temperature for 3–5 h. The structures of all compounds were proved through IR and NMR spectra and elemental analyses.

Experimental Section

All melting points are uncorrected. Infrared spectra were recorded using a Perkin-Elmer 377 spectrometer. NMR spectra were determined in a Hitachi Perkin-Elmer R-24 Model spectrometer. Elemental analyses were performed by "Istituto di chimica Farmaceutica dell'Università di Padova".

2-Amino-2′-[*N*-[(diethylamino)propyl]amino]biphenyl (Method A). Into a 500-mL round-bottomed flask were placed 14.7 g (0.080 mol) of 2,2′-diaminobiphenyl, 12.5 g (0.084 mol) of 3-(diethylamino)propyl chloride, and 130 mL of methanol. The mixture was refluxed for 20 h and, after cooling, was evaporated under reduced pressure. The oily residue was treated with slightly (HCl) acidic water and extracted twice with 50-mL portions of ether. The aqueous phase was basified with 80 mL of 1 N NaOH and extracted with two 50-mL portions of ether. The collected ethereal extract was dried overnight over KOH pellets and evaporated under reduced pressure to give 24 g of yellow-brown oil. This residue was found to be a 3:1 mixture of mono- and dialkylated 2,2′-diaminobiphenyl derivatives, besides some starting 2,2′-diaminobiphenyl. Using MeOH as the eluent, 15 g (0.050 mol, 63%) of the title compound was separated by column chromatography on silica gel.

2-[*N*-[(*p*-Nitrophenyl)acetyl]amino]-2′-[*N*-[(diethylamino)ethyl]amino]biphenyl (Method B, Compound 4). In a two-necked round-bottomed flask 3.24 g (0.02 mol) of *p*-nitrophenylacetonitrile, 60 mL of anhydrous chloroform, and 1.1 mL of absolute ethanol were mixed under dried N_2 flow. The solution was saturated with dry hydrogen chloride at 0 °C and allowed to stand overnight. The solvent was removed under vacuum at a temperature between 20 and 25 °C, and the residue was treated with 150 mL of acetic acid and 5.66 g (0.02 mol) of 2-amino-2′-[*N*-[(diethylamino)ethyl]amino]biphenyl. The mixture was heated at 60 °C for 16–20 h and then most of the acetic acid was evaporated under reduced pressure. The residue was treated with a 10% solution of hydrochloric acid and extracted with ether. The aqueous phase was basified with 1 N NaOH solution and again extracted with ether. The combined ethereal extract was dried over KOH pellets, and the solvent was removed to give an oily residue which crystallized within few hours at room temperature. By recrystallization from ethanol, 4.75 g of the yellow title compound was obtained.

2-[*N*-[(*p*-Hydroxyphenyl)acetyl]amino]-2′-aminobiphenyl (Method C, Compound 18). As previously described in method B, 6.5 g (0.05 mol) of *p*-hydroxyphenylacetonitrile, 2.5 mL of absolute ethanol, and 70 mL of anhydrous chloroform were mixed and treated with dry hydrogen chloride.

After ether was removed, the residue was combined with 150 mL of acetic acid and 6.42 g (0.03 mol) of 2-amino-2′-nitrobiphenyl.[2] The mixture was heated at 90 °C for about 16 h and, after cooling to about 30 °C, the solvent was removed under reduced pressure.

The residue was treated with 1 N hydrochloric acid and extracted twice with chloroform. The combined organic extract was washed with 5% sodium bicarbonate solution and then with water. Evaporation of the solvent gave an oil, which was chromatographed on a silica gel column using 1:1 cyclohexane–ethyl acetate as eluent; 5.76 g (yield 33%) of 2-[*N*-[(*p*-hydroxyphenyl)-acetyl]amino]-2′-nitrobiphenyl was obtained.

Using 0.5 g of 5% Pd/C as catalyst in 70 mL of a 4:1 THF–EtOH solvent mixture, 2.06 g of the latter nitro compound was reduced at room temperature with H_2 at 3 atm of pressure. Removing the solvent under reduced pressure and drying the residue at 60 °C in a vacuum oven gave 1.35 g of the title compound.

Pharmacology. Materials and Methods. CD-COBS albino rats and CD1 mice of both sexes purchased from Charles River (Italy) were used. Compounds were suspended in 1% acacia gum and orally administered in a volume of 10 mL/kg in the rat and 20 mL/kg in the mouse. The Dunnet's test was used for statistical evaluations. The LD_{50} was calculated with the method of probits.

The carrageenan foot edema test was performed following the method described by Winter et al.[3] (N = 5 rats per group).

Carrageenan-induced pleurisy was performed following the method described by Di Rosa et al.;[4] drugs were administered 48, 24, and 1 h before and the activity was assessed 6 h after the intrapleural injection of the irritant (N = 8 rats per group).

Adjuvant-induced polyarthritis was produced, in female rats (140–160 g), by intradermal injection into the tail of 0.1 mL of a fine suspension of *Mycobacterium butiricum* in mineral oil (6 mL/mL).[5] After 14 days, animals were chosen on the basis of their arthritic score and treated daily (oral route) for 14 days with the test compound. Activity was assessed by scoring the animals at the end of the treatment (N = 4 rats per group).

The analgesic activity was studied in female mice injected intraperitoneally with 0.2 mL of 0.2% phenylquinone;[6] drugs were administered 1 h prior to the injection of the irritant, and the animals, individually caged, were observed for abdominal contractions for a period of 20 min after the injection (N = 4 mice per group).

The LD_{50} was determined in female mice and calculated on the basis of the 7-day mortality (N = 4 mice per group).

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erythro-5-[1-Hydroxy-2-(isopropylamino)butyl]-7-hydroxycarbostyril, a Terbutaline-Type Derivative of the Bronchodilator Procaterol

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erythro-5-[1-Hydroxy-2-(isopropylamino)butyl]-7-hydroxycarbostyril (4), which is a terbutaline-type derivative of the bronchodilator procaterol, was synthesized by transfer of the 8-hydroxyl group of procaterol to the 7 position. Compound 4 showed less potent β-adrenoceptor stimulant activities than procaterol or terbutaline in an in vitro test using guinea pig tracheal muscle and right atrium. In an in vivo assay on anesthetized dogs, compound 4 showed 42 times less bronchodilator activity and 87 times less effect on the heart rate than *l*-isoproterenol.

The new β-adrenoceptor stimulant procaterol, *erythro*-5-[1-hydroxy-2-(isopropylamino)butyl]-8-hydroxycarbostyril (1),[1,2] was shown to have very potent bronchodilator activity and weak side effects in double blind tests on

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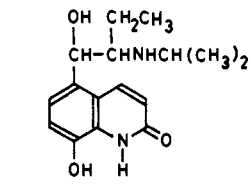

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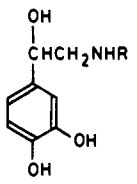

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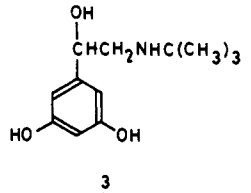

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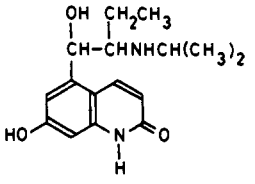

4

asthmatic patients at an oral dose of 0.05–0.1 mg twice a day. This indicates that the bronchodilator activity is not affected by replacing the *m*-hydroxyl group of catecholamines (2) by the amido group of the carbostyril nucleus, as well as a methanesulfonamido group,[3,4] a hydroxymethyl group,[5,6] or a ureido group.[7] So, it seemed interesting to see whether the amido group of the carbostyril nucleus could be substituted for one of the *m*-OH groups in terbutaline (3)[8] without affecting the β-adrenoceptor stimulant activities of the latter. The present report describes the synthesis and pharmacological activities of *erythro*-5-[1-hydroxy-2-(isopropylamino)butyl]-7-hydroxycarbostyril (4).

Chemistry. The synthesis of compound 4 was achieved by transfer of the 8-hydroxyl group of procaterol to the 7 position. Nitration of procaterol (1) with nitric acid in glacial acetic acid gave the 7-nitroprocaterol (5), which was catalytically reduced to the 7-aminoprocaterol (6). The phenolic hydroxyl group of 6 was removed by the method of Clauss and Jensen.[9] Thus, treatment of 6 with mesyl chloride, followed by catalytic reduction of the resulting 8-(mesyloxy) derivative (7) in alkaline medium, gave the 7-aminocarbostyril derivative (8). The NMR spectrum (Me_2SO-d_6) of 8 showed two doublets (J = 2.2 Hz) at 6.62 and 6.24 ppm due to meta coupling between the aromatic hydrogen atoms at the 6 and 8 positions. Conversion of the amino group of 8 to a hydroxyl group by diazotization, followed by hydrolysis, gave compound 4. Retention of the erythro configuration of starting material 1 during the above procedures was confirmed from the NMR spectrum (Me_2SO-d_6–D_2O) of 4, which showed a doublet (J = 4 Hz) at 5.69 ppm.[10] Catalytic reduction of 4 afforded the 3,4-dihydrocarbostyril derivative (9).[11]

Results and Discussion

Schwender et al.[12] reported that replacement of one of the *m*-OH groups of terbutaline by a hydroxymethyl group brought about a reduction in agonist potency at β-adrenoceptors but enhanced selectivity for the β_2 type over the β_1 type. Compound 4 has the structure in which an α-ethyl group is inserted in the side chain of terbutaline and one of the *m*-OH groups is replaced by the amido group of the carbostyril nucleus. This yielded a compound that showed weaker bronchorelaxing activity than procaterol or terbutaline in the guinea pig tracheal test and very weak effects in the atrial test, as shown in Table I. Compound 9, a 3,4-dihydrocarbostyril derivative of 4, also had weak bronchorelaxing activity and slight effects on the heart. In an in vivo assay on anesthetized dogs, compound 4 showed 42 times less bronchodilator activity and 87 times less effect on the heart rate than *l*-isoproterenol. These results indicate that compound 4 is a β-adrenoceptor stimulant with rather weak, but β-selective, pharmacological activities.

Experimental Section

Chemistry. All melting points are given as uncorrected values. Elemental microanalyses were done in a Yanagimoto MT-2 CHN recorder. Analytical values were within ±0.4% of the calculated ones. NMR spectra were recorded with a Hitachi R-20A spectrometer.

5-[1-Hydroxy-2-(isopropylamino)butyl]-8-hydroxy-7-nitrocarbostyril (5). To a solution of 20 g (64.9 mmol) of 5-[1-hydroxy-2-(isopropylamino)butyl]-8-hydroxycarbostyril monohydrate (1) in 160 mL of glacial acetic acid was added dropwise a solution of 10 mL (134 mmol) of nitric acid (d 1.38) in 10 mL of glacial acetic acid with stirring and cooling in ice–water. After 5 min, 500 mL of Et_2O was added. The nitrate that precipitated was dissolved in aqueous NaOH solution, and the resulting solution was acidified with concentrated hydrochloric acid. The precipitate was recrystallized from MeOH–acetone–Et_2O to give 16.8 g (66%) of 5 as the hydrochloride monohydrate, mp 169–171 °C dec. Anal. ($C_{16}H_{24}N_3O_6Cl$) C, H, N.

7-Amino-5-[1-hydroxy-2-(isopropylamino)butyl]-8-hydroxycarbostyril (6). A suspension of 2.9 g (7.44 mmol) of 5 and 0.1 g of Palladium black in 30 mL of water was reduced at room temperature for 3 h in a Paar hydrogenator. Hot water (200 mL) was added to the reduction mixture to dissolve the precipitate. The aqueous solution was decolorized with active carbon, filtered, and evaporated. The residue was recrystallized from water–MeOH to give 1.2 g (49%) of 6 as the hydrochloride, mp 257–258 °C dec. Anal. ($C_{16}H_{24}N_3O_3Cl$) C, H, N.

7-Amino-5-[1-hydroxy-2-(isopropylamino)butyl]-8-(mesyloxy)carbostyril (7). To a solution of 3.60 g (10.5 mmol) of 6 and 1.4 g of KOH in 80 mL of water was added 1.20 g (10.5 mmol) of mesyl chloride in small portions with stirring and cooling in ice–water. After 1 h, the precipitate was collected, washed with water and MeOH, and recrystallized from MeOH after filtering off unsoluble material to give 2.07 g (51%) of 7, mp 231–232 °C dec. Anal. ($C_{17}H_{25}N_3O_5S$) C, H, N.

7-Amino-5-[1-hydroxy-2-(isopropylamino)butyl]carbostyril (8). A suspension of 1.0 g (2.6 mmol) of 7 and 0.5 g of KOH in 50 mL of water was reduced in the presence of 0.1 g of Palladium black at room temperature for 20 h in a Paar hydrogenator. Concentrated hydrochloric acid was added to the reduction mixture and the catalyst was removed. The aqueous solution was made alkaline with aqueous KOH solution under cooling with ice–water. The precipitate was collected, washed with water, and recrystallized from MeOH–water to give 0.75 g (99%) of 8 as the monohydrate: mp 125–127 °C; NMR (Me_2SO-d_6) δ 6.62 and 6.24 (1 H, d, J = 2.2 Hz, aromatic CH). Anal. ($C_{16}H_{25}N_3O_3$) C, H, N.

5-[1-Hydroxy-2-(isopropylamino)butyl]-7-hydroxycarbostyril (4). To a solution of 2.61 g (8.5 mmol) of 8 in 1.60 mL of

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Table I. Pharmacological Results

compd	guinea pig[a] tracheal test ED_{50}[b] (SE)	intrinsic act.[c]	atrial test ED_{25}[d] (SE)	intrinsic act.[c]	anesthetized dogs[a] inhibn of bronchoconstriction, equipotent dose at ED_{50}[e]	increase in heart rate, equipotent dose at ED_{25}[e]
l-isoproterenol					1.00[g] (0.65–1.52)	1.00[h] (0.29–1.95)
procaterol	$1.10 (\pm 0.22) \times 10^{-8}$	0.98	$5.70 (\pm 1.63) \times 10^{-9}$	0.60		
terbutaline	$1.04 (\pm 0.19) \times 10^{-6}$	0.70	$2.64 (\pm 1.13) \times 10^{-6}$	0.73		
4	$2.25 (\pm 0.26) \times 10^{-7}$	0.50	*f*	0.09	41.7 (23.5–83.0)	87.1 (13.8–283.8)
9	$1.22 (\pm 0.28) \times 10^{-6}$	0.60	$5.66 (\pm 2.10) \times 10^{-7}$	0.27		

[a] $n = 5$. [b] Molar concentration for 50% of the maximum response to each compound. [c] *l*-Isoproterenol = 1. [d] Molar concentration for 25% of the maximum response to each compound. [e] Relative to *l*-isoproterenol = 1.00; 95% confidence limits in parentheses. [f] Maximal activity was obtained at 1×10^{-5} mol/L. [g] Mean ED_{50} value, obtained at a dose of 0.054 μg/kg. [h] Mean ED_{25} value, obtained at 0.021 μg/kg.

concentrated sulfuric acid and 10 mL of water was added dropwise a solution of 0.645 g (9.35 mmol) of sodium nitrite in 2 mL of water at below 5 °C. Stirring was continued for a further 10 min and 15 g of ice–water was added to the solution. Then the solution was heated at 80–90 °C for 2 h. The reaction mixture was cooled, and the precipitate was collected and washed with water. The crystalline sulfate obtained was dissolved in aqueous KOH solution with stirring and cooling in ice–water, and the resulting solution was adjusted to pH 8.5 with concentrated hydrochloric acid. The precipitate was collected, washed with water, and suspended in MeOH. The suspension was acidified to pH 1 with concentrated hydrochloric acid and evaporated. The residue was washed with cold MeOH and recrystallized from water to give 1.34 g (48%) of 4 as the hydrochloride 0.25-hydrate: mp 279–281 °C dec. NMR (Me_2SO-d_6–D_2O) δ 7.04 and 6.80 (1 H, d, J = 2.2 Hz, aromatic CH) and 5.69 [1 H, br d, J = 4 Hz, >C*H*OH]. Anal. ($C_{16}H_{23.5}N_2O_{3.25}Cl$) C, H, N.

3,4-Dihydro-5-[1-hydroxy-2-(isopropylamino)butyl]-7-hydroxycarbostyril (9). A solution of 0.48 g (1.45 mmol) of 4 in 50 mL of water was reduced in the presence of 0.1 g of Palladium black in a Paar hydrogenator at 70 °C under a hydrogen atmosphere of 6 kg/cm² for 12 h. The catalyst was removed and the aqueous solution was evaporated. The residue was recrystallized from water to give 0.23 g (46%) of 9 as the hydrochloride monohydrate, mp 177–180 °C. Anal. ($C_{16}H_{27}N_2O_4Cl$) C, H, N.

Pharmacology. Methods. A. Guinea Pig Tracheal Test. This test was performed as described previously,[1] without the expression of ED_{50} values. ED_{50} values were determined from dose–response curves as the values for 50% of the maximum response to each compound. The concentration–response curve for compound 4 on tracheal strips was shifted in a parallel fashion to the right in the presence of propranolol as follows (molar concentration of propranolol, ED_{50} value and maximum response to 4 with *l*-isoproterenol): 1×10^{-8}, 2.3×10^{-6}, 0.58; 3×10^{-8}, 4.8×10^{-6}, 0.42; 1×10^{-7}, 1.1×10^{-5}, 0.27.

B. Guinea Pig Atrial Test. This test was carried out as described previously,[1] using the cumulative method of drug administration described by Van Rossum.[13] The effects of the test compounds were expressed as percentages of the maximum response to *l*-isoproterenol. ED_{25} values were obtained from dose–response curves as the value for 25% of the maximum response to each compound.

C. In Vivo Assay Using Anesthetized Dogs. This test was performed as described previously.[2]

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Toxicity Quantitative Structure–Activity Relationships of Colchicines

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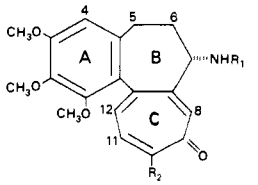
A method for extracting LD_{50} values from antitumor test data is described. A quantitative structure–activity relationship (QSAR) for 7- and 10-substituted colchicines is presented. This correlation equation closely parallels that which had been derived earlier for potency. This result indicates that attempts to modify 7- and 10-substituted colchicines in order to decrease toxicity will likely produce a simultaneous decrease in potency. Ring A modified colchicines do not obey the potency and toxicity correlations. 4-Substituted colchicines appear promising in terms of decreased toxicity, greater ILS, and a broader therapeutic range.

Colchicine (1), a potent mitotic inhibitor, has long been known to have antitumor properties.[1] As an agent against human tumors, however, its performance has been disappointing.[2] Major factors which preclude the use of colchicine as a clinical agent are severe toxicity and an extremely narrow therapeutic range. While P388-tumored mice treated with colchicine at the optimal dose (0.50



1 (colchicine), $R_1 = COCH_3$; $R_2 = CH_3O$

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