Perhydrogenation of Tabersonine, an *Aspidosperma* Indole Alkaloid

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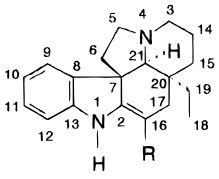
The natural indole alkaloid (−)-tabersonine (**1**) easily provided (−)-decahydrotabersonine (**4a**), isolated as dihydrochloride (**4b**), by catalytic hydrogenation. Saponification of **4a** led to the *β*-amino acid **5**. A binding study of **1**, **4b**, and **5** on various receptors and ionic channels showed that none of the compounds had a strong affinity for the receptors tested.

The *Aspidosperma* alkaloids constitute a large family of natural products in which biologically active structures account for the continuing interest in these compounds. The pivotal position of the readily available tabersonine (**1**)[1,2] and vincadifformine (**2**)[3] in the biosynthesis of various structural types of indole alkaloids have already elicited many important studies.[4] In the course of previous hydrogenation attempts of the 16-nitro analogue (**3**) into the expected 2,16-dihydro-16-amino compound,[5] the reduction of the aromatic ring was occasionally observed in moderate yield (unpublished results). Very few papers deal with perhydrogenated indole alkaloids;[6−9] therefore, we considered the feasibility of perhydrogenation on tabersonine (**1**).



	R	
1	CO_2Me	Δ 14-15
2	CO_2Me	
3	NO_2	

Hydrogenation of (−)-tabersonine (**1**) in 1 N methanolic $HClO_4$ over PtO_2 (40 h, room temperature, 1 atm) provided in 60% yield the decahydrotabersonine (**4a**) (EIMS *m*/*z* M^+ 346; no UV absorption above 210 nm) isolated as its dihydrochloride (**4b**). 1D ^{1}H- and ^{13}C-NMR spectra of **4a** indicated the presence of a pure compound and not a mixture of isomers. Both 2D homonuclear (COSY, HOHAHA) and heteronuclear (HMQC) experiments allowed assignments for all the protons and carbons and confirmed the structure (Table 1). Relative stereochemistry was inferred from rotating Overhauser spectroscopy (ROESY) experiments: significant ROE were observed between H-2 and H-8, H-13, H-16 but not between H-16 and H-21. This information is in agreement with the hydrogenation of the C2−C16 bond via the indoleninium form, then of the aromatic ring on the less hindered face to form a *cis* junction of the perhydro indole ring. Furthermore, ROE observed between H-21 and both H-19 are consistent with the stereochemistry of the starting compound **1**. A stoichiometric saponification of **4a** (THF−0.1 N NaOH 1:1) afforded in 90% yield the *β*-amino acid **5** isolated as its sodium carboxylate salt.

Table 1. ^{1}H- and ^{13}C-NMR Chemical Shift Values of Compunds **4a** and **5** in DMSO-d_6

	4a		**5**	
atom	δ_H	δ_C	δ_H	δ_C
2	3.15	67.5	3.10	69.2
3	1.80/2.90	48.8	1.80/2.90	48.7
5	1.70/2.65	53.6	1.65/2.60	53.4
6	1.70/1.80	45.6	1.65/1.75	46.1
7		51.8		51.8
8	1.55	51.2	1.45	51.2
9	1.10/1.65	25.4	1.35/1.45	25.9
10	1.30/1.45	19.8	1.30/1.45	20.2
11	1.30/1.50	24.3	1.05/1.65	24.9
12	1.50/1.65	27.1	1.50/1.65	27.7
13	3.05	55.6	3.02	55.2
14	1.60	18.4	1.45/1.65	18.4
15	1.05/1.60	27.6	1.00/1.60	26.9
16	2.82	39.1	2.25	39.9
17	1.30/1.60	28.4	1.40	30.6
18	0.80	7.8	0.77	8.0
19	1.30/1.80	32.0	1.25/1.65	32.1
20		34.3		34.9
21	1.40	71.8	1.35	72.3
CO_2CH_3	3.60	50.5		
CO_2CH_3		174.8		
$CO_2^-Na^+$				178.6

Table 2. Relative Affinities to Rat α_1, α_2 Adrenoreceptors and Batrachotoxin Channel Sodium Site

	α_1		α_2		batrachotoxin	
compds	K_i (μM)	*n* Hill	K_i (μM)	*n* Hill	K_i (μM)	*n* Hill
1, HCl	5.1	0.68	4.2	0.79	4.65	1.10
4b	>100		>100		3.70	0.98
5	>100		>100		>10	
reference	0.0035[a]	1.02	0.16[b]	1	2.10[c]	0.62

[a] Prazosin, HCl. [b] Adrenaline. [c] Veratridine.

The very easy access to these new perhydrogenated compounds prompted us to undertake a comparison, with **1**, **4b**, and **5** in a binding evaluation on some receptors (particularly adrenergic and serotoninergic receptors) and ionic channels. Compounds **1**, **4b**, and **5** demonstrated a poor affinity on 5-HT_{1A}, 5-HT_2 (IC_{50} > 10^{-5} M), and 5-HT_3 (IC_{50} > 10^{-4} M) receptors. The lack of aromaticity resulted in a decrease of affinity for α_1 and α_2 adrenoreceptors with **4b** and **5** and for the batrachotoxin sodium channel site only with compound **5** (Table 2). Unfortunately, no interesting activity toward other receptors studied (D_1, D_2, H_1, M, $GABA_A$,

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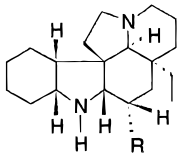
$GABA_B$, AMPA, NMDA, glycine, glucocorticoids) or ionic channels (K, Ca) was observed either for the parent compound **1** or for **4b** and **5**.



	R
4 a	CO_2Me
4 b	CO_2Me, 2 HCl
5	CO_2Na

Experimental Section

General Experimental Procedures. IR spectra were acquired on a Perkin-Elmer 457 spectrophotometer and optical rotations on a Schmidt-Haensch polarimeter. EI (70 eV) direct introduction mass spectra were obtained on a Finnigan MAT 95Q (BEqQ geometry). NMR spectra of **4a** and **5** were performed at 300 K for solutions of *ca.* 2−3 mg of compounds dissolved in 0.5 mL of DMSO d_6 on a Bruker AMX-500 equipped with a 5-mm QNP probe and a X32 computer, operating at 500.13 and 125.77 MHz for ^1H and ^{13}C, respectively. The homonuclear $^1H-^1H$ and heteronuclear $^1H-^{13}C$ chemical shift correlated 2D diagrams were obtained using the standard COSY 90, HOHAHA (2.5 ms for spin lock), and HMQC pulse sequences, respectively. Finally, 2D rotating-frame Overhauser spectroscopy (ROESY) spectra were recorded in the phase-sensitive mode TPPI. A mixing time of 800 ms was used. TLC data were obtained with Merck 60 F_{254} Si gel and Merck 60 F_{254} aluminum oxide neutral (Typ E) precoated on aluminum sheets. Compounds were visualized with a Dragendorff solution as spray reagent.

Catalytic Hydrogenation of Tabersonine (1). A solution of **1** HCl (1.863 g, 5 mmol) in 1 N methanolic $HClO_4$ (170 mL) was hydrogenated under 1 atm of pressure of hydrogen with PtO_2 (90 mg) at room temperature for 40 h. The catalyst was separated, and the filtrate was diluted with ice−H_2O, treated with 2 N aqueous NaOH to pH 10, and extracted with CH_2Cl_2. The organic layer provided crude **4a** (1.7 g) after standard treatment. Salt formation of this dry residue by gaseous HCl in MeOH and then precipitation of the crude salt in MeOH−Me_2CO afforded pure **4b** by filtration as an amorphous powder (1.250 g, 60%).

Compound 4a: colorless glass; TLC (alumina, CH_2Cl_2−MeOH, 99.5−0.5 R_f 0.30); (SiO_2, CH_2Cl_2−MeOH, 90−10 R_f 0.15); IR (CH_2Cl_2) ν_{max} 3400 (NH) 1730 (C=O, ester) cm^{-1}; EIMS *m/z* (rel int) 346 (42, M^+), 248 (64), 124 (100).

Compound 4b: amorphous pale yellow powder; $[\alpha]_D$ −48.3 (H_2O, *c* 1.5); IR (Nujol) ν_{max} 3370−2600 (N^+H, N^+H_2), 1730 (C=O, ester) cm^{-1}; *anal.* C 59.73%, H 8.10%, N 6.28%, Cl 16.62%; calcd for $C_{21}H_{36}N_2O_2Cl_2$, C 60.14%, H 8.65%, N 6.68%, Cl 16.90%.

Saponification of 4a. A solution of **4a** (346 mg, 1 mmol) in 19 mL of a mixture of THF and 0.1 N aqueous NaOH (1:1) was heated under nitrogen for 8 h at 130 °C. The solution was diluted with distilled H_2O, then washed twice with CH_2Cl_2. The aqueous layer was then evaporated to dryness, and the residue, purified by precipitation in CH_2Cl_2−EtOAc, afforded pure **5** by filtration as an amorphous powder (320 mg, 90%).

Compound 5: amorphous, pale orange powder; $[\alpha]_D$ −26.9 (H_2O, *c* 1.3); IR (Nujol) ν_{max} 3500−2800 (NH), 1575 (C=O, carboxylate) cm^{-1}; *anal.* C 67.50%, H 8.82%, N 7.74%, calcd for $C_{20}H_{31}N_2O_2Na$, C 67.77%, H 8.82%, N 7.90%.

Receptor Binding Assays. Binding assays were initiated by the addition of receptor membrane protein in an appropriate buffer containing the specific radioligand for the tested receptor. Reactions were incubated and bound ligands were separated from free ligands by vacuum filtration. The bound radioligand was estimated by liquid scintillation spectrometry. Compounds **1**, **4b**, and **5** were tested on the listed receptors in various concentrations from 10^{-10} to 10^{-4} M. The radioligand displacement curves were analyzed to calculate the IC_{50} (concentration of inhibitor displacing 50% of specifically bound radioligand) using a nonlinear least-squares program. K_i (inhibition constant) values were directly calculated using the Cheng and Prusoff equation: $K_i = IC_{50}/[1 + (L)/K_d)$.[10]

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References and Notes

(1) Plat, M.; Le Men, J.; Janot, M.-M.; Wilson, J. M.; Budzikiewicz, H.; Durham, L.-J.; Nakagawa, Y.; Djerassi, C. *Tetrahedron Lett.*, **1962**, 271−276.
(2) Numbering system proposed by Le Men, J.; Taylor, W. I. *Experientia* **1965**, *21*, 508−510.
(3) Djerassi, C.; Budzikiewicz, H.; Wilson, J. M.; Gosset, J.; Le Men, J.; Janot, M.-M. *Tetrahedron Lett.* **1962**, 235−239.
(4) For an updated review on the chemistry of *Aspidosperma* alkaloids, see Saxton, J. E. "Aspidospermine Group," in *Monoterpenoid Indole Alkaloids*; Saxton, J. E., Ed., John Wiley: NewYork, 1994; Suppl. to Vol. 25, part IV, pp 377−391.
(5) Lewin, G.; Poisson, J.; Toffoli, P. *Tetrahedron* **1987**, *43*, 493−500.
(6) Philpott, P. G.; Parsons, A. M. *J. Chem. Soc.* **1958**, 3018−3021.
(7) Siddiqui, S.; Warsi, S. A.; Allauddin, M.; Ahmad, V. *Pakistan J. Sci. Ind. Res.* **1959**, *2*, 86−92.
(8) Gorman, M.; Neuss, N.; Cone, N. J. *J. Am. Chem. Soc.* **1965**, *87*, 93−99.
(9) Malik, A.; Afza, N.; Sultana, N.; Siddiqui, S. *Heterocycles* **1981**, *16*, 1101−1113.
(10) Cheng, Y. C.; Prusoff, W. H. *Biochem. Pharmacol.* **1973**, *22*, 3099−3108.

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