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OLIGOMERIC POLYPHLOROGLUCINOLS FROM *FUCUS VESICULOSUS*: PHOTOPLATE MASS SPECTROMETRIC INVESTIGATION*

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Key Word Index—*Fucus vesiculosus*; Fucaceae; algae; photoplate; mass spectrometry; polyphenols; phloroglucinol; tannins; physodes.

Abstract—*Fucus vesiculosus* accumulates polyphenols consisting of four–seven phloroglucinol units. These 'oligomers' are primarily ether-linked; no evidence could be found for entirely phenyl-linked 'oligomers' having more than four phloroglucinol units, nor for any 'oligomer' with more than three phenyl linkages, nor for vicinally trihydroxylated polyphloroglucinols.

INTRODUCTION

Marine brown algae (class Phaeophyceae) accumulate a variety of low-, intermediate- and high MW phloroglucinol-based polyphenols containing both phenyl and phenoxy units[1]. Several of the low-MW polyphloroglucinols have been isolated and characterized, including phloroglucinol itself[2, 3], 'dimers' joined by phenyl bonds (e.g. 2, 2′, 4, 4′, 6, 6′-hexahydroxybiphenyl) [2, 4] or by ether bonds (e.g. 2, 3′, 4, 5′, 6 - pentahydroxybiphenyl ether) [5], 'trimers' (e.g. 2, 2′, 4, 6, 6′ - pentahydroxy - 4′ - (2, 4, 6 - trihydroxyphenoxy)biphenyl)[1, 6], 'tetramers'[1, 6], etc. At the other extreme, a high-MW 'polymeric' polyphloroglucinol fraction not diffusible during dialysis has been purified and examined spectroscopically (IR, ^{1}H- and ^{13}C NMR), and by capillary column GC-EIMS after calcium–ammonia degradation [Ragan, M. A.; and Ragan, M. A., McInnes, A. G., Walter, J. A., Smith, D. G. and Craigie, J. S., unpublished results]. Individual 'oligomeric' polyphloroglucinols intermediate in MW between these extremes cannot usually be resolved by TLC or HPLC, and consequently have received attention only when one isomeric 'oligomer' happens to be unusually abundant (e.g. ref. [7]).

An alternative approach, not requiring the separation of individual 'oligomers' prior to characterization, could provide more comprehensive and direct information on this fraction. Stepwise distillation of suitably derivatized polyphloroglucinols directly into high resolution mass spectrometry does not require prior separation of 'oligomers', but presents constraints regarding volatility, efficiency of derivative formation and MW range. Methylation of polyphloroglucinols prior to mass spectrometry[8] produces volatile derivatives of relatively low MW, but unavoidably methylates some of the phloroglucinyl ring carbons, producing multiple derivatives from each isomer. On the other hand, trimethylsilylation yields single, volatile derivatives, albeit of relatively high MW. The multiple exposure photoplate mass spectrometry procedure described earlier[9] allows mass assignments of high MW ions, and has now been applied to the analysis of trimethylsilylated 'oligomeric' polyphloroglucinols from the brown alga *Fucus vesiculosus* L.

RESULTS AND DISCUSSION

Preliminary experiments with a variety of polyphenols [1,2-, 1,3- and 1,4-dihydroxybenzenes; 1,2,3- and 1,3,5-trihydroxybenzenes; 2, 2′, 4, 4′, 6, 6′-hexahydroxybiphenyl; 2, 2′, 4, 6, 6′-pentahydroxy - 4′ - (2,4,6 - trihydroxyphenoxy)biphenyl] indicated that trimethylsilylation was essentially quantitative under the conditions employed, that the expected molecular ion (with its isotopic ions) was always by far the most intense ion observed (100%), and that only low levels ($\leq 3\%$) of [M − 15 m/z] (−Me) and [M − 89 m/z] (−OTMS) fragmentation occurred. Loss of TMS with proton transfer ([−72 m/z]), which was distinguished from incomplete trimethylsilylation by GC/MS, was rarely observed (and then only at intensities $\leq 1\%$) with these reference compounds.

Distillation of the trimethylsilylated 'oligomer' fraction in the mass spectrometer probe at increasing temperatures yielded four series of ions detected by electron multiplier (Table 1): above *ca* 145°, m/z 1362, 1290, 1218 and 1146, from 'tetrameric' polyphloroglucinol TMS ethers; above *ca* 165°, m/z 1630, 1558, 1486, 1414, 1342 and 1270, from 'pentamers'; above *ca* 185°, m/z 1898, 1826, 1754, 1682, 1610 and 1538, from 'hexamers'; and above *ca* 220°, m/z 2094,

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2022, 1950 and 1878, from 'heptamers'. The corresponding doubly charged ions and [M − 15 *m/z*] fragments were of much lower intensities. The masses of these ions could be assigned more accurately from the photoplate data (Table 1). Ions of *m/z* 270, 342, 610 and 682, also observed on the photoplate (Table 1), probably represent monomeric and dimeric phloroglucinyl fragments derived from larger 'oligomers'; if so, the absence of ions at *m/z* 934, 950 and 1022 suggests that terphenyl side chains are not common among the 'oligomeric' polyphloroglucinols. No evidence was found for entirely phenyl-linked phloroglucinols with DP > 4, nor for any 'oligomer' with >3 phenyl linkages, nor for the vicinally trihydroxylated polyphloroglucinols reported [1] in *Bifurcaria bifurcata, Cystoseira baccata, C. tamariscifolia, Halidrys siliquosa* and *Sargassum muticum*.

A more detailed analysis of these data depends on whether the 'oligomer' TMS ether ions (Table 1) are: (a) molecular ions of fully trimethylsilylated molecules; (b) incompletely derivatized; or (c) fragments of larger molecules. Several lines of evidence point to the former (a) interpretation: (i) the trimethylsilylation- and EIMS-behaviour of reference polyphenols and of lower-MW algal polyphloroglucinols under the same conditions (see above); (ii) the lack of evidence for loss of OTMS ([M − 89 *m/z*]), which would have been easily distinguished from loss of TMS (with proton transfer) and a methyl group ([M − 87 *m/z*]); (iii) the absence of metastable ions; (iv) the correspondence of the most intense ions in each series with polyphloroglucinol isomers having zero or one phenyl linkages; and (v) the presumed nonvolatility of partially derivatized compounds or (thermal) fragments having numerous free hydroxyl groups. However, the ions at *m/z* 1074, 1270, 1342, 1466, 1538, 1610, 1806 and 1878, which cannot be explained as fully trimethylsilylated derivatives of polyphloroglucinols of any known structural type, suggest that some incomplete derivatization, thermal decomposition and/or dibenzofuran formation[3, 10] occur-

Table 1. Polyphloroglucinol-TMS ether ions in the photoplate mass spectrum of trimethylsilylated *F. vesiculosus* 'oligomer' fraction

Degree of polymerization	Expected mass (monoisotopic)	Experimental mass (monoisotopic)	Relative abundances with EMD*	TMS ether units	Diaryl ether bonds	Direct (phenyl) linkages
1	342.150	342.144	0%	(3)†	(0)†	(0)†
	270.111	270.112	0	(2)	(0)	(0)
2	682.285	682.270	0	(6)	(0)	(1)
	610.245	610.264	0	(5)	(1)	(0)
4	1362.55	—	11	12	0	3
	1290.51	1290.60	66	11	1	2
	1218.47	1218.57	100	10	2	1
	1146.44	1146.47	50	9	3	0
	1074.40	—	17	(8)	(3)	(0)
5	1630.65	—	5	14	1	3
	1558.61	1558.54	27	13	2	2
	1486.57	1486.53	100	12	3	1
	1414.53	1414.51	80	11	4	0
	1342.49	1342.49	34	(10)	(4)	(0)
	1270.45	1270.46	6	(9)	(4)	(0)
6	1898.74	—	≤1	16	2	3
	1826.70	(+)‡	20	15	3	2
	1754.66	1754.61	58	14	4	1
	1682.63	1682.63	100	13	5	0
	1610.59	1610.63	65	(12)	(5)	(0)
	1538.55	1538.61	14	(11)	(5)	(0)
	1466.51	—	≤1	(10)	(5)	(0)
7	2094.80	—	19	17	4	2
	2022.76	(+)‡	59	16	5	1
	1950.72	1950.79	100	15	6	0
	1878.68	1878.77	67	(14)	(6)	(0)
	1806.64	—	18	(13)	(6)	(0)

*Electron multiplier detection; probe temps. 179° (tetramers), 226° (pentamers), 257° (hexamers and heptamers).

†Numbers in parentheses indicate TMS ether unit, diaryl ether bond, or phenyl linkage equivalents for ions probably arising from incomplete derivatization or thermal decomposition of 'oligomeric' polyphloroglucinols.

‡Weak line present.

red. Resolution of this point must await the separation and individual mass spectral characterization of the numerous isomers involved, perhaps by combined HPLC–mass spectrometry.

The 'polymeric' fraction (MW > 10^4) from *F. vesiculosus* has recently been shown to contain several times as many ether as phenyl linkages [Ragan, M. A., McInnes, A. G., Walter, J. A., Smith, D. G. and Craigie, J. S., unpublished results]. The much smaller 'oligomeric' fraction examined here (MW 498–870) is likewise primarily ether-linked, making it possible that one of these fractions is a biogenetic precursor to the other.

EXPERIMENTAL

Fucus vesiculosus L., together with a small amount of *F. distichus* L. subsp. *edentatus* (Pyl.) Powell, (10.5 kg fr. wt) was collected on 14 January 1975 at Fink Cove (Halifax Co., N.S.), cleaned of macroscopic epiphytes, and extracted with 85% EtOH for 60 hr at 3° under N_2. The extract was evaporated *in vacuo*, filtered and dialysed first against 80% EtOH (briefly), then 50% EtOH, then H_2O. The latter two dialysates were combined, evaporated *in vacuo*, redissolved in MeOH and fractionated on a silicic acid (Mallinckrodt)–Si gel (Baker) (1 : 1) column (increasing gradient of MeOH in $CHCl_3$). Fractions containing 'oligomeric' phloroglucinols were collected, and 'oligomers' were further separated from lower- (DP < 4) and higher-MW congeners by Si gel TLC (MeOH–$CHCl_3$). TMS ethers were formed using 'Tri-Sil' (Pierce Chemical Co.)[11].

Direct probe EI (70 eV, 11 kG) was done on a Dupont-CEC 21-110B MS equipped with electron multiplier and photoplate detection systems. Photoplate exposures (using Ionomet photoplates) were made in four steps: (i) with the MS accelerating voltage at ≈2 kV, the trimethylsilylated sample was distilled by increasing the probe temp. from 59 to 320° over 46 min; (ii) immediately thereafter, reference tris(perfluoroheptyl) - *s* - triazine (Peninsular Chemresearch) was introduced via the indirect sample introduction oven for 3 min on the same exposure; (iii) the photoplate was then shifted to an adjacent band position for a 3 min exposure of the reference; and (iv) the photoplate was returned almost to the original position (offset by ≈0.2 band width), and a 3 min exposure of the reference was made at ≈4 kV. Comparison of reference lines gave the actual '4 kV–2 kV' ratio = 1.991908 ± 0.000029 (n = 8 pairs), allowing the assignment of pseudomasses[9]. The total integrated ion current of the sample was 1430 pC. The developed photoplates were scanned with a Gaertner M1205 PC microcomparator-densitometer controlled through a Varian 620/L-100 minicomputor; the time–opacity data so acquired were reformatted on this minicomputer, then transferred to a Finnigan/INCOS data system[12, 13]. Data from repeated scans of the photoplate were merged, then corrected for a systematic error (361.3 ± 30.3 ppm, directly proportional to mass) arising from a drift in the kinetic energy of the ion beam during the exposure caused, most probably, by progressive contamination of the ionization chamber and focussing lenses with material derived from the sample. The corr. data were further analysed using modified INCOS software[13].

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