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Alkaloids from The Roots of *Tabernaemontana Macrocarpa* Jack

Khairana Husain[1], Ikram M. Said[1,*], Laily B. Din[1], Hiromitsu Takayama[2], Mariko Kitajima[2], and Norio Aimi[2]

[1]Department of Chemistry, Universiti Kebangsaan Malaysia, 43600 Bangi, Malaysia
[2]Faculty of Pharmaceutical Sciences, Chiba University, Japan

Abstract – The roots of *Tabernaemontana macrocarpa* Jack (Apocynaceae) yielded nine indole alkaloids which were identified as coronaridine (**2**), voacangine hydroxyindolenine (**3**), 3-oxo-coronaridine (**4**), 19-R-heyneanine (**7**), coronaridine pseudoindoxyl (**8**) and voacangine pseudoindoxyl (**9**) while 3-(2-oxopropyl)-coronaridine (**1**), 3-(2-oxopropyl)-coronaridine-pseudoindoxyl (**5**) and 3-(2-oxopropyl)-voacangine-pseudoindoxyl (**6**) were isolated as artefacts formed during the isolation process. All of the alkaloids were identified by spectroscopic methods (UV, MS, ^{1}H-NMR & ^{13}C-NMR) and in comparison with the literature data.

Keywords – *Tabernaemontana macrocarpa, Apocynaceae,* roots, indole alkaloids, I-boga-type alkaloid.

Introduction

Tabernaemontana macrocarpa Jack (Apocynaceae) is one of ten species of *Tabernaemontana* that can be found in Malaysia (Corner 1988), most of which are used in traditional medicine (Burkill 1966) for various ailments such as beri-beri and syphyllis. *T. macrocarpa* is a big tree that can grow up to 10 metres and well distributed in the lowland forests especially in Sabah and Sarawak (Ashton 1988). Its fruits are used for relieving toothache and headache (Van Beek *et al.* 1984a) and has been reported to contain indole alkaloids (Miet & Poisson 1977). This paper deals with the isolation and identification of nine indole alkaloids from the roots of *T. macrocarpa* of which 3-oxo-coronaridine, 19-R-heyneanine, coronari- dine pseudoindoxyl and voacangine pseudo- indoxyl are isolated for the first time from this species. All the compounds were identified mainly based on their NMR spectral data using various 2-D techniques.

*Author for correspondence.

Experimental

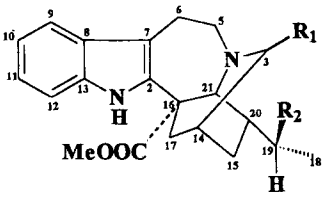
General experimental procedures – The UV spectra was measured with a UV-260 Shimadzu spectrophotometer using Et OH as solvent. The mass spectrum was obtained using a GC-MS model Hewlett Packard 5890 Series II spectrometer. The ^{1}H and ^{13}C-NMR spectra were determined with JEOL JNM A-500 or Bruker AM 300 spectrometer. Thin-layer chromatogtaphy was performed on Merck precoated silica gel 60 F254 plates while column chromatography was carried out using Fluka silica gel 60 (230-400 mesh) as absorbent. The alkaloids were detected with an UV lamp, Dragendorff and $FeCl_3$/$HClO_4$ spray reagents.

Plant material – The roots of *T. macrocarpa* were collected from trees found in Sar-

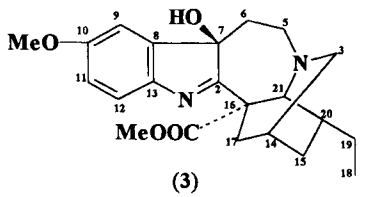
awak. The plant was authenticated at source and is deposited as a voucher specimen in the herbarium of UNIMAS, Sarawak.

Extraction and isolation of alkaloids – The dry powdered sample of the roots (1.0 kg) was extracted with MeOH (3 L) for 3 days at room temperature and concentrated under reduced pressure to yield 54 g of the crude extract. The crude MeOH extract was then triturated in 5% aqueous H_2SO_4 (2× 100 ml) followed by basification with 10% Na_2CO_3 and then extracted into $CHCl_3$. The organic layer was washed with distilled water, dried (Na_2SO_4) and concentrated to yield 3.0 g of crude alkaloids. The alkaloid mixture (2.8 g) was applied onto a column (silica gel, Fluka 230-400 mesh) and eluted with n-hexane (6 L), EtOAc (3 L) and MeOH (2 L) with increasing polarity. Fractions (50 ml) were collected, analysed by TLC and grouped accordingly. From the combined fractions nine alkaloids were obtained pure with the aid of repeated column chromatography or preparative TLC. Alkaloid (**1**) was in fractions 14-15 and (**2**) in fractions 34-61 eluated with n-hexane (100%). Alkaloid (**3**) was obtained from fractions 146-235, (**4**) in fractions 570-639, (**5**) in fractions 670-701, (**6**) in fractions 719-735, and (**7**) in fractions 736-742 on elution with increasing amounts of EtOAc (20%, 30%, 50%, 60% and 70% respectively). Alkaloids (**8**) and (**9**) were isolated from fractions 835-837 and 838-844 respectively. Elution was performed with EtOAc: MeOH (5%). Acetone was also used in the bulking and washing processes of fractions containing alkaloids (**1**), (**5**), and (**6**).

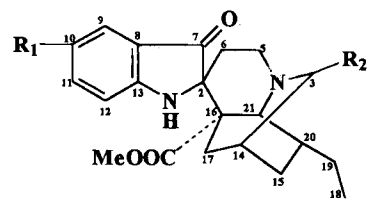
3-(2-oxopropyl)-coronaridine (**1**). Colorless amorphous powder (4.6 mg). TLC : R_f 0.69 (n-hexane: EtOAc, 7:3); greyblack with $FeCl_3$/$HClO_4$ reagent; UV (EtOH) λ_{max} nm: 223, 284; EIMS (70 eV) m/z (rel. int.) 394 (61%), 338 (24%), 337 (100%), 264 (22%), 214 (12%), 169 (7%), 154 (27%), 149 (1%), 144 (10%) 122 (15%); ^{1}H-NMR ($CDCl_3$, 270 MHz) δ7.76 (brs, N-H), 7.46 (brd, J=7.3 Hz, H-9), 7.20 (brd, J= 7.8, 1.5 Hz, H-12), 7.14 (dd, J=6.8, 1.5 Hz, H-10), 7.10 (dd, J=7.8, 1.5 Hz, H-11), 3.70 (s, COOMe), 3.58 (s, H-21), 2.48 (d, CH_2COMe), 2.17 (s, CH_2COMe), 0.89 (t, J=7.3 Hz, H-18);



	R1	R2
(1)	CH_2COCH_3	H
(2)	H	H
(4)	=O	H
(7)	H	OH



(3)



	R1	R2
(5)	H	CH_2COCH_3
(6)	OMe	CH_2COCH_3
(8)	H	H
(9)	OMe	H

Fig. 1. Alkaloids from the roots of *T. macrocarpa* Jack.

^{13}C-NMR ($CDCl_3$, 100 MHz) δ208.6 (CH_2 COMe), 175.5 (COOMe), 136.4 (C-2), 135.4 (C-13), 129.7 (C-8), 122.0 (C-11), 118.4 (C-9), 119.2 (C-10), 110.3 (C-12), 110.1 (C-7),58.2 (C-21), 55.2 (C-16), 54.7 (C-3), 52.6 (C-5), 51. 4 (COOMe), 46.6 (C-16), 37.6 (C-17),38.4 (C-20), 30.9 (C-15), 30.9 (CH_2COMe), 26.9 (C-14), 26.7 (C-19), 22.0 (C-6), 11.6 (C-18) (Perera *et al.* 1984 & Gunasekera *et al.* 1980).

Coronaridine (**2**) . Yellow amorphous powder (3.4 mg). TLC: R_f 0.87 (n-hexane: EtOAc, 7:3); grey with $FeCl_3$/ $HClO_4$ reagent; UV (EtOH) λ_{max} nm: 222, 284; EIMS (70 eV) m/z (rel. int.) 338 (100%), 322, (14%), 253 (2%), 214 (14%), 208 (13%), 195 (6%), 154 (27%), 136 (47%), 124 (22%), 122 (22%); ^{1}H-NMR ($CDCl_3$, 300MHz) δ: 7.80 (brs, NH), 7.47 (d, J=7.3 Hz, H-9), 7.00-7.14 (m, 2H, H-10, H-11), 7.25 (d, J=7.3 Hz, H-12), 3.70 (brs, COOMe), 3.55 (s, H-21), 0.88 (t, J=7.0 Hz, H-18) (Van Beek *et al.* 1984b).

Voacangine hydroxyindolenine (**3**). Pale yellow amorphous powder (2.9 mg). TLC: R_f 0.54 (n-hexane: EtOAc, 7:3); grey brown with $FeCl_3$/ $HClO_4$ reagent; UV (EtOH) λ_{max} nm: 211, 275; EIMS (70 eV) m/z (rel. int.) 384 (100%), 367 (67%), 355 (8%), 325 (10%), 323 (10%), 260 (8%), 218 (11%), 190 (11%), 162 (12%); ^{1}H-NMR, see Table 1; ^{13}C-NMR, see Table 3 (Madinaveitia *et al.* 1996).

3-oxo-coronaridine (4). Pale brown-yellow amorphous powder (6.2 mg). TLC: R_f 0.75 ($CHCl_3$: MeOH, 9.9:0.1); grey → green black with $FeCl_3$/ $HClO_4$ reagent; UV (EtOH) λ_{max} nm: 222, 284, 293; EIMS (70 eV) m/z (rel. int.) 352 (25%), 214 (15%), 197 (42%), 195 (19%), 154 (39%), 143 (13%), 138 (11%), 124 (100%); ^{1}H-NMR, see Table 1; ^{13}C-NMR, see Table 3 (Perera *et al.* 1985).

3-(2-oxopropyl)-coronaridine-pseudoindoxyl (**5**). Pale yellow-green fluorescent amorphous substance (1.7 mg). TLC: R_f 0.14 (n-hexane : EtOAc, 7:3); orange → pink → brown with Fe Cl_3/$HClO_4$ reagent; UV (EtOH) λ_{max} nm: 230,

Table 1. ^{1}H-NMR (500 MHz) data for compounds (**3**), (**4**), and (**7**).

Proton	Compound (**3**)		Compound (**4**)		Compound (**7**)	
	δ (ppm)	J (Hz)	δ (ppm)	J (Hz)	δ (ppm)	J (Hz)
H-3α	2.74 s	-	-	-	2.98-3.14 m	-
H-3β	2.74 s	-	-	-	2.81 brd	9.0
H-5α	2.97 dm	14.8	4.46-4.50 m	-	3.42-3.45 m	-
H-5β	3.49 ddd	12.5, 11.5, 3.4	3.20 m	-	3.10-3.20 m	-
H-6α	1.94 m	-	3.13-3.19 m	-	3.10-3.20 m	-
H-6β	1.87 m	-	3.21-3.23 m	-	-	-
H-9	6.90 d	2.4	7.48 brd	7.3	7.47 brd	7.8
H-10	-	8.3, 2.4	7.10 ddd	7.1, 7.1, 1.0	7.11 ddd	7.8, 7.8, 1.0
H-11	6.80 dd	8.3	7.15 ddd	7.1, 7.1, 1.2	7.18 ddd	7.2, 7.2, 1.2
H-12	7.35 d	-	7.25 brd	6.4	7.26 brd	7.1
H-14	1.91 brs	-	2.62 brs	-	2.05 m	-
H-15α	1.77 m	-	1.97-2.02 ddd	13.2, 10.0, 3.2	1.70-1.90 m	-
H-15β	1.11 m	13.9	1.38-1.42 m	-	1.42 m	-
H-17α	2.47 dm	14.2	2.66 d	1.7	2.60 dt	11.5, 2.0
H-17β	2.71 d	7.1	2.30-2.32 m	-	1.97 m	-
H-18	0.86 t	-	0.98 t	7.5	1.28 d	6.6
H-19α	1.40-1.44 m		1.52-1.56 m	-	3.88 brqd	6.1, 2.2
H-19β		-	1.40-1.43 m	-		
H-20	1.42-1.43 m	-	1.72-1.78 m	-	1.96-2.00 m	-
H-21	3.76 s	-	4.51 brs	-	4.08 brs	-
ArOMe	3.82 s	-	-	-	-	-
N-H	-	-	7.96 brs	-	7.79 brs	-
COOMe	3.70 s		3.87 s	-	3.75 s	-

Table 2. ^{1}H-NMR (500 MHz) data for compounds (**5**), (**6**), (**8**), and (**9**)

Proton	Compound (**5**)		Compound (**6**)	
	δ (ppm)	J (Hz)	δ (ppm)	J (Hz)
H-3	3.16-3.28 m	-	3.16-3.29 m	-
H-5α	3.66 ddd	13.0, 13.0, 3.4	3.67 ddd	13.7, 13.7, 3.7
H-5β	2.78-2.80 m	-	2.79-2.80 m	-
H-6α	2.12 ddd	13.2, 13.2, 5.1	2.12 ddd	13.3, 13.3, 5.1
H-6β	1.42 m	-	1.41 brd	2.4
H-9	7.56 brd	7.6	7.01 brd	2.7
H-10	6.80 brt	7.1	-	-
H-11	7.38 ddd	7.3, 7.3, 1.5	7.06 dd	8.8, 2.2
H-12	6.78 brd	6.8	6.75 brd	8.8
H-14	1.79-1.80 m	-	1.78-1.79 m	-
H-15α	1.45-1.48 m	-	1.44-1.47 m	-
H-15β	1.24-1.25 m	-	1.24-1.25 m	-
H-17α	2.71 dd	14.2, 3.1	2.70 dd	14.2, 3.2
H-17β	1.60 m	-	1.58-1.61 m	-
H-18	0.90 t	7.3	0.90 t	7.5
H-19α	1.66-1.71 m	-	1.68-1.72 m	-
H-19β				
H-20	1.21-1.22 m	-	1.22 m	-
H-21	3.81 brd	2.0	3.86 brd	1.5
ArOMe	-	-	3.75 s	-
N-H	4.50 brs	-	4.22 brs	-
COOMe	3.25 s	-	3.29 s	-
CH_2COMe	2.21 s	-	2.20 s	-
CH_2COMe	2.50 dd	15.8, 6.1 (1'β, 3β)	2.50 dd	16.1, 6.1
CH_2COMe	2.76 dd	15.8, 7.1 (1'α, 3β)	2.76 dd	16.1, 6.8

Proton	Compound (**8**)		Compound (**9**)	
H-3	3.00 brd	11.5	3.01 brd	11.2
H-5α	3.82 ddd	13.7, 13.7, 3.6	3.84 ddd	1.39, 13.9, 3.7
H-5β	2.72 dd	14.4, 3.9	2.73 dd	14.2, 3.7
H-6α	2.08 ddd	13.7, 13.7, 5.1	2.08 ddd	13.4, 13.4, 5.1
H-6β	-	-	1.47 m	-
H-9	7.56 brd	7.1	7.02 brd	2.7
H-10	6.80 brt	7.1	-	-
H-11	7.38 ddd	7.1, 7.1, 1.2	7.06 dd	8.6, 2.8
H-12	6.77 brd	8.3	6.74 brd	8.6
H-14	1.05-1.09 m	-	1.05-1.08 m	-
H-15α	1.40-1.42 m	-	1.42-1.44 m	-
H-15β	-	-	-	-
H-17α	2.63 m	-	2.61-2.63 m	-
H-17β	1.65-1.70 m	-	1.66-1.70 m	-
H-18	0.90 t	7.3	0.90 t	7.3
H-19α	1.60-1.65 m	-	1.59-1.61 m	-
H-19β	1.44-1.45 m	-	1.46-1.47 m	-
H-20	1.31-1.34 m	-	1.25-1.34 m	-
H-21	3.87 brs	-	3.90 s	-
ArOMe	-	-	3.76 s	-
N-H	4.52 brs	-	4.27 brs	-
COOMe	3.26 s	-	3.30 s	-
CH_2COMe	-	-	-	-
CH_2COMe	-	-	-	-

388; EIMS (70 eV) m/z (rel. int.) 410 (100%), 354 (27%), 353 (48%), 364 (4%), 194 (17%), 164 (9%), 136 (16%), 122(18%); ^{1}H-NMR, see Table 2; ^{13}C-NMR, see Table 3.

3-(2-oxopropyl)-voacangine-pseudoindoxyl (**6**). Pale green-yellow fluorescent amorphous substance (2.4 mg). TLC: R_f 0.43 (n-hexane: EtOAc, 3:2); green → grey → brown with $FeCl_3$ /$HClO_4$ reagent; UV (EtOH) λ_{max} nm: 227, 409; EIMS (70 eV) m/z (rel. int.) 440 (100%), 384 (22%), 383 (53%), 265 (23%), 252 (15%), 196 (12%), 186 (13%), 164 (15%), 136 (36%), 122 (44%); ^{1}H-NMR, see Table 2; ^{13}C-NMR, see Table 3.

19-R-heyneanine (**7**). Brown yellow amorphous powder (5.7 mg). TLC: R_f 0.57 (n-hexane: EtOAc, 7:3); grey with $FeCl_3/HClO_4$ reagent; EIMS (70 eV) m/z (rel. int.) 354 (100%), 353 (10%), 340 (18%), 339 (45%), 337 (35%), 336 (52%), 310 (7%), 309 (9%), 224 (10%), 214 (41%), 154 (44%), 140 (25 %); ^{1}H-NMR, see Table 1; ^{13}C-NMR, see Table 3 (Clivio 1989 , Wehrli & Nishida 1978).

Coronaridine pseudoindoxyl (**8**). Green-yellow fluorescent amorphous substance (3.8 mg). TLC: R_f 0.17 ($CHCl_3$: MeOH, 9.8:0.2); orange pink brown with $FeCl_3/HClO_4$ reagent; UV (EtOH) λ_{max} nm: 230, 388; EIMS (70 eV) m/z (rel. int.) 354 (27%), 353 (100%), 296 (1%), 294 (19%), 209 (12%), 194 (5%), 184 (11%), 151 (16%), 138 (49%), 137 (11%), 136 (12%), 122 (38%), 109 (65%); ^{1}H-NMR, see Table 2; ^{13}C-NMR, see Table 3.

Voacangine pseudoindoxyl (**9**). Yellow-green fluorescent amorphous substance (7.2 mg). TLC: R_f 0.56 ($CHCl_3$:MeOH, 9.4:0.6); green grey brown with $FeCl_3/HClO_4$ reagent; UV (EtOH) λmax nm: 227, 409; EIMS (70 eV) m/z (rel. int.) 383 (100%), 353 (28%), 247 (2%), 209 (27%), 138 (34%), 109 (40%); ^{1}H-NMR,

Table 3. ^{13}C-NMR (100 MHz) data of compounds (**3**), (**4**), (**7**), (**5**), (**6**), (**8**) and (**9**)

Carbon	(3)	(4)	(7)	(5)	(6)	(8)	(9)
	δ (ppm)	δ (ppm)	δ (ppm)	δ (ppm)	δ (ppm)	δ (ppm)	δ (ppm)
2	186.8	133.8	135.6	67.3	68.3	67.5	68.5
3	48.6	175.7	52.8	55.5	55.5	-	52.1
5	49.0	42.7	50.9	46.6	46.6	47.4	47.4
6	34.1	21.0	22.2	25.5	25.6	26.1	26.1
7	88.3	109.7	109.7	202.7	203.1	202.6	202.9
8	144.4	127.8	128.5	121.2	121.6	121.3	121.7
9	107.9	118.4	118.4	124.2	104.5	124.2	104.5
10	159.1	119.6	119.4	119.1	153.6	119.2	153.6
11	113.6	122.4	122.3	136.5	126.6	136.4	126.5
12	121.3	110.5	110.4	112.1	113.9	112.1	112.1
13	144.8	135.6	135.4	158.4	154.0	158.3	154.0
14	26.9	38.1	26.9	29.2	29.2	30.7	30.7
15	32.0	30.9	28.6	24.7	24.7	25.5	25.7
16	48.5	55.5	53.0	52.2	52.3	51.9	51.9
17	34.4	35.9	36.7	32.4	32.4	31.1	31.1
18	11.5	11.3	21.6	12.2	12.2	12.0	12.0
19	26.4	27.6	70.8	27.5	27.5	28.6	28.6
20	37.5	35.4	40.0	35.6	35.6	35.8	35.8
21	58.5	56.1	54.2	51.6	51.7	51.0	51.0
ArOMe	55.7	-	-	-	55.7	-	55.7
COOMe	53.2	53.0	51.9	52.0	52.0	51.6	51.7
COOMe	173.9	173.0	174.9	174.7	174.8	174.8	174.8
CH_2COMe	-	-	-	31.6	31.6	-	-
CH_2COMe	-	-	-	48.8	48.8	-	-
CH_2COMe	-	-	-	209.1	209.1	-	-

see Table 2; ^{13}C-NMR, see Table 3.

Results and Discussion

The crude alkaloid mixture from the roots of *T. macrocarpa* was isolated using column chromatography on silica gel to afford several fractions. Purifications were carried out on these fractions by repeated column chromatography, yielding nine pure compounds.

Coronaridine (**2**) which is ubiquitous in most *Tabernaemontana* species and four other known compounds have been identified as 3-(2-oxopropyl)-coronaridine (**1**), voacangine hydroxyindolenine (**3**), 3-oxo-coronaridine (**4**) and 19-R-heyneanine (**7**). Compound (**1**) is most likely an artefact produced from 3-hydroxy-coronaridine during the isolation process. All of the above alkaloids belong to the iboga group of subgroup coronaridine (**2**) and their spectral data are identical to those that have been reported in the literature.

The four other isolated alkaloids belong to the pseudoindoxyl-ibogan group whose structures are known but their high field NMR spectral data are reported here for the first time (see Tables 2 and 3).

Compounds (**5**), (**6**), (**8**) and (**9**) posseses in solution an intense yellow-green or green-yellow fluorescent coloration which indicates a pseudoindoxyl chromophore (Clivio *et al.* 1991). Compound (**5**) and (**8**) gave UV spectrum that displayed the two typical maxima at 230, 388 nm, while compound (**6**) and (**9**) absorbs at 227 and 409 nm.

The molecular ion of (**8**) was detected at m/z 354 ($C_{21}H_{26}N_2O_3$) together with common mass fragments m/z 353, 294, 138, 136, 122 and 109. Based on the appearence of the NH proton (NMR spectra) at δ4.52 and the presence of aromatic protons at δ7.56 (brd, J =7.1 Hz, H-9), δ6.80 (brt, J=7.1 Hz, H-10), δ 7.38 (ddd, J=7.1, 7.1, 1.2 Hz, H-11) and δ6.77 (brd, J=8.3, H-12), structure (**8**) was assigned to this compound. The ^{13}C-NMR spectrum of (**8**) (Table 3) indicated the presence of 6 aromatic carbons due to the pseudoindoxyl nucleus, two carbonyl groups, two quartenary carbons and nine other aliphatic carbons. From the above spectroscopic data compound (**8**) was concluded to be coronaridine pseudoindoxyl.

The mass spectrum of (**9**) showed a molecular ion at m/z 384 ($C_{22}H_{28}N_2O_4$) with typical fragments corresponding to loss of methoxy group in benzene ring at m/z 353 ([M^+-31]). In the ^{1}H-NMR spectra, the singlet at δ4.27 was assigned to the NH proton, the 3H singlet at δ3.30 indicated the presence of carbomethoxy group and a 3H triplet at δ0.90 showed the presence of a ethyl group in the side chain. The singlet (3H) at δ3.76 was assigned to an aromatic methoxy group. The position of the methoxy group was assigned to the 10-position in the benzene ring using the following data. In the aromatic region the doublet-doublets (J=8.7, 2.8 Hz) at δ7.06 was assigned to H-11 proton and the two broad doublet at δ7.02 (J=2.7 Hz) and δ6.74 (J=8.6 Hz) were assigned to H-9 and H-12 protons. The splitting pattern of the aromatic protons were similar to those in voacangine which also has a methoxy group in the 10-position. In the HMBC spectrum, three bond connectivity between the H-9 (δ7.02, J= 2.7 Hz) and the C-7 carbonyl carbon was observed. The ^{13}C-NMR spectra of the compound revealed the presence of 6 aromatic carbons due to the pseudoindoxyl nucleus, one methoxy carbon group in the benzene ring, two carbonyl groups, two quartenary carbons and nine other aliphatic carbons. Unambiguous assignments of all carbons and protons were obtained by using COSY, HMQC and HMBC spectra (see Table 2 and 3). Considering all these spectral data the structure voacangine-pseudoindoxyl (**9**) was assigned to this compound.

The mass spectrum of (**5**) and (**6**) showed a molecular ion at m/z 410 ($C_{24}H_{30}N_2O_4$) and m/z 440 ($C_{25}H_{32}N_2O_5$) respectively; i.e 57 mass units higher than in (**8**) and (**9**), suggesting

an additional CH_2COCH_3 moeity in (**5**) and (**6**), while m/z 354 and m/z 384 are the original signal for coronaridine pseudoindoxyl and voacangine pseudoindoxyl in (**5**) and (**6**) respectively. These were confirmed by both ^{13}C-NMR spectra in (**5**) and (**6**) assignment of CH_2COMe, CH_2COMe and CH_2COMe to signals at δ209.1, δ48.8 and δ31.6, respectively. These alkaloids were identified as 3-(2-oxopropyl)-coronaridine pseudoindoxyl (**5**) and 3-(2-oxopropyl)-voacangine pseudoindoxyl (6) which would be artefacts formed from reaction with acetone during the isolation process.

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