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FLAVONOIDS FROM *MERRILLIA CALOXYLON**†

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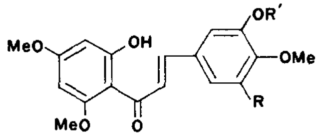
Key Word Index—*Merrillia caloxylon*, Rutaceae, penta- and tetra-oxygenated chalcones, flavones

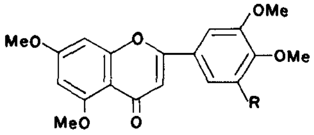
Abstract—Three chalcones and three flavones isolated from the fruit of *Merrillia caloxylon* (Rutaceae) have been characterised Two of the flavones and two of the chalcones are related structurally, i e 3',4',5,7-tetramethoxyflavone with 2'-hydroxy-3,4,4',6'-tetramethoxychalcone and 3',4',5,5',7-pentamethoxyflavone with 2',3-dihydroxy-4,4',6'-trimethoxychalcone A minor constituent was tentatively characterized as 5-hydroxy-3',4',5',6,7-pentamethoxyflavone and this is accompanied by 2-hydroxy-3,4,4',5,6'-pentamethoxychalcone and 5-hydroxy-3',4',6,7-tetramethoxyflavone

INTRODUCTION

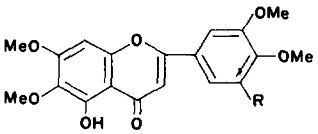
THE GENUS *Merrillia* is a monotypic member of the family Rutaceae and of rare occurrence in Malaysia.[1] We obtained quantities of the fruit of *M. caloxylon*, which grows near the Forest Research Institute, Kepong, Selangor, in order to extend our studies on S.E. Asian Rutaceae. The fruit is usually green ripening to yellow with the size and shape of a large lemon. Numerous oil glands lie under the thick rind and in cutting the fruit quite large amounts (*ca.* 20% wt) of essential oil is exuded; this oil was not examined. An ethanol soluble extract of the fresh fruit was prepared and this was partitioned between hexane and chloroform. From the chloroform extract three chalcones and three flavones were isolated, their structures being assigned through spectral analysis. By chromatographic separation, the three most mobile components, 2'-hydroxy-3,4,4',6'-tetramethoxychalcone (**1**), 2'-hydroxy-3,4,4',5,6'-pentamethoxychalcone (**2**) and 2',3-dihydroxy-4,4',6'-trimethoxychalcone (**3**) were isolated. The less mobile fractions yielded 3',4',5,7-tetramethoxyflavone (**4**), 3',4',5,5',7-pentamethoxyflavone (**5**) and 5-hydroxy3',4',6,7-tetramethoxyflavone (**6**). A minor component was tentatively identified as 5-hydroxy-3',4',5',6,7-pentamethoxyflavone (**7**). All these compounds are reported for the first time as natural products, and confirmation of their structures was obtained by comparison with synthetic samples.



(**1**) R = H; R' = Me
(**2**) R = OMe; R' = Me
(**3**) R = R' = H



(**4**) R = H
(**5**) R = OMe



(**6**) R = H
(**7**) R = OMe

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* Part 4 in the series "Rutaceae Constituents." For Part III see FRASER, A W and LEWIS, J R, (1973) *Chem Commun*, 615

† Some of this work was presented at a Symposium of the Phytochemical Society in Dublin, September 1971

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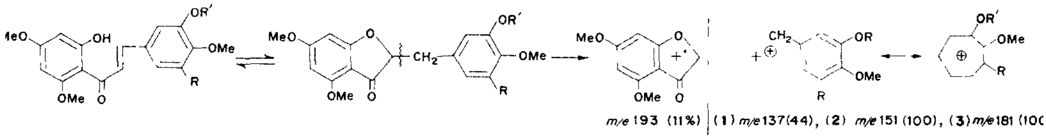
RESULTS

TLC separation of the chloroform soluble material gave (**1**)–(**3**) with high mobility on silica gel. Their UV spectra gave maxima in the ranges 245–254 and 365 377 nm shifting to 253–268; 323–346 and to 406–430 nm in the presence of $AlCl_3$ indicating the presence of a chalcone chromophore [2] Their IR spectra showed the presence of a hydroxyl group hydrogen bonded to a carbonyl group while the NMR spectra gave valuable structural information (see Experimental).

The MS obtained from these chalcones could be interpreted in terms of cleavage either side of the carbonyl group, as had been found in the MS of simple chalcones[3] and for echinatin.[4] Thermal isomerisation of chalcones to flavanones has been reported to occur in the mass spectrometer[3 5,6] and we have observed a series of ions in our spectra which would be expected for this type of compound* (see Experimental). The presence of additional ions in the MS suggested an alternative type of chalcone isomerization. Accurate mass measurement of the ion at *m/e* 151 obtained from (II) gave $C_9H_{11}O_2$ and from (III) at *m/e* 181, $C_{10}H_{13}O_3$, both ions could be attributed to the stable benzyl-tropylium cation as indicated in Scheme 1.



SCHEME 1

The synthesis of each chalcone was accomplished by published methods.[7-9]

Further chromatography gave four additional compounds having medium or low mobilities on silica gel Two of these compounds were easily purified and identified as 3′,4′,5,7-tetramethoxyflavone (**4**) and 3′,4′,5,5′,7-pentamethoxyflavone (**5**). The NMR spectrum of the third component suggested that another compound (with identical chromatographic behaviour) was present to *ca.* 10% Conversion to their acetates followed by rechromatography allowed the major component to be identified as 5-hydroxy-3′,4′,6,7-tetramethoxyflavone (**6**); the minor component had an NMR spectrum consistent with that attributed to 5-hydroxy-3′,4′,5′,6,7-pentamethoxyflavone (**7**)[9]

DISCUSSION

The co-isolation of chalcones with the related flavones gives additional evidence to the proposal by Wong,[10] that chalcones are direct precursors of flavones rather than by their conversion to flavanones followed by oxidation We were unable to detect any flavanones

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* We thank a referee for informing us of a recent publication[6]

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[3] Itagaki, V , Kurokawa, T , Sasaki, S Chang, C and Chen, F (1966) *Bull Chim Soc Japan* **39,** 538

[4] Furuya, T , Matsumoto, K and Hikichi, M (1971) *Tetrahedron Letters* 2567

[5] Kingston, D G I (1971) *Tetrahedron* **27,** 2691

[6] Sandl, C Van die, Serum, J W and Vandervalle, M (1972) *Org Mass Spectrosc* **6,** 1333

[7] Reidel V L Burkart, W and Muller K (1942), *Ann* **550,** 146

[8] Sonn, A , Muller V E , Bulow W and Meyer, W (1925), *Berichte* **58,** 1103

[9] Hoerhammer, L , Farkas, L , Wagner, H and Ostermayer I (1964) *Magyar Kem Folyoirat* **70,** 392

[10] Wong, E (1968) *Phytochemistry* **7,** 1751

in our extract and we also found great difficulty in converting our chalcones to the corresponding flavanones, although simple chalcones appear to isomerise easily.[11,12]

Mass spectral investigation of our chalcones confirmed previous observations,[3,5,6] that chalcone breakdown or isomerism to the flavanone followed by fragmentation, gives rise to most of the ions which appeared in the MS. The appearance of an additional intense ion in each chalcone spectrum was shown, by accurate mass measurement, to correspond to a benzylic (or tropylium) ion which we consider to be derived from ring B of the chalcone and its β-carbon atom (Scheme 1). The known stability associated with this type of ion could account for the intensity deserved and suggested that isomerization to a coumaran-3-one was taking place.

The mass spectral isomerisation of chalcones to coumaran-3-ones has been briefly commented upon by Scheinmann *et al.* in their investigation of the constituents of *Garcinia buchananii*[13] and others,[14] but it has not been previously suggested as a *major* pathway since the simple 2-hydroxychalcones previously studied in detail gave rise to low intensity benzylic ions by an alternative pathway (see Refs 3 and 6). The presence of methoxy or hydroxy substitution on ring B would appear to assist this type of isomerization and thus lead to the relatively high intensities observed for these ions.

EXPERIMENTAL

Extraction and purification of flavonoid constituents Fresh fruit of *M caloxylon* (6·5 kg) was sliced and extracted with EtOH (3 l) at R_t for 3 days Evaporation of the solvent left a residue (216 g) which was treated successively with C_6H_{12}, $CHCl_3$, EtOAc and EtOH in a Soxhlet The $CHCl_3$ soluble material showed three groups of compound on TLC examination (silica gel; EtOH/$CHCl_3$, 1 99) The fastest moving group was separated into its three individual components by preparative TLC using multiple development in silica gel with EtOH/$CHCl_3$ (1 99) to give (**1**) which crystallized from acetone/C_6H_{12} as yellow prisms m.p 151–153° Using $CHCl_3$/C_6H_{12} (4 1) (**2**) was purified to give orange needles m p 176–178° while multiple development using C_6H_6/EtOAc (4 1) gave (**3**) crystallizing as orange needles m p 163–165° The components with intermediate mobility were rechromatographed on silica gel (TLC) using EtOH–$CHCl_3$ (1 99) and compounds (**5**) and (**6**) isolated; (**5**) crystallized from C_6H_6–petrol (b p 60–80°) as colourless prisms, m p 176–178° while (**6**) gave colourless prisms from $CHCl_3$ m.p 190–192° (**5**) was found to be more easily purified by TLC on alumina using petrol (b p 60–80°)–$CHCl_3$ (100 0–3.1) The more polar and least mobile mixture from the original TLC was rechromatographed on an alumina column using $CHCl_3$ as eluant to give a crude sample of (**4**) Further purification by TLC on alumina, using petrol (b p 60–80°)/$CHCl_3$ in increasing ratio, gave pure (**4**), crystallizing from $CHCl_3$ as colourless prisms m p 190–192°

2′-Hydroxy-3,4,4′,6′-tetramethoxychalcone (**1**) was obtained in 0 0006% yield λ_{max} (EtOH) 252 *sh*, 326 *sh* and 376 nm (MeOH + $AlCl_3$) 265, 346 *sh* and 423 nm ν_{max} (KBr) 3420 (OH) and 1625 cm^{-1} (C=O) δ ($CDCl_3$) 3 82 (3 H, *s*, OMe), 3 91 (9 H, *s*, 3 × OMe), 5·95 and 6 10 (1 H, *d*, *J* 3 Hz, H-3′ and H-5′), 6 88 (1 H, *d*, *J* 9 Hz, H-5), 7 12 (1 H, *d*, *J* 2 Hz, H-2), 7 19 (1 H, *dd*, *J* 9 and 2 Hz, H-6), 7 75 (2 H, *s*, H_α and H_β), and 14 34 (1 H, *s*, OH) Irradiation at δ 5 95 collapsed the signal at δ 6 10 as it did at δ 6·88 for δ 7 19 MS showed the following peaks [parent ion 344 (50), base peak 151 (100)] corresponding to a chalcone fragmentation, peaks were obtained at 343 (42), 329 (3), 191 (8), 181 (21), 163 (8), 137 (9) and to flavanone fragmentation 327 (8), 207 (29), 180 (7), 164 (58), 137 (9) The coumaranone intermediate gave rise to peaks at 193 (3) and 151 (100) For the ion *m/e* 151, Found 151 0756 $C_9H_{11}O_2$ requires 151 0758 The synthetic chalcone[7] had similar physical and spectral properties

2′-Hydroxy-3,4,4′,5,6′-pentamethoxychalcone (**2**) obtained in 0 0003% yield was crystallized from C_6H_6 and had λ_{max} (MeOH) 245 *sh* and 366 nm, (MeOH + $AlCl_3$) 257, 339 *sh*, and 408 nm, ν_{max} (KBr) 3400 (OH) and 1640 cm^{-1} (CO) δ ($CDCl_3$) 3 84 (3 H, *s*, OMe), 3 90 (12 H, *s*, 4 × OMe), 5 98 and 6 12 (each 1 H, *d*, *J* 3 Hz, H-3′ and -5′), 6 84 (2 H, *s*, H-2 and -6), 7 74 and 7 76 (each 1 H, *s*, H-α and -β) and 14 26 (1 H, *s* OH) MS [parent ion 374 (50), base peak 181 (100)], with ions emanating from a chalcone fragmentation at 373 (15), 343 (7), 331 (12), 179 (18), and from a flavanone fragmentation at 357 (2), 207 (14), 194 (18) and 167 (2) The coumaranone intermediate

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[13] JACKSON, B , LOCKSLEY, H D , SCHEINMANN, F and WOLSTENHOLM, W A (1967) *Tetrahedron Letters*, 3049

[14] AUDIER, H (1966) *Bull Chem Soc France* **39,** 538

[13] GEISMANN, T A (1958) *Australian J Chem* **11,** 376

[14] MATSUKAWA, S , KOTAKE, H and YAMAGUCHI, M (1963) *Nippon Kagaku Zasshi* **84,** 359

gave rise to peaks at 193 (11) and 181[*] (100) The synthetic chalcone[8] had similar physical and spectral characteristics

2',3-Dihydroxy-4,4',6'-trimethoxychalcone (**3**) was isolated in 0 0002% yield with λ_{max} (MeOH) 254 *sh* and 377 nm, (MeOH + $AlCl_3$) 268, 344 *sh* and 430 nm, ν_{max} (KBr) 3360 (OH) and 1625 cm^{-1} (C=O), δ ($CDCl_3$) 3 84 (3 H, s, OMe), 3 92 (3 H, s, OMe), 3 96 (3 H, s, OMe), 5 90 (broad OH), 5 98 and 6 12 (each 1 H, *d*, *J* 3 Hz, H-3' and 5'), 6 95 (1 H, *d*, *J* 8 Hz, H-5), 7 10 (1 H, *d*, *J* 2 Hz, H-2), 7 23 (1 H, *dd*, *J* 8 and 2 Hz, H-6), 7 66 (2 H, s, Hα and β) and 14 38 (1 H, s, OH) MS [parent ion 330 (96) and base peak 329 (100)], corresponding to chalcone fragmentation at 329 (100), 315 (7), 314 (10), 302 (16), 177 (10), 181 (96), 150 (45), 123 (5) and to flavanone fragmentation at 313 (22), 207 (73) 180 (19), 150 (45), 123 (5) The coumaranone fragmentation corresponded to peaks at 191 (7) and 137[†] (44) The synthetic chalcone[9] was identical in all respects with that isolated herein

3',4',5,7-Tetramethoxyflavone (**4**) was purified as described (0 02% yield) and showed λ_{max} (MeOH) 244, 267 *sh*, and 338 nm, no change on addition of $AlCl_3$ or NaOMe ν_{max} (KBr) 1655 cm^{-1} (C=O) δ ($CDCl_3$) 3 95 (12 H, s, 4 × OMe), 6 35 (1 H, *d*, *J* 2 Hz, H-6), 6 54 (1 H *d* *J* 2 Hz H-8), 6 58 (1 H s H-3), 6 95 (1 H, *d* *J* 8 H, H-5'), 7 30 (1 H, *d*, *J* 2 Hz, H-2') and 7 44 (1 H, *dd*, *J* 8 and 2 Hz, H-6') Irradiation at δ 7 30 collapsed the signal centred at δ 7 44 to a doublet *J* 8 Hz and vice versa MS [parent ion 342 (100)] 341 (55), 325 (10) 313 (27), 312 (13), 311 (13), 297 (10), 296 (29), 85 (15) 83 (24) *m** 340 0 (324 → 341), 308 8 (342 → 328) and 286 5 (342 → 313) The synthetic compound was identical in all respects[13]

3',4',5,5',7-Pentamethoxyflavone (**5**) was obtained pure (0 0015% yield) as indicated previously and gave λ_{max} (MeOH) 237, 267 and 328 nm, no change with NaOMe or $AlCl_3$ ν_{max} (KBr) 1645 cm^{-1} (C=O), δ ($CDCl_3$) 3 92 (6 H, s, 2 × OMe), 3 94 (9 H, s, 3 × OMe), 6 38 (1 H, *d*, *J* 2 Hz, H-6), 6 56 (1 H, *d*, *J* 2 Hz, H-8), 6 72 (1 H, s, H-3) and 7 07 (2 H, s, H-2' and 6') MS [parent ion at 372 (100)], 371 (63) 355 (12) 343 (31), 342 (18), 341 (21) 327 (19), 326 (33) *m** 370 0 (372 → 371), 316 3 (372 → 343) and 312 6 (341 → 312) Its identity was confirmed by comparison with a sample prepared by the method described by Matsukawa *et al*[14]

5-Hydroxy-3',4',6,7-tetramethoxyflavone (**6**) was obtained as a crude sample from TLC (0·0012% yield); it was acetylated with Ac_2O, py at R_t to give a mixed acetate which crystallized from $CHCl_3$ C_6H_{12} to give pure 5-acetoxy-3',4',6,7-tetramethoxyflavone as prisms m p 159–161 ν_{max} (KBr) 1765 and 1630 cm^{-1} (C=O) δ ($CDCl_3$) 2·48 (3 H, s, COMe); 3·86; 3·94; 3·96; 4·00 (each 3 H, s, OMe); 6·50 (1 H, s H-3); 6·90 (1 H, s, H-8); 6·96 (1 H, *d*; *J* 8 Hz H-5'), 7 28 (1 H, *d*, *J* 2 Hz, H-2') and 7 48 (1 H, *dd*, *J* 8 and 2 Hz H-6') Irradiation at δ 7 48 collapsed the double doublet which was formally centred at δ 6 98 Hydrolysis (dilute methanolic NaOH for 3 hr at reflux) followed by acidification gave the flavone (**6**) which crystallised from C_6H_6–petrol (60–80) as yellow prisms m p 190–194 It showed similar spectral characteristics to that described in the literature[17] and had MS [parent ion 358 (100)], 357 (40), 345 (12) 344 (21) 343 (82), 324 (12), 341 (15), 329 (24), 328 (10), 315 (25), 312 (26) 381 (18), 163 (19), 153 (40), 69 (14), *m** 302 3 (358 → 329), 289 3 (343 → 315), and 271 9 (358 → 312)

5-Hydroxy-3',4',5',6,7-pentamethoxyflavone (**7**) was tentatively identified by comparison of the MS "impurity" peaks obtained from the original sample of (**6**) These ions were associated with +30 units i e –OCH_2– at *m/e* [parent ion at 388 (32)], 387 (7) 374 (8), 373 (30), 371 (2), 360 (6), 327 (13) 313 (10), 299 (6), 297 (3), 285 (3), 282 (10) The NMR spectrum ($CDCl_3$) of the mixture containing mainly **6** together with some **7** showed the following peaks 3 93 (*s*, OMe), 3 97 (s OMe), 6 55 (*s*, H-3), 6 59 (s, H-8), 6 97 (*d*, *J* 8 Hz H-5') 7 09 (*d*), 7 34 (*d* *J* 2 Hz, H-2'), 7 53 (*dd*, *J* 8 and 2 Hz, H-6'), 12 66 (s, OH), and 12 71 (s OH) of these peaks the small doublet at δ 7 09 could be assigned to the meta coupled protons H-2'/H-6' [cf (**5**) at δ 7 07 (*d*)] thus favouring structure (**7**) for the 'impurity' originally present in sample (**6**)

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[*] Accurate mass measurement gave 181 0864 corresponding to $C_{10}H_{13}O_3$

[†] Accurate mass measurement gave 137 0602 corresponding to $C_8H_9O_2$

[17] Barna A K, Chakrabarti, P and Sanyal, P K (1969) *J Ind Chem Soc* **46**, 271