Hawaiian Plant Studies. Part XVI.[1] Coumarins and Flavones from *Pelea barbigera* (Gray) Hillebrand (Rutaceae)[2]

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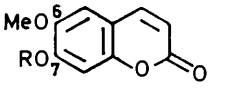
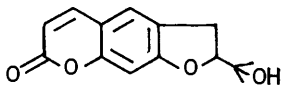
One sample of the endemic Hawaiian rutaceous shrub *Pelea barbigera* has yielded the known coumarins scopoletin (1a), marmesin (2), and psoralene (3a), and another collection yielded *p*-hydroxybenzaldehyde, a new furocoumarin 2-(1-hydroxy-1-methylethyl)-4-methoxyfuro[3,2-*g*][1]benzopyran-7-one (4), and two new flavones 5-hydroxy-3,7,8-trimethoxy-2-(3,4-methylenedioxyphenyl)-1-benzopyran-4-one (5a) and 5-hydroxy-3,6,7,8-tetramethoxy-2-(3,4-methylenedioxyphenyl)-1-benzopyran-4-one (6).

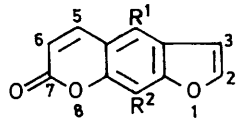
ENDEMIC Hawaiian Rutaceae include the genus *Pelea*, which comprises some 70 species of shrubs and trees, two of which have been described from the Marquesas, all the others from the Hawaiian islands.[3] Most *Peleae* are fragrant and only marginally alkaloidal. These properties are reversed in *P. barbigera*, which is a shrub native to the island of Kauai, and from which we isolated the furoquinoline alkaloids kokusaginine, isoplatydesmine, and edulinine.[1] Further fractionation of the methanolic plant extract, after removal of the alkaloids, yielded *p*-hydroxybenzaldehyde and several coumarins and flavones, which we now describe.



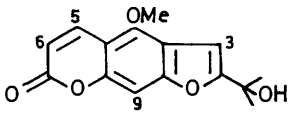
(1) a, R = H
b, R = Me



(2)



(3) a; $R^1 = R^2 = H$
b; $R^1 = OMe, R^2 = H$
c; $R^1 = H, R^2 = OMe$



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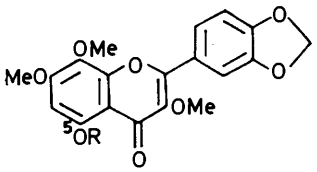
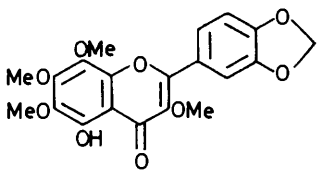


(5) a, R = H
b, R = Me



(6)

Two separate plant collections yielded two sets of constituents. *P. barbigera* collected along the Nualolo trail furnished scopoletin (1a), marmesin (2), and psoralene (3a), while plant material from Makaha ridge, Kokee, yielded *p*-hydroxybenzaldehyde, a new furocoumarin (4), and two new flavones (5a) and (6). Two of the known coumarins, scopoletin (1a) and marmesin (2), were isolated from 1 kg of dried plant. Scopoletin (0·5 mg) was identified by comparison of its u.v. and mass spectra with those of scoparone (1b),[4-6] prepared from a trace of scopoletin (1a) that had been treated with diazomethane, yielding a mixture of scoparone (1b) and scopoletin (1a), as seen by mass spectral determination.

Marmesin (2) was identified by comparison of its mass and n.m.r. spectra with those reported in the literature.[7,8]

The third known coumarin, psoralene (3a), had mass, i.r., and n.m.r. spectra identical with corresponding literature data.[9-12]

Our second plant sample yielded non-alkaloidal constituents from hexane and ether extracts of an aqueous acidic solution that was generated by partition with a concentrated, filtered methanol extract of 1 kg of dried plant. The hexane extract (see below) yielded flavones (5a) and (6). The ethereal extract was further fractionated on a column of Bio-Sil A. Purification of the petroleum eluate by t.l.c. yielded crystalline *p*-hydroxybenzaldehyde, with i.r. and mass spectra identical with those of an authentic sample.

The ether eluate of the Bio-Sil column after preparative t.l.c. (p.l.c.) and two recrystallizations from benzene afforded the new furocoumarin (4) (8 mg), m.p. 165—166°. Combustion analysis suggested a composition $C_{15}H_{14}O_5$, which was supported by a mass spectral molecular weight of 274. Structural assignment was based on comparison of its n.m.r. spectrum with those of psoralene (3a), bergapten (3b),[12] and xanthotoxin (3c).[12] Chemical shifts of the protons at C-5, C-6, and C-9 and coupling constants $J_{5,6}$, $J_{3,9}$, and $J_{5,9}$ were virtually identical with those of bergapten (3b). The n.m.r. signal at δ 6·84 assigned to H-3 is slightly upfield from the H-3 signal of bergapten (δ 7·00), but it coincides with the H-3 signals of psoralene (3a)

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(δ 6·83) and of xanthotoxin (3c) (δ 6·81). The corresponding chemical shifts for H-2 are well outside this range: δ 7·69 for (3a), 7·58 for (3b), and 7·68 for (3c). Clearly, therefore, the C_3H_7O residue is a C-2 substituent. Its nature as a 1-hydroxy-1-methylethyl group is secured by the six-proton singlet at δ 1·72. I.r. bands at 3597 and 1723 cm^{-1} confirmed the presence of a hydroxy-group and of an αβ-unsaturated δ-lactone.

The hexane extract of the aqueous acid after Bio-Sil A chromatography, silica gel t.l.c., and recrystallization from benzene, ethanol, and benzene again, afforded yellow crystals, m.p. 221·5—222°. Combustion and mass spectral data pointed to a $C_{19}H_{16}O_8$ formulation. The n.m.r. spectrum of flavone (5a) showed δ 3·88, 3·91, and 3·95 (3s, 3 × OMe), 6·07 (s, OCH_2O), and 6·41 (s) and three signals in the ABC pattern (total 4H, aromatic). A hydroxy-group and a conjugated carbonyl were shown by i.r. bands at 3450 and 1647 cm^{-1}. U.v. spectra [reported in Supplementary Publication No. SUP 20966 (4 pp.) *] in methanol, methanol–aluminum chloride, and methanol–aluminum chloride–HCl were typically flavonoid. A C-3 or C-5 hydroxy-group appeared likely from the aluminum chloride induced bathochromic shift, which was not readily reversible by HCl addition, which is diagnostic for such a functionality.[13] The methanol spectrum strongly resembled that of meliternin (5b),[14] a constituent of the closely related New Zealand rutaceous tree *Melicope ternata*. The splitting pattern of the three aromatic protons placed the methylenedioxy-group at C-3′ and C-4′ of ring B. Of the eight possible 3- or 5-hydroxy-3′,4′-methylenedioxytrimethoxyflavones five were known [*cf.* data in Supplementary Publication No. SUP 20966 (4 pp.) *]. Of the three remaining compounds two lack oxygenation at C-7 and are therefore unlikely natural compounds. The last isomer is 5-hydroxy-3,7,8-trimethoxy-3′,4′-methylenedioxyflavone, which is the structure that has been assigned to the *Pelea* flavone (5a). Further substantiation of this assignment comes from the following considerations. Although flavone (5a) and a known flavonol, 3-hydroxy-5,7,8-trimethoxy-3′,4′-methylenedioxyflavone,[15] possess the same hydroxylation pattern, the two compounds differ in m.p. and u.v. spectra, thus pointing to 5-hydroxy- rather than 3-hydroxy-substitution for flavone (5a). The assignment of the n.m.r. singlet at δ 6·41 to the proton at C-6 of flavone (5a) is virtually identical with the reported chemical shift (δ 6·42) of the corresponding proton of 5-hydroxy-3,3′,4′,7,8-pentamethoxyflavone.[16]

Preparative silica gel t.l.c. that had yielded (5a) afforded a second yellow band which, after two recrystallizations from cyclohexane–carbon tetrachloride, gave fine yellow needles, m.p. 153°. Combustion data and mass spectrum established a $C_{20}H_{18}O_9$ (*m/e* 402) formulation. Structure (6) could be assigned from n.m.r. data. Four methoxy-groups at δ 3·86 (3H), 3·88 (6H), and 3·99 (3H), and a methylenedioxy-singlet at δ 6·03 accounted for all five carbons in addition to a C_{15} flavone skeleton. The ABC pattern of the three aromatic protons was identical with that of flavone (5a), thereby placing the methylenedioxy-group at C-3′,4′ of ring B. A singlet at δ 12·18 is characteristic of a strongly H-bonded 5-hydroxy-group.[16] U.v. data [reported in Supplementary Publication No. SUP 20966 (4 pp.) *] further support the structure 5-hydroxy-3,6,7,8-tetramethoxy-3′,4′-methylenedioxyflavone for the *Pelea* flavone (6). The reported u.v. spectrum (EtOH) of 5-hydroxy-3,3′,4′,6,7,8-hexamethoxyflavone, which is a derivative of a *Citrus* flavone, with maxima at 258, 280, and 345 nm [17] is essentially identical with the spectrum (MeOH) of (6), having maxima at 259, 278, and 345 nm. In methanol–aluminum chloride–hydrochloric acid the 345 nm band of our *Pelea* flavone shifts bathochromatically to 364 nm. This small (19 nm) shift is characteristic for 5-hydroxy-, but not for 3-hydroxyflavones.[18] Normal bathochromic shifts for 3-hydroxyflavones are *ca.* 60 nm, while the range for 5-hydroxyflavones is 35—55 nm. Highly oxygenated 5-hydroxyflavones with a vicinal hydroxy- or methoxy-group at C-6 average unusually small shifts, *ca.* 21 nm.[19]

EXPERIMENTAL

M.p.s were determined on a Fisher–Johns block. Combustion analyses were performed by University of California Chemical Analytical Services. U.v. spectra were measured on a Beckman DK-2 and i.r. spectra on a Beckman IR-10 instrument. Mass spectra were recorded by Sr. M. R. Brennan on a Hitachi–Perkin-Elmer RMU-6D and n.m.r. spectra by Mr. J. Loo on a Varian HA-100 instrument.

First Extraction.—Dried and milled *P. barbigera* (1 kg) collected along the Nualolo Trail, Kokee, Kauai, in March 1972,[20] was extracted with hexane (16 h), then methanol (72 h). To the methanol extract was added an equal volume of 0·5N-HCl. The mixture was filtered through Celite and methanol was evaporated off. The remaining aqueous solution was extracted with ether (5 × 200 ml) yielding 4·4 g of residue.

Scopoletin (1a) *and marmesin* (2). This residue was placed on an alumina column and eluted with chloroform (20 × 50 ml). The product of fractions 6—10 was further separated by t.l.c. (silica gel G, chloroform–methanol 20 : 1) to furnish a crystalline component (9 mg) which on recrystallization from benzene–chloroform yielded scopoletin (0·5 mg), m.p. 192—195° (lit.,[4] 204—205°), $\lambda_{max.}$ (EtOH) 204, 228, 252sh, 258sh, 297, and 346 nm, $\lambda_{min.}$ 217, 270, and 307 nm, *m/e* 192 (100%), 177 (73), 164 (31), 149 (64), 121 (33), 79 (30), 69 (100), 65 (23), 53 (20), 51 (45), 50 (23), and 39 (19).

* For details of Supplementary Publications, see Notice to Authors No. 7 in *J.C.S. Perkin I*, 1972, index issue.

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Methylation of scopoletin (trace amount) with diazomethane in ether gave a mixture of scoparone (1b) (*m/e* 206, 191, 178, 163, 135, 120, 107, and 79) and scopoletin (*m/e* 192, 177, 149, 121, 79, and 69).

Preparative t.l.c. of fractions 1—5 on alumina G (chloroform) and on silica gel G (chloroform) gave a crystalline product which was recrystallized from benzene to afford crude marmesin (2) (3·5 mg), m.p. 136—142°. Another recrystallization from ethanol gave a purer product, m.p. 164—170° (lit.,[21] 189·5°), $\lambda_{max.}$ (EtOH) 205·5, 224, 247, 296sh, and 334 nm, $\lambda_{min.}$ 214, 237·5, and 267 nm, *m/e* 246 (53%, M^+), 228 (5·5, $M - H_2O$), 213 (21·5, $M - H_2O - CH_3$), 188 (84, $M - C_3H_6O$), 187 (100, $M - C_3H_7O$), 175 (16), 160 (27, $M - C_3H_6O - CO$), 159 (11, $M - C_3H_7O - CO$), 131 (16, $M - C_3H_7O - 2CO$), and 59 (40, $C_3H_7O^+$), δ ($CDCl_3$) 1·70 (s), 3·22 (m), 4·74 (m), 6·20 (d, *J* 9·6 Hz), 6·72 (s), 7·40 (s), and 7·77 (d, *J* 9·6 Hz).

Psoralene (3a). The aqueous acidic solution was then successively extracted at gradually increasing pH values. The residue resulting from continuous (72 h) chloroform extraction of the aqueous phase at pH 12 was subjected to t.l.c. on alumina G plates (MeOH–$CHCl_3$; 1 : 50), where it was separated into four major bands as seen under u.v. light. The least polar band furnished a solid (25 mg) which by repeated identical t.l.c. gave crystals (18 mg), R_F 0·93 (alumina G, chloroform), m.p. 162—166°. Recrystallization from 95% ethanol gave a product, m.p. 164—166°, *m/e* 186 (100%), 158 (74), 130 (17), 129 (10), 102 (28), 76 (15), 75 (14), 51 (24), and 50 (15), $\lambda_{max.}$ (95% EtOH) 210, 241sh, 245·5, 288, and 332·5sh nm, $\nu_{max.}$ (KBr) 3160, 3125, and 1720 cm^{-1}, δ ($CDCl_3$) 7·69 (H-2), 6·83 (H-3), 7·68 (H-4), 7·78 (H-5), 6·38 (H-6), and 7·47 (H-9).

Second Extraction.—Dried and milled *P. barbigera* (1 kg), collected along Makaha ridge, Kokee, Kauai, in July 1972,[22] was extracted with absolute methanol for 72 h.

The extract was concentrated to *ca.* 1 l and mixed with 5% HCl (1 l). A resinous precipitate was removed by filtration through Celite and the filtrate was extracted with hexane (2 × 300 ml), dried ($MgSO_4$), and concentrated to furnish a brown-yellow glass (A) (1·40 g). After removal of the remaining methanol, the aqueous solution was extracted with ether (3 × 300 ml). The ethereal extract was successively washed with water and saturated sodium hydrogen carbonate solution, dried, and evaporated to afford a brown-yellow glass (B) (3·10 g).

Residue (B) was adsorbed on Bio-Sil A (90 g) and eluted successively with petroleum–ether 5 : 1 (fractions 1—4, 100 ml each), 5 : 2 (fractions 4—7), 1 : 1 (fractions 8—14), ether (fractions 15—25), ether–chloroform 5 : 2 (fractions 26—38), chloroform (fractions 29—34), and chloroform–methanol 20 : 1 (fractions 35—37).

p-*Hydroxybenzaldehyde.* Fractions 8 and 9 were combined and separated by t.l.c. (Brinkmann silica gel HF_{254}, $CHCl_3$–MeOH 50 : 1). A band, R_F 0·4—0·7, gave a crystalline product which was recrystallized from benzene to yield *p*-hydroxybenzaldehyde (4 mg), m.p. 117—118° (lit.,[23] 115—116°) *m/e* 122 (87%), 121 (100), 93 (39), 65 (30), and 39 (24). The i.r. spectrum (KBr) was identical with that of an authentic sample (Eastman).

Furocoumarin (4). Bio-Sil fraction 17 (230 mg) was further separated by t.l.c. (silica gel G, $CHCl_3$–MeOH 25 : 1) to yield a crystalline product (30 mg) which upon recrystallization from benzene afforded 2-(1-*hydroxy*-1-*methylethyl*)-4-*methoxyfuro*[3,2-g][1]*benzopyran*-7-*one* (4) as fine crystals (8·2 mg), m.p. 160—163° (raised by one more recrystallization from the same solvent to m.p. 165—166°), $\nu_{max.}$ ($CHCl_3$) 3597, 1723, 1627, 1597, 1577, and 1123 cm^{-1}, $\lambda_{max.}$ (EtOH) 223 (log ε 4·25), 252 (4·25), 257infl (4·20), 268·5 (4·21), and 310 nm (4·07), $\lambda_{min.}$ 236 (log ε 4·02), 264 (4·08), and 279 nm (3·58), δ ($CDCl_3$) 1·72 (6H, s), 4·27 (3H, s), 6·27 (1H, d, *J* 10 Hz), 6·84 (1H, d, *J* 1·0 Hz), 7·09 (1H, m), and 8·14 (1H, dd, *J* 10 and 0·65 Hz), *m/e* 274 (40%, M^+), 259 (100, $M - CH_3$), 256 (20, $M - H_2O$), 244 (17, $M - 2CH_3$), 241 (13, $M - CH_3 - H_2O$), 217 (10, $M - CH_3 - C_3H_6$), 216 (13, $M - 2CH_3 - CO$), 213 (6, $M - CH_3 - H_2O - CO$), 185 (6, $M - CH_3 - H_2O - 2CO$), 115·5 (4, 231^{2+}, $M - CH_3 - CO$), 108·5 (8, 217^{2+}), 69 (5), 51 (5), and 43 (29) (Found: C, 65·85; H, 5·2. $C_{15}H_{14}O_5$ requires C, 65·7; H, 5·15%).

Flavone (5a). Residue (A), remaining from the hexane extract of the aqueous acid was chromatographed on a Bio-Sil A (30 g) column by eluting with petroleum–ether 5 : 1 (15 × 50 ml) and ether (3 × 100 ml). Fractions 8—11 (158 mg) gave a yellow solid which on t.l.c. (silica gel HF_{254}, $CHCl_3$), yielded two yellow bands. Band 1 (R_F 0·50) was recrystallized from benzene to furnish 5-*hydroxy*-3,7,8-*trimethoxy*-2-(3,4-*methylenedioxyphenyl*)-1-*benzopyran*-4-*one* (5a), as yellow, fine crystals (4·5 mg), m.p. 211—215°. Two more recrystallizations from ethanol, then benzene gave yellow needles, m.p. 221·5—222°; u.v. data listed in SUP 20966, $\nu_{max.}$ (KBr) 3450, 1647, 1598, 1495, 1446, 1367, 1336, 1300, 1246, 1210, and 1030 cm^{-1}, δ ($CDCl_3$) 3·88 (3H, s), 3·91 (3H, s), 3·95 (3H, s), 6·07 (2H, s), 6·41 (1H, s), 6·96 (1H, dd, *J* 8·6 and 0·4 Hz), 7·67 (1H, dd, *J* 1·9 and 0·4 Hz), and 7·77 (1H, dd, *J* 8·4 and 1·9 Hz), *m/e* 372 (60%, M^+), 357 (100, $M - CH_3$), 342 (3, $M - 2CH_3$), 329 (5, $M - CH_3 - CO$), 314 (3, $M - 2CH_3 - CO$), 309 (3, $M - CH_3 - CH_2O - CO$), 286 (5), 186 (5), 181 (4), 178·5 (5, 357^{2+}), 173 (3), 172 (4), 171 (5), 164·5 (329^{2+}), 163·5 (327^{2+}), 162 (5), 157 (4), 156 (5), 153 (8), 149 (11), 142 (3), 135 (3), 134 (3), 133 (4), 125 (4), 121 (4), 119 (3·5), 75 (4·5), and 69 (6) (Found: C, 61·4; H, 4·35. $C_{19}H_{16}O_8$ requires C, 61·3; H, 4·35%).

Flavone (6). The second band (R_F 0·43) from t.l.c. separation gave yellow crystals (6·0 mg) after recrystallization from cyclohexane, m.p. 120—140°. Two more crystallizations from cyclohexane–carbon tetrachloride gave 5-*hydroxy*-3,6,7,8-*tetramethoxy*-2-(3,4-*methylenedioxyphenyl*)-1-*benzopyran*-4-*one* (6) (2·6 mg), as fine, yellow needles, m.p. 153°, u.v. data listed in SUP 20966, $\nu_{max.}$ (CCl_4) 3540, 3180, 2937, 2890, 1647, 1598, 1476, 1445, 1422, 1373, 1342, 1305, 1275, 1253, 1203, 1173, 1150, and 1048 cm^{-1}, δ 3·86 (3H, s), 3·88 (6H, s), 3·99 (3H, s), 6·03 (2H, s), 6·87 (1H, dd, *J* 8·6 and 0·4 Hz), 7·59 (1H, dd, *J* 1·9 and 0·4 Hz), 7·69 (1H, dd, *J* 8·5 and 1·9 Hz), and 12·18 (1H, s), *m/e* (70 eV) 402 (84%, M^+), 387 (100, $M - CH_3$), 372 (8·5, $M - 2CH_3$), 357 (10, $M - 3CH_3$), 329 (5·5, $M - 3CH_3 - CO$), 301 (3·5), 263 (4), 211 (8·5), 201 (6), 183 (7), 162 (5), 157 (5), and 149 (11·5), *m/e* (20 eV) 402 (100%), 387 (41), 372 (6), and 357 (3) (Found: C, 60·05; H, 4·2. $C_{20}H_{18}O_9$ requires C, 59·7; H, 4·5%).

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