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PROCEEDINGS OF THE PHYTOCHEMICAL SOCIETY

A meeting of the Society was held at the University College of Swansea on 18–20 September 1974 when the following papers were presented, under the general title:

Nitrogenous Compounds of Current Interest in Plant Biochemistry

REVIEWS

Cytokinins

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The cytokinins are plant growth hormones which can promote cell division in plant callus cultures. With the exception of diphenyl urea all the naturally occurring cytokinins have been shown to be derivatives of adenine.

By the use of several different chromatographic techniques in conjunction with bioassay, it has been possible to demonstrate that many plants contain a complex mixture of cytokinins. Preparative GLC and GC–MS have been used to isolate and identify some of the components of these mixtures. GC–MS has proved most useful in confirming the presence of known cytokinins [1]. The structure of a new cytokinin has been determined on the μg scale using a combination of GC–MS, preparative GLC, UV and direct insertion MS. This compound was shown to be N^6-(*o*-hydroxybenzyl)adenosine [2, 3]. Work on the identification of further unknown cytokinins as well as the results of studies on the metabolism of cytokinins in plants will be presented.

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Biosynthesis and Catabolism of Caffeine

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The metabolism of caffeine (1,3,7-trimethylxanthine), first isolated in pure form from tea leaves more than 150 years ago, is only partially known. It was shown in the early 1960s that the purine ring of this secondary plant metabolite is formed according to the purine nucleotide biosynthesis in animals. More recently, scientists have been attracted to the question of whether caffeine is synthesized *de novo*, or whether it arises as a breakdown product of nucleic acid metabolism. Recently we were able to follow caffeine biosynthesis stepwise as far back as the nucleoside, 7-methylxanthosine [1]. There are, however, experimental problems which still make it difficult to distinguish between these two hypotheses.

Although caffeine breakdown has been well studied in animals, the first steps in its degradation in plants is little understood. It is well documented in the literature that in coffee leaves this purine alkaloid is finally degraded to allantoin, allantoic acid and urea. Fortuitously, we have discovered three further purines in young leaves of different *Coffea* species. These compounds, identified as highly methylated uric acids, are considered to be first products of caffeine breakdown [2].

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Occurrence and Metabolism of Adenosine 3′:5′-Cyclic monophosphate in Higher Plants

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That cyclic AMP acts as a mediator of the action of several mammalian hormones is well established (see, e.g. [1]). The role of cyclic AMP in the regulation of bacterial gene activity [2] and as 'acrasin' in *Dictyostelium discoideum* [3] is also recognized. Until recently, the occurrence of cyclic AMP in higher plants was uncertain, since much of the evidence was purely presumptive and based on observed physiological effects of exogenous cyclic AMP [4, 5]. Reports that administration of phytohormones elevates cyclic AMP concentrations also exist (*inter alia*, [6]), but the identification procedures employed often lack sufficient resolution.

Unequivocal evidence for the occurrence of cyclic AMP in *Phaseolus* has recently been presented [7]. This method involved several chromatographic and electrophoretic purification steps, demonstrably sufficient to separate cyclic AMP from all other naturally occurring adenine nucleotides, before spectrophotometric estimation. Later work [8] has utilized the binding protein assay of Brown *et al.* [9]. Cyclic AMP has been

demonstrated in other plant species by a binding protein assay [10–13], by radioimmunoassay [14] and by bioluminescence and protein kinase assays. Although the concentrations of cyclic AMP reported in these independent investigations are in reasonable agreement, these findings are disputed by other workers, e.g. Keates [15], and Amrhein [16].

Adenylate cyclase has been demonstrated in higher plants [8, 12, 14], as has cyclic nucleotide phosphodiesterase [12, 13, 16, 17]. Recent observations of the possible role of cyclic AMP as a 'secondary messenger' in plants is discussed.

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Pyrimidine Derivatives in Higher Plants

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In addition to the base constituents of their nucleic acids, the tissues of higher plants contain various other pyrimidine derivatives. Many early reports of the occurrence of free pyrimidine bases in these tissues, however, are considered to be the result of the use of extraction methods which permitted hydrolysis of nucleic acids, nucleotides and nucleosides. The biochemistry is reviewed of some of the more unusual pyrimidine derivatives which appear to be peculiar to higher plants. Special attention is paid to the pyrimidine amino-acids, lathyrine [1], willardiine and isowillardiine [2–4]. Their metabolic origins are discussed and evidence presented of a biosynthetic relationship between willardiine, isowillardiine and the orotate pathway of pyrimidine biosynthesis. Evidence for the production of the pyrimidine moiety of thiamine and the formation of lathyrine, by pathways other than that involving orotate, is discussed. Recent work with seedlings of *Lathyrus tingitanus* L. [5] has, however, shown that there is a significant incorporation of radioactivity from 6-^{14}C-orotate into lathyrine although this does not exclude the possible parallel operation of the γ-hydroxyhomoarginine route proposed by Bell [6]. Preliminary work outlining the origin of pyrimidine glucosides, vicine and convicine, in the orotate pathway is also discussed.

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Isoxazolinones in Higher Plants

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From *Pisum sativum* seedlings two amino acids have been isolated with some characteristic properties: instability in alkaline solution and high sensitivity towards UV irradiation. The structures of these compounds were found to be β-isoxazolin-5-one-2-yl)-alanine (**1**) and β-(2-β-D-glucopyranosyl-isoxazolin-5-one-4-yl)-alanine (**2**).

High concentrations of **1** (2% of dry wt) were found in 6-day-old pea seedlings (root + shoot) grown either in the dark or under continuous light. Both **1** and **2** were also found in seedlings of *Lens culinaris*, *Pisum arvense* and *Lathyrus odoratus*. From *Lathyrus odoratus* six new isoxazolinone derivatives have been isolated; together with **1** and **2** they account for 10% of the dry weight of 10-day-old seedlings (shoot + root).

The structures of these new compounds are: 2-γ-glutamyl-amino-ethyl-isoxazolin-5-one (**3**), α-amino-γ-isoxazolin-5-one-2-yl)-butyric acid (**4**), 2-amino-ethyl-isoxazolin-5-one (**5**), 2-cyanoethyl-isoxazolin-5-one (**6**), 2-β-D-glucopyranosyl-isoxazolin-5-one (**7**) and 2-carboxymethyl-isoxazolin-5-one (**8**). The structures of **5** and **7** have been confirmed by chemical synthesis [1].

The free isoxazolin-5-one ring and *o*-acetylserine are precursors for the enzymic synthesis *in vitro* of **1** [2]. The free ring and UDP-glucose are precursors for the *in vitro* enzymic synthesis of **7**, while **7** and *o*-acetylserine are precursors for the *in vitro* enzymic synthesis of **2**.*

In rats, compound **6** has the same lathyrogenic properties as its photoproduct, β-aminopropionitrile.†

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* In co-operation with Murakoshi *et al.*, University of Chiba, Japan.

† In co-operation with Ressler Ch., Inst. for Muscle Disease, New York, U.S.A.