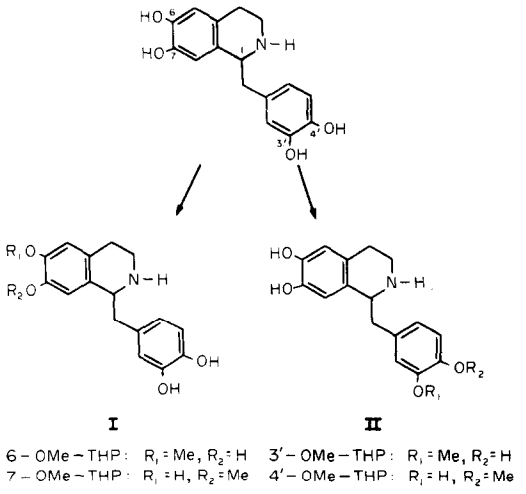


Fig. 1. Structural representation of THP and its mono-*O*-methylated positional isomers.

rat brain can distinguish between the two enantiomers of THP. Figure 2B shows that 3'-OMe-THP (peak 2) and 7-OMe-THP (peak 3) were the major metabolites of *R*-(+)-THP and that 6-OMe-THP (peak 4) was a minor product. However, with *S*-(−)-THP as the precursor (Fig. 2C), 6-OMe-THP became a major metabolite, and the relative quantities of 3'-OMe-THP and 7-OMe-THP showed a significant decrease. Chromatograms of brain extracts obtained from control animals produced no significant responses above the baseline. The absence of peaks equivalent to those of the reference standards (Fig. 2A) in the HPLC profile of control brain extracts provides evidence that the *O*-methylated products depicted in panels B and C of Fig. 2 must be formed *in vivo* from the injected alkaloids. Although 4'-OMe-THP was not detected in the initial rat brain extract (Fig. 2B and C), further concentration (10-fold) of the extracts revealed trace amounts of 4'-OMe-THP in brain extract from rats that received (±)-THP or *S*-(−)-THP. However, 4'-OMe-THP was not detected in brain extract concentrates from rats that received *R*-(+)-THP (chromatograms not shown). The recovery of reference mixtures of THP and its mono-*O*-methyl derivatives from rat brain homogenates obtained in eight separate determinations was: THP, 86.9 ± 4.6%; 6-OMe-THP, 86.1 ± 7.6%; 7-OMe-THP, 80.7 ± 4.0%; 3'-OMe-THP, 82.8 ± 6.2%; and 4'-OMe-THP, 85 ± 10% (mean ± S.D.).

The quantitative significance of the data shown graphically in Fig. 2 is illustrated in Table 1, which further highlights the ability of the *O*-methylating enzymes in rat brain to differentiate between the enantiomers of THP. These data show that the relative amount of each mono-*O*-methylated positional isomer formed *in vivo* in rat brain is markedly influenced by the specific enantiomer of THP administered. With *R*-(+)-THP as precursor, 51.6% of the *O*-methylated products was 7-OMe-THP, 46.1% was 3'-OMe-THP, and 6-OMe-THP represented only 2.3% of the *O*-methylated metabolites. In contrast, with *S*-(−)-THP as precursor, 7-OMe-THP represented only 31.5% of the *O*-methylated products and 3'-OMe-THP showed a modest but significant decrease to 39.3% whereas 6-OMe-THP exhibited a marked increase to 29.2%. With the racemic mixture of THP, as might be expected, the *O*-methylated products showed a distribution pattern intermediate between that of the resolved isomers. Differences between the values obtained for each of the mono-*O*-methylated products of *R*-(+)-THP and *S*-(−)-THP were significant (P values less than 0.001).

Although the isomeric form of THP administered had a significant influence on the distribution of

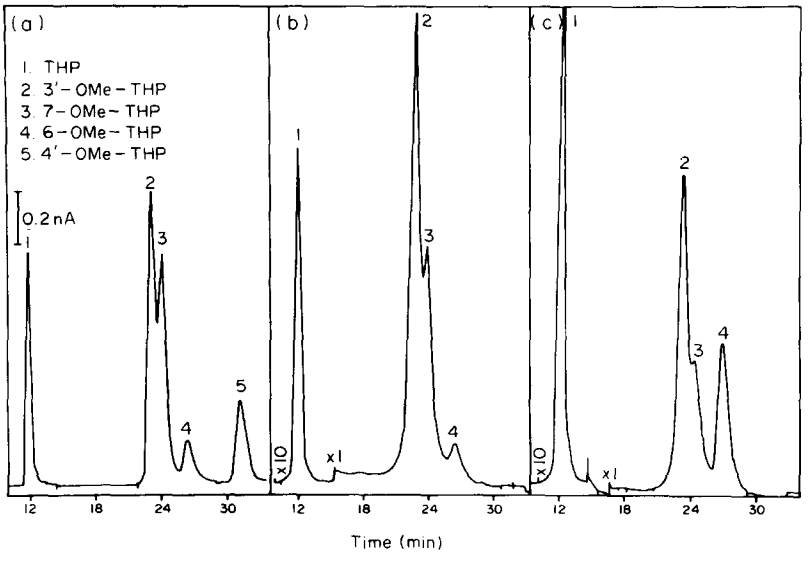


Fig. 2. HPLC profiles of: (A) a reference mixture of THP (4.0 pmoles), 3'-OMe-THP (27.0 pmoles), 7-OMe-THP (27.0 pmoles), 6-OMe-THP (4.5 pmoles) and 4'-OMe-THP (5.0 pmoles); (B) and (C) extracts equivalent to 12 mg of rat brain obtained 1 hr after intracerebroventricular injection of 50 nmoles of *R*-(+)-THP and *S*-(−)-THP respectively. Conditions for the isolation and HPLC analysis of THP and its *O*-methylated metabolites are described in Materials and Methods.

Table 1. Percent distribution of mono-methylated metabolites of THP in rat brain*

Alkaloid administered	7-OMe-THP (%)	6-OMe-THP (%)	3'-OMe-THP (%)
(±)-THP	43.4 ± 2.1	12.4 ± 1.6	43.5 ± 2.5
R-(+)-THP	51.6 ± 1.7	2.3 ± 0.9	46.1 ± 2.5
S-(−)-THP	31.5 ± 3.0†	29.2 ± 2.8†	39.3 ± 2.2†

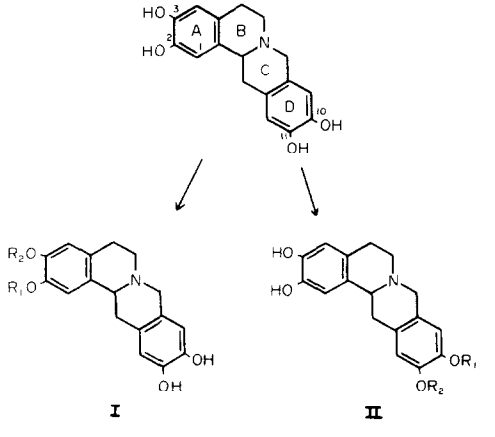
* Rats were injected intracerebroventricularly with 50 nmoles of (±)-THP, *R*-(+)-THP, or *S*-(−)-THP. One hour later, the animals were killed, and the amount of each *O*-methylated metabolite was determined by HPLC. Values are reported as mean ± S.D. for six animals.

† $P < 0.001$, compared with the respective metabolite obtained with the *R*-(+)-THP isomer.

metabolites, it had little influence on the amount of THP and metabolic products of THP recovered from rat brain after 1 hr. The amounts of THP recovered 1 hr after the i.c.v. injection of 50 nmoles of (±)-THP, *R*-(+)-THP and *S*-(−)-THP were (mean ± S.D.) 5.1 ± 1.5, 3.1 ± 1.6 and 4.3 ± 3.0 nmoles respectively. Likewise, the total amount of mono-*O*-methylated metabolites recovered 1 hr after the i.c.v. administration of (±)-THP, *R*-(+)-THP or *S*-(−)-THP was 2.8 ± 0.5, 2.2 ± 1.1 and 3.4 ± 0.67 nmoles respectively.

O-Methylation of (±)-*THB,* R-(+)-*THB and* S-(−)-*THB.* The structures of 2,3,10,11-THB and the four positional isomers of mono-*O*-methyl-THB are shown in Fig. 3. A chromatographic profile illustrating the resolution of a reference mixture of these alkaloids, i.e. (1) 2,3,10,11-THB, (2) 2-OMe-THB, (3) 11-OMe-THB, (4) 3-OMe-THB and (5) 10-OMe-THB, appears in Fig. 4A.

The intracerebroventricular administration of (±)-THB, *R*-(+)-THB or *S*-(−)-THB resulted in the formation of the four positional isomers of mono-*O*-methyl-THB. High performance liquid chromatograms of rat brain extracts (equivalent to 12 mg wet weight of tissue) after administration of *R*-(+)-THB and *S*-(−)-THB are shown in Fig. 4, panels B and C respectively. Each of these profiles shows five components with retention times identical to those of the corresponding compounds in Fig. 4A, i.e. (1) 2,3,10,11-THB, (2) 2-OMe-THB, (3) 11-OMe-THB, (4) 3-OMe-THB and (5) 10-OMe-THB.



2 − OMe − THB: R_1 = Me, R_2 = H 10 − OMe − THB: R_1 = Me, R_2 = H
3 − OMe − THB: R_1 = H, R_2 = Me 11 − OMe − THB: R_1 = H, R_2 = Me

Fig. 3. Structural representation of 2,3,10,11-THB and its mono-*O*-methylated positional isomers.

The differences in relative peak areas (peaks 2–5) in Fig. 4B as compared to Fig. 4C (peaks 2–5) demonstrate the ability of enzymatic systems in the intact rat brain to differentiate between the enantiomers of THB. The chromatographic profile in Fig. 4B shows that the major metabolites of *R*-(+)-THB were 2-OMe-THB (peak 2) and 11-OMe-THB (peak 3) and that 3-OMe-THB (peak 4) and 10-OMe-THB (peak 5) were minor products. By comparison, with *S*-(−)-THB as the precursor (Fig. 4C), a significant increase was seen in the amounts of 3-OMe-THB (peak 4) and 10-OMe-THB (peak 5) although 2-OMe-THB and 11-OMe-THB remained the major products. As in the case of THP, the absence of peaks equivalent to those of the reference standards (Fig. 4A) in the HPLC profile of control rat brain extracts provides evidence that the *O*-methylated products represented in panels B and C of Fig. 4 must be formed *in vivo* from the injected alkaloids.

A reference standard for each metabolite is particularly crucial for the quantitative determination of *O*-methylated metabolites of THB with the electrochemical detector because the molar response of each metabolite to the electrochemical detector differs. The molar response of 10-OMe-THB to the electrochemical detector was considerably greater than that of the other *O*-methylated products. In Fig. 4C, 10-OMe-THB is represented by the largest peak (peak 5) in the profile but the concentration of 10-OMe-THB was less than that of 2-OMe-THB or 11-OMe-THB in the extract (Table 2). The recovery of these alkaloids from rat brain homogenates was: THB, 65.9 ± 6.2%; 2-OMe-THB, 71.9 ± 9.5%; 3-OMe-THB, 75.5 ± 3.8%; 10-OMe-THB, 73.1 ± 3.3%; and 11-OMe-THB, 79.9 ± 8.2% (mean ± S.D. for eight determinations).

Table 2 further shows the distribution of the various THB *O*-methylated products formed *in vivo* from the *R*-(+)-THB and *S*-(−)-THB isomers, calculated as a percentage of total *O*-methylated products measured. These data show the marked influence of the specific enantiomer of THB on the pattern of *O*-methylation. Approximately 99% of the metabolites of *R*-(+)-THB were represented by 2-OMe-THB (73.4 ± 5.1%) and 11-OMe-THB (25.9 ± 5.0%) with 3-OMe-THB and 10-OMe-THB

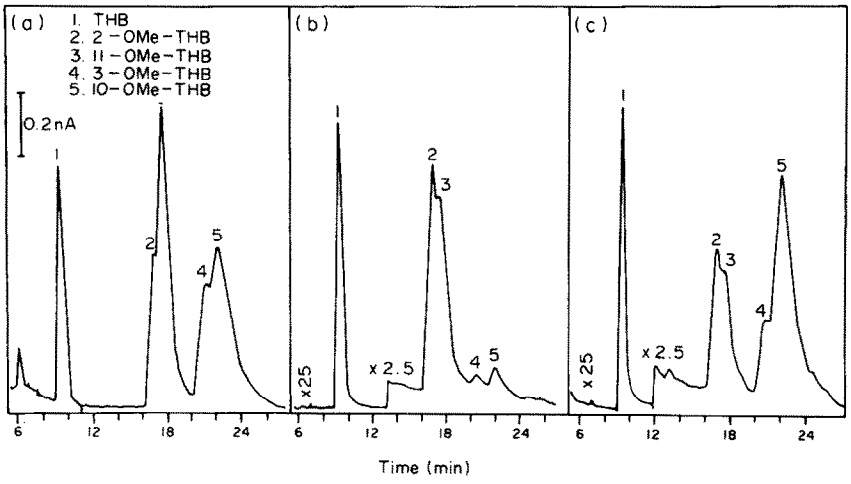


Fig. 4. HPLC profiles of: (A) a reference mixture of 2,3,10,11-THB (4 pmoles), 2-OMe-THB (80 pmoles), 11-OMe-THB (80 pmoles), 3-OMe-THB (10 pmoles) and 10-OMe-THB (10 pmoles); (B) and (C) extracts equivalent to 12 mg of rat brain obtained 1 hr after intracerebroventricular injection of 50 nmoles of *R*-(+)-THB AND *S*-(−)-THB respectively. Conditions for the isolation and HPLC analysis of THB and its *O*-methylated metabolites are described in Materials and Methods.

combined representing less than 1% of the *O*-methylated products. Replacing *R*-(+)-THB as precursor with *S*-(−)-THB yielded a 19-fold increase in 3-OMe-THB and a 35-fold increase in 10-OMe-THB with 6.6% of the *O*-methylated products as 3-OMe-THB and 13.4% as 10-OMe-THB. The 2-OMe-THB and 11-OMe-THB metabolites of *S*-(−)-THB represented 59.4 and 21.4% respectively. Differences between the values obtained for three of the mono-*O*-methylated products, i.e. 2-OMe-THB, 3-OMe-THB and 10-OMe-THB, of *R*-(+)- and *S*-(−)-THB were highly significant (P values less than 0.001). The chiral form of the administered alkaloid had little effect on the relative amount of the 11-OMe-THB metabolite produced (Table 2). As with the racemic form of THP, the values obtained for the *O*-methylated products of racemic THB were intermediate between those observed for the *R*-(+)- and *S*-(−)-enantiomers of THB.

The total amount of the *O*-methylated products of each isomeric form of THB exceeded that of the parent alkaloid 1 hr after its i.c.v. administration. The amounts of the parent alkaloids present in rat brain 1 hr after administration of 50 nmoles were: (±)-THB, 6.3 ± 1.2; *R*-(+)-THB, 8.7 ± 1.6; and *S*-(−)-THB, 3.4 ± 3.1 nmoles (mean ± S.D.). The total *O*-methylated products of (±)-THB, *R*-(+)-THB and *S*-(−)-THB, expressed as mean ± S.D., were 13.5 ± 5.1, 22.72 ± 2.2 and 6.24 ± 3.9 nmoles respectively.

DISCUSSION

The preferential enzymatic *O*-methylation of catecholamines at the position *meta* to the ethylamine side chain is well documented [10–13]. However, the formation of tetrahydroisoquinolines by the condensation of catecholamines with aldehydes imparts structural modifications which may affect the relative propensity for *O*-methylation at positions *meta* and *para* to the ethylamine side chain (i.e. 6 and 7 positions of THIQs respectively). In addition to introduction of the chiral center at C-1 in the case of C-1 substituted derivatives, other notable changes include: reduction in the conformational mobility of the amine group; conversion of a primary amine to a secondary amine, thereby altering the basicity of the amine group; and attachment of a nitrogen to the catechol moiety through a one carbon unit (at the 1 position of the tetrahydroisoquinoline moiety) which may partially alter the directional effect of the amine group. Indeed, Creveling *et al.* [12] have reported that 6,7-dihydroxytetrahydroisoquinoline, which is formed by the condensation of dopamine

Table 2. Percent distribution of mono-methylated metabolites of THB in rat brain*

Alkaloid administered	N	2-OMe-THB (%)	3-OMe-THB (%)	10-OMe-THB (%)	11-OMe-THB (%)
(±)-THB	5	69.2 ± 2.4	2.06 ± 0.19	6.33 ± 1.04	23.9 ± 2.2
R-(+)-THB	6	73.4 ± 5.1	0.34 ± 0.08	0.38 ± 0.10	25.9 ± 5.0
S-(−)-THB	6	59.4 ± 5.1†	6.61 ± 1.9†	13.4 ± 1.3†	21.4 ± 3.2

* Rats were injected intracerebroventricularly with 50 nmoles of (±)-THB, *R*-(+)-THB or *S*-(−)-THB. One hour later, the animals were killed, and the amount of each *O*-methylated metabolite was determined by HPLC. Values reported are means ± S.D.

† $P < 0.001$, compared with the respective metabolite obtained with the *R*-(+)-THB isomer.

with formaldehyde, is a substrate for COMT and is preferentially *O*-methylated at the 7 position by a COMT preparation from rat liver. Additionally, Smissman *et al.* [14] found that 6,7-dihydroxytetrahydroisoquinoline and epinephrine have similar kinetic parameters as substrates for rat liver COMT and that 'substitution of methyl groups in the 2 and/or 4 positions' of the alkaloid has minimal effect on the enzyme affinity for or the rate of *O*-methylation of these alkaloids. However, since the products of the *O*-methylation reactions were not identified, the effect of methyl substituents in the 2 and/or 4 positions of 6,7-dihydroxy-1,2,3,4-tetrahydroisoquinoline on the ratio of positional isomers formed could not be evaluated. Two groups of investigators [15, 16] have independently reported that salsolinol is metabolized *in vivo* in the brain of rat primarily to 7-*O*-methylsalsolinol, further demonstrating that the *O*-methylation pattern of the tetrahydroisoquinolines may differ from that of catecholamines. Since it is presumed that the racemate of salsolinol was used in both of these investigations, no information on possible stereoselectivity of the *O*-methylating enzymes for the resolved isomers can be deduced from these reports.

Our results demonstrate that the enzymatic system(s) in the intact brain of the rat can metabolize THP and THB alkaloids by *O*-methylation and that the chiral recognition mechanism responsible for stereoselective *O*-methylation *in vivo* in rat brain can differentiate between the enantiomers of THP and THB as reflected by regioselective *O*-methylation. This work also indicates that the change of the C–N bond from the open side chain of catecholamines to the tetrahydroisoquinoline ring, the introduction of a chiral center at C-1, and the presence of four phenolic groups in 1-benzyltetrahydroisoquinolines such as THP increase the diversity of the *O*-methylation reaction products.

In brain of the intact rat, the racemate of THP was mono-*O*-methylated primarily at the 6 or 7 position of the isoquinoline ring and the 3′ position of the benzylic catechol moiety. The racemate of THB was mono-*O*-methylated in rings A and D at either positions 2, 3, 10 or 11 of the protoberberine molecule yielding the four possible positional isomeric mono-*O*-methylated products. The possible formation of di-*O*-methylated products of THP or THB was not resolved in the present investigation. The innate low sensitivity of these compounds to the electrochemical detector and the specific HPLC conditions employed, which resulted in long retention times, precluded the evaluation of their formation.

It is noteworthy that the isoquinoline moiety of *R*-(+)-THP was *O*-methylated predominantly at the 7 position while *O*-methylation of *S*-(−)-THP resulted in the formation of approximately equal amounts of 6-OMe-THP and 7-OMe-THP (Table 1). The 3′-*O*-methyl ether of THP was a major metabolite of both *R*-(+)- THP and *S*-(−)-THP in rat brain (Table 1). The THP molecule maintains a freely rotating benzylic carbon–carbon bond, and *O*-methylation of this portion of the molecule may not be influenced to the same extent as the isoquinoline moiety by the chiral centers of the enantiomers of THP. The observed decrease in *O*-methylation of *S*-(−)-THP at the 3′-position as compared to *R*-(+)-THP, although significant ($P < 0.001$), may be a reflection of the marked increase in *O*-methylation of the (−)-enantiomer at the 6 position.

THB is formed by the insertion of a methylene unit (berberine bridge) into the THP molecule [1] and positions 6, 7, 3′ and 4′ of THP correspond to positions 3, 2, 11 and 10 of THB respectively (Figs. 1 and 3). Thus, some parallels in the *O*-methylation profiles of THP and THB are anticipated. Indeed, mono-*O*-methylation of *R*-(+)-THB in ring A (Fig. 3 and Table 2) occurred predominantly at the 2 position, and an increase in *O*-methylation in ring A at the 3 position was observed on substitution of *S*-(−)-THB for *R*-(+)-THB as the precursor. The *O*-methylation pattern of the enantiomers of THB in ring D is particularly interesting because this portion of the THB molecule is conformationally less mobile than the corresponding benzyl ring of the THP molecule. Although *O*-methylation of *R*-(+)-THB in ring D occurred predominantly at the 11 position (equivalent to the 3′ position of THP), substitution of *S*-(−)-THB as precursor resulted in a 35-fold increase in *O*-methylation at the 10 position (10-OMe-THB) (Table 2). In comparison to THP, the four mono-*O*-methylated metabolites of THB were readily detected in rat brain extract after the administration of any isomeric form of the conformationally less mobile THB alkaloid.

Furthermore, the amount of the *O*-methylated metabolites of THB present 1 hr after the administration of any of the various isomeric forms of THB was greater than that of the precursor while the amount of the metabolites of the different stereoisomers of THP present was less than that of the administered compound. Another interesting finding is that the levels of the administered THB alkaloids remaining in the rat brain after 1 hr were higher than those of the administered THP alkaloids. Additional studies will be required to determine whether the higher levels of THB and its metabolites observed indicate that THB and the monomethyl ethers of THB have a relatively slow rate of metabolism in the brain and/or a relatively slow rate of egress from the brain.

Although the mechanism(s) responsible for the regioselective *O*-methylation of the enantiomers of THP and THB is not presently apparent, the results obtained in this study may represent a series of sequential events in the intact rat brain. It is plausible that *O*-methylation of these alkaloids is preceded by selective uptake and storage of the enantiomers in specific regions of the brain which differ in enzyme activity. Kaplan *et al.* have reported variations in the pattern of COMT localization in regions of specialized circumventricular organs of the ventricular system in rat brain [17] and that the specific activities of COMT evaluated in two brain areas, i.e. piaarachnoid and the choroid plexus, are 2-fold and 9-fold, respectively, greater than that in the whole brain of rats [18]. Hence, the selective localization of the antipodes of these alkaloids into specific brain regions, which may differ in the localization of COMT as well as the level of COMT specific activity, may determine to some degree the products which result from the *O*-methylation of these alkaloids.

Additionally, the localization of demethylases in specific brain regions may also influence the product distribution in these *O*-methylation reactions. The demethylation of metanephrine [19] and the interconversion of *meta*- and *para*-catechol monomethyl ethers [20] have been demonstrated *in vivo* in rats.

It is noteworthy that the patterns of *O*-methylation of the enantiomers of THP at the 6 and 7 positions *in vivo* were similar to the pattern of *O*-methylation observed for these enantiomers *in vitro* with rat liver COMT preparations [8]. Likewise, the *in vivo O*-methylation patterns of the enantiomers of THB at the 2 and 3 positions were similar to those observed with rat liver COMT *in vitro* [8]. Therefore, a significant component in the regioselective *O*-methylation of enantiomers of THP and THB *in vivo* may involve presently unknown stereochemical factors.

Some parallels between the stereoselective biotransformation and the stereodependent biological activity of THP-related alkaloids are beginning to emerge. Rueffer *et al.* [21] recently reported the isolation of an enzyme, (*S*)-norlaudanosoline synthase, from three different plant families containing benzylisoquinoline alkaloids, which catalyzes the condensation of dopamine and dopaldehyde (3,4-dihydroxyphenylacetaldehyde) to form (*S*)-norlaudanosoline (*S*-(−)-THP). Additionally, *S*-(−)-THP has been reported to be more active biologically than *R*-(+)-THP [22, 23], especially in its action on adrenergic and dopaminergic receptors [24]. Similarly, the *S*-(−)-isomer of THB has been reported to be a more potent antagonist of dopamine-sensitive adenylate cyclase activity than the *R*-(+)-isomer [25]. Further, a comparison of the results presented in this report with concurrent studies in our laboratory on the interaction of tetrahydroisoquinolines and related alkaloids with receptors from rat cerebral cortex indicates that *O*-methylation of *S*-(−)-THP or *S*-(−)-THB, as compared to their *R*-(+)-enantiomers, results in a preponderance of mono-*O*-methylated products which have greater adrenergic and dopaminergic activity (Nimit *et al.*, unpublished results). For example, 6-OMe-THP, which is preferentially derived from *S*-(−)-THP, is a potent inhibitor of β-adrenergic and dopaminergic receptor binding. In contrast, 7-OMe-THP, which is preferentially derived from *R*-(+)-THP (Table 1), has only modest adrenergic activity and is devoid of dopamine agonist or antagonist activity. Of the four monomethyl ethers of THB, the 10-OMe-THB isomer, whose formation is accentuated in the *O*-methylation of *S*-(−)-THB, exhibited the most pronounced affinity for adrenergic and dopaminergic receptors whereas 2-OMe-THB, which is preferentially formed from *R*-(+)-THB, was the least active at these receptor sites.

In summary, this study demonstrates that both enantiomers of THP and THB are *O*-methylated *in vivo* in the rat brain. It further demonstrates that the chiral centers influence the position of *O*-methylation of these alkaloids, illustrating the regioselectivity of these *O*-methylation reactions. Additionally, as with catecholamines, *O*-methylation seems to be a primary mode of metabolism of THP and THB *in vivo* in rat brain. However, unlike catecholamines, which are deactivated by *O*-methylation, *O*-methylation of THP and THB appears to result in products which have distinct biological activities.

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REFERENCES

1. M. Shamma, in *The Isoquinoline Alkaloids: Chemistry and Pharmacology* (Eds. A. T. Blomquist and H. Wasserman), Vol. 25. Academic Press, New York (1972).
2. A. J. Turner, K. M. Baker, S. Algeri, A. Frigerio and S. Garattini, *Life Sci.* **14**, 2247 (1974).
3. J. L. Cashaw, K. D. McMurtrey, H. Brown and V. E. Davis, *J. Chromat.* **99**, 567 (1974).
4. T. Kametani, M. Ihara and K. Takahashi, *Chem. pharm. Bull., Tokyo* **20**, 1587 (1972).
5. C. L. Melchior, A. Mueller and R. A. Deitrich, *Biochem. Pharmac.* **29**, 657 (1980).
6. T. Kametani, M. Takemura, M. Ihara, K. Takahashi and K. Fukumoto, *J. Am. chem. Soc.* **98**, 1956 (1976).
7. A. C. Collins, J. L. Cashaw and V. E. Davis, *Biochem. Pharmac.* **22**, 2337 (1973).
8. L. R. Meyerson, J. L. Cashaw, K. D. McMurtrey and V. E. Davis, *Biochem. Pharmac.* **28**, 1745 (1979).
9. E. P. Noble, R. J. Wurtman and J. Axelrod, *Life Sci.* **6**, 281 (1967).
10. J. Axelrod and R. Tomchick, *J. biol. Chem.* **233**, 702 (1958).
11. J. Axelrod, *Pharmac. Rev.* **18**, 95 (1966).
12. C. R. Creveling, N. Morris, H. Shimizu, H. H. Ong and J. Daly, *Molec. Pharmac.* **8**, 398 (1972).
13. C. R. Creveling, E. T. McNeal, D. Cantacuzene and K. L. Kirk, *J. med. Chem.* **24**, 1395 (1981).
14. E. E. Smissman, J. R. Reid, D. A. Walsh and R. T. Borchardt, *J. med. Chem.* **19**, 127 (1976).
15. M. Bail, S. Miller and G. Cohen, *Life Sci.* **26**, 2051 (1980).
16. T. C. Origitano and M. A. Collins, *Life Sci.* **26**, 2061 (1980).
17. G. P. Kaplan, B. K. Hartman and C. R. Creveling, *Brain Res.* **229**, 323 (1981).
18. G. P. Kaplan, B. K. Hartman and C. R. Creveling, *Brain Res.* **204**, 353 (1980).
19. I. J. Kopin, J. Axelrod and E. Gordon, *J. biol. Chem.* **236** (7), 2109 (1961).
20. J. W. Daly, J. Axelrod and B. Witkop, *J. biol. Chem.* **235** (4), 1155 (1960).
21. M. Rueffer, H. El-Shagi, N. Nagakura and M. H. Zenk, *Fedn Eur. Biochem. Soc. Lett.* **129** (1), 5 (1981).
22. G. Cohen, R. E. Heikkila, D. Dembiec, D. Sang, S. Teitel and A. Brossi, *Eur. J. Pharmac.* **29**, 292 (1974)
23. G. Cohen, in *Membrane Mechanisms of Drugs of Abuse* (Eds. C. Sharp and L. G. Abood), p. 73. Alan R. Liss, New York (1979).
24. A. Brossi, K. C. Rice, C. P. Mak, J. Reden, A. E. Jacobson, Y. Nimitkitpaisan, P. Skolnick and J. Daly, *J. med. Chem.* **23**, 648 (1980).
25. Y. C. Clement-Cormier, L. R. Meyerson, H. Phillips and V. E. Davis, *Biochem. Pharmac.* **28**, 3123 (1979).