230–231° for the picrate. That the compound obtained in the present work was 5,6-benzolepidine and not the linear isomer was shown by the color of its hydrochloride and by the fact that it did not react with maleic anhydride.[18]

Summary

1. A modification of the Doebner–Miller reaction has been developed for the preparation of 4-methylquinolines from methyl vinyl ketone.

2. The yields have been improved by the use of mild conditions, oxidizing agents and condensing agents.

3. A new synthesis of 4-methylquinolines from 1,3,3-trimethoxybutane has been found.

4. The method has been applied to the preparation of the following substances: lepidine, 6-methoxylepidine, 8-chlorolepidine, 5,6-benzolepidine, 4,6-dimethylquinoline and 2,4-dimethylquinoline.

(18) Johnson and Mathews, THIS JOURNAL, 66, 210 (1944).

NOTRE DAME, INDIANA RECEIVED OCTOBER 5, 1944

[CONTRIBUTION FROM THE NOYES CHEMICAL LABORATORY, UNIVERSITY OF ILLINOIS]

The Structure of Leucenol. I

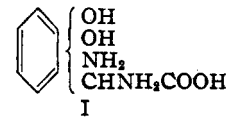
BY ROGER ADAMS, STANLEY J. CRISTOL, ARTHUR A. ANDERSON AND ALFRED A. ALBERT

Leucaena is a genus of tree similar to acacia and mimosa, one of the most common of which is *Leucaena glauca benth.* It is small, leguminous and native to tropical America, now widely distributed in southern Asia and neighboring islands. The leaves and seeds are reported to be a valuable fodder for cattle, but cause an irritation of the skin when ingested by horses.[1]

Mascré[2] isolated an optically inactive crystalline solid, m. p. 287°, by aqueous extraction of ground *Leucaena glauca* seed and named this substance leucenol. Its empirical formula was shown to be $(C_4H_5O_2N)_x$ and further experimental evidence demonstrated that it was an α-amino acid and contained a phenolic hydroxyl.

Mimosine, a substance with very similar properties to leucenol, was isolated from the sap of the sprouts and roots of *Mimosa pudica benth* by Renz.[3] This compound has the same empirical formula, $(C_4H_5O_2N)_x$, gives similar reactions and solubilities to those described by Mascré for leucenol, but has a different melting point (228°) and is optically active. Renz, on the basis of titrations in water and in ethanol, suggested the formula $C_{16}H_{20}O_8N_4$, although no molecular weight data could be obtained due to the insolubility of mimosine in organic solvents. It would appear to us that mimosine may be an optically active form of leucenol.

Mimosine was also described by Nienburg and Tauböck,[4] who repeated similar tests to those of Renz and in addition isolated a copper salt having the analysis $C_8H_8O_4N_2Cu\cdot 2H_2O$. From these data, the authors concluded that the formula of mimosine was $C_8H_{10}O_4N_2$ and speculated on two possible structures (I) and (II). They concluded that structure I was not possible for a substance that would titrate one equivalent of base to a phenolphthalein end-point and therefore that



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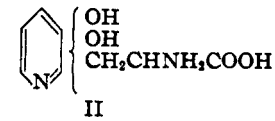


II

structure II, a dihydroxypyridylalanine, was the more likely formulation.

The objective in this investigation was the elucidation of the structure of leucenol. The isolation and purification of leucenol by the method of Mascré[2] was tedious and a continuous extraction of the ground seed with 90% ethanol was found to be more satisfactory. The product, recrystallized from water, melted at 291° with decomposition (Maquenne block). The empirical formula and other reactions described by Mascré were confirmed; a Van Slyke determination showed half the nitrogen to be primary amino, the ninhydrin test indicated an α-amino acid and the color with ferric chloride or Folin reagent a phenolic hydroxyl.

The chemical study of leucenol is hampered by its insolubility in practically all organic solvents except methanol and ethanol. It can be recovered unchanged from dilute acid or base solutions by adjusting the *p*H so that it is just acidic to brom cresol green (*p*H range, 3.8–5.4).

A difference in basicity of the two nitrogen atoms was demonstrated by the formation of monobasic salts with hydrochloric, hydrobromic and hydriodic acid. Treatment of leucenol with methanolic hydrogen chloride resulted in the formation of the dihydrochloride of the methyl ester of leucenol, indicating that both nitrogen atoms in this derivative were sufficiently basic to form salts. The ester gave a red-violet coloration with aqueous ferric chloride, showing the phenolic hydroxyl still to be present but, as expected, the ninhydrin test was negative.

Attempts to synthesize simple derivatives other than the methyl ester failed or resulted in noncrystalline products, *e. g.*, acetylation, benzoylation, catalytic hydrogenation under various conditions, formation of a β-naphthalene sulfonate or a

(1) Anon., Dept. Agr. Ceylon, Leaflet No. 7 (1918); *C. A.*, 13, 1108 (1919).

(2) Mascré, *Compt. rend.*, 204, 890 (1937).

(3) Renz, *Z. physiol. Chem.*, 244, 153 (1936).

(4) Nienburg and Tauböck, *ibid.*, 247, 80 (1937).

phenylthiohydantoic acid. The biuret and pyrrole pine-splinter tests were negative. Methylation with diazomethane in ether gave only an oil which did not crystallize; on oxidation of the product no ether-soluble acid was isolated.

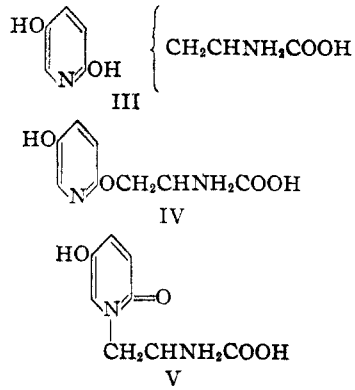
The ultraviolet absorption spectra of leucenol in water and in dilute hydrochloric acid (*p*H 2.2) were taken. In aqueous solution, leucenol had an absorption maximum at 282 mμ with log ϵ = 4.23 (ϵ = 17,000) whereas at *p*H 2.2 the maximum had shifted to 276 mμ with log ϵ = 3.97 (ϵ = 9300). These data are in accord with those of known hydroxypyridine derivatives.[5]

Leucenol was pyrolyzed at 2 mm. in a bath at 220–250° and the pale yellow sublimate, after recrystallization from absolute ethanol, melted at 242–244° (evac. tube). The same product was obtained when leucenol was pyrolyzed with zinc dust or when the crude pyrolyzate was resublimed from zinc dust.

The product had the empirical formula $C_5H_5O_2N$, and its aqueous solution was neutral to litmus and gave a violet ferric chloride test. It formed a monohydrochloride, hydrobromide and hydroiodide, $C_5H_6O_2NX$, and when treated with acetic anhydride, either with or without sodium acetate, gave a diacetate, $C_9H_9O_4N$. The aqueous solution of this latter product when first dissolved was neutral to litmus and gave no ferric chloride test, but rapidly developed an acid reaction and gave a violet color with ferric chloride due to hydrolysis. The product decomposed in moist air, liberating acetic acid.

The analysis and properties of the substance $C_5H_5O_2N$ and its derivatives seem best explained by the assumption that the compound is a dihydroxypyridine. Of the six possible isomeric dihydroxypyridines five have been reported in the literature.[6] The product isolated from leucenol has many properties resembling these compounds but the properties do not check completely with those of any one of them. For this reason it has been tentatively assigned the structure of 2,5-dihydroxypyridine which has not been described previously. Final proof of its structure must await ultimate synthesis.

These results demonstrate rather convincingly that a substituted pyridine ring is present in leucenol, just as was suggested (II) by Renz in mimosine merely from its empirical formula after eliminating groups that could be identified. Structure II, however, which may now be more explicitly written as III, is not the only one that will agree reasonably well with the available facts. Others are shown in IV and V formulated also on the assumption that the pyrolysis product is 2,5-dihydroxypyridine.



In formula IV, the side-chain has been substituted on the 2-hydroxyl because of the blue color that leucenol gives with Folin reagent. This color suggests that the hydroxyl group in the 5-position is unsubstituted, since Kuhn and Wendt[5a] have shown that β-hydroxypyridines give blue colorations with this reagent whereas α- and γ-hydroxypyridines give no color.

The isolation of a dihydroxypyridine from leucenol by pyrolysis at such a relatively low temperature as 220–250° seems to justify the assumption that the α-amino acid side-chain in leucenol probably is not bonded to a carbon atom in the pyridine ring, thus indicating III as a less likely structure. Cleavage of a carbon–oxygen bond or of a carbon–nitrogen bond at 25° might be anticipated.

Attempts were made to carry out hydrolysis of leucenol by refluxing with 48% aqueous hydrobromic acid for eight and one-half hours and with constant boiling hydriodic acid for thirty-six hours. Leucenol hydrobromide and leucenol hydroiodide were recovered from the reaction mixtures and no ethanol-soluble degradation products were obtained. Failure to hydrolyze leucenol under these conditions makes it appear unlikely that leucenol is an oxygen-linked ether as in IV since such a compound would be the derivative of a β-hydroxy acid and should react. On the other hand, it has been shown[7] that alkyl groups are not as easily hydrolyzed when attached to nitrogen as when attached to oxygen, and that normally hydrolysis of —NCH_3 is not effected by refluxing with constant boiling hydriodic acid.[7b,8]

The evidence thus far obtained is most satisfactorily explained on the basis of the formulation for leucenol as V. The pyridone structure is tentatively chosen because of the negative results in the hydrolysis experiments.

(5) See among others (a) Kuhn and Wendt, *Ber.*, **72**, 305 (1939); (b) Stiller, Keresztesy and Stevens, THIS JOURNAL, **61**, 1237 (1939).

(6) (a) 2,3-, erroneously reported as 2,5-, Kudernatsch, *Monatsh.*, **18**, 613 (1897); Schickh, Binz and Shulz, *Ber.*, **69**, 2593 (1936). (b) 2,4-, Errera, *Ber.*, **31**, 1690 (1898); *Gazz. chim. ital.*, **28**, I, 495 (1898). (c) 2,6-, Errera, *Ber.*, **31**, 1241 (1898); Gattermann and Skita, *ibid.*, **49**, 494 (1916); Ruhemann, *J. Chem. Soc.*, **73**, 350 (1898). (d) 3,4-, Ost, *J. prakt. Chem.*, [2] **27**, 270 (1883); Peratoner and Tamburello, *Gazz. chim. ital.*, **36**, I, 56 (1906); *Chem. Zentr.*, **76**, II, 681 (1905). (e) 3,5-, Koenigs and Geigy, *Ber.*, **17**, 1836 (1884); Weidel and Blau, *Monatsh.*, **6**, 656 (1885).

(7) See among others (a) Herzig and Meyer, *Monatsh.*, **15**, 613 (1894); **16**, 599 (1895). (b) Goldschmidt, *ibid.*, **27**, 849 (1906). (c) Haitinger and Lieben, *ibid.*, **6**, 311 (1885).

(8) Niederl and Niederl, "Organic Quantitative Microanalysis," John Wiley and Sons, Inc., New York, N. Y., 1938, p. 187.

When leucenol was treated with dry ethereal diazomethane, an ether-soluble substance was formed slowly. When, on the other hand, leucenol was suspended in water and shaken with a large excess of ethereal diazomethane, there was a rapid reaction with formation of a neutral water-soluble product. The product was soluble in methanol and insoluble in ether and was an oil which could not be induced to crystallize, but which formed a hygroscopic solid hydrochloride. It gave only a slight yellow color with ferric chloride, suggesting that the hydroxyl group or groups were methylated. It appears possible that this compound will prove to be the methylated betaine related to leucenol.

Experimental

Isolation of Leucenol.—Fifty grams of freshly-ground *Leucaena glauca* seed was extracted in a Soxhlet extractor with 90% ethanol or methanol for twelve hours. A mixture of crystals and oil began to separate after about one hour. The hot ethanolic solution was decanted from the separated crystals and oil as soon as the extraction was stopped. (If this were not done, the oil dissolved in the ethanol separates and renders the purification considerably more difficult.) The crystalline solid was washed with acetone to remove the oil, leaving crude leucenol. The average yield was 570 mg. (1.1%). The use of 70% ethanol gave only a trace of leucenol by this method. Several recrystallizations from water gave pure leucenol, m. p. 291° (cor.) with decomposition (bloc Maquenne). The temperature recorded is the lowest at which instantaneous melting occurs. In an evacuated tube, the melting point with decomposition is about 240–245°.

Anal. Calcd. for $(C_4H_5O_2N)_x$: C, 48.48; H, 5.05; N, 14.14. Found: C, 48.58; H, 5.06; N, 13.82, 13.94, 13.89.

Rotation. 0.1012 g. in 1 cc. of 10% hydrochloric acid made up to 5 ml. at 25° gave zero rotation; this was unchanged when the solution was diluted to 15 ml.

Primary Amine Determination (Van Slyke nitrogen). Calcd. for $(C_8H_8O_4N)NH_2$: N, 7.07. Found: N, 7.53, 7.57.

Leucenol and its derivatives, if analyzed for nitrogen without very special precautions, gave low values for this element.

Leucenol was found to be slightly soluble in water, much less soluble in methanol and ethanol and insoluble in the higher alcohols, dioxane, ethyl acetate, ether, benzene, chloroform, glacial acetic acid, pyridine and cellosolve. It is soluble both in dilute acids and bases and may be recovered from these solutions by adjusting the *p*H so that it is just acidic to brom cresol green.

Tests with Classification Reagents.—The tests referred to in the introduction were carried out in the standard way: (a) diazotization and attempted coupling with β-naphthol, (b) ninhydrin reaction, (c) ferric chloride, (d) Folin reagent, (e) biuret test, (f) pyrrole pine-splinter test.

Leucenol Hydrochloride.—The monohydrochloride of leucenol was prepared by dissolving leucenol in 0.1 *N* hydrochloric and evaporating to dryness in an air stream. After washing with hot absolute ethanol and drying in an Abderhalden over boiling methanol, it melted at 174.5–175° (cor.) with decomposition; soluble in cold water, insoluble in boiling ethanol or ether.

Anal. Calcd. for $C_8H_{11}O_4N_2Cl$: C, 40.95; H, 4.73. Found: C, 40.89; H, 5.12.

Leucenol Hydrobromide.—Preparation was the same as for the hydrochloride except that 0.1 *N* hydrobromic acid was used: m. p. 179.5° (cor.) with decomposition; soluble in water, insoluble in boiling ethanol.

Anal. Calcd. for $C_8H_{11}O_4N_2Br$: C, 34.42; H, 3.97. Found: C, 34.54; H, 4.08.

Leucenol Hydroiodide.—Preparation the same as for the hydrochloride except that 0.1 *N* hydriodic acid was used: m. p. 183–183.5° (cor.) with decomposition; soluble in water, insoluble in boiling ethanol.

Anal. Calcd. for $C_8H_{11}O_4N_2I$: C, 29.46; H, 3.40; I, 38.92. Found: C, 30.09; H, 3.56; I, 38.86.

Methyl Ester of Leucenol.—A suspension of 100 mg. of leucenol in 10 ml. of absolute methanol was saturated with gaseous hydrogen chloride and the resulting solution was heated at reflux for three hours. The solvent was removed *in vacuo* leaving about 115 mg. of pink solid which, after three recrystallizations from methanol–ether, gave 60 mg. of the dihydrochloride of the methyl ester of leucenol; m. p. 180–181° (cor.), with decomposition.

Anal. Calcd. for $C_9H_{14}O_4N_2Cl_2$: C, 37.91; H, 4.95; Cl, 24.87. Found: C, 38.27; H, 5.19; Cl, 24.39.

This salt was soluble in water, methanol and ethanol and insoluble in ether. It gave a red-violet coloration with ferric chloride solution. A ninhydrin test was negative.

Pyrolysis of Leucenol to a Dihydroxypyridine.—One gram of leucenol was pyrolyzed with or without zinc dust in a vacuum sublimation apparatus at 2 mm., heated in a Woods metal bath at 220–250°. The residue formed a black glass after cooling and could not be crystallized. The sublimate, which weighed 475 mg., was an orange-yellow solid, which exhibited polymorphism, melting at 60–90°, then resolidifying and melting at 175–190°. By repeated recrystallization from absolute ethanol (Darco), pale yellow needles were obtained, m. p. 242–244° (cor., evac. tube, sinters at 235–238°).

Anal. Calcd. for $C_5H_5O_2N$: C, 54.03; H, 4.54; N, 12.68. Found: C, 53.77; H, 4.75; N, 12.87.

The product was soluble in water, hot ethanol and glacial acetic acid, insoluble in ether, chloroform, acetone and petroleum ether. No insoluble picrate or picrolonate was formed in ethanol. A sample of the solid was pyrolyzed over zinc dust, but no pyridine odor was noted. Its aqueous solution was neutral to litmus and gave a violet color with ferric chloride.

Dihydroxypyridine Hydrochloride.—The hydrochloride was prepared by evaporation from a solution in 0.15 *N* hydrochloric acid in an air stream. It crystallized without water of crystallization, and several recrystallizations from ethanol and ether gave a yellow solid, m. p. 177–179.5° (cor.) with decomposition; soluble in water and ethanol, insoluble in ether.

Anal. Calcd. for $C_5H_6O_2NCl$: Cl, 24.03; Found: Cl, 23.69.

Dihydroxypyridine Hydrobromide.—The hydrobromide was prepared in a similar fashion, m. p. 174.5–176.5° (cor.) with decomposition; soluble in water and ethanol, insoluble in ether.

Anal. Calcd. for $C_5H_6O_2NBr$: C, 31.27; H, 3.15. Found: C, 30.75; H, 3.21.

Dihydroxypyridine Hydroiodide.—The hydroiodide was prepared in a similar manner, m. p. 155.5° (cor.) with decomposition; soluble in water and in ethanol, insoluble in ether.

Dihydroxypyridine Diacetate.—The diacetate was prepared by heating at reflux 300 mg. of the dihydroxypyridine with 300 mg. of anhydrous sodium acetate and 2.0 ml. of acetic anhydride. The excess acetic anhydride and acetic acid were removed *in vacuo* and the solid residue was extracted with dry ethyl acetate. The colorless product was obtained by concentration of the extract and cooling in an ice-bath. It was recrystallized (Darco) from dry ethyl acetate, or dry benzene, m. p. 139–140.5° (cor.). The same product is formed when the sodium acetate is omitted.

Anal. Calcd. for $C_9H_9O_4N$: C, 55.38; H, 4.65; N, 7.18. Found: C, 55.66; H, 4.53; N, 7.22.

The diacetate decomposed in moist air, liberating acetic acid. The aqueous solution when freshly prepared was neutral to litmus and gave no ferric chloride test; however, hydrolysis took place rapidly with appearance of an acid reaction and a violet color with ferric chloride.

Attempted Hydrolysis of Leucenol.—One hundred milligrams of leucenol was refluxed with 2 ml. of 47.3% hydrobromic acid for eight and one-half hours. The aqueous solution was evaporated to dryness *in vacuo*. The product dissolved in 5 ml. of absolute ethanol, but after precipitation with ether was no longer soluble in ethanol, m. p. after washing with absolute ethanol, 176–179° (cor.); melting point of a mixture with leucenol hydrobromide, 177.5–179° (cor.).

Anal. Calcd. for $C_5H_{11}O_4N_2Br$: C, 34.42; H, 3.96. Found: C, 34.37; H, 4.09.

No product was obtained which was soluble in absolute ethanol.

One hundred milligrams of leucenol was heated at reflux with 2 ml. of constant-boiling hydriodic acid for thirty-six hours. After removal of the excess acid and water *in vacuo*, the product was taken up in 2 ml. of absolute ethanol and precipitated with ether. The product, insoluble in boiling absolute ethanol, was apparently impure leucenol hydroiodide. No product soluble in ethanol was obtained.

Methylation of Leucenol.—(a) To a suspension of 200 mg. of leucenol in 50 cc. of ether, a large excess of an ether solution of diazomethane was added and the mixture allowed to stand at room temperature for two days. At the end of this time, most of the leucenol had reacted and gone into solution. Upon evaporation of the ether, an oily residue remained which could not be crystallized. Oxidation of the oil by refluxing with 50 cc. of 2% aqueous potassium permanganate for an hour failed to give a product which could be isolated.

(b) One hundred milligrams (1 mole equiv.) of leucenol was suspended in 0.2 ml. of water and an ethereal solution of diazomethane containing about 250 mg. (6 mole equiv.) of diazomethane was added. Nitrogen was evolved and the yellow color was bleached rapidly. Two more additions of 125-mg. portions of diazomethane were necessary before the yellow color persisted for one hour. The entire product was in the aqueous phase which was neutral to litmus. The water was removed *in vacuo* leaving a thick yellow sirup, which could not be induced to crystallize. The product was insoluble in ether and highly soluble in methanol and in water.

The hydrochloride of this product was obtained as a tan solid, soluble in water and in methanol. It was highly hygroscopic and therefore not suitable as a derivative.

The chloroaurate, picrate and picrolonate were prepared, but were not crystalline.

The product gave a pale yellow color with aqueous ferric chloride.

Oxidation with aqueous 1% potassium permanganate (5 atoms of oxygen and 11 atoms of oxygen per estimated mole of product) was carried out at room temperature. Permanganate equivalent to five atoms of oxygen was decolorized almost instantaneously, whereas the larger amount took several hours to react. No ether-soluble product could be isolated from either reaction.

Ultraviolet Absorption Spectrum of Leucenol.—The ultraviolet absorption spectra of leucenol in water and in dilute hydrochloric acid were determined in a Hilger spectroscope.

Summary

1. Leucenol was extracted from *Leucaena glauca benth* by means of 90% ethanol. It was shown to have the same empirical formula $(C_4H_5O_2N)_x$ as that reported by Mascré.

2. The presence of a phenolic group was confirmed by ferric chloride and Folin reagent tests; an α-amino acid by the ninhydrin test; half of the nitrogen as a primary amino group by Van Slyke analysis.

3. A methyl ester dihydrochloride was prepared.

4. Absorption spectra in aqueous and dilute hydrochloric acid resembled the spectra of hydroxypyridines.

5. Leucenol was pyrolyzed and yielded a compound which, by its properties and analyses, appears to be 2,5-dihydroxypyridine. The product forms a diacetate and salts which were characterized.

6. Leucenol was unaffected by long refluxing with hydrobromic or hydriodic acids.

7. Various possible structures for leucenol were discussed. The blue color with Folin reagent which is typical of a β-hydroxypyridine, along with the other available facts, leads to the conclusion that leucenol is probably β-N-(3-hydroxy-6-pyridone)-α-aminopropionic acid, although β-(3-hydroxy-6-pyridoxy)-α-aminopropionic acid is not excluded.

URBANA, ILLINOIS RECEIVED AUGUST 7, 1944

[CONTRIBUTION FROM THE RESEARCH LABORATORIES, THE UPJOHN COMPANY]

An Improved Synthesis of β-Alanine

BY SAUL R. BUC, JARED H. FORD AND E. C. WISE

A convenient laboratory method for the preparation of substantial quantities of β-alanine was desired since none of the earlier methods[1] appeared suitable.

In the present investigation we have obtained β-alanine by means of the following reactions:

(1) $CH_2{=}CHCN + NH_3 \longrightarrow NH_2CH_2CH_2CN + NH(CH_2CH_2CN)_2$

(2) $NH_2CH_2CH_2CN + 2HCl + 2H_2O \longrightarrow NH_2CH_2CH_2COOH{\cdot}HCl + NH_4Cl$

(3) $NH_2CH_2CH_2COOH{\cdot}HCl \xrightarrow[\text{resin}]{\text{anion exchange}} NH_2CH_2CH_2COOH$

The first step, addition of ammonia to acrylonitrile, has been described by Hoffmann and Jacobi,[2] and by Whitmore, *et al.*[3] These authors

(1) For a summary of the literature prior to 1942 see "Organic Syntheses," Coll. Vol. II, John Wiley and Sons, Inc., New York, N. Y., 1943. After the completion of our experimental work the following patents describing the preparation of β-alanine from acrylonitrile and several of its derivatives by heating with aqueous ammonia at 150–225° were issued: Carlson and Hotchkiss, U. S. Patent 2,335,997; Carlson, U. S. Patent 2,336,067; Kirk, U. S. Patent 2,334,163; Paden and Kirk, U. S. Patent 2,335,605; Dean, U. S. Patent 2,335,653.

(2) Hoffmann and Jacobi, U. S. Patent 1,992,615.

(3) Whitmore, Mosher, Adams, Taylor, Chapin, Weisel and Yanko, THIS JOURNAL, **66**, 725 (1944).