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COUMARIN GLYCOSIDES FROM *CITRUS* FLAVEDO

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Key Word Index—*Citrus aurantium*; *Citrus paradisi*; *Citrus grandis*; Rutaceae; bitter orange; grapefruit; pummelo; flavedo; isolation; structure determination; coumarin glycoside; meranzin; meranzin hydrate; isomeranzin.

Abstract—Two coumarin glycosides of possible taxonomic significance have been isolated from aqueous extracts of bitter orange flavedo and shown to be the new natural products 8-(1-β-D-glucopyranosyloxy-1-methylethyl)-8,9-dihydro-2H-furo-[2,3-h]-1-benzopyran-2-one and 8-(3-β-D-glucopyranosyloxy-2-hydroxy-3-methylbutyl)-7-methoxy-2H-1-benzopyran-2-one. The latter glycoside was also present in aqueous extracts of grapefruit and pummelo flavedo but at lower levels. All the flavedo extracts contained meranzin, meranzin hydrate and isomeranzin.

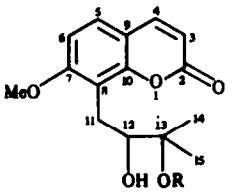
INTRODUCTION

The oxygen heterocyclic components present in *Citrus* peel oils have been investigated extensively and are useful taxonomic markers [1]. These components include a range of coumarins which can be determined conveniently by high pressure liquid chromatography (HPLC) with fluorescence detection [2]. The observation that aqueous extracts of bitter orange peel also contained fluorescent substances prompted the present work.

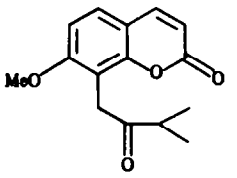
RESULTS AND DISCUSSION

Examination of an aqueous extract of bitter orange flavedo by HPLC with dual UV/fluorescence detection revealed the presence of a number of components. Four intensely fluorescent substances were isolated from the extract by chromatography and found to be closely related 7,8-disubstituted coumarins. Two of these were identified as meranzin hydrate (**1**) and isomeranzin (**2**) by comparison of their spectral properties with published data. Compound **1** has been reported from a variety of plant species, including grapefruit [1]. However, the isolation of **1** from bitter orange oil by thin layer chromatography was considered to be a consequence of hydration of meranzin (**3**) during chromatography [3]. Compound **2** may be an artifact formed from the known bitter orange coumarin, auraptenol (**4**) [4], although it is a reported constituent of *Skimmia japonica* [5]. The distribution of coumarins, including **1**, **2** and **3**, in the *Rutales* has been reviewed [6].

The two more polar components isolated were both glycosides. One of them, namely columbianetin-*O*-β-D-glucopyranoside (**5**) is a known compound. Structure **5** was originally proposed for columbianin, a coumarin glycoside of *Lomatium columbianum* [7]. Columbianin was later shown to be the β-D-gentiobiosyl rather than the β-D-glucopyranosyl derivative of columbianetin (**6**) in the course of a study which included the preparation of authentic **5** [8]. The spectral properties of **5**, **6** and their acetylated derivatives are well documented and match those of materials obtained in the present work. The remaining glycosidic component was identified as **7** (the 13-*O*-β-D-glucopyranoside of **1**) on the basis of its facile conversion to **1** on treatment with an enzyme possessing β-glucosidase activity and on NMR and mass spectral evidence. In particular, comparison of ^{13}C NMR data for **1**, **7** and **5** confirmed the presence of an *O*-β-D-glucopyranoside moiety at C-13 in **7** (Table 1).



1 R = H
7 R = β-D-glucopyranosyl



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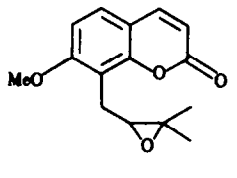

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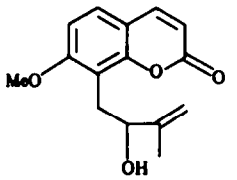

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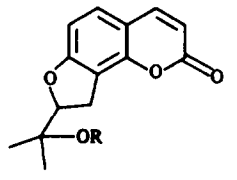

5 R = β-D-glucopyranosyl
6 R = H

Table 1. Carbon shifts of coumarins 1, 7 and 5 (δ values, CD_3OD containing TMS standard)

Carbon	1	7	5‡
C-2	163.8	163.8	165.5
3	113.0	113.0	112.3
4	146.4	146.4	146.3
5	128.4	128.4	130.3
6	109.1	109.1	107.9
7	162.5	162.7	163.0
8	114.3*	114.4*	114.3*
9	117.2*	117.3*	116.2*
10	154.8	154.5	152.5
11	26.3	26.3	28.5
12	78.8	77.7	77.7
13	74.1	81.9	79.3
14	25.6	22.7†	21.5†
15	25.6	23.8†	23.7†
OMe	56.8	56.8	—
1′		98.7	98.8
2′		75.2	75.1
3′		78.2	78.2
4′		71.8	71.7
5′		78.2	78.2
6′		62.7	62.9

‡Numbering of carbons is by analogy with 7.

*†Assignments may be reversed in each vertical column.

The chemical shift difference between the C-13 signals of 1 (at δ74.1) and 7 (at δ81.9) closely parallels that observed for C-2 in the case of the model compounds 2-methyl-2-butanol (at δ71.0) and its *O*-β-D-glucopyranoside (at δ79.4).

A relatively intense peak observed on HPLC analysis of fresh flavedo extracts was assigned to meranzin (3), on the basis of spiking experiments and the observation that, on standing, the intensity of the peak decreased with a corresponding increase in the intensity of the peak assigned to 1. This assignment was confirmed by dichloromethane extraction of a fresh flavedo extract which yielded a mixture consisting essentially of 2 and 3 in the approximate ratio 1:3 (^{1}H NMR evidence).

A preliminary HPLC examination of aqueous extracts of grapefruit flavedo indicated that 1, 2, 3 and 7 were present. The estimated concentrations of coumarins in bitter orange and grapefruit flavedo were compared (Table 2). The concentration of 7 in grapefruit was an order of magnitude less than in bitter orange and 5 was apparently absent. The latter substance has a more intense fluorescence than the other coumarins and could have been detected at very low levels.

Table 2. Approximate concentrations of coumarins in flavedo (ppm $\times 10^{-2}$)

Compound	1	2	3	5	7
Bitter orange	1	8	25	6	31
Grapefruit	1	4	16	—	4
Pummelo	2	4	28	—	17

Aqueous extractions of albedo were also undertaken. The concentrations of coumarins observed in the albedo extracts were sufficiently low to confirm that these substances are essentially flavedo constituents.

A number of coumarins closely related to 1–4 were isolated during a recent chemotaxonomic study of the rutaceous genus *Murraya* [9]. However, the genus was more conveniently divided into groups on the basis of the alkaloids rather than the coumarins present in individual species. Recent chemotaxonomic studies of *Citrus* have also involved compounds other than coumarins. An examination of certain enzymes and substrates present in *Citrus* species indicated that bitter orange is a hybrid of pummelo (*C. grandis*) and mandarin (*C. reticulata*) whereas grapefruit is a hybrid of pummelo and sweet orange (*C. sinensis*) [10]. Further evidence for a close relationship between pummelo, grapefruit and bitter orange is the occurrence of bitter flavanone neohesperidoses in these species but in no other *Citrus* species except Ponderosa lemon [11]. Our observations on the coumarin distributions in bitter orange and grapefruit flavedo extracts appeared consistent with other evidence on *Citrus* taxonomy and further supporting evidence was obtained on examination of pummelo flavedo extracts. Concentrations of 3 and 7 comparable with those present in bitter orange were observed (Table 2). As expected, aqueous extracts of sweet orange flavedo contained no coumarins or coumarin glycosides.

EXPERIMENTAL

Flavedo scrapings (269 g) were stirred with H_2O (2.7 l) at room temp. for 4 hr. The filtered extract was checked by HPLC (C-18, 30 cm × 3.9 mm; MeOH–H_2O, 45:55; UV, 315 nm and fluorescence, 313/425 nm) then evaporated to dryness (42 g). A soln of an aliquot (24 g) of the extract in MeOH (10 ml) was diluted with H_2O (400 ml). The soluble fraction (13 g) was separated by HPLC (Waters prep 500; C-18 prep-pak; step gradient, H_2O, MeOH–H_2O 7:3, MeOH). Material eluting with MeOH–H_2O was subjected to semi-prep. HPLC (C-18, 25 cm × 10 mm; MeOH–H_2O, 48:52 followed by MeOH; fluorescence, 313/425 nm) in 10 aliquots and gave 35 mg 5, 88 mg 7 and a further fraction (270 mg, eluted by MeOH). An aliquot (120 mg) of the latter was separated by prep. TLC (silica, 1 mm; MeOH–CH_2Cl_2, 1:9) affording 24 mg 1 and 6 mg 2. The mass and ^{1}H NMR spectra of 1 and 2 corresponded to published data [4–6]. The sample of 1 had mp 126–28° (CH_2Cl_2–Et_2O; lit. [12] 129°).

Compound 5. ^{1}H NMR (90 MHz, CD_3OD): δ1.26 (*s*, 3H), 1.42 (*s*, 3H), 6.16 (*d*, 1H, H-3, $J_{3,4}$ = 9.5 Hz), 6.73 (*d*, 1H, H-6, $J_{5,6}$ = 8.5 Hz) 7.36 (*d*, 1H, H-5), 7.83 (*d*, 1H, H-4); CIMS (NH_3) *m/z* (rel. int.): 426 $[MNH_4]^+$ (64), 180 (100); UV λ_{max}^{MeOH} nm (log ε): 326 (3.84) Conventional acetylation of 5 yielded a fully acetylated derivative of mp 157–159° (CH_2Cl_2–Et_2O; lit. [8] 163.5–164.5°) having spectral properties in accord with published data [8].

Compound 7. ^{1}H NMR (90 MHz, CD_3OD): δ1.38 (*s*, 3H), 1.40 (*s*, 3H), 3.05 (*m*, 2H), 3.93 (*s*, 3H), 6.22 (*d*, 1H, H-3, $J_{3,4}$ = 9.4 Hz) 7.01 (*d*, 1H, H-6, $J_{5,6}$ = 8.8 Hz), 7.48 (*d*, 1H, H-5), 7.86 (*d*, 1H, H-4); CIMS (NH_3) *m/z* (rel. int.): 458 $[MNH_4]^+$ (100), 296 (64), 180 (53); UV λ_{max}^{MeOH} nm (log ε): 320 (3.99). Conventional acetylation of 7 yielded a fully acetylated product with mp 198–201° (CH_2Cl_2–Et_2O).

Enzymic cleavage of 7. A soln of 7 (20 mg) in EtOH–H_2O (1:1, 0.1 ml) was added to a soln of naringinase (50 mg) in H_2O (2.5 ml) at pH 5.6. After 4 days at 37° the mixture was diluted with H_2O (50 ml) and extracted with CH_2Cl_2 (1 × 30 ml, 2 × 20 ml). The combined CH_2Cl_2 extracts were washed (H_2O), dried (Na_2SO_4)

and evaporated to yield 5 mg **1** having spectral data identical to that of material isolated directly from the flavedo extract.

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