

Supporting Information

Rec. Nat. Prod. 20:1 (2026):e25063553

A new isoflavone from the ethyl acetate extracts of *Apios fortunei* Maxim and anti-inflammatory activity

Jie Xiao^{1#}, Duzhun Zou^{1, 2#}, Hongliang Zeng¹, Yibing Hu¹, Shuihan
Zhang^{1*} and Fei Cheng^{1*}

¹*Institute of Chinese Materia Medica, Hunan Academy of Chinese Medicine, Changsha, Hunan
410013, P. R. China*

²*Department of Pharmacy, The First Traditional Chinese Medicine Hospital of Yiyang, Changsha,
Hunan 413000, P. R. China*

Table of Contents	Page
General experimental procedures	2
Cytotoxicity evaluation	2
The levels of NO TNF- α released by RAW264.7 cells were detected by the kit.	2
Figure S1: HRESIMS spectrum of compound 1	3
Figure S2: ¹ H NMR spectrum of compound 1 (CD ₃ OD, 600MHz)	3
Figure S3: ¹³ C NMR spectrum of compound 1 (CD ₃ OD, 150MHz)	4
Figure S4: DEPT135 spectrum of compound 1 (CD ₃ OD, 150MHz)	4
Figure S5: ¹ H- ¹ H COSY spectrum of compound 1	5
Figure S6: HSQC spectrum of compound 1	5
Figure S7: HMBC spectrum of compound 1	6
Figure S8: Scifinder similarity report for compound 1	6
Table S1 NMR data of compound 1 (Apiosisoflavone A) and Licoagroisoflavone	7

General experimental procedures

NMR spectra were measured on a Bruker AV-600 MHz spectrometer (Bruker, Karlsruhe, Germany) using CD₃OD as solvent and tetramethylsilane (TMS) as an internal standard. CD spectra were recorded on an Applied Photophysics spectrometer (Chirascan, New Haven, USA). Mass spectroscopy (HRESIMS) was recorded with an Agilent Technologies liquid chromatograph connected to Q-TOF mass spectra. Analytical HPLC was conducted on an Agilent 1100 series equipped with a DAD detector and a SilGreen C₁₈ column (5 µm, 250 mm × 4.6 mm). Semi-preparative HPLC was performed using an Agilent 1120 system equipped with a SilGreen C₁₈ column (5 µm, 250 mm × 10 mm). Silica gel (200-300 or 80-100 mesh; Qingdao Peremanent Sea Silica Ltd., Qingdao, China) and Sephadex HW-40C (TOYOPEARL TOSOH, Tokyo, Japan) were applied in column chromatography. Unless stated otherwise, all the chemical solvents were analytical grade (Cologne Chemical Co., Ltd., Chengdu, China).

Cytotoxicity evaluation

The RAW264.7 cell suspension was inoculated into 96-well plates at a rate of 100 µl per well for 3×10⁴ cells. After adhesion, different concentrations (0 µm, 25 µm, 50 µm, 100 µm) of flavonoid compounds were added and treated for 24 hours. The supernatant was then discarded. Add 100 µl of cck8 solution diluted 10 times to each well. Incubate in the incubator for 1 hour, detect the absorbance at a wavelength of 450 nm using an enzyme-linked immunosorbent assay (ELISA) reader, and calculate the cell viability of different treatments. Cell viability (%)=(experimental group OD - blank group OD)/(control group OD - blank group OD) × 100.

The levels of NO TNF-α released by RAW264.7 cells were detected by the kit

According to the cytotoxicity test, RAW264.7 cells were seeded in 6-well plates, with 5×10⁵ cells in each well. Two hours after adhesion, the patients were divided into the blank control group, the LPS group, the LPS+ dexamethasone group, the LPS+ low-dose flavonoid group, and the LPS+ high-dose flavonoid group. After continuing to culture for 24 hours, collect the cell supernatant and centrifuge at 15,000 r/min for 20 minutes. Calculate the contents of secreted NO and TNF-α according to the operation instructions of the kit.



Figure S1: HRESIMS spectrum of compound **1**

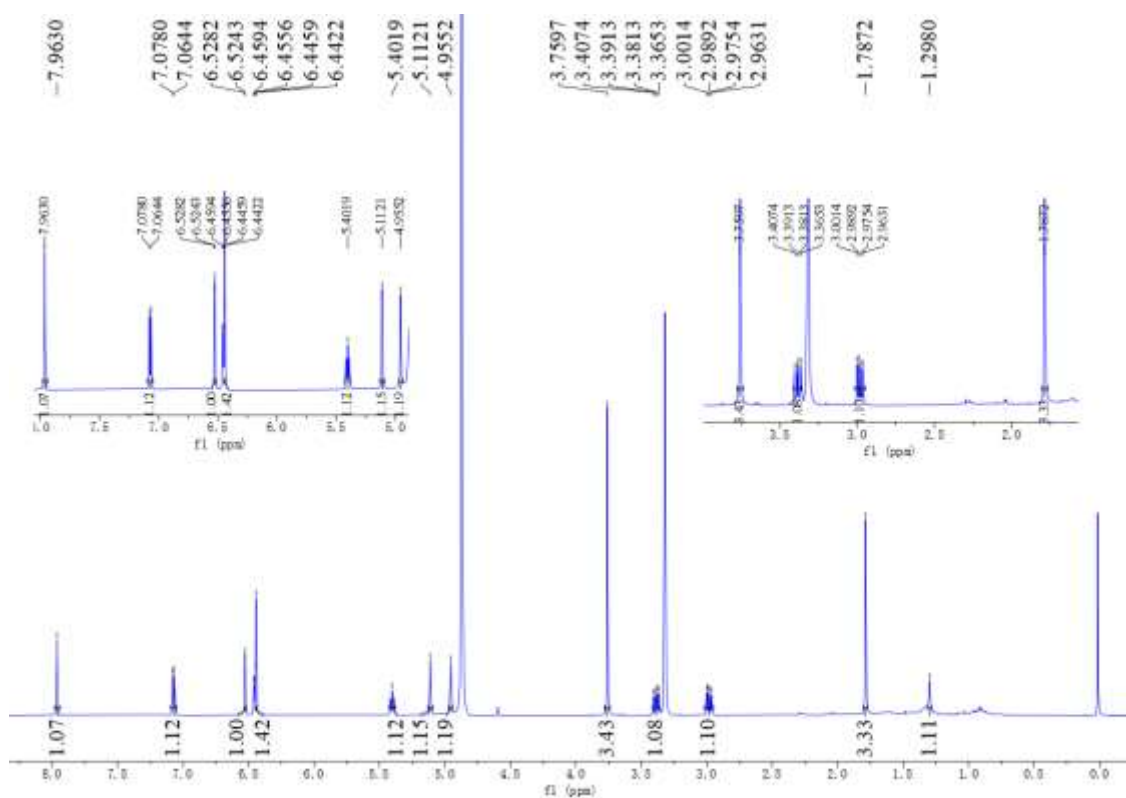


Figure S2: ^1H NMR spectrum of compound **1** (CD_3OD , 600MHz)

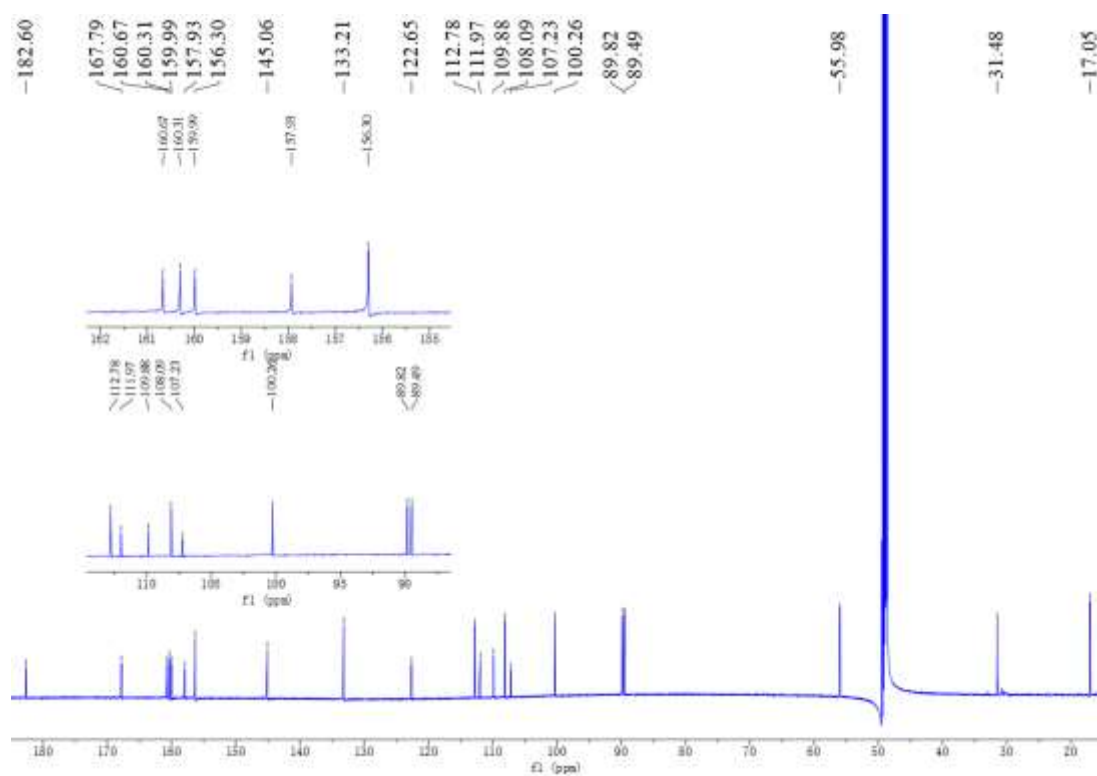


Figure S3: ^{13}C NMR spectrum of compound **1** (CD_3OD , 150MHz)

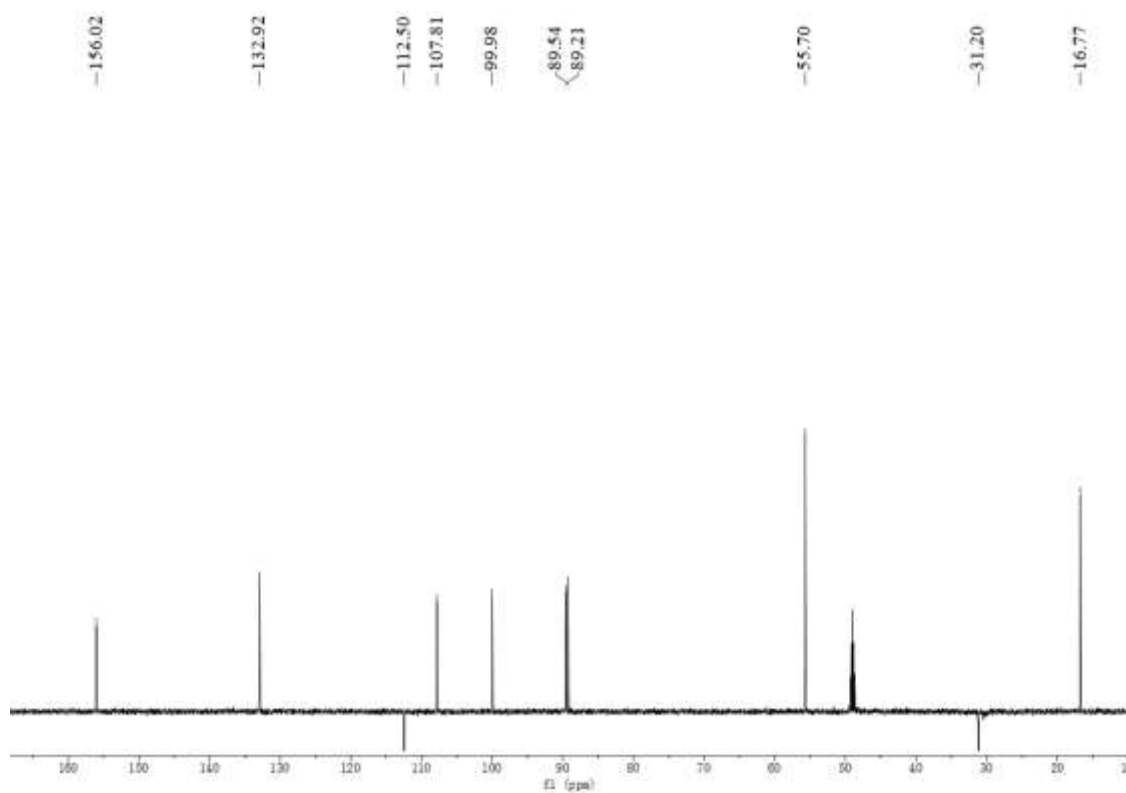


Figure S4: DEPT135 spectrum of compound **1** (CD_3OD , 150MHz)

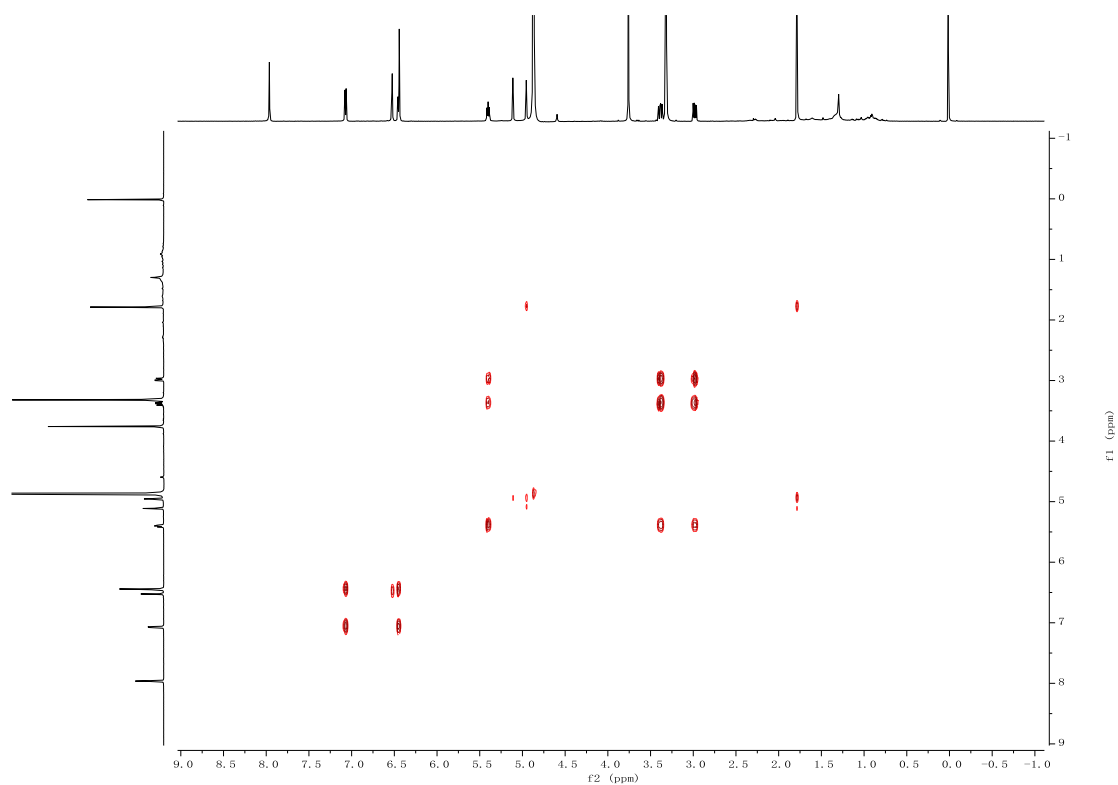


Figure S5: ^1H - ^1H COSY spectrum of compound **1**

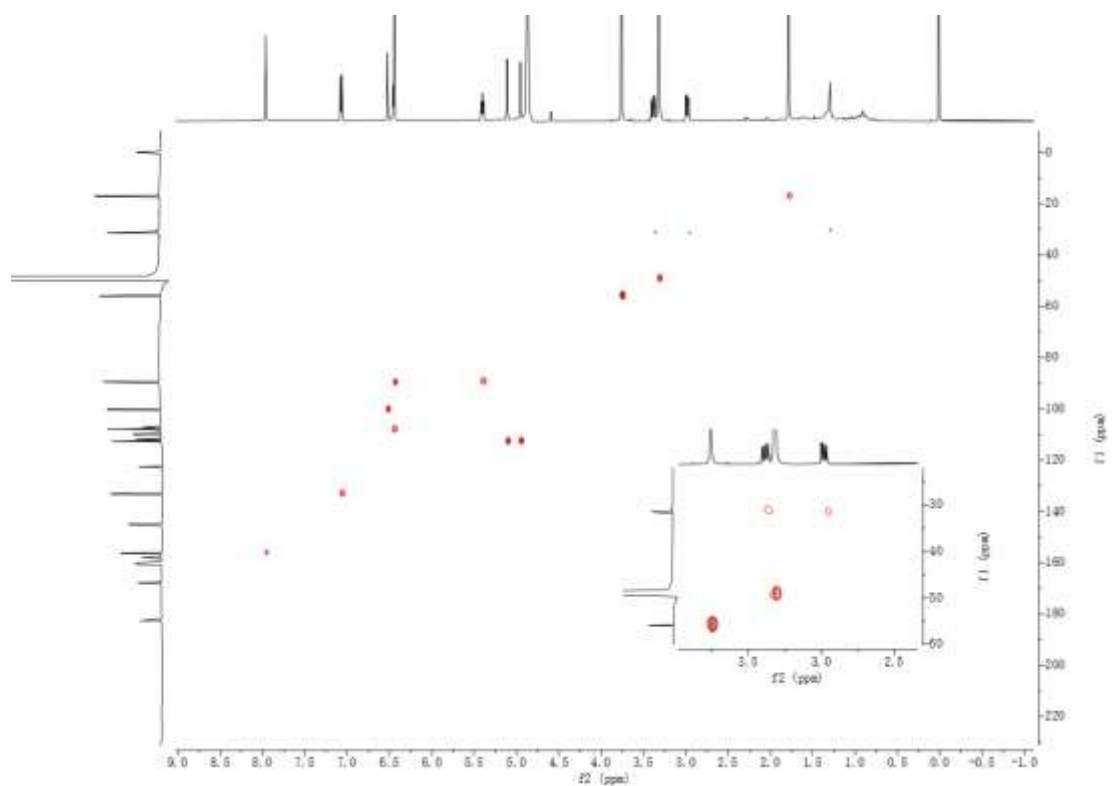


Figure S6: HSQC spectrum of compound **1**

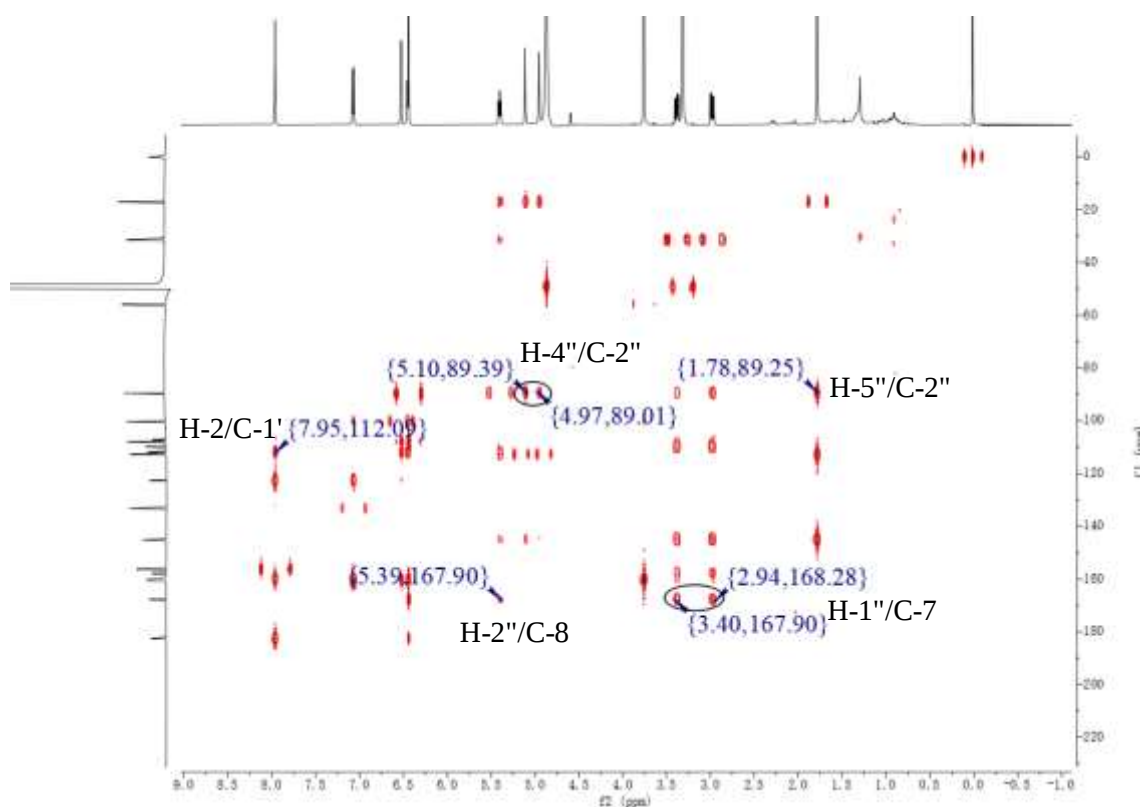


Figure S7: HMBC spectrum of compound **1**

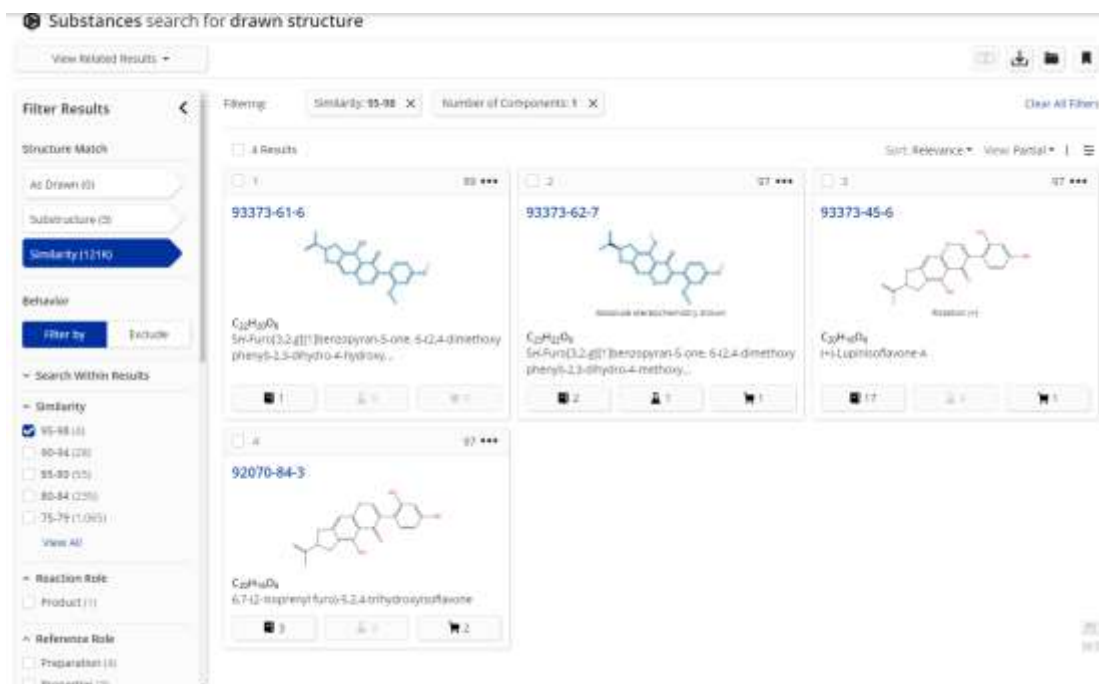


Figure S8: Scifinder similarity report for compound **1**

Table S1 NMR data of compound **1** (**apiosisoiflavone A**) and **licoagroisoflavone**

Name	apiosisoiflavone A		licoagroisoflavone	
Structure				
Position	δ_H (CD ₃ OD, 600MHz)	δ_C (CD ₃ OD, 150MHz)	δ_H (DMSO- <i>d</i> ₆ , 500MHz)	δ_C (DMSO- <i>d</i> ₆ , 125MHz)
2	7.95 s	156.0	8.37 s	154.3
3		122.5		122.5
4		182.6		180.7
5		157.9		156.5
6		109.9		108.6
7		167.8		165.9
8	6.43 s	89.5	6.60 s	89.2
9		160.0		158.0
10		107.2		105.7
1'		112.0		120.9
2'		160.3	7.37d (8.5)	130.6
3'	6.51 d (2.3)	100.3	6.80 d (8.5)	115.2
4'		160.7		157.6
5'	6.44 dd (8.2, 2.3)	107.2	6.80 d (8.5)	115.2
6'	7.06 d (8.2)	133.2	7.37d (8.5)	130.6
1''	2.95 dd (9.6, 15.6) 3.36 dd (7.3, 15.6)	31.4	2.92 dd (7.7, 15.6) 3.35 dd (9.2, 15.6)	29.9
2''	5.39 dd (7.9, 8.8)	89.2	5.44 dd (7.7, 9.2)	87.8
3''		145.1		143.0
4''	4.94 brs 5.10 brs	112.5	4.94 brs 5.08 brs	112.8
5''	1.78 s	16.9	1.72 s	17.0
-OCH ₃	3.75 s	55.7		