A New Flavanone with Antifungal Activity Isolated from Hops[†]

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6-Isopentenylnaringenin **1**, which has previously been synthesized by other workers, was isolated together with xanthohumol **2** and isoxanthohumol **3** from hard resins of hops (*Humulus lupulus* L.). The structures of **1** and sophoraflavanone B were examined; that of the latter previously reported as 6-isopentenylnaringenin, has been revised to 8-isopentenylnaringenin. **1**, **2** and **3** were found to have antifungal activities.

During a study of the antimicrobial activities of bitter resins of hops (*Humulus lupulus* L.) and related compounds, we found that constituents of hard resins of hops inhibited the growth of *Trichophyton* spp., pathogenic fungi in humans, and other microorganisms.

This paper reports the structural elucidation of **1** isolated from the hard resins of hops, and gives a revised structure for sophoraflavanone B.¹⁾ The antifungal activities of **1** and related compounds are also discussed.

MATERIALS AND METHODS

All melting points are given as uncorrected values. UV spectra were determined by a Hitachi 624 spectrometer. ¹H-NMR spectra were recorded on a JEOL FX-100 spectrometer at 100 MHz with tetramethylsilane as an internal standard. Most ¹³C-NMR spectra were recorded on a JEOL FX-100 at 25 MHz, and some ¹³C-NMR spectra were recorded on a JEOL JX-400 spectrometer at 100 MHz in the same way. Mass spectra were obtained with a Hitachi M-80 spectrometer (direct inlet system). The optical rotations were determined with a JASCO DIP-140 polarimeter. Thin-layer chromatography (TLC) was carried out on Merck silica gel plates (art. 5731).

The minimum inhibitory concentrations of each compound were determined by the dilution method using nutrient agar medium. For fungi, a medium consisting of 1% peptone, 4% glucose and 1.5% agar was used, and the cultures were incubated at 27°C for 7 days. For bacteria, the medium consisted of 1% peptone, 0.7% meat extract,

0.3% NaCl and 1.5% agar, and the cultures were incubated at 37°C for 20 hr. The organisms tested are listed in Table I.

1. Isolation of xanthohumol **2 and isoxanthohumol **3**.** Hard resins (8 g), prepared from Saaz hops (700 g), were chromatographed on Dowex 1-X4 (AcOH form). After the column had been eluted with 80% MeOH, the fractions which were eluted with 1% AcOH in 80% MeOH and 5% AcOH in 80% MeOH were evaporated to obtain a brown solid (195 mg) and a yellow solid (1.8 g), respectively. The yellow solid was washed with cold ether (50 ml) to obtain a pale yellow powder (550 mg). The washing solutions were combined and evaporated to obtain a crude product which was recrystallized from aqueous acetic acid to give **2** (630 mg) as yellow crystals: mp 171~172.5°C (lit.,^{2,3)} 171°C).

The pale yellow powder (550 mg) was recrystallized from aqueous acetic acid to give **3** (340 mg) as colorless prisms: mp 197.5~198°C (lit.,^{2,3)} 197~198°C).

2. Isolation of **1 from hard resins of hops.** The brown solid (195 mg) mentioned above was dissolved in ether (100 ml) and extracted with aqueous 10% Na₂CO₃ (3 × 30 ml). The extract was acidified with 1N HCl and re-extracted with ether (100 ml). The ether layer was washed with water, dried over Na₂SO₄ and concentrated to about 50 ml. Hexane was added to the concentrate at 0°C to obtain **1** (19 mg) as colorless crystals: mp 209~209.5°C (recrystallized from ether-hexane); $[\alpha]_D^{25} = 0^\circ$ (*c* = 0.1, MeOH); MS *m/z*: 340 (M⁺, 85%), 297 (20), 285 (15), 220 (20), 205 (45), 192 (32), 177 (22), 165 (100), 120 (26); IR $\gamma_{\max}^{\text{KBr}}$ cm⁻¹: 3250 (OH), 1630 (C=O), 1585, 1520 (arom. C=C); UV $\lambda_{\max}^{\text{MeOH}}$ nm (ϵ): 294 (17,700); $\lambda_{\max}^{\text{MeOH} + \text{NaOH}}$ nm: 332; $\lambda_{\max}^{\text{MeOH} + \text{AlCl}_3}$ nm: 315; $\lambda_{\max}^{\text{MeOH} + \text{AlCl}_3 + \text{HCl}}$ nm: 313;

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$\lambda_{\text{max}}^{\text{MeOH} + \text{AcONa}}$ nm: 330; $\lambda_{\text{max}}^{\text{MeOH} + \text{AcONa} + \text{H}_3\text{BO}_3}$ nm: 292; $\lambda_{\text{max}}^{\text{MeOH} + \text{MeONa}}$ nm: 332; $^1\text{H-NMR } \delta$ (acetone- d_6): 1.65, 1.76 (each 3H, 2s, =C(CH₃)₂), 2.71 (1H, dd, J =17.1, 3.4 Hz, 3-Ha), 3.20 (1H, dd, J =17.1, 12.7 Hz, 3-Hb), 3.24 (2H, d, J =8.0 Hz, Ar-CH₂), 5.24 (1H, br.t, J =8.0 Hz, =CH), 5.40 (1H, dd, J =12.7, 3.4 Hz, 2-H), 6.03 (1H, s, 8-H), 6.90 (2H, d, J =8.8 Hz, 3'- and 5'-H), 7.39 (2H, d, J =8.8 Hz, 2'- and 6'-H), 8.50, 9.54 (2H, 2s, OH $\times$ 2), 12.47 (1H, s, 5-OH); δ (CDCl₃): 1.77, 1.84 (each 3H, 2s, =C(CH₃)₂), 2.76 (1H, dd, 3-Ha), 3.09 (1H, dd, 3-Hb), 3.35 (2H, d, Ar-CH₂), 5.25 (1H, br.t, =CH), 5.33 (1H, dd, 2-H), 6.00 (1H, s, 8-H), 6.87 (2H, d, 3'- and 5'-H), 7.33 (2H, d, 2'- and 6'-H), 12.39 (1H, s, 5-OH); $^{13}\text{C-NMR } \delta$ (DMSO- d_6): 17.5 (q, 14-C), 20.6 (t, 11-C), 25.4 (q, 15-C), 42.1 (t, 3-C), 78.4 (d, 2-C), 94.5 (d, 8-C), 101.7 (s, 10-C), 107.7 (s, 6-C), 115.3 (d, 3'- and 5'-C), 122.8 (d, 12-C), 128.2 (d, 2'- and 6'-C), 129.2 (s, 1'-C), 130.2 (s, 13-C), 157.9 (s, 4'-C), 160.7 (s, 5- and 9-C), 164.4 (s, 7-C), 196.5 (s, 4-C); $^{13}\text{C-NMR } \delta$ (DMSO- d_6 , proton coupled): 94.5 (d, J =161.5 Hz, 8-C), 101.7 (br.t, J =4.5 Hz, 10-C), 107.7 (br.t, J =4.5 Hz, 6-C); $^{13}\text{C-NMR } \delta$ (DMSO- d_6 , decoupled at the 5-OH proton frequency): 94.5 (d, J =161.5 Hz, 8-C), 101.7 (d, J =4.5 Hz, 10-C), 107.7 (d, J =4.5 Hz, 6-C); R_f =0.51 (CHCl₃-MeOH = 9:1); R_f =0.54 (benzene-AcOEt = 1:1); blue-black with ferric chloride in EtOH.

3. *Synthesis of 1 and sophoraflavanone B 4.* A mixture of naringenin (2.5 g) and sodium methoxide (540 ng) in methanol (50 ml) and ether (100 ml) was treated with isopentenyl bromide (1.7 g) in the usual way. The reaction mixture was concentrated to dryness, and extracted with cold ether. The ether extract (about 1 g) was chromatographed on Dowex 1-X4 in the same way as described in Section 1 to obtain compound B (330 mg, from the fraction eluted with 0.5% AcOH/80% MeOH) and compound A (210 mg, from the fraction eluted with 1% AcOH/80% MeOH).

These compounds had the following characteristics. Compound B: mp 193~193.5°C (colorless crystals from aqueous methanol); MS m/z : 340 (M^+ , 100%), 297 (20), 285 (46), 220 (24), 205 (50), 192 (36), 177 (32), 165 (96), 120 (30); IR $\gamma_{\text{max}}^{\text{KBr}}$ cm⁻¹: 3350 (OH), 1630 (C=O), 1600, 1520 (arom. C=C); UV $\lambda_{\text{max}}^{\text{MeOH}}$ nm (ϵ): 295 (18,100); $\lambda_{\text{max}}^{\text{MeOH} + \text{NaOH}}$ nm: 336; $\lambda_{\text{max}}^{\text{MeOH} + \text{AlCl}_3 + \text{HCl}}$ nm: 314; $\lambda_{\text{max}}^{\text{MeOH} + \text{AlCl}_3 + \text{HCl}}$ nm: 315; $\lambda_{\text{max}}^{\text{MeOH} + \text{AcONa}}$ nm: 332; $\lambda_{\text{max}}^{\text{MeOH} + \text{AcONa} + \text{H}_3\text{BO}_3}$ nm: 291; $\lambda_{\text{max}}^{\text{MeOH} + \text{MeONa}}$ nm: 336; $^1\text{H-NMR } \delta$ (acetone- d_6): 1.60 (6H, s, =C(CH₃)₂), 2.75 (1H, dd, J =17.3, 3.5 Hz, 3-Ha), 3.15 (1H, dd, J =17.3, 12.3 Hz, 3-Hb), 3.22 (2H, d, J =8.3 Hz, Ar-CH₂), 5.19 (1H, br. t, J =8.3 Hz, =CH), 5.43 (1H, dd, J =12.3, 3.5 Hz, 2-H), 6.02 (1H, s, 6-H), 6.91 (2H, d, J =8.6 Hz, 3'- and 5'-H), 7.42 (2H, d, J =8.6 Hz, 2'- and 6'-H), 8.54, 9.62 (2H, 2s, OH $\times$ 2), 12.14 (1H, s, 5-OH); δ (DCDCl₃): 1.73 (6H, s, =C(CH₃)₂), 2.78 (1H, dd, 3-Ha), 3.07 (1H, dd, 3-Hb), 3.31 (2H, d, Ar-CH₂), 5.21 (1H, br.t, =CH), 5.36 (1H, dd, 2-H), 6.03 (1H, s, 6-H), 6.88 (2H, d, 3'- and 5'-H), 7.34 (2H, 2'- and 6'-H), 11.99 (1H, s, 5-OH); ^{13}C -

NMR δ (DMSO- d_6): 17.5 (q, 14-C), 21.2 (t, 11-C), 25.4 (q, 15-C), 42.2 (t, 3-C), 78.2 (d, 2-C), 95.5 (d, 6-C), 101.9 (s, 10-C), 107.1 (s, 8-C), 115.3 (d, 3'- and 5'-C), 122.9 (d, 12-C), 128.0 (d, 2'- and 6'-C), 129.4 (s, 1'-C), 130.2 (d, 13-C), 157.7 (s, 4'-C), 159.9 (s, 9-C), 161.4 (s, 5-C), 164.5 (s, 7-C), 196.7 (s, 4-C); R_f =0.55 (CHCl₃-MeOH = 9:1); R_f =0.59 (benzene-AcOEt = 1:1); blue-black with ferric chloride in EtOH.

These analytical data were in good agreement with those of sophoraflavanone B.¹⁾ Compound A: The compound obtained in this way had the same characteristics as 1 isolated from hard resins of hops.

$^{13}\text{C-NMR } \delta$ (DMSO- d_6) of naringenin 9: 41.9 (t, 3-C), 78.3 (d, 2-C), 94.9 (d, 8-C), 95.7 (d, 6-C), 101.8 (s, 10-C), 115.2 (d, 3'- and 5'-C), 128.1 (d, 2'- and 6'-C), 128.9 (s, 1'-C), 157.7 (s, 4'-C), 162.9 (s, 9-C), 163.5 (s, 5-C), 166.7 (s, 7-C), 196.3 (s, 4-C); UV of naringenin 9: $\lambda_{\text{max}}^{\text{MeOH}}$ nm (ϵ): 288 (16,500); $\lambda_{\text{max}}^{\text{MeOH} + \text{NaOH}}$ nm: 324; $\lambda_{\text{max}}^{\text{MeOH} + \text{AlCl}_3}$ nm: 311; $\lambda_{\text{max}}^{\text{MeOH} + \text{AlCl}_3 + \text{HCl}}$ nm: 310; $\lambda_{\text{max}}^{\text{MeOH} + \text{AcONa}}$ nm: 323; $\lambda_{\text{max}}^{\text{MeOH} + \text{AcONa} + \text{H}_3\text{BO}_3}$ nm: 289; $\lambda_{\text{max}}^{\text{MeOH} + \text{MeONa}}$ nm: 324.]

4. *Preparation of dihydroisoxanthohumol 5.* A mixture of isoxanthohumol 3 (60 mg) and platinum oxide (8 mg) in methanol (30 ml) was treated in an atmosphere of hydrogen to obtain 5 (51 mg, mp 176~177°C, colorless needles from aq. MeOH).

5. *Preparation of 6-isopentylnaringenin 6.* Compound A (30 mg) described in Section 3 was hydrogenated in the presence of platinum oxide (6 mg) to obtain 6 (26 mg, mp 223.5~224°C, colorless crystals from aq. MeOH). MS m/z : 342 (M^+ , 73%), 165 (100); IR $\gamma_{\text{max}}^{\text{KBr}}$ cm⁻¹: 3150 (OH), 1630 (C=O), 1590, 1520 (arom. C=C); UV $\lambda_{\text{max}}^{\text{MeOH}}$ nm (ϵ): 294 (17,800); $^1\text{H-NMR } \delta$ (acetone- d_6): 0.92 (6H, d, J =6.1 Hz, CH₃ $\times$ 2), 1.24~1.80 (3H, m, -CH₂-CH), 2.58 (2H, t, J =7.5 Hz, Ar-CH₂).

6. *Preparation of 8-isopentylnaringenin 7.* Dihydroisoxanthohumol 5 (40 mg) was dissolved in dry nitrobenzene (6 ml). Freshly powdered aluminum chloride (30 mg) was then added in three portions and the mixture was kept at 90~100°C for 1.5 hr. The mixture was diluted with water (50 ml), and the precipitated solid was chromatographed on Dowex 1-X4 in the same way as described in Section 1 to obtain 7 (16 mg, from the fraction eluted with 0.5% AcOH/80% MeOH).

7 was also obtained by hydrogenation of compound B (30 mg) in the presence of platinum oxide (5 mg) as described in Section 4. The product was recrystallized from aqueous methanol to obtain 4 (26 mg) as colorless flakes: mp 195.5~196°C; MS m/z : 342 (M^+ , 84%), 165 (100); IR $\gamma_{\text{max}}^{\text{KBr}}$ cm⁻¹: 3300 (OH), 1630 (C=O), 1600, 1510 (arom. C=C); UV $\lambda_{\text{max}}^{\text{MeOH}}$ nm (ϵ): 294 (17,100); $^1\text{H-NMR } \delta$ (acetone- d_6): 0.87 (6H, d, J =6.0 Hz, CH₃ $\times$ 2), 1.20-1.75 (3H, m, -CH₂-CH), 2.56 (2H, t, J =7.5 Hz, Ar-CH₂).

7. *Preparation of 8-isopentyl-5,7,4'-trimethoxyxynarin-*

genin **8**. A mixture of dihydroisoxanthohumol **5** (45 mg), dimethyl sulfate (0.05 ml) and anhydrous K_2CO_3 (0.5 g) in acetone (15 ml) was refluxed for 6 hr. The product was chromatographed on a silica gel TLC plate with a mixture of $CHCl_3$ -hexane (1:1) as the solvent, and a colorless band with no ferric reaction at $R_f=0.2$ was extracted with chloroform and concentrated to obtain a white solid. The solid was recrystallized from aqueous methanol to obtain **8** (11 mg) as colorless prisms: mp $95.5 \sim 96^\circ C$; MS m/z : 384 (M^+ , 51%), 193 (100); IR $\nu_{max}^{CHCl_3}$ cm^{-1} : 1660 (C=O), 1600, 1570, 1510 (arom. C=C); UV λ_{max}^{MeOH} nm (ϵ): 288 (17,100); 1H -NMR δ ($CDCl_3$): 0.88 (6H, d, $J=6.0$ Hz, $CH_3 \times 2$), 1.2-1.6 (3H, m, $-CH_2-CH$), 2.57 (t, $J=7.6$ Hz, Ar- CH_2), 2.78 (1H, dd, $J=16.6, 4.9$ Hz, 3-Ha), 3.00 (1H, dd, $J=16.6, 11.03$ Hz, 3-Hb), 3.83, 3.89, 3.93 (each 3H, 3s, $OCH_3 \times 3$), 5.33 (1H, dd, $J=11.03, 4.9$ Hz, 2-H), 6.12 (1H, s, 6-H), 6.93 (2H, d, $J=8.8$ Hz, 3'- and 5'-H), 7.38 (2H, d, $J=8.8$ Hz, 2'- and 6'-H).

8 (13 mg) was also obtained by methylation of 8-isopentenylnaringenin **7** (110 mg) with dimethyl sulfate (0.1 ml) and anhydrous K_2CO_3 (1 g) in acetone (15 ml) in the same way.

RESULTS AND DISCUSSION

1. Structure of **1** isolated from hard resins

Compound **1** was obtained as colorless crystals (mp $209 \sim 209.5^\circ C$, $M^+ = m/z$ 340). Its IR gave absorption bands for the hydroxyl, conjugated carbonyl and benzene groups. The 1H -NMR of **1** (acetone- d_6) revealed the presence of an isopentenyl group, the 2- and 3-proton of flavanone, four aromatic protons of 1', 4'-disubstituted benzene, one aromatic proton [δ

6.03 (1H, s)], and three hydroxyl protons, one being chelated at $\delta=12.47$. The MS of **1** showed fragments by the retro-Diels-Alder fragmentation of flavanone⁴) at m/z 220 (A-ring) and at m/z 120 (B-ring), suggesting the presence of an isopentenyl group on the A-ring, and one hydroxyl group on the B-ring, respectively. The UV shifts of **1** after adding shifting reagents⁵) suggested the presence of a naringenin (5,7,4'-trihydroxyflavanone) structure. The possibility of this naringenin structure is also supported by the appearance in the ^{13}C -NMR spectrum of fifteen signals attributable to the carbons of naringenin.

These findings show that **1** is a naringenin derivative with one isopentenyl group at 6-C or 8-C.

The position of the substituent was determined from the long-range decoupled ^{13}C -NMR spectra of **1**. Irradiation at the 5-OH proton frequency changed two broad triplets at δ 101.7 ($J=4.5$ Hz, 10-C) and δ 107.7 ($J=4.5$ Hz, 6-C) in the proton-coupled spectrum, respectively, into two doublets ($J=4.5$ Hz), but did not change the doublet at δ 94.5 ($J=161.5$ Hz, 8-C).

Wehrli⁶) has reported that the proton-coupled ^{13}C -NMR spectra of naringenin exhibited spin-spin coupling between the 6-C and 10-C carbons and the chelated 5-OH proton, but not between the 8-C carbon and the 5-OH proton, and thus permitting identification of these carbon atoms. Therefore, the structure of **1** is represented as 6-isopentenylnaringenin.

This is the same structure as that proposed for sophoraflavanone B by other workers.¹⁾ However, the spectral data for sophoraflavanone B were not identical with those for **1**. Nevertheless, it is clear that the two compounds are structurally very similar, each having the same naringenin structure and isopentenyl group, as revealed by 1H and ^{13}C -NMR data. A possible difference between the two compounds is in the position of the isopentenyl group, and sophoraflavanone B is assumed to be an isomer of **1**, that is, 8-isopentenylnaringenin **4**.

6- and 8-isopentenylnaringenin have pre-

FIG. 1.

TABLE I. ANTIFUNGAL ACTIVITIES OF 1~4 AND 9

Organisms tested	Minimum inhibitory concentrations ($\mu\text{g/ml}$)					Griseofulvin
	1	2	3	4	9	
<i>Trichophyton mentagrophytes</i>	3.13	3.13	200	6.25	200	6.25
<i>Trichophyton rubrum</i>	3.13	3.13	>200	12.5	>200	6.25
<i>Candida albicans</i>	>200	>200	>200	>200	>200	>200
<i>Fusarium oxysporum</i>	>200	>200	>200	200	>200	>200
<i>Mucor rouxianus</i>	50	50	>200	12.5	100	>200
<i>Staphylococcus aureus</i>	6.25	6.25	50	25	100	>200
<i>Escherichia coli</i>	>200	>200	>200	>200	>200	>200

group into naringenin increases its antifungal activities against *Trichophyton* and *Mucor* spp., and the introduction of a methyl group into 5-OH significantly decreases the activity. Furthermore, 6-isopentenylnaringenin **1** was more active than 8-isopentenylnaringenin **4** against *Trichophyton* spp., but less active than the latter against *Fusarium* and *Mucor* spp. All compounds **1**~**4** and **9** were active against *Staphylococcus* spp.

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REFERENCES

- 1) M. Komatu, I. Yokoe and Y. Shirataki, *Chem. Pharm. Bull.*, **26**, 3863 (1978).
- 2) M. Verzele, J. Stockx, F. Fontijn and M. Anteunis, *Bull. Soc. Chim. Belges*, **66**, 452 (1957).
- 3) P. R. Ashurst, J. Laws and R. Stevens, *J. Inst. Brew.*, **71**, 492 (1965).
- 4) J. B. Harborne, T. J. Mabry and H. Mabry, "The Flavonoids," Chapman and Hall Ltd., London, 1975, pp. 78~126.
- 5) T. J. Mabry, K. R. Markham and M. B. Thomas, "The Systematic Identification of Flavonoids," Springer-Verlag Ltd., New York, 1970, pp. 165~226; Y. Tomita, "Zoku Jikken Kagaku Koza," Vol. 5, ed. by the Chemical Society of Japan, Maruzen Co., Ltd., Tokyo, 1966, pp. 940~942.
- 6) F. W. Wehrli, *J. Chem. Soc., Chem. Commun.*, 663 (1975).
- 7) A. C. Jain, R. C. Gupta and P. D. Sarpal, *Tetrahedron*, **34**, 3563 (1978).
- 8) A. C. Jain, R. C. Gupta and P. D. Sarpal, *Chem. Lett.*, 995 (1978).
- 9) A. Nagar, V. K. Gujral and S. R. Guputa, *Tetrahedron Lett.*, 2031 (1978).
- 10) T. R. Seshadri, "The Chemistry of Flavonoid Compounds," ed. by T. A. Geissman, Pergamon Press Inc., Oxford, 1962, p. 184.
- 11) R. C. Shah, C. R. Mehta and T. S. Wheeler, *J. Chem. Soc.*, **38**, 1555 (1938).