# 173. Mammalian Alkaloids: *O*-Methylation of (*S*)- and (*R*)-Dideoxynorlaudanosoline-1-carboxylic Acid by Catechol *O*-Methyltransferase and Identification of a Yellow Pigment Obtained at Physiological pH

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*O*-Methylation of optically active 3′,4′-dideoxynorlaudanosoline-1-carboxylic acids **1** with *O*-methyltransferase *in vitro* afforded almost exclusively the 7-*O*-methylated acids **3**. A similar result was obtained with the yellow quinonemethide **4A** obtained from **1** at neutral or slightly alkaline pH by oxidative decarboxylation and affording the 3,4-dihydroisoquinoline **15** on methylation with catechol *O*-methyltransferase (COMT). The structure of quinonemethide **4A** was determined on the basis of spectral data, by its conversion into isoquinolines of established structure, and by synthesis. Quinonemethide **4A** was found to be a weak inhibitor of monoamine oxidase A (MAO A) but not a substrate. Nonenzymatic oxidative decarboxylation of dopamine-derived tetrahydroisoquinoline-1-carboxylic acids to quinonemethides may be a major factor in biochemical experimentation and should be considered in the interpretation of data.

**Introduction.** – Mammalian alkaloids are formed in mammals under physiological conditions apparently by *Pictet-Spengler* cyclization of catecholamines (*e.g.* dopamine and norepinephrine) or indolethylamines (*e.g.* tryptamine and 5-hydroxytryptamine) with α-keto analogs of aromatic amino acids or aldehydes [1–8]. There is substantial evidence that some 1,2,3,4-tetrahydroisoquinolines, 1,2,3,4-tetrahydroisoquinoline-1-carboxylic acids, and β-carbolines, prepared by *Hahn et al.* more than 30 years ago [9] [10], are present in small amounts *in vivo* and occur at increased levels in certain pathological conditions [11]. Tetrahydroisoquinoline-1-carboxylic acid **1**, which is found in higher concentrations in phenylketonurics than in healthy subjects [11], has been thought to contribute to the neurological changes manifested in this disease [12] [13]. Acid **1** was originally prepared by *Hahn et al.* as a racemic mixture [9], but its optical isomers have recently been obtained [14]. Since optically active β-carbolines have been reported to occur in mammalian tissues [15] [16] and optically active 1-benzyl-tetrahydroisoquinolines were found to be highly potent in adrenergic and dopaminergic receptor binding assays [17], we chose to investigate the optical isomers of acid **1** to determine whether they would behave differently in biochemical assays and provide data which would ultimately clarify the origin of the mammalian material. *Bobbitt* [18] reported that 6-hydroxy-tetrahydroisoquinoline-1-carbroxylic acids spontaneously decarboxylate at pH 7 or above in

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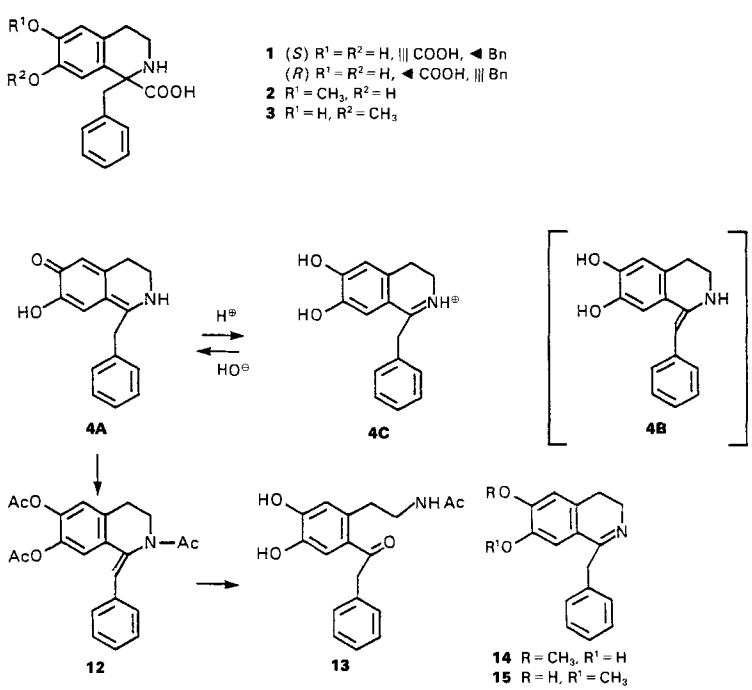
the presence of air to 3,4-dihydroisoquinolines. This finding suggests that the optically active tetrahydroisoquinoline-1-carboxylic acids are not converted into optically active tetrahydroisoquinolines primarily by enzymic decarboxylation.

Enzymatic methylation of (±)-salsolinol-1-carboxylic acid (1-methyl analog of **1**) has been examined, and the 7-methyl ether analog was found as the exclusive *O*-methyl metabolite [19]. However, this high regiospecificity in *O*-methylation of a tetrahydroisoquinoline-1-carboxylic acid with catechol *O*-methyltransferase (COMT) contrasts with findings reported on optically active tetrahydropapaverolines lacking the 1-carboxy group [20] which afforded several *O*-methylated products [21].

In this study, we examined the capacity of optically active **1** and of quinonemethide **4A** obtained by oxidative decarboxylation of **1** to serve as substrates for methylation by COMT and as inhibitors of monoamine oxidase A and B (MAO A and B). *O*-Methylation of (*R*)- or (*S*)-**1** with COMT at pH 7–9 was always accompanied by formation of a yellow pigment, identical with material formed from **1** upon standing in hydrogencarbonate solution. This yellow crystalline pigment was fully characterized by spectral data and identified as the isoquinoline quinonemethide **4A**. Quinonemethide **4A** is probably identical with material reported previously [22], but it was not characterized sufficiently for definitive identification. The existence of similar quinonemethides, but not isolated as such [18] [23] [24], support the notion that phenolic 3,4-dihydroisoquinolines and their quinonemethide tautomers belong to a relatively unknown class of isoquinolines which only in few instances were properly characterized [25]. Chemical conversions of **4A** fully supported its structure.

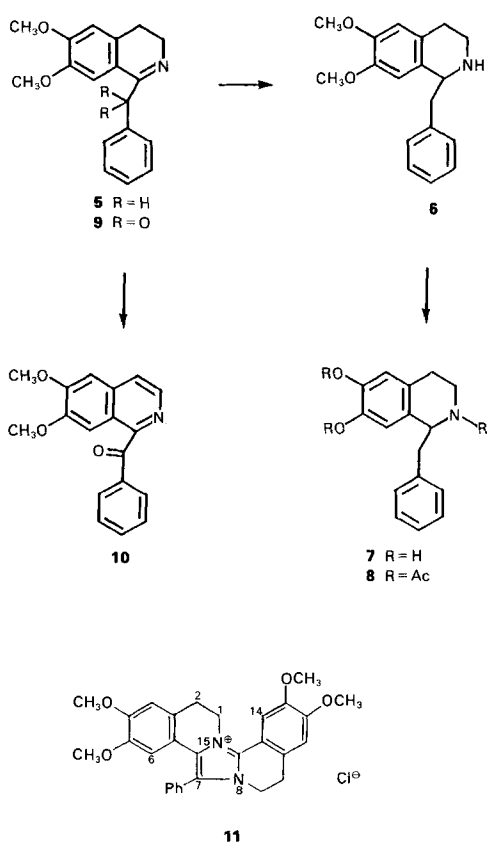
*Scheme 1*



**Chemistry.** – Racemic and optically active **1** [9] [14], (±)-6-methyl ether **2** [24], and (±)-7-methyl ether **3** [23] *(Scheme 1)* were prepared by published procedures. Products of the oxidative decarboxylation were easily recognized by their yellow color showing a characteristic UV maximum at 408 nm, which shifted to 367 nm on addition of 0.1N HCl; this is typical for isoquinoline quinonemethides of structure **4A** when converted with acid into iminium salts **4C** [25] [26] *(Scheme 1)*. The $^1$H-NMR spectrum of **4A** (obtained from **1**) in pyridine showed the benzylic protons at 4.15 ppm, exchanging rapidly with 2 D-atoms when $D_2O$ was added. This suggests that **4A** in protic solvents is in rapid equilibrium with the benzylidene tautomer **4B** *(Scheme 1)*. A fumarate salt **4A**·$C_4H_4O_4$ (of structure **4C**) was obtained when **1** was left standing in $NaHCO_3$ solution for 48 h and after usual workup and treatment of the extract with ethanolic fumaric-acid solution. The fumarate **4C** showed UV maxima (0.1N HCl/EtOH) at 206, 250, 314, and 357 nm and was found to be identical in every respect with a sample prepared from the known dihydroisoquinoline **5** [26–28] (see below *Scheme 2*) by demethylation with refluxing 48% HBr solution and conversion of the hydrobromide into the fumarate.

Structure **4A** was also confirmed by the transformations shown in *Scheme 2:* Reduction of **4A**·HBr with $NaBH_4$ afforded tetrahydroisoquinoline **7**, from which triacetate **8**

*Scheme 2*







**11**

was obtained after acetylation with $Ac_2O$ in pyridine. Compounds **7** and **8** also were obtained from **5** after $NaBH_4$ reduction to **6**, demethylation with 48% HBr to **7**, and acetylation to **8**.

Benzoyl derivative **9** [29–33] was prepared from **5** by a published procedure [31] and converted by dehydrogenation over Pd/C in refluxing toluene [34] into isoquinoline **10**. Imidazolinium chloride **11** *(Scheme 2)*, quantitatively obtained from **9** in 0.5N HCl after removal of benzoic acid formed in the dimerization reaction, showed the expected spectral data (MS, $^1$H-NMR; *cf.* [35]).

In addition, triacetate **12** *(Scheme 1)* prepared from **4C** (HBr) with $Ac_2O$/pyridine, probably a mixture of (*E*/*Z*) isomers and rotamers as shown by its complex $^1$H-NMR spectrum [36], has a UV maximum at 289 nm which is typical for a stilbene chromophore [37]. This was confirmed by heating **12** with 5% aq. HCl solution to afford deoxybenzoin **13** [38].

The two 3,4-dihydroisoquinolines **14** [39] and **15** [40] *(Scheme 1)* needed for the identification of products obtained in the *O*-methylation of **4A** by COMT were prepared by the published procedures. For the preparation of **15** (only its parent benzyl ether is described [40]), the *Bischler-Napieralski* cyclization of phenacylhomovanillylamide was preferred. Dihydroisoquinoline **15** was characterized by its spectra, and the UV showed the expected shifts of maxima when measured in acidic and alkaline solution.

**Biochemical Methods.** – *Isolation and Purification of Monoamine Oxidase A and B.* Purification of the enzymes used in these studies was performed by previously established methods [41] [42]. Preparations of MAO A were routinely isolated from mitochondria of 6–8 placenta according to *Weyler* and *Salach* [42]. SDS (sodium decyl sulfate)-gel analysis of the enzyme preparation showed a major band (judged to represent 90% or more of the stained proteins) of *ca.* 60000 daltons. MAO A was stored in 50% glycerol, 50 mM $Na_3PO_4$, pH 7.6, and 1 mM 2-mercaptoethanol at −100°.

MAO B was isolated from human autopsy liver using a monoclonal antibody *(MAO B-1C2)* that recognizes MAO B but not MAO A, by the method of *Patel et al.* [41]. The catalytically active enzyme-monoclonal antibody complex was stored at −20° in 50% glycerol, 50 mM $Na_3PO_4$, pH 7.6, and 0.1 mM DTT (dithiothreitol).

*Inhibition Studies of MAO by Yellow Quinonemethide* **4A**. The fumarate **4C** of yellow quinonemethide **4A** was dissolved in $H_2O$ to a final concentration of 80 mM, and UV-spectral analysis was performed on a *Beckman DU7-HS* spectrophotometer. A 0.1 mM soln. of the compound displayed no spectral changes when incubated in 50 mM $Na_3PO_4$, pH 7.6, at 30° for 1 h.

The inhibition constant of the yellow quinonemethide **4A** *vs.* MAO A was obtained by kinetic analysis. Briefly, MAO A was incubated in the presence or absence of the compound along with six concentrations (in duplicate) of the substrate kynuramine. Initial rates of oxidation of kynuramine were measured by following the change in absorption at 314 nm at 30° for 3 min. Double reciprocal plots of the kinetic analysis were then compared both in the absence and presence of the yellow quinonemethide **4A** (*ca.* 0.5 $K_i$–5 $K_i$). The type of inhibition was ascertained from the series of curves and then replotted as the slope of the line *vs.* the concentration of inhibitor to obtain the $K_i$.

The rate of oxidation of 0.1 mM kynuramine was assessed for each enzyme by monitoring the change in absorption at 314 nm. MAO A but not MAO B was inhibited *ca.* 30% by yellow quinonemethide **4A**. Incubation of 1 mM **4A** with either MAO A or B

did not produce UV-spectral changes of the compound, suggesting that this compound is not a substrate for MAO. MAO A, but not MAO B, showed decreases in the oxidation rate of kynuramine when incubated in the presence of **4A**. This compound gave weak mixed inhibition with an apparent $K_i$ of *ca.* 0.22 mM. Under similar conditions, the optically active tetrahydroisoquinolinecarboxylic acid **1** did not produce any UV-spectral changes when incubated with MAO A and B preparations.

*Purification of Catechol* O-*Methyltransferase (COMT).* Purification of COMT used in these studies was performed by previously established methods [43] [44]. Preparations of the soluble form of COMT were routinely obtained from the livers of male *Sprague-Dawley* rats and carried through the calcium phosphate gel step of the purification procedure [43] [44] to yield a preparation with a specific activity of 1–2 mol/mg protein/min (substrate, 3,4-dihydroxybenzoic acid). SDS analysis of the enzyme preparation indicated the 23000-dalton protein represented 85% of the *Coumassie*-blue-positive proteins.

*Procedures for Kinetic-Analysis Studies with COMT.* Kinetic analysis of the *O*-methylation of the racemic and optically active forms of **1** were performed.

Aliquots from 20 mM stock solns. of **1** in 2% MeOH to yield final concentrations ranging from 0.02 to 2.0 mM were added to an incubation mixture containing the following components (mM): $MgCl_2$ (1.2), dithiothreitol (4), *S*-adenosyl-L-methionine (0.025), *S*-adenosyl-L-[*methyl*-$^{14}$C]methionine (0.76 μCi(*SA* = 58.6 mCi/mmol)), *Tris* buffer of pH 9 (0.02), and 50 μg of enzyme in a final volume of 0.05 ml. The reaction was allowed to proceed for 5 and 20 min at 37°, stopped by the addition on 0.05 ml of 1N HCl, and extracted with 1 ml of toluene/isopentyl alcohol 7:3 (*v/v*). Following centrifugation (2 min, 1000 rpm, *Fischer* microfuge), 0.9 ml of the org. layer was added to counting vials containing 3 ml of scintillation fluid (*Hydrofluor, National Diagnostics,* Manville, NJ) and the $^{14}$C content measured in a *Beckman* scintillation counter, *LS 5801,* with an efficiency of 85%. Values (dpm) were corrected for substrate blanks obtained from identical reaction mixtures except for the omission of **1**. Results were expressed as nmol of product formed per mg enzyme protein per min.

Double reciprocal plots of initial reaction velocity *vs.* substrate concentration were analyzed by non-linear regression analysis (ENZFITTER, *R.J. Leatherbarrow,* London). $K_m$ and $V_{max}$ values are reported as the mean ±S.E.M. of three or more separate determinations.

*Enzymatic Formation of Methyl Ether* **3** *from Tetrahydroisoquinolinecarboxylic Acid* **1** *and Dihydroisoquinoline* **15** *from Quinonemethide* **4A**. Racemic and optically active **1** and **4A** were incubated with COMT under conditions designed to give the maximum product formation.

The catechols **1** or **4A** were added at a final concentration of 2 mM to reaction mixture containing the following components (mM): $MgCl_2$ (1.2), dithiothreitol (4), *S*-adenosyl-L-methionine (0.025), *S*-adenosyl-L-[*methyl*-$^{14}$C]-methionine (1.4 μ Ci), *Tris* buffer of pH 9 (0.04), and 0.5 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 0.02 ml of 60% $CCl_3COOH$. The precipitated protein was sedimented by centrifugation (5 min, 1000 rpm, *Fischer* microfuge), and the supernatant fraction filtered through at 45-μ filter. The supernatant fractions were subjected to HPLC and analyzed by a post-column, liquid-scintillation monitor. The system consisted of a *Perkin-Elmer* HPLC (series *4*) and *LC-95* spectrometer, set at 270 nm, followed by a *Ramona 4S* scintillation counter ($^{14}$C efficiency 35%). Separation of the components in the reaction mixture was achieved using a cation-exchange column (*Alltech,* mixed-mode *RP/8,* 5 μ, 150 × 4.6 mm) and an isocratic mobile phase of 5% MeOH in 0.1M $K_2HPO_4$ buffer, pH 3.0.

Kinetic analysis of the enzymatic *O*-methylation by COMT of (+)-(*S*)- and (−)-(*R*)-**1** indicated that the $K_m$ for (*S*)-**1** was 74.9 ± 6.5 μM while the value for (*R*)-**1** was 487 ± 13 μM. Thus, the affinity of (*S*)-**1** for COMT is 6 times greater than the affinity for (*R*)-**1**. The

$V_{max}$ values for (*S*)- and (*R*)-**1** were nearly equivalent, 3.4 ± 0.1 and 3.7 ± 0.5 nmol/min/mg protein, respectively. Thus, the relative rate constants ($V_{max}/K_m$) for (*S*)- and (*R*)-**1** of 43 and 7.4 $min^{-1}$, respectively, suggested that the *O*-methylation rate for (*S*)-**1** is *ca.* 6 times greater than the *O*-methylation rate for (*R*)-**1**. An analogous analysis of (*RS*)-**1** yielded values similar to the ones obtained for (*S*)-**1**, suggesting the *O*-methylation of (*S*)-**1** occurred preferentially, even in the presence of equimolar concentrations of (*R*)-**1**.

Analysis of the *O*-methylation products by HPLC demonstrated that in the system used the labeled methyl donor, *S*-adenosyl-L-methionine ($t_R$ = 1.1 min), and a small $^{14}$C-labeled impurity in the methyl donor ($t_R$ = 4.2 min) were completely separated from the products, the 6-*O*-methyl derivative **2** ($t_R$ = 18.3 min) and the 7-*O*-methyl derivative **3** ($t_R$ = 20.6 min). The catechol substrates (*S*)-, (*R*)-, and (*RS*)-**1** all had the same retention time of 8.8 min.

Examination of the profile of $^{14}$C-labeled products clearly demonstrated that *O*-methylation preferentially occurred on the 7-hydroxy group of (*S*)-**1**. The 7-methyl ether **3** accounted for 94.2% of the enzymatic products, while the 6-methyl ether **2** made only a minor contribution of 5.8%. The 7-*O*/6-*O* methylation ratio is, accordingly, 16.2. The same predominance of 7-*O*-methylation was observed with (*R*)-**1** and with (*RS*)-**1**.

Examination of the products from the enzymatic reaction mixture of **4A** with COMT revealed two radioactive products with HPLC retention times of 16.3 and 18.5 min, respectively. The product at 16.3 min was a very minor product representing only 8%, while the product with the longer retention time, 18.5 min, was the major product accounting for 92% of the total. As in the case of **1** above, both *S*-adenosyl-L-methionine and **4A** ($t_R$ = 9.4 min) were completely separated from the radioactive products. Chromatography of a mixture of authentic 7-methyl ether **15** derived from **4A** with the enzymatic reaction products clearly demonstrated that the major product co-migrated with the 7-methyl ether **15**, whereas authentic 6-methyl ether **14** did not. The contribution of the minor enzymatic product was too small to more than tentatively suggest its identity as the 6-methyl ether **14**.

**Conclusion.** – The almost regiospecific *O*-methylation of the dopamine-derived isoquinolines **1** and **4A** with COMT *in vitro* at the 7-OH group to afford 7-methyl ethers **3** and **15**, respectively, precludes in the 1-benzyl series the formation of 6-*O*-methylated isoquinolines as major reaction products. In this respect, the 1-benzyl series behaves similarly as the 1-methyl-substituted series of tetrahydroisoquinolinecarboxylic acids. Whether this also holds for tetrahydroisoquinolinecarboxylic acids and derived 3,4-dihydroisoquinolines which are oxygenated in the aromatic ring of the 1-benzyl group remains to be seen. The 6-times greater methylation rate of (*S*)-**1** over (*R*)-**1** may well be of diagnostic value.

Quinonemethide **4A** proved to be quite unstable towards air oxidation, as were the corresponding dimethyl ether **5** and the mono methyl ethers **14** and **15** which converted on TLC plates into less polar and faster moving decomposition products. In case of **5**, this was shown to be the well known 1-benzoyl-dihydroisoquinoline **9** which was further oxidized to isoquinoline **10** [45]. The instability of **9** in acidic milieu and during the preparation of salts is noteworthy, since it may have biological implications. We confirm that this reaction occurs with ease at pH 6 and quantitatively, as already noted by *Gardent* [31], affords the imidazolinium salt **11** which was now characterized by additional spectral data. Quinonemethide **4A** showed weak inhibitory activity of MAO A, whereas

the optically active or the racemic tetrahydroisoquinolinecarboxylic acids **1** were devoid of such activity.

## Experimental Part

1. *General.* TLC: plates from *Analtech Inc.* Flash chromatography (FC): columns packed with *Merck* silica gel *60* (0.015–0.040 mm) from *EM Laboratories*. M.p.: *Fisher-Johns* apparatus; uncorrected. UV spectra ($\lambda_{max}$ in nm, *A*): *Hewlett-Packard-8450-A* UV/VIS spectrophotometer; in MeOH, MeOH + 0.1N HCl (pH 2), and MeOH + 0.1N NaOH (pH 9). IR spectra (KBr, $cm^{-1}$): *Beckman IR 4230* instrument. $^1$H-NMR spectra ($CDCl_3$, $\delta$): *Varian-XL-300* spectrometer with $(CH_3)_4Si$ as the internal standard. MS (*m/z*, %): chemical-ionization (CI) MS using a *Finnigan-1015D* spectrometer with a model *6000* data collection system. Elemental analyses were performed by *Atlantic Microlab Inc.* (Atlanta, Georgia).

2. *1-Benzyl-3,4-dihydroisoquinoline-6,7-diol* (**4**). 2.1. *From 1-Benzyl-1,2,3,4-tetrahydro-6,7-dihydroxyisoquinoline-1-carboxylic Acid* (**1**). The hydrobromide of (±)-**1** [9] (107 mg, 0.28 mmol) was dissolved in 0.1M $NaHCO_3$ in MeOH/$H_2O$ 2:1 (7 ml, pH 9.6) and the yellow soln. left standing at r.t. for 48 h. MeOH was evaporated and the aq. soln. extracted with $CHCl_3$ (4 × 25 ml). A MeOH soln. of the material obtained after solvent evaporation showed UV maxima at 222 (2.65), 271 (1.09), 322 (0.53), and 410 (2.50). The combined $CHCl_3$ extracts were extracted with 0.1N HCl (3 × 20 ml), and the combined aq. extracts were concentrated *in vacuo* to give a residue which was dissolved in $H_2O$ (1 ml) followed by 10% aq. $NaHCO_3$ soln. added at 0° (pH 7.5). The yellow precipitate was filtered and dissolved in a sat. EtOH soln. of fumaric acid (1 ml). After standing in the refrigerator for 48 h, the fumarate **4C** ( = **4A**·$C_4H_4O_4$) was filtered and recrystallized from MeOH to afford 20 mg of crystals. TLC ($CHCl_3$/MeOH/$NH_4OH$ 90:9:1): yellow polar spot coloring grey when sprayed with 1% $FeCl_3$ soln. in EtOH. M.p. 212–213°. UV: 212 (2.44), 249 (1.30), 272 (0.529), 315 (0.737), 395 (0.948). IR: 3160 (br.), 1700 (br.), 1635. This fumarate is in every respect identical with the fumarate sample prepared from **5** (see below).

The same results were obtained with (*S*)- and (*R*)-**1**.

2.2. *From 1-Benzyl-3,4-dihydro-6,7-dimethoxyisoquinoline* (**5**). Compound **5** was prepared from *N*-(3,4-dimethoxyphenethyl)-2-phenylacetamide by *Bischler-Napieralski* condensation according to [46] in 81% yield. M.p. 86.5–90°. UV: 212 (2.11), 228 (2.037), 275 (0.792), 311 (0.719). IR: 1625 (sh), 1600. $^1$H-NMR: 2.65, 3.73 (2*t*, *J* = 7, Ar$CH_2CH_2$N); 3.71, 3.87 (2*s*, 2 $CH_3O$); 4.04 (*s*, Ph$CH_2$); 6.65, 6.94 (2*s*, 2 arom. H); 7.11–7.32 (*m*, 5 arom. H). MS: 282 (100, $M^+ + 1$). Anal. calc. for $C_{18}H_{19}NO_2$: C 76.84, H 6.81, N 4.98; found: C 76.65, H 6.90, N 4.89.

**5**·HBr: M.p. 195–196°.

Dihydroisoquinoline **5** (2.9 g, 10.3 mmol) was refluxed with 48% HBr soln. (50 ml) for 6 h under Ar. The mixture was concentrated under vacuum and left for crystallization to give **4A**·HBr ( = hydrochloride **4C**; 2.53 g, 98%). M.p. 112° and 229°, after recrystallization from 95% EtOH. UV: 211 (2.38), 250 (2.025), 313 (1.243), 369 (1.31). IR: 3100 (br.), 1645, 1615, 1580. $^1$H-NMR ((D$_6$)DMSO): 2.92, 3.80 (2*t*, *J* = 8, Ar$CH_2CH_2$N); 4.35 (*s*, Ph$CH_2$, disappears on treatment with $D_2O$ after 48 h); 6.79 (*s*, 1 arom. H); 6.79–7.31 (*m*, 6 arom. H); 9.56, 10.99, 12.49 (3*s*, 1 H each, disappear on treatment with $D_2O$, ArO*H*, N*H*). MS: 254 (100, $M^+ + 1$), 238 (10), 164 (10). Anal. calc. for $C_{16}H_{16}BrNO_2 \cdot \frac{1}{2} H_2O$: C 55.99, H 4.99, Br 23.28, N 4.08; found: C 56.18, H 4.96, Br 23.30, N 4.08.

2.3. *Quinonemethide* **4A** *from* **4A**·*HBr*. To a soln. of **4A**·HBr (0.9 g 2.7 mmol) in $H_2O$ (30 ml), 10% $NaHCO_3$ soln. was added at 0°, until no more yellow precipitate was formed (pH *ca.* 7.5). The precipitate was filtered off, washed with cold $H_2O$, and dried to give 0.62 g (91%) of **4A**. An anal. sample was recrystallized from 95% EtOH. M.p. 222–225°. UV: 271 (0.623), 321 (0.306), 408 (1.456). IR: 3300–2100, 1635, 1600. $^1$H-NMR ((D$_5$)pyridine): 2.57, 3.72 (2*t*, *J* = 7, Ar$CH_2CH_2$N); 4.15 (*s*, Ph$CH_2$, disappears on treatment with $D_2O$ after 24 h); 6.97 (*s*, 1 arom. H); 7.39–7.55 (*m*, 6 arom. H). MS: 254 (100, $M^+ + 1$), 238 (20), 164 (10). Anal. calc. for $C_{16}H_{15}NO_2$: C 75.87, H 5.97, N 5.53; found: C 75.89, H 6.02, N 5.53.

2.4. *Fumarate* **4C** *from* **4A**. To a soln. of **4A** (1.234 g, 4.9 mmol) in MeOH (19 ml), a soln. of fumaric acid (0.65 g, 5.6 mmol) in EtOH (10 ml) was added and left for crystallization to afford 0.934 g (52%) of **4A**·$C_4H_4O_4$ ( = fumarate **4C**). An anal. sample was recrystallized from MeOH. M.p. 214–216°. UV: 213 (2.55), 249 (1.466), 272 (0.583), 315 (0.834), 395 (1.092). IR: 3400–2400 (br.), 1720 (sh), 1700, 1635. $^1$H-NMR ((D$_6$)DMSO): 2.65, 3.61 (2 br. *s*, Ar$CH_2CH_2$N); 4.08 (br. *s*, Ph$CH_2$, disappears on treatment with $D_2O$ after 48 h); 6.54 (*s*, 1 arom. H); 6.58 (*s*, CH=CH); 7.09–7.29 (*m*, 6 arom. H). MS: 254 (100, $M^+ + 1$), 238 (10), 164 (5). Anal. calc. for $C_{20}H_{19}NO_6$: C 65.03, H 5.18, N 3.79; found: C 64.96, H 5.21, N 3.73.

3. *2-Acetyl-1-benzylidene-1,2,3,4-tetrahydroisoquinoline-6,7-diyl Diacetate* (**12**). A mixture of **4** (0.69 g, 2.7 mmol) and pyridine/$Ac_2O$ 1:1 (16 ml) was stirred at r.t. under Ar overnight. Solvents were evaporated, the remainder dissolved in $CH_2Cl_2$ and washed with 1% HCl and 1% NaOH soln., then worked up in the usual way. The oily product (0.94 g, 91%) was crystallized from $Et_2O$ to give crystalline **12**. M.p. 156–158°. UV: 215 (2.22), 289 (1.402). IR: 1780, 1765 (sh). $^1$H-NMR: 1.74 (*s*, AcN); 2.30, 2.33 (2*s*, 2 AcO); 2.76–2.81, 3.16–3.20, 5.00–5.02 (3*m*, ArC$H_2$C$H_2$N); 6.86 (*s*, PhC*H*=C); 7.01 (*s*, 1 arom. H); 7.26–7.54 (*m*, 6 arom. H). MS: 380 (100, $M^+ + 1$). Anal. calc. for $C_{22}H_{21}NO_5$: C 69.65, H 5.58, N 3.69; found: C 69.61, H 5.62, N 3.64.

4. *2-[2-(Acetylamino)ethyl]-4,5-dihydroxyphenyl Benzyl Ketone* (**13**). A soln. of **12** (0.19 g, 0.5 mmol) in THF (1 ml) and 5% HCl soln. (0.5 ml) was kept at 65° for 3 h. THF was evaporated and the residue neutralized to pH 7–8 and extracted with $CHCl_3$/i-PrOH 3:1, dried, and evaporated to give 0.14 g (89%) of **13** which was crystallized from AcOEt. M.p. 96–98°. IR: 3400 (br.), 1670 (sh), 1655 (sh), 1630. $^1$H-NMR: 1.92 (*s*, AcN); 2.78, 3.45 (2*m*, ArC$H_2$C$H_2$N); 4.20 (*s*, PhC$H_2$); 6.86, 7.37 (2*s*, 2 arom. H); 7.21–7.36 (*m*, 5 arom. H). MS: 314 (100, $M^+ + 1$). Anal. calc. for $C_{18}H_{19}NO_4 \cdot \frac{1}{2} H_2O$: C 67.07, H 6.25, N 4.35; found: C 67.32, H 6.46, N 4.17.

5. *1-Benzyl-3,4-dihydro-6-methoxyisoquinolin-7-ol* (**14**). Compound **14** was prepared by a similar procedure as **15** (see *Exper. 6*) from *N*-(4-hydroxy-3-methoxyphenethyl)-2-phenylacetamide obtained from homovanillylamine and phenylacetyl chloride, except that during the workup procedure after neutralization, the product was extracted with AcOEt. Amine **14** was characterized as the fumarate **14**·$C_4H_4O_4$ obtained in 69% yield. M.p. 169–171° (dec.; from EtOH/(i-Pr)$_2$O). UV: 211 (2.53), 241 (1.965), 310 (1.178), 354 (0.7551). IR: 3450 (br.), 2800 (br.), 1700, 1655, 1635, 1605. $^1$H-NMR: 2.58, 3.57 (2*t*, $J$ = 7, ArC$H_2$C$H_2$N); 3.78 (*s*, $CH_3O$); 3.97 (*s*, PhC$H_2$); 6.60 (*s*, CH=CH); 6.82, 7.02 (2*s*, 2 arom. H); 7.15–7.27 (*m*, 5 arom. H). MS: 268 (100, $M^+ + 1$). Anal. calc. for $C_{21}H_{21}NO_6 \cdot \frac{1}{2} H_2O$: C 64.28, H 5.65, N 3.57; found: C 64.56, H 5.72, N 3.61.

6. *1-Benzyl-3,4-dihydro-7-methoxyisoquinolin-6-ol* (**15**). A soln. of *N*-(3-hydroxy-4-methoxyphenethyl)-2-phenylacetamide (0.57 g, 2 mmol), prepared from homoisovanillylamine and phenylacetyl chloride, in MeCN (20 ml) and $POCl_3$ (1.5 ml) was refluxed under Ar for 1.5 h. Solvents were evaporated, and to the dry residue, $H_2O$ (2 ml) was added and the mixture refluxed under Ar for 1.5 h. It was then cooled in an ice-bath and neutralized with 10% $NaHCO_3$ soln. under Ar. The yellow precipitate was filtered off, washed with AcOEt, then with $Et_2O$, dissolved in 95% EtOH (40 ml) and treated with fumaric acid (0.23 g, 2 mmol) in EtOH (10 ml). The fumarate **15**·$C_4H_4O_4$ was obtained in 73% yield. M.p. 260–261° (dec.). UV: 212 (2.38), 241 (1.121), 274 (0.682), 313 (0.657), 402 (1.45). IR: 3450 (br.), 2600 (br.), 1660, 1605. $^1$H-NMR: 2.52, 3.54 (2*t*, $J$ = 7, ArC$H_2$C$H_2$N); 3.68 (*s*, $CH_3O$); 4.05 (*s*, PhC$H_2$); 6.58 (*m*, CH=CH); 7.12–7.33 (*m*, 7 arom. H). MS: 268 (100, $M^+ + 1$). Anal. calc. for $C_{19}H_{19}NO_4$: C 70.14, H 5.89, N 4.22; found: C 70.00, H 5.94, N 4.22.

7. *3,4-Dihydro-6,7-dimethoxyisoquinolin-1-yl Phenyl Ketone* (**9**). A soln. of **5** (0.562 g, 2 mmol) in benzene (10 ml) was left overnight at r.t. in an open flask with stirring. The soln. was evaporated and chromatographed on a silica-gel column (6 g) with benzene/$Et_2O$ 9:1: 0.42 g (71%) of pure **9**, oil ([31]: oil; [33]: m.p. 78.8–79.4°). UV: 209 (2.16), 232 (2.03), 255 (sh. 1.65), 316 (0.80). IR: 1680*w*, 1620*w*. $^1$H-NMR: 2.75, 3.86 (2*m*, ArC$H_2$C$H_2$N); 3.72, 3.87 (2*s*, 2 $CH_3O$); 6.68, 6.88 (2*s*, 2 arom. H); 7.38–7.53, 7.95–7.98 (2*m*, 5 arom. H). MS: 296 (100, $M^+ + 1$), 282 (20), 190 (10). Anal. calc. for $C_{18}H_{17}NO_3$: C 73.20, H 5.80, N 4.74; found: C 73.25, H 5.82, N 4.73.

8. *1-Benzyl-1,2,3,4-tetrahydroisoquinoline-6,7-diol* (**7**). 8.1. *From* **4A**·*HBr*. A soln. of **4A**·HBr (0.166 g, 0.5 mmol) in MeOH (3 ml) was treated with $NaBH_4$ (0.038 g, 1 mmol). This mixture was stirred at r.t. for 2 h, then $H_2O$ (1 ml) was added and stirred for additional 0.5 h. Solvents were evaporated and the remainder dissolved in 1% HCl soln. then neutralized with 10% $NaHCO_3$ soln. to deposit 108 mg (85%) of **7**, which was treated with 4.8% HBr in MeOH to give **7**·HBr. M.p. 235–238°, identical by TLC, $^1$H-NMR, and MS with a specimen obtained from **6** (see *Exper. 8.2*).

8.2. *From* **6**. From **5**·HBr (0.724 g, 2 mmol), **6**·HBr was obtained in 71% yield by reduction with $NaBH_4$ (0.1 g, 2.6 mmol) in MeOH (15 ml) and treatment of the crude products with 5% HBr in acetone. M.p. 189–190°. $^1$H-NMR ((D$_6$)DMSO): 2.91–2.97, 3.15–3.47 (2*m*, ArC$H_2$C$H_2$N, PhC$H_2$); 3.50, 3.73 (2*s*, 2 $CH_3O$); 4.71 (*t*, $J$ = 7.5, H–C(1)); 6.41, 6.80 (2*s*, 2 arom. H); 7.29–7.41 (*m*, 5 arom. H); 8.80, 9.09 (br. 2*s*, 1 H each, disappear on treatment with $D_2O$, NH). MS: 284 (100, $M^+ + 1$), 192 (35). Anal. calc. for $C_{18}H_{22}BrNO_2$: C 59.35, H 6.09, Br 21.93, N 3.85; found: C 59.23, H 6.10, Br 21.99, N 3.83.

Compound **6**·HBr (0.364 g, 1 mmol) was refluxed with 48% HBr soln. (6 ml) for 2 h. After cooling, the crystalline **7**·HBr was collected (0.282 g, 83%). M.p. 234–235°. $^1$H-NMR: 2.76–3.31 (*m*, ArC$H_2$C$H_2$N, PhC$H_2$); 4.62 (br. *s*, H–C(1)); 6.50, 6.57 (2*s*, 2 arom. H); 7.29–7.40 (*m*, 5 arom. H); 8.65, 8.85, 8.90, 9.12 (4*s*, 4 H, disappear on treatment with $D_2O$, NH, OH). MS: 256 (80, $M^+ + 1$), 164 (83). Anal. calc. for $C_{16}H_{18}BrNO_2 \cdot \frac{1}{2} H_2O$: C 55.66, H 5.55, Br 23.14, N 4.06; found: C 55.93, H 5.52, Br 23.21, N 4.10.

9. *2-Acetyl-1-benzyl-1,2,3,4-tetrahydroisoquinoline-6,7-diyl Diacetate* (**8**). 9.1. *From* **4A**·$C_4H_4O_4$. A soln. of **4A**·$C_4H_4O_4$ (0.1 g, 0.27 mmol) in MeOH (3 ml) containing $H_2O$ (0.25 ml) was treated with $NaBH_4$ (0.05 g, 1.3 mmol) at 0°, then stirred for 2 h at r.t. Solvents were evaporated and to the dry residue, pyridine (1 ml) and $Ac_2O$ (1.5 ml) were added under Ar. The mixture was left at r.t. overnight and then evaporated. The remainder was partitioned between $CH_2Cl_2$ and $H_2O$, the org. phase washed with 1% HCl and 1% NaOH soln. and worked up in the usual way: **8** was obtained as an oil (0.1 g, 97%); treatment with $Et_2O$ gave crystalline solid. M.p. 127.5–129°. IR: 1775 (sh), 1760. $^1$H-NMR: mixture of rotamers; 1.43, 1.62 (2*s*, 3 H, AcN); 2.10, 2.25, 2.27, 2.30 (4*s*, 6 H, AcO); 2.63–3.19, 3.40–3.46, 3.61–3.68 (3*m*, 5 H, Ar$CH_2CH_2$N, Ph$CH_2$); 4.80–4.89, 5.72–5.77 (2*m*, 2 H, H–C(1), Ar$CH_2CH_2$N); 6.67–7.35 (*m*, 7 arom. H). MS: 382 (100, $M^+ + 1$). Anal. calc. for $C_{22}H_{23}NO_5$: C 69.27, H 6.08, N 3.67; found: C 69.18, H 6.08, N 3.65.

9.2. *From* **7**. A mixture of **7**·HBr (0.128 g, 0.38 mmol), pyridine (1 ml), $Ac_2O$ (1 ml), and $Et_3N$ (0.2 ml) was stirred at r.t. for 24 h under Ar, then evaporated. The residue was partitioned between $Et_2O$ and $H_2O$ and the org. phase washed with 1% HCl soln. and worked up to give **8** in 72% yield. M.p. 128–129°; identical with the sample from *Exper. 9.1.*

10. *6,7-Dimethoxyisoquinolin-1-yl Phenyl Ketone* (**10**). Dihydroisoquinoline **9** (0.27 g, 0.92 mmol) in toluene (30 ml) was refluxed with 10% Pd/C (0.135 g) under Ar for 24 h. Another aliquot of catalyst (0.135 g) was added and the mixture refluxed for additional 10 h. The catalyst was filtered off hot and washed with refluxing toluene and the solvent evaporated to give **10** (0.23 g, 86%) as a crystalline solid. It was washed with $Et_2O$. M.p. 129–130° ([47]: 131°; [33]: 123.5–124.5°). UV: 235 (2.29), 328 (0.21). IR: 1660. $^1$H-NMR: 3.96, 4.06 (2*s*, 2 $CH_3O$); 7.14–8.47 (*m*, 9 arom. H). MS: 294 (100, $M^+ + 1$). Anal. calc. for $C_{18}H_{15}NO_3$: C 73.71, H 5.15, N 4.78; found: C 73.59, H 5.22, N 4.70.

11. *1,2,9,10-Tetrahydro-4,5,12,13-tetramethoxy-7-phenyldiisoquinolino[2,1-a:2′,1′-c]imidazol-15-ium Chloride* (**11**). A soln. of **9** (0.098 g, 0.33 mmol) in MeOH (5 ml) was treated with fumaric acid (0.043 g, 0.37 mmol) in EtOH (0.5 ml) and left for crystallization. The yellow precipitate was recrystallized several times from 3.6% HCl in MeOH. M.p. 284–286° (dec.). UV: 215 (2.50), 280 (1.318), 337 (2.261). $^1$H-NMR ($(D_6)$DMSO): 3.16, 4.11, 4.76 (3*m*, 2 Ar$CH_2CH_2$N); 3.27, 3.81, 3.90, 3.94 (4*s*, 4 $CH_3O$); 6.43, 7.14, 7.30, 7.42 (4*s*, 4 arom. H); 7.67 (*m*, 5 arom. H). MS: 469 (100, $M^+ - HCl - Cl$). Anal. calc. for **11**·HCl $C_{29}H_{30}Cl_2N_2O_4$: Cl 13.10; found: Cl 12.91.

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