PROCEEDINGS OF THE BIOCHEMICAL SOCIETY

The 494th Meeting of the Society was held at the Shell Centre, London S.E.1, on Friday, 16 May 1969, when the following papers were presented:

COLLOQUIUM ON 'NATURALLY OCCURRING MATERIALS OF POTENTIAL USE FOR CONTROL OF PLANTS AND ANIMALS'

Some Chemical and Biological Studies on an Exotoxin from *Bacillus thuringiensis* var. *thuringiensis* Berliner

By R. P. M. BOND, C. B. C. BOYCE, V. K. BROWN and J. D. TIPTON. ('Shell' Research Ltd., Milstead Laboratory of Chemical Enzymology, Sittingbourne, Kent)

Bacillus thuringiensis exerts its pathogenic effect on insects with two well defined toxins: a crystal protein endotoxin and an exotoxin whose activity survives brief autoclaving. Other toxins may well be produced by the bacterium (Heimpel, 1967). The adjective 'thermostable' normally applied to the exotoxin can be justified because no detectable change of activity occurs when the bacterial supernatant is heated at 110° for 4 hr. However, heating at 110° for 24 hr. destroyed approximately 80% of the activity. The toxin is effective against many insects. It is usually lethal to juvenile forms between instars or at pupation, but high doses can kill adult houseflies (*Musca domestica*). The method of administration of the toxin can be important. In some insects, the toxin is much more potent when given parenterally than when given orally. This difference has been investigated in detail for the case of the wax moth (*Galleria mellonella*) by Benz & Schmid (1969).

The exotoxin is also toxic to mice when given by intraperitoneal injection. Male mice are more susceptible to the toxin than are females.

The toxin is an adenine nucleotide inasmuch as it contains adenine and phosphate in a molar ratio of 1:1. The phosphate is removed by treatment with acid or alkaline phosphatase or by chemical hydrolysis at pH 4, and so is taken to be bound as a phosphomonoester. Spectroscopic evidence establishes that the adenine is substituted at position 9 and suggests that the substitution is by a β -ribofuranosyl moiety. Glucose and allomucic acid (Bond, 1969) have been identified as hydrolysis products of the exotoxin.

There are many points of similarity and some of difference between these results and those of de

Barjac & Dedonder (1968) and, more particularly, those of Sebesta and his associates (Sebesta, Horska & Vankova, 1969a,b; Farkas *et al.* 1969).

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Current Research on Absciscic Acid

By B. V. MILBORROW. ('Shell' Research Ltd., Milstead Laboratory of Chemical Enzymology, Sittingbourne, Kent)

The plant hormone absciscic acid (ABA) was characterized (Ohkuma, Addicott, Smith & Thiessen, 1965) and synthesized (Cornforth, Milborrow & Ryback, 1965) in 1965 and since that time the availability of the synthetic compound and its intrinsic interest have ensured that it is the subject of research in a large number of laboratories.

It has been isolated in crystalline form from cotton (Ohkuma, Lyon, Addicott & Smith, 1963), sycamore (Cornforth, Milborrow, Ryback & Wareing, 1965), yellow lupin (Rothwell & Wain, 1964; Cornforth, Milborrow, Ryback, Rothwell & Wain, 1966), pea (Isogai, Okamoto & Komoda, 1967) and yam (Hashimoto, Ikai & Tamura, 1968) and identified in extracts of some thirty other species of plant (Milborrow, 1967a). Most of these identifications have relied on four criteria: (i) general physical properties; (ii) co-chromatography with authentic material; (iii) production of the same physiological responses in test plants; (iv) demonstration of the characteristic optical-rotatory-dispersion spectrum. In addition, gas-liquid chromatography of methylated (Lenton, Bowen & Saunders, 1968) and trimethylsilylated (Davis,

Heinz & Addicott, 1968) plant extracts produce peaks which correspond with the retention time of the abscisic acid derivatives that show the fragmentation pattern of abscisic acid when introduced into a mass spectrometer (Gaskin & MacMillan, 1968).

Abscisic acid was originally isolated from cotton bolls, where it is involved in the abscission of the young fruit, and from sycamore, where it is concerned with the regulation of the bud dormancy. The growth-inhibitory factor in extracts of potato tubers and plum shoots has been identified as abscisic acid, and one of the roles of the compound appears to be the modification and regulation of the action of the growth-promoting hormones, gibberellin, cytokinin and auxin. Abscisic acid is present at its highest concentrations in ripe fruit and, although usually present at much lower concentrations in the seeds, it has been shown to regulate dormancy of rose embryos (Jackson & Blundell, 1963); during germination there is a considerable decrease in the amount of free abscisic acid.

Synthetic abscisic acid has been applied to plants and can duplicate all the effects that had been attributed to it *in vivo* (El Antably, Wareing & Hillman, 1967); in addition it has been found to accelerate the senescence of leaves and to promote flowering, but as yet there is little evidence to suggest that these are normal functions.

The biochemical investigation of abscisic acid has centred on its effects on inhibition of enzyme synthesis, and the site of action has been sought at the nucleic acid level. Abscisic acid has been shown by Varner & Johri (1967) to inhibit the synthesis of α -amylase by barley aleurone, but it has also been demonstrated to prevent the incorporation of labelled thymidine and uridine into the root tips of germinating ash seeds (Villiers, 1968). The role of abscisic acid, like that of the other plant hormones, is complex and it probably exerts a primary regulatory action at several different sites.

The terpenoid nature of abscisic acid suggests that biosynthesis occurs by the condensation of three isoprene residues, but the molecule could arise in this way either from a C₁₅ unit, such as farnesyl pyrophosphate, after a specific cyclization reaction, or it could be formed by the degradation of a carotenoid. This latter hypothesis is an attractive one because some carotenoids, such as violoxanthin, possess terminal rings with the carbon skeleton of abscisic acid, have oxygen functions at the appropriate positions and give a growth-inhibitory product on photolysis (Taylor & Smith, 1967). Experiments with labelled precursors have shown that quite rapid rates of synthesis of abscisic acid occur (Noddle & Robinson, 1969) and, although at present no decisive experiment has been carried out, the direct and specific route of biosynthesis is

favoured. The incorporation of tritium from stereospecifically tritiated (4*R*)- and (4*S*)-mevalonic acid into abscisic acid indicated that the carbon skeleton had been assembled from mevalonic acid subunits and that the double bonds of the C₁₅ precursor had been made in the *trans* configuration (Robinson in preparation). This labelling pattern is consistent with either direct synthesis or the carotenoid pathway.

The *cis*- $\Delta^{2,3}$ double bond of abscisic acid isomerizes under the action of sunlight to give a 1:1 equilibrium mixture with the biologically inactive *trans*- $\Delta^{2,3}$ isomer (*trans*-abscisic acid). This reaction was expected to occur *in vivo*, and *trans*-abscisic acid was found at a concentration of 4% of that of the abscisic acid in rose leaves when non-radioactive racemic *trans*-abscisic acid and racemic [2-¹⁴C]-abscisic acid were added to the methanol in which the tissue was extracted. The natural, and therefore optically active, (+)-*trans*-abscisic acid was detected by spectropolarimetry, and the possibility that the (+)-*trans*-abscisic acid had formed by isomerization during the isolation procedures was excluded by the virtual absence of ¹⁴C from the *trans*-abscisic acid. No isomerase activity was detected in plants and the low concentrations of *trans*-abscisic acid must therefore result from either (a) its preferential destruction or (b) continuous dilution by abscisic acid and an equal rate of destruction. When 1:1 mixtures of ($\pm$)-[2-¹⁴C]-abscisic acid and ($\pm$)-*trans*-[2-¹⁴C]abscisic acid were supplied to tomato plants the *trans* material was more rapidly converted into *trans*-abscisyl- β -D-glucopyranoside (Koshimizu, Inui, Fukui & Mitsui, 1968) than the *cis*. Most of the (+)-abscisic acid was converted into another material, 'metabolite C' (Milborrow, 1967b). This marked difference between the fate of the two enantiomers provides a striking contrast with their identical growth-inhibitory activity.

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Gibberellins and their Biosynthesis

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The gibberellins were first discovered in culture filtrates of the phytopathological fungus *Gibberella fujikuroi* as the products that stimulated internode elongation in rice seedlings. Subsequent investigations of the physiological activities of the gibberellins have revealed a wide range of developmental processes in flowering plants, including germination, dormancy, mobilization of food reserves in the endosperm, stem elongation, leaf growth and morphology, floral induction, parthenocarp and fruit growth, that can be influenced under appropriate circumstances by exogenous gibberellins. A number of field applications of the gibberellins in controlling the growth of economic plants are in use.

The gibberellins have been shown to occur naturally in a wide variety of angiosperms as well as in the fungus. These findings have led to the generally held view that the gibberellins are natural growth regulators or hormones in flowering plants. To date, 24 different gibberellins of defined structure have been isolated from *G. fujikuroi* culture filtrates and angiosperm sources, and it seems certain from both published and unpublished reports that additional members of this family will be identified. All of these gibberellins are diterpenoids based on the *enantio*-gibberellane carbon skeleton (gibbane nucleus).

The production of gibberellins in plants, and hence the course of plant development, appears to be under the control of a number of environmental as well as genetic factors. A detailed understanding

of the biosynthetic pathway and the characteristics of the enzymes involved in gibberellin production should thus provide a basis for investigations of natural regulation of some aspects of plant development. It may also provide a more rational basis for the development of methods of artificial control of these processes.

The results of radioactive-tracer studies with [1^{14}C]acetate and [2^{14}C]mevalonate (Birch, Richards & Smith, 1958) confirmed the suggested isoprenoid origin of the gibberellins. The co-occurrence of (–)-kaurene and gibberellins in culture filtrates and the conversion of (–)-[17^{14}C]-kaurene into [17^{14}C]gibberellic acid in *G. fujikuroi* cultures (Cross, Galt & Hanson, 1964) established the intermediary role of this diterpene hydrocarbon in the biosynthetic pathway. Information about intermediates and the enzymes participating in the biosynthesis and further metabolism of (–)-kaurene has come from studies of higher-plant cell-free systems from wild cucumber (*Echinocystis macrocarpa*) endosperm and castor bean (*Ricinus communis*) seedlings. Soluble enzymes catalyse the synthesis of the acyclic diterpene precursor *trans*-geranylgeranyl pyrophosphate from mevalonate (Oster & West, 1968). Another soluble enzyme system mediates the cyclization of *trans*-geranylgeranyl pyrophosphate to (–)-kaurene (Upper & West, 1967). A bicyclic intermediate, *trans*-copalyl pyrophosphate, has recently been shown to be an intermediate in this conversion. (–)-Kaurene is then converted successively into a series of oxidation products (–)-kauren-19-ol, (–)-kauren-19-al, (–)-kauren-19-oic acid and (–)-7 β -hydroxy-kauren-19-oic acid (Dennis & West, 1967). These reactions are catalysed by a particulate cell fraction requiring NADPH and oxygen, and have the general characteristics of mixed-function oxidases.

Some suggestions about intermediates and the sequence of reactions in later stages of gibberellin biosynthesis have come from tracer studies in *G. fujikuroi* cultures (see, e.g., Cross, Norton & Stewart, 1968; Verbiscar, Cragg, Geissman & Phinney, 1967).

The enzymic site of action of several plant-growth retardants that have been shown to interfere with the overall synthesis of gibberellins (Kende, Ninnemann & Lang, 1963; Ninnemann, Zeevaart, Kende & Lang, 1964; Dennis, Upper & West, 1965) have been investigated in cell-free systems. In extracts of *E. macrocarpa* endosperm, *R. communis* seedlings and *G. fujikuroi* mycelia 2'-isopropyl-4'-(trimethylammonium chloride)-5'-methylphenyl piperidine-1-carboxylate (Amo 1618) selectively inhibits, among the reactions between mevalonate and (–)-kaurene, the conversion of *trans*-geranylgeranyl pyrophosphate into copalyl pyrophosphate. β -Chloroethyltrimethylammonium chloride (CCC)