Title: Biotransformation of major flavonoid glycosides in herb epimedii by the fungus *Cunninghamella blakesleana*

Author: XiuLan Xin Guang-Jun Fan Zheng Sun Ning Zhang Ye Li Rong Lan Liang Chen PeiPei Dong

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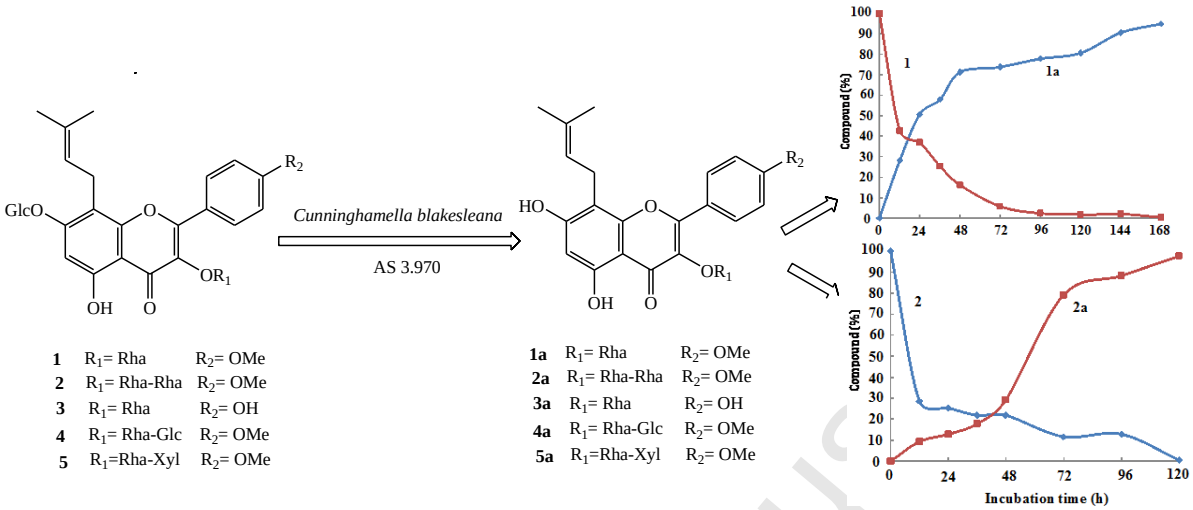
Graphical abstract



1 R_1= Rha R_2= OMe
2 R_1= Rha-Rha R_2= OMe
3 R_1= Rha R_2= OH
4 R_1= Rha-Glc R_2= OMe
5 R_1=Rha-Xyl R_2= OMe

1a R_1= Rha R_2= OMe
2a R_1= Rha-Rha R_2= OMe
3a R_1= Rha R_2= OH
4a R_1= Rha-Glc R_2= OMe
5a R_1=Rha-Xyl R_2= OMe

Highlight:

(1) Icariin (**1**), epimedin C (**2**), epimedoside A (**3**), epimedin A (**4**) and epimidin B (**5**) could be transformed to rare and bioactive products with the yield of above 95%.

(2) Some transformed products showed exhibited the more significant anti-osteoporosis activity.

(3) Our investigation provided an efficient biotransformation approach to enrich the rare and more biological active flavonoids in herb epimedi.

Biotransformation of major flavonoid glycosides in herb epimedii by the fungus *Cunninghamella blakesleana*

XiuLan Xin[a,+], Guang-Jun Fan [b,*], Zheng Sun[c], Ning Zhang [b,c], Ye Li [a], Rong Lan [a], Liang Chen[a], PeiPei Dong [c,*]

[a] College of Bioengineering, Beijing Polytechnic, Beijing 100029, China;

[b] The Second Affiliated Hospital of Dalian Medical University, Dalian 116044, China;

[c] College of Pharmacy, Dalian Medical University, Dalian 116044, P. R.China.

ABSTRACT:

Biotransformation of icariin (**1**), epimedin C (**2**), epimedoside A (**3**), epimedin A (**4**) and epimidin B (**5**), five major components of *E. koreanum*, were performed by using *Cunninghamella blakesleana*. And they could be metabolized efficiently to icariside II (**1a**), 2″-*O*-rhamnosylikarisoside II (**2a**), epimedoside b (**3a**), baohuoside VII (**4a**) and sagittatoside B (**5a**) with high yields of 95.1%, 97.7%, 93.7%, 95.8% and 96.4%, respectively. And these transformed products as major forms of herb epimedii *in vivo* exhibited the more significant anti-osteoporosis activities. Our method could be applied for enriching these rare flavonoids in herb epimedii, for further development of anti-osteoporosis medicines or functional foods.

Keywords: Biotransformation; Icariin; epimedin C; *Cunninghamella blakesleana;* epimedii

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Correspondence: Dr. Guang-Jun Fan, the Second Affiliated Hospital of Dalian Medical University, Dalian 116044, China. E-mail: faguangjun2014@163.com (G.J. Fan); maxc1978@126.com (P. P. Dong). + These authors contributed equally to this work.

1. Introduction

Herba Epimedii (Berberidaceae) is an important and frequently used as traditional Chinese medicine [1]. Its major bioactive constituents are flavonoid glycosides with the isopentene group at C-8, such as icariin, epimedin C, epimedoside A, epimedin A and epimidin B [2]. Among them, icariin and epimedin C have the natural abundance with the contents of 0.5% and 1% in herba epimedii, respectively [3]. However, due to their hydrophilicity of sugar moieties, these flavonoid glycosides are hardly absorbed from the small intestine of human, which resulting in the poor oral availabilities [4]. Only their secondary glycosides such as icariside II and 2″-O-rhamnosylikarisoside II, could be transported into the plasma and played an important bioactive role *in vivo* [5-8]. Meantime, these secondary glycosides, exhibited the more significant bioactivities including differentiation and proliferation of osteoblasts [9]; anti-osteoporosis [10], immunological function modulation [11], and anti-cancer [12]. So these secondary glycosides of epimedii had been paid more and more attention. Unfortunately, their contents were very low in crude material of herba epimedii, and limited their wide application in areas of medicines and foods. In previous investigation, some attempts for obtaining these secondary flavonoids had been reported. The chemical hydrolysis methods usually formed the cyclization products such as β-anhydroicaritin. And the enzymatic hydrolysis method could transform icariin to icariside II with the low yield of 47.5%, however other major epimedii flavonoid glycosides such as epimedin A, epimidin B and epimedin C, could not be transformed efficiently [13]. So it is very necessary to establish a systemic and efficient method to obtain the series of rare and bioactive secondary flavonoids glycosides of epimedii for development of new drugs or functional foods.

Biotransformation is a useful technique to modify structures of biologically

active compounds [14-21]. It has the advantage of high region-selectivity, mild reactions and avoiding the various steps for protection and deprotection to complicated structures and chiral centers of natural products [22-23].

In this study, biotransformation of icariin (**1**), epimedin C (**2**), epimedoside A (**3**), epimedin A (**4**) and epimidin B (**5**) by cell suspension of *C. blakesleana* were performed. And highly selective hydrolysis of C-7 was observed in the biotransformation process. Totally, icariside II (**1a**) and 2″-O-rhamnosylikarisoside II (**2a**), epimedoside b (**3a**), baohuoside VII (**4a**) and sagittatoside B (**5a**) were prepared with high yields of 95.1%, 97.7%, 93.7%, 95.8% and 96.4%, respectively. All metabolites were isolated and identified accurately by NMR and MS techniques. In addition, their cell proliferation activity for MC3T3-E1 cell was also investigated, and some metabolites (**1a**, **2a** and **5a**) exhibited the potential anti-osteoporosis activity.

2. Materials and methods

2.1. Apparatus and Reagents

^{1}H- and ^{13}C-NMR (nuclear magnetic resonance) spectra were recorded in DMSO-*d*6 using TMS as internal standard on a DRX-600 spectrometer (Bruker, Karlsruhe). ESIMS data were obtained by API3200-MS (AB SCIEX, New York, USA). Agilent 1100 series HPLC equipped with Diode array detector at 254 nm was used (Agilent, New York, USA). Silica gel (200-300 mesh) was purchased from Qingdao Marine Chemical Group, Qingdao, China. ODS (Chromatorex, size 100-200 mesh) was purchased from Fujian Silysia Chemical Group, Fujian, China. All solvents were AR grade from Tianjin Kermel Company. The crude materials of *E. koreanum* were purchased from Hongjiu BioTech Co. Jilin Province of China.

2.2. Microorganisms and Culture medium

C. blakesleeana was purchased from Chinese General Microbiological Culture Collection Center in Beijing, China. Biotransformation experiments were performed in potato medium, which was made by the following composition (L): 200 g potato and 20 g glucose.

2.3. Culture and biotransformation procedures

Mycelia of *C. blakesleeana* from agar slants (2 cm^2) were transferred to 100 mL of medium and cultured at 30 °C with 175 rpm for 36 h to make a stock inoculum. Then 10 mL volume of the inoculum was added to 1000 mL of liquid medium, and the flask was placed on rotary shaker operating at 175 rpm and 30 °C. After 36h of preculture, 100 mg of icariin with 2 mL methanol added into 2L of culture medium for preparative biotransformation with the final concentration of 50mg/L. The incubation was continued for 5 additional days. The culture was filtered, and the filtrate was extracted with same volume of EtOAc for five times. And the suspension cells of *C. blakesleeana* (5.2 g) were also extracted by using EtOAc (1000 mL). Then all extraction of EtOAc were collected and concentrated under the reduced pressure. The residues (1.6 g) were applied to an ODS column (200g, 10×50cm) and eluted with MeOH-H_2O (in a gradient manner from 10% to 90%, at a flow rate of 2.0 mL/min). Totally, 90.1 mg of icariside II (96.5% purity) was obtained from fraction of 60% MeOH (500 mL). Similarly, 100 mg of epimedin C (**2**), epimedoside a (**3**), epimedin A (**4**) and epimidin B (**5**), respectively, were added into 2L of culture medium of *C. blakesleeana*. Other processes of collection and isolation for the target compounds were same with that of icariin. Finally, 2″-*O*-rhamnosyli- karisoside II (**2a**, 95mg, 95% purity, Rt=16.5min), epimedoside B (**3a**, 83.5 mg, 94% purity, Rt=14.1min), baohuoside VII (**4a**, 89.5mg, 96% purity, Rt=10.2min) and sagittatoside B (**5a**, 85.1mg, 95% purity, Rt=7.5min) were prepared by preparative

HPLC in an isocratic manner of 60% MeOH with a flow rate of 2.0 mL/min.

2.4. HPLC analysis

The samples were analyzed on a DINOEX Ultimate 3000 instrument HPLC equipped with a Kromasil C-18 column, 4.6 mm × 250 mm (5μm), and diode array detector (DAD) at 270 nm, and the mobile phase was composed by solvents A (methanol) and B (0.3% acetic acid) in gradient elution. The gradient program was as follows: initial 0-5min, using a constant elution A-B (45: 55, *v/v*), then 5-10 min, linear change from A-B (45: 55, *v/v*) to A-B (80: 20, *v/v*); and then 10-20 min, using a constant elution A-B (80: 20, *v/v*); next 20-30 min, linear change to A-B (80: 20, *v/v*) to A-B (90: 10, *v/v*); then 30-35 min, using a constant elution A-B (90: 10, *v/v*). The flow rate was 0.8 mL/min and column temperature was 30℃.

2.5. Bioassay

Mouse osteoblastic cell line (MC3T3-E1, purchased from the Chinese Academy of Sciences Cell Bank) was cultured under a humidified atmosphere of 5% CO_2 at 37 °C with the Dulbecco's modified Eagle's medium (DMEM) containing 1% penicillin and 1% streptomycin and 10% fetal bovine serum. In this study, cell proliferation of MC3T3-E1 cell was measured by MTT assay. Briefly, cells were maintained in growth media for 24 h at 5% CO_2. And then, the cells were treated with the three different concentrations (1, 10 and 100μM) of the test compounds for 24 h. Finally, MTT (5 mg/mL) was added to the cell cultures (1:10) and samples were incubated at 37°C for 4h. In addition, the cells in normal culture medium were treated with same volume of DMSO as control condition, the values of which was set as 100%.

. The absorbance was measured in an optical 96-well microplate reader at the wavelength of 570 nm. All the data was present as the mean of three experiments

(n=3).

3. Results and discussion

3.1. Preliminary screening for biotransformation

15 strains of filamentous fungi from nine genera were initially screened for their abilities to transform icariin by TLC and HPLC chromatography. Among the cultures screened, *M. spinosus*, *F. solani* and *P. janthinellum*, showed the ability of transforming icariin into more polar metabolites. However, *C. blakesleeana* was found to be able to convert substrates into one main non-polar metabolite with high yield and low yield of by-products. Therefore, it was selected for the preparative biotransformation of substrates including icariin (**1**), epimedin C (**2**), epimedoside A (**3**), epimedin A (**4**) and epimidin B (**5**) in this study. (Figs 2 and 3)

3.2. Time course of biotransformation of substrates

Fig.4A showed the biotransformaton kinetic of icariin after administration of 50 mg/L. After incubation for 168h with *C. blakesleeana* it was completely transformed to icariside II (**1a**) with the conversion of 95.06% by HPLC analysis. In order to investigate the capability of *C. blakesleeana* to metabolize icariin, the effects of pH, culture temperature, substrate concentration and reaction specificity in the biotransformation process were also investigated (Fig. 4). Our results suggested that the optimum pH value should be at 6–7 with the highest transformation rate. And the preferred temperature was 28-30℃. However, when the concentration was increased to 80 mg/L, the substrates were not completely transformed with the rate of 63.18%.

Similarly, *C. blakesleeana* also exhibited the good ability to transform epimedin C to 2″-*O*-rhamnosylikarisoside II (**2a**). After incubation for 120 h with *C. blakesleeana*, epimedin C was completely transformed with the yield of 97.67% by HPLC analysis, which suggesting that *C. blakesleeana* had the high selectivity for

glucose hydrolysis of epimedium flavonoids at C-7. Finally, the optimum pH value, culture temperature and substrate concentration were determined at pH=7, 28-30℃ and 100 mg/L (supplement data, Figs 4).

In order to elucidate the relationship of biotransformation ability and substrate structures of epimedium flavonoids. The ability of *C. blakesleeana* to transform epimedoside A (**3**), epimedin A (**4**) and epimidin B (**5**), three major derivatives of icariin in epimedium, were also investigated. After incubation for 168h, they were completely transformed to epimedoside B (**3a**), baohuoside VII (**4a**) and sagittatoside B (**5a**) with the yield rates of 93.7%, 95.8% and 96.4%, respectively. The results were illustrated in Fig. 3.

3.3. Identification of biotransformation products

Incubation of icariin (**1**) and other four flavonoid glycosides epimedin C (**2**), epimedoside A (**3**), epimedin A (**4**) and epimidin B (**5**) with *C. blakesleeana* for 7 days yielded five products and their structures were identified as icariside II (**1a**), 2″-O- rhamnosylikarisoside II (**2a**), sagittatoside B (**3a**) baohuoside VII (**4a**) and epimedoside b (**5a**) (Fig. 1), respectively, and by comparing with the literatures [24].

3.3.1. Icariside (**1a**): yellow needle crystal, ^{1}H-NMR (DMSO-d_6, 600MHz): ^{1}H-NMR (DMSO-d_6, 600MHz): 5.16 (1H,t, J=6.0 Hz, H-12), 5.27 (1H, d, J=1.6 Hz, H-1´´), 6.32(1H, s, H-6), 7.13 (2H, d, J=9.2 Hz, H-3´, H-5´), 7.87 (2H, d, J=9.2 Hz, H-2´,H-6´), 10.84(1H, br. s, 7-OH), 12.53 (1H, s, 5-OH), 3.85 (3H,s, OCH_3), 1.69 (3H, s, H-15), 1.61 (3H, s, H-14) and 0.78 (3H, d, J = 6.6 Hz, H-6´´). ^{13}C-NMR (DMSO-d_6, 150 MHz): 156.6 (C-2), 134.4 (C-3), 178.0 (C-4), 161.6 (C-5), 98.3 (C-6), 161.2 (C-7), 106.0 (C-8), 153.7 (C-9), 104.1 (C-10), 21.1 (C-11), 122.3 (C-12), 131.1 (C-13), 25.4 (C-14), 17.8 (C-15), 122.4 (C-1´), 130.43 (C-2´, C-6´), 114.1 (C-3´ and C-5´), 158.8 (C-4´), 55.5 (OCH_3), 101.1 (Rha-1´), 70.7 (Rha-2´), 70.4

(Rha-3´), 71.2 (Rha-4´), 70.4 (Rha-5´), 17.4 (Rha-6´).

3.3.2. 2″-O-rhamnosylikarisoside II (**2a**): yellow powder (methanol), ^{1}H-NMR (DMSO-d_6,600MHz):12.57(1H,s,OH-5), 7.86 (2H, d, J=8.4Hz, H-2', H-6'), 7.13 (2H, d, J = 4.5 Hz, H-3', H-5'), 6.93 (1H, s, H-6'), 6.34 (1H,s), 5.37 (1H, s), 5.15 (1H, d, J = 7.0 Hz), 3.85 (3H, s, OCH_3-4'),1.68 (3H, s), 1.62 (3H, s), 0.81 (3H, d, J = 5.4Hz). ^{13}C-NMR (DMSO-d_6, 150 MHz): 156.8 (C-2), 134.5 (C-3), 178.0 (C-4), 161.4 (C-5), 98.5 (C-6), 161.8 (C-7), 106.0 (C-8), 153.8 (C-9), 104.2 (C-10), 21.3 (C-11), 122.3 (C-12), 131.1 (C-13), 25.5 (C-14), 17.9 (C-15), 122.3(C-1'), 130.5 (C-2', C-6'), 114.2 (C-3' and C-5'), 130.5 (C-4'), 55.6 (OCH_3), 101.7 (Rha-1″), 75.6 (Rha-2″), 70.3 (Rha-3″), 72.0 (Rha-4″), 70.2 (Rha-5″), 17.7 (Rha-6″), 100.8 (Rha'-1‴), 70.6 (Rha'-2‴), 70.7 (Rha'-3‴), 71.4 (Rha'-4‴), 68.9 (Rha'-5‴) and 17.6 (Rha'-6‴).

3.3.3 Epimedoside B (**3a**): yellow powder (methanol), ^{1}H-NMR (DMSO-d_6, 600MHz): 12.56(1H, s, OH-5), 10.90 (1H, s, OH-7), 10.27 (1H, s, OH-4'), 7.76 (1H, d, J=8.4Hz, H-2'), 7.14(2H, d, J= 4.5Hz, H-3', H-5'), 6.93(1H,s,H-6'), 6.32 (1H, s), 5.27 (1H, s), 5.14 (1H, t, J = 6.6Hz), 1.67(3H,s), 1.62(3H, s), 0.80 (3H,d, J=6.0 Hz). ^{13}C-NMR (DMSO-d_6, 150 MHz): 157.73 (C-2), 134.2 (C-3), 178.1 (C-4), 160.1 (C-5), 98.4 (C-6), 161.7 (C-7), 106.1 (C-8), 153.9 (C-9), 104.2 (C-10), 21.3 (C-11), 122.4 (C-12), 130.5 (C-13), 25.6 (C-14), 17.9 (C-15), 120.9(C-1'), 130.7 (C-2', C-6'), 115.5 (C-3' and C-5'), 160.0 (C-4'), 102.0 (Rha-1″), 70.5 (Rha-2″), 70.8 (Rha-3″), 71.2 (Rha-4″), 70.2 (Rha-5″) and 17.6(Rha-6″).

3.3.4. Baohuoside VII (**4a**): yellow powder (methanol), ^{1}H-NMR (DMSO-d_6, 600MHz): 6.34 (1H,s), 5.54 (1H,s), 5.16(1H, t, J = 7.2Hz), 4.25 (1H, d, J=7.8Hz), 3.86 (3H, s), 1.68 (3H,s), 1.62 (3H,s), 0.86 (3H, d, J = 6.0Hz). ^{13}C-NMR (DMSO-d_6, 150 MHz): 156.7 (C-2), 134.2 (C-3), 178.0 (C-4), 161.5 (C-5), 98.5 (C-6), 161.8

(C-7), 106.0 (C-8), 153.9 (C-9), 104.9 (C-10), 21.4 (C-11), 122.4 (C-12), 131.1 (C-13), 25.5 (C-14), 17.9 (C-15), 122.4(C-1'), 130.6 (C-2', C-6'), 114.2 (C-3' and C-5'), 158.9 (C-4'), 55.6 (OCH_3), 101.8 (Rha-1''), 81.2 (Rha-2''), 70.4 (Rha-3''), 71.3 (Rha-4''), 69.9 (Rha-5''), 17.6 (Rha-6''), 104.2 (Glc-1'), 73.8 (Glc-2'), 76.9 (Glc-3'), 69.2 (Glc-4'), 76.3 (Glc-5') and 61.1(Glc-6').

3.3.5: Sagittatoside B (**5a**): yellow powder (methanol), ^{1}H-NMR (DMSO-d_6, 600MHz): 6.34(1H,s), 5.34(1H,s), 5.16(1H,t, J=6.6Hz),4.17(1H,d, J=1.8Hz), 3.86 (3H, s), 1.68(3H,s), 1.62(3H,s), 0.86(3H, d, J=6.6Hz). ^{13}C-NMR (DMSO-d_6, 150 MHz): 156.6 (C-2), 134.6 (C-3), 178.1 (C-4), 161.5 (C-5), 98.5 (C-6), 161.8 (C-7), 106.1 (C-8), 153.8 (C-9), 104.1 (C-10), 21.3 (C-11), 122.3 (C-12), 131.1 (C-13), 25.5 (C-14), 17.8 (C-15), 122.4(C-1'), 130.4 (C-2', C-6'), 114.2 (C-3' and C-5'), 158.9 (C-4'), 55.5 (OCH_3), 101.1 (Rha-1''), 80.7 (Rha-2''), 70.4 (Rha-3''), 71.8 (Rha-4''), 70.4 (Rha-5''), 17.5 (Rha-6''), 106.5 (Xyl-1'), 73.8 (Xyl-2'), 76.3 (Xyl-3'), 69.4 (Xyl-4') and 65.9 (Xyl-5').

3.4 Bioassay

In this study, we evaluated the effects of compounds (**1-5** and **1a-5a**) on the osteoblast function using MC3T3-E1 cells. Our results indicated that the bioactivities of secondary aglycones were more significant than these of flavonoid glycosides of *E. koreanum* (Fig. **5**). At the concentration of 100μM, the activity of transformed products (**1a-5a**) exhibited the significant anti-osteoporosis activity than these of substrates (**1-5**), especially for **1a**, **2a** and **5a** with the mean survival rates of 137%, 143% and 158%, respectively (n=3). According to our results, compounds **1a**, **2a** and **5a** may be used new natural resources to develop the anti-osteoporosis agent.

4. Conclusions

C. blakesleana exhibited the high selectivity of hydrolysis glucose of C-7 such as

icariin (**1**), epimedin C (**2**), epimedoside A (**3**), epimedin A (**4**) and epimidin B (**5**), to produce some rare flavonoid glycosides of epimedii including icariside II (**1a**), 2″-O-rhamnosylikarisoside II (**2a**), sagittatoside B (**3a**) baohuoside VII (**4a**) and epimedoside b (**5a**), with the yield rates of above 95%. And these transformed products showed more anti-osteoporosis than these of substrates (**1**-**5**). Our investigation provided an efficient biotransformation approach to enrich the rare and better bioactive flavonoids in herb epimedii, for further development of anti-osteoporosis medicines and foods.

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Figure captions

Figure 1. The biotransoformation pathway of major epimedii flavonoids by *C. blakesleeana*

Figure 2. HPLC chromatograms of the blank of M. spinosus AS 3.3450 (A) and icariin (B), administrating 1 for 5 days (C), epimedin C (D), administrating 1 for 5 days (E).The DAD spectra of compounds1-2 and 1a-2a.

Figure 3. HPLC chromatograms of compound **3** (A), administrating **3** for 120h (B), compound 4 (C), administrating **4** for 120h (D), Compound **5** (E), administrating **5** for 120h (F).

Figure 4. The time course and effects of pH and temperature for icariin (**1**) and epimedin C (**2**) by *Cunninghamella blakesleana*. **(A)** Time course of biotransformation of icariin; **(B)** Time course of biotransformation of epimedin C; **(C)** Effects of pH value on the biotransformation of **1** and **2**; **(D)** Effects of temperature values.

Figure 5. Survival rate of compounds1-5 and 1a-5a on MC3T3-E1 cells by MTT method. Comparison test, *$p < 0.05$; **$p < 0.01$

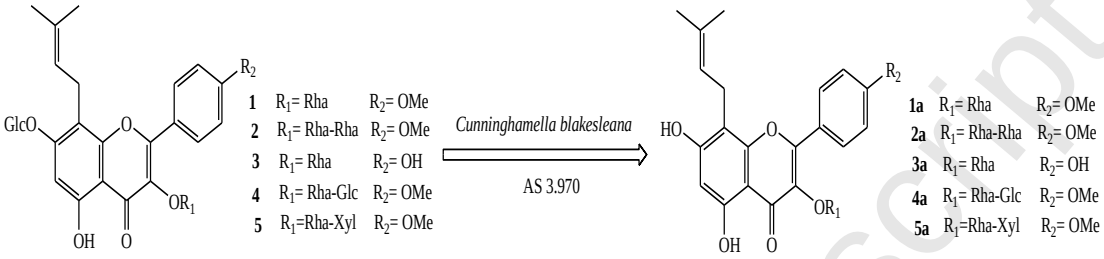


Figure **1**. The biotransoformation pathway of major epimedii flavonoids by *C. blakesleeana*

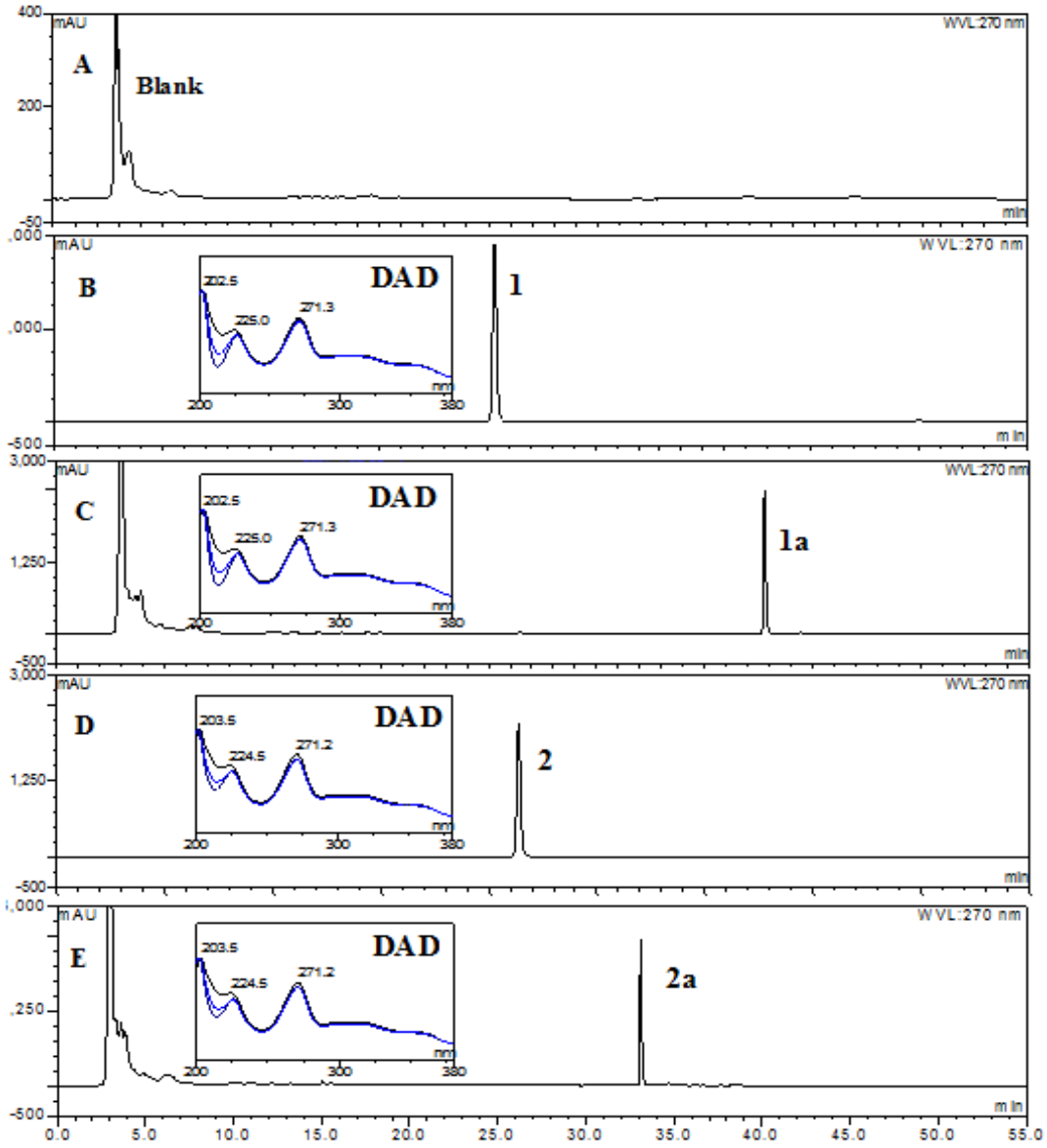


Figure. 2. HPLC chromatograms of the blank of M. spinosus AS 3.3450 (A) and icariin (B), administrating 1 for 5 days (C), epimedin C (D), administrating 1 for 5 days (E).The DAD spectra of compounds1-2 and 1a-2a.

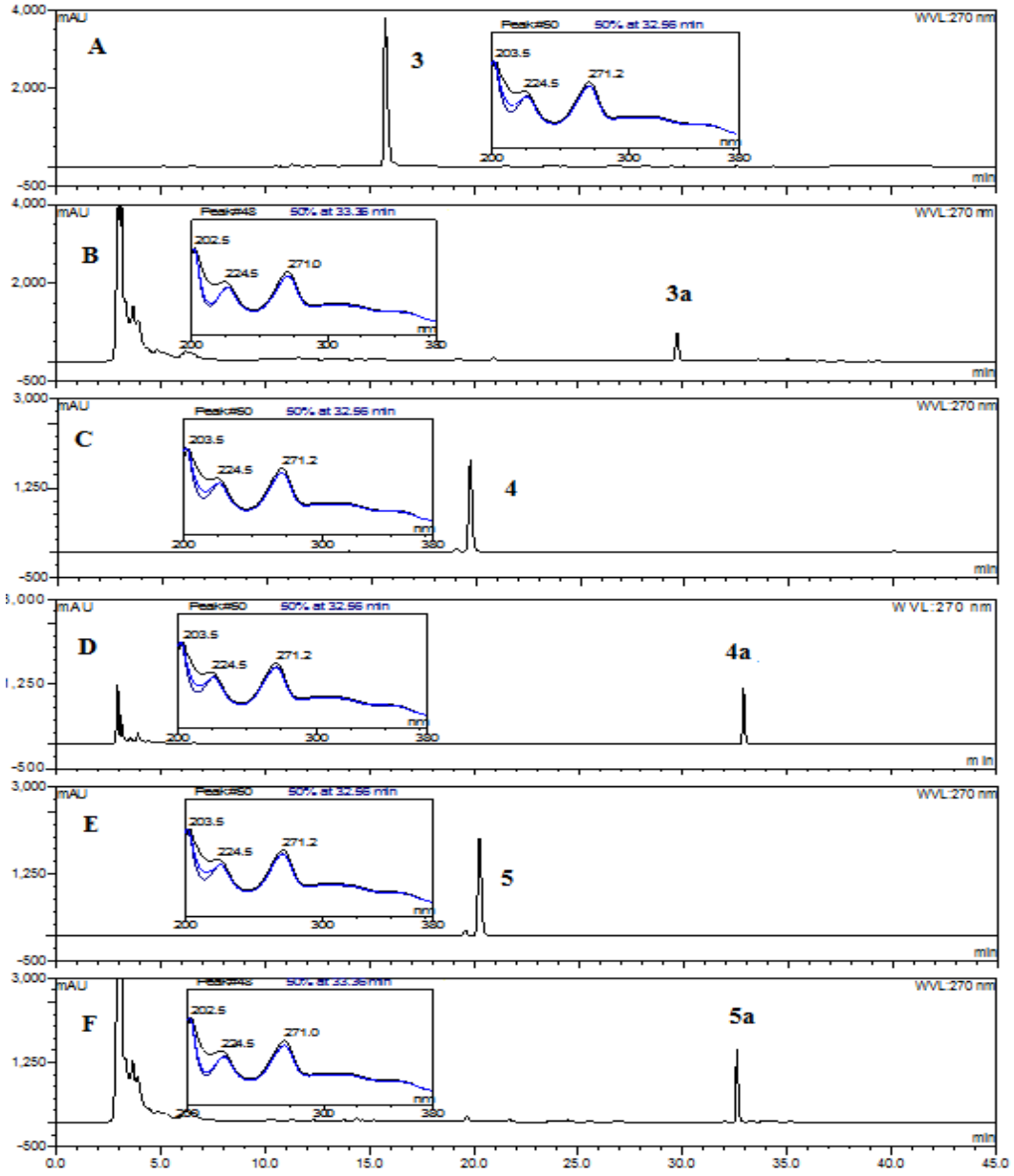


Figure 3. HPLC chromatograms of compound **3** (A), administrating **3** for 120h (B), compound 4 (C), administrating **4** for 120h (D), Compound **5** (E), administrating **5** for 120h (F).

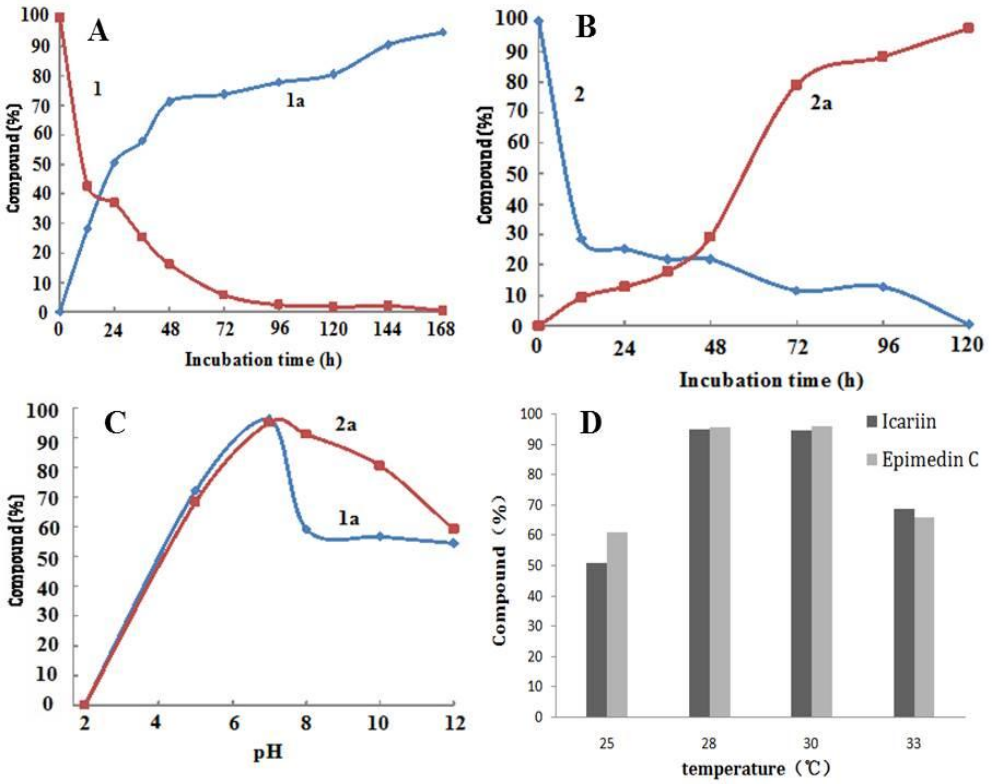


Figure 4. The time course and effects of pH and temperature for icariin (**1**) and epimedin C (**2**) by *Cunninghamella blakesleana*. **(A)** Time course of biotransformation of icariin; **(B)** Time course of biotransformation of epimedin C; **(C)** Effects of pH value on the biotransformation of **1** and **2**; **(D)** Effects of temperature values.

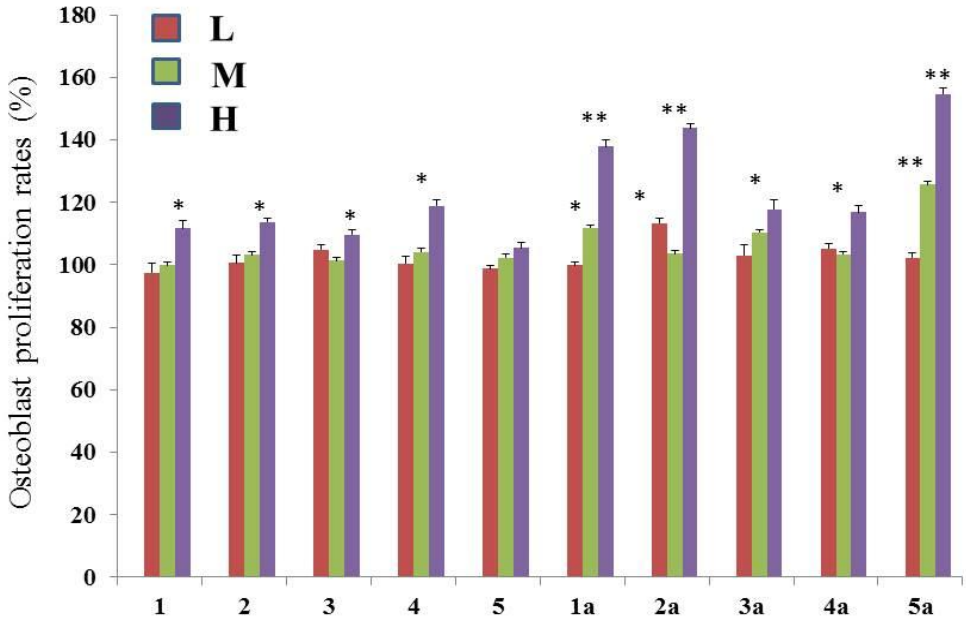
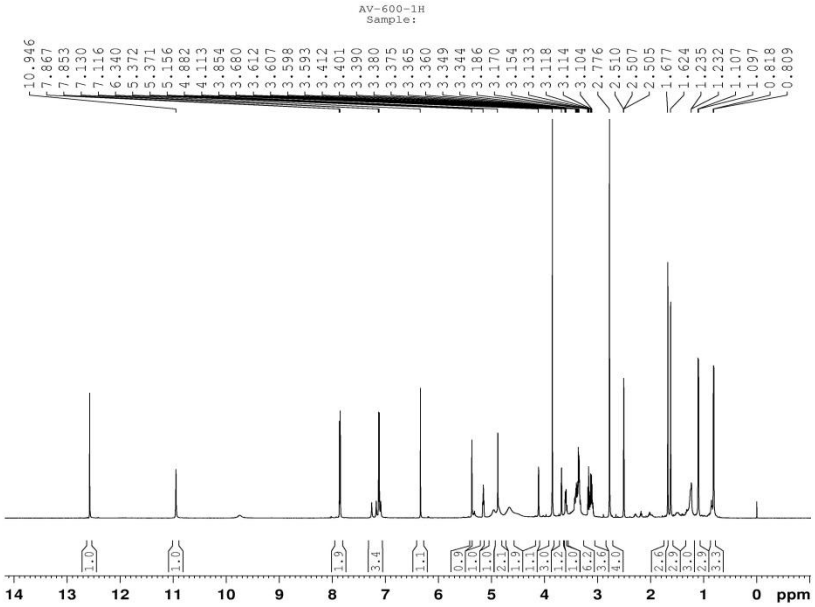


Figure 5. Survival rate of compounds1-5 and 1a-5a on MC3T3-E1 cells by MTT method. Comparison test, *$p < 0.05$; **$p < 0.01$

Supplementary data

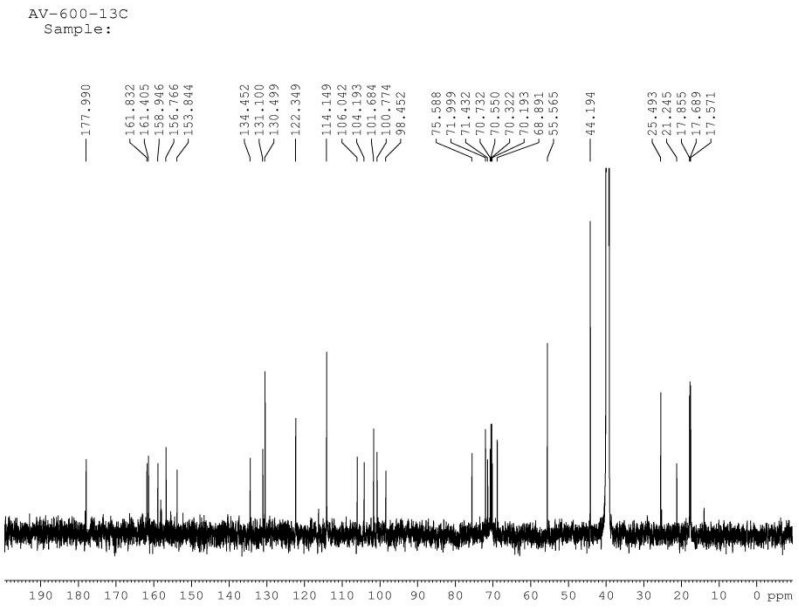
Compound 4a ^{1}H-NMR spectrum



BRUKER

NAME	WGZH-3
EXPNO	3
PROCNO	1
Date_	20101109
Time	16.15
INSTRUM	spect
PROBHD	5 mm PABBO BB-
PULPROG	zg30
TD	65536
SOLVENT	DMSO
NS	8
DS	2
SWH	13227.514 Hz
FIDRES	0.201836 Hz
AQ	2.4773486 sec
RG	181
DW	37.800 usec
DE	6.50 usec
TE	298.2 K
D1	1.00000000 sec
TD0	1
======== CHANNEL f1 ========	
NUC1	1H
P1	11.10 usec
PL1	-4.00 dB
PL1W	34.70265579 W
SFO1	600.1330006 MHz
SI	32768
SF	600.1299957 MHz
WDW	EM
SSB	0
LB	0.30 Hz
GB	0
PC	1.00

Compound 4a ^{13}C-NMR spectrum



BRUKER

NAME	WGZH-3
EXPNO	2
PROCNO	1
Date_	20101109
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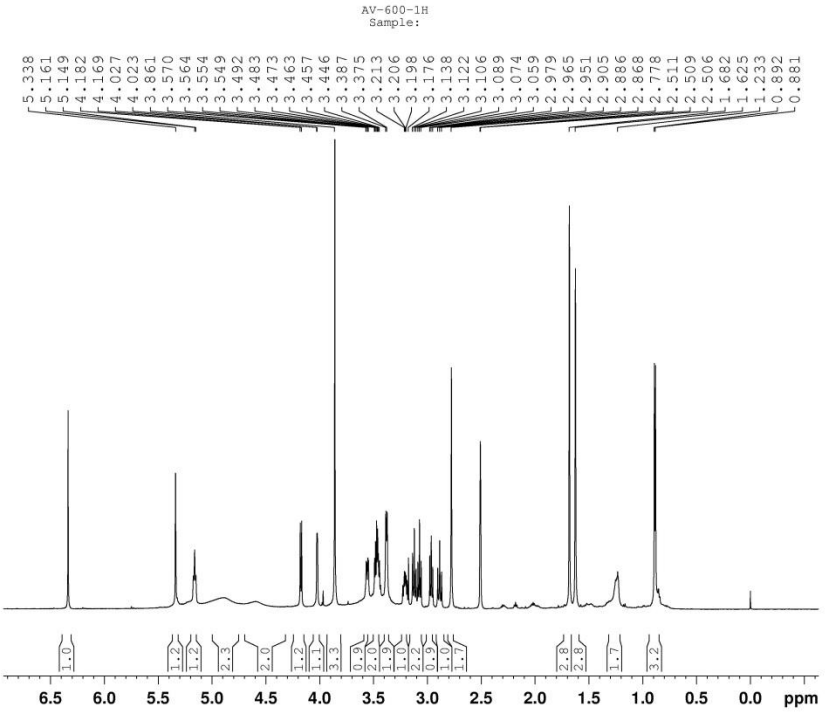
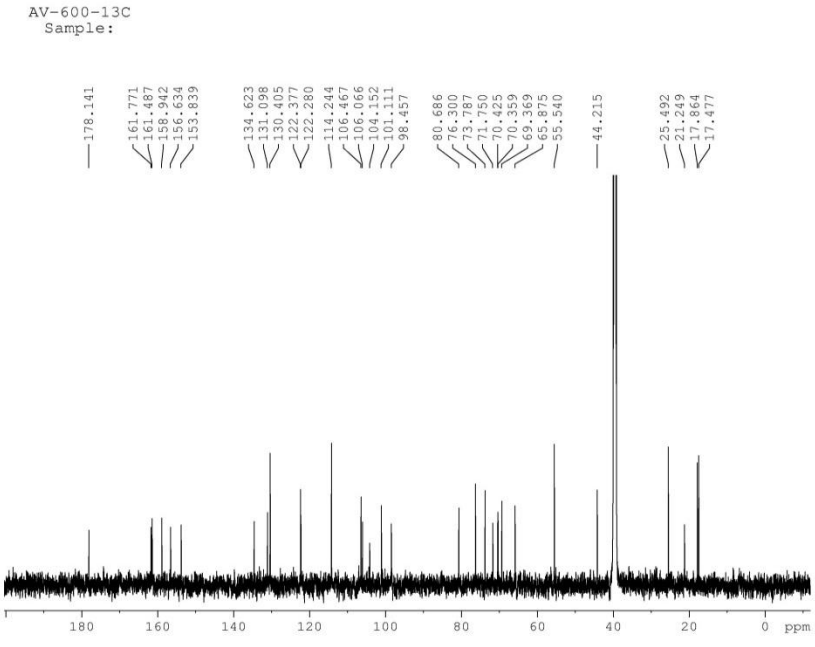
INSTRUM	spect
PROBHD	5 mm PABBO BB-
PULPROG	zgpg30
TD	65536
SOLVENT	DMSO
NS	878
DS	2
SWH	45454.547 Hz
FIDRES	0.693581 Hz
AQ	0.7209570 sec
RG	20600
DW	11.000 usec
DE	6.50 usec
TE	298.2 K
D1	2.00000000 sec
D11	0.03000000 sec
TD0	1
========= CHANNEL f1 ========	
NUC1	13C
P1	6.00 usec
PL1	1.00 dB
PL1W	83.20243835 W
SFO1	150.9178993 MHz
========= CHANNEL f2 ========	
CPDPRG2	waltz16
NUC2	1H
PCPD2	80.00 usec
PL2	-4.00 dB
PL12	13.16 dB
PL13	16.00 dB
PL2W	34.70265579 W
PL12W	0.66736388 W
PL13W	0.34702653 W
SFO2	600.1324005 MHz
SI	32768
SF	150.9028664 MHz
WDW	EM
SSB	0
LB	3.00 Hz
GB	0
PC	1.40

Compound 5a ^{1}H-NMR spectrum



NAME	WGZH-2
EXPNO	3
PROCNO	1
Date_	20101109
Time	17.10
INSTRUM	spect
PROBHD	5 mm PABBO BB-
PULPROG	zg30
TD	65536
SOLVENT	DMSO
NS	8
DS	2
SWH	13227.514 Hz
FIDRES	0.201836 Hz
AQ	2.4773486 sec
RG	181
DW	37.800 usec
DE	6.50 usec
TE	298.2 K
D1	1.00000000 sec
TD0	1
======== CHANNEL f1 ========	
NUC1	1H
P1	11.10 usec
PL1	-4.00 dB
PL1W	34.70265579 W
SFO1	600.1330006 MHz
SI	32768
SF	600.1299948 MHz
WDW	EM
SSB	0
LB	0.30 Hz
GB	0
PC	1.00

Compound 5a ^{13}C-NMR spectrum



NAME	WGZH-2
EXPNO	2
PROCNO	1
Date_	20101109
Time	17.00
INSTRUM	spect
PROBHD	5 mm PABBO BB-
PULPROG	zgpg30
TD	65536
SOLVENT	DMSO
NS	405
DS	2
SWH	45454.547 Hz
FIDRES	0.693581 Hz
AQ	0.7209570 sec
RG	20600
DW	11.000 usec
DE	6.50 usec
TE	298.2 K
D1	2.00000000 sec
D11	0.03000000 sec
TD0	1
======== CHANNEL f1 ========	
NUC1	13C
P1	6.00 usec
PL1	1.00 dB
PL1W	83.20243835 W
SFO1	150.9178993 MHz
======== CHANNEL f2 ========	
CPDPRG2	waltz16
NUC2	1H
PCPD2	80.00 usec
PL2	-4.00 dB
PL12	13.16 dB
PL13	16.00 dB
PL2W	34.70265579 W
PL12W	0.66736388 W
PL13W	0.34702653 W
SFO2	600.1324005 MHz
SI	32768
SF	150.9028656 MHz
WDW	EM
SSB	0
LB	3.00 Hz
GB	0
PC	1.40