Heat Deterioration of Phospholipids. V. 
A New Rearrangement Reaction of Sugars and Phosphatidylethanolamine

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Abstract: Novel four 2,3-dihydro-1H-imidazo[1,2-a]pyridine-4-yl derivatives were obtained with increase of UV absorption at 350 nm and browning of the solution by heating paste lecithin from soybean (SL) in octane. These four derivatives were formed by reaction of one molar of any sugar except 2-deoxysugars with two molar of phosphatidylethanolamines (PE) in SL. To confirm the reaction mechanism, several 13C-labeled-sugars were reacted with 1,2-di-O-stearoylsn-glycero-3-phosphatidylethanolamine (DSPE), respectively. These reactants clearly showed that five carbons of the pyridinium ring and one carbon of the substituted group were based on those of a sugar and that the formation of the pyridinium derivatives was accompanied with cleaving between the carbons of 1- and 2-positions in the sugar and rearrangement. This reaction is a new rearrangement reaction and we named it “new pseudo Maillard rearrangement reaction”.

Key words: soybean lecithin, heat decomposition, 2,3-dihydro-1H-imidazo[1,2-a]pyridine-4-yl derivatives, rearrangement reaction, new pseudo Maillard rearrangement reaction

1 INTRODUCTION

A lecithin is extremely a useful material as an emulsifier. It has been very widely used in the food and non-food fields and the application of it has made rapid progress at present. With the application, a thermally deterioration of lecithin sometimes causes the fall in the qualities of goods1,2). It is very important to investigate the mechanism of the thermally deterioration of lecithin and to develop the method for prevention of the thermally deterioration of lecithin.

We recently discovered the absorption bands appeared at 350 nm besides those of 240 nm and 280 nm during the heating process of paste lecithin from soybean (SL)3. Novel four 2,3-dihydro-1H-imidazo[1,2-a]pyridine-4-yl derivatives that have two molar of PE moieties in their structures, namely compound A: 1-[2′-(2′,3′-dilinoleoylsn-glycero-1″-phosphoethyl)]-6-methyl-2,3-dihydro-1H-imidazol[1,2-a]pyridine-4-yl, compound B: 1-[2′-(2″-linoleoyl*3″-palmitoyl*sn-glycero-1″-phosphoethyl)]-6-hydroxymethyl-2,3-dihydro-1H-imidazol[1,2-a]pyridine-4-yl, compound C: 1-[2′-(2″,3″-dilinoleoylsn-glycero-1″-phosphoethyl)]-6-hydroxymethyl-2,3-dihydro-1H-imidazol[1,2-a]pyridine-4-yl, compound D: 1-[2′-(2″-linoleoyl*3″-palmitoyl*sn-glycero-1″-phosphoethyl)]-6-hydroxymethyl-2,3-dihydro-1H-imidazol[1,2-a]pyridine-4-yl, were isolated and identified as the compounds involving the increase of the UV absorption at 350 nm of SL by heating in octane (* means interchangeable, shown in Fig. 1)4.

These pyridinium derivatives are formed by reaction one molar of sugar and two molar of phosphatidylethanolamines (PE) with change of UV absorption and browning of the solution5. Any sugar except 2-deoxysugars can react with PE to generate the derivatives, regardless of a kind of sugar6. This reaction mechanism has been not clarified. In this paper, we report about elucidation of the reaction mechanism by using several 13C-labeled-sugars.

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2 EXPERIMENTAL

2.1 Instruments

\(^1\)H- and \(^{13}\)C-NMR data were recorded by a JOEL JNM-AL400 (400MHz) spectrometer in CDCl\(_3\) with TMS as an internal standard. Letters (br-)s, (br-)d, t, q, and m represent (broad-)singlet, (broad-)doublet, triplet, quartet, and multiplet, respectively, and coupling constants are given in Hz. HPLC were carried out with a Shimadzu LC-10AD pumps equipped with a Shimadzu RID-10A and a Shimadzu SPD-M10A diode array detector. UV data were measured by a Shimadzu UV2550 spectrophotometer.

2.2 Material

1,2-Di-\(O\)-stearoyl-\(sn\)-glycero-3-phosphatidylethanolamine (DSPE), 1,2-Di-\(O\)-palmitoyl-\(sn\)-glycero-3-phosphatidylethanolamine (DPPE) and \(\nu\)-glucose (2-\(^{13}\)C) were purchased from SIGMA (Missouri, USA). \(\nu\)-Glucose (1-\(^{13}\)C) and \(\nu\)-glucose (6-\(^{13}\)C) were purchased from Aldrich (Milwaukee, USA). \(\nu\)-Glucose (1,2,3,4,5,6-\(^{13}\)C\(_6\)) was purchased from ISOTEC (Sigma-Aldrich Co.). \(\nu\)-Arabinose (2-\(^{13}\)C) was purchased from Wako (Osaka, Japan).

2.3 Extraction and Isolation

2.3.1 Methods for heating of DSPE or DPPE with \(^{13}\)C-labeled sugars

A mixture of DSPE (100 mg, 133.7 \(\mu\)mol) and each of \(^{13}\)C-labeled \(\nu\)-glucoses (24.2 mg, 134.3 \(\mu\)mol) was dissolved into octane and heated for 9 h, respectively. A mixture of DPPE (100 mg, 144.5 \(\mu\)mol) and \(\nu\)-arabinose (2-\(^{13}\)C) (21.4 mg, 142.6 \(\mu\)mol) was also dissolved into octane and heated for 9 h.

2.3.2 Isolation of reactants of DSPE and each of \(^{13}\)C-labeled \(\nu\)-glucoses

After heating for 9 h, the reactants of DSPE and each of \(^{13}\)C-labeled \(\nu\)-glucoses were chromatographed on a silica gel column (Wakogel C-100, 260 mm \(\times\) 20 mm i.d.), eluting in sequence with an increasing concentration of methanol in chloroform to obtain the chloroform, 25% methanol in chloroform, and 50% methanol in chloroform frs, respectively. The compounds 1~4 (\(Rt=26.9\) min) were isolated from the 50% methanol in chloroform fr. by using HPLC (column: Senshu Pak. AQUASIL SS-4251(60) 250 mm \(\times\) 10mm i.d, Senshu, Japan, flow rate: 3.0 mL/min, solvent: chloroform:methanol:water=70:30:1, Detector: UV at 350 nm). The yield of compound 1 formed by reaction of DSPE and \(\nu\)-glucose (1,2,3,4,5,6-\(^{13}\)C\(_6\)) was 6.4 mg. The yield of compound 2 formed by using \(\nu\)-glucose (6-\(^{13}\)C) was 2.1 mg. The yield of the compound 3 formed by reaction of \(\nu\)-glucose(1-\(^{13}\)C) was 2.0 mg. The yield of compound 4 formed by using \(\nu\)-glucose (2-\(^{13}\)C) was 2.1 mg.

Each reaction generated two compounds (main compound with \(Rt=26.9\) min and minor compound with \(Rt=36.0\) min). We, however, focused on the main compound in this paper.

2.3.3 Isolation of reactants of DPPE and \(\nu\)-arabinose (2-\(^{13}\)C)

According to the same manner of mentioned above, compound 5 (\(Rt=38.8\) min) was isolated from the reactant of DPPE and \(\nu\)-arabinose (2-\(^{13}\)C). The yield of the compound 5 was 23.7 mg.

The spectral data of compounds A and 1~5 were given as follows.
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Compound A [A part of alteration of identification of 6- and 9-positions in 13C-NMR of literature4,5,6]

\[ R_t = 8.14 \text{ min.} \lambda \text{ max } 352 \text{ nm} \]

1H-NMR (CDCl3) \( \delta \): 0.88(6H, m, J=6.8), 1.29(*, m), 1.57(4H, m), 2.02(8H, q, J=6.8), 2.27(4H, q, J=7.3), 2.36(3H, s), 2.77(4H, t, J=6.3), 3.95(2H, m), 4.11(2H, m)**, 4.11(1H, m)**, 4.22(2H, m)**, 4.22(2H, m)**, 4.38(2H, m)**, 4.38(1H, m)**, 5.17(1H, m), 5.34(4H, m), 5.37(4H, m), 6.23(1H, d, J=4.2), 7.25(1H, d, J=4.2), 8.81(1H, s).

* means not clear.

** were confirmed by C-H COSY.

13C-NMR (CDCl3) \( \delta \): 12.03(q, C-10), 40.61(t, C-2), 47.33(t, C-3), 58.88(t, C-1', J=5.4), 61.56(t, C-2', J=3.4), 115.40(d, C-8), 122.70(s, C-9), 127.33(d, C-7), 145.10(s, C-6), 151.41(d, C-5).

The signals of two molar of linoleic acid moieties were not clear.

Compound 1 [Reactant of DSPE and D-glucose(1,2,3,4,5,6-13C6)]

\[ R_t = 26.9 \text{ min.} \]

13C-NMR (CDCl3) \( \delta \): 12.03(q, C-10), 40.61(t, C-2), 47.33(t, C-3), 58.88(t, C-1', J=5.4), 61.56(t, C-2', J=3.4), 115.40(d, C-8), 122.70(s, C-9), 127.33(d, C-7), 145.10(s, C-6), 151.41(d, C-5).

The signals in aliphatic field were not clear.

Compound 2 [Reactant of DSPE and D-glucose(6-13C)]

\[ R_t = 26.9 \text{ min.} \]

13C-NMR (CDCl3) \( \delta \): 12.21(C-10), The other signals were not clear.

Compound 3 [Reactant of DSPE and D-glucose(1-13C)]

\[ R_t = 26.9 \text{ min.} \]

13C-NMR (CDCl3) \( \delta \): 151.10(C-5), The other signals were not clear.

Compound 4 [Reactant of DSPE and D-glucose(2-13C)]

\[ R_t = 26.9 \text{ min.} \]

13C-NMR (CDCl3) \( \delta \): 122.51(C-9), The other signals were not clear.

Compound 5 [Reactant of DPPE and D-arabinose(2-13C)]

\[ R_t = 38.8 \text{ min.} \]

13C-NMR (CDCl3) \( \delta \): 122.74(C-9). The other signals were not clear.

3 RESULTS AND DISCUSSION

Four 2,3-dihydro-1H-imidazo[1,2-a]pyridine-4-ylium derivatives were generated by heating paste lecithin from soybean (SL) in octane4). These four derivatives were formed by reaction of one molar of any sugar except 2-deoxysugars with two molar of phosphatidylethanolamines (PE) in SL6). To confirm the reaction mechanism, several 13C-labeled-sugars were reacted with 1,2-di-O-stearoyl-sn-glycero-3-phosphatidylethanolamine (DSPE), respectively.

When D-glucose (1,2,3,4,5,6-13C6) was reacted with DSPE, six of 13C-labeled carbons originated from the glucose were introduced into the pyridinium derivatives as shown in Fig. 2. This result clearly shows that all of sugar's carbons were rearranged and composed to form 6-methyl-2,3-dihydro-1H-imidazo[1,2-a]pyridine-4-ylium or 6-hydroxymethyl-2,3-dihydro-1H-imidazo[1,2-a]pyridine-4-ylium.

Next, several 13C-labeled-D-glucoses were reacted with DSPE in order to elucidate the mechanism of rearrangement reaction. In the reaction of D-glucose (1,2,3,4,5,6-13C6) with DSPE, the labeled-carbon was only introduced into the substituted group (10-position) on the pyridinium ring, as shown in Fig. 3. In the reaction of D-glucose (1,2,3,4,5,6-13C6) with

![Fig. 2 13C-NMR (CDCl3) Spectra of Compounds A and 1.](image-url)
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DSPE, the labeled-carbon was only introduced into the 5-position at 151.10 ppm of the pyridinium ring. Moreover, only the carbon at 9-position (122.51 ppm) of the pyridinium ring was labeled by reaction of D-glucose (2-13C) with DSPE.

In addition to these results, reaction of D-arabinose (2-13C) with 1,2-di-O-palmitoyl-sn-glycero-3-phosphatidylethanolamine (DPPE), the carbon at 9-position of the pyridinium ring without any substituted group at 6-position on the ring was only labeled.

These results show without any doubt that the formation of the pyridinium derivatives was accompanied with cleaving between the 1- and 2-positions of carbons in the sugar and rearrangement. The carbons of 1- and 2-positions in a sugar are always introduced into the 5- and 9-positions on the pyridinium derivatives, respectively. The carbons of 3-, 4-, and 5 positions in a sugar must be always introduced into the 8-, 7-, and 6-positions on the pyridinium derivatives, respectively. Moreover, the carbon of 6-position in a sugar is always introduced into the substituted group (10-position) at 6-position on the ring. These results are also suggested by facts that any pentose reacted with PE to form the pyridinium derivatives without the substituted group at 6-position on the ring and that 2-deoxysugars never react with PE to form the pyridinium derivatives because this rearrangement reaction requires to cleave between the 1- and 2-positions of a sugar and because there is no functional group at 2-position in 2-deoxysugar.

These experiments by using 13C-labeled-sugars also reveal incorrect identification of 6- and 9-positions in 13C-NMR about the pyridinium derivatives in previous reports. The correct identification is given in the Experimental section.

This formation of the pyridinium derivatives is occurred under very mild condition only by heating any sugar except 2-deoxysugars with PE in non polar solvent, accompanied with cleaving between the carbons of 1- and 2-positions in the sugar. This is a new rearrangement reaction and we named it "new pseudo Maillard rearrangement reaction".

We will investigate the mechanism of this rearrangement reaction in detail by isolation and identification of the intermediates which is formed during the reaction of one molar of a sugar and two molar of PE in the near future.

Fig. 3 13C-NMR (CDCl3) Spectra of Reactants of Several 13C-labeled Sugars with DSPE and DPPE.

References