[CONTRIBUTION FROM THE LABORATORIES OF THE ROCKEFELLER INSTITUTE FOR MEDICAL RESEARCH]

The Aconite Alkaloids. XXV. The Oxygen-containing Groups of Delphinine

BY WALTER A. JACOBS AND S. W. PELLETIER

RECEIVED JUNE 29, 1953

The oxygen atoms in the alkaloid delphinine (XV) have been characterized as follows. The four methoxyls, in the order in which they have been demethylated, are derivatives of a tertiary, a primary, a tertiary and a secondary hydroxyl group. The first and third hydroxyl groups to be exposed are shown to be of tertiary character by their relative resistance to oxidation. The second hydroxyl is primary since it can be oxidized successively to a stable aldehyde III and an acid IV. The relative resistance of the methyl ester IVa of this acid to saponification suggests a tertiary character for the carbomethoxy group and therefore for the parent $-CH_2OCH_3$ group. That the fourth hydroxyl to be exposed is secondary and adjacent to a $-CH_2$ group was shown by the successive oxidation of desmethylanhydroisopyroöxodelphinine (XI) to a ketone XIX and a dibasic acid XX which was not cyclized by the methods used. The hindered character of one of the ester groups of the dimethyl ester of XX suggests that this carbomethoxy group and therefore its apparent precursor, the carbinol group containing the fourth hydroxyl group to be exposed, is attached to a tertiary carbon atom. The benzoyl group of delphinine covers a secondary hydroxyl which is vicinal to a free, tertiary, and acylable hydroxyl group. Hydrolysis of the acetoxy group of delphinine furnished benzoyldelphonine (XVI) which was oxidized in turn to a benzoyloxodelphonine (XVII). The hydroxyl group exposed on deacetylation is considered to be tertiary since it could not be reacetylated. An uncertainty exists as to whether the position and (or) configuration of this hydroxyl are the same as in the case of delphinine itself. Several other new alkaloid derivatives and transformations are discussed.

In the alkaloid delphinine, $C_{33}H_{45}NO_9$, the oxygen atoms have been accounted for in four methoxyl groups: an acetoxy, a benzoxy and a free hydroxyl group.[1] On the basis of oxidative studies on the lactam, isopyroöxodelphonine,[1a] and its dihydro derivative, it has been shown that the benzoyl group in delphinine covers a secondary hydroxyl group which is vicinal to a free tertiary hydroxyl group.[2] It has been possible to demethylate the methoxyl groups of a number of delphinine derivatives to varying degrees. Complete, stepwise demethylation of isopyroöxodelphinine[3,4] has been effected with dehydration accompanying the demethylation of the second methoxyl group to give an oxidic bridge between the two hydroxyls thus liberated. The third methyl group to be removed was shown to expose a tertiary hydroxyl since the liberated substance was relatively inert to chromic acid. That the fourth methyl covers a secondary hydroxyl group was shown by oxidation of the latter to a ketone. In the present work it has been possible to characterize more fully the oxygen atoms in the original alkaloid delphinine.

As previously reported,[3] isopyroöxodelphinine, $C_{31}H_{39}NO_8$ (I),[5] readily loses one methyl group when dissolved in nitric acid. Recent oxidative studies on the resulting substance, $C_{30}H_{37}NO_8$ (II), have shown that the liberated hydroxyl group is tertiary since its reaction with chromic oxide is much more gradual than in the case of related substances of the type which can be oxidized to oxo derivatives. The complex oxidation mixture consisted of further demethylated neutral and acidic products together with unchanged starting material. Because of the generally obscure outcome of this work it is not recorded in the Experimental part. However, the tertiary character of this hydroxyl group was substantiated by its inert behavior in the following oxidation studies.

A dimethoxy derivative of uncertain formulation has been described as a product of the further action of nitric acid on isopyroöxodelphinine.[3] More recent study has shown this compound to be of special interest and to have the formulation of $C_{29}H_{33}NO_8$. It is a benzoxydihydroxydimethoxyaldehydolactam (III) which results from the demethylation of a second methoxyl group with accompanying oxidation of the liberated primary hydroxyl to an aldehydic carbonyl. Its study was at first complicated by variations in melting points due in part to its tendency to crystallize with solvent. However, after purification through alumina it was obtained in a solvent-free form which could then be obtained directly by seeding of the crude material. This substance readily gave an oxime. Saponification of the aldehyde III yielded the crystalline trihydroxydimethoxyaldehydolactam, $C_{22}H_{29}NO_7$ (IIIa). The presence of the carbonyl group in the latter was confirmed by the ultraviolet absorption data (λ_{max}^{EtOH} 260–290 mμ, log ϵ 1.46). The aldehydic character of the newly formed carbonyl was shown by the further oxidation of the unsaponified aldehyde III with chromic acid to the benzoxydihydroxydimethoxylactam carboxylic acid, $C_{29}H_{33}NO_9$ (IV). Titration showed that this compound is a monobasic acid in which the benzoxy group has been retained. This same acid resulted from the use of sufficient potassium permanganate to furnish one atom of oxygen. Treatment with diazomethane readily gave the methyl ester, $C_{30}H_{35}NO_9$ (IVa). Of special interest was the relatively hindered character of the ester group. When heated for several hours with an excess of 0.1 *N* alkali only one equivalent was consumed owing to saponification of the benzoyl group. This furnished the crystalline trihydroxydimethoxylactam carboxylic methyl ester, $C_{23}H_{31}NO_8$ (V). The corresponding acid, $C_{22}H_{29}NO_8$, (Va), was obtained in an amorphous form by saponification of the previously discussed benzoxy acid IV. The stability of both the aldehyde and the carbomethoxy groups is suggestive of

(1) W. A. Jacobs and L. C. Craig, *J. Biol. Chem.*, **127**, 361 (1939).

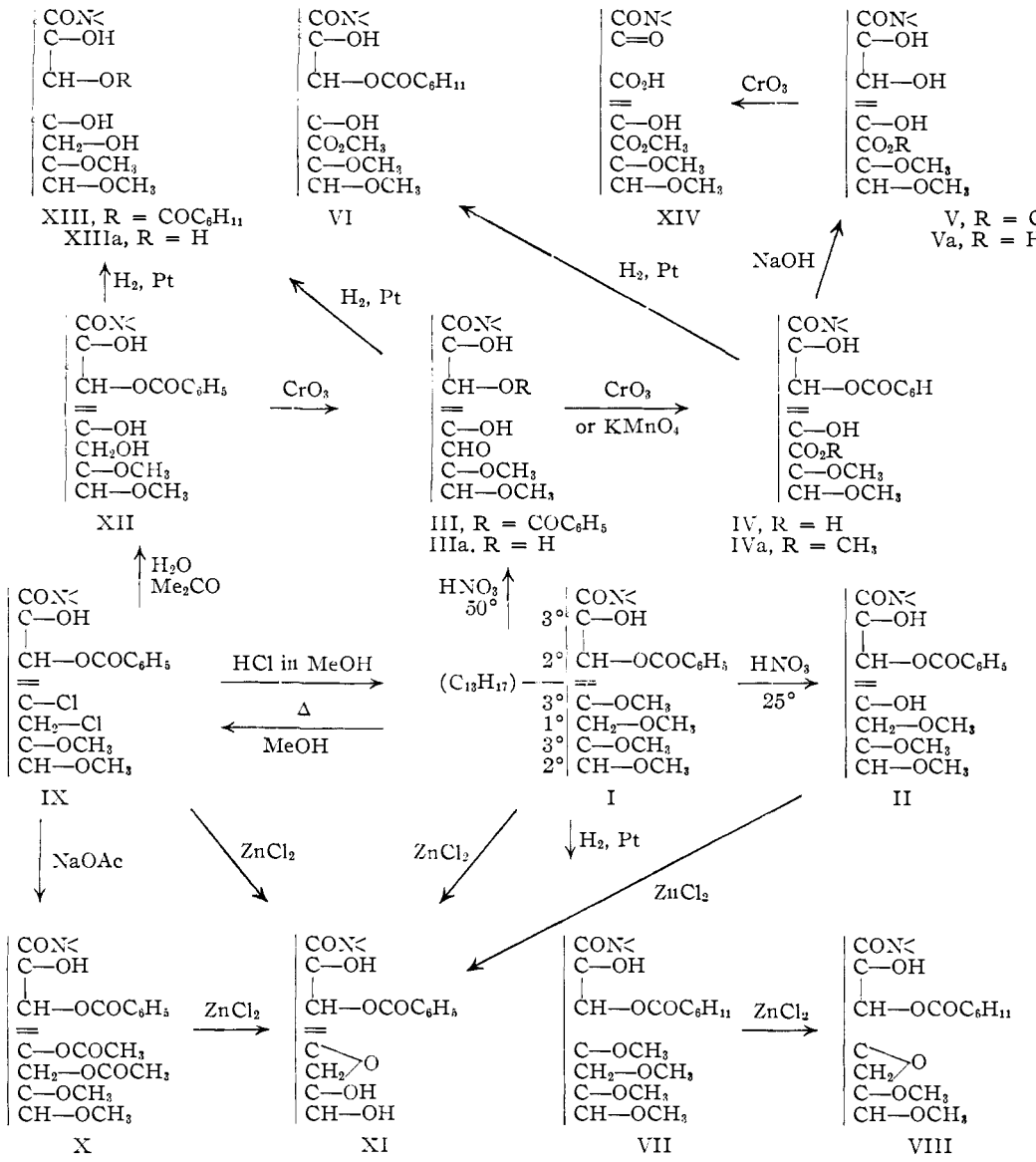
(1a) As in previous papers the term "delphonine" refers to the parent alkamine of delphinine, the ester alkaloid.

(2) W. A. Jacobs and Y. Sato, *ibid.*, **180**, 479 (1949).

(3) W. A. Jacobs and L. C. Craig, *ibid.*, **136**, 303 (1940).

(4) W. A. Jacobs and Y. Sato, *ibid.*, **180**, 133 (1949).

(5) The order in which the groups appear in the formulas is not meant to imply the positional relationship the groups may bear to each other except where definitely stated in the text. The numbers bearing degree signs in formula I anticipate the conclusions of this paper in that they denote whether the designated alcoholic functions are of primary, secondary or tertiary character.



their possible tertiary character. When hydrogenated, the methyl ester IVa absorbed four moles to give a saturated octahydrobenzoxydihydroxydimethoxylactam carboxylic methyl ester, $C_{30}H_{43}NO_9$ (VI).

It was of interest to further demethylate these oxidation products in order to characterize the liberated hydroxyls by oxidative studies and to determine other possible structural relationships. Unfortunately, such a simple change as the formation of the aldehyde or carboxyl group was found to affect greatly the lability of the two remaining methoxyl groups. This same stability of the methoxyls has already been shown to result when the double bond of isopyroöxodelphinine (I) is hydrogenated.[4] From the resulting hydrogenation product VII only the corresponding dimethoxyanhydro derivative VIII was obtained on demethylation. Treatment of the benzoxydimethoxyaldehydo derivative III with zinc chloride gave a product which still contained about two methoxyl groups but was contaminated with a small amount of chlorine. Further studies with this substance were abandoned. The aldehyde III was also recovered unchanged from concentrated hydrochloric acid. Similarly the dimethoxy acid IV was recovered unchanged after treatment with zinc chloride. A possible explanation for this stability of methoxyl groups involves the assumption that during formation of the aldehyde by the rather severe treatment with nitric acid, either rearrangements occur or the order of progressive demethylation is different from that followed when zinc chloride is employed, thus involving different methoxyls and exposing different hydroxyl groups. That this is not the case, however, was shown by the following parallel studies.

In a previous communication[3] a dichlorodimethoxy derivative, $C_{29}H_{33}Cl_2NO_6$ (IX), obtained from isopyroöxodelphinine (I) was described. The lability of the halogens was shown by its facile reconversion to isopyroöxodelphinine when heated in methanol. Also treatment with sodium acetate readily gave a diacetoxydimethoxy derivative,

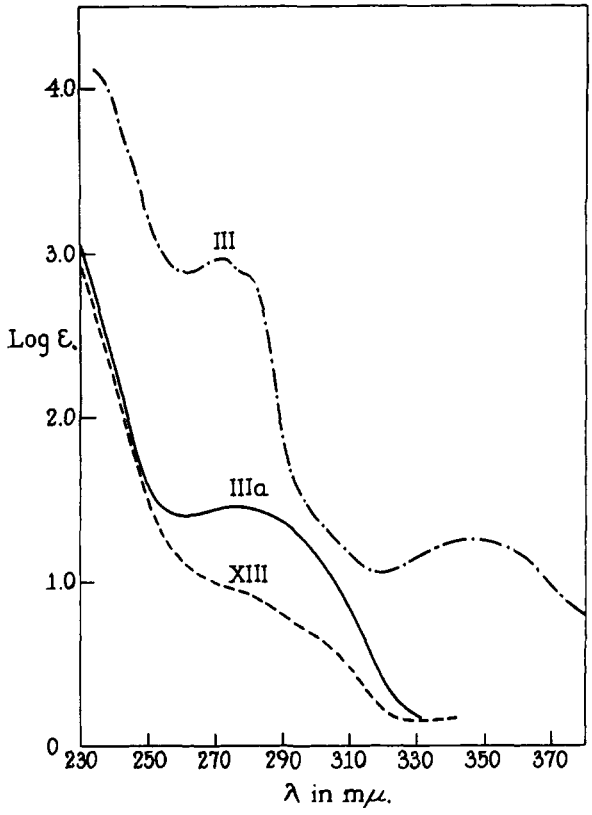


Fig. 1.—Ultraviolet absorption spectra in ethanol: III, the benzoxydihydroxydimethoxyaldehydolactam; IIIa, the trihydroxydimethoxyaldehydolactam; XIII, the octahydrobenzoxytrihydroxydimethoxylactam.

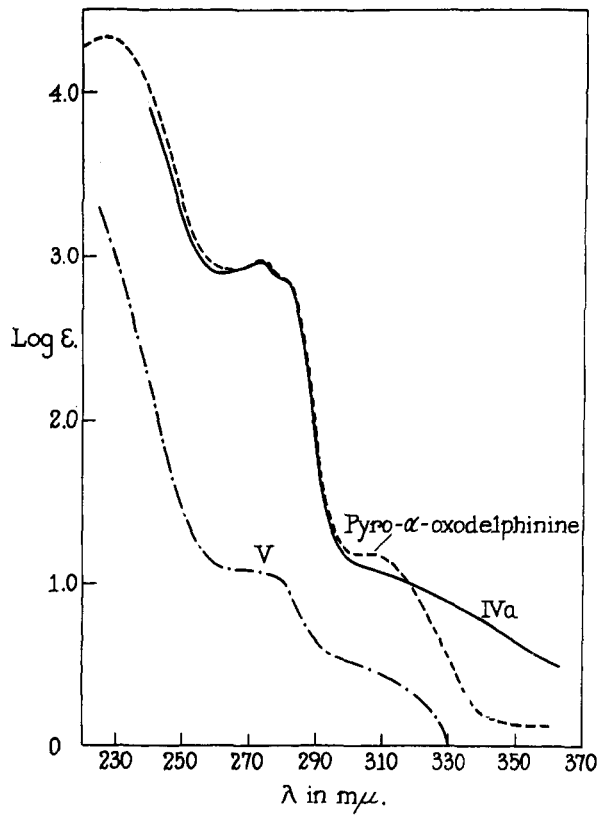


Fig. 2.—Ultraviolet absorption spectra, in ethanol: IVa, the benzoxydihydroxydimethoxylactam carboxylic acid methyl ester; V, the trihydroxydimethoxylactam carboxylic acid methyl ester.

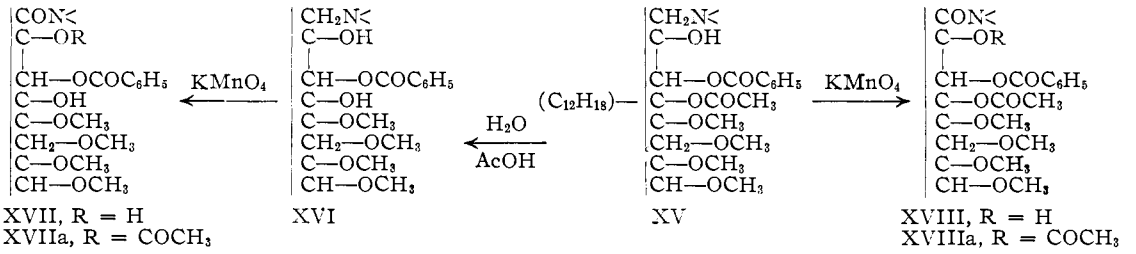
$C_{33}H_{39}NO_{10}$ (X).[6] More recently it has been possible to completely demethylate both of these derivatives with zinc chloride, as in the case of isopyroöxodelphinine, to give desmethylanhydroisopyroöxodelphinine (XI). Furthermore, when the dichlorodimethoxy compound was boiled in dilute acetone, both chlorine atoms were replaced by hydroxy groups to give the benzoxytrihydroxydimethoxylactam, $C_{29}H_{35}NO_8$ (XII). The latter is therefore partially demethylated isopyroöxodelphinine. Oxidation of this compound with chromic acid has yielded both the aldehyde III, and the corresponding acid IV. The direct relationship of these products to isopyroöxodelphinine is thus demonstrated. This relationship was demonstrated in still another way. Hydrogenation of the aldehyde III yielded the octahydrobenzoxytrihydroxydimethoxylactam, $C_{29}H_{43}NO_8$ (XIII), in which the double bond and benzoyl group have been hydrogenated and the carbonyl group reduced to a carbinol. The same substance resulted from the hydrogenation of the benzoxytrihydroxydimethoxylactam (XII). Removal of the hexahydrobenzoyl group from the hydrogenation product in turn gave the dihydrotetrahydroxydimethoxylactam, $C_{22}H_{33}NO_7$ (XIIIa), the melting point of which proved to be unusually high (313.5–315°).

The ultraviolet absorption data presented in Figs. 1 and 2 are in general agreement with the conclusions which have been drawn. Thus in Fig. 1 the curve of the benzoxyaldehyde III shows a peak at 273 mμ, attributed to the benzoxy group, and a second, lower peak at 330–360 mμ which possibly represents a displaced aldehydic carbonyl band. The saponification product IIIa no longer shows benzoxy absorption but does show absorption at 260–290 mμ characteristic of the aldehydic carbonyl. The octahydro derivative of III (XIII) as expected shows no characteristic absorption. The curve of the benzoxycarboxylic methyl ester (Fig. 2, IVa) shows absorption due to the benzoxy group and is practically identical with that obtained with the parent pyro-α-oxodelphinine (isopyroöxodelphinine is too insoluble to get absorption data). The curve of methyl ester V, obtained by saponification of IVa, shows a low shoulder at 265–285 mμ.

It was anticipated that the hexahydrobenzoxycarbinol (XIII) on treatment with zinc chloride would yield by simple dehydration the same anhydro derivative VIII which had been previously[4] obtained from the closely related, fully methylated octahydroisopyroöxodelphinine (VII). The latter with zinc chloride had given a dimethoxyanhydro derivative, $C_{29}H_{41}NO_7$ (VIII), owing to dehydration accompanying partial demethylation. However, it was found that XIII was merely isomerized when treated with zinc chloride.

A study of the chromic acid oxidation of the trihydroxydimethoxylactam carboxylic methyl ester,

(6) W. A. Jacobs and C. F. Huebner, *J. Biol. Chem.*, **170**, 209 (1947).



XVII, R = H
XVIIa, R = $COCH_3$

XVI

XV

XVIII, R = H
XVIIIa, R = $COCH_3$

$C_{23}H_{31}NO_8$ (V), has shown an expected parallelism with the results obtained in earlier work with isopyroöxodelphonine.[2] As with the latter, oxidation apparently occurred between the vicinal tertiary and secondary hydroxyl (from the benzoxy ester) groups to give an amorphous ketoacid, $C_{23}H_{29}NO_9$ (XIV).

The foregoing experiments with the isopyroöxodelphinine derivatives show that the methoxyl groups, in the order in which they are demethylated, are derivatives of a tertiary, a primary, a tertiary and finally a secondary alcohol. Furthermore, the free hydroxyl of delphinine is tertiary and vicinal to a secondary hydroxyl which is benzoylated.

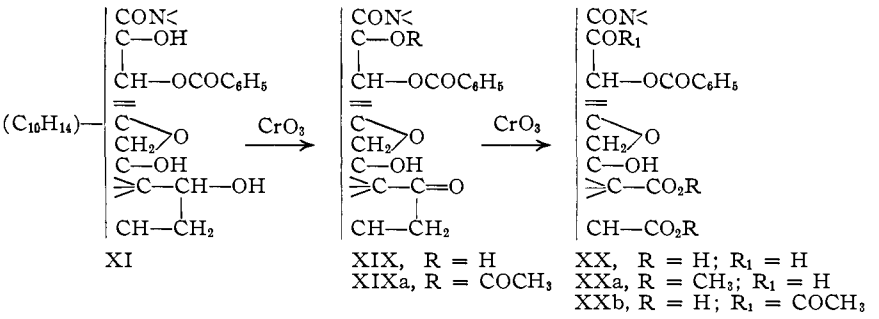
There remained the determination of the character of the hydroxyl group which is acetylated in delphinine. α-Oxodelphinine (XVIII) was first selected for this study but it was soon apparent that selective saponification of this substance was not feasible. With the use of aqueous neutral solvents or of one mole of alkali there was not sufficient difference in the ease of hydrolysis between the benzoxy and acetoxy groups. However, with delphinine (XV) itself, it was found possible to use a method somewhat similar to that already employed in the study of aconitine.[7] This consisted in the hydrolysis of delphinine in water containing one equivalent of acetic acid. The resulting benzoyldelphonine, $C_{31}H_{43}NO_8$ (XVI), was readily separated from unhydrolyzed delphinine over alumina. This substance and its hydrochloride were obtained only in an amorphous form. However, when it was oxidized with potassium permanganate, a crystalline neutral lactam, benzoyloxodelphonine (XVII) with the formulation $C_{31}H_{41}NO_9$ was obtained. Attempted oxidation of the latter with chromic acid gave unchanged starting material and obscure oxidation products. All evidence points to the tertiary character of the hydroxyl group which results from deacetylation. Acetylation with boiling acetic anhydride yielded a monoacetylbenzoxyloxodelphonine, $C_{33}H_{43}NO_{10}$ (XVIIa) (m.p. 155–156°; $[\alpha]D$ −34.2° in CH_3OH) which proved to be isomeric with α-oxodelphinine (XVIII). It is reasonably certain that in XVII the original, free, tertiary hydroxyl group of the alkaloid has been acetylated and not the hydroxyl which arises on deacetylation of delphinine. Recent studies of derivatives of delphinine, containing the original, free tertiary hydroxyl group, have shown that the latter is acetylated when heated with acetic anhydride. Thus, contrary to early experience in this Laboratory,[3] the tertiary hydroxyl of α-oxodelphinine is readily acetylated to give acetyl-α-oxodelphinine, $C_{35}H_{45}NO_{11}$ (XVIIIa), (m.p. 239–243°, $[\alpha]D$ −23.2° in methanol). Similarly, isopyroöxodelphinine (I) yields the acetyl derivative, $C_{33}H_{41}NO_9$ (m.p. 145–147°). In the case of benzoyldelphonine the position as well as the configuration of the new free hydroxyl group remains to be unambiguously proved. It is possible that rather than being a simple hydrolysis product of delphinine, it results from the addition of water in a different direction to an intermediate unsaturated compound which may arise by elimination of acetic acid during the reaction.

As previously discussed,[4] the last methoxyl group to be demethylated in isopyroöxodelphinine (I) exposes a secondary hydroxyl group since the resulting desmethylanhydroisopyroöxodelphinine (XI) furnished a desmethylanhydro ketone, $C_{27}H_{27}NO_7$ (XIX), on oxidation. Recently further oxidation studies with the latter have been made. Oxidation with chromic acid proceeds gradually to furnish a good yield of a dibasic acid, $C_{27}H_{27}NO_{10}$ (XX), $[\alpha]D$ −6° (50% EtOH) which consumed two equivalents of alkali on direct titration and another equivalent during saponification of the benzoyl group. The dibasic acid resulting from saponification and the corresponding dimethyl ester were obtained only in amorphous form. A report on these compounds and on their further oxidation will be reserved for a later communication. On the other hand, the dimethyl ester XXa of the $C_{27}H_{27}NO_{10}$ acid readily crystallized, m.p. 175–176°, $[\alpha]D$ +27.5° (CH_3OH). Owing to the relative resistance to saponification of one of the ester groups only two equivalents of alkali were consumed when heated with 0.1 *N* alkali.

In an effort to determine the size of the ring opened by this oxidation, unsuccessful attempts to pyrolyze the acid XX and its ester XXa were made. Similarly unfruitful were preliminary attempts to form an anhydride by heating XX with acetic anhydride. This resulted only in acetylation of the tertiary hydroxyl group to give an amorphous acetate of the dibasic acid, $C_{29}H_{29}NO_{11}$ (XXb). As a check on this acylation procedure, treatment of the desmethylanhydro ketone (XIX), under identical conditions gave an acetate, $C_{29}H_{29}NO_8$ (XIXa), m.p. 251–252°. The comparative stability of one of the ester groups of the dimethyl ester XXa toward alkali suggests its tertiary character. If such is the case, the intermediate carbonyl and therefore its precursor, the most resistant methoxyl group of delphinine, is attached to a carbon which is vicinal to a quaternary carbon atom.

Further discussion of the probable ring system in these alkaloids will be deferred to a report of work now in progress. A revision favoring a

(7) H. Schulze, *Arch. Pharm.*, **244**, 136, 165 (1906).



XI XIX, R = H; XIXa, R = $COCH_3$ XX, R = H; R_1 = H; XXa, R = CH_3; R_1 = H; XXb, R = H; R_1 = $COCH_3$

saturated hexacyclic structure over the unsaturated system suggested some years ago[8] may be necessary.

Experimental[9]

The Oxidation of Isopyroöxodelphinine (I) to the Benzoxydihydroxydimethoxyaldehydolactam, $C_{29}H_{33}NO_8$ (III).—The method employed for the nitric acid oxidation of pyro-α-oxodelphinine was used essentially as described[3] except that some alterations in the isolation procedure were made.

Thus 1.0 g. was dissolved in 10 ml. of nitric acid (d. 1.42) and warmed at 50° for 1 hour. The chilled reaction mixture when diluted with 40 ml. of water yielded material which readily crystallized. After standing at 0°, the mixture was diluted with an additional 40 ml. of water and the product was collected with water. Ten grams of starting material yielded 6.16 g. of product. The latter was digested at room temperature with a small volume of acetone which removed a yellow contaminant. The undissolved crystals when collected with acetone weighed 4.32 g., m.p. 295–296.5°.

The dilute acidic filtrate was then extracted 5 times with a total of 1500 ml. of chloroform. The extract was shaken with sufficient dilute sodium bicarbonate solution to remove free acid, dried and then concentrated *in vacuo*, to give 2.5 g. of residue. This readily crystallized from acetone when seeded and after collection weighed 1.17 g., m.p. 296–297°. When boiled in a large volume of acetone the product dissolved on the gradual addition of a minimal amount of water. After concentration to smaller volume it again crystallized as larger crystals and often as triangular platelets, m.p. 299–301° after sintering at 290°, although some samples showed m.p. 301–305° after sintering at 290°. This high melting form was solvent free, $[\alpha]^{27}D$ −70° (*c* 1.03 in pyridine).

Anal. Calcd. for $C_{29}H_{33}NO_8$: C, 66.52; H, 6.35; OCH_3, 11.86. Found: C, 66.35, 66.84; H, 6.60, 6.35; OCH_3, 11.91, 12.07.

When dissolved in boiling methanol and partly concentrated it gradually crystallized as minute prisms which retained solvent, even at 120° and 0.2 mm., m.p. 246–248°.

Anal. Calcd. for $C_{29}H_{33}NO_8 \cdot CH_3OH$: C, 64.85; H, 6.71; OCH_3, 16.76. Found: C, 64.45; H, 6.51; OCH_3, 16.48.

When this form was dissolved in a large volume of boiling acetone and concentrated it separated again in solvent free form, m.p. 307–313°.

A study of this substance was at first complicated by melting point irregularities. As originally recorded it sintered to a resin at 200° and melted gradually at 235–240°. In more recent studies it occasionally melted at 199–201° and other times it sintered at 140° and partly melted (10%) at 215–216°, with the major part melting at 247–249°. After recrystallization from acetone it then melted at 245–246°. An equal mixture of the latter with the 199° material melted at 241–246°.

When a solution of the low melting substance in a small volume of chloroform was added to a column of alumina suspended in benzene and then eluted with a 1:1 benzene–chloroform mixture, material appeared very gradually. It was speeded by addition of 1% methanol to the solvent mixture. The material recovered from the initial, intermediate, and later fractions all proved to be solvent free and melted about 300°. When this had once been obtained it was found in all later experiments that such high-melting, solvent-free material was the form in which III directly crystallized from acetone without chromatographing, as described above.

The aldehydo derivative (0.1 g.) readily dissolved in the cold in 2 ml. of HCl (sp. gr. 1.19). After 1 hour at room temperature the solution was chilled and diluted. The precipitate soon crystallized; yield 85 mg. When dissolved in a good volume of acetone and concentrated the first fraction yielded 61 mg. of unchanged aldehyde, m.p. 301–303.5° after slight preliminary sintering.

Anal. Calcd. $C_{29}H_{33}NO_8$: C, 66.52; H, 6.35. Found: C, 66.16; H, 6.21.

The Oxime.—The aldehyde (50 mg.) was refluxed for 4 hours in methanol with sufficient hydroxylamine hydrochloride and sodium acetate. After concentration and addition of water it readily crystallized; 44 mg. It separated from methanol as short stout prisms which contained solvent. This was not readily removed on drying at 120°; m.p. 272.5–274°. The desiccator-dried substance showed 1 mole of methanol.

Anal. Calcd. for $C_{29}H_{34}N_2O_8 \cdot CH_3OH$: OCH_3, 16.32. Found: OCH_3, 16.03.

When recrystallized by solution in acetone followed by concentration and dilution with water, it formed a microcrystalline powder which melted 277–283°. For analysis it was dried at 120° and 0.2 mm.

Anal. Calcd. for $C_{29}H_{34}N_2O_8$: C, 64.67; H, 6.36; N, 5.20; OCH_3, 11.52. Found: C, 64.90; H, 6.51; N, 4.86; OCH_3, 11.63.

The Saponification of III to the Trihydroxydimethoxyaldehydolactam, $C_{22}H_{29}NO_7$ (IIIa).—When 0.2 g. of the aldehyde (III) was treated with 4 ml. of methanol and 2 ml. of *N* NaOH and occasionally warmed, it gradually dissolved. After 2.5 hours the slightly colored solution was diluted with 2 ml. of water and the methanol removed *in vacuo*. The mixture was extracted 17 times with chloroform, which after washing with a minimum of water and drying, yielded 0.113 g. of amorphous residue. When dissolved in a small volume of acetone it soon crystallized without solvent as micro-needles or flat six-sided prisms, m.p. 231–232°, $[\alpha]^{29}D$ −12° (*c* 0.72 in methanol).

Anal. Calcd. for $C_{22}H_{29}NO_7$: C, 62.99; H, 6.97. Found: C, 62.72; H, 6.95.

The ultraviolet absorption curve given in Fig. 1 was obtained with the dry amorphous powder before crystallization from acetone to avoid the possibility that the absorption could be attributed to solvent.

The Oxidation of III to the Benzoxydihydroxydimethoxylactam Carboxylic Acid, $C_{29}H_{33}NO_9$. A. With Chromic Acid.—Two grams of the aldehyde derivative (III) was dissolved in a mixture of 60 ml. of acetic acid, 10 ml. of water, and 10 ml. of Kiliani chromic acid solution.[10] Oxidation proceeded very gradually at room temperature and after 20 hours, although some reagent remained, the mixture was

(8) L. C. Craig, L. Michaelis, S. Granick and W. A. Jacobs, *J. Biol. Chem.*, **154**, 293 (1944).

(9) Melting points were taken with a hot-stage microscope equipped with a polarizer. The thermometer was calibrated against known compounds.

(10) This is a solution of 53 g. of chromic oxide and 80 g. of sulfuric acid in 400 ml. of water.

diluted with 360 ml. of water. It was extracted 8 times with 40-ml. portions of chloroform and then cleared with sodium sulfate. Concentration *in vacuo* yielded 1.8 g. The latter was dissolved in a few ml. of acetone and carefully diluted with water. The resinous material which separated could not be made to crystallize until most of the acetone had been removed and then only when seeded. It formed aggregates of micro, boat-shaped or small pointed platelets, m.p. 220–221.5°. When freshly obtained, the material melted occasionally at 211° but after long standing showed the higher melting point. Whether this was due to incomplete crystallization or retention of solvent was not determined. For analysis a small portion was suspended in water and dissolved by the addition of sufficient ammonia. The clear solution when treated with acetic acid yielded a resinous suspension which crystallized as micro, diamond-shaped platelets when warmed; m.p. 219–223°, $[\alpha]^{26}D$ −5.4° (*c* 1.02 in 50% EtOH). For analysis it was dried at 110° and 0.2 mm.

Anal. Calcd. for $C_{29}H_{33}NO_9$: C, 64.55; H, 6.17; OCH_3, 11.50. Found: C, 64.20, 64.29; H, 6.00, 6.17; OCH_3, 11.46.

1.227 mg. in 0.05 ml. of EtOH on direct titration against phenolphthalein required 0.0232 ml. of 0.1 *N* NaOH. After refluxing for 2.25 hours with excess reagent an additional 0.0193 ml. was consumed. Calcd. for 1 equivalent: 0.0227 ml.

B. With Potassium Permanganate.—A solution of 50 mg. of the aldehyde III in a warm mixture of 5 ml. of acetone and 0.5 ml. of water was cooled and treated with 0.22 ml. of a fresh 5% permanganate solution. This contained about 13% in excess of one equivalent of oxygen. After several hours at room temperature the reagent was consumed. The concentrated filtrate and washings were acidified and extracted with chloroform. The chloroform extract was in turn extracted twice with a solution of 10 mg. of sodium bicarbonate. Acidification of the latter with dilute sulfuric acid and extraction with chloroform yielded 40 mg. When purified as described the acid melted at 219–220°.

Anal. Found: C, 64.28; H, 6.34.

The Methyl Ester (IVa).—This ester was prepared by treating a solution of the acid IV in acetone with diazomethane. It crystallized readily from acetone without solvent as rather sparingly soluble, micro prisms or triangular platelets; m.p. 267–272°, $[\alpha]^{25}D$ −3.7° (*c* 0.95 in methanol).

Anal. Calcd. for $C_{30}H_{35}NO_9$: C, 65.08; H, 6.37. Found: C, 65.24; H, 6.28.

Only the benzoyl group was readily saponified with alkali. For the titration 1.558 mg. in a mixture of 0.05 ml. of EtOH and 0.10 ml. of 0.1 *N* NaOH was refluxed for 2.5 hours and back titrated against phenolphthalein: 0.0254 ml. was consumed; calcd. for 1 equivalent, 0.0281 ml.

This ester was recovered unchanged after treatment with hydroxylamine.

Saponification of IV to the Trihydroxydimethyloxylactam Carboxylic Acid, $C_{22}H_{29}NO_8$ (Va).—A solution of 0.25 g. of the previous acid IV in 2.5 ml. of *N* NaOH was left at room temperature for 2 hours. After addition of 1 equivalent of *N* H_2SO_4, the color became paler. After the benzoic acid was extracted with petroleum ether, addition of several volumes of acetone precipitated most of the sodium sulfate. The concentrated filtrate yielded a resin which was extracted from residual impurities with boiling acetone. The combined extracts when concentrated yielded an amorphous residue. All attempts to induce crystallization were unsuccessful. For analysis it was dried at 110° and 0.2 mm.

Anal. Calcd. for $C_{22}H_{29}NO_8$: C, 60.68; H, 6.71. Found: C, 60.87; H, 6.90.

The Methyl Ester (V).—A suspension of 0.4 g. of methyl ester IVa in 4.0 ml. of *N* NaOH and 4.0 ml. of methanol was left at room temperature with occasional stirring. Although solution was complete in about 5 minutes, the solution was left for 1 hour before the addition of 1 equivalent of H_2SO_4. Most of the methanol was removed *in vacuo* and the mixture was extracted with petroleum ether to remove benzoic acid. Although during the operation partial crystallization occurred, the mixture was treated with acetone to precipitate sodium sulfate. The concentrated filtrate yielded a residue from which the product was again extracted from residual salts with boiling acetone. When concentrated to small volume crystallization occurred; yield 0.266 g. For recrystallization the substance was dissolved in boiling acetone to which a few drops of water were added and again concentrated. It separated as micro-needles or leaflets, m.p. 276–277° after preliminary sintering, $[\alpha]^{26}D$ −2° (*c* 0.95 in methanol).

Anal. Calcd. for $C_{23}H_{31}NO_8$: C, 61.45; H, 6.95. Found: C, 61.29; H, 6.89.

Hydrogenation of IVa to the Octahydrobenzoxydihydroxydimethoxylactam Carboxylic Methyl Ester (VI).—The benzoxy methyl ester (IVa) when hydrogenated with platinum oxide in methanol absorbed about 4 moles in 80 minutes, with slight additional absorption when continued overnight. The filtered reaction mixture yielded material which was at first difficult to crystallize but, when once obtained, separated from benzene or acetone as needles, m.p. 183.5°, $[\alpha]^{27}D$ −68° (*c* 1.04 in methanol). For analysis it was dried at 110° at 0.2 mm.

Anal. Calcd. for $C_{30}H_{43}NO_9$: C, 64.15; H, 7.72. Found: C, 64.02; H, 7.64.

Attempted Demethylation of IV.—When 60 mg. of IV was treated with a solution of 2.4 g. of zinc chloride in 0.84 ml. of 5% hydrochloric acid a resin formed. When heated at 40° for 1 hour solution was practically complete. The cooled, diluted solution required about 200 ml. of chloroform for extraction of all flocculent material. Concentration of the cleared extract gave the theoretical recovery. The substance was precipitated with water from acetone as a resin and only after removal of the acetone was crystallization of starting material induced, m.p. 218–221°. For analysis it was dried at 120° and 0.2 mm.

Anal. Calcd. for $C_{29}H_{33}NO_9$: C, 64.55; H, 6.17; OCH_3, 11.50. Found: C, 64.23; H, 6.09; OCH_3, 11.51.

The Oxidation of V to the Ketoacid, $C_{23}H_{29}NO_9$ (XIV).—A solution of 0.1 g. of V in 3 ml. of acetic acid was carefully treated with Kiliani chromic acid solution.[10] Although the reaction slowed up after about 0.30–0.35 ml. had been added, a total of 0.40 ml. was used. The diluted reaction mixture was repeatedly extracted with chloroform and the dried extract was concentrated to dryness *in vacuo;* yield 80 mg. of amorphous residue. A chloroform solution of this was carefully extracted with the minimum of sodium bicarbonate solution and the latter with washings was quickly reacidified with dilute H_2SO_4. No precipitate appeared and the solution was re-extracted repeatedly with chloroform. The latter on concentration yielded 60 mg. of residue. Since all attempts at crystallization failed, the amorphous powder was analyzed as such after drying at 120° and 0.2 mm.

Anal. Calcd. for $C_{23}H_{29}NO_9$: C, 59.60; H, 6.31. Found: C, 59.83; H, 6.46.

1.458 mg. of substance in 0.1 ml. of 50% EtOH was directly titrated against phenolphthalein with 0.1 *N* NaOH. Calcd. for 1 equivalent: 0.0315 ml. Found: 0.0273 ml. An excess of 0.1 *N* NaOH was added and heated at 100° for 2.5 hours and back titrated. Found: 0.0022 ml.

Demethylation of the Diacetoxy Derivative $C_{33}H_{39}NO_{10}$ (X) to Desmethylanhydroisopyroöxodelphinine (XI).—The diacetoxy derivative (X) used in the following reaction was prepared as previously described[8] with the exception that for its isolation, extraction of the product was not necessary. On seeding the diluted reaction mixture the product from 0.4 g. of starting material gradually separated as a microcrystalline powder; m.p. 299.5–303.5°, yield 0.27 g., $[\alpha]^{26}D$ −72° (*c* 1.38 in pyridine), $[\alpha]^{26}D$ −31° (*c* 0.91 in methanol).

Anal. Calcd. for $C_{33}H_{39}NO_{10}$: C, 65.01; H, 6.45. Found: C, 65.17; H, 6.33.

Addition of 0.1 g. of X to a solution of 4 g. of zinc chloride in 1.4 ml. of 5% hydrochloric acid gave a resin which gradually dissolved when heated at 60°. After 90 minutes the cooled solution was extracted 4 times with chloroform and yielded 29 mg. of residue which was not further studied. Further continuous extraction with chloroform for 43 hours yielded a gelatinous mass which was dried *in vacuo*. A solution of the material in warm acetone when seeded gave a wooly mass of delicate needles which was collected with acetone; yield 46 mg., m.p. 288–291°, $[\alpha]^{26}D$ −45° (*c* 0.80 in 50% EtOH); this substance was identical with the anhydro derivative ($[\alpha]$ −48.5°) previously obtained by the demethylation of isopyroöxodelphinine.[4] A mixture with

the latter (m.p. 290–291°)[11] melted at 288.5–290°. For analysis the substance was dried at 80° and 0.2 mm.

Anal. Calcd. for $C_{27}H_{29}NO_7$: C, 67.63; H, 6.10. Found: C, 66.87; H, 6.02.

Demethylation of the Dichloro Derivative $C_{29}H_{33}NO_6Cl_2$ (IX) to XI.—The dichloro derivative (IX) (0.1 g.) was heated at 60° for 70 minutes with a solution of 4 g. of zinc chloride in 1.4 ml. of 5% hydrochloric acid. The solution remained clear but became somewhat colored. The clear, diluted mixture was first extracted 6 times with chloroform. The latter yielded 57 mg. of residue which partly crystallized from acetone, m.p. 250–254.5°, and gave a strong positive halogen test. The analysis indicated it consisted largely of unchanged starting material.

Anal. Calcd. for $C_{29}H_{33}NO_6Cl_2$: C, 61.92; H, 5.91. Found: C, 62.74; H, 6.20.

The aqueous phase when extracted 8 times with chloroform yielded an additional 5 mg. of material. Continuous extraction of the aqueous phase with boiling chloroform yielded an appreciable fraction. From acetone this separated at first in gelatinous form, but gradually crystallized as a wooly mass of delicate needles; 18 mg., m.p. 286–289°. The mother liquor on further concentration gradually yielded an additional 10 mg. of less pure material. The main fraction was directly analyzed and found to be free of methoxyl and chlorine. This substance, m.p. 289–290°, was identical with the anhydro derivative prepared by demethylation of isopyroöxodelphinine.[4] The mixture with authentic material[11] melted at 288.5–290.5°, $[\alpha]^{27}D$ −48° (*c* 0.50 in 50% EtOH); previously found with desmethylanhydroisopyrooxodelphinine, −48.5°.

Anal. Calcd. for $C_{27}H_{29}NO_7$: C, 67.63; H, 6.10. Found: C, 66.88; H, 6.28.

Conversion of IX to the Benzoxytrihydroxydimethoxylactam, $C_{29}H_{35}NO_8$ (XII).—A suspension of 0.53 g. of the dichloro derivative IX[3] was refluxed in a mixture of 50 ml. of acetone and 50 ml. of water. Although complete solution gradually occurred overnight, the operation was continued for 49 hours. After addition of 25 ml. of water, the acetone was boiled off and on cooling the product crystallized as short, flat, micro-needles or rods. After collection with water the yield of halogen-free product was 0.37 g., m.p. 284–286.5°, $[\alpha]^{25}D$ −55.5° (*c* 0.77 in pyridine), $[\alpha]^{26}D$ 0.0° (*c* 0.52 in methanol). For analysis it was dried at 20° and 0.2 mm.

Anal. Calcd. for $C_{29}H_{35}NO_8$: C, 66.27; H, 6.71. Found: C, 66.50; H, 6.54.

Oxidation of XII to III and IV.—A solution of 85 mg. of XII in 4 ml. of acetic acid and 0.4 ml. of water was treated with 0.1 ml. of Kiliani solution[10] (about 1 mole of oxygen). In 5 to 10 minutes oxidation was complete and the diluted mixture was extracted with chloroform. The solvent was removed from the dried extract *in vacuo* with the aid of benzene. A solution of the residue in a small volume of acetone readily crystallized as a micro-crystalline powder when seeded; 38 mg., m.p. 299.5–304.5°, $[\alpha]^{29}D$ −67° (*c* 0.73 in pyridine). It was indistinguishable in properties from the aldehyde III obtained by oxidizing pyro-α-oxodelphinine with nitric acid.

Anal. Calcd. for $C_{29}H_{33}NO_8$: C, 66.52; H, 6.35. Found: C, 66.13; H, 6.26.

In an experiment in which an excess of reagent was used, but without waiting for completion of the reaction, a mixture of the above aldehydo derivative III and the corresponding acid IV was obtained. The acid fraction was carefully extracted with dilute ammonia and reprecipitated by prompt acidification with acetic acid. The initial resinous material gradually crystallized after several days standing. The collected material melted at 211–214°. For analysis it was dried at 120° and 0.2 mm.

Anal. Calcd. for $C_{29}H_{33}NO_9$: C, 64.55; H, 6.17. Found: C, 64.55; H, 6.29.

The Octahydrobenzoxytrihydroxydimethoxylactam, $C_{29}H_{43}NO_8$ (XIII). **A. By Catalytic Reduction of Aldehyde III.**—A solution of 0.1 g. of III in acetic acid was hydrogenated with 50 mg. of platinum oxide catalyst. Absorption progressed steadily and after several hours about 5 moles had been absorbed in excess of the catalyst requirement. The filtrate from the catalyst with chloroform washings was concentrated *in vacuo*. The residue rapidly crystallized in acetone as sparingly soluble, compact aggregates of micro-crystals, yield 86 mg. The product was sparingly soluble in the usual neutral solvents. It was dissolved in acetic acid and after concentration *in vacuo* it was induced to crystallize by addition of acetone, m.p. 286.5–290.5°. It separated also from methanol on concentration as foursided, micro-platelets, $[\alpha]^{27}D$ −60° (*c* 0.495 in methanol).

Anal. Calcd. for $C_{29}H_{43}NO_8$: C, 65.27; H, 8.12. Found: C, 65.20; H, 8.01.

B. By Catalytic Reduction of XII.—In a similar manner 90 mg. of the benzoxytrihydroxydimethoxylactam XII was hydrogenated in acetic acid. The reaction was completed in 45 minutes after absorption of about 4 moles. The product crystallized readily and was indistinguishable from the substance obtained by the previous route; yield 60 mg., m.p. 287–292.5°, $[\alpha]^{27}D$ −60° (*c* 0.50 in methanol). When mixed with the previous substance no depression in melting point was observed.

Anal. Calcd. for $C_{29}H_{43}NO_8$: C, 65.27; H, 8.12. Found: C, 64.90; H, 8.00.

Saponification of XIII to the Dihydrotetrahydroxydimethoxylactam, $C_{22}H_{33}NO_7$ (XIIIa).—A suspension of 0.1 g. of XIII (obtained from III) in 2 ml. of methanol and 1 ml. of *N* NaOH gradually dissolved when warmed at 40–45°. After about 75 minutes at room temperature 1 equivalent of *N* sulfuric acid was added and most of the methanol was removed *in vacuo*. An additional 0.25 ml. of *N* H_2SO_4 was added and the hexahydrobenzoic acid was extracted with petroleum ether. The aqueous phase was neutralized with 0.25 ml. of *N* NaOH and then treated with several volumes of acetone. The concentrated filtrate yielded needles of XIIIa which were collected with water in which it is appreciably soluble; m.p. 313.5–315°, $[\alpha]^{26}D$ −38° (*c* 0.47 in methanol).

Anal. Calcd. for $C_{22}H_{33}NO_7$: C, 62.39; H, 7.85. Found: C, 62.60; H, 7.75.

The Attempted Demethylation of XIII with Zinc Chloride.—The octahydro derivative (0.1 g., XIII) was treated with a solution of 4 g. of zinc chloride in 1.4 ml. of 5% HCl. The resinous material very gradually dissolved at 40° when constantly worked with a rod. Solution was complete after 50 minutes. The clear solution was diluted and continuously extracted with chloroform. After several hours the concentrated extract yielded about 0.1 g. of material which formed a gelatinous mass in acetone. Neither did it crystallize from a small volume of methanol when seeded with starting material. On dilution with water it gradually separated as micro-needles, 24 mg., m.p. 152°. When recrystallized by dilution of a methanol solution, it slowly separated as micro-crystals which were free of halogen; m.p. 150°, $[\alpha]^{28}D$ −78° (*c* 0.48 in methanol). For analysis it was dried at 110° and 0.2 mm.

Anal. Calcd. for $C_{29}H_{43}NO_8$: C, 65.27; H, 8.12. Found: C, 65.37; H, 8.17.

The mother liquor yielded on long standing a second fraction of 8 mg., m.p. 152–153° after sintering at 148°.

The filtrate from the second fraction on evaporation in the desiccator gradually deposited apparently amorphous or gelatinous material which contained some crystals. After longer standing it was suspended for collection in a minimum of water; 42 mg. It gradually melted at 145–153° after sintering about 130°, $[\alpha]^{25}D$ −108° (*c* 0.48 in methanol). The analysis and rotation showed this to be all or in part of isomeric character.

Anal. Found: C, 65.81; H, 7.80.

Benzoyldelphonine (XVI).—A suspension of 4 g. of delphinine (XV) in 100 ml. of water containing 6.7 ml. (about 1 mole) of *N* acetic acid was refluxed for 6 hours. Since some of the alkaloid was still undissolved the heating was continued on the steam-bath for an additional 17 hours. The clear, chilled solution was treated with a solution of 1.25 g. of sodium bicarbonate and extracted with chloroform. The latter yielded 3.85 g. of amorphous base. Its solution in benzene was chromatographed through 120 g. of alumina,

(11) The m.p. of desmethylanhydroisopyroöxodelphinine was originally recorded as 299–300°. The m.p. has since been found to be dependent on the conditions of crystallization, the rate of heating and the temperature at which the sample is introduced on the hot-stage. The above values were obtained by placing a sample on the stage at 280° and raising the temperature at the rate of 2° per minute.

Following elution of the column with 250 ml. of benzene and 250 ml. of 1% methanol in benzene, material began to appear in the eluant. The next 105 ml. furnished 0.80 g. of material consisting partly of unchanged delphinine. The following 20 ml. gave 0.28 g. of material which was discarded. Continued elution with 160 ml. of solvent gave 2.35 g. of benzoyldelphonine. All attempts to crystallize the deacetylated base were unsuccessful and analytical data were obtained with the amorphous substance; $[\alpha]^{27}D$ +33° (*c* 1.0 in EtOH). For the analysis it was dried at 100° and 0.2 mm.

Anal. Calcd. for $C_{31}H_{43}NO_8$: C, 66.76; H, 7.77. Found: C, 66.45; H, 7.66.

Attempts to prepare a crystalline hydrochloride also failed. The latter was obtained as an amorphous powder by precipitation from acetone solution with ether.

Anal. Calcd. for $C_{31}H_{43}NO_8 \cdot HCl$: C, 62.67; H, 7.46; Cl, 5.97. Found: C, 62.90; H, 7.15; Cl, 5.89.

Benzoyloxodelphonine (XVII).—A solution of 0.96 g. of benzoyldelphonine (XVI) in 100 ml. of acetone containing 0.96 ml. of acetic acid was treated with 0.58 g. of potassium permanganate and left at 30° for one day. The colorless concentrated filtrate was evaporated *in vacuo* with the aid of benzene and redissolved in chloroform. The latter after washing with dilute sulfuric acid and water, drying, and concentrating *in vacuo*, gave nearly a quantitative yield of neutral material. This crystallized gradually from a small volume of acetone as flat needles or blades. Because of its appreciable solubility the substance was collected in the cold with acetone. It was recrystallized from acetone, m.p. 224–225°, after sintering at 150–152°. For analysis it was dried at 110° and 0.2 mm.

Anal. Calcd. for $C_{31}H_{41}NO_9$: C, 65.13; H, 7.23. Found: C, 64.83; H, 7.13.

Because of persistently slightly low carbon analyses, the substance was purified by chromatographing in benzene through alumina with subsequent elution with 1% methanol in benzene. The major portion was recovered in practically a single band and was recrystallized from acetone; it partly melted at 152–155°, resolidified, and then melted at 224–227°; $[\alpha]^{25}D$ −27.4° (*c* 0.92° in 95% ethanol).

Anal. Found: C, 65.02; H, 7.31.

For titration 1.808 mg. was refluxed for 3 hours in 0.1 ml. of ethanol and 0.1206 ml. of 0.1 *N* NaOH and back titrated against phenolphthalein. Calcd. for 1 equivalent: 0.0316 ml. Found: 0.028 ml.

The Monoacetyl Derivative XVIIa.—Benzoyloxodelphonine (70 mg.) was boiled in acetic anhydride for 5 hours. After removal of solvent and solution in acetone all attempts to induce crystallization with α-oxodelphinine failed. When slowly evaporated the solution deposited delicate needles. After resuspension in a minimum of ether or acetone, in which it proved quite soluble, 17 mg. was collected, m.p. 155–156°, $[\alpha]^{29}D$ −34.2° (*c* 0.76 in methanol). This material is isomeric with α-oxodelphinine. For analysis it was dried at 110° and 0.2 mm.

Anal. Calcd. for $C_{33}H_{43}NO_{10}$: C, 64.58; H, 7.06. Found: C, 64.39; H, 6.84.

After heating for 2.5 hours with excess 0.1 *N* NaOH, 1.89 mg. used 0.0594 ml.; calcd. for 2 equivalents, 0.0616 ml.

Acetyl-α-oxodelphinine (XVIIIa).—α-Oxodelphinine (XVIII) was boiled for 10 hours with acetic anhydride. The product formed broad needles or leaflets from acetone in which it was quite soluble. It contained solvent and under the microscope sintered at 165 and partly melted at 195–200° with subsequent crystallization and melting at 239–243°; $[\alpha]^{29}D$ −23.2° (*c* 1.10 in methanol). For analysis it was dried at 110° and 0.2 mm.

Anal. Calcd. for $C_{35}H_{45}NO_{11}$: C, 64.11; H, 6.92. Found: C, 64.17; H, 6.82.

When heated for 2.5 hours with excess 0.1 *N* NaOH, 1.682 mg. used 0.0702 ml.; calcd. for 3 equivalents, 0.0769 ml.

The Oxidation of Desmethylanhydroisopyroöxodelphinine (XI) to the Dibasic Acid, $C_{27}H_{27}NO_{10}$ (XX).—For these studies the intermediate anhydroketo derivative **XIX**[4] was first employed but most of the data were obtained with desmethylanhydroisopyroöxodelphinine (XI)[4] as starting material. A solution of 1.02 g. of the latter in a mixture of 17 ml. of acetone and 17 ml. of 10% sulfuric acid was treated with 10.5 ml. of Kiliani chromic acid solution[10] and left at room temperature. Following initial oxidation to the ketone the reaction was very gradual. After 22 hours, although a slight excess of reagent persisted, the mixture was diluted with an equal volume of water and extracted 5 times with chloroform to remove undesired material. Extraction of the aqueous phase was continued 12 times more with a total of 500 ml. of methyl propionate. The extract was cleared with sodium sulfate and concentrated to dryness *in vacuo*. After evaporating with benzene, 0.83 g. was obtained. The largely crystalline material was suspended in a small volume of acetone and collected; yield 0.714 g., m.p. 230–233°. Ten additional extractions yielded 75 mg. more. For recrystallization the acid was suspended in acetone and dissolved by heating and adding the minimum of water. By repeated concentration with acetone most of the water was again removed, followed by separation of a copious mass of delicate needles. These contained solvent and melted at 234–236° with a few crystals persisting to 242°, $[\alpha]^{27}D$ −6° (*c* 1.04 in 50% EtOH). For analysis it was dried at 140° and 0.2 mm.

Anal. Calcd. for $C_{27}H_{27}NO_{10}$: C, 61.71; H, 5.18. Found: C, 61.85, 61.45; H, 5.38, 5.40.

A mixture of 1.275 mg. of substance with 0.1 95% of ethanol on direct titration against phenolphthalein required 0.0463 ml. of 0.1 *N* NaOH; calcd. for 2 equivalents: 0.0485 ml.

When performed on a preparative scale, the saponification yielded the dibasic acid $C_{20}H_{23}NO_9$ which was obtained only in amorphous form. The dimethyl ester of the latter also could not be obtained crystalline. However, the crystalline ester of the unsaponified acid XX was obtained as follows:

The dimethyl ester XXa was prepared by treating a solution of the acid XX in acetone with diazomethane. It crystallized from methanol as rather sparingly soluble needles, which after recrystallization melted at 175–176°, $[\alpha]^{27}D$ +27.5 (*c* 0.69 in methanol). For analysis it was dried at 110° and 0.2 mm.

Anal. Calcd. for $C_{29}H_{31}NO_{10}$: C, 62.92; H, 5.65; OCH_3, 11.21. Found: C, 63.29; H, 5.69; OCH_3, 11.10.

Due to the relative resistance of one of the ester groups to saponification, only 2 equivalents of alkali was consumed when titrated as follows: a mixture of 1.794 mg. of substance with 0.1207 ml. of 0.1 *N* NaOH and 0.05 ml. of ethanol was refluxed for 2 hours and back titrated against phenolphthalein. Calcd. for 2 equivalents: 0.0648 ml. Found: 0.0631 ml.

The ester slowly and incompletely sublimed as a resinous film at 0.05 mm. in a bath held at 230–245°. The sublimate appeared to be unchanged ester.

Anal. Found: C, 63.04; H, 5.34; OCH_3, 10.95.

The Acetyl Derivative XXb.—The dibasic acid XX was refluxed for 2 hours in acetic anhydride. Concentration *in vacuo* yielded a resin which could not be crystallized.

Anal. Calcd. for $C_{29}H_{29}NO_{11}$: C, 61.37; H, 5.15. Found: C, 61.53; H, 5.11.

A mixture of 1.224 mg. in 0.05 ml. of ethanol on direct titration against phenolphthalein with 0.1 *N* NaOH required 0.0387 ml. After addition of excess reagent and heating for 2 hours, back titration showed consumption of 0.0482 ml. more of alkali; calcd. for 2 equivalents, 0.0431 ml.

The Acetate of the Desmethylanhydroketone XIX (XIXa).—To remove solvent of crystallization 0.11 g. of the previously described anhydroketone[4] XIX was first dissolved in benzene and all solvent removed *in vacuo*. The residue was boiled in 3 ml. of acetic anhydride for 2 hours. Following concentration and solution in benzene the product crystallized copiously as micro-leaflets, m.p. 251–252°. For analysis it was dried at 110° and 0.2 mm.

Anal. Calcd. for $C_{29}H_{29}NO_8$: C, 67.04; H, 5.63. Found: C, 66.96; H, 5.83.

A mixture of 1.588 mg. of substance in 0.1202 ml. of 0.1 *N* NaOH and 0.05 ml. of ethanol was refluxed for 2 hours and back titrated against phenolphthalein. Calcd. for 2 equivalents: 0.0612 ml. Found: 0.0625 ml.

Isopyroöxodelphinine Acetate.—A suspension of 0.1 g. of isopyroöxodelphinine (I) in 3 ml. of acetic anhydride was

boiled for 4.5 hours. Complete solution occurred after an hour. The clear solution after concentration *in vacuo* and evaporation with benzene yielded a resin. On long standing in dilute acetone this finally crystallized as rather soluble, delicate needles, m.p. 145–147° after preliminary sintering at 135°. For analysis it was dried at 110° and 0.2 mm.

Anal. Calcd. for $C_{33}H_{41}NO_9$: C, 66.54; H, 6.94. Found: C, 66.25; H, 7.06.

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

NEW YORK 21, NEW YORK

[CONTRIBUTION FROM NATIONAL CANCER INSTITUTE, NATIONAL INSTITUTES OF HEALTH]

Preparation of Crystalline Phosphorylated Derivatives of Vitamin B_6

BY ELBERT A. PETERSON AND HERBERT A. SOBER

RECEIVED AUGUST 13, 1953

With a view to their use in projected enzyme experiments, the following derivatives of vitamin B_6 have been prepared in crystalline form: pyridoxamine-5-phosphate, pyridoxal-5-phosphate, pyridoxine-5-phosphate and deoxypyridoxine-5-phosphate. Phosphorylation was accomplished by heating pyridoxamine or deoxypyridoxine with a mixture of phosphoric acid and phosphorus pentoxide. Pyridoxal phosphate was prepared by the oxidation of pyridoxamine phosphate with finely divided manganese dioxide at room temperature, and pyridoxine phosphate was obtained from the same compound by deamination with nitrous acid. Purification was achieved in each case by chromatography on a weak cation-exchange resin and verified by paper chromatography in several solvent systems. The effectiveness of a similar column procedure in separating the phosphates of pyridoxamine, pyridoxal and pyridoxine from each other and from their unphosphorylated counterparts has been demonstrated. The ultraviolet absorption spectra of these compounds are essentially identical, with the exception of that of pyridoxal phosphate, which is distinguished by having a maximum at 385–390 mμ in neutral or alkaline solution, as has been reported by other investigators. These esters are relatively resistant to hydrolysis; little or no inorganic phosphate was split off on storage in aqueous solution in a refrigerator or freezer for 54 days. Data are presented to show their stability in acid and in alkali at 25 and 100°. The pure compounds were obtained in good yields, ranging from 40% for pyridoxal phosphate to 85% for deoxypyridoxine phosphate.

It is generally accepted that the biological activity of vitamin B_6 is due, in great part, to the participation of a phosphorylated derivative, pyridoxal-5-phosphate[1,2] as the coenzyme in many enzymatic reactions involving amino acids, namely, transamination,[3,4] decarboxylation,[5] desmolysis,[6] racemization,[7] etc. Although these systems have received considerable attention, the cofactors involved have, until recently, only been available as impure preparations. With the recognition that a clear understanding of the mechanisms by which the cofactors operate in the various reactions cited can best be reached by the employment of such cofactors in states of high purity, we have developed procedures leading to their preparation and to those of related compounds in pure crystalline condition.

The present paper describes the preparation and some properties of the free crystalline inner salts of pyridoxal-5-phosphate (PLP), pyridoxamine-5-phosphate (PMP), pyridoxine-5-phosphate (PNP) and deoxypyridoxine-5-phosphate (DPP). The very high activity of the first two compounds, PMP and PLP, as coenzymes and of the last two PNP and DPP, as enzyme inhibitors will be described in detail in a subsequent report.[8] The preparation of crystalline PMP and its ability to activate purified pig heart apotransaminase to the same extent as crystalline PLP have been noted in preliminary reports.[9–11]

Previous Methods of Preparation.—Pyridoxamine phosphate has been prepared by heating solutions of pyridoxal phosphate with glutamic acid[12] and by treating pyridoxamine in aqueous solution with phosphorus oxychloride.[13] Viscontini, Ebnother and Karrer have reported the preparation of the crystalline hydrochloride of pyridoxamine-5-phosphate by reaction of pyridoxamine dihydrochloride with metaphosphoric acid followed by mild hydrolysis.[14]

Gunsalus and co-workers have obtained pyridoxal phosphate as the crude barium salt after treatment of pyridoxal with phosphorus oxychloride.[15] Wilson and Harris[16] have phosphorylated pyridoxamine dihydrochloride with a phosphoric acid–phosphorus pentoxide mixture at room temperature. The pyridoxamine phosphate was not isolated but was converted to the impure ammonium salt of pyridoxal phosphate by oxidation with manganese dioxide, followed by charcoal adsorption and elution with ammonia. The barium and calcium salts of pyridoxal phosphate as well as the crystalline acridine salt were prepared[17,18] by the phos-

(1) W. W. Umbreit and I. C. Gunsalus, *J. Biol. Chem.*, **179**, 279 (1949).

(2) D. Heyl, E. Luz, S. A. Harris and K. Folkers, THIS JOURNAL, **73**, 3430 (1951).

(3) H. C. Lichstein, I. C. Gunsalus and W. W. Umbreit, *J. Biol. Chem.*, **161**, 311 (1945).

(4) D. E. O'Kane and I. C. Gunsalus, *ibid.*, **170**, 425 (1947).

(5) W. W. Umbreit, W. D. Bellamy and I. C. Gunsalus, *Arch. Biochem.*, **7**, 185 (1945).

(6) W. A. Wood, I. C. Gunsalus and W. W. Umbreit, *J. Biol. Chem.*, **170**, 313 (1947).

(7) W. A. Wood and I. C. Gunsalus, *ibid.*, **190**, 403 (1951).

(8) A. Meister, H. A. Sober and E. A. Peterson, *ibid.*, **206**, 89 (1954).

(9) E. A. Peterson, H. A. Sober and A. Meister, THIS JOURNAL, **74**, 570 (1952).

(10) A. Meister, H. A. Sober and E. A. Peterson, *ibid.*, **74**, 2385 (1952).

(11) E. A. Peterson, H. A. Sober and A. Meister, *Federation Proc.*, **11**, 268 (1952).

(12) E. E. Snell, THIS JOURNAL, **67**, 194 (1945).

(13) D. Heyl, D. Luz, S. A. Harris and K. Folkers, *ibid.*, **73**, 3436 (1951).

(14) M. Viscontini, C. Ebnother and P. Karrer, *Helv. Chim. Acta*, **34**, 2199 (1951).

(15) I. C. Gunsalus, W. W. Umbreit, W. D. Bellamy and C. E. Foust, *J. Biol. Chem.*, **161**, 743 (1945).

(16) A. N. Wilson and S. A. Harris, THIS JOURNAL, **73**, 4693 (1951).

(17) M. Viscontini, C. Ebnother and P. Karrer, *Helv. Chim. Acta*, **34**, 1834 (1951); **34**, 2199 (1951).

(18) M. Viscontini and P. Karrer, *ibid.*, **35**, 1924 (1952).