THE ACONITE ALKALOIDS*

II. THE FORMULA OF OXONITINE

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Since the discovery by Carr (2) in 1912 of oxonitine, the neutral, sparingly soluble product of the oxidation of aconitine with permanganate and for which he proposed a formula \( C_{23}H_{42}O_9N \), difference of opinion has prevailed as to the correct formulation of this substance. This interpretation assumed a new aspect when Späth and Galinovsky (3) in 1930 suggested the formula \( C_{35}H_{45}O_{12}N \) on the basis not only of the C and H determinations but also on those of N, methoxyl, and benzoic acid. This formulation was supported by the more recent analyses of Lawson (4). However, a further slight modification of the formula to \( C_{35}H_{41}O_{13}N \) was made by Majima and Tamura (5) on the basis of evidence reported by them, which appeared to be conclusive. Both aconitine, \( C_{34}H_{47}O_{11}N \), and mesaconitine (6), \( C_{33}H_{46}O_{11}N \), had been found to yield on oxidation the same oxonitine. Aconitine, on the one hand, has been shown to behave as if it contained an N-ethyl group (1, 5) by the formation of ethyl iodide in the N-alkyl determination and of ethylamine on fusion with KOH. On the other hand, mesaconitine (5) appears to contain an N-methyl group, since after saponification to mesaconine and subsequent demethylation (of \( OCH_3 \) groups) methylamine was the principal volatile base obtained on alkali treatment of the resulting demethylated mesaconine. Oxonitine, when similarly transformed to a demethylated oxonine, gave mainly ammonia and since it no longer shows an N-alkyl group in the alkyl determination Majima and Tamura conclude that the transformation of

* Earlier work on the aconite alkaloids was reported in 1936 (1).
aconitine and mesaconitine to oxonitine involves oxidative removal of the N-ethyl and N-methyl groups in these alkaloids with simultaneous oxidation of an adjoining CH₂ to CO group, with the formation of a lactam. The long known formation of acetaldehyde during the oxidation of aconitine to oxonitine and the detection by Majima and Tamura of formaldehyde among the oxidation products of mesaconitine seem to fit in with this interpretation. A precedent for the oxidative removal of an N-alkyl group is found in the conversion of tropine to tropigenine.

Thus a strong case appears to have been made for the nature of the transformation of these alkaloids into oxonitine. However, our recent experience may reopen this question. The analytical values which we have obtained with oxonitine prepared directly from Merck's crystalline aconitine as well as with aconitine purified over the hydrobromide according to the method of Majima and Suginome (7) have been consistently somewhat higher than those required by the formula C₃₂H₄₁O₁₂N and in closer agreement with that of C₃₃H₄₃O₁₂N. This was supported by the analytical results obtained with the "isomer (?)" previously described by us (1) and substantiated by our more recent work. At first we were reluctant to attribute too much significance to such analytical results until the experience with delphinine, as reported in the previous paper, had been encountered. Here from the analytical data the transformation of delphinine to oxodelphinine appears to proceed without loss of carbon atoms. Since this appeared to fit in with the oxonitine analyses, it was of importance to obtain other evidence. Accordingly, other derivatives of oxonitine were studied. On hydrogenation a hexahydrooxonitine was prepared. The analyses of this material supported a formula C₃₃H₄₉O₁₂N.

¹ Recent analyses of this more soluble oxidation product as well as those of its hexahydro derivative are in agreement with a formula C₃₆H₄₆O₁₂N for the former. It must be formed from aconitine without loss of carbon atoms and is therefore an oxoaconitine. It is produced in larger amount than oxonitine. This at once brings up the question as to whether both oxonitine and oxoaconitine are simultaneously formed from aconitine itself by different reactions or whether the aconitine reported by different workers to yield oxonitine has not been homogeneous but contaminated by a C₃₂ alkaloid which has been the source of oxonitine. This question is now under careful investigation.
Pyrooxonitine was then prepared according to Majima and Suginome (8). Contrary to the recent assumptions of Tamura (9), many analytical results which we have obtained with pyrooxonitine are in close agreement with a formula $C_{31}H_{39}O_{10}N$ rather than $C_{30}H_{37}O_{10}N$. Finally, on hydrogenation a crystalline hexahydropyrooxonitine was prepared, analysis of which supported the formula $C_{31}H_{45}O_{10}N$.

Thus from our experience the question of the exact relationship of oxonitine to aconitine has been reopened and can be finally settled only when the exact nature of the steps involved in the transformation of mesaconitine and aconitine to oxonitine has been determined.

In accordance with this, attempts at the degradation of oxonitine have been in progress. At this point we wish to report the results of a preliminary study of the cleavage of oxonitine with methyl alcoholic HCl. Roughly 40 to 50 per cent of a crystalline base has been obtained. Analyses indicate the presence of five methoxyl groups and at least one N-methyl group. Since $CO_2$ was formed during the production of the substance, the possibility appears that lactam cleavage occurs with loss of $CO_2$ and either with or without methylation of a resulting secondary amino group. Since in the case of the reaction of oxodelphinine with methyl alcoholic HCl the acetyl group is replaced by methyl, such a reaction may also occur here. The resulting base should then have a formula $C_{35}H_{45}O_{10}N$ or $C_{36}H_{47}O_{10}N$. However, this interpretation can be merely provisional and must await the results of further study. Attempts at further degradation (exhaustive methylation) of the substance are now in progress.

EXPERIMENTAL

Oxonitine—For the preparation of this substance we have used Merck's crystalline aconitine directly as such, and aconitine obtained from it by purification over the hydrobromide according to Majima and Suginome (7). In the latter case the recrystallized hydrobromide was reconverted to the base which was then recrystallized from methyl alcohol. This material then melted at 202–205°, depending on the rate of heating.

$C_{34}H_{44}O_{11}N$. Calculated, C 63.22, H 7.34; found, C 63.40, H 7.28
The purified alkaloid was oxidized with permanganate in acetone and acetic acid solution essentially according to the procedure of Barger and Field (10). After completion of the oxidation, the collected mixture of MnO₂ and oxonitine which had crystallized was treated in aqueous suspension with SO₂ to remove the MnO₂. From 5 gm. of purified aconitine, 0.83 gm. of oxonitine was directly obtained. After recrystallization by addition of acetone to the solution in hot acetic acid, it separated as the usual heavy crystalline powder and melted at 279-282° after preliminary softening.

\[ [\alpha]_D \text{[^0]} = -45^\circ (c = 0.956 \text{ in chloroform}) \]

\[ C_{13}H_{19}O_{12}N. \text{ Calculated. } C \ 61.36, \ H \ 6.72 \]
\[ C_{13}H_{19}O_{12}N. \text{ Found. } C \ 61.67, \ H \ 6.61 \]

Other preparations of equivalent character gave the following results.

Found. C 61.39, H 6.71

When Merck’s crystalline aconitine was directly employed without purification, similar results were obtained.

Found. C 61.45, H 6.65

From 20 gm. of Merck’s alkaloid, the yield of crude oxonitine was 5.5 gm. and 4.1 gm. of the following substance.

The acetone filtrate from the mixture of MnO₂ and oxonitine obtained above from the oxidation of recrystallized aconitine was concentrated to about 20 cc. On dilution with water an appreciable precipitate formed, which was collected with water. It proved to be a mixture. The filtrate from this after neutralization with sodium carbonate solution was extracted with chloroform. The washed chloroform extract was dried and concentrated to dryness. The residue when dissolved in a small volume of methyl alcohol slowly deposited prisms and rods. The yield of collected material was 1.3 gm. After recrystallization
from methyl alcohol it melted at 261°. The substance, like oxonitine, is not dissolved by dilute acid or alkali.

\[^{\text{a}}\alpha\]^{\text{a}} = -98° (c = 0.956 in chloroform)  
\text{C}_{27}\text{H}_{43}\text{O}_{12}\text{N. Calculated. C 61.38, H 6.72, N 2.17, OCHa 19.22}  
\text{Found. (a) " 61.40, " 6.69, " 2.57, " 19.18}  
\text{" (b) " 61.45, " 6.75, " 2.42, " 19.07}  

From a run of 20 gm. of Merck's crystalline aconitine the yield of this substance was 4.1 gm. After recrystallization from methyl alcohol it melted at 261°.

\[^{\text{a}}\alpha\]^{\text{a}} = -99° (c = 1.01 in chloroform)  
\text{Found. C 61.69, H 6.51}  

\text{Hexahydroaconitine}—0.5 gm. of purified aconitine was dissolved in 5 cc. of alcohol with a few drops of HCl and hydrogenated with 50 mg. of platinum oxide catalyst under 3 atmospheres pressure. The absorption of H_{2} was prompt and appeared to be complete in about 30 minutes. The absorption corresponded to 3 moles. The hydrogenated alkaloid as such was not obtained in crystalline form but was isolated as the beautifully crystalline perchlorate. This was obtained by addition of sodium perchlorate solution to the aqueous solution of the very soluble hydrochloride. Recrystallized by dilution of its concentrated solution in alcohol, it formed square-ended prisms which melted at 209–210° after preliminary sintering.

\text{C}_{26}\text{H}_{43}\text{O}_{11}\text{N-HClO}_{4}. Calculated. C 54.26, H 7.24  
\text{Found. " 53.84, " 7.35}  

On hydrolysis with water at 160–165° hexahydrobenzoic acid was cleaved from it. After acidification with HCl and extraction with ether, this acid was obtained as an oil with characteristic odor. The concentrated HCl solution after addition of acetone crystallized on seeding with aconine hydrochloride. The collected material melted at 174–176° and gave no depression with aconine hydrochloride.

\text{Hexahydrooxonitine}—0.15 gm. of oxonitine was suspended in

\footnote{Without isolation of the products, Freudenberg (11) demonstrated the absorption of 3 moles of H_{2} by the benzoxy group in aconitine and oxonitine, and not in aconine.}
acetic acid and shaken with 50 mg. of platinum oxide catalyst in hydrogen under 3 atmospheres pressure. Although absorption occurred promptly and all oxonitine dissolved within several hours, the operation was continued overnight. The filtrate from the catalyst left on concentration a crystalline residue. The substance formed prisms or minute rods from 95 per cent alcohol, which melted at 253° on rapid heating.

Pyrooxonitine—This was prepared essentially according to Majima and Suginome (8). 0.4 gm. of oxonitine was heated in an atmosphere of \( \text{H}_2 \) at 280–285°. As soon as the substance melted, the tube was withdrawn from the bath. After cooling, the melt was dissolved in a few cc. of methyl alcohol and allowed to stand at 25° overnight. A small amount of unchanged oxonitine separated. The concentrated filtrate gave a resin which crystallized readily under chloroform. The collected material was recrystallized from chloroform and contained solvent. It was collected with chloroform and washed with a mixture of chloroform and petroleum ether.

For analysis it was dried at 120° and 20 mm.

\[
\begin{align*}
\text{C}_{32}\text{H}_{48}\text{O}_{12}\text{N} & : \quad \text{Calculated.} \quad \text{C} 60.80, \text{H} 7.58 \\
& \quad \text{“} 60.25, \quad \text{“} 7.43 \\
& \quad \text{Found.} \quad \text{“} 60.74, \quad \text{“} 7.49 \\
& \quad \quad \quad \quad \quad \quad \text{“} 60.94, \quad \text{“} 7.30 \\
\end{align*}
\]

In another experiment successive fractions were obtained from chloroform, which after recrystallization from chloroform gave the following figures.

\[
\begin{align*}
\text{C}_{36}\text{H}_{56}\text{O}_{16}\text{N} & : \quad \text{Calculated.} \quad \text{C} 63.55, \text{H} 6.72 \\
& \quad \text{“} 63.01, \quad \text{“} 6.53 \\
& \quad \text{Found.} \quad \text{“} 63.48, \quad \text{“} 6.77 \\
& \quad \quad \quad \quad \quad \quad \text{“} 63.68, \quad \text{“} 6.90 \\
\end{align*}
\]

Fraction (a) was then recrystallized from a mixture of alcohol and ether.

\[
\begin{align*}
\text{C}_{30}\text{H}_{46}\text{O}_{10}\text{N} & \quad \text{Found.} \quad \text{C} 63.30, \text{H} 6.45 \\
& \quad \quad \quad \quad \quad \quad \text{“} 63.34, \quad \text{“} 6.85 \\
\end{align*}
\]

Majima and Suginome have reported a melting point of 231° for pyrooxonitine. In no case have we been able to duplicate this. Our substance as it separated from chloroform melted slowly to a
colorless resin at 160–170°. The material from alcohol-ether, which lost about 1 per cent in weight on drying, softened gradually to a resin at 170–180° which melted on further heating. Finally, when recrystallized from methyl alcohol the substance melted sharply at 180°. In the latter case a rotation was found of \([\alpha]_D^{25} = -127°\) \((c = 1.14\) in methyl alcohol). This agrees closely with the values reported by Majima and Suginome, which approximated \([\alpha]_D = -128°\) in methyl alcohol.

**Hexahydropyrooxonitine**—0.15 gm. of pyrooxonitine was hydrogenated in alcoholic solution under 3 atmospheres pressure with 50 mg. of platinum oxide catalyst. After 2½ hours absorption had practically stopped. The absorption due to the substance was slightly in excess of 3 moles. The concentrated filtrate crystallized readily under ether. The collected material was recrystallized from acetone-ether and formed platelets which melted slowly at 160–163° to a viscous mass.

For analysis it was dried at 110° and 20 mm.

\[
\begin{align*}
\text{C}_9\text{H}_{18}\text{O}_{10}\text{N. Calculated.} & \quad \text{C 62.91, H 7.67} \\
\text{C}_9\text{H}_{19}\text{O}_{10}\text{N.} & \quad " 62.35, " 7.51 \\
\text{Found.} & \quad " 62.81, " 7.67 \\
& \quad " 62.76, " 7.53
\end{align*}
\]

**Base, from Oxonitine**—50 mg. of oxonitine were heated in a tube with 2 cc. of a 6 per cent solution of HCl in dry methyl alcohol at 100° for 18 hours. A clear solution resulted which was concentrated in vacuo to dryness. The resinous residue was dissolved in water and after being made alkaline with dilute Na₂CO₃ the mixture was extracted with chloroform. The dried extract on concentration gave a residue which crystallized as platelets from ethyl acetate. The yield was 25 mg. The substance proved to be a base and dissolved readily in dilute acid. It melted at 250° after preliminary sintering. It was easily soluble in alcohol, acetone, less readily in ethyl acetate, and but sparingly soluble in ether.

\[
\begin{align*}
\text{C}_9\text{H}_{18}\text{O}_{10}\text{N. Calculated.} & \quad \text{C 62.91, H 7.67, N 2.37} \\
\text{C}_9\text{H}_{19}\text{O}_{10}\text{N.} & \quad " 63.43, " 7.83, " 2.31 \\
\text{Found.} & \quad " 62.49, " 7.58 \\
& \quad " 62.97, " 7.61, " 2.61
\end{align*}
\]

\[
\begin{align*}
\text{C}_9\text{H}_{18}\text{O}_{10}\text{N. Calculated.} & \quad \text{5(OCH) 26.22, N(CH₃) 2.54} \\
\text{C}_9\text{H}_{19}\text{O}_{10}\text{N.} & \quad " 25.62, " 2.49 \\
\text{Found.} & \quad " 24.94, " 3.19
\end{align*}
\]
In another experiment in which 1 gm. of oxonitine was heated with 25 cc. of 4.7 per cent methyl alcoholic HCl, the tube was chilled to low temperature before opening. On opening, it was at once equipped with a tube leading into Ba(OH)₂ solution. As the tube warmed up, the presence of CO₂ in the evolved gases was readily noted. No attempt at a quantitative estimation was made. In this experiment the yield of base was 0.36 gm.

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