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Note

Unambiguous NMR spectral assignments of salvinorin A

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The complete assignments of the ^{1}H and ^{13}C NMR spectra of the hallucinogenic neoclerodane diterpenoid salvinorin A were determined in three different NMR solvents using HSQC, HMBC and COSY. Solvent systems are described that allow the resolution of all ^{1}H signals. Virtual coupling was observed for the protons at C-2, C-3 and C-4 in the 600 MHz ^{1}H spectrum in $CDCl_3$. The complete assignments of the ^{1}H and ^{13}C NMR spectra of salvinorin B are also reported. Copyright © 2007 John Wiley & Sons, Ltd.

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The Mexican herb *Salvia divinorum* contains the neoclerodane diterpenoid salvinorin A (**1**), a highly unusual drug that exerts its hallucinogenic effects as a potent agonist of the κ-opioid receptor.[1–4] In connection with our biosynthetic studies of this molecule, the complete assignment of its ^{1}H and ^{13}C NMR spectra was necessary. In order to resolve inconsistencies between the NMR spectral assignments found in the two original reports of this substance[5,6] and a later publication,[7] detailed NMR analysis was carried out using a 600-MHz instrument.

The complete resolution of all NMR signals is desirable for the interpretation of isotopic labeling patterns resulting from biosynthetic experiments. However, while the signals of the ^{13}C NMR spectrum in $CDCl_3$ are well separated, considerable overlap is observed in the ^{1}H spectrum even at 600 MHz. A general strategy for resolving overlapping signals is to change the NMR solvent or the temperature. However, the changes in the spectrum are difficult to predict and must be determined experimentally. With the goal of finding conditions in which the ^{1}H signals were completely resolved, the solvents $CDCl_3$, C_6D_6, C_5D_5N and $(CD_3)_2CO$ were evaluated at different temperatures. Acetone was not an optimal solvent because there was limited solubility and the residual solvent signal overlapped with signals of the sample. The H_2O peak in acetone also appeared close to the signals of the sample. Pyridine, with three residual solvent peaks in the aromatic region, was difficult to shim. Again, the residual solvent signals overlapped with the signals of **1**. Binary combinations of equal volumes of the four different solvents were tested with the exception of $C_5D_5N/(CD_3)_2CO$. The combination $(CD_3)_2CO/CDCl_3$ 1:1 at 30 °C was found to provide excellent resolution of all of the signals of **1**, but the residual acetone peak came very close to the signal of the acetate methyl group and partly obscured another signal. Further combinations of variable compositions of $CDCl_3$ and C_6D_6 were explored, but in all cases the two signals near 1.15 ppm overlapped. The best resolution was finally achieved with a ternary mixture of equal volumes of $CDCl_3$, C_6D_6, and C_5D_5N at 20 °C.

To obtain the spectral assignments, two-dimensional NMR experiments (heteronuclear single quantum correlation (HSQC), heteronuclear multiple bond correlation (HMBC) and $^{1}H–^{1}H$ correlation spectrometry (COSY)) were carried out in three different solvents: in $CDCl_3$ at 30 °C, $(CD_3)_2CO/CDCl_3$ 1:1 at 30 °C and in $CDCl_3/C_6D_6/C_5D_5N$ 1 : 1:1 at 20 °C. The HSQC spectrum provided correlations of the signals of all H atoms with their directly attached C atoms. The assignments of the signals were largely based on the HMBC spectrum which gives the 2 and 3 bond connectivities (Fig. 1). As a starting point, the correlations of the C-19 and C-20 methyl groups were determined. These two methyl groups, however, were not easy to distinguish since both had correlations to one quaternary center, two methines and one methylene; and of these one methine group (C-4 or C-8) was in both cases flanked by an ester carbonyl and a methylene group, while the other methine (C-10) was common to both methyl groups. The assignment of C-19 and C-20 was made on the basis of a strong correlation observed between one of the methylene groups (C-11) and the hydrogen at C-12. The C-12 hydrogen also showed HMBC correlations to C-13, -14, and -15 of the furan ring, as well as the lactone carbonyl (C-17). The signal of the C-2 hydrogen, on the other hand, showed correlations to the ketone carbonyl (C-1) and the acetate carbonyl (C-21), which in turn was correlated to the acetate methyl group (C-22). These assignments were

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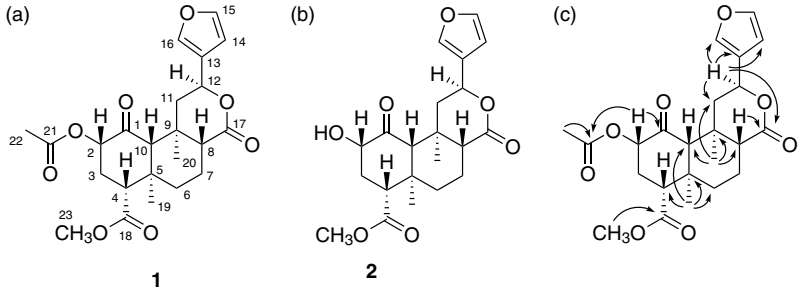

Figure 1. (a) Structure of salvinorin A (**1**) and the numbering scheme; (b) Structure of salvinorin B (**2**); (c) Selected HMBC correlations for salvinorin A (**1**).

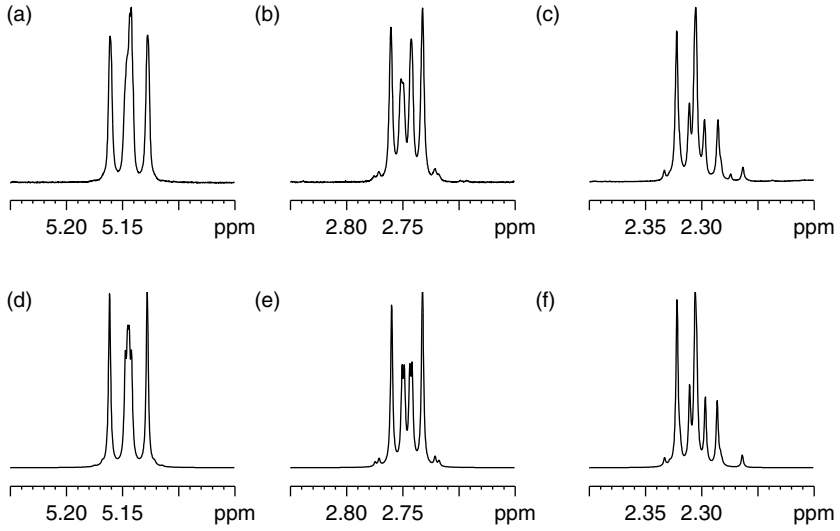

Figure 2. (a–c) Observed ^{1}H-NMR signals for salvinorin A (**1**) (600 MHz, $CDCl_3$, 30 C); (d–f) Simulated ^{1}H-NMR signals; (a) and (d) 2β-H; (b) and (e) 4β-H; (c) and (f) 3α-H and 3α-H.

confirmed by additional HMBC correlations of the hydrogen signal of the methoxy group (C-23) with the C-18 ester carbonyl, and of the hydrogen signal of C-8 with the lactone carbonyl (C-17). The ^{1}H signals of C-7 were clearly identified through correlations in the COSY spectrum with the C-8 methine signal. Useful COSY correlations were also observed between the C-15 and C-14 methines, and between the C-3 methylene group and the C-2 and C-4 methines. In this way, the assignments of the NMR signals were obtained in the three different solvents (Table 1).

The assignment of the diastereotopic protons of the methylene groups (C-3, 6, 7 and 11) was made on the basis of *J*-values that were obtained by direct inspection of the ^{1}H NMR spectrum. Thus, the axial H atoms displayed higher vicinal coupling constants than the equatorial H atoms as described by the Karplus relationship.[8] The larger coupling constants between H atoms in anti-periplanar relationships were also evident in the COSY spectrum as stronger correlations between 3α-H and the axial hydrogens at C-2 and C-4; the 11β-H atom and 12α-H; and between 7α-H and 8β-H. In general, the axial H atoms also displayed greater upfield chemical shifts than the equatorial H atoms.

The signals of 2β-H and 4β-H in the $CDCl_3$ spectrum displayed different apparent coupling constants (J = 11.0, 8.8 and J = 10.8, 6.0, respectively) than those observed in the other two solvents. This was shown to be a case of virtual coupling,[8] which is due to the small difference between the chemical shifts of the diastereomeric H atoms at position 3 in this solvent. Simulation of the spin system using Bruker NMR-Sim showed that the real *J*-values are similar to those obtained in the other two solvents (Fig. 2).

Comparison with the spectral data published by Ortega *et al*.[5] and by Valdes *et al*.[6] showed a much better agreement of the present assignments with those of the latter paper. The tentative assignments of the ^{13}C spectrum by Ortega *et al*. had the C-19 and C-20 methyls reversed, the C1- and C-18 carbonyls reversed, and C-11 incorrectly assigned, the only methylene signal to be assigned in that publication.[5] However, the assignment of the ^{1}H spectrum, though incomplete, was correct. The ^{1}H and ^{13}C assignments of Valdes *et al*. are in excellent agreement with our data, and differed significantly only at positions 6 and 7, where the signal of the 7β-H was assigned to 6β-H.[6] More recently published ^{1}H NMR data had the assignments of the 7α and 7β H atoms reversed.[7]

The NMR spectra of salvinorin B (2-desacetyl salvinorin A) were also assigned (Table 1). Although some ^{1}H signals overlapped in $CDCl_3$, a good separation of signals was achieved using $(CD_3)_2CO$ at 40 °C, in which only one ^{1}H-signal was partially obscured by the residual solvent signal.

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Table 1. 600 Mz ^{1}H and 151 Mz ^{13}C NMR assignments. All ^{1}H signals are 1 H unless specified otherwise, the coupling constants are given in Hz and all ^{13}C data are in boldface. The assignments were determined by HSQC, HMBC and COSY 2D NMR experiments

Position	Salvinorin A[a,b] $CDCl_3$ 30 °C	Salvinorin A[c] $CDCl_3/(CD_3)_2CO$ 1 : 1 30 °C	Salvinorin A[d] $CDCl_3/C_6D_6/C_5D_5N$ 1 : 1 : 1 20 °C	Salvinorin B[c] $(CD_3)_2CO$ 40 °C
1	**201.96**	**202.77**	**202.36**	**210.40**
2	5.145 dd 12.6,7.5*	5.180 dd 12.5,7.5	5.116 dd 12.5,7.4	4.216 ddd 11.8,7.7,4.0
	75.04	**75.43**	**75.38**	**75.16**
3α	2.301 ddd 13.4,13.0,12.6*	2.186 q 13.0	2.179 q 13.0	1.946 q 12.8
3β	2.313 ddd 13.4,7.5,3.5*	2.279 ddd 13.1,7.5,3.6	2.065 ddd 13.1,7.3,3.3	2.365 ddd 13.2,7.6,3.4
	30.82	**31.13**	**30.98**	**35.74**
4	2.746 dd 13.0,3.5*	2.918 dd 13.4,3.5	2.607 dd 13.3,3.0	2.905 dd 13.5,3.3
	53.65	**53.30**	**53.24**	**53.76**
5	**42.12**	**42.21**	**42.04**	**43.05**
6α	1.803 dt 13.2,2.9	1.734 dt 13.5,3.5	1.538 dt 13.3,2.6	1.738 dt 13.0,3.4
6β	1.573 dt 3.7,13.3	1.647 dt 3.5,13.2	1.347 dt 3.4,13.2	1.678 dt 3.4,13.0
	38.21	**38.17**	**38.11**	**38.76**
7α	1.640 ddt 12.8,2.8,13.1	1.574 dq 3.5,13.2	1.455 dq 2.8,13.3	1.613 dq 3.8,12.8
7β	2.166 dq 14.0,3.4	2.038 dq 14.2,3.3	2.007 dq 14.1,2.9	2.017 dq 13.9,3.4
	18.17	**18.50**	**18.50**	**19.23**
8	2.067 dd 11.9,3.0	2.241 dd 11.7,2.8	1.791 dd 12.0,2.6	2.334 dd 11.8,2.8
	51.44	**51.04**	**51.09**	**51.42**
9	**35.50**	**35.68**	**35.52**	**36.26**
10	2.165 s	2.547 s	2.050 s	2.629 s
	64.17	**63.30**	**63.27**	**63.45**
11α	2.514 dd 13.4,5.2	2.372 dd 13.5,5.3	2.300 dd 13.4,5.1	2.448 dd 13.5,5.4
11β	1.571 t 12.8	1.700 t 12.7	1.331 t 12.4	1.786 t 12.5
	43.48	**43.34**	**43.11**	**44.08**
12	5.526 dd 11.6,5.1	5.510 dd 11.6,5.2	5.259 dd 11.8,5.1	5.597 dd 11.6,5.4
	73.03	**72.00**	**71.87**	**72.28**
13	**125.30**	**126.15**	**125.93**	**127.45**
14	6.373 s	6.413 s	6.148 s	6.527 s
	108.38	**108.90**	**108.80**	**109.69**
15	7.390 t 1.7	7.394 t 1.5	7.164 t 1.5	7.518 t 1.6
	143.71	**143.90**	**143.79**	**144.67**
16	7.408 s	7.460 s	7.174 s	7.596 s
	139.41	**140.01**	**139.72**	**140.81**
17	**171.04**	**171.36**	**171.01**	**171.54**
18	**171.52**	**172.02**	**171.75**	**173.08**
19	1.121 s (3H)	1.065 s (3H)	0.899 s (3H)	1.077 s (3H)
	16.40	**16.42**	**16.34**	**16.76**
20	1.455 s (3H)	1.395 s (3H)	1.247 s (3H)	1.446 s (3H)
	15.22	**15.24**	**15.16**	**15.63**
21	**169.92**	**169.62**	**169.66**	–
22	2.168 s (3H)	2.068 s (3H)	1.898 s (3H)	–
	20.54	**20.44**	**20.48**	–
23	3.726 s (3H)	3.667 s (3H)	3.444 s (3H)	3.683 s (3H)
	51.93	**51.73**	**51.66**	**51.81**

[a] Coupling constants were obtained by direct inspection of the spectra, except those marked with an asterisk, which were obtained by simulation (see text).

[b] ^{1}H spectrum referenced to residual $CHCl_3$ (7.26 ppm). ^{13}C spectrum referenced to $CDCl_3$ (77.0 ppm).

[c] ^{1}H spectrum referenced to residual CD_3COCD_2H (2.05 ppm). ^{13}C spectrum referenced to $(CD_3)_2CO$ (29.84 ppm).

[d] ^{1}H spectrum referenced to residual C_6D_5H (7.15 ppm). ^{13}C spectrum referenced to C_6D_6 (128.0 ppm).

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REFERENCES

1. Prisinzano TE. *Life Sci.* 2005; **78**: 527.
2. Yan F, Roth BL. *Life Sci.* 2004; **75**: 2615.
3. Sheffler DJ, Roth BL. *Trends Pharmacol. Sci.* 2003; **24**: 107.
4. Roth BL, Baner K, Westkaemper R, Siebert D, Rice KC, Steinberg S, Ernsberg P, Rothman RB. *Proc. Natl. Acad. Sci.* 2002; **99**: 11934.
5. Ortega A, Blount JF, Manchand PS. *J. Chem. Soc., Perkin Trans. I* 1982; 2505.
6. Valdes LJ, Butler WM, Hatfield GM, Paul AG, Koreeda M. *J. Org. Chem.* 1984; **49**: 4716.
7. Munro TA, Rizzacasa MA, Roth BL, Toth BA, Yan F. *J. Med. Chem.* 2005; **48**: 345, (in supporting information).
8. Silverstein RM, Webster FX, Kiemle DJ. *Spectrometric Identification of Organic Compounds* (7th edn). Wiley: Hoboken, 2005.

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