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Short communication

Reversed-phase liquid chromatography of 1,3-benzodioxanes in *Piper mullesua*

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Abstract

A simple, rapid method for the direct determination of five 1,3-benzodioxanes, fargesin (**1**), sesamin (**2**), asarinin (**3**), 1,3-benzodioxole-5-(2,4,8-triene-isobutyl nonaoate) (**4**) and 1,3-benzodioxole-5-(2,4,8-triene-methyl nonaoate) (**5**), in *Piper mullesua* extracts by reversed-phase liquid chromatography with UV detection is described. The separation of these compounds was performed with acetonitrile–water (65:35) using a μBondapak C_{18} column with 10-μm particles (300 mm×3.9 mm I.D.).

Keywords: Piper mullesua; 1,3-Benzodioxanes

1. Introduction

The species of genus Piper (Fam. Piperaceae) have high commercial, economic and medicinal importance due to the presence of methylene dioxy phenyl containing compounds [1]. During our study on insecticidal chemicals from *Piper mullesua* (syn. *P. brachystachyum*) we reported it to be a potent source of methylene dioxy phenyl compounds including two new compounds **4** and **5** [2,3]. Sesamin, the major 1,3-benzodioxane, possesses potent antifeedant and growth inhibitory activities towards the larvae of *Spilarctia obliqua* [4]. Pharmacological activities of *Piper* species have been reviewed [1,5]. In our efforts towards developing liquid chromatographic procedures for plant drug analysis [6–9] we report here a simple reversed-phase HPLC (RPLC) method to analyse five major 1,3-benzodioxanes [(**1**)–(**5**)] (Fig. 1) by employing a binary mobile phase. This is

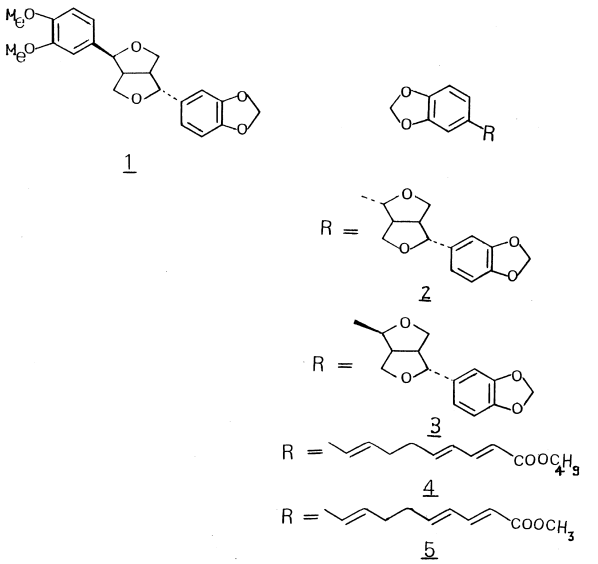

Fig. 1. Structures of the five 1,3-benzodioxanes in *Piper mullesua* extract.

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the first report on the quantitation of these 1,3-benzodioxane derivatives [(**1**)–(**5**)] by RPLC.

2. Experimental

2.1. Materials and reagents

Compounds **1**–**5** were isolated from *P. mullesua* fruit-bearing inflorescence material collected from Tamenglong district, Manipur, India, using silica gel column chromatography and their structures were confirmed by spectral analysis [3]. Reagents used were HPLC grade (E. Merck, Germany).

2.2. Chromatographic apparatus and conditions

A Shimadzu (Japan) LC-10A gradient high-performance liquid chromatography instrument equipped with two LC-10AD pumps controlled by a CBM-10 interface module, a Model 7725 i manual injector valve (Rheodyne), a 20 μl sample loop and a multidimensional UV–VIS detector SPD-10 A was used for the analysis. A SPD-M10AVP (Shimadzu) Photodiode array detector was used for peak purity tests of the compounds. Data were collected and analysed using a Class LC-10 work station equipped with a Pentium computer (Datamini, Singapore) and a HP deskjet printer. Solvents were filtered using a Millipore system and analysis was performed on a Waters μBondapak C_{18} column (300 mm×3.9 mm I.D. 10 μm). A constant flow-rate of 1 ml/min was used during analysis. The composition of the mobile phase was optimized by varying the percentage of acetonitrile in water, resulting in the following operating conditions: acetonitrile–water (65:35, v/v); flow-rate, 1 ml/min; column temperature, 26°C; detector wavelength, 220 nm; absorption maxima close to all the compounds.

2.3. Sample preparation

Air-dried and powdered plant material (1 g) was extracted with acetone three times (10 ml each time for 4 h), the combined extracts were filtered and concentrated under vacuum and the volume was made up to 5 ml in acetone. Samples were filtered through a Millipore filter and a known amount was subjected to HPLC under the above conditions. The content of each 1,3-benzodioxane derivative [(**1**)–(**5**)] was calculated using an external standard.

3. Results and discussion

The composition of the mobile phase was optimized using different proportions of acetonitrile in water, the final result being acetonitrile–water (65:35). Fig. 2 illustrates the separation of the 1,3-benzodioxanes in a standard mixture and a plant sample extract. Peaks corresponding to compounds **1**–**5**, checked via addition of standards, were symmetrical. The peak purity of compounds **1**–**5** was tested using a photodiode array detector: compound **1**, purity up 0.9922, down 0.9995; compound **2**, purity up 0.9980, down 0.9987; compound **3**, purity up 0.9947, down 0.9992; compound **4**, purity up 0.9950, down 0.9986; compound **5**, purity up 0.9990, down 0.9986. The similarity of compounds **1**–**5** in the sample and standards was also checked and found to be 0.9927, 0.9942, 0.9965, 0.9979 and 0.9928 for compound **1**, **2**, **3**, **4** and **5**, respectively. Peak purity test results using PDA are satisfactory. Recoveries of compounds **1**, **2**, **3**, **4** and **5** were 96, 97, 97, 97 and 96%, respectively. For the examination of recovery, known amounts of stock solutions of pure compounds **1**–**5** were added to the *P. mullesua* plant extract and the quantitation was repeated three times. The method reported here was applied for the analysis of a few samples of *Piper Species*. The upper and lower contents of compounds **1**–**5** in four different *P. mullesua* samples were fargesin (0.015–0.022%), sesamin (0.124–0.160%), asarinin (0.052–0.084%), 1-3-benzodioxole-5-(2,4,8-triene-isobutyl nonaoate) (0.015–0.019%) and 1,3-benzodioxole-5-(2,4,8-triene-methyl nonaoate) (0.016–0.024%). This method was also used in the analysis of three market samples of *P. longum*. Compounds **4** and **5** were absent from all three samples, whereas compounds **1**, **2** and **3** were present at 0.002–0.003%, 0.050–0.072% and 0.005–0.006%, respectively.

Calibration graphs for the compounds studied were linear in the range 2–20 μg; the regression equations are given in Table 1.

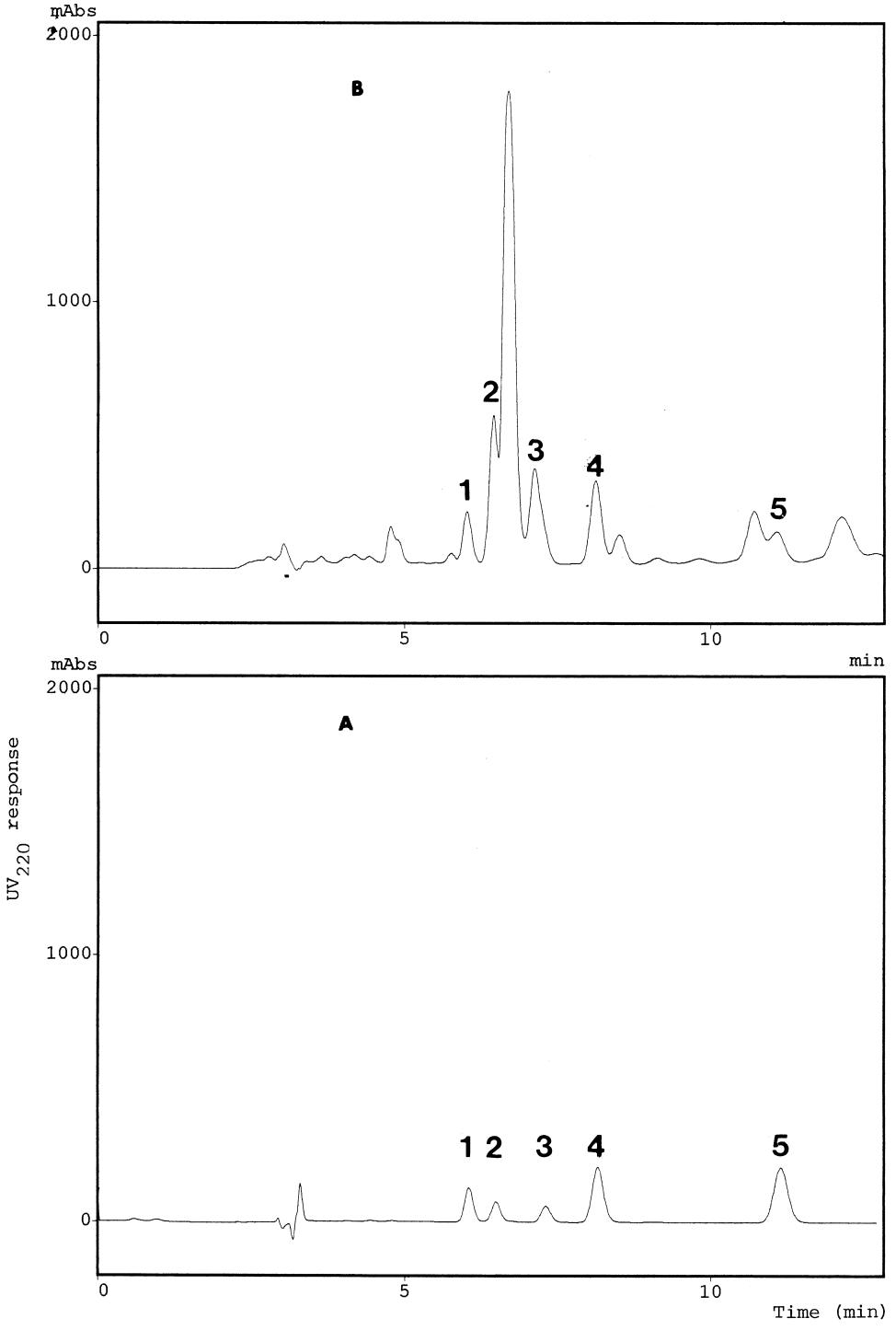

Fig. 2. RPLC separation of 1,3-benzodioxanes in an artificial mixture of pure compounds (A); 1 mg/ml and a *Piper mullesua* plant extract (B). Conditions: μBondapak C_{18} column; UV detection at 220 nm; mobile phase, acetonitrile–water (65:35); flow-rate, 1 ml/min. (**1**) Fargesin; (**2**) sesamin; (**3**) asarinin; (**4**) 1,3-benzodioxole-5-(2,4,8-triene-isobutyl nonaoate); (**5**) 1,3-benzodioxole-5-(2,4,8-triene-methyl nonaoate).

Table 1
Column performance and linear regression data for 1,3-benzodioxanes

Compound	Rt	Recovery (%)	Capacity factor	Equation[a]	r
1	6.01	96	1.04	$Y = 3.433 \times 10^{-6}X - 0.013$	0.999
2	6.45	97	1.19	$Y = 5.454 \times 10^{-6}X + 0.009$	0.999
3	7.25	97	1.46	$Y = 6.289 \times 10^{-6}X + 0.030$	0.999
4	8.11	97	1.75	$Y = 1.607 \times 10^{-6}X - 0.014$	0.999
5	11.05	96	2.75	$Y = 1.314 \times 10^{-6}X - 0.038$	0.999

[a] Number of data points, 5; number of replicates, 3.

4. Conclusion

The described method allows a simple and rapid separation of five 1,3-benzodioxanes present in *P. mullesua* plant extract. This method can be used for the rapid screening for the quality assessment of different *Piper* species for their insecticidal compounds.

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