SYNTHESIS AND SPECTROSCOPIC STEREOSPECIFICITY ASSAY OF THE DEUTERATED QUINOLIZIDINE ALKALOIDS (2S)-[^{2}H]- AND (2R)-[^{2}H]-SPARTEINE

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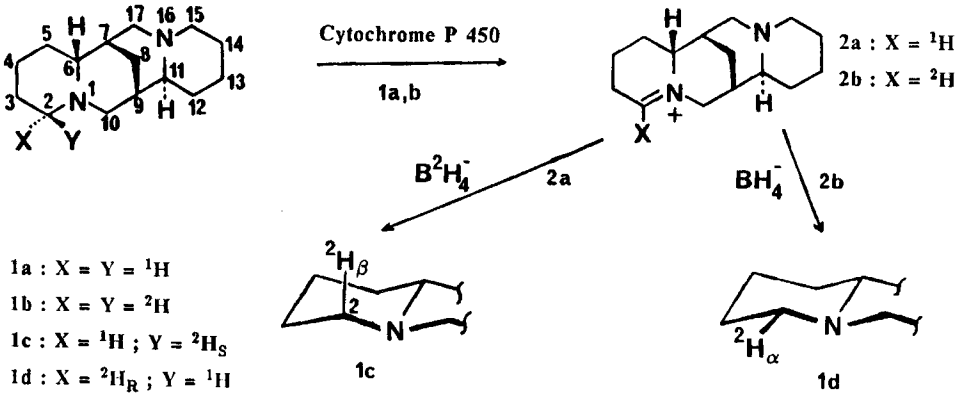
SUMMARY

Borohydride reduction of the (+)-1,2-dehydrosparteinium salts **2a,b** *proceeds almost exclusively from the* Si *side, yielding, respectively, the stereoselectively (2S)(ß)-deuterated (-)-sparteine* **1c** *from* **2a** *and* NaB^2H_4, *and the (2R)(α)-deuterated (-)-sparteine* **1d** *from* **2b** *and* $NaBH_4$. *Stereochemistry and isotopic purity of the deuterium label (≥98%) are established unequivocally by, in conjunction,* ^1H, ^2H, *and* ^{13}C *NMR spectroscopy.*

Key words: sparteine, deuteration, ^{2}H-NMR, ^{13}C-NMR.

(-)-Sparteine (**1a**) is a typical, biologically active quinolizidine lupin alkaloid found in most of the papilionaceous alkaloid synthesizing plants. In spite of numerous efforts, neither biosynthesis nor metabolism of **1a** in mammalians could be fully elucidated with respect to either key intermediates or stereochemistry of the conversions. Sparteine, which has been used for some time as an antiarrhythmic agent, has more recently received pronounced pharmacological interest as a prototype drug for uncovering polymorphic cytochrome P-450 catalyzed oxidation of various other drugs in humans[1]. The major metabolite of **1a** has tentatively been assigned a 1,2-dehydrosparteinium structure (**2a**) since, after administration of **1b** to human subjects, mass spectrometry shows the loss of one deuterium atom in the respective metabolite (**2b**)[2].



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Metabolite **2a** is now available on a preparative scale by chemical synthesis. From the known reactivity of iminium compounds towards hydride donors[4], reduction of the CN double bond of **2** could be expected to yield sparteine (**1**) in a retro-biomimetic manner. We accordingly treated synthetic (+)-**2a** and (+)-**2b** with borodeuteride and borohydride, respectively, and obtained monodeuterated (≥ 98% ^2H) (-)-sparteines **1** in 73-80% isolated yield[5]. The stereochemistry of the products was established by a combination of NMR methods.

For compound **1b**, di-deuterated at C-2, two signals appear in the ^2H-NMR spectrum (see Figure 1), separated by 0.74 ppm and corresponding to 2α and 2ß deuterium, respectively. The anisochrony between the axial and equatorial position at C-2 corresponds exactly to that reported by Golebiewski[6] for a two-dimensional correlation analysis of the ^1H spectrum of sparteine; the absolute shifts in our spectrum (0.15 molar in cyclohexane), however, are 0.05 ppm to lower field than the values given for **1a** (0.07 molar in benzene[6]). The second trace in Figure 1 shows the ^2H spectrum of the compound, prepared from the 2-deuterio iminium salt **2b** with borohydride (i.e. **1d**). The uppermost trace displays the spectrum of the compound, obtained from the sparteinium salt **2a** with NaB^2H_4 (i.e. **1c**). The ^2H spectra present striking proof that the mechanistic rationale, i.e. exclusive attack of the hydride reducing agent from the ß(*Si*) side, in fact holds true. At the same time, these spectra demonstrate a configurational selectivity better than the NMR detection threshold (≥98%).

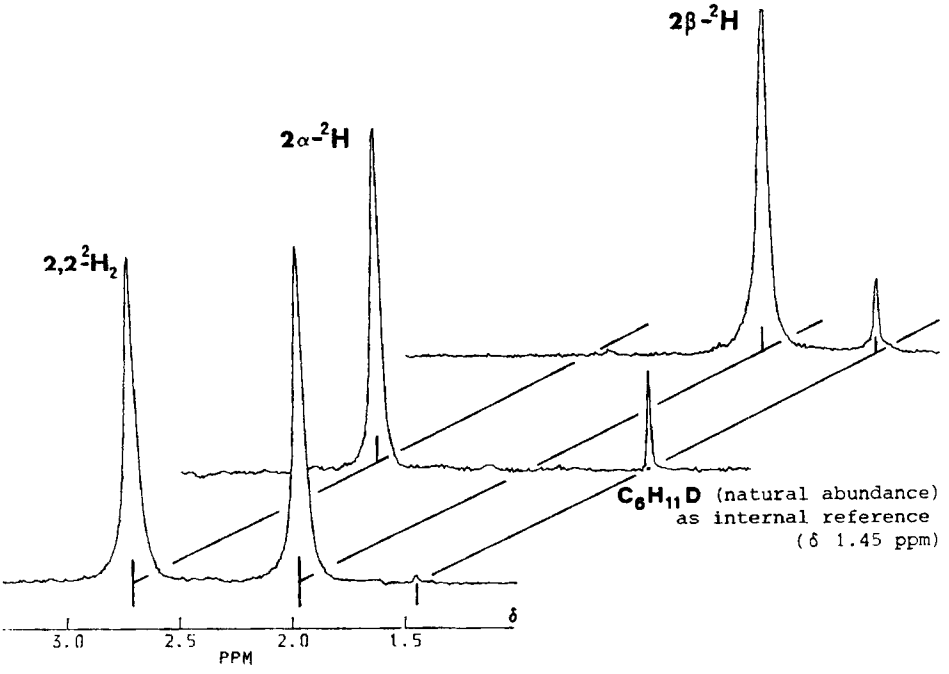

Figure 1. *^2H-NMR spectra (^1H noise-decoupled) of 2,2-($^2H_2$)-, 2α-(^2H)-, and 2ß-(^2H)-sparteine, respectively [0.15 molar in C_6H_{12}, with natural abundance $C_6{}^1H_{11}{}^2H$ as internal reference at δ 1.45 ppm, 20-50 scans, sweep width 1501.502 Hz, 8k interferograms, digital resolution 0.307 Hz = 0.01 ppm].*

In the case of sparteine, fortunately, a complete and reliable analysis of the ^{1}H-NMR spectrum is available on which the assignment of α-and ß-^{2}H resonances can be based[6]. If no full spin analysis of the proton spectrum is available, ^{13}C-NMR spectroscopy offers an alternative route for unequivocally establishing the stereochemistry of a deuterium label in this type of alkaloid structure.

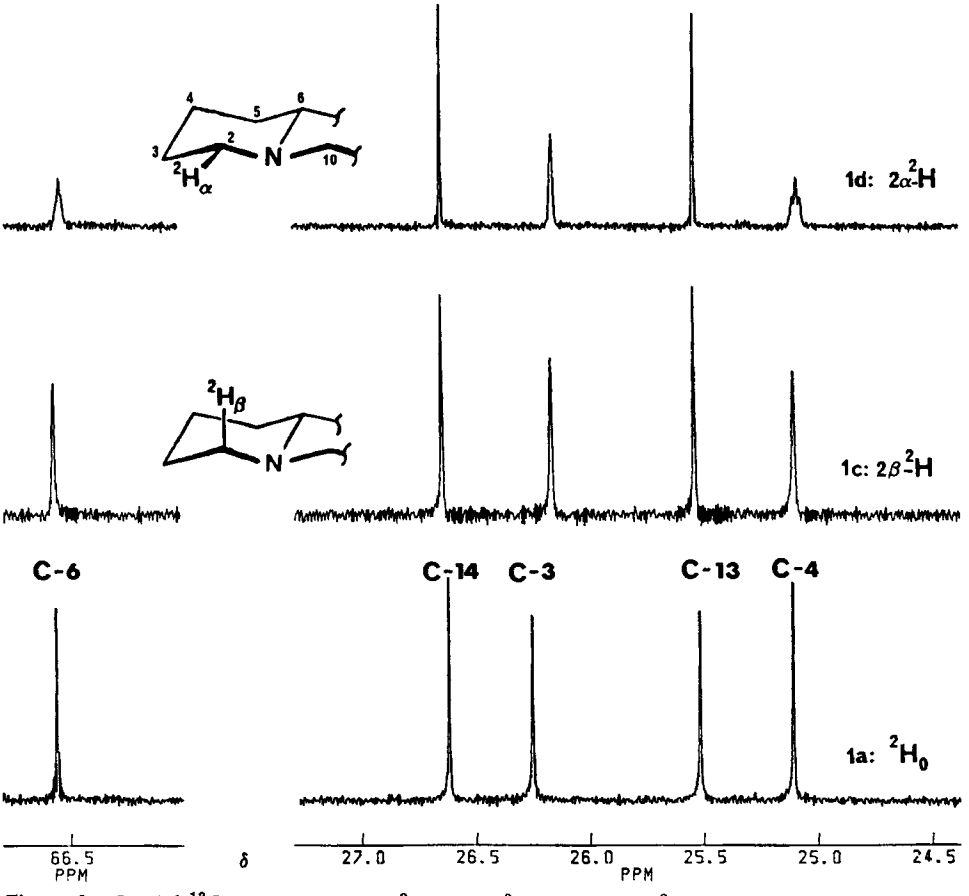

Figure 2. *Partial ^{13}C-NMR spectra of $^2H_0$-, 2ß-(^{2}H)-, and 2α-(^{2}H)-sparteine [0.15 molar in $C_6{}^2H_6$, with TMS as internal reference, ^{1}H noise-decoupled, 200-1000 scans, FID 32 k, FT 64k, digital resolution 0.198 Hz = 0.003 ppm, resolution enhancement by Gaussian multiplication].*

Figure 2 shows, in part, the ^{1}H noise-decoupled ^{13}C spectra of $^2H_0$-sparteine (**1a**, lower trace) and of the two reduction products, mono-deuterated, respectively, at C-2. In the uppermost trace, the C-13 and C-14 resonances (assignment according to ref.[7]) remain unchanged relative to **1a** while C-4 and C-6 are split by long-range ^{13}C,^{2}H coupling. Severe overlap of the individual multiplet components blurs the appearance of what actually should be 1:1:1 triplets. However, by careful Gaussian treatment of the FIDs, ^{3}J coupling constants of 1.0 and 0.8 Hz can be extracted from the spectra (not shown). Unresolved ^{2}J(C,^{2}H) coupling is responsible for the substantial broadening of the C-3 signal. In the other regioisomer (Figure 2, middle trace), C-6, C-3, and C-4 appear slightly broadened, each to roughly the same extent, while the C-13

and C-14 resonances once again remain unimpaired.

For the A ring of sparteine, a true chair conformation is safely established[8]. From a molecular model, it becomes immediately apparent that deuterium in an equatorial, i.e. α-position at C-2 is perfectly *s-trans* oriented with respect to both C-6 and C-4, and thus in the optimum configuration for ^3J coupling across a σ pathway (dihedral angle 180^0)[9]. For the ß-isomer, on the other hand, the dihedral angles between the deuterium in axial position and both C-6 and C-4 is 60^0, with a concurrent reduction of $^3J(C,^2H)$ which no longer is resolved in the ^{13}C spectrum. This stereochemical distinction becomes apparent, though to a lesser extent, even at C-10 in ring B, with dihedral angles of 30^0 and 60^0, respectively, for α- and ß-^2H.

Thus, stereoselectively labelled (*2S*-^2H)-sparteine (2ß-(^2H)-**1c**) and (*2R*-^2H)-sparteine (2α-(^2H)-**1d**) are now conveniently available in high diastereotopic purity for studies of the biosynthesis and metabolism of this alkaloid[10]. The preparative strategy and spectroscopic methodology presented here are expected to prove amenable also to the stereoselective α-deuterium labelling of other suitably structured tertiary amines.

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EXPERIMENTAL

The equipment used has been described in detail elsewhere[5]. ^2H- and ^{13}C-NMR spectra were recorded on a Bruker CXP 300 / ASPECT 2000 instrument in PFT mode at 300.13, 46.07, and 75.47 MHz nominal frequency (^2H spectra without deuterium lock). Interferogram and Fourier Transform size, as well as digital resolution are given in the individual figure captions.

(+)-1,2-Dehydrosparteinium monoperchlorate (2a) and **(+)-2-[^2H]-1,2-dehydrosparteinium monoperchlorate (2b)** were prepared in about 50% yield by reduction of (+)-lupanine ((+)-2-oxosparteine) with diisobutylaluminium-hydride and -deuteride, respectively[3].

2a: m.p. 168°C (dec.), $[\alpha]^{21}_D$ +36.3 (c = 1.0, CH_3CN). Calc. for $C_{15}H_{24}N_2 \cdot HClO_4$ (332.8) C 54.13%, H 7.57%, N 8.42%, Cl 10.65%; found C 54.21%, H 7.56%, N 8.34%, Cl 10.61%.-

2b: m.p. 165°C (dec.), calc. for $C_{15}{}^1H_{23}{}^2H_1N_2 \cdot HClO_4$ (333.8) C 53.9%, $^1H+^2H$ 7.85%, N 8.39%, Cl 10.62%; found C 53.91%, $^1H+^2H$ 8.08%, N 8.33%, Cl 10.82%.- **MS** (PI/EI, 70eV): $\geq$ 98% ^2H.

(-)-(2S)-[^2H]-Sparteine, (1c). To a cooled and stirred solution of (+)-1,2-dehydrosparteinium perchlorate (**2a**, 8.1 mmol) in 100 ml of methanol solid sodium borodeuteride (32.5 mmol, 98% ^2H) was added in portions. After 15 min the mixture was concentrated under reduced pressure, diluted with excess aqueous sodium hydroxide (pH> 10), and then extracted several times with diethyl ether. The combined extracts were dried ($MgSO_4$), concentrated *in vacuo*, and fractionated in a short-path distillation apparatus (bp 94°C/0.02mbar) to give **1c**, colourless oil, in 73-80% yield, $[\alpha]^{21}_D$ -16.3 (c 2.2, EtOH).- **MS**(70 eV, PI/EI), *m/z*(rel.abund.,%): 235(22.9,$M^{+\cdot}$), 194(39.8), 138(100), 99(63.4).-

Sulphate of **1c**: calc. for $C_{15}{}^1H_{25}{}^2HN_2 \cdot H_2SO_4 \cdot 5H_2O$ (423.5) C 42.54%, $^1H+^2H$ 9.27%, N 6.61%, S 7.57%; found C 42.57%, $^1H+^2H$ 9.46%, N 6.64%, S 7.59% .Mp 135-136°C, $[\alpha]^{21}_D$ -11.2 (c 1.76, EtOH).

(-)-(2R)-[^{2}H]-Sparteine, 1d. In like manner **1d** was prepared from **2b** using sodium borohydride (MS:235(36.5, $M^{+\cdot}$), 194(48.9), 138(100), 99(67.7)) and converted to its sulphate: mp 133°C, $[\alpha]^{21}_D$ -10.7 (c 0.85, EtOH); calc. for $C_{15}{}^1H_{25}{}^2H_1N_2 \cdot H_2SO_4 \cdot H_2O$ (423.5) C 42.54%, $^1H+^2H$ 9.27%, N 6.61%, S 7.57%; found C 42.78%, $^1H+^2H$ 9.46%, N 6.57%, S 7.60%.

The sulphates of **1c** and **1d** were prepared as described[5]. The isotopic purity of **1c** and **1d** was determined from the PI/CI(NH_3) mass spectra in the SIM mode using the $[M + 1]^{+\cdot}$ ion at m/z 236: **1c** and **1d** $\geq$ 98% $^2H_1$.

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