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PRENYLFLAVONOIDS FROM *HUMULUS LUPULUS*

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Key Word Index—*Humulus lupulus*; Cannabinaceae; hop; resin; flavonoids; prenylated chalcones and flavanones; 2′,4′,6′,4-tetrahydroxy-3′-*C*-geranylchalcone; mass spectrometry.

Abstract—Five flavonoids were isolated from the resin part of the female inflorescences of *Humulus lupulus*, together with four known hop flavonoids, i.e. xanthohumol, 2′,4′,6′,4-tetrahydroxy-3′-*C*-prenylchalcone, isoxanthohumol and 6-prenylnaringenin. The new hop compounds were identified as 2′,4′,6′,4-tetrahydroxy-3′-*C*-geranylchalcone, 5′-prenylxanthohumol, 6″,6″-dimethylpyrano (2″,3″: 3′,4′)-2′,4-dihydroxy-6′-methoxychalcone, its hydrate and 8-prenylnaringenin; apart from 8-prenylnaringenin, these are new flavonoids. Their mass fragmentation patterns were studied by mass spectrometry using atmospheric pressure chemical ionization in combination with collision-activated decomposition. Loss of the isoprenoid substituent in the positive ion mode and retro Diels–Alder fission in both the positive and negative ion modes provided useful information on the substitution patterns of the A and B rings. Nine hop varieties were qualitatively and quantatively characterized by HPLC-mass spectrometry. The flavonoid profiles of the samples examined were uniform and proved to be of little value in hop variety identification. Xanthohumol was the principal flavonoid in all samples (80–90% of the total of flavonoids) and was accompanied by minor amounts of the other eight flavonoids in virtually all samples. Copyright © 1997 Elsevier Science Ltd

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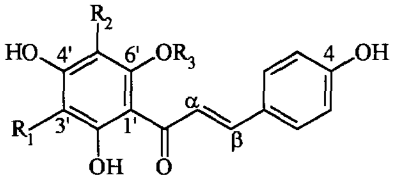
INTRODUCTION

The hop plant (*Humulus lupulus* L., Cannabinaceae) is a dioecious twining perennial which is cultivated widely throughout the temperate zones of the world. In the U.S.A. hop growing is concentrated in the Pacific northwest. The inflorescences (hop cones or 'hops') are used in the brewing industry to give beer its characteristic flavour and aroma. While the bitter acids and essential oils have received much attention in this respect, the brewing value of hop flavonoids is not well understood [1]. Xanthohumol, the principal flavonoid of hop resins, has been isolated by a number of research groups [2–5]. This prenylated chalcone is accompanied by its flavanone isomer, isoxanthohumol, and minor quantities of 2′,4′,6′,4-tetrahydroxy-3′-*C*-prenylchalcone (desmethylxanthohumol) [6], 2′,4-dihydroxy-4′,6′-dimethoxy-3′-*C*-prenylchalcone, 4′,4-dihydroxy-2′,6′-dimethoxychalcone [5] and 6-prenylnaringenin [4]. Xanthohumol and 6-prenylnaringenin were found to display antifungal activities [4]. Further, *H. lupulus* has repeatedly been reported as a rich source of oestrogens [7], but neither the alleged 'hop hormone' xanthohumol nor the bitter acids exhibited oestrogenic activity *in vitro* [8].

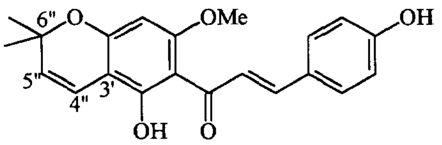
In the present study we have identified five flavonoids from hop resins in addition to four previously isolated flavonoids from *H. lupulus*. Of these five, four chalcones are reported here as new natural products while the remaining compound, 8-prenylnaringenin, is a new hop constituent. Nine hop varieties were examined to determine whether the resin flavonoids have any value in hop variety identification.

RESULTS AND DISCUSSION

The resin flavonoids were extracted by brief immersion of hop cones in chloroform. Separation of the phenolics from resinous materials and other lipophilic constituents was achieved by precipitation from methanol and chromatography of the methanol-soluble portion on Sephadex LH-20. This procedure yielded a crude mixture of flavonoids which was examined by chromatographic techniques. Individual flavonoids were separated by column chromatography on silica gel and RP-18. In addition to the known hop flavonoids, i.e. xanthohumol (**1**), desmethylxanthohumol (**2**), isoxanthohumol (**7**) and 6-prenylnaringenin (**9**), we obtained small quantities of 2′,4′,6′,4-tetrahydroxy-3′-*C*-geranylchalcone (**3**), 5′-prenylxanthohumol (**4**), dehydrocycloxanthohumol (**5**), dehydrocycloxanthohumol hydrate (**6**) and 8-prenylnaringenin (**8**). To our knowledge, **3**–**6** have not hitherto been reported from natural sources.



	R1	R2	R3
1	prenyl	H	Me
2	prenyl	H	H
3	geranyl	H	H
4	prenyl	prenyl	Me



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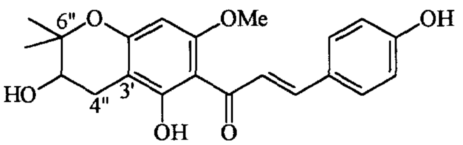

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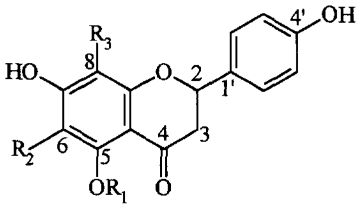
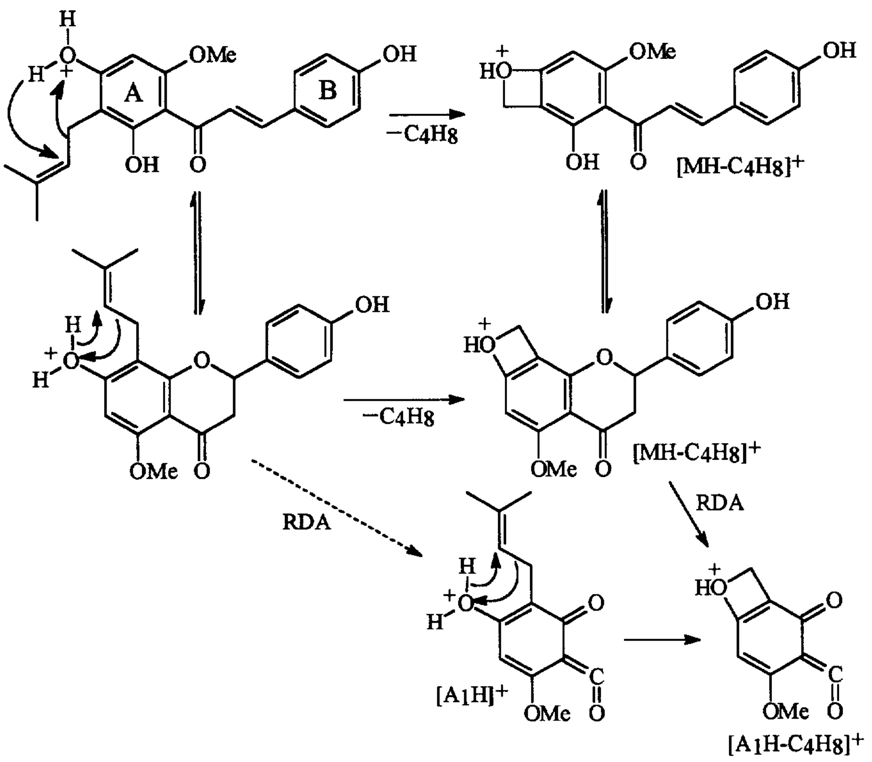


	R1	R2	R3
7	Me	H	prenyl
8	H	H	prenyl
9	H	prenyl	H

Mass spectrometry

UV spectroscopy proved useful to distinguish between substituted chalcones and flavanones [9], which is not possible by EI mass spectrometry due to thermal isomerization of 2′-hydroxychalcones in the ion source [10]. Cleavage of the heterocyclic C ring via a retro Diels–Alder (RDA) mechanism represents an important fragmentation pathway in both the flavanones and chalcones. RDA fission leads to $[A_1]^{+\cdot}$ and $[B_1]^{+\cdot}$ fragments [11], which provides useful information as to the number of hydroxyl, methoxyl and prenyl substituents on each ring. The same information was also obtained by a HPLC-tandem mass spectrometer system equipped with a heated nebulizer–atmospheric pressure chemical ionization (APCI) interface [12]. In the single mass spectrometry mode, the flavonoids investigated yielded chiefly pseudomolecular ions without significant fragmentation. Fragmentation was induced by collision-activated decomposition (CAD) using argon as the target gas in the following manner. The pseudomolecular ion was selected by the first quadrupole analyser and passed through the collision cell (the second quadrupole) where collisions between argon molecules and pseudomolecular ions resulted in the formation of fragment ions. The fragments were analysed by the third quadrupole mass analyser. With positive ion CAD mass fragmentation, prenylated chalcones and flavanones both yield intense prenyl cleavage ions, $[MH-C_4H_8]^+$ (Fig. 1). The appearance of A-ring fragment ions $[A_1H-C_4H_8]^+$ in both the flavanones and the chalcones must be attributed to RDA fission. This indicates that isomerization of 2′-hydroxychalcones into their corresponding flavanones takes place in the heated nebulizer interface or in the APCI source, or in both. Direct RDA cleavage of the pseudomolecular ion represents a minor fragmentation pathway. For instance, **1** yields an intense ion at *m/z* 299 due to loss of the prenyl substituent whereas the intensity of the directly formed RDA fragment $[A_1H]^-$ at *m/z* 235 has an abundance of only 5%. Clearly, the electron-donating properties of the isoprenoid function favours nucleophilic attack at the 4′-OH_2^+ oxygen by C-1″ (Fig. 1). The results are summarized in Table 1.

Without modification of the chromatographic conditions, negative ion APCI-MS yielded chiefly $[M-H]^-$ ions which were collisionally activated to give predominantly the RDA fragment ions, $[A_1-H]^-$ and $[B_1-H]^-$ (Fig. 1, Table 1). Cleavage of the isoprenoid substituent, the major fragmentation pathway in positive ion CAD, proved highly unfavourable in the negative mode due to the nucleophilic nature of the isoprenoid function, regardless of the location of the negative charge (Fig. 1). These observations indicate that cleavage of the isoprenoid function is charge-driven in the positive ion mode via the mechanism depicted in Fig. 1.


A. Positive ion APCI-CAD

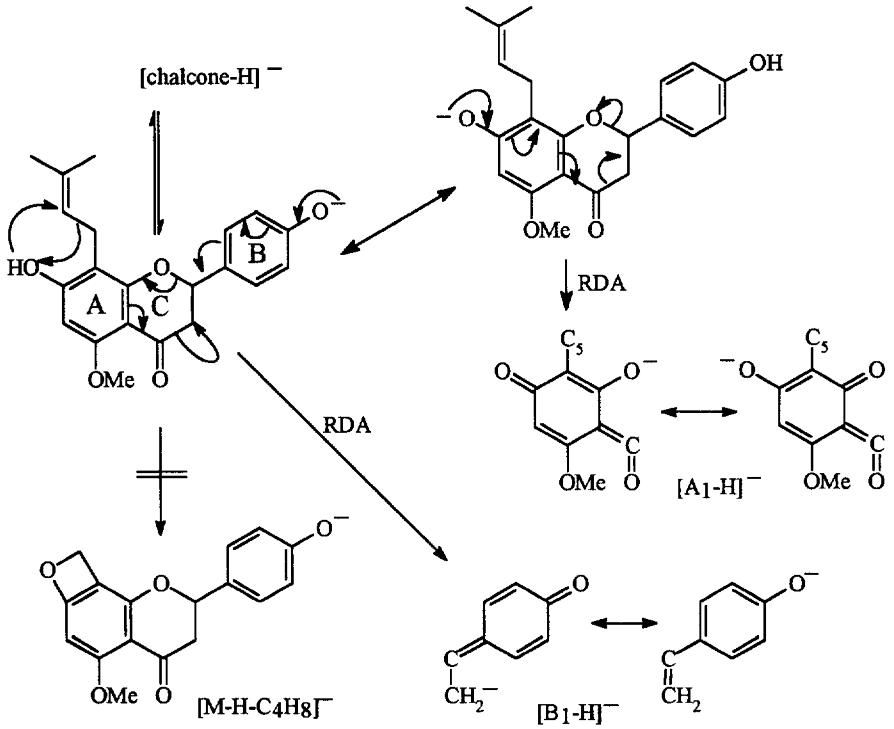

B. Negative ion APCI-CAD

Fig. 1. APCI-CAD mass fragmentation of prenylated chalcones and flavanones in the positive and negative ion mode. For instrumental conditions see Experimental.

Table 1. APCI-CAD* mass spectral data for flavonoids **1**–**9** [*m/z* (rel. int.)]

Compound			Positive ion mode				Negative ion mode	
no.	Flavonoid	M_r	$[MH-C_4H_8]^+$	$[A_1H]^+$	$[A_1H-C_4H_8]^+$	$[B_3]^+$	$[A_1-H]^-$	$[B_1-H]$
1	Xanthohumol	354	299 (100)	235 (5)	179 (65)	147 (3)	233 (100)	119 (92)
2	2′,4′,6′,4-Tetrahydroxy-3′-prenylchalcone	340	285 (100)	221 (20)	165 (89)	147 (3)	219 (100)	119 (92)
3	2′,4′,6′,4-Tetrahydroxy-3′-geranylchalcone	408	285† (64)	289 —	165‡ (21)	147	287 (93)	119 (16)
4	5′-Prenylxanthohumol§	422	367 (100)	303 (6)	247 (51)	147 —	301 —	119 (100)
5	Dehydrocycloxanthohumol	352	—	233 (100)	—	147 (20)	231 (15)	119 (100)
6	Dehydrocycloxanthohumol hydrate	370	Fig. 3				249 (23)	119 (100)
7	Isoxanthohumol	354	299 (59)	235 (17)	179 (85)	147 (6)	233 (57)	119 (100)
8	8-Prenylnaringenin	340	285 (39)	221 (4)	165 (12)	147 (1)	219 (100)	119 (72)
9	6-Prenylnaringenin	340	285 (100)	221 (2)	165 (34)	147 (1)	219 (100)	119 (82)

*Atmospheric pressure chemical ionization-collision activated decomposition.

†Represents $[MH-C_9H_{16}]^+$ ion.

‡Represents $[A_1H-C_9H_{16}]^+$ ion.

§Additional fragment ions observed at *m/z* 311 $[MH-C_4H_8-56]^+$ (14) and *m/z* 191 $[A_1H-C_4H_8-56]^+$ [21].

Identification of the flavonoids

The UV spectrum (maximum at 367 nm) of **3** was similar to that of **1**, suggesting that **3** was a substituted chalcone. To account for its molecular weight (M_r 408, $C_{25}H_{28}O_5$) there were two possibilities: (1) a diprenylated or (2) a geranylated tetrahydroxychalcone. The first possibility was ruled out since the EI mass spectrum lacked the expected $[M-C_4H_7]^+$ and $[M-C_4H_7-56]^+$ ions as demonstrated by Takayama *et al.* [10] for a number of diprenylated flavonoids. Instead, prominent peaks appeared at *m/z* 285 $[M-C_9H_{15}]^+$, 219 $[C_{12}H_{11}O_4]^+$ and 165 $[C_8H_5O_4]^+$, which have previously been observed for 5,7,4′-trihydroxy-6-*C*-geranylflavanone, bonannione A [13]. This leads us to suggest that **3** is the chalcone analogue of bonannione A, i.e. 2′,4′,6′,4-tetrahydroxy-3′-*C*-geranylchalcone. The presence of a geranyl substituent was also evident from APCI-tandem mass spectrometry when the pseudomolecular ion was collisionally activated with argon to give a prominent ion, $[MH-C_9H_{16}]^+$, with *m/z* 285 in the positive ion mode. The structure was confirmed by ^1H–^1H correlation, ^1H–^{13}C heteronuclear multiple quantum coherence (HMQC) and multiple bond correlation (HMBC) spectroscopies. In the HMBC spectrum (Table 4), the H-1″ proton provided a good starting point for the assignment of the proton and carbon resonances from the geranyl function. Proton H-1″ exhibited correlations with the C-2′, C-3′, C-4′, C-2″ and C-3″ carbons. This permitted assignment of the methyl protons at δ 1.71 to H-4″ because proton H-4″ interacts strongly with C-2″, C-3″ and C-5″. The carbon resonating at δ 40.0 was identified as C-5″, which led to the assignment of the proton triplets at δ 1.90 and 2.00 to H-5″ and H-6″, respectively. The identity of the H-6″ triplet was verified from its ^1H–^1H correlation with H-7″. The carbons to which H-5″ and H-6″ are attached were determined from the HMQC data. Finally, published carbon shifts for a number of geranylated flavonoids [14] led to the assignment of the carbon resonances at δ 18.4 and 26.3 to C-9″ and C-10″, respectively, which also showed correlations with H-7″.

Compound **4** was obtained in small amounts. Its structure was elucidated on the basis of UV and mass spectral data alone. The UV maximum (371 nm) pointed to a chalcone nucleus. From molecular weight information (M_r 422, $C_{26}H_{30}O_5$) two structures seemed possible: (1) a prenylated xanthohumol or (2) a methylated derivative of **3**. The latter possibility was ruled out since no loss of a C_9H_{16} species from a geranyl group could be observed in its APCI-CAD mass spectrum. Instead, the appearance of ions at *m/z* 367 $[MH-C_4H_8]^+$ and 311 $[MH-C_4H_8-56]^+$ was suggestive of the first possibility. Loss of one and two prenyl groups from RDA fragment $[A_1H]^+$ with *m/z* 303 gave rise to ions with *m/z* 247 $[303-56]^+$ and 191 $[303-56-56]^+$. These fragments were taken as evidence for a diprenylated phloroglucinol A ring with

Table 2. ^{1}H NMR data for chalcones **3**, **5** and **6** [δ_H ppm, mult. (J in Hz)]

H	**3***	**5**	**6**
H-β	7.67 *d*(15.6)	7.72 *s*	7.71 *d* (15.5)
H-α	8.00 *d* (15.6)	7.72 *s*	7.78 *d* (15.5)
2,6	7.53 *d* (8.4)	7.60 *d* (8.4)	7.58 *d* (8.3)
3,5	6.85 *d* (8.4)	6.85 *d* (8.4)	6.85 *d* (8.0)
5′	6.04 *s*	6.11 *s*	6.01 *s*
1″	3.12 *d* (7.0)		
2″	5.15 *t* (6.8)		
4″	1.71 *s*	6.56 *d* (10.0)	2.72 *dd* (16.6, 5) and 2.39 *dd* (16.5, 6)
5″	1.90 *t* (7.6)	5.61 *d* (10.0)	3.67 *t* (6.0)
6″	2.00 *dd* (14.7, 7.3)	1.42 *s* (2 × Me)	1.30, 1.23 (*s*, 2 × Me)
7″	5.05 *t* (6.8)		
9″	1.53 *s*		
10″	1.60 *s*		
4-OH	10.04 *s*	10.11 *s*	10.0 *br s*
2′-OH	14.54 *s*	14.61 *s*	14.78 *s*
4′-OH	10.32 *s*		
6′-OH	10.63 *s*		
6′-OMe		3.93 *s*	3.89 *s*

*These signals were assigned based on ^{1}H–^{1}H correlations.

one *O*-methyl group, while the presence of a B ring with one phenolic hydroxyl group followed from loss of 120 mass units from the pseudomolecular ion ($[A_1H]^+$ with m/z 303) via RDA fission. The compound was thus identified as 5′-prenylxanthohumol.

Compound **5** was also characterized as a chalcone (UV maximum at 371 nm). Its molecular weight (M_r 352, $C_{21}H_{20}O_5$) suggested a dehydro derivative of xanthohumol. The compound was resistant to loss of C_4H_8, but instead gave a prominent RDA fragment $[A_1H]^+$ with m/z 233 on collisional activation. From this mass spectral behaviour, cyclization of a prenyl group involving the 4′-OH function to form a dimethylpyrano ring with loss of two hydrogens was hypothesized. A 2,2-dimethylpyrano moiety was also evident from ^{1}H NMR (Table 2): the olefinic protons H-4″ and H-5″ appeared as doublets at δ 6.56 and 5.61 ($J = 10$ Hz), respectively. These chemical shifts are in agreement with those extracted from recent literature [15, 16]. However, no doublets were observed for protons H-α and H-β. These protons gave rise to a singlet at δ 7.72, integrating for two protons. This singlet showed correlations with two carbons in the HMQC spectrum which were identified as C-α (124.2 ppm) and C-β (144.3 ppm) from the HMBC data and by comparison with ^{13}C shift data for the other chalcones (Table 3). The ^{1}H–^{13}C connectivities observed in the HMBC spectrum are listed in Table 4. The compound was thus identified as 6″,6″-dimethylpyrano (2″,3″:3′,4′)-2′,4-dihydroxy-6-methoxychalcone, or, trivially, dehydrocycloxanthohumol. Proof of structure was obtained from conversion of xanthohumol into the putative compound by cyclodehydrogenation with 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ) using a procedure described by Jain *et al.* [17]. The semi-synthetic product was identical to the natural chalcone by TLC, HPLC, mass spectrometry and ^{1}H NMR, thus confirming structure **5**.

Compound **6** resembled **5** by UV and collision-activated mass fragmentation analysis (Fig. 2). From its molecular weight (M_r 370, $C_{21}H_{22}O_6$) it was suggested that **6** represents the hydrate of **5**. Indeed, loss of H_2O from the pseudomolecular ion (m/z 371 → 353) and the RDA fragment ion $[A_1H]^+$ (m/z 251 → 233) proved to be the major fragmentation pathway of **6**. Moreover, the APCI-CAD mass spectrum of **5** could be 'copied' by passing the $[MH-H_2O]^+$ ion of **6** (dehydration also occurred in the ion source), selected by the first quadrupole, into the argon collision cell and analysing the fragments with the third quadrupole. Compound **6** differed from **5** in its resistance to degradation of the dimethylpyrano ring. In fact, the 5″-position of the hydroxyl function in the pyrano ring of **6** followed from loss of $(HO)HC{=}C(Me)_2$ from the pseudomolecular ion, giving rise to a prominent ion with m/z 299. The ^{1}H NMR spectrum also supports this structure for **6**, for which we suggest the name dehydrocycloxanthohumol hydrate. The H-4″ protons each appeared as a pair of doublets at δ 2.72 and 2.39 ($J = 16.5$ and 6 Hz) while H-5″ appeared as a triplet at δ 3.67 ($J = 6$ Hz). These data are in good agreement with those reported for a structurally similar chalcone with a 2,2-dimethyl-3-hydroxypyrano substituent, isolated from the roots of *Helichrysum krausii* [18].

Compound **1** was isolated from Saazer × Zattler and Spalter hops in yields of *ca* 0.1%. The structure was determined by UV, mass spectrometry and NMR spectroscopies. The 3′-C position of the prenyl group was inferred from the HMBC spectrum (Table 4) in which the 2′-OH proton (δ 14.62, hydrogen bonded with the carbonyl oxygen) displayed couplings with

Table 3. ^{13}C NMR data for chalcones **1**, **2**, **3** and **5** (δ_C ppm)*

C	**1**†	**2**	**3**†	**5**†
C-β	142.5	142.0	142.8	144.3
C-α	123.8	123.4	125.0	124.2
C=O	191.6	191.8	192.7	192.7
1	126.0	126.2	127.1	126.7
2,6	130.4	130.3	131.1	131.6
3,5	115.9	116.0	116.8	116.9
4	159.9	159.2	160.4	160.5
1′	104.6	104.1	105.0	106.3
2′	164.6	164.1	165.1	163.3
3′	107.3	106.0	106.9	102.9
4′	162.3	162.4	163.3	162.1
5′	90.9	94.4	95.2	92.8
6′	160.5	159.8	160.6	161.1
1″	21.0	21.0	21.8	
2″	123.0	124.1	124.0	
3″	129.8	129.5	134.0	
4″	17.6	17.6	16.8	116.1
5″	25.4	25.5	40.0	126.7
6″			27.1	79.0
7″			125.0	
8″			131.4	
9″			18.4	
10″			26.3	
6″-Me				28.9
6′-OMe	55.7			57.2

*Chemical shifts were assigned from HMBC spectra (Table 4).
†These resonances were also assigned based on HMQC data.

Table 4. HMBC connectivities observed for chalcones **1**, **2**, **3** and **5**

H	**1**	**2**	**3**	**5**
H-β	C=O, 1, 2/6	C=O, α, 1, 2/6	C=O, 1, 2/6	C=O, 1, 2/6
H-α	C=O, 1	C=O, 1	C=O, 1	C=O, 1, 2/6
2/6	β, 2/6, 4	β, 2/6, 3/5, 4	β, 2/6, 3/5, 4	α/β, 2/6, 4
3/5	1, 3/5, 4	1, 4, 2/6, 3/5	1, 2/6, 3/5, 4	1, 3/5, 4
5′	1′, 3′, 4′, 6′	1′, 3′, 4′, 6′	1′, 3′, 4′, 6′	1′, 3′, 4′, 6′
1″	2′, 3′, 4′, 2″, 3″	2′, 3′, 4′, 2″, 3″	2′, 3′, 4′, 2″, 3″	
2″	1″, 4″, 5″	1″, 4″, 5″	1″, 4″, 5″	
4″	2″, 3″, 5″	2″, 3″, 5″	2″, 3″, 5″	2′, 3′, 4′, 6″
5″	2″, 3″, 4″	2″, 3″, 4″	2″, 3″, 4″, 6″	3′, 6″-Mes, 6″
6″			3″, 5″, 7″, 8″	
7″			9″, 10″	
9″			7″, 8″, 10″	
10″			7″, 8″, 9″	
4-OH	3/5, 4	3/5	3/5	
2′-OH	1′, 2′, 3′	1′, 2′, 3′	1′, 2′, 3′, C=O	1′, 2′, 3′
4′-OH	3′, 4′, 5′	3′, 4′, 5′	3′, 4′, 5′	
6′-OH		1′, 5′, 6′	1′, 2′, 5, 6′	
6′-OMe	6′			6′
6″-Me				5″, 6″

C-1′, C-2′ and C-3′, but not with C-5′ bearing the aromatic proton. As the C-3′ carbon also showed long-range interactions with the 4′-OH and H-1″ protons, C-3′ was identified as the carbon bearing the prenyl substituent.

Compound **2** yielded a UV spectrum with a maximum at 366 nm similar to that of **1**, indicating a chalcone nucleus. Mass spectrometry pointed to the empirical formula $C_{20}H_{20}O_5$. On electron impact, the compound lost $CH_3\cdot$, $C_3H_7\cdot$ and $C_4H_7\cdot$ radicals indicative of a prenyl substituent. Furthermore, RDA fission of the heterocyclic C ring after thermal iso-

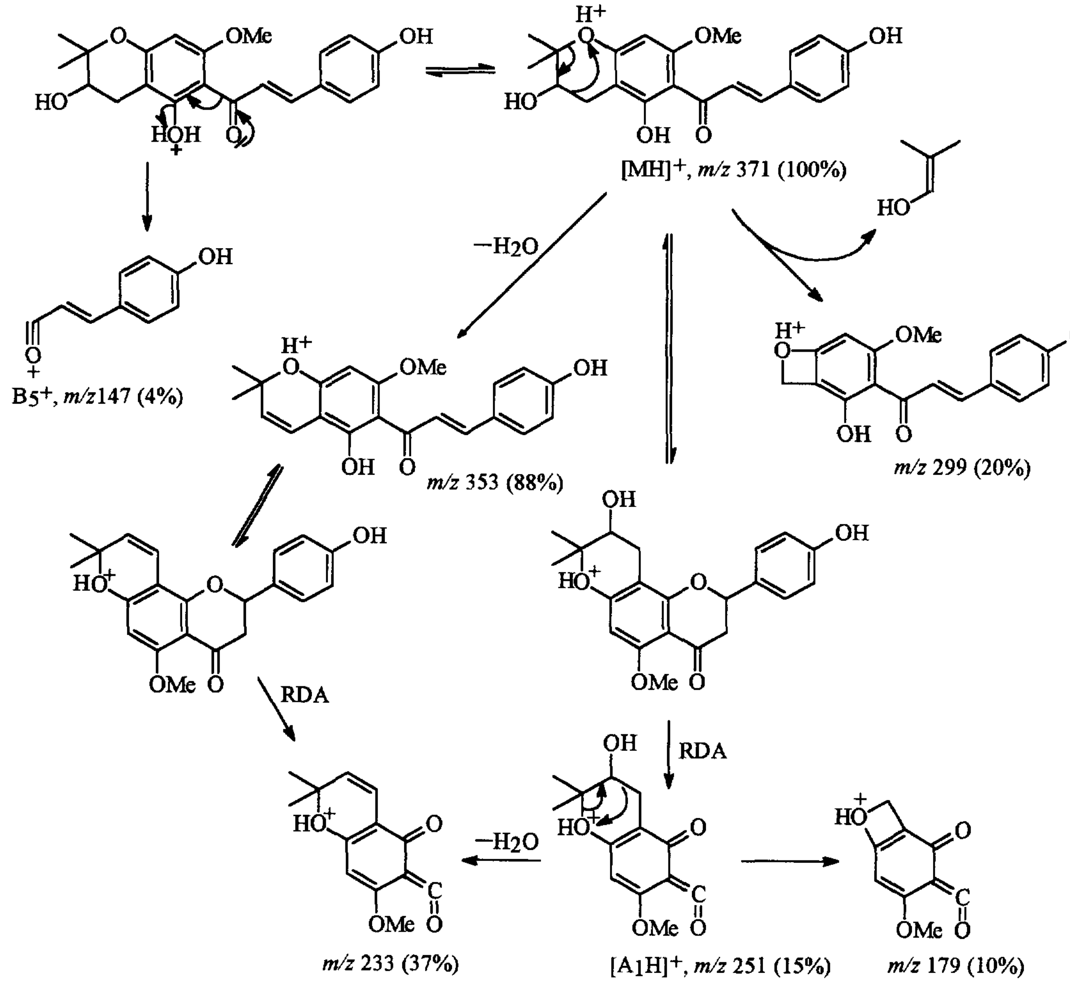


Fig. 2. APCI-CAD mass fragmentation of dehydrocycloxanthohumol hydrate (**6**). For instrumental conditions see Experimental.

merization to the corresponding prenylflavanones yielded a prenylated phloroglucinol A ring fragment $[A_1]^+$ with *m/z* 220 and a monophenolic B-ring fragment $[B_1]^+$ with *m/z* 120. The substitution patterns of the A and B rings was also evident from APCI-CAD mass spectrometry (Table 1). The compound was thus identified as 2′,4′,6′,4-tetrahydroxy-3′-C-prenylchalcone or desmethylxanthohumol. The structure was fully supported by ^1H and ^{13}C NMR data (Table 3) as well as by HMBC (Table 4). Desmethylxanthohumol was previously isolated from hops by Hänsel and Schulz [6].

Compound **7** was only a trace constituent in our samples. It was obtained by treatment of **1** with sodium acetate in methanolic solution, following a general procedure described by Shimokoriyama [19]. The EI mass spectrum of **7** was virtually identical to that of **1** due to thermal isomerization in the ion source [10]. By contrast, with APCI-single mass spectrometry, using an orifice plate voltage of 55 V, **1** differed from **7** in the abundance of the $[MH-56]^+$ ion arising from loss of isobutene from the prenyl substituent. This ion was prominent in the APCI mass spectrum of **1** whereas **7** showed no degradation of the prenyl function under these operating conditions.

In addition to the previously reported 6-prenylnaringenin (**9**) from hops [4], 8-prenylnaringenin (**8**) was also isolated. The position of the prenyl substituents was determined from HMBC data. The 5-OH proton of **8** (δ 12.08) showed long-range couplings with C-5, C-6 and C-10. From the observation that H-1″ was correlated with C-8, C-7, C-9, C-2″ and C-3″, but not with C-6, the prenyl substituent was located at C-8. Likewise, the position of the prenyl group in **9** was established starting from the 5-OH signal at δ 12.41. For this flavanone, no correlation between 5-OH and the —CH= carbon was observed. Both isomers could also be distinguished by APCI-single mass spectrometry when the orifice plate voltage was set at 55 V in the positive mode. Like **7**, **8** was resistent to loss of isobutene whereas **9** showed a prominent $[MH-56]^+$ ion with *m/z* 285 due to degradation of the prenyl group. Though less pronounced, the same trend was observed on collisional activation of both prenylnaringenins (compare entries 8 and 9 in Table 1). Preferential loss of isobutene from the 6-position

Table 5. Flavonoid profiles of some European hop varieties

		Flavonoid pattern*								
Sample no.	Variety	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**
1	Alsace	89	3	+	–	2	3	+	+	+
2	Hallertauer	87	3	+	+	2	5	+	+	1
3	Tradition	86	2	+	1	2	4	2	+	+
4	Hersbrucker	87	3	1	+	3	3	+	+	1
5	Jura	86	2	1	+	4	5	+	+	1
6	Lubliner	82	3	1	1	4	5	2	+	1
7	Saazer	87	3	1	+	2	4	+	+	1
8	Saazer	85	2	+	+	3	5	+	+	1
9	Spalter	87	2	+	+	3	4	1	+	1
10†	Spalter	88	2	+	+	2	4	+	+	1
11	Tettnanger	86	2	2	–	2	5	1	–	2

*Abundance (in %) of total resin flavonoids determined by LC-MS. For key to flavonoid numbers see structures; signs: – = not detected; + = less than 1%.

†Duplicate analyses.

was previously observed in the FAB mass spectra of prenylated flavonoids [10]. The reason for this phenomenon is not yet clear, but it is obvious that in the case of **9** there are two *ortho* phenolic hydroxyl groups available for cyclization with C-1″. It seems that 'soft' ionization techniques like FAB and APCI can be used to distinguish between 6- and 8-prenylated flavanones.

Flavonoid variation

Table 5 summarizes a flavonoid survey of nine European hop varieties. Compound **1** is the dominant resin flavonoid in all samples, making up 80–90% of the total of flavonoids identified. On the whole, this principal flavonoid is accompanied by small quantities of **2**–**9**. As can be seen from the table, the flavonoid profiles are rather similar and seem to have little value in regard to variety identification. Also, there is no evidence that the flavonoid profiles are affected by geographic origin as exemplified by the two Saazer samples, one from the Saazer region (sample no. 7) and one from Trsice (sample no. 8). It is not clear, however, whether the chemical diversity encountered reflects the species' secondary metabolism or whether the array of prenylflavonoids are in part the result of decomposition during drying and storage. For example, 2′-hydroxychalcones are known to isomerize chemically even *in vivo* when the enzyme chalcone isomerase is absent [20]. This would explain the small amounts of **7** and the prenylnaringenins in comparison to their chalcone precursors, **1** and desmethylxanthohumol (**2**). On the other hand, the presence of kaempferol and quercetin glycosides in hops [21] indicates that a chalcone isomerase should be present, but this enzyme does not necessarily have to be located in the lupulin glands where the resin flavonoids are likely to be biosynthesized. The reported high yield of **7** (0.18%) as compared to **1** (0.0012%) [5] is probably associated with exposure to alkali during the extraction and purification process. Earlier, Verzele *et al.* [3] demonstrated that **1** can be readily converted into **7** in dilute aqueous solutions of sodium hydroxide [3].

We have repeated this experiment and also observed rapid isomerization of **1** in 1% aqueous sodium hydroxide at 0° in the absence of air (50% conversion after 10 min). In a study of the stability of hops, Hänsel and Schulz [22] observed a 50% decrease in **1** levels within six months of storage, but did not investigate the fate of **1**. Earlier studies by Ashurst *et al.* [23] have shown that a large part of **1** is lost during drying. The loss of **1**, however, could not be accounted for by increased levels of **7** and other minor products detected.

EXPERIMENTAL

Plant material. Hop cones were grown in Europe and harvested in 1994 or 1995. The materials were dried at *ca* 50° to final moisture contents of *ca* 12% prior to shipment. Samples (Table 5) were supplied by Anheuser Busch Co. (St. Louis, MO) and stored at −20° until analysis.

NMR. ^1H (400 or 600 MHz) and ^{13}C (100.6 or 150.9 MHz) NMR spectra were recorded in DMSO-d_6 at room temp. DMSO resonances were used as int. chemical shift refs. ^1H–^1H COSY and ^1H–^{13}C HMQC and HMBC experiments were performed using standard pulse sequences. Spectral widths of 16 and 200 ppm were used in the H and C dimensions, respectively.

Mass spectrometry. EIMS (70 eV) were recorded by sample introduction via solid probe at 100–150°. APCI MS were run on a PE Sciex API III Plus triple quadrupole instrument. Samples were introduced by loop injection or by HPLC via the heated nebulizer interface kept at 480° which heats the column effluent to *ca* 120°. Ionization of the analyte vapour mixt. was initiated by a corona discharge needle at *ca* 8 kV and a discharge current of *ca* 3 μA. The orifice plate voltage was set at 45 V in the positive ion mode and at −55 V in the negative ion mode unless otherwise stated. CAD experiments were performed with Ar–N_2 (9:1) as target gas at a thickness of *ca* 1.9×10^{14} atoms cm^{-2}. The collision energy was 11 V. Other operating conditions were standard. The multiple ion scan mode was employed for selective detection and quantitation of flavonoids in hop extracts. With the aim of making a quantitative comparison of flavonoid variation, we assumed equal molar responses for all compounds, i.e. the proportion of molecules protonated and detected was assumed to vary little between structurally related flavonoids. Molar responses were converted into mass responses by multiplication with M_r. Integration of peak areas was carried out within the linear detection range using a computer program provided by the instrument's manufacturer.

Chromatography. TLC was run on silica gel with toluene–dioxane–HOAc (18:5:1). Spots were visualized by spraying with (1) anisaldehyde–H_2SO_4 followed by heating at 100–110° for *ca* 5 min, (2) 2% $FeCl_3$ in MeOH and (3) a 1% soln of diphenylboric acid–ethanolamine complex in MeOH ('Naturstoffreagenz A'). Analyt. RP-HPLC sepns were achieved on a 5 μm LiChrospher RP-18 column (250 × 4 mm) at 1 ml min^{-1} using solvent system 3 (see below). Compounds **1**–**9** had the following R_ts under these conditions: 10.8 min (**7**), 17.4 min (**8**), 18.2 min (**6**), 20.5 min (**2**), 22.5 min (**9**), 26.0 min (**1**), 32.4 min (**3**), 34.3 min (**5**) and 40.3 min (**4**). Semi-prep. sepns were carried out on a 10 μm Econosil RP-18 column at 5 ml min^{-1}. The following solvent systems were used: (system 1) MeCN–H_2O (3:2), (system 2) a linear solvent gradient from 40 to 80% aq. MeCN containing 1% HCO_2H over 20 min or (system 3) over 40 min. The UV trace was recorded at 290 and/or 370 nm.

General procedure for extraction and purification of resin flavonoids. Hop cones were immersed in $CHCl_3$ for *ca* 1 min. The $CHCl_3$ extract was filtered and evapd. After addition of MeOH to the oily residue, the mixt. was boiled under reflux conditions for *ca* 5 min and then left at −20° in order to ppt. the major part of resinous materials and other lipophilic constituents. The ppt. was sepd from the supernatant by filtration *in vacuo*. The fitrate was concd and directly chromatographed over Sephadex LH-20 with MeOH as eluent. Frs of 5–10 ml were collected and monitored by silica gel TLC. The flavonoid-containing frs were combined and evapd. Individual compounds were isolated by silica gel CC and prep HPLC. For a comparison of hop varieties by HPLC-MS, 10.0 g whole or coarsely ground hops were processed as described. Crude flavonoid mixts were diluted with MeOH to a concn corresponding to 40–100 mg plant material ml^{-1}.

Xanthohumol (**1**). The crude mixt. of flavonoids obtained from 500 g whole Saazer × Zattler hops was dissolved in a small vol. of MeOH. After addition of 150 ml H_2O, the resulting ppt. was redissolved by heating under reflux conditions and addition of small portions of MeOH. On cooling, crude **1** pptd, which was further purified by recrystallization from aq. MeOH. Yield: 445 mg yellow–orange crystals. UV λ_{max}^{MeOH}: 368 nm. ^{1}H NMR spectral data are in agreement with those reported for xanthohumol [6]. ^{13}C NMR: Table 3. EIMS *m/z* (rel.int.): 354 $[M]^+$ (100), 299 $[M-C_4H_7]^+$ (20), 234 $[A_1]^+$ (17), 179 $[A_1-C_4H_7]^+$ (79), 120 $[B_1]^+$ (17).

Isoxanthohumol (**7**). Compound **7** was obtained by chemical conversion of **1**. A soln of **1** (10 mg) and NaOAc (0.5 g) in MeOH (5 ml) was kept at 60° for 3 days. The reaction mixt. was then rendered slightly acidic with HOAc. Prep. HPLC (system 2) of the reaction mixt. yielded a few mg of isoxanthohumol (white powder after lyophilization). UV λ_{max}^{MeOH} nm: 287, 325sh. ^{1}H NMR: δ 5.31 (1H, *dd*, J = 12.4, 2.9 Hz, H-2), 2.92 (1H, *dd*, J = 16.3, 12.5 Hz, H-3ax), 2.56 (1H, *dd*, J = 16.4, 3.0 Hz, H-3eq), 6.13 (1H, *s*, H-6), 7.28 (2H, *d*, J = 8.5 Hz, H-2′, H-6′), 6.77 (2H, *d*, J = 8.6 Hz, H-3′, H-5′), 3.11 (1H, *d*, J = 7.1 Hz, H-1″), 5.09 (1H, *t*, J = 6.8 Hz, H-2″), 1.54 (3H, *s*, H-5″), 1.58 (3H, *s*, H-4″), 9.50 (*br s*, 4′-OH), 3.69 (3H, *s*, OMe). EIMS *m/z* (rel. int.): 354 $[M]^+$ (100); fragmentation pattern very similar to that of **1**.

Isolation of prenylnaringenins. The **1** mother liquors were taken to dryness by evapn *in vacuo* and lyophilization. The residue was fractionated by silica gel CC. Elution was started with hexane (100 ml), followed by increasing proportions of EtOAc in hexane. The 30% EtOAc fr. contained **8** and **9**. A few mg of both compounds were obtained by prep. HPLC using system 1.

8-*Prenylnaringenin* (**8**). Pale yellow powder after lyophilization. UV λ_{max}^{MeOH} nm: 291, 334sh. ^{1}H NMR: δ 5.42 (1H, *dd*, J = 12.4, 2.7 Hz, H-2), 3.19 (1H, *dd*, J = 17.1, 12.5 Hz, H-3ax), 2.72 (1H, *dd*, J = 17.3, 3.2 Hz, H-3eq), 5.97 (1H, *s*, H-6), 7.30 (2H, *d*, J = 8.3 Hz, H-2′ and H-6′), 6.79 (2H, *d*, J = 8.2 Hz, H-3′, H-5′), 3.08 (2H, *d*, J = 7.1 Hz, H-1″), 5.08 (1H, *t*, J = 7.1 Hz, H-2″), 1.59 (3H, *s*, H-4″), 1.54 (3H, *s*, H-5″), 9.50 (*s*, 4′-OH), 10.70 (*br s*, 7-OH), 12.08 (*s*, 5-OH). ^{13}C NMR: δ 78.2 (C-2), 41.9 (C-3), 196.6 (C-4), 161.1 (C-5), 95.2 (C-6), 164.3 (C-7), 106.9 (C-8), 159.7 (C-9), 101.7 (C-10), 129.2 (C-1′), 128.0 (C-2′), 115.1 (C-3′), 157.5 (C-4′), 115.1 (C-5′), 21.2 (C-1″), 122.7 (C-2″), 130.1 (C-3″), 17.5 (C-4″), 25.5 (C-5″). EIMS *m/z* (rel.int.): 340 $[M]^+$ (100), 285 $[M-C_4H_7]^+$ (20), 220 $[A_1]^+$ (10), 165 $[A_1-C_4H_7]^+$ (41), 120 $[B_1]^+$ (16).

6-*Prenylnaringenin* (**9**). Yellow powder after lyophilization. UV λ_{max}^{MeOH} nm: 291, 334sh. ^{1}H NMR: δ 5.39 (1H, *dd*, J = 12.7, 2.9 Hz, H-2), 3.22 (1H, *dd*, J = 17.1, 12.7 Hz, H-3ax), 2.67 (1H, *dd*, J = 17.1 Hz, 3.0, H-3eq), 5.97 (1H, *s*, H-8), 7.30 (2H, *d*, J = 8.5 Hz, H-2′, H-6′), 6.79 (2H, *d*, J = 8.4 Hz, H-3′, H-5′), 3.11 (2H, *d*, J = 7.1 Hz, H-1″), 5.13 (1H, *t*, J = 7.1 Hz, H-2″), 1.69 (3H, *s*, H-4″), 1.61 (3H, *s*, H-5″), 9.57 (*s*, 4′-OH), 10.73 (*br s*, 7-OH), 12.41 (*s*, 5-OH). ^{13}C NMR: δ 78.3 (C-2), 42.0 (C-3), 196.4 (C-4), 160.53 (C-5), 107.5 (C-

6), 164.2 (C-7), 94.3 (C-8), 160.49 (C-9), 101.6 (C-10), 129.0 (C-1′), 128.2 (C-2′), 115.1 (C-3′), 157.7 (C-4′), 115.1 (C-5′), 128.2 (C-6′), 20.6 (C-1″), 122.6 (C-2″), 130.2 (C-3″), 17.6 (C-4″), 25.4 (C-5″). EIMS *m/z* (rel. int.): 340 $[M]^+$ (100); fragmentation pattern very similar to that of **8**.

Isolation of compounds **2**, **3** *and* **5**. The mixt. of resin flavonoids obtained from 520 g whole Saazer × Zattler hops was fractionated by prep. HPLC using system 3. Repeated HPLC runs gave **1** (450 mg), **2** (not pure), **3** (13 mg) and **5** (1.5 mg). Compound **2** was further purified by prep. RP-HPLC (system 3) and finally by straight-phase HPLC on a 10 μm 250 × 10 mm silica gel column with hexane–EtOAc–HOAc (50:49:1) as eluent. This step furnished 10 mg **2**.

2′,4′,6′,4-*Tetrahydroxy*-3′-C-*prenylchalcone* (**2**). Yellow powder after lyophilization. UV λ_{max}^{MeOH}: 366 nm. ^{1}H NMR spectral data are in agreement with those reported for desmethylxanthohumol [6]. ^{13}C NMR: Table 3. EIMS *m/z* (rel. int.): 340 $[M]^+$ (100), 285 $[M-C_4H_7]^+$ (26), 220 $[A_1]^+$ (13), 165 $[A_1-C_4H_7]^+$ (51), 120 $[B_1]^+$ (16).

2′,4′,6′,4-*Tetrahydroxy*-3′-C-*geranylchalcone* (**3**). Yellow powder after lyophilization. UV λ_{max}^{MeOH} nm: 367, 310sh. ^{1}H and ^{13}C NMR: Tables 2 and 3. MS data are in good agreement with those reported for its flavanone analogue, bonannione A, isolated from *Bonannia graeca* [13].

Dehydrocycloxanthohumol (**5**). Red needles. UV λ_{max}^{MeOH} nm: 371, 298sh, 285. ^{1}H and ^{13}C NMR: Tables 2 and 3. APCI-MS (positive mode), *m/z* 353 $[MH]^+$. APCI-CAD-MS: Table 1.

Cyclodehydrogenation of xanthohumol (**1**). A soln of **1** (10.8 mg) and DDQ, (8.1 mg) in dry toluene (20 ml) was refluxed for 2.5 hr under N_2. The soln was decanted and evapd. Compound **5** proved to be the main reaction product and was sepd from by-products with prep. HPLC using system 3. The yield on lyophilization amounted to 1.9 mg. The semi-synthetic product was identical with natural **5** by TLC, HPLC, APCI-MS and ^{1}H NMR analysis.

Compounds **4** *and* **6**. The mixt. of flavonoids obtained from 150 g Spalter hops was submitted to prep. HPLC using system 3. Repeated runs afforded **4** (1 mg) and **6** (3.5 mg).

5′-*Prenylxanthohumol* (**4**). Dark-yellow powder after lyophilization. UV λ_{max}^{MeOH}: 371 nm. APCI-MS (positive mode) *m/z*: 423 $[MH]^+$; APCI-CAD-MS: Table 1.

Dehydrocycloxanthohumol hydrate (**6**). Yellow powder after lyophilization. UV λ_{max}^{MeOH}: 372 nm. ^{1}H NMR: Table 2. APCI-MS (positive mode) *m/z*: 371 $[MH]^+$. APCI-CAD-MS: Fig. 2.

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