Supporting Information

***Rec. Nat. Prod*. 10:6 (2016) 761-765**

Calotroposide S, New Oxypregnane Oligoglycoside from *Calotropis procera* Root Bark

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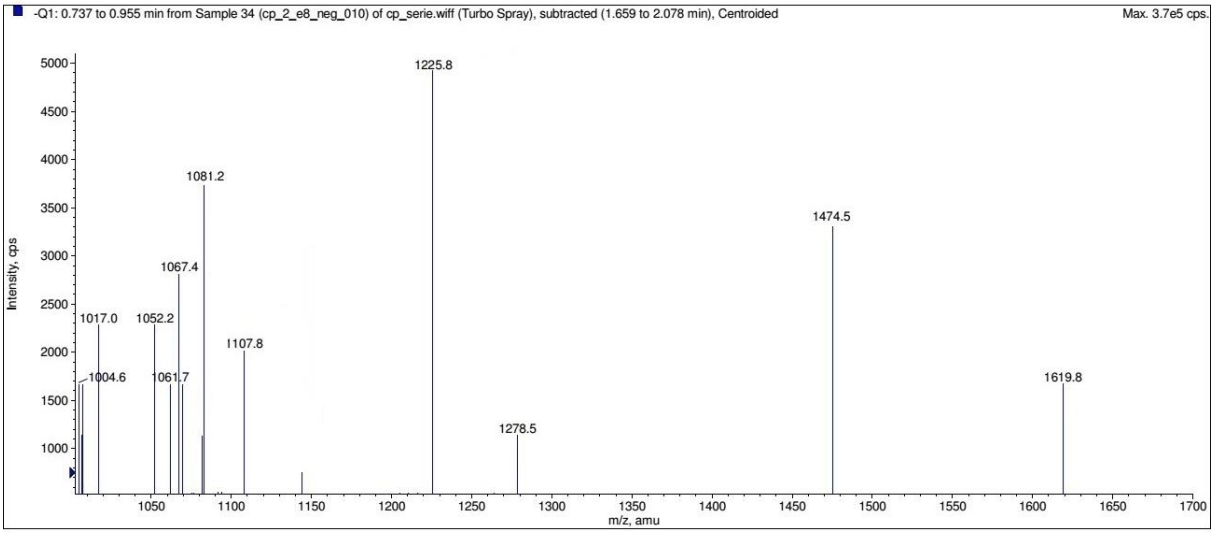
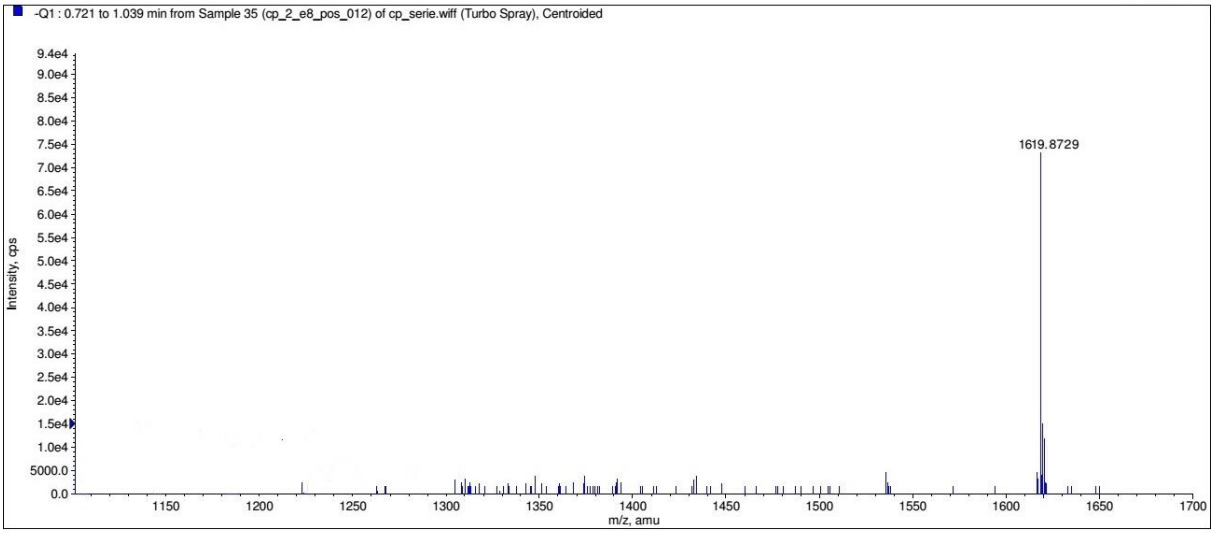
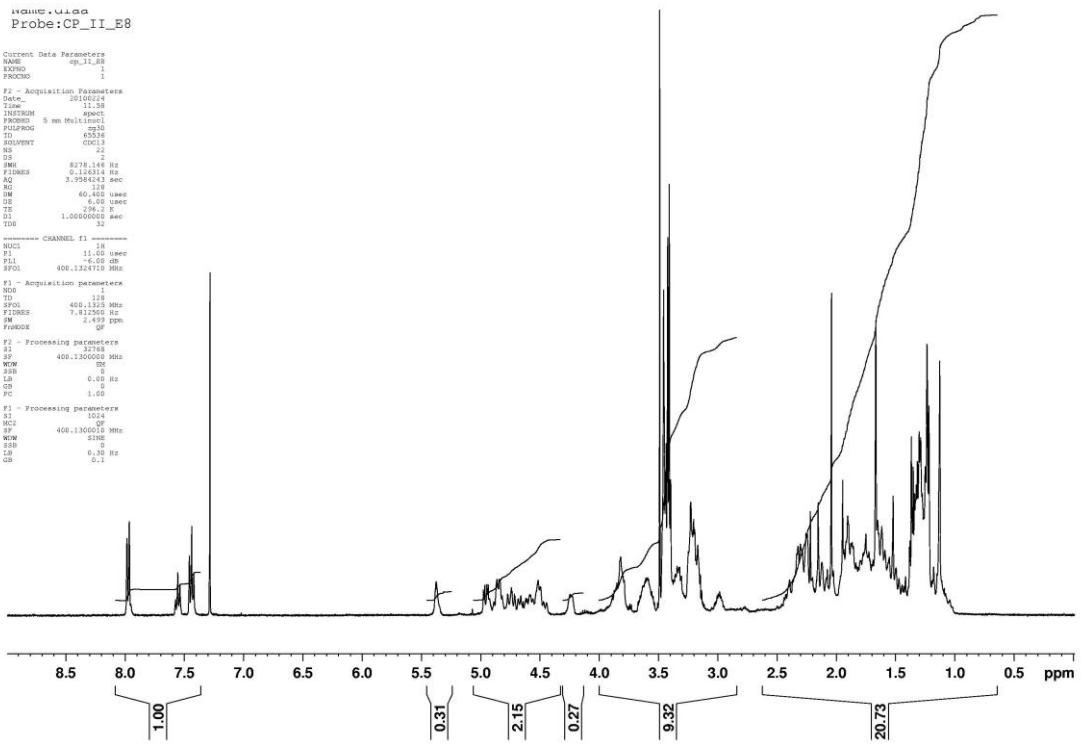


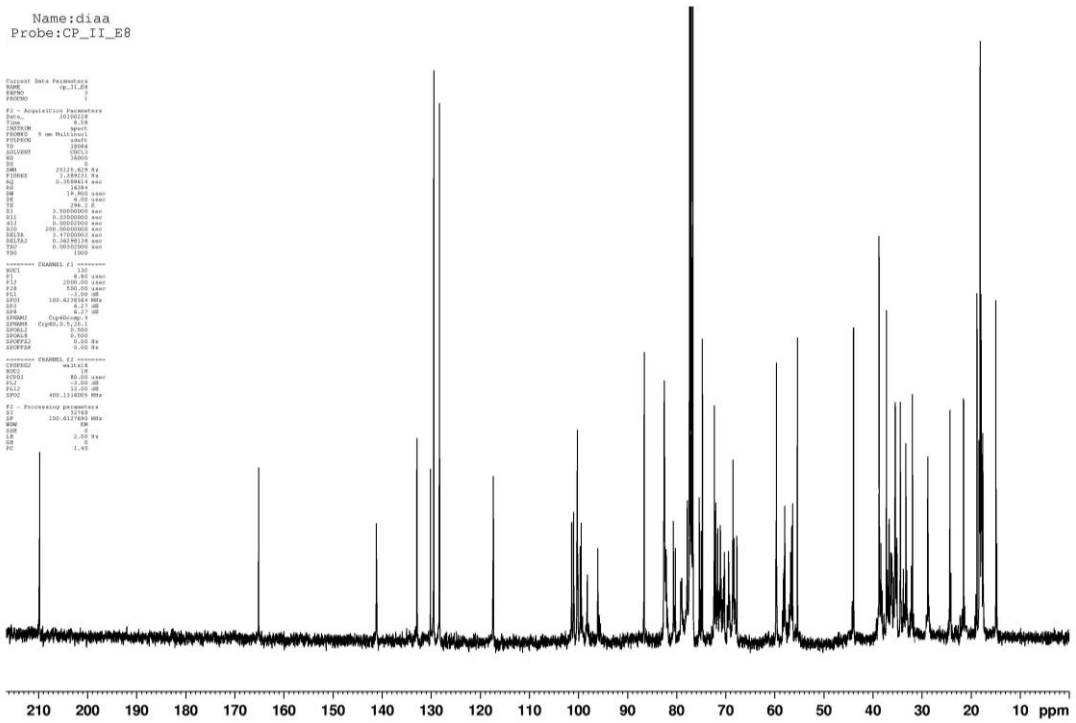
S1: ESI-MS spectrum of compound **1**



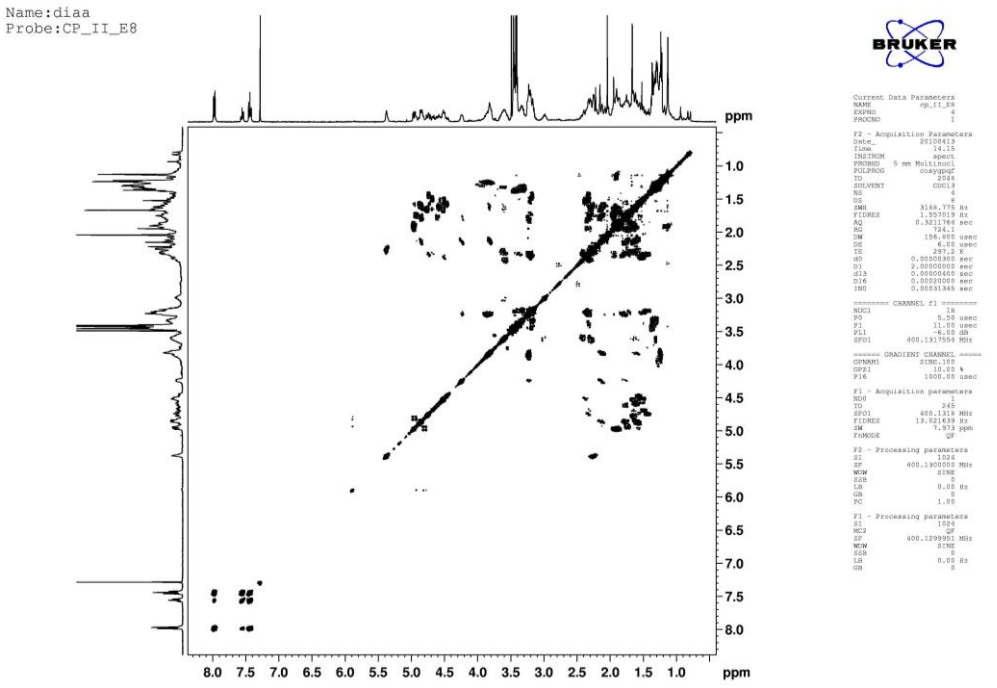
S2: HRESI-MS spectrum of compound **1**



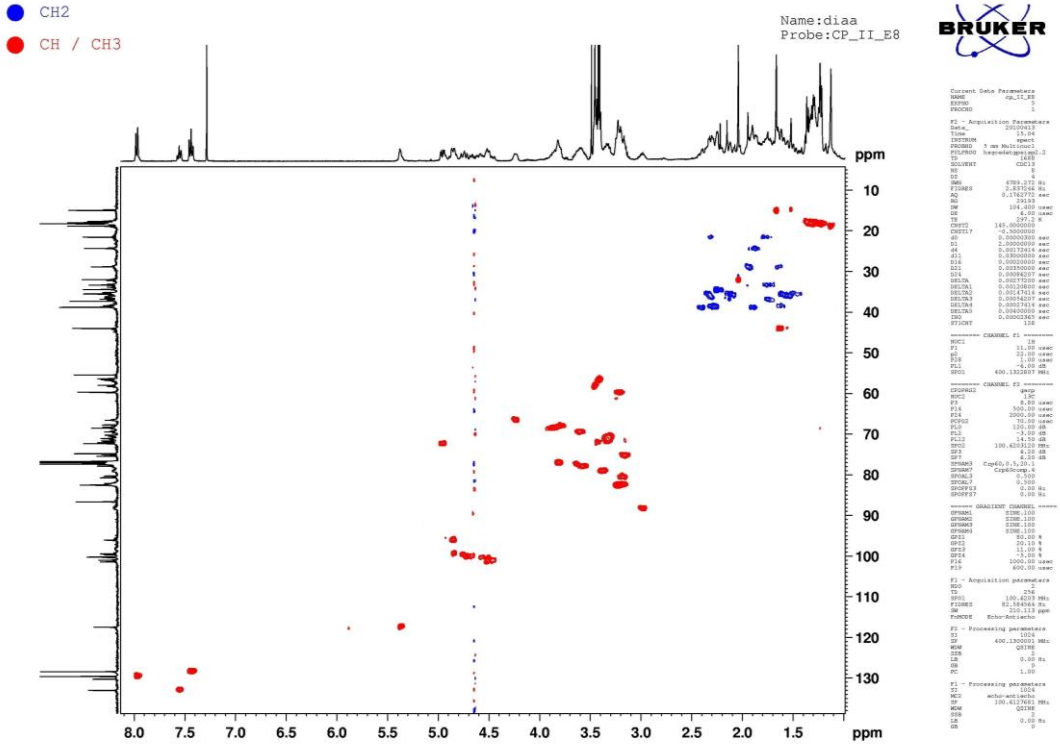
S3: ^{1}H NMR Spectrum of compound **1** (400 MHz, $CDCl_3$).



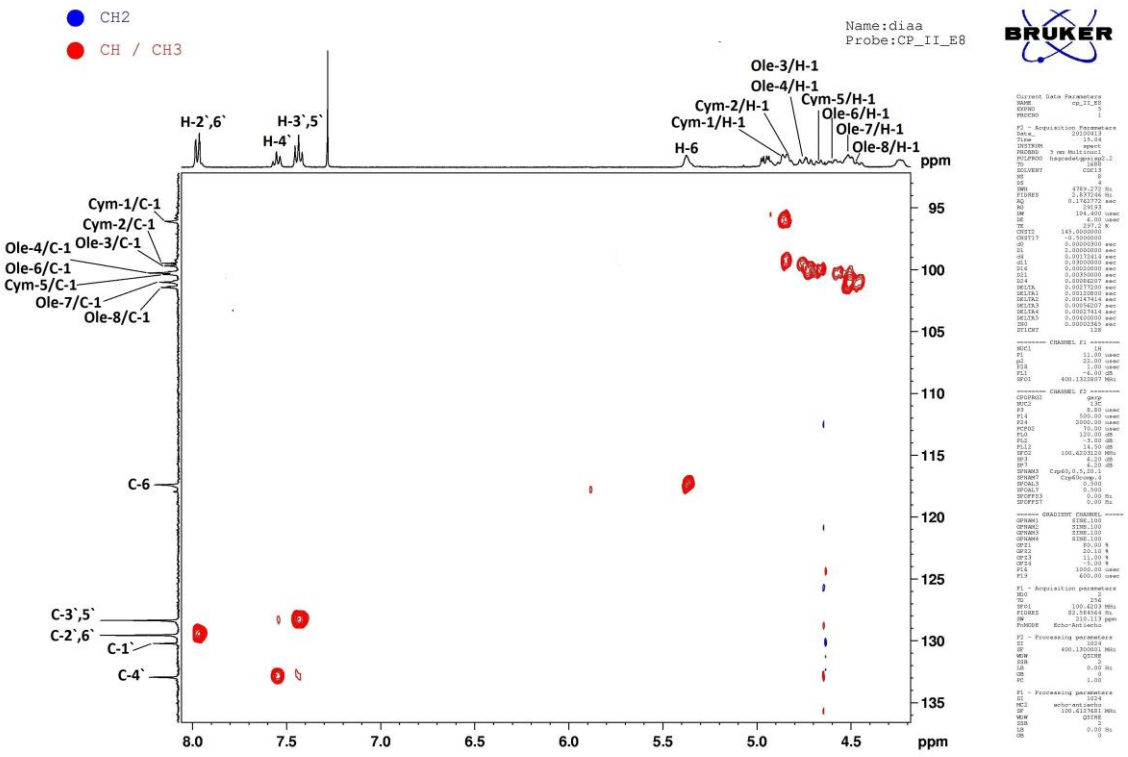
S4: ^{13}C NMR Spectrum of compound **1** (100 MHz, $CDCl_3$).



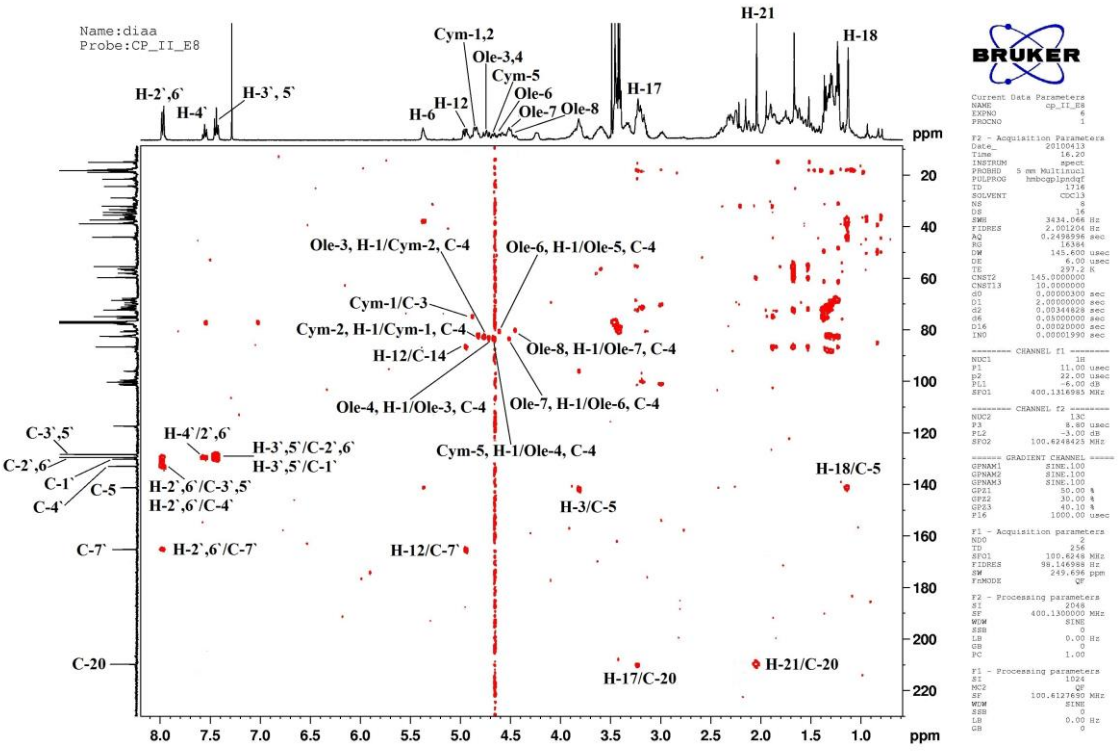
S5: ^{1}H-^{1}H COSY Spectrum of compound **1**.



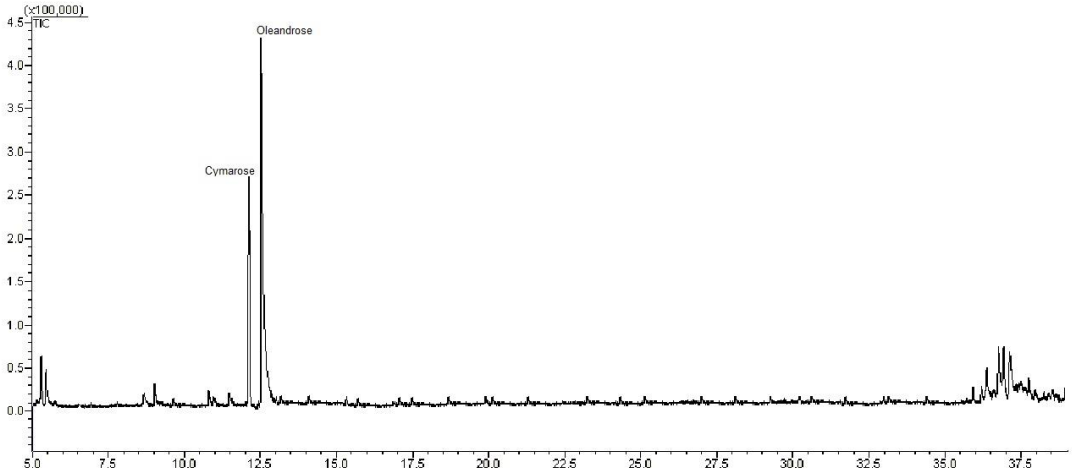
S6: HMQC Spectrum of compound **1**.



S7: Expanded HMQC Spectrum of compound **1**.



S8: HMBC Spectrum of compound **1**.



S9: GC/MS chromatogramme of compound **1** sugars.

S.1.1. General Experimental Procedures

A polarimeter Perkin-Elmer Model 341 LC was utilized for optical rotation measurement. A spectrophotometer Perkin-Elmer Lambda 25 UV/VIS was used for UV spectrum measurement. Shimadzu Infrared-400 spectrophotometer was used to record the IR spectral data. The mass spectrometer Finnigan MAT TSQ-7000 triple stage quadrupole was used to measure ESIMS spectrum. HRESIMS spectrum was obtained using an LTQ Orbitrap mass spectrometer. A Clarus 500 GC/MS was used to perform the GCMS analysis as previously described [1]. One and two dimensional NMR spectral data analyses were carried out on a Bruker DRX 400. Compounds separations were carried out using Sephadex LH-20 (Merck, 0.25-0.1 mm) and SiO_2 60 (Merck, 0.04-0.063 mm). The pre-coated SiO_2 F_{254} sheets were used for thin layer chromatography (Merck, Darmstadt, Germany). Detection of compounds was achieved by using *p*-anisaldehyde/H_2SO_4 spraying reagent and heating for 1-2 min at 110 °C. Authentic sugars samples were purchased from Haihang Industry Co., Ltd. (South Gongye Rd, Jinan City, China).

S.1.2. Acid Hydrolysis and Determination of the Absolute Configuration of the Sugar Moieties

The methanolic solution was subjected to acid hydrolysis as previously outlined [1]. Each sugar was mixed with 0.3 mL pyridine and treated with 0.5 mL *bis*(trimethylsilyl)trifluoroacetamide for 15 min at room temperature. Silylated sugars were subjected to GCMS analysis. By comparison with authentic samples, they were identified as D-oleandrose (t_R = 12.6 min) and cymarose (t_R = 12.1 min). The D-configuration of the sugars was assigned based on their optical rotation and comparison with literature as D-Cymarose: $[\alpha]_D$ +52.4 (*c* 0.15, H_2O, 24 h) (Lit. $[\alpha]_D$ +52.6 (*c* 0.15, H_2O, 24 h) and D-Oleandrose: $[\alpha]_D$ -12.7 (*c* 0.18, H_2O, 24 h) (Lit. $[\alpha]_D$ -12.8 (*c* 0.18, H_2O, 24 h) [1-3]. SiO_2 column of the $CHCl_3$ layer using *n*-hexane:EtOAc gradient gave 12-*O*-benzoylisolineolon which was determined by Co-TLC with standard sample.

References

[1] S. R. M. Ibrahim, G. A. Mohamed, L. A. Shaala, L. M. Banuls, R. Kiss and D. T. A. Youssef (2015). Calotroposides H-N, new cytotoxic oxypregnane oligoglycosides from the root bark of *Calotropis procera*, *Steroids* **96**, 63-72

[2] X. Y. Li, H. X. Sun, Y. P. Ye, F. Y. Chen, Y. J. Pan (2006). C-21 steroidal glycosides from the roots of *Cynanchum chekiangense* and their immunosuppressive activities, *Steroids* **71**, 61-66.

[3] I. M. Kuroda, **S.** Kubo, **S.** Uchida, H. Sakagami and Y. Mimaki (2010). Amurensiosides A-K, 11 new pregnane glycosides from the roots of *Adonis amurensis*, *Steroids* **75**, 83-94.