## REFERENCES

1. Yaozu Chen, Jianmin Yue, Suming Hua, Haiquan Li and Ning Chen, (1987) *Org Chem* **6**, 469.
2. Yaozu Chen, Jianmin Yue and Suming Hua, (1987) *Chem J Chinese Univ* **8**, 447.
3. Daisuke Takaoka, Keiko Watanabe and Misturu Hiroi, (1976) *J Chem Soc Jpn* **49**, 3564
4. Riggs, N. V. and Stevens, J D (1962) *Aust J Chem* **15**, 305.
5. Mcalpine, J B, Riggs, N V and Gordon, P G (1968) *Aust. J Chem* **21**, 2095
6. Jiasen Liu, Meifen Huang, Yaoliang Gao and Findlay, J A. (1981) *Can. J Chem* **59**, 1680


*Phytochemistry*, Vol 28, No 6, pp 1776 1777, 1989
Printed in Great Britain

0031-9422/89 $3 00+0 00


# CHALCONES FROM *HUMULUS LUPULUS*

SUN SONG-SAN, SATORU WATANABE*† and TAIICHI SAITO*

Department of Pharmacology, Capital Institute of Medicine, Peking, People's Republic of China, *Department of Pharmacology, Kawasaki Medical School, Kurashiki, Okayama 701-01, Japan

(*Received in revised form* 13 *December* 1988)

**Key Word Index**— *Humulus lupulus*, Moraceae, plant chemistry, isoxanthohumol, xanthohumol, 3'-(isoprenyl)-2',4-dihydroxy-4',6'-dimethoxychalcone, 2',6'-dimethoxy-4,4'-dihydroxychalcone

**Abstract**—Extracts of *Humulus lupulus* yielded two known compounds, isoxanthohumol and xanthohumol, and two new chalcones, 3'-(isoprenyl)-2',4-dihydroxy-4',6'-dimethoxychalcone and 2',6'-dimethoxy-4,4'-dihydroxychalcone. Their structures were established by spectral methods.

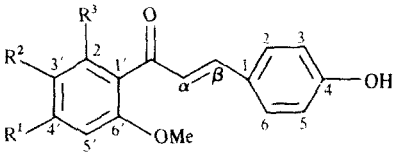
## INTRODUCTION

Two new chalcone derivatives· 3'-(isoprenyl)-2',4-dihydroxy-4',6'-dimethoxy chalcone and 2',6'-dimethoxy-4,4'-dihydroxychalcone, together with two already known compounds: isoxanthohumol and xanthohumol [1, 2] were isolated from hop cone extract The structures of the isoxanthohumol and xanthohumol were determined by comparing their spectra with those of references, while the new compounds were identified mainly by their $^1$H NMR and MS spectra

## RESULTS AND DISCUSSION

The MS spectra of xanthohumol (**3**) and isoxanthohumol (**4**) showed a similar fragmentation pattern and the same molecular formula $C_{21}H_{22}O_5$ ([M]$^+$ *m/z* 354), although the $R_f$ values on TLC differed. These similarities in the MS of the two compounds provided the necessary information for the determination of their chemical structures. The MS spectrum of **3**, which was cyclized to **4** by electron impact, exhibited exactly the same fragmentation pattern as **4** due to retro-Diels–Alder rearrangement The $^1$H NMR and other spectra of **3** and **4** were identical with those reported for iso- and xanthohumol, respectively, which were synthesized [3] and isolated from hops [1, 2].

3'-(Isoprenyl)-2',4-Dihydroxy-4',6'-dimethoxychalcone (**1**) mp 152–153° [M]$^+$ at *m/z* 368 ($C_{22}H_{24}O_5$) closely resembled xanthohumol in its UV, IR and MS spectral characteristics The IR spectrum 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 sodium methoxide, confirming the presence of the phenolic hydroxy group




**1** $R^1$ = OMe, $R^2$ = $CH_2CH{=}CMe_2$, $R^3$ = OH
**2** $R^1$ = OH, $R^2$ = H, $R^3$ = OMe
**3** $R^1$ = OH, $R^2$ = $CH_2CH{=}CMe_2$, $R^3$ = OH

† Author to whom correspondence should be addressed

[4]. Peaks for **1** were obtained at *m/z* 248 (15%) and 120 (24%) by retro-Diels–Alder rearrangement, which occurred as a result of cyclization of **1** following the MS electron 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 methyl 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 (Me + $CO_2$ + *p*-ethylenylphenol) from $[M]^+$. Fragments at *m/z* 353 (25%) and *m/z* 325 (75%) arose, respectively, due to loss of Me and (Me + 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 $[M]^+$. The $^1$H NMR spectrum of **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, and C-3 and C-5 protons of *p*-unsubstituted 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. 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 [5].

2′,6′-dimethoxy-4,4′-dihydroxychalcone (**2**), mp 192–193°, $[M]^+$ 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 sodium methoxide [4]. Its MS spectrum showed a [M − ethylenyl phenol]$^+$ at *m/z* 181 (100%). The fragment at *m/z* 207 (40%) caused by loss of *m/z* 93 in the $[M]^+$ indicated the presene of the phenol group. The MS spectrum of **2** did not show any retro-Diels–Alder fragmentation pattern. These results indicated the absence of a free hydroxyl group at positions of C-2′ and C-6′ in the chalcone. The $^1$H NMR spectrum of **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 7.62 (*d*, *J* = 9 Hz) were assigned to C-3 and C-5, and C-2 and C-6 protons, respectively. Two aromatic protons at 6.09 (*d*, *J* = 2 Hz) and 6.13 (*d*, *J* = 2 Hz), respectively, were assigned to C-3′ and C-5′ with reference to the *J* value of *meta* protons in phenol. The stereochemistry of the double bond at C-α and C-β were determined to be a *trans* from the *J* value between α-H and β-H (7.70 and 7.90, *d*, *J* = 16 Hz). The spectral data for compounds **1**–**4** were in agreement with the suggested structures.

## EXPERIMENTAL

Mps uncorr. $^1$H NMR values are given in ppm downfield from TMS. TLC spots were detected with conc. sulphuric acid and/or UV light after development in a mixture of $CH_2Cl_2$ and $Me_2CO$.

*Plant materials.* Cones of *Humulus lupulus* L. or Shinshu-Wase were collected from Yamagata (the northern part of Japan) at the end of August, 1985. These were frozen, crushed and sieved, 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 Ashai Brewery Co. (Mr J. Miyata).

*Isolation of chalcones.* The pellets (800 g) of *Humulus lupulus*, were extracted with *n*-hexane (3 l) under reflux for 12 hr and then with boiling MeOH (3 l) for 24 hr. Each extraction with *n*-hexane and MeOH was repeated × 3. Evaporation of MeOH to dryness yielded 114 g of tarry residue. The residue was dissolved in $H_2O$ and extracted with $Et_2O$. Evaporation of the ether dried over $Na_2SO_4$ yielded 51 g of green-black residue. The residue was dissolved in $CH_2Cl_2$ and extracted with 1.0 M NaOH. The aq. layer was neutralized with 6 M HCl and extracted with $Et_2O$. The $Et_2O$ layer was washed with $H_2O$, dried and yielded 9.4 g (1.17% yield) of phenolic residue after evapn of the ether.

*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. After crude separation on the column, compounds **1**–**4** were further sepd and/or purified on prep. TLC plates or on the column. The compounds in order of elution were **1**, **2**, **3** and **4**.

*3′-(isoprenyl)-2′,4-Dihydroxy-4′,6′-dimethoxychalcone* (**1**) *and 2′,6′-dimethoxy-4,4′-dihydroxychalcone* (**2**). Fraction 3 eluted with EtOAc–*n*-hexane (2.3) gave a mixture of **1** and **2**. The separation and purification of the mixture on CC in $CH_2Cl_2$–$Me_2CO$ (4·1) gave **1** and **2**. **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). $\lambda_{max}^{MeOH}$ nm (log ε): 210 (4.4) and 370 (4.6), $\lambda_{max}^{MeOH+MeONa}$ nm (log ε) 446 (4.6), $\lambda_{max}^{MeOH+AlCl_3}$ nm (log ε).210 (4.4) and 370 (4.5), $\lambda_{max}^{MeOH+AlCl_3+HCl}$ nm (log ε): 210 (4.4) and 414 (4.7), compound **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). $\lambda_{max}^{MeOH}$ nm (log ε): 210 (4.4) and 368 (4.4), $\lambda_{max}^{MeOH+MeONa}$ nm (log ε): 210 (4.3) and 446 (4.4), $\lambda_{max}^{MeOH+AlCl_3}$ nm (log ε): 210 (4.3) and 416 (4.5) $\lambda_{max}^{MeOH+AlCl_3+HCl}$ nm (log ε): 210 (4.3) and 416 (4.6).

*Xanthohumol* (**3**). Fraction 5 from the main column was eluted with EtOAc–*n*-hexane (3:2) and was purified on a prep. TLC plate with $CH_2Cl_2$–$Me_2CO$ (4:1) (× 3). **3** (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* (**4**). Fraction 9 from the main column was eluted with EtOAc–*n*-hexane (9·1) and gave **4** (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 **3** with $Ac_2O$–pyridine gave diacetylisoxanthohumol, mp 142–143°.

## REFERENCES


1. Verzele, M., Stockx, J., Fontijin, F. and Anteunis, M. (1957) *Bull. Soc. Chim. Belg.* **66**, 452.
2. Ashurst, P. R., Laws, D. R. J. and Stevens, R. (1965) *J. Inst. Brew.* **71**, 492.
3. Jain, A. C., Gupta, R. C. and Sarpal, P. D. (1978) *Tetrahedron* **34**, 3563.
4. Mabry, T. J., Markham, K. R. and Thomas, M. B. (1970) *The Systematic Identification of Flavonoids*. Springer, New York.