Chlorophyl Biodegradation Product from *Hamamelis Virginiana* Autumnal Leaves

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The biodegradation of bioorganic solid waste involves several million tons of senescent plant leaves every autumn. Chemical evidence about bioorganic matter contained in the senescent leaves remains undetermined. The biodegradation of senescent leaves comprises a series of biodegradation transitions that bring about changes in leaf texture, metabolic changes and colour. Leaves turn yellow as a result of chlorophyll biodegradation. Chlorophyll biodegradation products, in the autumnal leaves of Hamamelis virginiana, Hamamelidaceae, were investigated. Here is a report on one chlorophyll biodegradation product isolated from yellow Hamamelis virginiana, Hamamelidaceae autumnal leaves. The structure of the isolated chlorophyl biodegradation product was elucidated by spectroscopic and spectrometric data. The isolated chlorophyl biodegradation product was an UNCC (Urobilinogenic Non – fluorescent Chlorophyll Catabolite).

Keywords: UNCC, biodegradation, Hamamelis virginiana, Hamamelidaceae

Chlorophyl biodegradation has been observed in senescent leaves of several plant families: Altingiaceae[1], Amaranthaceae [2, 3], Brassicaceae [4], Cercidiphyllaceae [5, 6], Gramineae [7], Hamamelidaceae [8] and Solanaceae [9, 10]. From the practical point of view, colourless chlorophyll biodegradation products isolated can be divided into two groups: non – fluorescent chlorophyll catabolites (NCCs) [1 - 7, 9, 10] and urobilinogenic non – fluorescent chlorophyll catabolites (UNCCs) [8]. NCCs can further be subdivided into two groups: glucosylated ones [4, 9, 10] and aglicons [1- 3, 5-7]. Aglicons can further be subdivided into two group: the ones where modification on the lateral vinyl group has not occurred [1, 2, 5, 6] and others where the lateral vinyl group was oxidized into 1, 2 – diols [3, 7]. The chlorophyll biodegradation product isolated from *Parrotia persica*, Hamamelidaceae was an UNCC [8]. The purpose of this paper is to compare chlorophyll biodegradation product isolated from *Hamamelis virginiana*, Hamamelidaceae autumnal leaves with the chlorophyll biodegradation product isolated from *Parrotia persica*, Hamamelidaceae autumnal leaves and to observe the differences.

Experimental part

General

General part is the same as previously described [8].

Plant material

Hamamelis virginiana, Hamamelidaceae, leaves were collected during the autumn (2004) from the Botanical Garden of Fribourg, Switzerland.

Extraction and Isolation

Extraction and isolation were the same as for *Parrotia persica* autumnal leaves[8], with the following differences: *Hamamelis virginiana* Hamamelidaceae leaves (120.92 g dry weight, 175.00 g "fresh" weight) were frozen with liquid nitrogen, grinded and extracted with methanol. Crude *Hamamelis virginiana* extract obtained after evaporation of dichloromethane ($t<40^0C$) yielded 230 mg. Crude extract revealed the presence of a chlorophyll biodegradation product with a spot at R_f= 0.52 on TLC. Prepurification was done on MPLC and 11.8 mg of the prepurified chlorophyll biodegradation product was obtained. Final purification was done by semi – preparative HPLC and the chlorophyll

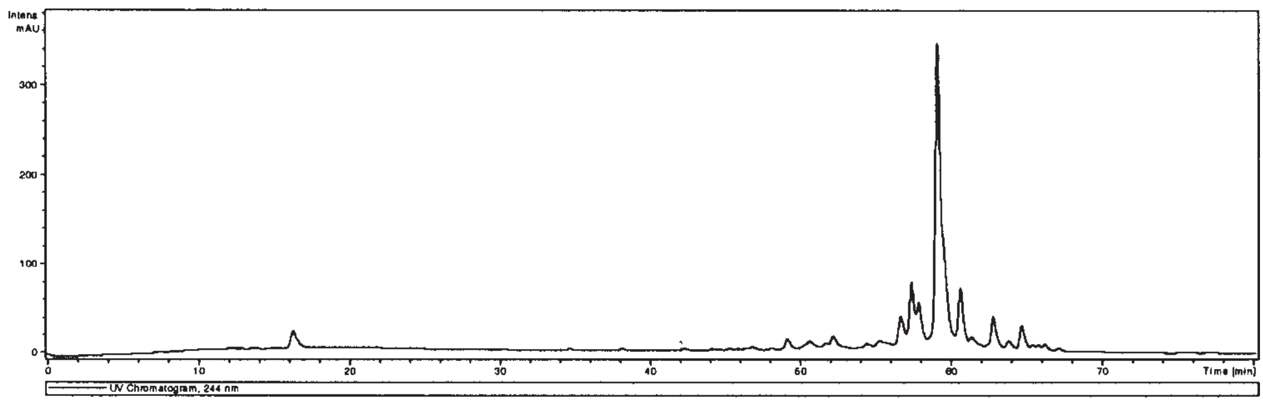

Fig. 1. UV chromatogram of *Hamamelis virginiana* crude leaves' extract, extracted at λ= 244 nm

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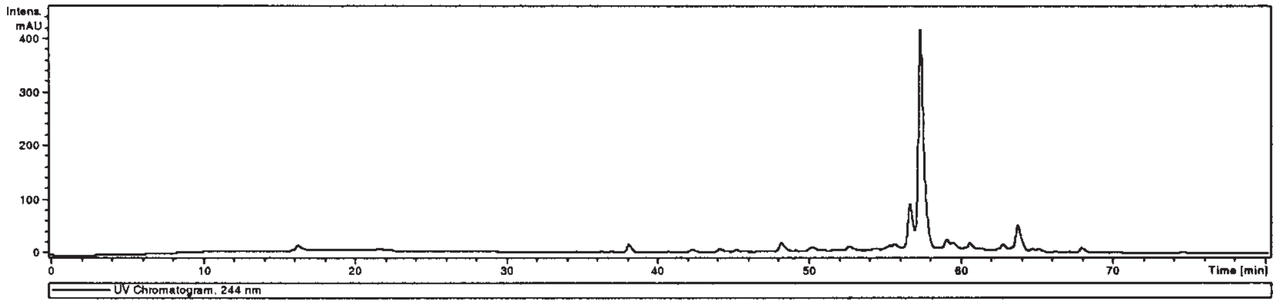

Fig. 2. UV chromatogram of *Parrotia persica* crude leaves' extract, extracted at λ= 244 nm

biodegradation product eluted at 73 min. was collected to obtain 1.07 mg of the pure chlorophyll biodegradation product.

Results and discussion

The isolation procedure for *Hamamelis virginiana* autumnal leaves was the same as described for the *Parrotia persica* autumnal leaves[8]. The LC–MS analysis of the crude *Hamamelis virginiana* autumnal leaves extract was subjected to RP – C_8 analytical column under the same acquisition parameters and elution solvent mixture as for the *Parrotia persica* autumnal leaves' extract [8]. The major compound in LC – MS chromatogram of *Hamamelis virginiana* autumnal leaves' extract gave molecular ion $[M+H]^+$ corresponding to *m/z* 633 like the UNCC present in *Parrotia persica* autumnal leaves' extract[8]. The *Hamamelis virginiana* UNCC eluted at 59.3 min. (fig. 1) and *Parrotia persica* UNCC eluted at 57.5 min. (fig. 2).

UNCC present in *Hamamelis virginiana* autumnal leaves (fig. 1) eluting at 57.4 min. had the *m/z* 633 (fig. 4) and corresponded to the *Parrotia persica* UNCC (fig. 2), in further text UNCC–*Pp* acronym will be used. The major UNCC in *Hamamelis virginiana* eluted at 59.3 minutes had also the *m/z* 633 (in further text UNCC–*Hvir* acronym will be used) (fig. 3). Another UNCC with the *m/z* 633 was present in *Hamamelis virginiana* crude leaf extract (fig. 5), along with the NCC with the *m/z* 645, also called *Cj*-NCC-1 [5, 6], major chlorophyll biodegradation product from *Cercidiphyllum japonicum*, Cercidiphillaceae (fig. 6). The compound with the *m/z* 631 has yet not been characterized (fig. 7).

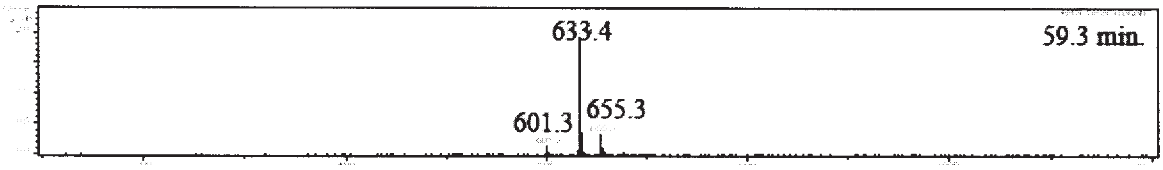

Fig. 3. ESIMS of *Hamamelis virginiana* crude leaves' extract, extracted at 59.3min

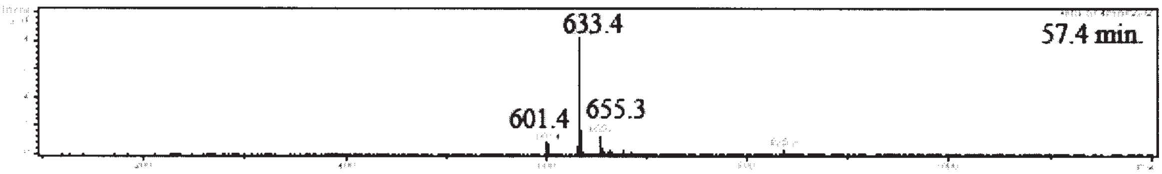

Fig. 4. ESIMS of *Hamamelis virginiana* crude leaves' extract, extracted at 57.4min

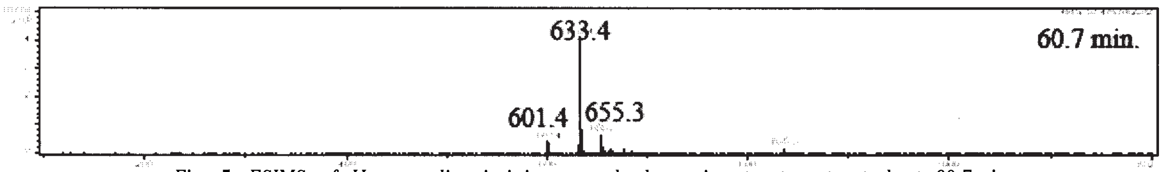

Fig. 5. ESIMS of *Hamamelis virginiana* crude leaves' extract, extracted at 60.7min

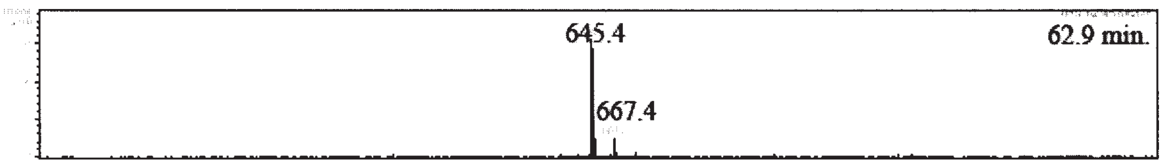

Fig. 6. ESIMS of *Hamamelis virginiana* crude leaves' extract, extracted at 62.9min

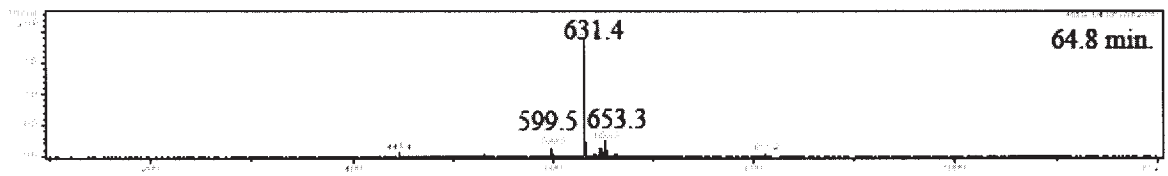

Fig. 7. ESIMS of *Hamamelis virginiana* crude leaves' extract, extracted at 64.8min

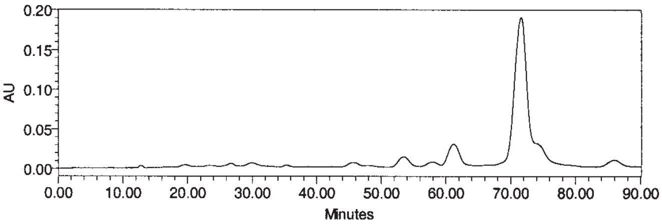

Fig. 8. UV chromatogram of the prepurified *Hamamelis virginiana* leaves' extract, extracted at λ = 244 nm

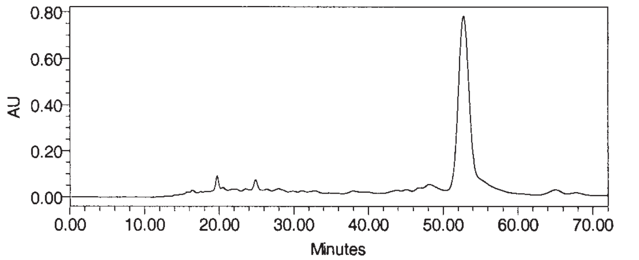

Fig. 9. UV chromatogram of the prepurified *Parrotia persica* leaves' extract, extracted at λ = 244 nm [8]

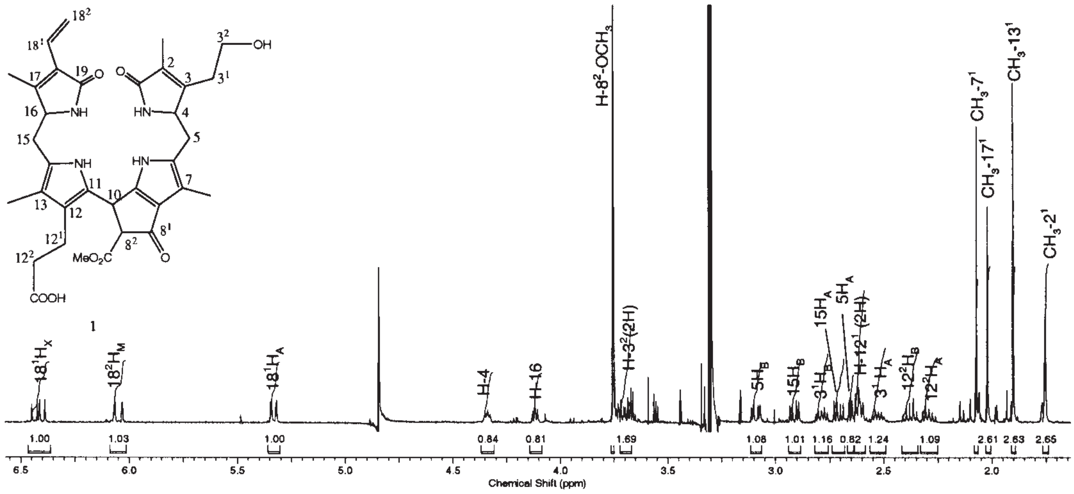

Fig. 10. The high resolution proton spectrum of the UNCC – *Hvir*

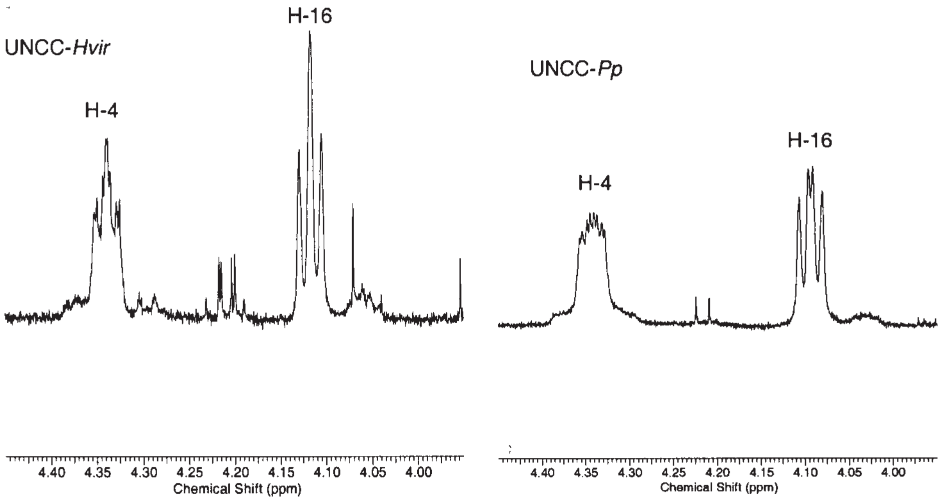

Fig. 11. Proton spectra in the region of H-4 and H-16 of the UNCC – *Hvir* (left) and UNCC – *Pp* (right)

The major UNCC present in *Hamamelis virginiana* crude leaves' extract was subjected to MPLC (230 mg) to yield 11.8 mg of the prepurified UNCC. The final purification was performed by means of the semi – preparative HPLC to give 1.07 mg of the pure UNCC–*Hvir*. The UNCC – *Hvir* eluted at 73 min. (fig. 8). The UNCC – *Pp* eluted at 52 min. (fig. 9).

Constitution of UNCC – *Hvir*

The UNCC – *Hvir* was obtained as orange amorphous solid. The High Resolution ElectroSpray Ionisation Mass Spectrometry (HRESIMS) sprectra showed a molecular ion at *m/z* 655.2738 for the molecular formula $C_{34}H_{40}N_4O_8Na$ $[M+Na]^+$, calculated *m/z* 655.2738, Δ 0.00 ppm.

Elucidation of the UNCC – Hvir structure by NMR data

In the proton spectrum, there were slight differences in the chemical shifts between the two UNCCs isolated, one from *Parrotia persica* and the other from *Hamamelis virginiana* autumnal leaves. The NMR spectra of the two UNCCs were measured at the same temperature and in the same solvent CD_3OD-d_4. The compound isolated from Hamamelis virginiana autumnal leaves was an isomer of UNCC – Pp [8]. The UNCC – Hvir proton spectrum is shown in the figure 10.

The multiplicity of the proton H-16 signal of the UNCC – Hvir was a triplet while in the UNCC – Pp proton spectrum a doublet of doublets was observed (fig. 11). When small interprotonal couplings are underestimated, the multiplicity of the UNCC – Hvir H-4 proton signal is triplet, while in case of UNCC – Pp it is the doublet of doublets.

The difference between two isomers was observed in CD spectra. The chromophore absorbing at λ=244 nm had Δε positive in case of UNCC-*Pp* and in case of UNCC-*Hvir* the Δε was negative (figs. 12 and 13)

Table 1
^{1}H (500 MHz) AND ^{13}C (125 MHz) NMR DATA IN CD_3OD-d_4 OF THE UNCC-*Hvir*

H/C	δ_H, multiplicity	J(Hz)	δ_C	DEPT	COSY (H→H)
1					
2					
2^1	1.75 *s*		8.5	↑	3^1H_A
3					
3^1	2.52 *dd* H_A 2.79 *dd* H_B	6.4;13.9 6.4;13.3	31.1	↓	2^1, 3^1H_B, 3^2; 3^1H_A, 3^2
3^2	3.67-3.74 *m* H_A and H_B	6.8;13.4 7.3;13.9	61.4	↓	3^1H_A, 3^1H_B
4	4.34 *tt*	4.8; 1.4	60.6	↑	$5H_A$, $5H_B$
5	2.65 *dd* H_A 3.09 *dd* H_B	8.5;14.9 14.9;4.8	29.8	↓	4, $5H_A$; 4, $5H_B$
6					
7					
7^1	2.07 *s*		9.6	↑	
8					
8^1					
8^2	3.79 *d*	3.2			
8^3					
8^4	3.75 *s*		52.9	↑	
9					
10	overlapped by the water peak		37.4	↑	
11					
12					
12^1	2.62 *t* H_A and H_B	7.7	36.7	↓	12^1H_B; 12^1H_A
12^2	2.29 *dt* H_A 2.38 *dt* H_B	7.5;15.6 8.0;15.9	20.8	↓	12^1H_B, 12^2H_B; 12^1H_A, 12^2H_A
12^3					
13					
13^1	1.90 *s*		9.5	↑	
14					
15	2.71 *dd* H_A 2.91 *dd* H_B	6.9;14.7 5.4;14.7	29.5	↓	$15H_B$, 16; $15H_B$, 16
16	4.12 *t*	6.0	61.6	↑	15 H_A, 15 H_B, 17^1*
17					
17^1	2.02 *s*		12.8	↑	16*
18					
18^1	6.42 *dd*	17.8; 11.7	127.0	↑	18^2H_A, 18^2H_B
18^2	5.33 *dd* H_A 6.05 *dd* H_B	11.7,2.4 17.8,2.4	119.2	↓	18^2H_B, 18^1; 18^2H_A, 18^1
19					

DEPT data: CH and CH_3 are phased up, CH_2 is phased down
*cross peak of low intensity

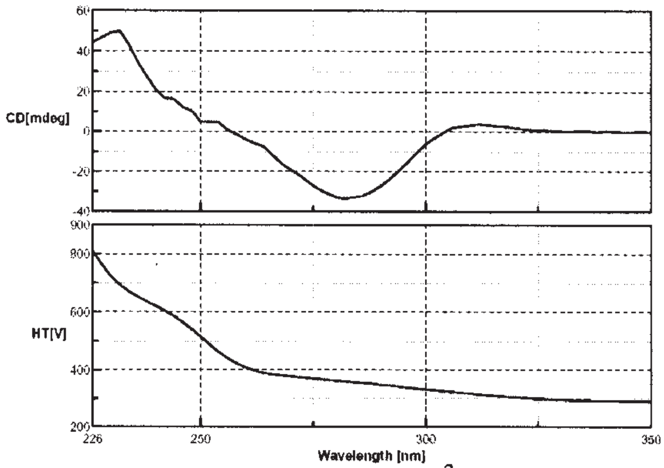

Fig. 12. CD and UV spectra of 0.1 μmoldm^{-3} UNCC-*Pp* in methanol

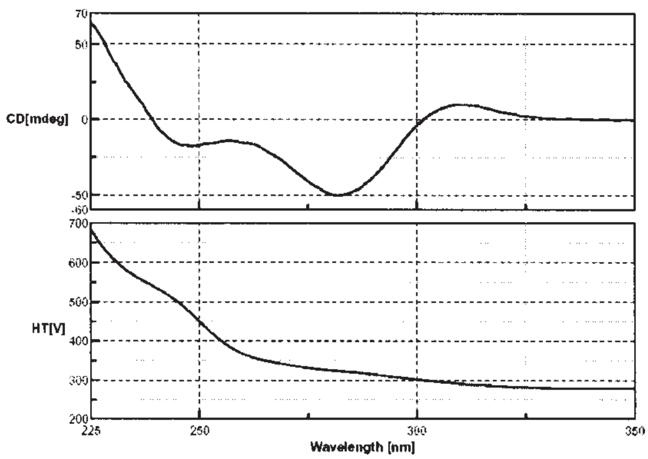

Fig. 13. CD and UV spectra of 0.03 μmoldm^{-3} UNCC-*Hvir* in methanol

Spectroscopic data

UV – Vis in methanol, $C = 3\cdot10^{-8}$ mol dm^{-3}; λ_{max}[nm] (log ε): 244 (7.24), 283 (7.03).

CD in methanol, $C = 3\cdot10^{-8}$ mol dm^{-3}; λ_{max}[nm] (Δε): 244 (-16.7), 283 (-51).

Conclusions

The UNCC was isolated from *Hamamelis virginiana* autumnal leaves and was named UNCC – *Hvir*. Its structure was determined by spectroscopic and spectrometric data. The UNCC – *Hvir* was compared to the UNCC isolated from *Parrotia persica* (UNCC – *Pp*) autumnal leaves. The isolated UNCC – Hvir differs from UNCC – Pp in few physico – chemical characteristics. Under the same elution conditions, on the same stationary phase, on the analytical scale, the UNCC – Hvir eluted at 59.0 min. (the capacity factor 2.60) and UNCC – Pp at 57.3 min. (the capacity factor 2.49). Under the same elution conditions, on the same stationary phase, on the semi – preparative scale, UNCC – Hvir eluted at 73 min. and UNCC – Pp at 52 min. The main difference in the proton NMR spectrum was the multiplicity of the proton H-4. When the small interprotonal couplings are underestimated the multiplicity of UNCC – Hvir H-4 is a triplet and the multiplicity of UNCC – Pp is a doublet of doubles. The other difference in the proton spectrum was the multiplicity of the proton H-16. In the case of the UNCC – Hvir the multiplicity of the proton H-16 was a triplet and in the case of the UNCC – Pp the multiplicity was doublet of doublets. Consequently, the UNCC – Hvir isolated from Hamamelis virginiana autumnal leaves is an isomer of the UNCC – Pp isolated from Parrotia persica autumnal leaves[8].

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