Chalcones from Methanol Extract of *Humulus Lupulus*

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Accepted for publication on September 12, 1990

ABSTRACT. Extracts of *Humulus Lupulus* yielded two known compounds, isoxanthohumol and xanthohumol, and two new chalcone derivatives, 3′-(isoprenyl)-2′,4-dihydroxy-4′,6′-dimethoxychalcone and 2′,6′-dimethoxy-4,4′-dihydroxychalcone. Their structures were established by spectral methods.

Key words: *Humulus Lupulus* — Moraceae — plant chemistry — isoxanthohumol — xanthohumol — 3′-(isoprenyl)-2′,4-dihydroxy-4′,6′-dimethoxychalcone — 2′,6′-dimethoxy-4,4′-dihydroxychalcone

In spite of several investigations[1-4] of estrogenic activity in Hop extract, no accorded conclusion has been drawn as to the activity in their results. Therefore, it should be worthwhile to determine the chemical component in various extracts of Hops. In the course of an identification study of the compound in a phenolic extract of Hops, we isolated already known isoxanthohumol and xanthohumol,[5,6] together with new two chalcone derivatives, 3′-(isoprenyl)-2′,4-dihydroxy-4′,6′-dimethoxychalcone and 2′,6′-dimethoxy-4,4′-dihydroxychalcone. The isolated compounds were identified by their spectroscopic (IR, UV, ^{1}HNMR and MS) data and by comparison with their data in references.

EXPERIMENTAL

Mps are uncorrected. ^{1}HNMR spectra were recorded on a Varian superconducting XL-200 FT-NMR spectrometer at 200 MHz with tetramethylsilane as an internal standard. Mass spectra were obtained with a Hitachi M-80B spectrometer (direct inlet system). UV spectra were determined by a Carry 118C spectrometer. TLC spots were detected with conc. Sulfulic acid and/or UV light after development in a mixture of CH_2Cl_2 and Me_2CO.

Plant materials. Cones of *Humulus lupulus L.* cultivar. *Shinshu-Wase* were collected from Yamagata (the northern part of Japan) at the end of August, 1985. These were frozen, crushed, sieved, and then made into pellets. Lupulin contained 5-6% α-acids before treatment, and 15 ± 0.5% in the pellets. The pellets were purchased from the Asahi Brewery Co. (Kyobashi, Tokyo) in 1986. A voucher has been deposited at the Asahi Brewery Co. (c/o MR. J. Miyata).

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Isolation of chalcones. The extract procedure for Hops is shown in Fig. 1. The pellets (800g) of *Humulus lupulus,* were extracted with n-hexane (3 ℓ) at reflux for 12 hrs and then with boiling MeOH (3 ℓ) for 24 hrs. Each extraction with n-hexane and MeOH was repeated three times. Evaporation of MeOH to dryness yielded 114 g of tarry residue. The residue was dissolved in water and extracted with diethylether. Evaporation of the ether dried over Na_2SO_4 yielded 51 g of green-black residue, which was dissolved in CH_2Cl_2 and extracted with 1.0 M NaOH. The aqueous layer was neutralized with 6 M HCl and then was extracted with ether. The ether layer was washed with water, dried over Na_2SO_4 and yielded 9.4 g (1.17 % yield) of phenolic residue after evaporation of the ether.

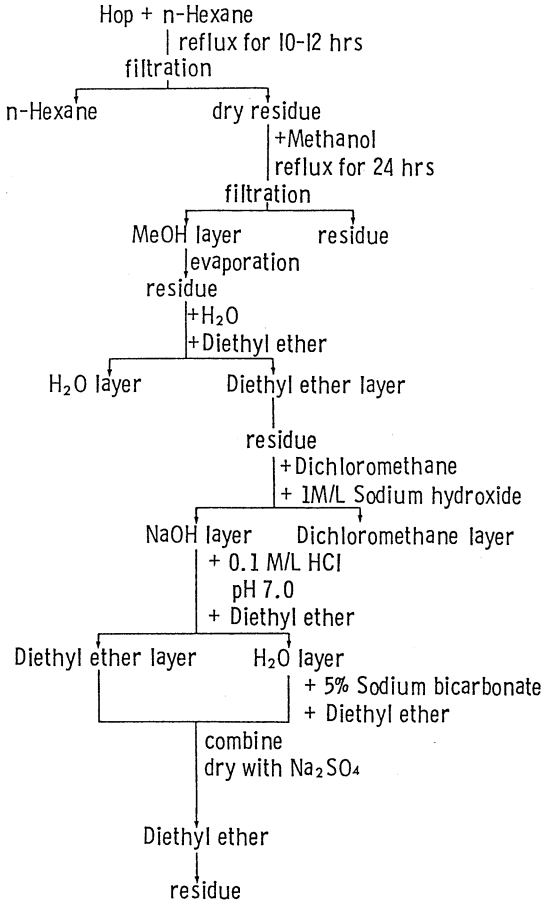

Fig. 1. Extract procedure for Hops.

Separation of chalcones. The phenolic residue of 9.4 g fractionated on a silica gel (Merk No. 7734) column (5×70 cm) was eluted with mixtures of n-hexane and EtOAc of increasing polarities, as shown in Table 1. After crude separation on the column, compounds F_3-1, F_3-2, F_5 and F_9 were further separated and/or purified on preparation TLC plates or on the column. The

compounds in the order of elution were: 3′-(isoprenyl)-2′,4-dihydroxy-4′,6′-dimethoxychalcone (F_3-1), 2′,6′-dimethoxy-4,4′-dihydroxychalcone (F_3-2), xanthohumol (F_5), and isoxanthohumol (F_9).

Xanthohumol (F_5) and isoxanthohumol (F_9) have been previously identified by their spectroscopic (IR, UV, ^{1}HNMR and MS) data and by comparison with their data in references.[5,6]

TABLE 1. Result of column chromatography of 7.06 g of the final Hop residue in Fig. 1 on silica gel.

No.	Solvent systems (volume ratios)	Weight(g)	Colour
1- 4 (F_1)	Ethyl acetate : Hexane (2 : 8)	—	—
5- 53 (F_2)	〃 〃 (2 : 8) (3 : 7)	1.57	Dark brown
54- 71 (F_3)	〃 〃 (4 : 6)	0.69	Brown
72-102 (F_4)	〃 〃 (5 : 5)	0.59	〃
103-118 (F_5)	〃 〃 (6 : 4)	0.45	〃
119-142 (F_6)	〃 〃 (6 : 4) (7 : 3)	0.34	〃
143-171 (F_7)	〃 〃 (7 : 3) (8 : 2)	0.29	〃
172-177 (F_8)	〃 〃 (8 : 2) (9 : 1)	0.57	Yellow
178-223 (F_9)	〃 〃 (9 : 1)	1.86	Yellow
224-	Methanol	0.51	Dark brown

3′-(isoprenyl)-2′,4-dihydroxy-4′,6′-dimethoxychalcone (F_3-1) and 2′,6′-dimethoxy-4,4′-dihydroxychalcone (F_3-2). Fraction F_3 eluted with EtOAc — n-hexane (4 : 6) gave a mixture of the compounds F_3-1 and F_3-2. The separation and purification of the mixture by column chromatography in CH_2Cl_2 — Me_2CO (8 : 2) gave F_3-1 and F_3-2. F_3-1; (22 mg, 0.0027 % yield: from EtOAc — n-hexane), mp 152-153°, MS m/z 368 $[M]^+$ $C_{22}H_{24}O_5$ (80), 358 (25), 325 (73), 313 (27), 283 (11), 261 (16), 248 (15), 233 (77), 219 (17), 205 (45), 193 (100), 181 (15), 163 (11), 147 (22), 119 (24), 107 (24), 91 (44), 77 (21), and 65 (26), λ_{max}^{MeOH} nm ($\log\varepsilon$): 210 (4.4) and 370 (4.6), $\lambda_{max}^{MeOH+MeONa}$ nm ($\log\varepsilon$): 446 (4.6), $\lambda_{max}^{MeOH+AlCl_3}$ nm ($\log\varepsilon$): 210 (4.4) and 370 (4.5), $\lambda_{max}^{MeOH+AlCl_3+HCl}$ nm ($\log\varepsilon$): 210 (4.4) and 414 (4.7), IR $_{max}^{KBr}$ cm^{-1} 3350, 2930, 1610, 1590, 1535, 1520, 1490, 1450, 1428, 1359, 1340, 1230, 1175, 1150, 1128, 1080, 1020, 988, 875, 840, and 550. ^{1}HNMR (200 MHz, CD_3Cl):

F_3-2: (60 mg, 0.0075 % yield: from EtOAc — n-hexane), mp 192-193°, MS m/z 300 $[M]^+$ $C_{17}H_{16}O_5$ (71), 283 (10), 272 (14), 229 (4), 207 (44), 194 (20), 181 (100), 166 (6), 154 (20), 136 (16), 120 (21), 107 (17), 91 (21), 79 (10), and 69(20). λ_{max}^{MeOH} nm ($\log\varepsilon$): 210 (4.4) and 368 (4.4), $\lambda_{max}^{MeOH+MeONa}$ nm ($\log\varepsilon$): 210 (4.3) 446 (4.4), $\lambda_{max}^{MeOH+AlCl_3}$ nm ($\log\varepsilon$): 210 (4.3) and 416 (4.6). IR $_{max}^{KBr}$ cm^{-1} 3240, 1630, 1610, 1585, 1550, 1520, 1490, 1440, 1350, 1290, 1220, 1170, 1120, 1060, 1032, 985, 830, 700, and 625. ^{1}HNMR (200MHz, $(CD_3)_2$ CO):

Xanthohumol (F_5). Fraction 5 from the main column was eluted with EtOAc — n-hexane (6 : 4) and was purified on a preparation TLC plate with CH_2Cl_2 — Me_2CO (8 : 2) at three time developments. F_5 (12 mg, 0.0012 % yield; from EtOAc — n-hexane) mp 157-159° [lit, 171-172°], MS m/z: 354 $[M]^+$ $C_{21}H_{22}O_5$.

Isoxanthohumol (F_9). Fraction 9 from the main column was eluted with

EtOAc — n-hexane (9 : 1) and gave F_9 (1.47 g, 0.18 % yield ; from EtOAc — n-hexane) mp 147–148°, [lit. 165–175°]. MS m/z : 300 $[M]^+$ $C_{21}H_{22}O_5$. Acetylation of compound F_9 with Ac_2O-pyridine gave diacetylisoxanthohumol, mp 142–143°.

RESULTS AND DISCUSSION

The MS spectra of xanthohumol (F_5) and isoxanthohumol (F_9) showed a quite similar fragmentation pattern and the same molecular formula $C_{21}H_{22}O_5$ ($[M]^+$ m/z 354), as shown in Fig. 2 ((A) and (B)), although the R_f values of the two compounds on TLC differed. These similarities in the MS of the two compounds provided us with the most information for the determination of their chemical structures. The MS spectrum of compound F_5, which was cyclized to compound F_9 by electrone impact, exhibited exactly the same fragmentation pattern as a compound F_9 by electrone impact following the retro-Diels-Alder reaction (Fig. 3). Other spectral properties (UV : Fig. 4 (A)

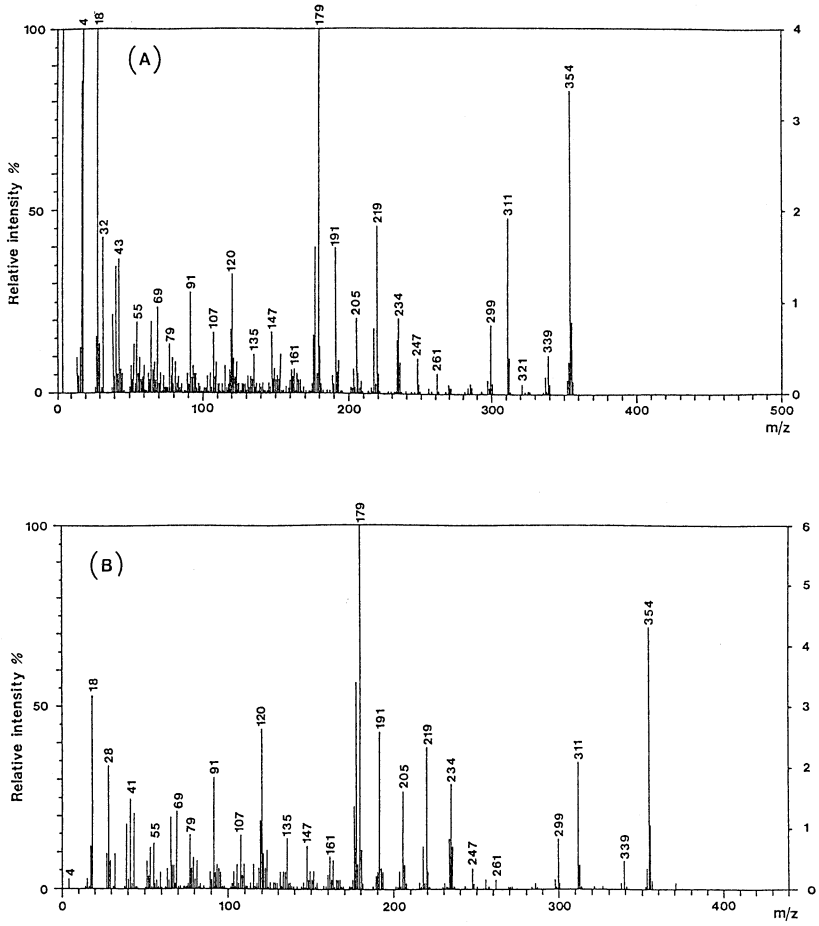

Fig. 2. Mass spectra of the compounds F_9 (A) and F_5 (B).

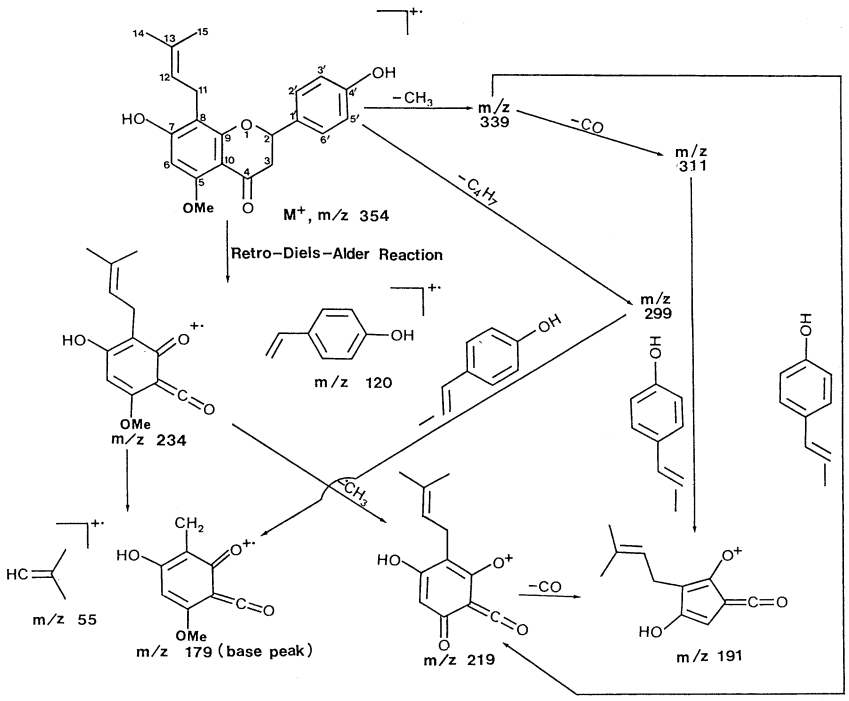

Fig. 3. Mass fragmentation pattern of the compound F_9.

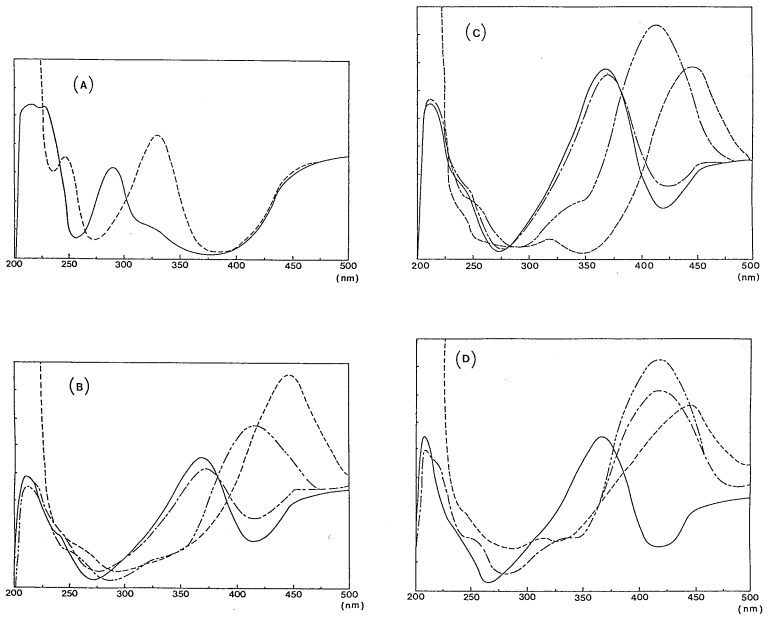

Fig. 4. UV spectra of the compounds F_9 (A), F_5 (B), F_3-1 (C) and F_3-2 (D) in a wave length of 200 — 500 nm. MeOH : ————, MeOH + MeONa : -----, MeOH + $AlCl_3$: ——-——, MeOH + $AlCl_3$ + HCl : ——-——.

TABLE 2. ^{1}H-NMR data of the chemical shift (ppm) and coupling constant (Hz) of the compounds F_9 in $(CD_3)_2C=O$ and CD_3OD, F_5 in $(CD_3)_2C=O$, F_3-1 in (CD_3Cl) and F_3-2 in $(CD_3)_2C=O$.

Proton	F_9 $((CD_3)_2C=O)$		$F_9(CD_3OD)$	
2	5.36	see Fig. 5	5.28	$J^{2}H$-^{3}Htrans=12.6Hz, $J^{2}H$-^{3}Hcis=3.0Hz
3	2.62(cls)	see Flg. 5	2.67	$J^{3}H$cis-^{3}Htrans=16.7Hz, $J^{3}H$cis-^{2}H=3.0Hz
	2.96(trans)	see Fig. 5	2.98	$J^{3}H$trans-^{3}Hcis=16.7Hz, $J^{3}H$trans-^{2}H=12.6Hz
6	6.23	singlet	6.14	singlet
11	3.27	doublet, $J^{11}H$-^{12}H=7.05Hz	3.21	doublet, $J^{11}H$-$J^{12}H$=7.54Hz
12	5.21	multiplet	5.10	multiplet
14 and 15	1.61	singlet	1.62and1.56	singlet
2′ and 6′	7.40	doublet $J^{2'}H$-$^{3'}H$ and$^{5'}H$-$^{6'}H$=8.60Hz	7.32	doublet $J^{2'}H$-$^{3'}H$ and$^{5'}H$-$^{6'}H$=8.60Hz
3′ and 5′	6.90		6.83	
OMe	3.74	singlet	3.81	singlet

Proton	$F_5((CD_3)_2C=O)$		F_3-1(CD_3Cl)		F_3-2$(CD_3)_2C=O$	
α	7.66	doublet $J^{\alpha}H$-βH=16.00Hz	7.75	singlet	7.70	doublet $J^{\alpha}H$-βH=15.50Hz
β	7.81		7.75		7.90	
3′					6.09	doublet $J^{3'}H$-$^{5'}H$=2.30Hz
5′	6.05	singlet	6.00	singlet	6.13	
7′	3.24	doublet, $J^{7'}H$-$^{8'}H$=7.00Hz	3.30	doublet, $J^{7'}H$-$^{8'}H$=8.00Hz		
8′	5.21	multiplet	5.21	multiplet		
10′ and 11′	1.75 and 1.65	singlet	1.68 and 1.67	singlet		
2 and 6	7.50	doublet $J^{2}H$-^{3}H and $J^{5}H$-^{5}H=8.00Hz	7.49	doublet $J^{2}H$-^{3}H and $J^{5}H$-^{5}H=8.00Hz	7.62	doublet $J^{2}H$-^{3}H and $J^{5}H$-^{6}H=8.69Hz
3 and 5	6.86		6.56		6.93	
OMe	3.91	singlet	3.94 and 3.90	singlet	4.01 and 3.88	singlet

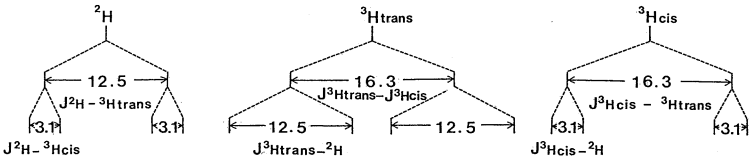

Fig. 5. Coupling constants obtained from double irradiation of Hcis, Htrans and ^{2}H in the compound F_9 (unit: Hz).

and (B)) and the data of ^{1}HNMR spectra are summarized in Table 2 with the coupling constants obtained from double irradiation experiments (Fig. 5) for compound F_9 in $(CD_3)_2C=O$ solvent. These were identical with those reported for iso- and xanthohumol, respectively, which were synthesized[7] and isolated from Hops[5,6].

3′-(isoprenyl)-2′,4-dihydroxy-4′,6′-dimethoxychalcone (F_3-1) mp 152-153°C, $[M]^+$ at m/z 368 ($C_{22}H_{24}O_5$) closely resembled xanthohumol in its UV, IR and MS (Fig. 6 (A)) spectral characteristics. The IR spectra of compound F_3-1 showed a hydroxyl band at 3350 cm^{-1} and a α, β-unsaturated carbonyl group at 1610 cm^{-1}. It had a UV maximum at 370 nm, which shifted to 446

nm on the addition of MeONa, confirming the presence of the phenolic hydroxy group[8] (Fig. 4 (C)). Peaks for compound F_3-1 were obtained at m/z 248 (15%) and 120 (24%) by the retro-Diels-Alder reaction of flavanone, which occurred as a result of cyclization of compound F_3-1 following the MS electrone impact. Intense peaks of m/z 193 (100%) and m/z 233 (77%) were obtained from a loss of 2-methylpropene (m/z 55) and CH_3 from m/z 248, respectively. Another peak at m/z 205 (5%) arose due to the loss of a CO from m/z 233 and/or the loss of (CH_3+CO_2+para-ethylenylphenol) from $[M]^+$. Fragments at m/z 353 (25%) and m/z 325 (75%) arose, respectively, due to loss of CH_3 and (CH_3+CO) from M^+, which did not undergo the retro-Diels-Alder reaction. m/z 313 (27%) showed a direct loss of 2-methylpropene (m/z 55) from M^+. Thus, the MS fragmentation pattern of compound F_3-1 could be superimposed on that of compound F_5 when m/z 14 was added to each fragment related to the A-ring in compound F_5, as shown in Fig. 6 (A). The ^{1}HNMR spectrum (Table 2) of compound F_3-1 showed the presence of two methoxy groups at 3.90 and 3.94, and five aromatic protons at 5.56 (d, J=8 Hz) and 7.49 (d, J=8 Hz) and 6.00 (s). The doublets integrating the two protons of 5.56 and the two of 7.49 were assigned to the C-2 and C-6 protons, and the C-3 and C-5 protons of para-nonsubstituted phenol, respectively. The singlet proton showed the presence of a five-substituted aromatic ring. The presence of the isoprenyl group was indicated by two methyl groups at 1.67 and 1.68 (C-10′ and 11′), methylene protons at 3.30 (d, J=8 Hz, C-7′) and a multiplet at 5.21 (C-8′). Another signal of a singlet at 7.25 indicated the presence of α, β-unsaturated ketone. Thus, the ^{1}HNMR spectrum of compound F_3-1 was quite similar to that of compound F_5 except for one OMe signal. Judging from these results on MS and ^{1}HNMR spectra, compound F_3-1 was evidently a methoxy derivative of compound F_5. The stereochemistry of the double bond at C-α and C-β was identified as a trans, judging from the fact that the α, β-protons of 2′-hydroxy-4,4′,6′-trimethoxychalcone did not split to a doublet.[8]

2′,6′-dimethoxy-4,4′-dihydroxychalcone (F_3-2) mp 192-193°C, $[M]^+$ at m/z 300 ($C_{19}H_{16}C_5$) had UV maxima at 210 and 368 nm. The IR spectrum showed a hydroxyl band at 3525 cm^{-1}. The hydroxyl function was shown to be phenolic by the presence of a shift in its UV maximum at 446 nm following the addition of MeONa,[8] as shown in Fig. 4 (D). Its MS spectrum (Fig. 6 (B)) showed a $[M]^+$-ethylenylphenol at m/z 181 (100%). The fragment at m/z 207 (40%) caused by loss of m/z 93 in the $[M]^+$ indicated the presence of the phenol group. The MS spectrum of compound F_3-2 did not show any fragmentation pattern as a result of the retro-Diels-Alder reaction, as seen in the cases of xanthohumol and compound F_3-1. These results indicated the absence of the free hydroxyl group at positions C-2′ and C-6′ in chalcone. The ^{1}HNMR spectrum (Table 2) of compound F_3-2 was similar to that of compound F_5, indicating the presence of isoprenyl group. The ^{1}HNMR spectrum of compound F_3-2 showed the presence of two methoxy groups at 4.01 and 3.88. The doublets integrating with two protons at 6.93 (d, J=9 Hz) and two at 7.62 (d, J=9 Hz) were assigned to the C-3 and C-5 protons and the C-2 and C-6 protons, respectively. Two aromatic protons at 6.09 (d, J=2 Hz) and 6.13 (d, J=2 Hz) were assigned to C-3′ and C-5′, respectively, with reference to the J value of meta protons in phenol. The stereochemistry of the double bond at C-α and C-β was determined to be trans from the J value

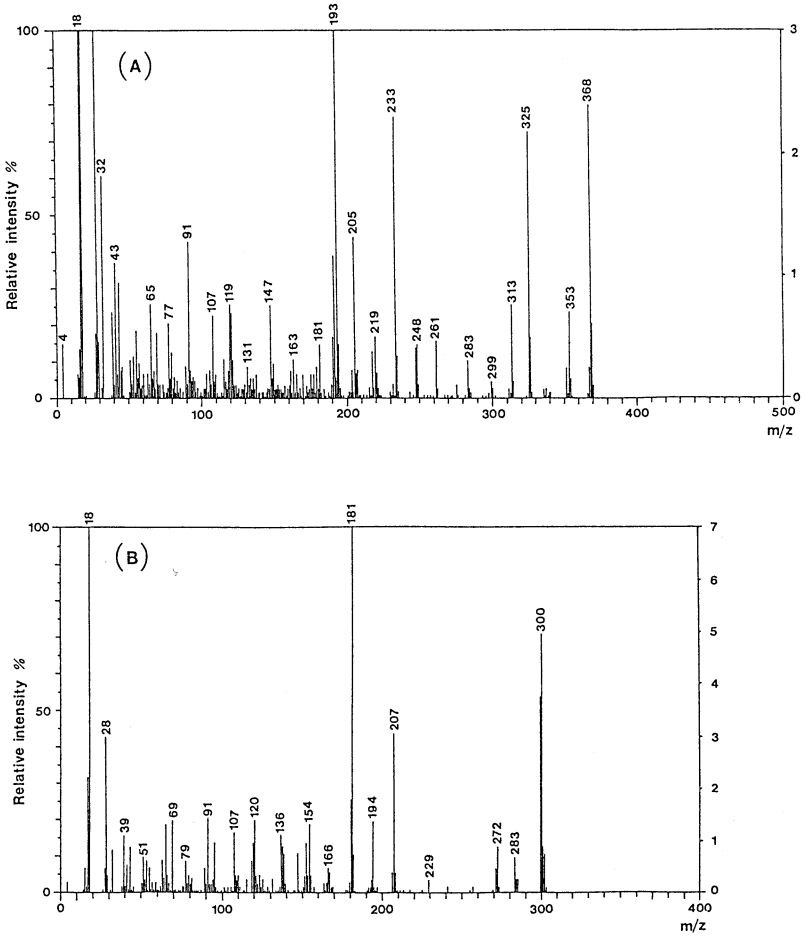

Fig. 6. Mass spectra of the compounds F_3-1 (A) and F_3-2 (B).

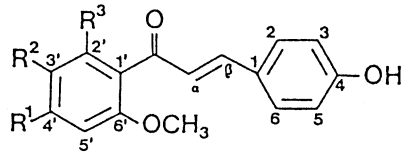

Fig. 7. Chemical structures of compounds identified by the present experiment.
F_3-1: R^2=OMe, R^2=CH_2CH=CMe_2, R^3=OH
F_3-2: R^1=OH, R^2=H, R^3=OMe
F_5 : R^1=OH, R^2=CH_2CH=CMe_2, R^3=OH

between α-H and β-H (7.70 and 7.90, d, J=16 Hz). Thus, the chemical structure of compound F_3-2 could be easily identified by comparison of the MS and ^{1}HNMR spectra of compounds F_3-1 and F_5 with those of compound

F_3-2. The spectral data for compounds F_3-1, F_3-2, F_5 and F_9 were in agreement with the suggested structures as shown in Fig. 7. A part of this paper was presented in the preliminary report.[9)]

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