Phytochemistry, Vol. 31, No. 4, pp. 1363–1366, 1992
Printed in Great Britain.

0031-9422/92 $5.00+0.00

7-HYDROXYCOUMARIN DERIVATIVES FROM THE JUICE OIL OF *CITRUS HASSAKU*

TOSHIYA MASUDA,* YUKARI MUROYA and NOBUJI NAKATANI

Laboratory of Food Chemistry, Faculty of Science of Living, Osaka City University, Sumiyoshi, Osaka 558, Japan

(*Received in revised form* 24 *August* 1991)

Key Word Index—*Citrus hassaku*; Rutaceae; fruits; juice oil.

Abstract—Three new 7-hydroxycoumarin derivatives have been isolated from the juice oil of whole fruits of *Citrus hassaku*, and their structures determined to be 7-(6*R*-hydroxy-3,7-dimethyl-2*E*,7-octadienyloxy)coumarin, (±)-7-hydroxy-6-linalylcoumarin and (*R*)-6-*O*-(4-geranyloxy-2-hydroxy)cinnamoylmarmin by spectral data and chemical evidence.

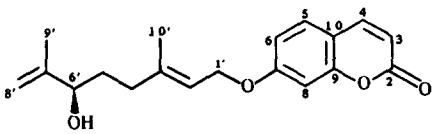
INTRODUCTION

Citrus fruits are cultivated widely as foods. They are also used widely in traditional medicine in east Asia. We are interested in the bioactive constituents in the new *Citrus* species, *Citrus hassaku*, and one of the authors (N. N.) has already reported on the presence, in juice oil of the fruits of this species, of three known 7-geranyloxycoumarin derivatives (aurapten, epoxyaurapten and marmin) [1] having spasmolytic activity [2]. We now report on the isolation and structure determination of three new 7-hydroxycoumarin derivatives (**1**–**3**) from juice oil.

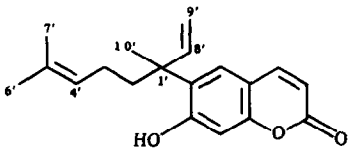
RESULTS AND DISCUSSION

The juice oil of *Citrus hassaku* obtained from fruits cultivated in Wakayama Prefecture of Japan was steam distilled to give a non-volatile fraction. The fraction was crystallized repeatedly from methanol to remove a main substance, aurapten (7-geranyloxycoumarin, **4**). The mother liquor was purified by combinations of silica gel CC and Sephadex LH-20 CC (see Experimental) to give three new 7-hydroxycoumarin derivatives (**1**–**3**).

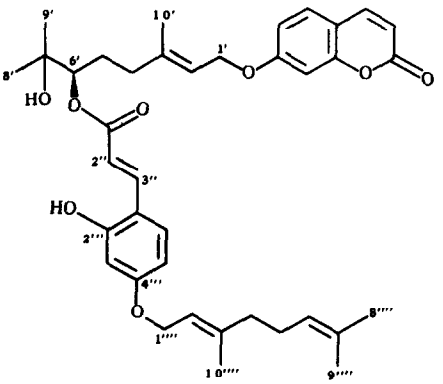
Compound **1** has the molecular formula $C_{19}H_{22}O_4$ (EIMS 20 eV; ^1H and ^{13}C NMR). An absorption at 320 nm in its UV spectrum suggested the presence of a coumarin moiety, and an absorption at 3403 cm^{-1} in its IR spectrum, the presence of a hydroxyl group. In its ^1H NMR spectrum, five aromatic signals [δ6.24 (1H, *d*, *J* = 9.5 Hz), 6.81 (1H, *d*, *J* = 2.4 Hz), 6.84 (1H, *dd*, *J* = 8.6 and 2.4 Hz), 7.36 (1H, *d*, *J* = 8.6 Hz), 7.63 (1H, *d*, *J* = 9.5 Hz)] were observed, indicating a 6- or 7-substituted coumarin. From the ^1H NMR signals due to the substituted group at position-6 or -7, the presence of two olefins, one of which was at a terminal position, [δ5.50 (1H, *br t*, *J* = 6.4 Hz), 4.85 (1H, *br s*), 4.94 (1H, *br s*)], an allyl-alcoholic methylene [δ4.60 (2H, *d*, *J* = 6.7 Hz)], an allyl-alcoholic methine [4.06 (1H, *br t*, *J* = 6.4 Hz)], two methyl groups attached to olefinic carbons [δ1.73 (3H, *s*), 1.78 (3H, *s*)] and two methylenes, one of which was attached to an olefinic carbon [δ1.60–1.78 (2H, *m*), 2.05–2.23 (2H, *m*)] were indicated. The substituent was determined to be a 4- or 6-hydroxy-3,7-dimethyl-2,7-octadienyloxyl group by examination of the ^1H NMR data (Table 1) and mass spectral fragment ions (*m/z* 162 and 151). Further confirmation of the positions of the substituents in compound **1** was provided by a derivation of **1** from aurapten



1



2



3

*Author to whom correspondence should be addressed.

Table 1. ^{1}H NMR spectral data (400 MHz) of compounds **1**, **6** and **7** ($CDCl_3$–TMS)

H	1	6	7
3	6.24 *d* (9.5)	6.25 *d* (9.8)	6.25 *d* (9.8)
4	7.63 *d* (9.5)	7.64 *d* (9.8)	7.64 *d* (9.8)
5	7.36 *d* (8.6)	7.30–7.50 *m*	7.30–7.50 *m*
6	6.84 *dd* (8.6, 2.4)	6.84 *dd* (8.6, 2.4)	6.83 *dd* (8.5, 2.4)
8	6.81 *d* (2.4)	6.80 *d* (2.4)	6.78 *d* (2.4)
1′	4.60 *d* (6.7)	4.58 *d* (6.7)	4.50 *d* (6.7)
2′	5.50 *br t* (6.7)	5.40 *t* (6.7)	5.15 *m*
4′	2.05–2.23 *m*	2.01 *br t* (7.5)	1.60
5′	1.60–1.78 *m*	1.70–1.90 *m*	1.80 *m*
6′	4.06 *br t* (6.4)	5.18 *t* (7.0)	5.15 *m*
8′	4.85 *br s*	4.68 *br s*	4.89 *br s*
	4.94 *br s*	4.73 *br s*	4.93 *br s*
9′	1.73 *s*	1.48 *s*	1.69 *s*
10′	1.78 *s*	1.61 *s*	1.59 *s*
MeO		3.42 *s*	3.41 *s*
Ph		7.30–7.50 *m*	7.30–7.50 *m*
α-H		4.77 *s*	4.77 *s*

Coupling constants (J in Hz) are given in parentheses.

(7-geranyloxycoumarin, **4**). The stereochemistry of the hydroxyl group at position 6 was determined by Trost's method [3]. Thus the shielding effects in the ^{1}H NMR caused by the phenyl group of (*R*)- and (*S*)-*O*-methylmandelic acids substituents in the (*R*)- and (*S*)-*O*-methylmandelic esters of **1** (**6**, **7**, respectively) (Table 1) confirmed that the configuration of the hydroxyl group at position 6 is *R* (Fig. 1). Thus, compound **1** was determined to be 7-(6*R*-hydroxy-3,7-dimethyl-2*E*,7-octadienyloxy) coumarin.

Compound **2** has the molecular formula $C_{19}H_{22}O_3$ (EIMS, 20 eV; ^{1}H and ^{13}C NMR). In the ^{1}H NMR of **2** (Table 2), four aromatic signals due to a 6,7-disubstitued coumarin [δ6.25 (1H, *dd*, J=9.5 Hz), 6.87 (1H, *s*), 7.30 (1H, *s*), 7.65 (1H, *d*, J=9.5 Hz)] were observed. The presence of a phenolic proton signal [δ6.90 (1H, *s*)] showed that one of the substituents was a hydroxyl group. The other was shown to be a linalyl group by the ^{1}H NMR data and the coupling networks observed in the HH-COSY spectrum of **2** [δ1.46 (3H, *s*), 1.51 (3H, *s*), 1.65 (3H, *s*), 1.69 (1H, *m*), 1.78 (1H, *dt*, J=11.9 and 3.1 Hz), 1.90 (1H, *m*), 1.99 (1H, *dt*, J=11.9 and 4.3 Hz), 5.07 (1H, *br t*, J=6.6 Hz), 5.31 (1H, *d*, J=17.7 Hz), 5.37 (1H, *d*, J=10.4 Hz), 6.20 (1H, *dd*, J=17.7 and 10.4 Hz)]. The positions of the linalyl and hydroxyl groups were determined to be 6 and 7, respectively, based on the ^{13}C chemical shifts of the coumarin ring and the calculated chemical shifts of 6-*t*-butyl-7-hydroxycoumarin [δ161.0 (C-2), 111.6 (C-3), 144.5 (C-4), 126.4 (C-5), 135.5 (C-6), 157.0 (C-7), 102.1 (C-8), 152.7 (C-9), 111.2 (C-10); 7-*t*-butyl-6-hydroxycoumarin [δ160.1 (C-2), 116.1 (C-3), 143.8 (C-4), 112.0 (C-5), 150.3 (C-6), 141.8 (C-7), 113.5 (C-8), 146.3 (C-9), 116.0 (C-10)] [4]. Although **2** has an asymmetric centre in the linalyl group, it was considered to be a racemic mixture because (*S*)-*O*-methylmandelylation of **2** gave two diastereomers as shown by TLC and ^{1}H NMR. Thus, **2** was determined to be (±)-6-linalyl-7-hydroxycoumarin.

Compound **3**, showed a pseudo-molecular ion at m/z 653 $[M+Na]^+$ in the SI mass spectrum and was thus

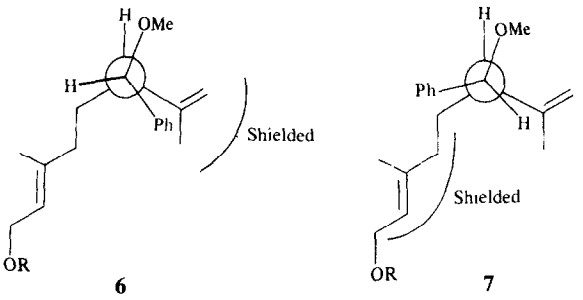


Fig. 1. Newman projections of (*R*)-*O*-methylmandelate of **1** (**6**) and (*S*)-*O*-methylmandelate of **1** (**7**), showing shielding effects caused by the phenyl group. *R* = 7-coumaryl group.

assigned the molecular formula $C_{38}H_{46}O_8$. In the ^{13}C NMR spectrum, the signals of one ester carbonyl carbon, one lactone carbonyl carbon, 22 olefinic carbons, eight carbons of which and the lactone carbon were assignable to a 7-hydroxycoumarin moiety, four oxygenated carbons and 10 aliphatic carbons were observed (see Experimental). Alkaline hydrolysis of **3** gave marmin (**5**), indicating that **3** is an ester derivative of **5**. The downfield shifted signal of the position 6′ of the marmin moiety [δ4.92 (1H, *dd*, J=8.6 and 4.3 Hz)] showed that the ester group was at the position 6′ of marmin. In the ^{1}H NMR spectral, region for aromatic part of **3**, signals for two sets of 1,2,4-trisubstituted benzene rings conjugated to olefins [δ6.47 (2H, *m*), 6.80 (2H, *m*), 7.33 (1H, *d*, J=8.5 Hz), 7.36 (1H, *d*, J=7.9 Hz), 6.24 (1H, *d*, J=9.5 Hz), 6.49 (1H, *d*, J=15.9 Hz), 7.63 (1H, *d*, J=9.5 Hz), 7.93 (1H, *d*, J=15.9 Hz)] were observed. These indicated that **5** is esterified with a 2′,4′-disubstituted *trans*-cinnamoyl group. Of the non-aromatic signals of **3**, the signals of a geranyl group [δ1.60 (3H, *s*), 1.67 (3H, *s*), 1.73 or 1.75 (3H, *s*), 2.09–2.20 (4H, *m*), 4.53 (2H, *d*, J=6.7 Hz)] were observed along with the signals due to marmin, suggesting the

Table 2. ^1H (400 MHz) and ^{13}C (100 MHz) NMR spectral data of compound **2** ($CDCl_3$–TMS)

Position	C	H
2	161.6	
3	113.1	6.25 *d* (9.5)
4	143.9	7.65 *d* (9.5)
5	126.9	7.30 *s*
6	132.1	
7	158.7	
8	105.3	6.87 *s*
9	154.6	
10	112.4	
1′	43.4	
2′	38.2	1.78 *dt* (11.9, 3.1)
		1.99 *dt* (11.9, 4.3)
3′	23.1	1.69 *m*, 1.90 *m*
4′	123.9	5.07 *br t* (6.6)
5′	129.1	
6′	25.7	1.65 *s*
7′	17.6	1.51 *s*
8′	146.1	6.20 *dd* (17.7, 10.4)
9′	115.2	5.31 *d* (17.7)
		5.37 *d* (10.4)
10′	24.6	1.46 *s*

Coupling constants (*J* in Hz) are given in parentheses. All assignments were confirmed by CH-COSY.

Table 3. ^1H NMR (400 MHz) spectral data of compound **3** ($CDCl_3$–TMS)

H	3
3	6.24 *d* (9.5)
4	7.63 *d* (9.5)
5	7.33 *d* (8.5)
6	6.80 *m*
8	6.80 *m*
1′	4.53 *d* (6.7)
2′	5.46 *br t* (6.6)
4′	2.09–2.20 *m*
5′	1.70–1.85 *m*
6′	4.92 *dd* (8.6, 4.3)
8′	1.25 *s*
9′	1.25 *s*
10′	1.73 *s*[a]
2″	6.49 *d* (15.9)
3″	7.93 *d* (15.9)
3‴	6.47 *m*
5‴	7.36 *d* (7.9)
6‴	6.47 *m*
1⁗	4.53 *d* (6.7)
2⁗	5.46 *m*
4⁗	2.09–2.20 *m*
5⁗	2.09–2.20 *m*
6⁗	5.03 *br t* (6.6)
8⁗	1.60 *s*[b]
9⁗	1.67 *s*[b]
10⁗	1.75 *s*[a]

Coupling constants (*J* in Hz) are given in parentheses.
[a,b]Assignments may be interchangable.

geranyl group was attached at the position 2′ or 4′ of the cinnamoyl group (Table 3). To determine the position, we photodegraded **3**. We presumed a *cis*-cinnamoyl group produced by photoirradiation of **3** would be unstable and would form a coumarin ring easily. In fact the photoreaction gave equal amounts of **4** and **5** which established that the substituted position is at 4′ (Fig. 2). As for the stereochemistry of **3**, the orientation of the cinnamoyloxy group was shown to be *R* by comparison of $[\alpha]_D$ of the produced marmin from **3** with authentic (+)-marmin (**5**) [1]. Thus, compound **3** is (*R*)-6-*O*-(4-geranyloxy-2-hydroxy) cinnamoylmarmin.

EXPERIMENTAL

NMR: ^{13}C at 100 MHz, ^1H at 400 MHz; EIMS: 20 eV, SIMS: glycerin matrix; IR: film.

Isolation. The juice oil of *Citrus hassaku* was supplied by the Nankaikako Company. The preparation procedure was as previously reported [1]. The juice oil (149 g) was steam distilled to give a non-volatile fraction which was crystallized repeatedly with MeOH to give aurapten (**4**) (106 g). The mother liquor (7 g) was subjected to silica gel CC eluted with 20–60% Me_2CO in hexane. Nine fractions were collected. From fr. 2, **1** (69 mg) was isolated after silica gel CC (25% Me_2CO in hexane) and silica gel TLC (30% Et_2O in CH_2Cl_2). From fr. 5, **2** (870 mg) was isolated after silica gel CC (2% Me_2CO in CH_2Cl_2). From fr. 7, **3** (200 mg) was isolated after Sephadex LH-20 CC (Me_2CO).

Compound **1**. Mp 75°; $[\alpha]_D^{26}$ +10° (EtOH; *c* 1.0); UV λ_{max}^{MeOH} nm: 320; IR ν_{max} cm^{-1}: 3403, 2922, 1731, 1708, 1613; EIMS *m/z* (rel. int.): 314 $[M]^+$ (40), 162 (100), 151 (15), 135 (70); ^1H NMR: see Table 1; ^{13}C NMR ($CDCl_3$) δ: 16.7, 17.5, 32.8, 35.4, 65.4, 75.4, 101.6, 111.2, 112.5, 113.0, 113.2, 118.7, 128.6, 142.0, 143.4, 147.3, 155.7, 161.2, 162.1.

Compound **2**. Mp 65°; $[\alpha]_D^{26}$ ±0° (EtOH; *c* 1.0); UV λ_{max}^{MeOH} nm: 332; IR ν_{max} cm^{-1}: 3274, 1696, 1616, 1568. EIMS *m/z* (rel. int.): 298 $[M]^+$ (100), 215 (85); ^{13}C and ^1H NMR see Table 2.

Compound **3**. Viscous oil; $[\alpha]_D^{26}$ +72° (EtOH; *c* 1.0); IR ν_{max} cm^{-1}: 3420, 1734, 1717, 1701, 1611; SIMS *m/z*: 653 $[M+Na]^+$; ^1H NMR: see Table 3; ^{13}C NMR ($CDCl_3$) δ: 16.6, 16.7, 17.7, 25.2, 25.6, 26.4, 26.7, 27.5, 36.2, 39.5, 65.1, 65.5, 72.9, 79.3, 101.7, 102.8, 107.7, 112.5, 112.7, 113.4, 114.8, 115.1, 119.1, 119.2, 123.8, 128.6, 130.5, 131.8, 141.3, 141.4, 141.5, 143.6, 155.9, 157.4, 161.8, 162.0, 162.3, 168.6.

Chemical derivation of **1** *from aurapten* (**4**). To a soln of **4** (1 g) in CH_2Cl_2 (50 ml), *m*-CPBA (0.55 g) was added at 0°. After stirring for 5 min at 0°, the mixture was poured into aq. $Na_2S_2O_3$ and $NaHCO_3$, extracted (×4) with CH_2Cl_2, dried over Na_2SO_4 and concd. The residue was treated with Et_2O to give 6′,7′-epoxyaurapten (0.4 g) as a powder. To a soln of 6′,7′-epoxyaurapten (50 mg) in MeCN and H_2O (4:1, 2.5 ml) was added *p*-TsOH (5 mg) at 23°. After standing for 30 min, the mixture was poured into aq. $NaHCO_3$, extracted (×4) with CH_2Cl_2, dried over Na_2SO_4 and concd. The residue was crystallized from Et_2O to remove (±)-marmin (6′,7′-dihydroxyaurapten) (19 mg). The mother liquor was purified by silica gel TLC (40% EtOAc in hexane) to give racemic **1** (2 mg).

(R)- *and* (S)-O-*Methylmandelates of* **1** (**6** and **7**, *respectively*). To a soln of **1** (5 mg), (*R*)-*O*-methylmandelic acid (8 mg) and dicyclohexylcarbodiimide (8 mg) in 1,2-dichloroethane (0.2 ml), dimethylaminopyridine (0.1 mg) was added at 0°. After stirring for 12 hr, the mixture was filtered. The filtrate was purified by

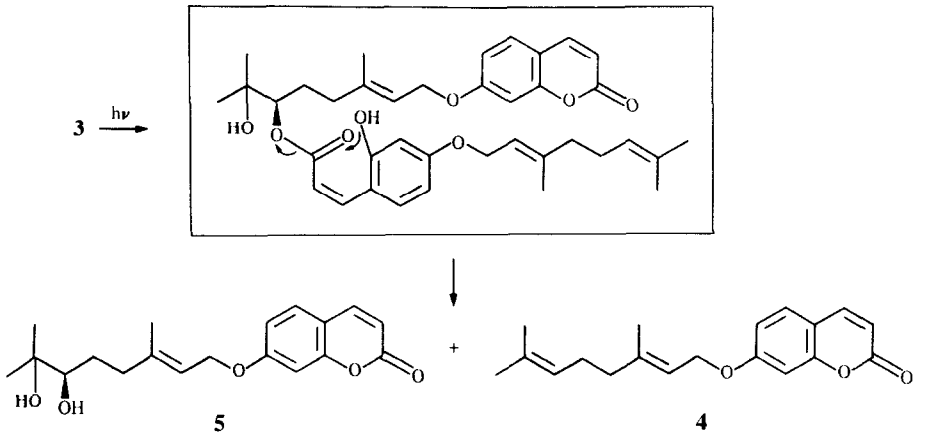


Fig. 2. Mechanism of photodegradation of 3 to 4 and 5.

silica gel TLC (CH_2Cl_2) to give **6** (14 mg). By the same method, **7** was synthesized from **1** and (*S*)-*O*-methylmandelic acid. ^{1}H NMR of **6** and **7**: see Table 1.

(S)-O-*Methylmandelylation of* **2**. To a soln of **2** (0.3 mg), (*S*)-*O*-methylmandelic acid (1.8 mg) and dicyclohexylcarbodiimide (3.3 mg) in 1,2-dichloroethane (0.2 ml) was added dimethylaminopyridine (0.3 mg) at 0°. The mixture was filtered after stirring for 1.5 hr at 0°. The filtrate was purified by silica gel TLC (CH_2Cl_2) to give the diastereomers of the (*S*)-*O*-methylmandelate of **2**, which were detected as two spots on silica gel TLC developed with Et_2O.

Alkaline hydrolysis of compound **3**. To a soln of **3** (20 mg) in MeOH (1 ml) was added 6 M NaOH (1 ml) at 23°. After stirring for 3 hr, the mixture was poured into 1M HCl, extracted (× 4) with CH_2Cl_2, dried over Na_2SO_4 and concd. The residue was purified by silica gel TLC (40% EtOAc in hexane) to give (+)-marmin (**5**) (8.6 mg), $[\alpha]_D^{26}$ +22° (EtOH; *c* 1.0).

Photodegradation of compound **3**. A CH_2Cl_2 soln (2 ml) of **3** (5 mg) was irradiated with sunlight at 0° for 7 hr. After concn, the residue was purified by silica gel TLC (40% EtOAc in hexane) to give aurapten (**4**, 1.0 mg) and marmin (**5**, 1.2 mg).

Acknowledgement—The authors thank Dr T. Hattori (a president of Nankaikako Company) for supplying juice of *Citrus hassaku*.

REFERENCES

1. Nakatani, N., Yamada, Y. and Fuwa, H. (1987) *Agric. Biol. Chem.* **51**, 419.
2. Yamada, Y., Nakatani, N. and Fuwa, H. (1987) *Agric. Biol. Chem.* **51**, 1711.
3. Trost, B. M., Belletire, J. L., Godleski, S., McDougal, P. G., Balkovec, J. M., Baldwin, J. J., Christy, M. E., Ponticello, G. S., Varga, S. L. and Springer, P. (1986) *J. Org. Chem.* **51**, 2370.
4. Kalinowski, H., Berger, S. and Braun, S. (1984) in *Carbon-13 NMR Spectroscopy*. John Wiley.