A New Flavanone with Antifungal Activity Isolated from Hops1

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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 sophorarflavanone 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 sophorarflavanone 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 (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 37°C for 20 hr. The organisms tested are listed in Table I.

I. 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) 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.,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 x 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); [a]D25 = 0° (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 ν max cm⁻¹: 3250 (OH), 1630 (C=O), 1585, 1520 (arom. C=C); UV λmax (MeOH) nm (ε): 294 (17,700); λmax (MeOH + NaOH) nm: 332; λmax (MeOH + AlCl3) nm: 315; λmax (MeOH + AlCl3 + HCl) nm: 313;

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These compounds had the following characteristics.

3. Synthesis of 1 and sophoraflavanone B 4. A mixture of naringenin, 2.5 g, and sodium methoxide, 0.4 g, 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, 1 g, was chromatographed on Dowex 1-X4 in the same way as described in Section 4. The product was recrystallized from aqueous methanol to obtain 4, mp 223.5-224°C, colorless crystals from aq. MeOH). MS m/z: 342 (M+, 73%), 165 (100); IRγ ££cm⁻¹: 3150 (OH), 1630, 1520, arom. C=C); UV λmax nm (ε): 304 (16,500) AλH+NaOH nm: 324; λmax NH+AlCl₃ nm: 311; λmax NH+NaOH nm: 324; λmax NH+MeOH nm: 332; λmax NH+AcONa nm: 323; λmax NH+MeOH+AcONa nm: 328; λmax NH+MeOH+H₂O nm: 342.

4. Preparation of dihydroisoxanthohumol 5. A mixture of isoxanthohumol, 30 mg, and platinum oxide, 5 mg, in methanol (30 ml) was treated in an atmosphere of hydrogen to obtain 5, mp 190-191°C, colorless needles from aq. MeOH).

5. Preparation of 6-isopentynaringenin 6. Compound A, 30 mg, described in Section 3 was hydrogenated in the presence of platinum oxide (6 mg) to obtain 6, mp 226.5-227°C (colorless crystals from aq. MeOH). MS m/z: 342 (M+, 73%), 165 (100); IRγ ££cm⁻¹: 3150 (OH), 1630, 1520, arom. C=C); UV λmax nm (ε): 294 (17,800). ¹H-NMR δ (acetone-d₆): 0.92 (6H, d, J= 16,500) AλH+NaOH nm: 324; λmax NH+AlCl₃ nm: 311; λmax NH+MeOH nm: 324; λmax NH+AcONa nm: 323; λmax NH+MeOH+AcONa nm: 328; λmax NH+MeOH+H₂O nm: 342.

6. Preparation of 8-isopentynaringenin 7. Dihydroisoxanthohumol, 50 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 80-90°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, mp 190-191°C, from the fraction eluted with 10% AcOH/80% MeOH and compound A (210 mg, from the fraction eluted with 1% AcOH/80% MeOH).

7. Preparation of 8-isopentyl-5,7,4-trimethoxynarigenin
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8. A mixture of dihydroisoxanthohumol 5 (45 mg), dimethyl sulfate (0.05 ml) and anhydrous K$_2$CO$_3$ (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 95.5~96°C; MS m/z: 384 (M$^+$, 51%), 193 (100); IR $\nu_{\text{max}}$ cm$^{-1}$: 1660 (C=O), 1600, 1570, 1510 (arom. C=C); UV $\lambda_{\text{max}}$ nm ($\varepsilon$): 288 (17,100); $^1$H-NMR $\delta$ (CDCl$_3$): 0.88 (6H, d, $\delta=6.0$Hz, CH$_3$-2), 1.2-1.6 (3H, m, -CH$_2$-CH), 2.57 (t, $\delta=7.6$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$_3$-3), 5.33 (1H, dd, $J=11.0$, 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-isopentynaringenin 7 (100 mg) with dimethyl sulfate (0.1 ml) and anhydrous K$_2$CO$_3$ (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 209~209.5°C, M$^+$ = m/z 340). Its IR gave absorption bands for the hydroxyl, conjugated carbonyl and benzene groups. The $^1$H-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 [$\delta$ 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 flavanone$^4$ 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 reagents$^5$ suggested the presence of a naringenin (5,7,4'-tri hydroxyflavanone) 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 $\delta$ 101.7 ($J=4.5$ Hz, 10-C) and $\delta$ 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 $\delta$ 94.5 ($J=161.5$ Hz, 8-C).

Wehrli$^6$ 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 sophorafavanone B by other workers.$^1$ However, the spectral data for sophorafavanone 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 $^1$H and $^{13}$C-NMR data. A possible difference between the two compounds is in the position of the isopentenyl group, and sophorafavanone B is assumed to be an isomer of 1, that is, 8-isopentenylnaringenin 4.

6- and 8-isopentenylnaringenin have pre-
viously been synthesized by Jain et al.\textsuperscript{7,8}) and Nagar et al.\textsuperscript{9}) But their reported \textsuperscript{1}H-NMR data do not agree with each other. Moreover, \textsuperscript{1}H-chemical shifts in CDCl\textsubscript{3} for 6-isopentenylnaringenin were reported to be the same as those in acetone-\textit{d}_{6},\textsuperscript{7,8}) but \textsuperscript{1}H-chemical shifts in these two solvents are not the same according to our experiments. It is therefore impossible to identify 6- and 8-isopentenylnaringenin only by comparing the reported data.

To investigate the structure of sophoraflavanone B, naringenin was isopentenylated to give two monoisopentenylated compounds, A and B. The melting point, mixed melting point and the spectral data for A were completely identical with those for 1 isolated from hops, and all the spectral data for B, except the specific rotation, were in good agreement with those for sophoraflavanone B. A direct comparison of the two compounds was carried out as follows:

As shown in Chart 1, isoxanthohumol 3 was hydrogenated in methanol over platinum oxide to give dihydroisoxanthohumol 5, which was then demethylated by aluminum chloride in nitrobenzene to give 8-isopentenylnaringenin 7. The product so obtained from isoxanthohumol was found to be identical with the compound obtained by hydrogenation of B (= sophoraflavanone B), but not with the compound obtained by hydrogenation of A (= flavanone 1 isolated from hops).

No Wessely-Moser rearrangement occurred on demethylation of dihydroisoxanthohumol 5 with aluminum chloride,\textsuperscript{10,11}) because methylation of the demethylated product of dihydroisoxanthohumol and dihydroisoxanthohumol gave the same product, 8-isopentyl-5, 7, 4'-trimethoxynaringenin 8.

From these findings, the isopentenyl group of sophoraflavanone B (=compound B) is concluded to be attached at 8-C, and sophoraflavanone B is 8-isopentenylnaringenin, as represented by 4.

2. Antifungal activities of constituents of hard resins and related compound 1 ~ 4 and 9

The antifungal activities of these compounds are shown in Table I in comparison with griseofulvin.

6-Isopentenylnaringenin 1 and xanthohumol 2 showed higher antifungal activities than griseofulvin against \textit{Trichophyton} spp., and these compounds also showed slight activity against \textit{Mucor} spp. However, isoxanthohumol 3 showed only slight activity against \textit{Trichophyton} spp.

From a comparison of the activities of 6-isopentenylnaringenin 1, isoxanthohumol 3, 8-isopentenylnaringenin 4 and naringenin 9, it is clear that the introduction of an isopentenyl
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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