

CHARACTERIZATION OF FLAVONOID DERIVATIVES FROM *BOESENBERGIA ROTUNDA* (L.)

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Keyword: *Boesenbergia rotunda*, *Boesenbergia pandurata*, Zingiberaceae, Flavonoids

Abstract

Three flavanones, pinostrobin (1), pinocembrin (2) and alpinetin (3) and two chalcones, cardamonin (4) and boesenbergin A (5) were isolated from the hexane and chloroform extracts of rhizomes of *Boesenbergia rotunda* (L.). The structures of all compounds were elucidated on the basis of spectroscopic data (IR, MS, and NMR) and 2-D NMR was carried out for the structure elucidation of boesenbergin A as a complementary to the spectroscopic data reported previously.

Abstrak

Tiga sebatian flavanones iaitu pinostrobin (1), pinocembrin (2), dan alpinetin (3) serta dua sebatian chalcones, cardamonin (4) dan boesenbergin A (5) telah diperolehi daripada pemencilan ekstrak heksana dan kloroform daripada rizom *Boesenbergia rotunda* (L.). Struktur-struktur bagi semua sebatian kimia dikenalpasti berdasarkan data spektroskopi (IR, MS dan NMR) dan 2-D NMR dijalankan untuk mengenalpasti struktur boesenbergin A sebagai maklumat pelengkap kepada data spektroskopi yang pernah dilaporkan dalam penyelidikan yang lepas.

Introduction

The Zingiberaceae is one of the largest families from the order Zingiberales, with approximately 50 genera and over 1,000 species. It is estimated about 150 species of ginger belonging to 23 genera found in Peninsular Malaysia [1]. Zingiberaceae species grow naturally in damp, shaded parts of the low-land or on hill slopes, as scattered plants or thickets. Most members of the family are easily recognized by the characteristic aromatic leaves and fleshy rhizome when both of them are crushed and also by the elliptic to elliptic-oblong leaves arranged in two ranks on the leaf-shoot. Various ginger rhizobia provide health-promoting effects and have been utilized to treat certain illnesses such as nausea, motion sickness, stomachache, asthma, diarrhea, digestive disorder, vomiting, rheumatism, swelling, common cold, cough and other disorders. Several studies have revealed that the members of the Zingiberaceae family consist of a wide variety of active phytochemicals and possess antioxidative, anti-inflammatory, anticancer and anti-tumour promoting activity [2].

Boesenbergia rotunda (L.) Mansf. Kulturpfl. (syn. *Boesenbergia pandurata* (Robx.) Schltr.), locally known in Thai as kra-chai, is a perennial herb belonging to the Zingiberaceae family. It is also known as 'temu kunci' in Malaysia. *Boesenbergia rotunda* is the most abundant *Boesenbergia* species in Malaysia. *Boesenbergia rotunda* is a small herbaceous plant with short, fleshy or slender rhizomes, one to a few leaves. The fresh rhizomes have a characteristic aroma and a slightly pungent taste. It is commonly used in Southeast Asia as a food ingredient, a folk medicine for the treatment of several diseases such as aphthous ulcer, dry mouth, stomach discomfort, leucorrhoea and dysentery. The rhizomes are given as tonics, to women in mixtures after childbirth; added into lotions for rheumatism and muscular pains, and into pastes for application to the body after confinement [3].

As regards its biological activities, *Boesenbergia rotunda* exhibits antimutagenic, antitumour, antibacterial, antifungal, analgesic, antipyretic, antispasmodic, anti-inflammatory and insecticidal activities [4]. Previous investigations have revealed the isolation of pinostrobin (1), pinocembrin (2), cardamonin (3), alpinetin (4) and boesenbergin A (5) from the rhizomes of *Boesenbergia rotunda* [5,6]. 2-D NMR technique was used for the structure elucidation of boesenbergin A to complement the data reported previously [5,7].

Experimental

General

Melting points (uncorrected) were determined on Kohfler melting points apparatus. Infrared spectra were recorded on Perkin Elmer FTIR model 1725X spectrophotometer; using potassium bromide (KBr) disc. MS was measured on equipped Shimadzu model QP5050A at 70 eV. ^1H and ^{13}C NMR spectra were recorded on JEOL FTNMR 400 and 100 MHz transformed spectrometer, respectively with tetramethylsilane (TMS) as internal standard. Chemical shifts are given in δ (ppm) values relative to that of the solvents used. Column chromatography was carried out using silica gel (Merck 7749) and Merck silica gel 60 PF₂₅₄ was used for TLC analysis.

Plant Material

The rhizomes of *Boesenbergia rotunda* were purchased from wholesale market in Puchong, Selangor Darul Ehsan.

Extraction and Isolation

1.0 kg finely ground air-dried rhizomes of *Boesenbergia rotunda* was three times extracted with hexane and chloroform sequentially for seventy two hours for each solvent at room temperature. The solvents were removed under reduced pressure to give 36.2 g of an orange coloured semi-solid of hexane extract and 42.6 g brown coloured semi-solid of chloroform extract. Around 34 g of hexane and chloroform extracts were subjected to flash column chromatography separation and eluted stepwise with various ratios of hexane, hexane/ethyl acetate, ethyl acetate, ethyl acetate/methanol and methanol to give 60 fractions for hexane extract and 70 fractions for chloroform extract.

Fractions 20-24 were obtained from separation of hexane extract were combined based on the same TLC profiles and evaporated, and the solid obtained was recrystallized from methanol to give colorless crystals of pinostrobin (1), $\text{C}_{16}\text{H}_{14}\text{O}_4$, m.p 96-98°C (lit. 100-101°C [5]). IR ν_{max} (cm^{-1} , KBr disc): 3444 (OH), 1646 (C=O), 1382, 1340, 1302, 1210, 1158 (C-O) 768, 742, 718. ^1H NMR (400 MHz, CDCl_3): δ 12.00 (1H, s, 5-OH), 7.41 (5H, m, H-2', H-3', H-5', H-6'), 6.05 (2H, d, $J = 2.72$ Hz, H-6, H-8), 5.39 (1H, dd, $J = 12.84, 2.76$ Hz, H-2), 3.79 (3H, s, 7-OMe), 3.06 (1H, dd, $J = 15.14, 12.84$ Hz, H-3), 2.79 (1H, dd, $J = 14.68, 2.76$ Hz, H-3). ^{13}C NMR (100 MHz, CDCl_3): 195.7 (C-4), 167.8 (C-7), 164.0 (C-9), 162.7 (C-5), 138.3 (C-1'), 128.8 (C-4'), 126.0 (C-2', C-3', C-5', C-6'), 103.0 (C-10), 95.0 (C-6), 94.1 (C-8), 79.0 (C-2), 55.6 (C-7-OMe), 43.2 (C-3). MS (m/z , rel. int.): m/z 270 ($[\text{M}]^+$, 100%), 269 ($[\text{M} - \text{H}]^+$, 67), 252 ($[\text{M} - \text{C}_6\text{H}_5]^+$, 8), 193 ($[\text{M} - \text{C}_6\text{H}_5]^+$, 99), 166 (68), 138 (41), 114 (14), 110 (18), 95 (34), 77 (20), 69 (19), 51 (19).

Fractions 29-35 yielded pinocembrin (2) as yellow needle-shaped crystals from column chromatography of chloroform extract, $\text{C}_{15}\text{H}_{12}\text{O}_4$, m.p 191-193°C (lit. 200-201°C [5]). IR ν_{max} (cm^{-1} , KBr disc): 3436 (OH), 3092, 1632 (C=O), 1584, 1488, 1358, 1168 (C-O). ^1H NMR (400 MHz, MeOD): δ 12.00 (1H, s, 5-OH, 7-OH), 7.34 (5H, m, H-3', H-4', H-5', H-6'), 5.87 (2H, d, $J = 1.84$ Hz, H-6, H-8), 5.33 (1H, dd, $J = 12.84, 3.68$ Hz, H-2), 2.98 (1H, dd, $J = 17.40, 12.88$ Hz, H-3), 2.67 (1H, dd, $J = 16.96, 3.68$ Hz, H-3). ^{13}C NMR (100 MHz, MeOD): 197.3 (C-4), 168.3 (C-7), 165.4 (C-5), 164.6 (C-9), 140.3 (C-1'), 129.7 (C-4'), 129.6 (C-3', C-5'), 127.3 (C-2', C-6'), 103.3 (C-10), 97.2 (C-8), 96.2 (C-6), 80.4 (C-2), 44.1 (C-3). MS (m/z , rel. int.): m/z 256 ($[\text{M}]^+$, 100%), 238 (14), 179 (92), 152 (77), 124 (52), 103 (18), 96 (20), 78 (29), 69 (31), 51 (16).

Fraction 59 gave alpinetin (3) as colorless needle-shaped crystals from chloroform extract, $\text{C}_{16}\text{H}_{14}\text{O}_4$, m.p 222-224°C (lit. 223-227°C [6]). IR ν_{max} (cm^{-1} , KBr disc): 3520 (OH), 3362, 3090, 1612 (C=O), 1578, 1436, 1362, 1108 (C-O), 1072, 702. ^1H NMR (400 MHz, MeOD): δ 7.32 (5H, m, H-2', H-3', H-4', H-5', H-6'), 6.01 (1H, d, $J = 1.84$ Hz), 5.96 (1H, d, $J = 2.76$ Hz, H-6), 5.33 (1H, dd, $J = 10.08, 2.80$ Hz, H-2), 3.74 (3H, s, 5-OMe), 2.90 (1H, dd, $J = 12.84, 4.60$ Hz, H-3), 2.63 (1H, dd, $J = 12.84, 3.68$ Hz, H-3). ^{13}C NMR (100 MHz, MeOD): 191.8 (C-4), 167.2 (C-7), 166.6 (C-5), 164.3 (C-9), 140.6 (C-1'), 129.7 (C-3', C-5'), 129.5 (H-4'), 127.3 (C-2', C-6'), 105.7 (C-10), 97.2 (C-6), 94.3 (C-8), 80.2 (C-2), 56.2 (C-5-OMe), 46.4 (C-3). MS (m/z , rel. int.): m/z 270 ($[\text{M}]^+$, 51%), 269 ($[\text{M} - \text{H}]^+$, 14), 252 ($[\text{M} - \text{C}_6\text{H}_5]^+$, 1), 242 (2), 193 ($[\text{M} - \text{C}_6\text{H}_5]^+$, 22), 166 (100), 138 (51), 104 (15), 95 (16).

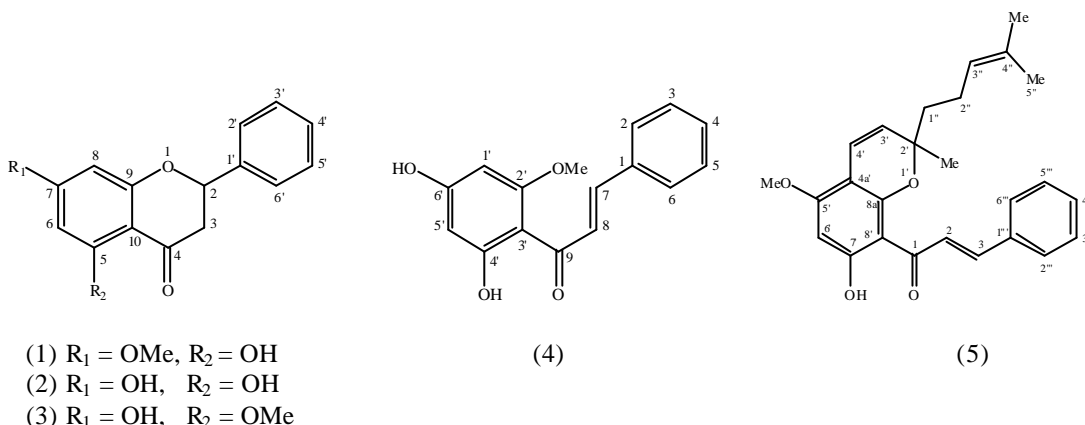
Fraction 41 yielded yellow needle-shaped crystals of cardamonin (4) from the column chromatography of chloroform extract, $\text{C}_{16}\text{H}_{14}\text{O}_4$, m.p 186-188°C (lit. 199-200°C [5]). IR ν_{max} (cm^{-1} , KBr disc): 3154 (OH), 1628 (C=O), 1542, 1486, 1286, 1320, 1224, 1188, 1114, 926. ^1H NMR (400 MHz, MeOD): δ 7.84 (1H, d, $J = 15.6$ Hz, H-7), 7.59 (1H, d, $J = 15.6$ Hz, H-8), 7.43 (5H, m, H-2, H-3, H-4, H-5, H-6), 5.93 (1H, d, $J = 2.76$ Hz, H-

1'), 5.85 (1H, *d*, *J* = 2.72 Hz, H-5'), 4.54 (1H, *s*, 4'-OH, 6'-OH), 3.84 (3H, *s*, 2'-OMe). ¹³C NMR (100 MHz, MeOD): 193.9 (C-9), 168.8 (C-2'), 167.0 (C-4'), 164.8 (C-6'), 142.9 (C-8), 137.0 (C-1), 131.2 (C-4), 130.1 (C-3, C-5), 129.3 (C-2, C-6), 128.9 (C-7), 106.6 (C-3'), 97.1 (C-5'), 92.6 (C-1'), 56.3 (C-2'-OMe). MS (*m/z*, rel. int.): *m/z* 270 ([M⁺], 52%), 269 (57), 253 (9), 193 (100), 131 (7), 103 (22), 77 (31).

Work-up procedure on fraction 19 of hexane extract yielded boesenbergin A (5) as orange needle shaped crystals, C₂₆H₂₈O₄, m.p 87-89°C (lit. 89-91°C [5]). Boesenbergin A was initially obtained as orange coloured oil and orange needle-shaped crystals was obtained after recrystallization with methanol. IR ν_{\max} (cm⁻¹, KBr disc): 3442 (OH), 2962, 1636 (C=O), 1590, 1546, 1446, 1344, 1152 (C-O). ¹H NMR (400 MHz, MeOD): *d* 14.23 (1H, *s*, 7'-OH), 8.18 (1H, *d*, *J* = 15.60 Hz, H-2), 7.78 (1H, *d*, *J* = 8.00 Hz, H-3), 7.59 (2H, *m*, H-2''', H-6'''), 7.39 (3H, *m*, H-3''', H-4''', H-5'''), 6.62 (1H, *d*, *J* = 10.12 Hz, H-4'), 6.05 (1H, *s*, H-6'), 5.42 (1H, *d*, *J* = 10.12 Hz, H-3'), 5.08 (1H, *t*, *J* = 6.40 Hz, H-3''), 3.86 (3H, *s*, 5'-OMe), 1.72-2.23 (4H, *m*, H-1'', H-2''), 1.63 (3H, *s*, 2'-Me), 1.46 (6H, *d*, *J* = 7.32 Hz, 4''-Me, H-5''). ¹³C NMR (100 MHz, MeOD): 192.8 (C-1), 167.4 (C-5'), 161.2 (C-8a'), 155.9 (C-7'), 142.2 (C-3), 135.6 (C-1'''), 132.1 (C-4''), 130.0 (C-4'''), 128.9 (C-3''', C-5'''), 128.3 (C-2''', C-6'''), 127.5 (C-2), 123.6 (C-3''), 123.4 (C-3'), 117.2 (C-4'), 106.1 (C-8'), 102.8 (C-4a'), 92.5 (C-6'), 77.3 (C-2'), 55.7 (C-5'-OMe), 41.4 (C-1''), 26.6 (C-2'-Me), 25.6 (C-4''-Me), 23.0 (C-2''), 17.5 (C-5''). MS (*m/z*, rel. int.): *m/z* 404 ([M⁺], 20%), 389 ([M - CH₃]⁺, 4), 321 ([M - C₆H₅]⁺, 100), 217 (99), 77 (10), 55 (6).

Results and Discussion

The extraction and separation on the hexane and chloroform extracts of *Boesenbergia rotunda* have led to the isolation and characterization of five flavonoids, which were identified and characterized as pinostrobin (1), pinocembrin (2), alpinetin (3), cardamonin (4) and boesenbergin A (5). The compounds were identified by comparison of their spectral data with those reported in the literature. As for the isolated compound of boesenbergin A, the high field NMR study including HMQC, HMBC and COSY correlation techniques was therefore undertaken to complement the published NMR data, with respect to the assignment of the peaks.



Compound 5 was obtained as orange needle-shaped crystal and analyzed as C₂₆H₂₈O₄. The EI-MS of compound 5 indicated the presence of molecular ion peak at *m/z* 404 with the base peak of at *m/z* 321. The IR spectrum displayed a strong absorption band at 1636 cm⁻¹ which was characterized for a conjugated ketone with intramolecular hydrogen bonding and a C-O stretching band at 1152 cm⁻¹. The IR spectra suggested compound 5 to be a chalcone derivative.

Furthermore, in the ¹H NMR spectrum of compound 5, a sharp singlet at *d* 14.23 ppm was observed due to a strong hydrogen-bonded hydroxyl group. An AB system (*J* = 15.60 Hz) at *d* 7.78 ppm and *d* 8.18 ppm were assigned to the (*E*)-vinylic protons of a chalcone moiety which are assigned to H-2 and H-3. Two multiplets at *d* 7.37 ppm and *d* 7.60 ppm indicates the protons on a monosubstituted benzene ring (H-2''', H-3''', H-4''', H-5''', H-6'''). The AB pattern at *d* 5.42 ppm and *d* 6.62 ppm (*J* = 10.12 Hz) suggested the presence of a vicinal coupling in a chromene ring (H-3', H-4'). A singlet at 6.05 ppm was assigned to H-6' which is on the second aromatic ring. An olefinic proton at *d* 5.08 ppm appeared as triplet with *J* = 6.40 Hz. The peak of a singlet at *d* 3.86 ppm indicates the presence of methoxyl protons (5'-OMe). Two methylene (H-1'', H-2'') and three methyl groups (4''-Me, H-5'', 2'-Me) were presents at *d* 1.72-2.23 ppm as multiplets and at *d* 1.63 ppm (singlet) and *d* 1.46 ppm (doublet, *J* = 7.32 Hz) respectively.

The ^{13}C NMR and DEPT spectrum indicated the presence of 26 carbons as 12 aliphatic carbons (3Me, 2CH₂, 1C, 1C=CH-, 2-CH=CH), 12 aromatic carbons (6CH, 4C, 2C-O), one carbonyl and one methoxyl carbon. From the previous report [7], 4 signals in ^{13}C NMR had been assigned incorrectly. The signals were indicated as C-2 at δ 123.4 ppm, C-3' at δ 117.2 ppm, C-4' at δ 130.0 ppm and C-4''' at δ 127.5 ppm. In this report, 2-D NMR (HMQC, HMBC, DEPT and COSY) was carried out for the re-assignment of 4 signals in ^{13}C NMR for compound 5 to complement the previous report. The correlations observed in HMQC and HMBC spectrum (Figure 1 and 2) confirmed the signals of C-2 present at δ 127.5 ppm, C-3' present at δ 123.4 ppm, C-4' present at δ 117.2 ppm and C-4''' at δ 130.0 ppm. The correlations observed in COSY and HMBC spectra linked all the connectivities in the structure (Table 1). The structure of compound 5 was established on the basis of its spectral data, making use of DEPT, COSY, HMQC and HMBC experiments in combination with ^1H and ^{13}C NMR spectroscopic data.

Table 1: NMR spectral data for compound 5 (boesenbergin A)

Position	$\delta^1\text{H}$	$\delta^{13}\text{C}$	DEPT	HMQC correlations	HMBC correlations	COSY
1	-	192.7	C	-	H-2, H-3	-
2	8.18, <i>d</i> ($J=15.60$ Hz)	127.5	CH	H-2	-	H-3
3	7.78, <i>d</i> ($J=15.60$ Hz)	142.2	CH	H-3	H-2''', H-6'''	H-2
1'	-	-	-	-	-	-
2'	-	80.6	C	-	2'-Me, H-3', H-4', H-1''	-
2'-Me	1.63, <i>s</i>	26.6	CH ₃	H-2'-Me	H-5''	H-5''
3'	5.42, <i>d</i> ($J=10.12$ Hz)	123.4	CH	H-3'	2'-Me, H-5''	H-4'
4'	6.62, <i>d</i> ($J=10.12$ Hz)	117.2	CH	H-4'	-	H-3'
4a'	-	102.8	C	-	H-3', H-4', H-6'	-
5'	-	167.4	C	-	H-6', 7'-OH	-
5'-OMe	3.86, <i>s</i>	55.8	CH ₃	H-5'-OMe	-	-
6'	6.05, <i>s</i>	92.5	CH	H-6'	7'-OH	-
7'	-	155.9	C	-	H-4'	-
7'-OH	14.23, <i>s</i>	-	-	-	-	-
8'	-	106.1	C	-	H-6', 7'-OH	-
8a'	-	161.2	C	-	H-4', 5'-OMe, H-6', 7'-OH	-
1''	1.72-2.23, <i>m</i>	41.4	CH ₂	H-1''	2'-Me, H-5''	H-2''
2''	1.72-2.23, <i>m</i>	23.0	CH ₂	H-2''	H-1'', H-3''	H-1''
3''	5.08, <i>t</i> ($J=6.40$ Hz)	123.6	CH	H-3''	2'-Me, H-1'', H-2'', H-5''	-
4''	-	132.1	C	-	2'-Me, H-2'', H-5''	-
4''-Me	1.46, <i>d</i> ($J=7.32$ Hz)	25.6	CH ₃	-	2'-Me, H-3''	-
5''	1.46, <i>d</i> ($J=7.32$ Hz)	17.5	CH ₃	H-5''	H-3''	2'-Me
1'''	-	135.6	C	-	H-2, H-3, H-3''', H-4''', H-5'''	-
2'''	7.59, <i>m</i>	128.3	CH	H-2'''	H-3, H-3''', H-4''', H-5'''	H-3''', H-4''', H-5'''
3'''	7.39, <i>m</i>	128.9	CH	H-3'''	H-2''', H-6'''	H-2''', H-6'''
4'''	7.39, <i>m</i>	130.0	CH	H-4'''	H-2''', H-3''', H-5''', H-6'''	H-2''', H-6'''
5'''	7.39, <i>m</i>	128.9	CH	H-5'''	H-2''', H-6'''	H-2''', H-6'''
6'''	7.59, <i>m</i>	128.3	CH	H-6'''	H-3, H-3''', H-4''', H-5'''	H-3''', H-4''', H-5'''

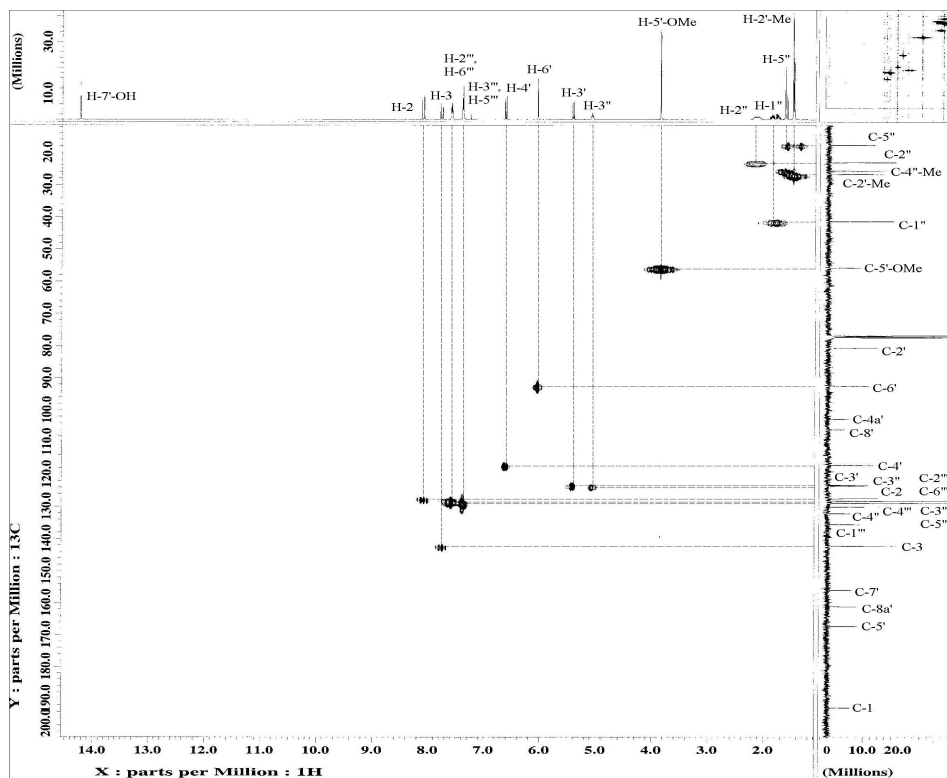


Figure 1: HMBC NMR spectrum of compound 5 (boesenbergin A)

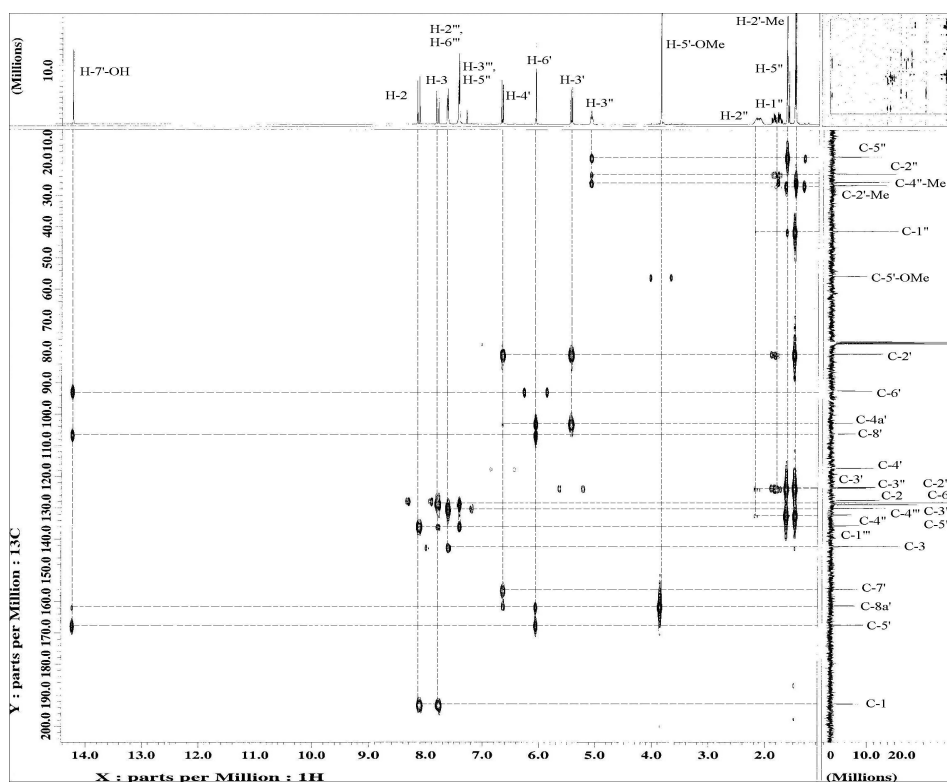


Figure 2: HMBC NMR spectrum of compound 5 (boesenbergin A)

Acknowledgement

We thank The Ministry of Science Technology and Innovation for the fund under Intensified Research in Priority Area (IRPA) Program

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