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News Letter

Brief survey of *Glycyrrhiza* plant resources in Xinjiang, China

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It has been reported that three glycyrrhizin-producing *Glycyrrhiza* species, i.e., *G. inflata, G. glabra* and *G. uralensis,* grow wild in Xinjiang Uygur Autonomous Region, on the northwestern border of China. We made a brief survey in Xinjiang focusing on the comparison of the characteristics of the fruits of these three species of *Glycyrrhiza* plants. We found that the falcate fruits with prickly hairs of *G. uralensis* were clearly distinguishable from the swollen fruits of *G. inflata* and from the long, glossy fruits of *G. glabra.* Based on the findings of the present survey, the *Glycyrrhiza* plants used in our cultivation study in eastern Inner Mongolia could be identified as *G. uralensis.*

The present brief survey also proved that Xinjiang is well endowed with an abundance of *G. inflata* resources, which are used as raw materials for making sweet food additives and glycyrrhizin, an active ingredient of licorice. Furthermore, we inspected *Ephedra* plant clusters in sandy wastes of the Tianshan Mountains, cultivated fields of *Carthamus tinctorius* and processing works of Cistanches Herba, which are representative medicinal resources produced in Xinjiang.

Key words *Glycyrrhiza inflata, Glycyrrhiza uralensis,* glycyrrhizin, licorice.

Abbreviations Dongbei-Gancao (Tohoku-Kanzo: 東北甘草), Xibei-Gancao (Seihoku-Kanzo: 西北甘草), Xinjiang (Xinjiang Uygur Autonomous Region: 新疆維吾爾自治区), Xinjiang-Gancao (新疆甘草：Shinkyo-kanzo).

1. Introduction

Licorice (Glycyrrhizae Radix) is prepared from the dried roots of *Glycyrrhiza* plants (Leguminosae), which grows widely throughout the Eurasian continent, but not in Japan. Licorice is one of the most important natural drugs[1)] and food additives[2)] throughout the world. Extensive studies have revealed that licorice contains glycyrrhizin (glycyrrhizic acid) as a major bioactive triterpenoid glycoside and many phenolic compounds such as glycycoumarin, glabridin, and licochalcone A.[3)]

The greater part of medicinal licorice (Kanzo in Japanese) compounded in traditional Chinese formulations (Kampo-formulations) used in Japanese medical care has been imported from China.[4)] Since licorice is the most frequently used crude drug in Kampo-formulations described in the old Chinese formulary, ShangHanLun (傷寒論),[5)] a stable supply of licorice is an essential issue. However, because of the risk of desertification in the northern region of China and the need to protect wild medicinal plant resources, the collection of wild *Glycyrrhiza* plants has recently been restricted by the Chinese government.

In an effort to compensate for the reduced supply of wild *Glycyrrhiza* plants, we initiated a cultivation study in eastern Inner Mongolia (NeiMengGu) of China in 1998. The study has already yielded 4-year-old cultivated roots that meet the Japanese Pharmacopoeia XIV (JP XIV) standard requirements, including the required glycyrrhizin content.[6)] In addition, the pharmacological and biopharmaceutical properties of the cultivated roots have been shown to be similar to those of existing medicinal licorice, conforming to the JP XIV standards.[7)] The botanical origin of the cultivated plants was identified as *G. uralensis* by examining the characteristic appearance of the fruits[8)] (Fig.1) obtained from July to August from the 3rd to 5th year in the cultivated field and from the results of an on-the-spot survey in the habitat of wild *G. uralensis* in China.[9)]

In order to confirm the identification, we made a brief survey in Xinjiang Uygur Autonomous Region (新疆維吾爾自治区) of China, which is the habitat of *G. uralensis* as well as *G. glabra* and *G. inflata.* These three plant species are listed as the sources of licorice (Gancao in Chinese) in the Chinese Pharmacopoeia (2005 edition). Of these three plants, the former two plants are also listed in the JP XIV as raw materials of medicinal licorice (Kanzo in Japanese).

In the present report, the results of a brief survey in Xinjiang Uygur Autonomous Region are described focusing on the comparison of three species of *Glycyrrhiza* plants. Xinjiang is located on the northwestern border of China and is bounded on the west by Kyrgyz and Kazakhstan, on the south by Pakistan and India, and on the northeast by Outer Mongolia. Our survey started from Wulumuqi (烏魯木斉), the capital city of the Autonomous Region, and was conducted on the northern and southern peripheries of the Tianshan Mountains (天山山脈) from 1 to 6 August 2002

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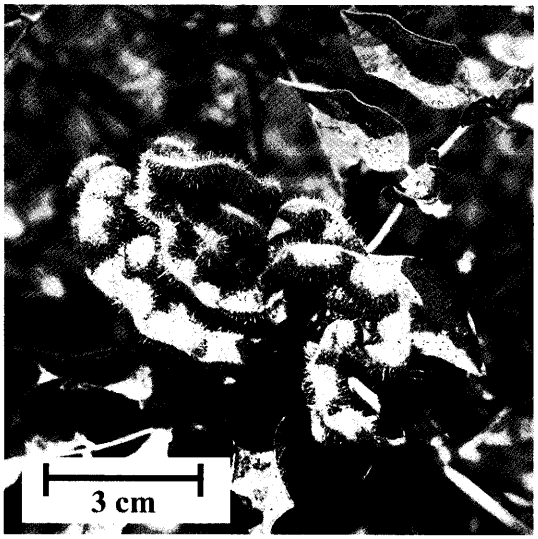

Fig. 1. Typical appearance of fruits of *Glycyrrhiza uralensis*
Cultivated at Yuanbaoshan-qu in eastern Inner Mongolia (内蒙古自治区元宝山区)

(Fig.2).

2. Fruits of *Glycyrrhiza* plants in Xinjiang

Since *Glycyrrhiza* plants are classified mainly according to the characteristics of their fruits,[8] we focused on observing the morphological variations of the fruits and flowers of *Glycyrrhiza* plants.

G. inflata is the typical *Glycyrrhiza* plant growing naturally in Xinjiang. We observed its clusters (Fig.3) in a somewhat fertile plain on the north side of the Tarim River, which runs between the southern periphery of the Tianshan Mountains and the Takelamagan desert. The Chinese name of the *G. inflata* plant is ZhangGuo-Gancao (脹果甘草), meaning Gancao (licorice) with swollen fruits (Fig.4), which are clearly distinguishable from the fruits of of *G. uralensis* (Fig.1).

G. glabra grows from southern Europe (Spain, France, and Italy) to Central Asia, and therefore it is called Spanish licorice (スペイン甘草) in Japan. Its Chinese name is

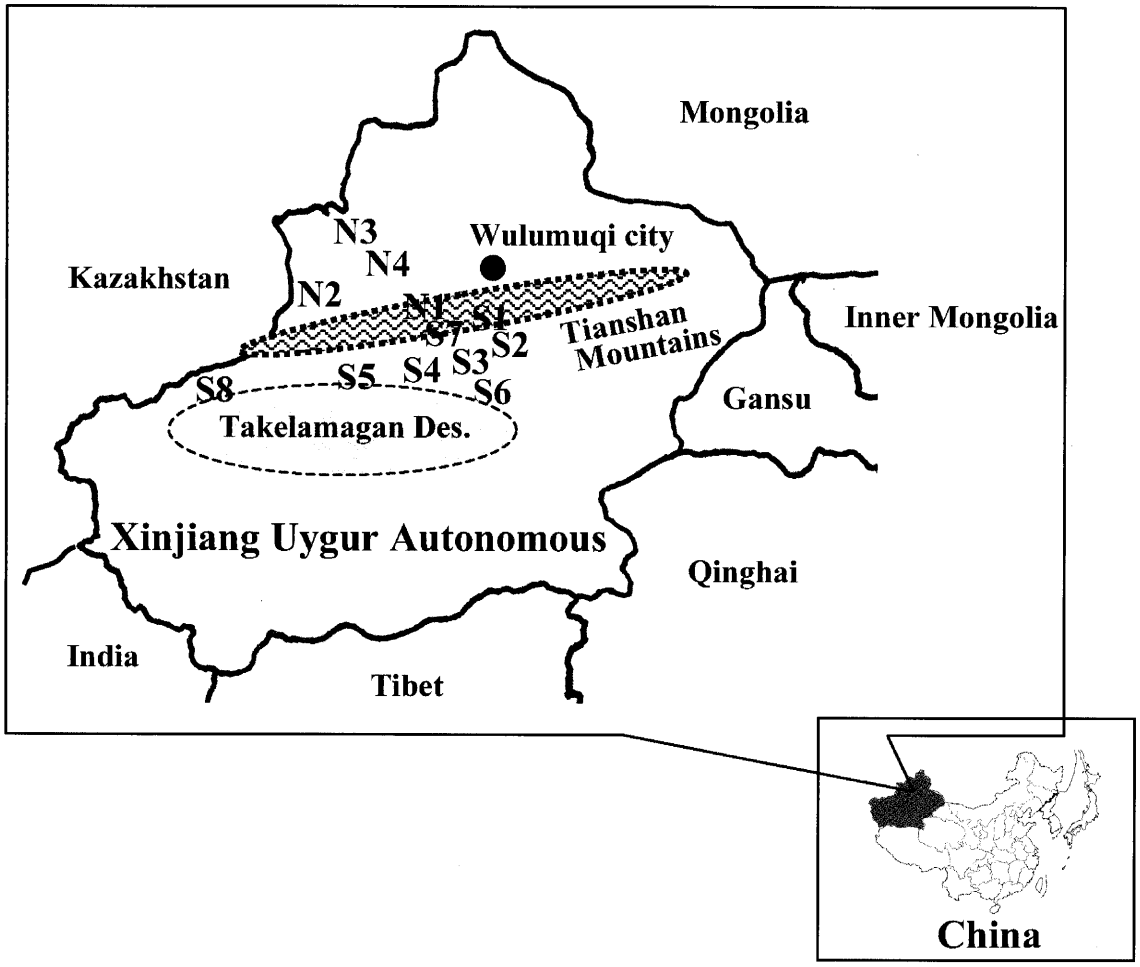

Fig. 2. Field survey in Xinjiang Uygur Autonomous Region of China
Survey route: Wulumuqi city ⇒ S1 ⇒ S2 ⇒ S3 ⇒ S4 ⇒ S5 ⇒ S3 ⇒ S6 ⇒ N1 ⇒ N2 ⇒ N3 ⇒ N4 ⇒ Wulumuqi city,
S1:Wushitala, Heshuo Prefecture (和碩県烏什塔拉), S2:Wuhaoqu, Yanqihuizuzizhi Prefecture (焉耆回族自治県五号渠), S3:Kuerle City (庫爾勒市), S4:Dunshili, Luntai Prefecture (輪台県墩実里), S5:Talimu, Shaya Prefecture (沙雅県塔里木), S6: Xinier, yuli Prefecture (尉犁県西尼爾), S7: Haermodun, Hejing Prefecture (和静県哈爾莫墩), S8: Akesu City (阿克蘇市), N1: Wulanbuluke (烏蘭布魯克), N2:Chabuchaerxibozizhi Prefecture (察布査爾錫伯自治県), N3:Bodong City (博東市), N4:Mangding, Jinghe Prefecture (精河県芒丁)

Fig. 3. *G. inflata* growing in clusters in Xinjiang
At Talimu, Shaya Prefecture (沙雅県塔里木 : S5 of Fig. 2)

Fig. 5. Typical appearance of fruits of *G. glabra* growing in Xinjiang
At Mangding, Jinghe Prefecture (精河県芒丁 : N4 of Fig. 2)

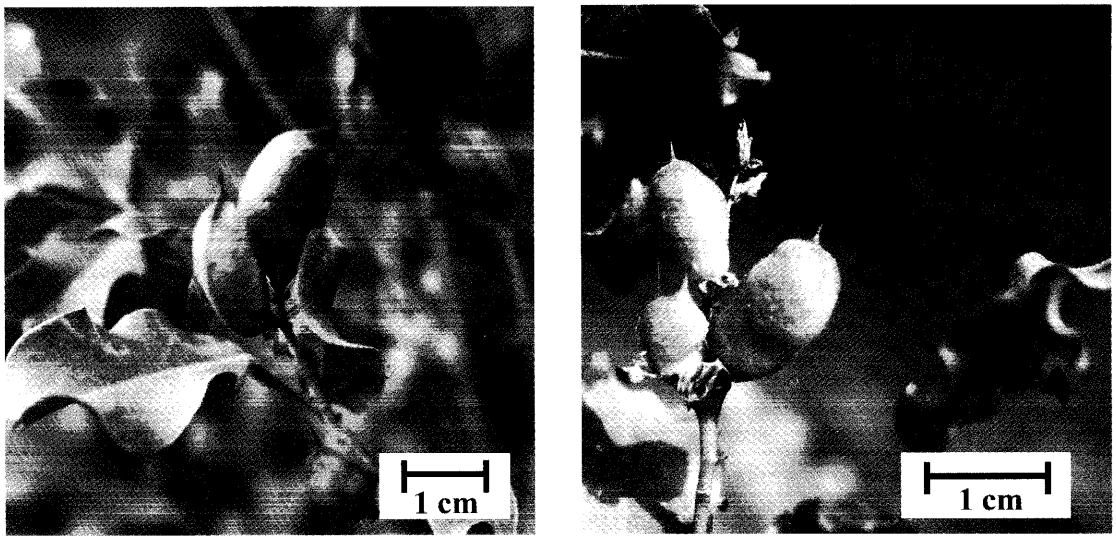

Fig. 4. Typical appearance of fruits of *G. inflata* growing in Xinjiang
Left : at Dunshili, Luntai Prefecture (輪台県墩実里 : S4 of Fig. 2) ; Right : at Talimu, Shaya Prefecture (沙雅県塔里木 : S5 of Fig. 2)

GuangGuo-Gancao (光果甘草), where GuangGuo means glossy or polished fruits (as shown in Fig.5). The fruits of *G. glabra* are longer than those of *G. uralensis* (Fig.1) and *G. inflata* (Fig.4). Furthermore, we observed that the lanceolate leaflets of *G. glabra* are longer than those of *G. uralensis*.

G. uralensis grows wild from Xinjiang to Mongolia, eastern Inner Mongolia and eastern Siberia, and is the main raw material of medicinal Kanzo, including Dongbei-Gancao (東北甘草), which is used mainly in Japan. We observed wild *G. uralensis* plants possessing falcate fruits with prickly hairs in both the southern and northern peripheries of the Tianshan Mountains (S2, S6, S7 and N4 of Fig.2). By comparison with the three types of *Glycyrrhiza* fruits observed in this survey, the *Glycyrrhiza* plants with fruits (as shown in Fig. 1) used in our cultivation study in the eastern Inner Mongolia Autonomous Region of China[6] could be confirmed to be *G. uralensis*.

It has already been reported that putative intermediates between *G. uralensis* and *G. glabra* have been observed in Kazakhstan,[10] which is located on the western border of Xinjiang. In this survey, we also observed remarkable variation of *Glycyrrhiza* fruits, as shown in Fig. 6, in which fruits X and Y were judged to be intermediate fruits between *G. uralensis* and *G. glabra*, and between *G. uralensis* and *G. inflata*, respectively. The manager of the licorice extract-producing works (Yuli Prefecture: 尉犁県,S6 of Fig.2) told in an interview that three or more types of *Glycyrrhiza* plants are available in Xinjiang. As shown in Fig. 7, we also observed a mixture of the fruits of *G. uralensis* and *G.*

glabra sold in a wholesale firm in Chabuchaerxibo Autonomous Prefecture (察布查爾錫伯自治県, N2 of Fig.2). Therefore, when evaluating the *Glycyrrhiza* plant resources in Xinjiang, the morphological, chemical, pharmacological and pharmaceutical characteristics of all of these varieties of *Glycyrrhiza* plants should be evaluated.

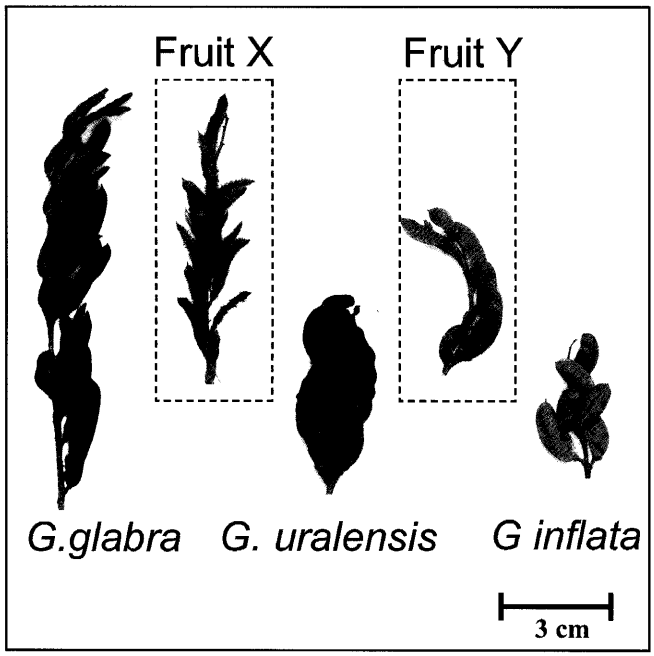

Fig. 6. Variation of *Glycyrrhiza* fruits collected in Xinjiang
G. glabra and *G. uralensis* collected at warehouse of crude drug company in Chabuchaerxibozizhi Prefecture (察布查爾錫伯自治県 : N2 of Fig. 2).
Fruit X collected at Anding, Chabuchaerxibozizhi Prefecture (察布查爾錫伯自治県安定 : N2 of Fig. 2)
Fruit Y collected at Wuhaoqu, Yanqihuizuzizhi Prefecture (焉耆回族自治県五号渠 : S2 of Fig. 2)
G. inflata collected at Dunshili, Luntai Prefecture (輪台県墩実里 : S4 of Fig. 2)

3. Roots of *G. inflata* in Xinjiang

Although the local government of Xinjiang Uygur Autonomous Region restricts the collection of wild *Glycyrrhiza* plants, we observed plentiful wild *G. inflata* roots gathered in areas in the southern periphery of the Tianshan Mountains, such as Kuerle (庫爾勒, S3 of Fig.2) and Akesu (阿克蘇, S8 of Fig.2). We visited licorice extract producing works (Fig. 8 in Yuli Prefecture: 尉犁県,S6 of Fig.2), in which approximately 7,000 tons of *G. inflata* roots were used for extraction, according to the managers. Part of the product was used in China for the purification of glycyrrhizin and part was exported to Europe, USA and Japan.

G. inflata roots were also partially processed to produce peeled licorice (Fig. 9, Shaya Prefecture: 沙雅県, S5 of Fig.2), which is exported to Europe (Germany) for use as a food additive. *G. inflata* roots are listed in the Chinese Pharmacopoeia (2005 edition) as raw material of medicinal licorice, but are not listed in the JP XIV. They are considered to be the main raw material of Xinjiang-Gancao (新疆甘草 : Shinkyo-kanzo in Japanese), and are imported as a raw material of the licorice extract used for the purification of glycyrrhizin and food additives.

Locally, *G. inflata* roots (Fig. 10) are called HuangPi-Gancao (黄皮甘草), meaning Gancao (licorice) with yellowish-brown peels, which differ from *G. uralensis* and *G. glabra* roots with reddish-brown peels. *G. inflata* roots (so-called Xinjiang-Gancao) are slightly woody and stout and differ from *G. uralensis* roots (a raw material of Dongbei-,

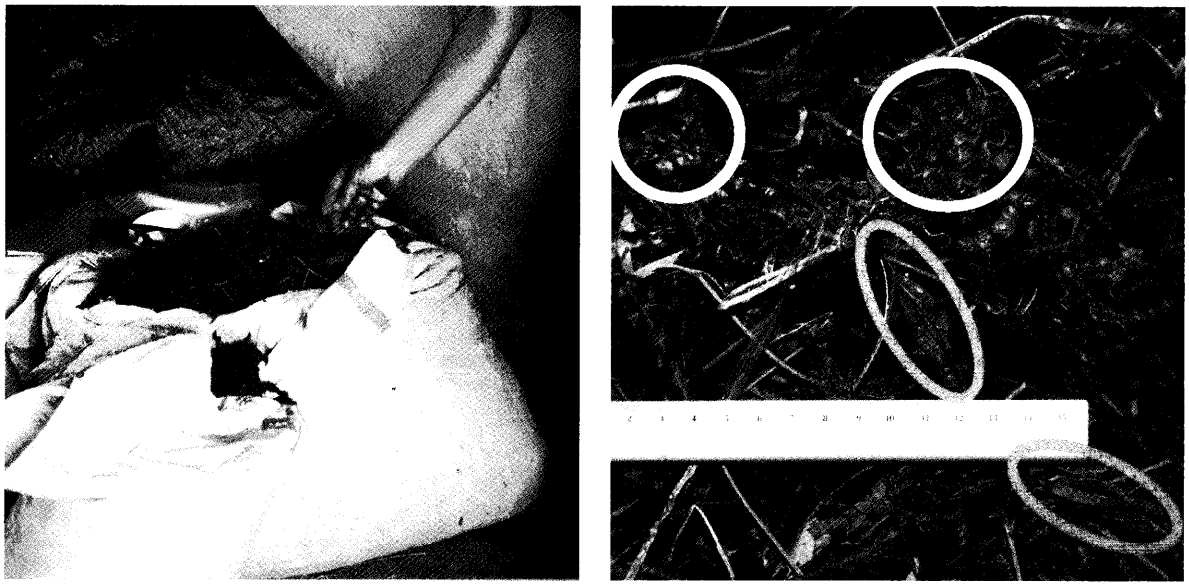

Fig. 7. Commercial fruits and seeds
collected at warehouse of crude drug company in Chabuchaerxibozizhi Prefecture (察布查爾錫伯自治県 : N2 of Fig. 2).
White circles : *G. uralensis* fruits; yellow circles : *G. glabra* fruits

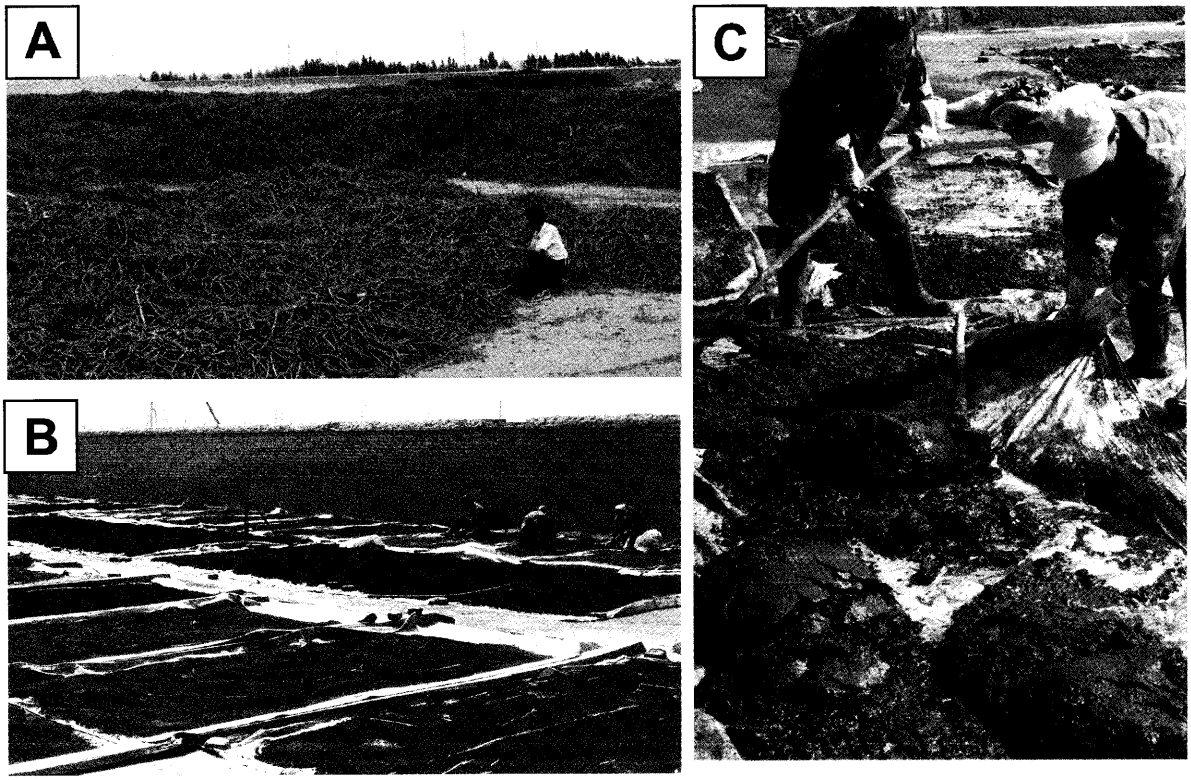

Fig. 8. Licorice extract producing works
A: Raw material of *G. inflata* roots; **B**: Drying off extract mass under sunlight; **C**: drying operations at Xinier, Yuli Prefecture (尉犁県西尼爾 : S6 of Fig. 2)

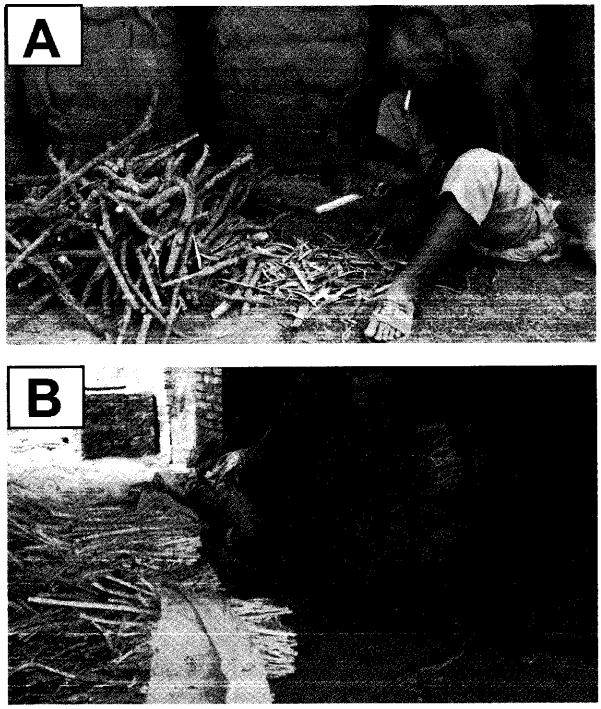

Fig. 9. Peeled licorice prepared from *G. inflata* roots
A: peeling *G. inflata* roots; **B**: peeled licorice
At Talimu, Shaya Prefecture (沙雅県塔里木 : S5 of Fig. 2)

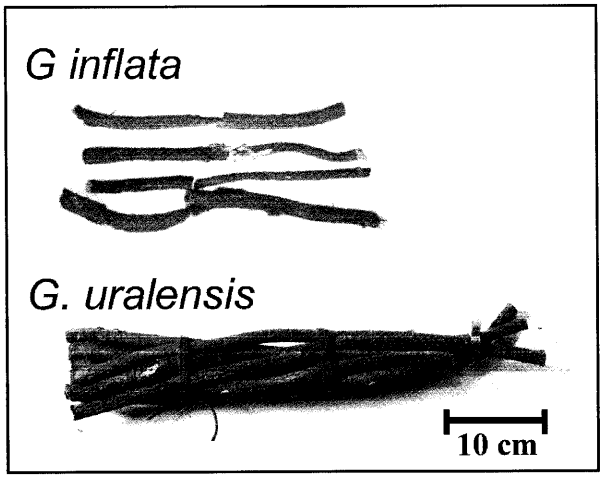

Fig. 10. Roots of *G. inflata* and *G. uralensis*

Xibei- and Daitou-Gancao: 帯頭甘草),[11] which are obviously longitudinally wrinkled and furrowed. Furthermore, it has been reported that *G. inflata* roots contain glycyrrhizin as well as species-specific retrochalcones such as licochalcone A,[12] which is not found in *G. uralensis* or *G. glabra*.[13,14] These distinct morphological and chemical featuresd of *G. inflata* are among the reasons that it does not meet the requirement of the JP XIV standards.

We recently reported a comparison of Radix Glycyrrhizae (licorice) from Europe and China by capillary-

zone electrophoresis (CZE),[15] which has the environmentally friendly advantage that the use of harmful solvents is greatly reduced compared to HPLC. As shown in Fig. 11, the electropherogram of *G. inflata* is characterized by the presence of licochalcone A (peak LC_A) and it is distinct from those of *G. uralensis* and *G. glabra*. In that report, it was confirmed that licochalcone A, glycycoumarin,[16] and glabridin are species-specific ingredients of the roots of *G. inflata*, *G. uralensis* and *G. glabra*, respectively.

4. Wild and cultivated *G. uralensis* roots in Xinjiang

We also collected pieces of commercial licorice (Fig.12). The results of chemical analyses carried out in Japan indicated that it contained 3.46 ± 0.85% glycyrrhizin, and thus fulfilled the JP XIV standards (glycyrrhizin content: not less than 2.5%). It contained no licochalcone A or glabridin, and therefore it was thought likely that it was prepared from *G. uralensis* roots. Therefore, it is probable that some licorices fulfilling the JP XIV standards can be obtained from Xinjiang, although detailed chemical analyses of species-specific ingredients would be needed to confirm this.

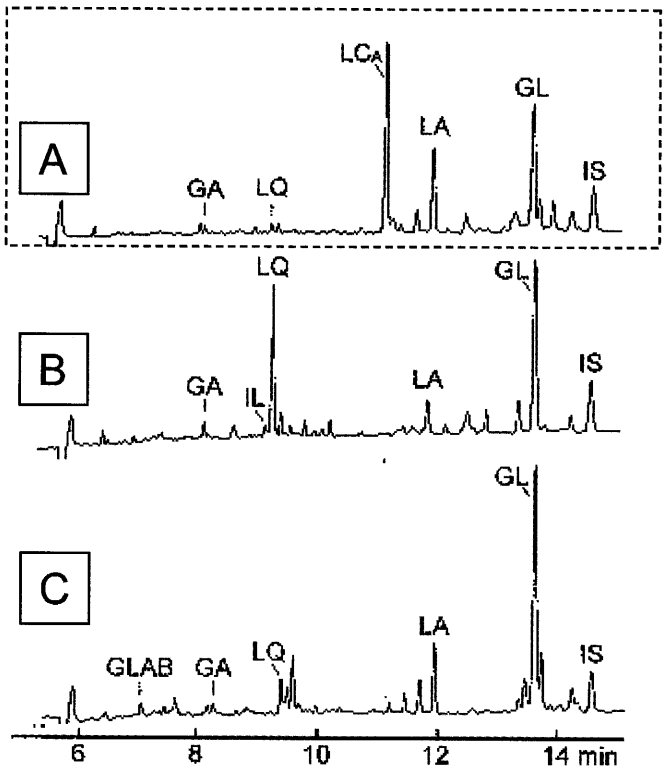

Fig. 11. Electropherograms of ethanol extracts of *G. inflata* (A), *G. uralensis* (B), and *G. glabra* (C)
GLAB: glabridin, GA: glycyrrhetic acid, IL: isoliquiritin, LQ: liquiritin, LC_A: licochalcone A, LA: liquiritin apioside, GL: glycyrrhizin, IS: internal standard (cinnamic acid).
Capillary-zone electrophoresis (CZE) conditions: fused silica capillary [57 cm (50 cm effective length), 50 μm I.D.]; running buffer, 70 mM borate (pH 9.22); injection, pressure, 0.034 atm for 5 s; voltage, 25 kV (constant voltage, positive to negative polarity); temperature, 20oC; detection, UV at 254 nm; 【modified from Ref. 15)】

As shown in the left panel of Fig.13, we also observed a cultivated field of *G. uralensis* in Yuli Prefecture (尉犁県, S6 of Fig.2). We confirmed that the original species of the cultivated plants was *G. uralensis* based on the morphological characteristics of flowers and fruits (the right panel of Fig. 13). According to interviews we conducted the cultivation of *G. uralensis* in Xinjiang was started in 1999. The general cultivation conditions were as follows: wild *G. uralensis* seeds, but not *G. inflata* seeds, purchased from Inner Mongolia were sowed in April. The next spring, taproots were transplanted, and then roots were harvested in the April and May of the 3rd year. Cultivated taproots with the head diameter (approximately 15 mm) of 2-year-old plants were obtained. This diameter is somewhat thinner than that obtained in our cultivated field in eastern Inner Mongolia (the suburbs of Chifeng: 赤峰).[6] We heard that these 3-year-old cultivated roots are mainly used in China and partly exported to Korea.

5. Some other medicinal resources in Xinjiang

In the present brief survey in Xinjiang, we passed through highlands (at an altitude of over 2000 m in the Tianshan Mountains), grasslands and sandy wastes (Fig.14). Besides licorice, the vast area of Xinjiang with its various environmental conditions has various medicinal resources, including Ephedrae Herba (麻黄), Carthami Flos (紅花), Cistanches Herba (肉蓯蓉), Fritillariae Bulbus (貝母), Arctii Fructus(牛蒡子), and Lupli Strobilus (ホップ).

We observed clusters of *Ephedra* plants in the sandy wastes of the Tianshan Mountains (S1 of Fig.2). These plants were growing in less watery wastes than *Glycyrrhiza* plants. Based on the shape of the scaly leaves, as shown in the close-up in Fig 15, the plants were judged to be *E. intermedia,* a Chinese plant name Zhong-Mahung (中麻黄). *Ephedra* plants, including *E. intermedia,* in Xinjiang have already been described in the survey report.[17] Although *Ephedra* plants were observed frequently in the course of our survey, Ephedrae Herba produced in Xinjiang is a not

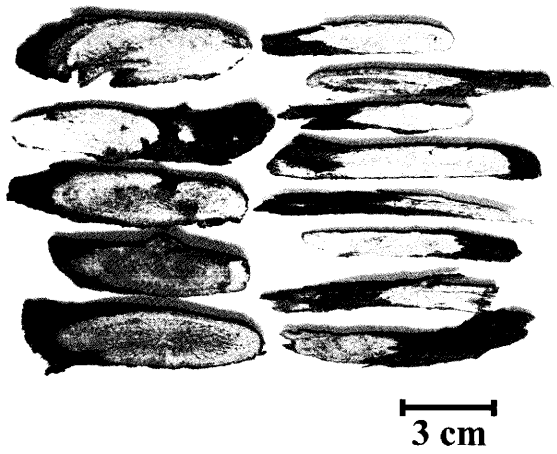

Fig. 12. Pieces of commercial licorice
Collected at a pharmaceutical company in Chabuchaerxibo Autonomous Prefecture (察布查爾錫伯自治県, N2 of Fig.2).

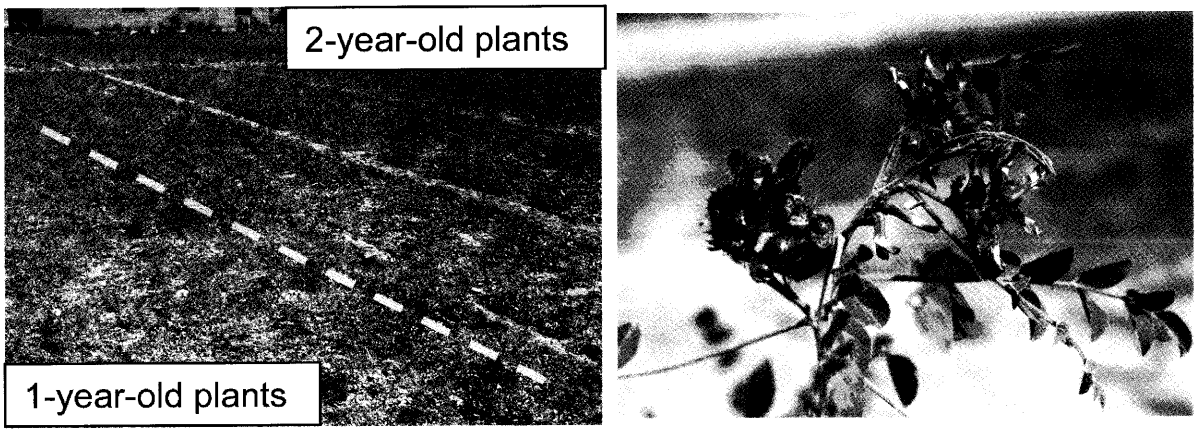

Fig. 13. Cultivated field of *G. uralensis* (left panel) and its flowers and fruits (right panel)
At Xinier, Yuli Prefecture (尉犁県西尼爾 : S6 of Fig. 2)

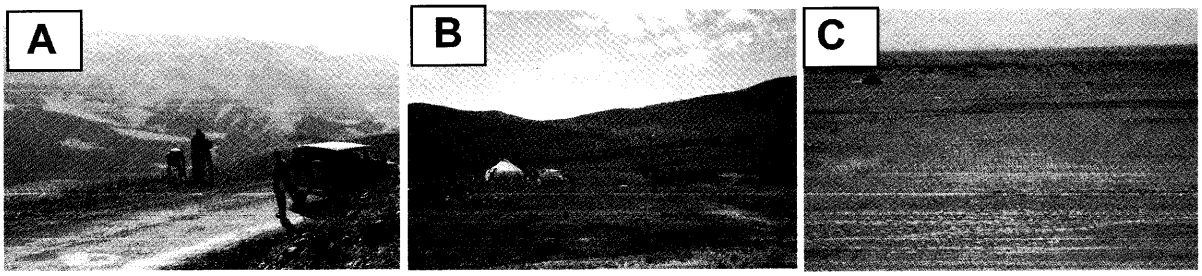

Fig. 14. Scenery of Xinjiang Uygur Autonomous Region
A : Wulanbuluke (烏蘭布魯克 : N1 of Fig. 2) in the Tianshan Mountains (天山山脈) ; **B** : Bodong City (博東市 : N3 of Fig. 2);
C : Shaya Prefecture (沙雅県 S5 of Fig. 2)

Fig. 15. *Ephedra* plant and scaly leaf
At Wushitala, Heshuo Prefecture (和碩県烏什塔拉 : S1 of Fig. 2)

Fig. 16. Cultivated field of *Carthamus tinctorius*
At Geremu, Chabuchaerxibozizhi Prefecture (察布査爾錫伯自治県格熱木 : N2 of Fig. 2)

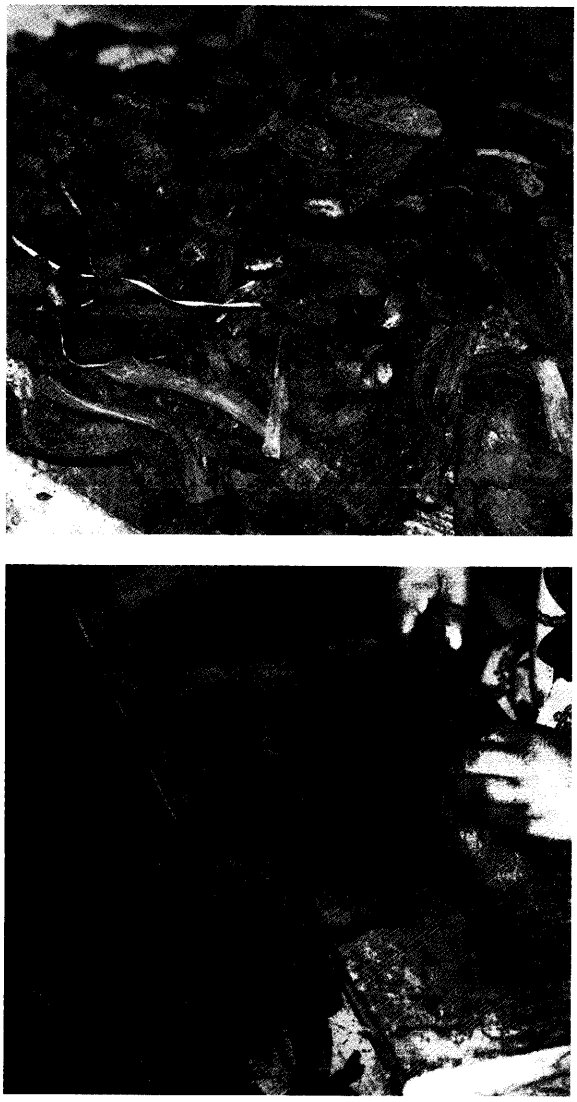

Fig. 17. Cistanches Herba processing works
At Mangding, Jinghe Prefecture (精河県芒丁 : N4 of Fig. 2)

staple medicinal resources in Japan.[18)]

Cultivated fields of *Carthamus tinctorius* (Fig.16) were observed in the northern part of Xinjiang, Chabuchaerxibo Autonomous Prefecture (察布查爾錫伯自治県, N2 of Fig.2). Medicinal Carthami Flos used in Japan is mainly imported from Xinjiang and Gansu Province (甘粛省).

Processing works (Fig. 17) preparing Cistanches Herba from succulent stems of Cistanche plants were observed in Jinghe Prefecture (精河県, N4 of Fig.2). The sources of Cistanches Herba are listed as *Cistanche deserticola* and *C. tubulosa* in the Chinese Pharmacopoeia (2005 edition). Although we heard from the manager of the processing works that the raw material was imported from Kazakhstan, the plant species used in the work is not clear.

6. General discussion

We made a brief survey in Xinjiang Uygur Autonomous Region (新疆維吾爾自治区), on the northwestern border of China, where three species of *Glycyrrhiza* plants, i.e., *G. inflata*, *G. glabra* and *G. uralensis*, are growing spontaneously. Wild-growing *G. inflata* with swollen fruits is mainly observed in the southern periphery of the Tianshan Mountains (S4, S5 and S6 of Fig.2). *G. glabra* with long, glossy fruits is observed in the northern periphery of the Tianshan Mountains (N2 and N4 of Fig.2). On the other hand, *G. uralensis* with prickly haired falcate fruits is observed growing in both peripheries of the Tianshan Mountains (S2, S6, S7 and N4 of Fig.2). By comparing the fruits of the three species of *Glycyrrhiza* plants, it was possible to confirm that the botanical origin of our cultivated plants in eastern Inner Mongolia was *G. uralensis*, which is the main raw material of medicinal Kanzo (甘草) used in Japan.

In an interview survey, we obtained the information that there are regional differences in the restriction of the collection of wild *Glycyrrhiza* plants in Xinjiang. We have the impression that the restriction in Xinjiang seems to be milder than those enforced by the local governments in neighboring regions such as Gansu (甘粛省), Shanxi (陝西省), Ningxia (寧夏回族自治区) and Neimenggu (内蒙古自治区), where we have already conducted surveys.[8)]

Furthermore, among various medicinal resources produced in Xinjiang, we have inspected three other representative resources such as *Ephedra* plants (estimated to be *E. intermedia*) in sandy wastes of Tianshan Mountains, *Carthamus tinctorius* cultivated in northern periphery of Tianshan Mountains, and processing work of Cistanches Herba.

At the present time, Xinjiang is supplying large quantities of *G. inflata* roots, which are used as raw materials to isolate glycyrrhizin, and as food additives. However, cultivation of *G. uralensis* has been initiated in order to maintain a stable supply of licorice. In our brief survey in Xinjiang, we had the impression that it is important to concomitantly foster the development of natural medicinal resources and the protection of the environment from destruction.

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Japanese abstract

我々は中国内蒙古自治区で栽培甘草の実生産を目指した研究を行っている。我々が栽培している植物は，その花と果実の形態から *Glycyrrhiza uralensis* であることを推定している。今回，基原植物を確認するために中国で薬用にされている 3 種の *Glycyrrhiza* 属植物（*G. uralensis, G. inflata, G. glabra*）が自生している新疆維吾爾自治区（Xinjiang Uygur Autonomous）を調査した。3 種の *Glycyrrhiza* 属植物の果実の形態を見比べた結果，我々が栽培している植物が *G. uralensis* であることを確認した。

今回の調査において，日本の薬局方に適合する甘草が新疆維吾爾自治区で流通していることも明らかになり，さらに *G. uralensis* の栽培生産も行われていることを確認した。このようなことから収穫地を管理し *G. inflata* 根に特異的な成分（licochalcone A）を含まないことを確認すれば新疆維吾爾自治区で産する *G. uralensis* 根を薬用甘草として輸入することが可能である。

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