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CONIFERIN—AN ASTRINGENT GLYCOSIDE IN CITRUS

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Key Word Index – *Citrus sinensis*; *C. paradisi*; *C. paradisi* × *C. reticulata*; Rutaceae; coniferin; astringent glycoside.

Abstract – From the peel of *C. sinensis* cv. Valencia, *C. paradisi* cv. Duncan and *C. paradisi* × *C. reticulata* cv. Murcott, coniferin, an astringent glycoside, was identified. This compound was found to be present in concentrated Valencia juice, Murcott concentrate and orange pulp wash concentrates.

INTRODUCTION

Because some citrus juices are excessively bitter or astringent we have been trying to isolate and identify the compounds responsible for these undesirable traits. Limonin and naringin are known bitterness factors found in citrus juices but there are probably other components contributing to the bitterness/astringency of juices [1, 2]. During these studies we isolated and now report the identification of coniferin, an astringent glycoside, from three varieties of citrus; Valencia, Duncan, Murcott, that are used in producing citrus juices.

Coniferin was first isolated from conifers (*Larix* and *Abies*) [3], and is present in comfrey root, sugar beet and asparagus [4]. Coniferin is widely distributed among conifers and other plants and was once used in cough syrups. The whole plant of *Balanophora polyandra* Griff. was claimed by some Thai doctors as antiasthmatic and was found to contain 0.72% coniferin [5].

RESULTS AND DISCUSSION

The methanol extract of *C. paradisi* × *C. reticulata* cv. Murcott citrus peel yielded naringenin 7-β-rutinoside. This compound was isolated in crystalline form and when tasted it was astringent. The rutinoside was first isolated from *C. sinensis* by Horowitz and Gentili who stated that it is tasteless [6]. Nishiura *et al.* isolated this rutinoside in 1971 and described it as bitter [7]. Thin-layer chromatography of our sample in various solvent systems finally showed a trace impurity which was collected and was found to be astringent. When the rutinoside was pure it was found to be tasteless, as stated by Horowitz and Gentili [6]. The impurity was coniferin [4-(3-hydroxy-1-propenyl)-2-methoxyphenyl-D-glucopyranoside]. The identity of this compound was based on comparison of spectral data published by Falshaw *et al.* [8]. Their NMR spectrum was of the pentaacetate, while ours was of coniferin itself. They obtained a parent peak at *m/z* 342 for coniferin in the mass spectrum while we did not. Our EI mass spectrum of coniferin showed a cleavage with hydrogen transfer to give *m/z* 180 $(C_{10}H_{12}O_3)^+$ for the aglycone while CI gave an *m/z* 163 $(C_{10}H_{10}O_3{}^+1)$ for glucose, and no ion for the aglycone.

Taste tests in water that had been adjusted to pH 3.25 with critric acid showed coniferin could be detected at 100 ppm [9]. HPLC analyses on seven samples of concentrated organge pulp wash that had been reconstituted to 12° Brix showed that they contained from 3.7 to 11.5 ppm coniferin. A sample of Valencia orange juice contained 7.2 ppm while a Murcott tangelo juice had 4.6 ppm coniferin. These values are well below the taste threshold for coniferin, but this compound may have an additive effect with other components found in citrus juices.

Many people confuse bitterness, sourness and astringency when tasting citrus juices. The °Brix/acid ratio of a juice affects the perception of bitterness [1, 2] and probably also affects the astringency of juices. 'Feeling, palpable or tactile sensations in the mouth, includes the sting, bite, piquancy, poignancy, or authority of a strong spice, such as mustard or pepper, the astringency of alum or of an unripe persimmon' [10].

EXPERIMENTAL

TLC solvent systems. (A) C_6H_6 nitromethane–MeOH (3:5:2); (B) C_6H_6–nitromethane MeOH–H_2O (6:10:4:0.4); (C) $CHCl_3$–MeOH (4:1); (D) C_6H_6 HOAc–H_2O–nitromethane (34:32:5:18), TLC plates were 250 m silica Gel Gf.

Column chromatography. (A) Silica gel (100–200 mesh type 60A Silicar) 5 × 20 cm. The silica was deactivated with HOAc and H_2O, washed with Me_2CO and then $CHCl_3$. The sample to be chromatographed was dissolved in MeOH and added to 1.5 × 5.0 cm silica gel, the mixture taken to dryness on a rotary evaporator, and transferred to the column. The column was eluted with $CHCl_3$, $CHCl_3$ MeOH 10 and 15%, then stripped with MeOH. In each case 125 ml fractions were collected and chekced by TLC. (B) Amberlite XAD-7 (Rohm & Haas, Philadelphia, PA) 4.5 × 35 cm. The sample was loaded in H_2O, washed with one column volume of H_2O and eluted with MeOH.

Samples. Citrus peel flavedo plus albedo in 5 kg batches was cut up and ground in a Waring blendor. The peel was placed in 2-l. beakers and covered with 50% MeOH in H_2O and warmed to 50°C. The solvent was decanted and the peel extracted again. The combined extracts were concentrated to a heavy syrup, taken up in H_2O and passed through column B to remove sugars and citric acid. The MeOH eluate from B was taken to dryness, the residue

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taken up in a minimum volume of MeOH and 10 vols of isopropanol were added to cause precipitation. The ppt was removed by filtration and the procedure repeated on the ppt. The filtrates were taken to dryness and then taken up in MeOH. These extracts were separated on column A and the fractions containing coniferin were separated by TLC using solvents B and C.

HPLC analyses were carried out using a Waters model 6000A pump, U6K injector with a 24 ml loading loop, a model 440 absorbance detector equipped with a 254 nm filter and Waters data module A Brownlee (5 μm C-18 packing) 10 cm column equipped with a 5 μm C-18 guard column were used. The eluting solvent was 11% acetonitrile in water at a flow rate of 0.5 ml/min. Reproducibility was 15.0%.

Sample preparation. All juice samples were prepared for HPLC by centrifuging 20 ml samples 54 K × g for 30 min and clear serum was obtained. The serum for analysis was passed through a 0.45 m filter. The pulp sample was extracted × 3 with 20 ml portions of MeOH, conc. and transferred to a 10 ml volumetric flask for HPLC analyses. About 15% of the coniferin was found in the centrifuged pulp.

Taste panel. In taste panels conducted by paired comparison, the 12-member experienced panel was asked to taste the control on the left side of the tray and then taste the sample on the right which was a random control or experimental. They were asked if the sample was the same or more astringent. Each taster was presented two pairs of samples. Taste tests in water that had been adjusted to pH 3.25 with citric acid showed coniferin could be detected at 100 ppm at the 95% confidence level [9].

^{1}H NMR 270 MHz, ^{13}C NMR 67.9 MHz DEPT sequence, TMS as int. standard in CD_3OD and MS were obtained through the Chemistry Department of Florida State University. Mps are uncorr.

Coniferin. MS m/z 180 [aglycone]$^+$; MS (CI isobutane) m/z 163 [glucose + 1]$^+$. Crystals from MeOH, 184–185° reported 186° [2]; ^{1}H NMR: (aglycone) 3.86 (3H, s, MeO-10), 4.20 (2H, *dd*, J = 6, 1.5, H-9), 6.26 (1H, *dt*, J = 16, 6, H-8), 6.54 (1H, *d*, J = 16, H-7), 7.07 (1H, *d*, J = 2, H-3), 6.93 (1H, *dd*, J = 8, 2, H-5), 7.10 (1H, *d*, J = 8, H-6), (glucose) 3.34–3.53 (4H-m), 3.67 (1H-m), 3.86 (1H-m), 4.87 (1H, *d*, J = 7.5, H-1); ^{13}C NMR (aglycone) 150.95 (c-1), 147.65 (c-2), 111.53 (CH-3), 133.75 (c-4), 120.75 (CH-5), 118.07 (CH-6), 131.28 (CH-7), 128.95 (CH-8), 63.71 (CH_2-9), 56.77 (CH_3-10), (glucose) 102.83 (CH-1), 74.93 (CH-2), 77.87 (CH-3), 71.38 (CH-4), 78.23 (CH-5), 62.55 (CH_2-6); UV λ_{max}^{MeOH} 259, 290 sh.

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