LETTERS TO THE EDITORS

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A Synthetic Differential Growth Inhibitor

IT has been shown[1,2,3] that malt, ungerminated grain and oranges contain a water-soluble, thermostable substance which permits the free growth of epithelial tissues *in vitro* at concentrations at which it inhibits totally the growth of fibroblasts and other mesenchyme cells. In the present investigation, commercial malt extract has been employed, and the methods of biological assay with fragments of ten-day chick's heart have already been described[2,3]. Vigorous steam distillation of malt extract affords a distillate which contains a variety of substances among which, after suitable treatment, a differential inhibitor can be detected.

This was presumably derived from the original factor by hydrolytic fission, but, as it should be a relatively simple substance, attention was focused on its study as likely to provide a valuable clue. The compound is produced in such small quantity that it has not yet been possible to identify it with certainty; its properties and the analysis of derivatives indicated the probability that it is an unsaturated lactone, $C_6H_8O_2$.

A natural product of this composition and character is the so-called parasorbic acid (δ-$\Delta^{\alpha\beta}$-hexenolactone) which occurs in the berries of the mountain ash[4]; this dextro-rotatory substance has not yet been synthesized so far as we are aware. We have obtained *dl*-δ-$\Delta^{\alpha\beta}$-hexenolactone in small yield by condensation of acetaldol with malonic acid in pyridine solution (b.p. 116°/16 mm. Found: C, 64·6; H, 7·3. $C_6H_8O_2$ requires C, 64·3; H, 7·1 per cent); sorbic acid is the main product[5]. Benzoylation of aldol in pyridine solution followed by condensation with malonic acid afforded a solid mixture of acids, and from these by steam distillation and gradual addition of sodium hydroxide a mixture of δ-hexenolactone and benzaldehyde was obtained (Found after removal of benzaldehyde: C, 64·7; H, 6·8 per cent). δ-Hexenolactone exhibits the usual properties of an unsaturated substance and slowly develops acidity in cold aqueous solutions as the result of hydrolysis; it is unstable, becoming orange-coloured in air and light, and it displays a marked tendency to polymerize both as a lactone and as a derivative of an αβ-unsaturated acid. It readily forms a complex condensation product with 2 : 4-dinitrophenylhydrazine (3 : 2 – $3H_2O$). It is completely removed from aqueous solution by charcoal, possibly in the form of a polymeride. In all these properties it resembles the lactone present in the malt extract steam-distillate.

Synthetic δ-hexenolactone exhibits the differential growth-inhibitory property: the two analysed specimens were active at a concentration of 0·011 mgm./c.c.; a specimen made by the first method and not distilled (avoidance of toxic decomposition products) was active at 0·006 mgm./c.c. A rough quantitative comparison with the malt distillate factor shows, however, that the latter is the more active. Three possibilities therefore arise: (*a*) that the malt distillate factor is optically active δ-hexenolactone, (*b*) that it is δ-hexenolactone and that other substances are accessory to the effect, or that the synthetic compound contains traces of toxic impurities; or (*c*) that it is another substance closely resembling δ-hexenolactone.

The discovery of the differential growth inhibition produced by δ-hexenolactone suggests that it is able to inhibit a growth factor which is more specifically required by fibroblasts than by epithelial cells, and an obvious provisional hypothesis is that the factor may be pantothenic acid, or an analogous substance. We are engaged in experiments designed to test this theory, the first step being the synthesis of δ-hydroxy-$\Delta^{\alpha\beta}$-hexenoyl-β-alanine.

A few further observations are that δ-valerolactone (dihydro-δ-hexenolactone) is non-toxic in the special sense and non-inhibitory. The malt extract distillate contains a highly toxic substance of salicylic acid type and several aldehydes (for example, acetaldehyde and furaldehyde) which are non-differential inhibitors.

Aldol and acrolein are toxic; crotonaldehyde inhibits all growth at a concentration between *M*/3,000 and *M*/3,600, whereas propionaldehyde and furaldehyde are much weaker inhibitors, the limiting concentrations lying between *M*/150 and *M*/200.

P. B. MEDAWAR.

Department of Zoology and Comparative Anatomy,

G. M. ROBINSON.
R. ROBINSON.

Dyson Perrins Laboratory, Oxford.
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[1] Heaton, *J. Path. Bact.*, **29**, 293 (1926).
[2] Heaton, *J. Path. Bact.*, **32**, 565 (1929).
[3] Medawar, *Quart. J. Exp. Physiol.*, **27**, 147 (1937).
[4] Hofmann, *Annalen*, **110**, 129 (1859); Doebner, *Berichte*, **27**, 345 (1894).
[5] The acid, $C_{12}H_{18}O_5$, described by Riedel (*Annalen*, **361**, 89; 1908) is doubtless a dimeride of δ-hexenolactone + H_2O, that is, *O*-δ-hydroxy-$\Delta^{\alpha\beta}$-hexenoyl-δ-hydroxy-$\Delta^{\alpha\beta}$-hexenoic acid.

The Saponin of Fenugreek Seeds

THE presence of a saponin in the seeds of *Trigonella Foenum græcum* (Leguminosæ) was first reported by Wunschendorff[1], who had subjected the defatted seeds to a process of extraction with alcohol. He obtained from the alcoholic extract a gelatinous precipitate which was afterwards dissolved in alcohol and reprecipitated with ether. Wunschendorff described the product so obtained as a white semi-crystalline powder, m.p. 214–215°, which gave a yellow colour test with concentrated sulphuric acid, and a white precipitate with barium hydroxide solution. However, he could not assign a definite formula to the saponin, but showed that it gives by hydrolysis a reducing sugar and water-insoluble sapogenin.

In view of the fact that very little is known of the chemical nature of the sapogenin, we have systematically investigated the seeds, and succeeded in isolating the sapogenin in a pure state. The powdered seeds of Trigonella were defatted by extraction with light petroleum, then extracted three times with alcohol. Concentration of the combined alcoholic extracts yielded a brownish syrup which dissolved freely in water, and the solution exhibited considerable frothing on shaking. A solution of the syrup in water was treated with a saturated solution of barium hydroxide; thus a saponin complex was obtained in