Synthesis of Metabolites and Related Compounds of 3-Isobutyryl-2-isopropylpyrazolo[1,5-*a*]pyridine (Ibudilast). III. 6,7-Dihydrodiol

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6,7-Dihydrodiol (1) has been isolated as a principal metabolite of ibudilast. We determined the structural formula of this compound (1) and made the stereochemical behavior in solution clear by the results of nuclear magnetic resonance (NMR) spectra. From these results, 6,7-dihydrodiol (1) exists as the equilibrium mixture of diastereomers and this equilibrium is influenced by the polarity of the solvent. It is concluded that 6,7-dihydrodiol (1) exists mainly as a *cis quasi*-equatorial-*quasi*-axial conformation (7-OH *quasi*-axial) in a non- or weak-polar solvent and mainly exists as a *trans quasi*-diaxial conformation in a polar solvent.

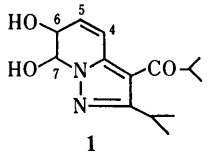
Keywords 6,7-dihydrodiol; ibudilast; metabolite; NMR spectra; solvent polarity; equilibrium relation; anomeric effect

In the preceding paper[1] we reported the synthesis of hydroxylated alkyl side chains and hydroxylated or methanesulfonated pyridine rings of ibudilast (3-isobutyryl-2-isopropylpyrazolo-[1,5-*a*]pyridine) as authentic references for metabolites of ibudilast. The urinary excretion of ibudilast has been studied[2] in animals and a principal metabolite has been isolated. We purified and characterized this metabolite, designated 6,7-dihydrodiol (**1**). It was proved that 6,7-dihydrodiol (**1**) exists as an equilibrium mixture of the *cis* form and *trans* form. The present study was undertaken in order to determine the chemical structure and make the stereochemical behavior in solution clear by the results of nuclear magnetic resonance (NMR) spectra.

Structure Determination of 6,7-Dihydrodiol (1) Urine collected from rabbits following oral administration of ibudilast was treated with gluculonidase[2] or diluted alkali to obtain the predominant metabolite as a white powder. Its mass spectrum (MS) (*m/z*: 264 (M^+)) and elemental analysis suggested that it contains two more hydroxy groups than ibudilast. Further confirmation of the presence of the hydroxy groups is given by infrared (IR) spectrum which, on comparison with the ibudilast spectrum, shows a broad band between 3400 and 3150 cm^{-1}. Treatment of this metabolite with azodicarboxylic acid diethyl ester and triphenylphosphine gave an epoxy compound (**2**, Chart 2). Moreover, on heating with $KHSO_4$ in methanol, it gave 6-hydroxy-3-isobutyryl-2-isopropylpyrazolo[1,5-*a*]pyridine[3] (**3**), but not a 4-hydroxy compound. We may conclude that this metabolite has the basic structural formula: 6,7-dihydrodiol (**1**) as shown in Chart 1 and that it is 6,7-dihydro-6,7-dihydroxy-3-isobutyryl-2-isopropylpyrazolo[1,5-*a*]pyridine.

Further confirmation of this structural hypothesis and the complete determination of the metabolite formula came from its NMR spectrum in $CDCl_3$ and from the spectrum obtained through the addition of D_2O as shown in Chart 1. The addition of D_2O causes the disappearance of the two broad peaks, which means that these signals were due to two hydroxy groups. However this spectrum shows existence of two kinds of compounds (compounds A and B) as shown in Chart 1. We presumed that these compounds were the mixture of diastereoisomers which came from two asymmetric carbon atoms, C^6 and C^7. Each peak was assigned on the basis of the spindecoupling technique. In order to investigate the stereochemistry of these compounds we tried to separate them by the preparation of derivatives.

Acetonide Derivative of 6,7-Dihydrodiol (1) We tried to prepare an acetonide derivative of 6,7-dihydrodiol (**1**) with a view to obtaining a *cis* form. The acetonide derivative (**3**) was prepared by the reaction of 6,7-dihydrodiol (**1**) with acetone in the presence of $CuSO_4$ and a catalytic amount



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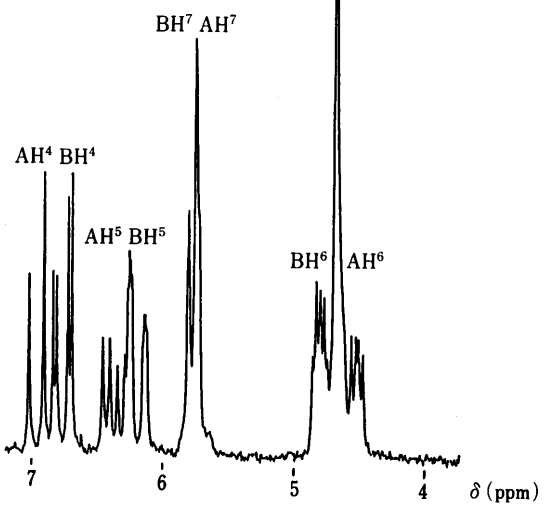

Chart 1. ^{1}H-NMR Spectrum of 6,7-Dihydrodiol (**1**) ($CDCl_3$, D_2O)

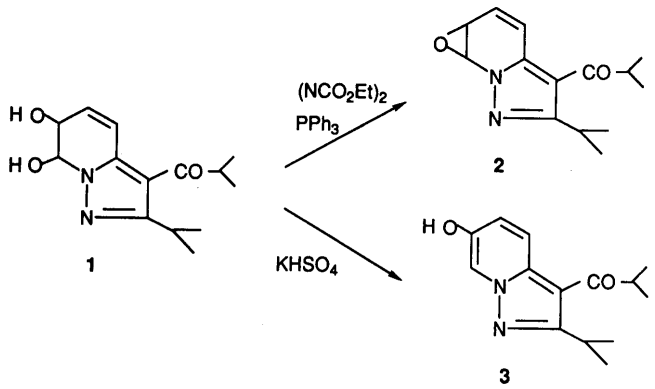

Chart 2. Derivatives of 6,7-Dihydrodiol (**1**)

of conc. H_2SO_4 at room temperature. The NMR spectrum ($CDCl_3$) of the obtained acetonide showed a single isomer and was similar to these of compound B. The vicinal coupling constant ($J_{6,7}$) is 6.5 Hz and the allylic coupling constant ($J_{4,6}$) between H^4 and H^6 was 1.0 Hz. This indicates that the allylic angle between the two hydrogens is very large and, therefore, H^6 lies perpendicular to the plane of the double bond $C^4=C^5$. Consequently, the ether group at C^6 will assume *quasi*-equatorial orientation, practically on the plane of the double bond. As shown in Chart 3, the acetonide derivative has two possible conformation: *trans quasi*-diequatorial and *cis quasi*-axial-*quasi*-equatorial. We concluded that the acetonide derivative (**4**) has a *cis quasi*-axial-*quasi*-equatorial conformation by observation of the nuclear Overhauser effect (NOE) between H^6 and H^7.

Pivaloyl Derivative of 6,7-Dihydrodiol (1) Furthermore we tried to prepare a pivaloyl derivative of 6,7-dihydrodiol (**1**) with the purpose of obtaining a *trans* form. The pivaloyl derivative was prepared by the reaction of 6,7-dihydrodiol (**1**) with pivaloyl chloride in pyridine. The pivaloyl derivative obtained was a single isomer based on its NMR spectrum ($CDCl_3$) and its similarity to compound A (Chart 1). The vicinal coupling constant ($J_{6,7}$) was 2.0 Hz and its contrariness to the acetonide derivative allylic coupling constant between H^4 and H^6 is zero. This indicates that the allylic angle between the two hydrogens (H^4 and H^6) is very small, therefore H^6 lies on the plane to the double bond $C^4=C^5$. Consequently the ester group at C^6 will assume *quasi*-axial orientation. As shown in Chart 4, the pivaloyl derivative (**5**) has two possible conformations, *trans quasi*-diaxial and *cis quasi*-axial-*quasi*-equatorial. We concluded that the pivaloyl derivative (**5**) has a *trans quasi*-diaxial conformation based on the steric disadvantage of the *cis* conformation and the distinction between the NMR spectra of **4** and **5**.

Conformational Analysis of 6,7-Dihydrodiol (1) Table I shows that the *cis* form has an allylic coupling between H^4 and H^6 whereas the *trans* form does not. In view of the similarity between the NMR spectra of compound A and the pivaloyl derivative, we concluded that compound A is a *trans* form. The *cis* form compound B was also determined in a similar way. The *cis* form exists as (b) form (Chart 5, *quasi*-axial(7-OH)-*quasi*-equatorial(6-OH)) because it has a stabilization which contributes to the anomeric effect between axial 7-OH and bridge-head nitrogen.

The effects of the orientation of electronegative substituents on the chemical shifts of ring protons have been studied and the following empirical rules proposed:[4] a) axial substituents (OH) exert a shielding effect on equatorial protons attached to adjacent carbon atoms; b) axial sub-

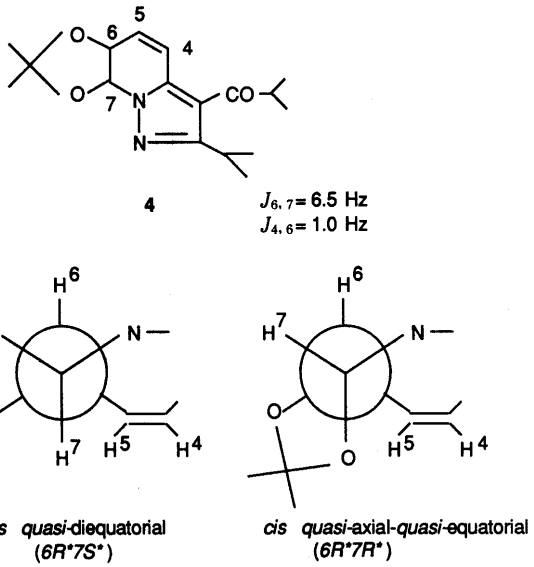

Chart 3. Acetonide Derivative of 6,7-Dihydrodiol (**1**)

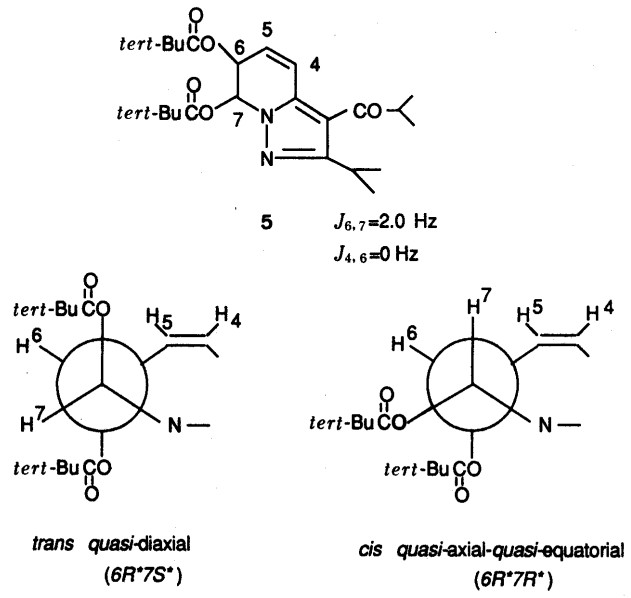

Chart 4. Pivaloyl Derivative of 6,7-Dihydrodiol (**1**)

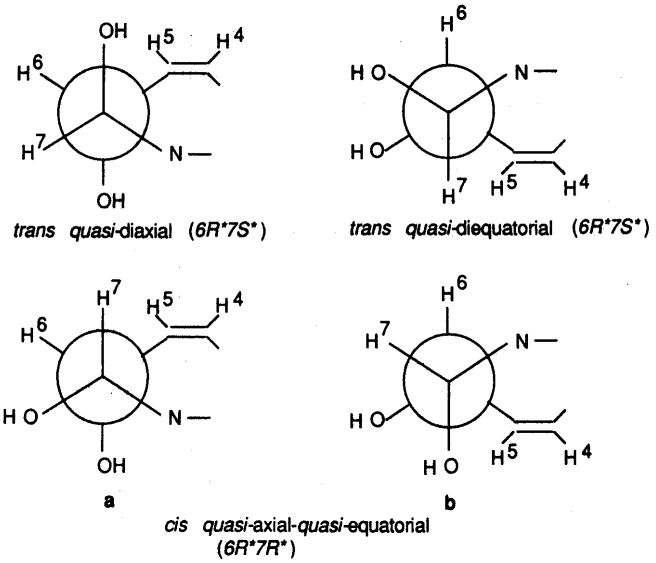

Chart 5. Conformational Analysis of 6,7-Dihydrodiol (**1**)

TABLE I. Coupling Constants of 6,7-Dihydrodiol (**1**) Derivatives (^{1}H-NMR, $CDCl_3$)

Derivatives		J value	
		$J_{6,7}$ (Hz)	$J_{4,6}$ (Hz)
Epoxy	(**2**)	3.5	1.8
Acetonide	(**4**)	6.5	1.0
Pivaloyl	(**5**)	2.0	
Compd. (**1**) (*trans*)	A	3.0	
Compd. (**1**) (*cis*)	B	4.6	2.0

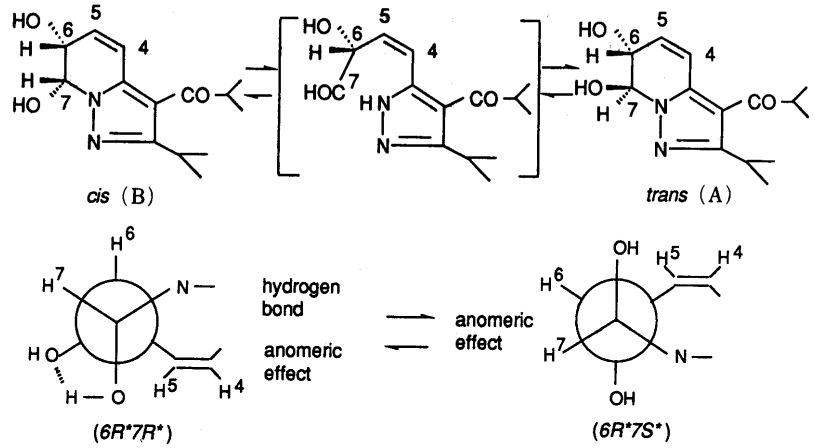

Chart 6. Structure of 6,7-Dihydrodiol (**1**) in Solution

TABLE II. Equilibrium Compositions for 6,7-Dihydrodiol (**1**) in Various Solvents

Solvent	*cis* (%)	*trans* (%)
CCl_4	69	31
$CDCl_3$	68	32
Acetone-d_6	25	75
DMSO-d_6	24	76
Pyridine-d_5	21	79

Calcd from integration of 6-H (^{1}H-NMR).

stituents (OH) deshield vicinal axial protons. These rules are in good agreement with the results of *trans* form A and *cis* form B in Chart 1.

Equilibrium Compositions for 6,7-Dihydrodiol (1) in Various Solvents As the above experimental results show, it is evident that 6,7-dihydrodiol (**1**) exists as a mixture of *cis* form and *trans* form in solution. Therefore, we investigated a change of the composition for 6,7-dihydrodiol (**1**) in various solvents by the use of NMR. The ratios of the *cis* and *trans* forms were calculated from integration of H^6. As shown in Table II, 6,7-dihydrodiol (**1**) exists principally as a *cis* isomer in non or low polar solvents ($CDCl_3$, CCl_4), while in polar solvents (acetone-d_6, DMSO-d_6, pyridine-d_5) 6,7-dihydrodiol (**1**) exists principally as a *trans* isomer. This indicates that 6,7-dihydrodiol (**1**) exists as an equilibrium mixture of *cis* and *trans* forms. When a polar solvent (DMSO-d_6) was added to a low polar solution ($CDCl_3$) of 6,7-dihydrodiol (**1**), the equilibrium shifted rapidly to the side of the *trans* form. The reverse was also the same. But temperature did not have a large effect on these equilibriums (from $-65\,^\circ C$ to $90\,^\circ C$). It is considered that the *cis* form has the stability of a hydrogen bond between two adjacent hydroxy groups in non- or low polar solvents, while in the case of polar solvents, this hydrogen bond is inhibited by solvation, and these two adjacent hydroxy groups sterically repel each other. Therefore, equilibrium is shifted to the side of *trans* form in polar solvents. Since the *trans* form is able to stabilize the anomeric effect between 7-OH and bridge-head nitrogen, the energy barrier of these conformations is small and these diastereomers are easily transformed into each other.

Consideration It has become apparent that 6,7-dihydrodiol (**1**) exists as an equilibrium mixture of *cis* and *trans* forms in solution. We suppose that the equilibrium relation occurs through an aldehyde intermediate as shown in Chart 6, but the existence of the aldehyde could not be confirmed by a qualitative analysis of aldehyde (dimedon[5]). It was considered that the equilibration of these diastereomers is very fast. 6,7-Dihydrodiol (**1**) exists mainly as a *cis quasi*-equatorial-*quasi*-axial conformation (7-OH axial) in non- or low-polar solvents and mainly as a *trans quasi*-diaxial conformation in polar solvents. Optical rotations of 6,7-dihydrodiol (**1**) and its derivatives were shown in Table III. In spite of the racemization of C^7, 6,7-dihydrodiol has optical rotation. It is indicated that the conformation of C^6 is maintained in an equilibrium relation.

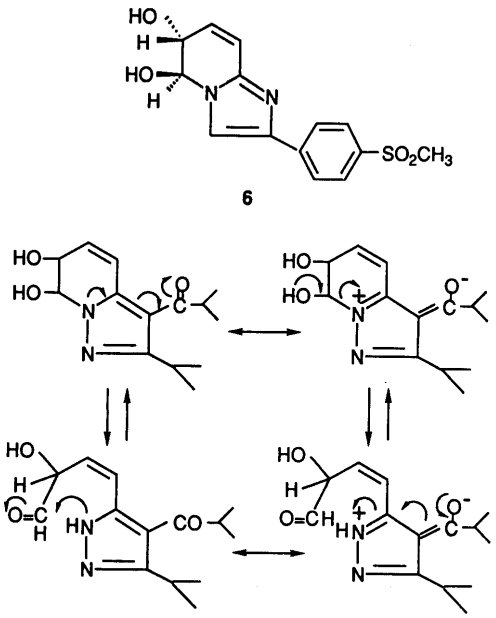

Chart 7. 6,7-Dihydrodiol and Metabolite of Zolimidine (**6**)

TABLE III. Optical Rotations

Compound	Solvent	$[\alpha]_D^{20}$ (°)	*c*
6,7-Dihydrodiol (**1**)	EtOH	+96.0	1.0
6,7-Dihydrodiol (**1**)	Pyridine	+176.6	0.9
6,7-Dihydrodiol (**1**)	$CHCl_3$	+61.3	1.0
Acetonide (**4**)	EtOH	+86.7	0.6
Pivaloyl (**5**)	EtOH	+204.3	4.2

A similar metabolite is observed for Zolimidine,[6] but the metabolite (**6**) exists only as a *trans* diaxial and does not have the equilibrium relation observed in 6,7-dihydrodiol (**1**). We presumed that the equilibrium relation in the case of 6,7-dihydrodiol (**1**) stemmed from the decrease of the electron density of bridge-head nitrogen which is influenced by the electron-withdrawing resonance effect of a 3-carbonyl group. For this reason, the bond of C^7–N breaks easily and the equilibrium relation is formed as shown in Chart 7.

Experimental

Apparatus Melting points were measured with a Yanagimoto melting point apparatus and are uncorrected. NMR spectra were measured with a JNM-FX 90Q FT NMR or JNM-EX 400 FT NMR spectrometer using tetramethylsilane as an internal standard. Column chromatography was carried out on silica gel (Wakogel C-200). MS and IR spectra were measured with a JMS-D 300 mass spectrometer and a Hitachi 260-10 infrared spectrophotometer, respectively. Optical rotations were obtained with a DIP-360 polarimeter at 20 °C.

Isolation of 6,7-Dihydro-6,7-dihydroxy-3-isobutyryl-2-isopropylpyrazolo[1,5-*a*]pyridine (6,7-Dihydrodiol (1)) The collected urine (1.9 l) after oral administration of ibudilast in rabbit (100 mg/kg) was treated with NaOH (2.0 N) and allowed to stand at room temperature for 3 h. The urine was extracted with AcOEt after treatment with an active carbon, dried over anhyd. Na_2SO_4, and purified by column chromatography on silica gel employing AcOEt as an eluent to give an oil, which solidified on standing. The solid was recrystallized from benzene to give 0.18 g of 6,7-dihydrodiol (**1**) in the form of a white powder, mp 126—127 °C. ^{1}H-NMR ($CDCl_3$) δ: 1.14 and 1.17 (6H × 2, d, J=6.8 Hz, $CH(C\underline{H}_3)_2$), 3.07 and 3.41 (1H × 2, m, J=6.8 Hz, $C\underline{H}(CH_3)_2$), 6.60 (2H, br, OH); compound A: 4.98 (1H, dd, J=5.0, 3.0 Hz, pyrazolopyridine-6-H), 5.73 (1H, d, J=3.0 Hz, pyrazolopyridine-7-H), 6.37 (1H, dd, J=10.1, 5.0 Hz, pyrazolopyridine-5-H), 6.91 (1H, d, J=10.1 Hz, pyrazolopyridine-4-H); compound B: 4.80 (1H, ddd, J=4.6, 2.0, 2.0 Hz, pyrazolopyridine-6-H), 5.77 (1H, d, J=4.6 Hz, pyrazolopyridine-7-H), 6.19 (1H, dd, J=10.0 Hz, pyrazolopyridine-5-H), 6.76 (1H, dd, J=10.0, 2.0 Hz, pyrazolopyridine-4-H). MS m/z: 264 (M^+). IR (KBr): 3400-3150 (OH), 1669 cm^{-1} (C=O). *Anal.* Calcd for $C_{14}H_{20}N_2O_3$: C, 63.62; H, 7.63; N, 10.60. Found: C, 63.61; H, 7.69; N, 10.59.

Epoxy Compound (2) Triphenylphosphine (0.30 g) and then azodicarboxylic acid diethylester (0.20 g) were added to the solution of dihydrodiol (**1**, 0.20 g) in dry benzene (10 ml). The reaction mixture was allowed to stand overnight at room temperature. The mixture was concentrated under reduced pressure at 35 °C (bath temperature), a small amount of ether was added to the residue, the insoluble material (triphenylphosphine oxide) was filtered off, and the filtrate was concentrated to dryness. The residue was purified by flash column chromatography and eluted with benzene–AcOEt (2:1) to afford 0.10 g (54%) of epoxy compound (**2**). ^{1}H-NMR ($CDCl_3$) δ: 1.17 (6H, d, J=6.6 Hz, $COCH(C\underline{H}_3)_2$), 1.32 (6H, d, J=7.0 Hz, $C\underline{H}(CH_3)_2$), 3.14 (1H, m, J=6.6 Hz, $COC\underline{H}(CH_3)_2$, 3.46 (1H, m, J=7.0 Hz, $C\underline{H}(CH_3)_2$), 4.10 (1H, ddd, J=4.0, 3.5, 1.8 Hz, pyrazolopyridine-6-H), 5.77 (1H, d, J=3.5 Hz, pyrazolopyridine-7-H), 6.60 (1H, dd, J=10.0, 4.0 Hz, pyrazolopyridine-5-H), 7.21 (1H, dd, J=10.0, 1.8 Hz, pyrazolopyridine-4-H).

Dehydration of 6,7-Dihydrodiol (1) A mixture of 6,7-dihydrodiol (**1**) (0.50 g) and $KHSO_4$ (1.75 g) in acetonitrile (20 ml) was refluxed for 28 h (10 ml more of acetonitrile was added during this time) and then the insoluble materials were filtered off. The filtrate was concentrated to dryness and dissolved in CH_2Cl_2 (*ca.* 10 ml). The insoluble materials were removed by filtration and evaporated to dryness. The residue was subjected to silica gel column chromatography and eluted with CH_2Cl_2. The eluate was evaporated to dryness and the residue was recrystallized from benzene–hexane to afford 0.35 g (75%) of 6-hydroxy-3-isobutyryl-2-isopropylpyrazolo[1,5-*a*]pyridine (**3**), mp 87—90 °C. ^{1}H-NMR ($CDCl_3$) δ: 1.32 and 1.40 (6H × 2, d, J=6.8 Hz, $CH(C\underline{H}_3)_2$), 3.34 and 3.76 (1H × 2, m, J=6.8 Hz, $C\underline{H}(CH_3)_2$), 5.60 (1H, br, OH), 6.96 (1H × 2, dd, J=9.0, 1.2 Hz, pyrazolopyridine-5-H), 7.76 (1H, d, J=9.0 Hz, pyrazolopyridine-4-H), 8.02 (1H, d, J=1.2 Hz, pyrazolopyridine-7-H). MS m/z: 246 (M^+). IR (KBr): 1618 cm^{-1} (C=O). *Anal.* Calcd for $C_{14}H_{18}N_2O_2$: C, 68.27; H, 7.37; N, 11.37. Found: C, 68.27; H, 7.49; N, 11.23.

Acetonide Compound (4) A mixture of 6,7-dihydrodiol (**1**) (0.10 g), dry acetone (14 ml), $CuSO_4$ (0.80 g) and conc. H_2SO_4 (2 drops) was stirred at room temperature for 3 d. The insoluble materials were filtered off and the filtrate was concentrated *in vacuo*. The residue was chromatographed over silica gel employing CH_2Cl_2 as the eluent. The eluate was evaporated to dryness to afford 66 mg (57%) of acetonide compound (**4**). ^{1}H-NMR($CDCl_3$) δ: 1.18 and 1.32 (6H × 2, d, J=7.0 Hz, $CH(C\underline{H}_3)_2$), 1.39 and 1.50 (3H × 2, s, acetonide-CH_3), 3.13 and 3.42 (1H × 2, m, J=7.0 Hz, $C\underline{H}(CH_3)_2$), 5.39 (1H, ddd, J=6.5, 3.5, 1.0 Hz, pyrazolopyridine-6-H), 6.04 (1H, d, J=6.5 Hz, pyrazolopyridine-7-H), 6.18 (1H, dd, J=10.0, 3.5 Hz, pyrazolopyridine-5-H), 6.92 (1H, dd, J=10.0, 1.0 Hz, pyrazolopyridine-4-H). MS m/z: 304 (M^+). IR (KBr): 1654 (C=O) cm^{-1}. *Anal.* Calcd for $C_{17}H_{24}N_2O_3$: C, 67.08; H, 7.95, N, 9.20. Found: C, 66.94; H, 7.96; N, 9.15.

Pivaloyl Compound (5) Pivaloyl chloride (0.20 g) was added dropwise to the solution of 6,7-dihydrodiol (**1**) (0.20 g) in pyridine (5 ml) under cooling and stirred at room temperature for 3 h. The mixture was poured into ice-cold water, made slightly acidic with dil. HCl, and extracted with CH_2Cl_2. The organic layer was washed successively with water, an aqueous K_2CO_3 solution and water again, and then dried over anhyd. Na_2SO_4. The obtained organic layer was concentrated to dryness and the residue was purified by column chromatography on silica gel employing CH_2Cl_2 as an eluent to afford 0.15 g (46%) of the pivaloyl compound (**5**) as a colorless oil. ^{1}H-NMR ($CDCl_3$) δ: 1.18 and 1.20 (18 H, s, $C(CH_3)_3$ × 2), 1.22 and 1.36 (6H × 2, d, J=7.0 Hz, $CH(C\underline{H}_3)_2$), 2.16 and 2.42 (1H × 2, m, J=7.0 Hz, $C\underline{H}(CH_3)_2$ and $COC\underline{H}(CH_3)_2$), 5.39 (1H, dd, J=5.3, 2.0 Hz, pyrazolopyridine-6-H), 6.29 (1H, d, J=10.0, 5.3 Hz, pyrazolopyridine-5-H), 6.82 (1H, d, J=2.0 Hz, pyrazolopyridine-7-H), 7.20 (1H, d, J=10.0 Hz, pyrazolopyridine-4-H). MS m/z: 432 (M^+). IR (KBr): 1725, 1723, 1652 (C=O) cm^{-1}. *Anal.* Calcd for $C_{24}H_{36}N_2O_5$: C, 66.64; H, 8.39; N, 6.48. Found: C, 66.44; H, 8.39; N, 6.39.

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