J Sci Food Agric 85:598–602 (2005)
DOI: 10.1002/jsfa.2011

Influence of drying mode on iridoid bitter constituent levels in gentian root

Andrée Carnat, Didier Fraisse, André-Paul Carnat, Catherine Felgines, Denis Chaud and Jean-Louis Lamaison*

Laboratoire de Pharmacognosie, Faculté de Pharmacie, Université d' Auvergne, Clermont-Ferrand, France

Abstract: Root samples of wild gentian (*Gentiana lutea* L) were harvested from six localities (altitude 970 m to 1350 m) from May to November 2000. Each batch of roots was split into three: fresh roots, naturally dried roots (ambient air) and artificially dried roots (40 °C). In all the samples, levels of iridoid bitter constituents and of xanthone coloured compounds were determined by HPLC. The mean total iridoid content in the fresh roots was 102.4 g kg^{-1} in dry matter (DM). The mean level of the principal bitter compound gentiopicroside was particularly high at 81 g kg^{-1} DM. Loganic acid, not previously reported in *G lutea*, was the second most abundant bitter compound at a mean level of 14.3 g kg^{-1} DM. Swertiamarin was present at 5.4 g kg^{-1}, with another minor unidentified iridoid. Levels of iridoid compounds were strongly dependent of the drying mode. These amounts were 88.5 g kg^{-1} DM in artificially dried roots and 62.5 g kg^{-1} DM in naturally dried roots, mostly owing to a marked decrease in gentiopicroside. The temperature of 40 °C preserved the bitter compounds and the bitterness of fresh gentian roots. The amount of coloured xanthones was relatively low at 3.3 g kg^{-1} and did not change with the drying mode.

Keywords: gentian; *Gentiana lutea*; iridoids; gentiopicroside; xanthones; fresh root; dried root; HPLC

INTRODUCTION

Gentian roots are widely used in bitter beverages, in food products and also in traditional medicine to stimulate the appetite and improve digestion.[1] The roots of wild gentian (*Gentiana lutea* L, Gentianaceae) are collected in France, principally in Auvergne in the uplands of the Cantal and Puy-de-Dôme. Gentian roots contain various characteristic compounds, mainly iridoids, its bitter constituents, and also xanthones, which give colour. Much work has been done on the bitter constituents: in the earliest studies, the main iridoids, gentiopicroside, amarogentin and swertiamarin, were isolated and quantified in the roots.[2] Other authors have assayed these components using various analytical methods, eg HPLC,[3–8] TLC densitometry,[9,10] micellar electrokinetic capillary chromatography,[11] HPLC with diode-array and mass spectrometric detection.[12] In Gentianaceae, the presence of loganic acid was reported in *Swertia caroliniensis*,[13] *G tibetica*[14] and *G septemfida*.[15] *G pedicellata* contains 4′-(4-coumaroyl) loganic acid.[16] Other derivatives of loganic acid, 7-*O*-coumaroyl loganic acid and 7-*O*-(4″-*O*-glucosyl)coumaroyl loganic acid were identified in *G linearis*.[17] To our knowledge, the presence of loganic acid in *G lutea* has not previously been described. For the coloured constituents, some data on the xanthone aglycones, gentisine and isogentisine, has been published.[18,19] Some xanthone glycosides have been studied by several authors.[20–22] A study using HPLC separated of three xanthone glycosides: gentioside, 7-hydroxy-3-methoxy-1-*O*-primeverosyl xanthone and 1-hydroxy-3-methoxy 7-hydroxy-primeverosyl xanthone.[23]

Variations of levels of each compound were related to geographical origin, stage of development and age of roots. Some authors have reported an increase in gentiopicroside levels during plant growth, followed by a decrease after flowering until dehiscence of fruits.[24,25] Other authors have reported conflicting results for gentiopicroside levels according to altitude.[26,27] Amarogentin levels varied with the ecotype from trace amounts to low percentages.[28,29] The levels of amarogentin and gentiopicroside changed inversely with stage of development and sugar accumulation in roots.[25] Results of a study carried out on cultivated plants indicated high amounts of gentiopicroside and amarogentin in the one-year roots, and a decrease in roots cultivated for 5 years.[30] In contrast, xanthones levels did not change significantly with either altitude or age of roots.[27,31] A maximum xanthone level was observed during the flowering period and a minimum during the non-vegetative period.[27]

* Correspondence to: Jean-Louis Lamaison, Laboratoire de Pharmacognosie, Faculté de Pharmacie, Université d' Auvergne, 28 place Henri Dunant, 63000 Clermont-Ferrand, France
E-mail: j-louis.lamaison@u-clermont1.fr
(*Received 25 November 2003; revised version received 11 May 2004; accepted 27 July 2004*)
Published online 26 November 2004

The aim of our study was to compare the effect of the drying method, natural (ambient air) or artificial (air flow at 40 °C), on the composition of gentian roots. As this plant is valued for its bitterness, we focused on iridoids, its main bitter constituents.

MATERIALS AND METHODS

Plant material

Wild yellow gentian is a perennial herb that grows in mountain meadows of the Massif Central (France). Roots were harvested by a collector of medicinal plants (SICARAPPAM, Aubiat, France). Batches of roots were collected from six localities of the department of Puy-de-Dôme, at altitudes ranging from 970 m to 1350 m, in the period May to November 2000: one batch in May, one in June, one in July, one in September, six in October and one in November. Each batch was divided into three parts: a fresh fraction, a fraction dried in a thin layer in ambient air in the shade (natural drying) and a fraction dried under a flow of air at 40 °C for 5 days (artificial drying). The fresh roots were analysed at the laboratory on the day they were collected.

Moisture content of the different batches varied; about 785 g kg^{-1} in fresh roots and about 85 g kg^{-1} in dried roots. The moisture content of each batch was therefore taken into account for the calculation of constituent percentages. To quantify the loss during drying, dry matter of the ground samples was measured by oven drying at about 110 °C to constant weight according to the pharmacopoeial requirements.[32]

Chemicals

Standards (gentiopicroside, swertiamarin and loganic acid) were purchased from Extrasynthese (Genay, France). Xanthone glycosides, gentisin and isogentisin were isolated as described elsewhere.[21,23] All organic solvents were of HPLC grade (Merck, Darmstadt, Germany).

Extraction procedure

Fresh roots were cut into pieces and reduced with an electric grater. Fresh root material (15 g) was refluxed with 180 ml of methanol for 30 min. After filtration, the solution was adjusted to a final volume of 200 ml in a volumetric flask. Dried roots were chopped into small fragments (about 1 cm × 1.5 cm), and pulverised in a laboratory crusher (IKA A10 type, Bioblock, Illkirch, France). The powder was sieved (mesh 0.85 mm) and 1.5 g of ground sample was refluxed with 95 ml of methanol for 30 min. After filtration, the solution was adjusted to a final volume of 100 ml in a volumetric flask. Before HPLC analysis, the samples were filtered through a 0.45-μm filter (Acrodisc GPH, Gelman, Ann Arbor, MI, USA).

HPLC analysis of iridoids and xanthones

Qualitative and quantitative HPLC analysis was carried out with an apparatus comprising two 510 pumps, a 680 solvent programmer and a 991 photodiode-array detector (Waters Associates, Milford, MA, USA). A 10-μl aliquot was injected onto a Lichrocart 125-4 Superspher RP8-E 5 μm column (Merck, Darmstadt, Germany). The mobile phase consisted of solvent A: water/phosphoric acid 85% (100:0.3 v/v) and solvent B: acetonitrile/water/phosphoric acid 85% (80:20:0.3 v/v/v). Separation was performed by linear gradient of B in A at a flow rate of 2 ml min^{-1} as follows: 0–15 min, 10–20% B; 15–30 min, 20–45% B; 30–40 min, 45–50% B; 40–45 min, 50% B. UV detection was at 239 nm.

In these conditions, standards compounds were well separated and eluted at approximate retention times (R_t): loganic acid 3.0 min, swertiamarin 5.4 min, gentiopicroside 7.3 min, 7-hydroxy-3-methoxy-1-*O*-primeverosylxanthone 21.7 min, 1-hydroxy-3-methoxy-3-*O*-primeverosylxanthone (gentioside) 23.8 min, 1-hydroxy-3-methoxy-7-*O*-prime verosylxanthone 24.5 min, gentisin 37.0 min and isogentisin 37.2 min.

The specificity of the method was verified for each iridoid and xanthone constituent with the assistance of a photodiode-array detector by comparison of their UV spectra with those of standard compounds. UV absorption maxima were at 239 nm for loganic acid and swertiamarin, at 245 and 275 nm for gentiopicroside, and at 236, 257, 308 and 374 nm for all xanthones. Detection was therefore carried out at 239 nm for all compounds. Linearity and fidelity standard deviations of iridoid compounds were <5%. The linearity correlation coefficient was higher than 0.99 (five points; three assays). All samples were run in triplicate and quantification was carried out using external standards.

The content of each compound was calculated and expressed as g kg^{-1} of dry matter.

TLC of loganic acid

TLC of loganic acid was carried out with an accepted technique.[33] The plates used were Silicagel 60F_{254} (Merck, Darmstadt, Germany). The mobile phase was as follows: ethyl acetate/methanol/water (77:15:8 v/v/v). A 10-μl aliquot of sample solution (0.5 g of powdered gentian roots extracted with 10 ml of methanol) and 10 μl of loganic acid standard solution (0.5 mg ml^{-1}) were applied comparatively on the layer. Loganic acid was visualised as a blue-violet (visible) spot at R_f 0.25 after spraying with vanillin sulfuric acid reagent.

Statistical analysis

Results were expressed as means ± SEM, and statistical analysis was performed by one-way analysis of variance (ANOVA), followed by Student–Newman–Keuls multiple comparisons test (Graph Pad Instat, San Diego, CA, USA). Values of $p < 0.05$ were considered significant.

RESULTS AND DISCUSSION

In all the samples, the principal bitter constituents, three major iridoids, and also the minor coloured constituents, three xanthone glycosides and two xanthone aglycones, were identified. The HPLC profiles of all samples presented also a relatively high peak (R_t 3.0 min; λ_{max} 239 nm) of an unidentified iridoid. This compound was co-chromatographed (TLC and HPLC) with a standard substance and was identified as loganic acid, an iridoid reported therefore for the first time in *G lutea* root. The other major bitter and coloured compounds were identified by HPLC. In all samples, gentiopicroside was always the main iridoid, loganic acid the second most abundant and swertiamarin a minor component. Another minor iridoid (R_t 7.9 min) was unidentified. Its spectrum (λ_{max} 239 nm) was the same as that of swertiamarin; quantification of this unidentified iridoid was therefore carried out by comparison with swertiamarin. Amarogentin was previously observed by Sancin *et al*[28] and by Franz and Fritz[29] but, in our work, only some extracts of fresh roots contained traces of this compound. Hence, the iridoid composition of yellow gentian root from Auvergne origin presents some peculiarities.

This comparative study concerns fresh roots and those dried (artificially and naturally). Minimum, maximum and mean levels of individual and total iridoids and of coloured xanthones are reported (Table 1).

In fresh roots, gentiopicroside represented about 76%, loganic acid about 17%, swertiamarin about 5.3%, and the other minor iridoid about 1.5% of total iridoids. A significant decrease (about 16%) was observed for gentiopicroside in artificial dried roots versus fresh ones ($p < 0.05$) and a much more marked decrease (about 43%) in natural dried roots versus fresh ones ($p < 0.001$). A significant difference was also observed between the two modes of desiccation ($p < 0.001$). Compared with fresh roots, loganic acid levels decreased by about 3.5% and about 24.5% after artificial drying and natural drying, respectively. However, this decrease was not statistically significant. Swertiamarin levels were similar in fresh and in artificially dried roots ($p > 0.05$). On the contrary, a significant decrease (about 24%) was observed in natural dried roots versus fresh roots ($p < 0.001$), and between the two modes of drying ($p < 0.001$).

Thus, total iridoid content decreased by about 13.5% after artificial drying versus fresh roots ($p < 0.05$), and about 39% after natural drying versus fresh roots ($p < 0.001$). These results show that, whatever the drying mode, the total amount of iridoids was significantly decreased ($p < 0.001$), mostly owing to wide variation of gentiopicroside levels. It has been reported that the lability of gentiopicroside required its isolation from fresh roots.[34] However, preparation of food commercial extracts is not always possible from fresh roots. During the natural drying mode, water is not rapidly removed and degradation by enzymatic hydrolysis or oxidation is possible, which might explain the losses of iridoids. The molecular formula of gentiopicroside shows a greater number of double bonds than are present in swertiamarin or loganic acid. These double bonds can be cleaved, producing structural changes. Natural drying thus greatly depressed the bitter constituent level (−39%), while artificial drying conserved most of iridoids present in fresh roots (86%).

Amounts of total xanthones (glycosides and aglycones) were also studied in terms of to the drying mode. Mean amounts of aglycones (gentisin and isogentisin) were low and relatively stable in both fresh (0.9 g kg^{-1}) and artificially or naturally dried roots (0.8 and 0.7 g kg^{-1}, respectively) (ANOVA test non-significant). Levels of xanthone glycosides were not significantly affected by drying (2.4 g kg^{-1} in fresh roots and 2.5 and 2.2 g kg^{-1}, respectively, in artificially or naturally dried roots, ANOVA test non-significant).

Total xanthones were calculated as the sum of analysed glycosides and aglycones. The ratio of aglycones to total xanthones was approximately 27, 24.5 and 24%, for fresh, artificially dried and naturally dried roots, respectively. Thus the mode of drying did not produce significant hydrolysis of xanthone glycosides to free aglycones. Previous authors have shown no significant variation of xanthone levels according to stage of growth or altitude.[31] Hence

Table 1. Effect of drying method on concentration (g kg^{-1} on dry matter) of iridoids and xanthones in gentian roots (mean values from 11 batches)[a]

	Fresh roots				Artificially dried roots				Naturally dried roots			
	Min	Max	Mean	SEM	Min	Max	Mean	SEM	Min	Max	Mean	SEM
Gentiopicroside	66.6	97.7	81.1a	3.0	52.7	90.8	67.8b	3.8	28.2	62.6	46.3c	3.6
Loganic acid	6.4	28.3	14.3	1.9	6.4	27.7	13.8	1.8	4.8	15.5	10.8	1.0
Swertiamarin	4.4	6.3	5.4a	0.2	4.8	6.2	5.5a	0.1	3.1	5.5	4.1b	0.2
Unidentified iridoid	0.8	3.4	1.7	0.2	0.4	3.2	1.3	0.2	0.4	2.3	1.2	0.2
Total iridoids[b]	81.2	122.6	102.4a	3.5	67.2	113.7	88.5b	4.5	43.8	82.1	62.5c	3.8
Xanthone glycosides	1.6	3.1	2.4	0.2	1.2	3.8	2.5	0.2	1.6	3.4	2.2	0.1
Xanthone aglycones	0.6	1.8	0.9	0.1	0.3	1.6	0.8	0.1	0.3	1.6	0.7	0.1
Total xanthones[c]	2.1	4.5	3.3	0.2	1.5	4.6	3.3	0.2	2.4	4.0	3.1	0.1

[a] Means followed by a different letter within a row are significantly different ($p < 0.05$).
[b] Total iridoids: the sum of iridoids analysed.
[c] Total xanthones: the sum of glycosides and aglycones analysed.

the amount of these xanthones also seems to be independent of the drying mode.

CONCLUSION

In conclusion, this work showed that artificial drying at 40 °C can be used to minimize the loss of iridoids during desiccation and preserve the bitterness of fresh gentian roots. After artificial drying, the mean percentage of preserved gentiopicroside, the principal bitter compound, was about 83.5% versus fresh roots. In contrast, natural drying induced marked changes: only 57% of gentiopicroside was preserved. Artificial drying thus conserved about 86% of the iridoids present in fresh roots, whereas natural drying preserved only 61% of total iridoids.

Knowledge of the qualitative and quantitative composition of *G lutea* roots was obtained by identification of loganic acid, previously mentioned in other *Gentiana* species.

However, the cost of artificial drying is relatively high, which might limit its use for gentian roots, even though the losses of bitter principles are minimized. Conversely, natural drying is cheap, which is a good argument for its use. This mode of drying may induce other chemical modifications that might affect the flavour of gentian roots. A study of flavouring compounds would thus have been interesting, but was difficult to carry out because of the very low levels of the volatiles present.[35]

ACKNOWLEDGEMENTS

The authors thank the producers and the members of the collectors association (SICARAPPAM, 63260 Aubiat, France).

REFERENCES

1 Leung AY and Foster S, *Encyclopedia of common natural ingredients used in food, drugs and cosmetics*, 2nd edn. Wiley-Interscience, New York, pp 267–269 (1996).
2 Bricout J, Identification et dosage des constituants amers des racines de *Gentiana lutea* L. *Phytochemistry* **13**:2819–2823 (1974).
3 Sticher O and Meier B, Identification of the bitter substance from gentian root with high performance liquid chromatography (HPLC). *Pharm Acta Helv* **53**:40–45 (1978).
4 Sticher O and Meier B, Quantitative determination of the bitter principles in the roots of *Gentiana lutea* and *Gentiana purpurea* with HPLC. *Planta Med* **40**:55–67 (1980).
5 Takino Y, Koshioka M, Kawaguchi M, Miyahara T, Tanizawa H, Ishii Y, Higashino M and Hayashi T, Quantitative determination of bitter components in Gentianaceous plants. Studies on the evaluation of crude drugs VIII. *Planta Med* **38**:344–350 (1980).
6 Ma L, Wu F and Song WZ, Determination of two main bitter secoiridoid glycosides in Gentianaceae. *Chin J Pharm Anal* **17**:241–244 (1997).
7 Quercia V, Battaglino G, Pierini N and Turchetto L, Determination of the bitter constituents of the gentiana root by high-performance liquid chromatography. *J Chromatogr* **193**:163–169 (1980).
8 Hayashi T and Yamagishi T, Quantitative determination of amarogentin in *Gentiana radix* by high performance liquid chromatography. *Hokkaidoritsu Eisei Kenkyushoho* **36**:83–84 (1986).
9 Krupinska A, Segiet-Kujawa E, Skrypczak L and Ellnain-Wojtaszel M, Quantitative determination of amarogentin by TLC-densitometry. *Sci Pharm* **59**:135–138 (1991).
10 Bodart P, Poukens-Renwart P, Wauters JN and Angenot L, Densitometric evaluation of gentiopicroside in the roots of *Gentiana lutea* L. *J Planar Chromatogr Modern TLC* **9**:143–145 (1996).
11 Glatz Z, Pospisilova J and Musil P, Determination of gentiopicroside in extracts of *Centaurium erythreae* and *Gentiana lutea* by micellar electrokinetic capillary chromatography. *J Liq Chromatogr Relat Technol* **23**:1831–1839 (2000).
12 Szucs Z, Danos B and Nyiredy S, Comparative analysis of the underground parts of *Gentiana* species by HPLC with diode-array and mass spectrometric detection. *Chromatographia* **56**:S19–S23 (2002).
13 Coscia CJ, Guarnaccia R and Botta L, Monoterpene biosynthesis. I. Occurrence and mevalonoid origin of gentiopicroside and loganic acid in *Swertia caroliniensis*. *Biochemistry* **8**:5036–5043 (1969).
14 Tan RX, Kong LD and Wei HX, Secoiridoid glycosides and an antifungal anthranilate derivative from *Gentiana tibetica*. *Phytochemistry* **47**:1223–1226 (1998).
15 Calis I, Ersoz T, Chulia AJ and Ruedi P, Septemfidoside: a new bis-iridoid diglucoside from *Gentiana septemfida*. *J Nat Prod* **55**:385–388 (1992).
16 Garcia J and Chulia AJ, 4′-*p*-Coumaroyl iridoid glucosides from *Gentiana pedicellata*. *Planta Med* **53**:101–103 (1987).
17 Bergeron C, Marston A, Gautier R and Hostettmann K, Iridoids and secoiridoids from *Gentiana linearis*. *Phytochemistry* **44**:633–637 (1997).
18 Canonica L and Pelizzoni F, Chemical investigations of gentians. I. Xanthone pigments of the root of *Gentiana lutea*. *Gaz Chim Ital* **85**:1007–1024 (1955).
19 Atkinson JE, Gupta P and Lewis JR, Phenolic constituents of *Gentiana lutea*. *Tetrahedron* **25**:1507–1511 (1969).
20 Nikolaeva GG, Glyzin VI, Mladentseva MS, Sheichenko VI and Patudin AV, Xanthones from *Gentiana lutea*. *Khim Prir Soedin* **1**:107–108 (1983).
21 Verney AM and Debelmas AM, Various xanthones of *Gentiana lutea* L, *G purpurea* L, *G punctata* L, *G pannonica* Scop. *Ann Pharm Fr* **31**:415–420 (1973).
22 Hostettmann K and Wagner H, Xanthone glycosides. *Phytochemistry* **16**:821–829 (1977).
23 Hayashi T and Yamagishi T, Two xanthone glycosides from *Gentiana lutea*. *Phytochemistry* **27**:3696–3699 (1988).
24 Rossetti V, Lombard A, Sancin P, Buffa M and Borgarello E, Seasonal variations in components of dried *Gentiana lutea* roots from the Western Alpine region. *Quat J Crude Drug Res* **19**:37–46 (1981).
25 Franz C, Franz G, Fritz D and Schultze J, Seasonal variations in the content of bitter substances and soluble sugars in *Gentiana lutea* L. *Sci Pharm* **53**:31–38 (1985).
26 Schultze J and Franz C, Sugars, bitter substances and essential oils of *Gentiana lutea* L in dependance of ecotype, plant age and stage of development. *Herba Hung* **18**:311–315 (1979).
27 Rossetti V, Lombard A, Sancin P, Buffa M and Menghini A, Composition of *Gentiana lutea* L dried roots harvested at different altitudes. *Plant Med Phytother* **18**:15–23 (1984).
28 Sancin P, Rossetti V, Lombard A, Buffa M and Lanzone L, Composition of fluid extracts of *Gentiana lutea* L roots harvested at different altitudes. *Boll Chim Farm* **124**:7–12 (1985).
29 Franz C and Fritz D, Cultivation of *Gentiana lutea* and main substances of some ecotypes. *Planta Med* **28**:289–300 (1975).
30 Hayashi T, Minamiyama Y, Miura T, Yamagishi T and Kaneshina H, Cultivation of *Gentiana lutea* and chemical evaluation of *Gentiana radix*. *Hokkaidoritsu Eisei Kenkyushoho* **40**:103–106 (1990).

31 Verney AM, Ozenda P and Debelmas AM, Stability of xanthones in the rhizomes of *Gentiana lutea* during different stages of vegetation and at different altitudes. *Bull Trav Soc Pharm Lyon* **16**:3–15 (1972).
32 *European Pharmacopeia*, 4th edn. EDQM, Strasbourg, pp 50–51 (2002).
33 Wagner H and Bladt S, *Plant Drug analysis: a thin layer chromatographic atlas*, 2nd edn. Springer, Berlin, pp 86–87 (1996).
34 Verotta L, Isolation and HPLC determination of the active principles of *Rosmarinus officinalis* and *Gentiana lutea*. *Fitoterapia* **56**:25–29 (1985).
35 Chialva VF, Frattini C and Martelli A, Unusual essential oils with aromatic properties. *Z Lebensm Unters Forsch* **182**:212–214 (1986).