

and which could also be used as a health food supplement, it could have a significant clinical benefit in the control of hypertension.

Stevioside is a glycoside isolated from the plant *Stevia rebaudiana* Bertoni, which has been widely used as a sweetening agent in Japan for more than 20 years.^{8,9} In pure form, stevioside is a white crystalline material with a melting point of 196-198 EC, an optical rotation of -39.3E in water, and an elemental composition of $C_{38}H_{60}O_{18}$ (Fig. 1). There are several reports concerning the effects of stevioside and other natural products from *S. rebaudiana* on the cardiovascular system. Previous investigators has shown that purified stevioside induces blood pressure reduction, diuresis, and natriuresis in rats.¹⁰ Our recent studies have shown that intravenous administration of stevioside results in a significant hypotensive effect in spontaneously hypertensive rats without adverse effects on heart rate or serum catecholamine levels.¹¹ Another human study revealed that stevioside powder is a relatively safe and effective hypotensive agent.¹²

However, previous studies have not systematically evaluated the dose-dependent effects of stevioside on blood pressure in conscious and anesthetized animals. This study was undertaken to investigate the dose-dependent effect on blood pressure in conscious and anesthetized SHR and anesthetized dogs.

MATERIALS AND METHODS

Animals

Male spontaneously hypertensive rats (SHRs, 4-8 weeks old, 250-450 g) and male mongrel dogs (3-9 years old, 10-15 kg) were used. They were obtained from the Animal Center of National Cheng Kung University Medical College. Animals were housed in a temperature-controlled room (25 ± 1 EC) and kept on a 12: 12-h light-dark cycle (light on at 0600). Food (Purina Animal Chow) and water were available ad libitum throughout the experiment.

Preparation of Stevioside

Stevioside was prepared from dried *S. rebaudiana* leaves by the method of Kelmer et al., which has been

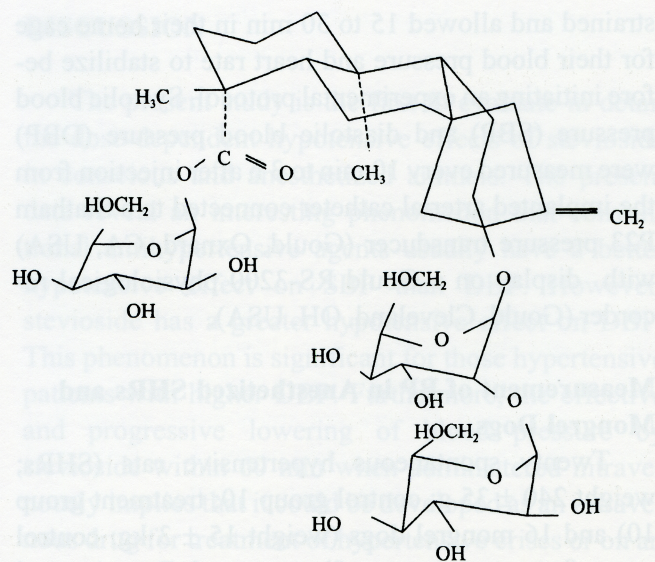


Fig. 1. Structural formula of stevioside ($C_{38}H_{60}O_{18}$)

reported to provide a product which is 95% pure.¹³ It was provided as a generous gift from Dr. Feng-Lin Hsu of Taipei Medical University. The remaining impurity (about 5%) mainly includes other sweetening principles like rebaudioside A, B, C, D and E, mucilage, and pigments from the leaves. These impurities can easily be removed by recrystallization but the less pure stevioside is more soluble in water and thus is easier to manipulate.

Measurement of Arterial Blood Pressure in Conscious SHRs

Experiments were performed on male spontaneously hypertensive rats (SHRs). Ten rats per group were studied, each weighing 230 to 300 g. The animals were maintained individually in metabolic cages. Water, food consumption, and body weight gain were recorded daily. Before the experiment, carotid artery and jugular vein catheters (PE50) were implanted under ether general anesthesia. The catheters were passed subcutaneously, brought out at the back of the neck, and fixed on the skin. When not in use, they were filled with heparinized saline and plugged with a stainless-steel stylet. Each animal was placed in a separate cage ($25 \times 15 \times 15$ cm) and allowed to recover from the surgery for at least 10 h. Animals were left unre-