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Note

High-performance liquid chromatography of some naturally occurring naphthoquinones

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Naphthoquinones are important constituents of plant families such as Plumbaginaceae, Juglandaceae and Ebenaceae, the last family including many commercially important tropical hardwood species. Many of the naphthoquinones isolated have interesting biological activities and are also used as dyestuffs[1].

During our investigations into the molluscicidal and fungicidal activities of naphthoquinones from African medicinal plants[2] we required a suitable method for the rapid screening of plant extracts for their naphthoquinone content. Although a brief report on the separation of quinones by high-performance liquid chromatography (HPLC) has appeared[3], to our knowledge, no HPLC of naturally occurring non-isoprenoid naphthoquinones has been published. Most analytical work on naphthoquinones has been confined to prenylquinones and K vitamins[4]. These isoprenoid naphthoquinones are best resolved by reversed-phase HPLC, using aqueous methanol mobile phases[4]. However, non-isoprenoid naphthoquinones require a high water content for separation by reversed-phase HPLC with methanol–water systems[5]. Xanthones have been successfully separated by CN bonded phase columns[6] and here we extend the method to the separation of a series of simple plant-derived naphthoquinones (Fig. 1).

EXPERIMENTAL

Materials

n-Hexane was of LiChrosolv grade (Merck, Darmstadt, F.R.G.) and acetic acid (Merck) was of analytical purity. Juglone and plumbagin were purchased from Roth (Karlsruhe, F.R.G.) and lawsone from Fluka (Buchs, Switzerland). 7-Methyljuglone, mamegakinone and isodiospyrin were isolated from the root bark of *Diospyros usambarensis*, as previously described[2]. Samples were dissolved in chloroform to give a concentration of 0.1 mg/ml.

Apparatus

The liquid chromatograph consisted of a Waters solvent-delivery system with Model U6K injector, coupled to a Waters Model 720 system controller and Model 730 data module. A Waters μBondapak CN column (3.9 mm I.D. × 30 cm), particle

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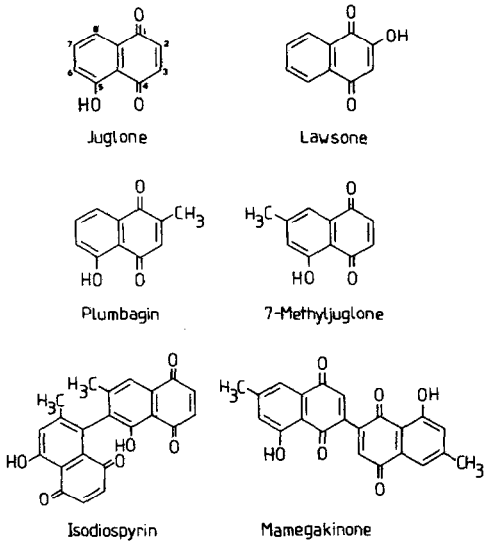

Fig. 1. Structures of naphthoquinones.

size 10 μm, was used for the separations, with UV detection at 254 nm carried out by a Waters Model 480LC spectrophotometer.

RESULTS AND DISCUSSION

Although the HPLC analysis of xanthones using Varian Micropak CN columns was very successful with *n*-hexane–chloroform as the mobile phase, separations of naphthoquinones on CN chemically bonded phases using *n*-hexane–chloroform and *n*-hexane–isopropanol mixtures gave rise to considerable tailing. Consequently, *n*-hexane with a trace of acetic acid was employed as the mobile phase.

The separation of the two pairs of isomers plumbagin and 7-methyljuglone, mamegakinone and isodiospyrin is shown in Fig. 2. The monomers are eluted much more rapidly than the dimers, providing an ideal means of distinction, especially as 7-methyljuglone and its dimers often occur in the same plant and thin-layer chromatographic separation is sometimes difficult. In the Malawi medicinal plant *Diospyros usambarensis* (Ebenaceae), for example, both 7-methyljuglone and the dimeric naphthoquinones mamegakinone and isodiospyrin are present in a ligroin extract of the root bark (Fig. 3). 7-Methyljuglone, the active molluscicidal principle of *D. usambarensis*[2], is by far the most predominant naphthoquinone (0.11% dry weight), but the other naphthoquinones can easily be identified. HPLC analysis of ligroin extracts from *D. usambarensis* and *D. lotus* ripe berries showed the presence of isodiospyrin and the total absence of 7-methyljuglone.

The same solvent system (1% acetic acid in *n*-hexane) gives a good separation of the six naphthoquinones as shown in Fig. 4. Notable is the separation of the two isomers juglone (peak 3) and lawsone (peak 4). Simply changing the hydroxyl group from position 5 in juglone to position 2 in lawsone gives a dramatic increase in retention time. Consequently, this isocratic analytical HPLC system would seem suit-

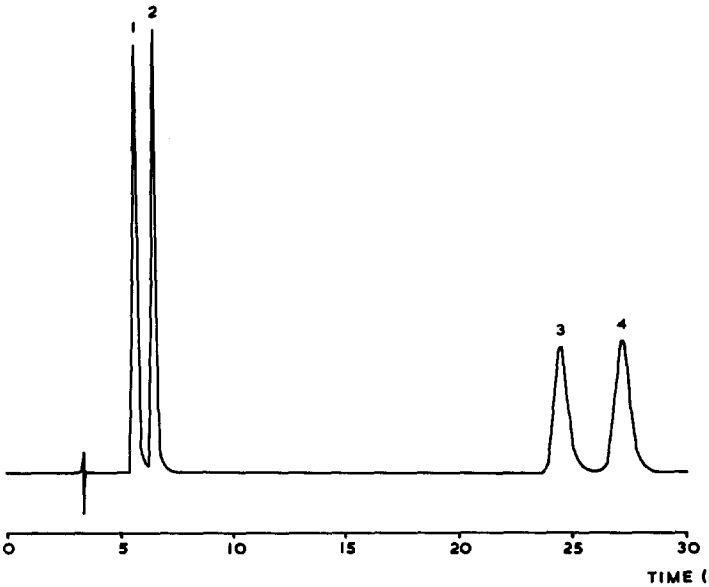

Fig. 2. Separation of the pairs of naphthoquinone isomers plumbagin (1), 7-methyljuglone (2) and mamegakinone (3), isodiospyrin (4) on a μBondapak CN column. UV detection at 254 nm. Solvent: 1% acetic acid in *n*-hexane. Flow-rate: 1 ml/min. Sample: 5 μl (*ca.* 0.5 μg of each compound).

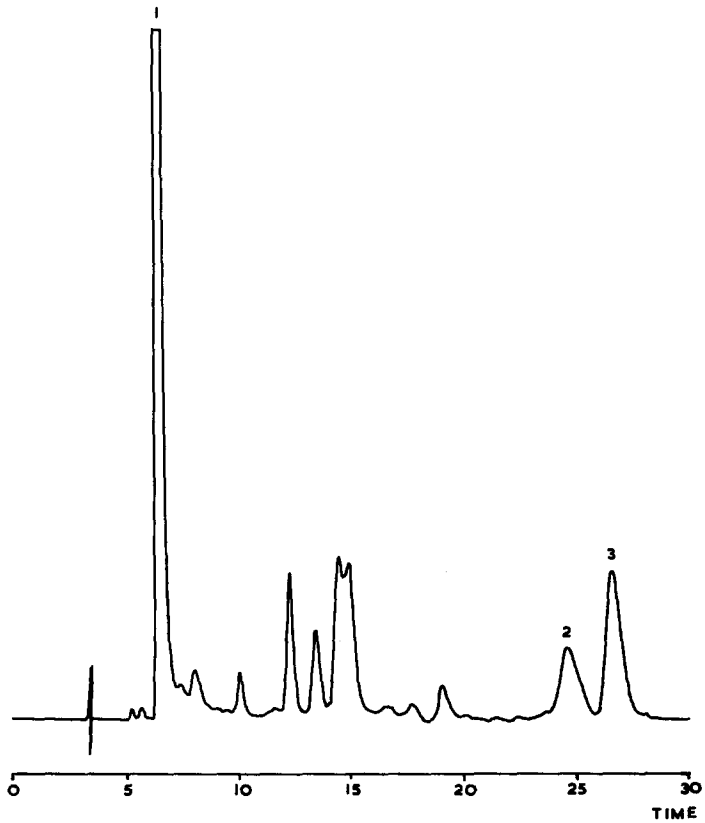

Fig. 3. HPLC of a *Diospyros usambarensis* ligroin extract on a μBondapak CN column. Conditions as Fig. 2. Sample: 5 μl of a solution of a 1 mg/ml extract in chloroform. Peaks: 1 = 7-methyljuglone; 2 = mamegakinone; 3 = isodiospyrin.

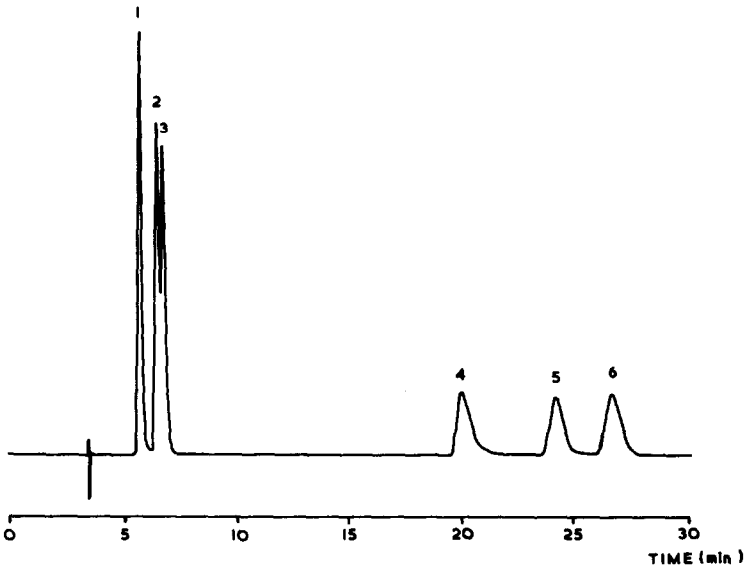

Fig. 4. HPLC separation of naphthoquinones on a μBondapak CN column. Conditions as Fig. 2. Peaks: 1 = plumbagin; 2 = 7-methyljuglone; 3 = juglone; 4 = lawsone; 5 = mamegakinone; 6 = isodiospyrin.

able for the rapid monitoring of naphthoquinones from plant sources. Screening of plant extracts for molluscicidal naphthoquinones is carried out by us as an important part of our research programmes for the control of schistosomiasis[2].

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