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FLAVONOID GLYCOSIDE AND ALKALOID FROM *AGERATUM CONYZOIDES L.*

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SUMMARY:

From Ageratum conyzoides L. growing in Thua Thien Hue province, two compounds, including an alkaloid: uracil (1), and a flavonoid glycoside: 6''-O-trans-p-coumaroylastragalin (2) were isolated. The structures of these compounds were characterized on the basis of IR, MS, NMR spectroscopic techniques. This is the first time uracil (1) was isolated from Ageratum genus and 6''-O-trans-p-coumaroylastragalin (2) was firstly isolated from this specices. The isolaed compounda were tested for their inhibitory effects on lipopolysaccharide (LPS)-induced nitric oxide (NO) production in RAW264.7 macrophages. Results showed that compound 2 exhibited a inhibitory effect on the NO production with IC50 value 93.69 ± 6.82 μg/ml.

Keywords: *Ageratum conyzoides L., 6''-O-trans-p-coumaroylastragalin, uracil, nitric oxide inhibition.*

I. INTRODUCTION

Ageratum conyzoides L. (Asteraceae) is a herb is distributed in tropical and subtropical regions. A. conyzoides has imparted numerous ethnomedicinal uses because it has been used to cure various ailments that include leprosy, skin disorders, sleeping sickness, rheumatism, headaches, dyspnea, toothache, pneumonia and many more[1, 2]. A wide range of chemical compounds including alkaloids, flavonoids, chromenes, benzofurans and terpenoids have been isolated from almost every part of this plant[3-5].

This research describes the isolation and structural elucidation of uracil (**1**) and 6''-O-*trans*-p-coumaroylastragalin (tiliroside, **2**) were isolated from methanol extract of *A. conyzoides*. The structures of these compounds were determined by IR, MS, NMR techniques and comparisons of their spectra with reported data.

II. EXPERIMENTAL

1. General

ESI-MS: LC-MSD-Trap-SL Agilent. NMR: Bruker Avance 500, 499.84MHz (^{1}H-) and 125MHz (^{13}C-, ^{13}C-DEPT). TMS (δ = 0.0, ^{1}H) and $CDCl_3$ (δ= 77.3, ^{13}C) were references. Column chromatography: silica gel 60, 0.06-0.2mm (Merck) for the first column and silica gel 60, 40-63μm (Merck), for the following columns. TLC: silica gel 60 F_{254} (Merck); the spots on the plates were observed under UV light and by spraying with solution of vanillin/sulfuric acid and heating for 5 minutes.

2. Plant material

The whole of plant material was collected in Thua Thien Hue province, in December, 2021. The species, *Ageratum conyzoides* L. was identified by Hoang Xuan Thao, Hue University of Education. A voucher specimen (AC12/2021) was deposited at the Laboratory of Natural Products, Department of Chemistry, Hue University of Education.

3. Extraction and isolation

The dried power of *A. conyzoides L.* (1.1kg) was extracted with *n*-hexane followed by chloroform ethyl acetat and methanol to afford 6.4; 9.4; 10.7 and 10.4 gram extracts, respectively. The methanol extract was chromatographed over silica gel with gradient chloroform/methanol (10:0 → 0:10, v/v) to give 12 fractions (ACM1 - ACM7).

Uracil (1): The compound **1** (10 mg) was isolated from ACM2 fraction (200 mg) and further purified by CC on silica gel (chloroform/methanol 95/5, v/v). IR (KBr) n_{max}, cm^{-1}: 3346 (N-H), 1637 (conj. C=O). ^{13}C-NMR (125MHz, CD_3OD) and ^{1}H-NMR (500MHz, CD_3OD) spectroscopic data, see Table 1.

6''-O-*trans-p*-coumaroylastragalin (2): The compound **2** (20mg) was isolated from ACM4 fraction (950mg) and further purified CC on silica gel (chloroform/methanol 90/10, v/v). IR (KBr) n_{max}, cm^{-1}: 3444,87 (OH), 1653 (O-C=O), 1504 (C=C). ^{13}C-NMR (125 MHz, CD_3OD) and ^{1}H- NMR (500MHz, CD_3OD) spectroscopic data, see Table 2.

4. Assay for NO inhibitory effect using RAW264.7 cells

RAW 274.7 cells were seeded in a 96 well-plate at a density of a 5.10^4 cells/well and incubated in 5% CO_2 incubator at 37°C for 24 h. The medium was then aspirated from each well and replaced with fresh FBS-free DMEM medium for 3h. After pretreatment with various concentrations of the essential oil for 2h, the cells were stimulated with LPS (1g/ml) for a further 24h. the accumulation of nitrite (NO_2^-) in the culture medium, was measured as an indicator of NO production using a colorimetric assay based on the Griess Reagent System (Promega Coporation, USA). Briefly, 100µl of cell culture medium was mixed with an equal volume of Griess Reagent: 50µl of 1% (w/v) sulfanilamide in 5% (v/v) phosphoric acid and 50µl of 0.1% (w/v) *N*-1-naphthylethylenediamine dihydrochloride in water and, incubated for 10 min at room temperature. Nitrite concentration was determined by measuring the absorbance at 540 nm using a microplate reader. Fresh culture medium was used as a blank for all experiments. L-NMMA was used as a positive control. The amount of nitrite in the samples was obtained from the $NaNO_2$ serial dilution concentration standard curve and nitrite production was measured. The NO inhibition capacity of the sample was calculated by the following formula:

The value of half maximal inhibitory concentration (IC_{50}) was determined by using the Table Curve 2Dv4 software (Systat Software Inc., USA)[6].

III. RESULTS AND DISCUSSION

Compound **1 (**12mg**)** was obtained as white amorphous powder. R_f = 0.5 (chloroform/methanol 10/1, v/v). Its molecular formula ($C_4H_4N_2O_2$) was deduced from a combine of the positive ESI-MS at *m/z* 113 $[M + H]^+$ and ^{1}H-, ^{13}C-NMR spectra.

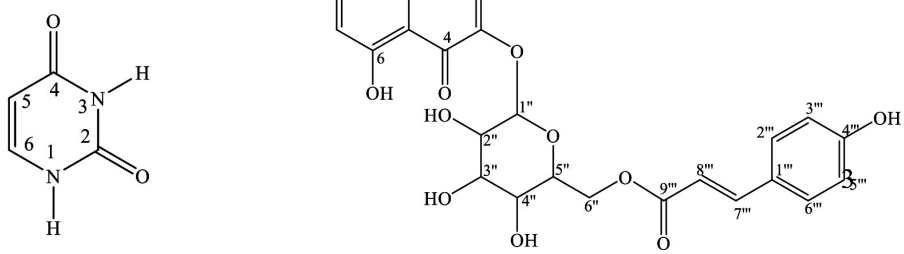
The IR spectrum of **1** showed the characteristic absorption frequencies at 3346 and 1.637cm^{-1} typical of -N-H and conjugated C=O bond vibration, respectively.

^{1}H-NMR spectrum of **1** only showed two signals of olefinic protons at δ_H 5.62ppm and 7.40ppm with coupling constants 7.5 and 8.0Hz, respectively. These coupling constants imply that olefinic protons has *cis* configuration in this compound. ^{13}C-NMR and HSQC of **1** indicated that the signals of olefinic carbon at δ_C 101.7; 143.5ppm and two signals characteristic of carbons in the aromatic compound at δ_C 153.5; 167.3ppm.

On the basis of analysis spectra, compound **1** was identified as heterocyclic with ammine and hydroxyl functional groups. Combination spectral data and comparison with reported data[7] (Table 1) confirmed the structure of **1** as pyrimidin-2,4(1H,3H)-dion (uracil) (Fig. 1). Uracil was isolated from the *Ageratum* genus for the first time.

Table 1: ^{1}H-, ^{13}C-NMR of 1 (CD_3OD) and uracil (D_2O), 125/500MHz

C	δ_C (ppm) 1	δ_C (ppm) Uracil [7]	δ_H (ppm) 1	δ_H (ppm) Uracil [7]
2	153.5	153.9	-	-
4	167.3	168.2	-	-
5	101.7	101.9	5.62 (d, 7.5Hz)	5.87 (d, 7.7Hz)
6	143.5	144.2	7.40 (d, 7.5Hz)	7.60 (d, 7.8Hz)



1: pyrimidin-2,4(1H,3H)-dion *2. tiliroside*

Fig.1. Structures of compounds 1 and 2 isolated from Ageratum conyzoides

Compound **2** (20mg) was obtained as yellow amophous powder; R_f = 0.45 (chloroform/metanol 9/1, v/v). Its molecular formula ($C_{30}H_{26}O_{13}$) was deduced from a combine of NMR spectra and the positive HR-ESI-MS at *m/z* 617,12971 $[M+Na]^+$ (calcd. $C_{30}H_{26}O_{13}Na$, 617,12711).

^1H- and ^{13}C-NMR displaed **2** is a derivatve of kaempferol. The typical signals of the kaempferol nucleus precense at δ_H 6.13ppm (1H, *d*, 1.7 Hz, H-6) and 6.30ppm (1H, *d*, *J* = 1.7Hz, H-8); a pair two doublets of 4 protons at δ_H 8.00 và 6.82ppm (each 2H, *d*) with *ortho* coupling constant (*J* = 8.7Hz) and two signals at δ_C 116.0; 132.2ppm (each 2C) showed that ring B has only one substitued group at C-4'.

The signals of 4 of methine protons at δ_H 335 - 3.50ppm (δ_C 71.7; 75.7; 75.8; 78.0ppm) and anomeric methine (δ_H 5.26ppm; δ_C 104.0ppm), methylen group at δ_H 4.22ppm (*dd*; 6.7; 11.8Hz), δ_H 4.33 ppm (*dd*; 1.6; 11.8Hz); δ_C 64.3ppm) suggested that **2** contains glucopyranosyl moiety.

Additionally, NMR spectra of **2** indicated the presence of *p*-coumaric acid, this conclusion confirmed by doublet of 2 olefinic protons at δ_H 7.40; 6.08ppm (each 1H, *d*) with coupling constant of two *trans* protons (16Hz) and doublet of 4 aromatic protons at 7.30; 6.79ppm with coupling constant of two *ortho* protons (each 2H, *d*, 8.5Hz and δ_C 116.8; 131.2ppm; each 2C). The glucopyranosyl moiety linked to C-3 of aglycon was determined by interaction on of H-1''/C-3 in HMBC spectrum; H-6''/C-9''' interaction on HMBC showed that ester linkage was formed between carboxyl of *p*-coumaric acid and hydroxyl at C-6 of glucopyranosyl moiety.

Combination spectral data and comparison with reported data[8] (Table 2), compound **2** was determined as 6''-O-*trans*-*p*-coumaroylastragalin (tiliroside) (Fig. 1b). This is the first time, tiliroside (**2**) was isolated from *Ageratum conyzoides L.*

Table 2: ^1H-, ^{13}C-NMR of 2 and tiliroside, (125/500MHz, CD_3OD)

C	δ_C (ppm) 2	δ_C (ppm) Tiliroside[8]	δ_H (ppm) 2	δ_H (ppm) Tiliroside[8]
2	159.2	159.2	-	-
3	135.2	135.2	-	-
4	179.4	179.2	-	-
5	162.9	162.7	-	-
6	100.0	99.9	6.13 (*d*, 1.7Hz)	6.05 (*d*, 2.0Hz)
7	165.9	165.7		
8	94.8	94.9	6.30 (*d*, 1.7Hz)	6.19 (*d*, 2.0Hz)
9	158.3	158.2		
10	105.6	105.0		
1'	122.7	122.6		
2', 6'	132.2	132.2	8.00 (*d*, 8.7Hz)	7.91 (*d*, 8.6Hz)
3', 5'	116.8	116.7	6.82 (*d*, 8.7Hz)	6.76 (*d*, 8.6Hz)
4'	161.5	161.4		
1''	104.0	104.1	5.26 (*d*, 7.0Hz)	5.19 (*d*, 7.6Hz)
2''	75.7	75.7	3.35 - 3.50(*m*)	3.45 - 3.50(*m*)
3''	78.0	78.0		
4''	71.7	71.7		
5''	75.8	75.7		
6''	64.3	64.4	4.33 (*dd*, 11.8; 1.6Hz) 4.22 (*dd*, 11.8; 6.7Hz)	4.29 (*dd*, 11.9; 1.9Hz) 4.15 (*dd*, 11.9; 6.8Hz)
1'''	127.0	127.0		
2''',6'''	131.2	131.1	7.30 (*d*, 8.5Hz)	7.21 (*d*, 8.5Hz)
3''',5'''	116.0	116.0	6.79 (*d*, 8.5Hz)	6.71 (*d*, 8.5Hz)
4'''	161.2	161.0		
7'''	146.5	146.5	7.40 (*d*, 16.0Hz)	7.34 (*d*, 15.9Hz)
8'''	114.7	114.7	6.08 (*d*, 16.0Hz)	6.02 (*d*, 15.9Hz)
9'''	168.8	168.8	-	-

The anti-inflammatory activity of tiliroside (**2**) as measured by inhibition of NO production in LPS-RAW 264.7 cells. The result showed that tiliroside (**2**) has anti-inflammatory activity with IC_{50} values: 93.69 ± 6.82μg/ml.

(Contineu reading page 41)

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Phản biện: PGS, TS MẠC ĐÌNH HÙNG

FLAVONOID GLYCOSIDE AND ALKALOID FROM *AGERATUM CONYZOIDES L.*

(Next page 31)

III. CONCLUSION

From *Ageratum conyzoides L.* growing in Thua Thien Hue province, an alkaloid uracil (**1**) and flavonoid glycoside 6''-O-*trans*-*p*-coumaroylastragalin (**2**) were isolated and structural idendified. This is the first time compound **1** isolated from *Ageratum* genus, while **2** from this species.

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