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

JAMES P. KUTNEY and JUDITH E. HALL

Department of Chemistry, University of British Columbia, Vancouver 8, Canada

(*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.

INTRODUCTION

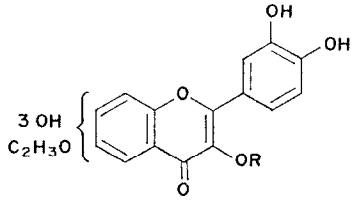
THE SPORES of *Equisetum telmateia* Ehrh. (syn. *E. maximum* auct.) were investigated by Sosa in 1949.[1] Several natural products were isolated at that time but since the proposed structures were incomplete it was of interest to re-examine the spores of this species. An earlier publication[2] has revealed that Sosa's 'equisetolic acid' is a mixture of octacosanedioic (C_{28}) and triacontanedioic (C_{30}) acids. The present report summarizes our work on equisporoside.

RESULTS AND DISCUSSION

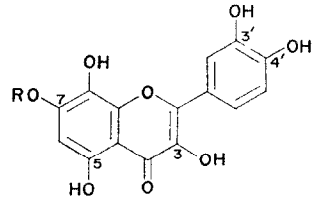
Equisporoside which had been assigned the partial structure (I)[1] was isolated from the methanol extracts of the spores. Hydrolysis of equisporoside with dilute aq. acid gave the corresponding aglycone, equisporol, and glucose, the latter being identified by paper chromatography in three solvents. Equisporol, m.p. 302–304° (dec.) also possessed a very characteristic UV spectrum (λ_{max}^{EtOH} 384, 341, 276 and 263 nm) which when compared with other spectral data suggested it to be a flavonoid.[3] Indeed, subsequent comparison of this data with that reported for gossypetin (II) and gossypitrin (III)[4] revealed that Sosa's equisporoside could be simply the known flavonoid, gossypitrin.

The derivatives of equisporol (hexaacetate, m.p. 226–230°, hexamethyl ether, m.p. 170–171·5°) also corresponded well to those previously reported.[4] Finally, comparison (TLC, mixed m.p. and IR) of equisporol and its hexamethyl ether with authentic samples of gossypetin and its ether derivative left no doubt that they were identical.

The position of attachment of the carbohydrate residue was finally settled when comparison (TLC, mixed m.p. and IR) of equisporoside with an authentic sample of gossypitrin, established their identity. On this basis the names, equisporoside, equisporol and, for that matter, equisetolic acid,[2] should be stricken from the literature since they represent previously known compounds.



I, R = glucose



II, R = H
III, R = glucose

EXPERIMENTAL

Isolation. The strobili (cones) of *Equisetum telmateia* were collected in Vancouver and Squamish, B.C. After allowing the strobili to dry for 3–4 days at room temp., the spores (4·407 kg) were shaken out.

The spores (4·407 kg) were extracted (Soxhlet) with ether for approximately 8 hr. Upon concentration of the combined ether extracts, equisetolic acid[2] (8 g) separated as a white solid.

The spores which had already been extracted with ether were then submitted either to procedure A or B below:

(A) The spores (721 g) were extracted with MeOH (Soxhlet) for 4 days, the extracts concentrated to a small volume and ether added. A yellow gum which separated was removed from the solution by decantation, and

[1] A. SOSA, *Bull. Soc. Chim. Biol.* **31**, 57 (1949).
[2] K. R. ADAMS, R. BONNETT, J. HALL and J. P. KUTNEY, *Chem. Commun.* 456 (1969).
[3] *The Chemistry of Flavonoid Compounds* (edited by T. A. GEISSMAN), pp. 107–155, MacMillan, New York (1962).
[4] T. A. GEISSMAN and C. STELLINK, *J. Org. Chem.* **22**, 946 (1957).

then dissolved in H_2O. The filtered solution was allowed to stand during which time a light brown solid (2·49 g) separated.

(B) The spores (750 g) were extracted (Soxhlet) for 10 hr with MeOH and the extract evaporated nearly to dryness. The residue was taken up in petroleum and extracted with H_2O. After concentration of the combined aq. layers yellow-brown needles (2·17 g) separated. Extraction of the spores with methanol for a further 10 hr followed by the same work-up gave a further 1·10 g of yellow-brown needles.

Paper chromatography using Whatman No. 3 paper and 5% HOAc or *n*-BuOH–HOAc–H_2O (4:1:5) showed that the yellow-brown solids were almost pure but the aq. solutions from which they separated were complex mixtures of up to six components. In the former solvent, equisporoside scarcely moved from the baseline while in the latter it had an R_f of 0·43.

Purification. Equisporoside (788 mg) was dissolved in MeOH–H_2O (1:1, 40 ml) and applied to a column of polyamide–celite (10 g) which had been packed with H_2O. Elution with MeOH–H_2O (1:1) yielded equisporoside (608 mg, 77% recovery). Equisporoside crystallized as small yellow needles from aq. HOAc. After drying at 85° under vacuum for 12 hr, it melted at 202–204°. An authentic sample of gossypitrin (obtained from Geissman), melted at 199–201°; mixed m.p. 199–201°; ν_{max} (KBr): 3400, 2900 (shoulder), 1650, 1605, 1557 and 1510 cm^{-1}; superimposable on that of gossypitrin.

Hydrolysis of equisporoside. Equisporoside (66·7 mg) in MeOH (20 ml) and 2 N H_2SO_4 (20 ml) was refluxed for 4 hr, cooled and the solution extracted with EtOAc. Evaporation of the combined EtOAc extracts yielded the aglycone, equisporol, (43 mg). It crystallized from aq. HOAc as yellow needles, m.p. 301–304° (dec., Kofler preheated to 290°). There was no depression of the m.p. when mixed with authentic gossypetin.

Equisporol hexamethyl ether. The procedure used here was essentially that of Geissman.[4] The product obtained after crystallization from MeOH–$CHCl_3$ melted at 170–171·5°; gossypetin hexamethyl ether (obtained from Geissman), 166–168°; mixed m.p. 166–169°. IR spectra (KBr) of both compounds were superimposable.

Equisporol hexaacetate. The hexaacetate obtained via the previously described procedure[4] had an unusual behaviour during the m.p. determination. The compound first sintered at 190°, again at 216° and finally melted at 226–230°. Again comparison with authentic sample from Geissman showed no depression.

Acknowledgements—Financial aid from the National Research Council of Canada is gratefully acknowledged. We also thank Professor G. H. N. Towers, University of British Columbia, for help in collecting the species and Professor T. A. Geissman, University of California at Los Angeles, for supplying us with authentic samples mentioned above.

Phytochemistry, 1971, Vol. 10, pp. 3289 to 3292. Pergamon Press. Printed in England.

GYMNOSPERMAE

PINACEAE

NEW LABDANE RESIN ACIDS FROM *PINUS ELLIOTTII**

B. P. SPALDING and D. F. ZINKEL

Forest Products Laboratory,† Forest Service, USDA, Madison, Wis. 53705, U.S.A.

and

D. R. ROBERTS

Southeastern Forest Experiment Station, Forest Service, USDA, Olustee, Fla. 32072, U.S.A.

(*Received* 6 *May* 1971)

Abstract—Slash pine needles and cortex oleoresin have been found to contain a new major diterpene constituent, imbricataloic acid. The closely related imbricatoloic acid, previously reported only in *Araucaria imbricata*, was found to be present in small amounts in slash pine needle extract. Spectral data are given for an unidentified diterpene alcohol isolated from the cortex oleoresin.

* Presented in part in "Pine Chemicals: The Naval Stores Industry", at the Wood Extractives Symposium, 161st Meeting, American Chemical Society, Los Angeles, Calif., March 1971 and at the 5th Great Lakes Regional Meeting, American Chemical Society, Peoria, Ill., June 1971.

† Maintained at Madison, Wis., U.S.A., in cooperation with the University of Wisconsin.