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# Chemical Investigation of the Constitutive Flavonoid Glycosides of the Leaves of *Crataegus sinaica*

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**Abstract** – Leaves of *Crataegus sinaica* Boiss, contain the new C-glycosyl flavone 3''',4'''-di-O-acetyl-2''-O-α-rhamnosylvitexin, together with the hitherto unknown, dihydroflavonol 3-O-xyloside, (2R:3R)-dihydroquercetin-3-O-β-xylopyranoside. The known compounds (+)-catechin, (-)-epicatechin, vicenin-II, 2''-O-α-rhamnosylvitexin, and 4'''-O-acetyl-2''-O-α-rhamnosylvitexin were also characterized. Structures were established by conventional methods of analysis and confirmed by $^{1}$H-, $^{13}$C-NMR, and ESI-MS.

**Key words** – *Crataegus sinaica,* Rosaceae, leaves, C-glycosyl flavones, 3''',4'''-di-O-acetyl-2''-O-α-rhamnosylvitexin, dihydroflavonol 3-O-xyloside, (2R:3R)-dihydroquercetin 3-O-β-xylopyranoside, NMR, ESI-MS.

## Introduction

Leaf extract of different *Crataegus* species (Rosaceae) is a typical plant derived medicament known as Crataegi folium gun flore (hawthorn extract) which is used mainly for treating cardiac insufficiency corresponding to stages I and II of the NYHA (Bisset, N.G. ed., 1994).

Previous phytochemical investigation proved that the official *Crataegus* species, *C. oxyacantha, C. monogyna, C. pentagyna, C. nigra* and *C. azarolus* are capale on sythesizing and accumulating, in their leaves, high contents of oligomeric procyanidines, vitexin 2''-O-rhamnosyl in addition to its 4'''-monoacetyl derivative, together with trace amount of rutin and spiraeoside as well. Some compounds were recently reported to occur in the leaves of *C. sinaica* (Shahat, A.A. *et al*, 1996). In the present study, the isolation and structure elucidation of the novel flavonoids 3''',4'''-di-O-acetyl-2''-O-α-rhamnosylvitexin (**6**) and (2R,3R)-dihydroquercetin-3-O-xylopyranoside (**7**) from the aqueous ethanolic *C. sinaica* leaf extract were described. The known compound, (+)-catechin **1**, (-)-epicatechin **2**, vicenin II **3**, 2''-O-α-rhamnosylvitexin **4** and 4'''-O-acetyl-2''-O-α-rhamnosylvitexin **5** were also isolated and identified.

It should be mentioned however, that flavananols and their glycosides were not known in *Crataegus* plants, before the characterization of compound **7** in the present study. However, the flavanone glycosides, naringenin 5,7-diglucoside and eriodictoyl 5,3'-diglucoside were identified before, to occur in the leaves of *C. phenophyrum* (Bisset, N.G., 1994).

## Results and Discussion

The fresh leaves of *Crataegus sinaica* Boiss (Rosaceae), a much branched 2-5 m tall thorny shrub, bearing rounded-ovoid fruits of pea-size, were exhaustively extracted with aqueous ethanol mixture (3:1). The dried extract was shown by preliminary 2D-PC screening to contain a phenolic mixture (positive res-

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ponse towards $FeCl_3$ spray reagent), among which compounds **1-7** were isolated and purified by standard methods (polyamide and Sephadex LH-20 CC, followed by prep. PC). The known compounds **1-5** gave $[\alpha]_D^{27}$, chromatographic, UV spectral (Table 1), FAB-MS, $^1$H- and $^{13}$C-NMR spectral analytical data typical for (+)-catechin , (-)-epicatechin (Shen, C. C. *et al.*, 1993), vicenin II (Hussein, S. A. *et al.*, 1997), 2"-O-$\alpha$-rhamnosylvitexin (Palme, E. *et al.*, 1994) and 4'''-O-acetyl-2"-O-$\alpha$-rhamnosylvitexin (Harborne, J. *et al.*, 1982).

The new compound **6** was isolated as an off-white amorphous powder and was found to possess chromatographic and colour properties [dark purple spot on PC under UV light turning light yellow with Naturstoff spray reagent, specific for hydroxylated flavonoid (Neu, R., 1957)] similar to those exhibited by glycosylated apigenin derivatives, mostly of the C-glycosylated type as could be concluded from its UV spectral analysis with diagnostic reagents (Mabry, T. *et al.*, 1970).

It showed a molecular weight of 662 amu in negative ESI-MS ($[M-H]^-$: 661) and a strong absorption at 1650 $cm^{-1}$ in its IR spectrum consistent with chelated carbonyl group.

Complete acid hydrolysis (2N aqueous methanolic HCl 1:1, 7 hours, 100℃) **6** yielded vitexin (major spot), isovitexin (minor spot) (CoPC) as the only phenolic hydrolysis products and rhamnose (CoPC) as the released sugar. This result when incorporated with the received UV spectral data (bathochromic shift of 45 nm for band I with $AlCl_3$/HCl, indicative of a free 5-hydroxyvitexin, bathocheromic shift of 8 nm for band II with NaOAc, indicative of unsubstituted 7-hydroxyvitexin group and a shift of 50 nm with NaOMe for band I, indicative of free 4'-hydroxyvitexin group) suggested that compound **6** is built up through rhamnosylation of one of the glucosyl hydroxyl groups of the vitexin moiety of **6**. However, the measured molecular weight of **6** (662 amu) together with the presence of a strong chelated carbonyl absorption band in its IR spectrum and the distinct Rf-values which are quite different from those of the isolated known compounds 2"-O-$\alpha$-rhamnosylvitexin **4** and 4'''-O-acetyl-2"-O-$\alpha$-rhamnosylvitexin **5** as well suggested a diacetylrhamnosylvitexin structure for compound **6**.

To find out the site of attachment of the O-rhamnosyl and acetyl moieties to the vitexin moiety in the molecule of compound **6**, $^1$H-NMR spectral analysis was then engaged. The recorded proton resonances in the received spectrum ($CD_3OD$, room temp.) were

**Table 1.** Chromatographic and UV data of phenolic compounds isolated from the leaves of *Crataegus sinaica*

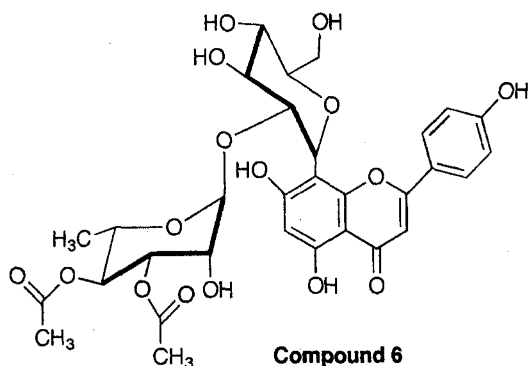
| Compounds | Chromatographic properties | | | UV Spectral data λmax (nm) | | | | |
|---|---|---|---|---|---|---|---|---|
| | $H_2O$ | HOAc | BAW | MeOH | NaOMe | $AlCl_3$ | NaOAc | NaOAc/$H_3BO_3$ |
| (+)-Catachen | 65 | 70 | 64 | 280 | | | | |
| (-)-Epicatechin | 58 | 67 | 60 | 280 | | | | |
| Vicenin II | 25 | 45 | 33 | 272, 333 | 275, 361, 400 | 280, 393<br>345, 383 | 282, 393 | 283, 390 |
| 2"-O-α-Rhamnosyl-vitexin | 53 | 56 | 55 | 272, 301*, 333 | 273, 330, 385 | 277, 305,<br>348, 380 | 280, 302*,<br>388 | 280, 282*,<br>305*, 390 |
| 4''''-Acetyl-2"-O-α-rhamnosylvitexin | 50 | 53 | 57 | 270, 302*, 335 | 270, 332, 385 | 275, 305,<br>350, 380 | 280, 305*,<br>388 | 280, 280*,<br>305*, 390 |
| 3''',4'''-di-O-Acetyl-2"-O-α-rhamnosylvitexin | 48 | 50 | 59 | 270, 302*, 335 | 272, 330, 383 | 275, 305,<br>352, 382 | 280, 305*,<br>389 | 280, 280*,<br>305*, 390 |
| (2R:3R)-Dihydroquercetin 3-O-xyloside | 50 | 55 | 58 | 292, 342* | 293, 368 | 313, 385 | 398, 345* | 396, 342* |

*Inflection.

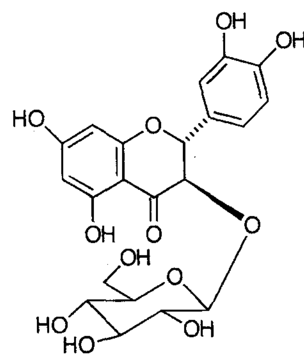
assigned, mostly by comparison with the corresponding resonances in the $^{1}$H-NMR spectra of 2″-O-α-rhamnosylvitexin **4** and 4‴-O-acetyl-2″-O-α-rhamnosylvitexin **5**. A one-proton downfield triplet ($J$=10 Hz) assignable to the α-rhamnose H-4‴ proton was recognized at δ 4.42 to prove acetylation at the hydroxy group OH-4‴. Additional one proton double doublet ($J$=3.5 and 10 Hz) revealed itself more downfield at δ 4.68. The characteristic mode of splitting of this signal proved that it belongs to the α-rhamnosyl H-3‴ proton (Altona *et al.*, 1980), thus confirming the acetylation of the corresponding hydroxy group, OH-3‴. This finding, together with the recognized proton resonances pattern of 2″-O-α-rhamnosylvitexin proved that compound **6** is 3‴,4‴-di-O-acetyl-2″-O-α-rhamnosylvitexin.

$^{13}$C-NMR analysis of **6** further confirmed that achieved structure. In the received spectrum, the presence of two acetyl groups followed from the two carbonyl carbon resonances at δ 173.4 and 173.5 ppm and from the two methyl carbon resonances at δ 20.2 and 20.3 as well. The remarkable changes in the chemical shift values of the C-3‴, C-4‴ and C-5‴ α-rhamnosyl carbon resonances, on comparison with those of the corresponding resonances in the spectrum of 4‴-O-acetyl-2″-O-α-rhamnosylvitexin (see experimental) confirmed acetylation of the α-rhamnosylhydroxy group No. 3‴ which brings the α-rhamnosyl C-3‴ carbon resonances downfield to δ 74.5 (α-effect) but shift the resonances of the two vicinal carbon (C-4‴ and C-2‴) resonances upfield to δ 70.8 and 73.2 ppm, respectively. Consequently the structure of compound **6** is finally confirmed to be 3‴,4‴-di-O-acetyl-2″-O-α-rhamnosylvitexin, which represents to the best of our knowledge a new natural flavonoid, not reported before in literature.

The new phenolic **7** (greenish blue $FeCl_3$ test), isolated as a light brown amorphous powder of chromatographic properties (dark purple spot on PC under UV light unchanged with ammonia vapour) and UV spectral data (Table 1) consistent with dihydroquercetin 3-O-glycoside (Markham, K. *et al.*, 1984). It exhibited a molecular weight of 436 amu in negative ESI-MS, ($[M-H]^-$: 435). On complete acid hydrolysis (2N aqueous HCl, 100 ℃, 3 hrs) it yielded xylose (CoPC) and dihydroquercetin (CoPC, UV and $^{1}$H-NMR analysis). However, a trace amount of the flavonol quercetin (CoPC) was also detected in the hydrolysate (Kenneth, R. *et al.*, 1984). The $^{1}$H-NMR spectrum of 7 (Acetone-$d_6$, room temp.) exhibited the characteristic resonance pattern of (2R:3R)-dihydroquercetin, whereby the trans-diaxial relationship between H-2 and H-3 protons (see formulae) was evident from the 11 Hz coupling constant of the two doublet resonance located in this spectrum at δ 5.28 and 4.72 ppm, attributed to H-2 and H-3 respectively. This recognizable downfield shifts on comparison with the chemical



**Compound 6**



**Compound 7**

shifts of the corresponding signals in the spectrum of (+)-dihydroquercetin, confirmed glycosylation of the flavanonol hydroxyl group No. 3. The 3-O-linked β-xylopyranoside in **7** was further concluded from the recognized β-anomeric proton doublet ($J$=7 Hz) located at δ 4.72. Other protons in this spectrum agreed well with the proposed structure of **7** as (2R:3R)-dihydroquercetin 3-O-xyloside.

The $^{13}$C-NMR spectral data of **7** further confirmed its achieved structure and showed chemical shifts for the recorded fifteen aglycone carbon resonances essentially similar to those of the corresponding signals in the spectrum of (2R:3R)-dihydroquercetin (Shen, C. C. *et al.*, 1993). However, a distinction can be made, whereby the resonance of C-3 was found shifted downfield to δ 76.8 (C-3 resonance in dihydroquercetin present at δ 71.6) due to xylosidation of the connected hydroxyl group (α-effect).

The β-anomeric xylose carbon revealed its resonance in this spectrum at δ 102.4. Other resonance, in this spectrum, possessed chemical shifts which were in consistent with the achieved structure of **7** as (2R:3R)-dihydroquercetin 3-O-β-xylopyranoside, a new natural flavonoid which was not reported before to occur in nature.

## Experimental

**General** – $^1$H-NMR spectra were measured on a JEOL 270 MHz NMR spectrometer, and the chemical shifts were measured relative to TMS. $^{13}$C-NMR resonance were measured relative to $CD_3OD$ or $(CD_3)_2CO$ and converted to the TMS scale by adding 49 or 29.8, respectively. Typical conditions. Spectral width=4000 Hz for $^1$H and 15000 Hz for $^{13}$C, 32 K data points and a flip angle of 45°. ESI-MS (negative mode). The direct flow injection technique was applied. Sample in MeOH was introduced (1.25 ml min$^{-1}$) together with MeOH sheath liquid (5 ml min$^{-1}$) by Havard infusion pump 9 ml min$^{-1}$ $SF_6$ sheath gas into the ESI ion source of a Finnigan MAT 4600 spectrometer. PC was carried out on Whatman No. 1 paper, using solvent system: (1) $H_2O$; (2) HOAc-$H_2O$ (3:17); (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 Whatmann No. 3 MM paper and solvent 3 and 4 for sugar analysis.

**Plant material** – Sample from the leaves of *Crataegus sinaica* Boiss, mature tree growing wild in the South of Sinai peninsula, Egypt, were collected in Jan. 1996 and authenticated by Dr. N. El-Hadeady, Prof. of Botany, Faculty of Science, Cairo University.

**Isolation and identification** – Powdered leaves (dried in shadow in an air stream) were exhaustively extracted with EtOH-$H_2O$ (3:1), filtered on, concentrated *in vacuo* and the concentrated extract was applied to a polyamide 6S CC (Riedel-De Häen AG, Seelze Hanover, Germany) and eluted with $H_2O$ followed by $H_2O$-EtOH mixts of decreasing polarities to yield seven major frs. (I-VII). Compound **1** (89 mg) and **2** (62 mg) were individually isolated from fr. II (eluted by 20% EtOH) by Sephadex LH-20 fractionation using EtOH for elution and compound **3** (122 mg) from fr. III (eluted by 30% EtOH) by repeated crystallization from aqu. alcohol. Compound **4** (146 mg) was separated from fr. IV (eluted by 50% EtOH) by CC on Sephadex LH-20 using EtOH and EtOH cntaining 1:1 mixture of $Me_2CO$-$H_2O$ (7:1), followed by prep. PC using BAW as solvent system. Compound **5** (98 mg) was separated from fr. V (eluted by 60% EtOH) by prep. PC using BAW as solvent system. Compound **6** (112 mg) and **7** (202 mg) were individually separated from fr. VI (eluted by 70% EtOH) through polyamide column fractionation, using a mixture of MeOH-toluene-$H_2O$ (60:38:2) as an eluent, followed by prep. PC of the eluted crude materials using BAW as solvent in both cases.

*3''',4'''-di-O-acetyl-2''-O-α-rhamnosylvitexin* **6**: Rfs: (Table 1). UV (MeOH) $\lambda_{max}$: (Table 1). ESI-MS (-ve) : m/z 661 $[M-H]^-$. Normal complete acid hydrolysis (18 mg in 10 ml 2N aq. methanolic HCl, 1:1, 100°, 7 hr) of **6** yielded vitexin (major component), isovitexin (minor component) and rhamnose (CoPC). $^1$H-NMR (270 MHz, $CD_3OD$) δ: vitexin moiety: 6.3 (s, H-6), 6.65 (s, H-3), 6.95 (d, $J$=7.5 Hz, H-3' and H-5'), 8.02 (d, $J$=7.5 Hz, H-2', H-6'), 5.04 (d, $J$=8 Hz glucosyl anomeric proton), 3.45-4.15 (m, remaining glucosyl protons together with rhamnosyl H-2 and H-5); rhamnosyl moiety; 5.1 (d, $J$=1.5 Hz, anomeric proton), 4.68 (dd, $J$=3.5 and 10 Hz, H-3), 4.42 (t, $J$=10 Hz, H-4), 3.45-4.15 (m, glucosyl and H-2 and H-5 rhamnosyl protons), 0.68 (d, $J$=6 Hz, Me protons); acetyl moieties: 1.97 and 1.95 (two s, two OAc methyl groups).

$^{13}$C-NMR δppm: Vitexin moiety : 167.5 (C-2), 104.5 (C-3), 184.96 (C-4), 167.0 (C-5), 101.94 (C-6), 165.13 (C-7), 106.8 (C-8), 158.7 (C-9), 106.6 (C-10), 124.33 (C-1'), 130.92 (C-2' and C-6'), 117.85 (C-3' and C-5'), 163.6 (C-4'), 74.8 (C-1''), 78.6 (C-2''), 82.2 (C-3''), 73.2 (C-4''), 83.65 (C-5''), 63.8 (C-6''); rhamnosyl moiety: 101.94 (C-1'''), 70.8 (C-2'''), 74.5 (C-3'''), 73.2 (C-4'''), 68.0 (C-5'''), 17.5 (Me group); acetyl moieties: 173.5 and 173.4 (two C=O), 20.0 and 20.08 (two Me groups).

*(2R:3R)-Dihydroquercetin 3-O-β-xyloside* **7**, Rfs: (Table 1). UV (MeOH) $\lambda_{max}$: (Table 1). ESI-MS (-ve): m/z 435 $[M-H]^-$. Complete acid hydrolysis (33 mg in 15 ml 2N aq. HCl, 1:1, 100°, 3 hr) of **7** yielded dihydroquercetin (major component), quercetin (trace amount) and xylose (CoPC). $^1$H-NMR (270 MHz $(CD_3)_2CO$, room temp.) δ: (2R:3R)-dihydroquercetin moiety, 5.28 (d, $J$=11 Hz, H-2), 4.72 (d, $J$=11 Hz, H-3), 5.92 (broad, s, $\Delta\gamma_{1/2}$=3 Hz, 2H, H-6 and H-8), 6.82 (s, 2H, H-5' and H-6'), 7.02 (s, 1H, H-2'); β-xylose moiety: 4.72 (d, $J$=7 Hz, anomeric proton), 3.15-4.05 (m, xylose and hydroxy protons). $^{13}$C-NMR δ: (2R:3R)-dihydroquercetin moiety; 82.43 (C-2), 76.8 (C-3), 194.43 (C-4), 164.4 (C-5), 96.9 (C-6), 168.17 (C-7), 96.0 (C-8), 163.03 (C-9), 101.8 (C-10), 127.95 (C-1'), 115.9 (C-2'), 145.6 (C-3'), 146.33 (C-4'), 115.7 (C-5'), 119.93 (C-6'); β-xylopyranoside moiety: 102.4 (C-1''), 72.4 (C-2''), 76.8 (C-3''), 69.8 (C-4''), 64.9 (C-5'').

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