EXPERIMENTAL

Isolation of constituents. Fractionation of a seed extract [2] was carried out by dry CC. The column was cut into 33 portions. Portions 15–23, purified by repeated TLC, gave a major constituent (2.1 g [2]). Elution of one of the remaining bands on the plate gave **1** (35 mg). An additional quantity of fruits was collected from a specimen growing near Cajuru, São Paulo State, by Hipolito F. Paulino Filho, UNESP, Araraquara. The seeds were removed and the pericarp was dried, reduced to powder (1 kg) and extracted with C_6H_6 at room temp. The solvent was evaporated and the residue (31 g) crystallized from MeOH to give acylglycerols (10 g). The mother liquor was evaporated and the residue (21 g) submitted to dry CC (400 g Si gel, $CHCl_3$–EtOAc, 19:1). The column was cut into three equal portions which were extracted with MeOH. Evaporation of the solns gave three residues corresponding to the lower (L), the middle (M) and the top (T) portions of the column. The residue L (7.1 g) was, in part (1.6 g), separated by TLC (Si gel, $CHCl_3$–EtOAc, 9:1) into **2a** (130 mg) and **2c** (120 mg). The residue M (6.8 g), chromatographed on a Si gel column, gave **2b** (300 mg); besides other products which, as also the residue T (5.5 g), were not further examined.

(2R, 3S)-3-(3,4-*Dimethoxybenzyl*)-2-(3,4-*methylenedioxybenzyl*)-*butyrolactone* (**1**). Mp 125–126° (MeOH). UV λ_{max}^{MeOH} nm: 230, 279 (ε 22 000, 10 900). IRν_{max}^{film} cm^{-1}: 1767, 1590, 1485, 1440, 1235, 935. $[\alpha]_D^{25}$ $-8.8°$ ($CHCl_3$; *c* 0.114). ORD (dioxane; *c* 0.003): $[\phi]_{240}^{tr}$ -13600, $[\phi]_{276}^{pk}$ -500, $[\phi]_{300}^{tr}$ -2700.

(2R, 3R)-3-(3,4-*Dimethoxybenzyl*)-2-(3,4-*methylenedioxybenzyl*)-*butyrolactone* (**2a**). Viscous oil. UV λ_{max}^{MeOH} nm: 229, 280 (ε 15 800, 8250). IR ν_{max}^{film} cm^{-1}: 1774, 1590, 1500, 1450, 1250, 945. $[\alpha]_D^{25}$ $-26.3°$ ($CHCl_3$; *c* 0.144). ORD (dioxane; *c* 0.004): $[\phi]_{240}^{tr}$ -13650, $[\phi]_{283}^{pk}$ -250, $[\phi]_{300}^{tr}$ -2950.

(2R, 3R)-2,3-*Di*-(3,4-*dimethoxybenzyl*)-*butyrolactone* (**2b**). Mp 127–128° (MeOH). UV λ_{max}^{MeOH} nm: 229, 277 (ε 17 400, 7150). IR ν_{max}^{film} cm^{-1}: 1778, 1590, 1510, 1450, 1250. $[\alpha]_D^{25}$ $-39.0°$($CHCl_3$; *c* 0.180). ORD (dioxane; *c* 0.006): $[\phi]_{243}^{tr}$ -12700, $[\phi]_{280}^{pk}$ -600, $[\phi]_{297}^{tr}$ -3200.

(2R, 3R)-2,3-*Di*-(3,4-*methylenedioxybenzyl*)-*butyrolactone* ((−)-*hinokinin* [7], **2c**). Mp 92–95° (MeOH). UV λ_{max}^{MeOH} nm: 232, 284 (ε 13 500, 10 600). IR ν_{max}^{film} cm^{-1}: 1778, 1590, 1500, 1250. $[\alpha]_D^{25}$ $-26.3°$ ($CHCl_3$; *c* 0.123). ORD (dioxane; *c* 0.004): $[\phi]_{250}^{tr}$ -10900, $[\phi]_{282}$ 0, $[\phi]_{285}^{pk}$ $+400$, $[\phi]_{287}$ 0, $[\phi]_{300}^{tr}$ -4850.

Acknowledgements—We wish to thank Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) and Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP) for fellowships and financial aid.

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Phytochemistry, Vol. 22, No. 6, pp. 1518–1520, 1983.
Printed in Great Britain.

0031-9422/83/061518-03$03.00/0
Pergamon Press Ltd.

A BIFLAVONOID FROM *SEMECARPUS ANACARDIUM*[*]

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(*Received* 24 *September* 1982)

Key Word Index—*Semecarpus anacardium*; Anacardiaceae; biflavanones; ^{1}H NMR and mass spectra; semecarpuflavanone.

Abstract—A new biflavonoid, semecarpuflavanone, has been isolated from the nut shells of *Semecarpus anacardium*. Its structure has been assigned on the basis of chemical and spectroscopic evidence.

Two new compounds **4** and **5**, besides the three known biflavanones [1] **1–3**, have been isolated from the acetone soluble fraction of an ethanolic extract of the defatted nut shells of *Semecarpus anacardium* L. The structure of **4** has already been assigned [2]. The present study deals with the structural determination of **5**.

Compound **5** appeared as a micro-crystalline pale yellow powder from acetone, $C_{30}H_{22}O_{10}$, mp 248–249° and has been named semecarpuflavanone. It gave a greenish-violet ferric reaction, a pinkish-red colour with

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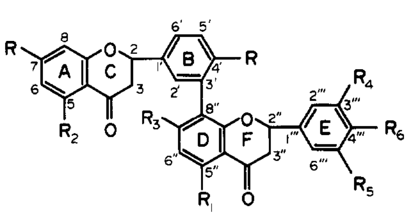
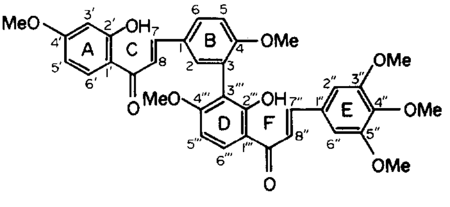
[*] Part 4 in the series "Naturally Occurring Biflavonoid Derivatives". For Part 3 see ref. [2].

[†] Chemical shifts throughout this communication in δ values.



1 R = R₁ = R₂ = R₄ = R₆ = OH, R₃ = R₅ = H
2 R = R₁ = R₂ = R₃ = R₆ = OH, R₄ = R₅ = H
3 R = R₃ = R₆ = OH, R₁ = R₂ = R₄ = R₅ = H
4 R = R₁ = R₂ = R₃ = R₄ = R₆ = OH, R₅ = H
5 R = R₃ = R₄ = R₅ = R₆ = OH, R₁ = R₂ = H
6 R = R₃ = R₄ = R₅ = OMe, R₆ = OH, R₁ = R₂ = H
7 R = R₃ = R₄ = R₅ = R₆ = OAc, R₁ = R₂ = H



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magnesium–hydrochloric acid and an orange colour with sodium borohydride–hydrochloric acid characteristic of a flavanone. The compound exhibited UV maxima in ethanol at 291 nm, addition of sodium acetate gave a bathochromic shift (291 → 312 nm) but with aluminium chloride there was no shift indicating the absence of chelated hydroxyl groups in the molecule. Also, in the ^{1}H NMR spectrum of 5 no low field proton was observed. The biflavanone showed the presence of hydroxyl groups at 3520–3470(*br*), a flavanone carbonyl at 1680 and benzene rings at 1600 and 1580 cm^{-1}. The above observations revealed that there is at least one 7-hydroxyflavanone system [3, 4] in the molecule.

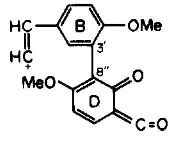
The ^{1}H NMR spectrum (270 MHz, acetone-d_6, TMS as internal standard) of semecarpuflavanone (5) showed signals† due to four methylene protons (C-3, F-3″) at 2.73 (2H, *dd*, *J* = 3.0, 17.0 Hz, *cis*) and 3.08 (2H, *m*, *trans*) and two benzylic methine protons (C-2, F-2″) at 5.42 (2H, *dd*, *J* = 4.0, 12.0 Hz). The three signals at 7.15 (1H, *d*, *J* = 8.5 Hz), 7.37 (1H, *d*, *J* = 2.0 Hz) and 7.46 (1H, *dd*, *J* = 2.0, 8.5 Hz) corresponded, respectively, to the protons at the 5′, 2′ and 6′ positions of ring B. The three protons of ring A, corresponding to the 5, 6 and 8 positions, were readily seen at 6.34 (1H, *d*, *J* = 8.0 Hz), 6.14 (1H, *dd*, *J* = 2.0, 8.0 Hz) and 6.22 (1H, *d*, *J* = 2.0 Hz), respectively. The two signals at 6.84 (*d*, *J* = 2.0 Hz) and 6.92 (*d*, *J* = 2.0 Hz), each integrating for one proton, corresponded to the two *meta*-coupled protons at the 2‴ and 6‴ positions of ring E. Further, the ^{1}H NMR spectrum showed the presence of six non-chelated hydroxyl groups which are exchanged with D_2O at 7.26 (2H, *s*), 7.64 (2H, *s*), 7.76 (1H, *s*) and 8.50 (1H, *s*) and these could be ascribed to the A-7, B-4′, D-7″ and the E-3‴, 4‴, 5‴ positions. There are two further signals at 6.52 (1H, *d*, *J* = 8.0 Hz) and 6.72 (1H, *d*, *J* = 8.0 Hz), which correspond to the two *ortho*-coupled protons at the 6″ and 5″ positions of ring D, respectively.

All 10 oxygens in 5 are accounted for by the six non-chelated hydroxyl groups and four pyranone oxygens. Hence, the two flavanone units must be linked by a C–C linkage only. Oxidation of semecarpuflavanone with neutral permanganate furnished only 1 mol gallic acid, suggesting that one of the side-phenyls is involved in the biflavonoid linkage.

On methylation with diazomethane, semecarpuflavanone afforded a pentamethyl ether (6), $C_{35}H_{32}O_{10}$, mp 161–162° whose ^{1}H NMR spectrum in $CDCl_3$ at 80 MHz (TMS as internal standard) showed signals due to five methoxyl groups at 3.75 (3 × 3H, *s*) and 3.84 (2 × 3H, *s*). There is a singlet signal at 8.52 (1H, exchanged with D_2O) corresponding to the non-chelated hydroxyl group at position E-4‴. Further, 5 on acetylation with acetic anhydride–pyridine gave a hexa-acetate (7), $C_{42}H_{34}O_{16}$, mp 174–176° while with DMS–potassium carbonate it furnished a bichalcone hexamethyl ether (8), $C_{36}H_{34}O_{10}$, mp 199–201°. The ^{1}H NMR spectra in $CDCl_3$ at 80 MHz (TMS as internal standard) revealed the presence of six acetoxyl groups (2.20–2.30) in 7 while in 8 six methoxyls at 3.74 (4 × 3H, *s*) and 3.80 (2 × 3H, *s*) were observed. The ^{1}H NMR spectrum of 8 also showed the presence of two chelated hydroxyl groups at 14.20*s* and 14.30*s*, corresponding to the A-2′ and D-2‴ positions, respectively [1] and the four chalcone protons could readily be found at 7.78 (2H, *d*, *J* = 16 Hz, C-7, F-7″) and 6.54 (2H, *d*, *J* = 16 Hz, C-8, F-8″).

Oxidation of semecarpuflavanone pentamethyl ether (6) with neutral permanganate furnished both syringic acid and 2-hydroxy-4-methoxybenzoic acid (mmp and IR). Hence, the biphenyl system must contain the remaining two methoxyl groups which are placed by anology and ^{1}H NMR at the B-4′ and D-7″ positions. Consequently, the C–C linkage must be either at the B-3′–D-8″ position or at the B-3′–D-6″ position. Since the biflavanone contains no chelated hydroxyl groups, the positions at A-5 and D-5″ are free. Further, the ^{1}H NMR spectra of semecarpuflavanone and its three derivatives (6–8) clearly pointed to the presence of two *ortho*-coupled protons in ring D. On this basis the linkage at the B-3′–D-6″ position is eliminated from consideration and, hence, semecarpuflavanone must have a C–C linkage at the B-3′–D-8″ position. This is also in concurrence with the biflavanones already reported from this plant [1].

The semecarpuflavanone pentamethyl ether (6) in its mass spectrum showed a molecular ion at M^+ 612 (42%). The peak at m/z 179 [(64.6%), 3,5-$(MeO)_2$-4-OH–C_6H_2–CH=$\overset{+}{C}$H] indicated that the rings E and F did



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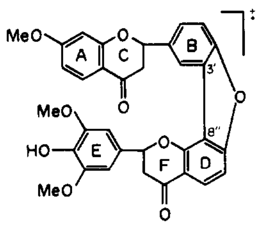

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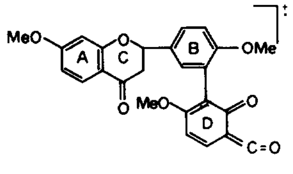

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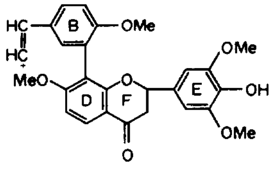

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not carry the biflavonoid linkage. The ion at m/z 281 (29 %) corresponding to the crucial fragment (**9**) is formed after two RDA fragmentations. Compound **6** showed a peak at m/z 566 (8 %), which is formed by the loss of 46 mass units. This fragment can be formulated as **10** in which the *ortho*-methoxyl groups to the biphenyl linkage cyclize to a furan ring [5]. Perhaps the most characteristic feature is the formation of the two fragments **9** and **10**, which could be indicative of a C–C linkage. There are two more ions at m/z 432 (10 %) and 461 (11 %) corresponding to **11** and **12**, respectively. It may be mentioned here that similar fragments have been reported in tetrahydroamentoflavone [6] and (±)-fukugetin heptamethyl ether [7]. Overall, the mass spectral fragmentation exhibits a close similarity to that of 2,3-dihydroamentoflavone hexamethyl ether [8] and GB-2 [9, 10].

From the foregoing spectral and chemical studies, structure **5** has been assigned for semecarpuflavanone with the interflavonoid linkage at the B-3′–D-8″ position.

EXPERIMENTAL

Oxidation of semecarpuflavanone (**5**) *with neutral permanganate*. A mixture of **5** (100 mg), $KMnO_4$ (100 mg) in dry Me_2CO (20 ml) was left at room temp. for 1 hr. The solvent was then evaporated and diluted with H_2O. The manganous salts were decomposed with SO_2 and extracted with Et_2O. The Et_2O extract was shaken with 1 % aq. $NaHCO_3$, acidified with dilute HCl and again extracted with Et_2O, dried and evaporated. The residue on crystallization from H_2O gave needles, mp 250–251° (decomp.), yield: 14 mg; identical with authentic gallic acid (mmp and IR). As the theoretical yield, calculated on the basis of structure **5** (molecular formula, $C_{30}H_{22}O_{10}$), is 31.36 mg, it can be easily seen that 1 mol of the acid resulted in this oxidation.

Oxidation of semecarpuflavanone pentamethyl ether (**6**) *with neutral permanganate*. Compound **6** (100 mg) was oxidized with $KMnO_4$ (100 mg) in dry Me_2CO (20 ml) for 1 hr and worked-up as above. The residue was extracted with hot C_6H_6 and concd resulting in a crystalline solid, mp 204°, yield: 12 mg; identical with authentic syringic acid (mmp and IR). The residual solid was crystallized from MeOH giving a colourless solid, mp 155–156°, yield: 10 mg; identical with authentic 2-hydroxy-4-methoxybenzoic acid (mmp and IR). Compound **6**, on methoxyl estimation (Zeisel's method), gave 4.96 as the methoxyl value, which indicated that only five methoxyls are present.

Acknowledgements—I am grateful to Professor L. R. Row for encouragement. My thanks are also due to Drs. P. A. Ramaiah and M. Bapuji for recording the ^1H NMR and mass spectra.

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Phytochemistry, Vol. 22, No. 6, pp. 1520–1521, 1983.
Printed in Great Britain.

0031-9422/83/061520-02$03.00/0

FLAVONOIDS IN THE SEEDS OF *ARGEMONE MEXICANA*: A REAPPRAISAL

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(*Received* 22 *November* 1982)

Key Word Index—*Argemone mexicana*; Papaveraceae; seeds; flavonoids; luteolin; eriodictyol.

Abstract—Re-examination of the seed extract of *Argemone mexicana* for the newly reported 5,7,2′,6′-tetrahydroxyflavone failed to indicate the presence of any novel flavone. The major seed flavone is, in fact, luteolin and this is accompanied by the related flavanone, eriodictyol. The danger of relying entirely on spectral measurements for identifying new flavonoids is highlighted by these findings.

In the process of identifying a novel tetrahydroxyflavone in some *Lupinus* species, we were interested in obtaining a sample of 5,7,2′,6′-tetrahydroxyflavone for comparative purposes. This substance has recently been reported as a new natural flavone, independently, from *Argemone mexicana* seed [1] and from *Scutellaria baicalensis* root [2].