Note

5-*n*-Alkylresorcinols as biomarkers of sedges in an ombrotrophic peat section

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Abstract

5-*n*-Alkylresorcinols with alkyl chain lengths varying from C_{19} to C_{25} were identified solely in bog-forming plant species classified as sedges (*Eriophorum vaginatum*, *E. angustifolium*, *Trichophorum cespitosum* and *Rhynchospora alba*). These compounds were then identified throughout the peat deposit on which these plants grow. Total abundances of 5-*n*-alkylresorcinols were found to reflect macrofossil abundances of sedges in a 40 cm peat profile from Bolton Fell Moss, Cumbria, UK, thereby complementing macrofossil data in palaeoclimate studies. © 2002 Published by Elsevier Science Ltd.

1. Introduction

The remains of peat-forming plants can be used to determine past communities growing on the surface of a peat bog. These macrofossil records can be used to estimate which plants were dominant during the development of a peat bog and, as the plants respond to different growth conditions, such records can be used as a proxy for climatic variation (Barber, 1985). However, the preservation of these macrofossils is not always adequate to accurately determine past plant communities and therefore other techniques must be sought, especially in the case of highly humified peats.

Attempts have been made to use lipid biomarkers to determine dominant plant species and relate these to environmental reconstruction studies (e.g. Farrimond and Flanagan, 1996; Ficken et al., 1998; Nott et al., 2000), but the studies were of limited success owing to the low specificity of the biomarkers used (e.g. *n*-alkanes, fatty acids, long-chain alcohols). However, our recent work has shown that even simple compounds like tricosane (*n*-C_{23}), when combined with compound-specific δD stratigraphy, can aid palaeoenvironmental reconstruction (Xie et al., 2000).

5-*n*-Alkylresorcinols have previously been identified in lipid extracts from rye, barley grains, wheat bran (Kozubek, 1985; Tyman, 1991), cashew nuts (e.g. Tyman, 1980) and Protaceae (Cirigottis et al., 1974) and in some bacteria (Reusch and Sadoff, 1979; Kozubek et al., 1996) and algae (Zarnowski et al., 2000). Their function in plants is uncertain, but in the epicuticular waxes of *Hordeum vulgare* (cereal) seeds they appear to be responsible for resistance to pathogenic fungi (García et al., 1997). They have also been associated with the fungal resistance of unripe mango fruit (Tsuge et al., 1991).

Here we report the presence of 5-*n*-alkylresorcinols in sedges and peat from an ombrotrophic mire and discuss their potential use in palaeoclimate reconstruction.

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2. Samples and methods

A 50 cm peat profile was taken, using a monolith tin, from the centre of Bolton Fell Moss, Cumbria, UK. Samples of the modern day covering vegetation were collected and stored, along with the peat monolith, in a freezer at −20 °C until required for analysis. Macro-fossil stratigraphy of an adjacent profile from the bog was assigned on the basis of microscopic quantification (Barber et al., 1994).

The core was sampled by taking contiguous 1 cm slices (discarding the outer layer to eliminate possible contamination) over the top 40 cm, which were then freeze dried. Dried samples were ground to pass through a 0.5 mm sieve before Soxhlet (peat) or ultrasonic (plant) extraction with DCM/acetone (9:1 v/v). The total lipid extracts were separated into acidic and neutral fractions using amino propyl Bond Elut cartridges (Varian Chromatography). The neutral components were eluted with DCM/isopropanol (2:1 v/v), then further fractionated by column chromatography (silica gel 60) using successive elution with hexane, hexane/DCM (9:1 v/v), DCM, DCM/MeOH (1:1 v/v) to yield the total alcohol fraction and MeOH to yield polar compounds. Gas chromatography (GC) and gas chromatography-mass spectrometry (GC/MS) were performed as described previously (Avsejs et al., 1998). Quantification of resorcinols was based on m/z 268 mass chromatograms and comparison with 5-n-pentylresorcinol as internal standard.

3. Results

The total alcohol fraction was obtained from 21 plant specimens (all present either on the surface or identified within peat sampled from Bolton Fell Moss) and components identified by GC and GC/MS. Plant species included eight species of *Sphagnum* moss, three other types of bog moss, *Cladonia* sp. (lichen), *Andromeda polifolia* (bog rosemary), *Calluna vulgaris* (common heather), *Erica tetralix* (bog heather), *Vaccinium oxycoccus* (cranberry), *Empetrum nigrum* (crowberry), *Eriophorum*

vaginatum (sheathed cotton sedge), *E. angustifolium* (many headed cotton sedge), *Trichophorum cespitosum* (deer sedge) and *Rhynchospora alba* (white beaked sedge).

GC/MS analysis of the total alcohol fraction of all the sedge species revealed an homologous series of components characterised by mass spectra of the form shown in Fig. 1. Fig. 1a shows the EI mass spectrum of an authentic sample of 5-n-pentylresorcinol (Sigma-Aldrich, UK), as its bis-TMS ether, which is characterised by fragment ions at m/z 268 (base peak), m/z 310, m/z 281, m/z 73 ($[(CH_3)_3Si]^+$) and $M^{+\cdot}$ 324. Fig. 1b shows a mass spectrum typical of 5-n-alkylresorcinols found in plant and peat samples. The spectrum is identical in all respects to that of the authentic 5-n-pentylresorcinol except that $M^{+\cdot}$ appears at m/z 520 indicating the presence of a longer, C_{19}, n-alkyl chain. Both plant and peat extracts displayed a regular series of components exhibiting analogous spectra but displaying $M^{+\cdot}$ ions at m/z 534, 548, 562, 576, 590 and 604, indicating the presence of additional C_{20} to C_{25} n-alkyl substituted components. The m/z 268 base peak, which is common to all 5-n-alkylresorcinols where the alkyl chain length is $>C_2$, provides the basis for profiling the distributions of these compounds, e.g. Fig. 2.

The 5-n-alkylresorcinols were detected solely in those plants classified as sedges (*E. vaginatum*, *E. angustifolium* etc.) with their distributions varying with species (Table 1). For example, in *E. vaginatum* the 5-n-alkylresorcinols ranged from C_{19} to C_{25}, with the major homologue bearing an n-henicosanyl chain, whilst in *E. angustifolium* and *T. cespitosum* the alkyl substituents ranged from C_{21} to C_{27}, maximising at C_{25}. Therefore, in view of their absence from the other major peat forming plants, i.e. *Sphagnum*, and their high abundance and characteristic distributions, 5-n-alkylresorcinols are of value as bio-markers for sedges in peat, potentially even at the species level, provided these compounds are preserved.

The 5-n-alkylresorcinols with substituent chains of length C_{19}, C_{21}, C_{23} and C_{25} were found to be present at all depths within the peat monolith. Odd carbon number homologues dominated throughout, although distributions varied somewhat down the 40 cm core (Table 2). In the upper parts of the profile the C_{21} and

Table 1
5-n-Alkylresorcinol compositions of modern plants. Abundance data from m/z 268 mass chromatograms

Plant species	Alkyl resorcinols µg g^{-1} dry weight									
	C_{19}[a]	C_{20}	C_{21}	C_{22}	C_{23}	C_{24}	C_{25}	C_{26}	C_{27}	Total
E. vaginatum	38.5	0.1	133.1	1.5	48.3	0.1	17.7	0.1	0.1	240
E. angustifolium	nd[b]	nd	9.5	0.1	49.6	8.3	55.0	2.4	13.9	139
T. cespitosum	nd	nd	11.3	0.1	48.2	5.9	82.2	5.0	24.1	177
R. alba	nd	nd	17.6	0.1	22.7	0.1	5.8	nd	nd	46

[a] C_n denotes carbon number of n-alkyl substituents.
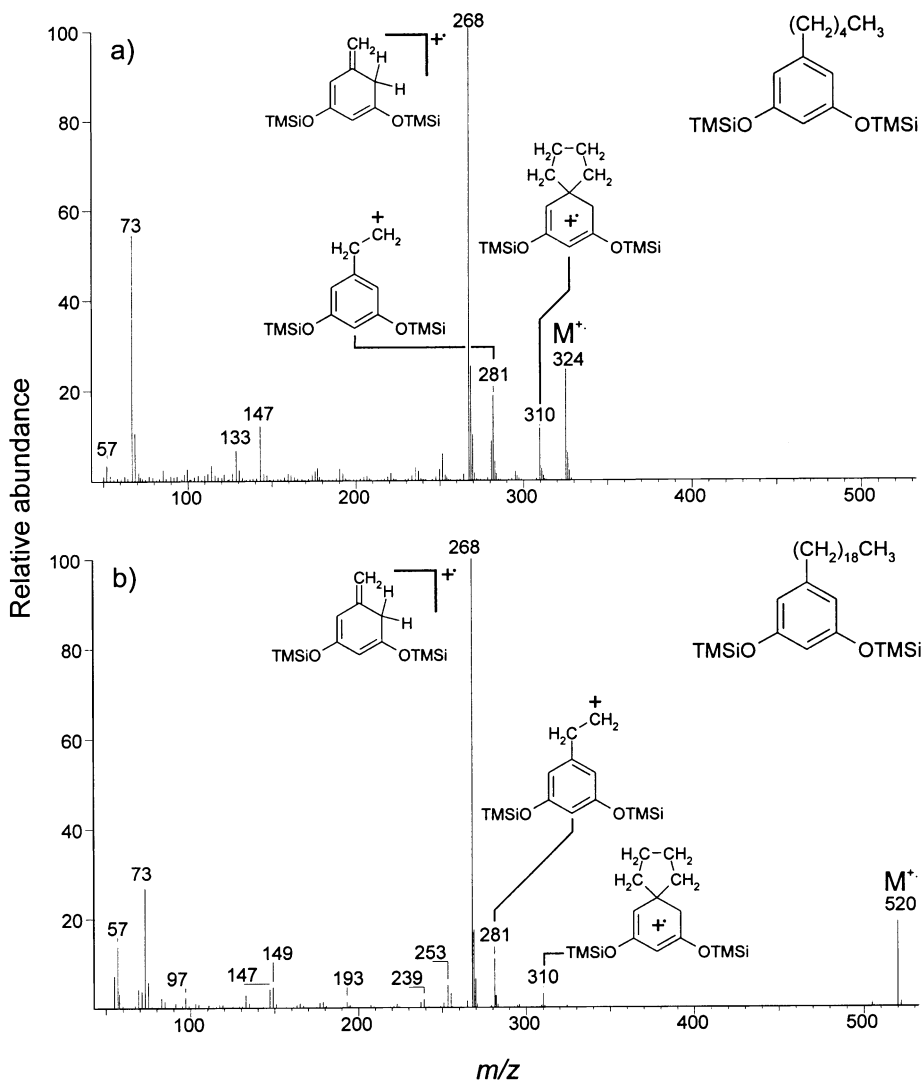[b] nd = Not detected.

Fig. 1. Mass spectra of (a) 5-*n*-pentylresorcinol (Sigma-Aldrich, UK) and (b) 5-*n*-nonadecylresorcinol (peat at depth 21 cm) as bis-trimethylsilyl ethers.

C_{19} *n*-alkylsubstituents dominate (0–7 and 10–24 cm, respectively), while in the lower section (25–30 cm) the C_{21} and C_{23} members are predominant. In the deeper section the 25–26, 30–31 and 39–40 cm horizons showed bimodal distributions maximising at C_{19} and C_{23}. A comparison of the variations of total 5-*n*-alkylresorcinol concentration and sedge macrofossil abundances is depicted in Fig. 3 and shows a strong positive correlation to exist between these two records.

4. Discussion and conclusions

The results presented above confirm that 5-*n*-alkylresorcinols with alkyl substituent chain lengths ranging from C_{19} to C_{25} were present at all depths in the peat profile. Interestingly, their distributional maxima varied, with coherent trends being seen between contiguous samples taken within certain depth ranges. Analysis of three sedge species revealed a characteristic distribution for the most common peat-forming sedge at Bolton Fell Moss, *E. vaginatum,* in comparison to the other species examined. *E. vaginatum* contained a distribution ranging from C_{19} to C_{25} maximising at C_{21}, a pattern similar to the those seen in the upper section of the profile, suggesting a direct contribution from this species. At the depth of 8–9 cm, however, a change in pattern was seen whereby the relative abundance of the C_{19} component increased with the maximum becoming the C_{23} homologue, a distribution similar to that of *R. alba* (Table 1)
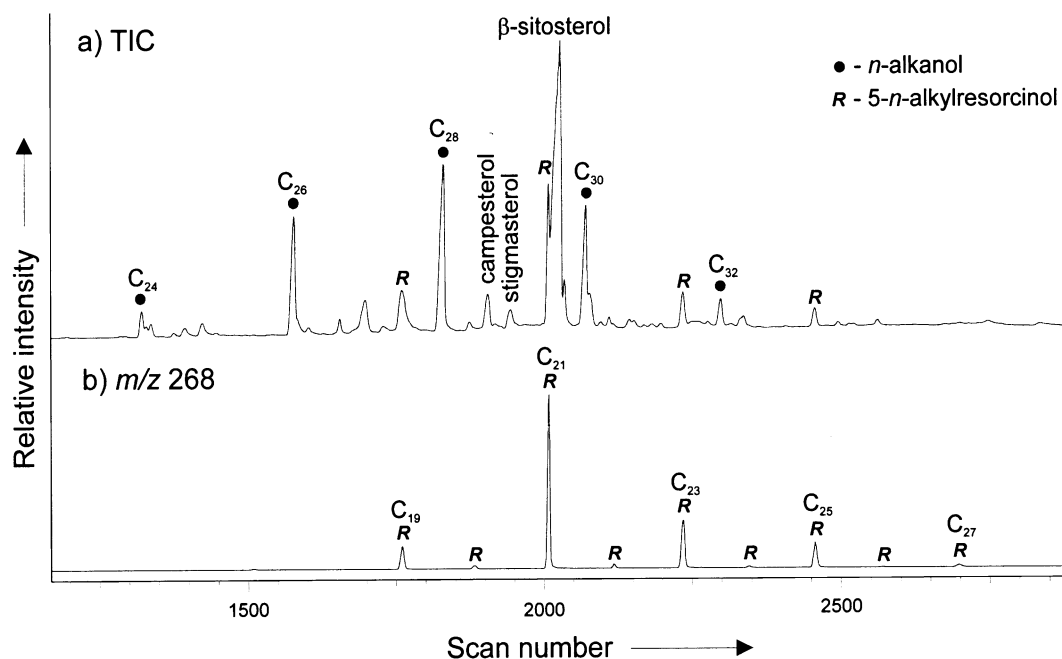
Fig. 2. Partial (a) total ion current (TIC) and (b) mass chromatogram of m/z 268 of the total alcohol fraction (as TMSi ethers) of *E. vaginatum*. $C_n = n$-alkyl C number.

Table 2
Relative abundances of the various homologues in the 5-n-alkylresorcinol fractions of the various peat horizons sampled from Bolton Fell Moss; abundance data from m/z 268 mass chromatograms

Sample depth (cm)	Alkyl resorcinols (%)			
	C_{19}[a]	C_{21}	C_{23}	C_{25}
0–1	6.1	89.4	2.8	1.8
2–3	3.5	91.3	4.7	0.5
4–5	5.1	65.2	27.3	2.5
6–7	9.6	47.4	37.6	5.4
8–9	27.2	31.9	33.1	7.8
10–11	41.0	28.7	13.1	17.2
13–14	49.2	25.2	20.8	4.9
15–16	69.9	13.9	12.2	4.0
16–17	76.4	3.0	17.0	3.6
17–18	56.7	20.9	22.4	nd[b]
18–19	83.7	7.4	6.7	2.2
19–20	41.8	37.3	13.4	7.5
21–22	54.6	31.2	7.3	6.9
23–24	54.0	19.0	20.8	6.2
25–26	39.2	17.7	32.5	10.6
27–28	22.0	41.0	26.7	10.4
30–31	33.0	8.0	40.7	18.3
32–33	20.1	44.4	26.0	9.5
34–35	28.2	16.5	38.7	16.5
36–37	18.9	47.3	24.3	9.5
39–40	38.7	11.0	38.0	12.3

[a] C_n denotes carbon number of n-alkyl substituents.
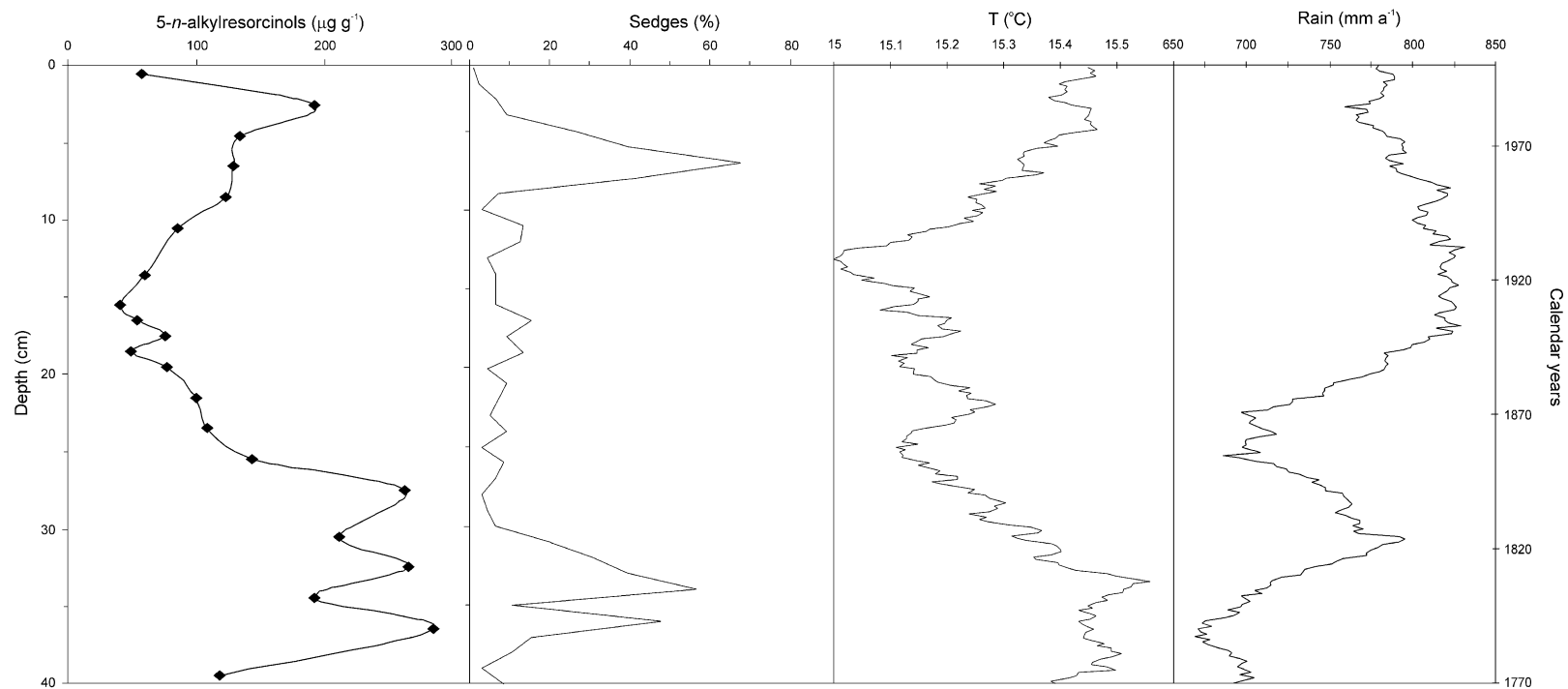[b] nd = Not detected.

Fig. 3. Plot of the abundance of 5-*n*-alkylresorcinols together with the total identified sedges from the peat core between 0 and 40 cm. The calendar dates, mean summer temperature and precipitation records are taken from Xie et al. (2000). Ten samples from the top 30 cm were submitted for ^{210}Pb dating (Flynn, 1968; Robbins, 1978).

if the C_{19} homologue is ignored. Thereafter, the prominence of 5-*n*-nonadecylresorcinol (C_{19}) cannot be explained by a direct input from a sedge, based on our analyses of the modern specimens common to this peat bog.

One possible explanation for the apparently anomalously high relative abundance of the C_{19} component is that an unknown species of sedge, producing such a mixture of resorcinols, dominated over this period of peat formation. Given the detailed macrofossil analyses that have been undertaken previously from this site (Barber et al., 1998), an unidentified sedge species is unlikely. Another explanation is that an entirely different source organism has contributed 5-*n*-alkylresorcinols to the peat. For example, such compounds have been reported as being constituents of certain bacteria, in particular *Azotobacter chroococcum* and *Pseudomonas* species (Reusch and Sadoff., 1979; Kozubek et al., 1996) and the C_{19}, C_{21} and C_{23} homologues are reported to be components of the latter. Thus, there exists the possibility that the resorcinols seen in the peat have a dual source and that the enhanced abundance of the C_{19} homologue observed are a direct result of a contribution from an as yet undefined bacterial source.

The concentrations of resorcinols reported in bacteria (2000–8000 µg g^{-1}) are substantially higher than those recorded for the plants and in the peat studied herein (Kozubek et al., 1996). However, the concentrations of the resorcinols in the peat are consistent with a plant source, taking into account such factors as selective preservation of recalcitrant biomarkers and degradative losses of labile plant components, e.g. carbohydrates, via microbial degradation. The strong positive correlation that exists between the total 5-*n*-alkylresorcinol concentration and sedge macrofossil abundances (Fig. 3) appears to rule out a substantial bacterial contribution throughout the profile, although there may be some localised inputs from this source. This conclusion is supported by our previously reported *Sphagnum* biomarker record for this peat profile based on *n*-alkanes (Nott et al., 2000) which mirrors the data obtained for sedges reported herein.

It is especially noteworthy that the sedge-specific biomarker and macrofossil records show a strong positive correlation with the mean summer temperature recorded at Carlisle over the past 220 years. In particular the trend toward low sedge abundance in the later part of the 19th and early part of the 20th centuries coincides with a minimum in the 5-*n*-alkylresorcinol concentration record across the period known as the Second Little Ice Age (Libby et al., 1976). Precipitation records for the same period show less correlation with the resorcinol record, and this suggests that the plants respond more to temperature driven changes than precipitation. The combined biomarker and macrofossil stratigraphy reported herein indicate that 5-*n*-alkylresorcinols can be

valuable biomarkers for sedges in highly humified peats and other sedimentary deposits where macrofossils are absent.

References

Avsejs, L.A., Nott, C.J., Maxwell, J.R., Evershed, R.P., 1998. Hydroxy and ketonic androstanes: a new class of sterol diagenetic product in peat. Organic Geochemistry 28, 749–755.

Barber, K.E., 1985. Peat stratigraphy and climatic changes: some speculations. In: Tooley, M.J., Sheail, G.M. (Eds.), The Climatic Scene: Essays in Honour of Gordon Manley. Allen and Unwin, London, pp. 175–185.

Barber, K.E., Chambers, F.M., Maddy, D., Stoneman, R., Brew, J.S., 1994. A sensitive high-resolution record of late Holocene climatic change from a raised bog in northern England. The Holocene 4, 198–205.

Barber, K.E., Dumayne-Peaty, L., Hughes, P., Mauquoy, D., Scaife, R., 1998. Replicability and variability of the recent macrofossil and proxy-climate record from raised bogs: field stratigraphy and macrofossil data from Bolton Fell Moss and Walton Moss, Cumbria, England. Journal of Quaternary Science 13, 515–528.

Cirigottis, K.A., Cleaver, L., Corrie, J.E.T., Grasby, R.G., Green, G.H., Mock, J., Nimgirawath, S., Read, R.W., Ritchie, E., Taylor, W.C., Vadasz, A., Webb, W.R.G., 1974. Chemical studies of the Protaceae. VII. An examination of the woods of 17 species for resorcinol derivatives. Australian Journal of Chemistry 27, 345–355.

Farrimond, P., Flanagan, R.L., 1996. Lipid stratigraphy of a Flandrian peat bed (Northumberland, UK): comparison with the pollen record. The Holocene 6, 69–74.

Ficken, K.F., Barber, K.E., Eglinton, G., 1998. Lipid biomarker, δ^{13}C and plant macrofossil stratigraphy of a Scottish montane peat bog over the last two millennia. Organic Geochemistry 28, 217–237.

Flynn, W.W., 1968. Determination of low levels of polonium-210 in environmental materials. Analytica Chemica Acta 43, 221–227.

García, S., García, C., Heinzen, H., Moyna, P., 1997. Chemical basis of the resistance of barley seeds to pathogenic fungi. Phytochemistry 44, 415–418.

Kozubek, A., 1985. Isolation of 5-*n*-alkyl, 5-*n*-alkenyl- and 5-*n*-alkdienyl-resorcinol homologues from rye grains. Acta Alimentaria Polonica 9, 185–189.

Kozubek, A., Pietr, S., Czerwonka, A., 1996. Alkylresorcinols are abundant lipid components in different strains of *Azotobacter chroococcum* and *Pseudomanas* spp. Journal of Bacteriology 178, 4027–4030.

Libby, L.M., Pandolfi, L.J., Payton, P.H., Marshal III, J., Becker, B., Sienbenlist, V.G., 1976. Isotopic tree thermometers. Nature 261, 284–288.

Nott, C.J., Xie, S., Avsejs, L.A., Maddy, D., Chambers, F.M., Evershed, R.P., 2000. *n*-Alkane distributions in ombrotrophic mires as indicators of vegetation change related to climatic variation. Organic Geochemistry 31, 231–235.

Reusch, R.N., Sadoff, H.L., 1979. 5-*n*-Alkylresorcinols from encysting *Azobacter vinelandii*: isolation and characterization. Journal of Bacteriology 139, 448–453.

Robbins, J.A., 1978. Geochemical and geophysical applications of radioactive lead. In: Nriagu, J.O. (Ed.), The Biogeochemistry of Lead in the Environment. Part A. Elsevier/North Holland Biomedical Press, Amsterdam, pp. 285–293.

Tsuge, N., Mizokami, M., Imai, S., Shimazu, A., Seto, H., 1991. Adipostatin-A and adipostatin-B, new inhibitors of glycerol-3-phosphate dehydrogenase. Journal of Antibiotics 45, 886–991.

Tyman, J.H.P., 1980. Cultivation, processing and utilisation of the cashew. Chemistry and Industry (London), 59–62.

Tyman, J.H.P., 1991. The chemistry of non-isoprenoid phenolic lipid. In: Atta-ur-Rahman (Ed.), Studies in Natural Products Chemistry, Vol 9. Elsevier, Amsterdam, pp. 313–381.

Xie, S., Nott, C.J., Avsejs, L.A., Volders, F., Maddy, D., Chambers, F.M., Gledhill, A., Carter, J.F., Evershed, R.P., 2000. Palaeoclimate records in compound-specific δD values of a lipid biomarker in ombrotrophic peat. Organic Geochemistry 31, 1053–1057.

Zarnowski, R., Suzuki, Y., Esumi, Y., Pietr, S., 2000. 5-*n*-Alkylresorcinols from the green microalga *Apatococcus constipatus*. Phytochemistry 55, 975–977.