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Note

Determination of the bitter constituents of the Gentiana root by high-performance liquid chromatography*

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The Gentiana has been known and used for therapeutic purposes for a long time[1], and is mentioned in most important European pharmacopoeias. It is, however, not adequately characterized for routine control, and its description in the European Pharmacopoeia is limited to the anatomy of the roots and of the powder, and assays for non-carbonizing and water-soluble substances. The bitter substances identified and isolated from different commercially available Gentiana species are listed in Table I together with the extents of their bitter taste and their concentrations in the roots[2]. Amarogentin, amaroswerin and amaropanin cause most of the bitter taste of Gentiana, although they are present in the drug only in low concentrations.

TABLE I

CONCENTRATION AND BITTER TASTE OF THE BITTER COMPOUNDS CONTAINED IN GENTIANA ROOTS

Substance	*Dilution which still has a bitter taste*	*% Contents in the roots*
Amarogentin	58,000,000	0.05–0.25
Amaroswerin	58,000,000	0.05
Amaropanin	20,000,000	0.05–0.15
Gentiopicroside	12,000	3–8
Gentiobiose	120	8–10

In addition to *Gentiana lutea*, most European pharmacopoeias mention, and therefore consider as official plants, the roots of *Gentiana pannonica* Scop., *Gentiana punctata* L. and *Gentiana purpurea* L., which grow in alpine habitats.

Because of the difficulties in determining the bitter taste by organoleptic

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assays, which give results with a relative standard deviation of ± 24%, it was necessary to design a method for the exact chemical determination of the concentration of the bitter substances. There have been many previous attempts to identify and determine the bitter substances 3–15. In the present study we analyzed exclusively the contents of amarogentin and amaropanin, the only compounds for which standards were relatively easy to find.

Gentiana lutea, the most used commercially, contains only amarogentin and gentiopicroside, the latter being responsible for only *ca.* 1% of the bitter taste. In this case, the evaluation of the bitter taste can be limited to the determination of the contents of amarogentin. To estimate the bitter taste of *Gentiana pannonica*, *Gentiana punctata* and *Gentiana purpurea* it is sufficient to determine the amounts of amarogentin and amaropanin; amaroswerin, although highly bitter, is of secondary importance because it is seldom present in large quantities.

EXPERIMENTAL AND RESULTS

Apparatus

A Perkin-Elmer Series 3 liquid chromatograph equipped with a variable-wavelength Model LC-65 T UV detector and Model 56 potentiometric recorder was used. Chromatographic column: ODS-HC SIL-X-1 (25 × 0.26 cm) (Perkin-Elmer).

Microsyringes: Hamilton Model 701 (10 μl).

Materials

Amarogentin and amaropanin standards were supplied by Professor H. Wagner of the Institut für Pharmazeutische Arzneimittellehre der Universität München (Munich, G.F.R.) Methanol for chromatography was obtained from Merck (Darmstadt, G.F.R.) and sodium phosphate for buffer solutions according to Sörensen from Carlo Erba (Milan, Italy).

Qualitative analysis

Methanol solutions of the standards were injected in the chromatograph under the conditions described in Table II; the graph of the concentration gradient of the mobile phase is shown in Fig. 1.

TABLE II

CHROMATOGRAPHIC CONDITIONS USED FOR THE SEPARATION OF THE AMAROGENTIN AND AMAROPANIN STANDARDS

Column	ODS-HC SIL-X-1, 25 × 0.26 cm (Perkin-Elmer)
Mobile phase:	
(A) methanol	10% A to 25% A in 5 min (T_1)
(B) phosphate buffer, 0.01 *M*, pH 5	25% A constant for 15 min (T_2)
Flow-rate (ml/min)	1
Temperature	60°
Wavelength (nm)	254
Attenuation	64
Recorder (mV)	10
Speed of paper (mm/min)	5

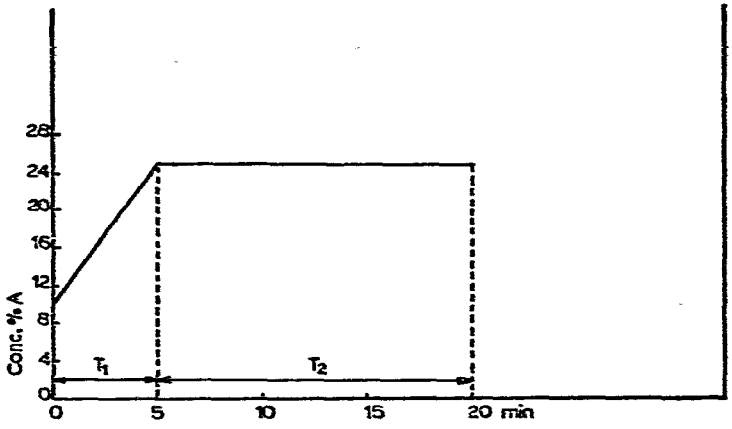

Fig. 1. Graph showing the concentration gradient of the mobile phase.

Fig. 2 shows one of the chromatograms obtained for the separation of the amarogentin and amaropanin standards under the conditions described in Table II. The time of analysis is *ca.* 18 min; the retention times and the respective chromatographic parameters are given in Table III.

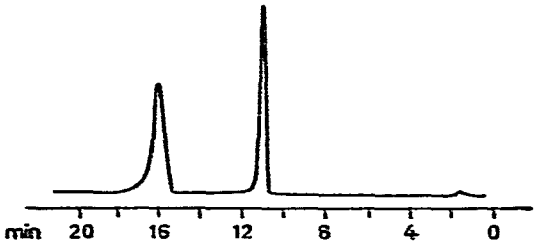

Fig. 2. Separation of the standards amarogentin (retention time = 11 min) and amaropanin (retention time = 16 min).

TABLE III

CHROMATOGRAPHIC PARAMETERS

Peak	*Retention time (min)*	*Capacity factor, k'*	*Separation factor, α*	*Theoretical plates, N*	*Effective plates, N_{eff}*	*Resolution, R_s*
Amarogentin	11	10	1.5	3951	3265	4.76
Amaropanin	16	15		2090	1837	

Quantitative determination

The standard solutions used are methanol solutions containing 0.037% amarogentin or 0.024% amaropanin. Chromatographic assays have been carried out by injecting gradually increasing quantities of these two compounds and measuring their respective peak areas.

TABLE IV

EQUATIONS OF THE CALIBRATION LINES AND CORRELATION COEFFICIENTS

x = Amount (μg) injected; y = peak area (mm^2).

Compound	*Equation*	*Correlation coefficient, r*	*Interval of calibration (μg injected)*
Amarogentin	$y = 22.4x + 0.77$	0.999	0.186–2.604
Amaropanin	$y = 93.07x + 1.12$	0.999	0.122–1.464

By plotting the peak areas against the amounts of amarogentin and amaropanin injected a straight line was obtained. Table IV gives the equations of the lines obtained with the respective correlation coefficients, r.

The repeatability of the chromatographic assay has been examined by analyzing ten injections of sample solutions. Table V lists the values obtained from the analyses of the two solutions using the calibration equations of Table IV.

TABLE V

REPEATABILITY OF THE CHROMATOGRAPHIC ANALYSES

Ten injections of every sample solution were made.

Compound	*Amount injected (μg)*	$\bar{X}$	*S.D.*	*C.V. (%)*
Amarogentin	1.116	1.122	0.018	1.60
Amaropanin	0.488	0.479	0.0017	0.35

The amounts of amarogentin and amaropanin in a dry freeze-dried extract and in a soft extract were then determined. These extracts were prepared by treating 5 g Gentiana with methanol, filtering and concentrating (at <60°), then resuspending with methanol and bringing to 25 ml in a volumetric flask. For the identification of the active compounds standards of these compounds were added to obtain higher peaks in the chromatogram. Figs. 3 and 4 show representative chromatograms obtained by injecting 10 μl of the methanol solution resulting from the extraction of the dry extract and 10 μl of the methanol solution resulting from the soft extract, respectively.

For a more correct evaluation of the peak areas of amaropanin, an extract was injected under the conditions of Table II but with an eight-fold reduction in the attenuation (Fig. 5).

Table VI gives the quantitative analytical results.

TABLE VI

QUANTITATIVE ANALYTICAL RESULTS

Compound	*Dry extract*			*Soft extract*		
	$\bar{X}$	*S.D.*	*C.V. (%)*	$\bar{X}$	*S.D.*	*C.V. (%)*
Amarogentin	0.0475	0.0013	2.83	0.1078	0.0050	4.64
Amaropanin	0.0265	0.0011	4.08	0.0417	9.79	

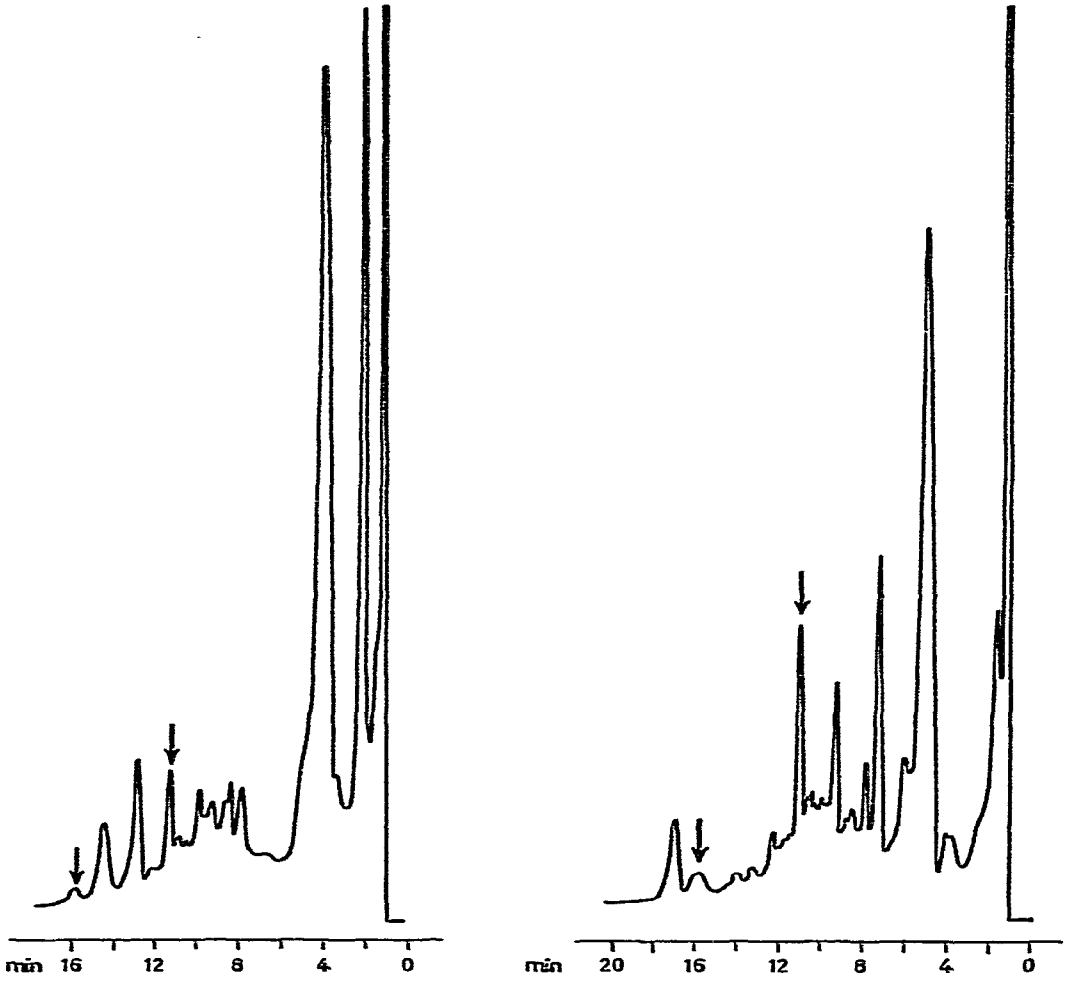

Fig. 3. Chromatogram obtained from the extraction of a dry extract of Gentiana under the conditions described in Table II. The arrows indicate the peaks resulting from the active compounds under examination.

Fig. 4. Chromatogram obtained from the extraction of a soft extract of Gentiana under the conditions described in Table II. The arrows indicate the peaks resulting from the active compounds under examination.

CONCLUSIONS

A quantitative determination of some of the active compounds that characterize the bitter taste of the Gentiana is reported. The problems in completing this study arose from the difficulty of obtaining other active compounds in Gentiana as standard. We believe, however, that a continuation of this investigation with suitable standards will lead to a better identification and determination of the active compounds of Gentiana.

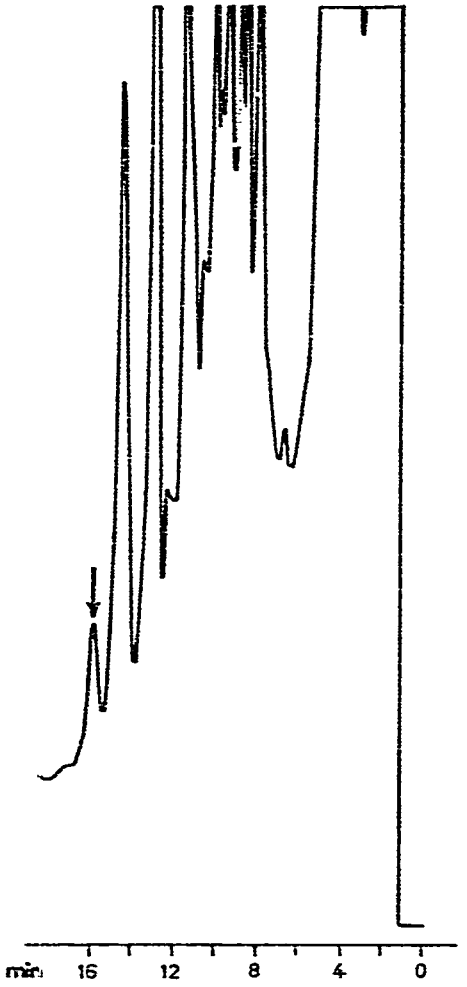

Fig. 5. Chromatogram obtained from the extraction of a Gentiana extract under the conditions described in Table II but reducing the attenuation eight-fold.

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