

Article

Total Syntheses of (\pm)-Gusanlung A, (\pm)-Gusanlung D and 8-Oxyberberrubine and the Uncertainty Concerning the Structures of (-)-Gusanlung A, (-)-Gusanlung D and 8-Oxyberberrubine

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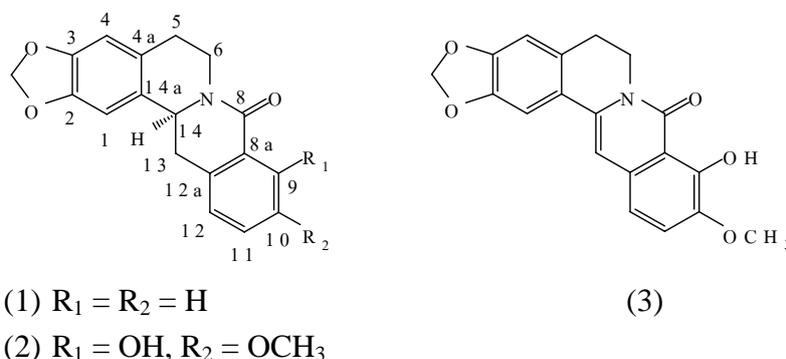
Abstract: (\pm)-Gusanlung A, 8-oxyberberrubine and (\pm)-gusanlung D have been synthesized by radical cyclisation of the corresponding 2-aroyle-1-methylenetetrahydroisoquinolines. The ¹H and ¹³C spectra of (-)-gusanlung D were found to be different from those of synthetic (\pm)-gusanlung D. Careful analyses of the ¹³C spectra of (-)-gusanlung A and natural 8-oxyberberrubine also cast doubt on the correctness of the structures previously assigned to these two compounds. (\pm)-Gusanlung A and (\pm)-gusanlung D were inactive against *Staphylococcus aureus* ATCC25932, *Escherichia coli* ATCC10536 and *Candida albicans* ATCC90028.

Keywords: Alkaloid; Protoberberine; Isoquinoline; Synthesis; Antimicrobial activity.

Introduction

(-)-Gusanlung D, isolated from *Acangelisia gusanlung* H. S. Lo (Menispermaceae), is the first natural 8-oxotetrahydroprotoberberine alkaloid with an unoxygenated ring D [1]. Based on spectral data analysis, structure **1** was proposed for (-)-gusanlung D. Prior to the isolation of (-)-gusanlung D, Kessar *et al.* synthesized in 1992 a compound which is essentially (\pm)-gusanlung D [2]. However, a close comparison of the $^1\text{H-NMR}$ data of (\pm)-gusanlung D with those reported for (-)-gusanlung D revealed significant differences. In 2003 Reimann, Grasberger and Polborn reported another synthesis of (\pm)-gusanlung D [3]; in this case the $^{13}\text{C-NMR}$ spectral data were found to show significant differences to those reported for (-)-gusanlung D. Subsequently, an unsymmetric synthesis of (-)-gusanlung D was achieved by Chrzanowska, Dreas and Razwadowska in 2004 [4]. Comparison of the $^1\text{H-}$ and $^{13}\text{C-NMR}$ data of synthetic (-)-gusanlung D with those of natural (-)-gusanlung D also showed significant differences. Finally, Chang and Chang reported a total synthesis of (\pm)-gusanlung D [5], whose spectral data were said to agree with those in references [1-4]. This last conclusion added further confusion to the matter since, if the spectral data of (\pm)-gusanlung D [5] are in good agreement with those reported for (\pm)-gusanlung D [2-3] and synthetic (-)-gusanlung D [4], they cannot also be consistent with those reported for natural (-)-gusanlung D [1]. In view of these discrepancies in the $^1\text{H-}$ and $^{13}\text{C-NMR}$ data of natural (-)-gusanlung D [1] and the synthetic alkaloids, it was therefore highly desirable to perform another independent synthesis of (\pm)-gusanlung D to shed further light on the possible structure of (-)-gusanlung D.

Figure 1. Structures of (-)-gusanlung D (**1**), (-)-gusanlung A (**2**) and 8-oxyberberrubine (**3**).



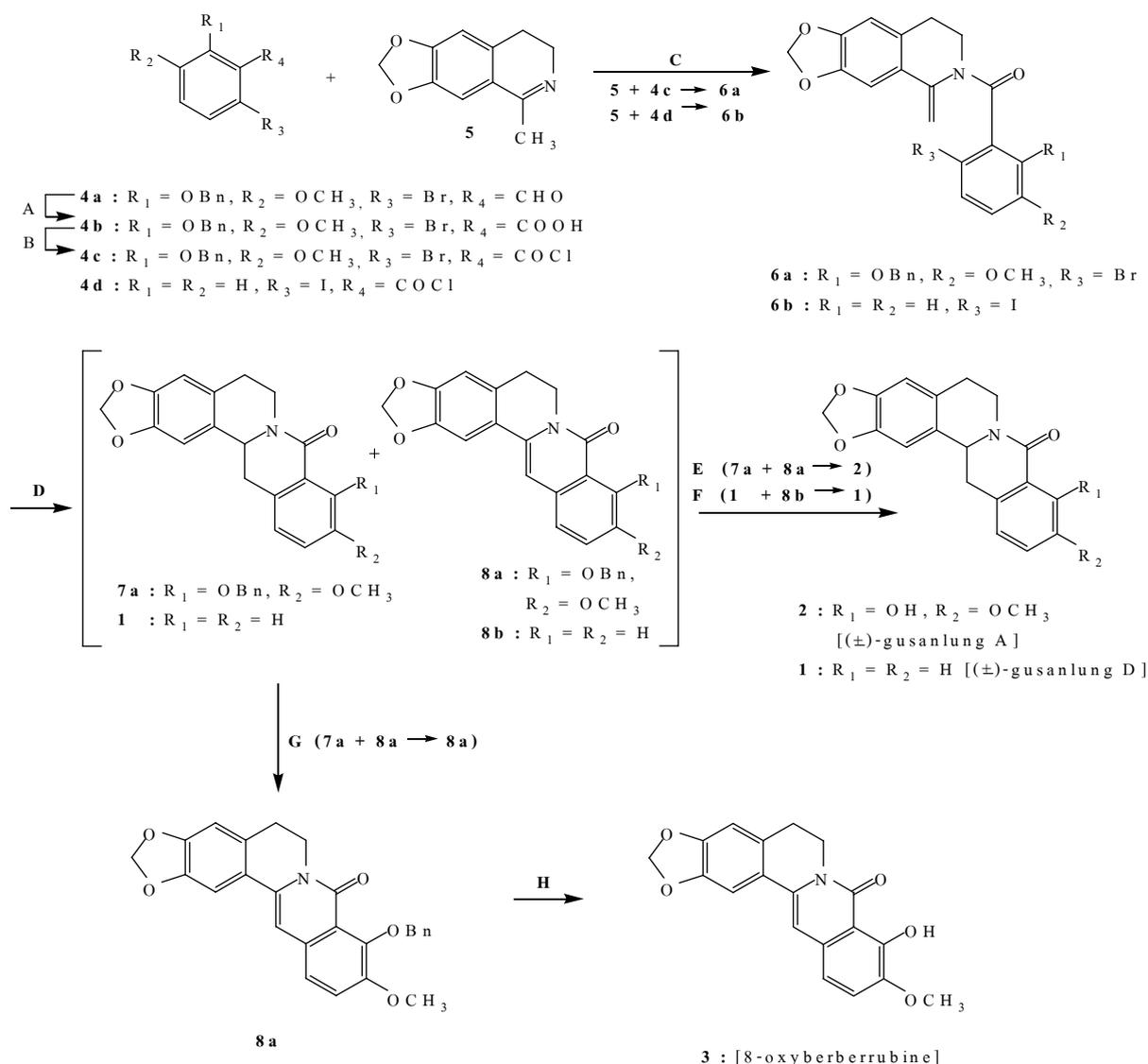
Furthermore, two new related alkaloids: (-)-gusalung A [**6**] and 8-oxyberberrubine [**1**], for which structures **2** and **3** were proposed based on spectral analysis, were isolated from *Acangelisia gusanlung* H. S. Lo. In view of the uncertainty regarding the correct structure of (-)-gusanlung D (**1**), it was therefore highly desirable to also confirm the correctness of the structures proposed for (-)-gusanlung A (**2**) and 8-oxyberberrubine (**3**) by total syntheses.

Results and Discussion

Syntheses of (\pm)-gusanlung A (**2**) and 8-oxyberberrubine (**3**)

The synthesis of (\pm)-gusanlung A (**2**) was based on the radical-initiated cyclization of 2-(2'-benzyloxy-6'-bromo-3'-methoxybenzoyl)-1-methylene-6,7-methylenedioxy-1,2,3,4-tetrahydroisoquinoline (**6a**), as outlined in Scheme 1, with subsequent catalytic hydrogenolysis of the benzyl protecting group.

Scheme 1. Synthetic routes to (\pm)-gusanlung A (**1**), (\pm)-gusanlung D (**2**), and 8-oxyberberrubine (**3**).



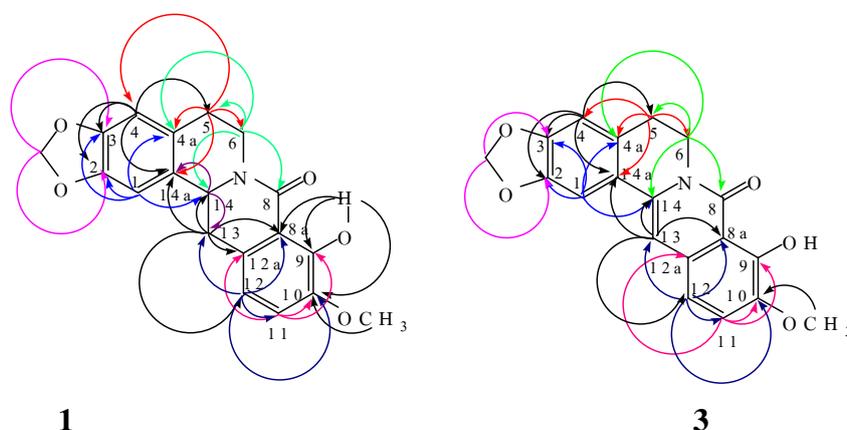
Reagents and Conditions A) NaClO_2 , sulphamic acid/ *tert*-butanol- H_2O ; B) SOCl_2 / benzene; C) Et_3N / dry benzene; D) Bu_3SnH , AIBN/ dry benzene; E) H_2 , Pd/C/ ethanol; F) hydrazine, Pd/C/ ethyl acetate-ethanol; G) I_2 / dioxane; H) conc. HCl/ ethanol.

Thus, oxidation of 2-benzyloxy-6-bromo-3-methoxybenzaldehyde (**4a**) [7] with sodium chlorite gave 2-benzyloxy-6-bromo-3-methoxybenzoic acid (**4b**), whose acid chloride (**4c**) was then reacted

with 6,7-methylenedioxy-1-methyl-3,4-dihydroisoquinoline (**5**) [8] in the presence of triethylamine to give the moderately stable compound **6a**. Treatment of **6a** with tributyltin hydride in the presence of a catalytic amount of 2,2'-azobis(isobutyronitrile) gave a 31.3% yield of a mixture of (\pm)-9-benzylgusanlung A (**7a**) and 9-benzyl-8-oxyberberrubine (**8a**) in a ratio of 78:22 according to $^1\text{H-NMR}$ analysis. Catalytic hydrogenolysis of the mixture of **7a** and **8a** to remove the benzyl protecting group also resulted in the concurrent hydrogenation of the C-C double bond to give pure (\pm)-gusanlung A (**2**). On the other hand, oxidation of the mixture of **7a** and **8a** with iodine gave 9-benzyl-8-oxyberberrubine (**8a**), whose benzyl protecting group was removed by acid treatment to give 8-oxyberberrubine (**3**).

The $^1\text{H-NMR}$ data of synthetic (\pm)-gusanlung A (**2**) were in reasonably good agreement with those reported for natural ($-$)-gusanlung A (**2**). However, a number of carbons in the $^{13}\text{C-NMR}$ spectrum of natural ($-$)-gusanlung A (**2**) were found to have quite different chemical shifts from the corresponding carbons in the spectrum of (\pm)-gusanlung A (**2**). We therefore carried out $^1\text{H-}^1\text{H-COSY}$, HMQC and HMBC experiments to allow complete assignments of chemical shifts of (\pm)-gusanlung A (**2**). Details of the HMBC correlations are shown in Figure 2 and Table 4. The $^1\text{H-NMR}$ spectral data of natural 8-oxyberberrubine (**3**) were found to be in good agreement with those of synthetic 8-oxyberberrubine (**3**). However, from HMBC correlation experiment, it was possible to establish that the chemical shifts of H-1 and H-13 previously assigned should be interchanged. On the other hand, the ^{13}C spectrum of natural 8-oxyberberrubine (**3**) had a number of features which were quite different from those of synthetic 8-oxyberberrubine (**3**). These differences were highlighted and the HMBC correlations were shown in Figure 2 and Table 5. In summary, it can be concluded that while the $^1\text{H-NMR}$ analysis lent good support to the structures proposed for ($-$)-gusanlung A (**2**) and 8-oxyberberrubine (**3**), in view of the discrepancies in a number of carbon chemical shifts in the $^{13}\text{C-NMR}$ spectra of ($-$)-gusanlung A (**2**) versus those of (\pm)-gusanlung A (**2**) on the one hand, and natural 8-oxyberberrubine (**3**) versus synthetic 8-oxyberberrubine (**3**) on the other, no definite conclusions can be drawn at this time concerning the correctness of the structures previously assigned to ($-$)-gusanlung A (**2**) and 8-oxyberberrubine (**3**).

Figure 2. HMBC correlations of (\pm)-gusanlung A (**1**) and 8-oxyberberrubine (**3**).



Synthesis of (\pm)-gusanlung D

The synthesis of (\pm)-gusanlung D (**1**) was uneventful. Thus, 2-iodobenzoyl chloride (**4d**) was reacted with **5** [**8**] in the presence of triethylamine to give the highly unstable 2-(2'-iodobenzoyl)-1-methylene-6,7-methylenedioxy-1,2,3,4-tetrahydroisoquinoline (**6b**). Treatment of **6b** with tributyltin hydride in presence of a catalytic amount of 2,2'-azobis(isobutyronitrile) gave a 39.0% yield of a mixture of **1** and **8b** in a ratio of 87:23 from $^1\text{H-NMR}$ analysis. Treatment of the mixture with hydrazine and palladium/charcoal gave (\pm)-gusanlung D (**1**), whose $^1\text{H-}$ and $^{13}\text{C-NMR}$ data were in good agreement with those of (\pm)-gusanlung D (**1**) and ($-$)-gusanlung D obtained from previous syntheses [2,3,4] but differed significantly from those of natural ($-$)-gusanlung D [1]. The structure previously assigned to ($-$)-gusanlung D [1] therefore remains uncertain.

Table 1. Comparison of $^1\text{H-NMR}$ spectral data between natural ($-$)-gusanlung D [**1**], synthetic ($-$)-gusanlung D [**4**] and synthetic (\pm)-gusanlung D [**2**] and [**this work**].

(position)	($-$)-gusanlung D CDCl ₃ [1] m.p. 250-251 °C	($-$)-gusanlung D CDCl ₃ [4] m.p. 195-197 °C	(\pm)-gusanlung D CDCl ₃ [2] m.p. 175-177 °C	(\pm)-gusanlung D CDCl ₃ [this work] m.p. 175-176 °C
	^1H	^1H	^1H	^1H
1	7.35 (s)	6.71 (s)	6.76 (d)	6.72 (s)
4	6.80 (s)	6.67 (s)	6.76 (d)	6.67 (s)
5 α	2.70-3.40 (m)	2.7-2.8 (m)	2.83-3.35 (m)	2.70-2.82 (m)
5 β	2.70-3.40 (m)	2.82-3.02 (m)	2.83-3.35 (m)	2.87-3.07 (m)
6 α	2.70-3.40 (m)	2.82-3.02 (m)	2.83-3.35 (m)	2.87-3.07 (m)
6 β	4.8 (m)	4.93-4.99 (m)	4.7-5.1 (m)	4.88-4.99 (m)
9	8.07 (d, 8.0)	8.13 (d, 7.4)	8.1-8.37 (m)	8.13 (dd, 7.6, 1.4)
10	7.29-7.41 (m)	7.34-7.40 (m)	7.25-7.65 (m)	7.39 (br t, 7.4)
11	7.29-7.41 (m)	7.41-7.49 (m)	7.25-7.65 (m)	7.46 (dt, 7.4, 1.5)
12	7.29-7.41 (m)	7.24 (d, 7.4)	7.25-7.65 (m)	7.22-7.29 (m)
13 α	2.70-3.40 (m)	2.82-3.02 (m)	2.83-3.35 (m)	2.87-3.07 (m)
13 β	2.70-3.40 (m)	3.18 (dd, 15.3, 3.7)	2.83-3.35 (m)	3.18 (dd, 15.7, 3.7)
14	3.95 (m)	4.83 (dd, 13.3, 3.7)	4.7-5.1 (m)	4.84 (dd, 13.3, 3.7)
OCH ₂ O	6.20, 6.06 (s)	5.96 (s)	5.93 (s)	5.96 (s)

Table 2. Comparison of $^{13}\text{C-NMR}$ spectral data between natural ($-$)-gusanlung D [**1**], synthetic ($-$)-gusanlung D [**4**] and synthetic (\pm)-gusanlung D [**3**] and [**this work**].

(position)	($-$)-gusanlung D CDCl ₃ [1] m.p. 250-251 °C	($-$)-gusanlung D CDCl ₃ [4] m.p. 195-197 °C	(\pm)-gusanlung D CDCl ₃ [3] m.p. 175-177 °C	(\pm)-gusanlung D CDCl ₃ [this work] m.p. 175-176 °C
	^{13}C	^{13}C	^{13}C	^{13}C
1	107.3	105.8	105.97	105.9
2	135.0	146.5^b	146.57	146.6^c
3	147.0	146.7 ^b	146.77	146.8 ^c
4	107.5	108.6	108.81	108.7

Table 2. Cont.

(position)	(-)-gusanlung D CDCl ₃ [1] m.p. 250-251 °C	(-)-gusanlung D CDCl ₃ [4] m.p. 195-197 °C	(±)-gusanlung D CDCl ₃ [3] m.p. 175-177 °C	(±)-gusanlung D CDCl ₃ [this work] m.p. 175-176 °C
4a	126.5	128.8	128.85	128.9
5	29.7	29.6	29.61	29.7
6	42.0	38.7	38.49	38.8
8	162.0	164.5	158.67	164.6
8a	117.3	137.2	137.24	137.3
9	128.7 ^a	128.6	128.60	128.6
10	127.9^a	127.3	127.37	127.4[*]
11	127.1^a	131.8	132.33	131.9[*]
12	126.8 ^a	126.8	126.87	126.9
12a	124.6	129.0	131.81	129.1
13	33.5	38.1	37.78	38.1
14	49.4	55.2	55.18	55.3
14a	126.5	128.5	128.55	128.6
OCH ₂ O	100.9	101.1	101.00	101.1

a, b, c, * assignments may be interchangeable.

Table 3. Comparison of ¹H-NMR spectral data between natural (-)-gusanlung A [1] and synthetic (±)-gusanlung A [this work].

(position)	(-)-gusanlung A (DMSO- <i>d</i> ₆) [6] m.p. 260-262 °C	(±)-gusanlung A (DMSO- <i>d</i> ₆) [this work] m.p. 188-189 °C	(±)-gusanlung A (CDCl ₃) [this work] m.p. 188-189 °C
	¹ H	¹ H	¹ H
1	6.96 (s)	7.00 (s)	6.71 (s)
4	6.80 (s)	6.79 (s)	6.66 (s)
5	2.73-2.81 (m)	2.75-2.89 (m)	2.72-2.84 (m)
6α	2.73-2.81 (m)	2.89-3.01 (m)	2.94-3.40 (m)
6β	4.71 (m)	4.69-4.59 (m)	4.80-4.87 (m)
11	6.99 (d, 8.1)	7.09 (d, 8.1)	6.94 (d, 8.1)
12	6.86 (d, 8.1)	6.71 (d, 8.1)	6.63 (d, 8.1)
13α	3.13 (dd, 15.3, 3.1)	3.36 (dd, 15.2, 3.6)	3.14 (dd, 15.2, 3.8)
13β	2.62 (dd, 15.3, 13.3)	2.66-2.75 (m)	2.80-2.94 (m)
14	4.68 (dd, 13.3, 3.1)	4.84 (dd, 13.3, 3.4)	4.80 (dd, 13.6, 3.5)
C ₁₀ -OCH ₃	3.76 (s)	3.78 (s)	3.90 (s)
OCH ₂ O	5.98, 5.99 (s)	5.98, 6.00 (s)	5.96 (s)
OH	-	12.88 (s)	12.83 (s)

Table 4. Comparison of ^{13}C -NMR spectral data between natural (-)-gusanlung A [6] and synthetic (-)-gusanlung A [this work] and HMBC correlations of (\pm)-gusanlung A [this work].

(position)	(-)-gusanlung A (DMSO- d_6) [6]	(\pm)-gusanlung A (DMSO- d_6) [this work]	(\pm)-gusanlung A (CDCl $_3$) [this work]	(±)-gusanlung A (DMSO- d_6) [this work]	
	m.p. 260-262 °C	m.p. 188-189 °C	m.p. 188-189 °C	HMBC	
	^{13}C	^{13}C	^{13}C	2J	3J
1	106.1	106.6	105.8	C-2	C-3, 4a, 14
2	145.9 ^a	146.7 ^c	146.8*	-	-
3	147.7 ^a	146.5 ^c	146.7*	-	-
4	107.8	108.7	108.6	C-3	C-2, 5, 14a
4a	129.1 ^b	128.3	128.1	-	-
5	29.0	28.9	29.4	C-4a, 6	C-4, 14a
6	37.8	38.5	38.4	C-5	C-4a, 8, 14
8	161.4	168.4	168.6	-	-
8a	122.3^b	111.4	111.4	-	-
9	149.7 ^a	151.4	151.8	-	-
10	145.7 ^a	147.2	147.5	-	-
11	118.9	116.7	115.4	C-10	C-9, 12a
12	122.1	116.9	116.1	C-11	C-8a, 10, 13
12a	128.2^b	129.6	128.7	-	-
13	37.7	35.9	37.1	C-12a, 14	C-8a, 12, 14a
14	54.4	55.4	55.7	C-13, 14a	-
14a	129.3^b	129.1	128.4	-	-
C $_{10}$ -OCH $_3$	60.5	56.3	56.3	-	C-10
OCH $_2$ O	100.5	101.3	101.2	-	C-2, 3
OH				C-9	C-8a, 10

a, b, c, * assignments may be interchangeable.

Table 5. Comparison of ^1H - and ^{13}C -NMR spectral data between natural 8-oxyberberubine (3) [1], synthetic 8-oxyberberubine (3) [this work] and HMBC correlations of 8-oxyberberubine [this work].

(position)	natural 8-oxy- berberubine (3) CDCl $_3$ [1]	synthetic 8-oxy- berberubine (3) CDCl $_3$ [this work]	natural 8-oxy- berberubine (3) CDCl $_3$ [1]	synthetic 8-oxy- berberubine (3) CDCl $_3$ [this work]	synthetic 8-oxyberberubine (3) (CDCl $_3$) [this work]	
	m.p. 240-241 °C	m.p. 238-239 °C	m.p. 240-241 °C	m.p. 238-239 °C	HMBC	
	^1H	^1H	^{13}C	^{13}C	2J	3J
1	6.83 (s)	7.21 (s)	104.0	104.8	C-2	C-3, 4a, 14
2			141.6	147.5*	-	-
3			146.4	148.6*	-	-
4	6.72 (s)	6.71 (s)	107.1	108.0	C-3	C-2, 5, 14a
4a			109.6	129.5	-	-
5	2.91 (t, 7.2)	2.92 (t, 6.1)	28.4	28.4	C-4a, 6	C-4, 14a
6	4.27 (t, 7.2)	4.27 (t, 6.1)	39.1	39.1	C-5	C-4a, 8, 14

Table 5. Cont.

(position)	natural 8-oxy-berberrubine (3) CDCl ₃ [1] m.p. 240-241 °C	synthetic 8-oxy-berberrubine (3) CDCl ₃ [this work] m.p. 238-239 °C	natural 8-oxy-berberrubine (3) CDCl ₃ [1] m.p. 240-241 °C	synthetic 8-oxy-berberrubine (3) CDCl ₃ [this work] m.p. 238-239 °C	synthetic 8-oxyberberrubine (3) (CDCl ₃) [this work] HMBC	
	8			164.0	165.4	-
8a			129.9	111.0	-	-
9			149.0	150.3	-	-
10			147.5	144.9	-	-
11	7.30 (AB q, 8.0)	7.28 (d, 8.5)	114.9	119.1	C-10	C-9, 12a
12	7.00 (AB q, 8.0)	6.99 (d, 8.5)	120.0	115.3	C-11	C-8a, 10, 13
12a			128.9	130.5	-	-
13	7.21 (s)	6.83 (s)	103.6	103.6	C-14	C-8a, 12, 14a
14			133.6	134.6	-	-
14a			122.1	123.5	-	-
C ₁₀ -OCH ₃	3.96 (s)	3.97 (s)	56.7	56.7	-	C-10
OCH ₂ O	6.02 (s)	6.02 (s)	100.6	101.5	-	C-2, 3
OH	-	13.14	-	-	-	-

* assignments may be interchangeable.

Antimicrobial activity

(±)-Gusanlung D (**1**) and (±)-gusanlung A (**2**) at the concentration value 256 µg/mL were inactive against *Staphylococcus aureus* ATCC25932, *Escherichia coli* ATCC10536 and *Candida albicans* ATCC90028.

Conclusions

Based on spectral analysis, there were significant discrepancies between the spectral data of natural (-)-gusanlung D and synthetic (±)-gusanlung D. Hence, the structure previously proposed for (-)-gusanlung D remains doubtful. While the ¹H spectral data of natural (-)-gusanlung A and 8-oxyberberrubine were in reasonably good agreement with those of synthetic (±)-gusanlung A and 8-oxyberberrubine, the ¹³C spectral data of natural (-)-gusanlung A and 8-oxyberberrubine were not entirely in good agreement with those of synthetic (±)-gusanlung A and 8-oxyberberrubine. The structures previously proposed for natural (-)-gusanlung A and 8-oxyberberrubine must therefore be treated with caution.

Experimental

General

Melting points were determined on a SMP 2 Stuart Scientific melting point apparatus and are uncorrected. Infrared spectra were recorded on CH₂Cl₂-films with a Perkin Elmer Spectrum GX FT-IR

spectrophotometer. Ultraviolet spectra were recorded on methanol solutions with a Perkin Elmer Lambda 35 UV-VIS spectrophotometer. ^1H - and ^{13}C -NMR spectra were recorded on (D) chloroform solutions at 300 MHz for ^1H and 75 MHz for ^{13}C with a Bruker AVANCE 300 spectrometer. Tetramethylsilane was used as the internal standard. MS spectra were recorded on a POLARIS Q mass spectrometer.

2-Benzyloxy-6-bromo-3-methoxybenzoic acid (4b). A solution of sodium chlorite (0.36 g, 3.6 mmol) in H_2O (5 mL) was added to a solution of 2-benzyloxy-6-bromo-3-methoxybenzaldehyde (**4a**) [7] (1.0 g, 3.1 mmol) and sulfamic acid (0.5 g) in *tert*-butanol (10 mL) and H_2O (3 mL). The solution was stirred for 1 h. The mixture was shaken with ethyl acetate (20 mL) and the ethyl acetate layer was extracted with 5% sodium carbonate (3×20 mL). The aqueous layer was then acidified with concentrated hydrochloric acid and extracted with chloroform (3×20 mL). The chloroform layer was dried over anhydrous sodium sulfate. Removal of the solvent under vacuum gave a solid which was recrystallized from benzene-hexane to give **4b** as pale white crystals (0.8 g, 76.2%), m.p. 112-115 °C; ^1H -NMR: δ 7.47-7.42 (2H, m, Ph-H); 7.38-7.25 (4H, m, Ph-H \times 3 and Ar-H); 6.88, (1H, d, $J = 8.9$ Hz, Ar-H); 5.10 (2H, s, CH_2Ph); 3.89 (3H, s, OCH_3). ^{13}C -NMR: δ 171.0 (C), 152.2 (C), 145.9 (C), 136.7 (C), 130.6 (C), 128.4 (CH), 128.3 (CH), 128.2 (CH), 114.9 (CH), 108.7 (C), 76.0 (CH_2), 56.2 (OCH_3).

2-(2'-Benzyloxy-6'-bromo-3'-methoxybenzoyl)-1-methylene-6,7-methylenedioxy-1,2,3,4-tetrahydroisoquinoline (6a). A solution of acid **4b** (3.6 g, 10.0 mmol) and thionyl chloride (3.9 g, 32.8 mmol) in benzene (20 mL) was refluxed for 1 h. The solvent and excess thionyl chloride were removed under vacuum to give acid chloride **4c** as a yellow oil (3.7 g, 94.9%) which was used in the next step without further purification. A solution of acid chloride **4c** (1.9 g, 5.3 mmol) in dry benzene (20 mL) was added dropwise over 10 min. to a solution of isoquinoline **5** [8] (1.0 g, 5.3 mmol) and triethylamine (1.0 g) in dry benzene (20 mL), then the mixture was refluxed for 2 h. On cooling, the precipitated triethylamine hydrochloride was filtered off. The filtrate was evaporated under vacuum to give enamide **6a** as a yellow oil (2.6 g, 84.4%) which was unstable and decomposed on standing. It was immediately used in the next step without further purification. ^1H -NMR: δ 7.38-7.23 (5H, m, Ph-H), 7.18(1H, d, $J = 8.8$ Hz, H-5'), 6.89 (1H, s, H-8), 6.75 (1H, d, $J = 8.8$ Hz, H-4'), 6.41 (1H, s, H-5), 5.90 (2H, AB q, $J = 1.3$ Hz, OCH_2O), 5.14 (1H, d, $J = 1.3$ Hz, $=\text{CH}_2$), 5.00 (2H, AB q, $J = 10.8$ Hz, CH_2Ph), 4.81 (1H, d, $J = 1.3$ Hz, $=\text{CH}_2$), 4.13-4.02, 3.57-3.50 (2H, 2 m, CH_2 -3), 3.80 (3H, s, OCH_3), 2.90-2.59 (2H, m, CH_2 -4); ^{13}C -NMR: δ 165.0 (C), 152.1 (C), 147.8 (C), 146.5 (C), 145.3 (C), 141.4 (C), 137.4 (C), 134.3 (C), 129.0 (C), 128.4 (CH), 128.1 (CH), 127.9 (CH), 127.7 (CH), 125.1 (C), 113.4 (CH), 110.0 (C), 108.4 (CH), 104.4 (CH_2), 103.8 (CH), 101.1 (CH_2), 75.4 (CH_2), 55.9 (OCH_3), 41.6 (CH_2), 28.8 (CH_2).

(±)-Gusanlung A (1) and 9-benzyl-8-oxyberberrubine (8a). A solution of enamide **6a** (2.7 g, 5.3 mmol), tributyltin hydride (3.4 g, 11.7 mmol) and 2,2'-azobis(isobutyronitrile) (0.2 g, 0.7 mmol) in dry benzene (50 mL) was refluxed with stirring for 3 h., then the solvent was removed under vacuum. The residue was washed with hexane (4×15 mL) and dissolved in chloroform (30 mL). The chloroform layer was washed with brine, then dried over anhydrous sodium sulfate. The solvent was removed under vacuum to give a yellow solid which was recrystallized from ethanol to give a 31.3% yield of a

mixture of (\pm)-9-benzylgusanlung A (**7a**) and 9-benzyl-8-oxyberberrubine (**8a**) in a ratio of 78:22 from $^1\text{H-NMR}$ analysis.

A solution of the mixture of **8a** and **7a** (303.7 mg, 0.7 mmol) in ethanol (50 mL) was hydrogenated over 10% Pd/C (30.4 mg) at atmospheric pressure for 48 h. The catalyst was filtered off and the solvent was removed under vacuum to give a crude yellow solid. Recrystallization of the crude solid from ethanol gave (\pm)-gusanlung A (**2**) as a pale yellow solid (82.4 mg, 34.3%), m.p. 188-189 °C; UV (MeOH) λ_{max} nm (log ϵ): 219 (4.54), 271sh (3.87), 281 (3.98), 308 (4.16), 319 (4.15); IR ν_{max} (film): 3737, 3650, 3585, 2919, 2852, 1748, 1634, 1615, 1581, 1542, 1506, 1488, 1456, 1386, 1356, 1336, 1315, 1262, 1239, 1154, 1084, 1069, 1037, 1001, 933, 858, 804, 792, 728 cm^{-1} ; MS (EI) m/z (%): 339 (M^+ , 55), 176 (100). $^1\text{H-NMR}$ see Table 3, $^{13}\text{C-NMR}$ and HMBC see Table 4.

A solution of iodine (4.6 g, 18.3 mmol) in dioxane (100 mL) was added dropwise over 30 min. to a refluxing solution of the mixture of **7a** and **8a** (1.3 g, 3.0 mmol) and sodium acetate (1.5 g) in dioxane (50 mL), then the mixture was refluxed for 6 h. On cooling, the sodium acetate was filtered off and the precipitate was washed with chloroform (100 mL). The chloroform layer was washed with 5% NaHSO_3 (100 mL), dilute NH_3 (30 mL), H_2O (100 mL) then dried over anhydrous Na_2SO_4 . Removal of the solvent under vacuum gave a red solid which was recrystallized with ethanol to give 9-benzyl-8-oxyberberrubine (**8a**) as red crystals (0.6 g, 50.0%), m.p. 190-192 °C. UV (MeOH) λ_{max} nm (log ϵ): 206sh (4.62), 224 (6.31), 255sh (5.78), 312 (5.76), 342 (6.03), 369 (5.86), 387 (5.71); IR ν_{max} (film): 2938, 2898, 2841, 1651, 1619, 1599, 1494, 1484, 1386, 1372, 1317, 1277, 1225, 1176, 1100, 1083, 939, 871, 834, 777, 734 cm^{-1} ; $^1\text{H-NMR}$: δ 7.73-7.68 (2H, m, Ph-H); 7.43-7.32 (3H, m, Ph-H); 7.32-7.28 (2H, m, H-11 and H-12); 7.22 (1H, s, H-1), 6.72 (1H, s, H-13); 6.70 (1H, s, H-4); 6.00 (2H, s, OCH_2O); 5.16 (2H, s, CH_2Ph); 4.31 (2H, t, $J = 6.1$ Hz, CH_2-6); 3.88 (3H, s, OCH_3); 2.88 (2H, t, $J = 6.1$ Hz, CH_2-5); $^{13}\text{C-NMR}$: δ 160.2 (C), 151.7 (C), 148.4 (C), 148.2 (C), 147.3 (C), 138.1 (C), 135.6 (C), 132.4 (C), 130.1 (C), 128.7 (CH), 128.2 (CH), 127.7 (CH), 123.8 (C), 122.5 (CH), 119.8 (C), 119.1 (CH), 107.9 (CH), 104.7 (CH), 101.4 (CH_2), 101.3 (CH), 75.7 (CH_2), 56.9 (OCH_3), 39.5 (CH_2), 28.7 (CH_2).

8-Oxyberberrubine (3). A solution of **8a** (100.0 mg, 0.2 mmol) in ethanol (30 mL) and conc. HCl (30 mL) was refluxed for 3 h. On cooling, the solution was extracted with chloroform (50 mL). The extract was washed with water (50 mL), then dried over anhydrous Na_2SO_4 . Removal of the solvent under vacuum gave a yellow solid which was recrystallized with ethanol to give 8-oxyberberrubine (**3**) as pale yellow crystals (42.2 mg, 53.5%), m.p. 238-239 °C (Lit. [2] m.p. 240-241 °C); UV (MeOH) λ_{max} nm (log ϵ): 225 (4.44), 258sh (3.99), 270 (3.87), 288 (3.69), 345 (4.16), 369 (4.13); IR ν_{max} (film): 3011, 2893, 2836, 1645, 1594, 1490, 1393, 1320, 1267, 1228, 1181, 1087, 1033, 932, 826, 665 cm^{-1} . $^1\text{H-NMR}$, $^{13}\text{C-NMR}$ and HMBC see Table 5.

2-(2'-Iodobenzoyl)-1-methylene-6, 7-methylenedioxy-1,2,3,4-tetrahydroisoquinoline (6b). A solution of 2-iodobenzoyl chloride **4d** (1.4 g, 5.4 mmol) in dry benzene (20 mL) was added dropwise over 10 min. to a solution of isoquinoline **5** [8] (1.0 g, 5.3 mmol) and triethylamine (1.0 g) in dry benzene (20 mL), then the mixture was refluxed for 2 h. On cooling, the precipitated triethylamine hydrochloride was filtered off and the filtrate was evaporated under vacuum to give enamide **6b** as a yellow oil (2.2 g, 99.1%) which was unstable and decomposed on standing, so it was immediately used in the next

step without further purification. $^1\text{H-NMR}$: δ 8.07-6.84 (5H, m, Ar-H); 6.58 (1H, s, Ar-H); 5.92 (2H, s, OCH_2O); 5.18 (1H, br s, $=\text{CH}_2$); 4.50 (1H, br s, $=\text{CH}_2$); 4.12 (2H, br s, CH_2); 2.95 (2H, br s, CH_2); $^{13}\text{C-NMR}$: δ 169.0 (C), 161.2 (C), 148.2 (C), 146.8 (C), 142.6 (C), 142.2 (CH), 139.3 (CH), 135.9 (C), 132.5 (C), 129.9 (CH), 128.3 (CH), 125.0 (C), 108.4 (CH), 106.2 (CH_2), 103.9 (CH), 101.2 (CH_2), 41.8 (CH_2), 29.0 (CH_2).

(\pm)-Gusanlung D (**1**) and 13,14-didehydrogusanlung D (**8b**). A solution of enamide **6b** (2.9 g, 10.0 mmol) tributyltin hydride (11.7 g, 40.0 mmol) and 2,2'-azobis(isobutyronitrile) (1.6 g, 10.0 mmol) in dry benzene (50 mL) was refluxed with stirring for 3 h., then the solvent was removed under vacuum. The residue was washed with hexane (4 \times 15 mL) and dissolved in chloroform (30 mL). The chloroform layer was washed with brine, then dried over anhydrous sodium sulfate. The solvent was removed under vacuum to give a solid which was recrystallized from ethanol to give a 39.0% yield of a mixture of **1** and **8b** in a ratio of 23:87 from $^1\text{H-NMR}$ analysis.

A mixture of **1** and **8b** (200.0 mg, 0.7 mmol), Pd/C (300.0 mg), hydrazine (50 mL), ethanol (50 mL) and ethyl acetate (50 mL) was refluxed for 48 h. The Pd/C was filtered and the filtrate extracted with chloroform (80 mL). The extract was washed with 10% HCl (2 \times 50 mL), water (50 mL) then dried over anh. Na_2SO_4 . Removal of the solvent under vacuum gave a yellow solid which was recrystallized with ethanol to give pure (\pm)-gusanlung D (**1**) as pale yellow crystals (99.4 mg, 49.4%), m.p. 175-176 $^\circ\text{C}$ (lit. [5] m.p. 175-177 $^\circ\text{C}$). UV (MeOH) λ_{max} nm (log ϵ): 206 (6.27), 230 (5.78), 253sh (5.42), 290 (5.42), 335 (5.02), 365 (4.77); IR ν_{max} (film): 2922, 1646, 1602, 1576, 1487, 1412, 1362, 1333, 1285, 1241, 1218, 1178, 1141, 1038, 936, 906, 853, 743, 636, 505 cm^{-1} . $^1\text{H-NMR}$ and $^{13}\text{C-NMR}$ see Tables 1 and 2.

Minimum inhibitory concentration (MIC)

MIC of (\pm)-gusanlung A (**2**) and (\pm)-gusanlung D (**1**) were determined by NCCLS microbroth dilution methods [9]. (\pm)-Gusanlung A (**2**) and (\pm)-gusanlung D (**1**) were weighed and dissolved in DMSO to make a solution of concentration 2.56 mg/mL. From this stock solution two-fold serial dilution has been carried out to give a series of solutions from 256 $\mu\text{g/mL}$ to 0.50 $\mu\text{g/mL}$ with culture medium in 96-well microplates (100 μL of total volume). Three different microorganisms were selected *viz.* *Staphylococcus aureus* ATCC25932, *Escherichia coli* ATCC10536 and *Candida albicans* ATCC90028. They were subcultured on nutrient broth supplemented with 10% glucose (NBG) (for bacteria) or Sabouraud glucose broth (for yeast) and incubated at 37 $^\circ\text{C}$ for 24 h. A final concentration of 1×10^5 cfu/mL of test bacteria or yeast was added to each dilution. The plates were incubated at 37 $^\circ\text{C}$ for 48 h. MIC was defined as the lowest concentration of test agent that inhibited bacterial or yeast growth, as indicated by the absence of turbidity. Test agent-free broth containing 5% DMSO was incubated as growth control.

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Sample Availability: All stable products reported in this paper are available from the authors.

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