A Novel Hydrolyzable Tannin and Related Compounds Isolated from the Leaf Surface of Chrysolepis sempervirens

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A NOVEL HYDROLYZABLE TANNIN AND RELATED COMPOUNDS ISOLATED FROM THE LEAF SURFACE OF *CHRYSOLEPIS SEMPERVIRENS*

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ABSTRACT.—A novel hydrolyzable tannin, chinquapinic acid [**3**], was isolated from the MeOH extract of the epidermal powder found on the leaf underside of *Chrysolepis sempervirens* in addition to two known tannins, vescalagin [**1**] and castalagin [**2**]. The structure of chinquapinic acid was established through spectroscopic data and auto-conversion to vescalagin. Scanning electron microscopy showed the yellowish powder to be crystal-like in appearance. These tannins are first examples for this class of compounds found as exudates on a plant surface.

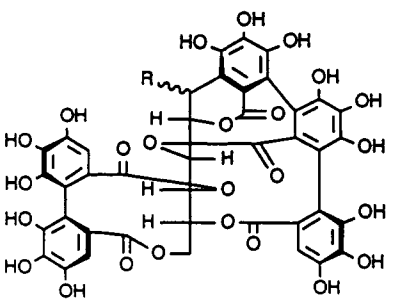
Chrysolepis sempervirens (Kellogg) Hjelmq. formerly *Castenopsis sempervirens* (Fagaceae), commonly referred to as bush chinquapin, is an evergreen shrub growing on rocky slopes of the Sierra Neveda and in central Oregon. We found the leaf underside of *Chr. sempervirens* densely covered with a gold-colored powder that was easily scraped off. This material was previously believed to be composed of felt-like hairs (1). However, scanning electron microscopy showed the leaves to be covered with crystal-like powders which dissolved in MeOH. This indicated that the powder consists of organic chemicals. Our interest was to discover what chemicals are exuded and what role they play in plants.

Many studies have been reported that plants secrete chemicals such as sugars, enzymes, and waxes, and that chemicals exuded onto the plant surface may act as defense substances against attack from insects (2) or microorganisms (3). We now report the isolation, structure determination, and biological activity of a novel hydrolyzable tannin and two known related tannins from *Chr. sempervirens*.

RESULTS AND DISCUSSION

The MeOH-soluble epidermal powder (2.0% yield, w/w, from the air-dried leaves of *Chr. sempervirens*) was scraped off from the leaf underside (Figure 1). The extract was purified on an hplc with an ods column to yield three major compounds: **1** (55.2% from the MeOH extract), **2** (18.0%), and **3** (6.8%). These three compounds in the samples collected in California and Oregon were distributed in a similar ratio.

All three compounds had similar ^{13}C-



1 R=OH
2 R=OH

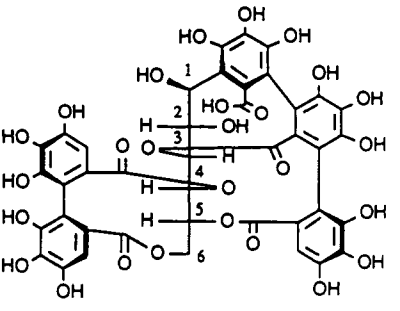


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FIGURE 1. The leaf underside surface of *Chrysolepis sempervirens*. Scanning electron microscope, magnification ×750.

nmr spectra of 41 carbon signals including six C-O, 30 aromatic carbons, and five carboxylic ester or acid carbonyl carbons in the range of δ 64 through 78, 105 through 149, and 166 through 171 ppm, respectively. In the similar ^{1}H-nmr spectra (Table 1), there were three characteristic singlet aromatic proton signals and signals corresponding to a glycosyl moiety which were assigned based on the ^{1}H-^{1}H and ^{1}H-^{13}C COSY experiments. The data indicated that these compounds are *C*-glycosidic ellagitannins. Compounds **1** and **2** were identified to known hydrolyzable tannins, vescalagin and castalagin (4), respectively, by direct comparison

TABLE 1. ^{1}H-nmr Data of Compounds **1–3**.[a]

Proton	Compound		
	1	**2**	**3**
Glucose			
H-1	4.88 bs	5.70 d (4.5)	5.35 d (1.7)
H-2	5.26 bs	5.02 m	4.54 bd (1.7)
H-3	4.54 d (6.8)	5.09 dd (7.5, 2.1)	4.48 dd (7.3, 1.3)
H-4	5.18[b] (6.8, 7.3)	5.24 t (7.5)	5.16 t (7.3)
H-5	5.61 bd (7.3)	5.61 bd (7.5)	5.60 dd (7.3, 2.1)
H-6	4.03 d (12.8)	5.04 m	4.00 d (12.8)
H-6	5.03 d (12.8)	4.01 d (12.1)	4.95 dd (12.8, 2.1)
Aromatics			
	6.62 s	6.63 s	6.57 s
	6.72 s	6.78 s	6.63 s
	6.83 s	6.92 s	6.78 s

[a]At 500 MHz, δ in ppm from TMS, *J* (in parentheses) in Hz, data in CD_3OD for **3**, CD_3COCD_3-D_2O (9:1) for **1** and **2**.

[b]This signal resembles a triplet, but is not symmetrical.

with the authentic samples. Recently, the stereochemistry of **1** and **2** was revised (5).

In contrast, the negative fabms for **3**, *m/z* 951 corresponding to a molecular formula of $C_{41}H_{28}O_{27}$, was considered *m/z* 933 ($C_{41}H_{26}O_{26}$ for **1** or **2**)+H_2O. This observation was supported by a high field shift of the signal (H-2, δ 4.54) adjacent to the benzylic signal (H-1, δ 5.35) in the ^{1}H-nmr spectra of **3**. The corresponding signals (H-2) in **1** and **2** were δ 5.26 and δ 5.02, respectively. The coupling patterns of the glycosyl moiety of **3** were close to those of vescalagin [**1**], and H-1 had a small vicinal coupling constant, $W_{1/2}$ <3.5 Hz, to H-2 as well as the coupling constant in vescalagin [**1**] ($W_{1/2}$ <2 Hz). On the other hand, the coupling constant of H-1 to H-2 was 4.5 Hz in castalagin [**2**]. Hence, the hydroxy groups in C-1 and C-2 were assigned to a threo form and the stereochemistry of C-1 was the same as in **1**.

Although the structure of chinquapinic acid was supposed as **3**, some ambiguity concerning the position of the carboxyl group remained. This ambiguity was solved by the spontaneous and quantitative intramolecular lactonization of chinquapinic acid [**3**] to vescalagin [**1**] during storage at ambient conditions.

The fab mass spectra of the freshly collected powder (which was not treated with any solvent prior to the ms experiment) had two characteristic peaks of *m/z* 933 due to **1** or **2** and *m/z* 951 due to **3**, but no peak over *m/z* 951 corresponding to a salt of **3**. On the other hand, the mass spectra of the powder on the leaves stored over one year gave only *m/z* 933 and no peak at *m/z* 951. This indicated that newly biosynthesized chinquapinic acid [**3**] was gradually decomposing to vescalagin [**1**]. Vescalagin [**1**] and castalagin [**2**] have been found to be widely distributed in plants (5), but neither has been reported from *Cas. sempervirens*. In addition, all three tannins appear to be the first plant exudate hydrolyzable tannins isolated from a plant leaf surface, and chinquapinic acid [**3**] is the first example of an ellagitannin having a free carboxylic acid and a free hydroxy group in its glycosyl moiety.

Vescalagin [**1**], castalagin [**2**], and chinquapinic acid [**3**] did not inhibit *Staphylococcus aureus, Candida albicans,* and *Pseudomonas aeruginosa* at a concentration of 1000 μg/ml. Although no antimicrobial activity was observed, it is still assumed that these tannins play a significant role against attack by microorganisms because of their high concentration on the leaf surface. However, these compounds were moderately cytotoxic against HeLa cells, 70–90% growth inhibition at a concentration of 100 μg/ml.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—The scanning electron microscope photo was taken by JEOL, JSM T-200 Scanning Electron Microscope. Ir spectra were acquired on a Perkin-Elmer model 1310 IR Sprectrometer. Nmr spectra were recorded on a JEOL GSX-500 spectrometer (500 MHz for 1H, 125 MHz for ^{13}C). Fabms data were obtained on a JEOL JMS-DX303HF system. Hplc was performed on a JAI LC-908 (Japan Analytical Industry, Tokyo, Japan) with an ods column, JAIGEL ODS GI-15 (⌀ 2.5 cm×125 cm). All solvents used for hplc were hplc grade. The H_2O used for hplc was distilled and then deionized.

PLANT MATERIAL.—The leaves were collected in the mountain forest of the Bend, Oregon region and the Sierra Nevada, California, and then transported for extraction without any treatment. A voucher specimen is deposited at the University of California Berkeley Herbarium.

BIOASSAY.—All microorganisms used for bioassay were purchased from American Type Culture Collection (Rockville, Maryland). The following bacteria were used; *S. aureus* ATCC 6538, *P. aeruginosa* ATCC 25619, and *C. albicans* ATCC 18804. The activities were determined using the macro dilution broth culture method. The samples were dissolved in DMF. HeLa, ATCC CCL2, cells were also purchased from ATCC. The procedure for the cytotoxicity assay followed the literature (6).

EXTRACTION AND ISOLATION.—The underside of the freshly collected plant leaves (186.42 g) were scraped and yielded 3.82 g of the yellow powder. The powder was dissolved in MeOH and filtered through a membrane filter (Waters SEP

PAK, Millipore, Milford, MA), and the filtrate was evaporated to yield 2.74 g of a viscous oil. The oil was chromatographed on an ods column with H_2O-MeOH (9:1) and flow rate 3 ml/min to yield 1, 2, and 3.

Chinquapinic acid [3].—Colorless amorphous: $[\alpha]_D$ +68.3° (c=0.75, MeOH); ir ν (KBr) cm^{-1} 3100–2800, 1735 1605; ^1H nmr (δ ppm from TMS in CD_3OD and J in Hz) see Table 1; ^{13}C nmr (δ ppm from TMS in CD_3OD) 75.80 (C-1), 73.91 (C-2), 68.91 (C-3), 70.43 (C-4), 71.77 (C-5), 66.09 (C-6), 108.03, 108.75, 109.46, 114.10, 115.15, 115.23, 115.54, 115.64, 116.84, 117.02, 124.99, 125.34, 125.42, 126.97, 128.12, 136.18, 137.04, 137.64, 138.22, 138.65, 144.69, 144.98 (×2C), 145.21, 145.27, 146.12 (×3C), 146.22, 148.83, 165.97, 166.46, 167.48, 167.97, 170.34.

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