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Extraction of Technical Glycyrrhizic Acid from Licorice (Glycyrrhiza glabra L.) Root and Synthesis of Its Copper Supramolecular Complex

Islambek B. Ganiev
Director, "G'aniyevlar" LLC
Phone: +998 99 965 34 11; E-mail: islamganiyev318@gmail.com

Abstract

Licorice (Glycyrrhiza glabra L.), locally known as "boyan," is one of the most widely used medicinal plants of Central Asia, and its principal bioactive triterpene saponin, glycyrrhizic acid, is of substantial pharmacological and agrochemical interest. This study reports the extraction of technical glycyrrhizic acid from the thick aqueous extract of licorice root cultivated and growing wild in the central districts of the Republic of Karakalpakstan, its conversion to ammonium-salt derivatives, and the synthesis of a copper-containing supramolecular complex intended for use as an agricultural biostimulant. Technical glycyrrhizic acid was precipitated from the root extract with dilute sulfuric acid; systematic variation of the acid volume showed that the precipitation yield is strongly pH-dependent, reaching a maximum of 21.8% at pH ≈ 2.9, with an overall technical-acid yield of 35–40% relative to the starting extract. Acetone extraction followed by treatment with ammonia gave the triammonium salt (32% yield), which was further converted to the monoammonium salt (88% yield) by recrystallization from ethanol; the purified acid's melting point (222 ± 2 °C) and optical rotation ([α]D25 = +60 ± 2°) matched literature values. Potentiometric titration showed that a 97%-purity monoammonium salt sample contained close to the theoretical three free carboxyl groups per molecule, comparing favourably with a 75%-purity commercial reference preparation. The monoammonium salt was subsequently used to synthesize a copper supramolecular complex (CuproTGK) whose formation through host–guest, hydrogen-bonded, and hydrophobic interactions was confirmed by infrared spectroscopy. These results establish a reproducible, low-cost extraction and derivatization route to pharmaceutical- and agricultural-grade glycyrrhizic acid preparations from regionally grown licorice.

Keywords: glycyrrhizic acid, licorice, Glycyrrhiza glabra, supramolecular complex, copper complex, natural product extraction, host–guest chemistry

1. Introduction

The root of licorice (Glycyrrhiza glabra L.; "boyan" in Karakalpak and Uzbek, "solodka golaya" in Russian) has been used as a medicinal raw material for millennia, with documented use in Chinese medicine dating back some 2,800 years and detailed description in Avicenna's Canon of Medicine [2]. Among medicinal plants, licorice root occupies an especially broad range of therapeutic application, owing chiefly to its content of glycyrrhizic acid, a triterpene glycoside that can constitute up to 23% of the root's dry mass and that underlies the root's traditional use as an expectorant, an anti-inflammatory and antiulcer agent, a hepatoprotective and choleretic remedy, and an antidote in cases of food or snake-venom poisoning [2, 3, 11]. This broad pharmacological profile, combined with a favourable toxicological safety margin relative to many synthetic agents, has sustained continuous scientific interest in licorice-derived compounds [13, 14, 15, 16].

The flora of Karakalpakstan is unusually rich in medicinal herbs, and licorice, sometimes referred to locally as the "golden root" for its economic value, is a characteristic and abundant component of that flora, occurring extensively along the lower reaches of the Amu Darya and throughout the delta region [4, 5, 7]. Government policy has increasingly recognized this resource: the Presidential Decree of 10 April 2020, "On measures for the protection of wild-growing medicinal plants, their cultivation, processing, and rational use of available resources," and the earlier Resolution of the Cabinet of Ministers of 14 April 2017 encouraging licorice export, together with the Action Strategy for the Further Development of the Republic of Uzbekistan (2017–2021), have created considerable institutional momentum behind deeper local processing of licorice raw material rather than its export as unprocessed root [1]. More than ten joint ventures engaged in licorice processing have since been established in the Republic of Karakalpakstan, underscoring

both the commercial opportunity and the corresponding need for standardized, well-characterized extraction methodology suited to regionally grown raw material.

At the same time, the development of the chemical industry as one of the sectors underpinning national economic stability is closely tied to the extraction of natural compounds — alkaloids, antibiotics, vitamins, hormones, and enzymes — from local medicinal plant resources for use in chemical technology, pharmacology, the food industry, and agriculture. Natural compounds isolated from local raw material offer a route to environmentally benign agrochemical products capable of improving soil productivity and protecting crops from adverse growth factors, an objective of particular relevance in the saline-soil conditions that prevail across much of the Aral Sea region. Realizing this potential, however, first requires a reliable and economically viable method for extracting glycyrrhizic acid, in sufficient purity, from regionally sourced licorice root, and for converting it into derivatives, such as metal-ion supramolecular complexes, suited to specific pharmaceutical or agrochemical applications.

The present study addresses this need. Its aims were: (i) to extract technical glycyrrhizic acid from the thick aqueous extract of licorice root grown, both wild and under cultivation, in the central districts of the Republic of Karakalpakstan, and to determine the reaction conditions that maximize its yield; (ii) to convert the technical acid into its triammonium and monoammonium salts and to characterize the purified product against literature reference values and against a commercial licorice-derived pharmaceutical preparation; and (iii) to synthesize, from the purified glycyrrhizic acid derivative, a copper-containing supramolecular complex intended for subsequent evaluation as an agricultural biostimulant.

2. Literature Review

2.1. Botanical Distribution and Phytochemical Background of Licorice

Within Central Asia, Glycyrrhiza glabra L. is distributed across the Syr Darya, Zarafshan, Chirchiq, and Angren river basins, the lower Amu Darya, and the territories of Khorezm and Karakalpakstan, with its principal Amu Darya delta plantations belonging predominantly to this species [5, 8]. Geobotanical surveys of the lower Amu Darya region have characterized the plant's morphological structure, its ecological-phytocoenological affinities, and the composition of its natural formations, which in some surveyed areas have been shown to comprise as many as 27 families, 68 orders, and 99 species growing in association with licorice stands [5]. Comparable surveys in Turkmenistan, western Kazakhstan, and the Fergana Valley have documented the plant's biological properties and raw-material reserves and have informed methods for the cultivation and accelerated regeneration of licorice stands subject to anthropogenic pressure [6, 9, 12].

Phytochemically, glycyrrhizic acid is a triterpene saponin comprising a glycyrrhetinic acid aglycone linked to a diglucuronide moiety [17]; its salts and derivatives have been the subject of extensive pharmacological and structural characterization, including studies of membrane-active and interferon-inducing derivatives of glycyrrhetinic acid and of molecular complexes formed between the monoammonium salt of glycyrrhizic acid and various pharmaceutical agents [14, 15, 16]. This body of work establishes both the chemical versatility of the glycyrrhizic acid scaffold and the practical importance of reliable, reproducible methods for its extraction and purification from licorice root.

2.2. Extraction and Standardization of Glycyrrhizic Acid

The glycyrrhizic acid content of licorice root reported in the literature ranges from approximately 2% to 24% of dry mass, and a large number of extraction methods have been proposed, the majority of which rely on precipitating glycyrrhizic acid from an aqueous root extract using a mineral acid [10]. Muravyev and Ponomarev demonstrated the advantages of gravimetric, titrimetric, and chromatographic analytical methods for standardizing licorice root and its preparations against a defined content of pure glycyrrhizic acid, establishing an analytical framework subsequently adopted by later researchers, including the present study [10]. A central practical difficulty noted in this literature is that the choice of precipitating acid and its concentration strongly affects both the yield and the physical form of the precipitate: concentrated sulfuric acid precipitates the acid rapidly but yields a resinous mass heavily contaminated with ballast substances, whereas more dilute acid, added gradually, favours precipitation of the product in a cleaner, amorphous form.

Once isolated, technical glycyrrhizic acid is conventionally converted to its ammonium salts to facilitate further purification and characterization. Literature methods typically proceed via acetone extraction of the technical acid followed by treatment with concentrated aqueous ammonia to give the triammonium salt, which is subsequently converted, via reaction with glacial acetic acid and recrystallization from aqueous ethanol, to the more stable and pharmaceutically relevant monoammonium salt [10, 18]. Commercial preparations of the monoammonium salt — such

as “Gliciram,” produced by Chimkentbiofarm ICHB in the Republic of Kazakhstan — provide a useful benchmark of purity and physicochemical constants against which regionally extracted material can be compared.

2.3. Supramolecular Complexes of Glycyrrhizic Acid

Glycyrrhizic acid and its monoammonium salt are known to form molecular and supramolecular complexes with a range of guest molecules, including pharmaceutical agents, through a combination of hydrophobic inclusion and hydrogen-bonding interactions, several of which have been shown to possess enhanced biological activity, including interferon-inducing activity, relative to the parent compounds [16]. This “host–guest” complexation behaviour, well documented for glycyrrhizic acid’s organic guest complexes, extends in principle to complexation with metal ions, offering a route to metal–glycyrrhizate supramolecular complexes whose combined organic-carrier and metal-micronutrient character makes them attractive candidates for agricultural biostimulant applications, particularly in regions such as Karakalpakstan where soil salinity and micronutrient availability constrain crop productivity.

3. Materials and Methods

3.1. Plant Material

Five-year-old licorice root, both wild-growing and under cultivation, was collected from the central and southern districts of the Republic of Karakalpakstan (Nukus, Kegeyli, Beruniy, and Ellikqala districts). Root material was cleaned, air-dried, and disintegrated prior to extraction. A commercially available thick aqueous extract of licorice root, of the kind produced by the private firm “LAKRISA,” was used as the starting material for the acid-precipitation experiments described below.

3.2. Extraction of Technical Glycyrrhizic Acid

Technical glycyrrhizic acid (TGK) was precipitated from the thick licorice-root extract by acidification with dilute sulfuric acid. In a representative preparation, 500 g of thick extract was dissolved in water at a 1:15 ratio and treated, with stirring, with 1% sulfuric acid until the solution reached a weakly acidic pH (3.5–4.0) and precipitation of technical glycyrrhizic acid ceased. The initially resin-like precipitate was converted to an amorphous powder by repeated decantation washing with water, filtered, washed with water to neutrality, and dried first in air and then in a drying oven, giving a brown amorphous powder (175 g; 35% relative to the starting extract).

To identify the reaction conditions that maximize precipitation yield, the volume of concentrated sulfuric acid added to a fixed mass and concentration of extract (200 g extract, 10% concentration in solution) was systematically varied from 7 to 16 mL, and the resulting solution pH and TGK yield were recorded for each trial (Table 1).

3.3. Synthesis of the Ammonium Salts

The triammonium salt of glycyrrhizic acid was prepared by extracting 100 g of technical glycyrrhizic acid with 1 L of acetone at reflux for 3 hours in a three-necked, 2000 mL flask equipped with a mechanical stirrer and reflux condenser; after cooling, the mixture was filtered and the residue re-extracted twice more with 1 L of acetone. The combined acetone filtrates were treated with concentrated aqueous NH4OH until the extract reached pH 7.5–8.0, precipitating the yellow triammonium salt, which was collected on a Büchner funnel, washed with ethyl alcohol (3 × 30 mL), and dried first at room temperature and then at 100 °C (32 g; 32% yield; $[\alpha]D25 = +38 \pm 2°$, c = 0.02% in 50% C2H5OH).

The triammonium salt was converted to the monoammonium salt by boiling with glacial acetic acid, and the resulting monoammonium salt was recrystallized from 80% ethyl alcohol, giving the pure monoammonium salt in 88% yield relative to the triammonium salt. Two monoammonium salt samples of differing purity were used for comparative physicochemical characterization: a 97%-purity sample prepared in-house (designated GKMAD-2) and a 75%-purity commercial reference sample of “Gliciram,” produced by Chimkentbiofarm ICHB, Republic of Kazakhstan (designated GKMAD-1).

3.4. Physicochemical Characterization

Melting points were determined using a PTP TU 25-11-1144 apparatus. Optical rotation was measured with an SM-2 polarimeter (1 dm cell path length) in 50% ethyl alcohol containing 0.25% NH4OH. Refractive indices were determined with an IRF-22 refractometer. Potentiometric titration of the free carboxyl groups of the GKMAD-1 and GKMAD-2 monoammonium-salt samples was carried out on an EV-74 universal ionometer, at constant temperature with stirring, using 0.2% aqueous sample solutions titrated against standardized 0.1 M alkali; apparent pKa values were calculated from the titration curves using the Henderson–Hasselbalch equation. Infrared spectra were recorded on a Perkin-Elmer FT-IR System 2000 spectrometer using KBr pellets; ultraviolet spectra of compound solutions were recorded on a Perkin-Elmer Lambda-16 spectrometer (1 cm quartz cells); and proton magnetic resonance (PMR) spectra

were recorded at a working frequency of 100 MHz on a Varian XL-100 spectrometer, using 10% solutions of the samples in deuterated dimethyl sulfoxide (DMSO-d6) with tetramethylsilane (TMS) as internal standard.

3.5. Synthesis of the Copper Supramolecular Complex

The copper supramolecular complex of technical glycyrrhizic acid (designated CuproTGK) was synthesized from the purified glycyrrhizic acid derivative with the aim of obtaining a natural-product-based agricultural biostimulant. The structure of the resulting compound was confirmed by infrared spectroscopy, which indicated that the CuproTGK supramolecular complex forms on a "host–guest" structural basis, arising from mutual hydrophobic interaction between the glycyrrhizic acid framework and the coordinated copper species together with hydrogen bonding between them.

4. Results

Table 1 presents the effect of the concentrated sulfuric acid volume, and the resulting solution pH, on the yield of technical glycyrrhizic acid obtained from a fixed mass (200 g) and concentration (10%) of licorice root extract.

Table 1. Effect of the acidification medium on the yield of technical glycyrrhizic acid

Trial No.	Conc. H_2SO_4 added (mL)	Resulting pH	TGK yield (%)
1	7	4.00	17.0
2	8	3.87	17.5
3	9	3.73	17.8
4	10	3.50	18.8
5	11	3.32	19.5
6	12	3.20	20.7
7	13	2.91	21.8
8	14	2.62	19.5
9	15	2.40	17.5
10	16	2.10	15.0

As shown in Table 1, the yield of technical glycyrrhizic acid rises with increasing sulfuric acid volume up to a maximum of 21.8% at trial 7 (13 mL, pH 2.91), beyond which further acid addition (trials 8–10) results in a progressive decline in yield to as low as 15.0% at pH 2.10. The optimal precipitation window therefore corresponds to a solution pH of approximately 2.5–3.2, consistent with the general literature observation that dilute-acid, gradual precipitation favours a cleaner amorphous product and reduces co-precipitation of ballast material.

Under the representative preparative conditions described in Section 3.2, technical glycyrrhizic acid was obtained in 35–40% yield relative to the starting thick root extract (175 g from 500 g of extract; 35%), with a comparison of wild-growing and cultivated root sources confirming that cultivated licorice root yields substantially more extractable glycyrrhizic acid than wild-growing root collected from the same districts. Conversion of the technical acid to the triammonium salt via acetone extraction and ammonia treatment proceeded in 32% yield, and subsequent conversion of the triammonium to the monoammonium salt, via glacial acetic acid treatment and recrystallization from 80% ethanol, proceeded in 88% yield. Using the full sequence directly from crude root extract to pure crystalline glycyrrhizic acid, an overall yield of 8–10% was obtained, a figure that the analysis identifies as a significant determinant of the final product's cost. The purified crystalline acid had a melting point of 222 ± 2 °C and an optical rotation of $[\alpha]D25 = +60 \pm 2°$ (c = 0.05%, in ethyl alcohol), both in agreement with literature reference values for glycyrrhizic acid.

Table 2 presents the results of potentiometric titration of the free carboxyl groups in the two monoammonium salt samples, GKMAD-1 (75% purity, commercial "Gliciram" reference) and GKMAD-2 (97% purity, prepared in this study).

Table 2. Potentiometric titration of glycyrrhizic acid monoammonium salt samples of differing purity

Parameter	GKMAD-1 (75% purity)	GKMAD-2 (97% purity)
Free carboxyl groups (per molecule)	2.7	3.2
Apparent pKa	4.6	4.6

The potentiometric titration curves for both samples showed a clear inflection over the range 1.1–1.6 mL of consumed 0.1 M NaOH, confirming the presence of free carboxyl groups. The free carboxyl group content calculated

from these curves — 2.7 for GKMAD-1 and 3.2 for GKMAD-2, against a theoretical value of three free carboxyl groups per monoammonium salt molecule — indicates that the higher-purity, in-house-prepared sample (GKMAD-2) more closely approaches the theoretical stoichiometry than the 75%-purity commercial reference. The apparent pKa of 4.6 obtained for both samples is close to that of acetic acid (pKa = 4.76), consistent with the carboxylic acid character of the titrated groups.

Infrared spectroscopic analysis of the product obtained by reacting the purified glycyrrhizic acid derivative with a copper source confirmed formation of the CuproTGK supramolecular complex, with spectral changes consistent with a “host–guest” complexation mode arising from combined hydrophobic interaction and hydrogen bonding between the glycyrrhizic acid framework and the coordinated copper species, rather than from a simple physical mixture of the two components.

5. Discussion

The pH-dependence of technical glycyrrhizic acid precipitation observed in this study (Table 1) provides a practical basis for optimizing extraction yield under regional production conditions: operating at a controlled pH of approximately 2.9, rather than adding excess concentrated acid in a single step, increased yield by more than six percentage points relative to the least favourable condition tested (21.8% versus 15.0%) while avoiding the resinous, ballast-laden precipitate associated with over-acidification. Because the same acidification step also governs the physical form of the precipitate and, downstream, the efficiency of subsequent acetone extraction and ammonium-salt conversion, this optimization has consequences that extend beyond the immediate precipitation yield to the overall economics of the extraction sequence.

The finding that cultivated licorice root yields substantially more extractable glycyrrhizic acid than wild-growing root collected from the same districts is of direct practical relevance to regional agricultural and industrial policy: it supports the case, already reflected in national decrees promoting licorice cultivation and export, for directing raw-material sourcing toward cultivated plantations rather than relying solely on wild stands, both to secure a more concentrated and predictable source of glycyrrhizic acid and to relieve harvesting pressure on wild licorice populations, whose reserves in the lower Amu Darya region have previously been shown to be sensitive to anthropogenic disturbance.

The physicochemical comparison between the in-house 97%-purity monoammonium salt (GKMAD-2) and the 75%-purity commercial “Gliciram” reference (GKMAD-1) indicates that the extraction and purification sequence used in this study is capable of producing material that matches, and on the free-carboxyl-group metric slightly exceeds, an established regional pharmaceutical-grade benchmark. Because free carboxyl group content bears directly on the compound’s capacity for further derivatization — including, as demonstrated here, complexation with metal ions — this level of purity is a meaningful indicator of the material’s suitability for downstream supramolecular chemistry rather than a purely analytical curiosity.

The successful synthesis of the CuproTGK copper supramolecular complex, and its structural confirmation by infrared spectroscopy as a host–guest assembly stabilized by hydrophobic interaction and hydrogen bonding, extends the established precedent of glycyrrhizic acid’s complexation with organic guest molecules to a metal-ion guest. Because copper is both a plant micronutrient and, in supramolecular combination with an organic natural-product carrier, a candidate route to a biodegradable, low-toxicity agrochemical, this complex forms a natural bridge between the extraction chemistry reported here and the agronomic evaluation of CuproTGK as a seed and crop biostimulant, which is reported separately.

Several limitations of the present study should be noted. The reported yields and physicochemical constants derive from single representative preparative runs rather than replicate batches, so their variability has not been formally quantified; the wild-versus-cultivated yield comparison is reported qualitatively rather than as a matched, statistically analyzed dataset; and the infrared characterization of the CuproTGK complex, while sufficient to confirm host–guest complex formation, was not accompanied in this work by full peak assignment or by complementary structural techniques such as PMR or elemental analysis of the isolated complex itself. Addressing these points in future work would strengthen the quantitative basis for scaling the extraction and complexation methodology to larger production volumes.

6. Conclusion

This study establishes a reproducible extraction and derivatization route from regionally grown Karakalpakstan licorice root to purified glycyrrhizic acid and its copper supramolecular complex. Acidification of the thick root extract with dilute sulfuric acid at a controlled pH of approximately 2.9 maximized technical glycyrrhizic acid yield (21.8%),

and the subsequent ammonium-salt conversion sequence gave pure crystalline glycyrrhizic acid, with physicochemical constants matching literature values, in an overall yield of 8–10% from crude extract. Cultivated licorice root was found to be a substantially more productive source of extractable glycyrrhizic acid than wild-growing root, and the purified monoammonium salt prepared in this study compared favourably, in free carboxyl group content, with a commercial regional reference preparation. The resulting material was successfully used to synthesize a copper supramolecular complex, CuproTGK, whose host–guest structure was confirmed by infrared spectroscopy. Together, these findings support the technical and economic feasibility of local, value-added processing of Karakalpakstan licorice root into pharmaceutical- and agrochemical-grade glycyrrhizic acid derivatives, and they establish the chemical basis for the agronomic biostimulant evaluation of CuproTGK reported in the companion study.

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