TABLE I

| | Substituents P | R | R′ | Mol. formula | M.p., °C. | Mol. wt. Found | Mol. wt. Calcd. | N, % Found | N, % Calcd. | Crude yield[a] Proc. A | Crude yield[a] Proc. B | Ref. |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | H | H | Cyclohexyl | $C_{14}H_{20}N_2O$ | 115–117 | 237 | 232.3 | 11.88 | 12.06 | 15% | 33% | ... |
| 2 | H | $CH_3$ | $CH_3$ | $C_{10}H_{14}N_2O$ | 150–153 | 178 | 178.2 | 15.63 | 15.72 | | 60 | ... |
| 3 | H | $CH_3$ | Cyclohexyl | $C_{15}H_{22}N_2O$ | 158–160 | 248 | 246.3 | 11.48 | 11.37 | | 62 | ... |
| 4 | H | $C_2H_5$ | $C_2H_5$ | $C_{12}H_{18}N_2O$ | 140–142 | 206 | 206.3 | 13.72 | 13.58 | | 69 | 6, 12 |
| 5 | H | $-(CH_2)_3-CH_3$ | $-(CH_2)_3-CH_3$ | $C_{16}H_{26}N_2O$ | 145–146 | 264 | 262.4 | 10.88 | 10.68 | 68 | 97 | 6 |
| 6 | H | $-CH_2-CH-(CH_3)_2$ | $-CH_2-CH-(CH_3)_2$ | $C_{16}H_{26}N_2O$ | 109–110 | 260 | 262.4 | 10.59 | 10.68 | | 80 | ... |
| 7 | H | Piperidino | | $C_{13}H_{18}N_2O$ | 153–154 | 218.3 | 218.3 | 12.65 | 12.83 | 79 | 85 | 2, 3, 4 |
| 8 | H | Morpholino | | $C_{12}H_{16}N_2O_2$ | 155–157 | 226 | 220.3 | 12.68 | 12.72 | | 82 | 9 |
| 9 | $OCH_3$ | $CH_3$ | $CH_3$ | $C_{11}H_{16}N_2O_2$ | 174 | 204 | 208.3 | 13.10 | 13.45 | | 58 | ... |
| 10 | $OCH_3$ | $C_2H_5$ | $C_2H_5$ | $C_{13}H_{20}N_2O_2$ | 150–152 | 238 | 236.3 | 11.90 | 11.86 | 58 | 62 | ... |
| 11 | $OCH_3$ | $-(CH_2)_2-CH_3$ | $-(CH_2)_2-CH_3$ | $C_{15}H_{24}N_2O_2$ | 128 | 264 | 264.4 | 10.60 | 10.59 | | 73 | ... |
| 12 | $OCH_3$ | $-CH-(CH_3)_2$ | $-CH-(CH_3)_2$ | $C_{15}H_{24}N_2O_2$ | 133–134 | 259 | 264.4 | 10.10 | 10.59 | | 76 | ... |
| 13 | $OCH_3$ | $-(CH_2)_3-CH_3$ | $-(CH_2)_3-CH_3$ | $C_{17}H_{28}N_2O_2$ | 130 | 292.4 | 292.4 | 9.56 | 9.58 | 72 | 77 | ... |
| 14 | $OCH_3$ | $-CH_2-CH-(CH_3)_2$ | $-CH_2-CH-(CH_3)_2$ | $C_{17}H_{28}N_2O_2$ | 106–108 | 289 | 292.4 | 9.31 | 9.58 | | 65 | ... |
| 15 | $OCH_3$ | $CH_3$ | Cyclohexyl | $C_{16}H_{24}N_2O_2$ | 110 | 280 | 276.4 | 10.42 | 10.14 | | 32 | ... |
| 16 | $OCH_3$ | Piperidino | | $C_{14}H_{20}N_2O_2$ | 182–184 | 250 | 248.3 | 11.15 | 11.28 | 56 | 75 | ... |
| 17 | $OCH_3$ | H | $-(CH_2)_3-CH_3$ | $C_{13}H_{20}N_2O_2$ | 104 | 236 | 236.3 | 11.90 | 11.86 | | 58 | ... |
| 18 | $OCH_3$ | H | Cyclohexyl | $C_{15}H_{22}N_2O_2$ | 115 | 264 | 262.3 | 10.82 | 10.67 | | 30 | ... |
| 19 | $CH_3$ | Piperidino | | $C_{14}H_{20}N_2O$ | 148–150 | 232 | 232.3 | 11.83 | 12.06 | 555 | 62 | ... |
| 20 | $CH_3$ | Morphilino | | $C_{13}H_{18}N_2O_2$ | 152–153 | 232 | 234.3 | 11.75 | 11.96 | | 58 | ... |

[a] Based on the amount of benzaldehyde, anisaldehyde or *p*-methylbenzaldehyde used.

equals papaverine in this respect. It is however several times less toxic. A detailed description of the pharmacological properties of this series of amides will be published elsewhere.[1]

### Experimental Part

The amines, obtained in good yield by concentrated sulfuric acid hydrolysis of the corresponding nitriles for 10 to 60 minutes at 100°, were purified with charcoal in boiling acid solution, followed by repeated crystallization from ethanol–ether mixtures. Hydrolysis does not proceed beyond the amide stage in these conditions.

The corresponding α-amino-α-phenylacetonitriles were prepared by one or both of the following general procedures adapted from reported methods.[2-13] **First Procedure:** from the amine hydrochloride (0.1 mole), the corresponding benzaldehyde (0.1 mole) and potassium cyanide (0.11 mole).[2] **Second procedure:** from the amine (0.12 mole), the bisulfite addition product of the corresponding benzaldehyde (0.1 mole) and potassium cyanide (0.1 mole).[3,11,12]

The following analytical methods were used (Table I).

(a) Melting points were determined with the micro-apparatus described by Kofler.[14]

(b) Total nitrogen content was determined using a semi-micro Kjeldahl method.[15]

(c) The molecular weight was determined by potentiometric perchloric acid titration of the amine function of the amides, dissolved in anhydrous acetic acid, using a Metrohm Titroskop.[16]

(d) Ultraviolet spectrophotometry (Beckman DU spectrophotometer) served as an important criterion of purity. The ultraviolet spectra of the amides in isopropyl alcohol (2 mmoles per liter) were recorded at 20 ± 1° between 210 and 300 mμ. The molar absorption spectra of the amides listed in the table fall into three groups, depending on the nature of the substituent P. Within these three groups, identical spectra were recorded for the amides with varying N-alkyl-substituents R and R′.

The following examples illustrate these two procedures.

**A.**—0.1 mole of dibutylamine (12.9 g., 97% pure by titration) was neutralized with 20% hydrochloric acid; anisaldehyde (0.1 mole, 13.6 g.; b.p. 134–135° (12 mm.)) was added at once and potassium cyanide (0.11 mole, 7.16 g.) dissolved in 25 ml. of water added dropwise to the stirred mixture at room temperature. After a two-hour heating period (100–120°), the oily α-dibutylamino-α-(*p*-methoxyphenyl)-acetonitrile, which separated from the cooled solution, was mixed with 30 ml. of concentrated sulfuric acid. This mixture was heated at 100° for 10 minutes, cooled for one hour at room temperature, and treated with three volumes of water at 0°. When this solution was neutralized with concentrated ammonia, a brown solid precipitated immediately. It was collected on a filter, washed with 200 ml. of water, dried, weighed[17] and dissolved in 100 ml. of 10% hydrochloric acid. After addition of 2 g. of charcoal, the suspension was heated under reflux for 20 minutes and neutralized with ammonia. The white precipitate was collected by filtration, washed with 200 ml. of water and repeatedly recrystallized from ethanol containing 10% of ethyl ether. Three crystallizations were necessary to obtain pure α-dibutylamino-α-(*p*-methoxyphenyl)-acetamide (white rods, m.p. 125–127°, 14.6 g., 50%).

**B.**—To 13.6 g. (0.1 mole) of ice-cooled anisaldehyde, a saturated aqueous solution of 12.5 g. (0.12 mole) of sodium bisulfite was added. After the subsequent dropwise addition of 15.5 g. (0.12 mole) of dibutylamine, 6.5 g. (0.1 mole) of solid potassium cyanide was poured at once into the reaction mixture. The oily nitrile, which separates from the solution at room temperature, was converted to the pure amide as described in the first procedure; 17.0 g. (58%) of pure α-dibutylamino-α-(*p*-methoxyphenyl)-acetamide was obtained.

**Acknowledgment.**—The author wishes to express his gratitude to Mr. A. Jageneau and Mr. P. Demoen for valuable assistance.

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(15) United States Pharmacopeia, 14th ed., p. 740.

(16) Metrohm AG, Herisau, Switzerland.

(17) The crude yields recorded in the table are based on this weight.

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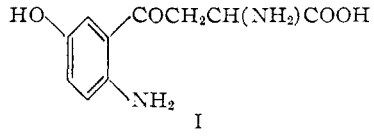
## The Conversion of 5-Hydroxykynurenine to 6-Hydroxykynurenic Acid and 6,4-Dihydroxyquinoline with Liver Homogenates

BY KATASHI MAKINO AND HITOSHI TAKAHASHI

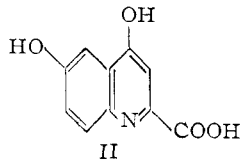
RECEIVED JUNE 7, 1954

5-Hydroxy-D,L-kynurenine (I), which was recently synthesized in our laboratory, has been con-

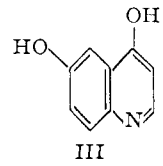
verted by rabbit, toad or mouse liver homogenate into 6-hydroxykynurenic acid (II) and 6,4-dihydroxyquinoline (III).[1] The incubated mixtures were examined by paper chromatography and spectrography. Compounds II and III required for identification were synthesized through ethyl 6-methoxy-4-hydroxyquinaldate.




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.

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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

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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°

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(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.

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