DEPARTMENT OF HEALTH & HUMAN SERVICES
FOOD AND DRUG ADMINISTRATION

Public Health Service

Memorandum

Date . OCT - 8 1999

9421 '99 OCT 13 P1:51

From Senior Regulatory Scientist, Regulatory Branch, Division of Programs & Enforcement Policy (DPEP), Office of Special Nutritionals, HFS-456

Subject 75-day Premarket Notification for New Dietary Ingredient

To Dockets Management Branch, HFA-305

New Dietary Ingredient:	Siraita Groxvenori (Lo Han Kuo)
Firm:	Nature's Marvel International
Date Received by FDA:	October 6, 1999
90-day Date:	December 24, 1999

In accordance with the requirements of section 413(a)(2) of the Federal Food, Drug, and Cosmetic Act, the attached 75-day premarket notification for the aforementioned new dietary ingredient should be placed on public display in docket number 95S-0316 after ~~December 24, 1999~~ 1/3/2000 jb

Robert J. Moore, Ph.D.

95S-0316

RPT 57

DEPARTMENT OF HEALTH & HUMAN SERVICES

Public Health Service

Food and Drug Administration
Washington, DC 20204

OCT - 8 1999

Nature's Marvel International
U.S. Division
1681 Alta La Jolla Drive
La Jolla, California 92037

Dear Sir:

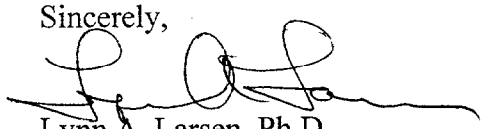
This is in response to your letter to the Food and Drug Administration (FDA) dated September 24, 1999, making a submission pursuant to 21 U.S.C. 350b(a)(2) (section 413 of the Federal Food, Drug, and Cosmetic Act (the Act)) and 21 CFR 190.6. In your letter, you notified FDA of your intent to market Lo Han Kuo Fruit Extract (*Siraitia grosvenorii* (swingle) C. Jeffrey), a substance that you assert is a new dietary ingredient.

The Act, as amended by the Dietary Supplement Health & Education Act of 1994, defines the term "dietary supplement" to exclude products represented for use as conventional foods. 21 U.S.C. 321(ff)(2)(B). In your submission, you state that the ingredient Lo Han Kuo is a new, natural food sweetener. You state in your submission that this ingredient is intended to be used as a sweetener in foods such as low calorie diet and drink supplements, it is a safe alternative to other sweeteners such as saccharine, duicin, and sodium cyclamate, and it can be used in place of sugar as a sweetener. Given the representations made for this product, as cited above, the product is not a dietary ingredient within the meaning of 21 U.S.C. 321(ff) and, therefore, cannot be a "new dietary ingredient" under 21 U.S.C. 350b. Because Lo Han Kuo is not a new dietary ingredient, it is not subject to the notification requirements in 21 CFR 190.6.

Instead, Lo Han Kuo is a conventional food ingredient. Under the Federal Food, Drug, and Cosmetic Act, any ingredient intentionally added to a conventional food must be used in accordance with a food additive regulation unless it is generally recognized as safe (GRAS) among qualified experts for its intended use in food. A food ingredient that is not GRAS or an approved food additive causes a food to be adulterated under 21 U.S.C. 342(a)(2)(C) and cannot be legally marketed in the U.S. If you intend to market Lo Han Kuo as an ingredient in food, it must be an approved food additive or it must be GRAS. Any questions regarding the marketing of this substance in conventional foods should be directed FDA's Office of Premarket Approval (HFS-200), 200 C St., SW, Washington, DC 20204.

Please contact us if we may be of further assistance.

Sincerely,



Lynn A. Larsen, Ph.D.
Director
Division of Programs and Enforcement Policy
Office of Special Nutritionals
Center for Food Safety
and Applied Nutrition

NATURE'S MARVEL

September 24,1999

Office of Special Nutritionals
Center for Food Safety and Applied Nutrition
Food and Drug Administration
200 C St. SW
Washington, DC. 20204

NEW DIATARY INGREDIENT PREMARKET NOTIFICATION

NAME OF DISTRIBUTOR. NATURE'S MARVEL INTERNATIONAL
ADDRESS. 1681 ALTA LA JOLLA DRIVE. LA JOLLA, CA. 92037
NAME OF THE DIETARY SUPPLEMENT. LO HAN KUO FRUIT EXTRACT. MOMORDICA GROSVENOR
SIRAITA GROSVENORI. LO HAN KUO GLUCOSIDE.
DESCRIPTION, CONDITIONS OF USE, LEVEL OF USE, PLEASE SEE ATTACHED
RECOMMENDED USE LEVEL IN ALL FOODS. 0.25%
SUGGESTED LABELING. EXTRACT OF THE FRUIT OF PARADISE, LO HAN KUO FRUIT EXTRAC

67540

NATURE'S MARVEL INTERNATIONAL
U.S. DIVISION
OFFICE OF STEVE BANYAN
1681 Alta La Jolla Drive • La Jolla, California 92037 • Phone 619.456.4501 Fax 619.459.2428

1. Brief Introduction of the Extract and the Major Technical Requirements

Lo-HAN-KUO [Siraitia grosvenorii (swingle) C. Jeffrey] is a calabash plant, whose ripe fruits are used for health purposes and as a dietary supplement. It is one of the traditional export items from China. After 1949, it's production has made a priority, especially during the 7th - 5 year planing period of the Chinese Government, funding was increased, more manpower and equipment were provided for producing the LO-HAN-KUO. Due to production increases, selling the extra fruit became a problem. This led to a desire to develop more applications where the LO-HAN-KUO can be used. Due to this urgent need to develop more uses for LO-HAN-KUO, and the research funding provided by the China Bank of Agriculture and Investment, led to a series of achievements. It is summarized as follows:

1) For the first time the chemical compositions of the fresh fruit of the LO-HAN-KUO have been determined. There are 5 calabash triterpene glucosides. From the structural studies. it was found that 3 of them are very sweet, and one of them is found the first time that it is the natural sweetner glucoside. NMR techniques were used for the structure determination. We also first time discovered the flavone compositions in the LO-HAN-KUO. We isolated and determined the structures of the grosvenorine from the new flavone glucoside. Those results are very useful for discovering the other applications.
2) New techniques for the extraction process have been developed. A resin extraction method was used which included deposing, regenerating the resins and product refinery. Large scale tests were done many times and then scaled up to a pilot plant with a 1.5 ton production scale. It has been proven that the technique has a short cycle, there is no need for special equipment, it is easy to operate, high production yields and batch to batch reproducibility is very high. The resin is easy to regenerate, produces less waste and generates no hazardous waste. This technique has been

approved for use in part of the production. The herbal extract manufactured from LO-HAN-KUO by using this process has 70% of the total glucosides content and the sweetness is 210 to 250 times sweeter than sugar, depending upon application. The recovery for fresh fruit is 1 % and 3 % by weight for the dried fruit.

3) First time regulated the quality assurance procedures, and the standard operating procedures and those are in effect.

2. Prospect of the Product and the Benefit

This study was based on using the fresh fruits. LO-HAN-KUO is specially grown in China. It grows around many southern Chinese provinces, and has a high yield and many suppliers. Due to the increasing growth of the plant, there is a large supply of the raw material.

Compare LO-HAN-KUO herbal extract with the synthetic sweetner, such as saccharine, duicin and sodium cyclamate, the biggest advantage of using LO-HAN-KUO is that LO-HAN-KUO is safe. is not harmful or toxic to the body. Regular sugar causes tooth decay. People gain weight from the calorie and human immune system is decreased. It can increase the fat content in the blood and cause blood vessel narrowing. For those synthetic sweetners, the toxicity has been tested and some of them have been limited or banned in some of the countries. LO-HAN-KUO has a high sweetness. It is about 210 - 250 times sweeter that sucrose so the caloric content of LO-HAN-KUO is minimal. It is very suitable for low calorie diet and drink supplements, for diabetes patients, people who are overweight and those people who have arteriosclerosis diseases. Meanwhile, LO-HAN-KUO is very soluble in water and ethanol and it is very thermostable. It does not decompose under continuous heating at 100°C for 5 hour and at 120 °C for 12 hour. This property made the process of beverage manufacturing and disinfective process as much easier. LO-HAN-KUO is non-fermentable and it is resistant to molding. It can be stored for long period of time, and has no strange smell, tastes good and smells good.

Since the fresh LO-HAN-KUO is quickly processed, it reduces the loss caused from moldy fresh fruits from storage. It requires less storage space and also cuts down the storage maintenance, disinfection and transportation. This reduces the cost. The by - product from the new technique can also be used for some other purposes, this becomes very economical.

3. Reviews from the Appraisal Committee

The extract of LO-HAN-KUO is an extract from the natural plant specially produced in China. The ripe fruits are used as food and in Chinese medicine. It has been an export item for China for a long time. Since the collaboration between GuangXi YongFu LO-HAN-KUO Manufacturer and the Department of Plant and Development from the Chinese Academy of Medical Sciences, they have discovered a new sweetner only available from China. LO-HAN-KUO's glucoside is 210 times sweeter than sucrose. A new natural sweetner is generated for both domestic markets and the international market, it will benefit the Children, elderly people, and people who need to reduce their sugar intake (such as diabetes patients, overweight people, high blood pressure patients, people having heart disease). LO-HAN-KUO glucoside has high sweetness, low caloric content, only use a small of amount. It tastes good and it is a substitute for sugar used for foodstuff, beverages, medicines, and other industries. It has a great future.

We have reviewed the production process and the quality assurance standards. By using the process which uses the resin to extract the glucoside from LO-HAN-KUO fresh fruits has generated better quality products. It also generated high yields from a number of large scale trials, and 3 production batches from the pilot plant. This process requires fewer production steps, less investment, and produces consistently high quality product from batch to batch. It is also easy to operate and produce no environmental pollutions. Scientifically and economically, it makes more sense.

We also reviewed the initial discovery of the chemical composition analysis from the fresh LO-HAN-KUO fruit. Five calabash triterpene glucoside and one flavone were found from the extraction of fresh fruit, a new natural glucoside was also found - a new LO-HAN-KUO type flavone. New technologies were used for determination the chemical structure.

It is a very difficult research. The organic and inorganic structures attach to the glucoside are also analyzed. This provided scientific evidence for the development and the utilization of this product.

This research is correct and reliable. It targeted the right thing. It has a full data to support the results. It is a new natural sweetner that can be used in the food industry. The new production process is advanced. The new discovery of the new natural sweetner and the utilization of the by -products will benefited the domestic and international markets. It also increases the economic value of the LO-HAN-KUO. It improves the economic development for the grown area and improve the living standards for the farms. It will create a new utilization of the natural resources.

Based on those considerations, we highly recommended to award this research.

Approved by Wang Zhenggang and Chen Xiaoshu

1/11/1993

4. Review Results form the Organizing Committee

Approved by the committee.

Sealed by

the Chinese Academy of Medical Sciences

6/4/1993

5. Review Results from the Appraisal Committee

Approved by the committee

Sealed by the Chinese Academy of Sciences

6/4/1993

6. Documentation Provider and the Name of the Company

A Corpus of Research and Development of LO-HAN-KUO Extract

GuangXi YongFu LO-HAN-KUO Manufacturing Standards
Q/452325 LGZ X50 02-92

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1. Scope and Contents

This procedure has set a number of standards for the manufacturing requirements, testing methods, QC and QA procedures, packaging, storage and transportation.

This procedure is suitable for fresh LO-HAN-KUO and dry LO-HAN-KUO as the raw materials.

2. Manufacturing Standards

GB 601 Manufacturing method for Chemical Reagents and Standard Solutions.

GB2760 Hygiene Standards for Food Additives

GB4789.2 Microbiological Testing for Food and Hygiene, Total Microbe Determination

GB4789.3 Microbiological Testing for Food and Hygiene, Salmonella Determination

GB4789.4 Microbiological Testing for Food and Hygiene. Pathogenic Bacteria Determination

GB5009.3-85 Water Content Determination in the foodstuff.

GB5009.4-85 Dust Determination in the foodstuff.

GB5009.11 Total Arsenic Determination in the foodstuff.

GB5009.12 Total Lead Determination in the foodstuff.

GB7718 Labeling Standards for Foodstuff

ZBX51003 Testing Standards, Regulations, Packaging, Transportation, Storage Standards for Fragrant Fruit Type Solid Drinks.

GuangXi YongFu LO-HAN-KUO Manufacturer Approved on 9/2/1992 and Effective on 9/2/1992

3. Technical Requirements

3.1 Apparent

It should be a light yellow or yellow power, has special fragrance, very sweet and very soluable in water and ethyl alcohol.

3.2 Physical Properties

Table 1

Items	Specifications
Content %	> 70
Sweetness	>210
Absorption $E^{3\%}_{1cm}$ 410 nm	<0.4
Dust %	<0.9
Water Content %	<9
Pb mg/kg	<1.0
As mg/kg	<0.5

3.3 Identification

Take exactly of 10 mg of the sample, dilute it in 1 ml of MeOH, make sure it is dissolved. make it as a sample solution. Take 10 mg of LO-HAN-KUO standard and make a standard solution as the one you made for the sample solution.

Using TLC test to make sure the sample TLC spots match the standard TLC results (Attachment 2: 1990, volume 1 Appendix, Page 57 TLC method).

3.4 Microbe Standard

Table 2

Items	Specifications
Total Microbe #/gram	<1000
Salmonella #/gram	Undetectable
Bacteria #/gram	Undetectable
Live mites, eggs #/gram	Undetectable

4. Testing Procedures

4.1 Test by Appearance and Taste:

Take 10 g of the sample, use eye to check it's color. It should be light yellow or yellow, it should have a nice fragrance. Take 5 g of it, dissolve it in water. The resulting solution should be tasted very sweet.

4.2 Physical Test:

4.2.1 Content determination

Generate a standard curve:

Weigh exactly 30 mg of LO-HAN-KUO reference standard and add to a 5 ml volumetric flask, add 70% ethanol in water and dilute to mark and shake. Take exactly 10, 20, 30, 40, 50, ul to 10 ml test tubes which can be capped. Use hot air to evaporate out the solvent (Not too high temperature) add 0.2 ml of freshly made 5% vanillin - glacial acetic acid solution and add 0.8 ml of perchloric acid, heat it in 60 ^{0}C water bath for 15 minutes, take it out and cool it with cold water immediately. Add 5 ml of glacial acetic acid, shake. Use the solvent as the blank, to generate a concentration vs. absorbance curve at 590 nm using spectroscopy (the spectroscopic method is described in Appendix 1: Volume 1, page 51, 1990 Chinese Pharmacopoeia). All the test should be done with one hour.

Concentration Determination:

Weigh exactly 30 mg of sample, dissolve it in 5 ml volumetric flask with 70% ethanol, diluted to mark, shake it well. Dilute 40 ul of the solution to a 10 ml test tube with cap and determine the absorbance. The concentration of the sample from the standard curve and calculate the concentration as follows:

Content X = c /(8 * W) x 100%

c: Concentration obtained from the standard curve (Unit, μg)

W: Sample weight (unit: mg)

4.2.2 Sweetness Test:

Compare the 2% sucrose solution with 210 fold dilution of the 2% of the LO-HAN-KUO solution.

4.2.3 Absorption Determination:

Weigh sample and make up a 2 mg/ml solution and determine the absorbance (Spectroscopic method is in Appendix 1: Volume 1, page 51, 1990 Chinese Phamacopoeir) at 410 nm.

4.2.4 Dust Determination:

Under the guidelines of GB 5009.4 -85

4.2.5 Water Content Determination:

Under the guidelines of GB 5009.3 -85

4.2.6 Lead Content Determination:

Under the guidelines of GB 5009.12

4.2.7 Arsenic Content Determination

Under the guidelines of GB 5009.11

4.3 Identification:

Weigh 10 mg of the sample, add 1 ml of MeOH to make sure it dissolves. Use it as sample solution. Weigh 10 mg of the LO-HAN-KUO reference standard and make up the solution in the same way.

Run TLC tests (Attachment 2: 1990, volume 1 Appendix, Page 57 TLC method). Spot both reference standard and sample, develop in $CHCl_3$ - MeOH - H_2O (40: 23: 5). Spray with 10% phosphomolybdic acid in ethyl alcohol, heat at 110°C for 5 minutes. The spots shown on the TLC plate for the sample should match Rf the spots of the reference standard.

4.4.1 Total Microbe Determination

Under the guidelines of GB4789.2

4.4.2 Salmonella Determination:

Under the guidelines of GB4789.3

4.4.2 Pathogenic Bacteria Determination

Under the guidelines of GB4789.4

5. Quality Assurance Regulations:

5.1 Each cycle of the production has one batch number.

5.2 Samples are random tested with each batch and once it passes all the requirements, it will be issued a pass certificate before the product can go out of the factory.

5.3 Under the normal production conditions, bacteria level, dust content, water content, absorption, and sugar content are mandatory tested routinely on every batch. Other tests are done randomly at a regular basis.

5.4 If is test is failed, a second test can be done. If the second test still failed, then those batches will be failed.

5.5 During the guarantee period, if there is concern from the customer about the quality. The problem can be discussed or brought to an arbitrator. If the quality problem is due to the inappropriate transportation, or storage, the manufacturer will not be responsible for the loss. The transportation, storage firm should be responsible.

6. Label, Packaging, Transportation and Storage

6.1 Label

The label should have the product name, manufacturer name, address, registered trade mark, production date (or batch #), expiration date, product standard code and net weight.

6.2 Packaging

All the packing material should meet the requirements of " The People's Republic of China Food and Hygiene Regulations (trial version)", Under the guideline of GB 10790.

6.3 Quality Guaranteed.

Name List of the Major Contributions

#	Name	Age	Education	Major	Title	Company	Major Contributions
1	Chen Dihua	51	BS or above	Organic Chemistry	Scientist	Plant Dept. of the Chinese Academy of Sciences	Project leader for chemical analysis and quality assurance and production design
2	Chang Qi	30	BS or above	Pharmacy	Associate Scientist	Plant Dept. of the Chinese Academy of Sciences	Chemical analysis, technical requirements and quality research
3	Jiang Chunfa	29	BS or above	Chemical Engineering	Engineering	Plant Dept. of the Chinese Academy of Sciences	Technical requirements
4	Si Jianyong	30	BS or above	Pharmacy	Associate Scientist	Plant Dept. of the Chinese Academy of Sciences	Chemical analysis and production research
5	Liu Xibin	58	BS or above	Mechanics	Senior Scientist	Plant Dept. of the Chinese Academy of Sciences	Coordinator and process testing
6	Shen Liangang	22	Vocational School		Technician	Plant Dept. of the Chinese Academy of Sciences	Process research
7	Huong Bin	26	BS or above	Chinese Medicine	Associate Scientist	Plant Dept. of the Chinese Academy of Sciences	Process testing

Name List of the Appraisal Committee

Title in the Committee	Name	Company	Major	Title	Signature
Chairman	Wang Zhenggang	Medical Research Institute of the Chinese Academy of Sciences	Pharmacy	Scientist	Signed
Vice Chairman	Chen Xiaoshu	Nutrition and Foodstuff Research Institute of China Academy of Prevention Medicinal Science	Nutrition	Scientist	Signed
Member	Shen Guohua	Beijing Foodstuff Research Institute	Foodstuff	Senior Scientist	Signed
Member	Zhang Yuzhong	Chinese Medicine Research Institute	Analytical Chemistry	Scientist	Signed
Member	Sha Shiyan	Medicinal Research Institute of the Chinese Academy of Medical Sciences	Analytical Chemistry	Scientist	Signed
Member	Sun Weillian	Plant Research Institute of the Chines Academy of Medical Sciences	Plant Chemistry	Scientist	Signed
Member	Bi Zhi	Sino-Japanese Hospital Clinical Research Institute	Plant Chemistry	Scientist	Signed

The Certificate of the Glucoside of the LO-HAN-KUO as a Dietary Supplement

Glucoside of the LO-HAN-KUO is an extract from the fresh fruit LO-HAN-KUO. It is a dietary supplement. It is co-developed by the Plant Resources Research Institute of the Chinese Academy of Sciences and GuangXi YongFu Manufacturer. It is a dietary supplement and only available in China.

LO-HAN-KUO has been used as a fruit, summer beverages and it has been used as a Chinese healthful herb by the people of GuangXi and GuangDong Provinces. It was recorded in 1997 in the China Pharmacopoeia as a Chinese herbal extract. In 1987, LO-HAN-KUO was listed as the first group of items that can be used as a dietary supplement by the Administration of Chinese Medicine of the Ministry of Hygiene. It showed that LO-HAN-KUO is safe for human consumption. The glucoside is the main content in the LO-HAN-KUO, and is useful as a dietary supplement.

GuangXi YongFu Manufacturer

6/3/1997

Attachments:

LO-HAN-KUO glucoside production diagram

2. The Modern Research of LO-HAN-KUO

2.1 In 1975, it was reported by Lee, et al[1] that there was a triterpene contained in LO-HAN-KUO, but there was no structure. In 1983, Takekimatsuhar from the University of Dedao in Japan discovered that LO-HAN-KUO contains much fructose. They also found that 3 kind of sweet LO-HAN-KUO glucosides. All those discoveries were done with the commercial available LO-HAN-KUO sold in Hong Kong. Later he isolated and structurally determined 7 components from the LO-HAN-KUO purchased in Macao. In 1992, Chen Dihua from the Department of Plant Resources and Development of the Chinese Academy of Medicinal Sciences did further research on the chemical composition. He was able to isolate 6 kinds of glucoside. There were also results on isolated glucoside and its contents of low molecular weight sugar, hydrolysis amino acids, some of the vitamins, fatty acid and inorganic compounds. Before Chen's research, Xu Weiqun from GuangXi Plant Research Institute also did the measurement of the amino acids content. Zhao Jifu et al did the sweetness testing: Chen Hongbing (from the Plant Resources and Development of the Chinese Academy of Sciences) did the toxicity studies on the LO-HAN-KUO extract.

2.2 Takekeharu [2] determined there are mogrosides IV, formula $C_{54}H_{92}O_24 \cdot H_2O$, mogroside V, formula $C_{60}H_{102}O_29 \cdot H_2O$ and mogroside VI, formula $C_{66}H_{112}O_{34}$ from the dry LO-HAN-KUO. They also determined the glucoside on V - is mogrol and its glucoside structure [3]. it is approved as triterpene.

2.3 Matsuki [4] separated and determined 7 components. Including the structures mentioned above, mogrosides IV, and mogroside V, there was also siamenoside I, formula $C_{54}H_{92}O_{24} \cdot 7/2\ H_2O$,11 - DXO - mogroside V, formula $C_{60}H_{100}O_{29} \cdot 7/2\ H_2O$, mogroside II E, formula $C_{42}H_{82}O_{19}$ and a very small amount of mogroside III, formula $C_{48}H_{82}O_{19}$ (compare with the mogroside III E, they are sterioisomers, they have different optical properties). Siamenoside I was determined as the sweetest triterpene, the sweetness is 563 times sweeter than 5 % of regular sugar when the concentration is diluted to 1/1,000,000.

2.4 Chen Dihua, et al[6] did further research with the fresh LO-HAN-KUO. They obtained the total glucoside and then isolated the mogroside II E, mogroside III, mogroside IV, mogroside V and the new discovery of the neomogroside, formula $C_{66}H_{112}O_{34}$. Among them, mogroside is the main component of the LO-HAN-KUO, which takes up to 0.5 % of the total weight of the fresh LO-HAN-KUO. They also found two kinds of flavones, the structure is -3-O-α–L- rhamnose- 7 - O- β - D-Glucosido-(1-2)- α -L- Rhamnose and phenyl -3, 7- α -L- dirrhamnoside. The first one is the natural product. Later on they determined the remaining product other than the extract. They were able to determine the low molecular weight sugar, hydrolyzed amino acids, some of the vitamins, fatty acids, and some of the inorganic component. The oil extracted from the solid waste takes about 0.8 % of the total fresh weight. It contains unsaturated fatty acid (49.9 %), saturated fatty acid (7.7 %), palmic acid (7.2 %) and stearic acid (4.4 %). Vitamin A in the oil is 8 IU/g. Other than the extracts, there are glucose which takes up 0.8 % of the total weight of the fresh fruit, fructose which takes up to 1.5 % of the total fresh weight, vitamin B1, B2 which take up to 3.38 % and 1.23 % (mg/g). The hydrolysis of various amino acids and the content is listed in Table 1.

Table 1 - Hydrolysis of the Various Amino Acid and their Content:

	Contents g/100 g		
Amino Acids	Fresh LO-HAN-KUO after removal water	water after removed the sugar content	solid waste
aspartic acid	0.9	1.56	0.61
threonine	0.25	0.28	0.25
serine	0.35	0.39	0.37
glutamic acid	0.55	0.97	0.87
glycine	0.36	0.32	0.44
alanine	0.53	0.82	0.40
cystine	0.24	0.23	0.16
valine	0.47	0.48	0.48
methionine	0.2	0.15	0.14
isoleucine	0.4	0.38	0.38
leucine	0.5	0.41	0.54
tyrosine	0.28	0.34	0.29
phenylalanine	0.32	0.31	0.41
lysine	0.31	0.22	0.37
histine	0.18	0.20	0.14
arginine	1.28	1.08	0.68
proline	0.27	0.19	0.27
total	7.38	8.36	6.87

The inorganic contents are listed in Table 2.

Table 2 - Inorganic Contents

	Content ppm	
Elements	Fresh LO-HAN-GUO after removal the water	water after removed the sugar content
Al	16.7	18.63
B	12.9	51.79
Ba	15.4	13.88
Ca	2221	11049
Cr	2.25	4.59
Cu	7.70	14.77
Fe	159	97.36
K	16089	44314
Mg	1138	6971
Mn	20.82	30.2
Cu	1.26	4.4
Na	86.5	4280
Pb	6.08	13.84
Zn	11.4	265
S	1314	5889

2.5 In 1986, Xu Shenchun [7] did many chemical composition studies, he reported that the protein is 7.1 - 7.8 % of the total dried fruit. He also reported the contents of the 18 out of the 19 amino acids from the hydrolysis for four different LO-HAN-GUO (different grower). In 1980, he measured 339 - 487 mg of Vitamin C per 100 g of fresh fruit. He also measured 0.1864 ppm of selenium (Se) which is 2 - 4 times more than the grain [8].

Selenium was reported that it has anti-heart disease, anti aging and anticancer effects.

2.6 In 1992, Zhao Jifu et al from the Tanjing Engineering Institute did the LO-HAN-KUO extract sweetness testing. The test was used sucrose as the threshold value (0.5 %, weight percentage). Using the international threshold stimulant testing method, it was reported that the extract from LO-HAN-KUO is 210 times sweeter than sucrose (t test, 5 % standard deviation) [9]. It was reported that the extract did not decompose when heated at 120 °C for 12 hours.

2.7 There has also been progress on the pharmacology and toxicity studies. In 1983, Takekimatsuharu [10] used mice, rabbits and dogs for tests. The LO-HAN-KUO extract resists the intestines shrinking cause by BaCl or acetyl chlorine, he makes the intestines move easily. At a high dosage, it reduced the blood pressure. In his toxicity study reports, it stated LD 50 testing (Half fatality number) on mice that LO-HAN-KUO extract (freeze dry) is greater than 10 g/kg; Chen Hongbin [11] used mice for LD 50 testing, he used the maximum concentration (60 %) and maximum volume (0.4 ml/ 10g wt) poured the solution (equivalent to 24 g/ kg body weight) down the throat, and observed the activity of the animal and found no fatalities. The animals were under observation for two weeks after the dosage and there was no abnormal behavior and no fatalities observed. Based on the regulations of the Ministry of the Hygiene which stated that if there is no fatality during the first stage of the rapid toxicity testing with a dosage of 10 g/ kg of the body weight, it is not necessary to do the LD 50 testing (Half fatality testing).

3. LO-HAN-KUO Extract's Future

In the southern part of the China, drinking LO-HAN-KUO tea is as a status of the person for more than hundred of years. Due to it's good taste, people like it. Recent years, LO-HAN-KUO has been used in herbal remedies. There are more than 20 years of history of people using dry LO-HAN-KUO as a tea. Since the 80's, LO-HAN-KUO has always been a product requested by the foreign trade department. It has been exported to most countries in Southeast Asia.

References

[1] L. H. Lee, Experientia, 1975; 31: 533.

[2] Takekimatsuharu, 3, Journal of Pharmacology, 1983; 103: 1151.

[3] ibid 1983, 103 : 1151; 1983; 103: 1167.

[4] K. Matsumoto et al, 1990; Chem. Pharm. Bull. 38 (7): 2030.

[5] R. Sasai et al, 1989; Agric. Biol. Chem, 53 (12): 3347.

[6] Chen Dihua et al; A Corpus of the Research, Development and the Utilization of Chinese Medicine LO-HAN-KUO; 1993, Beijing.

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The Chemical Composition Analysis of Fresh LO-HAN-KUO (I) The Isolation of Triterpenoid Glycosides and Their Structural Determination

Abstract: Chinese fruit LO-HAN-KUO [Siraitia grosvenori (swingle) C. Jeffrey] is specially cultivated in China and it is used as an herb. In this article, 6 glucoside compounds were extracted from the fresh fruit LO-HAN-KUO: Neomogroside **5**, and the known cucurbitane glucoside-mogroside II E, **1**, mogroside III, **2**, mogroside IV, **3**, mogroside V, **4** and a non-cucurbitane glucoside **7**. **3 - 5** are very sweet. **4** is the major component of the LO-HAN-KUO and has about 0.5% of the fruit weight. **5** is a new sweet glucoside, there is only trace amount in LO-HAN-KUO. The structures of **1 - 5** were determined by using their spectral data (^{1}H and ^{13}NMR, ^{13}C-^{1}H COSY, ^{1}H-^{1}H COSY and NOE Difference Spectroscopy) plus chemical reaction methods. The non-cucurbitane triglycoside is not sweet, its structural determination is in progress.

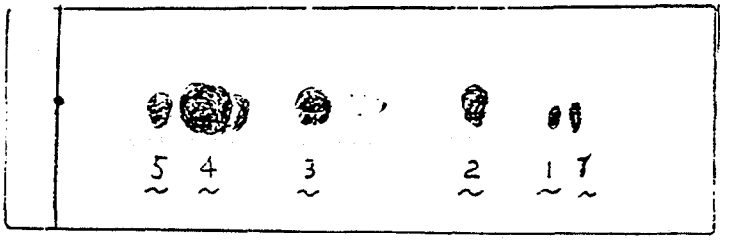
Keywords: LO-HAN-KUO genus, cucurbitane triglycoside, LO-HAN-KUO, herbal extract.

LO-HAN-KUO is classified as the calabash family [Siraitia grosvenori (swingle) C. Jeffrey] and is special vine plant cultivated in China. It is cultivated in YongFu and other counties of the northern part of GuangXi autonomous region. The ripe fruit is used as Chinese herb and tastes sweet. It is beneficial to the respiratory and digestive systems. The fruit is used as a dietary supplement and made into a summer drink by the people in GuangXi and GuangDong provinces. The healthful efficacy of LO-HAN-KUO is recorded in all editions of Chinese pharmacopoeia after 1977[1]. Japanese scientists Takemoto[2] and Matsumoto[3] had studied the chemical compositions of the dry fruit from markets in Hong Kong and Macao. They found 6 glycosides and one aglycone, all

of them are cucurbitane glycosides. According to literature[4], those compounds are the effective components of LO-HAN-KUO. During the course of study the utility of LO-HAN-KUO, we have found that there are many advantages of extracting the glycosides from the fresh fruit over the dried one. From the same quantity of the fresh fruit, the product extracted from the fresh fruit has better yield and lighter color than that from the dried fruit. The operation process is easier for fresh fruit than for the dried one. In addition, due to the lower price of the fresh fruit, it costs less to make the sweet glycosides, most of the production process is starting from the fresh fruit. It has not been reported yet whether the major sweetener composition in the fresh fruit is the same as that in dry fruit. Further study has to be done on this issue. Therefore we have carried out isolation process from the enriched glycoside mixture and we have found 5 cucurbitane glycosides and one non-cucurbitane glycoside. Upon analyzing their spectroscopy data and carrying out chemical tests, we have determined the structures of 5 components. They are mogroside II E (**1**), mogroside III (**2**), mogroside IV (**3**), mogroside V (**4**), and meomeogogroside (**5**). All of them are glucoside of mogrol (**6**) and they are diglucoside, triglucoside, tetraglucoside, pentaglucoside and hexaglucoside of **6** respectively. Components **3**, **4** and **5** are very sweet and they contribute to the sweetness of the LO-HAN-KUO. Component **4** is the major content of the LO-HAN-KUO and it takes up to 0.5% of the fresh fruit weight. Due to components **3**, **4** and **5** contain more sugar groups and they are all the same glycoside, it is very difficult to separate them as well as to determine their structure. The assignment of the NMR spectra is very difficult due to the heavily overlap of the signals on their ^{1}H or ^{13}C NMR spectra. It is very hard to determine the connecting position between sugar and aglycone as well as and between sugar groups. Even though components 3 and 4 are known compounds, all literatures [2, 3, 5] did not give assignments of their NMR spectra for the sugar part. In our study, we not only took the 1D NMR spectra (^{1}H and ^{13}C) and 2D NMR spectra (^{13}C-^{1}H COSY and ^{1}H-^{1}H COSY), but also applied the NOE difference spectroscopy. Some signal overlaps of

the sugar part have been effectively separated and assigned. This result is never achieved in the study on the glycoside on the LO-HAN-KUO before. We have compared the ^{1}H and ^{13}C data of **1 - 5** with those of **6**, the ^{1}H NMR chemical shifts of 4, 5 are listed in Tables 1, 2, 3 and the ^{13}C shifts of **1 - 5** are listed in Table 4 and 5. The non-cucurbitane glycoside **7** is the trace component in LO-HAN-KUO and not sweet, its structure assignment is in progress.

TLC analysis of the glycoside components from the fresh LO-HAN-KUO

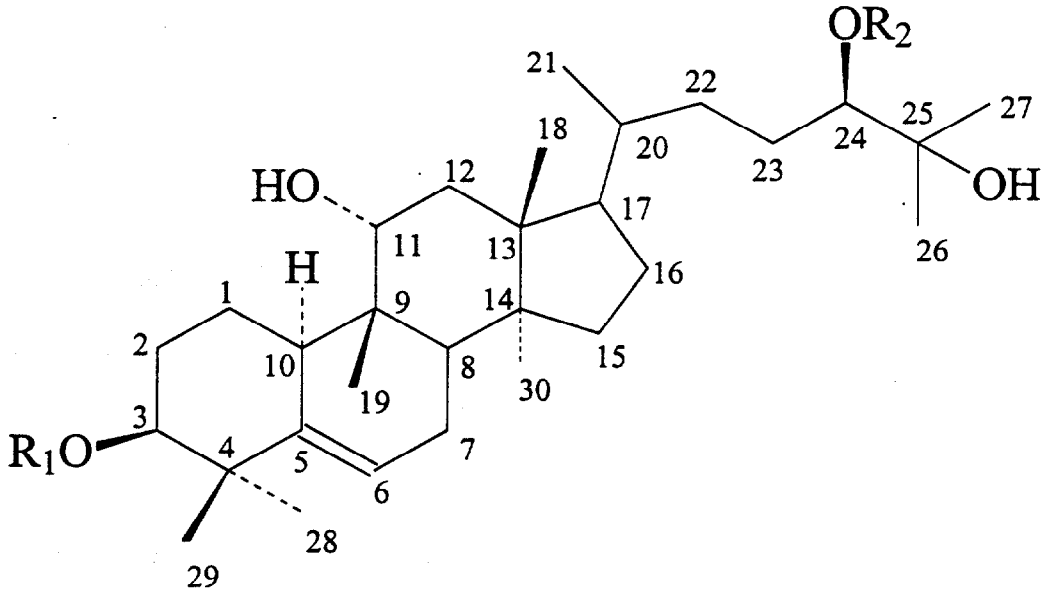


Silica Gel plate

$CHCL_3$-MeOH-H_2O (40:23:5, homogeneous phase)

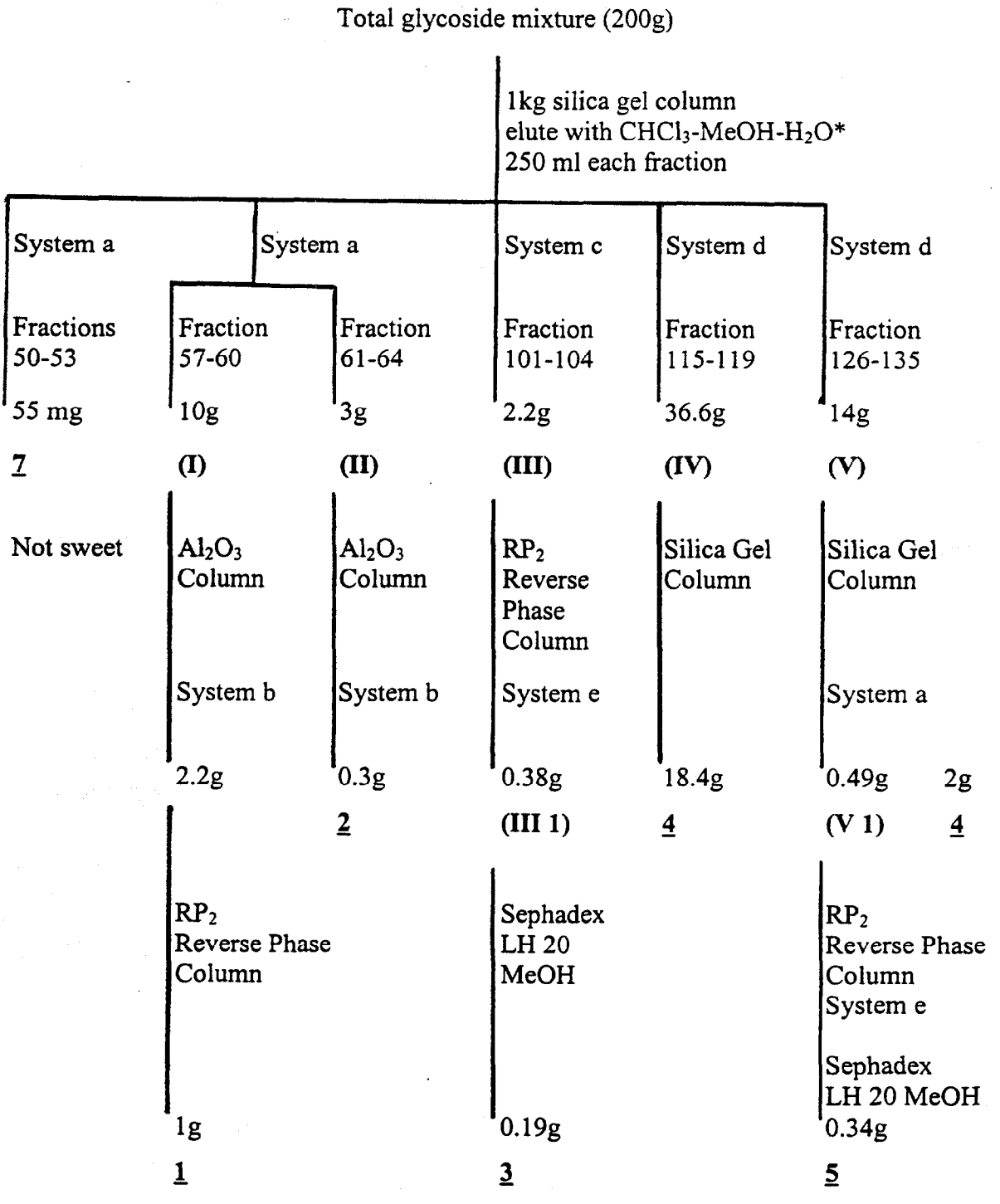
Spray with 10% phosphomolybdic acid in ethyl alcohol and heat to stain

Relative ratio 1:2:3:4 = 2.7: 12.2: 18.4: 66.7 (TLC scan results)



	R1	R2
1	Glc	Glc
2	Glc	Glc—(6-1)—Glc
3	Glc (I)—(6-1)—Glc (II)	Glc (III)—(2-1)—Glc (IV)
4	Glc (I)—(6-1)—Glc (II)	Glc (IV)—(6-1)—Glc (V); Glc (IV)—(2-1)—Glc (VI)
5	Glc (I)—(6-1)—Glc (II); Glc (II)—(2-1)—Glc (III)	Glc (IV)—(6-1)—Glc (V); Glc (IV)—(2-1)—Glc (VI)
6	H	H

Figure 1 Separation Flow Chart for LO-HAN-KUO Glycosides



*Elute system: a: 6.5: 3.5: 1 d: 5: 5:1
b: 7.5:2.5:1 e: 60% MeOH-H_2O
c: 6:4:1

1, Formula $C_{42}H_{72}O_{14}$, white powder, M/Z = 800 by FDMS; NMR results (Table 4) indicate that **1** has the same skeleton as **6**, in its ^{13}C NMR spectrum, δ 107.4 and δ 106.0 ppm indicate **1** is diglucoside. TLC acid hydrolysis indicates its sugar is glucose. ^{1}H NMR gives terminal protons δ 4.87 (1H, d, J = 7.8 Hz) and δ 4.98 (1H, d, J = 8.0 Hz) ppm, this indicates that the glucosidic bond is in β configuration. Compare the ^{13}C NMR data of **1** and **6**, chemical shifts of C_3 (δ 88.0ppm) and C_{24} (δ 90.8ppm) is obviously down field, this indicates the glucosidic bond is between C_3 and C_{24}. Therefore, we assign **1** as mogroside II E.

2, white powder, m/z = 986 $[M+H+Na]^+$, has a formula of $C_{48}H_{82}O_{19}$, Compare the ^{13}C NMR spectra of **2** with **1** (Table 4), they all have the similar ^{13}C chemical shift for their main frame. This means that **1** and **2** have the same skeleton of mogrol. The only difference is that **2** has three sugar terminal groups, which means **2** is a triglucoside. Result from TLC acid hydrolysis indicates that all three sugars are all glucose. The same as **1**, δ 88.0ppm in ^{13}C NMR spectra of **2** indicates that C_3 is connected to a glucose. For the remaining two sugar, δ 92.6 ppm indicates that the glucosidic bond is located at C_{24} position. δ 70.5ppm indicates a 6-1 connection between two sugars, that is, the are gentiobiose. ^{1}H NMR give sugar terminal protons at δ 4.84, 4.92 and 4.98 ppm, coupling constant J = 7.7, 7.4, 7.5 Hz respectively, they are all in β configuration. So, **2** should be mogrolyl-3-O-β-glucopyranose-24-O-β-gentiobios, it is named mogroside III.

3. $C_{54}H_{92}O_{24}$, ^{1}H NMR and ^{13}C NMR have shown that it has 4 sugar groups, this indicates that **3** is a tetraglucoside. Compare ^{13}C NMR data of **3** and **2** (Table 4), **3** and **2** have the same aglycone part, **3** also should be the glucoside of mogrol. TLC acid hydrolysis indicates all sugars in **3** are glucose. The same as **2**, chemical shifts of C_3 (δ 87.4ppm) and C_{24} (δ 92.6ppm) indicate the glucosidic bonds are located at C_3 and C_{24}. Analysis of ^{13}C-^{1}H COSY and NOE difference spectroscopy, the connecting pattern between sugar in **3** is determined. From the ^{13}C NMR spectrum of **3**, δ 62.5, 62.5, 63.2,

70.2 ppm represent the four terminal carbons, δ 70.2 also indicates a 1-6 connection. Chemical shift at δ 82.8 ppm indicates a 1-2 connection[3]. From NOE difference spectroscopy of **3**, irradiation at terminal proton (δ 4.78ppm), give enhancement at 6 positions, the broad single peak at δ 3.64 is C_3H of aglycone, it has a cross section with δ 87.4ppm in ^{13}C-^{1}H COSY. Therefore, the sugar with terminal proton at δ 4.78 ppm should be located inside and is connected at C_3 position. In addition, signals at δ 4.14 (I_{6b}), 4.64 (I_{6a}) are also enhanced, and δ 4.14 (I_{6b}), 4.64 (I_{6a}) have cross section with δ 70.0 ppm (-CH_2O-), this indicate they are the two protons of C_6 of sugar I and sugar I and sugar II have 1-6 connection. Irradiation at δ 5.05 ppm (II $_1$) also results signal enhancement at δ 4.14 and 4.64 ppm. This gives additional evidence for a 1-6 connection between I and II. Upon irradiation the terminal proton at δ 4.80 ppm (III $_1$) signal enhancement at δ 3.65 (C_{24}-H) is observed, δ 3.65 (C_{24}-H) has a cross section with δ 91.7ppm (C_{24}) in ^{13}C-^{1}H COSY, it indicates that sugar III is connected at C_{24}. Signal at δ 4.10ppm is also enhanced and peak at 4.10ppm has cross section with δ 82.8ppm, so sugar IV and sugar III has 1-2 connection. Therefore, **3** is a compound formed by C_3 of mogrol connected to gentiobiose, and C_{24} of mogrol connected to sophorose, it is mogroside IV.

4. White power, m/z = 1310 $[M+H+Na]^+$ with FDMS, ^{13}C and ^{1}H NMR indicates it has five sugar groups, so **4** is a pentaglucoside. Acid hydrolysis has approved all sugar groups are glucose. Comparison ^{13}C NMR data of **4** with **3** indicates that **4** is also glucoside of mogrol. Therefore **4** has formula of $C_{60}H_{102}O_{28}$. The aglycone part of **4** gives similar or equivalent chemical shifts to that of mogrol (except C_3 and C_{24}), this indicates that the glucosidic bonds are formed at C_3 and C_{24} positions. To elucidate the structure of **4**, its glucosidic bond configuration and the connection pattern between sugars have to be determined. From the coupling constant of the five terminal protons (J = 7.7-8.0 Hz), all glucosidic bonds have β configuration. The connection pattern

between sugars are determined by analysis ^{13}C-^{1}H COSY, ^{1}H-^{1}H COSY, and NOE difference spectroscopy. Chemiscal shifts of aglycone part are listed in Table 4 by analysis all spectra of **4** and comparison the ^{13}C data of mogrol. All remaining signals are belong to sugar parts of **4**, the chemical shifts of five terminal carbons is assigned as δ 62.8, 63.0, 63.7, 70.2, 70.4 ppm. δ 70.2 and 70.4 ppm indicate that there are two 1-6 connections (chemical shifts of $-CH_2O-$) and δ 82.7 ppm indicates there is a 2-1 connection.

From the NOE difference spectroscopy of **4**, signal enhancement is observed at 6 locations upon irradiation at δ 4.77 results. The broad single peak at δ 3.67 is C_3-H of aglycone, it has a cross section with δ 87.4 (C_3) in ^{13}C-^{1}H COSY. The sugar with terminal proton at δ 4.47 is the inner sugar connected to C_3 position of aglycone. Other enhancements are located at δ 3.87 (I_2), 4.13 (I_3), 4.03 (I_5), 4.28 (I_{6b}), 4.72 (I_{6a}). The last two have cross section with δ 70.4 ($-CH_2O-$) in ^{13}C-^{1}H COSY, this indicates they are protons at 6 position of sugar I, sugar I and Sugar II have 6-1 connection. Enhancement at δ 4.00 (II_2), 3.93 (II_3), 4.24 (II_5), 4.28 (II_{6b}), 4.72 (I_{6a}) is observed by irradiation at δ 5.12 (II_1) (refer to Figure), enhancement at 4.28 (II_{6b}), 4.72 (I_{6a}) further proves the 6-1 connection of sugar I and II.

In the ^{1}H-^{1}H COSY spectrum, δ 3.78 is correlated to δ 4.77, the proton at δ 3,78 is belong to I_2. The carbon at δ 75.4 is correlated to δ 3.78 in ^{13}C-^{1}H COSY, it is belong to sugar I_2. The assignment of I_3, I_5 and their protons is based on the enhancement (δ 4.13, 4.03) by NOE and the corresponding carbons (δ 78.4, 77.3) is assigned according to the cross over in ^{13}C-^{1}H COSY in combination the fact of carbon chemical shifts of glucose (chemical shift of C_3 is downfield relative to that of C_5[3]). It is determined that carbon I_3 at δ 78.4, carbon I_5 at δ 77.3 ppm, the proton I_3 at δ 4.13, and the proton I_3 at δ 4.03. The proton at C_4 position of glucose is far apart from the terminal proton[4], there is no NOE enhancement observed. Except C_6, C_4 of glucose appears at the most upfield, which is around 73.0 ppm[3], therefore the carbon at δ 71.7 is assigned as I_4 carbon. Further, the

corresponding to I_4 proton is assigned at δ 3.91 according to ^{13}C-^{1}H COSY. Other ^{13}C and ^{1}H chemical shifts are also assigned by the same means, we are not list all of them out.

Further irradiation at δ 4.88 (IV_1) results in enhancement at δ 3.74 (C_{24}-H) and δ 3.74 is correlated to δ 91.7 (C_{24}) in ^{13}C-^{1}H COSY, this indicates sugar IV is connected to C_{24} of aglycone. In addition, obvious enhancement at δ 3.93 (IV_{6b}), 4.02 (IV_5), 4.13 (IV_2), 4.21 (IV_3), is also observed. Since δ 4.13 is correlated to δ 82.7 (IV_2) in ^{13}C-^{1}H COSY, position 2 of sugar IV is connected to sugar VI and. δ 3.93 is correlated to δ 70.2 (IV_6), position 6 of sugar IV is connected to sugar V. Irradiation at the last terminal proton δ 4.83 (IV_1) in NOE difference spectroscopy results in enhancement at δ 3.93 (IV_{6b}), 4.01 (IV_3), 4.02 (IV_1), and 4.20 (IV_5) (refer to figure). These results further prove that sugar IV and sugar VI have 2-1 connection, sugar IV and sugar V have 6-1 connection.

From the results obtained, the C_3 position of aglycone is connected to diglucoside (I 6-1 II) and the C_{24} position is connected to triglucoside (IV 6-1 V, -IV 2-1 VI). Therefore **4** is determined as mogrolyl-3-O-[β-D-glucopyranosido (6-1)-β-D-glucopyranose]-24-O-{[β-D-glucopyranosido (2-1)][β-D-glucopyranosido (6-1)-β-D-glucopyranose]}, mogroside V in short.

5 is white powder, FBMS give m/z 1471 $[M+Na]^+$, acid hydrolysis gives only glucose, the aglycone part is determined as mogrol, its formula is $C_{66}H_{112}O_{34}$. ^{13}C NMR of **5** gives 6 terminal carbons and this indicates **5** is the hexaglucoside of mogrol. Compare the ^{13}C chemical shifts of **5** and mogrol (**6**), chemical shifts of C_3 and C_{24} of **5** are obviously downfield. This indicates the glucosidic bonds are also formed at C_3 and C_{24} positions. The connection pattern between sugar and aglycone, sugar and sugar is determined through ^{13}C-^{1}H COSY, NOE difference spectroscopy of **5** as well as comparison with those of **4**. ^{1}H NMR indicates coupling constant of the terminal protons of all sugar are

between 7.4~7.8Hz, so all sugars have β configuration. ^{13}C NMR indicates that there are two 6-1 connections (δ 70.2 and 70.5 ppm) and two 2-1 connections (δ 82.5, 82.6 ppm). ^{13}C chemical shifts of aglycone of **5** is assigned in Table 4 according to cross over in ^{13}C-^{1}H COSY and comparison to spectral data of **4**. δ 87.6 and 91.9 ppm are assigned as C_3 and C_{24} respectively and these two carbons are connected to sugar. In NOE difference spectroscopy, irradiation of the proton at δ 4.79 (sugar I $_1$) results in obvious enhancement of signal for proton correlated to C_3 of aglycone. This result indicates this proton is the terminal proton of the inner sugar. In addition, Enhancement is also observed at δ 4.30 (I $_{6a}$), 4.15 (I $_3$), 4.04 (I $_5$), 3.87 (I $_2$), 4.21 (I $_4$). δ 4.30 is correlated to δ 70.5 ppm (-CH_2O) in ^{13}C-^{1}H COSY, this indicates position 6 of sugar I is connected to sugar II. Due to the chemical shift of I $_{6b}$ proton is overlapped with that of terminal proton of sugar I (δ 4.78), I $_{6b}$ proton is also excited. Therefore, signal of I $_4$ is affected and signal enhancement is observed for I $_{6a}$ and I $_5$.

Enhancement is observed at δ 4.18 (II $_2$), 4.22 (II $_3$), 3.85 (II $_5$), 4.02 (II $_6$), 4.30 (I $_{6a}$), and 4.78 (I $_{6b}$) upon irradiation at δ 5.16 (II $_1$). δ 4.18 (II $_2$) is correlated to δ 82.5 ppm (carbon II $_2$) in ^{13}C-^{1}H COSY, 4.30 (I $_{6a}$) and 4.78 (I $_{6b}$) are correlated to δ 70.5 ppm (-CH_2O-). These results indicate connection between sugar II $_2$ and III and further prove the connection between sugar II and I $_6$. δ 4.02 is correlated to δ 61.7 (-CH_2OH) and it indicates II $_6$ is not connected to sugar.

Upon irradiation, signal enhancement is observed at δ 4.04 (III $_2$), 4.22 (III $_3$), 3.89 (III $_3$), 3.83 (III $_5$), 4.32 (III $_6$), it indicates sugar III is connected to sugar II $_2$. δ 4.32 (III $_6$) is correlated to δ 61.7 (-CH_2OH), it indicates III 6 is not connected to sugar.

Irradiation the terminal proton of IV (δ 4.89) results in signal enhancement at δ 3.74 (C_{24} of aglycone), 3.94 (IV $_{6a}$), 4.05 (IV $_3$), 4.21 (IV $_3$), and 4.18 (IV $_2$) ppm. The first enhancement indicates IV is an inner sugar connected to C_{24} of aglycone. 4.18 (IV $_2$) is correlated to δ 82.6 ppm (carbon IV $_2$) in ^{13}C-^{1}H COSY, a connection between IV $_2$ and sugar VI is determined. In addition, 3.94 (IV $_{6a}$) is correlated to δ 70.2 ppm (-CH_2O-) in

^{13}C-^{1}H COSY, indicates connection between sugar IV $_6$ and sugar V. These results are further proved by irradiation the two sugar terminal protons δ 5.48 (IV $_1$) and 4.85 (V $_1$) ppm, obvious enhancement is observed at δ 4.18 (IV $_1$) and 3.91 (IV $_6$) ppm.

From all results obtained, C_3 and C_{24} of **5** are connected to three sugar groups, the sequence of connection is I 6-1 II 2-1 III, IV 6-1 V, IV 2-1 VI. **5** is mogrolyl-3-O-[β-D-glucopyranosido (6-1)-β-D-glucopyranosido (2-1) -β-D-glucopyranose]-24-O-{[β-D-glucopyranosido (6-1)][β-D-glucopyranosido (2-1)-β-D-glucopyranose]. **5** is a new nature sweet glucoside, it is named as neomogroside.

Experimental

IR (KBr) is taken on Perkin-Elmer 983G; NMR's are taken on FX-100 or Bruker AM-500, C_5D_5N as solvent NMR solvent, TMS as intern al standard; FDMS was taken on MAT-90 Mass spectrometer.

Silica is from QingDao Ocean Chemical Plant; AlO_3 is from Shanghai Wusi Chemical Reagent Manufacturer; RP_2 reverse phase silica and RP_{18} reverse phase TLC plates are from Merck; Sephadex LH 20 is from Shanghai Chemical Reagent Manufacturer.

Raw materials and extractives of LO-HAN-KUO used in experiment are provided by YongFu Pharmaceutical Manufacturer form the GuangXi Autonomous Region.

Solvent systems used for TLC are mix solvent of $CHCl_3$-MeOH-H_2O, a: 6.5:3.5:1; b: 7.5:2.5:1; c: 6:4:1 (homogeneous); d: 5:5:1 (homogeneous); e: 60% MeOH-H_2O.

Extraction:

5.5 kg black LO-HAN-KUO paste (mobile, equivalent to 30 kg fresh fruit) form YongFu Pharmaceutical Manufacturer form the GuangXi Autonomous Region is added 3 times

water. The mixture is passed through the pre-treated enriching resin column, 317g light yellow glycoside mixture is obtained.

Apply 200g glycoside mixture to a column with 1kg silica gel, elute with various solvent system according to the flow chart. Components 1-6 are obtained.

Composition analysis

1, $C_{42}H_{72}O_{14}$, white powder, IR ν max (cm^{-1}): 3210 (OH), 1640 (C=C). FDMS: m/z 800, ^1H NMR (100 MHz): δ 4.87 (1H, d, J = 8.7 Hz), 4.98 (1H, d, J = 8.0Hz) (terminal protons). ^{13}C NMR (refer to Table 5, 4)

TLC acid hydrolysis[6]: silica G plate is made with 0.4% CMC-Na and is backed at 105°C for a hour and cooled to room temperature. Sample is dissolved in water and is spotted onto the plate, the plate is placed into the developing chamber containing concentrated HCl at room temperature for 50 minutes. The plate is taken out and placed under IR lamp to remove HCl moisture. Glucose is spotted as standard and the plate is developed with n-BuOH-HAc-H_2O (3:1:1). The plate is sprayed with aniline (0.93g)-benzene dicarboxylic acid (1.66g)-n-BuOH saturated with water (100ml) and baked at 105°C for 10 minutes to stain. Only the brown spot corresponding to glucose is visualized.

2, $C_{46}H_{82}O_{19}$, white powder, IR ν max (cm^{-1}): 3400 (br, OH), 1640 (C=C). FDMS m/z 986 $[M+H+Na]^+$, 824 $[M+H+Na\text{-}Glc]^+$. ^1H NMR (100M Hz): δ 4.84 (1H, d, J = 7.7 Hz), 4.92 (1H, d, J = 7.4 Hz), 4.98 (1H, d, J = 7.5 Hz) (terminal protons), 0.85, 0.92, 0.92, 1.16, 1.31, 1.31, 1.43 and 1.43 ppm (8x3H, d, $8CH_3$). ^{13}C NMR (refer to Table 4, 5).

TLC acid hydrolysis: the same as for **1**, only the brown spot corresponding to glucose is found.

3, $C_{54}H_{92}O_{24}$, white powder, IR ν max (cm^{-1}): 3400 (OH), 1640, 890 (C=C), ^1H NMR (500 MHz): 5.05 (1H, d, J = 7.8 Hz), 4.78 (1H, d, J = 8.0 Hz), 4.74 (1H, d, J = 7.4 Hz), 4.73 (1H, d, J = 7.1 Hz), (terminal protons). ^{13}C NMR data (refer to Table 4, 5).

TLC acid hydrolysis: the same as for **1**, only the brown spot corresponding to glucose is found.

4, $C_{60}H_{102}O_{29}$, white powder, IR ν max (cm^{-1}): 3400 (OH), 1640, 890 (C=C), FDMS m/z 1310$[M+H+Na]^+$, 1286 $[M]^+$, 1147 $[M+Na-Glc]^+$, 1129$[M-Glc-H2O]^+$, 985$[M+Na-2Glc]^+$, 823$[M+Na-3Glc]^+$, ^1H NMR (500 MHz) (refre to Table 1, 2), ^{13}C NMR data (refer to Tbale 4, 5).

Acid Hydrolysis: In a safety bottle, it is added 100mg of **4** and 1.5 ml of 5% H_2SO_4, the bottle is heated at 70°C for 6 hours. The reaction mixture was added 3ml water and extracted with n-BuOH saturated with water. The aqueous phase was neutralized with 10% NaOH and all solvent is removed by heating, TLC analysis indicates that only spot corresponding to glucose is found.

5, $C_{66}H_{112}O_{34}$, white powder, IR ν max (cm^{-1}): 3400 (OH), 1660 (C=C); FABMS m/z 1471 $[M+Na]^+$, ^1H NMR and ^{13}C NMR data (refer to Table 1, 3, 4, 5).

Acid Hydrolysis: the same method for **4**, TLC analysis indicates that only spot corresponding to glucose is found.

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LO-HAN-KUO Chemical Composition Analysis (II)
Flavone Glycoside Composition and Structural Determination

Abstract: Two flavone glycosides were obtained from the fresh Chinese fruit LO-HAN-KUO. From the analysis of their ^{1}H-^{1}H COSY, ^{13}C-^{1}H COSY and NOE difference spectroscopy, the structures were determined as Kaempferol-3-O-α-L-Rhamnose-7-O-[β-D-Glucosido-(1-2)-α-L-Rhamnoside] (VI) and Kaempferol-3,7-α-L-dirrhamnoside (VII). VI is a new natural component and named as grosvenorine.

Keywords: LO-HAN-KUO, grosvenorine.

LO-HAN-KUO is classified as the calabash family [Siraitia grosvenori (swingle) C. Jeffrey] and is special vine plant cultivated in China. The major chemical composition of dry LO-HAN-KUO is triglucoside. For comparison the chemical composition of the fresh fruit and that of dried one, we have carried out the study of chemical composition of the fresh LO-HAN-KUO. In addition to isolation of 5 cucurbitane triglucosides[1], two flavone glycosides were first isolated, Kaempferol-3-O-α-L-Rhamnose-7-O-[β-D-Glucosido-(1-2)-α-L-Rhamnoside] (VI) and Kaempferol-3,7-α-L-dirrhamnoside (VII). The study of their structures is reported in this article.

Grosvenorine (VI): light yellow needle crystal, mp 218-220°C. Formula is determined as $C_{33}H_{40}O_{19}$ according to Mass, ^{13}C NMR and ^{1}H NMR analysis. IR analysis indicates OH group (3200 cm^{-1}), C=O group (1860 cm^{-1}). It reacts with HCl-Mg and a deep red color is resulted. Molish reaction is positive. UV λmax in MeOH: 245 (sh), 265, 315 (sh), 345 nm, they are the absorption peaks of flavone type compound, so VI is flovane triglycoside. ^{13}C NMR gives three terminal carbons (δ 105.5, δ 101.8, and 97.3 ppm) and indicates VI is a triglycoside. Paper chromatography after acid hydrolysis of VI indicates the sugars are glucose and rhamnose. The coupling constant of the terminal proton of

glucose is J = 8 Hz in ^{1}H NMR, this indicates glucose adopts a β configuration. The coupling constant of the terminal proton of rhamnose is J = 2 Hz, so rhamnose adopts a α configuration. There are 19 O's in its formula, the sugar parts contain 13 O's atoms and two O's from the skeleton of flavone. No evidence of OCH_3 and other groups containing oxygen from ^{13}C NMR, so the remaining 4 O's should be in OH groups. ^{1}H NMR only gives two free phenol OH groups at δ 12.5 and 10.2 ppm (disappear upon D_2O exchange), therefore another two OH groups are glycosidated. VI is flavone glycoside containing two sugar chains and two free phenol OH groups. Upon addition of NaOMe into the solution, band I of the UV spectrum of VI is shifted to red by 40 nm and its intensity does not change, there should be a OH group at position 4'. Addition of $AlCl_3$/HCl results the red shift 40 nm of band I indicates there is OH group at 5 position. From δ 6.8 (1H, d, J = 2Hz) and 6.5 (1H, d, J = 2Hz) ppm from ^{1}H NMR, there two protons at meta-position on the aromatic ring and there should be a substituent at position 7. δ 7.8 (2H, d, J = 8.5 Hz) and 6.9 (2H, d, J = 8.5 Hz) indicate an A_2B_2 system and there is only substituent on ring B because another sugar chain only can be at position 3. From these results, the aglycone of VI should be 3, 5, 7, 4'-tetrahydroxyl flavone. The connection pattern of the sugar is determined according to the analysis of ^{1}H-^{1}H COSY and NOE difference spectroscopy. Upon irradiation of the terminal proton at δ 5.5 ppm on rhamnose, protons at 6, 8 positions of ring A receive stronger signal enhancement, the inner sugar at position 7 should be rhamnose. Irradiation of the terminal proton at δ 4.40 ppm on glucose also results in signal enhancement of H_6, H_8, this indicate position 7 is connected to a disaccharide. Irradiation of the terminal proton at δ 5.0 ppm on rhamnose results in weak signal enhancement at positions 5' and 6' on ring B. This indicates that there is a rhamnose connected to position 3. The proton at position 2 of rhamnose which correlated to the terminal proton (δ 5.30 ppm) of rhamnose at position 7 is at δ 3.92 ppm. From the ^{13}C-^{1}H cosy, the corresponding carbon at position 2 is at δ 79.7 ppm, it is shifted to downfied by 9.7 ppm. Therefore the glucose is connected at the position 2 of

the rhamnose. Irradiation of the terminal proton of glucose also results in signal enhancement at δ 3.97, this indicates it is a 1-2 connection between glucose and rhamnose. We conclude that VI is Kaempferol-3-α-L-rhamnose-7-O-[β-D-glucosido-(1-2)-α-L-rhamnoside, refer to scheme for its structure.

Mogroside VII: light yellow needle crystal, mp 187-189°C, HCl-Mg reaction give positive and molish reaction is also positive. The UV spectrum in anhydrous MeOH gives bands at 264 and 345 nm, they are the characteristic bands for of flavone type compound. This indicates that VII is a flavone glycoside. Comparison of the ^{13}C NMR data of VII and VI indicates that VI has additional six carbons from glucose and all other carbons have similar chemical shifts for both. Therefore VII is dirhamnose glycoside of kaempferol. Upon acid hydrolysis of VII, the aqueous phase is extracted with ethyl acetate and the resulted product is the aglycone of the glycoside. TLC analysis and mp determination of this aglycone with kaempferol as standard have proved it is kaempferol. TLC analysis of the aqueous phase only give the color spot corresponding to rhamnose standard. By comparison the ^{13}C NMR data of VII and kaempferol, chemical shifts of C_3, C_7 positions are upfield –0.8 and 0.9 ppm, the chemical shifts of C_2, C_6, C_8 are downfield 10.0, 1.2, 1.2 ppm. These results indicate C_3 and C_7 are connected to rhamnose. Therefore VII is kaempferol-3, 7-α-Ldirhamnose.

Experimental

Melting point is taken on a Fisher-Johne apparatus (not calibrated); NMR is taken on Bruker 500, DMSO-d_6 is used as solvent and internal standard; Mass spectroscopy is taken on a MaT-711; IR (KBr) is taken on Perkin-Elmer 983; UV is taken on Philips Phy Unicam PU 800.

Extraction

200g glycoside mixture (supplied by GuangXi Yongfu Pharmaceutical Manufacturer) is applied to a short column with 1kg silica gel. Elute with $CHCl_3$:MeOH:H_2O (7:3:1) and collect every 200 ml as fractions. A light yellow crystal from fractions 88 to 93 is obtained and it is recrystalized in CH_3OH and H_2O (3:1) to yield VI (70mg). Combine fractions 68-72 and apply it to a polyamide column. Elute with 60% MeOH and yield a yellow powder. It was recrystalized in MeOH to yield VII (62mg).

Composition Analysis

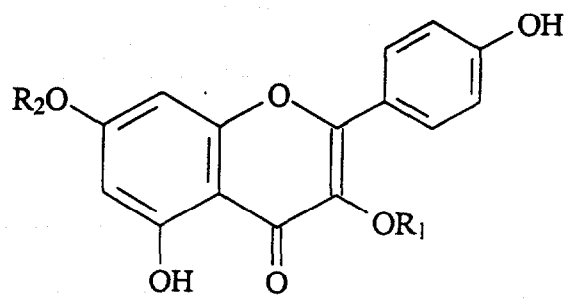
Grosvenorine (VI): mp 218-220°C, HCl-Mg reaction give positive, molish reaction is also positive; IR, ν_{max}^{KBr} (cm^{-1}): 3410, 1660, 1600, 1500, 1210, 1190, 840; UV, λ_{max}^{MeOH} (nm): 265, 345, addition of NaOMe: 245 (sh), 268, 385, addition of $AlCl_3$: 234 (sh), 275, 300 (sh), 342, 395, addition of $AlCl_3$/HCl: 255 (sh), 275, 300 (sh), 342, 395, addition of NaOAc: 265, 370, addition of NaOAc/H_3BO_3: 265, 345; FAB-MS m/z: 763 $(M+Na)^+$, 741 $(M+H)^+$, 595 $(M\text{-}Rha + 1)^+$, 286 $(M\text{-}2Rha\text{-}glu)^+$. EI-MS: m/z (%) 286 (100), 285 (27), 258 (6.4), 229 (5), 213 (3.3), 153 (4), 146 (13); ^1H NMR (DMSO-d_6) δ (ppm): 7.8 (2H, d, J = 8.5 Hz, H2', H6'), 6.9 (2H, d, J = 8.8 Hz, H3', H5'), 6.8 (1H, d, J = 2Hz, H8), 6.5 (1H, d, J = 2Hz, H6), 10.3 (4-OH), 12.6 (5-OH), 3-4 (m, proton form sugar), 5.9 (1H, d, J = 22 Hz, Rha-H1), 5.2 (1H, d, J = 2Hz, Rha'-H1), 4.4 (1H, d, J = 8.0 Hz, Glc-H1), 0.8 (3H, s, Rha-H6), 0.79 (3H, s, Rha'-H6). ^{13}C NMR (DMSO-d_6), δ (ppm): 156.1 (C-2), 134.6 (C-3), 177.9 (C-4), 160.9 (C-5), 99.5 (C-6), 161.4 (C-7), 94.6 (C-8), 157.7 (C-9), 105.8 (C-10), 120.3 (C-1'), 130.6 (C-2', C-6'), 115.6 (C-3', C-5'), 160.1 (C-4'). Glc: 105.2, 73.8, 76.7, 69.3, 76.7, 60.8, 7-Rha: 97.3, 79.7, 70.4, 71.9, 70.6, 17.7. 3-Rha: 101.9, 70.0, 70.8, 71.2, 69.8, 17.3.

Acid hydrolysis: 20 mg of VI was added 5% H_2SO_4/ethanol (1:1), the mixture is refluxed for 4 hours, TLC analysis indicates the completion of hydrolysis. It is extracted with

ethyl acetate three times, the recovered aglycone has the same R_f value as that of kaempferol. Paper chromatography of the aqueous phase only gives Glc and Rha.

VII: light yellow needle crystal, mp 187-189°C, HCl-Mg reaction gives positive and molish reaction is positive. UV, λ_{max}^{MeOH} (nm): 246 (sh), 267, 385, addition of $AlCl_3$: 274, 300 (sh), 345, 395, addition of $AlCl_3$/HCl: 274, 300 (sh), 345, 395, addition of NaOAc: 265, 358, 400 (sh), addition of NaOAc/H_3BO_3: 265, 345. EI-MS m/z (%): 286 (100), 185 (27), 258 (6), 229 (5), 213 (3.3), 153 (4), 146 (13). ^{13}C NMR (DMSO-d_6) δ (ppm): 156.1 (C-2), 134.5 (C-3), 177.9 (C-4), 160.9 (C-5), 99.5 (C-6), 161.7 (C-7), 94.6 (C-8), 157.8 (C-9), 105.8 (C-10), 120.4 (C-1'), 130.8 (C-2', C-6'), 115.4 (C-3', C-5'), 160.1 (C-4'), 3-Rha: 101.8, 70.2, 70.7, 71.8, 70.1, 17.5, 7-Rha: 98.4, 70.0, 70.7, 71.6, 69.8, 17.9.

Acid hydrolysis: hydrolysis as normal method, extracted with ethyl acetate four times, the recovered aglycone has the same R_f value as that of kaempferol. Paper chromatography of the aqueous phase only gives Rha.



	R1	R2
grosvenorine	Rha	Rha$\overset{2\text{-}1}{\text{—}}$Glc
mogroside VII	Rha	Rha

Table 1 ^{1}H NMR chemical shifts (ppm) of aglycone of **4**, **5** in C_5D_5N

Proton	**4** (J, Hz)	**5** (J, Hz)
1-H	2.96 (1H, d, 10.4)	2.96 (1H, br. s)
	2.00 (1H, dd, 10.5, 12.0)	2.00 (1H, dd, 10.5, 12.0)
2-H	2.43 (1H, dd, 10.5, 3.0)	2.44 (1H, br. s)
	2.10 (1H, m)	2.10 (1H,m)
3-H	3.67 (1H, br. s)	3.67 (1H, br. s)
6-H	5.43 (1H, d, 6.4)	5.45 (1H, d, 6.0)
7-H	2.28 (1H, dd, 7.2, 6.4)	2.25 (1H, m)
	1.65 (1H, d, 7.3, -)	1.65 (1H, m)
8-H	1.61 (1H, d, 7.3)	1.59 (1H, d, 7.3)
10-H	2.78 (1H, d, 12.1)	2.77 (1H, d, 12.0)
11-H	4.17 (1H, dd, 5.0, 11.5)	4.18 (1H, dd, 5.0, 11.5)
12-H	2.15 (2H, m)	2.15 (2H, m)
15-H	1.15 (2H, m)	1.15 (2H, m)
16-H	1.49 (1H, m)	1.49 (1H, m)
	2.15 (1H, m)	2.15 (1H, m)
17-H	1.87 (1H, m)	1.89 (1H, m)
18-H	0.91 (3H, s)	0.91 (3H, s)
19-H	1.09 (3H, s)	1.10 (3H, s)
20-H	1.52 (1H, m)	1.52 (1H, m)
21-H	1.07 (3H, d, 7.7)	1.10 (3H, d, 7.7)
22-H	1.78 (2H, m)	1.78 (2H, m)
23-H	2.43 (2H, m)	2.45 (2H, m)
24-H	3.74 (1H, d, 9.0)	3.73 (1H, d, 9.1)
26-H	1.44 (3H, s)	1.44 (3H, s)
27-H	1.33 (3H, s)	1.31 (3H, s)
28-H	0.92 (3H. s)	0.90 (3H, s)
29-H	1.09 (3H, s)	1.10 (3H, s)
30-H	1.49 (3H, s)	1.50 (3H, s)

Table 2 ^{1}H NMR chemical shifts (ppm) of the sugar of **4** in C_5D_5N

	Sugar				
Proton	I (J, Hz)	II (J, Hz)	IV (J, Hz)	VI (J, Hz)	V (J, Hz)
1	4.77 (J, 7.8)	5.12 (d, 8.0)	4.88 (d, 7.8)	5.42 (d, 7.8)	4.83 (d, 7.7)
2	3.87 (dd, 8.0, 8.5)	4.00 (dd, 8.0, 8.5)	4.13 (dd, 8.5, 8.0)	4.06 (dd, 8.0, 9.3)	4.02 (m, 8.0, 8.5)
3	4.13 (dd, 8.5, 8.5)	3.93 (dd, 8.5, 11.3)	4.21 (dd, 9.0, 8.5)	4.17 (dd, 9.3, 8.0)	4.01 (m, 8.5, 8.0)
4	3.91 (dd, 8.5, 9.3)	4.02 (m, 8.0, 8.5)	4.21 (dd, 8.5, 9.0)	4.08 (dd, 8.5, 9.3)	3.91 (dd, 9.3, 8.5)
5	4.03 (m, 4.7, 8.0, 8.5)	4.23 (m, 8.5, 9.0)	4.02 (m, 7.4, 8.0, 8.5)	3.91 (m, 3.5, 8.5, 9.3)	4.20 (m, 8.5, 9.0)
6a	4.28 (dd, 11.2, 4.8)	4.28 (dd, 11.2, 4.8)	4.86 (dd, 7.8, 11.3)	4.48 (dd, 12.4, 10.4)	4.45 (d, 12.4)
6b	4.72 (d, 11.2)	4.42 (d, 12.4)	3.93 (dd, 11.3, 8.5)	4.30 (dd, 3.5, 11.2)	4.30 (dd, 3.5, 11.2)

Table 3 ^{1}H NMR chemical shifts (ppm) of the sugar of **5** in C_5D_5N

	Sugar					
Proton	I (J, Hz)	II (J, Hz)	III (J, Hz)	IV (J, Hz)	V (J, Hz)	VI (J, Hz)
1	4.79 (d, 7.8)	5.16 (d, 7.8)	5.05 (d, 7.7)	4.89 (d, 7.8)	4.85 (d, 7.6)	5.48 (d, 7.7)
2	3.87 (dd, 7.7, 9.5)	4.18 (dd, 8.0, 10.5)	4.04 (br, s)	4.18 (dd, 8.0, 10.5)	4.02 (br, s)	4.08 (dd, 8.0, 9.5)
3	4.15 (dd, 9.0, 10.5)	4.22 (d, 8.5)	4.22 (dd, 8.5, 10.0)	4.21 (dd, 8.5, 10.5)	4.22 (dd, 8.5, 10.5)	4.19 (dd, 9.0, 10.5)
4	4.21 (dd, 9.0, 10.0)	4.10 (dd, 9.0, 10.0)	4.10 (dd, 9.0, 10.0)	4.21 (dd, 9.0, 10.0)	3.92 (dd, 10.5, 7.0)	4.10 (dd, 9.0, 10.0)
5	4.04 (m)	3.85 (dd, 9.0, 7.0)	3.88 (dd, 10.0, 7.5)	4.05 (dd, 9.0, 8.4)	3.85 (dd, 9.0, 7.0)	3.92 (9.0, 7.0)
6a	4.30 (dd, 9.0, 7.8)	4.02 (m)	4.28 (m)	3.94 (8.0, 7.5)	4.28 (m)	4.30 (m)
6a	4.78 (dd, 9.0, 7.7)		4.50 (t, 12.0, 13.0)	4.90 (9.0)	4.50 (t, 12.0, 13.0)	4.50 (t, 12.0, 13.0)

Table 4: ^{13}C NMR chemical shifts of aglycone of **1**, **2**, **3**, **4**, **5**, and **6** (ppm, 125 MHz, C_5D_5N)

Glucoside

Carbon	1	2	3	4	5	6
1	26.5	26.7	26.4	26.6	26.9	25.0
2	29.8	29.8	29.5	29.4	29.5	30.0
3	88.0	88.0	87.4	87.4	87.4	76.2
4	42.6	42.8	42.4	42.3	42.4	42.2
5	144.1	144.6	144.3	144.5	144.5	144.3
6	118.5	118.9	118.4	118.2	118.5	119.1
7	24.8	24.9	24.7	24.7	24.9	24.5
8	43.6	44.1	44.7	43.7	43.7	43.6
9	40.3	40.6	40.2	40.3	40.3	40.2
10	37.0	36.7	36.9	36.8	36.6	36.9
11	77.9	78.1	78.2	78.0	78.0	77.8
12	41.3	41.3	40.9	41.2	41.2	41.2
13	47.5	47.8	47.5	47.6	47.6	47.4
14	49.8	50.2	49.8	49.8	49.8	49.8
15	34.8	35.1	34.7	34.7	34.7	34.5
16	28.5	28.1	28.0	28.6	28.6	28.4
17	51.1	51.5	51.1	51.5	51.2	51.0
18	17.3	17.6	17.3	17.2	17.1	17.3
19	27.2	27.3	26.7	27.1	27.2	26.7
20	36.7	36.7	36.5	36.5	36.5	36.3
21	19.1	19.4	19.3	19.2	19.1	18.9
22	33.7	33.3	33.4	33.4	33.4	34.2
23	29.8	29.8	29.7	29.6	29.5	29.0
24	90.8	92.6	92.6	91.7	91.6	79.0
25	72.2	73.1	72.8	72.8	72.9	72.7
26	25.5	24.7	24.4	24.7	24.7	25.8
27	26.6	26.7	26.4	26.3	27.8	26.3
28	19.6	19.9	19.6	19.5	19.5	19.3
29	28.0	27.3	27.8	27.8	26.4	27.3
30	26.5	26.7	26.4	26.3	26.4	26.2

Table 5: ^{13}C NMR chemical shifts of the sugar part of 1, 2, 3, 4, and 5 (ppm, 125 MHz, C_5D_5N)

Glucoside

Carbon		1	2	3	4	5
C_3-O-Glc	1'	107.4	107.1	106.6	106.8	106.7
(Inner)	2'	75.4	75.4	75.2	75.4	75.3
(I)	3'	78.7	78.3	78.3	78.4	78.4
	4'	71.8	71.6	71.4	71.5	71.7
	5'	78.2	78.1	78.2	77.3	77.9
	6'	63.1	63.1	63.2	70.4	70.5
C_3-sugar	1'			105.1	105.3	105.5
(Terminal,	2'			75.2	76.0	76.0
6-1	3'			76.4	78.1	78.3
(II)	4'			71.4	71.9	71.8
	5'			78.3	78.6	78.3
	6'			62.5	63.0	62.9
C_3-sugar	1'					105.4
(Terminal,	2'					75.1
2-1)	3'					76.7
(III)	4'					71.6
	5'					78.4
	6'					62.8
C_{24}-O-Glc	1'	106.0	104.7	104.8	103.5	105.6
(Inner)	2'	75.4	75.6	82.9	82.7	82.6
(IV)	3'	78.2	78.1	78.2	78.7	78.6
	4'	71.8	71.9	72.9	72.8	72.9
	5'	78.2	76.6	77.2	77.3	77.2
	6'	62.8	70.5	70.2	70.2	70.2
C_{24}-sugar	1'		106.3	106.1	106.4	106.3
(Terminal,	2'		75.4	79.2	78.2	77.9
6-1	3'		78.5	78.3	78.4	78.4
(V)	4'		71.9	72.6	72.1	72.6
	5'		78.5	78.2	78.7	78.7
	6'		62.9	62.5	63.2	63.7
C_{24}-sugar	1'				104.9	104.8
(Terminal,	2'				75.3	75.2
2-1)	3'				78.5	78.5
(VI)	4'				71.8	73.2
	5'				78.4	77.9
	6'				62.8	61.7

Package B:
Regulatory Issues of the Lo-Han-Kuo Project

Document Number: NLA-Bf3-011999
Submitted to Nature's Marvel International by Sinotech
January 31, 1999

Project Scope and Background

Nature's Marvel International (NMI) intends to submit a self-affirmed GRAS (Generally Regard as Safe) petition to FDA. Preliminary information containing the following 5 Chinese documents (referred as Package A) were provided by the manufacturer of Lo-Han-Kuo extract in China:

- AC-1: Result Certificate -- Research & Development of Lo-Han-Kuo Product (by Institute of Chinese Medicine, 1/11/93)
- AC-2: Manufacturing Standard Operating Procedures (by Guangxi Yongfu Lo-Han-Kuo Factory, 9/2/92)
- AC-3: Certificate of Lo-Han-Kuo Glucosides as a Food Additive (by Guangxi Yongfu Lo-Han-Kuo Factory, 6/3/97)
- AC-4: Chemical Composition Analysis
- AC-5: Toxicity Studies (by Institute of Chinese Medicine, 2/20/92)

These 5 documents have been translated to English and split into 8 Sections (referred as AE-1 to AE-8) by NMI's previous translators. The translated package was reviewed by NMI and its regulatory consultant, CanTox. Fourteen questions on Package A were raised by CanTox to clarify some regulatory issues. These questions were translated into Chinese and send to the Chinese manufacturer of Lo-Han-Kuo extract. The response to these questions contains multiple Chinese and Japanese documents (referred as Package B). Sinotech was contracted to review this Package, to answer these 14 regulatory questions based on the Chinese documents provided in Package B, and to translate only those portions of Chinese documents in Package B needed to answer these questions.

Package B contains the following documents:

- Q&A: 14 Questions in English, the Chinese translation, and the answers in Chinese referring the following Attachments (3 pages)
- Attachment 1: Inspection records for 5 batches of extract, miscellaneous analysis of 2 batches (7 pages in Chinese)
- Attachment 2: Toxicity report of Lo-Han-Kuo extract (2 pages in Chinese)
- Attachment 3: Effects on immune response of Lo-Han-Kuo extract (3 pages in Chinese)
- Attachment 4: Assay and specifications of Lo-Han-Kuo extract (8 pages in Chinese)
- Attachment 5: Assay for total mogrosides in Lo-Han-Kuo (2 pages in Chinese)
- Attachment 6: Lo-Han-Kuo fruit listed in Chinese Pharmacopoeia (1 pages in Chinese)
- Attachment 6-1: Lo-Han-Kuo fruit listed in Chinese Herb Dictionary (3 pages in Chinese)
- Attachment 7: Pharmacological effects of Lo-Han-Kuo extract (1 pages in Chinese)

- Attachment 8: Study on the extraction process (2 pages in Chinese)
- Attachment 9: Marketing data of Lo-Han-Kuo fruit (1 pages in Chinese)
- Attachment 10: Assay of mannitol in Lo-Han-Kuo (2 pages in Chinese)
- Attachment 11: Assay of mogrosides in Lo-Han-Kuo (6 pages in Chinese)
- Attachment 12: Assay of grovenorine in Lo-Han-Kuo (3 pages in Chinese)
- Attachment 13: Study of sweeteners in Lo-Han-Kuo (6 pages in Chinese)
- Other information not directly related to Q&A: A Japanese vendor's technical information regarding a sweetener product containing Lo-Han-Kuo extract: (23 pages)

These Attachments in Package B contain 2 to 3 times more information than the 5 Chinese documents in Package A. However, some questions were not answered. A preliminary report was submitted by Sinotech to NMI and CanTox on 12/13/98 for their immediate reference. Sinotech wrote multiple letters to request the Chinese manufacturer to clarify the answers or provide more information. Based on the new information, a final report was completed here. Information between the "< >" sign were inserted by Sinotech for clarification.

Sinotech was also contracted to prepare the Material Safety Data Sheet (MSDS) for Lo-Han-Kuo Extract to its best capability using the currently available Chinese information in Package B. The MSDS is attached in Section B-3 of this document.

Section B-1: Certificate of Analysis

Question #1: "Certificates of analysis on 5 batches of extract to assess the variability within the limits <of> the <product> specifications."

Answer:

The "Inspection Records", equivalent to Certificate of Analysis (COA), of five batches of product were provided by the Chinese Manufacturer (Attachment 1). The assay Standard Operating Procedures (SOP) referred in the "Inspection Record" appeared to be what contained in Attachment 4 (c.f. Question #13).

Attachment 1 also contains other assay information for 2 batches. One data sheet reports the contents of crude fat (0.38%), total nitrogen (2.19%), organic carbon (61.05%), and crude fiber (0%) in the product sample of Batch #981016. The second data sheet reports the contents of carbon (53.13%), hydrogen (7.36%), and nitrogen (3.81%) in the product sample of Batch #980206.

The specification of the Lo-Han-Kuo Extract COA defines ~77% of the contents. The rest of ~23% material were not assayed routinely and were not defined in the COA. The assay results of these five COA cover 87%-90% of materials. The rest of 10%-13% material in the final product might be glucosides, fat, protein, pigment, ketone, etc.

Here is the translation of the COA on 5 batches:

Guilin Siter New Technology Company
Natural Botanical Product Factory
Product Quality Inspection Record

Product Name: Lo-Han-Kuo Glucosides
Inspection Date: 10/18/98
Batch Quantity: 5 Kg
Batch Number: 981016
Inspection Method: Standard Operating Procedures
Inspection Items: All items

Product Specifications and Inspection Results:

Items	Specifications	Inspection Results
Color	Yellow	Yellow
Appearance	Powder	Powder
Odor	Light Fragrance	Light Fragrance
Solubility	Easily dissolved in water or diluted ethanol	Passed
Glucoside Content	> 70%	82.3 %
Sweetness	>210 folds	215 folds
Water Content	< 6%	4.8%
Ash Content	< 1%	0.8%
Heavy Metal Ion Content	< 10 ppm	3 ppm

Inspected by: ID 303, ID 309
Approved by: ID 310
Date: 10/22/1998
Assay & Inspection Room
Guilin Siter New Technology Company, Natural Botanical Product Factory

Guilin Siter New Technology Company
Natural Botanical Product Factory
Product Quality Inspection Record

Product Name: Lo-Han-Kuo Glucosides
Inspection Date: 2/7/98
Batch Quantity: 100 Kg
Batch Number: 980206
Inspection Method: Standard Operating Procedures
Inspection Items: All items

Product Specifications and Inspection Results:

Items	Specifications	Inspection Results
Color	Yellow	Yellow
Appearance	Powder	Powder
Odor	Light Fragrance	Light Fragrance
Solubility	Easily dissolved in water or diluted ethanol	Passed
Glucoside Content	> 70%	81.5 %
Sweetness	>210 folds	215 folds
Water Content	< 6%	5.7%
Ash Content	< 1%	0.6%
Heavy Metal Ion Content	< 10 ppm	3 ppm

Inspected by: ID 304, ID 310
Approved by: ID 305
Date: 2/10/1998
Assay & Inspection Room
Guilin Siter New Technology Company, Natural Botanical Product Factory

Guilin Siter New Technology Company
Natural Botanical Product Factory
Product Quality Inspection Record

Product Name: Lo-Han-Kuo Glucosides
Inspection Date: 4/15/97
Batch Quantity: 100 Kg
Batch Number: 980410
Inspection Method: Standard Operating Procedures
Inspection Items: All items

Product Specifications and Inspection Results:

Items	Specifications	Inspection Results
Color	Yellow	Yellow
Appearance	Powder	Powder
Odor	Light Fragrance	Light Fragrance
Solubility	Easily dissolved in water or diluted ethanol	Passed
Glucoside Content	> 70%	85.7 %
Sweetness	>210 folds	215 folds
Water Content	< 6%	4.2%
Ash Content	< 1%	0.75%
Heavy Metal Ion Content	< 10 ppm	1 ppm

Inspected by: ID 304, ID 309
Approved by: ID 305
Date: 6/5/1997
Assay & Inspection Room
Guilin Siter New Technology Company, Natural Botanical Product Factory

Guilin Siter New Technology Company
Natural Botanical Product Factory
Product Quality Inspection Record

Product Name: Lo-Han-Kuo Glucosides
Inspection Date: 5/20/96
Batch Quantity: 300 Kg
Batch Number: 960518
Inspection Method: Standard Operating Procedures
Inspection Items: All items
Product Specifications and Inspection Results:

Items	Specifications	Inspection Results
Color	Yellow	Yellow
Appearance	Powder	Powder
Odor	Light Fragrance	Light Fragrance
Solubility	Easily dissolved in water or diluted ethanol	Passed
Glucoside Content	> 70%	81.5 %
Sweetness	>210 folds	215 folds
Water Content	< 6%	4.8%
Ash Content	< 1%	0.7%
Heavy Metal Ion Content	< 10 ppm	3 ppm

Inspected by: ID 304, ID 310
Approved by: ID 305
Date: 5/25/1996
Assay & Inspection Room
Guilin Siter New Technology Company, Natural Botanical Product Factory

Guilin Siter New Technology Company
Natural Botanical Product Factory
Product Quality Inspection Record

Product Name: Lo-Han-Kuo Glucosides
Inspection Date: 11/25/95
Batch Quantity: 100 Kg
Batch Number: 951120
Inspection Method: Standard Operating Procedures
Inspection Items: All items
Product Specifications and Inspection Results:

Items	Specifications	Inspection Results
Color	Yellow	Yellow
Appearance	Powder	Powder
Odor	Light Fragrance	Light Fragrance
Solubility	Easily dissolved in water or diluted ethanol	Passed
Glucoside Content	> 70%	82.3 %
Sweetness	>210 folds	220 folds
Water Content	< 6%	3.7%
Ash Content	< 1%	0.8%
Heavy Metal Ion Content	< 10 ppm	2 ppm

Inspected by: ID 304, ID 309
Approved by: ID 310
Date: 12/1/95
Assay & Inspection Room
Guilin Siter New Technology Company, Natural Botanical Product Factory

Section B-2: Residual Pesticide

Question #2: "Name of which pesticides that are applied on the crop and tree. Pesticide test results."

Answer:

The seedlings were applied with small amount of pesticides referred by the Chinese Manufacturer as "ester-likes-from-insect-propelling-Chrysanthemum", which is defined as pesticides extracted from the Chrysanthemum family such as permethrin, fenvalerate, tetramethrin, allethrin, cypermethrin, etc. The usage of pesticide depends on the degree of pest infection. The pesticide is usually sprayed between April and May, while Lo-Han-Kuo blooms in August and the fruits are harvested in October. There is no reason to expect there would be any pesticide in the product extracted from the fruits since the spray occurs months before the blooming season.

Section B-3: Material Safety Data Sheet

Question #3: "Material Safety Data Sheet <for the Lo-Han-Kuo Extract Product>"

Answer:

An MSDS for Lo-Han-Kuo Extract was prepared by Sinotech using information from the Chinese documents in Package B. The MSDS format was created based on the MSDS form suggested by U.S. Department of Labor and the guidelines set up by Office of Safety and Hygiene Administration (OSHA). The MSDS of chemicals or biochemical agents sold by several US companies were also used as a reference. A conservative approach was taken to prepare the MSDS. Multiple carcinogenic lists published by government offices have been checked to confirm that the active ingredients of Lo-Han-Kuo extract are not listed in these tables and are not considered as carcinogenic or toxic.

Here is the MSDS:

Nature's Marvel International

1681 Alta La Jolla Drive
La Jolla, CA 92037, USA
Emergency 1-619-456-4501
Fax 1-619-459-2428

MATERIAL SAFETY DATA SHEET MSDS NLE-1.3 1-19-99

SECTION 1 - PRODUCT IDENTIFICATION

Product Name: Lo-Han-Kuo Glucosides
Catalog #: NLE-001-10
Product Description: Extract of Lo-Han-Kuo fruits (Siraitia grosvenori, Swingle)
Appearance: Dry powder

SECTION 2 - COMPONENTS/INGREDIENT INFORMATION

Common Name: Mogrosides
Trade Name: to be determined
Composition: Mogrosides

SECTION 3 - HAZARDS IDENTIFICATION

Label Precautionary Statements:
Avoid inhalation. Inhalation may cause irritation or allergic reaction.
Avoid contact with skin and eyes.

SECTION 4 - FIRST AID MEASURES

In case of contact with eyes, immediately flush eyes with copious amounts of water for at least 15 minutes.
If ingested, rinse mouth with water followed by drinking water.
If inhaled remove person to fresh air. If allergic reaction occurs seek medical help.
In case of contact with skin, wash skin with soap and water.

SECTION 5 - FIRE FIGHTING MEASURES

Extinguishing media : Water spray, carbon dioxide, dry chemical powder or appropriate foam.
Combustion Products : Carbon monoxide and carbon dioxide
Special Fire-fighting Procedures : Wear self-contained breathing apparatus and protective clothing to prevent contact with skin and eyes or inhalation.

SECTION 6 - ACCIDENTAL RELEASE MEASURES

Wear respirator, rubber gloves, chemical safety goggles and protective clothing. Sweep up gently, minimizing the raising of particulate, place in bag and hold for waste disposal. Wash spill site with soap and water after material pickup is complete, then ventilate area.

SECTION 7 - HANDLING AND STORAGE

Storage: Store in tightly sealed containers. Although the product may be stored at room temperature, storage at 4 ∞C is recommended.
Handling : Do not handle without the proper safety equipment outlined in Section 8.

SECTION 8 - EXPOSURE CONTROLS/PERSONAL PROTECTION

Wear appropriate NIOSH approved respirator, chemical resistant gloves, safety goggles, and other protective clothing. Use only in a chemical fume hood.
Do not breathe dust.
Do not get in eyes, on skin, or on clothing.
Wash thoroughly after handling.

SECTION 9 - PHYSICAL AND CHEMICAL DATA

Physical state : Dry powder
Color: Ranges from yellow to light yellow
Percent Volatile: N/A
Vapor Pressure: N/A
Solubility in Water: N/A
Evaporation Rate: N/A
Odor : Light fragrance
Vapor Density: N/A
Specific Gravity: N/A
Melting Point: N/A
NFPA Rating: N/A
Flash point: N/A

SECTION 10 - STABILITY AND REACTIVITY

Stability: Stable
Incompatibilities: Strong oxidizing agents
Hazardous Decomposition Products: Thermal decomposition may produce carbon monoxide, carbon dioxide, nitrogen oxides and sulfur oxides.

SECTION 11 - TOXICOLOGICAL INFORMATION

Not listed as a carcinogen by the National Toxicological Program (NTP), the International Agency for Research on Cancer (IARC), or by OSHA. Not listed by the Registry of Toxic Effects of Chemical Substances (RTECS).

TOXICITY DATA
Oral-Rat LD50: > 24 mg/kg
Intravenous-Mouse LD50: N/A

SECTION 12 - ECOLOGICAL INFORMATION

No evidence of ecological toxicity, mobility, degradability, or bio-accumulation. Further data not yet available.

SECTION 13 - DISPOSAL CONSIDERATIONS

Combine material with combustible solvent and incinerate in a chemical incinerator which is properly equipped with afterburner and scrubber.

SECTION 14 - TRANSPORT INFORMATION

Contact Nature's Marvel International for transportation information.

SECTION 15 - REGULATORY INFORMATION

The product is not listed as a hazardous substance under 40 CFR section 302.4 pursuant to CERCLA; it is not listed as an extremely hazardous substance under Appendix A to 40 CFR 355; it is not listed as a hazardous waste under 40 CFR section 261, pursuant to RCRA; it is not listed in the 29 CFR part 1910, subpart Z list under OSHA; and is not considered a hazard under the List of Threshold Limit Values for Chemical Substances and Physical Agents in the Work Environment published by ACGIH. Other regulatory requirements are unknown at this time.

SECTION 16 - OTHER INFORMATION

The enclosed information is based on information Nature's Marvel International believes to be accurate, however; it should not be considered all inclusive, as it is to serve only as a guide. Proper handling should be exercised at all times. Nature's Marvel International shall not be held liable for any damage resulting from the handling or contact with this product.

Section B-4: Content Analysis for Food

Question #4: "Analytical method to verify the quantity of Lo-Han-Kuo <active ingredients> in food"

Answer:

This question is redundant to Question #11, as confirmed by CanTox on 11/30/98. Please refer to Section B-11 for details.

Section B-5: Usage before 1958

Question #5: "Report from Chinese government and/or the factory which shows the production of the extract and sales of extract before 1958"

Answer:

According to Attachment 9, Lo-Han-Kuo fruits had been used as a supplement of Chinese herb medicine before 1970, and the commercialization of Lo-Han-Kuo extract products was started from 1980 (c.f. Section B-8). The usage of Lo-Han-Kuo fruits before 1958 was described in Document AC-3, Certificate of Lo-Han-Kuo Glucosides as a Food Additive, but was not translated by NMI's previous translator. The related portions were translated here:

> "Lo-Han-Kuo has been used by Chinese for drink and medicine for more than 300 years. Its value as a natural sweetener and a herb medicine has been well recognized."
>
> "Lo-Han-Kuo is an unique herb only found at the south part of China, especially around Yongfu, Lingqua, Longshen areas at the north of Guangxi Province. As the production center of Lo-Han-Kuo, Guangxi Yongfu area produces approximately 70% of Lo-Han-Kuo in China."
>
> "According to the County History of Guangxi Yongfu, local people has cultivated Lo-Han-Kuo crops and collected the fruits for more than three hundred years. The Guangxi Chinese Medicine printed in 1963 described the detailed record of using Lo-Han-Kuo as a medicine in 1885 at Guangxi Yongfu."
>
> "According to the Guangxi Chinese Medicine, Lo-Han-Kuo is sweet, not toxic, beneficial to Lung and Spleen Channels <of Qi>. It can stop coughing, improve digestion, and serve as a refrigerant. It can be used to cure coughing, constipation, etc."

The historical record of the usage of Lo-Han-Kuo fruits by Chinese for at least 300 years can help justifying the GRAS status of Lo-Han-Kuo extract.

Section B-6: Pharmacopoeia Listing

Question #6: "A copy of the 1997 China Pharmacopoeia listing"

Answer:

The Lo-Han-Kuo section in the China Pharmacopoeia 1995 edition (Attachment 6) was translated below.

Important Notes: This Chinese document contains some Chinese medicine concepts which are very different to those of the Western medical science. Chinese medicine is based on "Qi" which is the internal energy flowing in the "Channels" of human body and can be felt, controlled, and enhanced by practicing "Qi-Gong". According to the Chinese medicine: when the "ying" and "yang" Qi are not balanced or the flow of Qi is blocked, illness will occur. To cure the illness, one must balance the ying and yang by herb medicine, acupuncture, practicing Qi-Gong, etc. The original description of Lo-Han-Kuo as a Chinese herb medicine in these documents can be very confusing to most Western scientists. To avoid raising more questions than answering them, Sinotech has translated the document in such a way which can be better understood by regular Western scientists. For example: the original statement of "It can put off the FIRE <i.e. too much yang Qi> in lung" was translated into "It can help the function of lung", which is not what the Chinese document literally said but is what it means, at least to Sinotech's best knowledge. Sinotech can translate it in a more literally "accurate" (but confusing) way if it is what NMI and CanTox prefer.

Here is the translation of Attachment 6:

China Pharmacopoeia, 1995 edition, page 185

Luo-han-kuo (Fructus Momordicae)

The Subject is the dried fruit of a cucurbitaceous plant, Lo-Han-Kuo (Momordica grosvenori Swingle). The fruit is harvested in the Fall when it turns from light green to dark green. The fruit is partially dried in the shade for several days and further dried by baking at a medium temperature.

[Appearance]: The Subject is oval or spherical with a height of 4.5 to 8.5 cm and a diameter of 3.5 to 6 cm. The surface is brown, light brown, or greenish brown with dark spots and yellow fuzz. It might have 6 to 11 strips, with residual style at the top and the scar of peduncle at the bottom. It is light and crispy. The (outer) pericarp is thin and can be easily broken. The pulp (i.e. middle and inner pericarps) is spongy and light brown. Numerous seeds are flat, circular, pink to brownish red with a length of ~1.5 cm and a width of ~1.2 cm. The seed is concave in the center with radial streaks on the side and a groove on the edge of the seed. The Subject has a light odor and is sweet.

[Identification]:

1. The powder product of this Subject is brown. The pericarp cells usually remain in groups, and are yellowish and in square or oval shape with diameters of 7-38 micrometers. The cell walls are thick with clear holes and grooves. The cells of seed skin are in rectangular or irregular shape with thin cell wall, holes and streaks on the surface. The fibers are in long shuttle shape with diameter of 16-42 micrometers, large cell chamber, clear holes on the cell wall. The xylem vessels contain step-wise or helical streaks. "Thin wall" cells are in irregular shapes with streaks.

2. Mix 2 g of the powder product with 20 mL of 50% ethanol. Heat for 30 minutes with vapor condensed and recycled. Filter the mixture and concentrate the filtrate to 5 mL by evaporation. Extract the concentrate with 10 mL and repeat with 5 mL of n-butanol. Completely evaporate the combined butanol solution and add 0.5 mL of methanol to dissolve the residual for assay. Repeat the above step using standard product to prepare the "control" sample for assay. Following the standard thin layer chromatography procedures (Appendix VI-B) to load 10 micro-liters of both methanol samples on the silicon-G plate and elute with chloroform:methanol:water (60:10:1). After elution and drying, spray the plate with 10% sulfuric acid / ethanol solution and dry with hot air until the color of spots is clear. The sample should have the same spot at the same location on the chromatogram comparing with the control.

[Characteristics]: Sweet and refrigerant. Beneficial to Lung and Intestine Channels <of Qi>.

[Functions]: It can help the function of lung and serve as a refrigerant. It can resolve constipation by improving the function of intestines. It can be used to cure hacking cough, throat pain, aphasia, constipation, etc.

[Dosage]: 9-15 g

[Storage]: Keep it dry. Prevent mold growing and insect biting.

Section B-7: Listing as Food and Medicine by Chinese Government

Question #7: "Copy of 1987 listing of Lo-Han-Kuo as a medicine or herb or dietary supplement by the Chinese Academy of Medicine of the Ministry of Hygiene"

Answer:

The 1987 listing of Lo-Han-Kuo as a health enhancing medicine by the Ministry of Hygiene, People's Republic of China is translated below. The information inside of the sign, < >, was added by Sinotech for clarification.

Regulation of Chinese Health Enhancing Medicine

Ministry of Hygiene, People's Republic of China
Document ID: Wei-Yao (87) #70
October 28, 1987

1. This regulation was formulated based on the "Drug Administration Law of People's Republic of China" to enforce the regulation of "Chinese health enhancing medicine" and to protect the health of Chinese people.

2. "Chinese health enhancing medicine" refers to the medicine which has definite benefits on enhancing human health or curing health problems, is nutritious, and has no toxic effect on human body even after being taken regularly for a long time.

3. "Chinese health enhancing medicine" must not contain medicine with toxicity, radioactivity, psychotic effect, or anesthetic effect. In general, it does not use materials from endangered animals, endangered plants, or importing materials as its major raw materials or components.

4. All additives or ingredients used for "Chinese health enhancing medicine" must follow the requirements and regulations for food and drugs.

5. The production and clinical trials of "Chinese health enhancing medicine" must be inspected and approved by the Office of Hygiene of each province, autonomous region, or city. The approval document must be submitted to the Ministry of Hygiene, with a approval ID following the format of "province-Wei-Yao-Chan (year)Z-number". For example: "Herpei-Wei-Yao-Chan (87)Z-01". The medicine approved by the Office of Hygiene of each province, autonomous region, or city will not be granted with a "Certificate of New Drug" and will not be entitled to the grace period of New Drug. To obtain the "Certificate of New Drug" for a "Chinese health enhancing medicine", the local Office of Hygiene must submit the documents to the Ministry of Hygiene for approval. The approval process will be identical to "Class III (Chinese Medicine) New Drug Application". The approval ID will follow the format of "(year)-Wei-Yao-Chan Z-number".

6. The application information package of "Chinese health enhancing medicine" must follow the requirement of "Class III (Chinese Medicine) New Drug Application". Since "Chinese health enhancing medicine" is usually taken on a routine basis for a long time, the safety issue must be the focus.

7. The production and management organizations of "Chinese health enhancing medicine" must be regulated following Chapter 2 and 3 of "Drug Administration Law".

8. The packaging, advertisement, labeling of "Chinese health enhancing medicine" must be regulated in the same way as "Therapeutic Medicine" following "Drug Administration Law".

9. The expenses of using "Chinese health enhancing medicine" should not be reimbursed by the government.

10. This regulation is effective immediately. Any violation of this regulation must be prosecuted according to "Drug Administration Law".

(First Batch)

Under Item #8 of "The Food Hygiene Law of People's Republic of China", the following listed materials are both food and medicine according to Chinese tradition:

A. Species listed in both "Pharmacopoeia of People's Republic of China, 1985 edition" and "Food Ingredients Table, 1981 edition #3" (excluding wild vegetables) published by the Institute of Hygiene of the Chinese Academy of Medicine.

B. The following items:

1. Wu-Shau snake <body of Zaocys dhumnades Cantor>
2. Viper <body of Agkistrodon halys Pallas>
3. Chinese jujube <seed of Zizyphus jujuba Mill. or Z. vulgaris Lamarck var. spinosus Bunge>
4. Oyster shell <shell of Ostrea gigas Thunb, O. rivularis Gould, O. talienwhanensis Crosse>
5. Gardenia <fruit of Gardenia jasminoides Ellis>
6. Licorice <root of Glycyrrhizae radix, G. uralensis, or G. glabra>
7. Dai-Dai flower <flower of Citrus aurantium L. var. amara Engl.>
8. **Lo-Han-Kuo** <fruit of Siraitia grosvenorii (Swingle) C. Jeffrey ex A.M. Lu et Z. Y. Zhang>
9. Cassia <bark of Cinnamomum cassia Presl>
10. Sickle senna <seed of Cassia tora L.>
11. Tsai-Lou-Zi <seed of Raphanus sativus L.>
12. Dried orange peel < fruit skin of Citrus reticulata Blanco>
13. Sha-Ren < fruit of Amomum longiligulare T. L. Wu>
14. Black plum <fruit of Prunus mume Sieb. et Zucc.>
15. Ro-Do-Kuo <seed of Myristica fragrans Houtt>
16. Angelica < root of Angelica dahurica Benth. et Hook>
17. Winter aster <flower of Chrysanthemum morifolium Ramat or C. indicum L.>
18. Bishop wort <plant of Agastache rugosa (Fisch. et Mey.) O. Ktze.>
19. Sha-Ji <fruit of Hippophae rhamnoides L. subsp. (yunnanensis, turkestanica, mongolica, or sinensis) Rousi>
20. Yu-Li-Ren <seed of Prunus humilis Bge or P. japonica Thunb.>

21. Chinese white olive <fruit of Canarium album raeusch>
22. Longstamen onion leaf <leaf of Allium macrostemon bge>
23. Peppermint<plant of Mentha haplocalyx Briq.>
24. Clove < flower bud of Eugenia aromatica Merr.l et Perry, E. caryophyllata Thunb., Syzygium aromaticum, or Caryophyllus aromaticus>
25. Kao-Lian-Jiang <seed of Sorghum vulgare pers>
26. White nut < nut of Ginkgo bilobal>
27. Xiang-Xiu <plant of Rabdosia rosthornii (Diels) Hara, Mosla chinensis Maxim., M. dianthera (Buch. Ham.) Maxim., Origanum vulgare L., Elsholtzia densa Benth, E. stanuntonii Benth., or E. eriostachya Benth>
28. Huo-Ma-Ren <fruit of Cannabis sativa I.>
29. Mandarine orange <skin of the fruit of Citrus tangeriana Hort. et Tanaka, C. erythrosa Tanaka, C. grandis Osbeck var. tomentosa Hort., or C. chachiensis Hort.>
30. Tuckahoe < fruit body of Poria cocos Wolff = Pachyma hoelen Rumph>
31. Xiong Yuan <fruit of Citrus wilsonii Tanaka>
32. Safflower <flower of Carthamus tinctorius L.>
33. Purple perilla <leave of Perilla frutescens Britton var. crispa Decaisne or P. frutescens Britton var. acuta Kudo>

Section B-8: Consumption

Question #8: "Any survey, report or scientific publications which estimates daily, weekly, monthly or yearly consumption of Lo-Han-Kuo"

Answer:

Attachment 9 (Journal of Chinese Medicine Information 1996(9)13) contains some marketing information about the consumption of Lo-Han-Kuo. Please note that the units are very different from what in the Western world. Some conversion factors are listed here:

- Weight: 1 "Jin" is 0.5 kilogram
- Area: 1 "Mu" is 0.1647 acre
- Currency: 1 "Yuan" in Ren-Min-Bi (RMB) is equivalent to approximately 1/8 US dollars.

Here is the translation of Attachment 9:

Journal of Chinese Medicine Information, 1996
Volume 3, Number 9, page 13

Market of Chinese Herb Medicine: Analysis of the Production and Sales of Lo-Han-Kuo

Lo-Han-Kuo, or Han-Kuo, is the dried fruit of a cucurbitaceous plant. It can help the function of lung and serve as a refrigerant. It can resolve constipation by improving the function of intestines. It is mainly produced in Guangxi, especially at the Yongfu and Lingqua areas, which are known as "the county of Lo-Han-Kuo". It is not only sued as a supplement of herb medicine, but also used as the major components of many over-the-counter Chinese medicine. It is also a traditional exporting item, among many other Chinese herb medicine. Lately, many local government offices have considered Lo-Han-Kuo as a major business. The production of Lo-Han-Kuo has increased very significant. The total cultivation area is over 10,000 "Mu" and the annual production volume is around 50-60 million "Jin". 1995 was a bumper year for Lo-Han-Kuo, with the annual production over 60 million Jin. Despite of the increase of production, the sale was even better than before and the price was doubled. The current price at production site of Lo-Han-Kuo fruit has increased from 0.62 Yuan in last year to 1.2 Yuan in this year for each large-size fruit; from 0.45 to 1.0 Yuan for each medium-size fruit; and from 0.26 to 0.5 Yuan for each small-size fruit. Based on the author's investigation, the price increase was due to the following reasons:

1. The medical value was confirmed and the usage volume increased: Before 1970, Lo-Han-Kuo had only been used in the Guangxi and Guangdong areas as a local Chinese herb medicine. It was seldom used in other areas. The annual production and sale volume was less than 10 million Jin. After the medial application of Lo-Han-Kuo was recorded in the 1991 edition of Pharmacopoeia of People's Republic of China, the application has been spread to the whole China and the medical effects have been confirmed again and again. With the confirmed medial value, Lo-Han-Kuo started to be sold in the whole China. Sine 1980, the sale volume increased year after year. In the 1990's, the sales increase dramatically. According to data gathered by government, the annual domestic sales were 5 million piece of fruits in 1970's, 15 million pieces in late 1980's, 25 million pieces lately, and over 30 million pieces in 1995.

2. The types of applications increased: For a long time before 1970's, Lo-Han-Kuo had only been used as a supplement of herb medicine. Since 1980, the medical usage of Lo-Han-Kuo has been increased with the growth of Chinese medicine industry. To date, there are over 20 over-the-counter Chinese medicine products and approximately 10 health products using Lo-Han-Kuo as the main raw material. There are more than 50% of domestic sales of Lo-Han-Kuo was as the raw material for the above mentioned products. In addition, Lo-Han-Kuo is also used to prepare drink. It is sweet, tasty, refrigerant, and known to be helpful

for the lung. The Lo-Han-Kuo drink is very popular and contributes to the sales of Lo-Han-Kuo.

3. The export volume increased: Lo-Han-Kuo is a traditional exporting item among many other Chinese herb. It is very welcomed by other countries. Since 1980, Lo-Han-Kuo and its over-the-counter medicine products have entered the Europe and America markets. According to related government offices, the export amount of Lo-Han-Kuo increases 2% per year. Annual export amount increases more than 6 folds, from 5 million pieces in 1970 to 35 million pieces of Lo-Han-Kuo fruit in 1995. Lo-Han-Kuo is mainly exported from Guangzo, Shanghai, and Tianjin

Due to the above mentioned reasons, the recent production volume of Lo-Han-Kuo cannot meet the demand of domestic and foreign markets, causing the price to increase. According to the market trends, we must increase production to fulfill the market demand by improving cultivation techniques and area productivity.

Section B-9: Composition Measured

Question #9: "On what basis is the chemical composition of Lo-Han-Kuo Extract standardized (Total Mogrosides? An individual Mogroside?)"

Answer: Total Mogrosides

Section B-10: Specifications

Question #10: "What are the chemical composition specification limits"

Answer:

The chemical composition specifications have been provided and translated in the 5 COA under Question #1.

Section B-11: Assay Methods and Validation

Question #11: "What method (complete detailed steps) is used to measure the amount of Lo-Han-Kuo extract in foods to which it has been added and what procedure is used to validate the method (details)"

Answer:

The Chinese manufacturer provided a procedure for measuring the amount of Lo-Han-Kuo extract in foods (referred as "Lo-Han-Kuo Products" by the Chinese manufacturer) to which it has been added. The information is translated below:

Assay Methods for the Contents of Lo-Han-Kuo Glucosides in Lo-Han-Kuo Products

China Guilin Siter New Technology Company
Natural Botanical Product Manufacturer
1-4-1999

1. Instrument and Reagents

1.1 UV Spectrometer
1.2 Electronic Balance (1/100,000 sensitivity)
1.3 Lead acetate
1.4 Methanol
1.5 Ethanol, anhydrous
1.6 Vanillin
1.7 Sulfuric Acid
1.8 Standard sample of Lo-Han-Kuo Extract

2. Establishment of Standard Curve

Weigh exactly 30 mg of Lo-Han-Kuo Extract Standard and add into a 10 mL volumetric bottle. Add 70% ethanol to dissolve and Q.S. to the final volume<of 10 mL>. Mix well and pipette exactly 25, 50, 75, 100, 125 microliters of solution to 10 mL test tubes with sealing caps. Add 70% ethanol to each tube and Q.S. to 0.5 mL. Add 0.5 mL of 10% vanillin - ethanol solution to each tube. Mix well and sit the tubes in ice bath. Add 5 mL of 75% sulfuric acid solution and mix well. Heat the tubes to 50 C for 20 minutes then put tubes back to the ice bath immediately. After 10 minutes in ice bath, measure the absorbence at 530 nm, which is the wave length of peak absorbence. Plot the amount of Standard vs. the absorbence to establish the Standard Curve.

3. Determination of the Content <of Lo-Han-Kuo Extract> in the Sample

3.1 Sample preparation:

Weigh exactly 20 g of Lo-Han-Kuo product and suspend with distilled water. Transfer the suspension into a 250 mL flask. After soaking for 10-15 minutes, add 2-5 mL of neutralized lead acetate to remove protein. Mix well for 10 minutes and add water to 150 mL. Filter to remove precipitate. Rinse the precipitates with 30 mL of water for 3 times (90 mL total amount of water)Combine all filtrate solutions. Add the filtrate to a 500 mL round bottom bottle and concentrate until it is dry by rotating film evaporation. Add 100 mL of methanol and 5 gram of anhydrous sodium sulfate and extract the dried material by heating with reflux condensation for 1 hour. Filter the extraction suspension after cooling and repeat

the above methanol-sodium sulfate extraction process for a total of three times. Combine three filtrates and concentrate by a K-D concentrator until it is dry.

3.2 Measurement of Lo-Han-Kuo glucosides

Add 70% ethanol to dissolve the dry material in the K-D concentrator and remove the solution to a 10 mL volumetric bottle. Add 70% ethanol and Q.S. to the final volume of 10 mL. Mix well and pipette exactly 75 microliters of solution to a 10 mL test tube with sealing caps. Follow the steps in Section 2 to measure the absorbence. Use the Standard Curve to convert the absorbence to the equivalent amount of Standard in microgram, "C". The Content <of Lo-Han-Kuo Extract> in the Sample can be determined by the following equation:

$$\text{Content in Sample (\%)} = C / (7.5 \times W) \times 100\%$$

where C = the amount obtained from the Standard Curve, micro-gram
W = the amount of Sample, mg

Section B-12: Chemical Abstract System Registry Number

Question #12: "Have chemical abstract system registry numbers been assigned to Lo-Han-Kuo or any of it's constituents (Mogrosides, etc.). If so, please indicate those numbers."

Answer:

The chemical abstract service registration numbers of mogrosides were identified by Sinotech:

Mogroside IV: 89590-95-4
Mogroside V: 88901-36-4
Mogroside VI: 89590-98-7

Section B-13: Assay Standards

Question #13: "What is the source, chemical composition and method of standardization for Lo-Han-Kuo "Standard" used in chromatographic and other analysis <at the Manufacturer's site>"

Answer:

Attachment 4 is the SOP of Guilin Siter Factory which describes the assay and standard used to generate the COA of 5 batches provided for Question 1. It contains most information needed to answer this Question:

- Method of Standardization: See Item 4.2.2 of the SOP (Attachment 4) for details.
- Chemical composition of Standard: Lo-Han-Kuo mogrosides, etc. (c.f. Item 3.2 of the SOP)
- Source of Standard: Lo-Han-Kuo Extract produced in Guilin Siter Factory (c.f. Item 4.2.1.6)

Here is the translation of Attachment 4:

Guilin Siter New Technology Company
Natural Botanical Product Manufacturer
Standard Operating Procedures

Q/TRP002-1995

Lo-Han-Kuo Glucosides

Guangxi Zuang Tribe Autonomous Region
Technology Inspection Bureau
Standard Operating Procedures Filing & Registration Stamp
#450000

Publishing Date: 1995-07-10 Effective Date: 1995-08-05
Published by: Guilin Siter New Technology Company, Natural Botanical Product Factory

1.0 Scope and Contents

This Standard Operating Procedures (SOP) apply to the Lo-Han-Kuo Glucoside Powder extracted and purified from fresh or dried Lo-Han-Kuo fruits.

2.0 <Reference> Procedures Cited

GB 5009.3	Assay Procedures for Water Content in Food
GB 5009.4	Assay Procedures for Ash Content in Food
GB 8451	Assay Procedures for Heavy Metal Content in Food
GB 8450	Assay Procedures for Arsenic Content in Food Additives
GB 7718	General Standards for Food Labeling

3.0 Specifications

3.1 Appearance Specification

3.1.1 The product should be light yellow or yellow powder and very soluble in water and ethanol.

3.1.2 The product should have the unique fragrance of Lo-Han-Kuo and is very sweet.

3.2 Physical Property Specifications

Physical Property Specifications of Lo-Han-Kuo Extract

Item	Grade 1 Specification	Grade 2 Specification
Glucosides	> 85 %	> 75 %
Sweetness	240	210
Ash	< 2.0 %	< 2.2 %
Water	< 5 %	< 6 %
Lead (Pb)	< 0.002 %	< 0.002 %
Arsenic (As)	< 0.0001 %	< 0.0001 %
Infra Red Characteristic Peaks (wave number) +/- 10 cm^{-1}	3430, 1651, 1454, 1377, 1166, 1075	3430, 1651, 1454, 1377, 1166, 1075

4.0 Assay Methods

Unless noted differently, only distilled water or equivalent is used. All reagents are "analysis" grade.

4.1 Sensational Inspection

Put the sample on white paper and inspect it under appropriate natural light. It should be in light yellow or yellow color with the unique fragrance of Lo-Han-Kuo. Dissolve the sample in water to 1% concentration, the solution should taste very sweet.

4.2 Quantitative Analysis

4.2.1 Instrument and Reagents

4.2.1.1 UV Spectrometer
4.2.1.2 Electronic Balance (1/100,000 sensitivity)
4.2.1.3 Ethanol, anhydrous
4.2.1.4 Vanillin
4.2.1.5 Sulfuric Acid
4.2.1.6 Standard sample of Lo-Han-Kuo Extract -- Provided by this Factory. Store the Standard in a brown sealed bottle and place the bottle in a desiccator. Replace the Standard every other year.

4.2.2 Establishment of Standard Curve

Weigh exactly 30 mg of Lo-Han-Kuo Extract Standard and add into a 10 mL volumetric bottle. Add 70% ethanol to dissolve and Q.S. to the final volume of 10 mL. Mix well and pipette exactly 25, 50, 75, 100, 125 microliters of solution to 10 mL test tubes with sealing caps. Add 70% ethanol to each tube and Q.S. to 0.5 mL. Add 0.5 mL of 10% vanillin - ethanol solution to each tube. Mix well and sit the tubes in ice bath. Add 5 mL of 75% sulfuric acid solution and mix well. Keep

the tubes in 50 C water batch for 20 minutes then put back to the ice bath immediately. After 10 minutes in ice bath, measure the absorbence at 530 nm, which is the wave length of peak absorbence. Plot the amount of Standard vs. the absorbence to establish the Standard Curve.

4.2.3 Determination of the Content <of Lo-Han-Kuo Extract> in the Sample

Weigh exactly 30 mg of Sample and add into a 10 mL volumetric bottle. Add 70% ethanol to dissolve and Q.S. to the final volume of 10 mL. Mix well and pipette exactly 75 microliters of solution to a 10 mL test tube with sealing caps. Follow the steps in Section 4.2.2 to measure the absorbence at 530 nm. Use the Standard Curve to convert the absorbence to the equivalent amount of Standard in microgram, "C". The Content <of Lo-Han-Kuo Extract> in the Sample can be determined by the following equation:

$$\text{Content in Sample (\%)} = C / (7.5 \times W) \times 100\%$$

where C = the amount of Standard obtained from the Standard Curve, micro-gram
W = the amount of Sample, mg

4.3 Determination of Sweetness

Weigh 2 g of sucrose and dissolve in 100 mL of distilled water to prepare a 2% solution. Weigh 2 g of Sample and also prepare a 2% solution. Dilute the 2% Sample solution for 210 folds with distilled water. Compare the dilution Sample solution with the sucrose solution <by tasting it>. When the sweetness of these two solutions are equivalent, the dilution factor <of the 2% Sample solution> is the 'Sweetness" of the Sample.

4.4 Determination of Water Content

Follow Procedure GB5009.3

4.5 Determination of Ash Content

Follow Procedure GB5009.4

4.6 Determination of Heavy Metal (based on lead, Pb) Content

Follow Procedure GB8451

4.7 Determination of Arsenic Content

Follow Procedure GB8450

4.8 Infra Red Spectrum

4.8.1 Principle:

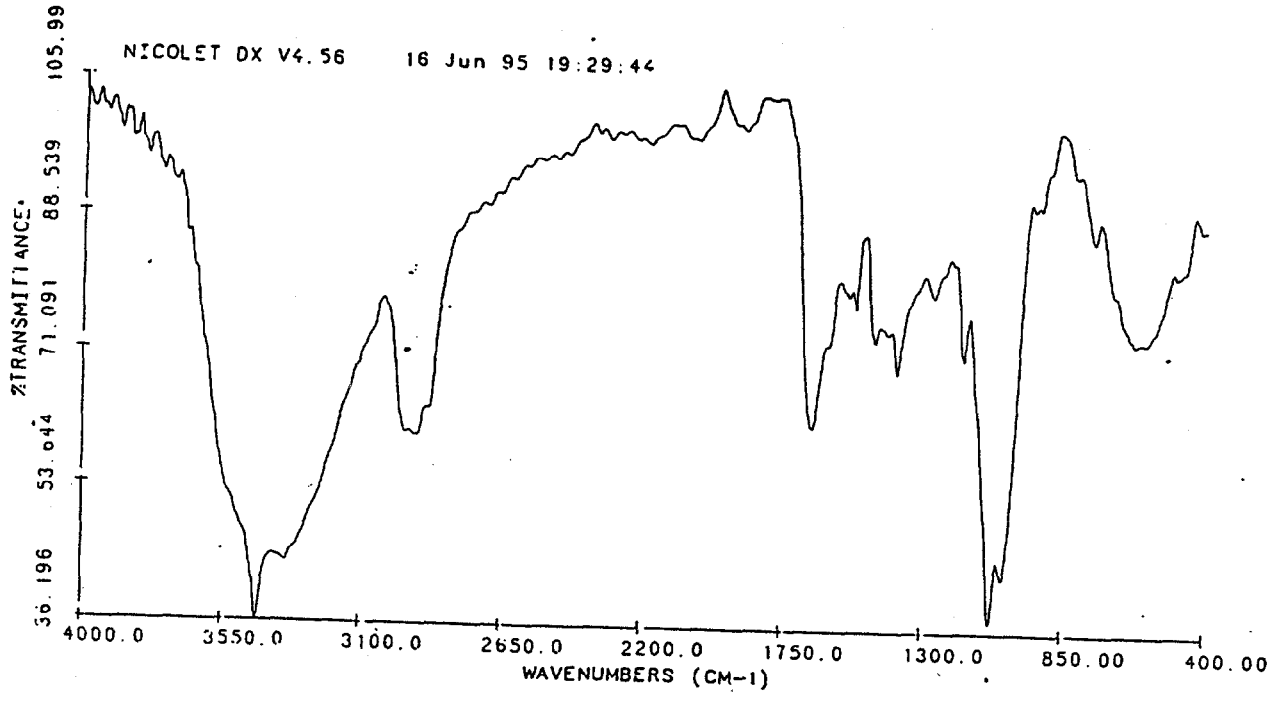
Mix the Sample with potassium bromide and press into a chip for Infra Red spectrum measurement. Compare the characteristic peaks on the spectrums of the Sample and the Standard.

4.8.2 Instrument:

Infra Red Spectrophotometer

4.8.3 Determination of the Wave Numbers of Infra Red Spectrum

Following the standard "Potassium bromide press chip method" of Infra Red Spectrum Measurement, mix the Sample with potassium bromide in a ratio of 1:100. Press the mixture into a chip and measure the Infra Red Spectrum. The Wave Numbers of the Sample spectrum should be consistent with what listed in the Physical Property Specification Table (Section 3.2). The Spectrum of Standard is attached in the following figure:



5.0 Inspection Guidelines

5.1 The product should be inspected by the Technical Inspection Department of the production factory. All products released from the factory must meet the specifications listed in this SOP. Each batch of product released from the factory must be accompanied with a Certificate of Quality Assurance confirming that the quality meets the specifications.

5.2 Take samples from more than 10% of packages in each batch, or at least 3 packages, whichever is larger. Remove 10 grams of sample from each package and mix all samples together. Divide the mixed sample into four groups and take 30 grams to put into two clean polyester bags. Put the each bag into another complex aluminum bag and seal. Label the bags with product name, batch number and sampling date. Take one bag for assay and another as the archive sample.

5.3 Every batch must be inspected against the Physical Property Specifications.

5.4 If the inspection results indicate that there is one item does not meet the specification, the inspection of the failed item can be repeated to determine the fate of this batch. If the repeated inspection still fails to meet the particular specification, the particular batch fails to meet the specifications.

5.5 When the customer and the factory disagree on the quality of product, both parties may negotiate or authorize an arbitrate organization to conduct the inspection according to this SOP.

5.6 Any product quality issues caused by inappropriate shipping or storage should not be the responsibility of the factory. It should be the responsibility of the organization handling shipping and storage.

6.0 Packaging, Labeling, Shipping, and Storage

6.1 Product should be first packaged in a polyester bag then packaged in another complex aluminum bag. The external package is a carton box with water-proof coating.

6.2 The label on the polyester bag should follow the guideline in GB7718.

6.3 A Certificate of Quality Assurance must be accompanied with the package. The Certificate should contain the following information: production factory, product name, address, batch number, net weight, production date, product specification ID number.

6.4 The external package must contain a robust label containing the following information: production factory, product name, address, batch number, net weight, production date, expiration date.

6.5 During the shipping process, the product should be kept from moisture, rain, over-pressure, sun light, heat, and contamination of toxic materials.

6.6 The product should be stored in a cool and dry shady area. It should be segregated from toxic, stink, or chemical materials.

6.7 The expiration date is two years from the production date.

Note: The SOP was drafted and published by Guilin Siter New Technology Company, Natural Botanical Product Factory.

SOP Originator: Lee Jin

Section B-14: Siamenoside

Question #14: "What is the sweetness of siamenoside triterpene and how much of occurs in the extract"

Answer:

A paper published by several researchers at Institute of Medicinal Plant Development, Chinese Academy of Medical Sciences reviewed studies on chemical principles and uses of natural non-sugar sweeteners from Lo-Han-Kuo (Research and Development of Natural Products, 1992(1)72, Attachment 13). According to the paper, the sweetness of 0.01% siamenoside I solution is 563 folds of 5% sucrose solution, and the yield of siamenoside I is 0.047%. The yield is presumably based on the dry weight of Lo-Han-Kuo fruit but it was not defined in the paper. There are no information in this paper regarding the amount of siamenoside I in Lo-Han-Kuo extract to answer the second part of Question #14. However, Package A stated that the yield of Lo-Han-Kuo extract is 1% of dried Lo-Han-Kuo fruits. Therefore, the content of siamenoside I in Lo-Han-Kuo extract should be around 4.7%. Since the sweetness of siamenoside I is ~280,000 (i.e. 500 x 563) folds of pure sucrose, the sweetness of Lo-Han-Kuo extract should be at least 13,000 (i.e. 280,000 x 4.7%) folds of sucrose, which is dramatically inconsistent with the measured sweetness of Lo-Han-Kuo extract, around 200 folds of sucrose. The above inconsistency suggests that these data are questionable.

The sweetness of siamenoside I was also mentioned in Package A but the concentration of siamenoside for sweetness measurement was translated incorrectly by the previous translator. The siamenoside solution was diluted for 10,000 folds instead of 1,000,000 folds when its sweetness was compared with 5% sucrose solution. An English scientific paper was cited as the original source of the sweetness information (c.f. R. Kasai et al, 1989; Agric. Biol. Chem., 53(12):3347). However, upon reviewing the cited paper, no information about the sweetness of siamenoside was found.

It is interesting to note that three other glucosides from Lo-Han-Kuo are also extremely sweet, according to Attachment 13. The table from Attachment 13 containing related sweetness information was enclosed. Again, these data are questionable since the sweetness of Lo-Han-Kuo extract calculated from these data is much too high.

Here is the table with the sweetness and yield data in Attachment 13:

表1 罗汉果中的三萜成分

Name	Formula	m·p·(C)	[α] D	Yield(%)	水中的浓度与甜度*	References
mogroside Ⅳ (1)	$C_{54}H_{92}O_{24} \cdot 2H_2O$	185—188(dec)	−4.2 −5.8	0.12	0.012% 392	10, 11
mogroside Ⅴ (2)	$C_{60}H_{102}O_{29} \cdot 2H_2O$	197—201(dec)	−11.7	0.029—1.0	0.012% 425	10, 11
siamenoside Ⅰ (3)	$C_{54}H_{92}O_{24} \cdot 7/2H_2O$	—	+4.9	0.047	0.01% 563	10, 11
11-oxo-mogroside Ⅴ (4)	$C_{60}H_{100}O_{29} \cdot 7/2H_2O$	—	+20.5	0.18	0.05% 84	8, 11
mogroside ⅡE (5)	$C_{42}H_{72}O_{14}$	—	+35.2		tastless	11
mogroside ⅢE (6)	$C_{48}H_{82}O_{19}$	—	+4.5		tastless	11
mogroside Ⅲ (7)	$C_{48}H_{82}O_{19}$		+2.5		tastless	11
mogrol (8)	$C_{30}H_{52}O_4 \cdot H_2O$	135—135.5	+70.0		tastless	9, 11
11-oxo-mogrol (9) (bryoducosigenin)	$C_{30}H_{50}O_4 \cdot 1/2H_2O$				tastless	9, 10
mogroside Ⅵ (10)	$C_{66}H_{112}O_{34}$	198—204	−4.2		0.01% 125	8

*甜度以5%的蔗糖溶液为1计。

REPORTS ON THE TOXICOLOGY TESTS OF LUOHANGUO EXTRACTS (GLYCOSIDES) FOR EXPORT TO NORTH AMERICA

Nov. 15, 1996

Acadamy Institute: Department of Pharmacology, Guilin Medical College
Address: 20 Lequn Rd
Guilin, Guangxi 541001
P. R. China

Coordinators: Nanhai Goldstar Industrail CO. LTD.
Natural Product Factory, Guilin S&T New Technology Company.

Date of test: Aug. 13, 1996——Nov. 11, 1996

Ⅰ. Extract Samples

Five samples tested from five batches of the products (batch No: 951120, 960116, 960206, 960410, 960518) were provided by the Natural Plant Product Factory, Guilin S&T New Technology Company. All samples from the five batches show a slightly brawn colour (for quality examination results of the five batches of produced, see Apendix Ⅰ)

Ⅱ. Sample Treatments

Upon administration to the dogs each time, extract sample was dissolved in distilled water to make a solution of 100ml containing 30g of the extract. Sample of each batches was used for 18 days.

Ⅲ. Methods

1. Animal housing and feeding

Sixteen hybrid dogs weighting 8.0～9.0 Kg, age of 24～30 weeks, with eight males and eight females, were perchased from the local farmer market, guarantines proved all healthy. Each dog was assigned to a number, from 1 to 16, and beared a metal card with the number assigned. Dogs were randomly divided into groups, eight dogs per group, with No. 1, 5, 6, 8, 9, 11, 13, and 15 in the experimental group, No. 2, 3, 4, 7, 10, 12, 14 and 16 in the control group. Animals were housed individually in a steel cage (80×60×80cm) at

the Experiment Animal House of Guilin Medical College, with room temperature of 22±2 C, relative humidity of 30~40% and 12 hour dark-light cycle. The animals were fed with cooked rice as main food, three times a day supplement with cooled pork meat or/and fish and vegetables. Distilled water was provided as drinking water. (for water analysis results, see Supplement Document 2)

2. Luohanguo Extract Dose and Administration

Before starting experiment, dogs housed under the condition described above were allowed to acclimate the envirdment for four days. After 12 hour fasting, dogs of experiment group were given Luohanguo extract 3..0g/kg (BWT), (in 5ml/kg), twice daily at 8 : 00~8 : 30 and 17 : 00~17 : 30 by gavage (tube feeding) for consecutive 90 days; control group were given distilled water by the same method, during the 90 days of the Luohanguo extract feeding period, all animals were observed for their food and water intake, urine and stool excretion, and general behavior changes (if any). Each dog was weighed once a week and body weight was recorded, meanwhile, blood sample was drawn and urine sample was collected for laboratory analysis. At the 91th day (the second day of discontinue of Luohanguo extract feeding) all dogs in both groups were killed and organs were disected for pathological examinations.

3. Examinations

(1) Hematology: RBC, WBC, HB;

(2) Blood Biochemistry; blood K^{-}, P^{4-}, Cl^{-}, Ca^{2+};

(3) Liver function: total protein (TP), albumin (ALB), globubmin (GLO), ALT, AST;

(4) Renal function: urine volume, urine PH, BUN, Creatinine (Cr), urine protein;

(5) Blood sugar and urine sugar;

(6) pathological examinations of heart, liver, spleen, lung and kidney.

4. Statistic analysis

All result data was statistically analysized by one way of ANOVA, a $p<0.05$ was considered significat difference.

Ⅳ. Results

All results are shown in the tables 1 to 6.

Ⅴ. Discussions and Conclusions

There was no evidence showing effect of Luohanguo extract, 3.0g/kg daily (equivatent to 360 folds of the dose for a human adult) for 90 consecutive days on the animals body weights, food consumption and, urine and feces secretions. As seen from the Tables A to G, statistical analysis also shows no effects on blood biochemistries, urine chemical and functions of liver, kidney as well as on the histology of organs examined.

Investigators: Zhunian Tang, Qin Xu, Yuxian Wei, Xiao Jian Su .

P. I: Xiao Jian Su

桂林医学院实验记录

8只狗子均值

Experiment Data Sheet

汉果提取物(甜甙)3 g/kg 白连续灌服 90 天 8 只狗(第 1,5,6,8,11,13,15,号狗)各项检查结果统计表

Statistic calculations of the results of Examinations of the eight dogs(No. 1, 5, 6, 8, 9, 11, 13, 15) fed with Luohan-guo(glycosides) for consecutive 90 days.

Tab.A.B.

指标 Index	动物数 (n)	日期(天) Date											
		0	7	14	21	28	35	42	49	56	63	77	90
体重(kg) Body Weight	8	8.39±0.30	8.50±0.24	8.61±0.22	8.83±0.25	9.05±0.24	9.36±0.87	9.60±0.86	9.81±0.89	10.11±1.02	10.36±1.08	10.60±1.15	10.95±1.34
外观毛色 Hair Appearance	8	Q	Q	Q	Q	Q	Q	Q	Q	Q	Q	Q	Q
活动情况 Active(Condition)	8	Q	Q	Q	Q	Q	Q	Q	Q	Q	Q	Q	Q
心率(次/分) Heart Rate(time/min)	8	167.75±16.37	170.25±11.12	170.38±11.21	171.38±15.13	173.25±18.23	169.63±14.89	168.88±12.33	168.75±18.05	173.00±13.16	173.88±11.10	170.25±17.51	171.75±14.21
血压(mmHg) Blood Presure	8	121.12/72.38 ±5.74/±7.56	119.88/70.88 ±6.62/±7.99	126.38/72.00 ±10.46/±6.44	128.13/76.75 ±13.10/±7.92	123.38/72.88 ±8.28/±8.58	121.75/76.25 ±9.65/±8.03	120.13/71.63 ±8.63/±7.52	127.50/74.13 ±7.78/±5.36	125.83/72.00 ±9.95/±4.81	125.88/73.00 ±5.77/±7.63	124.38/72.63 ±7.33/±7.31	124.63/72.38 ±6.02/±7.86
呼吸(次/分) Respiration(time/min)	8	29.50±3.96	29.13±3.04	31.38±4.63	31.63±5.26	31.63±3.96	32.25±3.65	31.25±5.47	31.38±4.24	31.13±5.87	29.00±3.78	31.13±4.58	30.38±3.74
大便 Stool	8	Q	Q	Q	Q	Q	Q	Q	Q	Q	Q	Q	Q
小便 Urine	8	Q	Q	Q	Q	Q	Q	Q	Q	Q	Q	Q	Q
RBC:×10⁶/mm³	8	6.55±0.45	6.39±0.26	6.53±0.46	6.29±0.27	6.44±0.49	6.62±0.67	6.53±0.66	6.28±0.75	6.34±0.73	7.05±0.57	6.31±0.58	6.84±0.50
WBC:×10³/mm³	8	10.25±0.54	10.83±1.10	11.02±0.52	10.96±1.34	11.66±0.99	11.59±1.30	10.84±1.13	11.14±0.77	10.77±0.71	11.23±1.26	11.15±1.20	11.58±0.74
Hb: g%	8	13.13±0.39	12.31±0.73	12.78±1.04	12.45±0.33	13.14±0.64	12.64±0.72	12.71±0.79	12.41±0.59	12.39±0.42	12.31±0.45	12.49±0.72	12.53±1.12

A: General Condition

B: Hematology examination

Q: Normal

桂林医学院实验记录

Experiment Data Sheet

罗汉果提取物(甜甙)3 g/kg/日连续灌胃 90 天 8 只狗(第 1,5,6,8,11,13,15,号狗)各项检查结果统计表

Statistic calculations of the results of Examinations of the eight dogs(No. 1, 5, 6, 8, 9, 11, 13, 15) fed with Luohan-guo(glycosides) for consecutive 90 days.

Tab.F.

指标 Index		动物数 (n)	日期(天) Date											
			0	7	14	21	28	35	42	49	56	63	77	
血液生化检验F	血钾(mmol/L) Blood K^+	8	4.22±0.08	4.19±0.06	4.11±0.09	4.21±0.09	4.32±0.12	4.30±4.29	4.25±0.19	4.20±0.08	4.19±0.09	4.49±0.43	4.33±0.20	4.3
	血磷(mmol/L) Blood P^{+1}	8	1.73±0.06	1.79±0.05	1.74±0.07	1.75±0.06	1.70±0.17	1.35±0.10	1.88±0.13	1.90±0.13	1.77±0.13	1.75±0.08	1.70±0.10	1.76
	血氯(mmol/L Blood CL^-	3	112.6±12.13	109.2±7.37	113.3±14.1	114.2±14.3	112.1±10.40	103.2±31.3	123.4±22.7	116.6±7.89	114.0±10.7	121.4±18.2	108.8±11.7	116.9
	血钙(mmol/L) Blood Ca^{2+}	8	1.65±0.12	1.74±0.13	1.71±0.21	1.87±0.13	2.09±0.19	2.04±0.25	1.72±0.26	1.67±0.09	1.74±0.14	1.85±0.17	1.75±0.26	1.86

F: Blood biochemistry examination

Tab. G.

器官 Organs	动物数 (n)	重量(g) Weight	外观 Appearance	浊肿 Turbid swelling	空泡变性 Vacuolar degeneration	炎症灶 Inflammatory focus	坏死灶 Necrotic focus
肝 Liver	8	354.4±12.16	无异常 without abnormer	无 None	无 None	无 None	无 None
心 Heart	8	101.7±7.20	无异常 〃	无 〃	无 〃	无 〃	无 〃
肾 kidney	8	42.4±3.42	无异常 〃	无 〃	无 〃	无 〃	无 〃
肺 Lung	8	228.1±17.08	无异常 〃	无 〃	无 〃	无 〃	无 〃
脾 Spleen	8	63.3±3.63	无异常 〃	无 〃	无 〃	无 〃	无 〃

G: Organ's pathology examination

桂林医学院实验记录

Experiment Data Sheet

罗汉果提取物(甜武)3 g/kg/日连续灌服 90 天 8 只狗(第 1,5,6,8,11,13,15,号狗)各项检查结果统计表

Statistic calculations of the results of Examinations of the eight dogs(No. 1, 5, 6, 8, 9, 11, 13, 15) fed with Luohan-guo(glycosides) for consecutive 90 days

Tab.C.D.E.

	指标 Index	动物数 (n)	日期(天) Date 0	7	14	21	28	35	42	49	56	63	77	90
肝功能检验 C	TP (g/L)	8	58.15±5.20	58.88±5.10	57.20±6.18	56.90±2.35	58.88±4.82	58.23±6.18	57.80±5.35	55.38±5.14	58.10±2.36	62.16±1.24	62.40±4.94	58.46±
	ALB(g/L)	8	26.89±1.23	27.10±0.81	26.00±2.46	27.25±2.80	26.22±1.47	27.92±2.48	26.68±2.27	28.34±2.81	27.11±1.42	27.81±1.32	28.12±3.17	29.01±
	GLO(g/L)	8	32.25±3.47	32.24±1.10	30.04±2.85	28.10±4.38	30.27±2.79	30.94±4.07	33.40±6.18	31.62±19.67	29.48±1.33	33.40±2.01	30.72±2.72	30.50±
	ALT(u/L)	8	26.83±3.49	27.14±3.13	30.62±5.02	27.20±2.05	27.94±3.81	29.10±5.92	27.33±4.48	27.05±2.79	27.21±2.64	28.97±1.59	28.73±1.75	28.76±
	AST(u/L)	8	28.21±3.58	30.58±5.71	28.71±4.39	31.00±3.41	27.12±28.08	29.03±2.11	27.89±3.49	27.17±2.21	29.68±1.78	28.62±3.01	31.17±3.64	30.23±
肾功能检验 D	尿量(ml/日) Urine Volume(ml/day)	8	211.25±61.15	239.00±28.60	217.63±22.69	210.50±43.12	212.00±40.60	280.38±61.76	210.00±31.80	227.25±24.18	235.63±20.45	208.25±60.18	202.63±38.45	200.25
	尿液 PH Urine PH	8	6.15±0.23	6.14±0.11	5.94±0.38	6.02±0.07	6.00±0.45	6.20±0.26	5.93±0.41	6.23±0.22	6.23±0.16	6.16±0.40	6.08±0.63	6.33±
	BUN(mmol/L)	8	3.40±0.33	3.36±0.35	4.08±0.49	3.33±0.25	3.31±0.46	3.83±0.64	3.87±0.43	4.23±1.00	4.06±0.67	3.93±0.34	4.07±0.80	3.83±
	Cr(mmol/L)	8	77.80±11.84	79.19±6.47	81.25±10.15	73.41±9.02	77.38±5.83	70.38±8.26	75.16±5.83	72.97±3.38	75.69±6.30	73.14±6.22	76.17±3.81	74.57±
	红蛋白 (g/L) Red Protein	8	0	0	0	0	0	0	0	0	0	0	0	0
血尿糖检验 E	血糖 (mmol/L) Blood sugar	8	5.44±0.81	5.23±0.69	5.19±0.67	5.23±0.33	5.12±0.50	5.53±0.60	5.07±0.21	5.06±0.46	5.39±0.63	5.29±0.45	5.23±0.32	5.21±
	尿糖 (mmol/L) Urine Sugar	8	0	0	0	0	0	0	0	0	0	0	0	0

C: Liver function examination .

D: Kidney function examination .

E: Blood sugar and urine sugar examination

一只的咽拔去8个

桂林医学院实验记录

Experiment Record of Guilin Medical College

(1)号狗试验记录表: Experiment Record Table of dog(1)

No. 1

	指标 Index	日期(天) Date											
		0	7	14	21	28	35	42	49	56	63	77	9
一般情况A	体重(kg) Body Weight	8.5	8.7	8.7	9.1	9.5	11.0	11.7	12.0	12.6	13.0	13.4	14.
	外观毛色 Hair Appearance	正常 Q	正常 Q	正常 Q	正常 Q	正常 Q	正常 Q	正常 Q	正常 Q	正常 Q	正常 Q	正常 Q	正常 Q
	活动情况 Aative(Condition)	正常 Q	正常 Q	正常 Q	正常 Q	正常 Q	正常 Q	正常 Q	正常 Q	正常 Q	正常 Q	正常 Q	正常 Q
	心率(次/分) Heart Rate(time/min)	186	175	182	190.	187	179	183	191	176	183	190	186
	血压(mmHg) Blood Presure	120/80	128/82	119/78	124/84	117/80	130/90	126/86	118/76	116/74	124/76	130/80	126/8
	呼吸(次/分) Respiration(time/min)	34	28	36	31	29	34	28	35	36	27	31	30
	大便 Stool	正常 Q	正常 Q	正常 Q	正常 Q	正常 Q	正常 Q	正常 Q	正常 Q	正常 Q	正常 Q	正常 Q	正常 Q
	小便 Urine	正常 Q	正常 Q	正常 Q	正常 Q	正常 Q	正常 Q	正常 Q	正常 Q	正常 Q	正常 Q	正常 Q	正常 Q
血液学检验B	RBC: $\times 10^6$/mm^3	6.34	5.76	6.47	7.23	6.75	7.34	6.58	5.92	6.87	7.35	6.92	7.29
	WBC: $\times 10^3$/mm^3	10.44	9.65	11.23	8.95	10.51	8.82	9.73	11.49	9.91	10.62	11.64	11.92
	Hb(g%)	13.61	12.74	13.52	11.94	12.83	11.66	12.23	12.56	13.14	12.43	13.75	12.87

A: General Condition

B: Hematology examine

Q: Normal

桂林医学院实验记录

Experiment Record of Guilin Medical College

罗汉果提取物 3g/kg/天灌服 90 天后对实验狗 (1) 五种器官病理学检查结果

Five organ's pathology examination results of the dog (1) with extract of Luohanguo 3g/kg/day i.g. for 90days.

No. 1

器官 Organs	重量(g) Weight	外观 Appearance		浊肿 Turbid swelling	空泡变性 Vacuolar degeneration	炎症灶 Inflammatory focus	坏死灶 Necrotic focus
肝 Liver	368.4	无异常 Without	Abnormal	无 None	无 None	无 None	无 None
心 Heart	97.32	无异常 〃	〃	无 None	无 None	无 〃	无 None
肾 kidney	38.60	无异常 〃	〃	无 〃	无 〃	无 〃	无 〃
肺 Lung	218.20	无异常 〃	〃	无 〃	无 〃	无 〃	无 〃
脾 Spleen	60.41	无异常 〃	〃	无 〃	无 〃	无 〃	无 〃

(1)号狗试验记录表:Experiment Record Table of dog (1).

	指标 Index	日期(天) Date											
		0	7	14	21	28	35	42	49	56	63	77	90
血液生化检验F	血钾(mmol/L) Blood K^+	4.30	4.27	4.08	4.11	4.32	4.05	4.07	4.12	4.20	4.25	4.30	4.16
	血磷(mmol/L) Blood P^{5+}	1.76	1.74	1.82	1.69	1.77	1.75	1.81	1.84	1.67	1.69	1.57	1.49
	血氯(mmol/L Blood Cl^-	98.60	100.00	97.50	99.28	98.10	92.20	112.30	115.40	120.50	132.10	118.10	120.20
	血钙(mmol/L) Blood Ca^{2+}	1.70	1.82	1.90	2.00	2.12	2.30	1.92	1.73	1.80	1.93	1.76	1.94

F: Blood biochemistry examine

桂林医学院实验记录

Experiment Record of Guilin Medical College

（1）号狗试验记录表：（接上表）Experiment Record Table of dog (1)

No. 1

	指标 Index	日期（天）Date											
		0	7	14	21	28	35	42	49	56	63	77	90
肝功能检验C	TP(g/L)	60.14	58.26	52.35	59.26	61.74	58.34	58.69	60.53	58.45	61.26	56.22	59.
	ALB(g/L)	26.58	27.12	26.46	25.66	24.78	28.45	26.24	25.67	26.12	27.24	24.59	26.
	GLO(g/L)	28.54	31.46	26.43	25.26	32.14	26.67	28.46	30.48	29.14	28.26	31.42	32.
	ALT(u/L)	31.44	28.67	30.25	28.58	29.64	31.27	30.54	28.44	31.25	28.41	28.48	31.
	AST(u/L)	26.41	30.12	28.42	31.23	26.91	28.42	32.43	26.96	30.24	28.68	27.61	29.
肾功能检验D	尿量（ml/日）Urine Volume(ml/day)	310.25	280.13	290.41	260.82	272.31	281.24	290.53	310.57	323.86	294.31	261.45	310.
	尿液 PH Urine PH	6.2	6.1	6.0	5.9	5.8	6.2	6.0	6.5	6.4	6.0	5.9	6.1
	BUN(mmol/L)	3.24	3.67	4.15	4.27	3.45	4.64	3.81	4.23	4.11	3.92	4.26	3.84
	Cr(mmol/L)	88.27	76.45	72.37	62.54	71.15	61.59	72.41	75.63	66.41	76.13	62.45	70.
	红蛋白（g/L）Red Protein	0	0	0	0	0	0	0	0	0	0	0	0
血尿常检验E	血糖（mmol/L）Blood sugar	5.24	5.57	4.33	4.90	5.26	4.85	5.10	4.64	5.21	5.68	5.28	4.87
	尿糖（mmol/L）Urine Sugar	0	0	0	0	0	0	0	0	0	0	0	0

C: Liver function examine.

D: Kidney function examine.

E: Blood sugar and urine sugar examine.