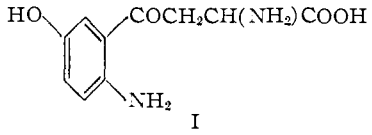
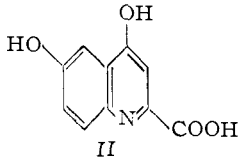
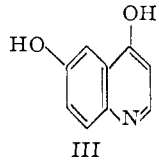
verted by rabbit, toad or mouse liver homogenate into 6-hydroxykynurenic acid (II) and 6,4-dihydroxyquinoline (III).[1] The incubated mixtures were examined by paper chromatography and spectrography. Compounds II and III required for identification were synthesized through ethyl 6-methoxy-4-hydroxyquinaldate.



I



II



III

Experimental

Incubation of 5-Hydroxy-D,L-kynurenine (I) with Liver Homogenates.—5-Hydroxy-D,L-kynurenine sulfate (2 mg.) was dissolved in 2 ml. of Krebs–Ringer phosphate solution (*p*H 7.4) and the *p*H adjusted to 7.4; 2 g. of liver homogenate, prepared by the homogenization of equal quantities of rabbit, toad or mouse liver and Krebs–Ringer phosphate (*p*H 7.4) in an ice-bath, was added and the mixture incubated at 38° for 3–4 hours. The mixture was then chromatographed (ascending method) on wide filter paper, No. 50 Tōyō Roshi (40 × 40 cm.), with a mixture of methanol, butanol, benzene and water (2:1:1:1). Two marked fluorescent bands of R_f 0.41 (A) and 0.78 (B) were obtained along with 5-hydroxy-D-kynurenine (R_f 0.21); the controls showed no such fluorescent bands. The bands were cut out; A was eluted with weak alkali and B with alcohol. The two eluates were examined chromatographically with various solvent systems and spectrographically. Finally A was identified as II and B as III. These results are summarized in Table I.

TABLE I

	A	6-Hydroxykynurenic acid	B	6,4-Dihydroxyquinoline	C[c]
Fluorescence	White pink	White pink	White green	White green	White green
Diazo reaction	Red	Red	Red	Red	Purple
$FeCl_3$ reaction	Brown	Brown	Reddish brown	Reddish brown	Brown
Absorption max., mμ	356	356	340	340	380
R_f[a]	0.41	0.41	0.78	0.78	0.61
R_f[b]	0.35	0.35	0.76	0.75	0.21
R_f (80% isopropyl alc.)	0.19	0.19	0.80	0.80	0.46[d]

[a] Methanol, butanol, benzene and water = 2:1:1:1. [b] Butanol, acetic acid and water = 4:1:5. [c] Orange color with Ehrlich's aldehyde reaction. [d] 70% isopropyl alcohol.

When the incubation was interrupted after one hour, another green fluorescent band (C) appeared between A and B; C had almost vanished at the end of 2 hours incubation.

Synthesis of Ethyl 6-Methoxy-4-hydroxyquinaldate.—*p*-Anisidine (12.5 g.) was condensed with 19 g. of ethyl oxalacetate on a water-bath for about 1.5 hours and the water which separated was evaporated *in vacuo*. The resulting dark red sirup was stirred in heated paraffin at 250° for about 10 minutes and cooled. After being decanted from the precipitated tar, it was again heated for a short time until yellowish brown crystals appeared. The crystals were washed with ether and finally recrystallized from boiling water to give tan needles, m.p. 215°, yield 2.4 g.

Anal. Calcd. for $C_{13}H_{13}NO_4$: C, 63.15; H, 5.26; N, 5.67. Found: C, 62.96; H, 5.33; N, 5.34.

6-Hydroxykynurenic Acid.—Ethyl 6-methoxy-4-hydroxyquinaldate (900 mg.) was refluxed with 20 ml. of hydroiodic acid (57%) under carbon dioxide for about 5 hours and cooled. The resulting crystals were separated on a glass filter and dried in a desiccator over alkali (HI salt; m.p. 285° dec., yellow plates). They were then dissolved in sodium carbonate solution; the solution was filtered and precipitated with dilute hydrochloric acid in the presence of a little bisulfite. The precipitate was dissolved in boiling 20% hydrochloric acid and filtered. The flat yellow crystals which separated on cooling were washed with very dilute hydrochloric acid to give the hydrochloride of 6-hydroxykynurenic acid, m.p. 298 ~ 300° dec., yield 670 mg.

Anal. Calcd. for $C_{10}H_7NO_4 \cdot HCl$: C, 49.69; H, 3.34; N, 5.79. Found: C, 49.90; H, 3.91; N, 5.86.

6,4-Dihydroxyquinoline (III).—II was decarboxylated by being heated above its melting point to III which was purified chromatographically.[2]

This work was aided by a grant from the scientific research fund of the Ministry of Education of Japan. We wish to thank the Takeda Research Laboratory for the elementary analyses.

(1) Dr. O. Hayaishi (personal communication) obtained II and 5-hydroxyanthranilic acid from our synthetic 5-hydroxykynurenine with purified enzyme.

(2) K. Makino and H. Takahashi, *Proc. Comm. Res. Animal Metabolism*, **23**, April (1954).

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Synthesis of 1,4,5,6,13,14-Hexahydro-5-methyl-8,9-methylenedioxyphenanthridine Hydrochloride

BY L. H. MASON AND W. C. WILDMAN

RECEIVED JULY 23, 1954

In the course of our research on the chemistry and pharmacological action of the alkaloids of the *Amaryllidaceae*, the title compound was required for study. This paper records its synthesis by a method similar to that used for its 6-methoxy analog.[1]

4-(3,4-Methylenedioxyphenyl)-3-nitrocyclohexene, prepared by the diene synthesis with butadiene and 3,4-methylenedioxy-β-nitrostyrene, was reduced to the corresponding amine with lithium aluminum hydride. Pictet–Spengler cyclization with formaldehyde and N-methylation gave the desired product in good yield.

2,3-Dimethyl-8,9-methylenedioxy-1,4,13,14-tetrahydrophenanthridine and its 8,9-dimethoxy-6-phenyl analog have been prepared by Sugasawa[2] using a slightly different method.

Experimental[3]

4-(3,4-Methylenedioxyphenyl)-3-nitrocyclohexene (I).—A Pyrex bomb was charged with 4.0 g. (0.02 mole) of 3,4-methylenedioxy-β-nitrostyrene,[4] 16 ml. of dry toluene, 10 g. (0.19 mole) of butadiene and a trace of hydroquinone. The bomb was sealed and heated gradually to 110° over a period of four days. The temperature was maintained at 110°

(1) W. C. Wildman and W. T. Norton, THIS JOURNAL, **76**, 152 (1954).

(2) S. Sugasawa and K. Kodama, *Ber.*, **72**, 675 (1939).

(3) All melting points were observed on a Kofler microscope hotstage equipped with polarizer and are corrected. The numbering of the phenanthridine ring system is in accord with that found in A. M. Patterson and L. T. Capell, "The Ring Index," Reinhold Publishing Corp., New York, N. Y., 1940, p. 267. Microanalyses were performed by Dr. W. C. Alford and his staff. Ultraviolet absorption spectra were determined by Mrs. I. J. Siewers and Miss F. C. Bateman.

(4) E. Knoevenagel and L. Walter, *Ber.*, **37**, 4502 (1904).

for eight days. The bomb was cooled and the brown liquid was concentrated in an air jet. Trituration with ethanol gave 4.08 g. of crude solid which was recrystallized from ethanol to yield 3.15 g. (61%) of light tan prisms, m.p. 97–99°. A portion was sublimed for analysis, m.p. 97–99°.

Anal. Calcd. for $C_{13}H_{13}NO_4$: C, 63.15; H, 5.30; N, 5.67. Found: C, 63.18; H, 5.23; N, 5.48.

The ultraviolet absorption spectrum (ethanol) showed maxima at 234 mμ (log ε 3.68) and 288 mμ (log ε 3.59).

3-Amino-4-(3,4-methylenedioxyphenyl)-cyclohexene Hydrochloride (II).—By the procedure of Wildman and Norton,[1] 3.0 g. of 4-(3,4-methylenedioxyphenyl)-4-nitrocyclohexene was reduced with excess lithium aluminum hydride to give 2.21 g. (72%) of amine hydrochloride, m.p. 230–238°. The analytical sample was recrystallized twice from ethanol, m.p. 240–242°.

Anal. Calcd. for $C_{13}H_{15}NO_2 \cdot HCl$: C, 61.54; H, 6.36; N, 5.52. Found: C, 61.37; H, 6.43; N, 5.72.

The ultraviolet absorption spectrum (ethanol) showed maxima at 238 mμ (log ε 3.60) and 288 mμ (log ε 3.60).

1.4.5.6.13,14-Hexahydro-8,9-methylenedioxyphenanthridine (III).—A solution of 0.80 g. of II in 27 ml. of water was made basic with 10% sodium hydroxide solution and the free base was extracted three times with ether. The ether was removed and the residual oil was treated with 1.1 ml. of formalin. The mixture was heated on a steam-bath for 20 minutes with occasional swirling. The mixture was extracted with benzene and the oily Schiff base was obtained upon concentration of the benzene solution. A solution of 2 ml. of 19% hydrochloric acid was added to the Schiff base and the solution was allowed to stand overnight. The product crystallized during this time and was removed by filtration, 0.71 g., m.p. 253–265° dec. Concentration of the filtrate gave an additional 0.03 g., m.p. 271–282° dec., after one recrystallization from methanol–ethanol. This was combined with the first crop and recrystallized from methanol–ethanol, 0.49 g. (59%), m.p. 279–282° dec. A small portion was recrystallized from the same solvent for analysis, m.p. 280–282° dec.

Anal. Calcd. for $C_{14}H_{15}NO_2 \cdot HCl$: C, 63.27; H, 6.07; N, 5.27. Found: C, 63.09; H, 6.01; N, 5.38.

The ultraviolet absorption spectrum (water) showed maxima at 234 mμ (log ε 3.55) and 292 mμ (log ε 3.65).

1,4,5,6,13,14-Hexahydro-5-methyl-8,9-methylenedioxyphenanthridine Hydrochloride (IV).—By the procedure of Wildman and Norton,[1] 0.489 g. of III gave 0.317 g. (62%) of IV, m.p. 239–243° dec. A portion was recrystallized from methanol–ether for analysis, m.p. 240–243° dec.

Anal. Calcd. for $C_{15}H_{17}NO_2 \cdot HCl$: C, 64.39; H, 6.48; N, 5.01. Found: C, 64.14; H, 6.49; N, 4.75.

The ultraviolet absorption spectrum (ethanol) showed maxima at 238 mμ (log ε 3.53) and 292 mμ (log ε 3.66).

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α-L-Formamidinoglutaric Acid, an Intermediate in Histidine Metabolism[1]

BY ALEXANDER MILLER AND HEINRICH WAELSCH

RECEIVED JULY 22, 1954

Isolation of a crystalline compound obtained upon incubation of histidine or urocanic acid with a phosphate extract of cat liver has recently been reported from this Laboratory.[2,3] Although not attacked by mammalian tissue preparations, the compound was rapidly degraded by cell-free extracts of histidine-adapted *Pseudomonas fluorescens* prepared according to Tabor and Hayaishi.[4] On the basis of the analytical data and properties of the crystalline compound and its mercury derivative, it was suggested that the compound was α-L-formamidinoglutaric acid. A compound with similar properties has recently been isolated from incubation mixtures of histidine and extracts of *Pseudomonas fluorescens*.[5]

Further characterization of the compound isolated from enzymatic digests was dependent upon comparison with synthetic α-L-formamidinoglutaric acid. Because of the necessity of distinguishing the formamidino compound from its tentatively postulated metabolic precursor, 4(5)-imidazolone-5(4)-propionic acid,[3,6] the synthesis used had to be one where cyclization of the formamidino compound to the imidazolone was unlikely. Compounds of these types are little known.

The synthesis of α-L-formamidinoglutaric acid with properties essentially identical with those of the isolated product has been accomplished. The γ-benzyl ester of L-glutamic acid suspended in formamide was treated with formamidine in the presence of silver carbonate. The resulting γ-benzyl ester of α-formamidinoglutaric acid was converted to free α-formamidinoglutaric acid by hydrogenolysis of the ester with palladium-black as a catalyst.

The analytical data and properties of synthetic α-L-formamidinoglutaric acid (A) and of the compound isolated from enzymatic digests (B) are given in Table I.

The synthetic compound, like that isolated from enzymatic digests,[2,3] was decomposed by extracts of *Pseudomonas fluorescens* more rapidly than urocanic acid.

TABLE I

PROPERTIES OF α-L-FORMAMIDINOGLUTARIC ACID

A synthetic; B from enzymatic digests[2,3]

Calcd. for	Found	
$C_6H_{10}N_2O_4 \cdot H_2O$	A	B
C 37.5	37.2[a]	37.5, 39.6
H 6.3	..	6.5, 6.7
N 14.6	14.6	14.0, 14.1
Alkali labile N, 7.3	7.3	7.0
pK_1'; pK_2'; pK_3'	2.7, 4.4, 11.3	2.4, 4.7, 11.1
$[\alpha]^{28}D$	−10.3[b]	−10.7
M.p., °C.	85–95	80–87
Infrared spectrum μ	3.1 (s),[c] 5.2 (w). 5.8(s), 6.2(s), 7.1 (m), 9.2 (w), 12.2 (w)	3.1 (s), 5.2 (w), 5.8 (s), 6.2 (s), 7.1 (m), 9.2(w), 12.2 (w)

[a] D. D. Van Slyke and J. Folch, *J. Biol. Chem.*, **136**, 509 (1940). [b] 0.8% in 1 *N* HCl. [c] (s) strong, (w) weak, (m) medium.

The γ-benzyl ester of formamidinoglutaric acid

(1) This work was supported in part by grants from the National Institute of Neurological Disease and Blindness (Grant B-226) of the National Institutes of Health, Public Health Service and by a contract between the Office of Naval Research and the Psychiatric Institute. Taken from a doctoral dissertation to be submitted by Alexander Miller.

(2) B. A. Borek and H. Waelsch, THIS JOURNAL, **75**, 1772 (1953).

(3) B. A. Borek and H. Waelsch, *J. Biol. Chem.*, **205**, 459 (1953).

(4) H. Tabor and O. Hayaishi, *ibid.*, **194**, 171 (1952).

(5) H. Tabor and A. H. Mehler, *ibid.*, in press; J. E. Seegmiller, M. Silverman, H. Tabor and A. H. Mehler, THIS JOURNAL, **76**, 6205 (1954).

(6) M. Suda, A. Nakaya, M. Hara, A. Kato and T. Ikenaka, *Med. J. Osaka Univ.*, **4**, 107 (1953).