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ROBUSTAFLAVONE FROM THE SEED-KERNELS OF *RHUS SUCCEDANEA*

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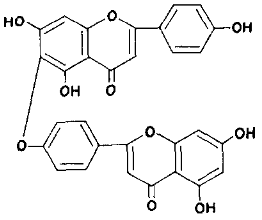
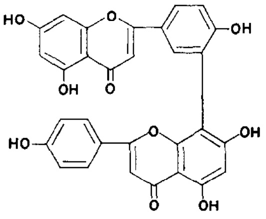
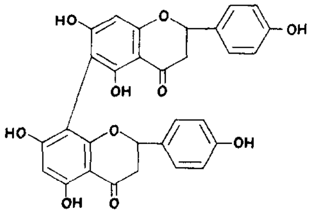
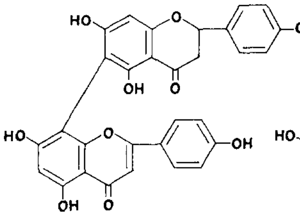
Key Word Index—*Rhus succedanea* Anacardiaceae, biflavone, robustaflavone, 3',6''-biapigenin

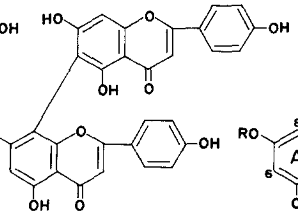
Recently we reported five biflavonyls, i.e. hinokiflavone (**1**), amentoflavone[1] (**2**), rhusflavanone[2] (**3**), rhusflavone[3] (**4**) and agathisflavone[4] (**5**) from the ethanol extract of the seed-kernels of *Rhus succedanea*. These materials were prepared by one of us (F.C.C.) some 30 yr ago.[5]

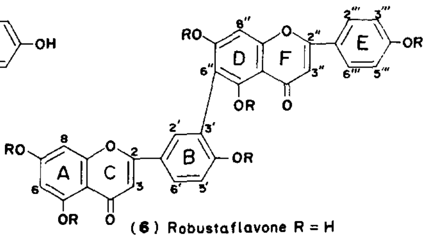

(**1**) Hinokiflavone


(**2**) Amentoflavone


(**3**) Rhusflavanone


(**4**) Rhusflavone


(**5**) Agathisflavone



(**6**) Robustaflavone R = H
(**6b**) R = AcO
(**6c**) R = Me

In the present paper we wish to report the isolation and characterization of a considerable amount of robustaflavone (**6**) along with the hinokiflavone-containing fraction. Although the isolation of minor amounts of hexa-*O*-methyl robustaflavone from *Agathis robusta* was reported recently,[6] the isolation of large quantities of robustaflavone has not yet been accomplished

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[1] CHEN, F C, LIN, Y M and LIANG, C M (1974) *Phytochemistry* **13**, 276

[2] LIN, Y. M and CHEN, F C (1973) *Tetrahedron Letters* 4747

[3] CHEN, F C, LIN, Y M and WU, J C (1973) *Anniversary Meeting of Chinese Chem Soc Abstract*, p 49, Taichung, Taiwan, Dec 8, 1973, (1974) *Phytochemistry* **13**, 1571

[4] LIN, Y M and CHEN, F C (1974) *Phytochemistry* **13**, 657

[5] This investigation was carried out at the Department of Applied Chemistry, Tainan Technical College, Tainan, 1940–43 Our thanks are due to the late Professors I Sakuma and I Momose, Messrs Wen Chung-san and Li An-chin for their co-operation in this work

[6] VARSHNEY, A K, RAHMAN, W, OKIGAWA, M and KAWANO, N (1973) *Experientia* **29**, 784

The fraction (pigment *A*) containing hinokiflavone was chromatographed on SiO_2 by dry-column procedure,[7] using C_6H_6–pyridine–HCOOH (20:5 1) as the developing solvent yielded two yellow bands. Hinokiflavone was obtained from the lower band Extraction of the upper band with EtOAc and subsequent concentration of the extract yielded yellow crystals which were recrystallized from pyridine–H_2O. m.p. 350–352° (dec.), $[\alpha]_D^{20}$ $-37\,5°$ (*c* 4·0, pyridine) The compound gave an orange–red colour in Mg–HCl test and a brown one with alcoholic $FeCl_3$ The IR spectrum showed a broad hydroxyl absorption at 3250 cm^{-1} and a conjugated carbonyl adsorption at 1650 cm^{-1} The UV spectrum in MeOH showed four maxima in the region of 347 (log ϵ 4 49), 300 (4·42), 275 (4·44) and 255 (4 71) nm, underwent a bathochromic shift on addition of NaOAc or $AlCl_3$ The UV spectrum in $AlCl_3$–MeOH was similar to that of in $AlCl_3$–MeOH on addition of HCl, indicating the presence of OH groups in 5, 7 and 4′ positions, and the absence of an *o*-dihydroxyl group[8] [$\lambda_{max}^{NaOAc-MeOH}$ (log ϵ) 378 (4·38), 313 (sh 4 41), 277 (4·48), 257 (4·66) nm $\lambda_{max}^{AlCl_3-MeOH}$ 388 (4 43), 352 (4·50), 300 (4 45), 278 (4·45), 254 (4 80) nm]

The NMR spectrum of the compound E (Table 1) showed six OH groups at δ 13·53 (*s*, 1H) 13 28 (*s*, 1H) and 11·23–8 63 (*br*, 4H); the four protons in the 1,4-disubstituted benzene ring appeared at δ 7 97 (*d*, *J* 9 Hz, 2H) and 7 03 (*d*, *J* 9 Hz, 2H), the three protons in the 1,3,4-trisubstituted benzene ring appeared at δ 7 87 (*d*, *J* 2 Hz, 1H), 7·94 (*q*, *J* 2 Hz, 9 Hz, 1H) and 7·09 (*d*, *J* 9 Hz, 1H); two aromatic protons appeared as *meta*-coupled doublets (*J* 2 Hz) at 6 23 (1H) and 6 52 (1H), three isolated protons appeared at δ 6·83 (*s*), 6 80 (*s*) and 6·68 (*s*) respectively The above evidence suggested that the structure of the compound was composed of two apigenin units joined by an interflavonyl linkage of $C_{3'}$–$C_{6''}$, i.e. robustaflavone (**6**), an isomer of amentoflavone.

This was further supported by examination of its acetate and methyl ether. Acetylation with pyridine–Ac_2O gave a colourless needle, m.p. 199–200° Methylation with Me_2SO_4–K_2CO_3 in dry acetone afforded a colourless ether, m.p 303–305°, $C_{36}H_{30}O_{10}$, M^+ *m/e* 622 The NMR spectra of these products (Table 1) were in accord with the structure **6b** and **6c** respectively The induced change in the chemical shifts (ppm) owing to the addition of $Eu(FOD)_3$ on compound **6c** represented by S-value[9] was also listed in Table 1 The S-values of MeO-5 and MeO-5″ were 10·85 ppm (largest) and 2 17 ppm respectively, whereas H-8″ was 0·34 ppm, indicating the presence of a linkage of $C_{3'}$–$C_{6''}$ as structure **6c** which was characterized as hexa-*O*-methylrobustaflavone by comparison with authentic sample (TLC, IR, NMR and MS)

EXPERIMENTAL

M ps were not corrected, NMR spectra were recorded on a Varian T60 instrument using TMS as internal reference in solvent ($CDCl_3$ or DMSO-d_6), MS were recorded by direct inlet system on Hitachi RMS-4-Mass spectrometer, UV and IR spectra were taken with a Cary-14 spectrophotometer and a Jasco IR-G spectrophotometer respectively

Extraction of biflavones from the seeds of Rhus succedanea The coarsely powdered and defatted seeds (16 kg) of *R succedanea* were exhaustively extracted with boiling 95% EtOH (150 l) Combined EtOH extract was concentrated *in vacuo* yielding crude yellow fractions *A* and *B* (each *ca* 0 2%), further conc to dryness to give fraction *C* (*ca* 2%)

Separation of robustaflavone and hinokiflavone SiO_2 (Kiselgel nach Stahl Type 60 Merck, 100 g) in a column (4 × 20 cm) was used and pigment *A* (1 g) in pyridine (10 ml) was mixed with SiO_2 (5 g) evaporated *in vacuo*, and packed on top of the column The whole was developed with 400 ml of solvent BPF (20 5 1) The column

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[7] LOEV, B and GOODMAN, M M (1967) *Chem Ind* 2026

[8] MABRY, T J, MARKHAM, K R and THOMAS, M B (1970) *The Systematic Identification of Flavonoids* pp 35–36, Springer New York

[9] COCKERILL, A F and RACKHAM D M (1970) *Tetrahedron Letters* 5149

TABLE 1 NMR SPECTRA (δ ppm) OF BIFLAVONE E (ROBUSTAFLAVONE) AND ITS ACETATE AND METHYL ETHER

Compound (solvent)	Position 2′	6′	5′	2‴,6‴	3‴,5‴	6	8
Biflavone E (DMSO d_6)	7 87*d* (*J* 2 *Hz*)	7 94*q* (*J* 2, 9)	7 09*d* (*J* 9)	7 97*d* (*J* 9)	7 03*d* (*J* 9)	6 23*d* (*J* 2)	6 52*d* (*J* 2)
Hexaacetate ($CDCl_3$)	7 93*d* (*J* 2)	8 00 *q* (*J* 2, 9)	7 50*d* (*J* 9)	7 99*d* (*J* 8)	7 48*d* (*J* 8)	6 93*d* (*J* 2)	7 30*d* (*J* 2)
Hexamethyl ether ($CDCl_3$)	7 83*d* (*J* 2)	7 91*q* (*J* 2, 9)	7 13*d* (*J* 9)	7 90*d* (*J* 9)	7 05*d* (*J* 9)	6 40*d* (*J* 9)	6 62*d* (*J* 2)
S-values of hexamethyl ether by Eu(FOD)$_3$	1 14	0 17	0 31	−0 10	−0 10	5 04	1 08

Compound (solvent)	8″	3,3″	5	5″	7	7″	4′	4″
Biflavone E (DMSO d_6)	6 68*s*	6 80*s* 6 83*s*	13 53*s*	13 28*s*		8 63–11 23 *br* (4 H)		
Hexaacetate ($CDCl_3$)	7 43*s*	6 70*s* 6 73*s*	2 43*s*	2 35*s*	2 35*s*	2 22*s*	2 13*s*	2 07*s*
Hexamethyl ether ($CDCl_3$)	6 92*s*	6 63*s* 6 65*s*	3 95*s*	3 63*s*	3 89*s*	3 86*s*	3 87*s*	3 89*s*
S-values of hexamethyl ether by Eu(FOD)$_3$	0 34	0 23 −0 03	10 85	2 17	0 33	0 85	0 29	−0 02

Spectra were taken on a Varian T60 instrument using TMS as international standard

was sliced into seven bands. The bands 3 and 4 and 6 and 7 were each extracted with EtOAc yielding robustaflavone (*ca* 200 mg) and hinokiflavone (*ca* 200 mg) respectively. Robustaflavone was obtained as yellow crystals from pyridine–H_2O, m p. 350–352° (Found: C, 66·7; H, 3·6. $C_{30}H_{18}O_{10}$ requires. C, 66 9; H, 3·4% $[\alpha]_D^{20}$ −37·5° (*c* 4, pyridine).

Robustaflavone hexaacetate (**6b**) A solution of the biflavone (**6a**; 70 mg) in pyridine (1 ml) and Ac_2O (1 ml) was kept at room temp. for 24 hr, then poured onto crushed ice The white solid (90 mg) was recrystallized from EtOAc–MeOH as colorless needles (50 mg), m p 199–200° (Found. C, 63 6, H, 3 6. $C_{42}H_{30}O_{16}$ requires· C, 63 8; H, 3·8%)

Hexa-O-methyl robustaflavone (**6c**) The biflavone (**6a**; 120 mg), anhyd K_2CO_3 (2 3 g) and Me_2SO_4 (1 ml) in dry acetone (70 ml) were refluxed for 48 hr The mixture on usual work up yielding a brown solid (118 mg) which was purified by preparative TLC, then crystallized from EtOAc–$CHCl_3$ to give colorless crystals (40 mg), m.p 303–305° (reported m p 305–308°)[6] (M^+ *m/e* 622). (Found C, 69 1; H, 4 7 Calc for $C_{36}H_{30}O_{10}$· C, 69 4, H. 4 9%)

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