[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY AND CHEMICAL ENGINEERING, UNIVERSITY OF CALIFORNIA]

Stereochemical Studies in the Morphine Series. The Relative Configuration at Carbons Thirteen and Fourteen[1]

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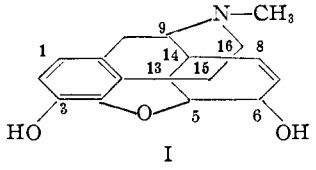
RECEIVED JANUARY 26, 1953

Previous work[2,3] has established the relative configuration at carbons 5, 6 and 13 in morphine. The present work relates the configuration at carbon 14 to 13, and thus completes the stereochemistry of the molecule. As starting material, dihydrothebainone (V) was chosen since its epimer at carbon 14, epi-dihydrothebainone (β-dihydrothebainone), was also available. Dihydrothebainone was degraded to thebenone (VIII) which on treating with isoamyl nitrite and potassium *t*-butoxide formed the dioximino compound. Rearrangement with *p*-toluenesulfonyl chloride in pyridine gave the dinitrile with loss of the carbonyl carbon, and hydrolysis to the acid amide and sublimation resulted in the imide, XV. When the same sequence was applied to epi-dihydrothebainone, the results were parallel except in the last cyclization, where polymeric material was formed rather than imide. This leads to the conclusion that in morphine the hydrogen at carbon 14 is *cis* to the ethanamine chain (confirming a previous assignment based on hydrogenation studies[5]) as are the hydrogens at carbons 5 and 6. The morphine molecule may thus be represented by Ib.

The stereochemistry of morphine (I) has been the subject of recent work[2,3] which established the configurations at carbons 5 and 6 relative to that at the quaternary carbon 13. Since the ethanamine bridge joining carbons 9 and 13 can be fused in only one manner (*cis*), this leaves carbon 14 as the one asymmetric center for which there is lacking incontrovertible chemical evidence relating its configuration to that at carbon 13. The purpose of the present report is to provide such evidence.



I

Several speculations as to the configuration at carbon 14 have appeared in the literature. Schöpf and Borkowsky[4] found that, in a compound with an hydroxyl at carbon 14, degradation to the nitrogen-free material resulted in cyclic ether formation with the ethane chain. This hydroxyl at carbon 14 therefore must be *cis* to the ethanamine chain, but whether this is true as well for the hydrogen it replaced is open to question

Stork[5] has concluded from hydrogenation data that the hydrogen at carbon 14 and the ethanamine chain are *cis*. This is based on the fact that catalytic hydrogenation of the 8,14-double bond in a variety of morphine derivatives leads to hydrogenation products with the natural configuration at carbon 14. He concluded that introduction of the hydrogen at carbon 14 must take place *trans* to the phenyl group, and therefore *cis* to the ethanamine chain.

However, since a study of models revealed only slight differences, we have sought independent chemical evidence. Also, there are several cases in which catalytic hydrogenation procedures have resulted in compounds with the unnatural, epimeric configuration at carbon 14. From the hydrogenation of thebaine (II) with a palladium-on-calcium carbonate catalyst, epi-dihydrothebainone (β-dihydrothebainone) (Ve), an isomer of dihydrothebainone, was isolated as the oxime.[6,7] Again, using a palladized strontium carbonate catalyst, epi-tetrahydrothebainone methine[8] (VIIe) was obtained from the hydrogenation of $\Delta^{8,9}$-thebainone methine (thebainone-B methine),[9] an observation we have confirmed.[14] It thus appears that hydrogenation does not provide the unequivocal answer as to carbon 14 stereochemistry.[10]

In seeking this answer, we sought a method which would be applicable to compounds in which both epimers at carbon 14 are available. An admirable pair of compounds for this purpose is thebenone (VIII) (which has the same configuration as morphine at carbon 14) and epi-thebenone (VIIIe),[11] nitrogen-free degradation products known to differ only in their configuration at carbon 14. Because of the keto group remaining at carbon 6, they appeared to be amenable to a stereochemical study

(1) Reported in part in Abstracts Papers *Am. Chem. Soc.*, **123**, 15L (1953).

(2) H. Rapoport and G. B. Payne, *J. Org. Chem.*, **15**, 1093 (1950).

(3) H. Rapoport and G. B. Payne, THIS JOURNAL, **74**, 2630 (1952).

(4) C. Schöpf and F. Borkowsky, *Ann.*, **452**, 249 (1927).

(5) G. Stork, THIS JOURNAL, **74**, 768 (1952).

(6) C. Schöpf and L. Winterhalder, *Ann.*, **452**, 232 (1927).

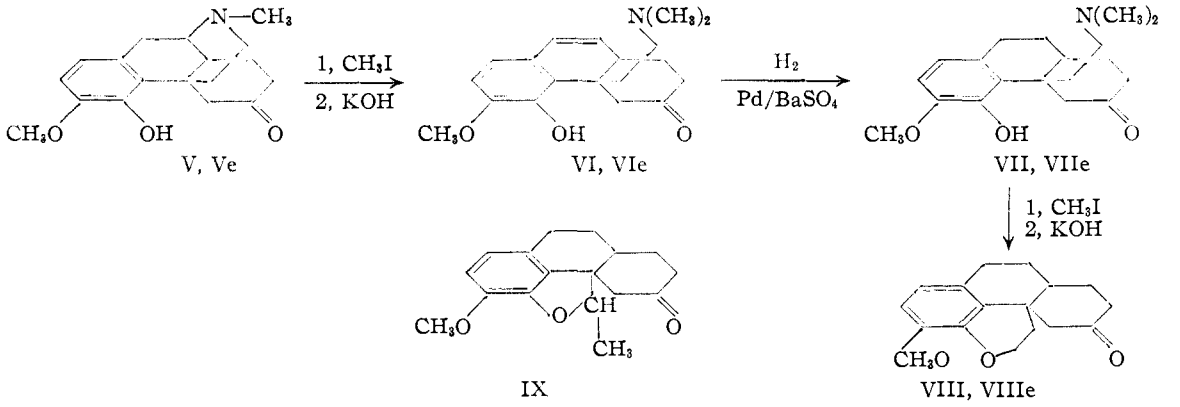
(7) C. Schöpf and F. Borkowsky, *ibid.*, **458**, 148 (1927).

(8) The nomenclature applied to compounds with the unnatural configuration at carbon 14 is somewhat confused. Schöpf and Borkowsky[7] originally used the prefix epi- to denote these compounds. Small and Browning[12] introduced the term β-, and Bentley and Wain [*J. Chem. Soc.*, 967 (1952)] used a complex system of trivial names. Since the β-terminology already is used in the morphine series for designating certain methines, and since little justification can be found for more trivial names, we have adopted the use of the prefix epi- in referring to all compounds with the unnatural carbon 14 configuration. This leads to a logical, extensible system of nomenclature in which the name to some extent connotes the structural feature involved. Also, the designation "Δ-" will be employed in all cases where the double bond position is known.

(9) K. W. Bentley, R. Robinson and A. E. Wain, *J. Chem. Soc.*, 958 (1952).

(10) Another argument, based on catalytic hydrogenation, has been advanced (G. Stork in Manske and Holmes, "The Alkaloids," Vol. II, Academic Press, Inc., New York, N. Y., 1952, p. 175). This is that the presence of dihydrocodeine methyl ether among the hydrogenation products of thebaine proves the hydrogen at carbon 14 is *cis* to the ethanamine bridge since the hydrogen at carbon 6 in this compound is known to be *cis*. If one assumes 1,4- addition, this would be true. However, evidence against 1,4-addition is provided by the isolation of neopine methyl ether from a hydrogenation of thebaine, interrupted before hydrogen absorption ceased (L. F. Small, personal communication). Since the 8,14-double bond in neopine is very easily reduced, it is conceivable that dihydrocodeine methyl ether resulted from further hydrogenation of neopine methyl ether, and no 1,4-addition need be involved. Although neopine methyl ether must have arisen by addition of hydrogen to the 6,7-double bond *cis* to the ethanamine bridge, this of course does not prove a parallel addition must take place at 8,14.

(11) For those compounds which were prepared in both configurations, the epimer with the natural configuration is referred to by the roman numeral, and the unnatural epimer by the additional suffix e.



by further degradation through the ketone ring. The problem thus became first the preparation of thebenone and epi-thebenone in sufficient quantity to permit further degradative work.

The intermediate necessary to prepare thebenone (VIII) is dihydrothebainone (V) and this was prepared by hydrogenation of thebaine in dilute acetic acid (using a palladized carbon catalyst) in a 78% yield of crude material, satisfactory for conversion to thebenone. By modification of the known procedures, dihydrothebainone was then degraded through Δ^9-dihydrothebainone methine (VI) and tetrahydrothebainone methine (VII) to thebenone (VIII) in a 41% over-all yield.

We have assigned thebenone the six-membered oxygen ring structure VIII rather than the five-membered ring as in IX. Small and Browning[12] originally postulated a six-membered ring, as does Stork.[5] However, Bentley, Robinson and Wain,[9] on the basis of an unlikely mechanism for its formation involving displacement of trimethylamine in the methohydroxide by a hydride ion from carbon 15, adopted the five-membered ring structure IX.

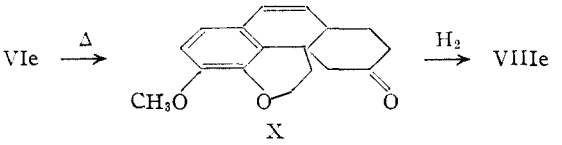
Confirmation for the six-membered ring structure VIII has been found in the fact that thebenone gives no evidence for the presence of C-CH_3 group in a Kuhn–Roth oxidation. Also, a more reasonable mechanism for the formation of the cyclic ether during the degradation, as suggested by Stork,[13] would be the direct displacement at carbon 16 of trimethylamine by the phenoxide ion due to (a) steric proximity and (b) the fact that carbon 16, being adjacent to the strongly electron-attracting ammonium ion, would be more susceptible to anionic attack. The marked ease of trimethylamine elimination in thebenone formation, as contrasted to those second-stage degradations where no cyclization can occur, is strong evidence that the usual mechanism, involving initial appearance of a vinyl group at carbon 13, is not operative.

The best method for preparing compounds with the unnatural configuration at carbon 14 is the hydrolysis of $\Delta^{5,8}$-dihydrothebaine (III) to epi-thebainone (IV), originally discovered by Small and Browning.[12] Since these investigators also had shown the conversion to epi-thebenone to be feasible, this path was examined in detail for the large scale preparation of epi-thebenone (IVe).

To prepare $\Delta^{5,8}$-dihydrothebaine (III) the excellent procedure of reduction of thebaine by sodium in liquid ammonia[9] was used and resulted in an 88% yield. Hydrolysis with potassium acid sulfate gave crystalline epi-thebainone (IV) which was hydrogenated in methanol using a platinum oxide catalyst. The product (Ve) has been previously[12] characterized as an oil which was purified by distillation. However, when a small sample was chromatographed on alumina, crystalline epi-dihydrothebainone (Ve), m.p. 116–118°, resulted from hexane in about 50% yield. Normally, however, the epi-dihydrothebainone was not isolated as such, but as the methiodide in an 80% yield from epi-thebainone.

The methiodide of Ve was then degraded, the product, Δ^9-epi-dihydrothebainone methine (VIe), was hydrogenated, and the pure epi-tetrahydrothebainone methine (VIIe),[14] was isolated by sublimation and crystallization. By degrading epi-tetrahydrothebainone methine methiodide, epi-thebenone (VIIIe) was obtained in essentially quantitative yield and the over-all conversion of thebaine to epi-thebenone was accomplished in 33% yield.

In one case where an attempt was made to purify the intermediate Δ^9-epi-dihydrothebainone methine (VIe) by sublimation, extensive decomposition occurred and a small amount of a neutral compound was isolated. This proved to be Δ^9-dehydro-epi-thebenone (X) since on hydrogenation epi-thebenone was obtained.

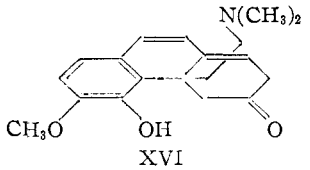


With thebenone and epi-thebenone thus readily available as the pair of epimers differing only in

(12) L. Small and G. L. Browning, *J. Org. Chem.*, **3**, 618 (1939).

(13) Reference 10, p. 192.

(14) An alternative method for the preparation of epi-tetrahydrothebainone methine was offered by the work of Bentley, Robinson and Wain.[9] Although no yields of purified material were given, their approach offered a shorter route and was investigated. $\Delta^{5,8}$-Dihydrothebaine (III) methiodide with hot sulfurous acid gave $\Delta^{8,9}$-thebainone methine (thebainone-B methine) (XVI) in 66% yield, but on hydrogenation only a poor yield of pure epi-tetrahydrothebainone methine (VIIe) could be isolated.



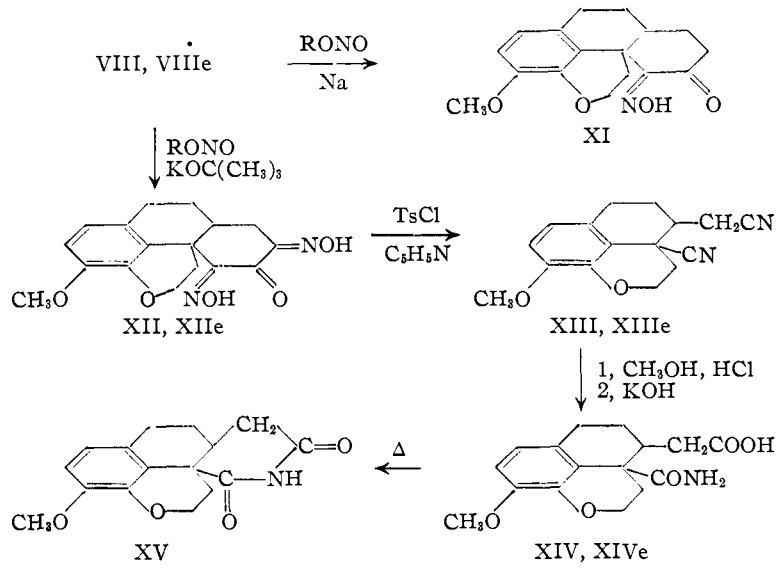
configuration at carbon 14, attention was turned to further degradation through the ketone ring to compounds in which the configuration might be established by cyclization reactions. The first problem, ring opening, seemed most attractively accomplished by a second-order Beckmann rearrangement on the α-oximino compound prepared by Wieland and Kotake[15] for which 5-(rather than 7-) oximinothebenone (XI) appears to be the more reasonable structure.[16] However, repetition of their work gave XI in very poor yield, precluding any ring-opening and further degradation.

Aside from the poor yield, an interesting point in the preparation of XI is that only one oximino group has entered the molecule. At one time, the fact that only monooximino-benzylidene and -piperonylidene derivatives of thebenone could be prepared, even under forced conditions, was interpreted as indicating the presence of only one α-methylene group, and thus as evidence supporting the postulated attachment of the ethanamine chain at carbon 5.[15] The recent elegant synthesis of morphine[17] completely eliminates this structure from consideration, but does raise the interesting question as to why only one of the α-methylene groups of thebenone underwent reaction.

In an effort to secure reaction at both methylene groups, we sought both a homogeneous reaction mixture and a strong alkaline condensing agent. These objectives were achieved by using potassium *t*-butoxide in *t*-butyl alcohol and, under these conditions, isoamyl nitrite reacted with thebenone to form dioximinothebenone (XII). The reaction proceeds in good yield and thus clears up an apparently anomalous reaction of thebenone.

The dioximinothebenone presented interesting ring-opening and degradative possibilities. Although no analogies could be found in the literature the dioximino compound was subject to the usual second-order Beckmann rearrangement reagents (*p*-toluenesulfonyl chloride in pyridine) in the expectation that, since a monoöximino ketone rearranges to a cyano acid, the dinitrile would result from the dioximino ketone, with the concurrent elimination of the carbonyl carbon. This was found to be the case as thebedinitrile (XIII)[18] resulted in a 55% yield. Application of this type of Beckmann rearrangement appears to offer interesting possibilities in ketone degradation. Not only is a carbon atom eliminated, but the two fragments produced have terminal cyano groups, potentially subject to further degradation.

Parallel reactions with the corresponding epimeric compounds provided epi-thebedinitrile (XIIIe), and the intent was to hydrolyze XIII and XIIIe to the respective dibasic acids and characterize the latter as *cis* or *trans* by a study of anhydride formation.



However, hydrolysis proved to be exceedingly difficult. Although potassium hydroxide in refluxing ethylene glycol led to a 90% evolution of the theoretical quantity of ammonia in 48 hours, no crystalline material could be isolated because of extensive ether cleavage at both the methyl and cyclic ether groups. A partial hydrolysis under the same conditions, terminated after one molar equivalent of ammonia had been evolved (eight hours) resulted in the isolation of acid amide (XIV) in 30% yield. However, attempts to continue the conversion of XIV to dibasic acid using an alkyl nitrite and hydrochloric acid[19] led to intractable tars.

Since the acid amide XIV offered promise for possible ring closure to the imide, its preparation in better yield through imido ester formation was investigated. Anhydrous hydrochloric acid in methanol followed by heating with water, gave a precipitate of ammonium chloride corresponding to the hydrolysis of one cyano group, a fact in line with the reported observation that hindered nitriles fail to undergo this reaction.[20] Assuming the isolated intermediate was ester-nitrile, it was first treated with ethanolic potassium hydroxide (to saponify ester and give aqueous alkali soluble material) and then with aqueous potassium hydroxide (to convert nitrile to amide). During these hydrolyses, no ammonia was evolved and the acid amide, thebedioic acid monoamide (XIV), was obtained crystalline in 80–90% yields. From epi-thebedinitrile

(15) H. Wieland and M. Kotake, *Ann.*, **444**, 69 (1925).

(16) The assignment of the oximino group to position 5 is made by analogy with the bromination of dihydrothebainone [C. Schöpf and T. Pfeifer, *Ann.*, **483**, 157 (1930)] which is known to occur at position 5 since the action of alkali closes the oxide ring. Since bromination and oximination in alkali both probably proceed by the same mechanism, the comparison seems valid. Wieland and Kotake[15] assigned the oximino group to the 7-position on the basis of the now disproved formula for morphine in which carbon 15 of the ethanamine chain was attached at carbon 5, and this assignment was supported by their inability to form the dioximino compound.

(17) M. Gates and G. Tschudi, THIS JOURNAL, **74**, 1109 (1952).

(18) Names designed to show the relationship to thebenone have been chosen for XIII (thebedinitrile), XIV (thebedioic acid monoamide) and XV (thebedioicimide).

(19) N. Sperber, D. Papa and E. Schwenk, THIS JOURNAL, **70**, 3091 (1948).

(20) A. Pinner, "Die Imidoäther und ihre Derivate," R. Oppenheim, Berlin, 1892.

(XIIIe), epi-thebedioic acid monoamide (XIVe) resulted in an identical fashion.

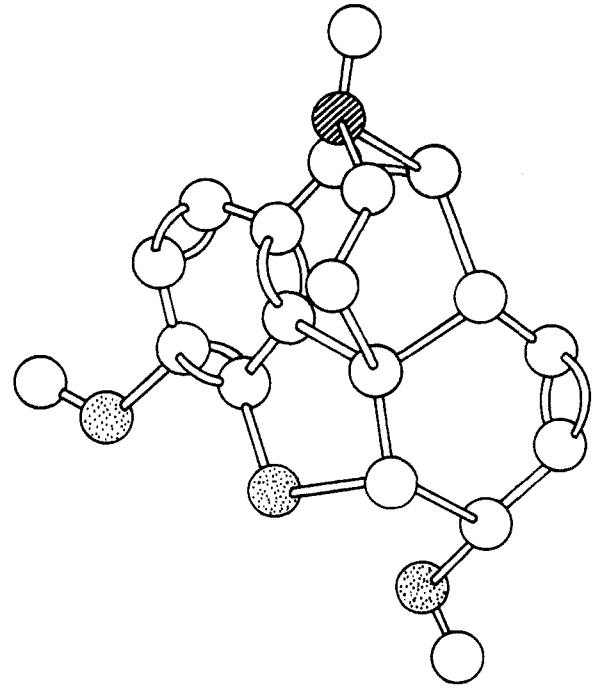
Originally, it was intended to degrade the acetic acid side-chain by one more carbon so that ring-closure would involve a five-membered ring. In this way the frequently encountered difficulty with fused six-membered rings in which both *cis* and *trans* isomers undergo ring-closure would be avoided. However, a study of the models of compounds XIV and XIVe revealed that this might not be necessary. Since carbons 10, 11, 12 and 13 are constrained in a plane, there is a marked difference between the isomers, making it extremely difficult if not impossible for cyclization to occur with the *trans* isomer whereas with the *cis* isomer ring closure is spatially favored.[21] Thus one would expect a striking difference in kind upon subjecting both isomers to ring-closure, and this was actually observed experimentally.

Two methods were used to effect ring closure of thebedioic acid monoamide (XIV) to the imide. A solution of the acid amide in liquid ammonia was evaporated, the residual ammonium salt was fused at 175° and then sublimed at 0.02 mm. pressure. The sublimate, on crystallization from ethanol, gave a 44% yield of imide (XV), characterized by insolubility in carbonate and solubility in hydroxide solution, and by analysis. Fusion and sublimation of the thebedioic acid monoamide itself gave a quantitative recovery of material as sublimate, which, by optical rotation analysis, appeared to be 16% unchanged amide and 84% imide. On crystallization a 60% yield of pure imide was isolated.

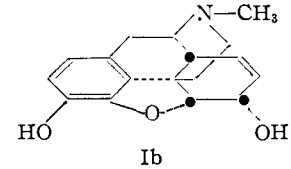
These same two procedures were applied to epi-thebedioic acid monoamide (XIVe). Fusion and sublimation of the ammonium salt gave a sublimate which consisted almost entirely of recovered epi-acid amide (43%). There was a large non-sublimable residue. Fusion and sublimation of the epi-acid amide itself resulted in a 49% recovery of starting material as crystalline sublimate, and the remainder was non-volatile, polymeric material.

This facile ring-closure to imide in the case of the natural isomer, thebedioic acid monoamide, as contrasted with the complete absence of imide and formation of considerable polymeric material when epi-thebedioic acid monoamide was subjected to the same conditions, coupled with the study of the models discussed above, allows a conclusion to be reached as to the relative configuration of the carboxamide and acetic acid groups, *viz.*, that they are *cis* in the natural isomer, and *trans* in its epimer. From this it follows that in morphine the hydrogen at carbon 14 is *cis* to the ethanamine chain and the two six-membered alicyclic rings are joined in a *cis* fusion[22] confirming the previous assignment.[5]

Incorporation of this deduction with those previously reached[2,3] leads to the conclusion that the hydrogens at carbons 5, 6 and 14 are all *cis* to the ethanamine chain. The relative configuration at all the asymmetric centers in morphine has thus been established. A model of the molecule is shown in Ia and a conventional line representation in Ib.



Ia



Ib

Experimental[23]

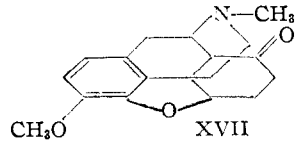
Thebenone (VIII).—Following known procedures with some variation, thebaine (II)[24] was hydrogenated in 2 *N* acetic acid using a palladium-on-carbon catalyst to dihydrothebainone (V), which was converted to methiodide and degraded. The resulting Δ^9-dihydrothebainone methine (VI) was hydrogenated to tetrahydrothebainone methine (VII) and this in turn was converted to methiodide and degraded[12] to thebenone, m.p. 134–135°; $[\alpha]^{25}D$ +64.7° (*c* 0.87, ethanol) [reported[12] m.p. 134–136°; $[\alpha]^{27}D$ +66.9° (*c* 0.508, ethanol)].

Anal. Calcd. for $C_{17}H_{20}O_3$: C, 75.0; H, 7.4; C-CH_3, 0. Found: C, 74.9; H, 7.5; C-CH_3, 0.

Epi-thebenone (VIIIe).—Thebaine was converted to epithebenone essentially by the procedure of Small and Brown-

(21) In view of the absence of stereochemical data on analogous complex fused ring systems, this interpretation, which is based on a study of models, while strongly suggestive, is not absolutely conclusive.

(22) This conclusion is interesting in regard to the classical work [W. Hückel and E. Brinkmann, *Ann.*, **441**, 21 (1925); W. Hückel, R. Danneel, A. Gross and H. Naab, *ibid.*, **502**, 99 (1933)] on the stability of decalin systems, in which the *trans* ring fusion was found to be the more stable. Only very occasional exceptions to this generalization have been found [*e.g.*, R. P. Linstead and R. R. Whetstone, *J. Chem. Soc.*, 1428 (1950)] and the possible cause for exception in the present case is being sought further. Dihydropseudocodeinone (XVII), which has the natural (*cis*) configuration at carbon 14, is stable to alcoholic alkali (unpublished work, this Laboratory), and the corresponding ketones with nitrogen and/or oxide rings opened are being examined to ascertain if this stability of the *cis* juncture might be due to the rigidity imparted by these additional fused rings [*cf.* W. S. Johnson, *Experientia*, **7**, 315 (1951)].



XVII

(23) All melting points are corrected, and those above 200° were taken in evacuated capillaries; microanalyses were performed by the Microchemical Laboratory, University of California.

(24) We are greatly indebted to Dr. A. H. Homeyer of the Mallinckrodt Chemical Works, St. Louis, Mo., for a very generous supply of thebaine.

ing,[12] except that thebaine was reduced with sodium in liquid ammonia[9] rather than in alcohol. The $\Delta^{5,8}$-dihydrothebaine (III) thus obtained was hydrolyzed with 2 *M* potassium bisulfate to epi-thebainone (IV)[25] and this was hydrogenated to epi-dihydrothebainone (Ve). Chromatography of the latter on alumina with benzene as an eluant gave crystalline material which served as seed for crystallization of the bulk of the epi-dihydrothebainone from hexane, m.p. 116–118°; $[\alpha]^{25}D$ −50.9° (*c* 0.55, ethanol) [reported[12] as an oil, $[\alpha]^{27}D$ −48.1° (*c* 0.50, ethanol)].

Anal. Calcd. for $C_{18}H_{23}O_3N$: C, 71.7; H, 7.7. Found: C, 71.5; H, 7.7.

Epi-dihydrothebainone methiodide [m.p. 236–237°, $[\alpha]^{25}D$ −32.9° (*c* 0.50, water). *Anal.* Calcd. for $C_{19}H_{26}O_3NI$: C, 51.5; H, 5.9; I, 28.6. Found: C, 51.2; H, 6.0; I, 27.8.[26] was degraded to the methine (VIe) which was hydrogenated, converted to methiodide, and degraded again to give epi-thebenone, m.p. 187–190° (reported m.p. 189–190°,[12] 188°[9]).

Δ^9-Dehydro-epi-thebenone (X).—When large-scale purification by sublimation of crude Δ^9-epi-dihydrothebainone methine (VIe) was attempted, extensive decomposition occurred. From 13.6 g. of crude material, heated at 160–180° and 0.05 mm. pressure for 18 hours, only 3.3 g. of sublimate was obtained. Partition between 2 *N* hydrochloric acid and chloroform gave a basic fraction (Δ^9-epi-dihydrothebainone methine, m.p. 185–187°) and a neutral fraction. The latter, after two crystallizations from ethanol and sublimation at 170° and 0.04 mm. pressure gave Δ^9-dehydro-epi-thebenone, m.p. 183–185°; $[\alpha]^{25}D$ +140° (*c* 0.50, ethanol). A mixture with epi-thebenone (m.p. 187–190°) melted at 165–172°.

Anal. Calcd. for $C_{17}H_{18}O_3$: C, 75.5; H, 6.7. Found: C, 75.2; H, 6.9.

The identity of this compound was confirmed by hydrogenation of 21.2 mg. at room temperature and atmospheric pressure in methanol in the presence of platinum oxide (10 mg.). Crude material after removal of the catalyst and solvent was crystallized twice from ethanol to give 7 mg. of epi-thebenone, m.p. 186–189°, mixed m.p. with authentic epi-thebenone (m.p. 187–190°), 186–189°.

Dioximinothebenone (XII).—A solution of potassium (8.3 g., 0.21 mole) in dry *t*-butyl alcohol (200 ml.) was cooled to 25° and thebenone (VIII) (22.7 g., 0.084 mole) was added and dissolved by stirring (*ca.* 30 min.). The yellow-brown solution was treated with isoamyl nitrite (30.0 g., 0.26 mole) in 1-ml. portions with stirring, resulting in a black solution and an exothermic reaction until *ca.* 20 ml. had been added. The remainder of the nitrite was then added at once, and the solution, after standing at room temperature overnight, was poured into one liter of water. The dark, red-brown solution thus obtained was extracted with ether (3 × 250 ml.), filtered, and poured with vigorous stirring into a mixture of water (100 ml.) and concd. hydrochloric acid (25 ml.). A yellow gelatinous precipitate formed and was washed by stirring with one liter of water. The washing was repeated twice and the material dried to constant weight at 60° *in vacuo*. Crude dioximinothebenone was obtained as a brown-yellow powder (23.6 g., 86% yield) and was best purified by crystallization from isopropyl alcohol from which it separated as the 2-propanolate, m.p. 125–127°, $[\alpha]^{25}D$ +186° (*c* 0.51, ethanol).

Anal. Calcd. for $C_{17}H_{18}O_5N_2 \cdot C_3H_8O$: C, 61.5; H, 6.7; N, 7.2; C–CH_3, 3.8. Found: C, 61.3; H, 6.4; N, 7.2; C–CH_3, 3.3.

The oxime of dioximinothebenone was prepared in the usual manner and recrystallized from ethanol, m.p. 211–212°, $[\alpha]^{25}D$ −21° (*c* 0.50, dimethylformamide).

Anal. Calcd. for $C_{17}H_{19}O_5N_3$: C, 59.1; H, 5.6; N, 12.2. Found: C, 59.6; H, 5.9; N, 12.5.

Dioximino-epi-thebenone (XIIe).—The epi-isomer was prepared in the same manner as dioximinothebenone, using epi-thebenone (VIIIe) (4.4 g., 0.016 mole), potassium (1.8 g., 0.046 mole) and isoamyl nitrite (6.8 g., 0.058 mole). The product, which could not be crystallized, was obtained by extraction of the acid solution with chloroform, washing, drying and concentration *in vacuo*, for a yield of 4.6 g. (87%). The crude dioximino-epi-thebenone was used directly for conversion to the nitrile.

Thebedinitrile (XIII).—Crude dioximinothebenone (XII) (3.3 g., 0.01 mole) was dissolved in dry pyridine (20 ml.) containing *p*-toluenesulfonyl chloride (4.80 g., 0.025 mole), and a vigorous exothermic reaction ensued, with the evolution of gas. After standing overnight, the solution was poured with swirling into 6 *N* sulfuric acid (50 ml.). The resultant mixture was extracted with chloroform (5 × 25 ml.) and the extract was washed, dried and concentrated on the steam-bath. The residue was taken up in methanol (50 ml.) and 2 *N* potassium carbonate (10 ml.) added. After heating under reflux for two hours, the black solution was diluted with water, concentrated *in vacuo* to remove the methanol, and partitioned between water and chloroform. The organic extract was washed and dried, the solvent was evaporated, and the crude product was sublimed at 160° and 0.05 mm. pressure to yield 1.43 g. of a light yellow, crystalline sublimate. Recrystallization from methanol gave 1.32 g., 50% yield, m.p. 181–182°.

A sample for analysis was recrystallized from ethanol; m.p. 181.5–182.5°, $[\alpha]^{25}D$ +53.5° (*c* 0.52, dioxane).

Anal. Calcd. for $C_{16}H_{16}O_2N_2$: C, 71.6; H, 6.0; N, 10.4. Found: C, 71.4; H, 6.0; N, 10.4.

Epi-thebedinitrile (XIIIe),—Preparation of this epimer was carried out exactly as described for thebedinitrile. Crude dioximino-epi-thebenone (XIIe) (4.6 g., 0.014 mole) was converted to 0.89 g. of epi-thebedinitrile, m.p. 165–166°, and 0.15 g., m.p. 157°–163°, for a total yield of 28%. A sample was recrystallized from ethanol and dried overnight at 100° and 1 mm. prior to analysis; m.p. 165–167°, $[\alpha]^{25}D$ −19.7° (*c* 0.50, ethanol).

Anal. Calcd. for $C_{16}H_{16}O_2N_2$: C, 71.6; H, 6.0; N, 10.4. Found: C, 71.8; H, 6.0; N, 10.2.

Thebedioic Acid Monoamide (XIV).—Thebedinitrile (XIII) (1.00 g., 3.7 millimoles) was suspended in dry methanol (50 ml.) and the suspension was cooled in ice while being saturated with anhydrous hydrogen chloride. The nitrile gradually passed into solution and the reaction mixture was kept at 0° overnight, after which it was heated under reflux for 45 min. Water (0.2 ml.) was then added and the reflux continued for two hours to hydrolyze the imido ester. The residue obtained by concentration *in vacuo* was dissolved in ethanol (100 ml.) and potassium hydroxide (12 g.) added. After heating under reflux in a nitrogen atmosphere overnight, the solution of acid nitrile was diluted with water (150 ml.) and solvent (150 ml.) distilled to remove the alcohol. Hydrolysis was then continued by boiling the aqueous solution for two days. After remethylating phenolic material by shaking the hydrolysate at room temperature with dimethyl sulfate (7.5 ml.), the *p*H was adjusted to 11, the solution was filtered, and acidification with concentrated hydrochloric acid continued to *p*H 1. The precipitate was collected after cooling and a yield of 1.02 g. (90%) of hygroscopic thebedioic acid monoamide, m.p. 180–181°, was obtained. A sample for analysis was recrystallized from water and dried at 100° *in vacuo*, m.p. 180–181°, $[\alpha]^{25}D$ +51.7° (*c* 0.50, ethanol).

Anal. Calcd. for $C_{16}H_{19}O_5N$: C, 62.9; H, 6.3; N, 4.6; equiv. wt., 305. Found: C, 62.7; H, 6.4; N, 4.7; equiv. wt., 306.

Epi-thebedioic Acid Monoamide (XIVe).—This hygroscopic acid was prepared in 81% yield by the same procedure as used above. A sample was recrystallized from isopropyl alcohol, sublimed at 180°, and 0.05 mm. pressure, and dried at 100° *in vacuo* for analysis; m.p. 209–211°, $[\alpha]^{25}D$ −20.5° (*c* 0.50, ethanol).

Anal. Calcd. for $C_{16}H_{19}O_5N$: C, 62.9; H, 6.3; N, 4.6; equiv. wt., 305. Found: C, 63.0; H, 6.4; N, 4.6; equiv. wt., 306.

Thebedioicimide (XV). A.—Thebedioic acid monoamide (XIV) (230 mg., 0.75 millimole) was converted to the ammonium salt by solution in liquid ammonia and evaporation of the solvent. This salt was fused at 0.5 mm. pressure for 15 minutes and then sublimed at 0.02 mm. (four hours), all at a bath temperature of 175°. The yellow crystalline sublimate (220 mg.) was recrystallized first from 50% eth-

(25) The rate of neutralization of the acid hydrolysate is of paramount importance in this preparation. The sodium carbonate solution was added with vigorous stirring over a 3.5-hour period, and crystalline epi-thebainone began to precipitate at *p*H 5.7 and was isolated when *p*H 6.9 was reached.

(26) This material has been previously reported[12] as the dihydrate, m.p. 149–154°.

anol (1.0 ml.) and then from ethanol (0.5 ml.) to yield 95 mg. (44%) of hygroscopic crystals, insoluble in carbonate but soluble in sodium hydroxide solution, which were dried at 100° *in vacuo* prior to analysis; m.p. 179–181°, $[\alpha]^{25}D$ +222° (*c* 0.50, ethanol).

Anal. Calcd. for $C_{16}H_{17}O_4N$: C, 66.9; H, 6.0; N, 4.9. Found: C, 66.8; H, 6.0; N, 5.0.

B.—Optimum conditions for the direct dehydration of the acid amide (100 mg., 0.33 millimole) to the imide were found to be fusion under nitrogen at 188–193° and atmospheric pressure for six hours, followed by sublimation at 0.1 mm. and 193°. The sublimate (95 mg., 101%) possessed $[\alpha]^{25}D$ +194° (*c* 0.50, ethanol), indicative of an imide content of 84%. Recrystallization two times from ethanol (0.5 ml., 1.0 ml.) gave 56 mg. (60%) of thebedioicimide, m.p. 177–178°, mixed m.p. with imide (m.p. 179–181°) prepared by method A, 177–180°.

Attempted Cyclization of Epi-thebedioic Acid Monoamide (XIVe) A.—The attempted preparation of the epi-imide by fusion of the ammonium salt of epi-thebedioic acid monoamide (200 mg., 0.66 millimole) in a manner identical with that used on the salt of thebedioic acid amide yielded a white crystalline sublimate. This was dissolved in chloroform and extracted successively with 1 *N* sodium carbonate and 1 *N* sodium hydroxide. Isolation of material from both extracts gave 85 mg. of carbonate soluble material, m.p. 210–214°, identified as recovered epi-thebedioic acid monoamide; and 7 mg. of an alkali soluble brown oil which could not be characterized. A large non-volatile residue was obtained.

B.—The direct fusion of epi-thebedioic acid monoamide was carried out exactly as for the natural series, except that, because of the high volatility of the starting material, hourly washings with chloroform were employed during the fusion to return volatile material from the cold finger to the melt. In addition, the compound was initially melted at 225° and the melt then kept at 193°. The sublimate of 27 mg., 49% of the starting material, was carbonate soluble and was identified as epi-thebedioic acid monoamide; $[\alpha]^{25}D$ −13.4° (*c* 0.54, ethanol). A non-volatile residue of 25 mg. also was obtained.

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[CONTRIBUTION FROM THE DEPARTMENT OF PHYSIOLOGICAL CHEMISTRY, THE JOHNS HOPKINS UNIVERSITY SCHOOL OF MEDICINE]

The Oxidation of L-Ascorbic Acid by *o*-Iodosobenzoic Acid[1,2]

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RECEIVED MARCH 25, 1953

In non-buffered aqueous solutions, ascorbic acid is oxidized by *o*-iodosobenzoic acid according to a second-order reaction expressed by the differential equation: $-d[AA]/dt = k_2[AA][o\text{-RIO}]$. The value of k_2 is 1.3 liters mole^{-1} min.$^{-1}$ at 25° over the *p*H range 4 to 8 (half-life of 13 hours for 0.001 *M* solutions). The reaction exhibits general acid catalysis in agreement with the assumption that *o*-iodosobenzoic acid and the acid catalyst combine reversibly through hydrogen bonding to form a complex which then reacts with ascorbic acid in a rate-controlling step. The dissociation constant (K_c) of the complex was shown to vary inversely as the dissociation constant (K_a) of the ligating acid. *o*-Iodobenzoic acid catalyzes the reaction in acetate buffer at *p*H 4.6 but not in phosphate buffer at *p*H 7. Dehydroascorbic acid has no effect on the rate of the reaction in either case. In phosphate buffer at *p*H 7, the reaction proceeds partially at a rate independent of the concentration of *o*-iodosobenzoic acid but directly proportional to the concentration of the buffer: $-d[AA]/dt = k_2[AA][o\text{-RIO}] + k'[AA][\text{buffer}]$. The second term in the rate equation does not appear in other buffer systems at *p*H 7. Cupric ion markedly catalyzes the reaction while ferrous ion is only about one-tenth as effective. Ascorbic acid is oxidized practically instantaneously by *m*-iodosobenzoic acid or *p*-iodosobenzoic acid. *o*-Iodoxybenzoic acid is rapidly reduced to *o*-iodosobenzoic acid by ascorbic acid. Iodide ion is also oxidized by *o*-iodosobenzoic acid according to a second-order reaction which exhibits general acid catalysis in agreement with that observed for the oxidation of ascorbic acid. Oxidized ascorbic acid, in non-buffered aqueous solution, undergoes transformation to an equivalent molar quantity of a product which is readily oxidized by iodine but not by *o*-iodosobenzoic acid. In phosphate buffer more extensive degradation occurs with formation of and up to three equivalent molar quantities of products capable of reducing *o*-iodosobenzoic acid. The reaction should serve as a model system for further studies on the end products of oxidation of ascorbic acid.

The kinetics of oxidation of ascorbic acid has been investigated repeatedly,[5] with respect particularly to vitamin C preservation in foods, the determination of its role in oxidation–reduction processes, and the mechanism of its action in the living organism.

The introduction of *o*-iodosobenzoic acid[6] as a reagent for the estimation of certain sulfhydryl compounds prompted a study of the oxidizing action of this compound toward ascorbic acid. This had been observed to be oxidized *slowly*[7] to dehydroascorbic acid while *o*-iodosobenzoic acid was reduced to *o*-iodobenzoic acid. The slow rate of reaction here is unique in that either *m*- or *p*-iodosobenzoic acid now has been found to oxidize ascorbic acid practically instantaneously. Further study demonstrated that the rate of the reaction is affected by the nature and the concentration of the buffer system in a manner suggestive of a rather unusual type of general acid catalysis. A thorough study of the kinetics of oxidation of ascorbic acid by *o*-iodosobenzoic acid was undertaken to elucidate the mechanism of the reaction.

Experimental

Materials.—Solutions of L-ascorbic acid were prepared and standardized daily. Dehydro-L-ascorbic acid and diketogulonic acid were prepared by the methods of Kenyon and Munro.[8] *o*-Iodosobenzoic acid, purified according to Loe-

(1) (a) From the doctoral dissertation of Wendell T. Caraway, The Johns Hopkins University, 1950. (b) Presented before the Annual Meeting of the American Society of Biological Chemists, Atlantic City, N. J., April, 1950.

(2) One of several investigations supported in part by a research grant from the National Cancer Institute, National Institutes of Health, United States Public Health Service.

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