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Note

Straight-phase and reversed-phase high-performance liquid chromatographic separations of valepotriate isomers and homologues

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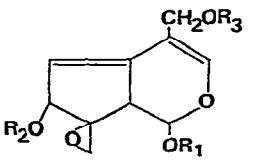
Valepotriates are ester iridoids discovered by Thies and Funke[1], and found so far exclusively in various species of the Valerianaceae. The valepotriates can be divided into two classes: monoene and diene valepotriates. Table I shows the valepotriates discussed in this paper.

TABLE I

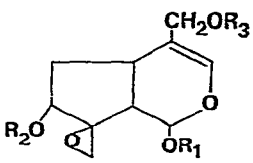
VALEPOTRIATE STRUCTURES

Diene valepotriates



	R_1	R_2	R_3
Valtrate	Iv	Iv	Ac
Isovaltrate	Iv	Ac	Iv
Homovaltrate I	Iv	Ic	Ac
Homovaltrate II	Ic	Ic	Ac
Acevaltrate	Aciv	Iv	Ac
or	Iv	Aciv	Ac
Homo acevaltrate	Acic	Iv	Ac
or	Iv	Acic	Ac

Monoene valepotriates



	R_1	R_2	R_3
Didrovaltrate	Iv	Ac	Iv
Homodidrovaltrate	Ic	Ac	Iv

Iv - Isovaleryl
Ic - Isocapryl
Ac - Acetyl
Aciv - Acetoxy-isovaleryl
Acic - Acetoxy-isocapryl

According to pharmacological[2,3] and especially clinical[4,5] experiments, these valepotriates are considered to be active constituents in the sedative effect of valerian preparations. However, none of these investigations was carried out with single or highly purified valepotriates. A thin-layer chromatographic (TLC) system developed

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by Stahl and Schild[6] has been widely used for the separation of some major occurring valepotriates (valtrate, didrovaltrate and acevaltrate). Recently a straight-phase high-performance liquid chromatographic (HPLC) system was described for the separation of these valepotriates[7].

Extensive chromatographic work in our laboratory has shown that naturally occurring valepotriates accumulate as complex mixtures of positional isomers and homologues[8–10]. HPLC separations using straight-phase and reversed-phase systems proved to be most appropriate for isolating and analysing valepotriate isomers and homologues. This paper reports the HPLC analysis of some of these compounds.

EXPERIMENTAL

Apparatus

The chromatograph used was a Chromatronix Model 3500 from Spectra-Physics, with an injection valve system and a 10-μl sample loop. In the straight-phase mode the stainless-steel column was 25 cm × 3.0 mm I.D., packed with 5-μm Partisil silica particles (Chrompack, Middelburg, The Netherlands). The column used in the reversed-phase mode was 25 cm × 3.0 mm I.D., packed with 5-μm Spherisorb ODS particles (Chrompack). The column outlet was connected to a Chromatronix Model 770 variable-wavelength UV detector (Spectra-Physics). The detection wavelengths were 206 nm for monoene valepotriates and 256 nm for diene valepotriates.

Solvents

n-Hexane, ethyl acetate, dichloromethane and methanol were of analytical-reagent grade (Merck, Darmstadt, G.F.R.) and acetonitrile was of spectrophotometric grade (Merck). The water used for the solvent system was deionized.

When using the straight-phase mode the HPLC system was operated isocratically with the binary solvent systems *n*-hexane–ethyl acetate (90:10 or 95:5) or *n*-hexane–ethanol (99.5:0.5). In the reversed-phase mode the HPLC system was operated with the binary solvent systems methanol–water (50:50) or acetonitrile–water (60:40).

Samples

Roots and rhizomes of *Valeriana officinalis* L. s.l. were collected near Utrecht, The Netherlands. Roots and rhizomes of *Centranthus angustifolius* D.C. were obtained from plants cultivated in our botanical garden in Maartensdijk, The Netherlands.

Valmane®* dragées were obtained from commercial sources.

Sample preparation and analysis

After collection, the plant material was immediately stored at −20°C and then freeze-dried. Extraction of the plant material was carried out by homogenizing 1.0 g of freeze-dried plant material with 25 ml of dichloromethane for 5 min. The homogenate was filtered through a filter-paper and the precipitate on the filter was washed with two 10-ml portions of dichloromethane. After drying over anhydrous sodium sulphate and evaporation of the solvent carefully below 30°C, by means of rotating distillation, the residue was dissolved in 1.0 ml of dichloromethane.

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* Valmane is a commercial product originating from *Valeriana wallichii* D.C. roots.

The same procedure was carried out for the extraction of one Valmane dragée, the extract residue being dissolved in 5.0 ml of dichloromethane.

A 10-μl portion of each filtered extract solution was injected into a prepared chromatograph.

Reference compounds

Valtrate, didrovaltrate and acevaltrate were obtained from Kali-Chemie (Hannover, G.F.R.). Isovaltrate, homovaltrate I, homovaltrate II, homodidrovaltrate and homoacevaltrate were isolated and identified in our laboratory[10,11]. The structural elucidation of these compounds will be published elsewhere[10].

RESULTS AND DISCUSSION

From the basic work on valepotriates carried out by Thies and Funke[1] and Thies[12,13], it became clear that different positional isomers and homologues co-occur in genuine mixtures, extracted from Valerianaceae species. Using the time-consuming open-column chromatography, Thies[12] succeeded in the isolation and structural elucidation of several valepotriates. HPLC, however, provides the possibility of the rapid and complete separation of these compounds. Especially by using columns packed with 5-μm particles, we have been able to separate valepotriate isomers and homologues.

Analytical separation of diene valepotriates

Diene valepotriates (see Table I) show an intense absorption maximum at 256 nm, due to their two conjugated double bonds, and can be detected with high sensitivity at this wavelength. Even a wavelength of 280 nm can be used for the detection. They can be separated by straight-phase HPLC using Partisil 5 silica columns and *n*-hexane–ethyl acetate solvent systems, as illustrated in Figs. 1–3.

Fig. 1 shows the separation between isovaltrate (peak 1) and valtrate (peak 2),

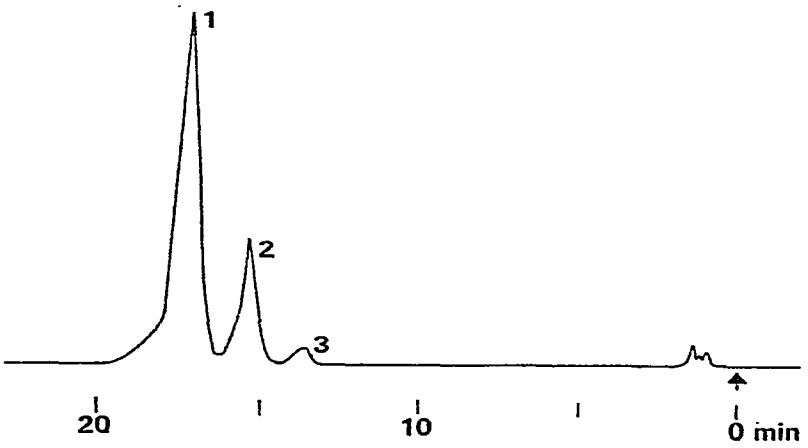

Fig. 1. Straight-phase HPLC separation of the "valtrate" fraction from *Valeriana officinalis* L. s.l. Partisil 5 silica column, 25 cm × 3.0 mm I.D.; solvent system, *n*-hexane–ethyl acetate (95:5); flow-rate, 1.0 ml/min; detection at 256 nm; sensitivity at 0.2 a.u.f.s. Peaks: 1 = isovaltrate; 2 = valtrate; 3 = homovaltrate I.

indicating that isovaltrate is the major valepotriate component in *Valeriana officinalis* L. s.l. Owing to inadequate TLC separations in the past[6], it was wrongly concluded that valtrate is the major valepotriate component in this species.

Fig. 2 displays the composition of a fraction from Valmane that, according to TLC data[6], was called "valtrate". It shows that this fraction is in fact a mixture of at least four components, which could be identified as valepotriates: isovaltrate, valtrate, homovaltrate I and homovaltrate II.

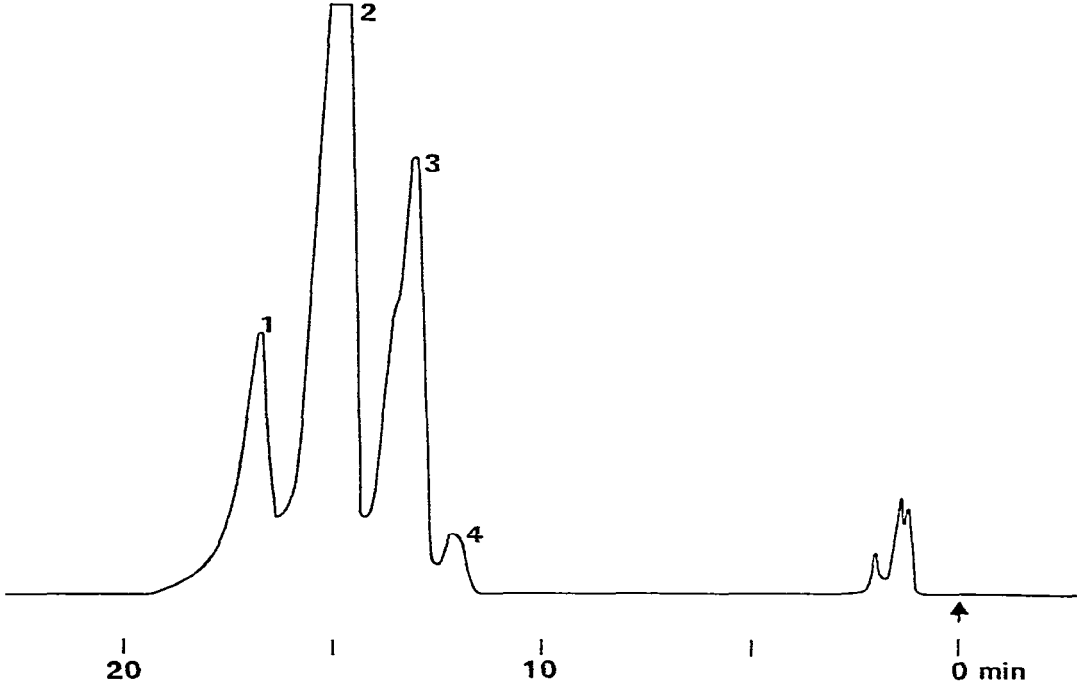

Fig. 2. Straight-phase HPLC separation of the "valtrate" fraction from Valmane. Partisil 5 silica column, 25 cm × 3.0 mm I.D.; solvent system, *n*-hexane–ethyl acetate (95:5 flow-rate); 1.0 ml/column, 25 cm × 3.0 mm I.D.; solvent system, *n*-hexane–ethyl acetate (95:5); flow-rate, 1.0 ml/min; detection at 256 nm; sensitivity at 0.2 a.u.f.s. Peaks: 1 = isovaltrate; 2 = valtrate; 3 = homovaltrate I; 4 = homovaltrate II.

Fig. 3 shows the straight-phase separation of the diene valepotriates from *Centranthus angustifolius* D.C., containing valtrate (peak 3) and homovaltrate I (peak 4) as major components.

By using reversed-phase HPLC, the separation of the valtrate homologues in particular could be improved tremendously in comparison with straight-phase HPLC. Using a Partisil 5 ODS column and water–methanol or water–acetonitrile solvent mixtures, excellent baseline separations (Fig. 4) between valtrate (peak 3), homovaltrate I (peak 4) and homovaltrate II (peak 5) could be achieved.

Acevaltrate and homoacevaltrate, also present in appreciable amounts in *Centranthus angustifolius* D.C., can be well separated by both straight-phase and reversed-phase HPLC systems (Figs. 3 and 4).

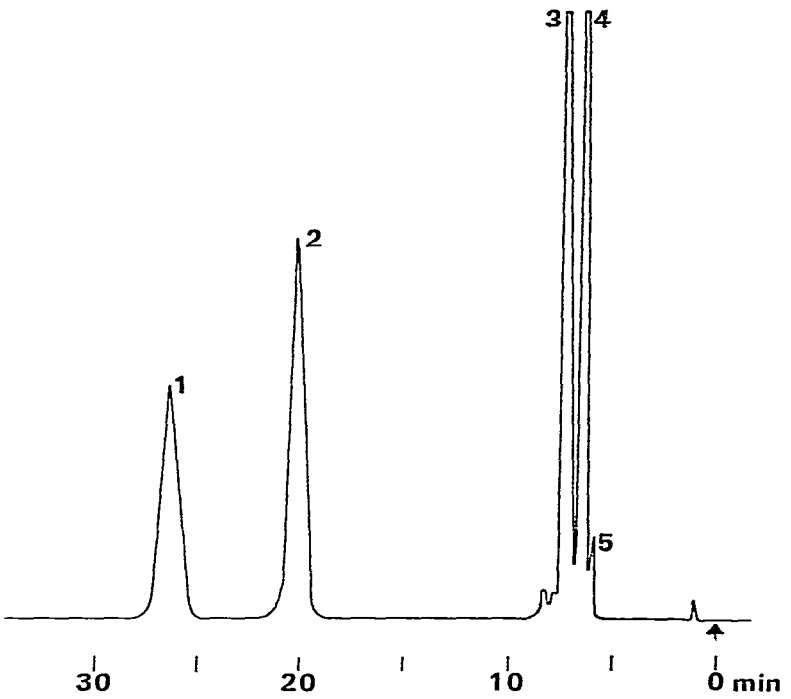

Fig. 3. Straight-phase HPLC separation of the diene valepotriate fraction from *Centranthus angustifolius* D.C. Partisil 5 silica column, 25 cm × 3.0 mm I.D.; solvent system, *n*-hexane–ethyl acetate (90:10); flow-rate, 1.0 ml/min; detection at 256 nm; sensitivity at 0.2 a.u.f.s. Peaks: 1 = acevaltrate; 2 = homoacevaltrate; 3 = valtrate; 4 = homovaltrate I; 5 = homovaltrate II.

Analytical separation of monoene valepotriates

The monoene valepotriates didrovaltrate and homodidrovaltrate (see Table I), which have a single double bond, can be detected spectrophotometrically only below 220 nm. Preferably they are detected at about 206 nm, where they show their absorption maximum. In relation to their UV detection limits, the number of solvent systems that can be applied for the separation of these compounds is restricted. However, with solvent systems consisting of *n*-hexane–isopropanol or *n*-hexane–ethanol mixtures and Partisil 5 silica columns, didrovaltrate and homodidrovaltrate could be separated. Fig. 5 shows the separation of these compounds in the relative concentrations found in Valmane samples.

As with the valtrate homologues, the separation of these didrovaltrate homologues could also be improved tremendously by using reversed-phase HPLC systems (Fig. 6).

The examples discussed show that straight-phase HPLC can be used for the separation of both valepotriate isomers and homologues. Reversed-phase HPLC, however, seems to be the method of choice for the separation of valtrate and didrovaltrate homologues in particular.

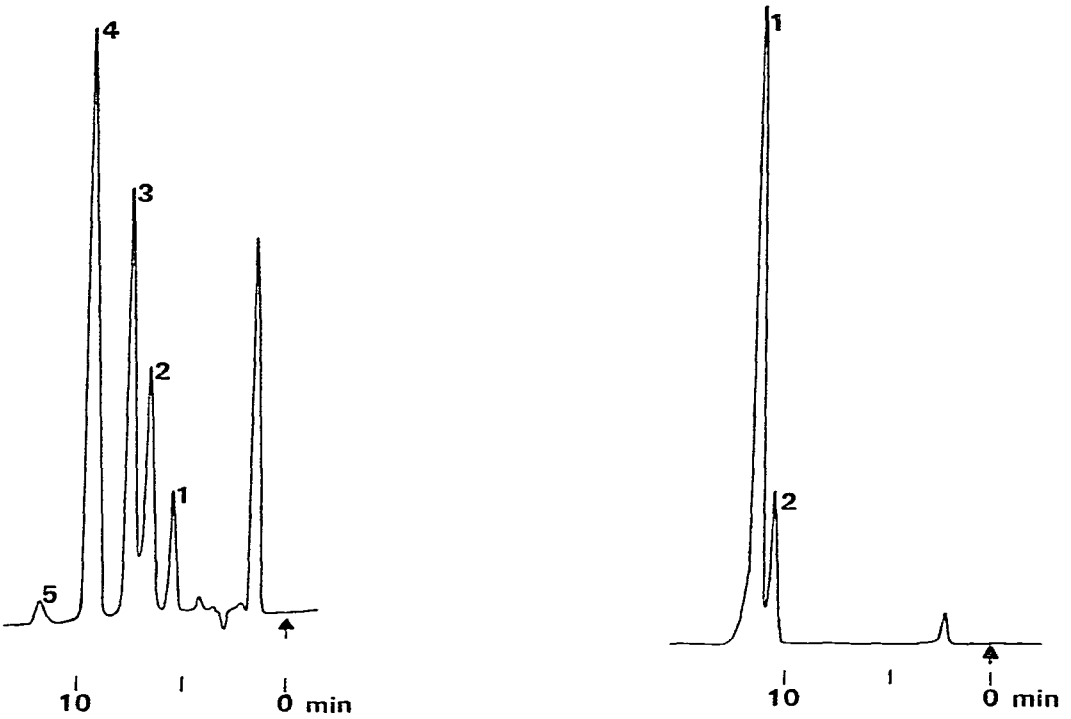

Fig. 4. Reversed-phase HPLC separation of the diene valepotriate fraction from *Centranthus angustifolius* D.C. Spherisorb 5 ODS column, 25 cm × 3.0 mm I.D.; solvent system, water–acetonitrile (40:60); flow-rate, 1.0 ml/min; detection at 256 nm; sensitivity at 0.4 a.u.f.s. Peaks: 1 = acevaltrate; 2 = homoacevaltrate; 3 = valtrate; 4 = homovaltrate I; 5 = homovaltrate II.

Fig. 5. Straight-phase HPLC separation of the "didrovaltrate" fraction from Valmane. Partisil 5 silica column, 25 cm × 3.0 mm I.D.; solvent system, *n*-hexane–ethanol (99.5:0.5); flow-rate, 1.0 ml/min; detection at 206 nm; sensitivity at 0.4 a.u.f.s. Peaks: 1 = didrovaltrate; 2 = homodidrovaltrate.

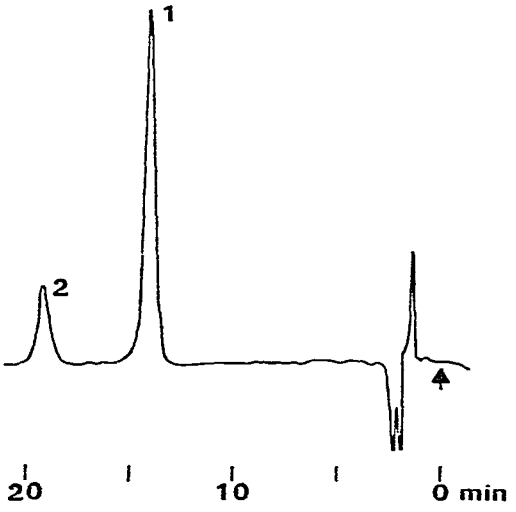

Fig. 6. Reversed-phase HPLC separation of the "didrovaltrate" fraction from Valmane. Spherisorb 5 ODS column, 25 cm × 3.0 mm I.D.; solvent system, methanol–water (50:50); detection at 206 nm; sensitivity at 0.4 a.u.f.s. Peaks: 1 = didrovaltrate; 2 = homodidrovaltrate.

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