Phytochemistry, Vol. 20, No. 5, pp. 1169–1170, 1981.
Printed in Great Britain.

0031-9422/81/051169-02 $02.00/0
© 1981 Pergamon Press Ltd.

TETRAHYDROAMENTOFLAVONE FROM NUTS OF *SEMECARPUS PRAINII*

IQBAL AHMAD,* KHWAJA ISHRATULLAH,* M. ILYAS,* W. RAHMAN,* OTTO SELIGMANN† and HILDEBERT WAGNER†

* Department of Chemistry, Aligarh Muslim University, Aligarh 202001, India; † Institut für Pharmazeutische Arzneimittellehre der Universität München, D-8000 München 2, Karlstr. 29, West Germany

(*Received* 18 *August* 1980)

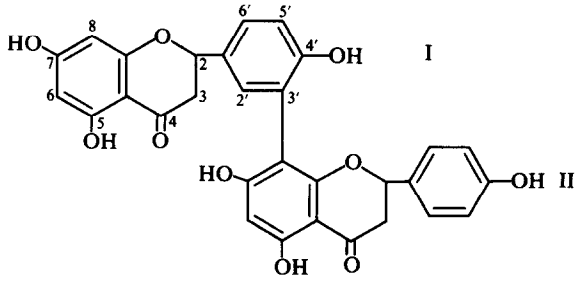
Key Word Index—*Semecarpus prainii*; Anacardiaceae; tetrahydroamentoflavone; naringenin.

INTRODUCTION

Semecarpus anacardium L. (Anacardiaceae) is reported to possess many medicinal properties [1]. *Semecarpus prainii* King, which is closely related to *S. anacardium*, grows in Andaman and Nicobar Island, but does not appear to have been investigated chemically or medicinally. We now wish to report the isolation and characterization of tetrahydroamentoflavone [2] and naringenin from the defatted nut extracts of *S. prainii* King. This is the first report of the isolation and characterization of tetrahydroamentoflavone in the genus *Semecarpus*, though it has been characterized indirectly by dehydrogenation and methylation [3, 4] of a biflavonoid mixture and fractional crystallization of a *bis*-naringenin-dimethyl ether.

RESULTS AND DISCUSSION

Two flavonoids, A and B, were isolated from the defatted fruit material by extraction with acetone and column chromatography on silica gel using ether, benzene and EtOAc–benzene (1:9) as eluants. Compound B (mp 248–251°) was identified as naringenin by mmp and co-chromatography. The main compound (A) showed a UV spectrum with a maximum at 285 and inflexion at 330 nm, characteristic of a flavanone. A molecular ion peak at 542, the $M^+ - 18$ peak at 524 and the fragment ions at 404, 389 and 252 were indicative of a C–C linked biflavonoid. The double signals for two 5-OH groups at δ 12.27 and 12.18, a singlet for 1 H (H-6-II) at 6.08 and 2 H at 5.92 (H-6, 8-II) were characteristic of an A–B-ring linkage. This was confirmed by the presence of seven side phenyl protons resonating between δ 6.6 and 7.4. The C-2 and C-3 protons of two hydrogenated γ-pyrone rings appeared in the region 3.55–2.60 and 5.45. The ^{13}C NMR spectrum of compound A at 90° showed great similarity to that of naringenin. However, there were differences in the chemical shifts of carbon atoms in the vicinity of the interflavonoid linkage. Thus the signals of II-8 and I-3′ were shifted downfield by 11.0 and 5.0 ppm respectively as in the case of amentoflavone [5, 6] whereas the I-2′, I-4′, II-7, and II-9 resonances moved upfield to a much lesser extent (*ca* < 2.5 ppm). The structure deduced for compound A (I-3′, II-8 binaringenin) was confirmed by its dehydrogenation to amentoflavone and subsequent methylation to hexa-*O*-methyl amentoflavone.



Compound A

EXPERIMENTAL

Mps are uncorr. Analytical and prep. TLC were performed on Si gel G (BDH) using C_6H_6–pyridine–HCO_2H (BPF, 36:9:5) and toluene–HCO_2Et–HCO_2H (TEF, 5:4:1) as the developing system [7]. DMSO was dried by distillation from calcium hydride under red. pres. ^1H NMR and ^{13}C NMR were recorded on a Bruker WP 80, the MS on a AEI MS 30 instrument.

Isolation procedure. Nuts (400 g) of *S. prainii* King procured from Calicut village, Port Blair (Andaman Island), India were extracted several times with petrol (60–80°) by cold percolation. The treated nuts were crushed and defatted again by repeated extraction with petrol and C_6H_6 successively, followed by boiling Me_2CO. The combined Me_2CO extracts were concd first at atmos. pres. and then under red. pres. to give a dark, viscous mass. This was treated successively with petrol (60–80°) and C_6H_6 to remove non-flavonoid and resinous matter. The dark brown residue (5.0 g) thus obtained responded to the usual colour test for flavonoids and showed the presence of two phenolic spots on TLC (BPF, 36:9:5) which were labelled as A (major, R_f 0.19) and B (minor R_f 0.70). The dark brown solid (5.0 g) was adsorbed on Si gel (50.0 g) set with petrol (40–60°). The column was successively eluted with petrol, C_6H_6 and EtOAc–C_6H_6 (1:9). Naringenin (0.02 g) was eluted first followed by tetrahydroamentoflavone (2.5 g, mp 234–238°).

2,3,2″,3″-Tetrahydroamentoflavone ($C_{30}H_{22}O_{10}$, M = 542). MS: (EI 70 eV, 4 kV, 100 μA, 250°; DI 10^{-6} T) *m/z* 542 M^+ (10% rel. int.), 254 (14), 404 (11), 389 (13), 378 (9), 312 (12), 311 (10), 270 (11), 253 (11), 252 (24), 230 (10), 226 (24), 213 (17), 186 (13), 179 (22), 160 (21), 153 (54), 152 (23), 147 (43), 132 (23), 126 (100), 120 (60), 119 (31), 94 (33), 91 (58), 85 (35), 78 (31), 77 (34), 69 (73), 65 (39), 58 (76), 57 (92). UV λ_{max}^{MeOH} nm (log ε): 285 (4.52) 225 (sh), 330 (sh). IR: ν_{max}^{KBr} cm^{-1}: 3400, 1605, 1445, 1310, 1235, 1148, 1078,

820. ^{1}H NMR (DMSO-d_6, TMS int., 80 MHz): δ 2.60–3.55 ppm (*m*, 4H) I-3,3; II-3,3; 5.45 (*dd*, J = 9 and 3 Hz, 2H) I-2; II-2; 5.92 (*s*, 2H) I-6,8; 6.08 (*s*, 1H) II-6; 6.71 (*d*, J = 9 Hz) II-3′,5′; 6.85 (*d*, J = 9 Hz), I-5′; 3H; 7.23 (*d*, J = 9 Hz) II-2′,6′; 7.15–7.30 (*m*) I-2′,6′; 4H; 9–11 (*br.*) I-OH-7,4′; II-OH-7,4′; 4H; 12.18 (*s*, 1H); 12.27 (*s*, 1H) I-OH-5; II-OH-5. ^{13}C NMR: (20.15 MHz, DMSO-d_6, 90°): δ 42.4 ppm I-3, II-3; 77.9 II-2; 78.5 I-2; 95.1 I-8; 95.9 I-6, II-6; 102.0 I-10; 102.2 II-10; 106.0 II-8; 115.2 II-3′,5′; 115.4 I-5′, 120.2 I-3′; 126.8 I-2′; 127.6 II-2′,6′; 128.5 I-6′; 129.2 II-1′; 131.1 I-1′; 155.9 I-4′; 157.4 II-4′; 160.2 II-9; 162.4 I-9; 163.1 II-5; 163.5 I-5; 164.4 II-7; 166.6 I-7; 195.9 I-4; 196.4 II-4.

Dehydrogenation of tetrahydroamentoflavone and methylation. Fraction A (1.0 g), iodine (0.1 g), DMSO (4 ml) and conc H_2SO_4 (0.4 ml) were heated in a 10 ml flask at 100° for 1 hr [8]. The mixture was then poured into ice water and the ppt. was filtered, washed with H_2O and dried to afford crude product (0.95 mg). TLC examination of the crude product on Si gel using TEF as the solvent system showed two flavonoid spots (A_1, A_2). The crude product was chromatographed on a column of Si gel using EtOAc–C_6H_6 (1:9) as eluant, where A_1 eluted first followed by A_2. Further purification was carried out by re-chromatography using the same system. The solvent mixture on evapn gave 400 mg A_2 and 450 mg A_1.

Methylation of 50 mg A_2 with Me_2SO_4 yielded a yellow residue which was washed (2–3 times) with petrol and then taken up in $CHCl_3$ (100 ml) and washed several times with H_2O. The $CHCl_3$-soluble fraction was concd and purified by prep. TLC. The solid crystallized from $CHCl_3$–MeOH as colourless cubes (30 mg), mp 180–181° (lit. [9], mp 170–173°), R_f 0.40. It was characterized as hexa-*O*-methyl amentoflavone by comparison (R_f value, fluorescence in UV light, mp, mmp) with an authentic sample.

Methylation of 50 mg A_1 with Me_2SO_4 resulted in a mixture which was purified by prep. TLC. The light yellow solid (35 mg) which crystallized from $CHCl_3$–MeOH was identified as dihydroamentoflavone hexamethyl ether. Dehydrogenation of this ether gave hexa-*O*-methyl amentoflavone identified (R_f value, fluorescence in UV light, mp and mmp) by comparison with an authentic sample. 30 mg A_1 on dehydrogenation as above and crystallization from MeOH gave amentoflavone which was identical (R_f value, fluorescence in UV light and mp) with A_2 and an authentic sample.

Naringenin. Fraction A_1 was characterized as naringenin by direct comparison with an authentic sample (R_f value, fluorescence in UV light, mp and mmp). It was further confirmed by its dehydrogenation followed by methylation and comparison with an authentic apigenin tri-*O*-methyl ether (R_f value, fluorescence in UV light).

Acknowledgements—One of us (I.A.) thanks the U.G.C., New Delhi, for a Teacher Fellowship through its Faculty Improvement Programme. Thanks are also due to the CSIR for financial assistance to Kh. I.

REFERENCES

1. Chopra, R. N., Nayar, S. L. and Chopra, I. C. (1956) in *Glossary of Indian Medicinal Plants*, p. 225. CSIR, New Delhi.
2. El Sohly, M. A., Craig, J. C., Coy, W. W. and Turner, C. E. (1978) *Phytochemistry* **17**, 2140.
3. Rao, N. S. P., Row, R. L. and Brown R. T. (1973) *Phytochemistry* **12**, 671.
4. Ishratullah, Kh., Ansari, W. H., Rahman, W., Okigawa, M. and Kawano, N. (1977) *Indian J. Chem.* **15B**, 615.
5. Chari, V. M., Wagner, H. and Neszmelyi, A. (1977) in *Flavonoids and Bioflavonoids, Proceedings of the Vth Hungarian Bioflavonoid Symposium*. Akademiai Kiado, Budapest.
6. Chari, V. M., Ilyas, M., Wagner, H., Neszmelyi, A., Fa-Ching Chen, Li-Huang Chen, Yu-Chi Lin and Yu-Meci Lin (1977) *Phytochemistry* **16**, 1273.
7. Chexal, K. K., Handa, B. K. and Rahman, W. (1970) *J. Chromatogr.* **48**, 484.
8. Fatima, W., Iqbal Iawaid, Ismail, H., Ishratullah, Kh., Shaida, W. A. and Rahman, W. (1979) *Chem. Ind. (London)* 315.
9. Pelter, A., Warren, R., Hameed, N., Khan Nizam, U., Ilyas, M. and Rahman, W. (1970) *Phytochemistry* **9**, 1897.