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SEPARATION OF MONOSACCHARIDES BY CHROMATOGRAPHY ON SILICA GEL*

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RESUMEN

Se describe un método de cromatografía en columna de gel de sílice con el cual se logra satisfactoriamente la separación de los monossacáridos: ribosa, glucosa, galactosa y sedoheptulosa, en cantidad suficiente. Los componentes de la mezcla, una vez separados, se pueden identificar por cromatografía en capa fina en la misma mezcla de disolventes para establecer la pureza de las fracciones que abarca cada azúcar aislado.
Al aplicarse esta técnica a hidrolizados ácidos de algunos glicósidos esteroidales, se logró el aislamiento de los azúcares puros. Este método puede ser usado probablemente con éxito en otras mezclas complejas de carbohidratos.

ABSTRACT

The described column chromatographic method using silica gel achieves a very satisfactory separation of the menosacharides: ribose, glucose, galactose and sedoheptulose in sufficient quantities as to permit their conversion to derivatives. The separation can be followed by tlc in the same solvent mixture to ascertain the purity of each isolated monosaccharide.
This method, when applied to acid hydrolysates of some steroidal glycosides, permitted isolation and further derivatization of the pure sugars obtained. This method can probably be applied with success to other complex barbohydrate mixtures.

INTRODUCTION

To determine the composition of complex sugar mixtures is an intricate chemical problem, particularly in vegetable matter. It is often desirable to isolate sufficient of the pure compounds for absolute identification by chemical and physical methods.

Since the discovery of paper chromatography (Consden *et al.*, 1944) which has proved to be an excellent aid for resolution of complex mixtures (Brimley and Barret, 1954) in minute quantities. The application of this technique to the separation of sugars have been so numerous as to make it very difficult to record them. The only drawback of this procedure is the limited amount separated and the time required for a good separation of homologous monosaccharides.

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Recent techniques make use of suitable volatile derivatives such as TMS-sugars (Hedgley and Overend, 1960; Sweeley *et al.*, 1963; Arreguín and Taboada, 1970), methly ethers, butane boronates (Wood and Siddiqui, 1971), etc. which permit separation by glc. Osazones have been chromatographed on paper impregnated with borate (Arreguín, 1959).

Other procedures used include ion exchange chromatography of the barbohydrates as borate (Khym and Zill, 1951; Khym and Zill, 1952; Fujita and Asakura, 1971), or bisulftite (Takasaki, 1972) adducts, but these offer the disadvantage of having to eliminate complexing reagents (Mackie and Perlin, 1965). Still others employ charcoal chromatography (Barker *et al.*, 1956).

Satisfactory results have also been obtained by tlc for qualitative identification, either with silica plates impregnated with borate (Pastuska, 1961), with cellulose (Raadsveld and Klomp, 1971; Jeffery *et al.*, 1969; Scherz *et al.*, 1968), or with Kieselguhr plates impregnated with sodium acetate. Non impregnated silica has been shown to be particularly useful in resolving sugar mixtures.

During a study of the structure and stereochemistry of some glycosides problems were encountered in separating the sugars in their free form in sufficient quantities to establish their identity by derivatization. The use of columns of silica gel offered definite advantages over the known methods.

RESULTS

In some experiments a mixture of ribose, glucose and galactose were eluted from a silica gel column of 1.6 x 125 cm with the following solvent mixture ethylacetate-methanol-waater 80/20/5, v/v. The results obtained after tlc of the fractions are presented in Figure 1. The sugars appeared well separate into three groups: ribose in fractions 56-61, glucose in 74-89 and galactose 92 102. Each group of fractions was pooled and lyophylized, converting each sugar to the corresponding osazone. The time of osazone formation agreed well with that of the pure sugar run simultaneously. Excellent agreement was found for the crytalline forms (Vogel, 1951) and likewise the melting points without further recrystallization were: Ribose F. 158-159, R 160; Glucose F. 203-205, R. 205; Galactose F. 196-198, R. 196-198 (Fisher Johans apparatus).

In other experiments using a model mixture of ribose, glucosa, galactose and sedoheptulose, the results obtained were plotted as a curve (Fig. 2).

Glass chromatography columns of sizes: 125 x 1.6 cms with 100 g silica and 190 x 2.2 cm with 360 g of silica achieved an efficient resolution of 100-200 mg of sugar mixture. The columns were loaded with silical gel (Grace Davison Chemical Grade 922) in the form of a slurry in the solvent mixture: AcOEt/MeOH/H_2O 80:20:5. The free sugars combined in model mixtures were ribose, glucose, galactose and sedoheptulose (10^{-4} moles of each sugar). The mixed sugars were ground thoroughly in a mortar with about 10 g of silica and 20-30 ml of elution mixture. Afterwards they were transferred to a round flash and evaporated to dryness under reduced pressure in a rotatory evaporator.

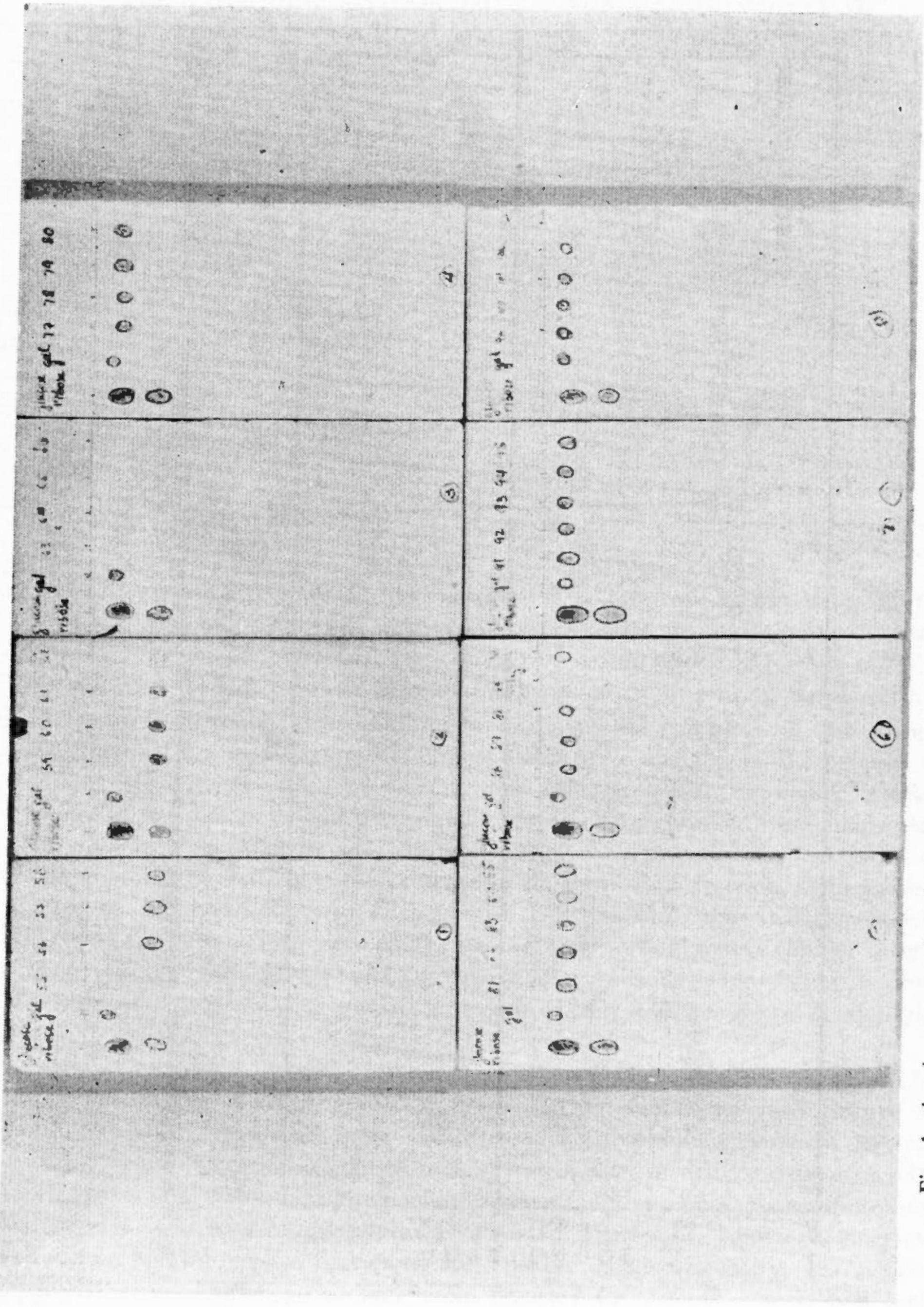

Fig. 1. tlc chromatography of the fractions obtained from the column compared with sugar standards.

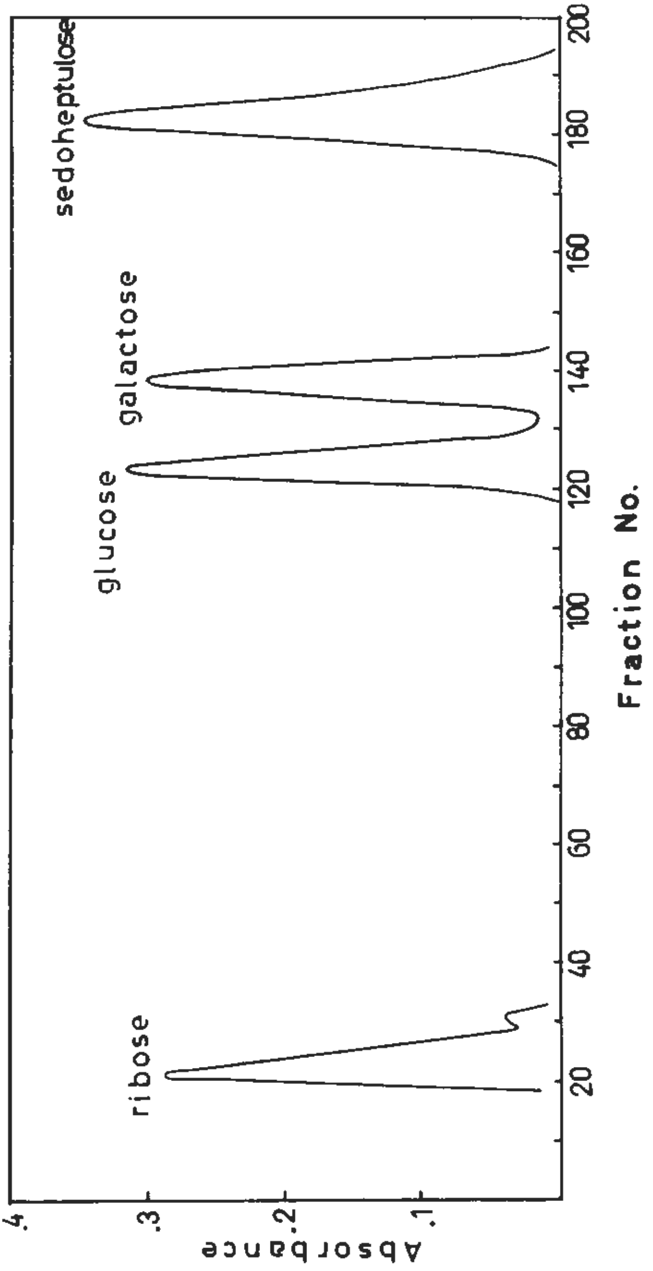

Fig. 2. Separation of sugar after column chromatography. Fraction number and absorbance in the X and Y axis. The recovery of sugars varied between 89-94%.

TABLE 1. GLC CHROMATOGRAPHY OF THE SUGARS MOIETY OF GLYCOSIDES A AND B OF YUCCA FILIFERA AS TMS DERIVATES 150°C

Suggars	R_t	$R_{t\,ribose}$
Standards		
ribose	2.85	1.00
xylose	2.65	0.93
galactose	8.50	3.00
glucose	9.50	3.33
Sugars separtted by column chromatography filiferin a		
xylose	2.65	0.93
galactose	8.80	3.09
Filiferin b		
galactose	8.60	3.02
glucose	9.60	3.37

The dry powder containing the sugars was transferred quantitatively to the top of the column (which had a layer of solvent above it) and allowed to settle uniformly, forming a horizontal layer of about 2 cm thick. A further layer of pure silica was added, followed by a disc of filter paper to prevent disturbance.

The chromatography was initiated immediately by eluting with the solvent mixture contained in a separatory funnel attached to the top of the column by a rubber stopper. Fractions of 10 ml were collected in an automatic fraction collector at the rate of 1.4 ml per minute. The fractions collected were evaporated to dryness in a steam bath and the contents of each tube dissolved in 2 ml of distilled water. To determine the presence of sugars in each fraction, aliquots were applied to thin-layer plates of silica gel (5 x 10 cm, 0.25 mm thickness-Merck, Kieselgel F_{234}). Plates were developed with the same solvent system (one or several times, dryin the thin chromatographic plates after each development wit a draft of warm air), then sprayed with ceric sulfate-H_2SO_4 and heated to develop the colour.

When quantitative experiments were carried out, the sugars were analyzed by Nelson's modified Somogy method (Nelson, 1957), employing pure sugars as standards in the calibration curves, and detected spectrophotometrically at 520 nm.

When this technique was applied to acid hydrolysates of two steroidal glycosides of *Yucca filifera*, their sugar components were cleanly separated by column chromatography and could be quantitatively analyzed. Filiferin a, contained galactose and xylose and filiferin b, galactose and glucose (Lemieux *et al.*, 1977). The separated sugars bad mps matching those reported for the pure sugars and their purity further tested by gas liquid chromatography (glc) at 150°C, after converting them to the corresponding trimethyl silyl derivatives (TMS).

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