EXPERIMENTAL

Plant material. Cyclotrichium niveum Boiss. (Labiatae) was collected from eastern Turkey (Sivas) in July 1987 and was identified by one of us (E. Tuzlaci), a voucher is deposited in the Herbarium of the Faculty of Pharmacy, University of Marmara (MARE 1293).

Isolation. The powdered whole plant (500 g) was extracted with Me_2CO in a Soxhlet. The extract was evapd *in vacuo* yielding 11 g of a residue. The residue was fractioned in a silica gel column (4 × 60 cm) eluting with petrol, a gradient of C_6H_6 was added up to 100% followed by $CHCl_3$ and EtOH both to 100%. The compounds were obtained in the following order: β-amyrin (90 mg), sitosterol (400 mg), vergatic acid (1 g), oleanolic acid (2 g), apigenin (34 mg), apigenin 7-methyl ether (43 mg), eriodictyol 7-*O*-glucoside (38 mg), isosakuranetin (125 mg), isosakuranetin 7-*O*-rhamnoside (260 mg), acacetin 7-*O*-rutinoside (15 mg).

Hydrolysis of **1**. Acid hydrolysis was carried out with 2 N HCl (3 hr at 100°) under reflux. Rhamnose was identified by Co-PC (BuOH–HOAc–H_2O 4:1:5 and pyridine–EtOAc–HOAc–H_2O 36:36:7:21) and on TLC silica gel plates (EtOH–$CHCl_3$ 1:19) and cellulose plates (30% HOAc; 45% HOAc; BuOH–pyridine–H_2O; 15:3:2).

Isosakuranetin 7-O-*rhamnoside* (**1**). Cream coloured crystals, mp 205°, UV given in the text. ^{1}H NMR given in Table 1.

Isosakuranetin 7-O-*rhamnoside tetra-acetate* (**1a**). Mp 195°, ^{1}H NMR given in Table 1. MS *m/z* (%), no mass peak was observed, 327 [isosakuranetin + 5 × OAc − H]$^+$ (2), 273 [rham + 3 × OAc − H]$^+$ (80), 213 [273-HOAc]$^+$ (30), 153 [213 − HOAc]$^+$ (100), 110 [153 − COMe]$^+$ (76), 151 [A_1 − H]$^+$ (30), 132 [B_3]$^+$ (10), 119 [B_3 − Me]$^+$ (8).

Isosakuranetin (**1b**). Mp 191°, UV, MS and ^{13}C NMR spectral data as the lit., ^{1}H NMR spectrum is given in Table 1.

Acknowledgements—The authors thank TUBITAK (Basic Science Research Institute) (Gebze, Turkey) for ^{1}H and ^{13}C NMR spectra and Prof. Dr F. Bohlmann (T.U. Berlin) for mass spectra.

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TWO FLAVANONES FROM *CITRUS* SPECIES

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Key Word Index—*Citrus*; Rutaceae; flavanones; hiravanone; yukovanol; 3-methylbut-2-enyl.

Abstract—The new flavanones, hiravanone and yukovanol were isolated from root extracts of some *Citrus* species and structures were determined by spectrometric and synthetic methods.

INTRODUCTION

In our phytochemical studies of the root of *Citrus* plants, we have isolated many kind of coumarins, acridones, and flavanones [1]. In a continuation of these studies two new flavanones named hiravanone (**1**) and yukovanol (**5**) were isolated and characterized from roots of *Citrus* species.

RESULTS AND DISCUSSION

Hiravanone (**1**) was obtained from the root extract of several hybrid seedlings resulting from crosses of *C. tamurana* and *C. kinokuni*, as a palc yellow oil, $[\alpha]_D$ 0° ($CHCl_3$). The IR and ^{1}H NMR spectra showed the presence of a conjugated carbonyl, a methoxyl, three hydroxyl, and two 3-methylbut-2-enyl moieties. An observation of a characteristic ABX signals at δ5.31 (*dd*, *J* = 3.4 and 12.8 Hz), 3.05 (*dd*, *J* = 12.8 and 17.2 Hz), and 2.80 (*dd*, *J* = 3.4 and 17.2 Hz) together with the hydrogen-bonded hydroxy proton signal suggested the presence of a flavanone nucleus in the molecule. A remaining 3H-multiplet at δ6.94 was attributable to protons of a 1,3,4-trisubstituted aromatic ring. The appearances of diagnostic mass fragment peaks at *m/z* 288 and 150 produced by a retro-Diels–Alder process at the B-ring in the flavanone skeleton [2], suggested the location of two 3-methylbut-2-enyl moieties on the A-ring and a hydroxyl and a methoxyl group on the C-ring. Based on these spectral

data coupled with the biogenetic considerations [2], we proposed the structure **1** or **2** for hiravanone. The yield of hiravanone from the plant was so small that we could not carry out further spectroscopic analyses of hiravanone itself and because of an overlapping of aromatic proton signals in the ^{1}H NMR spectrum of hiravanone, the NOE technique could not be applied. Thus, in order to confirm the relative location of the hydroxyl and methoxyl groups, syntheses of **1** and **2** were attemped. Treatment of homoeriodictyol (**3**) [3] and hesperetin (**4**) [4] derived from the reactions between phloroglucinol and the benzaldehydes having suitable substituents with 3-methylbut-2-enyl bromide on alumina surface [5] gave **1** and **2**, respectively. The IR and ^{1}H NMR spectra of **1** was found to be identical with those of hiravanone from natural sources. Consequently, the structure of hiravanone was confirmed as **1**.

Yukovanol (**5**), a colourless amorphous powder, $[\alpha]_D$ $-47°$ ($CHCl_3$), was isolated from root extracts of *C. funadoko*, *C. yuko*, and several hybrid seedlings resulting from crosses of *C. hassaku* and *C. grandis*. A molecular formula of $C_{20}H_{18}O_6$ was indicated by a HRMS. Compound **5** was shown to have a 3-hydroxy-4′,5,7-trioxygenated flavanone nucleus having a dimethylpyran ring by UV, IR, and ^{1}H NMR spectroscopy (see Experimental). The MS fragment peaks at *m/z* 219 and 136 produced by a retro-Diels–Alder process suggested the location of a dimethylpyran ring on the A-ring. To confirm the orientation of the dimethylpyran ring, a long-range selective proton decoupling (LSPD) method in the ^{1}H NMR spectrum was carried out. Irradiation of the hydrogen-bonded 5-OH proton at δ_H11.7 destroyed the double doublets at δ_C98.31 (J=164.4 and *ca* 5 Hz) having a proton at δ_H 5.93 to a doublet (J=164.4 Hz) indicating the angular orientation of the dimethylpyran ring. And the *trans* stereochemistry between H-2 and H-3 was deduced by J values (11.6 Hz) and the absence of NOE between these protons. From these results the structure **5**, corresponding to a 3′,4′-dehydroderivative of phellamuretin [6], was proposed for yukovanol except for the absolute stereochemistry. Bohlmann *et al.* also proposed structure **5** for 4-oxoobovatachromene but this was based only on biogenetic considerations [7] and spectral comparisons (IR, ^{1}H NMR, MS) did not allow confirmation that 4-oxoobovatachromene and yukovanol are indeed identical.

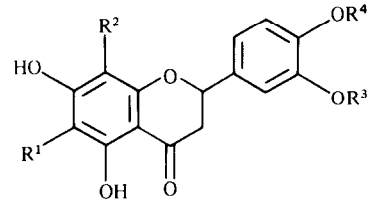
EXPERIMENTAL

Plants material was grown in the orchard of Okitsu Branch, Fruit Tree Research Station, Ministry of Agriculture, Forestry and Fisheries (Shizuoka). ^{1}H NMR (270 MHz) and ^{13}C NMR (100 MHz): in $CDCl_3$ (unless otherwise stated) with TMS as an int. standard. UV: in MeOH and IR: in $CHCl_3$.

Separation of flavanones. The Me_2CO extract of dried roots of each *Citrus* plant was subjected to silica gel CC and then prep. TLC to obtain **1** in 0.0017% yield from several hybrid seedlings resulting from *C. tamurana* Hort. ex Takahashi (hyuganatsu) × *C. kinokuni* Hort. ex Tanaka (hirakishu) and **5** in 0.0010, 0.0018, and 0.042% yields from several hybrid seedlings resulting from *C. hassaku* Hort. ex Tanaka (hassaku) × *C. granadis* Osbeck (hiratobuntan), *C. funadoko* Hort. ex Y. Tanaka (funadoko), and *C. yuko* Hort. ex Tanaka (yuko), respectively.

Hiravanone (**1**). Pale yellow oil, $[\alpha]_D$ 0° ($CHCl_3$). ^{1}H NMR δ: 12.34 (1H, *s*, hydrogen-bonded 5-OH), 6.94 (3H, *m*, arom. H), 6.37 (1H, *s*, OH), 5.68 (1H, *s*, OH), 5.31 (1H, *dd*, J=12.8 and 3.4 Hz, H-2), 3.92 (3H, *s*, OMe), 3.05 (1H, *dd*, J=12.8 and 17.2 Hz, H-3), 2.80 (1H, *dd*, J=3.4 and 17.2 Hz, H-3), and [δ5.24 (1H, *t*, J=7.1 Hz), 5.23 (1H, *t*, J=7.1 Hz), 3.35 (2H, *d*, J=7.1 Hz), 3.30 (2H, *d*, J=7.1 Hz), 1.82 (3H, *s*), 1.75 (3H, *s*), and 1.70 (6H, *s*): 2 × 3-methylbut-2-enyl]; IR ν_{max} cm^{-1}: 3550, 3400 (*br*), 1630; UV λ_{max} nm: 230, 294, 336; EIMS *m/z* 438 (M^+, 100%), 423 (20), 383 (48), 370 (12), 367 (24), 327 (24), 288 (8), 273 (20), 260 (12), 246 (16), 233 (32), 232 (12), 231 (12), 217 (20), 189 (28), 177 (24), 150 (8); HRMS *m/z* 438.2047 (M^+, calcd for $C_{26}H_{30}O_6$: 438.2040).

Yukovanol (**5**). Colourless amorphous, $[\alpha]_D-47°$ ($CHCl_3$: *c* 0.053). ^{1}H NMR (400 MHz, Me_2CO-d_6) δ: 11.78 (1H, *s*, hydrogen-bonded 5-OH), 8.58 (1H, *br*, OH), 5.93 (1H, *s*, H-6), 5.16 (1H, *d*, J=11.6 Hz, H-2), 4.78 (1H, *d*, J=4.0 Hz, disappeared with D_2O, 3-OH), 4.69 (1H, *dd*, J=4.0 and 11.6 Hz, H-3), [7.45 and 6.91 (each 2H, *d*, J=8.5 Hz): 1,4-disubst. arom. H], [6.44 (1H, *d*, J=10.4 Hz), 5.60 (1H, *d*, J=10.4 Hz), 1.43 (3H, *s*), 1.41 (3H, *s*): dimethyl pyran]; ^{1}H NMR (400 MHz) δ: 11.26 (1H, *s*, OH), 7.42 (2H, *d*, J=8.6 Hz), 6.88 (2H, *d*, J=8.6 Hz), 6.71 (1H, *s*),



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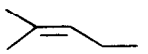
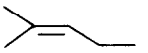
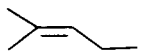
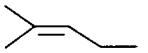
1			Me	H
2			H	Me
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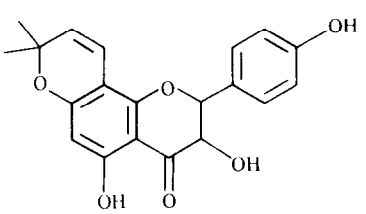

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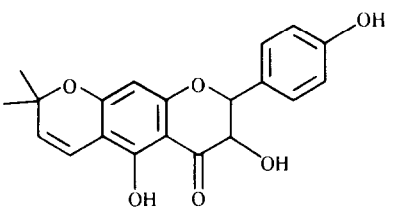

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6.48 (1H, *d*, $J = 10.4$ Hz), 6.04 (1H, *s*), 5.47 (1H, *d*, $J = 10.4$ Hz), 5.02 (1H, *d*, $J = 11.6$ Hz), 4.53 (1H, *d*, $J = 11.6$ Hz), 3.61 (1H, *br*), 1.45 (3H, *s*), 1.42 (3H, *s*); ^{13}C NMR (Me_2CO-d_6) δ: 199.15 (*s*), 164.81 (*s*), 163.61 (*s*), 159.40 (*s*), 158.33 (*s*), 151.66 (*s*), 130.73 (*d*), 129.40 (*s*), 128.09 (*s*), 117.08 (*d*), 116.47 (*d*), 116.32 (*d*), 103.16 (*s*), 102.44 (*s*), 98.31 (*d*), 84.90 (*d*), 79.53 (*s*), 73.58 (*d*), 29.12 (*q*), 28.87 (*q*); IR ν_{max} cm^{-1}: 3400, 1710, 1640, 1620, 1580; UV λ_{max} nm: 227, 272, 296, 310, 359; EIMS *m/z* 354 (M^+, 27%), 339 (43), 321 (18), 219 (59), 218 (8), 203 (100), 192 (10), 177 (30), 136 (8); HRMS *m/z* 354.1120 (M^+, calcd for $C_{20}H_{18}O_6$: 354.1103).

Synthesis of hiravanone (**1**) *from* **3**. A solution of **3** (0.10 g) in EtOAc was added to a basic Al_2O_3 (2.41 g). The solvent was evapd to dryness, and 3-methylbut-2-enyl bromide (0.28 g) in *n*-hexane–ether (1:1) (12 ml) added and left for 68 hr. Al_2O_3 was filtered off and washed with CH_2Cl_2. The combined organic layer was evapd and the residue subjected to prep. TLC to give **1** as a colourless oil (24 mg) (17% yield), which was found to be identical with natural hiravanone by IR, ^{1}H NMR and co-TLC comparisons.

Synthesis of **2** *from* **4**. A soln of **4** (0.10 g) in EtOAc was added to basic Al_2O_3 (2.41 g). The solvent was evapd to dryness, and 3-methylbut-2-enyl bromide (0.28 g) in *n*-hexane–Et_2O (1:1) (12 ml) was added and left overnight. Al_2O_3 was filtered off and washed with CH_2Cl_2. The combined organic layer was evapd and the residue subjected to prep. TLC to give **2** (7 mg) as a colourless oil; ^{1}H NMR δ: 12.33 (1H, *s*, OH), 7.04 (1H, *d*, $J = 1.5$ Hz), 6.91 (1H, *dd*, $J = 1.5$ and 8.8 Hz), 6.87 (1H, *d*, $J = 8.8$ Hz), 6.36 (1H, *s*, OH), 5.69 (1H, *br*, OH), 5.29 (1H, *dd*, $J = 12.5$ and 3.0 Hz), 5.23 (1H, *t*, $J = 7.1$ Hz), 5.19 (1H, *t*, $J = 7.1$ Hz), 3.92 (3H, *s*), 3.34 (2H, *d*, $J = 7.1$ Hz), 3.30 (2H, *d*, $J = 7.1$ Hz), 3.02 (1H, *dd*, $J = 12.5$ and 16.9 Hz), 2.79 (1H, *dd*, $J = 3.0$ and 16.9 Hz), 1.81 (3H, *s*), 1.74 (3H, *s*), 1.72 (6H, *s*); IR ν_{max} cm^{-1}: 3550, 3400 (*br*), 1640, 1520; UV λ_{max} nm: 231, 290, 340; EIMS *m/z* 438 (M^+, 100%), 423 (21), 395 (10), 383 (45), 370 (14), 367 (41), 339 (17), 327 (27), 288 (7), 273 (31), 260 (21), 246 (24), 233 (55), 232 (27), 231 (27), 217 (48), 189 (56), 177 (66), 150 (4).

Acknowledgements—We are grateful to Prof. Dr F. Bohlmann for providing the spectral data (IR, ^{1}H NMR, MS) of 4-oxoobovatachromene, and also thank Mr K. Masuda (Analytical Center of Meijo University) for measurements of HRMS. Financial support from the Ministry of Education, Science and Culture of Japan is gratefully acknowledged.

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TWO FLAVANONES FROM THE ROOT BARK OF *LESPEDEZA DAVIDII*

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Key Word Index—*Lespedeza davidii*; Leguminosae; root bark; lespedezaflavanone C; lespedezaflavanone D.

Abstract—Two new flavanones have been isolated from the root bark of *Lespedeza davidii* and their structures established as 8,3′-di-γ,γ-dimethylallyl-5,7,4′-trihydroxy-(2*R*,3*R*)-flavanonol and 8,5′-di-γ,γ-dimethylallyl-5,7,2′4′-tetrahydroxy-(2*S*)-flavanone on the basis of spectroscopic evidence.

INTRODUCTION

The roots and leaves of *Lespedeza davidii* Franch., which grows in Zhejiang province, have been used as a Chinese drug, he-xue-dan, for the treatment of dysentery and fever. In a previous communication, we reported that two new flavanones, lespedezaflavanone A and lespedezaflavanone B, had been obtained from this plant [1]. During further studies on the same species, we have now isolated two additional new flavanones.

RESULTS AND DISCUSSION

Lespedezaflavanone C (**1**). The IR spectrum showed strong absorptions at 1634 cm^{-1} (chelated C=O group) and 3430 cm^{-1} (OH). The UV spectrum (λ_{max}^{MeOH} 296 nm) suggested a flavanone structure [2]. Its ^{1}H NMR spectrum showed four hydroxy groups (C-5, C-7, C-4′ and C-3), four aromatic protons (C-6, C-2′, C-5′ and C-6′) [3]. It also indicated the presence of two γ,γ-dimethylallyl groups [4].