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DIHYDROFLAVONOLS AND OTHER FLAVONOIDS OF *EUPATORIUM* SPECIES

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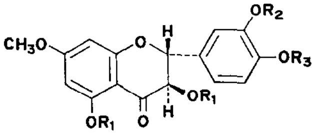
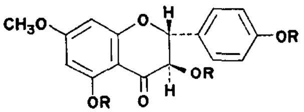
Key Word Index—*Eupatorium*; Compositae; dihydroflavonols; 7-*O*-methylaromadendrin; genkwanin; quercetin 3-methyl ether.

Abstract—Chloroform extracts of a hybrid of *Eupatorium capillifolium* (Lam.) Small and *E. perfoliatum* L. yielded the dihydroflavonols (2*R*, 3*R*)-7-methoxy-3,5,4′-trihydroxyflavanone (IIa), (2*R*, 3*R*)-3,4′-dihydroxy-5,7-dimethoxyflavanone (IIIa) and either (2*R*, 3*R*)-3,7-dimethoxy-3,5,4′-trihydroxy- or (2*R*, 3*R*)-7,4′-dimethoxy-3,5,3′-trihydroxyflavanone (Ia or Ic). Ia (or Ic) and IIIa are new; the stereochemistry of IIa has not been assigned previously. Genkwanin and quercetin 3-methyl ether were also found. *E. capillifolium* gave IIIa and IIa. *E. perfoliatum* gave no crystalline flavone fractions.

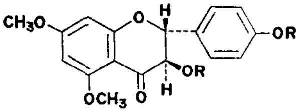
A previous report[1] on the constituents of the polar extract of various *Eupatorium* species revealed that astragalin (kaempferol 3-β-glucoside) and hyperoside (quercetin 3-β-galactoside), two flavonol 3-glycosides found in a naturally-occurring hybrid of *E. capillifolium* (Lam.) Small and *E. perfoliatum* L*., were also present in *E.capillifolium* and *E. perfoliatum*. The polar extract of *E. perfoliatum* also contained rutin (quercetin 3-rutinoside), kaempferol rutinoside and the parent flavonols quercetin and kaempferol.



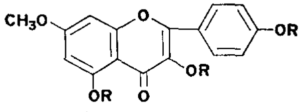
(Ia) $R_1,R_3 = H$, $R_2 = CH_3$
(Ib) $R_1,R_3 = Ac$, $R_2 = CH_3$
(Ic) $R_1,R_2 = H$, $R_3 = CH_3$
(Id) $R_1,R_2 = Ac$, $R_3 = CH_3$



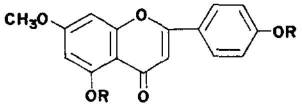
(IIa) R = H
(IIb) R = Ac



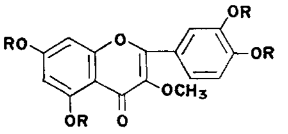
(IIIa) R = H
(IIIb) R = Ac



(IVa) R = H
(IVb) R = Ac



(Va) R = H
(Vb) R = Ac



(VIa) R = H
(VIb) R = Ac

These results made it of interest to compare the constituents of the non-polar fractions of the hybrid and the parents. This has led to the isolation of two new dihydroflavonols Ia (or Ic) and IIIa, the dihydroflavonol 7-*O*-methylaromadendrin (IIa) and the flavonols genkwanin (Va) and quercetin 3-methyl ether (VIa) from the chloroform extract of the hybrid. *E. capillifolium* yielded IIa and IIIa, but no crystalline flavones could be isolated from the chloroform extract of *E. perfoliatum*.

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* The hybrid is identifiable as *E. pinnatifidum* Elliott and *E. eugenii* Small according to published descriptions (private communication from Professor R. K. Godfrey).

[1] H. Wagner, M. A. Iyengar, L. Hörhammer and W. Herz, *Phytochem.* **11**, 1504 (1972).

That the least polar substance Ia or Ic, m.p. 188–191°, $[\alpha]_{Hg}^{23}$ + 16·2°, m.p. of triacetate 107–109°, was a dihydroflavonol was clear from the NMR spectrum which exhibited the typical *AB* system of H-2 and H-3 at 4·69 (H-2) and 5·12 ppm (H-3, $J_{2,3}$ = 12·5 Hz) where the H-3 signal was split further by coupling to the hydroxyl proton found as a doublet at 5·82 ppm. The four remaining substituents, two hydroxyls and two methoxyls, were distributed over C-3′, C-4′, C-5 and C-7 because of signals characteristic of H-2′ (doublet at 7·16 ppm coupled to H-6′), H-5′ (doublet at 6·18 ppm coupled to H-6′), H-6′ (doublet of doublets at 6·98 ppm coupled to H-2′ and H-5′), H-6 and H-8 (*AB* system centered at 5·12 ppm, $J_{6,8}$ = 1·5 Hz).

Since the UV spectrum was unaltered after addition of NaOAc, one of the two methoxyls was at C-7.[2] The NMR signal at 11·87 ppm and the usual bathochromic shifts of band I (47 nm) and II (24 nm) on addition of $AlCl_3$ established the presence of a hydroxyl group at C-5;[3] hence the substance was either Ia or Ic. Acetylation caused downfield shifts of H-2′ (0·26 ppm), H-5′ (0·34 ppm) and H-6′ (0·17 ppm); although the effect on H-5′ was somewhat larger than on H-2′, it seemed unwise to deduce from this observation that the new dihydroflavonol possessed formula Ia rather than Ic. An attempt to distinguish between these alternatives by oxidizing the remaining material with I_2–NaOAc in acetic acid[4] resulted in destruction of the substance.

Analytical data and NMR spectrum of the second dihydroflavonol, m.p. 181–183°, $[\alpha]_{Hg}^{24}$ −11·5°, indicated that it was a methoxytrihydroxy derivative. Ring *B* was monosubstituted at the 4′-position (A_2B_2 system of H-2′, H-3′, H-5′ and H-6′ at 7·28 and 6·73 ppm); the two remaining oxygen functions were attached to C-5 and C-7 because of a two proton singlet at 6·01 ppm (combined H-6 and H-8) which was resolved into a narrowly-split *AB* quartet ($J_{6,8}$ = 2 Hz) in the NMR spectrum of the triacetate, m.p. 134–135°. The single methoxyl group was sited at C-7 because the UV spectrum was unaltered on addition of NaOAc and because the usual shifts on addition of $AlCl_3$ established the presence of a hydroxyl group at C-5. Hence the substance was IIa (7-*O*-methylaromadendrin).

The same structure, without any reference to stereochemistry, has been assigned to material isolated previously by other workers.[5–7] Because the properties—and particularly the rotation—of our substance differed somewhat from those reported earlier, the structure was confirmed by oxidation of IIa with I_2–NaOAc[4] to 7-methoxy-3,5,4′-trihydroxyflavone (IVa). The oxidation product and its triacetate were identical with authentic IVa and IVb.[8]

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[2] T. J. MABRY, K. R. MARKHAM and M. B. THOMAS, *The Systematic Identification of Flavonoids*, p. 170, Springer Verlag, Berlin (1970).

[3] T. J. MABRY, K. R. MARKHAM and M. B. THOMAS, *The Systematic Identification of Flavonoids*, p. 171, Springer Verlag, Berlin (1970).

[4] V. B. MAHESH and T. R. SESHADRI, *Proc. Indian Acad. Sci.* **41A**, 219 (1955).

[5] J. CHOPIN and H. PACHECO, *Bull. Soc. Chim. Biol.* **40**, 1593 (1958), reported m.p. 185–187°, $[\alpha]_D$ +26°, m.p. of triacetate 128–130°, for a substance isolated from wild cherry wood. The structure assignment was based on synthesis by hydroxylation of sakuranetin.

[6] R. J. GELL, J. T. PINHEY and E. RITCHIE, *Austral. J. Chem.* **11**, 372 (1958), obtained a dihydroflavonol, m.p. near 193°, $[\alpha]_D$ 21°, from *Eucalyptus maculata* Hook. The structure assignment was based on oxidation to 7-methoxy-3,5,4′-trihydroxyflavone.

[7] L. CANONICA, B. RINDONE, C. SCOLASTICO, G. FERRARI and G. CASAGRANDE, *Gazz. Chim. Ital.* **99**, 260 (1969) reported m.p. 164–170°, $[\alpha]_D$ +11°, m.p. of triacetate 126–128°, for a dihydroflavonol from *Psiadia altissima* Benth. and Hook. Although the m.p. was considerably lower than that reported by the Australian workers (the only reference cited) they assumed, presumably because of the NMR spectrum, that they were dealing with IIa.

[8] M. GUIDER, T. H. SIMPSON and D. B. THOMAS, *J. Chem. Soc.* 170 (1955).

The NMR spectrum of the third substance, m.p. 238–242°, $[\alpha]_H^{23}$ +7·5°, m.p. of diacetate 135–136°, also showed that it was a dihydroflavonol (*AB* system of H-2 and H-3 at 5·06 and 4·41 ppm with H-3 being additionally coupled to the C-3 hydroxyl proton). Three other substituents—two methoxyl and one hydroxyl group—were distributed over C-4′ (A_2B_2 system of H-2′, H-3′, H-5′ and H-6′ at 7·39 and 6·86 ppm), C-5 and C-7 (*AB* quartet of H-6 and H-8 centered at 6·33 ppm). Since the UV spectrum was not affected by addition of NaOAc or $AlCl_3$, the methoxyl groups were located at C-5 and C-7 and the second free hydroxyl group was attached to C-4′. Thus the new dihydroflavonol was 3,4′-dihydroxy-5,7-dimethoxyflavanone (IIIa).

Racemates of IIa and IIIa have been synthesized,[8] but optically active forms of Ia (or Ic) and IIIa have to our knowledge not been isolated from natural sources nor was the stereochemistry of the samples of IIa isolated previously[5–7] determined. That the 2-aryl and 3-hydroxyl groups of Ia (or Ic), IIa and IIIa were diequatorially substituted on ring C was evident from the NMR spectra all of which exhibited $J_{2,3}$ = 11·5–12·5 typical of 1,2-diaxial coupling. Although the rotations of I and IIIa on the one hand, and IIa on the other at the Hg line were of opposite sign, the CD curves which are almost superimposable (Table 1) clearly show that the absolute configuration of I, IIa and IIIa are identical. Comparison of these curves with CD curves of dihydroflavonols of established absolute configuration[9] demonstrates that the configuration of I, IIa and IIIa is 2*R*, 3*R* as illustrated.

TABLE 1. CD CURVES OF DIHYDROFLAVONOLS*

Compound	Conc. ($\times 10^2$)	Readings
I	0·4	$[\theta]_{327}$ +6840 (max); $[\theta]_{290}$ −24 600 (min); $[\theta]_{250}$ +4380 (max); $[\theta]_{237}$ +2730 (min)
IIa	0·39	$[\theta]_{325}$ +8300 (max); $[\theta]_{285}$ −28100 (min); $[\theta]_{250}$ +6380 (sh); $[\theta]_{230}$ +13000†
IIIa	0·41	$[\theta]_{327}$ +6350 (max); $[\theta]_{285}$ −21600 (min); $[\theta]_{248}$ +8020 (sh); $[\theta]_{232}$ +15900†

* All measurements in methanol.
† Last reading.

Two known flavones were additionally isolated from the *Eupatorium* hybrid. The first of these was identified as genkwanin (5,4′-dihydroxy-7-methoxyflavone, Va) spectroscopically and through the diacetate (see Experimental). The second flavone was identified as quercetin 3-methyl ether (VIa). Although the m.ps of VIa and VIb were slightly higher than those recorded in the literature,[10,11] the NMR spectrum was as expected, the UV spectra duplicated those recorded for VIa[12] and a m.m.p. with an authentic sample of VIa was undepressed.[13]

The chloroform extract of *E. capillifolium* yielded IIa and IIIa as the only crystalline components. Extensive chromatography of the chloroform extract of *E. perfoliatum* failed to yield homogeneous fractions.

[9] W. GAFFIELD, *Tetrahedron* **26**, 4093 (1970).
[10] E. J. MIDDLETON and P. R. JEFFERIES, *Austral. J. Chem.* **81**, 2349 (1968).
[11] R. KUHN and I. LÖW, *Chem. Ber.* **77**, 211 (1944).
[12] T. J. MABRY, K. R. MARKHAM and M. B. THOMAS, *The Systematic Identification of Flavonoids*, p. 134, Springer, Berlin (1970).

EXPERIMENTAL

M.ps are uncorrected. NMR spectra were measured on Varian A60 or Bruker 90 mHz spectrometers; MS on a Nuclide 12 in medium or a MS 902 high resolution mass spectrometer, CD spectra on a Jasco ORD/UV recording spectrometer in MeOH, rotations on a Bendix automatic polarimeter at the Hg_{4358} line, UV spectra in 95% EtOH. Collections of *E. capillifolium* × *perfoliatum*, *E. capillifolium* (Lam.) Small and *E. perfoliatum* L. used in the present work were described in our previous report.[1]

Extraction of E. capillifolium × perfoliatum. Above-ground parts of the hybrid of *E. capillifolium* and *E. perfoliatum*,[1] wt 5·7 kg, were extracted with $CHCl_3$ in the usual fashion.[13] The crude gum, wt 98 g, was chromatographed over 1 kg of silicic acid (Mallinckrodt 100 mesh), the following fractions being collected: 1–25, benzene; 26–40, benzene–$CHCl_3$ (1:1), 41–75, $CHCl_3$; 76–110, $CHCl_3$–MeOH (99:1). Fraction 36 gave yellow crystals (Ia or Ic) which were purified by solution in EtOH, filtration, precipitation with H_2O and recrystallization from light petroleum-acetone, yield 0·06 g, m.p. 188–191°, $[\alpha]^{23}_{Hg}$ + 16·2° (dioxane, *C* 0·308), UV spectrum λ_{max} 288 and 333 nm, + NaOAc 288 and 333 nm, + $AlCl_3$ 312 and 380 nm, + NaOAc-H_3BO_3 288 and 333 nm, NMR signals (DMSO-d_6) at 11·87 br (5-O$\underline{H}$), 9·10 br (–O$\underline{H}$), 7·16d (J = 1·5, H-2′), 6·98dd (J = 8, 1·5, H-6′), 6·81d (J = 8, H-5′), 6·04 (almost a singlet, center of *AB* system, J = 1·5, H-6 and H-8), 5·82 dbr (J = 5, 3-O$\underline{H}$), 5·12d (J = 12) and 4·69 ddbr (J = 12, 5, *AB* system, H-2 and H-3), 3·89 ppm (two methoxyls). (Calc. for $C_{17}H_{16}O_7$: C, 61·44; H, 4·85; O, 33·70, MW 332. Found: C, 61·22; H, 4·53; O, 34·26; MW—MS 332.)

The triacetate Ib or Id was recrystallized from EtOAc-hexane and had m.p. 107–109°, NMR signals ($CDCl_3$) 7·02 m (H-2′, H-5′, H-6′), 6·44d and 6·35d (*AB* system, J = 2, H-6 and H-8), 5·73d and 5·38d (*AB* system, J = 12, H-3 and H02), 3·85, 3·83 (methoxyls), 2·36, 2·30 (aromatic acetates), and 2·02 ppm (3-acetate), NMR signals (DMSO-d_6) 7·42 br (H-2′), 7·15 br (H-5′, H-6′), 6·65d and 6·51d (J = 5, H-6 and H-8), 5·92d and 5·69d (J = 13, H-3 and H-2, 3·84, 3·81 (methoxyls), 2·30, 2·28 (aromatic acetates) and 1·99 ppm (3-acetate). Hydrolysis of the triacetate resulted in recovery of Ia (24 mg). An attempt to oxidize this with I_2-NaOAc gave a small amount of amorphous material which could not be characterized satisfactorily.

Fractions 42–44 gave solid material which was recrystallized from MeOH, yield 0·54 g, m.p. 289–290°, green $FeCl_3$ test. It was identified as genkwanin (Va, lit.[14] m.p. 285–287) through conversion to the diacetate, m.p. 199–200° (lit.[14] m.p. 204°) and through the NMR spectrum which had signals at 7·90d and 6·92d (A_2B_2 system, J = 9, H-2′, H-3′, H-5′ and H-6′), 6·75 (H-3), 6·68d and 6·30d (J = 2·5, H-6 and H-8) and 3·82 ppm (methoxyl). The diacetate VIb had NMR signals ($CDCl_3$) at 8·16d and 7·36d (J = 9, H-2′, H-3′, H-5′ and H-6′), 7·28d and 6·80d (J = 2, H-6 and H-8), 6·82 (H-3), 3·90 (methoxyl) and 2·32 ppm (two acetates).

Fractions 45–46 gave solid material which was recrystallized several times from EtOH–H_2O, yield of IIa 0·38 g, m.p. 181–183° $[\alpha]^{23}_{Hg}$ −11·5° ($CHCl_3$, (*c* 0·314), IR bands (KBr) at 3400 (broad), 1638 and 1548 nm^{-1}, UV λ_{max} 335 and 290 nm (unaltered on addition of NaOAc or NaOAc–H_3BO_3), + $AlCl_3$ 380 and 313 nm, NMR signals (DMSO-d_6) at 7·28d and 6·73d (J = 9, A_2B_2 system, H-2′, H-3′, H-5′ and H-6′), 6·01 (H-6 and H-8), 5·73d (J = 6, 3-O$\underline{H}$), 4·08d and 4·58dd (J = 11·5, *AB* system, *B* split by coupling to 3-OH, H-2 and H-3), and 3·74 ppm. (Calc. for $C_{16}H_{14}O_6$: C, 63·57; H, 4·67; O, 31·76. Found: C, 63·71; H, 4·67; O, 31·47.)

The triacetate IIb had m.p. 134–135°, NMR signals ($CDCl_3$) at 7·40 (center of A_2B_2 quartet, J = 9, H-2′, H-3′, H-5′ and H-6′), 6·45 (center of *AB* quartet, J = 2, H-6 and H-8), 5·60 (center of *AB* quartet, J = 11·5, H-2 and H-3), 3·88 (methoxyl) and 2·40, 2·32 and 2·02 ppm (three acetates). (Calc. for $C_{22}H_{20}O_9$: C, 61·68; H, 4·70; O, 33·60. Found: C, 61·90; H, 4·82; O, 33·90.)

Oxidation of 0·1 g of IIa with I_2–NaOAc in HOAc[4] and recrystallization furnished 7-methoxy-3,4′,5-trihydroxyflavone (IVa), yield 0·02 g, m.p. 220° (lit.[8] m.p. 221–223°) whose triacetate IVb melted at 208–210° (lit.[8] m.p. 202°). These substances were identical with authentic IVa and IVb.

Fractions 57–65 also solidified and were recrystallized several times from EtOH–H_2O, yield of IIIa 0·59 g, m.p. 238–242° $[\alpha]^{23}_{Hg}$ + 7·5° ($CHCl_3$, *c* 0·300), neg. $FeCl_3$ test, IR bands (KBr) at 3400, 1680, 1615, 1570 and 1521 nm, UV λ_{max} 285 and 320 nm (unaltered on addition of NaOAc, NaOAc–H_3BO_3 or $AlCl_3$), NMR signals (DMSO-d_6) at 7·30d and 6·86d (A_2B_2 system, J = 8·5, H-2′, H-3′, H-5′ and H-6′) 6·23 (center of *AB* quartet, J = 2·0, H-6 and H-8), 5·20d (J = 4,3-OH), 5·06d (J = 11·5, J-2), 4·41dd (11·5, 4·0, H-3) and 3·82 ppm (two methoxyls). (Calc. for $C_{17}H_{16}O_6$: 64·56; H, 5·09; O, 30·35; MW 316. Found: C, 65·26; H, 4·74; O, 30·12; MW–MS, 316.)

The diacetate IIIb had m.p. 135–136° and NMR signals ($CDCl_3$) at 7·42 (center of A_2B_2 quartet, J = 9, H-2′, H-3′, H-5′ and H-6′), 6·20 (H-6 and H-8), 5·57 (center of *AB* quartet, J = 12, H-2 and H-3), 3·95, 3·90 (two methoxyls), and 2·32 and 2·08 ppm (two acetates). (Calc. for $C_{21}H_{20}O_8$: C, 63·01; H, 5·03; O, 31·96. Found: C, 63·09; H, 5·18; O, 31·77.)

Fraction 104 gave a yellow solid which was recrystallized repeatedly from $CHCl_3$–light petroleum and

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[13] W. HERZ and G. HÖGENAUER, *J. Org. Chem.* **27**, 905 (1962).

[14] GRIPENBERG, in *The Chemistry of Flavonoid Compounds* (edited by T. A. GEISSMAN), p. 418, Macmillan, New York (1962).

was identified as quercetin 3-methyl ether (VIa), m.p. 282–284° (lit. m.p. 275–278°,[10] 273–275°[11]), yield 0·12 g, UV λ_{max} 257, 267(sh), 295(sh) and 359 nm, + NaOAc 270, 327 and 380 nm, + $AlCl_3$ 268, 276, 298 (sh), 360 and 402 nm, + NaOMe 271(sh), 330 and 410 nm and + NaOAc–H_3BO_3 265, 298(sh), 372 and 415 nm (sh), NMR signals (90 mHz, DMSO-d_6) 12·75 (5-OH), 10·5 (center of very broad band, three OH) 7·57d (J = 2·5, H-2′), 7·49dd (J = 9·0, 2·5, H-6′), 6·93d (J = 9·0, H-5′), 6·41 and 6·20d (*AB* system, J = 2·0, H-6 and H-8) and 3·81 ppm (methoxyl). The m.m.p. with rhamnetin, m.p. 292–294°,[15] was depressed, the m.m.p. with authentic quercetin 3-methyl ether,[10] m.p. in our hands 282–283°, was undepressed.

The triacetate (VIb) melted at 185–186° (lit. m.p. 180–182°,[10] 182–184°[11]), NMR signals (DMSO-d_6) at 8·01dd (J = 9, 2·5, H-6′) partially superimposed on 7·97 br (H-2′), 7·49d (J = 9, H-5′), 7·59d and 7·09d (*AB* system, J = 5, H-6 and H-8), 3·80 (methoxyl), 2·30 (acetate) and 2·33 ppm (three acetates).

Extraction of E. capillifolium. $CHCl_3$ extraction of 5·2 kg of *E. capillifolium* and work-up gave 28·7 g of crude gum which was chromatographed over 1 kg of silicic acid, 2 l. fractions being collected as follows fr. 1–25, benzene; fr. 26–40, benzene–$CHCl_3$ (1:1); fr. 41–75, $CHCl_3$; fr. 76–110, $CHCl_3$–MeOH (99:1). Fractions 55–58 showed a major spot on TLC and were combined and recrystallized. This resulted in 0·1 g of IIa, m.p. 181–183°. Fractions 80–82 also showed one major spot. Combination and recrystallization resulted in isolation of 0·55 g of IIIa, m.p. 238–240°.

Extraction of E. perfoliatum. $CHCl_3$ extraction of 4·1 kg of *E. perfoliatum* followed by the usual work-up gave 25 g of crude gum. Chromatography in the manner described above gave 111 fractions none of which were homogeneous by TLC criteria.

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[15] L. JURD, *J. Am. Chem. Soc.* **80**, 5531 (1958). We wish to thank Dr. Jurd for an authentic sample of this material.