his much helpful advice regarding the analytical techniques in the first stage of this work and to Mr. Takashima of this faculty for his help with the spectrophotometric measurements. Appreciation is expressed to the Research Laboratories of Dainihon Seiyaku Co. Ltd. and of Takeda Pharmaceutical Industries, Ltd. for providing some of the samples of ephedra alkaloids tested. Some of the analyzed samles of ephedra supplied by Amatsu Experimental Station of Forestry, Agricaltural Faculty, University of Tokyo, and the Botanical Research Laboratory of the Nippon Shinyaku Co. Ltd., are also gratefully acknowledged.

Summary

Alkaloids were extracted from a sample of ephedra with ammoniacal ether, transferred into acid solution, and diluted to a certain volume. Aliquots of this test solution were reacted under heating with ninhydrin in the presence of ascorbic acid and pyridine. The intensity of the color developed was measured at the maximum absorbancy peak at 570 mμ. Alkaloid content was calculated from the regression line, subtracting the blank from the observed value. It was found that this method was hardly interfered by amino acids and ammonium salt present in ephedra, through a control test with the quantity of these acids and salt about several times as much as those contained in ephedra.

For further check of the comparative accuracy and precision of this method of analysis, several sets of analytical data were evaluated. The results of estimation made according to this proposed procedure agreed well with those obtained by the method of Japanese Pharmacopoeia VI and their reproducibility was satisfactory.

(Received December 10, 1956)

UDC 547.759.3

54. Masayuki Onda and Mituo Sasamoto : Analogs of Rauwolfia Alkaloids. IV. Synthesis of 2-Substituted Tetrahydro-β-carbolines : Ring Closure of 2-Carboxy-3-indoleacetobenzylamide.

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

In the preceding paper of this series,[1] we reported on the synthesis of over 10 analogs of Rauwolfia alkaloids, several of which were found to possess reserpine-like activity. Therefore we attempted to synthesize 2-substituted tetrahydro-β-carbolines because of pharmacological interest.

The most common of the synthesis of tetrahydro-β-carboline will be the Pictet-Spengler reaction of tryptamine with aldehydes or α-ketocarboxylic acids and the Bischler-Napieralski reaction of acylated tryptamines, but these methods are not so easy as with isoqunolines and necessitate more steps via tryptamine synthesis.

This paper is concerned with the synthesis of 2-benzyl-1,2,3,4-tetrahydro-β-carboline from 2-carboxy-3-indoleacetic acid[2] (I) which was prepared easily in a good yield from α-ketoglutaric acid and phenylhydrazine according to Fischer's procedure.

Attempt was first made to synthesize tetrahydro-β-carboline by the route shown in Chart 1 〔(I)→(II)→(III)→(IV)〕 but it failed because of the sensitivity of 2-hydroxymethyltryptophol (II) to acids. Thionyl chloride and phosphorus bromide converted (II) into a black, high-melting substance, which did not dissolve in organic solvents, and the

* 731, Daita 1-Chome, Setagaya-ku, Tokyo (恩田政行, 笹本光雄).

1) Part III : J. Pharm. Soc. Japan, **76**, 966(1056).

2) R. Robinson, *et al.* : J. Chem. Soc., **1921**, 1602.

halide (III: X=Cl, Br.) was not formed. Benzenesulfonyl chloride also did not produce the sulfonate (III).

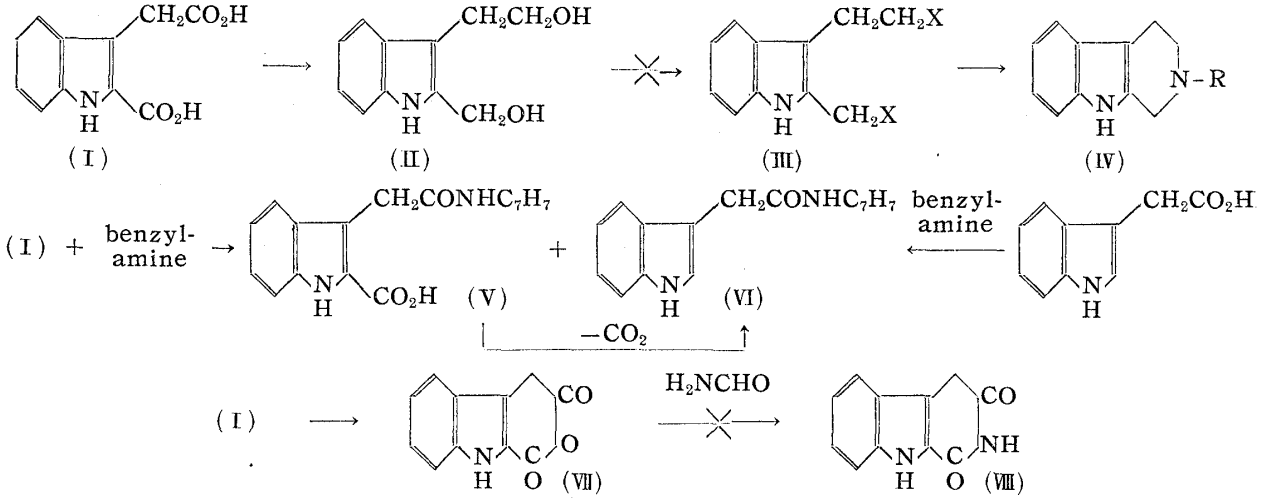

Chart 1.

In view of the fact that Taylor[3] pointed out that 2-hydroxymethylindole, especially its 3-substituted compound, was sensitive to acids this result seemed reasonable.

On heating with benzylamine at ca. 180°, (I) gave an acidic compound (V), m.p. 197°, $C_{18}H_{16}O_3N_2$, and a neutral compound (VI), m.p. 153~154°, $C_{17}H_{16}ON_2$. Although (VI) seemed to be the expected 1,3-dioxo-2-benzyltetrahydro-β-carboline, its composition indicated the absence of CO_2 from (V) instead of H_2O. Actually, on heating at 210°, (V) produced the neutral compound (VI) by decarboxylation. (VI) showed no depression when admixed with authentic 3-indoleacetobenzylamide, m.p. 153~154°, which was prepared from 3-indoleacetic acid and benzylamine. Therefore (V) must be 2-carboxy-3-indoleacetobenzylamide.

Generally, with formamide, anhydride produces an imide, but the anhydride[2](VII) of 2-carboxy-3-indoleacetic acid did not form its imide (VIII).

Robinson, *et al.*[2] reported that the anhydride (VII) gave 2-carbamoyl-3-indoleacetic acid (IX) on treating with ammonia and it was converted to 1-oxo-3-acetoxy-1,2-dihydro-β-carboline (X) with acetic anhydride and acetyl chloride and that similarly, aniline and (VII) gave (XI), then acetanhydride (XII).

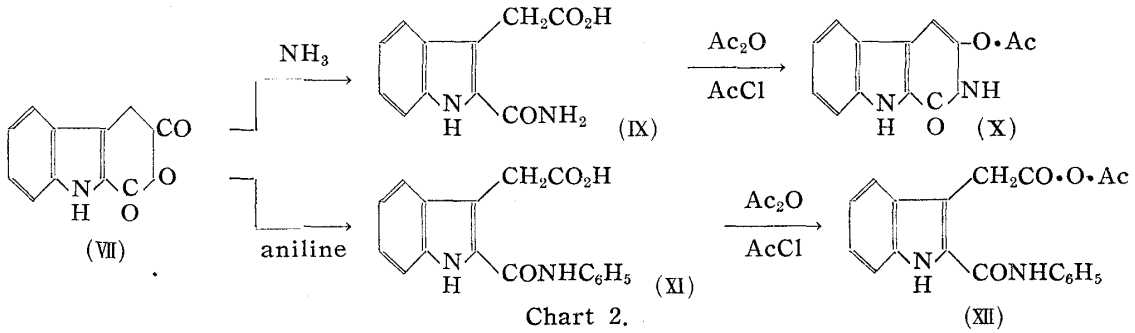

Chart 2.

When (VII) was reacted with benzylamine, an acidic compound was also produced. Since it was identified with (V), the positions of the acid and the amide in this compound were inverted to comform with Robinson's formulae (IX and XI). Therefore we repeated Robinson's procedure to confirm their structure.

The acidic compound (XI), which was formed by the method of Robinson and indicated

3) W. I. Taylor: Helv. Chim. Acta, **33**, 164(1950).

the same melting point of 216~217°, was converted to the neutral compound of m.p. 152~153°, by heating at 230°, and was indentified with authentic 3-indoleacetanilide.

Therefore, (XI) must be 2-carboxy-3-indoleacetanilide instead of the Robinson's formula. On treating with acetic anhydride and acetyl chloride, (XI) gave the neutral compound (XII) which possessed the same melting point, 182°, given in the literature. The fact that on treating with alcohol, (XII) returned to (XI) and its infrared spectra recorded bands at 5.6, 5.85, 5.95(C=0), 6.55(amide II), and 8.62 and 9.44 μ (anhydride -C•O•C-), indicated the presence of amide and acetanhydride in (XII), but their positions must be reversed to the Robinson's formula as mentioned above.

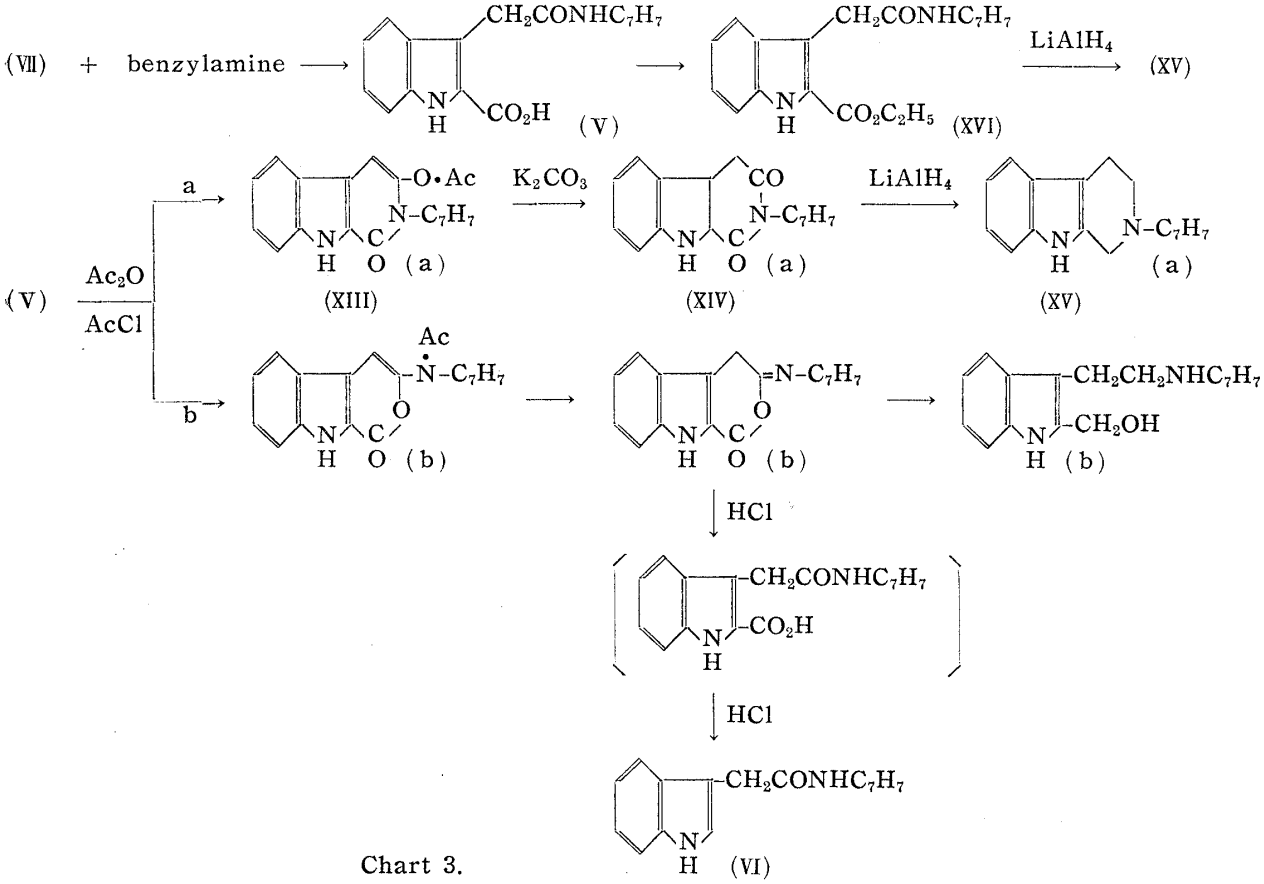

Chart 3.

On treating with acetic anhydride and acetyl chloride, (V) gave a neutral compound (XIII), $C_{20}H_{16}O_3N_2$, which was different from the anhydride, and saponification of (XIII) with potassium carbonate in hydrous methanol afforded the neutral compound (XIV), $C_{18}H_{14}O_2N_2 \cdot H_2O$. (XIV) did not return to (XIII) on treating with acetic anhydride and acetyl chloride or acetic anhydride and pyridine, but on boiling with 15% hydrochloric acid in acetic acid for several hours, 3-indoleacetobenzylamide was isolated. On being reduced with lithium aluminum hydride, (XIV) gave a basic compoud (XV), m.p. 138~140°, $C_{18}H_{20}ON_2$ (oxalate, m.p. 212~213°(decomp.)); U. V. λ_{max}^{EtOH} 224 mμ(log ε 4.51) and 282 mμ(3.88).

If the ring closure took place as shown in Chart 2 [(IX)→(X)], (XIII), (XIV), and (XV) will possess the structure of a-series in Chart 3. However, (XV) was not identified with authentic 2-benzyl-1,2,3,4-tetrahydro-β-carboline. Accordingly, the ring closure of imide-type did not occur in this case.

We propose the structure of b-series of lactone-type in Chart 3. The ultraviolet and infrared spectra of these compounds were as follows: (XIII): U. V. λ_{max}^{EtOH} 245 mμ(log ε 4.62), 308(4.07); I. R. λ_{max}^{Nujol} 5.85 μ, 5.95(C=O), 6.15(C=C), 7.95(lactonic C—O).

(XIV) : U. V. λ_{max}^{EtOH} 230 mμ(log ε 4.33), 298(4.24); I. R. λ_{max}^{Nujol} 5.89 μ(C=O), 6.08(C=N), 7.93 (lactonic C—O). These values agree well with the structures assumed. Fortunately, (XV) was identified with authentic 2-hydroxymethyl-N-benzyltryptamine, m.p. 138~140°, which was prepared from 2-ethoxycarbonyl-3-indoleacetobenzylamide (XVI) by reduction with lithium aluminum hydride. The ultraviolet spectra of its oxalate, m.p. 212~213° (decomp.), showed λ_{max}^{EtOH} 224 mμ(log ε 4.52) and 282(3.84) as (XV).

Although we did not confirm the structure of (X), it will also be the lactone-type. In view of the fact that in either case of nothing or benzyl radical on N of amide the ring closure occurred and of phenyl radical alternatively the dehydration with acetic acid resulted, this reaction seemed to be controlled by the electronic effect of the substituent, but it is a subject to be investigated henceforth.

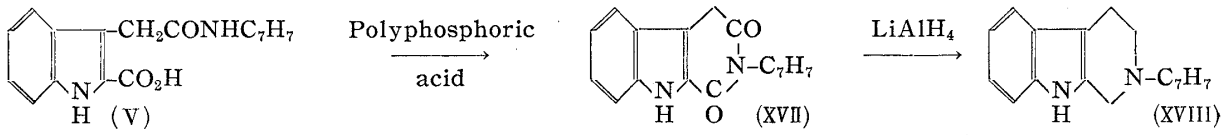

Chart 4.

On dehydration with polyphosphoric acid, (V) gave the neutral compound (XVII), m.p. 230~233°, $C_{18}H_{14}O_2N_2$; U. V. λ_{max}^{EtOH} 240 mμ(log ε 4.30) and 312(4.33); I. R. λ_{max}^{Nujol} 5.85 μ and 6.02μ(imide C=O). These spectral data support the imide-type structure. (XVII) gave a basic compound (XVIII), m.p. 140~142°, $C_{18}H_{18}N_2$, by lithium aluminium hydride reduction; oxalate, m.p. 226~227°(decomp.); U. V. λ_{max} 227 mμ(log ε 4.53) and 282(3.82). (XVIII) showed no depression on admixture with authentic 2-benzyl-1,2,3,4-tetrahydro-β-carboline, which was prepared as follows. Tryptamine (XIX) was formylated by heating with formic acid and the crude amide (XX) gave the base (XXI) by the Bischler-Napieralski reaction. On condensing with benzyl chloride, (XXI) gave the quaternary salt (XXII), which was converted into (XVIII), m.p. 140~142°, by catalytic hydrogenation. Oxalate of (XVIII), m.p. 226~227°(decomp.), U. V. λ_{max} 227 mμ(log ε 4.53) and 282(3.80).

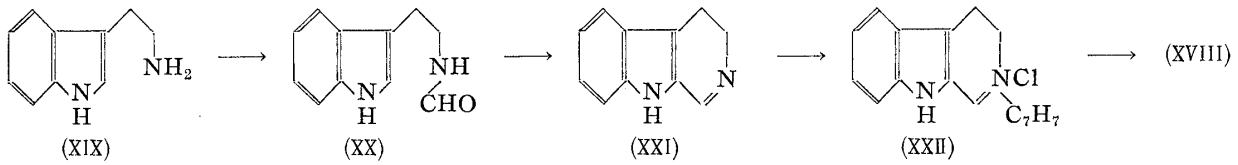

Chart 5.

Deep gratitude of the authors is expressed to Prof. S. Sugasawa of the University of Tokyo for his kind and unfailing guidance throughout the course of this work. Thanks are also due to Prof. T. Ukita and Dr. H. Watanabe of the University of Tokyo for advice on the infrared and ultraviolet spectra, to Mr. J. Hata, Director of this Laboratory, for encouragement, and to the members of the Analysis Room of this Laboratory for microanalytical data.

Experimental

Reaction of 2-Carboxy-3-indoleacetic Acid and Benzylamine (Formation of V and VI)—Acid (I, 1.0 g.) and benzylamine (0.5 g.) were mixed with EtOH (5 cc.) and then EtOH was removed. The residue was heated at 175~180° for 2 hrs. in aspirator vacuum. After cooling, the residue was dissolved in boiling MeOH (10 cc), filtered, concentrated, and separated 1.1 g. of colorless crystalline solid of m.p. 140~152°. On treating with dil. Na_2CO_3 solution, 0.63 g. of 2-carboxy-3-indoleacetobenzylamide (V) and 0.35 g. of 3-indoleacetobenzylamide (VI) separated. (V), colorless needles (from MeOH), m.p. 195~197° *Anal.* Calcd. for $C_{18}H_{16}O_3N_2$: C, 70.1; H, 5.2; N, 9.1. Found : C, 69.7; H, 5.4; N, 9.2. (VI), colorless plates (from MeOH), m.p. 153~154°. *Anal.* Calcd. for $C_{17}H_{16}ON_2$: C, 77.3; H, 6.1; N, 10.6. Found : C, 77.4; H, 6.1; N, 10.6.

Reaction of the Anhydride of 2-Carboxy-3-indoleacetic Acid and Benzylamine—The anhydride (VII, 7 g.) was mixed with dehyd. acetone (420 cc), refluxed, and then a clear solution resulted. Benzylamine (3.7 g.) was added to this solution and the whole was refluxed for 4 hrs. Acetone was removed *in vacuo*, the residue was dissolved in dil. Na_2CO_3 solution, acidified with HCl, and faint

yellow crystaline solid of m.p. 193~195° separated; yield, 10 g. Purified from MeOH, forming colorless needles of m.p. 195~197°, no depression on admixture with (V).

6-Acetobenzylaminoindolo[2,3-*c*]-2-pyrone (XIII)—A mixture of the acid amide (V, 4 g.), Ac_2O (24 cc.), and AcCl (4 cc.) was heated on water bath for 1 hr., mixed with water after cooling, the separated crystalline solid was filtered, washed with dil. Na_2CO_3 solution and water, and colorless crystalline solid of m.p. 170~172° was obtained; yield, 3.5 g. Recrystallization from EtOH gave colorless pillars, m.p. 176~177°. *Anal.* Calcd. for $C_{20}H_{16}O_3N_2$: C, 72.3; H, 4.9; N, 8.4. Found: C, 72.9; H, 5.3; N, 8.5.

6-Benzylimino-5, 6-dihydroindolo[2,3-*c*]-2-pyrone (XIV)—A mixture of the lactone amide (XIII, 1.0 g.), K_2CO_3 (0.2 g.), H_2O (1 cc.), and MeOH (5 cc.) was refluxed for 15 mins. After cooling, separated crystalline solid was collected, washed successively with MeOH, water, and again MeOH, and gave colorless crystalline solid of m.p. 186~188°; yield, 0.7 g. Purified from EtOH to colorless needles, m.p. 188~189°. *Anal.* Calcd. for $C_{18}H_{14}O_2N_2 \cdot H_2O$: C, 70.1; H, 5.2; N, 9.1. Found: C, 70.3; H, 5.3; N, 9.1.

The filtrate was concentrated, the residue was dissolved in water, filtered (decolorizing charcoal), acidified with HCl, and separated 0.09 g. of (V) of m.p. 194~196°.

2-Hydroxymethyl-N-benzyltryptamine (XV)—The foregoing compound (XIV, 0.5 g.) in tetrahydrofuran (25 cc.) was added slowly to a solution of $LiAlH_4$ (0.3 g.) in ether (15 cc.) with stirring and the whole was then refluxed for 3 hrs. The required amount of water was now added and boiled for 30 mins. The filtrate from inorganic salts was dried and evaporated. The reddish syrupy residue was dissolved in EtOH (1 cc.), anhyd. oxalic acid (0.15 g.) was added, and gave crystalline solid of m.p. 206~208°(decomp.); yield, 0.27 g. Purified from EtOH, forming almost colorless granules of m.p. 212~213°(decomp.). *Anal.* Calcd. for $C_{20}H_{22}O_5N_2$: C, 64.9; H, 5.9; N, 7.6. Found: C, 65.7; H, 5.9; N, 7.6.

Free base: Colorless pillars (from *iso*-PrOH), m.p. 138~140°, no depression on admixture with authentic sample.

2-Ethoxycarbonyl-3-indoleacetobenzylamide (XVI)—Mixture of acid amide (V, 2.0 g.), EtOH(20 g.), benzene (10 g.), and conc. H_2SO_4 (0.5 cc.) was refluxed for 2 hrs. The solvent was removed *in vacuo* and the residue was dissolved in AcOEt, washed with dil. Na_2CO_3 solution and water, dried, and evaporated. The residue, when mixed with ether, gave faint yellow crystalline solid of m.p. 178~180°; yield, 1.3 g. Purified from EtOH, forming colorless needles, m.p. 180~181°. *Anal.* Calcd. for $C_{20}H_{20}O_3N_2$: C, 71.4; H, 6.0; N, 8.3. Found: C, 71.0; H, 5.9; N, 8.2.

2-Hydroxymethyl-N-benzyltryptamine (XV)—Amide ester (XVI, 0.4 g.) in tetrahydrofuran (30 cc.) was added slowly to a solution of $LiAlH_4$ (0.3 g.) in ether (15 cc.) with stirring and the whole was then refluxed for 3 hrs. On working up the reaction product as above, oxalate of (XV) was obtained as faint yellow granules of m.p. 209~211°(decomp.); yield, 0.16 g. Purified from EtOH, forming almost colorless granules of m.p. 212~213°(decomp.). *Anal.* Calcd. for $C_{20}H_{22}O_5N_2$: C, 64.9; H, 5.9. Found: C, 65.6; H, 6.2.

Free base: Colorless pillars (from *iso*-PrOH), m.p. 138~140°.

1,3-Dioxo-2-benzyl-1,2,3,4-tetrahydro-β-carboline (XVII)—Acid amide (V, 3 g.) was added to polyphosphoric acid (from 31 g. of P_2O_5 and 20 cc. of conc. H_3PO_4) and the whole was heated on a water bath with stirring for 1.5 hrs. The reaction mixture was mixed with ice water, extracted with AcOEt, which was washed with dil. Na_2CO_3 solution and water, dried, and evaporated. The residue was triturated with a little EtOH, separating a crystalline solid, which was filtered, washed with EtOH, and furnished faint yellow needles, m.p. 227~230°; yield, 0.5 g. Purified from AcOEt to faint yellow needles, m.p. 230~233°. *Anal.* Calcd. for $C_{18}H_{14}O_2N_2$: C, 74.45; H, 4.85; N, 9.65. Found: C, 74.3; H, 4.85; N, 9.4.

2-Benzyl-1,2,3,4-tetrahydro-β-carboline (XVIII)—The imide (XVII, 0.45 g.) in tetrahydrofuran (20 cc.) was added slowly to a solution of $LiAlH_4$ (0.3 g.) in ether (15 cc.) with stirring and the whole was then refluxed for 3 hrs. On working up the reaction product as above, the oxalate of (XVIII) was obtained as faint yellow granules of m.p. 223~225°(decomp.); yield, 0.37 g. Purified from EtOH to almost colorless granules, m.p. 226~227°(decomp.). *Anal.* Calcd. for $C_{20}H_{20}O_4N_2$: C, 68.2; H, 5.7; N, 7.95. Found: C, 68.5; H, 5.9; N, 8.2. Free base: Colorless pillars (from MeOH), m.p. 140~142°, no depression on admixing with authentic sample. *Anal.* Calcd. for $C_{18}H_{18}N_2$: C, 82.45; H, 6.85. Found: C, 82.8; H, 6.75.

3,4-Dihydro-β-carboline (XXI)—Tryptamine carbonate (XIX, 1.5 g.) and formic acid (90%, 0.5 g.) were heated at 180° for 30 mins. The reaction product was dissolved in AcOEt, washed with dil. HCl, Na_2CO_3, and water, dried, and evaporated, furnishing the crude (XX) as faint yellow syrup; yield, 1.1 g. $POCl_3$ (4 cc.) was added slowly to the foregoing amide (XX, 1.1 g.) with cooling and then the whole was refluxed for 1.5 hrs. $POCl_3$ was removed *in vacuo*, the residue was washed with ether, dissolved in 10% AcOH (20 cc.), filtered (decolorizing charcoal), and basified with NaOH. The base liberated was collected in AcOEt, washed with water, dried, and evaporated, furnishing the crude (XXI) as yellow powder of m.p. 93~99°; yield, 0.5 g.

2-Benzyl-3,4-dihydro-β-carbolinium Chloride (XXII)—A mixture of the base (XXI, 0.5 g.), benzyl chloride (0.55 g.), and EtOH (2 cc.) was refluxed for 2 hrs., from which EtOH was removed *in vacuo*, and the residue was triturated with *iso*-PrOH, separating a crystalline solid of m.p. 188~190° (melted once at ca. 95°, then solidified at ca. 130°); yield, 0.71 g. Purified from *iso*-PrOH to yellowish orange needles, m.p. 192~194° (melted once at 95~97°, then solidified at 130°). *Anal.* Calcd. for $C_{18}H_{17}N_2Cl \cdot 1\frac{1}{2} H_2O$: C, 67.1; H, 6.25. Found : C, 67.5; H, 6.4.

2-Benzyl-1,2,3,4-tetrahydro-β-carboline(XVIII)—A solution of the chloride (XXII, 0.4 g.) in MeOH (15 cc.) was reduced at atmospheric pressure in the presence of the Adams' PtO_2 catalyst, 1 mole of H_2 being absorbed in a few mins. The filtrate from the catalyst was evaporated and the residue was triturated with EtOH, the hydrochloride of (XVIII) separating faint yellow crystalline solid of m.p. 244~246°; yield, 0.35 g. Purified from MeOH to colorless needles, m.p. 247~249°. *Anal.* Calcd. for $C_{18}H_{19}N_2Cl$: C, 72.3; H, 6.1; N, 9.35. Found : C, 72.0; H, 6.4; N, 8.9.

Free base : Colorless pillars (from MeOH) m.p. 140~142°.

Oxalate : Almost colorless granules (from EtOH), m.p. 226~227°(decomp.).

Summary

The ring closure of 2-carboxy-3-indoleacetobenzylamide (V) was investigated. On dehydration with acetic anhydride and acetyl chloride the ring closure of the lactone-type occurred, furnishing 6-acetobenzylaminoindolo[2,3-*c*]-2-pyrone (XIII) and 1,3-dioxo-2-benzyl-1,2,3,4-tetrahydro-β-carboline (XVII) with polyphosphoric acid in which the ring closure of the imide-type occurred instead of the lactone-type.

(Received March 15, 1957)

UDC 547.831.6

55. Eiji Ochiai, Akihiro Ohta, und Hiroaki Nomura : Polarisation der heterozyklischen Ringe mit aromatischem Charakter. CXVIII.[1] Über das 4-Hydroxyaminochinolin-N-oxyd.

(*Pharmazeut. Institut, Mediz. Fakultät, Universität Tokyo**)

Ochiai und Naito[2] erhielten bei der katalytischen Reduktion des 4-Nitrochinolin-N-oxydes mit Palladiumkohle in Äthanol-Lösung ein gelbes Pulver mit unklarem Zersetzungspunkt bei ca. 210°. Es war anscheinend nicht kristallinisch, in meisten organischen Lösungsmitteln schwer löslich, sodass es als nadelförmiges Chlorhydrat vom Zers. Pkt. 202° gereinigt und charakterisiert wurde. Seine Analysenzahlen stimmten mit einer Formel $C_9H_8N_2O \cdot HCl$ oder $(C_9H_7N_2O \cdot HCl \cdot H_2O)_2$, d.h. mit 4-Hydroxyaminochinolin-N-oxyd oder 4,4'-Hydrazochinolin-N,N'-dioxyd überein. Von den beiden Möglichkeiten für die Konstitution dieses Produktes hielten die beiden Autoren die Hydrazoformel als wahrscheinlicher, obwohl sein Molekulargewicht wegen der geringen Löslichkeit nicht bestimmen liess.

Da diese Annahme bis heute unbewiesen geblieben war, haben wir nun untersucht, um die sichere Entscheidung zwischen den beiden Möglichkeiten zu bringen. So haben wir dieses Produkt nach der früheren Vorschrift aus 4-Nitrochinolin-N-oxyd hergestellt. Der Zersetzungspunkt seines Chlorhydrates lag jedoch meistens bei 189°, und zwar konnte man ihn trotz mehrmaligem Umkristallisieren aus Methanol nicht höher als 192° bringen. Da das frühere Präparat noch vorhanden war, wurde sein Zersetzungspunkt aufs Neue gemessen und bestätigt, dass es sich wirklich auch bei 192° zersetzte.

---

* Hongo, Tokyo (落合英二, 太田明広, 野村容朗).

1) CXVII. Mitteilung : Dieses Bulletin,

2) E. Ochiai, T. Naito : J. Pharm. Soc. Japan, **64**, 204(1944).