過ヨウ素酸酸化によるトレオース誘導体のグリコシドジアステレオマーを経由した3,6-アンヒドロガラクトースの絶対配置の帰属

Assignment of the absolute configuration of 3,6-anhydrogalactose derivatives via diastereomeric glycosylation of threose derivatives obtained by periodate Oxidation.

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Abstract: In order to assign the absolute configuration of 3,6-anhydrogalactose and its 2-*O*-methyl ether, their dimethylacetals were subjected to periodate oxidation to yield 3-*O*-(2-hydroxy-methyl)threose dimethylacetal derivatives, which were further derivatized to the diastereomeric glycosides with *(R)*- or *(S)*-2-octanol. Their TMS derivatives were completely resolved by gas-liquid chromatography. Complication in the chromatogram due to contaminants or by-products, when this procedure was applied to total methanolysate of the red algal galactans, was overcome by monitoring the fragment ion of m/z = 116 in the mass spectra using selected ion monitor (SIM) chromatogram using GC-MS.

Keywords: absolute configuration, 3,6-anhydrogalactose, agar, carrageenan, red seaweed

Introduction

3,6-Anhydrogalactose is a component of the red algal galactans whose backbone consists of alternating repeat of 3-linked galactose and 4-linked galactose or 3,6-anhydro-galactose units (for review, Usov 2011, Chopin *et al*. 1999). While the absolute configuration of the former residue is always D-form, the latter residue may be of D- or L-form, the algal galactans thus being classified as carrageenan/ carrageenose or agaran/agarose, respectively (Knutsen *et al*. 1994). It is accordingly necessary to assign the absolute configuration of 4-linked galactose and 3,6-anhydrogalactose residues when studying the red algal

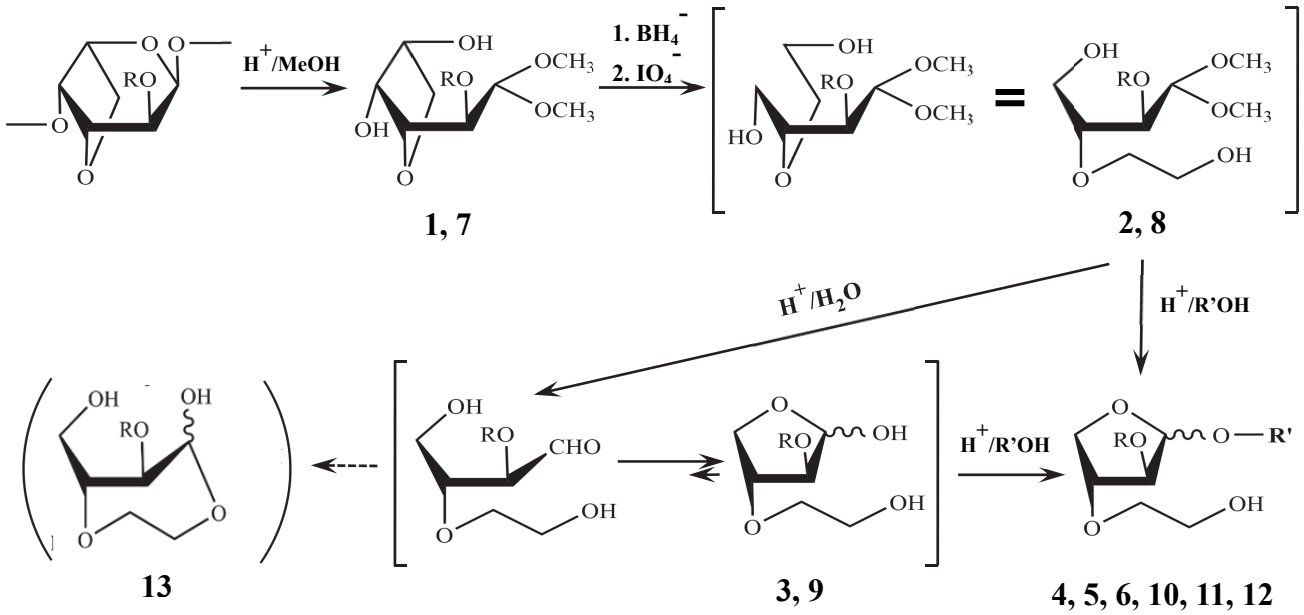

Fig. 1. Scheme for reactions of 3,6-anhydro-L-galactose and 3,6-anhydro-2-*O*-methyl-L-galactose derivatives.

R= -H (1, 2, 3, 4, 5, 6), -Me (7, 8, 9, 10, 11, 12)

R' = -Me (4, 10), -*(R)*-Oct (5, 11), -*(S)*-Oct (6, 12)

galactans. While the configuration of galactose and its methyl ethers can be assigned by GC as the volatile derivatives of diastereomeric glycosides of chiral alcohols (Gerwig *et al*. 1978, Leontein *et al*. 1978, Takano *et al*. 1993), this method is not applicable to 3,6-anhydrogalactose, the absolute configuration of which can be asigned by identifying derivatives of the disaccharides, 4-*O*-β-D-galactopyranosyl-3,6-anhydro-D- and -L-galactose (carrabiose and agarobiose, respectively) obtained by partial degradation. Under these conditions, however, such diad may remain linked to 4-linked galactose residue at the reducing end of the fragment surviving the degradation unless the diads are distributed consecutively. It is thus theoretically hard to assign the configuration of all the 3,6-anhydrides by this method.

Errea *et al*. (1998) successfully determined the D/L configuration of 3,6-anhydrogalactose using GLC by oxidizing the 3,6-anhydride with bromine into 3,6-anhydrogalactonic acid, which was then derivatized to acetylated diastereomeric 2-butylgalactonate via its acid chloride. The configuration was also chromatographically assigned by Navarro and Stortz (2003), who applied reductive amination of 3,6-anhydrogalactose with optically active 1-amino-2-propanol or 1-phenylethylamine.

We here report a new method for the assignment of the absolute configuration of 3,6-anhydrogalactose and its 2-*O*-methyl ether as diastereomeric 2-octyl glycosides of 3-*O*-(2-hydroxymethyl)threose and 3-*O*-(2-hydroxy-methyl)-2-*O*-methylthreose, respectively, via periodate oxidation as shown in Fig.1.

Experimental

Materials and general methods

Reagents used were purchased from Nacalai Tesque except for *(R)*-2-octanol (Wako), chlorotrimethylsilane (CTMS) (Shin-Etsu), trimethylsilylimidazol (TSIM) (Merck), pentaerythritol and D-threitol (Sigma), and Amberite IR120B NA and IRA96SB (Rhom and Haas).

Preparative HPLC was performed by a chromatograph LC-5 (Shimadzu) equipped with a refractive index detector RID-10A (Shimadzu) using a column TSKgel Amide-80 (7.8 mm × 30 cm, Tosoh) eluted with acetonitrile and water (93:7) at a flow rate of 1 mL/min at room temperature. GG-MS was performed using a chromatograph GCMS QP-2100 (Schimadzu) in EI mode (ionization potential of 70 eV) connected with the column DB-1 (0.25 mm × 50 m, Ajirent) operated at 180°C at a flow rate of 1 mL/min.

3,6-Anhydro-L-galactose dimethylacetal (1)

To agarose LE (100 mg) in 10 mL of methanol was added CTMS (128 μL). The mixture was heated for 16 h at 70°C, and then the solution was neutralized with silver carbonate. After removal of the precipitate of the resulting silver chloride and the excess silver carbonate, the resulting 3,6-anhydro-L-galactose dimethylacetal (**1**, L-AGM2) in the supernatant was isolated and purified by preparative TLC. The 0.5 mg portion of the product was trimethylsilylated with 50 μL of *N*-trimethylsilylimidazole (TSIM) in 50 μL of pyridine for 20 min at room temperature and then analyzed with GC-MS.

3-*O*-(2-Hydroxyethyl)-D-threose dimethylacetal (2)

To aqueous solution (0.5 mL) of **1** (5 mg) was added the sodium metaperiodate solution (12.9 mg/1 mL). After the mixture was kept for 1 h at room temperature, the solution was reduced with sodium borohydride (4. 6 mg) for 1 h at room temperature. The excess borohydride was decomposed with acetic acid (72 μL), the solution was evaporated to dryness, and the resulting borate was removed by evaporation with methanol (0.5 mL) for four times. The residue was dissolved in water, applied to serially connected columns of Amberlite IR 120B (H form, 2 mL) and Amberlite IRA96SB (free base form, 2 mL) to remove inorganic salts, and then concentrated to dryness to give a syrup (4 mg)of 3-*O*-(2-hydroxyethyl)-D-threose dimethylacetal (**2**, D-HTM2).

3-*O*-(2-Hydroxyethyl)-D-threose (3)

Syrup of **2** (3 mg) were hydrolyzed with 0.5 M TFA (0.5 mL) for 2 h at 100°C. The hydrolysate was evaporated to dryness to obtaine syrup (2 mg) of 3-*O*-(2-hydroxyethyl)-D-threose (**3**, D-HT).

Methyl 3-*O*-(2-hydroxyethyl)-D-threosides (4)

Syrup of **3** (0.5 mg) was heated with 0.5 M methanolic hydrogenchloride for 1 h at 70°C, the solution was neutralized with silver carbonate, centrifuged, concentrated to dryness, and the resulting syrup of the mixture of methyl α- and β-3-*O*-(2-hydroxyethyl)-D-threosides (**4**, D-HTM) were applied to GC-MS after TMS-derivatization.

2-Octyl glycosidation of D-HT (3)

Syrup of **3** (0.5 mg) was reacted with *(R)*-2-octanol or *(S)*-2-octanol (200 μL) and CTMS (20 μL) at 110°C for 90 min, the reaction mixture was neutralized with silver carbonate, centrifuged, evaporated to dryness, trimethylsilylated, and then analyzed using GC-MS to obtain the TMS derivatives of *(R)*-2-octyl 3-*O*-(2-hydroxyethyl)-D-threosides (**5**, D-HTR) and *(S)*-2-octyl 3-*O*-(2-hydroxyethyl)-D-threosides (**6**, D-HTS).

3,6-Anhydro-2-*O*-methyl-L-galactose dimethylacetal (7)

2,6'-Dimethylagarose extracted (100-121°C) and purified from the red seaweed *Gracilaria eucheumoides* (Takano *et al.* 1995) was methanolyzed for 16 h at 70°C, neutralized with silver carbonate, centrifuged, and the methanolyzate was applied to preparative HPLC using TSK-gel Amide 80. The fraction corresponding to 3,6-anhydro-2-*O*-methyl-L-galactose dimethylacetal (**7**, L-2MAGM2) was isolated as monitored by GLC of the TMS derivative.

3-*O*-(2-Hydroxyethyl)-2-*O*-methyl-D-threose derivatives (8 – 12)

L-2MAGM2 (**7**) was oxidized with sodium metaperiodate as already described to obtain 3-*O*-(2-hydroxyethyl)-2-*O*-methyl-D-threose dimethylacetal (**8**, D-2MHTM2). This product was hydrolysed to 3-*O*-(2-hydroxyethyl)-2-*O*-methyl-D-threose (**9**, D-2MHT). This was further treated with methanolic hydrogenchloride to obtain methyl 3-*O*-(2-hydroxyethyl)-2-*O*-methyl-D-threoside (D-2MHTM, **10**) Then **9** was treated with *(R)*-2-octanol or *(S)*-2-octanol and CTMS to obtain *(R)*-2-octyl 3-*O*-(2-hydroxyethyl)-2-*O*-methyl-D-threosides (**11**, D-2MHTR) and *(S)*-2-octyl 3-*O*-(2-hydroxyethyl)-2-*O*-methyl-D-threosides (**12**, D-2MHTS).

Configuration analysis of 3,6-anhydrogalactose derivatives in the polysaccharides from the red seaweeds.

Polysaccharide isolated, purified, and fractionated from the red seaweed *Gloiopeltis furcata* and *Rhodomela larix* were prepared as described elsewhere (Takano *et al*. 1998 and Takano *et al*. 1999). Each sample (5 mg) was totally methanolysed with 1 M methanolic hydrogenchloride (500 μL) for 16 h at 70°C, neutralized with silver carbonate, centrifuged, and then evaporated to dryness. To the methanolysis product dissolved in water (0.5 mL) was added the sodium metaperiodate solution (12.9 mg/1 mL). The mixture was kept for 1 h at room temperature and then reduced with sodium borohydride (4.6 mg) for 1 h at room temperature. After addition of acetic acid (72 μL), the solution was evaporated to dryness and co-evaporated with methanol (0.5 mL) for four times. The residue was dissolved in water, applied to serially connected columns of Amberlite IR 120B (H form) and Amberlite IRA96SB (free base form) (2 mL each), and then concentrated to dryness. The reaction mixture was then reacted with *(R)*-2-octanol (200 μL) and CTMS (20 μL) at 110°C for 90 min, neutralized with silver carbonate, centrifuged, evaporated to dryness, trimethylsilylated, and then applied to GC-MS to obtain total ion chromatogram and SIM chromatogram at m/z = 116 (**Fig. 3b** for *G. furcata* and **Fig. 3c** for *R. larix*).

Table. Relative retention time (T) of the compounds derived from 3,6-anhydrogalactose and 3,6-anhydro-2-*O*-methylgalactose.

Compound*	Entry*	T**		Mass spectrum
L-AGM2	**1**	2.28		Fig. 2a
D-HTM2	**2**	2.25		Fig. 2b
D-HT	**3**	1.61	1.76	Fig. 2c
D-HTM	**4**	1.17	1.22	Fig. 2d
D-HTR	**5**	1.65	1.69	Fig. 2e
D-HTS	**6**	1.60	1.78	Fig. 2e
L-2MAGM2	**7**	1.82		Fig. 2f
D-2MHTM2	**8**	1.75		Fig. 2g
D-2MHT	**9**	1.22	1.30	Fig. 2h
D-2MHTM	**10**	0.88	0.95	Fig. 2i
D-2MHTR	**11**	1.39	1.43	Fig. 2j
D-2MHTS	**12**	1.31	1.49	Fig. 2j

*See text and Fig.1 for abbreviation and entry.

** Relative to trimethylsilylated threitol.

Result and discussion

While the absolute configuration of usual monosaccharides can be determined by derivatization into their diastereomeric glycosides, this method is not applicable to 3,6-anhydrogalactose that tends to form the dialkylacetal instead of the glycosides due to distortion arising from the anhydro-ring. We then started from L-AGM2 and L-2MAGM2 (**1** and **7**), the vicinal OH groups between C-4 and C-5 of which make them susceptible to

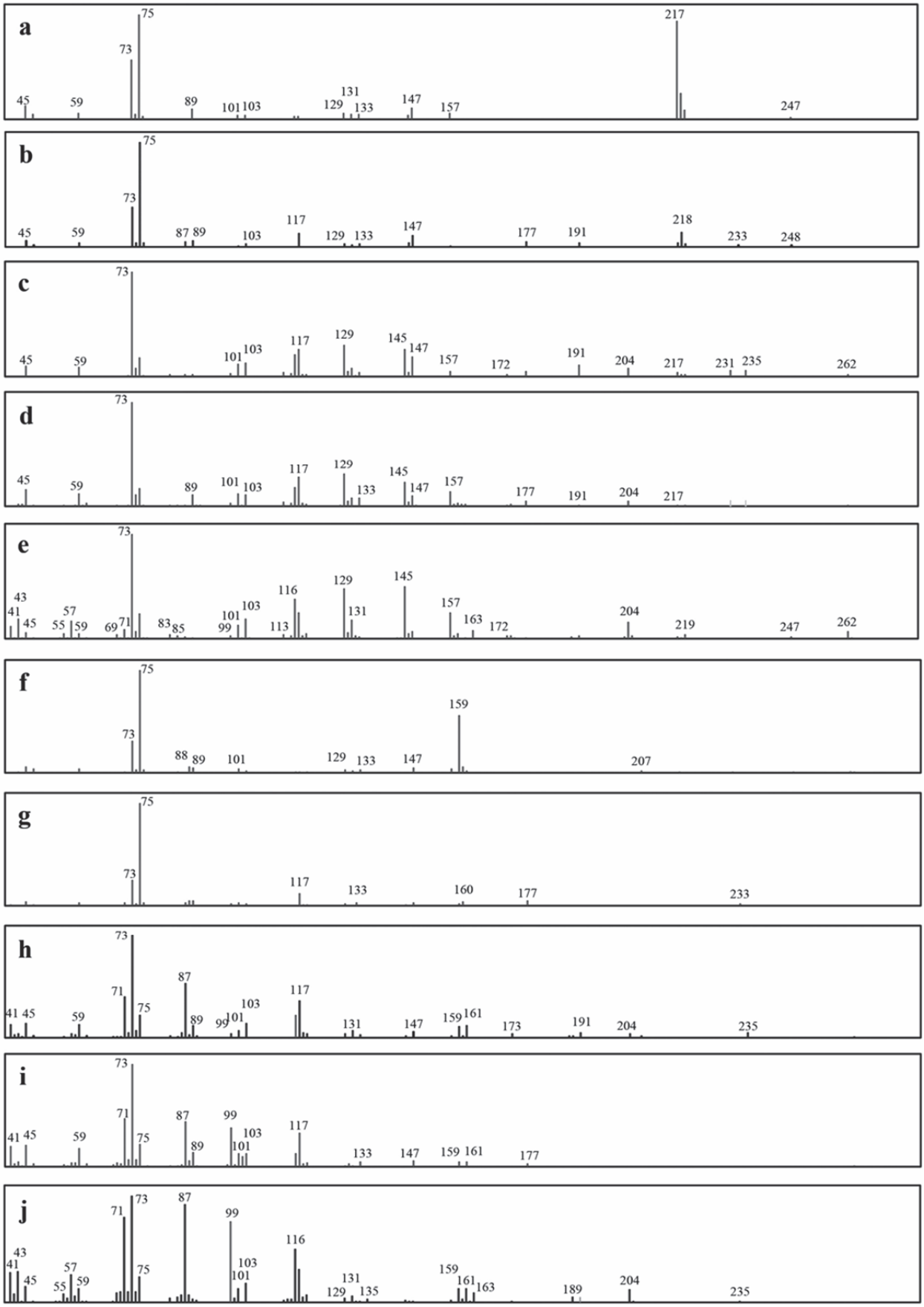

Fig. 2. Mass spectrum of the compounds derived from 3,6-anhydrogalactose and 3,6-anhydrogalactose-2-*O*-methylgalactose.

a; AGM2, **b**; HTM2, **c**; HT, **d**; HTM, **e**; HTR and HTS, **f**; 2MAGM2, **g**; 2MGHTM2, **h**; 2MGHT, **i**; 2MGHTM, **j**; 2MGHTR and 2MGHTS
For abbreviation, see text.

periodate oxidation leading to the threose derivatives that are expected to make glycosides with alcohols (Fig. 1).

Periodate oxidation products from **1** and **7** were reduced with sodium borohydride, and analyzed by GC-MS as the TMS derivative. The chromatogram showed the single peak corresponding to D-HTM2 (**2**) and D-2MHTM2 (**8**). Their mass spectra showed intense signal at the m/z value of 75 (Fig. 2b and g) due to the dimethylacetal structure, $-CH(OMe)_2$, common with the starting materials (Fig. 2a and 2f). They also showed the signals possibly due to the trimethylsilylated hydroxyethyl group, $-O(CH_2)_4OTMS$, at m/z values of 89, 103, 117, and 133. While the signals at m/z = 217 and 159 decreased as compared with the starting materials, the newly appeared signals at m/z = 218 and 160 are likely to have arisen from the fission of C4-C5 bond by the periodate-oxidation and the subsequent borohydride reduction of liberated formyl groups.

Hydrolysis and methanolysis of the dimethylacetal derivatives **2** and **4** followed by silylation afforded couples of the anomers of D-HT (**3**), D-2MHT (**9**), D-HTM (**4**) and D-2MHTM (**10**). Their mass spectra (Fig. 2c, h, d, and i, respectively) showed largely reduced signal at m/z = 75 from the dimethylacetal group as compared with starting materials (Fig. 2b and g) while retained those from silylated hydroxylethyl group as discussed above. Such fragments would not be given from the unfavorable seven-membered cyclic acetal **13**. The furanose structure is also supported from the signals at m/z = 262 and m/z = 204 which would be from the ions arising from fragmentation of C1-C2 and C4-O4 bonds as seen in the spectrum of trimethylsilylated threofuranose (Havlicek *et al*. 1972).

Then **5** and **6** were glycosylated with *(R)*- and *(S)*-2-octanol and trimethylsilylated to afford the couple of the peaks of diastereomeric glycosides. Their mass spectra include the fragment ions similar to their methyl glycosides in addition to those arising from the octyl group at m/z = 43, 57, 71, 85, 99, and 113 in addition to a relatively intense signal at m/z = 116 (Fig. 2e and j).

In the chromatogram of the mixture of all the diastereomeric glycoside obtained above, the eight peaks of each component are clearly separated (Fig 3a). Since *(S)*-octyl glycosides are chromatographically equivalent to *(R)*-octyl glycosides of its antipode, the configuration of 3,6-anhydrogalactose and its 2-*O*-methyl ether also can be assigned as shown in the following examples.

The present method was applied to the polysaccharides from the red seaweeds *Gloiopeltis furcata* (Takano *et al*. 1998) and *Rhodomela larix* (presently *Neorhodomela larix*) (Takano *et al.* 1999). Their total methanolysis products containing dimethylacetals of the anhydrides were periodate-oxidated and directly glycosylated with *(R)*-2-octanol, followed by trimethylsilylation before GC-MS analysis. The total ion chromatogram, analogous to ordinal FID gas-liquid chromatogram, were complicated due to contaminants or by-products. (Fig. 3b and c). However, this was overcome by monitoring the fragment ion of m/z = 116 in the mass spectra using selected ion monitor (SIM) chromatogram.

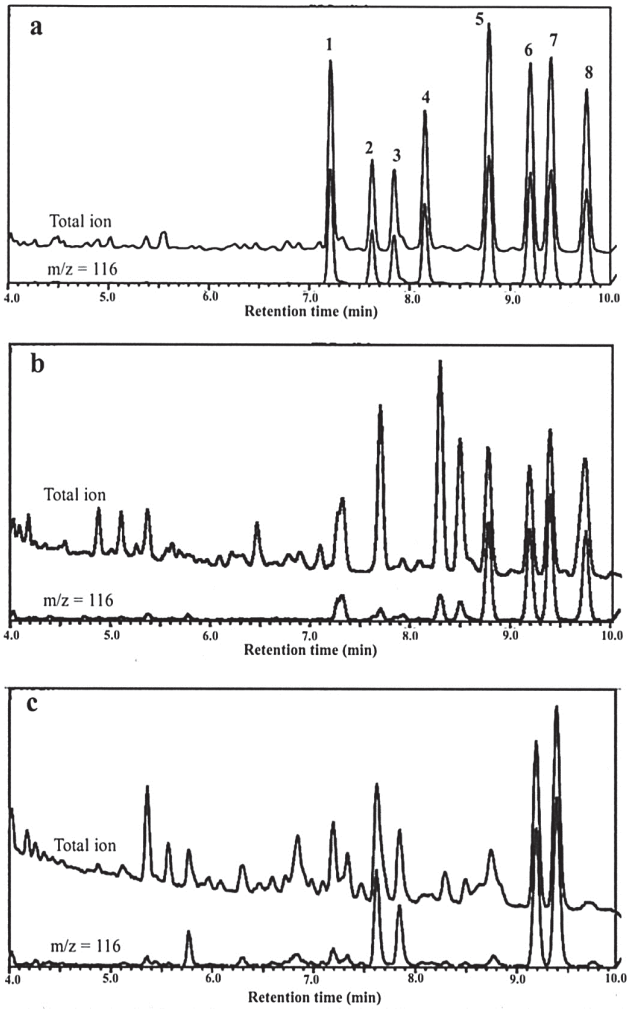

Fig. 3. Total ion and SIM chromatograms of TMS derivatives.

a. Mixture of D-HTR (**5**), D-HTS (**6**), D-2MHTR (**11**), and D-2MHTS (**12**).

b. Total methanolysate of the polysaccharide fraction PS-3 from

the red seaweed *Gloiopeltis furcata* (Takano *et al*. 1998).

c. Total methanolysate of the main polysaccharide from the red seaweed *Rhodomela larix* (Takano *et al*. 1999).

As shown in Fig. 3b, alkali-treated polysaccharide fraction PS-3 from the red seaweed *Gloiopeltis furcata* appeared to have approximately equal amount of both enantiomers of 3,6-anhydrogalactose and 3,6-anhydro-2-*O*-methylgalactose residues. The fraction PS-3 before the alkaline treatment is thus most likely to have cyclizable 4-linked D-galactose and 2-*O*-methyl-D-galactose residues as this fraction contains agarose/agaran/carrageenan backbone (Takano *et al*. 1998). In contrast, 3,6-anhydrogalactose and 3,6-anhydro-2-*O*-methylgalactose in the main polysaccharide fraction from *Rhodomela larix* was assigned to be virtually only of L-form (Fig 3c), while carrageenan backbone was detected from a minor fraction from the same alga.

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過ヨウ素酸酸化によるトレオース誘導体のグリコシドジアステレオマーを経由した3,6-アンヒドロガラクトースの絶対配置の帰属

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要約

紅藻の多糖の主要成分である 3,6-アンヒドロガラクトースの絶対配置を帰属するため、3,6-アンヒドロガラクトースジメチルアセタールを過ヨウ素酸酸化して 3-*O*-(2-ヒドロキシメチル)トレオースジメチルアセタールに変換し、さらにこのものを(*R*)-または(*S*)-2-オクタノールとのジアステレオマーのグリコシドに導いた。これらを TMS 誘導体として GLC 分析したところ、両ジアステレオマーは完全に分離された。また、3,6-アンヒドロ-2-*O*-メチルガラクトースも同様に分離することができた。この方法を紅藻多糖の完全メタノリシス化物に適用すると、クロマトグラムは夾雑物または副生成物により複雑化するが、GC-MS を用い、m/z = 116 のフラグメントイオンを用いた SIM クロマトグラフィーを適用することにより解決された。

キーワード : 絶対配置、3,6-アンヒドロガラクトース、寒天カラギーナン、紅藻