Glaucarubolone Glucoside, a Potential Fungicidal Agent for the Control of Grape Downy Mildew

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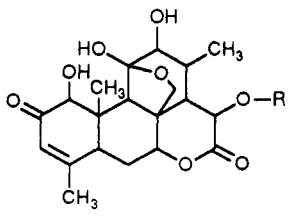
A methanol extract of *Castela emoryi* was active as both a preventive and a curative agent against grape downy mildew infestation. 15-Glucopyranosylglaucarubolone (1) was identified as the active constituent. In the same assay holacanthone (2) was found to be phytotoxic and glaucarubolone (3) was inactive.

In recent years quassinoids have received increased attention as potential insecticidal and antifeeding agents (Polonsky et al., 1989; Lidert et al., 1987). As a result of our ongoing search for potential agrichemicals from natural sources, we screened a methanol extract of *Castela emoryi* (Gray) Moran & Felger (Simaroubeaceae) for curative and preventive activity against grape downy mildew (*Plasmopara viticola*). Since the crude extract of this plant demonstrated 100% control of this pathogen at 500 ppm, a bioassay-directed fractionation was undertaken to determine the source of the activity. This resulted in the isolation of 15-glucopyranosylglaucarubolone (1) as the active moiety, which was identified by comparison of its spectral data with the previously published data (Bhatnagar et al., 1984).



	R
1	β-D-glucose
2	OAc
3	H

MATERIALS AND METHODS

Plant Material. The above-ground parts (primarily stems and spines) of *C. emoryi* were collected from three different populations: southwest of Casa Grande and southeast of Maricopa, both in Pinal Co., Arizona, and southeast of Ocotillo in Imperial Co., California. Voucher specimens (SPM 4454, 5513) are deposited in the herbarium at the University of Arizona. After all of the samples tested positive, they were pooled and worked up as a single sample.

Extraction and Preliminary Fractionation. The air-dried plant material was ground to 3 mm particle size in a hammer mill and stored at 5 °C prior to extraction. The milled plant matter (39.2 kg) was extracted with dichloromethane in a 85-L Lloyd type extracter followed by methanol. The volume of the methanol extract was reduced under pressure to 10 L; with constant stirring 20 L of acetone was added, and the resultant precipitate (1.32 kg) was removed by filtration. The mother liquor (~1.74 kg) was absorbed directly onto 3.5 kg of silica gel 60 (E. M. Merck Co.), divided into two equal portions, and placed onto two silica gel 60 columns (4 kg, 10 cm × 150 cm). Each column was eluted with dichloromethane (10 L) followed by dichloromethane/methanol (98:2, 5 L; 95:5, 10 L; 9:1, 15 L; 85:15, 40 L; 75:25, 10 L; 50:50, 5 L) and then washed with methanol (8 L). Fractions of 2 L were collected and combined on the basis of their TLC pictures (chloroform/methanol/water 65:25:10, lower phase). This resulted in the collection of 51 fractions that were combined to yield 9 mixtures (A–I). The activity was concentrated in mixture E (620 g, fractions 20–30).

Isolation of 15-Glucopyranosylglaucarubolone (1). A 1-g aliquot of fraction E was subjected to medium-pressure liquid chromatography (MPLC, Buchi 632a system, RP-18, 25 μm, 5 mm × 49 cm, methanol/water 3:97, with UV detector set at 254 nm). Fractions were collected on the basis of their chromatographic profile. The eight resultant fractions were tested with activity concentrated in fraction 3 (8 mg). To obtain more material, a 10-g aliquot of fraction E was subjected to flash chromatography (5 × 45 cm, SiO_2 60, 40 μm, chloroform/methanol/water 65:25:10, lower phase). These eight new fractions were tested with activity concentrated in fraction 3a (600 mg). To complete the testing, an additional aliquot of fraction E (50 g) was subjected to flash column chromatography (10 × 75 cm, SiO_2 60, 63–200 μm, chloroform/methanol/water 65:25:10, lower phase). Similar fractions were combined and subjected to bioassay. According to HPLC and bioassay, the activity was concentrated in fractions 14 and 15 (22.5 g), which were combined with fraction 3a. This material was subjected to MPLC (5 × 46 cm, silica gel 60, 40 μm, ethyl acetate/methanol/acetic acid 85:13:2). On the basis of the UV chromatographic profile, the active fraction was concentrated into a 4.4-g mixture, which was resubjected to a second run (chloroform/methanol/water 65:25:10, lower phase), yielding a 1.1-g mixture followed by a third run that yielded three relatively pure samples, which when subjected to crystallization from methanol yielded 140, 260, and 62 mg, respectively. All three sets gave identical NMR spectra. 1 was identified by comparison of its CMR, PMR, and FAB-MS data with the previously reported data (Bhatnagar et al., 1984). The crystals obtained melted at 231 °C.

Isolation of Holacanthone (2). A 1-g aliquot of fraction B was decolorized with activated charcoal and subjected to crystallization from methanol to yield betulin in the first crop followed by crude 2 in the second crop. The latter crystals were recrystallized from acetone followed by methanol to yield pure platelets of 2. Both compounds were identified by comparison with authentic samples.

Glaucarubolone (3). A 100-mg sample of 2 was deacetylated in the usual manner with $KHCO_3$ in MeOH to yield 3 after workup.

Bioassay. Seeds obtained from *Vitis vinifera* cv. Chardonnay were used to produce grape plants grown at 27 °C, 70% relative humidity (RH), 16-h photoperiod for 3 weeks (three or four true leaves). Sporangia of *P. viticola* (local isolate) were obtained by brushing from infected plants after incubation for 24 h at 20 °C, 100% RH. Sporangia were brushed into cold (4 °C) deionized water, and the titer was adjusted to 20 000 sporangia/mL. In the curative bioassay grape seedlings were

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inoculated 24 h before the extracts were applied, and in the preventive bioassay inoculation was 24 h after extract application. In both assays a Preval atomizer (Precision Valve Corp., Yonkers, NY) was used to apply approximately 2 mL of sporangial suspension per plant to the under surfaces of leaves. Plants were then immediately placed at 20 °C, 100% RH, for 24 h. Plants were then incubated for 5–7 days at 20 °C, 70% RH, and then placed at 20 °C, 100% RH, for 12 h to induce sporulation, and the percentage of total leaf area covered by sporulation was assessed. Extracts and other experimental compounds were applied to runoff using a belt drive sprayer with three nozzles directed at upper and lower leaf surfaces. Extracts were formulated in deionized water containing Trem 14 (Henkel Corp., Ambler, PA) surfactant (9 drops/L). Extracts with low water solubility were first dissolved in methanol which was then diluted with water to 10% or less of the final solution.

RESULTS AND DISCUSSION

Holacanthone (2), the major quassinoid found in this plant, was inactive in this assay with a pronounced phytotoxic activity at 100 ppm. The fungicidal activity appeared to be concentrated in the more polar materials. These latter fractions were also devoid of phytotoxic activity. Repetitive flash and medium-pressure column chromatography resulted in the isolation and identification of 15-glucopyranosylglaucarubolone (1) as the sole active moiety.

When compared to the commercial agent, curzate, 1 was as active or more active in both the curative and preventive assays for grape downy mildew. The final activity level was consistently between 10 and 20 ppm. Since 2 was inactive, the effect of removing the glucose molecule from 1 was evaluated by testing deacetylholacanthone or glaucarubolone (3). This latter compound was also found to be inactive in this assay at up to 500 ppm.

To date, the insecticidal activity of the quassinoids has for the most part paralleled their antitumor activity. Also, the more nonpolar compounds have usually been found to demonstrate better activity levels than their more polar counterparts. Thus, in an assay for tobacco budworm antifeedant activity, 1 was almost devoid of activity, with 3 displaying a slightly higher and longer duration of action (Lidert et al., 1987).

These results indicate that the more polar, especially glycosidic, quassinoids might have potential applications as fungicides in the area of general plant health. However, in the screen conducted during this study, the only activity detected was against grape downy mildew. This suggests that the glycoside may play an important function in the transport of the toxin to the fungus and in its selective toxicity.

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