# Preparation of Some Pentose Oximes

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

# Preparation of Some Pentose Oximes

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

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

*(Received May 8, 1975)*

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 hexadeuterodimethyl 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 oxiimino reducing enzyme, oximase, from the metabolic cycle of inorganic nitrogen compounds such as hydroxylamine. Simple aldoximes, 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 transoximascs. 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°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 $C_5H_{11}O_5N$ | 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 | Fouud | 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

*M. Iio et al.*

**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-buta-
nol-water  (3 : 4 : 7),  solvent  C ;  acetic  acid-butanol-pyridine-water  (1 : 3 : 2 : 4),  sol-
vent  D ;  acetic  acid-butanol-water  (1 : 4 : 2),  solvent  E ; butanol-benzene-pyridine-
water  (5 : 1 : 3 : 3).
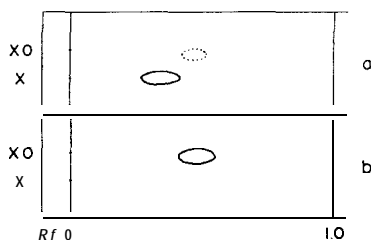


**Fig. 1.**   Paper  chromatograms  of  xylose  and  xylose  oxime.  Detection;  a)  aniline
hydrogen  phthalate,  b)  cupric  chloride.

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  chromato-
grams  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-l  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  ara-
binose- 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
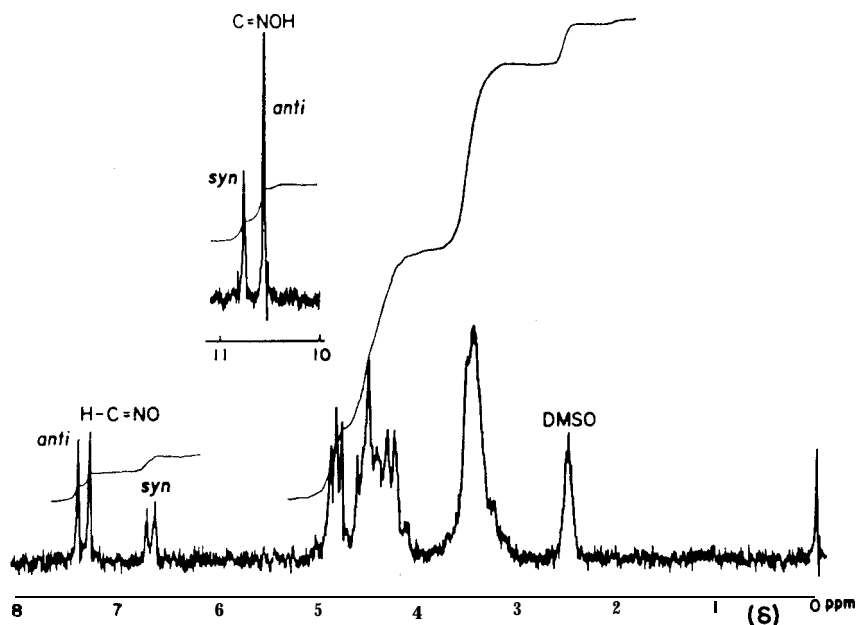
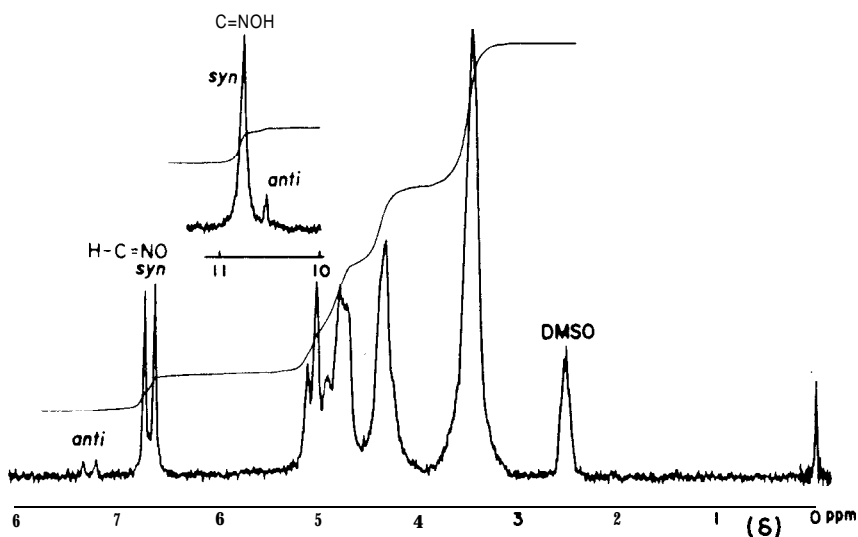Fig. 2.    **NMR** spectrum of arabinose oxime.



**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
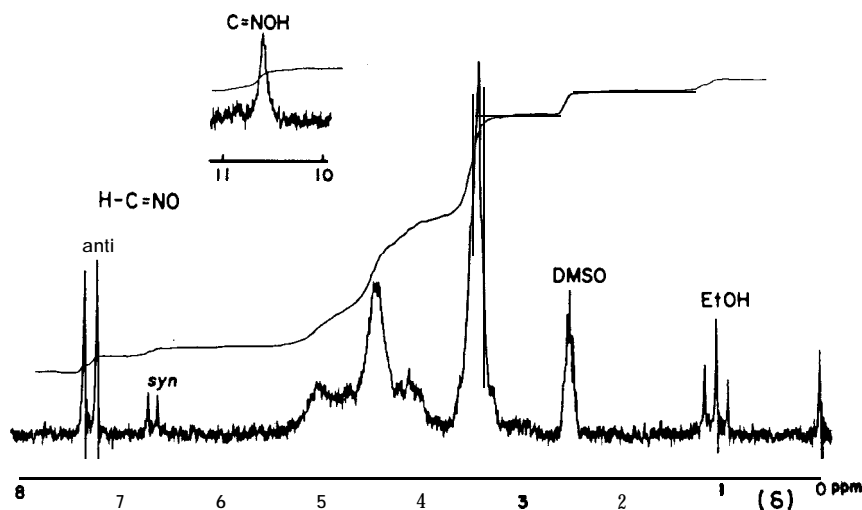
M. Iio *et al.*



**Fig. 4.** NMR spectrum of xylose oxime.

**Table 3.** Some NMR spectroscopic features of sugar oximes.

| Sugar oxime | *Anti* form | | *Syn* form | | Peak area ratio |
|---|---|---|---|---|---|
| | Chem. shift ($\delta$), J | | Chem. shift ($\delta$), J | | *anti/syn* |
| 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).

## REFERENCES

Hranisavljević-Jakovljević, M., I. Pejković-Tadlć and A. Stojkljkovit 1963 Thin-layer chromatography of isomeric oximes. *J. Chromatog., 12: 70-73*

Omura, H., Y. Osajima, S. Hatano, F. Yoshihara, K. Watanabe and K. Yamafuji 1963 On the estimation of inorganic nitrogenous compounds and oximes. *Sci. Bull. Fac. Agr., Kyushu Univ., 20: 179-198*

Partridge, S.M. 1949 Aniline hydrogen phthalate as a spraying reagent for chromatography. *Nature, 164: 443*

Tsutsumi, M., M. Iio and H. Omura 1969 Preparation of fructose oxime. *J. Jap.* Soc. *Food and Nutrition, 22: 462-467*

Yamafuji, K. 1953 Enzymatic transoximation. *Nature, 171: 745*