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Note

Tandem high-performance liquid chromatography methods for resolution of lapachol and related naphthaquinones

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Due to a lack of confidence in the taxonomic designation of herbal materials[1], as well as concern about the toxicity of certain potential naphthaquinone constituents in material marketed as Taheebo and Pau d'Arco, the need of a simple rapid procedure for screening plant extracts of such materials was perceived.

Consequently, an high-performance liquid chromatographic (HPLC) method was sought which could effect separation of: lapachol (I), the naphthaquinone found in several species of the Bignoniaceae botanical family and which has been of chief interest to investigators on account of its oncolytic activity[2]; β-lapachone (II), an isomer of lapachol, possessing antineoplastic and antibacterial[3], as well as antiviral[4] activity, but reportedly twenty times more toxic than its parent phenol[5]; α-lapachone (III), a positional isomer of II, which in the presence of light and air is readily converted to dehydro-α-lapachone (IV)[6,7]; IV and its positional isomer, dehydro-β-lapachone (V). The system to be developed would also be capable of resolving isolapachol (VI), the fully conjugated isomeric phenol, readily synthesized[8], and the synthetic precursor of the dehydrolapachones, as well as a potential —but as yet unobserved— constituent of extractives of natural material.

Compounds I–IV and three other naphthaquinone derivatives of lapachol (I), as well as nine additional anthraquinones, have been isolated from the heartwood of *Tabebuia avellanedae*[9], by a careful and tedious combination of column and thin-layer chromatography. Also, compounds I–IV have been isolated by similar methods from *Tabebuia chrysantha* heartwood and veneer of *Tabebuia donnell-smithi*, and I, its methyl ether and II, from the heartwood of *Paratecoma peroba*[10].

The presence of I and/or its related naphthaquinones in material from South American forests may reasonably be regarded as indicative of the Bignoniaceae family —which includes the genera *Tabebuia, Tecoma* and *Paratecoma*— since the only other genera where such presence has been confirmed are *Tectona* (teak)[11] and *Avicennia* (mangrove)[12] of the Verbenaceae family; the former occurs in the Philippines, Southeast Asia, and Indomalaysia, whereas the latter thrives in the saline intertidal zones of sheltered coastlines in tropical areas.

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EXPERIMENTAL

An SP8700 solvent delivery system (Spectra-Physics, Santa Clara, CA, U.S.A.) and a Valco injector (Valco, Houston, TX, U.S.A.) equipped with a 20-μl loop, were used for all HPLC determinations, in conjunction with an LKB 2140 rapid spectral (diode array) detector operating over the range 190–370 nm, and Wavescan (Version 1.04) software (LKB-Produkter, Bromma, Sweden). An IBM XT computer (IBM, Wallingford, CT, U.S.A.) interfaced to a Canon A1210 color printer (Canon Canada, Mississauga, Canada), was used for data collection and analysis.

Solvents

Acetonitrile and "hexanes" (95% *n*-hexane) were HPLC-grade, and glacial acetic acid was Baker-analyzed (J. T. Baker, Phillipsburg, NJ, U.S.A.). Isopropyl alcohol was of distilled-in-glass quality from Caledon Labs. (Georgetown, Canada).

HPLC system A

A reversed-phase Brownlee RP-18 Spheri-10 column (10 μm, 25 cm × 4.6 mm I.D., Brownlee Labs., Santa Clara, CA, U.S.A.) was used with a mobile phase consisting of acetonitrile–0.25% aqueous acetic acid (1:1). The flow-rate was 2 ml/min (800 p.s.i.), at ambient temperature.

Similar results were obtained with a Waters μBondapak C_{18} reversed-Phase column (30 cm × 3.9 mm I.D., Waters, Milford, MA, U.S.A.).

Measured at 254 nm, the limits of detectability for compounds I, II, III and IV are, respectively, 100, 15, 15 and 25 ng; isolapachol (VI) measured at 268 nm has a limit of detectability of 200 ng.

HPLC system B

A Pirkle Covalent Leucine chiral column (5 μm, 25 cm × 4.6 mm I.D., Regis Chemical, Morton Grove, IL, U.S.A.) was used with a mobile phase consisting of "hexanes"–isopropyl alcohol (70:30), isocratically; the gradient system ranged from 90:10 to 50:50, linearly, over 10 min. The flow-rate was 1 ml/min in both isocratic (600 p.s.i.) and gradient (450–880 p.s.i.) modes.

Measured at 254 nm, the limits of detectability for compounds II, III and IV are 5, 10 and 5 ng, respectively.

Naphthaquinones

Isolapachol[8], α-lapachone[6,7], β-lapachone[13], dehydro-α-lapachone[14], and dehydro-β-lapachone[14] were all prepared according to standard published methods. Lapachol was obtained from the Aldrich (Milwaukee, WI, U.S.A.) and its purity checked by HPLC prior to synthetic use. Each sample for HPLC analysis was dissolved in the appropriate mobile phase prior to injection.

RESULTS AND DISCUSSION

The first of two HPLC procedures, herein reported, permits a rapid screening for lapachol, isolapachol and lapachones (Fig. 1). A characteristic separation is shown in the chromatogram illustrated in Fig. 2, which accounts for all compounds

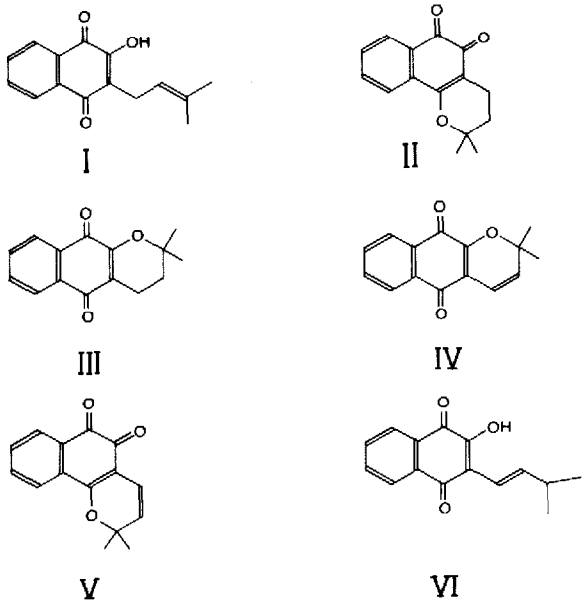

Fig. 1. Structural formulea of the compounds investigated. I = Lapachol; II = β-lapachone; III = α-lapachone; IV = dehydro-α-lapachone; V = dehydro-β-lapachone; VI = isolapachol.

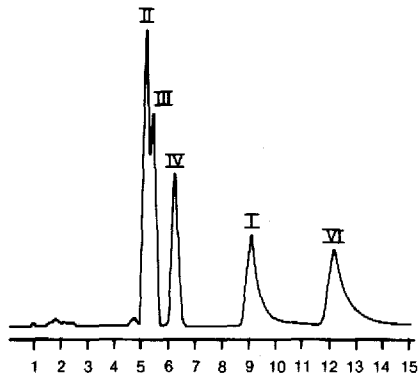

Fig. 2. HPLC system A chromatogram. Peaks: II = β-lapachone (1.6 μg); III = α-lapachone (1.0 μg); IV = dehydro-α-lapachone (1.5 μg); I = lapachol (1.7 μg); VI = isolapachol (3.1 μg).

of interest but dehydro-β-lapachone (V)*, which is coeluted with β-lapachone under these conditions. The resolution of peaks due to α- and β-lapachones is invariably achieved with mixtures of comparable proportions of these two isomers, but significant departure from equality (approaching 3:1 ratio) makes visual discrimination difficult. However, examination of the UV profile of mixtures of α- and β-lapachone allows visual detection of α-lapachone content above roughly 5%; the presence of β-lapachone associated with a predominance of α-lapachone is less readily appreci-

* Dehydro-β-lapachone (V) is prone to rearrange to its more stable α-isomer (IV) making its purification rather demanding; to the best of our knowledge, V has never been isolated from nature.

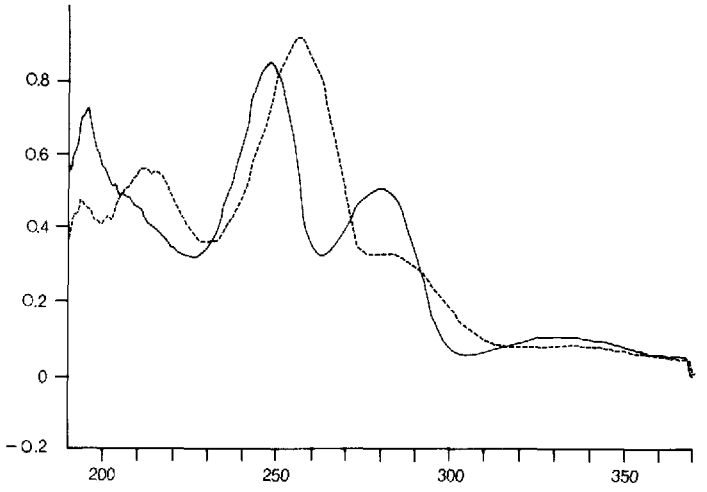

Fig. 3. UV profiles of α-lapachone (———) and β-lapachone (---------).

ated, but it may be confidently assessed from a determination of the ratio of absorbances in the vicinity of 250 and 280 nm: the α-isomer has maxima at 249 and 280 nm in the ratio 1.7, whereas the β-isomer absorbs mainly at 256 nm, with a shoulder at 280 nm, in the ratio 2.8, as shown in Fig. 3.

The isomeric lapachones and dehydrolapachones are completely resolved by the companion HPLC procedure, utilizing a Pirkle chiral column which retains the quinols, lapachol and isolapachol. Fig. 4 illustrates a representative chromatogram of the resolution of these four components; the separation between α- and dehydro-α-lapachones (III and IV) is improved in the gradient mobile phase mode. This latter system (B) would be employed after preliminary screening with HPLC system A for confirmation of α- and β-lapachones and/or semi-quantitative determinations.

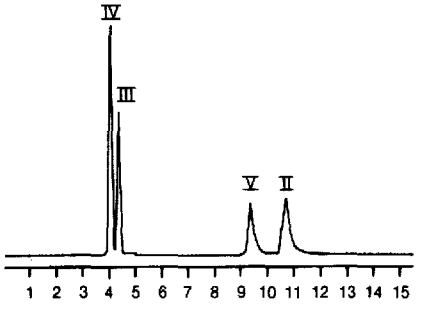

Fig. 4. HPLC system B chromatogram. Peaks: IV = dehydro-α-lapachone; III = α-lapachone; V = dehydro-β-lapachone; II = β-lapachone.

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