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OCCURRENCE OF GLYCOFLAVONES IN THE ACANTHACEAE

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Key Word Index—*Ecbolium linneanum*; Acanthaceae; glycoflavones; orientin, vitexin, isoorientin and isovitexin.

Plant. *Ecbolium linneanum* Kurz. (Syn. *E. viride* (Forsk) Merrill) (voucher specimen No. 8/74 deposited at JIPMER).

Uses. Medicinal [1,2]. *Previous work.* None other than phytochemical screening [3].

Present work. Shade-dried leaves, flowers and roots extracted separately with hot 90% EtOH and the solid residue from the concentrate purified by recrystallization from EtOAc and ethyl methyl ketone. The yellow flavonoid fraction was found to be a mixture of four flavone glycosides, (*A–D*) by PC which could not be purified by crystallization. However, they were separated into pure components (TLC) by preparative PC using *n*-BuOH–27% aq. HOAc (1:1). They had the following characteristics: (*A*) m.p. 258–60°, UV purple→yellow with NH_3; resistant to hydrolysis (2 N HCl, 3 hr) and giving luteolin on refluxing with HI in phenol, was identified as orientin by R_f, preparation of acetyl derivative and co-PC with an authentic sample (*B*) m.p. 250–52°, UV purple → light yellow with NH_3; identified similarly as vitexin. *C* and *D*, present in traces, were identified as isoorientin (6-*C*-glucosyl luteolin) and isovitexin (6-*C*-glucosyl apigenin) as above.

Comment. This is the first record of the occurrence of glycoflavones in the Acanthaceae. *E. linneanum* which contains orientin, vitexin, isoorientin and isovitexin in the ratio 5:5:1:1, and it is interesting that all the parts of the plant are rich in glycoflavones and devoid of the corresponding free aglycones or their *O*-glycosides. This is not in conformity with the general flavonoid pattern in the family [4,5]. Glycoflavones may be considered to occur atypically in this genus similar to their presence in *Vitex* sp. [4] in the Verbenaceae.

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SUCCEDANEAFLAVANONE—A NEW 6,6″-BINARINGENIN FROM *RHUS SUCCEDANEA**

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Key Word Index—*Rhus succedanea*; Anacardiaceae; biflavanone; 6,6″-binaringenin; MS and NMR data.

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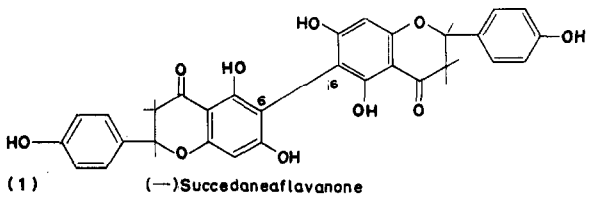
Previously, we reported the isolation of hinokiflavone, amentoflavone, robustaflavone, agathisfla-

vone, a new biflavanone, rhusflavanone (i.e. 6,8″-binaringenin), and a new flavanoflavone, rhusflavone (i.e. 6,8″-naringenylapigenin) from the seed-kernels of *Rhus succedanea* (Anacardiaceae) [1–5]. We have further studied the rhusflavanone-containing fraction C_1 [4] and isolated a new type of biflavanone (viz. 6,6″-linked binaringenin).



(1) (−)Succedaneaflavanone

The fraction C_1 was further chromatographed on polyamide yielded a colorless compound, succedaneaflavanone (**1**), mp 318–322° (dec.), $[\alpha]_D^{20}$ −13° (C = 2·15, C_5H_5N) $C_{30}H_{22}O_{10}$, M^+ *m/e* 542, the mass spectral fragmentations were similar to those of rhusflavanone. It gave a pink colour in the Mg–HCl test, and a violet one with alcoholic $FeCl_3$. The UV spectrum in MeOH was very similar to that of rhusflavanone showing maxima at 293 (log ε 4·54) and 338 (3·89) nm, which on addition of NaOAc or $AlCl_3$ underwent the characteristic bathochromic shift of 5,7-dihydroxyflavanone. [$\lambda_{max}^{NaOAc-MeOH}$ nm (log ε) 256 (4·41), 273 (4·44), 296 (4·44), 321 (4·38); $\lambda_{max}^{AlCl_3-MeOH}$ 222 (4·78), 254 (sh, 4·27), 314 (4·62), 388 (3·93).] Acetylation of **1** with C_5H_5N–Ac_2O gave a hexaacetate (**2**) as needles, mp 252–255°, $[\alpha]_D^{29}$ −9·4° (C = 0·8, $CHCl_3$).

The NMR spectra (Table 1) of **1** and **2** were clearly indicative of the symmetrical nature of linking between the two naringenin units; **1** in DMSO-d_6 showed six OH groups at δ 12·35 (*s*, 2H), 10·47 (*bs*, 2H), 9·62 (*bs*, 2H); eight aromatic protons of two 1,4-disubstituted benzene rings appeared as a set of A_2B_2 doublets (*J* 8) at δ 7·38 (4H) and 6·86 (4H); 2 aromatic protons at δ 6·05 (*s*); 6 protons in the 2 heterocyclic rings C and F appeared as multiplets at δ 5·33–5·77 (2H) and 2·70–3·33 (4H). The signal of the 4 protons at δ 6·86 shifted 0·39 ppm to lower field at δ 7·25 by acetylation indicating the presence of OH groups at 4′- and 4‴-positions [4]. From the above data it is clear that the structure of **1** is 6,6″- or 8,8″-binaringenin.

Methylation of **1** with Me_2SO_4–K_2CO_3 in dry acetone afforded a tetramethyl ether (**3**), mp 292–294° and a small quantity of pentamethyl ether (**4**), mp 272–275°, but the hexamethyl ether could not be obtained. Acetylation of **3** afforded a colorless diacetate (**5**), mp 267–269°. The IR spectrum of **3** indicated a CO absorption band of 5-hydroxyflavanone at 1633 cm^{-1}, whereas **4** showed two CO absorption bands of 5-hydroxy- and 5-*O*-substituted flavanones at 1645 and 1680 cm^{-1} respectively. The UV spectrum of **3** showed maximum at 293 and 345 nm, which on addition of $AlCl_3$ underwent bathochromic shift of Band II (293→314), but no change by NaOAc indicating the presence of 5-OH but no free 4′- and 7-OH groups in **3**. The NMR spectrum of **3** showed a signal of 5,5″-OH at δ 12·27 (s, 2H), whereas **5** showed a signal of the two acetoxyl groups at 2·18 (*s*, 6H). In general, 5-acetoxyl protons in monoflavanoids showed a signal at δ 2·46 distinguishable with the other acetoxyl groups at δ 2·34 [6]. The signals of 5- and 5″-acetoxyl groups of 3-8″-linked GB-1a, GB-2a, volkensiflavone and morelloflavone [7], 3′-8″-linked amentoflavone, 8-8″-linked cupressuflavone appeared at δ 2·35–2·53, while the signals of 5″-acetoxyl group of 3′-6″-linked robustaflavone appeared at δ 2·22; 5-acetoxyl groups of 6-8″-linked agathisflavone, rhusflavone and rhusflavanone appeared at δ 2·18, 2·20 and 2·15 respectively, indicating the anisotropy of the benzene nucleus substituent at 6-position caused the 5-acetoxyl protons to resonate at higher region. The signal of the two acetoxyl groups of **5** appeared at δ 2·18, indicative of the presence of a benzene nucleus substituent at 6-position, suggesting a 6,6″-linkage in **5**; therefore **1** must be 6,6″-binaringenin.

A comparison of the mp and optical rotation of **1** and its derivatives with those of other biflavanoids with a C–C linkage between rings A and D is given in Table 2.

Mass spectra of **3** and **4** showed M^+ ion at *m/e* 598 (47·5%) and 612 (41·8%) respectively. The successive retro-Diels–Alder (RDA) fragmentation around ring C and F of **3** gave the ion at *m/e* 330 as the base peak. The fragmentations of **3** and **4** are in accord with the structures suggested.

Dehydrogenation of the hexaacetate (**2**) in CCl_4 with NBS–KOAc under irradiation [8] for 15 min. gave the biflavone hexaacetate (**6**) mp 291–294°, which on hydrolysis by $NaHCO_3$ afforded a yellow biflavone (**7**), mp > 350°. $[\alpha]_D^{31}$ −24°

Table 1. NMR spectra (δ ppm) of succedaneaflavanone (i.e. 6,6″-binaringenin) and its derivatives
Compound

Position	Succedaneaflavanone† (1)	Succedaneaflavanone* hexaacetate (2)	Succedaneaflavanone† tetramethyl ether (3)
2′,6′ 2‴,6‴	7·38 (*d*, *J* 8 Hz, 4H)	7·58 (*d*, *J* 8 Hz, 4H)	7·65 (*d*, *J* 9 Hz, 4H)
3′,5′ 3‴,5‴	6·86 (*d*, *J* 8 Hz, 4H)	7·25 (*d*, *J* 8 Hz, 4H)	7·14 (*d*, *J* 9 Hz, 4H)
6,6″			
8,8″	6·05 (*s*, 2H)	6·97 (*s*, 2H)	6·40 (*s*, 2H)
2,2″	5·33 ~ 5·77 (*m*, 2H)	5·63 (*dd*, *J* 4 Hz, 12 Hz, 2H)	5·76 (*dd*, *J* 4 Hz, 13 Hz, 2H)
3,3″	2·70 ~ 3·33 (*m*, 4H)	2·83 ~ 3·27 (*m*, 4H)	~ 3·0 (*m*, 4H)
5,5″	12·35 (*s*, 2H)	2·17 (*s*, 6H)	12·27 (*s*, 2H)
7,7″	10·47 (*bs*, 2H)	2·10 (*s*, 6H)	3·85 (*s*, 6H)
4′,4‴	9·62 (*bs*, 2H)	2·33 (*s*, 6H)	3·78 (*s*, 6H)

Spectra were taken on a Varian T60 instrument using TMS as internal standard.
* Solvent: $CDCl_3$. † Solvent: DMSO-d_6.

C = 0·25, MeOH). The IR spectrum of **6** showed an acetoxy CO absorption band at 1775 cm^{-1} and a flavone CO band at 1653 cm^{-1}. The mass spectrum of **6**, however showed no M^+ ion peak but showed the fragment at *m/e* 622 (1·1%) which may result from the elimination of **4** acetoxy groups from M^+ ion. The fragmentation was thus consistent with the structure of biapigenin hexaacetate bearing a linkage of ring A to ring D. The NMR spectrum of **6** in $CDCl_3$ (Table 1) showed no signal of the protons of the flavanone ring C at δ 5·63 and 2·83–3·27 but showed 2 protons as a downfield singlet at δ 6·80 indicating clearly that the biflavanone hexaacetate (**2**) converted into the corresponding hexaacetate (**6**) of biflavone, viz. 6,6″- or 8,8″-biapigenin (viz. cupressuflavone) [9, 10]. As the biflavone (**7**) and its hexaacetate (**6**) were shown to be different from authentic 8,8″-linked cupressuflavone and its hexaacetate and 6,8″-linked agathisflavone and its hexaacetate, so **7** and **6** must be 6,6″-linked biapigenin and its hexaacetate respectively; hence succedaneaflavanone (**1**) is assigned to 6,6″-binaringenin. Studies on its synthesis are in progress.

EXPERIMENTAL

Mp's are uncorrected.

Isolation of succedaneaflavanone (**1**). The fraction C_1 (1 g) [4] was chromatographed on a column of polyamide (nylon 66, 100 g), eluting with 70% aq. MeOH to give rhusflavanone (500 mg) then eluting with MeOH to give succedaneaflavanone (**1**, 40 mg). Fractional recrystallization of the fraction C_1 (1 g) from MeOH also gave **1** (50 mg) as cubes, mp 318–322° (dec.), $[\alpha]_D^{20}$ −30° (C = 2·15, pyridine), M^+ *m/e* 542, IR: ν_{max} (KBr) 3300 (OH), 1635 (conj. CO), 1605, 1520, 1490 (arom. ring)

Table 2. Comparison of succedaneaflavanone with other biflavanoids

Parent compound		Acetate	Tetramethyl ether	Pentamethyl ether	Hexamethyl ether	Heptamethyl ether
Succedaneaflavanone (**1**)	318–322° (dec.) $[\alpha]_D^{31}$ −13°	252–255°	292–294°	272–275°		
Rhusflavanone	204–206° $[\alpha]_D^{20}$ −29°	130–131°	172–175°	226–228°	131–133°	
Rhusflavone	236–238° $[\alpha]_D^{25}$ −163°	140–142°				244–246°
Dehydrogenation product of **1**	>3500° $[\alpha]_D^{31}$ −24°	291–294°	>350°			
Cupressuflavone	>360°	252–254°	259–261°		295–297°	
Agathisflavone	>330° $[\alpha]_D^{20}$ +17°	154–156°			158–160°	

Table 1. (Contd.)
Compound

Diacetate of succedaneaflavanone* tetramethyl ether (5)	Dehydrogenation product of 2* 6,6″-Biapigenin hexaacetate (6)	Hydrolysis product of 6† (7)	Cupressuflavone hexaacetate*
7·50 (*d*, *J* 9 Hz, 4H)	8·07 (*d*, *J* 9 Hz, 4H)	7·95 (*d*, *J* 8 Hz, 4H)	7·40 (*d*, *J* 9 Hz, 4H)
7·05 (*d*, *J* 9 Hz, 4H)	7·42 (*d*, *J* 9 Hz, 4H)	6·94 (*d*, *J* 8 Hz, 4H)	7·15 (*d*, *J* 9 Hz, 4H) 7·18 (*s*, 2H)
6·57 (*s*, 2H) 5·56 (*dd*, *J* 4 Hz, 13 Hz, 2H)	7·63 (*s*, 2H)	6·63 (*s*, 2H)	
3·20 ~ 2·80 (*m*, 4H)	6·80 (*s*, 2H)	6·78 (*s*, 2H)	6·68 (*s*, 2H)
2·18 (*s*, 6H)	2·25 (*s*, 6H)	13·13 (*s*, 2H)	2·53 (*s*, 6H)
3·85 (*s*, 6H)	2·13 (*s*, 6H)		1·95 (*s*, 6H)
3·77 (*s*, 6H)	2·40 (*s*, 6H)	10·40 (*b*, 4H)	2·28 (*s*, 6H)

cm^{-1}. Found: C, 62·41; H, 4·42. $C_{30}H_{22}O_{10}.2H_2O$ requires: C, 62·28; H, 4·53%.

Succedaneaflavanone hexaacetate (**2**) was obtained as needles mp 252–255° (from $CHCl_3$–MeOH), $[\alpha]_D^{29}$ −9·4° (C = 0·8, CHCl$_3$). IR: ν_{max} (KBr) 3770 (OAc), 1688 (flavanone CO), 1613, 1560, 1510 (arom. ring) cm^{-1}. Found: C, 62·63; H, 3·87. $C_{42}H_{34}O_{16}.\frac{1}{2}H_2O$ requires: C, 62·76; H, 4·39%. The tetramethyl ether (**3**) was obtained as cubes mp 292–294°, M$^+$ *m/e* 598. IR: ν_{max} (KBr) 3600–2500 (OH), 3000, 2950, 2830 (OMe), 1633 (5-OH-flavanone CO), 1613, 1570, 1515, 1490 (arom. ring) cm^{-1}, and pentamethyl ether (**4**, 30 mg), mp 272–275°, M$^+$ *m/e* 612. IR: ν_{max} (KBr) 3600–2500 (OH), 3000, 2950, 2840 (OMe), 1680 (5-O-sub. flavanone CO), 1645 (5-OH-flavanone CO), 1613, 1603, 1575, 1520 (arom. ring) cm^{-1}. The diacetate of **3** was obtained as cubes, mp 267–269° (from $CHCl_3$–MeOH), IR: ν_{max} (KBr) 1775 (acetoxy CO), 1680 (flavanone CO), 1605, 1565, 1515 (arom. ring) cm^{-1}. Found: C, 66·59; C, 5·24. $C_{38}H_{34}O_{12}$ requires: C, 66·85; C, 5·02%.

Dehydrogenation of **2** *by means of NBS to biflavone hexaacetate* (**6**). The compound **2** (44 mg) in CCl_4 (200 ml) under irradiation was refluxed with NBS (30 mg) and benzoyl peroxide (8 mg) for 10 min. The product was recrystallized from MeOH as cubes (**6**), mp 291–294°, IR: ν_{max} (KBr) 1775 (acetoxy CO), 1653 (flavone CO), 1630, 1615, 1510 (arom. ring) cm^{-1}. Found: C, 63·57; H, 4·03, $C_{42}H_{30}O_{16}$ requires: C, 63·80; H, 3·81%.

Hydrolysis of the dehydrogenation product (**6**) *to 6,6″-biapigenin* (**7**). The above compound **6** (100 mg) was suspended in MeOH (100 ml) and refluxed with 2% aq. $NaHCO_3$ (50 ml) 45 min. The reaction mixture was evaporated *in vacuo* to 50 ml, then 5% HCl added, then extracted with EtOAc, yielding a yellow powder (**7**, 60 mg), mp > 350°, $[\alpha]_D^{31}$ −24° (C = 0·25, MeOH). IR: ν_{max} (KBr) 3200 (OH), 1645 (flavone CO), 1608, 1565, 1510, 1490 (arom. ring) cm^{-1}. Found: C, 64·49; H, 3·83. $C_{30}H_{18}O_{10}.H_2O$ requires: C, 64·75; H, 3·62%.

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