Isomers of *erythro*-5-(1-Hydroxy-2-isopropylaminobutyl)-8-hydroxycarbostyril, a New Bronchodilator

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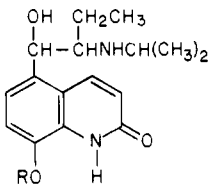
The isomers of *erythro*-5-(1-hydroxy-2-isopropylaminobutyl)-8-hydroxycarbostyril (1), a new potent and β_2-selective bronchodilator, were synthesized by optical resolution of compound 1 and inversion of the erythro to the threo isomers. The isomers were tested for activities to inhibit histamine-induced bronchospasm and to increase the heart rate of anesthetized dogs. Racemic and (−)-*erythro*-1 showed potent and β_2-selective bronchodilater activities. Among the isomers, (−)-*erythro*-1 showed the highest activities and (+)-*erythro*-1 showed the lowest.

The value of β-adrenoceptor stimulants for therapy of asthma is widely recognized and several new β_2-selective bronchodilators, such as carbuterol,[1,2] clenbuterol,[3-5] and bitolterol,[6] have been reported. Previously, we also reported a series of new sympathomimetic amines with a carbostyril nucleus that showed potent bronchorelaxing activities and high β_2-selectivities on isolated guinea pig tracheal and atrial tissue.[7] One of the compounds, *erythro*-5-(1-hydroxy-2-isopropylaminobutyl)-8-hydroxycarbostyril (1), was evaluated in our laboratories as a potent and β_2-selective bronchodilator agent (procaterol). This compound showed prolonged and significant bronchodilator activity when given orally at a dose of 0.05–0.1 mg/body in volunteer asthmatic patients.[8]

Several bronchodilators have been resolved into their optical isomers and reported with their pharmacological results.[1,9-11] We also synthesized the isomers of compound 1, which has two asymmetric centers, and examined the pharmacological activities of the isomers in anesthetized dogs.



1, R = H
2, R = $CH_2C_6H_5$

The erythro form of compound 1 was synthesized by the $NaBH_4$ reduction of the precursor amino ketone[7] and was converted into the 8-benzyloxy derivative 2 with benzyl chloride in alkaline solution. Compound 2 was treated with dibenzoyl-*l*- (or *d*-) tartaric acid[12] and the resulting salts were recrystallized from ethanol to constant rotation. The purified salts were neutralized and the resolved bases 2 were debenzylated catalytically to give (−)- and (+)-*erythro*-1. Inversion of the erythro isomers of compound 2 was achieved by N-acetylation, replacement of the OH group by Cl, and alkaline hydrolysis. The resulting threo isomers of compound 2 were also debenzylated to give the threo isomers of compound 1. The NMR spectra (D_2O) of the erythro isomers showed a doublet (J = 4.0–4.2 Hz) at 5.7 ppm and that of the threo isomers showed a doublet (J = 8.2–8.3 Hz) at 5.4 ppm.[11,13]

Results and Discussion

The pharmacological activities of the isomers of compound 1 were examined by in vivo assay in anesthetized dogs. The bronchodilator activities of the compounds and their effects on the heart were evaluated as inhibition of histamine-induced bronchospasm and increase in the heart rate, respectively. The results are shown in Table I.

rac-erythro-1 had half the bronchodilator activity of *l*-isoproterenol and 50 times less effect than the latter on the heart rate. The observed separation ratio of 24.9 indicates the high β_2-selectivity of this compound, in agreement with the previous results.[7] (−)-*erythro*-1 also showed high β_2-selectivity but other isomers possessed only weak β-adrenoceptor stimulant activities. The bronchodilator activities of the compounds decreased in the following order: (−)-*erythro*-1 > *rac-erythro*-1 > *rac-threo*-1 > (+)-*threo*-1 > (−)-*threo*-1 > (+)-*erythro*-1. The order of potencies of the erythro isomers was the same as that of the enantiomers of isoetharine on guinea pig tracheal tissue.[11] *rac-threo*-1 showed 73 times less bronchodilator activity than *rac-erythro*-1. Despite the poor relationship between the biological results of the threo isomers, the weak activity of the threo racemate probably resides in the (+)-*threo*-1. The potencies of the compounds on heart decreased in the following order: (−)-*erythro*-1 > *rac-erythro*-1 >> (−)-*threo*-1 > (+)-*threo*-1 > *rac-threo*-1 > (+)-*erythro*-1. The threo isomers and (+)-*erythro*-1 showed very low potencies. These results indicate that the bronchodilator activity of compound 1 and its potency on heart are due to its (−)-erythro isomer.

Experimental Section

Chemistry. Melting points (uncorrected) were determined by the capillary method using a thermometer with an immersion line as described in the Pharmacopoeia of Japan. Optical rotations were measured in a Union Giken PM-71 polarimeter using a methanolic solution at 20 °C and a 10-cm cell. Elemental microanalyses were done in a Yanagimoto MT-2 CHN recorder and analytical values were within ±0.4% of the calculated ones. NMR spectra were recorded with a Hitachi R-20B spectrometer.

***erythro*-8-Benzyloxy-5-(1-hydroxy-2-isopropylaminobutyl)carbostyril (2).** To a solution of 32.7 g (0.1 mol) of *rac-erythro*-1 hydrochloride in 105 mL of 2 N NaOH and 200 mL of MeOH was added 15.2 g (0.12 mol) of benzyl chloride and the mixture was refluxed for 3 h. The solvent was evaporated and the residue was extracted with $CHCl_3$. The $CHCl_3$ layer was washed with water, dried over anhydrous $MgSO_4$, and evaporated to give 35.8 g (94%) of compound 2: mp 142–143 °C (AcOEt); NMR ($CDCl_3$) δ 7.97 and 6.65 (d, 1, J = 10.2 Hz, C_4H and C_3H), 7.39 [s (br), 5, C_6H_5], 7.35 and 7.03 [d, 1, J = 8.4 Hz, CH (Ar)], 5.16 (s, 2, CH_2O), 5.15 (1, CHO, overlapped with CH_2O), 3.2–2.6 (m, 2, CHNCH), 1.15 [m (br), 2, CCH_2C], 1.13 [d, 6, J = 6.0 Hz, $C(CH_3)_2$], and 0.8 (t, 3, CH_3). Anal. ($C_{23}H_{28}N_2O_3$) C, H, N.

(−)-*erythro*-5-(1-Hydroxy-2-isopropylaminobutyl)-8-hydroxycarbostyril. A suspension of 79 g (0.208 mol) of *rac-erythro*-2 and 82 g (0.218 mol) of dibenzoyl-*l*-tartaric acid monohydrate in 500 mL of EtOH was refluxed to a clear solution, and this was left at room temperature for 4 days. The precipitate was collected and repeatedly recrystallized from EtOH to give

Table I. Pharmacological Results in Dogs

Compd	No. of dogs	Inhibn of bronchoconstriction, equipotent dose at ED_{50}[a]	Increase in heart rate, equipotent dose at ED_{25}[a]	Separation ratio[b]
rac-erythro-1	5	2.03 (1.74-3.04)	50.5 (27.9-111)	24.9
(−)-*erythro*-1	5	1.41 (1.13-1.74)	33.2 (9.47-8570)	23.5
(+)-*erythro*-1	5	18 500 (11 200-40 600)	*c*	
rac-threo-1	5	148 (49.9-280)	*d*	
(−)-*threo*-1	5	4110 (1820-13 700)	7560 (3580-17 700)	1.84
(+)-*threo*-1	5	566 (328-1050)	9550 (2780-263 000)	16.9
l-Isoproterenol	5	1.00[e] (0.84-1.16)	1.00[f] (0.63-1.42)	1.00

[a] Relative to *l*-isoproterenol = 1.00. 95% confidence limits in parentheses. [b] Increase in heart rate, ED_{25}, divided by inhibition of bronchoconstriction, ED_{50}. [c] $ED_{14.5}$ at 3000 μg/kg. [d] $ED_{19.5}$ at 300 μg/kg. [e] Mean ED_{50} value, obtained at a dose of 0.069 μg/kg. [f] Mean ED_{25} value, obtained at 0.019 μg/kg.

42 g (27 %) of (−)-*erythro*-**2** dibenzoyl-*l*-tartaric acid salt hemihydrate: mp 164–165 °C; $[\alpha]_D$ +62.0° (*c* 1, MeOH). Anal. ($C_{41}H_{43}N_2O_{11.5}$) C, H, N. This salt was neutralized with saturated $NaHCO_3$ solution and extracted with AcOEt. The extract was washed with water, dried over $MgSO_4$, and evaporated. The residue was recrystallized from Et_2O–petroleum ether to give 19.5 g (91%) of (−)-*erythro*-**2**: mp 103–104 °C; $[\alpha]_D$ −13.5° (*c* 1, MeOH). Anal. ($C_{23}H_{28}N_2O_3$) C, H, N. This was converted to the hydrochloride and debenzylated over 5% Pd/C at room temperature to give 14 g (81%) of (−)-*erythro*-1 as the hydrochloride hemihydrate: mp 195–196 °C (MeOH–Et_2O); $[\alpha]_D$ −10.4° (*c* 1, MeOH); NMR (D_2O) δ 8.18 and 6.76 (d, 1, *J* = 10.2 Hz, C_4H and C_3H), 7.46 and 7.18 [d, 1, *J* = 8.4 Hz, CH (Ar)], 5.71 (d, 1, *J* = 4.2 Hz, CHO), 4.0–3.4 [m (br), 2, CHNCH], 1.6 [m (br), 2, CH_2], 1.52 [d, 6, *J* = 6.6 Hz, $C(CH_3)_2$], and 0.76 (t, 3, CH_3). Anal. ($C_{16}H_{24}N_2O_{3.5}Cl$) C, H, N.

(+)-*erythro*-1 was synthesized in a similar manner to give the hydrochloride hemihydrate: mp 195–196 °C; $[\alpha]_D$ +10.3° (*c* 1, MeOH); NMR (D_2O) δ 5.71 (d, 1, *J* = 4.0 Hz, CHO). Anal. ($C_{16}H_{24}N_2O_{3.5}Cl$) C, H, N.

***threo*-5-(1-Hydroxy-2-isopropylaminobutyl)-8-hydroxycarbostyril.** To a solution of 11.4 g (0.03 mol) of **2** and 6.1 g (0.06 mol) of triethylamine in 120 mL of $CHCl_3$ was added dropwise 4.7 g (0.06 mol) of acetyl chloride with stirring and cooling in ice–water. After 1 h the $CHCl_3$ layer was washed with 10% Na_2CO_3 solution and water and dried over $MgSO_4$. The solvent was evaporated and the residue was crystallized from Et_2O to give 6.3 g (50%) of *erythro*-5-(*N*-acetyl-1-hydroxy-2-isopropylaminobutyl)-8-benzyloxycarbostyril, mp 171–172 °C. Anal. ($C_{25}H_{30}N_2O_4$) C, H, N. This was mixed with 20 mL of $SOCl_2$, and after 3 h the excess $SOCl_2$ was evaporated. The residue was dissolved in 240 mL of MeOH and 120 mL of 2 N NaOH. The resulting solution was stirred for 5 h at room temperature and acidified with concentrated HCl with cooling in ice–water. The solvent was evaporated, the residue was extracted with $CHCl_3$, and the $CHCl_3$ layer was washed with water. The solvent was evaporated and the crystalline residue was recrystallized from MeOH–Et_2O to give 5.0 g (79%) of *rac-threo*-**2** hydrochloride hemihydrate, mp 168 °C dec. Anal. ($C_{23}H_{30}N_2O_{3.5}Cl$) C, H, N. The catalytic debenzylation of 4.0 g of this compound over 10% Pd/C gave 2.2 g (72%) of *rac-threo*-1 hydrochloride: mp 208 °C dec (MeOH–Et_2O); NMR (D_2O) δ 8.40 and 6.78 (d, 1, *J* = 9.8 Hz, C_4H and C_3H), 7.47 and 7.22 [d, 1, *J* = 8.4 Hz, CH (Ar)], 5.40 (d, 1, *J* = 8.2 Hz, CHO), 3.9–3.4 (m, 2, CHNCH), 1.7 [m (br), 2, CH_2], 1.50 [q, 6, $C(CH_3)_2$], and 0.96 (t, 3, CH_3). Anal. ($C_{16}H_{23}N_2O_3Cl$) C, H, N.

(−)-*threo*-1 and (+)-*threo*-1 were synthesized similarly from (+)-*erythro*-**2** and (−)-*erythro*-**2**, respectively. (−)-*threo*-1 hydrochloride: mp 215–216 °C; $[\alpha]_D$ −46.5° (*c* 1, MeOH); NMR (D_2O) δ 5.42 (d, 1, *J* = 8.2 Hz, CHO). Anal. ($C_{16}H_{23}N_2O_3Cl$) C, H, N. (+)-*threo*-1 hydrochloride: mp 215–217 °C; $[\alpha]_D$ +50.0° (*c* 0.572, MeOH); NMR (D_2O) δ 5.47 (d, 1, *J* = 8.3 Hz, CHO). Anal. ($C_{16}H_{23}N_2O_3Cl$) C, H, N.

Pharmacology. Adult male mongrel dogs, weighing 10–15 kg, were anesthetized by intravenous injection of 30 mg/kg body weight of sodium pentobarbital. The anesthetized dogs were placed on their backs and a cannula was inserted into the trachea. Histamine at a dose of 10 μg/kg body weight was given as a bronchoconstrictor 1 min after injecting aqueous solutions of various concentrations of the test compounds through the femoral vein. Artificial respiration was carried out by the Konzett–Rössler method.[14] The volume of air inhaled was measured with a differential transducer (San-ei Sokki, Type 1236) to determine the bronchial resistance and the values obtained were recorded on a polygraph. The ED_{50} values of the test compounds were determined from dose–response curves and compared with that of *l*-isoproterenol. The heart rate was measured simultaneously with a heart rate meter triggered from the blood pressure through a pressure transducer (San-ei Sokki, Type 1236) attached to the cannulated femoral artery. The ED_{25} values of the test compounds (producing an increase in the heart rate of 25 beats/min) were determined from dose–response curves and compared with that of *l*-isoproterenol. To inhibit spontaneous respiration and to keep anesthetic conditions constant during the test period, sodium pentobarbital was infused continuously during the experiment at a dose of 4 mg/kg body weight per hour, using an automatic injector.

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