

Chapter 3

Results and Discussion

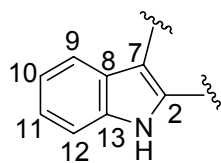
The crude alkaloid of the roots of *Tabernaemontana peduncularis* Wall. was separated by chromatographic methods yielding seven compounds, R9TP 01, R9TP 03, R9TP 05, R9TP 08, R9TP 13, R9TP 17 and R9TP 20. One of them is a new compound (R9TP 08). Their structures were determined base on the spectroscopic data. The known compounds were also accomplished by comparison of their spectroscopic data, especially ^1H NMR and ^{13}C NMR spectral data with those reported in the literatures.

3.1 The structure elucidation of R9TP 08

R9TP 08 was obtained as amorphous form, $[\alpha]_{\text{D}} -52.0^\circ$ (c 0.10, CHCl_3). The UV spectrum (**Figure A.1**) showed absorption maxima at 226, 254 and 286 nm ($\log \varepsilon$ 4.55, 3.48 and 3.81 respectively), typical of an indole chromophore. In addition, the IR spectrum (**Figure A.2**) showed bands due to OH and NH stretching (3375 cm^{-1}) and ester carbonyl (1720 cm^{-1}) functions. The HRFABMS showed a $[\text{M}+\text{H}]^+$ ion peak at m/z 369.2168 leading to the chemical formula as $\text{C}_{22}\text{H}_{29}\text{N}_2\text{O}_3$ (calculated for $\text{C}_{22}\text{H}_{29}\text{N}_2\text{O}_3$, 369.2178). Therefore, the chemical formula $\text{C}_{22}\text{H}_{29}\text{N}_2\text{O}_3$ was corresponding to the molecular formula $\text{C}_{22}\text{H}_{28}\text{N}_2\text{O}_3$.

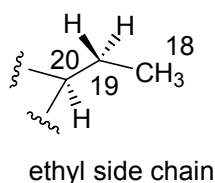
The ^{13}C NMR spectrum (**Table 3.1** and **Figure A.4**) showed 22 signals for 22 carbon atoms. Analysis by HMQC and ^1H - ^1H COSY spectra of R9TP 08 suggested the presence of two methyl carbon atoms (δ 52.67 and 11.69), six methylene carbon atoms (δ 62.22, 51.57, 38.28, 27.56, 26.44 and 21.76), nine methine carbon atoms (δ 122.05, 119.29, 110.40, 59.92, 57.92, 38.11 and 30.75) and six signals for quaternary carbon atoms (δ 175.44, 136.30, 135.50, 128.47, 109.95 and 54.96).

The ^1H NMR spectrum (**Table 3.1** and **Figure A.5**) showed signals attributable to four isolated protons of an aromatic system (δ 7.47, br dd, $J= 8.0$, 1.2 Hz; 7.26, br dd, $J= 8.1$, 1.1 Hz ; 7.16, ddd, $J= 8.0$, 7.1, 1.1 Hz; 7.10, ddd, $J= 8.0$, 7.1, 1.2 Hz) indicated the unsubstituted indole ring which supported by the UV spectrum. In addition, from the ^1H - ^1H COSY (**Figure A.6**) and HMQC (**Figure A.7**) spectra those aromatic proton (δ 7.47, δ 7.26, δ 7.16 and δ 7.10) were assigned to H-12, H-9, H-11 and H-10, respectively. Furthermore, aromatic methine carbons resonanced (δ 122.05, 119.29, 118.34 and 110.40) were determined to C-11, C-10, C-12 and C-9, respectively. Nonetheless, the other carbons of indole nucleus carbon resonanced (δ 136.30, 135.50, 128.47 and 109.95) were established by HMBC (**Figure A.8**) spectrum to C-2, C-13, C-8 and C-7, respectively.



indole nucleus

The observation of methyl protons signal (δ 0.92, dd, $J= 7.4, 7.4$ Hz) which coupling with two methylene protons resonated at δ 1.59 (ddq, $J= 13.7, 7.4, 6.1$ Hz) and δ 1.44 (ddq, $J= 13.7, 7.4, 6.6$ Hz) together with two carbon atoms resonated at δ 11.69 and 26.44 indicated the presence of ethyl side chain, C-18 and C-19. Moreover, the chemical shifts of C-18 and C-19 were characteristic to the iboga type indole alkaloids e.g. coronaridine C-19 (δ 27.0), C-18 (δ 11.6) (van Beek *et al.*, 1982). Indeed, from ^1H - ^1H COSY and HMQC spectral data, methyl protons (δ 0.92) and two methylene protons signals (δ 1.59 and 1.44) was readily determined to H-18, H-19a and H-19b, respectively, while two carbon atoms signals at δ 11.69 and 26.44 were belonging to C-18 and C-19 respectively.



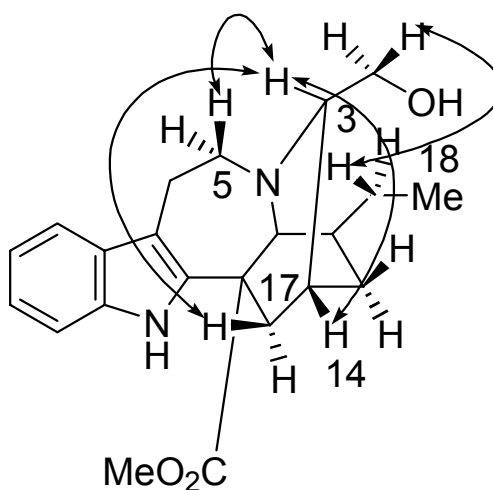
In the HMBC spectrum, the carbon resonance at δ 38.11 showed the correlation with methyl protons (H-18) while the HMQC spectrum showed the correlation with a proton signal at δ 1.32 (br dddd, $J= 7.4, 7.4, 7.2, 6.1$ Hz). Together with the ^1H - ^1H COSY spectrum showed the correlation with methylene proton of C-19. Consequently the ethyl side chain attached at the methine carbon resonated at δ 38.11. With in the iboga type of indole alkaloids, the ethyl side chain attaches at C-20 (van Beek *et al.*, 1984). Therefore, the carbon signal at δ 38.11 was assigned to be C-20.

The ^1H NMR and ^1H - ^1H COSY spectra showed two methylene protons which coupling within each others (δ 3.36, ddd, $J=$ 13.9, 7.6, 6.2 Hz; 3.28, ddd, $J=$ 13.9, 10.0, 6.0 Hz; 3.15, ddd, $J=$ 15.9, 10.0, 6.2 Hz and 3.09, ddd, $J=$ 15.9, 7.6, 6.0 Hz). Thus, these were two couple germinal protons, δ 3.36 & 3.28 and δ 3.15 & 3.09. In the HMQC spectrum the carbon resonances at δ 51.57 showed the correlation with the proton signals at δ 3.36 and 3.28 while the carbon resonances at δ 21.76 showed the correlation with the proton signals at δ 3.15 and 3.09. In addition, the carbon resonance at δ 51.57 was characteristic to a carbon atom which directly connect to an N atom.

Moreover, H-12 (δ 7.47) and H-9 (δ 7.26) showed the correlation with this carbon atom. Thus, this carbon was determined to C-5 which leading to establish a carbon signal at δ 21.76 to be C-6. The ^{13}C NMR also showed others carbon resonance that could be assigned to directly connect with N atom, δ 59.92 and 57.92. The methine carbon, δ 59.92, showed the correlation with a proton δ 2.92 (br dd, $J=$ 7.0, 2.6 Hz) in the HMQC spectrum. Furthermore, in the HMQC and ^1H - ^1H COSY spectra the proton signals δ 3.64 (dd, $J=$ 10.9, 2.6 Hz) and 3.54 (dd, $J=$ 10.9, 7.0 Hz) not only showed the correlation with the carbon value δ 62.22 but also showed the correlation with proton δ 2.92. Thus, the carbon resonance δ 62.22 was established to ($-\text{CH}_2\text{OH}$) group which directly connected with a carbon value δ 59.92. The presence of OH group was confirmed by the IR spectrum at 3375 cm^{-1} (OH stretching). Nevertheless, the HMBC spectrum showed the

correlation between proton signal δ 2.92 and C-5 (δ 51.57). Consequently, the methine carbon value δ 59.92 was determined to C-3. The remained methine carbon resonance (δ 57.92) which could be adjacent to N atom had the correlation with a broad singlet signal at δ 3.71 in the HMQC spectrum. In addition this proton (δ 3.71) also revealed the correlation with H-20 (δ 1.32), therefore the carbon resonanced δ 57.92 was assigned to be C-21.

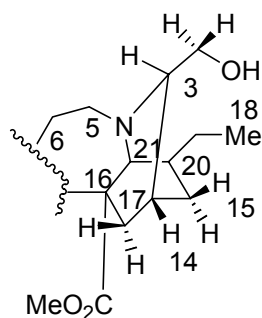
The stereochemistry of ($-\text{CH}_2\text{OH}$) group was investigated by NOE spectroscopy experiment (**Figure A.9-A.11**). The NOE spectrum exhibited the correlation of H-3 to H-5 *Pro-S*, H-3 to H-17 *exo*, H-3 to H-14 and ($-\text{CH}_2\text{OH}$) to H-19. These indicated that ($-\text{CH}_2\text{OH}$) group and ethyl side chain was located at the same side of the molecule. The isolation of R9TP 08 represented the first instance of ($-\text{CH}_2\text{OH}$) group function at position C-3 of the iboga skeleton.



Selected NOE correlation of R9TP 08

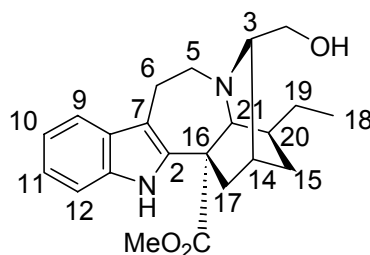
The ^1H NMR and ^1H - ^1H COSY spectra exhibited the correlation between five proton signals (δ 2.65, dd, $J=$ 13.5, 1.8 Hz; 2.00, br ddd, $J=$ 13.5, 4.1, 2.7 Hz; 1.92, m; 1.59, m; 1.47, br dd, $J=$ 16.2, 7.0, 2.2, 2.2 Hz). Furthermore, the HMQC spectrum showed the correlation between the carbon value 38.28 and the proton signal at δ 2.65 and 2.00, while the carbon value δ 27.56 had the correlation with the proton signal at δ 1.59 and 1.47.

Nonetheless, all those four proton signals also had the correlation with a proton at δ 1.92 which showed the correlation to C-3 (δ 59.92) in the HMBC spectrum. Thus, the carbon resonanced at δ 30.75 could be assigned to C-14. The 14 position in the iboga skeleton was connected to C-15 and C-17, therefore the carbon resonanced at δ 27.56 was deduced to C-15 since all methylene proton signals (δ 1.59 and 1.47) revealed the correlation with H-20 (δ 1.32) in the ^1H - ^1H COSY spectrum. On the other hand, the methylene protons (δ 2.05 and 2.00) had the correlation with H-14 and did not had the correlation with H-20 were readily determined to H-17.



The signal due to carbomethoxy function were characteristically observed in the ^1H NMR and ^{13}C NMR spectra data. The ester carbonyl carbon was exhibited at δ 175.44 and further proof of this was provided by the observed band at 1720 cm^{-1} in the IR spectrum. Addition, the singlet methyl protons at δ 3.72 and the methoxy carbon resonanced at δ 52.67 also supported the presence of this function. The carbomethoxy group was located at the quaternary carbon resonance at δ 54.96 that had the correlation with H-21 and H-17 in the HMBC spectrum. Therefore the carbon value 54.96 was established as C-16.

The above-mentioned data unambiguously confirmed the structure of R9TP 08 as (-)-3*S*-(hydroxymethyl)-coronaridine.



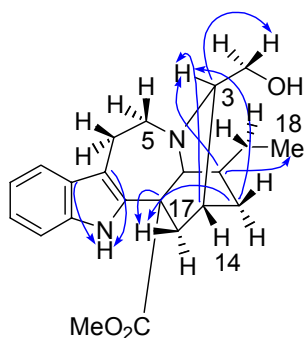
R9TP 08, (-)-3*S*-(hydroxymethyl)-coronaridine

Table 3.1 ^1H and ^{13}C NMR spectral data for R9TP 08 (500 and 125 MHz respectively in CDCl_3)

Position	δ C	δ H (J, Hz)	HMBC ($^{13}\text{C} \rightarrow ^1\text{H}$)
2	136.30		$6_{Pro-R\&S}$, $17_{exo\&endo}$, 21
3	59.92	2.92 br dd (7.0, 2.6)	$5_{Pro-R\&S}$, $15_{exo\&endo}$, 20, 21, CH_2OH
5	51.57	<i>pro-S</i> 3.28 ddd (13.9, 10.0, 6.0) <i>pro-R</i> 3.36 ddd (13.9, 7.6, 6.2)	$6_{Pro-R\&S}$
6	21.76	<i>pro-S</i> 3.09 ddd (15.9, 7.6, 6.0) <i>pro-R</i> 3.15 ddd (15.9, 10.0, 6.2)	$5_{Pro-R\&S}$
7	109.95		$5_{Pro-R\&S}$, $6_{Pro-R\&S}$, NH
8	128.47		9, 10, 12, NH

Table 3.1 (Continued)

Position	δ C	δ H (J, Hz)	HMBC ($^{13}\text{C} \rightarrow ^1\text{H}$)
9	110.40	7.26 br dd (8.1, 1.1)	10
10	119.29	7.10 ddd (8.1, 7.1, 1.2)	9, 12
11	122.05	7.16 ddd (8.0, 7.1, 1.1)	
12	118.34	7.47 br dd (8.0, 1.2)	10, 11
13	135.50		11, 12, NH
14	30.75	1.92 m	3, 15 _{exo, endo} , 17 _{exo, endo} , CH ₂ OH
15	27.56	<i>exo</i> 1.47 dddd (16.2, 7.4, 2.2, 1.5) <i>endo</i> 1.59 ddd (16.2, 7.4, 4.6)	3, 14, 17 _{exo, endo} , 19 _{a,b} , 20, 21
16	54.96		14, 17 _{exo, endo} , 21
17	38.28	<i>exo</i> 2.00 ddd (13.5, 4.1, 1.5) <i>endo</i> 2.65 dd (13.5, 1.8)	14, 15 _{exo, endo} , 21
18	11.69	0.92 dd (7.4, 7.4)	19 _{a,b} , 20
19	26.44	1.44 ddq (13.7, 7.4, 7.2) 1.59 ddq (13.7, 7.4, 6.1)	15 _{exo, endo} , 18
20	38.11	1.32 br dddd (7.4, 7.4, 7.2, 6.1)	3, 15 _{exo, endo} , 18, 19 _{a,b} , 21
21	57.92	3.71 br s	5 _{Pro-R&S} , 15, 17, 19
-CH ₂ OH	62.22	<i>pro-S</i> 3.54 dd (10.9, 7.0) <i>pro-R</i> 3.64 dd (10.9, 2.6)	
CO ₂ Me	52.67	3.72 s	
C=O	175.44		
NH		7.61 br s	



Selected HMBC correlation of R9TP 08