ANALYSIS OF THE BASIC NITROGEN
COMPONDS IN BONE OIL

A
THESIS
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OF THE
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OF
MASTER OF ARTS
BY
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HOUSTON TEXAS
MAY 1936
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HISTORICAL PART
Bone oil or animal tar is the product obtained by the dry distillation of bones in the preparation of bone black or animal charcoal. The first mention of an animal oil appeared in the writings of C. Gesner in 1552 and of A. Libavius in 1595 (Thorpe's Dictionary 1, 627, 1927). J. R. Glauber (1604-68) described the unpleasant smelling oil derived from the distillation of animal parts. The German Alchemist, Johann Dippel, in about 1711 was the first to examine bone oil (The Americana 9, 141, 1929). He obtained this "evil smelling" product by the destructive distillation of bones. It was refined by washing with potash and rectifying until it became limpid and aromatic. The resulting product was the "oleum animale Dippelli" as described in the older pharmacopoeias and was prescribed in doses of a few drops as an antispasmodic and also as a hypnotic.

Unverdorben, whose writings appear in Poggendorf's Annalen 8, 253 (1826) and 11, 59 (1827), was the next investigator of bone oil. He isolated four bases which he named odorin, animin, olanin, and ammonin. He first obtained odorin and found it to be entirely miscible with water. Its hydrochloride gave crystalline products with
several metallic salts including platinum chloride and gold chloride. Animin was Unverdorben's next base. It was not miscible with small volumes of water but dissolved in twenty parts of cold water. The solution of this base in hydrochloric acid gave a crystalline derivative with platinum chloride and oily derivatives with gold and mercuric chlorides. Olanin, the third base, had a persistent odor similar to that of animin but not as strong. It gave only oily derivatives. The fourth base, ammonin, was heavier than water and boiled at a very high temperature.

The next work of interest in the identification of the basic fraction of bone oil was done by Anderson in the middle of the nineteenth century (Trans. Roy. Edin. 16, 463, 1845; 20, 247, 1853; and 21, 219 and 571, 1857). He prepared the bases by extracting the animal oil with sulphuric acid, boiling to remove the pyrrol bodies which he collected, and then regaining the bases by adding potassium hydroxide and extracting with ether. The ether extract was dried and fractionally distilled.

Anderson first obtained a low boiling base which he called petinine. Subsequent analysis of the base itself and its platinum salt showed that was butyl amine as named by the new system of nomenclature just then being introduced.

In previous work on coal tar bases Anderson had
isolated a base which he called picoline. From the description of odorin in Unverdorben's articles he was lead to believe that the two were identical. He then isolated a base from bone oil and on comparison with his coal tar picoline, he found them to be the same.

Going back to the more volatile fractions Anderson found in very small quantities three more of the aliphatic series of amines - namely methyl, ethyl, and propyl.

Anderson isolated two more bases which he called pyridine and lutidine. From the platinum salts of these two and that of picoline he obtained their molecular formulae and discovered that he had an homologous series of compounds with pyridine being the lowest member. He called this the picoline series. Anderson noticed that the picoline series was isomeric with the aniline series but he was unable to give an explanation as to why they should have the same formulae and still be different bases. He mentioned that the most obvious explanation would be to call the picoline series nitrile bases. This would explain the difference from the aniline series but he also found that the picoline bases differed from the nitrile compounds that had been thus far examined in that they were very stable whereas the nitriles were not.

Having decided that he had an homologous series of
compounds, Anderson believed that there were still higher members, and he actually did get a base which he called collidine that was a higher homologue.

Anderson now did further work on the constitution of his picoline series of bases. He allowed ethyl iodide to react with pyridine, picoline, and collidine and analyzed the products. In each case he found that one molecule of ethyl iodide had been taken on. He did not realize, however, what the products obtained really were. Anderson thought that he had the iodide of a base corresponding to ammonium, which in the case of picoline he called ethylopicoline. This, then, only substantiated his idea that the picoline bases "must be," quoting directly from Anderson's articles, "considered as nitrile bases, that is, bases capable of taking up only one additional atom of ethyl or similar radical, by doing which they are converted into compounds of the class designated as ammonia bases (aliphatic amines). If this be their constitution, we must, according to the views at present entertained, assume that by replacement of its three atoms of hydrogen by as many different radicals."

He advanced such formulae as

\[
\begin{array}{c}
\text{C}_4\text{H}_2 \\
\text{C}_4\text{H}_2 \\
\text{C}_2\text{H}_2
\end{array}
\]

for pyridine.
The last base isolated by Anderson was amyl amine only a trace of which was present. He also showed that no higher homologues of the aliphatic series were present.

Several years later after the structure of pyridine had been placed on a sounder structural basis other workers were enabled to recognize more clearly the relationships of these compounds. Dewar shows in J. Chem. Soc. 24, 144 (1871) that pyridine is the lowest member of the series of bases and corresponds to benzene in the aromatic series. He proposed the theory that pyridine is the nucleus from which all other members of the series are derived and that picoline is methyl pyridine. Ramsay in J. Chem. Soc. 36, 262 (1879) confirmed these results and showed that lutidine is a dimethylpyridine. He also gave evidence of the ring structure of pyridine which had previously been advanced by Koerner.

Weidel (Ber. 12, 1989, 1879) obtained a picoline fraction with boiling-point 133°-139° from animal tar and oxidized it with potassium permanganate. He obtained two acids, one of which was picolinic acid indicating the presence of α-picoline and another that he said was nicotinic acid which indicate β-picoline. Weidel with Pick on further investigation of the bases of bone oil obtained a base from the fraction boiling between 170° and
180° which on oxidation gave lutidinic acid. This base had the composition C\textsubscript{8}H\textsubscript{11}N, and they concluded that it was 2:4-dimethylpyridine.

Ladenburg and Roth (Ber. 18, 913-920, 47, and 1590-93, 1885) identified three different lutidines in bone oil. Their first boiled at 158°-160° and gave a mercuric chloride salt melting at 130°. The oxidation product was pyridine 2:4 dicarboxylic acid. The base was, therefore, 2:4-dimethylpyridine. Another lutidine taken from the fraction boiling at 139°-142° was isolated by its mercuric chloride salt melting at 186°. It was found to be 2:6-dimethylpyridine.

Up to the time of the investigation of bone oil in this laboratory the following bases had been isolated and identified:

<table>
<thead>
<tr>
<th>Methylamine</th>
<th>2:6-dimethylpyridine</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ethylamine</td>
<td>2:4-dimethylpyridine</td>
</tr>
<tr>
<td>Propylamine</td>
<td>3:5-dimethylpyridine</td>
</tr>
<tr>
<td>Butylamine</td>
<td>2:4-methylethylpyridine</td>
</tr>
<tr>
<td>Amylamine</td>
<td>Aniline</td>
</tr>
<tr>
<td>Pyridine</td>
<td>Quinoline</td>
</tr>
<tr>
<td>2-methylpyridine</td>
<td></td>
</tr>
</tbody>
</table>
EXPERIMENTAL PART
Since no complete investigation of the basic fraction of bone oil has been made, we have undertaken to make one both quantitatively and qualitatively. In the preparation of the bases, eight and one-half liters of bone oil were extracted with enough 3 N sulphuric acid (six hundred and forty c.c. per liter of oil) to just take up all the bases. An excessive quantity of acid caused polymerization of the pyrrol bodies present, and this materially increased the difficulty of purification. The acid solution was made alkaline with potassium hydroxide and extracted with ether. The resulting ether solution of the bases was dried over potassium hydroxide. One and one-half liters of bases (including the pyrrol bodies present in the extract) were thus obtained.

The dried ether solution was subjected to fractional distillation. The first column used was one very similar to that described by Peters and Baker in Ind. and Eng. Chem. 18, 70 (1926). An adequate picture of it may be obtained from Fig. 1 on the following page. Fractions were collected over five degree ranges. Starting with 110 C. the following volumes were obtained boiling at the temperature indicated:
<table>
<thead>
<tr>
<th>Temperature (at atmospheric pressure)</th>
<th>Volume (in c.c.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>110°-115°</td>
<td>6</td>
</tr>
<tr>
<td>115°-120°</td>
<td>11</td>
</tr>
<tr>
<td>120°-125°</td>
<td>7</td>
</tr>
<tr>
<td>125°-130°</td>
<td>10</td>
</tr>
<tr>
<td>130°-135°</td>
<td>47</td>
</tr>
<tr>
<td>135°-140°</td>
<td>130</td>
</tr>
<tr>
<td>140°-145°</td>
<td>75</td>
</tr>
<tr>
<td>145°-150°</td>
<td>30</td>
</tr>
<tr>
<td>155°-157°</td>
<td>25</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Temperature (at 30 mm. pressure)</th>
<th>Volume (in c.c.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>60°-65°</td>
<td>65</td>
</tr>
<tr>
<td>65°-70°</td>
<td>13</td>
</tr>
<tr>
<td>70°-75°</td>
<td>53</td>
</tr>
<tr>
<td>75°-80°</td>
<td>53</td>
</tr>
<tr>
<td>80°-85°</td>
<td>28</td>
</tr>
<tr>
<td>85°-90°</td>
<td>55</td>
</tr>
<tr>
<td>90°-95°</td>
<td>35</td>
</tr>
<tr>
<td>95°-100°</td>
<td>18</td>
</tr>
<tr>
<td>100°-105°</td>
<td>42</td>
</tr>
<tr>
<td>105°-110°</td>
<td>36</td>
</tr>
<tr>
<td>110°-115°</td>
<td>24</td>
</tr>
<tr>
<td>115°-120°</td>
<td>23</td>
</tr>
</tbody>
</table>
Temperature (at 3 mm. pressure) | Volume (in c.c.)
---|---
67-70 | 26
70-75 | 4
75-80 | 32
80-85 | 41

A second fractional distillation was necessary to get further separation of the compounds. This was carried out on each five degree fraction using a Widmer column—see Fig. 2 on the next page. The temperature at which each c. c. distilled was recorded and this plotted against the total number of c. c. to give the distillation curve shown on pages 13-16. From the steps in the curve the number of fractions and volume of each were obtained.

The composition of each fraction is shown on the graph and is given in absolute per cent, that is, the per cent of each base calculated from its volume collected and the total volume of bases before distillation. A summary of the content of each base is:

- **Pyridine**: 1.7%
- **Pyrrol bodies**: 5.2%
- **α-picoline**: 6.9%
- **2:6-dimethylpyridine**: 5.5%
- **2:4-dimethylpyridine**: 7.3%
- **3:5-dimethylpyridine**: 2.4%
- **3:4-methylethylpyridine**: 3.3%
- **Aniline**: 1.4%
COMPOSITION:

Fraction 1
1.7% Pyridine

Fraction 2
3.3% Pyrrol
6.5% N-Picoline

Fraction 3
0.5% Pyrrol
0.4% N-Picoline
0.5% 2,6 Dimethylpyridine
<table>
<thead>
<tr>
<th>Fraction 7</th>
<th>Fraction 8</th>
<th>Fraction 9</th>
<th>Fraction 10</th>
<th>Fraction 11</th>
<th>Fraction 12</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.7% 2:4-Di-</td>
<td>0.3% 3:5-Di-</td>
<td>0.1% Aniline</td>
<td>0.7% Aniline</td>
<td>0.6% Aniline</td>
<td>1.5% 4-Aminopyridine</td>
</tr>
<tr>
<td>methylpyridine</td>
<td>methylpyridine</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1.8% 3:3-Di-</td>
<td>0.4% 2:3-Di-</td>
<td>0.1% 3:5-Di-</td>
<td>1.5% 2:4-Methyl-</td>
<td>0.7% 2:4-Methyl-</td>
<td>1.15 3:3:4-Trimethylpyridine</td>
</tr>
<tr>
<td>methylpyridine</td>
<td>methylpyridine</td>
<td>methylpyridine</td>
<td>methylpyridine</td>
<td>methylpyridine</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.7% 2:4-Methyl-</td>
<td>1.3% 2:3:4-Tri-</td>
<td>0.3% C9 H13 N</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>methylpyridine</td>
<td>methylpyridine</td>
<td>methylpyridine</td>
<td></td>
</tr>
<tr>
<td>Compound</td>
<td>Percentage</td>
<td></td>
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<td></td>
</tr>
<tr>
<td>--------------------------------</td>
<td>------------</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2:3:4-trimethylpyridine</td>
<td>2.3%</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>a-aminopyridine</td>
<td>3.8%</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C9H10N</td>
<td>2.8%</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Methylaminopyridine</td>
<td>2.0%</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Quinoline</td>
<td>4.4%</td>
<td></td>
<td></td>
<td></td>
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</tr>
</tbody>
</table>
DESCRIPTION OF ANALYSIS

FRACTION ONE:

The boiling-point of the fraction was 114°-118° and the density 0.971. The base was found to be a tertiary amine giving no reaction with acetyl chloride and no carbonyl amine test. A picrate was made by treating an alcoholic solution of the base with picric acid, and it melted at 163°-165°. This was found to be the melting-point of pyridine picrate ([C 80, (1), 1761(1909)], so a mixed melting-point with known pyridine picrate was taken and found to be the same.

FRACTION TWO:

Fifteen c. c. of this fraction were just neutralized with hydrochloric acid using congo red paper as an indicator. The resulting solution was distilled until all the pyrrol had gone over as shown by the failure of the pine splinter test for pyrrol (A pine splinter dipped in concentrated hydrochloric acid will turn red in the presence of pyrrol). About 5 c. c. of pyrrol were obtained making the fraction one-third pyrrol. Since the total volume of the fraction was 147 c. c., there were 49 c. c. or 3.3 % of pyrrol.

The remaining hydrochloric acid solution was made alkaline with potassium hydroxide and steam distilled.
Ten c. c. of a base were obtained. This was dried with potassium hydroxide. The boiling-point was 139°-133° and the density 0.948. Tests showed the base to be a tertiary amine. A picrate was formed, and after recrystallization, it gave a melting-point of 166°-167°. The chloroplatinate, formed by treating a solution of the base in hydrochloric acid with platinic chloride, melted at 192. These results correspond to those found for α-picoline in J. Chem. Soc. 61, 728 (1892) and J. Prakt. Chem., 2, 42, 423 (1890). The 10 c. c. of α-picoline obtained from the treatment described above represented two-thirds or 98 c. c. of the second fraction. This would be 6.5%.

FRACTION THREE:

Fifteen c. c. of this fraction were treated in a similar manner as fraction two to remove the pyrrol. Six c. c. of pyrrol were obtained. This would correspond to 9 c. c. of the total fraction or 0.6%. The bases recovered after distilling off the pyrrol were dried. The boiling-point was 134°-141°. This large range indicated that there was more than one base present. A picrate was made, but after repeated recrystallizations the melting-point, 150°-157°, indicated that very little separation was taking place. A chloroplatinate melting at 192 was made, and it was found to be that of α-picoline by a mixed melting-point with the one made from fraction two.
Trying to get a derivative by which a separation of the constituents could be carried out, mercuric chloride was added to a solution of the bases in hydrochloric acid. After two recrystallizations a pure salt was obtained that melted at 185°-186°. This was found to correspond to 2:6-dimethylpyridine described in J. Chem. Soc. 81, 454 (1902).

For further proof of the identity of 2:6-dimethylpyridine, four grams were oxidized. Twenty-eight grams of potassium permanganate, theoretical quantity necessary to oxidize four grams of a dimethylpyridine, were dissolved in one liter of water and the base added to this. The mixture was then refluxed several hours until the permanganate was all decomposed as evidenced by the disappearance of the purple colour of it. The manganese dioxide formed in the reaction was filtered off and the filtrate concentrated to about 100 c. c. This was neutralized with nitric acid. Lead nitrate was then added to precipitate the organic acid formed in the oxidation as its lead salt. This salt was put in boiling water and treated with hydrogen sulphide to regenerate the acid. The lead sulphide thus formed was filtered off and the filtrate concentrated until the acid crystallized out. It melted at 236° and this corresponds to pyridine 2:6-dicarboxylic acid as given in C. 102, (1), 1232(1931).
It was estimated that 6 c. c. or 0.4 % of this fraction was α-picoline and 3 c. c. or 0.5 % 2:6-dimethylpyridine.

FRACTION FOUR:

A small amount of pyrrol was obtained from this fraction - 4.5 c. c. or 0.3 %. The bases boiled at 142-147°. The salt with mercuric chloride melted at 186-187° after the first recrystallization and was the same as that of 2:6-dimethylpyridine in fraction three. Seventy-two c. c. or 4.8 % 2:6-dimethylpyridine were present in this fraction.

FRACTION FIVE:

A small amount of pyrrol bodies, about 0.2 %, was present in this fraction. The boiling-point was 153-156°. A small amount of 2:6-dimethylpyridine, 3 c. c. or 0.2 %, was present. A little of the mercuric chloride salt was formed melting at 137°, and it was the same one formed in fraction four. Sixteen and one-half c. c. or 1.1 % of a base giving a mercuric chloride salt melting at 130 are contained in this fraction. This corresponds to 2:4-dimethylpyridine(A. 247, 35(1888)). Further proof of the identity of this compound is given under the next fraction where there is a much larger quantity of it.

FRACTION SIX:

The boiling-point of fraction six was 158-165°. The mercuric chloride salt was made and it melted at 130°.
Some of the base was regenerated from the salt by steam distilling from an alkaline solution of potassium hydroxide. This gave the base in a pure form. It had a micro boiling-point of 159°. The micro boiling-point is taken by drawing out a capillary tube and allowing some of the liquid to rise in it. The tube is then sealed off leaving a small bubble of air at the bottom. The temperature at which the liquid reaches the surface of the oil bath in which it is being heated is the boiling-point. The picrate of the pure base melted at 179° and corresponds to that of 2:4-dimethylpyridine (A. 247, 37(1888)).

Four grams of the base were oxidized with potassium permanganate by the same procedure as that described for 2:6-dimethylpyridine in fraction three. The acid obtained melted at 241° and was pyridine 2:4-dicarboxylic acid (Mulliken's "Identification of Pure Organic Compounds", vol. 2, p. 77). It gave a blood red color with ferric chloride which differentiated it from pyridine 2:3 and 2:5-dicarboxylic acids, the latter two not giving a blood red color. Sixty c. c. or 4% 2:4-dimethylpyridine were in the fraction. A small amount of a base present in the higher boiling part of the fraction gave a picrate melting at 228°-233°. This occurred in a larger quantity in fraction seven.
FRACTION SEVEN:

The boiling-point of fraction seven was 165°-170°. It contained 27 c.c. or 1.8% of the base with a picrate melting at 226°-228° which was the same as that found in the previous fraction. This picrate corresponds to that of 3:5-dimethylpyridine as described in Ber. 7, 2964 (1874). No oxidation product of this base could be obtained as it apparently decomposed when treated with potassium permanganate. There was also some 2:4-dimethylpyridine in this fraction as evidenced by the formation of a mercuric chloride salt melting at 130° which was the same as that in fraction five.

FRACTION EIGHT:

The boiling-point of this fraction was 174°-177° and the density 0.932. There were about 4.5 c.c. or 0.3% 3:5-dimethylpyridine as shown by the picrate melting at 228°, and this gave no depression of the melting-point when mixed with the picrate formed in fraction seven. A second base was present and it gave a chloroplatinate melting at 200°. This was found to be 2:4-methyllethylpyridine the identification of which is given in the following fraction.

FRACTION NINE:

The boiling-point was 178.5°-181°. A small amount of a primary amine was present as evidenced by an isocyanide
odor in the carbonyl amine test. This test is made by adding a drop of a solution of one drop of the base in one c.c. of alcohol to a potassium hydroxide solution containing one drop of chloroform. On heating a primary amine will give an isocyanide odor which is very characteristic. The primary amine was removed by diazotization. The identification of it is given in the next fraction.

A small amount of 3:5-dimethylpyridine was present and it was removed by precipitating it with picric acid. Twelve and one-half c.c. or 0.7% of a base remained. The picrate of this base was very soluble in alcohol, and on concentration and cooling of its solution it came out as an oil. The chloroplatinate was made and it melted at 200°, the same as the one in fraction eight. A combustion was carried out on the platinum salt and the following results obtained on 4.647 mg. of the salt:

1.400 mg. or 30.13 % Platinum
1.303 mg. H₂O or 4.31 % Hydrogen
5.051 mg. CO₂ or 29.64 % Carbon

The molecular weight of the base was calculated from the amount of platinum present as follows:

100 g. of salt would give 30.13 g. platinum
x g. or the molecular weight of the salt would give 195.23 g. of platinum (195.23 is the atomic weight of platinum)
\[
x = \frac{195.23 \times 100}{30.13} = 648, \text{ the molecular weight of the chloroplatinate.}
\]

The molecular weight of chloroplatinic acid is 410, therefore, the molecular weight of the base is

\[
\frac{648 - 410}{2} \text{ or } 119
\]

since two molecules of base combine with one of chloroplatinic acid. This would correspond to a methylethylpyridine or a trimethylpyridine. The melting-point of the platinum salt corresponds to that of 2:4-methylethylpyridine (\textit{C. R.} (1), 524(1913)). The per cents of C and H that were obtained experimentally also agree with the theoretical values for methylethylpyridine which are 29.50 % for C and 3.68 % for H.

\text{FRACTION TWO:}

The boiling-point of this fraction was 182°-184° and the density 0.961. The carbyl amine test gave a very strong isocyanide odor indicating the presence of a primary amine. A chloroplatinate was made and it melted at 198°. Upon analysis of this salt 5.621 mg. gave 1.830 mg. platinum. This corresponds to 32.56 % platinum or a base with a molecular weight of 95. Three c.c. of the base were refluxed with acetic anhydride for about two hours. The resulting acetyl derivative of the primary amine was obtained by pouring the reaction mixture into
water. After two or three recrystallizations the product melted at 113°. This corresponds to acetanilide, so a mixed melting-point was taken with known acetanilide. There was no depression of the melting-point, therefore, the primary amine was aniline whose molecular weight is 93, a value corresponding to that obtained upon analysis of the platinum salt.

It was found that diazotization was the best method for the complete removal of the primary amine. Seven c.c. of this fraction were dissolved in concentrated hydrochloric acid and cooled to 0°. A solution of sodium nitrite was added and the mixture kept at 0° for about one-half hour. The mixture was then heated to decompose the diazonium salt and form a phenol. After making alkaline with potassium hydroxide the tertiary base which did not react with nitrous acid was steam distilled off and 4.75 c.c. thus obtained. Therefore, 2.25 c.c. of the 7 c.c. were aniline. That is, 32% of the 33 c.c. fraction or 10.5 c.c., representing 0.7% of the total basic fraction, was aniline.

There were 22.5 c.c. or 1.5% of the tertiary base after removing the aniline. The boiling-point was 179°-181° and the density 0.932. A chloroplatinate of this base was made and was found to melt at 200°. Upon taking a mixed melting-point with that in fraction nine the two
were found to be identical, and the base was, therefore, 2:4-methylethylpyridine.

FRACTION ELEVEN:

The boiling-point of this fraction was 184°-188°. The carbonyl amine test gave a strong isocyanide odor indicating the presence of a primary amine. The acetyl derivative of the primary amine was made by refluxing one gram of this fraction with acetic anhydride. The product melted at 113°. There was no depression of the melting-point when the acetyl derivative was mixed with acetonilide, therefore, the primary amine was aniline.

By diazotization, as carried out in fraction ten, there were 9 c.c. or 0.6 % aniline present in the 37 c.c. fraction. A picrate was made with the remaining tertiary base. After recrystallization it melted at 182°-183°. A combustion was run on this picrate; 8.120 mg. of the salt gave 14.372 mg. CO₂ which corresponds to 48.27 % C and 3.141 mg. H₂O which corresponds to 4.29 % H. These values agree with the theoretical ones, 48.00 % C and 4.00 % H, for C₁₂H₁₀N. The chloroplatinate of the base was made and it melted at 207°. Upon analysis 4.018 mg. of this platinum salt gave 1.195 mg. of platinum or 29.74 %. This makes the molecular weight of the salt 656 and that of the base 123. The molecular weight of
C₇H₆N is 181. The melting-points of the picrate and the chloroplatinate correspond to 2:3:4-trimethylpyridine ([C. 71. (1), 1161(1900) and 89. (1), 534(1918)].

After the removal of the picrate on adding an excess of picric acid, it was found that another tertiary base was present. This base was obtained by distilling off the alcohol from the picric acid solution, adding potassium hydroxide solution and steam distilling. The chloroplatinate of this base melted at 200° and was the same as that of 2:4-methylethylpyridine in fraction.

FRACTION TWELVE:

The boiling-point of this fraction was 193°-198°. The carbyl amine test indicated the presence of a primary amine. Before removing the primary amine, a picrate was made with a portion of this fraction. A very insoluble picrate was precipitated. After recrystallization it melted at 223°-223°. The base was regenerated from several grams of this picrate by boiling with potassium hydroxide solution. The base was distilled from the mixture and came over with several parts of water, it being very soluble in water. The water solution of the base was nearly saturated with potassium hydroxide and a solid base came out. It was filtered from the solution and recrystallized from ligroin. The melting-point of the
base was 56°-57°. The boiling-point was 204°-205°.

A gold salt of the base made by adding gold chloride to its solution in hydrochloric acid. The crystalline chloraurate melted at 252°-233°. The molecular weight was obtained by analyzing the gold salt.

7.187 mg. of salt gave 3.257 mg. of gold

\[
\frac{3.263}{7.187} = 45.40 \text{ % gold in the salt}
\]

100 gm. of salt would give 45.13 gm. of gold

\[x \text{ gm. or the molecular weight of the salt would give } 197.2 \text{(the atomic weight of gold) gm. of gold}
\]

\[x = \frac{197.2 \times 100}{45.40} = 434.3\]

The molecular weight of chlorauric acid is 340.2. Therefore, the molecular weight of the base is 434.3-340.2 or 94. The melting-point and boiling-points of the base and the melting-points of the picrate and chloraurate all correspond to those of α-aminopyridine (Ber. 27, 1320 (1894) and C. 73, (2), 373 (1902)). The molecular weight of α-aminopyridine is 94 and it corresponds to that obtained upon analysis of the gold salt.

Seven c.c. of this fraction were diazotized to determine how much α-aminopyridine was present. About 3.4 c.c. of tertiary base were obtained after the primary amine was removed. Therefore, 51 % or 22 c.c. of the
fraction were \(\alpha\)-aminopyridine. This represents 1.5\% of the total volume of bases. The tertiary amine present had a micro boiling-point of 190\(^\circ\). It gave a picrate melting at 182\(^\circ\) and a platinum salt melting at 207\(^\circ\). These were found to be the same as those of 2:3:4-trimethylpyridine in fraction eleven.

**FRACTION THIRTEEN:**

The boiling-point was 203\(^\circ\)-205\(^\circ\). The picrate of this fraction melted at 223\(^\circ\) and was the same as \(\alpha\)-aminopyridine picrate. The \(\alpha\)-aminopyridine was removed by diazotization. From 8 c.c., 2.8 c.c. of a tertiary base were obtained. This is 35\% or 13 c.c. of the fraction, representing 0.9\% absolute per cent. The remainder of 26 c.c. or 1.6\% was \(\alpha\)-aminopyridine.

A platinum salt was made with the tertiary base. It melted at 191\(^\circ\)-192\(^\circ\). An analysis of 8.120 mg. of this salt gave 2.344 mg. or 28.87\% platinum, 3.047 mg. H\(_2\)O or 4.20\% H, and 9.318 mg. CO\(_2\) or 31.26\% C. The molecular weight obtained from the per cent platinum was 133 and this corresponds to C\(_9\)H\(_{13}\)N. The theoretical values for the per cents of C and H, 31.76\% and 4.12\% respectively, in the platinum salt of C\(_9\)H\(_{13}\)N agree with those obtained experimentally. The picrate of the base came out as an oil and, therefore, no further knowledge could be obtained from it. All attempts to make it crystallize
were unsuccessful. The formula $C_4H_7$ corresponds to tetramethylpyridines, diethylpyridines, and dimethyl-ethylpyridines.

**FRACTION FOURTEEN:**

This fraction was found to contain the same bases as the above fraction. The $\alpha$-aminopyridine was removed by diazotization. Ten c.c. of the fraction or 0.7% were $\alpha$-aminopyridine. Fifteen c.c. or 1% were $C_4H_7$.

**FRACTION FIFTEEN:**

This fraction boiled from 214°-233°. A positive carbyl amine test indicated the presence of a primary amine. A picrate was formed and after recrystallization from acetone it melted at 231°-232°. An excess of picric acid solution in alcohol was added to 10 c.c. of this fraction. The resulting picrate was treated with potassium hydroxide and a base distilled off with water. The alcohol was evaporated from the picric acid mixture. The residue was treated with potassium hydroxide and a tertiary base steam distilled off. About 2.6 c.c. were thus obtained. That is, 25.7% or 9 c.c. of this fraction were tertiary bases and 74.3% or 26 c.c. were the base obtained from the picrate melting at 232. These volumes represent 0.6% and 1.7%, respectively, of the total volume of bases.
The base from the picrate melting at 232° was the primary amine that gave the carbyl amine test above. It had a micro boiling-point of 314°. Upon cooling it crystallized in long colorless rods and had a melting-point of 30°. A chloroplatinate was made and it melted at 317°. Upon analysis, 8.868 mg. of the salt gave 2.753 mg. or 31.05 % platinum, 2.345 mg. water or 2.94 % H, and 7.406 mg. carbon dioxide or 22.78 % C. The molecular weight calculated from the per cent platinum was 109.5. This agrees with the molecular weight of a methylaminopyridine which is 108. The theoretical values of C and H for the platinum salt of a methylaminopyridine are 23.00 % and 2.56 % respectively. These agree with the results obtained experimentally. Only four of the possible methylaminopyridines are known with their properties recorded in the literature. The isomer found in bone oil with a picrate melting at 231°-232° and a platinum salt melting at 217° does not agree with any of these four.

It was believed that the base might be 2:3 amino-methylpyridine made by Seide (Ber. 57, 1802-06 (1924)). This base had a picrate melting at 229°. To determine whether or not they were the same, 6 gm. of 2-picoline were treated with an equivalent amount of sodamide with xylene as the solvent. The mixture was heated at 130°
in an oil bath for 6 hours until the reaction (known as the Tschitschibabin reaction) was complete. The resulting 2:3-aminomethylpyridine was extracted with hydrochloric acid after pouring the reaction mixture into water. The hydrochloric acid extract was made alkaline with potassium hydroxide and the resulting mixture extracted with ether. A picrate was made with this ether extract of 2:3-aminomethylpyridine and it melted at 229°. Some of this picrate was mixed with the picrate of the base from bone oil. The melting-point was 205°-230°; therefore, it was concluded that the two were not the same.

The tertiary base obtained as described above gave a platinum salt which was at first an oil. It then became sticky and finally solidified after standing in a desiccator. On attempting to purify it by recrystallization much decomposition took place. A neutral equivalent was obtained by titrating the base with 0.191 M hydrochloric acid using thymol blue as an indicator (thymol blue was previously found to give an end-point corresponding to the correct neutral equivalent for pyridine). A sample of 0.110 gm. of base was weighed out and it took 8.12 c.c. of the acid to neutralize it. The neutral equivalent or molecular weight is equal to the weight of the sample times 1000 divided by the number of c.c. of normal acid
used to neutralize the sample. In the case of this base the neutral equivalent is
\[
\frac{0.110 \times 1000}{8.12 \times 0.101} \text{ or } 134
\]
which is the molecular weight of \( C_9H_8N \).

FRACTION SIXTEEN:

This fraction boiled at 228°-236°. A very small amount of methylaminopyridine was present. The remainder was a tertiary base giving a picrate melting at 204°. A mixed melting-point was taken with known quinoline picrate and no depression occurred, therefore, the base was quinoline.

FRACTION SEVENTEEN:

The boiling-point was 234°-237°. The picrate of this fraction melted at 204° and was the same as that in fraction sixteen. This fraction was practically all quinoline.
THEORETICAL PART
Pyridine bases are found to occur in the products of distillation of matter containing nitrogenous carbon compounds. In the case of bones there is protein material present which at the high temperature of distillation would undergo decomposition and give rise to ammonia. There are also some unsaponified fats present which would decompose to give glycerine. Glycerine, in turn, dehydrates to form acrolein.

Acrolein and ammonia at high temperatures condense to give α-picoline:

\[
\begin{align*}
\text{CH}_2 & \quad \text{CHO} \\
\text{CH} + \quad \text{CHO} & \quad \text{CH} = \text{CH}_2 \\
\text{CHO} & \quad \text{NH}_3
\end{align*}
\]

\[
\text{→} \quad \text{CH}_3 \quad + \quad 2 \text{H}_2 \text{O}
\]

This is one of the earliest pyridine syntheses and was carried out by von Baeyer (A. 55, 281 (1870)).

Since ammonia is undoubtedly present in the bone distillation chamber and since the temperature of the chamber is greater than that necessary to give acrolein from fats, it is quite plausible that this condensation reaction takes place and is one source of the α-picoline that is found to be present in the bone distillate.
Another source of pyridine compounds is from the condensation of ammonia with saturated aldehydes, say acetaldehyde. Acetaldehyde may be present as a result of the reaction of water and acetylene, the latter of which is nearly always present when certain organic compounds are exposed to intense heat.

Ethyl amine which has been shown by Anderson to be present in bone oil may be taken to illustrate the presence of acetylene. Ethylene is among the products of pyrolysis of ethyl amine (Hurd's "The Pyrolysis of Organic Compounds," chap. 12, p. 290):

\[
\text{CH}_3\text{CH}_2\text{NH}_2 \rightarrow \text{CH}_2 = \text{CH}_2 + \text{NH}_3
\]

Ethylene undergoes pyrolysis (loc. cit., p. 56) to form acetylene:

\[
\text{CH}_2 = \text{CH}_2 \rightarrow \text{CH} = \text{CH} + \text{H}_2
\]

Then the acetylene and water which comes from the bones when heated combine as follows to form acetaldehyde:

\[
\text{CH} = \text{CH} + \text{H}_2\text{O} \rightarrow \text{CH}_3 - \text{CHO}
\]

Assuming that these reactions occurred and that acetaldehyde was present during the bone distillation, \(\alpha\)-picolone could be formed as follows:
Tschitschibabin has obtained α-picoline by passing acetaldehyde and ammonia through a hot tube (J. Chem. Soc. 124, (1), 1123 (1923)).

Pyridine has been made by heating a mixture of acetaldehyde, acrolein, and ammonia (Hollins' "Synthesis of Nitrogen Ring Compounds", chap. 7, p. 219):

\[
\begin{align*}
\text{CH}_3\text{CHO} + \text{CHO} + \text{NH}_3 & \rightarrow \text{CH}_3 + 3\text{H}_2\text{O} + \text{H}_2 \\
\text{CH}_3\text{CHO} + \text{NH}_3 & \rightarrow \text{CH}_3 + 3\text{H}_2\text{O} + \text{H}_2
\end{align*}
\]

It is quite probable that some pyridine is formed in this manner during the bone distillation.

Another explanation of the formation of pyridines in the distillation of bones is the expansion of the pyrrole ring (Hollins' "Synthesis of Nitrogen Ring Compounds", p. 239). This widening of the pyrrole ring was first observed by Ciamician and Derrnstedt (Ber. 14, 1153 (1881)). They treated pyrrole with chloroform and sodium ethylate.
Dennstedt and Zimmermann (Ber. 19, 2198(1886)) obtained pyridine by heating methylpyrrole with concentrated hydrochloric acid in a sealed tube. Either 1- or 2-methylpyrrole gives pyridine.

Pictet obtained pyridine by distilling methylpyrrole through a tube heated to a dull red heat (Ber. 37, 2793 (1904)). Pictet also showed that the C atom entering the ring takes the α-position (Ber. 38, 1946(1905)). He proved this by passing N-benzylpyrrole through a tube heated to dull redness and obtained β-phenylpyridine as the product.

Weidel and Ciamician (Ber. 13, 77(1880)) showed that 1- and 2-methylpyrroles and 2:5-dimethylpyrrole were present in bone oil. Ciamician and Dennstedt (Ber. 14, 1339-42(1881)) showed that a 2:3:5-trimethylpyrrole occurred in the oil.

Considering the presence of these pyrrole bodies during the distillation and also the work that has been done on the expansion of the pyrrole ring, one can explain the formation of various pyridine compounds that were found in the bone distillate. The temperature of distillation of the bones is very high and is in all probability sufficient to cause the conversion of pyrroles to pyridines.

First, considering the methylpyrroles, pyridine may be formed according to the following equations:
Next, using 2,5-dimethylpyrrole, \( \alpha \)-picoline may be formed as follows:

\[
\begin{align*}
\text{2,5-dimethylpyrrole} & \quad \text{\( \alpha \)-picoline} \\
\text{\( CH_3-C-C-CH_3 \)} & \quad \text{\( CH_3-N-CH_3 \)} \\
\text{\(+ H_2 \)} & \\
\end{align*}
\]

2,3:5-trimethylpyrrole may give rise to three lutidines as follows:

\[
\begin{align*}
\text{1.} & \quad \text{2,3:5-trimethylpyrrole} \\
\text{\( CH_3-C-C-CCH_3 \)} & \quad \text{\( CH_3-N-CH_3 \)} \\
\text{\(+ H_2 \)} & \\
\end{align*}
\]
Apparently the latter of these three reactions did not take place as only 2:4- and 2:6-dimethylpyridines were found in the bone oil.

There are four pyrrole bodies which could be present during the distillation as a result of decomposition of blood pigments. These are 2:3-dimethyl-4-ethylpyrrole, 2:4-dimethyl-3-ethylpyrrole, 3:4-methylethylpyrrole, and 2:3:5-trimethyl-4-ethylpyrrole.

By a proper rearrangement of 3:4-methylethylpyrrole another lutidine may be formed as follows:

\[
\begin{align*}
\text{CH}_3-\text{C}-\text{C}-\text{CH}_3 & \quad \text{2:6-dimethylpyridine} \\
\text{NH} &
\end{align*}
\]

\[
\begin{align*}
\text{CH}_3-\text{C}-\text{C}-\text{CH}_3 & \rightarrow \text{C}_3\text{H}_7 - \text{CH}_3 + \text{H}_2 \\
\text{NH} &
\end{align*}
\]

\[
\begin{align*}
\text{CH}_3-\text{C}-\text{C}-\text{CH}_3 & \quad \text{2:3-dimethylpyridine} \\
\text{NH} &
\end{align*}
\]

3:4-methylethylpyrrole    3:5-dimethylpyridine

3:5-dimethylpyridine was found in bone oil.
The 2:3:5-trimethyl-4-ethylpyrrole may be the progenitor of several pyridines of the composition \( \text{C}_{9}\text{H}_{13}\text{N} \) as indicated by the equations:

1. \[
\begin{align*}
\text{CH}_{3}-\text{C} & \quad \text{C} \quad \text{C}_{2}\text{H}_{5} \\
\text{CH}_{3}-\text{C}  & \quad \text{C} \quad \text{CH}_{3} \quad \text{NH} \\
\end{align*}
\rightarrow
\begin{align*}
\text{C}_{2}\text{H}_{5} & + \text{H}_{2} \\
\text{C} \quad \text{C} \quad \text{CH}_{3} \quad \text{CH}_{3} \quad \text{N} \\
\end{align*}
\]

2:3-dimethyl-4-ethylpyridine

2. \[
\begin{align*}
\text{CH}_{3}-\text{C} & \quad \text{C} \quad \text{C}_{2}\text{H}_{5} \\
\text{CH}_{3}-\text{C}  & \quad \text{C} \quad \text{CH}_{3} \quad \text{NH} \\
\end{align*}
\rightarrow
\begin{align*}
\text{CH}_{3} & + \text{H}_{2} \\
\text{C} \quad \text{C} \quad \text{CH}_{3} \quad \text{CH}_{3} \quad \text{N} \\
\end{align*}
\]

2:4-dimethyl-3-ethylpyridine

3. \[
\begin{align*}
\text{CH}_{3}-\text{C} & \quad \text{C} \quad \text{C}_{2}\text{H}_{5} \\
\text{CH}_{3}-\text{C}  & \quad \text{C} \quad \text{CH}_{3} \quad \text{NH} \\
\end{align*}
\rightarrow
\begin{align*}
\text{CH}_{3} & + \text{H}_{2} \\
\text{C} \quad \text{C} \quad \text{CH}_{3} \quad \text{CH}_{3} \quad \text{N} \\
\end{align*}
\]

2:6-dimethyl-3-ethylpyridine

4. \[
\begin{align*}
\text{CH}_{3}-\text{C} & \quad \text{C} \quad \text{C}_{2}\text{H}_{5} \\
\text{CH}_{3}-\text{C}  & \quad \text{C} \quad \text{CH}_{3} \quad \text{NH} \\
\end{align*}
\rightarrow
\begin{align*}
\text{CH}_{3} & + \text{H}_{2} \\
\text{C} \quad \text{C} \quad \text{CH}_{3} \quad \text{CH}_{3} \quad \text{N} \\
\end{align*}
\]

2:3:5:6-tetramethylpyridine

The base of composition \( \text{C}_{9}\text{H}_{13}\text{N} \) found in bone oil but whose structure is still unknown may be one or more of these isomers.
Wibaut and van de Lande have obtained \( \alpha \)-aminopyridine (Rec. trav. chim. 48, 1005 (1929)) by passing pyridine and ammonia through a hot tube containing nickel or iron as a catalyst:

\[
\text{C_5H_5N} + \text{NH}_3 \rightarrow \text{C_5H_4N}_2H + \text{H}_2
\]

Since this reaction is known to take place, it would account for the formation of \( \alpha \)-aminopyridine in bone oil.

The same two investigators (Rec. trav. chim. 50, 1056 (1931)) found that 2:6-methylaminopyridine was formed when \( \alpha \)-picoline and ammonia were passed through the hot tube:

\[
\text{C_5H_4N}_2CH_3 + \text{NH}_3 \rightarrow \text{C_5H_4N}_2H + \text{CH}_3 + \text{H}_2
\]

This reaction would account for the presence of a methylaminopyridine in bone oil.

The aminopicoline actually isolated in our study was \( \text{C_5H_5N}_2CH_3(?) \). One could best account for the formation of this substance by assuming the interaction of ammonia and a picoline. The equation for the reaction would be:
Assuming that the picoline reacting in this case is β-picoline, this would account for the fact that β-picoline was absent in the basic fraction of the bone oil.

Since aniline was found to be present in bone oil and acrolein is undoubtedly present, the formation of quinoline may be accounted for by the well known Skraup synthesis which takes place according to the following mechanism (Hollins' "Synthesis of Nitrogen Ring Compounds", p. 245):

\[
\text{CH}_2\text{NH}_2 + CH_2\text{CHO} \rightarrow CH_2\text{NH}_2 \text{CHO}
\]

\[
\text{CH}_2\text{NH}_2 \rightarrow \text{CHO} + H_2O
\]

In the actual synthesis aniline, glycerine, and concentrated sulphuric acid are used together with an oxidizing agent.

Koenigs (Ber. 13, 911(1880)) has obtained quinoline by the dry distillation of acrolein aniline. The formation of quinoline in this case is due to the same mechanism.
as the Skraup reaction. This undoubtedly makes the formation of quinoline possible during the distillation of bones.