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Chemical Investigation of the Constitutive Phenolics of *Ailanthus altissima*; The Structure of a New Flavone Glycoside Gallate

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Abstract – The aqueous ethanolic leaf extract of *Ailanthus altissima* was found to contain the new natural product, luteolin 7-O-β-(6"-galloylglucopyranoside), **13**, along with fourteen known phenolic metabolites (**1-12, 14** and **15**). Structures of all compounds (**1-15**) were established by conventional methods of analysis and confirmed by FAB-MS, ^{1}H- and ^{13}C-NMR spectral analysis.

Key words – *Ailanthus altissima*, Simaroubaceae, phenolics, luteolin 7-O-β-(6"-galloylglucopyranoside), FAB-MS, ^{13}C-NMR.

Introduction

Leaves and bark of *Ailanthus altissima*, Mill. syn. *Ailanthus glandulosus*, Desf. (Simaroubaceae), known as the tree of heaven provide extracts which were found in traditional medicine to be emetic, cathartic, anthelmintic, and have been used as remedy for dysentery and diarrhea (Millspaugh, 1974). However, this plant has not been subjected previously to any comprehensive phytochemical investigations. The present study reports on the isolation and structure elucidation of fifteen phenolic constituents (**1-15**), including the new natural product, luteolin 7-O-β-(6"-galloylglucopyranoside), **13**, from an aqueous ethanolic leaf extract of *A. altissima*. The study, also proves the capability of this plant to synthesize and accumulate, in its leaves, high contents of gallic acid, ellagitannins, flavone and flavonol glycosides together with their 6"-galloylated derivatives as well.

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Results and Discussion

The meal of the dried leaves of *A. altissima* was exhaustively extracted with aqueous ethanol (3 : 1). Compounds (**1-15**) were individually isolated and purified from the received dried aqueous ethanol extract by polyamide column fractionation, followed by Sephadex LH-20 column chromatography and preparative paper chromatography. The known compounds **1-12, 14** and **15** gave Rfs and UV spectral data (Table 1), FAB-MS, ^{1}H- and ^{13}C-NMR analytical data as well as hydrolytic results identical with those of the ellagitannins; bervifolin carboxylic acid **1**; corilagin **3** (Nawwar *et al.*, 1994a) and 3,4,8,9,10-pentahydroxydibenzo[b,d]-pyran-6-one **15** (Barakat *et al.*, 1996) and to those of gallic acid **2** (Nawwar *et al.*, 1982); quercetin 3-O-rutinoside **4**; quercetin 3-O-rhamnoside **7**; quercetin 3-O-glucoside **8** (Nawwar *et al.*, 1984a); kaempferol 3-O-glucoside **5**; kaempferol 3-O-β-(6"-galloylglucoside) **6** (Wills *et al.*, 1980); quercetin 3-O-β-(6"-galloylglucoside) **9** (Collins *et al.*, 1975); apigenin 7-O-glucoside **10**; luteolin 4'-O-

Table 1. Chromatographic and UV data of compounds **1-15**

Compounds	Chromatographic properties, R_{fs} (×100)			UV Spectral data λ_{max} (nm)				
	H_2O	HOAc	BAW	MeOH	NaOAc	NaOAc-H_3BO_3	$AlCl_3$	MeONa
1	55	59	62	278, 350, 362*				
2	53	59	78	272				
3	55	59	32	276				
4	65	55	55	255, 267*, 360	255*, 270, 278	261, 380	266, 300*, 364*, 420	270, 330, 403
5	20	42	68	267, 353	273, 355	271, 355	272, 408	275, 310, 402
6	10	34	70	268*, 295*, 350	271, 305, 370	267, 280, 370	276, 302*, 420	274, 320, 400
7	20	52	72	259*, 297*, 348	276, 372	272, 382	268, 352, 408	270, 355, 402
8	8	38	60	258, 356	274, 362	272, 380, 420*	268, 430	275, 474
9	8	35	62	260, 295*, 356	272, 295*, 360	265, 300, 375	275, 300*, 425	275, 330, 410
10	5	22	68	268, 318	268, 318	268, 300*, 320, 382	278, 298, 328, 380	262, 308, 370
11	7	21	65	242*, 253, 266, 345	276, 322, 368	253, 267, 346	267*, 272, 296, 362, 390	270, 303*, 387
12	5	21	63	243*, 266*, 348	260, 369*, 405	252, 372	274, 329, 430	263, 300*, 387
13	5	20	66	245*, 267, 348	260, 290*, 368	260, 292*, 372	275, 330, 433	260, 300, 398
6-monogallol glucose	67	75	62	273	269, 326*, 348	259, 301*, 370, 430*	274, 300*, 328, 426	266*, 329*, 401
Luteolin	2	5	76	242*, 253, 267, 348, 349				
14	67	66	56	220, 245, 300, 330				
15	00	22	46	219, 264, 287*				

*Inflection

glucoside **11**; luteolin 7-O-glucoside **12** (Nawwar *et al.*, 1994b); chlorogenic acid **14** (Harborne, 1959).

The new compound **13**, isolated as an off-white amorphous powder was found to possess chromatographic and color properties (dark purple spot on PC under UV light, blue $FeCl_3$ colur reaction and a rose color with aqueous KIO_3, specific for galloyl esters), (haddok *et al.*, 1982) and UV absorption maxima consistent with galloylated luteolin 7-O-glycoside. It exhibited a molecular weight of 600 amu in negative FAB-MS ([M-H]-599). On complete acid hydrolysis, **13** yielded luteolin, gallic acid (CoPC, UV and ^{1}H-NMR analysis) and glucose (CoPC). On controlled acid hydrolysis, it yielded luteolin 7-O-β-glucoside and 6-monogalloyl-(α/β)-glucopyranose (CoPC, UV spectral, FAB-MS and ^{1}H-NMR, Nawwar, 1994c) as the two main controlled hydrolysis products. Both were individually separated from the concentrated aqueous hydrolysate by preparative paper chromatography (see experimental).

The ^{1}H-NMR spectrum of **13** (DMSO-d_6, room temp.) exhibited the characteristic resonance pattern of luteolin 7-O-β-glucopyranoside with the exception of the recognized two glucose proton signals, appearing as downfield shifted doublet (J= 12.5 Hz) at δ 4.46 ppm and as double doublet (J=12.5 and 5 Hz) at δ 4.3 ppm, attributable to two methylenic glucose protons whose geminal hydroxyl group is galloylated. In addition, a sharp singlet located in this spectrum at δ 6.97 ppm was assigned to the two equivalent H-2 and H-6 galloyl protons, thus proving the structure of **13** as luteolin 7-O-β-(6"-galloylglucopyranoside).

The ^{13}C-NMR spectrum, of **13** showed the characteristic fifteen distinct carbon resonances of a luteolin moiety. Gluco-

sidation at the 7-hydroxyl of luteolin was deduced from the recognizable upfield shift ($\Delta\delta$ ppm 2) of the C-7 carbon resonance (Table 2) as well as β-glucopyranose resonance in which that for the anomeric carbon appeared at δ ppm 99.9 Galloylation of the C-6 glucose hydroxyl group was evidenced from the downfield shift ($\Delta\delta$ ppm 2.4) of this sugar carbon resonance, in comparison with the resonance of the corresponding carbon in the spectrum of free luteolin 7-O-β-glucopyranoside (see experimental). The spectrum also proved the presence of a galloyl moiety in the molecule of **13** as shown by the distinct galloyl carboxylic carbon resonance at δ ppm 166.03 and by the characteristic pattern of the galloyl carbon resonances (Table 2). In this spectrum, resonances of the glucose carbons were assigned (Table 2) by comparison with the ^{13}C-NMR data reported for similar galloylglucose (Nawwar *et al.*, 1994a, c), as well as by cosideration of the known α and β effects (Nawwar *et al.*, 1984b) caused by esterification of the glucose hydroxyl groups. The β-anomeric carbon resonance was identified from its characteristic shift value (99.9 ppm). Other resonances in this spectrum exhibited chemical shifts which were in accordance with the structure of **13** as luteolin 7-O-β-(6"-galloyl-glucopyranoside), a natural product which has not been reported before to occur in nature.

Table 2. ^{13}C-NMR data of the new compound **13** and its hydrolysis products

Carbon No.	**13**	Luteolin 7-O-β-glucopyranoside	Luteolin
2	164.78	164.6	164.5
3	103.4	103.3	103.2
4	182.1	182.0	182.6
5	161.4	161.0	162.4
6	99.5	99.7	99.3
7	162.9	162.8	164.9
8	95.5	94.9	94.5
9	157.1	156.8	158.0
10	105.6	105.3	104.6
1'	121.7	121.3	122.5
2'	113.7	113.5	113.9
3'	145.7	145.5	146.5
4'	150.1	149.6	150.3
5'	16.2	116.0	116.7
6'	119.1	119.0	119.6
β-glucose moiety			
1	99.9	100.3	
2	73.3	73.1	
3	76.7	77.5	
4	69.5	69.8	
5	76.3	76.3	
6	63.4	61.0	
Galloyl moiety			
1	119.7		
2,6	108.9		
3,5	145.6		
4	138.7		
C=O	166.03		

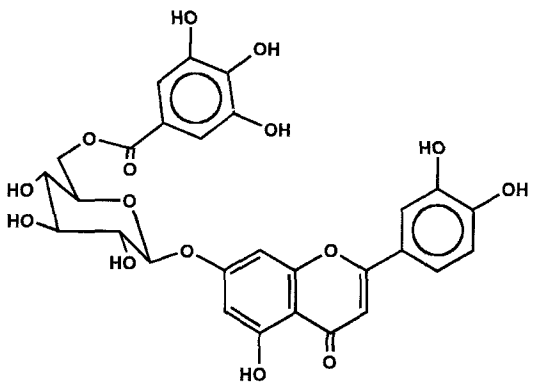
Experimental

General – NMR analysis: A JEOL EX-NMR spectrometer, 270 MHz for ^{1}H-NMR and 67.5 MHz for ^{13}C-NMR, was used with superconducting magnet from Oxford and 5 mm Dual probehead for ^{1}H- and ^{13}C-analysis. Typical conditions: spectral width=4000 Hz for ^{1}H and 16000 Hz for ^{13}C, 32 K data point and a flip angle of 45°. The UV spectra were recorded in MeOH and with shift reagents (NaOAC, NaOAc+H_3BO_3, $AlCl_3$ and NaOMe), using Shimadzu UV-240 spectrometer; FAB-MS (negative mode) were measured using MM 7070E spectrometer (VG analytical). PC was carried out on Whatman No. 1 paper using solvent systems: [1] H_2O; [2] HOAc-H_2O (3:50), [3] n-BuOH-HOAc-H_2O (4:1:5, upper layer); [4] C_6H_6-n-BuOH-H_2O-pyridine (1:5:3:3, upper layer). Solvent 2 and 3 were used for prep. PC on Whatman No. 3 MM paper and solvents 3 and 4 for sugar analysis

Plant material – A leaf sample of *A. altissima* was collected in March 1995, from a mature tree of 6 m height, growing in El-Orman garden, Cairo and authenticated by dr. N. El-Hadidi, Prof. of Botany, Faculty of science, Cairo University.

Extraction and isolation – Dried leaf material (2.5 kg) were refluxed with 75% aq.

ethanol over a boiling water-bath for four times, each lasts for 24 hrs. The collected extract, dried *in vacuo*, amounted to 500 g was then fractionated over a polyamide 6S (Riedel-De Häen AG, Seelze Hannover, Germany) and eluted by H_2O followed by H_2O-EtOH mixts of decreasing polarities to yield seven major fractions (I-VII). Compounds **1** (130 mg) and **2** (270 mg) were isolated from fr. I, eluted from the column with 20:80, EtOH-H_2O, by refractionation over Sephadex LH-20, using EtOH as an eluent, followed by crystallization from H_2O. Compound **3** (192 mg) was obtained from fr. II (30:70, EtOH-H_2O) by precipitation with ether from concd. acetone soln. of the dried fr. The process was repeated thrice to afford pure **3**. Compounds **4** (72 mg), **5** (81 mg), **6** (103 mg) and **7** (56 mg) were isolated by repeated prep. PC, using solvent systems 2 followed by 3, from fr. III (40:60, EtOH-H $_2$O). Compounds **8** (137 mg) and **9** (155 mg) were isolated from fr. IV (50:50, EtOH-H_2O) by Sephadex LH-20 column fractionation, using BuOH saturated with H_2O as solvent. Compound **10** (23 mg) was isolated from fr. V (70:30, EtOH-H_2O) through repeated crystallization thrice from aq. EtOH. Compounds **11** (68 mg), **12** (90 mg), **14** (29 mg) and **13** (111 mg) were seperated from fr. VI (80:20, EtOH-H_2O) through repeated Sephadex LH-20 column fractionation, using BuOH saturated with H_2O, followed by prep. PC, using BAW as solvent.



Compound **13**

luteolin 7-O-β-(6"-galloylglucopyranoside), **13**: R_{fs}: Table 1. UV (MeOH and MeOH + shift reagent) λ_{max}: Table 1. FAB-MS: neg. ion 499: $[M-H]^-$, Mr: 500. Complete acid hydrolysis of **13** (20 mg in 10 ml aq. methanolic 2N HCl, 100°, 7 hrs) yielded glucose (CoPC), gallic acid and luteolin. Gallic acid: R_{fs}: Table 1. UV (MeOH) λ_{max}: Table 1. ^{1}H-NMR: δ (ppm): 6.95 (s, H-2 and H-6); luteolin: R_{fs}: Table 1. UV (MeOH and MeOH + shift reagents) λ_{max}: Table 1. ^{1}H-NMR: δ (ppm): 6.2 (d, J=2.5 Hz, H-6), 6.5 (d, J=2.5 Hz, H-8), 6.82 (s, H-3), 6.9 (d, J=7.5 Hz, H-5'), 7.52 (dd, J=7.5 and 2.5 Hz, H-6'), 7.6 (d, J=2.5 Hz, H-2'). On controlled acid hydrolysis (37 mg of **13** in 25 ml aq. 0.5N HCl, 100°, 3 hrs), both luteolin 7-O-β-glucoside **12** and 6-monogalloyl glucose were released and individually isolated by prep. PC, using solvent system 2 for elution. Luteolin 7-O-β-glucoside: R_{fs}: Table 1. UV (MeOH and MeOH + shift reagents) λ_{max}: Table 1. ^{1}H-NMR: luteolin moiety: δ (ppm): 6.42 (d, J=2.5 Hz, H-6), 6.72 (s, H-3), 6.79 (d, J=2.5 Hz, H-8), 6.92 (d, J=7.5 Hz, H-5'), 7.4 (m, H-2' and H-6'); β-glucose moiety: δ (ppm): 5.08 (d, J=7.5 Hz, H-1β), 3.3-3.5 (m, sugar protons overlapped by hydroxyl protons); 6-monogalloyl-(α/β)-glucose: R_{fs}: Table 1. UV (MeOH) λ_{max}: Table 1. ^{1}H-NMR: α-glucose moiety: δ (ppm): 5.1 (d, J=3.5 Hz, H-1α), 3.5-3.9 (m, H-2, H-3 and H-4), 3.92 (m, H-5), 4.38 (d, J=12.5 Hz, H-6), 4.25 (dd, J=12.5 and 4.5 Hz, H-6'); β-glucose moiety: δ (ppm): 4.6 (d, J=8 Hz, H-1β), 3.5-3.9 (m, H-2, H-3 and H-4), 3.93 (m, H-5), 4.42 (d, J=12.5 Hz, H-6), 4.3 (dd, J=12.5 and 4.5 Hz, H-6); galloyl moieties: δ (ppm): 6.99 (s) and 7.0 (s). ^{1}H-NMR of **13**: δ (ppm): 6.44 (d, J=2.5 Hz, H-6), 6.68 (s, H-3), 6.75 (d, J=2.5 Hz, H-8), 6.9 (d, J=7.5 Hz, H-5'), 6.92 (s, H-2 and H-6 galloyl), 7.4 (m, H-2' and H-6'), 5.17 (d, J=8 Hz, H-1β), 4.42 (d, J=12.5 Hz, H-6), 4.3 (dd, J= 12.5 and 4.5 Hz, H-6'). ^{13}C-NMR of **13**: Table 2.

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