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INABILITY TO DETECT ORGANO-SILICON COMPOUNDS IN *EQUISETUM* AND *THUJA*

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Key Word Index—*Equisetum*; Equisetaceae; *Thuja plicata*; Cupressaceae β-thujaplicine; silica.

Abstract—Silicon in *Equisetum arvense* sap has low molar mass and is probably monomeric silicic acid. Tris(β-thujaplicine)silicon X complexes (X = Cl, I_3) were synthesized but could not be found in extracts of *Thuja plicata*.

INTRODUCTION

Silicon is believed to be essential for vascular plants [1, 2] as it certainly is for diatoms [3]. However very few natural silicon compounds have been reported from plant extracts. Lovering and Engel [4], on tenuous evidence, suggested that silicon transport in *Equisetum*, a silicon-accumulator [5], involved a silicon–phenyl complex. More recently Weiss and Herzog [6] have isolated a tris(β-thujaplicine)silicon complex by adding potassium hexafluorophosphate to extracts of *Thuja plicata*. We have not been able to confirm the existence of either of these silicon complexes in plant extracts.

RESULTS AND DISCUSSION

Equisetum arvense sap (1 ml) was eluted through columns of Sephadex G-200 and Bio-Gel P-2 with water or 0.1 M sodium chloride to give K_{av} values of 0.95 and 0.92 respectively. The molar mass exclusion ranges were 1000–200 000 (Sephadex) and 100–1800 (Bio-Gel). The fact that the elution volume equalled the bed volume in both cases implies that the silicon compound present has a molar mass less than 100. Both before and after elution all the silicon in the sap could be determined by the molybdenum blue method which is specific for monomers and dimers of silicic acid. Hence the most probable silicon compound in the sap is monomeric silicic acid, with a molar mass of 96. Moreover the silicon compound in the sap could not be extracted into diethyl ether. Our findings do not support the natural occurrence of phenyl–silicon compounds suggested by Lovering and Engel [4]. Silicon in *Equisetum* is transported as monomeric silicic acid, or as some derivative so labile that it is destroyed by exposure to air or sodium dodecyl sulphate. The brown organic matter in the sap was not separated from silicon by passage through Sephadex G-200, but was well separated using Bio-Gel P-2.

All attempts to isolate the β-thujaplicine (= TH) complex SiT_3^+ from leaves and bark of *Thuja plicata* were unsuccessful. We were not able to isolate crystals from extracts of the plant by adding potassium hexafluorophosphate as reported by Weiss and Herzog [6]. The complexes SiT_3Cl and SiT_3I_3 were prepared and characterised as described below. The nature of the anion associated with SiT_3^+ affects both solubility and chromatographic behaviour. Using thin-layer chromatography, no constituent of solvent extracts of carefully cleaned shoots and bark of *Thuja plicata* behaved like the highly coloured SiT_3Cl or SiT_3I_3. Unless further evidence is forthcoming, we believe that the β-thujaplicine complexes of silicon isolated by Weiss and Herzog were artefacts of the isolation procedure, since impurities of dissolved or particulate silica are able to react with β-thujaplicine *in vitro* to form the complex.

EXPERIMENTAL

Stems of *Equisetum arvense*, collected locally, were cut into 3 cm segments and spun in polypropylene centrifuge tubes fitted with a perforated plastic shelf, so that the dark brown sap could be collected. The sap was treated with 2 mg sodium dodecyl sulphate per ml to retard microbial growth and enzyme action, and stored at 5° in sealed plastic tubes.

Columns of polyethylene tubing, 35 cm long and 1.2 cm internal diameter and fitted with plastic taps, were filled with slurries of Sephadex G-200 or Bio-Gel P-2. The void volume of each column was determined using Blue Dextran 2000. One ml of sap was applied to each column and eluted with water or 0.1 M NaCl at a rate of 0.3 ml/min. The eluate was collected using an automatic fraction collector modified to take a fraction cutter and collection tubes made of polyethylene. The silicon content of the original sap and all fractions was measured by atomic absorption spectrometry at 251.6 nm, using a Perkin-Elmer Model 272 spectrometer with a sensitivity of 2.1 ppm Si. The presence of inorganic silica in the sap was confirmed by reacting it with ammonium molybdate at pH 1.0, in presence of tartaric acid to eliminate interference by phosphate. This gave yellow molybdosilicic acid which could be reduced to molybdenum blue and determined by spectrophotometry at 810 nm.

Samples of young shoots of *Thuja plicata*, collected locally, were cleaned by successive rinsings in distilled water (silica-free), MeOH and Et_2O. They were then frozen in liquid N_2 and ground to a powder with an iron pestle and mortar. Alternatively the clean shoots were dried at 50°, with a weight loss of about 70%, and ground in an electric grinder made of plastic with steel blades. Inner bark samples were ground in the same way. Powdered shoot (200 g), or 50 g powdered bark, were stirred for several hr in plastic beakers with the following solvents: Me_2CO, $CHCl_3$, CH_2Cl_2, EtOH, EtOAc, MeOH, $C_2H_2Cl_4$, THF and toluene. The extracts were evaporated to form dark green or dark red oils respectively. They were then subjected to TLC on 20 cm × 5 cm

plastic sheets pre-coated with alumina or cellulose, together with synthetic samples of SiT_3Cl and SiT_3I_3 prepared as described below. Et_2O–EtOAc (1 : 1) was found to be a satisfactory eluent.

Synthesis and characterization of silicon β-thujaplicine complexes. Plastic apparatus and dry conditions were used throughout this work. SiI_4 (0.3 g) (Lancaster Synthesis) was dissolved in 10 ml tetrahydrofuran and treated with 0.1 g β-thujaplicine. A reddish-brown compound A could be separated from the mixture by TLC or CC on alumina with Et_2O–EtOAc (1 : 1) as eluent. The yield was 45%. A was found to be insoluble in water, hydrocarbons, Et_2O and CCl_4, but was soluble in Me_2CO, alcohols, $CHCl_3$ and EtOAc. Its elemental composition was 2.99% Si, 39.6% C, 3.58% H and 40.2% I [Expected for $SiC_{30}H_{33}O_6I_3$: 3.13% Si, 40.1% C, 3.70% H and 42.4% I]. Its IR spectrum lacks the O–H stretching band at 3200 cm^{-1} found in β-thujaplicine, but has a new band at 800 cm^{-1} which can be ascribed to the Si–O bonds, since similar bands were found in the IR spectra of tetraethyl silicate and the tricatecholsilicon anion. The mass spectrum of A did not give a peak at mass 898 corresponding to the parent molecule, but gave peaks at masses 644, 572, 502 etc which could be assigned to SiT_3I, $SiTI_3$, (SiT_3–CH_3) etc. The ^1H NMR spectrum of A in $CDCl_3$ gave a strong doublet at δ1.5 due to its methyl groups, a septet at δ3.15 due to the isopropyl C–H groups and a broad multiplet at δ7.8 due to the aromatic protons of the tropolone ring, with no trace of the O–H peak found for β-thujaplicine. The iodine is thought to be present as the triodide anion.

β-thujaplicine (0.1 g) was dissolved in 10 ml $CHCl_3$ and stirred for 8 hr at 55° with 0.5 g $SiCl_4$, which produced a deep yellow soln. A yellow compound B could be precipitated by adding excess CCl_4, but even after five cycles of dissolution and precipitation the product was impure. B was found to be soluble in the same solvents as A. Its elemental composition was 5.41% Si, 68.1% C, 6.60% H and 6.17% Cl [Expected for $SiC_{30}H_{33}O_6Cl$: 5.07% Si, 65.1% C, 6.01% H and 6.41% Cl]. The IR, UV and NMR spectra of B were similar to those of A. Mass spectrometry gave no meaningful results due to the rapid decomposition of the sample after injection.

Attempts to make the SiT_3^+ species in aqueous solution by reacting β-thujaplicine with silica solns or sols gave poor yields, as the reagent is almost insoluble.

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