Macrocyclic Spermine Alkaloids from *Verbascum:* The (*E*/*Z*)-Isomeric Pairs (−)-(*S*)-Verbasitrine/(−)-(*S*)-Isoverbasitrine and (+)-(*S*)-Verbametrine/(+)-(*S*)-Isoverbametrine: Isolation, Structure Elucidation, and Synthesis

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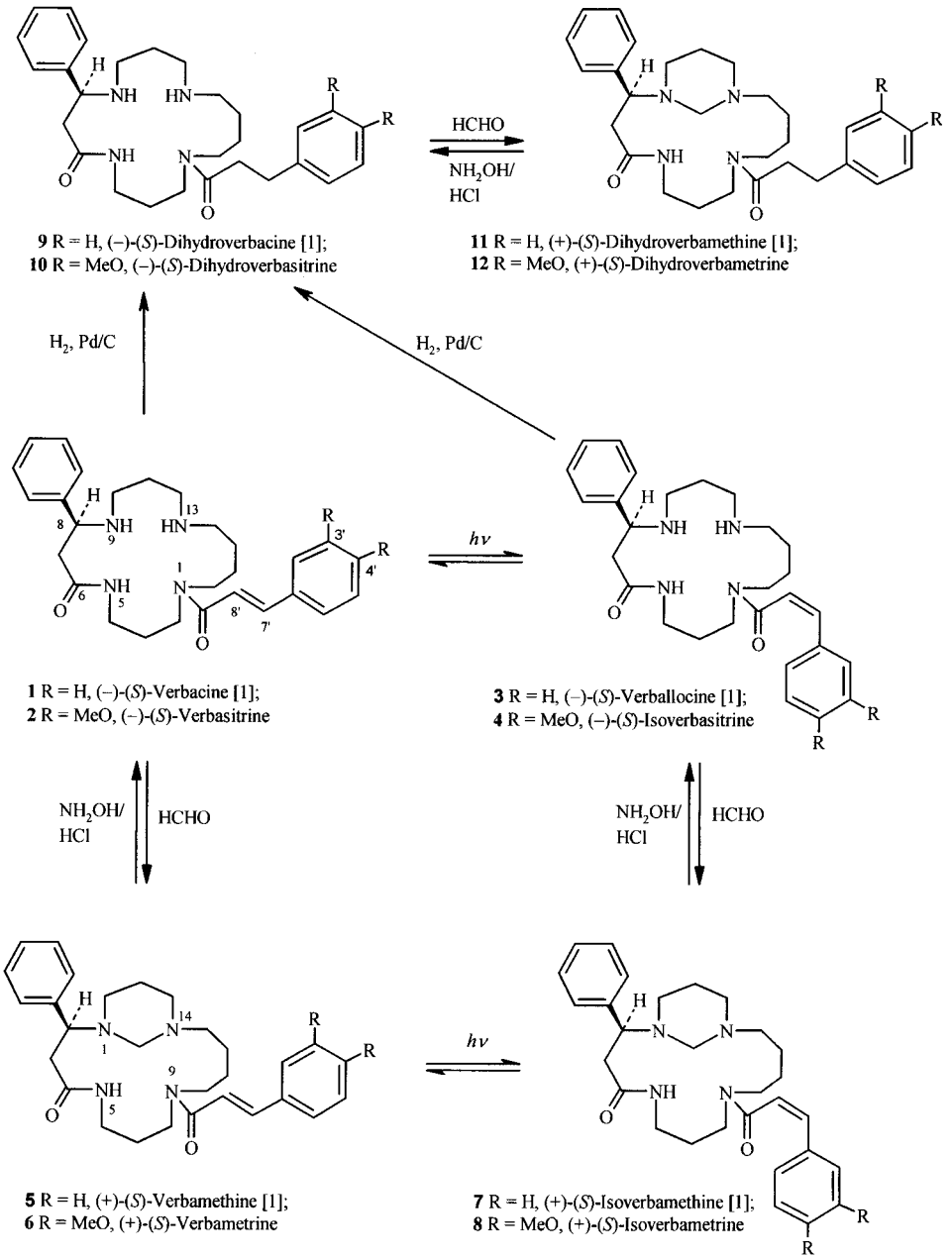
The isolation and structure elucidation of the 17-membered macrocyclic spermine alkaloids (−)-(*S*)-verbasitrine (**2**), (−)-(*S*)-isoverbasitrine (**4**), (+)-(*S*)-verbametrine (**6**), and (+)-(*S*)-isoverbametrine (**8**) is presented. The synthesis of their racemates is described.

Introduction. – The (*E*/*Z*)-isomeric pair of macrocyclic spermine alkaloids (−)-(*S*)-verbacine (**1**) and (−)-(*S*)-verballocine (**3**) has been isolated from the leaves of *Verbascum pseudonobile* STOJ. et STEF. (Scrophulariaceae), together with their corresponding *N*,*N*′-methylene-bridged derivatives, namely (+)-(*S*)-verbamethine (**5**), and (+)-(*S*)-isoverbamethine (**7**) (*Scheme 1*) [1]. In the same plant material, four other alkaloids were detected: (−)-verbasitrine (**2**), (−)-isoverbasitrine (**4**), (+)-verbametrine (**6**), and (+)-isoverbametrine (**8**) (*Scheme 1*). The structure elucidation of these alkaloids **2**, **4**, **6**, and **8**, subject of the present paper, established that they are dimethoxy analogues of **1**, **3**, **5**, and **7**, respectively.

Results and Discussion. – *Structure Elucidation.* From the alkaloid fraction containing (−)-(*S*)-verbacine (**1**) and (−)-(*S*)-verballocine (**3**) (see *Exper. Part*), two minor alkaloids (*ca.* 9% of the total alkaloid extract) were now isolated, named (−)-verbasitrine (**2**; 0.9% yield) and (−)-isoverbasitrine (**4**; 8.1%).

The vigorous acid hydrolysis (2N aq. HCl, 165°, 18 h) of (−)-isoverbasitrine (**4**) yielded spermine (= *N*,*N*′-bis(3-aminopropyl)butane-1,4-diamine; identified by TLC comparison with an authentic sample). The molecular-ion peak of **4** at *m/z* 522 (EI-MS) was found to be 60 amu (atomic mass units) higher than those of (−)-(*S*)-verbacine (**1**) and (−)-(*S*)-verballocine (**3**). This fact suggests for **4** a dimethoxy-substituted analogue of **1** and **3**. Its EI-MS fragmentation pattern is similar to those of **1** and **3**. The fragment ion at *m/z* 331, arising by cleavage of the acyl moiety at N(1) of **4**, corresponds to the 17-membered macrocyclic residue common to all of these alkaloids (*Scheme 1*). This fact, and the presence of a fragment ion at *m/z* 191 (base peak), clearly indicate that the additional substituents of **4** are localized on the peripheral acyl group and suggests a dimethoxycinnamoyl residue. The ^{1}H-NMR spectrum of **4** (room temperature, mixture of conformers) confirms the presence of two aromatic MeO groups (4 *s* at 3.873, 3.87, 3.85, and 3.848 ppm), and a typical *ABX* spin system in the aromatic region (7.06 (*m*, H−C(2′)), 6.97–6.94 (*m*, H−C(6′)), 6.56/

Scheme 1 [1])



9 R = H, (–)-(*S*)-Dihydroverbacine [1];
10 R = MeO, (–)-(*S*)-Dihydroverbasitrine

11 R = H, (+)-(*S*)-Dihydroverbamethine [1];
12 R = MeO, (+)-(*S*)-Dihydroverbametrine

1 R = H, (–)-(*S*)-Verbacine [1];
2 R = MeO, (–)-(*S*)-Verbasitrine

3 R = H, (–)-(*S*)-Verballocine [1];
4 R = MeO, (–)-(*S*)-Isoverbasitrine

5 R = H, (+)-(*S*)-Verbamethine [1];
6 R = MeO, (+)-(*S*)-Verbametrine

7 R = H, (+)-(*S*)-Isoverbamethine [1];
8 R = MeO, (+)-(*S*)-Isoverbametrine

[1]) Arbitrary numbering of the side chain.

6.55 ppm (2*d*, *J* = 8 Hz, H–C(5′)), established the 3,4-position of the dimethoxy substitution in the acyl residue at N(1).

Additionally, the saponification of (–)-isoverbasitrine (**4**) (KOH, ethylene glycol, reflux 1.5 h) gave a mixture of commensurate amounts of (*E*)-cinnamic and (*E*)-3,4-dimethoxycinnamic acids, which were identified by comparison with authentic samples (TLC, m.p., IR spectra). These acids are formed by cleavage of the benzylic C(8)–N(9) bond by β-elimination (*retro-Michael* reaction) and simultaneous saponification of the arising *N*,*N*′-diacylspermine.

The ^{1}H-NMR spectrum of (–)-isoverbasitrine (**4**) indicates (*Z*)-configuration of its 3,4-dimethoxycinnamoyl residue (*d* at 6.56 and 6.55 and *d* at 5.96 and 5.95 ppm with *J* = 13 Hz), but under the saponification conditions (1.5 h at 197°), a quantitative thermal (*Z*) → (*E*) isomerization took place, and only (*E*)-3,4-dimethoxycinnamic acid (together with (*E*)-cinnamic acid) was isolated.

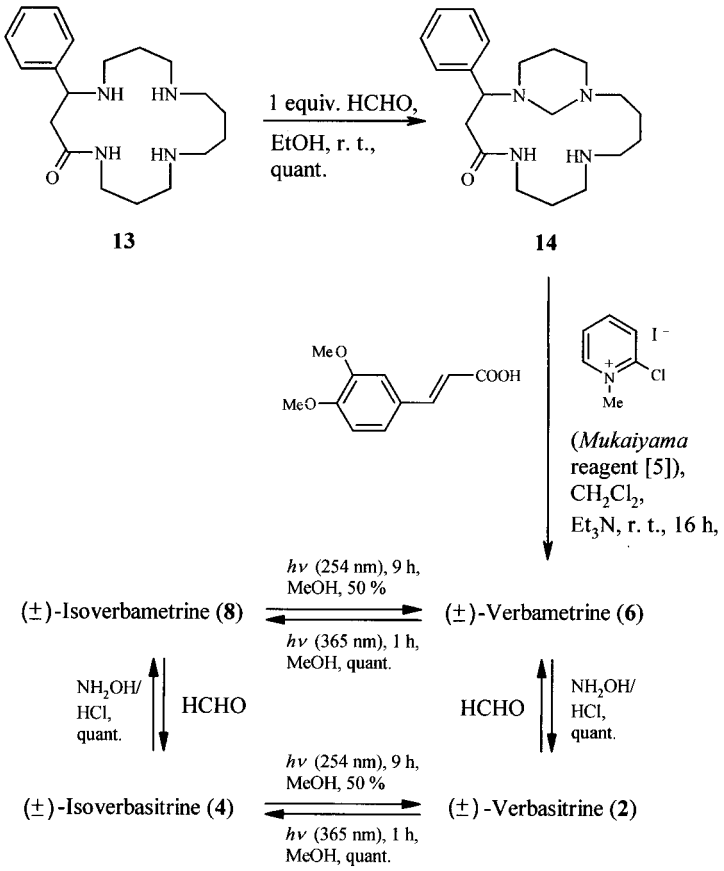
From the same alkaloid mixture, also the (*E*)-isomer of (–)-isoverbasitrine (**4**) was isolated, namely (–)-verbasitrine (**2**) (ESI-MS: $[M+H]^+$ *at m/z* 523; ^{1}H-NMR: 2 olef. H at 7.65 and 6.70 ppm (*J* = 15 Hz)). The catalytic hydrogenation of both (–)-verbasitrine (**2**) and (–)-isoverbasitrine (**4**) gave the same dihydro derivative **10** (*Scheme 1*).

(–)-Verbasitrine (**2**), (–)-isoverbasitrine (**4**), and (–)-dihydroverbasitrine (**10**) reacted quantitatively with formaldehyde, giving the bicyclic aminals **6**, **8**, and **12**, all with positive $[\alpha]_D$ value (*Scheme 1*). This fact indicates the same localization of the 3,4-dimethoxycinnamoyl residue (at N(1)) in these alkaloids as those of their non-substituted analogues (–)-verbacine (**1**), (–)-verballocine (**3**), (–)-dihydroverbacine (**9**), (+)-verbamethine (**5**), (+)-isoverbamethine (**7**), and (+)-dihydroverbamethine (**11**) (*Scheme 1*).

(+)-(*S*)-Verbamethine (**5**; 5% of the total alkaloid extract) and (+)-(*S*)-isoverbamethine (**7**, 4%) were isolated from the alkaloid mixture, but were first interpreted as artifacts [1]. Later it was established that they are of natural origin [2]. From the same alkaloid mixture, also the cyclic aminals (+)-verbametrine (**6**; 0.1%) and (+)-isoverbametrine (**8**; 0.9%) were isolated. By mild acid hydrolysis in the presence of NH_2OH (a HCHO interceptor) [1], **6** and **8** were converted quantitatively to **2** and **4**, respectively (*Scheme 1*).

Syntheses. The alkaloid (±)-verbacine (±)-(**1**) has been synthesized recently by an antimony-templated macrolactamization method [3]. An alternative synthetic strategy has been mentioned in [4]. Both methods led to the 17-membered macrocyclic compound **13** as an intermediate. According to [4], starting from compound **13** (prepared by both methods) and formaldehyde, the corresponding *N*,*N*′-methylene-bridged derivative **14** was obtained (*Scheme 2*). Following *N*-acylation with (*E*)-3,4-dimethoxycinnamic acid, using *Mukaiyama*'s procedure [5], (±)-verbametrine ((±)-**6**) was prepared from **14** in excellent yield. The mild acid hydrolysis of the latter, in the presence of NH_2OH, furnished (±)-verbasitrine ((±)-**2**). By (*E*) → (*Z*) photoisomerization, (±)-**6** was converted to (±)-isoverbametrine ((±)-**8**). Finally, ((±)-**8**) was hydrolyzed to (±)-isoverbasitrine ((±)-**4**) (*Scheme 2*). Most of these transformations proceeded almost quantitatively, and the synthetically prepared racemic alkaloids were spectroscopically (UV, IR, NMR, MS) and chromatographically identical with the natural alkaloids.

Scheme 2



Recently, the (*S*)-isomers of compounds **13** and **14** were isolated from *V. pseudonobile* as natural secondary metabolites and named (−)-(*S*)-protoverbine ((−)-**13**) and (+)-(*S*)-protomethine ((+)-**14**); they are biogenetic precursors of the present class of alkaloids, *i.e.*, of compounds **1**–**8**, (*Schemes 1* and *2*) [2].

Absolute Configuration. The CD spectra of (−)-dihydroverbasitrine (**10**) and (+)-dihydroverbametrine (**12**) (*Fig.*) are equally shaped as those of (−)-(*S*)-dihydroverbacine (**9**) and (+)-(*S*)-dihydroverbamethine (**11**), respectively [6]. Thus, the (*S*)-chirality of **10** and **12**, and of their unsaturated natural analogues **2**, **4**, **6**, and **8** is established.

The localization and the number of *Cotton* effects between 250 and 275 nm (same as in the case of **9** and **11** [6]) in the CD spectra of **10** and **12** is typical for a non-substituted benzene chromophore, directly attached to a chiral center [6] [7]. This fact independently confirms the localization of the MeO substitution in the side chain of the acyl residue. The chiroptical properties of this class of macrocyclic lactam alkaloids were discussed in detail in [6].

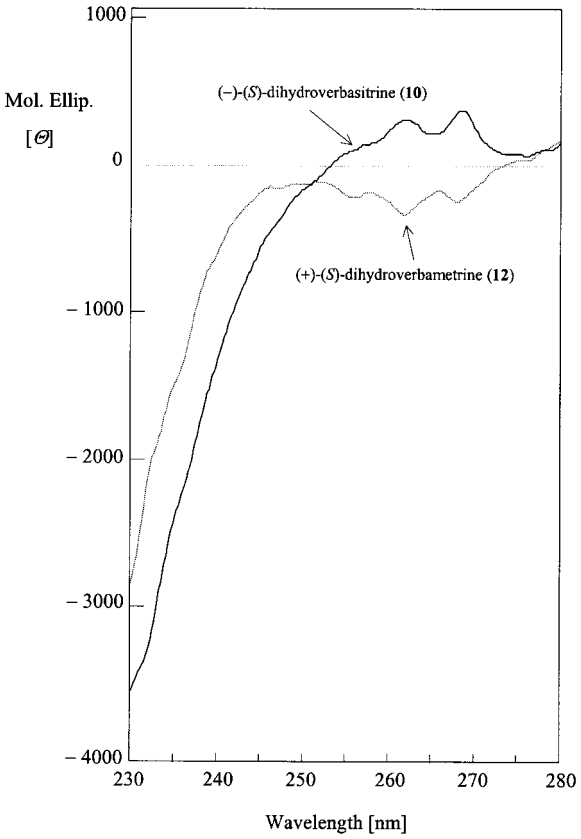


Fig. *CD Curves of (–)-(*S*)-dihydroverbasitrine (***10***) and (+)-(*S*)-dihydroverbametrine (***12***)*

Photoisomerization of the Alkaloids **1**–**8**. A number of natural compounds carrying a cinnamamide moiety have been isolated from different plant species in both (*E*)- and (*Z*)-forms [8]. In some cases, the presence of the (*Z*)-isomers along with their (*E*)-counterparts has been ascribed to an artificial isomerization under the isolation conditions [9]. In some cases of hydroxycinnamic acid derived alkaloids, only the (*Z*)-isomer was observed [10].

The exposure of either (+)-(*S*)-verbametrine (**6** (*E*) or (+)-(*S*)-isoverbametrine (**8** (*Z*) to light of 254 nm gave a *ca.* 1:1 (*E*)/(*Z*)-mixture. On the other hand, the exposure of **6** (*E*) for 1 h to light of 365 nm, or for 2 h to sunlight caused its almost quantitative photoisomerization to its (*Z*)-isomer **8**. (–)-(*S*)-Verbasitrine (**2** (*E*)) and (–)-(*S*)-isoverbasitrine (**4** (*Z*)) showed a similar photoisomerization behavior. The irradiation of MeOH solutions of the non-substituted cinnamic acid amides **1** (*E*), **3** (*Z*), **5** (*E*), and **7** (*Z*) at 254 (16 h) or 365 nm (45 h) resulted in *ca.* 1:1 (*E*)/(*Z*)-mixtures [1][2]).

[2]) The 160-h exposure of the 0.4% MeOH soln. of **5** (*E*) and **7** (*Z*) to light of 365 nm (under Ar) gave a *ca.* 1:4 (*E*)/(*Z*)-mixture

The isomeric pairs of the non-substituted cinnamamides (−)-(*S*)-verbacine (**1** (*E*))/(−)-(*S*)-verballocine (**3** (*Z*)) and (+)-(*S*)-verbamethine (**5** (*E*))/(+)-(*S*)-isoverbamethine (**7** (*Z*)) were established to occur in the alkaloid extract from *V. pseudonobile* in a (*E*/*Z*)-ratio of *ca.* 5:4, and the isomeric pairs of the 3,4-dimethoxycinnamamides (−)-(*S*)-verbasitrine (**2** (*E*))/(−)-(*S*)-isoverbasitrine (**4** (*Z*)) and (+)-(*S*)-verbametrine (**6** (*E*))/(+)-(*S*)-isoverbametrine (**8** (*Z*)) in a ratio of *ca.* 1:9. These ratios were similar to the mixtures observed after irradiation of these compounds by UV light at 365 nm or by sunlight. Thus, for the present class of alkaloids, we can conclude that the (*Z*)-isomers arise in the intact plant tissues (leaves) by sunlight-mediated (*E*) → (*Z*) photoisomerization of the corresponding (*E*)-isomers.

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Experimental Part

1. *General*. TLC: *Merck* precoated plates, *silica gel 60* F_{254}; detection by *Dragendorff*'s and ninhydrine reagents (No. D 156a and D 127a in [11]); for more details about TLC retention of the (*E*/*Z*)-isomers of macrocyclic spermine alkaloids, their dihydro derivatives, and simpler cinnamamides, see [12]. Prep. TLC: *silica gel* GF_{254} from *Merck*. CC: *silica gel 60* (70–230 mesh) from *Merck* and alumina *N* (act. 1) from *ICN Biomedicals*. Optical rotation: *Perkin-Elmer-241* polarimeter. UV: *Perkin-Elmer 555*; λ_{max} (log ε) in nm. CD Spectra: *JASCO J-715* spectropolarimeter at r.t. in EtOH, 1-cm quartz cell, between 230 and 280 nm; λ (molecular ellipticity [θ]) in nm. IR: *Perkin-Elmer 297*; film; in cm^{-1}. ^{1}H-NMR: *Bruker AC-300* or *ARX-300*. ^{13}C-NMR: *Bruker ARX-300* (75 MHz); chemical shifts δ in ppm rel. to $SiMe_4$ as internal standard; $CDCl_3$ solns. at r.t. EI-MS (70 eV): *Finnigan-MAT 90*. ESI-MS: *Finnigan-TSQ-700* mass spectrometer.

Plant Material and Extraction Procedure. Air-dried leaves of *Verbascum pseudonobile*, cultivated and collected in summer in south-west Bulgaria, were used. The plant material was extracted twice overnight with 3% aq. H_3PO_4 soln. The combined extracts were alkalinized (25% aq. NH_3 soln.) and extracted twice with $CHCl_3$. The $CHCl_3$ extracts were evaporated and reextracted with 3% aq. H_3PO_4 soln. The acidic aq. reextract was alkalinized (25% aq. NH_3 soln.), extracted with $CHCl_3$ to yield, after evaporation, the total alkaloid extract. All extraction procedures were performed in diffuse room light. From 500 g of dry leaves, 3 g (0.6%) of the alkaloid mixture were obtained.

Extractive Separation of the Secondary Amines **1**–**4** *from Their Aminals* **5**–**8**. The total alkaloid extract contained as main constituents the group of the secondary amines **1**–**4** (*ca.* 90%) and the group of their cyclic aminals **5**–**8** (*ca.* 10%), the former being quite stronger bases than the latter. This was the reason for their separation as groups by extraction from an aq. soln. at different pH. Therefore, the $CHCl_3$ soln. of the total alkaloid mixture was extracted with 3% aq. H_3PO_4 soln., the pH of the acidic aq. extract was corrected to 5 with dil. NaOH soln., and this aq. phase extracted with $CHCl_3$. The cyclic aminals **5**–**8** passed to the $CHCl_3$ phase (*Extract A*). The aq. phase containing **1**–**4** was alkalinized with NaOH and extracted with $CHCl_3$ (*Extract B*). *Extract A* (a soln. of the aminals **5**–**8**) was evaporated and the residue (10% from the starting amount of mixture) separated by prep. TLC on hand-made plates (5 g of silica gel + 15 ml of 1% aq. $NaHCO_3$ soln. for one 20 × 20 cm plate, activation 30 min at 110° before use, 30 mg of mixture in 0.5 ml of $CHCl_3$ per plate). The plates were developed twice in toluene/96% aq. EtOH soln. 9:1. Before the second development, the plates were impregnated for 1 min in a Et_3N atmosphere. From the separated scraped zones, *(+)-verbamethine* (**5**; R_f 0.45), *(+)-isoverbamethine* (**7**; R_f 0.35), *(+)-verbametrine* (**6**; blue fluorescence zone at 365 nm, R_f 0.25), and *(+)-isoverbametrine* (**8**; R_f 0.2) were recovered with EtOH satd. with NH_3 gas. Compounds **5** (5%), **6** (0.1%), **7** (4%), and **8** (0.9%) were purified by CC (alumina, AcOEt/MeOH 9:1).

The mixture of the secondary-amine derivatives *(−)-verbacine* (**1**; 45%), *(−)-verbasitrine* (**2**; 0.9%), *(−)-verballocine* (**3**; 36%), and *(−)-isoverbasitrine* (**4**; 8.1%) (*Extract B*) was not easily separated by prep. TLC. Thus, **1**–**4** were separated in form of their *N,N′*-methylene-bridged derivatives **5**–**8** after derivatization with formaldehyde. Mild acidic hydrolysis of **5**–**8** in the presence of NH_2OH easily reconverted them to **1**–**4** (*Scheme 1*), as reported earlier [1].

*(−)-(8*S*)-1-[(*E*)-3-(3,4-Dimethoxyphenyl)prop-2-enoyl]-8-phenyl-1,5,9,13-tetraazacycloheptadecan-6-one* (=*(−)-(*S*)-Verbasitrine*; **2**). Colorless glass-like solid. TLC (silica gel, BuOH/AcOH/H_2O 4:1:5): R_f 0.28.

$[\alpha]_D^{25} = -14$ ($c = 1.85$, $CHCl_3$). UV ($c = 0.004$, EtOH): max. 294 (sh, 3.66), 320 (3.71); min. 260 (3.45), 302 (3.66). IR: 1640*s* (C=O, amide I), 1595*s* (C=C), 1550*m* (CONH, amide II), 1510*s*, 1260*s*. ^{1}H-NMR (mixture of conformers)[1]): 8.45 (br. *t*, 0.5 H, CONH···N); 7.65 (*d*, *J* = 15, H–C(7′)); 7.42 (br. *t*, 0.5 H, CONH); 7.34–7.20 (*m*, 5 arom. H); 7.14 (*t*, *J* = 8, H–C(6′)); 7.01 (br. *s*, H–C(2′)); 6.88 (*t*, *J* = 8, H–C(5′)); 6.70 (*t*, *J* = 15, H–C(8′)); 3.95 (*m*, PhC*H*N); 3.92–3.8 (*m*, 7 H, incl. 2 arom. MeO at 3.92 and 3.91); 3.75–3.0 (*m*, 3 CH_2N); 2.80–1.4 (*m*, 17 H). ^{13}C-NMR (mixture of conformers): 171.2, 169.5 (2 C=O); 150.5, 149.0 (arom. quat. C), 142.7, 128.5, 128.2, 127.2, 126.6, 126.3, 121.4, 115.4, 111.2, 110.6, 110.2 (CH=CH); 59.6 (Ph*C*N); 55.9 (arom. MeO); 49.5, 48.8, 48.5, 48.3, 46.5, 45.8, 43.8, 43.2, 37.5, 36.5, 30.3, 29.4, 28.4, 28.2, 26.7 (CH_2). ESI-MS: 523 ($[M+H]^+$).

The synthetic *(±)-verbasitrine* ((±)-**2**) was prepared by acid hydrolysis of *(±)-verbametrine* ((±)-**6**) in the presence of NH_2OH, according to [1]. ESI-MS: 523 ($[M+H]^+$).

*(−)-(8*S*)-1-[(*Z*)-3-(3,4-Dimethoxyphenyl)prop-2-enoyl]-8-phenyl-1,5,9,13-tetraazacycloheptadecan-6-one* (*=(−)-(*S*)-Isoverbasitrine*; **4**). Colorless glass-like solid. TLC (silica gel, BuOH/AcOH/H_2O 4:1:5): R_f 0.27. $[\alpha]_D^{28} = -15$ ($c = 2$, $CHCl_3$). UV ($c = 0.002$, EtOH): max. 230 (sh, 4.19), 272 (4.08), 296 (sh. 3.97); min. 250 (3.93). IR: 1640*s* (C=O, amide I), 1600*s* (C=C), 1550*m* (CONH, amide II), 1510*s*, 1270*s*, 1140*s*, 1030*s*. ^{1}H-NMR (mixture of conformers)[1]): 8.25 (br. *t*, 0.5 H, CONH···N); 7.66 (br. *t*, 0.5 H, CONH); 7.35–7.15 (*m*, 5 arom. H); 7.06 (*m*, H–C(2′)); 6.97–6.94 (*m*, H–C(6′)); 6.81, 6.79 (2*d*, *J* = 8, H–C(5′)); 6.56, 6.55 (2*d*, *J* = 13, H–C(7′)); 5.96, 5.95 (2*d*, *J* = 13, H–C(8′)); 4.0–3.90 (*m*, PhC*H*N); 3.873, 3.87, 3.85, 3.848 (4*s*, 2 arom. MeO)[1]); 3.75–2.90 (*m*, 4 CH_2N); 2.80–2.40 (*m*, 8 H); 1.95–1.3 (*m*, 8 H). ^{13}C-NMR (mixture of conformers): 171.6, 171.4, 169.3 (2 C=O); 149.2, 148.6, 142.8, 142.5 (arom. quat. C); 132.9, 132.8, 128.7, 128.6, 127.3, 127.2, 126.5, 126.2, 121.8, 121.7, 111.3, 111.2, 110.8 (CH=CH); 59.6 (Ph*C*N), 55.8 (arom. MeO); 49.6, 48.5, 48.2, 47.2, 46.2, 45.5, 44.0, 43.7, 43.2, 37.1, 36.4, 29.6, 29.2, 28.4, 26.8, 26.5, 26.3, 24.9 (CH_2). ESI-MS: 523 ($[M+H]^+$). EI-MS: 522 (11, $M^{+\bullet}$), 479 (13), 331 (10, $[M-(MeO)_2C_6H_3-CH=CH-C\equiv O]^+$), 288 (10), 191 (100, $[(MeO)_2C_6H_3-CH=CH-C\equiv O]^+$), 146 (15), 131 (10), 127 (5).

(±)-Isoverbasitrine ((±)-**4**). A mixture of 50 mg of *(±)-isoverbametrine* ((±)-**8**) and 50 mg of NH_2OH in 3 ml of 1% aq. HCl soln. was heated at 60° for 1 h [1], alkalinized with 25% aq. NH_3 soln., and extracted with $CHCl_3$. The org. extract was washed with H_2O, dried (Na_2SO_4), and evaporated to give *(±)-isoverbasitrine* ((±)-**4**) almost quantitatively, as a colorless glass-like solid. ^{1}H- and ^{13}C-NMR: identical[3]) with those of the natural (−)-(*S*)-isoverbasitrine (**4**). ESI-MS: 523 ($[M+H]^+$).

*(+)-(2*S*)-9-[(*E*)-3-(3,4-Dimethoxyphenyl)prop-2-enoyl]-2-phenyl-1,5,9,14-tetraazabicyclo[12.3.1]octadecan-4-one* (*= (+)-(*S*)-Verbametrine*; **6**). Colorless glass-like solid. TLC (silica gel, toluene/EtOH/H_2O 90:9.6:0.4; blue fluorescence at 365 nm): R_f 0.13. $[\alpha]_D^{28} = +8$ ($c = 2$, $CHCl_3$). UV ($c = 0.0028$, EtOH): max. 232 (sh, 3.44), 296 (sh., 3.44), 320 (3.50); min. 260 (3.16). IR: 1660*s* (C=O, amide I), 1590*s* (C=C), 1540*m* (CONH, amide II), 1515*s*, 1260*s*. ^{1}H-NMR[1]): 9.53, 9.33 (2 br. *s*, CONH···N); 7.67, 7.66 (2 *d*, *J* = 15, H–C(7′)); 7.40–7.25 (*m*, 3 arom. H); 7.2–7.05 (*m*, 3 arom. H incl. H–C(6′)); 7.02 (*s*, H–C(2′)); 6.87 (*d*, *J* = 8, H–C(5′)); 6.68, 6.67 (2 *d*, *J* = 15, H–C(8′)); 4.02 (br. *d*, PhC*H*N); 3.95–3.85 (*m*, 7 H, incl. 2 arom. MeO); 3.75–2.5 (*m*, 5 CH_2N); 2.5–2.15 (*m*, 3 H); 2.0–1.4 (*m*, 10 H). ^{13}C-NMR: 171.8, 166.0 (2 C=O); 150.4, 149.0 (arom. quat. C); 142.5, 142.3, 135.8, 128.4, 128.2, 127.9, 121.3, 115.3, 115.0, 111.1, 110.5, 110.3 (CH=CH); 64.0 (Ph*C*N); 55.9 (arom. MeO); 50.8, 47.1, 45.7, 36.4, 29.5, 27.2, 25.3, 21.5, 21.2 (CH_2). ESI-MS/MS (−28 eV): 535 (26, $[M+H]^+$), 191 (100, $[(MeO)_2C_6H_3-CH=CH-C\equiv O]^+$).

(±)-Verbametrine ((±)-**6**). To a suspension of 64 mg (0.25 mmol) of 1-methyl-2-chloropyridinium iodide [5] and 44 mg (0.21 mmol) of (*E*)-3,4-dimethoxycinnamic acid in 2 ml of CH_2Cl_2 and 0.1 ml of Et_3N, a soln. of 72 mg (0.21 mmol) of **14** (prepared from **13** [2–4] and HCHO according to [2] [4]) was added dropwise under Ar. The mixture was stirred at r.t. for 16 h. Then it was washed with 10% aq. K_2CO_3 soln. and evaporated. The residue was purified by CC (alumina, AcOEt/MeOH 9:1): (±)-**6** (102 mg, 91%). Colorless glass-like solid. TLC (silica gel, toluene/EtOH/H_2O 90:9.6:0.4; blue fluorescence at 365 nm): R_f 0.13. IR, ^{1}H- and ^{13}C-NMR: identical with those of the natural (+)-verbametrine (**6**). ESI-MS/MS (−28 eV): 535 (24, $[M+H]^+$), 191 (100, $[(MeO)_2C_6H_3-CH=CH-C\equiv O]^+$).

*(+)-(2*S*)-9-[(*Z*)-3-(3,4-Dimethoxyphenyl)prop-2-enoyl]-2-phenyl-1,5,9,14-tetraazabicyclo[12.3.1]octadecan-4-one* (*= (+)-(*S*)-Isoverbametrine*; **8**). Colorless glass-like solid. TLC (silica gel, toluene/EtOH/H_2O 90:9.6:0.4): R_f 0.12. $[\alpha]_D^{28} = +5$ ($c = 1.08$, $CHCl_3$). UV ($c = 0.0028$, EtOH): max. 230 (sh, 4.07), 270 (3.94),

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[3]) To obtain reproducible superimposable NMR spectra, the samples of the natural (−)-(*S*)-isoverbasitrine (**4**) and the synthetic (±)-isoverbasitrine ((±)-**4**) were dissolved in $CHCl_3$, the solns. washed with aq. NH_3 soln., dried (Na_2SO_4), and evaporated, and the ^{1}H- and ^{13}C-NMR spectra measured immediately.

298 (sh, 3.77); min. 250 (3.77). IR: 1640*s* (C=O, amide I), 1600*s* (C=C), 1550*m* (CONH, amide II), 1515*s*, 1270, 1140*s*, 1025*s*. ^{1}H-NMR[1]): 9.53, 9.33 (2 br. *s*, CONH···N); 7.42–7.25 (*m*, 5 arom. H); 7.08 (*m*, H–C(2′)); 6.98, 6.97 (2*d*, *J* = 6, H–C(6′)); 6.81 (*d*, *J* = 8, H–C(5′)); 6.55, 6.53 (2*d*, *J* = 13, H–C(7′)); 5.98, 5.97 (2*d*, *J* = 13, H–C(8′)); 3.99 (br. *d*, PhC*H*N); 3.96–3.87 (*m*, 7 H, incl. 2 arom. MeO); 3.75–2.5 (*m*, 5 CH_2N); 2.5–2.15 (*m*, 3 H); 2.0–1.3 (*m*, 10 H). ^{13}C-NMR: 171.8, 168.8, 168.6 (2 C=O); 149.2, 148.6 (arom. quat. C); 136.0, 132.9, 128.4, 128.2, 127.9, 127.8, 121.9, 121.4, 111.4, 111.3, 110.2 (CH=CH); 64.0 (Ph*C*N); 55.8 (arom. MeO); 50.8, 47.7, 44.2, 36.3, 28.4, 25.5, 25.0, 21.5, 21.1 (CH_2). ESI-MS: 535 ([*M* + H]$^+$). EI-MS: 534 ($M^{+\cdot}$, 71), 533 (100, [*M* – H]$^+$), 343 (14, [*M* – $(MeO)_2C_6H_3$–CH=CH–C≡O]$^+$), 191 (54, [$(MeO)_2C_6H_3$–CH=CH–C≡O]$^+$), 146 (6), 139 (11), 131 (23).

The synthetic *(±)-isoverbametrine* (±)-(**8**) was prepared by photoisomerization of (±)-verbametrine ((±)-**6**) (see the procedure below).

*(–)-(8*S*)-1-[3-(3,4-Dimethoxyphenyl)propanoyl]-8-phenyl-1,5,9,13-tetraazacycloheptadecan-6-one* (= *(–)-(*S*)-Dihydroverbasitrine*; **10**). Prepared by cat. hydrogenation of **4** over 10% Pd/C according to [1]. Colorless glass-like solid. TLC (silica gel, BuOH/AcOH/H_2O 4:1:5): R_f 0.29. $[\alpha]_D^{28} = -18$ (*c* = 2, $CHCl_3$). UV (*c* = 0.006, EtOH): max. 228 (sh, 4.02), 278 (3.94); min. 252 (2.9). CD (*c* = 0.011%, EtOH): 230 (–3505), 232 (sh, –3227), 236 (sh, –2069), 247 (sh, –339), 251 (sh, –91), 252 (±0), 256 (max., +133), 258 (infl., +186), 262 (max., +333), 265 (min., +236), 269 (max., +400), 274 (+101). IR: 1635*s* (C=O, amide I), 1550*m* (CONH, amide II), 1515*s*, 1260*s*, 1235*m*, 1155*m*, 1140*m*, 1030*m*. ^{1}H-NMR (mixture of conformers): 8.3 (br. *t*, 0.5 H, CONH···N); 7.5 (br. *t*, 0.5 H, CONH); 7.37–7.15 (*m*, 5 arom. CH); 6.82–6.7 (*m*, 3 arom. H); 4.01–3.90 (*m*, PhC*H*N); 3.87–3.84 (*m*, 2 arom. MeO); 3.57–2.85 (*m*, 4 CH_2N); 2.75–2.4 (*m*, 12 H); 1.9–1.4 (*m*, 8 H). ^{13}C-NMR (mixture of conformers): 172.2, 172.0, 171.7, 171.3 (2 C=O); 148.8, 147.3, 142.8, 142.5, 134.0 (arom. quat. C); 128.7, 128.6, 128.4, 127.3, 127.2, 126.5, 126.3, 120.3, 120.2, 111.9, 111.4, 111.3 (CH=CH); 59.6 (Ph*C*N); 55.8, 55.7 (arom. MeO); 49.1, 48.5, 48.2, 46.6, 46.3, 46.1, 45.7, 45.2, 43.8, 43.2, 37.2, 36.4, 35.5, 31.1, 29.6, 29.2, 28.7, 26.8, 26.6, 25.1 (CH_2). ESI-MS: 525 ([*M* + H]$^+$).

*(+)-(2*S*)-9-[3-(3,4-Dimethoxyphenyl)propanoyl]-2-phenyl-1,5,9,14-tetraazabicyclo[12.3.1]octadecan-4-one* (= *(+)-(*S*)-Dihydroverbametrine*; **12**). To an EtOH soln. of **10** [6], a molar excess of 40% aq. HCHO soln. was added. After several min, the mixture was evaporated to give **12** which was purified by CC (alumina, AcOEt/MeOH 9:1). Colorless glass-like solid. TLC (silica gel, toluene/EtOH/H_2O 90:9.6:0.4): R_f 0.13. $[\alpha]_D^{28} = +8$ (*c* = 2, $CHCl_3$). UV (*c* = 0.004, EtOH): max. 228 (sh, 4.07), 278 (3.42); min. 254 (2.91). CD (*c* = 0.011%, EtOH): 230 (–2822), 234 (sh, –1781), 236 (sh, –1314), 240 (sh, –571), 244 (sh, –223), 247 (max., –135), 252 (–90), 252 (min., –90), 256 (max., –204), 258 (min., –161), 262 (max., –311), 266 (min., –155), 268 (max., –224), 272 (±0). IR: 1640*s* (C=O, amide I), 1550*m* (CONH, amide II), 1515*s*, 1260*s*, 1155*m*, 1030*m*. ^{1}H-NMR: 9.43, 9.30 (2 br. *s*, CONH···N); 7.40–7.25 (*m*, 3 arom. H); 7.09 (*d*, *J* = 8, 2 arom. H); 6.82–6.74 (*m*, 3 arom. H); 4.00 (*m*, PhC*H*N); 3.87, 3.86 (2 *s*, 2 arom. MeO); 3.8–2.2 (*m*, 18 H); 2.0–1.3 (*m*, 9 H). ^{13}C-NMR: 171.6, 171.5 (2 C=O); 148.8, 147.3, 135.7, 134.1, 134.0 (arom. quat. C); 128.4, 128.2, 127.9, 127.8, 120.2, 111.9, 111.3 (CH=CH); 64.0 (Ph*C*N); 55.9, 55.8 (arom. MeO); 50.7, 47.0, 45.0, 36.3, 35.0, 31.3, 31.1, 28.6, 27.0, 26.1, 25.4, 21.4, 21.1 (CH_2). ESI-MS: 537 ([*M* + H]$^+$).

Vigorous Acid Hydrolysis of **4**. A soln. of **4** (2 mg) in 2N aq. HCl (0.1 ml) in a sealed glass tube was heated at 165° for 18 h. The mixture was evaporated at r. t. in a vacuum exsiccator over granulated NaOH. The residue was splashed with EtOH (the black-violet compounds were dissolved). The EtOH insoluble residue (white solid, spermine tetrahydrochloride) was dissolved in 25% aq. NH_3 soln./EtOH 1:4 (0.1 ml) and analyzed by TLC (silica gel; MeOH/17% NH_4Cl in 25% aq. NH_3 soln. 1:1; ninhydrine): R_f 0.23 (spermine); for comparison: spermidine shows in the same system R_f 0.36.

Saponification of **4**. A soln. of **4** (20 mg) and KOH (30 mg) in ethylene glycol (2.5 ml) was refluxed for 1.5 h. The mixture was diluted with H_2O (3 ml), acidified with HCl, and extracted with $CHCl_3$. The org. extract was washed with H_2O and evaporated. The residue (6 mg) was separated by prep. TLC (silica gel; toluene/AcOEt/80% aq. HCOOH 6:1:0.2): R_f 0.49 ((*E*)-cinnamic acid), 0.28 ((*E*)-3,4-dimethoxycinnamic acid; blue fluorescence at 365 nm). From the scraped zones, the acids were recovered by extraction with AcOEt. After evaporation of the eluate, the acids crystallized. M. p. of (*E*)-3,4-dimethoxycinnamic acid 178–182° ([13]: 180°). IR ($CHCl_3$): spectra of isolated and authentic (*E*)-3,4-dimethoxycinnamic acid superimposible.

Photoisomerization Procedures: General. The photoisomerizations were performed in a quartz cell, using a standard *Camag* TLC UV-detection lamp at 254 or 365 nm (without filter) which was placed 10 cm above the cuvette.

Photoisomerization of **2** *to* **4** *and of* **6** *to* **8**. A $8.7 \cdot 10^{-7}$ M MeOH soln. of natural **6** or synthetic (±)-**6** was irradiated 1 h at 365 nm under Ar. TLC (silica gel, AcOEt/MeOH 8:2); R_f 0.61 (**6**), R_f 0.52 (**8**) showed almost quant. (*E*) → (*Z*) conversion.

The same result was obtained after 1 h irradiation of the MeOH soln. of **2** (the process of photoisomerization was followed by TLC of the corresponding aminals **6** and of **8**, after addition of formaldehyde to the sample of the reaction mixture).

The same results were obtained after 2 h irradiation of the MeOH solns. of **2** or **6** by sunlight.

Photoisomerization of **4** *to* **2** *and of* **8** *to* **6**. A 10^{-2} M MeOH soln. of natural **4** or synthetic (±)-**4**, or of **8** was irradiated for 9 h at 254 nm under Ar. TLC showed a *ca.* 1 : 1 ratio of **2**/**4** and **6**/**8**, resp.

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