Tetrahedron Letters,Vol.27,No.22,pp 2523-2526,1986 0040-4039/86 $3.00 + .00
Printed in Great Britain Pergamon Journals Ltd.

TRIFLUOROACETIC ACID, A ^{1}H-NMR SHIFT REAGENT FOR ALKALOIDS

J. Schripsema, R. Verpoorte and A. Baerheim Svendsen

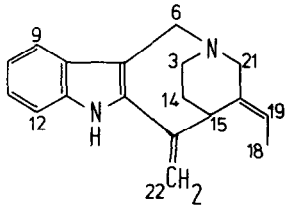
Department of Pharmacognosy, Center for Bio-Pharmaceutical Sciences, Leiden University, Gorlaeus Laboratories, P.O. Box 9502, 2300 RA Leiden, the Netherlands.

Summary: Acidic impurities may change the ^{1}H-NMR spectra of alkaloids drastically. The acid-induced shifts might be a useful tool in the structure elucidation of alkaloids.

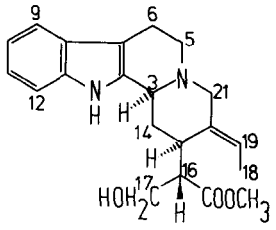
Recently several alkaloids were isolated from a cell-culture of a *Tabernaemontana* species (1). One of the alkaloids was isolated in a minute amount. In MS, UV and TLC behaviour the alkaloid was identical to apparicine (1). The ^{1}H-NMR spectrum also showed all signals present in the spectrum of apparicine. However, most signals displayed considerable shifts (see Fig. 1). After a thorough analysis of the spectrum it could be concluded that those signals due to protons close to N-4 showed the most pronounced downfield shifts. The signals of the aromatic protons of the indole-moiety hardly shifted at all. These shifts could not be explained by differences in conformation or stereochemistry. The fact that the protons in the neighbourhood of N-4 showed the largest shifts, suggested that the shifts might be a result of some sort of interaction at the nitrogen lone-pair.
The TLC and MS data ruled out the possibility of an N-oxide.
Addition of small amounts of water to an apparicine solution in $CDCl_3$ demonstrated that traces of water were not responsible for the observed shifts. However, considerable shifts were obtained when small amounts of trifluoroacetic acid or other acids were added. These shifts were similar to the ones observed.

Isolated from another source, a minute sample of 16-epi-isositsirikine (2) also showed a deviating ^{1}H-NMR spectrum, and again similar shifts could be obtained by addition of trifluoroacetic acid to a $CDCl_3$ solution of the pure alkaloid. It was therefore concluded that the changes in the ^{1}H-NMR spectra of the isolated apparicine and 16-epi-isositsirikine were caused by acidic impurities.

Trifluoroacetic acid forms an ion-pair with the alkaloids in which N-4 is protonated. The differences in the protonation shifts of the different protons cannot be explained just by the number of bonds between the proton



1. Apparicine



2. 16-Epi-isositsirikine

and the protonated nitrogen. Other factors are also important. Theoretically the protonation shifts can be influenced by the following factors:

1) the number of bonds between the proton and the protonated nitrogen (σ-bond induction);
2) the distance through space (direct induction);
3) the angles between the bonds (orbital interactions).

The latter two factors can be especially important in rigid structures, like many alkaloids. However, the contribution of these factors to the protonation shifts in ^{1}H-NMR have not yet been studied in detail. Protonation shifts themselves have been used several times, e.g. ^{1}H-NMR was used to study the protonation of polyaminocarboxylate compounds in aqueous solutions (2,3). A change of solvent from $CDCl_3$ to CF_3COOH is more commonly used to get resolution of overlapping signals. It has been described that in this way N-methyl groups can be detected (4).

As it seemed that trifluoroacetic acid could be a useful shift reagent for alkaloids in ^{1}H-NMR, the protonation shifts were studied in some more detail.

In table 1 the protonation shifts of all protons in apparicine and 16-epi-isositsirikine upon addition of about one equivalent of trifluoroacetic acid are given. Stepwise addition of the acid showed the shifts as being almost linear to the amount added, until complete protonation was achieved. The linearity has previously been described (5).

Protons attached to carbons 3,5 (or 6) & 21, adjacent to the protonated nitrogen, in both compounds show a strong downfield shift. However, the shifts observed for geminal protons are not always equally large. As has been shown by the investigation of models, this is a result of different angles between the C-H bond and the N-H bond, in the preferred conformations of the alkaloids (6,7). Generally speaking, the smaller the angle the larger the shift.

The signal of the N-1 proton in 16-epi-isositsirikine shows a very large downfield shift of 1.64 ppm, while in apparicine this signal only shifts 0.44 ppm. The much larger shift in 16-epi-isositsirikine is probably a result

TABLE 1: Protonation shifts[1] of appariceine (1) and 16-epi-isositsirikine (2).

appariceine (1)			16-epi-isositsirikine (2)		
H nr.	δ in ppm	protonation shift	H nr.	δ in ppm	protonation shift
NH	7.98	+0.44	NH	8.08	+1.64
3a	3.49	+0.34	3	3.97	+0.69
3b	3.09	+0.17	5a	2.85	+0.40
6a	4.56	+0.31	5b	3.19	+0.24
6b	4.28	+0.14	6a	2.97	+0.09
9	7.42	0.00	6b	2.70	+0.18
10	7.07	+0.06	9	7.48	-0.05
11	7.19	+0.07	10	7.09	+0.02
12	7.30	+0.05	11	7.15	+0.03
14a	2.20	+0.16	12	7.32	+0.05
14b	1.91	+0.11	14a	2.29	+0.13
15	3.93	+0.12	14b	2.25	+0.40
18	1.48	+0.10	15	3.41	-0.12
19	5.32	+0.33	16	2.65	-0.23
21a	3.86	+0.30	17a	3.94	+0.01
21b	3.26	+0.35	17b	3.88	0.00
22a	5.43	+0.18	18	1.63	-0.04
22b	5.29	+0.12	19	5.54	+0.20
			21a	3.81	+0.12
			21b	3.13	+0.31
			CO_2Me	3.59	-0.06

[1] + = downfield shift
- = upfield shift
Protonation shifts upon addition of about one equivalent of trifluoroacetic acid.

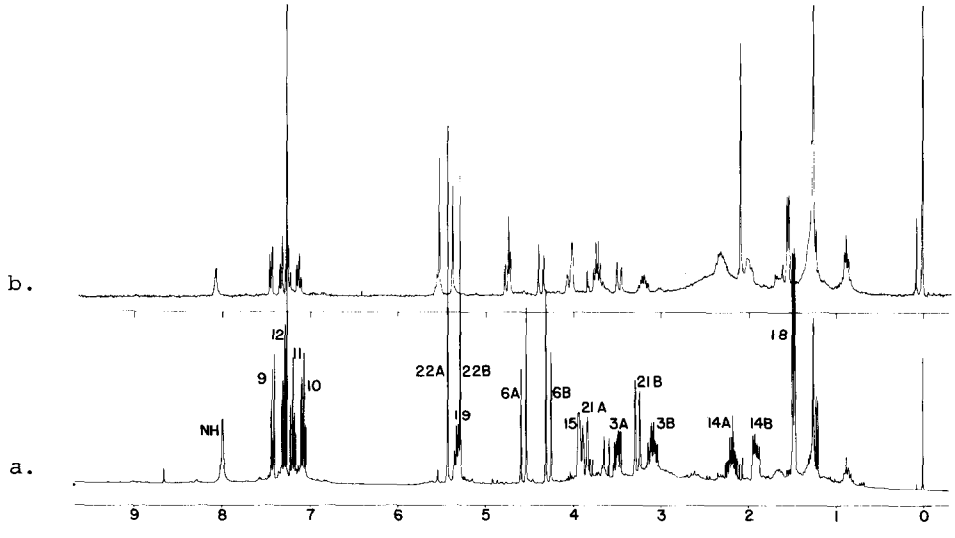


Fig. 1. The influence of an acidic impurity upon the ^{1}H-NMR spectrum of appariceine: a. normal spectrum;
b. with acidic impurity.

of a strong through-bond interaction between the two nitrogens, which in 16-epi-isositsirikine, where the nitrogens are separated by only two carbon-atoms, is expected to be larger than in apparicine, where they are separated by three carbon-atoms.

Protons attached to double bonds show relatively large downfield shifts as compared to aliphatic protons, which are equally remote to the protonated nitrogen. This is explained as the effect of the easier polarization of double bonds.

From these data, it is apparent that a lot of information can be gathered from these protonation shifts. However, further investigations are necessary to come to a better understanding of the factors influencing the shifts. Such investigations are now in progress and will be reported in due time.

We may summarize the advantages of trifluoroacetic acid as a shift reagent for alkaloids in ^{1}H-NMR as follows:

1) overlapping signals might be resolved;
2) protons in the neighbourhood of the protonated nitrogen are readily identifiable by large shifts;
3) information about stereochemistry and conformation can be obtained;
4) linear relation between shifts observed and concentration of trifluoro-acetic acid, until the equivalence point is reached;
5) relatively simple experiment, small amounts of alkaloid are sufficient;
6) cheap shift reagent.

References:

1. R. van der Heijden, R.L. Brouwer, R. Verpoorte, R. Wijnsma, T.A. van Beek, P.A.A. Harkes and A. Baerheim Svendsen, Phytochemistry, 25 (1986), in press.
2. J.L. Sudmeier and C.N. Reilley, Anal. Chem., 36, 1698 (1964).
3. J.L. Sudmeier and C.N. Reilley, Anal. Chem., 36, 1707 (1964).
4. J.C.N. Ma and E.W. Warnhoff, Can. J. Chem., 43, 1849 (1965).
5. E. Grunwald, A. Loewenstein and S. Meiboom, J. Chem. Phys., 27, 641 (1957).
6. F. Heatley, L. Akhter and R.T. Brown, J. Chem. Soc. Perkin Trans. 2, 919 (1980).
7. C. Kan, S.-K. Kan, M. Lounasmaa and H.P. Husson, Acta Chem. Scand., B35, 269 (1981).

(Received in UK 21 March 1986)