BIOSYNTHETIC PREPARATION OF ^{11}C-LABELLED GALACTOSE, GLYCEROL AND MANNITOL

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SUMMARY

^{11}C-labelled galactose, glycerol and mannitol have been prepared biosynthetically from ^{11}C-labelled carbon dioxide using species of marine algae. Following biosynthesis the labelled compounds are rapidly extracted from the algae and purified by liquid chromatography.

INTRODUCTION

Biosynthesis has been used extensively for the preparation of ^{14}C-labelled compounds. Recently this technique has been applied to the preparation of glucose and fructose labelled with the short lived isotope ^{11}C (half life 20.3 min, β^+) for use in in vivo metabolic studies, Swiss chard or broad bean leaves being used for the biosynthesis.[1,2] In 1958 Bidwell described the products of biosynthesis in fourteen species of brown, red and green marine algae using $^{14}CO_2$ and found that the major labelled product was invariably D-mannitol in the brown algae, galactosylglycerol (floridoside) or a glycoside of mannose in the reds, and sucrose in the green algae[3]. Since then these systems have been used for preparing ^{13}C and ^{14}C labelled products[4].

This paper describes the use of marine algae for ^{11}C labelling. The brown alga Fucus evesiculosus was used to prepare ^{11}C-mannitol while the red species Girgartina stellata produced ^{11}C-galactose and ^{11}C-glycerol. Carrier-free

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$^{11}CO_2$ in nitrogen is produced routinely on the Medical Research Council cyclotron by bombardment with 7.5 MeV protons ($^{14}N(p,\alpha)^{11}C$ reaction).

EXPERIMENTAL

Biosynthesis

The alga (1.0 - 2.5g) is placed in a cylindrical silica illumination chamber (25 cm x 2.5 cm diameter) illuminated by a Phillips 125 w "Powerwhite" fluorescent lamp at a distance of 10 cm. Carrier-free $^{11}CO_2$ in nitrogen is then swept directly from the cyclotron target, through the chamber at a flow rate of 50 ml min^{-1} for a period of about 20 min.

Separation of ^{11}C-Galactose and ^{11}C-Glycerol (G. stellata)

The alga is transferred to a 50 ml boiling tube containing 80% aqueous ethanol (10 ml) and the solution is boiled for 10 min on an oil bath at 130°C. The solution is then separated from the alga, cooled, extracted with diethyl ether (20 ml), and the aqueous layer boiled for a further 1-2 min to remove traces of ether. Dilute hydrochloric acid (2N, 1 ml) is then added and the solution is boiled for a further 15 min during which time the temperature of the oil bath is raised to 170°. After this time the solution is neutralised by the addition of 4 N lithium hydroxide and the volume is reduced to 1.0 - 1.5 ml by further boiling if necessary. The procedure up to this time may be carried out in about 40 min, and produces a solution of labelled galactose and glycerol together with other residual organic material.

The desired products are separated by liquid chromatography using a 96 cm x 0.8 cm column of AG50W-X8 (Li^+) 200 - 400 mesh resin eluting with water for injection at a flow rate of 0.75 ml min^{-1}. The sample volume applied to the column is 0.9 ml. The mass concentration in the eluant is monitored by a Waters Associates R 401 Differential Refractometer, and the radioactivity by a scintillation counter. A typical chromatogram is shown in Fig. 1. D-Galactose is eluted as a peak with a maximum at 25 ml and glycerol at 30 ml.

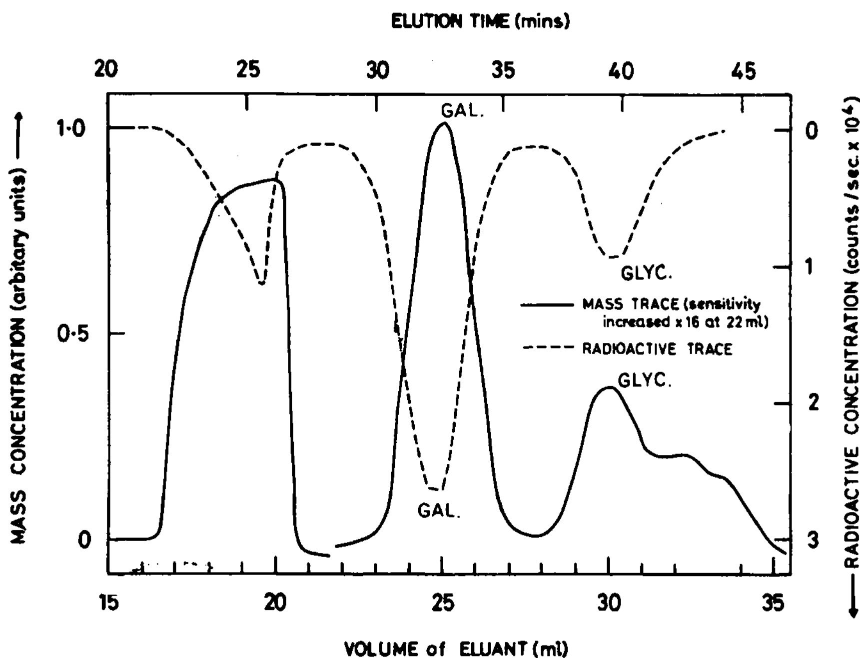

Fig. 1 Preparative liquid chromatogram for the separation of ^{11}C-galactose and ^{11}C-glycerol.

The fractions containing the active products are collected and passed through 0.22 μ filters into sterile vials.

Separation of ^{11}C-Mannitol (F.evesiculosus)

This is carried out by the same procedure as described above ommiting the acid hydrolysis and subsequent neutralisation stages. A typical chromatogram obtained in this case is shown in Fig. 2. D-Mannitol is eluted as a peak with a maximum at 25 ml, using a flow rate of 0.70 ml min^{-1}.

DISCUSSION

The algae were collected the previous day and stored damp in polythene

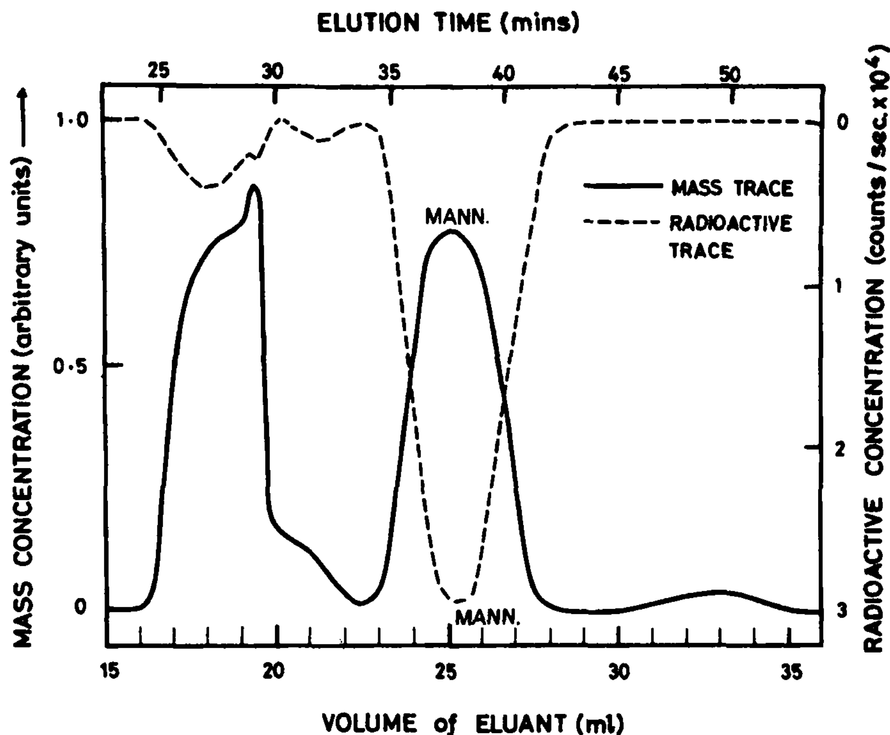

Fig. 2 Preparative liquid chromatogram for the separation of ^{11}C-mannitol.

bags overnight prior to use. No further treatment was necessary prior to biosynthesis and both the G. stellata and F.evesiculosus could be used up to one week after collection if they were kept damp and stored in the dark at a temperature between 5 and 10°C.

The products were identified by comparison with the retention volumes of authentic samples on the AG50W-X8 (Li^+) column, and also by gas chromatography of the silyl ether derivatives, again by comparison with authentic samples. These systems were also used to check that hydrolysis of the galactosylglycerol

produced in the case of G.stellata was complete. The glycoside and galactose could not be resolved on the preparative AG50W-X8 (Li^+) column. However, they were resolved on a 145 cm x 0.8 cm column of this resin in the Na^+ form and chromatograms of the hydrolysed and unhydrolysed solutions are shown in Fig. 3. The glycoside had a retention volume of 36 ml on this column, galactose

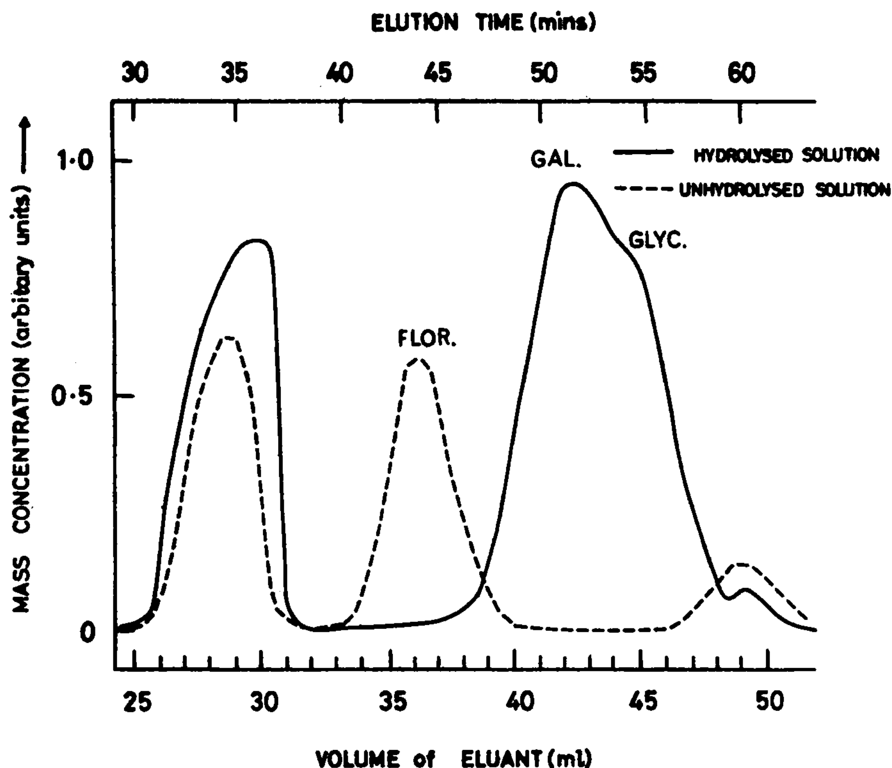

Fig. 3 Analytical liquid chromatograms of the hydrolysed and unhydrolysed extracts from G. stellata.

The radioactive traces are not corrected for decay.

Peaks, GAL galactose; GLYC glycerol; MANN mannitol, FLOR floridoside.

42.5 ml and glycerol 45.5 ml. Interestingly the two latter compounds were not completely resolved using the Na^+ form of the resin.

In all cases small multiple active peaks (10 - 20% of the soluble activity) were eluted from the preparative column with an elution volume near the non-sorbed volume. The chemical form(s) of this activity was not identified. A larger multiple peak due to inactive impurities, and also lithium chloride in the case of work with G.stellata, was also present in the mass trace in this region.

When the red alga Polysiphonia lanosa was used in place of G. stellata and the same procedure followed a very similar chromatogram was obtained. In this case however the major active peak was identified as D-mannose again by gas chromatography of the silyl ether derivatives. This alga appeared to be rather less effective at biosynthesis than the others and it was essential to use it on the day following collection. Colin and Augier[5] and Bouveng et al[6] have identified the glycoside present in Polysiphonia fastigiata as α-D-mannopyranosyl-2-glyceric acid.

Details of several typical preparations are given in Tables 1 and 2. The total times required for the complete procedures are 80 - 90 min. In addition the extractions are not started until about 10 min. after the end of cyclotron bombardment in order to allow time for most of the remaining $^{11}CO_2$ in the target to be flushed into the illumination chamber. The target system used has been described by Clark and Buckingham[7] and is similar to that reported by Finn and Wolf[8] However, the yield of $^{11}CO_2$ has not been measured for the conditions under which the target was run in the present series of experiments. Consequently no accurate estimate of the overall radiochemical yields of the labelled products can be made, since any $^{11}CO_2$ not taken up by the algae was not recovered. In order to reduce radiation dose to the worker the integrated beam current used in the present series of experiments was limited to a maximum of 10 μA hrs. By using longer bombardments at a higher beam current the activities of the final products

TABLE 1. Preparation of ^{11}C-galactose and, ^{11}C-glycerol

Wt. *G. stellata* g.	Activity of galactose μCi	Sp.Activity of galactose μCimg^{-1}	Total wt. of galactose ext. mg.	Activity of glycerol μCi	Sp.activity of glycerol μCi mg^{-1}	Total wt. glycerol ext. mg.	Int. Beam current μAhrs
2.2	135 (100)	25.8 (100)	8.2	72 (100)	26.8 (100)	4.2	10
2.1	110 (105)	14.8 (105)	11.7	64 (105)	18.2 (105)	5.4	10

TABLE 2. Preparation of ^{11}C-mannitol

Wt. *F. evesiculosus* g.	Activity of mannitol μCi	Specific activity of mannitol μCi mg^{-1}	Total wt. of mannitol extr. mg.	Int. beam current μA hrs
1.6	910 (90)	77.1 (90)	21.2	10
1.0	395 (91)	38.7 (91)	15.6	7

The total weights of the extracted products were calculated from the proportions of the solutions loaded onto the chromatograph column and are therefore different from the weights eluted. The activities of the products were measured at the times after EOB shown in parentheses (min), and are not corrected to EOB.

could be increased by at least a factor of three. The specific activity of each product was obtained directly by comparing the area of the peak in the preparative chromatogram with that of a known weight of an authentic sample run under identical conditions.

On a molar basis the specific activity of the galactose obtained from G.stellata was approximately 1.8 times that of glycerol. Hassid and co-workers proved the structure of floridoside obtained from Irideae laminarioides[9] to be α-D-galactopyranosyl-2-glycerol and also suggested a mechanism for its biosynthesis[10]. If this mechanism is correct in the case of G.stellata the galactose obtained in the present experiments would be expected to be labelled at the 3 or 4 positions while the glycerol would be labelled at the 1 position. As far as is known no mechanism has been proposed for the biosynthesis of mannitol, but it is likely that glycerol is a precursor[11].

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