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STUDIES ON THE CONSTITUENTS OF THE LEAVES OF *ACER NEGUNDO* (L.)

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تتميز النباتات من جنس الآسر بإستعمالاتها الطبية الشعبية المتعددة كقابض وفى علاج أمراض الكبد وكغسول للعين وفى إزالة القرح.

تم فى هذا البحث تحضير المستخلص الكحولى لأوراق هذا النبات ثم تجزئتها بإستخدام الهكسان وخلات الإيثيل وبعد ذلك تم فصل وفحص مكونات المستخلصين كل على حده بإستخدام كروماتوجرافيا العمود والطبقة الرقيقة.

كما تم دراسة الصفات الطبيعية والكيميائية للمركبات المفصولة بإستخدام مطياف الأشعة فوق البنفسجية ودون الحمراء ومطياف الكتلة والرنين النووى المغناطيسى الهيدروجينى والكربونى.

وقد أمكن التعرف على الكامبفيرول-٣،٧-ثنائى الجلوكوز ، الكامبفيرول-٣-روتينوزيد ، أيزورامنتين-٣-روتينوزيد ، حمض السينامك من خلاصة خلات الإيثيل بينما تم التعرف على ٤،'٣،٧،٥ -رباعى هيدروكسى أيزوفلافون ، ستيجماستيرول-٣-جلوكوزيد من مستخلص الهكسان.

Kaempferol-3,7-diglucoside (1), Kaempferol-3-O-ß-D-rutinoside (2), isorhamnetin-3-O-ß-D-rutinoside (3), cinnamic acid (4), 5,7,3`,4`-tetrahydroxyisoflavone(5) and stigmasterol-3-O-ß-D-glucoside (6) were isolated from ethyl acetate and hexane fractions of ethanol extract of the leaves of Acer negundo (L.). Identification of these compounds has been established by spectral evidence (UV, IR, MS, ^{1}H- and ^{13}C-NMR).

INTRODUCTION

The genus Acer (Maple Family,[1] Aceraceae) is cultivated for ornamental purposes and is reported to have several folkloric medicinal uses as astringent, to raise blisters,[2] for hepatic disorders, and as eye wash[3]. The plants of the genus have been subjected to many chemical investigations.

Previous literatures reported the isolation and identification of the chemical constituents of the leaves and stem bark of various *Acer* species. Methyl gallate and the flavonoids myricetin, cyanidin-3-glucoside, luteoline glucosides, kaempferol, quercetin, quercitrin, acylated flavonols and the biflavonoid agathisflavone were isolated from the leaves.[4-7] Some of these compounds showed interesting biological activity against human immuno-deficiency virus (HIV-1).[5] The phenolic diarylheptanoid acerogenin A-E and their glycosides, in addition to acerosides I-XIII were isolated from the stem bark.[8,9] ß-amyrin, ß-sitosterol, campesterol, stigmasterol, sitogluoside and ß-amyrin acetate were isolated from both the leaves and stem bark.[6] 2,6-Dimethoxy-p-benzoquinone was isolated from the aqueous methanolic extract from *Acer cratagifolium* steb root bark and tested as anti-inflammatory.[10]

This paper deals with the study of the chemical constituents of *Acer negundo* (L.) (Boxelder, Ash-leaved Maple)[11] and five phenolic compounds were reported for the first time from this plant.

EXPERIMENTAL

General experimental procedures

1- Melting points are uncorrected and were measured by Electrothermal 9100 Digital Melting Point Instrument (England Ltd., England).

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2- UV spectra are measured in methanol and different ionizing and complexing agents using a Uvidec-320 (Jasco, Tokyo, Japan) spectrophotometer with matched 1 cm quartz cells.
3- ^{1}H- and ^{13}C-NMR spectra were run in CD_3OD at 400 MHz using JEOL TNM-LA400, FT NMR system, Japan, using TMS as internal standard.
4- EIMS and FAB-MS were recorded by JEOL, JMS 600 H.
5- Column chromatography using silica gel G (E. Merck, 6.3-20μ).
6- TLC was performed on silica gel G F_{254} (E. Merck, Germany) activated layers using $CHCl_3$—MeOH—H_2O (75:22:3) (system I), CH_2Cl_2-MeOH-H_2O (40:10:1) (system II) and $CHCl_3$-MeOH-H_2O (65:30:5) (system III). The spots were detected under UV before and after exposure to ammonia.

Plant material

The leaves of *Acer negundo* (L.) were collected from the Faculty of Agriculture, Assiut University in May 1997 and identified by Prof. Dr. Gamal Taha, Department of Horticulture, Faculty of Agriculture, Assiut University.

Extraction and isolation

The air-dried leaves (2 Kg) of *Acer negundo* (L.) was extracted with ethanol (90 %) at room temperature by maceration. The residue left after evaporation of the solvent was diluted with water and successively extracted with n-hexane and ethyl acetate. The ethyl acetate soluble fraction (15 g) was chromatographed over silica gel using $CHCl_3$-MeOH gradients. The fraction eluted with $CHCl_3$-MeOH (8:2) was subjected to PTLC using solvent system I to afford compound **1** (R_f= 0.50). The fraction eluted with $CHCl_3$-MeOH (7:3) was subjected to PTLC using solvent system III and gave compounds **2,3** and **4** (R_f= 0.60, 0.48 and 0.30 respectively).

The n-hexane soluble fraction (20 g) was chromatographed over silica gel using n-hexane-ethyl acetate gradient. The fraction eluted with n-hexane-ethyl acetate (9:1) was rechromatographed over sephadex LH-20 using MeOH and then purified by PTLC using solvent system II yielded compound **5** (R_f= 0.70). The fraction eluted with n-hexane-ethyl acetate (8:2) yielded compound **6** (R_f= 0.20).

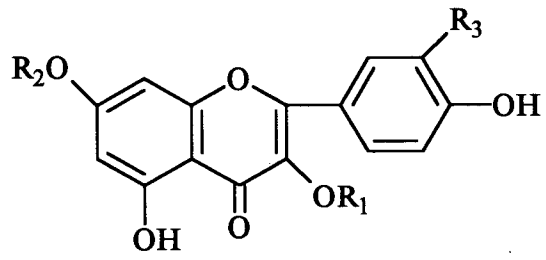
Acid hydrolysis

Five mg portion of each of the isolated glycosides (**1-3** and **5**) was dissolved in 5 ml of 5 % hydrochloric acid in methanol. The mixture was refluxed for 3 hours on a boiling water-bath, cooled, the aglycone was extracted with chloroform, purified and subjected to TLC. The produced sugars were identified by TLC using silica gel G and solvent system: n-butanol-acetone-formic acid-water (60:17:8:15).[12]

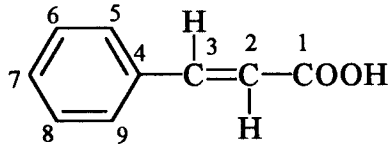
Compound 1: Yellow amorphous powder (15 mg), m.p 245-247°, UV (λ_{max}, nm, MeOH): 264, 301 sh, 352; NaOMe: 272, 320, 395; $AlCl_3$: 272, 301 sh, 355, 400; $AlCl_3$/HCl: 273, 300, 350, 395; NaOAc: 268, 357; NaOAc/H_3BO_3: 265, 301, 352. ^{1}H-NMR spectrum: δ 3.2-3.8 (sugar protons), 5.24 (1H, d, J= 7.0 Hz, H-1``), 5.32 (1H, d, J= 7.0 Hz, H-1```), 6.19 (1H, d, J= 2.0 Hz, H-6), 6.38 (1H, d, J= 2.0 Hz, H-8), 6.88 (2H, d, J= 8.0 Hz, H-3`,5`),8.04 (2H, d, J= 8.0 Hz, H-2`,6`). EI$^+$-MS, 286 [aglycone]$^+$.

Compound 2: Yellow amorphous powder (10 mg), m.p 258-260°, UV (λ_{max}, nm), MeOH: 265, 302 sh, 350; NaOMe: 273, 320, 400; $AlCl_3$: 273, 306 sh, 350, 400; NaOAc: 272, 304, 356; NaOAc/H_3BO_3: 266, 302, 350. FAB-MS; 595 [M+1]$^+$, 449 [(M+1)-rhamnosyl]$^+$, 287 [449-glycosyl]$^+$ = [aglycone+1]$^+$. ^{1}H-NMR spectrum: δ 1.11 (3H, d, J= 6.1 Hz, CH_3-rh), 3.22-3.82 (sugar protons), 4.51 (1H, d, J= 1.50 Hz, H-1```, anomeric rhamnose-H), 5.10 (1H, d, J= 7.1 Hz, H-1``, anomeric glucose-H), 6.19 (1H, d, J= 2.0 Hz, H-6), 6.38 (1H, d, J= 2.0 Hz, H-8), 6.87 (2H, d, J= 8.0 Hz, H-3`,5`), 8.04 (2H, d, J= 8.0 Hz, H-2`,6`).

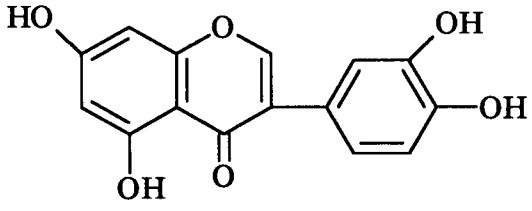
Compound 3: Yellow amorphous powder (8 mg), m.p 127-130°, UV (λ_{max}, nm), MeOH: 256, 269 sh, 309 sh, 354; NaOMe: 265, 320 sh,



Compound	R_1	R_2	R_3
1	glucose	glucose	H
2	glucose - rhamnose	H	H
3	glucose - rhamnose	H	OMe



Compound 4



Compound 5

405; $AlCl_3$: 267, 305 sh, 360 sh, 403; $AlCl_3$/HCl: 264, 298 sh, 356, 401; NaOAc: 274, 324, 380; NaOAc/H_3BO_3: 254, 267 sh, 302 sh, 357. FAB-MS; 625 $[M+1]^+$, 479 [(M+1)-rhamnosyl]$^+$, 317 [(M+1)-(glycosyl+rhamnosyl)]$^+$= [aglycone+1]$^+$. ^{1}H-NMR spectrum: δ 1.09 (3H, d, J= 6.1 Hz, CH_3-rh), 3.22-3.82 (sugar protons), 3.94 (s) 3`-OMe, 4.52 (1H, d, J= 1.50 Hz, H-1```, anomeric rhamnose-H), 5.21 (1H, d, J= 7.1 Hz, H-1``,anomeric glucose-H), 6.19 (1H, d, J= 2.0 Hz, H-6), 6.38 (1H, d, J= 2.0 Hz, H-8), 6.90 (1H, d, J= 8.4 Hz, H-5`),7.6 (1H, dd, J= 8.0, 2.0 Hz, H-6`), 7.94 (1H, d, J= 2.0 Hz, H-2`).

Compound 4: White powder (15 mg), UV (λ_{max}, nm), MeOH: 273, m.p 133-134°. IR (KBr, υ, cm^{-1}): 2645, 1686, 1624, 976, 763, 705. ^{1}H-NMR spectrum: δ= 6.21, (1H, d, J= 16.1 Hz, H-2), 6.67 (2H, dd, J= 8.3, 2 Hz, H-6,8), 6.84 (2H, dd, J= 8.3, 2 Hz, H-5,9), 6.95 (1H, m, H-7), 7.48 (1H, d, J= 16.1 Hz, H-3).

Compound 5: Yellow amorphous powder (10 mg), UV (λ_{max}, nm), MeOH: 262, 294 sh, 343 sh; $AlCl_3$: 277, 338, 405, +NaOAc: 274, 332; NaOMe: 273, 337 (dec.). ^{1}H-NMR spectrum: δ= 6.02 (1H, d, J= 2.0 Hz, H-6), 6.21 (1H, d, J= 2.0 Hz, H-8), 6.77 (1H, d, J= 8.6 Hz, H-5`),7.33 (1H, dd, J= 8.6, 3.15 Hz, H-6`),7.62 (1H, d, J= 3.15 Hz, H-2`), 8.45 (1H, s, H-2). EI$^+$-MS: 286 $[M]^+$, other peaks at m/z= 153, 152, 122 and 121.

Compound 6: White amorphous powder (150 mg) from $CHCl_3$, m.p 232-237°. IR (KBr, υ, cm^{-1}): 3400, 2945, 1370, 1610, 1070 and 1020.

RESULTS AND DISCUSSION

The air-dried powdered leaves of *Acer negundo* (L.) was extracted with ethanol 90 % and the concentrated extract was fractionated with n-hexane, and ethyl acetate. Each extract was concentrated and chromatographed on silica gel column. Ethyl acetate fraction afforded compounds **1**, **2**, **3**, and **4**.

Compounds **1** and **2** gave mass spectra with $[M]^+$ or $[M+1]^+$ peaks at m/z 286 or 287 respectively consistent with the formula $C_{15}H_{10}O_6$, which was confirmed with ^{13}C-NMR and ^{1}H-NMR data. In ^{1}H-NMR spectra of **1** and **2**, two doublets at δ= 6.88 and 8.04 (J= 8.0 Hz) were assigned to H-3`,5` and H-2`,6` respectively which were confirmed by two signals in ^{13}C-NMR at 116.88 and 133.08 for **1**; 116.93 and 133.15 for **2**, Table 1. Also the spectra gave two doublets (J= 2.0 Hz) characteristic for H-6 and H-8. The glycosidic nature of **1** was confirmed from the appearance of two anomeric protons, doublets at 5.24 and 5.32 (J= 7.0 Hz) and the high J values indicated a ß-linkage of the sugar.[13] The ^{13}C-NMR chemical shifts of the sugar carbons were consistent with the corresponding data for two separate glucose units[14] which was confirmed by the presence of two signals at 63.43 and 62.98 (for C-6`` and C-6```) in ^{13}C-NMR, Table 1.

The absence of bathochromic shift in band II of compound **1** with sodium acetate indicated the glycosylation at C-7. Compound **1** also has a purple fluorescent spot under UV and turned yellow with ammonia indicating the presence of free 5-OH and substitution in 3-position.[15]

Compound **2** revealed two anomeric protons at 4.50 (d, J= 1.5 Hz) and 5.10 (d, J= 7.1 Hz) characteristic for rhamnose and glucose respectively. The presence of rhamnose was confirmed by the appearance of CH_3 signal at 1.17 (d, J= 6.1 Hz) and at 18.69 in ^{13}C-NMR. The ^{13}C-NMR shifts of the C-6`` and C-1``` appeared at 69.39 and 103.20 respectively, Table 1, suggested that the interglycosidic linkage was (1→6). ^{1}H- and ^{13}C-NMR data, Table 1, are identical with the reported values.[16,17]

Acid hydrolysis of each of **1** and **2** with 5 % HCl in methanol gave glucose and kaempferol (**1**); glucose, rhamnose and kaempferol (**2**), which were identified by co-PC and co-TLC with authentic samples.

From the aforementioned data, it could be concluded that, compounds **1** and **2** are kaempferol-3,7-diglucoside and kaempferol-3-O-rutinoside respectively.

Compound **3** FAB-MS gave a mass spectrum with $[M+1]^+$ at m/z $[M+1]^+$= 625, 479 [M-rhamnosyl+1]$^+$, 317 [aglycone+1]$^+$ consistent with the molecular formula $C_{16}H_{12}O_7$.

The UV spectrum indicated a free hydroxyl group at C-7 evidenced by the bathochromic shift of band II (+18 nm) obtained by the addition of sodium acetate. The bathochromic shift in band I (+51 nm) with NaOMe indicated a free OH at C-4`.[4] Addition of $AlCl_3$ or $AlCl_3$/HCl produced a shift of 49 and 47 nm respectively in band I indicated the presence of free hydroxyl group at C-5 and absence of ortho-dihydroxy groups at ring B.

The presence of three aromatic protons in ^{1}H-NMR spectrum of **3**, was confirmed through an ABX-type coupling at δ 7.60 (1H, dd, J= 8.0, 2.0 Hz, H-6`), 6.90 (1H, d, J= 8.4 Hz, H-5`) and 7.94 (1H, d, J= 2.0 Hz, H-2`). In addition, two doublets with J= 2.0 Hz at 6.19 and 6.37 indicated H-6 and H-8 respectively. A singlet at 3.94 was assigned to a methoxyl group. The glycosidic nature of **3** was identical with that of **2**. ^{1}H-NMR data are in accordance with the reported values.[16]

Based on the above data, compound **3** was identified as isorhamnetin-3-O-rutinoside. Acid hydrolysis of **3** with 5 % HCl in methanol gave glucose, rhamnose and isorhamnetin, which were identified by co-PC and co-TLC with authentic samples.

^{1}H-NMR spectrum of **4** exhibited diagnostically valuable signals for cinnamic acid appeared at δ: 6.21 and 7.48 (each 1 H, J= 16.1 Hz) for H-2 and H-3, 6.67 (for H-6 and H-8), 6.84 (for H-5 and H-9), each dd, J= 8.3 and 2.0 Hz), at 6.95 (m) for H-7.

^{13}C-NMR spectrum indicated a carboxylic acid carbon at 169.65 and two carbons for CH= CH at δ 115.96 and 147.65. Other signals were in acccordance with the reported data for cinnamic acid.[18]

The n-hexane fraction afforded compounds **5** and **6**.

Compound **5** gave a mass spectrum with $[M]^+$ peak at m/z 286 consistent with the formula $C_{15}H_{10}O_6$, which was confirmed by ^{1}H-NMR and ^{13}C-NMR, Table 1.

Table 1: ^{13}C-NMR data of compounds (**1-5**) isolated from *Acer negundo* (L.), (100 MHz, CD_3OD, relative to TMS).

C - atoms	1	2	3	4	5
1	--	--	--	169.65	--
2	159.42	159.29	159.33	115.96	156.90
3	133.17	136.31	136.30	147.65	122.75
4	180.32	180.13	180.04	128.78	183.67
5	163.83	162.27	162.27	117.36	163.80
6	100.71	100.50	100.60	123.74	98.98
7	166.78	167.50	167.09	116.55	161.00
8	95.58	95.80	95.80	123.84	93.41
9	159.93	160.18	159.62	117.36	156.90
10	105.85	106.37	106.37	--	104.35
1`	124.40	123.54	123.76	--	120.30
2`	133.08	133.15	115.37	--	116.05
3`	116.88	116.93	151.65	--	144.86
4`	162.36	163.66	149.10	--	145.38
5`	116.88	116.93	116.93	--	114.80
6`	133.08	133.15	124.80	--	119.06
1``	104.61	105.46	105.28	--	--
2``	73.97	76.54	76.69	--	--
3``	76.50	78.94	78.94	--	--
4``	72.15	72.24	72.40	--	--
5``	79.18	77.99	78.13	--	--
6``	63.43	69.39	69.34	--	--
1```	104.50	103.20	103.29	--	--
2```	73.82	72.86	72.86	--	--
3```	75.81	73.10	73.10	--	--
4```	70.80	74.70	74.63	--	--
5```	78.83	70.51	70.56	--	--
6```	62.98	18.69	18.66	--	--
OMe on C-3`	--	--	57.58	--	--

The UV spectrum of compound **5** showed absorption maxima at 262 (band II), 294 sh and 343 (sh) nm (band I) which indicated an isoflavone skeleton.[19] A free hydroxyl group at C-7 was evidenced by the bathochromic shift in band II (+12 nm) obtained by the addition of sodium acetate. ^{1}H-NMR spectrum of **5** displayed a characteristic one proton singlet at δ= 8.45 for H-2 of an isoflavonoid nucleus, in addition to two doublets at 6.02 and 6.21 for H-6 and H-8 (each 1H, J= 2.0 Hz). Also the spectrum showed the ABX system (three signals) characteristic for 3`,4`-disubstituted benzene ring (ring B), each 1 H for H-5`, 2` and 6`. From the aforementioned data, it could be concluded that **5** is 5, 7, 3`,4`-tetrahydroxy-isoflavone.

Compound **6** was identified as stigmasterol-3-O-ß-D-glucoside after acid hydrolysis. The chromatographic study revealed that the sugar appeared as a single spot corresponding to authentic glucose (R_f = 0.43). The aglycone was identified as stigmasterol by comparing m.p, IR, and co-chromatography with reference sample.

In conclusion, the flavonoids myricetin, cyanidin-3-glucoside, luteoline glucosides, kaempferol, quercetin, quercitrin, acylated flavonols and the biflavonoid agathisflavone were prevously isolated from the leaves of different *Acer* species. Little work was found to be reported on *Acer negundo* L. In this study, isorhamnetin-3-O-rutinoside, 5, 7, 3`, 4`-tetra-hydroxyisoflavone and cinnamic acid have not been found so far in *Acer* species.

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