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Liquorice, *Glycyrrhiza glabra* L.—Composition, Uses and Analysis

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ABSTRACT

The liquorice plant (Glycyrrhiza glabra *L., formerly* Liquiritae officinalis *Moench) has been used by physicians and herbalists since the earliest of times. Many of the early claims for the effectiveness of liquorice extracts, decoctions and potions have been shown by modern science to be credible, a root component (glycyrrhizin) being generally regarded as the major biologically-active principle. Liquorice extracts (in pharmacy called* succus liquiritae*) are currently used mainly in the tobacco, pharmaceutical and confectionery industries; because such extracts contain glycyrrhizin there is a need for accurate methods for its estimation, in both crude isolates and finished products. The purpose of this review is to briefly describe the background to current interests in liquorice, to indicate the origins and usage of liquorice extracts and to discuss the composition of liquorice, with particular emphasis on glycyrrhizin. Current methods for the analysis of glycyrrhizin will be considered and the levels found in liquorice roots, extracts and a range of products indicated. Brief mention will also be made of human exposure levels and the possible risk to human health as a result of consuming liquorice-based products.*

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INTRODUCTION

Liquorice is one of the most widely investigated of the economically-important medicinal plants. Earliest recorded references to the plant relate to its wide-ranging therapeutic properties. Today it is harvested for its below-ground parts which form the basis of products used in the tobacco, confectionery and pharmaceutical industries (Leung, 1980).

In this paper the origins and uses of liquorice and its products are described and the chemical composition discussed. Particular emphasis will be placed on glycyrrhizin, which is of considerable economic, industrial and scientific importance as a result of its sweet taste, pharmacological properties and toxicity. Methods of glycyrrhizin analysis, its levels in foods, beverages and other products and its likely human exposure will be described. Following a brief mention of the biological properties of liquorice, its extracts and components, the possible safe exposure levels will be considered. In a complementary paper, the pharmacological and toxicological properties of liquorice will be reviewed in depth (Lutomski *et al.*, 1990).

HISTORICAL PERSPECTIVE

The interest and attraction of the liquorice plant (*Glycyrrhiza glabra* L.) to early man was, as in many other instances, more related to its medicinal properties than its culinary or flavour potential. Moore *et al.* (1984) have advocated the use of fecal odorgrams as a means of partially reconstructing ancient diets. The odours of liquorice, detected in coprolites dating back to beyond 4000 BC, was suggested by these authors to result from ingestion of *Glycyrrhiza lepidota* (American liquorice) or *Osmorhiza obtusa* (sweet cicely), both of which are believed to have featured in the diet of prehistoric American populations.

According to Wheelwright (1974) the earliest written reference to the use of liquorice as a medicine is contained in the Codex Hammurabi, dating from 2100 BC. The curative properties of liquorice were certainly well known to the Assyrians (Lucas, 1966) and Thorwald (1966) has reported that Arad-Nana, personal physician to King Asahadon (680–669 BC), son of Sennacherib, prescribed liquorice for the relief of rheumatic, and other, pain. References to the effectiveness of liquorice are contained in the 'Shen nung Pen t'sao King', the first Chinese herbal, where it is included in the list of drugs of the first class and recommended for its life-enhancing properties and godly influence on the body.

The fame of the plant spread westward from Mesopotamia, and

Hippocrates (430 BC), Theophrastus (322 BC) and, later, the Elder Pliny (AD79) all commented on its healing effect on ulcers. The writings of these authorities, and in particular the 'De Materia Medica Libri Quinque' of the 1st-century Greek military physician, Dioscorides, had great influence on successive generations of doctors and herbalists, including the Arab pharmacognosist Ibn Baithar (13th century), Conrad von Mengenberg (14th century), and the 16th and 17th century herbalists Paracelsus, Lonicerus, Gerard, Mattiolus and Culpepper. Gibson (1978) has indicated some of the uses ascribed to liquorice in these works and has emphasised the extent to which many early claims for its effectiveness have been substantiated by recent research.

Grocer–apothecaries, importing spices and medicinal herbs containing liquorice, were probably the first to combine the juice with honey and sugar, and liquorice pastilles, cast in crude moulds, later became familiar towards the end of the Middle Ages. Extruded liquorice sweets were said to be introduced into The Netherlands in the early 17th century, and a century later 'salty liquorice' containing ammonium chloride was produced in France (Nieman, 1974).

THE LIQUORICE PLANT

Liquorice, a leguminous shrub having a height of 70–200 cm, occurs mainly in sub-tropical regions where it grows wild and is also under cultivation. The commercially-important parts of the plant from which extracts are made are the long, tangled roots and creeping underground stems. According to Perrot (1944) there are four main varieties.

Glycyrrhiza glabra L. var. *typica* Reg. et Herd., having blue flowers and being found in the Mediterranean and Caucasian regions. Frequently termed Spanish or Italian liquorice, it was this variety that was cultured in Pontefract and used by Dunhill (see below).

G. glabra L. var. *violacea* Boiss. (Persian or Turkish liquorice) and *G. glabra* L. var. *pallida* Boiss., both found wild in the regions of old Mesopotamia.

G. glabra L. var. *glandulifera* (Waldst. et Kit.) Reg. et Herd. (sometimes called *G. glandulifera*, *G. hirsuta* or *G. brachycarpa*), having violet flowers and occurring in Hungary, Southern Siberia, Turkestan and Afghanistan, from which the common name Russian liquorice is derived.

In addition, reference is sometimes made to Mongolian or Chinese liquorice (*G. uralensis* Fisch.) and Manchurian liquorice (*G. pallidiflora* Maxim), but these are regarded as separate species of *Glycyrrhiza* (Amirova & Kir'yalov, 1989).

According to Čikov (1976), *G. glabra* L. var. *glandulifera*, *G. uralensis* Fisch. et Mey and *G. korshinski* Grig. are used in Soviet medicine. *G. uralensis* grows in the southern part of Asia between the Urals, Mongolia and China. It has bigger flowers, in thicker clusters than those of *G. glabra*. *G. korshinski*, which is endemic along the banks of the Volga, Ural Tobola, Iszima and Caryca rivers, has smaller leaves and fruit than does *G. uralensis* (Siskin, 1963). Multiplication is usually by root cuttings or division of the crown, although it may occasionally be achieved from seed. In the natural state, plants are found in deep, moist soil in environments where the late summer is both hot and dry. Cultivated crops are initially watered, but thereafter require little husbandry until being harvested in the third or fourth year. The roots and underground stems are cut into sections, the tops being used as a compost, the remaining root material being the basis for the next generation of plants. Depending upon local practice, the harvested roots may be peeled and dried before being processed to yield the extracts, powders or glycyrrhizin of industry (Morton, 1977).

COMPOSITION OF LIQUORICE ROOT

Proximate composition

Duke (1985) gives the proximate composition of liquorice root as 20% moisture, 5–24% glycyrrhizin (a triterpene glycoside considered to be the main biologically-active principle, see below), 3–16% sugars, 30% starch and 6% ash. However, the exact composition varies greatly according to species, cultivation conditions, growth environment, etc. Thus Vondenhof *et al.* (1973) have examined sixteen samples of liquorice from a variety of origins. Glucose contents varied between 0·15 and 4·15%, and those of fructose and sucrose between 0·5 and 4·1% and 1·5 and 20·3%, respectively. Four samples, including three from Turkey, contained maltose (0·1–0·6%). In addition to these low molecular weight carbohydrates, the authors noted the presence of polymeric substances, including soluble starches and dextrins. According to the results of an investigation of Moldavian liquorice (Muchnik, 1976), the contents of glycyrrhizin, sugars and extractives increased with the age of the plant and were highest in the horizontal roots and tap roots. Maximum levels were found in the hottest periods of the year, which also corresponded to the termination of flowering. Based upon these and other data, the author suggested that roots should be gathered at the end of the third season, when glycyrrhizin and sugar levels were ~11 and ~15%, respectively.

Of especial significance are the flavonoids and isoflavonoids, chalcones and coumarins whose structures are shown in Fig. 1 (Lutomski, 1983).

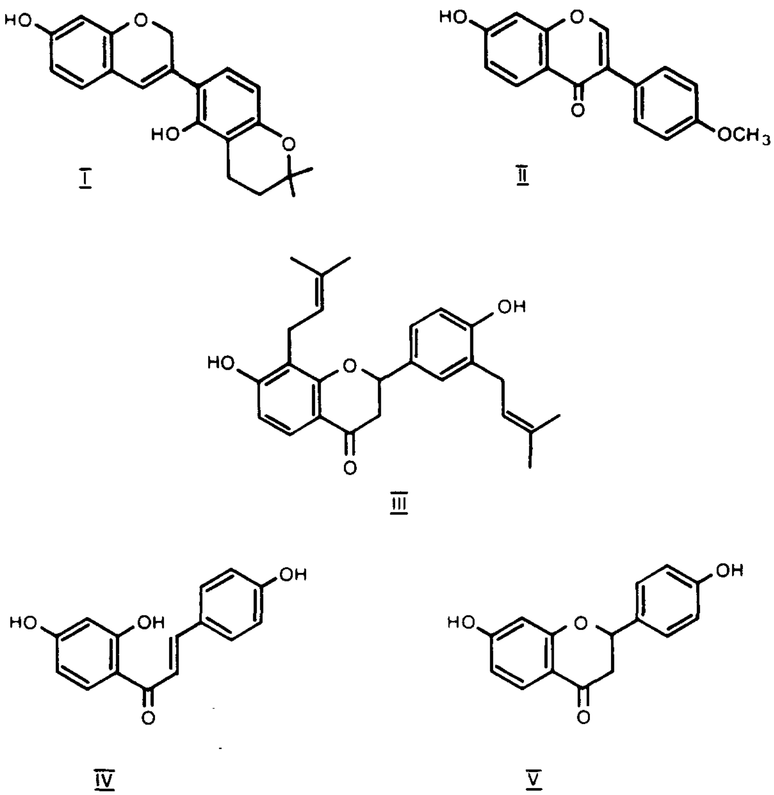

Fig. 1. Constituents of liquorice: glabrene (I), formononetin (II), glabrol (III), isoliquiritigenin (IV), liquiritigenin (V).

Glabrene (I) is unusual in possessing an isoflavene skeleton, whilst formononetin (II) possesses oestrogenic activity. Glabrol (III) shows potent activity against the *Staphylococcus aureus* and *Mycobacterium smegmais*, and the spasmolytic activities of isoliquiritigenin (IV) and liquiritigenin (V) are considered by many to be responsible for the gastric-ulcer-healing properties of liquorice (Lutomski *et al.*, 1990). A number of other phenolic constituents of liquorice are listed in Table 1.

Mitscher *et al.* (1980) have found *O*-acetylsalicylic acid in liquorice and have speculated that its presence might contribute to the therapeutic properties of the plant and its aqueous extracts. Whilst glycyrrhizin (VI, Fig. 2) is by far the major saponin in *Glycyrrhiza* species, Price *et al.* (1987) have listed twenty triterpenes which have been reported, often in trace amounts, suggesting that the saponin profile may actually be much more complex. In agreement with this, Kitagawa *et al.* (1988) have recently described five new oleanene-type saponins isolated from *G. uralensis* of Chinese origin. These totalled <0·06% of the dried root, compared with 3·6% glycyrrhizin. The authors further reported another five

TABLE 1
Phenolic Constituents of Liquorice

Flavonoid, Isoflavonoid	
Licoricidin[a]	Shibata & Saitoh (1968)
Glabridin[a]	Saitoh *et al.* (1976*a*)
3'-*O*-Methylglabridin	Mitscher *et al.* (1980)
4'-*O*-Methylglabridin	Mitscher *et al.* (1980)
Phaseollinisoflavan	Mitscher *et al.* (1980)
Hispaglabridin A	Mitscher *et al.* (1980)
Hispaglabridin B	Mitscher *et al.* (1980)
Glabrene	Kinoshita *et al.* (1976)
Licoisoflavone A[a]	Kinoshita *et al.* (1978*b*)
Licoisoflavone B[a]	Kinoshita *et al.* (1978*b*)
Licoricone[a]	Kaneda *et al.* (1973)
Neoliquiritin	Litwinienko & Obolencova (1964)
Prunetin	Kattaev & Nikonov (1974)
Formononetin	Elgamel & Fayez (1972)
Saponaretin (isovitexin)	Litwinienko & Nabiezyna (1972)
Kumatakenin	Saitoh *et al.* (1976*b*)
Licoflavonol	Saitoh *et al.* (1976*b*)
Liquiritigenin	Reiners (1964)
Liquiritin	Reiners (1964)
Liquiritin apioside	Kitagawa *et al.* (1989)
Glabrol[a]	Saitoh *et al.* (1976*a*)
3-Hydroxyglabrol	Mitscher *et al.* (1980)
Glabranin	Kattaev & Nikonov (1972)
Pinocembrin	Kattaev & Nikonov (1974)
Licoisoflavanone	Saitoh *et al.* (1978)
Genkwanin	Litwinienko & Nabiezyna (1972)
Licoflavone[b]	Yang & Liu (1988)
4'-7-Dihydryoxyflavone[b]	Yang & Liu (1988)
Coumestan	
Glycyrol[a,d]	Saitoh & Shibata (1969)
5-*O*-Methylglycyrol[a]	Saitoh & Shibata (1969)
Isoglycyrol[a,d]	Saitoh & Shibata (1969)
Licocoumarin	Bhardwaj *et al.* (1976)
Licocoumarone	Demizu *et al.* (1988)
Glycyrin	Kinoshita *et al.* (1978*a*)
Herniarin	Reiners (1964)
Umbelliferon	Reiners (1964)
Glycycoumarin	Hattori *et al.* (1986)
Chalcone	
Isoliquiritigenin	Litwinienko & Obolencova (1964)
Isoliquiritin	Puri & Seshadri (1954)
Neoisoliquiritin	Litwinienko & Obolencova (1964)
Licochalcone A	Saitoh & Shibata (1975)
Licochalcone B	Saitoh & Shibata (1975)
Echinatin[c]	Furuya *et al.* (1971)
Licuroside	Litwinienko & Obolencova (1964)
Neolicuroside	Miething & Speicher-Brinker (1989)

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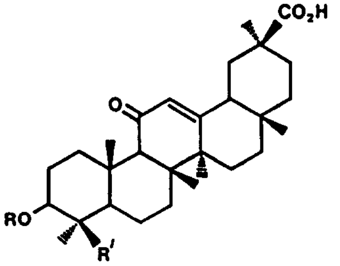
[a] *Glycyrrhiza uralensis* Fisch. et DC.
[b] *Glycyrrhiza inflata* Bat.
[c] *Glycyrrhiza echinata* L.
[d] Structures revised, Shiozawa *et al.* (1989).



VI R = gluA(1→2)gluA(1→), R′ = CH_3

VII R = H, R′ = CH_3

VIII R = gluA(1→2)gluA(1→), R′ = CH_2OH

Fig. 2. Constituents of liquorice: glycyrrhizin (VI), glycyrrhetinic acid (VII), sweet saponin (VIII) (Tokiwa Shokubutsu, 1981).

saponins (total 0·03%), the structures of which are under investigation. Two sweet saponins, apioglycyrrhizin and araboglycyrrhizin, have been isolated from *G. inflata* (Kitagawa *et al.*, 1989) whilst Uchida *et al.* (1988) have reported the isolation of the galacturonic acid (terminal analogue of glycyrrhizin from unspecified *Glycyrrhiza* species and determined this to be three times as sweet as glycyrrhizin.

Liquorice root has been shown to contain both free (proline, asparagine, asparaginic acid) and bound (e.g. 1-*N*-L-asparaginic acid-1-deoxy-D-fructose, 2-*N*-L-asparaginic acid-2-deoxy-D-glucose) amino acids (Vondenhof *et al.*, 1973), amines (asparagine, betain, choline), and sterols (β-sitosterol, stigmasterol, 22,23-dihydrostigmasterol).

Volatile oil

Few detailed studies have been reported into the composition of the volatile oil of liquorice. From the data available it is clear that the oil composition, and content, is markedly affected by environmental, cultural and, especially, processing factors. Thus Frattini *et al.* (1977) have reported compositional differences between the steam-distilled essential oils of heat-treated (130–150°C) liquorice (0·03% w/w) and macerated roots. Twenty-eight components were identified in the latter, compared with sixty-three in the former. Half of the volatiles identified in the heated product were nitrogenous or (mainly) furan derivatives, a finding consistent with the relatively low levels of amino acids and proteins in the root and the complex thermal reactions of the sugars known to be present. The major components of the heat-treated product were acetol, propionic acid, 2-acetylfuran, furfural and 2-acetylpyrrole, whilst only γ-nonalactone, α-terpineol, 2-phenylethanol, *ortho*-methoxyphenol and 2-methyl-tetrahydrofuran-3-one were common to both oils. Toulemonde *et al.* (1977) separated more than one hundred compounds comprising a volatile concentrate (0·0001%) obtained from dried *G. glabra* rhizomes and identified thirteen of these; two, *n*-pentanol and *n*-hexanol, comprised the major part of the product.

Recently, Japanese workers (Kameoka & Nakai, 1987) have separated more than one hundred and fifty components in the essential oil obtained in 0·047% yield by steam distillation of liquorice root. Eighty-two compounds were identified, with hexanoic acid (32%) dominating. Other compounds which contributed >1% to the essential oil were hexanol, 4-methyl-1-*iso*propyl-3-cyclohexen-1-ol, *N*-methyl-2-pyrrolidone, heptanoic acid, octanoic acid and γ-nonalactone. A fraction possessing the main sensory characteristics of liquorice was concentrated and shown to contain estragole, anethole, eugenol, indole, γ-nonalactone and cumic aldehyde.

Glycyrrhizin*

Glycyrrhizin (VI) is the major triterpenoid saponin in liquorice root, present in concentrations ranging up to 15%. The processing of liquorice root to provide more appropriate industrial products results in the concetration of glycyrrhizin. Thus block liquorice extract (prepared by macerating the roots, extracting with hot water and concentrating to a final moisture content of ~20%) contains ~20% glycyrrhizin, whilst the glycyrrhizin content of the spray-dried powdered extract (moisture ~5%) is nearer 25%. The composition of a range of liquorice block extracts from different countries of origin is shown in Table 2 and depends upon a variety of factors, including the root source and method of processing. The figures of glycyrrhizin content are undoubtedly over-estimates, being based upon gravimetric methods of analysis (see below).

Although the presence of glycyrrhizin in liquorice has been known for well over two hundred years and chemical investigations have been conducted since the beginning of the last century (Nieman, 1957), it is only following extensive studies on the aglycone (Ruzicka *et al.*, 1943*a*, *b*, *c*) and disaccharide (Lythgoe & Trippett, 1950) released following mild hydrolysis that the structure of the intact saponin was confirmed as VI. Tschirch and Cederberg (1907) isolated crystalline glycyrrhizin and described its physical and sensory properties. An intensely-sweet taste characterised the free compound, and its potassium and ammonium salts. This taste was completely removed by hydrolysis of the saponin to its crystalline aglycone, glycyrrhetinic acid (VII).

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* This saponin has also been called glycyrrhizic acid or glycyrrhizinic acid: its aglycone, glycyrrhetinic acid (VII), has also been termed glycyrrhetic acid.

TABLE 2
Average Composition (% w/w) of Block Liquorice Extract

Country of manufacture	*Moisture*	*Ash*	*Insolubles*	*Gums and starches*	*Reducing sugars*	*Sucrose*	*Glycyrrhizin*
Italy	15 (12–18)	4 (3–5)	8 (2–14)	42 (34–50)	5 (2–8)	4 (1–7)	12 (7–17)
Spain	14 (11–17)	—	16 (3–29)	36 (31–41)	4 (2–6)	3 (1–5)	13 (10–16)
Israel	16	6	4	30	5	6	16
Turkey	13 (11–15)	9 (6–12)	4 (1–7)	26 (16–36)	6 (3–9)	12 (6–18)	16 (11–21)
USA	16 (13–19)	6 (5–7)	3 (1–5)	28 (24–32)	4 (2–6)	6 (2–10)	21 (16–26)
Syria	15 (14–16)	8 (6–10)	3 (2–4)	21 (15–27)	6 (4–8)	11 (7–15)	21 (15–27)
Iran	17	7	3	38	7	5	25
China	13 (12–14)	13 (12–14)	4 (1–7)	25 (17–33)	5 (3–7)	10 (6–14)	26 (22–30)
USSR	12 (8–16)	8 (7–9)	2 (1–3)	19 (16–22)	9 (7–11)	5 (2–8)	32 (25–39)

Data taken from Nieman (1974). Glycyrrhizin content determined by gravimetric method; see text for comments on this method.

The amount of glycyrrhizin in the roots and underground stems of liquorice are markedly affected by variety, source and prevailing climate conditions. Serrano (1946) and Muchnik (1976) have reported data for Spanish and Russian liquorice, respectively, whilst Fuggersberger-Heinz and Franz (1984) have shown the concentration of this glycoside in Spanish liquorice to be higher in the main roots (1·77%, range 0·99–2·80%) than in the lateral stems (0·31%, range 0·28–0·34%). The latter authors also found no detectable glycyrrhizin in the green parts of the plant. Biosynthetic studies, using labelled substrates, indicated that the roots alone were capable of synthesising glycyrrhizin. ^{14}C-Acetate was most effectively incorporated and localised predominantly in the aglycone, glycyrrhetinic acid—a finding consistent with the expected acetate-mevalonate pathway. Feeding of ^{14}C-glucuronic acid resulted in exclusive labelling of the carbohydrate moiety (Fuggersberger-Heinz & Franz, 1984). Recent work by Hayashi *et al.* (1988), using callus and cell suspension cultures of *G. glabra*, suggests that the absence of glycyrrhizin in undifferentiated cultured cells may be due, in part, to interruption of the later reactions leading to the formation of this saponin from triterpenoid precursor(s).

Glycyrrhizin, and structurally-related saponins (e.g. VIII, Tokiwa Shokubutsu, 1981) possess a sweet taste and sweetness-potentiating characteristics and have been employed industrially for this reason. For example, ammoniated glycyrrhizin has long been used to mask the bitter taste of cough syrups, linctus and medicines. Following sensory studies on a range of (synthetic) glycyrrhetinic acid glycosides, Esaki *et al.* (1978) have related the sweetness of glycyrrhizin to the presence of two carboxylic acid groups in the sugar moiety. However, according to Hodge and Inglett (1974), the sweetness of glycyrrhizin may be ascribed to the presence of a disaccharide moiety possessing an internal $(1' \rightarrow 2)$-*o*-glycosidic linkage, an extended hydrophobic aglycone and, crucially, the presence of a second hydrophilic group at the opposite end of the molecule to the C_3 disaccharide (i.e. the carboxylic acid group attached to C_{20}).

Treatment of a hot water extract of liquorice root with sulphuric acid followed by neutralization with dilute ammonia affords ammoniated glycyrrhizin. This product, which has a liquorice taste and is brown in colour, is extensively used in industry but may be further purified by solvent extraction and other procedures to give colourless monoammonium glycyrrhizinate pentahydrate. Both compounds have the same degree of sweetness but vary markedly in their solubility characteristics and pH sensitivity. The GRAS status of these glycyrrhizin-containing products was originally defined in terms of the level of *each separate* product which was allowable in foods (Anon., 1977) but has subsequently been redefined in terms of *total* glycyrrhizin (Anon., 1983, 1985).

USES

Despite increasing demands, and interest, for 'natural' medicines and dietary supplements, including those containing liquorice, the plant and its extracts are mainly used as a condiment or as a flavouring for tobacco, pharmaceutical and confectionery products. According to Duke (1981) the tobacco industry is the predominant user of liquorice in the US with paste, juice, extract or syrup being added to cigarettes, cigars, smoking mixtures and, especially, chewing and snuff tobaccos. The liquorice imparts both desirable taste and flavour characteristics and also seems to control the moisture. This is supported by data (Anon., 1983) indicating that in the US, 90% of the liquorice supply is used by the tobacco industry, with the remainder being shared equally between the food and pharmaceutical sectors. By-products of the processing of liquorice also find use in such diverse areas as fibreboard insulation, mushroom compost, animal feedingstuffs and fire extinguisher components. The pharmaceutical and therapeutic uses of liquorice and glycyrrhizin will be covered in a separate paper (Lutomski *et al.*, 1990).

As long ago as 1970, the usage of liquorice and its products in food exceeded 1500 t in the US, comprising the dried root (60 t), extract (270 t), powdered extract (1100 t) and ammoniated glycyrrhizin (70 t). In 1983, liquorice products accounted for 5·4% of the UK sugar confectionery market (4·5% by value). Volume of sales was 13 400 t (having declined from 15 635 t in 1979), but their value increased from £25·6 m to £30·8 m over the same period (Hilliam, 1985). Current UK usages are estimated to be approximately 16 200 t.

Confectionery

The use of liquorice extract for the flavouring of confectionery may be dated to the 18th century, when an English chemist, Dunhill, included molasses, sugar, flour and liquorice in a recipe for Pontefract cakes. The nature, and consumption, of liquorice-flavoured confectionery varies from region to region, being especially popular in parts of Europe. In some countries there is food legislation as to the minimum content of liquorice extract in liquorice confectionery, and the proposed European Community directive on sugar confectionery, which the Community's Commission tried to establish in the years 1968–1976, required a minimum content of 0·5% glycyrrhizin for liquorice confections. In Germany the semi-official standards for sugar confectionery as drawn up by the industry itself formulate the minimum level in terms of liquorice extract of which at least 5% should be present. This requirement proved difficult to manage since the extract content cannot be determined and the glycyrrhizin content is no reliable indicator, the less so if a deglycyrrhinizinised extract is used.

Detailed aspects of liquorice confectionery and processing are beyond the brief of this review and the interested reader is referred to Lees and Jackson (1973), Nieman (1974), Seibl and Ludewig (1979), Minifie (1982) and, most recently, Hoffmann *et al.* (1985) for details about the different types of liquorice confectionery, their manufacture and recipes. Cast liquorice moulded in starch is manufactured by blending and cooking the ingredients and depositing the warm fluid or semifluid mixture into forms imprinted in moulding starch. The cast liquorice in the starch trays is heated in a drying room at approximately 50°C until the moisture content has been reduced to 11–13%. After cooling, the gumdrops are cleaned of starch, placed on screens, steam-dampened (to close the pores) and finished by polishing, for example with mineral oil.

Pressed, that is roller-shaped and cut, liquorice is usually manufactured by cold mixing liquorice extract and other flavouring materials (menthol, peppermint oil, laurel essence, anise oil, or sal-ammoniac) with dry carbohydrate sweeteners or some starch, with gum arabic and/or gum tragacanth (and sometimes a small proportion of wheat flour or gelatine as the binding agent). The paste is pressed between rollers into a sheet, dried and cut into small forms, or the dough may be pressed and shaped in one operation by two pairs of rollers, and dried afterwards. Articles belonging to this group, such as pellets, cachous, small rhombic lozenges, etc., generally have a high content of liquorice extract and are heavily flavoured.

Extruded liquorice, usually called liquorice paste in English, is actually made from a dough in which gelatinized soft wheat flour is the binding ingredient. The low-boiled mixture, cooked to around 19% moisture by the continuous method, emerges in endless strands which are cut into pieces of the required length, dried, and often coated with a glaze. Extruded centres for liquorice comfits of 'torpedos' are covered with a coloured coating by panning. These are often flavoured with oil of anise to such an extent that this flavour has become virtually synonymous with the liquorice taste in the US. Liquorice paste (usually coloured with caramel and of a more 'short' texture) can be sandwiched between layers of coloured cream pastes by means of a layering machine, which extrudes layered sheets, subsequently cut by a guillotine-type machine.

As in other branches of confectionery, a successful product is usually the result of a well-balanced recipe, suitable and efficient machinery and effective processing (Anon., 1987*a*, *b*). There are also, however, more traditional products which are processed by secretive and more labour-intensive methods.

Other food and beverage uses

As a sweetener, glycyrrhizin has the limitation of possessing a distinct, and lingering, liquorice taste and researches have been directed at reducing or suppressing the latter whilst maintaining the former (Cook & Gominger, 1974). Whilst ammonium glycyrrhizinate is fifty times sweeter than sucrose, in the presence of that sugar its sweetening power is doubled (Muller, 1966). In contrast, Kobuke *et al.* (1985) have stated that disodium glycyrrhizinate is two hundred times as sweet as refined sugar. Some uses of ammonium glycyrrhizinate have been described by Cook and Gominger (1974). According to these authors there was probably insufficient liquorice root grown and processed to meet the various requirements of industry, and this certainly remains true over a decade later.

The use of liquorice extracts in jams, marmalades and generally as a brown colouring matter has been reported (Nieman, 1957). Liquorice extracts have been employed in the production of beer and spirits, for flavouring purposes and for improving foam-stabilization, 'head-forming' characteristics. Submissions to the FDA in persuance of the protocols for GRAS affirmation (Anon., 1983) also mention the use of liquorice in the formation of a variety of non-liquorice flavours, such as 'fermented', fruit-pulpy', 'root', 'vanilla' and 'cooked, brown and roasted'.

Ammoniated glycyrrhizin has been used in chocolate, chewing gum, in diet cola (to mask the bitter aftertaste of saccharin) and as a sweetener enhancer in gelatin, puddings and cream. Sweet sauces, reconstituted vegetable proteins and herbs and seasons may also benefit from the inclusion of liquorice products as flavour agent or enhancer. Many herbal teas contain liquorice root in addition to a variety of other components, although it should be noted that the use of liquorice to make tea is not included within the provision of 21 CFT Part 184.1408 GRAS affirmation.

Various patents have been granted for the use of liquorice extracts in health drinks (for example, Sanyo-Kokusaku, 1985; Tanaka, 1986) and low-sodium condiments (Tanpei Seiyaku, 1982, 1983). Extracts of liquorice have food application as antioxidants (Maruzen, 1983, 1985; Takagaki, 1988*a*, *b*) and antimicrobial agents (Ueno *et al.*, 1985). In addition to forming the basis for such products, the liquorice residue after the extraction of glycyrrhizin is rich in readily-solubilized polysaccharides ($\sim$20%), relatively insoluble polysaccharides ($\sim$25%), pentosans (14%) and lignin (22%). Technological methods have been described (Akmamedov *et al.*, 1984) for the hydrolysis of such components to mixtures of glucose, xylose, arabinose, glucose and mannose, and for the subsequent use of this hydrolysate in single-cell protein manufacture.

ANALYSIS OF GLYCYRRHIZIN

Writing thirty years ago, Nieman (1957) emphasised the importance of the rapid and specific analysis of glycyrrhizin in liquorice and described a number of methods, almost all of which must now be consigned to the history books. Subsequently, spectrophotometric methods were used (Zwaving, 1975; Steinegger & Marty, 1976), which relied upon the conjugated dienone absorption of the saponin, or on its ability to form a coloured complex with, for example, vanillin/sulphuric acid reagent or modified Liebermann-Buchard reagent. Such methods are, however, time-consuming and not applicable to all liquorice products. Thus despite continued searches for improved accuracy and specificity (e.g. Manyak & Murav'ev, 1984; Liu *et al.*, 1985) attention was turned to gas chromatography, whereby the saponin is hydrolysed to the aglycone, glycyrrhetinic acid (VII) and estimated following derivatisation to the methyl ester or trimethylsilyl derivative. One such procedure has been validated by the AOAC (Association of Official Analytical Chemists, 1984), and a number of others have been used (Larry, 1972; Pohl & Haedrich, 1976; Ventura *et al.*, 1978).

Although the use of GC has been advocated for the simultaneous analysis of the 18α- and 18β- glycyrrhetinic acid hydrolysis products in *Glycyrrhizae radix* (Amagaya *et al.*, 1985), most workers now tend to use HPLC, which does not require a hydrolytic step and hence can measure both glycyrrhizin and glycyrrhetinic acid (Sadlej-Sosnowska, 1987). Amongst the plethora of methods which have been described (including Akada & Tanase, 1976; Ogawa *et al.*, 1976*a*, *b*; Sticher & Soldati, 1978; Beasley *et al.*, 1979; Bell, 1980; Pail *et al.*, 1981; Hirst *et al.*, 1983; Bennetti & Luise, 1985; Collinge *et al.*, 1985; Petitbois *et al.*, 1985; Sagara *et al.*, 1985; Li *et al.*, 1987) that of Vora (1982) has been subjected to collaborative testing, has been included by the Association of Official Analytical Chemists (1984) for 'final action' and is currently acceptable to the FDA in relation to the GRAS status of liquorice products (Anon., 1985). An indication of the probable overestimates of glycyrrhizin contents resulting from application of non-specific methodology is seen in the study of Bell (1980) who compared gravimetric and HPLC methods, the latter yielding figures approximately 30% of the former.

LEVELS OF GLYCYRRHIZIN IN LIQUORICE-CONTAINING PRODUCTS

Data published by the FDA (Anon., 1983) indicate that liquorice root (moisture content 4·5%) contains 11·8% glycyrrhizin whilst block liquorice extract (22·4% moisture) and liquorice extract powder (5·2% moisture) contain 19·5% and 23·7%, respectively. These figures will vary greatly with species, origin, nature of harvesting and subsequent processing. Sagara *et al.* (1985) found the glycyrrhizin contents of Spanish and Chinese liquorice to vary between 1 and 7·6%, with eight samples from China being within the range 2·1–7·6%. Sticher and Soldati (1978) have found 3·4, 6·0 and 5·8% glycyrrhizin in liquorice of Persian, Syrian and Spanish origin, respectively. According to Morton (1977) the glycyrrhizin content of liquorice root may vary from 5 to 20%, with the Russian variety (1–14%) possessing higher levels than that from Spain (6–8%). However, it is probable that the methods employed in the analysis of these samples have overestimated the true figure (see above). Bell (1980) quotes figures of 2·9 and 8·7% for the glycyrrhizin content of two oriental powdered extracts, 3% for root paste, 7·3% for Iranian liquorice extract and 8·3% for liquorice essence (all expressed on a dry weight basis). Three liquorice products analysed in the collaborative trial reported by Vora (1982) possessed glycyrrhizin levels of approximately 2·5, 5·5 and 7·2%.

Amongst thirteen samples of beer analysed by Hermesse *et al.* (1986), ten contained less than 0·6 ppm glycyrrhizin, the remainder having 0·9, 8 and 6 ppm. A range of non-alcoholic drinks contained between 214 and 812 ppm glycyrrhizin whilst drinks containing alcohol were generally rather lower (< 74 ppm), although two such drinks did contain 200 and 234 ppm glycyrrhizin. Confectionery products have been analysed by Hurst *et al.* (1983), Benetti and Luise (1985), Hermesse *et al.* (1986) and Spinks and Fenwick (1990). The former workers found glycyrrhizin levels between 110 and 1650 ppm in North American products, in general agreement with the levels in English confectionery products (Spinks & Fenwick, 1990). Of seventeen Belgian confectionery products examined by Hermesse *et al.* (1986), ten contained > 2200 ppm glycyrrhizin whilst one, liquorice and menthol pearls, contained 4·3% glycyrrhizin and a further 1200 ppm β-glycyrrhetic acid. Liquorice sticks contained 1·2–2·8% (Hermesse *et al.*, 1986) and 1·8–3·6% (Spinks & Fenwick, 1990). Klein and Blasek (1989) have employed isotachophoresis to determine levels of glycyrrhizin in liquorice confectionery and reported 0·2–0·5%; the same technique was used to analyse liquorice powder (2·0–13·7%) and block liquorice (2·2–11·4%).

In a recent survey of Japanese foods (Fujinuma *et al.*, 1988) glycyrrhizic acid was found in 16 out of 56 samples, in tare—a soy sauce product (6·12 ppm); takuan-zuke—a pickle (52–224 ppm); an unspecified sauce (3·8 ppm); smoked squid (135 ppm); the fish product, saki-ika (110 ppm) and macaroni salad (2 ppm). Bell (1980) has reported glycyrrhizin levels in five samples of chewing tobacco to be 1500–4100 ppm, the same general range as found for medicinal preparations (Hermesse *et al.*, 1986). However, one such

TABLE 3
Possible Mean Daily Intakes of Liquorice-Related Substances and Glycyrrhizin in the US (pre-1972)

Substances	*Intake (mg/person/day)*			
	0–5 months	*6–11 months*	*12–23 months*	*2–65+ yrs*
Liquorice root	4·01	83·80	166·38	369·44
Liquorice extract	6·29	67·72	114·53	283·06
Liquorice extract powder	10·79	118·22	206·09	541·86
Ammoniated glycyrrhizin	0·57	5·10	9·09	19·18
Total glycyrrhizin	4·83	56·2	99·8	246·4

Data from Anon. (1974), total glycyrrhizin data calculated by present authors on the basis of compositional data in Anon. (1983).

preparation, a liquorice infusion, contained very much higher (3·8%) levels. A single sample of herbal tea examined by Sadlej-Sosnowska (1987) contained 5000 ppm of glycyrrhizin and 480 ppm of 18β-glycyrrhetinic acid. The same worker found 8·4% glycyrrhizin and 8900 ppm 18β-glycyrrhizinic acid in concentrated extract of liquorice root (*Extractum Glycyrrhizae Siccum*).

DIETARY EXPOSURE TO LIQUORICE AND GLYCYRRHIZIN

Information on the likely daily intake of liquorice-containing products is difficult to obtain. As part of the detailed background information made available to the Food and Drug Administration (see Anon., 1977), data were provided which suggested the mean daily intakes shown in Table 3. Whilst there are undoubtedly individuals consuming very high levels of liquorice on a regular basis (see Reynolds, 1982) it is probable that the figures in Table 3 are the original suppliers indicated, very substantial overestimates since they were collected not in response to any particular concern about liquorice consumption but in regard to interest in overall US consumer dietary patterns.

Calculation of US daily intake data, for a decade ago, based upon the totals of liquorice products used, yields figures of 0·10, 0·44, 1·79 and 0·11 mg for liquorice root, extract, powdered extract and ammoniated glycyrrhizin, respectively. In combination, these correspond to an intake of 0·63 mg glycyrrhizin/person/day. If the availability of liquorice for food consumption is used as an index for calculating the US daily intakes, the figures obtained were 27·2 mg liquorice root and 0·61 mg liquorice extract, corresponding to 3·21 and 0·12 mg glycyrrhizin/person/day, respectively. It was considered by the Select Committee on GRAS Substances that these figures were probably more realistic than those presented earlier. Elsewhere, Nieman (1974) has reported that in The Netherlands, where liquorice confectionery is widely eaten, annual consumption exceeded 1 kg, suggesting perhaps a mean daily intake of 4–5 g. In the absence of detailed analytical data the glycyrrhizin content and, hence, intake cannot be reliably stated. However, based upon the later data of Hermesse *et al.* (1986), a mean daily intake of 5 mg glycyrrhizin may be suggested for Belgium, a similar figure recently being calculated for the UK (Spinks & Fenwick, 1990).

LEGAL STATUS OF LIQUORICE (GLYCYRRHIZIN) IN FOODS

The FDA (Anon., 1977) proposed affirmation of GRAS status for liquorice root, extract and ammoniated glycyrrhizin when used as a flavour agent to impart liquorice flavour to specified foods and subject to maximum levels of usage. Later (Anon., 1979) provision was made for the use of ammoniated glycyrrhizin as a surface-active agent in non-alcoholic beverages. However, usage as a sweetener, flavour enhancer or sweetening synergist was denied because the application was not self-limiting and a greater consumption could not be supported by the existing safety data.

The recognition that the major biologically-active principle in liquorice root, block, liquid or powdered extract is glycyrrhizin, and that this may be replaced by ammoniated glycyrrhizin preparations in certain product formulations, has resulted in the limits to the usage of liquorice products being defined for GRAS purposes in terms of total glycyrrhizin content (Table 4)—thereby permitting liquorice-derived products to be used interchangeably or in any combination (Anon., 1983, 1985).

The Council of Europe Report on Flavouring Substances and Natural Sources of Flavourings (1981) classifies liquorice as a 'plant or part thereof, including herbs, spices and seasonings commonly added to foodstuffs in small quantities, the use of which is considered acceptable with a possible limitation of an active principle in the final product'. Glycyrrhizin is also included (No. 2221) as a flavouring substance subject to a limit in foods and beverages of 50 ppm. Liquorice roots or wood is considered by the UK Food Additives and Contaminants Committee (1976) as a 'natural flavouring substance consisting of, or deriving from, vegetables, herbs or spices to be used in small quantities as additives to food provided that it is not used in amounts exceeding those occurring naturally in food'. In this context 'small quantities' were again defined as less than 50 ppm in the food.

Glycyrrhizin was assessed by the European Commission's Scientific Committee for Food as part of its review of safety aspects of the use of sweeteners. The UK Food Additives and Contaminants Committee in its

TABLE 4
Specific Limitations for GRAS Affirmation of Glycyrrhizin, 21 CFR Part 182 (Anon., 1983, 1985)

Category of food	*Max. level of glycyrrhizin in food (%)*	*Functional use*[a]
Baked goods	0·05 (500 ppm)	1, 2
Alcoholic beverages	0·1 (1 000)	1, 2, 3
Non-alcoholic beverages	0·15 (1 500)	1, 2, 3
Chewing gum	1·1 (11 000)	1, 2
Hard candy	16·0 (160 000)	1, 2
Soft candy	3·1 (31 000)	1, 2
Herbs and seasonings	0·15 (1 500)	1, 2
Plant protein products	0·15 (1 500)	1, 2
Vitamin or mineral dietary supplements	0·5 (5 000)	1, 2
All other foods, except sugar substitutes	0·1 (1 000)	1, 2

[a] 1 Flavour enhancer; 2 flavouring agent; 3 surface active agent.

evaluation of sweeteners in food had earlier (1976) decided that the primary function of glycyrrhizin was as a flavouring rather than a sweetener. The Scientific Committee's report (Poulsen, 1985) stated that the available data were inadequate for a toxicological evaluation of the substance as a sweetener, but the findings from clinical toxicology revealed a possible need to restrict consumption of liquorice. The Scientific Committee was unable to endorse the use of glycyrrhizin as a sweetener and suggested further assessment of usage.

The FDA has taken the view that 'adverse effects of glycyrrhizin are generally associated with the consumption of foods that are characterised by a distinctive liquorice flavour, such as liquorice-flavoured candies, liquors or other beverages. These foods contain higher levels of glycyrrhizin than do foods in which the liquorice or ammoniated glycyrrhizin has been added as a flavour enhancer. Persons who are sensitive to glycyrrhizin can avoid experiencing glycyrrhizin-induced symptoms by excluding liquorice-flavoured foods from their diets. FDA believe that the levels of glycyrrhizin contained in foods do not pose a hazard to the public provided that foods that contain glycyrrhizin are not consumed in excessive quantities or by individuals who are sensitive to low levels of glycyrrhizin' (Anon., 1983).

There have, however, been reports of human fatalities and illness following the consumption ('excessive' or 'prolonged') of liquorice-containing products (see Lutomski *et al.*, 1990) and this reinforces the need for public and medical awareness, especially since there is now an increasing range of confectionery, beverage, herbal and medicinal products available which may contain liquorice and, hence, contribute to the total glycyrrhizin intake.

Since virtually all such reports record consumption levels well over the toxicity threshold, they provide no information as to the level of liquorice confectionery that can be safely consumed. Calculation of the toxicity of glycyrrhizin is also difficult since, where levels of this chemical have been measured, they are now considered to be considerable over-estimates.

Recently, however, the Dutch Nutrition Information Bureau has advised against a daily glycyrrhizin consumption in excess of 200 mg, assumed to correspond to 150 g liquorice confectionery (Anon., 1988). This recommendation should be seen in the context of The Netherlands having the highest daily *per capita* consumption of liquorice confectionery. Assuming the maximum levels allowable according to GRAS legislation, the following daily consumption would be needed to provide a daily intake of 200 mg glycyrrhizin–400 g baked goods, 200 ml alcoholic beverages, 140 ml non-alcoholic beverages, 20 g chewing gum, 1 g hard candy, 6 g soft candy, 140 g herbs and seasonings, 140 g plant protein or 40 g vitamin or mineral supplement. Ten grams of liquorice stick (average content 20 000 ppm glycyrrhizin) would yield the same intake. In combination, of course, less would be required of each individual dietary component.

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REFERENCES

Akada, Y. & Tanase, Y. (1976). High speed chromatographic determination of glycyrrhizin in liquorice roots and extracts. *Yakugaku Zasshi*, **96**, 1035–7.

Akmamedov, K., Annadurdyev, K., Dovletmuradov, K., Varganov, L. A., Klimova, Z. K., Belen'kii, S. I. & Mamedniyazov, O. N. (1984). Chemical composition and hydrolysis of liquorice oilcake. *Izv. Akad. Nauk. Tadzh SSR, Otd, Biol. Nauk.*, **3**, 73–6. *Chemical Abstracts*, 1985, **102**, 22782h.

Amagaya, S., Sugishita, E., Ogihara, Y., Ogawa, S., Okada, K. & Aizawa, T. (1985). Separation and quantitative analysis of 18α-glycyrrhetinic acid and 18β-glycyrrhetinic acid in *Glycyrrhizae radix* by gas liquid chromatography. *J. Chromatogr.*, **320**, 430–4.

Amirova, G. S. & Kir'yalov, N. P. (1989). On the chemotaxonomy of *Glycyrrhiza* L. and *Meristotropis* Fisch. et Mey Genera. *Zh. Obshch. Biol.*, **50**, 184–8; *Chemical Abstracts* 1989, **111**, 36668y.

Anon. (1974). Evaluation of the health aspects of liquorice, *Glycyrrhiza* and ammoniated glycyrrhizin as food ingredients. Contract FDA 72-85, Life Science Research Office, FASEB, Bethesda, MD.

Anon. (1977). Licorice, glycyrrhiza and ammoniated glycyrrhizin. Proposed affirmation of GRAS status with special limitations as direct human food ingredients. *Federal Register*, **42**, 39117–20.

Anon. (1979). Ammoniated glycyrrhizin. Proposed affirmation of GRAS status as direct human food ingredient with specific limitations amendment. *Federal Register*, **44**, 28334–5.

Anon. (1983). GRAS status of licorice (*Glycyrrhiza*), ammoniated glycyrrhizin and monoammonium glycyrrhizinate. *Federal Register*, **48**, 54983–90.

Anon. (1985). GRAS status of licorice (*Glycyrrhiza*), ammoniated glycyrrhizin and monoammonium glycyrrhizinate. *Federal Register*, **50**, 21043–5.

Anon. (1987*a*). Preparing liquorice sheets, rods and shapes. *Confectionery Production*, **53**, 714–15.

Anon. (1987*b*). Recipes for liquorice sheets, rods and shapes. *Confectionery Production*, **53**, 782–7.

Anon. (1988). Suikerwerk (glycyrrhizinezuur). *Voedingsinform*, **11**, 22–3.

Association of Official Analytical Chemists (1984). *Official Methods of Analysis*. (14th edn), ed. S. Williams. AOAC, Arlington, VA, paras 19.136–19.145.

Beasley, T. H., Ziegler, H. W. & Bell, A. D. (1979). Separation of the major components in liquorice using high performance liquid chromatography. *J. Chromatogr.*, **175**, 350–5.

Bell, J. H. (1980). Determination of glycyrrhizic acid in liquorice extracts and chewing tobaccos. *Tobacco Science*, **24**, 126–9.

Benetti, G. & Luise, M. (1985). Determination of glycyrrhizic acid in liquorice products by HPLC. *Bull. Chim. Ig. Parte Sci.*, **36**, 81–5.

Bhardwaj, D. K., Murari, R., Seshadri, R. & Singhr, R. (1976). Liqcoumarin, a novel coumarin from *Glycyrrhiza glabra*. *Phytochem.*, **15**, 1182–3.

Čikov, P. S. (1976). Atlas arealov i resursov lekarstvennych rastenij SSSR. GUGK, Moskva, pp. 146–7, 301–2.

Collinge, A., Hermesse, B. & Noirfalise, A. (1985). Recherche et dosage part chromatographie liquide à haut pouvoir de resolution de la glycyrrhizine dans les denrées alimentaires. *Belg. J. Food Chem. Biotechnol.*, **40**, 143–5.

Council of Europe (1981). *Flavouring Substances and Natural Sources of Flavourings* (3rd edn). Council of Europe, Strasbourg, France.

Cook, M. K. & Gominger, B. H. (1974). Glycyrrhizin. In *Symposium: Sweeteners*, ed. G. E. Inglett. AVI, Westport, CT, pp. 211–15.

Demizu, S., Kajiyama, K., Takahashi, K., Hiraga, Y., Yamamoto, S., Tamura, Y., Okada, K. & Kinoshita, T. (1988). Oxidant and antimicrobial constituents of licorice: Isolation and structure elucidation of a new benzofuran derivative. *Chem. Pharm. Bull.*, **36**, 3474–9.

Duke, J. A. (1981). *Handbook of Legumes of World Economic Importance*. Plenum Press, New York.

Duke, J. A. (1985). *Handbook of Medicinal Herbs*. CRC Press, Boca Raton, FA.

Elgamel, M. H. A. & Fayez, M. B. E. (1972). Isolation of formononetin from roots of *Glycyrrhiza glabra* Linn. collected locally. *Ind. J. Chem.*, **19**, 128.

Esaki, S., Konishi, F. & Kamiya, S. (1978). Synthesis and taste of some glycosides of glycyrrhetic acid. *Agric. Biol. Chem.*, **42**, 1599–600.

Food Additives and Contaminants Committee (1976). *Report on the Review of Flavourings in Food.* FAC/REP/22, HMSO, London.

Frattini, C., Bicchi, C., Barettini, C. & Nano, G. M. (1977). Volatile flavor components of licorice. *J. Agric. Food Chem.*, **25**, 1238–41.

Fuggersberger-Heinz, R. & Franz, G. (1984). Formation of glycyrrhizinic acid in *Glycyrrhiza glabra* var. *typica. Planta Med.*, **50**, 409–13.

Fujinuma, K., Saito, K., Nakazato, M., Kikuchi, Y., Ibe, A. & Nishima, T. (1988). Determination of glycyrrhizic acid in foods. *Shokuhir Eiseigaku Zasshi*, **29**, 210–15; *Chemical Abstracts* 1988, **109**, 228867t.

Furuya, T., Matsumoto, K. & Hikichi, M. (1971). Echinatin, a new chalcone from tissue culture of *Glycyrrhiza echinata. Tetrahedron Letters*, 2567–9.

Gibson, M. R. (1978). Glycyrrhiza in old and new perspectives. *Lloydia*, **41**, 348–54.

Hattori, M., Miyachi, K., Shu, Y.-Z., Kakiuchi, N. & Namba, T. (1986). Studies on dental caries prevention by traditional medicines (IX). Potential antibacterial action of coumarin derivatives from licorice roots against *Streptococcus mutans. Shoyakugaku Zasshi*, **40**, 406–12.

Hayashi, H., Fukui, H. & Tabata, M. (1988). Examination of triterpenoids produced by callus and cell suspension cultures of *Glycyrrhiza glabra. Plant Cell Rep.*, **7**, 508–11.

Hermesse, B., Collinge, A. & Noirfalise, A. (1986). Taux de glycyrrhizine présents dans quelques denrées alimentaires, *Arch. B. Med. Soc. Hyg., Méd. Tr. et Méd. Lég.*, **44**, 60–7.

Hilliam, M. A. (1985). *Food Market Updates. 9. Confectionery in the UK.* Leatherhead Food RA, March.

Hodge, J. E. & Inglett, G. E. (1974). Structural aspects of glycosidic sweeteners containing (1→2)-linked disaccharides. In *Symposium: Sweeteners*, ed. G. E. Inglett. AVI, Westport, CT, pp. 216–34.

Hoffmann, H., Mauch, W. & Untze, W. (1985). *Zucker und Zuckerwaren.* Verlag Paul Parey, Berlin, Germany, pp. 204–10.

Hurst, W. J., KcKim, J. M. & Martin, R. A., Jr (1983). High performance liquid chromatographic determination of glycyrrhizin in liquorice products. *J. Agric. Food Chem.*, **31**, 387–9.

Kameoka, H. & Nakai, K. (1987). Components of essential oil from the root of *Glycyrrhiza glabra. Nippon Nogeikagaku Kaishi*, **61**, 119–21.

Kaneda, M., Saitoh, T., Itaka, Y. & Shibata, S. (1973). Chemical studies on the oriental plant drugs. XXXVI. Structure of the licoricone, a new isoflavone from liquorice root. *Chem. Pharm. Bull.*, **21**, 1338–41.

Kąttaev, N. Sh. & Nikonov, G. K. (1972). Glabranin–A new flavanone from *Glycyrrhiza glabra. Kkim. Prir. Soedin.*, 805–6.

Kattaev, N. Sh. & Nikonov, G. K. (1974). Flavonoids of *Glycyrrhiza glabra. Khim. Prir. Soedin.*, 93.

Kinoshita, T., Saitoh, S. & Shibata, S. (1976). The occurrence of an isoflavene and the corresponding isoflavone in liquorice root. *Chem. Pharm. Bull.*, **24**, 991–4.

Kinoshita, T., Saitoh, S. & Shibata, S. (1978*a*). A new 3-arylcoumarin from liquorice root. *Chem. Pharm. Bull.*, **26**, 135–40.

Kinoshita, T., Saitoh, S. & Shibata, S. (1978*b*). A new isoflavone from liquorice root. *Chem. Pharm. Bull.*, **26**, 141–3.

Kitagawa, I., Zhou, J. L., Sakagami, M., Taniyama, T. & Yochikawa, M. (1988). Licorice-saponins A3, B2, C2, D3 and E2, five new oleanene-type triterpene oligosaccharides from Chinese *Glycyrrhizae radix*. *Chem. Pharm. Bull.*, **36**, 3710–13.

Kitagawa, I., Sakagami, M., Hashiuchi, F., Zhou, J. L., Yoshikawa, M. & Ren, J. (1989). Apioglycyrrhizin and araboglycyrrhizin, two new sweet oleanene-type triterpene oligoglycosides from the root of *Glycyrriza inflata*. *Chem. Pharm. Bull.*, **37**, 551–3.

Klein, H. & Blasek, K. (1989). Beitrag zur Bestimmung von Glycyrrhizin in Lakritzwaren mittels Isotachophorese. *Ernährung*, **13**, 82–6.

Kobuke, T., Inai, S., Nambu, K., Ohe, T., Takemoto, T., Matsuki, K., Nishina, H., Huang, I. B. & Tokuoka, S. (1985). Tumorigenicity study of disodium glycyrrhizinate adminstered orally to mice. *Food Chem. Toxicol.*, **23**, 979–83.

Larry, D. M. (1972). Quantitative determination of ammonium glycyrrhizinate by gas liquid chromatography: Collaborative study. *J. Assoc. Off. Anal. Chem.*, **55**, 570–2.

Lees, R. & Jackson, E. B. (1973). *Sugar Confectionery and Chocolate Manufacture*. Leonard Hill, London, pp. 269–85.

Leung, A. Y. (1980). *Encyclopedia of Common Natural Ingredients Used in Foods, Drugs and Cosmetics*. John Wiley and Sons, New York.

Li, R., Wang, Q., Dou, Y. & Pei, Y. (1987). Determination of glycyrrhizin in liquorice (*Glycyrrhiza uralensis*) and its extraction by HPLC. *Zhongcaoyao*, **18**, 157–8; *Chemical Abstracts*, 1987, **108**, 104744r.

Litwinienko, W. I. & Nabiezyna, T. P. (1972). Flawonoidy nadzemnoj czasti solodko goloi. *Rast. Res.*, **8**, 35–42.

Litwinieko, W. I. & Obolencova, G. W. (1964). Chimiczeskoje I farmakologiczeskoje issledowanie flawonoidow solodki goloi (*Glycyrrhiza glabra* L.) I Solodki Uralskoj (*Glycyrrhiza uralensis* Fischm.). *Med. Prom.*, **18**, 20–3.

Liu, L., Pan, J., Yin, X. & Lu, Y. (1985). Quantitative determination of glycyrrhizin in *Glycyrrhiza* and its preparations by macro-reticular resin, *Zhongcaoyao*, **16**, 202–6, *Chemical Abtracts*, 1985, **103**, 59374y.

Lucas, R. (1966). *Nature's Medicines*. Parker Publishing Co., West Nyack, NY.

Lutomski, J. (1983). Chemie und therapeutische Verwendung von Süssholz (*Glycyrrhiza glabra* L.). *Pharmazie in unserer Zeit*, **12**, 49–54.

Lutomski, J., Nieman, C. & Fenwick, G. R. (1990). Chemical and biological properties of liquorice, *Glycyrrhiza glabra* L. Part II: Biological effects. *Human Nutrition, Food Sciences and Nutrition* (to be submitted).

Lythgoe, B. & Trippett, S. (1950). The constitution of the disaccharide of glycyrrhinic acid. *J. Chem. Soc.*, 1983–90.

Manyak, V. A. & Murav'ev, I. A. (1984). use of sulphostyrene cationic resin exchanger KU-2-8 for improved accuracy of spectrophotometric determination of glycyrrhizic acid in *Glycyrrhiza* roots and dry extract. *Farmatsiya (Moscow)*, **33**, 31–3.

Maruzen Chemical Co. Ltd (1983). Food antioxidants from liquorice. *Japanese Patent*, 58, 217, 583 (17 December 1983); *Chemical Abstracts* 1984, **100**, 119655r.

Maruzen Chemical Co. Ltd (1985). Food antioxidants from liquorice extracts and gallic acid. *Japanese Patent*, 60, 44, 580 (9 March 1985); *Chemical Abstracts* 1985, **103**, 86795t.

Miething, H. & Speicher-Brinker, A. (1989). Neolicurosid, ein neues Chalkonglykosid aus der Süssholzwurzel. *Arch. Pharm. (Weinheim)*, **322**, 141–3.

Minifie, B. N. (1982) *Chocolate, Cocoa and Confectionery: Science and Technology* (2nd edn). AVI, Westport, CT, pp. 468–70.

Mitscher, L. A., Park, Y. H., Clark, D. & Beal, J. L. (1980). Antimicrobial agents from higher plants. Antimicrobial isoflavonoids and related substances from *Glycyrrhiza glabra* L. var. *typica*. *J. Nat. Products*, **43**, 259–69.

Moore, J. G., Krotoszynski, B. K. & O'Neill, H. J. (1984). Fecal odorgrams. A method for partial reconstruction of ancient and modern diets. *Dig. Dis. Sciences*, **22**, 907–11.

Morton, J. (1977). *Major Medicinal Plants*. Charles C. Thomas, Springfield, IL.

Muchnik, Z. S. (1976). Content of glycyrrhizic acid, sugars and extractive substances from the subsoil organs of liquorice cultivated in Moldavia. *Ratit. Resur.*, **12**, 78–84.

Muller, R. E. (1966). Sucrose-ammoniated glycyrrhizin sweetening agent. *US Patent* 3, 282, 706, 1 November 1966.

Nieman, C. (1957). Licorice. *Advances in Food Research*, **7**, 339–81.

Nieman, C. (1974). Licorice confectionery. In *Encyclopaedia of Food Technology Volume 2*, ed. A. H. Johnson & M. S. Peterson. AVI, Westport, CT, pp. 550–3.

Ogawa, S., Yoshida, A. & Mitani, Y. (1976*a*). Determination of glycyrrhizin in *Glycyrrhizae radix* by high speed liquid chromatography. *Yakugaku Zasshi*, **96**, 122–4.

Ogawa, S., Yoshida, A. & Mitani, Y. (1976*b*). Determination of glycyrrhizin in pharmaceutical preparations including *Glycyrrhiza radix* by high speed liquid chromatography. *Yakugaku Zasshi*, **96**, 1488–91.

Paik, N. H., Park, M. K., Han, D. S., Kim, W. S. & Kang, T. L. (1981). Studies on glycyrrhizin and glycyrrhetinic acid content in liquorice-*Triticium-Zizyphus* compound examined by high performance liquid chromatography. *Seoul Taehakkyo Yakhak Nonmunjip*, **6**, 33–8; *Chemical Abstracts* 1983, **98**, 87789j.

Perrot, E. M. (1944). *Matières Premieres usuelles du Règne Végétal. Vol. 2*. Masson et Cie, Paris, France.

Petitbois, D., Hamann, Y., Lesgards, G. & Bozzi, R. (1985). Étude analytique des produits issus de la réglisse. Choix d'un système chromatographique pour la détermination de la teneur en acide glycyrrhizique comparaison avec la méthode gravimétrique traditionelle. *Ann. Fals. Chim.*, **78**, 19–31.

Pohl, P. & Haedrich, W. (1976). Eine neue Methode zur quantativen Bestimmung der Glycyrrhizinsäure in *Radix liquiritae*. *Deut. Apoth.-Zt.*, **116**, 625–7.

Poulsen, E. (1985). Report of the Scientific Committee for Food: Sweeteners. *Rep. Sci: Committee Food* (Brussels), **16**, 1–20.

Price, K. R., Fenwick, G. R. & Johnson, G. R. (1987). The chemistry and biological significance of saponins in foods and feeding stuffs. *CRC Crit. Rev. Food Sci. Nutr.*, **26**, 27–135.

Puri, B. & Seshadri, T. R. (1954). Survey of anthoxanthins: Part V—Colouring matter of liquorice roots. *J. Sci. Ind. Res.*, **13B**, 475–80.

Reiners, W. (1964). Cumarine und Hydroxyzimtsäuren aus Süssholzwurzel. *Naturwissenschaften*, **51**, 193.

Reynolds, J. E. F. (ed.) (1982). *Martindale, The Extra Pharmacopoeia* (28th edn). Pharmaceutical Press, London, pp. 691–2.

Ruzicka, L., Jeger, O. & Ingold, W. (1943*a*). Neuer Beweis für die verschiedene Lage der Carboxylgruppe bei der Oleanolsäure und der Glycyrrhetinsäure. *Helv. Chem. Acta*, **26**, 2278–82.

Ruzicka, L., Jeger, O. & Winter, M. (1943*b*). Zur Lage der Carboxylgruppe bei der Oleanolsäure und der Glycyrrhetinsäure. *Helv. Chim. Acta*, **26**, 265–79.

Ruzicka, L., Van der Sluys-Veer, F. C. & Jeger, O. (1943*c*). Uber die Pyrolyse des Isooleanon-lactondisäure-monomethylesters. *Helv. Chim. Acta*, **26**, 280–6.

Sadlej-Sosnowska, N. (1987). Determination of glycyrrhizinic and glycyrrhetinic acids in pharmaceuticals by high-performance liquid chromatography. *J. Pharmaceut. Biomed. Anal.*, **5**, 289–93.

Sagara, K., Ito, Y., Oshima, K., Kawaura, M. & Misaki, T. (1985). Application of ion-pair high performance liquid chromatography to the analysis of glycyrrhizin in *Glycyrrhizae radix*. *Chem. Pharm. Bull.*, **33**, 5364–68.

Saitoh, T. & Shibata, S. (1969). Chemical studies on the oriental plant drugs. XXII. Some new constitutents of liquorice root. 2. Glycerol, 5-*O*-methylglycerol and isoglycerol. *Chem. Pharm. Bull.*, **17**, 729–34.

Saitoh, T. & Shibata, S. (1975). New type chalcones from liquorice root. *Tetrahedron Letters*, 4461–2.

Saitoh, T., Kinoshita, T. & Shibata, S. (1976*a*). New isoflavan and flavanone from liquorice root. *Chem. Pharm. Bull.*, **24**, 752–5.

Saitoh, T., Kinoshita, T. & Shibata, S. (1976*b*). Flavonols of liquorice root. *Chem. Pharm. Bull.*, **24**, 1242–5.

Saitoh, T., Noguchi, T. & Shibata, S. (1978). A new isoflavone and the corresponding isoflavanone of liquorice root. *Chem. Pharm. Bull.*, **26**, 144–7.

Sanyo-Kokusaku Pulp Co. (1985). Health drink. *Japanese Patent 60*, 126, 074 (5 July 1985); *Chemical Abstracts* 1986, **104**, 4882c.

Seibl, W. & Ludewig, H.-G. (1979). Mehlqualität bei der Lakritzherstellung. *Zucker u. Süsswarenwirtsch.*, **32**, 424, 439–40.

Serrano, M. (1946). Contribución al estudio del regaliz español. *Farmacognosia*, **5**, 9–74.

Shibata, S. & Saitoh, T. (1968). The chemical studies on oriental plant drugs. XIX. Some new constituents of liquorice root 1. The structure of licoricidin. *Chem. Pharm. Bull.*, **16**, 1932–6.

Shiozawa, T., Urata, S., Kinoshita, T. & Saitoh, T. (1989). Revised structures of glycyrol and isoglycyrol, constituents of the roots of *Glycyrrhiza uralensis*. *Chem. Pharm. Bull.*, **37**, 2239–41.

Siskin, B. K. (1963). Botaniceskij Atlas, Izdatelstvo Selskochazajst. Literatury, Moskva, Leningrad, pp. 120–1.

Spinks, E. A. & Fenwick, G. R. (1990). The glycyrrhizin content of selected UK liquorice products. *Food Additives Contam.* (in press).

Steinegger, E. & Marty, S. (1976). Glycyrrhizinsäurebestimmung in *Radix liquiritae*. Vergleich bisheriger Methoden. *Pharm. Acta Helv.*, **51**, 374–7.

Sticher, O. & Soldati, F. (1978). Glycyrrhizinsäurebestimmung in *Radix liquiritae* mit Hochleistungs-Flussigkeitschromatographie (HPLC). *Pharm. Acta Helv.*, **53**, 46–52.

Takagaki, R. (1988*a*). Antioxidant activity of sankanon, an extract of licorice, and its use. *Gekkan Fudo Kemikaru*, **4**, 75–81; *Chemical Abstracts* 1988, **109**, 169024r.

Takagaki, R. (1988*b*). Antioxidant from liquorice roots and its use as a stabilizer for hardened oil. *Japanese Patent* 63, 110, 290 (14 May 1988); *Chemical Abstracts* 1989, **110**, 153082t.

Tanaka, K. (1986). Effervescent refreshing drink. *Japanese Patent*. 61, 92, 554 (10 May 1986); *Chemical Abstracts* 1986, **105**, 96298p.

Tanpei Seiyaku, K. K. (1982). Low-sodium salt substitute composition. *Japanese Patent*, 57, 186, 108 (16 November 1982); *Chemical Abstracts* 1983, **98**, 105997g.

Tanpei Seiyaku, K. K. (1983). Low-sodium condiment composition. *Japanese Patent* 58, 81, 758 (17 May 1983); *Chemical Abstracts* 1983. **99**, 193452z.

Thorwald, J. (1966). *Histoire Médecine Antiquité*. Hachette, Paris, France.

Tokiwa Shokubutsu Kagaku Kenkyusho Ltd (1981). A triterpenoid glyceride sweetener from liquorice. *Japanese Patent* 81, 161, 400 (11 December 1981); *Chemical Abstracts* 1982, **97**, 90729z.

Toulemonde, B., Mazza, M. & Bricout, J. (1977). Sur la composition de l'arome du rhizome de *Glycyrrhiza glabra* L. *Ind. Aliment. Agricol.*, 1179–82.

Tschirch, A. & Cederberg, H. (1907). Glycyrrhizin. *Arch. Pharmazie*, **21245**, 97–111.

Uchida, M., Wada, M., Nakamura, H., Ishihara, S., Mano, N. & Komoda, Y. (1988). Isolation of triterpene glycoside and sweeteners containing it. *Japanese Patent* 63,267,795 (4 November 1988); *Chemical Abstracts* 1989. **110**, 133965e.

Ueno, R., Matsuda, T., Tago, K., Kunimi, Y. & Kanayama, T. (1985). Antimicrobial agents with solubilizers for food preservation. *Japanese Patent*, 60,233,015 (19 November 1985); *Chemical Abstracts* 1986. **104**, 187375p.

Ventua, P., Visconti, M. & Pifferi, G. (1978). A simple GLC analysis of ammonium glycyrrhizinate. *Boll. Chim. Farm.*, **117**, 217–21.

Vondenhof, T., Glombitza, K.-W. & Steiner, M. (1973). Lösliche Kohlenhydrate in Rohlakritzen (*Succus liquiritiae*). *Z. Lebens. Unters. Forsch.*, **152**, 345–7.

Vora, P. S. (1982). High pressure liquid chromatographic determination of glycyrrhizic acid or glycyrrhizic acid salts in various liquorice products: Collaborative study. *J. Assoc. Off. Anal. Chem.*, **65**, 572–4.

Wheelwright, E. G. (1974). *Medicinal Plants and Their History*. Dover Publications, New York.

Yang, S.-L. & Liu, Y.-L. (1988). Chemical constituents of *Glycyrrhiza inflata* Bat. *Acta Bot. Sinica*, **30**, 176–82.

Zwaving, J. H. (1975). Comparative investigation of some methods for the determination of glycyrrhizin in liquorice root and its preparations. *Pharamaceut. Weekblad*, **110**, 873–9.