

THE OCCURRENCE AND ROLE OF α -KETO- δ -GUANIDINOVALERIC ACID
IN WHITE SPRUCE (*PICEA GLAUCA* (MOENCH) VOSS)

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Although it has long been known that appreciable quantities of arginine occur in conifers (1-4) its metabolic role has been poorly understood. In this communication evidence is provided for the occurrence of free α -keto- δ -guanidinovaleric acid in white spruce, and its significance in the metabolism of arginine in that plant is briefly discussed.

For a basis of comparison approximately 250 mg of α -keto- δ -guanidinovaleric acid was prepared by Meister's (5) enzymatic method. The keto product was recrystallized from hot water and made to react with Sakaguchi reagent (pink), 2,4-dinitrophenylhydrazine (yellow), and Jaffé reagent (orange-red). Chromatography of the keto acid on paper in two solvents, phenol-water (8:3, v/v) and *n*-butanol - acetic acid - water (9:1:2.9, v/v), for 48 hours yielded R_F values similar to an authentic sample of α -keto- δ -guanidinovaleric acid that was kindly provided by Dr. A. Meister. Elemental analysis of our crystalline product which darkened at 212 °C was found to be C, 37.33; H, 7.16; O, 33.17; and N, 22.13%. Theoretical values for the formula $C_6H_{11}O_3N_3 \cdot H_2O$ are C, 37.69; H, 6.85; O, 33.47; and N, 21.98%.

To demonstrate the occurrence of the free α -keto acid, a 70% ethanol extract made from 40 g of fresh spruce shoots harvested in September 1964 was first passed through a column of Dowex 50W X 4 (hydrogen form). The eluate with 2 *N* NH_4OH was taken to dryness and dissolved in 2 ml of distilled water. Development of one-directional chromatograms (butanol - acetic acid - water) revealed at least five Sakaguchi-positive compounds (Fig. 1), only one of which reacted with ninhydrin (arginine). Co-chromatography of the synthetic α -keto acid demonstrated the presence of a similar compound ($R_{(\text{guanidinobutyric acid})}$ 0.45) in the extract but in relatively small amounts.

Attempts to extract the 2,4-dinitrophenylhydrazone of the natural α -keto- δ -guanidinovaleric acid from a plant preparation by a general procedure (6) were at first unsuccessful. The hydrazone prepared from the synthetic α -keto acid was found to be insoluble in organic solvents (ethyl acetate, ether, *n*-butanol, isoamyl alcohol) and cold water, and it occupied the boundary between the organic and aqueous layers. The insolubility of hydrazones of keto acids which contain nitrogen in the usual extracting solvents, could account for previous failures to detect α -keto- δ -guanidinovaleric acid in higher plants.

The hydrazone of the naturally occurring α -keto acid was successfully detected as follows. Foliage collected in September from the leader shoots of spruce saplings was fixed in liquid nitrogen, and pulverized and homogenized

at 0 °C with 10% HPO_3 . An equal volume of 1% 2,4-dinitrophenylhydrazine in ethanolic 2 *N* HCl was added to the filtrate and the mixture allowed to stand for 1 hour at room temperature. A dark red precipitate was filtered off and washed with cold 70% ethanol and then with ether. Ethereal extraction is a convenient method for the separation of the hydrazones of non-nitrogenous keto acids from those which contain nitrogen. Approximately 250 mg of the washed residue was taken up in hot 3 *N* HCl, and the yellow-orange solution was chromatographed on paper with butanol - acetic acid - water, which showed the presence of a Sakaguchi-positive compound and the absence of ninhydrin-positive compounds which might be attributable to free amino acids. Reduction of the 3 *N* HCl fraction by an electrolytic desalter (6) yielded a brown solution which was further purified by passing the solution through Dowex 50W in the hydrogen form. Elution with 2 *M* NH_4OH yielded a small quantity of one ninhydrin-positive (violet) and Sakaguchi-positive (pink) compound. On a two-directional chromatogram (phenol-water; butanol - acetic acid - water) the compound clearly corresponded to arginine.

In the case of conifers, the role of arginine in the Krebs-Henseleit or ornithine cycle (7) has been emphasized (8, 9). However, application of uniformly labeled ^{14}C -arginine to white spruce buds showed that the ^{14}C moved to several guanidino compounds including γ -guanidinobutyric acid (10). The presence of free γ -guanidinobutyric acid derived from arginine suggests that either an oxidative deamination followed by decarboxylation, or a transamination and decarboxylation of arginine may occur. In animals, moreover, γ -guanidinobutyric acid is said to be formed by transamidation, in which arginine serves as a donor and γ -aminobutyric acid as an acceptor of the amidine moiety (11). In white spruce, the presence of the keto acid corresponding to arginine as detected by its chemical conversion to arginine, co-chromatography on paper with synthetic material, and autoradiography, appears to exclude the latter process. Stumpf and Green have demonstrated (12) that deamination occurs in the bacterium *Proteus vulgaris*. It is interesting that they, too, recorded the insolubility of the hydrazone of α -keto- δ -guanidinovaleric acid.

The occurrence of free α -keto- δ -guanidinovaleric acid in white spruce and its very recent demonstration in *Phlox decussata* (13) indicates that arginine may be metabolized in ways other than through the Krebs-Henseleit cycle or transamidinase reaction. Deamination or transamination of arginine to the corresponding α -keto acid are possible alternatives.

1. K. MOTHES. *Planta*, **7**, 585 (1929).
2. E. SCHULZE. *Z. Physiol. Chem.* **22**, 435 (1896).
3. E. SCHULZE. *Z. Physiol. Chem.* **24**, 276 (1898).
4. U. SUZUKI. *Bull. Coll. Agr. Tokyo Imperial Univ.* **4**, 25 (1900).
5. A. MEISTER. *J. Biol. Chem.* **206**, 577 (1954).
6. I. SMITH. *Chromatographic and electrophoretic techniques*. Vol. 1. Interscience, Inc., New York, 1960. p. 268.
7. H. A. KREBS and K. HENSELEIT. *Z. Physiol. Chem.* **210**, 33 (1932).
8. R. L. BARNES and A. W. NAVLOR. *Botan. Gaz.* **121**, 63 (1959).

PLATE I

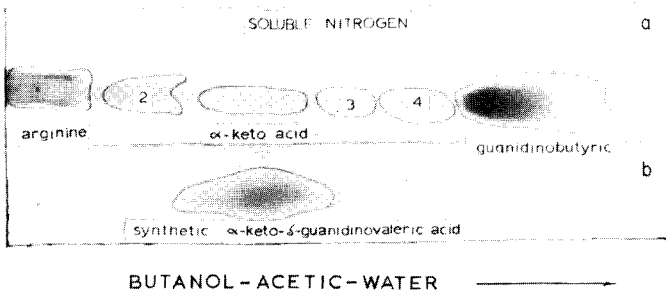


FIG. 1. (a) Naturally occurring α -keto- δ -guanidinovaleric acid on a paper chromatogram which shows the ethanol-soluble nitrogen of white spruce foliage.
 (b) Synthetic α -keto acid moving to a similar R_F value as the naturally occurring keto acid. The spots were developed by Sakaguchi reagent. Numbers 2-4 refer to unidentified guanidino compounds.

9. A. W. NAYLOR. Symp. Soc. Exptl. Biol. **13**, 193 (1959).
10. D. J. DURZAN and F. C. STEWARD. Plant Physiol. **38**, vi (1963).
11. F. IRREVERRE, R. L. EVANS, A. R. HAYDEN, and R. SILBER. Nature, **180**, 704 (1957).
12. P. K. STUMPF and D. E. GREEN. J. Biol. Chem. **153**, 387 (1944).
13. G. BRANDNER and A. I. VIRTANEN. Acta Chem. Scand. **18**, 574 (1964).

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