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# Synthesis of Deoxyneo-β-hydroxyaspergillic Acid

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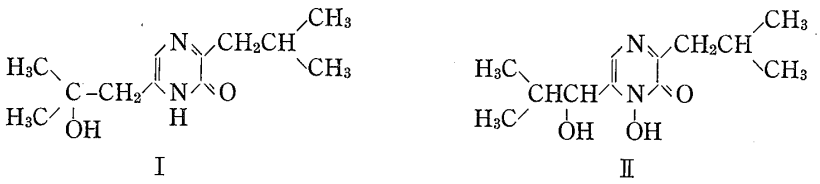
Deoxyneo-β-hydroxyaspergillic acid (I), originally isolated from *Aspergillus ochraceus* Wilh., was synthesized from 2-chloro-3,6-diisobutylpyrazine (III) through several steps. Methylmagnesium iodide was used for cleavage of the ether bond on the last step.

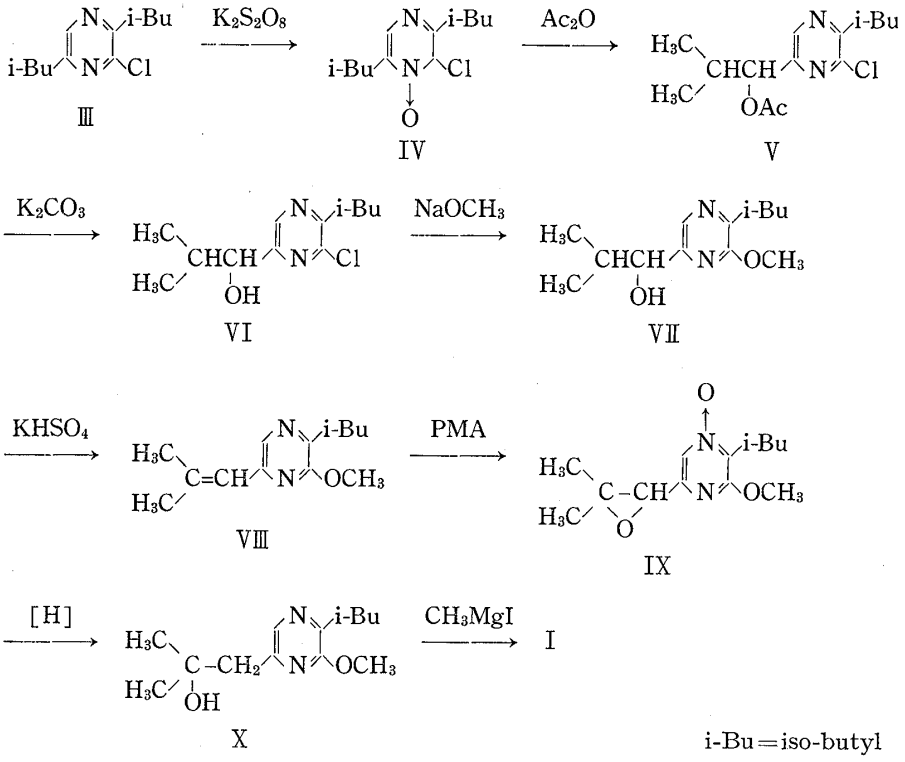
**Keywords**——deoxyneo-β-hydroxyaspergillic acid; *Aspergillus ochraceus*; 2-chloro-3,6-diisobutylpyrazine; potassium persulfate oxidation; methylmagnesium iodide; ether bond cleavage

Deoxyneo-β-hydroxyaspergillic acid (I) has been isolated as a fungal metabolite from the mycelia of *Aspergillus ochraceus* Wilh., and is characteristic in possessing a hydroxyl



I II

Chart 1



i-Bu=iso-butyl

Chart 2

1) Location: *1432-1 Horinouchi, Hachioji, Tokyo 192-03, Japan.*

group at the β-position of an isobutyl side chain.[2] We now report the synthesis of I by the route shown in Chart 2, starting from 6-(α-acetoxy)isobutyl-2-chloro-3-isobutylpyrazine (V), an intermediate in the synthesis of *dl*-neohydroxyaspergillic acid[3] (II).

In the present work, the preparation of V was carried out by a route different from the reported one, namely, *via* 2-chloro-3,6-diisobutylpyrazine 1-oxide (IV), which was obtained by oxidation of 2-chloro-3,6-diisobutylpyrazine[4] (III) with Caro's acid[5] in good yield. Transformation of IV to V was achieved by heating with acetic anhydride in a sealed tube in moderate yield. The preparation of V starting from III was achieved *via* seven steps in 0.65% overall yield in the reported work.[3] The procedure described in the present report is better than the previously reported one in every respect.

V was hydrolyzed by refluxing in a mixture of 10% potassium carbonate and methanol to afford 2-chloro-6-(α-hydroxy)isobutyl-3-isobutylpyrazine (VI); The proton magnetic resonance (PMR) spectrum of the product suggested the presence of a hydroxyl group at the α-position of an isobutyl group. Treatment of VI with sodium methoxide in methanol gave 6-(α-hydroxy)isobutyl-3-isobutyl-2-methoxypyrazine (VII) in nearly quantitative yield.

In order to introduce an epoxide group into the side chain, dehydration of VII and subsequent oxidation were investigated. VII was heated in the presence of *p*-toluenesulfonic acid or treated with thionyl chloride in pyridine solution, but these attempts were unsuccessful. It was observed in the GC-mass spectrum that the dehydration of VII occurred on treatment with a mixture of phosphoryl chloride and pyridine at room temperature or on heating with potassium hydrogensulfate at 200° in moderate yield, and the latter procedure was adopted. The structure of VIII was elucidated from the PMR spectrum, which exhibited two singlets at 1.95 and 2.22 ppm due to the methyl groups of a (β-methyl)propenyl group and a singlet at 6.16 ppm due to an ethylenic proton.

VIII was oxidized with permaleic acid to give a crystalline product, whose analytical and mass spectral data supported the proposed structure (IX). The PMR spectrum of the product showed three singlets at 1.22 (3H), 1.52 (3H), and 3.73 (1H) ppm, which were assignable to the methyl protons attached to the oxirane ring and the proton on the oxirane ring, respectively. The pyrazine ring proton of IX appeared as a singlet at 7.74 ppm. As will be described later, 6-(α,β-epoxy-β-methyl)propyl-3-isobutyl-2-methoxypyrazine (XIII) and 6-(β-hydroxy)isobutyl-3-isobutyl-2-methoxypyrazine 4-oxide (XIV) were obtained by the catalytic reduction of IX at room temperature in addition to 6-(β-hydroxy)isobutyl-3-isobutyl-2-methoxypyrazine (X). This result indicates that the N–O group is located as shown in the chart.

Catalytic reduction of IX in the presence of Raney nickel at 50° under high pressure gave an alcohol (X) as a pale yellowish oil, whose IR spectrum (film, 3400 $cm^{-1}$) suggested the presence of a hydroxyl group on an isobutyl group. In the PMR spectrum, a doublet at 2.66 ppm ($J$=8 Hz) due to the methylene protons and a doublet at 0.92 ppm ($J$=8 Hz) due

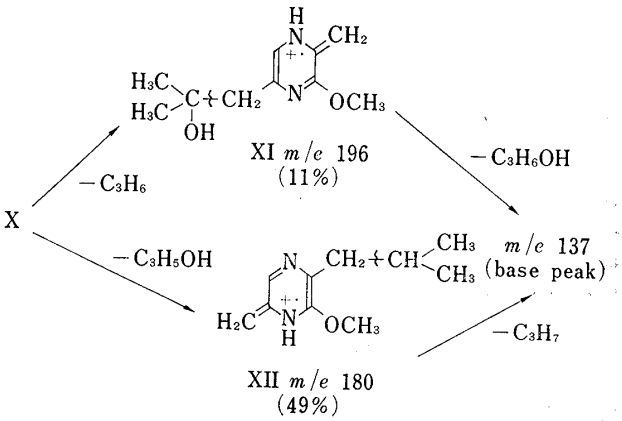



Chart 3

2) M. Yamazaki, Y. Maebayashi, and K. Miyaki, *Chem. Pharm. Bull.* (Tokyo), **20**, 2274 (1972).
3) A. Ohta, Y. Akita, A. Izumida, and I. Suzuki, *Chem. Pharm. Bull.* (Tokyo), **27**, 1316 (1979).
4) A. Ohta, *Chem. Pharm. Bull.* (Tokyo), **16**, 1160 (1968).
5) C.E. Mixan and R.G. Pews, *J. Org. Chem.*, **42**, 1869 (1977).

to the methyl protons of an isobutyl group were observed. On the other hand, the methyl and methylene proton signals of the isobutyl group carrying a hydroxyl group each appeared as a singlet at 1.21 and 2.80 ppm, respectively. In the mass spectrum of X, peaks were observed at *m/e* 238 ($M^+$), 223 ($M^+$-15), 196, 180, and 137 (base peak). The *m/e* 196 and 180 ions, resulting respectively from loss of $C_3H_6$ and $C_3H_5OH$ from the parent, are singnificant. These peaks may correspond to the ions XI and XII, resulting from McLafferty rearrangement[6] with hydrogen transfer to the nitrogen atom, and suggest the presence of a tertiary hydroxyl group. The *m/e* 137 peak (base peak) may be formed by $\beta$-cleavage of XI and XII.

By the catalytic reduction of IX at room temperature, X, XIII, and XIV were obtained in 23, 14, and 35% yields, respectively. A singlet at 3.80 ppm in the PMR spectrum of XIII suggested that the oxirane ring was not cleaved. The pyrazine ring proton (7.95 ppm) of XIII appeared at lower field than that (7.74 ppm) of IX. In the PMR spectrum of XIV, the pyrazine ring proton appeared at 7.65 ppm, namely, at higher field than that (7.86 ppm) of X. Moreover, the methylene protons of the isobutyl group were detected at 2.78 ppm as a doublet ($J$=8 Hz). Thus, the location of the N–O group of IX could be definitely determined as shown in the proposed structure.[7]

The ether bond of X was cleaved by heating with methylmagnesium iodide[8] to give colorless needles, whose mass, PMR, IR, and UV spectra and melting point were identical with those of an authentic specimen of I. Although demethylation was also carried out by heating in hydrochloric acid, the dehydration products, VIII and 2-hydroxy-3-isobutyl-6-($\beta$-methyl)propenylpyrazine (XV), were obtained in addition to I. Consequently, the above-mentioned method seemed to be the most suitable for this demethylation.

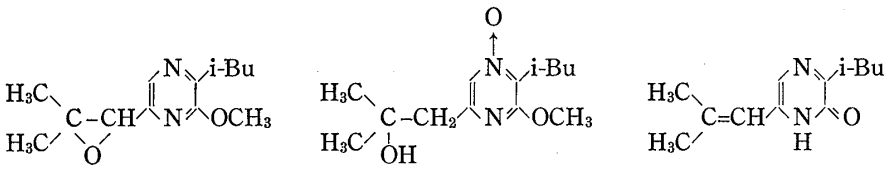



Chart 4

**Experimcntal**

All melting points were recorded on a Yanagimoto micro-melting point apparatus and are uncorrected. Boiling points are also uncorrected. Gas chromatograms were recorded on a Shimadzu GC-4B unit, UV spectra on a Hitachi 323 spectrometer, IR spectra on a Shimadzu IR-400 spectrometer, and PMR spectra on a JEOL JNM-PS-100 instrument with tetramethylsilane as an internal standard. Mass spectra were obtained on a Hitachi RMU-7L spectrometer.

**2-Chloro-3,6-diisobutylpyrazine 1-Oxide (IV)**——$K_2S_2O_8$ (1.62 g, 6 mmol) was added in small portions to a solution of 1.14 g (5 mmol) of III dissolved in 6 ml of conc. $H_2SO_4$ during 15 min under stirring and ice-cooling. The mixture was stirred at room temperature for a further 26 hr, then poured into 20 ml of ice-water, and extracted with $CHCl_3$. The $CHCl_3$ layer was washed successively with 5% $NaHCO_3$ and $H_2O$, and then dried over anhyd. $Na_2SO_4$. Removal of the solvent by evaporation gave a red-brown oil, which was chromatographed over silica gel (Wakogel C-200, 12 g) and eluted with $CHCl_3$ to afford IV as colorless crystals. Recrystallization from MeOH–$H_2O$ furnished 1.00 g (82%) of IV as colorless needles, mp 33—34°. *Anal.* Calcd. for $C_{12}H_{19}ClN_2O$: C, 59.37; H, 7.89; N, 11.54. Found: C, 59.16; H, 7.76; N, 11.37. MS *m/e*: 242 ($M^+$), 225 ($M^+$–OH). PMR ($CDCl_3$) $\delta$ ppm: 0.96 (12H, d, $J$=6 Hz), 2.22 (2H, m), 2.72 (2H, d, $J$=8 Hz), 2.80 (2H, d, $J$=8 Hz), 8.18 (1H, s). UV $\lambda_{max}^{95\%EtOH}$ nm (log $\varepsilon$): 234 (4.34), 271.5 (4.01), 302—305 (3.50), 314 (3.46, shoulder).

6) Q.N. Porter and J. Baldas, "Mass Spectrometry of Heterocyclic Compounds," Wiley-Interscience, a Division of John Wiley & Sons, Inc., New York, 1971, p. 382.
7) A. Ohta, Y. Akita, and C. Takagai, *Heterocycles*, **6**, 1881 (1977).
8) A. Ohta, S. Masano, E. Maeda, T. Ohwada, Y. Akita, and T. Watanabe, *Chem. Pharm. Bull.* (Tokyo), **25**, 817 (1977).

**6-(α-Acetoxy)isobutyl-2-chloro-3-isobutylpyrazine (V)**——A mixture of 990 mg (4.08 mmol) of IV and 4 ml of $Ac_2O$ was heated in a sealed tube at 190° for 2 hr, then the reaction mixture was poured into ice-water, made alkaline with $K_2CO_3$, and extracted with ether. The usual work-up of the ether extract gave a pale yellowish oil, which was subjected to column chromatography over Florisil (100 g), and eluted successively with hexane, benzene, and $CHCl_3$. The fractions eluted with a mixture of benzene and $CHCl_3$ (1: 1) were distilled to give a colorless oil (229 mg, 20%), bp 125—135° (bath temp.)/1 Torr (lit.[3] bp 149°/3 Torr).

**2-Chloro-6-(α-hydroxy)isobutyl-3-isobutylpyrazine (VI)**——A mixture of 4.50 g (18.7 mmol) of V, 10 ml of 10% $K_2CO_3$, and 20 ml of MeOH was refluxed for 20 min. MeOH was removed by distillation *in vacuo*, then the reaction mixture was extracted with ether. The ether layer was dried over anhyd. $Na_2SO_4$ and evaporated to dryness. The resulting oil was chromatographed on silica gel (Wakogel C-200, 100 g) eluting successively with hexane, benzene, and $CHCl_3$. The $CHCl_3$ fractions afforded a colorless oil, which was distilled at 140—142°/5 Torr to furnish 2.78 g (61%) of VI. *Anal.* Calcd. for $C_{12}H_{19}ClN_2O$: C, 59.37; H, 7.89; N, 11.54. Found: C, 59.15; H, 7.94; N, 11.65. MS *m/e*: 242 ($M^+$). PMR ($CDCl_3$) δ ppm: 0.88 (3H, d, $J$=4 Hz), 0.95 (3H, d, $J$=4 Hz), 0.96 (6H, d, $J$=6 Hz), 2.18 (2H, m), 2.83 (2H, d, $J$=8 Hz), 3.15 (1H, s, broad), 4.52 (1H, d, $J$=6 Hz), 8.42 (1H, s). UV $\lambda_{max}^{95\%EtOH}$ nm (log ε): 214.5 (4.01), 281 (3.94), 302 (3.53, shoulder). IR (film) $cm^{-1}$: 3400 (OH).

**6-(α-Hydroxy)isobutyl-3-isobutyl-2-methoxypyrazine (VII)**——A mixture of 3.60 g (15 mmol) of VI and NaOMe, prepared from 3.60 g (160 mg atom) of Na and 40 ml of MeOH, was heated in a sealed tube at 140° for 1.5 hr, then the reaction mixture was evaporated to dryness under reduced pressure. The residue was triturated with water and extracted with ether. The usual work-up of the ether layer gave a pale yellow oil, which was purified by distillation to give 3.56 g (99%) of VII as a pale yellowish oil, bp 133—135°/5 Torr. *Anal.* Calcd. for $C_{13}H_{22}N_2O_2$: C, 65.51; H, 9.31; N, 11.76. Found: C, 65.43; H, 9.41; N, 11.98. MS *m/e*: 238 ($M^+$), 223 ($M^+-CH_3$), 195 ($M^+-C_3H_7$, base peak). PMR ($CDCl_3$) δ ppm: 0.88 (12H, d, $J$=8 Hz), 2.08 (2H, m, $J$=8 Hz), 2.63 (2H, d, $J$=8 Hz), 3.40 (1H, s, broad), 3.92 (3H, s), 4.40 (1H, s, broad), 7.96 (1H, s). UV $\lambda_{max}^{95\%EtOH}$ nm (log ε): 216 (3.87), 280 (3.70, shoulder), 297 (3.78). IR (film) $cm^{-1}$: 3350 (OH).

**3-Isobutyl-2-methoxy-6-(β-methyl)propenylpyrazine (VIII)**——A mixture of 5.67 g (23.8 mmol) of VII and 7.48 g (55.0 mmol) of $KHSO_4$ was heated at 200° for 1 hr in a metal bath. After cooling, the reaction mixture was triturated with water and extracted repeatedly with ether. The usual work-up of the extract gave 2.07 g of a yellowish oil, which was chromatographed on silica gel (Wakogel C-200, 135 g) with hexane containing an increasing amount of benzene as an eluant. The fractions eluted with a mixture of hexane–benzene (1: 1) was distilled at 108°/3 Torr to furnish 2.01 g (38%) of VIII as a colorless oil. *Anal.* Calcd. for $C_{13}H_{20}N_2O$: C, 70.87; H, 9.15; N, 12.72. Found: C, 70.58; H, 9.35; N, 12.75. MS *m/e*: 220 ($M^+$), 205 ($M^+-CH_3$). PMR ($CDCl_3$) δ ppm: 0.93 (6H, d, $J$=6 Hz), 1.95 (3H, s), 2.15 (1H, m), 2.22 (3H, s), 2.65 (2H, d, $J$=7.5 Hz), 3.93 (3H, s), 6.16 (1H, s), 7.88 (1H, s). UV $\lambda_{max}^{95\%EtOH}$ nm (log ε): 248.5 (4.13), 323 (4.06).

**6-(α,β-Epoxy-β-methyl)propyl-3-isobutyl-2-methoxypyrazine 4-Oxide (IX)**——A solution of 385 mg (1.75 mmol) of VIII, 232 mg (6.12 mmol) of 90% $H_2O_2$, and 686 mg (7.00 mmol) of maleic anhydride in 20 ml of $CH_2Cl_2$ was refluxed for 1.5 hr and then washed successively with $H_2O$, 10% $KHCO_3$, and $H_2O$. The $CH_2Cl_2$ layer was worked up as usual to yield a pale yellowish viscous oil, which was purified by column chromatography on Florisil (9 g), eluting successively with hexane, benzene, and $CHCl_3$. The fractions eluted with $CHCl_3$ were recrystallized from hexane to give 323 mg (73%) of IX as colorless needles, mp 66—68°. *Anal.* Calcd. for $C_{13}H_{20}N_2O_3$: C, 61.88; H, 7.99; N, 11.10. Found: C, 61.92; H, 7.89; N, 11.22. MS *m/e*: 252 ($M^+$), 235 ($M^+-OH$), 193 (235-$C_3H_6$, base peak). PMR ($CDCl_3$) δ ppm: 0.94 (6H, d, $J$=7 Hz), 1.22 (3H, s), 1.52 (3H, s), 2.23 (1H, m), 2.80 (2H, d, $J$=7.5 Hz), 3.73 (1H, s), 3.99 (3H, s), 7.74 (1H, s). UV $\lambda_{max}^{95\%EtOH}$ nm (log ε): 231 (4.31), 269 (3.98), 312.5 (3.81).

**Reduction of 6-(α,β-Epoxy-β-methyl)propyl-3-isobutyl-2-methoxypyrazine 4-Oxide (IX)**——1) A solution of 1.264 g (5 mmol) of IX in 50 ml of MeOH was shaken in the presence of Raney Ni, prepared from 5 g of the alloy, in an $H_2$ stream under high pressure (50 $kg/cm^2$) at 50° for 23 hr. After removal of the catalyst by suction, the solvent was evaporated off *in vacuo* to give a pale yellowish oil, which was chromatographed over silica gel (Wakogel C-200, 36 g) and eluted with a mixture of $CHCl_3$ and AcOEt (4: 1) to yield 945 mg (79%) of colorless oil, bp 110—120° (bath temp.)/5 Torr. *Anal.* Calcd. for $C_{13}H_{22}N_2O_2$: C, 65.51; H, 11.76; N, 9.31. Found: C, 65.40; H, 11.55; N, 9.59. MS *m/e* 238 ($M^+$), 223 ($M^+-CH_3$), 196 ($M^+-C_3H_6$), 180 ($M^+-C_3H_5OH$), 137 ($M^+-C_6H_{12}OH$, base peak). PMR ($CDCl_3$) δ ppm: 0.92 (6H, d, $J$=8 Hz), 1.21 (6H, s), 2.18 (1H, m, $J$=8 Hz), 2.66 (2H, d, $J$=8 Hz), 2.80 (2H, s), 3.90 (3H, s), 4.62 (1H, s, broad), 7.86 (1H, s). UV $\lambda_{max}^{95\%EtOH}$ nm (log ε): 217.5 (4.01), 279 (3.82, shoulder), 297.5 (3.96). IR (film) $cm^{-1}$: 3400 (OH).

2) IX (126 mg, 0.5 mmol) dissolved in 50 ml of MeOH was treated at room temperature as described above in the presence of Raney Ni, prepared from 0.5 g of the alloy. The oily product was chromatographed on silica gel (Wakogel C-200, 3 g), eluting with hexane, benzene, $CHCl_3$, and AcOEt, successively. XIII: A colorless oil, which was eluted with a mixture of hexane/benzene (3: 2 and 1: 1). bp 105—115° (bath temp.)/4 Torr. Yield: 16.8 mg (14%). High-resolution mass spectrum calcd. for $C_{13}H_{20}N_2O_2$: 236.152454. Obs.: 236.152775. PMR ($CDCl_3$) ppm: 0.92 (6H, d, $J$=6 Hz), 1.22 (3H, s), 1.52 (3H, s), 2.16 (1H, m), 2.65 (2H, d, $J$=8 Hz), 3.80 (1H, s), 3.94 (3H, s), 7.95 (1H, s). UV $\lambda_{max}^{95\%EtOH}$ nm (log ε): 221.5 (3.98), 261 (3.80, shoulder), 295.5 (4.02). X: A colorless oil, which was eluted with a mixture of benzene/$CHCl_3$ (9: 1 and 4: 1). Yield: 27.9 mg (23%). XIV: A colorless oil, which was eluted with a mixture of $CHCl_3$/AcOEt (9: 1 and

1: 1). bp 158—165° (bath temp.)/4 Torr. Yield: 43.9 mg (35%). High-resolution mass spectrum calcd. for $C_{13}H_{22}N_2O_3$: 254.163016. Obs.: 254.160614. PMR ($CDCl_3$) $\delta$ ppm: 0.94 (6H, d, $J$=7 Hz), 1.26 (6H, s), 2.22 (1H, m), 2.73 (2H, s), 2.78 (2H, d, $J$=8 Hz), 3.94 (3H, s), 4.26 (1H, s, broad), 7.65 (1H, s). UV $\lambda_{max}^{95\%EtOH}$ nm (log $\varepsilon$): 222 (4.21, shoulder), 227 (4.23), 267.5 (3.93), 307.5 (3.79, shoulder), 312 (3.80). IR (film) $cm^{-1}$: 3400 (OH).

**Demethylation of 6-($\beta$-Hydroxyl)isobutyl-3-isobutyl-2-methoxypyrazine (X)**——1) A mixture of 122 mg (0.5 mmol) of X and MeMgI, prepared from 36 mg (1.5 mg atom) of Mg, was heated at 150° for 30 min. After cooling, a small amount of $H_2O$ was added to the reaction mixture, which was slightly acidified with AcOH and extracted with ether. The ether layer was worked up as usual to afford 133 mg of a pale pinkish solid. The product was chromatographed over 4 g of silica gel (Wakogel C-200) and eluted with $CHCl_3$ to give 43.4 mg (39%) of I, which was recrystallized from i-$Pr_2O$ to furnish 32 mg (29%) of I as colorless needles, mp 118—119.5° (lit.[2] mp 122.5—123°).

2) X (119 mg, 0.5 mmol) was heated under reflux for 2 hr in 10% HCl (5 ml), then the reaction mixture was made alkaline with solid $K_2CO_3$ and extracted with ether. The usual work-up of the ether extract gave a semi-solid product (119 mg), which was chromatographed over silica gel (Wakogel C-200, 3.5 g) and eluted with benzene containing an increasing amount of $CHCl_3$. The benzene fractions gave VIII (30 .2 mg, 27%). The benzene/$CHCl_3$ (4: 1) fractions gave 24.2 mg (22%) of the starting material and 10.1 mg (10%) of XV. The $CHCl_3$ fractions afforded 28.5 mg (25%) of I. XV: Colorless prisms (recrystallized from hexane), mp 95—96.5°. *Anal.* Calcd. for $C_{12}H_{18}N_2O$: C, 69.87; H, 8.79; N, 13.58. Found: C, 69.82; H, 8.95; N, 13.38. MS $m/e$: 206 ($M^+$), 191 ($M^+-CH_3$), 164 ($M^+-C_3H_6$, base peak). PMR ($CDCl_3$) $\delta$ ppm: 0.98 (6H, d, $J$=7 Hz), 1.99 (6H, s), 2.24 (1H, m), 2.50 (2H, d, $J$=8 Hz), 5.95 (1H, s, broad), 7.32 (1H, s). UV $\lambda_{max}^{95\%EtOH}$ nm (log $\varepsilon$): 247.5 (3.80), 342 (4.06). IR (KBr) $cm^{-1}$: 1640 (C=O).

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