[CONTRIBUTION FROM THE CHEMISTRY LABORATORY, UNIVERSITY OF NEW BRUNSWICK]

Annotinine. I. The Relationship of Functional Groups[1]

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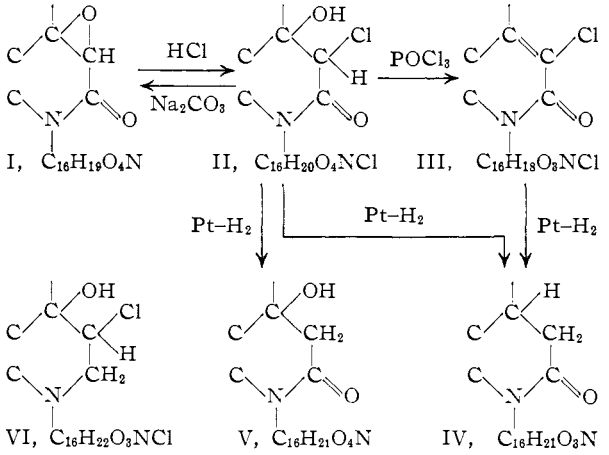
On the basis of various transformations the partial structure VII is proposed for annotinine lactam.

Orekhov,[2] in 1934, drew attention to the fact that the club moss *Lycopodium annotinum* was a source of alkaloids. Manske and Marion in a series of outstanding analytical studies[3] have isolated and characterized more than thirty alkaloids from various species of club mosses. Among these annotinine,[4] the major alkaloid of *Lycopodium annotinium*, was characterized as a base of the formula $C_{16}H_{21}O_3N$. It was shown to possess a tertiary basic nitrogen and a γ-lactone; the remaining oxygen was regarded as in an ether grouping, possibly epoxide. Treatment of annotinine with alkali followed by acid led to annotinine hydrate ($C_{16}H_{23}O_4N$) in which the ether group was thought to be replaced by two hydroxy groups. Similarly, hydrochloric acid converted annotinine into the corresponding chlorohydrin $C_{16}H_{22}O_3NCl$.

FORMULA SCHEME I



The nature of the immediate vicinity of the nitrogen was clarified in an interesting study by MacLean and Prime.[5] Annotinine may be oxidized by permanganate to a compound $C_{16}H_{19}O_4N$.[6] MacLean and Prime have shown this compound to be a lactam and to possess the partial formula I. Hydrochloric acid converted I into a chlorohydrin formulated as II, which on dehydration gave an anhydro compound III.

Hydrogenation of II gave a mixture of IV and V, whereas hydrogenation of III gave IV. The easy hydrogenolysis of II as well as its reconversion into I as compared with the failure of these reactions with annotinine chlorohydrin VI were given as an argument for the assignment of the structures portrayed in formula scheme I.

In the present communication we wish to corroborate MacLean's conclusions and to present evidence allowing the expansion of the partial structure I to VII. A preliminary account of some of the first experiments leading to this result has already appeared.[7] According to the partial structure VII, MacLean's anhydro compound $C_{16}H_{18}O_3NCl$ may be formulated as VIII. The ultraviolet spectrum of VIII (λ_{max} 227 mμ, log ϵ 3.9; λ_{max} 275 mμ, log ϵ 2.9) is in agreement with the postulated conjugated lactam group. The infrared spectrum shows a characteristic double peak in the carbonyl region (1662, 1616 cm.$^{-1}$) which we have found is always associated with the conjugated lactam group. Since the existence of this grouping in VIII will be proved by independent chemical means (*vide infra*), we shall refer to these spectroscopic characteristics as evidence for the presence of the conjugated lactam in all the subsequent derivatives. MacLean's arguments for the placement of the chlorine[8] in a position α to the lactam carbonyl are considered sound. On the basis of this assignment, an SN1 mechanism of the oxide ring opening would have to proceed *via* an unfavorable carbonium ion. An SN2 mechanism, on the other hand, explains the formation of the chlorohydrin satisfactorily. We have subjected VIII to a permanganate oxidation and isolated the amino acid IX as the sulfate in a 60% yield. The crystalline methyl ester X was obtained by treatment of IX with diazomethane. The pK_a values of the amino acid (4.03, 9.3) and of the methyl ester (6.8) are in agreement with the expected values for a β-amino acid derivative. It could also be shown that the carbomethoxy group of X is resistant to saponification; this allows the assumption that this group is tertiary. It is probable that the conjugated lactam group of VIII is in a six-membered or (less likely) in a seven-membered ring. This follows from the infrared frequencies of various derivatives possessing the lactam group, as well as from the pK_a values of the amino acid derivatives. In this respect it is significant that various attempts to dehydrogenate VIII into an α-pyridone were unsuccessful.

The relationship of the γ-lactone group to the conjugated lactam group in compound VIII was discovered by the following series of reactions. Mild reflux of compound VIII with alcoholic alkali gave the hydroxy acid XI. This compound may be

(1) Presented at the Seventh Summer Seminar on the Chemistry of Natural Products, August 17–21, 1955, University of New Brunswick, Fredericton, N. B.

(2) Orekhov, *Arch. Pharm.*, **272**, 673 (1934).

(3) For references see T. A. Henry, "The Plant Alkaloids," 4th Ed., The Blakiston Company, Philadelphia, Penna., 1949, p. 752.

(4) R. H. F. Manske and L. Marion, *Can. J. Res.*, **21B**, 92 (1943).

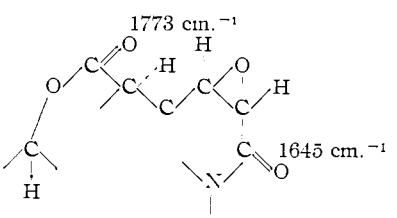
(5) D. B. MacLean and H. C. Prime, *Can. J. Chem.*, **31**, 543 (1953).

(6) R. H. F. Manske and L. Marion, THIS JOURNAL, **69**, 2126 (1947).

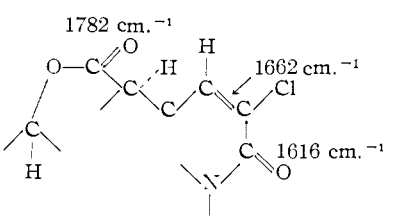
(7) D. R. Henderson, F. W. Stonner, Z. Valenta and K. Wiesner, "Chemistry and Industry," 1954, p. 544, 852. See also "Summaries of Dissertations," University of New Brunswick, F. W. Stonner, April, 1955, J. S. Little, September, 1955.

(8) For full discussion of these arguments the original article, Ref. 5, has to be consulted.

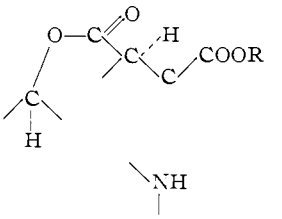
FORMULA SCHEME II



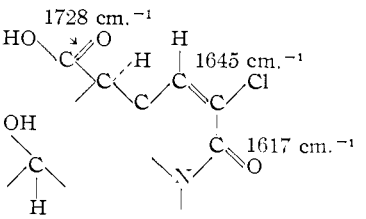
VII, $C_{16}H_{19}O_4N$



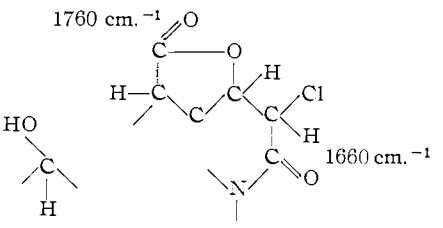
VIII, $C_{16}H_{18}O_3NCl$



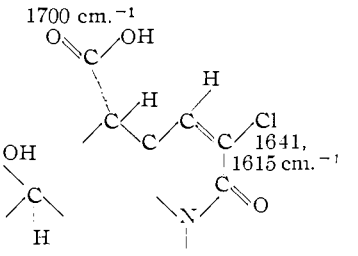
IX, R = H; $C_{14}H_{19}O_4N$
X, R = CH_3; $C_{15}H_{21}O_4N$



XI, $C_{16}H_{20}O_4NCl$



XII, $C_{16}H_{20}O_4NCl$



XIII, $C_{16}H_{20}O_4NCl$

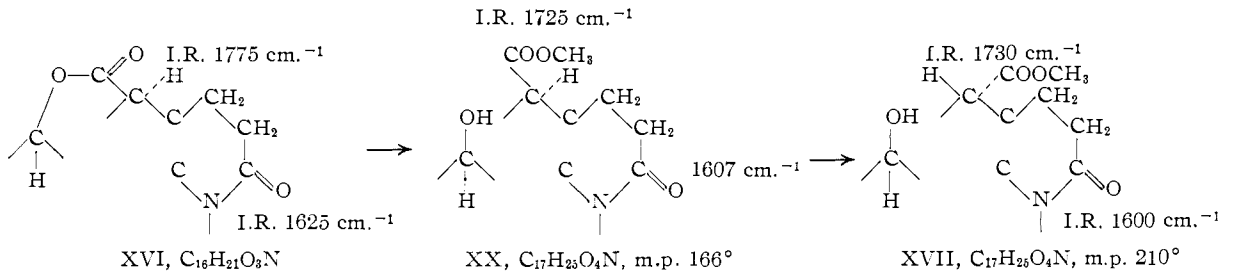
shown by its infrared characteristics still to possess the conjugated lactam grouping. Also, the ultraviolet spectrum of XI is almost identical with the spectrum of VIII. Refluxing of the hydroxy acid XI with a trace of *p*-toluenesulfonic acid in dry benzene gave the hydroxy lactone XII. The ultraviolet and infrared spectra of this compound show clearly the disappearance of the conjugated amide grouping. The nature of this hydroxy γ-lactone may be easily understood if we assume that the carboxy group has lactonized by addition across the conjugated double bond. Treatment of the hydroxy lactone XII with alkali causes a base-catalyzed β-elimination with the formation of the hydroxy acid XIII. This compound has the carboxy group in the opposite configuration with respect to the acid XI. It has an ultraviolet spectrum identical with that of compound XI; infrared spectra of the two hydroxy acids are very similar but clearly show non-identity. Relactonization of XIII with benzene and *p*-toluenesulfonic acid gives back the hydroxy-lactone XII. The correctness of the relationship of XI and XIII follows from subsequent correlations. It should be mentioned that the hydroxy acid XI is very labile and in the majority of experiments the hydroxy acid XIII is isolated directly from the saponification of the lactone VIII.

A confirmation of the existence of two different lactone rings in compounds VII and XII may be obtained by blocking the hydroxy group in the hydroxy acid and then effecting the relactonization. If the hydroxy acid XIII is acetylated for 48 hours with acetic anhydride and pyridine, acetoxy γ-lactone XIV, $C_{18}H_{22}O_5NCl$, is obtained. This compound has only end absorption in the ultraviolet and a single lactam peak at 1670 cm.$^{-1}$; furthermore, it possesses a γ-lactone peak at 1780 cm.$^{-1}$ and an acetoxy peak at 1740 cm.$^{-1}$. These properties can be best rationalized by assuming that compound XIV is simply the O-acetyl derivative of the hydroxy lactone XII. If now the hydroxy acid XIII is acetylated for three hours at 100°, the corresponding acetoxy acid XV, $C_{18}H_{22}O_5NCl$, may be isolated. The ultraviolet spectrum of this compound shows the presence of the conjugated lactam grouping, and the carbonyl region in its infrared spectrum contains four carbonyl bands (carboxyl 1710 cm.$^{-1}$, acetoxy 1740 cm.$^{-1}$, conjugated lactam 1625 cm.$^{-1}$, 1653 cm.$^{-1}$). Heating of compound XV with a trace of *p*-toluenesulfonic acid in benzene gave a quantitative yield of the acetoxy lactone XIV. While in view of the remote possibility of acetyl migration this does not constitute rigorous evidence for the structure of the relactonization products, it is still strong support for the original hypothesis.

The hydrogenation of compound VIII as already described by MacLean gives compound $C_{16}H_{21}O_3N$, which according to the present views must be represented by the partial structure XVI. This compound proved especially useful for the elucidation of some of the properties of the lactone ring. Mild reflux of compound XVI with potassium hydroxide in methanol gave a hydroxyl-methyl ester XVII (m.p. 210°). Saponification of this compound with an excess of ethanolic potassium hydroxide gave the corresponding hydroxy acid XVIII, which may be reconverted into the ester XVII by the action of ethereal diazomethane.

Direct saponification of the lactone XVI with ethanolic potassium hydroxide gives a hydroxy acid XIX. The analytical values of this acid were not reproducible, probably because of hydrate formation (low carbon values); it was therefore characterized by conversion with ethereal diazomethane into the well defined methyl ester XX. Treatment of ester XX with methanolic potassium hydroxide gives a quantitative yield of the ester XVII. The rationalization of these transformations is very simple. In methanolic potassium hydroxide there is a considerably higher concentration of methoxide than hydroxide ions. The methoxide ions cause the opening of the lactone ring of XVI to give the ester XX which is immediately isomerized under these conditions to XVII. This isomerization, as already mentioned, has been demonstrated in a

FORMULA SCHEME III



XVI, $C_{16}H_{21}O_3N$ XX, $C_{17}H_{23}O_4N$, m.p. 166° XVII, $C_{17}H_{25}O_4N$, m.p. 210°

separate experiment. While the formation of an ester under these conditions is unusual, it has been rigidly demonstrated in the above sequence. In ethanolic potassium hydroxide, the lactone group is attacked by a hydroxide rather than ethoxide ion. This gives the corresponding hydroxy acid which is present in the reaction mixture as a carboxylate ion and is stable to epimerization. This compound consequently gives with diazomethane the unstable ester XX. The present sequence establishes not only the secondary character of the lactone carboxyl but also the fact that *the original configuration of the carboxyl group in annotinine is less stable than the epimeric one.* The two hydroxy esters XVII and XX have been converted by acetylation into the corresponding acetoxy derivatives XXI and XXII. The difference in molecular rotations of XVII and XXI is practically identical in both magnitude and sign with rotation difference of XX and XXII (see Experimental part). This shows that the only difference between the two esters XX and XVII is the configuration of the carbomethoxy group.

Oxidation of the hydroxy esters XVII and XX with chromium trioxide in pyridine gives the corresponding ketoesters XXIII and XXIV. This corroborates the secondary nature of the hydroxy group contained in the original lactone of annotinine.

In order to obtain rigorous evidence for the partial formulas proposed in this communication it was thought desirable to obtain a direct correlation between the unsaturated hydroxy acid XIII and the two saturated hydroxy acids XVIII and XIX. The hydroxy acid XIII could indeed be converted into the saturated acid XVIII by hydrogenation; this identification is substantiated further by conversion into the methyl ester XVII by the action of diazomethane.

A further transformation of the unsaturated acid XIII which is relevant to the relationship of the carboxyl to the conjugated amide group was performed as follows: acid XIII was oxidized by the action of chromium trioxide in pyridine to give an oily product from which a ketolactone XXVI could be obtained by direct crystallization. Compound XXVI shows only end absorption in the ultraviolet and a single amide peak at 1665 cm.$^{-1}$ in the infrared, together with a ketonic carbonyl band at 1725 cm.$^{-1}$ and a γ-lactone band at 1790 cm.$^{-1}$. It is interesting that the accumulation of carbonyl groups in the molecule clearly has the tendency to shift all carbonyl peaks to higher wave numbers. Viewed from this perspective the ketonic carbonyl at 1725 cm.$^{-1}$ seems to indicate a keto group in a six-membered ring.

The ketolactone XXVI in alkaline solution has an ultraviolet spectrum practically identical with that of hydroxy acid XIII. This shows that in alkaline solution the α,β-unsaturated amide group is regenerated. Several hours boiling in alkaline solution does not change this spectrum and the corresponding uncharacterized ketoacid XXVII may be reconverted into the ketolactone XXVI by *p*-toluenesulfonic acid in benzene. This shows that the keto group is not in the α- or β-position with

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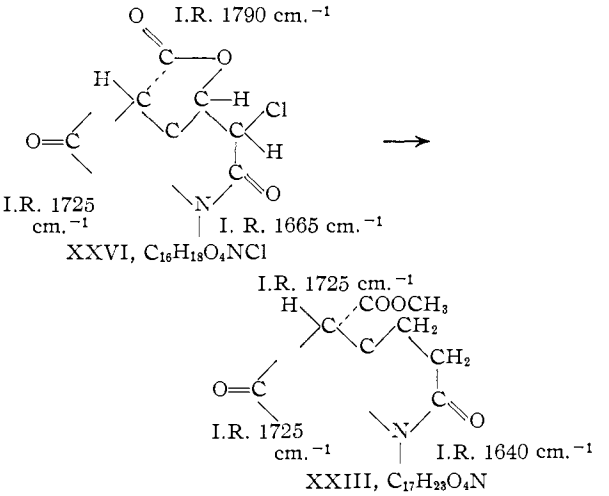


XXVI, $C_{16}H_{18}O_4NCl$ XXIII, $C_{17}H_{23}O_4N$

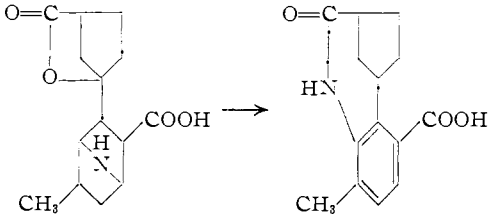
respect to the lactam carbonyl and can hardly be placed elsewhere than at the site of the original lactone hydroxyl.

Rigorous evidence that the keto group of the ketolactone XXVI actually is at the site of the original lactone hydroxyl was obtained by a direct conversion of XXVI into the ketoester XXIII. This was achieved by dissolution in alcoholic alkali which causes a β-elimination of the lactone followed by hydrogenation of this solution with a palladium–charcoal catalyst and esterification of the acidic product with diazomethane. This whole sequence was performed in a 50% over-all yield and *from the alternate mode of formation of XXIII (vide supra) there remains no doubt that it possesses the keto group at the site of the original lactone hydroxyl.*

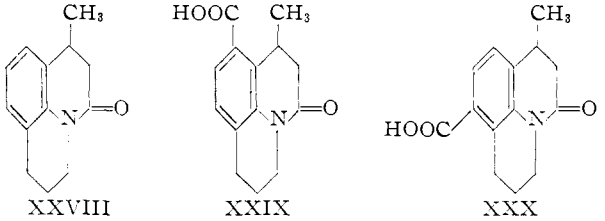
Anet and Marion[9] have recently subjected our amino acid IX to a mild dehydrogenation with a palladium catalyst and have isolated a lactam acid

(9) F. A. L. Anet and Léo Marion, *Can. J. Chem.*, **33**, 849 (1955).

$C_{14}H_{15}O_3N$ which was decarboxylated to a lactam $C_{13}H_{15}ON$. This reaction was rationalized by the scheme

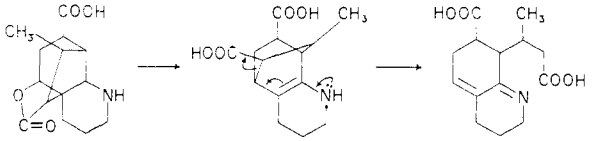


However, it has been shown in this Laboratory[10] that the lactam $C_{13}H_{15}ON$ actually has the structure XXVIIII and the lactam acid XXIX or XXX. Compound XXX has now been synthesized and

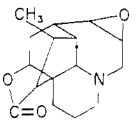


XXVIII XXIX XXX

proved non-identical with the lactam acid which leaves the structure XXIX to be assigned to the latter.[11] It is difficult to rationalize in a simple unexceptional manner the formation of XXIX from IX with all the limitations imposed by the present work. An exceedingly simple possibility emerges if one retains all the findings about the γ-lactone ring except its relative position to the oxide ring. In such a case, annotinine could be formulated as XXXIV and the amino acid as XXXI, which would then give XXIX in the manner indicated by structures XXXI, XXXII and XXXIII.



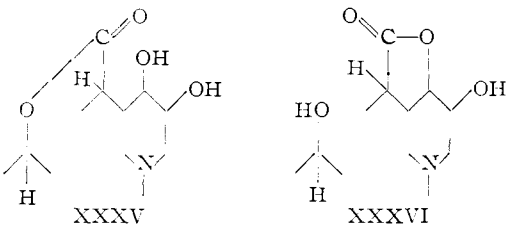
XXXI XXXII XXXIII



XXXIV

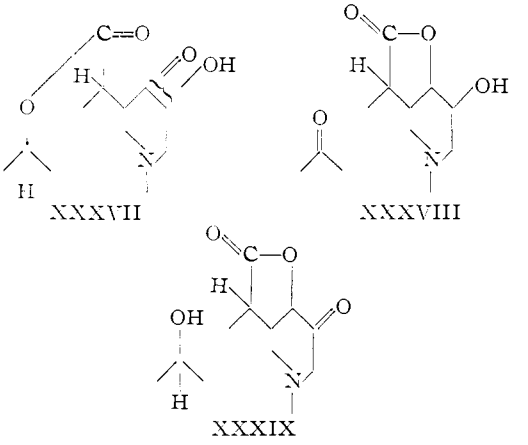
It was the existence of this simple possibility which led us to doubt our original concept of the existence of two alternate lactones and subject it to the tests reported in this communication. However, the large amount of independent evidence all pointing to the formation of an alternate γ-lactone and the rigorous nature of some of it, seems to exclude this simple possibility and indicate a more deep-seated rearrangement. We intend to discuss the possible courses of such a rearrangement in a later paper together with a synthetic structure proof of the lactam carboxylic acid XXIX.

The point which remains to be discussed is the structure of annotinine hydrate (*vide supra*). On the basis of the present results, two structures,



XXXV XXXVI

XXXV and XXXVI, must be considered. Several objections may be raised against the structure XXXV. The compound does not take up periodic acid, although this might be due to a *trans* diaxial configuration of the hydroxy groups. A more serious point is the oxidation product of annotinine hydrate described by Meier, Meister and Marion.[12] This product is a hydroxy keto lactone which must have the structure XXXVII, XXXVIII or XXXIX.



XXXVII XXXVIII XXXIX

We have repeated the preparation of this compound and found that it lacks completely the properties of an α-ketol. Thus formula XXXVI seems to be a better representation of annotinine hydrate.

If the formula XXXVIII for the oxidation product of annotinine hydrate is indeed correct, the reported ketonic maximum at 1712 cm.$^{-1}$ is in agreement with a keto group in a six-membered ring.

Acknowledgment.—We wish to thank the National Research Council, Ottawa, for the support of these investigations and the award of a Studentship to F. W. S. We also wish to thank Dr. F. J. Toole, Dean of the School of Graduate Studies, for his constant support and help. K. W. thanks the John Simon Guggenheim Foundation, New York, for the award of a fellowship, and Professor R. B. Woodward for the kind hospitality in his laboratory and many discussions of annotinine chemistry.

Experimental

Melting points were determined on a Kofler hot-stage and are uncorrected. All solvents were distilled. Ultraviolet spectra were taken in ethanol solution with a Beckman quartz spectrophotometer, model DU. Infrared spectra were determined by Dr. R. L. Bohon of the Anderson Physical Laboratory, Champaign, Ill. Microanalyses were carried out by Dr. S. M. Nagy of the microanalytical laboratory, M. I. T., and Dr. Robert Dietrich of the microanalytical laboratory, Zurich, Switzerland. Experiments marked by an asterisk have been performed by Dr. J. S. Little.

(10) Unpublished experiments, C. Bankiewicz; D. R. Henderson, Ph.D. thesis summary, April, 1955, Univ. New Brunswick.

(11) Unpublished experiments, C. Bankiewicz.

(12) H. L. Meier, P. D. Meister and Léo Marion, *Can. J. Chem.*, **32**, 268 (1954).

Amino Acid IX.—Potassium permanganate (1.4 g.) in acetone (140 ml.) was added over a period of 3.5 hours to a solution of compound VIII (1 g.) in acetone (60 ml.) under stirring and external cooling to 5°. The stirring was continued for an additional 18 hours. The precipitated manganese dioxide was then filtered off, suspended in water (50 ml.) and reduced with sulfur dioxide gas. The clear solution was continuously extracted with ether for 48 hours. Removal of the solvent yielded the amino acid sulfate (735 mg.) which after crystallization from methanol–ether melted at 232–233°.

Anal. Calcd. for $C_{14}H_{19}O_4N\cdot{}^1/_2H_2SO_4$: C, 53.48; H, 6.41. Found: C, 53.52; H, 6.50.

Amino Acid Methyl Ester X.—The sulfate of IX (100 mg.) was dissolved in methanol and treated with a few drops of concentrated hydrochloric acid. Careful addition of ether precipitated the amino acid hydrochloride, which after crystallization from methanol–ether melted at 219–220°. The hydrochloride of IX (60 mg.) was dissolved in dry methanol and treated with ethereal diazomethane in excess. The solvent was removed after 15 minutes and crystallization of the residue from ether–petroleum ether gave the analytical sample, m.p. 122°.

Anal. Calcd. for $C_{15}H_{21}O_4N$: C, 64.50; H, 7.58; N, 5.02; 1 OCH_3, 10.28; 1 act. H, 0.33. Found: C, 64.58; H, 7.87; N, 5.32; OCH_3, 8.75; act. H, 0.46.

Hydroxy Acid XI.—Compound VIII (420 mg.) was heated under reflux for 3.5 hours with potassium hydroxide in methanol (10%, 20 ml.). The solvent was evaporated to a small volume *in vacuo;* the residue was dissolved in water (50 ml.) and the aqueous solution was acidified with sulfuric acid and extracted with ether. The ether extract was washed with water and dried over sodium sulfate. Removal of the solvent yielded a crude acid (424 mg.) which after crystallization from ether melted at 183–184°; infrared spectrum (Nujol): bands at 3280 (OH), 1728 (carboxyl) and 1645, 1617 cm.$^{-1}$ (conjugated lactam); ultraviolet spectrum: λ_{max} 230 mμ (log ε 4.02), λ_{max} 280 mμ (log ε 3.1).

Anal. Calcd. for $C_{16}H_{20}O_4NCl$: C, 58.98; H, 6.19; N, 4.30. Found: C, 59.04; H, 6.40; N, 4.54.

Hydroxy Lactone XII.—A solution of acid XI (430 mg.) and *p*-toluenesulfonic acid (85 mg.) in dry benzene (60 ml.) was heated under reflux for 6 hours in a flask with a water separator attachment. The solvent was then evaporated *in vacuo*, the residue dissolved in chloroform and the chloroform solution was washed with a 5% sodium carbonate solution and dried. The removal of the solvent gave the analytical sample, m.p. 219–220°; infrared spectrum (Nujol): bands at 3400 (OH), 1760 (γ-lactone) and 1660 cm.$^{-1}$ (lactam).

Anal. Calcd. for $C_{16}H_{20}O_4NCl$: C, 58.98; H, 6.19; N, 4.30. Found: C, 58.47; H, 6.06; N, 4.04.

Hydroxy Acid XIII.—Lactone XII was treated under conditions identical with those described for the hydrolysis of compound VIII (*vide supra*). Crystallization from methanol–ether gave the analytical sample, m.p. 186–187°; infrared spectrum (Nujol): bands at 3400 (OH), 1700 (carboxyl), 1641 and 1615 cm.$^{-1}$ (conjugated lactam); ultraviolet spectrum: identical with spectrum of acid XI.

Anal. Calcd. for $C_{16}H_{20}O_4NCl$: C, 58.98; H, 6.19; N, 4.30. Found: C, 59.01; H, 6.27; N, 4.40.

Acetoxy Lactone XIV.*—A solution of hydroxy acid XIII (200 mg.) in dry pyridine (2 ml.) and acetic anhydride (3 ml.) was allowed to stand at room temperature for 48 hours. The solution was then evaporated to dryness *in vacuo*, the residue was treated with ice and 10% hydrochloric acid and the acidic solution was extracted with chloroform. The removal of the solvent gave an oil (243 mg.) which crystallized from methanol (50 mg.). Repeated crystallization from methanol gave the analytical sample, m.p. 227–229°; infrared spectrum (chloroform solution): bands at 1780 (γ-lactone), 1740 (acetoxyl) and 1670 cm.$^{-1}$ (lactam).

Anal. Calcd. for $C_{18}H_{22}O_5NCl$: C, 58.77; H, 6.03; N, 3.81; 1 $COCH_3$, 11.43. Found: C, 58.74; H, 6.28; N, 3.84; $COCH_3$, 9.11.

Acetoxy Acid XV.*—A solution of hydroxy acid XIII (494 mg.) in dry pyridine (3 ml.) and acetic anhydride (5 ml.) was heated with exclusion of moisture at 95–100° for 3.5 hours. Isolation with chloroform in the usual way, followed by crystallization from methanol gave the crystalline acid (229 mg.) which was recrystallized from ethyl acetate to the analytical sample, m.p. 203–205°; infrared spectrum ($CHCl_3$ solution): bands at 1740 (acetoxyl), 1710 (carboxyl), 1653 and 1625 cm.$^{-1}$ (conjugated lactam); ultraviolet spectrum: λ_{max} 230 mμ (log ε 4.0), 280 mμ (log ε 3.04).

Anal. Calcd. for $C_{18}H_{22}O_5NCl$: C, 58.77; H, 6.03; N, 3.81. Found: C, 59.01; H, 6.18; N, 3.86.

Reflux of compound XV (65 mg.) in dry benzene (15 ml.) with *p*-toluenesulfonic acid (10 mg.) (for experimental details see the preparation of hydroxy lactone XII) gave a quantitative yield of lactone XIV, characterized after crystallization from methanol by m.p., mixed m.p. and infrared spectrum.

Hydroxy Ester XVII.—Compound XVI (2 g.) was refluxed for 3 hours with an excess of potassium hydroxide in methanol. The solvent was then removed *in vacuo*, and the residue was suspended in water, acidified and extracted with chloroform. Removal of the solvent gave the crude ester (2.15 g.) which on crystallization from ethyl acetate gave the analytical sample, double m.p. 204°, 210°; infrared spectrum (Nujol): bands at 3220 (OH), 1730 (carbomethoxyl) and 1600 cm.$^{-1}$ (lactam); optical rotation, see Table I.

Anal. Calcd. for $C_{17}H_{25}O_4N$: C, 66.40; H, 8.20; N, 4.56; 1 OCH_3 10.10. Found: C, 66.71; H, 8.33; N, 4.60; OCH_3, 9.33.

Acetoxy Ester XXI.—Ester XVII (100 mg.) was treated with acetic anhydride (3 ml.) in dry pyridine (3 ml.) at room temperature for 24 hours. Isolation with chloroform in the usual way gave the crude product (103 mg.) which was crystallized from ether to analytical purity (m.p. 141–142°); infrared spectrum ($CHCl_3$ solution): bands at 1750 (acetoxy), 1730 (carbomethoxyl) and 1620 cm.$^{-1}$ (lactam); optical rotation, see Table I.

Anal. Calcd. for $C_{19}H_{27}O_5N$: C, 65.31; H, 7.79; N, 4.01. Found: C, 65.58; H, 7.91; N, 4.03.

Hydroxy Ester XX.—Acid XIX was dissolved in dry methanol and treated with an excess of ethereal diazomethane for 10 minutes. The solvent was then evaporated *in vacuo* and the residue crystallized from acetone–ether (m.p. 165–166°); infrared spectrum (Nujol): 3430 (OH), 1725 (carbomethoxyl) and 1607 cm.$^{-1}$ (lactam); optical rotation, see Table I.

Anal. Calcd. for $C_{17}H_{25}O_4N$: C, 66.40; H, 8.20; N, 4.56; 1 OCH_3 10.10. Found: C, 66.52; H, 8.37; N, 4.58; OCH_3 9.92.

Ester XX (120 mg.) was refluxed for 3 hours with potassium hydroxide (120 mg.) in methanol (50 ml.). Extraction with chloroform in the usual way and crystallization of the extract from ethyl acetate gave ester XVII (113 mg.), double m.p. 205°, 210° (no depression on admixture with authentic specimen).

Acetoxy Ester XXII.—Ester XX (100 mg.) was acetylated under conditions identical with those used for the preparation of compound XXI. The product (75 mg.) was crystallized from ether–petroleum ether and melted at 137–138°; infrared spectrum ($CHCl_3$ solution): broad band at 1750–1730 (acetoxyl and carbomethoxyl) and band at 1628 cm.$^{-1}$ (lactam).

Anal. Calcd. for $C_{19}H_{27}O_5N$: C, 65.31; H, 7.79; N, 4.01. Found: C, 65.34; H, 7.84; N, 4.11.

TABLE I

ROTATION DIFFERENCES

Compound	$[\alpha]^{24}D$	Δ^a	Solvent
XVII	−14.1°	3.0	Ethanol
XXI	−17.1°		Ethanol
XX	−45.6°	3.5	Ethanol
XXII	−49.1°		Ethanol

[a] Molecular weights of the esters XVII and XX on the one hand and of the corresponding acetates XXI and XX on the other are the same.

Hydroxy Acid XVIII.—Ester XVII (1 g.) was refluxed for 24 hours with potassium hydroxide (1 g.) in ethanol. The solvent was then evaporated *in vacuo* and the residue dissolved in water (50 ml.); the aqueous solution was then washed with chloroform, acidified with hydrochloric acid and extracted exhaustively with chloroform. Crystallization of the chloroform extract (840 mg.) from acetone gave

the analytical sample, m.p. 263–264°; infrared spectrum (Nujol): bands at 3450 (OH), 1690 (carboxyl) and 1565 cm.$^{-1}$ (lactam).

Anal. Calcd. for $C_{16}H_{23}O_4N$: C, 65.52; H, 7.90; N, 4.78. Found: C, 65.57; H, 8.20; N, 4.73.

Acid XVIII was dissolved in dry methanol and treated with an excess of ethereal diazomethane for 10 minutes. The solvent was then evaporated *in vacuo* and the residue crystallized from ethyl acetate. The product was identified as ester XVII by m.p., mixed m.p. and infrared spectrum.

Hydroxy Acid XIX.—Lactone XVI (1 g.) was refluxed for 22 hours with potassium hydroxide (1 g.) in ethanol (120 ml.). The solvent was then removed *in vacuo*, the residue dissolved in water (30 ml.); the aqueous solution was washed with chloroform, acidified and extracted with chloroform. Crystallization of the chloroform extract (850 mg.) from acetone gave the analytical sample, m.p. 264°. Analysis of this acid was not reproducible, possibly as a result of hydrate formation.

It is interesting to note that the treatment of this acid with *p*-toluenesulfonic acid in benzene under relactonizing conditions led to the recovery of starting material; infrared spectrum (Nujol): bands shifting while spectrum is taken. (For characterization of this acid through its methyl ester see under preparation of hydroxy ester XX.)

Keto Ester XXIII.—Hydroxy ester XVII (240 mg.) was dissolved in dry pyridine (3 ml.) and added to the complex prepared by addition of chromium trioxide (240 mg.) to dry pyridine (3 ml.). The mixture was shaken vigorously for a few minutes and then allowed to stand at room temperature for 20 hours. It was then decomposed with ice and extracted with ether. The solvent was removed *in vacuo* and the resulting oil was purified by chromatography on neutral alumina (Merck, "acid washed," 6 g.) using ether as eluent. Crystallization of the product (156 mg.) from acetone gave the analytical sample, m.p. 198–200°; infrared spectrum ($CHCl_3$ solution): bands at 1725 (ketone and carbomethoxyl) and 1640 cm.$^{-1}$ (lactam).

Anal. Calcd. for $C_{17}H_{23}O_4N$: C, 66.94; H, 7.60. Found: C, 67.02; H, 7.71.

Keto Ester XXIV.—Hydroxy ester XX (300 mg.) was oxidized under conditions identical with those described for the preparation of keto ester XXIII. Crystallization of the product (255 mg.) from acetone gave the analytical sample, m.p. 176–177°; infrared spectrum ($CHCl_3$ solution): bands at 1735 (carbomethoxyl), 1720 (ketone) and 1640 cm.$^{-1}$ (lactam).

Anal. Calcd. for $C_{17}H_{23}O_4N$: C, 66.94; H, 7.60. Found: C, 67.06; H, 7.64.

Hydroxy Ester XXV.—Acid XIII (100 mg.) was dissolved in methanol and treated with an excess of ethereal diazomethane for 10 minutes. The solvent was then evaporated *in vacuo* and the residue (87 mg.) was crystallized from acetone (m.p. 214–215°).

Anal. Calcd. for $C_{17}H_{22}O_4NCl$: C, 60.08; H, 6.53; N, 4.12; 1 OCH_3, 9.42. Found: C, 60.22; H, 6.64; N, 4.18; OCH_3, 9.18.

Conversion of Acid XIII to Ester XVII.*—Acid XIII (200 mg.) was hydrogenated with Adams catalyst in ethanol in the presence of a few drops of ammonium hydroxide. The catalyst was then filtered off, the filtrate was evaporated to a small volume *in vacuo* and dissolved in water (50 ml.); the aqueous solution was acidified and extracted exhaustively with chloroform. Removal of the solvent gave crystalline material (200 mg.), m.p. 265°, no depression of m.p. with an authentic specimen of acid XVIII.

This compound (200 mg.) was dissolved in dry methanol and treated with an excess of ethereal diazomethane for 15 minutes. Removal of the solvent and crystallization from ethyl acetate gave the hydroxy ester XVII (163 mg.), double m.p. 205, 210°, no depression of mixed m.p., identical infrared spectrum.

Anal. Calcd. for $C_{17}H_{25}O_4N$: C, 66.40; H, 8.20; N, 4.55. Found: C, 66.33; H, 8.21; N, 4.71.

Keto Lactone XXVI.*—Acid XIII (936 mg.) was treated with the complex, formed by the addition of chromium trioxide (1.0 g.) to pyridine (10 ml.). The mixture was shaken for 30 minutes and then allowed to stand at room temperature for 24 hours. The reaction mixture was then treated with a sodium carbonate solution (10%); the alkaline solution was washed thoroughly with chloroform and then acidified and extracted with ether. Evaporation of the solvent *in vacuo* gave a white foam (560 mg.) which on treatment with methanol deposited white crystals (90 mg.), m.p. 230–233°. Crystallization from methanol gave the analytical sample, m.p. 232–234°; infrared spectrum ($CHCl_3$ solution): bands at 1790 (γ-lactone), 1725 (ketone) and 1665 cm.$^{-1}$ (lactam).

Anal. Calcd. for $C_{16}H_{18}O_4NCl$: C, 59.35; H, 5.60; N, 4.33. Found: C, 59.43; H, 5.94; N, 4.28.

Conversion of XXVI to XXIII.—The keto lactone XXVI (200 mg.) was boiled with potassium hydroxide (200 mg.) in ethanol (10 ml.) under reflux for 10 minutes. The cooled hydrolysis mixture was then diluted with ethanol (40 ml.), palladium-on-charcoal catalyst (5%, 200 mg.) was added and the mixture was shaken in a hydrogen atmosphere until the theoretical amount of hydrogen was taken up (2 hours).

The filtered hydrogenation mixture was evaporated to a small volume, the residue was dissolved in chloroform and the chloroform solution was washed with dilute hydrochloric acid. Evaporation of the solvent gave a white foam (180 mg.) which was dissolved in ethanol and treated with an excess of ethereal diazomethane for 15 minutes. Evaporation to dryness *in vacuo* gave a crystalline residue (177 mg.) which on repeated crystallization from acetone melted at 201° either alone or on admixture to an authentic specimen of XXIII (*vide supra*). Its infrared spectrum confirmed the identity with XXIII.

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