Chemistry of Natural Compounds, Vol. 47, No. 1, March, 2011 [Russian original No. 1, January–February, 2011]

# AESCIN DETERMINATION IN SEEDS AND CAPSULES OF THREE PURE AND HYBRID *Aesculus* SPECIES

**M. Glensk,[1*] M. Wlodarczyk,[1] C. Bassarello,[2] and C. Pizza[2]**

UDC 547.918

The genus *Aesculus* L. consists of 13 species, distributed in the northern hemisphere, primarily in eastern Asia and eastern North America, with one species native to Europe, one to western North America, and one to northwestern Mexico [1]. No previous phytochemical studies were carried out on the investigated species. According to earlier reports on other species, *Aesculus hippocastanum* contains an aescin that is a natural mixture of triterpene saponins. The aglycons are derivatives of protoaescygenin, acylated by acetic acid at C-22 and by either angelic or tiglic acids at C-21. Two forms exists, $\alpha$ and $\beta$, which can be distinguished by melting point, specific rotation, hemolytic index, and solubility in water. A number of other products have been isolated from chestnut seeds, i.e., flavonoids such as quercetin, as well as antioxidants such as proanthocyanidin $A_2$ and the coumarins esculin and fraxin [2–6]. However, all of these products can be found in larger amounts in other sources; furthermore, it was concluded that the antiedematous, antiexudative, and vasoprotective activities of hippocastanus extracts (HCE) are exclusively due to aescin [4]. From the ethanolic extract of the roots of *Aesculus assamica* two novel triterpenoid saponins with insulin-like activity were isolated [7]. Also identification of novel saponins from edible seeds of Japanese horse chestnut (*Aesculus turbinata*) after treatment with wooden ashes and their nutraceutical activity were recently reported [8]. It is worth noting that sapogenols from *Aesculus hippocastanum* showed significant *in vitro* cytotoxicity in the 9-KB cell culture assay [9].

Aescin occurrence and quantitative determination in seeds and capsules of three *Aesculus* species was performed by TLC densitometry. Surprisingly the highest aescin content was found in *Aesculus × marylandica* capsules, not seeds, whereas there was a lack of this substance in *Aesculus parviflora* capsules. In case of *Aesculus × hemiacantha,* the seeds were a richer source than capsules (Table 1). Moreover, from capsules of *Aesculus × marylandica* two sapogenins were isolated: barringtogenol C and protoaescygenin. The latter two compounds are ubiquitious metabolites of the genus *Aesculus*; however, their presence in capsules of *Aesculus × marylandica* was not previously described. It is also worth noting that *Aesculus × marylandica*, especially *Aesculus parviflora*, is resistant to *Cameraria ohridella* – horse chestnut leafminer [10, 11]. However, our findings indicate that the high aescin content most probably does not determine their resistance against the moth.

**Plant Material**. Both mature seeds and capsules of *Aesculus × hemiacantha* Topa (hybrid of *A. hippocastanum* and *A. turbinata*) and *Aesculus × marylandica* Booth ex Kirchn. (hybrid of *A. flava* and *A. glabra*) were collected at the Botanical Garden of Wroclaw University in 2005. The mature seeds and capsules of *Aesculus parviflora* Walt. were collected at Wojslawice Arboretum in 2005. All plant material was dried in a dark place in an oven at 40°C. A voucher specimen is deposited at the Herbarium of the Department of Pharmacognosy, Wroclaw Medical University, Wroclaw, Poland.

**Densitometric Determination**. In recent years an analysis of the aescin content by densitometric thin-layer chromatography was developed [5, 12, 13]. The plant materials were air-dried at room temperature and ground to powder using a laboratory mill, then assayed for aescin content according to Costantini [5] using the TLC method. Chromatography was performed on analytical HPTLC silica gel 60 $F_{254}$ plates (10 × 20 cm) of 0.2 mm thickness (E. Merck, Darmstadt, Germany). Samples (2–3 μL) and standard solutions (1–6 μL) were applied to the plate as 5 mm bands (10 mm from the lower edge of the plate) by means of an autosampler. For each analysis, one single plate containing five levels of standard and six samples was used. Each sample was chromatographed in triplicate. The plates were developed for about 7.5 cm from the baseline in a saturated horizontal developing chamber with 1-PrOH–EtOAc–$H_2O$ 3.5:4.5:2 (v/v/v), dried with a stream of lukewarm air, and quantified by linear scanning at 212 nm using computer- assisted Proquant Desaga software for quantitative analysis.

1) Department of Pharmacognosy, Wroclaw Medical University, pl. Nankiera 1, 50-140 Wroclaw, Poland, fax: +48 71 7840218, e-mail: michalg@farmgn.am.wroc.pl; 2) Universito Ricercatore di Biologia Farmaceutica Dipartimento di Scienze Farmaceutiche Universita Degli studi di Salerno, via Ponte don Melillo, 84084-Fisciano, Italy. Published in Khimiya Prirodnykh Soedinenii, No. 1, p. 126–128, January–February, 2011. Original article submitted September 9, 2009.

 0009-3130/11/4701-0142 ©2011 Springer Science+Business Media, Inc.

TABLE 1. Results for the TLC Densitometric Determination of Aescin at Three *Aesculus* Species

| Sample name | Amount of substance (aescin/20 mg of dry extract), mg | Ratio, % |
|---|---|---|
| *Aesculus parviflora* seeds | 0.59 | 2.95 |
| *Aesculus* × *marylandica* seeds | 0.86 | 4.30 |
| *Aesculus* × *marylandica* capsules | **9.11** | **45.55** |
| *Aesculus* × *hemiacantha* seeds | 5.14 | 25.70 |
| *Aesculus* × *hemiacantha* capsules | 1.51 | 7.55 |

TABLE 2. $^{13}C$ and $^{1}H$ NMR Spectra of Barringtogenol C (δ, ppm, J/Hz)

| C atom | $\delta_C$ | $\delta_H$ | C atom | $\delta_C$ | $\delta_H$ |
|---|---|---|---|---|---|
| 1 | 40.3 | 1.69; 1.04 | 16 | 68.6 | 4.26 (br.s) |
| 2 | 27.9 | 1.68; 1.16 | 17 | 47.8 | – |
| 3 | 79.6 | 3.19 | 18 | 41.8 | 2.32 |
| 4 | 40.1 | – | 19 | 48.5 | 2.50; 1.09 |
| 5 | 56.8 | 0.80 | 20 | 36.9 | – |
| 6 | 19.7 | 1.62; 1.47 | 21 | 79.7 | 3.97 (d, J = 10.1) |
| 7 | 34.4 | 1.65; 1.42 | 22 | 77.2 | 3.79 (d, J = 10.1) |
| 8 | 40.8 | – | 23 | 28.9 | 1.02 |
| 9 | 48.3 | 1.70 | 24 | 16.7 | 0.82 |
| 10 | 38.3 | – | 25 | 16.4 | 1.01 |
| 11 | 24.9 | 1.94 | 26 | 17.2 | 0.98 |
| 12 | 124.3 | 5.33 | 27 | 27.8 | 1.46 |
| 13 | 143.9 | – | 28 | 68.4 | 3.43; 3.25 |
| 14 | 42.5 | – | 29 | 30.4 | 0.97 |
| 15 | 34.9 | 1.87; 1.40 | 30 | 19.0 | 0.94 |

The limits of detection (LOD) and quantification (LOQ) for densitometric analyses were calculated from calibration equations using signal-to-noise ratios S/N ≥ 3:1 and S/N ≥ 10:1 respectively: retention time of aescin, linear range, 1–12 μg/mL, correlation coefficients r, 0.996, LOD 0.21, and LOQ 0.41 μg/mL.

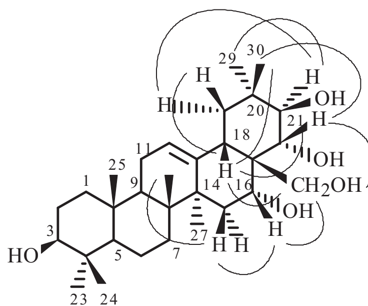
**Sample and Standard Preparation**. The dried and powdered seeds and capsules (5.0 g each) were sonicated with 10 mL of 80% MeOH at room temperature. The obtained extracts were evaporated under reduced pressure. 20 mg of each dry sample was suspended in methanol (1 mL) and agitated for 15 min in an ultrasonic bath. Later on, it was filtered through a 45 μm membrane filter. The filtrate was automatically deposited on the plate together with the standard solution for quantitative determination.

The standard solution was prepared through dissolution of 10 mg of aescin in 10 mL of methanol. The solution was freshly prepared before each analysis.

**Equipment**. A Desaga (Heidelberg, Germany) AS 30 automatic applicator was used as the application device. Densitograms were obtained using a Desaga CD 60 densitometer. Chromatograms were developed in a horizontal Teflon DS chamber (Chromdes, Lublin, Poland).

Solvents for TLC were from Chempur (Piekary Sl., Poland) and POCh (Gliwice, Poland). *β*-aescin for preparing standard solutions was obtained from Roth (Germany).

**Barringtogenol C and Protoaescygenin Isolation of *Aesculus* × *marylandica* Capsules**. The dried and powdered capsules from *Aesculus marylandica* (13 g) were sonicated with 80% MeOH at room temperature. The crude extract was evaporated under reduced pressure in order to distill methanol off. From the water remaining, a creamy precipitate was obtained (0.82 g). Later on, it was chromatographed over RP-18 (Bakerbond, octadecyl $C_{18}$, particle size 40 μm, 100 g). Elution with increasing polarity of MeOH–$H_2O$ (0–100% MeOH) mixtures yielded nine fractions, A–I. Of these, I was purified on Bakerbond SPE silica gel disposable extraction columns (500 mg, 6 mL) using aethyl acetate saturated with water to yield barringtogenol C (4.5 mg). The structure was elucidated on the basis of NMR data (Table 2), obtained by dissolving the sample in $CD_3OD$ on a Bruker DRX-600 spectrometer at 300 K, and direct comparison with published values [14–16]. Assignments were based on 2D COSY, HOHAHA, HSQC, and HMBC experiments. $^{1}H$-$^{1}H$ coupling constants were measured from the $^{1}H$ spectrum.



H16-H28b; H15$\beta$-Me26; H16-H15$\beta$ (strong effect)
H16-H15$\alpha$ (medium effect); H21-Me29; H21-H19$\alpha$
H18-H28a; H18-Me30; H18-H19$\beta$; H18-H22
H22-Me30; H22-H28a

Fig. 1. Barringtogenol C (ROE effects).

ROE effects (extracted from the ROESY spectrum acquired with a mixing time of 400 ms) for barringtogenol are presented in Fig. 1. Similarly, from fraction H a mixture (6 mg) of two aglycones was obtained. In this mixture barringtogenol C and protoaescigenin occurred in a *ca.* 3:2 ratio. We evaluated the ratio of intensities of the proton signals typical of barringtogenol and protoaescigenin in the $^1$H spectrum.


## ACKNOWLEGMENT

The authors thank MSc Tomasz Burycz (Herbapol Wroclaw Inc.) for his kind presence during the densitometric determination.


## REFERENCES


1. F. Forest, J. N. Drouin, R. Charest, L. Brouillet, and A. Bruneau, *Can. J. Bot.*, **79**, 154 (2001).
2. E. Bombardelli and P. Morazzoni, *Fitoterapia*, **6**, 483 (1996).
3. K. Hostettmann and A. Marston, *Saponins*, Cambridge University Press, 1995, 548 pp.
4. C. R. Sirtori, *Pharmacol. Res.*, **44**, 183 (2001).
5. A. Costantini, *Il Farmaco*, **54**, 728 (1999).
6. I. Kapusta, B. Janda, B. Szajwaj, A. Stochmal, S. Piacente, C. Pizza, F. Franceschi, C. Franz, and W. Oleszek, *J. Agric. Food Chem*., **55**, 8485 (2007).
7. T. Sakurai, T. Nishimura, N. Otake, Y. Xinsheng, K. Abe, M. Zeida, H. Nagasawa, and S. Sakuda, *Bioorg. Med. Chem. Lett.*, **12**, 807 (2002).
8. H. Kimura, S. Ogawa, M. Jisaka, Y. Kimura, T. Katsube, and K. Yokota, *J. Pharm. Biomed. Anal.*, **41**, 1657 (2006).
9. T. Konoshima and K-H. Lee, *J. Nat. Prod.*, **49**, 650 (1986).
10. M. Dziegielewska and G. Kaup, *Prog. Plant Protect.*, **47**, 218 (2007).
11. E. Szopinska, J. Mazurek, and A. Dradrach, Raport koncowy z projektu badawczego KBN nr. 3 P04G 034 24 2005 (unpublished data).
12. O. M. Kockar, M. Kara, S. Kara, B. Bozan, and K. H. C. Baser, *Fitoterapia*, **65**, 439 (1994).
13. S. Apers, T. Naessens, L. Pieters, and A. Vlietinck, *J. Chromatogr. A*, **1112**, 165 (2006).
14. L. Voutquenne, C. Kokougan, C. Lavaud, I. Pouny, and M. Litaudon, *Phytochemistry*, **59**, 825 (2002).
15. L. Voutquenne, P. Guinot, C. Froissard, O. Thoison, M. Litaudon, and C. Lavaud, *Phytochemistry*, **66**, 825 (2005).
16. Z. Zhang, Li. Shiyou, Z. Shanmin, and D. Gorestein, *Phytochemistry*, **67**, 784 (2006).