135. Mammalian Alkaloids: Synthesis and *O*-Methylation of (*S*)- and (*R*)-3′-Hydroxycoclaurine and Their *N*-Methylated Analogues with *S*-Adenosyl-L-[*methyl*-^{14}C]methionine in Presence of Mammalian Catechol *O*-Methyltransferase

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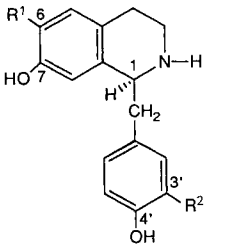
(23.VII.91)

O-Methylation of the optically active 3′-hydroxycoclaurines **3a** and **3b** and of the *N*-methylated analogs **5a,b** with *S*-adenosyl-L-[*methyl*-^{14}C]methionine in presence of mammalian COMT was investigated *in vitro*. The *N*-unsubstituted (1*S*)- and (1*R*)-isomers **3a** and **3b**, respectively, afforded almost equal amounts of the corresponding *N*-norreticuline **4** and *N*-nororientaline **19**, besides two unknown by-products (see *Fig.* and *Table 1*). The *N*-methylated (1*S*)-isoquinoline **5a**, on the other hand, afforded largely (*S*)-orientaline ((*S*)-**19**), while an almost equal mixture of (*R*)-reticuline (**6b**) and (*R*)-orientaline ((*R*)-**19**) was obtained from the (1*R*)-enantiomer **5b**. The isoquinolines **3a,b** and **5a,b** were prepared by a *Bischler-Napieralski* cyclization yielding *O*-benzyl-protected isoquinoline **10** (*Scheme 1*). The optical resolution of **10** was accomplished with 2′-bromotartranilic acid. The *N*-methylated isoquinolines were prepared by *N*-formylation of **10a,b** and reduction of the formamides **13a,b** with diborane (→ **14a,b**). Deblocking of the benzyl-ether moieties of **10a,b** and **14a,b** was accomplished by catalytic hydrogenation in presence of HCl, affording directly **3a,b**·HCl and **5a,b**·HCl, respectively.

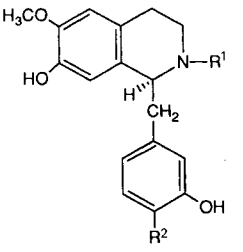
Introduction. – The biosynthesis of morphine in the opium poppy (*Papaver somniferum*) proceeds from (*S*)-reticuline *via* dehydroreticulinium salt to (*R*)-reticuline and then to morphine alkaloids, and this part is rather well elaborated [1] [2]. Details on the biosynthesis of (*S*)-reticuline (**6a**) itself, however, were only recently clarified: *Zenk* and coworkers [3] showed that the first isoquinoline formed in plants which produce benzyltetrahydroisoquinolines is (*S*)-norcoclaurine (**1a**), converted by an enzymatic *O*-methylation into (*S*)-coclaurine (**2a**), followed by two bioconversions into (*S*)-*N*-methylcoclaurine (not shown) and (*S*)-3′-hydroxy-*N*-methylcoclaurine (**5a**) as the ultimate precursor of (*S*)-reticuline (**6a**)[1]). In an earlier biosynthetic scheme, *Zenk* and coworkers [4] had proposed that **6a** was derived from **1a** *via* (*S*)-3′-hydroxycoclaurine (**3a**) and (*S*)-*N*-norreticuline (**4a**), followed by its *N*-methylation to **6a** as the last step.

The correctness of the now proposed scheme (see [3]) for the biosyntheses of **6a** from **1a** in benzylisoquinoline-producing plants is further supported by the complete absence of *N*-demethyl compounds in these plants [3].

[1]) Throughout this paper, the key numbers of tetrahydroisoquinolines with (1*S*)-configuration are followed by **a**; compounds of the **b** series (not all shown) have (1*R*)-configuration.



1a[1]) R^1 = OH, R^2 = H
2a[1]) R^1 = CH_3O, R^2 = H
3a[1]) R^1 = CH_3O, R^2 = OH



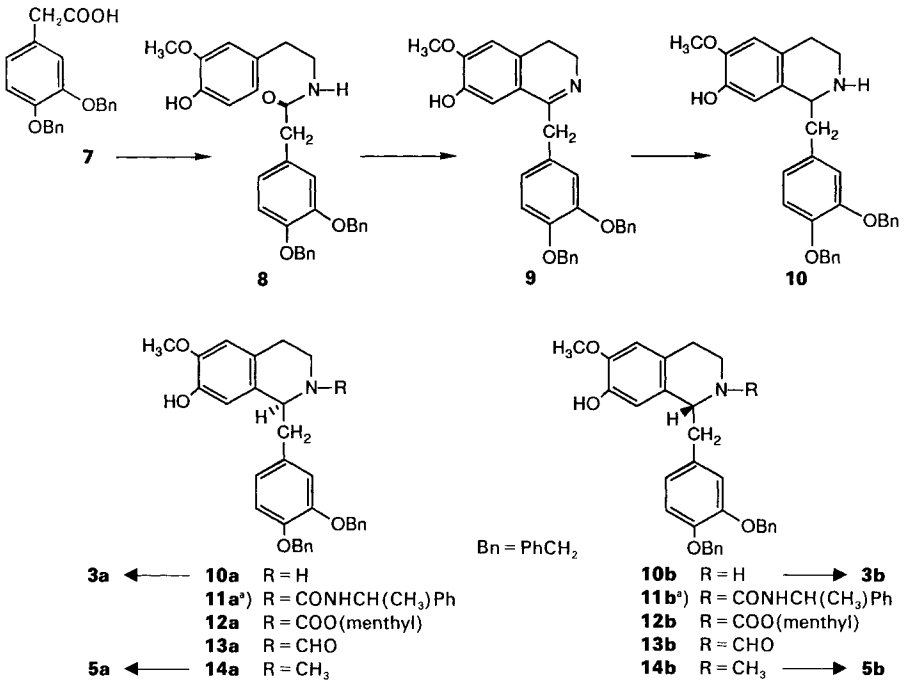
4a[1]) R^1 = H, R^2 = CH_3O
5a[1]) R^1 = CH_3, R^2 = OH
6a[1]) R^1 = CH_3, R^2 = CH_3O

Morphine is a normal constituent in man [5], and the amounts of mammalian morphine found in the urine of human volunteers, excluding conjugates, was found to reach pmol/ml levels [6]. This prompts speculation on its origin, and whether the isoquinolines of the plant biosynthesis of morphine could similarly be transformed by mammalian enzymes. We recently reported that *O*-methylation of norcoclaurines **1a,b** with *S*-adenosyl-L-[*methyl*-^{14}C]methionine in the presence of mammalian catechol *O*-methyltransferase (COMT) was highly enantio- and regiospecific [7]. The products obtained with **1a** as the ligand were highly enriched in the 6-*O*-methylated coclaurine **2a**, whereas with unnatural **1b**, products enriched in the 7-*O*-methylated isomer (not shown) were obtained. To further support the notion that a biosynthetic route to mammalian morphine might follow a similar pathway as that used by the opium poppy, prompted us to prepare the optical isomers **3a,b** and **5a,b** and to study their *O*-methylation with *S*-adenosyl-L-[*methyl*-^{14}C]methionine in the presence of COMT. The key intermediate for these syntheses was tetrahydroisoquinoline **10** which was separated into its antipodes. For analytical purposes, we also prepared the racemic tetrahydroisoquinolines **17–19** of the orientaline series.

Chemistry. – Racemic tetrahydroisoquinoline **10** was prepared from the benzyl-protected phenylacetamide **8** (obtained from **7**) by a *Bischler-Napieralski* cyclization, followed by reduction of imine **9** with $NaBH_4$ in MeOH (*Scheme 1*). The optical resolution of **10** was accomplished by salt formation with (–)- and (+)-2′-bromotartranilic acid (= *N*-(2′-bromophenyl)-D- and -L-tartarmonoamide) as described in the synthesis of optically active reticulines [8] (*Method A*), and by a urea diastereoisomerization followed by alcoholysis (*Method B*). In the latter case, the urea diastereoisomers **11a,b**, obtained from **10** and (+)-(*R*)-1-phenylethyl isocyanate, were separated by chromatography and hydrolyzed with NaOBu in BuOH [9]. Formation of ureas **11a** and **11b** was also used to control the enantiomeric purity of **10a** and **10b** by TLC. The enantiomeric purity was also checked by HPLC of the diastereoisomeric menthyl *N*-carboxylates **12a** and **12b**, obtained from **10a** and **10b**, respectively (see *Exper. Part*).

N-Methylation of the optical isomers **10a,b** was achieved *via* the *N*-carbaldehydes **13a,b** followed by their reduction with borane (→ **14a,b**), as described in the synthesis of reticulines [8]. Deprotection of the benzyl-ether functions in **10a,b** and **14a,b** was achieved by hydrogenation of their hydrochloride salts over $Pd(OH)_2$ catalyst in EtOH in the

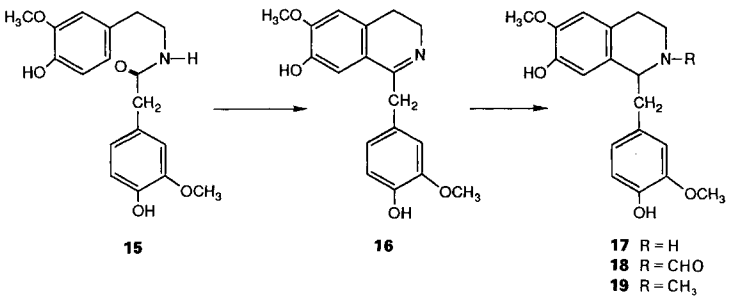
Scheme 1



a) Ureas prepared either with (+)-(*R*)- or with (−)-(*S*)-1-phenylethyl isocyanate.

presence of a slight excess of HCl to yield the desired optically active tetrahydroisoquinolines **3a,b** and **5a,b**, respectively. The enantiomers **3a,b** were found to be identical on TLC with a sample of racemic **3** prepared by a different route [10]. The enantiomeric purity of **3a,b** (prepared *via Method A*) was determined by analysis of the ureas obtained with (−)-(*S*)-1-phenylethyl isocyanate (TLC) and of the carbamates obtained with (−)-menthyl chloroformate (HPLC). According to this materials were at least 99% enantiomerically pure. It is clearly established on the basis of their specific rotations, and in going from a negative $[\alpha]_D$ for **3a** to a positive $[\alpha]_D$ for **5a**, that the **a** series has the absolute configuration *S* [11]. This was confirmed by a comparison of the CD spectra of **5b**·HCl

Scheme 2



and (+)-(*R*)-reticuline (**6b**; not shown), which both showed strong negative *Cotton* effects in the 270–290 nm region [12]. (−)-(*S*)-*N*-Norreticuline (**4a**) and (−)-(*S*)-reticuline (**6a**), required as analytical standards, were prepared by the published procedure [8].

As described above for the synthesis of **10** from **8**, the known (±)-*N*-nororientaline [13] (**17**) was prepared from the unprotected phenylacetamide **15** *via* **16** and transformed to (±)-orientaline [14] (**19**) *via* **18** (*Scheme 2*).

Biochemical Results. – As described in [7], (*S*)- and (*R*)-3′-hydroxycoclaurines (**3a** and **3b**, resp.) and (*S*)- and (*R*)-3′-hydroxy-*N*-methylcoclaurines (**5a** and **5b**, resp.), obtained *via Method A* (see above), were subjected 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 enzymatic reaction clearly indicated that the *N*-methylcoclaurines **5a,b** yielded a mixture of the two expected ^{14}labeled *O*-methyl ethers reticuline **6** and orientaline **19**. The relative amounts of the 3′-*O*- and 4-*O*-methyl ethers (*R*)-**19**[2]) and **6b**[2]), respectively, derived from the (*R*)-antipode **5b**, were 56 and 43.7%, respectively, and of the 3′-*O*-methyl and 4′-*O*-methyl ether (*S*)-**19**[2]) and **6a**[2]), respectively, derived from the (*S*)-antipode **5a**, 85.7 and 14.4%, respectively (see *Table 1*). In the case of the 3′-hydroxycoclaurines **3a,b**, not only were the expected *O*-methyl ethers *N*-norreticuline **4** and *N*-nororientaline **17** formed, but two additional unknown products as well. The relative amounts of 3′-*O*- and 4′-*O*-methyl ethers (*R*)-**17**[2]) and **4b**[2]), respectively, derived from the (*R*)-antipode **3b**, were 26.9 and 32.1%, respectively. A significant fraction (29.8%) of the *O*-methylated products derived from the (*R*)-antipode was the unknown product **C** (see *Table 1*). The second, less polar

Table 1. *Products of* (S)- *and* (R)-3′- *Hydroxycoclaurine* (**3a** and **3b**, resp.) *and* (S)- *and* (R)-3′-*Hydroxy*-N-*methylcoclaurines* (**5a** and **5b**, resp.) *Obtained by Enzymatic* O-*Methylation*

Substrate	Product	Yield [%][a])
(*S*)-3′-Hydroxycoclaurine (**3a**)	(*S*)-*N*-norreticuline (**4a**)[2])	31.2 ± 2.8 (10)
	(*S*)-*N*-nororientaline ((*S*)-**17**)[2])	45.1 ± 2.2 (10)
	Unknown **A**	12.2 ± 1.3 (9)
	Unknown **B**	13.2 ± 1.4 (9)
(*R*)-3′-Hydroxycoclaurine (**3b**)	(*R*)-*N*-norreticuline (**4b**)[2])	32.1 ± 2.1 (11)
	(*R*)-*N*-nororientaline ((*R*)-**17**)[2])	26.9 ± 2.1 (11)
	Unknown **C**	29.8 ± 1.9 (10)
	Unknown **D**	16.1 ± 0.9 (10)
(*S*)-3′-Hydroxy-*N*-methylcoclaurine (**5a**)	(*S*)-reticuline (**6a**)[2])	14.4 ± 0.5 (4)
	(*S*)-orientaline ((*S*)-**19**)[2])	85.7 ± 0.5 (4)
(*R*)-3′-Hydroxy-*N*-methylcoclaurine (**5b**)	(*R*)-reticuline (**6b**)[2])	43.7 ± 1.6 (4)
	(*R*)-orientaline ((*R*)-**19**)[2])	56.0 ± 1.8 (4)

[a]) Products formation is expressed as the mean of the yield ± standard error of the total *O*-methylated products (= 100%). Numbers in parentheses indicate the number of separate experiments performed.

[2]) The absolute configurations for the isoquinolines **4**, **6**, **17**, and **19**, formed in the *O*-methylations reported here and analyzed by a chromatographic comparison with standard (in part racemic) samples, is not established *per se*. It is reasonable to assume, however, that the products formed from **3a**, **b** and **5a**, **b** are optically active and have the same absolute configuration as that of the precursor alkaloids. A racemization which would require an oxidation, followed by a reduction, is unlikely to occur under the experimental conditions used.

unknown product **D** represented only 16.1% of the total. The relative amounts of the 3′-*O*- and 4′-*O*-methyl ethers (*S*)-**17**[2]) and **4a**[2]), respectively, derived from the (*S*)-antipode **3a**, were 45.1 and 31.2%, and the unknown compounds **A** and **B** (less polar) 12.2 and 13.2%, respectively, of the total *O*-methylated products. Examples of the TLC analysis of the radioactive products from each catechol are shown in the *Figure*.

Kinetic analysis of the enzymatic *O*-methylation (see *Exper. Part*) of **5a** and **5b** yielded the relative rate constants V_{max}/K_m of 0.40 min^{-1} and 0.15 min^{-1}, suggesting a greater reaction rate for the (*S*)-antipode **5a** as well a higher affinity for the enzyme (see *Table 2*). The same relationship held for **3a** and **3b**: the relative rate constant for the (*S*)-antipode **3a** was 0.30 min^{-1} compared to 0.11 min^{-1} for the (*R*)-antipode **3b**. Again

Table 2. *Kinetic Analysis of the Enzymatic* O-*Methylation of (*S*)- and (*R*)-3′-Hydroxycoclaurines* (**3a** and **3b**, resp.) *and (*S*)- and (*R*)-3′-Hydroxy-*N*-methylcoclaurines* (**5a** and **5b**, resp.)[a])

Substrate	K_m [μM]	V_{max} [nmol/min/mg protein]	V_{max}/K_m [min^{-1}]
(−)-(*S*)-3′-Hydroxycoclaurine (**3a**)	29 ± 2	5.9 ± 0.5	0.30
(+)-(*R*)-3′-Hydroxycoclaurine (**3b**)	58 ± 4	6.5 ± 0.6	0.11
(+)-(*S*)-3′-Hydroxy-*N*-methylcoclaurine (**5a**)	23 ± 1	9.3 ± 0.4	0.40
(−)-(*R*)-3′-Hydroxy-*N*-methylcoclaurine (**5b**)	46 ± 1	8.7 ± 0.6	0.15

[a]) Values for K_m and V_{max} are the mean ± s.e.m. of three separate determinations.

the (*S*)-antipode **3a** displayed a higher affinity (K_m = 29 μM) for the enzyme than the (*R*)-antipode **3b** (K_m = 58 μM). The kinetic analysis performed with **3a** and **3b** was complicated by the formation of the two additional products which were not formed with the *N*-methylated isoquinolines **5a,b**. Both **5a** and **5b** produced clear substrate inhibition at concentrations greater than 1 mM. No substrate inhibition was observed with the *N*-unsubstituted coclaurines at concentrations up to 4 mM.

Discussion. – The biosynthesis of (*S*)-reticuline (**6a**), in benzylisoquinoline-producing plants, including the opium poppy (*Papaver somniferum*), following the formation of (*S*)-coclaurine (**2a**) was recently revised [3]. Data collected with plant cell suspension cultures showed, contrary to earlier reports [4], that hydroxylation with an ascorbate-dependent phenolase takes place at C(3′) after *N*-methylation, to afford (*S*)-3′-hydroxy-*N*-methylcoclaurine [15] (**5a**). Conversion of the latter alkaloid into (*S*)-reticuline (**6a**) is accomplished by a highly enantiospecific and regiospecific *O*-methyltransferase [16]. The steps involved in converting (*S*)-reticuline (**6a**) into (*R*)-reticuline (**6b**), which are required for the construction of the morphinandienone skeleton and morphine [1], have now been reevaluated by *Zenk* and coworkers. It was shown that **6a** is first oxidized by an enantiospecific oxidase into a dehydroreticulinium species [17], which is then reduced stereospecifically with a $NADPH_2$-dependent enzyme [18]. The formation of 65% of (*S*)-coclaurine[2]) (**2a**) and 35% of (*S*)-isococlaurine[3]) on *O*-methylation of (*S*)-norcoclaurine (**1a**) with *S*-adenosyl-L-[*methyl*-^{14}C]methionine and mammalian COMT [7] concurs with results obtained with tissue cultures of benzylisoquinoline-producing plant species [3] [4]. The *O*-methylation of (*S*)-3′-hydroxy-*N*-methylcoclaurine (**5a**) with *S*-adenosyl-

[3]) Another name of isococlaurine is 1-benzyl-4′,6-dihydroxy-7-methoxytetrahydroisoquinoline [19].

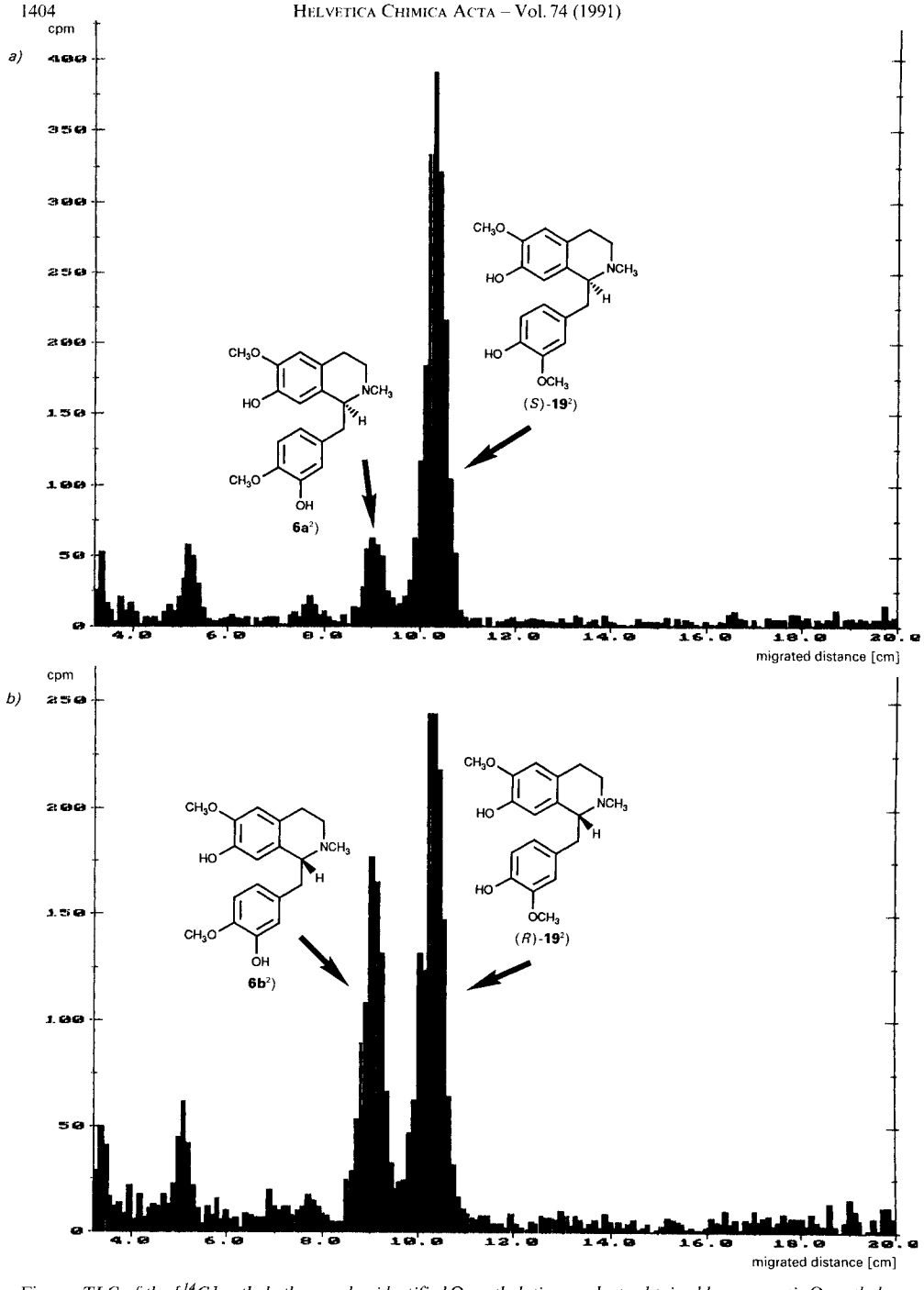
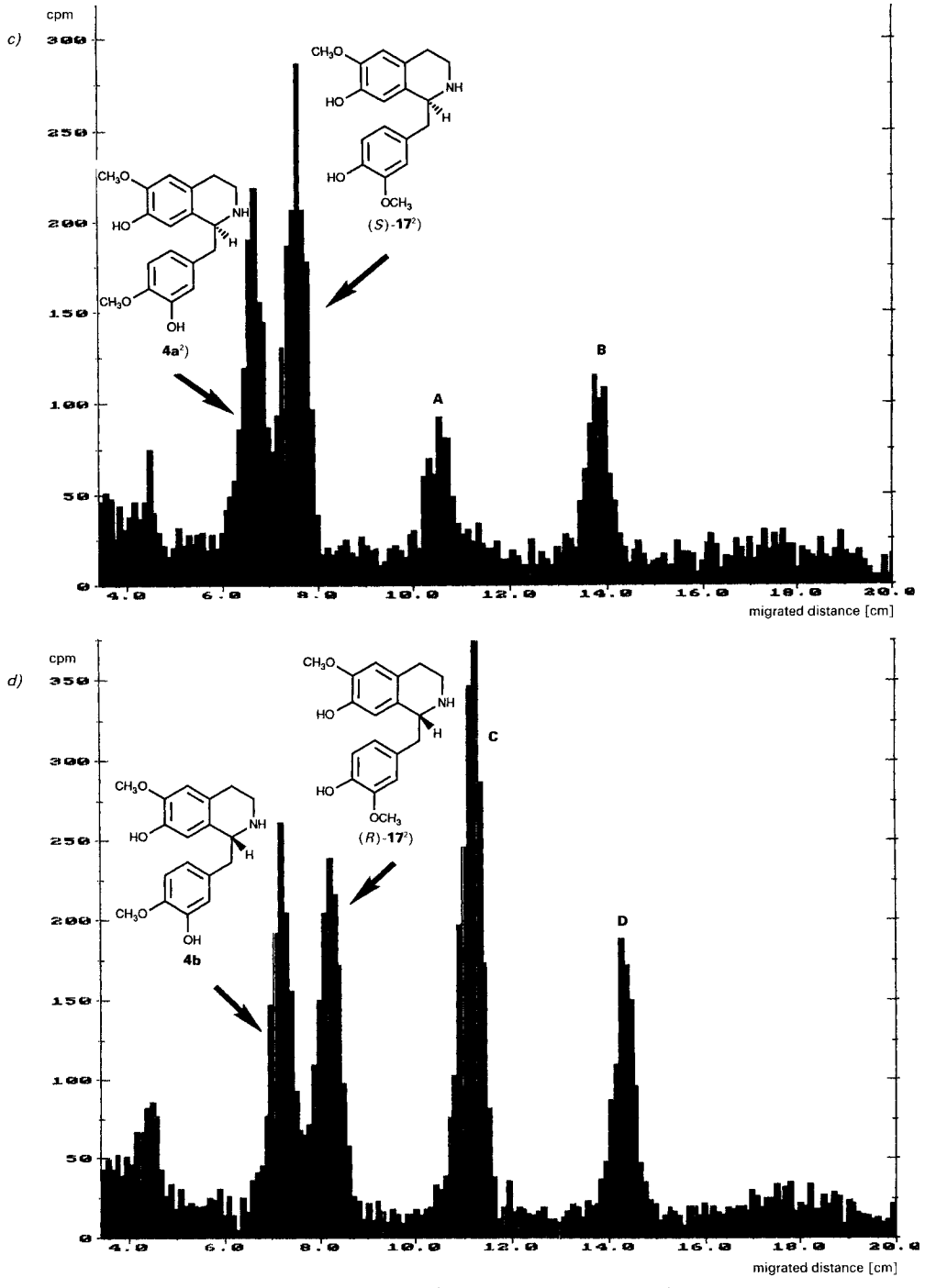


Figure. *TLC of the [*^{14}*C]methyl ethers and unidentified* O*-methylation products obtained by enzymatic* O*-methylation of* a) *(*S*)-3′-hydroxy-*N*-methylcoclaurine* (**5a**), b) *(*R*)-3′-hydroxy-*N*-methylcoclaurine* (**5b**), c) *(*S*)-3′-hydroxy-coclaurine* (**3a**), *and* d) *(*R*)-3′-hydroxycoclaurine* (**3b**)[a]). See *Table 1.*



[a]) Compounds of the **a** series have (1*S*)-configuration, compounds of the **b** series (not shown, C(1)◀H) the (1*R*)-configuration[1]). See *Table 1*.

L-[*methyl*-^{14}C]methionine and mammalian COMT, affording as reported here predominantly (*S*)-orientaline ((*S*)-**19**[2]), however, differs significantly from the highly stereospecific conversion of **5a** into (*S*)-reticuline (**6a**) observed in plants [16].

(*S*)-*N*-Norreticuline (**4a**), now discarded as an important intermediate in the plant biosynthesis of (*S*)-reticuline (**6a**) [3], was obtained here from **3a** on methylation with *S*-adenosyl-L-[*methyl*-^{14}C]methionine in the presence of mammalian COMT in 31% yield, besides 25% of unknown by-products. A high-yield conversion of (*S*)-*N*-norreticuline (**4a**) into (*R*)-*N*-norreticuline (**4b**; not shown) has been reported to occur with *Berberis* plant cell cultures, when followed by a chemical reduction of the intermediately formed dehydronorreticuline [20]. Since *N*-methylations of tetrahydroisoquinolines with *N*-methyltransferases are known to be unspecific [21], it is suggested that (*S*)- or (*R*)-*N*-norreticulines (**4a**,**b**) may well represent intermediates of the mammalian synthesis of morphine, with the conversion of (*R*)-reticuline (**6b**) into morphinandienones taking place in the liver [22]. The by-products formed in the *O*-methylation of **4a**,**b** with *S*-adenosyl-L-[*methyl*-^{14}C]methionine in the presence of mammalian COMT, but not with the *N*-methylated species **5a**,**b**, suggests that they are berbines. This possibility, and whether they are formed from exogenous or endogenous formaldehyde sources, is presently being explored.

We thank Dr. *Voldemar Toome* from the Research Division of *F. Hoffmann-La Roche Ltd.*, Nutley, N.J., USA, for having provided us with CD spectra of the various optically active isoquinolines reported here. We also would like to thank Dr. *Brian R. de Costa* for helping us in finalizing the manuscript.

Experimental Part

General. Porcine-liver catechol *O*-methyltransferase EC 2.1.1.6 from *Sigma Chemical Co.*; *S*-adenosyl-L-[*methyl*-^{14}C]methionine (SA = 55 mCi/mmol) from *New England Nuclear-Dupont Inc.*, Boston, MA. TLC: silica-gel plates from *Analtech Inc.*, Newark, NJ; silica gel *G*-preadsorbant *07191* for biological investigations; *A*, $CHCl_3$/MeOH/NH_4OH 90:9:1; *B*, $CHCl_3$/MeOH/NH_4OH 80:18:2; *C*, hexane/AcOEt/(i-Pr)$_2$O 1:1:1 containing 1% of AcOH; *D*, $CHCl_3$; *E*, $CHCl_3$/MeOH 98:2. HPLC: *Shimadzu LC-6A*, column *Alltech* (250 mm, i.d. 4–6 mm, fitting *B*). Radioactive analysis: *Bioscan* imaging scanner system *200-IBM*, *Bioscan Inc.*, Washington D.C.; *Beckman LS-7500* scintillation spectrometer. M.p. (uncorrected): *Fisher-Johns* apparatus. Optical rotation ($[\alpha]_D$): *Perkin-Elmer-241MC* automatic polarimeter. UV spectra (nm): *Hewlett-Packard-8450-A* UV/VIS spectrophotometer. CD spectra ($[\Phi]_{max}$): in EtOH, r.t.; *Jasco* model *J-500A* spectropolarimeter, model *DP-500N* data processor and *IBM-BC-XT* computer. ^{1}H-NMR spectra (300 MHz): *Varian XL-300* spectrometer, δ in ppm rel. to TMS (= 0.0 ppm) as internal standard. Chemical-ionization MS (CI-MS; *m/z*): *Finnigan-1015D* spectrometer; for high-resolution MS (HRMS), *VG-Micro Mass 7070F* spectrometer.

Reticulines. – *[3,4-Bis(benzyloxy)phenyl]acetic Acid* (**7**). To a stirred soln. of (3,4-dihydroxyphenyl)acetic acid (6.48 g, 38.5 mmol) in DMF (190 ml) was added K_2CO_3 (18 g, 130 mmol) and benzyl chloride (16.1 g, 127 mmol) in DMF (30 ml). The mixture was stirred at 70° under N_2 for 24 h, then cooled to r.t., and poured into H_2O (1000 ml). The aq. mixture was extracted with Et_2O. The combined Et_2O layer was washed with sat. $NaHCO_3$ soln. and H_2O, dried (Na_2SO_4), and evaporated: benzyl ester of **7** as white solid (16.7 g). The benzyl ester was dissolved in a warm EtOH (125 ml, 60°), and to the stirred soln. was added dropwise 10% aq. NaOH soln. (125 ml). The mixture was stirred at r.t. for 2 h (TLC (*D*) monitoring) and then poured into H_2O (1000 ml) and extracted with Et_2O to remove benzyl alcohol. The H_2O phase was rendered acidic (pH 4) with 12M HCl (ice-bath), the white solid that precipitated during acidification filtered, and the filter cake washed with H_2O, air dried, and crystallized from AcOH/H_2O: off-white crystals (11.8 g, 88.0%). M.p. 106–108° ([23]: m.p. 106–108°).

*2-[3′,4′-Bis(benzyloxy)phenyl]-*N*-[2-(4-hydroxy-3-methoxyphenyl)ethyl]acetamide* (**8**). A mixture of 3,4-bis(benzyloxy)phenylacetic acid (**7**; 11.79 g, 33.9 mmol) and [2-(4-hydroxy-3-methoxyphenyl)ethyl]amine (5.66 g, 33.9 mmol) was heated in an oil bath at 185–190° for 2 h, while passing a slow continuous stream of N_2 over the

melt to sweep out the H_2O formed. After cooling to r.t., the residue was taken up in CH_2Cl_2 (TLC (*A*) monitoring) and the soln. washed with 5% HCl soln., H_2O, sat. $NaHCO_3$ soln. and H_2O, dried (Na_2SO_4), and evaporated. The brown oil crystallized from 70% aq. AcOH: 10.1 g (60.4%) of **8**. Off-white crystals. M.p. 106–107°. ^{1}H-NMR ($CDCl_3$): 2.58–2.63 (*t*, 2H); 3.35–3.41 (*m*, 4H); 3.82 (*s*, MeO); 5.11–5.15 (*d*, 2 PhCH_2); 6.45–6.87 (*m*, 6 arom. H); 7.26–7.47 (*m*, 10 arom. H). CI-MS: 498 ($[M + 1]^+$). Anal. calc. for $C_{31}H_{31}NO_5$ (497.567): C 74.83, H 6.28, N 2.82; found: C 74.91, H 6.30, N 2.78.

1-[3′,4′-Bis(benzyloxy)benzyl]-3,4-dihydro-6-methoxyisoquinolin-7-ol Oxalate (**9**·$C_2H_2O_4$). To a stirred and refluxing soln. of **8** (9.94 g, 0.02 mol) in MeCN (250 ml) was added dropwise $POCl_3$ (11 ml, 0.12 mol). The mixture was refluxed for 1 h under N_2, cooled to r.t., and evaporated. The residue was dissolved in $CHCl_3$ and the soln. washed with 5% NH_4OH soln. and H_2O, dried (Na_2SO_4), and evaporated: **9** as a tan foam. The foam was dissolved in acetone, heated, and treated with oxalic acid (1.8 g, 0.02 mol) in i-PrOH (50 ml) to give **9**·$C_2H_2O_4$. Recrystallization from acetone/i-PrOH gave **9**·$C_2H_2O_4$ as yellow crystals (7.5 g, 64.8%). M.p. 186–187°. UV (EtOH): 236, 246 (sh), 308, 367. ^{1}H-NMR ((D$_6$)DMSO): 2.80–2.85 (*t*, 2H); 3.71–3.74 (*t*, 2H); 3.86 (*s*, MeO); 4.17 (*s*, 2H); 5.06–5.08 (*d*, 2 PhCH_2); 6.81–7.07 (*m*, 4 arom. H); 7.29–7.42 (*m*, 11 arom. H). CI-MS: 480 ($[M + 1]^+$). Anal. calc. for $C_{31}H_{29}NO_4 \cdot C_2H_2O_4 \cdot 0.5\ H_2O$ (578.597): C 68.50, H 5.58, N 2.42; found: C 68.32, H 5.54, N 2.54.

(±)-1-[3′,4′-Bis(benzyloxy)benzyl]-1,2,3,4-tetrahydro-6-methoxyisoquinolin-7-ol (**10**). A soln. of **9**·$C_2H_2O_4$ (6.7 g, 11.8 mmol) in MeOH/H_2O 1:1 (200 ml) was stirred and cooled to 0° under N_2. Then conc. NH_4OH soln. (50 ml) was added and after 15 min, $NaBH_4$ (1.6 g, 42.3 mmol) in small portions. The mixture was stirred for 15 min at 0° and for 1 h at r.t. (TLC (*A*) monitoring) and poured into H_2O/$CHCl_3$ 1:1 (400 ml). The $CHCl_3$ phase was removed and the H_2O phase reextracted twice with $CHCl_3$. The combined $CHCl_3$ extracts were washed with H_2O, dried (Na_2SO_4), and evaporated to give a white foam which was crystallized from EtOH: crystalline **10** (5.1 g, 89.7%). M.p. 131–132°. ^{1}H-NMR ($CDCl_3$): 2.64–2.82 (*m*, 4H); 3.07–3.13 (*m*, 2H); 3.84 (*s*, MeO); 3.97–4.01 (*dd*, 1H); 5.14–5.15 (*d*, 2 PhCH_2); 6.55–6.90 (*m*, 5 arom. H); 7.26–7.46 (*m*, 10 arom. H). CI-MS 482 ($[M + 1]^+$), 178. Anal. calc. for $C_{31}H_{31}NO_4$ (481.567): C 77.31, H 6.49, N 2.91; found: C 77.14, H 6.48, N 2.89.

Fumarate salt **10**·$C_4H_4O_4$ (from EtOH): M.p. 185–187°. Anal. calc. for $C_{31}H_{31}NO_4 \cdot C_4H_4O_4 \cdot 0.25\ H_2O$ (602.142): C 69.81, H 5.94, N 2.33; found: C 69.66, H 5.88, N 2.46.

*Optical Resolution of (±)-***10**. *Method A: Formation of Diastereoisomeric Salts.* To a stirred, boiling soln. of (±)-**10** (4.1 g, 8.5 mmol) in MeOH (125 ml) was added a warm soln. of (−)-2′-bromotartranilic acid hydrate [24] (= (−)-2′-BTA·H_2O; 2.7 g, 8.5 mmol) in MeOH (25 ml). The soln. was allowed to cool gradually and then seeded with crystalline material (from small-scale experiment). After standing at r.t. for 1 h, the crystalline mixture was filtered and the filter cake washed with MeOH (2 × 10 ml, r.t.) and Et_2O (2 × 10 ml) and dried: white crystals (3.45 g) which were recrystallized twice from MeOH to give colorless silky crystals of anal. and enantiomerically pure **10a**·(−)-2′-BTA·H_2O. M.p. 163–164°. $[\alpha]_D = -38.55$ ($c = 0.62$, DMSO). Anal. calc. for $C_{31}H_{31}NO_4 \cdot C_{10}H_{10}BrNO_5 \cdot H_2O$ (803.689): C 61.27, H 5.39, N 3.49; found: C 61.09, H 5.36, N 3.52.

The first filtrate was evaporated to give a syrup to which was added H_2O/NH_4OH to pH 9. The mixture was extracted with $CHCl_3$ and the $CHCl_3$ phase washed with brine, dried (Na_2SO_4), and evaporated: mixed bases as a foam (2.5 g). This was dissolved in MeOH (80 ml), and to the stirred, boiling soln. was added a warm soln. of (+)-2′-bromotartranilic acid hydrate [24] (= (+)-2′-BTA·H_2O; 1.67 g, 5.2 mmol) in MeOH (15 ml). The crystals were filtered after standing at r.t. for 1 h. The filter cake was washed with MeOH (2 × 6 ml, 3 ml, r.t.) and Et_2O (2 × 6 ml) to give colorless crystals which were recrystallized once from MeOH: colorless silky crystals of pure **10b**·(+)-2′-BTA·H_2O. M.p. 164–166°. $[\alpha]_D = +38.39$ ($c = 0.54$, DMSO). Anal. calc. for $C_{31}H_{31}NO_4 \cdot C_{10}H_{10}BrNO_5 \cdot H_2O$ (803.689): C 61.27, H 5.39, N 3.49; found: C 61.48, H 5.41, N 3.50.

A suspension of **10a**·(−)-2′-BTA·H_2O (1.45 g, 1.8 mmol) in H_2O/$CHCl_3$ 1:1 (200 ml) was adjusted to pH 9 with conc. NH_4OH. The $CHCl_3$ phase was removed and the aq. phase reextracted with $CHCl_3$ (2 × 50 ml). The combined $CHCl_3$ extracts were washed with brine, dried (Na_2SO_4), and evaporated to give a foam which was crystallized from i-PrOH: 0.77 g (88.8%) of **10a** ((*S*)). Colorless crystals. M.p. 88–90° $[\alpha]_D = -29.33$ ($c = 0.73$, $CHCl_3$). Anal. calc. for $C_{31}H_{31}NO_4$ (481.567): C 77.31, H 6.49, N 2.91; found: C 77.40, H 6.49, N 2.86.

Similarly **10b**·(+)-2′-BTA·H_2O (2.01 g, 2.5 mmol) was converted to 1.14 g (94.7%) of pure **10b** ((*R*)). M.p. 89–90°. $[\alpha]_D = +29.07$ ($c = 0.87$, $CHCl_3$). Anal. calc. for $C_{31}H_{31}NO_4$ (481.567): C 77.31, H 6.49, N 2.91; found: C 77.13, H 6.52, N 2.87.

Method B: a) *Formation of (1*S*)-1-[3′,4′-Bis(benzyloxy) benzyl]-1,2,3,4-tetrahydro-7-hydroxy-6-methoxy-*N*-[(1″*S*)-phenylethyl]isoquinoline-1-carboxamide* (**11a**) *and Its (1*R*,1″*S*)-Diastereoisomer* **11b** *from (±)-***10**. The fumarate of (±)-**10** (410 mg, 0.74 mmol) was dissolved in H_2O, the soln. basified with NH_4OH and the mixture extracted with toluene/i-PrOH 1:1: free base (±)-**10** (270 mg, 0.56 mmol). This material was dissolved in CH_2Cl_2 (10 ml) and treated with 10% (+)-(*R*)-1-phenylethyl isocyanate soln. in CH_2Cl_2 (0.8 ml, 0.54 mmol). The mixture

was stirred at r.t. for 1 h, washed with 1N HCl and H_2O, dried (Na_2SO_2), and evaporated. The residue obtained was purified by flash chromatography (SiO_2, CH_2Cl_2/AcOEt 3:1): **11a**/**11b** (300 mg, 62%). This mixture was separated by prep. TLC on SiO_2 plates (20 × 20 cm) eluting 3 times with solvent *C*, and the clearly separated ureas (UV) were extracted with CH_2Cl_2, affording the more polar **11b** (90 mg, 18.6%) as an oil (CI-MS: 629 ($[M + 1]^+$), 539, 482 (100)) and the less polar **11a** (86 mg, 17.8%) as crystals after crystallization from CH_2Cl_2/(i-Pr)$_2O$, (m.p. 72–73°, CI-MS identical with that of **11b**).

b) *Alcoholysis of* **11a** *and* **11b**. To the less polar **11a** (86 mg, 0.14 mmol) in BuOH (12 ml), Na (34 mg, 0.15 mmol) was added and the mixture refluxed under Ar for 3.5 h. The mixture was evaporated, H_2O and excess NH_4Cl were added, the aq. soln. was extracted with Et_2O/AcOEt (59 ml) and the org. phase dried (Na_2SO_4) and evaporated: 85 mg of an amorphous solid which was directly used for the next step. The material was dissolved in EtOH (5 ml), the soln. rendered acidic by addition of a few drops of 10% aq. HCl soln., $Pd(OH)_2$ catalyst (20 mg) was added and the hydrogenation carried out at r.t. for 3 h. After filtration of the catalyst, the soln. was concentrated to give crystals of **3a**·HCl which were collected by filtration, washed with acetone, and air-dried. M.p. 238–240°. $[\alpha]_D = -14.5$ ($c = 0.24$, MeOH). CI-MS: identical to material prepared by *Method A*[4]).

Alcoholysis of the more polar **11b** as described above for **3a**·HCl gave crystals of **3b**·HCl. M.p. 233–235°. $[\alpha]_D = +15.1$ ($c = 0.25$, MeOH). CI-MS: identical with **3a**·HCl prepared by *Method A*[4]).

Enantiomeric Purity of **10a** *and* **10b**. a) *Reaction of (±)-***10**, **10a**, *or* **10b** *with (−)-(*S*)-1-Phenylethyl Isocyanate: Formation of Diastereoisomers* **11a** *and* **11b**. To a soln. of the appropriate base (48 mg, 0.1 mmol) in $CHCl_3$ (3 ml, hydrochloride-stabilized) was added a soln. of optically pure (−)-(*S*)-1-phenylethyl isocyanate [9] (44 mg, 0.3 mmol) in $CHCl_3$ (3 ml). The mixture was worked up as in *Method B*. TLC (*C*): **11a** and **11b** (higher R_f) clearly separated.

b) *Reaction of (±)-***10**, **10a**, *or* **10b** *with (−)-(1*R*)-Menthyl Chloroformate: (1*R*)-Menthyl (1*S*)-1-[3′,4′-bis(benzyloxy)benzyl]-1,2,3,4-tetrahydro-7-hydroxy-6-methoxyisoquinoline-1-carboxylate* (**12a**) *and Its (1″*R*,1*R*)-Diastereoisomer* (**12b**). To a soln. of the appropriate base (50 mg, 0.1 mmol) in Et_2O (2.5 ml) was added (−)-menthyl chloroformate (0.0225 ml, 0.1 mmol) *via* syringe, followed by 0.03 ml (0.2 mmol) of Et_3N. The mixture was allowed to stir at r.t. for 1 h and then filtered. The filtrate was washed with 5% HCl soln. (5 ml) and H_2O (5 ml), dried (Na_2SO_4), and evaporated and the white foam utilized directly for HPLC. HPLC (hexane/AcOEt 8:2, 1 ml·min^{-1}): **12a**; purity 100.0%, t_R 7.59 min. **12b**; purity > 99.5%, t_R 8.61 min.

*(−)-(*S*)-1-(3′,4′-Dihydroxybenzyl)-1,2,3,4-tetrahydro-7-hydroxy-6-methoxyisoquinoline Hydrochloride* (**3a**·HCl). The soln. of 200 mg (0.42 mmol) of **10a** in EtOH (35 ml) was rendered acidic by addition of a few drops of 5% aq. HCl soln., and $Pd(OH)_2$ catalyst (60 mg) was added. After 2.5 h at r.t. (TLC (*B*) monitoring), the catalyst was filtered off and the soln. evaporated. The colorless oil was crystallized from i-PrOH: 125 mg (86.9%) of **3a**·HCl. Colorless crystals. M.p. 238–240°. $[\alpha]_D = -16.98$ ($c = 0.62$, MeOH). ^{1}H-NMR ((D$_6$)DMSO): 2.86–3.18 (*m*, 6H); 3.75 (*s*, MeO); 4.47 (*t*, 1H); 6.55–6.75 (*m*, 5 arom. H); 8.87–8.94 (*t*, 3H, exchange with D_2O); 8.85–9.00 (br., 1H, exchange with D_2O). CI-MS: 302 ($[M + 1]^+$), 178 (100), 123. Anal. calc. for $C_{17}H_{19}NO_4 \cdot HCl \cdot 0.25\ H_2O$ (342.295): C 59.65, H 6.04, N 4.09; found: C 59.34, H 5.88, N 4.10.

*(+)-(*R*)-1-(3′,4′-Dihydroxybenzyl)-1,2,3,4-tetrahydro-7-hydroxy-6-methoxyisoquinoline Hydrochloride* (**3b**·HCl). As described for **3a**·HCl, **10b** (350 mg, 0.73 mmol) was converted to 217 mg (88.1%) of **3b**·HCl. M.p. 238–240°. $[\alpha]_D = +18.1$ ($c = 0.50$, MeOH). CI-MS and ^{1}H-NMR: identical with **3a**·HCl. Anal. calc. for $C_{17}H_{19}NO_4 \cdot HCl$ (337.79): C 60.44, H 5.97, N 4.15, Cl 10.50; found: C 60.20, H 5.94, N 4.10, Cl 10.41.

*(+)-(*S*)-1-[3′,4′-Bis(benzyloxy)benzyl]-1,2,3,4-tetrahydro-7-hydroxy-6-methoxyisoquinoline-2-carbaldehyde* (**13a**). **10a** (481 mg, 1 mmol) in ethyl formate (60 ml) was refluxed for 24 h under N_2 (TLC (*A*) monitoring). After evaporation, the residue was dissolved in $CHCl_3$. The $CHCl_3$ soln. was washed with 5% aq. HCl soln. and brine, dried (Na_2SO_4), and evaporated to give a foam which was crystallized from i-PrOH: 472 mg (92.7%) of **13a**. Colorless crystals. M.p. 145–146°. $[\alpha]_D = +27.33$ ($c = 0.72$, $CHCl_3$). ^{1}H-NMR ($CDCl_3$): 2.41–3.29 (*m*, 6H); 3.85–3.88 (*d*, MeO); 4.37–4.42 (*m*, 1H); 5.02–5.19 (*m*, 2 PhCH_2); 5.51–5.55 (*d*, exchange with D_2O, OH); 6.65–6.88 (*m*, 5 arom. H); 7.25–7.46 (*m*, 10 arom. H): 7.93– (*s*, CHO); formamide-rotamer ratio 3:2. CI-MS: 510 ($[M + 1]^+$, 100), 206. Anal. calc. for $C_{32}H_{31}NO_5$ (509.577): C 75.42, H 6.13, N 2.75; found: C 75.17, H 6.19, N 2.73.

*(−)-(*R*)-1-[3′,4′-Bis(benzyloxy)benzyl]-1,2,3,4-tetrahydro-7-hydroxy-6-methoxyisoquinoline-2-carbaldehyde* (**13b**). As described above for **13a**, **10b** (600 mg, 1.25 mmol) was converted to 617 mg (97.0%) of **13b**. M.p.

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[4]) The optical isomers **3a,b**·HCl prepared by *Method B*, as judged by their specific optical rotations, were enantiomerically less pure than those prepared *via Method A*. Specific optical rotations of salts in this series of isoquinolines are extremely sensitive to solvent contamination, and it is not easy to get exact rotational values [12].

145–146°. $[\alpha]_D = -26.21$ ($c = 0.73$, $CHCl_3$). CI-MS and ^{1}H-NMR: identical with **13a**. Anal. calc. for $C_{32}H_{31}NO_5$ (509.57): C 75.42, H 6.13, N 2.75; found: C 75.27, H 6.19, N 2.70.

(+)-(S)-1-[3′,4′-Bis(benzyloxy)benzyl]-1,2,3,4-tetrahydro-6-methoxy-2-methylisoquinolin-7-ol Oxalate (**14a**·$C_2H_2O_4$). To a soln. of **13a** (420 mg, 0.83 mmol) in THF (3 ml) was added 1M BH_3 in THF (20 ml) under N_2. When H_2 evolution was nearly complete, the mixture was overnight (TLC (*E*) monitoring), cooled, and treated dropwise with MeOH cautiously. When no more H_2 was evolved, the mixture was rendered acidic (pH 1) with 5% aq. oxalic acid and refluxed (bath temp. 90–95°) for 3.5 h (TLC (*A*) monitoring). The mixture was cooled and evaporated to give a white solid which was dissolved in H_2O and extracted with Et_2O. The H_2O phase was adjusted to pH 9 with conc. NH_4OH and extracted with $CHCl_3$. The combined $CHCl_3$ extracts were washed with brine, dried (Na_2SO_4), and evaporated to give a colorless oil which was treated with oxalic acid (75 mg, 0.83 mmol) in i-PrOH: 420 mg (86.5%) of **14a**·$C_2H_2O_4$. Colorless crystals. M.p. 136–138°. $[\alpha]_D = +86.12$ ($c = 0.73$, MeOH). ^{1}H-NMR ((D$_6$)DMSO): 2.70 (*s*, MeN); 2.82–3.47 (*m*, 6H); 3.75 (*s*, MeO); 3.86 (br., 2H, exchange with D_2O, COOH); 4.02 (br., 1H); 5.03–5.09 (*d*, 2 PhC*H*$_2$); 6.19–7.00 (*m*, 5 arom. H); 7.30–7.75 (*m*, 10 arom. H). CI-MS: 496 ($[M + 1]^+$). Anal. calc. for $C_{32}H_{33}NO_4 \cdot C_2H_2O_4$ (585.627): C 69.73, H 6.02, N 2.39; found: C 69.55, H 6.07, N 2.37.

(−)-(R)-1-[3′,4′-Bis(benzyloxy)benzyl]-1,2,3,4-tetrahydro-6-methoxy-2-methylisoquinolin-7-ol Oxalate (**14b**·$C_2H_2O_4$). As described above for **14a**, **13b** (509 mg, 1 mmol) was converted to 430 mg (73.5%) of **14b**·$C_2H_2O_4$. M.p. 137–138°. $[\alpha]_D = -84.18$ ($c = 0.51$, MeOH). CI-MS and ^{1}H-NMR: identical with **14a**·$C_2H_2O_4$. Anal. calc. for $C_{32}H_{33}NO_4 \cdot C_2H_2O_4$ (585.627): C 69.73, H 6.02, N 2.39; found: C 69.58, H 6.04, N 2.35.

(+)-(S)-1-(3′,4′-Dihydroxybenzyl)-1,2,3,4-tetrahydro-6-methoxy-2-methylisoquinolin-7-ol Hydrochloride (**5a**·HCl). Similarly to the preparation of **3a**·HCl, **14a**·$C_2H_2O_4$ (350 mg, 0.6 mmol) was converted to 159 mg (66.8%) of **5a**·HCl·2.5 H_2O. Colorless crystals (from 2N HCl). M.p. 115–118° (melted and desolvated), > 220° (dec.). $[\alpha]_D = +80.87$ ($c = 0.30$, MeOH). ^{1}H-NMR ((D$_6$)DMSO): 2.74–2.75 (*d*, exchange with D_2O (→ *s*), MeN); 2.80–3.30 (*m*, 6H); 3.75 (*s*, MeO); 4.42–4.43 (*dt*, 1H); 6.08–6.78 (*m*, 5 arom. H); 8.93, 10.12, 10.31 (3*s*, each 1H, exchange with D_2O, 3 OH). CI-MS: 316 ($[M + 1]^+$), 192, 123. Anal. calc. for $C_{18}H_{21}NO_4 \cdot HCl \cdot 2.5\ H_2O$ (396.87): C 54.47, H 6.86, N 3.53; found: C 54.71, H 6.68, N 3.54.

(−)-(R)-1-(3′,4′-Dihydroxybenzyl)-1,2,3,4-tetrahydro-6-methoxy-2-methylisoquinolin-7-ol Hydrochloride (**5b**·HCl). As described above **14b**·$C_2H_2O_4$ (350 mg, 0.6 mmol) was converted to 152 mg (63.8%) of **5b**·HCl·2.5 H_2O. Colorless crystals (from 2N HCl). $[\alpha]_D = -81.27$ ($c = 0.26$, MeOH). M.p., CI-MS, and ^{1}H-NMR: identical with **5a**·HCl·2.5 H_2O. $[\phi]_{max}$: −12000 (290), −32400 (237), −57000 (210): *cf.* (−)-(*R*)-reticuline: −12000 (290), −29200 (238), −93000 (209). Anal. calc. for $C_{18}H_{21}NO_4 \cdot HCl \cdot 2.5\ H_2O$ (396.87): C 54.47, H 6.86, N 3.53; found: C 53.80, H 6.72, N 3.46.

(±)-Orientalines. – *(4′-Hydroxy-3′-methoxyphenyl)-*N*-[2-(4-hydroxy-3-methoxyphenyl)ethyl]acetamide* (**15**). Homovanillic acid (2 g, 11 mmol) and [2-(4-hydroxy-3-methoxyphenyl)ethyl]amine (1.86 g, 11 mmol) were treated as described for **8**. The crude material was crystallized from 50% aq. AcOH: 2.16 g (59.3%) of **15**. Colorless crystals. M.p. 136–137°. ^{1}H-NMR ($CDCl_3$): 2.64–2.68 (*t*, 2H); 3.39–3,46 (*m*, 4H); 3.82–3.82 (*d*, 2 MeO); 5.37, 5.50, 5.60 (3*s*, each 1H, exchange with D_2O, 2 OH, NH); 6.47–6.87 (*m*, 6 arom. H). CI-MS: 332 ($[M + 1]^+$). Anal. calc. for $C_{18}H_{21}NO_5$ (331.357): C 65.24, H 6.39, N 4.23; found: C 65.14, H 6.42, N 4.23.

3,4-Dihydro-1-(4′-hydroxy-3′-methoxybenzyl)-6-methoxyisoquinolin-7-ol (**16**). Prepared from 2 g (6 mmol) of **15** as described for **9**. The crude material was crystallized directly from EtOH to give 2.07 g of **16** as yellow crystals, which were used for the next step without further purification. UV (MeOH): 232, 305, 359. ^{1}H-NMR ((D$_6$)DMSO): 2.94–3.00 (*t*, 2H); 3.76 (*s*, MeO); 3.77–3.83 (*t*, 2H); 3.89 (*s*, MeO); 4.30 (*s*, 2H); 6.73–7.51 (*m*, 5 arom. H); 9.09, 9.73 (2*s*, each 1H, exchanges with D_2O, 2 OH). CI-MS: 314 ($[M + 1]^+$). HR-MS: 313.1299 M^+, $C_{18}H_{19}NO_4$, calc. 313.1314. Oxalate salt **16**·$C_2H_2O_4$ (from i-PrOH): M.p. 101–103°. Anal. calc. for $C_{18}H_{19}NO_4 \cdot 0.5\ C_2H_2O_4 \cdot 1.5\ H_2O$ (385.377): C 59.21, H 6.01, N 3.63; found: C 59.02, H 6.01, N 3.34.

(±)-1,2,3,4-Tetrahydro-1-(4′-hydroxy-3′-methoxybenzyl)-6-methoxyisoquinolin-7-ol (**17**). Prepared from 1.6 g (3.6 mmol) of **16** with $NaBH_4$ in H_2O/MeOH as described for (±)-**10**: 863 mg (75.6%) of **17**. Colorless crystals (recrystallized from i-PrOH). M.p. 162–164°. ^{1}H-NMR ($CDCl_3$): 2.62–3.22 (*m*, 6H); 3.86 (*s*, 2 MeO); 4.04–4.08 (*dd*, 1H); 5.22–5.92 (br., 2H, exchange with D_2O, 2 OH); 6.57–6.88 (*m*, 5 arom. H). CI-MS: 316 ($[M + 1]^+$). Anal. calc. for $C_{18}H_{21}NO_4 \cdot 0.25\ H_2O$ (319.862): C 67.59, H 6.78, N 4.38; found: C 67.72, H 6.75, N 4.37.

(±)-1,2,3,4-Tetrahydro-7-hydroxy-1-(4′-hydroxy-3′-methoxybenzyl)-6-methoxyisoquinoline-2-carbaldehyde (**18**). Prepared from 600 mg (1.9 mmol) of **17** with ethyl formate as described for the preparation of **13a** to give 625 mg (91.0%) of **18**. Colorless crystals (recrystallized from i-PrOH). M.p. 208–209°. ^{1}H-NMR ($CDCl_3$): 2.55–3.47 (*m*, 6H); 3,77–3.89 (*t*, 2 MeO); 4.46–4.53 (*m*, 1H); 5.46–5.55 (*m*, exchange with D_2O, 2 OH); 6.49–6.88 (*m*, 5 arom. H); 7.60, 8.10 (*s*, CHO); formamide-rotamer ratio 1:2. CI-MS: 344 ($[M + 1]^+$). Anal. calc. for $C_{19}H_{21}NO_5 \cdot H_2O$ (361.387): C 63.14, H 6.41, N 3.88; found: C 63.23, H 5.86, N 3.85.

(±)-1,2,3,4-Tetrahydro-1-(4'-hydroxy-3'-methoxybenzyl)-6-methoxy-2-methylisoquinolin-7-ol Perchlorate (= *(±)-Orientaline*[5]); **19**·$HClO_4$). As described for **14a**, 450 mg (1,3 mmol) of **18** were reduced with borane: 501 mg (86.1%) of **19**·$HClO_4 \cdot H_2O$ (crystallized with 60% aq. $HClO_4$ from i-PrOH). M.p. 135–136° ([14]: m.p. 127°). ^{1}H-NMR ((D_6)DMSO): 2.78 (*s*, MeN); 2.84–3.60 (*m*, 6H); 3.71–3.75 (*d*, 2 MeO); 4.53 (*s*, 1H); 6.15–6.79 (*m*, 5 arom. H); 8.95, 9.32, 9.84 (*s*, each 1 H, exchange with D_2O, 2 OH, $HClO_4$). CI-MS: 330 ([M + 1]$^+$). Anal. calc. for $C_{19}H_{23}NO_4 \cdot HClO_4 \cdot H_2O$ (447.86): C 50.95, H 5.85, N 3.13, Cl 7.92; found: C 50.92, H 5.88, N 3.15, Cl 8.00.

Biological Investigations. – *Procedures for Kinetic-Analysis Studies with COMT.* Kinetic analyses of the *O*-methylation of **3a,b** and **5a,b** were performed as described previously [26]. Double reciprocal plots of initial reaction velocity *vs.* substrate concentration were analyzed by a program for non-linear regression analysis (ENZFITTER, *Elsevier-Biosoft*). Kinetic values are reported as the mean ± the standard error of the mean (s.e.m.) for three or more separate determinations.

*Enzymatic Formation of the 3'-*O*- and 4'-*O*-Methyl Ethers.* Compounds **3a,b** and **5a,b** were incubated with COMT under conditions designed to yield maximum product formation. Soln. of the catechols in 0.001N HCl 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 8.5; 20), *S*-adenosyl-L-[methyl-^{14}C]methionine (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 60% CCl_3COOH. Authentic methyl ethers of the appropriate catechols were added, and the precipitated protein was sedimented by centrifugation (5 min, *Fischer* microfuge). Separation of the products was achieved by TLC of 3–5 μl of the supernatant fraction. For the solid phase, 20 × 20 cm plates precoated with silica gel *G 250* with a 3 cm preadsorbant area and scored into 19 lanes 0.9 cm in width were employed (mobile phase: toluene/AcOEt/Et_2NH 7:4:2). The TLC was repeated 2–3 times with the plates air-dried between each run. The ethers were visualized with I_2 vapor.

The extent of *O*-methylation was determined by scanning each lane of a plate with a gas flow (90% Ar, 10% CH) equipped with a detector anode of a C-coated quartz fiber. Counting efficiency for ^{14}C was 15%. On occasion, sections of silica gel were removed and the radioactive content confirmed by suspending the gel in 3 ml of scintillation fluid (*Hadroflor, National Diagnostics,* Manville, N.J.), and measuring the ^{14}C in a *Beckman* scintillation counter, with a counting efficiency of 65%. The R_f values of labeled and unlabeled methyl ethers were identical in each case: reticuline (**6**), 0.35; orientaline (**19**), 0.44; *N*-norreticuline (**4a**), 0.28; *N*-nororientaline (**17**), 0.36; unknown compounds from **3a** and **3b**, 0.59 and 0.83, resp. The authentic standards (+)-(*S*)-reticuline (**6a**) and (−)-(*S*)-*N*-norreticuline (**4a**) yielded a dark brown to black color with I_2 vapor, while authentic orientaline (**19**) and *N*-nororientaline (**17**) gave a bright yellow color.

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