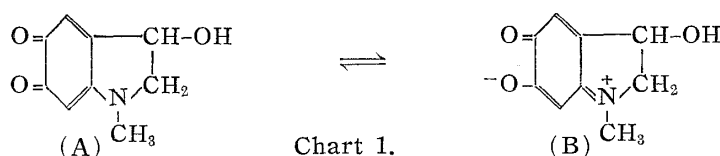


#### 46. Jun-ichi Iwao: Studies on Adrenochrome Derivatives. I. Determination of the Structure of Adrenochrome Monosemicarbazone.

(Osaka Research Laboratory, Gohei Tanabe & Co., Ltd.\*)

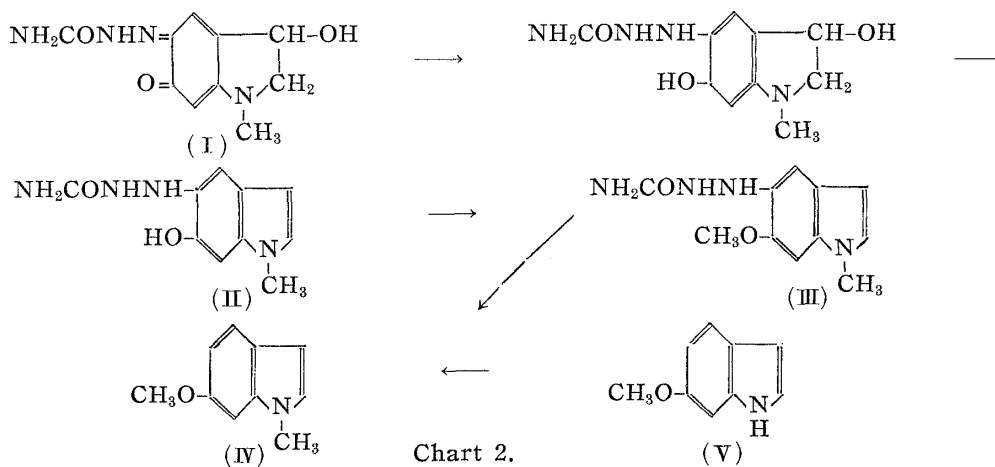
Adrenochrome, which was found to possess hemostatic activity by the work of Derouaux and others, is unstable but its monosemicarbazone is a stable hemostatic substance. The structure of this compound is given as 1-methyl-3-hydroxy-5-semicarbazono-6-oxo-2,3,5,6-tetrahydroindole (I) in U. S. Patent No. 2,581,850, and the Belgian Patent No. 510,295 also supports this formula. Recently, Ramirez and others<sup>1)</sup> discussed this compound as having this structure (I) from its ultraviolet and infrared spectra, but no direct synthetic evidence has been offered.

It is known from the works of Harley-Mason<sup>2,3)</sup> that the resonance system shown by Chart 1 exists in adrenochrome. Since the structure (B) possesses =C-O<sup>-</sup> group in the 6-position, >C=O group in the 6-position of (A) is assumed to be practically devoid of carbonyl properties, which may explain the fact that only a monosemicarbazone or monoxime is formed and not disemicarbazone or dioxime. It follows that the reaction of semicarbazide or hydroxylamine occurs with >C=O in the 5-position.



Kehrmann and Hoehn<sup>4)</sup> synthesized 4-acetamido-*o*-benzoquinone monoxime and proved that the reaction occurred in the position *para* to the acetamido group. The writer carried out the present work in order to prove the structure of adrenochrome monosemicarbazone by direct synthesis.

Harley-Mason<sup>3)</sup> obtained 5,6-dihydroxyindole and 3,5,6-trihydroxyindole by the catalytic reduction of adrenochrome. Catalytic reduction of adrenochrome monosemicarbazone (tentatively assumed as having the structure (I)) with palladium-carbon catalyst resulted in the absorption of 1 mole of hydrogen and microneedles of m.p.



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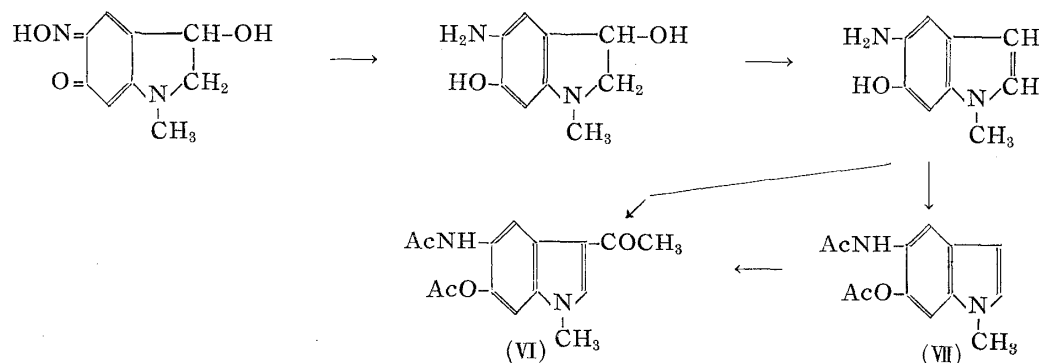
- 1) F. Ramirez, P. Ostwalden: J. Org. Chem., **20**, 1676(1955).
- 2) J. Harley-Mason: Experientia, **4**, 307(1948)(C.A., **43**, 3404(1949)).
- 3) J. Harley-Mason: J. Chem. Soc., **1950**, 1276.
- 4) F. Kehrmann, E. Hoehn: Helv. Chim. Acta, **8**, 218(1925).

205°(decomp.) were obtained. This compound dissolves in sodium hydroxide solution but not in sodium carbonate, and colors dark red with the Ehrlich reagent. It was thereby assumed that a reduction as shown in Chart 2 had occurred, forming 1-methyl-5-semicabazido-6-hydroxyindole (II) by subsequent dehydration. Methylation of (II) in methanol and dimethyl sulfate, adding sodium hydroxide at 20°, yielded 1-methyl-5-semicarbazido-6-methoxyindole (III), insoluble in sodium hydroxide solution. Pyrolysis of (III) by heating with sodium carbonate in glycerol at 185~195° afforded crystals of m.p. 30°, b.p.<sub>1</sub> 145°(bath temperature), with evolution of nitrogen gas. This compound was assumed to be 1-methyl-6-methoxyindole (IV) and its picrate is obtained as reddish brown silky crystals of m.p. 123°. Both (III) and (IV) color dark red with the Ehrlich reagent and their analytical values agree well with the assumed structures.

Kermack *et al.*<sup>5)</sup> synthesized 1-methyl-6(?)methoxyindole from pyruvic acid *N-m-methoxyphenyl-N-methylhydrazone* by the Fischer's indole synthesis and decarboxylation, and reported its picrate to be reddish brown needles melting at 123°, but left a question mark for the 6-position since two routes of cyclization are possible.

The writer obtained 1-methyl-6-methoxyindole (IV) almost quantitatively by the reaction of 6-methoxyindole (V), synthesized by the method of Harrey and Robson,<sup>6)</sup> with methyl iodide in liquid ammonia, in the presence of metallic sodium, in accordance with the method of Potts and Saxton.<sup>7)</sup> This compound (IV) was found by mixed melting point determination to be identical with the substance obtained by the pyrolysis of (III). Their ultraviolet spectra also agreed well. This has offered an unequivocal evidence that >C=O group in the 5-position of adrenochrome reacted with semicarbazide to form adrenochrome monosemicarbazone (1-methyl-3-hydroxy-5-semicarbazono-6-oxo-2,3,5,6-tetrahydroindole) and has also proved that the compound obtained by Kermack and others was actually 1-methyl-6-methoxyindole.

Catalytic reduction of adrenochrome monoxime in dehydrated ethanol with palladium-carbon catalyst results in absorption of two moles of hydrogen and slightly yellowish green scaly crystals, m.p. 220~222°(decomp.), are obtained as the product. This is an extremely labile substance and heating it with acetic anhydride converts it to pale yellow scaly crystals (VI), m.p. 169~171°. Further, catalytic reduction of the monoxime in glacial acetic acid and heating of the filtrate, obtained on the removal of the catalyst, with acetic anhydride yields needle crystals (VII) of m.p. 205~206°. Both (VI) and (VII) color dark red with the Ehrlich reagent, indicating the presence of an indole ring. (VI) is also obtained on heating (VII) with acetic anhydride. It is already known that the reaction of indole and acetic anhydride<sup>8)</sup> results in the formation of 1-acetyl- and 1,3-diacetylindoles. Based on the foregoing structural determination of



5) W. O. Kermack, W. H. Perkins, R. Robinson : J. Chem. Soc., **1922**, 1872.

6) D. G. Harrey, D. Robson : *Ibid.*, **1938**, 97.

7) K. T. Potts, J. E. Saxton : *Ibid.*, **1954**, 2641.

8) C. Zatti, A. Ferratini : *Ber.*, **23**, 1359(1890).

adrenochrome monosemicarbazone, (VII) is assumed to be 1-methyl-5-acetamino-6-acetyloxyindole and (VI), 1-methyl-3-acetyl-5-acetamino-6-acetyloxyindole. The analytical values of these compounds agreed well with these assumed structures.

The writer expresses his deep gratitude to Prof. Shigehiko Sugasawa of the University of Tokyo for his kind and unflinching guidance throughout the course of this work. He is also grateful to Mr. Shiro Tejima, Director of Preparations and Planning Section of this Company, Dr. Masao Fujisawa, Director of the Research Laboratory, Dr. Hikaru Ozawa, and Dr. Shun-ichi Yamada for their advices and encouragements, to Mr. Kôichi Tomino for technical help, and to Mrs. F. Hisamichi and Mr. T. Yoda for microanalytical data.

### Experimental

**1-Methyl-5-semicarbazido-6-hydroxyindole (II)**—A suspension of 1 g. of adrenochrome monosemicarbazone in 70 cc. of glacial AcOH, added with 0.5 g. of 10% Pd-C, was submitted to catalytic reduction at ordinary temperature and pressure and 100 cc. of H<sub>2</sub> was absorbed during 10~15 mins., the reaction mixture turning from orange red to colorless solution. The catalyst was filtered off, AcOH was distilled off under a reduced pressure at 40~50°, and the crude crystals thereby obtained were washed with a small amount of MeOH and ether (0.6 g.). This was recrystallized from MeOH to microneedles, m.p. 205°(decomp.), soluble in 10% HCl and 10% NaOH, insoluble in 10% Na<sub>2</sub>CO<sub>3</sub>. *Anal.* Calcd. for C<sub>10</sub>H<sub>12</sub>O<sub>2</sub>N<sub>4</sub>: C, 54.55; H, 5.46; N, 25.46. Found: C, 54.20; H, 5.35; N, 25.93.

**1-Methyl-5-semicarbazido-6-methoxyindole (III)**—To a suspension of 5 g. of (II) in 20 cc. MeOH, 4 g. of Me<sub>2</sub>SO<sub>4</sub> was added, and 5 g. of 40% NaOH solution was dropped in while stirring the mixture at 20~25°. The stirring was continued for some time after the completion of the dropwise addition, the crystals thereby formed were collected by suctional filtration, and washed thoroughly with 5% NaOH and water, affording 3 g. of crude crystals. Recrystallization from MeOH yielded colorless needles, m.p. 210°(decomp.). *Anal.* Calcd. for C<sub>11</sub>H<sub>14</sub>O<sub>2</sub>N<sub>4</sub>: C, 56.40; H, 6.02; N, 23.92. Found: C, 56.12; H, 5.75; N, 24.18.

**1-Methyl-6-methoxyindole (IV)**—i) A small amount of Fe(NO<sub>3</sub>)<sub>3</sub> was added to 50 cc. liq. NH<sub>3</sub>, 0.31 g. of metallic Na dissolved in it, and a solution of 1.8 g. of 6-methoxyindole (V) dissolved in 10 cc. dehyd. ether was added gradually. After 10 mins., 2.1 g. MeI was added, stirred for 15 mins., and liq. NH<sub>3</sub> was allowed to evaporate. Water and ether were added to the residue and the mixture shaken to extract the product in ether. After drying, the ether was evaporated and pale brown needle crystals melting at 29~30° were obtained. Its low-pressure distillation afforded 1.9 g. (96.5%) of an oil of b.p. 145°(bath temp.). This oil solidified immediately at room temp. (15°) to crystals of m.p. 30~32°. *Anal.* Calcd. for C<sub>10</sub>H<sub>11</sub>ON: C, 74.51; H, 6.88; N, 8.69. Found: C, 74.01; H, 6.89; N, 8.73. U. V.  $\lambda_{max}^{EtOH}$  m $\mu$  (log  $\epsilon$ ): 224(5.43), 275(4.68), 292(4.74).

Picrate: Reddish brown silky needles (from EtOH), m.p. 123°. *Anal.* Calcd. for C<sub>10</sub>H<sub>11</sub>ON·C<sub>6</sub>H<sub>3</sub>O<sub>7</sub>N<sub>3</sub>: C, 49.23; H, 3.62; N, 14.36. Found: C, 49.04; H, 3.63; N, 14.30.

ii) A mixture of 2.3 g. of (III), 4 g. of anhyd. Na<sub>2</sub>CO<sub>3</sub>, and 10 cc. glycerol was heated, by which the mixture began to melt at 160° and to generate gas at 185~195°. This gas was not absorbed either by 30% NaOH or 30% H<sub>2</sub>SO<sub>4</sub>, and burning charcoal placed in this gas was extinguished immediately, that it was assumed to be N<sub>2</sub> (ca. 50 cc. collected). After the generation of gas had ceased, the mixture was poured into water, extracted with ether, and the ether was distilled off after drying over anhyd. Na<sub>2</sub>SO<sub>4</sub>. The residue was again extracted with ligroine and 0.4 g. of reddish brown oily residue was obtained on evaporation of ligroine. The substance of b.p. 145°(bath temp.), solidified on cooling, and melted at 30°. Its picrate came as reddish brown silky needles, m.p. 123°. Both these substances showed no depression of m.p. on admixture with the corresponding compounds obtained in i), and their ultraviolet spectra were identical.

**Reduction of Adrenochrome Monoxime**—1) A solution of 0.5 g. of the oxime dissolved in 80 cc. dehyd. EtOH, added with 0.3 g. of 10% Pd-C, was submitted to catalytic reduction at ordinary temperature and pressure. During 7 mins., 150 cc. of H<sub>2</sub> was absorbed and the solution changed from red to yellow in color. After filtering off the catalyst, EtOH was evaporated under a reduced pressure and 0.35 g. of slightly yellowish green scaly crystals, m.p. 220~222°(decomp.), were obtained. Its solution was easily oxidized, turning dark brown, and recrystallization was impossible.

ii) A mixture of 0.5 g. of the crude crystals obtained in i) and 2 cc. Ac<sub>2</sub>O was refluxed for 1.5 hrs., decomposed by pouring this mixture into water, and 0.5 g. of crude crystals were obtained. Recrystallization from MeOH yielded pale yellow scaly crystals, m.p. 169~171°, of 1-methyl-3-acetyl-5-acetamino-6-acetyloxyindole (VI). *Anal.* Calcd. for C<sub>18</sub>H<sub>16</sub>O<sub>4</sub>N<sub>2</sub>: C, 62.50; H, 5.56; N, 9.72. Found: C, 62.43; H, 5.52; N, 9.70.

iii) A solution of 1 g. of the oxime dissolved in 12 cc. of glacial AcOH, added with 0.2 g. of 5% Pd-C, was submitted to catalytic reduction and the absorption of H<sub>2</sub> was completed in 10 mins.

After removal of the catalyst by filtration, 3 cc. of  $\text{Ac}_2\text{O}$  was added to the filtrate and the mixture was refluxed for 1.5 hrs. This reaction mixture was poured into water, decolorized, and neutralized with  $\text{NaHCO}_3$ , from which 0.2 g. of crystals were obtained. Recrystallization from  $\text{MeOH-EtOH}$  afforded colorless needles, m.p.  $205\sim 206^\circ$ , of 1-methyl-5-acetamino-6-acetyloxyindole (VII). *Anal.* Calcd. for  $\text{C}_{13}\text{H}_{14}\text{O}_3\text{N}_2$ : C, 63.42; H, 5.69; N, 11.39. Found: C, 63.90; H, 5.52; N, 11.15.

(VII) was formed even on the use of optically active oxime. (VII) is not optically active.

iv) A mixture of 0.2 g. of (VII) obtained in iii) and 1 cc. of  $\text{Ac}_2\text{O}$  was refluxed for 1.5 hrs., cooled, and the mixture was poured into ice water to effect decomposition. The crude crystals that separated out were collected by suctional filtration and recrystallized from  $\text{MeOH}$  to 0.2 g. of scaly crystals, m.p.  $169\sim 171^\circ$ , showing no depression on admixture with (VI) obtained in ii), proving it to be 1-methyl-3-acetyl-5-acetamino-6-acetoxyindole.

### Summary

Adrenochrome possesses  $>\text{C}=\text{O}$  group in the 5- and 6-positions but no synthetic evidence has been offered on which of these  $>\text{C}=\text{O}$  groups had reacted with semicarbazide to form adrenochrome monosemicarbazone. Catalytic reduction of the monosemicarbazone with palladium-carbon catalyst results in absorption of 1 mole of hydrogen to form 1-methyl-5-semicarbazido-6-hydroxyindole (II), whose methylation with dimethyl sulfate and sodium hydroxide affords 1-methyl-5-semicarbazido-6-methoxyindole (III). Pyrolysis of (III) by heating with sodium carbonate in glycerol at  $185\sim 195^\circ$  ended in liberation of nitrogen and 1-methyl-6-methoxyindole (IV) was formed. On the other hand, methylation of 6-methoxyindole (V) with methyl iodide in liquid ammonia with metallic sodium yielded (IV). This has proved that adrenochrome monosemicarbazone is 1-methyl-3-hydroxy-5-semicarbazono-6-oxo-2, 3, 5, 6-tetrahydroindole.

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*U.D.C. 547.756.07*

#### 47. Jun-ichi Iwao : Studies on Adrenochrome Derivatives. V.<sup>1)</sup> Solubilization of Adrenochrome Monosemicarbazone and Its Derivatives. (1). Synthesis of 4 $\beta$ -Hydroxyethylsemicarbazones.

(Osaka Research Laboratory, Gohei Tanabe & Co., Ltd.\*)

It has already been shown that adrenochrome monosemicarbazone (I) is sparingly soluble in solvents, its solubility in water being 0.3 mg./cc. at  $5^\circ$  and 0.4 mg./cc. at  $20^\circ$ . The excellent hemostatic action of adrenochrome had been reported by Derouaux<sup>2)</sup> but its actual medicinal usage had seemed difficult due to its instability. The more stable (I) is also known to possess excellent hemostatic effect but due to its difficulty of dissolving in water, preparation of higher concentrations had been impossible. In 1948, a patent was taken out for the molecular compound of sodium salicylate and (I),<sup>3)</sup> and its specification revealed the fact that a mixture of 25 : 1 of salicylic acid and (I) dissolved in water up to 25 mg./cc. The Belgian Patent<sup>4)</sup> seems to use sodium benzoate as the solubilization agent but no details are known.

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- 1) Part IV : J. Pharm. Soc. Japan, **76**, 814 (1956)
- 2) C. Derouaux : Compt. rend. soc. biol., **131**, 830 (1939) (C. A., **33**, 7887 (1939)).
- 3) U. S. Pat. 2,581,850 (C. A., **46**, 2759 (1952)).
- 4) Belgian Pat. 525,542 (through C. A., **48**, 14132 (1954)).
- 5) a) Part II. J. Pharm. Soc. Japan, **76**, 808 (1956). b) Part III. *Ibid.*, **76**, 811 (1956).