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Iio, Masayoshi

Food Chemistry Institute, Faculty of Agriculture, Kyushu University

Shimotokube, Toshiro

Food Chemistry Institute, Faculty of Agriculture, Kyushu University

Omura, Hirahisa

Food Chemistry Institute, Faculty of Agriculture, Kyushu University

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## Preparation of Some Pentose Oximes

**Masayoshi Iio, Toshiro Shimotokube and Hirahisa Omura**

Food Chemistry Institute, Faculty of Agriculture,  
Kyushu University, Fukuoka

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Oximes of arabinose, ribose and xylose have been prepared from each pentose and hydroxylamine in anhydrous ethanol. Free hydroxylamine prepared from its hydrochloride by neutralization with sodium ethoxide was added dropwise into a pentose solution. Water in the solvent and that formed during the reaction were removed by coevaporation with benzene. Arabinose and ribose gave crystalline oximes. Although crystallization could be also achieved with xylose oxime under the present reaction conditions, collected crystals melted into syrup when they were left stand at room temperature. The formation of pentose oximes was demonstrated by paper chromatography, elemental analysis and NMR spectrum measurement in hexadeuterio-dimethyl sulfoxide. NMR spectroscopy furnished the relative contents of *syn* and *anti* form in each oxime and some other interesting informations.

### INTRODUCTION

A group of enzymes termed transoximase were discovered by Yamafuji in 1953 and the distribution in organisms as well as their nature including the substrate specificity was studied along with the improvement of the enzyme activity assay. The function of the enzymes in biological system was also indicated: They might constitute a new biosynthetic pathway of amino acids together with oximinoreducing enzyme, oximase, from the metabolic cycle of inorganic nitrogen compounds such as hydroxylamine. Simple aldioximes, ketoximes, keto acid oximes and hexose oximes were found to be the substrate of the transoximase. It was not until the preparation of the hexose oximes that they could be investigated as substrate of the enzyme. Above all an improved method developed by Tsutsumi *et al.* (1969) for the preparation of fructose oxime advanced the study on the enzyme which exerts its action on the hexose oxime. The point of the method was the rather long-time reaction of fructose with hydroxylamine under anhydrous condition. The method could be employed in the preparation of glucose oxime, which otherwise could be obtained only after a fairly long time period. Xylose oxime, however, could not be prepared even by this method.

Among transoximases for sugar oximes, only those concerning hexose oxime had been studied. Therefore an investigation of transoximases for pentose oximes would be interesting, which might clarify the similarities and differences among various types of the transoximases. Pentose oximes including xylose oxime must be obtained in pure state in order to study the nature of the enzymes which use pentose oximes as substrate.

This paper deals with an attempt to improve the preparation method of pentose oximes and an investigation of some of their properties.

## EXPERIMENTAL

### General methods and materials

Pentoses and hydroxylamine hydrochloride were purchased from Wako Pure Chemicals, Paper chromatography was carried out with Toyo No. 51 paper using ascending technique and the detection methods were (1) cupric chloride spray for oximes (Hranisavljević-Jakovljević et al., 1963) and (2) aniline hydrogen phthalate spray for sugars (Partridge, 1949). The solvent systems used are indicated on the footnote of Table 2. NMR spectra were recorded on a Hitachi Perkin Elmer R-20 High Resolution NMR Spectrometer (60 MHz) with specimens in hexadeuterodimethyl sulfoxide using tetramethylsilane as internal standard.

### Free hydroxylamine solution

Hydroxylamine hydrochloride (2.8 g, 40 mmole) was dissolved in 70 ml of ethanol with stirring in a tree-necked flask equipped with a calcium chloride tube, into which sodium ethoxide solution (metallic sodium 0.92 g, 40 mmole in 50 ml of ethanol) in a dropping funnel was added to the neutral point using phenolphthalein as internal indicator. Sodium chloride formed was filtered off and washed with a little amount of ethanol.

### Pentose solution

Pentose (3 g, 20 mmole) in a little more amount of ethanol than to bring saturation was refluxed until a solution was obtained. Five ml of benzene was added to the solution and distilled for a short period to remove trace of water in the solution.

### Preparation of pentose oxime

The free hydroxylamine solution in ethanol containing 10 ml of benzene was added dropwise into the pentose solution, Distillation was continued throughout the oxime formation reaction. The amount of hydroxylamine solution introduced into the pentose solution was adjusted to cancel the amount distilled out. After all the hydroxylamine solution was added, the reaction mixture was concentrated in a rotary vacuum evaporator. In the case of arabinose or ribose, each oxime was deposited on evaporation. When xylose was used, the reaction mixture was concentrated into about 50 ml and chilled in a freezer at  $-30^{\circ}\text{C}$  overnight. White powdery crystals formed were collected by cold centrifugation or by filtration using a sintered-glass filter cooled either with calcium chloride-ice mixture or with dry ice-ethanol mixture. The crystals thus obtained melted

Table 1. Analytical data of pentose oximes.

| Oxime     |  | C     | H    | N    | mp   |
|-----------|--|-------|------|------|------|
| Pentose   | Calcd. for $\text{C}_5\text{H}_{11}\text{O}_5\text{N}$ | 36.36 | 6.72 | 8.48 |      |
| Arabinose | Found  | 36.53 | 6.75 | 8.49 | 142° |
| Ribose    | Found  | 36.15 | 6.67 | 8.43 | 142  |
| Xylose    | Found  | 36.16 | 6.94 | 7.93 | —    |

into syrup on standing at room temperature. Analytical data of pentose oximes are shown in Table 1.

## RESULTS AND DISCUSSION

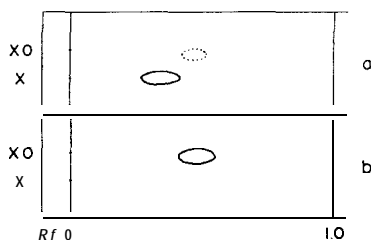
Crystals of arabinose or ribose oxime were obtained rather easily (Table 1), but xylose did not give its oxime in crystalline state. Since the reaction mixture of xylose and hydroxylamine did give a positive test for oxime, the problem seemed to lie in the method of crystallization. So procedures of crystallization as well as the reaction condition were examined. Oxime formation being the dehydration of carbonyl compounds and hydroxylamine, it is desirable that the solvent contains no water and that water formed during the reaction is removed from the reaction mixture. Many oximes of usual carbonyl compounds are relatively insoluble in water, which favors the oxime formation due to the deposition of the oxime from reaction mixture. Sugar oximes, however, are very soluble in water, making their crystallization difficult especially in the cases of fructose, glucose or xylose. As a result the utilization of anhydrous alcohol as solvent and hydroxylamine solution in anhydrous alcohol is favorable both for formation of oxime and for crystallization of the oxime produced. The procedure had been successfully employed in the preparation of fructose and glucose oxime, but still failed to synthesize crystalline xylose oxime. Hence a strictly anhydrous condition was applied in an attempt to prepare xylose oxime; water formed was removed by coevaporation with benzene in the course of the reaction. Another attempt was the continual supply of free hydroxylamine, which might be decomposed by heat, water or atmospheric carbon dioxide during the reaction. Formation of xylose oxime was followed by application of aliquots of the reaction mixture to paper chromatography and the relation of the amount of added hydroxylamine with the yield of the oxime was observed. When the ratio of xylose and hydroxylamine reached 1 : 1.8, all the sugar used was completely converted to the oxime and no by-product was observed. The reaction mixture thus obtained formed white powdery crystals when chilled, which could be collected either by cold centrifugation or by cold filtration. But they melted into syrup when left stand at room temperature. The syrup was dried over phosphorus pentoxide for a long time and the residual ethanol was removed as much as possible. The sample gave a negative test for xylose. Solvents other than ethanol, for example, methanol, propanols, butanols, ethylene glycol, tetrahydrofuran, 1,4-dioxane, ethyl ether or chloroform, could not give crystals. Difficulty of crystallization of xylose oxime could be attributed to its high solubility in polar solvents such as alcohols, as is the case with fructose oxime. It can be generally said that the more soluble the starting sugar is in alcohol, the harder crystallization of the oxime is to achieve in the solvent. A similar phenomenon is known for condensation products of sugars with another carbonyl compound, hydrazine. A mixture of xylose and arabinose can be distinguished from each other according to the difference of the solubilities of their hydrazones, since xylose hydrazone is more highly soluble than arabinose derivative.

Paper chromatography with several solvent systems showed that solvent E

**Table 2.** Rf's of sugars and their oximes.

| Compound   | Solvent system |      |      |      |      |
|------------|----------------|------|------|------|------|
|            | A              | B    | C    | D    | E    |
| Arabinose  | 0.36           | 0.67 | 0.91 | 0.36 | 0.24 |
| Ara. Oxime | 0.41           | 0.70 | 0.90 | 0.41 | 0.66 |
| Ribose     | 0.41           | 0.67 | 0.64 | 0.39 | 0.45 |
| Rib. Oxime | 0.39           | 0.75 | 0.70 | 0.37 | 0.46 |
| Xylose     | 0.38           | 0.74 | 0.64 | 0.35 | 0.33 |
| Xyl. Oxime | 0.43           | 0.76 | 0.64 | 0.36 | 0.46 |

Solvent A ; formic acid-pyridine-butanol-water (1: 2 : 3 : 4), solvent B ; Pyridine-butanol-water (3 : 4 : 7), solvent C ; acetic acid-butanol-pyridine-water (1 : 3 : 2 : 4), solvent D ; acetic acid-butanol-water (1 : 4 : 2), solvent E ; butanol-benzene-pyridine-water (5 : 1 : 3 : 3).

**Fig. 1.** Paper chromatograms of xylose and xylose oxime. Detection; a) aniline hydrogen phthalate, b) cupric chloride.

was most useful to separate arabinose from its oxime, as can be seen in Table 2. The formation of xylose oxime was apparently shown on paper chromatograms when the same developing solvent was used for the xylose-hydroxylamine reaction mixture in the present method, as shown in Fig. 1. Moreover there were no xylose remained in the mixture.

Although the formation of xylose oxime was thus indicated by the paper chromatography, it was shown more evidently by NMR spectroscopy in the present investigation. NMR spectrum of arabinose ( $\beta$ -L-) in hexadeuterodimethyl sulfoxide showed a doublet at 6.02 ppm, which could be assigned to protons of hydroxyl function bound to C-1 carbon. The doublet disappeared when the sugar was converted into oxime and two new doublets appeared at 6.68 and 7.34 ppm due to the presence of H-C=N group of *syn* and *anti* form, respectively (Fig. 2). Ribose oxime (Fig. 3) gave a spectrum consisting of a series of peaks of nearly the same chemical shifts as the case of arabinose oxime. There also appeared two singlets in 10-11 ppm region in the spectra of the oximes which were assigned to C=N-OH, since the singlets were extinguished on deuterium oxide exchange. The spectrum of xylose oxime (Fig. 4) also apparently showed two doublets around 7 ppm, although the degree of resolution in other respects was rather low probably due to ethanol remained in the syrup of the specimen, which gave a methyl proton peak at 1.07 ppm, one absent in the spectra of arabinose- and ribose oxime. The remaining ethanol also seemed to affect the peak pattern beyond 10 ppm. There is only one, rather broad singlet, indicating that

### Preparation of Penrose Oxime

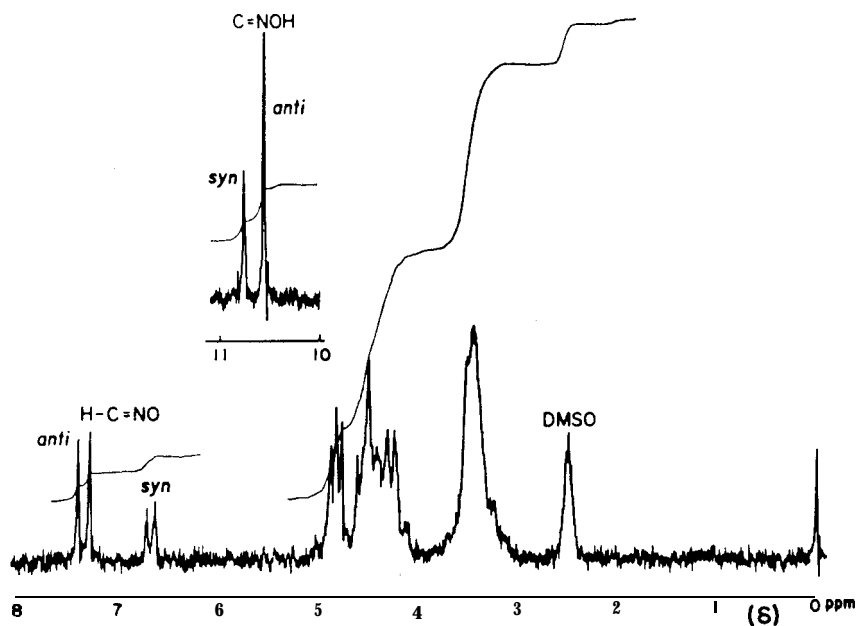


Fig. 2. NMR spectrum of arabinose oxime.

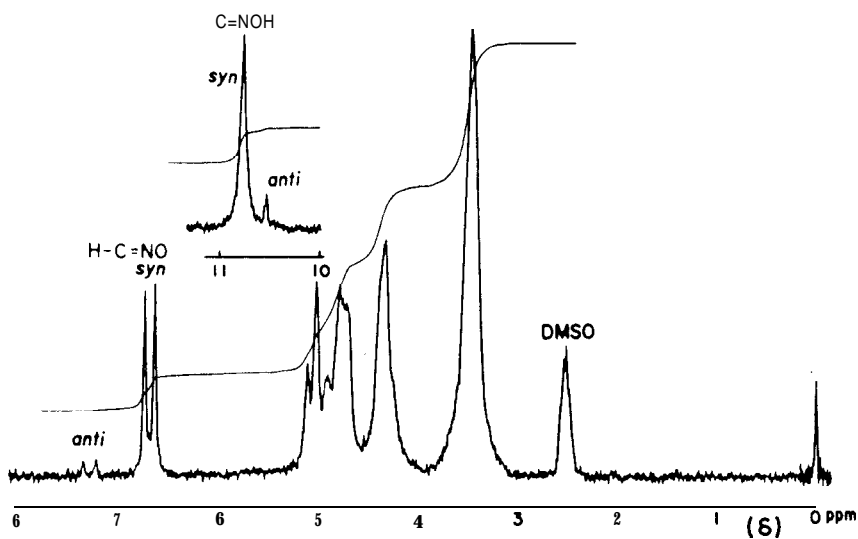


Fig. 3. NMR spectrum of ribose oxime.

protons of both *anti* and *syn* hydroxyimino group exchange for each other more rapidly in the presence of ethanol than in its absence.

Table 3 exhibits some other interesting features of NMR spectra of pentose oximes. The coupling constants of *anti* form of the doublet at about 7 ppm (H-C=N-) are always larger than those of *syn* form. By comparing peak area of

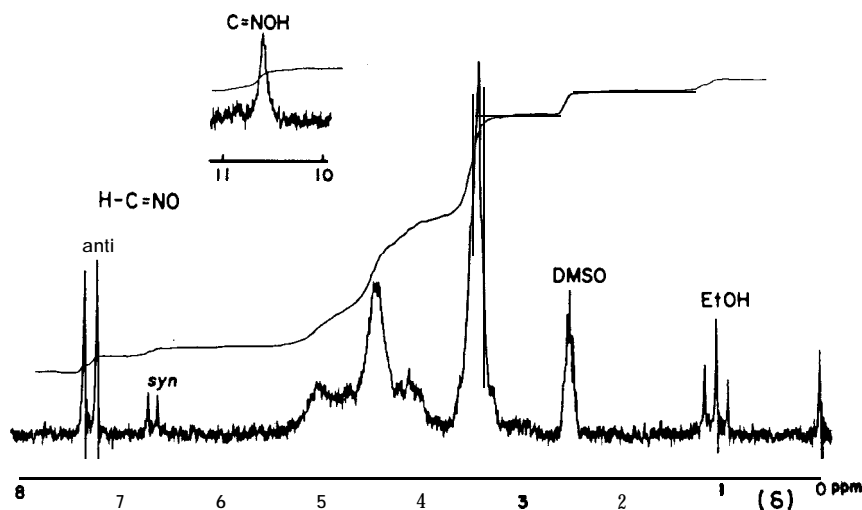


Fig. 4. NMR spectrum of xylose oxime.

Table 3. Some NMR spectroscopic features of sugar oximes.

| Sugar oxime | <i>Anti</i> form            |       | <i>Syn</i> form             |       | Peak area ratio |
|-------------|-----------------------------|-------|-----------------------------|-------|-----------------|
|             | Chem. shift ( $\delta$ ), J |       | Chem. shift ( $\delta$ ), J |       | <i>anti/syn</i> |
| Arabinose 1 | 7.34 ppm                    | 7 cps | 6.68 ppm                    | 5 cps | 1.8: 1          |
| Arabinose 2 | 7.36                        | 7     | 6.70                        | 5     | 1: 2.5          |
| Ribose      | 7.28                        | 8     | 6.68                        | 6     | 1: 10           |
| Xylose      | 7.30                        | 7     | 6.69                        | 6     | 3: 1            |

the two doublets of each oxime, relative amount of *anti* and *syn* form present in the specimens could be calculated. *Anti* form dominated in the specimen of arabinose oxime that crystallized first, while crystals recovered from mother liquor included more *syn* form. Syrup of xylose oxime always contained more *anti* form. On the other hand, most of ribose oxime existed as *syn* form.

All the results strongly indicated the formation of xylose oxime in pure state, although it existed as syrup at room temperature. The xylose oxime thus prepared could be used in enzymatic investigations, when the concentration of a solution of the oxime is estimated by a suitable method (Omura et al., 1963).

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