ther, 1β-H has an intense cross-peak with a proton at δ 2.20, which is then assigned 1α-H. The integration of ^{1}H NMR spectrum indicates two protons at δ 2.37; thus, 1β-H shows NOESY cross-peak with 2β-H too. Additionally, a NOESY cross-peak is observed between 1α-H and 11α-H, deduced from HETCOR, which proves the vicinity of the two protons. The normal conformation has 1β-H equatorial, which brings the 11α-H within the dipolar coupling distance, and hence should exhibit the NOESY cross-peak, while the inverted conformation requires dipolar coupling between 1α-H and 11α-H. Thus, the NOESY connectivity pattern (Figure 5c) suggests the 1β,2α inverted conformation.

It should be mentioned here that although the DQF-COSY experiment yielded the confirmation of various assignments, the cross-peaks were not analyzable due to intense strong coupling effects, making dihedral angle calculations difficult.

Conclusions

The study of conformational preferences of these steroids highlights the following:

(i) It is possible to utilize the 2D NOE technique for qualitative distance estimates for steroids of 19-functionalized type, although the technique is known to be fraught with problems for small molecules. Quantitative distance calculations would require rigorous growth curves and are not attempted.

(ii) The protocol—COSY 90 + HETCOR + NOESY at 300 MHz—serves to provide conformational preferences for these steroids rather successfully. More experiments need to be performed to generalize its applicability. In addition, the protocol demands careful analysis due to its nonrigorous approach.

(iii) The conformational preferences of 4-en-3-one steroids are not only governed by 1,3-diaxial interactions but also by the "tilt" of 19-carbon toward either of the rings. A formal tilting of 19-carbon toward ring A induces greater bias for normal 1α,2β conformation, while that toward ring B produces inverted 1β,2α conformation.

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Registry No. 1, 23925-63-5; 2, 133930-37-7; 3, 133930-38-8.

Supplementary Material Available: ^{1}H NMR spectra of steroids 2 and 3; COSY spectra of steroids 1–3; NOESY spectrum of steroid 2 and HETCOR spectra of steroids 2 and 3 (8 pages). Ordering information is given on any current masthead page.

Flow Thermolysis Rearrangements in the Indole Alkaloid Series: 1,2-Dehydroaspidospermidine

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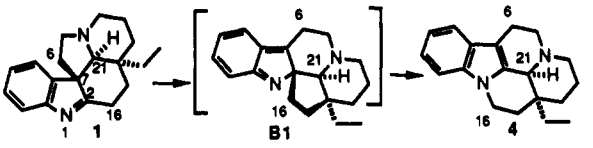
Flow thermolysis of 1,2-dehydroaspidospermidine (1) at various temperatures allowed isolation of all four predictable rearrangement products, namely indolenines 2 and 3 and indoles 4 and 5. The structures of the rearranged products were confirmed by chemical and spectroscopic means, particularly HMBC and HMQC NMR techniques.

Flow and flash thermolysis have scarcely ever been applied to alkaloidal materials,[1,2] although these methods combine several advantages: absence of extra reagent, easy separation of the products, and strictly unimolecular reactions. A limitation lies in the difficulty of rigid temperature control.

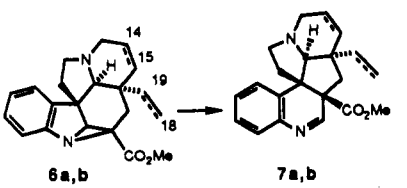
For several years, we have been interested in the flow thermolysis of indole alkaloids, after our attention had been drawn to the synthetic applications developed in Reims by Chuche et al.[3] In a preliminary study,[2] flow thermolysis of 1,2-dehydroaspidospermidine (1) at 580 °C was shown to yield vincane 4, which implied two successive [1,5] sigmatropic shifts via the *o*-quinonoid intermediate B1 (Scheme I).

Later, flow thermolysis of aziridines **6a,b** to dihydroquinolines **7a,b** was used as the key step in a synthetic entry into the *Melodinus* alkaloids[4,5] (Scheme II).

Scheme I



Scheme II



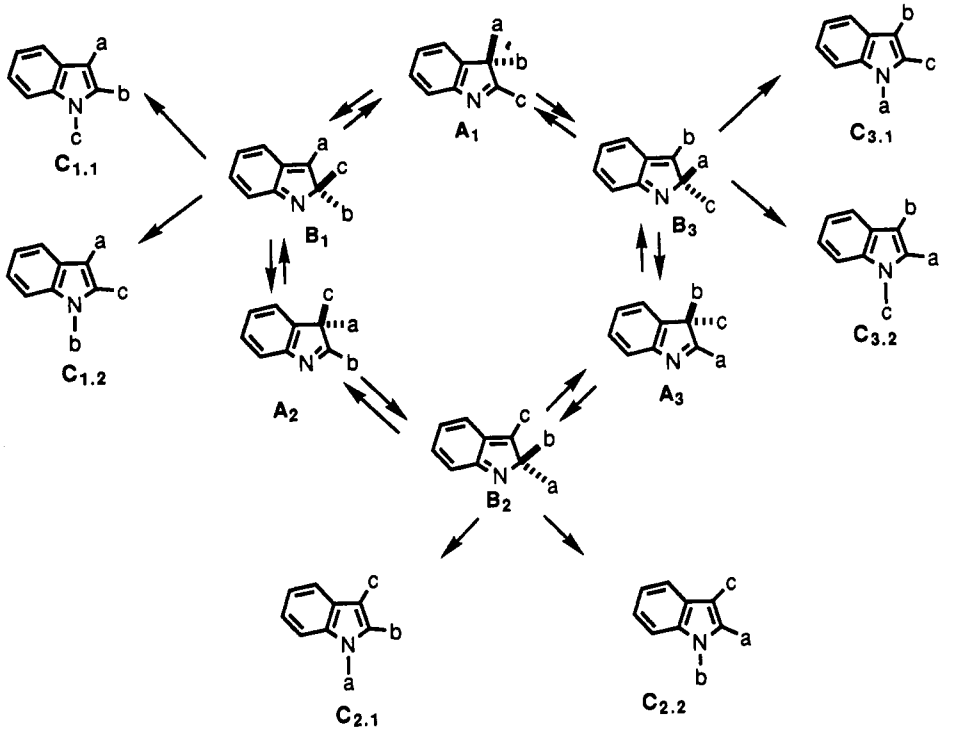
a: 14,15-H_2; 18,19-H_2
b: Δ^{14}; Δ^{18}

We have now thoroughly reexamined the flow thermolysis of 1,2-dehydroaspidospermidine (1) as a model for the thermal rearrangements of indolenines. Thermal rearrangements of indolenine A_1 (Scheme III) are expected to

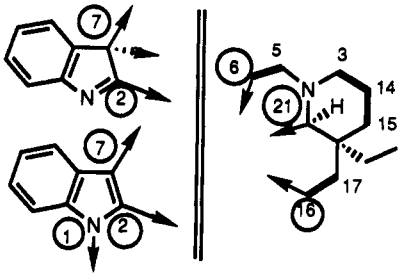
(1) Sisti, N. J.; Fowler, F. W. *Tetrahedron Lett.* **1989**, *30*, 5977.
(2) Hugel, G.; Lévy, J. *Tetrahedron* **1984**, *40*, 1067.
(3) (a) Manisse, N.; Chuche, J. *Bull. Soc. Chim. Fr.* **1972**, 2422. (b) Coqueret, X.; Bourelle-Wargnier, F.; Chuche, J. *Tetrahedron* **1986**, *42*, 2263.
(4) Hugel, G.; Lévy, J. *J. Org. Chem.* **1984**, *49*, 3275.
(5) Hugel, G.; Lévy, J. *J. Org. Chem.* **1986**, *51*, 1594.

0022-3263/91/1956-4631$02.50/0

Scheme III. Predictable Rearrangement Products of Indolenine A_1



Scheme IV



scramble the positions of the three different substituents (a, b, c) on the indolic C-2 and C-3 positions through reversible [1,5] shifts via *o*-quinonoid intermediates, such that two new indolenines (A_2, A_3) are likely to be formed through three possible different intermediates (B_1, B_2, B_3). As any given substituent will migrate in a suprafacial process, the depicted indolenine A_1 is related to the set of other indolenines A and intermediates B as featured in Scheme III, while the corresponding enantiomeric indolenine will equilibrate with the enantiomeric set of chemical species. Finally, besides equilibrating with indolenines, each quinonoid species B could possibly suffer an irreversible migration of either substituent on C-2 to N-1, and there would thus be generated six possible *indolic* derivatives (for instance, $C_{1.1}$ and $C_{1.2}$ from B_1).

Scheme III allows prediction of the behavior of 1,2-dehydroaspidospermidine (1) under flow thermolysis conditions by assigning substituents a, b, and c to C-6, C-21, and C-16, respectively, and further discarding those rearranged skeletons that are geometrically unprobable. Scheme IV summarizes the combinations to be considered between the indolenine vs indole system on one hand, and the aliphatic portion of the molecules on the other, by connecting the arrowed atoms. None of the three indolenines A_{1-3} and none of the three quinonoid intermediates B_{1-3} proved to be structurally improbable using Dreiding's models, while only indoles $C_{1.1}$ and $C_{2.1}$ met with the geometrical obligation of C-21 being attached between C-6 and C-16 on the adjacent atoms N-1, C-2, and C-7.

Table I. Yields of Products Isolated after Flow Thermolysis of Indolenine 1

	isolated compds, % yield (% transformation)				
	indolenines			indoles	
temp, °C	1[a]	2[a]	3[a]	4[a]	5[a]
580[b]	66			6 (17)	
620–30	42	5 (9)	2 (3)	11 (19)	18 (31)
650–60	32			17 (25)	27 (40)
670–80	10			12 (13)	31 (34)

[a] See structures in text. [b] From ref 2.

Consequently, 1,2-dehydroaspidospermidine (1; $=A_1$) was expected to uniquely rearrange to indolenines 2 ($=A_2$) and 3 ($=A_3$) and to indoles 4 ($=C_{1.1}$) and 5 ($=C_{2.1}$) with the depicted geometries (see structure in the following text). Moreover, the connection matrix of all atoms in the nonindolic part of these molecules will not predictably be affected: structure elucidation will then mainly depend on recognizing the three separate polymethylene fragments (bold bonds in Scheme IV), on specifying the chromophore, and on determining the attachments of C-6, C-16, and C-21 onto the said chromophore.

Flow thermolysis of 1,2-dehydroaspidospermidine (1) was performed in dry toluene solution at temperatures ranging from 580 to 680 °C (Table I).

The compounds were separated by TLC. At temperatures below 660 °C, ca. 75% of the material reacted was isolated. Higher temperatures produced more degradation. Indole 4 was the only isolable rearranged product formed at 580 °C; raising the temperature by only 40–50 °C gave one more indole (5) and two new indolenines, namely 2 and 3, albeit in low yields. Indolenines 2 and 3 were no longer produced at temperatures over 650 °C, when indoles 4 and 5 composed up to 65% of the transformation products. The isomeric indolenines then appear to result from reversible equilibria, while the indoles would be irreversibly formed from the quinonoid intermediates. Separately thermolizing indolenines 2 and 3 (610–620 °C) yielded

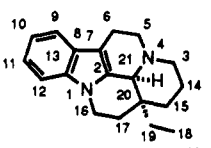
Table II. ^{13}C NMR Data for Compounds 1–5 and 12

C no.	4[a]	5[a]	1[b]	2[b]	3[b]	12[b]
2	132.54	132.51	191.95	186.04	186.52	186.70
3	44.59	44.96	51.68	51.87	50.15	40.81[c]
5	51.25	50.39	54.24	45.55	57.67	170.30
6	17.08	36.42	34.84	34.86	31.96	38.70[c]
7	104.29	107.93	60.99	51.09	66.35	53.87
8	127.95	127.23	146.60	145.80	144.19	145.57
9[d]	118.08	118.25	120.70	121.57	124.31	121.38
10[d]	119.28	119.36	124.80	125.04	124.53	126.13
11[d]	120.51	120.57	127.14	127.78	127.96	128.44
12[d]	109.19	108.88	119.76	121.34	120.40	120.82
13	136.30	137.74	154.16	155.27	155.22	154.52
14	20.70	21.02	21.73	14.92	18.13	20.21
15	23.81	24.54	32.93	33.24	27.12	22.88
16	38.39	17.62	23.41	31.97	34.03	24.49
17	31.88	32.38	27.00	26.97	37.98	33.93
18	7.53	7.70	7.02	6.61	8.61	7.08
19	28.92	29.20	29.43	29.91	28.59	29.25
20	34.08	35.06	36.20	40.84	47.60	36.94
21	59.23	58.80	78.59	64.53	81.44	70.13

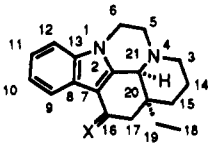
[a] Indole chromophore [b] Indolenine chromophore. [c] Labeled values may be interchanged. [d] See ref 17.

identical mixtures containing indole **5** (10–20%) and the regenerated indolenine **1** (10%). No indole **4** was detected under the conditions used in these last experiments.

All four possible rearranged products were then isolated and fully characterized. They were assigned the predicted isomeric structures mainly through spectroscopic means. COSY experiments allowed assignment of most of the protons. Refinement of the attributions was based on ^1H–^{13}C correlation experiments in the reverse mode:[6,7] HMQC for ^1J and HMBC for ^2J and ^3J.



eburnamenine (8): Δ^{-16}
vincane (4) : 16,17-H_2



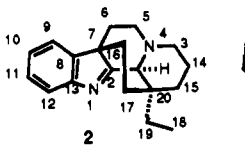
isovincane (5): X = H_2
9 : X = O

Indole **4** was found to be identical with (–)-vincane,[8] a compound initially prepared from (–)-eburnamenine (**8**) through catalytic hydrogenation.[2]

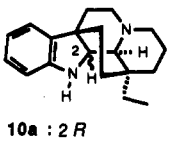
Indole **5** (isovincane) is structurally related to **4** by interchanging the connections of carbons 6 and 16 with N-1 and C-7 on the indole moiety. Accordingly, the ^{13}C NMR chemical shifts of the homologous carbon atoms in both compounds, as numbered after the biogenetic origin[9] are only slightly different, except for C-6 and C-16 (Table II). The ^1H NMR spectrum supported a similar observation: when comparing **5** to **4**, the most striking difference was the deshielding of the C-6 protons in the vicinity of N-1 and the reciprocal shielding of the C-16 protons, while the ^1H–^1H COSY spectrum unequivocally demonstrated the N-1–CH_2CH_2–N-4 part structure; in fact, the four corresponding coupled protons appeared at low field as well-individualized signals at δ 3.25, 3.41, 3.70 and 3.94. Complementary evidence was given by the HMBC spectrum of **5**, which exhibited ^2J and ^3J couplings of the C-16 protons with carbons 7, 2, 17, and 20 on one hand and of the C-17 protons with carbons 16, 7, 20, 19, and 15 on the other.

With a view to further confirmation of its structure, indole **5** was oxidized with DDQ[10] or with Jones' reagent to ketone **9**, which exhibited a 3-acylindole UV spectrum.[11] The ketonic C-16 was seen at δ 191.69 in the ^{13}C NMR spectrum and the now isolated C-17 methylene gave an AB system (δ 2.40 and 2.60, J = 15.7 Hz) in the ^1H NMR spectrum. These last protons coupled (HMBC) with carbons 16 and 20 (^2J) and with carbons 7, 21, 19, and 15 (^3J).

Indolenine **2** (UV; signal of C-2 at δ (186.02) was characterized by its H-21 proton appearing as a singlet at low field (δ 3.69) because of its vicinity to the imino double bond. In this region of the spectrum one of the two protons on C-5 suffered a shift to δ 3.75, due to anisotropy of the aromatic rings. Here again the HMBC spectrum ascertained the correlation matrix, as the C-16 protons coupled with carbons 17, 20, 6, 7, and 2, the C-6 protons with carbons 2, 7, and 16, and the C-5 protons with carbons 6, 7, 3, and 21.

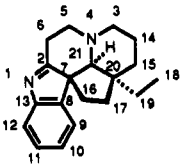


2

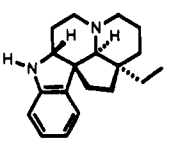


10a : 2*R*
10b : 2*S*

Reduction of indolenine **2** with LAH was not stereospecific: two isomeric indolines, **10a**, less polar, and **10b** were formed in a 2:11 ratio. The ^1H NMR spectra of the two indolines were only slightly different. In the minor compound **10a**, H-2 gave a triplet at δ 3.63, H-21 gave a doublet at δ 3.14 (J = 2.7 Hz), and H-1 (exchangeable with D_2O) gave a doublet at δ 4.04 (J = 3.5 Hz). The corresponding protons in **10b** were at δ 3.53 (part of a multiplet), 3.0 (J = 2.1 Hz), and 4.0 (J = 2.9 Hz), respectively. Like in the starting indolenine **1**, one of the two C-5 protons was submitted to the aromatic anisotropy and it resonated at δ 3.56 for **10a** and in the multiplet around δ 3.5 for **10b**. Although these findings are of little diagnostic value regarding the configuration of C-2, the minor compound **10a** is thought to be 2*R* and the major compound **10b** to be 2*S* with regard to the different accessibility of the reagent to either side of the imine group.



3



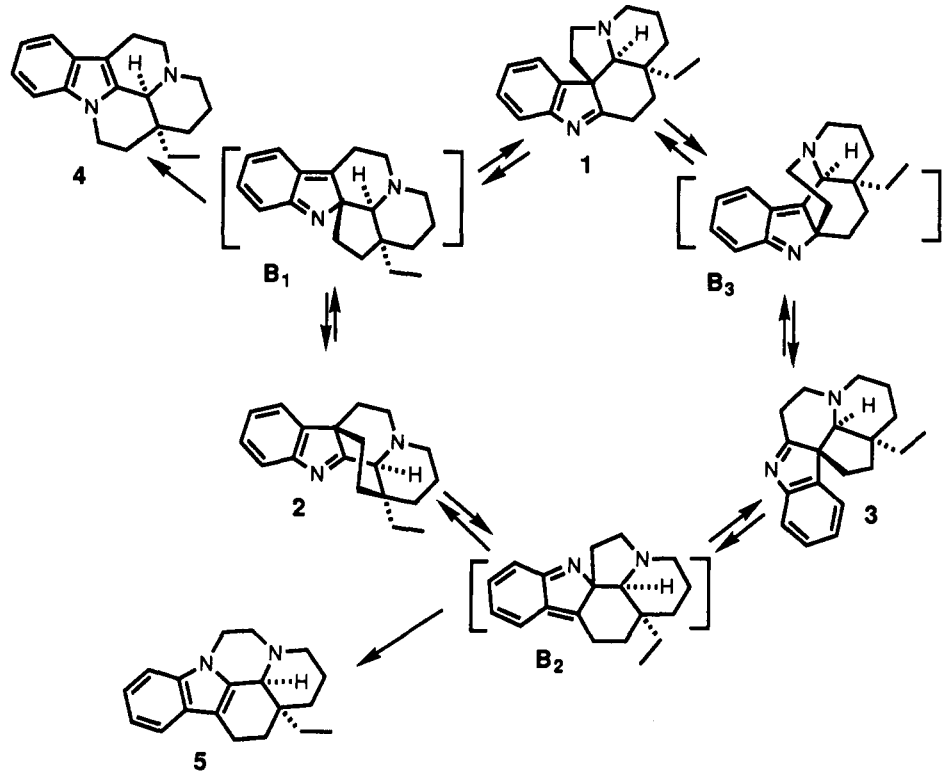
11

Indolenine **3** exhibited the quaternary C-2 at δ 186.5. All signals of the ^1H NMR spectrum could be attributed on the basis of a COSY experiment, which again showed the integracy of the three separate polymethylenic fragments. The HMBC spectrum showed the C-5 protons coupling with carbons 6, 2, and 21 and the C-6 protons with carbons 5, 2, and 7, thus demonstrating the C-2–C-6 bond. Similarly, the C-16 protons coupled with carbons 7, 17, 8, 2, 20, and 21, thus demonstrating the C-7–C-16 bond.

Of interest was the shielding of H-21 at δ 1.89 in **3**, as compared with 1,2-dehydroaspidospermidine (**1**; 2.38 ppm).

(6) Muller, L. *J. Am. Chem. Soc.* **1979**, *101*, 4481.
(7) Bax, A.; Summers, M. F. *J. Am. Chem. Soc.* **1986**, *108*, 2093. (HMQC/HMBC: Heteronuclear Multiple Quantum/Multiple Bond Correlation.)
(8) (a) Schnoes, H. K.; Burlingame, A. L.; Biemann, K. *Tetrahedron Lett.* **1962**, *22*, 993. (b) Thomas, D. W.; Achenbach, H.; Biemann, K. *J. Am. Chem. Soc.* **1966**, *88*, 1537.
(9) Le Men, J.; Taylor, W. I. *Experientia* **1965**, *21*, 508.
(10) (a) Oikawa, Y.; Yonemitsu, O. *Heterocycles* **1976**, *4*, 11. (b) Hagen, T. J.; Narayanan, K.; Names, J.; Cook, J. M. *J. Org. Chem.* **1989**, *54*, 2170.
(11) Oikawa, Y.; Yonemitsu, O. *J. Org. Chem.* **1977**, *42*, 1213.

Scheme V. Rearrangement Products of 1,2-Dehydroaspidospermidine (1)



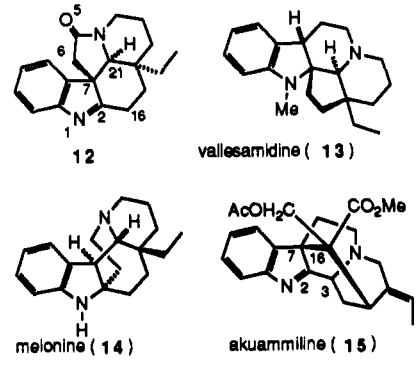
X-rays studies of such *aspidosperma* compounds as 1 have actually shown H-21 to be oriented in a trans relationship with the N-4 lone pair of electrons. As a result of a steric interaction between the 19-methylene group and H-9, compound 3 suffers inversion at N-4, which accounts for the above observation. Another consequence is the deshielding of the 19-methylene from 0.65 ppm in 1 to 1.7 ppm in 3, no longer subject to the aromatic anisotropy.

Reduction of 3 with LAH yielded indoline 11, $M^{\bullet+}$ 282, whose H-2 proton gave signals at δ 3.71 (dd, J = 4.5, 9.0 Hz). This compound had retained the conformation of 3 around N-4, as evidenced by the H-21 singlet at δ 1.95.

As a byproduct (~1%, lactam 12 resulting from oxidation of 1,2-dehydroaspidospermidine (1) during thermolysis was isolated in several runs. Its structure rests on UV, IR, and NMR measurements, the most salient feature being an IR band at 1685 cm^{-1}, a ^{13}C signal at δ 186.7, and an AB system in the ^1H NMR spectrum (C-6 methylene) with signals centered at δ 2.53 and 2.80 (J = 18 Hz). The HMBC NMR spectrum indicated that the original skeleton had not been altered. Introduction of the C-5 carbonyl group induced a relative flattening of the molecule, as the signals of the ethyl side chain appeared to be less biased by the aromatic anisotropy.

Scheme V finally summarizes the thermal rearrangements of 1,2-dehydroaspidospermidine 1 into indolenines 2 and 3 and indoles 4 and 5 and the pathways involved. From the above results, however, intermediacy of B_3 does not appear indispensable. Nevertheless, while 2–4 may result from 1 through only two successive [1,5] sigmatropic shifts, remarkably enough, formation of 5 necessitates *four* such successive [1,5] sigmatropic shifts.

The skeletons of compounds 2, 3, and 5 have not yet been encountered in nature. However, intermediate B_1 is related to vallesamidine (13);[12] previous work from these laboratories has performed the chemical rearrangement of 1 to the vallesamidine.[13] Intermediate B_3 is related to melonine (14).[14] Compound 3 formally constitutes the *aspidosperma* counterpart of the akuammiline (15) type of alkaloids[15,16] in the *corynanthe* series, as both are characterized by a C-7–C-16 linkage.



Experimental Section

General Procedures. Melting points were determined on a Reichert microscope and are uncorrected. Specific rotations were measured using a Perkin-Elmer Model 241 polarimeter. IR spectra were recorded on a Beckmann Acculab 4 and UV spectra on a Varian 634 spectrophotometer. ^1H (300-MHz) and ^{13}C (75-MHz) NMR were measured on a Brucker AC 300 spectrometer in $CDCl_3$ with Me_4Si as internal standard. J values are given in Hz. The AC 300 was modified to allow detection in the reverse mode:

(12) Walser, A.; Djerassi, C. *Helv. Chem. Acta.* **1965**, *48*, 391.

(13) Lévy, J.; Maupérin, P.; Dõé de Maindreville, M.; Le Men, J. *Tetrahedron Lett.* **1974**, 491.

(14) Baassou, S.; Mehri, H. M.; Rabaron, A.; Plat, M. *Tetrahedron Lett.* **1983**, *24*, 761.

(15) Olivier, L.; Lévy, J.; Le Men, J.; Janot, M.-M.; Budzikiewicz, H.; Djerassi, C. *Ann. Pharm. Fr.* **1964**, *22*, 35.

(16) Olivier, L.; Lévy, J.; Le Men, J.; Janot, M.-M.; Budzikiewicz, H.; Djerassi, C. *Bull. Soc. Chim. Fr.* **1965**, 868.

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HMBC and HMQC were measured in the reverse mode and ^1H pulses were emitted via the decoupler channel. Mass spectra were recorded on a JEOL D-300 spectrometer. Separations were carried out on TLC and with a Chromatotron Harrisson Research apparatus with Kieselgel 60 PF_{254} Merck, eluant CH_2Cl_2/MeOH and hexane.

Flow Thermolysis of (-)-1,2-Dehydroaspidospermidine (1). The apparatus was that described by Manisse and Chuche,[3] using an oven purchased from Herrmann-Moritz. Compound 1[18] (523 mg, 1.86 mmol) was dissolved in dry toluene (40 mL), and the solution was passed through a vertical column at 620 ± 5 °C. The effluent was condensed in a liquid nitrogen cooled trap at the bottom of the column. Centrifugal chromatography (silica plate; hexane to remove greases, then hexane–CH_2Cl_2, then CH_2Cl_2–MeOH) followed by TLC purification allowed successive separation of indole **5** (94 mg, 18%), indolenine **1** (220 mg, 42% recovered), vincane **4** (57 mg, 11%), indolenine **2** (9 mg, 2%), and indolenine **3** (30 mg, 6%). Along the TLC purification of **1**, compound **12** (4 mg, 1%) was further isolated. **Vincane 4**: amorphous; $[\alpha]_D$ -11.7° (*c* 0.2, MeOH); ^1H NMR ($CDCl_3$) δ 0.85 (t, 3 H, J = 7.5, CH_3), 1.00 (td, 1 H, J = 3.8, 13.6, H-15), 1.18 (m, 1 H, 15), 1.28 (m, 1 H, H-14), 1.48 (dq, 1 H, J = 7.5, 15.0, H-19), 1.72 (tq, 1 H, J = 13.6, 3.8, H-14), 1.87 and 1.89 (m, 2 H, H_2-17), 2.07 (dq, 1 H, J = 7.5, 15.0, H-19), 2.38 (td, 1 H, J = 2.5, 11.3, H-3), 2.50 (m, 2 H, H-6, H-3), 2.92 (m, 1 H, H-6), 3.20 and 3.23 (m, 2 H, H_2-5), 3.69 (dt, 1 H, J = 5.0, 11.3, H-16), 3.82 (s, 1 H, H-21), 4.07 (ddd, 1 H, J = 2.5, 5.0, 11.3, H-16), 7.02 and 7.09 (m, 2 H, H-10, H-11), 7.21 (dd, 1 H, J = 1.5, 8.8, H-12), 7.41 (td, 1 H, J = 1.5, 8.8, H-9). This compound was found by comparison (IR, UV, MS) to be identical with an authentic sample obtained from (-)-eburnamenine[2,8] by catalytic hydrogenation. **Isovincane 5**: mp 130–134 °C (CH_2Cl_2); $[\alpha]_D$ +14.4°(*c* 0.6, MeOH); IR (film) 2900, 1450 cm^{-1}; UV (MeOH) 230, 275, 283.5, 292 nm; ^1H NMR ($CDCl_3$) δ 0.83 (t, 3 H, J = 7.6, CH_3), 1.18, 1.23 and 1.26 (m, 3 H, H_2-15, H-14), 1.51 (dq, 1 H, J = 7.6, 14.0, H-19), 1.70 (m, 3 H, H-14, H_2-17), 1.88 (dq, 1 H, J = 7.6, 14.0, H-19), 2.10 (td, 1 H, J = 2.2, 11.0, H-3), 2.45 (dt, 1 H, J = 3.3, 11.0, H-3), 2.57 and 2.67 (m, 2 H, H_2-16), 3.25 (dd, 1 H, J = 5.5, 12.0, H-5), 3.41 (ddd, 1 H, J = 5.5, 12.0, 17.6, H-5), 3.70 and 3.71 (m, 2 H, H-6, H-21), 3.94 (td, 1 H, J = 5.5, 12.0, H-6), 7.04 (m, 2 H, H-11, H-10), 7.15 (dd, 1 H, J = 1.1, 9.9, H-9 or H-12), 7.39 (dd, 1 H, J = 2.0, 7.7, H-12 or H-9); MS (m/z) 280 ($M^{\bullet+}$), 251, 237; HRMS 280.1931, calcd for $C_{19}H_{24}N_2$ 280.1938. Anal. Calcd for $C_{19}H_{24}N_2$: C, 81.14; H, 8.63; N, 9.995. Found: C, 81.19; H, 8.74; N, 10.01. **Indolenine 3**: mp 85–87 °C; $[\alpha]_D$ +73.6° (*c* 0.5, MeOH); IR (film) 2945, 2800, 1595 cm^{-1}; UV (MeOH) 218, 270 nm; ^1H NMR ($CDCl_3$) δ 1.00 (t, 3 H, J = 6.7, CH_3), ~1.4 (m, 2 H, H-16, H-14), ~1.7 (m, 6 H, H-17, H_2-19, H_2-15, H-14), 1.89 (s, 1 H, H-21), 2.05 (m, 1 H, H-3), 2.19 (td, 1 H, J = 2.4, 11.5, H-5), 2.40 (td, 1 H, J = 6.8, 12.8, H-17), 2.67 (ddd, 1 H, J = 7.5, 10.5, 19.5, H-16), 2.79 (dt, 1 H, J = 2.3, 12.0, H-6), 2.94 (m, 2 H, H-3, H-6), 3.09 (ddd, 1 H, J = 2.3, 5.3, 11.3, H-5), 7.15 (td, 1 H, J = 0.3, 2.3, H-10), 7.32 (td, 1 H, J = 0.3, 2.3, H-11), 7.56 (m, 2 H, H-9, H-12); MS (m/z) 280 ($M^{\bullet+}$), 251, 156, 108; HRMS 280.1943, calcd for $C_{19}H_{24}N_2$ 280.1940. **Indolenine 2**: amorphous, $[\alpha]_D$ +34.0° (*c* 1.2, MeOH); IR (film) 2920, 1595, 1450 cm^{-1}; UV (MeOH) 215, 270 nm; ^1H NMR ($CDCl_3$) δ 0.73 (t, 3 H, J = 7.6, CH_3), 1.06 (q, 2 H, J = 7.6, H_2-19), ~1.4 (m, 5 H, H-17, H-15, H-14, H-16, H-6), 1.76 (m, 1 H, H-15), 2.23 (m, 2 H, H-14, H-17), 2.38 (dd, 1 H, J = 6.7, 13.4, H-16), 2.48 (dd, 1 H, J = 4.5, 13.4, H-6), 2.77 (dd, 1 H, J = 7.2, 11.6, H-5), 3.01 (td, 1 H, J = 2.7, 13.4, H-3), 3.14 (dd, 1 H, J = 4.5, 13.4, H-3), 3.69 (s, 1 H, H-21), 3.75 (td, 1 H, J = 4.5, 11.6, H-5), 7.18 (td, 1 H, J = 1.8, 5.8, H-10), 7.35 (m, 2 H, H-11, H-9), 7.70 (dd, 1 H, J = 1.8, 7.2, H-12); MS (m/z) 280 ($M^{\bullet+}$), 251 (100); HRMS 280.1939, calcd for $C_{19}H_{24}N_2$ 280.1994. **Indolenine 12**: amorphous, highy unstable in chlorinated solvents; $[\alpha]_D$ -81.0° (*c* 0.5, MeOH); IR (film) 2940, 1685, 1575 cm^{-1}; UV (MeOH) 215, 262 nm; ^1H NMR ($CDCl_3$) δ ~0.7 (m, 4 H, CH_3, H-19), 1.10 (dq, 1 H, J = 6.7, 13.5, H-19), 1.33 (td, 1 H, J = 4.5, 13.5, H-15), 1.60 (m, 3 H, H-17, H-15, H-14), 1.82 (b d, 1 H, H-14), 2.18 (dt, 1 H, J = 9.9, 13.5, H-17), 2.53 (dd, 1 H, J = 1.4, 18.0, H-6), 2.79 (m, 1 H, H-3), 2.80 (d, 1 H, J = 18.0, H-6), 2.95 (m, 2 H, H_2-16), 3.61 (d, 1 H, J = 1.4, H-21), 4.35 (m, 1 H, H-3), 7.20 (td, 1 H, J = 1.5, 6.0, H-10), 7.34 (m, 2 H, H-9, H-11), 7.53 (dd, 1 H, J = 1.5, 7.5, H-12); MS (m/z) 294 ($M^{\bullet+}$), 265, 156; HRMS 294.1742, calcd for $C_{19}H_{22}N_2O$ 294.1732.

Oxidation of Isovincane 5: Compound 9. (A). Compound **5** (7 mg, 0.025 mmol) was dissolved in a 9:1 mixture of THF and H_2O (1.5 mL), and DDQ (10 mg, 1 h; then 8 mg, 2 h; then 8 mg, 4 h) was added at 20 °C. Dilution with aqueous $NaHCO_3$ and extraction with CH_2Cl_2 followed by TLC separation yielded **5** (2 mg) and compound **9** (3 mg, 40%).

(B). Compound **5** (9 mg, 0.032 mmol) was dissolved in CH_2Cl_2 (2 mL) and reacted with Jones' reagent (0.027 mL) at 20 °C for 2 h. Extraction and separation as above gave **5** (5 mg) and **9** (3 mg, 30%). **16-Oxoindole 9**: mp 200–210 °C ($CHCl_3$, MeOH); $[\alpha]_D$ -82.0° (*c* 0.5, $CHCl_3$); IR (KBr) 2930, 1635 cm^{-1}; UV (MeOH) 208, 244, 268 sh, 299 nm;[11] ^1H NMR ($CDCl_3$) δ 0.95 (t, 3 H, J = 6.1, CH_3), ~1.4 (m, 3 H, H_2-15, H-14), 1.75 (m, 2 H, H-19, H-14), 2.00 (m, 1 H, H-19), 2.27 (td, 1 H, J = 11.2, 2.3, H-3), 2.40 (d, 1 H, J = 15.7, H-17), 2.60 (d, 1 H, J = 15.7, H-17), 2.70 (b d, 1 H, H-3), ~3.50 (m, 2 H, H_2-5), 3.85 (dd, 1 H, J = 4.5, 13.5, H-6), 4.11 (s, 1 H, H-21), 4.16 (td, 1 H, J = 4.5, 11.3, H-6), 7.30 (m, 3 H, H-10, H-11, H-9), 8.19 (dd, 1 H, J = 7.2, 1.4, H-12); ^{13}C NMR ($CDCl_3$) δ 7.94 (18), 20.76 (14), 26.86 (15), 28.88 (19), 35.72 (6), 41.52 (20), 45.58 (3), 49.20 (17), 49.74 (5), 58.49 (21), 109.23 (12), 110.91 (7), 121.73 (9), 122.95 (10 or 11), 123.04 (11 or 10), 124.28 (8), 137.62 (13), 148.17 (2), 191.69 (16); MS (m/z) 294 ($M^{\bullet+}$), 293, 266, 251, 237, 167; HRMS 294.1736, calcd for $C_{19}H_{22}N_2O$ 294.1731.

Flow Thermolysis of Indolenine 2. Compound **2** (10 mg, 0.035 mmol) in dry toluene (15 mL) was flow-thermolyzed at 610 °C as in the previous text. TLC separations gave indole **5** (1 mg) and 1,2-dehydroaspidospermidine (**1**; 1 mg), which were found to be identical (R_f, IR, UV) with authentic samples.

Flow Thermolysis of Indolenine 3. Compound **3** (10 mg, 0.035 mmol) in dry toluene (10 mL) was flow-thermolyzed at 620 °C as in the previous text. TLC separations allowed isolation of indole **5** (2 mg), and 1,2-dehydroaspidospermidine (**1**; 1 mg), which were both found to be identical (R_f, IR, UV) with authentic samples.

Reduction of Indolenine 2: Compounds 10a and 10b. Indolenine **2** (10 mg, 0.036 mmol) was dissolved in dry THF (3 mL) and LAH (25 mg) added. After the solution was refluxed for 1.5 h, water was added and the solution was extracted as in the previous text to yield a mixture (8.5 mg). TLC separation gave indoline **10a**, less polar (1 mg), and indoline **10b**, more polar (5.5 mg). **(2*R*)-Indoline 10a**: IR (film) 3250, 2900, 1600, 1450 cm^{-1}; UV (MeOH) 207, 240, 286 nm; ^1H NMR ($CDCl_3$) δ 0.82 (t, 3 H, J = 7.2, CH_3), 1.25 (m, 3 H), 1.50 (m, 3 H), 1.80 (m, 3 H), 2.03 (m, 1 H), 2.30 (m, 3 H), 3.00 (m, 2 H), 3.14 (d, 1 H, J = 2.3, H-21), 3.56 (m, 1 H, H-5), 3.63 (t, 1 H, J = 3.5, 2.3, H-2), 4.04 (d, 1 H, J = 3.5, NH), 6.74 (m, 2 H), 7.03 (m, 2 H). **(2*S*)-Indoline 10b**: amorphous, $[\alpha]_D$ -66.3° (*c* 0.5, MeOH), IR (film) 3360, 3260, 1615, 1460 cm^{-1}; UV (MeOH) 205, 241, 290 nm; ^1H NMR ($CDCl_3$) δ 0.80 (t, 3 H, J = 7.5, CH_3), 1.21 (m, 2 H), 1.50 (m, 3 H), 1.75 (m, 4 H), 2.03 (m, 1 H, H-14), 2.20 (m, 1 H, H-6), 2.28 (td, 1 H, J = 5.9, 12.2, H-6), 2.83 (dd, 1 H, J = 11.3, 7.2, H-5), 3.00 (d, 1 H, J = 2.3, H-21), 3.03 (m, 1 H, H-3), 3.53 (m, 2 H, H-2, H-5), 4.00 (d, 1 H, J = 2.9, NH), 6.72 (m, 2 H), 7.03 (m, 2 H); ^{13}C NMR ($CDCl_3$) δ 7.48 (18), 14.79 (14), 28.25 (16 or 17), 30.16 (15), 31.11 (19), 33.42 (6), 34.50 (20), 34.95 (17 or 16), 40.00 (7), 46.75 (5), 53.52 (3), 60.25 (21), 69.31 (2), 110.57 (12), 118.99 (10), 121.35 (9), 126.99 (11), 139.42 (8), 149.90 (13); MS (m/z) of **10a** + **10b** 282 ($M^{\bullet+}$), 281, 253, 124 (100); HRMS of **10b** 282.2076, calcd for $C_{19}H_{26}N_2$ 282.2094.

Reduction of Indolenine 3: Compound 11. Indolenine **3** (5 mg, 0.018 mmol) was dissolved in a saturated solution of LAH in dry THF (5 mL) and refluxed for 1 h. After addition of a few drops of water and CH_2Cl_2 (2 mL), filtration of the mixture over Hyflosupercel, evaporation, and TLC separation gave compound **11** (3 mg, 60%): amorphous, $[\alpha]_D$ +2.0° (*c* 0.8, MeOH), IR (film) 3360, 2920, 2790, 1600 cm^{-1}; UV (MeOH) 207, 238, 292 nm; ^1H NMR ($CDCl_3$) δ 0.90 (t, 3 H, J = 7.6, CH_3), ~1.6 (m, 6 H), ~1.8 (m, 5 H), 1.95 (s, 1 H, H-21), 2.20 (m, 2 H, H-3, H-5), ~2.7 (m, 2 H, H-3, H-5), ~3.5 (s, 1 H, NH), 3.71 (dd, 1 H, J = 4.5, 7.4, H-2), 6.63 (d, 1 H), 6.71 (td, 1 H), 7.04 (td, 1 H), 7.22 (d, 1 H); ^{13}C NMR ($CDCl_3$) δ 8.69 (18), 19.03, 26.54, 26.96, 30.96, 34.96, 36.01, 45.92 (20), 49.80 (3 or 5), 51.75 (5 or 3), 56.72 (7), 63.39 (2 or 21), 76.68 (21 or 2), 109.98 (12), 118.36 (10), 124.40 (9), 127.38

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(11), 137.58 (8), 150.07 (13); MS (m/z) 282 ($M^{\cdot+}$, 100), 254, 253, 225, 130; HRMS 282.2032; calcd for $C_{19}H_{26}N_2$ 282.2094.

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Supplementary Material Available: ^1H and ^{13}C NMR spectra of **2-5**, **9**, **10b**, **11**, and **12** and ^1H NMR spectrum of **10a** (9 pages). Ordering information is given on any current masthead page.

Synthesis of Huperzine A and Its Analogues and Their Anticholinesterase Activity

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Huperzine A is a new alkaloid isolated from the club moss *Huperzia serrata* (Thunb.) Trev., a Chinese folk medicine. This alkaloid exhibits potent activity as an inhibitor of acetylcholinesterase. Consequently, the compound is presently being investigated in China for the treatment of individuals suffering from various forms of memory impairment including Alzheimer's dementia. Details of the total synthesis of (±)-huperzine A are described as well as the preparation of a variety of huperzine analogues including its presumed pharmacophore. The extent of these new compounds to inhibit acetylcholinesterase is presented along with a discussion of the effects of the structural changes on biological activity.

Introduction

Huperzine A (1) and B (2) are two new alkaloids isolated from *Huperzia serrata* (Thunb.) Trev. = *Lycopodium serratum* Thunb., a Chinese folk medicine (Qian Ceng Ta).[1] The structures of 1 and 2 have been determined by chemical and spectroscopic studies to be as shown in Figure 1. While huperzine A would appear to be closely related to another pyridone-containing alkaloid, selagine (3), a compound whose structure was elucidated in 1960 by Wiesner and co-workers,[2] recent studies have revealed the earlier structural assignment, i.e., 3, to be incorrect. The alkaloid isolated from *L. selago* L. and named selagine is, in fact, *identical* with huperzine A.[2]

Pharmacologically, huperzines A and B have been found to exhibit potent anticholinesterase activity: the pI_{50}s (negative logarithm of the molar concentration causing 50% inhibition) of huperzine A toward erythrocyte membrane and caudate nuclei acetylcholinesterase are 7.2 and 7.9, respectively.[3] Huperzine A is thus about 3 times more potent than physostigmine as an inhibitor of acetylcholinesterase but is less potent than physostigmine when tested against butyrylcholinesterase. The pI_{50}s of huperzine B toward erythrocyte and caudate acetylcholinesterase are 6.1 and 6.2, respectively. The rank order of antiacetylcholinesterase activity of huperzines A and B, physostigmine, and neostigmine are huperzine A > physostigmine > neostigmine > huperzine B.

Inhibitors of AChE range from some of the most toxic agents ever synthesized by man (VX, Sarin, and Soman) to the useful therapeutic agents physostigmine and neostigmine, compounds that find use in the treatment of glaucoma and myasthenia gravis, respectively (Figure 2).[4] Since a deficiency in the cholinergic system is believed to constitute one of the hallmarks of Alzheimer's dementia, reversible inhibitors of AChE that can make their way into the central nervous system may serve as palliative agents in the treatment of the disease.[5] In this regard a number of promising studies have begun to appear in the literature that support the clinical utility of huperzine A.[6c,d]

In experiments using the Y-maze, 167 μg/kg intraperitoneal administration of huperzine A was found to facilitate learning and retrieval in rats.[6a] In squirrel monkeys the intramuscular injection of huperzine A was found to improve the accuracy of retention by 5-13% at doses of 0.003-0.03 mg/kg.[6b] Furthermore, compound 1 has been tested clinically in the treatment of human memory impairment[6c] and myasthenia gravis.[6d] In a clinical study of 100 individuals (46-87 years of age) suffering from various forms of memory impairment including Alzheimer's dementia, huperzine A improved memory 1-4 h after injection and exhibited a duration of action of ~8 h. In 128 cases of myasthenia gravis, 99% of patients treated with

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