ELSEVIER

Food Chemistry 73 (2001) 225–233

Food Chemistry

www.elsevier.com/locate/foodchem

Analytical, Nutritional and Clinical Methods Section

Structural analysis by ^{13}C-nuclear magnetic resonance spectroscopy of glucans elaborated by gum-producing bacteria isolated from palm wine

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Received 5 September 2000; accepted 28 September 2000

Abstract

The linkages of the glucans produced by palm wine bacteria in sterile palm sap and in sucrose broth were determined using ^{13}C nmr spectroscopy. The glucose units appeared to be linked α(-1–6) in the main chain. Therefore, the glucans are likely to be dextrans. There were branch linkages, and these differed between the genera of lactic acid bacteria (LAB) and even within one genus. However, branching by α(-1–3) was a feature common to all the dextrans of the three organisms employed. The dextran of *Leuconostoc dextranicum* appeared to branch mainly by α(1–3) linkages with minor α(1–4) ones; that of *Leconostoc mesenteroides*, mainly by α(-1–2) and that of the *Lactobacillus* spp. by only α(1–3) linkages. The organisms were found to elaborate more highly branched dextrans in sucrose broth than in palm sap probably due to nutrient differences, but the branch linkage types remained the same. The degree of branching did not appear to affect the viscosity. It was concluded that gums produced by palm wine glucan-producers were dextrans and that these different dextran-producing bacteria, in palm wine, each produced its own peculiar type of dextran in the beverage.

1. Introduction

Palm wine is an alcoholic beverage produced by the spontaneous yeast/lactic fermentation of the sugary sap of palms. It is usually white or milky in colour and the source of this white coloration had been assumed to be the yeast and bacteria cells therein (Okafor, 1977). Studies have however, shown that there are also gums in palm wine contributing to both its colour and consistency (Uzochukwu, Ngoddy & Balogh, 1994). Two major types of such gums were found in palm wine: one group composed entirely of glucose units and another containing only fructose units. When the different gum-producing bacteria isolated from palm wine were each made to produce gum in pure culture, analysis of the gums showed that the glucans were elaborated by one group of bacteria and the fructans by another (Uzochukwu et al. 1994). Elucidating the exact nature of the gums would make it possible to use the pure compound in the extension of palm wine or the formulation of its analogues. The present report presents the structural analysis by ^{13}C nmr of the glucans elaborated in pure culture by different gum-producing bacteria isolated from palm wine, in sterile sucrose medium and in sterile palm sap.

Colson, Jennings and Smith (1974) assigned the ^{13}C nmr spectra of several linear glucans by comparison with the spectra of glucose, some of its specifically *O*-methylated derivatives and a number of differently linked glucobioses and glucotrioses such as maltose (α-1–4); isomaltose (α-1–6); maltotriose (α-1–4,- α-1–6); nigerose (α-1–3) and laminaribiose (β-1–3). They were able to assign unambiguously, the chemical shifts of C1–C6 of linear α-1–6-linked glucans and establish the effect of branch linkages on the chemical shifts of the various carbon atoms. The elucidation of the composition, sequence and conformation of glucans with mixed linkages was achieved by the full assignment of these disaccharide and homoglucan resonances. The structures of dextrans established by methylation and by periodate oxidation, were shown to agree with that obtained in this way by ^{13}C nmr. Seymour et al. (1979a,

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0308-8146/01/$ - see front matter
PII: S0308-8146(00)00291-0

b,c,d,e,g) employed ^{13}C nmr spectroscopy to examine the structure of a series of dextrans and established that:

1. Linear dextran has six prominent ^{13}C-nmr resonances which they termed A, B, C, D, E, F.
2. Branching contributes additional resonances to the ^{13}C-nmr spectra of dextrans.
3. The positions of these additional resonances in the anomeric region (95–105 ppm) and the 80–85ppm region are diagnostic for the type of branch linkage present.
4. The relative peak heights of these diagnostic resonances resulting from branching are in general, proportional to the degree of branching.

The assignments in the present study are based mainly on the work of these two groups of workers.

2. Materials and methods

2.1. Cultures

All cultures used were gum-producers isolated from palm wine, identified and characterised as reported in Uzochukwu et al. (1994). They were maintained on MRS agar (Difco) slants at 4°C. The organisms were *Leuconostoc dextranicum* (34), *Leuconostoc mesenteroides* (35) and a *Lactobacillus* spp. (AW).

2.2. Gums

Gums were harvested from cultures of gum-producing bacteria (isolated from palm wine) in (1) sterile sucrose medium and (2) sterile palm sap. The details of media preparation, inoculation and/or incubation, extraction and purification of gums were as described in Uzochukwu et al. (1994). Each of the three glucan- producing bacteria isolated from palm wine (Uzochukwu et al., 1994) was cultivated for gum production on both the sterile palm sap and sterile sucrose medium, respectively.

2.3. Inoculation and Inoculum

Before use, cultures were revived by transferring cells from slants to 5 ml of either sterile palm sap or sterile sucrose medium and incubating for 24 h at 30°C; this inoculum, grown on the same medium was used to inoculate 250 ml of the appropriate media, such that the final concentration was 10^4 cfu/ml.

Inoculated flasks, as well as uninoculated controls, were incubated at 30°C for 24 h.

2.4. Efficiency of sterilization

Efficiency of sterilization was checked by examining a drop of each culture under the microscope and checking for cells different from those inoculated. Streak plates were also prepared on MRS and sucrose agars, and colonies obtained examined for colony characteristics different from those of the organism inoculated. The uninoculated controls were examined in a similar manner and also checked for turbidity after the incubation period.

2.5. Structural analysis

Proton-decoupled ^{13}C nmr spectra were obtained at natural abundance with a total carbohydrate concentration of 100 mg/ml of deuterium oxide. A Joel 9×400 spectrometer was employed. The spectral width was 25,000; acquisition time 0.655 s, pulse width 14 ms. Chemical shifts are expressed in ppm relative to external acetone. The samples were measured in 10 mm diameter tubes and spun at 12 Hz.

Spectra were taken with broad band proton decoupling. The solutions were passed through a fritted glass filter to remove any trace of particulate matter. The spectra were interpreted according to evidence in the literature, especially Colson et al. (1974), Gorin, (1981), Seymour, Knapp and Bishop (1976) and Seymour et al. (1979 a,b,c,e,f,g).

3. Results and discussion

Table 1 shows the corrected chemical shifts for ^{13}C nmr spectra of glucans produced by gum-producers isolated from palm wine. The chemical shifts of dextrans having specific linkages which have been reported are also included for comparison. The spectra were recorded at 20°C using acetone as external standard. For relative ease of comparison with values in the literature, where tetramethyl silane was the external standard and temperatures different, a correction factor was obtained by recording the chemical shifts for the ^{13}C nmr spectrum of β-cyclodextrin at 20°C, using acetone, and comparing with the values for the β-cyclodextrin spectrum obtained at 32°C in the literature, using tetramethylsilane (Colson et al., 1974). In this manner, a correction factor of 1.33 ppm was obtained which was added to the values obtained here. Furthermore, in order to compare the values here with others obtained for dextrans at 27°C (Seymour et al., 1976), a temperature correction factor of 0.017 ppm per degrees centigrade increase in temperature (Seymour et al.) was also used. Thus, after adding 1.33 to the original value, i.e. correcting it to tetramethylsilane at 32°C, it was corrected for temperature only, to 27°C, by subtracting $0.017 \times 5 = 0.085$ ppm, giving a final correction factor of 1.245. The spectra shown bear the uncorrected chemical shifts in ppm while the values in Table 1 are corrected values, shown in comparison with literature values. In the discussion of the individual spectra, references to

Table 1
Chemical shifts for glucans elaborated by gum-forming bacteria isolated from palm wine, compared with those of glucans with specific linkages published in literature[a]

	35_s	35_m	34_s	34_m	AW	Linear dextran B640	α(1→6) and α(1→2) linked D-glucan[c]B13992	α(1→6) and α(1→3) linked D-glucan[c]B13559	α(1→6) and α(1→4) linked D-glucan[c]B1254	Levan[e]
										105.68
										105.14
									101.03	
				100.609	100.655				100.76[d]	
								100.55		
	100.29	100.29	100.321	100.321	100.30			100.29		
				100.210						
				99.833					99.38	
				99.061				99.02		
A[b]	98.697	98.712	98.712	98.712	98.727	98.67	98.71	98.93	98.70	
	97.225	97.255					97.22			
	96.360	96.390					96.37			
				82.122						82.87
			81.636	81.682						81.80
	81.575	81.575	81.515	81.484	81.591			81.60		
				80.650						
										78.94
									79.54	78.74
										77.26
	76.449	76.491					76.50			76.82
B[b]	74.385	74.412	74.412	74.396	74.412	74.36	74.40	74.36	74.33	
				74.230						
	74.108	74.083	74.118	74.116	74.108					
	73.854	73.896	73.911	73.911	73.926					
	72.883	72.909	72.833	72.818	72.788					
	72.640	72.666	72.713	72.742	72.727					
			72.636	72.605						
			72.560	72.560						
C[b]	72.378	72.408	72.408	72.408	72.408	72.37	72.40	72.62	72.42	
					72.256					
			71.452	71.452						
					71.361					
	71.319	71.330								
D[b]	71.167	71.194	71.194	71.179	71.194	71.14	71.18	71.16	71.26	
			71.088	71.103	71.118					
			70.966	70.966						
			70.799	70.814						
E[b]	70.510	70.556	70.541	70.526	70.572	70.52	70.54	70.62	70.44	
					70.420					
	70.363	70.405	70.374	70.359	70.359					
									67.64	
	66.781	66.845								
F[b]	66.554	66.580	66.549	66.519	66.610	66.56	66.59	66.13	66.55	
			66.382	66.337						
										64.99
										64.23
										62.45
	61.470	61.515	61.510	61.495					61.55	
	61.389	61.404	61.338	61.328	61.389		61.38			
					61.328					

[a] PW_{50} = 50% ethanol-insoluble palm wine gum. 35_s and 35_m = glucan produced by *Leuconostoc mesenteroides* in palm sap and sucrose broth, respectively. 34s and 34m = glucan produced by *L. dextranicum* in palm sap and sucrose broth, respectively. AW = glucan produced by *Lactobacillus* species AW in palm sap. Value obtained at 20°C using acetone as external standard corrected to 27°C and tetramethyl silane by addition of 1.245.

[b] The letters A–F refer to the major resonances of linear dextran (Seymour et al., 1976).

[c] From Seymour et al., (1976).

[d] Observed in pullulan, also an α (1→6) ; α (1→4) linked glucan (Seymour et al., 1976).

[e] From Seymour et al. (1979c).

chemical shifts are corrected values, as found in Table 1, while the uncorrected values obtained at 20°C with acetone are in parentheses and are those found in the spectra. The ^{13}C nmr spectra of all gums studied are shown in Figs. 1–3 while a summary of their resonances is in Table 1.

3.1. Basis for assignments

All the gums used in this study had been shown to be glucans by hydrolysis and subsequent thin layer chromatography (Uzochukwu et al., 1994). It had been previously demonstrated, for carbohydrate compounds, that C-2, C-3, C-4 and C-5 chemical shifts are normally found in the 70–75 ppm region and that the anomeric (C-1) carbon displays a downfield chemical shift (about 90 ppm) while C-6 shows an upfield chemical shift (about 60 ppm; Gorin, 1973). Seymour et al. (1976) showed that glycoside bond formation causes the chemical shift of the two carbon atoms involved to be displaced downfield by about 10 ppm. They showed that

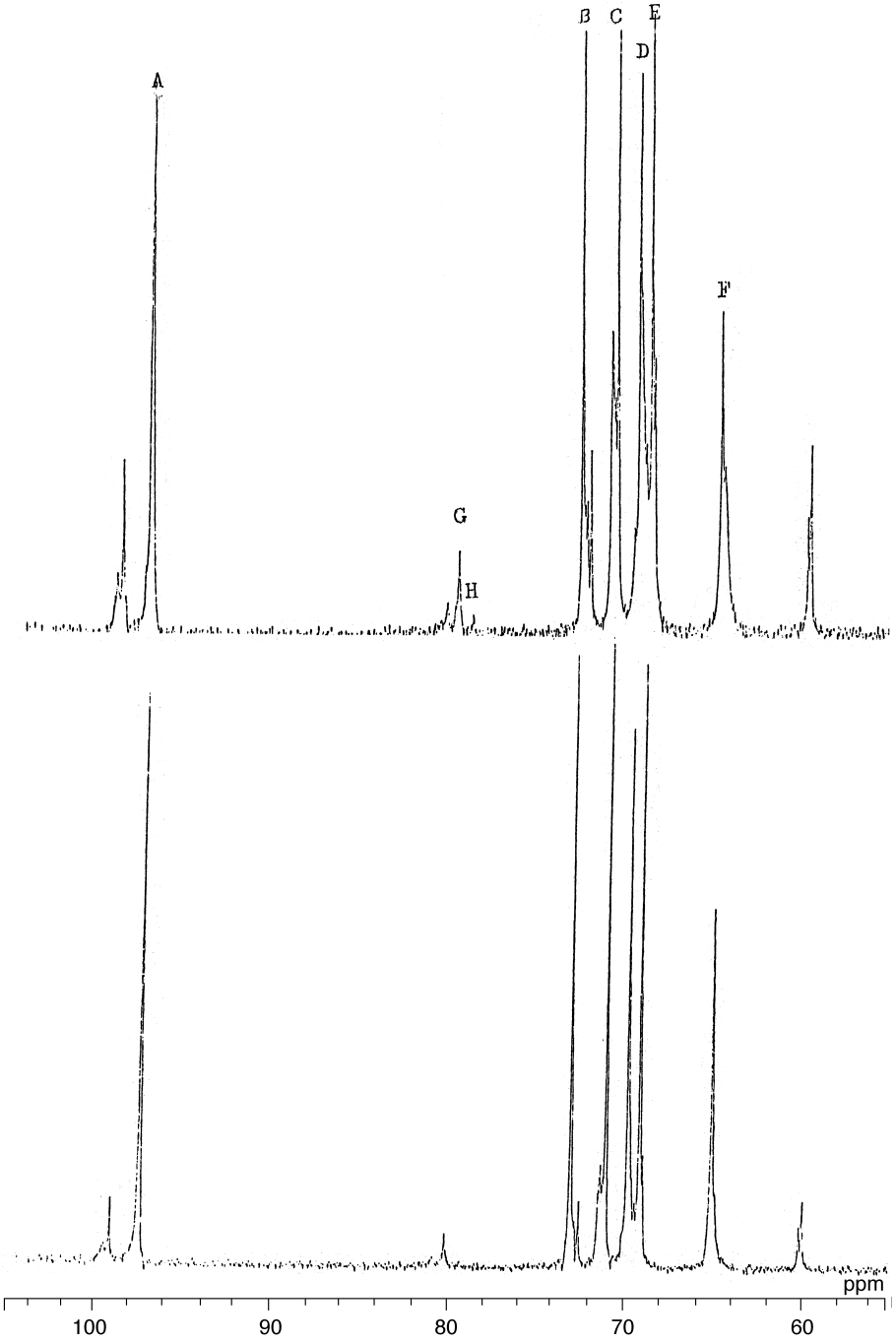

Fig. 1. ^{13}C nmr of glucans produced by *Leuconostoc dextranicum* in sucrose broth (above) and in sterile palm sap (below). The letters A–F refer to the major resonances of linear-type dextran.

the resonances of C2, C3, C4, C5, which were linked as a result of branching, are displaced downfield into the 75–85ppm region, and that these are diagnostic for recognizing branch linkages. Seymour et al. (1976, 1979a,b,c) have also shown that the ^{13}C nmr spectra of all dextrans contain six major resonances tagged A–F (Table 1, Fig. 1) and branched dextrans contain additional diagnostic resonances. Peaks A and F were assigned to the anomeric carbon and C-6, respectively, involved in the chain-elongating α-(1–6) linkages, while peaks B, C, D and E represent C3, C2, C5 and C4, respectively, which are not involved in branching (Colson et al., 1974). The resonances of α-anomeric carbons in D-glucans are usually in the 96–102 ppm area while those of β-anomeric carbons are downfield of 102 ppm (Seymour et al., 1976).

3.2. Chain extending linkages

Examination of Table 1 shows that, for all glucan samples in this study, virtually all the signals in the anomeric regions of their spectra are upfield of 102

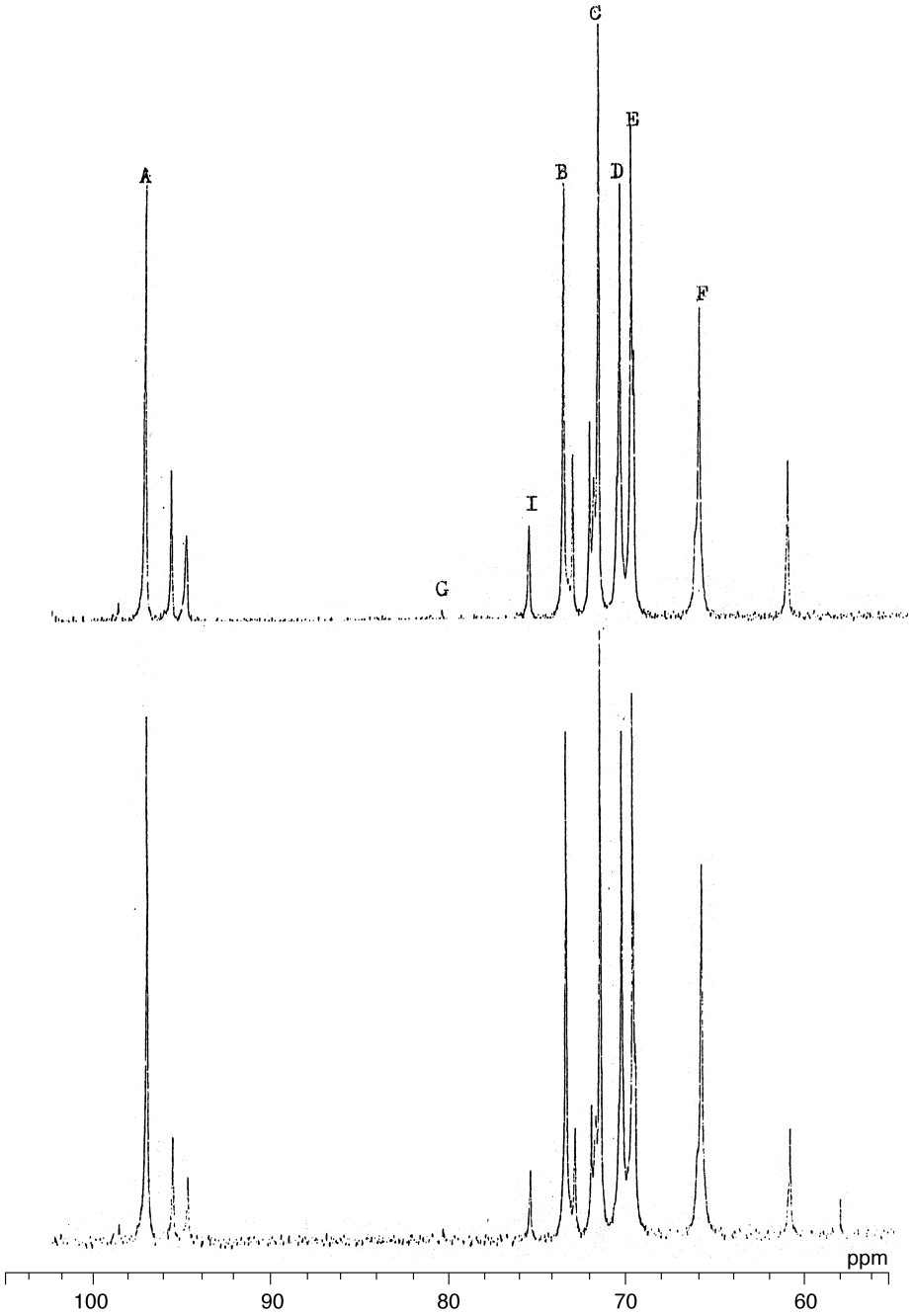

Fig. 2. ^{13}C nmr of glucans produced by *Leuconostoc mesenteroides* in sucrose broth (above) and in sterile palm sap (below). The letters A–F refer to the major resonances of linear-type dextran.

ppm. This indicates that all the glucans in this study are α-linked. There are resonances in the 70–75 ppm region, labelled B, C, D, E, with chemical shifts as would be expected for unlinked C-3, C-2, C-5, and C-4, respectively (Colson et al., 1974; Seymour et al., 1976). This is diagnostic for the D-glucopyranoid ring (Seymour et al., 1979a). The major resonance in the anomeric regions occurs at 98.7 (97.46) ppm rather than at about 90 ppm, showing that the C-1 is linked. An equally intense signal at 66.5 (65.3) ppm rather than at 60 ppm indicates that most of the C-6 is also linked (Seymour et al., 1976). There are no other similarly intense signals that could be due to linkage, suggesting that the glucose units are linked α-(1–6). Thus, the glucose units are in the pyranoid form and are linked mainly α-(1–6). In addition, the resonances mentioned correlate with those assigned to the anomeric and C-6 carbons, respectively, involved in α-(1–6) linkages (Seymour et al., 1976). Each spectrum also displays all the characteristic resonances of linear dextran (A–F) as assigned by Gorin (1973) and adopted by Seymour et al. (1976). Therefore these glucans are all dextrans. They also all display characteristic branching resonances in the regions expected for dextrans (75–85 ppm; Seymour et al., 1976).

The ^{13}C nmr spectra of the individual dextrans will now be considered in order to determine their branch linkages and degrees of linearity. It is easy to see this by comparing the chemical shifts of the samples with those containing different branch linkages reported in the literature and included in Table 1.

3.3. Branch linkages

3.3.1. Dextrans produced by Leuconostoc dextranicum (34) isolated from palm wine

The ^{13}C nmr spectrum of the dextrans produced by *Leuconostoc dextrancum* (34) isolated from palm wine in sucrose medium (34m) and in palm sap (34s) are shown in Fig. 1. Their chemical shifts (Table 1) correlate excellently, showing them to be qualitatively the same. The shifts also correlate well with those of dextran B1355S reported to branch by α(1–3) linkages by both

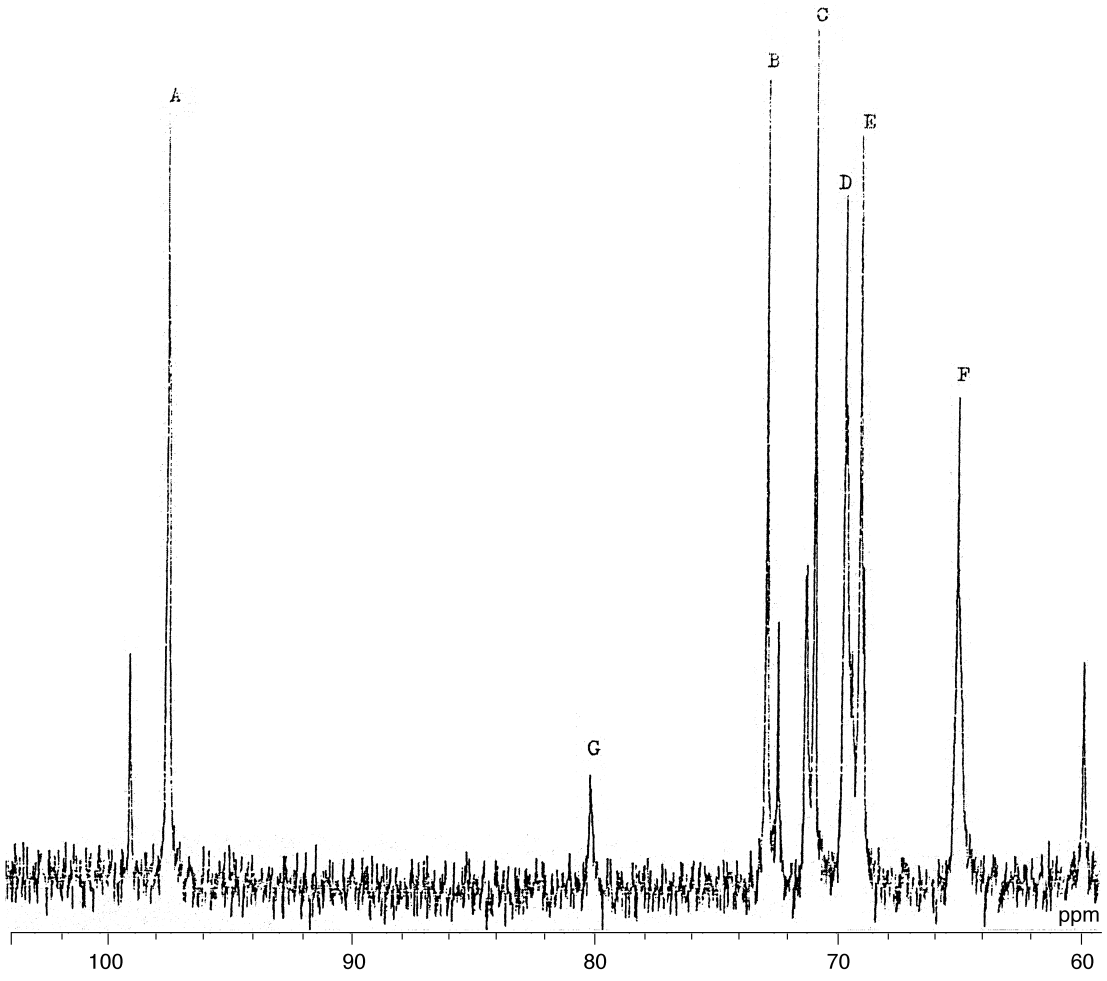

Fig. 3. ^{13}C nmr of glucans produced by *Lactobacillus* spp. (AW), in sterile palm sap. The letters A–F refer to the major resonances of linear-type dextran.

methylation and ^{13}C nmr data (Seymour et al., 1979d), shown in the same table as α(1–6) and α(1–3)-linked glucan. Thus, apart from the peaks A to F which correlate with the six signals of linear dextran in Table 1, the spectra contain minor peaks indicative of branching. In the anomeric regions, apart from the major resonance A at 98.71 (97.46), which represents the C-1 of linear α-(1–6) linked residues, there are two other prominent, though less-intense, signals. The peak at 100.3 (99.06) ppm occurs 1.609 ppm downfield of peak A. There is also a weaker signal at 99.06 ppm which is 0.349 ppm downfield of peak A. Seymour et al. (1979d) have shown that the ^{13}C nmr spectra of dextrans branching through 3,6-di-*O*-substituted residues which also contain 3-mono-*O*-substituted residues, contain two anomeric resonance, 0.4 and 1.6 ppm, respectively, downfield of peak A, while those containing only 3, 6-di-*O*-substituted residues have only one peak downfield of peak A. Therefore, the two signals at 100.32 (99.0) ppm and 99.06 (97.82) represent the two anomeric resonances due to C-1 when both 3-mono and 3, 6-di-*O*-substituted residues are present. The dextran B1355S, earlier indicated to branch by α(1–3) linkages, was also shown to contain intra-chain α(1–3) linkages. That there are two types of α(1–3) linkages in 34s and 34m dextrans is further substantiated by the presence of signals at 82.12 (80.877) ppm and 81.48 (80.239) ppm in the 75–85 ppm region. The peaks occur in the area designated the "G" region (in continuation of the A–F linear dextran series), which is diagnostic for the presence of α(1–3) linkages (Seymour et al., 1979e). This G region separates into two groups corresponding to the C-3 resonance of the 3-mono-*O*-substituted (intrachain) residue found in the present study at 82.1 (80.877) and the C-3 resonance of 3, 6-di-*O*-substituted (branching) residue at 81.6 (80.2) ppm. Seymour et al. (1979e) found these resonances at 83.3 and 82.9ppm, respectively, when recording at 90°C. They also observed that the difference between the chemical shifts of these two resonances was consistently about 0.5 ppm. In the present study, the difference is 0.6 ppm, which is in good agreement. Thus, the 75–85 ppm region also shows an α(1–3) intra chain resonance just like the anomeric region, thereby confirming the presence of these two residues in the 34 series dextrans. A branch terminating C-1 resonance should be present also at the anomeric region but this is usually not observed and it is thought that this is because the chemical shift of that resonance coincides with that of peak A (Seymour et al., 1976, 1979a). There is a peak virtually indistinquishable from peak A at 98.83 (97.588) ppm which represents this resonance. The shoulder at 100.6 (99.41) ppm suggests the presence of an α-(1–4) linkage, but no peak characteristic of this type of linkage is found at about 79.5 ppm. However, Colson et al. (1974) found a similar shoulder on the 100.5 ppm peak of the dextran B742 spectrum, representing the C-1 of α(1–3)-linked residue, and assigned it to the C-1 of an α(1–4) residue. The rest of the signals of the α-(1–4) linked glucosyl unit were not found in either the present study or that of Colson et al. (1974). This was probably due to overlap with the resonances of glucosyl units involved in other types of linkages. The dextran B742 has been shown by these workers to contain mainly α-(1–3) branch linkages with less than 10% α-(1–4), by methylation analysis. Furthermore, an inspection of Table 1 shows that the shoulder at 100.6 ppm in the spectra of the 34 series dextrans in the present study appears to correlate well with the C-4 anomeric resonance observed in pullulan — a polysaccharide containing only α-(1–6) and α-(1–4) linkages), at 100.76 ppm, and assigned to C-1 of α-(1–4) linked residues (Seymour et al., 1976). In the C-6 region of the *L. dextranicum* dextrans' spectra, there is a small peak at 61.3 (60.0) apart from the major F resonance which is due to C-1 of α-(1–6) linked residues. The smaller peak represents the free C-6 of the branch terminating residues. Both of these C-6 peaks have shoulders depicting a minor C-6 in a slightly different chemical environment. These are probably from the linked and free C-6 of the α-(1–4) branch point and branch-terminating residues, respectively.

The absence of any resonance upfield of peak A in the anomeric region or at about 76.5 ppm, in the 80.85 ppm region, is significant, in that it suggests the complete absence or negligible presence of α-(1–2) linkages (Seymour et al., 1979b).

3.3.2. Degree of linearity

In determining the relative concentration of the linkages in the 34 series dextrans, the concept of anomeric ratios was used. The anomeric ratio is used to calculate a value, *n*, which is the number of chain-extending residues per branch point residue. It is obtained by dividing the peak height of peak A by the peak height of the anomeric branch point resonance and multiplying by the factor 1.503 (Seymour et al., 1979b,d). The use of the factor arose because Seymour et al. (1979b) assigned $n = 2$ to dextrans with an anomeric ratio between 1.33 and 2. Therefore, to normalize ratios to this value, one multiplies by 1.503. The anomeric ratios and degree of branching calculated using this method in the present study are shown in Table 2. Thus, dextran 34m with an *n* value of 4.76, for α-(1–3) branching, branches by this linkages once every five glucose residues and by α-(1–4) once every 14 glucose residues. On the other hand, for dextran 34s these are every 14 and 21 residues, respectively. The intensities of all resonances due to branching are much less in the sap-grown dextran. This shows that *L. dextranicum* elaborates a more branched dextran in sucrose broth than in palm sap. The sap-grown dextran in fact shows a strong tendency towards linearity. The contribution of linear α(1–3) linkages was ignored in

calculating anomeric ratios as the evidence suggests that they occur only in traces.

Comparing the two 34 spectra, the decrease in intensity of the 81.6 (80.3) ppm resonance, thought to be due to linear α-(1–3) linkages along with the branching resonances, suggests that the linear α-(1–3) linkages might be within the side chains rather than within the main backbone chain. The decrease in intensity of the anomeric resonance due to α-(1–4) linked carbon at 100.6 ppm in sap-grown dextran, along with signals due to branching, further suggests that it is a branch linkage rather than an intrachain one. It is interesting to note that, for *L. dextranicum*, the ratio of α-(1–3) and α-(1–4) linkages remains constant in both sucrose and palm sap. The ratio in both is 2.9:1 or 3:1. It is possible that such linkage to linkage ratios are strain-specific.

3.3.3. *Dextrans produced by* Leuconostoc mesenterorides *(35) isolated from palm wine*

The spectra of the dextrans produced by *L. mesenteroides* in both 35m and 35s are shown in Fig. 2. They show prominent A–F peaks of linear dextran as well as additional peaks indicative of branching (Seymour et al. 1976). As in *L. dextranicum* (34) dextrans, there is very good correlation between the chemical shifts of the two *L. mesenteroides* dextrans (35m and 35s), showing them to be basically the same except that they differ in the intensities of branching resonances. Thus, this organism also elaborates a more branched dextran in sucrose broth than in palm sap. The anomeric resonances at 97.2 (96.01) ppm and 96.3 (95.1) ppm, as well as the signal at 76.4 (75.24) ppm (due to linked C-2), are specifically unique to dextrans having 2, 6-di-*O*-substituted α-D-glucopyranosyl residues. The 96.4 (95.24) ppm signal occurs in the "1" region diagnostic for linked C-2 (Seymour et al., 1979b). Of the two minor anomeric resonances, the more intense (97.2 (96.01) ppm) resonance represents the C-1 of the branch-terminating residue while the 96.3 (95.19) ppm resonance represents the C-1 of the branch point residue. The argument is that terminal side-chain residues have greater mobility than branch point residues which are incorporated into the dextran backbone and, usually, carbon atoms with greater mobility have narrower, more intense resonances. As there must be an exact one-to-one correspondence of branch point to terminal residues, the more intense signal must be due to C-1 of the terminal (branch-terminating) residues (Seymour et al., 1979b). Significantly absent is the 79.5 (78.2) ppm resonance, diagnostic for 4, 6-di-*O*-subtituted residues in D glucans (Seymour et al., 1979e). There is a weak anomeric peak at 100.29 (99.04) ppm which indicates a trace of α-(1–3) linked residues, as this resonance has been assigned to the C-1 of α-(1–3) linked branch point residues. There is also a correspondingly weak signal at 81.5 (80.3) ppm which appears to be in the "G" region diagnostic for 3-6-di-*O*-substituted residues (Seymour et al., 1979d). This confirms the presence of a trace of α-(1–3) linked branch points in these dextrans. This is not unusual, as most dextrans so far studied have been found to have some α-(1–3) branching, irrespective of the nature of the major branching linkage (Seymour et al., 1979e). As in the *L. dextranicum* dextrans, both the F resonance (from linked C-6) and the 61.40 (60.15) ppm signal (from free C-6) have barely visible shoulders representing the signal, possibly, of other linked and free C-6, respectively, in a slightly different chemical environment, such as the linked C-6 of branch point residues and the free C-6 of branch-terminating residues. A comparison of the chemical shifts of the 35 series dextrans with those of B 1399 shown in Table 1, shows clearly that 35m and 35s like 1399, branch mainly through α-(1–2) linkages.

The anomeric ratios and *n* values representing degree of branching are shown in Table 2. The values are $n = 4.45$ for 35m and 7.59 for 35s, for the α-(1–2) branching residues. For the trace α-(1–3) branching, they are 41.28 for 35m and 45.5 for 35s. It does appear that the intensity of the signals due C-1 and C-3, involved in α-(1–3) branching in the 35m and 35s dextrans, does not change much, making it a more fixed feature of the dextran. Perhaps the amount of α-(1–3) branch linkage in a dextran is constant when another linkage constitutes the major linkage. The difference in degree of branching exhibited by dextrans elaborated by the same organisms in sucrose broth and palm sap, probably explains the differences in viscosity between these dextrans. The dextran elaborated in palm sap (which is less branched) is always much less viscous than that elaborated by the same organism in sucrose broth. This is probably due to nutrient differences, e.g. phosphates could be limiting to dextran synthesis (Jeanes et al., 1954).

Table 2
Degree of linearity calculated from ^{13}C nmr spectra at 20°C for dextrans produced in sucrose broth and palm sap, respectively, by bacteria isolated from palm wine

Dextran sample[b]	13C nmr anomeric ratios			*n* Calculated from 13C nmr data		
	α- (1→3)[a]	α- (1→4)[a]	α- (1→2)[a]	α- (1→3)[a]	α- (1→4)[a]	α- (1→2)[a]
34m[b]	3.14	9.2	–	4.72	13.82	–
34s	9.28	27.85	–	13.95	41.6	–
34m	27.45	–	2.96	41.28	–	4.45
35s	30.33	–	5.05	45.5	–	7.59
AW	3.17	–	–	4.77	–	–

[a] Type of branching.

[b] 34m and 34s = dextran produced by *Leuconostoc dextranicum* in sucrose broth and palm sap, respectively. 35m and 35s = dextran produced by *L. mesenteroides* in sucrose broth and palm sap, respectively. AW = dextran produced by the *Lactobacillus* species AW. *n* = Number of linear residues per branch point residue.

3.3.4. Dextran produced by Lactobacilluss *spp. (Aw) isolated from palm wine*

The ^{13}C nmr spectra of the *Lactobacillus* spp. (Aw) is shown in Fig 3. The chemical shifts of the signals are shown in Table 1. The anomeric region of this dextran is very simple. Apart from peak A which as usual represents C-1 of chain-extending α-(1–6) linked residues, there is only one minor peak situated at 100.3 (99.06) ppm which is the position for signals of C-1 of α-(1–3) linked residues. The position of the resonance in the "G" region diagnostic for α-(1–3) linkages at 81:59 (80.346) ppm, shows that the linked C-3 signal is from a 3, 6-di-*O*-subtituted residue. This coupled with the fact that there is only one α-(1–3) C-1 resonance in the anomeric region shows that the α-(1–3) linkages in this dextran are branching linkages only. If 3-mono-*0*-substituted residues were present there would be two anomeric resonances for C-1 of α-(1–3) linked residues (Seymour et al 1979a) as in the *L. dextranicum* dextran-spectra in Fig. 1. In the C-6 area, the free C-6 resonance is at 61.3 (60.14) ppm and it has no shoulder, suggesting the presence of only one type of secondary linkage. There is no indication, either in the anomeric region or in the 74–84 ppm region, of any other type of branching or intrachain secondary linkages. The peaks in the 69–74 ppm region are, as usual, the peaks B–E of chain-extending residues of linear dextran due to unlinked C2–C5 as well as minor resonances of these carbons at branch points and branch-terminating residues.

The anomeric ratio and *n* value for the dextrans are included in Table 2 and are 3.17 and 4.77, respectively, for sucrose broth- and sap-grown dextrans. This is not surprising because this dextran is very viscous and a bit difficult to dissolve in water so it was suspected that it would be more highly branched than the others. It is known that, the more α-(1–3) linkages there are in a dextran the less easily soluble it is (Jeanes et al., 1954). It is also possible that, in this case, it is the complete absence of α-(1–2) and α-(1–4) linkages that is responsible for the reduced solubility.

4. Conclusion

It is concluded that the glucans produced by *L. dextranicum, L. mesenteroides* and *Lacbaccillus* spp. in natural palm sap, are all dextrans and that each organism produces a different type of dextran. They also produce, in sucrose broth, the same types of dextrans as they produce in natural palm sap. Thus, if employed in a sucrose solution for the preparation of a simulated or extended palm wine, they will reproduce the same dextran that they elaborate in natural palm wine and, by so doing, reproduce the colour and consistency of palm wine.

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