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DISTRIBUTION OF EQUISETOLIC ACID IN THE EQUISETALES

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Abstract—Equisetolic acid has been found in the spores and cones of *E. telmateia* and *E. arvense* but not in other parts of these species. It could not be detected in five other contemporary *Equisetum* species, nor in two fossil compressions (*E. columnare, E. lyelli*)

AS PART of an investigation into the chemistry of fossil compressions, we are undertaking a comparison of the chemical constituents of contemporary and fossil members of the *Equisetaceae*. This family is particularly attractive in this respect since, although the number of species of the one surviving genus, *Equisetum*, is limited, the family is well represented in the fossil record, Moreover, Knocke and Ourisson have reported[1] a striking correlation, with respect to hydrocarbon constituents, between the contemporary *E. sylvaticum* and the fossil *E. brongniarti.*

The identification of equisetolic acid,[2] obtained from the spores of *E. telmateia* and *E. arvense*, as triacontanedioic acid ($HO_2C\cdot(CH_2)_{28}CO_2H$),[3,4] has led us to examine the distribution of this unusual lipid. The spores, cones, fertile stems and sterile stems of these two species have now been investigated, together with various available parts of five other contemporary species (*E. fluviatile, E. hyemale, E. palustre, E. sylvaticum* and *E. variegatum*). The dicarboxylic acid appeared to have physcial properties which might lead to its persistance and this encouraged us to examine two fossil compressions. Fossil stems of *E. columnare* were obtained from coastal exposures of the Lower Deltaic Series (Middle Jurassic) at Cloughton Wyke and Hayburn Wyke, Yorkshire, England, while similar compressions of *E. lyelli* were extracted from the Weald clay (Lower Cretaceous) at Horam Brick Works, near Hailsham, Sussex, England.

Two methods of examination were employed. In the first the air-dried comminuted material was washed briefly with dichloromethane, and then extracted with ether. In those cases where equisetolic acid was present in appreciable quantity it crystallized from the boiling ether. Where this method failed, the acidic materials were extracted from the organic solution, converted to methyl esters with diazomethane and submitted to preparative TLC. Material running with the same R_f as dimethyl equisetolate was separated, and was examined by GLC and MS.

Application of the first method demonstrated the presence of 0·45 and 0·09% equisetolic acid in the spores and empty cones respectively of *E. telmateia* and 0·37 and 0·17 %of the

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[1] H. KNOCKE and G. OURISSON, *Angew. Chem. Inter. Edn.* **6**, 1085 (1967).

[2] A. SOSA, *Bull. Soc. Chim. Biol.* **31**, 57 (1949); *idem. Ann. Sci. Nat. Bot.* **10**, 201 (1949).

[3] K. R. ADAMS, R. BONNETT, J. HALL and J. P. KUTNEY, *Chem. Commun.* 465 (1969).

[4] K. R. ADAMS and R. BONNETT, *Phytochem.* **10**, 1885 (1971).

acid in the spores and empty cones of *E. arvense*. While it is probable that the 'empty' cones contained some residual spores, the yields obtained suggests that these were not the principal source of equisetolic acid obtained on extracting the cones.

The effectiveness of the second method of examination was confirmed by investigating the mother liquor from the ethereal extract from the cones of *E. telmateia*. However, equisetolic acid (as its ester) could not be detected in the stems (fertile and sterile) of *E. telmateia* and *E. arvense*, nor in ten fractions from the five other contemporary and two fossil species. In two cases (*E. telmateia* fertile stems, *E. variegatum* spores and cones) the GLC results suggested that dimethyl equisetolate was present, but this finding was not confirmed by MS. An incidental examination of spores from *Selaginella kraussiana* (Selaginellales) showed no detectable amounts of equisetolic acid.

The restricted location of equisetolic acid in *Equisetum* is of some taxonomic interest since the two species in which it has been found are distinguished morphologically from the other examples that we have examined. As far as fossil material is concerned, the results suggest that an examination of fossil *Equisetum* spores[5] for the presence of equisetolic acid would be of interest.

EXPERIMENTAL

General experimental conditions were described earlier.[4]

Plant and fossil material. The horsetails were collected from the following locations in England: *E. telmateia* Ehrh.–Epping, Essex; *E. sylvaticum* L.–Lamberhurst, Kent; *E. arvense* L.–Coxtie Green, Essex; *E. fluviatile* L. and *E. variegatum* Schleich.–Harlech, N. Wales; *E. palustre* L.–Paxtol, Kent; *E. hyemale* L.–Southampton, Hants; *E. columnare* Brongniart–Hayburn Wyke, Yorks; and *Equisetites lyelli* Mantell–Horam, Sussex. The living species were air dried and powdered with an Apex cutter mill. *E. lyelli* fossils were powdered using an agate cone grinder. The surface layer of the large *E. columnare* stems was removed using a ball mill. *Extraction Procedure*. The following procedure is typical. All solvents were redistilled. Powdered air dried fertile stem (102·3 g) of *E. telmateia* was kept with CH_2Cl_2 (700 ml) for 10 min at room temp. and then extracted (Soxhlet) with Et_2O for 7 days. No solid precipitated. (In extractions from spores and cones of *E. arvense* and *E. telmateia* equisetolic acid crystallized out—see Discussion.) The solvent was removed and the residual dark paste was dissolved in Et_2O (150 ml) and extracted with 2 N KOH (8 × 20 ml). The aqueous solution was washed with Et_2O and then acidified (HCl). Extraction with Et_2O (10 × 20 ml) and removal of the organic solvent gave the acid fraction as a pale yellow paste (14·3 mg). This was esterified (CH_2N_2, MeOH, Et_2O) and the crude esters were submitted to TLC (silica gel HF_{254}, 0·1 × 20 × 20 cm, hexane–Et_2O = 1 : 2). The material with R_f 0·4–0·8 was extracted with Et_2O to give a pale yellow oil (6·1 mg) which was submitted to GLC analyses (Pye Argon instrument, 60 × 0·6 cm, 5% Apiezon L on 80–100 mesh Gas-Chrom Z. pretreated with hexamethyldisilazane, 240°, 1·4 kg/cm^2 argon). In two cases (*E. telmateia* fertile stems, *E. variegatum* stems and cones) where GLC gave a positive indication the appropriate fraction was submitted to MS. This did not confirm the identification but indicated the presence of C_{22}–C_{27} alkanoic acid esters.

Positive results have been summarized in the Discussion. No equisetolic acid was detected in the following: *E. telmateia*–fertile stem, sterile stem; *E. arvense*–fertile stem, sterile stem; *E. sylvaticum*, *E. fluviatile*, *E. palustre*,–stems; *E. variegatum*–stem, spore and cone; *E. hyemale*–spore, cone, stem; *E. lyelli* and *E. columnare*–stems.

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[5] R. W. Baxter and G. A. Leisman, *Am. J. Bot.* **54**, 748 (1967).