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COUMARINS AND PSORALENS IN GRAPEFRUIT PEEL OIL

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Key Word Index—*Citrus paradisi*; Rutaceae; grapefruit; coumarins; psoralens.

Abstract—Four coumarins, four psoralens and two methoxyflavones were isolated and identified from the peel oil of grapefruit. Five of these compounds are reported as constituents of grapefruit oil for the first time, one of which, 5[(3,7-dimethyl-6-epoxy-2-octenyl)oxy]psoralen is a new natural product.

INTRODUCTION

Coumarins, methoxyflavones and flavanones have been found useful as taxonomic markers in *Citrus* species [1, 2]. For example, *Citrus paradisi* consistently contained three unknown coumarins, while hybrids of *C. paradisi* × *C. reticulata* contained from 0 to 3 of these compounds some of which were also observed in hybrids of *C. sinensis* × *C. sinensis* which was unexpected. Grapefruit peel oil was chosen as a source of coumarins to aid in the identification of these unknown coumarins in the leaf extracts. A review of the coumarins and psoralens present in citrus was published in 1971 by Stanley and Jurd [3]; the most recent review was by Gray and Waterman [4].

RESULTS AND DISCUSSION

Table 1 lists the compounds isolated and identified in this work. Bergamottin **6**, bergaptol **7**, 7-geranyloxycoumarin **10**, 5,6,7,8,4′-pentamethoxyflavone **13**, and 5,6,7,8,3′,4′-hexamethoxyflavone **14** were isolated and identified. Compounds **6**, **7** and **10** had been isolated previously [5] while **13** and **14** have also been found in orange and mandarin [1].

Table 1. Components of grapefruit oil and their related produts

1	5[(3,7-Dimethyl-6-epoxy-2-octenyl)oxy] psoralen
2	5[(6,7-Dihydroxy-3,7-dimethyl-2-octenyl)oxy] psoralen
3	5[(6-Hydroxy-3,7-dimethyl-2,7-octadienyl)oxy] psoralen*
4	5[(3,7-Dimethyl-6-keto-2-octenyl)oxy] psoralen*
5	5[(3-Methyl-5-formyl-2-pentenyl)oxy] psoralen*
6	5[(3,7-Dimethyl-2,7-octadienyl)oxy] psoralen (bergamottin)
7	5-Hydroxypsoralen (bergaptol)
8	7[(3,7-Dimethyl-6-epoxy-*trans*-2-octenyl)oxy] coumarin
9	7[(6,7-Dihydroxy-3,7-dimethyl-*trans*-2-octenyl)oxy] coumarin (marmin)*
10	7[(3,7-Dimethyl-2,7-octadienyl)oxy] coumarin (7-geranyloxycoumarin)
11	7-Methoxy-8 (2,3-epoxy-isopentyl) coumarin
12	7-Methoxy-8 (2,3-dihydroxy-isopentyl) coumarin
13	5,6,7,8,4′-Pentamethoxyflavone
14	5,6,7,8,3′,4′-Hexamethoxyflavone

* Synthetic compounds.

The compounds **1**, **2**, **8**, **11** and **12**, are reported as constituents of grapefruit peel oil for the first time (Table 1, Fig. 1). Compound **1** is a new natural product.

Compound **2** was synthesized from **1** by two procedures; the epoxide was opened by either (a) perchloric acid [6] which gave **2** as the major product or (b) by oxalic acid [7, 8] where a number of products were formed of which four were identified. They were compounds **2**, **3**, **4** and **7**. Compound **1** was oxidized to **5** with lead tetraacetate [6].

Marmin **9** was obtained from **8** using perchloric acid. Marmin has previously been found in grapefruit oil and synthesized [5, 6]. We did not observe marmin in the oil but it might have been present as an impurity along with **2** since it had the same R_f values in all TLC solvents tried. Compounds **8**, **9** and **10** are apparently the three coumarins observed in *C. paradisi* leaf extracts. The TLC of these three compounds compared to the unknowns in the leaf extracts in 5 different solvent systems show them to have the same R_fs.

Compound **11** had previously been isolated from citrus oil [8] and on treatment with oxalic acid gave **12** another diol which was present in the oil. The three epoxides **1**, **8** and **11** which were isolated from grapefruit oil on treatment with acid, gave three diols; **2**, **9**, and **12**. Fisher *et al.* [9] isolated **15**. 5[(3,6-dimethyl-6-formyl-2-heptenyl)oxy] psoralen and **16**, 7-methoxy-8(2-formyl-2-methyl-propyl) coumarin. Stanley and Jurd [3] postulated that **2** and **12** were the probable precursors for **15** and **16** which were probably formed by a pinacol rearrangement. Compounds **15** and **16** were not isolated in this study and if present would be trace components.

Compound **10** appears to be the major component present with **1** in about the same concentration. The rest of the compounds in approximate order of decreasing concentration are: **10** = **1** > **11** > **8** > **6** > **13** > **14** > **7** > **2** and **12**. Two other compounds were isolated in smaller amounts and appear to be coumarins. One of these might be 5,7-dimethoxycoumarin [10], based on TLC, R_f and color under UV and an IR spectrum on an impure sample.

EXPERIMENTAL

Thin layer chromatography. Preparative plates (1 mm thick) were prepared with Si gel HF-254. TLC plates (0.25 mm thick) were commercial Si gel GF type. Solvent systems were: hexane–Me$_2$CO (17:3) (1), (8:2) (2), CH$_2$Cl$_2$–Me$_2$O (37:3) (3), CH$_2$Cl$_2$

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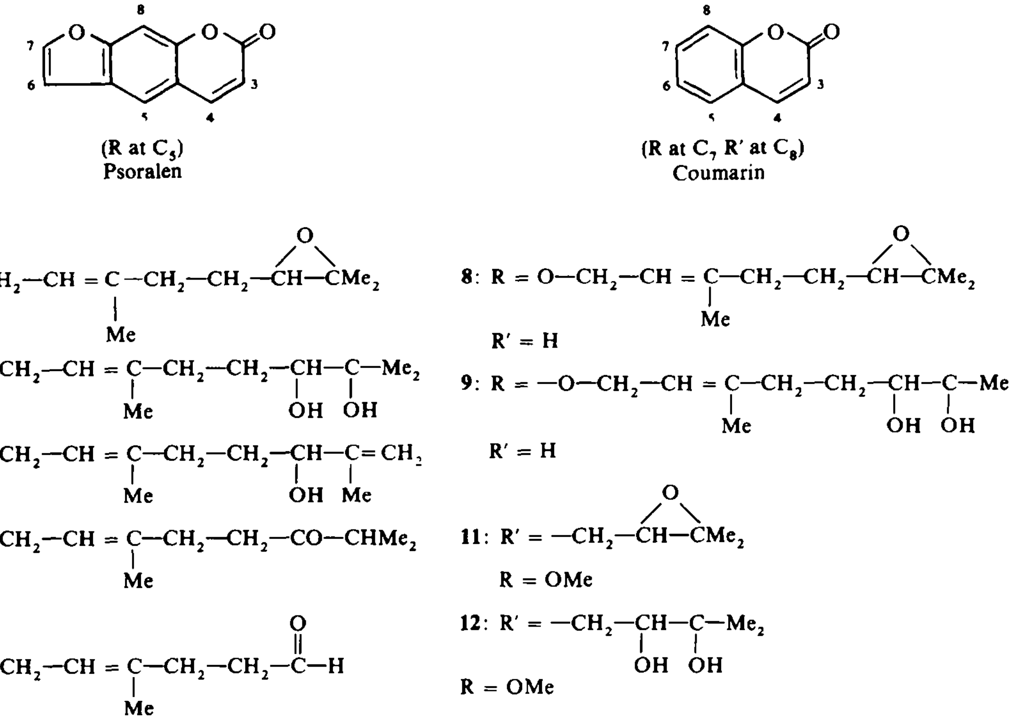

Fig. 1.

(4), hexane–$CHCl_3$–Et_2O (6:1:1) (5), (2:1:1) (6), C_6H_6–Me_2CO (3:1) (7).

Materials. Four l. of grapefruit oil that had not been dewaxed were placed into −20° and after 4 months the supernatant oil was decanted from the solid and sludge which were filtered. The solid was recrystallized from hexane–Me_2CO. Three batches of crystals were obtained.

Both Duncan and Marsh varieties of grapefruit flavedo were examined. Flavedo (15 g) was removed from the fruit and ground 1 min with 50 ml MeOH and filtered. TLC comparison of whole oil, the crystals, mother liquor, Duncan extract and Marsh extract showed them to contain the same components.

Isolation of samples. The crystals and mother liquor from the grapefruit oil were separated by preparative TLC by the procedures outlined below, origin at 2 cm and solvent travel 18 cm.

Band	R_f* TLC solvent 1	Principal compound	Second and third TLC solvent
1	0.53	**6**	5
2	0.47	**10**	5
3	0.36	Unidentified	4
4	0.30	**1, 8**	6
5	0.22	**11**	2
6	0.11	**13, 7**	2, 3
7	0.06	**14**	2, 3
8	0	**2, 12**	2, 3

* 3 × developed.

Multiple development was required with all solvent systems. The compounds were eluted from the Si gel with C_6H_6–Me_2CO (4:1).

MS data. High resolution MS mass measurement data obtained through Dr. Dougherty at Florida State University. The NMR spectra were run in $CDCl_3$ with TMS as internal standard. All spectra were recorded at 270 MHz and obtained through Dr. G. Levy at Florida State University. Melting points mp are uncorr. The IR spectra were run as KBr pellets.

Compound **1** was isolated from grapefruit oil and crystallized from hexane–Me_2CO mp 82.5–83.5 (lit. [11] 67–70°). MS (*m/e*): 354.1467, Calc. mass 354.1467; NMR, $CDCl_3$: δ 8.15 (1H, *d*, J = 9.8, H-4), 7.59 (1H, *d*, J = 2, H-7), 7.14 (1H, *s*, H-8), 6.95 (1H, *d*, J = 1.7, H-6), 6.26 (1H, *d*, J = 9.8, *H*-3), 5.59 (1H, *t*, J = 6.5, H-2'), 4.95 (2H, *d*, J = 6.8, CH_2-1'), 2.70 (1H, *t*, J = 6.5, H-6'), 2.23 (2H, *m*, CH_2-5'), 1.73 (3H, *s*, CH_3-3'), 1.67 (2H, *m*, CH_2-4'), 1.29 (6H, *d*, J = 9, 2CH_3-7'); IR: 1725, 1620, 1600, 1575.

Compound **2** was isolated from grapefruit oil and crystallized from EtOAc–hexane, mp 70–74° (lit. [11] 104–106°). MS (*m/e*): 372.1573, calc. mass 372.1573; NMR, $CDCl_3$: δ 8.15 (1H, *d*, J = 9.8, H-4), 7.59 (1H, *d*, J = 2, H-7), 7.14 (1H, *s*, H-8), 6.95 (1H, *d*, J = 1.5, H-6), 6.26 (1H, *d*, J = 9.8, H-3), 5.59 (1H, *t*, J = 6.5, H-2'), 4.95 (2H, *d*, J = 6.8, CH_2-1'), 3.32 (1H, *ud*, J = 8, H-6'), 2.26 (4H, *brm*, CH_2-5', OH-6, 7), 1.71 (3H, *s*, CH_3-3'), 1.53 (2H, *brm*, CH_2-4'), 1.19 (6H, *d*, J = 10, 2CH_3-7'). Addition of D_2O causes a loss of 2H at 2.26 (2H, *brm*, CH_2-5'). IR: 3550-OH, 3500-OH, 1728, 1628, 1608, 1580. Hydrolysis of 500 mg **1** [6] gave 352 mg of **2** crystallized from EtOAc–hexane, mp 70–74°. Prep-TLC of mother liquor (solvent system 6) yielded 42 mg of **2**. See oxalic acid hydrolysis for synthesis of **2**.

Compound **3**. Oxalic acid hydrolysis of **1** gave **3** crystallized from hexane–EtOAc mp 113–115°. MS (*m/e*): 354.1467, calc. mass 354.1467; NMR: δ 8.15 (1-H, *d*, $J = 9.8$, H-4), 7.59 (1H, *d*, $J = 2$, H-7), 7.15 (1H, *s*, H-8), 6.95 (1H, *d*, $J = 2$, H-6), 6.26 (1H, *d*, $J = 9.8$, H-3) 5.57 (1H, *t*, $J = 6.5$, H-2′), three peaks at 4.96, 4.94, 4.86 (4H, *ud*, (1 : 1.5), $J = 6.6$, *d*, $J = 21$, $CH_2 = 1'$, 7′), 4.04 (1H, *t*, $J = 6$, H-6′), 2.13 (2H, *m*, CH_2-5′), 1.73 (3H, *s*, CH_3-3′), 1.71 (3H, *s*, CH_3-7′), 1.70 (2H, *m*, CH_2-4′), hydroxyl not observed in spectrum, addition of D_2O causes no change, IR: 3495-OH, 1700, 1620, 1605, 1575.

Compound **4**. Oxalic acid hydrolysis of **1** gave 32.2 mg **4** mp 69–70° from hexane–EtOAc. MS (*m/e*): 354.1467, Calc. mass 354.1467: NMR, $CDCl_3$: δ 8.13 (1H, *d*, $J = 9.3$, H-4), 7.59 (1H, *d*, $J = 2.5$, H-7), 7.14 (1H, s, H-8), 6.94 (1H, s, H-6), 6.26 (1H, *d*, $J = 10$, H-3), 5.53 (1H, *t*, $J = 6$, H-2′), 4.93 (2H, *d*, $J = 7$, CH_2-1′) 2,61 (3H, *m*, CH_2-5′, C-$(CH_3)_2$H-7′), 2.34 (2H, *ut*, $J = 7.5$, CH_2-4′), 1.70 (3H, *s*, CH_3-3′), 1.09 (6H, *d*, $J = 7.3$, $2CH_3$-7′), IR: 1720, 1620, 1600, 1575.

Compound **5**. To a solution of 50 mg of **1** in 1.1 ml HOAC was added 70 mg of $Pb(OAc)_4$ [6]. The reaction was stirred for 1 hr and then neutralized with $NaHCO_3$. The mixture was extracted with EtOAC, washed (H_2O) and dried. Crystallized from hexane–EtOAc, 21.4 mg of **5** mp 98–99°. NMR, $CDCl_3$: δ 9.78 (1H, *s*, $\overset{\overset{\mathrm{O}}{\|}}{\mathrm{C}}$—H), 8.13 (1H, *d* $J = 10$, H-4), 7.60 (1H, *d*, $J = 2.3$, H-7), 7.14 (1H, *s*, H-8), 6.93 (1H, *d*, $J = 2.1$, H-6), 6.27 (1H, *d*, $J = 9.6$, H-3), 5.56 (1H, *t*, $J = 6.5$, H-2′), 4.94 (2H, *d*, $J = 6.7$, CH_2-1′), 2.60 (2H, *ut*, $J = 8$, CH_2-5′), 2.41 (2H, *ut*, $J = 8$, CH_2-4′), 1.72 (3H, *s*, CH_3-3′); IR: 1720, 1615, 1595, 1575, 1540.

Oxalic acid hydrolysis of **1** [7]. Several variations were tried. (A) To 0.2 g of oxalic acid were added 50 ml H_2O. Heat was applied and when the soln reached 100°, 0.2 g of **1** was added and the mix. refluxed 10 min. The mixture was cooled to 0° and the soln extracted 4 × with 50 ml EtOAc. The EtOAc was removed, 2 ml EtOAc added and brought to boiling. The soluble portion was placed on three prep-TLC plates (solvent system 7, develop 3 ×). Four areas were collected, R_f 0.83 **4** 32.2 mg, R_f 0.76 **3** 7.6 mg, R_f 0.53 bergaptol and R_f 0.39 **2** 27 mg. The insoluble portion above was primarily bergaptol. (B) A 30-min reflux gave mainly bergaptol. (C) Ten min at 70° gave starting material **1** and 17.4 mg of **2**. (D) When the soln reached 50° add **1**, at 10 min temperature was 73°, at 25 min 84°, at 35 min 73° and at 40 min cool flask to 0°. This gave 23.5 mg of **4**, 4 mg of **3**, 9.5 mg of **2** and bergaptol. (E) When citric acid was used instead of oxalic and refluxed for 30 min bergaprol was the major product.

Compound **8** was isolated from grapefruit oil and crystallized from EtOAc–hexane, mp 44–45° (lit. [6] 55.3–57.5°). MS (*m/e*): 314.1463, calc. mass 314.1518, error 0.0055, NMR confirmed [6].

Compound **9**. Hydrolysis of 15 mg **8** [6] gave 5 mg of **9** crystallized from EtOAc–hexane, mp 116–119°, (lit. [6, 10] mp 114–117°, 125°). Prep-TLC of mother liquor yielded 3 mg. MS (*m/e*): 332.1624, calc. mass 332.1624; IR: 3470-OH, 3350-OH, 1717, and 1610.

Compound **11** was isolated from grapefruit oil and crystallized from hexane–EtOAc, mp 96–98° (lit. [8] 98°). MS (*m/e*): 260 M^+: NMR, $CDCl_3$: δ 7.63 (1H, *d*, $J = 9.5$, H-4), 7.35 (1H, *d*, $J = 8.6$, H-5), 6.86 (1H, *d*, $J = 8.6$, H-6), 6.24 (1H, *d*, $J = 9.5$, H-3), 3.93 (3H, *s*, CH_3-7), 3.19 (1H, *t*, $J = 8$, H-2′), 3.02 (2H, *m*, CH_2-1′), 1.49 (3H. *s*, CH_3-4′), 1.28 (3H, *s*, CH_3-4′). IR: 1710, 1600, 1560, 1490, 1455, 1425, 1390, 1295, 1275, 1225, 1175, 1115, 1100 sh, 1050, 1015, 890, 855, 825, 800, 775, 745, 695.

Compound **12** was isolated from grapefruit oil and crystallized from hexane–EtOAc, mp 116–117° (lit. [8] 128–129°). To 29 mg **11** in 20 ml H_2O was added 100 mg oxalic acid [7] and refluxed 10 min, cooled in an ice bath and extracted 3 × with EtOAc. Conc EtOAc and separated on prep-TLC (solvent system 7). Yield 11 mg of **12**, mp 118.5–119°. IR of synthetic and natural product identical. MS (*m/e*): 278 M^+, NMR confirmed [12]. IR: 3490-OH, 3390-OH, 1715, 1695, 1600, 1545.

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