Pergamon

S0031-9422(96)00020-9

Phytochemistry, Vol. 42, No. 4, pp. 1005–1010, 1996

0031-9422/96 $15.00 + 0.00

PHYTOTOXIC CONSTITUENTS FROM *BUNIAS ORIENTALIS* LEAVES

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(*Received in revised form* 14 *November* 1995)

Key Word Index—*Bunias orientalis*; Brassicaceae; allelopathy; germination inhibition; leaf leachates; cinnamates; norisoprenoids; glycoconjugates.

Abstract—Aqueous extracts of *Bunias orientalis* leaves showed considerable germination inhibition in petri-dish bioassays. As major *B. orientalis* leaf constituents with known allelopathic activity bound forms of *p*-coumaric, ferulic and sinapic acids as well as two C_{13}-norisoprenoids, i.e. 3-hydroxy-β-ionone and 3-hydroxy-5,6-epoxy-β-ionone, were identified in XAD-2 isolates. After separation of the glycosidic isolate by counter-current chromatography/HPLC, 3-hydroxy-5,6-epoxy-β-ionyl-β-D-glucoside and the glucose ester of sinapic acid were isolated in pure form and their structures elucidated from ^{1}H and ^{13}C NMR spectral data. The corresponding aglycones were found to be major constituents in leaf leachate solutions of *B. orientalis*, together with the known allelochemic loliolide.

INTRODUCTION

Bunias orientalis L., a perennial Brassicaceae probably native to the highlands of Armenia, was introduced to central Europe in the 18th century [1]. From there a westward migration took place and to date the species is naturalized in most parts of Europe [2]. The recent development is characterized by an increase in the occurrences of dominance stands [3] with *B. orientalis* cover of 50–90% and by colonization of meadows, vineyards and arable land, thus causing concern for farmers and winegrowers.

Many species of the Brassicaceae are known to be potentially allelopathic [4–6]. Since part of the interference of *B. orientalis* with the growth of other species could have been due to allelopathic effects, this study was directed to an examination of the phytotoxic compounds produced by this species. Although secondary compounds, to which allelochemicals belong, may vary considerably in content between various plant parts [7], in many cases they are not restricted to particular ones [e.g. 8]. Therefore, we have used the basal rosette leaves of *B. orientalis* for extraction because they constitute the main fraction of the aboveground biomass for this species. Our objectives in the present study were (i) to screen aqueous and methanolic extracts of *B. orientalis* leaves for their ability to inhibit germination of other plant species, and (ii) to elucidate the structure of the phytotoxic compounds causing these effects.

RESULTS AND DISCUSSION

Bioassay for phytotoxic compounds

As a first step in this study it was essential to demonstrate the presence of phytotoxic compounds in *B. orientalis*. For this purpose, leaves were homogenized in water to obtain a crude aqueous extract whose osmotic concentration of 75 mmol l^{-1} was below values which show inhibitory activity on germination or radicle growth by concentration alone [9]. In a separate test, we found that concentrations of 120 mmol l^{-1} sorbitol and above must be applied to obtain marked germination or radicle growth inhibition for the three species tested. The aqueous extract was subjected to germination inhibition tests (petri dish bioassay). In this initial test, seeds of *Matricaria inodora* L., *Lactuca sativa* L. and *Hordeum vulgare* L. were used because of their rapid and relatively uniform germination behaviour. For all three species, the crude aqueous extract showed considerable germination inhibition (Fig. 1A). In the case of *H. vulgare*, the effect was most prominent with *ca* 50% germination reduction as compared to the control. Radicle growth was even more strongly impaired by treatment with *B. orientalis* extracts in comparison to the control (Fig. 1B) resulting in significant differences for all species ($p < 0.001$, Mann–Whitney U-test). A similar test for allelopathic activity of a crude aqueous root extract from *B. orientalis* also yielded significant, but considerably weaker, inhibitory

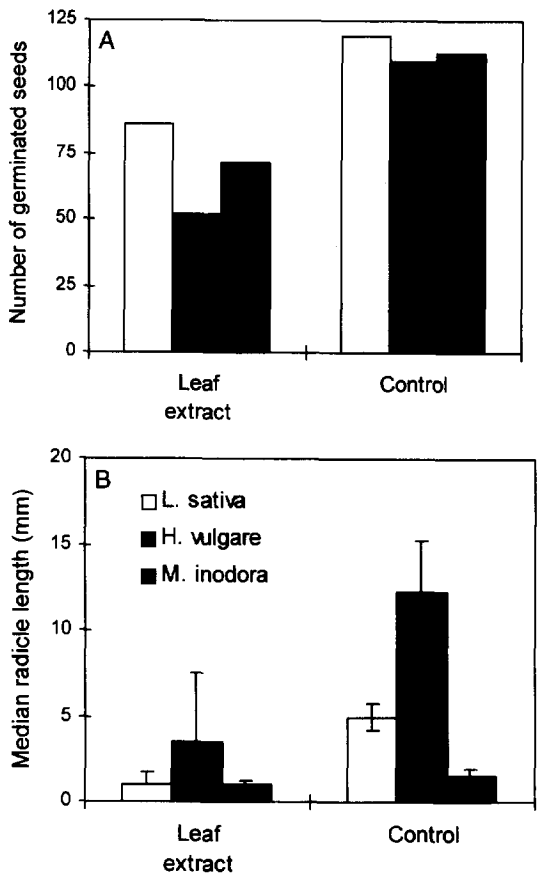

Fig. 1. Inhibition of germination (A) and radicle growth (B) of seeds of *L. sativa*, *H. vulgare* and *M. inodora* by aqueous *B. orientalis* leaf extracts in comparison to control treatments. Error bars in (B) indicate quartiles. Since no significant differences between replicates were obtained, data from corresponding Petri dishes were pooled.

effects on germination of the three indicator species (data not shown). Therefore, for the rest of the study, we concentrated on the analysis of above-ground plant parts.

XAD-2 adsorption of glycoconjugates and fractionation by rotation locular counter-current chromatography (RLCC)

In earlier investigations, it was observed that most of the secondary compounds involved in allelopathy (i.e. phenols and other water-soluble compounds, e.g. glycoconjugates) can be retained on XAD-columns [6, 10–12]. Therefore, in order to isolate the presumed phytotoxic substances from *B. orientalis* leaves, the XAD-2 procedure was used [13]. XAD-2, a polystyrene resin, is known selectively to retain non-polar as well as glycosidically bound constituents whereas little affinity is observed for the bulk of other more polar organic plant constituents, e.g. acids and sugars. The latter pass the column unretained and are found in the permeate. The retained glycosides and non-polar constituents were subsequently eluted with methanol. The methanolic isolate was concentrated *in vacuo* and the resultant concentrate was subsequently liquid extracted with diethyl ether to remove the non-polar constituents. The residue was then further fractionated with the aid of the all-liquid chromatographic technique of RLCC [14]. The three fraction groups obtained by RLCC (osmotic potential ranging between 50 and 80 mmol l^{-1}) were each almost completely able to prevent germination of the seeds of the above mentioned species (Table 1). In the rare cases where germination was observed, the radicles were only protruding. Therefore, the phytotoxic compounds from *B. orientalis* were efficiently concentrated in these fractions. The residual stationary phase of the RLCC as well as the permeate (osmotic potential 50 and 60 mmol l^{-1}, respectively) affected only germination of *M. inodora*. Hence, this species seems to be more sensitive to potential germination inhibitors.

On the basis of these findings, it could be concluded that allelochemicals which considerably inhibit seed germination are mainly found among the polar constituents, thus requiring a detailed analysis of these RLCC fraction groups.

Table 1. Inhibition of germination and radicle growth of seeds of *L. sativa*, *H. vulgare* and *M. inodora* by various fractions of methanolic *B. orientalis* leaf extracts in comparison with control treatments

	RLCC fraction groups			Residual stationary phase	XAD-2 permeate	De-ionized water (control)
L. sativa	−/-*	−/-	o/-	o/+	+/+	+/+
H. vulgare	−/-	−/-	-/-	o/-	o/o	+/+
M. inodora	−/-	−/-	-/-	+/+	+/+	+/+
	← Increasing polarity					

*Symbols before the slash indicate germination rate as a proportion of the total number of seeds. Symbols following the slash indicate radicle length relative to radicle length in the control (+ > 75%, o 25–27%, - < 25%, − no germination).

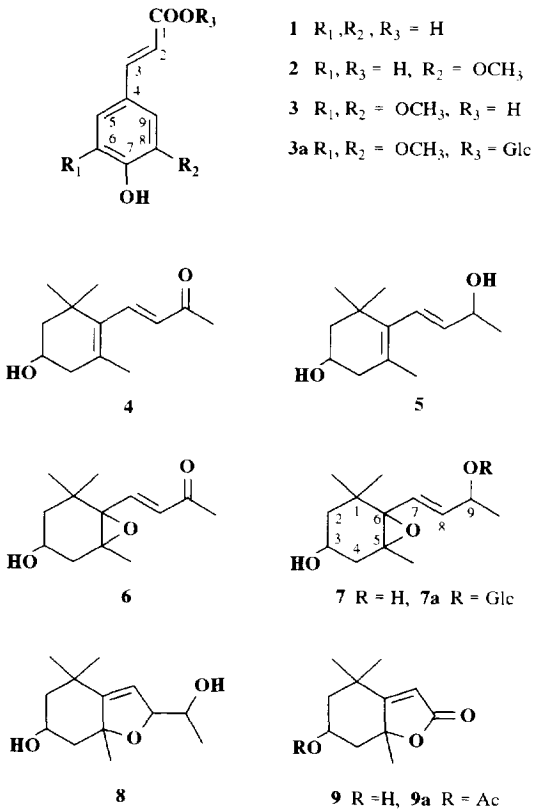

Fig. 2. Structures of *B. orientalis* leaf constituents.

Separation and characterization of phytotoxic B. orientalis *leaf constituents*

Aglycone analysis

A first insight into the structure of the presumed phytotoxic glycosides was obtained after enzymic hydrolysis of aliquots of fraction groups I–III using a non-selective glycosidase, Rohapect D5L. The aglycones obtained from this hydrolysis were then identified by GC–mass spectrometry. The GC–mass spectral analysis of the combined fractions in group I showed that phenolic constituents predominated. As major aglycones, *p*-coumaric acid (**1**), ferulic acid (**2**) and sinapic acid (**3**) were identified at concentrations of 10–50 mg kg^{-1} fr. wt. Ferulic acid and *p*-coumaric acid are among the most widespread and persistent allelochemicals [15].

In RLCC fraction group II, the majority of the enzymically liberated aglycones belonged to the class of norisoprenoids. The structures of these apparently carotenoid-derived compounds are depicted in Fig. 2. The composition of fraction group III was similar to that of fraction group II although the glycosides were less abundant. Besides several common plant constituents, e.g. vomifoliol and 3-oxo-α-ionol, RLCC fractions II and III were found to contain glycosidic forms of 3-hydroxy-β-ionone (**4**), 3-hydroxy-β-ionol (**5**), 3-hydroxy-5,6-epoxy-β-ionone (**6**), 3-hydroxy-5,6-epoxy-β-ionol (**7**) and 3-hydroxyactinidols (**8**). Epoxide **7** was the most abundant norisoprenoid in *B. orientalis* leaves with a concentration of *ca* 25 mg kg^{-1} fr. wt (standard-controlled quantification). From an allelochemical point of view, however, the finding of the oxidized forms **4** and **6** is more significant. Both ionone derivatives are known for their phytotoxic potential [16, 17].

Characterization of intact glycosides

In an attempt to characterize completely the intact glycoconjugates, RLCC fraction groups I and II were subfractionated by multilayer coil counter-current chromatography [18] and, after acetylation, finally purified by preparative HPLC. In this way, RLCC fraction group II yielded peracetylated 3-hydroxy-5,6-epoxy-β-ionyl-β-D-glucoside (**7a**) (30 mg). In the DCI mass spectrum of **7a** a strong pseudo-molecular ion of *m/z* 616 $[M + NH_4]^+$ was observed. The ^1H NMR spectrum included three three-proton singlets at δ 0.97, 1.14 and 1.18 as well as a three-proton doublet at δ 1.24 ($J = 6.5$ Hz). An $ABMX_3$ system assigned to the (*E*)-hydroxybutenyl side chain of **7a** was virtually identical in chemical shifts and coupling constants to that of structurally related C_{13}-norisoprenoids [19]. Also, the data for the tetraacetyl-β-D-glucopyranosyl moiety were in good agreement with those reported for other acetylated β-D-glucosides [20]. The location of the glucose moiety was deduced from the ^1H NMR spectrum. The signal for the proton at C-9 was less downfield shifted (δ 4.28) as observed for substitution with an acetoxy group (*ca* δ 5.3). The ^{13}C NMR data confirmed the presence of a glucose moiety, and the data for the aglycone part were in good agreement with those published for 3-hydroxy-5,6-epoxy-β-ionol [21]. β-Glucosidase (sweet almond emulsin) treatment of the deacetylated product liberated aglycone **7**, showing identical chromatographic and spectral data compared to those for an authentic sample. D-Glucose was identified in the hydrolysate by HPLC using simultaneous refractive index and polarimetric detection. The major glycoconjugate in fraction group I (150 mg) was found to be the β-D-glucose ester of sinapic acid. 1-*O*-Sinapoyl glucose, which is involved in the biosynthesis of sinapine, is a known constituent of most species of the Brassicaceae [22].

Analysis of leaf leachates

Analysis of the polar RLCC fractions showed that *B. orientalis* accumulates a considerable amount of known phytotoxic substances in glycoconjugated form. However, it was still not known to what extent these substances are released from the plant and how they interfere with other species. One important way of releasing phytotoxic substances is by leaching of decomposing plant material [15]. In order to have an insight into the structure of liberated plant products, intact *B. orientalis* leaves were kept immersed in water

at room temperature, thus simulating leaf leaching of first stage decaying leaves under wet conditions. After three days, the leachates were extracted stepwise with diethyl ether, and the neutral and acidic extracts were subsequently analysed by HR GC–mass spectrometry.

Again, hydroxycinnamic acid derivatives predominated, thus indicating the action of hydrolases in the decaying leaves. Ferulic and *p*-coumaric acids were present in concentrations of *ca* 5 and 19 mg l^{-1}, respectively, in the solution obtained by leaching the decaying leaves. A synergistic inhibitory effect of both compounds on sorghum seedling growth was reported [23] when both components were present at a concentration of 2.5×10^{-4} mol l^{-1} or 48 and 40 mg l^{-1}, respectively. Among the C_{13}-norisoprenoids, 3-hydroxy-5,6-epoxy-β-ionol was the major product. Moreover, a similar spectrum of norisoprenes as found by RLCC of fraction II was detected. More than 7.5 mg of 3-hydroxy-5,6-epoxy-β-ionol was leached from the *B. orientalis* leaves on immersing them in 800 ml of water. In the case of the oxidized form, 3-hydroxy-5,6-epoxy-β-ionone, similar concentrations were shown to cause inhibition (>50%) in wheat coleoptile section tests [16]. In soil, the oxidation of **7** to produce the allelochemical **6** can be assumed, a mechanism which is also proposed for the generation of the well-known allelochemical juglone [12]. This shikimate metabolite, which is responsible for the restricted growth around walnut trees, occurs *in vivo* as a hydroquinone (possibly as a glycoside) and after rain leaching from the leaves it is oxidized to the active form in the soil. Oxidation reactions are also likely to generate a further known allelopathic agent, the so-called loliolide (**9**). Hydroxyketone **9**, which was not detectable in *B. orientalis* leaves, was a major constituent (> 0.5 mg l^{-1}) in the leaf leachate. The earlier reported formation of loliolide from epoxide **7** via **8** is shown in Fig. 3 [21]. Compound **4**, which was shown to be an inhibitor for hypocotyl growth [17], was released at a concentration of >1.5 mg l^{-1}.

Although most of the amounts of individual phytotoxins obtained on leaching *B. orientalis* leaves are below those reported as inhibitory in the literature, the extracts of intact *B. orientalis* leaves (50 g fr. wt, 20% w/w), nevertheless, showed considerable inhibitory effects (Fig. 1A and B). Therefore, synergistic effects of the various phytotoxic compounds in the extract/leachate solution are plausible. The amounts of phytotoxins found in the leachate solution should merely be regarded as a first coarse estimation of their egression during the first days of the leaf rotting process. In this respect, it is important to bear in mind that the concentrations of egressed compounds depend on the proportion of plant biomass to solution volume. Moreover, the amounts of allelochemicals liberated to the soil solution by leaching under field conditions are contingent upon the standing biomass, rate of egression of the phytotoxins, soil texture and the decomposition rate of the respective allelochemicals [15].

In summary, our results show that *B. orientalis* produces a variety of known allelochemicals, most of which are liberated in appreciable amounts at the beginning of decomposition of leaves under wet conditions. Therefore, we can address questions more pertinent to the importance of allelopathic effects for the competitive ability of *B. orientalis* in the field, where environmental factors (e.g. nutrient levels, type of soil) are known to have a substantial influence on allelopathic effects [15, 24]. Thus, our current work focuses on further germination and growth tests, using seeds and seedlings of plant species naturally co-occurring with *B. orientalis* in the field, to assess ecologically relevant allelopathic effects of compounds egressed from intact (root exudates) or decaying *B. orientalis* plants in sand and soil. Additionally, further tests will be performed to assess the still unknown contribution of isothiocyanates produced by *B. orientalis* to the plants allelopathic potential.

EXPERIMENTAL

General. NMR: Fourier transform Bruker AC 200 and WM 400 spectrometers; flash chromatography (FC): Merck silica gel 60 (0.032–0.063 mm); TLC: silica gel 60 plates (Merck), RLCC stationary phase as developing solvent and H_2SO_4/vanillin detection; HRGC: J & W fused silica DB-5 capillary column (30 m × 0.25 mm i.d., film thickness 0.25 μm). Split injection (1:20) was used. The temp. programme was from 60° to 300° at 5° min^{-1}. Flow rates of carrier gas were 2.0 ml min^{-1} for He, for the make-up gas 30 ml min^{-1} of N_2 and for detector gases 30 ml min^{-1} of H_2 and 300 ml min^{-1} of air. The injector temp. was kept at 250° and detector temp. at 300°. HRGC-MS: Varian 3300 gas chromatograph equipped with a split injector was combined by direct coupling with a Finnigan MAT 44 mass spectrometer with PCDS data system. The same type of column and same temp.

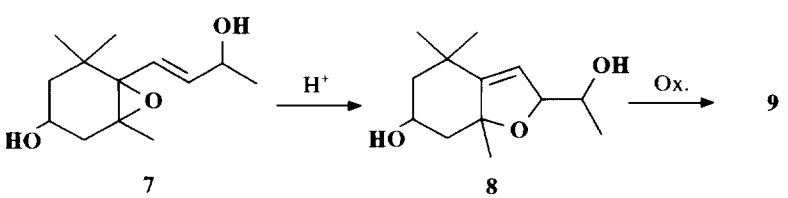

Fig. 3. Formation of loliolide from 3-hydroxy-5,6-epoxy-β-ionol (**7**) according to ref. [21].

programme as for HRGC analyses were used; electron energy 70 eV.

Plant material. B. orientalis L. plants were obtained from dense stands in the vicinity of Würzburg in May 1994. Seeds of *L. sativa* L. Kagraner Sommer/Duna, and *H. vulgare* L. were obtained from Carl Sperling (Lüneburg, Germany); seeds of *M. inodora* L. were collected at a nearby field site.

Petri-dish bioassay of water extracts. Intact *B. orientalis* leaves (50 g fr. wt) were immersed in H_2O (20% w/w) and kept in the dark at room temp. for 3 days. 25 seeds of *L. sativa*, *H. vulgare* and *M. inodora* were sown in separate petri dishes (ϕ 90 mm, height 15 mm) lined with filter paper discs (ϕ 80 mm). Each petri dish was supplemented with 3 ml of extract. Dishes with 3 ml H_2O served as controls. Every treatment was replicated (×5). The petri dishes were kept in a growth chamber with a 14:10 hr light/dark period and 26°:10° daily temp. change. After 72 hr, the proportion of germinated seeds was determined and lengths of the radicles measured.

Isolation of glycosidic extracts from B. orientalis *leaves.* Deep-frozen leaves (4 kg) were crushed in a Braun blender. Portions of 1 kg were worked up as follows: after addition of MeOH (1 l kg^{-1} of leaves), stirring overnight at room temp. and centrifugation (4000 rpm, 30 min), the MeOH extract was concd *in vacuo*. The aq. residue was extracted with pentane (200 ml) and passed through a column of Amberlite XAD-2. After rinsing with H_2O (500 ml), the retained glycosides were eluted with MeOH (500 ml). The combined MeOH eluates were extracted with Et_2O to remove any remaining non-polar substances and evapd to dryness. 5 g of the glycosidic-containing extract was fractionated by RLCC in portions of 1 g. The RLCC apparatus (Eyela RLCC P-60, Tokyo Rikakikai) was operated in the ascending mode employing a solvent system made up from the two phases produced by mixing $CHCl_3$–MeOH–H_2O (7:13:8) with the more dense layer being used as stationary phase. The flow rate was 1 ml min^{-1}, the rotation speed was 80 rpm, and the slope was 30°.

Screening of RLCC fractions. The RLCC sepn was checked by TLC. Closely spaced frs were pooled to yield 3 groups. 10 ml of 0.2 mol l^{-1} citric acid/Pi buffer (pH 5.2) together with 20 μl of a non-selective glycosidase (Rohapect DSL, Röhm, Darmstadt; int. standard: phenyl-β-D-glucose) were added to one-tenth of the respective solvent-free fr. groups. Liberated aglycones (neutral/acidic frs) were worked up according to ref. [25], dried over Na_2SO_4 and concd to 50 ml prior to HRGC and HRGC-MS analyses.

Petri-dish bioassay of extract fractions. 750 g deep-frozen leaves were worked up as described above. The obtained RLCC fr. groups and residual stationary phase were evapd to dryness, and the residue taken-up in 5 ml H_2O. The permeate of the XAD-2 column was diluted to 60 mmol l^{-1}. Ten seeds of each of the 3 indicator species were sown separately on filter paper discs within petri dishes (ϕ 35 mm, height 10 mm). 0.5 ml of the RLCC and XAD-2 solns were applied per petri dish for each of the species. Addition of 0.5 ml de-ionized H_2O served as control. All species × treatment combinations were run in triplicate. The petri dishes were kept in a growth chamber for 72 hr and germination was determined as described above. No considerable reduction of soln vol. in the petri dishes could be observed at the end of the experiment. Therefore, a significant increase of the osmotic pressure of the solns by evapn could be excluded.

MLCCC separations. RLCC fr. groups were re-chromatographed using an Ito multilayer coil separator–extractor (P.C. Inc., Potomac) equipped with 160 m × 1.6 mm PTFE tubing using EtOAc–BuOH–H_2O (3:2:5) as solvent system (mobile phase: upper layer; mode: tail to head; flow rate: 1 ml min^{-1}; rotational speed: 800 rpm). 10-ml frs were collected. After screening by TLC the major compounds were acetylated (Ac_2O/pyridine) and, after FC, finally purified by prep. HPLC (Eurospher 100-Si column, 5 μm, 250 × 16 mm, Knauer Säulentechnik, Berlin; eluent: Et_2O). Aglycone and sugar analysis were performed as in ref. [26].

Spectral data for **7a** (*pentaacetate*). DCI-MS: pseudo-molecular ion at *m/z* 616 $[M\ (598) + NH_4]^+$; ^1H NMR (400 MHz, $CDCl_3$, ppm, *J* in Hz): 0.97, 1.14 (2 × 3H, 2*s*, 2 × Me-1), 1.18 (3H, *s*, Me-5), 1.24 (3H, *d*, *J* = 6.5, Me-9), 1.32 (1H, *dd*, *J* = 13.4, 8.5, H_a-2), 1.64 (1H, *dd*, *J* = 13.4, 2.7, H_b-2), 1.74 (1H, *dd*, *J* = 14.8, 6.9, H_a-4), 2.00, 2.01, 2.02, 2.05, 2.08 (5 × 3H, 5*s*, acetates), 2.37 (1H, *dd*, *J* = 14.8, 5.7, H_b-4), 3.64 (1H, *ddd*, *J* = 9.8, 4.6, 2.3, H-5′), 4.06 (1H, *dd*, *J* = 12.2, 2.3, H_a-6′), 4.23 (1H, *dd*, *J* = 12.2, 4.6, H_b-6′), 4.28 (1H, *dq*, *J* = 6.5, 5.9, H-9), 4.57 (1H, *d*, *J* = 8.0, H-1′), 4.90 (1H, *m*, H-3), 5.00 (1H, *dd*, *J* = 9.5, 8.0, H-2′), 5.09 (1H, *dd*, *J* = 9.8, 9.5, H-4′), 5.18 (1H, *t*, *J* = 9.5, H-3′), 5.73 (1H, *dd*, *J* = 15.5, 5.9, H-8), 5.89 (1H, *br d*, *J* = 15.5, H-7); ^{13}C NMR (100 MHz, $CDCl_3$): 20.2, 21.3, 25.6, 28.5 (Me at C-1/C-5/C-9), 34.4 (C-1), 36.8, 41.3 (C-2/C-4), 64.9, 69.6 (C-5/C-6), 67.6 (C-3), 76.2 (C-9), 125.9, 135.3 (C-7/C-8), sugar part: 62.2 (C-6′), 68.7 (C-4′), 71.9, 72.0 (C-2′/C-5′), 73.0 (C-3′), 99.4 (C1′), 20.6–20.9, 169.1–170.5 (5 acetates). The signals were assigned on the basis of DEPT experiments. β-Glucosidase treatment of the deacetylated product liberated aglycone **7**; MS (70 eV) *m/z* (rel. int.): 208 $[M - H_2O]^+$ (5), 166 (4), 135 (3), 125 (22), 109 (12), 107 (11), 82 (15), 67 (8), 55 (12), 43 (100).

Spectral data for **3a** (*pentaacetate*). DCI-MS: pseudo-molecular ion at *m/z* 614 $[M\ (596) + NH_4]^+$; ^1H NMR (400 MHz, $CDCl_3$, ppm, *J* in Hz): 2.02, 2.03, 2.04, 2.08, 2.33 (5 × 3H, 5*s*, acetates), 3.85 (6H, *s*, OMe), 3.90 (1H, *ddd*, *J* = 9.9, 4.4, 2.2, H-5′), 4.13 (1H, *dd*, *J* = 12.2, 2.2, H_a-6′), 4.31 (1H, *dd*, *J* = 12.2, 4.4, H_b-6′), 5.16 (1H, *dd*, *J* = 9.8, 9.4, H-4′), 5.25 (1H, *dd*, *J* = 9.3, 8.0, H-2′), 5.30 (1H, *dd*, *J* = 9.4, 9.3, H-3′), 5.85 (1H, *d*, *J* = 8.0, H-1′), 6.35 (1H, *d*, *J* = 15.9, H-8), 6.77 (2H, *s*, H-2/6), 7.67 (1H, *d*, *J* = 15.9, H-7); ^{13}C NMR (100 MHz, $CDCl_3$): 56.3 (OMe), 61.6

(C-6'), 68.1 (C-4'), 70.5 (C-2'), 72.9 (C-3', C-5', not resolved), 92.1 (C-1'), 105.2 (C-2/C-6), 116.6 (C-8), 132.6 (C-1), 147.0 (C-3/C-5), 147.1 (C-7), 152.7 (C-4), 164.4 (C-9), 20.3–20.6, 168.2–170.5 (5 acetates).

Leaf-leachate analysis. Intact *B. orientalis* leaves (300 g fr. wt) were immersed in 800 ml H_2O and kept for 3 days at room temp., simulating leaf leaching of first stage decaying leaves under wet conditions. The extract was filtrated and continuously extracted with Et_2O. The neutral and acidic extracts [25] were concd and analysed by standard-controlled (2-octanol) HRGC and HRGC-MS. The unknown constituent, after acetylation (Ac_2O/pyridine), was isolated by normal phase HPLC.

Spectral data for **9a**. ^{1}H NMR (200 MHz, $CDCl_3$, ppm, J in Hz): 1.29, 1.39, 1.72 (3H, 3*s*, 2 × Me-1, Me-5), 1.56 (1H, *dd*, J = 14.8, 4.2, H_a-2), 1.82 (1H, *dd*, J = 14.8, 4.2, H_b-2), 2.06 (1H, *dt*, J = 14.5, 2.7, H_a-4), 2.10 (3H, *s*, OAc), 2.52 (1H, *dt*, 14.5, 2.7, H_b-4), 5.25 (1H, *m*, H-3), 5.74 (1H, *s*, H-7); ^{13}C NMR (100 MHz, $CDCl_3$): 21.3 (OAc), 25.9, 26.5, 30.5 (Me at C-1/C-5), 42.8, 44.1 (C-2/C-4), 68.6 (C-3), 113.5 (C-7); MS (70 eV) *m/z* (rel. int.): 238 $[M]^+$ (3), 196 (5), 178 (15), 163 (10), 150 (15), 135 (28), 121 (5), 107 (20), 67 (8), 43 (100). Deacetylation afforded **9**; spectral data: *cf.* ref. [27].

Acknowledgements—The authors thank Monika Messerer for her skillful technical assistance and Prof. I. Ullmann for her helpful comments on the manuscript.

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