[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY, UNIVERSITY OF CALIFORNIA, AND THE NAVAL MEDICAL RESEARCH INSTITUTE]

The Nitrogen-Containing Ring of Carpaine

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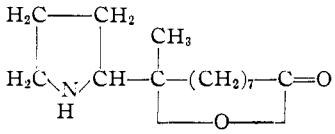
RECEIVED MAY 9, 1952

Examination of carpamic acid, the hydrolysis product from carpaine, by ninhydrin reaction and potentiometric titration indicates the carboxyl group and nitrogen-containing ring are at opposite ends of the molecule. Liquid phase catalytic dehydrogenation of carpaine gives desoxycarpyrinic acid, identified as 8-[2′-(6′-methylpyridyl)]-octanoic acid (X). Thus carpaine most reasonably contains a piperidine rather than a pyrrolidine ring and structure XI is proposed for the alkaloid.

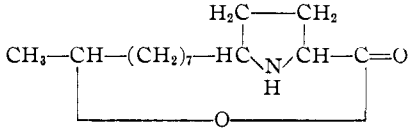
In a preceding publication,[2] the structure (I) proposed[3] for carpaine, the alkaloid from papaya leaves, was shown to be untenable, since from a two-stage Hofmann degradation the straight chain, fourteen-carbon acid, myristic acid, was obtained. In this report evidence is presented on a second structural feature, the position of the nitrogen atom.



I

That the nitrogen of carpaine is present as a secondary amine and in a ring has been established by the formation of typical secondary amine derivatives[3a,4] and the necessity of a two-stage Hofmann degradation for its elimination. Two points, however, on which further data would be highly desirable are the position of the nitrogen ring relative to the carboxyl group and the size of the nitrogen ring.

For example, in regard to the relative position of carboxyl group and nitrogen-containing ring, a structure such as II would be completely compatible with the previously established structural



II

features. This possibility may be easily investigated, since the amino acid resulting from lactone ring opening, carpamic acid, would be distinguishable as an α-amino acid. Carpamic acid was therefore subjected to the ninhydrin reaction and found to evolve no carbon dioxide, whereas proline readily evolved one mole.

Further information has been obtained by potentiometric titration of carpamic acid (Fig. 1)

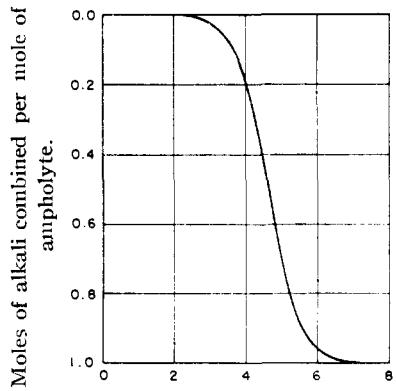
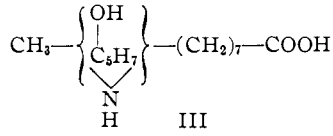


Fig. 1.—Potentiometric titration of carpamic acid.

from which the pK' of the carboxyl group was found to be 4.6.[5] Since it has been shown that, in amino acids, the pK' of the carboxyl group does not reach values of about 4.5 until the amino group is beyond the γ-position,[6] structures similar to II may be eliminated and only those formulations in which the carboxyl group and nitrogen ring are at opposite ends of the molecule need be considered. If two other structural elements are incorporated, *viz.*, the presence of one C-CH_3 group[3b] and the oxidation of carpaine to azelaic acid,[3a] a generalized formula for carpamic acid, $C_{14}H_{27}NO_3$, may be written as in III.



III

Previous conclusions as to the size of the nitrogen ring were based on dehydrogenations effected with selenium at 280° or above. The products were oils which gave positive Ehrlich tests and appeared to be substituted pyrroles. Hence a pyrrolidine nucleus was presumed to be present in the parent compound.[3a] In order to have more confidence that the dehydrogenation products reflect the structure present originally, we have sought to find milder dehydrogenation conditions, and at the same time to establish the structure of the products. Since at this stage, IV was considered to be a reasonable alternative to I as the structure of carpaine, the

(1) Naval Medical Research Institute, National Naval Medical Center, Bethesda, Md. The opinions contained herein are the writers' and are not necessarily those of the Navy Department.

(2) H. Rapoport and H. D. Baldridge, THIS JOURNAL, **73**, 343 (1951).

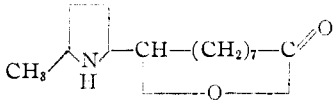
(3) (a) G. Barger, A. Girardet and R. Robinson, *Helv. Chim. Acta*, **16**, 90 (1933); (b) G. Barger, R. Robinson and T. S. Work, *J. Chem. Soc.*, 711 (1937).

(4) Although an N–H band was not detectable in the infrared absorption spectrum of carpaine in solution, a weak band was present at 3.0 μ when observed in an oil mull. Similar observations have been reported by L. Marion, D. A. Ramsay and R. N. Jones, THIS JOURNAL, **73**, 305 (1951).

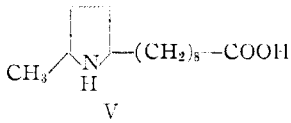
(5) For leading references and a discussion of the method used for this calculation see C. L. A. Schmidt, Editor, "Chemistry of the Amino Acids and Proteins," 2nd Ed., Charles C. Thomas, Springfield, Ill., 1949, Chapter XI (by D. I. Hitchcock), especially pp. 604–609.

(6) Reference 5, p. 614.

pyrrolidine V was synthesized as a model compound for studying dehydrogenation conditions.

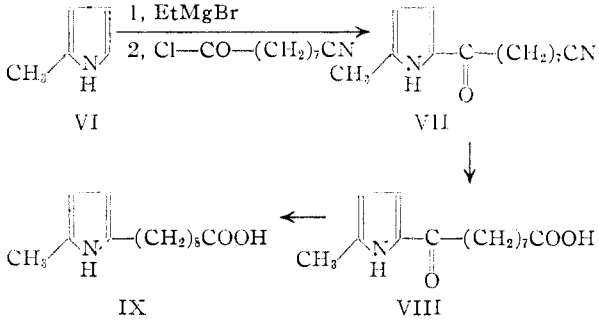


IV



V

The synthesis of V proceeded from 2-methylpyrrole (VI) as outlined in the sequence below. The pyrryl Grignard reagent was added to 8-cyano-



octanoyl chloride and 2-(8′-cyanoöctanoyl)-5-methylpyrrole (VII) was obtained in 38% yield. This could be hydrolyzed to the keto acid (VIII) which was then reduced, or VII could be directly converted to IX in 89% yield by modified Wolff–Kishner reduction. Hydrogenation of 9-[2′-(5′-methylpyrryl)]-nonanoic acid (IX) in glacial acetic acid with a platinum oxide catalyst ceased after absorption of two moles, and the pyrrolidine acid (V) after conversion to methyl ester and without attempting to separate isomers, was subjected to dehydrogenation experiments.

The mildest dehydrogenation conditions were found to be liquid phase dehydrogenation in boiling *p*-cymene with a 5% palladium-on-carbon catalyst. Under these conditions, the methyl ester of V evolved two moles of hydrogen in three hours after which evolution ceased completely and the pyrrole acid, IX, was isolated after saponification in an 82% yield.

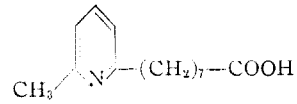
Carpaine was then subjected to these dehydrogenation conditions and found to smoothly evolve two moles of hydrogen in two hours, whereupon hydrogen evolution practically ceased. Examination of the reaction mixture by the usual procedure gave as the only product a water soluble hydrochloride, $C_{14}H_{21}NO_2H\cdot Cl$, to which the name desoxycarpyrinic acid hydrochloride has been assigned.

Two features of desoxycarpyrinic acid were quite unexpected. Since carpamic acid, the substance that results from opening the lactone ring of carpaine, is $C_{14}H_{27}NO_3$, the saponified dehydrogenation product of carpaine, assuming a pyrrolidine nucleus, was expected to be $C_{14}H_{23}NO_3$. Apparently, desoxycarpyrinic acid, $C_{14}H_{21}NO_2$, had lost the elements of water as well. Secondly, the presence of a basic nitrogen, as shown by formation of a hydrochloride, indicated desoxycarpyrinic acid was not a substituted pyrrole.

A potentiometric titration of desoxycarpyrinic acid hydrochloride gave the curve shown in Fig. 2 from which the presence of two groups of pK' 4.6 and 7.0 can be calculated.[5] The value of 4.6 is that expected for the carboxyl group; however, a value of 7.0 for the amine hydrochloride indicates the presence of a nitrogen with apparent basic dissociation constant, K'_B, equal to 10^{-7}. Thus the nitrogen cannot be present as a pyrrole. A very reasonable alternative is an α,α'-dialkylpyridine since 2,6-lutidine has been reported to have a K'_B of 10^{-7},[7a] 7.9×10^{-8},[7b] and 4.2×10^{-8}.[7c]

However, if desoxycarpyrinic acid is a substituted pyridine, an explanation for the origin of the third double bond must be found, since only two moles of hydrogen were evolved on dehydrogenation. The loss of water, as indicated by the empirical formula, was at first thought to account for this, but examination of the dehydrogenation reaction mixture revealed the product was present as a carboxylic acid and the saponification step, applied on the assumption that the product was a lactone, was unnecessary. Therefore, the lactone originally present in carpaine had cracked to give a carboxylic acid during the dehydrogenation, thus accounting for the third unsaturation. It is interesting in this regard to compare the facile dehydrogenation of carpaine with that of methyl hexahydrodesoxycarpyrinate. The latter compound, a piperidine in which the cracking possibility does not exist, dehydrogenated very slowly, and 12 hours was needed for the evolution of 2.9 moles of hydrogen.

These observations lead to the proposal of X as the most reasonable structure for desoxycarpyrinic acid.[8] Support for this structure is found in the



X

close similarity of the ultraviolet absorption spectrum of desoxycarpyrinic acid hydrochloride, with that of 2,6-lutidine hydrochloride, and the marked difference from that of 9-[2′-(5′-methylpyrryl)]-nonanoic acid (IX) (Fig. 3). Further evidence has been obtained by oxidation of desoxycarpyrinic acid to pyridine-2,6-dicarboxylic acid.

Since desoxycarpyrinic acid has been shown to contain a pyridine ring, the inference is very strong that carpaine is a substituted piperidine rather than a pyrrolidine. In order to maintain a pyrrolidine ring for carpaine, a rearrangement during the dehydrogenation reaction must be postulated.

(7) (a) H. C. Brown and R. B. Johannesen, THIS JOURNAL, **72**, 2934 (1950), footnote 1; (b) C. Golumbic and M. Orchin, *ibid.*, **72**, 4145 (1950); (c) A. Gero and J. J. Markham, *J. Org. Chem.*, **16**, 1835 (1951).

(8) Previous investigators (ref. 3a) have derived support for the presence of a pyrrole nucleus in the dehydrogenation products from the Ehrlich test (H. Fischer and H. Orth, "Die Chemie des Pyrrols," Vol. 1, Akad. Verlag., Leipzig, 1934, p. 66). However, we have found that 2,6-lutidine and desoxycarpyrinic acid give positive tests at room temperature. Thus the test may be misleading in the presence of α-alkylpyridines.

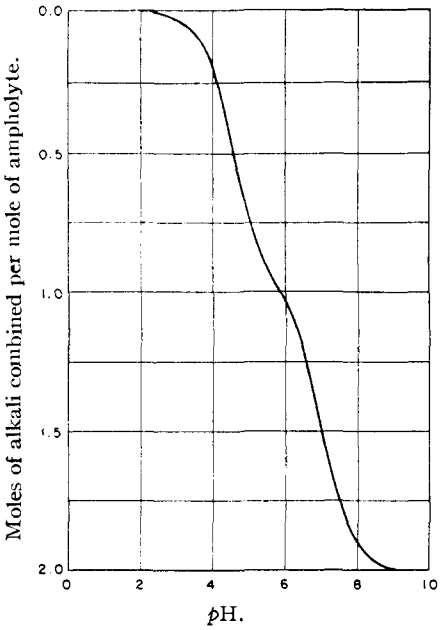
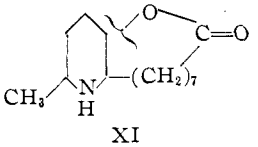


Fig. 2.—Potentiometric titration of desoxycarpyrinic acid hydrochloride.

Conceivably, dehydrogenation may proceed to a pyrrole which then rearranges to a pyridine. However, this seems unlikely and would result in a 2,5-dialkylpyridine whereas desoxycarpyrinic acid is a 2,6-substituted pyridine, as established by oxidation (see above). Another rearrangement to consider is that which might possibly occur if carpaine had the structure IV. Cracking might then give an intermediate which could rearrange to a six-membered ring[10] and dehydrogenate to a 2,6-disubstituted pyridine. However, a structure such as IV for carpaine, which would make carpamic acid an α,β-amino alcohol, seems unlikely on the basis of preliminary periodate oxidation experiments.[11]

Considering all the above evidence, the structure for carpaine which best accounts for its chemistry is XI, with the point of attachment of the lactone ring at some as yet undetermined position in the piperidine nucleus.



XI

Experimental[12]

Carpamic Acid.—After heating under reflux for two hours, a solution of 2.07 g. of carpaine in 50 ml. of 12 *N* hydrochloric acid was concentrated to dryness *in vacuo*. The concentration was repeated with two 20-ml. portions of water and the residue, after drying in a desiccator, was crystallized from an absolute ethanol–acetone mixture to give

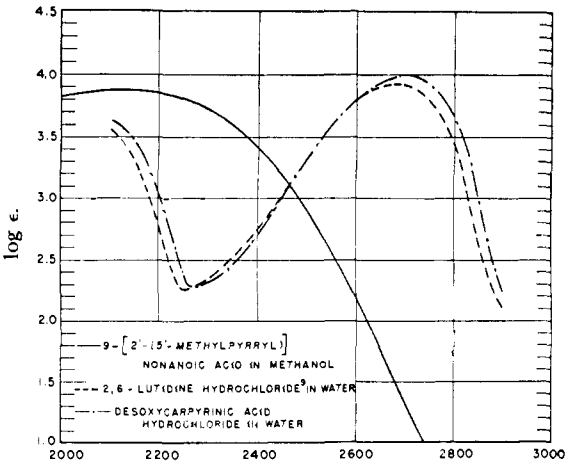


Fig. 3.—Ultraviolet absorption spectra: 10^{-4} *M* solutions.

2.18 g., 86%, of carpamic acid hydrochloride, m.p. 160.6–161.4° (reported[13] m.p. 161°).

To obtain the free amino acid, the crude hydrochloride from 2 g. of carpaine was dissolved in 30 ml. of water, shaken with silver oxide (freshly prepared from 5 g. of silver nitrate) and the mixture filtered. Evaporation of the filtrate after treatment with hydrogen sulfide left a residue which was dried (vacuum desiccator) and crystallized several times from 95% ethanol, yielding 0.8 g. of carpamic acid, m.p. 225–226° (dec.) (reported[13] m.p. 224°).

Ninhydrin Reaction with Carpamic Acid.—Using the procedure of Van Slyke, *et al.*,[14] carpamic acid was treated with ninhydrin at 100° in an aqueous solution buffered at *p*H 4.7. A very light yellow color appeared and only 2% of the theoretical quantity of carbon dioxide was evolved.

Under identical conditions, a solution of proline rapidly developed a deep red color and evolved 99.4% of the calculated amount of carbon dioxide.

2-Methylpyrrole (VI).—2-Methylpyrrole was prepared from pyrrole and ethyl diazoacetate through ethyl 2-pyrrylacetate according to the method of Nenitzescu and Solomonica[15]; b.p. 147–149°, $n^{25}D$ 1.5021 (reported[15] b.p. 149°).

2-(8'-Cyanoöctanoyl)-5-methylpyrrole (VII).—A solution of 2-methylpyrrylmagnesium bromide (0.116 mole, freshly prepared by addition of 9.4 g. of 2-methylpyrrole in 40 ml. of anhydrous ether to 65.5 ml. of 1.8 *M* ethereal ethylmagnesium bromide) was added over a three-hour period to a stirred solution of 21.6 g. (0.115 mole) of 8-cyanoöctanoyl chloride[2] in 25 ml. of anhydrous ether cooled in an ice-bath. Care had to be taken to prevent stoppage of the stirrer by the lumps produced during the addition period. With continuous stirring, the mixture was allowed to rise slowly to room temperature as the ice-bath melted (*ca.* four hours), after which stirring was continued for 15 hours. The reaction mixture was then heated under reflux for one hour, and, after cooling, was decomposed with 100 g. of ice and 150 ml. of saturated ammonium chloride solution. The layers were separated, the aqueous phase was washed with 100 ml. of ether and the combined ether extracts were washed with three 50-ml. portions of 3 *N* potassium carbonate solution and water. Evaporation of the ethereal solution after drying over magnesium sulfate left an oil (23.5 g.) which was distilled at reduced pressure. The fraction of b.p. 120–130° (0.1 mm.) solidified in the receiver and was crystallized from 95% ethanol to give 10.1 g. (38%) of material, m.p. 63–64°.

Anal. Calcd. for $C_{14}H_{20}ON_2$: C, 72.4; H, 8.7; N, 12.1. Found: C, 72.2; H, 8.6; N, 12.1.

2-(8'-Carboxyoctanoyl)-5-methylpyrrole (VIII).—A solution of 221 mg. (0.95 millimole) of 2-(8'-cyanoöctanoyl)-5-methylpyrrole in 2 ml. of 2 *N* potassium hydroxide in diethylene glycol was heated at 170–180° (bath temperature) for

(9) Purified by the method of W. Koenigs and G. Happe, *Ber.*, **36**, 2904 (1903).

(10) Similar rearrangements have been observed with 1,2-aminohalides, *e.g.*, R. C. Fuson and C. L. Zirkle, THIS JOURNAL, **70**, 2760 (1948).

(11) H. D. Baldridge, unpublished results; to be discussed in a subsequent publication.

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

(13) G. Barger, *J. Chem. Soc.*, **97**, 466 (1910).

(14) D. D. Van Slyke, D. A. MacFayden and P. Hamilton, *J. Biol. Chem.*, **141**, 671 (1941).

(15) C. D. Nenitzescu and E. Solomonica, *Ber.*, **64**, 1924 (1931).

four hours at which time hydrolysis was 95% completed as indicated by titration[16] of the evolved ammonia. After adding 50 ml. of water to the reaction mixture, it was washed thoroughly with ether, acidified to congo red with hydrochloric acid and extracted with ether. The extracts were washed free from mineral acid with water, dried over magnesium sulfate and concentrated. Crude keto acid (0.21 g., 86%) resulted which after crystallization from 60% aqueous ethanol and sublimation at 145–150° (0.1 mm.), melted at 143–144°.

Anal. Calcd. for $C_{14}H_{21}O_3N$: C, 66.9; H, 8.4; N, 5.6. Found: C, 67.1; H, 8.3; N, 5.6.

9-[2′-(5′-Methylpyrryl)]-nonanoic Acid (IX).—In accordance with the general procedure of Huang-Minlon,[17] a solution of 2.0 g. (8.6 millimoles) of 2-(8′-cyanoöctanoyl)-5-methylpyrrole, 2.5 g. of potassium hydroxide and 2 ml. of 85% hydrazine hydrate in 12 ml. of diethylene glycol was heated under reflux in a nitrogen atmosphere at 120–130° (internal temperature) for 1.5 hours and then at 195–200° for four hours. The cooled solution was added to 50 ml. of water, washed with two 50-ml. portions of ether, acidified to congo red with hydrochloric acid and extracted with ether. Evaporation of the ether extracts after washing free of mineral acid with water and drying over magnesium sulfate left 1.9 g. (93%) of a crystalline solid. After recrystallizing from 60% aqueous ethanol, a sample was distilled onto a cold finger at 130–140° (0.1 mm.) to give a white solid, m.p. 118.5–119°.

Anal. Calcd. for $C_{14}H_{23}O_2N$: C, 70.9; H, 9.8; N, 5.9; equiv. wt., 237. Found: C, 71.0; H, 9.7; N, 5.6; equiv. wt., 237.

Dehydrogenation Experiments.—Catalytic liquid phase dehydrogenation, as described by Fieser,[18] was employed in all the cases given below. A solution of one millimole of compound in 20 ml. of *p*-cymene (purified by distillation from phosphorus pentoxide) was heated under reflux with 70 mg. of 5% palladium-on-carbon and the evolved gas was collected in an eudiometer over 50% aqueous potassium hydroxide, using carbon dioxide as carrier. The collected gas was shown to be entirely hydrogen by using it to hydrogenate cinnamic acid.

A. Methyl 9-[2′-(5′-Methylpyrrolidyl)]-nonanoate.—Hydrogenation of 9-[2′-(5′-methylpyrryl)]-nonanoic acid (IX) in glacial acetic acid at room temperature and three atmospheres pressure using 10% by weight of platinum oxide as catalyst proceeded rapidly with absorption of two moles of hydrogen, then ceased. Filtration, evaporation of the solvent and esterification in methanol with diazomethane resulted in crude methyl 9-[2′-(5′-methylpyrrolidyl)]-nonanoate (as an oil) which was dehydrogenated according to the general procedure above. Hydrogen evolution reached 2 moles in three hours and then ceased. After removing the catalyst by filtration, 1 *N* ethanolic potassium hydroxide was added, the solution was heated under reflux for nine hours, and the alcohol was evaporated on the steam-bath. Water was added, the mixture was washed with ether and the aqueous phase was acidified and extracted thoroughly with ether. Evaporation of the ether solution after washing and drying gave an 82% yield of crude and a 60% yield of pure 9-[2′-(5-methylpyrryl)]-nonanoic acid, m.p. 118–119°.

B. Carpaine.—When subjected to the above dehydrogenation conditions, carpaine (in three different experiments) evolved from 2 to 2.04 moles of hydrogen in two hours, after which gas evolution practically ceased. Benzene, 50 ml., was added to the cooled mixture which was then filtered free of catalyst and washed successively with three 50-ml. portions of 5% sodium bicarbonate solution, three 50-ml. portions of 1 *N* acetic acid and 50 ml. of water. Neither the benzene or acetic acid solutions gave any appreciable residues on evaporation to dryness. The combined bicarbonate extracts were acidified to congo with hydrochloric acid and concentrated to dryness *in vacuo* at 50°. Repeated digestion of the dry residue with chloroform and evaporation of the combined chloroform extract left a residue, in 85% yield, which crystallized on standing in a vacuum desiccator. Recrystallization from anhydrous acetone and drying at 80° *in vacuo* overnight gave desoxycarpyrinic acid hydrochloride, m.p. 96–97°.

Anal. Calcd. for $C_{14}H_{22}O_2NCl$: C, 61.9; H, 8.2; Cl, 13.1; equiv. wt., 136. Found: C, 61.8; H, 8.2; Cl, 13.1; equiv. wt., 136.

C. Methyl Hexahydrodesoxycarpyrinate.—A sample of desoxycarpyrinic acid hydrochloride in glacial acetic acid was hydrogenated with 10% of platinum oxide as catalyst at room temperature and atmospheric pressure. Hydrogen absorption ceased after the uptake of three moles in two hours. After removal of the catalyst and distillation of the acetic acid, the residue was esterified with diazomethane and the oily methyl hexahydrodesoxycarpyrinate was dehydrogenated as above. Hydrogen evolution proceeded slowly and after 12 hours 2.9 moles had been evolved. The reaction mixture was refined as in the case of the pyrrolidyl ester above, and a 51% yield of recrystallized desoxycarpyrinic acid hydrochloride, m.p. 95–96°, was obtained.

Potentiometric Titrations.—A Beckman Model G *p*H meter equipped with glass and calomel electrodes was used. The *carpamic acid* solution contained 0.1 millimole of carpamic acid and slightly more than 0.1 millimole of hydrochloric acid in 3 ml. of solution. To this was added small aliquots of 1 *N* sodium hydroxide from a microburet and the *p*H of the resulting solution determined. From the data, the following values were calculated[5]: pK'(COOH), 4.6; pI', 7.5; $pK'(-NH_2^+)$, 10.4.

Desoxycarpyrinic acid hydrochloride, 0.05 millimole in 7.5 ml. of water, was titrated as above and gave the following values: pK'(COOH), 4.6; pI', 5.8; $pK'(\geq NH^+)$, 7.0.

Oxidation of Desoxycarpyrinic Acid[19] (X).—To a stirred solution of 272 mg. (1 millimole) of desoxycarpyrinic acid hydrochloride in 20 ml. of water, maintained at 70°, was added 1.58 g. (10 milliuoles) of potassium permanganate in ten equal portions over a period of 15 hours, each successive portion being added only after the previous one had been consumed. The hot solution was filtered, the precipitate was digested with two 10-ml. portions of water and the combined filtrate and digests concentrated to 2 ml. After careful acidification with 12 *N* hydrochloric acid to *p*H 1.5, the solution was cooled and filtered. The dried, precipitated crystalline material (containing potassium chloride) was heated under reflux for one hour with 5 ml. of purified thionyl chloride, the excess thionyl chloride was removed under reduced pressure and the residue was treated with 10 ml. of cold, concentrated aqueous ammonia. Crystallization of the resulting amide from nitrobenzene gave material melting at 305–307° (dec.).

An authentic sample of 2,6-pyridinedicarboxamide (dipicolinamide), prepared by the above procedure from dipicolinic acid,[20] melted at 304–306° (dec.) (reported[21] m.p. 302°) and on mixing did not depress the melting point of the material obtained from desoxycarpyrinic acid above. However, 2,5-pyridinedicarboxamide (isochinchomeronamide), prepared as above from isochinchomeronic acid,[20] melted at 314–316° (dec.) (reported[22] melting point 310–313°) and a mixture with the amide of the desoxycarpyrinic acid oxidation product melted at 275–281°. A mixture of authentic samples of the 2,6- and 2,5-diamides melted at 277–280°.

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(16) T. S. Ma and G. Zuazaga, *Anal. Chem.*, **14**, 280 (1942).

(17) Huang-Minlon, THIS JOURNAL, **68**, 2487 (1946).

(18) L. F. Fieser, "Experiments in Organic Chemistry," Second Edition, D. C. Heath and Company, New York, N. Y., 1941, p. 461.

(19) These experiments were performed by Ann Hollins.

(20) G. Black, E. Depp and B. B. Corson, *J. Org. Chem.*, **14**, 14 (1949).

(21) H. Meyer, *Monatsh.*, **24**, 207 (1903).

(22) T. O. Soine, *J. Am. Pharm. Assoc.*, **33**, 223 (1944).