UDC 615.771.7[547.852.9]-012

166. Takanobu Itai and Sachiko Suzuki: Potential Anti-cancer Agents. I. Synthesis of Imidazo[4,5-*d*]- and Triazolo[4,5-*d*]-pyridazine Derivatives. (1).

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A number of articles[1] concerning the purine derivatives which have anti-cancer activity stimulated interest in the preparation of imidazopyridazines and their aza derivatives. These types of compounds might be expected to possess outstanding biological activity, since their structures resemble those of purines.

There are four isomers of imidazopyridazine, viz. 1*H*-imidazo[4,5-*d*]pyridazine (A), 1*H*-imidazo[4,5-*c*]pyridazine (B), imidazo[1,2-*b*]pyridazine (C), and imidazo[3,4-*b*]pyridazine (D). The type (A) was first taken up. Two general ways are readily conceivable to reach the heterocyclic ring system. One method for that purpose, which has been reported recently,[2~4] is to start from the imidazole part and cyclize imidazole-4,5-dicarboxylic esters with hydrazine through the diacid hydrazides. Another possible way that consists of the route from diaminopyridazine has been attempted by two different workers but ended fruitless.[4,5]

(A) (B) (C) (D)

In this paper will be described the syntheses of 4,7-dimethoxy-1*H*-imidazo[4,5-*d*]-pyridazine, 4,7-dimethoxy-1*H*-*v*-triazolo[4,5-*d*]pyridazine, and some of their related compounds by the second of these two procedures, as shown in Chart 1. The present method seems to provide a new convenient route for achieving the imidazo[4,5-*d*]pyridazines or their aza derivatives substituted in the pyridazine moiety, whose preparation has been obviously difficult by the first reaction procedure.

3,6-Dimethoxy-4-aminopyridazine (II) was already prepared[6] by catalytic hydrogenation of 3,6-dimethoxy-4-nitropyridazine 1-oxide (I) over palladium-charcoal in acetic anhydride, followed by hydrolysis under a mild condition. In the present series, Raney nickel[7] was used in this reaction and (II) was obtained directly from (I) in a quantitative yield. (II) was smoothly nitrated with potassium nitrate in conc. sulfuric acid at room temperature and gave a mononitro compound (III), m.p. 232~233°. In contrast to the fact that 3-amino-6-methylpyridazine afforded the 3-nitramino derivative under the same condition,[8] 3,6-dimethoxy-4-amino-5-nitropyridazine (III) was deduced to be the nitration product in this case from the following reasons: i) Nearly quantitative recovery was observed when the mononitro compound was warmed in conc. sulfuric acid; ii) the compound showed sharp maxima at 3470 and 3320 cm^{-1}, attributable to primary amino group and at 1509 and 1302 cm^{-1} for C-nitro group in its infrared absorption spectrum; iii) the

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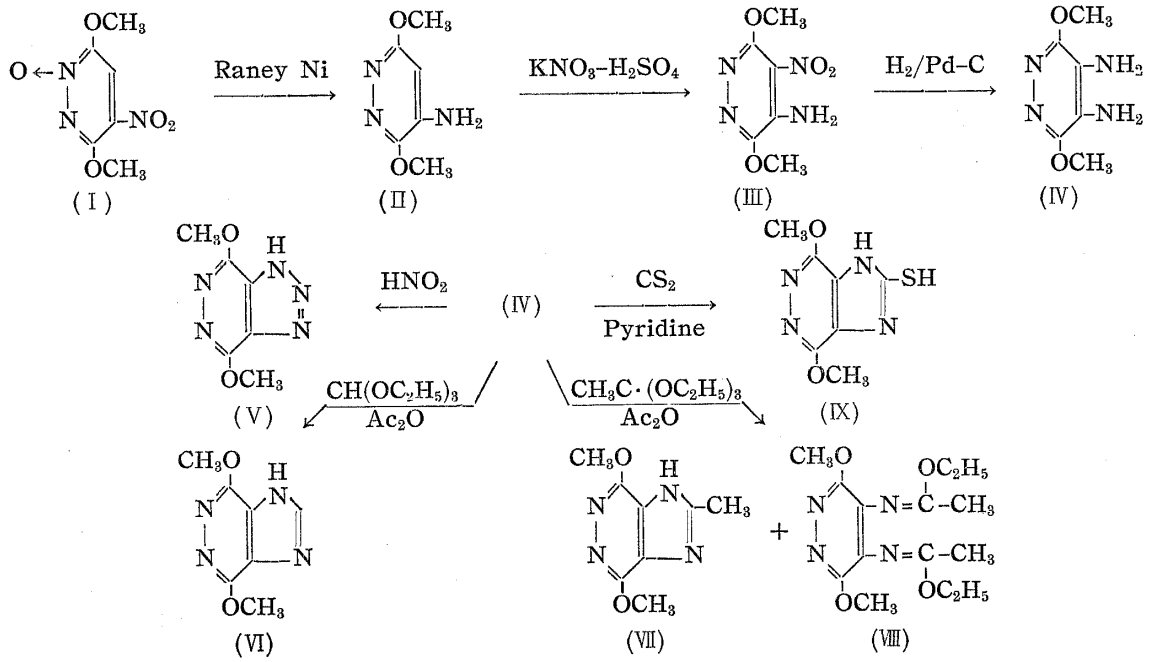

Chart 1.

reduction product, 3,6-dimethoxy-4,5-diaminopyridazine (IV), gave readily its diacetate and further showed no infrared absorption band in the longer wave-length, attributable to the CH out-of-plane vibration of aromatic systems.

The 4,5-diamino derivative (IV), which was obtained through catalytic hydrogenation of (III), was quite readily converted either to 4,7-dimethoxy-1*H*-*v*-triazolo[4,5-*d*]pyridazine (V) by diazotization by usual treatment or to 4,7-dimethoxy-1*H*-imidazo[4,5-*d*]pyridazine (VI) by heating in a mixture of ethyl orthoformate and an equal amount of acetic anhydride. Both reactions gave a fairly good yield. When (IV) was treated with ethyl orthoacetate, the expected 2-methyl-4,7-dimethoxy-1*H*-imidazo[4,5-*d*]pyridazine (VII) was detected only in a small amount and the major part of the reaction product came as colorless plates, melting at 101°. Its analytical data and the absence of NH band in its infrared spectrum suggested this compound to be a condensation product of (IV) and two molar equivalents of the reagent, and was formulated as (VIII). (VIII) remained unchanged after being treated with 10% ethanolic potassium hydroxide solution, whereas it was sensitive to acidic reagents and was hydrolysed to (IV) with dry hydrogen chloride in ether at room temperature or with picric acid in hot methanol. In the latter case the picrate of (IV) was produced in a very good yield.

Treatment of (IV) with thiourea at 180° by the usual method[9] failed to give the desired imidazopyridazine-2-thiol and in its stead there was obtained a small amount of a brown solid, melting above 360°, whose identification remains to be made. In connection with this object, it was found that (IV) underwent cyclization by treatment with carbon disulfide in hot pyridine to form 3,6-dimethoxy-1*H*-imidazo[4,5-*d*]pyridazine-2-thiol (IX) in 30% yield.

Treatment of (IV) with the acidic reagents, which cyclize diaminopyrimidines or *o*-phenylenediamines to the derivatives of 8-alkylpurine of 2-alkylbenzimidazole, respectively, was attempted under similar conditions described in past literature,[10] in order to obtain 2-alkylimidazopyridazine compounds. The reactions proceeded with loss of a methyl

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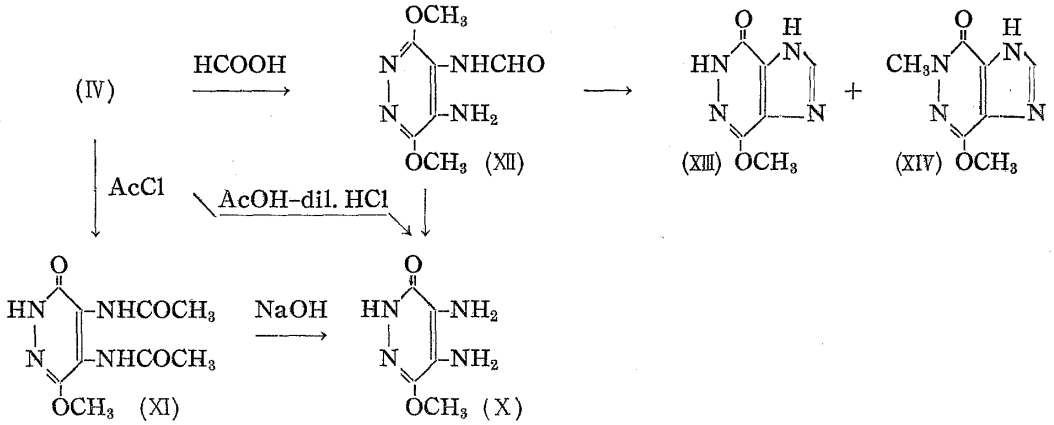
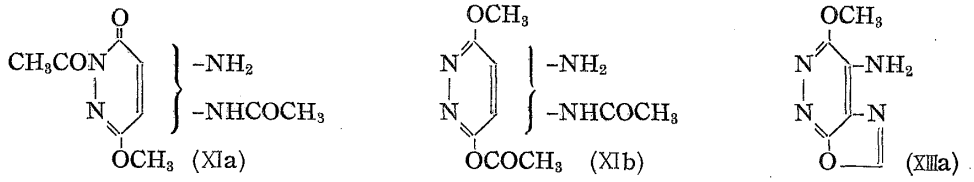


group from two methoxyl substituents. Thus, 4,5-diamino-6-methoxy-3(2*H*)-pyridazinone (X), m.p. 227°(decomp.), was the only product instead of the desired (VII), when (IV) was refluxed with acetic acid in dilute hydrochloric acid. Acetyl chloride was applied to (IV) in boiling xylene and the product of colorless needles, m.p. 273~275°, obtained here was subjected to alkaline hydrolysis affording (X) and gave analytical values corresponding to the diacetate of (X). These crystals were soluble in dilute caustic alkali and showed no infrared absorption bands corresponding to phenol acetate. These characters ruled out the structures (XIa, b) and the product was formulated as (XI). Formylation reaction of (IV) was accomplished by treating with hot formic acid for a short time and yielded the monoformate (XII), m.p. 202~203°, which sublimed at around 200° in vacuum. General procedure[11] for cyclization of monoacyl derivative of an *o*-diamine was applied to (XII) and (X) was formed by hydrochloric acid treatment instead of the expected cyclization product. When (IV) was treated with hot formic acid for a longer period, the reaction went further and three compounds were separated from the reaction mixture. The main product was alkali-soluble, fine needles, (XIII), $C_6H_6O_2N_4$, obtained in 30% yield. (XIII) was obviously 4-methoxy-1*H*-imidazo[4,5-*d*]pyridazin-7(6*H*)-one because of the absence of absorptions for primary aromatic amine in its infrared spectrum and its solubility in caustic alkali eliminated the isomeric structure of (XIIIa). The other product coming in colorless prisms, m.p. 262~264°, which was isolated in 3% yield, was assigned (XIV), an isomer of (VI). This



characterization was supported by both its analytical data and the absorption for pyridone-type carbonyl at 1665 cm^{-1} in its infrared spectrum. Usual migration[5,12] of a methyl group from 3,6-dimethoxypyridazine to 6-methoxy-3(2*H*)-pyridazinone seemed to take place here, catalyzed by the acid medium. Compound (XII), which was the single product in shorter reaction time, was detected in 3% yield in this case.

Biological activities of 1*H*-imidazo[4,5-*d*]pyridazine derivatives and also of their aza compounds which were prepared at the present time will be reported in near future.

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Experimental

3,6-Dimethoxy-4-aminopyridazine (II)—Raney Ni, freshly prepared from 3 g. of Ni-Al alloy, and 0.5 cc. of glacial AcOH were added to a suspension of 2 g. of (I) in 100 cc. of MeOH and the mixture was shaken in H_2 stream at an atmospheric pressure. The reduction stopped after 1050 cc. of H_2 uptake (4 mol. equiv.) at 29°. The catalyst was filtered off, the filtrate was evaporated to dryness under a reduced pressure, and the crystalline residue was recrystallized from H_2O, affording 1.4 g. (91%) of (II), m.p. 175~176°, undepressed on admixture with an authentic sample.

Nitration of 3,6-dimethoxy-4-aminopyridazine—To a solution of 6.2 g. of (II) dissolved in 4.2 cc. of conc. H_2SO_4 under cooling, finely powdered KNO_3 (5 g., 1.2 moles) was added slowly below 15° with stirring. The solution was kept at room temperature for 4 hr., poured into 800 cc. of ice-water, neutralized with $NaHCO_3$, and extracted repeatedly with $CHCl_3$. After drying over Na_2SO_4, the combined extract was evaporated. Recrystallization of the crystalline residue (7.5 g.) from Me_2CO gave 7.1 g. (89%) of light yellow plates (III), m.p. 232~233°. *Anal.* Calcd. for $C_6H_8O_4N_4$: C, 36.00; H, 4.03; N, 27.99. Found: C, 36.07; H, 3.87; N, 28.02. IR ν_{max}^{KBr} cm^{-1}: 3470, 3320, 1627, 1509, 1302. UV λ_{max}^{EtOH} mμ (log ε): 298 (3.54), 349~351 (3.59).

Treatment of (III) with conc. H_2SO_4: A solution of 0.14 g. of (III) dissolved in 1.5 cc. of conc. H_2SO_4 was warmed at 50° for 30 min. When cooled, the reddish-colored mixture was poured into 20 cc. of ice-water, neutralized with NH_4OH, and extracted with $CHCl_3$. After drying over Na_2SO_4, the extract was evaporated, and 0.13 g. of the starting material (m.p. 230°) was recovered.

Catalytic Hydrogenation of 3,6-Dimethoxy-4-amino-5-nitropyridazine—A suspension of 0.22 g. of (III) in 30 cc. of EtOH was submitted to reduction over Pd-C prepared from 1.9 cc. of 1% $PdCl_2$ solution and 0.1 g. of charcoal. The reduction stopped after 3 molar equivalents of H_2 uptake. The catalyst was filtered off, the filtrate was evaporated to dryness under a reduced pressure, and the residue was recrystallized from Me_2CO to 0.15 g. (80%) of colorless plates, m.p. 252~254° (decomp.). *Anal.* Calcd. for $C_6H_{10}O_2N_4$: C, 42.35; H, 5.92; N, 32.93. Found: C, 42.19; H, 5.85; N, 32.85. IR ν_{max}^{KBr} cm^{-1}: 3501; 3351, 3288, 3256, 1651, 1627~1621. UV: λ_{max}^{EtOH} 275 mμ (log ε 3.97).

Picrate: m.p. 202~203° (from MeOH). *Anal.* Calcd. for $C_6H_{10}O_2N_4 \cdot C_6H_3O_7N_3$: C, 36.10; H, 3.28; N, 24.56. Found: C, 36.65; H, 3.00; N, 24.42.

Diacetate: Prepared by heating with Ac_2O and recrystallized from MeOH to colorless needles, m.p. 254°. *Anal.* Calcd. for $C_{10}H_{14}O_4N_4$: C, 47.24; H, 5.55; N, 22.04. Found: C, 47.19; H, 5.19; N, 22.52. IR ν_{max}^{KBr} cm^{-1}: 3288, 1672.

4,7-Dimethoxy-1*H*-*v*-triazolo[4,5-*d*]pyridazine (V)—To a well-cooled solution of 0.51 g. of (IV) in 30 cc. of H_2O containing 3 cc. of glacial AcOH, a solution of 0.35 g. of $NaNO_2$ in 3 cc. of H_2O was added dropwise and pale yellow precipitate separated almost immediately. The reaction mixture was heated for 15 min. at 100°, cooled, and the product was collected by suction. Recrystallization of this material (0.53 g.) from MeOH containing 1% of H_2O gave 0.35 g. (65%) of pale yellow dice, m.p. 183~184° (became solid at ca. 200° and melted again at 212~213°). *Anal.* Calcd. for $C_6H_7O_2N_5$: C, 39.78; H, 3.89; N, 38.66. Found: C, 39.98; H, 3.85; N, 38.20. IR ν_{max}^{KBr} cm^{-1}: 3100, 3000~2550, 1930, 1676, 1597. UV: $\lambda_{max}^{0.1N\ KOH}$ 257~258 mμ (log ε 3.69).

4,7-Dimethoxy-1*H*-imidazo[4,5-*d*]pyridazine (VI)—A suspension of 0.2 g. of (IV) in a mixture of 2 cc. of ethyl orthoformate and 2 cc. of Ac_2O was heated slowly to reflux (the initial reaction is exothermic). In a few min., all of (IV) dissolved and nearly colorless product deposited. After 1 hr., the reaction mixture was cooled, the crude product (0.12 g.; m.p. 235~238°) was collected by filtration, treated with charcoal, and recrystallized from 90% EtOH to colorless fine needles, m.p. 238~239°; yield, 0.1 g.

From the mother liquor, the second crop of crystals (0.06 g.) was obtained after concentration *in vacuo* and addition of a small amount of H_2O. This was purified to give 0.04 g. of (VI), m.p. 238~239°. Total yield of (VI), 0.14 g. (66%). *Anal.* Calcd. for $C_7H_8O_2N_4$: C, 46.66; H, 4.48; N, 31.10. Found: C, 47.03; H, 4.15; N, 31.39. IR: ν_{max}^{KBr} cm^{-1}: 3160~2550, 1615, 1592, 844. UV: $\lambda_{max}^{0.1N\ KOH}$ 242~244 mμ (log ε 3.68).

Reaction of (IV) with Ethyl Orthoacetate in Ac_2O—A suspension of 0.2 g. of (IV) in a mixture of 0.8 cc. of ethyl orthoacetate and 1 cc. of Ac_2O was heated at 120~130°. An exothermic reaction took place and a clear solution was obtained. After being refluxed for 1 hr., the mixture was evaporated to dryness *in vacuo* and diluted with 2 cc. of H_2O. The colorless crystalline product that separated was collected and recrystallized from MeOH-H_2O to yield 175 mg. of plates (VII), m.p. 101°. *Anal.* Calcd. for $C_8H_6N_4(CH_3O)_2(C_2H_5O)_2$: C, 54.18; H, 7.15; N, 18.05, ($CH_3O + C_2H_5O$), 49.03. Found: C, 53.90; H, 6.90; N, 17.82, ($CH_3O + C_2H_5O$), 48.06. IR ν_{max}^{Nujol} cm^{-1}: 1674, 1664, 1282, 1257, 1085. 1055.

The mother liquor was extracted with $CHCl_3$, the extract was dried over Na_2SO_4, and evaporated. The residue was separated by Al_2O_3 chromatography into two fractions. The front fraction, m.p. 101°, eluted with $CHCl_3$, was identical with (VII); yield, 20 mg. Total yield of (VII), 195 mg. (54%).

The next fraction (50 mg.), eluted with $CHCl_3$, was recrystallized from MeOH-Me_2CO to colorless needles (Ⅶ), m.p. 212°; yield, 30 mg., soluble in 10% HCl and 10% NaOH. *Anal.* Calcd. for $C_8H_{10}O_2N_4$: C, 49.48; H, 5.19. Found: C, 49,47; H, 5.04. IR ν_{max}^{KBr} cm^{-1}: 3200～2850. UV: $\lambda_{max}^{0.1N\ KOH}$ 244～245 mμ (log ε 3.75).

Treatment of (Ⅷ) with dry HCl: Dry HCl was passed through an Et_2O solution of 50 mg. of (Ⅷ). The resulting hydrochloride was collected, washed with Et_2O, and treated with $NaHCO_3$. Thus, 25 mg. (91%) of crystalline product, m.p. 254°, was obtained, which was identified with (Ⅳ) by admixture.

Treatment of (Ⅷ) with picric acid: To a MeOH solution of 40 mg. of (Ⅷ), a solution of 30 mg. (1 mole) of picric acid in MeOH was added and the mixture was heated on a water bath. Yellow needles (50 mg.) precipitated, melting at 198～200°, undepressed on admixture with the picrate of (Ⅳ).

4,7-Dimethoxy-1*H*-imidazo[4,5-*d*]pyridazine-2-thiol (Ⅸ)—A mixture of 0.15 g. of (Ⅳ), 1.5 cc. of pyridine, 0.4 cc. of CS_2, and 0.05 g. of NaOH was refluxed for 5 hr., cooled, and acidified with 10% HCl. The precipitate that appeared was collected and washed with H_2O. This brownish solid (100 mg.) was dissolved in 10% NaOH, the solution was treated with charcoal, and acidified with 10% HCl. Colorless solid thus obtained was recrystallized from 80% EtOH to fine needles, m.p. 273～275° (decomp.); yield, 60 mg. (32%). *Anal.* Calcd. for $C_7H_8O_2N_4S$: C, 39.61; H, 3.80; N, 26.40. Found: C, 39.51; H, 3.75; N, 26.51. IR λ_{max}^{KBr} cm^{-1}: 3235, 3030～2610, 1657, 1593, 1553. UV $\lambda_{max}^{0.1N\ KOH}$ mμ (log ε): 226～227 (4.46), 260 (3.95).

Reaction of (Ⅳ) with AcOH—A mixture of 0.45 g. of (Ⅳ), 0.3 cc. of glacial AcOH, and 2 cc. of 10% HCl was refluxed for 40 min. After standing overnight, the mixture was concentrated *in vacuo* and neutralized with satd. $NaHCO_3$ solution. The crystalline product that deposited was collected and recrystallization from MeOH gave (Ⅹ) as colorless needles, m.p. 226～227° (decomp.); yield, 0.12 g. (29%), soluble in 10% HCl and 10% NaOH. *Anal.* Calcd. for $C_5H_8O_2N_4$: C, 38.46; H, 5.16; N, 35.88. Found: C, 38.35; H, 4.61; N, 35.30. IR ν_{max}^{KBr} cm^{-1}: 3466, 3335, 3300, 3170, 1669, 1640～1630. UV λ_{max}^{EtOH} 297 mμ (log ε 4.00).

Reaction of (Ⅳ) with AcCl—To a suspension of 0.2 g. of (Ⅳ) in 3 cc. of xylene, 0.25 cc. of AcCl was added and the mixture was refluxed for 6 hr. After standing overnight, separated colorless needles were collected by suction, which gave 135 mg. of (Ⅺ), m.p. 270～273° (decomp.), after two recrystallizations from MeOH, easily soluble in 10% NaOH. *Anal.* Calcd. for $C_9H_{12}O_4N_4$: C, 45.00; H, 5.04; N, 23.33. Found: C, 44.95; H, 4.74; N, 23.51. IR ν_{max}^{KBr} cm^{-1}: 3300, 3180, 3000, 1668～1619.

A solution of 60 mg. of (Ⅺ) in 3 cc. of 10% NaOH was heated on a boiling water bath for 1 hr. The mixture was concentrated *in vacuo*, neutralized with AcOH, and shaken with AcOEt. The combined AcOEt was dried over Na_2SO_4 and evaporated to yield 30 mg. of colorless solid, m.p. 193～210°. Recrystallization from MeOH raised its m.p. to 223～225°, undepressed on admixture with (Ⅹ).

Reaction of (Ⅳ) with HCOOH—a) 3,6-Dimethoxy-4-amino-5-formamidopyridazine (Ⅻ): A mixture of 0.45 g. of (Ⅳ) and 5 cc. of commercial HCOOH (98%) was refluxed for 15 min. The excess of HCOOH was evaporated *in vacuo*, the residue was neutralized with satd. $NaHCO_3$ solution, and the resulting colorless crystals (A), m.p. 195～200°, were collected by filtration.

The mother liquor was extracted with $CHCl_3$, the extract was dried over Na_2SO_4, and evaporated. The resulting crystals, m.p. 190～200°, were combined with (A) and recrystallized from MeOH to colorless needles (Ⅻ), m.p. 202～203°; yield, 0.37 g. (71%). *Anal.* Calcd. for $C_7H_{10}O_3N_4$: C, 42.42; H, 5.09; N, 28.27. Found: C, 42.65; H, 4.96; N, 28.09. IR ν_{max}^{KBr} cm^{-1}: 3415, 3310～3230, 1667, 1642.

Treatment of (Ⅻ) with dil. HCl: A solution of 0.3 g. of (Ⅻ) in 10% HCl was refluxed for 10 min. The mixture was evaporated *in vacuo* and neutralized with satd. $NaHCO_3$ solution. The resulting crystals, m.p. 220～225° (130 mg.), were collected by suction and recrystallization from MeOH gave colorless needles, m.p. 225～227°, undepressed on admixture with (Ⅹ).

b) 4-Methoxy-1*H*-imidazo[4,5-*d*]pyridazin-7(6*H*)-one (ⅩⅢ): A mixture of 1.2 g. of (Ⅳ) and 10 cc. of commercial HCOOH (98%) was heated under reflux for 4 hr. After standing overnight, the excess of HCOOH was removed *in vacuo*. The colorless precipitate obtained by addition of a small amount of H_2O was collected and recrystallized from EtOH to fine needles, m.p. >360° (brownish above 310°); yield, 0.35 g. (30.5%), soluble in 10% NaOH and regenerated by acidification with AcOH. *Anal.* Calcd. for $C_6H_6O_2N_4$: C, 43.37; H, 3.64; N, 33.73. Found: C, 43.58; H, 3.79; N, 33.49. IR ν_{max}^{KBr} cm^{-1}: 3115～2820, 1689. UV: λ_{max}^{EtOH} 248～253 mμ (log ε 3.62).

The mother liquor was neutralized with $NaHCO_3$ and extracted with $CHCl_3$. The extract was evaporated after drying over Na_2SO_4 and the residue was chromatographed on Al_2O_3 using $CHCl_3$. From the first fraction, 45 mg. (3.2%) of (Ⅻ) was obtained, m.p. 202～203°. The second fraction was recrystallized from EtOH to colorless prisms (ⅩⅣ) (30 mg., 2.3%), m.p. 262～264°. *Anal.* Calcd. for $C_7H_8O_2N_4$: C, 46.66; H, 4.48; N, 31.10. Found: C, 46.29; H, 4.00; N, 31.94. IR ν_{max}^{KBr} cm^{-1}: 3430, 3235, 3150, 1665. UV: λ_{max}^{EtOH} 265 mμ (log ε 3.60).

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Summary

4,7-Dimethoxy-1*H*-imidazo[4,5-*d*]- and 4,7-dimethoxy-1*H*-*v*-triazolo[4,5-*d*]-pyridazines and some of their derivatives were synthesized from 3,6-dimethoxy-4,5-diaminopyridazine (IV). Various reactions concerning the cyclization of (IV) were reported.

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167. Ken'ichi Takeda and Kaname Hamamoto : Studies on the Steroidal Components of Domestic Plants. XXII.*1 Structure of Metagenin. (2).[1]

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Previously, 5β,25D-spirostane-2β,3β,x-triol (I) was proposed as the structure of metagenin, a steroidal sapogenin isolated from *Metanarthecium luteo-viride* MAXIM.[1] and it was also reported that the position of the unknown hydroxyl group in metagenin was limited to C-6, C-7, or C-11 from infrared absorption spectrum of metagenin triacetate (Va) and by the properties of metagenic acid, an oxidation product of metagenin.

In order to replace the unknown hydroxyl group in metagenin by hydrogen, the Huang-Minlon reduction[2] of the x-keto derivative of metagenin was carried out. Metagenin acetonide (IIa), obtained in the usual manner as described earlier,[1] was converted to x-monoketo derivative (metagenone) (IIIb), m.p. 248°, $[\alpha]_D^{25}$ −4.0°, IR λ_{max}^{Nujol} 2.95 (OH) and 5.88 μ (C=O), via ketone acetonide (IIIa), m.p. 203~204°, by chromium trioxide oxidation followed by hydrolysis with dilute acetic acid. Metagenone (IIIb) formed a diacetate (IIIc), m.p. 240~242°, and this acetate again formed the unchanged parent ketone (IIIb) by the action of alkali.

Although metagenone (IIIb) and its diacetate (IIIc) underwent the Huang-Minlon reduction and afforded samogenin diacetate[3,4],*3 (IVb), m.p. 197~198°, in ca. 24% and 30% yield, respectively, when acetylated with acetic anhydride-pyridine, metagenone gave neither a semicarbazone nor hydrazone under the usual conditions. On the other hand, metagenone acetonide (IIIa) was not affected by the Huang-Minlon reduction and the unchanged starting material was recovered almost quantitatively.

From these results, it was confirmed that the two vicinal hydroxyl groups are located

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*2 Imafuku, Amagasaki, Hyogo-ken (武田健一, 浜元 要).
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