PLANT PHYSIOLOGY

Copper–Cellulose Complexes

Our attention has been directed to the fact that our recent paper[1] on the subject of timber preservation by copper compounds is being quoted in the literature[2] in support of the copper-cellulose hypothesis of Abrams and Bottoms[3].

These workers and their associates have concluded, largely from indirect evidence, that after treating wood or cellulose with a copper formate solution followed by autoclaving or heating, the copper is converted into a copper–cellulose complex and that this complex confers durability. This is known as the N.C.G. process, and its use has been advocated for wood, cotton and other cellulosic materials as a preservative measure.

During our examinations in the electron microscope[1,4] of wood treated with water-soluble preservatives or aqueous solutions of metal salts, characteristic electron diffraction patterns were noted which were interpreted as referring to metal–cellulose complexes in the wood. This interpretation was later confirmed[5,6], and the electron-diffraction diagrams were shown to arise from a two-dimensional array of metal atoms adsorbed on to the surface of cellulose microfibrils.

The properties of this metal–cellulose complex, however, do not appear to be consistent with the claims of Abrams and Bottoms. We have found that complexing takes place in the cold from aqueous solutions of copper salts so that no heat is necessary for the adsorption and, in addition, the electron diffraction pattern given by the complex remains unchanged after heat treatment. Cations from solutions of metal salts, other than those of copper, form a metal–cellulose complex so that the reaction is not restricted to copper formate solutions.

Abrams and Bottoms have claimed that N.C.G.-treated cellulose is resistant to hydrolysis and will not dissolve in concentrated hydrochloric acid, whereas we have found that, in the case of the complex detected by means of electron diffraction analysis, the 'bound' copper is instantly removed by dilute acids.

Thus, while our investigations have confirmed the existence of a metal–cellulose complex, the configuration of which has been elucidated by means of electron diffraction analysis, the general behaviour of this complex does not resemble that of the copper–cellulose complex postulated by Abrams and Bottoms, and our findings can in no way be taken to support their copper–cellulose hypothesis.

D. S. Belford
C. D. Cook
E. H. Nevard

Hickson's Timber Impregnation Co. (G.B.), Ltd.,
Castleford, Yorks.

R. D. Preston

Botany Department,
University of Leeds.

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Tetragonin : a Yeast Growth-regulating Substance from *Tetragonia expansa*

The number of antimicrobial substances isolated from higher plants is small. Recently, studies with *Tetragonia expansa* or New Zealand spinach have shown it to contain a water-soluble component which we have tentatively named 'tetragonin'. Tetragonin is characterized by a narrow spectrum, being active apparently specifically upon *Saccharomyces* spp.

Activity tests were conducted with the agar diffusion paper disk method[1] using 1-day-old cultures of *Saccharomyces carlsbergensis* ATEE 9080 as test organism. The medium containing 2 per cent malt extract and 1 per cent agar, inoculated with approximately 5×10^7 cells/ml., was spread on glass plates in layers 1 mm. thick.

A preparation was purified by addition of chloroform to the centrifuged juice obtained from the stems of well-developed plants. The supernate was kept on a steam-bath for 10 min. and then centrifuged. The addition of one part ethanol to two parts of the supernate resulted again in precipitation. This supernate was dried at 50° C. and dissolved in methanol. After drying, a hygroscopic, yellow, sweet-smelling substance was obtained.

A methanolic solution of this partially purified substance was chromatographed on Whatman No. 1 filter paper. The dried chromatograms were placed on top of agar plates inoculated with *Saccharomyces carlsbergensis*. The R_F values thus obtained with 3 : 2 mixtures of methanol–water were 0·44–0·46, with 3 : 2 mixtures of ethanol–water 0·39–0·40 and of 3 : 2 mixtures of acetone–water 0·38–0·39. The same R_F values were obtained using the eluate of the chromatogram and also with the crude leaf juice. Tetragonin is very soluble in water, soluble in methanol, scarcely soluble in absolute ethanol and *iso*propanol, and insoluble in benzene, carbon tetrachloride, toluol and chloroform. The compound is stable at 100° C. for about 20 min., then undergoing decomposition. This decomposition with respect to time is logarithmic. The relationship between zone diameter and the logarithm of concentration is a straight line. Tetragonin dried on filter paper retains its activity for at least one year.

Tetragonin in high concentration inhibited, but in lower concentration stimulated, the multiplication also of several types of *Saccharomyces cerevisiae* and of *S. cerevisiae* var. *ellipsoideus*, in addition to *S. carlsbergensis*. No activity was observed with the following micro-organisms :

Yeasts : *Schizosaccharomyces octosporus*, *Endomyces magnusii*, *Sporobolomyces* sp., *Endomycopsis fibuliger*, *Rhodotorula gracilis*, *Torulopsis utilis*.
Bacteria : *Escherichia coli*, *Serratia marcescens*, *Bacillus subtilis*, *B. cereus*.
Actinomycetes : *Actinomyces roseus*, *Streptomyces floridae*, *Proactinomyces roseus*, *Nocardia grisea globispora*.
Phycomycetes : *Rhizopus nigricans*, *Mucor* sp.
Ascomycetes : *Aspergillus ruber*, *Penicillium striatum*.
Basidiomycetes : *Puccinia graminis*
Fungi Imperfecti : *Aspergillus niger*, *A. flavipes*, *Penicillium chrysogenum*, *P. notatum*, *Trichothecium roseum*, *Colletotrichum lini*, *Botrytis cinerea*, *Cercospora beticola*, *Pullularia pullulans*.

Tetragonin is present throughout the plants, and though its concentration varies considerably during the period of cultivation, it is always the highest in the roots. In the young plants the leaf juice shows higher activity than that of the stem, but as the plant ages, the activity of the stem becomes higher than the activity of the leaves, while the activity of the lower stem parts approaches that of the roots. Green unripe fruit show an activity between that of the leaves and