

### 135. Mammalian Alkaloids: Synthesis and *O*-Methylation of (*S*)- and (*R*)-3'-Hydroxycoclaurine and Their *N*-Methylated Analogues with *S*-Adenosyl-L-[methyl-<sup>14</sup>C]methionine in Presence of Mammalian Catechol *O*-Methyltransferase

by Xiao-shu He, Dragana Tadić, Malgorzata Brzostowska, and Arnold Brossi\*

Section on Natural Products, Laboratory of Structural Biology, NIDDK, National Institutes of Health, Bethesda, Maryland 20892, USA

and Maureen Bell and Cyrus Creveling

Laboratory of Bioorganic Chemistry, NIDDK, National Institutes of Health, Bethesda, Maryland 20892, USA

(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-<sup>14</sup>C]methionine in presence of mammalian COMT was investigated *in vitro*. The *N*-unsubstituted (*1S*)- and (*1R*)-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 (*1S*)-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 (*1R*)-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'-bromotartronic 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.

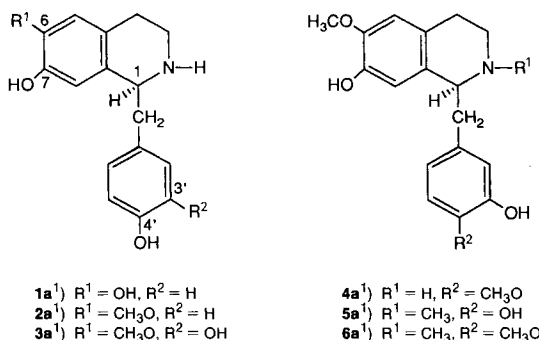
---

**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 benzyl-tetrahydroisoquinolines 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**)<sup>1</sup>. 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].

---

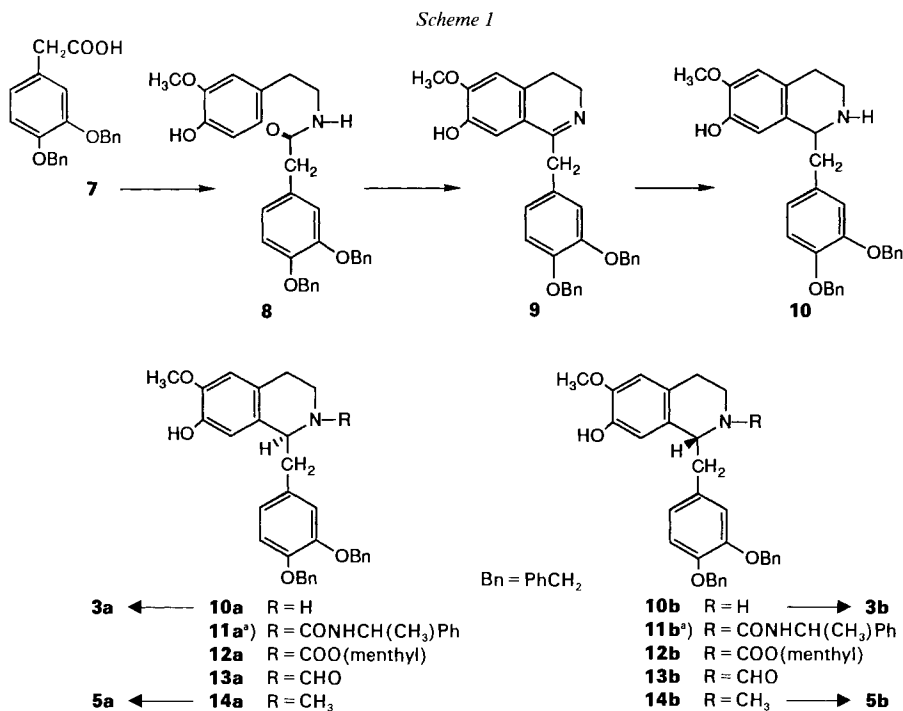
<sup>1</sup>) Throughout this paper, the key numbers of tetrahydroisoquinolines with (*1S*)-configuration are followed by **a**; compounds of the **b** series (not all shown) have (*1R*)-configuration.



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-<sup>14</sup>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-<sup>14</sup>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.

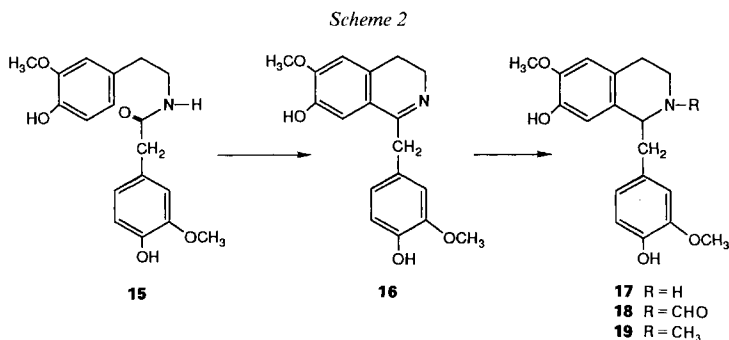
**Chemistry.** – Racemic tetrahydroisoquinoline **10** was prepared from the benzyl-protected phenylacetamide **8** (obtained from **7**) by a *Bischler-Napieralski* cyclization, followed by reduction of imine **9** with NaBH<sub>4</sub> in MeOH (*Scheme 1*). The optical resolution of **10** was accomplished by salt formation with (–)- and (+)-2'-bromotartronic 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)<sub>2</sub> catalyst in EtOH in the



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



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 ( $\pm$ )-*N*-nororientaline [13] (**17**) was prepared from the unprotected phenylacetamide **15** *via* **16** and transformed to ( $\pm$ )-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-<sup>14</sup>C]methionine in the presence of mammalian COMT (see *Exper. Part*). Examination of the <sup>14</sup>C-labeled products from the enzymatic reaction clearly indicated that the *N*-methylcoclaurines **5a,b** yielded a mixture of the two expected <sup>14</sup>C-labeled *O*-methyl ethers reticuline **6** and orientaline **19**. The relative amounts of the 3'-*O*- and 4'-*O*-methyl ethers (*R*)-**19**<sup>2</sup> and **6b**<sup>2</sup>, 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**<sup>2</sup> and **6a**<sup>2</sup>, 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**<sup>2</sup> and **4b**<sup>2</sup>, 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 [%] <sup>a</sup>
( <i>S</i> )-3'-Hydroxycoclaurine ( <b>3a</b> )	( <i>S</i> )- <i>N</i> -norreticuline ( <b>4a</b> ) <sup>2</sup>	31.2 ± 2.8 (10)
	( <i>S</i> )- <i>N</i> -nororientaline (( <i>S</i> )- <b>17</b> ) <sup>2</sup>	45.1 ± 2.2 (10)
	Unknown A	12.2 ± 1.3 (9)
	Unknown B	13.2 ± 1.4 (9)
( <i>R</i> )-3'-Hydroxycoclaurine ( <b>3b</b> )	( <i>R</i> )- <i>N</i> -norreticuline ( <b>4b</b> ) <sup>2</sup>	32.1 ± 2.1 (11)
	( <i>R</i> )- <i>N</i> -nororientaline (( <i>R</i> )- <b>17</b> ) <sup>2</sup>	26.9 ± 2.1 (11)
	Unknown C	29.8 ± 1.9 (10)
	Unknown D	16.1 ± 0.9 (10)
( <i>S</i> )-3'-Hydroxy- <i>N</i> -methylcoclaurine ( <b>5a</b> )	( <i>S</i> )-reticuline ( <b>6a</b> ) <sup>2</sup>	14.4 ± 0.5 (4)
	( <i>S</i> )-orientaline (( <i>S</i> )- <b>19</b> ) <sup>2</sup>	85.7 ± 0.5 (4)
( <i>R</i> )-3'-Hydroxy- <i>N</i> -methylcoclaurine ( <b>5b</b> )	( <i>R</i> )-reticuline ( <b>6b</b> ) <sup>2</sup>	43.7 ± 1.6 (4)
	( <i>R</i> )-orientaline (( <i>R</i> )- <b>19</b> ) <sup>2</sup>	56.0 ± 1.8 (4)

<sup>a</sup>) 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.

<sup>2</sup>) 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**<sup>2</sup> and **4a**<sup>2</sup>, 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<sup>-1</sup> and 0.15 min<sup>-1</sup>, 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<sup>-1</sup> compared to 0.11 min<sup>-1</sup> 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.)<sup>a)</sup>

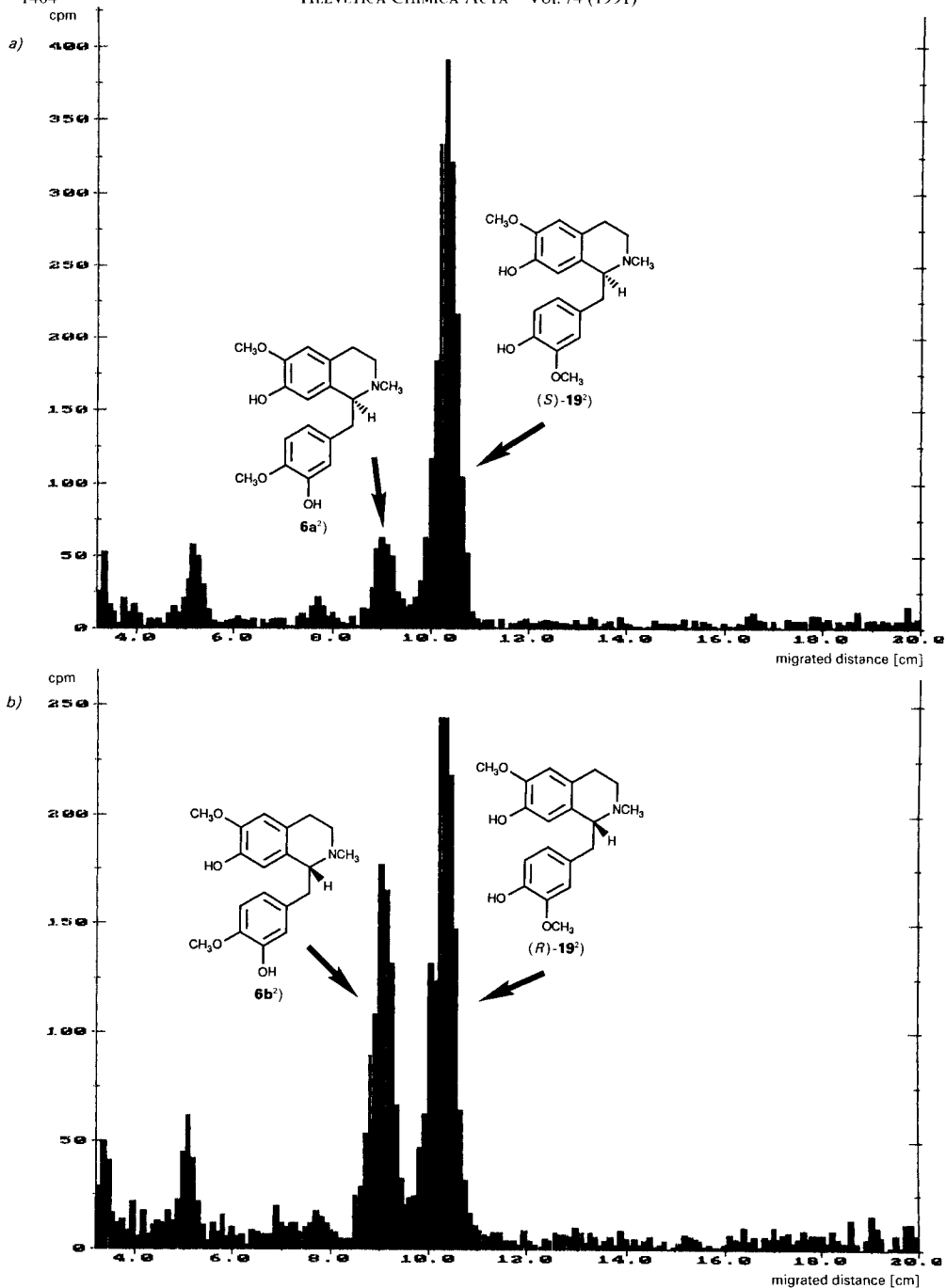
Substrate	$K_m$ [ $\mu\text{M}$ ]	$V_{\max}$ [nmol/min/ mg protein]	$V_{\max}/K_m$ [min <sup>-1</sup> ]
(-)-( <i>S</i> )-3'-Hydroxycoclaurine ( <b>3a</b> )	29 ± 2	5.9 ± 0.5	0.30
(+)-( <i>R</i> )-3'-Hydroxycoclaurine ( <b>3b</b> )	58 ± 4	6.5 ± 0.6	0.11
(+)-( <i>S</i> )-3'-Hydroxy- <i>N</i> -methylcoclaurine ( <b>5a</b> )	23 ± 1	9.3 ± 0.4	0.40
(-)-( <i>R</i> )-3'-Hydroxy- <i>N</i> -methylcoclaurine ( <b>5b</b> )	46 ± 1	8.7 ± 0.6	0.15

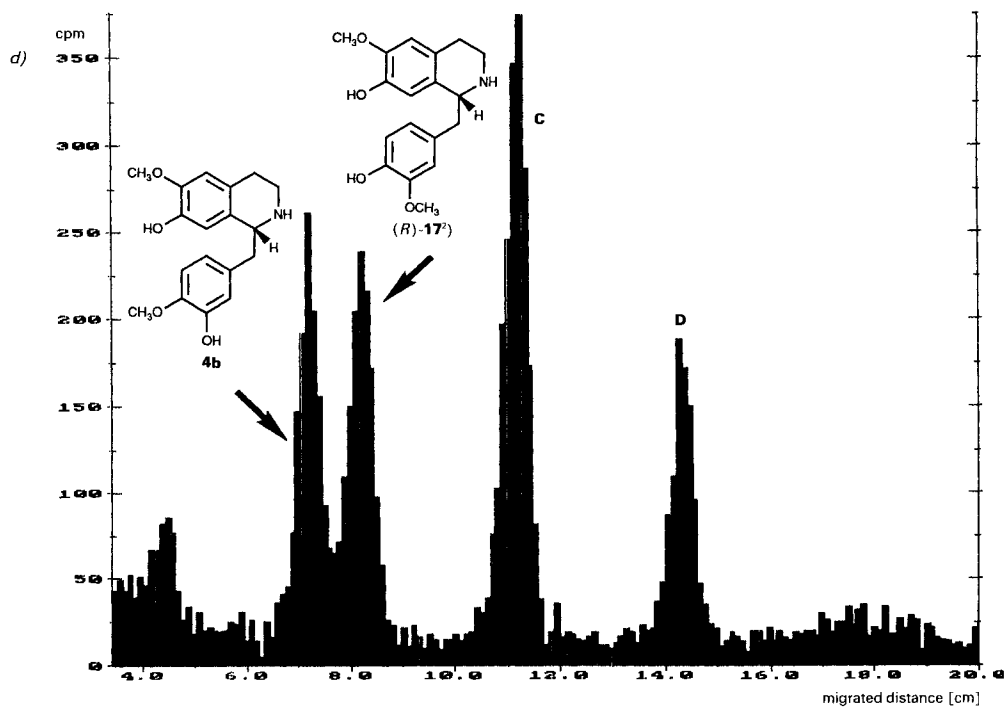
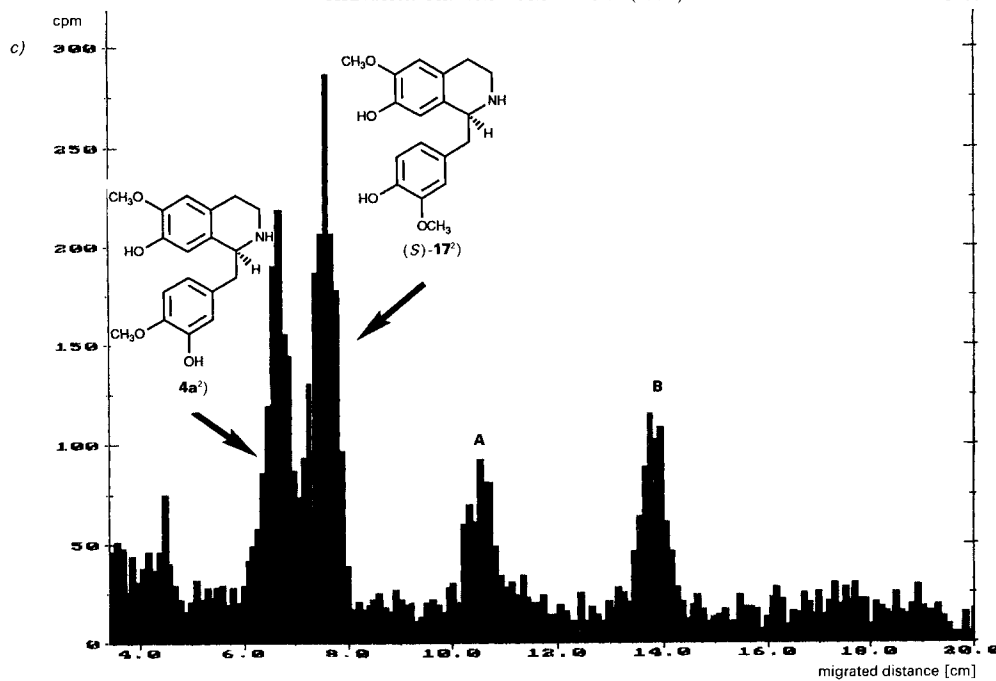
<sup>a)</sup> Values for  $K_m$  and  $V_{\max}$  are the mean ± s.e.m. of three separate determinations.

the (*S*)-antipode **3a** displayed a higher affinity ( $K_m = 29 \mu\text{M}$ ) for the enzyme than the (*R*)-antipode **3b** ( $K_m = 58 \mu\text{M}$ ). 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 benzyloquinoline-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 dehydroreticulium species [17], which is then reduced stereospecifically with a NADPH<sub>2</sub>-dependent enzyme [18]. The formation of 65% of (*S*)-coclaurine<sup>2</sup> (**2a**) and 35% of (*S*)-isococlaurine<sup>3</sup> on *O*-methylation of (*S*)-norcoclaurine (**1a**) with *S*-adenosyl-L-[methyl-<sup>14</sup>C]methionine and mammalian COMT [7] concurs with results obtained with tissue cultures of benzyloquinoline-producing plant species [3] [4]. The *O*-methylation of (*S*)-3'-hydroxy-*N*-methylcoclaurine (**5a**) with *S*-adenosyl-

<sup>3)</sup> Another name of isococlaurine is 1-benzyl-4',6-dihydroxy-7-methoxytetrahydroisoquinoline [19].





<sup>3)</sup> Compounds of the a series have (1*S*)-configuration, compounds of the b series (not shown, C(1)◀H) the (1*R*)-configuration<sup>1</sup>). See Table 1.

L-[methyl-<sup>14</sup>C]methionine and mammalian COMT, affording as reported here predominantly (*S*)-orientaline ((*S*)-**19**)<sup>2</sup>, 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-<sup>14</sup>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-<sup>14</sup>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-<sup>14</sup>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<sub>3</sub>/MeOH/NH<sub>4</sub>OH 90:9:1; *B*, CHCl<sub>3</sub>/MeOH/NH<sub>4</sub>OH 80:18:2; *C*, hexane/AcOEt/(*i*-Pr)<sub>2</sub>O 1:1:1 containing 1% of AcOH; *D*, CHCl<sub>3</sub>; *E*, CHCl<sub>3</sub>/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. <sup>1</sup>H-NMR spectra (300 MHz): *Varian XL-300* spectrometer,  $\delta$  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<sub>2</sub>CO<sub>3</sub> (18 g, 130 mmol) and benzyl chloride (16.1 g, 127 mmol) in DMF (30 ml). The mixture was stirred at 70° under N<sub>2</sub> for 24 h, then cooled to r.t., and poured into H<sub>2</sub>O (1000 ml). The aq. mixture was extracted with Et<sub>2</sub>O. The combined Et<sub>2</sub>O layer was washed with sat. NaHCO<sub>3</sub> soln. and H<sub>2</sub>O, dried (Na<sub>2</sub>SO<sub>4</sub>), 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<sub>2</sub>O (1000 ml) and extracted with Et<sub>2</sub>O to remove benzyl alcohol. The H<sub>2</sub>O 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<sub>2</sub>O, air dried, and crystallized from AcOH/H<sub>2</sub>O: 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<sub>2</sub> over the



melt to sweep out the H<sub>2</sub>O formed. After cooling to r.t., the residue was taken up in CH<sub>2</sub>Cl<sub>2</sub> (TLC (A) monitoring) and the soln. washed with 5% HCl soln., H<sub>2</sub>O, sat. NaHCO<sub>3</sub> soln. and H<sub>2</sub>O, dried (Na<sub>2</sub>SO<sub>4</sub>), and evaporated. The brown oil crystallized from 70% aq. AcOH: 10.1 g (60.4%) of **8**. Off-white crystals. M.p. 106–107°. <sup>1</sup>H-NMR (CDCl<sub>3</sub>): 2.58–2.63 (*t*, 2H); 3.35–3.41 (*m*, 4H); 3.82 (*s*, MeO); 5.11–5.15 (*d*, 2 PhCH<sub>2</sub>); 6.45–6.87 (*m*, 6 arom. H); 7.26–7.47 (*m*, 10 arom. H). CI-MS: 498 ([*M* + 1]<sup>+</sup>). Anal. calc. for C<sub>31</sub>H<sub>31</sub>NO<sub>5</sub> (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<sub>2</sub>H<sub>2</sub>O<sub>4</sub>). To a stirred and refluxing soln. of **8** (9.94 g, 0.02 mol) in MeCN (250 ml) was added dropwise POCl<sub>3</sub> (11 ml, 0.12 mol). The mixture was refluxed for 1 h under N<sub>2</sub>, cooled to r.t., and evaporated. The residue was dissolved in CHCl<sub>3</sub> and the soln. washed with 5% NH<sub>4</sub>OH soln. and H<sub>2</sub>O, dried (Na<sub>2</sub>SO<sub>4</sub>), 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<sub>2</sub>H<sub>2</sub>O<sub>4</sub>. Recrystallization from acetone/*i*-PrOH gave **9**·C<sub>2</sub>H<sub>2</sub>O<sub>4</sub> as yellow crystals (7.5 g, 64.8%). M.p. 186–187°. UV (EtOH): 236, 246 (sh), 308, 367. <sup>1</sup>H-NMR ((D<sub>6</sub>)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<sub>2</sub>); 6.81–7.07 (*m*, 4 arom. H); 7.29–7.42 (*m*, 11 arom. H). CI-MS: 480 ([*M* + 1]<sup>+</sup>). Anal. calc. for C<sub>31</sub>H<sub>29</sub>NO<sub>4</sub>·C<sub>2</sub>H<sub>2</sub>O<sub>4</sub>·0.5 H<sub>2</sub>O (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<sub>2</sub>H<sub>2</sub>O<sub>4</sub> (6.7 g, 11.8 mmol) in MeOH/H<sub>2</sub>O 1:1 (200 ml) was stirred and cooled to 0° under N<sub>2</sub>. Then conc. NH<sub>4</sub>OH soln. (50 ml) was added and after 15 min, NaBH<sub>4</sub> (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<sub>2</sub>O/CHCl<sub>3</sub> 1:1 (400 ml). The CHCl<sub>3</sub> phase was removed and the H<sub>2</sub>O phase reextracted twice with CHCl<sub>3</sub>. The combined CHCl<sub>3</sub> extracts were washed with H<sub>2</sub>O, dried (Na<sub>2</sub>SO<sub>4</sub>), and evaporated to give a white foam which was crystallized from EtOH: crystalline **10** (5.1 g, 89.7%). M.p. 131–132°. <sup>1</sup>H-NMR (CDCl<sub>3</sub>): 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<sub>2</sub>); 6.55–6.90 (*m*, 5 arom. H); 7.26–7.46 (*m*, 10 arom. H). CI-MS 482 ([*M* + 1]<sup>+</sup>), 178. Anal. calc. for C<sub>31</sub>H<sub>31</sub>NO<sub>4</sub> (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<sub>2</sub>H<sub>4</sub>O<sub>4</sub> (from EtOH): M.p. 185–187°. Anal. calc. for C<sub>31</sub>H<sub>31</sub>NO<sub>4</sub>·C<sub>4</sub>H<sub>4</sub>O<sub>4</sub>·0.25 H<sub>2</sub>O (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'-bromotartranic acid hydrate [24] (= (–)-2'-BTA·H<sub>2</sub>O; 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<sub>2</sub>O (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<sub>2</sub>O. M.p. 163–164°. [*α*]<sub>D</sub> = –38.55 (*c* = 0.62, DMSO). Anal. calc. for C<sub>31</sub>H<sub>31</sub>NO<sub>4</sub>·C<sub>10</sub>H<sub>10</sub>BrNO<sub>5</sub>·H<sub>2</sub>O (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<sub>2</sub>O/NH<sub>4</sub>OH to pH 9. The mixture was extracted with CHCl<sub>3</sub> and the CHCl<sub>3</sub> phase washed with brine, dried (Na<sub>2</sub>SO<sub>4</sub>), 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'-bromotartranic acid hydrate [24] (= (+)-2'-BTA·H<sub>2</sub>O; 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<sub>2</sub>O (2 × 6 ml) to give colorless crystals which were recrystallized once from MeOH: colorless silky crystals of pure **10b**·(+)-2'-BTA·H<sub>2</sub>O. M.p. 164–166°. [*α*]<sub>D</sub> = +38.39 (*c* = 0.54, DMSO). Anal. calc. for C<sub>31</sub>H<sub>31</sub>NO<sub>4</sub>·C<sub>10</sub>H<sub>10</sub>BrNO<sub>5</sub>·H<sub>2</sub>O (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<sub>2</sub>O (1.45 g, 1.8 mmol) in H<sub>2</sub>O/CHCl<sub>3</sub> 1:1 (200 ml) was adjusted to pH 9 with conc. NH<sub>4</sub>OH. The CHCl<sub>3</sub> phase was removed and the aq. phase reextracted with CHCl<sub>3</sub> (2 × 50 ml). The combined CHCl<sub>3</sub> extracts were washed with brine, dried (Na<sub>2</sub>SO<sub>4</sub>), 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° [*α*]<sub>D</sub> = –29.33 (*c* = 0.73, CHCl<sub>3</sub>). Anal. calc. for C<sub>31</sub>H<sub>31</sub>NO<sub>4</sub> (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<sub>2</sub>O (2.01 g, 2.5 mmol) was converted to 1.14 g (94.7%) of pure **10b** ((*R*)). M.p. 89–90°. [*α*]<sub>D</sub> = +29.07 (*c* = 0.87, CHCl<sub>3</sub>). Anal. calc. for C<sub>31</sub>H<sub>31</sub>NO<sub>4</sub> (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 (1S)-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 (1R,1''S)-Diastereoisomer 11b from (±)-10.* The fumarate of (±)-**10** (410 mg, 0.74 mmol) was dissolved in H<sub>2</sub>O, the soln. basified with NH<sub>4</sub>OH and the mixture extracted with toluene/*i*-PrOH 1:1: free base (±)-**10** (270 mg, 0.56 mmol). This material was dissolved in CH<sub>2</sub>Cl<sub>2</sub> (10 ml) and treated with 10% (+)-(*R*)-1-phenylethyl isocyanate soln. in CH<sub>2</sub>Cl<sub>2</sub> (0.8 ml, 0.54 mmol). The mixture

was stirred at r.t. for 1 h, washed with 1N HCl and H<sub>2</sub>O, dried (Na<sub>2</sub>SO<sub>2</sub>), and evaporated. The residue obtained was purified by flash chromatography (SiO<sub>2</sub>, CH<sub>2</sub>Cl<sub>2</sub>/AcOEt 3:1): **11a/11b** (300 mg, 62%). This mixture was separated by prep. TLC on SiO<sub>2</sub> plates (20 × 20 cm) eluting 3 times with solvent *C*, and the clearly separated ureas (UV) were extracted with CH<sub>2</sub>Cl<sub>2</sub>, affording the more polar **11b** (90 mg, 18.6%) as an oil (CI-MS: 629 ([*M* + 1]<sup>+</sup>), 539, 482 (100)) and the less polar **11a** (86 mg, 17.8%) as crystals after crystallization from CH<sub>2</sub>Cl<sub>2</sub>/(i-Pr)<sub>2</sub>O, (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<sub>2</sub>O and excess NH<sub>4</sub>Cl were added, the aq. soln. was extracted with Et<sub>3</sub>O/AcOEt (59 ml) and the org. phase dried (Na<sub>2</sub>SO<sub>4</sub>) 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)<sub>2</sub> 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°. [ $\alpha$ ]<sub>D</sub> = –14.5 (*c* = 0.24, MeOH). CI-MS: identical to material prepared by *Method A*<sup>4</sup>).

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

*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<sub>3</sub> (3 ml, hydrochloride-stabilized) was added a soln. of optically pure (–)-(S)-1-phenylethyl isocyanate [9] (44 mg, 0.3 mmol) in CHCl<sub>3</sub> (3 ml). The mixture was worked up as in *Method B*. TLC (*C*): **11a** and **11b** (higher *R<sub>f</sub>*) clearly separated.

b) *Reaction of (±)-10, 10a, or 10b with (–)-(1R)-Menthyl Chloroformate: (1R)-Menthyl (1S)-1-[3',4'-bis(benzyloxy)benzyl]-1,2,3,4-tetrahydro-7-hydroxy-6-methoxyisoquinoline-1-carboxylate (12a) and Its (1' R,1R)-Diastereoisomer (12b)*. To a soln. of the appropriate base (50 mg, 0.1 mmol) in Et<sub>2</sub>O (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<sub>3</sub>N. 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<sub>2</sub>O (5 ml), dried (Na<sub>2</sub>SO<sub>4</sub>), and evaporated and the white foam utilized directly for HPLC. HPLC (hexane/AcOEt 8:2, 1 ml·min<sup>–1</sup>): **12a**; purity 100.0%, *t<sub>R</sub>* 7.59 min. **12b**; purity > 99.5%, *t<sub>R</sub>* 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)<sub>2</sub> 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°. [ $\alpha$ ]<sub>D</sub> = –16.98 (*c* = 0.62, MeOH). <sup>1</sup>H-NMR ((D<sub>2</sub>)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<sub>2</sub>O); 8.85–9.00 (*br.*, 1H, exchange with D<sub>2</sub>O). CI-MS: 302 ([*M* + 1]<sup>+</sup>), 178 (100), 123. Anal. calc. for C<sub>17</sub>H<sub>19</sub>NO<sub>4</sub>·HCl·0.25 H<sub>2</sub>O (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°. [ $\alpha$ ]<sub>D</sub> = +18.1 (*c* = 0.50, MeOH). CI-MS and <sup>1</sup>H-NMR: identical with **3a**·HCl. Anal. calc. for C<sub>17</sub>H<sub>19</sub>NO<sub>4</sub>·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<sub>2</sub> (TLC (*A*) monitoring). After evaporation, the residue was dissolved in CHCl<sub>3</sub>. The CHCl<sub>3</sub> soln. was washed with 5% aq. HCl soln. and brine, dried (Na<sub>2</sub>SO<sub>4</sub>), and evaporated to give a foam which was crystallized from i-PrOH: 472 mg (92.7%) of **13a**. Colorless crystals. M.p. 145–146°. [ $\alpha$ ]<sub>D</sub> = +27.33 (*c* = 0.72, CHCl<sub>3</sub>). <sup>1</sup>H-NMR (CDCl<sub>3</sub>): 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<sub>2</sub>); 5.51–5.55 (*d*, exchange with D<sub>2</sub>O, 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]<sup>+</sup>, 100), 206. Anal. calc. for C<sub>32</sub>H<sub>31</sub>NO<sub>5</sub> (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.

<sup>4</sup>) 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°.  $[\alpha]_D = -26.21$  ( $c = 0.73$ ,  $\text{CHCl}_3$ ). CI-MS and  $^1\text{H-NMR}$ : identical with **13a**. Anal. calc. for  $\text{C}_{32}\text{H}_{31}\text{NO}_5$  (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**· $\text{C}_2\text{H}_2\text{O}_4$ ). To a soln. of **13a** (420 mg, 0.83 mmol) in THF (3 ml) was added 1M  $\text{BH}_3$  in THF (20 ml) under  $\text{N}_2$ . When  $\text{H}_2$  evolution was nearly complete, the mixture was overnight (TLC (*E*) monitoring), cooled, and treated dropwise with MeOH cautiously. When no more  $\text{H}_2$  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  $\text{H}_2\text{O}$  and extracted with  $\text{Et}_2\text{O}$ . The  $\text{H}_2\text{O}$  phase was adjusted to pH 9 with conc.  $\text{NH}_4\text{OH}$  and extracted with  $\text{CHCl}_3$ . The combined  $\text{CHCl}_3$  extracts were washed with brine, dried ( $\text{Na}_2\text{SO}_4$ ), 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**· $\text{C}_2\text{H}_2\text{O}_4$ . Colorless crystals. M.p. 136–138°.  $[\alpha]_D = +86.12$  ( $c = 0.73$ , MeOH).  $^1\text{H-NMR}$  ( $(\text{D}_6)$ DMSO): 2.70 (*s*, MeN); 2.82–3.47 (*m*, 6H); 3.75 (*s*, MeO); 3.86 (*br.*, 2H, exchange with  $\text{D}_2\text{O}$ , COOH); 4.02 (*br.*, 1H); 5.03–5.09 (*d*, 2 PhCH<sub>2</sub>); 6.19–7.00 (*m*, 5 arom. H); 7.30–7.75 (*m*, 10 arom. H). CI-MS: 496 ( $[M + 1]^+$ ). Anal. calc. for  $\text{C}_{32}\text{H}_{33}\text{NO}_4$ · $\text{C}_2\text{H}_2\text{O}_4$  (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**· $\text{C}_2\text{H}_2\text{O}_4$ ). As described above for **14a**, **13b** (509 mg, 1 mmol) was converted to 430 mg (73.5%) of **14b**· $\text{C}_2\text{H}_2\text{O}_4$ . M.p. 137–138°.  $[\alpha]_D = -84.18$  ( $c = 0.51$ , MeOH). CI-MS and  $^1\text{H-NMR}$ : identical with **14a**· $\text{C}_2\text{H}_2\text{O}_4$ . Anal. calc. for  $\text{C}_{32}\text{H}_{33}\text{NO}_4$ · $\text{C}_2\text{H}_2\text{O}_4$  (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**· $\text{C}_2\text{H}_2\text{O}_4$  (350 mg, 0.6 mmol) was converted to 159 mg (66.8%) of **5a**·HCl·2.5  $\text{H}_2\text{O}$ . Colorless crystals (from 2N HCl). M.p. 115–118° (melted and desolvated), > 220° (dec.).  $[\alpha]_D = +80.87$  ( $c = 0.30$ , MeOH).  $^1\text{H-NMR}$  ( $(\text{D}_6)$ DMSO): 2.74–2.75 (*d*, exchange with  $\text{D}_2\text{O}$  ( $\rightarrow$  *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  $\text{D}_2\text{O}$ , 3 OH). CI-MS: 316 ( $[M + 1]^+$ ), 192, 123. Anal. calc. for  $\text{C}_{18}\text{H}_{21}\text{NO}_4$ ·HCl·2.5  $\text{H}_2\text{O}$  (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**· $\text{C}_2\text{H}_2\text{O}_4$  (350 mg, 0.6 mmol) was converted to 152 mg (63.8%) of **5b**·HCl·2.5  $\text{H}_2\text{O}$ . Colorless crystals (from 2N HCl).  $[\alpha]_D = -81.27$  ( $c = 0.26$ , MeOH). M.p., CI-MS, and  $^1\text{H-NMR}$ : identical with **5a**·HCl·2.5  $\text{H}_2\text{O}$ .  $[\phi]_{\text{max}}$ : -12000 (290), -32400 (237), -57000 (210); cf. (-)-(*R*)-reticuline: -12000 (290), -29200 (238), -93000 (209). Anal. calc. for  $\text{C}_{18}\text{H}_{21}\text{NO}_4$ ·HCl·2.5  $\text{H}_2\text{O}$  (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°.  $^1\text{H-NMR}$  ( $\text{CDCl}_3$ ): 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  $\text{D}_2\text{O}$ , 2 OH, NH); 6.47–6.87 (*m*, 6 arom. H). CI-MS: 332 ( $[M + 1]^+$ ). Anal. calc. for  $\text{C}_{18}\text{H}_{21}\text{NO}_5$  (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.  $^1\text{H-NMR}$  ( $(\text{D}_6)$ 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  $\text{D}_2\text{O}$ , 2 OH). CI-MS: 314 ( $[M + 1]^+$ ). HR-MS: 313.1299  $M^+$ ,  $\text{C}_{18}\text{H}_{19}\text{NO}_4$ , calc. 313.1314. Oxalate salt **16**· $\text{C}_2\text{H}_2\text{O}_4$  (from *i*-PrOH): M.p. 101–103°. Anal. calc. for  $\text{C}_{18}\text{H}_{19}\text{NO}_4$ ·0.5  $\text{C}_2\text{H}_2\text{O}_4$ ·1.5  $\text{H}_2\text{O}$  (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  $\text{NaBH}_4$  in  $\text{H}_2\text{O}$ /MeOH as described for (±)-**10**: 863 mg (75.6%) of **17**. Colorless crystals (recrystallized from *i*-PrOH). M.p. 162–164°.  $^1\text{H-NMR}$  ( $\text{CDCl}_3$ ): 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  $\text{D}_2\text{O}$ , 2 OH); 6.57–6.88 (*m*, 5 arom. H). CI-MS: 316 ( $[M + 1]^+$ ). Anal. calc. for  $\text{C}_{18}\text{H}_{21}\text{NO}_4$ ·0.25  $\text{H}_2\text{O}$  (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°.  $^1\text{H-NMR}$  ( $\text{CDCl}_3$ ): 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  $\text{D}_2\text{O}$ , 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  $\text{C}_{19}\text{H}_{21}\text{NO}_5$ · $\text{H}_2\text{O}$  (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<sup>5</sup>); **19**·HClO<sub>4</sub>. As described for **14a**, 450 mg (1,3 mmol) of **18** were reduced with borane: 501 mg (86.1%) of **19**·HClO<sub>4</sub>·H<sub>2</sub>O (crystallized with 60% aq. HClO<sub>4</sub> from i-PrOH). M.p. 135–136° ([14]: m.p. 127°). <sup>1</sup>H-NMR ((D<sub>6</sub>)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 1H, exchange with D<sub>2</sub>O, 2 OH, HClO<sub>4</sub>). CI-MS: 330 ([M + 1]<sup>+</sup>). Anal. calc. for C<sub>19</sub>H<sub>23</sub>NO<sub>4</sub>·HClO<sub>4</sub>·H<sub>2</sub>O (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<sub>2</sub> (1.2), dithiothreitol (4), *S*-adenosyl-L-methionine (0.05), Tris buffer (pH 8.5; 20), *S*-adenosyl-L-[methyl-<sup>14</sup>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<sub>3</sub>COOH. 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<sub>2</sub>NH 7:4:2). The TLC was repeated 2–3 times with the plates air-dried between each run. The ethers were visualized with I<sub>2</sub> 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 <sup>14</sup>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 <sup>14</sup>C in a Beckman scintillation counter, with a counting efficiency of 65%. The R<sub>f</sub> 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<sub>2</sub> vapor, while authentic orientaline (**19**) and *N*-nororientaline (**17**) gave a bright yellow color.

#### REFERENCES

- [1] E. Brochmann-Hanssen, 'The Chemistry and Biology of Isoquinoline Alkaloids', Eds. J. D. Phillipson, M. F. Roberts, and M. H. Zenk, Springer Verlag, New York, 1985, pp. 229–239.
- [2] M. H. Zenk, R. Gerardy, R. Stadler, *J. Chem. Soc., Chem. Commun.* **1989**, 1725, and ref. cit. therein.
- [3] R. Stadler, M. H. Zenk, *Liebigs Ann. Chem.* **1990**, 555.
- [4] R. Stadler, T. M. Kutchan, S. Loeffler, N. Nagakura, B. Cassels, M. H. Zenk, *Tetrahedron Lett.* **1987**, 28, 1251.
- [5] H. W. Kosterlitz, *Nature (London)* **1987**, 330, 606, and ref. cit. therein.
- [6] K. Matsubara, S. Fukushima, T. Shikami, A. Akane, M. Hasegawa and H. Shino, *Jpn. J. Tox. Environ. Health* **1989**, 35, 354.
- [7] Y. Sekine, C. Creveling, N. Bell, A. Brossi, *Helv. Chim. Acta* **1990**, 73, 426.
- [8] K. C. Rice, A. Brossi, *J. Org. Chem.* **1980**, 45, 592.
- [9] B. Schönenberger, A. Brossi, *Helv. Chim. Acta* **1986**, 69, 1486.
- [10] A. Brossi, K. C. Rice, C. P. Mak, J. Reden, Y. Nimikitaisan, P. Skolnick, J. Daly, *J. Med. Chem.* **1980**, 23, 648.
- [11] J. C. Craig, S. K. Roy, *Tetrahedron* **1965**, 21, 401.
- [12] M. Chrzanowska, B. Schönenberger, A. Brossi, *Helv. Chim. Acta* **1987**, 70, 1721.
- [13] M. Tomita, J. Kunitomo, *J. Pharm. Soc. Jpn.* **1960**, 80, 1238.
- [14] J. Kunitomo, *J. Pharm. Soc. Jpn.* **1961**, 81, 1253.

<sup>5</sup>) Another name for orientaline is 4',7-dihydroxy-3',6-dimethoxy-*N*-methyltetrahydroisoquinoline [25].

- [15] S. Loeffler, M. H. Zenk, *Phytochemistry* **1990**, *29*, 3499.
- [16] T. Frenzel, M. H. Zenk, *ibid.* **1990**, *29*, 3505.
- [17] S. Loeffler, R. Stadler, M. H. Zenk, *Tetrahedron Lett.* **1990**, *31*, 4853.
- [18] W. De-Eknamul, M. H. Zenk, *Tetrahedron Lett.* **1990**, *31*, 4855.
- [19] I. W. Southon, J. Buckingham, in 'Dictionary of Alkaloids', Chapman and Hall, New York, 1989, pp. 578–579.
- [20] M. Amann, M. H. Zenk, *Phytochemistry* **1987**, *26*, 3235.
- [21] C. K. Wat, P. Steffens, M. H. Zenk, *Z. Naturforsch., C* **1968**, *41*, 210.
- [22] C. J. Weitz, K. F. Faull, A. Goldstein, *Nature (London)* **1987**, *330*, 676.
- [23] C. Schöpf, H. Perrey, I. Jackh, *Liebigs Ann. Chem.* **1932**, *497*, 47.
- [24] T. A. Montzka, R. T. L. Pindell, J. D. Matisckella, *J. Org. Chem.* **1968**, *33*, 3993.
- [25] I. W. Southon, J. Buckingham, in 'Dictionary of Alkaloids', Chapman and Hall, New York, 1989, p. 795.
- [26] M. D. Rozwadowska, M. Chrzanowska, A. Brossi, C. R. Creveling, M. E. Bembenek, C. W. Abell, *Helv. Chim. Acta* **1988**, *71*, 1598.