Chapter 12

CHEMISTRY AND BIOACTIVITY OF SAPONINS FROM SOME SRI LANKAN PLANTS

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1. Introduction

The flora of Sri Lanka comprises about 3500 flowering plants of which about 850 species are endemic to the island [1]. Of these 3500 species, about 750 are claimed to have uses in the indigenous system of medicine [2]. In a continuation of our studies towards the discovery of biologically active saponins from Sri Lankan plants, we have chemically investigated *Diploclisia glaucescens* and *Anamirta cocculus* (Menispermaceae), *Pometia eximia* and *Filicium decipiens* (Sapindaceae), *Terminalia catappa* (Combretaceae) and *Uncaria elliptica* (Rubiaceae). Chemical investigation of the methanol extracts of these plants led to the isolation of over twenty five saponins. Of them fifteen triterpenoidal saponins were found to be new natural products. Some of them showed insecticidal, anti-inflammatory and strong molluscicidal activity.

2. Saponins from Sri Lankan Plant Species

Diploclisia glaucescens (Bl.) Diels (= *Cocculus macrocarpus* W. & A.) is a liana of the family Menispermaceae, growing in the mid-country regions of South India and Sri Lanka. The leaves have been used in the treatment of biliousness and venereal diseases [3]. The presence of alkaloids in the leaves and twigs of the plant has been indicated in a preliminary survey [4]. Chemical investigation of the seeds of the plant gave five phytoecdysteroids showing activity against larvae of the European corn borer, *Ostrinia nubialis*. The principal phytoecdysteroid ecdysterone (**1**) was isolated in a yield of 0.46% from seed [5] and 0.5% yield from the roots of *D. glaucescens* [6].

The methanol extract of the stem of *D. glaucescens* showed molluscicidal activity and a highly positive response for saponins in both the froth and haemolysis tests. Chromatographic separation of the methanol extract over silica gel furnished stigmasterol (**2**) [7], serjanic acid (3β-hydroxy-30-methoxycarbonylolean-12-en-28-oic acid) (**30**) (Figure 1), phytolaccagenic acid (3β, 23-dihydroxy-30-methoxycarbonylolean-12-en-28-oic acid)(**4**) [8], ecdysterone (**1**) [7] and two new

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saponins 3-*O*-β-D-glucopyranoside phytolaccagenic acid (**5**) [8] and 3,28-di-*O*-β-D-glucopyranoside phytolaccagenic acid (diploclisin **6**) [9].

Ecdysterone (**1**) an arthropod moulting hormone has been isolated by us from the stem of *D. glaucescens* in a yield over 3%, the highest recorded for the isolation of any moulting hormone from a natural source [7]. The procedure for the isolation of **1** has been extended to a large scale by a combination of vacuum liquid chromatography (VLC) and medium pressure liquid chromatography (MPLC) [10]. Ecdysterone showed significant spermicidal activity, moderate insecticidal activity and also gave a highly positive response for the froth and haemolysis tests [7].

After complete elution of **6**, the column was washed with methanol. Concentration of the methanol extract gave *vibo*-quercitol (**7**) as colourless needles. The residual methanol extract was partitioned between *n*-butanol and water. The *n*-butanol extract showed strongly positive froth and hemolysis tests. It showed high spermicidal activity (100% immotility of spermatozoa of fresh human semen at 8mg/mL within 20 sec.) and showed potential as a molluscicide (100% lethality to *Biomphalaria glabrata* snails at a minimum concentration of 50 ppm). The extract also showed mild anti-inflammatory activity (40% inhibition of carrageenan induced rat paw edema at a dose of 100mg/kg). Repeated chromatography over silica gel of the *n*-butanol extract gave four new saponins 3-*O*-β-D-glucuronopyranosylserjanic acid (**8**), 3-*O*-β-D-glucuronopyranoside phytolaccagenic acid (**9**), 3-*O*-β-D-glucuronopyranosyl-28-*O*-β-D-glucopyranoside serjanic acid (**10**) and 3-*O*-β-D-glucuronopyranosyl-28-*O*-β-D-glucopyranoside phytolaccagenic acid (**11**). The minimum concentration for 100% lethality in *Biomphalaria glabrata* snails was found to be 12 ppm, 30 ppm and 50 ppm for **8**, **9** and **10** respectively. No activity was observed at 100 ppm for **11**. Saponin **9** showed mild anti-inflammatory activity [11,12].

Anamirta cocculus (L) Wight et Arn. is a liana occurring in several regions of South-East Asia. The sesquiterpene mixture picrotoxin is commercially isolated from the berries [13]. Berries of the plant are used as fish poison. The seeds are used externally to destroy head lice. The bruised fresh bark is applied on wounds caused by snake bite. Primitive tribes of the Malay Peninsula use the plant as an arrow poison [14].

The *n*-butanol extract of the methanol extract of the defatted stem of *A. cocculus* showed strongly positive froth and haemolysis tests for saponins. Although the extract did not show any antifungal activity against *Cladosporium cladosporioides*, it showed marginal molluscicidal activity (100% lethal to *B. glabrata* snails at a minimum concentration of 200 ppm). A chromatographic separation over silica gel of the *n*-butanol extract gave two triterpenoids and three triterpenoidal saponins. Out of the two triterpenoids, the less polar is a new natural product identified as a 2α,3β,23-trihydroxy-11α,12α-epoxyolean-28,13β-olide (**12**). The other is arjunolic acid (2α,3β,23-trihydroxyolean-12-en-28-oic acid) (**13**) isolated for the first time from the family Menispermaceae and reported to be useful in the treatment of skin cancer. Out of the three saponins one is a known compound, 28-*O*-β-D-glucopyranoside arjunolic acid (**14**) obtained for the first time from the family Menispermaceae and other two saponins are 23-*O*-β-D-glucopyranoside arjunolic acid (**15**) and a 28-*O*-β-D-glucopyranoside