FLAVONOID GLUCURONIDES FROM *ALYSSUM MINIMUM*

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Alyssum minimum, Willd. (syn. *Alyssum desertorum* Stapf.) (Cruciferae) is distributed throughout Europe, Asia, and the Middle East. In Iran seeds are used as a treatment for fevers and other ailments. No previous investigation of the phenolic constituents of *A. minimum* has been carried out; however, the glucosinolates, hydrocarbons, and fatty acids of whole plants and cell cultures have been identified and quantified (1). The occurrence of three new flavonol 7-glucuronides in this cruciferous species is now reported. Aguinadalde *et al.* (2) found nine acylated and some related non-acylated glycosides of kaempferol and quercetin in the related *Sisymbrium gilliesii*, along with *Crambe tartaria, Crambe cordifolia*, and *Crambe scamberrima*. The literature records the presence of either kaempferol, quercetin, rhamnetin, or isorhamnetin glycosides in four other crucifers (3-6). The 7-glucuronides of luteolin (7-8), agipenin (9), and diosmetin (9) have been previously reported, whereas the 7-glucuronides of these well documented aglycones (10) listed above have not been previously identified.

EXPERIMENTAL

PLANT MATERIAL.—Seeds of *A. minimum* were obtained from a commercial market in Isfahan, Iran, in 1984. They were grown, and the fully developed plants were harvested and identified at the Botany Department, University of Manchester Museum, where voucher specimen No. 11.34.M.-S.A85/1 is deposited.

EXTRACTION, ISOLATION, AND IDENTIFICATION.—Crushed, defatted seeds were extracted with boiling MeOH, and the concentrated extracts were fractionated using large scale preparative cellulose tlc. Separation of the major bands was effected using BuOH-HOAc-H_2O (4:1:5), followed by H_2O-HOAc (95:5) as solvent systems. Eluted flavonoids were then further purified by hplc using Hypersil ODS, eluted with MeOH.

Glycone moieties of phenolic glycosides were released by hydrolysis with 1N HCl at 100° for 45-180 min and identified by cellulose tlc using EtOAC-C_5H_5N-H_2O (12:5:4) as the solvent system.

The minor component was kaempferol, 4′-methylether 5-glucoside, 7-glucuronide; uv MeOH λ max 248, 269, 357; 80 MHz ^{1}H nmr (CD_3OD) δ3.44-3.83 [10H(sugar)], 3.88 (s, 3H, OCH_3), 5.24 (d, 1H, J=7 Hz), 5.40 (d, 1H, J=4 Hz), 6.36 (d, 1H, J=2.4 Hz), 6.63 (d, 1H, J=2.4 Hz), 7.27 (d, 2H, J=8.4 Hz), 7.32 (d, 2H, J=8.4 Hz); eims *m/z* (% rel. int.) 300 (M^+ aglycone, 55) 299 (10), 283 (7), 275 (18), 153 (9), 137 (15), 135 (18), 107 (20); glucose+glucuronic acid released by hydrolysis and identified by tlc.

Two quercetin-5-glucoside-7-glucuronide methylethers (3′, 4′, and 4′) were unresolved by tlc; 80 and 300 MHz ^{1}H nmr (CD_3OD) showed doublets with J=7 Hz near δ5.0 but not at δ5.8, indicating the attachment of glucose at C-5 (10). Release of glucose by hydrolysis (45 min) (11) yielded quercetin methylether glucuronides: quercetin-3′, 4′-dimethylether-7-glucuronide; uv MeOH λ max 254, 270, 366; 80 MHz ^{1}H nmr (CD_3OD) δ3.44-3.74 [4H(sugar)], 3.88 (s, 3H, OCH_3), 3.94 (s, 3H, OCH_3), 5.44 (d, 1H, J=4 Hz), 6.30 (d, 1H, J=2.4 Hz), 6.60 (d, 1H, J=2.4 Hz), 6.92 (d, 1H, J=8 Hz), 7.55-7.86 (2H); eims *m/z* (% rel. int.) 330 (M^+ aglycone, 30), 315 (10), 313 (15), 165 (10), 153 (10), 137 (12); quercetin-4′-methylether-7-glucuronide; uv MeOH λ max 269, 324, 363; 80 MHz ^{1}H nmr (CD_3OD) δ3.44-3.74 [4H (sugar)], 3.88 (s, 3H, OCH_3), 5.4 (d, 1H, J=4 Hz), 6.32 (d, 1H, J=12.4 Hz), 6.62 (d, 1H, J=2.4 Hz), 7.62 (d, 1H, J=2.4 Hz), 6.92 (d, 1H, J=8.5 Hz), 8.14 (q, 1H, J=2.5 and J=8.5 Hz); eims *m/z* (% rel. int.) 316 (M^+ aglycone, 6), 153(2), 137(3), 123(4).

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LITERATURE CITED

1. G.B. Lockwood and S. Afsharypuor, *J. Chromatogr.*, **356**, 438 (1986).
2. I. Aguinalde and M.A. Del Pero Martinez, *Phytochemistry*, **21**, 2875 (1981).
3. J.B. Bacon, *Rhodora*, **80**, 159 (1979).
4. I. Matlawska and Z. Kowalewski, *Herba Pol.*, **22**, 138 (1976).
5. I. Matlawska and Z. Kowalewski, *Herba Pol.*, **22**, 11 (1976).
6. B. Stengel and H. Greiger, *Z. Naturforsch.*, **31**, 662 (1976).

7. M. Shimizu, T. Ito, S. Terashima, T. Hagashi, M. Arisawa, N. Morita, S. Kurokawa, K. Ito, and Y. Hashimoto, *Phytochemistry*, **23**, 1885 (1984).
8. H. Teubert and K. Herrmann, *Z. Lebensm.-Unters. Forsch.*, **165**, 147 (1977).
9. C.A. Williams, J.H. Fronczyk, and J.B. Harborne, *Phytochemistry*, **22**, 1953 (1983).
10. T.J. Mabry, K.R. Markham, and M.B. Thomas, "The Systematic Identification of Flavonoids," Springer-Verlag, Berlin, 1970, p. 268.
11. J.B. Harborne, *Phytochemistry*, **4**, 107 (1965).

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COMPOSITION OF THE ESSENTIAL OIL OF *OCIMUM TRICHODON* GROWN IN RWANDA

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Ocimum trichodon Baker ex Guerke (Lamiaceae) is reported to occur in eastern tropical Africa, in Cameroon and, less frequently, in Rwanda (1). In the latter country the plant, called Ihonoranzobe or Umwenya w'ifumbi, is extensively used in traditional medicine, e.g., to cure cough and headache and as an analgesic after childbirth (2). In a previous study the essential oil of *O. trichodon* showed an antimicrobial activity against some bacteria and a yeast (3). Further studies on this subject are in hand. Because no chemical data on the species in question could be found, and because of the statement of Hegnauer (4) that striking differences in the essential oil composition within species of the genus *Ocimum* may exist, we were interested in analyzing some samples of the volatile oil of *O. trichodon* grown in Rwanda.

MATERIALS AND METHODS

Leaves and flowers of *O. trichodon* growing wild in Butare (Southern Rwanda; samples 213C, 342C, 343C, 395C) and in Kibungo (Eastern Rwanda; samples 268J, 280J, 377J) were collected in January/February 1984. Voucher specimens were deposited in the Herbarium of Curphametra, the National University of Rwanda, at Butare, in that of the National Institute of Scientific Research (INRS) at Butare, and in that of the Botanical Institute, University of Liège, Belgium, under the numbers Ayobangira 1660 and 1697.

The samples of fresh plant material were subjected to hydrodistillation for 2 h using a Clevenger-type apparatus. The yield of oil varied from 0.4% to 0.8% (v/w). Since preliminary gc analyses showed that one sample (268J) differed qualitatively from the other six samples, its was submitted to liquid-solid chromatography (lsc) over silica deactivated by addition to 5% H_2O (5) in order to separate the hydrocarbons from the oxygen-containing compounds (6). The same lsc procedure was carried out with one of the six samples, viz., sample 213C.

Glc was performed on a gas chromatograph Packard 436S equipped with FID and connected with a digital integrator Packard 603 (Packard Instrument BV, Delft, the Netherlands). Gc conditions: column fused silica, 60 m × 0.25 mm i.d., coated with Durabond-DB 1 (J&W Scientific); film thickness 0.25 μm; oven temperature programmed, 60-200° (3°/min); carrier gas N_2; splitting ratio 1:100; gas velocity 16 cm/sec; injector and detector 200°. The seven oil samples and the fractions obtained by lsc were also analyzed by glc on packed columns under conditions comparable to those described previously (7).

The identity of the compounds was assigned by comparing their gc retention times with those of authentic samples. For this purpose also another 50 m capillary, fused silica column coated with CP-Wax 57cb (Chrompack BV, Middelburg, the Netherlands) was used. When necessary, gc/ms was performed as described before (8). The percentage composition of the oil samples was computed from the gc peak areas without correction factors.

RESULTS AND DISCUSSION

The percentage composition of the seven essential oil samples was determined using the 60 m fused silica Durabond-DB 1 column (see Table 1). The components are listed according to the elution on this column. All samples were dominated by eugenol (44-81%). Oct-1-en-3-ol and β-caryophyllene epoxide were