46. Mammalian Alkaloids: *O*-Methylation of (±)-Norcoclaurine-1-carboxylic Acid and Related Isoquinolines Including (*S*)- and (*R*)-Norcoclaurine with ^{14}C-Labeled *S*-Adenosyl-L-Methionine in Presence of Mammalian Catechol *O*-Methyltransferase

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(±)-Norcoclaurine-1-carboxylic acid (**5**) and the derived dihydroisoquinolinone **6** (present as quinonemethide **6a** at pH 7) afforded, on methylation with ^{14}C-labeled *S*-adenosyl-L-methionine in the presence of mammalian catechol *O*-methyltransferase, exclusively the 7-*O*-methylated congeners **7** and **9**, respectively. High stereoselectivity of the *O*-methylation was observed with (−)-(*S*)- and (+)-(*R*)-norcoclaurine (**2a** and **2b**, resp.), affording 80% of 6-*O*-methylated isoquinoline **12** and 20% of the 7-*O*-methylated isomer **11** from **2a**, and the reversed proportion of **12** and **11** from **2b**. Synthesis of the reference amino acid **8** was achieved by *Pictet-Spengler* condensation of *O*-benzyl-protected dopamine **17** with benzyl-protected keto acid **20** (→**21**) followed by methylation with diazomethane (→**22** + **23**) and removal of the protecting groups by acid hydrolysis. It is considered unlikely that amino acids such as **5** constitute important precursors in the biosynthesis of isoquinolines related to reticuline.

Introduction. – Reports that L-dopa is converted in callus cultures of poppy plants into norlaudanosoline-1-carboxylic acid (**1**) as the first isoquinoline formed in the biosynthesis of reticuline and derived morphine alkaloids [1–4] were recently disputed on the basis of *in vivo* [5] and *in vitro* experiments [6] [7]. Data collected by *Zenk* and his group clearly show that (*S*)-norcoclaurine (**2a**), derived from an enzymatic and stereospecific condensation of dopamine with (4-hydroxyphenyl)acetaldehyde, is the first isoquinoline in the biosynthesis of reticuline [8] [9]. The finding of tiny amounts of morphine in rat brain [10] [11] and reports that mammalian liver can carry out the critical conversion of reticuline into salutaridine [12][2]) [13] renewed interest in the mammalian origin of reticuline which by itself has not yet been detected in mammals [13].

We recently showed that (*S*)- and (*R*)-dideoxynorlaudanosoline-1-carboxylic acids ((*S*)- and (*R*)-**3**) and quinonemethide **4a** (derived from **3** by oxidative decarboxylation; *Scheme 1*) were *O*-methylated *in vitro* by ^{14}C-labeled *S*-adenosyl-L-methionine in the presence of mammalian catechol *O*-methyltransferase (COMT) almost exclusively at the 7-OH group and not at the 6-OH group as required for further conversion into isoquinolines related to reticuline [14]. This result obtained with both optical isomers of **3** supports the conclusion reached by the *Zenk* group that tetrahydroisoquinoline-1-carboxylic acids are unlikely intermediates in the biosynthesis of 1-benzylisoquinoline alkaloids related to reticuline [15].

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[1]) On leave from Tanabe, Seiyaku Co., Saitama, Japan.

[2]) This is the first report on the conversion of radiolabeled reticuline by rat-liver homogenates into the morphinandienone alkaloid sinacutine.

Scheme 1

1 **2a** (*S*) **2b** (*R*)

3 $R^1 = OH, R^2 = H$
5 $R^1 = R^2 = OH$
7 $R^1 = CH_3O, R^2 = OH$

4a $R^1 = OH, R^2 = H$
6a $R^1 = R^2 = OH$
9a $R^1 = CH_3O, R^2 = OH$

4 $R^1 = OH, R^2 = H$
6 $R^1 = R^2 = OH$
9 $R^1 = CH_3O, R^2 = OH$

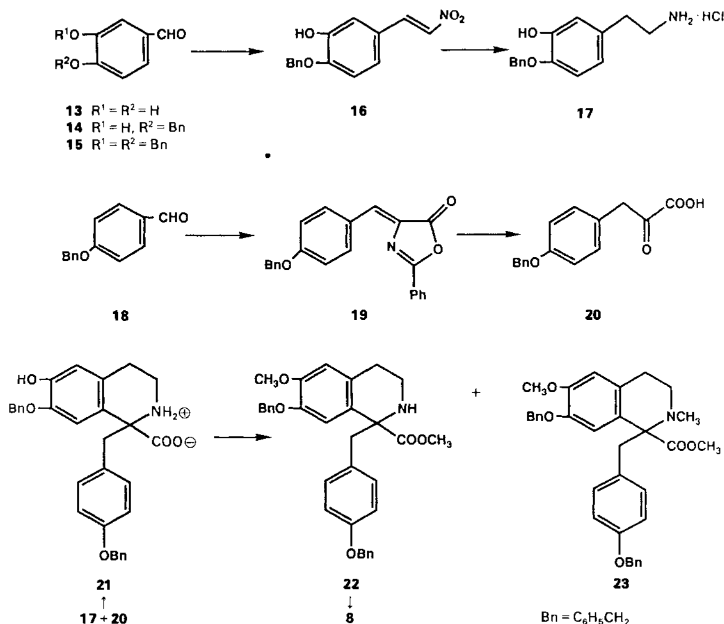
8 **10**

11 $R^1 = H, R^2 = CH_3$
12 $R^1 = CH_3, R^2 = H$

To further support this conclusion, we now have subjected (±)-norcoclaurine-1-carboxylic acid (**5**) and dihydroisoquinolinium salt **6**, obtained from **5** by oxidative decarboxylation and present above pH 7 as quinonemethide **6a**, to similar *O*-methylation. Furthermore, we have included the racemic norcoclaurine (**2**) and its antipodes **2a** and **2b** in the investigation. The products obtained in their methylation with COMT were separated by TLC and identified by comparison of the ^{14}C-labeled products with *O*-methylated standards. These reference compounds included the *O*-methylated carboxylic acids **7** and **8**, the dihydroisoquinolinium salts **9** and **10**, and the tetrahydroisoquinolines **11** and **12**.

Chemistry. – Hydrochlorides of amino acids **5** [2] and **7** [16] and hydrobromides **6**, **9**, and **10** [17] were prepared by published procedures, and quinonemethides **6a** and **9a**, present in solution at pH 7, were isolated and characterized by spectral data. Also isolated and characterized was the free base of **10** which did not give a quinonemethide at pH 7. The optically active norcoclaurines **2a** and **2b** [18] and racemic methyl ethers **11** and **12** [17] were prepared by published methods.

Scheme 2



Amino acid **8** was prepared as shown in *Scheme 2* by a *Pictet-Spengler* condensation of 4-*O*-benzyldopamine (**17**) with [4-(benzyloxy)phenyl]pyruvic acid (**20**). Attempts to prepare **17** requires comment: selective acid hydrolysis of bis(benzyloxy) derivative **15** afforded a mixture of products which were difficult to separate. Selective benzylation of 3,4-dihydroxybenzaldehyde (**13**) with benzyl chloride proved to be a superior method to prepare the desired **14**. The latter afforded nitrostyrene **16** on condensation with nitromethane, and reduction of **16** with $LiAlH_4$ in THF yielded amine **17** which was fully characterized as its hydrochloride. Acid **20** was obtained from 4-(benzyloxy)benzaldehyde (**18**) by condensation with hippuric acid (→azalactone **19**) followed by alkaline hydrolysis. Conversion of amino acid **21**, obtained from **17** and **20** at pH 5.5, was accomplished by methylation with diazomethane (→**22** + **23**). Chromatographic separation of the more polar **22** and its acid hydrolysis with 20% HCl solution yielded the hydrochloride of **8** which was fully characterized.

Biochemical Results. – As described in [14] for **3**, (±)-norcoclaurine (**2**) and its optical salt **2a** and **2b**, (±)-norcoclaurine-1-carboxylic acid (**5**), and dihydroisoquinolinium salt **6** were submitted to enzymatic *O*-methylation with *S*-adenosyl-L-[*methyl*-^{14}C]methionine in the presence of mammalian COMT (see *Exper. Part*). Examination of the ^{14}C-labeled products from the reaction of **2a** clearly demonstrated that *O*-methylation preferentially occurred on the 6-OH group: the 6-methyl ether [^{14}C]-**12** accounted for 80% of the enzymatic products and the 7-methyl ether [^{14}C]-**11** for 20% (see the *Table*). Similarly, **2b** yielded 76% of the 7-methyl ether [^{14}C]-**11** and 24% of the 6-methyl ether [^{14}C]-**12**. In the

Table. *Enzymatic O-Methylation of* **2a**, **2b**, **2**, **5**, *and* **6** *by Mammalian Catechol O-Methyltransferase*

Starting catechol	6-MeO product	7-MeO product [%]	K_m [µM] [%]	V_{max} [nmol/min/ mg protein]	V_{max}/K_m [min^{-1}]
(–)-(*S*)-Norcoclaurine (**2a**)	80 ± 5	20 ± 5	18.2 ± 0.4	4.9 + 0.3	0.27
(+)-(*R*)-Norcoclaurine (**2b**)	24 ± 2	76 ± 2	19.6 ± 0.5	4.3 + 0.2	0.22
(±)-Norcoclaurine (**2**)	55 ± 6	45 ± 7	44.8 ± 0.8	3.0 + 0.2	0.07
1,2-Didehydronorcoclaurine (**6**)	2 ± 5	98 ± 2	34.2 ± 1.0	2.9 + 0.2	0.09
(±)-Norcoclaurine-1-carboxylic acid (**5**)	–	+++	22.0 ± 0.7	0.89 + 0.3	0.04

case of racemic norcoclaurine (**2**), the yield of [^{14}C]-**12** was 55% and that of [^{14}C]-**11** 45%. An almost complete preference for methylation on the 7-OH group was observed with (**6**): the 7-methyl ether [^{14}C]-**9** accounted for 98% of the enzymatic products and the 6-methyl ether [^{14}C]-**10** for only 2%. The preference for the enzymatic *O*-methylation at the 7-OH group of carboxylic acid **5** was estimated to be *ca.* 90% based upon the color change of both the reference compound **7** and the enzymatic reaction products in base, this color originating from quinonemethide **9a**, formed from **7** by oxidative decarboxylation (*cf.* [14]). The isomeric 6-methyl ether **8** did not undergo oxidative decarboxylation under these conditions, and the solution remained colorless.

Kinetic analysis of the enzymatic *O*-methylation (see *Exper. Part* and [14]) of **2a** and **2b** yielded the relative rate constants (V_{max}/K_m) 0.22 and 0.27 min^{-1}, respectively, suggesting nearly equivalent *O*-methylation rates for the two antipodes (see the *Table*). The racemic compound **2** had a slightly lower relative rate constant (0.7 min^{-1}). Compound **6** clearly exhibited substrate inhibition at concentrations greater than 50 µM (V_{max}/K_m = 0.09 min^{-1}), and the relative rate constant of **5** was 0.041 min^{-1}.

Conclusions. – *O*-Methylation of the antipodes of dideoxynorlaudanosoline-1-carboxylic acid (**3**) with *S*-adenosyl-L-methionine, catalyzed by COMT, afforded exclusively 7-*O*-methylated products [14]. Similar regioselectivity is demonstrated here with (±)-norcoclaurine-1-carboxylic acid (**5**) which suggests that isoquinoline-1-carboxylic acids are not likely intermediates in the synthesis of (*S*)-reticuline, as already demonstrated by *Zenk et al.* using a different methodology [15]. The formation of 80% of 6-*O*-methylated products from the (*S*)-antipode **2a** is in excellent accord with the methylation of similar benzylisoquinolines in plant species [19] and suggests that non-mammalian COMT and mammalian COMT are very similar enzymes. 'COMT' activity has been demonstrated in various plants and yeasts [20–26]. Many of the properties of non-mammalian COMT are similar to those of mammalian COMT with regard to the requirement for *S*-adenosyl-L-methionine as the methyl donor, activation by Mg^{2+} ion, response to inhibitors, and substrate specificity [26] [27].

Experimental Part

General. TLC: plates from *Analtech Inc.* Column chromatography: *Fluka* silica gel *60* (230–400 mesh). M.p. (uncorrected): *Fisher-Johns* apparatus. UV spectra (λ_{max} in nm, log ε): *Hewlett-Packard-8450-A* UV/VIS spectrophotometer; in EtOH if not otherwise indicated. IR spectra (cm$^{2-1}$): *Beckman-IR-4230* instrument. ^{1}H-NMR spectra: *Varian-XL-300* (300 Hz) spectrometer; δ values in ppm relative to internal TMS, coupling constants (*J*) in Hz. MS (*m/z*): for chemical ionization (CI-MS), *Finnigan-1015D*, and for electron impact (EI-MS) *Hitachi-Perkin-Elmer RMU-6E* instrument.

*3,4-Dihydro-7-hydroxy-1-(4-hydroxybenzyl)isoquinolin-6(2*H*)-one* (**6a**) *from* **6**. The salt **6** (100 mg, 0.286 mmol) was dissolved in hot H_2O and the soln. brought to pH 7–8 with sat. $NaHCO_3$ soln. The yellow precipitate was filtered and washed with H_2O; 49.8 mg (64.8%) of **6a**. Recrystallization from MeOH gave an anal. sample. M.p. 238–240° (dec.). UV: 208 (0.83), 221 (0.76), 273 (0.41), 318 (0.18), 410 (0.71). UV (EtOH with 0.1N HCl): 218 (0.75), 251 (0.72), 308 (0.42), 367 (0.46). IR (KBr): 1585. ^{1}H-NMR ((D_5)pyridin): 2.58 (*t*, *J* = 7.5, 2 H–C(4)); 3.72 (*t*, *J* = 7.5, 2 H–C(3)); 4.13 (*s*, $C_6H_4CH_2$); 6.96 (*s*, 1 arom. H); 7.04 (*d*, *J* = 8.3, 2 arom. H); 7.34 (*d*, *J* = 8.3, 2 arom. H); 7.56 (*s*, OH). CI-MS: 270 (100, $[M + 1]^+$). Anal. calc. for $C_{16}H_{15}NO_3 \cdot 1/4\ H_2O$ (273.80): C 70.19, H 5.70, N 5.11; found: C 70.42, H 5.75, N 5.08.

6a *from* **5**. A soln. of **5** (67 mg, 0.194 mmol) in 60% aq. MeOH (30 ml) was brought to pH 9–10 with aq. Na_2CO_3 soln. After 1 h standing at r.t., MeOH was evaporated and the aq. soln. extracted with AcOEt. The org. phase was dried (Na_2SO_4) and evaporated: 15 mg (28.7%) of **6a**, identical by TLC and UV with **6a** prepared from **6**.

3,4-Dihydro-6-hydroxy-1-(4-hydroxybenzyl)-7-methoxyisoquinoline Hydrobromide (**9**). M.p. 224–226° (EtOH/PrOH) UV: 210 (2.17), 222 (2.05), 247 (2.04), 305 (1.18), 359 (1.52). IR (KBr): 1640, 1610, 1560, 1510. ^{1}H-NMR ((D_6)DMSO): 2.91 (*t*, *J* = 7.8, CH_2); 3.77 (*t*, *J* = 7.8, CH_2); 3.79 (*s*, CH_3O); 4.35 (*s*, $C_6H_4CH_2$); 6.72 (*d*, *J* = 8.3, 2 arom. H); 6.82 (*s*, 1 arom. H); 7.20 (*d*, *J* = 8.3, 2 arom. H); 7.53 (*s*, 1 arom. H); 9.48, 11.01, 12.31 (3*s*, 2 OH, HBr). CI-MS: 364,362 ($[M - 1]^+$), 284 (100, $[M - HBr + 1]^+$). Anal. calc. for $C_{17}H_{17}NO_3 \cdot HBr \cdot 1/4\ H_2O$ (368.76): C 55.37, H 5.06, N 3.80, Br 21.67; found: C 55.20, H 5.39, N 3.60, Br 21.52.

*3,4-Dihydro-1-(4-hydroxybenzyl)-7-methoxyisoquinolin-6(2*H*)-one* (**9a**) *from* **9**. As described for **8a**. Yellow crystals. M.p. 153–155°. UV: 216 (2.36), 230 (2.71), 276 (1.82), 310 (1.15), 408 (2.65). UV (EtOH with 0.1N HCl): 213 (2.29), 227 (2.45), 247 (2.75), 306 (1.72), 356 (2.23). IR (KBr): 1620, 1600. ^{1}H-NMR ((D_6)DMSO): 2.55 (*t*, *J* = 7.5, 2 H–C(4)); 3.53 (*t*, *J* = 7.5, 2 H–C(3)); 3.69 (*s*, CH_3O arom); 3.95 (*s*, $C_6H_4CH_2$); 6.56 (*s*, 1 arom. H); 6.65 (*d*, *J* = 8.5, 2 arom. H); 7.10 (*d*, *J* = 8.5, 2 arom. H); 7.14 (*s*, 1 arom. H); 9.23 (*s*, OH). CI-MS: 284 (100, $[M + 1]^+$).

9a *from* **7**. A soln. of **7** (41 mg, 0.125 mmol) in 60% aq. MeOH (30 ml) was brought to pH 10 with aq. Na_2CO_3 soln. and left standing at r.t. for 3 days. Workup as described for **6a** from **5**: 3 mg (8.2%) of **9a**, identical by TLC and UV with **9a** prepared from **9**.

3,4-Dihydro-7-hydroxy-1-(4-hydroxybenzyl)-6-methoxyisoquinoline Hydrobromide (**10**). M.p. 219–220°. UV: 204 (1.23), 247 (0.95), 305 (0.57), 363 (0.45). IR (KBr): 3200, 1640, 1600, 1560. ^{1}H-NMR ((D_6)DMSO): 2.98 (*t*, *J* = 7.9, 2 H–C(4)); 3.82 (*t*, *J* = 7.9, 2 H–C(3)); 3.89 (*s*, CH_3O); 4.25 (*s*, $C_6H_4CH_2$); 6.73 (*d*, *J* = 8.5, 2 arom. H); 7.08 (*s*, 1 arom. H); 7.15 (*d*, *J* = 8.5, 2 arom. H); 7.40 (*s*, 1 arom. H); 9.50 (*s*, 1 H); 9.61 (*s*, 1 H, 2 OH). CI-MS: 284 (100, $[M - HBr + 1]^+$). Anal. calc. for $C_{17}H_{17}NO_3 \cdot HBr$ (364.25): C 56.06, H 4.98, N 3.85, Br 21.94; found: C 55.79, H 5.02, N 3.82, Br 21.83.

The free base was prepared by neutralization of salt **10** in H_2O. M.p. 196–198° (dec.). UV: 232 (2.78), 275 (1.31), 314 (0.99). IR (KBr): 3520, 1630. ^{1}H-NMR ((D_6)DMSO): 2.46 (*m*, 2 H–C(4)); 3.50 (*m*, 2 H–C(3)); 3.75 (*s*, CH_3O); 3.76 (*s*, $C_6H_4CH_2$); 6.62 (*d*, *J* = 8.4, 2 arom.H); 6.76 (*s*, 1 arom. H); 6.93 (*s*, 1 arom. H); 7.01 (*d*, *J* = 8.4, 2 arom. H); 9.02 (br., 2 OH). CI-MS: 284 (100, $[M + 1]^+$).

4-(Benzyloxy)-3-hydroxybenzaldehyde (**14**). A mixture of **13** (7.13 g, 0.0517 mol), $NaHCO_3$ (4.78 g, 1.1 equiv), and benzyl chloride (6.54 g, 1.0 equiv.) in DMF (70 ml) was stirred at 60° for 3 days. The solvent was evaporated, the crude product extracted with AcOEt, washed with sat. aq. $NaHCO_3$ soln. and brine, and dried ($MgSO_4$). The residue, after evaporation of the solvent, was chromatographed on silica gel (hexane/AcOEt 4:1): **14** (6.70 g, 56.8%). Recrystallization from i-PrOH gave 4.56 g. M.p. 120–121°. IR (KBr): 1665. ^{1}H-NMR ($CDCl_3$): 5.18 (*s*, $C_6H_5CH_2$); 6.24 (br. *s*, 1 arom. H); 7.07 (*d*, *J* = 8.2, 1 arom. H); 7.38–7.52 (*m*, 6 arom. H); 9.82 (*s*, CHO). EI-MS: 228 ($M^{+\cdot}$), 91 (100). Anal. calc. for $C_{14}H_{12}O_3$ (228.25): C 73.67, H 5.30; found: C 73.58, H 5.33.

2-(Benzyloxy)-5-(2-nitroethenyl)phenol (**16**). A soln. of **14** (1.38 g, 6.50 mmol) and NH_4OAc (0.23 g, 0.5 equiv.) in nitromethane (40 ml) was refluxed for 3 h, then left at r.t. over night. Nitromethane was evaporated the residue extracted with AcOEt, and the extract washed with H_2O, dried (Na_2SO_4), and evaporated. The residual solid was recrystallized from i-PrOH: 1.16 g (70.8%). M.p. 127–129°. IR (KBR): 3430, 1690, 1640, 1580. ^{1}H-NMR ($CDCl_3$): 5.02 (*s*, $C_6H_5CH_2$); 5.65 (br. *s*, OH); 6.80 (*d*, *J* = 8.3, 1 arom. H); 6.90 (*dd*, *J* = 2.0, 8.3, 1 arom. H); 6.98 (*d*, *J* = 2.0, 1 arom. H); 7.26 (br. *s*, 5 arom. H); 7.32 (*d*, *J* = 13.6, $CHNO_2$); 7.75 (*d*, *J* = 13.6, 1 olef. H). CI-MS: 289 ($[M + NH_3 + 1]^+$), 272 (100, $[M + 1]^+$). Anal. calc. for $C_{15}H_{13}NO_4$ (271.28): C 66.44, H 4.83, N 5.16; found: C 66.22, H 4.89, N 5.15.

*4-*O*-Benzyldopamine Hydrochloride* (= *2-(Benzyloxy)-5-(2-aminoethyl)phenol Hydrochloride*; **17**). A soln. of **16** (0.05 g, 1.85 mmol) in THF (10 ml) was added to $LiAlH_4$ (1.0 g) in THF (15 ml) with stirring under ice cooling. The mixture was refluxed for 2 h, then quenched with sat. Na_2SO_4 soln. The inorg. compounds were filtered off and washed with THF and H_2O. The filtrate was evaporated and the resulting aq. soln. made acidic with 10% HCl soln.

and washed with Et_2O. The aq. soln. was then made alkaline with conc. NH_4OH soln. and extracted with Et_2O. The org. phase was washed with sat. NaCl soln., dried (Na_2SO_4), and evaporated. The crude amine was treated with conc. HCl soln. in EtOH, and the hydrochloride, after evaporation of the solvent, was recrystallized from $EtOH/Et_2O$: 0.22 g (42.5%). M.p. 190–195°. Recrystallization from i-PrOH gave an anal. sample. M.p. 201–203°. IR (KBr): 3540, 3400, 2000–1980, 1590. ^1H-NMR (D_2O): 2.85 (*t*, *J* = 7.2, CH_2CH_2N); 3.19 (*t*, *J* = 7.2, CH_2CH_2); 5.19 (*s*, $C_6H_5CH_2$); 6.74 (*dd*, *J* = 2.1, 8.3, 1 arom. H); 6.83 (*d*, *J* = 2.1, 1 arom. H); 7.01 (*d*, *J* = 8.3, 1 arom. H). CI-MS: 244 (100, $[M + 1]^+$). Anal. calc. for $C_{15}H_{17}NO_2 \cdot HCl$ (179.77): C 64.40, H 6.49, N 5.01, Cl 12.67; found: C 64.21, H 6.55, N 5.00, Cl 12.73.

*4-[4-(Benzyloxy)benzylidene]-2-phenyloxazol-5(4*H*)-one* (**19**). A mixture of **18** (5.0 g, 0.0236 mol), hippuric acid (4.22 g, 0.0236 mol), and NaOAc (1.93 g, 0.0236 mol) in Ac_2O (7.2 g, 3 equiv.) was stirred at 95° for 1.5 h. After cooling, EtOH (10 ml) was added and the mixture allowed to stand over night. The precipitate was filtered and washed with H_2O and EtOH. Recrystallization from benzene gave 5.30 g (63.3%). M.p. 159–161°. IR (KBr): 1790, 1650, 1600. ^1H-NMR ($CDCl_3$): 5.15 (*s*, $C_6H_5CH_2$); 7.06 (*d*, *J* = 89, 2 arom. H); 7.21 (*s*, C_6H_4CH=); 7.35–7.59 (*m*, 7 arom. H); 8.15–8.20 (*m*, 5 arom. H). CI-MS: 356 (100, $[M + 1]^+$). Anal. calc. for $C_{23}H_{17}NO_3$ (355.40): C 77.73, H 4.82, N 3.94; found: C 77.57, H 4.84, N 3.90.

(4-Benzyloxy)pyruvic Acid (= 3-[4-(Benzyloxy)phenyl]-2-oxopropanoic Acid; **20**). A mixture of **19** (2.15 g, 6.06 mmol) and 20% NaOH soln. (50 ml) was refluxed for 2 h. After cooling, sat. $NaHSO_3$ soln. was added and the resulting precipitate collected and washed with H_2O and Et_2O. A suspension of this adduct in H_2O was acidified with 10% HCl soln. and extracted with Et_2O. The extract was washed with 10% HCl soln. and H_2O, dried ($MgSO_4$), and evaporated. The residual solid was recrystallized from Et_2O hexane: 1.08 g (66.0%). M.p. 183–185°. IR (KBr): 1760, 1730. ^1H-NMR ($CDCl_3$): 4.16 (*s*, 2 H–C(3)); 5.06 (*s*, $C_6H_5CH_2$); 6.00 (br., COOH); 6.96 (*d*, *J* = 8.2, 2 arom. H); 7.18 (*d*, *J* = 8.6, 2 arom. H); 7.30–7.45 (*m*, 5 arom. H): CI-MS: 288 (100, $[M + NH_3 + 1]$). Anal. calc. for $C_{16}H_{14}O_4$ (270.29): C 71.10, H 5.22; found: C 71.19, H 5.28.

7-(Benzyloxy)-1-[4-(benzyloxy)benzyl]-1,2,3,4-tetrahydro-6-hydroxyisoquinoline-1-carboxylic Acid (**21**). A soln. of **17** (0.25 g, 0.894 mmol) and **20** (0.25 g, 0.894 mmol) in EtOH (15 ml) was brought to pH 5–5.5 with dil. NH_4OH soln. The resulting soln. was allowed to stand at r.t. for 5 days, and the precipitate was collected by filtration and washed with H_2O, EtOH, and Et_2O: 0.19 g (42.9%). M.p. 213–214°. IR (KBr): 3420, 1620. ^1H-NMR ((D_6)DMSO): 2.50–2.57 (*m*); 3.14–5.02 (*s*, $C_6H_5CH_2$); 5.11 (*s*, $C_6H_5CH_2$); 6.46 (*s*, 1 arom. H); 6.78 (*d*, *J* = 8.6, 2 arom. H); 6.94 (*d*, *J* = 8.6, 2 arom. H); 7.28–7.57 (*m*, 12 arom. H); 9.06 (br. *s*, 1 H). CI-MS: 496 ($[M + 1]^+$), 450 (100). Anal. calc. for $C_{31}H_{29}NO_5$ (495.58): C 75.13, H 5.90, N 2.83; found: C 74.90, H 5.96, N 2.79.

Methyl 7-(Benzyloxy)-1-[4-(benzyloxy)benzyl]-1,2,3,4-tetrahydro-6-methoxyisoquinoline-1-carboxylate (**22**) *and Methyl 7-(Benzyloxy)-1-[4-(benzyloxy)benzyl]-1,2,3,4-tetrahydro-6-methoxy-2-methylisoquinoline-1-carboxylate* (**23**). To a suspension of **21** (127 mg, 0.257 mmol) in MeOH (30 ml), CH_2N_2 in Et_2O was added under ice cooling. The mixture was allowed to stand at r.t. over night, then evaporated. The residue was chromatographed on silica gel (benzene/AcOEt 17:3): less polar **23** (21 mg, 15.2%; oil) and polar **22** (81 mg, 60.2%; oil).

23: IR ($CHCl_3$): 1715. ^1H-NMR ($CDCl_3$): 2.21–2.31 (*m*, $ArCH_2$); 2.47 (*s*, CH_3N); 2.64–2.69 (*m*, 1 H); 3.02–3.10 (*m*, 1 H); 2.97, 3.25 (*d*, *J* = 14.1, $ArCH_2$); 3.60 (*s*, $COOCH_3$); 3.82 (*s*, CH_3O); 4.94 (*s*, $C_6H_5CH_2$); 5.14 (*s*, $C_6H_5CH_2$); 6.38 (*s*, 1 arom. H); 6.43 (*d*, *J* = 8.7, 2 arom. H); 6.57 (*d*, *J* = 8.7, 2 arom. H); 6.60 (*s*, 1 arom. H); 7.24–7.46 (*m*, 10 arom. H). CI-MS: 538 (100, $[M + 1]^+$).

22: IR ($CHCl_3$): 1715. ^1H-NMR ($CDCl_3$): 1.79 (br., NH); 2.50–2.70 (*m*, $ArCH_2$); 2.95, 3.32 (*d*, *J* = 13.5, $ArCH_2$); 3.02–3.06 (*m*, CH_2N); 3.58 (*s*, $COOCH_3$); 3.87 (*s*, CH_3O); 5.01 (*s*, $C_6H_5CH_2$); 5.21 (*s*, $C_6H_5CH_2$); 6.55 (*s*, 1 arom. H); 6.82 (*d*, *J* = 8.6, 2 arom. H); 6.93 (*d*, *J* = 8.6, 2 arom. H); 7.24–7.49 (*m*, 11 arom. H). CI-MS: 524 (100, $[M + 1]$).

1,2,3,4-Tetrahydro-7-hydroxy-1-(4-hydroxybenzyl)-6-methoxyisoquinoline-1-carboxylic Acid Hydrochloride (**8**). A soln. of **22** (110 mg, 0.210 mmol) in 20% HCl soln. (11 ml) was refluxed for 2 h. After cooling, the precipitate was collected by filtration and washed with H_2O to give 51.8 mg (75%): Recrystallization from 5% HCl soln. gave an anal. sample. M.p. 250–252° (dec.). IR (KBr): 3470, 1740. ^1H-NMR ((D_6)DMSO): 2.78–2.84, 3.61–3.66 (2 *m*, 1 H each, $ArCH_2$); 2.96–3.08 (*m*, $ArCH_2$); 3.23–3.28 (*m*, 2 H–C(3)); 3.76 (*s*, CH_3O); 6.72 (*d*, *J* = 8.4, 2 arom. H); 6.75 (1 arom. H); 7.14 (*d*, *J* = 8.4, 2 arom. H); 7.24 (*s*, 1 arom. H); 8.90 (br. *s*, OH); 9.16 (*s*, OH); 9.52 (br., NH, COOH). CI-MS: 330 (100, $[M + 1]^+$). Anal. calc. for $C_{18}H_{19}NO_5 \cdot HCl$ (365.82): C 59.10, H 5.51, N 3.83, Cl 9.69; found: C 58.86, H 5.60, N 3.77, Cl 9.61.

Purification of Catechol O-*Methyltransferase* (COMT; see [13]). Purification of COMT used in these studies was performed by previously established methods [28]. Preparations of the soluble form of COMT were obtained from the livers of male, *Sprague-Dawley* rats and carried through the calcium phosphate gel step of the purification procedure to yield a preparation with a specific activity of 1–2 μmol/min/mg protein.

Procedures for Kinetic-Analysis Studies with COMT. Kinetic analysis of the *O*-methylation of racemic **2**, optically active **2a** and **2b**, racemic **5**, and dihydroisoquinolinium salt **6** were performed as described previously [14]. Double reciprocal plots of initial reaction velocity *vs.* substrate concentration were analyzed by non-linear regression analysis. K_m and V_{max} values are reported as the mean ± the standard error of the mean (s.e.m.) of three or more separation determinations.

Enzymatic Formation of the 6- and 7-Methyl Ethers. Racemic **2** optically active **2a** and **2b**, dihydroisoquinolinium salt **6**, and racemic **5** were incubated with COMT under conditions designed to yield maximum product formation. The catechols were added at a final concentration of 2 mM to a reaction mixture containing the following components (mM): $MgCl_2$ (1.2), dithiothreitol (4), *S*-adenosyl-L-methionine (0.05), *Tris* buffer (pH 9; 20), *S*-adenosyl-L-[*methyl*-^{14}C]methionine (0.2 μCi), and 0.3 mg of enzyme in a final volume of 0.25 ml. The reaction was allowed to continue for 60 min at 37° and then stopped by the addition of 0.2 ml of 60% CCl_3COOH. The precipitated protein was sedimented by centrifugation (5 min, *Fischer* microfuge). Authentic methyl ethers of the appropriate catechols were added to the supernatant fluid. Separation of the reaction products was achieved by TLC (5 × 20 cm plates precoated with silica gel *G 250* microns (*Analtech, Uniplate*)). The mobile phase consisted of $CHCl_3$/EtOH/conc. NH_4OH soln. 20:5:0.05. The methyl ethers were visualized with I_2 vapor.

The extent of enzymatic *O*-methylation was determined by removing sections of silica gel (0.5 cm), suspending it in 3 ml of scintillation fluid (*Hydrofluor, National Diagnostics*, Manville, N.J.), and measuring its ^{14}C content in a *Beckman* scintillation counter, with a counting efficiency of 65% (see the *Table*). Radioactivity from *S*-adenosyl-L-[*methyl*-^{14}C]methionine remained at the origin. The R_f's of labeled and unlabeled methyl ethers were identical in each case. Products form **2**: R_f 0.51 ([^{14}C]-**11**), 0.45 ([^{14}C]-**12**). Products from **6**: R_f 0.5 (^{14}C]-**10**), 0.15 ([^{14}C]-**9**). Products from **5**: methyl ethers [^{14}C]-**7**, and [^{14}C]-**8** or the products could not be separated by TLC; however, in the presence of base (0.1N NaOH), the reference compound **7** (7-MeO) and the enzymatic reaction product turned yellow, while **8** (6-MeH) under the same conditions remained colorless (*cf.* [14]).

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