THE CARDENOLIDES OF *THEVETIA PERUVIANA* GROWN IN MALAYSIA

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ABSTRACT

The cardiac glycosides found in the seed kernels of *Thevetia peruviana* grown in Malaysia was investigated. Thevetin, a triglycoside, was separated into thevetin A and thevetin B by DCCC while the monoglycosides obtained after incubation of the seeds were identified as neriifolin, neriifolin monoacetate, and peruvoside.

INTRODUCTION

Thevetia peruviana (fam. Apocynaceae) is grown throughout Malaysia as an ornamental. The yellow flowered variety of this large evergreen shrub is more commonly found than the orange and white flowered variety. *Thevetia* is highly toxic to animals (Kingsbury, 1964) and is mainly due to the presence of cardiotonic glycosides (Roa, 1973) which are known to occur in all parts of the plant except the flowers. They have been used in folkloric medicine (Perry, 1980) and several clinical trials have also been carried out on the glycosides (Arora and Rangaswami, 1972; Huang *et al.*, 1986). The seeds of *Thevetia* have also been known to have insecticidal properties (McLaughlin, 1980). Several polar glycosides (triosides) and secondary glycosides (monosides) have since been isolated from the seed kernels (Rao, 1973).

Our interest in the constituents of medicinal and poisonous plants of Malaysia had led us to investigate the seed kernels of *Thevetia peruviana* found in Malaysia.

RESULTS AND DISCUSSION

From the defatted seed kernels we obtained 3 – 4% thevetin, a triglycoside that was shown by tlc (n-BuOH: HOAc: H₂O; 8:1:1) and hplc (C₁₈ column, 80% aq. methanol) to consist of two components namely thevetin A and thevetin B (Fig. 1), and had melting point of 188 – 190°C which is in agreement with the literature (Sharma, 1981).

Fig. 1 : Thevetin

The yield of thevetin obtained in this study was comparatively lower than previous works (Sharma, 1981; Helfenberger, 1948) which gave a average yield of 5 – 7%. The two components were separated by means of droplet counter-current chromatography (DCCC), a technique that is becoming increasingly popular in natural product chemistry (Hostettman *et. al*, 1985). This is the first time that DCCC has been used in the separation of thevetin. Thevetin A and thevetin B were both obtained as white crystals from water and had melting point and spectra that were consistent with its structure.

The defatted seed kernels were also subjected to enzymatic hydrolysis by incubation at 37°C for 4 days. The white solid (6.1%) obtained after work-up was shown to contain three major components by tlc in CHCl₃ – MeOH (9:1) and was then isolated in pure form by using the chromatotron. From their melting points and spectral data the three compounds were identified as neriifolin (47.5%), monoacetylneriifolin (5.2%) and peruvoside (22.5%), Fig. 2, all of which have been known to occur in the seed kernels (Rao, 1973, Vorglaender *et. al*, 1969).

III: R = CH₃; R' = COCH₃; neriifolin 2'-monoacetate

IV: R = CH₃; R' = H; neriifolin

V: R = CHO, R' = H, peruvoside

Fig 2 : Monoglycosides of *T. peruviana*

MATERIALS AND METHODS

The seed of *Thevetia peruviana* were collected in the campus of Universiti Kebangsaan Malaysia, Bangi. Voucher specimens are deposited in the herbarium of the Botany Department, UKM.

ISOLATION OF THE GLYCOSIDES

All the glycosides were obtained from the defatted seed kernels according to the method of Helfenberger and Reichstein (1948).

Thevetin was obtained as white crystals (1.4g, 4%) from 34g of defatted seed kernels and had m.p. 188 – 190°C. Tlc (0.25 mm plates, silica gel G) in BuOH – HOAc – H₂O (8:1:1) indicated the presence of thevetin A (I) and thevetin B (II). Likewise hplc (Waters Assoc., C-18 column, 80% aq. MeOH) showed the presence of thevetin A and thevetin B

and in the ratio 35:65. Crude thevetin (0.25 g) was separated by DCCC (Tokyo Rikakikai, Model A, CHCl_3 - MeOH - H_2O ; 35:65:45; ascending mode) to give thevetin A (0.08 g, 32% mp 210–212, octaacetate mp 143 – 149) and thevetin B (0.15 g, 60%, mp 187 – 190°, octaacetate mp 145 – 149°).

Incubation of the defatted seed kernels (50 g) gave a mixture of monoglycosides (3.1 g, 6.2%) as a white solid. Separation of the mixture was carried out on a chromatotron (Model 7924T Harrison Research Palo Alto, 2 mm plate, 0.2 g material), eluting with chloroform - methanol mixtures and the following compounds were isolated in succession.

1. Nerifolin 2'-monoacetate, III, (0.0104 g, 5.2%), white crystals, m.p. 219 – 221° similar to that reported (mp 218 – 220, Cruz *et. al.*, 1977), and had ir and nmr spectra that are similar with literature (McLaughlin, 1980).
2. Nerifolin, IV, (0.0949 g, 47.5%), mp 222–225, (lit. mp 226 – 230°, Rao, 1973), ir and nmr spectra consistent with literature (McLaughlin, 1980).
3. Peruvoside, V, (.0554 g, 22.5%) had mp 200 – 202° and ir and nmr spectra consistent with the above monoglycosides.

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