138. NMR Studies of Alkaloids

Assignment of the Configuration at C(20) in Tubotaiwine (Dihydrocondylocarpine)

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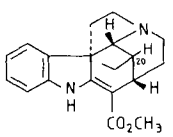
Two-dimensional, high-field NMR methods are employed in confirming the relative configuration at the C(20) ethyl side-chain junction as *S** in the natural alkaloid tubotaiwine (dihydrocondylocarpine).

1. Introduction. – Tubotaiwine and dihydrocondylocarpine are two names used in the current literature to denote compound **1** originally isolated by *Pinar* and *Schmid* in 1963 [1]. The alkaloid was given the name tubotaiwine and observed to be identical with dihydrocondylocarpine prepared by catalytic hydrogenation of condylocarpine **2** [2]. The material has since then been shown to possess interesting pharmacological activities, ranging from antimicrobial activity [3] [4] to cytotoxic activity [5] [6]. Also, weak clonic convulsions have been detected in pharmacological screenings [7]. The ambiguity concerning the configuration at C(20) has led to two different representations for tubotaiwine and dihydrocondylocarpine. This has further led to several confusions in the literature [5] [7–10]. In this paper, we will settle the configuration at C(20) unambiguously using modern two-dimensional (2D) NMR methods [11] to obtain direct information.



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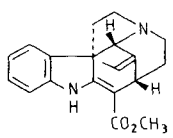

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2. ^{1}H-NMR Spectra. – The ^{1}H-NMR spectrum of tubotaiwine (**1**) resolves cleanly at 500 MHz. Apart from the trivial NH, $COOCH_3$, and aromatic protons, the assignments were based on the 2D methods. To begin with, proton-proton connectivity was elucidated through COSY experiments. *Fig. 1* exhibits the phase-sensitive COSY spectrum [12–14], wherefrom the connectivity information was extracted.

An unambiguous starting point is the CH_3(18) signal at 0,68 ppm, which is coupled only with the 2H multiplet at 0.8 ppm. The latter must, therefore, be CH_2(19). The only other cross peak of this latter signal is shared with the 1H signal at 1.94 ppm, which is due to H–C(20). The methine proton H–C(20) is further connected to two other methine

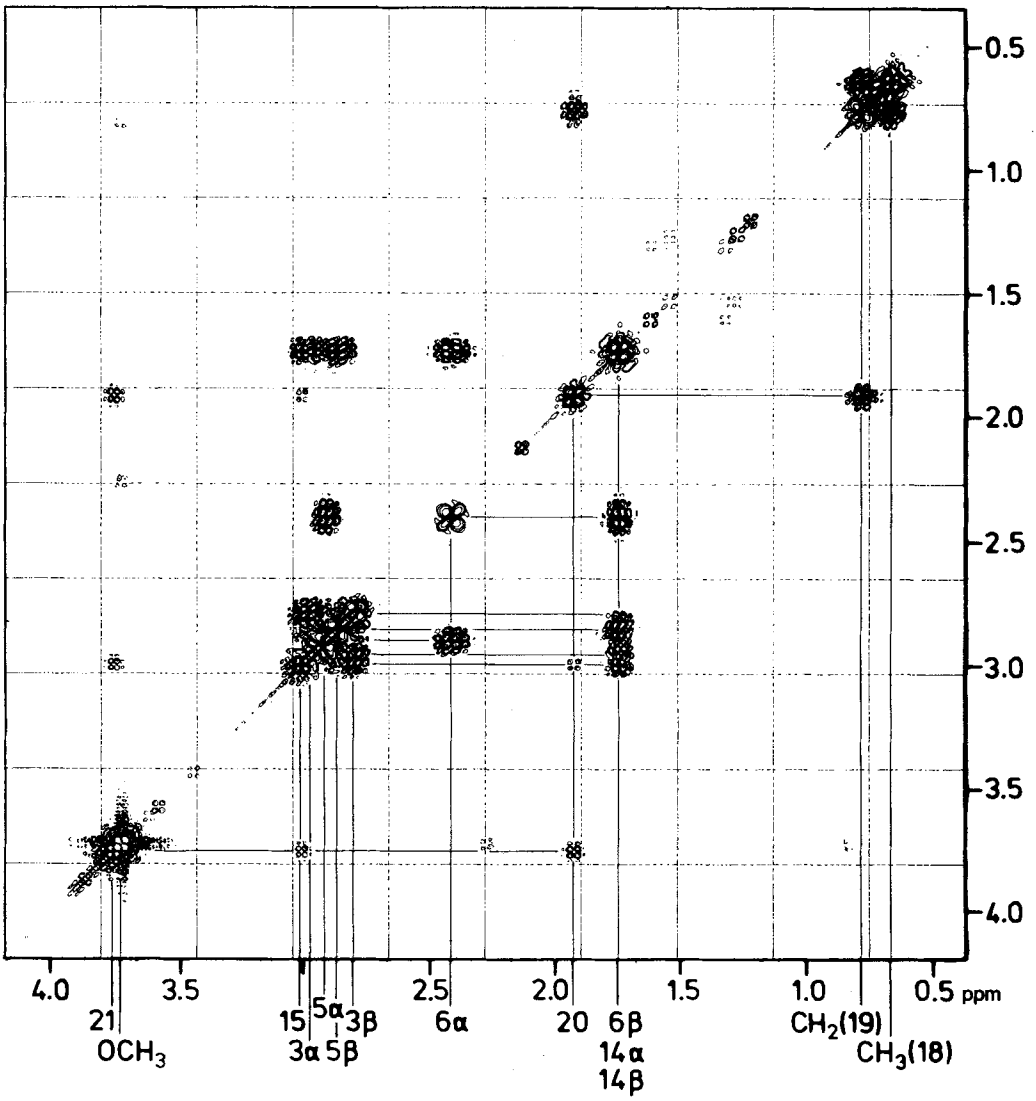


Fig. 1. *Aliphatic region of the phase-sensitive $^1H,^1H$-COSY-NMR spectrum* (500 MHz) *of* **1** *in* $CDCl_3$

protons, H–C(15) and H–C(21), the latter of which should occur at substantially lower field than the former one due to the N(4) being bonded to the same C-atom. The signal due to H–C(20) indeed exhibits further two cross peaks, one with the signal at 3.77 and one with the signal at 3.02 ppm.

Furthermore, these two protons feature a non-vanishing J coupling due to a W coupling. Based on the above considerations, the lowest-field aliphatic signal at 3.77 ppm is due to H–C(21) and the one at 3.02 ppm is due to H–C(15).

Of the remaining eight signals, four are located in the region between 2.8 and 3.05, one at 2.43, and three in the unresolvable multiplet at 1.76 ppm. However, some further

refinement can be obtained from the COSY spectrum. First, H–C(15) exhibits a cross peak with the 3H signal at 1.76 ppm, implying that at least one H–C(14) is located in this region. Secondly, inside the down-field, 4H complex, a coupling is observed between the signals at 3.0 and 2.8 ppm.

Based on chemical-shift criteria, H–C(3α), H–C(3β), H–C(5α), and H–C(5β) located α to the N-atom should be the ones associated with the 4H, low-field region. At this point, only tentative assignments for the eight protons can be made without recourse to the NOE information. The following assignments will be corroborated in the NOESY data later in this paper. Because of the greater conformational flexibility of the D ring

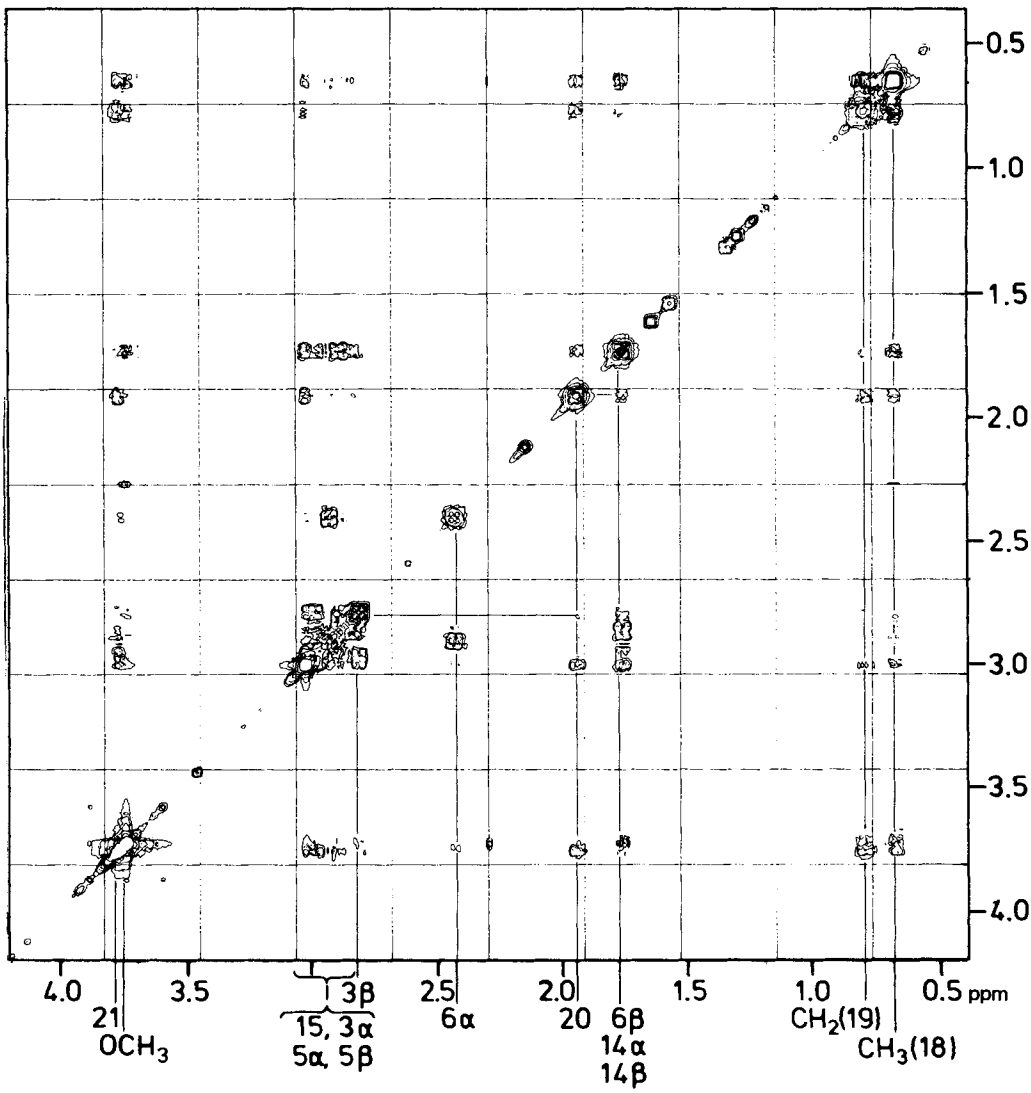


Fig. 2. *Aliphatic region of the $^1H,^1H$-NOESY-NMR spectrum* (500 MHz) *of* **1** *in* $CDCl_3$

(C(3)–N(4)–C(21)–C(20)–C(15)–C(14)) [15], one would expect the H–C(14) protons to experience a similar magnetic environment, causing them to coalesce at the 3H multiplet at 1.76 ppm. Of the remaining two upfield protons, H–C(6β) is expected to reside at higher field because of the spatial proximity to the anisotropic ring current of the aromatic region shielding this proton relative to H–C(6α) which will feel only the deshielding effect of the proximal C lattice. Thus, H–C(6α) exhibits a cross peak with H–C(6β) at 1.76 and with H–C(5α) at 2.94 ppm. Slightly upfield of this proton must be H–C(5β) at 2.86 ppm, connected to H–C(6β). The remaining two protons, H–C(3α)

Table. *NMR Data of Tubotaiwine* (**1**, $CDCl_3$, TMS = 0)

^{1}H-NMR		^{13}C-NMR		
Proton	δ [ppm]	C-Atom	δ [ppm]	DEPT[a])
H–C(3α)	2.98	C(2)	169.8	(C)
H–C(3β)	2.82	C(3)	44.0	(CH_2)
H–C(5α)	2.94	C(5)	54.0	(CH_2)
H–C(5β)	2.86	C(6)	45.2	(CH_2)
H–C(6α)	2.43	C(7)	55.1	(CH)
H–C(6β)	1,76	C(8)	137.2	(C)
H–C(9)	7.13	C(9)	120.9	(CH)
H–C(10)	6.85	C(10)	119.5	(CH)
H–C(11)	7.08	C(11)	127.0	(CH)
H–C(12)	6.78	C(12)	109.5	(CH)
H–C(14α)	1.76	C(13)	143.6	(C)
H–C(14β)	1.76	C(14)	28.4	(CH_2)
H–C(15)	3.02	C(15)	30.9	(CH)
H–C(18)	0.68	C(16)	95.6	(C)
H–C(19)	0.80	C(18)	11.5	(CH_3)
H–C(20)	1.94	C(19)	23.8	(CH_2)
H–C(21)	3.77	C(20)	41.2	(CH)
$COOCH_3$	3.74	C(21)	65.5	(CH)
N*H*	8.84	$COOCH_3$	51.0	(CH_3)
		$COOCH_3$	170.7	(C)

[a]) Multiplicity information for the ^{13}C-NMR data was obtained from DEPT spectra.

and H–C(3β), are only mutually coupled and, therefore, cannot be discerned from one another, except for chemical-shift criteria. The effects of the lone pair of N(4) on H–C(3α) and H–C(3β) are equal. However, H–C(3β) experiences a shielding effect caused by the proximity of the flagpole proton H–C(20). Conclusive evidence to distinguish these protons from each other is only possible through NOE data. From *Fig. 2*, one can see a weak cross-peak between H–C(20) and one H–C(3), at 2.82 ppm, which must necessarily be H_β. Thereby, we are led to the assignments collected in the *Table*. As mentioned above, the assignments of the protons at C(3), C(5), C(6), and C(14) must be judged with caution based only on COSY data.

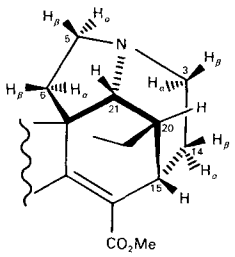
However, in the following the NOESY data will be presented to support our assignments. More importantly, the conclusions concerning the relative orientation of the ethyl side chain are unambiguous and independent of the assignments of the above protons.

The symmetrised NOESY spectrum of **1** is shown in *Fig. 2*. Besides the cross peaks already discernible in the COSY spectrum *(Fig. 1)* due to scalar coupling, one can observe that CH_2(19) does not have a dipole-dipole interaction with either of the H–C(3) protons, thereby suggesting β-orientation of the ethyl side chain.

Also, if the ethyl side chain were α-oriented, *i.e.* *R** configuration at C(20), CH_2(19) would exhibit a dipolar coupling with H–C(14β), which is not observed. The only cross peaks of CH_2(19) seen in the NOESY spectrum are associated with H–C(20), H–C(15), and H–C(21), whose assignments were unambiguous from the COSY data.

Further support for the β orientation, *i.e.* *S** configuration at C(20), of the ethyl appendage is obtained from the occurrence of the cross peak of H–C(20) with one H–C(14), or H–C(6β). This obviously must be due to H–C(14β) through a 1,3-diaxial disposition of the two protons. Also, a weak off-diagonal signal is observed between the signals of H–C(20) and H–C(3β), at 2.82 ppm, arising from flagpole interaction between the nuclei in the boat conformation of the D ring.

Were the ethyl side chain α-oriented (*R** configuration at C(20)), H–C(20) would reside in an isolated environment not participating in dipolar couplings with any distant protons. Thus, one arrives at the conclusion that the C(18)–C(19) chain is β-oriented (*S** configuration at C(20)) as depicted in stereostructure **1'**.



1'

3. Measurement Conditions. – 3.1. *General.* All the NMR spectra were recorded on a *Bruker AM-500* spectrometer (operating at 500.13 MHz for protons) equipped with an *Aspect 3000* system equipped with an *Array* processor. All measurements were performed at r.t. in $CDCl_3$ (concentration *ca.* 0.19M). One-dimensional ^{1}H-NMR and ^{1}H,^{1}H-COSY spectrum were recorded using a 5-mm broad-band probe (90° = 24 μsec). Then, ^{1}H,^{1}H-NOESY spectrum was recorded using a dedicated ^{1}H probe (90° = 9 μsec).

3.2. *One-Dimensional ^{1}H-NMR Spectrum.* Size: 32K, sweep width: 5494 Hz, digital resolution: 0.335 Hz/pt; relaxation delay: 1 sec; 15 μsec pulse (*ca.* 65°); 121 acquisitions, *Gaussian-Lorentzian* apodization of FID ($LB = -0.2$, $GB = 0.5$).

3.3. *^{1}H,^{1}H-COSY-NMR Spectrum.* TPPI phase-sensitive method [13] [14]. Sequence: Delay $-90° - t_1 - 90° - t_2$. Delay: 1.25 sec, 90° pulse, 24 μsec; acquisition time: 0.06681 sec, spectral width in f_1 and f_2 dimensions 1915.7 Hz, size: 256 words, 256 increments, zero-fill to 1K in both dimensions prior to *Fourier* transform, quadrature detection; 4 acquisitions, 2 dummy acquisitions. For spectrum workup, non-shifted sine bell apodization was used in both dimensions. Digital resolution: 3.742 Hz/pt.

3.4. *^{1}H,^{1}H-NOESY-NMR Spectrum.* Sequence: Delay $-90° - t_1 - 90° - t_{mix} - 90° - t_2$. Delay: 1.25 sec, 90° pulse 9 μsec; acquisition time: 0.2672 sec, spectral width in f_1 and f_2 dimensions 1915.709 Hz, size: 1K, 256 increments, zero-fill to 1K in f_1 dimension prior to *Fourier* transform; quadrature detection, 32 acquisitions, 2 dummy acquisitions, mixing time t_{mix} 1.25 sec, 20% random variation of mixing time, non-shifted sine bell apodization in both dimensions. Digital resolution: 3.742 Hz/pt.

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