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CARBON-13 NUCLEAR MAGNETIC RESONANCE SPECTRUM OF COLCHICINE : A REASSIGNMENT

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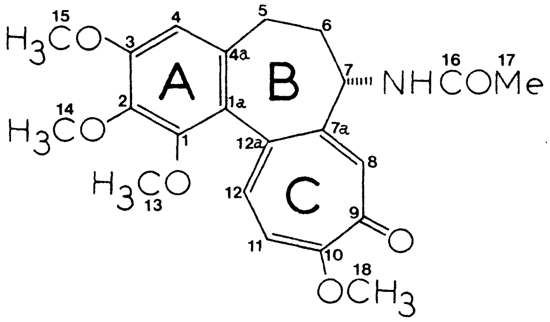
A new NMR signal assignment of the twenty-two carbon atoms of colchicine is proposed that differs considerably from that of Singh, Palmar, Stenberg and Farnum [Spectroscopy Letters, 10, 1001 (1977)]. Some reasons of those discrepancies are analyzed.

The great biological and pharmacological interest of colchicine[1] justifies its study by ^{13}C NMR spectroscopy. A correct and complete assignment is furthermore absolutely necessary if the chemical shifts have to be used for a subsequent study of the interaction between colchicine and the tubuline enzyme or for the identification of its metabolites.

Recently, a paper from Singh, Palmar, Stenberg and Farnum[2] has been published in which the ^{13}C NMR spectrum of colchicine in $CDCl_3$ is given and a complete assignment of the 22 carbon signals proposed. We had in progress a similar study of colchicine and a dozen of its derivatives. The fact that our assignment and that of Singh et al.[2] agree only for 12 of the 22 colchicine carbon atoms, prompts us to publish our assignment and to explain briefly the reasons which in our opinion, have misled the quoted authors.

Like Singh et al.[2] we have recorded the proton-noise-decoupled and the SFORD (single frequency off-resonance decoupled) ^{13}C NMR spectra (but in DMSO-d_6 instead of $CDCl_3$) and compared colchicine C ring to tropolone methyl ether. In addition we have studied many other colchicinic and isocolchicinic derivatives and measured the T_1 relaxation times of the different carbon atoms. The results are gathered in Table 1.



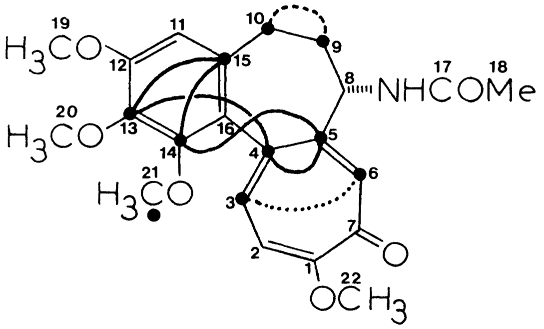


TABLE 1. Carbon-13 chemical shifts of colchicine[3] (δ in ppm relative to TMS)

This work:	Carbon No	Assignment	Singh et al.[2] Assignment	(No)	Difference
	13	60.9*	56.4	(21)	4.5 ●
	14	60.7*	61.3	(20)	-0.6
	15	56.0**	56.4	(19)	-0.4
	16	168.9	170.3	(17)	-1.4
	17	22.4	22.6	(18)	-0.2
	18	55.9**	56.1	(22)	-0.2

TABLE 1 (Continued)

Ring A	1a	125.7	125.7	(16)	0.0	
	1	150.7	152.9	(14)	-2.2	●
	2	141.1	134.5	(13)	6.6	●
	3	153.2	153.6	(12)	-0.4	
	4	108.0	107.5	(11)	0.5	
	4a	134.4	151.1	(15)	-16.7	●
Ring B	5	29.4	36.2	(10)	-6.8	●
	6	36.0	30.0	(9)	6.0	●
	7	51.7	52.9	(8)	-1.2	
Ring C	7a	151.2	137.2	(5)	14.0	●
	8	134.7	130.5	(6)	4.2	●
	9	178.4	179.6	(7)	-1.2	
	10	163.8	164.1	(1)	-0.3	
	11	112.3	113.1	(2)	-0.8	
	12	130.7	135.8	(3)	-5.1	●
	12a	135.6	141.6	(4)	-6.0	●

Origin of the mistakes of Singh et al.[2]

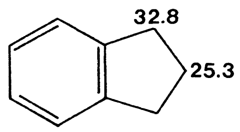
Methyl carbon atom, C_{13}.

Due to the lack of resolution (the authors worked at 15.03 MHz instead of 20.0 MHz) they observed only three OCH_3 signals at δ 56.1, 56.4, and 61.3. They supposed the missing signal, corresponding to C_{13} to be mixed with C_{15} (56.4), but actually it was under the C_{14} signal (61.3). That the coincidence of C_{13} and C_{14} signals was due to the lower resolution and not to a solvent effect, is proved by the fact that Battersby *et al.*,[4] in a previous publication, observed in $CDCl_3$, but not assigned, four signals for the four OCH_3 groups at δ 55.8, 56.1, 61.0, and 61.2.

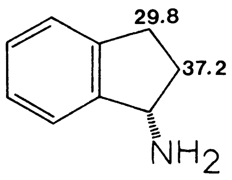
Methylene carbon atoms, C_5 and C_6.

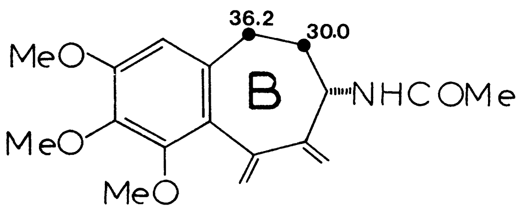
Singh *et al.*[2] assigned the signals of these two carbon atoms of ring B by comparison with indane. They forgot the β and γ effects produced by an amino group; actually, the comparison with 1-aminoindane[5] leads to the inverse assignment:

Indane

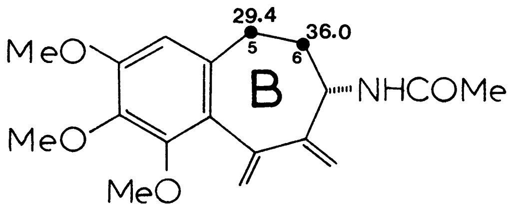


1-Aminoindane[5]



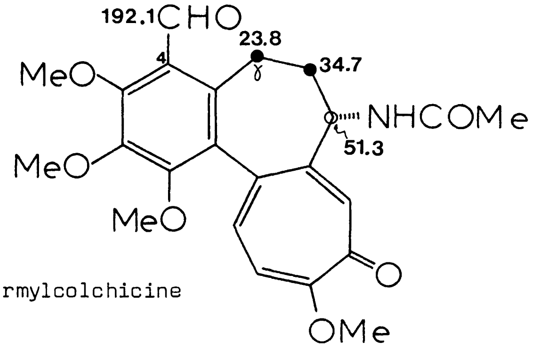


Singh *et al.*[2] assignment

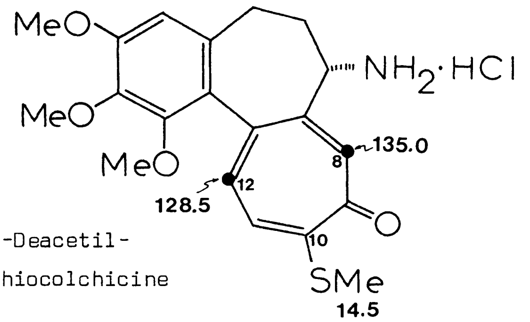


Our assignment

Moreover, our assignment of C_5 and C_6 signals is not founded on the 1-aminoindane similarities but on the effects produced by a substituent ($CHCl_2$ or CHO) in position 4: steric γ effect on C_5.



4-Formylcolchicine



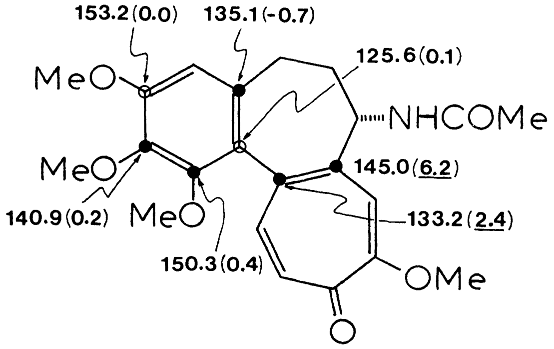
N-Deacetil-thiocolchicine

Methine carbon atoms, C_8 and C_{12}.

The assignment of signals at δ 130.5 and 135.8 ($CDCl_3$) by Singh et al.[2] is arbitrary. In fact they are too close to be assigned by comparison with tropolone methyl ether, because of the influence of ring C substituents in positions 7a and 12a. Our assignment, inverted in regard to Singh et al.,[2] is supported by the study of N-deacetylthiocolchicine (a derivative with a SCH_3 group instead of a OCH_3 group in position 10).

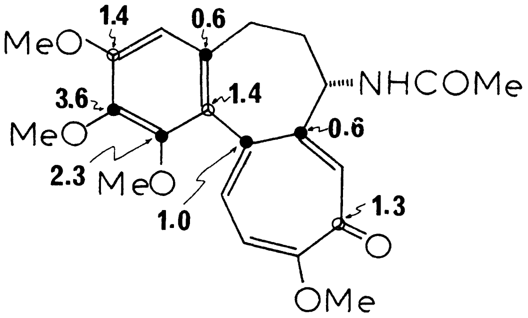
Quaternary carbon atoms, C_1, C_2, C_{4a}, C_{7a}, and C_{12a}.

Due to the fact that Singh et al.[2] studied only colchicine itself, they could not differentiate between aromatic quaternary carbon atoms belonging to rings A (benzenic) and C (tropolonic). Because of the study of differently substituted colchicines (substituents in positions 4, 9, and 10 are particularly relevant), we could classify the benzenic and tropolonic carbon atoms into two different groups. After this classification has been done, the assignment is based on T_1 values.[6,7]



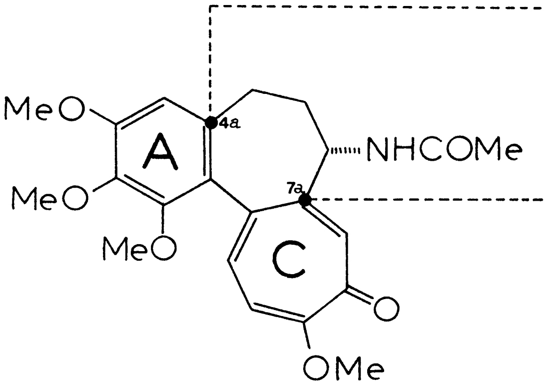
Isocolchicine

$(\Delta\delta) = \delta_{\text{isocolchicine}} - \delta_{\text{colchicine}}$



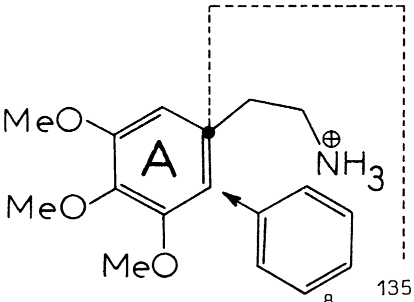
Colchicine: T_1 values (sec)

The most important differences between both assignments are found for quaternary carbon atoms, and amongst them for C_{4a} (-16.7 ppm) and C_{7a} (+14.0 ppm). It is possible to estimate chemical shifts of those two carbons from the literature values of mescaline[7] and tropolone methyl ether.[2]

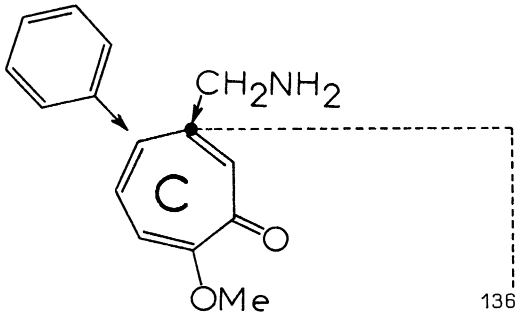


Singh et al.^{2} : 151.1
This work : 134.4*

Singh et al.^{2} : 137.2
This work : 151.2†



135.5 ppm[7]
Phenyl ortho-effect[8] : -1.1
134.4*



136.7 ppm[2]
Phenyl ortho-effect[8] : -1.1
CH_2NH_2 ipso-effect[5] : 15.6
(both on benzene) 151.2†

Beyond the accidental coincidence between the experimental and the above calculated values for C_{4a} and C_{7a} carbon atoms, this rough calculations allows no doubt concerning what assignment is the correct one.

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