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A cyanoglucoside stored by a *Sedum*-feeding Apollo butterfly, *Parnassius phoebus*

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Abstract. A bitter tasting cyanoglucoside, sarmentosin, was isolated from an aposematic Apollo butterfly, *Parnassius phoebus*, and from its plant-host, *Sedum stenopetalum*. The content of sarmentosin in the body tissues was as high as 500 µg/insect, suggesting a defensive role for this substance; a high concentration was detected in the wings. Sarmentosin was also present in the eggs.
Key words. *Parnassius phoebus*; Apollo butterfly; Papilionidae; *Sedum*; defense substance; cyanoglucoside; sarmentosin; sequestration.

A clue to the nature of a long sought-after deterrent, assumed to be present in the aposematic (warningly coloured) Apollo butterflies, was provided by the Magpie moth (*Abraxas grossulariata*). The imago contains high concentrations of sarmentosin (fig. 1)[1] and the larva has been recorded as feeding on *Sedum*, hitherto the only known plant source of this cyanoglucoside[2].
The genus *Parnassius* (Papilionidae) has been described as a relic of the glacial epoch. *P. phoebus* inhabits sub-Alpine situations at around 1500 m and above. The specimens one of us (R. Nishida) collected in Washington State, in the U.S., were flying in high mountain meadows where one of its host-plants, *Sedum stenopetalum* (Crassulaceae), was exceedingly common. In Europe, *Sempervirum montanum* (Crassulaceae) and *Saxifraga aizioides* (Saxifragaceae) are recorded as its food plants[3]. The butterfly exhibits classical warning colouration, both the dorsal surface and underside being strikingly aposematic.
We originally examined *P. apollo*, since in Europe it feeds exclusively on stonecrop (*Sedum* spp.), but only preserved specimens were available, which proved unsuitable for the detection of sarmentosin. Fresh material was unobtainable, since the insect is declining in numbers and strictly protected. It is of historical interest that *P. apollo* was the first butterfly to be officially protected; this was achieved by the efforts of the Emperor of Germany, William I, and it was thereafter included under the Convention of International Trade in Endangered Species of Wild Fauna and Flora (CITES)[4].

Materials and methods

Adults of *P. phoebus* were captured at a field site in Washington State (Kittitas County) in mid-June 1994. Ten males were immediately extracted with ethanol (20 ml) and kept below 0 °C. After removing the first extract, the body tissues were finely ground and re-extracted with a mixture of benzene and ethanol (1:2, 10 ml × 2). The combined extracts (159 mg) were subjected to solvent extraction with chloroform and water. The water layer (28 mg) was chromatographed on a reverse-phase column (Sep-pak cartridge C18, Waters Associates), by eluting successively with water (2 ml + 2 ml), 20% methanol (3 ml) and 50% methanol (4 ml). The bitter component was found mainly in the second 2 ml water-eluate (4 mg) after elution of the initial 2 ml of water. Portions of the second eluate were purified by high performance liquid chromatography (HPLC) with YMC-Pack ODS-AM (150 × 6 mm i.d.) eluting with 3% methanol (1.5 ml/min), monitored by a photodiode array detector (λ200–400 nm). The compound was isolated at a retention time of 6.5 min. A similar analytical procedure was employed for the detection of the compound in the female specimens, eggs and leaves of host *S. stenopetalum*. Eggs were obtained from wild-caught females and were crushed and extracted in ethanol within a week of oviposition. *S. stenopetalum* was collected at a higher elevation than the *P. phoebus* samples, where the leaves were green, since the plants at the *P. phoebus* site were mostly flowering or withering at the time of the butterfly's emergence. The fresh leaves were homogenized below −5 °C in ethanol for extraction.

Results

A bitter component with a characteristic UV absorption band at λ_{max} 207 nm (in 3% methanol-water) was isolated from the body of *P. phoebus* and from its host-plant *S. stenopetalum* (fig. 2). The compound was identified as sarmentosin [4-(β-D-glucopyranosyloxy)-2-hydroxymethyl-2Z-butenenitrile] (fig. 1) from its diag-

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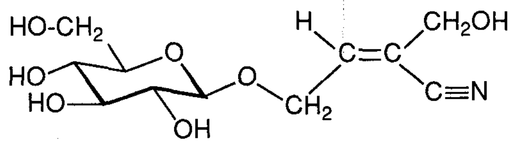

Figure 1. Sarmentosin.

nostic spectrometric data[1,2]: Mass spectrum (Hitachi M-1000 LC API) m/z 276 (M + H), 114 (aglycone + H); ^{1}H-NMR (90 MHz, MeOH-d_4): δ 6.68 (1H, triple triplet, $J = 6.5$ and 1 Hz), 4.60 (2H, multiplet), 4.37 (1H, doublet, $J = 7$ Hz), 4.16 (2H, doublet, $J = 1$ Hz), 4.1 – 3.1 (6H, multiplet) and ^{13}C-NMR (22.5 MHz, MeOH-d_4) δ 144.6, 118.1, 116.8, 104.1, 78.1 (2C), 74.9, 71.5, 68.2, 63.1, 62.6.

The contents of sarmentosin in the adult butterflies were approximately 420 µg/male and 490 µg/female. The compound was also detected from eggs freshly oviposited (approximately 1 µg/egg). The quantity of sarmentosin in *S. stenopetalum* was estimated to be 800 µg/g fresh leaf, although a portion of the compound appeared to have decomposed during extraction.

Discussion

The imago of *P. phoebus* stores sarmentosin and we can presume this cyanoglucoside is sequestered from its food plant. It is significant that *P. glacialis*, a species belonging to the group of *Parnassius* which feeds on *Corydalis* (Papaveraceae) was found (with a preliminary chemical analysis) not to contain sarmentosin in the body tissues. Hitherto isolated from *S. sarmentosum*[2], sarmentosin has now been found in several other related species of *Sedum* (*S. lanceolatum*, *S. oryzifolium*, *S. japonicum* etc.) (Nishida, unpubl.), suggesting that it is a fairly characteristic constituent of the genus. Sarmentosin epoxide, found as a constituent of *S. cepaea*, liberates HCN in aqueous media[5], and sarmentosin may well undergo the same process if the double bond is enzymatically epoxidized. Since this substance tastes bitter[1], it probably acts as a deterrent before its toxic properties are experienced by predators.

The quantities of sarmentosin found in each specimen range from 360 to 500 µg/insect. Substantial quantities were detected in the wings (approximately a quarter of

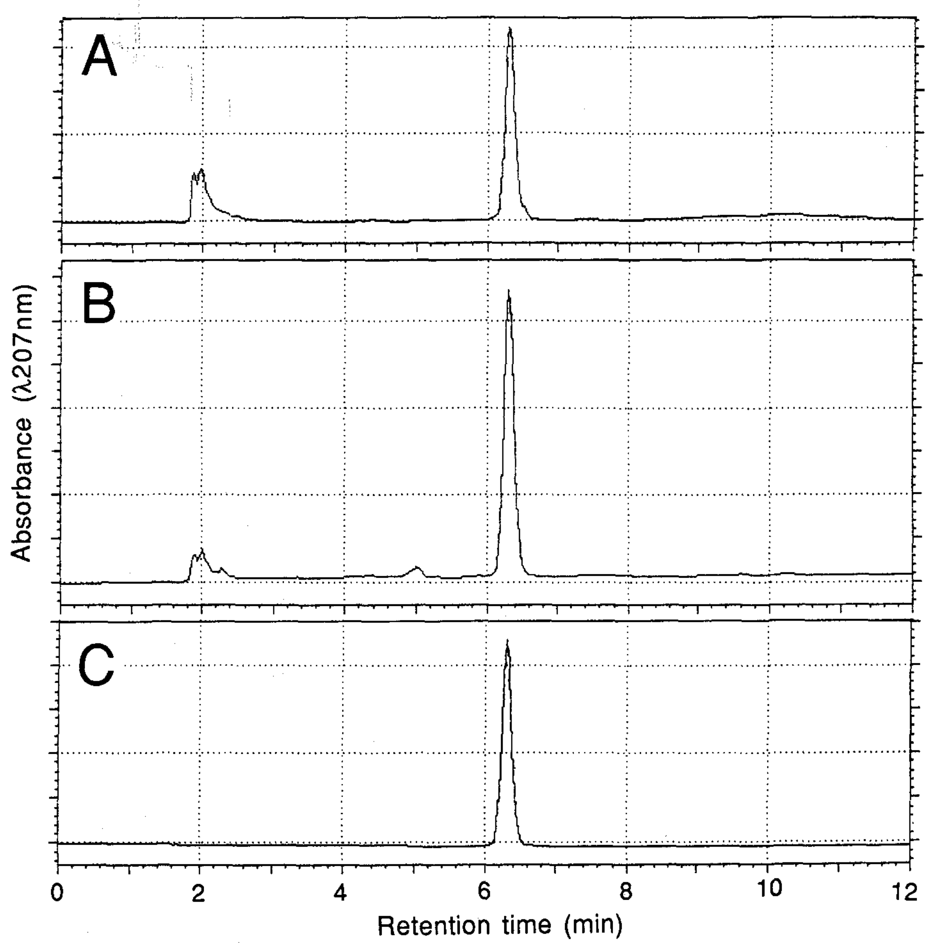

Figure 2. Liquid chromatograms of fractions in the fresh leaf extract of *Sedum stenopetalum* (A), whole body extract of *Parnassius phoebus* (B), and standard sarmentosin (C). The chromatogram was done on a YMC-Pack ODS-AM (150 × 6 mm i.d.), eluting with 3% methanol in water at a flow of 1.5 ml/min, monitored by UV detector (207 nm).

the total content of whole body in males). Parsons demonstrated that the cardiac glycosides sequestered and stored by the Monarch butterfly (*Danaus plexippus*) were also concentrated in the wings[6].

The aposematic colour scheme of the imago of *P. phoebus* is particularly striking, red spots and circles are distributed on creamy white wings in the male, offset with black and grey markings. In the female, large areas are completely transparent. Although the red spots and circles vary in tone from bright scarlet to salmon pink, they are often well displayed when the insect is basking or collecting nectar. In both sexes there is a characteristic collar of yellow hairs above the cervical glands.

The larvae are also aposematic; they are hairy, with double lines of red or yellow tubercles along the black dorsal surface. They feed only in bright sunshine and at other times conceal themselves under stones or leaves. Aposematic insects often behave differently in light or darkness; thus *Caenocoris nerii*, a brilliant red and black plant bug, congretates in dense aggregations on the exposed seed-pods of *Nerium* in bright sunlight, but at night scatters singly all over the plant (Rothschild, pers. observ.)

Sarmentosin has previously been identified in a genus of aposematic geometrid moths, *Abraxas*[1] (Nishida, unpubl.). More recently, a zygaenid moth, *Pryeria sinica*, has been found to incorporate sarmentosin from its food plant *Euonymus japonicus* (Celastraceae) (Nishida, unpubl.). Sarmentosin may eventually prove to be not uncommon as a sequestered deterrent.

It is interesting to find how versatile the aposematic Papilionidae are in acquiring different defensive toxins, such as cardenolides (*Papilio antimachus*[7]), aristolochic acids (*Zerynthia*[8], *Pachliopta*[9] and *Atrophaneura*[10]) and a cyanoglycoside (*Parnassius*).

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