Carbohydrate Research, 103 (1982) 186–189
Elsevier Scientific Publishing Company, Amsterdam – Printed in The Netherlands

Note

Assignment of the L configuration to the fucose elaborated by brown seaweeds

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(Received September 3rd, 1981; accepted for publication, October 13th, 1981)

Both D- and L-fucose occur naturally. β-D-Fucopyranose occurs in the plant kingdom in several cardiac glycosides[1], *e.g.*, in *Digitalis purpurea*[2]. L-Fucose occurs in both the plant and animal kingdoms. It occurs[3] in seaweeds, especially brown seaweeds, where the α-pyranoid form is the major constituent of fucans. *Macrocystis pyrifera* is a major source[4] of L-fucose. In the animal kingdom, α-L-fucopyranose is a common constituent of glycoproteins[5] (including blood-group substances[5a]) and of human-milk oligosaccharides[5b].

It is generally assumed[6a] that brown seaweeds elaborate L-fucose, but the absolute configuration is often not established, particularly as most identifications are now[6b] made by forms of chromatography that do not achieve recognition of chirality. There is a possibility that some brown seaweeds might elaborate D- and L-fucose, especially since their homomorphs D- and L-galactose, respectively, occur together in agarose[6c] and in the polysaccharides of *Porphyridium aerugineum*[7] and of *P. cruentum*[7].

Therefore, we have investigated the fucoses which are constituents of fucans extracted from *Ascophyllum nodosum*[8], *Bifurcaria bifurcata*[9], *Dictyopteris plagiogramma*[10], *Himanthalia lorea*[9], *Macrocystis pyrifera*[11], *Padina pavonia*[9], and *Pelvetia canaliculata*[12]. The compositions of the hydrolysates of the fucans are shown in Table I.

Capillary g.l.c. of derivatised, diastereoisomeric glycosides obtained from enantiomeric monosaccharides by using a chiral alcohol (2-butanol[13] or 2-octanol[14]) can be used to determine the absolute configuration. We have used (−)-(*R*)-2-butanol[13] and trifluoroacetic acid as the glycosidation catalyst, followed by capillary g.l.c. on SE-30 of the trimethylsilylated glycosides, for the determination of the absolute configuration of the fucose isolated from the seaweeds in Table I.

The retention times of the eight products formed from synthetic D-fucose and from the fucoses of the fucan hydrolysates with either (*R*,*S*)-2-butanol or commercial (*R*)-2-butanol are shown in Table II. The retention times at 152° were virtually those reported[13] for 150°.

TABLE I

COMPOSITION OF HYDROLYSATES OF FUCANS OF BROWN SEAWEEDS

Origin of fucan (seaweed)	*Monosaccharides in hydrolysates (mol. ratio)*					
	Fuc	*Gal*	*Glc*	*Man*	*Xyl*	*GlcA*
Ascophyllum nodosum[8]	4.9				1.0	1.1
Bifurcaria bifurcata[9]	13.0	trace			1.0	2.3
Dictyopteris plagiogramma (de-sulphated)[10]	13.3	5.0	1.0	2.7	3.3	6.7
Himanthalia lorea[9]	14.0				1.0	2.0
Macrocystis pyrifera[11] (extracellular mucilage)	18.8	2.5		1.0	1.3	1.4
Padina pavonia[9]	12.5	trace			1.0	3.0
Pelvetia canaliculata[12]	112	5.0	1.0	2.4	2.0	

TABLE II

G.L.C. OF TRIMETHYLSILYLATED 2-BUTYL FUCOSIDES

Component[a]	T[b]		*Absolute configuration of parent 2-butyl fucosides*[c]	*Mole fraction*[d]		
	152°	*170°*		D-*Fuc*	*B*[e]	*M*[f]
1	0.58	0.64	(*S*)L- and/or (*R*)D-*f*	0.409	0.029	0.042
2	0.60	0.66	(*R*)L- and/or (*S*)D-*f*	0.023	0.414	0.433
3	0.65	0.72	(*R*)L- and/or (*S*)D-*p*	0.007	0.174	0.204
4	0.66	0.73	(*S*)L- and/or (*R*)D-*p*	0.145	0.022	0.040
5	0.71	0.77	(*S*)L- and/or (*R*)D-*p*	0.118	0.007	0.009
6	0.76	0.81	(*R*)L- and/or (*S*)D-*p*	0.006	0.108	0.108
7	0.80	0.83	(*R*)L- and/or (*S*)D-*f*	0.013	0.239	0.163
8	0.81	0.85	(*S*)L- and/or (*R*)D-*f*	0.279	0.007	0.001

[a]Components **1–8** were obtained on glycosidation of D-fucose or fucan fucoses with either (*R,S*)-2-butanol or commercial (*R*)-2-butanol. [b]Retention time relative to that of trimethylsilylated methyl α-D-galactopyranoside. [c]The absolute configuration of the butyl group is indicated by (*R*) and (*S*), that of fucose by D and L; furanosides[13] and pyranosides[13] are indicated by *f* and *p*, respectively. [d]Mole fraction of product (at 170°) of glycosidation of fucose with commercial (*R*)-2-butanol. [e]Fucose of *Bifurcaria bifurcata*. [f]Fucose of *Macrocystis pyrifera*.

Commercial (*R*)-2-butanol is not easily obtained free of the *S* enantiomer, and for the purpose of this investigation it was necessary to know the *S,R*-ratio. As each α- and β-pyranoside and α- and β-furanoside is produced as a diastereoisomeric pair and as the glycosidation reactions are usually 85–90% complete, this ratio would only be meaningful if the diastereomers are produced at equal rates. This was established by glycosidations with (*R,S*)-2-butanol. The components of each pair of diastereomers (*i.e.*, **1/2**, **3/4**, **5/6**, and **7/8**) were produced in equal amounts.

In the chromatograms obtained from synthetic D-fucose with commercial (*R*)-2-butanol, the components **1**, **4**, **5**, and **8** ($\sum$ mole fraction, 0.951) correspond to the major products and are the (*R*)-2-butyl D-fucosides. Therefore, the 2-butanol used had an *S*,*R*-ratio of 0.052:1.

The major products of the glycosidation of the fucoses of the fucan hydrolysates with the (*R*)-2-butanol were the (*R*)-2-butyl L-fucosides (corresponding to components **2**, **3**, **6**, and **7**). The components **1**, **4**, **5**, and **8** could be the trimethylsilyl derivatives of (*R*)-2-butyl D-fucosides and/or (*S*)-2-butyl L-fucosides, the latter arising from the contaminant (*S*)-2-butanol. The ratio of the $\sum$ mole fraction of the apparent (*R*)-2-butyl D-fucosides and/or (*S*)-2-butyl L-fucosides (components **1**, **4**, **5**, and **8**) to that of the (*R*)-2-butyl L-fucosides (components **2**, **3**, **6**, and **7**) obtained from the fucans listed in Table I ranged from 0.070:1 (from fucose of *Bifurcaria bifurcata*) to 0.101:1 (from fucose of *Macrocystis pyrifera*).

These data establish that brown seaweeds elaborate L-fucose. That the ratios are greater than 0.052:1 (*cf.* results obtained with synthetic D-fucose) could indicate that the fucans contain ~2–6% of D-fucose. However, it is more likely that the relative peak areas are the result of incomplete resolution. All the fucans examined contain small proportions of xylose (*cf.* Table I) and, under the conditions of g.l.c. used, trimethylsilylated xyloses and 2-butyl xylosides are not completely resolved from the trimethylsilylated 2-butyl fucosides. The corresponding galactose, glucose, mannose, and glucuronic acid derivatives have $T \gg 1$ and, therefore, did not interfere. Thus, it is concluded that brown seaweeds elaborate only the L enantiomer of fucose.

EXPERIMENTAL

Materials. — The fucans studied were from our Departmental collection of polysaccharides. (−)-(*R*)-2-Butanol and D-fucose were commercial materials.

Hydrolysis and analysis of fucans. — A solution of fucan (~10 mg) in aqueous 90% formic acid (1 ml) was heated at 100° for 6 h, diluted with water (5 ml), and heated at 100° for 2 h. The solvent was co-distilled with methanol, and the residue was dried over $CaCl_2$.

A solution of the residue in either (*R*,*S*)-2-butanol or (*R*)-2-butanol (0.25 ml) containing trifluoroacetic acid (2 drops/ml) was heated in a sealed tube at 80° for 20 h, and then concentrated to dryness. The residue was dried over P_2O_5 and treated with hexamethyldisilazane–chlorotrimethylsilane–pyridine (0.1 ml; 2:1:5) at room temperature for 24 h, and then subjected to g.l.c.

G.l.c. was performed on (*a*) a Varian 3770 Capillary Gas Chromatograph, equipped with a flame-ionisation detector and a glass-capillary column (25 m, wall-coated with SE-30), and linked to a Varian CDS-111C Chromatography Data System; and (*b*) a Pye 104 Chromatograph (converted with Pye–Unicam accessories into a capillary gas chromatograph), equipped with a flame-ionisation detector and a glass-capillary column (25 m, wall-coated with SE-30).

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