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Biochemical Systematics and Ecology 33 (2005) 749–752
biochemical systematics and ecology
www.elsevier.com/locate/biochemsyseco

Hirsutanonol, oregonin and genkwanin from the seeds of *Alnus glutinosa* (Betulaceae)

Ciara O'Rourke [a], Maureen Byres [a], Abbas Delazar [a], Yashodharan Kumarasamy [a], Lutfun Nahar [b], Fiona Stewart [a], Satyajit D. Sarker [a,*]

[a] *Phytopharmaceutical Research Laboratory, School of Pharmacy, The Robert Gordon University, Schoolhill, Aberdeen AB10 1FR, Scotland, UK*
[b] *School of Life Sciences, The Robert Gordon University, St. Andrew Street, Aberdeen AB25 1HG, Scotland, UK*

Received 24 December 2003; accepted 8 October 2004

Keywords: *Alnus glutinosa*; Betulaceae; Flavone; Hirsutanonol; Oregonin; Genkwanin; Chemotaxonomy

1. Subject and source

Alnus glutinosa (L.) Gaertn. (family: Betulaceae), commonly known as 'black alder' or 'European alder', native to a number of countries in northern Africa, temperate Asia and Europe, is one of the ca. 30 species of trees and shrubs of the genus *Alnus* (GRIN Database, 2003; Mitchell and Wilkinson, 1997). Seeds of *A. glutinosa* (catalogue no. 225) were purchased from B&T World Seeds sarl, Pauguignan, 34210 Olonzac, France, and a voucher specimen (PH00171103-2-SDS) has been kept in the Plant and Soil Science Department, University of Aberdeen, UK.

* Corresponding author. Tel.: +44 1224 262547; fax: +44 1224 262555.
E-mail address: s.sarker@rgu.ac.uk (S.D. Sarker).

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doi:10.1016/j.bse.2004.10.005

2. Previous work

Various types of plant secondary metabolites including anthraquinones, phenolic glycosides, flavonol glycoside, terpenoids, xanthones, etc. have previously been reported from the barks, buds, leaves and pollens of *A. glutinosa* (Phytochemical and Ethnobotanical Database, 2003; DNP CD-ROM, 2001; Meurer et al., 1988; Khvorost et al., 1987). To our knowledge, there is no report on any detail phytochemical study on the seeds of this species available to date.

3. Present study

Ground seeds of *A. glutinosa* (50 g) were Soxhlet-extracted, successively, with *n*-hexane, dichloromethane and methanol (1 L each). The methanol extract was subjected to fractionation by solid phase extraction method using a Sep-Pak C_{18} (10 g) cartridge eluting with a step gradient: 30, 60, 80 and 100% MeOH in water (200 mL each). Preparative-HPLC (Luna C_{18} column 10 μm, 250 mm × 21.2 mm, eluted with a linear gradient – water:ACN = 90:10 to 30:70 over 50 min followed by 70% ACN for 10 min, 20 mL/min, monitored by photo-diode-array detector) of the Sep-Pak fraction, which was eluted with 60% MeOH, yielded two diarylheptanoid derivatives, hirsutanonol (**1**, 4.5 mg, ret. time: 10.0 min) (Aoki et al., 1990; Ohta et al., 1984) and oregonin (**2**, 8.1 mg, ret. time: 8.0 min) (Lee et al., 1998; Ohta et al., 1984; Suga et al., 1982). Similar purification (linear gradient – water:ACN = 90:10 to 00:100 over 50 min followed by 100% ACN for 10 min, 20 mL/min) of the Sep-Pak fraction eluted with eluted with 60% MeOH, yielded the flavone derivative, genkwanin (**3**, 3.1 mg, ret. time: 15.2 min) (Bosabalidis et al., 1998; Zahid et al., 2002). Compounds (**1**–**3**) were identified by direct comparison of their UV, ESIMS, ^{1}H and ^{13}C NMR data with those of respective published data.

3.1. Hirsutanonol (*1*)

Pale brown amorphous powder UV λ_{max} (MeOH) nm: 278; ESIMS *m*/*z*: 369 $[M + Na]^+$; ^{1}H NMR and ^{13}C NMR data (Aoki et al., 1990; Ohta et al., 1984).

3.2. Oregonin (*2*)

Brown amorphous powder UV λ_{max} (MeOH): 278; ESIMS *m*/*z*: 501 $[M + Na]^+$; ^{1}H NMR and ^{13}C NMR data (Lee et al., 1998; Ohta et al., 1984; Suga et al., 1982).

3.3. Genkwanin (*3*)

Yellow amorphous powder; UV λ_{max} (MeOH) nm: 268, 300 sh and 334, +NaOMe 273, 300 sh and 384, +$AlCl_3$ 275, 301, 349 and 382 sh, +$AlCl_3$·HCl 278, 299, 341 and 381 sh, +NaOAc 267, 299 sh, 369 and 380, +H_3BO_3 267, 299 sh and

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