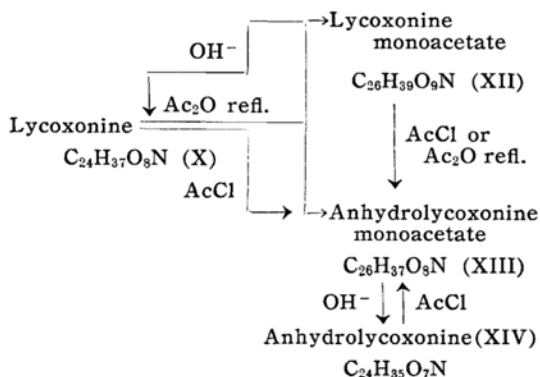


On oxidation of anhydrolycoctonamic acid (VII)⁴ with potassium permanganate in 0.5 per cent potassium carbonate solution, a new acid, apo-anhydrolycoctonamic acid (XI), $C_{24}H_{31}O_8N$, m. p. 183~184°C, $[\alpha]_D + 37^\circ$, was yielded.

Lycxonine (X) contains an *N*-ethyl and four methoxyl groups as does lycoctonam (III). In the infrared spectra of both compounds no decisive difference could be detected in the hydroxyl region because of the broad absorption. On the other hand, the bands due to lactam carbonyl were distinctly different (lycxonine showed at 1631 cm^{-1} and lycoctonam at 1618 cm^{-1}). The same shift has been observed⁴ between lycoctonam and the compounds in which the primary hydroxyl group has been acetylated or replaced by hydrogen. Since, from the above observations, the properties of the primary hydroxyl group were considered to be different, the acetylation of lycxonine was examined.

Lycxonine was acetylated with acetyl chloride at room temperature yielding anhydrolycxonine monoacetate (XIII), m. p. 232~234°C, $[\alpha]_D + 37.8^\circ$, or with acetic anhydride under reflux yielding the same anhydro monoacetate (XIII) and lycxonine monoacetate (XII), m. p. 222~225°C. The compound XIII gave anhydrolycxonine (XIV), m. p. 160~162°C, by hydrolysis.

However, lycxonine is not acetylated on treatment with acetic anhydride and pyridine at room temperature, but lycoctonam (III) or other lycoctonine derivatives containing original the primary hydroxyl group have been readily acetylated⁴ under the same conditions. Thus the formation of anhydro monoacetate (XIII) indicates the existence of the original glycol system and at least another hydroxyl group in lycxonine (X). However, the hydroxyl group to be acetylated in lycxonine is no longer the original primary one in view of the difficulty of acetylation.



This interpretation is in concordance not only with the properties of lycxonine (whilst lycoctonam has been oxidized readily, lycxonine is stable), but also with the observation of the infrared spectra of anhydrolycxonine (XIV) and anhydrolycoctonam (VI) (the latter compound is derived from lycoctonam (III) by pinacolic dehydration). The absorption band at 3413 cm^{-1} , characteristic of the hydroxyl in compound VI, disappeared and a new band at 3534 cm^{-1} appeared in compound XIV. From the above results of acetylation, oxidation, and examination of spectra and compositions, it may be concluded that the properties of lycxonine (X) are probably consistent with a structure in which the hydroxymethylene of lycoctonam (III) has been removed and a hydroxyl group has been produced.

It has been reported²⁻⁴ that the substituent group, which is located at the bridgehead of the azabicyclo [3,3,1]-nonane system in the skeleton of lycoctonine, is readily removed. For example, de-(hydroxymethylene)-lycoctonam (V) has been formed during the permanganate oxidation of lycoctonine, and it has been obtained also by decarboxylation of lycoctonamic acid (IV). Thus, on the basis of the ready removal of the hydroxymethylene group, it may be considered that compound V is an intermediate in the formation of lycxonine from lycoctonam. If this consideration is correct, the formation of formic acid during the oxidation will be explained by the analogous mechanism which has been suggested by Edwards, Marion and Stewart².

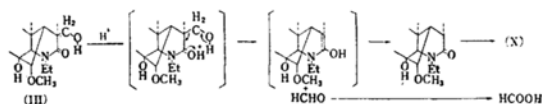


Fig. 3.

However, on the same treatment with chromic anhydride in *N*-sulfuric acid solution, de-(hydroxymethylene)-lycoctonam (V) is very stable against the reagents. On more vigorous treatment of lycoctonam (III) with the acid only, the hydroxymethylene group is left intact while lycoctonam undergoes extensive change⁵. It is, therefore, unlikely that de-(hydroxymethylene)-lycoctonam (V) is an intermediate. An attempt was made to determine the location of the new hydroxyl

5) O. E. Edwards, Léo Marion and R. A. McIvor, *Can. J. Chem.*, **32**, 708 (1954).

group, but decisive conclusions have not yet been obtained.

Recently an interesting compound, which may be related to the change from lycotoctonam to lycoxonine, has been reported by Edwards, Marion and Stewart²⁾. This compound was obtained by the action of lead tetraacetate on hydroxylycoctonam (VIII). It was suggested that it had a structure in which the hydroxymethylene group of compound VIII had been replaced by a new hydroxyl group and the glycol system had been cleaved.

Therefore, except for the cleavage of glycol, the properties of this compound appear to be closely analogous to those of lycoxonine. The same investigators²⁾ suggested in addition that it is difficult to demonstrate whether the skeleton of hydroxylycoctonine⁶⁾ (VIII) is or is not the same as that of lycotoctonine. Hence, it is of interest to compare the action of chromic anhydride upon lycotoctonam (lycotoctonine skeleton) with that upon hydroxylycoctonam (hydroxylycoctonine skeleton), and to investigate whether the new hydroxyl group in lycoxonine is or is not located at the same position as that of the compound obtained by the above workers.

On treatment of hydroxylycoctonam (IX) with chromic anhydride in *N* sulfuric acid solution, two acids, Y-acid, m. p. 193~195°C, and C₁-acid, m. p. 220~222°C, and two neutral compounds, Y, m. p. 177~180°C, and Z, m. p. 226~227°C, were obtained. The properties of these compounds will be reported later.

The new acid, apo-anhydrolycoctonamic acid (XI), obtained by alkaline permanganate oxidation of anhydrolycoctonamic acid (VII), still contains an *N*-ethyl group. It shows the lactam band at 1597 cm⁻¹ in the infrared spectrum, and gives a decarboxy-compound, m. p. 105~110°C. These evidences have been observed⁴⁾ analogously as in the case of the starting acid (VII), but the apo-acid (XI) contains only three methoxyl groups while the starting acid contains four.

In the ultraviolet spectrum the apo-acid

(XI) shows the maximum at 305 mμ (log ε 2.07) which is usual in position but unusual in intensity; the maximum of dehydro-compounds from lycotoctonine has been shown at 305 mμ (log ε 1.89). In addition, an oxime of the decarboxy-compound derived from the apo-acid shows the ultraviolet maximum still at 305 mμ, but the intensity is usual (log ε 1.85).

From the above observations, the conversion of anhydro-lycoctonamic acid (VII) to apo-anhydrolycoctonamic acid (XI) seems to involve the loss of one methoxyl and the formation of one carbonyl group. The detection of the produced carbonyl group was unsuccessful in the infrared spectrum, but the above consideration is supported by the chemical evidence that, whilst the starting acid VII is resistant to carbonyl reagents, the apo-acid consumes bromine quickly although the starting acid does not. The decarboxy compound from the apo-acid gave a 2,4-dinitrophenyl-hydrazone, m. p. 221~222°C, and an oxime, m. p. 140~142°C.

In the apo-acid (XI), the location of the removed methoxyl and the newly produced carbonyl group have not yet been determined. However, it is sure that the carbonyl group in anhydrolycoctonamic acid (VII) exerted some effects upon this conversion, because the analogous conversion was not observed in lycoctonamic acid (IV).

Experimental

Lycotoctonam (III).—To a solution of 1 g. of lycotoctonine in 70 cc. of acetone containing 7 cc. of water, was added 0.6 g. of powdered potassium permanganate in small quantities at room temperature; the resulting solution was left at room temperature overnight. After filtration of manganese dioxide, the filtrate was evaporated to syrup under reduced pressure. When the syrup was further concentrated in a desiccator, crystalline lycotoctonam was separated. A further amount was deposited by the addition of ether. Thus 0.7 g. of crystal was obtained. This substance was recrystallized from ethyl acetate in needles, m. p. 95~98°C, $[\alpha]_D^{25} + 67.5^\circ$ (C, 1.10 in water).

Anal. Found: C, 58.05; H, 8.24; N, 2.74; H₂O, 6.94. Calcd. for C₂₅H₃₉O₈N·2H₂O: C, 58.01; H, 8.37; N, 2.71; H₂O, 6.96%.

In the infrared spectrum in Nujol, this compound showed broad bands at 3367, 3401 and 3460 cm⁻¹ in the region of hydroxyl group. This compound was identical with lycotoctonam, obtained in the same way as reported by Edwards and Marion⁴⁾.

Oxidation of Lycotoctonam with Chromic Acid.—To a solution of 4 g. of lycotoctonam in

6) It was reported in a previous paper⁷⁾ that hydroxy-lycoctonine was obtained from lycotoctonine on oxidation with Ag₂O; Cookson and Trevett⁸⁾ reported that they had obtained also the same compound by means of lead tetraacetate. The present authors found that this compound was produced by treatment not only with the above reagents but also with potassium ferricyanide.

7) H. Sugimoto and K. Ohno, *J. Fac. Sci., Hokkaido Univ., Ser. III. Chem.*, 4, 36 (1950).

8) R. C. Cookson and M. E. Trevett., *Chem. & Ind.*, 1956, 276.

100 cc. of *N* sulfuric acid was added a solution of chromic anhydride in a small volume of water with ice-cooling. When the resulting solution was left at room temperature overnight, an acidic product *a* was separated in needles. After filtration of the acid *a*, the filtrate was extracted with chloroform. The chloroform extract containing the acidic and neutral materials was shaken with diluted aqueous ammonia. On evaporation the chloroform solution gave 1.15 g. of the neutral product, lycoxonine (see below).

When the aqueous ammonia layer was acidified with dilute sulfuric acid, an acidic product *b* was obtained. Thus the combined acidic product, *a* and *b*, weighed 1.45 g. After recrystallization from aqueous ethanol, this acid was confirmed to be identical with lycoctonamic acid (IV)⁴, m. p. 200~203°C, after softening at 95~100°C, $[\alpha]_D^{25} + 94.2^\circ$ (C, 1.66 in ethanol).

Estimation of Formic Acid Formed during Oxidation.—Lycoctonam was oxidized with chromic anhydride in the same way as described above. After being kept overnight, the major amount of the solution was distilled. When the distillate was just neutralized with 0.05 *N* sodium hydroxide and evaporated to dryness, crystals were obtained which decomposed at 250~253°C (authentic sodium formate decomposed at 255°C). The acidic material was confirmed to be formic acid by the chromotropic acid test after it was reduced to formaldehyde.

Lycoxonine (X).—This compound was obtained as described under oxidation of lycoctonam. It crystallized from water as a tetrahydrate in rhombic forms. After recrystallization it melted at 80~83°C and had $[\alpha]_D^{25} + 40.8^\circ$ (C, 1.66 in ethanol). In the infrared spectrum in Nujol, lycoxonine showed the broad band at 3448 cm^{-1} in the region of hydroxyl group.

Anal. Found: C, 53.75; H, 8.48; H_2O , 13.52. Calcd. for $\text{C}_{24}\text{H}_{37}\text{O}_8\text{N} \cdot 4\text{H}_2\text{O}$: C, 53.42; H, 8.41; H_2O , 13.35%.

This compound was subjected to the Micro-Zeisel methoxyl, Herzig-Meyer *N*-alkyl and Ginger *C*-methyl⁹ determinations.

Anal. Found: C_2H_5 , 4.83; OCH_3 , 23.20, CH_3 , 2.45. Calcd. for $\text{C}_{18}\text{H}_{28}\text{O}_8\text{N}(\text{CH}_2 \cdot \text{CH}_3)(\text{OCH}_3)_4$: C_2H_5 , 5.36; 4-OCH_3 , 23.00; CH_3 , 2.78%.

De-(hydroxymethylene)-lycoctonam (V).—Since this compound could not be isolated directly from the oxidation products of lycoctonine, the present authors obtained it by pyrolysis of lycoctonamic acid in the same manner as described in Edwards and Marion's report⁴. The compound obtained was recrystallized from water as a trihydrate, m. p. 110~113°C.

The melting point and the form of the trihydrate were not in agreement with the above report⁴. However, the derivatives of this compound, de-(hydroxymethylene)-lycoctonine perchlorate and anhydro-de-(hydromethylene)-lycoctonam, were well in agreement with those of the same report.

Upon subjection to the same treatment with chromic anhydride as described under oxidation of lycoctonam, this compound V did not react

for more than three days.

Acetylation of Lycoxonine.—(i) Lycoxonine tetrahydrate (0.5 g) was treated with 2 cc. of acetyl chloride in a sealed tube at room temperature overnight. Excessive reagent was removed under reduced pressure, and water was added. On standing, the resulting aqueous mixture gave 0.2 g. of a crystalline product, anhydrolycoxonine monoacetate (XIII). It was recrystallized readily from ethanol in rosette forms, m. p. 225~230°C, $[\alpha]_D^{25} + 37.8^\circ$ (C, 3.30 in ethanol). The ultraviolet absorption spectrum showed the maximum at 305 $\text{m}\mu$ ($\log \epsilon$ 1.85).

Anal. Found: C, 63.38; H, 7.72; N, 2.67. Calcd. for $\text{C}_{24}\text{H}_{34}\text{O}_8\text{N}(\text{OCOCH}_3)$: C, 63.52; H, 7.59; N, 2.85%.

(ii) Anhydrous lycoxonine (0.1 g.), obtained by drying the hydrate under reduced pressure over phosphorous pentoxide, was treated with 1 cc. of acetic anhydride and 0.5 cc. of pyridine in a sealed tube at room temperature, but lycoxonine was recovered unchanged after more than 80 hr.

(iii) A solution of 0.5 g. of anhydrous lycoxonine in 0.7 g. of acetic anhydride was refluxed for 3 hr. and the solution was then concentrated to a syrup in a desiccator under reduced pressure. When a small amount of water was added to the resulting syrup, a crystalline product was obtained. This product could be separated into two parts by treating it with hot water. The first part was insoluble in hot water whilst the second was readily soluble. The insoluble material was identical with anhydrolycoxonine monoacetate which was obtained by acetyl chloride treatment (i). The readily soluble material was lycoxonine monoacetate (XII); it was recrystallized from water in prisms, m. p. 222~225°C.

Anal. Found: C, 61.35; H, 7.78; OCOCH_3 , 10.43. Calcd. for $\text{C}_{24}\text{H}_{36}\text{O}_7 \cdot (\text{OCOCH}_3)$: C, 61.28; H, 7.71; OCOCH_3 , 10.02%.

Lycoxonine monoacetate gave anhydrolycoxonine monoacetate upon further treatment with acetyl chloride or acetic anhydride as described above.

Anhydrolycoxonine (XIV).—Anhydrolycoxonine monoacetate was saponified by 50% potassium hydroxide in aqueous methanol on a steam bath for 30 min. The solution was neutralized with dilute hydrochloric acid and evaporated under reduced pressure to dryness. The resulting residue was extracted with ethanol. When the alcoholic solution was evaporated at room temperature, anhydrolycoxonine was separated in plates. After recrystallization from aqueous ethanol, it melted at 160~162°C and showed the maximum at 305 $\text{m}\mu$ ($\log \epsilon$ 1.85) in ultraviolet spectrum.

Anal. Found: C, 64.38; H, 7.90; N, 3.21. Calcd. for $\text{C}_{24}\text{H}_{35}\text{O}_7\text{N}$: C, 64.12; H, 7.85; N, 3.12%.

Anhydrolycoctonamic Acid (VII).—Lycoctonamic acid (1 g.) was treated with 4 cc. of acetyl chloride in a sealed tube overnight. After evaporation of excess of the reagent, water was

9) L. G. Ginger, *J. Biol. Chem.*, **156**, 453 (1944).

added to the resulting residue. Then, 0.5 g. of an amorphous material was obtained, which crystallized readily from ethanol in prisms, m. p. $177\sim 178^{\circ}\text{C}$, $[\alpha]_D^{25} + 52.8^{\circ}$ (C, 1.00 in ethanol), λ_{max} 305 $\text{m}\mu$ ($\log \epsilon$ 1.87). This product was identical with anhydrolycoctonamic acid reported by Edwards and Marion⁴.

Anal. Found: C, 62.47; H, 7.38. Calcd. for $\text{C}_{25}\text{H}_{35}\text{O}_8\text{N}$: C, 62.87; H, 7.39%.

Apo-anhydrolycoctonamic Acid (XI).—To a solution of 0.5 g. of anhydrolycoctonamic acid in 100 cc. of water containing 0.5 g. of potassium carbonate, 0.3 g. of powdered potassium permanganate was added in portions at room temperature. The potassium permanganate was consumed very slowly in three days.

The manganese dioxide was filtered off and washed with 0.1% potassium carbonate solution. When the combined filtrate and washings were acidified with diluted sulfuric acid, apo-anhydrolycoctonamic acid was obtained. Yield, 0.19 g. It was recrystallized from ethanol in thin plates, m. p. $183\sim 184^{\circ}\text{C}$, $[\alpha]_D^{25} + 37^{\circ}$ (C, 1.00 in ethanol). This acid was considerably less soluble in ethanol than anhydrolycoctonamic acid. The ultraviolet spectrum of this acid showed the maximum at 305 $\text{m}\mu$ ($\log \epsilon$ 2.07).

Anal. Found: C, 62.14; H, 6.69; OCH_3 , 20.20. Calcd. for $\text{C}_{24}\text{H}_{31}\text{O}_8\text{N}$: C, 62.45; H, 6.77; 3-OCH_3 , 20.17%.

On oxidation in the same way as described above, lycoctonamic acid was not affected.

The apo-acid decolorized one mole of bromine within 30 min. in acetic acid, while anhydrolycoctonamic acid did not do so within 24 hr.

Decarboxy Compound of Apo-anhydrolycoctonamic Acid.—(i) On treatment of apo-anhydrolycoctonamic acid in the same way as reported by Edwards and Marion⁴, no crystalline product was isolated.

(ii) A solution of 40 mg. of the apo-acid in 0.3 cc. of acetic anhydride was refluxed for 3 hr. The solution was poured into 10 cc. of water, and the resulting aqueous solution was extracted with chloroform. After being washed with diluted potassium hydroxide and then with water, the chloroform extract was evaporated to dryness. When ether was added to the residue, crystals were deposited from the ether solution in prisms. The substance was recrystallized from water as a polyhydrate, which melted at $110\sim 113^{\circ}\text{C}$ after softening at 100°C .

Formation of Oxime and 2,4-Dinitrophenylhydrazone from the Decarboxy Compound.—

(i) To a solution of 40 mg. of the decarboxy compound in 1 cc. of ethanol was added a solution of 40 mg. of hydroxyl amine hydrochloride in 0.4 cc. of water containing 40 mg. of potassium carbonate; the resulting solution was warmed on a steam bath for 4 hr. When the alcoholic solution was concentrated under reduced pressure, the oxime of the decarboxy compound was separated in needles, m. p. $140\sim 142^{\circ}\text{C}$. In the ultraviolet spectrum, this oxime showed the maximum at 305 $\text{m}\mu$ ($\log \epsilon$ 1.85).

Anal. Found: N, 6.54. Calcd. for $\text{C}_{23}\text{H}_{32}\text{O}_6\text{N}$:

N, 6.48%.

(ii) To a solution of 20 mg. of the decarboxy compound in 2 cc. of ethanol was added four drops of the solution of 0.5 g. of 2,4-dinitrophenylhydrazine and 1 cc. of 90% sulfuric acid in 7 cc. of ethanol. When the resulting solution was allowed to stand at room temperature for several hours, it deposited 2,4-dinitrophenylhydrazone of the decarboxy compound in yellow needles, m. p. $221\sim 222^{\circ}\text{C}$.

Anal. Found: N, 11.54. Calcd. for $\text{C}_{29}\text{H}_{35}\text{O}_9\text{N}_5$: N, 11.72%.

On treatment of anhydro-de-(hydroxymethylene)-lycoctonam with either of the above reagents, the starting compound was recovered without change.

Hydroxylycoctonine (VIII).—A solution of lycoctonine in 20 cc. of methanol was mixed with a solution of 1.2 g. of potassium hydroxide in 20 cc. of methanol. Then, a solution of 5.4 g. of potassium ferricyanide in 25 cc. of water was added to the above alcoholic solution and cooled in water. Then the resulting solution was left at room temperature overnight. After evaporation of the methanol under reduced pressure, the solution was extracted with chloroform. The chloroform extract was evaporated to dryness after being washed with water and then dried with anhydrous sodium sulfate. When ether was added to the resulting residue, 2 g. of the product was obtained. It crystallized readily from ethyl acetate in little buttons. After recrystallization from the same solvent, the compound melted at $152\sim 154^{\circ}\text{C}$ and did not depress the melting point of an authentic hydroxylycoctonine, prepared by silver oxide oxidation⁷.

Hydroxylycoctonam (IX).—This compound was obtained by treatment analogous to that reported by Edwards and Marion¹⁰. To a solution of 5.32 g. of hydroxylycoctonine in 400 cc. of water was added 3.45 g. of powdered potassium permanganate in small portions at room temperature. After the addition of the entire amount of the oxidant, the reaction mixture was left at room temperature overnight, and the resulting manganese dioxide was filtered off. The filtrate was extracted with chloroform, and the resulting extract was washed with diluted hydrochloric acid and then with water. When the washed chloroform solution was evaporated, a resinous material remained. This material was crystallized by the addition of acetone-ether. It was recrystallized from acetone as a monohydrate, m. p. $200\sim 203^{\circ}\text{C}$.

Anal. Found: C, 58.31; H, 7.98. Calcd. for $\text{C}_{25}\text{H}_{39}\text{O}_9\text{N}\cdot\text{H}_2\text{O}$: C, 58.24; H, 8.01%.

Oxidation of Hydroxylycoctonam (IX) with Chromic Anhydride.—In 10 cc. of 5% sulfuric acid solution, 0.51 g. of hydroxylycoctonam was treated with 0.4 g. of chromic anhydride in just the same way as described under oxidation of lycoctonam. The oxidation of this compound was slower than that of lycoctonam, and required more than a week. After reducing the oxidant

10) O. E. Edwards and Léo Marion; *Can. J. Chem.* **32**, 1146 (1954).

the solution was extracted with chloroform, and the extract was shaken with dilute aqueous ammonia. When the chloroform layer was evaporated, there remained resinous products a. Yield, 0.20 g. (see below).

The aqueous layer was acidified with dilute hydrochloric acid and then extracted with chloroform. On evaporation of the solvent, the chloroform extract gave acidic products which were crystallized by the addition of ether. These products were separated into two acids, one readily soluble in acetone and the other less soluble. The readily soluble acid was recrystallized from acetone-ethanol in prisms melting at 220~222°C (decomp.) after softening at 200°C. This acid was named C₁-acid. The less soluble acid was crystallized from hot water in plates, melting at 193~195°C. This acid was named

Y-acid.

The neutral product a was separated into two parts, one insoluble in benzene and the other soluble. The insoluble part crystallized from acetone in small buttons, which weighed 80 mg. each and melted at 177~180°C after recrystallization from acetone. This neutral product was named Y. The benzene-soluble part, weighing 100 mg., was subjected to chromatography on alumina. Elution with 5% methanol in benzene gave a product which crystallized from acetone in rhombic forms, m. p. 226~227°C. This product was named Z.

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