Tetrahedron, 1960, Vol 9, pp. 254 to 270. Pergamon Press Ltd. Printed in Northern Ireland

THE STRUCTURE OF DELPHININE

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(*Received* 2 *November* 1959)

Abstract—The complete structure XLIIIa has been proved for delphinine.

AMONG the alkaloids of the Aconitum–Delphinium–Garrya group, delphinine occupies a central position. It belongs to the most complicated representatives of this class and has been, since its isolation in 1819, repeatedly the target of structural studies in several laboratories.[1,2]

An additional stimulus to an attack on delphinine chemistry was provided by the similarity of many reactions of this alkaloid and those of the even more complex and extensively studied alkaloid, aconitine.[1] This similarity led us to believe that a solution of the delphinine problem might aid considerably in the task of unravelling the structure of aconitine.

We wish to describe in this Communication the details of our studies on delphinine which enabled us to propose the structure XLIII for this alkaloid.[3*]

The initial phase in the structure determination of delphinine was facilitated by the large amount of precise experimental work accumulated over a number of years by W. A. Jacobs *et al.*[4] Thus, Jacobs has clearly shown that delphinine has the empirical formula $C_{33}H_{45}O_9N$, and that it has six rings, a tertiary nitrogen carrying a N-methyl group, four methoxyls, a benzoxy, acetoxy and a tertiary hydroxy group. Jacobs has also performed a considerable amount of oxidative degradations.[4,5]

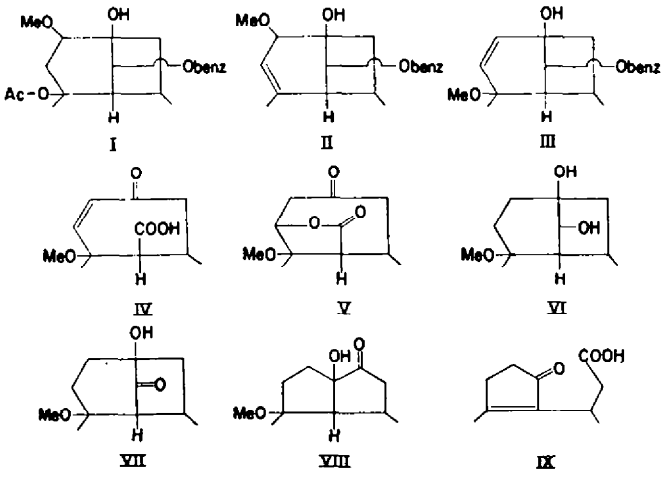
A careful analysis and repetition of this work, and our own additional experiments, enabled us first to deduce the partial structure I for delphinine.[6,7] The same partial structure also represents α-oxodelphinine ($C_{33}H_{43}O_{10}N$) which is a N-formyl compound.

Pyro-α-oxodelphinine ($C_{31}H_{39}O_8N$), which is obtainable from α-oxodelphinine by a pyrolytic loss of acetic acid, may be represented by the partial structure II, and the isomerization product of II, isopyro-α-oxodelphinine, is represented by the formula III. The isomerization II → III, which is carried out in methanolic hydrochloric acid,

* The structure XLIII was proposed for the first time as one of four possibilities for delphinine in a lecture by one of us (K. W.) at the Gordon Conference for steroids and related natural products, New Hampton, 30 July 1958. (See also ref. 7.)

[1] E. S. Stern, *The Aconitum and Delphinium Alkaloids* in: R. H. F. Manske and H. L. Holmes, *The Alkaloids, Chemistry and Physiology* Vol. IV, p. 275. Academic Press, New York (1954).
[2] K. Wiesner and Z. Valenta in L. Zechmeister, *Progress in the Chemistry of Organic Natural Products* Vol. XVI, p. 26. Springer, Wien (1958).
[3] K. Wiesner, F. Bickelhaupt, D. R. Babin and M. Götz, *Tetrahedron Letters* No. 3, 11 (1959).
[4] For a summarizing reference, see: W. A. Jacobs and S. W. Pelletier, *J. Amer. Chem. Soc.* **78**, 3542 (1956).
[5] W. A. Jacobs and S. W. Pelletier, *J. Org. Chem.* **22**, 1428 (1957).
[6] K. Wiesner, F. Bickelhaupt and Z. Valenta, *Tetrahedron* **4**, 418 (1958).
[7] K. Wiesner, F. Bickelhaupt and D. R. Babin, *Experientia* **15**, 93 (1959).

is thus portrayed as an allylic rearrangement of a methoxyl. The transformations to be described in the sequel prove rigorously the correctness of our formulations.



We shall first focus our attention on the isopyro series and describe the structure proof of III. Hydrolysis of the benzoxy group in III gives the corresponding diol which Jacobs[8] oxidized to an α,β-unsaturated keto acid represented by us as IV. Compound IV shows an ultraviolet maximum at 235 mμ (log $\varepsilon = 3{\cdot}8$) and is easily isomerized into the γ-lactone V. Compound V shows two maxima in the carbonyl region of the infrared spectrum: 1706 cm^{-1} (ketone) and 1783 cm^{-1} (γ-lactone).

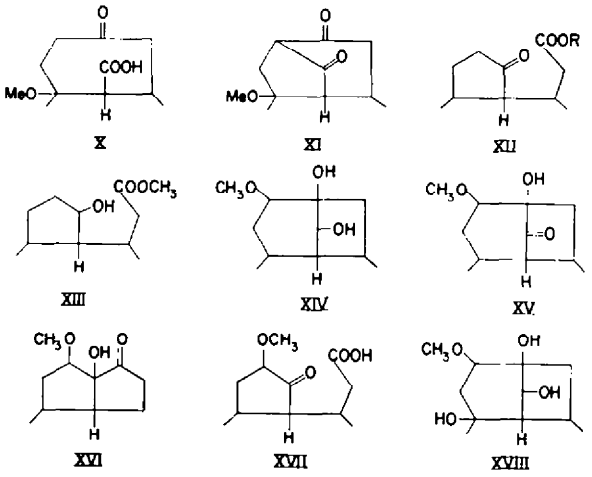
Hydrogenation of isopyro-α-oxodelphinine III results in the saturation of the double bond and of the benzoyl group. The octahydro derivative may now be hydrolyzed to the corresponding diol, dihydro-α-oxoisopyrodelphonine VI, and this last compound may be oxidized to the dihydro-α-oxoisopyrodelphonone VII.[5] Compound VII shows a peak at 1754 cm^{-1} in the carbonyl region of the infrared spectrum in agreement with a five-membered ring ketone.

Jacobs[5] has noticed that VII may be isomerized by treatment with alkali or on alumina to a more levorotatory isomer ($\Delta\alpha_D$ approx $= -110°$), which shows in the infrared spectrum a carbonyl band (1747 cm^{-1}) also in agreement with a five-membered ring ketone. Jacobs regarded this isomerization tentatively as an epimerization of an asymmetric centre adjacent to the carbonyl group. Such an explanation is, however, not admissible if we are to retain our partial structures, since both positions α to the keto group in VII are bridgeheads. We formulate the isomerization product of VII as VIII, and the isomerization is thus a simple acyloin rearrangement.

We have performed further experiments in order to test the formula VIII. Compound VIII takes up one mole of periodic acid and the expected seco keto acid eliminates spontaneously, on workup, a mole of methanol and gives a crystalline α,β-unsaturated keto acid, $C_{23}H_{31}O_7N$, formulated as IX. Compound IX shows an ultraviolet spectrum ($\lambda_{max} = 250$ mμ, log $\varepsilon = 4$) which is shifted by approx 10-15 mμ to higher wavelength with respect to our expectations based on spectra of many known cyclopentenones. However, it seems that cyclopentenones are especially susceptible to spectral anomalies due to strain.

[8] W. A. Jacobs and Y. Sato, *J. Biol. Chem.* **180**, 479 (1949).

Thus, Fajkoš[9] has prepared in the steroid series a derivative in which an unsubstituted cyclopentenone chromophore absorbs at 235 mμ. If we add to this value the expected increment for two substituents, we arrive at a value 257 mμ which is even higher than the one observed with compound IX. The infrared spectrum of IX is in agreement with the assigned constitution and shows a ketonic absorption at 1695 cm^{-1} and a carboxyl at 1730 cm^{-1}.



Dihydro-α-oxoisopyrodelphonine VI may be converted by oxidation with chromic acid into a seco keto acid, $C_{24}H_{35}O_8N$, which has already been described by Jacobs[4] and was formulated by us as X.[7] The same author has shown[4] that compound X may be converted into a diketone, $C_{24}H_{33}O_7N$, by sublimation in high vacuo. We have formulated this last compound as XI. The infrared spectrum of XI shows ketonic carbonyl peaks at 1768 cm^{-1} (five-membered) and 1727 cm^{-1} (six-membered). If we take into account the strong dipole–dipole interaction of both keto groups due to the rigid structure of XI, we may expect a shift of both carbonyl peaks to higher wave numbers and the observed values for both carbonyls are thus in good agreement with the postulated structure.

As a further corroboration of the structure XI, we have found that this diketone gives quantitatively the seco acid IX by treatment with dilute aqueous alkali. Another way to obtain compound IX is the reflux of the oily methylester of X with methanolic sodium methoxide, followed by hydrolysis on workup. The intermediate in this reaction must be again the diketone XI. The significant point about this mode of formation of IX is the fact that the methylester of X does, as the first reaction, undergo a cyclization rather than an elimination of methanol. This fact can be explained by the assumption that a double bond obtained by a β-elimination of methanol in the structure X is probibited and suggests directly that the two points of attachment of the cycloheptane ring to the rest of the molecule are as indicated in formula X.

The exploration of the isopyro system was finally concluded by the following experiment. The seco acid IX was reduced by sodium amalgam in refluxing alcohol, the products esterified with diazomethane and separated by chromatography on alumina. One product was the oily ester XII (R = CH_3). This compound showed no

[9] J. Fajkoš, *Coll. Czech. Chem. Comm.* **23**, 1559 (1958).

hydroxyl band and a peak at 1739 cm^{-1} (cyclopentanone and ester) in the infrared spectrum. Hydrolysis of this ester gave the corresponding acid XII (R = H). This compound also remained amorphous. It showed in the infrared spectrum a peak at 1731 cm^{-1} (cyclopentanone) and 1710 cm^{-1} (carboxyl). The fact that the peak at 1731 cm^{-1} indeed belongs to the cyclopentanone follows not only from the spectrum of the corresponding ester XII (R = CH_3), but also from the finding that this peak is unchanged on salt formation.

The second product separated from the chromatogram was a crystalline hydroxy ester, $C_{24}H_{37}O_7N$, to which we ascribe the partial structure XIII. This compound was proved to be of special importance since it enabled us to correlate directly the isopyro series with a delphinine derivative which has not undergone the pyro-isopyro rearrangement (*vide infra*).

The transformations described up to this point prove conclusively the partial structure III for α-oxoisopyrodelphinine. The position of the benzoyl group in III follows from the fact that no derivative of III can be oxidized to a ketone before the removal of the benzoyl group.

We shall now turn to the description of a few experiments designed to show that α-oxopyrodelphinine II has the same skeletal structure as α-oxoisopyrodelphinine III, but differs from this last compound in the site of attachment on one methoxyl.*

Hydrogenation of II saturates the double bond and the benzoyl group, and by saponification of the resulting octahydro compound, dihydro-α-oxopyrodelphonine may be obtained.[4] We have formulated this compound as XIV.

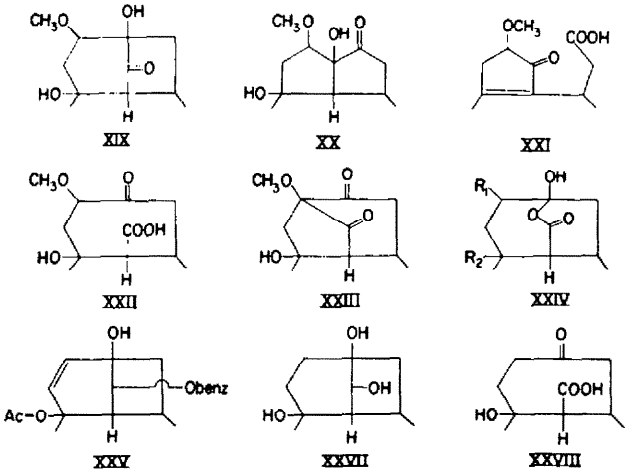
Compound XIV may now be subjected to a series of reactions which are completely analogous to the series VI → VII → VIII. Oxidation with chromium trioxide gives the ketone XV which may be isomerized with base to XVI.[5] The values of the ketonic carbonyl maxima in XV and XVI (1755 cm^{-1}, 1747 cm^{-1}) are quite similar to the corresponding values in compounds VII and VIII. Also the molecular rotation differences for the reactions VII → VIII and XV → XVI are approximately the same ($\Delta\alpha_D$ = approx −110°). The only difference between XVI and VIII, according to our formulation, is the site of attachment of one methoxy group. This is corroborated by the cleavage of both compounds with periodic acid.

As we have already discussed, the keto acid which is obtained in this manner from compound VIII eliminates spontaneously a mole of methanol and gives the cyclopentenone carboxylic acid IX. Compound XVI, on the other hand, is cleaved with periodic acid to the amorphous, but completely stable, keto acid XVII. The infrared spectrum of XVII shows a peak at 1744 cm^{-1} (cyclopentanone) and 1709 cm^{-1} (carboxyl). The fact that the 1744 cm^{-1} peak indeed belongs to the cyclopentanone is easily demonstrated by esterification with diazomethane. The oily ester shows a single peak at 1743 cm^{-1} which belongs both to the cyclopentanone and ester group.

The limited amount of data which we have collected on the pyro system II are thus in complete agreement with the structures proposed. The rigorous proof that no skeletal rearrangement has occurred in the change I → II → III was obtained by the study of α-oxodelphinine I. This compound was hydrolyzed to α-oxodelphonine XVIII, compound XVIII oxidized to α-oxodelphonone XIX (ketonic maximum in

* It should be pointed out that with the elucidation of the isopyro system as III the pyro-isopyro change must be a rearrangement and not merely a shift of a double bond as assumed by Jacobs.[4] The system III could be formed either by a skeletal rearrangement or an allylic rearrangement of the methoxyl.

IR 1751 cm^{-1}) and compound XIX finally isomerized by base to XX (ketonic maximum in IR 1747 cm^{-1}) according to the experimental procedures of Jacobs.[5] Again the rotation difference for the reaction XIX → XX ($\Delta\alpha_D$ approx = −110°) was approximately the same as the rotation difference for the rearrangement VII → VIII in the isopyro series.



Compound XX took up one mole of periodic acid and yielded a crystalline acid, $C_{24}H_{33}O_8N$, which must have been formed by a spontaneous β-elimination of a hydroxyl from the original seco acid. We have formulated this compound as XXI. It shows an ultraviolet spectrum (λ_{max} 257 mμ, log ε = 4·1) very similar to the abnormal chromophore of compound IX. The small shift of the maximum of XXI to higher wavelength with respect to IX must be ascribed to an influence of the methoxyl in XXI which is absent in IX.

At this point we were able to correlate directly compound XXI with compound IX and thus to check all the partial structure assignments in the oxodelphinine and pyro-oxodelphinine series. Compound XXI was subjected to a sodium amalgam reduction in refluxing alcohol and the products were esterified with diazomethane and separated by chromatography on alumina. They were identified as the oily keto ester XII and crystalline hydroxy ester XIII which have been previously obtained by us in an analogous reduction experiment performed on compound IX (*vide supra*).

Jacobs has shown[4] that chromium trioxide oxidation of compound XIX gives a seco acid which we have formulated as XXII. We have also obtained this compound by treatment of compound XVIII with periodic acid, followed by neutralization and oxidation with permanganate.

In complete analogy to the transformations already discussed in the isopyro series, the methyl ester of XXII may be converted to the seco acid XXI by reflux with methanolic sodium methoxide followed by saponification on workup. Also in this case the diketone XXIII is presumably the intermediate. The same argument as in the isopyro series may be repeated in the present case; i.e. the transformation XXII → XXI is made possible by the remarkable reluctance of the ester of compound XXII to eliminate the β-hydroxyl. The simplest explanation of this situation is again the assumption that the resulting double bond would be impossibly strained due to the

mode of attachment of the cycloheptane system XXII to the rest of the molecule.

The infrared spectra of the seco acids X (1736 cm^{-1} δ-lactone, sharp hydroxyl peak at 3300 cm^{-1}) and XXII (1730 cm^{-1} δ-lactone, 3500 cm^{-1} hydroxyl) deserve a brief remark. It appears that the keto acid forms X and XXII are in equilibrium with the pseudoacid forms XXIV ($R_1 = H$, $R_2 = OCH_3$; $R_1 = OCH_3$, $R_2 = OH$ respectively) and that these latter predominate in chloroform solution. On the other hand, the esters of X (1750 cm^{-1} ester, 1703 cm^{-1} ketone) and XXII (1741 cm^{-1} ester, 1715 cm^{-1} ketone, 3500 cm^{-1} hydroxyl) are normal keto esters.

From all the transformations discussed up to now, the partial structures I, II and III for α-oxodelphinine, pyro-α-oxodelphinine and isopyro-α-oxodelphinine follow rigorously. Consequently, it is clear that the pyro-isopyro change must, in fact, be an allylic rearrangement of a methoxyl. However, before proceeding with the elucidation of the remaining part of the delphinine molecule we have decided to subject this conclusion to a direct test. We have isomerized α-oxopyrodelphinine II in radioactive methanol, and as expected radioactivity corresponding to one mole of methanol was incorporated into the resulting α-oxoisopyrodelphinine III.

We have also found that it is not possible to prepare III in any medium other than methanol. Thus, for instance, α-oxopyrodelphinine II, when treated with *p*-toluenesulfonic acid in glacial acetic acid, exchanges quantitatively a methoxyl for an acetoxy group and yields a compound, $C_{32}H_{39}O_9N$, formulated as XXV. Hydrogenation of XXV gives an octahydrocderivative XXVI which may be saponified to the triol $C_{23}H_{35}O_7N$, XXVII. Compound XXVII was then converted to the crystalline acid IX by oxidation with chromium trioxide, esterification of the resulting uncharacterized seco acid XXVIII to the corresponding methyl ester and treatment of this last compound by refluxing methanolic sodium methoxide followed by hydrolysis. It is clear that this new mode of formation of IX is entirely analogous to the route VI → X → IX.

The series of experiments just described proves directly:

(a) that the pyro system rearranges to the isopyro system with a simultaneous exchange of a methoxyl for a nucleophylic solvent molecule,

(b) that, as postulated by our scheme, the group which replaces a methoxyl in the pyro-isopyro rearrangement is the one which ultimately eliminates in the formation of the unsaturated seco acid IX.

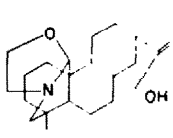
After the completion of all these studies, it was clear that there is no doubt left about the correctness of the partial formula I for delphinine, and that the stage is set for an attempt to extend it into a complete structure.

Having considered the problem carefully, we have decided to abandon the systematic approach pursued until now and to set up a hypothesis for the complete structure, which could be confronted with the mass of scattered data in the literature,[1,3] as well as with new experiments designed to test it. Fortunately, we were in possession of a certain amount of purely chemical information which has enabled us to write down readily two very plausible delphinine structures.

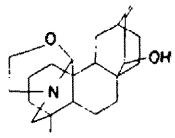
In 1953 we deduced the structures of the first two alkaloids belonging to the aconite–garrya class.[1,10] They were the alkaloids, veatchine XXIX and atisine XXX. The key evidence which gave the idea for the proposal of the two diterpenoid alkaloid skeleta was the formation of the compounds XXXI and XXXII on dehydrogenation of veatchine. Similarly, the dehydrogenation of atisine gave the phenanthrene XXXIV

[10] K. Wiesner, J. R. Armstrong, M. F. Bartlett and J. A. Edwards, *Chem. & Ind.* 132 (1954).

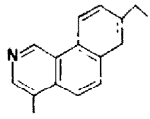
and a base which we, at that time, postulated to be XXXIII. Later the structure of the atisine dehydrogenation base XXXIII was confirmed by synthesis.[11]



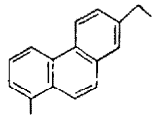
XXIX



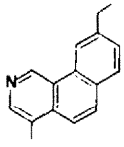
XXX



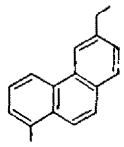
XXXI



XXXII

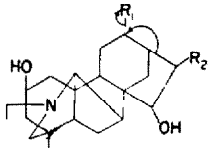


XXXIII

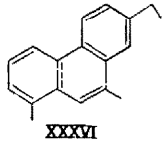


XXXIV

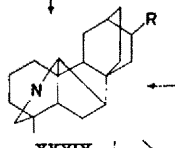
This reasoning by which the skeleton of a diterpenoid alkaloid is revealed from the structures of two "overlapping" dehydrogenation products is applicable also to hexacyclic representatives of this group. We have postulated the structure XXXV[12] ($R_1 = O=$; $R_2 = CH_2=$) for the alkaloid songorine. This compound yields as



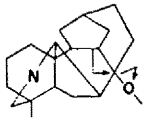
XXXV



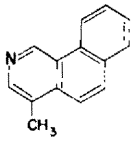
XXXVI



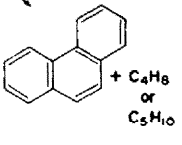
XXXIX
(R = H or CH_3)



XL



XXXVII



XXXVIII

[11] D. M. Locke and S. W. Pelletier, *J. Amer. Chem. Soc.* **80**, 2588 (1958).
[12] K. Wiesner, Shô Itô and Z. Valenta, *Experientia* **14**, 167 (1958).

characteristic dehydrogenation products the veatchine azaphenanthrene XXXI[12] and the dimethyl ethyl phenanthrene XXXVI.[13]

We have naturally attempted a dehydrogenation of delphinine in order to ascertain whether the typical dehydrogenation products of the simpler aconite alkaloids can be isolated also in this case.

While it was possible to demonstrate spectroscopically traces of phenanthrenes and azaphenanthrenes on chromatography of the dehydrogenation mixture, no compound of this type was isolated in pure form. However we have obtained a good indication of the skeletal system of delphinine by the study of the alkaloid neoline ($C_{24}H_{39}O_6N$) which accompanies aconitine in Aconitum napellus. The parallelism of many transformations of neoline and delphinine, has convinced us that the two alkaloids must possess the same skeleton.[14] Especially the presence in neoline of the delphinine (1-2-3) bicyclo-octane system I in which the tertiary hydroxyl was missing and the two ester groups were replaced by hydroxyls was assured.

The dehydrogenation of neoline has now, possibly because of the lesser substitution of this alkaloid, yielded positive results.[14] It gave the azaphenanthrene XXXVII and a phenanthrene XXXVIII which had the empirical composition $C_{19}H_{20}$ or $C_{18}H_{18}$.

In the course of our studies on songorine (XXXV, $R_1 = O=$; $R_2 = CH_2=$) and napelline (XXXV, $R_1 = -OH$; $R_2 = CH_2=$) which accompany neoline in the mother liquors of Aconitum napellus, Maudgal has performed a dehydrogenation of dihydronapelline (XXXV, $R_1 = OH$; $R_2 = -CH_3$). He was able to isolate from the dehydrogenation mixture a phenanthrene $C_{19}H_{20}$ or $C_{18}H_{18}$ which was later recognized as identical with XXXVIII from neoline. This finding has, of course, a profound significance for the tentative deduction of a possible neoline and delphinine skeleton, since dihydronapelline is a compound of known structure.

A further point to be emphasized is that the connection between napelline and neoline, established through the common dehydrogenation product XXXVIII, extends clearly to the earliest representative of the garrya–aconite class, veatchine XXIX. As already stated, veatchine and napelline yield by dehydrogenation the same azaphenanthrene XXXI.

It is clear that delphinine and neoline must possess a skeleton which is capable of yielding the same dehydrogenation products as XXXV ($R_1 = —OH$; $R_2 = —CH_3$). Both neoline and XXXV ($R_1 = —OH$; $R_2 = —CH_3$) may of course be assumed to dehydrogenante with or without a plausible rearrangement.

A further limitation imposed on the neoline skeleton is the requirement that it must accommodate the properly substituted 1,2,3-bicyclo-octane system and have an oxygen function correctly placed for any postulated rearrangement.

It is very easy to see that there are two schemes that satisfy these requirements. If neoline is XL, it may rearrange to XXXIX (R = H) and this intermediate may give the dehydrogenation products XXXVII and XXXVIII.

The same dehydrogenation phenanthrene XXXVIII may be obtained from XXXV ($R_1 = -OH$; $R_2 = —CH_3$) via a rearrangement to XXXIX ($R = —CH_3$) in a manner indicated by the arrows in formula XXXV.

[13] A. D. Kuzovkov, *Zh. Obshch. Khim.* **28**, 2283 (1958); E. Ochiai, T. Okamoto, S. Sakai, T. Sugasawa and T. Onouchi, In press.

[14] K. Wiesner, H. W. Brewer, D. L. Simmons, D. R. Babin, F. Bickelhaupt, J. Kallos and T. Bogri, *Tetrahedron Letters* No. 3, 17 (1960).

The second possibility is the skeleton XLI for neoline. This system may rearrange, as indicated, to XLII. Finally, this last intermediate may yield on dehydrogenation the azaphenanthrene XXXVII and the phenanthrene XXXVIII, which may also be obtained from XXXV by a dehydrogenation without rearrangement.

Both XL and XLI contain the 1,2,3-bicyclo-octane system attached and substituted in a manner compatible with the rigorously established partial structure of delphinine and neoline.

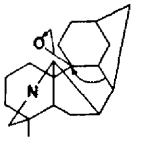
For these reasons we have decided to consider seriously XL and XLI as very plausible working hypotheses for the complete skeletons of delphinine and neoline.

The skeleton XL has of course an important precedent. The brilliant crystallographer, Przybylska, solved the structure of lycoctonine which was shown by her to be based on this system.[15] It was then recognized independently in our laboratory[16] and by Cookson[17] that the system XL may arise by a simple rearrangement, bridging, and loss of a methyl group from the atisine skeleton.

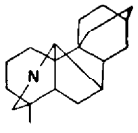
The preceding arguments show that one can readily deduce the skeleton XL by purely chemical reasoning.

We shall now turn our attention to the disposition of the remaining functional groups in the skeletons XL or XLI, as well as to the proof that one of these structures is really correct.

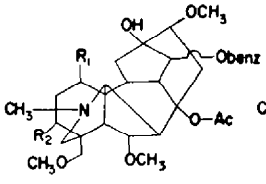
The disposition of functional groups in the 1,2,3-bicyclo-octane system of XL or XLI follows clearly from the partial structure I. The remaining functions were distributed in such a manner as to accommodate experimental work already available in the literature.[18] This led to the four structures for delphinine XLIII (a and b) and XLIV (a and b). Most of the reactions discussed in the sequel can be fitted equally well into all four structures. Since, however, the structure XLIIIa was ultimately



XLI

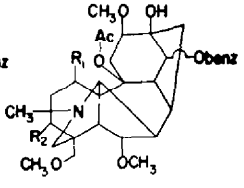


XLII



XLIII

(a) $R_1 = OCH_3$, $R_2 = H$
or
(b) $R_1 = H$, $R_2 = OCH_3$



XLIV

(a) $R_1 = OCH_3$, $R_2 = H$

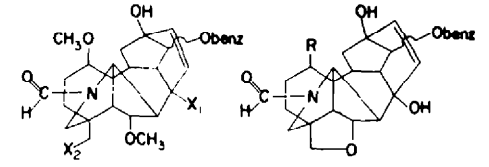
(b) $R_1 = H$, $R_2 = OCH_3$

[15] M. Przybylska and L. Marion. *Canad. J. Chem.* **34**, 185 (1956).
[16] Z. Valenta and K. Wiesner, *Chem. & Ind.* 354 (1956).
[17] R. C. Cookson and M. E. Trevett, *J. Chem. Soc.* 3121 (1956).
[18] W. A. Jacobs and S. W. Pelletier, *J. Amer. Chem. Soc.* **76**, 161 (1954).

shown to be correct, we shall use only this particular representation in order to conserve space.

Jacobs[18] has shown that in α-oxoisopyrodelphinine a tertiary and a primary methoxyl are readily displaced by chlorine. The resulting dichloro derivative may be converted by methanol back into α-oxoisopyrodelphinine, or by water into the corresponding dihydroxy derivative. This last compound may be oxidized into a hydroxy acid in which the carboxyl seems to be tertiary.

It is clear that the tertiary methoxyl displaced by chlorine must be the one engaged in the pyro-isopyro rearrangement. The fact that the same dichloride is obtained both from pyro- and isopyro-α-oxodelphinine is sufficient proof of this assumption. We may, consequently, formulate the dichloride as XLVa and the hydroxy acid as XLVb.



XLV

(a) $X_1, X_2 = Cl$

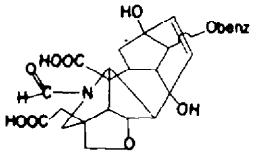
(b) $X_1 = OH$, $X_2 =$ 

(c) $X_1, X_2 = OCD_3$

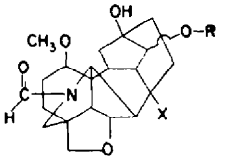
XLVI

(a) R = −OH

(b) R = =O



XLVII



XLVIII

(a) R=hexahydrobenzoyl, X=−OCH_3

(b) R=hexahydrobenzoyl, X=−OCD_3

Jacobs[18] has further shown that treatment of α-oxoisopyrodelphinine with aqueous zinc chloride will result in the replacement of two methoxyls by hydroxy groups and the remaining two methoxyls are lost with the simultaneous formation of a cyclic ether. We formulate the product of this complete demethoxylation as XLVIa. Compound XLVIa has been oxidized by Jacobs to the corresponding ketone formulated here as XLVIb. The infrared absorption of the keto group in XLVIb (1712 cm^{-1}) is in agreement with a keto group in a six-membered ring.[4] A more vigorous oxidation of XLVIb by chromium trioxide gave [18] a dicarboxylic acid formulated here as XLVII. Compound XLVII was esterified and one of the two ester groups in the resulting dimethylester was found to be resistant to saponification.

We shall now consider the identity of the two methoxyls which are engaged in the formation of the cyclic ether. Already Jacobs[18] has correctly deduced that one of them is the primary methoxyl which may be displaced by chlorine. This follows clearly from the fact that compound XLVIa (according to the results of its oxidation to XLVIb and XVLII) does not possess a primary hydroxyl. Jacobs has further postulated[4] that the second methoxyl engaged in the cyclic ether formation is identical with the

tertiary methoxyl displaced by chlorine in the formation of the dichloride XLVa. However, a careful analysis of the data shows that this assumption has no experimental basis and, in fact, is quite unlikely. Thus, according to our structure XLVIa, a new methoxyl which is not identical with either of the two methoxyls displaced by chlorine forms the second terminus of the cyclic ether.

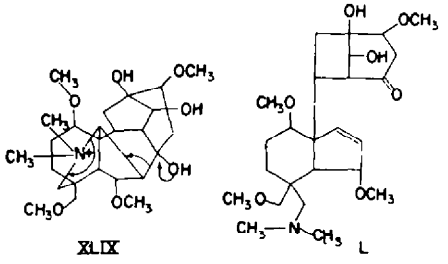
The key to the experimental decision of this point was a compound described by Jacobs[18] and formulated by us as XLVIIIa. It may be obtained by treatment of octahydro-α-oxoisopyrodelphinine with aqueous zinc chloride and it contains the cyclic ether with retention of the remaining two methoxyls.

We have prepared hexadeutero-α-oxoisopyrodelphinine XLVc by treatment of the dichloride XLVa with CD_3OH. The octahydroderivative of XLVc was then converted to XLVIIIb which still contained one deuterated methoxyl. This shows conclusively that as predicted by our scheme one of the two methoxyls which may be displaced by chlorine does not participate in the cyclic ether formation. All the deuterated delphinine derivatives were practically indistinguishable in properties from their only hydrogen containing analogs, but they gave correct deuterium analyses and showed deuterium bands in the infrared spectrum (see experimental part).

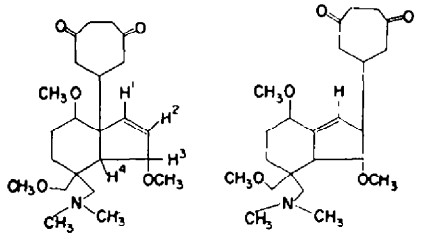
All the evidence we have discussed up to now has convinced us that one of the two delphinine structures XLIII or XLIV is correct, however, none of the data enabled us to differentiate between them rigorously.

A rigorous decision between XLIII and XLIV, which at the same time provided us with a dramatic corroboration of XLIII, was achieved by the clarification of the Hofmann degradation of delphonine. A successful Hofmann degradation of this compound has been reported by Schneider.[19] As it is clear that this finding cannot be explained by the structures XLIII or XLIV in a simple manner, we have decided to reinvestigate it.

Delphonine formed a crystalline methiodide XLIX which melted at 208–211°. The



XLIX L



LI LII

[19] W. Schneider, *Archiv. Pharm.* **283**, 86, 281 (1950).

methiodide was converted into a methohydroxide on a column of Amberlite IRA-400, and the methohydroxide was heated with 10 per cent aqueous sodium hydroxide until most of the water evaporated. From the resulting products a crystalline base, $C_{23}H_{37}O_5N$, was isolated by extensive countercurrent distribution. The infrared spectrum of this compound shows a strong ketonic peak at 1707 cm^{-1} and no peak in the hydroxyl region. The analysis indicated three methoxyls, and the presence of two keto groups was corroborated by the preparation of a bis-2, 4-dinitrophenylhydrazone.

We ascribe to this product the structure LI. It can be formed by an initial 1-3 cleavage as indicated by the arrows in formula XLIX. The product of this reaction, L, may now be converted to LI by a reverse aldol reaction, β-elimination of a methoxyl and vinilogous β-keto aldehyde cleavage. The order in which these last three steps occur can, of course, not be specified.

In this way the supposed Hofmann degradation of delphinine is explained in terms of an extensive base catalysed breakdown of the molecule and the "methine" of Schneider[19] may be conceivably identical with our compound LI.

The properties of LI discussed so far do not rule out, of course, the structure XLIV for delphinine. This last formula has all the required relationships of functional groups to undergo a similar degradation to LII. However, it was possible to decide between the two structures LI and LII for our "Hofmann product" on the basis of the NMR spectrum.

The "Hofmann product" shows in the NMR spectrum (toluene scale) a doublet with an area 1H at 1014, 1019 cps which we assign to H_2 split by the adjacent H_1. Both peaks of this doublet show a further slight splitting of approximately 1·1 cps which we ascribe to the adjacent hydrogen H_3. A further doublet at 1029, 1034 cps, which has the same splitting as the first one and an area of 1H, may be ascribed to H_1 split by the adjacent hydrogen H_2. It is characteristic that this second doublet (1029, 1034 cps) is a clean doublet without the secondary splitting.

A third doublet with an area 1H is located at 1091, 1096·5 cps and ascribed to H_3 unshielded by the methoxyl and split by the adjacent hydrogen H_4. Both peaks of this doublet again show secondary splitting of 1·1 cps caused by the adjacent hydrogen H_2. It is clear that this situation is incompatible with the formula LII which could show in the NMR spectrum only one doublet in the region of vinylic hydrogens. Consequently, we may assign the structure LI to our "Hofmann product", and structure XLIII to delphinine itself.[3] The last problem which had to be solved was the decision between the two possible locations of the ring A methoxyl in delphinine (i.e. the structures XLIII a and b).

An analogous uncertainty remained also in the complete structure of aconitine proposed some time ago.[20] In aconitine, ring A was known to be substituted by a methoxyl and hydroxyl in the 1 and 3 position, but no conclusive argument was available whether the locations of these substituents should not be exchanged. This problem was recently solved in aconitine by chemical studies[21] and also X-ray crystallography.[22] Both methods in agreement assigned the position 1 to the methoxyl and 3 to the hydroxy group. We have recently been able to convert aconitine and delphinine into an identical derivative. This correlation shows that the ring A methoxyl

[20] K. Wiesner, M. Götz, D. L. Simmons, L. R. Fowler, F. W. Bachelor, R. F. C. Brown and G. Büchi, *Tetrahedron Letters* No. 2, 15 (1959).
[21] G. Büchi, private communication.
[22] M. Przybylska and L. Marion, *Canad. J. Chem.* 37, 1116 (1959).

of delphinine is located in the same position as the corresponding substituent of aconitine, and that consequently the structure XLIIIa is the correct representation of delphinine.

The correlation of aconitine and delphine was reported by a preliminary letter[23] and the details of this work will be described in a complete article on aconitine chemistry.

EXPERIMENTAL

Secoacid IX

(a) *From ketol* VIII. Dihydro-α-oxoisopyrodelphonone VII was prepared and isomerized to VIII according to the procedure given by Jacobs.[5] The ketol VIII (104 mg) was dissolved in 10 ml ethanol and treated for 5 hr with a solution of 150 mg periodic acid in 10 ml water. The alcohol was then evaporated *in vacuo* at room temp and the remaining aqueous phase was extracted exhaustively with chloroform. The acidic fraction from this chloroform extract was separated in the usual way and crystallized from benzene–ether. The yield was 23 mg and the final melting point 138°. This material showed no m.p. depression with a sample of IX described in the subsequent experiment. Both samples also showed superimposable infrared spectra.

(b) *From secoacid* X. The secoacid X was prepared according to Jacobs[5] and remained amorphous. It was purified by chromatography on silicic acid and converted into the oily methyl ester with diazomethane. This last compound (117 mg) was dissolved in 10 ml absolute methanol and refluxed with a solution of 0·6 g sodium in 30 ml absolute methanol. After 2 hr an equal volume of water was added and the methanol swirled off *in vacuo*. The alkaline solution was exhaustively extracted with chloroform, acidified and extracted again. The yield of acidic material was 90 mg and it was recrystallized from benzene–ether to a constant m.p., 138°. (Found: C, 61·38; H, 7·24; N, 2·92; OCH_3, 19·99. Calc. for $C_{23}H_{31}O_7N \cdot H_2O$: C, 61·18; H, 7·37; N, 3·10; $3OCH_3$, 20·62%). Infrared spectrum, (KBr) 1730, 1695, 1668, 1641 cm^{-1}. Ultraviolet spectrum (ethanol) λ_{max} 250 mμ, log $\varepsilon = 4·0$.

(c) *From diketone* XI. The diketone XI was prepared by sublimation of the secoacid X according to Jacobs.[4] The diketone (31 mg) dissolved in 5 ml ethanol and 20 ml 0·01 M aqueous sodium hydroxide was added. After 30 min at room temp the solution was acidified and extracted with chloroform. The yield (36 mg) of foam which crystallized from benzene–ether and was recrystallized to a constant m.p., 138°. This material has been found to be identical with compound IX obtained in experiment (b) by mixed m.p., ultraviolet and infrared spectrum.

Reduction of secoacid IX

Compound IX (50 mg was refluxed in 10 ml ethanol with 500 mg 8% sodium amalgam for 12 hr. The solution was decanted and the amalgam washed with water. The solution and washing were combined and the ethanol evaporated *in vacuo*. The acidic fraction was separated in the usual manner and yielded 50 mg of material. The acids were esterified with diazomethane and the esters chromatographed on 5 g alumina. The ester XII (R = CH_3, 19 mg) was eluted with 0·5% methanol in benzene. The hydroxy ester XIII (22 mg) was eluted in a subsequent fraction with 1% methanol in benzene. Ester XII (R = CH_3) infrared spectrum (CCl_4) 1739, 1672 cm^{-1}.

The acid XII (R = H) was prepared by saponification of the ester with 5% aqueous methanolic potassium hydroxide under reflux, followed by a separation of the acidic material in the usual manner. Infrared spectrum (acid), (KBr) 1730, 1713, 1660 cm^{-1}. Infrared spectrum (sodium salt), (KBr) 1730. 1659, 1580 cm^{-1}.

The hydroxy ester XIII was recrystallized to a constant m.p. of 197° from benzene–ether. (Found: C, 63·99, 63·74; H, 8·16, 8·01; OCH_3, 27·22. Calc. for $C_{24}H_{37}O_7N$: C, 63·83; H, 8·26; $4OCH_3$, 27·48%). Infrared spectrum (KBr) 1729, 1653 cm^{-1}.

The sample was found to be identical by mixed m.p. and infrared spectrum with the ester XIII prepared by reduction of the acid XXI (*vide infra*).

Secoacid XVII

The ketone XVI was prepared according to Jacobs[5] by isomerization of dihydro-α-oxopyrodelphonone XV. Compound XVI (20 mg) was dissolved in 5 ml ethanol and a solution of 42 mg

[23] K. Wiesner, D. L. Simmons and L. R. Fowler, *Tetrahedron Letters* No. 18, 1 (1959).

periodic acid in 4 ml water was added. After 2 hr the reaction mixture was diluted with an equal volume of water and the ethanol evaporated *in vacuo* at room temp. The solution was then exhaustively extracted with chloroform and the acidic fraction from the chloroform extract was separated in the usual manner. It yielded 14 mg of colorless glass. This material did not have any high extinction ultraviolet chromophore and it did not develop such a chromophore on treatment with acid or base on the steam bath. Infrared spectrum ($CHCl_3$) 1744, 1709, 1660 cm^{-1}. The acid was esterified with diazomethane to the oily ester. Infrared spectrum of ester, (CCl_4) 1743, 1678 cm^{-1}.

Secoacid XXI

(a) *From ketol* XX. α-Oxodelphonone XIX was prepared and isomerized to XX according to the prescription of Jacobs.[6] Compound XX (70 mg) was dissolved in 3 ml water and 10 ml of a 1% aqueous solution of periodic acid was added to it. After 19 hr the aqueous layer was exhaustively extracted with chloroform and the acidic fraction from the chloroform extract was separated in the usual manner. The yield of acid was 57 mg and it was recrystallized to a constant m.p. of 187° from ethanol. (Found: C, 61·70; H, 7·58; O, 28·02; N, 3·00. Calc. for $C_{24}H_{33}O_8N \cdot \frac{1}{2}C_2H_5OH$): C, 61·70; H, 7·46; O, 27·99; N, 2·88%). Infrared spectrum, (KBr) 1731, 1709, 1644, 1628 cm^{-1}. Ultraviolet spectrum (ethanol), $\lambda_{max} = 257$ mμ, log $\varepsilon = 4.07$.

Esterification of acid XXI with diazomethane gave the oily methyl ester. Infrared spectrum (CCl_4), no OH band; 1742, 1710, 1677, 1655 cm^{-1}.

(b) *From secoacid* XXII. The secoacid XXII was obtained according to Jacobs[5] as the acidic by-product in the oxidation of α-oxodelphonine XVIII to α-oxodelphonone XIX with chromic acid. Compound XXII (280 mg) was esterified with ethereal diazomethane to the oily methyl ester. This last compound was dissolved in a solution of 0·6 g sodium in 50 ml absolute methanol and refluxed for 2 hr. The methanol was then evaporated to a small volume *in vacuo* at room temp and water added. From the aqueous solution the acidic material was isolated in the usual manner. The yield of acid was 156 mg and it was esterified with diazomethane and chromatographed on 6 g alumina. Chloroform eluted 50 mg of an oil which was, according to its infrared spectrum, identical with the methylester of compound XXI prepared in the previous experiment. It was saponified with 5% methanolic sodium hydroxide in a quantitative yield to the acid which was recrystallized from ethanol to the constant m.p. of 187°. This material gave no m.p. depression with compound XXI prepared in experiment (a), and the two samples showed identical ultraviolet and infrared spectra.

Sodium amalgam reduction of the secoacid XXI

The secoacid XXI (108 mg) was dissolved in 29 ml ethanol, 2·84 g 8% sodium amalgam was added and the suspension was heated under reflux for 7 hr. After this period the solution was decanted and worked up exactly as in the reduction of the secoacid IX. The yield of acidic material was 92 mg and it was esterified with ethereal diazomethane. The esters were chromatographed on 10 g alumina. An oily fraction of 24 mg was eluted with 0·5% methanol in benzene. The infrared spectrum of this material was identical with the corresponding spectrum of compound XII (R = CH_3) previously obtained by the analogous reduction of the secoacid IX.

On further elution of the chromatograph with 1% methanol in benzene, 50 mg of oil was eluted. This material crystallized from benzene and after recrystallization from the same solvent reached the constant m.p. of 197°. The substance proved to be identical by mixed m.p. and infrared spectrum with the ester XIII previously obtained by a reduction of the secoacid IX.

Acetoxyisopyro-α-oxodelphinine XXV

To 2·03 g pyro-α-oxodelphinine dissolved in 53 ml glacial acetic acid was added a solution of 810 mg *p*-toluenesulphonic acid in 30 ml glacial acetic acid. After being allowed to stand for 15 min at room temp the mixture was heated to 60–70° for 30 min. At the end of this period 200 ml water were added and the solution extracted exhaustively with chloroform. The yield of material thus obtained was 1·91 g and it was recrystallized from chloroform–ethanol to a constant m.p. of 305°. (Found: C, 66·35; H, 6·75; OCH_3, 15·51. Saponification equivalent 271. Calc. for $C_{32}H_{39}O_9N$: C, 66·07; H, 6·76; $3OCH_3$, 16·00%. Saponification equivalent 290·8). Infrared spectrum, (KBr) 1732, 1714, 1643 cm^{-1}.

Octahydroacetoxyisopyro-α-oxodelphinine

Compound XXV (1·53 g) was hydrogenated in 50 ml glacial acetic acid with 200 mg prehydrogenated platinum oxide. The uptake of four molecules of hydrogen was completed in 9 hr. The solution was then evaporated to dryness and the hydrogenation product recrystallized from chloroform–ether to a constant m.p. of 229°. The yield of once recrystallized material was 1·42 g. (Found: C, 65·45; H, 7·97; OCH_3, 15·96. Calc. for $C_{33}H_{47}O_8N$: C, 65·17; H, 8·03; $3OCH_3$, 15·82%). Infrared spectrum, (KBr) 1735, 1648 cm^{-1}.

Desmethoxy-α-oxodelphonine XXVII

The above octahydro compound (1·11 g) was dissolved in 32 ml methanol and 8 ml of 10% aqueous sodium hydroxide was added. The solution was heated under reflux for 2 hr. After this time the methanol was evaporated *in vacuo* and the aqueous layer extracted with chloroform. The chloroform extract was evaporated to dryness and the residue recrystallized from methanol to a constant m.p. of 239°. The yield after one crystallization was 678 mg. (Found: C, 63·29; H, 8·04; OCH_3, 20·87. Calc. for $C_{32}H_{35}O_7N$: C, 63·14; H, 8·06; $3OCH_3$, 21·28%). Infrared spectrum, (KBr) 1648 cm^{-1}.

The secoacid XXVIII

The compound XXVII (504 mg) was dissolved in 30 ml glacial acetic acid and 0·5 ml of a 10% aqueous solution of chromic acid was added dropwise over a period of 15 min with stirring. After 30 min, 2 hr and 6 hr additional 0·5 ml portions of the same chromic acid solution were added in the same manner. After the last addition the solution was allowed to stand at room temp for 20 hr. At the end of this period the mixture was diluted with 50 ml water and the acidic oxidation product was separated in the usual way, using chloroform for the extractions. The yield was 108 mg of a white foamy acid which did not crystallize. The infrared spectrum of this material was typical of the seven-membered secoacids of this series and was compatible with the formula XXIV (R_1 = H, R_2 = OH) rather than XXVIII. Infrared spectrum, ($CHCl_3$) 3600, 3400 (hydroxyl), 1725 (lactone), 1660 cm^{-1} (amide).

Secoacid IX

The above acid (108 mg) was esterified with ethereal diazomethane. The oily ester was dissolved in 10 ml absolute methanol and added to a solution of 194 mg sodium in 68 ml of the same solvent. The mixture was heated under reflux for 2 hr. At the end of this period 60 ml water was added and the methanol evaporated *in vacuo*. The alkaline aqueous solution was extracted with chloroform, acidified and extracted again. The residue of the second chloroform extract was recrystallized from benzene–ether to give 32 mg of the secoacid IX. After several crystallizations the substance melted at 138° and showed no depression of m.p. on admixture of a sample of the secoacid IX prepared from α-oxoisopyrodelphinine. Also the ultraviolet and infrared spectra of both samples were identical.

Pyro-isopyro rearrangement in labelled methanol

(a) *In deuterated methanol.* α-Oxopyrodelphinine (193 mg) was heated to 60° in 6 ml deuterated methanol (CD_3OH) and 0·35 ml conc hydrochloric acid for 15 min. The crystalline product was then filtered off, washed with ordinary methanol and recrystallized from chloroform–ether and chloroform–ordinary methanol until it reached a constant m.p. of 287°. The infrared spectrum of this material was superimposable with the spectrum of α-oxoisopyrodelphinine except that the deuterated material showed additional bands at 2245, 2215 and 2070 cm^{-1}, not present in α-oxoisopyrodelphinine. (Found: 6·44 atom per cent XSS D. Calc. for $C_{31}H_{36}D_3O_8N$: 7·69 atom per cent XSS D.)

(b) *In* C^{14}*-methanol.* α-Oxopyrodelphinine (408 mg) was dissolved in 8·5 ml C^{14}-methanol (molar activity—$2·0 \times 10^{-3}$ mc/mole). After an addition of 0·5 ml 38% hydrochloric acid the solution was kept at 60° for 10 min. The crystalline labelled α-oxoisopyrodelphinine was filtered off and recrystallized from chloroform–ether and chloroform–ordinary methanol to a constant m.p. of 287°. The yield was 181 mg. The counting of the labelled sample was done as $BaCO_3$ and compared with a calibration curve constructed with samples of $BaCO_3$ of known activity. The combustion was performed according to the Van Slyke method.[24]

[24] M. Calvin, C. Heidelberger, J. C. Reid, B. M. Tolbert and P. E. Yankwich, *Isotopic Carbon* p. 92. Wiley, New York (1949).

In three separate combustions the labelled α-oxoisopyrodelphinine was found to have a molar activity 106, 102 and 99% of the molar activity of the labelled methanol used for the rearrangement. This corresponds to the incorporation of exactly one mole of methanol.

Dichloro-α-oxoisopyrodelphinine XLVa

This compound was prepared according to Jacobs[18] in quantitative yield. α-Oxopyrodelphinine was dissolved in absolute methanol saturated with hydrochloric acid at 0°. The reaction mixture was allowed to stand overnight at room temp. The highly insoluble compound XLVa was filtered off and recrystallized from chloroform–ether. It started decomposing without melting at about 270°. (Found: C, 61·79; H, 5·95; Cl, 13·42; OCH_3, 12·10. Calc. for $C_{29}H_{33}O_6NCl_2$: C, 61·90; H, 5·92; Cl, 12·61; $2OCH_3$, 11·03%.)

Hexadeutero-α-oxoisopyrodelphinine XLVc

A suspension of 756 mg of compound XLVa and 1 g silver nitrate in 25 ml CD_3OH was heated under reflux for 18 hr. The solution was then evaporated to dryness and the residue was extracted with chloroform. After one crystallization from chloroform–ether 500 mg labelled α-oxoisopyrodelphinine XLVc was obtained. The compound reached after several crystallizations, a constant m.p. of 285° and gave no m.p. depression with α-oxoisopyrodelphinine. The infrared spectra of both samples were identical except for the presence in the spectrum of the labelled compound of additional bands at 2050, 2180 and 2245 cm^{-1}. (Found: 13·0 atom per cent XSS D. Calc. for $C_{31}H_{33}D_6O_8N$: 15·4 atom per cent XSS D.)

Hydrogenation of compound XLVc

Compound XLVc (492 mg) was hydrogenated in 25 ml glacial acetic acid with 170 mg prehydrogenated platinum oxide. Four moles of hydrogen were taken up in 9 hr. The catalyst was filtered off and the solution evaporated to dryness *in vacuo*. The residue was recrystallized from ether to give 369 mg material m.p. 204–209° which was used for the conversion into the compound XLVIIIb without further purification.

Compound XLVIIIb

The octahydro hexadeutero-α-oxoisopyrodelphinine (369 mg), prepared in the previous experiment, was heated with a solution of 16 g zinc chloride in 6 ml 5% hydrochloric acid to 40–60° for 90 min. The reaction mixture was diluted with water and continuously extracted with chloroform. The residue of the chloroform extract (332 mg) was crystallized from methanol–water until it reached a constant m.p. of 266°. This m.p. is the same as the m.p. of compound XLVIIIa which we have prepared according to Jacobs[18] in the same manner from unlabelled octahydo-α-oxoisopyrodelphinine. The two samples gave also no m.p. depression and had identical infrared spectra except for the presence of bands at 2065, 2210, 2240 cm^{-1} in the spectrum of the labelled compound XLVIIIb. (Found: OCH_3, 10·71; 6·83 atom per cent XSS D. Calc. for $C_{29}H_{38}D_8O_7N$: $2OCH_3$, 11·97; 7·3 atom per cent XSS D.)

Delphonine methiodide XLIX

Delphonine (30 g) was heated under reflux with 200 ml n-butyl ether and 30 ml methyl iodide for 6 hr. The precipitate which has formed at the end of this period was filtered off and washed with ether. The yield was 36 g. This crude material was recrystallized several times from acetone until it reached the constant m.p. of 208–211°. (Found: C, 50·73; H, 7·02; I, 21·25; OCH_3, 20·44. Calc. for $C_{25}H_{42}O_7NI$: C, 50·42; H, 7·11; I, 21·33; $4OCH_3$, 20·77%.)

The methiodide was converted into the methohydroxide by filtering a methanolic solution through a column of amberlite IRA-400 in the hydroxyl form.

Base degradation of delphonine methohydroxide to LI

Delphonine methohydroxide (27·5 g) was divided into five equal portions and each of them was subjected to the following treatment. It was dissolved in 25 ml water and 10 ml saturated sodium hydroxide. This solution was heated on an oil bath (130–190°) until most of the water evaporated. This required a period of 2–4 hr, after which the mixture was diluted with water and extracted with

ether. The total yield of all five runs was 20·4 g of dark red-brown resin. This material was subjected to 3 countercurrent distributions in 9 funnels as follows:

(1) A distribution between 500 ml phosphate buffer pH 5·8 and 500 ml chloroform. Fractions 5, 6, 7 and 8 were combined and yielded 4 g of material which was subjected to the next distribution.

(2) A distribution between 500 ml phosphate buffer pH 5·45 and 500 ml chloroform. The combined fractions 3, 4, 5, 6 and 7 yielded 2·6 g of material which was subjected to the third distribution.

(3) A distribution between 400 ml phosphate buffer pH 5·45 and 400 ml chloroform. The fractions 5, 6 and 7 contained 370 mg of colorless oil which crystallized and was recrystallized from ether to a constant m.p. of 153°. (Found: C, 67·52, 67·52; H, 9·30, 9·02; OCH_3, 22·48. Calc. for $C_{23}H_{37}O_5N$: C, 67·78; H, 9·15; $3OCH_3$, 22·85%.) Infrared spectrum, ($HCCl_3$) no hydroxyl, 1706 cm^{-1}.

The bis-2,4-dinitrophenylhydrazone of LI

2,4-Dinitrophenylhydrazine (22·7 mg) was dissolved in a mixture of 0·5 ml ethanol, 5 drops conc sulphuric acid and 5 drops water. A solution of 25·5 mg of compound LI in 1 ml ethanol was then added and the mixture heated for 10 min on the steam bath. The entire reaction mixture was then washed with benzene on a column of 10 g basic alumina and eluted with 1% methanol in benzene. The substance was precipitated with chloroform from an ethanolic solution. It was microcrystalline m.p. 157°. (Found: C, 53·70; H, 5·73; N, 16·45; OCH_3, 12·37, 10·22. Calc. for $C_{35}H_{45}O_{11}N_9$: C, 54·74; H, 5·90; N, 16·41; $3OCH_3$, 12·12%).

Acknowledgements—The present work was supported at various stages by the National Research Council, Ottawa, The Eli Lilly and Company, Indianapolis, Indiana, and the Research Corporation, New York.

We wish to thank Dr. R. S. Stuart, Manager, Scientific Division, Merck and Company Limited, Montreal for generously putting at our disposal the facilities of his radioactive tracer laboratory, and Mr. P. Egli for his help and advice in the actual tracer experiment. We also wish to thank Mr. J. Atkinson (Chemistry Department, M.I.T., Cambridge, Mass.) for recording the first NMR spectrum of compound LI), and to Dr. R. Kaiser (Chemistry Department, U.N.B.) for the final recording of a high resolution NMR spectrum of the same compound.