AN ALTERNATIVE SYNTHESIS OF 2-BENZYLOXY-6-HYDROXYMEYHYL-3-ISOBUTYL-5-METHOXYPYRAZINE 4-OXIDE, A KEY INTERMEDIATE FOR SYNTHESIS OF OPC-15161

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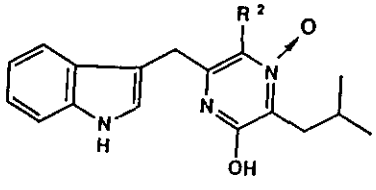
Abstract ——— 2-Benzyloxy-6-hydroxymethyl-3-isobutyl-5-methoxypyrazine 4-oxide (5e), a key intermediate for synthesis of OPC-15161 (1a) having an inhibitory activity against superoxide anion generation, was prepared from 5-amino-2-benzyloxy-3-isobutyl-6-methoxycarbonylpyrazine 4-oxide (5b) by 3-step reaction.

Active oxygen species cause a variety of diseases, such as inflamation, autoimmune disease, diabetes, cardiovascular disease and cancer-initiation. [1,2]

OPC-15161 (1a), a major degradation product of naturally occurring OPC-15160, was reported to show an inhibitory activity against superoxide anion generation and hence expected to have possibility to control these diseases. [3] After the structure of 1a was determined by X-ray crystallographic analysis, three synthetic methods have been reported starting respectively from tryptophan methyl ester, [4] 2-hydroxyimino-4-methylpentanoic



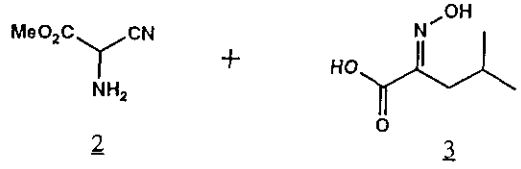
1a (OPC-15161) : $R^2 = OMe$
1b : $R^2 = OH$

acid and ethyl aminocyanoacetate,[5] and 5-chloro-3-isobutyl-6-methylpyrazine.[6] However, these methods bear either a low yield in the selective methylation at the C-5 hydroxy group of 1b,[4] or tedious many-step reaction to obtain 1a from the keto oxime (3) *via* methoxy compound (5e).[5]

The authors were interested in exploring an alternative efficient synthesis of 2-benzyloxy-6-hydroxymethyl-3-isobutyl-5-methoxypyrazine 4-oxide (5e) from 5-amino-2-benzyloxy-3-isobutyl-6-methoxycarbonylpyrazine 4-oxide (5b), because 5e serves as a key intermediate not only for synthesis of 1a, but also for a variety of chemical modification of OPC-15161. In this paper, our new 3-step synthesis of 5e from 5b is described.

According to the method reported by Ito,[5] 5b was obtained from amino ester (5a) by the treatment with benzyl bromide. 5a was easily prepared by the cyclization of amide (4) obtainable from methyl aminocyanoacetate (2)[7] and 2-hydroxyimino-4-methylpentanoic acid (3).[5]

Scheme *Synthetic pathway of pyrazine 4-oxide derivatives (5)*



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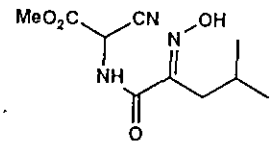

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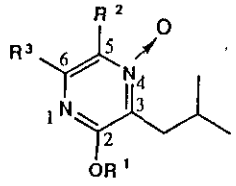

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	R^1	R^2	R^3
5a :	H	NH_2	CO_2Me
b :	Bn	NH_2	CO_2Me
c :	Bn	Cl	CO_2Me
d :	Bn	OMe	CO_2Me
e :	Bn	OMe	CH_2OH
f :	Bn	Cl	CH_2OTHP
g :	Bn	H	CO_2Me
h :	Bn	H	CH_2OH

Table 1 Effect of molar ratio of copper salts in the conversion of 5b to 5c

Entry	$CuCl_2$: CuCl	Product and Yield(%)* 5c	5g
1	5 : 0	30	26
2	3 : 2	78	27
3	2 : 3	54	20
4	0 : 5	5	2

* Yields were assayed by hplc (Wakosil 5C18, MeCN : H_2O = 4 : 6, Uv 254 nm).

Table 2 Reaction of 5d with various reducing agents

Entry	Reducing agent (mol. eq.)	Solvent	Temperature (℃)	Time (h)	Product and Yield(%)* 5e	5h	5g	5d
1	DIBAL (2.5)	CH_2Cl_2	−70	1	1	0	0	8
2	Red−Al (2.5)	Toluene	25	20	6	5	4	23
3	$LiBH_4$ (2.0)	THF	25	1	37	5	3	0
4	$NaBH_4$ (2.5)	MeOH	25	20	10	0	25	6
5	$LiAl(OBu-t)_3H$ (4.0)	THF	25	20	51	7	0	0
6	$Ca(BH_4)_2$ (2.0)	THF	25	3	14	1	0	0
7	$Zn(BH_4)_2$ (2.0)	THF	25	20	1	0	0	81

* Yields were assayed by hplc (Wakosil 5C18, MeCN : H_2O = 4 : 6, Uv 254 nm).

Diazotizaiton of the amino group of 5b followed by methanolysis of the diazonium salt to give methoxy compound (5d) and subsequent reduction of the ester moiety of 5d seemed to be a possible pathway to obtain 5e from 5b. However, treatment of 5b with $NaNO_2$ in the presence of an acid such as H_2SO_4, HCl or AcOH gave a mixture of unidentified products. 5b was recovered unreacted when treated with isoamyl nitrite in MeOH or MeCN. On the contrary, the use of CuCl/$CuCl_2$ with isoamyl nitrite in MeCN afforded the desired chloro compound (5c) together with the reductively deaminated product (5g). The molar ratio of CuCl and $CuCl_2$ in this reaction appeared to be critical (Table 1). When 5b was treated only with either CuCl or $CuCl_2$, almost equal amount of 5c and 5g was obtained in low yields (Entries 1 and 4). However, by using a mixture of the copper salts (Entries 2 and 3), 5c was obtained rather predominantly.

Introduction of a methoxy moiety at C-5 of 5c was next tried. Although it has been reported that direct nucleophilic replacement of the chloro group at C-5 with a methoxy group was unsuccessful in the case of 5f, [5] the existense of an electron-withdrawing group (CO_2Me) at C-6 of 5c was expected to increase the reactivity of the C-5 chloro group. As expected, 5c was found to react with sodium methoxide to give the desired methoxy compound (5d) in a moderate yield (65%).

Selective reduction of the methoxycarbonyl group of 5d was tried using a variety of reducing agents (Table 2). When 5d was treated with $LiAl(OBu\text{-}t)_3H$ in THF, desired 5e was obtained in a good yield. Reduction of 5d with $LiBH_4$ (Entry 3) also gave 5e but with two undesirable demethoxylated products (5g and 5h). These by-products were probably formed by the preferential attack of hydride to C-5 of the pyrazine ring because 5h was converted from 5g, but not from 5e under the reduction conditions employed. The compound (5e), thus obtained, was identical in all respects with the sample prepared according to the method reported by Y. Ito. [5]

In conclusion, 2-benzyloxy-6-hydroxymethyl-3-isobutyl-5-methoxypyrazine 4-oxide (5e) was prepared in only 3-step reaction starting from 5-amino-2-benzyloxy-3-isobutyl-6-methoxycarbonylpyrazine 4-oxide (5b). Thus, a formal synthesis of 1a was accomplished, and our facile synthesis of 5e will make it easier a variety of chemical modification of OPC-15161 (1a).

EXPERIMENTAL

All melting points were determined on a Yanagimoto hot-stage apparatus and are uncorrected. Ir spectra were measured with a Janssen Micro FTIR spectrophotometer using KBr disk. ^{1}H-Nmr spectra were recorded on a JEOL GSX-270 with tetramethylsilane as an internal standard and $CDCl_3$ was used as solvent for all substances. Chemical shifts are expressed in δ (ppm) value. Wakogel-C200 was used for silica gel column chromatography.

5-Amino-2-hydoxy-3-isobutyl-6-methoxycarbonylpyrazine 4-oxide (5a)

To a stirred mixture of 2-hydroxyimino-4-methylpentanoic acid (3) (17.9 g, 123 mmol) and *N*-hydroxy-succinimide (15.6 g, 136 mmol) in DMF (160 ml) was added DCC (28 g, 136 mmol) in DMF (40 ml) at 0 °C under nitrogen. After 20 min, methyl aminocyanoacetate (2) (16.9 g, 148mmol) and 4-dimethyl-aminopyridine (1.51 g, 12.3 mmol) were added in portions to the mixture at room temperature and then the mixture was stirred at 50 °C for 14 h. The precipitated urea was removed by filtration and the filtrate was concentrated *in vacuo*. The residue was diluted with AcOEt (300 ml) and washed with water (200 ml), 10 % HCl (150 ml), sat. aq. $NaHCO_3$ (150 ml) and sat. aq. NaCl (150 ml), respectively. The dried ($MgSO_4$) organic layer was concentrated *in vacuo* to give crude oil (23 g) of the amide (4). The oil was stirred at 70 °C for 3 h in AcOH (200 ml). The mixture was concentrated under reduced pressure to give brown solid. Trituration of the solid with MeOH afforded the title compound (5a) (9.65 g, 32 % from 3) as pale yellow needles; mp 208.5-209.5 °C; ir: 3450, 3330, 1720, 1623 cm^{-1}; ^{1}H-nmr: 0.98(d, J=7 Hz, 6H), 2.35(m, 1H), 2.95(d, J=7 Hz, 2H), 3.96(s, 3H), 6.74(br s, 2H, disappeared by D_2O), 9.25(br s, 1H, disappeaed by D_2O). *Anal.* Calcd for $C_{10}H_{15}N_3O_4$: C, 49.79; H, 6.27; N, 17.42. Found: C, 49.86; H, 6.27; N, 17.65.

5-Amino-2-benzyloxy-3-isobutyl-6-methoxycarbonylpyrazine 4-oxide (5b)

A mixture of 5a (10 g, 41.5 mmol), benzyl bromide (9.9 ml, 82.9 mmol) and $KHCO_3$ (12.45 g, 124 mmol) in DMF (200 ml) was stirred at room temperature for 16 h under nitrogen. The mixture was poured into water (2 l) and separated solid was collected by filtration. Crystallization of the solid from *n*-hexane gave 5b (10.2g, 74 %) as pale yellow needles; mp 139-140 °C; ir: 3450, 3330, 1700 cm^{-1}; ^{1}H-nmr: 0.95(d, J=7 Hz, 6H), 2.30(m, 1H), 2.92(d, J=7 Hz, 2H), 4.00(s, 3H), 5.37(s, 2H), 7.00(br s, 2H, disappeaed by D_2O), 7.34-7.48(m, 5H). *Anal.* Calcd for $C_{17}H_{21}N_3O_4$: C, 61.62; H, 6.39; N, 12.68. Found: C, 61.33;

H, 6.56; N, 12.58.

Estimation of diazotization products (5c and 5g) by hplc analysis

Isoamyl nitrite (120 μl, 1 mmol) was added to 5b (100 mg, 0.3 mmol) in MeCN (2 ml) in one portion in the presence of appropriate moles of $CuCl_2$ (1.5 – 0 mmol) and CuCl (0 – 1.5 mmol) (see Table 1) at room temperature under nitrogen. After being stirred for 1 h, the reaction was quenched with 10 % HCl (2 ml). The ratios of the products were determined by hplc (column, Wakosil 5C18; mobile phase, MeCN : H_2O = 6 : 4; flow rate, 1 ml/min; detection, uv 254 nm; retention times, 5g, 7.2 min, 5c, 12.6 min). The results are summarized in Table 1.

Diazotization of 5b with isoamyl nitrite in the presence of $CuCl_2$ and CuCl

Isoamyl nitrite (7.42 g, 63.3 mmol) was added to a mixture of 5b (7 g, 21.1 mmol), $CuCl_2$ (8.52 g, 63.4 mmol) and CuCl (4.18 g, 42.2 mmol) in MeCN (140 ml) at room temperature under nitrogen in a period of 30 min. After being stirred for 1 h, the mixture was diluted with 2 % HCl (200 ml) and extracted twice with AcOEt (200 ml). The extracts were dried over $MgSO_4$ and concentrated *in vacuo* to give brown oil, which was subjected to column chromatography on silica gel. Elution with a mixed solvent of AcOEt and *n*–hexane (1 : 5) afforded 5c (4.55 g, 61%) as colorless needles along with the reduced product (5g) (1.67 g, 25 %). 5c: mp 79.5–80.5 °C; ir: 1740 cm^{-1}; ^{1}H–nmr: 0.94(d, J= 7Hz, 6H), 2.25(m, 1H), 2.88(d, J= 7Hz, 2H), 4.03(s, 3H), 5.43(s, 2H), 7.36–7.44(m, 5H). *Anal.* Calcd for $C_{17}H_{19}N_2O_4Cl$: C, 58.21; H, 5.46; N, 7.99. Found: C, 58.28; H, 5.36; N, 8.04. 5g: mp 103.5–104.5 °C; ir: 1730 cm^{-1}; ^{1}H–nmr: 0.93(d, J=7 Hz, 6H), 2.25(m, 1H), 2.86(d, J=7 Hz, 2H), 3.99(s, 3H), 5.50(s, 2H), 7.30–7.50(m, 5H), 8.46(s, 1H). *Anal.* Calcd for $C_{17}H_{20}N_2O_4$: C, 64.54; H, 6.37; N, 8.86. Found: C, 64.44; H, 6.28; N, 8.84.

2–Benzyloxy–3–isobutyl–5–methoxy–6–methoxycarbonylpyrazine 4–oxide (5d)

A 28 % MeOH solution of NaOMe (5.5 g, 29 mmol) was added dropwise to 5c (1 g, 2.9 mmol) in MeOH (10 ml) at room temperature. The mixture was stirred for 30 min, diluted with 10 % HCl (50 ml) and extracted once with AcOEt (50 ml). The extract was dried over $MgSO_4$ and concentrated to give yellow oil, which was purified by column chromatography on silica gel. Elution with a mixture of AcOEt and *n*–hexane (1 : 3) afforded 5d (0.7 g, 71 %) as colorless prisms: mp 51–52 °C; ir: 1740 cm^{-1}; ^{1}H–nmr: 0.94(d, J=7 Hz, 6H), 2.24

(m, 1H), 2.86(d, J=7 Hz, 2H), 4.00(s, 3H), 4.11(s, 3H), 5.42(s, 2H), 7.36–7.48(m, 5H). *Anal.* Calcd for $C_{18}H_{22}N_2O_5$: C, 62.42; H, 6.40; N, 8.09. Found: C, 62.45; H, 6.44; N, 8.16.

Estimation of reduction products (5e, 5h and 5g) with various reducing agents by hplc analysis

An appropriate reducing agent was added in one portion to a 5 ml solution of 5d (100 mg, 0.29 mmol) (see Table 2 for reducing agents, solvents, reaction temperatures and times). After being stirred, 1 ml of the mixture was diluted with 10 % HCl (1 ml) and then made up in a volume of 10 ml with a mixed solvent of MeCN and water (4 : 6). The solution was analyzed by hplc (column, Wakosil 5C18; mobile phase, MeCN : H_2O = 4 : 6; flow rate, 1.5 ml; detection, uv 254 nm; retention times, 5h, 8.6 min, 5e, 9.7 min, 5g, 22.4 min, 5d, 31.2 min). And the results were summarized in Table 2.

2–Benzyloxy–6–hydroxymethyl–3–isobutyl–5–methoxypyrazine 4–oxide (5e)

To a stirred mituture of 5d (1g, 2.89 mmol) in THF (50 ml) was added $LiAl(OBu\text{-}t)_3H$ (5.87g, 23 mmol) in a period of 15 min at 0 °C. After being kept at 7 °C for 18 h, the mixture was diluted with AcOEt (100 ml), washed with 10 % HCl (50 ml) and sat. aq. NaCl (30 ml), dried over $MgSO_4$ and evaporated. The residue was subjected to column chromatography on silica gel, which was eluted with a mixed solvent of AcOEt and *n*–hexane (1:2) to give 5e (350 mg, 38 %); mp 68–69 °C(lit.,[5] 68.0–69.5 °C). Specral data were identical in all respects with a sample prepared according to the reported method. [5]

Reduction of 5d with $LiBH_4$

To a solution of 5d (350 mg, 1 mmol) in THF (10 ml) was added $LiBH_4$ (46 mg, 2.1 mmol) at 0 °C. After being stirred for 3 h at 0 °C, the mixture was diluted with 10 % HCl (10 ml) and extracted once with AcOEt (20ml). The extract was washed with water (5 ml), dried over $MgSO_4$ and concentrated to dryness. The residue was subjected to preparative tlc on silica gel plate, which was developed with a mixed solvent of AcOEt and *n*–hexane (1 : 2). Elution of each of 3 bands with AcOEt–*n*–hexane (2 : 1) followed by concentration gave 5e (60 mg, 19 %), 5g (42 mg, 13 % and 5h (12 mg, 4 %). 5h: mp 116–117 °C; ir: 3300, 1600 cm^{-1}; ^1H–nmr: 0.93(d, J=7 Hz, 6H), 2.24(m, 1H), 2.82(d, J=7 Hz, 2H), 4.62(s, 2H), 5.40(s, 2H), 7.30–7.50(m, 5H), 7.89(s, 1H). *Anal.* Calcd for $C_{16}H_{20}N_2O_3$: C, 66.65; H, 6.99; N, 9.72. Found: C, 66.

41; H, 6.84; N, 9.72.

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