Phytochemistry, Vol. 30, No. 12, pp. 4198 4200, 1991
Printed in Great Britain.

0031-9422/91 $3.00 + 0.00
Pergamon Press plc

A MALONIC ACID ESTER DERIVATIVE OF NARINGIN IN GRAPEFRUIT

MARK A. BERHOW, RAYMOND D. BENNETT, KATHERINE KANES, STEPHEN M. POLING and CARL E. VANDERCOOK*

U.S. Department of Agriculture, Agricultural Research Service, Fruit and Vegetable Chemistry Laboratory, Pasadena, CA 91106, U.S.A.

(*Received in revised form* 13 *May* 1991)

Key Word Index—*Citrus paradisi*; Rutaceae; grapefruit; flavonoids; naringin.

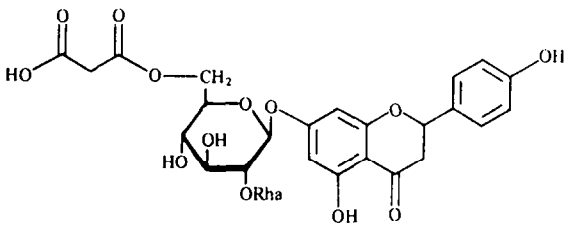
Abstract-- A malonic acid ester derivative of the flavanone naringin was abundant in the young leaves and fruits of grapefruit plants, but not in the mature leaves and fruits. After isolation, the structure of this compound was established as naringin 6″-malonate (naringenin 7-(2″-*O*-α-L-rhamnosyl)-β-D-glucoside 6″-malonate).

INTRODUCTION

Accumulation of the bitter flavanone naringin in grapefruit can adversely affect the quality of the ripe fruit and juice [1]. Developing an understanding of the plant's regulatory control of the accumulation of this compound may yield a way to artificially modulate its levels in the plant. Naringin is biosynthesized in young leaves and fruit of grapefruit during times of active cell division [2, 3]. Yet, little is known about the metabolism of naringin in grapefruit. It is thought that esterification of flavonoids with acyl groups such as malonic acid and cinnamic acid may act to mediate between different metabolic fates for the individual flavonoids [4]. Few, if any, of these flavonoid acyl derivatives have been shown to be present in citrus. We have found that young leaves and fruit of the grapefruit contain an acidic flavanone in concentrations similar to those of the better known flavanones. We describe the isolation and identification of naringin 6″-malonate (**1**) from young grapefruit leaves.

RESULTS AND DISCUSSION

HPLC analysis of the flavonoid composition at different stages during the development and maturation of the fruit and leaves of grapefruit plants showed the presence of a relatively abundant flavanone glycoside in immature leaves and fruit other than naringin, narirutin, hesperidin and neohesperidin. In fully mature leaves and fruit, however, little or none of this compound is present. The compound could be removed from a methanol extract of young grapefruit leaves by passing the extract through an amino anion exchange column. It was then eluted from the ion exchange column with a weak acid, indicating the presence of an acidic group in the structure. Spectral analysis using diode array HPLC indicated that it was a flavanone, but with a R_t several minutes longer than that of either narirutin or naringin. Purification of the compound was then achieved by a combination of DEAE-Sephacel, C_{18} reverse phase, and Sepralyte diol chromatography. The isolated compound could be hydrolysed under mild alkaline conditions to yield naringin, as identified by UV spectra, HPLC and TLC R_ts.



1

The ^{1}H NMR spectra of the compound was very similar to that of naringin, with two exceptions. The glucose H-1 signal was slightly further downfield (δ5.16) and two mutually coupled proton signals (a doublet and a doublet of doublets) were observed between δ4.0 and 4.5. In naringin the latter signals were not distinguishable, being hidden under the other sugar resonances which are found between δ3.1 and 3.8. The ^{13}C NMR spectrum was also very similar to that of naringin, but three additional resonances were observed. Two of these represented quaternary carbons, at δ166.7 and 167.6, while the third was a methylene, at δ41.3. Given the above evidence that the compound is an ester of naringin, this NMR data suggested that the acid involved was malonic acid. The point of attachment was established by examining the ^{13}C sugar resonances. These signals had been previously assigned for naringin [5], and the assignments were confirmed by a ^{1}H–^{13}C COSY spectrum of the latter. The positions of the rhamnose signals and four of the glucose signals in the ester were identical to those of naringin, but the glucose C-6 signal was shifted 2.8 ppm downfield and the glucose C-5 signal was shifted 3.6 ppm upfield. As hydrolysis of the ester to naringin establishes the rhamnose linkage at C-2 of glucose, these shifts show unequivocally that the ester is attached at the glucose 6-position. Furthermore, they are consistent with the shifts observed in the case of naringin 6″-butanoate (glucose C-6 + 2.8 and glucose C-5 − 3.1 ppm) [5]. The downfield

*Retired.

sugar resonances observed in the ^{1}H NMR spectrum of the ester can then be ascribed to the glucose H-6 protons.

Malonic esters of flavonoid glycosides are known to decarboxylate readily to acetate esters when heated in DMSO [6]. This reaction was used to confirm that the naringin ester is indeed a malonate. In the ^{13}C NMR spectrum of the product the methylene signal was replaced by a methyl, one of the carbonyl peaks disappeared, and the other moved slightly downfield. The ^{1}H NMR spectrum showed two acetate methyl peaks which together integrated for three protons. This was due to the presence of two conformers, since upon heating the sample the two peaks gradually merged and completely coalesced at 175°. Thus, the NMR data allow us to assign the structure naringenin 7-(2″-*O*-α-L-rhamnosyl)-β-D-glucoside 6″-malonate to the original compound.

The FAB mass spectrum of **1** showed peaks at *m/z* 667 $[M+H]^+$, 521 $[M-Rha+H]^+$, 273 [naringenin + H]$^+$, and 250 [glucosy 6″-malonate + H]$^+$ which is in agreement with the proposed structure and its proposed M_r of 666.

Many important plant secondary metabolites and growth regulators have been found to have acylated ester forms, including abscisic acid, the gibberellic acids, and the flavonoids. The flavonoids have many documented acyl ester forms in many different plant species, including a large number of malonyl esters [7]. Under certain physiological conditions, the various flavonoids in a plant can be in either a metabolically 'inactive' pool or metabolically 'active' pool [8]. The biological controls over this process have not been completely characterized. It is possible that naringin 6″-malonate is involved in the conversion of naringin from one of these states to the other. Enzymes for the acylation of a specific site on the glucoside have been purified from parsley [9]. Acylation has been reported to be required for the uptake of flavonoids into parsley vacuoles [10]. In grapefruit, naringin 6″-malonate may be involved in this translocation process in young leaves and fruit, while the flavonoids are being actively synthesized. After deposition in the vacuole, the malonyl group would be removed. Thus, in mature fruit where flavonoids are no longer being actively synthesized and the naringin is sequestered in the cell vacuoles, naringin 6″-malonate is not found.

EXPERIMENTAL

Plant material. Young leaves of *Citrus paradisi* [L.] MacFad. cv 'Duncan' and 'Marsh' were gathered from mature trees at the University of California Citrus Research Center and Agricultural Experimental Station in Riverside, California.

Extraction and purification. Oven-dried (65°) leaves (100 g dry wt) were ground to a powder and extracted × 3 with 200 ml MeOH. The extract was applied to a DEAE Sephacel (Pharmacia) column (200 ml) that was previously washed with 1 M HCO_2NH_4, H_2O and MeOH. After rinsing with MeOH, the column was eluted with 0.1 M Pi (pH 6). The eluate was then applied to a C_{18} 'flash column' (1.5 × 15 cm) equilibrated with H_2O. The column was then washed with H_2O and eluted with MeOH. The eluate was concd and filtered through a 0.45 μm filter prior to injection on a preparative HPLC system. The chromatographic system consisted of dual pumps, an automatic sample injector, a preparative C_{18} reverse phase column (40 × 1 cm), and a diode array detector. At a flow rate of 4 ml min^{-1} the gradient elution consisted of an initial 2 min of 80% 0.01 M H_3PO_4 and 20% MeOH followed by a linear gradient to 100% MeOH in 55 min. Frs containing the acid ester were collected, pooled, evapd to remove residual MeOH and concd on a C_{18} SepPak, which was subsequently eluted with MeOH. After evapn of the MeOH, the residue (11 mg) was dissolved in DMF (200 μl) and adsorbed in 5 portions on Sepralyte diol (120 mg) (Analytichem), the solvent being removed in vacuum after adding each portion. The adsorbant was then placed on top of a 2 ml diol column packed in EtOAc. The column was eluted with a linear gradient of EtOAc–Me_2CO–H_2O–HOAc (78:20:1:1–68:28:3:1). Frs containing the ester were combined and evapd, yielding **1** (6 mg).

Mass spectral analysis. FAB-MS were determined in the positive ion mode using a glycerol matrix and Xe as the fast atom beam (20 μA at 8 kV).

Naringin 6″-malonate (**1**) UV λ_{max}^{MeOH} nm: 282, 310 (sh), (NaOH) 283.5, 412. Base hydrolysis (pH 10, 75°, 4 hr, neutralized with H_2SO_4) yielded naringin as identified by HPLC and TLC methods outlined in [3]. UV λ_{max}^{MeOH} (after base hydrolysis) nm: 282, 310 (sh). (NaOH) 256, 283.5, 424.5, identical to the spectra of naringin. Acid hydrolysis (10% HOAc at 100°, 2 hr) of either the ester itself or the base hydrolysed ester yielded naringenin as determined by HPLC. ^{1}H NMR (270 MHz, DMSO-d_6, 35°): δ1.16 (3H, *d*, *J* = 6 Hz, H-6‴), 2.73 (1H, *d*, *J* = 17 Hz, H-3), 4.11 (1H, *dd*, *J* = 7 and 12 Hz, H-6″), 4.30 (1H, *d*, *J* = 12 Hz, H-6″), 5.10 (1H, *s*, H-1‴), 5.16 (1H, *d*, *J* = 7 Hz, H-1″), 5.50 (1H, *m*, H-2), 6.08 (1H, *s*, H-6), 6.09 (1H, *s*, H-8), 6.79 (2H, *d*, *J* = 8.5 Hz, H-3′, H-5′), 7.32 (2H, *d*, *J* = 8.5 Hz, H-2′, H-6′). ^{13}C NMR (67.8 MHz, DMSO-d_6, 35°): δ17.9 (C-6‴), 41.3 (malonate CH_2), 42.0 (C-3), 63.8 (C-6″), 68.2 (C-5‴), 69.7 (C-4″), 70.3 (C-2‴), 70.4 (C-3‴), 71.8 (C-4‴), 73.5 (C-5″), 76.0 (C-2″), 76.8 (C-3″), 78.6 (C-2), 95.1 (C-8), 96.3 (C-6), 97.2 (C-1″), 100.4 (C-1‴), 103.3 (C-10), 115.1 (C-3′, C-5′), 128.3 (C-2′, C-6′), 128.5 (C-1′), 157.7 (C-4′), 162.7 (C-9), 162.8 (C-5), 164.5 (C-7), 166.7 (malonate CO_2R), 167.6 (malonate CO_2H), 197.0 (C-4).

Naringin 6″-acetate. The above sample, in DMSO-d_6 in a NMR tube, was heated at 95°. Decarboxylation was complete after 15 min. ^{1}H NMR (270 MHz, DMSO-d_6, 35°): δ1.16 (3H, *d*, *J* = 6 Hz, H-6‴), 1.93 and 1.96 (3H, 2*s*, acetate Me conformers), 4.02 (1H, *dd*, *J* = 7 and 10.5 Hz, H-6″), 4.27 (1H, *d*, *J* = 10.5 Hz, H-6″), 5.10 (1H, *s*, H-1‴), 5.14 (1H, *d*, *J* = 7 Hz, H-1″), 5.50 (1H, *m*, H-2), 6.06 (1H, *s*, H-6), 6.11 (1H, *s*, H-8), 6.79 (2H, *d*, *J* = 8.5 Hz, H-3′, H-5′), 7.31 (2H, *d*, *J* = 8.5 Hz, H-2′, H-6′). ^{13}C NMR (67.8 MHz, DMSO-d_6, 35°): δ17.9 (C-6‴), 20.4 (acetate Me), 42.0 (C-3), 63.2 (C-6″), 68.2 (C-5‴), 69.9 (C-4″), 70.3 (C-2‴), 70.4 (C-3‴), 71.8 (C-4‴), 73.6 (C-5″), 76.0 (C-3″), 76.9 (C-2″), 78.7 (C-2), 95.2 (C-8), 96.2 (C-6), 97.3 (C-1″), 100.4 (C-1‴), 103.3 (C-10), 115.1 (C-3′, C-5′), 128.3 (C-2′, C-6′), 128.5 (C-1′), 157.7 (C-4′), 162.7 (C-9), 162.8 (C-5), 164.4 (C-7), 170.0 (acetate CO_2R), 197.2 (C-4).

Acknowledgement—The authors would like to thank Dr Robert Horowitz for this helpful discussions and insights during this work.

REFERENCES

1. Maier, V. P. (1969) *Proc. Int. Citrus Symp.* **1**, 235.
2. Fisher, J. F. (1968) *Phytochemistry* **7**, 769.
3. Berhow, M. A. and Vandercook, C. E. (1989) *Phytochemistry* **28**, 1677.
4. Stafford, H. A. (1990) *Flavonoid Metabolism*. CRC Press, Boca Raton, FL.
5. Danieli, B., De Bellis, P., Carrea, G. and Riva, S. (1990) *Helv. Chim. Acta* **73**, 1837.
6. Horowitz, R. M. and Asen, S. (1989) *Phytochemistry* **28**, 2531.
7. Harborne, J. B. and Williams, C. A. (1982) in *The Flavonoids: Advances in Research* (Harborne, J. B. and Mabry, T. J., eds), p. 261. Chapman & Hall, London.

8. Barz, W. and Hosel, W. (1978) in *The Flavonoids* (Harborne, J. B., Mabry, T. J. and Mabry, H., eds), p. 916. Chapman & Hall, London.
9. Heller, W. and Forkmann, G. (1988) in *The Flavonoids, Advances in Research Since* 1980 (Harborne, J. B., ed), p. 399. Chapman & Hall, London.
10. Maturn, U., Reichenbach, C. and Heller, W. (1986) *Planta* **167**, 183.

Phytochemistry, Vol. 30, No. 12, pp. 4200 4201, 1991
Printed in Great Britain

0031 9422/91 $3.00 + 0.00

A CINNAMOYLGLUCOSE FROM *GOMORTEGA KEULE**

JANETE H. YARIWAKE VILEGAS, OTTO R. GOTTLIEB and HUGO E. GOTTLIEB†

Instituto de Química, Universidade de São Paulo, 05508, São Paulo, SP, Brazil; †Department of Chemistry, Bar-Ilan University, Ramat Gan 52100, Israel

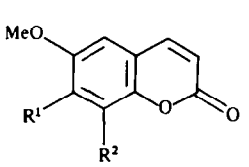
(*Received* 8 *April* 1991)

Key Word Index—*Gomortega keule*; Gomortegaceae bark; trunk wood; cinnamoylglucose; coumarins.

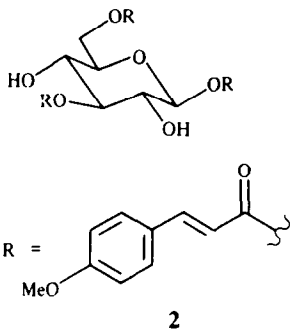
Abstract—Examination of bark and trunk wood of *Gomortega keule* resulted in the isolation of 1,3,6-tri-*O*-*p*-methoxycinnamoyl-β-D-glucose.

INTRODUCTION

The monotypic Gomortegaceae and the Lauraceae are closely related. The latter family is known for the presence of aryl- and styrylpyrones, allyl- and propenylphenols, lignans and neolignans, as well as alkaloids [1]. Previous papers on *Gomortega keule* Mol. [2, 3] reported the isolation of the coumarins **1a–d**. During the present work on this Chilean plant the presence of **1a** and **b** was confirmed and, while no trace of **1c** and **d** was found, the novel cinnamoylglucose **2** was isolated.



	R^1	R^2
1a	OMe	H
1b	OH	H
1c	H	OMe
1d	H	H



2

RESULTS AND DISCUSSION

Acid hydrolysis of **2** yielded *p*-methoxycinnamic acid as the sole aglycone. As shown by the NMR spectra of **2**, three units of this acid must esterify one unit of a hexose. This was identified with glucose by ^{1}H NMR (including double irradiation experiments) and ^{13}C NMR (including 2D COSY heteronuclear correlations). The proton coupling constant of 8 Hz for the doublet at δ5.92 indicated the β-configuration of the anomeric carbon. The presence of three chemically non-equivalent *p*-methoxycinnamoyl groups was evidenced by multiple signals for identical part structures of these groups. For instance the ^{1}H spectrum included signals for three methoxyls at δ3.85 (3H) and 3.95 (6H), and the ^{13}C spectrum included signals for three carbonyls at δ165.23, 167.32 and 167.54. Furthermore, the hydroxyls at positions 1 (δ5.98, *d*, $J = 8$ Hz) and 6 (δ4.56, *dd*, $J = 10$ and 2 Hz; 4.45, *dd*, $J = 10$ and 5 Hz), and at one additional position of the glucose unit (δ5.35, *t*, $J = 9$ Hz) are esterified, in contrast to two other carbinols (δ3.94 and 3.85, multiplets). The location of the additional ester at position 3 was established by conventional proton decoupling techniques [4].

EXPERIMENTAL

Isolation of the constituents. Powdered bark and trunk wood (140 g) of *Gomortega keule* (collected at Concepción, Chile, and

*Taken from part of the Doctorate thesis presented by J.H.Y.V. (present address: Centro de Pesquisas de Paulínia, Rhodia S. A., C. Postal 7, 13140, Paulinia, SP, Brazil) to Universidade de São Paulo (1990).