

AN INVESTIGATION OF THE ALKALOIDS OF SOME BRITISH SPECIES OF *EQUISETUM*

BY J. D. PHILLIPSON AND C. MELVILLE

From the Department of Pharmacy, University of Manchester

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The alkaloids nicotine and palustrine have been separated from authentic mixtures by paper partition chromatography, paper electrophoresis and the use of buffered cellulose columns. These techniques were applied to extracts of *E. arvense*, *E. telmateia* and *E. sylvaticum* from the last two of which no alkaloids have previously been isolated. Nicotine was found in all three species but palustrine was not detected.

Equisetum species, popularly referred to as "Horsetails", have been known to medicine for centuries and *E. arvense* (common horsetail) has been included in several European pharmacopoeias. The principal use of the herb is as a diuretic, but it has also been recommended for haemoptysis, haemorrhoids, varicose ulcers and tuberculosis. In the field and as contaminants of hay, *Equisetums* have caused fatalities among farm stock, the toxicity being attributed to alkaloids¹, although more recent work suggests that a thiaminase may be responsible²⁻⁴. The compounds which have been identified in *Equisetums* include alkaloids, flavonoids, amino-acids, thymine, 3-methoxypyridine, dimethylsulphone, tannins, saponins, silica, silicic acids, vitamin C and several organic acids.

The presence of alkaloids in *Equisetum* species was first reported by Lohmann¹, in 1903, but it was not until 1936, that Glet and others⁵, obtained from *E. palustre* 0.95 per cent of an alkaloid which they named palustrine. This was isolated from the same species in pure form by Karrer and Eugster⁶, in 1948, and given the molecular formula $C_{17}H_{29}O_2N_3$. In the following year they obtained it from *E. arvense* and *E. hiemale* together with traces of nicotine⁷. In 1950, Wohlbiel and Beckman⁸ obtained from *E. palustre* 0.01 per cent of an alkaloid equisetine which proved to be identical with palustrine. They also found traces (0.0002 per cent) of a second alkaloid equisetonine. Further work by Eugster and others⁹, 1953, recorded the isolation from *E. palustre* of another alkaloid palustridine, molecular formula $C_{18}H_{31}O_3N_3$, which was thought to be identical with equisetonine. Both palustrine (equisetine) and palustridine (equisetonine) yielded degradation products related to the lupinane alkaloids.

The only record of alkaloids in *E. telmateia* and *E. sylvaticum* appears to be the early report of Lohmann, 1903; hence the present work was undertaken to investigate and compare these species with *E. arvense*. All three are abundant in the Manchester area.

EXPERIMENTAL METHODS

Separation of Nicotine and Palustrine

Owing to the low yields of alkaloids previously reported⁶⁻¹¹, methods for the microseparation of nicotine from palustrine were investigated.

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Paper partition chromatography by the descending method using butanol : concentrated hydrochloric acid : glacial acetic acid : water (100 : 5 : 5 : 22) (solvent system A) gave results similar to those of Eugster⁹, R_F nicotine 0.08, R_F palustrine 0.15–0.20. A better separation was obtained with butanol : glacial acetic acid : water (4 : 1 : 5) (solvent system B), R_F nicotine 0.5–0.56, R_F palustrine 0.37–0.44.

Electrophoresis on acid impregnated paper produced well separated round compact spots. The best separations were obtained on Whatman No. 1 paper impregnated with 0.1M tartaric acid using initial currents of 3 to 5 mA at 500 to 850 V. An initial current of 3 mA at 540 V moved nicotine 14.2 cm. and palustrine 10.4 cm. after 60 minutes.

Chromatograms were sprayed with freshly prepared Dragendorff's reagent (potassium bismuth iodide) which revealed the alkaloids as orange-red spots on a yellow background.

TABLE I

R_F VALUES AND CHARACTERS OF SPOTS AFTER CHROMATOGRAPHY OF EXTRACTS FROM THREE SPECIES OF *Equisetum*. REFERENCE COMPOUNDS NICOTINE AND PALUSTRINE

Solvent system	Butanol : hydrochloric acid : acetic acid : water	Butanol : acetic acid : water
Main compact spots	0.08	0.53
Small diffuse spots	0.56	0.59
Small diffuse spots	—	0.69
Large diffuse spots	0.89	0.90–0.93
Nicotine compact spots	0.08	0.53
Palustrine compact spots	0.19–0.21	0.38

Extraction of the Alkaloids

The material used consisted of the sterile aerial stems of *E. arvense*, *E. telmateia* and *E. sylvaticum* collected locally during June to September, dried at room temperature and coarsely powdered.

Soft extracts were prepared from 5 kg. quantities by percolation with 20 l. of 95 per cent ethanol, evaporation to low volume at 55°, precipitation of non-alkaloidal matter in the presence of 1 per cent hydrochloric acid and extraction with ether after making alkaline.

Examination of the Extracts

Chromatography of the extracts using the equivalent of 100 g. of dry plant in ethanolic solution yielded spots corresponding to nicotine but not to palustrine. Typical results are given in Table I.

Preliminary trials with nicotine on buffered cellulose columns¹² using ether saturated with water as eluant showed that the alkaloid remained on the column at pH 5.1 but was readily eluted after addition of a small amount of diethylamine. Recovery estimated by ultra-violet absorption was about 80 per cent. Application of this technique to the extracts using quantities equivalent to 5 kg. of dry plant gave ultra-violet absorption spectra similar to that of nicotine eluted from a comparable column.

Electrophoresis of the eluate produced spots with the same mobility as nicotine.

Further portions of the eluate were spotted on filter paper bearing reference spots of nicotine and (a) sprayed with 1 per cent *p*-aminobenzoic acid in ethanol, (b) moistened with 30 per cent aqueous acetic acid and sprayed with 1 per cent α -naphthylamine in ethanol. The papers were dried and after exposure to cyanogen bromide, the extracts developed colours similar to the nicotine. Another portion of the eluate after heating with epichlorhydrin gave the same colour as nicotine similarly treated.

RESULTS

Palustrine, the main alkaloid of *Equisetum*, was not detected in the three British species examined, but all contained small amounts of nicotine.

DISCUSSION

Alkaloids have been isolated from *Equisetum* species of continental origin, but no reference to their presence in British species appears to exist. The absence of alkaloids (other than traces of nicotine) from the British species reported in this work may be due to climatic effects or may be genotypical.

Paper electrophoresis proved to be a useful technique for the separation of mixtures of alkaloids, its main advantage compared with paper partition chromatography being a saving of time. It was less suitable for plant extracts which tended to form streaks the full length of the paper, unless previously purified on buffered cellulose columns. Paper partition chromatography of the same extracts without special purification produced well defined spots with reproducible R_F values.

The amount of nicotine obtained from 5 kg. of dried plant material was estimated by ultra-violet absorption spectrophotometry to be not more than 2 mg. Such small quantities were readily separated from much larger amounts of impurities by the use of buffered cellulose columns following the technique of Carless¹².

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