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N-Hydroxypyridone alkaloids, chromone derivatives, and tetrahydroxanthones from the scale-insect pathogenic fungus *Orbiocrella* sp. BCC 33248

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ABSTRACT

Six new compounds, an *N*-hydroxypyridone glucoside, orbiocrellin A (**1**), its aglycone orbiocrellin B (**2**), chromone glucosides **3** and **4**, a dihydrochromone **5a**/**5b**, and a chromone **6**, were isolated from the scale-insect pathogenic fungus *Orbiocrella* sp. BCC 33248. Orbiocrellin A (**1**) exhibited antimalarial activity against *Plasmodium falciparum* K1 (IC_{50} 3.1 μg/mL) while it was non-cytotoxic. In contrast, orbiocrellin B (**2**) showed both antimalarial (IC_{50} 2.1 μg/mL) and cytotoxic (NCI-H187 cells, IC_{50} 0.70 μg/mL) activities.

Keywords: *Orbiocrella*; Invertebrate pathogenic fungi; Antimalarial activity; Cytotoxicity

1. Introduction

Invertebrate pathogenic fungi have recently been proved to be sources of structurally diverse bioactive compounds.[1] Although a number of compounds with unique skeletons and significant biological activities have been isolated from several large genera such as *Cordyceps*, *Beauveria*, *Aschersonia*, *Isaria*, and *Hirsutella*,[2] there are still many genera/species of entomogenous fungi that remain chemically poorly explored or unexplored. *Orbiocrella* is a very small genus of specific scale-insect pathogen in the family Clavicipitaceae, recently described as a part of taxonomic reclassification of *Torrubiella*.[3] There is only one currently recorded species, *Orbiocrella petchii*, which was formerly named as *Torrubiella petchii*. There has been still no report of the secondary metabolites from *Orbiocrella*, in contrast to the fact that a variety of bioactive compounds have been isolated from *Conoideocrella* (also moved from *Torrubiella*),[4-8] another genus of specific scale-insect pathogen of the family Clavicipitaceae. As part of our research program on novel bioactive compounds from invertebrate pathogenic fungi, primarily aimed for utilization of the fungal sources in Thailand, we selected a strain (BCC 28517) of *Orbiocrella* sp. for large scale fermentation and chemical studies. A culture broth extract (screening sample) of this strain exhibited cytotoxicity to small-cell lung cancer cells (NCI-H187) with an IC_{50} value of 2.9 μg/mL. We report here the isolation, structure elucidation, and biological activities of five new compounds, an *N*-hydroxypyridone glucoside, orbiocrellin A (**1**), its aglycone orbiocrellin B (**2**), chromone glucosides **3** and **4**, a dihydrochromone **5a**/**5b**, and a chromone **6** (Fig. 1). Eugenitol (**7**)[9] and isoeugenitol (**8**),[10] aglycones of **3** and **4**, respectively, were also isolated from the same extract.

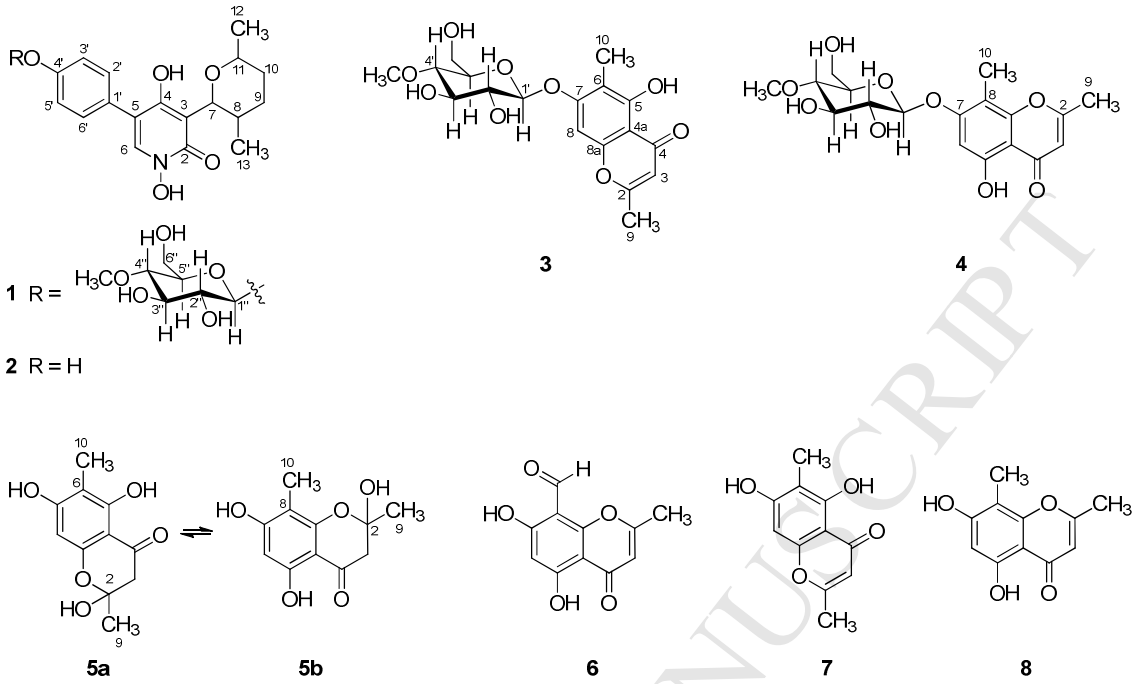


Fig. 1. Structures of compounds **1**–**4**, **5a**/**5b**, and **6**–**8** isolated from *Orbiocrella* sp. BCC 33248.

2. Results and discussion

Orbiocrellin A (**1**) was isolated as a yellow solid, and its molecular formula was determined to be $C_{25}H_{33}NO_{10}$ on the basis of the HR-ESI-MS. Interpretation of 2D NMR spectroscopic data suggested the presence of a pyranose unit (Table 1). The proton at δ_H 4.97 (d, J = 7.8 Hz, H-1″), which was attached to the downfield carbon at δ_C 100.9 (C-1″), was assigned to the anomeric position. The connectivity from C-1″ to C-6″ was revealed by analyses of COSY and HMQC spectra. Intense HMBC correlation from methoxy protons (δ_H 3.57, 3H, s) to C-4″ and the correlation from H-4″ to the methoxy carbon (δ_C 59.7) indicated that 4″-OH was methylated. Vicinal coupling constants of $J_{1'',2''}$ = 7.8, $J_{2'',3''}$ = 9.1, $J_{3'',4''}$ = 9.3, and $J_{4'',5''}$ = 9.3 Hz indicated axial orientations of these protons, which was further confirmed by the NOESY correlations

from H-1″ to H-3″ and H-5″. Consequently, the sugar unit was identified as 4″-*O*-methyl-β-glucopyranose. The rest of the ^{1}H and ^{13}C NMR resonances were assigned to those of the aglycone, a C_{18} alkaloid. The ^{13}C NMR, DEPT135, and HMQC spectroscopic data indicated that the aglycone contained five sp^2 quaternary carbons, five sp^2 methines, two oxygenated methines (δ_C 80.8 and 75.4), a methine, two methylenes, and two methyl groups. The presence of a *para*-oxygenated phenyl group was revealed by two pairs of *ortho*-coupled (J = 8.7 Hz) symmetrical aromatic methines (H-2′/H-6′ and H-3′/H-5′) and a downfield sp^2 quaternary carbon (δ_C 157.3, C-4′). An intense HMBC correlation from the sugar anomeric proton (H-1″) to C-4′ indicated the location of the sugar unit, which was further confirmed by the NOESY correlations from H-1″ to H-3′/H-5′. A terminal tetrahydropyran ring bearing two methyl groups was demonstrated by COSY correlations. A key HMBC correlation from H-7 (δ_H 4.71) to C-11 (δ_C 75.4) indicated the ether linkage to form a tetrahydropyran ring. The remaining five sp^2 carbons, together with one nitrogen and three oxygen atoms, should constitute the central unit of the aglycone. Analysis of the HMBC correlations revealed 1,4-dihydroxy-2-pyridone substituted at C-3 and C-5 (Fig. 2). Key HMBC correlations were those from H-6 to C-2, C-3 (weak, ^{4}J), C-4, C-5, and C-1, from H-7 to C-2, C-3, and C-4, and from H-1′/H-6′ to C-5. The C-3–C-1 bond was further supported by the NOESY correlations from H-6 to H-1′/H-6′.

The relative configuration of the tetrahydropyran unit was assigned to be 7*S**, 8*S**, and 11*S** on the basis of the vicinal coupling constant values and NOESY correlations (Fig. 3). The axial orientations (antiperiplanar relation) of H-7 and H-8 was evident from the large *J*-value (10.1 Hz) and a weaker intensity of the NOESY cross-peak of these protons relative to that for H-7 and H_3-13. NOESY correlations

from H-7 to H_{ax}-9 and H-11, and the correlation from H_{ax}-9 to H-11 demonstrated the coplanar relation and that the tetrahydropyran ring adopts a chair conformation, wherein the pyridone ring and two methyl groups are equatorial. The D-configuration of the 4-*O*-methylglucopyranose was determined by acid hydrolysis of **1**.[11] The anomeric mixture of sugar fragment obtained from the hydrolysate aqueous layer showed positive sign of specific rotation, $[\alpha]^{26}{}_{D}$ +71 (*c* 0.30, MeOH), which was consistent with the literature data for 4-*O*-methylglucopyranose, $[\alpha]^{20}{}_{D}$ +80 (*c* 1.3, MeOH).[12]

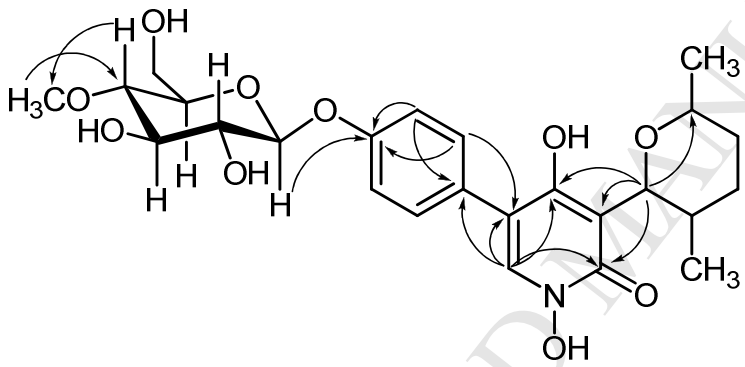


Fig. 2. Selected HMBC correlations for **1**.

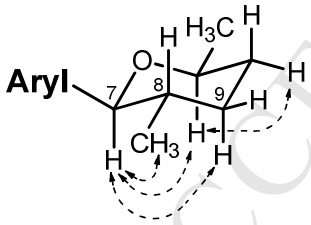


Fig. 3. Key NOESY correlations for the tetrahydropyran unit of **1**.

Table 1

^{13}C (100 MHz) and ^{1}H (400 MHz) NMR data for **1** and **2** in acetone-d_6

Position	Orbiocrellin A (**1**)			Orbiocrellin B (**2**)	
	δ_C, mult.	δ_H, mult. (*J* in Hz)	HMBC	δ_C, mult.	δ_H, mult. (*J* in Hz)
2	157.0,[a] C			157.1,[b] C	
3	109.3, C			109.5, C	
4	160.6,[a] C			160.8,[b] C	
5	112.2, C			112.9, C	
6	130.8, CH	7.83, s	2, 4, 5, 1′	130.8, CH	7.78, s
7	80.8, CH	4.71, d (10.1)	2, 3, 4, 8, 9, 11	80.9, CH	4.71, d (10.1)
8	35.5, CH	1.82, m		35.7, CH	1.80, m
9	32.4, CH_2	1.87, m; 1.41, m	7, 8, 10, 11	32.5, CH_2	1.87, m; 1.41, m
10	33.5, CH_2	1.76, m; 1.47, m	8	33.7, CH_2	1.76, m; 1.48, m
11	75.4, CH	3.68, m		75.5, CH	3.68, m
12	21.1, CH_3	1.24, d (6.1)	10, 11	21.3, CH_3	1.24, d (6.1)
13	17.1, CH_3	0.76, d (6.2)	7, 8, 9	17.2, CH_3	0.77, d (6.3)
1′	127.4, C			124.9, C	
2′,6′	130.3, CH	7.43, d (8.7)	5, 2′, 4′, 6′	130.6, CH	7.34, d (8.4)
3′,5′	116.3, CH	7.08, d (8.7)	1′, 3′, 4′, 5′	115.1, CH	6.87, d (8.4)
4′	157.3, C			157.0, C	
1″	100.9, CH	4.97, d (7.8)	4′, 2″, 5″		
2″	74.1, CH	3.47, m	1″, 3″		
3″	77.1, CH	3.64, t (9.1)	2″, 4″		
4″	79.3, CH	3.23, t (9.3)	2″, 5″, 6″, 4″-OCH_3		
5″	76.2, CH	3.49, m	6″		
6″	61.2, CH_2	3.84, dd (12.0, 1.9)	4″		
		3.69, dd (12.0, 4.8)			
4″-OCH_3	59.7, CH_3	3.57, s	4″		

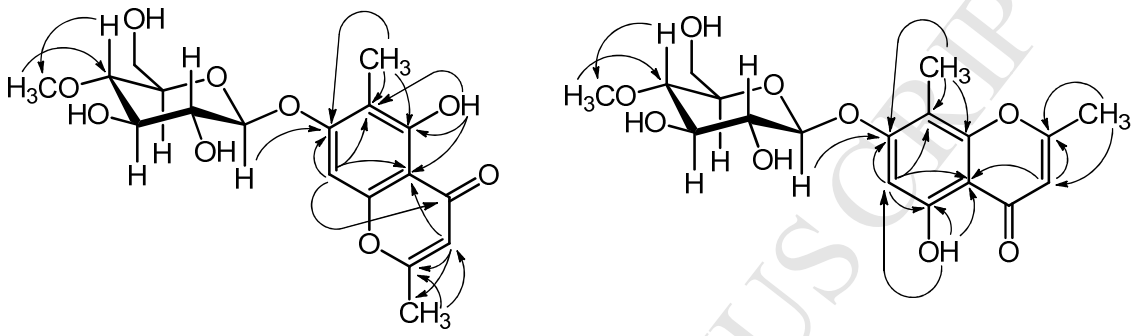
[a,b] The assignment of carbons can be interchanged.

Orbiocrellin B (**2**), the aglycone of **1**, was also isolated in relatively lower quantity. The 2D NMR spectroscopic data were consistent with those of the aglycone unit in **1**. 4-Hydroxy-2-pyridone alkaloids are a family of fungal secondary metabolites that are rich in diversity and biological activity.[13] Biosynthetically, orbiocrellin B (**2**) is a tetraketide fused to L-tyrosine.[14] A number of 4-hydroxy-2-pyridone alkaloids possessing the same L-tyrosine-derived unit as **2**, such as tenellins, bassianins, militarinones, farinosones, and torrubiellones, have been isolated from invertebrate pathogenic fungi of the genera *Beauveria*, *Cordyceps*, *Isaria*, *Torrubiella*, all are in the family Cordycipitaceae. These compounds possess a larger polyketide 3-acyl chain, while compounds **1** and **2** from *Orbiocrella* (family Clavicipitaceae) have a C-7 reduced structure forming a tetrahydropyran ring. To the best of our knowledge, orbiocrellide A (**1**) is the first fungal 4-hydroxy-2-pyridone alkaloid possessing a sugar unit.

The molecular formula of a new chromone glycoside **3** was determined to be $C_{18}H_{22}O_9$ by HR-ESI-MS. Interpretation of the NMR spectroscopic data revealed that the sugar unit was identical to that of **1**, 4-*O*-methyl-β-glucopyranose (Table 2). The aglycone was identified as 5,7-dihydroxy-2,6-dimethylchromone (eugenitol) based on the HMBC correlations (Fig. 4). An intense HMBC correlation from the glucose anomeric proton (H-1′) to C-7 (δ_C 161.1) and a NOESY correlation from H-1′ to H-8 indicated the location of the sugar linkage.

The molecular formula of another chromone glycoside **4** was the same as **3** (HR-ESI-MS). Analysis of 2D NMR spectroscopic data revealed the same sugar moiety as **3**, 4′-*O*-methyl-β-glucopyranose, while the aglycone was identified to be 5,7-dihydroxy-2,8-dimethylchromone (isoeugenitol). An HMBC correlation from the H-1′ to C-7 (δ_C 160.9) and an NOESY correlation from H-1′ to H-6 indicated the

glucosidation at C-7. The D-configuration of the 4′-*O*-methyl-β-glucopyranose in **4** was confirmed by acid hydrolysis. The sugar fragment obtained from the hydrolysate aqueous layer showed positive sign of specific rotation, $[\alpha]^{27}_{D}$ +93 (*c* 0.48, MeOH).



3 **4**

Fig. 4. Selected HMBC correlations for **3** amd **4**.

Table 2

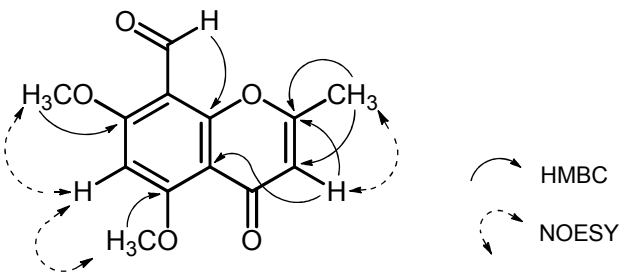
^{13}C (125 MHz) and ^{1}H (500 MHz) NMR data for **3** and **4** in DMSO-d_6

Position	**3**			**4**		
	δ_C, mult.	δ_H, mult. (*J* in Hz)	HMBC	δ_C, mult.	δ_H, mult. (*J* in Hz)	HMBC
2	168.6, C			168.7, C		
3	108.7, CH	6.25, s	2, 4a, 9	108.4, CH	6.24, s	2, 4, 4a, 9
4	182.5, C			182.9, C		
4a	105.1, C			105.2, C		
5	158.4, C			159.5, C		
6	109.1, C			98.3, CH	6.57, s	4a, 5, 7, 8
7	161.1, C			160.9, C		
8	93.3, CH	6.75, s	4, 4a, 6, 7, 8a	104.6, C		
8a	155.9, C			155.0, C		
9	20.5, CH_3	2.38, s	2, 3	20.5, CH_3	2.41, s	2, 3
10	7.9, CH_3	2.03, s	5, 6, 7	8.1, CH_3	2.16, s	7, 8, 8a
5-O*H*		13.03, s	4a, 5, 6		12.78, s	4a, 5, 6
1′	100.2, CH	5.05, d (7.8)	7	100.3, CH	5.02, d (7.8)	7
2′	73.8, CH	3.30, m	1′, 3′	73.9, CH	3.30, m	1′, 3′
3′	76.5, CH	3.43, m	2′, 4′	76.5, CH	3.44, m	2′
4′	79.4, CH	3.05, t (9.1)	5′, 4′-O*C*H_3	79.4, CH	3.05, t (9.2)	5′, 4′-O*C*H_3
5′	76.1, CH	3.49, m	4′	76.1, CH	3.47, m	
6′	60.7, CH_2	3.63, m; 3.50, m		60.7, CH_2	3.63, m; 3.50, m	
2′-O*H*		5.50, d (5.3)	1′, 2′, 3′		5.47, d (5.4)	1′, 2′, 3′
3′-O*H*		5.31, d (5.5)	2′, 3′, 4′		5.29, d (5.5)	2′, 3′, 4′
4′-O*C*H_3	60.1, CH_3	3.46, s	4′	60.1, CH_3	3.46, s	4′
6′-O*H*		4.74, dd (6.0, 5.1)	5′, 6′		4.73, dd (5.8, 4.7)	5′, 6′

A pair of dihydrochromone isomers, **5a/5b**, was obtained as a 3:2 mixture by silica gel column chromatography. Clear peak separation could be achieved by preparative HPLC; however, concentration of the fractions corresponding to each peak gave the same mixture of **5a** and **5b**. These results strongly suggested their interconversion during the concentration of the aqueous solution. Thus, structure elucidation was achieved using a mixture. Both isomers had a hemiacetal functionality (δ_C 100.9 and 100.8, C-2) whose location was indicated by the HMBC correlations from the methyl protons (H_3-9) and diastereotopic methylene protons (H_2-3) to the hemiacetal quaternary carbon. The structure of **5a** was assigned by the HMBC correlations from the aromatic methine proton H-8 (δ_H 5.98) to C-4a, C-6, C-7, and C-8a, and the correlations from the benzylic methyl protons H_3-10 (δ_H 1.95) to C-5, C-6, and C-7. On the other hand, **5b** showed key HMBC correlations from H-6 (δ_H 5.99) to C-4a, C-5, C-7, and C-8, and the correlations from H_3-10 to C-7, C-8, and C-8a. The interconversion of **5a**/**5b** indicated that the hemiacetal formations are reversible. Hydrated chromones **5a**/**5b** should be the biosynthetic precursors for the chromones **7** and **8** and their glucoside derivatives **3** and **4**. The interconversion of **3**/**4** was not observed during the isolation procedures. Even under the acid hydrolysis conditions for **4**, the chromone isomerisation from the aglycone **8** to **7** was not observed, which indicated the absence of the chromone hydration/dehydration equilibrium.

The molecular formula of compound **6** was determined as $C_{11}H_8O_5$ based on its HR-ESI-MS and NMR data. The ^1H and ^{13}C NMR spectroscopic data suggested a chromone skeleton similar to **7** and **8**. A significant difference was the presence of an aldehyde (δ_C 191.1; δ_H 10.35, s), replacing the benzylic methyl group in **7** and **8**. Since the HMBC and NOESY spectroscopic data for **6** were not informative enough to assign

the location of the formyl group of **6**, this compound was converted into its 5,7-*O*-dimethyl derivative **9** (Fig. 5). The HMBC correlation from the formyl proton to C-8a (δ_C 160.8) and the NOESY correlations from H-6 (δ_H 6.72) to both two methoxy groups (δ_H 4.09 and 4.02) indicated the connection of the formyl group to C-8. Therefore, compound **6** was identified as 5,7-dihydroxy-8-formyl-2-methychromone.



9

Fig. 5. Key HMBC and NOESY correlations for **9**.

The new compounds, except for **6** (sample shortage), were tested for cytotoxicity to cancer cell-lines (NCI-H187, MCF-7, and KB) and nonmalignant Vero cells, and antimalarial activity against *Plasmodium falciparum* K1 (Table 3). Orbiocrellide A (**1**) was non-cytotoxic up to a concentration of 50 μg/mL, while it exhibited antimalarial activity (IC_{50} 3.1 μg/mL). By contrast, orbiocrellin B (**2**) showed both cytotoxicity and antimalarial activity. Compounds **3**, **4**, and **5a/5b**, were inactive in these assays.

Table 3
Biological activities of compounds **1**–**4** and **5a**/**5b**

Compound	Cytotoxicity (IC_{50}, μg/mL)[a]				Anti-malaria[b] (IC_{50}, μg/mL)
	NCI-H187	MCF-7	KB	Vero	
1	>50	>50	>50	>50	3.1
2	0.70	7.3	>50	0.92	2.1
3	>50	>50	>50	>50	>10
4	>50	>50	>50	>50	>10
5a/**5b**	>50	>50	>50	>50	>10

[a] The IC_{50} values of a standard compound, doxorubicin hydrochloride, against NCI-H187, MCF-7, and KB cells were 0.18, 9.3, and 0.96 μg/mL, respectively. Ellipticine was used as a standard compound for the cytotoxicity assay against Vero cells (IC_{50} 0.73 μg/mL).
[b] Antimalarial activity against *Plasmodium falciparum* K1. The standard antimalarial drug, dihydroartemisinin, showed an IC_{50} value of 0.75 ng/mL.

3. Conclusion

The present study demonstrates that the genus *Orbiocrella* is a unique source of novel bioactive compounds. The biological activities of the *N*-hydroxypyridones **1** and **2** are noteworthy. Orbiocrellin A (**1**) selectively inhibited the proliferation of malarial parasite, while orbiocrellin B (**2**) deserves further biological evaluation as an anticancer agent.

4. Experimental Section

4.1. General procedures

Melting points were measured with an Electrothermal IA9100 digital melting point apparatus. Optical rotations were measured with a JASCO P-1030 digital polarimeter. UV spectra were recorded on a GBC Cintra 404 spectrophotometer. IR spectra were taken on a Bruker ALPHA spectrometer. NMR spectra were recorded on Bruker AV500D and DRX400 spectrometers. ESITOF mass spectra were measured with

a Bruker micrOTOF mass spectrometer.

4.2. Fungal material

The fungus used in this study was isolated from a scale insect (Hemiptera) collected in Khlong Lan National Park, Kamphaeng Phet Province, Thailand, on September 26, 2008 by one of the authors (S.M.). This fungus was deposited in the BIOTEC Culture Collection (BCC) as BCC 33248. On the basis of the ITS rDNA (GenBank accession number, KJ138267) and LSU rRNA (GenBank accession number, KJ138268) gene sequence data and the results of the BLAST search, this strain was assigned to the genus *Orbiocrella* within the family Clavicipitaceae.

4.3. Fermentation and isolation

The fungus BCC 33248 was maintained on Potato Dextrose Agar (PDA) at 25°C. The agar was cut into small plugs and inoculated in 4 × 250 mL Erlenmeyer flasks containing 25 mL of Difco™ Potato Dextrose Broth (PDB; composition, potato starch 4 g/L, dextrose 20 g/L). After incubation at 25°C for 5 days on a rotary shaker (200 rpm), each primary culture was transferred into a 1000 mL Erlenmeyer flask containing 250 mL of PDB, and incubated at 25°C for 5 days on a rotary shaker (200 rpm). These secondary cultures were pooled and each 25 mL portion was transferred into 40 × 1000 mL Erlenmeyer flasks, each containing 250 mL of M102 medium (composition, sucrose 30 g/L, malt extract 20 g/L, Bacto-peptone 2.0 g/L, yeast extract 1.0 g/L, KCl 0.5 g/L, $MgSO_4 \cdot 7H_2O$ 0.5 g/L, KH_2PO_4 0.5 g/L), and final fermentation was carried out at 25°C for 33 days under static conditions. The cultures were filtered to separate mycelia and filtrate. The filtrate was extracted with ethyl acetate (3 × 10 L),

and concentrated under reduced pressure to leave a brown gum (5.8 g). This extract was triturated in MeOH at room temperature and filtered. The residual solid was recrystallized from MeOH-H_2O to give **4** (92 mg). The filtrate from trituration (MeOH solution) was subjected to column chromatography on Sephadex LH-20 (3.8 × 60 cm, MeOH) to obtain ten pooled fractions. Fractions 3 (756 mg) and 4 (929 mg) were combined and fractionated again by Sephadex LH-20 (MeOH), and the fractions were purified by preparative HPLC (Dionex SunFire C18 OBD, 19 × 150 mm, 5 μm; step gradient elution with 20-100% MeCN/H_2O, 0–30 min, then MeCN 100%; flow rate 10 ml/min) to furnish **1** (133 mg, retention time 19 min). Fractions 5 (1.20 g) and 6 (599 mg) were combined and further fractionated and purified by Sephadex LH-20 (MeOH) and preparative HPLC (MeCN/H_2O) to give **2** (7.2 mg). Fraction 7 (501 mg) from the Sephadex LH-20 column chromatography was recrystallized from MeOH/CH_2Cl_2 to furnish **3** (31 mg). Fraction 8 (792 mg) was chromatographed again on Sephadex LH-20 (MeOH) and the subfractions were further separated by preparative HPLC (MeCN/H_2O) to furnish **5a**/**5b** (17 mg), **6** (4.9 mg), **7** (2.2 mg), and **8** (4.4 mg).

*4.3.1. Orbiocrellin A (**1**)*

Yellow solid; mp 134–135 °C; $[\alpha]^{28}_D$ –131 (*c* 0.12, MeOH); UV (MeOH) λ_{max} (log ε) 214 (3.99), 247 (4.10), 310 sh (3.37) nm; IR (ATR) ν_{max} 3455, 1739, 1641, 1511, 1367, 1230, 1217, 1061 cm^{-1}; ^1H NMR (400 MHz, acetone-d_6) and ^{13}C NMR (100 MHz, acetone-d_6) data, see Table 1; HRMS (ESI-TOF) *m/z* 508.2172 $[M + H]^+$ (calcd for $C_{25}H_{34}NO_{10}$, 508.2177).

*4.3.2. Orbiocrellin B (**2**)*

Yellow solid; mp 137–138 °C; $[\alpha]^{28}_{D}$ –89 (*c* 0.12, MeOH); UV (MeOH) λ_{max} (log ε) 214 (3.90), 254 (3.92), 317 sh (3.34) nm; IR (ATR) ν_{max} 1740, 1366, 1229, 1217 cm^{-1}; ^{1}H NMR (400 MHz, acetone-d_6) and ^{13}C NMR (100 MHz, acetone-d_6) data, see Table 1; HRMS (ESI-TOF) *m/z* 332.1499 $[M + H]^+$ (calcd for $C_{18}H_{22}NO_5$, 332.1492).

*4.3.3. 7-O-(4-O-Methyl-β-glucopyranosyl)eugenitol (**3**)*

Colorless solid; mp 167–168 °C; $[\alpha]^{27}_{D}$ –81 (*c* 0.11, MeOH); UV (MeOH) λ_{max} (log ε) 213 (3.98), 231 (4.05), 254 (4.05), 288 (3.75), 324 sh (3.42) nm; IR (ATR) ν_{max} 3375, 1662, 1626, 1341, 1119, 1093 cm^{-1}; ^{1}H NMR (500 MHz, DMSO-d_6) and ^{13}C NMR (125 MHz, DMSO-d_6) data, see Table 2; HRMS (ESI-TOF) *m/z* 383.1335 $[M + H]^+$ (calcd for $C_{18}H_{23}O_9$, 383.1337).

*4.3.4. 7-O-(4-O-Methyl-β-glucopyranosyl)isoeugenitol (**4**)*

Pale yellow solid; mp 237–238 °C; $[\alpha]^{27}_{D}$ –81 (*c* 0.11, MeOH); UV (MeOH) λ_{max} (log ε) 227 sh (3.81), 253 (3.94), 293 sh (3.32), 326 (3.32) nm; IR (ATR) ν_{max} 3307, 1667, 1621, 1595, 1424, 1315, 1276, 1109, 1083, 818 cm^{-1}; ^{1}H NMR (500 MHz, DMSO-d_6) and ^{13}C NMR (125 MHz, DMSO-d_6) data, see Table 2; HRMS (ESI-TOF) *m/z* 383.1344 $[M + H]^+$ (calcd for $C_{18}H_{23}O_9$, 383.1337).

*4.3.5. Compound **5a/5b***

Yellow solid; UV (MeCN/H_2O) λ_{max} 210, 259, 273 sh nm; IR (ATR) ν_{max} 3444, 1738, 1638, 1366, 1229, 1217, 1161, 1112 cm^{-1}; ^{1}H NMR (400 MHz, acetone-d_6) δ **5a**, 12.36 (1H, s, 5-O*H*), 5.98 (1H, s, H-8), 2.99 (1H, d, *J* = 16.8 Hz, H_a-3), 2.71 (1H, d, *J* = 16.8 Hz, H_b-3), 1.95 (3H, s, H-10), 1.66 (3H, s, H-9), **5b**, 12.04 (1H, s, 5-O*H*), 5.99 (1H,

s, H-6), 2.99 (1H, d, J = 16.8 Hz, H_a-3), 2.71 (1H, d, J = 16.8 Hz, H_b-3), 1.95 (3H, s, H-10), 1.72 (3H, s, H-9); ^{13}C NMR (100 MHz, acetone-d_6) δ **5a**, 195.9 (C, C-4), 164.0 (C, C-7), 161.1 (C, C-5), 158.5 (C, C-8a), 103.5 (C, C-6), 101.9 (C, C-4a), 100.9 (C, C-2), 95.1 (CH, C-8), 27.5 (CH_3, C-9), 6.3 (CH_3, C-10), **5b**, 196.3 (C, C-4), 164.3 (C, C-7), 161.4 (C, C-5), 157.9 (C, 8a), 103.7 (C, C-8), 102.1 (C, C-4a), 100.8 (C, C-2), 95.1 (CH, C-6), 27.6 (CH_3, C-9), 7.1 (CH_3, C-10); HRMS (ESI-TOF) *m/z* 247.0578 $[M + Na]^+$ (calcd for $C_{11}H_{12}O_5Na$, 247.0577).

*4.3.6. Compound **6***

Pale yellow solid; UV (MeOH) λ_{max} (log ε) 240 (3.83), 265 (3.86), 300 (3.82) nm; IR (ATR) ν_{max} 3399, 1666, 1615, 1588, 1433, 1360, 1286, 1209 cm^{-1}; ^{1}H NMR (400 MHz, acetone-d_6) δ 10.35 (1H, s, H-10), 6.34 (1H, s, H-3), 6.21 (1H, s, H-6), 2.53 (3H, s, H-9); ^{13}C NMR (100 MHz, acetone-d_6) δ 191.1 (CH, C-10), 182.1 (C, C-4), 169.2 (C) and 168.6 (C) (C-5 and C-7), 168.4 (C, C-2), 160.3 (C, C-8a), 109.8 (CH, C-3), 104.3 (C, C-4a), 98.9 (CH, C-6), 19.4 (CH_3, C-9); HRMS (ESI-TOF) *m/z* 243.0277 $[M + Na]^+$ (calcd for $C_{11}H_8O_5Na$, 243.0264).

4.4. Hydrolysis of 1.

Compound **1** (20.5 mg) was hydrolyzed in 5% aqueous hydrochloric acid (4 mL) at 90 °C for 15 h. The mixture was washed with EtOAc (2 × 3 mL), and the aqueous layer was concentrated under reduced pressure and purified by column chromatography on Sephadex LH-20 (MeOH) to furnish an anomeric mixture of 4-*O*-methyl-D-glucopyranose (5.9 mg, ^{1}H NMR in D_2O–acetone-d_6); $[\alpha]^{26}_D$ +71 (*c* 0.30, MeOH). The EtOAc layer was concentrated in vacuo to give a yellow gum (12.7 mg),

whose ^{1}H NMR spectrum ($CDCl_3$) indicated that compound **2** was the major component.

4.5. Hydrolysis of 4.

The same acid hydrolysis procedure as described above for **1** was applied to **4** (20.0 mg). The aqueous layer was concentrated in vacuo to yield 4-*O*-methyl-D-glucopyranose (9.5 mg, ^{1}H NMR in D_2O–acetone-d_6); $[\alpha]^{27}_{D}$ +93 (*c* 0.48, MeOH). The EtOAc layer (11.3 mg) was identified as isoeugenitol (^{1}H NMR in $CDCl_3$).

4.6. Synthesis of 9.

A mixture of compound **6** (1.6 mg), MeI (100 μL), and K_2CO_3 (30 mg) in DMSO-d_6 (0.5 ml) was stirred at room temperature for 15 hours. The mixture was diluted with EtOAc and washed with H_2O. The organic layer was concentrated in vacuo to obtain a yellow gum, which was purified by preparative HPLC (MeCN/H_2O) to furnish the 5,7-*O*-dimethyl derivative **9** (0.8 mg).

*4.6.1. Compound **9***

Pale yellow solid; ^{1}H NMR (400 MHz, acetone-d_6) δ 10.45 (1H, s, H-10), 6.72 (1H, s, H-6), 5.99 (1H, s, H-3), 4.09 (3H, s) and 4.02 (3H, s) (5-OCH_3 and 7-OCH_3), 2.32 (3H, s, H-9); ^{13}C NMR (100 MHz, acetone-d_6) δ 185.4 (CH, C-10), 175.6 (C, C-4), 167.1 (C) and 166.5 (C) (C-5 and C-7), 164.1 (C, C-2), 160.8 (C, C-8a), 112.8 (CH, C-3), 109.3 (C, C-4a), 92.6 (CH, C-6), 57.1 (CH_3) and 56.9 (CH_3) (5-OCH_3 and 7-OCH_3),, 19.4 (CH_3, C-9); HRMS (ESI-TOF) *m/z* 271.0578 $[M + Na]^+$ (calcd for $C_{13}H_{12}O_5Na$, 271.0577).

4.6. Biological assays

Cytotoxic activities against human cancer cell-lines, NCI-H187 cells (small-cell lung cancer), MCF-7 cells (breast cancer), and KB cells (oral cavity cancer), were evaluated using the resazurin microplate assay.[15] Cytotoxicity to Vero cells (African green monkey kidney fibroblasts) was performed using the green fluorescent protein microplate assay (GFPMA).[16] Assay for activity against *Plasmodium falciparum* (K1, multi-drug resistant strain) was performed in duplicate using the microculture radioisotope technique.[17]

Acknowledgments

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Supplementary data

NMR spectra of compounds **1**–**4**, **5a/5b**, **6**, and **9**. Supplementary data associated with this article can be found in the online version, at doi:.

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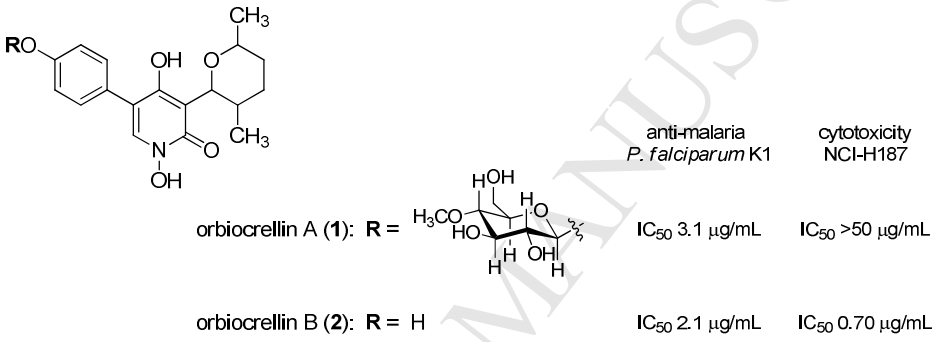
Graphical Abstract

N-Hydroxypyridone alkaloids, chromone derivatives, and tetrahydroxanthones from the scale-insect pathogenic fungus *Orbiocrella* sp. BCC 33248

Masahiko Isaka*, Rachada Haritakun, Sumalee Supothina, Wilunda Choowong, Suchada Mongkolsamrit



Supporting Information for:

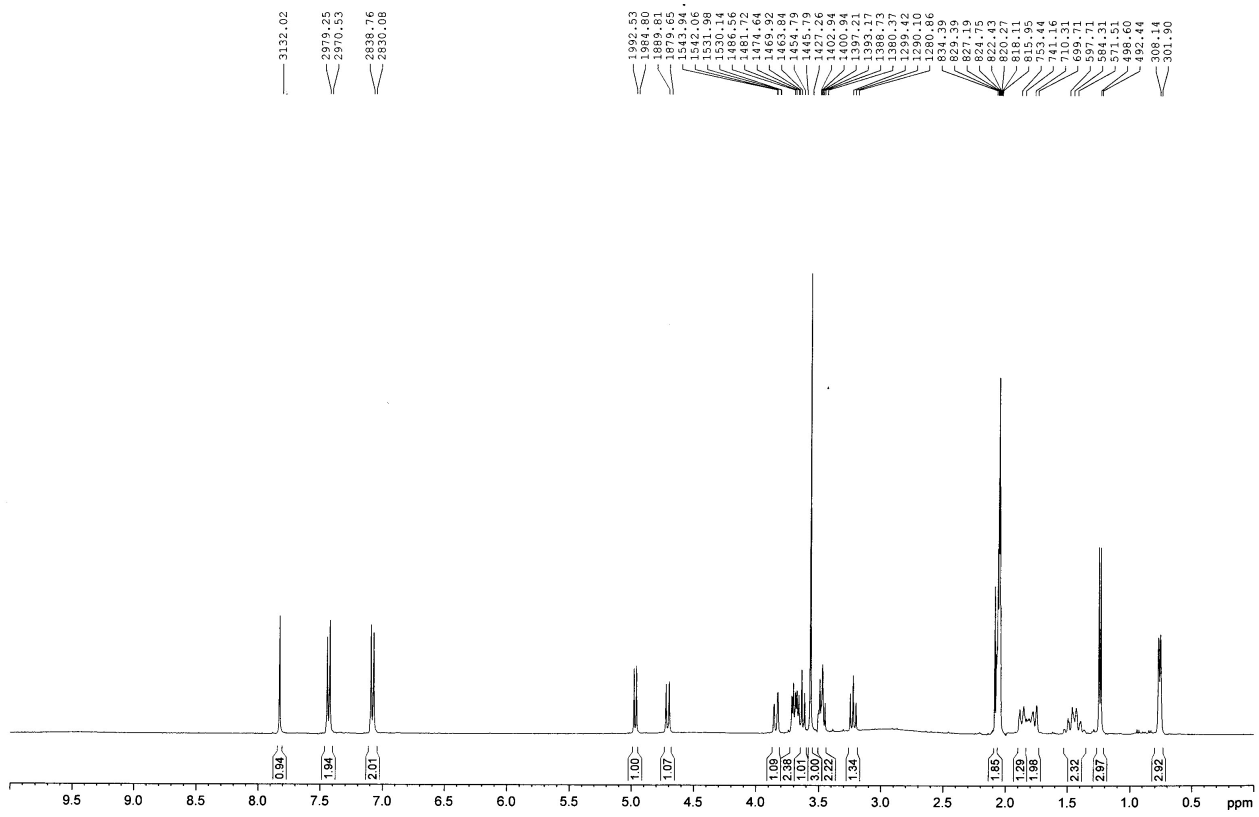
N-Hydroxypyridone alkaloids, chromone derivatives, and tetrahydroxanthones from the scale-insect pathogenic fungus *Orbiocrella* sp. BCC 33248

Masahiko Isaka*, Rachada Haritakun, Sumalee Supothina, Wilunda Choowong, Suchada Mongkolsamrit

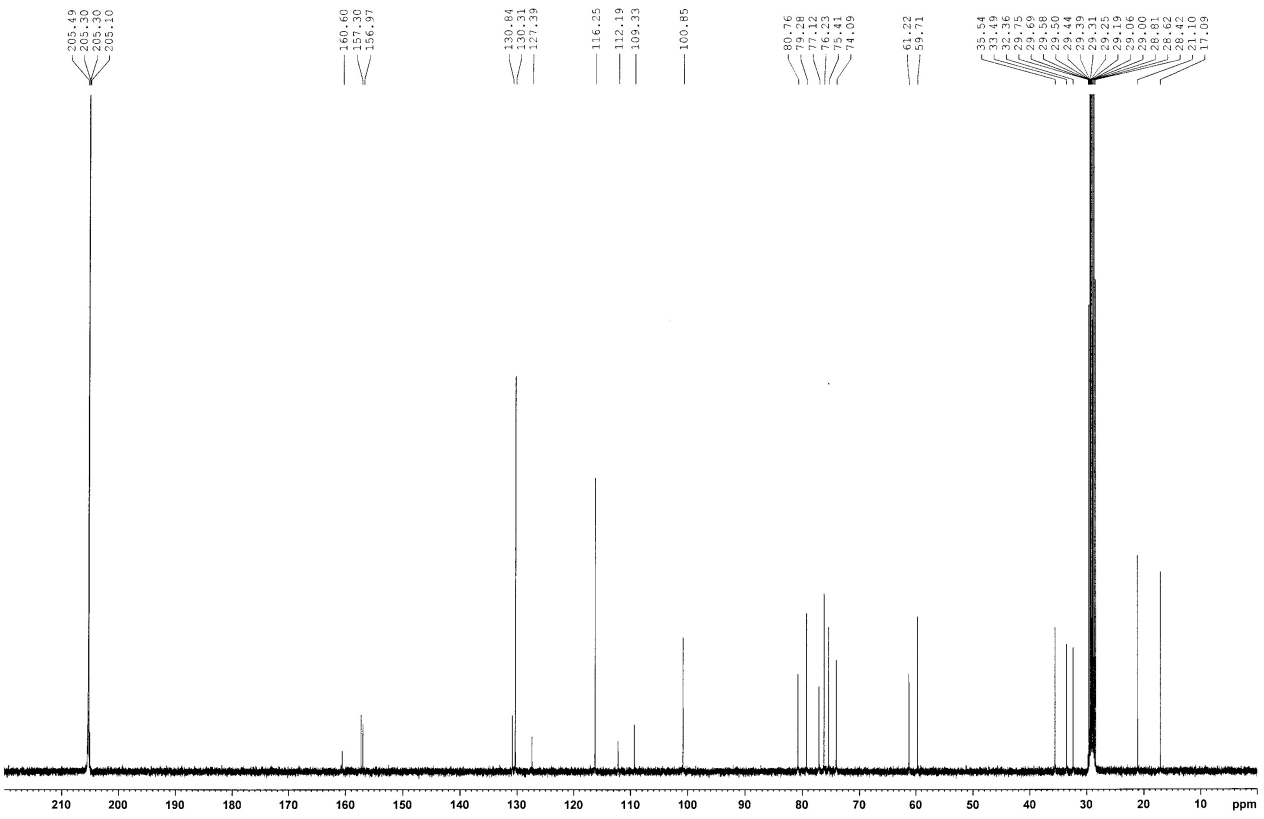
Contents

NMR Spectra

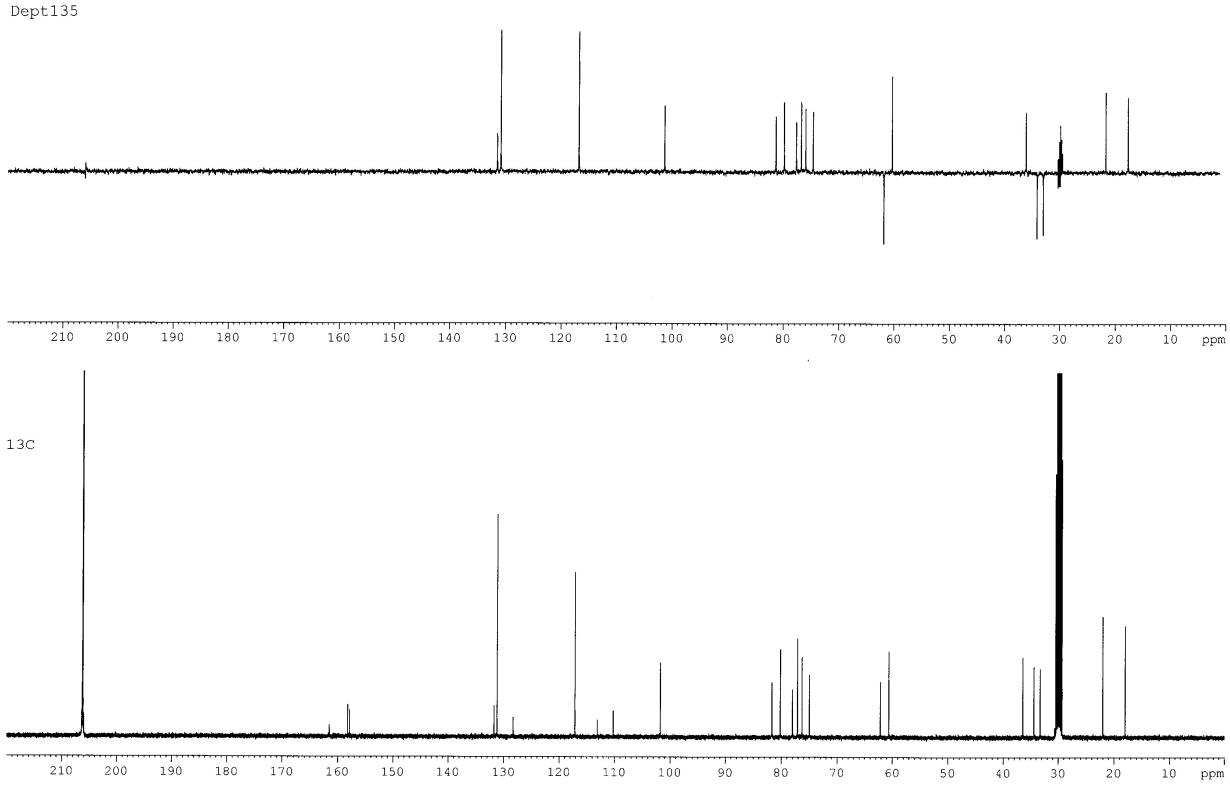
S1 ^{1}H NMR spectrum of orbiocrellin A (**1**) (acetone-d_6, 400 MHz)



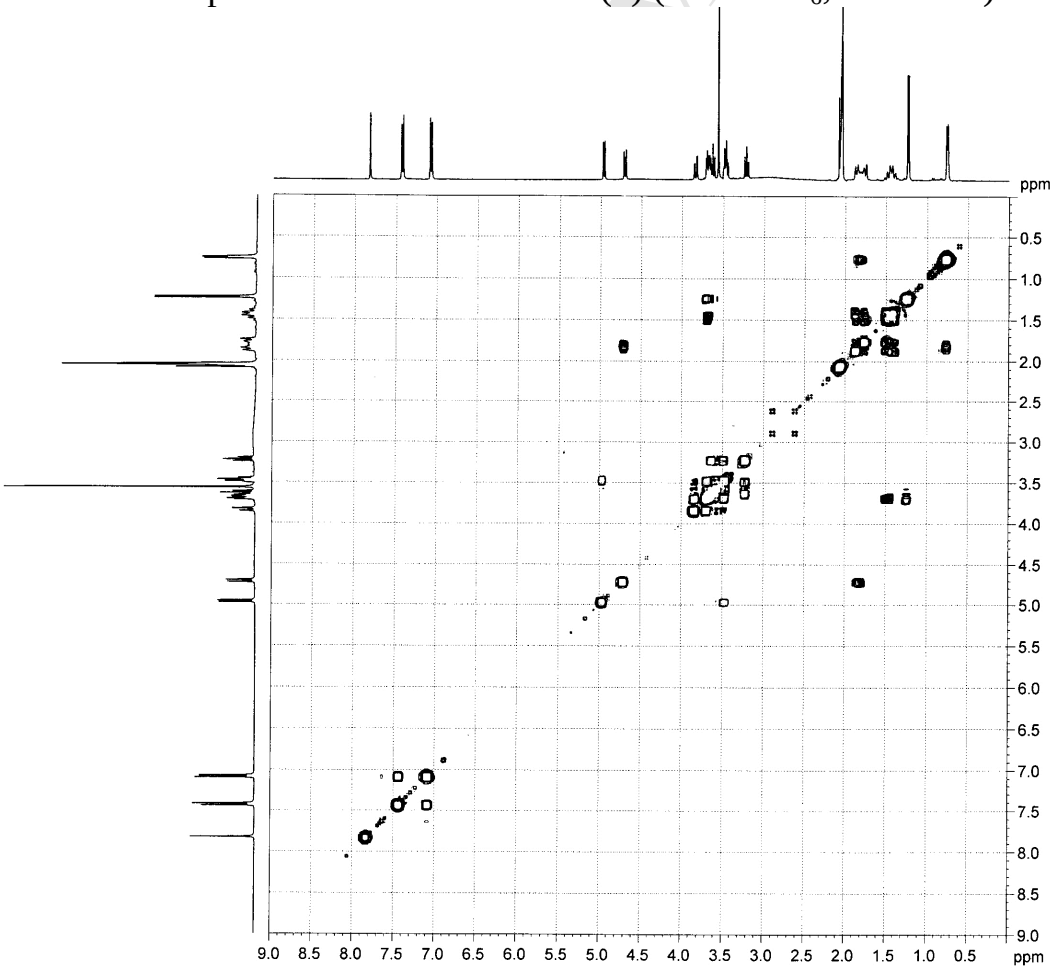
S2 ^{13}C NMR spectrum of orbiocrellin A (**1**) (acetone-d_6, 100 MHz)



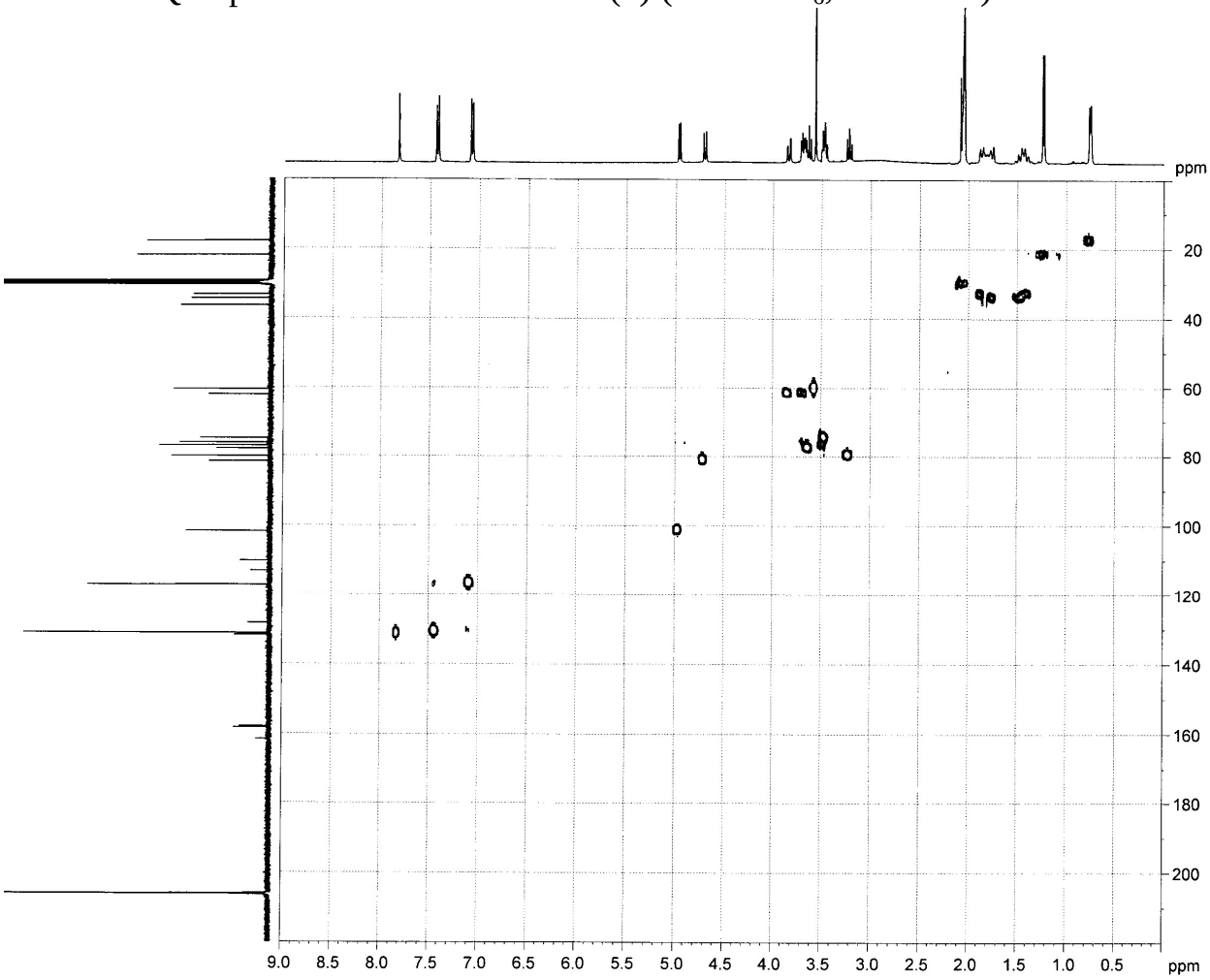
S3 DEPT135 spectrum of orbiocrellin A (**1**) (acetone-d_6, 100 MHz)



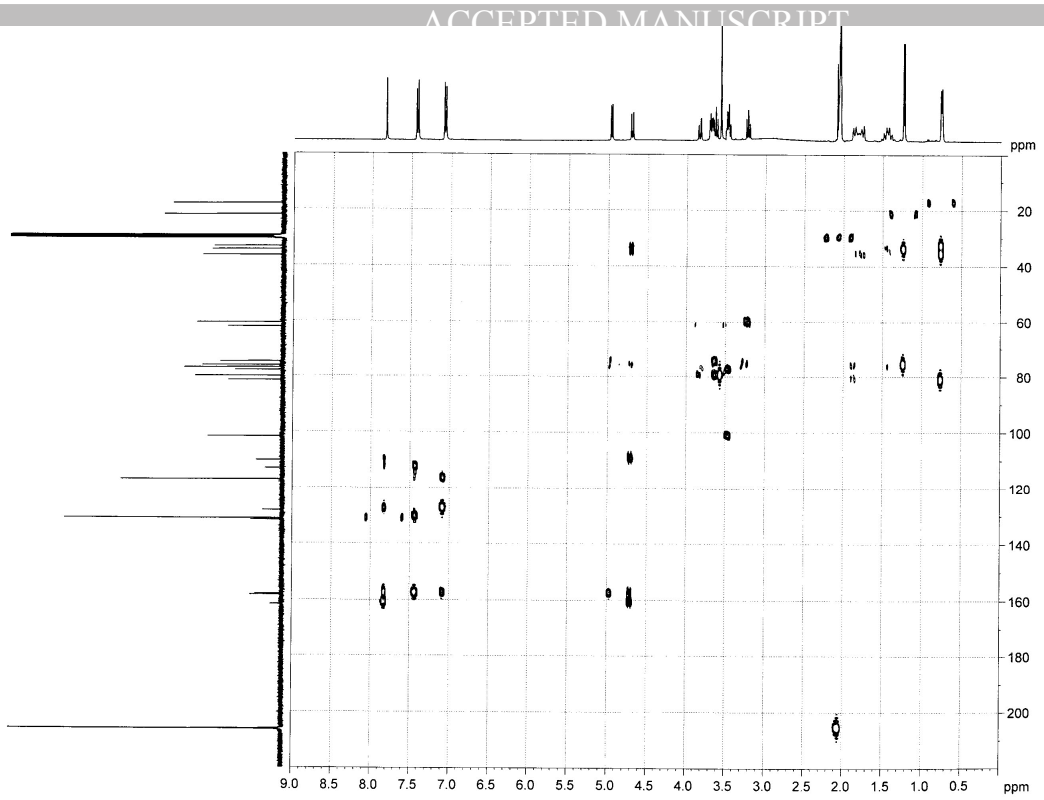
S4 COSY spectrum of orbiocrellin A (**1**) (acetone-d_6, 400 MHz)



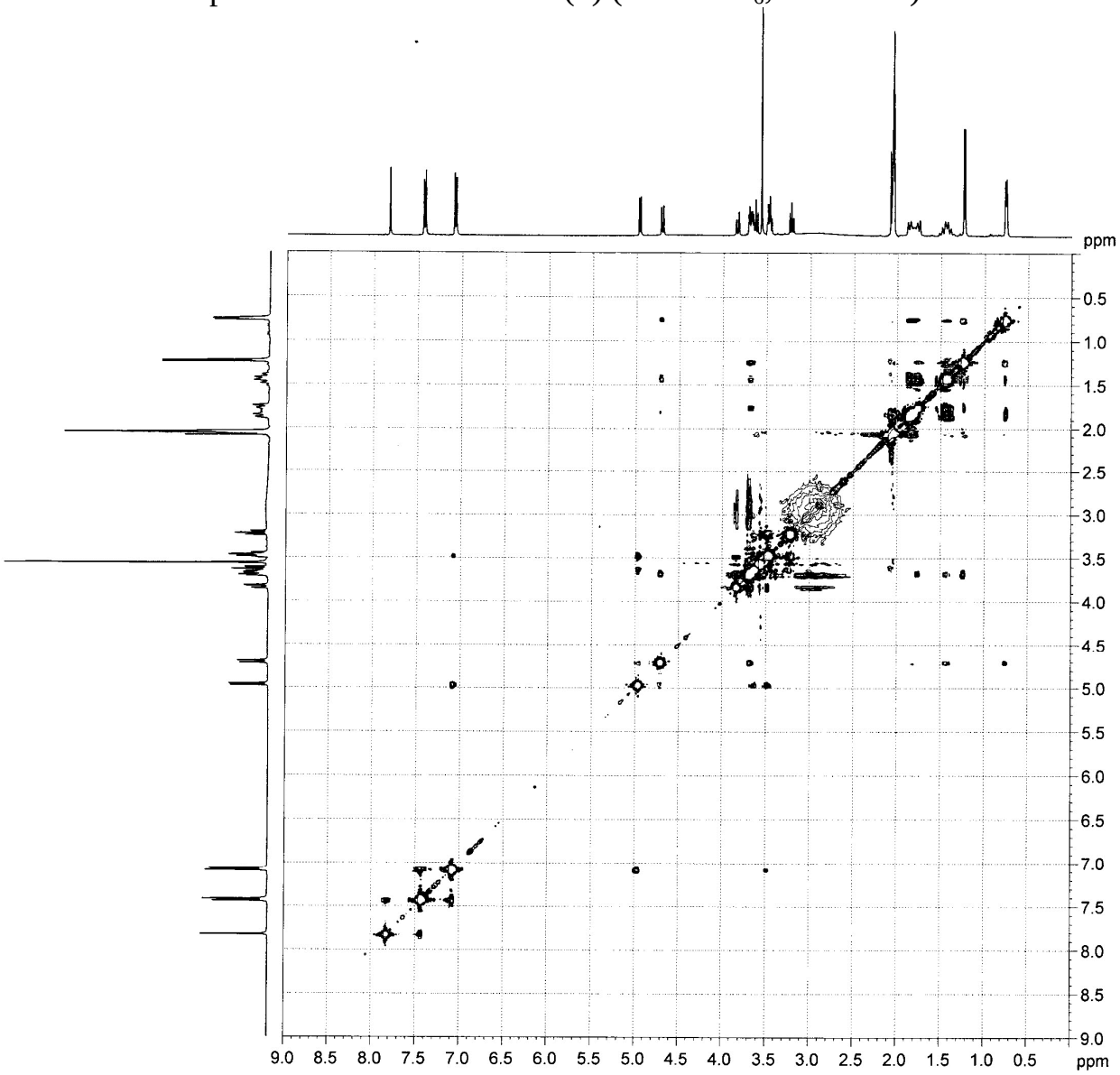
S5 HMQC spectrum of orbiocrellin A (**1**) (acetone-d_6, 400 MHz)



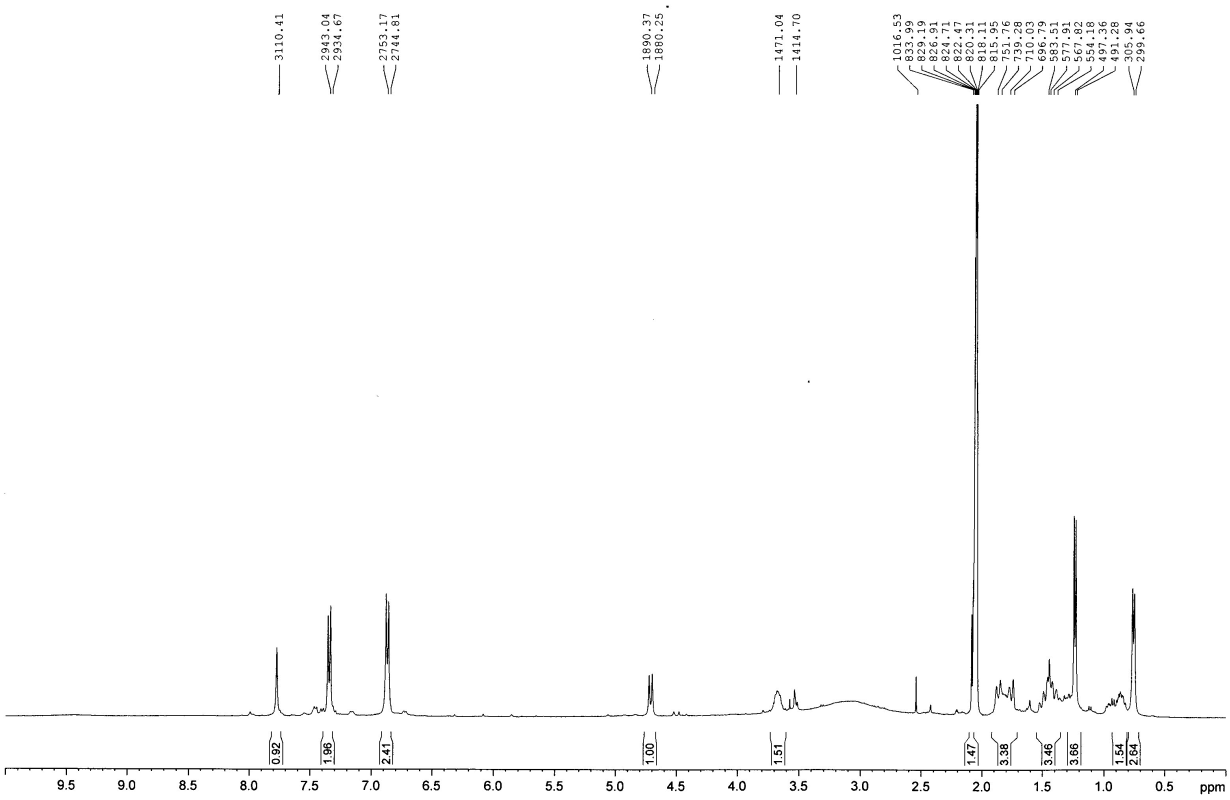
S6 HMBC spectrum of orbiocrellin A (**1**) (acetone-d_6, 400 MHz)



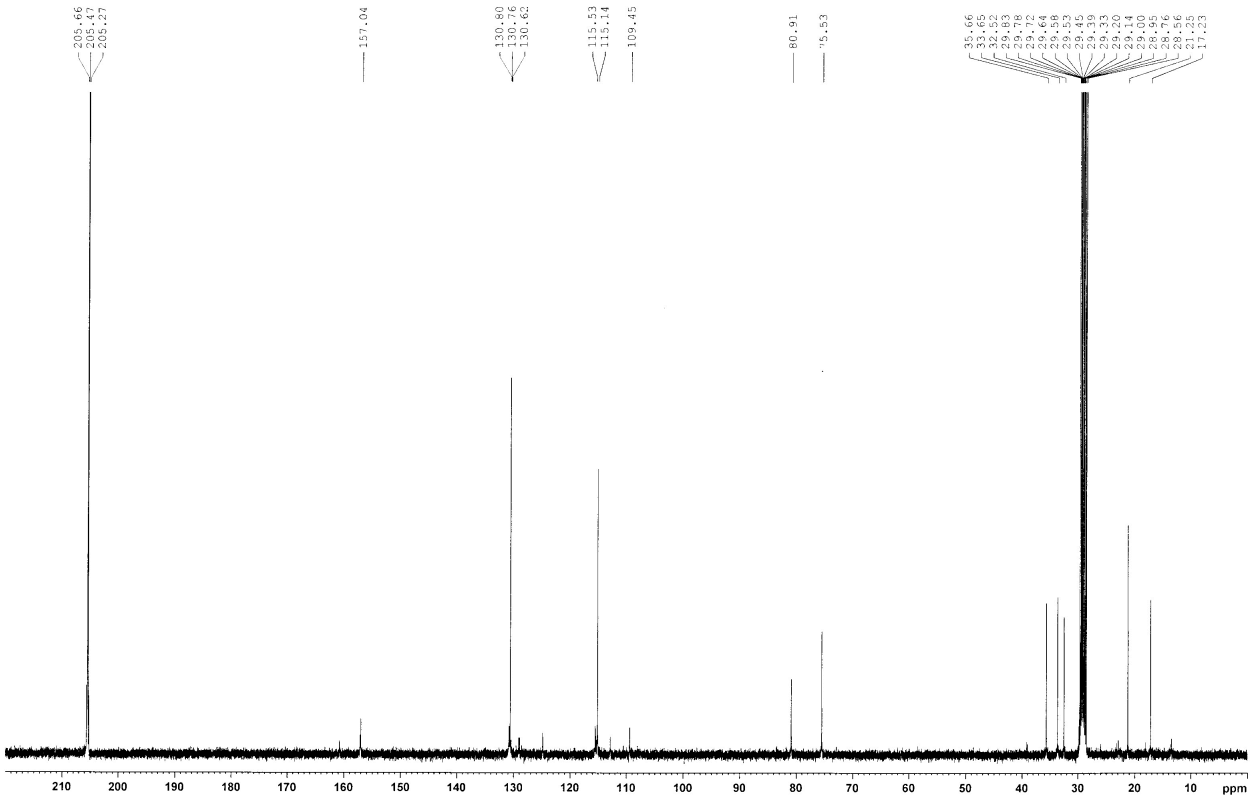
S7 NOESY spectrum of orbiocrellin A (**1**) (acetone-d_6, 400 MHz)



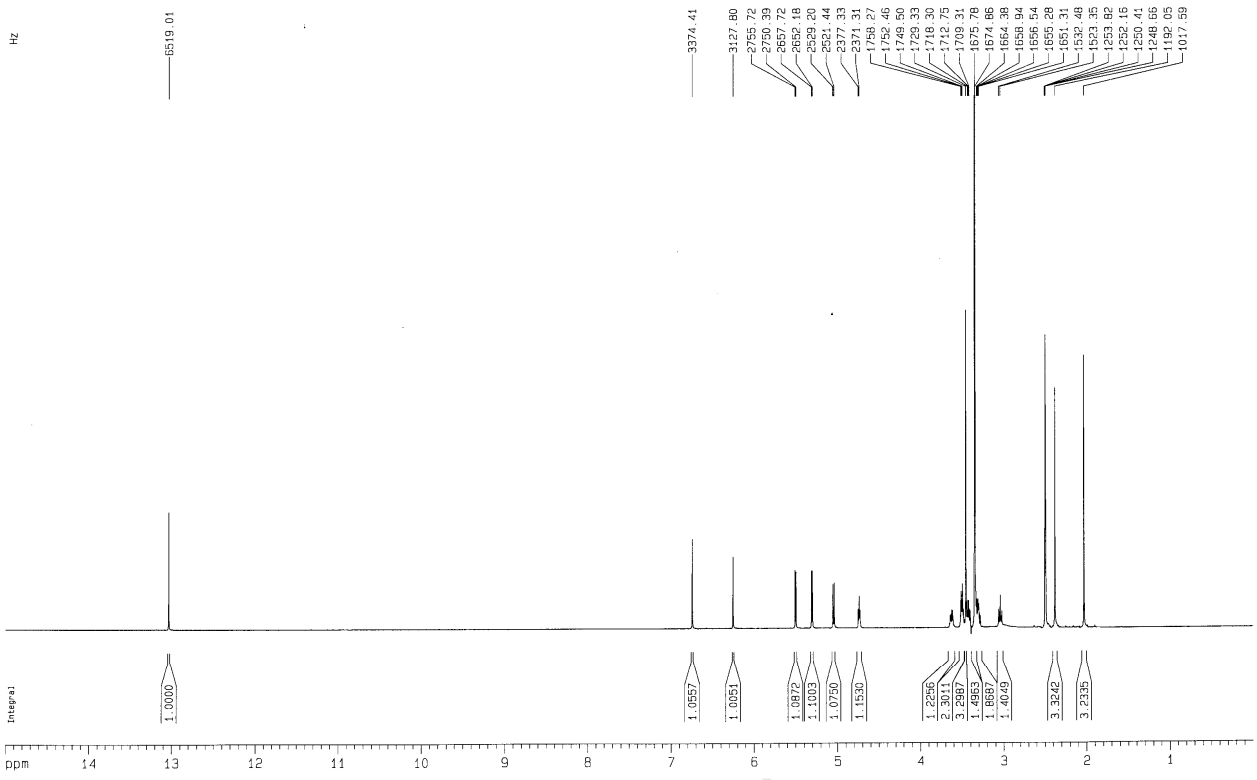
S8 ^{1}H NMR spectrum of orbiocrellin B (**2**) (acetone-d_6, 400 MHz)



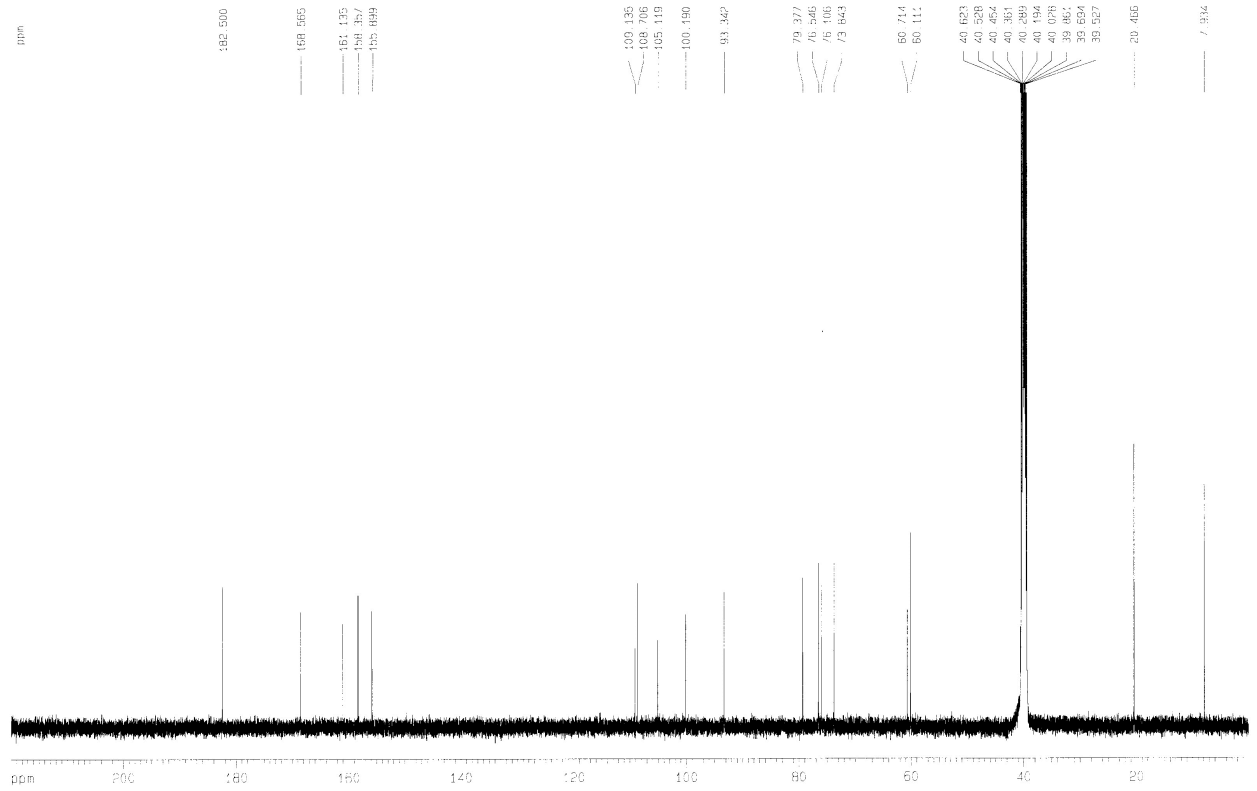
S9 ^{13}C NMR spectrum of orbiocrellin B (**2**) (acetone-d_6, 100 MHz)



S10 ^{1}H NMR spectrum of compound **3** (DMSO-d_6, 500 MHz)



S11 ^{13}C NMR spectrum of compound **3** (DMSO-d_6, 125 MHz)



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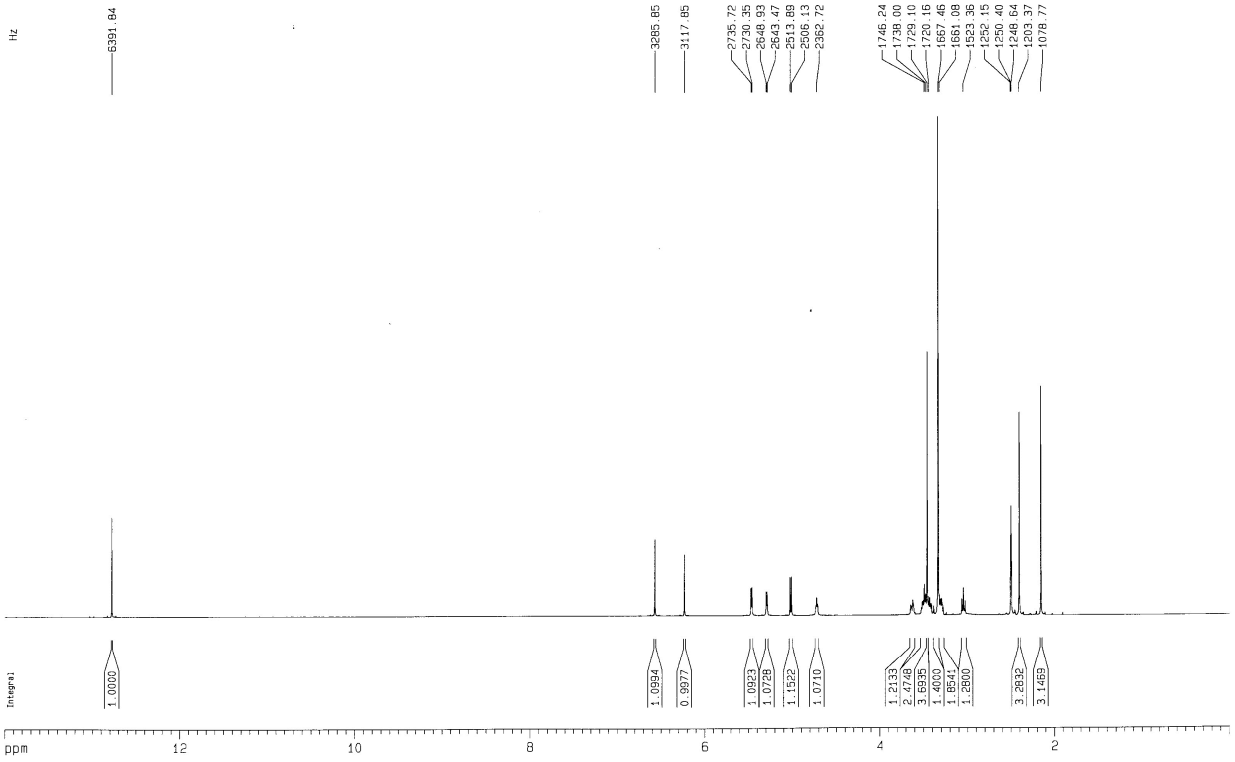
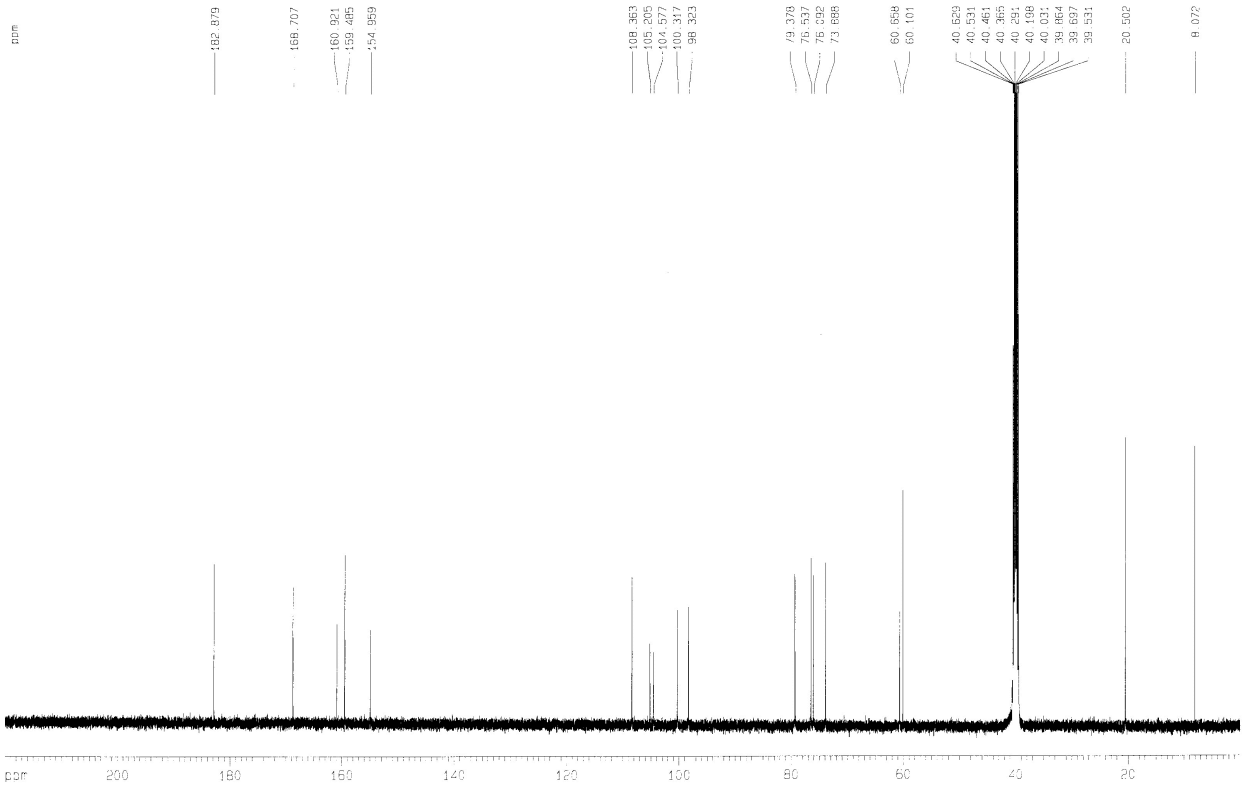
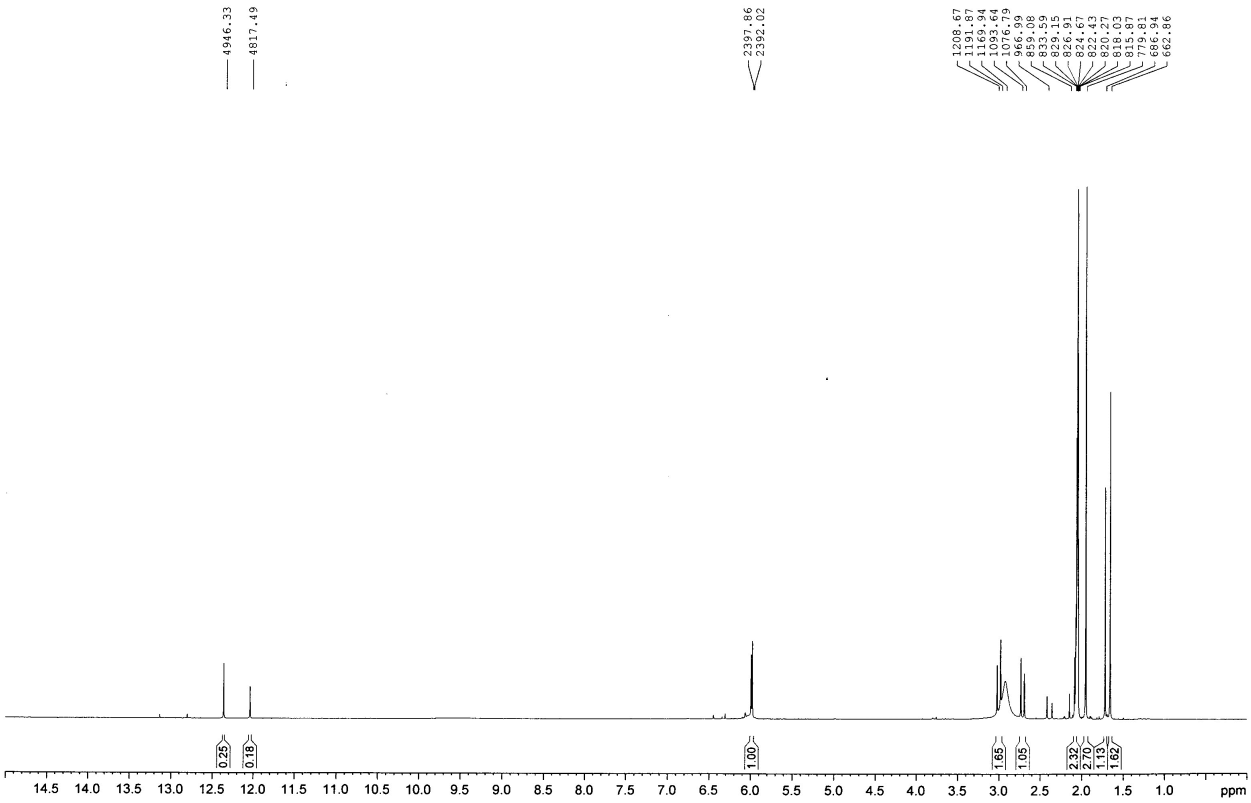


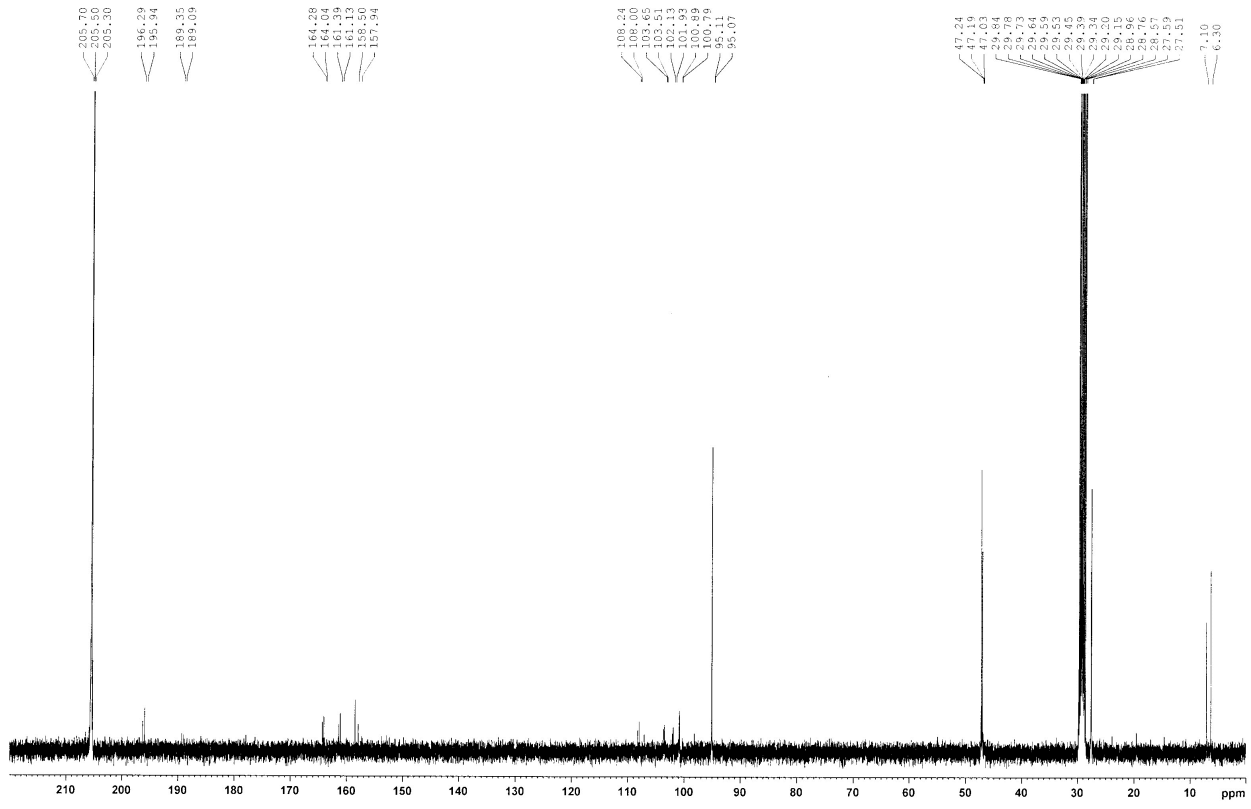
S13 ^{13}C NMR spectrum of compound **4** (DMSO-d_6, 125 MHz)



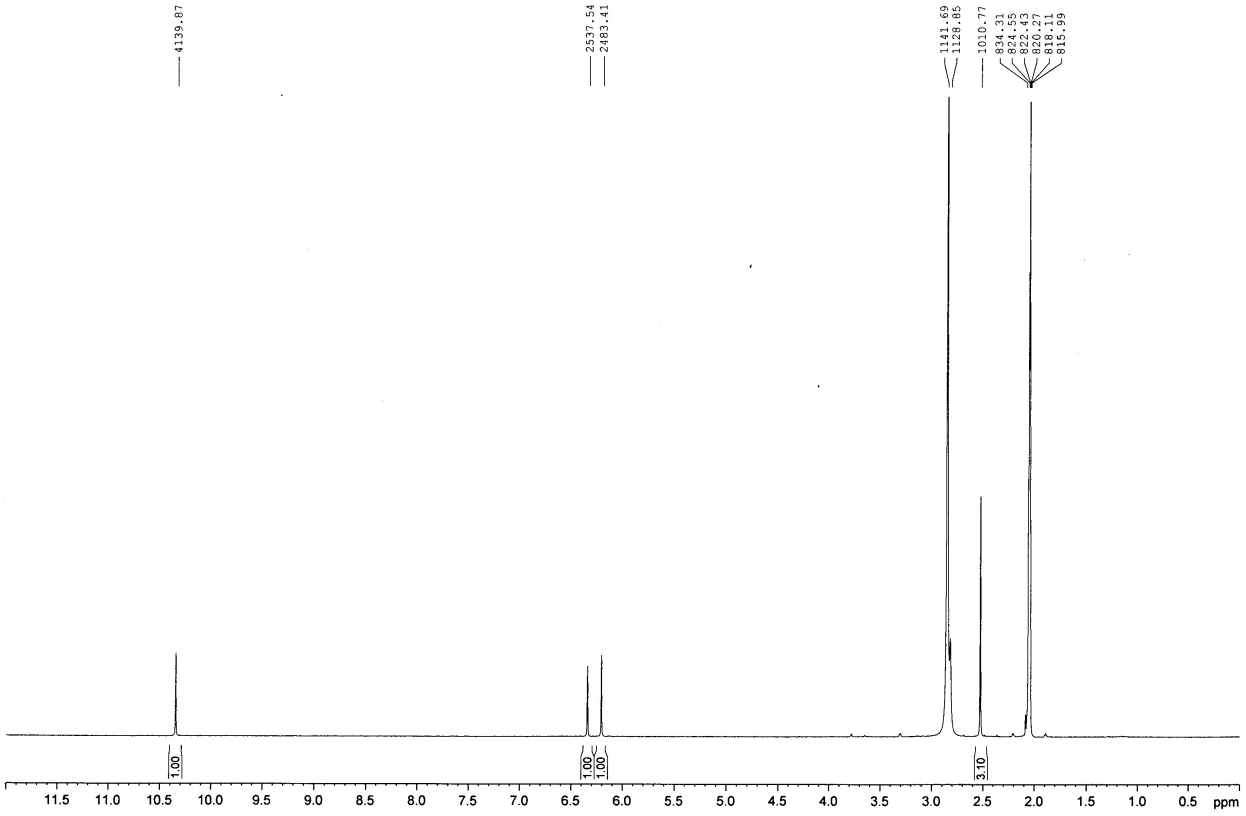
S14 ^{1}H NMR spectrum of compound **5a**/**5b** (acetone-d_6, 400 MHz)



S15 ^{13}C NMR spectrum of compound **5a**/**5b** (acetone-d_6, 100 MHz)



S16 ^{1}H NMR spectrum of compound **6** (acetone-d_6, 400 MHz)



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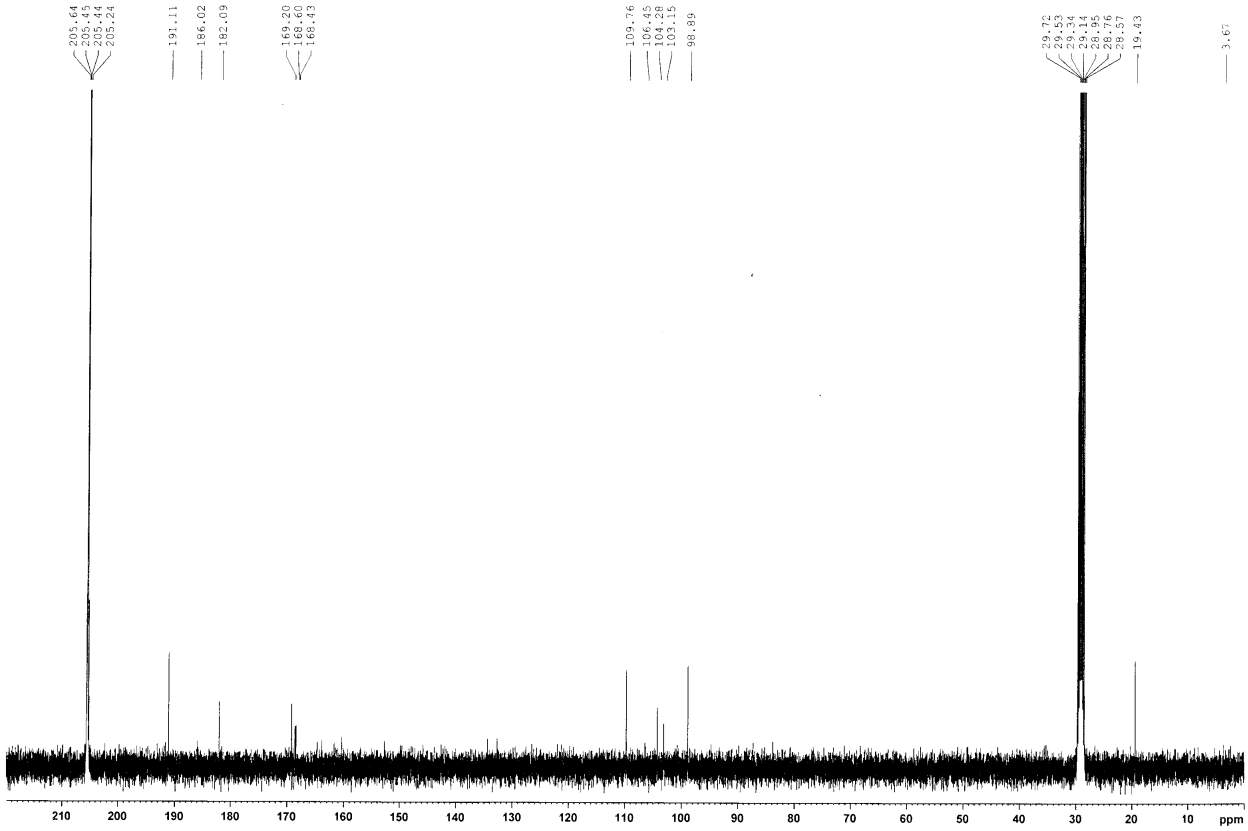

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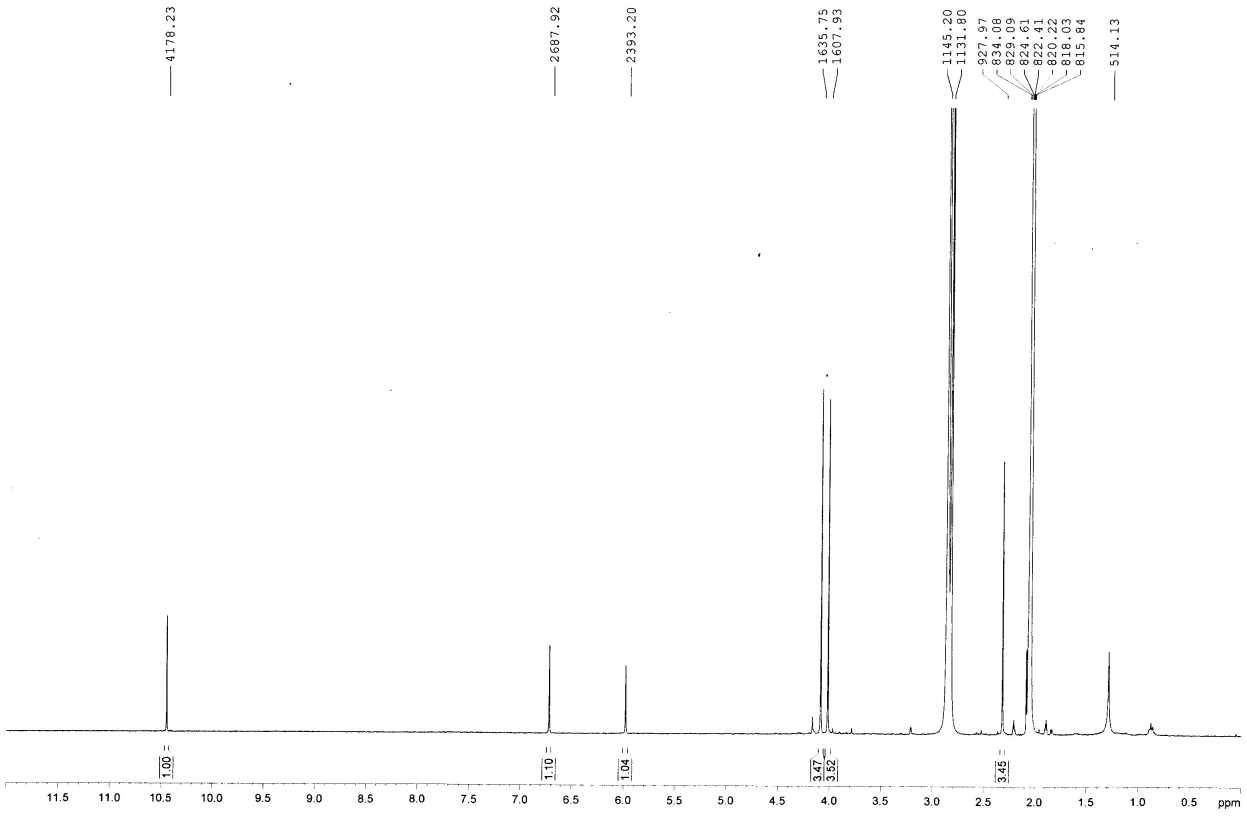

S19 ^{13}C NMR spectrum of compound **6** (acetone-d_6, 100 MHz)