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ANNOTININE—II

THE COMPLETE STRUCTURE

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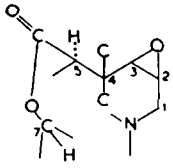
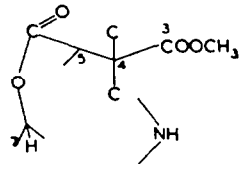
Abstract—The structure (LII) is shown unambiguously to be the correct representation of annotinine.

IN the first full account[1] of our studies on annotinine we showed conclusively that this compound is represented by the partial structure (I). In this partial structure the lactone carboxyl is in the less stable configuration, and annotinine derivatives, in which this is possible, readily epimerise at carbon 5.

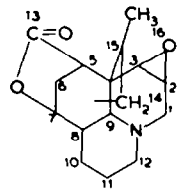
It has been further demonstrated that C_4 is probably a quaternary carbon, since the amino ester (II) proved resistant to saponification.



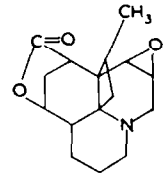
I



II



III



IV

In a preliminary communication[2] we extended the partial formula (I) to (III).

In the partial structure (III) only one carbon–carbon bond between C_{14} and C_9, C_8 or C_6 remained to be closed.

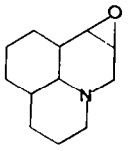
Early in 1957 we presented evidence in another preliminary communication,[3] which rigorously corroborated the partial structure (III) and permitted us unambiguously to extend it to (IV). Finally, in a paper submitted after the appearance in print of our complete structure (IV),[3] Przybylska and Marion[4] corroborated this formula by X-ray crystallography.

In the present communication we wish to give a detailed account of the experiments that permit the derivation of the complete structure (IV) for annotinine. An interpretation of these data makes possible also the assignment of configuration to most of the asymmetric centres.

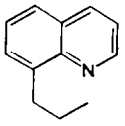
* Holder of an Eli-Lilly Postdoctoral Fellowship.
† Holder of a National Research Council Studentship.

[1] Z. Valenta, F. W. Stonner, C. Bankiewicz and K. Wiesner, *J. Amer. Chem. Soc.* **78**, 2867 (1956).
[2] K. Wiesner, Z. Valenta, W. A. Ayer and C. Bankiewicz, *Chem. & Ind.* 1019 (1956).
[3] K. Wiesner, W. A. Ayer, L. R. Fowler and Z. Valenta, *Chem. & Ind.* 564 (1957).
[4] M. Przybylska and L. Marion, *Canad. J. Chem.* **35**, 1075 (1957).

The first indication that annotinine contains the perhydrojulolidine system (V) came from dehydrogenation reactions.



V

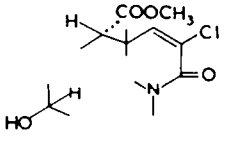


VI

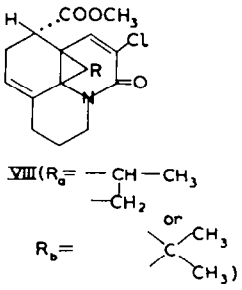
At the very beginning of our work we showed that 8-*n*-propylquinoline (VI) is formed in good yield besides other products in a selenium dehydrogenation of annotinine.[5]

Compound (VI) may be obviously derived from annotinine in two different ways, if the alkaloid contains the partial structure (V).

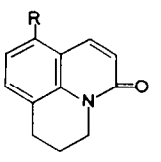
We have obtained an even more striking confirmation of the presence of system (V) in annotinine in the following manner. The hydroxy ester, $C_{17}H_{22}NO_4Cl$, which we have described previously[1] and which is represented by the partial structure (VII), was subjected to dehydration with phosphorus pentoxide in boiling xylene.



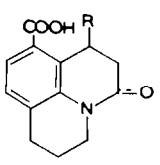
VII



VIII (R_a = —CH(CH$_3$)CH$_2$ or R_b = C(CH$_3$)$_3$)



IX (R = —COOH)
X (R = H)



XI (R = H)
XII (R = CH$_3$)

The product of this reaction was a crystalline anhydro ester (VIII), $C_{17}H_{20}NO_3Cl$, obtained in a yield of 60 per cent. Compound (VIII) had practically the same ultra-violet absorption spectrum [λ_{max} 227 mμ (log ε 4), shoulder 280 mμ (log ε 2·9)] as compound (VII) and showed also very similar peaks in the carbonyl region of the infra-red [1735 cm^{-1} (ester), 1658, 1620 cm^{-1} (conjugated lactam)]. It has been possible to show rigorously (*vide infra*) that (VIII) was formed merely by elimination of water and that it has the same skeletal structure as annotinine.

Palladium dehydrogenation of the anhydro-ester (VIII) gave a good yield of the quinolone acid (IX). The structure of (IX) follows from the decarboxylation of this compound to the known trimethylenequinolone (X), which was identified by comparison with an authentic specimen.[6] It is reasonable to assume that the carboxy group in (IX) is located at C_5, since this position corresponds to the rigorously established partial formula of the compounds (VII) and (VIII). This assumption is supported by the spectroscopic properties of the dihydroquinolone acid (XI), which may be prepared from (IX) by a reduction with sodium amalgam. Compound (XI) has an ultra-violet spectrum [λ_{max} 236 mμ (log ε 4·2), shoulder 258 mμ (log ε 4·1),

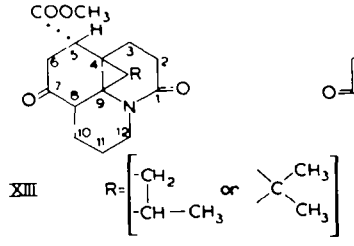
[5] C. Bankiewicz, D. R. Henderson, F. W. Stonner, Z. Valenta and K. Wiesner, *Chem. & Ind.* 544 (1954).
[6] L. G. S. Brooker and D. W. Hezeltine, *Chem. Abstr.* **48**, 1184 (1954).

λ_{max} 300 mμ (log ε 3·3)] identical and an infra-red spectrum very similar to the compound (XII), which we have previously synthesised.[7]

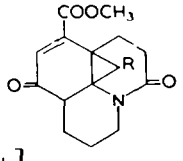
If we assume that no rearrangement has taken place in the palladium dehydrogenation, three out of the four rings of annotinine are defined in the quinolone acid (IX). The fourth ring must now be added by attaching a bridge at two points to the skeleton of compound (IX). Since annotinine is known to have one C-methyl group, this bridge may have either the structure Ra or Rb [in formula (VIII)].

One point at which the bridge must be attached is C_4, since, as we already stated, this carbon has been shown to be quaternary.

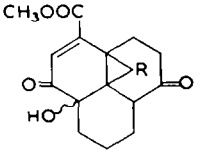
The experiments to be described in the sequel show rigorously that the second point of attachment is at C_9. They also provide us with a rigorous confirmation of the skeletal structure suggested by the results of the dehydrogenation reactions just described. Thus we may formulate the anhydro ester by the structural formula (VIII). The position of the double bond in (VIII) follows from oxidation studies (*vide infra*).



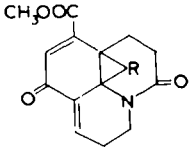
XIII



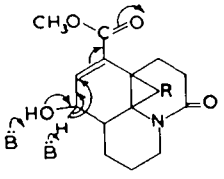
XIV



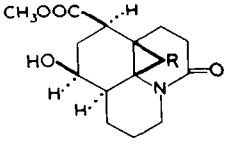
XV (-OH, R *cis*)
XVI (-OH, R *trans*)



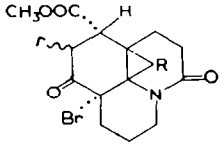
XVII



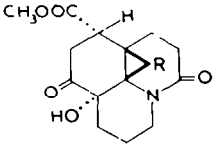
XVIII



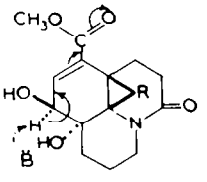
XIX



XX (r = Br)
XXI (r = H)

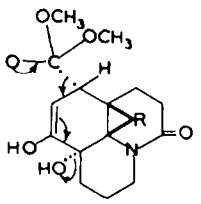


XXII

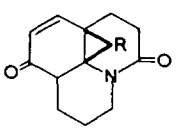


XXIII

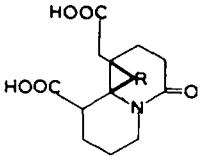
[7] Z. Valenta, K. Wiesner, C. Bankiewicz, D. R. Henderson and J. S. Little, *Chem. & Ind.* (*B.I.F. Review No.*) R40 (1956).



XXIV



XXV



XXVII

The key compound in the series of experiments to be discussed is the keto ester $C_{17}H_{23}O_4N$ (m.p. 200°), which we have described previously[1] and which may now be formulated as (XIII). It will be noted that the carbomethoxy group of (XIII) has a configuration epimeric to the natural configuration of the lactonic group in annotinine.

The oxidation of (XIII) with selenium dioxide in dioxan gave a mixture of four products, which were separated by chromatography on alumina. The first of these compounds was a keto ester, $C_{17}H_{21}O_4N$, m.p. 144–145° [ultra-violet spectrum, λ_{max} 242 mμ (log ε 3·9); infra-red spectrum, 1725 cm^{-1} (ester), 1698 cm^{-1} (ketone), 1640 cm^{-1} (lactam)], to which we assigned the structure (XIV).

While the ultra-violet and infra-red data were in good agreement with a conjugated keto ester such as is portrayed in (XIV), it was nevertheless not possible to exclude on the basis of spectra alone an $\alpha\beta$-unsaturated ketone with an unconjugated ester group. The following chemical transformations prove rigorously the presence of the conjugated keto ester chromophore. Compound (XIV) was reduced with sodium borohydride to the corresponding allylic alcohol (XVIII), m.p. 235–236° [ultra-violet spectrum, λ_{max} 222 mμ (log ε 4·0); infra-red spectrum 1718 cm^{-1} (ester), 1624 cm^{-1} (lactam)]. Treatment of (XVIII) with sodium methoxide in methanol under reflux gave the original keto ester (XIII). Now it is not conceivable that an unconjugated allylic alcohol could be isomerised by base to the corresponding ketone. On the other hand, in the case of a conjugated allylic alcohol ester such as (XVIII), one can easily envisage two mechanisms by which such a transformation may be accomplished. They are indicated by arrows in the formula (XVIII) and are self-explanatory.

The second product of the selenium dioxide treatment of compound (XIII) was a keto ester, $C_{17}H_{19}O_4N$, m.p. 127–128° [ultra-violet spectrum, λ_{max} 251 mμ (log ε 4·0); infra-red spectrum, 1731 cm^{-1} (ester), 1672 cm^{-1} (ketone), 1625 cm^{-1} (lactam)]. On the basis of the ultra-violet and infra-red spectra, the structure (XVII) was assigned to this compound. A striking confirmation of this structure, which at the same time clarified the configuration of three asymmetric centres of annotinine, was achieved as follows. Compound (XVII) was vigorously hydrogenated over platinum oxide in glacial acetic acid. In this hydrogenation it is reasonable to assume that the hydrogen has been added from one side of the molecule and opposite to the C_4–C_9 bridge. Thus the relative configuration of C_5, C_7 and C_8 in the hydrogenation product must be as indicated in the structure (XIX). Now the hydrogenation product (XIX) is identical with a hydroxy ester $C_{17}H_{25}O_4N$, m.p. 166°, which we described previously and which has been shown[1] to possess the carbomethoxy group (and of course all other asymmetric centres) in the natural configuration of annotinine. Consequently, the lactone ring in annotinine must be *cis* to the C_4–C_9 bridge and *trans* to the hydrogen at C_8.

The last two products of the selenium dioxide reaction were two isomeric hydroxy-keto esters $C_{17}H_{21}O_5N$. These compounds are represented by the structures (XV) and (XVI) and they are regarded as C_8 epimers. The properties of the two compounds are as follows:

(XV), m.p. 212–213°; ultra-violet spectrum, λ_{max} 244 mμ (log ε 3·9); infra-red spectrum, 1726 cm^{-1} (ester), 1709 cm^{-1} (ketone), 1640 cm^{-1} (lactam).

(XVI), m.p. 246·5–247°C; ultra-violet spectrum, λ_{max} 242 mμ (log ε 3·8); infra-red spectrum, 1732 cm^{-1} (ester), 1701 cm^{-1} (ketone), 1628 cm^{-1} (lactam).

A much more convenient method of preparation of compound (XV) is the alkaline hydrolysis of the dibromide (XX), which gives (XV) in a yield of 60 per cent. The dibromide (XX), m.p. 163–163·5° [infra-red spectrum ($CHCl_3$), 1741 cm^{-1} (ester and ketone), 1635 cm^{-1} (lactam)] in turn may be obtained by bromination of (XIII) in glacial acetic acid. In this bromination we believe that the bromine at C_8 is the first one to be introduced and consequently most certainly is *trans* to the C_4–C_9 bridge. A displacement of the bromine at C_8 with inversion of this asymmetric centre and an elimination of the bromine at C_6 then leads to (XV), which consequently must have the configuration indicated.

Treatment of the keto ester (XIII) with 1 mole of bromine in glacial acetic acid gave rise to the monobromide (XXI), m.p. 129–130° [infra-red spectrum in CCl_4, 1745 cm^{-1} (ester), 1725 cm^{-1} (ketone), 1650 cm^{-1} (lactam)]. Alkaline hydrolysis of (XXI) gave an uncharacterised oily material, which definitely on spectroscopic grounds did not contain even traces of compound (XIV). This finding enables us to place the bromine in the monobromide at C_8, since a bromine at C_6 would be certainly easily eliminated with the formation of the conjugated keto ester chromophore.

Thus the structure and configuration of the selenium dioxide oxidation product (XV) may be deduced from its alternate formation via the dibromide (XX). It is now of considerable importance for further confirmation of the peripheral structure of annotinine to demonstrate that the oxidation product (XVI) is indeed a C_8 epimer of (XV).

There are two independent pieces of evidence, both supporting this assignment. Compound (XVI) was reduced with zinc and acetic acid to the saturated ketol, $C_{17}H_{23}O_5N$ (XXII), m.p. 218° [infra-red spectrum, 1724 cm^{-1} (ester and ketone), 1630 cm^{-1} (lactam)]. Bromination of this compound, even with a very large excess of bromine in acetic acid, gave in good yield only the monobromide $C_{17}H_{22}O_5NBr$, m.p. 215–216° [infra-red spectrum, 1730 cm^{-1} (ester and ketone), 1642 cm^{-1} (lactam)]. On attempted further bromination, the monobromide was recovered unchanged. When this behaviour of the saturated ketol (XXII) is contrasted with the easy dibromination of (XIII), it is clear that the hydroxy group in (XXII) must be located at C_8.

The second piece of evidence is an exceedingly unusual reaction, which, nevertheless, in spite of its unprecedented type provides support for the structure (XVI). Reduction of (XVI) with sodium borohydride gave the diol $C_{17}H_{23}O_5N$, formulated as (XXIII), m.p. 249–250° [infra-red spectrum, 1723 cm^{-1} (ester), 1621 cm^{-1} (lactam)].

The two hydroxy groups in (XXIII) are assumed to be *trans* to explain the extremely slow uptake of periodic acid by this material. Compound (XXIII) was next treated with sodium methoxide in methanol under reflux with the expectation that a vinylogous β-elimination would convert it to (XIV). However, this reaction did not take place, probably because it is not favoured by the *cis* arrangement of the hydrogen at C_7 and

hydroxyl at C_8. Instead, an $\alpha\beta$-unsaturated ketone $C_{15}H_{19}O_2N$, m.p. 140·5–141·5° [ultra-violet, spectrum, shoulder 230 mμ (log ε 4); infra-red spectrum, 1687 cm^{-1} (ketone), 1623 cm^{-1} (lactam)], formulated as (XXV), was obtainable in a yield of about 10 per cent.

We believe that a reaction analogous to the isomerisation of (XVIII) into (XIII) must have taken precedence. This reaction is portrayed by the arrows in formula (XXIII) and would lead to the enol of (XXII). If this compound is attacked by a methoxide ion, the intermediate (XXIV) may form and decompose [as indicated by the arrows in (XXIV)] to give dimethyl carbonate and the conjugated ketone (XXV).

Since compound (XV) was available in somewhat larger quantities via the dibromide, it was selected for further oxidation studies. Significant results from all the methods tried were obtainable only on application of the ingenious periodate–permanganate method of Lemieux and von Rudloff.[8] The oxidation products were separated by partition chromatography on silica gel and formic and succinic acid were identified. It is clear that the formic acid must originate from C_6 and succinic acid from C_8, C_{10}, C_{11} and C_{12}. Thus, in spite of the fact that the larger fragment of the oxidation still remains oily, the two compounds identified complete together with the work described in this and the previous[1] communication a rigorous verification of the skeletal structures used in the present discussion [(XIII)–(XXIV)].

Further confirmatory evidence on the structure of the area between C_7 and C_5 was obtained as follows. Compound (XIII) gave a quantitative yield of a benzylidene derivative (XXVI), $C_{24}H_{27}O_4N$, m.p. 212–213° [ultra-violet spectrum, λ_{max} 295 mμ (log ε 4·28); infra-red spectrum, 1741 cm^{-1} (ester), 1696 cm^{-1} (ketone), 1648 cm^{-1} (lactam), 1610, 1570 cm^{-1}]. A spectroscopically entirely similar (see under "Experimental") but oily furfurylidene derivative was prepared and ozonised. Decomposition of the ozonide gave an amorphous acid, which readily decarboxylated and yielded the crystalline dicarboxylic acid (XXVII), m.p. 304°.

We shall next turn our attention to the study of the anhydro ester (VIII), the dehydrogenation of which to the quinolone acid (IX) provided us with the most important clue for the ultimate elucidation of the structure.

From the origin of the anhydro ester (VIII) it is safe to assume that the carbomethoxy group is in the more stable configuration. On the other hand, in the annotinine compounds encountered previously, the unnatural configuration of the carboxyl (i.e., *trans* to the C_4–C_9 bridge) was always far more stable.

It was consequently surprising that on alkaline hydrolysis of (VIII) two acids were obtained and separated by fractional crystallisation in roughly equal quantities.

It was demonstrated that these two acids are C_5 epimers. One of them (the anhydro acid) gave on treatment with diazomethane the anhydro ester (VIII), the other (the *iso*anhydro acid) gave an isomeric *iso*anhydro ester (XXVIII), which must be regarded as the C_5 epimer of (VIII). Treatment of (VIII) and (XXVIII) in methanol with sodium methoxide gave in both cases the same equilibrium mixture. The ratio of (VIII) to (XXVIII) in this equilibrium mixture estimated from infra-red spectra is about 15 : 1.

Thus in order to explain the formation of the anhydro and *iso*anhydro acids on alkaline hydrolysis of (VIII) in approximately equal quantities, one must assume that either the less stable ester is hydrolysed more rapidly or else that the acids in the anion

[8] R. U. Lemieux and E. von Rudloff. *Canad. J. Chem.* 33, 1701 (1955).

form are equilibrated and that in this equilibrium the *iso*anhydro acid anion is more favoured than the *iso*anhydro ester in the ester equilibrium.

It is not easy to make predictions about the relative stabilities of (VIII) and (XXVIII), since in both C_5 epimers the carboxyl may assume the quasi-equatorial conformation and in both there are strong non-bonded interactions with the C_4–C_9 bridge and the C_3 hydrogen. As a matter of fact, (VIII) might be even more stable in the quasi-axial than the quasi-equatorial conformation. This is borne out by the finding that the anhydro acid [corresponding to the ester (VIII)] forms exceedingly easily the lactone (XIX), $C_{16}H_{18}O_3NCl$, m.p. 305° [ultra-violet spectrum, end absorption; infra-red spectrum, no OH band, 1792 cm^{-1} (lactone), 1668 cm^{-1} (lactam)]. The lactone (XXIX) may be easily converted into the anhydro ester (VIII) by mild-base catalysed β-elimination followed by diazomethane treatment of the resulting acid.

The next point that had to be established about the anhydro ester is the connexion with an authentically unrearranged annotinine derivative. Such a derivative was the acid (XXX) obtained by a vigorous Wolff–Kishner reduction of the keto ester (XIII).

The anhydro ester (VIII) gave on reduction with sodium amalgam in refluxing ethanol a mixture of the acids (XXXI) and (XXXII), which were separated by fractional crystallisation. They are C_5 epimers, since the methyl esters of (XXXI) and (XXXII) on treatment with sodium methoxide in methanol give the identical equilibrium mixture, as ascertained by infra-red spectroscopy.

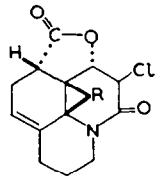
Vigorous hydrogenation of the high-melting acid (XXXII) with platinum oxide in glacial acetic acid gave the fully saturated acid (XXXIII). This compound was not identical with the acid (XXX). Moreover, esterification of (XXX) and (XXXIII) with diazomethane and equilibration of the esters with sodium methoxide in methanol resulted in non-identical equilibrium mixtures. The non-identity must be consequently due to the configuration at the asymmetric centre C_8.

If we assume that in the catalytic hydrogenation [(XXXII)→(XXXIII)], the hydrogen has been added *trans* to the C_4–C_9 bridge, then in the acid (XXX) the C_8 hydrogen must be *cis* to the C_4–C_9 bridge. Since the acid (XXX) was obtained from the ketone (XIII) in which the C_8 asymmetric centre is adjacent to a keto group, it follows that both (XIII) and (XXX) actually have the unnatural configuration at C_8 and that the keto ester (XIII) must have epimerised at C_8 in the course of the isolation, since it is stable to alkali.

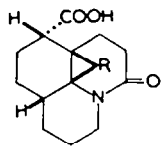
The finding that the configuration at C_8 as in (XXX) is more stable than as in (XXXIII) is not too surprising. First of all, we can deduce that the ring which originally carried the lactone must even after the opening of the lactone group remain in a quasi-chair form. If this was not the case and the ring changed into a quasi-boat, then in the boat form the natural configuration of the carboxyl would have become quasi-equatorial and more stable, which is contrary to experimental evidence.[1] If we now construct models of (XXX) and (XXXIII) with the carboxyl-carrying ring in a quasi-chair form, we see immediately that there is serious non-bonded interaction between the hydrogens of the C_4–C_9 bridge and the hydrogen at C_{10} in (XXXIII). This interaction makes (XXXIII) less stable than (XXX) in spite of the fact that in (XXX) C_{10} is quasi-axial with respect to the carboxyl-carrying ring.

This reasoning was finally corroborated and a correlation of (VIII) and (XXX) accomplished in the following manner. The methyl ester of (XXXII) was oxidised with selenium dioxide in glacial acetic acid to the oily acetoxy ester (XXXIV), which

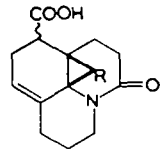
was saponified and one diastereoisomer of the hydroxy acid (XXXV), m.p. 275°, was isolated in crystalline form. Oxidation of (XXXV) with chromium trioxide in pyridine gave the amorphous keto acid (XXXVI) [ultra-violet spectrum, λ_{max} 236 mμ (log ε 3·99); infra-red spectrum (KBr pellet), 1705 cm^{-1} (ketone and carboxyl), 1635 cm^{-1} (lactam)].



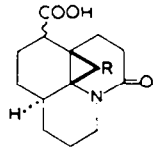
XXIX



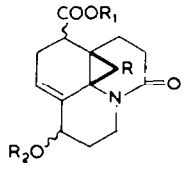
XXX



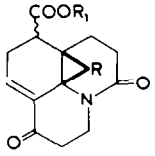
XXXI (m.p. 220°C)
XXXII (m.p. 271°C)



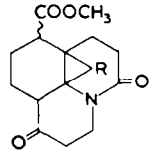
XXXIII



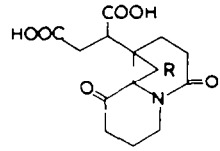
XXXIV ($R_1 = -CH_3$, $R_2 = -CO-CH_3$)
XXXVI ($R_1 = H$, $R_2 = H$)
XXXVIII ($R_1 = -CH_3$, $R_2 = H$)



XXXVI ($R_1 = H$)
XXXVIII ($R_1 = CH_3$)



XXXIX



XL, XLI

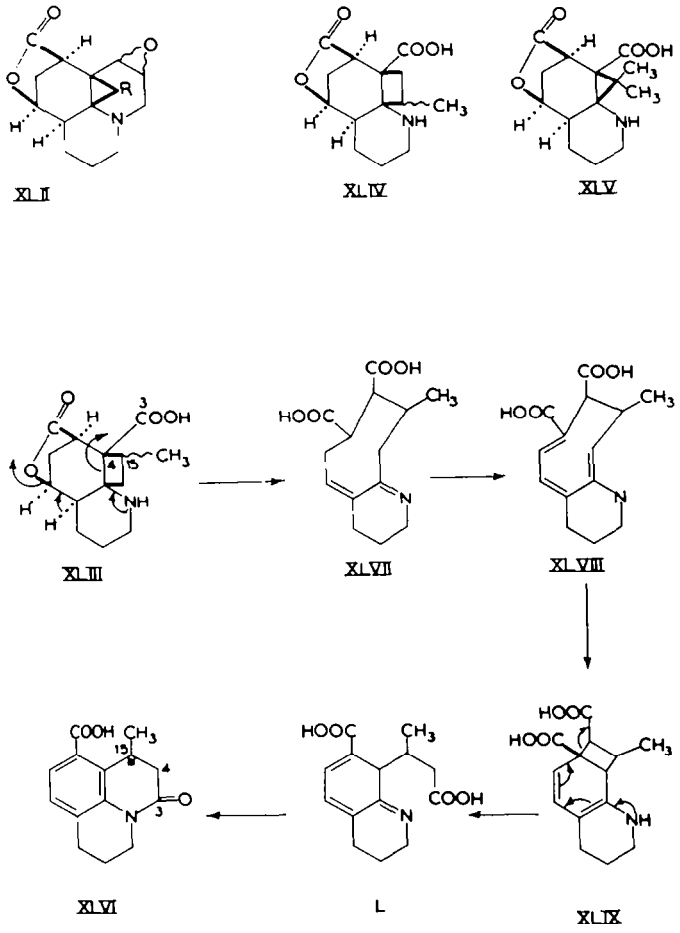
Compound (XXXVI) failed to decarboxylate on heating alone or on being heated under reflux with acid. This enables us to place the new substituent in (XXXVI), (XXXV) and (XXXIV) at C_{10}. Esterification of (XXXV) with diazomethane gave the oily methyl ester (XXXVII), which by oxidation (chromium trioxide–pyridine) gave the amorphous ester (XXXVIII) in good yield [ultra-violet spectrum, λ_{max} 236 mμ (log ε 3·94); infra-red spectrum, (CCl_4), 1740 cm^{-1} (ester), 1710 cm^{-1} (ketone), 1650 cm^{-1} (lactam)].

Hydrogenation of (XXXVIII) with palladium on calcium carbonate in alcohol gave a quantitative yield of the oily saturated keto ester (XXXIX) [ultra-violet spectrum, end absorption; infra-red spectrum, (CCl_4), 1735 cm^{-1} (ester), 1723 cm^{-1} (ketone), 1650 cm^{-1} (lactam); infra-red spectrum distinctly non-identical with keto ester (XIII)].

Compound (XXXIX), irrespective of the fact that it has been obtained via several oily intermediates, is derived directly from the anhydro ester (VIII). Moreover, (XXXIX) also has a ketone adjacent to the C_8 asymmetric centre and consequently, if our reasoning is correct, it should by a vigorous Wolff–Kishner reduction give the same product as the keto ester (XIII), that is the acid (XXX). This expectation was borne out by experiment. A Wolff–Kishner reduction of (XXXIX) gave a crystalline acid, which was identical by mixed melting point and infra-red spectrum with (XXX),

prepared by the Wolff–Kishner reduction of the keto ester (XIII). Thus it has been rigorously shown that the anhydro ester (VIII) is formed without any skeletal rearrangement.

The only structural feature of the anhydro ester (VIII) that still possibly requires corroboration is the location of the double bond. This was achieved by applying the Lemieux oxidation[8] (periodate–permanganate) to the mixture of the two diastereoisomeric acids (XXXI) and (XXXII). It was possible to isolate from this oxidation by chromatography on silica gel two crystalline diastereoisomeric *seco*-keto acids (XL) and (XLI). The fact that they indeed are diastereoisomers follows rigorously from the finding that esterification with diazomethane and equilibration (sodium methoxide–methanol) convert both (XL) and (XLI) into the identical ester, as ascertained by infra-red spectroscopy.



We have now shown that annotinine must be represented by the formula (XLII), in which the configuration of the oxide ring and the structure and configuration of the C_4–C_9 bridge remain to be determined. First of all, we wish to deal with the

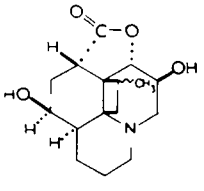
problem of the structure of the bridge R. It is obvious that (XLII) is a representation of three possible structural formulae. At the outset of our work[9] on annotinine we degraded this alkaloid to an amino acid $C_{14}H_{19}O_4N$, which according to the present discussion must be represented by (XLIII), (XLIV) or (XLV).

This amino acid was later subjected to a very mild dehydrogenation by Anet and Marion.[10] These authors have isolated an acid $C_{14}H_{15}O_3N$ as the major product of the dehydrogenation. We have later repeated the dehydrogenation and confirmed the properties of the dehydrogenation acid as reported by Anet and Marion. Moreover, we have succeeded to elucidate the structure of the dehydrogenation acid and to prove by synthesis that it has the formula (XLVI).[7]

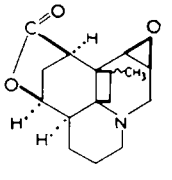
The dehydrogenation acid is optically active owing to the asymmetric carbon marked by an asterisk in (XLVI). It is clear that (XLVI) must have been formed by rearrangement from the amino acid represented by (XLIII), (XLIV) or (XLV). Of these structures, only (XLIII) has the correct arrangement of carboxyl and C-methyl to give rise to the lactam ring in (XLVI) with preservation of optical activity. Thus it can be seen that the carbons 3, 4 and 15 of (XLIII) form the lactam ring in (XLVI). From the several plausible ways to arrive from (XLIII) to (XLVI) we give only one, which does not necessarily represent the best pathway, but clearly demonstrates that of the three structures (XLIII), (XLIV) and (XLV) only (XLIII) may be converted to (XLVI) by an acceptable route.

The amino acid (XLIII) may be converted to (XLVII) by a reverse Mannich reaction, followed by β-elimination of the lactone ring. Dehydrogenation and tautomerisation of the imino group then give the *cyclo*octatriene derivative (XLVIII). The latter is converted to (XLIX) by transannular bridging and finally the new *cyclo*butane ring in (XLIX) is opened by a vinylogous reverse Mannich reaction and the product (L) is obtained. The conversion of (L) into the dehydrogenation acid (XLVI) does not require any comment.

The next point that may be clarified by a discussion of the reported behaviour of annotinine is the configuration of the oxide ring. We have shown previously[1] that annotinine hydrate, obtained by treatment of annotinine with alkali followed by acid,



LI



LII

has a new lactone ring formed between the epimerised carboxyl and hydroxy group at C_3, which has been generated by the opening of the oxide ring. Consequently, the structure of annotinine hydrate is portrayed by formula (LI). Since an intermediate oxido-hydroxy acid may be isolated[9] on shorter treatment with alkali, it becomes exceedingly probable that the actual opening of the oxide ring is performed by an intramolecular attack of the carboxylate ion. Consequently, we may represent

[9] D. R. Henderson, F. W. Stonner, Z. Valenta and K. Wiesner, *Chem. & Ind.* 544, 852 (1954).
[10] F. A. L. Anet and L. Marion, *Chem. & Ind.* 1232 (1954).

annotinine by the structure (LII), in which the configuration of the oxide ring is not proven but extremely probable and the configuration of the C-methyl group is unknown.

The confirmation of (LII) by X-ray crystallography in the National Research Council Laboratories in Ottawa[4] has finally revealed even this last point and shown that the methyl group is on the side of the lactone ring.

In the course of our work we have encountered a large number of rearrangement products that have no direct significance in the structural argument, but are interesting in themselves, as they illustrate the quite expected ability of annotinine to perform molecular acrobatics reminiscent of morphine chemistry.

We intend to report on some of these compounds in a separate communication.

EXPERIMENTAL

Anhydro ester (VIII). The hydroxy ester (VII)[1] (2 g) was heated under reflux for 2 hr with 2 g of phosphorus pentoxide on 100 ml of absolute xylene. The resulting solution was washed with water, dried and evaporated to dryness. From the oily product a 60 per cent yield of the anhydro ester (VIII) was obtained by direct crystallisation from light petroleum. After several crystallisations from the same solvent and from methanol, the sample melted at 159° and was sublimed for analysis (Found: C, 63·51; H, 6·13; N, 4·35; Cl, 11·23. Calc. for $C_{17}H_{20}O_3NCl$: C, 63·44; H, 6·26; N, 4·35; Cl, 11·02 per cent).

Dehydrogenation of the anhydro ester. The anhydro ester (VIII) (2 g) was thoroughly ground with 10% palladium on charcoal (1·5 g) and placed in a small bulb. A further 0·5 g of palladium on charcoal was spread over the mixture and the bulb was heated to 290–295° for 2 hr. The bulb with the contents was then ground up and extracted with ether in a continuous extractor. The acidic fraction was separated from this material and recrystallised to a constant m.p. of 286–286·5°; the yield was 0·22 g of the quinolone acid (IX) (Found: C, 68·22; H, 4·82. Calc. for $C_{13}H_{11}O_3N$: C, 68·11; H, 4·84 per cent). Ultra-violet spectrum, λ_{max} 236 (log ε 4·39), λ_{max} 283 mμ (log ε 3·77), λ_{max} 340 mμ (log ε 3·58). The rest of the material escaped dehydrogenation by subliming out of the bulb and was recovered.

The quinolone acid (IX) was further characterised by its methyl ester, which was prepared by treating (IX) with ethereal diazomethane. It crystallised from ether–light petroleum and melted constantly at 120–121° (Found: C, 68·66; H, 5·35; Calc. for $C_{14}H_{13}O_3N$: C, 69·11; H, 5·38 per cent). Infra-red spectrum, 1715, 1655, 1610, 1580 cm^{-1}.

Decarboxylation of the quinolone acid (IX). The acid (IX) (98 mg) was dissolved in quinolone (1 ml) and copper powder (80 mg) was added. The mixture was heated under reflux for 45 min, cooled, diluted with chloroform and washed three times with 20 per cent sulphuric acid, once with water, twice with 5 per cent sodium hydroxide, again with water, and then dried and evaporated to dryness. The residue was sublimed *in vacuo* at 100° with a cold finger. The sublimate (74 mg) was recrystallised to a constant melting point of 106–108°, which was not depressed on admixture of an authentic sample of 1-8-trimethylene-2-quinolone.[6] The infra-red spectra of the decarboxylation product and the authentic sample were identical in every detail.

Sodium amalgam reduction of the quinolone acid (IX). Acid (IX) (65 mg) was dissolved in ethanol (40 ml) and heated under reflux with stirring, while 15 g of

sodium amalgam (8% Na) was added over a period of 8 hr. The mixture was heated under reflux overnight. The alcohol solution was then decanted from the remaining amalgam and evaporated to a small volume *in vacuo*, and water was added. The solution was extracted with chloroform, acidified and again extracted with the same solvent. The yield was 46 mg of the dihydroquinolone acid (XI). After several crystallisations from methanol it melted at 266–267° (Found: C, 67·44; H, 5·73. Calc. for $C_{13}H_{13}O_3N$: C, 67·51; H, 5·67 per cent).

For infra-red spectroscopy the acid (XI) was converted to the methyl ester by treatment with ethereal diazomethane. The ester showed infra-red bands at 1728, 1665, 1605 and 1578 cm^{-1}.

Oxidation of keto ester (XIII) *with selenium dioxide.* The keto ester[1] $C_{17}H_{23}O_4N$, m.p. 200° (0·5 g), was dissolved in dioxan (12 ml) and a solution of selenium dioxide (0·25 g) in 12 ml of aqueous dioxan was added to it. The solution was heated under reflux with stirring for 16 hr and, after cooling, the precipitated selenium was filtered off. The solution was then evaporated to dryness, the residue was dissolved in chloroform and the solution was filtered through a column of neutral alumina (10 g). The oil thus obtained (0·6 g) was crystallised from acetone–ether to yield 124 mg of crystalline starting material. The mother liquors were chromatographed on neutral alumina (15 g). Benzene–ether (1 + 1) eluted 0·22 g of compound (XIV), which crystallised from ether in large blocks and melted after several crystallisations constantly at 144–145° (Found: C, 67·37; H, 6·93; N, 4·67. Calc. for $C_{17}H_{21}O_4N$: C, 67·31; H, 6·98; N, 4·62 per cent).

In a second experiment the keto ester (XIII) (1·35 g) was heated under reflux in 50 ml of dioxan with 1·35 g of selenium dioxide for 22 hr. Selenium was removed by filtration and the material was chromatographed on 25 g of acid-washed alumina.

The fractions eluted by benzene gave after several crystallisations from ether–light petroleum 80 mg of compound (XVII), which melted constantly at 127–128° (Found: C, 67·84; H, 6·52. Calc. for $C_{17}H_{19}O_4N$: C, 67·76; H, 6·35 per cent). Elution with benzene–ether (1 + 1) yielded 400 mg of the already described compound (XIV). Finally elution with chloroform–methanol (200 + 1) yielded 400 mg of oily material, from which compounds (XVI) and (XV) were separated by crystallisation from ethyl acetate and ether. Compound (XVI) was obtained pure in a yield of 0·19 g. It melted constantly at 246·5–247° (Found: C, 64·14; H, 6·67; N, 4·45; Calc. for $C_{17}H_{21}O_5N$: C, 63·93; H, 6·63; N, 4·39 per cent).

Compound (XV) was obtained in a yield of 70 mg and melted constantly at 212–213°. The mixed melting point and infra-red spectrum of this product were identical with compound (XV), obtained in good yield by hydrolysis of the dibromide (XX).

The dibromide (XX). The keto ester (XIII) (50 mg) was dissolved in glacial acetic acid (35 ml) and 0·5 ml of glacial acetic acid saturated with hydrogen bromide was added to it. A solution of 0·56 g of bromine in glacial acetic acid was added and it was decolorised in 6 hr. When more bromine was added, the solution remained brown indefinitely. The reaction solution was then poured into water and the precipitated dibromide was filtered off. After several recrystallisations from ethanol, it melted constantly at 163–163·5° (Found: C, 44·51; H, 4·71; Br, 32·24. Calc. for $C_{17}H_{21}O_4NBr_2$: C, 44·10; H, 4·58; Br, 34·51 per cent).

The monobromide (XXI). The keto ester (XIII) (0·67 g) was dissolved in 40 ml of acetic acid and glacial acetic acid (0·5 ml) freshly saturated with hydrogen bromide was added. Bromine (0·41 g) in 3 ml of acetic acid was then added and was decolorised in 40 min. The solution was poured into ice-water and the aqueous layer was extracted exhaustively with ether. The ether layer was washed with dilute bicarbonate solution and water and, after being dried, evaporated to dryness. The product (0·75 g) was recrystallised from methanol to a constant melting point of 129–130° (Found: C, 53·39; H, 5·80; Br, 20·87. Calc. for $C_{17}H_{22}O_4NBr$: C, 53·14; H, 5·77; Br, 21·77 per cent).

Hydrolysis of the dibromide (XX) *to compound* (XV). The dibromide (XX) (0·91 g) was dissolved in dioxan (25 ml) and 5 per cent aqueous potassium hydroxide (25 ml) was added. The resulting solution was heated under reflux for 1½ hr and cooled, and most of the solvent was removed *in vacuo*. Water was added and the aqueous solution was extracted with chloroform, acidified and continuously extracted with ether. The ether solution was dried, evaporated to a small volume, treated with ethereal diazomethane and evaporated to dryness. From the residue 288 mg of compound (XV) was obtained by crystallisation from benzene. After several crystallisations it melted constantly at 213° (Found: C, 64·11; H, 6·60. Calc. for $C_{17}H_{21}O_5N$: C, 63·93; H, 6·63 per cent).

Borohydride reduction of compound (XIV). Compound (XIV) (95 mg) was dissolved in methanol (5 ml) and 130 mg of sodium borohydride was added. The solution was kept at room temperature for 45 min and then treated as usual. Several crystallisations from ethyl acetate gave 56 mg of compound (XVIII), which melted at 235–236° (Found: C, 67·38; H, 7·59. Calc. for $C_{17}H_{23}O_4N$: C, 66·94; H, 7·60 per cent).

Isomerisation of (XVIII) *to* (XIII). Compound (XVIII) (35 mg) was heated under reflux overnight with 6 ml of 2 per cent methanolic sodium methoxide. The methanol was then removed *in vacuo* and water was added. The aqueous solution was extracted with ether, and the ether extract was dried and evaporated to dryness. The yield was 30 mg of brown oil, which was chromatographed on 1·2 g of neutral alumina. Elution with benzene–ether (1 + 1) gave 13 mg of a crystalline fraction. This material melted after two recrystallisations from acetone at 200° and was identical in infra-red spectrum and mixed melting point with compound (XIII).

The benzilidene derivative of (XIII). The keto ester (XIII) (100 mg) was dissolved in a solution of 60 mg of sodium in 10 ml of methanol. Benzaldehyde (0·1 g) was added and the solution was heated under reflux for 1 hr, cooled, concentrated and diluted with water. The remaining methanol was removed *in vacuo* and the aqueous solution was washed with chloroform and acidified. Extraction of the acidic solution with chloroform gave on evaporation of the chloroform 140 mg of a white foam. This material was esterified with diazomethane and recrystallised from ether to a constant melting point of 212–213° (Found: C, 73·20; H, 6·91; N, 3·71. Calc. for $C_{24}H_{27}O_4N$: C, 73·27; H, 6·91; N, 3·56 per cent).

The furfurylidene derivative of (XIII). The keto ester (XIII) (84 mg) was treated in exactly the same way as in the previous experiment, except that furfural (0·4 ml) was substituted for benzaldehyde. The endproduct after esterification was 0·15 g of a brown gum, which did not crystallise. It was chromatographed on 6 g of alumina. Chloroform eluted 0·1 g of a light yellow oil, which again did not crystallise. However, it exhibited the expected spectroscopic properties. Infra-red spectrum (CCl_4),

1742, 1697, 1652, 1607, 1540 cm^{-1}. Ultra-violet spectrum, λ_{max} (328 mμ (log ε 4·15).

Ozonolysis of the furfurylidene derivative. The oily furfurylidene derivative (0·73 g) was dissolved in ethyl acetate (100 ml) and cooled to −70°. Ozone was bubbled in at the rate of about 55 mg/min for 6 min, after which time the solution turned pale violet. The solution was warmed to room temperature and the ethyl acetate was removed by evaporation *in vacuo* below 20°. The oily residue was dissolved in glacial acetic acid (50 ml), and water (25 ml) concentrated hydrochloric acid (2 drops) and 30 per cent hydrogen peroxide (10 ml) were added. The resulting solution was stirred for 13 hr at room temperature and then the solvent was removed *in vacuo* below 50°. The residue was taken up in water and solid sodium bicarbonate was added until the solution became alkaline. The solution was then extracted with chloroform, acidified and extracted continuously with ether. The continuous extract gave 0·65 g of foam. This material was decarboxylated by being slowly heated up to 190° in a stream of nitrogen, which was then bubbled through a solution of barium hydroxide. The heating was discontinued when precipitation of barium carbonate has stopped. The residue (0·51 g) was chromatographed on a partition column of silica gel. The peak fraction 12, eluted with 5 per cent butanol in chloroform, contained 73 mg of the acid (XXVII) and was crystalline. It was recrystallised from acetone to a constant melting point of 302–304° (Found: C, 61·05; H, 7·12; Calc. for $C_{15}H_{21}O_5N$: C, 61·01; H, 7·17 per cent). The infra-red spectrum (KBr pellet) showed peaks at 1727 cm^{-1} (carboxyl) and 1600 cm^{-1} (lactam).

Hydrogenation of compound (XVII). Compound (XVII) (103 mg) was hydrogenated in 10 ml of glacial acetic acid with 50 mg of prehydrogenated platinum oxide for 12 hr. The product (XIX) was recrystallised from ether to a constant m.p. of 165–167°. A mixed melting point with the previously described[1] ester $C_{17}H_{25}O_4N$, m.p. 165–166° [compound (XX) in Valenta *et al.*[1]] showed no depression. Also the infra-red spectra of both materials were identical.

Borohydride reduction of (XVI). Compound (XVI) (470 mg) was reduced exactly in the same manner as described for compound (XIV). The product (XXIII) was obtained in a yield of 392 mg and was recrystallised to a constant m.p. of 249–250° from chloroform (Found: C, 63·55; H, 7·38. Calc. for $C_{17}H_{23}O_5N$: C, 63·48; H, 7·21 per cent). Ultra-violet spectrum, shoulder 210–220 mμ (log ε 4·0).

Compound (XXV). Compound (XXIII) (0·36 g) was dissolved in 50 ml of 2 per cent methanolic sodium methoxide and the solution was heated under reflux overnight. The methanol was taken to a small volume *in vacuo* and water was added. The aqueous solution was extracted several times with chloroform. Evaporation of the chloroform extracts to dryness gave 0·18 g of a colorless oil, from which 53 mg of starting material was obtained by crystallisation from chloroform. The mother liquors were chromatographed on 3·3 g of neutral alumina. Elution with benzene–ether (1 + 1) afforded 70 mg of a colorless oil, from which compound (XXV) crystallised on treatment with ether. It was recrystallised from ether–light petroleum to a constant m.p. of 140–141·5° (Found: C, 73·64; H, 7·79. Calc. for $C_{15}H_{19}O_2N$: C, 73·45; H, 7·81 per cent).

Zinc and acetic acid reduction of (XVI). Compound (XVI) (0·32 g) was dissolved in glacial acetic acid (50 ml) and heated to reflux temperature, and zinc dust (3 g) was gradually added to it. The heating was continued for 1½ hr, in the course of which time a second portion of zinc dust (3 g) was added. The remaining zinc dust was

filtered off and the filtrate was evaporated to dryness *in vacuo*. The residue was taken up in water and extracted with chloroform. Evaporation of the chloroform gave 0·30 g of compound (XXII), which was recrystallised to a constant m.p. of 218° from methanol (Found: C, 63·62; H, 7·18. Calc. for $C_{17}H_{23}O_5N$: C, 63·52; H, 7·21 per cent).

Bromination of (XXII). Compound (XXII) (95 mg) was dissolved in glacial acetic acid (5 ml) and a few drops of acetic acid saturated with hydrogen bromide were added. Bromine (150 mg) was then added and the mixture was set aside for several hours. The reaction mixture was then diluted with water, bromine was decolorised by a few crystals of sodium sulphite and the aqueous solution was extracted with chloroform. The chloroform extract gave on evaporation to dryness 115 mg of the monobromo derivative, which was crystallised from ether to a constant m.p. of 215–216° (Found: C, 51·70; H, 5·50; Br, 21·53. Calc. for $C_{17}H_{22}O_5NBr$: C, 51·00; H, 5·54; Br, 19·96 per cent).

Periodate-permanganate oxidation of (XV). Compound (XV) was saponified by being heated on the steam-bath with 5 per cent methanolic potassium hydroxide for 6 hr. The crystalline acid was isolated in the usual way and used directly for the oxidation experiment. It was dissolved (1·6 g) in 1 M aqueous potassium hydroxide (6·5 ml) and aqueous potassium carbonate (400 ml of 0·02 M) was added to it. A solution of sodium metaperiodate (13·75 g) in water (300 ml) was added and the acidity was adjusted to pH 8 by addition of potassium hydroxide. After the addition of 0·2 g of potassium permanganate, the solution was kept at 30° for 30 hr. The reaction mixture was then acidified strongly with sulphuric acid and continuously extracted with ether. The ether was distilled off through a column and the residue was subjected to a short steam distillation. The distillate was again extracted with ether, the ether was removed and the residue was identified as formic acid by partition chromatography on a silica gel–water column according to Marvel and Rands.[11] The non-volatile portion from the steam distillation was extracted continuously with ether and the ether was evaporated to dryness. It yielded 1·06 g of a brownish gum. This material was subjected to partition chromatography on 320 g of silica gel. Only fractions 28 and 29, which are eluted with 20 per cent *n*-butanol in chloroform, crystallised. Recrystallisation from methanol–ethyl acetate gave 36 mg of material melting at 185–189°. This substance was crystallised until the constant m.p. of 197° was reached. This melting point showed no depression on admixture of authentic succinic acid. The infra-red spectra (taken in KBr pellets) of the oxidation product and authentic succinic acid were identical in every detail.

Saponification of the anhydro ester (VIII). The anhydro ester (VIII) (1·2 g) was heated under reflux for 2 hr with 5 per cent ethanolic potassium hydroxide. The hydrolysis mixture was worked up in the usual way and yielded 1·1 g of a crystalline acidic product. Crystallisation from ethyl acetate gave the crude anhydro acid (0·59 g), m.p. 285–290°. Recrystallisation of this material did not improve its purity, since the acid was on crystallisation spontaneously converted into the γ-lactone (XXIX). However, the anhydro acid was quantitatively converted by treatment with ethereal diazomethane into the anhydro ester (VIII) m.p. 159°. This latter compound was identified by mixed melting point and infra-red spectrum. Concentration of the mother liquors

[11] C. S. Marvel and R. D. Rands, Jr., *J. Amer. Chem. Soc.* 72, 2642, (1950).

gave a crop of the *iso*anhydro acid (0·40 g), which was recrystallised from benzene to a constant m.p. of 238–241° (Found: C, 62·39; H, 6·13; Cl, 11·44. Calc. for $C_{16}H_{18}O_3NCl$: C, 62·42; H, 5·89; Cl, 11·52 per cent).

The *iso*anhydro acid was converted into the *iso*anhydro ester (XXVIII) by treatment with ethereal diazomethane. This latter compound was recrystallised from ether–light petroleum to a constant m.p. of 142·5–143·5° (Found: C, 62·76; H, 5·99. Calc. for $C_{17}H_{20}O_3NCl$: C, 63·43; H, 6·26 per cent). Infra-red spectrum, 1735, 1660, 1630 cm^{-1}. The mixed m.p. with compound (VIII) was 120–125°.

Equilibration of (VIII) *and* (XXVIII). Each compound (VIII) and (XXVIII) (100 mg) was heated under reflux overnight with 15 ml of 2 per cent methanolic sodium methoxide. The solutions were evaporated *in vacuo* to a small volume, poured into ice-water and immediately extracted with chloroform. The esters thus obtained showed infra-red spectra identical in every detail.

Lactonisation of the anhydro acid. The anhydro acid (500 mg) was heated under reflux for 3 hr in absolute benzene (100 ml) containing 100 mg of toluene-*p*-sulphonic acid. The cooled reaction mixture was evaporated to a small volume and 150 ml of chloroform were added. The chloroform solution was washed with 5 per cent sodium carbonate and water, dried and evaporated to dryness. The crystalline lactone (XXIX) was obtained in a yield of 430 mg and it was recrystallised to a constant m.p. of 305° from chloroform (Found: C, 62·32; H, 5·92; Cl, 11·74. Calc. for $C_{16}H_{18}O_3NCl$: C, 62·42; H, 5·89; Cl, 11·52 per cent).

Conversion of the lactone (XXIX) *into the anhydro ester* (VIII). The lactone (XXIX) (90 mg) was dissolved in 5 per cent methanolic potassium hydroxide (10 ml) and heated under reflux for 30 min. The cooled solution was taken to a small volume, water was added, and the aqueous solution was extracted with chloroform. The aqueous layer was then cooled with ice, acidified with 10 per cent hydrochloric acid and extracted immediately with chloroform. The chloroform extract was dried and evaporated to dryness, and the residue was treated with ethereal diazomethane. Removal of the solvent and crystallisation from ether gave 70 mg of a compound m.p. 159°. It was identified by mixed melting point and infra-red spectrum as the anhydro ester (VIII).

Sodium amalgam reduction of the anhydro ester (VIII). The anhydro ester (VIII) 0·55 g) was dissolved in ethanol (100 ml) and treated at reflux temperature with 8% sodium amalgam for 23 hr. The solution was then decanted, evaporated to a small volume *in vacuo* and diluted with water. The aqueous solution was washed with chloroform, acidified and extracted with chloroform. Evaporation of the chloroform gave 0·54 g of crystalline acidic material, which was separated into the two epimeric acids (XXXI) and (XXXII) by fractional crystallisation from methanol–ether [compound (XXXI), m.p. 219–220° (Found: C, 69·91; H, 7·98); compound (XXXII), m.p. 270–271° (Found: C, 69·89; H, 8·00. Calc. for $C_{16}H_{21}O_3N$: C, 69·80; H, 7·69 per cent)]. Both acids were converted by treatment with ethereal diazomethane into the oily esters and these were equilibrated in the already described manner. Both acids (XXXI) and (XXXII) thus gave identical esters and ascertained by infra-red spectroscopy.

Catalytic hydrogenation of (XXXII). Compound (XXXII) (90 mg) was hydrogenated in glacial acetic acid over 50 mg of prehydrogenated platinum oxide. The uptake of hydrogen was finished in 6 hr and it was 90 per cent of the theory for 1 mole.

The hydrogenation mixture was worked up as usual and it yielded 80 mg of the perhydro acid (XXXIII). This compound was recrystallised to a constant m.p. of 256–257° from methanol–ether (Found: C, 69·37; H, 8·48. Calc. for $C_{16}H_{23}O_3N$: C, 69·28; H, 8·36 per cent).

Wolff–Kishner reduction of the ester (XIII). The keto ester (XIII), m.p. 200° (0·4 g), was reduced by the Barton modification of the Wolff–Kishner procedure.[12] The reaction mixture was poured into ice and water and washed with chloroform. The aqueous layer was then acidified and exhaustively extracted with chloroform. The extract on evaporation to dryness gave 0·33 g of semi-crystalline material, from which 83 mg of compound (XXX) crystallised on treatment with ether. It was recrystallised from ether and acetone to a constant m.p. of 271° (Found: C, 68·82; H, 8·01. Calc. for $C_{16}H_{23}O_3N$: C, 69·28; H, 8·36 per cent). Compound (XXX) was clearly not identical in melting point and mixed melting point with the isomeric acid (XXXIII). The non-identity of the two products was corroborated by the distinct differences in their infra-red spectra (KBr pellets). Both compounds (XXX) and (XXXIII) were esterified with ethereal diazomethane and the esters were equilibrated as described for compounds (VIII) and (XXVIII). The equilibrated esters thus obtained were clearly non-identical, as ascertained by infra-red spectroscopy.

The seco-*ketodicarboxylic acids* (XL) *and* (XLI). The crystalline mixture of the two epimeric acids (XXXI) and (XXXII) (1·38 g) was suspended in 50 ml of water and dissolved by dropwise addition of 5 per cent aqueous potassium hydroxide. A solution of potassium carbonate (2·1 g in 30 ml of water) and a solution of potassium meta-periodate (8·7 g) and potassium permanganate (0·11 g) in 400 ml of water were then added. The mixture was left at room temperature for 6 hr, after which time it was acidified by addition of sulphuric acid and continuously extracted with ether. The continuous extract gave on evaporation to dryness 1·12 g of a white foam, which was subjected to partition chromatography on 350 g of silica gel.[11] Fraction 11 was eluted with *n*-butanol–chloroform (1 + 10) and contained 195 mg of crystalline material. Fractional crystallisation of this material from a mixture of methanol and chloroform gave the two *seco* acids (XL) and (XLI) [(XL), m.p. 230° (Found: C, 59·49; H, 6·55); (XLI), m.p. 258° (Found: C, 59·37; H, 6·56; N, 4·36. Calc. for $C_{16}H_{21}O_6N$: C, 59·44; H, 6·55; N, 4·33 per cent)]. Both acids showed an ultra-violet spectrum typical of a keto group λ_{max} 302 mμ (log ε 1·7). Both acids (XL) and (XLI) were esterified with ethereal diazomethane. The ester of (XLI) was crystalline and it was recrystallised from ether to a constant m.p. of 123·5–124° (Found: C, 62·02; H, 7·15; N, 4·31; OCH_3, 18·42. Calc. for $C_{18}H_{25}O_6N$: C, 61·52; H, 7·17; N, 3·99; OCH_3, 17·66 per cent). The esters of (XL) and (XLI) were equilibrated as described for compounds (VIII) and (XXVIII). The infra-red spectra of the equilibrated esters were identical in every detail.

Conversion of the anhydro ester (VIII) *to the acid* (XXX). The mixture of the epimeric acids (XXXI) and (XXXII) obtained by sodium amalgam reduction of (VIII) (*vide supra*) was esterified by ethereal diazomethane and subjected to selenium dioxide oxidation. The esters (2·12 g) were heated in glacial acetic acid (135 ml) under reflux with (2·1 g) of selenium dioxide for 24 hr. The reaction mixture was then filtered and evaporated to dryness *in vacuo*. The residue was taken up in chloroform and

[12] D. H. R. Barton, D. H. Y. Ives and B. R. Thomas, *J. Chem. Soc.* 2056 (1955).

washed with water, and the chloroform layer was evaporated to dryness. The yield was 2·2 g of a dark brown foam, which was purified by chromatography on neutral alumina (80 g). Chloroform eluted 1·465 g of oily material. According to its infra-red spectrum (1730, 1628 cm^{-1}) and subsequent transformations, it was assumed to be essentially the acetoxy ester (XXXIV). The oily ester (XXXIV) was saponified by heating under reflux with 5 per cent ethanolic potassium hydroxide (20 ml) for 3 hr. The hydrolysis mixture was worked up as usual and a 96 per cent yield of the crude acid (XXXV) was obtained. This product was recrystallised to a constant m.p. of 275° from methanol–ethyl acetate (Found: C, 65·81; H, 7·25. Calc. for $C_{16}H_{21}O_4N$: C, 65·96; H, 7·27 per cent). The compound (XXXV) (70 mg) was oxidised with chromium trioxide (100 mg) in pyridine (2 ml). The mixture was shaken for 21 hr and the acidic oxidation product separated as usual. The yield was 47 mg of the keto acid (XXXVI), which was amorphous (for spectra: see p. 94). Esterification of (XXXV) with ethereal diazomethane gave the oily ester (XXXVII). This compound (2·25 g) was shaken for 1 hr with 2 g of chromium trioxide in 45 ml of pyridine. The mixture was then allowed to stand for 17 hr and the neutral oxidation products were isolated as usual and purified by chromatography on 60 g of neutral alumina. Benzene–ether (1 + 1) eluted 1·36 g of compound (XXXVIII), which did not crystallise (for spectra, see p. 94). Hydrogenation of (XXXVIII) in ethanol with prehydrogenated palladium on calcium carbonate as catalyst resulted in an uptake of 1 mole of hydrogen in 13 hr and it gave a quantitative yield of the saturated ketone (XXXIX). This compound remained also oily and had an infra-red spectrum (see p. 94) distinctly different from the keto ester (XIII). Equilibration of (XXXIX) with methanolic sodium methoxide did not produce a significant change in the infra-red spectrum. The oily compound (XXXIX) (717 mg) was reduced by the Barton modification of the Wolff–Kishner reaction exactly in the same manner as compound (XIII) (*vide supra*). The acidic reduction product isolated from the reaction mixture was a semi-crystalline brown material (672 mg), from which 330 mg of crystals were obtained on treatment with ether. The substance was recrystallised to a constant m.p. of 271° from a mixture of methanol and acetone (Found: C, 69·55, 69·23; H, 8·34, 8·56; N, 4·91, 4·84; Calc. for $C_{16}H_{23}O_2N$: C, 69·28; H, 8·36; N, 5·05 per cent). The melting point of the product was not depressed on admixture of compound (XXX) [obtained by the reduction of compound (XIII)]. The infra-red spectra (KBr pellets) of the product and of compound (XXX) were identical in every detail.

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