*Phytochemistry*, 1976, Vol. 15, pp 135–136 Pergamon Press. Printed in England.

# THE SUGAR COMPONENTS OF THE GALACTOSYL DIGLYCERIDES FROM GREEN PLANTS

G. R. JAMIESON and ELIZ. H. REID

Chemistry Department, The Paisley College of Technology, High Street, Paisley, Scotland

(*Received* 21 *January* 1975)

**Key Word Index**—Green plants; chemotaxonomy; galactosyl diglycerides; sugars.

**Abstract**—Preparative TLC was used to separate the MGDG and DGDG fractions from 22 green plant species. Methanolysis of the separated fractions gave methyl glycosides which were analysed by GLC as trimethylsilyl derivatives. Galactose was found to be the major component in the fractions of all the species examined and glucose was present as a minor component in 15 of the species. From the results it was possible to calculate the MGDG/DGDG ratio.

## INTRODUCTION

Considerable interest has been shown in the lipid components of green plants and, in particular, in the galactosyl diglycerides, the major lipids of photosynthetic tissue [1–4]. The role of the galactosyl diglycerides within the chloroplast still remains a topic of much controversy [5].

The acyl groups of the galactosyl diglycerides have been extensively studied and those from photosynthesising tissues have been found to be highly unsaturated, e.g. in many angiosperm species 90% of the fatty acids of the monogalactosyl diglyceride from green leaves consists of linolenic and other $\omega 3$ acids [6–9]. Although such information is available for the nature of the acyl components of galactosyl diglycerides, little work has been carried out on the nature of the sugar components. The purpose of the present investigation was to determine the nature of the sugar components of the galactosyl diglycerides and to determine the relative proportions of the mono- and di-galactosyl diglycerides in a range of green plants.

## RESULTS AND DISCUSSION

The complete separation and isolation of the individual lipids from plant material is a difficult and time-consuming task. It is often possible, however, to use less extensive separation procedures if only a particular class of lipid is being investigated [5]. Both column and thin-layer chromatographic procedures have been used to separate and isolate the mono- and di-galactosyl diglycerides [3,10–12]. The method used in the present investigation was by preparative TLC of the total lipid extract using a solvent based on acetone [13,14], and the separated fractions were checked for purity by analytical TLC. The sugar components, obtained after methanolysis of the fractions, were analysed by GLC as their trimethylsilyl derivatives. The results obtained from 22 species of green plants are shown in Table 1. The main sugar in all 22 species studied was galactose, but glucose was found as a minor component in 15 of the galactolipid fractions usually being present in both. A small amount of glucose was found in the MGDG fraction of *Anthriscus sylvestris* but not in the DGDG fraction and conversely in the fractions from *Petasites hybridus*. Sastry and Kates [10] found small amounts of glucose in the galactosyl diglycerides of runner bean leaves and found that the lipids from these leaves contained sterol glucosides and glucose-containing cerebrosides. They assumed that the presence of glucose in the galactolipids was due to contamination caused by tailing of the glucose-containing lipids during their chromatographic separation. In the present work, however, all the fractions from the preparative TLC separation were checked for purity and, in all the species studied, the MGDG fraction contained no other lipid. It was found, however, that the DGDG fraction from certain species did contain an additional unidentified glycolipid, and in order to obtain a pure DGDG fraction, a second preparative TLC separation was carried out with a different solvent system before sugar determination.

In the present investigation the MGDG and DGDG fractions were obtained from one aliquot of the total lipid extract after separation on a single TLC plate. Since equal amounts of mannitol as the internal standard [15] were added to each separated fraction, it was possible to calculate from the GLC analysis the relative amounts of MGDG and DGDG (Table 1).

The relative proportions of MGDG and DGDG vary from 0·74 to 3·21 with a mean value of 1·76. This value compares with 1·50 calculated from the results of Roughan and Batt [3] who used a non-specific colorimetric method to determine the sugar components of the galactolipids from 20 green plants. Since it has been shown that there is a diurnal and seasonal variation in the MGDG/DGDG ratio for *Pteridium aquilinum* with a maximum value for the ratio in the middle of the growing season, the ratios shown in Table 1 were calculated

*Abbreviations used*: MGDG—monogalactosyl diglycerides; DGDG—digalactosyl diglycerides; gal— galactose; glu—glucose.

Table 1. Relative proportions of sugars found in the MGDG and DGDG fractions from green plants and ratio of fractions

| Species | MGDG % gal | MGDG % glu | DGDG % gal | DGDG % glu | MGDG/DGDG |
|---|---|---|---|---|---|
| *Angiosperms* | | | | | |
| *Anthriscus sylvestris* | 97·7 | 2·3 | 100·0 | — | 1·72 |
| *Caltha palustris* | 100·0 | | 100·0 | — | 3·03 |
| *Cheiranthus cheiri* | 99·0 | 1·0 | 98·1 | 1·9 | 2·44 |
| *Chrysosplenium oppositifolium* | 96·3 | 3·7 | 99·1 | 0·9 | 1·18 |
| *Montia perfoliata* | 100·0 | — | 100·0 | — | 1·79 |
| *Narcissus pseudonarcissus* | 100·0 | — | 100·0 | — | 1·59 |
| *Petasites hybridus* | 100·0 | — | 99·1 | 0·9 | 1·67 |
| *Rhododendron ponticum* | 99·0 | 1·0 | 97·4 | 2·6 | 0·96 |
| *Ribes sanguineum* | 98·6 | 1·4 | 95·1 | 4·9 | 2·80 |
| *Rubus ulmifolius* | 99·6 | 0·4 | 99·4 | 0·6 | 2·22 |
| *Rumex obtusifolium* | 98·4 | 1·6 | 99·0 | 1·0 | 1·96 |
| *Sambucus nigra* | 93·3 | 6·7 | 98·4 | 1·6 | 1·56 |
| *Symphytum officinale* | 98·7 | 1·3 | 99·1 | 0·9 | 1·45 |
| *Tulipa gesneriana* | 99·1 | 0·9 | 97·7 | 2·3 | 1·30 |
| *Gymnosperms* | | | | | |
| *Larix decidua* | 99·6 | 0·4 | 98·6 | 1·4 | 3·21 |
| *Picea abies* | 93·3 | 6·7 | 87·1 | 12·9 | 1·96 |
| *Ferns etc* | | | | | |
| *Dryopteris filix-mas* | 100·0 | — | 100·0 | — | 2·76 |
| *Equisetum arvense* | 97·4 | 2·6 | 98·1 | 1·9 | 2·85 |
| *Pteridium aquilinum* | 98·2 | 1·8 | 97·5 | 2·5 | 2·17 |
| *Algae* | | | | | |
| *Ascophyllum nodosum* | 100·0 | — | 100·0 | — | 0·74 |
| *Cladophora rupestris* | 100·0 | — | 100·0 | — | 1·69 |
| *Cryptopleura ramosum* | 100·0 | — | 100·0 | — | 2·78 |

from lipids extracted from plants which had been harvested in the middle of their growing season and thus probably represent maximum values.

## EXPERIMENTAL

Leaves of angiosperms, conifers, ferns and horsetail were collected in the surrounding districts of Paisley and marine algae from the shores of the Firth of Clyde at Seamill, Ayrshire. The lipids were separated into classes using preparative TLC on 0·75 mm layers of silica (without binder) with acetone as the solvent in paper-lined tanks. The separated components were monitored for purity on 0·25 mm layers of silica (without binder) using two different solvent systems in paper-lined tanks: (a) $CHCl_3$–MeOH–HOAc–$H_2O$, (85:15:10:3).

(b) development to 6 cm with hexane–$Et_2O$–MeOH, 90:30:5, followed by development to 15 cm with $CHCl_3$–MeOH–$NH_3$ (0·880)–$H_2O$, (70:30:5:2·5) [5]. Sugar components of the MGDG and DGDG fractions were analysed by GLC of the trimethylsilyl-derivatives of the methanolysis products with mannitol as internal standard [5,15]. GLC analysis was carried out on a PE 800 chromatograph using SE-30 and XE-60 SCOT columns 50ft. × 0·02 in stainless steel.

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