FUNGAL CELLULASES

XIV. THE SYNTHESIS OF PENTAFLUOROPHENYL β -d-GLUCOPYRANOSIDE AND ITS INTERACTION WITH β -GLUCOSIDASE AND THE β -GLUCOSIDASE INDUCTION SYSTEM IN STACHYBOTRYS ATRA*

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A number of experiments showed no trace of any ability on the part of pentafluorophenyl β -glucoside to induce β -glucosidase formation. The results of a typical experiment in which this substance is compared with phenyl β -glucoside and β -thioglucoside are set out in Table 1. The limits of the concentration of putative

TABLE 1

β -glucosidase induction by various β -glucosides

Washed 3-day-old S. atra mycelium (Jermyn 1965) was resuspended in water made up to the indicated concentration of putative inducer. After shaking for 16 hr at 28°C, an aliquot of the suspension was subjected to ultrasonic disintegration and centrifuged, and the enzyme activity in the supernatant measured

| Addition | Concentration (M) | Enzyme Activity (units/ml) |
|--------------------------------------|----------------------|-------------------------------|
| None (0 hr) | | $1 \cdot 15, 1 \cdot 05$ |
| None (16 hr) | | 0.95, 1.20 |
| Phenyl β -glucoside | $2	imes 10^{-3}$ | $23 \cdot 5$ |
| Phenyl β -thioglucoside | $2	imes 10^{-3}$ | $48 \cdot 6$ |
| Pentafluorophenyl β -glucoside | $2	imes 10^{-2}$ | $1 \cdot 16, 1 \cdot 24$ |
| Pentafluorophenyl β -glucoside | $2	imes 10^{-3}$ | $1 \cdot 16, 1 \cdot 30$ |

inducers that can be compared are set by solubility considerations, and the lack of any inductive effect cannot be stated as an absolute property, since still higher concentrations might theoretically show some effect. None the less it is apparent that the pentafluorophenyl compound must be several orders of magnitude less effective an inducer than the phenyl compounds.

The lag period in enzyme induction at 28°C is some 4 hr (Jermyn 1965). Experiment showed that spontaneous decomposition of pentafluorophenyl β -glucoside in aqueous solution over this time at 28°C was negligible. Nor is this compound likely to be completely destroyed by enzyme action under circumstances where the phenylglucosides remain effective. The most likely explanation of the observation is that the glucoside or the released pentafluorophenol are acting as metabolic inhibitors. Compare the effectiveness of pentachlorophenol as an uncoupler of oxidative phosphorylation.

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SHORT COMMUNICATIONS

Table 2 sets out some derived kinetic constants for the action of *S. atra* β -glucosidase on pentafluorophenyl and 2,4,6-trichlorophenyl β -D-glucopyranosides. The kinetics of the inhibition of this enzyme by *ortho*-substitution in the aglycone are most simply explained by supposing (Jermyn 1955) that the substitution produces its principal effect on the binding of substrate and enzyme by hindering whatever rotation of the aglycone around the glycosidic linkage is involved in the formation of the Michaelis complex, but has less effect on the subsequent decomposition of this complex to products. The van der Waals radius of the fluorine atom $(1\cdot35 \text{ Å})$ is the next smallest after that of hydrogen $(1\cdot2 \text{ Å})$; if the earlier hypothesis is correct, the inhibitory effects of 2,6-substitution in phenyl β -glucoside by fluorine should be much less than those of substitution by chlorine $(1\cdot8 \text{ Å})$.

TABLE 2

KINETIC CONSTANTS FOR THE ACTION OF S. ATRA β -GLUCOSIDASE ON PENTAFLUOROPHENYL AND 2,4,6-TRICHLOROPHENYL β -GLUCOSIDES COLLATED WITH RELEVANT DATA FROM EARLIER WORK All observations at pH 5 and 28°C. Since the kinetics of the enzyme are of the ternary complex type, it is not to be expected that K_m and K_i will be identical

| Glucoside | 10 ⁵ К _т (м) | Relative V (phenyl β -glucoside = 1.00) | 105 <i>K</i> i* (м) | Van der Waals Radii (Å) | Source of Data |
|---------------------------|---------------------------------------|---|------------------------|--------------------------------|----------------------|
| Phenyl | $7 \cdot 2$ | 1.00 | 33 | 1.2 (H) | h |
| 2-Iodophenyl | 34 | 0.38 | 22 | $2 \cdot 15$ (I) | |
| 2,4-Diiodo-6-methylphenyl | † | + | + | (-) | Jermyn |
| 2-Methylphenyl | 66 | 0.32 | 217 | $2 \cdot 0$ (CH ₂) | (1955) |
| 2,6-Dimethylphenyl | 680 | 0.002 | + | (01-3) | (1000) |
| 2-Chlorophenyl | 34 | 0.38 | 22 | 1.80 (Cl) | |
| 2,4,6-Trichlorophenyl | 110 | 0.50 | 27 | () | К |
| 2,3,4,5,6-Pentafluoro- | | | | | Present |
| phenyl | 16 | $1\cdot 22$ | $8 \cdot 3$ | 1.35 (F) | ∫ work |

* Against *p*-nitrophenyl β -glucoside. † Activity too low for measurement.

The data may best be summarized by the statement that as the van der Waals radius of the substituent falls, the dominating effect of diortho-substitution in the aglycone on the values of the kinetic constants becomes less until for the smaller substituents (H, F) other factors determine the relative values, i.e. in the present state of knowledge they become unpredictable. It may be noted that the value of V for the pentafluorophenyl compound is one of the highest so far observed, but it is no more than an attractive speculation to connect this with the high electron-attractive effect of the C₆F₅O group revealed by the ready ionization of C₆F₅OH.

Chemical Syntheses

Pentafluorothiophenol (1·1 molecular equivalents) reacts in aqueous acetone at room temperature with acetobromoglucose and sodium hydroxide (1 molecular equivalent of each) to give a 92% yield of *pentafluorophenyl* 2,3,4,6-tetra-O-acetyl- β -D-thioglucopyranoside, white needles from ethanol, m.p. 155°C, $[\alpha]_{D}^{20} - 5 \cdot 9^{\circ}$ [c, 3 in HCON(CH₃)₂]. C₂₀H₁₉F₅O₉S requires C, 45·3; H, 3·6; F, 17·9; S, 6·0. Found: C, 45·9; H, 3·8; F, 18·1; S, 6·2%.

Pentafluorophenol, under conditions identical except for the inclusion of 30 min boiling under reflux, gives a 42% yield of *pentafluorophenyl* 2,3,4,6-tetra-O-acetyl- β -D-glucopyranoside, coarse white needles from ethanol, m.p. 143°C, $[\alpha]_{20}^{20} - 3 \cdot 4^{\circ}$ [c, 3 in HCON(CH₃)₂]. C₂₀H₁₉F₅O₁₀ requires C, 46.7; H, 3.7; F, 18.5. Found: C, 46.4; H, 3.8; F, 17.7%.

Pentachlorophenyl 2,3,4,6-tetra-O-acetyl β -D-glucopyranoside may be synthesized in 22% yield from sodium pentachlorophenate and acetobromoglucose by refluxing in aqueous ethanol. Recrystallization from acetone gives soft white needles, m.p. 165°C, $[\alpha]_{D}^{20} + 9\cdot6^{\circ}$ [c, 5·5 in HCON(CH₃)₂]. C₂₀H₁₉Cl₅O₁₀ requires C, 40·2; H, 3·2; Cl, 29·7. Found: C, 40·3; H, 3·5; Cl, 29·5%. This compound resembles the 2,4-dinitrophenyl compound (Glaser and Thaler 1926) in giving rise to the free phenol instead of the glucoside when attempts are made to deacetylate it by any of the usual methods. 2,4,6-Trichlorophenyl β -D-2,3,4,6-tetra-O-acetyl-glucopyranoside, m.p. 170°C [Rosenmund and Güssow (1954) give 166°C] was deacetylated with sodium in ethanol and the product recrystallized from ethanol-ether to give white needles of 2,4,6-trichlorophenyl β -D-glucopyranoside, m.p. 196–197°C, $[\alpha]_{D}^{20} - 14\cdot4^{\circ}$ [c, 10 in HCON(CH₃)₂]. C₁₂H₁₃Cl₃O₆ requires C, 40·1; H, 3·6; Cl, 29·6. Found: C, 40·0; H, 3·7; Cl, 29·7%. Solubility in water at 28°C, 5×10^{-4} M.

Deacetylation of the tetraacetyl pentafluorophenyl glucoside by vigorous shaking with a solution of sodium in ethanol, with immediate neutralization of the solution by acetic acid when the last crystals of the acetate disappeared, gave a nearly quantitative yield of *pentafluorophenyl* β -D-glucopyranoside, white needles from water, m.p. 166–168°C, $[\alpha]_{20}^{20} - 15 \cdot 3^{\circ}$ [c, 10 in HCON(CH₃)₂]. C₁₂H₁₁F₅O₆ requires C, 41 \cdot 6; H, 3 \cdot 2; F, 27 \cdot 4. Found: C, 41 \cdot 3; H, 3 \cdot 3; F, 27 \cdot 6%. Solubility in water at 28°C, 2×10^{-2} M.

The extremely rapid (about 3 min at 25°) deacetylation procedure was adopted because an initial experiment in which 2–3 hr of reaction with sodium ethoxide at room temperature was allowed gave an 80% yield of an unidentified compound, easily crystallized as white needles from ethanol-ether, m.p. 143° C, $[\alpha]_{D}^{20} - 40^{\circ}$ [c, 3 in HCON(CH₃)₂], with an analysis: C, $39 \cdot 0$; H, $4 \cdot 3$; F, $17 \cdot 9\%$. The compound is presumably a β -glucoside, being water-soluble and attacked by β -glucosidase with the liberation of reducing power, but the analysis seems to be incompatible with any simple defluorination product of the pentafluorophenyl glucoside. Attempts to deacetylate the tetraacetylthioglucoside by a variety of procedures invariably led to partly defluorinated products.

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