Phytochemistry, 1971, Vol. 10, pp. 3286 to 3287. Pergamon Press. Printed in England.

LYCOPSIDA

SELAGINELLACEAE

BIFLAVONES IN *SELAGINELLA* SPECIES

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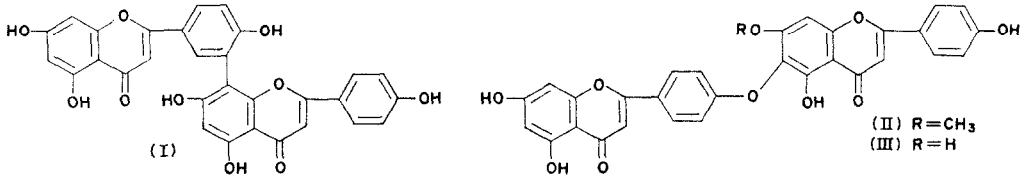
(*Received* 3 *March* 1971, *in revised form* 4 *June* 1971)

Abstract—Amentoflavone, hinokiflavone and isocryptomerin have been isolated from *Selaginella tamariscina* (Beauv.) Spring and two other *Selaginella* species were shown to contain biflavones.

NATURALLY occurring biflavones have been mostly isolated from Gymnospermae, though they have also been found in *Hevea* (Euphorbiaceae),[1] *Viburnum* (Caprifoliaceae)[2] and *Psilotum* (Archegoniatae).[3] Amentoflavone and sotetsuflavone were formerly reported in *Selaginella tamariscina*.[4] However, this finding has been lacking in experimental details and sotetsuflavone was later found to be a mixture.[5] *Selaginella* plants were, therefore, re-investigated.

Amentoflavone (I), isocryptomerin (II)[6] and hinokiflavone (III)[7] were isolated and identified from the leaves of *Selaginella tamariscina* (Beauv.) Spring (Selaginellaceae, iwahiba in Japanese) as described in the Experimental. In addition to the three biflavones, TLC showed the presence of a dimethyl ether of hinokiflavone in the same plant; this was not fully characterized because of the small quantity but it was methylated to hinokiflavone pentamethyl ether, detected by TLC.



[1] R. MADHAV, *Tetrahedron Letters* 2017 (1969).
[2] L. HÖRHAMMER, H. WAGNER and H. REINHARDT, *Bot. Mag. Tokyo* **79**, 510 (1966).
[3] B. VOIRIN and P. LEBRETON, *Compt. Rend.* **262D**, 707 (1966).
[4] H. Y. HSU, *Bull. Taiwan Prov. Hyg. Lab.* 1 (1959) (quoted by T. SWAIN, in *Chemical Plant Taxonomy*, 1963, p. 104); T. KARIYONE *et al.*, *Japan J. Pharmacog.* **16**, 8 (1962).
[5] Sotetsuflavone has been reported as the sole biflavone of *Cycas revoluta*, but recently it was found to be a mixture of amentoflavone (major) and methyl ethers. Details will be published separately.
[6] H. MIURA and N. KAWANO, *Chem. Pharm. Bull. Tokyo* **15**, 232 (1967).
[7] K. NAKAZAWA, *Chem. Pharm. Bull. Tokyo* **16**, 2503 (1968).

Two other *Selaginella* species were also examined for biflavones by TLC. Amentoflavone and its mono- and dimethyl ethers were detected in the leaves of *S. pachystachys* Koidz. (katahiba in Japanese) and *S. nipponica* Franch. et Savat. (tachikuramagoke in Japanese). Amentoflavone in *S. pachystachys* was isolated and identified.

EXPERIMENTAL*

Isolation of biflavones from S. tamariscina. The MeOH extracts (100 g) of dried leaves (1 kg) were treated with hot H_2O repeatedly to remove water-soluble substances and refluxed with trichloroethylene. Insoluble parts were collected and washed with trichloroethylene until washings were almost colourless. The brownish solid (15 g) was refluxed with 30% EtOH (600 ml) for 2 hr, when the solid was almost dissolved. After cooling dark yellow deposits were collected, dissolved in acetone and filtered. The filtrate was concentrated and left for standing to give a mixture of biflavones (4 g).

TLC. TLC analysis was performed on silica gel G according to Stahl (Merck) using toluene–HCO_2Et–HCO_2H (5:4:1). The above mixture gave four spots corresponding to amentoflavone, hinokiflavone and its mono- and dimethyl ethers. When methylated, it gave two spots corresponding to hexa-*O*-methylamentoflavone and penta-*O*-methylhinokiflavone.

Countercurrent distribution. The above mixture (500 mg) was subjected to countercurrent distribution between MeCOEt (10 ml, equilibrated) and borate buffer (Clark-Lubs, pH 9·8, 10 ml). After 60 transfers the following fractions were collected, acidified with HCl and MeCOEt was distilled off to give pale yellow precipitates; fraction 1 (tubes 5–18, hinokiflavone detected by TLC, 30 mg), fraction 2 (20–38, amentoflavone, 180 mg), fraction 3 (42–47, isocryptomerin, 65 mg) and fraction 4 (51–54, hinokiflavone dimethyl ether, 2–3 mg, impure). Fraction 1 (30 mg) was recrystallized from pyridine–MeOH, acetylated with Ac_2O and NaOAc and recrystallized from EtOAc to give colourless minute crystals (20 mg), m.p. 238–240°, which was identified with an authentic sample of hinokiflavone pentaacetate by mixed m.p. and comparison of their NMR spectra. Fraction 2 (180 mg) was recrystallized similarly to give yellow crystals (150 mg), m.p. > 300°. The acetate was prepared and identified with amentoflavone hexaacetate (mixed m.p. and NMR spectra). Fraction 3 (65 mg) was recrystallized and acetylated to give the acetate, m.p. 205–210°, which was identified with isocryptomerin tetraacetate (mixed m.p. and NMR spectra). Fraction 4 was methylated with dimethyl sulfate and penta-*O*-methylhinokiflavone was detected by TLC.

Biflavones in two other Selaginella *species.* Air-dried leaves (500 g) of *S. pachystachys* were similarly treated as described above to give a crude mixture of biflavones (2·2 g), which gave three spots by TLC corresponding to amentoflavone and its mono- and dimethyl ethers. Amentoflavone was isolated and identified by similar countercurrent distribution separation. A small amount (10 g) of *S. nipponica* was extracted similarly and examined by TLC to detect amentoflavone and its mono- and dimethyl ethers.

* M.ps were uncorrected. NMR spectra were recorded on a Hitachi H-60 instrument in pyridine and $CDCl_3$ solutions with TMS as internal standard.

Phytochemistry, 1971, Vol. 10, pp. 3287 to 3289. Pergamon Press. Printed in England.

SPHENOPSIDA

EQUISETACEAE

CONSTITUENTS FROM *EQUISETUM TELMATEIA*: THE STRUCTURES OF EQUISPOROSIDE AND EQUISPOROL

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(*Received* 4 *January* 1971, *in revised form* 14 *May* 1971)

Abstract—An investigation of the spores of *E. telmateia* Ehrh. (the Giant Horsetail) has established that the previously isolated natural product, equisporoside, is identical with the known flavonoid, gossypitrin.