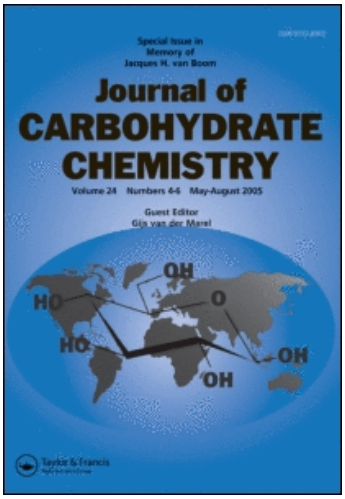

Journal of Carbohydrate Chemistry

Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713617200

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To cite this Article Tanaka, Mutsuo , Nakajima, Yoshikazu , Nishio, Koji and Hashimoto, Hironobu(1993) 'Structure of Oligosaccharides Prepared by Acidic Condensation of Palatinose', Journal of Carbohydrate Chemistry, 12: 1, 49 — 61
To link to this Article: DOI: 10.1080/07328309308018540
URL: http://dx.doi.org/10.1080/07328309308018540

J. CARBOHYDRATE CHEMISTRY, 12(1), 49-61 (1993)

STRUCTURE OF OLIGOSACCHARIDES PREPARED BY ACIDIC CONDENSATION OF PALATINOSE[1]

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Received March 12, 1992 - Final Form September 18, 1992

ABSTRACT

Structures of dimers to tetramers of palatinose formed by its acidic self-condensation were elucidated. Dimers are composed of almost all possible isomers such as α2'→1 and β2'→1 linked tetraoses, α2'→3 and α2'→4 linked tetraoses, and α2'→1: β2→1'-dianhydrides. However, trimer and tetramer mixtures were rather simple and proved to be composed of α2→1 and β2→1 linked oligomers as major and minor components, respectively.

INTRODUCTION

Palatinose (6-*O*-α-D-glucopyranosyl-D-fructofuranose) is produced by treatment of sucrose with certain immobilized microorganisms[2] involving transfer of the α-D-glucopyranosyl residue. The use of palatinose as a non-cariogenic nutritive sweetener has been reported.[3,4] Acidic condensates[5] of palatinose were found to increase bifidobacteria in human intestine[6,7] and recently also proved to be utilized only by Bifidobacterium spp.[8] In this paper we describe the chemical structures of the palatinose condensates.

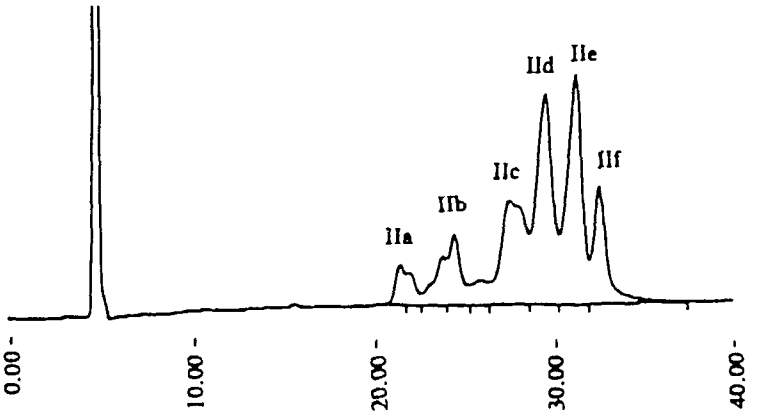

Fig. 1. Fractionation of dimeric fraction II by HPLC on a column of amido 80 (Tosoh) eluted with CH_3CN-H_2O (68:32).

RESULTS AND DISCUSSION

Condensation of palatinose in the presence of citric acid gave a mixture of palatinose (52.4%) and palatinose oligomers (dimers 26.0%, trimers 12.0% and tetramers 5.7%) with other minor decomposed components (4%).[5] Palatinose oligomers were separated as dimeric (II), trimeric (III), and tetrameric (IV) fractions by gel permeation chromatography on Toyopearl HW40-S. The dimeric fractions were further fractionated by HPLC on a column of amide 80 (Tosoh) using acetonitrile-water (68:32) as eluant into 6 fractions (IIa-f), whose elution pattern and purities were checked by rechromatography, Fig. 1 and Fig. 2a-f, respectively.

Structures of these oligomers were elucidated from ^{13}C NMR data summarized in Tables 1 and 2, and FAB mass spectral data in Tables 3 and 4. Although dimers show a complex product distribution as described later, both trimer and tetramer mixtures were simply composed of α2→1 and β2→1 linked palatinose as shown in Fig. 3. These linkages were confirmed by ^{13}C NMR spectra, in which all the non-reducing acetal carbons of α- and β-fructofuranosyl residues have the same chemical shifts of 111.0 and 106.5 ppm, respectively. On the other hand, the β-fructofuranose residue at the reducing end has a chemical shift of 104.4 ppm. The corresponding signal of the α-anomer which should appear at 107 ppm could scarcely be observed in the cases of trimers and tetramers. The signals belong to the non-reducing and reducing fructose moieties and could be easily

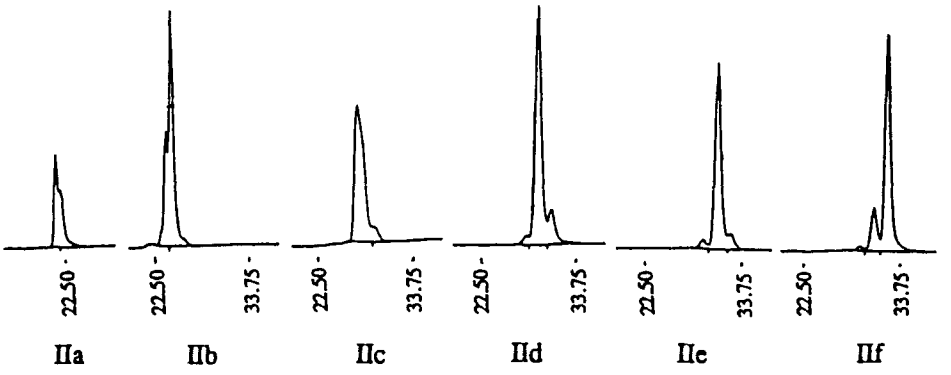

Fig. 2. Rechromatograms of separated dimeric fractions IIa~IIf under the same conditions as described in Fig. 1.

classified due to the difference in their intensities. These four kinds of signals were helpful for the preliminary classification of the glycosidic linkages, and their assignments are reasonable based on reported ^{13}C NMR data of fructofuranoses, methyl fructofuranosides,[9] and palatinose.[10] Thus, major and minor components of trimers and tetramers were confirmed as α2→1 and β2→1 linked palatinoses, respectively and designated as $\alpha^{21}\alpha^{21}\alpha^{21}\beta$ **1** and $\alpha^{21}\alpha^{21}\beta$ **2**. Minor components were designated as $\beta^{21}\beta^{21}\beta^{21}\beta$ **4** and $\beta^{21}\beta^{21}\beta$ **5** (Fig. 3), where α and β indicate 6-*O*-(α-D-glucopyranosyl)ated α- and β-fructofuranosyl moieties. In Table 1 and 2 the assigned residues are shown with shadowed letters.

Among 6 fractions of palatinose dimers, 2'→1-linked isomers can be easily assigned based on ^{13}C NMR data of trimers and tetramers. Namely, the 5th (IIe) and 6th (IIf) fractions were elucidated to be α2'→1 and β2'→1 linked dimers due to the above described characteristic acetal signals at 111.0 and 106.5 ppm, respectively. In the ^{13}C NMR spectra of IIe and IIf, not only the major α-anomers ($\alpha^{21}\beta$ **3** and $\beta^{21}\beta$ **4**, 104.3 and 104.4 ppm, respectively) but also their minor β-anomers ($\alpha^{21}\alpha$ and $\beta^{21}\alpha$, both 107.4 ppm) could be detected and assigned as shown in Table 1. Furthermore, each glucose moiety of the major anomers has slightly different chemical shifts. The third fraction, IIc, and the major components of the 4th fraction, IId, were deduced to be α2'→4 and α2'→3 linked isomers, *i.e.*, $\alpha^{24}\beta$ **7** and $\alpha^{23}\beta$ **8** based on the following data (Table 2): 1) The chemical shifts of α-fructofuranosyl moieties at the non-reducing end (111.5 and 111.1 ppm, respectively) and β-fructofuranosyl moieties at the reducing end (104.4 and 103.6 ppm) are almost the same as those of $\alpha^{21}\beta$. In particular, both the acetal and the C-1 carbons of β-fructofuranosyl moiety have characteristic chemical shifts. 2) The chemical shifts of C-4 in $\alpha^{24}\beta$ **7** and C-3 in $\alpha^{23}\beta$

Table 1. ^{13}C NMR Data from Palatinose Oligomer Fractions with 2→1 Linkages

Oligomer Fractions		Assignments of Linkage and Moiety [a]	Fructose Moiety 1	2	3	4	5	6	Glucose Moiety 1	2	3	4	5	6
IV (major)	1	$\alpha^{21}\alpha^{21}\alpha^{21}\beta$	61.23	111.03	82.97	79.86	84.43	69.52	101.08	74.00	75.72	72.14	74.59	63.16
		$\alpha^{21}\alpha^{21}\alpha^{21}\beta$	65.39	104.35	78.02	77.21	81.86	70.46						
IV (minor)	4	$\beta^{21}\beta^{21}\beta^{21}\beta$	62.54	106.49	79.32	77.73	81.59	71.55	— not assigned —					
III (major)	2	$\alpha^{21}\alpha^{21}\beta$	61.18	111.01	82.96	79.86	84.40	69.51	101.17	73.98	75.70	72.12	74.57	63.14
		$\alpha^{21}\alpha^{21}\beta$	65.36	104.46	77.99	77.17	81.84	70.43						
III (minor)	5	$\beta^{21}\beta^{21}\beta$	62.54	106.48	79.32	77.69	81.53	71.37	— not assigned —					
IIf	6	$\beta^{21}\beta$ $\beta^{21}\alpha$	63.32	106.48	79.32	77.70	81.85	71.52	101.04	73.99	75.66	72.17	74.60	63.17
		$\beta^{21}\beta$	65.41	104.32	78.04	77.23	81.48	70.47	100.98	74.02	75.70	72.135	73.55	63.12
		$\beta^{21}\alpha$		107.37	82.67	78.91	84.40	69.56	101.18	— not assigned —				
IIe [b]	3	$\alpha^{21}\beta$ $\alpha^{21}\alpha$	61.19	111.04	82.96	79.91	84.43	69.54	101.18	74.00	75.72	72.14	74.59	63.16
		$\alpha^{21}\beta$	65.39	104.36	78.015	77.26	81.56	70.63	101.00	73.99	75.78	72.37	74.53	63.29
		$\alpha^{21}\alpha$	61.22	107.37	82.66	78.83	84.40	69.65	101.22	— not assigned —				

a. The anomeric configuration of glycosylated positions are shown left to right from the non-reducing end. ^{13}C NMR chemical shifts were assigned to the moieties designated with shadowed letters.

b. A minor component deduced to be $\beta^{23}\beta$ **9** is a contaminant. The following signals were assigned to C-1 to C-6 of the fructose moiety at non-reducing and reducing ends: (β^2) 62.61, 106.34, 79.13, 77.50, 81.87, 71.24; ($^3\beta$) 66.29, 103.74, 79.40, 77.26, 81.56, 70.26; and C-1 and C-6 of the glucose moiety: 100.96, 101.22, 63.11, 63.20.

Table 2. ^{13}C NMR Data from Dimeric Fractions IIa, IIb, IIc, and IId

Oligomer Fractions		Assignments of Linkage and Moiety	Fructose Moiety						Glucose Moiety					
			1	2	3	4	5	6	1	2	3	4	5	6
IId [a]	**8**	$\alpha^{23}\beta$	61.22	111.12	82.99	79.91	84.76	69.56	101.18	74.06	75.71	72.14	74.59	63.16
		$\alpha^{23}\beta$	65.60	103.63	79.075	77.20	81.56	70.49	100.90	74.05	72.25	72.25	74.54	63.27
IIc	**7**	$\alpha^{24}\beta$	63.19	111.53	82.67	78.6	84.40	69.56	101.07	73.94	75.73	72.18	74.59	63.13
		$\alpha^{24}\beta$	65.35	104.44	78.1	77.49	81.57	70.88	100.81		75.68	72.10	74.31	63.18
IIb [b]	**10**	$\alpha^{21}_{12}\beta$	63.38	105.47	82.34	79.65	85.14	69.73	101.47	74.24	75.92	72.37	74.89	63.31
		$\alpha^{21}_{12}\beta$	65.38	101.96	80.61	77.76	84.66	69.68	101.35		75.83		74.83	63.43
IIa	**13**	$\alpha^{21}_{32}\beta$	63.655	111.59	83.49	78.85	84.55	69.87	101.315	74.21	75.98	72.41	74.84	63.42
		$\alpha^{21}_{32}\beta$		106.18	78.29	77.51	81.69	71.62	101.16	74.42	75.94		74.70	

a. Two minor monoanhydro dimers deduced to be $\alpha^{22}\alpha$ and $\alpha^{22}\beta$ (**12**) were contaminants. The following signals were assigned to C-2 to C-5 of the fructose moieties show in parentheses: 111.49, 83.565, 79.32, 85.58(α^2); 111.45, 81.82, 78.60, 84.15(α^2); 107.14, 82.715, 79.445, 84.53($^2\beta$); The signals of glucose moiety: 101.26, 101.16, 101.08(C-1); 73.98(C-2), 74.10(C-3), 72.08(C-4), 74.62(C-5); 63.64, 64.78(C-6).

b. A minor dianhydro dimer deduced to be $\alpha^{21}_{12}\alpha$ **11** is a contaminant. The following signals were assigned to C-1 to C-6 of the fructose moiety and, C-1 and C-6 of the glucose moiety, respectively: 64.70, 106.90, 82.52, 80.05, 84.87, 72.10, 101.96, 63.86.

Table 3. Positive and Negative FAB Mass Spectral Data from Dimeric Fractions IIa~IIf.

Fraction	Charge	m/z	High Mass	Molecular Formula
IIa	+	649:$[M_1+H]^+$, 671:$[M_1+Na]^+$, 689:$[M_2+Na]^+$	647.2044: $[M_1-H]^-$	$C_{24}H_{39}O_{20}$
	-	647:$[M_1-H]^-$ (major), 665:$[M_2-H]^-$ (minor) [a]		
IIb	+	649:$[M_1+H]^+$, 671:$[M_1+Na]^+$, 687:$[M_1+K]^+$	647.2013: $[M_1-H]^-$	$C_{24}H_{39}O_{20}$
	-	647:$[M_1-H]^-$		
IIc	+	649:$[M_1+H]^+$, 667:$[M_2+H]^+$, 689:$[M_2+Na]^+$, 705:$[M_2+K]^+$	647.2044: $[M_1-H]^-$	$C_{24}H_{39}O_{20}$
	-	647:$[M_1-H]^-$ (minor) [b], 665:$[M_2-H]^-$ (major)	665.2156: $[M_2-H]^-$	$C_{24}H_{41}O_{21}$
IId	+	649:$[M_1+H]^+$, 689:$[M_2+Na]^+$, 705:$[M_2+K]^+$	665.2163: $[M_2-H]^-$	$C_{24}H_{41}O_{21}$
	-	665:$[M_2-H]^-$		
IIe	+	649:$[M_1+H]^+$, 667:$[M_2+H]^+$, 689:$[M_2+Na]^+$	665.2176: $[M_2-H]^-$	$C_{24}H_{41}O_{21}$
	-	665:$[M_2-H]^-$		
IIf	+	649:$[M_1+H]^+$, 667:$[M_2+H]^+$, 689:$[M_2+Na]^+$, 705:$[M_2+K]^+$	665.2176: $[M_2-H]^-$	$C_{24}H_{41}O_{21}$
	-	665:$[M_2-H]^-$		

a. Relative abundance was about one-third of the major peak.
b. Relative abundance was about one-fourth of the major peak.

Table 4. Major Negative and Positive FAB Mass Spectral Peaks from Dimeric Fractions IIa~IIf

Negative m/z																			
IIa	665w	647.4s		545.3w	503.3w	485w	391.3w	369m	367.3m	341m	329.3s	313m	299m	277vs	275.2*				
IIb		647.4s				485.2w			367.3w	341.1w		313w			275.2s				
IIc	665.3m	647.3w	551.4w			459.3w	383w		367.3s	341.1m		313m-s			275.2*	273s			
IId	665.3s				503.3w	399.2w	383w			341.1vs	324.5m-s				275.2s				
IIe	665.3m		551w		503.3w	459.3w	383w		367.3s	341.2m					275.2s	273m			
IIf	665.3s	575.2w		545.3m	503m	459.3w	383s		367s	341.2s		313.1m			275.2*	273vs			

Positive m/z																			
IIa	689.0w	671.0s	649.0m	553vw	527.0w	487.1w	461w	409.1w	392m	369.1s	325m	315.1m	299vs	277.1*	241.1s	222*	207.1*		
IIb	686.9w	671.0m	649.1m	553w		487w	461.2w			369m	325.1m	315w	299m	277.1*	241.1m	222s	207.1*		
IIc	689.0m	667.0m	649.1m	553.1w		487.0w	461m		392w	369.1s	325.1s	315w-m	299m	277.1*	241.1s	222s	207.1*		
IId	689.0s		649.0w	553w	527vw	487.0w	423w			365.0w	325.1s		289.1m	259.1m		222m	207.1*		
IIe	689.0m	667.0m	649.1s	553vw		487.0m	461w	417.1w		369.1s	325.1s		299w	277.1*	241.1s		207.1s		
IIf	689.0s	667.1w	649.1m	553vw		487.0w	461w	405.1w	392w	369.1m	325m	313.1m	299m	277.1*	241.1m	222*	207.1*		

Relative abundance: * >95% , vs 60-95%, s 30-60%, m 15-30%, w< 15%

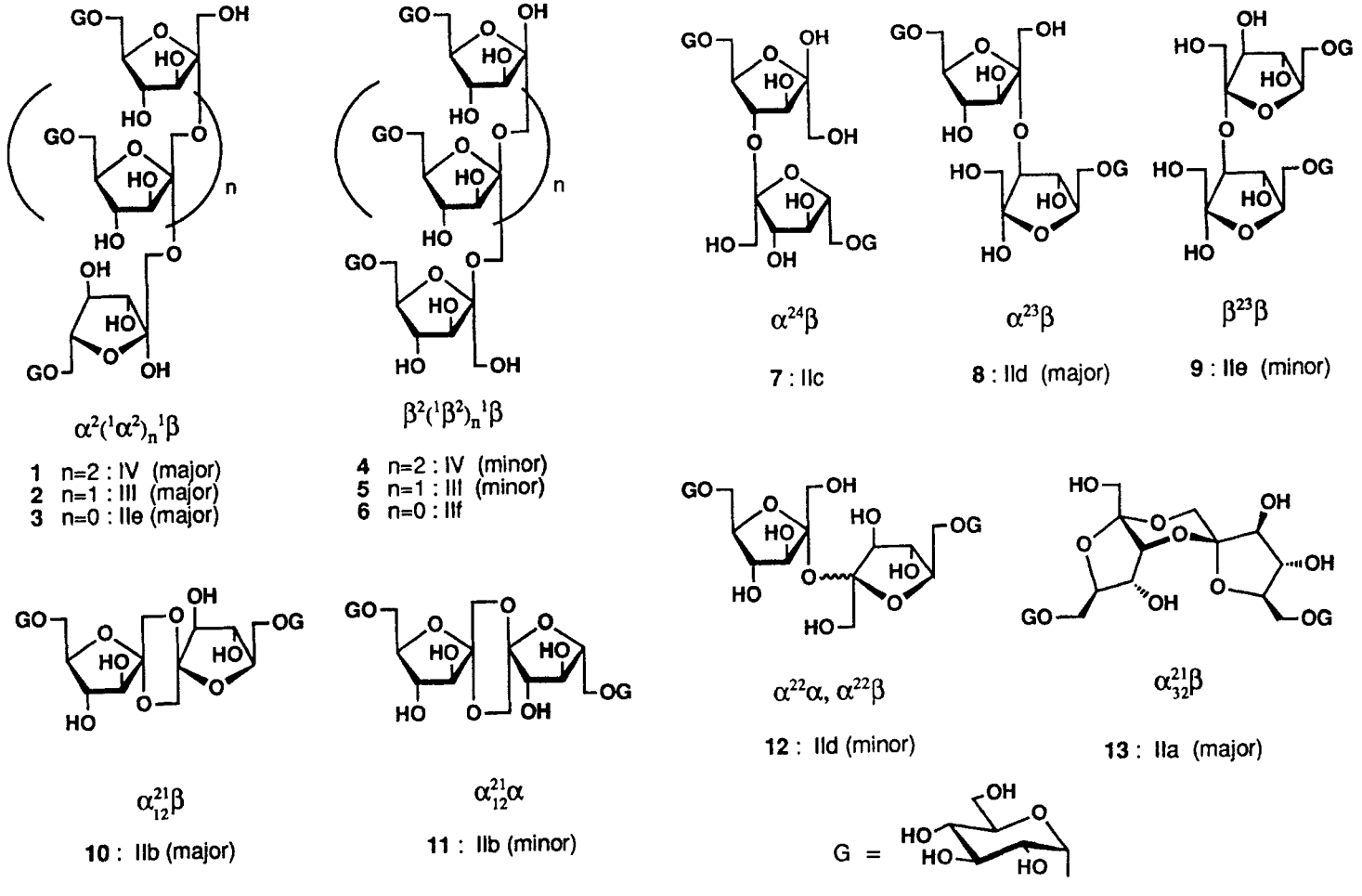

Fig. 3 Proposed structures of dimeric, trimeric, and tetrameric palatinose oligomers.

8 are shifted slightly to a lower field. Furthermore, contamination of β2'→3 linked dimer, *i.e.*, $\beta^{23}\beta$ **9** in fraction IIe, was also deduced from the spectral data (see the footnote of Table 1).

In addition to these reducing dimers, non-reducing dimers: dianhydrides and 2→2 linked dimers, were also confirmed. The major component of the second fraction IIb was confirmed as α2'→1 : β2→1' dianhydro dimer $\alpha^{21}_{12}\beta$ **10**, whose acetal carbons have characteristic chemical shifts at 105.5 and 102.0 ppm, respectively, as reported for the non-glucosylated skeleton.[11] Furthermore, α2'→1 : α2→1' dianhydro dimer $\alpha^{21}_{12}\alpha$ **11**, was deduced to be contaminated (Table 2). In the spectrum of fraction IId, four other sets of fructose signals were observed, presumably due to the α-anomer of **8**, monoanhydro dimers $\alpha^{22}\alpha$ and $\alpha^{22}\beta$ (**12**). These types of fructose dimers have never been reported. The major component of the first fraction IIa was deduced from ^{13}C NMR signals to be a dianhydro dimer $\alpha^{21}_{32}\beta$ **13**.

Negative and positive FAB mass spectral data from all dimeric fractions IIa-IIf, summarized in Tables 3 and 4, gave additional support to the above described structural assignments. Two kinds of molecular ions were detected, one due to the monoanhydride dimer (M_2 = 666) and the other to the dianhydride dimer (M_1 = 648). In the positive FAB mass spectra only the fraction IIb showed M_1 as M_1+H (649), M_1+Na (671), M_1+K (687), while other fractions showed both M_1 and M_2 (Table 3). In contrast, in the negative FAB mass spectra, only fractions IIa and IIc contained two molecular ions M_1 and M_2, fraction IIb contained only M_1 and the other three fractions only M_2. Although positive FAB mass spectra molecular ions gave no information about the structures due to the dehydration that occurred during ionization, the results from the negative FAB mass spectra indicated that fractions IIa and IIc were composed of two kinds of dimers. The dianhydride and monoanhydride dimers are the major components, respectively. Fraction IIb contains only dianhydride dimers (**10** and **11**), and others fractions (IId, IIe, and IIf) only monoanhydride dimers (**8** and **12**, **3** and **9**, and **6**, respectively). Furthermore, the high mass values from negative FAB confirmed the expected elemental compositions as shown in the last column of Table 3.

Possible mass spectral fragmentations are shown in Fig. 4 using the 2→1 linked isomer as an example according to nomenclature recently proposed.[12] In addition to the cleavage of glycosidic bonds (represented by Bi and Yj), various sugar ring cleavages (represented by ^{k,l}Ai and ^{k,l}Xj) were observed. Fragmentations $Y_{2\alpha}$, $X_{2\alpha}$, and $X_{1\alpha}$ can be alternatively substituted with $Y_{1\beta}$, $X_{1\beta}$ and X_0, respectively. Although mechanisms of positive and negative ions geneses are different and not shown in this paper, these fragmentations can be explained reasonably. As shown in Table 4, distinct differences in the signals attributed to the ring cleavages represented by $X_{1\alpha}$ were observed indicating that

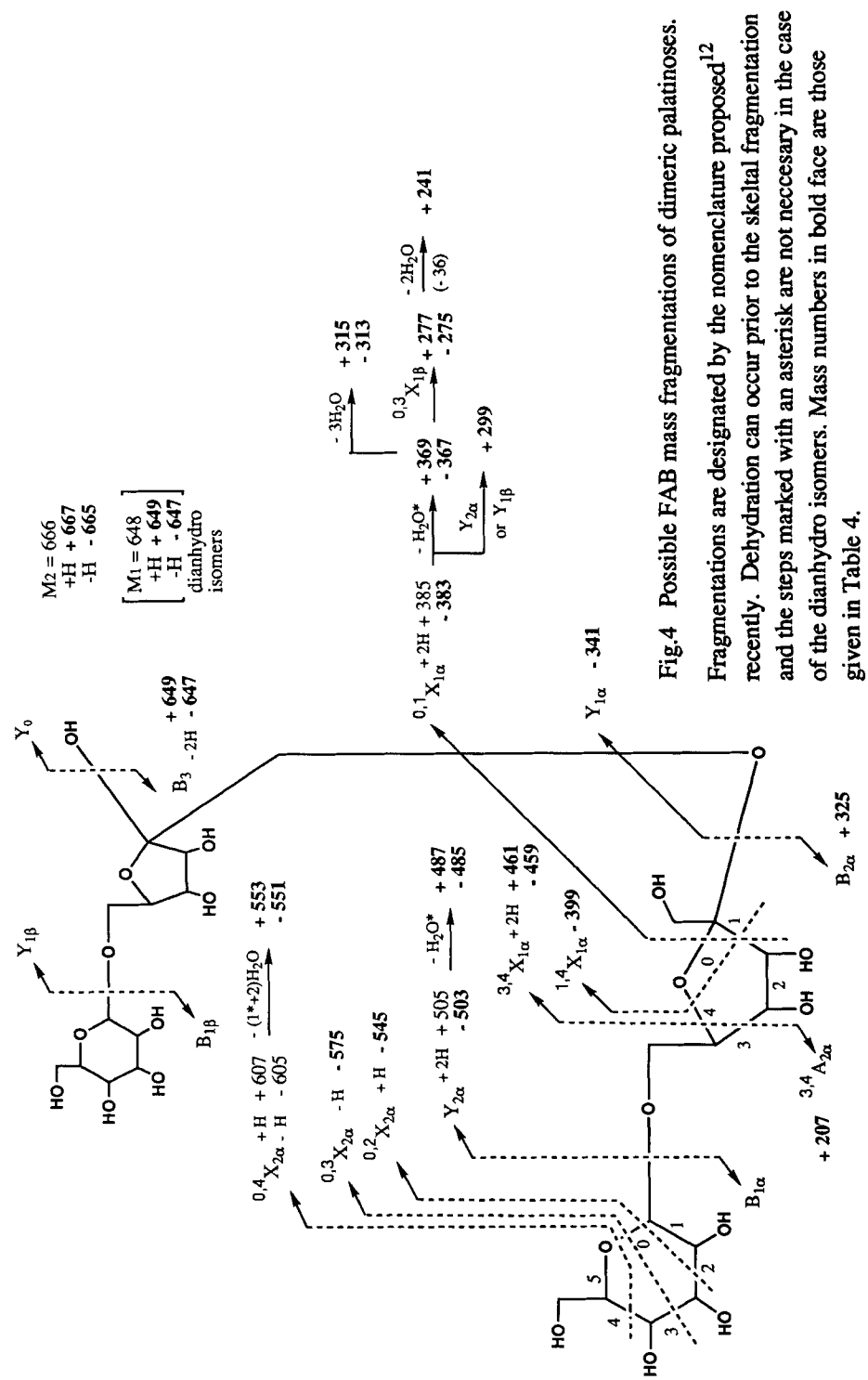

Fig.4 Possible FAB mass fragmentations of dimeric palatinoses.

Fragmentations are designated by the nomenclature proposed[12] recently. Dehydration can occur prior to the skeltal fragmentation and the steps marked with an asterisk are not neccesary in the case of the dianhydro isomers. Mass numbers in bold face are those given in Table 4.

the fragmentations in the furanose ring should be sensitive to the type of the glycosidic linkages between two fructose residues.

Treatment of D-fructose with strong acid[11,13,14] as well as chemical[11,13] and enzymic[15] hydrolysis of its β2,1-linked polymer inulin have been rather well studied. Those reactions gave mixtures of several di-D-fructose dianhydrides as the main products or by-products. Analysis of the product distribution has revealed the preferential formation of an α-fructofuranosyl structure over a β-fructofuranosyl one with approximate ratios of two to four.[11] Although β-fructopyranosyl is the most stable ring structure, it is not the fructose ring form of palatinose. Thus formation of α2→1 and β2→1-linked trimers and tetramers as well as the predominance of the former anomers were rationalized. It is noteworthy that the α-fructofuranosyl linkage was formed predominantly in spite of preferential existence of β-furanose anomers in the cases of D-fructose[16] and palatinose,[10] whose β-furanose anomers are about 3 times as abundant as the α-furanose anomers even at elevated temperatures.[16]

As mentioned above, formation of 2',4-linked and 2',3-linked dimers was suggested as a characteristic of the palatinose condensation. Furthermore, in trimerization and tetramerization steps relatively strict stereoselection was indicated. Among three kind of linkages, *i.e.*, 2'→1-, 2'→3- and 2'→4-linkages, only the first ones have a suitable conformation for oligomerization. These results represent an interesting example of stereochemical proliferation, which might have had an important role in formation of biologically active chiral polymers in nature.

EXPERIMENTAL

Preparation of Palatinose Oligomers. Crystalline palatinose (100 parts) was dissolved in water (30 parts) by boiling at atmospheric pressure in the presence of citric acid (0.01 part : pH of the solution was 4.3). The mixture was then heated under reduced pressure in an oil bath at 160 °C and the heating was stopped when the temperature of the reaction mixture reached 130 °C. Rapid cooling of the mixture gave a candy-like residue, which was crushed and used for the following fractionations.

Fractionation of Palatinose Oligomers. A crude mixture of palatinose oligomers (500 mg) was fractionated on a column (ϕ 22 mm x 90 cm) of Toyopearl HW40-S by eluting with water (0.4 mL/min) at 60 °C to give fractions II (92 mg), III (56 mg) and IV (24 mg). The purity of each fraction was proved to be over 98% by rechromatography.

Fractionation of Dimeric Fraction. A mixture of palatinose dimers was separated by HPLC using a column (ϕ 4.6 mm x 25 cm) of amido 80 (Tosoh) with acetonitrile-water

(68:32) to give IIa (3 mg), IIb (4.6mg), IIc (8.3 mg), IId (14.1 mg), IIe (15.8 mg), and IIf (6.8 mg). The chromatograms are shown in Fig. 2.

FAB mass spectra were measured by using a JEOL JMS-AX505H double-focusing mass spectrometer with a JMA-DA5000 mass data system. The compounds were dissolved in water and ionized by fast atom bombardment (FAB) with glycerol as the liquid matrix. A 5.0KeV beam of xenon atoms was used as the primary ion beam. The accelerating potential for the secondary ions produced from the sample was 3.0kV. The normal mass spectra were measured in positive- and negative-ion modes by magnetic field scans (resolution:1000). The exact masses were measured in negative-ion mode by narrow accelerating voltage scans (resolution:3000). Poly (ethylene glycol)-600 was used as the calibrant for exact mass measurement.

ACKNOWLEDGEMENTS

The authors thank A. Kusai (JEOL Co.) for recording the FAB-mass spectra and Dr. K. Ajisaka (Meiji Institute of Health Science) for recording the 400 MHz NMR spectra.

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