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Syntheses of Mutaaspergillic and *dl*-Hydroxyaspergillic Acids

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Mutaaspergillic and *dl*-hydroxyaspergillic acids, metabolites of aspergilli, were prepared by base-catalyzed hydroxylation of the corresponding 2-chloro-3,6-dialkylpyrazine 1-oxides derived from DL-leucyl-valyl and DL-isoleucyl-leucyl anhydrides.

Keywords——leucyl-valyl anhydride; isoleucyl-leucyl anhydride; 2-chloro-3-isobutyl-6-isopropylpyrazine 1-oxide; 2-chloro-3-isobutyl-6-*sec*-butylpyrazine 1-oxide; base-catalyzed hydroxylation; mutaaspergillic acid; hydroxyaspergillic acid

There are some naturally occurring pyrazines which carry hydroxyl groups on the alkyl side chains.[1-7] Among these hydroxyalkylpyrazines, deoxyneo-β-hydroxyaspergillic acid (I),[8] *dl*-neohydroxyaspergillic acid (II)[9] and mutaaspergillic acid (IIIa)[10] have already been synthesized. In the case of the former two compounds, a hydroxyl group was introduced into the side chain by the reaction of 2-chloro-3,6-diisobutylpyrazine 1- and 4-oxides with acetic anhydride.[8,9] On the other hand, ring closure of 4-methyl-2-(3-hydroxy-3-methyl-2-oxobutylamino)valerohydroxamic acid was undertaken for the synthesis of IIIa, which carries a tertiary hydroxyl group on the isopropyl side chain.[10] In a previous communication,[11] we described a hydroxylation of the α-position of the alkyl groups of alkylpyrazines by oxygen in the presence of a base,and a convenient synthesis of II. The present paper reports a continuation of our study on the hydroxylation of the side chain of alkylpyrazines and describes the syntheses of IIIa and *dl*-hydroxyaspergillic acid (IIIb).[1,6]

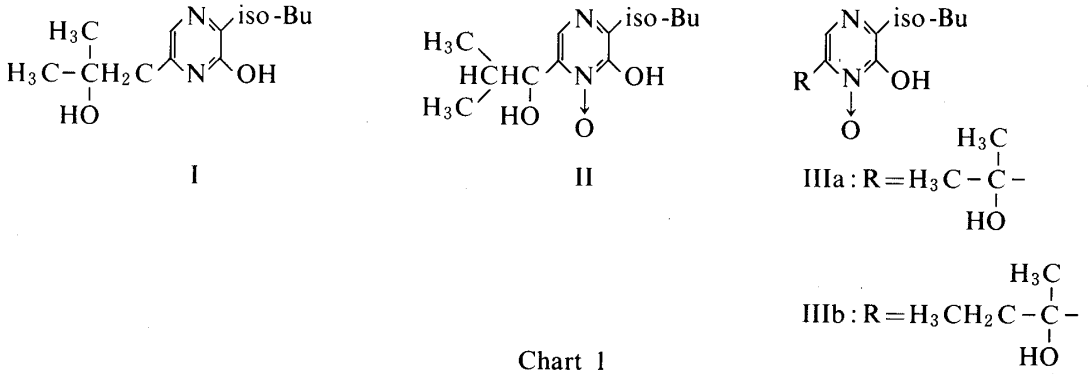

Chart 1

2-Chloro-3-isobutyl-6-isopropylpyrazine (Va) was obtained in the reported manner.[12] 2-Chloro-3-isobutyl-6-*sec*-butylpyrazine (Vb)[13] was similarly prepared by the reaction of DL-isoleucyl-leucyl anhydride (IVb)[14] with a mixture of phosphoryl chloride and phosphorus pentachloride, and purified by careful column chromatography on silica gel. Both 2-chloropyrazines were oxidized with persulfate in concentrated sulfuric acid[15] to afford the corresponding 1-oxides (VIIIa and VIIIb). In order to determine their structures, the products (VIIIa and VIIIb) were hydrolyzed in an alkaline medium to afford the corresponding hydroxypyrazines (IXa and IXb),[15] which gave a positive hydroxamic acid coloration with ferric chloride. Compounds IXa and IXb were shown to be identical with authentic specimens of 2-hydroxy-3-isobutyl-6-isopropylpyrazine 1-oxide[12] and *dl*-aspergillic acid[13] respectively, by

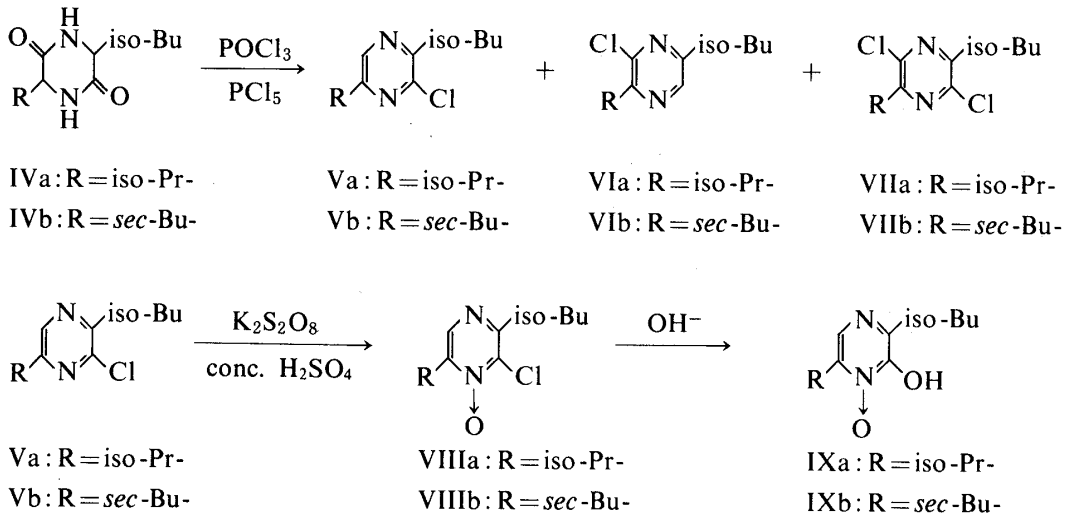

Chart 2

comparing the infrared (IR) spectra and measuring the mixed melting points. Thus, it was clarified that the N-oxidation of Va and Vb occurred at N-1, adjacent to C-2 carrying the chlorine atom.

As described in the previous report,[11] treatment of 2-chloro-3,6-dialkylpyrazine 1-oxides with oxygen in the presence of lithium diisopropylamide (LDA) led to successful syntheses of 3-alkyl-2-chloro-6-(α-hydroxy)alkylpyrazine 1-oxides. Accordingly, the hydroxylation of 2-chloro-3-isobutyl-6-isopropylpyrazine 1-oxide (VIIIa) and 2-chloro-3-isobutyl-6-*sec*-butylpyrazine 1-oxide (VIIIb) was carried out under the same conditions as reported. Namely, dry oxygen was bubbled into tetrahydrofuran (THF) solutions of the monoxides (VIIIa and VIIIb) in the presence of LDA at −78 °C. The acidic fractions of the products gave a mixture of the desired hydroxylated compounds (IIIa and IIIb) and the hydrolyzed compounds (IXa and IXb). These compounds were separated by the reported method,[11] *i.e.*, chloroform solutions of these compounds (IIIa and IXa, IIIb and IXb) were extracted with 0.03 M sodium bicarbonate to isolate IIIa and IIIb. On the other hand, from the alkali-insoluble fractions of the products, a mixture of the starting materials (VIIIa and VIIIb) and the hydroxyalkyl-chloropyrazines (Xa and Xb) was obtained, and each substance was isolated by silica gel column chromatography. Alkaline hydrolysis of the latter products led to the desired compounds (IIIa and IIIb), which were shown to be identical with the corresponding authentic specimens[6,10] by comparison of IR spectra. The ^{1}H-nuclear magnetic resonance (NMR) spectra of the products were consistent with the reported structures.

Thus, mutaaspergillic and *dl*-hydroxyaspergillic acids were synthesized conveniently, starting from the corresponding 2,5-dioxopiperazines.

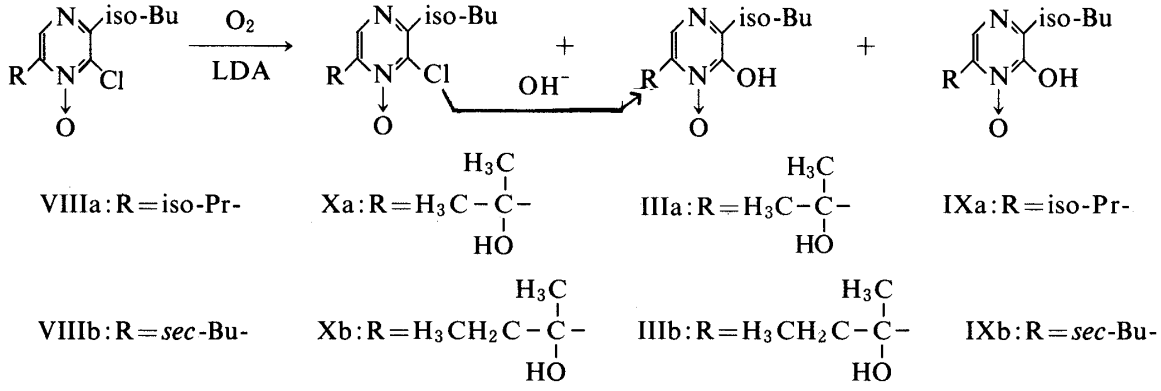

Chart 3

Experimental

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, ultraviolet (UV) spectra on a Hitachi 557 spectrophotometer, IR spectra on a Shimadzu IR-400 spectrometer, and ^{1}H-NMR spectra on a Varian EM-390 instrument with tetramethylsilane as an internal standard. Mass spectra (MS) were obtained with a Hitachi M-80 spectrometer.

2-Chloro-3-isobutyl-6-*sec*-butylpyrazine (Vb)——A mixture of DL-isoleucyl-leucyl anhydride[14] (20 g, 88.5 mmol), $POCl_3$ (50 ml), and PCl_5 (10 g) was heated at 130—140°C for 1 h in a sealed tube, then poured into ice-water, made alkaline with K_2CO_3 and extracted with Et_2O. The Et_2O layer was dried over Na_2SO_4 and the solvent was evaporated off to give a brown oil, which was dissolved in hexane. The hexane solution was extracted with conc. HCl. The HCl layer was made alkaline with K_2CO_3 and extracted with Et_2O to give a brown oil, which was applied to a silica gel (Wakogel C-200, 400 g) column and eluted with hexane containing increasing amounts of Et_2O. The hexane–Et_2O (58:1) fractions gave VIb as a colorless oil (7.1 g, 32%), bp 105—106°C (4 Torr). Further elution with the same developer gave a mixture of VIb and Vb (4.2 g) and then Vb as a colorless oil (4.7 g, 21%), bp 110—111°C (4 Torr). Both monochloropyrazines were shown to be identical with authentic samples[13] by a comparison of IR spectra.

The hexane layer was washed with H_2O and dried over Na_2SO_4. Evaporaion of the solvent gave VIIb as a pale yellow oil, which was purified by distillation to furnish a colorless oil, bp 115—116°C (4 Torr). *Anal.* Calcd for $C_{12}H_{18}Cl_2N_2$: C, 55.18; H, 6.95; N, 10.73. Found: C, 55.04; H, 6.95; N, 10.61. MS *m/e*: 264 (M^++4), 262 (M^++2), 260 (M^+). ^{1}H-NMR ($CDCl_3$) δ ppm: 0.86 [3H, t, J=7.5 Hz, $CH(CH_3)CH_2C\underline{H}_3$], 0.97 [6H, d, J=6 Hz, $CH_2CH(C\underline{H}_3)_2$], 1.26 [3H, d, J=7 Hz, $CH(C\underline{H}_3)CH_2CH_3$], 1.45—1.94 [2H, m, $CH(CH_3)C\underline{H}_2CH_3$], 2.01—2.46 [1H, m, $CH_2C\underline{H}(CH_3)_2$], 2.77 [2H, d, J=6 Hz, $C\underline{H}_2CH(CH_3)_2$], 3.05—3.56 [1H, m, $C\underline{H}(CH_3)CH_2CH_3$)]. UV $\lambda_{max}^{ethanol}$ nm (log ϵ): 221 (4.13), 285 (3.92, sh), 297 (3.99).

2-Chloro-3-isobutyl-6-isopropylpyrazine 1-Oxide (VIIIa)——$K_2S_2O_8$ (7.7 g, 28 mmol) was added portionwise to a solution of Va (5.0 g, 23.5 mmol) in conc. H_2SO_4 (25 ml), over a period of 30 min under stirring at room temperature. The reaction mixture was stirred for a further 24 h, poured into ice-water (80 ml) and extracted with $CHCl_3$. The $CHCl_3$ layer was washed successively with 10% $KHCO_3$ and water, and dried over Na_2SO_4. Evaporation of the $CHCl_3$ gave a pale yellow oil (*ca.* 5 g), which was chromatographed on silica gel (Wakogel C-200, 55 g) with hexane–Et_2O (7:3) and Et_2O. From the former eluate, the starting material (2.8 g, 56%) was recovered. The Et_2O fractions gave VIIIa (2.2 g, 41%), which was distilled to furnish a colorless oil, bp 115°C (1 Torr). *Anal.* Calcd for $C_{11}H_{17}ClN_2O$: C, 57.76; H, 7.49; N, 12.25. Found: C,57.58; H, 7.50; N, 12.10. MS *m/e*: 228 (M^+). ^{1}H-NMR ($CDCl_3$) δ ppm: 0.99 [6H, d, J=6 Hz, $CH_2CH(C\underline{H}_3)_2$], 1.33 [6H, d, J=6 Hz, $CH(C\underline{H}_3)_2$], 2.01—2.46 [1H, m, J= 6 Hz, $CH_2C\underline{H}(CH_3)$], 2.83 [2H, d, J= 6 Hz, $C\underline{H}_2(CH_3)_2$], 3.40—3.85 [1H, m, J= 6 Hz, $C\underline{H}(CH_3)_2$], 8.30 (1H, s, pyrazine $\underline{H}$). UV $\lambda_{max}^{ethanol}$ nm (log ϵ): 232 (4.39), 270—272 (4.06), 299.5—302 (3.52), 311—312 (3.51, sh).

2-Chloro-3-isobutyl-6-*sec*-butylpyrazine 1-Oxide (VIIIb)——A solution of Vb (2.5 g, 11 mmol) in conc. H_2SO_4 (12 ml) was treated with $K_2S_2O_8$ (3.6 g, 13 mmol) and worked up as described for the synthesis of VIIIa. The products were chromatographed on Wakogel C-200 (30 g). The fractions eluted with hexane–Et_2O (7:3) gave the starting material (0.98 g, 40%) and those eluted with Et_2O gave VIIIb (1.5 g, 57%). The product (VIIIb) was purified by distillation to furnish a colorless oil, bp 130—131°C (4 Torr). *Anal.* Calcd for $C_{12}H_{19}ClN_2O$: C 59.37; H, 7.89; N, 11.54. Found: C, 59.65; H, 7.90; N, 11.51. MS *m/e*: 242 (M^+). ^{1}H-NMR ($CDCl_3$) δ ppm: 0.95 [3H, t, J=7 Hz, $CH(CH_3)CH_2C\underline{H}_3$], 1.00 [6H, d, J=7 Hz, $CH_2CH(C\underline{H}_3)_2$, 1.32 [3H, d, J=7 Hz, $CH(C\underline{H}_3)CH_2CH_3$], 1.55—2.03 [2H, m, J=7 Hz, $CH(CH_3)C\underline{H}_2CH_3$], 2.03—2.46 [1H, m, J=7 Hz, $CH_2C\underline{H}(CH_3)_2$], 2.81 [2H, d, J=7 Hz, $C\underline{H}_2CH(CH_3)_2$], 3.27—3.65 [1H, m, J=7 Hz, $C\underline{H}(CH_3)CH_2CH_3$], 8.25 (1H, s, pyrazine $\underline{H}$). UV $\lambda_{max}^{ethanol}$ nm (log ϵ): 307 (4.40), 270—271 (4.06), 301—302 (3.56), 311—312 (3.51, sh).

2-Hydroxy-3-isobutyl-6-isopropylpyrazine 1-Oxide (IXa)——A solution of VIIIa (83 mg, 0.36 mmol) in a mixture of 20% KOH (3 ml) and MeOH (3 ml) was refluxed for 2 h, then the MeOH was removed by distilltion *in vacuo*. The residual alkaline solution was extracted with Et_2O. The water layer was acidified with 2 N HCl and extracted with CH_2Cl_2. The extract was dried over Na_2SO_4, and the solvent was distilled off to give IXa as a pale yellow solid (64 mg, 84%), which was recrystallized from MeOH–H_2O to furnish pale yellow needles, mp 88—91°C, undepressed on admixture with an authentic sample (mp 92—93°C).[12]

***dl*-Aspergillic Acid (IXb)**——A solution of VIIIb (46 mg, 0.19 mmol) in a mixture of 20% KOH (3 ml) and MeOH (3 ml) was worked up as described in the above-mentioned experiment, to afford IXb (36 mg, 86%) as pale yellow needles, mp 92—94°C, undepressed on admixtue wlth an authentic sample (mp 93—94.5°C).[13]

Hydroxylation of 2-Chloro-3-isobutyl-6-isopropylpyrazine 1-Oxide (VIIIa)——Oxygen was bubbled into a THF (30 ml) solution of VIIIa (490 mg, 2.1 mmol) and LDA [prepared from 4.1 ml (2.6 mmol) of a BuLi–hexane solution (0.64 M) and 260 mg (2.6 mmol) of diisopropylamine] for 5 h at −78°C, then the solvent was distilled off under reduced pressure. The resulting brown oily substance was triturated with water (30 ml) and extracted with Et_2O. The water layer was acidified with 2 N HCl and extracted with CH_2Cl_2.

The Et_2O extract was dried over Na_2SO_4 and concentrated to give an oily residue (*ca.* 500 mg), which was

applied to a silica gel (Wakogel C-200, 50 g) column and eluted with benzene containing increasing amounts of Me_2CO. The benzene–Me_2CO (80:1) eluate gave the starting material (106 mg, 22%). From the benzene–Me_2CO (30:1) fractions, Xa (98 mg, 19%) was obtained as a colorless oil, bp 120—125°C (3 Torr) (oil bath temp.).

The CH_2Cl_2 extract was shaken with 0.05 M $NaHCO_3$. The organic layer was dried over Na_2SO_4, and the solvent was evaporated off to give IXa (40 mg, 9%) as a pale yellow solid. The aqueous layer was acidified with 2 N HCl and extracted with $CHCl_3$. The extract was dried over Na_2SO_4. Evaporation of the solvent afforded IIIa (28 mg, 6%) as pale yellow crystals, which were recrystallized from MeOH–H_2O to furnish yellow prisms, mp 170—173°C (lit.[6] mp 173—174°C, lit.[10] mp 167—168°C).

IIIa: MS *m/e;* 226 (M^+), 211 (M^+-CH_3), 209 (M^+-OH). ^{1}H-NMR ($CDCl_3$) δ ppm: 0.97 [6H, d, J=6 Hz, $CH_2CH(C\underline{H}_3)_2$], 1.70 [6H, s, $C(OH)(C\underline{H}_3)_2$], 1.90—2.55 [1H, m, J=6 Hz, $CH_2C\underline{H}(CH_3)_2$], 2.75 [2H, d, J=6 Hz, $C\underline{H}_2CH(CH_3)_2$], 5.85—6.26 [2H, br s, $C(O\underline{H})(CH_3)_2$ and pyrazinol-O$\underline{H}$], 7.84 (1H, s, pyrazine $\underline{H}$). UV $\lambda_{max}^{ethanol}$ nm (log ϵ): 235.5 (3.89), 338—339 (3.66).

Xa: High resolution MS Calcd for $C_{11}H_{17}ClN_2O_2$: 244.0978. Obsd: 244.0981. MS *m/e*: 244 (M^+), 229 (M^+-CH_3). ^{1}H-NMR ($CDCl_3$) δ ppm: 1.00 [6H, d, J=6 Hz, $CH_2CH(C\underline{H}_3)_2$], 1.84 [6H, s, $C(OH)(C\underline{H}_3)_2$], 2.04—2.50 [1H, m, J=6 Hz, $CH_2C\underline{H}(CH_3)_2$], 2.85 [2H, d, J=6 Hz, $C\underline{H}_2CH(CH_3)_2$], 6.05 [1H, br s, $C(O\underline{H})(CH_3)_2$], 8.50 (1H, s, pyrazine H). UV $\lambda_{max}^{ethanol}$ nm (log ϵ): 231 (4.27), 271 (3.96), 297—301 (3.48, sh), 311 (3.41, sh). IR (liq. film) cm^{-1}: 3430 (OH).

Hydrolysis of 2-Chloro-3-isobutyl-6-(α-hydroxy)isopropylpyrazine 1-Oxide (Xa)——A solution of Xa (94 mg, 0.38 mmol) in a mixture of MeOH (3 ml) and 20% KOH (3 ml) was refluxed for 2 h, then worked up as described for the hydrolysis of VIIIa to give IIIa (80 mg, 92%) as pale yellow prisms.

Hydroxylaion of 2-Chloro-3-isobutyl-6-*sec*-butylpyrazine l-Oxide (VIIIb)——A THF (30 ml) solution of VIIIb (508 mg, 2.1 mmol) was treated with oxygen in the presence of LDA [prepared from 3.9 ml (2.5 mmol) of a BuLi–hexane solution (0.64 M) and 248 mg (2.5 mmol) of diisopropylamine]. The reaction mixture was worked up as described for the hydroxylation of VIIIa to afford Xb (110 mg, 20%) as a colorless oil, bp 120—121°C (3 Torr) (oil bath temp.), IIIb (44 mg, 9%) as pale yellow prisms (recrystallized from hexane), mp 148—151°C (lit.[1] mp 149°C, lit.[6] mp 155°C), and IXb (68 mg, 15%) as pale yellow prisms.

IIIb: MS *m/e* 240 (M^+), 224 (M^+-O), 223 (M^+-OH), 211 ($M^+-C_2H_5$). ^{1}H-NMR ($CDCl_3$) δ ppm: 0.90 [3H, t, J=8 Hz, $C(OH)(CH_3)CH_2C\underline{H}_3$], 0.98 [6H, d, J=6 Hz, $CH_2CH(C\underline{H}_3)_2$], 1.67 [3H, s, $C(OH)(C\underline{H}_3)$-CH_2CH_3], 1.91—2.46 [3H, m, $CH_2C\underline{H}(CH_3)_2$, $C(OH)(CH_3)C\underline{H}_2CH_3$], 2.76 [2H, d, J=6 Hz, $C\underline{H}_2CH(CH_3)_2$], 5.30—5.80 [2H, br s, $C(O\underline{H})(CH_3)CH_2CH_3$, pyrazinol-O$\underline{H}$], 7.75 (1H, s, pyrazine H). UV $\lambda_{max}^{ethanol}$ nm (log ϵ): 235 (4.16), 333 (3.84).

Xb: High resolution MS Calcd for $C_{12}H_{19}ClN_2O_2$: 258.1135. Obsd: 258.1104. MS *m/e*: 243 (M^+-CH_3), 241 (M^+-OH), 229 ($M^+-C_2H_5$). ^{1}H-NMR ($CDCl_3$) δ ppm: 0.92 [3H, t, J=7.5 Hz, $C(OH)(CH_3)CH_2C\underline{H}_3$], 1.00 [6H, d, J=6 Hz, $CH_2CH(C\underline{H}_3)_2$], 1.65 [3H, s, $C(OH)(C\underline{H}_3)CH_2CH_3$], 2.10 [2H, q, J=7.5 Hz, $C(OH)(CH_3)C\underline{H}_2CH_3$], 2.08—2.50 [1H, m, $CH_2C\underline{H}(CH_3)_2$], 2.87 [2H, d, J=7.5 Hz, $C\underline{H}_2CH(CH_3)_2$], 5.93 [1H, s, $C(O\underline{H})(CH_3)CH_2CH_3$], 8.43 (1H, s, pyrazine $\underline{H}$). UV $\lambda_{max}^{ethanol}$ nm (log ϵ): 232.5 (4.22), 271—273 (3.86), 300—302 (3.41, sh).

Hydrolysis of 2-Chloro-3-isobutyl-6-(α-hydroxy)-*sec*-butylpyrazine 1-Oxide (Xb)——Compound Xb (29 mg, 0.11 mmol) was hydrolyzed in a mixture of MeOH (2 ml) and 20% KOH (2 ml), as described for the hydrolysis of VIIIa, to give IIIb (23 mg, 89%) as pale yellow needles.

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