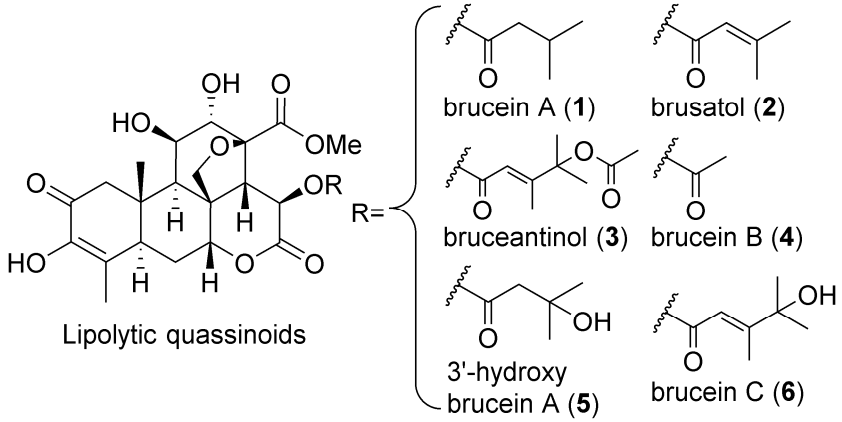
*Post-print manuscript

Graphical abstract



Research paper

Quassinoids in *Brucea javanica* are potent stimulators of lipolysis in adipocytes

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Abstract

Obesity is associated with a number of metabolic disorders. Lipolysis is the initial step in the metabolism of lipids stored in adipocytes and is therefore considered a therapeutic target for obesity. Quassinoids are unique terpenes found in plants of the Simaroubaceae family, which were recently reported to have lipolytic activity and to suppress weight gain. *Brucea javanica* is a plant employed in traditional medicines in Asia, which is known to contain various quassinoids. Here, we investigated the lipolytic activity of *B. javanica* extracts, and identified six quassinoids: brucein A, brucein B, brucein C, 3'-hydroxybrucein A, brusatol, and bruceantinol, which represent the bioactive principals. The quassinoids contained in *B. javanica* demonstrated lipolytic activity at nanomolar concentrations, which were an order of magnitude lower than those of the previously reported quassinoids, suggesting that they may be useful for the treatment of obesity.

Keywords

lipolysis, obesity, adipocyte, quassinoid, medicinal plant, *Brucea javanica*

1. Introduction

Lipolysis is the initial step in the metabolism of lipids stored in adipose tissue. The regulation of the lipolytic process directly influences the amount of lipid stored in adipocytes, and thus lipolysis is considered to be a therapeutic target for obesity. β3-adrenergic receptor agonists are known to induce lipolysis in adipocytes, and due to their effect to induce weight loss in rodents without a change in food consumption, these agonists were once thought of as promising candidates in anti-obesity medicine. However, the anti-obesity effect of β3-adrenergic receptor agonists could not be reproduced in humans [1].

The lack of effect of β3-adrenergic receptor agonists on human body mass is presumed to be due to lower expression of their receptor in human white adipose tissue, relative to rodents, which express it abundantly [2]. Therefore, the pro-lipolytic effect of β3-adrenergic receptor activation is currently not a target for anti-obesity drug development. However, lipolysis is essential for the metabolism of lipids in white adipose tissue, and polymorphisms in genes expressing proteins involved in the lipolytic pathway have been suggested to influence the efficacy of physical exercise for weight loss [3]. Therefore, pro-lipolytic compounds other than β3-adrenergic receptor agonists

still have potential as anti-obesity drugs.

Brucea javanica (family Simaroubaceae) is a ~3-meter-tall shrub, called *buah makassar* in Indonesia. This shrub is found from Southeast Asia to Northern Australia and its fruits have been used in traditional medicines in Asia for the treatment of dysentery, malaria, cancer, and diabetes [4]. Various chemical compounds have been isolated from *B. javanica*, including alkaloids [5], lignans, terpenoids [6], and alkaloid glycosides [7], but the most extensively studied are the triterpene lactones, known as "quassinoids". Quassinoids are triterpenes that are unique to the Simaroubaceae family, and nearly 50 have been identified in *B. javanica* extracts, including bruceins A–C, bruceantinol, javanicolides A–D, javanicosides A–F [8–12], and their glycosides (bruceosides and yadanziosides) [13–16].

Quassinoids exhibit a wide range of biological activities, and have been shown to have antimalarial [4], anti-babesial [17,18], amoebicidal [19], antileukemic [20], anti-protozoal [21], anti-human immunodeficiency virus [22], and anti-inflammatory effect [23]. In addition, we have recently explored the pro-lipolytic effects of eurycomanone and 13β,21-epoxyeurycomanone, the two quassinoids found in *Eurycoma longifolia* Jack [24]. A quassinoid-enriched extract of *E. longifolia* was also later reported to suppress weight gain in C57BL/6J mice, suggesting that quassinoids may have anti-

obesity effects [25].

Due to the presence of many quassinoids in *B. javanica*, we suspected that extracts of this plant would also possess a pro-lipolytic effect. Therefore, we aimed to characterize the pro-lipolytic activity of a *B. javanica* fruit extract, and have confirmed that several quassinoids in this plant are potent lipolytic compounds that have potential as lead compounds for the treatment of obesity.

2. Materials and Methods

2.1. Materials and equipment

Commercially available chemicals were purchased from Fujifilm Wako Pure Chemical Co. (Osaka, Japan), unless otherwise stated. Absorbance was measured using a Synergy™ MX microplate reader (BioTek Instruments, Inc., Winooski, VT, USA). A Bruker AMX 500 instrument (Bruker BioSpin K.K., Bruker Instruments, Billerica, MA, USA) was used to obtain nuclear magnetic resonance (NMR) spectra and residual solvents were used as internal standards (pyridine-d_5: ^{1}H 7.22 ppm, ^{13}C 135.91 ppm). Mass spectra were obtained using an LCT-Premier mass spectrometer (Waters Corp., Milford, MA, USA) or a Jeol JMS-T100GCV (Jeol Ltd., Tokyo, Japan). For ultra-high-

performance liquid chromatography (UPLC)-mass spectrometry (MS) analysis, a Waters Acquity UPLC system (Waters Corp., Milford, MA, USA) was linked to an LCT-Premier mass spectrometer.

2.2. Plant material

Dried *B. javanica* seeds were purchased from Merapi Farma Herbal Co. (Yogyakarta, Indonesia) in February 2015. The plants were certified by the company's herbalist and the voucher specimens (SL. 1A. 2015. BMKS) were deposited with the same company.

2.3. Lipolysis assay

2.3.1. Cell culture

3T3-L1 cells (JCRB9014) were obtained from the Japanese Collection of Research Bioresources Cell Bank (Osaka, Japan). They were cultured at 37°C in 10% CO_2 atmosphere in Dulbecco's-modified Eagle's medium (DMEM), supplemented with 10% fetal bovine serum (FBS) and antibiotics (100 units/mL penicillin, 100 µg/mL streptomycin sulfate, and 50 µg/mL gentamicin sulfate). For assays, the cells were seeded into 48-well plates and differentiation was induced 1 day after they reached

confluence (day 0) by changing the medium to 10% FBS/DMEM supplemented with 0.5 mM 3-isobutyl-1-methylxanthine, 0.25 μM dexamethasone, and 5 μg/mL insulin. On days 2 and 4, the medium was changed to 10% FBS/DMEM supplemented with 10 μg/mL insulin, and on day 6 to 10% FBS/DMEM. These cells were then used in lipolytic assays on day 8.

2.3.2. Assay procedure

The samples were dissolved in dimethyl sulfoxide (DMSO) and diluted in phenol-red-free DMEM immediately before use. The final DMSO concentrations were kept below 0.1%. Differentiated 3T3-L1 cells were treated with samples in medium for 24 h, and then the medium was recovered and mixed with free glycerol reagent (F6428; Sigma-Aldrich Co., St Louis, MO, USA) to quantify the glycerol released, in accordance with manufacturer's instructions. Isoproterenol hydrochloride (IPT, Sigma-Aldrich Co., St Louis, MO, USA) was used as a positive control and 0.1% DMSO alone was used as the control.

2.4. Cell viability

Cell Counting Kit-8 reagent (Dojindo Lab., Kumamoto, Japan) was added to the

treated cells and they were incubated for 3 h. The absorbance of the medium at 450 nm was measured and the cell viability was calculated in accordance with the manufacturer's instructions. Triton X-100 (0.1%) was used as a positive control.

2.5. Isolation of quassinoids

Dried *B. javanica* seeds (500 g) were powdered and extracted using 50% methanol in water for 24 h. The extract (69.5 g) was suspended in water and sequentially extracted three times using ethyl acetate and 1-butanol to obtain an ethyl acetate layer (7.99 g), a butanol layer (3.65 g), and a water layer (50.85 g).

The ethyl acetate layer (0.92 g) was loaded onto a silica-gel column (ø30 × 220 mm) and eluted stepwise using 300 mL each of hexane/ethyl acetate at ratios of 2/1, 1/1, 1/2, 1/5, and 0/1 and chloroform/methanol at a ratio of 2/1, to obtain fractions 1–6.

Fraction 2 (225.8 mg) was loaded onto silica-gel column (ø15 × 120 mm) and eluted stepwise using 75 mL each of chloroform/methanol at ratios of 80/1, 40/1, 20/1, 10/1, and 2/1, to obtain fractions 2-1 to 2-5. Fraction 2-2 was then sequentially separated by preparative thin-layer chromatography (TLC) (chloroform/methanol, 20/1 and hexane/acetone, 5/4) to obtain fractions A and B. Fraction A (19.2 mg) was recrystallized from methanol to obtain brucein A (**1**, 5.5 mg) as a colorless solid.

Fraction B (16.0 mg) was separated by high-performance liquid chromatography (HPLC) using an InertSustain C8 (ø20 × 250 mm, GL Sciences Inc., Tokyo, Japan) column and 45% methanol in water as the eluent to obtain brusatol (**2**, 1.6 mg) and bruceantinol (**3**, 4.5 mg).

Fraction 3 (73.7 mg) was loaded onto a silica-gel column (ø10 × 60 mm) and eluted stepwise with 30 mL each of hexane/ethyl acetate at ratios of 1/1, 1/3, 1/5, 1/10, and 0/1 to obtain fractions 3-1 to 3-5. Fraction 3-3 was then separated by preparative TLC (hexane/acetone, 1/1) to obtain fraction C. Fraction C (15.5 mg) was separated by HPLC using an InertSustain C8 (ø20×250 mm) column and 45% methanol in water as the eluent to obtain brucein B (**4**, 4.0 mg).

Fraction 5 (44.3 mg) was loaded onto a silica-gel column (ø15 × 120 mm) and eluted stepwise with 75 mL each of chloroform/methanol at ratios of 80/1, 40/1, 20/1, 10/1, and 2/1 to obtain fractions 5-1 to 5-5. Fraction 5-3 was then separated by preparative TLC (chloroform/methanol, 20/1) to obtain fraction D. Fraction D (12.0 mg) was separated by HPLC using an InertSustain C8 (ø20 × 250 mm) column and 45% methanol in water as an eluent to obtain 3'-hydroxybrucein A (**5**, 2.2 mg), brucein C (**6**, 5.4 mg), and peak E. Peak E (3.0 mg) was then separated by preparative TLC (toluene/methanol, 6/1) to obtain 3'-hydroxydehydrobrucein A (**7**, 1.9 mg).

The butanol layer (3.65 g) was adsorbed onto Diaion HP-20 (Mitsubishi Chemical Co., Tokyo, Japan) and eluted stepwise using water, 50% methanol/water, and methanol. The methanol-eluted fraction (0.75 g) was charged onto a Cosmosil 75C18-OPN column (Nakalai Tesque, Inc., Kyoto, Japan, ø30 × 150 mm) and eluted stepwise with 225 mL each of water, 10% methanol, 20% methanol, 30% methanol, 50% methanol, and methanol to obtain fractions BM-1 to BM-6. Fractions BM-5 and BM-6 were mixed (397.4 mg) and again separated using a Cosmosil 75C18-OPN column (ø20 × 150 mm) with stepwise elution by 120 mL each of 30% methanol, 40% methanol, 50% methanol, 60% methanol, and methanol to obtain fractions BM-5/6-1 to BM-5/6-5. Fraction BM-5/6-3 (20 mg) was then separated by HPLC using an InertSustain C18 (ø20×250 mm) column and 50% methanol as an eluent to obtain yadanzioside B (**8**, 2.2 mg), and peak F. Finally, peak F was separated by HPLC using an InertSustain C18 (ø20 × 250 mm) column and 50% methanol containing 0.1% trifluoroacetic acid as an eluent to obtain brucein A (**1**, 0.5 mg).

2.6. Methanolysis of brucein A (1)

Brucein A (**1**, 37.4 mg) was dissolved in methanol (4.1 mL) and 28% sodium methoxide/methanol solution (0.32 mL) was added. The solution was stirred under a

nitrogen atmosphere for 2 h, and then Dowex 50W-X8 was added to neutralize the reaction mixture. The suspension was filtered and concentrated. The residue was purified by HPLC using an InertSustain C18 (ø20 × 250 mm) column and 35% methanol as an eluent to obtain bruceolide (**9**, 20.2 mg, 64%) [26].

2.7. UPLC-MS analysis

The extract was prepared as a 1 mg/mL solution in methanol and 4 μL was injected for the analysis. An InertSustain C18 (ø 2.1 × 100 mm, particle size 2 μm, GL Science Inc., Tokyo, Japan) was used and a gradient elution was performed from 5% to 90% methanol in water within 15 min, using a flow rate of 0.2 mL/min at room temperature. The isolated compounds were used as standards. The following ions were used for the quantification: $[M+H]^+$ for brucein A (**1**) and brucein B (**4**); and $[M+Na]^+$ for brusatol (**2**), bruceantinol (**3**), 3'-hydroxybrucein A (**5**), and brucein C (**6**).

2.8. Statistics

The lipolysis assay was performed at least twice, with six replicates per assay, and representative results are shown in graphs. EZR software was used for the statistical analysis of the results [27]. Statistical significance was determined using either t-test or

one-way ANOVA coupled with Tukey test, and $p<0.01$ was considered significant.

3. Results and Discussion

3.1. Lipolytic activity of B. javanica *fruit extract*

The lipolytic activity of the dried *B. javanica* fruit extract was evaluated in 3T3-L1 adipocytes. The extract showed significant activity at concentrations above 3.13 μg/mL, indicating its relative potency compared with the previously studied *E. longifolia* root extract, which demonstrated lipolytic activity at concentrations above 100 μg/mL (Figure 1). Cell viability testing excluded the potential influence of a cytotoxic effect of the extract (Figure S1).

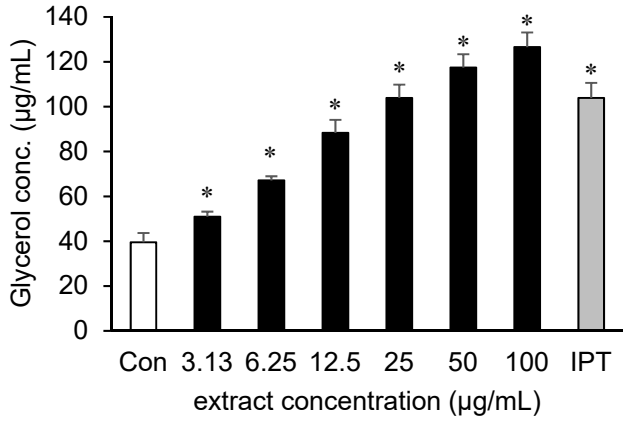

Figure 1. Lipolytic activity of an extract of dried *B. javanica* fruit in 3T3-L1

adipocytes

Differentiated 3T3-L1 adipocytes were treated with the indicated concentrations of the extract for 24 h and the glycerol released into the medium was quantified as an indicator of lipolysis. Each bar represents the mean ± SEM. *p< 0.01 (t-test). Con: Control, IPT: isoproterenol (2.5 μM).

3.2. Isolation of quassinoids from B. javanica *fruit extract*

Bioactive compounds in the extract were isolated using activity-guided fractionation. The extract suspended in water was partitioned with ethyl acetate and then 1-buthanol. The ethyl acetate-soluble and 1-butanol-soluble compounds that showed lipolytic activity were repeatedly separated using silica-gel column chromatography and preparative TLC. The obtained active fractions were then separated by reverse-phase HPLC to obtain compounds **1**-**8**.

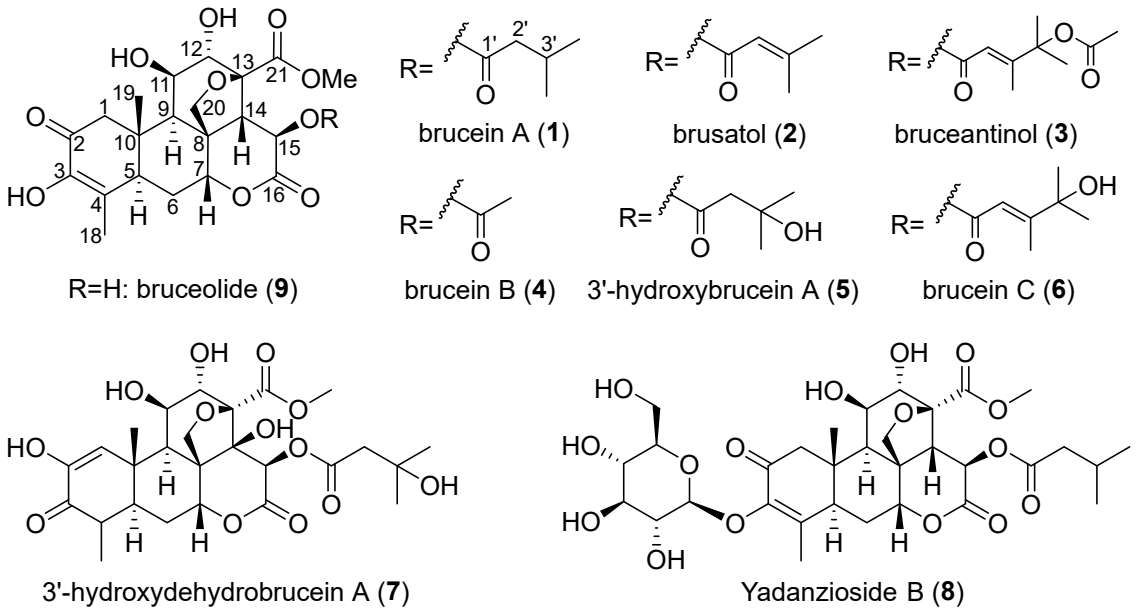

Figure 2. Structures of the quassinoids

Brucein A (**1**) [15], brusatol (**2**) [28], bruceantinol (**3**) [16], brucein B (**4**) [29], 3'-hydroxybrucein A (**5**) [14], brucein C (**6**) [14], and yadanzioside B (**8**) [15] are known compounds, and their structures were confirmed by comparing the NMR spectra with previously reported data (Figure 2, Table S1–S7, Figure S2–S7,S9). The NMR spectrum of compound **7** did not match the published data and it was therefore considered to be a newly identified quassinoid. The ^{1}H-NMR spectrum of **7** showed a characteristic signal at 6.5 ppm, indicative of the core quassinoid structure being a dehydrobrucein moiety [15], and the side-chain signals were similar to **4**, implicating 3'-hydroxydehydrobrucein A (**7**) as the candidate structure. Further analysis using several 2D-NMR techniques (^{1}H-^{1}H correlated spectroscopy, heteronuclear single

quantum coherence, and heteronuclear multiple bond correlation) and high resolution field desorption-mass analysis confirmed the structure (found *m*/*z* =536.1896, $C_{26}H_{32}O_{12}$, calculated molecular weight 536.1894) (Table 1, Figure 2).

Table 1. NMR assignments of 3'-hydroxydehydrobrucein A (7) (pyridine-d_5, 323 K)

No.	^{1}H-NMR (ppm)	^{13}C-NMR (ppm)
1	6.55 (s)	121.74
2	-	145.80
3	-	180.79
4	-	130.93
5	-	155.95
6	3.33 (dd, *J*=3.0, 15.5 Hz)	32.30
6	2.60 (br d, *J*=15.5 Hz)	32.30
7	4.83 (br s)	84.11
8	-	46.05
9	1.98 (br s)	41.59
10	-	43.15
11	4.46 (dd, *J*=5.6, 6.4 Hz)	73.40 or 73.30
12	4.23 (s)	75.85
13	-	81.69
14	3.10 (d, *J*=11.0 Hz)	50.97
15	6.15 (br s)	66.63
16	-	166.26
18	2.02 (s)	10.93
19	1.66 (s)	23.56
20	4.88 (d, *J*=8.0 Hz) 3.90 (d, *J*=8.0 Hz)	73.40 or 73.30
21	-	171.70

OMe	3.85 (s)	53.30
1'	-	170.56
2'	2.49 (d, *J*=15.0 Hz)	46.40
2'	2.45 (d, *J*=15.0 Hz)	46.40
3'	-	69.45
4'	1.314 (s)	29.26
5'	1.307 (s)	29.11
2-OH	6.52 (s)	-
11-OH	2.43 (d, *J*=6.4 Hz)	-
12-OH	3.35 (s)	-
3'-OH	3.02 (s)	-

3.3. Lipolytic activity of the quassinoids

The lipolytic activity of quassinoids **1**–**8** was tested in 3T3-L1 adipocytes. Quassinoids **1**–**6** demonstrated concentration-dependent increases in glycerol release into the medium between 0.16 and 10 μM, indicative of lipolytic activity (Figure S11–S16). Quassinoid **7** did not show potent lipolytic activity at any of the concentrations tested (0.16–100 μM), but the glycerol concentration in the medium gradually increased as the concentration of **7** was increased, which may indicate slight activity (Figure S17). The glycosylated quassinoid **8** showed no activity at concentrations between 1 and 100 μM (Figure S18). The differences in lipolytic activity identified between **1**–**6** and **7–8** imply that 3-OH or the structure of ring A may be important for this activity.

To directly compare the lipolytic activity of more potent compounds, the lipolytic activity of quassinoids **1**–**6** was tested at 0.16 μM (Figure 3). The highest

activity was shown by bruceantinol (**3**) (a 531% increase over control) and the lowest by brucein B (4) (a 171% increase, p=0.012 *vs*. control). Quassinoids **1**–**6** differ in the structure of the acyl chains esterified to 15-OH. Quassinoids **3** and **4** have the shortest and longest acyl side chains, respectively, implying that acyl group length is important for the potency of the lipolytic activity. This contention is also supported by comparing the lipolytic activities of **2** and **6,** which possess acyl side chains of medium length and moderate activity (267% and 269% increase over control). In addition to the length of acyl side chain, the double bond between 2'C and 3'C may also influence lipolytic activity, given the difference between **1** and **2**.

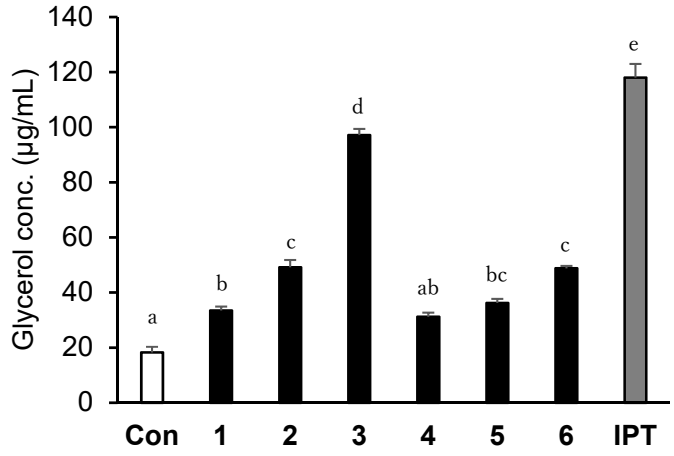

Figure 2. Lipolytic activity of the isolated quassinoids 1–6 in 3T3-L1 adipocytes

Differentiated 3T3-L1 adipocytes were treated with 160 nM of each compound for 24 h and the glycerol released into the medium was quantified as an indicator of lipolysis.

Each bar is the mean ± SEM. Different letters indicate that compounds had significantly different activities (Tukey test, $p<0.01$). Con: Control, IPT: isoproterenol (2.5 μM).

To confirm the importance of the acyl moiety at 15-OH, brucein A (**1**) was treated with sodium methoxide to remove its acyl moiety, yielding bruceolide (**9**). Bruceolide (**9**) showed no lipolytic activity at 1 μM, but a concentration-dependent lipolytic activity was observed between 5 and 100 μM (Figure S19). This increase in the concentration required for a lipolytic effect confirms the importance of the acyl side chain. This result is also consistent with the importance of the core quassinoid moiety of **1**–**6**, because **7**, which has a different structure, and **8**, in which the quassinoid structure is decorated by glucose, showed little or no activity at any of the tested concentrations, whereas **9** retained some activity. Additionally, comparable activity of the eurycomanone and 13β,21-epoxyeurycomanone, the previously isolated lipolytic quassinoids from *E. longifolia* [24], to **9** also supports that quassinoid structure is important for the activity except the position of ether bond between C20-C13 or C20-C11 are both allowed to retain the activity. In the same study, 13β,21-dihydroxyeurycomanone was found as an inactive compound which may suggests the importance of methoxycarbonyl group attached to C13 for the activity of **1**-**6**, and **9**.

The above results gave some indications to the structure-activity relationship, but how the quassinoids **1**-**6** induce lipolysis to 3T3-L1 adipocytes remains to be clarified. As written in the introduction section, β3-adrenergic receptor is the major receptor concerned in lipolysis. But our previous result showed that the lipolytic activity of eurycomanone and 13β,21-epoxyeurycomanone is not blocked by the co-treatment with propranolol, the non-selective β-adrenergic receptor antagonist. Thus, β3-adrenergic receptor is not likely the target of quassinoids. Low *et al* suggested eurycomanone as the inhibitor of phosphodiesterase, the enzyme involved in the lipolytic pathway downstream of β3-adrenergic receptor, except this was from indirect evidence combined with *in silico* study [30]. Recently, expression of bitter taste receptors (taste receptor type 2) are reported in white adipose tissue of mice as well as in 3T3-L1 adipocytes, and their involvement in adipocyte metabolism and differentiation process is suggested [31,32]. Quassinoids are well-known for their strong bitter taste and thus, bitter taste receptors may be a target to induce lipolytic activity.

3.4. Quantification of quassinoids 1–6 in the B. javanica seed extract

To confirm that quassinoids **1**–**6** are the principle bioactive compounds responsible for the lipolytic activity of the *B. javanica* seed extract, each compound was

quantified by UPLC-MS analysis (Table 2, Figure S20, S21). These quassinoids were present in 1 mg/mL of *B. javanica* seed extract at concentrations of 1.42–21.6 μM, with a total concentration of 53.7 μM, which is sufficient to explain the activity of the extract (Figure 1; 3.13 μg/mL extract will contain 168 nM of active quassinoids).

Table 2. Quantitation of lipolytic quassinoids in the *B. javanica* seed extract

Compound	μM in extract (1 mg/mL)	wt% in the dry seed
Brucein A (**1**)	21.6±0.1	0.157%
Brusatol (**2**)	4.44±0.05	0.032%
Bruceantinol (**3**)	5.26±0.1	0.044%
Brucein B (**4**)	17.7±0.3	0.118%
3'-Hydroxybrucein A (**5**)	1.42±0.02	0.011%
Brucein C (**6**)	3.25±0.04	0.025%
Total	53.7	0.387%

4. Conclusions

Quassinoids have demonstrated a variety of bioactivities, but their lipolytic activity is attracting attention because of its potential anti-obesity effect [24,25]. We have shown the quassinoids present in *B. javanica* to possess potent lipolytic activities, at concentrations as low as 160 nM, which is an order of magnitude lower concentration than that required by the previously studied compound eurycomanone, which is present

in *E. longifolia*. Brucein A (**1**), brucein B (**2**), brucein C (**3**), 3'-hydroxybrucein A (**4**), brusatol (**5**), and bruceantinol (**6**) are the principal quassinoids in *B. javanica* seeds that contribute to its lipolytic activity. The mechanism involved in the lipolytic activity of these compounds is not known, but further studies of this and the *in vivo* effect of these compounds in obesity may demonstrate applications for *B. javanica* seeds or quassinoids **1**–**6** in the prevention or treatment of obesity.

Acknowledgements

We thank Ryota Hirosawa (Graduate School of Agriculture, Hokkaido University), who was involved in the isolation, but passed away in Jan 2017. We thank Mr. Yusuke Takata and Dr. Eri Fukushi of the GC-MS and NMR Laboratory, Faculty of Agriculture, Hokkaido University, for their skillful quantification of mass spectra and advice regarding NMR analysis. We also thank Mark Cleasby, PhD, from Edanz Group (www.edanzediting.com/ac) for editing a draft of this manuscript. This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Declarations of interest: none

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Supplementary material

Quassinoids in *Brucea javanica* are potent stimulators of lipolysis in adipocytes

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Content

Cell viability test of *B. javanica* fruit extract

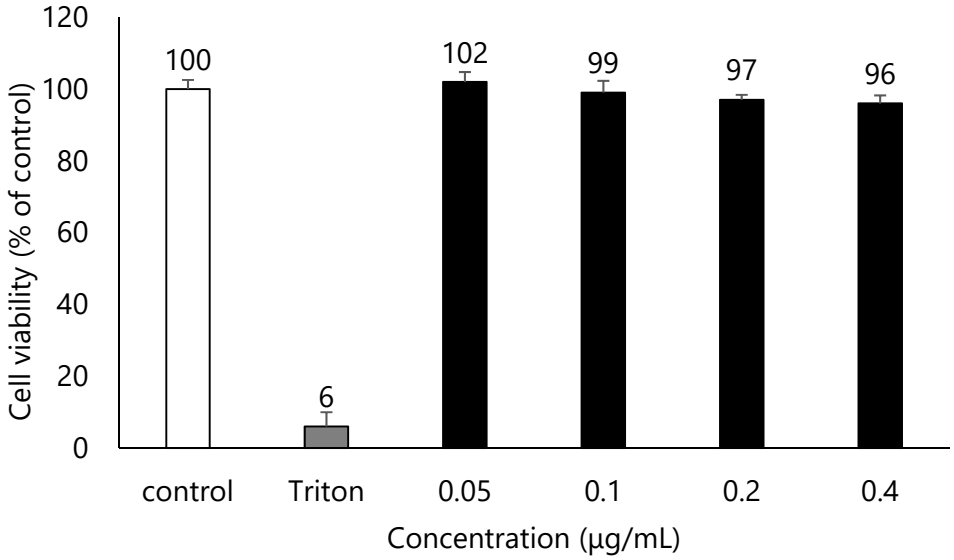
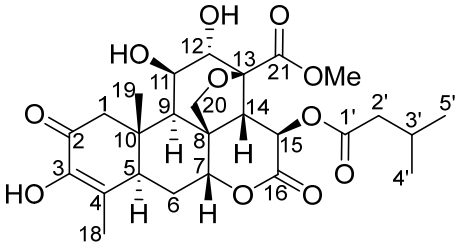


Figure S1. Cell viability test showed no effect of *B. javanica* fruit extract. Differentiated 3T3-L1 adipocytes were treated with the indicated concentrations of the extract for 24 hr and Cell Counting Kit-8 reagent (Dojindo Lab., Kumamoto, Japan) was added to the medium and was incubated for 3 h. The absorbance of the medium at 450 nm was measured and the cell viability was calculated according to the manufacturer's instructions. Triton X-100 (0.1%) was used as positive control.

Spectral data of isolated compounds

Brucein A (**1**)



ESI-MS (positive) : *m/z* = 545 $[M+Na]^+$

Table S1. NMR assignments (pyridine-d_5, 323 K)

No.	^{1}H-NMR (ppm)	Coupling (Hz)	^{13}C-NMR (ppm)
1	3.25	d, 16.0	50.51
	2.48	d, 16.0	
2	-		193.34
3	-		146.38
4	-		128.55
5	3.02	br d, 13.2	42.93
6	1.77	ddd, 2.6, 13.2, 14.1	30.05
	2.31	td, 2.8, 14.1	
7	5.02	m	84.12
8	-		46.71
9	2.55	d, 4.8	42.87
10	-		41.83
11	4.73	d, 4.2	73.57
12	4.98	br s	76.32
13	-		83.26
14	3.84	m	51.27
15	6.91	d, 13.5	68.85
16	-		168.50
18	1.96	d, 1.6	13.72
19	1.62	s	16.18
20	3.92	d, 7.6	74.22
	5.08	d, 7.6	
21	-		172.02
OMe	3.84	s	52.76
1'	-		171.75
2'	2.39	dd, 6.8, 14.8	43.79
	2.34	dd, 6.8, 14.8	
3'	2.23	nonet, 6.8	26.30
4'/5'	1.01	d, 6.8	22.88
4'/5'	0.99	d, 6.8	22.95

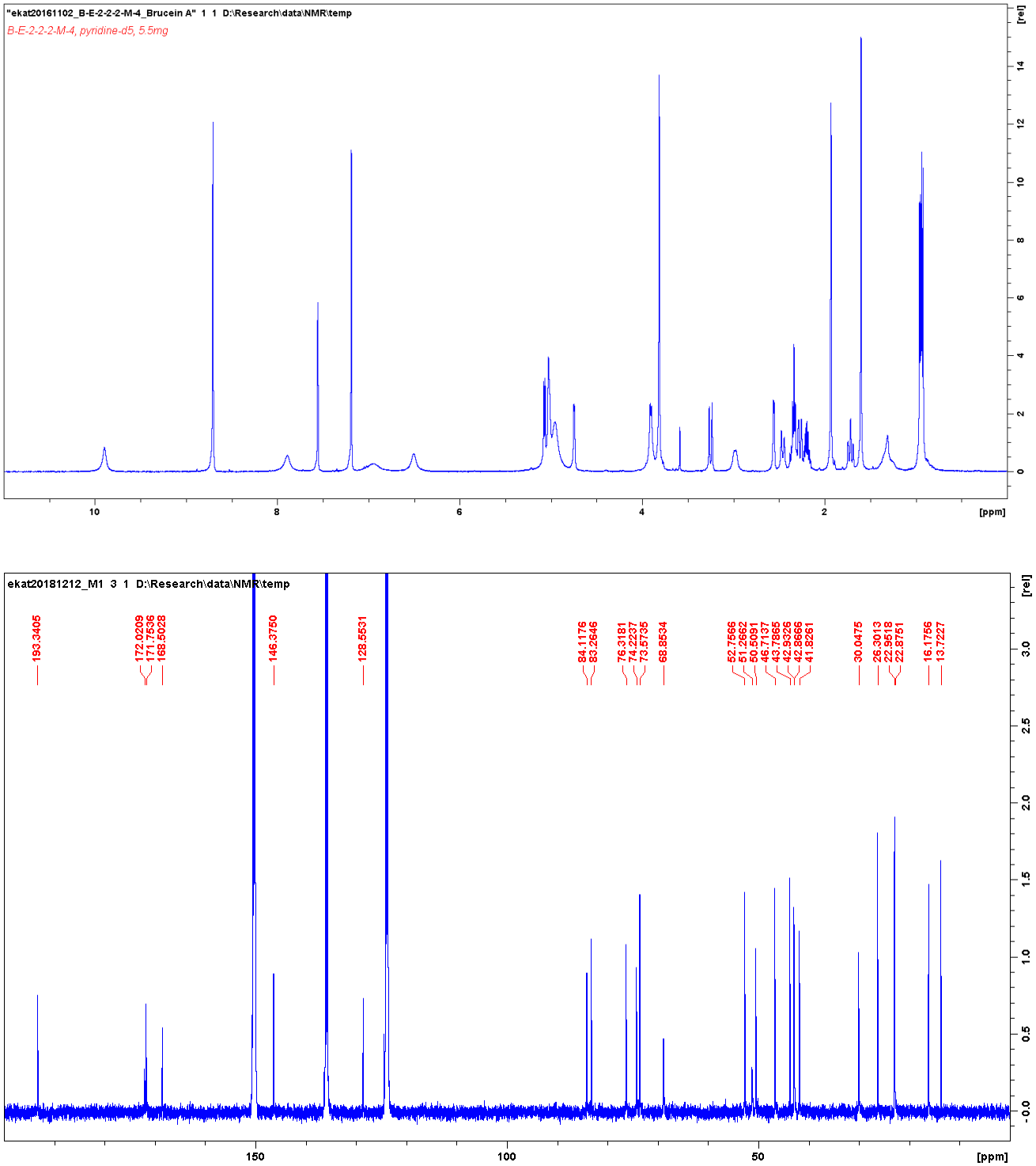
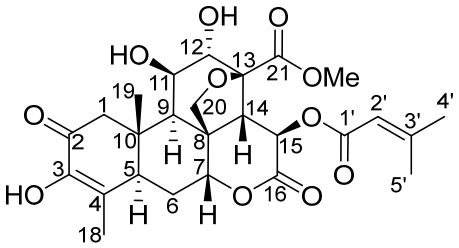


Figure S2. NMR spectrums of Brucein A (**1**)

Brusatol (**2**)



ESI-MS (positive) : *m/z* = 543 $[M+Na]^+$

Table S2. NMR assignments (pyridine-d_5, 323 K)

No.	^{1}H-NMR (ppm)	Coupling (Hz)	^{13}C-NMR (ppm)
1	2.53	d, 16.0	50.61
	3.28	d, 16.0	
2			193.40
3			146.44
4			128.63
5	3.06	br d, 13.5	43.00
6	1.78	ddd, 2.9, 13.7, 14.3	30.15
	2.32	td, 2.7, 14.3	
7	5.03	br s	84.01
8			41.92
9	2.58	d, 4.4	42.76
10			46.69
11	4.77	d, 4.4	73.59
12	5.06	br s	76.47
13			83.27
14	3.96	br s	50.66
15	6.88	br s	68.68
16			168.61
18	1.96	d, 1.8	13.77
19	1.64	s	16.24
20	3.93	d, 7.3	74.26
	5.10	d, 7.3	
21			171.80
OMe	3.78	s	52.75
1'			165.83
2'	5.87	br s	116.54
3'			158.65
4'/5′	2.18	s	20.68
4'/5'	1.67	s	27.42

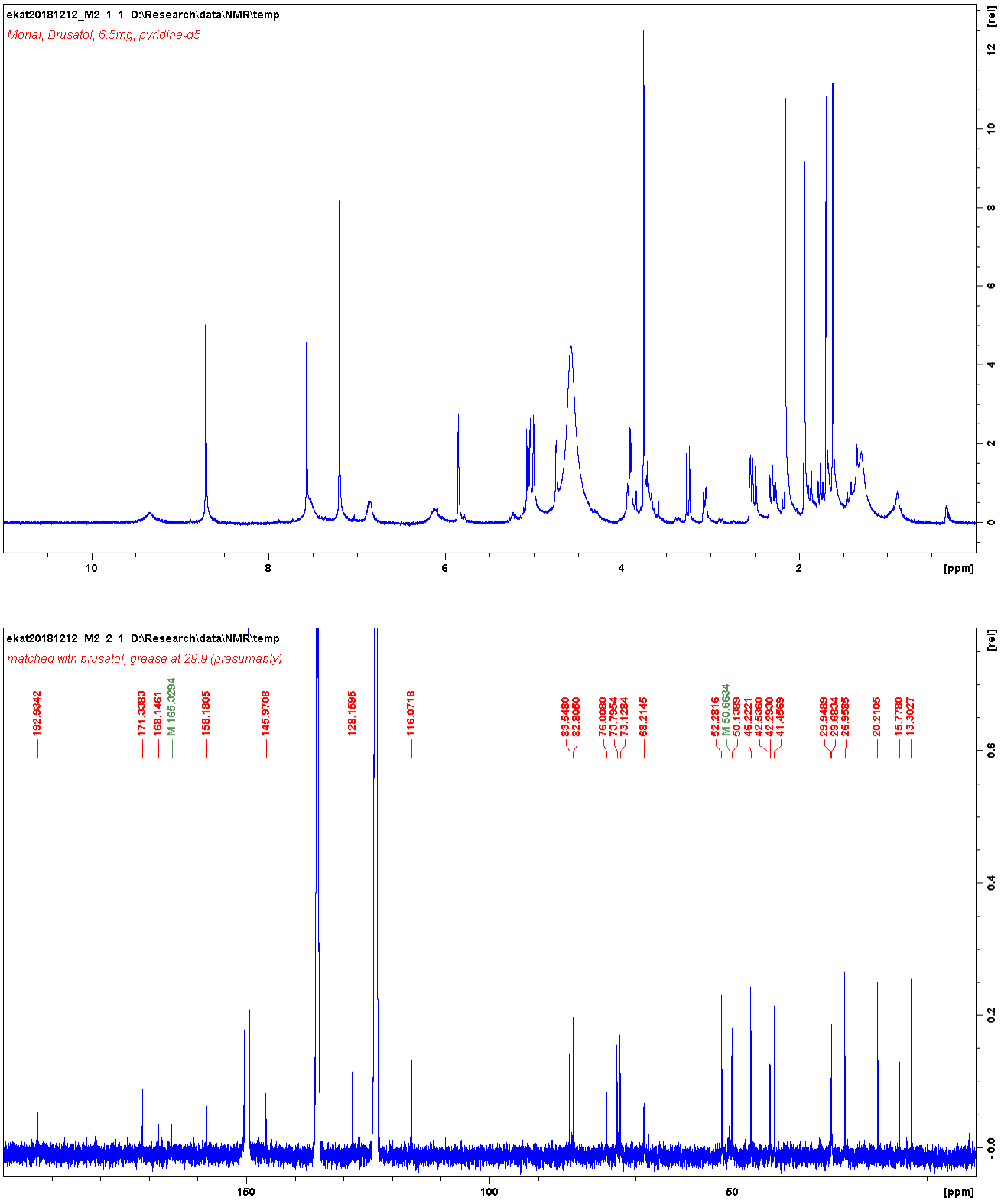
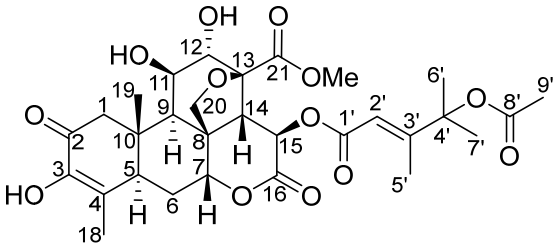


Figure S3. NMR spectrums of Brusatol (**2**)

Bruceantinol (**3**)



ESI-MS (positive) : *m/z* = 629 $[M+Na]^+$

Table S3. NMR assignments ($CDCl_3$, rt)

No.	^{1}H-NMR (ppm)	Coupling (Hz)	^{13}C-NMR (ppm)
1	2.40	d, 16.0	48.59
	2.98	d, 16.0	-
2	-		191.95
3	-		144.05
4	-		127.64
5	2.96	m	41.82
6	1.77	dt, 2.4, 13.9	29.07
	2.39	m	-
7	4.81	br s	82.36
8	-		45.40
9	2.11	br s	41.82
10	-		41.12
11	4.26	br s	70.96
12	4.21	s	75.68
13	-		81.30
14	ND		51.20
15	ND		66.10
16	-		166.84
18	1.85	d, 1.6	13.29
19	1.40	s	15.41
20	3.81	d, 7.7	74.06
	4.73	d, 7.7	-
21	-		171.71
OMe	3.82	s	53.30
1'	-		164.70
2'	5.77	s	111.78
3'	-		169.55
4'	-		82.15
5'	2.14	d, 1.0	14.56
6′	1.53	s	26.12
7′	1.52	s	26.20
8′	-		165.40
9′	2.02	s	21.62

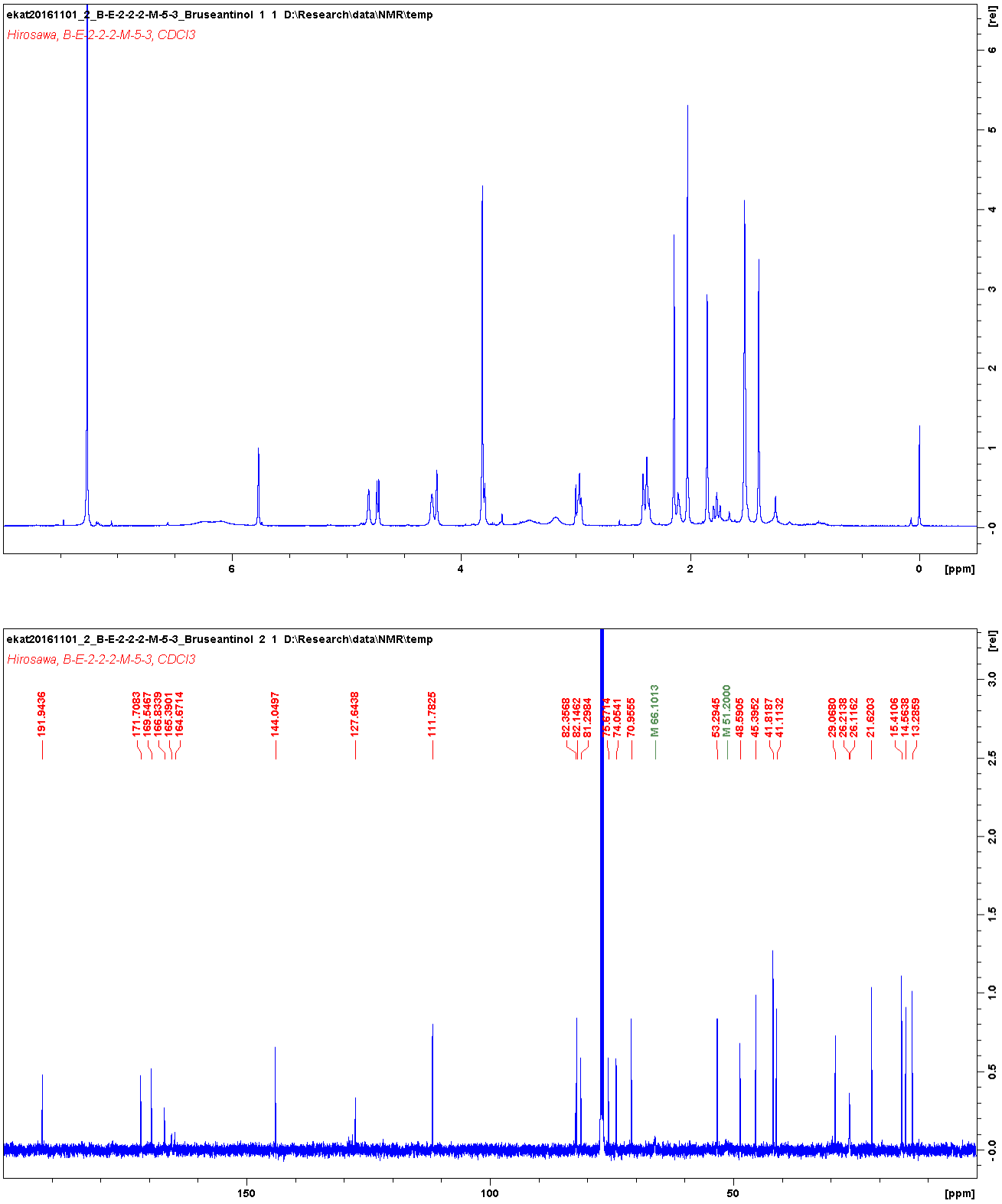
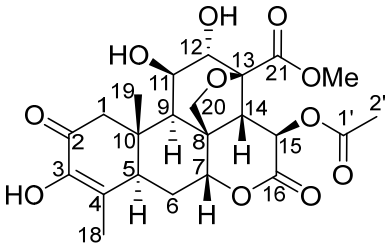


Figure S4. NMR spectrums of Bruceantinol (**3**)

Brucein B (**4**)



ESI-MS (positive) : *m/z* = 503 $[M+Na]^+$

Table S4. NMR assignments ($CDCl_3$, rt)

No.	^{1}H-NMR (ppm)	Coupling (Hz)	^{13}C-NMR (ppm)
1	2.43	d, 16.5	48.56
	2.97	d, 16.5	48.56
2	-		192.0
3	-		144.1
4	-		127.7
5	2.95	d, 14.0	41.92
6	1.77	dd, 14.0, 14.7	29.07
	2.38	d, 14.7	29.07
7	4.76	s	82.59
8	-		45.40
9	2.16	br s	41.84
10	-		41.10
11	4.25	br s	70.98
12	4.19	s	75.82
13	-		81.30
14	3.04	d, 12.0	51.76
15	6.35	br s	66.40
16	-		166.70
18	1.85	s	13.25
19	1.39	s	15.44
20	3.79	d, 7.7	74.04
	4.73	d, 8.3	74.04
21	-		172.10
OMe	3.85	s	53.03
1'	-		169.10
2'	2.09	s	20.28

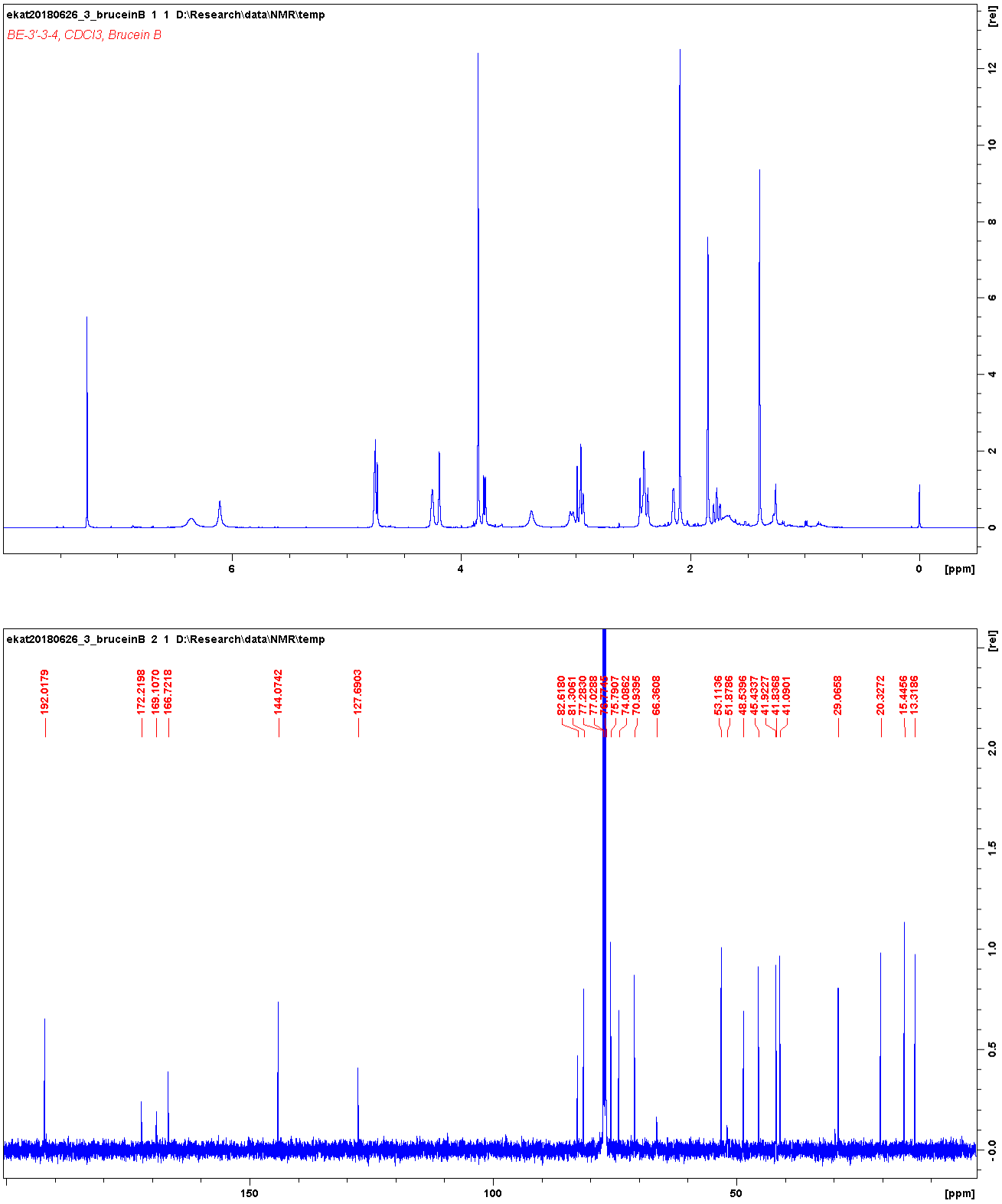
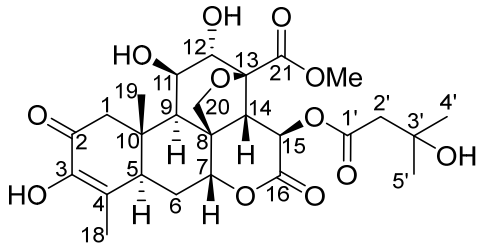


Figure S5. NMR spectrums of Brucein B (**4**)

3′-Hydroxybrucein A (**5**)



ESI-MS (positive) : *m/z* = 561 $[M+Na]^+$

Table S5. NMR assignments (pyridine-d_5, 323K)

No.	^{1}H-NMR (ppm)	Coupling (Hz)	^{13}C-NMR (ppm)
1	2.50	d, 16.2	50.54
	3.28	d, 16.2	
2			193.36
3			146.42
4			128.56
5	3.00	br d, 13.0	42.97
6	1.75	ddd, 2.3, 13.0, 14.4	30.06
	2.3	br d, 14.4	
7	5.06	m	84.23
8			46.80
9	2.59	d, 4.4	42.91
10			41.86
11	4.78	br s	73.56
12	5.05	m	76.38
13			83.33
14	3.94	m	51.14
15	6.99	br s	69.09
16			168.52
18	1.95	d, 1.3	13.74
19	1.63	s	16.20
20	3.94	d, 7.3	74.23
	5.10	d, 7.3	
21			171.78
OMe	3.81	s	52.85
1'			171.18
2'	2.96	d, 14.2	49.02
	2.89	br d, 14.2	
3'			69.64
4'/5′	1.61	s	30.24
4'/5′	1.64	s	30.33

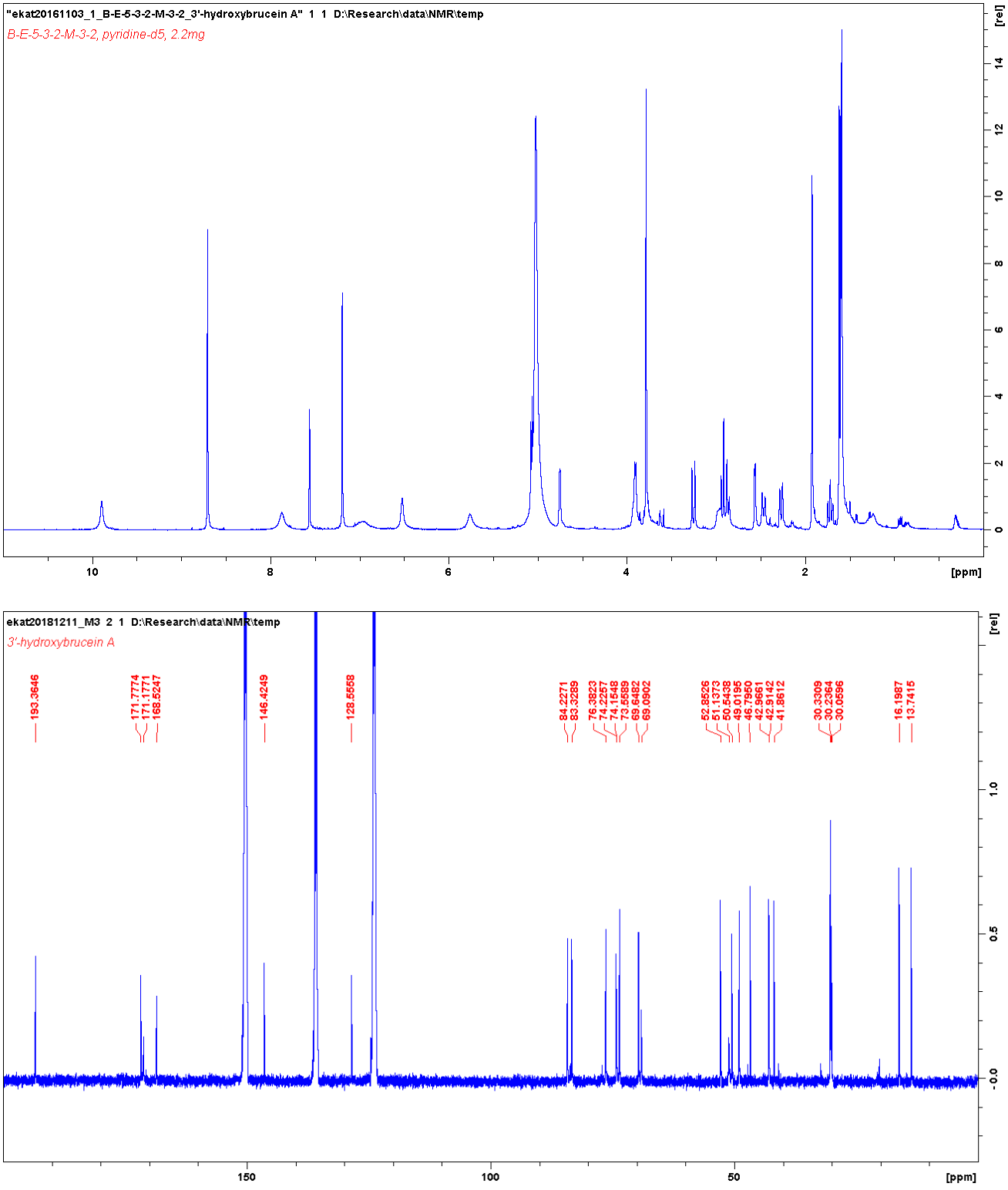
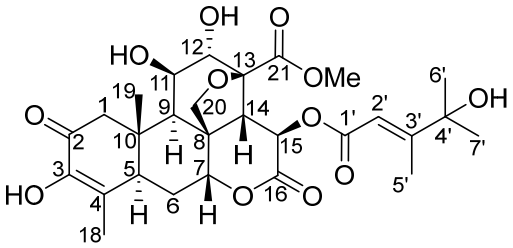


Figure S6. NMR spectrums of 3′-hydroxybrucein A (**5**)

Brucein C (**6**)



ESI-MS (positive) : *m/z* = 587 $[M+Na]^+$

Table S6. NMR assignments ($CDCl_3$, rt)

No.	^{1}H-NMR (ppm)	Coupling (Hz)	^{13}C-NMR (ppm)
1	2.45	d, 16.8	48.48
	2.95	d, 16.8	
2			192.50
3			144.01
4			128.62
5	2.98	br d, 13.0	41.50
6	1.76	ddd, 2.4, 13.0, 14.2	29.02
	2.38	td, 2.7, 14.2	
7	4.82	s	82.55
8			45.34
9	2.14	s	41.75
10			41.05
11	4.25	d, 4.2	71.06
12	4.22	s	75.51
13			81.34
14	ND		ND
15	ND		66.20
16			167.28
18	1.85	d, 1.5	13.35
19	1.38	s	15.38
20	3.80	d, 8.0	73.94
	4.72	d, 8.0	
21			168.13
OMe	3.76	s	53.08
1'			165.34
2'	6.08	s	111.26
3'			171.62
4'			74.20
5'	2.17	s	15.64
6′/7′	1.366	s	28.28
6′/7′	1.371	s	28.28

ND: not detected

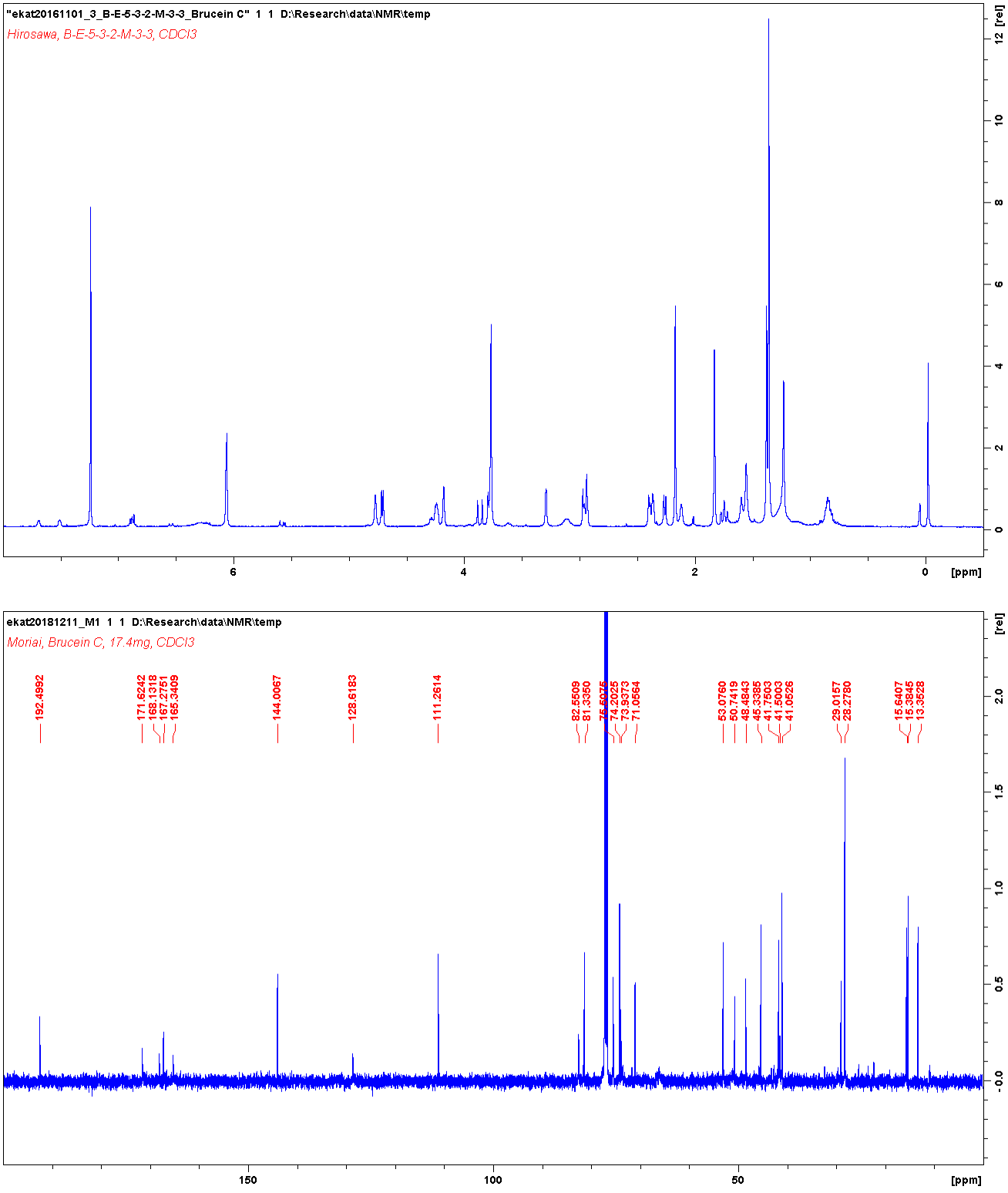


Figure S7. NMR spectrums of Brucein C (**6**)

3′-hydroxydehydrobrucein A (7)

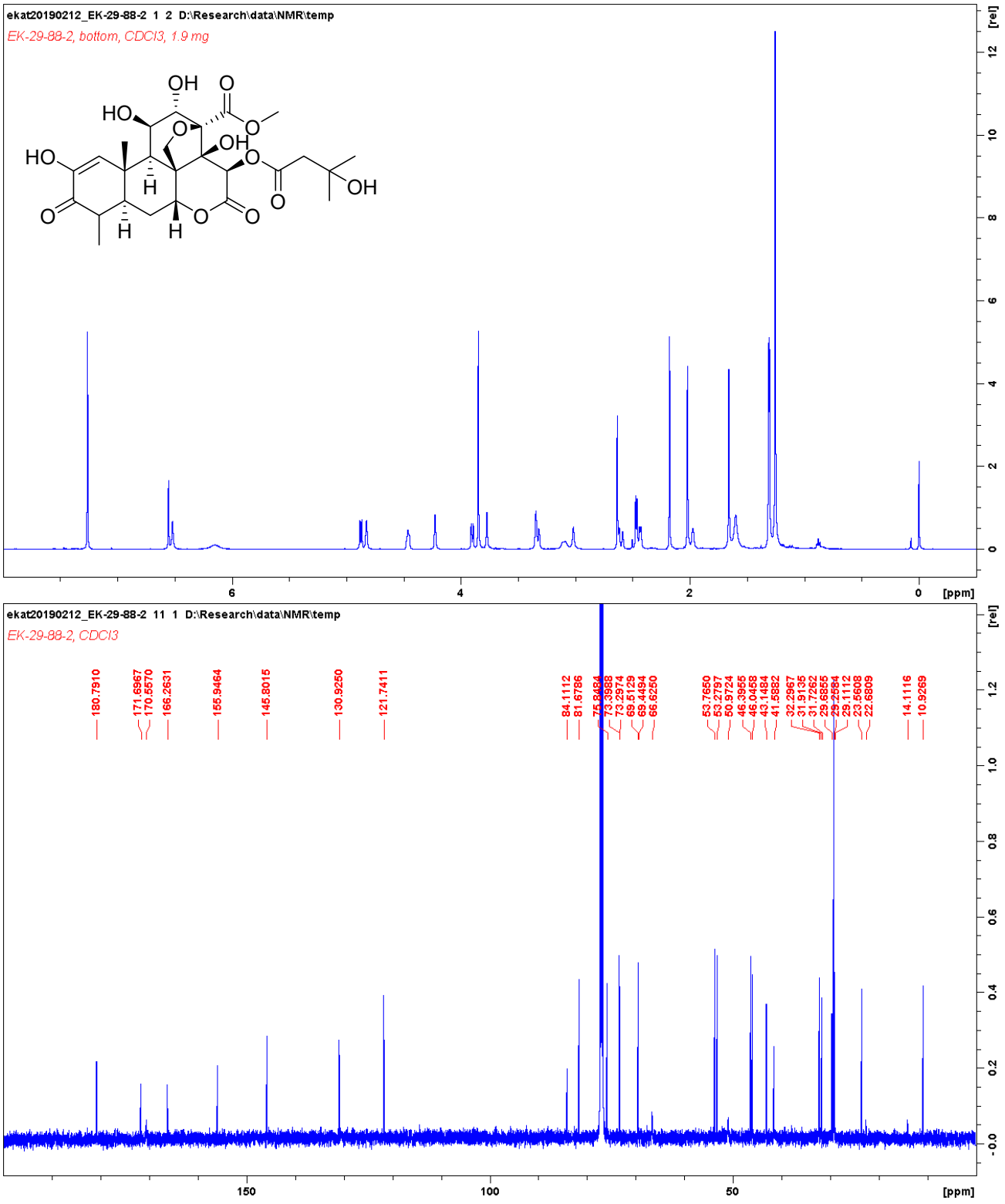
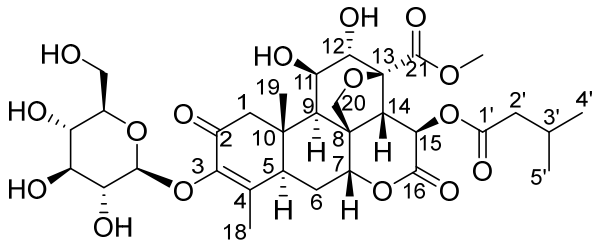


Figure S8. NMR spectrums of 3′-hydroxydehydrobrucein A (**7**)

Yadanzioside B (8)



ESI-MS (negative) *m/z* = 425 $[M-H]^-$

Table S7. NMR assignments (pyridine-d_5, 325K)

No.	^{1}H-NMR (ppm)	Coupling (Hz)	^{13}C-NMR (ppm)
1	2.48	d, 16.3	51.63
	3.23	d, 16.3	
2			194.01
3			147.21
4			148.20
5	3.03	d, 12.7	43.92
6	1.74	m	29.87
	2.28	m	
7	4.99	m	83.93
8			46.61
9	2.50	br s	42.81
10			41.31
11	4.71	d, 4.4	73.55
12	4.98	d, 4.4	76.34
13			83.27
14	3.83	d, 11.8	51.30
15	6.90	d, 11.8	68.87
16			168.46
18	2.05	s	15.67
19	1.70	s	16.32
20	3.90	d, 7.4	74.09
	5.06	d, 7.4	
21			171.73
OMe	3.84	s	52.77
1'			172.03
2'	2.35	dd, 6.8, 14.5	43.80
	2.40	dd, 7.2, 14.5	43.80
3'	2.24	m	26.32
4'/5'	1.00	d, 6.8	22.89
4'/5'	1.02	d, 6.8	22.96
G1	5.37	d, 7.4	105.41
G2	4.18	m	76.48
G3	4.20	m	78.91
G4	4.20	m	72.21
G5	3.83	m	79.02
G6'	4.28	dd, 5.2, 11.6	63.45
'	4.40	dd, 2.7, 11.6	

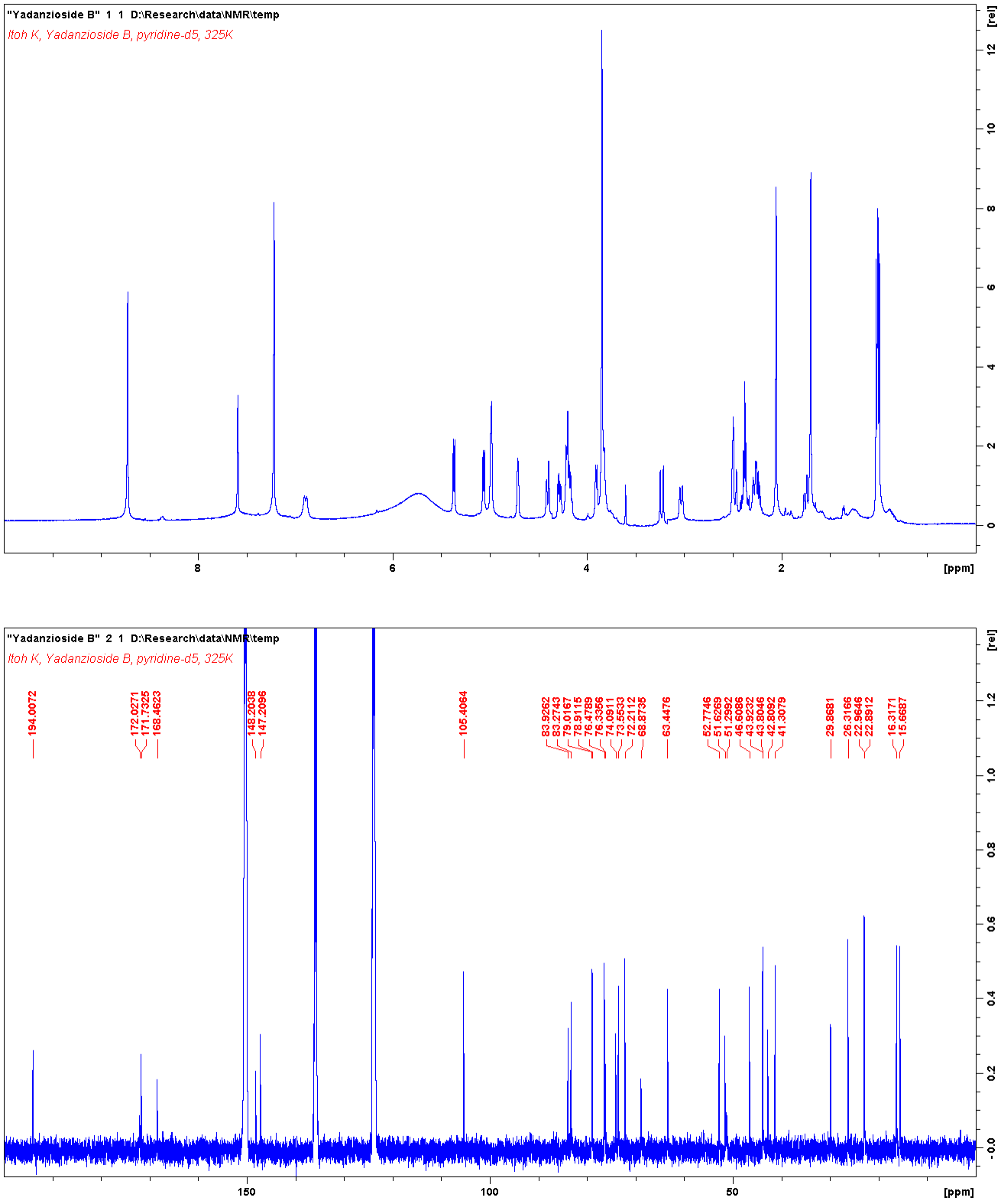
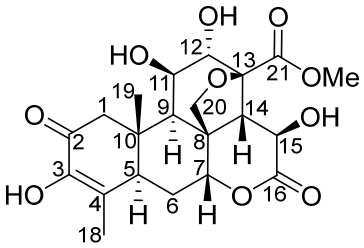


Figure S9. NMR spectrums of Yadanzioside B (**8**)

Bruceolide (9)



ESI-MS (positive) : *m/z* = 461 $[M+Na]^+$

Table S8. NMR assignments (pyridine-d_5, 323K)

No.	^{1}H-NMR (ppm)	Coupling (Hz)	^{13}C-NMR (ppm)
1	2.54	d, 15.5	50.56
	3.26	d, 15.5	
2			193.40
3			146.25
4			128.75
5	3.07	br d, 13.8	42.96
6	1.74	ddd, 2.6, 13.8, 14.8	30.13
	2.30	ddd, 2.6, 2.6, 14.8	
7	4.88	br s	83.52
8			46.27
9	2.56	s	43.12
10			41.79
11	4.73	d, 4.3	73.33
12	4.94	s	77.37
13			83.19
14	3.52	d, 11.9	55.86
15	5.96	d, 11.9	66.61
16			173.83
18	1.94	d, 1.7	13.70
19	1.61	s	16.11
20	3.93	d, 7.3	74.57
	5.06	d, 7.3	
21			173.14
OMe	3.82	s	52.67

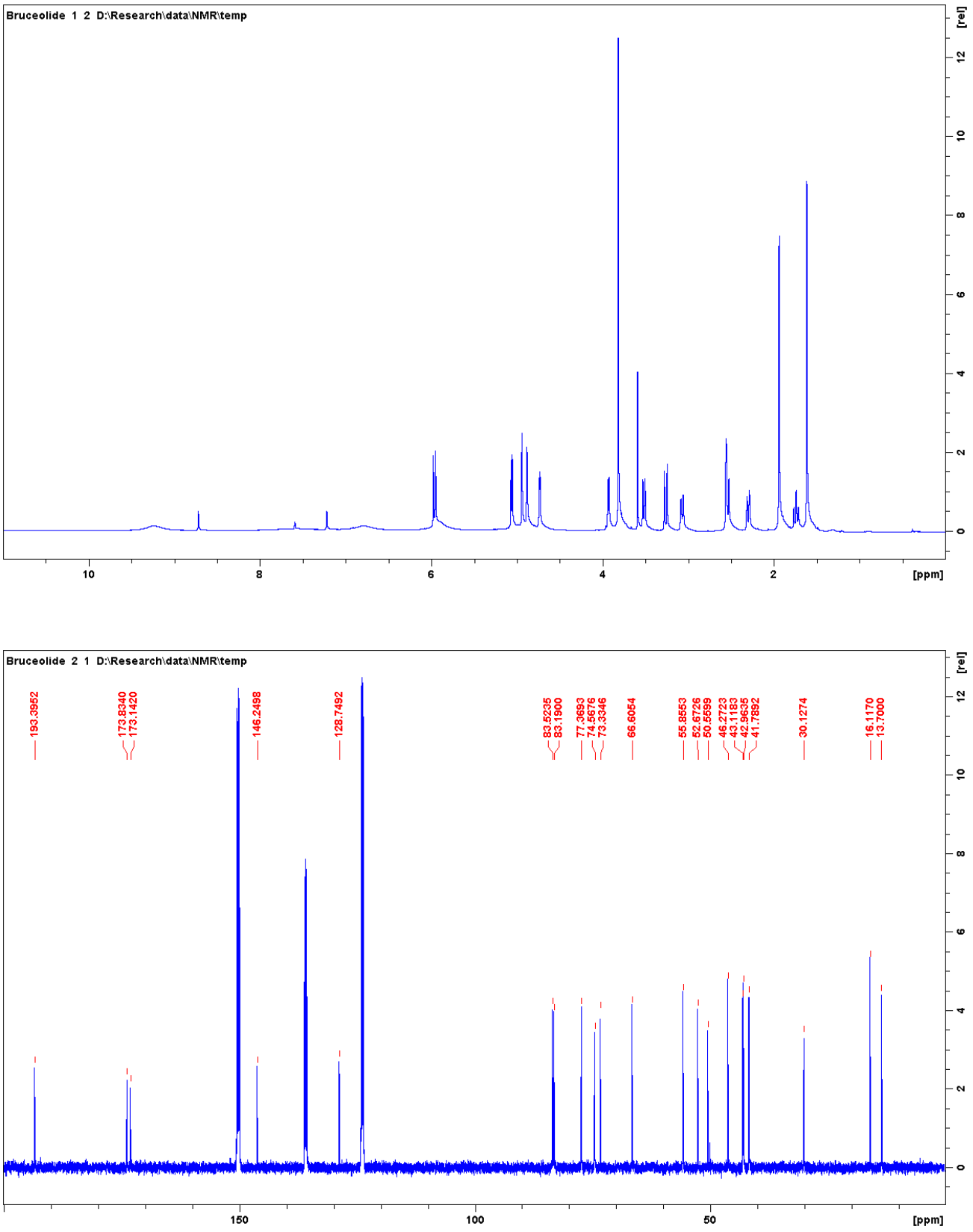


Figure S10. NMR spectrums of bruceolide (**9**)

Lipolytic activity of quassinoids

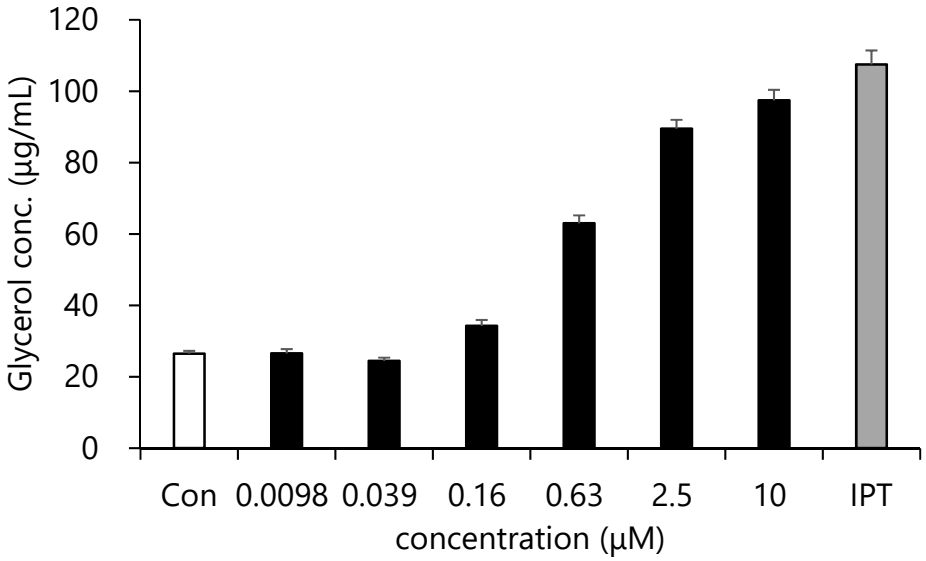


Figure S11. Lipolytic activity of brucein A (**1**)

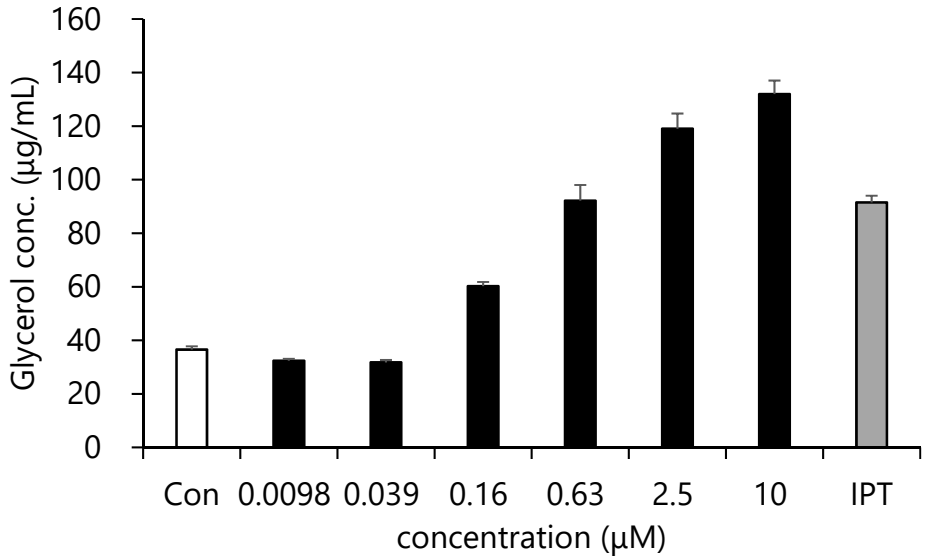


Figure S12. Lipolytic activity of brusatol (**2**)

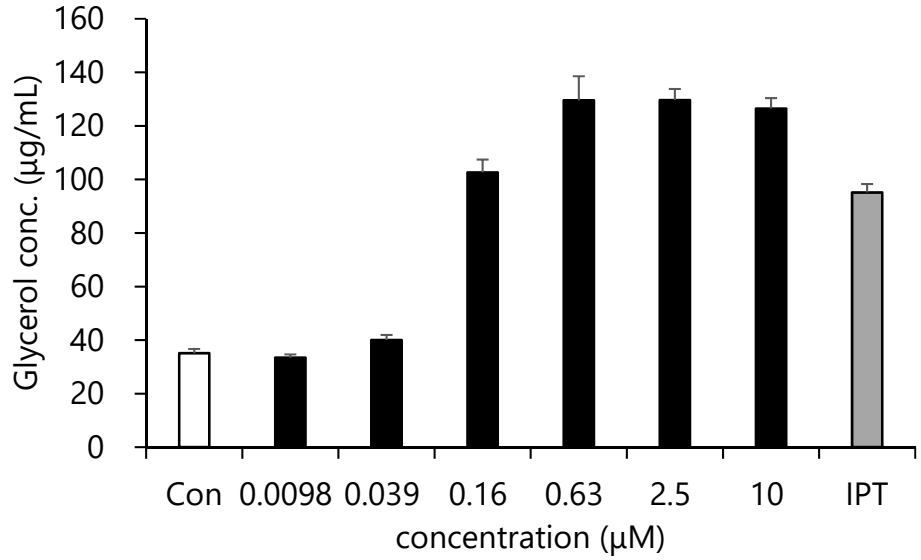


Figure S13. Lipolytic activity of bruceantinol (**3**)

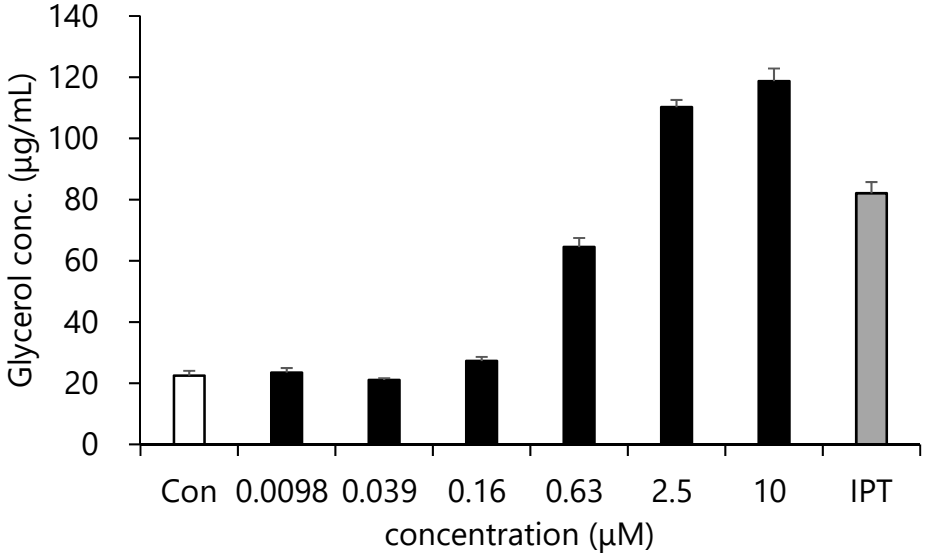


Figure S14. Lipolytic activity of brucein B (**4**)

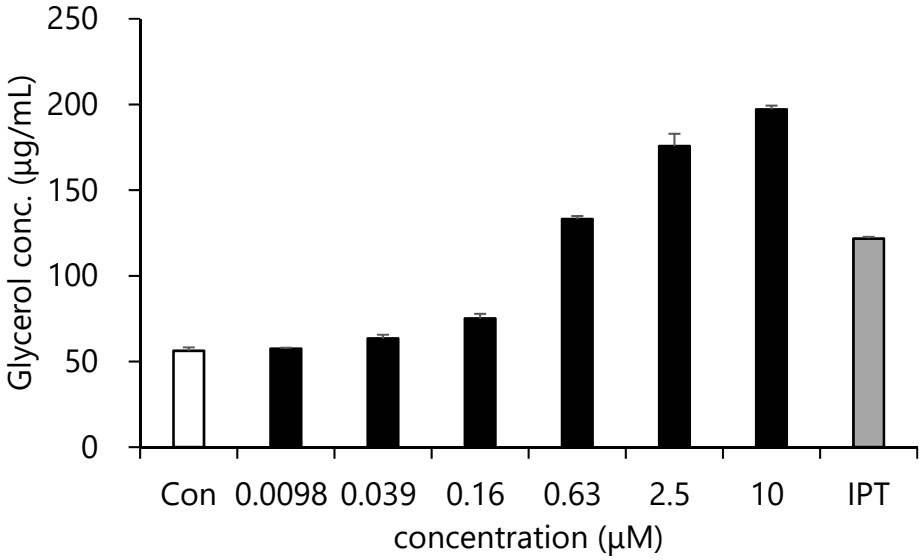


Figure S15. Lipolytic activity of 3′-hydroxybrucein A (**5**)

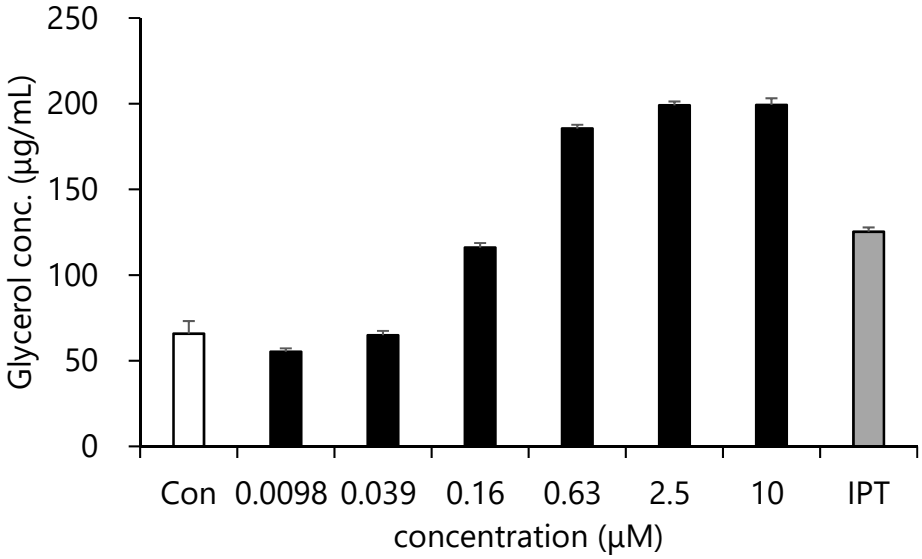


Figure S16. Lipolytic activity of brucein C (**6**)

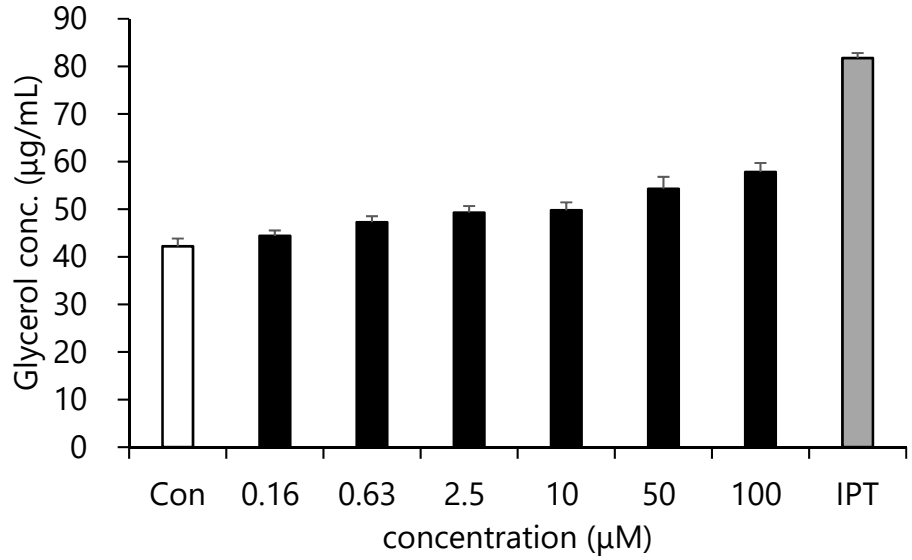


Figure S17. Lipolytic activity of 3′-hydroxydehydrobrucein A (**7**)

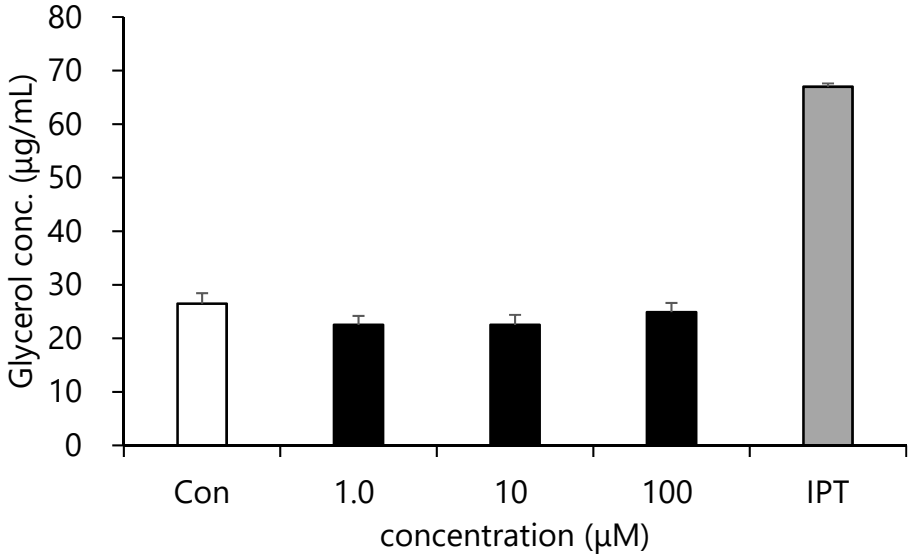


Figure S18. Lipolytic activity of yadanzioside B (**8**)

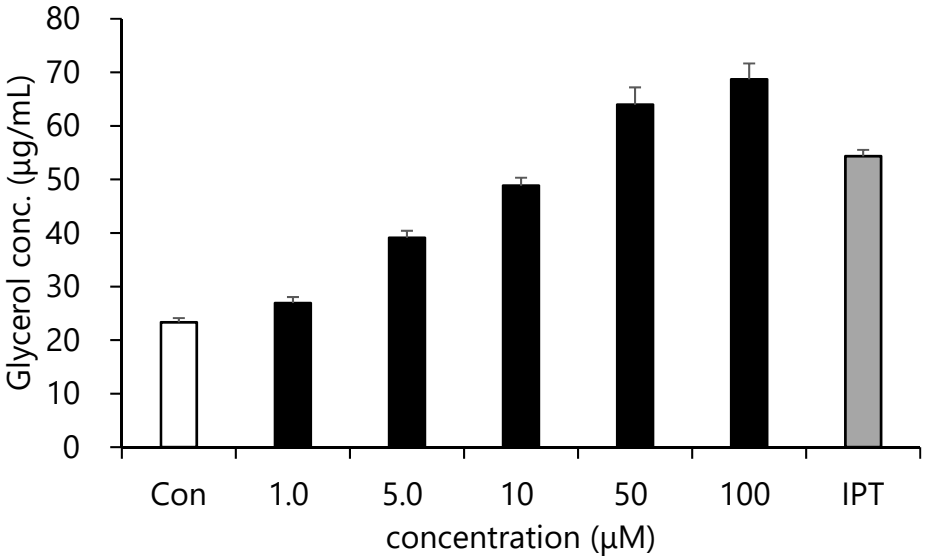
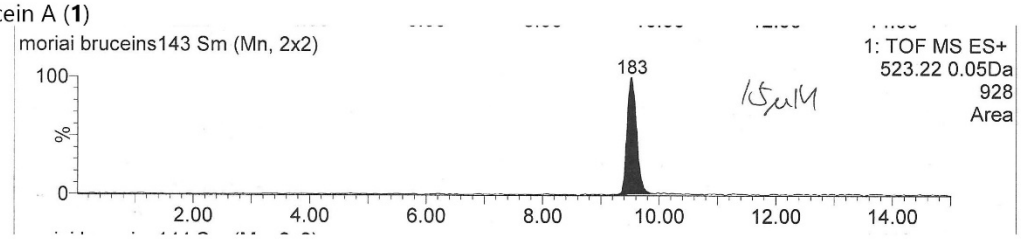


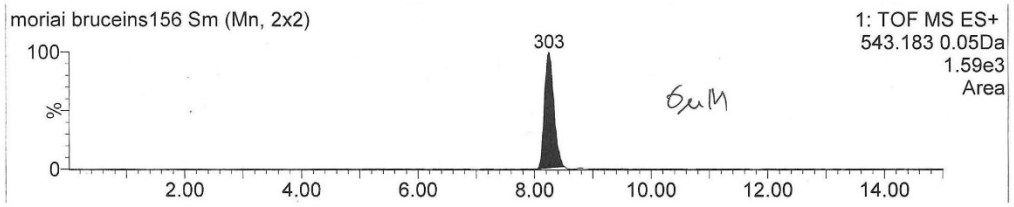
Figure S19. Lipolytic activity of bruceolide (**9**)

UPLC-MS chromatogram of quassinoids

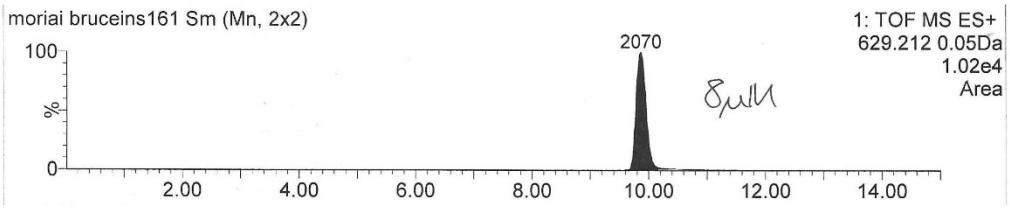
Brucein A (**1**)



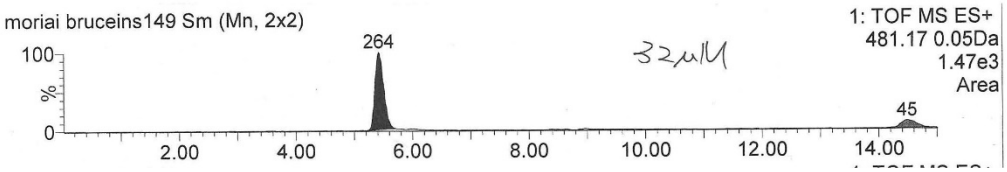
Brusatol (**2**)



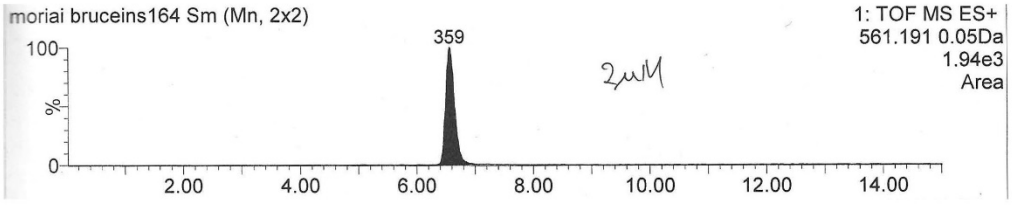
Bruceantinol (**3**)



Brucein B (**4**)



3′-Hydroxybrucein A (**5**)



Brucein C (**6**)

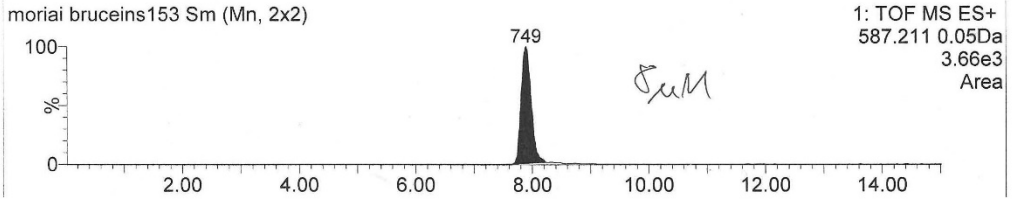
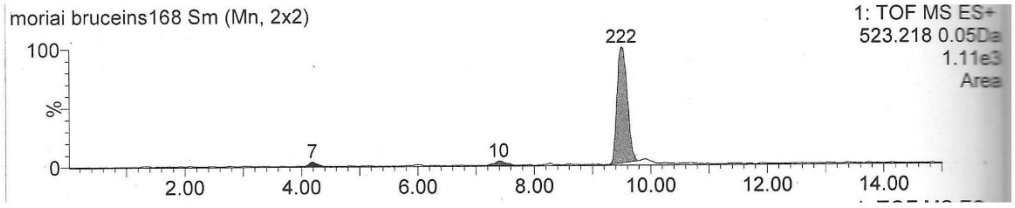
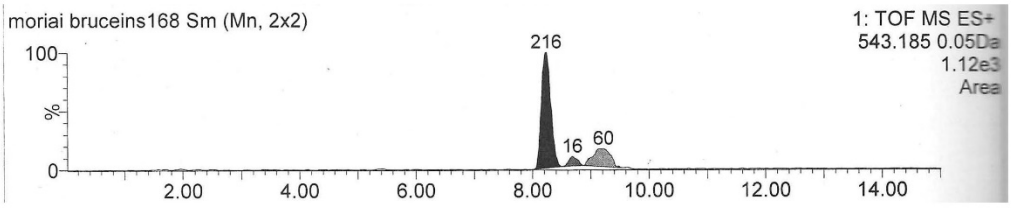


Figure S20. Extracted ion chromatogram of standard quassinoids.

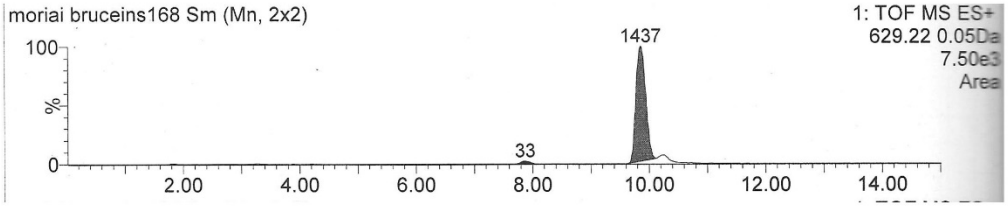
Brucein A (**1**)



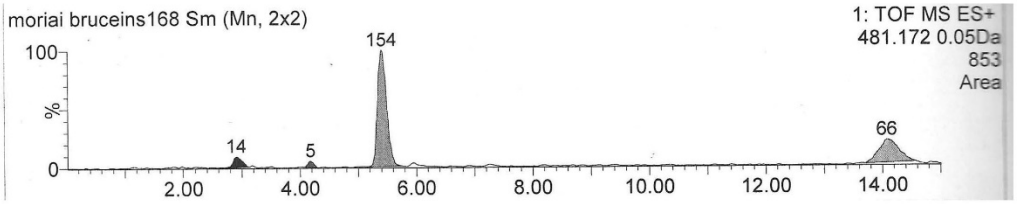
Brusatol (**2**)



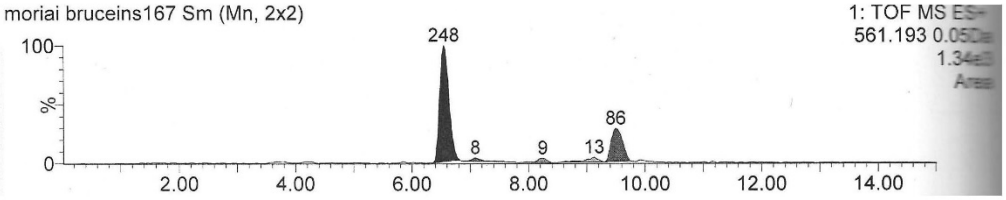
Bruceantinol (**3**)



Brucein B (**4**)



3′-Hydroxybrucein A (**5**)



Brucein C (**6**)

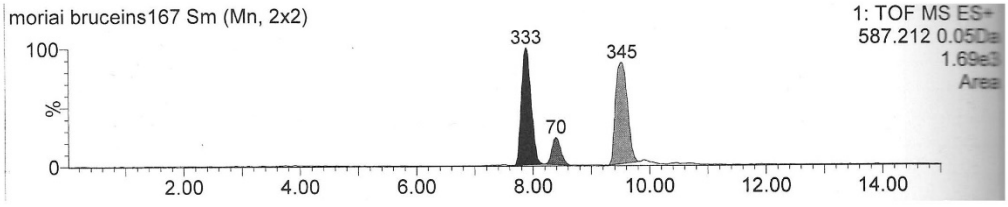


Figure S21. Extracted ion chromatogram of *B. javanica* seed extract.