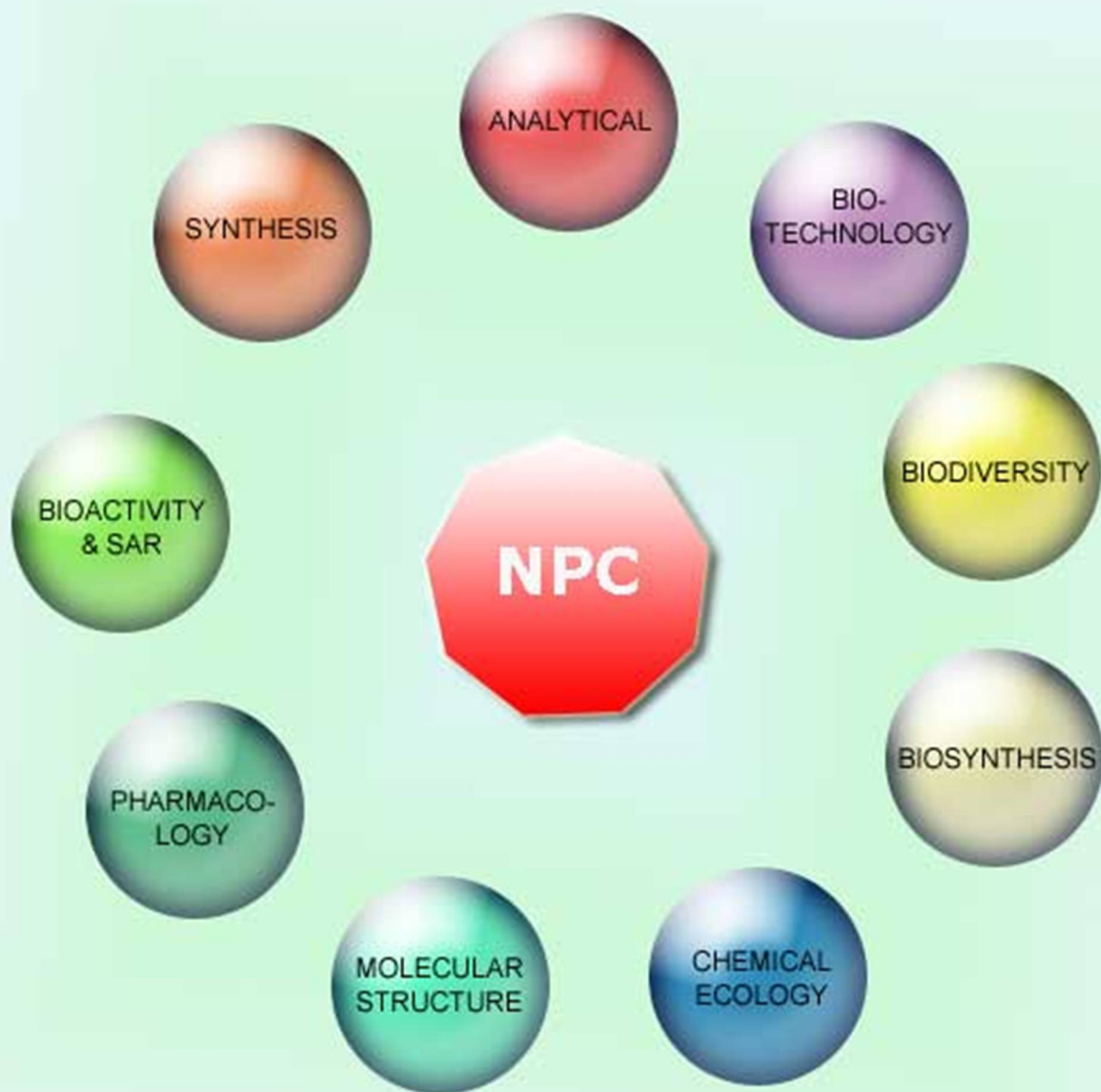


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A New Chromene Isolated from *Ageratum conyzoides*Abiodun Humphrey Adebayo^{a,b,d,*}, Chang-Jiu Ji^{a,d}, Yu-Mei Zhang^a, Wen-Jun He^a, Guang-Zhi Zeng^a, Hong-Jin Han^a, Jun-Ju Xu^a, Afolabi Akintunde Akindahunsi^c and Ning-Hua Tan^{a,*}^aState Key Laboratory of Phytochemistry and Plant Resources in West China, Kunming Institute of Botany, Chinese Academy of Sciences, Kunming 650204, P. R. China^bDepartment of Biological Sciences, College of Science and Technology, Covenant University, PMB 1023, Ota, Ogun State, Nigeria^cDepartment of Biochemistry, Federal University of Technology, Akure, Nigeria^dContributed equally to this work

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From the ethanol extract of the whole plant of *Ageratum conyzoides* L. (Compositae), one new chromene, 2,2-dimethylchromene 7-methoxy-6-*O*- β -D-glucopyranoside, was isolated, together with thirteen known compounds, seven of which were being reported for the first time. The compounds were all characterized by MS, IR, 1D- and 2D-NMR spectroscopy. 7,3',5'-Tri-*O*-methyltricetin (**7**), precocene II (**9**), 3,5,7,4'-tetrahydroxyflavone (**13**) and 5,6,7,3',4',5'-hexamethoxyflavone (**14**) exhibited inhibitory activity on the P-388 cancer cell line with IC₅₀ values of 12.8, 24.8, 3.5 and 7.8 μ M respectively, while compound **9** exhibited inhibitory activity on the HT-29 cancer cell line with an IC₅₀ value of 61 μ M; the others showed no significant cytotoxic activity on the cell lines tested.

Keywords: *Ageratum conyzoides*, Compositae, 2,2-dimethylchromene 7-methoxy-6-*O*- β -D-glucopyranoside, cytotoxicity.

The Compositae family has been employed for diverse beneficial purposes due to its wide distribution across the world. *Ageratum conyzoides* L. belongs to this family and is native to Central America, the Caribbean, Florida (USA), Southeast Asia, South China, India, West Africa (including Nigeria), Australia and South America [1,2]. It has been used in folklore for the treatment of fever, pneumonia, cold, rheumatism, spasm, headache, and curing wounds [3,4]. Its gastroprotective [4], antibacterial [5], anti-inflammatory, analgesic, antipyretic [1], anticonvulsant [6], antischistosomal [7], and anticoccidial [8] activities have been reported, and the bioactive compounds isolated include flavonoids, tannins, saponins, triterpenoids, sesquiterpenes, chromenes, and benzofurans [1]. Precocene I and II are the principal components of this plant. As part of our search for new bioactive constituents, we investigated the whole plant of *A. conyzoides*, which led to the isolation of one new compound (**1**) and thirteen known ones, seven of which were identified from this plant for the first time. Herein, we report the structure elucidation as well as the cytotoxicity of these compounds on cancer cell lines.

Compound **1** was obtained as colorless oil and its molecular formula was assigned as C₁₈H₂₅O₈ by HREI-MS showing an [M]⁺ at *m/z* 369.1542 [M+H]⁺ (calcd for 369.1549 [M+H]⁺). This was confirmed by ¹³C and DEPT NMR spectra. Its IR spectrum showed a broad band at

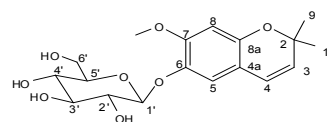


Figure 1: Structure of compound 1.

3424 cm⁻¹, which indicated the presence of a hydroxyl group, and the absorption at 1618 cm⁻¹ suggested an aromatic ring. One glucopyranosyl moiety was evident from the series of signals at δ 104.0, 75.0, 78.1, 71.4, 77.8 and 62.5 in the ¹³C NMR spectrum. The assignment of the β -D configuration of the sugar moiety was supported by the signals at δ 4.73 (1H, d, *J* = 7.8 Hz, H-1'), 3.42-3.44 (2H, m, H-2', 5'), 3.34-3.46 (2H, m, H-3', 4'), 3.86 (1H, d, *J* = 12.0 Hz, H-6'a), and 3.68 (1H, dd, *J* = 12.0, 5.0 Hz, H-6'b), in the ¹H NMR spectrum. The data of the aglycone of **1** in the ¹³C NMR spectra were consistent with those of chromene [9], and δ_{H} 6.88 (1H, s, H-5), 6.43 (1H, s, H-8), and 4.73 (1H, d, *J* = 7.8 Hz, H-1') were consistent with those of the tetraacetate of 6-monodemethylated precocene II -6-*O*- β -D-glucoside in the ¹H NMR spectrum [10]. The HMBC correlation of δ_{H} 4.73 (1H, d, *J* = 7.8 Hz, H-1') and δ_{C} 141.8 (d, C-6) confirmed that the attachment of the glucosyl group was at C-6. Similarly, δ_{H} 3.80 (3H, OCH₃) was found to correlate with δ_{C} 151.8 (s, C-7) from HMBC, which suggests that the methoxy must be attached to C-7. Thus, the structure of **1** was established as 2,2-dimethylchromene 7-methoxy-6-*O*- β -D-glucopyranoside.

Thirteen known compounds, seven (**2**, **3**, **4**, **5**, **6**, **7** and **8**) of which are being reported for the first time for this species, were also isolated and characterized from *A. conyzoides*. These were eugenyl-*O*- β -D-glucopyranoside (**2**) [11,12], eugenyl-*O*- β -D-apiofuranosyl-(1" \rightarrow 6')- β -D-glucopyranoside (**3**) [12], 3-(2'-*O*- β -D-glucopyranosyl)-phenyl-2-*trans*-propenoic acid (**4**) [13], (2*S*)-2,3-*O*-di-(9,12,15-octadecatrienoyl)-glyceryl-6-*O*-(α -D-galactopyranosyl)-(1" \rightarrow 6')- β -D-galactopyranoside (**5**) [14,15], ((9*Z*,12*Z*,15*Z*)-octadeca-9,12,15-trienoic acid) (**6**) [14,15], 7,3',5'-tri-*O*-methyltricetin (**7**) [16], and cirsilineol (**8**) [17]. The other known compounds isolated were precocene II (**9**) [18-20], precocene I (**10**) [20], 6-(1-methoxyethyl)-7-methoxy-2,2-dimethylchromene (**11**) [20], 2,2-dimethylchromene-7-*O*- β -D-glucopyranoside (**12**) [9], 3,5,7,4'-tetrahydroxyflavone (**13**) [21,22], and 5,6,7,3',4',5'-hexamethoxyflavone (**14**) [18].

Compounds **7**, **9**, **13** and **14** exhibited cytotoxic activity against mouse leukemia (P-388) and human colon adenocarcinoma (HT-29) cancer cell lines, but there was no inhibitory activity on human non-small cell lung carcinoma (A549) (Table 1). Compound **1** showed no activity at the tested concentration of 10 μ g/mL on these three cancer cell lines.

Table 1: IC₅₀ values of P-388, HT-29 and A549 cancer cell lines.

Compounds	P-388 cells (μ M)	HT-29 cells (μ M)	A-549 cells (μ M)
7	12.9 \pm 1.24	-	-
9	24.8 \pm 3.57	61.3 \pm 2.90	-
13	3.5 \pm 0.06	-	-
14	7.8 \pm 0.33	-	-
Taxol	0.00008 \pm 0.000001	0.005 \pm 0.0002	0.02 \pm 0.0004

- no activity at the tested concentration of 10 μ g/mL.

Values represent mean \pm SD and were calculated from at least three sets of independent experimental data.

Experimental

General: Optical rotations were measured with a Horbia SEAP-300 polarimeter. IR spectra were obtained on a Bio-Rad FTS 135 spectrophotometer with KBr pellets. UV spectra were taken on a Shimadzu 2401PC spectrophotometer. FAB-MS and HR-TOF-MS were recorded on a VG Auto Spec-3000 spectrometer. 1D- and 2D-NMR spectra were respectively recorded on Bruker AM-400 and DRX-500 spectrometers with TMS as internal standard. Column chromatography (CC) was carried out over silica gel (200-300 mesh, Qingdao Marine Chemical Inc., China), Sephadex LH-20 (Pharmacia Fine Chemical Co., Ltd., Sweden), and MCI (Mitsubishi Chemicals, Japan). HPLC was carried out on an Agilent 1100 (USA) instrument.

Plant material: The whole plant of *A. conyzoides* was obtained in April, 2008 from Xishuangbanna, South Western China and authenticated by Dr You-Kai Xu of Xishuangbanna Tropical Botanical Garden, Chinese Academy of Sciences, China. A voucher specimen (KUN 0486260) was deposited in the State Key Laboratory of

Phytochemistry and Plant Resources in West China, Kunming Institute of Botany, CAS, China.

Extraction and isolation: The dried whole plant (7 kg) of *A. conyzoides* was extracted 3 times with 95% ethanol (each for one week) at room temperature. After concentration of the combined extracts under reduced pressure, the residue was dissolved in hot water and extracted successively with light petroleum, ethyl acetate and *n*-butanol. The light petroleum extract (201 g) was fractionated by CC (268 g silica gel: light petroleum/acetone mixtures of increasing polarity) to afford fractions (Frs.) 1-5. Frs. 2, 3 and 4 were respectively subjected to CC (Sephadex LH-20: CHCl₃/ MeOH 2:1; silica gel: light petroleum/CHCl₃ mixtures of increasing polarity) to afford **9** (2.3g), **10** (41 mg), **7** (9.3 mg), **8** (5.0 mg), **11** (2.3 mg), **12** (4.0 mg), **14** (8.0 mg), **2** (25.0 mg) and **3** (6.0 mg). Similarly, the ethyl acetate extract (173 g) was purified by CC (1.4 kg silica gel: CHCl₃/MeOH mixtures of increasing polarity) giving Frs. 1-7. Frs. 2 and 3 were repeatedly purified by CC (Sephadex LH-20: CHCl₃/MeOH 1:1) to yield **6** (5.0mg) and **13** (21mg). Fr 4 was repeatedly purified by HPLC (H₂O/MeOH 2.5:7.5) to afford **1** (3.0 mg). Fr.5 was fractionated by CC (MCI: H₂O/MeOH in increasing polarity; Silica gel: CHCl₃/MeOH 4:1 and 3:1) to give **4** (2.1mg) and **5** (36.6mg).

2,2-Dimethylchromene-7-methoxy-6-*O*- β -D-glucopyranoside (**1**)

$[\alpha]_D^{17}$: -53.57 (*c* 0.028, MeOH)

IR (KBr) ν_{\max} : 3424, 2925, 1618, 1505, 1071 cm⁻¹.

UV/Vis λ_{\max} (MeOH) nm (log ϵ): 195.40 (4.15), 220.40 (4.35), 275.40 (3.65), 316.80 (3.77).

¹H NMR: (500 MHz, CDCl₃) δ : 5.53 (1H, d, *J* = 9.8 Hz, H-3), 6.26 (1H, d, *J* = 9.8 Hz, H-4), 6.88 (1H, s, H-5), 6.43 (1H, s, H-8), 1.36 (3H, s, CH₃), 1.37 (3H, s, CH₃), 4.73 (1H, d, *J* = 7.8 Hz, H-1'), 3.42-3.44 (2H, m, H-2', 5'), 3.34-3.46 (2H, m, H-3', 4'), 3.86 (1H, d, *J* = 12.0 Hz, H-6'a), 3.68 (1H, dd, *J* = 12.0, 5.0 Hz, H-6'b), 3.80 (3H, s, -OCH₃). ¹³C NMR: (100 MHz, CD₃OD) δ : 77.2 (s, C-2), 129.6 (d, C-3), 123.0 (d, C-4), 115.2 (s, C-4a), 117.3 (d, C-5), 141.8 (s, C-6), 151.8 (s, C-7), 102.4 (d, C-8), 150.4 (s, C-8a), 28.0 (q, C-9), 27.9 (q, C-10), 104.0 (d, C-1'), 75.0 (d, C-2'), 78.1 (d, C-3'), 71.4 (d, C-4'), 77.8 (d, C-5'), 62.5 (t, C-6'), 56.7 (q, -OCH₃ at C-7).

EIMS *m/z* (%): 368 [M]⁺ (1), 206 [M-162]⁺ (42), 191 (100).

HREIMS: *m/z* 369.1542 [M+H]⁺ (calcd. 369.1549 [M+H]⁺ for C₁₈H₂₅O₈)

Cancer cell growth inhibition assay: The procedure described by [23] was followed. In brief: the sulphorhodamine B (SRB) assay was adopted for a measurement of cell growth and viability [24]. Mouse leukemia (P-388), human colon adenocarcinoma (HT-29) and human non-small cell lung carcinoma (A549) cells were seeded in 96-well microtiter plates at 3000-7000 cells per well. After 24 h, compounds were added to a final

concentration of 10 µg/mL and incubated for 48 h. Cells were then fixed by the addition of 50% ice-cold CCl₃COOH and then left at 4°C for 1 h. After washing, air-drying and staining for 15 min with 100 µL 0.4% SRB in 1% glacial AcOH, excessive dye was removed by washing with 1% glacial AcOH. The absorbance values of resuspended SRB in 10 mM Tris buffer were read at 560 nm on a microplate spectrophotometer (SPECTRA MAX 340, USA). If the cell growth inhibition was >50% at the highest tested concentration of 10 µg/mL, further assessment was carried out with at least 4 diluted concentrations (dilution ratio 1:2) to calculate the IC₅₀ values (50% inhibitory concentration). Each sample concentration was tested in triplicate on the plate. Results

were expressed as mean IC₅₀ values ± standard deviation. Taxol was used as the positive compound.

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