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Study of amino acids in petrified plants from the Rajmahal Hills

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Chemistry of fossil plants collected from the Rajmahal Hills, Bihar (India) is studied. Amino acids extracted from the petrified woods, rachides and fructifications are identified with the help of paper chromatography. Implication of the chemical study of fossil plants in relation to taxonomy and evolution is discussed.

Key-words—Palaeochemistry, Amino acids, Petrified fossils, Rajmahal Hills (India).

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सारांश

राजमहल पहाड़ियों से प्राप्त अश्मीभूत पौधों में अमीनो-अम्लों का अध्ययन

बी० डी० शर्मा एवं आर० हर्ष

राजमहल पहाड़ियों से एकत्र अश्मित पौधों का रासायनिक अध्ययन किया गया है। कागज-वर्ण-लेखी विधि द्वारा अश्मीभूत काष्ठों, फलनों एवं रेकाइडों से निकर्षित अमीनो अम्लों का अभिनिर्धारण किया गया है। वर्गिकी एवं विकास को ध्यान में रखते हुए अश्मित पौधों के रासायनिक महत्व का भी विवेचन किया गया है।

CHEMICAL analysis of fossil plants and associated sediments is currently providing biochemical information to palaeobotanists for relating different groups of extinct plants and in the formulation of phylogenetic classifications. Many compounds previously considered so mobile as to prevent their preservation in ancient sediments, may infact be found in fossil material (Dilcher *et al.*, 1970; Niklas, 1982; Niklas & Chaloner, 1976; Niklas & Giannasi, 1977; Hohn & Meinschein, 1976; Wehmiller *et al.*, 1976). Chemicals recovered, e.g., amino acids, flavonoids, lignin, fatty acids. etc. from the fossil samples have helped in establishing relationship among the genera and species of the extinct plants. Amino acids have been recovered from fossil samples of animals and sedimentary rocks (Bada *et al.*, 1973; Dungworth, 1976).

In the Rajmahal Hills, petrified fossils are found either embedded in ferruginous rocks as at Amarjola or in the form of hard silicified cherts as at Sonajori, Nipania, Chilgujari and Hiranduba localities. Amino acids have been extracted from some of the petrified materials and identified tentatively with the help of paper chromatography.

MATERIAL AND METHODS

Petrified *Bucklandia* stems, *Ptilophyllum* rachides, *Williamsonia* (seed-bearing) naked receptacles, *Pentoxylon* stems and *Coniferocaulon* stems were collected from the well-known ferruginous rock of Amarjola, while decorticated, silicified woods were obtained from Sonajori. The specimens were washed several times with distilled

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water and then cooked in a furnace at 200°C for 24 hours to kill all micro-organisms present on the material. After repeated washing with distilled water, dried. The material was crushed into small pieces using separate pestal/mortar for each sample. Extractions were made through soxhlet in 80 per cent alcohol for 48 hours. The solutions were evaporated and to the dried extracts was added 5 ml of 20 per cent alcohol and centrifuged for 10 minutes. The supernatant of each sample was kept in properly labelled Corning glass tubes in a freezer.

Amino acids were recovered through paper chromatography using the method of Hanes *et al.* (1961). The solvent used for chromatography was prepared by mixing butanol, glacial acetic acid and water in the ratio of 5 : 1 : 4 respectively. Different concentrations of extracts, i.e., 50λ, 100λ, 150λ, 200λ and 300λ were spotted on Whatman no. 1 chromatographic paper. 150λ and 200λ concentrations gave satisfactory results. The spots were put at proper distances and 'run' into chromatographic chamber at room temperature (35°C) for approximately 6 hours. The chromatogram was dried and sprayed with a mixture of 200 mg ninhydrin dissolved in 100 ml of acetone, again dried and kept in an oven at 80°C for 10 minutes for colour development. Distinct spots of different colours appeared representing various amino acids present in the samples. Amino acids were identified tentatively on the basis of R_f values.

OBSERVATIONS

Five to nine amino acids appeared in six samples used for the present investigation. Maximum number of amino acids appeared in *Coniferocaulon* stem, while minimum in the naked receptacles of seed-bearing *Williamsonia*. In all, on the basis of R_f value, 17 amino acids could be identified; a number of others remain unknown. The chromatograms show the amino acids in each sample as under:

Bucklandia Stem

L-Arginine
DL-Serine
DL-Alanine
L-Tyrosine
unknown
unknown
unknown

Ptilophyllum rachis

L-Cystine
unknown
DL-Serine
L-Glutamic acid
unknown
L-Cystine hydrochloride
unknown

Williamsonia naked receptacle
unknown

DL-3, 4 Dihydroxyphenylealanine
unknown
DL-Methionine
unknown

Pentoxylon stem

L-Cystine
DL-Aspartic acid
L-Glutamic acid
unknown
L-Cystine hydrochloride
L-Leucine

Coniferocaulon stem
unknown

L-Ornithine monochloride
unknown
DL-Serine
L-Glutamic acid
unknown
unknown
unknown
L-Leucine

Decorticated silicified coniferous wood (from Sonajori)

L-Histidine monochloride
unknown
DL-Threonine
DL-2-Aminobutyric acid
L-Tyrosine
DL-Valine
DL-nor-leucine

A comparison of the known 17 amino acids in the six samples (Table 1) shows that none is common in all the samples. Related organs of a bennettitalean plant, i.e., *Bucklandia* stem, *Ptilophyllum* leaf and *Williamsonia* fructification (Sahni, 1932) possess different amino acids. Similarly the two conifers, selected for the present purpose (samples 5 and 6) do not possess any common amino acid. While taxonomically separated plants preserve some common amino acids, e.g., L-cystine, L-Glutamic acid, and L-Cystine hydrochloride are present in *Ptilophyllum* rachis and

Table 1—Amino acids identified in six samples of petrified plants collected from the Rajmahal Hills, India

	1	2	3	4	5	6
3.55 L-Cystine		+		+		
9.77 L-Ornithine-monochloride					+	
12-L Histidine monochloride						+
13.9 L-Arginine	+					
17.7 DL-Aspartic acid				+		
20.44 DL-serine	+	+			+	
24.44 L-Glutamic acid		+		+	+	
26.00 DL-3-4 Dihydroxy-phenylealanine			+			
27.3 DL-Alanine	+					
30.8 DL-Threonine						+
39.1 DL-2-Aminobutyric acid						+
40.88 L-Cystinehydrochloride		+		+		
46.2 L-Tyrosine	+					+
50 DL-Methionine			+			
55 DL-Valine						+
67.11 L-Leucine				+	+	
70 DL-nor-Leucine						+

1. *Bucklandia* stem, 2. *Ptilophyllum* rachis, 3. *Williamsonia* naked receptacle, 4. *Pentoxylon* stem, 5. *Coniferocaulon* stem, 6. Decorticated silicified coniferous wood.

Pentoxylon stem. Similarly, L-Glutamic acid, and L-Leucine are present in both *Pentoxylon* and *Coniferocaulon*. Present study is a preliminary investigation and needs further work to draw any conclusion regarding the utility of palaeochemistry in taxonomy and phylogeny of extinct plants. However, such a study certainly advances the frontiers of our knowledge about the fossil plants and associated sediments.

There is no effect of kind of preservation in the presence of amino acids. Except the sixth sample (decorticated silicified coniferous wood) from Sonajori, all others have been collected from Amarjola and are preserved in an identical manner. But they possess different amino acids. Amino acids are also very well preserved in the hard silicified wood from Sonajori.

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