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Short Communication

Isolation and identification via high-performance liquid chromatography and thin-layer chromatography of benzoxazolinone precursors from *Consolida orientalis* flowers

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ABSTRACT

From the flowers of *Consolida orientalis* (Ranunculaceae) benzoxazolinone (BOA) and 2,4-dihydroxy-1,4-benzoxazin-3-one (DIBOA) were isolated and identified by thin-layer chromatography, high-performance liquid chromatography, infrared spectroscopy and ^1H nuclear magnetic resonance. Also, the presence of 2-O-glucosyl-2,4-dihydroxy-1,4-benzoxazin-3-one was confirmed by thin-layer chromatography and high-performance liquid chromatography. These compounds are reported for the first time from the Ranunculaceae family.

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INTRODUCTION

Benzoxazolinone (BOA) was first isolated from rye seedlings; it possesses antifungal, anti-inflammatory and plant growth activities [1–3]. It is reported to be a degradation product produced by heating the hydroxamic acid, 2,4-dihydroxy-1,4-benzoxazine-3-one (DIBOA). DIBOA also exists in the plant as its 2-glucoside form [4–8]. The sugar is enzymatically removed during the isolation procedure. The reactions responsible for the formation of DIBOA and BOA can be summarized as shown in Fig. 1.

BOA, DIBOA and its glucoside have also been found in certain species of Gramineae and Acanthaceae [1,3–5,7,9]. *Consolida orientalis* (Gay) Schröd (synonym: *Delphinium orientale*) is one of the 23 *Consolida* species found widely in Turkey [10]. *Delphinium* and *Consolida* species are used in folk medicine as pediculicides, insecticides for cattle and as antirheumatics [11]. These plants are rich sources of alkaloids, and there are many studies of their alkaloids [12]. There is only one report on the organic acids [13] and anthocyanins [14] of *C. orientalis*.

After isolating and identifying BOA from the flowers of *C. orientalis*, because this product is derived from DIBOA and its glucoside, we also searched for these products. In this study, BOA, DIBOA and its glucoside were identified by thin-layer chromatography (TLC) and high-performance liquid chromatography (HPLC). In the identification, standard materials used were isolated from the seeds of *Secale cereale* L [4,5]. After identification, BOA and DIBOA were isolated by using column chromatography and preparative TLC and their structures were elucidated by their m.p. and IR and ^{1}H NMR spectra. These compounds have been isolated from the Ranunculaceae species for the first time.

Plant material from *C. orientalis* was collected from two different parts of Turkey and it was found that both types contained DIBOA and its glucoside.

EXPERIMENTAL

Plant material

C. orientalis was collected from Ankara-Ayaş and from Gümüşhane-Bayburt in July–August 1990, and *Aqulegia olympica* from Maçka-Trabzon, *Delphinium formosum* from Arsin-Trabzon and *Consolida glandulosa* from Gümüşhane-Bayburt were collected in July 1990.

Secale cereale L., used as a source of standard materials, was collected from Trabzon in August 1990.

Chemicals

Methanol (LiChrosolv) and acetic acid were obtained from Merck (Darmstadt, Germany). ^{1}H NMR solvents and authentic BOA were purchased from Aldrich (Milwaukee, WI, USA).

General procedure

All melting points were determined on an electrothermal digital melting point apparatus and are not corrected. ^{1}H NMR spectra were obtained using a Bruker AC 200 FT NMR spectrometer, using [$^2H_6$]dimethyl sulphoxide (DMSO-d_6) as the solvent and tetramethylsilane (TMS) as internal standard. IR spectra were determined on a Perkin-Elmer 177 IR spectrophotometer in potassium bromide pellets. TLC and preparative TLC were performed on silica gel 60 G and HF_{254} (1:1) (Merck), thickness 0.25 and 0.5 mm (Camag instruments, Muttenz, Switzerland).

The solvent systems were as follows: (I) chloroform–methanol–water (75:25:3, v/v/v); (II) *n*-butanol saturated with water; (III) ethyl acetate–methanol–water (100:13.5:10, v/v/v); (IV) chloroform–

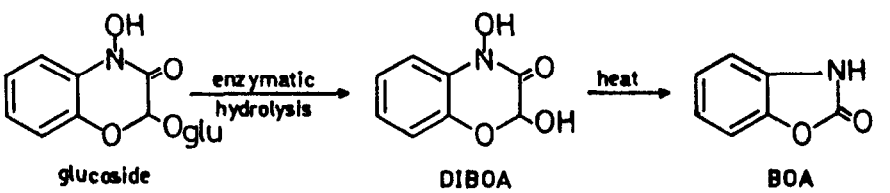

Fig. 1. Formation of DIBOA and BOA.

acetic acid (90:10, v/v). The spots were visualized under short-wavelength UV light and ferric chloride was used for the determination of hydroxamic acids. Column chromatography was carried out using silica gel 60 (70–230 mesh) (Merck).

The HPLC system consisted of a Shimadzu (Tokyo, Japan) liquid chromatograph fitted with two Model LC 9A solvent delivery systems, a Model SPD 6AV UV absorbance detector, a Model C-R5A computing integrator and a Rheodoyne 7125 injector valve with a 20-μl sample loading loop. Chromatography was carried out on a Shim-pack CLC-ODS (M) silica stainless-steel column (150 × 4 mm I.D., 5 μm particle size) (Shimadzu) eluted with water–methanol–acetic acid (73:27:3, v/v) at 254 nm and flow-rate was 0.8 ml/min. The sample volume injected was 20 μl and separations were performed at ambient temperature under normal laboratory lighting conditions. The mobile phase was degassed with helium and chromatographic analysis was performed isocratically.

Extraction and isolation of compounds

Extraction of DIBOA and BOA from C. orientalis flowers. A 50-g aliquot of plant material was ground with water in a Waring blender (300 ml) after standing for 3 h at room temperature to allow enzymatic hydrolysis of the glucoside, heated on a water bath for 1 h at 70°C and filtered. The filtrate (approximately 250 ml) was extracted successively with several portions of *n*-hexane and diethyl ether and combined diethyl ether extracts were dried over anhydrous magnesium sulphate and evaporated to dryness on a rotary evaporator under reduced pressure. The residue was placed on a silica gel column and eluted successively with *n*-hexane, *n*-hexane–chloroform (90:10 to 50:50), chloroform and chloroform–diethyl ether (90:10 to 80:20). Fractions of 25 ml were collected and examined by TLC and HPLC.

In some fractions BOA and DIBOA were identified and their R_F values and retention time were identical to the authentic BOA and DIBOA isolated from *S. cereale* L. From these fractions DIBOA and BOA were isolated by preparative TLC using solvent system I. The identities of BOA and DIBOA were confirmed by m.p. and IR and ^{1}H NMR spectra.

BOA: m.p. 138°C (*n*-hexane–diethyl ether); IR (cm^{-1}) 3234, 1885, 1778, 1731, 1484, 1372, 1011, 941, 698, ^{1}H NMR (DMSO-d_6), δ (ppm) 7.31 (s, 4H Ar-H), 9.04 (s, 1H, N-H).

DIBOA: m.p. 155°C (diethyl ether); IR (cm^{-1}) 3200, 2870, 1650, 1590, 1475, 1270, 1210, 1085, 1052, 1030, 973, 825, 747. ^{1}H NMR (DMSO-d_6), δ (ppm) 7.10 (s, 4H, Ar-H), 8.17 (s, 1H, OH), 10.90 (s, 1H, N-OH).

Extraction of glucoside from C. orientalis flowers. A 5-g aliquot of dried plant material was ground in a Waring blender with methanol (150 ml), refluxed for 6 h and the slurry filtered through filter paper. The extract was evaporated to dryness under reduced pressure. The residue was taken up in water and then extracted successively with *n*-hexane, diethyl ether and *n*-butanol. The combined *n*-butanol phases were evaporated to dryness under reduced pressure and the residue (0.05 g) was dissolved in methanol. From the methanolic solution, glucoside was determined by co-chromatography with standard material isolated from *S. cereale* in both TLC and HPLC. At the same time both this glucoside and the DIBOA glucoside isolated from *S. cereale* gave the same colours with ferric chloride reagent in TLC.

RESULTS AND DISCUSSION

Two different extracts of *C. orientalis*, with water and methanol, were prepared and both of the extracts were tested on TLC with authentic BOA and DIBOA obtained from *S. cereale*.

In the water extract, the amount of BOA and DIBOA was higher, because of the heat and enzymatic hydrolysis. In the methanol extract, the amount of the glucoside was higher, but there was a small amount of DIBOA since boiling methanol destroys the enzymes.

In TLC, the spots of DIBOA and its glucoside were easily detected as dark zones at 254 nm UV light and visualized with ferric chloride reagent, which gives a violet colour with hydroxamic acids. Thus, it is apparent that BOA does not occur naturally in *C. orientalis* flowers but is a degradation product of DIBOA as mentioned in the literature [4–8].

After isolating DIBOA, it was heated in water and confirmed by HPLC that it turns to BOA.

BOA and DIBOA were isolated from column and preparative TLC. Since it is well known that

TABLE I

TLC R_F VALUES AND HPLC RETENTION TIMES OF BOA, DIBOA AND DIBOA GLUCOSIDE

Compound	TLC R_F values in system				Colour with ferric chloride	Retention time (min)
	I	II	III	IV		
BOA	0.80	0.91	0.77	0.48	–	20.73
DIBOA	0.44	0.88	0.63	0.18	Violet	11.89
Glucoside	0.11	0.59	0.14	–	Violet	9.47

DIBOA occurs in intact plants as its 2-O-glucosyl derivative, and since the presence of BOA and DIBOA was confirmed by spectroscopic methods, the identification of the glucoside was made only with TLC and HPLC.

The spectroscopic data and m.p. were identical to those of authentic BOA and DIBOA isolated from *S. cereale* and also fit the results reported previously [4,6,7,15,16].

The R_F values obtained by TLC and retention times obtained by HPLC of BOA, DIBOA and its glucoside are given in Table I.

The HPLC separation of the mixture of these three compounds is shown in Fig. 2.

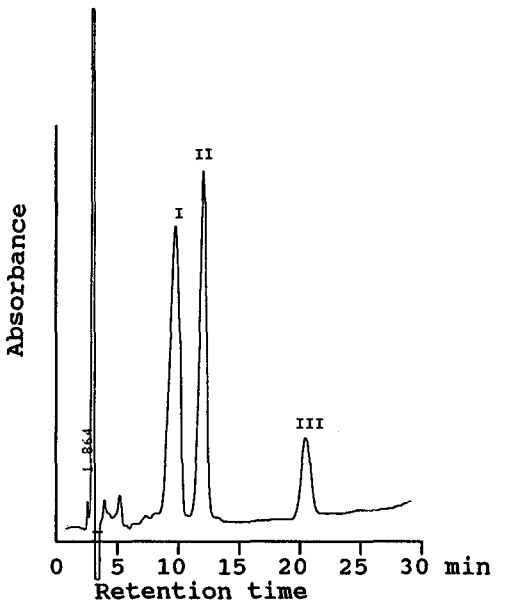

Fig. 2. Chromatogram of an artificial mixture of purified BOA (III), DIBOA (II) and its glucoside (I). Injection volume, 20 μl.

In this study, the complete separation of these three products with a CLC–ODS silica column using UV detection (254 nm) and the solvent system water–methanol–acetic acid (73:27:3, v/v) is also realized for the first time. The method is simple and easily applicable.

DIBOA and its glucoside were also sought in *Aqulegia olympica*, *Consolida armeniaca*, *Delphinium formosum* and *Consolida glandulosa* flowers by co-chromatography with standards in both TLC and HPLC, but it was found that they are not present in these plants.

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