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Synthesis of *dl*-Neohydroxyaspergillic Acid

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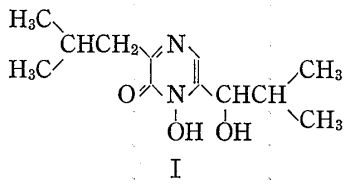
dl-Neohydroxyaspergillic acid (I), the *l*-isomer of which has been isolated from *Aspergillus flavus*, was synthesized from DL-leucine anhydride. By treatment of 2-chloro-3,6-diisobutylpyrazine 4-oxide (II) with acetic anhydride, an acetoxyl group was introduced into the isobutyl group. Deoxygenation of the N-oxide group at the 4-position of a 2-hydroxypyrazine 1,4-dioxide derivative (XII), prepared by hydrolysis of a 2-chloropyrazine 1,4-dioxide derivative (XI), was achieved using titanium(III) chloride to afford I.

Keywords——neohydroxyaspergillic acid; *Aspergillus flavus*; pyrazinol; hydroxamic acid; DL-leucine anhydride

A large number of 3,6-disubstituted 2-hydroxypyrazines and their 1-oxides have been isolated as fungal metabolites, especially from *Aspergillus* and *Candida* spp.[2] It is already known that these pyrazine metabolites are derived biosynthetically from the corresponding amino acids.[2] We have synthesized some of the naturally occurring pyrazines,[3–6] and would now like to report the synthesis of *dl*-neohydroxyaspergillic acid (I), whose *l*-isomer was isolated from *Aspergillus flavus* by Weiss *et al.*,[7] and characterized by Micetich and MacDonald.[8]



I

Chart 1

2-Chloro-3,6-diisobutylpyrazine 4-oxide[4] (II), prepared from DL-leucine through several steps, was heated with acetic anhydride in a sealed tube at 190° to afford a brown oil. This oily product was purified by distillation *in vacuo* and column chromatography on Florisil to give a colorless oil, whose infrared (IR) spectrum suggested the presence of an acetoxyl group (1740 cm^{-1}). In the proton magnetic resonance (PMR) spectrum of the product, a doublet (1H, J=8 Hz) is observed at 5.70 ppm, indicating substitution at the α-position of one of the isobutyl groups. On the other hand, methylene protons of the other isobutyl group resonated at 2.62 ppm as a doublet. Signals for the two methylene groups in 2-chloro-3,6-diisobutylpyrazine[4] appeared at 2.58 and 2.76 ppm. The former might be ascribed to the methylene protons of the butyl group at C-6 and the latter to those at C-3.[6] These spectral data suggest that the acetoxylation of II might occur at the butyl group on C-3. As will be mentioned later, the signal for methylene protons in 6-(α-acetoxy)isobutyl-2-chloro-3-isobutylpyrazine (IX) was observed at 2.78 ppm, at lower field than that of the oily product.

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2) *a*) P.G. Sammes, "Fortschritte der Chemie Organischer Naturstoffe," Vol. 32, Springer-Verlag, Wien, 1975, p. 51; *b*) W.B. Turner, "Fungal Metabolites," Academic Press, New York, 1971, p. 320.
3) A. Ohta, *Chem. Pharm. Bull.* (Tokyo), **12**, 125 (1964).
4) A. Ohta, *Chem. Pharm. Bull.* (Tokyo), **16**, 1160 (1968).
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6) A. Ohta, Y. Akita, K. Takizawa, M. Kurihara, S. Masano, and T. Watanabe, *Chem. Pharm. Bull.* (Tokyo), **26**, 2046 (1978).
7) U. Weiss, F. Strelitz, H. Flon, and I.N. Asheshov, *Arch. Biochem. Biophys.*, **74**, 150 (1958).
8) R.G. Micetich and J.C. MacDonald. *J. Chem. Soc.*, **1964**, 1507.

On the basis of these observations, it seemed reasonable to assign the structure of the product as 3-(α-acetoxy)isobutyl-2-chloro-6-isobutylpyrazine (III).

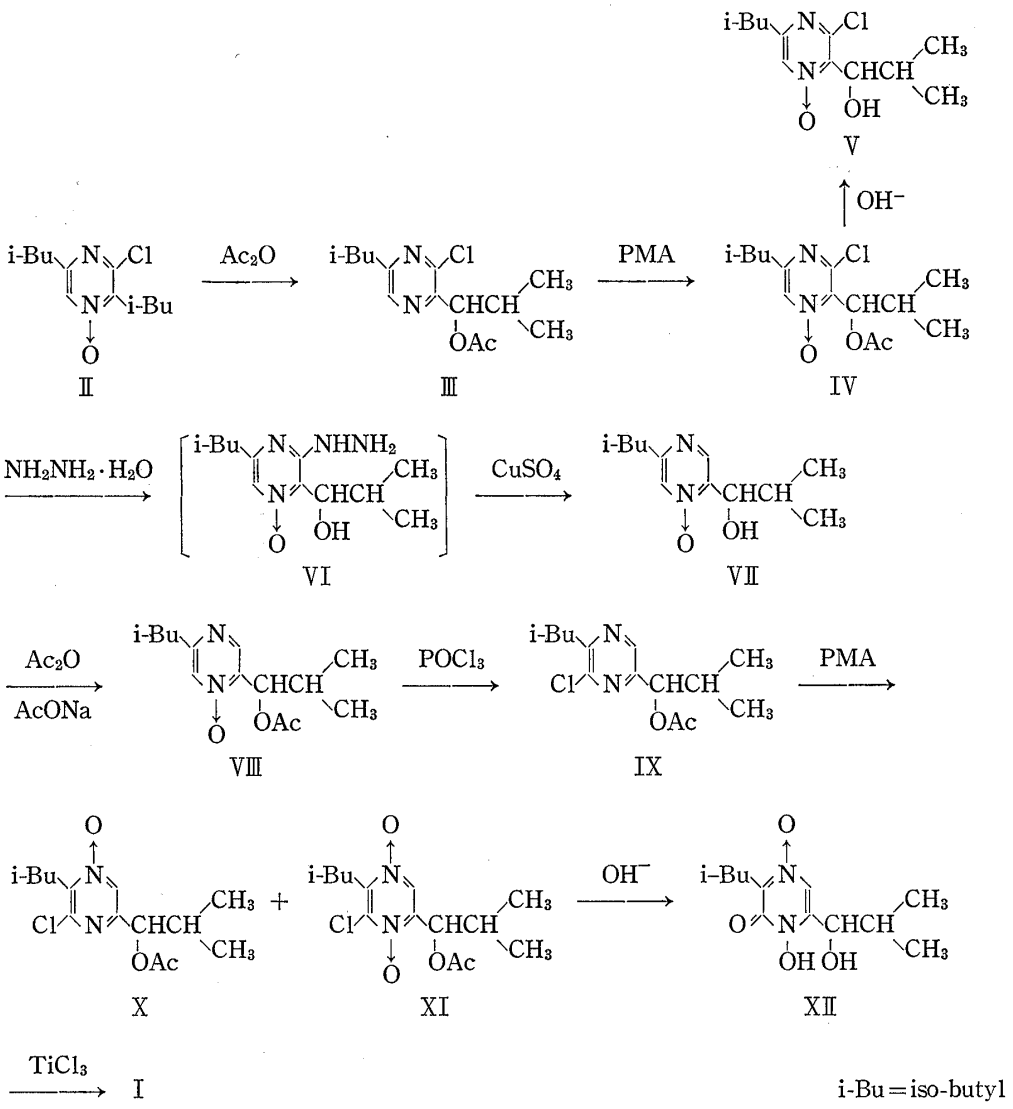

Chart 2

III was treated with permaleic acid in methylene chloride at room temperature and subjected to column chromatography to yield an N-oxide, 3-(α-acetoxy)isobutyl-2-chloro-6-isobutylpyrazine 4-oxide (IV), as a colorless oil, which gave colorless needles (V) on alkaline hydrolysis. In the PMR spectrum of IV, the ring proton resonates at higher field than that of III, and this indicates that the oxidation proceeded as shown in Chart 2.

Dechlorination of IV was successfully achieved *via* a hydrazino compound (VI). IV was heated with hydrazine hydrate in a sealed tube to afford the hydrazino compound, and subsequent oxidation using copper sulfate in dilute acetic acid gave colorless crystals, whose ultraviolet (UV) spectrum was similar to those of 2,5-dialkylpyrazine 1-oxides.[9] Based on these results and other analytical data the structure of the product was elucidated as 2-(α-hydroxy)isobutyl-5-isobutylpyrazine 1-oxide (VII).

The acetate (VIII), prepared from VII by heating with acetic anhydride and sodium acetate, was heated with phosphoryl chloride in a sealed tube at 140—150° and purified by column chromatography to give a colorless oil (IX). IX is an isomer of III and the UV

9) B. Klein and J. Berkowitz, *J. Am. Chem. Soc.*, **81**, 5150 (1959).

spectra of the two compounds were found to be similar. As mentioned above, the methylene protons of IX resonated at lower field than those of III, while the proton attached to the acetoxyl group of IX appeared at higher field than the corresponding one of III. These phenomena were in good agreement with those noted in the case of monochloro-2-isopropyl-5-isobutylpyrazines,[6] and this seems consistent with the proposed structures of III and IX.

N-Oxidation of IX was achieved under slightly stronger conditions than in the case of III, with permaleic acid in 1,2-dichloroethane under reflux, to afford a monoxide (X) and the required dioxide (XI) in 37 and 45% yield, respectively. These two oxides were separated by chromatography and characterized by comparing their UV spectra with those of 2-chloro-3,6-diisobutylpyrazine 4-oxide and 1,4-dioxide.[3] As shown in Table I, the monoxides showed four absorption maxima, while the dioxides gave two maxima in the UV region.

Table I. UV Absorption Maxima of 2-Chloro-3,6-diisobutylpyrazine N-Oxides

Compounds	$\lambda_{max}^{95\%EtOH}$ nm (log ε)			
2-Chloro-3,6-diisobutylpyrazine 4-oxide	237 (4.27)	275 (4.13)	304.5 (3.54)	314 (3.51)
X	238 (4.21)	275—277 (3.98)	303 (3.55)	312—314 (3.46)
2-Chloro-3,6-diisobutylpyrazine 1,4-dioxide	247 (4.43)	313 (4.29)		
XI	246.5 (4.37)	314.5 (4.27)		

The compound XI was hydrolyzed under heating in an alkaline medium to give a cyclic hydroxamic acid (XII), which gave a red coloration with ferric chloride in methanolic solution. The structure of XII was elucidated as 2-hydroxy-6-(α-hydroxy)isobutyl-3-isobutylpyrazine 1,4-dioxide on the basis of elemental analysis and the spectral data.

Conversion of XII to I was planned by the same route used in the synthesis of neoaspergillic acid, *via* three steps consisting of methylation, deoxygenation, and demethylation.[4] However, the yield of the last step was poor, because of the occurrence of dehydration. Therefore, direct deoxygenation of XII was carried out with titanium (III) chloride. The product was purified by column chromatography over silica gel followed by recrystallization from hexane to give pale yellow prisms, whose PMR, UV, and IR (Fig. 1) spectra were identical with those of *l*-neohydroxyaspergillic acid. No depression in melting point was observed in an admixture of the synthetic and naturally occurring samples.

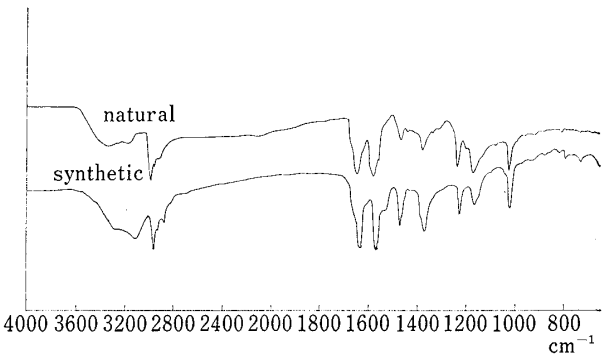

Fig. 1. IR Spectra of Natural and Synthetic Neohydroxyaspergillic Acids

Experimental

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 with a Hitachi RMU-7L spectrometer.

Treatment of 2-Chloro-3,6-diisobutylpyrazine 4-Oxide (II) with Ac_2O——A mixture of 18.7 g (82.7 mmol) of II and 40 ml of Ac_2O was heated in a sealed tube at 190° for 2 hr. After removal of Ac_2O *in vacuo*, the resulting brown residue was distilled to collect a yellowish oil (21.1 g), bp 115—140°/2 Torr. This oil was shown to be composed of many kinds of products by GLC (1.5% OV-17 on Shimalite; column temp., 170°; N_2 flow rate, 50 ml/min) and was accordingly chromatographed on Florisil (210 g), eluting with a mixture of hexane and benzene to yield 4.58 g (20%) of III as a colorless oil, bp 113—123°/2 Torr. *Anal.* Calcd. for

$C_{14}H_{21}ClN_2O_2$: C, 59.05; H, 7.43; N, 9.83. Found: C, 59.35; H, 7.50; N, 10.02. MS *m/e*: 284 (M^+). PMR (CCl_4) δ ppm: 0.96 (6H, d, $J=8$ Hz), 0.98 (6H, d, $J=8$ Hz), 2.04 (3H, s), 2.30 (2H, m, $J=8$ Hz), 2.62 (2H, d, $J=8$ Hz), 5.70 (1H, d, $J=8$ Hz), 8.22 (1H, s). UV $\lambda_{max}^{95\%EtOH}$ nm (log ε): 216 (4.03), 280.5 (3.94), 299 (3.59, shoulder). IR (film) cm^{-1}: 1740 (C=O).

3-(α-Acetoxy)isobutyl-2-chloro-6-isobutylpyrazine 4-Oxide (IV)——A mixture of 4.58 g (16.1 mmol) of III, 0.90 g (23.7 mmol) of 90% H_2O_2, and 2.40 g (24.5 mmol) of maleic anhydride dissolved in 70 ml of CH_2Cl_2 was allowed to stand overnight at room temperature. The reaction mixture was washed successively with H_2O, 10% $KHCO_3$, and H_2O, and dried over Na_2SO_4. Removal of CH_2Cl_2 by evaporation gave a yellowish oil (5.20 g), which was chromatographed on Florisil (100 g) and eluted with a mixture of hexane and benzene (1: 1) to give 2.28 g (47%) of IV as a colorless oil, bp 145—150°/3 Torr. *Anal.* Calcd. for $C_{14}H_{21}ClN_2O_3$: C, 55.91; H, 7.04; N, 9.31. Found: C, 55.91; H, 7.11; N, 9.30. MS *m/e*: 300 (M^+). PMR (CCl_4) δ ppm: 1.00 (12H, m), 2.08 (3H, s), 2.20 (1H, m), 2.50 (2H, d, $J=8$ Hz), 2.70 (1H, m), 5.96 (1H, d, $J=10$ Hz), 7.80 (1H, s). UV $\lambda_{max}^{95\%EtOH}$ nm (log ε): 238 (4.20), 274—277 (3.96). IR (film) cm^{-1}: 1750 (C=O).

2-Chloro-3-(α-hydroxy)isobutyl-6-isobutylpyrazine 4-Oxide (V)——A solution of 288 mg (0.96 mmol) of IV dissolved in a mixture of 1 ml of 10% K_2CO_3 and 2 ml of EtOH was heated under reflux for 30 min. After removal of EtOH *in vacuo*, extraction of the residue with ether gave V (229 mg, 93%), which was recrystallized from cyclohexane to afford colorless needles, mp 134—134.5°. *Anal.* Calcd. for $C_{12}H_{19}ClN_2O_2$: C, 55.70; H, 7.40; N, 10.83. Found: C, 55.67; H, 7.40; N, 10.72. MS *m/e*: 258 (M^+), 241 (M^+-OH). PMR (CCl_4) δ ppm: 0.80 (3H, d, $J=8$ Hz), 0.98 (6H, d, $J=8$ Hz), 1.12 (3H, d, $J=8$ Hz), 2.20 (2H, m, $J=8$ Hz), 2.52 (2H, d, $J=8$ Hz), 4.60 (2H, broad s), 7.78 (1H, s). UV $\lambda_{max}^{95\%EtOH}$ nm (log ε): 210 (4.16), 236 (4.23), 276 (3.99). IR (KBr) cm^{-1}: 3400 (OH).

3-(α-Hydroxy)isobutyl-6-isobutylpyrazine 4-Oxide (VII)——A mixture of 2.40 g (8 mmol) of IV, 2.40 ml of 80% hydrazine hydrate and 10 ml of EtOH was heated at 130° in a sealed tube for 3 hr. Evaporation of the solvent *in vacuo* gave a yellow oil, which was dissolved in CH_2Cl_2, washed with H_2O, and dried over Na_2SO_4. After removal of CH_2Cl_2, the resulting yellow oil was dissolved in a mixture of 7.4 ml of AcOH and 7.4 ml of H_2O, and heated on a water bath. $CuSO_4$ solution, prepared from 2.32 g of $CuSO_4 \cdot 5H_2O$ and 7.4 ml of H_2O, was added dropwise to the above-mentioned solution during 15 min and the mixture was heated further for 30 min with occasional shaking. After cooling, the reaction mixture was made alkaline with powdered K_2CO_3 and extracted with ether. The usual work-up of the ether layer gave brown crystals (1.10 g), which were chromatographed over silica gel (Wakogel C-200, 40 g), eluting with a mixture of CH_2Cl_2 and AcOEt (10: 1), to give 0.91 g (51%) of VII as pale brown crystals. Recrystallization from hexane furnished colorless prisms, mp 114—115°. *Anal.* Calcd. for $C_{12}H_{20}N_2O_2$: C, 64.25; H, 8.99; N, 12.49. Found: C, 64.28; H, 9.18; N, 12.53. MS *m/e*: 224 (M^+), 207 (M^+-OH). PMR (CCl_4) δ ppm: 0.92 (12H, m), 2.20 (2H, m, $J=8$ Hz), 2.54 (2H, d, $J=8$ Hz), 4.44 (1H, s), 4.48 (1H, q, $J=8$ Hz), 7.80 (1H, s), 8.34 (1H, s). UV $\lambda_{max}^{95\%EtOH}$ nm (log ε): 229 (4.22), 270 (4.02). IR (KBr) cm^{-1}: 3200 (OH).

3-(α-Acetoxy)isobutyl-6-isobutylpyrazine 4-Oxide (VIII)——A mixture of 8.00 g (35.7 mmol) of VII, 8 g of AcONa and 80 ml of Ac_2O was heated on a water bath for 1.5 hr. After removing Ac_2O *in vacuo*, the resulting oil was poured into ice-water and the brownish precipitates (9.06 g) were collected by suction. Recrystallization from MeOH–H_2O gave colorless prisms of VIII (7.15 g, 75%), mp 42—43°. *Anal.* Calcd. for $C_{14}H_{22}N_2O_3$: C, 63.13; H, 8.33; N, 10.52. Found: C, 63.01; H, 8.35; N, 10.84. MS *m/e*: 266 (M^+). PMR (CCl_4) δ ppm: 0.96 (12H, m), 2.10 (3H, s), 2.30 (2H, m), 2.52 (2H, d, $J=8$ Hz), 6.04 (1H, d, $J=6$ Hz), 7.80 (1H, s), 8.20 (1H, s). UV $\lambda_{max}^{95\%EtOH}$ nm (log ε): 230 (4.20), 270 (3.95). IR (film) cm^{-1}: 1740 (C=O).

6-(α-Acetoxy)isobutyl-2-chloro-3-isobutylpyrazine (IX)——After heating 8 g (30.1 mmol) of VIII with 80 ml of $POCl_3$ in a sealed tube at 140—150° for 1 hr, the reaction mixture was poured into ice-water, and made alkaline with powdered K_2CO_3. Extraction with ether gave a brown oil (7.2 g), which was chromatographed over Florisil (140 g) and eluted with a mixture of benzene and AcOEt (10: 1) to give a colorless oil (2.91 g, 34%), bp 149°/3 Torr. *Anal.* Calcd. for $C_{14}H_{21}ClN_2O_2$: C, 59.05; H, 7.43; N, 9.83. Found: C, 58.86; H, 7.46; N, 9.83. MS *m/e*: 284 (M^+), 269 (M^+-CH_3). PMR (CCl_4) δ ppm: 0.98 (12H, m), 2.09 (3H, s), 2.30 (2H, m), 2.78 (2H, d, $J=8$ Hz), 5.50 (1H, d, $J=8$ Hz), 8.30 (1H, s). UV $\lambda_{max}^{95\%EtOH}$ nm (log ε): 213—214 (4.00), 280.5 (3.91), 300 (3.55, shoulder). IR (film) cm^{-1}: 1740 (C=O).

Oxidation of 6-(α-Acetoxy)isobutyl-2-chloro-3-isobutylpyrazine (IX)——A solution of 1.23 g (4.33 mmol) of IX, 1.58 g (41.58 mmol) of 90% H_2O_2, and 5.28 g (53.88 mmol) of maleic anhydride in 50 ml of CH_2Cl–CH_2Cl was heated under reflux for 3.5 hr, then the reaction mixture was washed successively with H_2O, 5% $KHCO_3$ and H_2O, and dried over Na_2SO_4. The usual work-up gave a brownish oil, which was chromatographed over Florisil (50 g) and eluted successively with benzene, $CHCl_3$, and AcOEt. The fraction eluted with a mixture of benzene and $CHCl_3$ (4: 1 and 1: 1) gave 475 mg (37%) of the monoxide (X) as a colorless oil (bp 175—180°/3 Torr) and the fraction eluted with AcOEt gave 610 mg (45%) of the required dioxide (XI) as colorless crystals, which were recrystallized from hexane to furnish colorless prisms, mp 111—112°. X: *Anal.* Calcd. for $C_{14}H_{21}ClN_2O_3$: C, 55.91; H, 7.04; N, 9.31. Found: C, 55.89; H, 6.97; N, 9.48. MS *m/e*: 300 (M^+), 283 (M^+-OH). PMR ($CDCl_3$) δ ppm: 0.94 (6H, d, $J=8$ Hz), 0.99 (6H, d, $J=8$ Hz), 2.16 (3H, s), 2.24 (2H, m, $J=8$ Hz), 2.94 (2H, d, $J=8$ Hz), 5.50 (1H, d, $J=8$ Hz), 8.00 (1H, s). UV $\lambda_{max}^{95\%EtOH}$ nm (log ε): 238 (4.21), 275—277 (3.98), 303 (3.55, shoulder), 312—314 (3.46). IR (film) cm^{-1}: 1740 (C=O). XI: *Anal.* Calcd. for $C_{14}H_{21}ClN_2O_4$: C, 53.08; H, 6.68; N, 8.84. Found: C, 53.19; H, 6.61; N, 9.06. MS *m/e*: 316 (M^+),

299 (M^+-OH). PMR ($CDCl_3$) δ ppm: 0.90 (3H, d, $J=8$ Hz), 1.00 (6H, d, $J=8$ Hz), 1.02 (3H, d, $J=8$ Hz), 2.16 (3H, s), 2.40 (2H, m), 2.96 (2H, d, $J=8$ Hz), 6.12 (1H, d, $J=6$ Hz), 7.96 (1H, s). UV $\lambda_{max}^{95\%EtOH}$ nm (log ε): 246.5 (4.37), 314.5 (4.27). IR (KBr) cm^{-1}: 1750 (C=O).

2-Hydroxy-6-(α-hydroxy)isobutyl-3-isobutylpyrazine 1,4-Dioxide (XII)——A mixture of 758 mg (2.4 mmol) of XI, 5 ml of 10% KOH, and 5 ml of EtOH was heated under reflux for 1 hr. After removal of EtOH *in vacuo*, the reaction mixture was extracted with CH_2Cl_2 and the CH_2Cl_2 layer was discarded. The H_2O layer was acidified with dil. HCl under ice-cooling and extracted with CH_2Cl_2. The usual work-up gave 330 mg (54%) of XII as brownish crystals, which were recrystallized from AcOEt to furnish pale brownish prisms, mp 177—178°. *Anal.* Calcd. for $C_{12}H_{20}N_2O_4$: C, 56.23; H, 7.87; N, 10.93. Found: C, 56.25; H, 7.71; N, 10.93. MS *m/e*: 256 (M^+), 239 (M^+-OH). PMR (CF_3COOD) δ ppm: 1.08 (3H, d, $J=8$ Hz), 1.10 (6H, d, $J=8$ Hz), 1.18 (3H, d, $J=8$ Hz), 2.48 (2H, m, $J=8$ Hz), 3.14 (2H, d, $J=8$ Hz), 5.10 (1H, d, $J=8$ Hz), 8.04 (1H, s). UV $\lambda_{max}^{95\%EtOH}$ nm (log ε): 229.5 (4.18), 258.5—259.5 (4.09), 295.5 (3.85), 362 (3.96). IR (KBr) cm^{-1}: 1640 (C=O).

***dl*-Neohydroxyaspergillic Acid (I)**——A solution of 64 mg (0.25 mmol) of XII and 77 mg (0.5 mmol) of $TiCl_3$ in 20 ml of dry tetrahydrofuran (THF) was stirred for 2 hr in an N_2 stream at room temperature. H_2O (4 ml) was added and THF was evaporated off under reduced pressure. The residual solution was made basic with 2 N NaOH and the precipitate was removed by suction. After the filtrate had been extracted with ether, the aqueous layer was acidified with 2 N HCl and extracted with CH_2Cl_2. The CH_2Cl_2 solution was dried over Na_2SO_4 and the solvent was removed by distillation to give a straw-yellow solid. The product was chromatographed over silica gel and eluted with a mixture of $CHCl_3$ and MeOH (100: 1) to give 9 mg (15%) of I, which was recrystallized from hexane to furnish pale yellowish-brown prisms, mp 170—171° (lit.[8] mp 170—171°). MS *m/e*: 240 (M^+), 223 (M^+-OH). PMR (CF_3COOD) δ ppm: 1.52 (12H, d, $J=8$ Hz), 2.40 (2H, m), 3.10 (2H, d, $J=8$ Hz), 5.30 (1H, broad s), 7.24 (1H, s). UV $\lambda_{max}^{95\%EtOH}$ nm (log ε): 238 (3.95), 344.5 (3.63). IR (KBr) cm^{-1}: 1630 (C=O).

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