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Technical Papers

Structure of Pan's Crystalline Trisaccharide[1]

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Recently Pan, Nicholson, and Kolachov have described the isolation in crystalline form of a nonfermentable reducing trisaccharide (*1*) produced from maltose by the action of an enzyme system from *Aspergillus niger* (*2*). Through the kindness of Dr. Pan and his associates this trisaccharide has been placed at our disposal, and we wish to report preliminary studies pointing to its constitution.

The trisaccharide (I) was partly hydrolyzed by heating 1 ml of a 1% solution together with 0.67 ml of 0.5 *N* H_2SO_4 for 1 hr in a boiling water bath. An excess of $BaCO_3$ was added, the insoluble salts were removed by filtration and washed with 1.33 ml of water (II).

Further, 10 ml of a 1% solution, 0.06 g of iodine, and 0.4 ml of 1 *M* KOH were shaken together and allowed to stand for 30 min. At this point 0.2 ml more 1 *M* KOH was added, and the mixture allowed to stand overnight at room temperature. One ml of this solution was then hydrolyzed with sulfuric acid as described above (III). I, II, and III were then compared on a paper chromatogram (*3*) with the amylose series of saccharides (IV) prepared by partial acid hydrolysis of a low molecular weight amylose, the dextran series of oligosaccharides (V) similarly prepared from dextran, and a crude isomaltose preparation containing homologous, nonfermentable or difficultly fermentable higher saccharides (VI) supplied by Edna Montgomery (*4*). The chromatogram is reproduced in Fig. 1.

From a perusal of this chromatogram, it appears that Pan's trisaccharide has a considerably slower chromatographic mobility than the trisaccharide of the amylose series, yet it is by no means as slow as the trisaccharide of the dextran series. Partial acid hydrolysis produces reducing saccharides which in all probability are isomaltose and maltose, together with glucose. Partial acid hydrolysis of the aldonic acid

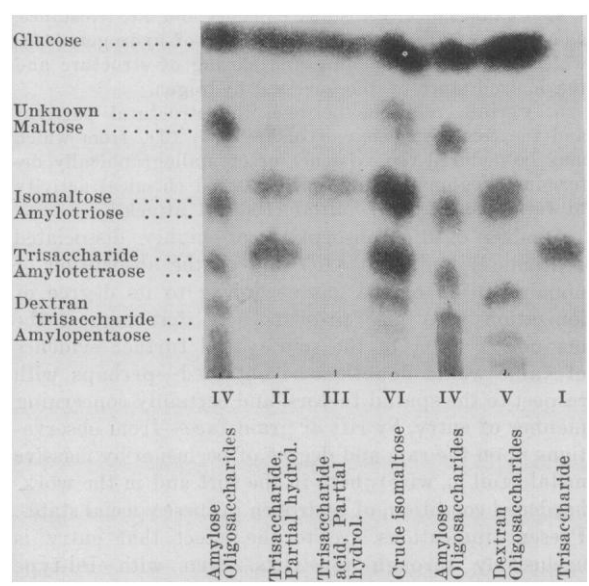

FIG. 1. Triple ascent chromatogram of Pan's trisaccharide, degradation products, and reference saccharides.

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gives isomaltose and glucose, *but no maltose*. These findings mean, first, that the trisaccharide must consist of three glucose residues united by one α-1, 4 bond and by one α-1,6 bond (the high positive rotation, $[\alpha]_D + 154°$, excludes the possibility of β-linkages); second, of the three possible structures for such a trisaccharide, the only one that could give rise to isomaltose on partial hydrolysis of the aldonic acid is that in which a glucosyl unit is joined to the sixth position in the nonreducing glucose residue of maltose; i.e., 4-[6-(α-D-glucopyranosyl)α-D-glucopyranosyl] D-glucose.

The more exact identification of the products of partial acid hydrolysis, together with confirmatory tests for other aspects of the structure, will be reported elsewhere.

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Physiological Mechanisms of Color Blindness

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Retinal processes of color-blind subjects were investigated by Motokawa's method (*1*). The electrical excitability of the eye (the reciprocal of threshold) is determined with an electrical phosphene as an index after preliminary dark adaptation of about 20 min. Stimulating currents, single constant current pulses of 100 msec in duration are applied to the eye through a pair of silver electrodes of 2×1.5 cm² in size, placed one on the forehead slightly above the eyebrow and the other on the homolateral temple of the subject. The electrical excitability of the eye is measured at varying intervals after 2 sec exposure of the eye to a preilluminating patch of 2° in visual angle. The excitability recovers in general very rapidly, becomes supernormal, and then gradually decreases to the initial level. It is, however, sometimes observed that a few humps appear in the recovery curve, especially in those obtained from color-anomalous subjects. The time course depends upon the wavelength of the light used for preillumination and upon the kind of color blindness, but little on the intensity of the preilluminating light; the latter changes only the height of the excitability curve without altering its form. Examples of excitability curves obtained from the fovea of typical dichromats as tested by Ishihara's test, as well as by Nagel's anomaloscope, are shown in Fig. 1, in which percentage increases in electrical excitability above the resting level are plotted as ordinates against time

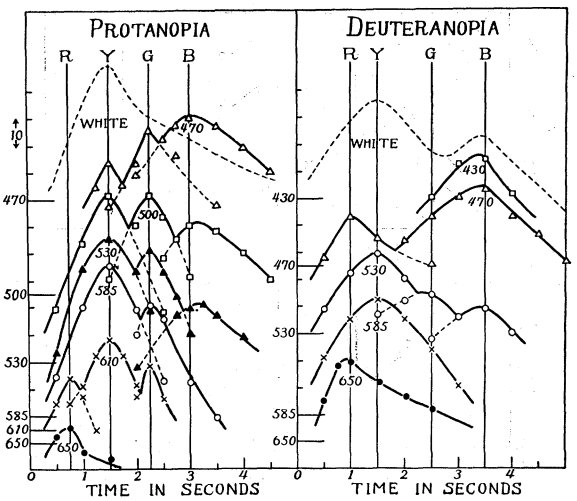

FIG. 1. Excitability curves of dichromats. Ordinates: percentage increases in electrical excitability above the resting level. Abscissae: time after the end of preillumination. Figure by each curve indicates wavelength of light used for preillumination. Zero level for each curve is indicated on the axis of ordinate.

after removal of the preilluminating light as abscissae. As can be seen in the figure, the curves generally show distinct humps, which may be classified into four groups according to their temporal location. It is very probable that these humps represent different retinal processes, but not different phases of one and the same process, because for one and the same abscissa 2 different values of threshold can be obtained, one of which belongs to the one hump, and the other to the neighboring hump, as indicated by broken lines in Fig. 1. In such cases, the subject, at a certain definite voltage, finds it very difficult, or almost impossible, to distinguish the stimulus in question from one far below the threshold, but on further decreasing the voltage, discrimination becomes easier, and thus the second threshold is reached.

The four kinds of processes may be denoted by *R, Y, G,* and *B* in the order of rapidity of processes. In color-blind subjects, any one of these processes is especially weak, e.g., the *R* process is weak in the protanope, the *G* in the deuteranope. It is another characteristic common to the two kinds of color blindness that a dominant *Y* process can be seen in the fovea. Motokawa (*2*) has provided evidence that the *Y* process, an independent process underlying a sensation of yellow, does exist in the periphery of the retina of normal and color-blind subjects, but he has not yet succeeded in proving the same process in the fovea itself of normal human subjects. The relative magnitudes of the processes are naturally different according to the wavelength of the preilluminating light. This relation is most clearly shown in the curves for spectral distribution of the four processes shown in Fig. 2, in which the ordinates represent the magnitudes of each process or the heights of each kind of hump plotted against the wavelengths of the spectral lights used for preillumination as abscissae. Fig. 2