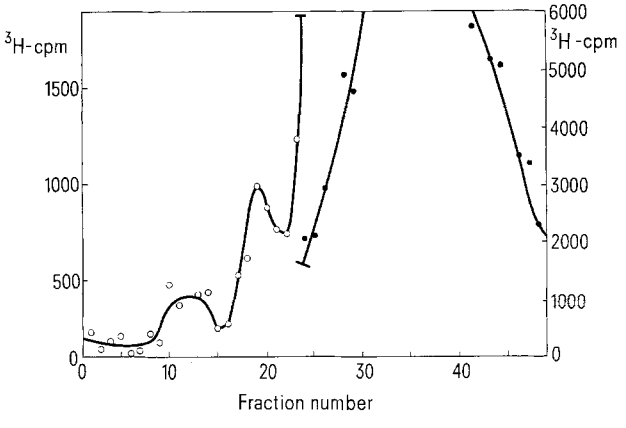
Bacteria growing in nutrient broth (*8* g/l) supplemented with 0.2% mannitol were used to inoculate the labeling medium, either nutrient broth with mannitol or minimal media consisting of 3.6 g K_2HPO_4, 0.4 g KH_2PO_4, 0.05 g $MgSO_4$-$7H_2O$, 0.5 g NaCl, 1.0 g $(NH_4)_2SO_4$ and 4.0 mg ferric citrate in 1 l of double-distilled water.

The pH of the minimal media was adjusted to 7.0 with NaOH and 10 g/l of mannitol was added after sterilization. The radioactive labels utilized were $^{32}PO_4$ (0.1 mCi/ml) or ^{3}H-adenine (0.001 mCi/ml). ^{3}H-thymidine was used but good incorporation was not obtained with this strain.

The bacteria were allowed to grow to late log or stationary phase before harvesting by centrifugation. The pellet was washed with TKE buffer (0.05 *M Tris*-HCl, pH 8.0, 0.6 *M* KCl, 0.05 *M* EDTA) and resuspended in a small volume of the same buffer. The suspension was quick frozen on acetone dry-ice and thawed in water at room temperature 2 times. Lysozyme (100 µg) was added to the suspension and the freeze-thaw repeated twice. The lysate was brought to 1% Sarkosyl and the freeze-thaw repeated again. The lysate was then layered on a gradient (Sarkosyl lysate) or cleared by centrifugation at 27,000 × *g* for 30 min (cleared lysate). Caesium chloride, ethidium bromide (200 µg/ml final concentration) and 0.01 *M Tris*-HCl buffer, pH 8.0 were added to the cleared lysate to a final volume of 4 ml and a refractive index of 1.393. A gradient was then formed by centrifugation in a SW 50 rotor at 29,000 rpm for 48 h at 20 °C.



Equilibrium density gradient centrifugation of a cleared-lysate of *R. japonicum* 10324 DNA. Fractions were collected from the bottom of the tube. 50 µl samples were removed from each fraction for refractive index determination and KOH hydrolysis. The KOH-hydrolyzed TCA-precipitable material was collected on GF/A filters, washed with ethanol and ether, and its radioactivity determined.

The Figure shows the satellite peak (fractions 16–22) found at the expected location for covalently-closed circular plasmid DNA. The chromosomal DNA banded at the location expected for linear DNA (fractions 30–40). The peak observed in fractions 11–14 was present occasionally and has not yet been identified. Similar profiles were obtained from either late-log or stationary cultures and with either the $^{32}PO_4$ or ^{3}H-adenine label. Gradients formed with cleared lysates as in the Figure were less ambiguous than gradients formed with Sarkosyl lysates. Treatment of the lysate with 50 µg/ml DNAase I (Sigma) in 10 m*M* $MgCl_2$ for a short time resulted in a decrease in the plasmid DNA peak and a porportional increase in the main genome peak. CsCl gradients in the absence of ethidium bromide showed a single peak of DNA at a density of 1.72.

The plasmid peak was observed in numerous independent experiments carried out in a variety of conditions. Quantitative determinations of molecular weight and percent plasmid DNA (of total DNA) were hampered by the low level of plasmid observed and the resulting difficulty in reproducibility. The upper and lower limits of plasmid DNA/chromosomal DNA are estimated to be 0.5% and 0.2% respectively.

Treatment of cultures under a variety of conditions with acridine orange or ethidium bromide had no effect on their antibiotic resistance. Treatment of cultures growing in minimal medium with 60 µg/ml atabrine for 3 to 4 days resulted in an increased frequency of susceptibility to the antibiotics chloramphenicol (less than 1% increased to 3%) and streptomycin (1% increased to 10%), as would be expected if atabrine acts to select cells which have lost an R-factor[6]. However, no effect of atabrine treatment on the presence or absence of plasmid DNA or the ability to produce nodules on soy beans was observed.

Résumé. L'ADN de *Rhizobium japonicum* 10324 montre une bande plasmide lors d'une centrifugation à l'équilibre sur CsCl/bromure d'éthidium. Nous ne pouvons pas corréler la présence de cette bande d'une part, la résistance aux antibiotiques ou la production de nodules de l'autre part.

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[6] M. Yoshikama and M. G. Sevag, J. Bact. *93*, 245 (1967).

[7] We are grateful to Dr. D. A. Phillips, Indiana State University, for assaying some of our bacterial isolates for the ability to produce nodules.

Intense Sweetener from Lo Han Kuo (*Momordica grosvenori*)

The search for non-sugar sweeteners from natural sources has led to the discovery of many interesting substances which possess either intense sweet taste[1] or taste-modifying properties[2]. This communication reports another source of a natural sweetening material.

Lo Han Kuo (Lo Han fruit), from *Momordica grosvenori* Swingle, is a dried fruit produced in Southern China. The fruits are gourd-like, 6–11 cm long by 3–4 cm broad, dark brown, broadly ellipsoid, ovoid or subglobose, with broadly rounded ends. The rind is very thin (0.5–0.8 mm

[1] For example: Glycyrrhizin, R. E. Muller, Forest Prod. J. *16*, 41 (1966). Monellin, J. A. Morris, R. Martenson, G. Deibler and R. H. Cagan, J. biol. Chem. *248*, 534 (1973). Osladin, J. Jizba, L. Dolejs, V. Herout and F. Sorm, Tetrahedron Lett. *1971*, 1329. Phyllodulcin, M. Yamato, K. Sato, K. Hashigaki, M. Oki and T. Koyama, Chem. Pharm. Bull., Japan *22*, 475 (1974). Stevioside, F. Bell, Chemy Ind. *1954*, 897. Thaumatin, H. van der Wel, FEBS Lett. *21*, 88 (1972).

[2] For example: Miraculin, K. Kurihara and L. M. Beidler, Nature, Lond. *222*, 1176 (1969). Gymnemic acid, G. P. Dateo and L. Long, J. agric. Food Chem. *21*, 899 (1973).

thick). The 3 double locules (compartments or cavities) each have 2 rows of seeds (about 10–12 in a row). The brownish-gray pulp is dried to a light fibrous mass. The seeds are light brownish-gray, flattened (15–18 mm long, 10–12 mm broad, and 3–4 mm at edges), and have a depressed area in the center of each side. A detailed characterization of the tissue, as well as the taxonomic status of the plant was described by SWINGLE[3], who reported that the species was introduced to the United States through the Division of Plant Exploration and Introduction, Bureau of Plant Industry. SWINGLE also stated that 1000 tons of the green fruits were delivered every year to the drying sheds at Kweilin (Kwangsi Province). The fruits lost much weight in drying and were then carefully packed in boxes and shipped to Canton, where most of the crop was used, but large numbers were also exported to Chinese communities outside China.

Folk medicine valued them as household remedies for colds, sore throats, and minor stomach and intestinal troubles. Memory from the author's own childhood experience that the cooked broth of this fruit tasted both very sweet and bitter prompted the author to investigate the constituents which gives rise to such taste qualities.

The sweet principle can be extracted by water from either the fibrous pulps or from the thin rinds[4] of Lo Han Kuo. 50% ethanol was also found to be a good extractant. In general, the rinds afforded a more easily purified extract. The pulps or rinds (or both) were suspended with the extractant and stirred in a Waring blender. The suspension was centrifuged to afford a very dark brown extract. Initial fractionation was effected by ultrafiltration through an Amicon PM-10 membrane filter. The concentrated colored filtrate was then passed through a column of Sephadex G-25. The sweet tasting fractions, well separated from the bitter fractions, were found to give little or no UV- absorption by the monitoring spectrophotometer (Uvicord II). These fractions were lyophilized and examined by thin layer chromatography (ethyl acetate: methanol: water, 5:3:1, v/v). A major component was revealed by spraying and heating the plates with 50% H_2SO_4. It had an Rg (relative to glucose) value of 0.67. Stevioside, the sweetener from *Stevia rebaudiana*, under the same conditions, have an Rg value of 1.1, while glycyrrhizin (the free acid form) had an Rg value of 0.27.

It was later found that if an aqueous extract of the fruit was passed through an Amberlite XAD-2 resin (Rohm and Haas Company), the sweetener was retained by the resin, but easily eluted with 50% ethanol. Both the Sephadex G-25 and Amberlite XAD-2 treated materials still showed several spots on TLC. The major component at Rg 0.67 could be isolated by preparative layer chromatography for organoleptic evaluation and chemical studies.

The sweetness of Lo Han sweetener was accompanied by a lingering taste described as licorice-like, somewhat similar to that of stevioside, glycyrrhizin, and the dihydrochalcones[5]. The potency was estimated to be about 150 times sweeter than sucrose, but more accurate determination has to be made when a larger quantity of the pure sweetener is available. The sweetener appears to be stable in boiling water for 5 h, as shown by tasting and by TLC.

Preliminary toxicological studies on a lyophilized extract with sweetness intensity equal to that of ammonium glycyrrhizinate showed that the LD_{50} was in excess of 10 g/kg mice[6], which is not surprizing in view of the common household usage of the dried fruits.

Chemical and spectroscopic studies indicate the sweetener component to be a glycoside of a triterpenoid. Structural elucidation is underway and will be the subject of subsequent communication[7].

Zusammenfassung. Die getrockneten Früchte, Lo Han Kuo, von *Momordica grosvenori* Swingle, enthalten einen Süss-Stoff, der mit Hilfe von Dünnschicht-Chromatographie abgesondert wurde.

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17 October 1974.

[3] W. T. SWINGLE, J. Arnold Arboretum *22*, 198 (1941). I am grateful to Dr. SHIU YING HU of Harvard University for supplying this reference.

[4] The paper by SWINGLE[3] noted the intense sweet taste of Lo Han Kuo pulps, but no mention was made of the taste of its rinds. It is quite noteworthy that the rinds also contain the sweetener.

[5] R. M. HOROWITZ and B. GENTILI, J. agric. Food Chem. *17*, 696 (1969).

[6] Male albino mice (Charles River Laboratory) weighing 19–24 g were dosed orally with aqueous solutions of the lyophilized extracts at a volume of 0.3 ml/10 g body weight and were kept for observation for 1 week. A very crude extract (without any processing) as well as the Sephadex G-25 treated extract were tested with 10 animals per group. None of the mice died. At a dose of 15 g crude extract per kg mice, the animals exhibited a mild sedation and some diarrhea; however, these effects were transient as all animals appeared normal within 30–60 min. I thank P. HOLMES and A. PRITCHARD for these results.

[7] The technical assistance of J. WEINBERGER is appreciated.

The Glair Glands and Oosetae of *Austropotamobius pallipes* (Lereboullet)

The origin and function of the glair exuded by the crayfish just prior to egg laying is obscure. In this connection a number of studies have been made of spawning in the European[1], Australian and American crayfishes, but not of *A. pallipes*, the endemic British species.

In mid-September, sexually mature females of *A. pallipes* are conspicuous by the presence of creamy-white patches on the pleura, and sterna of the abdomen, and on the pleopods and uropods, but never on the telson. A closer examination of these cream coloured areas, using the scanning electronmicroscope, discloses the presence of numerous pores in the overlying integument (Figure 1a). These pores are grouped together in roughly circular patches, presenting the appearance of 'pepper-pot' tops (Figures 1b, c and d). Observations in the field show that it is through these pores that the glair is exuded (Figure 1e), and the cream colouration is due to the very large groups of glair glands underneath the areas of perforated integument. On the pleopod the pores occur in much smaller groups (Figure 1f).

In *A. pallipes* the distribution pattern of the pores is constant; they are very numerous on the anterior faces of the pleura, less so on the anterior faces of the protopodites, and first segments of the endopodites and

[1] Z. MALACZYNSKA-SUCHCITZ, Bull. Soc. scient. Lett., Poznan, *13B*, 39 (1956).