[Contribution from the Laboratories of the Rockefeller Institute for Medical Research]

THE ACONITE ALKALOIDS. XXIV. THE DEGRADATION OF ATISINE AND ISOATISINE

WALTER A. JACOBS

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The oxidation of isoatisine, $C_{22}H_{33}NO_2$, with potassium permanganate to the lactamtricarboxylic acid, $C_{21}H_{29}NO_7$, has been previously described (1). A first stage was shown to be the formation of the neutral lactam oxoisoatisine, $C_{22}H_{33}NO_3$, by addition of one oxygen atom with the disappearance of a double bond (2). The conversion of oxoisoatisine to the tricarboxylic acid was the result of further oxidation at several additional points external to a possible perhydrophenanthrene portion of the structure and involved the remaining double bond and hydroxyl groups of oxoisoatisine as well as the loss of a carbon and four hydrogen atoms. A somewhat clearer picture has been obtained more recently by the interception of the reaction at intermediate stages. By a modification of the oxidation procedure as presented in the experimental part, a reaction mixture was obtained which was separated into neutral and acid fractions. A neutral crystalline product was isolated which was shown to be dihydroxyoxoisoatisine, $C_{22}H_{35}NO_5$, and was formed by the addition of two hydroxyl groups to the remaining double bond of the intermediate oxoisoatisine. The acid fraction yielded a crystalline lactamdicarboxylic acid, $C_{21}H_{31}NO_6$. This substance was also obtained by a repetition of the oxidation procedure with dihydroxyoxoisoatisine and thus showed the latter to be an intermediate stage in its formation. Five of the oxygen atoms of the acid are thus accounted for by the lactam and the two carboxyl groups. The remaining oxygen is contained in the unaffected hydroxyl group of isoatisine (and therefore of atisine) and was shown to be of primary character by the further oxidation of the dicarboxylic acid to the lactamtricarboxylic acid, $C_{21}H_{29}NO_7$.

The dicarboxylic acid yielded with diazomethane a resinous dimethyl ester which could be distilled at low pressure. Since one of the ester groups proved to be relatively resistant to alkali a crystalline monomethyl ester was readily obtained. In the earlier work the trimethyl ester of the tricarboxylic acid was also shown to yield a resistant monomethyl ester and the latter must therefore involve one of the carboxyl groups produced in the formation of the dicarboxylic acid. This was confirmed by a study of the oxidation of dihydroöxoisoatisine with permanganate. Since this has no double bond, the oxidation proceeded much more gradually than with oxoisoatisine and the acid isolated was found to be monobasic and resulted from oxidation only of the primary alcoholic group to carboxyl. The crystalline acid, $C_{22}H_{33}NO_4$, was purified as the crystalline methyl ester which could be readily saponified to the acid in contrast to the monomethyl ester of the dicarboxylic acid. The hydroxyl group which is associated with the double bond of oxoisoatisine now remains unaffected after hydrogenation of the double bond. This hydroxyl was shown to be of secondary character by oxidation of the methyl ester of the acid $C_{22}H_{33}NO_4$ to the keto ester, which on saponification yielded the crystalline keto acid, $C_{22}H_{31}NO_4$. The ultraviolet absorption spectrum curve obtained with the keto ester (5.6 mg. in 5 cc. of ethanol) as shown in Fig. 1 is characteristic of ketonic absorption in contrast to the uneventful curve obtained with its precursor (6.3 mg. in 5 cc. of ethanol).[1]

The formation of the lactamdicarboxylic acid, $C_{21}H_{29}NO_6$, from atisine which was previously described (1) has also been more readily interpreted. Since the earlier work the yield of this substance has been considerably improved and its isolation facilitated as presented in the experimental part. The production of the two carboxyl groups with loss of a carbon atom must parallel that phase of the oxidation of oxoisoatisine which involves the secondary hydroxyl group and the neighboring double bond. The oxidation to the lactam stage is apparently different. This must result from the oxidation to CO of a CH_2 group attached to the nitrogen atom, with retention of the double bond which is in the neighborhood of the nitrogen atom. The presence of this double bond was confirmed by subsequent hydrogenation and the product isolated was indistinguishable from and apparently identical with the lactamdicarboxylic acid, $C_{21}H_{31}NO_6$, obtained directly from isoatisine. The previously described study of the esters of the acid $C_{21}H_{29}NO_6$ is similarly in agreement, since the dimethyl ester could be readily saponified to a more resistant monomethyl ester.

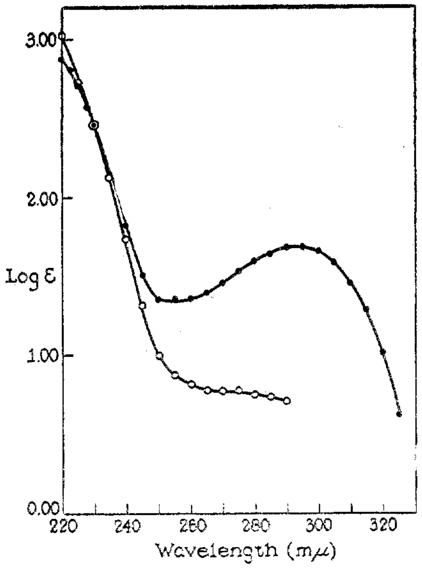

FIGURE 1. ULTRAVIOLET ABSORPTION SPECTRA. ● = the keto ester $C_{23}H_{33}NO_4$; ○ = the hydroxy ester $C_{23}H_{35}NO_4$.

In this connection the previously reported tricarboxylic acid, $C_{19}H_{27}NO_6$, isolated after more vigorous oxidation of atisine, requires reinterpretation. It is

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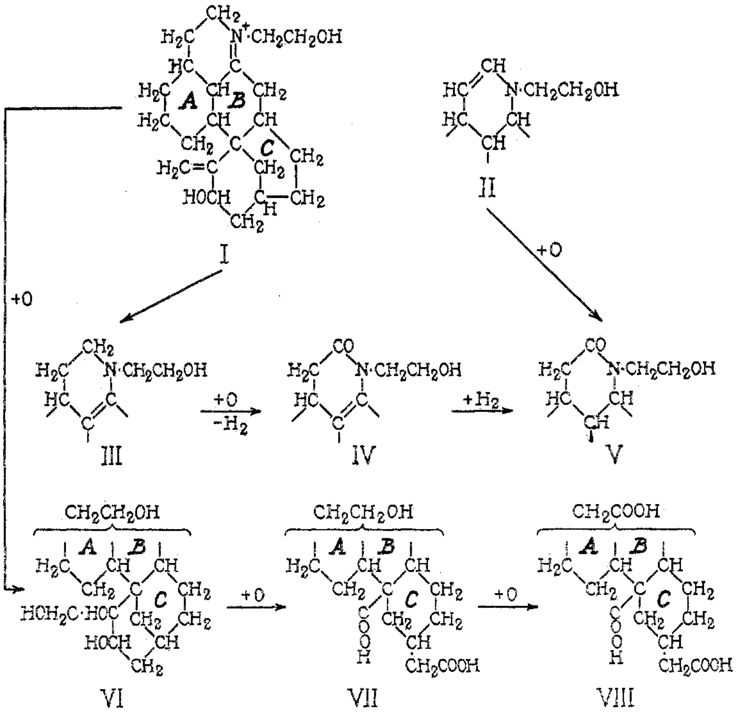
[1] These curves were kindly obtained by Dr. Yoshio Sato.

probably the result of further oxidation of the unsaturated dicarboxylic acid in which the primary alcoholic group is changed to carboxyl. It would therefore be the unsaturated lactamtricarboxylic acid, $C_{21}H_{27}NO_7$. (Calc'd: C, 62.19; H, 6.72; Found: C, 62.35; H, 7.14).

The earlier work has shown that the lactamtricarboxylic acid $C_{21}H_{29}NO_7$ on dehydrogenation yields 1,6-dimethylphenanthrene whereas the characteristic product from atisine is 1-methyl-6-ethylphenanthrene. In the attempt to limit the origin of the 6-ethyl group, and of the 6-methyl group following oxidation, additional dehydrogenation studies with selenium have been made. Oxoisoatisine like atisine still yields 1-methyl-6-ethylphenanthrene. The same hydrocarbon has also been obtained from tetrahydroatisine. Elimination of the double bonds or isomerization of atisine or the oxidation to oxoisoatisine have therefore not affected the 6-ethyl-producing structural feature. On the other hand, dehydrogenation of the lactamdicarboxylic acid, $C_{21}H_{31}NO_6$, has yielded 1,6-dimethylphenanthrene. This has shown that the portion of the structure bearing the double bond, the secondary hydroxyl group, and the carbon atom lost in the oxidation to the dibasic acid are a part of the structural feature which yields the 6-ethyl group on dehydrogenation of atisine.

The origin of the 1-methyl group of the hydrocarbons has not been completely determined. However, since in the tribasic lactamacid, $C_{21}H_{29}NO_7$, 18 carbon atoms are required for the perhydrophenanthrene portion of the structure, the three carboxyl groups, and the 6-methyl group, its origin is limited to the lactam side chain and the primary alcohol-bearing side chain. Although this phase of the work is still in progress, there is some reason to believe that its source will be found in the nitrogen-bearing side chain. In the previous paper the conversion of the lactamtricarboxylic acid with thionyl chloride to a lactam dibasic acid, $C_{18}H_{25}NO_5$, was described. A more recent study has given reason to question this formulation. The acid itself remained of questionable homogeneity but its dimethyl ester showed retention of the stable ester group and was readily saponified to the crystalline monomethyl ester which possessed excellent physical properties. The analytical data obtained with both of the esters were as satisfactory for derivatives of an acid $C_{19}H_{27}NO_5$. Such an acid would result by removal of a $CH_2COO(H)$ group from the tribasic acid $C_{21}H_{29}NO_7$. It is now planned to study the dehydrogenation of this acid to determine whether only 3-methylphenanthrene or 1,6-dimethylphenanthrene will result.

The available data are still insufficient to permit final conclusions regarding the complete structure of atisine. The scheme presented in Formula I with which there are possible objections is only tentative and is given rather as an aid in visualizing the data. It is also based on the assumption that the production of 1,6-disubstituted phenanthrenes on dehydrogenation is not the result of rearrangements or a ring closure. Atisine especially in the form of its salts is assumed to contain a pentavalent nitrogen since its basicity has been previously shown (2). It can be completely liberated from its salts only with NaOH, whereas isoatisine is liberated readily with sodium carbonate. A recent study has shown that tetrahydroatisine is also a weaker base. It is probable therefore that one of the double bonds is associated with the nitrogen in atisine as shown in I. In isoatisine the double bond is assumed to have shifted as shown in II to explain its oxidation to the lactam isooxoatisine (V). In the oxidation of atisine the carbon atom vicinal to the nitrogen also becomes carbonyl but with loss of two hydrogen atoms (IV). The double bond which is retained must have shifted elsewhere away from the nitrogen. The structure involved in the oxidation to the dicarboxylic acid must be an allylic structure containing a secondary hydroxyl and perhaps an exocyclic methylene group which are part of a ring external to the perhydrophenanthrene portion as indicated in I. In order to explain its relatively resistant ester group, one carboxyl group of the dicarboxylic acid is assigned a tertiary character while the other carboxyl group is attached by CH_2 to a position which permits the production of the 6-methyl group on dehydrogenation. The various stages of the oxidation are indicated in VI, VII, and VIII.



It is still possible that the phenanthrene hydrocarbons may prove to be artefacts due to ring closure during dehydrogenation and not products of an original perhydrophenanthrene partial structure in atisine.

The action of HCl on oxoisoatisine was previously shown (2) to yield a substance in which the ultraviolet absorption curve indicated the presence of a carbonyl group and for which a formulation $C_{20}H_{29}NO_3$ was derived. More recent work has shown that this formulation is incorrect.

The analytical data have been complicated by the presence of chloro compounds, the removal of which has proved difficult. The data obtained indicate that no loss of carbon has occurred and that the reaction of interest is that of isomerization of an unsaturated hydroxy to the isomeric keto derivative, $C_{22}H_{33}NO_3$. A report of this phase of the work will be withheld until it is completed.

EXPERIMENTAL

The lactam dicarboxylic acid, $C_{21}H_{31}NO_6$. A solution of 8 g. of isoatisine in 160 cc. of pyridine was treated with 160 cc. of a 1.25% aqueous NaOH solution. To the chilled mixture was added in portions 16.27 g. of powdered potassium permanganate or about 10% in excess of the requirements for six oxygen atoms. After about 20 minutes the reaction became more gradual and required about 1¼ hours for completion. The diluted mixture was filtered through Norit followed by resuspension for washing. The filtrate was extracted with chloroform and the extract, which contained some suspended aqueous phase, was washed once with water. The chloroform extract will be described later. The clear aqueous phase was concentrated *in vacuo* at 30–40° to about 100 cc. Acidification with excess acetic acid caused CO_2 effervescence followed by gradual crystallization; 3.54 g. of the acid was collected with water. It was recrystallized by solution in dilute ammonia and reacidification with acetic acid. It separated as aggregates of minute flat microneedles which melted at 253–255° after preliminary sintering. The melting point varied somewhat with individual preparations. Evaporation of a diluted ethanol solution yielded flat microneedles or leaflets which melted at 255–257°; $[\alpha]_D^{29}$ −17° (*c*, 0.99 in 95% ethanol). For analysis it was dried at 110° and 0.2 mm.

Anal. Calc'd for $C_{21}H_{31}NO_6$: C, 64.08; H, 7.94.
Found: C, 64.03; H, 7.90.

In addition, 0.1 g. of the following dihydroxyoxoisoatisine was similarly oxidized with 0.13 g. of permanganate. The reagent remained in excess following the main reaction. After dilution and filtering the filtrate was worked up as above. A first crop of 39 mg. of flat microneedles which melted at 251–259° was collected; $[\alpha]_D^{27}$ −19° (*c*, 0.86 in 95% ethanol).

Anal. Found: C, 64.13; H, 7.86.

When 0.1 g. of oxoisoatisine was used instead of isoatisine it yielded the same dibasic acid which melted at 255–259°.

Anal. Found: C, 63.80; H, 8.07.

The dimethyl ester and monomethyl ester. A suspension of 0.15 g. of the dibasic acid in acetone readily dissolved with excess diazomethane. Concentration yielded a resin which could not be crystallized. At 0.02 mm. the major portion distilled from a bath at 168–178°; 0.13 g. of colorless resin was obtained.

Anal. Calc'd for $C_{23}H_{35}NO_6$: C, 65.51; H, 8.37.
Found: C, 64.94; H, 8.08.

When 1.805 mg. of substance was heated at 100° for 1 hour in 0.1 cc. of 50% ethanol and 0.1128 cc. of 0.1 *N* NaOH it consumed 0.0411 cc.; Calculated for 1 equivalent, 0.0428 cc.

A solution of 50 mg. of the dimethyl ester in 1 cc. of methanol was treated with 2 cc. of 0.1 *N* NaOH and heated on the bath for 5–10 minutes. The cooled mixture was filtered and acidified with acetic acid. On removal of methanol *in vacuo* the monomethyl ester separated as delicate micro needles which melted at 129.5–131°.

Anal. Calc'd for $C_{22}H_{33}NO_6$: C, 64.82; H, 8.17; OCH_3, 7.62.
Found: C, 64.60; H, 8.00; OCH_3, 7.84.

When 0.1 g. of the dibasic acid, $C_{21}H_{31}NO_6$ was treated with 2 cc. of thionyl chloride it reacted quickly and dissolved. The mixture was heated on the water-bath for 40 minutes and excess reagent was flashed off *in vacuo* with benzene. The residue was treated with

150 cc. of H_2O and heated until most had dissolved. The filtrate when concentrated on the water-bath to about 15 cc. yielded a trace of resin. On further concentration of the filtrate the resin which separated crystallized on cooling. A first fraction of 20 mg. separated which melted at 238–244° and proved to be the dibasic acid. This was followed by a succeeding, partly crystalline fraction of similar material.

Anal. Found: C, 64.04; H, 7.89.

Dihydroxyoxoisoatisine. The chloroform extract from the previous oxidation of isoatisine contained most of the pyridine. It was dried with sodium sulfate and concentrated *in vacuo* to remove solvent and pyridine. The residue when treated with benzene began to crystallize, and repeated flashing off of solvent to remove residual pyridine caused further crystallization. The substance which was collected with benzene amounted to 0.91 g. It crystallized from acetone as microleaflets or flat needles which melted at 253.5–258.5°; $[\alpha]_D^{23}$ −33° (*c*, 0.91 in 95% ethanol).

Anal. Calc'd for $C_{22}H_{35}NO_5$: C, 67.13; H, 8.97; N, 3.56.
Found: C, 67.34; H, 8.76; N, 3.99.

No alkali was consumed by the substance on direct titration or after heating with excess reagent.

Oxidation of the dibasic acid $C_{21}H_{31}NO_6$. A solution of 60 mg. of the dibasic acid in 1 cc. of 1.25% NaOH was treated with 40 mg. of potassium permanganate. At room temperature the reaction was very slow and after 27 hours the reagent remained in excess. The solution was carefully decolorized with sodium sulfite. Acidification of the filtrate with H_2SO_4 caused separation of some amorphous material followed by gradual crystallization; the product was collected the next day and was recrystallized by solution in acetone and dilution. The excess acetone was boiled off to turbidity which caused gradual separation of microcrystalline aggregates of the tricarboxylic acid. The yield was 17 mg. followed by an additional 4 mg. It melted at 250–253°.

Anal. Calc'd for $C_{21}H_{29}NO_7$: C, 61.88; H, 7.18.
Found: C, 62.52, 62.21; H, 7.29, 7.23.

The acid $C_{22}H_{33}NO_4$ and its methyl ester. A solution of 0.5 g. of dihydroöxoisoatisine in 10 cc. of pyridine and 10 cc. of 1% NaOH was treated with 0.63 g. of potassium permanganate. After standing several days the reagent was used up. Following dilution and filtration the filtrate was extracted with chloroform. From this a neutral fraction was recovered which consisted partly of unreacted starting material. The aqueous phase was acidified with H_2SO_4 and extracted further with chloroform. The washed and dried extract yielded 0.23 g. of an acid fraction. Its purification was accomplished through the methyl ester which was prepared with diazomethane in acetone. The ester crystallized from dilute methanol as needles which melted gradually from 202–217° after preliminary sintering. The yield was 0.1 g. When crystallized from a small volume of chilled methanol it melted under the microscope at 228–231° after sintering above 220°.

Anal. Calc'd for $C_{23}H_{35}NO_4$: C, 70.90; H, 9.06.
Found: C, 70.62; H, 8.87.

Titration: 1.67 mg. of substance was refluxed for 2 hours in 0.15 cc. of 50% ethanol and 0.123 cc. of 0.1 *N* NaOH and titrated back against phenolphthalein. Calc'd for 1 equivalent: 0.0428 cc.; Found: 0.0422 cc.

The ultraviolet absorption curve obtained with the ester is shown in Fig. 1.

A solution of 0.1 g. of the ester in 2 cc. of methanol was treated with an excess of 10% NaOH and 2 cc. of H_2O. After heating a few minutes it was allowed to stand for 1 hour. After acidification and further dilution with warming crystallization began and upon cooling it was gradually completed. Yield, 77 mg. of micro needles which melted at 240–244° after preliminary sintering.

Anal. Calc'd for $C_{22}H_{33}NO_4$: C, 70.35; H, 8.86.
Found: C, 70.25; H, 8.78.

The keto acid $C_{22}H_{31}NO_4$ and methyl ester. The previous methyl ester ($C_{23}H_{35}NO_4$, 0.1 g.) in 1.5 cc. of acetic acid and 0.5 cc. of H_2O was treated with 0.5 cc. of Kiliani chromic acid solution. After 1 hour the diluted mixture was extracted with benzene and the extract was washed in turn with water, Na_2CO_3 solution, and water. The dried benzene phase on concentration yielded 90 mg. of residue. Crystallization attempts were unsuccessful. On sublimation at 0.02 mm. the major portion sublimed from a bath above 150°.

Anal. Calc'd for $C_{23}H_{33}NO_4$: C, 71.27; H, 8.59.
Found: C, 70.92; H, 8.35.

The ultraviolet absorption curve obtained with the ester is given in Fig. 1.

Saponification occurred readily in dilute methanol with an excess of NaOH. After acidification with acetic acid and dilution the keto acid crystallized as flat microneedles. Acidification with dilute H_2SO_4 caused immediate precipitation of resinous material which gradually crystallized. After recrystallization from dilute acetone, it melted at 223.5–229°.

Anal. Calc'd for $C_{22}H_{31}NO_4$: C, 70.73; H, 8.37.
Found: C, 70.61; H, 8.29.

The lactamdicarboxylic acid, $C_{19}H_{27}NO_5$ (?). This substance, previously (1) assigned the formula $C_{18}H_{25}NO_5$, was prepared from the lactamtricarboxylic acid $C_{21}H_{29}NO_7$ with thionyl chloride essentially as before and gave similar analytical data.

The dimethyl ester. The dibasic acid (74 mg.) was esterified in acetone with diazomethane. After removal of solvent the resin was sublimed at 0.02 mm. and at about 180–210°; yield, 60 mg.

Anal. Calc'd for $C_{21}H_{31}NO_5$: C, 66.80; H, 8.28.
Calc'd for $C_{20}H_{29}NO_5$: C, 66.07; H, 8.05.
Found: C, 66.48; H, 8.29.

When 1.71 mg. of the ester was heated on the bath for 1 hour in 0.1 cc. of 50% ethanol and 0.105 cc. of 0.1 *N* NaOH it consumed 0.0485 cc.; Calc'd for 1 mole, 0.0453 cc.

The monomethyl ester. The ester (50 mg.) was dissolved in a small volume of methanol and treated with some excess of 10% NaOH. After standing about an hour further dilution caused no turbidity. Acidification with acetic acid produced a voluminous wooly mass of delicate needles which was collected with water. The yield was 45 mg., m.p. 301–309°.

Anal. Calc'd for $C_{20}H_{29}NO_5$: C, 66.07; H, 8.05.
Calc'd for $C_{19}H_{27}NO_5$: C, 65.29; H, 7.79.
Found: C, 65.64, 65.63; H, 7.93, 8.17.

The lactonedicarboxylic acid $C_{21}H_{29}NO_6$ from atisine. To 5 g. of atisine hydrochloride dissolved in 56 cc. of H_2O was added 76 cc. of pyridine followed by 56 cc. of 10% NaOH with cooling which caused partial formation of an emulsion. Then 12 g. of potassium permanganate was added in portions with thorough mixing; as it was gradually used an additional 15 cc. of H_2O was required. The reaction was completed in about an hour. After dilution the coagulated manganese dioxide was centrifuged and washed by resuspension in H_2O. The clear alkaline solution was extracted with benzene and yielded 0.43 g. of a mixture of neutral and basic material which was left for future study. The alkaline aqueous phase after acidification with H_2SO_4 was extracted with ethyl acetate. The washed and dried extract when concentrated yielded 2.74 g. of resinous residue. After solution in warm acetone it gradually crystallized as micro prisms which amounted to 0.97 g. The mother liquor after concentration gradually yielded a second fraction of 73 mg. For recrystallization it was dissolved in a good volume of acetone containing a few cc. of H_2O because of insolubility in the dry solvent. After concentration most was recovered in successive, practically indistinguishable fractions of minute micro prisms and four-sided leaflets. It gradually melted from 306–325° after slight preliminary sintering; $[\alpha]_D^{27}$ $-26°$ (*c*, 1.04 in 95% ethanol).

Anal. Calc'd for $C_{21}H_{29}NO_6$: C, 64.41; H, 7.47.
Found: C, 64.07; H, 7.44.

The hydrogenation product $C_{21}H_{31}NO_6$. Hydrogenation of the acid, $C_{21}H_{29}NO_6$ in 70% acetic acid proceeded very slowly in the course of a number of days. The reaction remained incomplete in glacial acetic acid and failed in dilute aqueous ammonia. The product from 0.1 g. of substance and 0.1 g. of platinum oxide catalyst was obtained after removal of the solvent. On acidification of the solution in dilute alkali with acetic acid it separated as aggregates of micro needles and leaflets; yield, 56 mg. After recrystallization from dilute alcohol it showed m.p. 256–259° which varied with the state of division. No depression was obtained when mixed with the acid $C_{21}H_{31}NO_6$ from isoatisine; $[\alpha]_D^{25}$ −22.5° (*c*, 0.92 in 95% ethanol).

Anal: Calc'd for $C_{21}H_{31}NO_6$: C, 64.08; H, 7.94.

Found: C, 64.14; H, 8.00.

Dehydrogenation of oxoisoatisine. A ground mixture of 3.3 g. of oxoisoatisine and 9 g. of selenium was heated in a flask from which air was displaced by nitrogen and which was connected by a side-arm with a receiving flask. The temperature was gradually raised within about 80 minutes to 340° and maintained for 2 hours. Only a little condensate collected in the receiver. After cooling, this fraction and the condensate above the melt were rinsed out with ether and the pulverized solidified melt was extracted overnight in a Soxhlet. The combined ether extracts on concentration yielded 1.6 g. of a dark resin which was redissolved in ether. The filtrate from the insoluble selenium-containing resin was concentrated to about 10 cc. and treated with 10 cc. of 10% HCl. On thorough mixing an appreciable red resinous precipitate of salts separated which was not further examined. The ether phase with the rinsings was cleared with sodium sulfate and on concentration yielded 1.02 g. of colored resin.

The resin was added with 8 cc. of benzene to a column of 40 g. of alumina prepared with benzene and at once developed with petroleum ether (30–60°). After 40 cc. had passed through, eluted material appeared and was collected in 2-cc. fractions in successive amounts of 27 mg., 84 mg., 60 mg., 30 mg., and 20 mg. This material (0.22 g.) was recombined and after solution in 2 cc. of petroleum ether was rechromatographed through 10 g. of alumina also using this solvent.

CHROMATOGRAPHIC FRACTIONATION

NO. OF FRACTION	SOLVENT	EXTRACTED SUBSTANCE, MG.	APPEARANCE OF RESIDUE
Forerun	15 cc. petroleum ether (30–60°)	—	—
2	5 cc. petroleum ether (30–60°)	23	Partly crystalline when chilled
3	1 cc. petroleum ether (30–60°)	14	Resinous
4–14	1 cc. each of petroleum ether	98	Resinous
15	2 cc. of petroleum ether	7	Resinous
16–23	1 cc. each of petroleum ether	23.5	Resinous
24	10 cc. benzene-petroleum ether, (1:10)	11.5	Resinous
25	5 cc. benzene-petroleum ether, (1:10)	5	Resinous
26	5 cc. benzene-petroleum ether, (1:10)	7	Resinous

Fraction 25 when combined with 6 mg. of trinitrobenzene in a small volume of 95% ethanol readily yielded delicate yellow needles. Some 7 mg. were collected in the cold; m.p. 139.5–140.5°. Recrystallization from 95% ethanol gave 4.5 mg.; m.p. 139.5–142.5°.

Anal. Calc'd for $C_{17}H_{16} \cdot C_6H_3N_3O_6$: C, 63.71; H, 4.42.

Found: C, 63.54; H, 4.31.

Fraction 15 similarly yielded an indistinguishable trinitrobenzene compound which melted at 140.5–143.5°. A mixture with the trinitrobenzene derivative of synthetic (3) 1-methyl-6-ethylphenanthrene (m.p. 143.5–147°) melted at 140.5–146°.

Fractions 16–23 (23.5 mg.) with trinitrobenzene in 95% ethanol gave 34 mg. of compound; m.p. 139–144°. This derivative was dissolved in 2 cc. of benzene and chromatographed through 4 g. of alumina suspended in petroleum ether. On development with 10% benzene in petroleum ether the color descended and as it approached the lower portion of the column petroleum ether alone was used and soon yielded 20 mg. of oil which crystallized in ice or from 95% ethanol. It yielded from ethanol a picrate which melted at 131.5–136°.

Anal. Calc'd for $C_{17}H_{16} \cdot C_6H_3N_3O_7$: C, 61.44; H, 4.26.
Found: C, 61.57; H, 4.12.

Dehydrogenation of tetrahydroatisine. Tetrahydroatisine (3.6 g.) was heated with 10 g. of selenium essentially as in the case of oxoisoatisine. Some dark red condensate which accumulated in the receiver was extracted with ether and after drying yielded 0.2 g. of a dark red oil which was added to the main reaction material. The latter was obtained by continuous ether extraction of the ground reaction mixture. The combined material in about 10 cc. of ether was extracted with excess 10% HCl followed by water. The ether phase yielded 1.47 g. of a dark red oily resin. The latter was dissolved in 10 cc. of benzene and chromatographed through 50 g. of alumina and developed with benzene. Following the first 60 cc., colored material emerged. In the next 5 cc. 20 mg. of oil was eluted and succeeding 2-cc. cuts yielded successively 0.171 g., 0.214 g., 0.21 g., 0.2 g., 0.12 g., 70 mg., 30 mg., and 21 mg. The next 10 cc. gave 40 mg. None of these fractions crystallized. Although appreciable material continued to emerge in subsequent fractions, it was discarded. The combined fractions dissolved in petroleum ether (30–60°) were rechromatographed through 50 g. of alumina using only the same solvent. Following an initial 25 cc., the desired substance was eluted and was collected in 2-cc. cuts. Of these only fractions 21 to 38 were studied. Fraction 21 gave 25 mg. of colored oil which did not crystallize when chilled and seeded. With 30 mg. of picric acid in 95% ethanol 17 mg. of yellow needles were obtained, m.p. 120–130°.

Anal. Calc'd for $C_{17}H_{16} \cdot C_6H_3N_3O_7$: C, 61.44; H, 4.26.
Found: C, 62.15, H, 4.38.

Fraction 23 gave 20 mg. This yielded from absolute ethanol 15 mg. of a *trinitrobenzene compound* which melted at 139–140° after preliminary softening.

Anal. Calc'd for $C_{17}H_{16} \cdot C_6H_3N_3O_6$: C, 63.71; H, 4.42.
Found: C, 63.75; H, 4.56.

Fraction 24 (23 mg.) when chilled in ice partly crystallized on seeding with methylethylphenanthrene.

Fractions 37 and 38 gave 25 mg. of oil which crystallized on chilling.

Fractions 25 to 38 were therefore recombined and yielded 0.21 g. of a practically colorless oil which crystallized partly on chilling. It was dissolved in 10 cc. of 95% ethanol and treated with 0.22 g. of trinitrobenzene in ethanol. Yield, 0.355 g. of a compound which after recrystallization melted at 139–140°. This material in 10 cc. of benzene was added to a column of 40 g. of alumina prepared with petroleum ether (30–60°) and at once developed with this solvent. A first fraction of about 100 cc. on concentration yielded most of the colorless hydrocarbon which crystallized on chilling. This was dissolved in sufficient 95% ethanol and crystallized in the cold as micro leaflets, m.p. 32–33°. After repeated recrystallization from this solvent 25 mg. of lustrous leaflets were finally obtained, m.p. 38–39°.

Anal. Calc'd for $C_{17}H_{16}$: C, 92.68; H, 7.32.
Found: C, 92.41; H, 7.30.

The *trinitrobenzene compound* from this material melted at 149–149.5°.

Anal. Found: C, 64.11; H, 4.34.

The *picrate* similarly prepared melted at 131.5–134.5°.

Anal. Found: C, 61.43; H, 4.28.

Dehydrogenation of the acid $C_{21}H_{31}NO_6$. The dicarboxylic acid $C_{21}H_{31}NO_6$ (3.1 g.) was heated with 9 g. of selenium as in the previous cases. The ether extract of the ground reaction product after concentration was extracted with 10% HCl. The remaining fluorescent ether solution yielded 0.22 g. of the crude hydrocarbon-containing fraction. This was chromatographed through 4 g. of alumina and benzene was used as solvent. When the material did emerge the elution was rapid and a total of 22 cc. of eluent yielded 0.12 g. of partly crystalline hydrocarbon. The latter was rechromatographed through 4 g. of alumina and only petroleum ether (30–60°) was used as the solvent. Following the elution of a small amount of oil successive, readily-crystallizing fractions of 70 mg., 13 mg., and 5 mg., respectively were collected.

The first fraction after repeated recrystallization from 95% ethanol was obtained as leaflets which melted at 82.5–83.5°. When mixed with a synthetic sample of 1,6-dimethylphenanthrene obtained from Prof. R. D. Haworth, the mixture m.p. was 82.5–84°. The Haworth sample melted at 90–93°. The mixture with 1,7-dimethylphenanthrene (m.p. 87–91°) showed a marked depression and melted from 59–86°.

Anal. Calc'd for $C_{16}H_{14}$: C, 93.15; H, 6.85.
Found: C, 93.46; H, 6.75.

The *trinitrobenzene derivative* formed delicate yellow needles from 95% ethanol and was indistinguishable from a sample obtained from Prof. Haworth (m.p. 158.5–162.5°). It melted at 159.5–160.5° and showed no depression with the former.

Anal. Calc'd for $C_{16}H_{14} \cdot C_6H_3N_3O_6$: C, 62.98; H, 4.09.
Found: C, 62.88; H, 3.91.

The *picrate* formed orange needles which melted at 140.5–144.5°.

Anal. Calc'd for $C_{16}H_{14} \cdot C_6H_3N_3O_7$: C, 60.66; H, 3.93.
Found: C, 60.41; H, 3.87.

All analytical data have been obtained by Mr. D. Rigakos of this laboratory.

SUMMARY

The previously described oxidation of isoatisine through the intermediate oxoisoatisine to the lactamtricarboxylic acid, $C_{21}H_{29}NO_7$, has been intercepted at the successive stages of a dihydroxyoxoisoatisine, $C_{22}H_{35}NO_5$, and a lactamdicarboxylic acid, $C_{21}H_{31}NO_6$. Dihydroöxoisoatisine behaves differently and has yielded a monobasic lactamacid, $C_{22}H_{33}NO_4$, by oxidation of a terminal primary carbinol group. The remaining hydroxyl was characterized as secondary by further oxidation to the keto acid $C_{22}H_{31}NO_4$.

In the case of atisine itself, there were also successive oxidation stages. Those isolated were the unsaturated lactamdicarboxylic acid, $C_{21}H_{29}NO_6$, and the unsaturated lactamtricarboxylic acid, $C_{21}H_{27}NO_7$, in which a double bond has been retained. Hydrogenation of the dibasic acid yields a substance apparently identical with the above lactamdicarboxylic acid obtained directly from isoatisine.

1-Methyl-6-ethylphenanthrene, the characteristic dehydrogenation product of atisine has been obtained also from oxoisoatisine and tetrahydroatisine. On the other hand, 1,6-dimethylphenanthrene, the hydrocarbon obtained from the lactamtricarboxylic acid, $C_{21}H_{29}NO_7$, is also the product from the lactamdicarboxylic acid, $C_{21}H_{31}NO_6$.

Other observations have been made and, to explain accumulated data, tentative structures for atisine and its derivatives are discussed.

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