rapidly in the cold or warm to give purple or blue products. A variety of steroid drugs, especially those containing a 17α -ethynyl group, give coloured and/or fluorescent products. If conditions are carefully standardized, particular sequences of colour/fluorescence change help to distinguish different groups of steroids. Recommended experimental conditions are described elsewhere (Jeffery, 1971, 1972). The convenience of the techniques is demonstrated for (1) detection of the synthetic oestrogen mestranol [3-methoxy-19-nor-17 α -pregna-1,3,5(10)-trien-20-yn-17 β -ol], (2) distinguishing the products of selective reduction of progesterone (pregn-4-ene-3,20-dione) and (3) distinguishing 7-oxopregnenolone (3 β -hydroxypregn-5-ene-7,20-dione) from common steroids absorbing in the same region of the u.v., e.g. 4-ene-3-ones. These reactions work best on silica-gel layers. Other common staining reagents such as 2,4-dinitrophenylhydrazine and dodecamolybdo-phosphoric acid can be used after the HCl-vapour procedure.

The interest and encouragement of Professor S. C. Frazer and gifts of steroids from many friends are gratefully acknowledged.

Jeffery, J. (1971) *J. Chromatogr.* **59**, 216

Jeffery, J. (1972) *J. Chromatogr.* **67**, 188

A Method for the Analysis of ^{15}N -Labelled Amino Acids in the Effluent from Preparative Amino Acid-Analysis Columns

By SUSAN E. HAWKES, PETER J. BUTTERY and DYFED LEWIS (*Department of Applied Biochemistry and Nutrition, University of Nottingham, Sutton Bonington, Loughborough, Leics. LE12 5RD, U.K.*)

Analysis of micromolar quantities of ^{15}N -labelled amino acids in effluents from preparative amino acid systems presents considerable problems. Conventional methods, which require about $70\mu\text{mol}$ of N (Anon., 1970), employ the Kjeldahl technique and subsequent conversion of the NH_3 produced into N_2 gas by using hypobromite before analysis in a mass spectrometer. A technique is reported here employing emission spectrometry (Leickman *et al.*, 1968), which is suitable for quantities as small as $0.5\mu\text{mol}$ of amino acid contained in large volumes of citric acid buffer.

The fraction, up to 50 ml, containing the amino acid is adjusted to pH 5.5 and heated with half its volume of ninhydrin reagent (1 g of ninhydrin, 0.15 g of hydrindantin, 70 ml of 2-methoxyethanol and 30 ml of water) for 15 min at 100°C . The diketohydrindylidene-diketohydrindamine, which contains the α -amino N from the amino acid, is then extracted from the citric acid buffer with chloroform. It is essential to adjust the pH of the solution to 3.3 to facilitate

extraction. Below pH 3.0 the diketohydrindylidene-diketohydrindamine is destroyed (Kennedy, 1965). The chloroform is then evaporated to dryness under reduced pressure in a glass bubble of approx. 5 ml capacity. The bubble is crushed and the powder added to a thick-walled Pyrex-glass tube together with 1.5 g of CuO and 1 g of CaO. The tube is evacuated to a pressure of $0.133\text{ N}\cdot\text{m}^{-2}$ (10^{-3} Torr), sealed and heated at 550°C overnight. The ^{15}N content of the N_2 gas produced (Dumas reaction) is determined by emission spectrometry, by using the Dumas tube as an electrodeless discharge tube (Leicknam *et al.*, 1968).

Phenol is not used in the analyser buffers because it is extracted into the chloroform and causes the Dumas tube to explode. It is not possible to use the column effluent directly since the high carbon content gives rise to too much gas within the tube.

During the manipulation of samples a certain amount of contamination is unavoidable; for example the % atoms excess of $1\mu\text{mol}$ of amino acid can be underestimated by up to 25%. Preliminary experiments indicate that the contamination can be determined by assay of a [^{15}N]glycine sample of known % atoms excess with each batch of samples.

S. E. H. holds a Ministry of Agriculture, Fisheries and Food Studentship.

Anon. (1970) *Tech. Rep. Ser. Int. At. Energy Ag.* **111**, 19

Kennedy, I. R. (1965) *Anal. Biochem.* **11**, 105–110

Leicknam, J. P., Middelboe, V. & Proksch, G. (1968) *Anal. Chim. Acta* **40**, 487–502

Degradation of Sclerotin, a β -(1 $\rightarrow$ 3)-Glucan, by Enzymes from Fungi Parasitic on Sclerotia

By JOHN S. D. BACON, A. H. GORDON and D. JONES (*Macaulay Institute for Soil Research, Craigiebuckler, Aberdeen AB9 2QJ, U.K.*)

The resting bodies, sclerotia, of a number of fungi pathogenic towards plants contain a gel-forming polysaccharide, first found in *Sclerotinia libertiana*, named 'sclerotin', and shown by Kitahara & Takeuchi (1961) to be a β -(1 $\rightarrow$ 3)-linked glucan with single glucose residues attached at intervals by β -(1 $\rightarrow$ 6)-linkage. Studies of its enzymic degradation and biosynthesis (Johnson *et al.*, 1963; Batra *et al.*, 1969) have emphasized the regularity of its structure.

We have examined the composition of cell walls of several sclerotia-forming plant pathogens (Jones, 1970; Jones *et al.*, 1972). Culture fluids from two fungi parasitic on *Sclerotinia sclerotiorum* will lyse cell-wall preparations from this species, in which sclerotin is the predominant wall component (Jones & Watson, 1969). When the culture fluid was fractionated (cf. Bacon *et al.*, 1970) several β -(1 $\rightarrow$ 3)-glucanases were found, but none would lyse cell walls

when acting alone. In both parasitic fungi, *Coniothyrium minitans* and *Trichoderma viride*, the main component was an exo- β -(1 $\rightarrow$ 3)-glucanase (cf. Chesters & Bull, 1963). Since an exoglucanase from a basidiomycete had been shown by others to degrade sclerotan completely (Buck *et al.*, 1968), we have tested the glucanases on glucan extracted from the fungus.

Sclerotia of *Sclerotinia sclerotiorum* or autoclaved mycelia of *Sclerotium rolfii* were homogenized and centrifuged at 100000g. A water-clear colourless gel was decanted, which with 0.2 vol. of ethanol could be compressed to a small volume at 100000g. Resuspension and repetition of the process gave a product that could be freeze-dried without affecting its properties.

The purified exo- β -(1 $\rightarrow$ 3)-glucanase from *Coniothyrium minitans* acted on it to give the expected products (glucose and gentiobiose in molar ratio 2:1) but degradation beyond 30–35% was very slow. After dissolution in dimethyl sulphoxide and recovery the resistance was unchanged.

Purified endo- β -(1 $\rightarrow$ 3)-glucanase, from *Coniothyrium minitans* or *Cytophaga johnsonii*, had a very restricted action on the preparation, the products being mainly glucose and a suspected trisaccharide, but no gentiobiose; the viscosity was not affected.

Simultaneous action of the two enzymes brought about almost 100% conversion into glucose and gentiobiose, with small amounts of other oligosaccharides.

The resistance of the cell walls to the β -glucanases used singly is thus partly attributable to resistance by the sclerotan component. The resistance shown by our preparations (and by one from *Claviceps purpurea* kindly provided by Dr. K. W. Buck) may be due to occasional structural 'errors' that impede the exoglucanase, or possibly to non-glycosidic linkages; treatment with hot 3% (w/v) NaOH renders the glucan more susceptible to exoglucanase.

- Bacon, J. S. D., Gordon, A. H., Jones, D., Taylor, I. F. & Webley, D. M. (1970) *Biochem. J.* **120**, 67–78
 Batra, K. K., Nordin, J. H. & Kirkwood, S. (1969) *Carbohydr. Res.* **9**, 221–229
 Buck, K. W., Chen, A. B., Dickerson, A. G. & Chain, E. B. (1968) *J. Gen. Microbiol.* **51**, 337–352
 Chesters, C. G. C. & Bull, A. T. (1963) *Biochem. J.* **86**, 31–38
 Johnson, J., Kirkwood, S., Misaki, A., Nelson, T. E., Scaletti, J. V. & Smith, F. (1963) *Chem. Ind. (London)* 820–822
 Jones, D. (1970) *Trans. Brit. Mycol. Soc.* **54**, 351–360
 Jones, D. & Watson, D. (1969) *Nature (London)* **224**, 287–288
 Jones, D., Farmer, V. C., Bacon, J. S. D. & Wilson, M. J. (1972) *Trans. Brit. Mycol. Soc.* **58**, in the press
 Kitahara, M. & Takeuchi, Y. (1961) *Nippon Nogei Kagaku Kaishi* **35**, 468–474

Double-Helical Conformations of Sulphated Polysaccharides and their Potential Biological Function

By FRANK B. WILLIAMSON (*Department of Biochemistry, University of Aberdeen, Marischal College, Aberdeen AB9 1AS, U.K.*)

Many ionic polysaccharides exist naturally in a gel state. With the carrageenans, which are a group of sulphated polysaccharides present in the intercellular matrix of certain species of red seaweeds, the predominant mechanism of gel formation involves the twisting together of linear polysaccharide molecules to form double-helical tie points (Rees *et al.*, 1969). The extent and stability of the double-helical regions are dependent on the primary structure of the polysaccharide. Enzymic alteration of the primary structure would seem to provide a means of metabolically controlling the polysaccharide conformation and hence the properties of the intercellular gel matrix.

The primary structures of the carrageenans can also be chemically altered to yield sulphated regular linear co-polymers of 3,6-anhydro- α -D-galactopyranose and β -D-galactopyranose, linked 1,3 and 1,4 respectively. The different carrageenans each have their own characteristic sulphation pattern, and the non-sulphated hydroxyl groups are available for hydrogen-bonding, which is responsible for holding the double helices together. Breaking of these hydrogen bonds results in a denaturation of the double helix to yield two separate polysaccharide chains with more random conformations. These conformations and conformational transitions can be conveniently studied by the use of optical-rotation techniques, which have been shown to give good correlations between theoretical and observed rotation values (Rees *et al.*, 1970).

The acidic sulphate half ester groups confer interesting ion-exchange properties to the gels. Studies of ligand-induced conformational transitions and selective cation-binding suggest that the double-helical carrageenans may have a biological function in maintaining a suitable intercellular ionic environment, and they may act as allosteric ion-exchange agents involved in the uptake and transport of specific ions. Calcium binding studies suggest that certain carrageenans may play an active role in the calcification of these seaweeds.

Studies of the distribution and conformations of ionic polysaccharides within the native tissue are possible by using metachromatic fluorescent 'reporter' molecules, which are cationic histochemical dyes that bind to polyanions and exhibit changes in their optical properties that reflect the nature of the polymer binding site.

- Rees, D. A., Steele, I. W. & Williamson, F. B. (1969) *J. Polym. Sci. Part C* **28**, 261
 Rees, D. A., Scott, W. E. & Williamson, F. B. (1970) *Nature (London)* **227**, 390