

OCCURRENCE OF *N*-PHOSPHORYLARGININE IN THE SPRUCE BUDWORM, *CHORISTONEURA* *FUMIFERANA*

D. J. DURZAN* and J. A. PITEL†

Biochemistry Unit, Forest Ecology Research Institute, Department of the Environment,
Ottawa, K1A 0W5, Ontario, Canada

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Abstract—Evidence is reported for the occurrence of a phosphagen, *N*-phosphorylarginine, in spruce budworm *Choristoneura fumiferana*. The occurrence of this compound relates the energy requirements of the insect to one of the main dietary nitrogen-rich amino acids, arginine, found in abundance in the host coniferous trees.

INTRODUCTION

N-PHOSPHORYLARGININE has been found in the majority of invertebrates, including Lepidoptera (cf. VAN THOAI and ROBIN, 1969), but it has never been reported in the phytophagous spruce budworm, Canada's major forest insect pest. *N*-phosphorylarginine is of special interest to us because it contains high proportions of nitrogen (four atoms) and a high energy phosphorus bond. These elements can be ultimately derived from the soil and soil amendments, e.g. urea and its metabolic product, carbamyl phosphate (DURZAN, 1973). The carbon of *N*-phosphorylarginine is derived from arginine with the α -keto carbon skeleton synthesized by the host tree ultimately through photosynthesis. The nitrogenous α -keto analogue occurs in spruce (cf. DURZAN and RICHARDSON, 1966) and may be further combined with nitrogen at the α -amino position to form arginine. Arginine is essential in the diet of most animals and hence a factor that establishes the nutritional value of food chains. The corresponding α -keto acid analogue can replace arginine and points the essential requirement of the carbon skeleton in the diet (MEISTER, 1965). Since under Canadian conditions, nitrogen and phosphorus are factors that limit productivity of host coniferous trees, e.g. *Abies balsamea* and *Picea glauca*, in eastern forests, and are used as soil amendments, the compound we describe takes on added significance from a nutritional viewpoint.

MATERIALS AND METHODS

Spruce budworm larvae were collected from the field from a white spruce forest or reared on artificial diet

(MCMORRAN, 1965) by the method of GRIDDALE (1970) until they were 2 to 3 days from the larval-pupal ecdysis. From each source the live insects (20 g) were then ground in liquid nitrogen and the phosphagens extracted by the method of ROBIN (1964). Phosphagens of arginine were detected by their reaction with ninhydrin and with the modified Sakaguchi reagent of Satake and Luck using an automatic analyzer for guanidines developed by DURZAN (1969a). The phosphagens were mixed with an equal volume of sodium citrate buffer, pH 2.2 (0.2 N Na) and applied to a 14 × 0.9 cm column of PA-35 resin at 30°C. As the compounds eluted, absorbance of the reaction products was determined in a flow cell at 495 nm using a Spectronic 20. The fraction that eluted during the first 15 min was collected on ice and divided into two equal volumes. One part was hydrolyzed with 0.1 M HCl for 1 min at 100°C while the other part was untreated to establish a control and measure the effectiveness of the hydrolytic reaction.

Our identification of *N*-phosphorylarginine is based on (1) isolation and extraction procedures for phosphagens, (2) on well-established hydrolysis rates of high energy phosphate bonds characteristic of phosphagens, (3) on chemical properties and chromatographic behavior on ion-exchange columns of the compound consistent with the authentic compound, (4) the examination of the hydrolysis degradation product, i.e. arginine, formed by acid hydrolysis (0.1 N HCl, 1 min, 100°C), (5) on the reaction of specific moieties of the molecule with diagnostic test reagents such as the Sakaguchi reaction for monosubstituted guanidines and ninhydrin for amino compounds, and (6) on the derivation of the δ -guanidinovaleric acid structure from UL-¹⁴C-L-arginine (cf. DURZAN, 1968, 1969b).

RESULTS AND DISCUSSION

In ion exchange chromatograms, Fig. 1(A), the G-7 fraction (i.e. guanidine compound that eluted at 7 min) has identical mobility to that of commercial and authentic *N*-phosphorylarginine. When this fraction was collected before entry into the reagent reaction coil, traces of free arginine were always present.

* Current Address: Senior Advisor, Policy & Program Development Environmental Management Service, Ottawa, K1A 0H3, to whom reprint requests should be addressed.

† Current Address: Petawawa Forest Experiment Station, Chalk River, Ontario, KOJ 1J0.

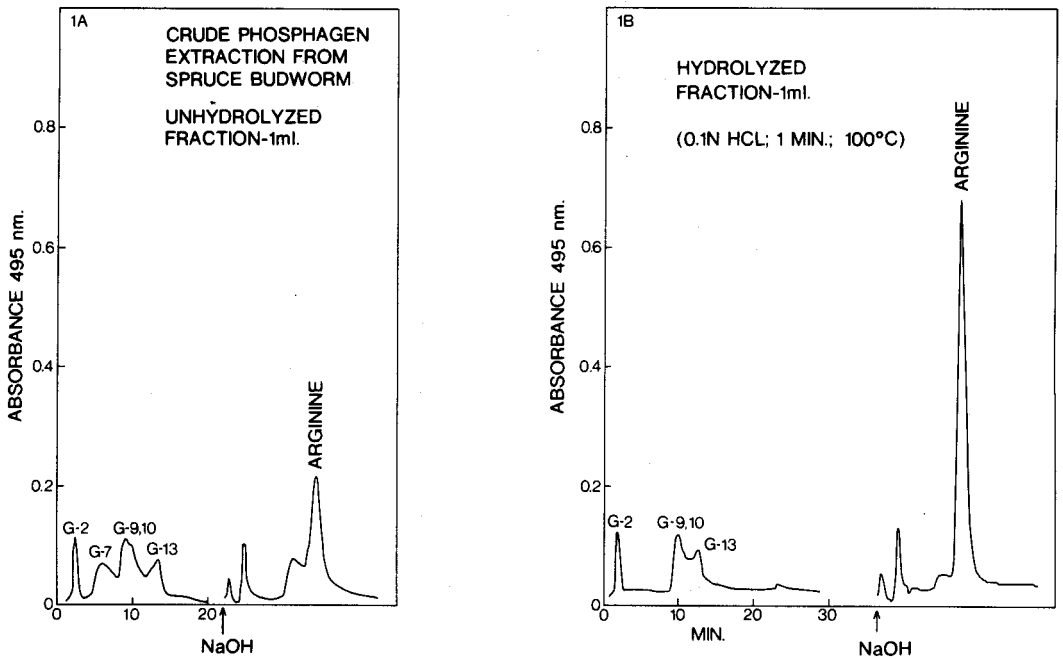
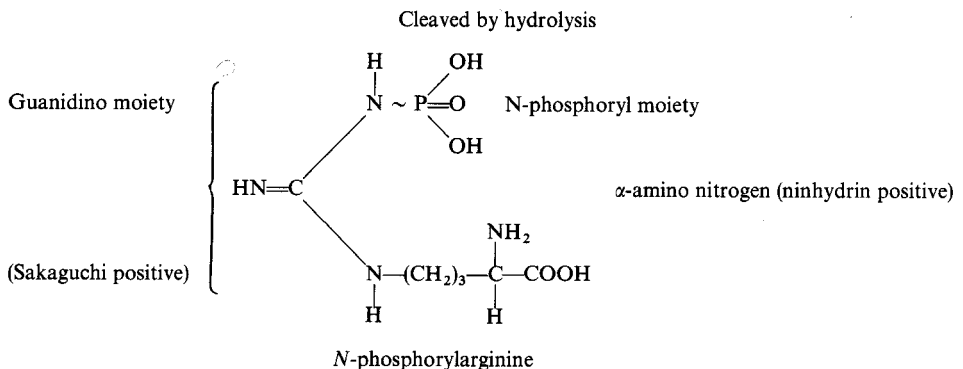


Fig. 1. Phosphagen extract from spruce budworm larvae reared on artificial diet and analyzed on a 14×0.9 cm column of PA-35 resin. In Fig. 1A one half of the sample was placed on the column untreated. In Fig. 1B the other half was hydrolyzed with 0.1 N HCl for 1 min at 100°C before analysis.

The derivation of arginine from N-phosphorylarginine is consistent with the known lability of the phosphagen. Acid hydrolysis of the G-7 fraction destroyed the postulated phosphagen with a concomitant increase in the level of arginine (Fig. 1(B)). Other fractions: G-2, G-9, G-10, G-13, reacted with the Sakaguchi reagent and were not affected by the hydrolysis. The test on budworm tissues from the field and *in vitro* have been repeated successfully several times over a 1-year period. The reaction of the G-7 fraction with Sakaguchi and ninhydrin reagents was positive in all cases. Evidence for the presence of other Sakaguchi reactive compounds in this fraction could not be found by chromatographic means.

The test for the occurrence of phosphate, which is important in the identification of phosphorylarginine, was positive. However, the test was not unequivocal because of the possible natural occurrence of other phosphorylated compounds in the fraction which would also yield a positive test for inorganic P. Nevertheless, our suspicions on the occurrence of N-phosphorylarginine were supported by paper chromatographic studies with authentic N-phosphorylarginine and by studies with ^{14}C -L-arginine, which show radioactivity, derived from arginine, in the G-7 fraction (DURZAN, 1969b).

As far as we are aware, organisms containing phosphagens contain the enzyme arginine phosphokinase.



This enzyme catalyzes the formation of *N*-phosphorylarginine from arginine and ATP (adenosine triphosphate).

The only tissue known to contain comparatively large amounts of *N*-phosphorylated guanidines is muscle. In insects the main function of *N*-phosphorylarginine seems to be a reservoir of readily available phosphate-bond energy. The energy is conveyed to adenosine triphosphate during muscular contraction as the insect disperses and develops normal body functions. Small amounts of phosphagens are also found in tissues where sudden and extensive demands for energy are not needed (ENNOR and ROSENBERG, 1952). Here the molecule is useful for other vital processes such as morphogenesis and growth. In the budworm the levels of *N*-phosphorylarginine vary with the stage of insect development and are highest in the very early instars and before pupation (unpublished). As for other naturally occurring phosphagens, we have never been able to detect phosphocreatine in budworm extracts. While phosphagens have not been reported in plants, it is of interest that we now have evidence (DURZAN and CHALUPA, 1976) for the occurrence of other, as yet unidentified, phosphorylated guanidines in seeds of jack pine (*Pinus banksiana* Lamb.).

L-arginine is essential to the diet of most insects, but the requirements for the spruce budworm have not yet been firmly established (DURZAN and LOPUSHANSKI, 1968). The *N*-phosphorylarginine in the budworm is postulated as being derived from the L-arginine found in abundance in the host conifers (BIDWELL and DURZAN, 1975). *N*-phosphorylarginine appears to serve as the main phosphagen in the spruce budworm and the relationship to ATP and muscle activity deserves further consideration. This information develops a possible metabolic link in the food chain and provides a fuller understanding of the molecular and nutritional basis of budworm development.

REFERENCES

BIDWELL R. G. S. and DURZAN D. J. (1975) In *Historical and Current Aspects of Plant Physiology* pp. 152-225. N.Y. State Coll. Agric. Life Sci., Cornell University.

- DURZAN D. J. (1968) Nitrogen metabolism of *Picea glauca* (Moench) Voss—I. Seasonal changes of free amino acids in buds, shoot apices and leaves, and the metabolism of uniformly labelled ^{14}C -L-arginine by buds during the onset of dormancy. *Can. J. Bot.* **46**, 909-919.
- DURZAN D. J. (1969a) Automated chromatographic analysis of free monosubstituted guanidines in physiological fluids. *Can. J. Biochem.* **47**, 657-664.
- DURZAN D. J. (1969b) Nitrogen metabolism of *Picea glauca*. IV. Metabolism of uniformly labelled ^{14}C -L-arginine, carbamyl- ^{14}C -L-citrulline, and 1, 2, 3, 4- ^{14}C - γ -guanidinobutyric acid during changes in the soluble and protein nitrogen associated with the onset of expansion of spruce buds. *Can. J. Biochem.* **47**, 771-783.
- DURZAN D. J. (1973) The metabolism of ^{14}C -urea by white spruce seedlings in light and darkness. *Can. J. Bot.* **51**, 1197-1211.
- DURZAN D. J. and CHALUPA V. (1976) Growth and metabolism of cells and tissue of jack pine (*Pinus banksiana* Lamb.). 5. Changes in free arginine and Sakaguchi-reactive compounds during callus growth and in germinating seedlings of similar origin. *Can. J. Bot.* **54**, 483-495.
- DURZAN D. J. and LOPUSHANSKI S. M. (1968) Free and bound amino-acids of spruce budworm larvae feeding on balsam fir and red and white spruce. *J. Insect Physiol.* **14**, 1485-1497.
- DURZAN D. J. and RICHARDSON R. G. (1966) The occurrence and role of α -keto- δ -guanidinovaleric acid in white spruce (*Picea glauca* (Moench) Voss). *Can. J. Biochem.* **44**, 141-143.
- ENNOR A. H. and ROSENBERG H. (1952) The determination and distribution of phosphocreatine in animal tissues. *Biochem. J.* **51**, 606-610.
- GRISDALE D. (1970) An improved laboratory method for rearing large numbers of spruce budworm, *Choristoneura fumiferana* (Lepidoptera: Tortricidae). *Can. Ent.* **102**, 1111-1117.
- McMORRAN A. R. (1965) A synthetic diet for the spruce budworm, *Choristoneura fumiferana* (Clem.). (Lepidoptera: Tortricidae). *Can. Ent.* **97**, 58-62.
- MEISTER A. (1965) *Biochemistry of the Amino Acids*. Academic Press, New York.
- ROBIN Y. (1964) Biological distribution of guanidines and phosphagens in marine Annelida and related phyla from California, with a note of pluriphosphagens. *Comp. Biochem. Physiol.* **12**, 347-367.
- VAN THOAI N. and ROBIN Y. (1969) In *Chemical Zoology* (Ed. by FLORKIN M. and SCHEER B. T.), **4**, 169-203. Academic Press, N.Y.