Notes

Modified celite partition chromatography

In studies on the chromatography of metabolism fluids resulting from the action of *Acetobacter acetigenum* on glycerol, in a suitably defined medium, it became necessary to examine these fluids for their carbohydrate content in order to gain an insight into the mode of formation of bacterial cellulose. Application of Ultrasorb charcoal chromatography to these fluids by RAMAMURTI AND JACKSON[1] resulted in the isolation of eight water fractions, designated W_1 through W_8, and six aqueous ethanolic fractions A_1 through A_6. These fractions were further resolved by means of the celite partition chromatographic procedure of LEMIEUX, BISHOP AND PELLETIER[2] with modifications, the details of which are given below.

These experiments were carried out during the summer months when the variation between the day and night temperature was considerable, and it was observed that when celite columns were prepared by the slurry method of NEISH[3] and left running overnight (at the rate of 16 ml/h) a change in the equilibrium conditions of the solvent system (water-saturated *n*-butanol) seemed to occur. The appearance of the packed material at the top of the column underwent a progressive change rather as though it had become wetter. This effect was observed even under carefully controlled temperature conditions and after an overnight run extended some 2 cm down the column. Attempts to resolve one of the aqueous ethanolic fractions A_3 (approximate composition: glucose, fructose and traces of a pentose) on such a column were unsuccessful. When the chromatogram was stopped after 16 h and the column extruded and sprayed, no band was visible on the celite column. However, when the eluant (*ca.* 300 ml) was concentrated and examined by paper chromatography the picture was quite similar to that obtained from fraction A_3. The failure of the method to resolve fraction A_3, as seems probable, may be due to the leaching effect of water that might have separated from the solvent system (water-saturated *n*-butanol) during an overnight run. The following procedure was devised to counter the foregoing difficulty.

Experimental

One litre of re-destilled *n*-butanol was allowed to stand overnight in contact with 500 ml distilled water. A portion of the stationary phase was separated from the organic phase, and used in preparing the celite column, while the organic phase served as the developing medium in the procedure. 70 ml of stationary phase was absorbed on to dry acid washed celite No. 535 (70 g), which was then slurried with the developing phase; the celite column (32 × 2.8 cm) was constructed according to NEISH[3]. The sample to be fractionated, A_3, after deionisation with Zeo-Karb 225 and Deacidite E (10 % solution, wt./vol., in the stationary phase) was absorbed on dry celite No 535 (1 ml/g) and the resulting powder packed to the top of the packed celite column

which was just filled with the developing phase. The column was connected to a reservoir containing water-saturated *n*-butanol and the chromatogram was run for an hour to allow the developing solvent to percolate through the column. Then the column was disconnected from the reservoir and allowed to drain. When most of the solvent had drained off, the delivery end of the column was closed. Dry celite (5 g) was absorbed in 5 ml stationary phase and a thick slurry of this with the developing phase was packed tight on the top of the celite column, giving an extra height of 2.5 cm. The celite column was again connected to the reservoir containing water-saturated *n*-butanol and the chromatogram run for 16 h, the rate of flow of the solvent being 20 ml/h. Owing to the increased length of the column the change in the equilibrium conditions of the solvent system in the top 2 cm of the celite column did not impede the resolution of the sample to be fractionated. At the end of 16 h the celite column was disconnected from the reservoir and allowed to drain for 2 h. The column was extruded by a special technique that was developed during the course of this work.

Extrusion technique

Excess solvent was removed from the celite column by the application of gentle suction. The column was then placed in a slightly larger glass jacket tightly stoppered at one end by a rubber bung and compressed air was carefully blown through the celite column. As the celite was slowly dislodged, it was received into the wider glass jacket, while the tube which had contained the celite was gently withdrawn at the same time. The column of extruded celite material was carefully transferred to a large sheet of clean glass by means of a glass plunger, and then sprayed with alkaline potassium permanganate (1 % aqueous potassium permanganate containing 2 % sodium carbonate) through a mask in the form of a narrow slit. Eight bands of varying colour intensity appeared on the column. The portions of celite material, corresponding to the eight bands were sectioned with a clean knife and eluted with distilled water. Invariably the fourth band from the top of the celite column comprised nearly pure glucose. Enrichment of this component was sought by repeated partition of the deionised fraction A_3 on celite columns using the above procedure.

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