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Summary

Quinoxalines derived from D-glucose and maltose with O-phenylenediamine (OPD) in alkaline media under deoxygenated and refluxed conditions were studied using GLC, GC-MS, HPLC and NMR measurements. Seven quinoxaline derivatives, (G-6, G-5, G-4, G-3, G-2, G-1 and M-1), were separated from the reaction mixture of both glucose and maltose, and their structures were determined.

Quinoxaline G-6 was identified as 2-methylquinoxaline, likewise, G-5, 2-hydroxymethyl-3-methylquinoxaline; G-4, 2-(2′-hydroxyethyl) quinoxaline; G-3, 2-(1′, 2′-dihydroxyethyl)-3-methylquinoxaline; G-2, 2-(2′, 3′-dihydroxypropyl) quinoxaline; G-1, 2-(2′, 3′, 4′-trihydroxybutyl) quinoxaline; M-1, 2-(2′, 3′-dihydroxypropyl)-3-hydroxymethylquinoxaline.

Introduction

Studies on the degradation of carbohydrates, including mono- and oligosaccharides, are very important in the field of carbohydrate and food chemistry. There have been many papers on the degradation of sugars in alkaline media.[1-5] However, few of them seem to contain quantitative analysis of the degradation products[6] or degradation pathways,[7] since no suitable and practical analytical methods have been available, except for the rather complicated methylation method[8] or Smith's degradation analysis,[9] *etc.*

Takagi and his colleagues have reported an alkaline *o*-phenylenediamine (OPD) method for the analysis of starches[10-12] and some hetero-type carbohydrates.[13] That is to say, when these carbohydrates are treated by this method, the first step of the alkaline degradation starts with the formation of an enediol or dicarbonyl compound which is specific to the structure of the original carbohydrate, followed by condensation with OPD when heated to 100°C under deoxygenated conditions. Degradation of sugars, especially, in alkaline media, is generally considered to proceed *via* enediol formation, followed by dehydration to make dicarbonyl compounds. The mutual transition between enediol and dicarbonyl may have a certain regularity, but the phenomenon is still ambiguous because of deficience of appropriate analytical methods. When the alkaline OPD method was applied to pentoses or hexoses, several quinoxaline derivatives were generally obtained, as it was reported as TLC method.[14] However, the TLC method can not pro-

vide quantitative analysis of the degradation products.

Since we succeeded in the separation of these quinoxaline derivatives by gas-liquid chromatography (GLC),[15] we made quantitative analyses of quinoxalines formed from D-glucose and maltose.

In this paper, we describe the preparation and physicochemical characteristics of quinoxalines derived from D-glucose and maltose, and discuss the mechanism of quinoxaline formation in alkaline media preliminarily, compared to that in acidic media.

Materials and Methods

Chemicals

o-Phenylenediamine was obtained from Merck, AG, and β-phenyl-D-glucoside, from Sigma Chemicals Co. Other reagents were of analytical grade, unless otherwise stated.

Reactions of D-glucose and maltose with OPD

The reaction conditions for the formation of quinoxaline derivatives were essentially the same as described previously.[10,15] 4 mM of D-glucose or maltose was dissolved in 0.2 M carbonate buffer (pH 10) under bubbling of nitrogen gas. After the addition of Na_2SO_3 and OPD to make 0.3 M and 20 mM in the final solution, respectively, the solution was filled up to 5 ml with the same buffer under N_2 bubbling, followed by heating to 100°C under deoxygenated conditions for 60 min. The reaction mixture was cooled in an ice-water bath, then an aliquot (0.1 ml) of the solution was withdrawn and diluted 16-fold with the same carbonate buffer, for the estimation of the polarographic limiting current. To another aliquot (1 ml) of the reaction mixture was added 1 ml of *n*-butanol, and then it was shaken vigorously for 5 min with a vortex mixer. This butanol extraction was repeated two more times. After evaporation of the pooled butanol layers, the residue was subjected to GLC.

To obtain each quinoxaline derivative on a large scale, equimolar amounts (0.5 mol) of D-glucose or maltose and OPD were dissolved in carbonate buffer (300 ml) and refluxed at 100°C under bubbling of N_2 gas in a three-necked flask. This reaction mixture was applied to high-performance liquid chromatography (HPLC) for the separation of the quinoxalines.

Analytical methods

All evaporations were conducted under reduced pressure and below 45°C. Melting points were measured with a Kofler hot stage apparatus. The spectra were recorded on the following apparatus: IR spectra, JASCO IRA-400; UV spectra, Hitachi 200-10; Specific rotation, Union Giken PM-60; Mass spectra: Hitachi RMU-6MG, GLC column 2% OV-210; NMR spectra, JEOL FX-100 for ^1H and Hitachi R-90H for ^{13}C. Other analytical apparatus were as follows: GLC, JEOL JGC-1100, column 2% OV-210; Elementary analysis, Yanagimoto CHN-corder MT-1; Polarography, Yanagimoto Voltammetric Analyzer P-1000; HPLC: Irica S-553, column LiChrosorb RP-18, eluent MeOH-acetonitrile-deionized water (30:5:65, v/v), UV monitoring 320 nm, flow rate 0.6 ml/min.

TLC was performed using Merck silica gel TLC plates with a solvent system of *n*-hexane-ether-EtOH (2:1:1, v/v). The same solvent system was also employed for silica gel column chromatography. Quinoxalines were detected as fluorescent and/or colored spots under UV light after heating the TLC plates which had been treated with 10% sulfuric acid. Acetylation of quinoxaline derivatives (*ca* 20 mg) was carried out with 2 ml of pyridine-acetic anhydride (1:1, v/v) overnight at room temperature.

Results

Polarography of quinoxalines derived from D-glucose and maltose

The time-course of quinoxaline formation from D-glucose and maltose was obtained by following the polarographic limiting current due to quinoxalines. The quinoxaline limiting current at 0.87 V *vs* SCE increased with time, and reached the maximal values of 33.1 μA for D-glucose and 42.7 μA for maltose after 1 hr of reaction. From these values, the total amounts of quinoxalines were estimated to be 1.38 mol for 1 mol glucose, and 1.78 mol for 1 mol maltose, by calculating the concentration of quinoxalines from the total limiting current as that of pure M-1 for convenience.

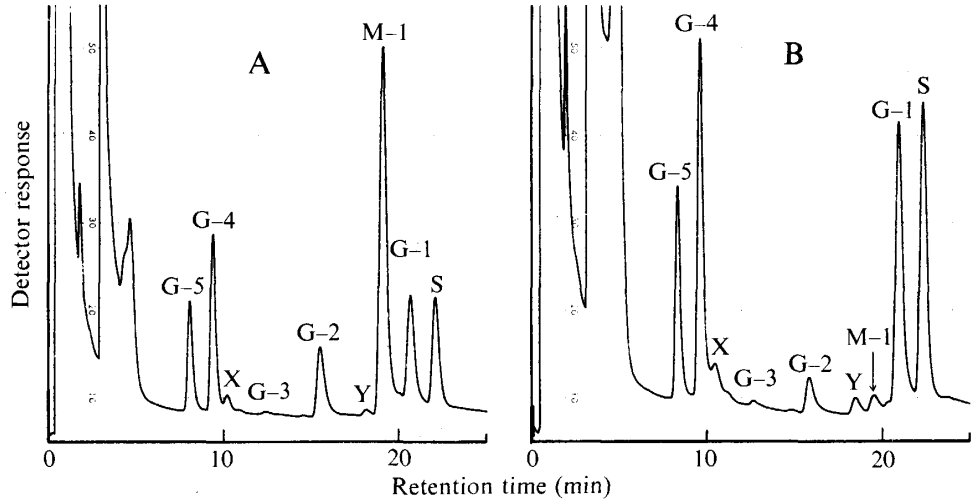


Fig. 1. Gas Chromatograms of TMS-Ethers of Quinoxaline Derivatives Derived from D-Glucose (A) and Maltose (B).
Conditions of GLC: column (glass, 0.3 × 200 cm), 2% OV-210 (Gaschrom Q, 100/200); temperature, 130°C – 200°C (3°C/min); carrier gas (N_2), 1 kg/cm^2; detector FID. G-6, 2-methylquinoxaline; G-5, 2-hydroxymethyl-3-methylquinoxaline; G-4, 2-(2'-hydroxyethyl) quinoxaline; G-3, 2-(1', 2'-dihydroxyethyl)-3-methylquinoxaline; G-2, 2-(2', 3'-dihydroxypropyl) quinoxaline; G-1, 2-(2', 3', 4'-trihydroxybutyl) quinoxaline; M-1, 2-(2', 3'-dihydroxypropyl)-3-hydroxymethylquinoxaline; s, internal standard (β-phenyl-D-glucoside).

Quinoxalines derived from D-glucose and maltose

Figure 1A shows the result of GLC analysis of quinoxalines obtained from D-glucose. Nine quinoxaline derivatives, designated G-5, G-4, G-3, G-2, G-1, M-1, X and Y were obtained from D-glucose in alkaline media. The largest product, G-6, which has been known previously from ion-exchange chromatography and TLC[10,12] is not observed in the present GLC, due to its high volatility. However, G-6 has been known as the simplest quinoxaline.[10,12]

Minor peak X has a molecular ion peak at m/z 260, and other fragment ions at m/z 245, 215, 170, 156, 137 (base peak), 117, and 73 by GC-MS analysis, but we could not determine its structure at present. Likewise, peak Y has a molecular ion peak at m/z 450, and a base ion peak at m/z 73. The M^+ value is the same as those of M-1 and G-1 presented in the section "analytical data of quinoxaline derivatives." This quinoxaline, Y most probably is a structural isomer of M-1 and G-1, and gives a dark green spot and an R_f value of 0.15 on TLC.

Figure 1B shows the GLC of quinoxalines derived from maltose. The same kinds of quinoxalines are observed as those from D-glucose. As is evident from the figure, M-1 was the predominant derivative, the amount of which has been demonstrated to be proportional to the amount of 1, 4-linkage of maltose in the reaction mixture.[12]

For the determination of G-6, the diluted reaction mixtures were applied to HPLC, and the results are shown in Table 2.

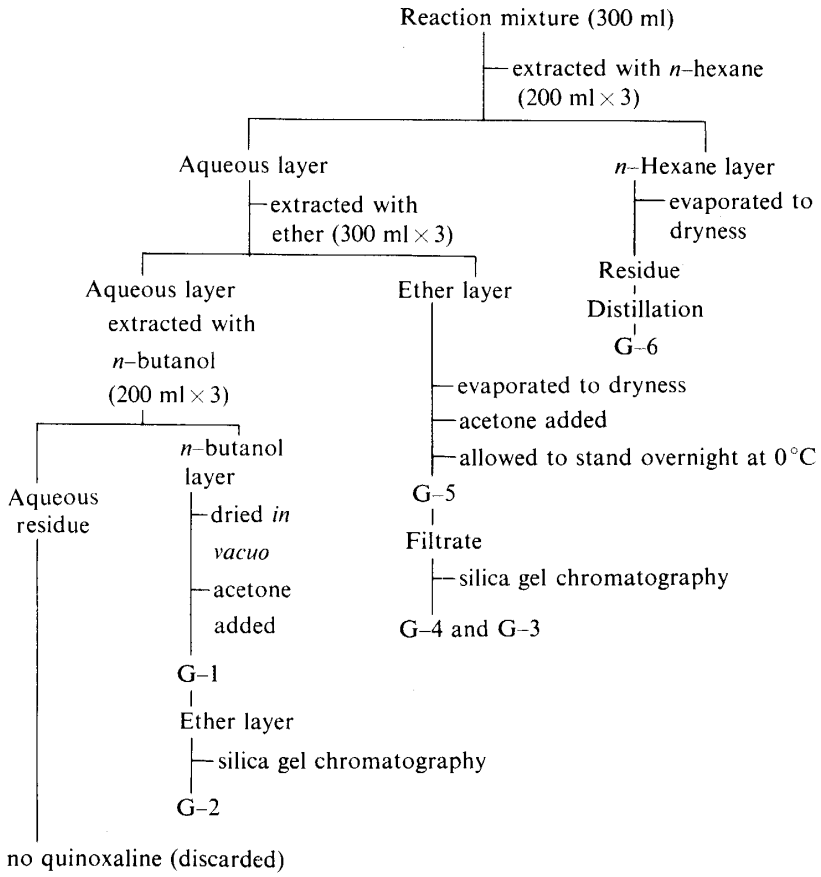


Fig. 2. Isolation Procedure for Quinoxalines Derived from D-Glucose in Alkaline Media

Separation of quinoxaline derivatives

As the quinoxalines derived from D-glucose or maltose in alkaline media differ in the structure of the side chains of the quinoxaline ring, the solubility of these compounds in organic solvents should vary considerably with their structures. The separation of these quinoxaline derivatives was done by a combination of solvent extraction and silica gel column chromatography. The outline of the procedure is shown in Fig. 2. Since G-6 has the lowest vapor pressure, it is difficult to estimate its content by the present GLC method. This quinoxaline, G-6, was easily extractable with *n*-hexane, and could be prepared in liquid form by distillation under reduced pressure. This specimen was found to be 2-methylquinoxaline by the agreement of analytical data as reported by TAKAGI *et al.*[11]

G-5 was crystallized from the ether-soluble fraction as follows: an ether extraction was performed on the *n*-hexane-insoluble layer. After evaporation of the ether solution, a minimal volume of acetone was added, followed by standing in a refrigerator overnight.

The crystals of G-5 thus formed were washed with chilled acetone.

After the removal of the acetone-insoluble crystals and precipitates, the acetone-soluble fraction containing G-4 and G-3 was concentrated to a small volume, followed by silica gel chromatography. The fractions containing G-4 as detected by TLC were pooled and evaporated to dryness. The syrup was sublimated at 100°C under reduced pressure. Fine needles were obtained.

G-3 was eluted more slowly than G-4 by the same silica gel column as above. The main fractions of G-3 were collected and evaporated to dryness, followed by addition of a minimal volume of acetone. By standing overnight in a refrigerator, colorless fine needles were formed. The crystals were washed with acetone and recrystallized from the same solvent.

G-1 and G-2 were prepared as follows: after extraction with *n*-butanol of the ether-insoluble layer, the butanol layer was evaporated to dryness. To the residue was added a small volume of acetone, and the mixture was allowed to stand overnight in a refrigerator. Since the crystals of G-1 thus formed contained some impurities, we recrystallized from acetone. For the separation of G-2, after the removal of the crystallized G-1 through a glass filter, the combined filtrates were concentrated to a small volume, and applied to a silica gel column. The main fractions containing G-2 were pooled and evaporated to dryness, followed by addition of a minimal volume of acetone. After standing overnight in a refrigerator, G-2 was crystallized, and then it was washed with acetone and ether.

For the separation of M-1, quinoxalines derived from maltose were used, because of the large amounts of M-1 formed, as shown in Fig. 1B. After the removal of the *n*-hexane- and ether-soluble fractions, *n*-butanol extraction was performed on the aqueous layer, followed by evaporation under reduced pressure. To the residue was added a minimal volume of ethanol, and the solution was allowed to stand overnight in a refrigerator. Lightly yellow crystals were obtained. For further purification, silica gel column chromatography was performed.

Analytical data of quinoxaline derivatives

Table 1 and Fig. 3 show the ^{1}H-NMR spectral data of acetylated quinoxaline derivatives. The structures of quinoxalines M-1 and G-1 were confirmed by ^{13}C-NMR spectroscopy. Fig. 4 shows the result of ^{13}C FT-NMR of G-1 in DMSO-d_6. The analytical data are as follows: G-1 δ(ppm): 39.9(t), 63.4(t), 71.8(d), 75.2(d), 128.5(d), 128.8(d), 129.0(d), 140.6(s), 141.6(s), 147.2(d), and 156.7(s); M-1 δ(ppm): 38.4(t), 63.4(t) 66.0(t), 76.8(d), 128.3(d), 128.5(d), 129.3(d), 139.7(s), 141.0(s), 155.5(s), and 155.8(s).

Table 2 is a summary of the physical properties of the quinoxaline derivatives derived from D-glucose and maltose. These results indicate that the pairs of quinoxalines G-5 and G-4, G-3 and G-2, and M-1 and G-1, were found to be structural isomers.

Structures of quinoxaline derivatives

Quinoxaline G-6 G-6 shows a molecular ion peak at m/z 144 (base peak), and other characteristic fragment ions at m/z 137, 117, 77, and 76. IR (Nujol, cm^{-1}) : 3400(br), 3050, 1560, 1490(s), 1365, 1290, 1200(s), 1120, 960(s), 760(s), and 600. These results are consistent with the previous description of G-6 in which it was identified as 2-methylquinoxaline.

Quinoxaline G-5 The *O*-TMS ether of G-5 shows a molecular ion peak at m/z 246, and other characteristic fragment ions at m/z 231 (base peak), 156, 129, and 73. IR (KBr, cm^{-1}) : 3200(br), 2850, 1490(s), 1375, 1160, 1020(s), 900, 820, 760(s), and 660. From

Table 1. ^{1}H-NMR Spectral Data of Acetylated Quinoxaline Derivatives

Quinoxalines	H-3	H-5,8	H-6,7	H-1′	H-2′	H-3′	H-4′	H-1″	Acetoxyl**
G-1*	8.74 (1H,s)	8.05 (2H,m)	7.75 (2H,m)	3.36 (2H,d) $J_{1',2'}$=6.2	5.66 (1H,dd) $J_{2',3'}$=5.4 $J_{1',2'}$=6.6	5.33 (1H,m)	4.42 (1H,dd) $J_{3,4a'}$=3.6 4.22(1H,dd) $J_{3,4b'}$=6.0 $J_{4a'4b'}$=12		2.10(2′) 2.08(3′) 1.97(4′)
M-1*		8.02 (2H,m)	7.74 (2H,m)	3.36 (2H,d) $J_{1',2'}$=6.8	5.78 (1H,m)	4.55(1H,dd) $J_{2'3a'}$=3.5 4.32(1H,dd) $J_{2',3b'}$=5.3 $J_{3a'3b'}$=12		5.47 (2H,s)	2.19(2′) 2.08(3′) 2.00(1″)
G-2	8.72 (1H,s)	8.02 (2H,m)	7.70 (2H,m)	3.33 (2H,d) $J_{1',2'}$=6.9	5.61 (1H,m)	4.44(1H,dd) $J_{2'3a'}$=3.6 4.19(1H,dd) $J_{2',3b'}$=5.4 $J_{3a',3b'}$=12			2.06(2′) 1.99(3′)
G-3		8.04 (2H,m)	7.74 (2H,m)	6.39(1H,t) $J_{1',2a'}$=5.6 $J_{1'2b'}$=6.3	4.67(2H,d) $J_{2a',2b'}$=5.6			2.86 (3H,s)	2.15(1′) 2.06(2′)
G-4	8.73 (1H,s)	8.05 (2H,m)	7.72 (2H,m)	3.33(2H,t) $J_{1',2'}$=6.6	4.59(2H,t)				1.99(2′)
G-5		8.05 (2H,m)	7.84 (2H,m)	5.43 (2H,s)				2.78 (3H,s)	2.18(1′)
G-6	8.70 (1H,s)	8.00 (2H,m)	7.69 (2H,m)	2.70 (3H,s)					

The spectra were determined in $CDCl_3$ with tetramethyl silane as an internal standard at 100 MHz except * marked compound at 90 MHz. The chemical shifts and coupling constants (J) are given as ppm and Hz, respectively.

** The figures in parentheses show the position of acetoxyl residue singlet = s, doublet = d, triplet = t, quartet = q, and multiplet = m.

these results and other analytical data, G-5 was finally identified as 2-hydroxymethyl-3-methylquinoxaline.

Quinoxaline G-4 The *O*-TMS ether of G-4 shows a molecular ion peak at m/z 246, and other characteristic fragment ions at m/z 231, 201, 156(base peak), 129, 104, and 73. IR (KBr, cm^{-1}): 3250(br), 2900, 2800, 1555, 1490(s), 1305(s), 1200, 1160, 1130, 1110, 1050(s), 1040(s), 970, 890, and 760(s). Found: C, 69.11; H, 5.44; N, 16.33. Calcd. for $C_{10}H_{10}N_2O$: C, 69.00; H, 5.78; N, 16.10%.

From these results and other analytical data, G-4 was identified as 2-(2′-hydroxyethyl) quinoxaline.

Quinoxaline G-3 The *O*-TMS ether of G-3 shows a molecular ion peak at m/z 348, and other characteristic fragment ions at m/z 333, 245(base peak), 147, and 73. IR (KBr, cm^{-1}): 3250(br), 1560, 1480, 1420, 1320, 1200(s), 1080(s), 1020(s), 1000, 915(s),

880, 855, 760(s), and 600. Found: C, 62.58; H, 5.95; N, 13.27. Calcd. for $C_{11}H_{12}N_2O_2$: C, 64.69; H, 5.92; N, 13.72%.

From these results and other analytical data, G-3 was identified as 2-(1′, 2′-dihydroxyethyl)-3-methylquinoxaline.

Quinoxaline G-2 The *O*-TMS ether of G-2 shows a molecular ion peak at m/z 348, and other characteristic fragment ions at m/z 333, 257(base peak), 245, 205, 169, 147, and 73. IR (KBr, cm^{-1}): 3200(br), 2850, 1580, 1555(s), 1485, 1405, 1285, 1250, 1200(s), 1160, 1090, 1030(s), 970(s), 900, 865(s), and 755(s). Found: C, 65.16; H, 6.10; N, 13.66. Calcd. for $C_{11}H_{12}N_2O_2$: C, 64.69; H, 5.92; N, 13.72%.

From these results and other analytical data, G-2 was identified as 2-(2′, 3′-dihydroxypropyl) quinoxaline.

Quinoxaline G-1 The *O*-TMS ether of G-1 shows a molecular ion peak at m/z 450, and other characteristic fragment ions at m/z 435, 360, 245(base peak), 217, 205, 147, 117, and 73. IR (KBr, cm^{-1}): 3250(br), 2870, 1550, 1490, 1360, 1200, 1110(s), 1065(s), 1000(s), 980, 875, 855, and 755(s). Found: C, 60.55, H, 6.30; N, 11.74; Calcd. for $C_{12}H_{14}N_2O_3$: C, 61.52; H, 6.02; N, 11.96%

From these results and other analytical data, G-1 was finally identified as 2-(2′, 3′, 4′-trihydroxybutyl)quinoxaline.

Quinoxaline M-1 The *O*-TMS ether of M-1 shows a molecular ion peak at m/z 450, and other characteristic fragment ions at m/z 435, 360, 347, 270, 246, 217, 205, 147, 117, and 73. IR (KBr, cm^{-1}): 3200(br), 2880, 2820, 1565, 1480, 1455(s), 1320, 1295, 1240,

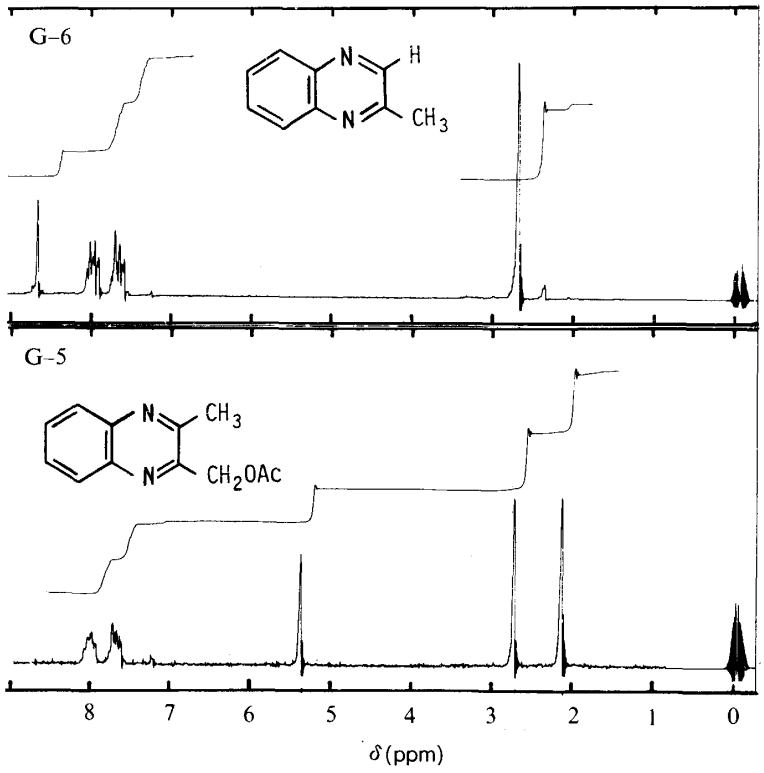


Fig. 3-(A). ^{1}H-NMR Spectra of Acetylated Quinoxaline Derivatives in $CDCl_3$ at 100 MHz. G-6, 2-methylquinoxaline; G-5, 2-hydroxymethyl-3-methylquinoxaline.

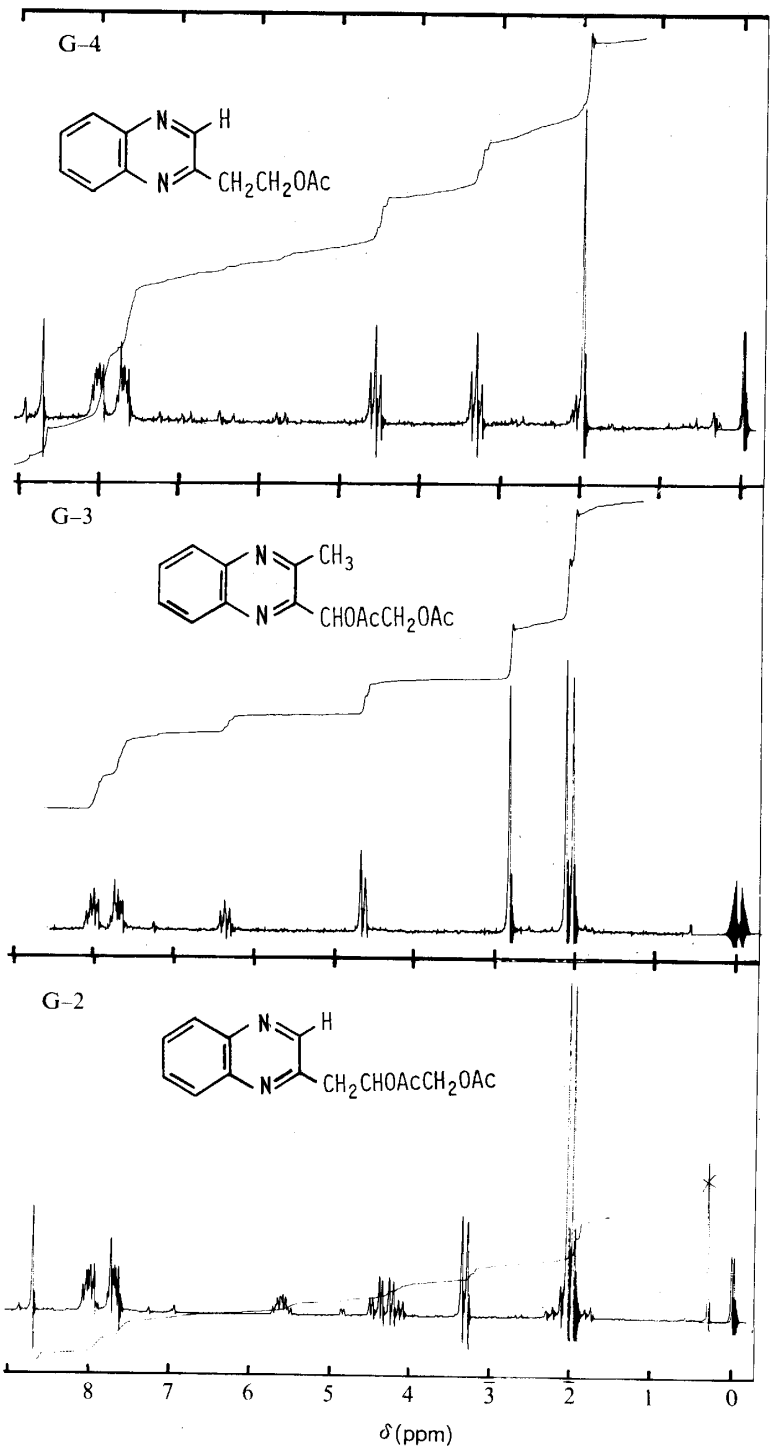


Fig. 3-(B). ^{1}H-NMR Spectra of Acetylated Quinoxaline Derivatives in $CDCl_3$ at 100 MHz. G-4, 2-(2′-hydroxyethyl) quinoxaline; G-3, 2-(1′, 2′-dihydroxyethyl)-3-methylquinoxaline; G-2, 2-(2′, 3′-dihydroxypropyl) quinoxaline.

1200, 1140, 1090, 1040(s), 980, 960, 935, 905, 820, 775, and 675. Found: C, 61.37; H, 6.32; N, 11.98. Calcd. for $C_{12}H_{14}N_2O_3$: C, 61.52; H, 6.02; N, 11.96%.

From these results and other analytical data, M-1 was finally identified as 2-(2′, 3′-dihydroxypropyl)-3-hydroxymethylquinoxaline.

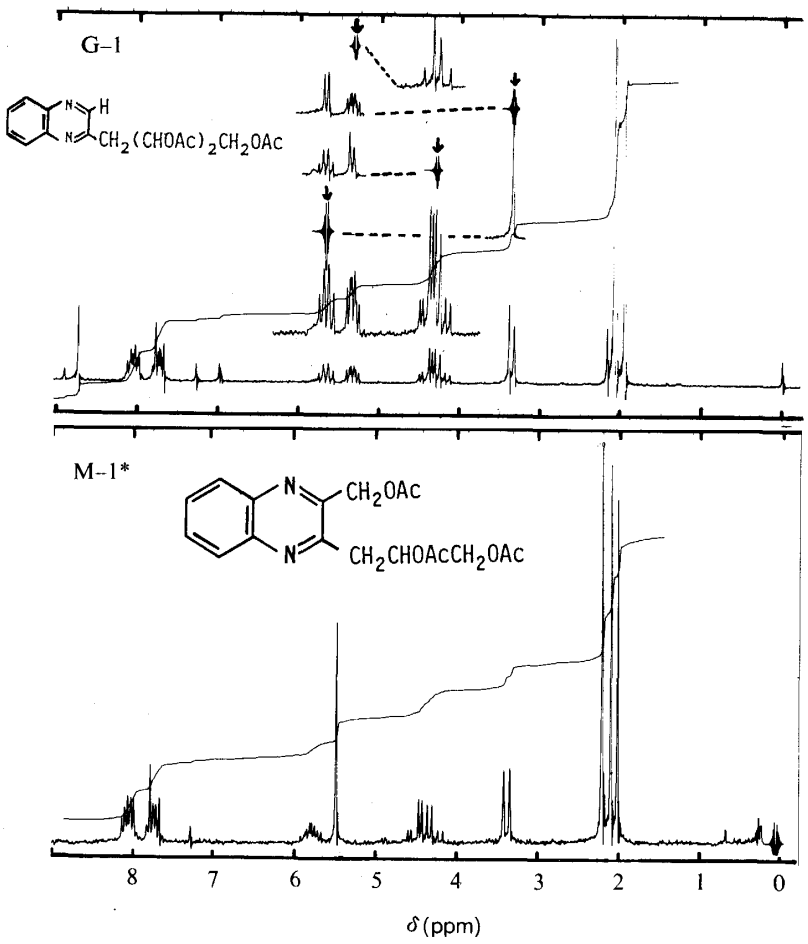


Fig. 3-(C). ^{1}H-NMR Spectra of Acetylated Quinoxaline Derivatives in $CDCl_3$ at 100 MHz or *90 MHz.
G-1, 2-(2′, 3′, 4′-trihydroxybutyl) quinoxaline, M-1, 2-(2′, 3′-dihydroxypropyl)-3-hydroxymethylquinoxaline.

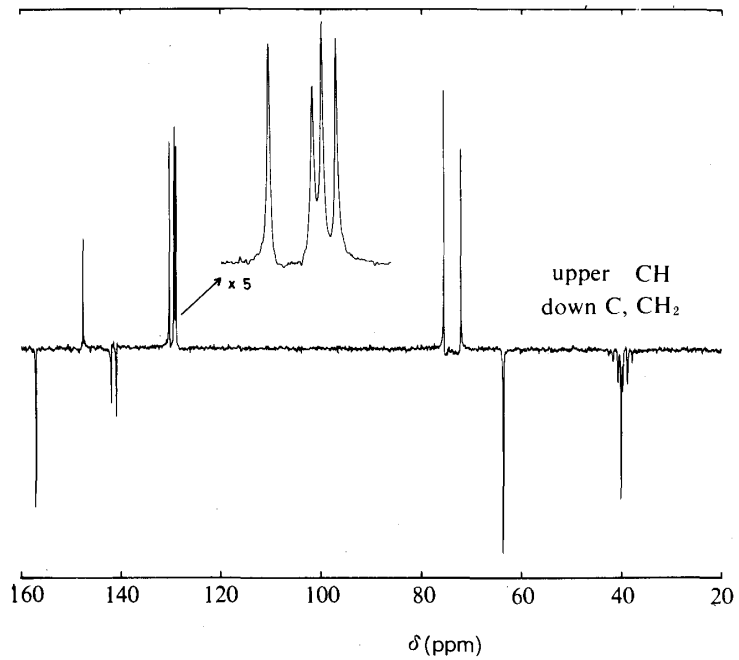


Fig. 4. Proton Noise-Decoupled ^{13}C FT-NMR Spectrum of Quinoxaline G-1 in DMSO-d_6.

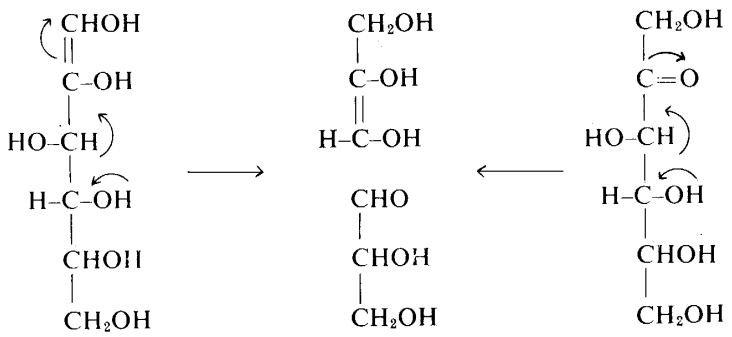
Table 2. Physical Properties of Quinoxalines Derived from D-Glucose and Maltose in Alkaline Media

Quinoxalines	G-6	G-5	G-4	G-3	G-2	G-1	M-1
R_f-values*	0.70	0.60	0.46	0.37	0.33	0.21	0.30
Color of spot	violet	brown	blue	reddish yellow	green	dark green	brown
mp (°C)	125–127**	sublimates	73–74	128–129	98.5	220–222	128–130
M.W.	144	174	174	204	204	234	234
$[\alpha]_D^{25}$ (C=0.4, MeOH)		–	–	+2.9°	−91.4°	−76.8°	−84.4°
$\varepsilon_{237}^{H_2O}$	24,700	27,800	29,030	27,080	29,100	27,800	28,950
(317 nm)	6,450	7,210	7,430	7,240	7,510	7,640	7,410
		6,260[a]					6,030[b]
Molar ratio***	0.868	0.093	0.153	0.003	0.018	0.156	0.013

* TLC was carried out on silica gel 60 plates with *n*-hexane-ether-EtOH (2 : 1 : 1)
** bp at 11 mmHg; [a] ε at 326 nm, [b] ε at 329 nm.
*** The molar ratio was calculated from the combination of GLC and HPLC results. The ratio is the value *vs* the mother molecule, *i.e.* molar production from one mole glucose.

Discussion

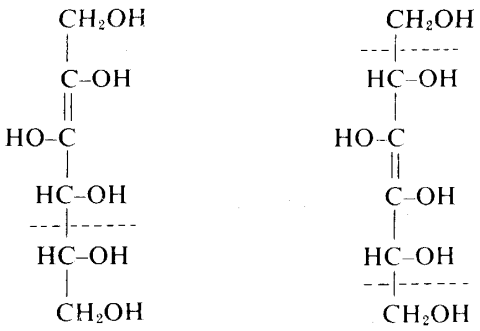
G-6 is the quinoxaline derivative of D-glucose of which the largest amount is formed. The main reason is that two moles of endiol or dicarbonyl compounds composed of three carbon atoms, such as 1, 2, 3-trihydroxypropene or methylglyoxal, may be predominantly produced from one mole of D-glucose residue by alkaline degradation, but for other quinoxalines only one mole of quinoxaline is obtainable from one mole D-glucose. The formation of 1, 2, 3-trihydroxypropene or methylglyoxal from D-glucose may occur by reverse aldolization,[16] as follows.



This reaction mechanism also suggests that a C-C bond having threo-type hydroxyl residues at the C_3 and C_4 of free D-glucose is readily cleaved in alkaline media, whereas this does not occur in the D-glucose residue linked by 1, 4-linkage with another D-glucose residue. This is quite different from quinoxaline in acidic media, where several kinds of quinoxaline derivatives are formed, but all are condensation products of OPD with

1, 2-dicarbonyl compounds from sugar residues,[17] in which none of the C-C linkages are cleaved.

For the formation of G-2 and G-3 or G-4 and G-5, reverse aldolization is a possible mechanism. However, before cleavage of the C-C bond, enediol-forms may be produced in alkaline media as below.



On the other hand, when G-4 and G-5 are obtained by the cleavage of the C-C bond, two-carbon-atom-possessing compounds may be formed from the rest of the cleaved compounds, which might be followed by the formation of quinoxalines, since such a simple quinoxaline without substitution was formed from oxyamylose prepared by oxidizing amylose with sodium metaperiodate, as reported previously.[11] Under the present deoxygenated alkaline conditions, no such simple quinoxaline could be obtained.

As to the reactivity of some other sugars with OPD in alkaline media, we tested several compounds such as 2-deoxyglucose, phenyl glucoside, glucitol, cyclodextrin and reducing-end reduced amylose, but no quinoxaline formation was observed. The formation of dicarbonyl compounds of sugar may be essential for this alkaline OPD method.

Acknowledgements

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