Journal of Natural Products
Vol. 58, No. 11, pp. 1727-1729, November 1995

GLYCYRRHIZIN VARIABILITY IN SUBTERRANEAN ORGANS OF SARDINIAN *GLYCYRRHIZA GLABRA* SUBSPECIES *GLABRA* VAR. *GLABRA*

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ABSTRACT.—Concentration levels of the triterpene saponin glycyrrhizin have been determined in underground organs of *Glycyrrhiza glabra* of Sardinian origin. The highest concentrations of this compound were found in the roots (diameter >1 cm). No more than 0.02% w/w of glycyrrhizin was found in the axes of stems above the soil level.

Glycyrrhiza glabra L. (Leguminosae) is native to the Mediterranean area, central and southern Russia, the Anatolian peninsula, and Iran (1). Licorice is the common name for the drug extracted from different *Glycyrrhiza* species. The principal constituents of licorice roots and stolons are a number of triterpenoid saponins (2–15% w/w), and among these saponins glycyrrhizin is the most important and abundant component (2). Glycyrrhizin is a triterpenoid saponin formed from an aglycone (glycyrrhetinic acid) and a diglucuronide saccharide unit. Glycyrrhizin is known to be effective as an anti-inflammatory (3–4), antiallergenic (5), and antiulcer agent (6–7), and inhibits migration of leukocytes to the sites of inflammation (8). The administration of high levels of licorice extracts (more than 50 g/day) produces an increase in blood pressure (9). Glycyrrhizin inhibits the activity of phospholipase A and the production of prostaglandin E_2 (8) in peritoneal macrophages, and also exhibits antihepatotoxic (10) and anticarcinogenic (11) activities.

Licorice is used as ingredient in many food products due primarily to its sweet taste (12), which is from glycyrrhizin and other triterpene glycosides (8); in fact, these constituents are 50–150 times more potently sweet than sucrose (8). The biosynthetic route of formation of glycyrrhizin is poorly understood and still under investigation (13).

This paper reports the quantitative distribution of glycyrrhizin (measured as glycyrrhetinic acid) in different parts of the subterranean organs of wild specimens of *Glycyrrhiza glabra* L. ssp. *glabra* var. *glabra* (=var. *typica* Regel et Herder), growing in Sardinia, Italy. In order to carry out a quantitative determination of glycyrrhizin in different parts of a wild specimen of *G. glabra*, several portions of each part of the plant were extracted with $MeOH/H_2O$, and subjected to solvent partitioning, and to acidic hydrolysis. A $CHCl_3$ fraction obtained after hydrolysis was subjected to hplc analysis on a C_{18} column.

The hplc analyses carried out on the hypogeal parts of *Glycyrrhiza glabra* L. gave percentage glycyrrhizin levels of between 0.12% and 2.24% w/w of dry material. The highest concentration of glycyrrhizin was found in the roots (Table 1), particularly in organs of diameter greater than 1 cm, where the concentration levels of the saponin were a minimum of 1.96% and a maximum of 2.24% (average 2.1%). In roots having a diameter of less than 1 cm, the concentration of glycyrrhizin was between 1.30% and 0.93% (average 1.1%). In all roots, it is evident that a positive correlation exists between the diameter of organs (and therefore the age) and the percentage of glycyrrhizin.

The underground stems (Table 1) were characterized by a high content of glycyrrhizin (1.27–1.97%, average of 1.4%). This concentration was superior to that of the stolons also, when the stem diameter was between 0.30 and 0.70 cm.

TABLE 1. Concentration Levels of Glycyrrhizin in the Hypogeous Organs of *Glycyrrhiza glabra* ssp. *glabra* var. *glabra*.

Roots		Stolons		Underground stems		Stems at the soil level	
Diameter (cm)	Glycyrrhizin (%)	Diameter (cm)	Glycyrrhizin (%)	Diameter (cm)	Glycyrrhizin (%)	Diameter (cm)	Glycyrrhizin (%)
1.70	2.24	1.40	0.74	0.55	1.27	0.20	0.39
1.70	1.96	0.90	0.75	0.35	1.57	0.20	0.39
0.60	1.30	0.80	1.77	0.45	1.97	0.80	0.57
0.55	1.30	0.60	1.77	0.40	1.97	0.55	0.57
0.30	0.93	1.20	1.30	0.70	1.63	0.45	0.12
0.20	0.93	0.50	1.31	0.40	1.44	0.50	0.93
0.20	0.93	0.40	1.31	0.30	1.44	0.40	0.93
0.20	0.93	—	—	—	—	0.20	0.74
—	—	—	—	—	—	0.20	0.74
—	—	—	—	—	—	0.50	0.95

Concentrations of glycyrrhizin were quite variable among the stolons (Table 1) and apear to be unrelated to the age of the plant. A maximum concentration was found (1.77%) in stolons having a diameter of between 0.60 and 0.80 cm, and a minimum concentration was found (0.74%) in stolons with a diameter of 1.40 cm. In the stems at soil level (Table 1) the concentration of glycyrrhizin was never found to be higher than 0.95%; also in this case there was no correlation between the concentration of glycyrrhizin and diameter of organ. In stems above soil level the maximum content of glycyrrhizin was 0.02%.

This study has allowed us to conclude that glycyrrhizin is present in only the below-ground parts of *Glycyrrhiza glabra*. The organs in which the active constituents are more abundant are the oldest roots, followed by the underground stems. When making a vertical transect, passing through a rhizome having both a fully grown root and an emerging stem, it is possible to observe a direct correlation between the percentage of glycyrrhizin and the root depth. In the aerial parts of licorice, glycyrrhizin was detected only in trace amounts. The present data are in agreement with indications that the biosynthesis of the saponin takes place in the roots and not in the green parts of the plant (14).

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Hplc analysis was performed with the use of an HP 1090 solvent-delivery system, equipped with a uv detector (λ 254 nm) and an HP 3394A integrator. The column used was an Alltech Adsorbsphere C_{18} column, particle size 5 mm, 25 cm×4.6 mm i.d. Solvents within this study were all hplc grade.

PLANT MATERIAL.—*Glycyrrhiza glabra* L. ssp. *glabra* var. *glabra* (=var. *typica* Regel et Herder) was obtained as a wild specimen growing in the delta region of the Cedrino River, East Sardinia, Italy. The plant material was identified by two of us (A.D.A. and V.P.). The plant material has been deposited in the Herbarium SASSA (Istituto di Botanica Farmaceutica, Università degli Studi di Sassari).

EXTRACTION AND ISOLATION.—The plant material was air-dried, powdered, and extracted for 9 h in a Soxhlet apparatus using MeOH-H_2O (80:20) according to a literature procedure with minor modification (14). The concentrated crude extract was subjected to partition (15) with, successively, $CHCl_3$-MeOH-H_2O (30.76:38.46:30.76); $CHCl_3$-*i*-PrOH-H_2O (38.46:30.76), and $CHCl_3$-H_2O (61.45:38.46). This last step was added to be sure that all the glycyrrhizin (H_2O-soluble) was extracted. The aqueous layer (16, slightly modified) was heated under reflux for a few min, after which 10% H_2SO_4 was added, and reflux continued for a further 4 h. After cooling, with $CHCl_3$ added, and heating under reflux for 20 min, the aqueous layer was washed with $CHCl_3$ (4×30 ml). The $CHCl_3$ fraction from the hydrolysis was purified by Si gel cc with $CHCl_3$-MeOH (95:5) as eluent. The fractions (1–7) containing glycyrrhetinic acid were subjected to hplc analysis on a C_{18} column.

ANALYTICAL PROCEDURE.—Several analyti-

cal methods have been employed for the determination of glycyrrhizin and glycyrrhetinic acid in plant material and pharmaceutical and food preparations, such as gc (17), hplc (18, 19), tlc (20), and hptlc (21). In the present study, an hplc method was used, with the solvent system (16) being $MeOH_2$-H_2O (80:20), R_f 0.6 ml/min. The threshold of detection of the method was 0.1 ppm. The retention time of each eluted sample was reproducible.

To verify the fitness of the proposed method, we performed recovery experiments using several *G. glabra* samples (1 g), to which were added 100 mg of glycyrrhizinic acid ammonium salt (Aldrich); all the experiments gave recovery precentages of at least 90%. Moreover, we applied the above-described analytical procedure to several samples of licorice, with a known content of glycyrrhizin, kindly furnished by D. Ulrich S.p.A. (Torino, Italy); the percentages of glycyrrhetinic acid obtained were in accordance with expected values. The concentrations of glycyrrhizin were obtained using a tare curve for glycyrrhetinic acid ($y=407.530+170816.996x$; $r^2=0.99983$) and converting the values to percentages of glycyrrhizin, using a correction factor, derived from the recovery percentage and the compound molecular weights.

ACKNOWLEDGMENTS

The work was financially supported by M.R.A.A.F. (Ministero delle Risorse Agricole Alimentari e Forestali) and M.U.R.S.T. (Ministero dell'Università della Ricerca Scientifica e Tecnologica, 40%). The authors gratefully acknowledge Dott.ssa Galliano-Raspino for the samples of *Glycyrrhiza glabra* L. with a known content of glycyrrhizin, and Mr. Gianuario Manca for technical assistance.

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Received 13 December 1994