Triterpenoids as Major Components of the Insect-Trapping Glue of *Roridula* Species

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Roridula dentata and *R. gorgonias*, two South African plants that were formerly believed to be carnivorous, exhibit an extremely sticky exudate at the tips of secretory trichomes. Unlike the trapping mucilage of Droseraceae, it does not consist of acidic polysaccharides. The *Roridula* trapping glue was found to be a mutual solution of mainly dihydroxytriterpenoids, instead. All samples contain two isomers of ring A dihydroxyolean-12-enes and dihydroxyurs-12-enes. The difference between the two species is the additional presence of taraxeradiol in the glue of *R. gorgonias*. The absolute chemical structures of the reported triterpenoids still need confirmation.

Key words: Roridula, Trapping Glue, Triterpenoids

Introduction

The leaves of the South African plants *Roridula dentata* L. and *R. gorgonias* Planch. bear numerous glandular hairs with droplets of an extremely sticky liquid at their tips. These hairs trap insects very successfully and, therefore, these plants have been considered to be carnivorous. However, they produce no digestive enzymes. The plants are frequently inhabited by specialized small bugs (*Pameridea marlothii* on *Roridula dentata* and *P. roridulae* on *R. gorgonias*; Hemiptera: Miridae), which nimbly move around on the leaves and stems and rapidly consume trapped insects. The bugs' capability to run over the sticky plant surfaces may be due to the presence of specialized ultrastructural properties of their legs (W. Barthlott, personal communication). There is evidence from ^{15}N labeling experiments that *Roridula* does derive significant amounts of nitrogen from trapped prey, not by direct uptake but by indirect uptake apparently via exudations from the mutualistic hemipterans onto the plant leaf (Ellis and Midgley, 1996; Anderson and Midgley, 2002). The bugs' feces and undigested remainders of their prey may further contribute to the plant nitrogen supply. According to a recent paper, uptake of nitrogen compounds is facilitated by the existence of cuticular gaps in the epidermal cells (Anderson, 2005).

The sticky gland product of *Roridula* is not a trapping mucilage, nor an aqueous solution of acidic polysaccharides, containing proteolytic enzymes as in Droseraceae, but a lipophilic resin that is extremely viscous. This material contains very small amounts of flavonoid aglycones (Wollenweber, 2007). It does not contain proteolytic enzymes, so trapped insects are not digested directly. The term "indirect carnivory" was therefore introduced. The trapping glue consists mostly of triterpenoids, which may be responsible for its sticky property. Some of the major compounds have been isolated and analyzed. Their structure elucidation is reported here.

Material and Methods

Plant material and extraction

Dried old leaves of *Roridula dentata* and *R. gorgonias*, respectively, were rinsed briefly with dichloromethane/methanol. Another sample of *R. dentata* leaves was rinsed with acetone. The solvent was evaporated to yield a very sticky resinous gum (samples #1, #2, #3). This material was "defat-

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ted" by solution in a small volume of hot methanol, cooling to −10 °C, and removal of precipitated material by centrifugation (sample #7). The supernatant was evaporated to yield an extremely sticky residue. Part of this material remained as a mushy glue, insoluble even in boiling methanol (sample #6). The soluble portion was chromatographed on a Sephadex LH-20 column (Pharmacia, Freiburg, Germany) eluted with methanol to separate flavonoids from the predominant terpenoids, and to further separate the latter. Fractions were monitored by thin-layer chromatography (TLC) on silica gel-coated plates (SIL G 25, Macherey-Nagel), developed with toluene/butan-2-one (9:2 v/v). Terpenoids were visualized by spraying the TLC plates with $MnCl_2$ reagent, followed by heating (Jork *et al.*, 1989). The bands were scraped off, eluted with acetone and ethanol, and the extracts concentrated by rotary evaporation.

Hydrogenation

Some of the extract fractions were subjected to hydrogenation to aid in their characterization. The hydrogenation reaction was performed at 1 atm H_2 with PtO_2 (Acros Organics, Pittsburgh, PA, USA; 83% Pt) as catalyst in 10% hexane in ethyl acetate. The reaction was carried out at room temperature with constant stirring for 12 h. The catalyst was removed by filtration after reaction and the products were concentrated by rotary evaporation. Aliquots of extracts or fractions were converted to their trimethylsilyl derivatives using *N,O*-bis-(trimethylsilyl)trifluoroacetamide (BSTFA) containing 1% trimethylchlorosilane (TMCS) and pyridine (Pierce Chemical Co., Rockford, IL., USA) for 3 h at 70 °C prior to gas chromatography-mass spectrometry (GC-MS) analysis.

GC-MS analysis

GC-MS was carried out on a Hewlett-Packard Model HP6890 gas chromatograph interfaced with a HP5973 mass selective detector (MSD). The injector and ion source temperatures were set at 280 °C and 230 °C, respectively. A DB5-MS capillary column (30 m × 0.25 mm i. d., 0.25 μm film thickness, Agilent) was used with helium as the carrier gas. The GC operating program consisted of injection (splitless) at 65 °C, hold for 2 min, temperature increase of 6 °C min^{-1} to 300 °C, followed by an isothermal hold at 300 °C for 15 min. The MSD was operated in the electron impact mode with an ionization energy of 70 eV and scan range from 50 to 650 Da.

Data were acquired and processed with the HP-Chemstation software. Individual compounds were identified by comparison of mass spectra with literature and library data, comparison of mass spectra and GC retention times with those of authentic standards and/or interpretations of mass spectrometric fragmentation patterns. Compounds were quantified, using the total ion current (TIC) peak area, and converted to compound mass, using calibration curves of external standards. A procedural blank was run in sequence to the samples and presented no significant background interferences.

Results and Discussion

The total extracts and the various fractions are all relatively simple mixtures, and typical examples of the GC-MS data are shown in Fig. 1. The composition of the *Roridula dentata* extracts are comprised mainly of dihydroxyolean-12-ene and dihydroxyurs-12-ene, whereas the *R. gorgonias* extracts have a dominance of taraxeradiol with the prior and minor other compounds (Table I).

Mass spectra of the major triterpenols

All mixtures were analyzed as the silylated derivatives and the mass spectra to be discussed as illustrative examples are shown in Fig. 2. All major compounds are diols with ursane, oleanane and taraxerane skeletons and two hydroxy groups on ring A. Two extracts were analyzed as the underivatized mixtures by GC-MS confirming the triterpenediols; however, the response and GC peak resolution were poor.

The mass spectra of the triterpenols as the trimethylsilyl (TMS) ethers have a molecular ion at *m/z* 586 and a characteristic double loss of 90 Da (trimethylsilanol) to *m/z* 496 and 406, respectively (Figs. 2a–c). Specifically, the mass spectra of dihydroxyolean-12-ene and dihydroxyurs-12-ene have the base peak at *m/z* 218 ($C_{16}H_{26}$) from the retro-Diels-Alder rearrangement of the C-12 double bond. That, coupled with the ratios of the fragment ions at *m/z* 203 and 189, indicates that rings C and D have no functional group as in the amyrins. The even ion at *m/z* 278 ($C_{17}H_{28}OSi$) is the rings A and B part of the molecules from the same

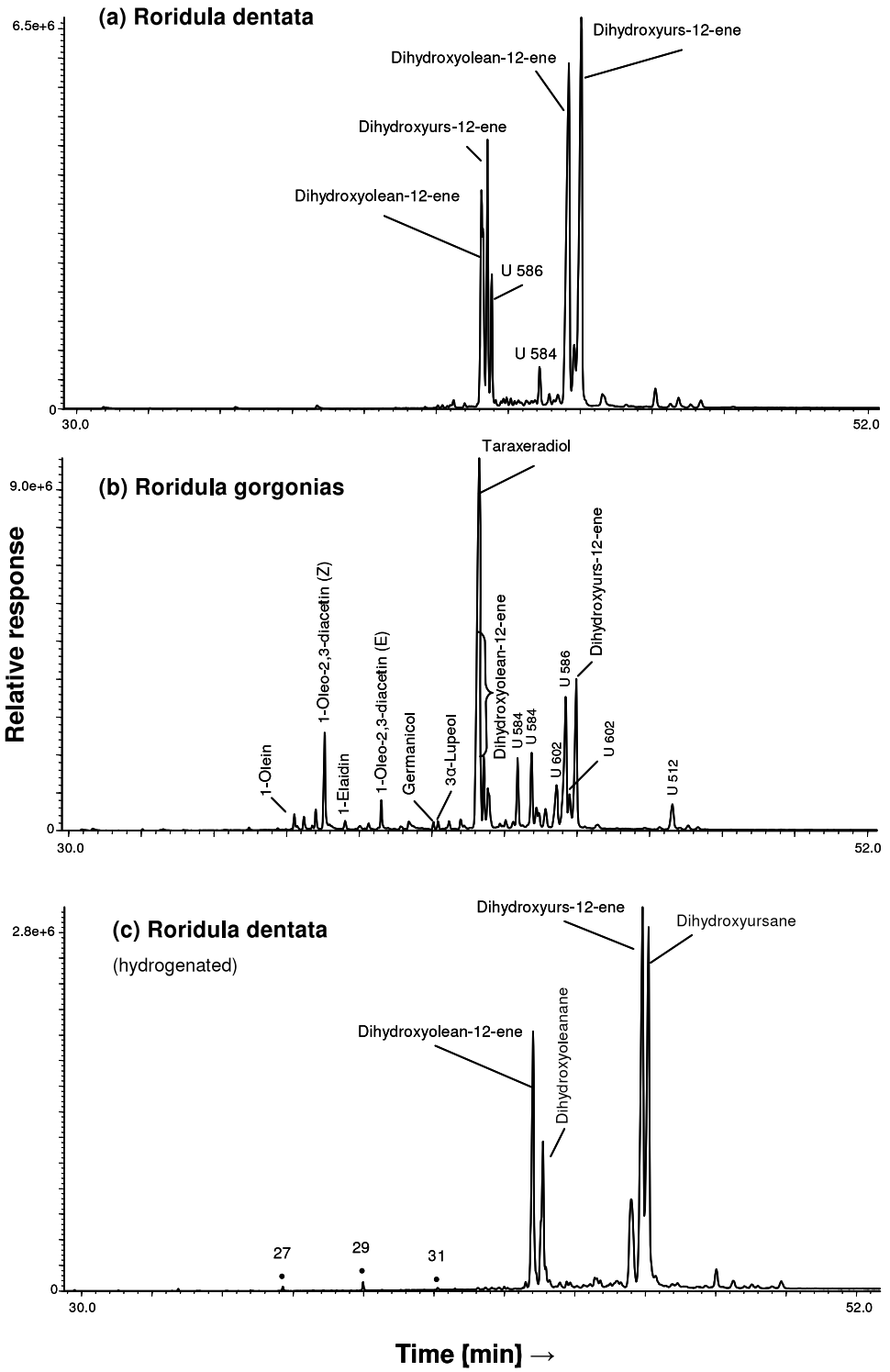

Fig. 1. GC-MS total ion current (TIC) traces for total extracts from the Roridulaceae: (a) *Roridula dentata*, silylated; (b) *R. gorgonias*, silylated; (c) *R. dentata*, hydrogenated and silylated. U *i*, unknown with molecular weight *i*; dots designate *n*-alkanes.

Table I. Ratio percent of major compounds found in extracts of *Roridula* species.

Compound	Empirical formula	M_r	*R. dentata*	*R. gorgonias*
Taraxeradiol (olean-18-en-2,3-diol)	$C_{30}H_{50}O_2$	442		100
Dihydroxyolean-12-ene	$C_{30}H_{50}O_2$	442	55	10
Dihydroxyurs-12-ene	$C_{30}H_{50}O_2$	442	100	30
Germanicol + lupeol	$C_{30}H_{50}O$	426		2
Acyl glycerides				20
Unknown triterpenols			25	50

retro-Diels-Alder rearrangement with charge retention and subsequent loss of trimethylsilanol.

Based on the prevalence of amyrins in nature we assign one of the hydroxy groups as 3β, the same as in the amyrins. The position of the second hydroxy group may be on ring A or B as discussed next. In all samples there are two peaks for each α- and β-amyrin derivative separated by about 2 min in elution time (cf. Fig. 1a), and their mass spectra are virtually identical. This indicates that the position of the second hydroxy group is probably at C-2 for the isomers eluting first (I and II, structures of all compounds cited are shown in Fig. 3) and at C-1 or possibly at C-6 or C-7 for the isomers eluting second (III, IV). This C-1, C-6 or C-7 position is the preferred assignment rather than the epimer at C-3. The location of the second hydroxy group at C-23 or C-24 is excluded because no cyclo-TMS derivatives were detected. The cyclo-TMS derivative forms with standard compounds such as aescigenin (V) or 3α-hydroxy-*ent*-kaur-16-en-19-oic acid (VI). We are still searching for appropriate standards or published data to aid this structural identification.

The mass spectrum of the dominant compound in the *Roridula gorgonias* extract is shown in Fig. 2c. The molecular ion at *m/z* 586 also indicates a triterpenediol-diTMS, with successive losses of trimethylsilanol to *m/z* 496 and 406. The base peak at *m/z* 204 with the ions at *m/z* 177 and 189 are characteristic for rings C and E of taraxerene or possibly olean-18-ene, *i.e.*, there is no functional group on rings C and E. Analogous to the previous diols, the ion at *m/z* 278 ($C_{17}H_{28}OSi$) is the ring A and B part of the molecule with charge retention and subsequent loss of TMSOH. Thus, the structural assignment is 2,3β-taraxeradiol (VII), and the stereochemistry of the 2-hydroxy group may be α or β.

The mass spectra of the hydrogenated natural products confirm the presence of the C-12 double bond in the dihydroxy-oleanenes/ursenes (Figs. 2d and e). The molecular ion is at *m/z* 588 ($C_{36}H_{68}O_2Si_2$) with fragments indicating the double losses of TMSOH at *m/z* 498 and 408. Thus, the preferred structure assignment is as indicated by VIII and IX (Fig. 3).

Extract compositions

The *R. dentata* extract (Fig. 1a) consists of mainly two isomers each of dihydroxyolean-12-ene and dihydroxyurs-12-ene, with minor amounts of unknown triterpenediols (U 586, U 584) and a 3,4-*seco*-triterpenoid ($C_{30}H_{50}O_2$, U 602). No saccharides or acyl glycerols are detectable.

The *R. gorgonias* extracts and fractions have a slightly more complex composition, with taraxeradiol as the major component and lesser amounts of the two isomers dihydroxyolean-12-ene and dihydroxyurs-12-ene (Table I). The total extract and fractions 8–10 contain significant concentrations of mono- and diacylglycerides, minor amounts of the same unknown triterpenediols (U 586, U 584 and U 602) as described above, with traces of germanicol and 3α-lupeol (Fig. 1b). No saccharides are detectable.

The compositions of both sticky gland products count for mainly free dihydroxytriterpenoids. Individually, the pure natural products should be crystalline solids, but as a mixture they are a sticky syrup, somewhat analogous to freshly bled conifer resin which is a solution of diterpenoid resin acids (also crystalline solids individually) in mono- and sesquiterpenes. Although some structural aspects of the reported triterpenoids still need confirmation, we deem the present result an important step in understanding the extreme stickiness of the *Roridula* trapping glue. It also underlines the segregation of the Roridulaceae from the Droseraceae, in which *Roridula* was formerly included.

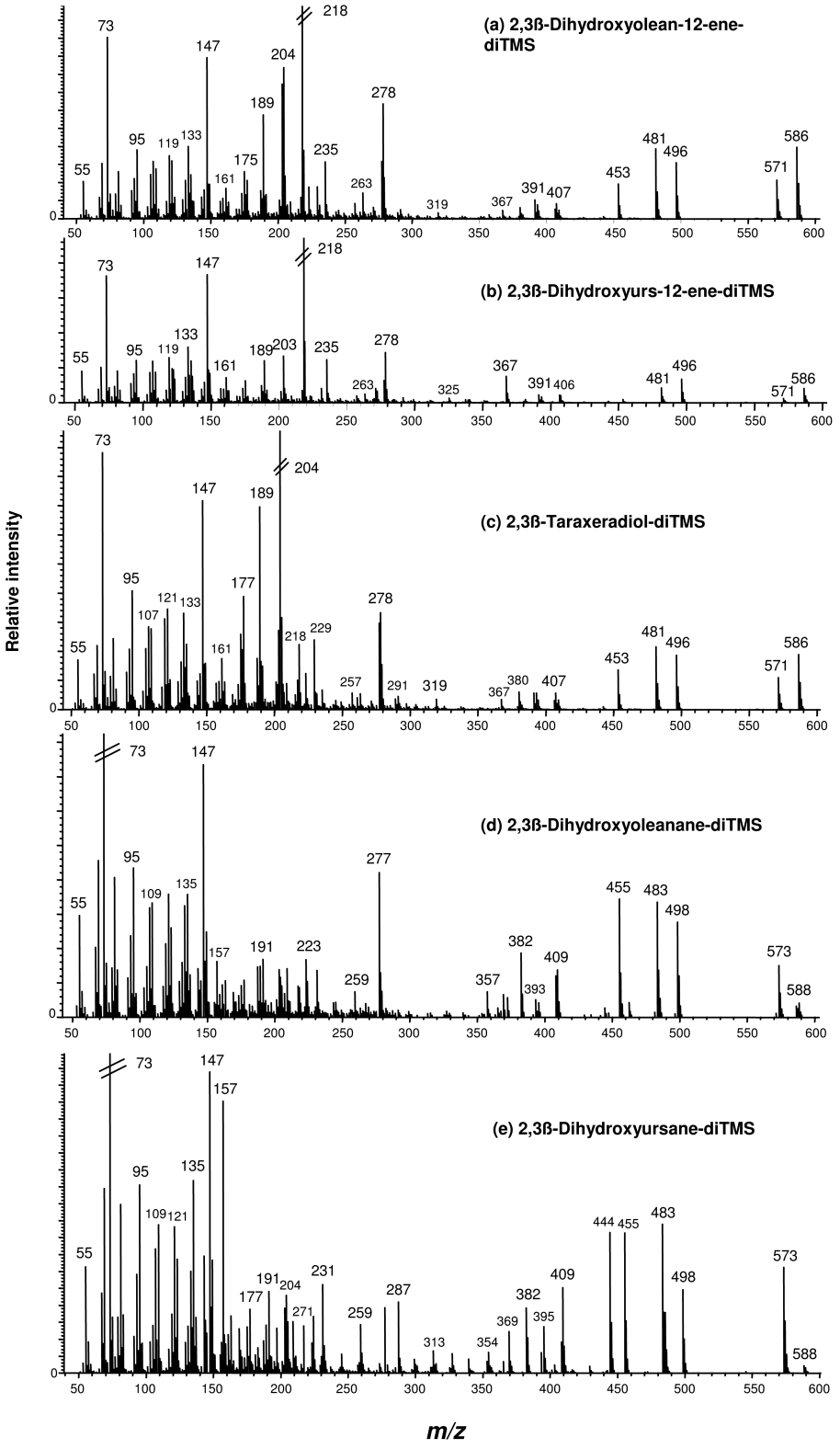

Fig. 2. Mass spectra of the major compounds in the extracts as trimethylsilyl derivatives: (a) 2,3β-Dihydroxyolean-12-ene-diTMS (I); (b) 2,3β-dihydroxyurs-12-ene-diTMS (II); (c) 2,3β-taraxeradiol-diTMS (VII); (d) 2,3β-dihydroxyoleanane-diTMS (VIII); (e) 2,3β-dihydroxyursane-diTMS (IX).

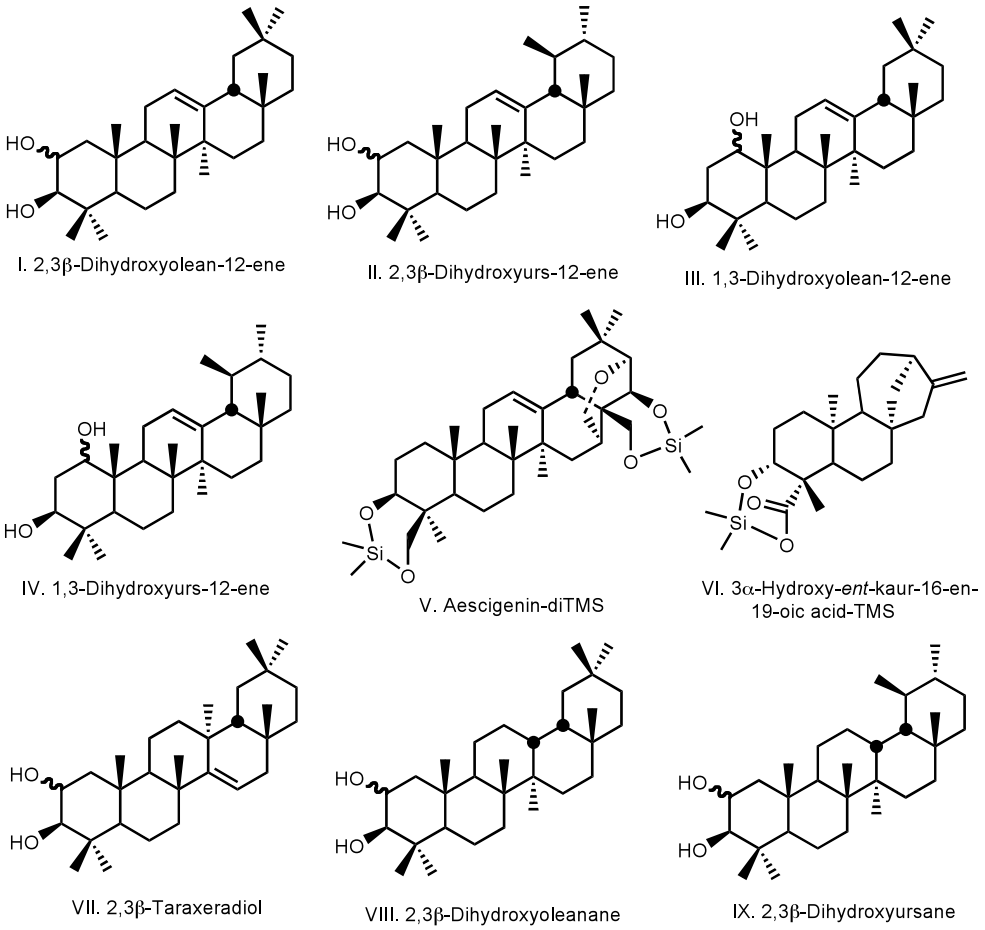

Fig. 3. Chemical structures of the compounds cited.

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