THIO SUGARS

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The generally accepted biological significance of substances containing a thiol group is sufficient justification for undertaking a detailed study of the thio sugars. Only one naturally occurring thio sugar in which the thio group is attached to a carbon atom in other than the reducing position is known. This is a thiomethyl pentose which is a component of an adenine nucleoside occurring in yeast. Two papers appeared almost simultaneously on the structure of this sugar; Suzuki, Odake, and Mori assigned to the sugar the structure of a 5-thiomethyl aldopentose, whereas Levene and Sobotka regarded it as a 3- or 4-thiomethyl ketopentose. Neither of these theories appears to have been adequately tested, and further investigation was suggested by Dr. Levene.

In view of the scarcity of the material, the synthetic method of approach seemed most promising. The theory of Suzuki et al. is particularly susceptible of investigation by this method, for there are only four possible 5-thiomethyl aldopentoses of either series (d- or l-) and these would give only two osazones. Thus their theory requires that the osazone of the natural sugar be identical with the osazone of 5-thiomethyl xylose (lyxose) or 5-thiomethyl ribose (arabinose).

The synthesis of 5-thioethyl and 5-thiomethyl xylose was accomplished by heating 5-tolylsulfo monoacetone xylose with the potassium salt of ethyl or methyl mercaptan, followed by hydrolysis of the acetone derivative to the nicely crystalline alkylthio xyloses. By analogy with 5-methyl xylose in method of preparation, these compounds would be expected to have the thio

groups attached to carbon atom (5) and this assumption was confirmed by examination of the glycoside formation. The formation of only one glycoside, of the typical γ type, was indicated. The substances may therefore be designated 5-thiomethyl and 5-thioethyl xylose. They rapidly reduce Fehling's solution on warming, and reduce it slowly at room temperature. Since the only ring form in which they can exist is the furanose, it is interesting that these free sugars are so nicely crystalline.

The synthetic 5-thiomethyl xylose differed from the natural sugar in that it was crystalline, had a different specific rotation, and gave a phenylosazone which differed in melting point and optical properties from the osazone of the natural thiomethyl pentose. Thus, 5-thiomethyl xylose, 5-thiomethyl lyxose, and 5-thiomethyl-2-ketoxylose (or -lyxose) are definitely excluded as possible structures for the natural sugar.

On attempting to apply these same methods to the p-tolylsulfo 2,3-monoacetone methylribofuranoside described by Levene and Stiller, the reaction apparently followed a different course and after hydrolysis only a small amount of a syrupy reducing substance was obtained. From this material, however, there was prepared a nicely crystalline p-bromophenylosazone of correct composition. In its properties this substance also differed from the corresponding osazone of the natural sugar. Had the structure of the syrupy material been definitely established, then position (5) would have been excluded for the allocation of the thiomethyl group in the natural sugar. An attempt was therefore made to prepare 5-thiomethyl ribosazone in a manner that would assure its structure. Since the osazone of 5-thiomethyl arabinose would be identical with that of 5-thiomethyl ribose, it would serve equally well, but unfortunately no derivatives of arabinose which permit substitution in position (5) are easily available. For this reason attention was directed to the possibility of preparing 6-thiomethyl glucose with a view to degrading it to 5-thiomethyl arabinose. The 6-thiomethyl glucose was in fact prepared (from 6-p-tolylsulfo monoacetone glucose) and its structure was confirmed by glucoside formation, which was analogous in type to that of glucose and of 6-methyl glucose. The degradation of this derivative to the arabinose compound has not as yet been completed.

Because of the further possibility that, in the naturally occurring thio sugar, the thiomethyl group might be attached in a position other than (5), attention was directed to the development of methods for the introduction of thio groups into such positions. The only previous work along this line is that of Freudenberg, who synthesized 3-thiomethyl glucose by isomerizing the xanthate; but this method would be difficult to extend to the substances which we desired to synthesize. In view of the fact that 2-bromo-β-methylglucoside has been shown to give 3-amino-β-methylglucoside on treatment with ammonia, it was hoped that the 3-thiomethyl derivatives might be synthesized analogously. In preliminary experiments, 2-bromo-methylglucoside was treated with potassium methyl mercaptan, giving a thiomethyl hexoside. However, on hydrolysis to the free reducing hexose, it was found that an osazone could not be prepared. Though negative evidence is open to question, it is felt that the experiments were convincing in this regard, and that the thiomethyl group is in all probability attached to carbon atom (2). From the mother liquors of the thiomethyl hexoside preparation, an isomeric, non-crystalline material was isolated by acetylation and distillation. On hydrolysis to the reducing hexose, this material was likewise found to yield no osazone, again indicating substitution in position (2).

In connection with the work on the synthetic thio sugars, their behavior toward oxidizing agents was studied because of its bearing on the possible keto structure of the natural sugar. It was found that the 5-thiomethyl monoacetone xylose, in which the aldehyde group was protected against oxidation, was practically non-reducing in the Lehma,nn-Maquenne determination, indicating no oxidation of the thio group by this reagent. In the Willstätter determination, on the other hand, exactly 1 atom of oxygen was used, indicating quantitative oxidation of the CH₃S— to CH₃SO—. The free thiomethyl xylose required 1.92 atoms of oxygen in the Willstätter oxidation so that both aldehyde and thio groups are almost quantitatively oxidized. In the Lehmann-Maquenne determination the free thio sugar used 1.39 atoms of oxygen, 1 mg. of the thio pentose being equivalent to 0.42 mg. of glucose.

**EXPERIMENTAL**

5-Thioethyl Monoacetone Xylose—12 gm. of 5-p-tolylsulfo monoacetone xylose and 6.0 gm. (2 moles) of dry sodium ethyl mercap-
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Tan were added to 70 cc. of dry acetone and sealed in a Pyrex flask body. The mixture was heated in the boiling water bath for 2 hours, cooled, and opened. Without filtering, the mixture was concentrated under reduced pressure, water being added from time to time to keep the sodium toluenesulfonate in solution. The aqueous solution was extracted with 600 cc. of chloroform in three portions, the extract washed with dilute, ice-cold sulfuric acid, and then three times with ice water. The chloroform extracts were dried with sodium sulfate and concentrated under reduced pressure to a thick syrup. This was dissolved in hot hexane and cooled in a solid carbon dioxide-alcohol bath. The product crystallized on stirring. It was washed with cold hexane and air-dried. By reworking the mother liquors a second crop was secured, the total yield of air-dry material being 6.1 gm. or 75 per cent of the theoretical. The product was three times recrystallized from hexane and it then melted at 66°-67° and had a rotation, in absolute alcohol, of

$$[\alpha]_D^\text{a} = \frac{-2.35^\circ \times 100}{2 \times 2.04} = -57.5^\circ$$

The composition agreed with that of a thioethyl monoacetone pentose.

4.585 mg. substance: 8.600 mg. CO₂ and 3.100 mg. H₂O
8.164 " " : 8.030 " BaSO₄
7.585 " " : 7.570 " AgI
C₁₀H₁₄O₆S. Calculated. C 51.24, H 7.75, S 13.69, OC₂H₅ 19.21

The substance is very soluble in methyl or ethyl alcohol, ether, acetone, ethyl acetate, and chloroform; fairly soluble in hot water, boiling heptane, or hexane; little soluble in boiling pentane; and almost insoluble in water, heptane, hexane, and pentane in the cold.

5-Thioethyl Xylose—2 gm. of 5-thioethyl monoacetone xylose were added to 40 cc. of 1 N H₂SO₄ and the mixture was heated at 50° with frequent shaking until dissolved (about 1 hour). The solution was then heated to 80°, maintained at this temperature for 2 hours, cooled, and shaken with an excess of barium carbonate. The mixture was filtered and the filtrate evaporated to dryness in a vacuum desiccator over phosphorus pentoxide. The product
crystallized spontaneously. It was extracted several times with boiling ether, leaving a small syrupy residue, which was discarded. The ether solutions were concentrated to a small volume and cooled. On seeding and stirring, the product crystallized. A second crop was obtained from the mother liquors, the yield of crystalline material being 1.4 gm. or 84 per cent of the theoretical. The material was re-crystallized from ether in the same manner and then melted at 81–82° and had a rotation, in absolute alcohol, of

\[
\alpha = \frac{-1.69 \times 100}{2 \times 2.00} = -42.2^\circ
\]

3 minutes after dissolving and was unchanged after 2 hours. The rotation, in water, was

\[
\alpha = \frac{+1.20 \times 100}{2 \times 2.012} = +29.8^\circ
\]

1 minute after dissolving, +20.6° (2 minutes), +17.1° (3 minutes), +16.4° (5 minutes), +15.4° (10 minutes, 20 minutes, and 1½ hours).

The composition agreed with that of a thioethyl pentose.

<table>
<thead>
<tr>
<th>Substance</th>
<th>Carbon Dioxide</th>
<th>Water</th>
<th>BaSO₄</th>
<th>AgI</th>
</tr>
</thead>
<tbody>
<tr>
<td>4.030 mg.</td>
<td>6.450 mg.</td>
<td>2.710 mg.</td>
<td>10.840 “</td>
<td>8.471 “</td>
</tr>
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<td>4.030 mg.</td>
<td>6.450 mg.</td>
<td>2.710 mg.</td>
<td>10.840 “</td>
<td>8.471 “</td>
</tr>
<tr>
<td>C₅H₇O₄S</td>
<td>43.65</td>
<td>7.53</td>
<td>16.24</td>
<td>21.48</td>
</tr>
<tr>
<td>Calculated</td>
<td>43.26</td>
<td>7.27</td>
<td>16.51</td>
<td>23.17</td>
</tr>
</tbody>
</table>

The substance is very soluble in water, methyl or ethyl alcohol, chloroform, and acetone. It is fairly soluble in hot ether or ethyl acetate, but not very soluble in the cold. It is very little soluble even at the boiling point, in heptane, hexane, or pentane.

5-Thiomethyl Monooacetone Xylose---15 gm. of 5-p-tolylsulfo monoacetone xylose, 7.6 gm. (2 moles) of dry potassium methyl mercaptan, and 75 cc. of dry acetone were heated, in a sealed flask, in the boiling water bath for 4 hours. The subsequent procedure was the same as for the ethyl derivative. The washed, dried chloroform extracts were concentrated under reduced pressure to a syrup which was dissolved in hexane. The product crystallized immediately and was filtered off and washed with hexane. By reworking the mother liquors, two additional crops of material
were secured, the total weight of air-dry substance being 7.2 gm.
(75 per cent of the theoretical). The product was recrystallized
from hexane containing 8 per cent of ethyl acetate, and then twice
recrystallized from hexane containing 5 per cent of ethyl acetate.
The material then melted at 91°–92° and had a rotation, in
absolute alcohol, of

$$\left[\alpha\right]_{D}^{20} = \frac{-2.20° \times 100}{2 \times 2.04} = -53.9°$$

The composition agreed with that of a thiomethyl monoacetone
pentose.

5.244 mg. substance: 9.500 mg. CO₂ and 3.480 mg. H₂O
6.990 “ “ : 7.580 “ AgI
C₆H₁₀O₃S. Calculated. C 49.05, H 7.33, S 14.56, OCH₂ 14.07

The solubilities of the substance are similar to those of the
corresponding ethyl derivative.

5-Thiomethyl Xylose—2 gm. of 5-thiomethyl monoacetone xylose
were added to 40 cc. of 1 N H₂SO₄, heated to 50°, and shaken fre-
quently until dissolved (about 30 minutes). The subsequent
treatment was the same as for the thioethyl derivative. The yield
of crude crystalline product was 1.4 gm. (85 per cent of the theo-
retical). The product, twice crystallized from ether, had a melting
point of 74–75°. The rotation, in absolute alcohol, was

$$\left[\alpha\right]_{D}^{20} = \frac{-0.46° \times 100}{2 \times 1.0} = -23.0°$$

3 minutes after dissolving and −39.0° at equilibrium. In water
it was

$$\left[\alpha\right]_{D}^{20} = \frac{+1.45° \times 100}{2 \times 1.992} = +36.4°$$

1 minute after dissolving, 27.8° at 2 minutes, 22.8° at 3 minutes,
22.6° at 4½ minutes, 22.1° at 6 minutes, 20.6° at 9 minutes, 24 min-
utes, and 3 hours.

The composition agreed with that of a thiomethyl pentose.
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5.018 mg. substance: 7.370 mg. CO₂ and 3.100 mg. H₂O
5.390 “ “ : 6.740 “ AgI
C₆H₁₅O₃S. Calculated. C 39.96, H 6.72, S 17.79, OCH₃ 17.20

The solubilities of the substance are similar to those of the corresponding thioethyl derivative.

5-Thiomethyl Xylosazone—0.5 gm. of crude crystalline 5-thiomethyl xylose was dissolved in water and 1.0 cc. (3.3 moles) of phenylhydrazine dissolved in 1 cc. of glacial acetic acid was added. The mixture was heated on the steam bath for 2 hours and cooled. The osazone was filtered off, washed with methyl alcohol, and then recrystallized from methyl alcohol. The final material was a felt of coarse lemon-yellow needles. The composition corresponded to that of a thiomethyl pentosazone.

4.712 mg. substance: 0.633 cc. N (23° and 766 mm.)
7.400 “ “ : 4.730 mg. BaSO₄
C₁₈H₂₂O₂SN. Calculated. N 15.65, S 8.94
358.3 Found. “ 15.62, “ 8.78

The product sintered slightly at 151° and melted without decomposition at 162–163°. The rotation in pyridine-absolute alcohol (2:3 by volume) was

\[
\left[\alpha\right]_D^{20} = \frac{-0.17 \times 100}{0.5 \times 1.036} = -32.8°
\]

2 minutes after dissolving, and −21.2° at equilibrium. The osazone from the natural product was reported by Levene and Sobotka as having an initial rotation in the above solvent of +6° and +7° at equilibrium. A newly prepared sample of the osazone of the natural product was found to melt at 160–161° and to have a mixed melting point with the osazone of the synthetic material of 143–145°. Thus there can be no doubt as to the non-identity of the two osazones.

Glycoside Formation on 5-Thiomethyl and 5-Thioethyl Xylose—
The method and technique were those previously employed, the glycoside formation being conducted at room temperature and at 76°. The results are plotted in Fig. 1, 5-methyl xylose being
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included for comparison. It will be seen that in all cases only one glycoside appears to be formed and that this is of the typical $\gamma$ type.

**Fig. 1.** Glycoside formation on 5-thiomethyl, 5-thioethyl, and 5-methyl xylose.

**Table I**

*Oxidation Equivalents of 5-Thiomethyl Xylose and 5-Thiomethyl Monoacetone Xylose*

<table>
<thead>
<tr>
<th>Method</th>
<th>Substance</th>
<th>Amount of Substance (mg.)</th>
<th>0.1 N thiosulfate (cc.)</th>
<th>Atoms C per mole</th>
<th>Glucose equivalent to 1 mg. (mg.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lehmann-Maquenne</td>
<td>Thiomethyl xylose</td>
<td>7.97</td>
<td>1.22</td>
<td>1.39</td>
<td>0.42</td>
</tr>
<tr>
<td>&quot;</td>
<td>&quot; monoacetone xylose</td>
<td>50.7</td>
<td>0.04</td>
<td>0.01</td>
<td></td>
</tr>
<tr>
<td>Willstätter</td>
<td>xylose</td>
<td>31.8</td>
<td>0.76</td>
<td>1.92</td>
<td></td>
</tr>
<tr>
<td>&quot;</td>
<td>monoacetone xylose</td>
<td>50.7</td>
<td>4.63</td>
<td>1.00</td>
<td></td>
</tr>
</tbody>
</table>
Oxidation Determinations—The thiomethyl xylose and its monoacetone derivative were used for oxidation determinations by both the Lehmann-Maquenne and Willstätter methods. The results are given in Table I.

6-Thiomethyl Monoacetone Glucose—45 gm. of 6-tolylsulfo monoacetone glucose and 22 gm. of dry potassium methyl mercaptan were added to 200 cc. of dry, peroxide-free dioxane, and sealed in a Pyrex flask body. The mixture was shaken overnight at room temperature and was then heated in the steam bath, with occasional shaking, for 1 1/4 hours. The mixture was cooled, the bulb opened, and considerable ether was added. The precipitate was filtered off and extracted several times with boiling ether. The ethereal filtrate and extracts were combined, concentrated under reduced pressure to a small volume, and part of the dioxane was distilled off. 800 cc. of ether were added to the residue, the solution filtered with charcoal, and concentrated under reduced pressure to a syrup. This was distilled (at about 0.1 mm.), the boiling point being 140-155°. Four fractions (total weight 23.3 gm.) were obtained and of these the third crystallized spontaneously. When this was used for nucleation, all the fractions crystallized to a hard mass; but re-crystallization from a solvent has not as yet been effected. The second fraction had a rotation, in absolute alcohol, of 

\[
[a]_D^\circ = \frac{-0.16 \times 100}{2 \times 2.282} = -3.5^\circ
\]

and the last fraction, in the same solvent, a rotation of \(-7.6^\circ\).

The composition of the product agreed with that of a thiomethyl monoacetone hexose.

4.498 mg. substance: 7.920 mg. CO₂ and 2.810 mg. H₂O
7.180 " " : 6.648 " BaSO₄
5.840 " " : 5.485 " AgI

\[C_{19}H_{23}O_5S\] Calculated. C 47.96, H 7.25, S 12.81, OCH₃ 12.39
250.2 Found. " 48.02, " 7.00, " 12.72, " 12.40

6-Thiomethyl Glucose—5 gm. of 6-thiomethyl monoacetone glucose were added to 30 cc. of 1 N sulfuric acid, the mixture warmed and shaken until dissolved, and then heated at 80° for 2 hours.
The solution was cooled, the sulfuric acid neutralized with barium carbonate, and the filtrate concentrated under reduced pressure to a syrup. Despite repeated attempts, crystallization of this syrup has not as yet been effected. It strongly reduced hot Fehling’s solution.

1 gm. of this syrup was heated on the steam bath with 3 moles of p-bromophenylhydrazine and acetic acid, and the osazone which formed was filtered off. A second crop was secured by heating for a further time and then cooling in the refrigerator. The combined material was extracted twice with hot methyl alcohol in which the osazone is practically insoluble. It was then a felt of beautiful, yellow needles. It darkened, on rapid heating, at 177° and melted at 184–185°. The rotation, in pyridine–absolute alcohol (3:2 by volume) was

\[
[a]_D^\infty = \frac{-0.24^\circ \times 100}{0.5 \times 1.00} = -48^\circ
\]

2 minutes after dissolving, and −16° at equilibrium after 5 hours.

The composition agreed with that of a bromophenylosazone of a thiomethyl hexose.

4.600 mg. substance: 7.060 mg. CO₂ and 1.650 mg. H₂O

6.299 " " : 0.564 cc. N (at 27° and 759.5 mm.)

C₃₅H₂₂O₃N₄Br₂. Calculated. C 41.75, H 4.06, N 10.26

546.1 Found. " 41.86, " 4.02, " 10.19

Glucoside Formation on 6-Thiomethyl Glucose—The method and technique were identical with those for the xylose derivative. The results are plotted in Fig. 2, 6-methyl glucose being included for comparison.

Thiomethyl Methylhexoside from 2-Bromo-β-Methylglucoside I—Bromine was added to triacetylglucal, and the bromo sugar converted to the glucoside, substantially as described by Fischer et al. The first form crystallized in good yield, was recrystallized, and deacetylated.

30 gm. of dry, recrystallized 2-bromo-methylglucoside I and 15 gm. of dry potassium methyl mercaptan were added to 350 cc. of dry acetone and sealed in a Pyrex flask body. This was heated in

The steam bath for 5 hours, cooled, and opened. The insoluble material was filtered off and washed several times with hot acetone, the combined acetone solutions were concentrated as far as possible, and considerable chloroform was added. The mixture was boiled for several minutes and filtered hot. The residue was washed with hot chloroform, and the combined solutions were concentrated under reduced pressure to a syrup. This material crystallized spontaneously on standing. It was triturated with acetone to remove the syrupy fraction and the crystalline material which remained weighed, on drying, 8.6 gm. It was recrystallized from 45 volumes of hot acetone. After two recrystallizations it had a melting point of 128$\frac{1}{2}$–129$\frac{1}{2}$° and a rotation, in water, of

$$[\alpha]_b^\circ = \frac{-2.99^\circ \times 100}{2 \times 1.988} = -52.6^\circ$$
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The composition agreed with that of a thiomethyl methyl-
hexoside.

4.600 mg. substance: 7.220 mg. CO₂ and 2.930 mg. H₂O
9.150 “ “ : 59.505 “ BaSO₄
5.470 “ “ : 10.970 “ AgI

C₅H₁₈O₃S. Calculated. C 42.82, H 7.19, S 14.30, OCH₃ 27.65

A portion of this material was hydrolyzed with 1.7 N hydro-
chloric acid until the rotation became constant. The solution
was not very strongly reducing and no osazone was formed on
heating with either phenylhydrazine or p-bromophenylhydrazine.
The non-crystalline mother liquors resulting from the isolation
of the thiomethyl methylhexoside were combined, acetylated, and
distilled under greatly reduced pressure. The syrup was deacety-
lated and hydrolyzed with hydrochloric acid until the rotation
became constant. The solution was slightly reducing but no
osazone was obtained on heating with either phenylhydrazine or
p-bromophenylhydrazine.
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