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Natural products for the improvement of the quality of life

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Abstract: Work on the utility of some Thai plants as the source of food is presented including toxicological studies of stevioside and chemical investigations of cassava (*Manihot esculenta* Crantz).

Increasing interest has been focused on the utilization of natural products as a source of fine chemicals for various uses. Prominent among these uses is the medicinal property of natural products. It is well established that a large number of modern medicines are derived from natural sources in general and from plant origins in particular. Herbal drugs have been used in Thailand for centuries as an integral part of Thai culture. A great potential is foreseen for indigenous plants to be used as a source of new drugs. Our research goal at the Chulabhorn Research Institute is to utilize science and technology to improve "Quality of Life", a concept first propounded and practiced by His Majesty the King in the Royal Initiated Projects. This includes research on natural products. Some selected examples of Thai medicinal plants that are reputed to have antiparasitic, antimalarial and other properties have been recently surveyed (1). Attention will now be focused on some ongoing work in Thailand on the use of plants as source of food.

Considerable effort (2,3) has been expended on the search for organic molecules that are potently sweet, non-nutritive and noncarcinogenic especially for use in diabetic products. Moreover, the fact that sucrose in the main dietary source is now well recognized to be the cause of dental caries makes the substitutes of sucrose more desirable.

The need for new non-sugar sweeteners was reinforced by the introduction of the "Delaney Clause" in the Food, Drug and Cosmetic Act in the USA, prohibiting the use of any food additive which has been shown to produce cancer in laboratory animals. As a result many chemicals used as food additives, which include food flavouring agents such as sweeteners, have been banned. Toxicological problems associated with cyclamate and some concerns over saccharins have prompted the interests of scientists to search for non-nutritive, high intensity sweeteners to provide alternatives to sugar.

The "non-sugar" sweeteners are found in a variety of plants which contain chemicals with a wide range of chemical structures. Among these sweeteners, glycerrhizin from licorice, steviosides from *Stevia rebaudiana* (compositae), phyllodulcin from hydrangea, abrusosides from crab's eye vine, monellin from *Dioscorephyllum cumminsii* and muraculin from African miracle berries have been extensively studied.

Stevioside (3) is a diterpenoid glycoside isolated from *Stevia rebaudiana* bertoni (compositae). Stevioside is 200-300 times sweeter than sugar. Stevia is a perennial plant, originally grown in South America (Paraguay and Brazil), and was first identified in 1899. The leaves of the plant were known to have sweetening power by the natives and have been used as a sweetener for a long time. The plant is now cultivated commercially in Brazil, Israel, Korea, China and Japan. The plant was introduced to Thailand in 1977 and since then it has become an economic crop.

Although stevioside has already been used as a sweetener in many food products in Japan, toxicity studies of stevioside (4,5) have been extensively conducted in Thailand since 1987 using hamster as test animal while previous toxicity testing conducted elsewhere was in other rodents. Stevioside is more acutely toxic when administered intravenously, intraperitoneally and subcutaneously, but demonstrates no acute toxic effect when given orally. Acute toxicity is manifested by tremor, slow respiratory rate and anuria which is most pronounced in the hamster with the subsequent increase in plasma urea creatinine and uric acid.

Administration of stevioside at 1.05, 0.5 or 2.5 gm/kg. in food for a period of 6 days/week for 6 months slowed growth rate of the hamster as compared to the control and sucrose fed animals. This feeding regimen did not cause any pathological changes nor induce any neoplastic nodule.

A recent report (5) revealed the relationships between urinary enzyme levels and changes in blood urea nitrogen (BUN) and plasma creatinine levels, along with simultaneous ultrastructural changes of the kidney. The study was conducted in rats treated with stevioside. BUN levels increased at 3 h. onward after subcutaneous injection (s.c.) with stevioside (1.5 g/kg BW). The maximum increases in BUN and creatinine were approximately 180% and 132% respectively at 9 h. after stevioside injection. At this time, stevioside also caused significant increases in glucosuria, alkaline phosphatase (AP) and *g*-glutamyl transpeptidase (*g*-GTP) but no significant changes in proteinuria, N-acetyl-β-D-glucuronidase (NAG) or glutathione-S-transferase (GSH-S-TF). Histopathological examination of the kidney induced by stevioside revealed degeneration of the proximal convoluted tubule cells but no relation to lipid peroxide formation was detected. These results suggest that stevioside induced nephrotoxicity at the proximal convoluted tubules rather than at the glomeruli and other tubules, presumably by a defect of cell volume regulation due to depletion of intracellular ATP and disruption of microvilli, and nuclear dysfunction.

The effects of stevioside (6) on intestinal glucose absorption were examined in hamsters. Oral administration by gavage of a high dose of stevioside at 2.5 g/kg BW/day for 12 weeks caused inhibition of glucose absorption, but lower doses of 0.5 and 1 g/kg BW/day had no effect. Reductions in the activity of intestinal Na^+-, K^+-ATPase and absorption surface area were responsible for the inhibition of glucose absorption by stevioside. In addition, stevioside at a dose of 2.5 g/kg BW/day for 12 weeks also caused a reduction in body weight and an increase in sucrase activity of the jejunum. These results suggest that inhibition of glucose absorption by stevioside in hamsters is due to the inhibition of intestinal mucosal Na^+- K^+-ATPase and an alteration of the morphology of the intestinal absorptive cells which would lead to reductions in body weight of hamsters.

The effects of stevioside and steviol (7) on intestinal glucose absorption were examined in the hamster jejunum *in vitro*. By using the jejunal rings technique, it was found that stevioside at a high dose of 5 mM had no inhibitory effect on glucose absorption. In contrast, glucose absorption was inhibited 43% by 1 mM steviol. The inhibition of glucose absorption by steviol was related to steviol concentration and incubation time. The inhibitory effect of steviol as compared to phlorizin and ouabain was also investigated. Steviol, which caused a decrease in glucose accumulation in the intestinal ring tissues, possibly acts on the brush border membrane as does phlorizin. Furthermore, it was also found that steviol altered the morphology of the intestinal absorptive cells. These results suggest that the possible site of inhibitory action of steviol might be on the mucosal side and/or at the intracellular organelles of intestinal absorptive cells.

It should be noted that these toxicity studies utilized high dose of stevioside which may be well over the level that human beings are likely to be exposed to. Stevioside is still considered "safe" for normal consumption as an artificial sweetener.

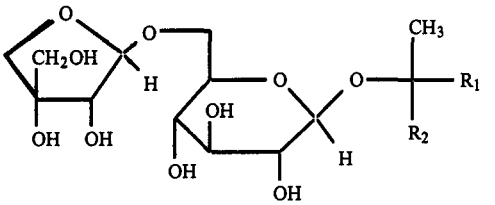
We are also interested in research on other agricultural products which are of vital importance to Thailand. One of the most important agricultural products in Thailand is cassava.

Storage roots of cassava (*Manihot esculenta* Crantz) (8), commonly known as tapioca, is an important source of carbohydrate for half a billion people in the tropical zone. About two-thirds of cassava is consumed as human food and one-third as animal feed. For human consumption, about half is eaten as cooked fresh roots and the remainder as processed flour or meal. The use of cassava as a staple food is associated with chronic cyanide toxicity which may cause such health problems as goiter, cretinism, tropical toxic neuropathy and tropical diabetes. Cassava is known to contain cyanogenic glycosides (8,9) which can break down to release the toxic cyanide. At present there are about 200 plants which are known to contain cyanogenic glycosides. Although traditional food processing and idustrial extraction of cassava starch can effectively remove the toxic cyanogens, they add cost to this caloric source of the poor. Thus, understanding of the biotransformation (10) of these cyanogens in cassava is essential for future manipulation to develop a cyanide-free cassava cultivar. However, before the biotransformation can be studied, the structures of these cyanogens should be investigated and identified. Before we began our work (11), two cyanogenic glycosides were known i.e. linamarin and lotaustralin.

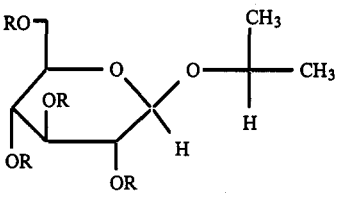
From the ethanol extract of fresh root cortex of cassava (*Manihot esculenta Crantz* ; Euphorbiaceae), two cyanogenic glucosides were isolated i.e (*R*)-1-cyano-1-methylpropyl-β-D-glucopyranoside (Lotaustralin) and 1-cyano-1-methylethyl-β-D-glucopyranoside (Linamarin). Apart from two cyanogenic glucosides, lotaustralin and linamarin and simple glucoside, ethyl-β-D-glucopyranoside, a novel cyanogenic glycoside i.e. 1-cyano-1-methylpropyl-β-D-apiofuranosyl(1→6)-β-D-glucopyranoside (I) was also isolated and identified.

We also were able to isolate two novel glycosides, one of which is the decyanated product of the previous cyanogenic glycoside. These two compounds are (*S*)-1-methylpropyl-β-D-apiofuranosyl (1→6)-β-D-glucopyranoside (II) and 1-methylethyl-β-D-apiofuranosyl(1→6)-β-D-glucopyranoside(III)

The three glycosides are novel and unique glycosides; these rare glycosides have the apiose moiety linking with the glucose unit at the 6th position.



(I) R_1 = CH_3CH_2, R_2 = CN (IV) R = H
(II) R_1 = CH_3CH_2, R_2 = H
(III) R_1 = CH_3, R_2 = H



(V) R = Ac

Apart from the extract from the root cortex, we have also investigated the methanol extract of fresh leaves of cassava. From the methanol extract of the fresh leaves, a mixture of cyanogenic glucosides, lotaustralin and linamarin previously isolated from the root cortex were again isolated. Apart from lotaustralin and linamarin, two flavonoid glycosides, i.e kaempferol-3-*O*-rutinoside and quercetin-3-*O*-rutinoside were isolated.

The structures of these compounds were deduced from the spectroscopic methods, especially NMR as well as some chemical transformations. Acid hydrolysis of the glycoside (III) gave D-glucose and apiose. However, partial hydrolysis of the compound could be accomplished by heating with 1% sulfuric acid at about 60 °C to give another smaller glycoside (IV) as shown. The resulting glycoside was further acetylated by the action of acetic anhydride in pyridine to give the corresponding acetate derivative (V). Both the glycoside and the corresponding acetate derivative were fully characterized by all spectroscopic methods to have the proposed structures.

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