# FLAVONOIDS OCCURRING IN THE STICKY RESIN ON *RORIDULA DENTATA* AND *RORIDULA GORGONIAS* (RORIDULACEAE)

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## Introduction

The leaves of *Roridula dentata* L. and *Roridula gorgonias* Planch., two South African plant species, bear numerous glandular hairs, with droplets of sticky liquid at their tips. The plants' habitus thus largely resembles that of many *Drosera* species, and also *Drosophyllum lusitanicum* (L.) Link (Droseraceae)[1]. The resemblance is further indicated by the fact that *Roridula* plants trap insects very successfully. Flower morphology, however, led botanists to place *Roridula* in a separate family, Roridulaceae. In the scope of ongoing research on structures and distribution of flavonoid aglycones in plant exudates (see e.g. Wollenweber *et al*. 2005), the insect-trapping glue of *Roridula dentata* and *Roridula gorgonias* has now been studied for the presence of flavonoids. Flavonoids are phenolic compounds with a basic three ring C15-skeleton, that can display numerous variations, depending on the state of C-ring oxydation, number and position of hydroxyl and methoxyl groups and other substituents (see Figure 1 for examples). Natural products of this type are ubiquitous throughout the plant kingdom in glycosidic form, i.e., they are linked with sugar molecules and are therefore water soluble and located within the plant tissue. The occurrence of sugar-free molecules, called aglycones, on the other hand, is restricted and

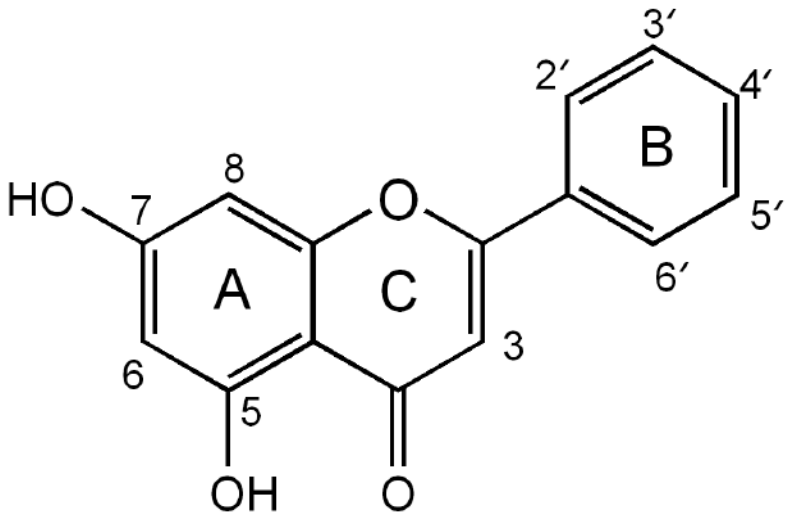


Figure 1: Flavones: 5,7,4'-OH=Apigenin; 5,7,3',4'-OH=Luteolin; 5,7,3',4',5'-OH=Tricetin. Flavonols: 3,5,7,4'-OH=Kaempferol; 3,5,7,3',4'-OH=Quercetin.

[1]According to recent molecular studies, *Drosophyllum* is more closely related to Dioncophyllaceae/Ancistrocladaceae and should be excluded from Droseraceae (J. Schlauer, pers. comm.).

appears to be correlated with the presence of secretory structures and the formation of lipophilic exudates (leaf resins, leaf waxes, essential oils). The detection and identification of flavonoid aglycones in the mucilage of *Roridula* is reported here.

## Experimental

One dried plant of *Roridula gorgonias* was obtained from the Botanischer Garten der TU Darmstadt, but most of the material of *R. dentata* and *R. gorgonias* was kindly supplied by three people, cultivating these plants and breeding the obligately associated carnivorous hemipteran insect, *Pameridea roridulae* as a hobby (see Acknowledgements section). Seeds were from different origin. The flavonoid patterns observed in different samples of each species exhibited the same flavonoid pattern and were, therefore, combined for analysis. Voucher specimens are kept in the herbarium of the Botanischer Garten Darmstadt.

On rinsing with acetone, 106 g of dried old *Roridula gorgonias* leaves yielded 15.3 g of sticky resinous exudate. This material was "defatted", by solution in a small volume of hot MeOH, cooling to -10°C, and removal of precipitated material by centrifugation. The supernatant was evaporated to yield 13.1 g of residue. Ca. 3.6 g of material remained as a mushy glue, insoluble even in boiling MeOH. The soluble portion was chromatographed on Sephadex LH-20 (Pharmacia) eluted with methanol to separate flavonoids from the predominant terpenoids. Fractions were monitored by thin layer chromatograpy (tlc) on silica, and those containing flavonoids were combined. The flavonoid mixture was further purified by preparative TLC on silica plates (SIL G 25, Macherey-Nagel), developed with toluene/MeCOEt 9:1 (v/v). Flavonoid bands were marked under UV light and scraped off. Elution of the silica with ethanol yielded individual flavonoids. Fractions were monitored and comparison with markers was achieved by TLC on polyamide (DC 11, Macherey-Nagel) with the following solvents:
i) PE 100-140/toluene/MeCOEt/MeOH 12:6:1:1 (v/v/v/v);
ii) toluene/PE100-140/ MeCOEt /MeOH 12:6:2:1 (v/v/v/v);
iii) toluene-dioxane-MeOH 8:1:1 (v/v/v), and on silica with solvent;
iv) toluene/MeCOEt 9:1 (v/v).

Chromatograms were viewed under UV (366 nm) before and after spraying with Natural Product Reagent A (0.2 % of diphenyl-boric acid 2-aminoethyl ester in MeOH). One of the isolated flavonoids was further characterized by its mass spectrum (Varian MAT 212 spectrometer at 70 eV) and by its $^{1}$H NMR and $^{13}$C NMR spectra (DMSO-$d_6$, Brucker ARX 400 spectrometer at 400 MHz and 100 MHz, respectively). $^{1}$H NMR: $\delta$ =6.98 (s, H-3), 6.40 (d, J = 2.0 Hz, H-6), 6.78 (d, J = 2.0 Hz, H-8), 7.19 (d, J = 2.0 Hz, H-2'), 7.22 (d, J = 2.0 Hz, H-6'), 12.88 (s, 5-OH), 9.73 (s, 5'-OH), 3.77 (s, OMe), 3.88 (s, OMe), 3.90 (s, OMe). $^{13}$C NMR: 163.4 (C-2), 104.8 (C-3), 181.9 (C-4), 161.0 (C-5), 98.0 (C-6), 165.2 (C-7), 92.6 (C-8), 157.2 (C-9), 104.7 (C-10), 125.6 (C-1'), 104.8 (C-2'), 153.5 (C-3'), 139.7 (C-4'), 150.9 (C-5'), 107.8 (C-6'), 56.0 (7-OMe), 56.1 (3'-OMe), 60.0 (4'-OMe). These data are in accordance with those reported for tricetin-7,3',4'-trimethyl ether (Zahir *et al*. 1996).

In the case of *Roridula dentata*, only a small amount of leaf material was available. The exudate was recovered and further processed as described above. The flavonoid portion obtained after column chromatography on Sephadex LH-20 was immediately analyzed by comparative TLC with markers.

## Results and Discussion

With one exception, the flavonoids were readily and unambiguously identified by direct TLC comparisons with authentic markers available in the author's lab. In the exudate of *R. gorgonias*, apigenin and its 4'-monomethyl ether (acacetin) and 7,4'-dimethyl ether, luteolin 7,3'-dimethyl ether (velutin) and luteolin 7,3',4'-trimethyl ether, and the 3',4'-dimethyl (apometzgerin), 3',4',5'-trimethyl and 7,3',4',5'-tetramethyl ether (corymbosin) of tricetin were thus

identified. For structural formulas see Figure 1. Based on its molecular mass (M+ at m/z 344, indicating a flavone with two OH and three OMe groups) and on extensive experience with the thin layer chomatographic behaviour of flavonoid spots as related to chemical structures, one further flavone was assumed to be the 7,3',4'-trimethyl ether of tricetin. Its identity was proven by its NMR spectra (see "Experimental" section).

The apigenin and luteolin derivatives present in the resinous exudate of *R. gorgonias* are widespread flavones whereas the tricetin derivatives, on the other hand, are rare plant constituents. Tricetin 3',4'-dimethyl ether, first reported as a *C*-glycoside in a liverwort (Theodor *et al*. 1980), has been found only three times previously as an aglycone (c.f. Valant-Vetschera & Wollenweber 2006). Tricetin 7,3',4'-trimethyl ether has been reported only once before, from *Lethedon tannensis* Spreng (Thymelaeaceae; Zahir *et al*. 1996, as *L. tannaensis* Forst.). The 3',4',5'-trimethyl ether, first reported from several Poaceae (Kaneta & Sugiyama 1973), was later found on the leaf surface of *Nonea pulla* (Boraginaceae; Wollenweber *et al.* 2002) and in the exudate of *Asarina procumbens* (Scrophulariaceae; Wollenweber unpublished). Tricetin 7,3',4',5'-tetramethyl ether, originally reported from *Webera corymbosa* (Rubiaceae; Joshi & Rane 1967), was found recently on *Centaurea incana* (Asteraceae) and on *Walsura piscidia* (Meliaceae) (for references, see Valant-Vetschera & Wollenweber 2006).

In the exudate of *R. dentata*, kaempferol 3,4'-dimethyl ether and traces of kaempferol 3-methyl ether, quercetin 3,3',4'-trimethyl ether and traces of quercetin 3,3'-dimethyl ether were identified. Two minor components remain thus far unidentified, due to lack of material. The four flavonols found are widespread compounds.

It is striking that the trapping gland exudate of *R. gorgonias* contains exclusively flavones, whereas in *R. dentata* it contains flavonols only. It should also be mentioned that, in both species, no difference was detected between the flavonoid profiles of fresh leaves on the one hand, and of wilted or dried leaves on the other hand.

As was already mentioned in the Introduction, classical taxonomic characters led botanists to segregate Roridulaceae from Droseraceae. The different character of the insect-catching exudates has been used to support the distinctness of these two families (cf. Marloth 1925). However, to the best of our knowledge the chemical composition of *Roridula* exudate has not yet been studied. The insect-trapping mucilage of *Drosera* and *Drosophyllum* is an aqueous solution of acidic polyasaccharides, and hence hydrophilic. The exudate of *Roridula*, on the other hand, is lipophilic material, consisting primarily of triterpenoids, natural products that are often found as major components of plant resins. As shown in this paper, the exudate also contains very small amounts of flavonoids. Analysis of the exudate terpenoids, which make up the bulk of the extremely sticky resin secreted by stalked glands on *Roridula* leaves, is in progress and will be published in the future.

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## Literature Reviews

By Doug Darnowski

Reut M.S., and Fineran, B.A. 2000. Ecology and vegetative morphology of the carnivorous plant *Utricularia dichotoma* (Lentibulariaceae) in New Zealand. New Zealand J. of Bot. 38: 433-450.

The authors examined eight sites spread across New Zealand to better understand the ecology of *Utricularia dichotoma*, defined broadly to include *U. monanthos* and *U. novae-zealandiae*. Essentially they found that *U. dichotoma* prefers open, nutrient-poor, brightly lit, wet sites and could often be associated with a few species of plants, especially various bryophytes and *Juncus articulatus*. It could, however, extend its range into other habitats once well established, such as permanently flooded areas, but not without limits—not surprisingly, *U. dichotoma* was never found growing in calcareous soils. Those trying to grow *U. dichotoma* or any other members of its Australia/New Zealand species complex will find these data helpful, and this paper also presents some valuable insights into the plasticity of one widespread group of terrestrial bladderworts. (DWD)

Sanabria-Aranda L., Gonzalez-Bermudez A., Torres N.N., Guisande C., Manjarres-Hernandez A., Voloyes-Valois V., Diaz-Olarte J., Andrade-Sossa C., and Duque S. R. 2006. Predation by the tropical plant *Utricularia foliosa*. Fresh. Biol. 51: 1999-2008.

In this paper, Sanabria-Aranda *et al*. examined prey capture by the large, pan-American *Utricularia foliosa* for the importance of bladder size, the type of prey captured, and the strategies used for trapping. This is a species which can be found as far north as Florida, easily identified by its flattened stems. In this paper, the work was done in Colombian Amazon. Increasing trap sizes did not provide disproportionate benefits in terms of nutrients obtained from prey, and the prey trapped most commonly were those types whose behavior made them most likely to contact the traps and the trap hairs, similar to what has been found by several other studies. Perhaps most interesting and novel was the finding that longer trap hairs/"antennae" led to significantly improved trapping rates, confirming in the field previous experimental studies. (DWD)