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# 3-DEOXY-D-*lyxo*-2-HEPTULOSARIC ACID, A COMPONENT OF THE PLANT CELL-WALL POLYSACCHARIDE RHAMNOGALACTURONAN-II*†‡

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## ABSTRACT

3-Deoxy-D-*lyxo*-heptulosaric acid (DHA) has been identified as a component of plant cell walls. This sugar was found in the pectic polysaccharide rhamnogalacturonan-II as a component of the disaccharide β-L-Ara*f*-(1→5)-D-DHA*p*. The structural formula and linkage position of DHA were determined by mass spectrometry of several derivatives. The *lyxo* configuration was determined by $^1$H-n.m.r. spectroscopy of a derivative of the isolated disaccharide. The D configuration was determined by partial degradation of DHA to L-glyceric acid. DHA was found to be present in the primary cell walls of many higher plants, including several dicots, two monocots, and a gymnosperm, but was not detected in two bacterial lipopolysaccharides.

## INTRODUCTION

Rhamnogalacturonan-II (RG-II) is a small, complex, pectic polysaccharide released from sycamore cell walls[2] by treatment with endo-1,4-α-polygalacturonase[3]. RG-II has also been isolated from the cell walls of Douglas fir[4] and rice[5], from sycamore extracellular polysaccharides[6], and from Pectinol AC[7,8].

Pectinol AC is a commercially available preparation of enzymes obtained from the culture filtrate of the fungus *Aspergillus niger*, grown on various plant tissues. The RG-II present in Pectinol AC is undoubtedly a fungal-enzyme-resistant residue of the plants used as a carbon source. RG-II isolated from Pectinol and RG-II isolated from sycamore cell walls have similar glycosyl and glycosyl-linkage

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compositions and similar elution volumes on a Bio-Gel P-10 gel-permeation column[8]. Thus, Pectinol RG-II (PRG-II) has served as an easily isolable model for structural studies of RG-II.

RG-II contains a variety of unusual sugar residues, including 2-*O*-methylfucose, 2-*O*-methylxylose, apiose, 3-*C*-carboxy-5-deoxy-L-xylose (aceric acid)[7], and 3-deoxy-D-*manno*-2-octulosonic acid (KDO)[8].

KDO (detected by the thiobarbituric acid assay[8,9]) was released from Pectinol and sycamore RG-II in the form of the disaccharide α-L-Rha*p*-(1→5)-D-KDO[8] by treatment with dilute acid. However, only ~50% of the thiobarbituric-acid-positive material was released from RG-II by this treatment. We now report on a second thiobarbituric-acid-positive sugar in RG-II, namely, 3-deoxy-D-*lyxo*-2-heptulosaric acid (DHA), and show that, in Pectinol and sycamore RG-II, DHA can be isolated as the disaccharide β-L-Ara*f*-(1→5)-D-DHA*p*.

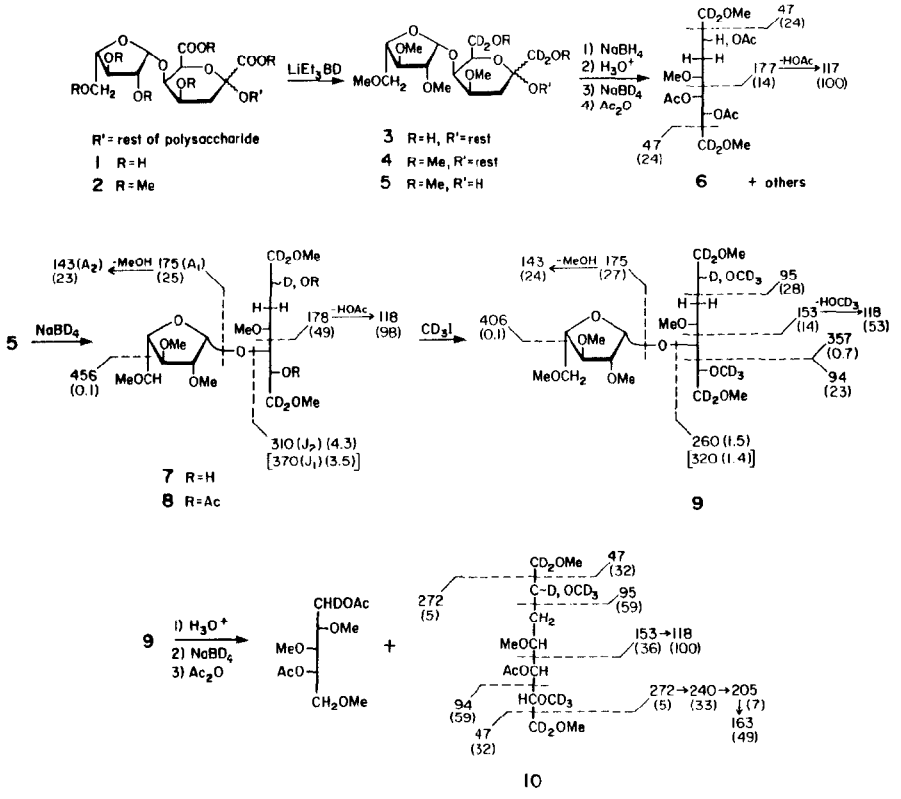
## RESULTS AND DISCUSSION

*Methylation of PRG-II.* — Pectinol RG-II (PRG-II, **1**) was methylated (→**2**, Scheme 1) by a modified Hakomori procedure[10]. Attempted methylation of dry PRG-II in dimethyl sulfoxide resulted in almost no methylation. However, if PRG-II was first dissolved in a minimal amount of water, followed by the addition of dimethyl sulfoxide, methylation proceeded in a satisfactory manner. Glycosyl-linkage analysis still showed some undermethylation of apiosyl and galactosyl residues (data not shown) as indicated by the presence of per-*O*-acetylapiitol and 1,2,4,5,6-penta-*O*-acetyl-3-*O*-methylgalactitol.

The methyl-esterified carboxyl groups of methylated PRG-II were reduced with $LiEt_3BD$, yielding **3**, which was re-methylated to give **4**, thus converting carboxyl groups in the native polysaccharide into methoxydideuteriomethyl groups. Glycosyl-linkage analysis of **4** showed only very minor amounts of the undermethylated products.

*Identification of DHA as a component of PRG-II.* — KDO and related sugar residues are degraded under the conditions normally used for acid-catalyzed hydrolysis of glycosidic linkages. For this reason, the per-*O*-methylated PRG-II (**4**) was first hydrolyzed under mild acidic conditions sufficient to cleave the ketosidic linkages of derivatized KDO and KDO-like residues (0.1M $CF_3CO_2H$, 0.5 h, 60°), but not the glycosidic linkages of furanosyl residues. These conditions did not significantly degrade the KDO-derived residue of **4**. After acid hydrolysis, the methylated carbohydrate was extracted into $CH_2Cl_2$. The carbonyl groups of a portion of the $CH_2Cl_2$ extract were reduced with $NaBH_4$, thereby protecting the KDO and KDO-like residues from acid-catalyzed degradation. The remaining glycosidic linkages in the $NaBH_4$-reduced portion were then fully hydrolyzed, and the resulting monosaccharides reduced to alditols with $NaBD_4$, which were then acetylated.

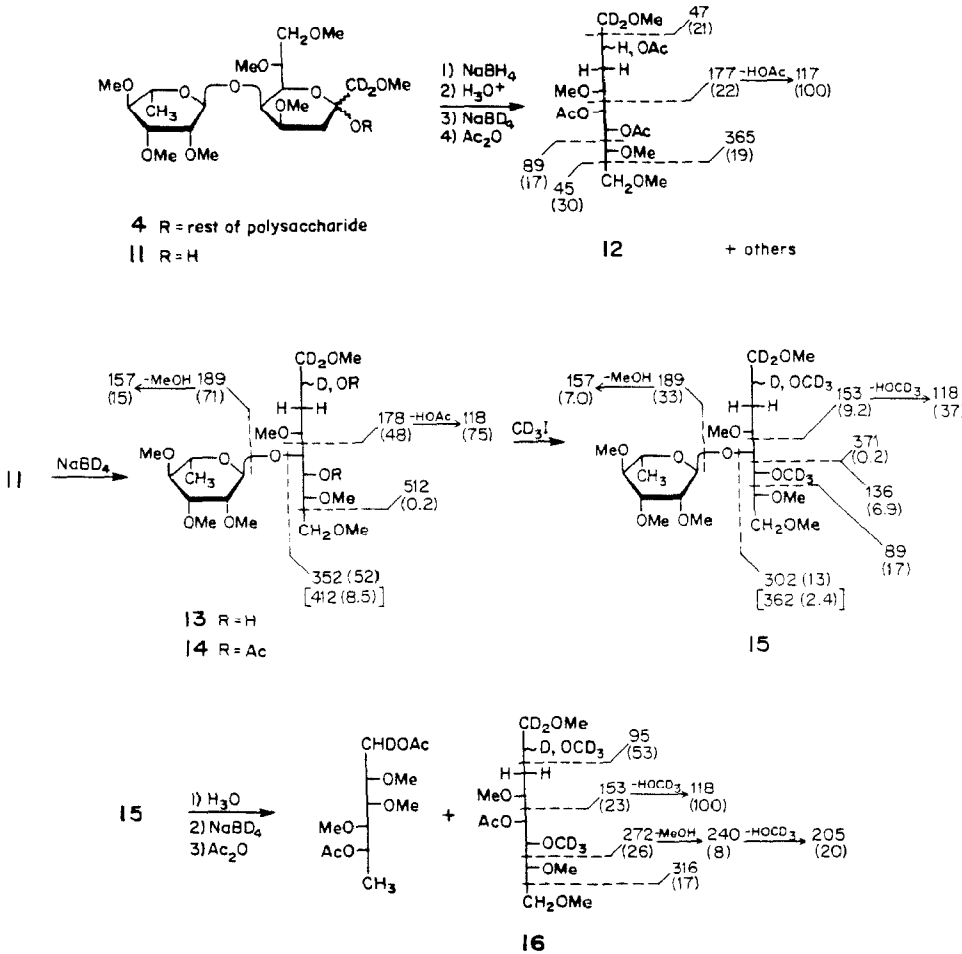
The above procedure allowed the acid-labile KDO and any glycosyl residue



Scheme 1. Formation of DHA derivatives, starting from PRG-II (**1**). Compounds **1–4** are polysaccharides and only a small portion is depicted here, with the rest of the polysaccharide indicated by R'. "Others" indicates that **6** comprises two components in a complex mixture. E.i.-mass-spectral fragmentations of **6, 8, 9**, and **10** are shown with relative abundances in parentheses.

with similar properties to be characterized as volatile products. Analysis of the mixture of carboxyl-reduced, partially methylated alditol acetates by g.l.c. and g.l.c.–m.s. gave two peaks with the e.i.- and c.i.-m.s. fragmentation patterns expected of the two diastereomers of the KDO-derivative **12** (Scheme 2). Two earlier-eluting components gave (Scheme 1) the e.i.-m.s. fragment ions *m/z* at 177 and 117 (177 − HOAc) that were indicative of the substitution pattern exhibited by the heptitol derivative **6**. C.i.-m.s. analysis of these earlier-eluting components established their molecular weights as 368, suggesting derivatives of a deoxydicarboxyheptulose. When the first reduction (Scheme 1) was performed with $LiEt_3BH$, rather than $LiEt_3BD$, the molecular weight of **6** decreased by 4, confirming that two carboxyl groups had been reduced to hydroxydideuteriomethyl groups (Scheme 1). Thus, it was established that the earlier-eluting components were derived from a 3-deoxy-2-heptulosaric acid (DHA).

*Isolation of DHA- and KDO-containing disaccharides* **5** *and* **11**. — Another




Scheme 2. Formation of KDO derivatives (see Scheme 1); different portions of **4** are depicted here and in Scheme 1. E.i.-mass-spectral fragmentations of **12, 14, 15,** and **16** are shown with relative abundances in parentheses.

portion of the $CH_2Cl_2$ extract (Scheme 1) was deuterioreduced, acetylated, and analyzed by g.l.c.–m.s., revealing two volatile products. These gave mass spectra (Schemes 1 and 2) consistent with monoglycosylalditols **8**, derived from pentosyl→DHA, and **14**, derived from the previously[8] characterized rhamnosyl→KDO. The masses of the "A" fragment ions[11] established that the non-reducing terminal residue of each monoglycosylalditol was fully methylated, and the masses of the "J" fragment ions[11] established that each alditol had only two acetyl groups. If either pentosyl→DHA or rhamnosyl→KDO were part of the backbone or the internal portion of a side chain, at least one more acetyl group would have been present in **8** or **14**. Thus, these spectra established that, in PRG-II, both disaccharides exist as disaccharide side-chains or as the terminal portion of larger side-chains.

The remainder of the $CH_2Cl_2$ extract was then fractionated by chromatography on a C-18 cartridge, using aqueous 20% $CH_3CN$, followed by 50% and 100% $CH_3CN$. Colorimetric analysis[12] of the eluants indicated that most of the hexosyl-containing material was eluted in the 50% $CH_3CN$ fraction, while reduction, acetylation, and g.l.c.–m.s. analysis showed that most of the disaccharides were eluted in the 20% $CH_3CN$. The 20% $CH_3CN$ eluate was further fractionated on a C-18 reverse-phase h.p.l.c. column, yielding purified disaccharide **5** (Scheme 1) and **11** (Scheme 2).

DHA-containing disaccharide **5** (Scheme 1) was characterized to determine (*a*) the substitution and relative configuration of the DHA residue and (*b*) the identity, absolute configuration, and anomeric configuration of the pentosyl residue. Disaccharide **11** (Scheme 2) was characterized to aid in the interpretation of the spectra obtained from **5** and its derivatives. Disaccharide **11** was derived from the glycosyl sequence α-L-Rha-(1→5)-D-KDO, which had been characterized previously[8] as the underivatized disaccharide, released from native RG-II by mild hydrolysis with acid.

*The substitution and ring-form of DHA.* — Reduction of **11** to **13** (Scheme 2) and glycosyl-linkage analysis yielded 1,5-di-*O*-acetyl-1-deuterio-2,3,4-tri-*O*-methylrhamnitol (derived from terminal rhamnose) and the two 2-deuterio-labeled diastereomers of **12** in the molar ratio 1:0.8. Similarly, reduction of **5** to **7** (Scheme 1) and glycosyl-linkage analysis yielded 1,4-di-*O*-acetyl-1-deuterio-2,3,5-tri-*O*-methylarabinitol and the two 2-deuterio-labeled diastereomers of **6** in the molar ratio 1:0.7. These results established that the DHA-derived residue of **5** was substituted with a terminal, non-reducing arabinofuranosyl residue.

Portions of **5** and **11** were deuterioreduced and trideuteriomethylated, giving monoglycosylalditols **9** and **15** (Schemes 1 and 2). The e.i.-mass spectra of **9** (Scheme 1) and **15** (Scheme 2) are consistent with the structures shown. The alditol fragment ions at *m/z* 357 and 94 (Scheme 1) suggested that the DHA alditol of **9** was derived from a 5-linked pyranosyl residue. Analogous fragment ions were found in the spectrum of **15**.

Glycosyl-linkage analysis of **15** (Scheme 2) gave the expected derivatives from terminal rhamnose and 5-linked KDO in the pyranose ring form (**16**) in the molar ratio of 1:1. Glycosyl-linkage analysis of **9** (Scheme 1) gave derivatives from the terminal arabinofuranose and 5-linked DHA in the pyranose ring form (**10**) in the molar ratio 1:0.8. The e.i.-m.s. fragmentation pattern of the DHA-derived alditol **10** (Scheme 1) was consistent with 5-linked DHA in the pyranose ring form. The strong fragment ion at *m/z* 94 corresponded to the mass of the C-6–C-7 fragment. Had DHA existed in the 6-linked furanose ring form, then the substituents on C-5 and C-6 would have been reversed and a strong ion at *m/z* 166 corresponding to the mass of the C-5–C-7 fragment would have been expected. This ion was present in very minor abundance in the spectrum. Thus, in PRG-II, DHA existed as the disaccharide Ara*f*-(1→5)-DHA*p*.

*The relative configuration of DHA.* — Preliminary $^1$H-n.m.r. spectra of **5** and

TABLE I

$^1$H-N.M.R. CHEMICAL SHIFTS[a] FOR **5** AND **11** IN BENZENE

| *Chemical shifts (p.p.m.)*[b] | | | | | |
|---|---|---|---|---|---|
| *Proton* | **5** | **11** | *Proton* | **5** | **11** |
| OH-2[c] | 3.22[d] | 3.134 | H-4′ | 4.282 | 3.548 |
| H-3*a* | 2.048 | 1.87 | H-5a′ | 3.867 | 4.100 |
| H-3*e* | 1.890 | 1.83 | H-5b′ | 3.733 | — |
| H-4 | 3.763 | 3.681 | H-6′ | — | 1.456 |
| H-5 | 4.091 | 4.462 | Me singlets | 3.303 | 3.556 |
| H-6 | 4.184 | 4.163 | | 3.289 | 3.513 |
| H-7 | — | 3.825 | | 3.240 | 3.397 |
| H-8a | — | 3.743 | | 3.225 | 3.335 |
| H-8b | — | 3.610 | | 3.115 | 3.202 |
| H-1′ | 5.223 | 5.480 | | 3.036 | 3.073 |
| H-2′ | 3.783 | 3.926 | | | 2.956 |
| H-3′ | 3.999 | 3.825 | | | |

[a]500 MHz. [b]Relative to benzene-$d_5$ at $\delta$ 7.15. [c]See Fig. 1 for numbering. [d]Obscured by other signals, assigned from COSY-90 experiment.

**11** were recorded for solutions in both acetone-$d_6$ and benzene-$d_6$. Benzene was chosen for more detailed studies because the signals of the samples appeared to be better resolved and those from incompletely deuterated solvent and residual water were removed from the region of interest. The proton resonances (Table I) were assigned with the aid of two-dimensional, COSY spectra.

The $^1$H-n.m.r. spectrum of **5** was consistent with the *lyxo* configuration of DHA (Fig. 1, Tables I and II). The two geminally-coupled upfield multiplets at $\delta$ 2.048 and 1.890 were clearly identified as the signals for H-3*a* and H-3*e*, respectively, of the DHA-derived residue of **5**. The signal for H-3*a* showed two large coupling constants (12.1 Hz), one geminal ($J_{3a,3e}$) and the other consistent only with a *trans*-diaxial configuration ($J_{3a,4}$), establishing that H-4 was axial and MeO-4 equatorial. A small four-bond coupling between the anomeric hydroxyl group and H-3*a* (2.0 Hz) was observed, indicating that the DHA-derived residue was in the $\alpha$-anomeric configuration; only an $\alpha$ hydroxyl group could obtain the required planar "W" configuration[13].

The chair form of the DHA-derived residue is determined by the stereochemistry of C-6[14], with the bulky substituent on C-6 in the equatorial position. Thus, H-6 is axial. The small coupling between H-5 and H-6 is consistent only with an axial–equatorial arrangement. Furthermore, the small "W" coupling (0.9 Hz) observed between H-3*e* and H-5 confirmed H-5 to be equatorial. The expected small coupling between H-4 and H-5 was found to be 2.7 Hz. Thus, H-5 was equatorial and the substituent at C-5 axial.

The $^1$H-n.m.r. spectrum of **11** was fully consistent with the known *manno* configuration of KDO. The upfield signal for H-3*a* of **11** showed large geminal and

TABLE II

PROTON COUPLING CONSTANTS FOR 5 AND **11**[a]

*Coupling constants (Hz)*

| *Interaction* | **5** | **11** | *Interaction* | **5** | **11** |
|---|---|---|---|---|---|
| $J_{OH\text{-}2,3a}$ | 2.0 | 1.9 | $J_{7,8b}$ | — | 4.6 |
| $J_{3a,3e}$ | 12,1 | ~12.0 | $J_{8a,8b}$ | — | 10.7 |
| $J_{3a,4}$ | 12.1 | ~11.5 | $J_{1',2'}$ | 4.5 | 2.0 |
| $J_{3e,4}$ | ~4.5 | 5.4 | $J_{2',3'}$ | 7.1 | 3.0 |
| $J_{3e,5}$ | 0.9 | small | $J_{3',4'}$ | 5.4 | 9.5 |
| $J_{4,5}$ | 2.7 | 2.3 | $J_{4',5a'}$ | 6.3 | 9.4 |
| $J_{5,6}$ | small | 0.9 | $J_{4',5b'}$ | 6.5 | — |
| $J_{6,7}$ | — | 9.2 | $J_{5a',5b'}$ | 9.8 | — |
| $J_{7,8a}$ | — | 1.9 | $J_{5a',6'}$ | — | 6.2 |

[a]See footnotes, Table I.

vicinal couplings (~12 Hz) in agreement with the axial configuration of H-4. The small "W" coupling ($J_{OH,3a}$ 1.9 Hz) showed that **11** also adopted the $\alpha$ configuration. The small coupling between the axial H-6 and H-5 established that H-5 was equatorial; this orientation was further substantiated by the small "W" coupling between H-3*e* and H-5.

The ring configuration and conformation of the DHA-derived residue of **5**, deduced from the $^1$H-n.m.r. coupling constants, were the same as those of the KDO-derived residue of **11**. The ring proton coupling constants of the ketodeoxy

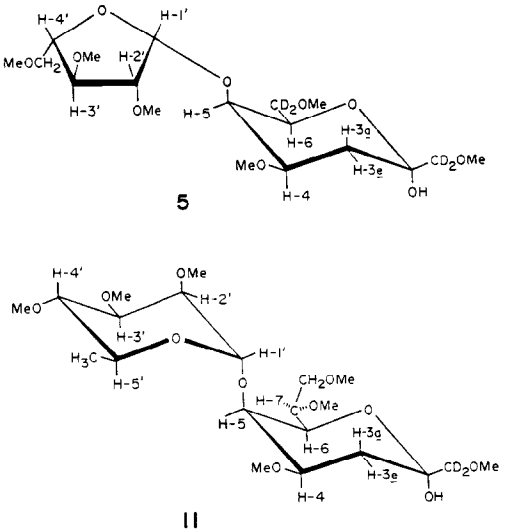



Fig. 1. Atomic numbering system for **5** and **11**.

residues of **5** and **11** were very similar (Table II). Thus, the relative configuration of DHA was determined to be *lyxo*.

*The anomeric and absolute configurations of the arabinofuranosyl residue of* **5**. — It is difficult to determine the anomeric configurations of furanosides by $^1$H-n.m.r. spectroscopy[15,16]. Studies of methyl glycofuranosides in $D_2O$ have shown[17] that the chemical shift of H-1 is not a good indicator of the anomeric configuration and that while the $J_{1,2}$ values of the $\alpha$ and $\beta$ anomers differ, the difference can be small. The chemical shift of C-1 is considered to be a reasonably good indicator of the anomeric configuration[15], but it can be misleading. For example, the anomeric resonances of an $\alpha$-arabinan were found at $\delta$ 109–110, values similar to that of methyl $\alpha$-arabinofuranoside ($\delta$ 111.0) and dissimilar to that of methyl $\beta$-arabinofuranoside ($\delta$ 104.8)[15]. However, the resonances of the anomeric carbons of the methylated polysaccharide were shifted to $\delta$ 105.1–107.1 (in $CDCl_3$), values that could erroneously suggest the $\beta$ configuration, if compared to unmethylated standards. For this reason, the anomeric configuration of the arabinofuranosyl residue of **5** was determined by n.m.r. spectroscopy, using methyl 2,3,5-tri-*O*-methyl-$\alpha$- and -$\beta$-L-arabinofuranosides as standards (Table III).

The chemical shifts for H-1′ of the $\alpha$ and $\beta$ standards were very similar. In addition, there was a strong solvent effect on the chemical shifts. However, the $J_{1,2}$ coupling constants of the two standards differed significantly, as did the chemical shifts of C-1′. The C-1′ resonance of **5** at $\delta$ 102.6 agrees well with that of the $\beta$ standard ($\delta$ 101.9) and differs from that of the $\alpha$ standard ($\delta$ 107.2). In addition, the $J_{1,2}$ coupling constant of **5** (4–4.5 Hz) agrees with that of the $\beta$ standard and not that of the $\alpha$ standard. Thus, the anomeric configuration of the arabinofuranosyl residue of **5** is $\beta$.

The absolute configuration of the arabinofuranosyl residue of **5** was found to be L by a modification of the procedure of Gerwig *et al.*[18]. Diastereomers of the arabinofuranosyl residue of **5** were produced by solvolysis with (*R*)- and (*S*)-2-butanol, and the retention times in g.l.c. of the diastereomers agreed with those of butanolyzed methyl 2,3,5-tri-*O*-methyl-$\alpha$-L-arabinofuranoside.

*Development of a g.l.c. assay for DHA*. — DHA gives a positive response in the thiobarbituric acid assay[8,9]. However, the presence of DHA in plant material

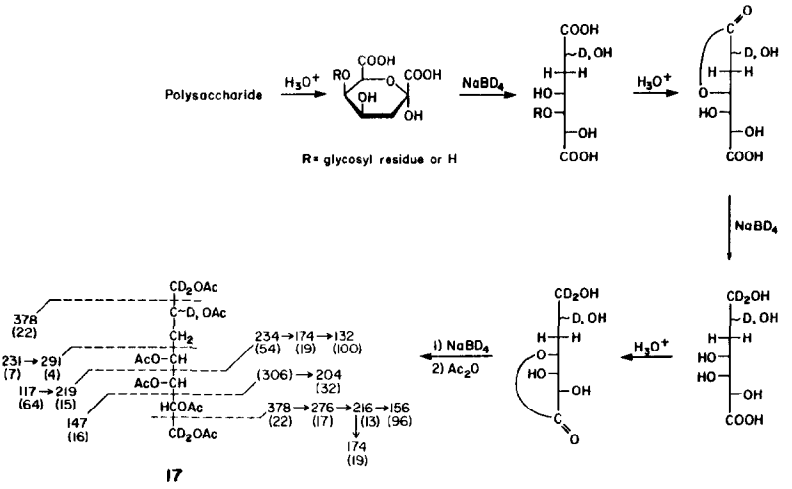
TABLE III

SELECTED N.M.R. DATA FOR **5** AND FOR METHYL 2,3,4-TRI-*O*-METHYL-$\alpha$- AND -$\beta$-L-ARABINOFURANOSIDES

| | *Chemical shift (p.p.m.)* | | |
|---|---|---|---|
| | $\alpha$ | $\beta$ | **5** |
| H-1′ (acetone) | 4.80 (1.1)[a] | 4.88 (4.2) | 5.09 (4.0) |
| H-1′ (benzene) | 4.93 (1.1) | 4.67 (4.3) | 5.22 (4.5) |
| C-1′ (benzene) | 107.2 | 101.9 | 102.6 |

[a]Values in parentheses are coupling constants ($J_{1,2}$) in Hz.

could not be assumed from a positive thiobarbituric acid response, since KDO, which is present in plant walls[8], also gives a positive response. Therefore, a g.l.c. assay was developed that is specific for DHA, but that can also detect KDO. The procedure is a modification of that developed for KDO by York *et al.*[8]. The sample to be analyzed was partially hydrolyzed under mild conditions (0.1M $CF_3CO_2H$, 100°, 1 h) and the ketoses thus formed were converted into alditols by reduction with alkaline $NaBD_4$ (Scheme 3). The sample was then fully hydrolyzed, cleaving all remaining glycosyl linkages and converting the reduced DHA into a lactone. The lactone was reduced with $NaBD_4$ under neutral conditions. The sample was then acidified a third time, the DHA derivative now forming a second lactone that was reduced with $NaBD_4$ under neutral conditions, providing two diastereomers resulting from reduction of the carbonyl group of 3-deoxy-1,1,2,7,7-pentadeuterioheptitol. Acetylation gave volatile derivatives **17**, which were analyzed by g.l.c. and g.l.c.–m.s. The e.i.-mass spectrum of **17** is shown in Scheme 3. The two diastereomers of **17** were not separated by the chromatographic conditions used, but were readily separated from the alditol acetates of KDO and other sugars.




Scheme 3. Synthesis of the two diastereomers of per-*O*-acetyl-3-deoxy-1,1,2,7,7-pentadeuterioheptitol (**17**) from a DHA residue. The ring forms and the order in which the lactones were formed and reduced are hypothetical and do not influence the products formed.

*Isolation of DHA from PRG-II.* — G.l.c. analysis of the products of hydrolysis of PRG-II by 0.1M $CF_3CO_2H$ for 1 h at various temperatures showed that the glycosidic linkage of DHA was more acid stable than that of KDO (M HOAc, 6 h, 40°; conditions used in ref. 8) and that hydrolysis for 1 h at 100° gave optimal yields of cleavage of the DHA glycosidic linkage (data not shown). Cleavage of the residues attached to KDO and DHA could not be measured by the g.l.c. analysis.

To isolate native DHA, PRG-II was hydrolyzed (0.1M $CF_3CO_2H$, 1 h, 100°)

and the products were fractionated on a Bio-Gel P-2 gel-permeation column (Fig. 2). G.l.c. analysis showed that fractions 52 and 58 contained KDO and no DHA (probably containing Rhamnosyl→KDO and KDO, respectively), while fraction 62 contained both. Fractions 60–64 were combined and eluted from a QAE-Sephadex anion-exchange column with a linear gradient of $NH_4OAc$ (Fig. 3). Two thiobarbituric-acid-positive peaks were shown by g.l.c. and g.l.c.–m.s. analyses to contain KDO (earlier eluting) and DHA (later eluting). The $^1$H-n.m.r. spectrum of the DHA-containing peak was complex, having at least three separate spin systems, probably from the $\alpha$- and $\beta$-furanose (major systems) and the $\alpha$-pyranose form (minor system) of DHA. The lack of any resonances from anomeric protons showed that the arabinofuranosyl residues had been cleaved and that the DHA had been isolated as a monosaccharide.

*The absolute configuration of DHA.* — This was determined by degrading DHA with sodium periodate to glyceric acid, the absolute configuration of which was ascertained by converting it into (*R*)-2-butyl 2,3-di-*O*-acetylglycerate and analyzing by g.l.c.

DHA was converted into diastereomeric heptitols by reduction with $NaBD_4$ (Scheme 4). The heptitols were dissolved in saturated aqueous $Ca(OH)_2$ to avoid the formation of lactones. Earlier experiments indicated that the use of $Ca(OH)_2$ instead of NaOH gave greatly increased yields of glyceric acid. The concentration of reduced DHA was determined by periodate oxidation of a small aliquot; the amount of periodate consumption was determined spectrophotometrically[21]. Reduced DHA was then oxidized with a limited amount of sodium periodate (1 mol. equiv.), providing a mixture of 2-, 3-, 4-, and 5-carbon fragments as well as residual, 7-carbon, reduced DHA. The aldehyde groups were then reduced, the acid groups esterified with (*R*)-2-butanol, and the alcohol groups acetylated.

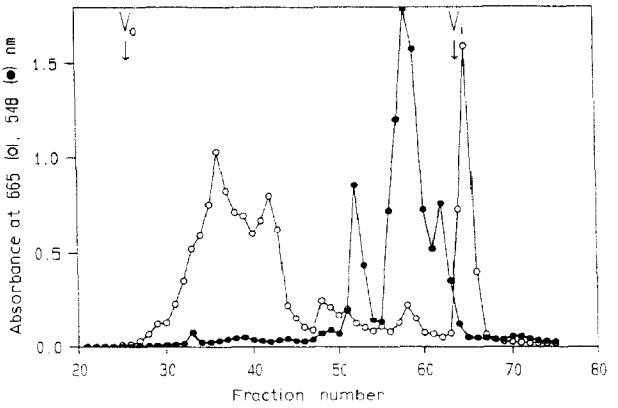



Fig. 2. Elution profile of the partially acid-hydrolyzed (0.1M$CF_3CO_2H$, 100°, 1 h) PRG-II (100 mg) on Bio-Gel P-2 (95 × 1.6 cm, −400 mesh) eluted with NaOAc buffer (50mM, pH 5.2). Fractions (2.5 mL) were collected and portions assayed for pentosyl residues (●) by the orcinol method[19,20], and for DHA and KDO (○) by the thiobarbituric acid method[8,9].

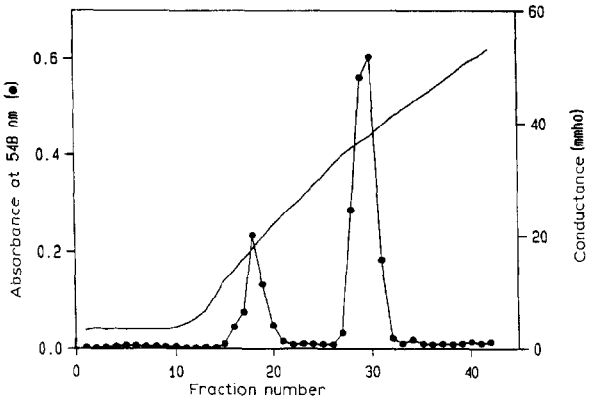
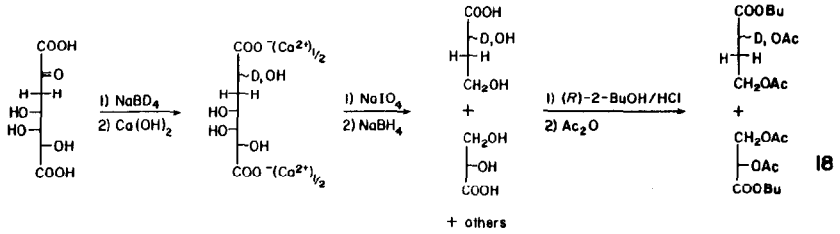



Fig. 3. Elution profile of Bio-Gel P-2 fractions 60–64 on QAE-Sephadex. The column was eluted with a linear gradient of $NH_4OAc$ buffer from 50mM (175 mL, pH 4.95) to 4M (175 mL, pH 5.15). Fractions (~3 mL) were collected and assayed by the thiobarbituric acid method[8,9]. Only the first third of the collected fractions are shown, later fractions contained no thiobarbituric-acid-positive material.




Scheme 4. Formation of (*R*)-butyl 2,3-di-*O*-acetyl-L-glycerate (**18**) from D-DHA.

The esterification of D- and L-glyceric acid with the chiral alcohol allowed the diastereomers (*R*)-2-butyl 2,3-di-*O*-acetyl-D- and -L-glycerate (**18**) to be readily separated by g.l.c. G.l.c.–m.s. of the reaction mixture (containing periodate-oxidized DHA) established the presence of a butyl 2,3-di-*O*-acetylglycerate. When analyzed by g.l.c., only the L-glycerate derivative (**18**) was found. L-Glycerate is the product expected from D-DHA. Thus, the absolute configuration of DHA was D.

*Identification of arabinosyl→DHA in sycamore RG-II.* — Carboxy-reduced and per-*O*-methylated sycamore RG-II was produced in an analogous manner to per-*O*-methylated PRG-II (Scheme 1). The methylated sycamore RG-II was then hydrolyzed with dilute acid, and the products were eluted through a C-18 cartridge with aqueous 20% $CH_3CN$, deuterioreduced, acetylated, and analyzed by g.l.c. and g.l.c.–m.s. as described (Scheme 1 and text). The chromatograms showed major components with retention times and mass spectra the same as those of **8** and **14**. Glycosyl-linkage analysis yielded compounds with retention times identical to those of **6** and **12** and mass spectra consistent with the 2-deuterio analogs of **6** and **12**. The volatile derivatives of terminal arabinofuranosyl and rhamnopyranosyl

TABLE IV

THE IDENTIFICATION OF DHA AND KDO IN VARIOUS PLANT AND BACTERIAL SOURCES

| *Source*[a] | *DHA* | *KDO* |
|---|---|---|
| Pectinol RG-II | +++[b] | +++ |
| Sycamore RG-II[c] | +++ | +++ |
| Sycamore xyloglucan[d] | – | – |
| Sycamore Rhamnogalacturonan-I[c] | + | + |
| Sycamore walls[c] | ++ | ++ |
| Red-kidney-bean leaf walls | ++ | ++ |
| REd-kidney-bean epicotyl walls | ++ | ++ |
| Red-kidney-bean hypocotyl walls | ++ | ++ |
| Red-kidney-bean root walls | ++ | ++ |
| Potato-tuber walls | ++ | + |
| Apple-parenchyma walls | + | + |
| Runner-bean-parenchyma walls | ++ | ++ |
| Runner-bean-string walls | –[e] | –[e] |
| Cabbage-leaf walls | ++ | + |
| Onion-corm walls | ++ | + |
| Rice walls[c] | + | + |
| Douglas-fir walls[c] | ++ | ++ |
| *Salmonella* lipopolysaccharide | – | +++ |
| *E. coli* lipopolysaccharide | – | ++ |

[a]Sycamore walls, Rhamnogalacturonan-I RG-II, and xyloglucan were isolated as described[20]. Walls of red-kidney-bean leaves, epicotyl, hypocotyl, and roots were obtained from the eight-day-old seedlings of a previous study[22]. Walls of potato[23], apple[24], runner-bean parenchymal[25], runner-bean strings[26], cabbage[27], and onion[28] were a gift of R. R. Selvendran. Rice[5] and Douglas-fir[4] walls were from J. R. Thomas of this laboratory, and *Salmonella typhimurium* (Type III) and *E. coli* (Serotype O26:B6) lipopolysaccharides were purchased from Sigma Chemical Co. [b]–, undetected; + present as a minor constituent; ++, present in easily detectable amounts; +++, abundantly present. [c]Cell wall or polysaccharide isolated from suspension-cultured cells. [d]Isolated from extracellular polysaccharides. [e]A trace detected in one of two analyses.

groups were also present. Thus, arabinosyl→DHA and rhamnosyl→KDO are both present in sycamore RG-II as terminal disaccharides.

*The presence of DHA and KDO in other plant and bacterial materials.* — The g.l.c. assay for DHA (Scheme 3) was used to determine whether DHA or KDO was present in plant cell walls, polysaccharides isolated from suspension-cultured sycamore cells, and bacterial lipopolysaccharides (Table IV). The volatile derivatives of DHA and KDO were identified by g.l.c.–m.s. operating in the selected-ion-monitor mode, thus providing detection that was selective and sufficiently sensitive to establish the presence of either sugar in 2-mg samples of isolated sycamore cell walls. Due to the uncertain yields obtained upon hydrolyzing an insoluble substrate and forming and reducing lactones, the results are expressed semi-quantitatively, that is, as undetected (–), present as a minor constituent (+), present in easily detectable amounts (++), and abundantly present (+++). As expected, DHA and KDO were abundantly present in RG-II obtained from both Pectinol and sycamore. Neither keto sugar was found in the hemicellulose xyloglucan. Rhamnogalacturo-

nan-I apparently contained both sugars. However, at least one preparation of rhamnogalacturonan-I has been shown[29] to contain RG-II as a contaminant, and this may have been the source of the minor amounts of DHA and KDO detected in the rhamnogalacturonan-I sample that was analyzed. DHA and KDO were present in the cell walls isolated from all dicots examined, including samples of stem, leaf, root, fruit, and suspension-cultured cells. DHA and KDO were absent from, or present in very limited quantities in, the secondary walls isolated from runner-bean strings, an organ particularly rich in secondary walls. DHA and KDO were found in the isolated primary cell walls of the monocots (onion and rice) and gymnosperm (Douglas fir) examined. On the other hand, KDO, but not DHA, was found in the two bacterial lipopolysaccharides examined.

## CONCLUSIONS

A new sugar, 3-deoxy-D-*lyxo*-heptulosaric acid (DHA), and KDO were found to be components of the cell walls of six dicots, two monocots, and a gymnosperm, including samples of stem, leaf, root, fruit, and suspension-cultured cells. In two commercially available lipopolysaccharides, DHA was not found, but KDO was abundant. DHA and KDO were released from plant cell walls by treatment with mild acid.

A 3-deoxy-2-heptulosaric acid has been isolated previously from the lipopolysaccharide of the Gram-negative bacterium *Acinetobacter calcoaceticus*[30], but neither the relative nor the absolute configuration of the sugar was determined. The 3-deoxy-2-heptulosaric acid in that study[30] was isolated from the lipopolysaccharide as a monosaccharide and as the disaccharide Glc*p*-(1→4)-3-deoxy-2-heptulosaric acid. The 3-deoxy-2-heptulosaric acid isolated from *A. calcoaceticus* and that now found in RG-II may be the same sugar.

DHA was found to be a constituent of the pectic polysaccharide RG-II isolated either from suspension-cultured sycamore cells or from Pectinol AC. In sycamore and Pectinol RG-II, DHA was found in the terminal disaccharide β-L-Ara*f*-(1→5)-D-DHA*p*. The previously characterized[8] disaccharide α-L-Rha*p*-(1→5)-D-KDO*p* has now also been shown to be a terminal disaccharide.

RG-II is even more complex than was previously believed. With the discovery of DHA as a component, RG-II is now known to contain at least 12 different sugars. The elucidation of the structure of RG-II remains a goal of this laboratory.

## EXPERIMENTAL

*General methods.* — The following g.l.c. and g.l.c.–m.s. conditions were used: (*A*) SP-2330 (30 m × 0.25 mm) fused-silica capillary column, split injection, oven programmed from 170° (2-min hold) at 4°/min to 235° with a 15-min final hold, flame-ionization detection; (*B*) column from *A*, splitless injection, oven programmed from 80° (2-min hold) at 30°/min to 240° with a 28-min final hold, e.i.

mass-selective detection; (*C*) column from *A*, except 15-m long, split injection, operated at 240° isothermally, flame-ionization detection; (*D*) DB-1 (15 m × 0.25 mm) fused-silica capillary column, on-column injection, oven programmed from 50° (1-min hold) at 30°/min to 170° then at 10°/min to 330° with a 4-min final hold, flame-ionization detection; (*E*) column and detection from *D*, split injection, oven programmed from 80° (1-min hold) at 5°/min to 150° then at 30°/min to 275°; (*F*) column from *D* except 30 m × 0.32 mm, on-column injection, oven programmed from 50° (0.5-min hold) at 30°/min to 170° then at 10°/min to 340°, e.i.-mass spectrometer detection. Mass spectra were recorded on a Hewlett–Packard 5970 mass selective detector (e.i. spectra of monosaccharide derivatives) or on a Hewlett–Packard 5985 mass spectrometer (e.i. spectra of disaccharide derivatives and all c.i. spectra). C.i.-m.s. was performed using methane as the reagent gas.

N.m.r. spectra were recorded at 27° on either a Bruker AM 250-MHz or AM 500-MHz spectrometer. Chemical shifts are reported in p.p.m. relative to sodium trimethylsilylpropionate ($\delta$ 0.0, $D_2O$), acetone-$d_5$ ($\delta$ 2.04), or benzene-$d_5$ ($\delta$ 7.15) for $^1$H-n.m.r. spectra and benzene-$d_6$ ($\delta$ 128.0) for $^{13}$C-n.m.r. spectra. COSY-90 spectra were recorded in benzene-$d_6$.

Glycosyl-linkage analysis of methylated samples consisted of hydrolysis (2M $CF_3CO_2H$, 120°, 1 h), reduction ($NaBD_4$), and acetylation ($Ac_2O$, 120°, 3 h), as described[20], followed by g.l.c. (conditions *A*) and g.l.c.–m.s. analyses. Molar ratios were calculated by the appropriate effective carbon response factors[31].

Reductions and acetylations of methylated samples were performed as described[20]. If reduction was followed by a reaction other than acetylation, sodium ions were removed with cation-exchange resin (Dowex 50W-X12 or BioRex 70) and boric acid was removed by codistillation with MeOH (5×).

*Isolation of PRG-II.* — Pectinol-AC (Corning, 500 g) in water (2 L) was stirred for 15 min and filtered through a GF/A glass-fiber filter (Whatman). The residue was resuspended in water (700 mL) and filtered again. To precipitate proteins, 2-propanol (560 mL) and $CH_2Cl_2$ (2.2 L) were added to the combined filtrates (2.2 L), the mixture was swirled violently and centrifuged (40 g, 20 min), and the upper aqueous layers were centrifuged again (16,000*g*, 30 min). Polysaccharides were precipitated by addition of absolute EtOH to the combined supernatant solutions to a final concentration of 70% at 4°, and isolated by centrifugation (16,000*g*, 30 min). A solution of the pellet in water (2 L) was chromatographed on a QAE-Sephadex column (350 mL) with a linear gradient of imidazolium chloride buffer (pH 7, from 10mM to M, 1.5-L total volume). Fractions containing *m*-hydroxybiphenyl-positive material[32] were combined and dialyzed against water. The resulting black solution (1.2 L) was stirred overnight with activated charcoal (~250 mL) and then passed through a GF/A filter, and finally through a Nylon-66, 0.22-$\mu$m filter. The light-brown filtrate was lyophilized, and a solution of the residue in buffer was divided into five parts, which were chromatographed separately on a Bio-Gel P-10 gel-permeation column (500 mL, 200–400 mesh) with Na acetate buffer (pH 5.2, 50mM). Collected fractions were analyzed for neutral

sugars by g.l.c. of their alditol acetates. Those fractions containing largely PRG-II were combined, dialyzed, and lyophilized, yielding ~3 g of crude PRG-II. This material was further purified in 100-mg portions on a high-resolution Bio-Gel P-10 column (1.6 × 95 cm, ~400 mesh) eluted with NaOAc buffer (pH 5.2, 50mM)[20], giving the nearly colorless PRG-II used in this study.

*Methylation of PRG-II.* — PRG-II (60 mg, see Scheme 1) was placed in a three-neck flask, and water (110 μL) was added to give a viscous, homogeneous solution. After 1 h, dry dimethyl sulfoxide (11 mL) was added and the contents of the flask were stirred at room temperature for 2 h. The flask was purged with dry argon, and lithium methylsulfinylmethanide (11 mL, 2M in dimethyl sulfoxide), prepared as described[10], was added. The solution was stirred for 4 h and then cooled on an ice bath until solidified. Methyl iodide (8 mL) was added and the mixture was stirred under argon overnight at room temperature, after which water (~40 mL) was added and argon bubbled through the liquid to remove unreacted methyl iodide. The mixture was then dialyzed against water and lyophilized, yielding methylated PRG-II (**2**).

The next procedure was to reduce the carboxyl groups of the methylated PRG-II (**2**). A solution of $LiEt_3BD$ in tetrahydrofuran (M, 4 mL) was added to the methylated PRG-II and the suspension was stirred for 1 h at room temperature. The remaining reductant was quenched with HOAc (230 μL) and the solvent removed by evaporation in a stream of dry air. Lithium acetate and residual $BEt_3$ were removed by dialysis against water, and the water was removed by lyophilization. The resulting methylated and carboxyl-reduced PRG-II (**3**) was methylated a second time as described above, except that no water was used and 1 mL each of dimethyl sulfoxide and lithium methylsulfinylmethanide solution and 0.75 mL of $CH_3I$ were used, giving **4**.

*Identification of the DHA-derivative* **6** *from* **4**. — A solution of **4** (~16 mg) in $CF_3CO_2H$ (0.1M, 30 mL) was heated in a water bath (0.5 h, 60°). The reaction was quenched by the addition of saturated aqueous $NaHCO_3$ (30 mL) and the solution extracted with $CH_2Cl_2$ (3 × 30 mL). The $CH_2Cl_2$ layers were combined and concentrated to dryness by rotary evaporation.

An aliquot of the $CH_2Cl_2$ extract was reduced with $NaBH_4$. Glycosyl-linkage analysis showed the presence of two diastereomeric pairs of 3-deoxyalditols. The diastereomers of 2,5,6-tri-*O*-acetyl-3-deoxy-1,1,7,7-tetradeuterio-1,4,7-tri-*O*-methylheptitol (**6**) were eluted at 16.8 and 16.9 min. For the e.i.-m.s. data, see Scheme 1. C.i.-m.s. data: *m/z* 369 (M + 1, 12%), 337 (M + 1 − MeOH, 100), 309 (M + 1 − HOAc, 57). The diastereomers of 2,5,6-tri-*O*-acetyl-3-deoxy-1,1-dideuterio-1,4,7,8-tetra-*O*-methyloctitol (**12**) were eluted at 18.8 and 18.9 min. For the e.i.-m.s. data, see Scheme 2. C.i.-m.s. data: *m/z* 411 (M + 1, 11%), 379 (M + 1 − MeOH, 100), 351 (M + 1 − HOAc, 45).

The above experiment was repeated, using material that was prepared from PRG-II in an equivalent manner, except that carboxyl groups were reduced with $LiEt_3BH$ instead of $LiEt_3BD$, following the initial methylation. DHA derivative **6**

without deuterio-labeling gave c.i.-m.s. data: *m/z* 365 (M + 1, 11%), 333 (M + 1 − MeOH, 100), 305 (M + 1 − HOAc, 42). KDO derivative **12** without deuterio-labeling gave c.i.-m.s. data: *m/z* 409 (M + 1, 13%), 377 (M + 1 − MeOH, 100), 349 (M + 1 − HOAc, 40).

*Isolation of disaccharides* **5** *and* **11**. — A portion of the $CH_2Cl_2$ extract was reduced ($NaBD_4$) and acetylated. G.l.c.–m.s. analysis (conditions *F*) revealed two diastereomers of **8** co-eluting at 10.1 min, and two diastereomers of **14** co-eluting at 10.7 min. For the e.i.-m.s. data, see Schemes 1 and 2.

Disaccharides **5** and **11** were isolated from the $CH_2Cl_2$ extract by reverse-phase chromatography. The $CH_2Cl_2$ extract was loaded onto a C-18 cartridge (Rainin, 1-mL volume) with aqueous 20% $CH_3CN$ (6 mL) and washed with 50% $CH_3CN$ (6 mL) and 100% $CH_3CN$ (6 mL). When assayed by the anthrone method[12], the 20, 50, and 100% $CH_3CN$ fractions had absorbances at 620 nm of 0.02, 0.21, and 0.02, respectively, indicating that most of the carbohydrate was eluted with 50% $CH_3CN$. Aliquots of each fraction were reduced, acetylated, and analyzed by g.l.c.–m.s. for monoglycosylalditols **8** and **14**. Most of disaccharide **5** and ~2/3 of disaccharide **11** were eluted with 20% $CH_3CN$; the remainder was eluted in the 50% fraction.

The 20% $CH_3CN$ fraction was applied to a C-18 reverse-phase l.c. column (Beckman, 5-μm particles, 4.6 mm × 25 cm) and eluted with a linear gradient of water containing from 5% to 35% of $CH_3CN$ over a period of 30 min at 1 mL/min. Collected fractions were assayed by spotting on a silica gel t.l.c. plate, spraying with ethanolic $H_2SO_4$, and heating in an oven. Disaccharides **5** and **11** were eluted at ~26% and ~30% $CH_3CN$, respectively. For the n.m.r. data, see Tables I–III.

*The substitution and ring-form of DHA*. — Disaccharides **5** and **11** were reduced to **7** and **13**, respectively (Schemes 1 and 2). The glycosyl-linkage analysis of **7** gave 1,4-di-*O*-acetyl-1-deuterio-2,3,5-tri-*O*-methylarabinitol (*T* 8.55 min) and the two diastereomers of 2-deuterio-labeled **6** (*T* 16.8 and 16.9 min) in the molar ratio 1:0.7. Glycosyl-linkage analysis of **13** gave 1,5-di-*O*-acetyl-1-deuterio-2,3,4-tri-*O*-methylrhamnitol (*T* 8.1 min) and the two diastereomers of 2-deuterio-labeled **12** (*T* 18.8 and 18.9 min) in the molar ratio 1:0.8.

Disaccharides **5** and **11** were deuterioreduced to **7** and **13**, and trideuteriomethylated to give **9** and **15**. Glycosyl-linkage analysis of monoglycosylalditol **9** gave 1,4-di-*O*-acetyl-1-deuterio-2,3,5-tri-*O*-methylarabinitol and the two diastereomers of 5-*O*-acetyl-3-deoxy-1,1,2,7,7-pentadeuterio-1,4,7-tri-*O*-methyl-2,6-di-*O*-trideuteriomethylheptitol (**10**, *T* 8.8 and 9.05 min) in the molar ratio 1:0.8. For the e.i.-m.s. data for **10**, see Scheme 2. Glycosyl-linkage analysis of monoglycosylalditol **15** gave 1,5-di-*O*-acetyl-1-deuterio-2,3,4-tri-*O*-methylrhamnitol and the two diastereomers of 5-*O*-acetyl-3-deoxy-1,1,2-trideuterio-1,4,7,8-tetra-*O*-methyl-2,6-di-*O*-trideuteriomethyloctitol (**16**, diastereomers co-eluting at *T* 11.0 min) in the molar ratio 1:1. For the e.i.-m.s. data for **16**, see Scheme 2.

*Methyl α- and β-arabinofuranosides*. — L-Arabinose (5 g) was treated[33] with methanolic HCl (100 mL, ~0.3M) for 5 h at room temperature. Pyridine (5 mL) was added and the reaction mixture concentrated to dryness by rotary evaporation.

The arabinofuranosides were purified by chromatography on a column of silica gel eluted with a step gradient of water-saturated EtOAc containing from 0 to 30% of MeOH. Methyl α-L-arabinofuranoside was the earliest eluting glycoside and was isolated as a chromatographically pure oil. The $^1$H- and $^{13}$C-n.m.r. spectra were in agreement with previous studies[15,34].

Methyl α-L-arabinofuranoside was methylated using two preliminary methylations and a final methylation as described[35], except that lithium methylsulfinylmethanide was used instead of potassium, the base treatment was for 0.5 h for the preliminary methylations and 1 h for the final methylation, and the mixture was stirred with $CH_3I$ for 1 h for the preliminary methylations and 4 h for the final methylation. The reaction mixture was quenched with water and partitioned between water and $CH_2Cl_2$. The $CH_2Cl_2$ layer was concentrated to dryness and the residue was eluted through a silica-gel cartridge (Whatman, 1-mL volume) with hexane, to yield methyl 2,3,5-tri-*O*-methyl-α-L-arabinofuranoside as a colorless oil. $^1$H-N.m.r. (acetone, 500 MHz): δ 4.795 (br. s, 1 H, H-1), 3.936 (ddd, 1 H, H-4, $J_{3,4}$ 6.8, $J_{4,5a}$ 3.7, $J_{4,5b}$ 5.7 Hz), 3.621 (dd, 1 H, H-2, $J_{1,2}$ 1.1, $J_{2,3}$ 2.9 Hz), 3.49 (dd, 1 H, H-5a, $J_{5a,5b}$ 10.8 Hz), 3.488 (dd, 1 H, H-3), 3.46 (dd, 1 H, H-5b), 3.340, 3.329, 3.316, 3.284 (4 s, 12 H, 4 OMe); (benzene, 250 MHz): δ 4.929 (br. s, 1 H, H-1), 4.319 (dt, 1 H, H-4, $J_{3,4}$ 6.8, $J_{4,5a} = J_{4,5b}$ 4.6 Hz), 3.849 (dd, 1 H, H-2, $J_{1,2}$ 1.1, $J_{2,3}$ 3.1 Hz), 3.739 (dd, 1 H, H-3), 3.493 (d, 2 H, H-5a,5b), 3.189, 3.183, 3.137, 3.083 (4 s, 12 H, 4 OMe). $^{13}$C-N.m.r. (benzene, 62.9 MHz): δ 107.2 (C-1), 90.8, 86.5, 81.2, 73.0, 58.9, 57.7, 57.2, 54.4.

Fractions (from the silica-gel column) containing methyl β-L-arabinofuranoside were combined and re-chromatographed on silica gel using EtOAc–acetone–water (5:4:1) as eluant. Fractions judged by $^1$H-n.m.r. spectroscopy to contain mostly methyl β-L-arabinofuranoside were combined and concentrated, giving a colorless oil with ~80% desired product and ~10% each of the methyl α- and β-L-arabinopyranosides. The later components did not interfere with spectral interpretations. The $^{13}$C-n.m.r. spectrum was in agreement with published data[15]. $^1$H-N.m.r. ($D_2O$, 250 MHz): δ 4.904 (d, 1 H, H-1, $J_{1,2}$ 4.5 Hz)[15], 4.148 (dd, 1 H, H-2, $J_{2,3}$ 7.8 Hz), 4.015 (dd, 1 H, H-3), 3.894 (ddd, 1 H, H-4, $J_{3,4}$ 7.0 Hz, $J_{4,5a}$ 3.4, $J_{4,5b}$ 7.0 Hz), 3.77 (dd, 1 H, H-5a, $J_{5a,5b}$ 12.1 Hz), 3.63 (dd, 1 H, H-5b), 3.430 (s, 3 H, OMe).

Methyl β-L-arabinofuranoside was methylated and purified as described above, to give methyl 2,3,5-tri-*O*-methyl-β-L-arabinofuranoside as a colorless oil. $^1$H-N.m.r. (acetone, 250 MHz): δ 4.882 (d, 1 H, H-1, $J_{1,2}$ 4.2 Hz), 3.854 (ddd, 1 H, H-4, $J_{3,4}$ 5.2, $J_{4,5a}$ 5.9, $J_{4,5b}$ 6.7 Hz), 3.724 (dd, 1 H, H-2, $J_{2,3}$ 6.7 Hz), 3.629 (dd, 1 H, H-3), 3.415 (d, 1 H, H-5a), 3.410 (d, 1 H, H-5b), 3.334, 3.330, 3.326, 3.305 (4 s, 12 H, 4 OMe); (benzene, 250 MHz): δ 4.673 (d, 1 H, H-1, $J_{1,2}$ 4.3 Hz), 4.218 (dt, 1 H, H-4, $J_{3,4}$ 5.3, $J_{4,5a} = J_{4,5b}$ = 6.3 Hz), 3.932 (dd, 1 H, H-3, $J_{2,3}$ 6.7 Hz), 3.729 (dd, 1 H, H-2), 3.438 (d, 2 H, H5a,5b), 3.290, 3.180, 3.126, 3.102 (4 s, 12 H, 4 OMe). $^{13}$C-N.m.r. (benzene, 62.9 MHz): 101.9 (C-1), 87.0, 85.9, 80.9, 75.6, 58.7, 57.9, 57.5, 54.5.

*The absolute configuration of the arabinofuranosyl residue of* **5**. — This was determined by a modification of the procedure of Gerwig *et al.*[18]. Methyl 2,3,5-tri-*O*-methyl-L-arabinoside was solvolyzed in (*R*)- and separately in (*S*)-2-butanol containing M HCl (1 h, 120°). The samples were concentrated to dryness and analyzed by g.l.c. (conditions *E*). The anomers of the (*S*) diastereomer had *T* 11.14 and 11.74 min. The anomers of the (*R*) diastereomer had *T* 10.93 and 11.82 min. Compound **5**, solvolyzed in (*R*)-2-butanol, had *T* 10.92 and 11.82 min.

*G.l.c. assay for DHA*. — The sample to be analyzed (see Scheme 3) was hydrolyzed (0.25 mL of 0.1M $CF_3CO_2H$, 1 h, 100°) and the acid removed by evaporation with a stream of filtered air. Reduction, hydrolysis, and reduction were as described[8]. The sample was then hydrolyzed a third time (300 μL of 2M $CF_3CO_2H$, 5 min, 120°) and the aqueous acid removed by evaporation with air and subsequent codistillation with toluene. The sample was reduced a third time with $NaBD_4$ (10 mg/mL in water) as described[8]. This reduction typically required 1 mL of reductant due to residual acid that is difficult to remove from the accumulated salts in the reaction mixture. After 1 h, the reductant was quenched with HOAc and the mixture was passed through a column (~3 mL) of Dowex 50W-X12 ($H^+$) resin (100–200 mesh). The solution was concentrated to dryness by evaporation with air and the boric acid removed by codistillation with MeOH. The alditols were acetylated[8], and analyzed for **17** using g.l.c. conditions *C*.

When insoluble plant cell walls were to be assayed for DHA, the assay described above was modified slightly. The initial hydrolysis was performed using 1 mL of acid in a boiling water bath, with stirring. The sample was filtered through a GF/A glass-fiber filter and concentrated to dryness. Following derivatization as described above, the alditol acetates were analyzed by g.l.c.–m.s., using g.l.c. conditions *B*. To increase sensitivity, samples were injected in the splitless mode, and the instrument was operated in the selected-ion-monitor mode. For acetylated DHA-alditols **17**, *m/z* 43, 72, 132, 156, and 378 were scanned from 20 to 24 min; for acetylated KDO-alditols, *m/z* 43, 72, 124, 132, and 448 were scanned from 30 to 34 min.

*The absolute configuration of DHA*. — The carbonyl group of DHA was reduced with $NaBD_4$ (Scheme 4). The sodium ions were removed with cation-exchange resin, and the boric acid was removed by codistillation with MeOH. The product was dissolved in saturated aqueous $Ca(OH)_2$, and calculated, using the method of Dixon and Lipkin[21], to contain ~8 mmol of reduced DHA. The reduced-DHA solution (100 μL, ~0.8 μmol) was then added to mM sodium periodate (0.75 mL, 0.75 μmol) and the reaction allowed to proceed for 5 h at room temperature. Solid $NaBH_4$ (12 mg) was added. After 1 h, the reaction was quenched with HOAc, and cations and boric acid were removed as described above. (*R*)-2-Butanol containing M HCl (200 μL) was added and the sample heated (3 h, 80°). Solid silver carbonate was added, followed by MeOH (2 mL). The suspension was stirred and centrifuged, and the supernatant solution concentrated to dryness in a stream of dry, filtered air. Acetic anhydride (100 μL) and pyridine (100 μL) were added and

the reaction was allowed to proceed for 1 h at room temperature. The pyridine and acetic anhydride were then removed by azeotropic distillation with toluene. The sample was dissolved in acetone (~10 μL) and analyzed by g.l.c.–m.s. and g.l.c.

Reference standards of (*R*)-butyl 2,3-di-*O*-acetyl-D- and -L-glycerate (**18**) were prepared separately, as described above, from commercially available D- and L-glyceric acid (Sigma Chemical Co.).

M.s. analysis of the partially periodate-degraded DHA established the presence of either **18** or its D isomer. E.i.-m.s. data for **18**: *m/z* 173 (45%), 145 (15), 131 (44), 103 (100), 86 (42), 57 (51). G.l.c. analysis (30 m × 0.25 mm SP2330 fused-silica column, 150° isothermally) of the degraded DHA showed a peak at *T* 10.00 min. Reference **18** had *T* 10.03 min and reference (*R*)-2-butyl 2,3-di-*O*-acetyl-D-glycerate had *T* 10.26 min. Degraded DHA was mixed with each of the two reference compounds. The peak at *T* 10.00 min co-eluted with reference **18**, derived from L-glyceric acid, and did not co-elute with the D isomer.

*Identification of arabinosyl→DHA in sycamore RG-II.* — Sycamore RG-II was isolated from the walls of suspension-cultured sycamore cells as described[20]. Methylation, reduction, remethylation, hydrolysis with dilute acid, partial purification by chromatography on a C-18 cartridge, deuterioreduction, and acetylation were as described above (Scheme 1). Analysis of the resulting mixture by g.l.c. (conditions *D*) gave three peaks, *T* 15.22, 15.82, and 15.88 min. Compound **8** from PRG-II had *T* 15.25 min, and **11** had *T* 15.85 and 15.91 min. The e.i.-mass spectra of the components eluted at 15.22 min were nearly identical to those of **8**, and the spectra of the components eluted at 15.82 and 15.88 were nearly identical to those of **11**.

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