Classification of Grass Fructans by ^{13}C NMR Spectroscopy

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Fructans have been extracted with water from rough meadowgrass and bromegrass and from roots of reed canarygrass and quackgrass. The fructans were precipitated by the addition of ethanol to the extracts. ^{13}C NMR spectroscopy showed that the reed canarygrass fructan, like that from rough meadowgrass, is a linear, long-chain levan. The spectrum of the fructan from quackgrass is in accordance with an extensively branched structure, whereas the bromegrass fructan is a more moderately branched short-chain levan.

The reserve polysaccharides in grasses of temperate origin are fructans. The majority of the grass fructans are linear polysaccharides with β-2,6-linkages (levans),[1] but the degree of polymerisation varies (Fig. 1). An investigation of the solubility of fructans from a great number of grass species resulted in the classification of the different grasses as long- or short-chain fructan accumulators,[2] though short- and long-chain fructans may be present in the same plant in different parts and at different growth stages. Some of the grasses that accumulate long-chain fructans have been found to lack oligosaccharides of the fructosylsucrose type and their homologues,[3,4] and since such oligosaccharides are found in a number of short-chain fructan-accumulating grasses,[5,6] differences in the biosynthesis leading to the two types of fructans have been suggested.[6]

In addition to grasses accumulating linear fructans, species that accumulate branched fructans also exist. An example is quackgrass [*Agropyron repens* (L.) Beauv.].[7] Also, wheat (*Triticum aestivum* L.) and rye (*Secale cereale* L.) are reported to contain branched fructans.[8]

^{13}C NMR spectroscopy has been used to show that a grass fructan from ryegrass (*Lolium perenne* L.) is a short-chain, linear levan,[9] and spectra of fructans of inulin (β-2,1-linked) and levan type show characteristic differences.[9–15] These observations suggest the use of ^{13}C NMR spectroscopy in the classification of grass fructans as short- or long-chain, and as branched or linear levans. In this paper we report the application of this method to fructans from four grass species and the comparison of the results with those from methylation analyses.

Results and discussion

The fructan fraction of rough meadowgrass (*Poa trivialis* L.) and bromegrass (*Bromus inermis* Leyss), and from roots of quackgrass and reed canarygrass (*Phalaris arundinacea* L.) was extracted with water from plant material that had been pre-extracted with 80 % ethanol. The fructans were precipitated from the water solutions by the addition of ethanol, and they were purified by repeated dissolution and precipitation. All resonances observed in the ^{13}C NMR spectra could be assigned to the carbons of the fructans (Table 1). The low-intensity signals from the ring carbons of the terminal glucose unit in the fructans are well separated from those of the fructosyl carbons, and on the basis of the intensities of the glucosyl carbon signals relative to those from the fructosyl carbons, the fructans can be classified as short- or long-chain levans.

The proton-decoupled spectra of the fructan fractions from rough meadowgrass (Fig. 2) and reed canarygrass are very similar to that reported for the short-chain levan from ryegrass,[9] except for a much lower intensity of the resonances from the glucosyl carbons than in the ryegrass spectrum. This is in accordance with the classification of *poa* and *phalaris* species as long-chain frutan accumulators,[2] and that the fructans are linear levans. Methylation analyses, previously carried out for the *poa* fructan[16] and now for that from reed canarygrass, confirm this classification.

Spectra of slightly branched bacterial levans show a weak C-3 signal about 0.2 ppm downfield from the major levan C-3 signal.[11] In the spectrum of the quackgrass fructan (Fig. 2) the dominating C-3 signal appears 0.3 ppm downfield from the ordinary, weak levan C-3 signal. A weak C-3 signal is also observed with a chemical shift corresponding to 1-*O*-monosubstitution. It is obvious that C-3 correspond-

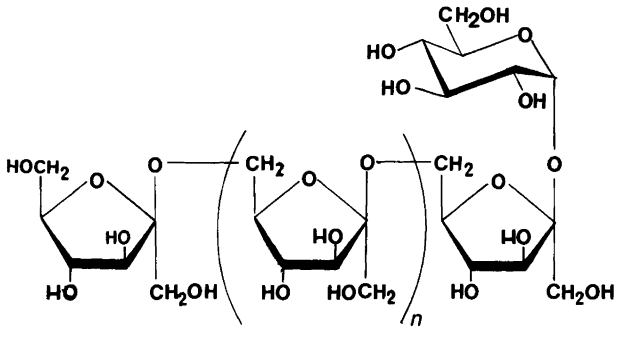

Fig. 1. Linear β-2,6-linked fructan (levan).

Table 1. ^{13}C NMR chemical shifts in ppm downfield from tetramethylsilane for β-D-fructofuranoside units of grass fructans and some model compounds. The assignments are tentative, based on those in Refs. 9–12.

Carbon	Compound					
	Sucrose	Isokestose (1-Kestose)	Inulin	Reed canary- and rough meadow-grass fructan	Quackgrass fructan	Bromegrass fructan
1a		62.0	61.8			
b				60.8		60.9
c					61.0–62.0[e]	61.0–62.0[e]
d	62.2	61.5			61.3	61.3
2a		104.6	104.1		103.9[f]	
b				105.1	104.9[f]	105.0
c					104.2	104.3
d	104.5	104.2			104.5	104.5
3a		77.7	77.9		78.1[f]	
b				77.2	77.3[f]	77.2
c					77.6	77.6
d	77.3	77.7			77.6	77.6
4a		74.9	75.2			75.5
b				76.1		76.0
c					75.7	75.8
d	74.8	75.5			75.2	75.1
5a		82.1	81.9			81.9
b				81.2		81.1
c					81.1	81.1
d	82.2	82.1			81.9	81.9
6a		63.3[g]	63.0			63.3[f]
b				64.3		64.2
c					64.0	64.0
d	63.2	63.2[g]			63.1	63.1

[a]1-*O*-Substitution. [b]6-*O*-Substitution. [c]1,6-Di-*O*-Substitution. [d]Unsubstituted (terminal) unit. [e]Unresolved from the other C-1 signals. [f]Weak. [g]Assignments might be reversed.

ing to 1,6-disubstitution and that corresponding to unsubstituted fructosyl units resonates with approximately the same chemical shift. In accordance with the previously reported methylation analysis of quackgrass fructan,[7] the spectrum reveals a highly branched structure with mainly 1,6-di-*O*-substituted and unsubstituted terminal fructosyl units in addition to a small number of 1-*O*- and 6-*O*-monosubstituted units in the fructan. The C-2 region of the spectrum is also in agreement with this structure, showing weak signals corresponding to levan (δ 104.9 ppm) and inulin (δ 104.0 ppm) resonances, and between them two major signals. The assignment of the signals in the spectrum to the respective carbon atoms is based on those previously reported by others for fructans and other fructosyl compounds.[9–12].

The bromegrass fructan gives a more complicated spectrum than the other fructans (Fig. 2). The signals found in the quackgrass fructan spectrum are observed, but the branching is obviously somewhat less extensive in this fructan since the signals characteristic of linear levans are of relatively higher intensity than in the quackgrass fructan spectrum. Signals characteristic of the carbons of 1-*O*-monosubstituted fructosyl units are, on the other hand, also very weak in this spectrum. Furthermore, the signals from the carbons of the terminal glucosyl unit are considerably more intense in this spectrum than in the other ones, and the bromegrass fructan is conclusively a short-chain levan with extensive, but not complete, branching.

The results presented support the use of ^{13}C NMR spectroscopy for the classification of the polysaccharides in the fructan fractions from grasses as short- or long-chain, and as branched or linear levans.

Experimental

General methods. Gas chromatography was carried out on a Perkin–Elmer F 11 gas chromatograph, equipped with a flame ionisation detector and a glass column (1.8 m×2 mm i.d.) filled with 3 % SE 30 on Chromosorb G AW-DMCS, the temperature program was 3° min^{-1} from 90 → 160 °C. Thin layer chromatography (TLC) was performed on silica gel G plates in: A, chloroform–methanol (15:1) and B,

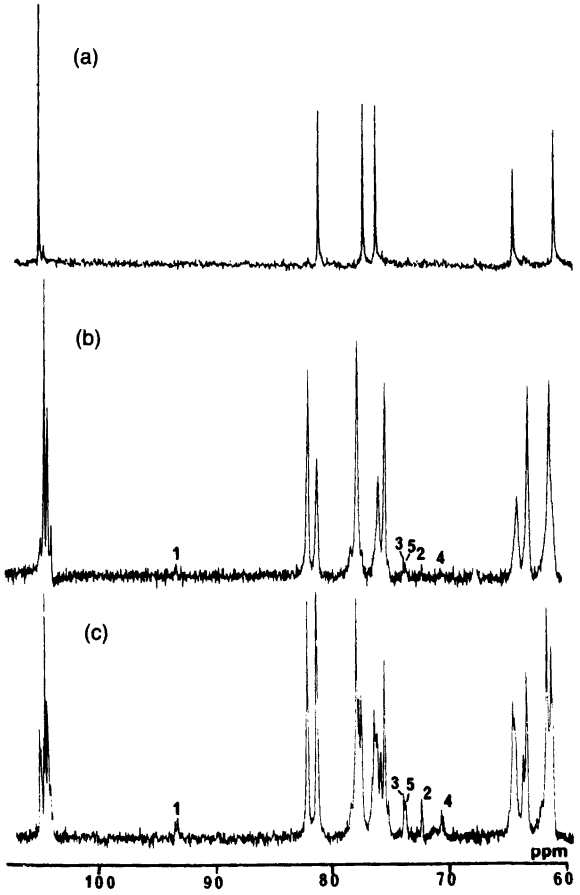

Fig. 2. Proton decoupled ^{13}C NMR spectra of fructans from (a) rough meadowgrass, (b) quackgrass and (c) bromegrass. The numbered signals originate from the respective carbons of the terminal glucose unit.

propanol–ethyl acetate–water (6:2:2). The compounds were detected with diphenylamine–aniline–phosphoric acid[17] and hydroxylamine–ferric chloride.[18] NMR spectra were recorded with a Jeol JNM-GX 270 spectrometer in deuterium oxide at 21 °C with 1,4-dioxane (δ 67.40) as an internal standard.

Isolation of the fructan fractions from plant material. The plant material was collected in Ås, southern Norway. From rough meadowgrass (10 g, harvested on June 13, 1989) and bromegrass (10 g, harvested August 5, 1989) the spikes were removed, and the rest of the plant material was cut into pieces and treated with aqueous 80 % ethanol (200 ml) on a boiling-water bath for a few min. The residues were washed once with a small volume of cold 80 % ethanol and then homogenised mechanically in hot acetone. Roots of reed canarygrass and roots of quackgrass (10 g, collected September 16, 1989) were treated briefly with 80 % ethanol on a boiling water-bath and then cut roughly into pieces and dried. The residues from the above treatments were extracted separately with water (100–200 ml) at room temperature for 3 h. The extract from quackgrass roots (150 ml) was shaken with chloroform (38 ml) and butanol (15 ml) to remove protein,[7] and the process was repeated 3 times. The water extracts from the different plants were then concentrated (to 20 ml or less) under reduced pressure at 40 °C, and the fructans were precipitated by the addition of 5 parts of ethanol. Redissolution of the precipitate in water and precipitation with ethanol was repeated until TLC (solvent B) showed no oligosaccharide in the precipitate. Finally, the material was freeze-dried.

Methylation analyses. The fructan fractions from reed canarygrass and bromegrass were methylated according to Hakomori.[19] The methylated fructans were hydrolysed in 60 % aqueous formic acid at 90 °C for 1 h and then, after removal of the formic acid and water under reduced pressure, the hydrolysis was completed in 0.01 M sulfuric acid for 45 min at 90 °C. The solutions were neutralised with Dowex 1 (HCO_3^-) ion-exchange resin, and the solvent was removed at reduced pressure. The *O*-methyl sugars from methylated reed canarygrass fructan were analysed by TLC (solvent A) and GLC after treatment with silver carbonate on Celite as described previously for the rough meadowgrass fructan,[16] showing the presence of 1,3,4-tri-*O*-methyl-D-fructose as the major methyl sugar and in addition, traces of 1,3,4,6-tetra-*O*-methyl-D-fructose and 2,3,4,6-tetra-*O*-methyl-D-glucose were detected. From the methylated bromegrass fructan were obtained (TLC) di-, tri- and tetra-*O*-methyl-fructose in roughly equimolar amounts.

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