in addition to (1), (2) and (3). The hyperfine coupling constants were derived from the spectrum as:

$$A_N = 14.2G \qquad A_{H\gamma} = 3.4G \qquad A_{H\delta} = 0.4G$$

The reliability of our interpretation is still under investigations in comparison with the hyperifine coupling constants derived from the other radical intermediates containing azido group.

[Chem. Pharm. Bull. 20(5)1063—1067(1972)]

UDC 547.298.1.04 : 546.185.131—3.04

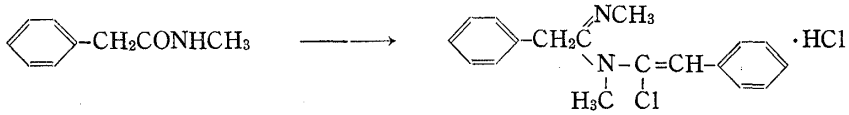
Reaction of Amide Homologs. XXVI.[1] On the Products obtained from the Reaction of N-Alkylphenylacetamides with Phosphoryl Chloride

Akira Hara, Jiro Suzuki and Minoru Sekiya

Shizuoka College of Pharmacy[2]

(Received November 4, 1971)

The reaction of N-ethylphenylacetamide with phosphorus pentachloride has been reported in 1932 by Heymons[3] to give N,N′-diethyl-N-(β-chlorostyryl)phenylacetamidine as the chloroplatinate. Analogous N,N′-dimethyl-N-(β-chlorostyryl)phenylacetamidine was obtained as a hydrochloride (I) in 36% yield by a reaction of N-methylphenylacetamide with phosphoryl chloride.



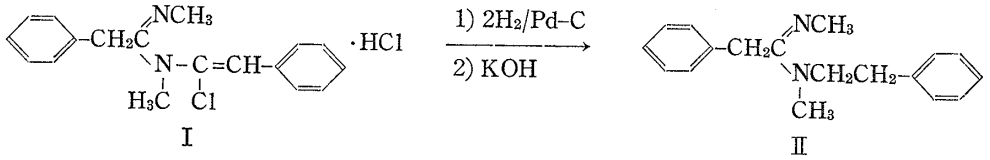
I

Lack of data of this compound prompts us to study the physical and chemical properties. The hydrochloride was isolated as prisms, mp 174°. Its nuclear magnetic resonance (NMR) spectrum was interpreted to fit the structure by the following assignments: the doublet at τ 6.75 to the CH_3N, the singlet at τ 6.18 to the $CH_3N=$, the singlet at τ 5.76 to the $-CH_2-$, and the singlet at τ 3.22 to the CH=. The structure was also reasonably interpreted from its infrared (IR) spectrum.

The free base could not be isolated because of its instability. However, without isolation of the free base addition of ammonia to a solution of the hydrochloride in chloroform followed by treatment with hydrogen bromide and hydrogen iodide gave the hydrobromide, mp 175° (decomp.), and the hydroiodide, mp 146—147° (decomp.), respectively, indicating the presence of one ionic chlorine atom different from the other in the hydrochloride. The hydrochloride in ethanol suffered catalytic hydrogenation over palladium-on-charcoal at ordinary pressure and temperature resulting in up-take of two molar equiv. of hydrogen. The product

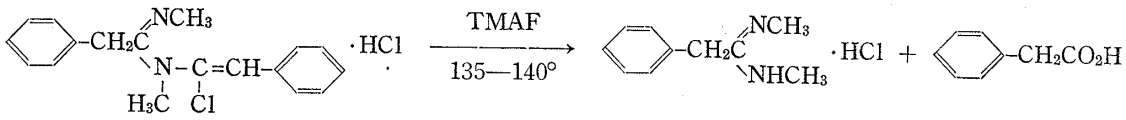
1) Part XXV: M. Sekiya and Y. Terao, *Chem. Pharm. Bull.* (Tokyo), **19**, 391 (1971).
2) Location: *2-2-1, Oshika, Shizuoka.*
3) Albrecht Heymons, *Chem. Ber.*, **65**, 320 (1932).

was obtained as free base oil, bp 159—161° (0.2 mmHg). Analysis and IR and NMR spectra of this compound are in agreement with N,N′-dimethyl-N-(phenethyl)phenylacetamidine (II). The following proton signals appeared in the NMR spectrum: the triplet at τ 7.36 to the $C_6H_5C\underline{H}_2CH_2$, the singlet at τ 7.13 to the CH_3N, the singlet at τ 6.95 to the $CH_3N=$, the triplet at τ 6.58 to the NCH_2-, the singlet at τ 6.37 to the $C_6H_5C\underline{H}_2C=$, and the multiplet at τ 3.10—2.46 to the aromatic protons.



I II

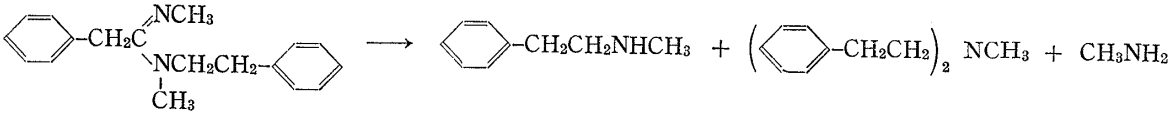
This structure was further confirmed from the results of its hydrolysis and catalytic reduction under high hydrogen pressure. The hydrolysis was carried out by heating with 30% sulfuric acid at 200° for 4 hr in an autoclave giving phenylacetic acid, N-methylphenethylamine and methylamine. The latter two were identified as the phenylthioureas. In addition, a trial of partial hydrolysis was successful on heating II with the formate reagent, (TMAF), which has been known as a constant boiling liquid given by $5HCO_2H \cdot 2NMe_3$.[4]



I

The identification of N,N′-dimethylphenylacetamidine hydrochloride was made by analysis and by noting well correspondence of its NMR and IR spectra.

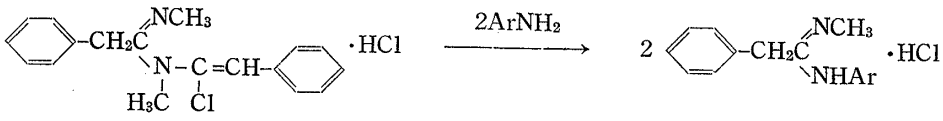
The catalytic reduction of I could be performed under the following conditions: catalyst, Raney Ni; initial hydrogen pressure, 110 kg/cm² (23°); temperature, 120—130°. Resulting in up-take of two molar equiv. of hydrogen, the hydrogenation gave products, N-methylphenethylamine, N-methyl-N,N-diphenethylamine, and methylamine. They were identified as the phenylthioureas or hydrochlorides.



II

These product formations appeared to be in agreement with the possible paths, hydrogenation of the C=N bond and hydrogenolysis at both the C–N bonds of such amidine structure.

Scattered papers have reported the replacements of the amine residue of amidines by primary amines.[5] The action of aromatic primary amine upon the compound I was noticeable to follow the equation described bellow, giving two molar equiv. of amidine possessing the aromatic amine as one of the amine residues.



I

4) Minoru Sekiya and Keiichi Ito, *Chem. Pharm. Bull.* (Tokyo), **12**, 677 (1964).
5) R.L. Shriner and Fred W. Neumann, *Chem. Reviews*, **35**, 351 (1944).

By refluxing the methanolic solution the reaction was carried out with several representative anilines so as to see effect of substituent of aniline. The following yields were obtained by varying the substituent: H, 72%; *p*-CH_3, 77%; *p*-CH_3O, 77%; *p*-Cl, 22%; *p*-NO_2, 0%. From this result, aniline with electron-releasing group proceeded with greater efficiency. All these amidine products have not been described previously, which were identified by analysis and by noting well correspondence of their IR spectra.

Chemical reaction described above seemed to be useful of preparations of phenylacetamidine derivatives. Several representative N-alkylphenylacetamides were then allowed to react with phosphoryl chloride in the same manner as described in the foregoing, but in most cases the product, N,N′-dialkyl-N-(β-chlorostyryl)phenylacetamidine hydrochloride was hardly crystallized. In the case of the N-ethyl analog the crude oily hydrochloride was directly hydrogenated catalytically using palladium-on-charcoal catalyst under ordinary pressure of hydrogen to give N,N′-diethyl-N-(phenethyl)phenylacetamidine as a liquid, bp 151° (0.015 mmHg) which was identified by analysis and by noting good correspondence of the NMR spectrum.

Experimental

N,N′-Dimethyl-N-(β-chlorostyryl)phenylacetamidine Hydrochloride (I)——To a boiling solution of 7.4 g (0.05 mole) of N-methylphenylacetamide in 45 ml of chloroform, 7.6 g (0.05 mole) of phosphoryl chloride was dropwise added over 7 min. and the resulting mixture was refluxed for one hour. The organic layer was washed with concd. HCl and dried over $MgSO_4$. Removal of the drying agent and chloroform afforded gluey material which was scratched after addition of a small amount of benzene to deposit white powders. The recrystallization from ethanol gave 3 g (36% yield) of prisms, mp 174°. IR ν_{max}^{KBr} cm^{-1}: 3000, 2900, 2850, 1610, 768, 742, 707. NMR (τ in $CDCl_3$): 6.75 (3H, d, J=4 Hz, CH_3N), 6.18 (3H, s, CH_3N–), 5.76 (2H, s, CH_2), 3.22 (1H, s, CH–), 3.00—2.38 (10H, m, aromatic), −1.75 (1H, m, –NH). *Anal.* Calcd. for $C_{18}H_{20}N_2Cl_2$: C, 64.43; H, 6.01; N, 8.35. Found: C, 64.52; H, 6.04; N, 8.32.

Formations of the Hydrobromide and Hydroiodide——The Hydrobromide: Into a solution of 1 g of the initial hydrochloride (I) in 6 ml of chloroform, dry ammonia was bubbled with an external ice-water cooling. The deposited ammonium chloride was removed by filtration and the filtrate concentrated under reduced pressure to dryness. The residual oil was dissolved in 5 ml of chlorofrom and, into the solution dry hydrogen bromide was bubbled to saturation. The resulting acidic solution was evaporated to give 1.1 g (97% yield) of glassy residue, which was recrystallized form ethanol to prisms, mp 175° (decomp.). *Anal.* Calcd. for $C_{18}H_{20}N_2$ ClBr: C, 56.93; H, 5.30; N, 7.37. Found: C, 56.67; H, 5.21; N, 7.73.

The Hydroiodide: Obtained in the same manner as described above by using hydrogen iodide in place of hydrogen bromide. Yield 79%, mp 146—147° (decomp.) (from ethanol). *Anal.* Calcd. for $C_{18}H_{20}N_2$ ClI: C, 50.66; H, 4.69; N, 6.56. Found: C, 50.41; H, 4.61; N, 6.49.

N,N′-Dimethyl-N-(phenethyl)phenylacetamidine(II)——N,N′-Dimethyl-N-(β-chlorostyryl)phenylacetamidine hydrochloride (I) (20.2 g, 0.06 mole) in 200 ml of ethanol was hydrogenated with 3 g of 5% palladium-on-charcoal as catalyst in atmospheric pressure of hydrogen and at room temperature. The compound absorbed nearly 0.12 mole of hydrogen in 67 hr. The reaction mixture was filtered and the filtrate was concentrated to dryness to give 19.2 g of pale yellow and viscous oil. The oil was dissolved in 30 ml of water and the solution was strongly basified by addition of solid KOH with an external water-cooling. The liberated free base was extracted with benzene, and the benzene solution was dried over KOH. After removal of the drying agent and benzene, the residual oil was distilled under reduced pressure to give 13.6 g (85% yield) of glutinous distillate, N,N′-dimethyl-N-(phenethyl) phenylacetamidine, bp 156—161° (0.2-mmHg). *Anal.* Calcd. for $C_{18}H_{22}N_2$: C, 81.11; H, 8.28; N, 10.67. Found: C, 81.16; H, 8.33; N, 10.52. IR $\nu_{max}^{liq.}$ cm^{-1}: 3000, 2820, 2900, 1620, 750, 730, 700. NMR (τ in $CDCl_3$): 7.36 (2H, t, J=7.5 Hz, $NCH_2\underline{CH_2}$-C_6H_5), 7.13 (3H, s, CH_3N), 6.95 (3H, s, CH_3N=), 6.58 (2H, t, J=7.5 Hz, NCH_2), 6.37 (2H, s, $C_6H_5\underline{CH_2}C$=), 3.1—2.46 (10H, m, aromatic).

Hydrolysis of N,N′-dimethyl-N-(phenethyl)phenylacetamidine (II)——A mixture of 1.9 g (7.3 mmole) of N,N′-dimethyl-N-(phenethyl)phenylacetamidine and 7 ml of 30% sulfuric acid was heated at 200° in an autoclave for 4 hr. The reaction mixture contained scaly crystals, which were taken up by suction filtration, 0.3 g, mp 77°, and showed no melting point depression on admixture with an authentic sample of phenylacetic acid. The filtrate was extracted with ether and the ethereal solution was wahed with a small amount of water followed by drying over $MgSO_4$. Removal of the drying agent and ether afforded 0.5 g of additional crystals of phenylacetic acid. Total yield of phenylacetic acid was 0.8 g.

The aqueous acidic solution was shown to contain N-methylphenethylamine and methylamine, which were separated by fractional neutralization. To the acidic solution, equivalent amount of 5% KOH was

added in divided portions. After each addition, distillation was followed and the distillates were sepatately collected in 5% HCl. N-Methylphenethylamine hydrochloride was obtained from the earlier distillates. Recrystallization from ethyl acetate gave scales, mp 160—161° (lit.[6] mp 161—162°), weighing 0.8 g. *Anal.* Calcd. for $C_9H_{14}NCl$: C, 62.99; H, 8.22; N, 8.16. Found: C, 62.30; H, 8.13; N, 8.43. Phenylthiourea, 0.8 g, mp 113° (from 90% ethanol) (lit.[7] mp 113—114°). *Anal.* Calcd. for $C_{16}H_{18}N_2S$: N, 10.36; S, 11.81. Found: M, 10.39; S, 11.80.

From the later distillates, the second product, methylamine hydrochloride, mp 226° (from ethanol), weighing 0.3 g, was obtained. Phenylthiourea, mp 112 (from ethanol) (lit.[8] mp 113°). *Anal.* Calcd. for $C_8H_{10}N_2S$: N, 16.85; S, 19.28. Found: N, 16.74; S, 19.23. Admixture with an authentic sample of N-methyl-N′-phenylthiourea showed no depression of the melting point.

Catalytic Hydrogenation of N,N′-Dimethyl-N-(phenethyl)phenylacetamidine (II)——N,N′-Dimethyl-N-(phenethyl)phenylacetamidine (II) (5 g, 19 mmole) in 50 ml of ethanol was hydrogenated with Raney nickel (1.5 g as 50% alloy) as catalyst at 120—134° under initial hydrogen pressure of 110 kg/cm² (23°). The compound absorbed almost equimolar hydrogen in 3.3 hr. The catalyst was filtered off and the filtrate was evaporated to distil the solvent along with the produced methylamine into 50 ml of 1 N HCl solution. The solution was concentrated to dryness affording 0.3 g of hygroscopic methylamine hydrochloride. The phenylthiourea, mp 110° (from ethanol), showed no depression of the melting point on admixture with an authentic sample of N-methyl-N-phenylthiourea. The foregoing residual oil free of methylamine and ethanol was distilled under reduced pressure to give two fractions: bp 95—100° (21 mmHg), 1.2 g, and bp 125° (2.5 mmHg), 2.0 g. The first fraction was shown to be N-methylphenethylamine, which was identified as the phenylthiourea, mp 113—114° (from 90% ethanol). *Anal.* Calcd. for $C_{16}H_{18}N_2S$: C, 71.06; H, 6.71; N, 10.36. Found: C, 70.94; H, 6.72; N, 10.30. No depression of the melting point was observed on admixture with the above obtained N-methyl-N′-phenyl-N-phenethylthiourea. The secondary fraction was converted to the hydrochloride, which was identified with N-methyl-N,N-di-phenethylamine hydrochloride, mp 160° (from benzene-chloroform) (lit.[9] mp. 163—165°). *Anal.* Calcd. for $C_{17}H_{22}NCl$: C, 74.04; H, 8.02; N, 5.08. Found: C, 73.69; H, 8.09; N, 5.49.

N,N′-Dimethylphenylacetamidine Hydrochloride——A mixture of N,N′-dimethyl-N-(β-chlorostyryl)-phenylacetamidine hydrochloride (I) (10 g, 0.03 mole) and 52.5 g of TMAF given by $5HCO_2H \cdot 2NMe_3$ was refluxed (135—140°) with vigorous stirring for 10 hr. Removal of excess of TMAF under reduced pressure gave a viscous liquid which crystallized on standing. After washing with acetone, the crystals weighed 3.8 g (64% yield). Recrystallization from a mixture of benzene and a small amount of ethanol gave prisms, which were shown to be identical with N,N′-dimethylphenylacetamidine hydrochloride, mp 205—206° (lit.[10] mp 210°). IR ν_{max}^{KBr} cm^{-1}: 3106, 2896, 2740, 1658, 733, 708. NMR (τ in $CDCl_3$): 7.14 (3H, d, J= 4.8 Hz, $C\underline{H}_3NH$), 6.92 (3H, d, J=4.8 Hz, $C\underline{H}_3NH$=), 6.02 (2H, s, CH_2), 2.71 (5H, s, aromatic), 0.6 (1H, m, NH), −0.4 (1H, m, HN=). *Anal.* Calcd. for $C_{10}H_{15}N_2Cl$: C, 60.45; H, 7.61; N, 14.10; Cl, 17.84. Found: C, 60.51; H, 7.64; N, 14.26, Cl, 17.51.

Concentration of the foregoing acetone rinsings and distillation of the residual liquid under reduced pressure gave a crude distillate, which was washed with 50% H_2SO_4 and dissolved in ether. The ethereal solution was dried over $MgSO_4$. Removal of the drying agent and ether led to deposition of plates, which were recrystallized from petroleum ether to give 2.6 g (64% yeild) of phenylacetic acid, mp 69—72°. No depression of the melting point was shown on admixture with an authentic sample of phenylacetic acid.

Reaction of N,N′-Dimethyl-N-(β-chlorostyryl)phenylacetamidine Hydrochloride (I) with Aromatic Primary Amines——A solution of 0.01 mole of N,N′-dimethyl-N-(β-chlorostyryl)phenylacetamidine hydrochloridine (I) and 0.04 mole of aromatic primary amine in 5 ml of methanol was refluxed for 15 min. The reaction mixture was concentrated to remove excess of the free amine. The deposited crystals were recrystallized from appropriate solvent to give the product, N-methyl-N′-(aryl)phenylacetamidine hydrochloride. The yields and identifications of the product obtained are shown in the following.

N-Methyl-N′-(phenyl)phenylacetamidine Hydrochloride——Obtained from the reaction with aniline, mp 261—262° (from methanol). Yield, 72%. *Anal.* Calcd. for $C_{15}H_{17}N_2Cl$: C, 69.08; H, 6.57; N, 10.76. Found: C, 69.08; H, 6.68; N, 10.76. IR ν_{max}^{KBr} cm^{-1}: 1650, 770, 740, 705.

N-Methyl-N′-(*p*-tolyl)phenylacetamidine Hydrochloride——Obtained from the reaction with *p*-toluidine, mp 168—169° (from ligroin). Yield, 77%. *Anal.* Calcd. for $C_{16}H_{19}N_2Cl$: C, 69.91; H, 6.97; N, 10.19; Cl, 12.90. Found: C, 69.86; H, 7.20; N, 10.32; Cl, 13.37. IR ν_{max}^{KBr} cm^{-1}: 1642, 811, 750, 715.

6) Tadashi Suyama and Seizo Kanao, *Yakugaku Zasshi*, **84**, 1014 (1964).
7) Bernhard Prager. Paul Jacobson, Paul Schmidt and Dora Stern, "Beilsteins Handbuch der Organischen Chemie," **XII**, 1100 (1929).
8) Shriner, R.L., Fuson, R.C. and Curtin, D.Y., "The Systematic Identification of Organic Compounds" John Wiley and Sons, Inc., New York, 1956, p. 288.
9) *C.A.*, **53**, 10523d (1959).
10) Pierre Reynaud, Robert Cesar Moreau and Jean Claude Tetard, *Compt. Rend., Ser, C.* **262** (8), 665 (1966); *C.A.*, **64**, 15791a (1966).

N-Methyl-N'-(*p*-anisyl)phenylacetamidine Hydrochloride——Obtained from the reaction with *p*-anisidine, mp 136—140° (from acetone–ethanol). Yield, 77.3%. *Anal.* Calcd. for $C_{16}H_{19}N_2OCl$: C, 66.08; H, 6.58; N. 9.63; Cl, 12.19. Found: C, 66.13; H, 6.64; N, 9.82; Cl, 12.51. IR ν_{max}^{KBr} c^{-1}: 1650, 1212, 1023, 825, 750, 720.

N-Methyl-N'-(*p*-chlorophenyl)phenylacetamidine Hydrochloride——Obtained from the reaction with *p*-chloroaniline, mp 243—245° (from acetone–ethanol). Yield, 22%. *Anal.* Calcd. for $C_{15}H_{16}N_2Cl$: C, 61.03; H, 5.46; N, 9.46; Cl, 24.08. Found: C, 61.53; H, 5.59; N, 9.55; Cl, 23.41. IR ν_{max}^{KBr} cm^{-1}: 1650, 810, 742, 704.

N,N'-Diethyl-N-(phenethyl)phenylacetamidine——To a boiling solution of 16.3 g (0.1 mole of N-ethylphenylacetamide in 98 ml of chloroform, 15.4 g (0.1 mole) of phosphoryl chloride was dropwise added in 5 min. The resulting mixture was washed several times with concd. HCl containing 5% $FeCl_3$. The separated chloroform layer was dried over $MgSO_4$. Removal of the drying agent and chloroform gave a viscous liquid, which hardly crystallized. This was dissolved in 100 ml of ethanol and hydrogenated with 3.2 g of 5% palladium-on-charcoal as catalyst under atmospheric hydrogen pressure and at room temperature. After up-take of nearly two molar equiv. of hydrogen, the catalyst was filterd off and the filtrate was concentrated to dryness. The oily residue was treated with cold saturated KOH solution to liberate the free amidine, which were extracted with benzene and the benzene solution was dried over K_2CO_3. Removal of the drying agent and benzene afforded a brown liquid, which was distilled under reduced pressure to give an oil, bp 151—153° (0.018 mmHg), 3.7 g (25% yield) of the product, N,N'-diethyl-N-(phenethyl)phenylacetamide. *Anal.* Calcd. for $C_{20}H_{26}N_2$: C, 81.58; H, 8.90; N, 9.52. Found: C, 81.34; H, 8.77; N, 9.54. NMR (τ in $CDCl_3$): 8.98 (3H, t, J=7.5 Hz, $CH_3CH_2N<$), 8.84 (3H, t, J=7.5 Hz, $CH_3CH_2N=$), 7.14 (2H, q, J=7.5 Hz, $CH_3CH_2N<$), 6.69 (2H, q, J=7.5 Hz, $CH_3CH_2N=$), 6.64 (2H, t, J=6.15 Hz, $C_6H_5CH_2CH_2N$), 6.61 (2H, t, J=6.15 Hz, $C_6H_5CH_2CH_2N$). 6.32 (2H, s, $C_6H_5CH_2$), 2.68 (10H, s, aromatic).

Acknowledgement We are grateful to the members of Analytical Center of this colloge for their elemental analyses and spectral measurements.

[Chem. Pharm. Bull. 20(5)1067—1069(1972)]

UDC 547.92.02 : 615.33.011.5 : 581.192

Fusidic Acid, a Steroidal Antibiotic from *Isaria kogane*[1]

Hiroshi Hikino, Yoko Asada, Shigenobu Arihara and Tsunematsu Takemoto

Pharmaceutical Institute, Tohoku University[2]

(Received November 15, 1971)

In an attempt to obtain physiologically active metabilites from Basidiomycetes, we have carried out submerged culture of a number of Basidiomycetes. The fermentation brothes were filtered and the filtrates extracted with ethyl acetate. The extracts from the water and ethyl acetate layers were submitted to screeing tests for the antibacterial activity by the paper disc method using *Staphylococcus aureus* 209P, *Sarcina lutea*, *Bacillus subtilis*, and *Escherichia coli*. As a result, it was found that the ethyl acetate extract of the fermented broth of a Basidiomycetes, *Isaria kogane* Hasegawa et Koyama (IFO 5299) (Hypocreaceae), a mushroom parasitic mainly on larvae and imagos of beetles,[3] showed the strongest antibacterial activity against the Gram-positive bacteria and, in particular, against a strain of

1) This paper is Part II in the series on Fungal Metabolites. Part I: H. Hikino, D. Kuwano, and T. Takemoto, *Yakugaku Zasshi*, **89**, 1149 (1969); This also forms Part XVI in the series on Steroids. Part XV: H. Hikino, Y. Ohizumi, and T. Takemoto, *Yakugaku Zasshi*, in press.
2) Location: Aoba-yama, Sendai.
3) K. Hasegawa and R. Koyama, Ringyo Shikenjo Hokoku, **4**, 1 (1941).