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6,7-DIMETHOXYCOUMARIN IN THE PEELS OF *CITRUS*

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Key Word Index—*Citrus*; Rutaceae; fruit peels; 6,7-dimethoxycoumarin; chemotaxonomy.

Abstract—6,7-Dimethoxycoumarin was isolated and identified from the peels of *Citrus sinensis* cv. Pineapple orange, of *C. paradisi* × *C. paradisi* × *C. reticulata* cv. Wekiwa and of *C. mitis* cv. Clamondin. The coumarin was tentatively identified in two other sweet oranges, *C. sinensis* cv. Mediterranean Sweet and Waite Parson Brown, and in *C. aurantifolia* cv. West Indian lime and in *C. aurantium* cv. Bitter Sweet orange.

INTRODUCTION

Until recently, 6,7-dimethoxycoumarin had only been reported once in citrus when Riov [1] isolated it from the peel of gamma-irradiated mature grapefruit, but not from non-irradiated fruit. In a review, Kefford and Chandler [2] stated that five other coumarins had been isolated from sweet orange. Their references do not show unequivocal proof that coumarins are present in *C. sinensis*. The isolation of a coumarin from *C. sinensis* or other citrus would show that the allele for the production of coumarins was present.

Such information would aid in chemotaxonomy, help separate nucellar and zygotic seedlings [3,4] and provide more background information on citrus for the production of new hybrids.

RESULTS AND DISCUSSION

6,7-Dimethoxycoumarin was found in the peels of several different sweet orange varieties and other citrus species. These included peels of fruit from seven different Pineapple trees, one Mediterranean Sweet and one Parson Brown tree *C. sinensis* Osbeck, one Wekiwa tree *C. paradisi* × *C. reticulata* × *C. paradisi*, one Calamondin tree (*C. mitis* Blanko), one Bitter Sweet orange tree (*C. aurantium* L.) and one West Indian lime tree (*C. aurantifolia* Christm.). The compound was first observed in the peel and juice from Pineapple oranges on December 4, 1975. 6,7-Dimethoxycoumarin was not always detected when the fruit was picked. A portion of the peel was removed and examined by TLC. If the coumarin was not visible, the fruit was placed in storage at 9° for six days, usually the coumarin was present in the stored fruit. The peels of fruit from seven different Pineapple orange trees were tested in December. Two contained 6,7-dimethoxycoumarin when the fruit was picked, the other five contained the compound after storage. One Mediterranean Sweet and one Parson Brown sample contained the coumarin after storage. Two immature Valencia samples did not contain the coumarin before or after storage. Two Hamilins were tested and the coumarin was found in one after storage. The peel of Wekiwa was tested when picked on January 29, March 4 and 12, and April 5. Each time, it contained the coumarin but in progressively lower concentration. The peels of Calamondin and West Indian lime were examined on March 4, and both contained the coumarin when picked. A possible precursor of 6,7-dimethoxycoumarin, 6-methoxy-7-hydroxycoumarin was isolated from *C. limon* by Horowitz and Gentili [5].

Forty-two orange oils were examined for the presence of 6,7-dimethoxycoumarin. There were seven control and nine experimental oils of Pineapple, eight control and ten experimental oils of Valencia and four control and four experimental oils of Hamlin. The experimental oils were prepared from fruit that had been sprayed with abscission agents [6]. 6,7-Dimethoxycoumarin was detected in all experimental oils but not in any control sample even when concentrated oil samples were examined.

This is the first report of 6,7-dimethoxycoumarin presence in five different species of citrus. We did not observe this compound each time sweet orange fruit was tested. As stated above, it was present in some fruit the day it was picked and developed in others several days after picking. The coumarin may be an intermediate since its concentration appeared to decrease as the fruit matured. Its presence in 23 experimental orange oils indicate that 6,7-dimethoxycoumarin is formed when the fruit is under senescence or stress since it was not found in the 19 control oils. Senescence occurs rapidly after fruit is sprayed with abscission agents [6].

EXPERIMENTAL

Thin-layer chromatography. Preparative plates (1 mm thick) were prepared with Si gel HF-254. Thin-layer plates (0.25 mm thick) were commercial Si gel GF type. Solvent systems were: C_6H_6–Me_2CO (4:1) (1),(19:1) (2), C_6H_6–MeOH (39:1) (3) and C_6H_6–Me_2CO–HOAc (43:5:2) (4).

Preparation of samples for TLC survey. 10 g flavedo was removed from fruit and diced. Whole peel was used (10 g) from the Calamondin and West Indian lime. The diced peel was blended 1 min in 50 ml MeOH and filtered. Ten μl of this extract was spotted on a 20 channel 0.25 mm Si gel GF plate below a 2 cm mark on the plate. To concentrate all samples at a common starting mark, the plate was placed in a tank containing Me_2CO, developed to the 2 cm mark and air dried. This procedure was repeated and the plate air dried again before developing to the

top of the plate (18 cm) with solvent (4). The R_f was 0.33 for 6,7-dimethoxycoumarin if developed one time. If the plate were dried and developed again, the R_f was 0.47. When solvent (1) was used for survey the R_f was 0.41. When whole juice was tested the fruit were lightly hand extracted. The juice was filtered through cheese cloth and then spotted (25 μl) using a 3 cm origin, air dried, spotted (25 μl) for a total of 50 μl of juice, and treated as above.

Preparation of samples for isolation. The flavedo from 6 Pineapple oranges was removed with a potato peeler, ground in a Waring Blendor and transferred to a beaker with C_6H_6. It was extracted in a counter-rotating mixer for 5 min twice with C_6H_6, then once with 0.4 l. of Me_2CO. The extracts were filtered, combined, concentrated and placed on 4 preparative plates and developed twice with C_6H_6–Me_2CO (17:3). A blue fluorescent band at R_f 0.58 was collected and eluted with solvent (1), rechromatographed with solvent (2), developed 3 times and collected, rechromatographed with solvent (3), developed 2 times and collected. 6,7-Dimethoxycoumarin recrystallized from EtOAc (×4) had mp 142–3°. For isolation of the coumarin from Calamondin, 1200 g of whole peel was used; from Wekiwa, 1200 g flavedo was used. For isolation of the coumarin from one experimental orange oil, 100 ml whole oil was stripped of (+)-limonene at 50° and 1 mm Hg. A portion of the residue was placed on 8 preparative plates and separated as above.

Synthesis of 6,7-dimethoxycoumarin. 6-Methoxy-7-hydroxycoumarin was methylated according to DeBoer and Backer [7]. The product, 6,7-dimethoxycoumarin, was isolated by preparative TLC with solvent (3). Mp 142–3°, MS: *m/e* 206 (m+), 191, 178, 163, 135. 6,7-Dimethoxycoumarin, isolated from the peels of the Pineapple orange, Calamondin and Wekiwa, was identified by comparison (mp, IR, MS, TLC) with the synthetic compound. 6,7-Dimethoxycoumarin in the other samples was identified by colour under long wave UV light and TLC in two solvents.

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NEUARTIGE CUMARIN-DERIVATIVE AUS *ETHULIA CONYZOIDES**

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Key Word Index—*Ethulia conyzoides*; Compositae; new coumarin derivatives.

—The aereal parts of *Ethulia conyzoides* contain two new coumarins which are related to those of *Erlangea* and *Bothriocline*. They are, however, condensed with a terpene. The structures have been elucidated by spectroscopic methods.

Vertreter der Gattung *Ethulia* (Tribus Vernonieae) sind bisher noch nicht eingehend untersucht worden. Die Wurzeln von *E. conyzoides* enthalten das weit verbreitete Pentainen **1** und das Entetrainen **2** [1]. Die oberirdischen Teile enthalten jedoch zwei neue Cumarin-Derivate mit den Summenformeln $C_{20}H_{20}O_5$ und $C_{20}H_{22}O_5$. Die ^{1}H-NMR-Spektren zeigen, daß wie bei *Erlangea* [2] und *Bothriocline* [3] Methylcumarine vorliegen müssen, die in 3,4-Stellung substituiert sind. Wie schon die Summenformeln deuten auch die NMR-

*16 Mitt. in der Serie Natürlich vorkommende Cumarin-Derivate; 15 Mitt. Bohlmann, F. und Zdero, C. (1977) *Phytochemistry*, in press. † Höheren und das 1'- und 2'-Signal 2⁻ tieferen Feldern verschoben.