Phytochemistry, 1974 Vol 13, pp 1571 to 1574 Pergamon Press Printed in England

RHUSFLAVONE: A NEW FLAVANOFLAVONE FROM *RHUS SUCCEDANEA*

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(*Received* 7 *November* 1973)

Key Word Index—*Rhus succedanea*, Anacardiaceae; flavanoflavone, 6,8″-naringeninylapigenin, NMR data

Abstract—From the drupes of *Rhus succedanea* has been isolated a new flavanoflavone, rhusflavone, which has the structure 6,8″-naringeninylapigenin.

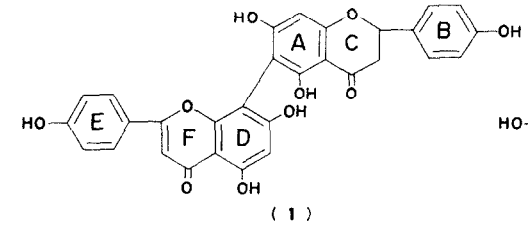
IN EARLIER communication,[1] we reported that hinokiflavone and amentoflavone were present in an ethanol extract of the drupes of *Rhus succedanea* L. Further concentration yielded a crude yellow pigment which was subjected to column chromatography on SiO_2 eluting with C_6H_6–EtOAc, giving three fractions C_I, C_{II} and C_{III}. From the fraction C_I and C_{III} a new biflavanone, rhusflavanone (i.e. 6,8″-binaringenin[2]), and agathisflavone (i.e. 6,8″-biapigenin[3]) were isolated respectively and characterized by chemical and spectral evidence.

In the present paper we wish to report the isolation and characterization of a new biflavonoid from the fraction C_{II}. This was further chromatographed on SiO_2 yielded a yellow compound, rhusflavone, m.p. 236–238°, $C_{30}H_{20}H_{10}$, M^+ *m/e* 540, $[\alpha]_D^{25}$ − 163 (*c* = 0·39, EtOH). It gave an orange colour in the Mg–HCl test, and a reddish brown one with alcoholic $FeCl_3$. The UV spectrum in MeOH showed maxima at 227 (log ε 4·65), 275 (4·41), 292 (4·42) and 324 (4·29) nm, which on addition of NaOAc or $AlCl_3$ underwent the characteristic bathochromic shift of 5,7-dihydroxyflavonoid system.[4] [$\lambda_{max}^{NaOAc-MeOH}$ 250 (*sh*, 4·38), 257 (4·50), 292 (4·42), 324 (4·29) nm. $\lambda_{max}^{AlCl_3-MeOH}$ 230 (4·68), 255 (4·42), 280 (4·36), 307 (4·48), 360 (4·27), 380 (*sh*, 4·0) nm.]

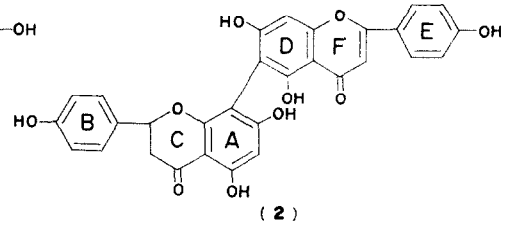
Acetylation of rhusflavone with pyridine–acetic anhydride gave a hexaacetate as colorless needles, m.p. 140–142°, $C_{42}H_{32}O_{16}$, M^+ *m/e* 792. The IR spectrum of rhusflavone indicated a broad OH absorption band at 3300 cm^{-1} and a chelated carbonyl band at 1640 cm^{-1}. The latter was resolved into two bands at 1692 and 1650 cm^{-1} in the spectrum of the hexaacetate, indicating the presence of both a 5-hydroxyflavone and a 5-hydroxyflavanone nucleus in rhusflavone.[5]

[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] LIN, Y M and CHEN, F C (1974) *Phytochemistry* **13**, 657
[4] MABRY, T J, MARKHAM, K R and THOMAS, M B (1970) *The Systematic Identification of Flavonoids*, pp 48–55, Springer, New York.
[5] PELTER, A., WARREN, R., CHEXAL, K K, HANDA, B K. and RAHMAN, W (1971) *Tetrahedron* **27**, 1625

The NMR spectrum of rhusflavone showed six OH groups at δ13·27 (*s*), 12·68 (*s*), 10·78 (*br*. 2H), 10·47 (*s*) and 9·77 (*s*). Two signals at the down-field δ13·27 (*s*), and 12·68 (*s*) indicated the two chelating OH groups at 5″ and 5-positions. The signal at δ5·68 (*dd*, *J* 12 Hz, 3 Hz) was assigned to the H-2 of the flavanone unit. The multiplet at δ2·8–3·3 was attributed to the two protons of H-3 (*trans*) and H-3 (*cis*) of the flavanone unit. Eight of the 11 aromatic protons appeared as two sets of A_2B_2 type doublets at δ7·54, 6·98 (*J* 8 Hz) and 7·73, 7·02 (*J* 9 Hz) indicating H-2′, H-6′, H-3′, H-5′ of ring B and H-2‴, H-6‴, H-3‴ and 5‴ of ring E. The remaining three aromatic protons showed as singlets at δ6·90, 6·50 and 6·28 which were assigned to H-3″, H-6″ and H-8 respectively. The considerable downfield shifts (*ca* 0·3 ppm) of H-3′, H-5′ (δ6·98 → 7·26) and H-3‴, H-5‴ (δ7·02 → 7·35) in rhusflavone hexaacetate supported the presence of OH groups at 4′ and 4‴-positions in rhusflavone.[6] This was further supported by the mass spectra of rhusflavone and its hexaacetate which showed peaks of *m/e* 94 (due to C_6H_5–OH), 118 ($CH\equiv C$–C_6H_4–OH), 121 $^+O\equiv C$–C_6H_4–OH), 160 ($CH\equiv C$–C_6H_4–OAc), 162 (CH_2=CH–C_6H_4–OAc), 163 ($^+O\equiv C$–C_6H_4–OAc). The above results supported a flavanone–flavone structure with a interflavonyl linkage between ring A and D, i.e. 6-6″, 8-8″ or 6-8″.



(1)



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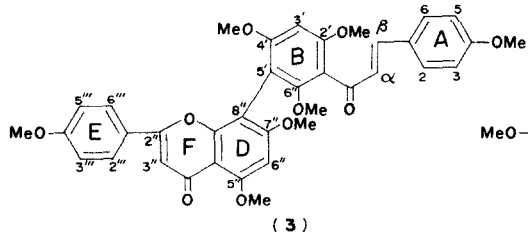

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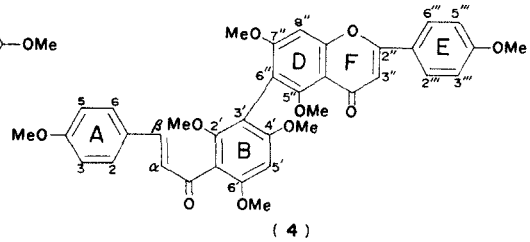

(4)

Dehydrogenation of rhusflavone with iodine and potassium acetate in acetic acid yielded[7] a biflavone, m.p. >300°, which identical with agathisflavone by comparison of TLC, IR, UV and NMR. Consequently the possibility of 6-6″ and 8-8″ linkages can be ruled out. Rhusflavone should, therefore, be assigned the structure of (**1**) or (**2**).

Methylation of rhusflavone with dimethyl sulfate and potassium carbonate in dry acetone gave a heptamethyl ether as colorless needles, m.p. 244–246°, $C_{37}H_{34}O_{10}$, M^+ *m/e* 638. The IR spectrum showed the presence of a conjugated carbonyl absorption at 1643 cm^{-1}, and the absence OH groups and no absorption around 1690 cm^{-1}, suggested strongly that during methylation, opening of the chromanone ring C had occurred transforming the flavanone to the corresponding chalcone of the structure (**3**) or (**4**), which was further supported by NMR spectra.

The NMR spectrum of the heptamethyl ether showed seven methoxyl groups at δ4·10 (*s*), 3·93 (*s*, 6H), 3·85 (*s*), 3·80 (*s*), 3·73 (*s*) and 3·40 (*s*). The signals at δ5·68 and 2·8–3·3 due

[6] KHAN, N. U., ILYAS, M., RAHMAN, W., OKIGAWA, M. and KAWANO, N. (1970) *Tetrahedron Letters* 2942.
[7] GOEL, R. N., MAHESH, V. B. and SESHADRI, T. R. (1958) *Proc. Ind. Acad. Sci.* **47A**, 184.

to H-2 and H-3 of the flavanone unit had disappeared and instead a downfield doublets appeared at δ7·50 (*d*, *J* 16 Hz, 1H) and 6·97 (*d*, *J* 16 Hz, 1H), indicating the β-H and α-H of the chalcone unit. Two sets of A_2B_2 type doublets at δ7·54 (*d*, *J* 9 Hz), 6·94 (*d*, *J* 9 Hz), and 7·43 (*d*, *J* 9 Hz), 6·89 (*d*, *J* 9 Hz), attributed to the eight aromatic protons at 2, 6, 3, 5 and 2‴, 6‴, 3‴, 5‴ positions of rings A and E respectively. The three singlets at δ6·53, 6·58 and 6·60 attributed to the three isolated protons in rings B, D and F, i.e. 3′, 3″, 6″ of the structure (**3**) or 5′, 3″, 8″ of the structure (**4**).

Recently it was reported[8] that (a) the shift values of the MeO-5 and 5″ in full methylated biflavones, on addition of $Eu(FOD)_3$, showed the largest downfield shift value, (b) H-8 and 8″ are much smaller than those of H-6 and 6″. This makes it possible to distinguish a proton attached to either C-6 or C-8. On addition of $Eu(FOD)_3$, the chemical shift changes, represented by *s*-value,[9] found in each proton of rhusflavone heptamethyl ether are listed in Table 1. The *s*-values of MeO-5″ and H-6″ are 12·65 (largest) and 5·98 (considerable shift), whereas MeO-6′ is 0·55 (small one), indicating the presence of a linkage C_6–C_8 as structure (**3**) or (**4**).

TABLE 1 NMR SPECTRAL DATA AND *s*-VALUES FOR RHUSFLAVONE HEPTAMETHYL ETHER

Assigned position	5″	6′	7″	4′	2′	4	4‴	6″
δ-value (ppm from TMS)	4·10 (*s* 3H)	3·40 (*s* 3H)	[3·93 (*s* 3H)	3·93 (*s* 3H)	3·85 (*s* 3H)	3·80 (*s* 3H)	3·73 (*s* 3H)]	6·52 (*s* 1H)
s-value by $Eu(FOD)_3$	12·65	0·55	0·87	0·37	0·25	0·35	0	5·98

Assigned position	3′	3″	2,6	2″,6‴	3, 5	3″,5‴	α-	β-
δ-value (ppm from TMS)	6·58 (*s* 3H)	6·62 (*s* 3H)	7·43 (*d* 2H, *J* 9)	7·54 (*d* 2H, *J* 9)	6·89 (*d* 2H, *J* 9)	6·94 (*d* 2H, *J* 9)	6·97 (*d* 1H, *J* 16)	7·50 (*d* 1H, *J* 16)
s-value by $Eu(FOD)_3$	0·33	−0·22	0·23	0·23	0·23	0·20	0·50	0·40

s = singlet, *d* = doublet, *J* = coupling constant in Hz
Spectra were taken on a Varian T60 instrument using TMS as internal standard

The problem of deciding between structures (**1**) or (**2**) for rhusflavone was solved by solvent induced shifts[10] studies of methoxy resonances of rhusflavone heptamethyl ether. On change of solvent from $CDCl_3$ to 80% $C_6D_6/CDCl_3$, all the methoxyl groups except the one most upfield (δ3·40) moved upfield (*ca* 26–32 cps) showing that only one methoxyl group was no *ortho* proton. Two methoxyl groups at 2′ and 5″-positions in the structure (**4**) have no *ortho* proton, whereas in the structure (**3**) only one at 6′-position. Therefore structure (**3**) rather than (**4**) is more likely for rhusflavone methyl ether. This is also in accordance with recent findings[11] that the methoxyl group at position 6′ (chalcone numbering) in the structure (**3**), flanked by ring D on one side and a carbonyl group on the other, showed the chemical shift at considerable high field (δ3·40). The above evidence clearly indicate that rhusflavone is 6,8″-naringeninylapigenin (**1**).

EXPERIMENTAL

M ps were determined in a Yanagimoto apparatus and not corrected, NMR spectra were recorded on a Varian T60 instrument using TMS as internal reference in solvent ($CDCl_3$, C_6D_6 or DMSO-d_6), MS were recorded

[8] OKIGAWA, M, KAWANO, N, RAHMAN, W and DHAR, M M (1972) *Tetrahedron Letters* 4125
[9] COCKERILL, A F and RACKHAM, D M (1970) *Tetrahedron Letters* 5149
[10] PELTER, A, WARREN, R, USMANI, J N, ILYAS, M and RAHMAN, W (1969) *Tetrahedron Letters* 4259
[11] PELTER, A, WARREN, R, USMANI, J N, RIZVI, R H, ILYAS, M and RAHMAN, W (1969) *Experimentia* **25**, 351

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

Plant material and the extraction of biflavonoids. The fruits of *Rhus succedanea*, obtained from Fukuoka, Japan were treated as described in earlier communication [1]. The coarsely powdered and defatted drupes (98.4 kg) were completely exhausted with 95% EtOH (1080 l.). The EtOH extract was concentrated *in vacuo* yielding pigment A (*ca* 0.25%, hinokiflavone) and pigment B (*ca* 0.25%, amentoflavone). Further concentration yielded crude yellow pigment C (*ca* 2%).

Isolation of rhusflavone. The EtOAc soluble part of the crude pigment C (38 g) was subjected to column chromatography on SiO_2 (1:70 w/w), eluting with C_6H_6–EtOAc (1:1) yielded fractions C_I (rhusflavanone[2]) C_{II} and C_{III} (agathisflavone[3]). The fraction C_{II} was rechromatographed on SiO_2 yielding rhusflavone as a yellow amorphous powder (400 mg), m.p. 236–238°.

Dehydrogenation of rhusflavone. Rhusflavone (55 mg), KOAc (0.4 g) and I_2 (90 mg) in HOAc (10 ml) were refluxed for 4 hr. After cooling, the solution was poured into water to give a precipitate which was extracted with EtOAc. Removal of the solvent, then washed with CCl_4 to remove I_2 yielded a solid (48 mg) which was subjected to column chromatography on SiO_2, eluting with C_6H_6–EtOAc (1:2), giving a yellow solid (5 mg), m.p. > 300°, which was identical with an authentic specimen of agathisflavone (TLC, IR, UV and NMR).

Acknowledgements—The authors are indebted to Professor M. Yasue, Nagoya City University, for elementary analysis; to Professor N. Kawano, Nagasaki University, for a generous gift of authentic specimens, copies of IR and NMR spectra, to Professors W. C. Lin, C. H. Yang, T. M. Hseu, L. C. Lin and T. S. Shih for measurement of NMR, UV, IR, MS and technical assistance. This work was supported by National Council on Science Development as the research project of the Chemistry Research Center, National Taiwan University.