Biomimetic Formation of Macrocyclic Spermine Alkaloids

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Dedicated to Professor *Heinz Heimgartner* on the occasion of his 60th birthday

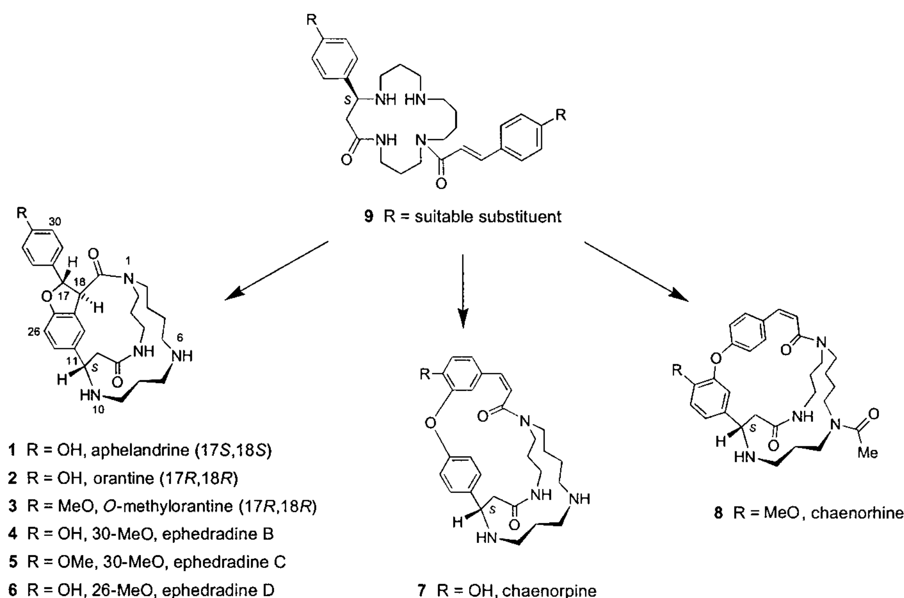
Dihydroxyverbacine (**10**), a precursor for oxidative phenol coupling, was obtained *via* (±)-buchnerine (**14**), whose synthesis is described. The oxidizing system hemin (ferriprotoporphyrin IX chloride)/H_2O_2 promoted intramolecular coupling of **10** to give the alkaloids aphelandrine (**1**), orantine (**2**), and chaenorpine (**7**). The alkaloids were identified by on-line coupled HPLC and atmospheric-pressure chemical-ionization (APCI) mass spectrometry.

Introduction. – The polyamines spermine and spermidine, which naturally occur in a variety of living organisms, have been shown to be crucial, *e.g.*, for cell growth, carcinogenesis, and neurotransmission [1][2]. The polyamine derivatives have been observed as alkaloids in linear conjugates or as macrocyclic lactams [1]. In the case of putrescine alkaloids, the base is preferably incorporated in a chain, whereas, in spermidine and spermine alkaloids, the cyclic form is predominant mostly in 13- or 17-membered lactam rings [1].

Aphelandrine (**1**), its diastereoisomer orantine (**2**), and chaenorpine (**7**) are examples of spermine alkaloids (*Scheme 1*). Both alkaloids **1** and **2** have been found in several *Aphelandra* species [3–5]. Aphelandrine was identified also in *Encephalosphaera lasiandra* (Acanthaceae) [4]. Orantine was further observed in *Chaenorhinum minus* [6], *Schweinfurthia papilionacea* (Scrophulariaceae) [7], and in *Ephedra sp.* (Ephedraceae) [8]. Finally, the alkaloid chaenorpine was identified in the basic extract of *Chaenorhinum minus* [6]. The biosynthesis of these alkaloids, in particular that of aphelandrine (**1**), has long been in the center of our interest [9]. The biogenetic precursor should be a macrocyclic compound of type **9**, which provides, after oxidation and oxidative phenol coupling, the alkaloids presented in *Scheme 1*. The hypothesis of *Barton* and *Cohen* was that the biosynthesis of a variety of natural products includes oxidative coupling of phenols [10]. This hypothesis has been confirmed by the identification of the enzyme, membrane-bound cytochrome P-450, that catalyzes the transformation of the benzyltetrahydroisoquinoline alkaloid (*R*)-reticuline into salutaridine; the latter is a key intermediate in morphine synthesis [11].

For the biogenesis of the alkaloids shown in *Scheme 1*, (*S*)-dihydroxyverbacine ((*S*)-**10**) seems to be very suitable as a precursor. Several pathways are possible for the formation of (*S*)-**10** (*Scheme 2*). The open-chained species **11a** and **11b** can lead, after aza-*Michael* addition, to protoverbine (**12**) and prelandrine (**13**), respectively. In an additional step, prelandrine has to be transformed into (*S*)-dihydroxyverbacine ((*S*)-**10**). Another possibility is the formation of buchnerine (**14**) from **11c**, after aza-*Michael*

Scheme 1



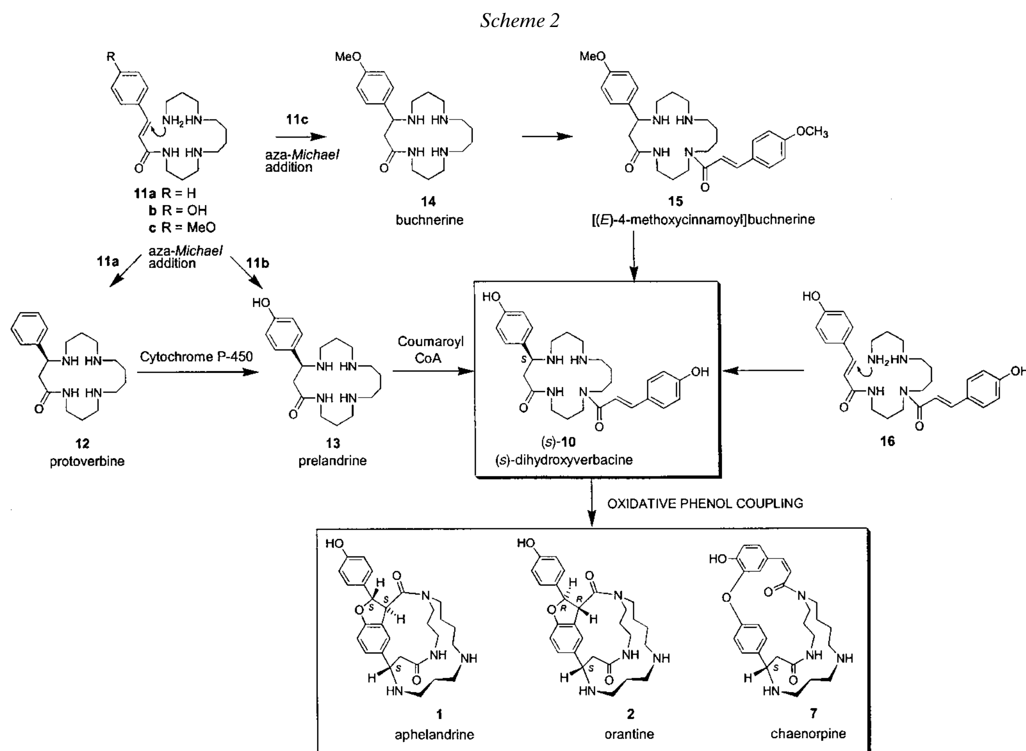
addition, its further acylation to [(*E*)-4-methoxycinnamoyl]buchnerine (**15**), followed by transformation to **10**. It is also possible that **10** is formed by an aza-*Michael* addition of the *N*,*N'*-dicoumaroylspermine (**16**) [9]. Actually, some of the compounds presented in *Scheme 2* are alkaloids that have been identified in different plants. Protoverbine (**12**) was isolated from *Verbascum pseudonobile* SOJ. *et* STEF. (Scrophulariaceae) [12], and buchnerine (**14**) was found in *Clerodendrum buchneri* (Verbenaceae) [13]. An analogue of dihydroxyverbacine (**10**), the [(*Z*)-4-methoxycinnamoyl]buchnerine, was identified in *C. buchneri* [13].

Three recent important findings considerably support the biosynthetic pathway of aphelandrine (**1**) proceeding *via* compounds **12**, **13**, and (*S*)-**10**. Protoverbine (**12**) could be transformed to prelandrine (**13**) by incubation with cytochrome P-450-containing microsomes from the roots of *A. squarrosa* in the presence of NADPH [14]. Prelandrine (**13**) was detected in the roots of *A. squarrosa* [14]. The formation of aphelandrine (**1**) was realized after microsome-assisted oxidation of (*S*)-dihydroxy-verbacine ((*S*)-**10**) and its D_8-labeled analogue [15][16].

The task of the present work was to find a biomimetic way for oxidative phenol coupling of **10** leading to the formation of any of the above discussed alkaloids by means of the simple 'instruments' of synthetic organic chemistry. In this paper, we describe the results of the nonenzymatic oxidative phenol coupling of dihydroxyver-bacine (**10**) leading to aphelandrine (**1**), orantine (**2**), and chaenorpine (**7**).

Results and Discussion. – *Dihydroxyverbacine* (**10**) *as Precursor for Oxidative Phenol Coupling*. There are two possible synthetic pathways to **10** proceeding *via* buchnerine (**14**): *a*) the route to the racemic product **10**, and *b*) those providing the

Scheme 2

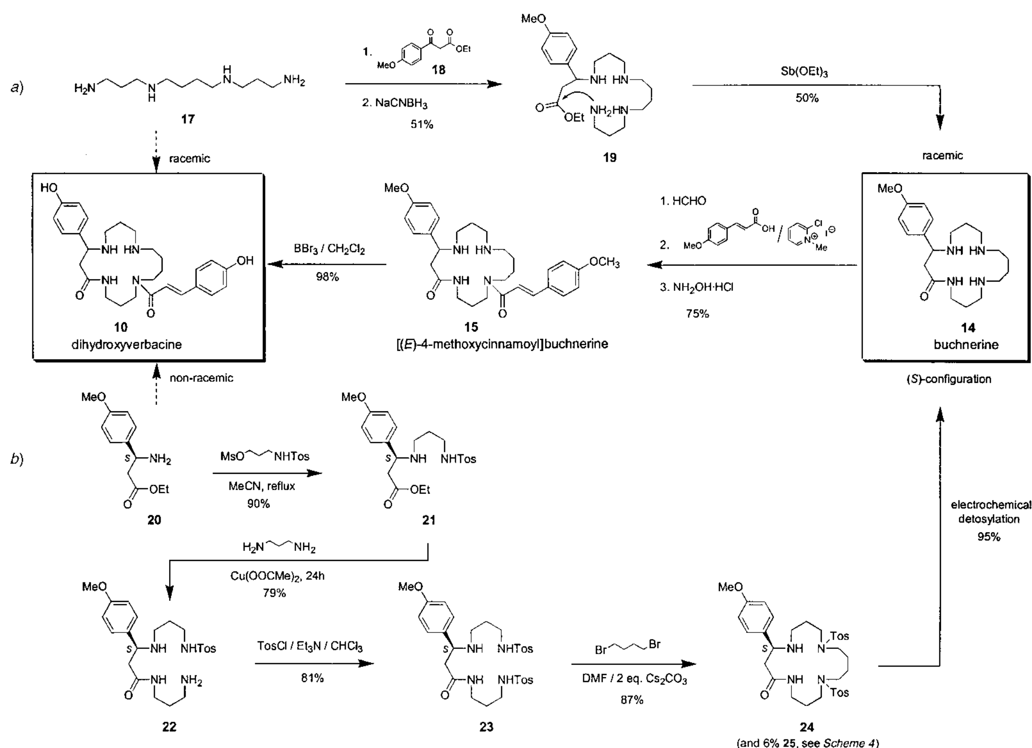


possibility to prepare (*S*)-dihydroxyverbacine ((*S*)-**10**) (*Scheme 3*). Racemic buchnerine (**14**) was synthesized in two steps *via* **19** from spermine (**17**) by the reaction with β-keto ester **18** [17], followed by $Sb(OEt)_3$-promoted macrolactamization, as described by *Yamamoto* and co-workers [18]. (*S*)-Buchnerine ((*S*)-**14**) was prepared starting from (*S*)-β-amino-β-(4-methoxyphenyl) ester **20** *via* **21**–**24**, which was further transformed in four steps to (*S*)-dihydroxyverbacine ((*S*)-**10**) according to the recently published procedures [16][19].

In the synthetic step from **23** to **24**, the formation of an interesting by-product was observed, which is worth mentioning. The transformation of **23** was performed on a 1–2 g scale, and, therefore, it was possible to isolate the by-product **25** in sufficient quantity. We suggest for **25** the structure of a carbamic acid (see *Scheme 4*), based on the arguments presented below. Two feats were critical for the structure elucidation of **25**, namely to establish the presence of the (N)COOH group as a substituent and to determine the place of the substitution. The occurrence of the (N)COOH group at the macrocyclic framework of **25** was deduced by means of chemical transformations and MS investigations. The MS experiments with **25** showed the presence of an additional 44-amu moiety, which we presume to be CO_2 in view of the reagents used in the reaction **23** → **24**. Indeed, the formation of **25** may be understood to be the result of CO_2 insertion into the N–H bond leading to the carbamic-acid derivative. Similar insertion reactions with CO_2 have recently been reported [20]. The molecular mass of **25** was determined by ESI-MS. The quasi-molecular ions at *m/z* 849 ($[M+H]^+$), 871 ($[M+Na]^+$), and 887 ($[M+K]^+$) showed the characteristic isotope pattern resulting from the presence of one Br-atom, which was confirmed by microanalysis. The ESI-MS/MS experiments with **25** gave the fragments shown in *Scheme 4*, that, with *m/z* 805, indicate the loss of CO_2.

Compound **25** was treated with morpholine to afford **26**, which was detosylated to **27**. The quasi-molecular ions at *m/z* 856 ($[M+H]^+$) and 548 ($[M+H]^+$) in the ESI-MS of **26** and **27**, respectively, were in accordance

Scheme 3

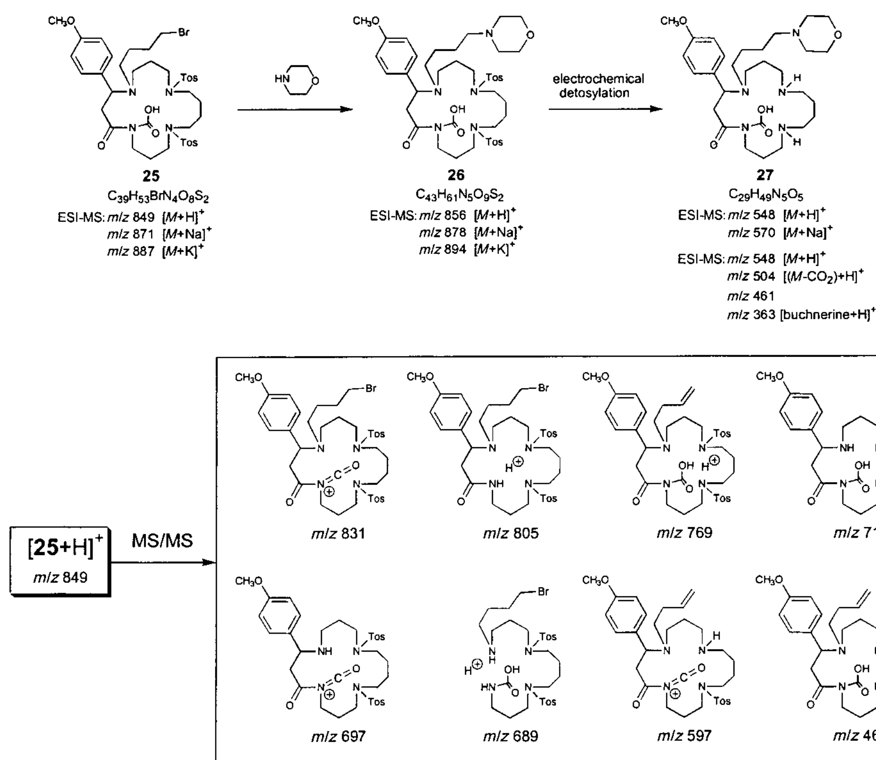


with the 'nitrogen rule' (odd number of N-atoms). The loss of CO_2 (*m/z* 504), was also observed in the CI-MS of **27**, and the CI-MS was in accordance with the ESI investigations.

The NMR data (^1H, ^{13}C, HSQC, HMBC, NOESY, and TOCSY of **25**–**27** in different solvents ($CDCl_3$, CD_3OD, (D_6)DMSO, (D_8)toluene, and (D_5)pyridine) and at different temperatures (from $-50°$ up to $80°$) were difficult to analyze. The spectra were temperature-dependent and exhibited broad signals in all cases. Obviously, several possible conformations of **25**–**27** are present, due to the flexibility of the macrocyclic ring, which prevented access to detailed structure information. However, the NMR data established the presence of two different carbonyl C-atoms, which is an additional argument for the suggested COOH group. However, no conclusions concerning the position of the COOH and C_4H_8X (X=Br or morpholino) substituents could be drawn. Moreover, **25**–**27** could not be crystallized, and, thus, the question of the exact structure of **25**–**27** remains open.

Oxidative Phenol Coupling and On-Line HPLC/APCI-MS and HPLC/APCI-MS/MS Investigations. We checked several reagents known for their potential to induce oxidative phenol coupling [21–24]. Thus, the complex CuCl(OH)·TMEDA (TMEDA = *N,N,N',N'*-tetramethylethylenediamine) is an excellent catalyst for the oxidative binaphthyl coupling [21]. For the oxidation of dihydroxyverbacine (**10**), its CH_2Cl_2 solution at 0° was saturated with O_2 in the presence of 10 mol-% of CuCl(OH)·TMEDA, according to the conditions that were recently used for the preparation of steroidal binaphthyl derivatives [22]. However, after two days stirring at room temperature, HPLC coupled on-line with atmospheric-pressure chemical-

Scheme 4



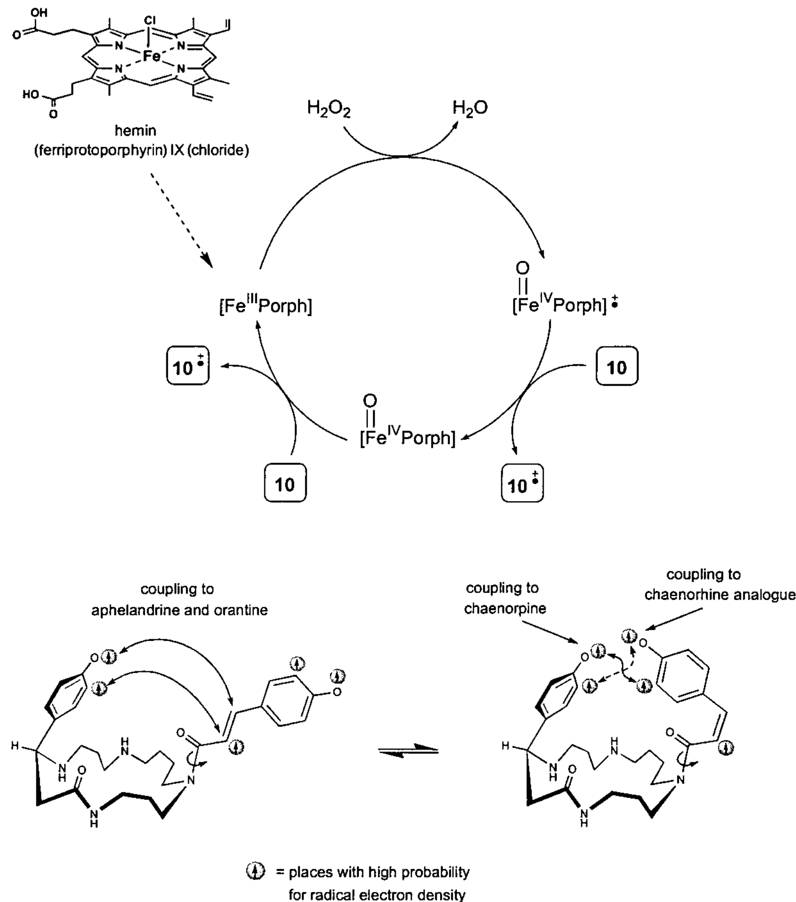
ionization mass spectrometry (APCI-MS) showed that **10** ($[M+H]^+$ at *m/z* 495) had not reacted. Thus, we prepared an analogous Cu complex with **10** itself as ligand instead of TMEDA, anticipating a possible template effect, the generation of radicals within the macrocyclic framework, and the participation of the metal center in close proximity to the phenol moiety of the molecule. For this purpose **10** was treated with CuCl in 95% aqueous MeOH under the same conditions as for the preparation of CuCl(OH) · TMEDA [21]. The formation of a complex CuCl · **10** was strongly suggested by the observed color change from initially slightly yellow to blue-green and finally blue-violet, in accordance with the observations made on formation of CuCl(OH) · TMEDA. After solvent evaporation, CH_2Cl_2 was added to CuCl · **10** and the mixture saturated with O_2 at 0° and stirred for two days at room temperature. But, also in this case, the precursor **10** remained unchanged. The use of other metal salts, $CuCl_2$ and $FeCl_3$ in $CHCl_3$/MeOH or 0.1M aqueous K_2CO_3/MeCN did not produce any coupling products.

It is known that oxoammonium salts, such as 4-(acetylamino)-2,2,6,6-tetramethyl-1-oxopiperidinium tetrafluoroborate, are reagents for oxidative phenol coupling in $MeCN/H_2O$ in the presence of $KHCO_3$ [23]. However, when the oxoammonium oxidizing reagent was applied to **10**, no coupling products could be detected, and **10**

remained unchanged after 3 days. Thus, dihydroxyverbacine (**10**) seems to be surprisingly stable under oxidative conditions.

In further experiments, we used hemin (ferriprotoporphyrin IX chloride) in combination with H_2O_2 as oxidizing agent. The following arguments led to this choice. There are several types of heme proteins that contain the iron protoporphyrin IX group – hemoglobins and myoglobins that are responsible for the storage and the transport of O_2, and cytochromes that are involved in the transfer of electrons. The ability of cytochrome P-450 to promote the oxidative phenol coupling of **10** to aphelandrine (**1**) has recently been demonstrated [15]. Although, to the best of our knowledge, neither hemin (see *Scheme 5*) nor any analogous heme protein has been used for oxidative phenol coupling, it seemed appropriate to apply it as reagent in H_2O_2 oxidation experiments. Metalloporphyrin-catalyzed oxidations, also those with H_2O_2, have been the object of a variety of investigations [24]. Thus, a dark red-brown solution of dihydroxyverbacine (**10**) and 30 mol-% of hemin in 0.1M aqueous K_2CO_3/MeCN 1:1

Scheme 5



was treated with 10% aqueous H_2O_2 solution at room temperature. On-line coupled HPLC/APCI-MS and HPLC/APCI-MS/MS (reversed-phase HPLC column and conditions) of the homogeneous reaction mixture, revealed the formation of the desired coupling products at *m/z* 493 ($[M+H]^+$), already 15 min after the addition of H_2O_2 (M_r 492 for the isomeric alkaloids aphelandrine (**1**), orantine (**2**), and chaenorpine (**7**)). The main component of the reaction mixture during the first 1.5 h was unreacted **10** (*m/z* 495 ($[M+H]^+$), t_R 8.60 min). Besides the desired products at *m/z* 493, some decomposition products with t_R between 13 and 21 min originating from **10**, but possibly also from hemin, were observed. The by-product at *m/z* 347 (13.03 min), formed from **10** by loss of the cinnamoyl fragment, became the main component after 3 h reaction time. It must be pointed out that, when **10** was stirred with H_2O_2 alone for several hours, the main component in the mixture was unchanged **10**, and only an insufficient quantity of an oxidation product at *m/z* 527 (t_R 4.57 min; $[(\mathbf{10}+32)+H]^+$) was observed.

The aim of the HPLC/MS investigations was to detect coupling products with M_r 492. The best way to observe the products of oxidative phenol coupling was to detect them selectively in the selected-ion-monitoring mode (SIM). However, the additional use of MS/MS techniques (tandem mass spectrometry) provided more structural information. The triple-stage quadrupole mass spectrometer allowed the isolation of the quasi-molecular ions with the first quadrupole, the collision-induced dissociation (CID) with the second quadrupole used as collision cell, and the recording of the corresponding daughter-ion spectra with the third quadrupole [25]. Several products possessing M_r 492 and MS/MS closely similar to each other were thus observed (*Fig.*) and the alkaloids aphelandrine (**1**), orantine (**2**), and chaenorpine (**7**) could unambiguously be identified by the addition of authentic **1**, **2**, or **7**, respectively, to the reaction mixture. The on-line MS/MS of the coupling products obtained and those of **1**, **2**, and **7** were almost identical (*Table*). The fragments at *m/z* 251, 265, and 476 (*Fig.*) were the most intense signals in the MS/MS of the coupling products and are typical for aphelandrine (**1**), orantine (**2**), and chaenorpine (**7**). The fragment ions B2

Table. *MS/MS Data of the Coupling Products of Dihydroxyverbacine* (**10**) *and of Aphelandrine* (**1**), *Orantine* (**2**) *and Chaenorpine* (**7**)

Peaks in the HPLC/APCI-MS/MS						Pure alkaloids		
A1 *m/z*	A2 (Orantine) *m/z*	A3 *m/z*	A4 *m/z*	A5 (Aphelandrine) *m/z*	A6 (Chaenorpine) *m/z*	**1** (Aphelandrine) *m/z*	**2** (Orantine) *m/z*	**7** (Chaenorpine) *m/z*
476	476	–	–	476	476	476	476	476
464	–	–	464	–	–	464	464	464
419	419	–	–	–	419	419	419	419
348	348	348	348	348	348	348	348	348
322	322	–	–	–	–	322	322	322
265	265	265	265	265	265	265	265	265
251	251	251	251	251	251	251	251	251
–	198	–	–	198	–	198	198	–
–	155	155	–	–	–	155	155	155
129	129	129	–	129	129	129	129	129
112	112	–	112	112	112	112	112	112

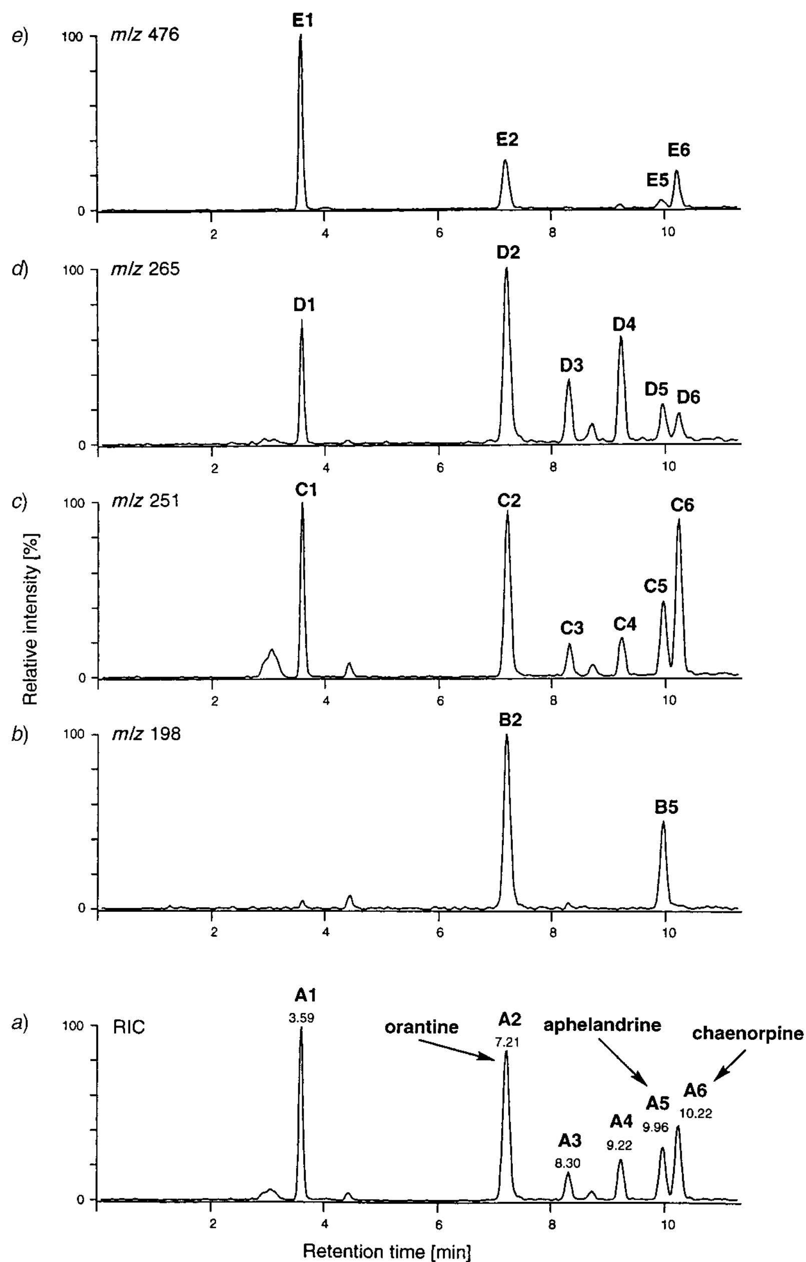


Figure. *HPLC/APCI-MS Chromatogram of the coupling products of dihydroxyverbacine* (**10**): a) *reconstructed ion chromatogram* (RIC) *after CID of the quasi-molecular ions* m/z *493 and* b) – d) *extracted-ion chromatograms of daughter fragment ions* m/z *198, 251, 265, and 476*

and B5 at *m*/*z* 198 were characteristic only for **1** and **2** and can, therefore, be used as a finger print for these alkaloids. The structure of the coupling products A1 (3.59 min), A3 (8.30 min), and A4 (9.22 min) could not definitively be elucidated (see also below).

Some conclusions and propositions according to the coupling possibilities seem appropriate. The hemin forms, after reaction with H_2O_2, an oxoiron(IV) radical cation that can act as a two-electron oxidizing reagent [24][26]. The heme proteins perform their oxidations within a catalytic cycle, which is possible also for the hemin (*Scheme 5*). In the case of the precursor **10**, radical cations can be formed in both phenol moieties of the molecule. Concerning the coupling reaction, *Barton* and *Cohen* [10] favored the 'radical pairing hypothesis', although they envisaged as well the 'oxidation substitution oxidation' mechanism. However, it is possible that in the case of compound **10**, both mechanisms play a role, *e.g.,* the 'oxidation substitution oxidation' mechanism would provide **1** and **2**, whereas the radical pairing would be more appropriate for the formation of chaenorpine or chaenorhine analogue (*Scheme 5*). It is reasonable to assume that the coupling occurs predominantly at positions with higher electron density. Computer calculations suggested the location of the electron density at the positions shown in *Scheme 5*. Additionally, a high electron density is located at the *para* position to the OH group, which is, however, highly hindered. These calculations are in accordance with the observed coupling products. For the formation of aphelandrine (**1**) and orantine (**2**), the configuration of the double bond (*E* or *Z*) in the precursor **10** should not affect the cyclization. In this case, the orientation (*Re* or *Si* face of the C=C bond) should play a significant role. It must be pointed out that in the observed chaenorpine (**7**), the C=C bond possesses the (*Z*)-configuration. That, from the (*E*)-configurated precursor **10** chaenorpine (**7**) was obtained, indicates an isomerization after the formation of the radical-cation species (*Scheme 5*). The coupling products A1, A3, and A4 (see above and *Fig.*) are probably chaenorhine analogues ((*E*) and (*Z*) isomers are possible) and (*E*)-chaenorpine. It should be further pointed out that the oxidative phenol coupling presented was relatively fast, but obviously not selective, according to the reagents and conditions applied.

In conclusion, it was demonstrated that the oxidative phenol coupling of dihydroxyverbacine (**10**), according to the biogenetic pathway, leading to the naturally occurring aphelandrine (**1**), orantine (**2**), and chaenorpine (**7**) is possible by means of the simple 'instruments' of synthetic organic chemistry.

We thank the *Swiss National Science Foundation* and the *Dr. Helmut Legerlotz Stiftung* of the Organisch-chemisches Institut der Universität Zürich for generous financial support. *V. D.* thanks Dr. *K. Drandarov* for providing his experience for the synthesis of dihydroxyverbacine and Dr. *L. Bigler* for the introduction in the secrets of mass spectrometry. We thank Dr. *R. Kunz* for the computer calculations.

Experimental Part

General. Materials and solvents: (−)-(8*S*)-1-[(*E*)-3-(4-Hydroxyphenyl)prop-2-enoyl]-8-(4-hydroxyphenyl)-1,5,9,13-tetraazacycloheptadecan-6-one ((*S*)-**10**) was prepared according to [16][19]; MeCN (HPLC-grade, *Scharlau*, E-Barcelona); AcOH (*Fluka*, *purum*, Switzerland); the water was purified with an *Milli-*Q_{RG} apparatus (*Millipore*, Milford, MA, USA). TLC: precoated silica gel *60* F_{254} plates (*Merck*); visualization by irradiation with UV light, by *Schlittler*'s reagent, and $Ce(SO_4)_2/H_2SO_4$ soln. Column chromatography: silica gel *Merck 60* (230–400 mesh). IR Spectra: *Perkin-Elmer 781*; $\tilde{\nu}$ in cm^{-1}. ^1H- and ^{13}C-NMR Spectra: *Bruker ARX-300*; *Bruker DRX-500*; δ in ppm rel. to Me_4Si as internal standard. HPLC: *Waters 626-LC* system, with *996-*

photodiode-array detector, *600S* controller, and *Millenium Chromatography Manager 2010* v. 2.15 (*Waters Corp.*, Milford, MA, USA); *Rheodyne 77251* rotary valve fitted with a 5-μl loop (*Rheodyne*, Cotati, CA, USA); *Waters Symmetry™-C8* column with integrated guard column (5 μm, 3.9 × 150 mm); flow rate 0.5 ml min^{-1}; mobile phase: *A*, H_2O; *B*, MeCN; *C* 10% AcOH in H_2O; linear gradient *A*/*B*/*C* 87 : 3 : 10 → 10 : 80 : 10 within 30 min. APCI-MS and APCI-MS/MS: *Finnigan TSQ-700* triple-stage quadrupole instrument equipped with an atmospheric pressure chemical ionization (APCI) ion source (*Finnigan*, San José, CA, USA); APCI operating conditions in the positive mode: vaporizer temp. 450°, corona voltage 5 kV, heated capillary temp. 220°, cheat gas N_2 with an inlet pressure of 40 psi, conversion dynode − 15 kV; MS/MS experiments: collision gas Ar with a relative pressure 2.5 – 3.3 mTorr, collision-induced dissociation offset (Coff) − 35 eV.

Ethyl (±)-3-{{3-{{4-[(3-Aminopropyl)amino]butyl}amino}propyl}amino}-3-(4-methoxyphenyl)propanoate (**19**). To a soln. of spermine (**17**; 20.23 g, 99.98 mmol) in EtOH (100 ml) were added ethyl 3-(4-methoxyphenyl)-3-oxopropanoate (**18**; 7.41 g, 33.34 mmol) in EtOH (35 ml) and AcOH (24.60 g, 409.66 mmol). The mixture was refluxed for 2 h, cooled to r.t., and then MeOH (100 ml), AcOH (16.70 g, 278.10 mmol), and a soln. of $NaCNBH_3$ (2.40 g, 38.19 mmol) in MeOH (30 ml) were added. The mixture was stirred overnight at r.t. After evaporation H_2O (100 ml) was added, and the mixture was extracted with $CHCl_3$ (3 × 100 ml). The aq. phase was alkalinized with K_2CO_3 and extracted with $CHCl_3$ (1 ×) and $CHCl_3$/i-PrOH 4 : 1 (5 ×). After evaporation of the extract, the residue was dissolved in EtOH and the soln. acidified with 32% aq. HCl soln. The precipitated tetrahydrochloride of **19** was filtered off, washed with EtOH and Et_2O, and recrystallized from AcOH (40 ml): 9.35 g (51% rel. to **18**) of **19** · 2 HCl. TLC (silica gel, $CHCl_3$/MeOH/25% aq. NH_3 soln. 7 : 3 : 1): R_f 0.40. IR (KBr): 3435, 2986, 1735, 1613, 1515, 1463, 1300, 1255, 1188, 1180, 1112, 1030, 992, 845, 825, 750. ^{1}H-NMR (CD_3OD): 7.48 (*d*, *J* = 8.8, 2 arom. H); 7.02 (*d*, *J* = 8.8, 2 arom. H); 4.66 (*m*, H−C(3)); 4.06 (*q*, *J* = 7.1, MeC*H*$_2$); 3.82 (*s*, MeO); 3.18 – 2.84 (*m*, 14 H); 2.24 – 2.04 (*m*, 4 H); 1.83 (br. *m*, 4 H); 1.14 (*t*, *J* = 7.1, *Me*CH$_2$. ^{13}C-NMR: 172.5 (*s*, C=O); 162.5 (*s*, C_p); 131.2 (*d*, arom. C); 126.4 (*s*, C_{ipso}); 115.8 (*d*, arom. C); 62.4 (*t*, C(2)); 60.2 (*d*, C(3)); 56.0 (*q*, MeO); 48.3, 48.2, 46.0, 44.1, 38.8, 38.0, 25.4, 24.2, 24.1 (9*t*, 9 CH_2); 14.3 (*q*, Me). CI-MS: 409 (38, [*M* + 1]$^+$), 337 (12), 224 (26), 207 (86), 203 (100, [spermine + H]$^+$).

(±)-8-(4-Methoxyphenyl)-1,5,9,13-tetraazacycloheptadecan-6-one (= *(±)-Buchnerine*; **14**). For the preparation of the free base, **19** · 4 HCl (4.18 g, 7.54 mmol) was dissolved in H_2O (85 ml), the soln. alkalinized with solid K_2CO_3 and extracted with $CHCl_3$/i-PrOH 4 : 1 (5 × 50 ml), the extract evaporated, the residue dissolved in $CHCl_3$, and the soln. filtered and evaporated. The free base **19** was refluxed with benzene (200 ml) for 2 h in a flask equipped with a H_2O remover. The soln. was cooled to 0°, 1M $Sb(OEt)_3$ in toluene (9.65 mmol) was added, and the mixture was refluxed for 14 h. After cooling to 0°, the mixture was quenched with MeOH (50 ml) and chromatographed (silica gel, $CHCl_3$/MeOH/25% aq. NH_3 soln. 78 : 19 : 3 → 7 : 3 : 1): 1.46 g (53%) of **14**. Colorless oil. TLC (silica gel, $CHCl_3$/MeOH/25% aq. NH_3 soln. 7 : 3 : 1): R_f 0.45. IR ($CHCl_3$): 2995, 2930, 2840, 2675, 1650, 1612, 1510, 1465, 1305, 1250, 1178, 1120, 1035, 909, 833, 658. ^{1}H-NMR ($CDCl_3$): 8.48 (*t*, *J* = 4.8, CONH); 7.26 – 7.12 (*m*, 2 arom. H); 6.90 – 6.79 (*m*, 2 arom. H); 4.03 – 2.27 (*m*, 21 H); 1.89 – 1.36 (*m*, 8 H). ^{13}C-NMR ($CDCl_3$): 171.7 (*s*, C=O); 158.6 (*s*, C_p); 135.4 (*s*, C_{ipso}); 127.8, 113.9 (2*d*, arom. C); 59.7 (*d*, C(8)); 55.2 (*q*, MeO); 48.8, 48.6, 48.2, 47.7, 46.7, 45.7, 38.6, 28.2, 27.9, 27.3, 27.0 (11*t*, 11 CH_2). ESI-MS: 363 ([*M* + H]$^+$).

8-(4-Methoxyphenyl)-1,13-(4-tolylsulfonyl)-1,5,9,13-tetraazacycloheptadecan-6-one (**24**) *and 9-(4-Bromobutyl)-8-(4-methoxyphenyl)-6-oxo-1,13-(4-tolylsulfonyl)-1,5,9,13-tetraazacycloheptadecane-5-carboxylic Acid* (**25**). To a soln. of **23** (2.74 g, 4.44 mmol) in dry DMF (445 ml) Cs_2CO_3 (3.25 g, 9.97 mmol) was added. The mixture was heated at 60° for 40 min and stirred for a further 40 min at r.t. Then 1,4-dibromobutane (1.07 g, 4.96 mmol) in DMF (94 ml) was added dropwise. The mixture was stirred for 30 h at r.t. and evaporated. The residue was chromatographed (AcOH, then AcOH/MeOH 12 : 1, 10 : 1, and 9 : 1): 2.59 g (87%) of **24** and 0.22 g (6%) of **25**.

Data of **24**: TLC (silica gel, AcOH/MeOH 6 : 1): R_f 0.38. ESI-MS: 671 ([*M* + H]$^+$). Spectroscopic data: see [16][19].

Data of **25**: Colorless glass-like solid. TLC (silica gel, AcOH/MeOH 6 : 1): R_f 0.57. ^{1}H-NMR: br. signals, no interpretation possible. ^{13}C-NMR ($CDCl_3$): 180.00 – 131.18 (br. *s*, several C); 129.65, 128.36, 126.98, 113.93 (*d*, arom. CH); 62.82 (br. *d*, C(8)); 55.19 (*q*, MeO); 50.00 – 24.15 (br. *t*, several CH_2); 28.38 (*q*, Me). ESI-MS: 849 ([*M* + H]$^+$), 871 ([*M* + Na]$^+$), 887 ([*M* + K]$^+$). Anal. calc. for $C_{39}H_{53}BrN_4O_8S_2$ (849.90): C 55.11, H 6.29, Br 9.40, N 6.59, S 7.55; found: C 56.43, H 6.29, Br 8.84, N 6.87, S 7.56.

Hemin/H_2O_2 Oxidation of Dihydroxyverbacine (**10**). Dihydroxyverbacine (**10**) (3 mg, 0.0060 mmol) and hemin (1.3 mg, 0.0019 mmol) were dissolved in MeCN (0.15 ml) and 0.1M eq. K_2CO_3 (0.15 ml). Then 10% H_2O_2 soln. (20 μl, *ca.* 0.0588 mmol) was added, and the mixture was shaken for 10 min. For analysis, the mixture was filtered through a 0.45-μm centrifugal filter tube (*Eppendorf-Netheler-Hinz GmbH*, Hamburg). The mixture was analyzed by HPLC/MS and HPLC/MS/MS (aliquots of 5 μl were injected; see *Fig.*).

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