ON SPHINGOSINE.¹

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(Received for publication, May 2, 1912.)

Sphingosine was discovered by Thudichum on hydrolysis of a cerebroside, phrenosine. Discussing the chemical properties of the substance, its behavior towards bases and acids, the author took into consideration the possibility of the substance having the structure of an amino-acid or of an alkaloidal base. In his final conclusion he expressed preference to the view of the basic nature of the substance.

In later years Thierfelder repeated the work of Thudichum, and in the main substantiated his views. The work of Thierfelder, however, was directed principally to the study of the properties of

¹ A report on the results of the present investigation has appeared in the Proceedings of the Meeting of the American Society of Biological Chemists, held December 28th to December 30th, 1911, published in the March number of this Journal. In Heft 6, vol. lxxvii of Hoppe-Seyler’s Zeitschrift für physiologische Chemie, published on April 9, there appeared two articles by Thierfelder, Riesser and Thomas in which the authors arrived at the same conclusions as reported by us. The appearance of the two articles was caused undoubtedly by the publication of our report, since Heft 5 of vol. lxxvii of Hoppe-Seyler’s Zeitschrift contained no mention of Thierfelder’s name among the authors of twenty-six articles received for publication. In a footnote to one of the articles Professor Thierfelder claims the sole privilege for work on sphingosine and related substances, for the reason that Thudichum’s work had been half forgotten at the time when Thierfelder directed his attention to cerebrosides. We do not feel that this justifies the request made by Thierfelder, that the work which had been in progress in our laboratory for more than a year should be abandoned before it is completed.

² Die chemische Konstitution des Gehirns, Tübingen, 1901.
On Sphingosine

the cerebroside, which he named "cerebron." The author expressed no definite view regarding the chemical structure of the base.

The results of the present investigation have made certain that sphingosine is an unsaturated monoaminodihydroxyalcohol.

This conclusion is based on the following data:

1. The substance contains all its nitrogen in form of primary amino nitrogen.

2. The presence of a double binding in the molecule is demonstrated by the readiness with which sphingosine absorbs hydrogen when treated according to the method of Paal. A substance is thus formed which has the composition of dihydrosphingosine. It was analyzed in the form of a sulphate and a triacetylderivative.

3. The presence of two hydroxyl groups in the molecule is evident from the fact that sphingosine forms a triacetylderivative which no longer contains the original primary amino group. The substance forms a dimethylether, and finally it can be reduced to an amine, sphingamine.

As yet it is not certain whether or not the carbon atoms are linked in a normal chain. Attempts were made to reduce the dihydrosphingosine to the corresponding amine, but instead of the heptadecylamine there was always obtained the unsaturated sphingamine. Efforts to obtain the saturated amine are now in progress. Also work is in progress on the respective position of the double bond, and of the hydroxyl groups.

EXPERIMENTAL PART.

Sphingosine.

The base was obtained on hydrolysis of "cerebrin" prepared by a slight modification of the process described by Parcus.\(^4\) The conditions of hydrolysis were similar, but not identical with those described by Thierfelder. The base was prepared in crude form as the sulphate, which was then transformed into the free base and into the acetate. The discoverer of the base mentioned that it could be made to crystallize out of ether. It was found in course

of this work that crystallization proceeded much more readily out of petrolic ether.

The sulphate was obtained in the form of a white crystalline powder. It melted with decomposition at 233°C to 234°C. (uncorrected). A great many samples were analyzed. The analysis of one of these gave the following results:

0.0996 gram of the substance dried in chloroform-vacuum bath over phosphorus pentoxide gave on combustion 0.0940 gram of H₂O and 0.2280 gram of CO₂.

0.2400 gram of the substance, employed for a Kjeldahl nitrogen estimation, required for neutralization 6.9 cc. of N/10 acid.

Calculated for (\(\text{C}_7\text{H}_8\text{N}_2\text{O}_{12}\text{H}_2\text{SO}_4\)): Found:

\[
\begin{array}{ll}
\text{C} & 61.08 \quad 61.05 \\
\text{H} & 10.78 \quad 10.60 \\
\text{N} & 4.19 \quad 4.06 \\
\end{array}
\]

The optical activity of the substance was the following:

0.5304 gram of the sulphate was dissolved in a mixture of 5 cc. of chloroform and 1 cc. of glacial acetic acid. The total weight of solution was 8.7514 grams. The rotation in pure D-light was -1.50°, hence

\[\left[\alpha\right]_{D}^{20} = -13.12° (+0.00)\].

Diacetate. Dissolved in glacial acetic acid and petrolic ether, the substance crystallized in form of very long needles of the following composition.

0.1402 gram of the substance gave on combustion 0.1290 gram H₂O and 0.3206 gram of CO₂.

Calculated for (\(\text{C}_7\text{H}_8\text{N}_2\text{O}_{12}\)): Found:

\[
\begin{array}{ll}
\text{C} & 62.22 \quad 62.36 \\
\text{H} & 10.61 \quad 10.22 \\
\end{array}
\]

Amino nitrogen estimation. A solution of 0.300 gram of the sulphate in 10.0 cc. of glacial acetic acid was employed for an amino nitrogen estimation according to the method of Van Slyke. Five cc. were used for each experiment. All nitrogen was given off in thirty minutes. In each experiment 11 cc. of nitrogen were formed at \(t = 21°C\) and \(p = 760\) mm.

Calculated for (\(\text{C}_7\text{H}_8\text{N}_2\text{O}_{12}\)): Found:

\[
\begin{array}{ll}
\text{N} & 4.19 \quad 4.17 \\
\end{array}
\]
On Sphingosine

Dihydrosphingosine.

The hydrogen absorption value of sphingosine is obtained most conveniently when the free base is dissolved in ether and shaken with aqueous colloidal palladium prepared according to Paal. 0.100 gram of palladium to about 0.500 gram of the base dissolved in about 150 cc. of absolutely pure ether gave the most satisfactory results. The absorption was completed in about thirty minutes. The velocity of the operation was greatly increased by the addition of 1 cc. of glacial acetic acid to the ethereal solution.

0.500 gram of the substance absorbed 50 cc. of hydrogen (without correction for t and p). Theory requires 45 cc. of H.

0.6480 gram of the substance absorbed 59 cc. of H; theory requires 56 cc.

The ethereal solution of dihydrosphingosine was evaporated to dryness and the substance converted into the sulphate and into the triacetyl derivative.

The sulphate was obtained in form of a white crystalline powder. Its melting point was only slightly different from the unsaturated compound, being 235°C.

The optical activity of the substance was difficult to determine for the lack of a sufficiently satisfactory solvent. Approximately it was as follows:

0.0776 gram of the substance dissolved in about 3 cc. of alcohol containing sulphuric acid, and weighing 2.8640 grams gave a rotation of $-0.39^\circ$ in a 2 dm. tube.

\[ [\alpha]_D^{20} = -10.67^\circ \]

0.1214 gram of the substance gave on combustion 0.1153 gram of H₂O and 0.2948 gram of CO₂.

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0.1214 gram of the substance gave on combustion 0.1153 gram of H₂O and 0.2948 gram of CO₂.

\[ C_{17}H_{34}N_2O_8 \] (Found: 
\[ C \].......................... 66.76 66.76
\[ H \].......................... 10.60 10.60

\[ C_{17}H_{34}N_2O_8 \] (Found: 
\[ C \].......................... 66.76 66.76
\[ H \].......................... 10.60 10.60
Acetylderivatives.

On treatment of the free base with acetic anhydride di- or triacetylsphingosine can be obtained. The first is obtained by dissolving the base in boiling acetic anhydride and evaporating the solution under diminished pressure. The triacetylderivative is prepared by allowing the base to digest with acetic anhydride in a boiling water bath with return condenser for one hour and only then evaporating the solution to dryness. The further treatment in both instances is identical. The residue obtained on evaporating the solution under diminished pressure is taken up in chloroform and again evaporated under diminished pressure. This residue is taken up in hot acetone and the substance allowed to crystallize. For analysis the substances were dried in a vacuum-chloroform bath over phosphorus pentoxide.

Diacetylderivative. 0.1317 gram of the substance gave on combustion 0.1256 gram of H₂O and 0.3283 gram of CO₂.

0.2720 gram of the substance was dissolved in 10 cc. of glacial acetic acid and used for amino nitrogen estimation according to Van Slyke. Five cubic centimeters of the solution were employed for each experiment. There was formed 9.3 cc. nitrogen at 24°C. and 758 mm. pressure. The substance was allowed to react one hour, although the reaction was practically completed in twenty minutes.

Calculated for
\[ (C₁₅H₂₆O₇·NH₃)·(CH₃CO)₂ \]

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<thead>
<tr>
<th></th>
<th>Calculated</th>
<th>Found</th>
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</thead>
<tbody>
<tr>
<td>C</td>
<td>68.1</td>
<td>68.16</td>
</tr>
<tr>
<td>H</td>
<td>10.8</td>
<td>10.59</td>
</tr>
<tr>
<td>N</td>
<td>3.4</td>
<td>3.78</td>
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The physical constants and saponification value of this substance were not determined.

Triacetylderivative. 0.1194 gram of the substance gave on combustion 0.1064 gram of H₂O and 0.2950 gram of CO₂.

0.2500 gram of the substance was dissolved in 10 cc. of glacial acetic acid and employed for an amino nitrogen estimation. No formation of nitrogen took place.

Calculated for
\[ C₁₇H₂₇NO₈·(CH₃CO)₃ \]

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<th></th>
<th>Calculated</th>
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<tbody>
<tr>
<td>C</td>
<td>67.15</td>
<td>67.38</td>
</tr>
<tr>
<td>H</td>
<td>9.98</td>
<td>9.98</td>
</tr>
</tbody>
</table>

The substance melted sharply at 102° to 103° C. (uncorr.).

0.3383 gram of the substance was dissolved in 60 cc. of methyl alcohol, containing 10 cc. of a \( \frac{N}{5} \) solution of sodium hydrate in methyl alcohol. The solution was heated on boiling water bath for two hours, allowed to stand over night and titrated. It required 24.90 cc. of \( \frac{N}{5} \) alkali to neutralize the acetic acid formed on saponification. The theory required 24.75 cc.
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Dimethylsphingosine.

This substance is formed in course of hydrolysis of cerebrosides by means of methylalcohol and mineral acid. Thierfelder, who was the first to have the substance in his hands, erroneously regarded it as a new base. Since our first communication, Thierfelder and Riesser\(^5\) substantiated our view on the substance. The methyl derivative was obtained in form of a sulphate on concentrating the mother liquors from the crude sphingosine sulphate. The sulphate was then transformed into the free base and this again transformed into the hydrochloride. The hydrochloride crystallizes out of alcohol in the form of large glittering plates.

The substance was identified by the fact that, similarly to sphingosine, it contained all its nitrogen in form of primary amino nitrogen; it contained one unsaturated bond and on boiling with hydroiodic acid formed the required amount of methyl iodide.

0.1615 gram of the substance gave on combustion 0.1654 gram \(\text{H}_2\text{O}\) and 0.3926 gram of \(\text{CO}_2\).

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<th>Calculated for</th>
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<tbody>
<tr>
<td>(\text{C}_n\text{H}_m\text{NO}_a\cdot\text{HCl})</td>
<td></td>
</tr>
<tr>
<td>C</td>
<td>65.18</td>
</tr>
<tr>
<td>H</td>
<td>11.52</td>
</tr>
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</table>

*Hydrogen absorption value.* One gram of the substance dissolved in ether containing 2 cc. of glacial acetic acid. On treatment with palladium according to Paal it absorbed 67 cc. of hydrogen. Theory requires 72.6 cc.

*Amino nitrogen estimation.* 0.3500 gram of the hydrochloride dissolved in 10 cc. of glacial acetic acid. Five cc. of this solution used for amino nitrogen estimation according to Van Slyke. There formed 12.5 cc. of nitrogen at \(t = 24^\circ\) and \(p = 758 \text{mm}\).

<table>
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<tr>
<th>Calculated for</th>
<th>Found:</th>
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<tbody>
<tr>
<td>(\text{C}_n\text{H}_m\text{NO}_a\cdot\text{H}_2\text{HCl})</td>
<td></td>
</tr>
<tr>
<td>N</td>
<td>4.01</td>
</tr>
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</table>

*Methyl estimation.* 0.1206 gram of the hydrochloride boiled with hydroiodic acid of specific gravity = 1.71 in the apparatus of Zeisel and Fanto. There was obtained 0.1286 gram of silver iodide.

<table>
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<th>Calculated for</th>
<th>Found:</th>
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<tbody>
<tr>
<td>(\text{C}_n\text{H}_m\text{NO}_a\cdot\text{CH}_3\text{H}_2\text{HCl})</td>
<td></td>
</tr>
<tr>
<td>(\text{CH}_3)</td>
<td>8.45</td>
</tr>
</tbody>
</table>

The physical properties of the substance did not permit a more accurate estimation. In a control experiment with sphingosine.

crystallized out of petrolic ether no silver iodide was formed. On
the other hand, the base obtained directly after removing the sul-
phuric acid from apparently pure sphingosine sulphate still caused
the formation of some silver iodide. The highest value obtained
in this manner was equivalent to $\text{CH}_3 = 2.32$ per cent.

Sphingamine.

Attempts were made to reduce dihydrosphingosine to the corre-
sponding amine. The normal heptyldecylamine has been obtained
synthetically. Hence a comparison of the two bodies should have
determined the fact whether or not the substances were identical.
In several experiments the reduction was attempted by means of
hydroiodic acid, and in one experiment the dihydrosphingosine
was transformed into the dihydrodichlorsphingosine, which was
then reduced by means of metallic sodium and alcohol.

However, under all conditions the unsaturated substance was
formed.

Reduction with hydroiodic acid was carried out in sealed tubes at
$125^\circ\text{C}$. The reaction-product was dissolved in ether. The ethe-
real solution was dried with anhydrous sodium sulphate, and then
diluted with one-third of its volume of 98 per cent alcohol, and the
solution treated with metallic sodium.

The substance obtained from the solution was transformed into
the sulphate. The analysis of the substance obtained from three
different experiments follows:

I. 0.1042 gram of the substance gave 0.1100 gram $\text{H}_2\text{O}$ and 0.2562 gram
of $\text{CO}_2$.

II. 0.1014 gram of the substance gave 0.0998 gram $\text{H}_2\text{O}$ and 0.2494 gram
of $\text{CO}_2$.

III. 0.1133 gram of the substance gave 0.1233 gram $\text{H}_2\text{O}$ and 0.2800 gram
of $\text{CO}_2$.

0.1480 gram of sample I was used for Kjeldahl nitrogen estimation. It
required for neutralization 4.1 cc. of $\text{H}_2\text{SO}_4$.

<table>
<thead>
<tr>
<th>Calculated for (C$<em>7$H$</em>{15}$N)$_2$H$_2$SO$_4$:</th>
<th>Found:</th>
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<tbody>
<tr>
<td></td>
<td>I</td>
</tr>
<tr>
<td>C</td>
<td>67.46</td>
</tr>
<tr>
<td>H</td>
<td>12.02</td>
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<tr>
<td>N</td>
<td>4.52</td>
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Reduction of dihydrodichlorsphingosine. The chlorderivative was obtained by digesting dihydro sphingosine with thionylchloride in a water bath at 50°. The crude substance without purification was dissolved in a mixture consisting of two parts of ether and one of 98 per cent alcohol and reduced with metallic sodium. The substance obtained in this manner was transformed into the sulphate and analyzed.

0.1251 gram of the substance gave on combustion 0.1310 gram of $\text{H}_2\text{O}$ and 0.3097 gram of $\text{CO}_2$.

<table>
<thead>
<tr>
<th></th>
<th>Calculated for $(\text{C}<em>7\text{H}</em>{16}\text{N}_3\text{H}_2\text{O}_4$)</th>
<th>Found:</th>
</tr>
</thead>
<tbody>
<tr>
<td>C</td>
<td>67.46</td>
<td>67.51</td>
</tr>
<tr>
<td>H</td>
<td>12.02</td>
<td>11.72</td>
</tr>
</tbody>
</table>

ERRATUM.

On page 217 of this volume, No. 3, tenth line from the top, for strict read generic.
ON SPHINGOSINE
P. A. Levene and W. A. Jacobs

J. Biol. Chem. 1912, 11:547-554.

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