Phytochemistry, 1967, Vol. 6, pp. 455 to 464. Pergamon Press Ltd. Printed in England

PROCEEDINGS OF THE PHYTOCHEMICAL GROUP

A meeting of the Group was held at Royal Holloway College, University of London, on 20 and 21 September, 1966, when the following papers were presented.

Symposium on Nucleic Acids and Nucleotides in Higher Plants

The Chemistry of Nucleic Acids

T. L. V. ULBRICHT

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THE covalent structure of nucleic acids was determined in 1952 and the double-helical structure for DNA proposed the following year, thereby initiating the rapid development of molecular biology which we have witnessed these last years. Two aspects of the *chemistry* of nucleic acids which are currently attracting attention will be discussed:

(1) The determination of nucleotide sequence and the complete primary structure of nucleic acids, with special reference to transfer RNA (s-RNA). The minor nucleotide components of s-RNA will be mentioned, and the complete sequences of alanine-, serine- and tyrosine-s-RNA discussed.

(2) The secondary structure of polynucleotides, including RNA. It is now apparent that the main factor in stabilizing helical polynucleotides is base-stacking, not hydrogen-bonding.[1] Experimental work has established that even simple nucleotides have a conformation in solution similar to that which they have in polymers. Secondary structure is very sensitive to environmental factors such as temperature, pH, solvent, and the nature and concentration of counter-ions (Na^+, Mg^{++} etc.). It is probable that the possibility of taking up different conformations in slightly different environments is one of the important factors in the biological role of s-RNA.

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Quantitative Extraction and Estimation of Nucleic Acids from Higher Plants

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THE quantitative analysis of nucleic acids in higher plant tissues presents many problems to the analyst. Care must be taken at every stage to ensure that loss of RNA and/or DNA does not occur, and that complete removal of substances which may interfere with the selected procedure(s) of estimation has been obtained.[1]

The three most frequently used procedures for nucleic acid analysis, namely the Ogur and Rosen,[2] the Schneider,[3] and the Schmidt and Thannhauser[4] procedures, must always be preceded by a series of extractions to remove various compounds which may interfere with subsequent estimations. It has become the practice to start the extractions by treating the tissue homogenate with cold acid. Some observations concerning the various acids and their concentrations which have been used will be presented. Cold acid extraction is followed by the extraction of lipids. Again the sequence and combination of solvents used has tended to vary with the worker, but a much favoured combination has been ethanol, ethanol:ether (two or three times) and finally ether. Evidence will be presented which strongly suggests that this sequence of solvents tends to be inefficient and generally unsatisfactory.

Nucleic acids are extracted from the cold-acid soluble and lipid-free residue by one of the procedures mentioned above. The merits of the three methods will be discussed, and it will be shown that the Schmidt and Thannhauser procedure is the most suitable for the analysis of RNA and DNA in higher plants. The Schmidt and Thannhauser RNA extract frequently requires purification; procedures for purification will be discussed

together with the methods available for estimation. The extraction and estimation of DNA by the Schmidt and Thannhauser procedure will be discussed.

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Nucleic Acid Changes during Cell Growth in Plants

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IT HAS been shown that, during cell expansion in the pea root, there is not only an increase in the quantity of most cellular components, but also a change in the type of many components synthesized. Such a change can be observed in the cell wall components deposited during growth and in the enzyme complement which, as activity determinations show, continues to change throughout the life of a cell.[1] At least part of this change in the enzyme complement is accompanied by a change in the nature of the proteins synthesized during cell growth and development.

Analysis has shown that the over-all composition and stability of both the DNA and RNA changes during cell growth[2] and that the sub-cellular distribution of both RNA and protein also changes.[3] Such observations, although indicative of changes in the nucleic acid complement, do not enable the type of RNA mediating the observed change in protein synthesis to be identified.

Using excised root tissue the effect of purine and pyrimidine analogues has been studied. The excised root tip, grown in sterile culture, has been used to study the effect of 8-azaguanine upon growth and metabolism subsequent to its incorporation into RNA.[4] The observed inhibition of cell division and concomitant slight stimulation of cell expansion makes the interpretation of the results difficult, but proteins are synthesized without the normal degree of enzyme activity.

The sub-apical 2 mm segment excised from seedling pea roots has the capacity for considerable growth which is entirely by cell expansion when provided with an energy source. 2-Thiouracil enhances cell growth considerably and the relationship between the altered pattern of RNA synthesis in this excised tissue and the effects of the analogue on protein and RNA synthesis have been examined.[5]

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The Role of RNA Metabolism in the Regulation of Growth and Development of Higher Plants

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GROWTH and development involves changes in complement of enzyme activities in the cell. Such activity changes may be regulated at different levels in the flow of information from the genome; this may be at the transcription level between DNA and messenger-RNA, at the translation level between messenger-RNA and protein, or by inhibition or activation of the resulting enzyme activity. Experiments using the inhibitor of RNA synthesis, actinomycin D, indicated that much of the regulation of growth and development of plant tissues was at the DNA–messenger-RNA transcription level. Consequently studies were initiated to determine the types of RNA synthesized in plant tissue, with particular interest in messenger-RNA. The synthesis of a fraction of RNA, which had properties very different from either soluble- or ribosomal-RNA, was demonstrated during the growth of excised hypocotyl tissue. This fraction of RNA was called DNA-like, or D-RNA, and the properties of the D-RNA, its composition, its molecular size, its rate of synthesis and breakdown, resembled those of the messenger-RNA described from bacterial systems.

A fraction of RNA very similar to the D-RNA of the excised hypocotyl tissue was synthesized in intact roots. In the root tip D-RNA was found in association with polyribosomes; a further indication of its role as messenger-RNA.

Some aspects of the biological importance of the D-RNA in the regulation of growth and development have been studied.

(*a*) Selective inhibition of the synthesis of soluble- and ribosomal-RNA by the analogue 5-fluorouracil has shown that growth of excised hypocotyl tissue required the continual synthesis of D-RNA, but not of soluble- and ribosomal-RNA. There was a general correlation, in both intact and excised tissues, between the growth potential of the tissue and the relative amount of D-RNA synthesized, and differences within the D-RNA population from different tissues could be detected.

(*b*) The induction of two enzymes involved in the assimilation of nitrate by higher plants, nitrate reductase and nitrite reductase, required the synthesis of protein and RNA. Again 5-fluorouracil, which selectively inhibited the synthesis of soluble- and ribosomal-RNA, did not prevent the induction, indicating the requirement for D-RNA synthesis for induction of specific protein synthesis.

The Nucleic Acids of Photosynthetic Cells

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A study has been made of the nucleic acids of a number of photosynthetic cells and in particular those of broad bean leaves (*Vicia faba* var. Sutton). Sub-cellular organelles were separated by differential centrifugation and their nucleic acids isolated and fractionated by chromatography on methylated serum albumin–kieselguhr columns.

Four distinct types of low molecular weight (LMW) RNA could be distinguished in preparations of broad bean leaf nucleic acids. The cytoplasmic and chloroplast ribosomes each contain distinct LMW RNA components. There is a "soluble" RNA (i.e. RNA which is not precipitated by centrifugal forces which sediment ribosomes) which apparently only occurs in chloroplasts. In addition there is a soluble component, probably transfer RNA, which is found in both the chloroplasts and cytoplasm.

The chloroplast LMW RNA's are present only in small amounts in etiolated leaves and are absent in non-photosynthetic tissues of roots and flowers. They are present in some albino mutants i.e. they can apparently be synthesized without the concomitant production of chlorophyll. RNA of broad bean chloroplasts and that of *Anabaena variabilis* var. kützing were found to be dissimilar.

The DNA of isolated broad bean chloroplasts is firmly bound to the lamellae, and prolonged sonic disintegration only partly removes it. Added chloroplast DNA is adsorbed onto isolated chloroplast lamellae.

This study was performed in collaboration with Dr. Rachel M. Leech with able technical assistance from Miss Christine Poole.

The Nucleic Acids of Plastids

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In the past few years a great deal of evidence has accumulated indicating that plastids contain the full range of nucleic acids commonly found in living systems. Electron microscope and biochemical studies have demonstrated the presence of ribosomes within plastids.[1–4] Plastid ribosomes have a lower sedimentation constant than the cytoplasmic ribosomes from the same cells.[3, 5, 6] Transfer RNA[6] and template RNA[7] have also been isolated from chloroplasts. It has been shown that chloroplasts have the ability to carry out protein synthesis,[6, 8, 9] and it appears that the function of the RNA species so far found in chloroplasts is to take part in this process.

Electron microscope and autoradiographic studies indicate the presence of DNA in plastids.[10–13] DNA has been estimated chemically in chloroplasts isolated from enucleated cells of *Acetabularia*.[14, 15] In *Chlorella* two types of DNA, differing in base ratio and metabolic activity, have been found,[16] one of which is thought to be chloroplast DNA. DNA from chloroplasts of broad bean,[17] *Chlamydomonas reinhardi*,[18] *Euglena gracilis*[19–21] and *Antirrhinum majus*,[22] has been shown to have a base ratio different from that of the corresponding nuclear DNA. DNA from chloroplasts of spinach[23] and Swiss chard[11] has been shown to have a different density to that of the nuclear DNA. In its general physicochemical properties, the plastid DNA

resembles genetic DNA from other systems. There is enough DNA in a plastid to code for the structure of cetainly hundreds, and possibly thousands, of proteins. Isolated chloroplasts appear to be able to carry out DNA-directed RNA synthesis[24–27] indicating that chloroplasts have the machinery for translating a genetic message. It thus seems a plausible assumption that plastid DNA contains genetic information. There is some evidence that plastid DNA includes the information for the synthesis of plastid ribosomal RNA. However, genetic studies suggest that many of the enzymes of chlorophyll synthesis, carotenoid synthesis and photosynthesis are under nuclear control. The function of plastid DNA is, therefore, still almost completely unknown.

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Some Effects of Light on Plant Nucleic Acids

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The growth of a tissue is controlled by its protein content, and it is possible therefore that light influences growth through effects (involving a pigment system) on the nucleic acids. This is the case with hypocotyl growth in seedlings of *Lupinus albus* (studied by two former research students, Dr. K. W. Giles and Dr. R. J. Lewington, and myself). Lupin hypocotyls reach a final length of about 25 cm in 14 days' continuous dark, an increase of 100-fold which involves cell enlargement only. In continuous light hypocotyls can only manage 5 cm, again without cell division. Light also strongly inhibits net RNA and to a smaller extent DNA synthesis, and protein synthesis is found to be proportional to growth. When light-grown plants are transferred to the dark the inhibition is lifted and the rate of synthesis of nucleic acid is increased to the dark value, final contents being progressively lower the longer the plants remain in the light before transfer. Transfer of plants from dark to light results in immediate cessation of growth and of net protein synthesis, and this correlates with a reduced rate of DNA synthesis and more strikingly with loss of an RNA fraction (which seems likely to be polysomal), in some cases amounting to 50 per cent of the total RNA. Associated with this is a 3·5 times increase in total RNAase activity (but not in DNAase) during the first 2 days after transfer. The sequence of events therefore is that under the influence of light, lupin cells produce a large increase in RNAase activity resulting in loss of susceptible RNA, so that protein synthesis is restricted and growth stopped. Preliminary experiments in which lupin seedlings were immersed in RNAase solution showed cessation of growth even in the dark. The process by which light brings about increase in RNAase activity is under investigation.

Minor Components in RNA from Soluble and Particulate Fractions of Leaves

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WITH several organisms it has been shown that the majority of the minor bases are to be found in the RNA of the soluble fraction[1–5] but pseudouridine and 2-*O*-methylribose-containing nucleotides have been detected in significant amounts in ribosomal RNA.[1, 2, 4, 6–9] Examination of the minor components present in RNA from ribosomes and from the soluble fraction of leaves of *Brassica chinensis* and *Nicotiana tabacum* gives further support for these general conclusions.

Ribosomes centrifuged out of clarified sap were purified by differential centrifugation using in some instances deoxycholate,[10] or were adsorbed onto magnesium bentonite[11] and washed free of s-RNA while adsorbed on the clay. RNA was isolated from both types of preparation by treatment with phenol. With free ribosomes this was carried out in the presence of lauryl sulphate[12] and sodium bentonite,[13] while with bentonite-adsorbed particles the extraction was made in the presence of 0·5 M NaCl and 0·1 M tris-HCl, pH 7·5. All procedures gave RNA which contained very similar proportions of pseudouridine and 2-*O*-methylribose-containing nucleotides. These proportions remained unaltered when the RNA was precipitated at 0° by M NaCl.

While the proportion of pseudouridine in s-RNA does not show great variation between different groups of organisms, this does not apply to the pseudouridine and 2-*O*-methylribose residues in ribosomal RNA which have so far been shown to vary from 1·9 and 1·7 per cent, respectively, in high molecular weight RNA from wheat germ[7, 8] to 0·15 and 0·1 per cent in ribosomal RNA from *Escherichia coli*.[3, 9] The proportions of these two components found in the leaf ribosomal RNA's (1·1–1·4% pseudouridine and 1·4–1·7% 2-*O*-methylribose) were only slightly lower than those detected in the wheat germ RNA and may indicate that ribosomes from higher plants are particularly rich in these minor components.

RNA was isolated as previously described[10] from the 105,000 *g* sap supernatant or from sap clarified by magnesium bentonite,[14] a method which avoids the necessity for high speed centrifugation. The two methods gave similar yields of RNA and analyses showed that the s-RNA's were indistinguishable in their content of minor components. These components were the same as those detected in s-RNA from yeast and *Neurospora* but were mostly present in lower proportion, pseudouridine representing 13–16% uridine. In addition to the components previously identified in the *B. chinensis* s-RNA,[10] inosine was detected in the s-RNA from *N. tabacum*. Most components were isolated from alkaline hydrolysates of the RNA but in order to confirm the presence of 1-methyladenosine and 7-methylguanosine residues in the *B. chinensis* s-RNA hydrolysis with N $HClO_4$ at 28° was used.[15]

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Nucleotides in Plant Metabolism

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IN ADDITION to being nucleic acid components, nucleotides participate in, and control, major metabolic pathways. Thus, as discussed by Schmitz,[1] a close relationship exists between the nucleotide pattern of a

tissue and the type and extent of the predominant metabolism. Studies have been made of this relationship in higher plants with the object of obtaining information regarding the main biochemical changes accompanying germination, growth, and differentiation. Two main sources of difficulty in the work were (*a*) presence in plant extracts of non-nucleotide, u.v. light-absorbing impurities[2, 3] and (*b*) widespread occurrence of extremely active and persistent non-specific phosphatase activity.[4, 5]

In germinating pea seeds, the main change during the imbibition phase (0–16 hr) is a rapid increase in ATP concentration at the expense of AMP concentration; that of ADP remains constant. After this phase, there is a slow fall in concentration of all three adenine derivatives and, as growth starts, a rapid increase occurs in the concentration of UDP–glycosyl compounds.[6] Several nucleotide-like substances, none of which could be identified with any known compound, were present in seeds in small amounts. One unusual substance was identified as acetyl-3′-dephosphocoenzyme A; its concentration in seeds fell during the stage at which the radicle penetrates the testa.[6, 7]

Attempts to induce an exaggeration in certain aspects of nucleotide metabolism by infiltration of seedlings with various nucleotides, resulted in a rapid degradation of the infiltrated substances to their nucleosides and corresponding free bases. Guanosine 5′-monophosphate differed in behaviour to the other nucleotides examined and consistently stimulated production of an unusual uracil derivative.[8]

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The Intracellular Distribution of Free Nucleotides in Leaves

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FREE nucleotides in tobacco leaf chloroplasts have been determined by ion-exchange chromatography. Similar estimations were made on the whole tissue and "chloroplast depleted" tissue, so that the distribution of nucleotides between chloroplasts and the other cell material might be assessed. The effect of light on the intracellular distribution is being studied.

Leaves of plants which were destarched by a prolonged period in the dark contained a low level of nucleotides. Exposure to 30 sec of intense light caused considerable changes in the nucleotide content of the chloroplasts. These changes were not observed in the non-chloroplast cell material. Prolonged illumination (10 min or more) of the destarched leaves caused a general increase in the free nucleotides, and especially in ATP. This increase took place in both the chloroplast and non-chloroplast cell fractions.

The results are to be used to determine how readily free nucleotides, especially ATP, can pass into and out of the chloroplast. The importance of this problem will be discussed in relation to the synthesis, by plant tissues, of starch from an exogenous supply of sugar.

Nucleotides and Oligosaccharides

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THE isolation of UDP-glucose from yeast[1] initiated investigations of the role of nucleoside diphosphate sugar derivatives in the biochemistry of carbohydrates. Since 1950 it has become apparent that they are intimately involved in the formation of mono-, oligo- and poly-saccharides in plants and animals. Using higher plant enzyme preparations it has been firmly established that sucrose can be synthesized from UDP-glucose and fructose, and sucrose-6′-phosphate from UDP-glucose and fructose-6-phosphate. The latter reaction coupled with that of a specific sucrose phosphatase may be the more important for the synthesis of sucrose *in vivo*.[2] Raffinose in plants would appear to result from the galactosylation of sucrose by UDP-galactose.[3, 4]

Sucrose synthetase is probably involved in the conversion of sucrose to starch in maturing seeds.[5, 6] Similarly, in germinating seeds the enzyme may be responsible for the conversion of sucrose to UDP-glucose.

This system has been studied at Royal Holloway College using *Vicia faba* seedlings[7] and its probable role in the utilization of sucrose and raffinose in the early stages of germination will be discussed.

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Communications

The Effect of Kinetin on the Incorporation of Labelled Orotate into Various Fractions of Ribonucleic Acid from Excised Radish Leaf Discs

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Discs from senescing radish leaves were floated either on water or on a solution of kinetin and incubated in the dark for 11 or 21 hr. They were then fed with labelled orotic acid for 4 hr and afterwards two fractions of RNA were extracted by the phenol method. One fraction contained soluble, light and heavy ribosomal RNA which were separated by sedimentation on a sucrose density gradient; the other fraction contained RNA with similar sedimentation properties on a sucrose density gradient to messenger RNA extracted from some mammalian tissues.[1] It was found that 21 hr treatment with kinetin causes a small but consistent stimulation of labelled precursor incorporation into soluble, light and heavy ribosomal RNA. Also there is a considerably greater stimulation of incorporation into the messenger RNA fraction. A comparable differential effect on soluble and ribosomal RNA on the one hand and messenger RNA on the other was found in discs treated with kinetin for only 11 hr.

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Nucleotides of Potato Starch Grains

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LELOIR[1] has suggested that the nucleotides ADP-glucose and UDP-glucose are involved in starch synthesis. Experimental evidence has shown however that ADP-glucose is much more active than UDP-glucose in the synthesis of starch. The enzyme for amylose synthesis is firmly bound to the starch grain and Rees[2] has found that there are also a number of nucleotides bound to starch. From commercial starches he has been able to extract and isolate small quantities of ADP-glucose, the principal nucleotide components present being AMP and ADP.

In the present study kilogram amounts of fresh starch were extracted from potato tubers at different times in the growing season. The nucleotides were precipitated as mercury salts and fractionated on a Dowex column (4 per cent cross-linked, 50–100 mesh) by elution with a linear gradient of lithium chloride.

The principal nucleotide found was ADP-glucose which accounted for 70 per cent of the total. Small amounts of AMP and ADP were also detected but these appeared to be degradation products of the ADP-glucose. Another major component was found by comparison with authentic samples to be NAD. As reported by Rees, no UDP-glucose was found at any time. Traces of a component which could have been UMP were detected.

In view of the above result we are prompted to suggest that ADP-glucose is the main sugar nucleotide involved in starch synthesis.

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*Recent Work on the Enzymic Synthesis of Plant Xylan and Cellulose from Nucleotide Sugars**

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PARTICULATE preparations from immature corn cobs contain enzymes which catalyse the epimerization of UDP-D-xylose^{14}-C to UDP-L-arabinose^{14}-C and the transfer of both of these sugar nucleotides to polysaccharide.[1] The synthesized polysaccharide has been shown to be similar to natural corn xylan as it is alkali-soluble and precipitated from solution by acid, and contains acid labile L-arabinofuranoside^{14}-C units and chains of 1,4-linked D-xylosyl-^{14}C units. Linkages between the xylosyl units appear to have a β-configuration. The xylan synthesizing activity of the particulate preparation is considerably enhanced by preparation in the presence of albumin (1%) and by assay in the presence of both albumin (1%) and sucrose (0·4 M). Activity is markedly depressed by UTP, UDP and UMP but not by uridine, ATP or GTP.

A mung bean (*Phaseolus aureus*) particulate preparation was able to incorporate D-glucose-^{14}C from GDP-D-glucose-^{14}C but not from UDP-D-glucose-^{14}C into an alkali-insoluble polymer which was shown to be cellulose.[2] *Lupinus albus* preparations[3] have, however, recently been reported to incorporate D-glucose from both of these sugar nucleotides into polymer which contains alkali-insoluble material which may be cellulose. Results now show that crude mung bean particles[2] at pH 7·0 do incorporate glucose from UDP-D-glucose into alkali-insoluble material provided Mg^{++} and cysteine are added to the incubation mixture; this incorporation rate is markedly improved by preparation of the particles in the presence of 1% albumin. Synthesis of cellulose from GDP-D-glucose by the same unwashed particles is not affected by Mg^{++}, cysteine or albumin. The alkali-insoluble polymers synthesized from both substrates were not hydrolysed by a hemicellulase which readily hydrolysed laminarin and β-1,3-β1,4-glucan but not cellulose. Polymer synthesized from UDP-D-glucose may therefore possibly contain cellulose.

* Investigation supported by research grant A-1418 from the National Institutes of Health, U.S. Public Health Service and Research grant G-23763 from the National Science Foundation. Support by the Agricultural Experiment Station is also acknowledged.

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The Biosynthesis of Saligenin Glucosides

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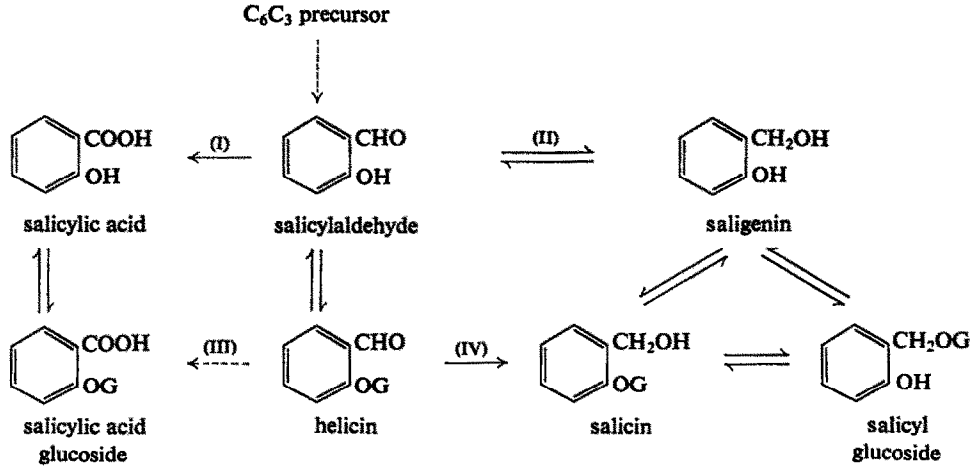
THE glucoside of saligenin, salicin, has been known for well over a century in species of willow, but its isomer, salicyl glucoside, has not been found in nature. The latter compound is now reported to occur in considerable quantities in the flower oil of a number of species of *Spiraea* (Rosaceae).

Although rare in nature, salicyl glucoside is known to be the major product when saligenin is fed to many higher plants, including willow. This led to the suggestion that helicin and not saligenin is the direct precursor of salicin *in vivo*.[1, 2] Some experiments are reported which support this theory.

The probable preliminary step in the formation of saligenin derivatives, is the conversion of a C_6C_3 compound to salicylaldehyde. In the Salicaceae, this salicylaldehyde and its glucoside, helicin, are probably metabolized rapidly and the end products are salicylic acid glucoside and salicin. In *Spiraea* flowers, there is an accumulation of salicylaldehyde, helicin and salicyl and salicylic acid glucosides.

Careful examination of willow tissues shows that trace amounts of salicyl glucoside are present. Its presence has been confirmed in *S. fragilis*, *S. babylonica* and *S. vitellina*. The methods in general use for scanning phenolic glucosides, do not show up trace amounts of one saligenin isomer in the presence of the other.

It is known that salicin can be partially converted to salicyl glucoside in the presence of β-D-glucoside glucohydrolase preparations from almond and willow.[3] This type of reaction may occur *in vivo* or there may be a direct glucosylation of trace amounts of saligenin.



Preliminary studies have been made on the enzymes catalysing reactions (I) and (II). These enzymes appear to occur in most higher plants and consist of a pair of NAD-linked dehydrogenases. It is suggested that the same or a similar pair of enzymes may catalyse reactions (III) and IV).

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Biosynthesis of Carotenoids in Verticillium albo-atrum

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Verticillium albo-atrum Reinke and Berthold is a colourless fungus and usually produces a dark melanoid pigment in culture.[1] Valadon and Heale[2] isolated after u.v. irradiation of the fungus an orange mutant M 5 in which the following C_{40} polyenes were characterized: phytoene, β-carotene, γ-carotene, neo-lycopene A, lycopene, neurosporaxanthin and four unidentified fractions. M 5 was subjected to further irradiation and a number of secondary mutants were obtained, and these were analysed with respect to their carotenoid contents. Three of these mutants had the same pigments as M 5 but in different proportions; a fourth produced a new pigment, torulene, and lacked neo-lycopene A and lycopene, and a fifth was colourless containing only phytoene. The phytoene level was more or less constant in wild type, M 5 and in all the secondary mutants analysed, even though the amount of carotenoid pigments present varied considerably. This does not seem to support the Porter-Anderson hypothesis[3] that phytoene is an important precursor in carotenoid synthesis; and also since not all of the intermediates phytoene$\longrightarrow\beta$-carotene were identified.

However, it is well known that precursor–product relationships are not always reflected in their quantities or even in the detectability of the compounds involved. Thus, one of the mutants was treated with various inhibitors of carotenoid synthesis, i.e. diphenylamine, β-ionone and methylheptenone, in an attempt to obtain more information relating to carotenoid synthesis in this organism. With these compounds, all the intermediates, phytoene$\longrightarrow\beta$-carotene, in the Porter–Anderson series were identified but in different amounts. Also, an inhibition of the production of the most unsaturated polyenes (β-, γ-carotene, lycopene and neurosporaxanthin) and a stimulation of the more saturated ones (phytoene, phytofluene, β-zeacarotene, ζ-carotene and neurosporene) were observed. Furthermore, when the mutant was grown in the presence of diphenylamine, then washed and regrown on 1% glucose in M/15–KH_2PO_4, the unsaturated polyenes seemed to be formed at the expense of the saturated ones. These results strongly suggest that carotenoid biosynthesis in *V. albo-atrum* takes place along the Porter–Anderson pathway.

This research has been aided by a grant from the Central Research Fund of the University of London.

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Crocin and Linoleate Oxidation by Isolated Sugar Beet Chloroplasts

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THE oxidation of the carotenoid glycoside crocin by isolated sugar beet chloroplasts at pH 7·6[1] and by deoxycholate extracts of chloroplasts was found to be stimulated by the addition of partially peroxidized linoleate.[2] The effects of adding linoleate to isolated chloroplasts have therefore been examined in more detail and a close similarity in the patterns of linoleate and crocin oxidation has been found. Both oxidations are inhibited by potassium cyanide and ethylene diamine tetraacetate and are stimulated by light. Chloroplasts were found to catalyse both the formation and the breakdown of linoleate hydroperoxide and crocin oxidation can be coupled to either process. The deoxycholate extracts appear to be more effective in catalysing the breakdown of linoleate hydroperoxide and this activity, and the anaerobic destruction of crocin in the presence of linoleate hydroperoxide, is retained after boiling. From a comparison of the pH activity curves and the response to inhibitors, it seems that the formation of linoleate hydroperoxide by chloroplasts is not catalysed by either lipoxygenase or haematin compounds; it is suggested that a copper protein may be the catalyst concerned.

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