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DETERMINATION OF TAURINE AND OTHER ACIDIC AMINO ACIDS IN PLANTS

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Key Word Index—*Phaseolus vulgaris*; Leguminosae; *Pinus sylvestris*; Pinaceae; taurine; phosphoethanolamine; phosphoserine.

Abstract—Micromolar concentrations of taurine (about 10 μmol per kg fresh weight) were obtained in the green tissues of higher plants and in the seeds of *Phaseolus vulgaris* (about 50 μmol/kg). Fruits in general, and also potatoes and fungal sporophores, contained very little taurine. Phosphoserine and phosphoethanolamine showed much higher concentrations in the seeds of *Phaseolus vulgaris* and in the winter buds of *Pinus sylvestris*. Their concentrations changed considerably during the growth of the shoot, while that of taurine was more constant.

Taurine (2-aminoethanesulphonic acid) occurs ubiquitously in living matter [1], but positive results have been reported only very rarely in plant material. This may result in part from difficulties in determining the concentrations of taurine, since it may overlap with a number of other highly ionized or acidic compounds in the chromatographic methods available [2, 3]. We have used several chromatographic methods to analyse this fraction of acidic ninhydrin-positive compounds further in mammalian tissues [2–5], and the present paper shows that the same analytical methods are applicable to the determination of the concentrations of taurine and other individual acidic amino compounds in this fraction in a variety of plant material.

The plant tissue extracts contained a number of unknown acidic ninhydrin-positive compounds eluting in the region of the most acidic amino acids in ion-exchange chromatography. A great part of this material was hydrolysable and could be removed by HCl hydrolysis. The fraction of phosphoserine in particular was originally impure, and the fractions of taurine and phosphoethanolamine overlapped seriously.

All the plant samples studied in the present paper contained taurine, the highest concentrations being obtained in the leguminous plant tissues, onion bulbs and the winter buds and shoots of the Scots pine (Table 1). The potato tuber and the fruit and fungal tissues contained relatively little taurine. The most satisfactory taurine concentrations were obtained with the amino acid analyser.

The concentrations of phosphoethanolamine, phosphoserine and taurine decreased markedly as the seedlings of *Phaseolus vulgaris* developed, the decrease being particularly high in the concentration of phosphoserine (Fig. 1). The winter buds of the Scots pine contained 10–14 times more phosphoethanolamine and phosphoserine than taurine (Fig. 2), but while the concentrations of the former compounds decreased to about 1–2 μmol as the shoots grew, that of taurine remained relatively constant throughout the growth season.

There are millimolar concentrations of taurine in mammalian tissues, but only micromolar or less in many higher plants, moulds and bacteria, although taurine is widely distributed in these organisms [6]. Taurine is a

Table 1. Taurine concentrations (μmol/kg fr. wt) in various plant materials determined with an automatic amino acid analyser (I) or the method of Garvin (1960) (II)

Material	Method I	Method II
Phaseolus vulgaris seed	53	205
seedling leaf	14.0	88
Pisum sativum seed	8.1	8.4
Trifolium repens leaf	6.8	9.0
Pinus sylvestris winter bud	6.8	22
shoot	9.0	9.3
needle	2.1	2.4
Phleum pratense leaf	1.8	1.9
Solanum tuberosum tuber	0.2	0.2
Allium cepa bulb	12.8	19.6
Lycopersicon esculentum fruit	0.9	1.1
Vaccinium vitis-idaea berry	0.2	0.3
Lactarius rufus sporophore	2.1	2.2
Leccinum scabrum sporophore	0.9	0.8
Saccharomyces cerevisiae	1.9	2.2

Results are means from 4–5 experiments. S.d. varied in general from 6 to 9%.

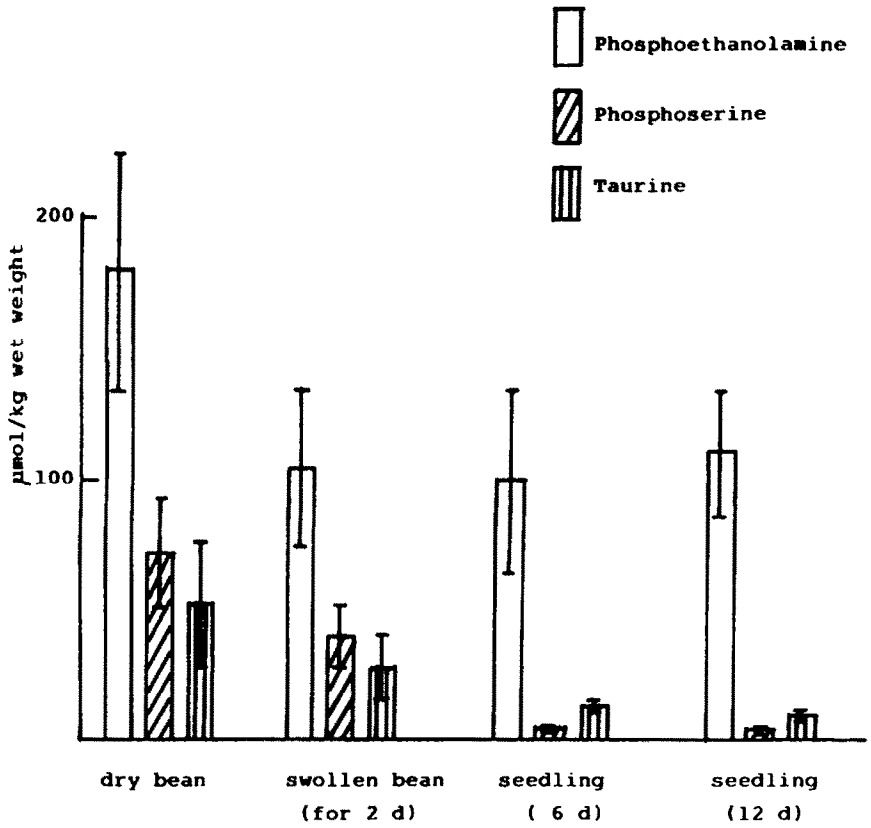

Fig. 1. Concentrations of phosphoethanolamine, phosphoserine and taurine (μmol/kg wet weight) in beans and bean seedlings determined using an automatic amino acid analyser. Results (means ± s.d.) are from 4–6 experiments.

known component of bile acids, an osmoregulator in some marine invertebrates and possibly a neuronal transmitter or modulator in higher animals [1, 7]. In plants it has been reported only in a few cases. It is nevertheless common in plant tissues, as the random examples studied in this paper show. The concentrations of taurine are only 1/100–1/1000 of those of the most abundant amino acids, such as glutamate and aspartate [8, 9], but the concentrations of phosphoethanolamine and phosphoserine, the potential cellular phosphate donors in phospholipid biosynthesis, for instance, are much higher than those of taurine [8, 9]. Particularly high values for these were obtained in winter pine buds, which suggests that they may have a cryoprotectant role [10–12]. The concentrations of phosphoethanolamine and phosphoserine decreased very drastically as the shoots grew, possibly an indication of the transfer of the phosphate groups to biosynthetic processes. Similar changes in the concentrations of these amino acids could be observed as the bean seedlings grew. In all cases the concentration of taurine remained relatively constant.

The direct analysis of plant tissue extracts does not give correct results regarding the concentrations of all acidic amino acids. As shown earlier with mammalian tissues [2, 3], about 15 acidic amino compounds overlap in the chromatograms, and only taurine, phosphoethanolamine and phosphoserine can be satisfactorily separated and analysed quantitatively with an amino acid analyser. In some cases the results are more satisfactory after hydrolysis of the sample with 6 mol/l HCl. A number of amino compounds appear in the same fractions, and qualitative thin layer or paper chromatography is sometimes essential for their identification [2].

EXPERIMENTAL

Plant tissue samples collected in the surroundings of the Oulu University campus at Linnanmaa in August and September 1981–1983 or obtained commercially (see Table 1) were homogenized in cold 5% trichloroacetic acid (TCA) or 15% perchloric acid (PCA) and the precipitated proteins removed by centrifugation. TCA was then removed by shaking with Et_2O and PCA as a $KClO_4$ precipitate after neutralization with KOH. The samples of pine tissues were further treated several times with hot Et_2O and C_6H_6. The residual solns were then lyophilized and the amino compounds then dissolved in H_2O.

The amino acid samples were analysed quantitatively in an automatic amino acid analyser (Kontron Liquimat III), equipped with a two-channel peak integrator. Both native and hydrolysed (6 mol/l HCl, 20 hr at 110°) samples were analysed. Ion-exchange chromatography in separate short columns according to Garvin [13] was also used. TLC on 0.25 mm thick silica gel plates (Kieselgel G) together with known standards was used for the identification of individual amino acids [2].

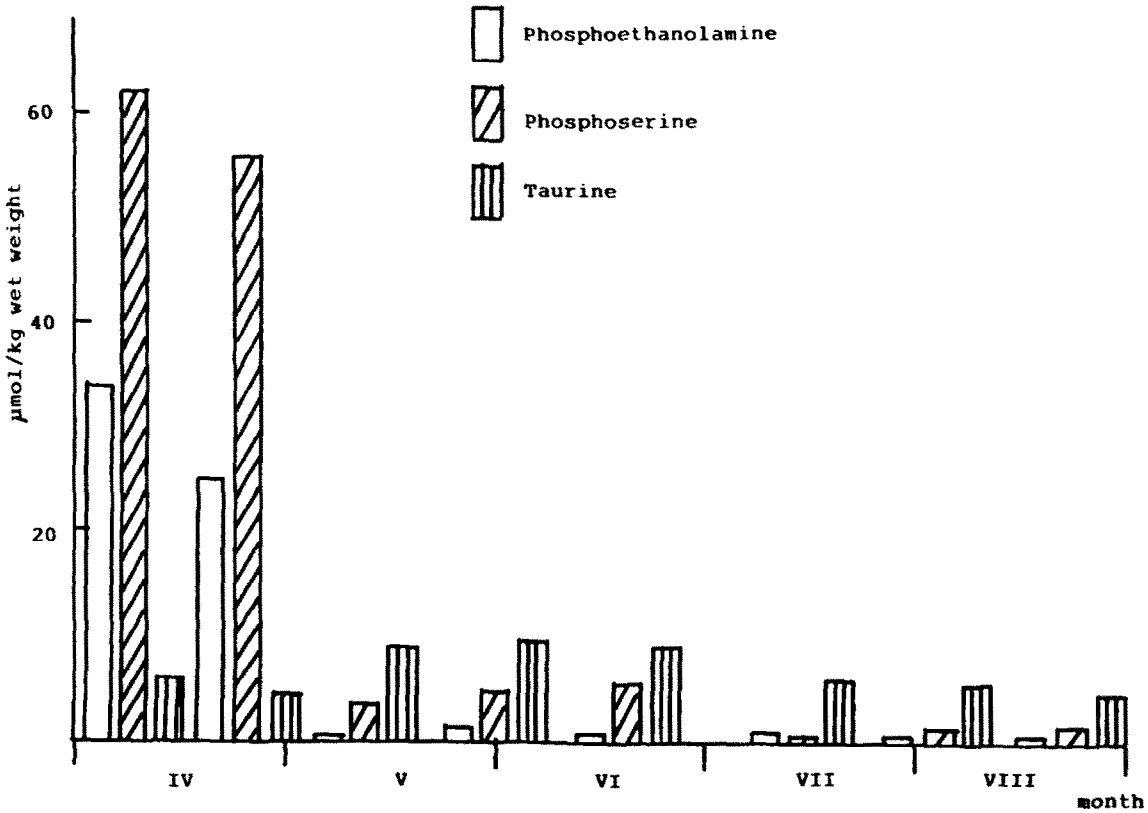

Fig. 2. Concentrations (µmol/kg wet weight) of phosphoethanolamine, phosphoserine and taurine in pine apical buds and shoots during the growth season. Results are means from 3–7 determinations. S.d. varied in general from 8 to 10%.

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