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Structural analysis by ^{13}C- nuclear magnetic resonance spectroscopy of glucan extracted from natural palm wine

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Abstract

The linkages of the glucan produced in palm wine during fermentation were determined by ^{13}C NMR spectroscopy. The glucan was found to be linked α(1–6) in the main chain, showing it to be a dextran. The dextran appeared to be a mixture of the dextran types elaborated individually in pure culture by dextran-producing bacteria isolated from palm wine in a previous study. All branch linkages [α(1–3), α(1–2), α(1–4)] found in the spectra of the dextrans of the respective palm wine bacteria, were present in the spectrum of the palm wine glucan. There was also evidence of the presence of a dextran branching mainly through α(1–4) linkages, thus differing from all the dextran-producing bacteria so far isolated from palm wine. It is concluded that at least 4 types of dextrans are produced concomitantly in palm wine by different bacteria and that the bacteria producing the dextran branching mainly by α(1–4) linkages in palm wine are yet to be isolated. Formulations of analogues of palm wine may therefore need to use a mixture of dextrans in order to simulate, more exactly, the connsistency and colour of natural palm wine.

Keywords: Natural palm wine; Glucan; dextrans

1. Introduction

Palm wine is an alcoholic beverage produced by the spontaneous yeast/lactic fermentation of the sap of palms. It is usually white or milky in colour and the source of this white coloration had been assumed to be the yeasts and bacteria 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 by these workers. One group was made up entirely of glucose units and insoluble in 50% ethanol and another, contained only fructose units and was insoluble in 60% ethanol. The lactic acid bacteria in palm wine produce glucans in pure culture in both palm sap and sucrose broth, (Uzochukwu et al., 1994) and the glucans are all dextrans, differing in their secondary linkages, depending on the producing species (Uzochukwu, Sylvia, Balogh, Loefler, & Ngoddy, 2001). From these previous results it is expected that the glucan extracted from palm wine would be a mixture of the dextrans of the dextran-producing bacteria isolated from it. It is not known whether all or only some of these bacteria produce their dextran in palm wine and it is not possible, in practice, to separate the palm wine glucan by fractional precipitation without cross-contamination. The hypothesis now, is that the secondary linkages detected in palm wine glucan would be a good indication of the type of dextrans present, i.e. which of the bacteria actually produces its dextran in native palm wine. Thus if the secondary linkages found in the different dextrans elaborated by dextran—producing lactics isolated from palm wine are present in the glucan extracted from natural palm wine, it could be assumed that the bacteria do elaborate the dextrans in natural palm wine concomitantly. Elucidating the nature of the palm wine gum, would make it possible to use the pure compounds in the extension of natural palm wine and its analogues. The analysis reported here is on the 50% ethanol-insoluble glucan fraction extracted from palm wine. Struc-

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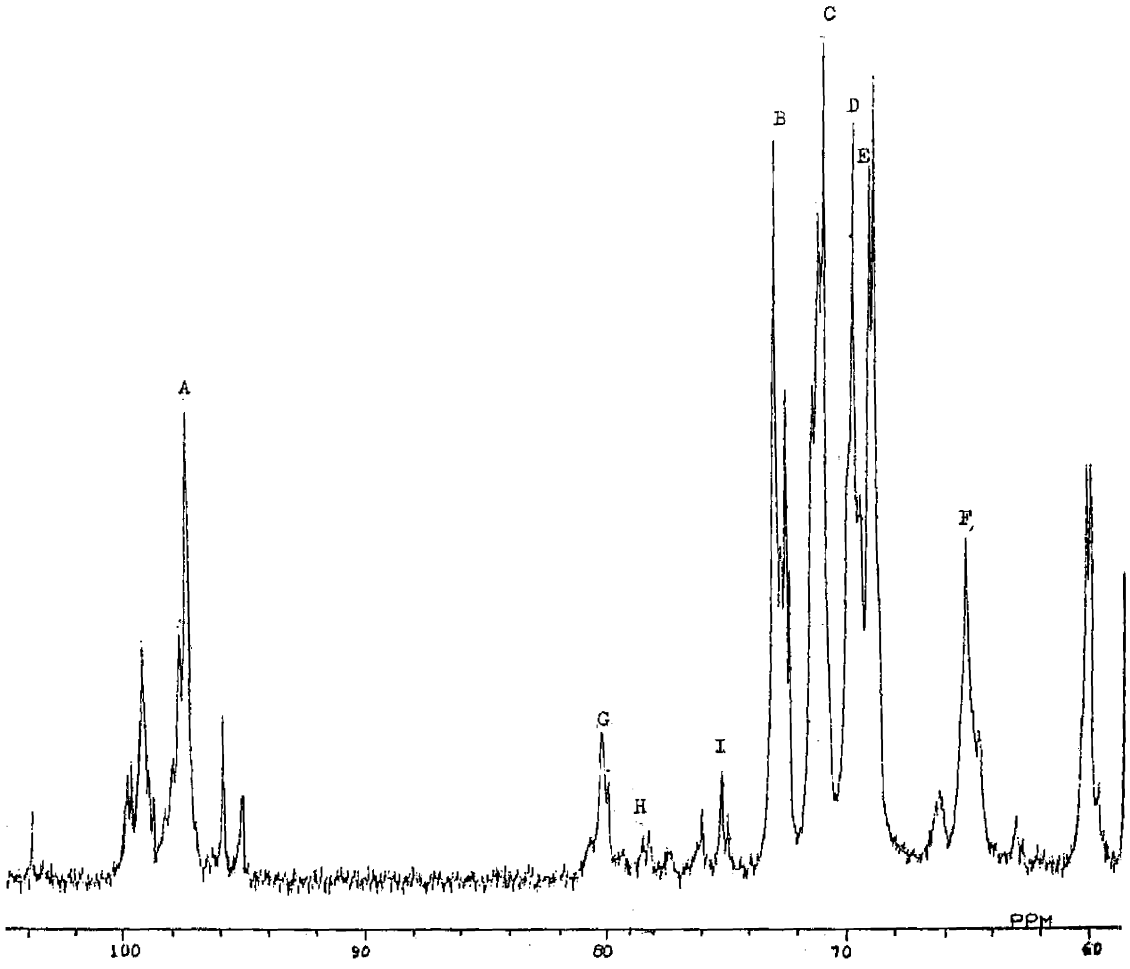

Fig 1. ^{13}C NMR. spectrum of 50%-ethanol- insoluble glucan extracted from palm wine.

tural analysis of this glucan by ^{13}C NMR spectroscopy is presented.

2. Materials and methods

2.1. Gum recovery and purification

Palm sap collection, fermentation, gum extraction and purification, as well as associated precautions were as described in Jeans (1965) and Uzochukwu et al. (1994).

2.2. Structural analysis

Procedure for the structural analysis was as described in Uzochukwu et al. (2001). The spectra were interpreted according to evidence in literature especially Colson et al. (1974); Seymour, Knapp, and Bishop (1976); Seymour, Knapp, Bishop, and Jeans, (1979a); Seymour, Knapp, Bishop, and Jeans, (1979b); Seymour, Knapp, Bishop, and Jeans, (1979c); Seymour, Knapp, Bishop, and Jeans, (1979d); Seymour, Knapp, Bishop, and Jeans, (1979e) and Gorin (1981), as explained in Uzochukwu et al. (2001).

3. Results and discussion

3.1. General

The ^{13}C NMR spectrum of palm wine glucan is shown in Fig. 1. Table 1 shows the corrected chemical shifts for the $^{13}Cn.m.r.$ spectrum of the same glucan. The chemical shifts of the 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 (27 °C), a correction factor of 1.245 ppm was obtained as described in Uzochukwu et al. (2001). The

Table 1
Chemical shifts for ^{13}C NMR spectra of dextrans from palm wine (PW50), compared with those of dextrans with specific linkages published in literature[a]

	Palm wine glucan PW50	Linear dextran B640	α(1–6) and α(1–2) linked dextran B13992	α(1–6) and α(1–3) linked dextran[b] B13559	α(1–6) and α(1–4) linked dextran[b] B1254	Levan[c]
						105.68
	105.19*					105.14
	101.25					
	101.05				101.03	
	100.69				100.76[d]	
	100.57			100.55		
	100.34			100.29		
	100.15					
	99.32				99.38	
	99.09			99.02		
A[e]	98.74	98.67	98.71	98.93	98.70	
	97.26		97.22			
	96.42		96.37			
						82.87
						81.80
	81.58			81.60		
	81.27					
	79.82					78.94
	79.54				79.54	78.74
	77.36					77.26
	76.49		76.50			76.82
	76.22					
B	74.40	74.36	74.40	74.36	74.33	
	74.11					
	73.93					
	73.72					
	72.83					
	72.64					
C	72.41	72.37	72.40	72.62	72.42	
	71.35					
D	71.19	71.14	71.18	71.16	71.26	
	71.16					
E	70.54	70.52	70.54	70.62	70.44	
	70.39					
	67.61				67.64	
	66.76					
F	66.56	66.56	66.59	66.13	66.55	
	65.99					64.99
	64.38					64.23
	61.69					62.43
	61.53				61.55	
	61.37		61.38			
	60.96					

*Value obtained at 20 °C using acetone as external standard and corrected to 27 °C and tetramethyl silane by addition of 1.245 (Uzochukwu et al., 2001)

[a] PW50 = 50% ethanol insoluble palm wine gum i.e. palm wine dextran.

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

[b] From Seymour et al. (1976)

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

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

spectrum bears the uncorrected chemical shifts while the values in Table 1 are corrected values shown in comparison with literature values. In the discussion of the spectrum, reference to 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 spectrum.

3.2. Basis for assignments

The gum used in this study had been shown to be a glucan by hydrolysis and subsequent thin layer chromatography (Uzochukwu et al., 1994). The basis for assignment of chemical shifts was as explained in Uzochukwu et al. (2001), according to Colson et al. (1974),

Seymour et al. (1976) and Seymour et al. (1979a, 1979b, 1979c, 1979d, 1979e).

3.3. Chain extending linkages

As was the case for the pure culture dextrans described in Uzochukwu et al. (2001), examination of Table 1 and Fig. 1 shows that, for the glucan sample in this study, virtually all signals in the anomeric region are upfield of 102 ppm. This indicates that the glucan is α-linked. There are resonances in the 70–75ppm regions with chemical shifts as would be expected for unlinked C-2, C-3, C-4 and C-5 (Seymour et al., 1976). This is diagnostic for the D-glucopyranoid ring (Seymour et al., 1979a). The major resonance in the anomeric region occurs at 98.7 (97.46) ppm rather than at 90 ppm, showing that the C-1 is linked. An equally intense signal at 66.5 (65.30), 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 mainly 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). The spectrum also displays all the characteristic resonances of a linear dextran (A–F) (Fig. 1 & Table 1), as assigned by Colson et al. (1974), adopted by Seymour et al. (1976) and employed in Uzochukwu et al. (2001). Therefore, this glucan is a dextran. It also displays all the characteristic branching resonances expected for dextrans, suggesting that it is a mixture.

3.4. Branch linkages

The complex nature of the spectrum (Fig. 1; Table 1), especially as regards the resonances characteristic of branch linkages, suggests strongly that the sample is a mixture. The usual linear dextran signals are marked A–F. It has been shown that the introduction of branch points into the dextran chain would result in two additional anomeric resonances (Seymour et al., 1979b). Prominent, in the anomeric region of this spectrum, are the characteristic α-(1–3) - linked C-1 peaks found in the spectra of dextran B1355S (Seymour et al., 1979d) and palm wine *Leuconostoc dextranicum* dextrans 34m and 34s (Uzochukwu et al., 2001), indicating that both branch point and intra chain linkages are present. These signals are found here at 100.34(99.09) and 100.57(99.33)ppm, respectively. The diagnostic α-(1–2) linked C-1 signals found in the spectra of dextran B13992 (Seymour et al., 1976) and palm wine *Leuconostoc mesenteroides* dextrans 35m & 35s are present here at 97.25 and 96.42 ppm. In addition, there is a weak resonance at 105.19 (103.94) ppm which is indicative of levan contamination (Seymour et al., 1979c). This is not unexpected as palm wine contains a levan fraction. (The signal of the anomeric carbon of β-linked polysaccharides is normally found downfield of 102 ppm.) Comparison with the spectrum of B1254 (Seymour et al., 1979e) shows that the signals at 101.04 (99.8) ppm and 100.68 (99.44) ppm are due to α(1–4) - linked C-1. They are associated with branching at the 4- position. Thus the second resonance for α(1–4) - linked C-1 (100.68 ppm), that was missing in the dextran of *L. dextranicum* (34 ms; Uzochukwu et al., 2001), is present here. There appeared to be only a trace of α-(1–4) branching in that dextran. Here, however, the signals are complete and intense.

Similarly, in the 75–85ppm region, there are signals in the "G" and "H" and "I" regions diagnostic respectively for linked C3 and C4 and C2 at branch points, as also found in B1355S B1254 and B1399L spectra respectively (Seymour et al., 1976, 1979d). Each of these characteristic branching resonances is split in two in this spectrum and they are found here in the G region at 81.27 (80.0290) and 81.57 (80.3310) ppm; in the "H" region at 79.54 (78.29) and 79.81 (78.56) ppm; and in the "I" region at 76.21 (74.96) and 76.49 (75.24) ppm (Seymour, 1979d, 1979e). The split indicates that the linkage is present both as branch point and intra chain linkages. The branchpoint resonance in the "H" region for α-(1–4) branching was also absent in the palm wine *L. dextranicum* dextrans that appeared to contain this linkage, but it is present here. The presence of the three characteristic resonances for 4,6-di-*O*-substituted glucopyranosyl residues in the spectrum of this sample is significant. No organism that elaborates a dextran branching mainly through this residue has been isolated in these studies. Yet it is clear from the intensity and completeness of the signals characteristic for these residues in this spectrum, that there is such a dextran in palm wine in good quantity relative to the other types. This means that the organism elabourating this dextran in palm wine is yet to be isolated from it.

The C-6 region is complex, like the rest of the spectrum. The split in the free C-6 signal at 61.50 (60.28) ppm depicts free C-6 of equal proportions in different chemical environments. There are three possible sources of free C-6 from the sample: the C-6 of branch—terminating residues in α(1–3), α(1–2), and α(1–4) branching. These free C6 signals, associated with branching at C2, C4 and C3, occur here at 61.37 (60.44), 61.52 (60.00), and 61.69 (60.44) ppm, respectively. Thus, the signals for free C6 from C3 and C4 branching appear to have overlapped. Though the C6 of main-chain terminating residues are considered negligible, the shoulder at 60.96 (59.70) ppm may represent this usually undetected peak or may be part of the interfering levan spectrum.

The multiplicity of minor resonances in the 85–62 ppm region of this spectrum is attributable to levan contamination. An examination of the chemical shifts of levans, also included in Table 1, shows that resonances

are expected for levans in that area and palm wine has been shown to contain fructans likely to be levans (Uzochukwu et al., 1994). It proved difficult to fractionally precipitate and purify dextrans from palm wine without levan contamination.

It appears from the foregoing, that the dextran of palm wine is made up of the dextrans of the palm wine bacteria *L. dextranicum* (34 m & s), *L. mesenteroides* (35 m & s) and *Lactsbacillia spp*. (AW) discussed in Uzochukwu et al. (2001) There are strong indications that there is also another microorganism in palm wine which produces dextrans branching predominantly through 4,6-di-*O*-substituted residues, but which has not been isolated from palm wine in these studies.

3.5. Degree of linearity

Using the concept of anomeric ratios, as explained in Uzochukwu et al. (2001), the degree of linearity was estimated. Because of the likely presence of more than one dextran, branching by α(1–3), and α(1–4) linkages, in palm wine, degree of linearity was estimated for only the dextran branching mainly by α(1–2) linkages, as this linkage has been observed only for the dextran of *L. mesenteroides* among the dextran producers of palm wine. This gave an anomeric ratio of 2.83 and so, an *n* value of 4.25 which is very close to the value of 4.45 obtained for this dextran when produced in pure culture by palm wine *L. mesenteroides* in sucrose broth (Uzochukwu et al., 2001). Thus, this dextran, when produced in palm wine, also branches by this linkage once every five glucose residues, indicating that it is *L. mesenteriodes* that produces it in palm wine.

4. Conclusion

It is concluded that there are at least four types of dextrans in palm wine, elaborated concomitantly by the dextran-producing bacteria therein and that the organism producing one of these dextrans is yet to be isolated from palm wine.

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