the subsequent formation of ·OH by a metal-ion-catalyzed Haber–Weiss reaction. As O_2^- is an obligatory intermediate in the activation of molecular oxygen in these model system, the active oxidant for hydroxylation is probably ·OH or singlet oxygen. The hemin-cysteine system is not inhibited strongly by any of these inhibitors. Thus aniline hydroxylation in this system probably does not involve ·OH, O_2^-, $^1O_2$ or H_2O_2, but may have a different reactive intermediate.

In view of the present findings and the facts that the hemin-cysteine system retains the thiolate-heme iron linkage and requires molecular oxygen, excess cysteine and an acidic pH for aniline hydroxylation, we propose that the hydroxylation sequence can be explained as follows. Thiolate-heme complex formed from the thiolate-hemin complex by reduction with excess cysteine reacts with molecular oxygen to from an intermediate in which oxygen is bound to the heme iron directly. The electron generated by the oxidation of cysteine to cystine is used to reduce the intermediate in our model system, then the oxygen bound to the thiolate-heme complex reacts with a proton to cleave the dioxygen bond and afford the reactive intermediate containing atomic oxygen. The heme iron in the intermediate is probably in equilibrium among various multivalent states. In this form, the thiolate group can push the electron through the heme iron to oxygen.[3] This clarifies the possible significance of retaining the thiolate-heme iron linkage in connection with the hydroxylation of aniline. The intermediate should itself be an active oxygen form. The proposed reaction cycle should continue in air until all the added cysteine has been oxidized.

Experimental

Hemin (Type I, bovine), superoxide dismutase (Type I, bovine) and catalase (bovine liver, 2x crystallized) were obtained from Sigma Chemical Co. 1,3-Diphenylisobenzofuran was from Aldrich Chemical Inc. Hydroxylation of aniline with model systems was carried out as reported previously.[4] The reaction products, *p*- and *o*-aminophenol (*p*- and *o*-AP) were determined by liquid chromatography.[4] Buffer solutions used were as follows: pH 3, 1 M HCl–1 M CH_3COONa; pH 4—7, 1 M CH_3COOH–1 M CH_3COONa; pH 7—8, 1 M tris(hydroxymethyl)aminomethane–0.5 M HCl; pH 8—9, 1 M $NaCO_3$–1 M $NaHCO_3$.

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Synthesis of 5-(2-Amino-1-hydroxybutyl)-8-hydroxycarbostyril, One of the Major Metabolites of Procaterol

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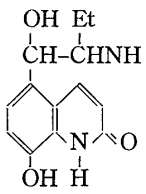
The two isomers of 5-(2-amino-1-hydroxybutyl)-8-hydroxycarbostyril (2), which is a major metabolite of procaterol, were synthesized.

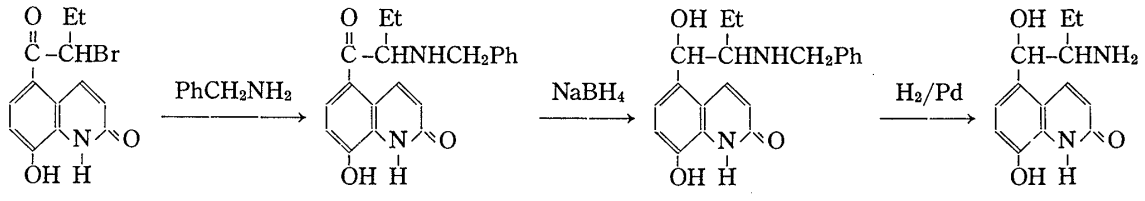
Keywords——procaterol; metabolite; 5-(2-amino-1-hydroxybutyl)-8-hydroxycarbostyril; amination; reduction

1) Location: *Kagasuno, Kawauchi-cho, Tokushima.*



1

Previously we reported on procaterol, *erythro*-5-(1-hydroxy-2-isopropylaminobutyl)-8-hydroxycarbostyril (**1**), which is a very potent and highly selective β-adrenoceptor stimulant.[2] Procaterol showed very potent bronchodilatory activity and weak side effects in double blind tests at an oral dose of 0.05—0.1 mg/body. In the course of development of procaterol, we synthesized 5-(2-amino-1-hydroxybutyl)-8-hydroxycarbostyril (**2**), which is a major metabolite of procaterol.[3] We did not clarify the stereoconfiguration of compound **2**, but we synthesized its two isomers.



a : *erythro*-form
b : *threo*-form

Chart 1

The synthesis of these two isomers is outlined in Chart 1. 5-(2-Bromo-1-oxobutyl)-8-hydroxycarbostyril (**3**) was synthesized from 8-hydroxycarbostyril as reported previously.[2a] Crude **3** was treated with excess benzylamine to give 5-(2-benzylamino-1-oxobutyl)-8-hydroxycarbostril (**4**). Compound **4** in methanolic solution was reduced with sodium borohydride to afford 5-(2-benzylamino-1-hydroxybutyl)-8-hydroxycarbosytril (**5**). The nuclear magnetic resonance (NMR) spectrum of crude **5** showed that it was a mixture of the *erythro* and *threo* isomers (*erythro*: *threo*=4: 1).[4] Recrystallization of crude **5** from methanol gave the *erythro* isomer **5a** in 56% yield, and the *threo* isomer **5b** containing a small amount of **5a** was obtained from the mother liquor. Catalytic reduction of compounds **5a** and crude **5b** gave *erythro*-5-(2-amino-1-hydroxybutyl)-8-hydroxycarbostyril (**2a**) and *threo*-5-(2-amino-1-hydroxybutyl)-8-hydroxycarbostyril (**2b**), respectively, in good yields. The stereoconfigurations of compounds **2a** and **2b** were confirmed by their NMR spectra.[4] Compound **2a** showed a doublet at 5.72 ppm (J=4.2 Hz) and compound **2b** showed a doublet at 5.37 ppm (J=7.6 Hz). These results are in good agreement with those on the isomers of procaterol.[2b] The *Rf* values of **2a** and **2b** on thin layer chromatography (TLC) in chloroform-methanol-ammonium hydroxide (20:10:1) were 0.30 and 0.26, respectively.

The metabolite **2** may be the *erythro* isomer because this material is produced in the metabolism of procaterol, which has the *erythro* configuration. The acute toxicity and pharmacological properties of compound **2a** were investigated in our laboratories.

Experimental[5]

5-(2-Benzylamino-1-oxobutyl)-8-hydroxycarbostyril (4)——Crude 5-(2-bromo-1-oxobutyl)-8-hydroxycarbostyril **3** (500 g) was added in small portions to 1750 g of benzylamine with stirring and cooling in ice-water.

2) *a*) S. Yoshizaki, K. Tanimura, S. Tamada, Y. Yabuuchi, and K. Nakagawa, *J. Med. Chem.*, **19**, 1138 (1976); *b*) S. Yoshizaki, Y. Manabe, S. Tamada, K. Nakagawa, and S. Tei, *ibid.*, **20**, 1103 (1977).
3) T. Shimizu, H. Mori, E. Tabusa, S. Morita, G. Miyamoto, Y. Yasuda, and K. Nakagawa, *Xenobiotica*, **8**, 349 (1978).
4) P.S. Portoghese, *J. Med. Chem.*, **10**, 1057 (1967).
5) Melting points (uncorrected) were determined by the capillary method. Elemental microanalyses were done in a Yanagimoto MT-2 CHN recorder. NMR spectra were recorded with a Hitachi R-20B spectrometer. TLC was carried out on E. Merck Kieselgel F_{254} with $CHCl_3$–MeOH–NH_4OH (20:10:1).

Stirring was continued for 4 hr at room temperature and then excess amine was washed out with Et_2O. The residue was washed with H_2O and dissolved in MeOH. Insoluble material was removed by filtration. The filtrate was adjusted to pH 1—2 with concentrated hydrochloric acid and the resulting solution was evaporated to dryness. The residue was extracted with H_2O (active C), concentrated and recrystallized from H_2O to give 175 g (27% from 8-hydroxycarbostyril) of compound **4** as the hydrochloride, mp 241—243° (dec.). *Anal.* Calcd for $C_{20}H_{21}ClN_2O_3$: C, 64.43; H, 5.68; N, 7.51. Found: C, 64.51; H, 5.51; N, 7.39.

5-(2-Benzylamino-1-hydroxybutyl)-8-hydroxycarbostyril (5)——A solution of 110 g (0.295 mol) of **4** in 2.3 l of MeOH was adjusted to pH 9 with aqueous 10 N NaOH, with stirring and cooling in ice-water. Sodium borohydride (40 g) was added in small portions to this solution and stirring was continued for 5 hr at room temperature. The resulting solution was cooled in ice-water with stirring, adjusted to pH 2 with concentrated hydrochloric acid and concentrated. The residue was dissolved in MeOH, insoluble material was filtered off and the filtrate was evaporated to dryness. The residue was then dissolved in MeOH and the solution was concentrated to remove boron as methyl borate. The residue was recrystallized from MeOH to give 65 g (56%) of *erythro*-5-(2-benzylamino-1-hydroxybutyl)-8-hydroxycarbostyril (**5a**) as the hydrochloride monohydrate, mp 182—184°. *Anal.* Calcd for $C_{20}H_{25}ClN_2O_4$: C, 61.14; H, 6.41; N, 7.13. Found: C, 61.36; H, 6.37; N, 7.18. NMR (Me_2SO-d_6–D_2O) δ: 5.61 (1H, d, J=3.4 Hz, >C<u>H</u>–OH).

The mother liquor after recrystallization of compound **5** was concentrated and the residue was crystallized from acetone to give 15 g of crude *threo*-5-(2-benzylamino-1-hydroxybutyl)-8-hydroxycarbostyril (**5b**). NMR (Me_2SO-d_6-D_2O) δ: 5.34 (1H, d, J=7.5 Hz, >C<u>H</u>–OH).

***erythro*-5-(2-Amino-1-hydroxybutyl)-8-hydroxycarbostyril (2a)**——Palladium black (3 g) was added to a solution of 30 g (0.076 mol) of **5a** in 500 ml of MeOH and 100 ml of H_2O, and reduction was carried out in a Paar hydrogenator for 2 days at room temperature. The catalyst was removed and the solvent was evaporated off. The residual crystalline solid was recrystallized from H_2O to give 20 g (87%) of **2a** as the hydrochloride monohydrate, mp 170—171° (dec.). *Anal.* Calcd for $C_{13}H_{19}ClN_2O_4$: C, 51.57; H, 6.33; N, 9.25. Found: C, 51.41; H, 6.66; N, 9.41. NMR (D_2O) δ: 8.29 and 6.76 (1H, d, J=10.0 Hz, C_4–H and C_3–H), 7.48 and 7.18 (1H, d, J=8.2 Hz, aromatic CH), 5.72 (1H, d, J=4.2 Hz, >C<u>H</u>–OH), 3.76 (1H, m, >C<u>H</u>–N), 1.75 (2H, m, C<u>H_2</u>CH_3) and 1.13 (3H, t, CH_3). TLC: *Rf* 0.30.

***threo*-5-(2-Amino-1-hydroxybutyl)-8-hydroxycarbostyril (2b)**——Palladium black (1 g) was added to a solution of 10 g of crude **5b** in 170 ml of MeOH and 30 ml of H_2O, and reduction was carried out in a Paar hydrogenator for 40 hr at room temperature. The catalyst was removed, the solvent was evaporated off and the residual crystalline solid was recrystallized from H_2O to give 4.5 g (62%) of **2b** as the hydrochloride, mp 220—222° (dec.). *Anal.* Calcd for $C_{13}H_{17}ClN_2O_3$: C, 54.84; H, 6.02; N, 9.84. Found: C, 54.76; H, 5.95; N, 9.58. NMR (D_2O) δ: 8.33 and 6.72 (1H, d, J=10.0 Hz, C_4–H and C_3–H), 7.38 and 7.15 (1H, d, J=8.2 Hz, aromatic CH), 5.37 (1H, d, J=7.6 Hz, >C<u>H</u>–OH), 3.70 (1H, m, >C<u>H</u>–N), 1.68 (2H, m, C<u>H_2</u>CH_3) and 1.14 (3H, t, CH_3). TLC: *Rf* 0.26.

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Synthesis of 8-Hydroxycarbostyril

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8-Hydroxycarbostyril (**4b**), which is a starting material for the synthesis of procaterol, was synthesized by two new routes.

Keywords——8-hydroxycarbostyril; 8-methoxycarbostyril; 3-ethoxyacrylanilides; 3,3-di-*n*-butoxypropionanilide; condensation

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1) Location: *a) Kagasuno, Kawauchi-cho, Tokushima; b) 133-1 Yamadakami, Suita, Osaka.*