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Short communication

High-performance liquid chromatographic separation of some naturally occurring naphthoquinones and anthraquinones

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Abstract

A high-performance liquid chromatographic method is described which allows the separation of thirteen naturally occurring naphthoquinones and anthraquinones as well as a pyranonaphthalene derivative on a reversed-phase column. The separation was achieved under isocratic and gradient conditions. The compounds are known as constituents of the heartwood of *Tabebuia avellanedae* (Bignoniaceae). Their chromatographic behaviour is compared to that of furanonaphthoquinones known from the inner bark of *T. avellanedae*.

Keywords: Tabebuia avellanedae; Naphthoquinones; Anthraquinones

1. Introduction

In a previous study we reported a HPLC method for the separation of some naphtho[2,3-*b*]furan-4,9-diones occurring in the inner bark of *Tabebuia avellanedae* (Bignoniaceae) [1]. Preparations of this inner bark are used as adjuvants in cancer therapy in South America and in the USA.

However, while the inner bark is only a small part of a trunk the main part (80–85%) is the heartwood. The heartwood of *T. avellanedae* was thoroughly investigated by Burnett and Thomson [2]. They identified seven naphthoquinones, nine anthraquinones and a pyranonaphthalene derivative amongst other substances. Their investigations included extraction of the finely ground heartwood with various organic solvents, followed by preparative chromatographic techniques and identification of the pure compounds by means of melting point, infrared spectroscopy and elemental analysis.

The quinone pattern of the heartwood differs from that of the inner bark. Thus, lapachol is a major constituent of the heartwood and a minor constituent of the inner bark; anthraquinones only occur in the heartwood while furanonaphthoquinones only occur in the inner bark.

This study reports a HPLC method, which separates fourteen compounds known as constituents of the heartwood of *T. avellanedae* (Table 1). Their chromatographic behaviour was also compared to that of some furanonaphthoquinones known from the inner bark, in order to see whether it is possible to distinguish the

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Table 1
Numbering and names of the investigated compounds (anthraquinone = anthracene-9,10-dione; furanonaphthoquinone = naphtho[2,3-*b*]furan-4,9-dione)

Number	Name
1	Lapachol
2	Dehydro-α-lapachon [3]
3	α-Lapachone [4]
4	β-Lapachone [4]
5	Lapacholmethylether [5]
6	Menaquinone-1 [5]
7	Desoxylapachol [5]
8	1-Hydroxyanthraquinone [6]
9	1-Methoxyanthraquinone [7]
10	2-Methylanthraquinone
11	2-Hydroxymethylanthraquinone
12	2-Acetoxymethylantraquinone [8]
13	Anthraquinone-2-carboxylic acid
14	Lapachenol [9]
15	2-Acetyl-furanonaphthoquinone
16	2-Hydroxyethyl-furanonaphthoquinone
17	8-Hydroxy-2-acetyl-furanonaphthoquinone
18	8-Hydroxy-2-hydroxyethyl-furanonaphthoquinone
19	2-Ethyl-furanonaphthoquinone
20	2-Isopropyl-furanonaphthoquinone
21	2,3-Dihydro-2-(2-methylethenyl)-furanonaphthoquinone

Compounds **1–14** are constituents of the heartwood *T. avellanedae*; compounds **1**, **2**, **15–21** are constituents of the inner bark of *T. avellanedae*; numbers between square brackets refer to references according to which compounds were prepared.

quinone pattern of the heartwood from that of the inner bark.

2. Experimental

2.1. Apparatus

A Model 2249 liquid chromatograph (LKB Pharmacia, Bromma, Sweden) was used, fitted with a LKB 2141 UV–Vis detector and a Rheodyne injection valve (20-μl loop), connected with a LKB 2221 integrator with a two-channel module.

2.2. Chromatography

A Spherisorb ODS-2 column (250 × 4 mm I.D.; 5 μm particle size; Grom, Herrenberg, Germany) was used. The mobile phases consisted of water–methanol–methyl-*tert.*-butyl ether mixtures in various ratios. Water was always acidified with phosphoric acid. The detector was set at 254 nm. The detailed chromatographic conditions are given in each figure.

2.3. Chemicals and materials

Methanol (MeOH), acetonitrile (ACN), water (all purchased from Merck, Darmstadt, Germany), methyl-*tert.*-butyl ether (MTBE; from Fluka, Neu-Ulm, Germany) were of HPLC-grade. Acetone and phosphoric acid (85%) were purchased from Merck and were of analytical grade.

Lapachol (**1**) and anthraquinone-2-carboxylic acid (**13**) were purchased from Aldrich (Steinheim, Germany), 2-hydroxymethyl-anthraquinone (**11**) and 2-methyl-anthraquinone (**10**) were from Fluka.

The other compounds were prepared according to published procedures (see Table 1).

Stock standard solutions were prepared by dissolving compounds in methanol except for

compounds **8** and **13**, which were dissolved in MeOH–acetone (4:1, v/v). Working standard solutions (5–50 μg/ml) were obtained by dilution with MeOH.

3. Results and discussion

Fig. 1 shows the separation of the compounds **1–14** using a mixture of MeOH, diluted H_3PO_4 and MTBE as eluent. The separation was achieved in about 20 min. The isomers **1**, **3** and **4** were completely resolved. Until now they could only be separated on a chiral phase [10]. For their separation on a reversed-phase (RP) column it is necessary to use mobile phases containing an ether, e.g. MTBE or THF. Only **7** + **8** and **9** + **2** were not completely resolved under these isocratic conditions.

The resolution could be improved by using a MeOH–H_2O–H_3PO_4–MTBE gradient (Fig. 2A). Moreover, the peaks eluting last were sharper than those obtained using isocratic conditions.

It was important to use an ODS-column which was "endcapped", because otherwise the peaks of the hydroxyquinones **1** and **8** tended to tail even by using acidified eluents. This might be due to counteractions of the hydroxy group neighbouring the quinone system with the free silanol groups of the modified silica gel in the stationary phase (especially if it has a basic surface like the Spherisorb silica).

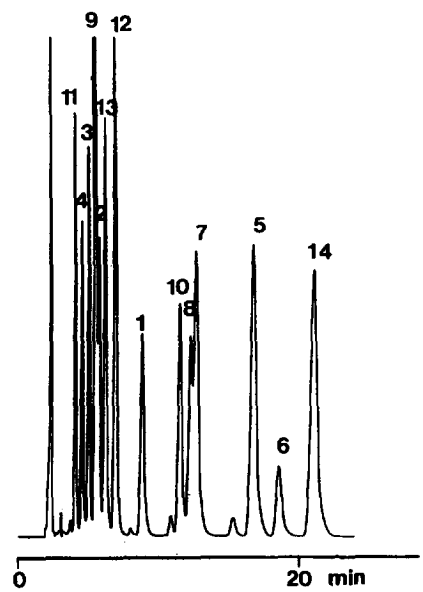

Fig. 1. HPLC separation of compounds **1–14** (1.2 ml/min MeOH–H_2O–H_3PO_4–MTBE, 65:30:0.1:5, v/v); 0.10 AUFS.

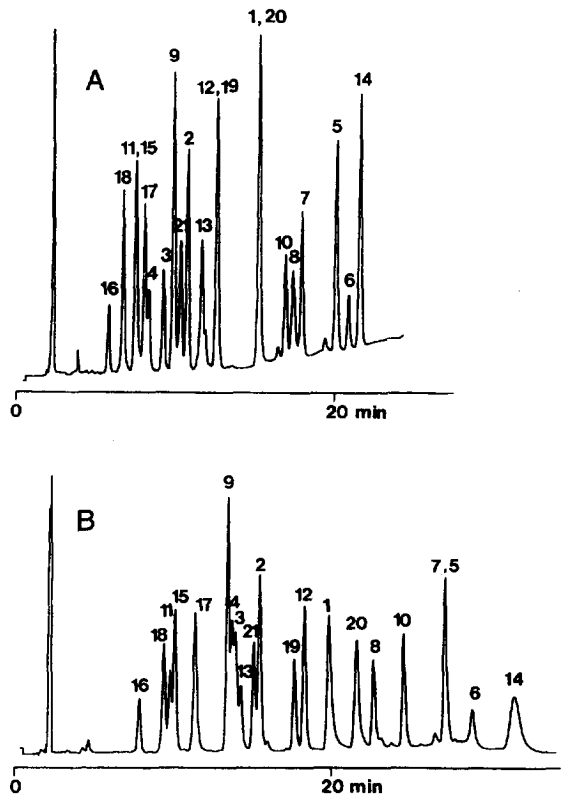

Fig. 2. HPLC separation of compounds **1–21** with gradient elution. (A) Conditions optimized for separation of quinones from the heartwood. Mobile phase A: MeOH; B: MeOH–H_2O–H_3PO_4–MTBE (65:30:0.1:5, v/v); C: H_2O. Gradient: 75% B and 25% C for 5 min, then in 25 min to 50% A and 50% B with linear increase and holding for 5 min. Flow: 1.2 ml/min; 0.10 AUFS. (B) Conditions optimized for separation of quinones from the inner bark. Gradient starting with 1.3 ml/min MeOH–ACN–H_2O–H_3PO_4 (25:20:55:0.1, v/v) for 5 min, then to MeOH–ACN–H_2O–H_3PO_4 (25:45:20:0.1, v/v) in 25 min with linear increase; 0.10 AUFS.

Calibration graphs for each compound were constructed by plotting concentration vs. peak area. Good linearities were achieved in the range from 0.1 to 27 mg/l with correlation coefficients between 0.990 and 0.999. The linearities are

suitable for the expected quinone content (about 20–200 μg/g for compounds **2–14** according to Ref. [2]) in combination with the method proposed for extraction and sample clean-up [1]. The dual λ-detection is useful as a simple means for peak identification. We found that peak-area ratios ($A_{280\ nm}/A_{254\ nm}$) differed over a wide range: from 0.16 (**4**) to 0.96 (**6**).

In a previous study we reported the HPLC separation of seven furanonaphthoquinones (FNQs) together with **1** and **2**. Separation of these compounds was achieved with MeOH–ACN–H_2O–H_3PO_4 mixtures [1]. In order to compare the chromatographic behaviour of these FNQs (**15–21**; Table 1) with that of the quinones from the heartwood (**1–14**) mixtures of all 21 compounds were subjected to HPLC analysis with gradient elution (Fig. 2). It is obvious that almost all peaks are resolved. Under conditions optimized for the separation of quinones from the heartwood, **11** + **15**, **12** + **19**, and **1** + **20** were coeluting (Fig. 2A). Resolution was better under gradient conditions optimized for quinones from the inner bark: only **7** + **5** were coeluting and **3** + **4** were hardly resolved (Fig. 2B).

Furthermore, the FNQs seem to be more polar than the naphthoquinones and anthraquinones: **15–18** and **21** (from the inner bark) appear in the first part of the chromatograms while **5–7** and **14** (from heartwood) appear at the end. Compounds **1** and **2** (constituents of both the heartwood and the inner bark) are eluted in the middle of the chromatograms.

The higher polarity of the FNQs might be attributed to their higher oxygen content compared with that of the naphthoquinones and anthraquinones. The average oxygen content of **15–21** is 25.2%, whereas **1–14** contain 18.8% oxygen. It is known that oxygen from air diffuses through the bark and oxidation of quinonoid systems may occur [11]. This results in a different quinone pattern in the inner bark and in the heartwood, respectively, which is distinguishable by HPLC.

4. Conclusions

Fourteen quinonoid compounds, known as constituents of the heartwood of *T. avellanedae* could be separated on a RP column with MeOH–H_2O–H_3PO_4–MTBE eluents. In order to separate the isomers α- and β-lapachone the mobile phase had to contain an ether (e.g. MTBE). The chromatographic behaviour of these compounds differed from that of FNQs known from the inner bark of *T. avellanedae*. Therefore both gradient elutions permit rapid screening for most of the quinonoid constituents of *T. avellanedae*.

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