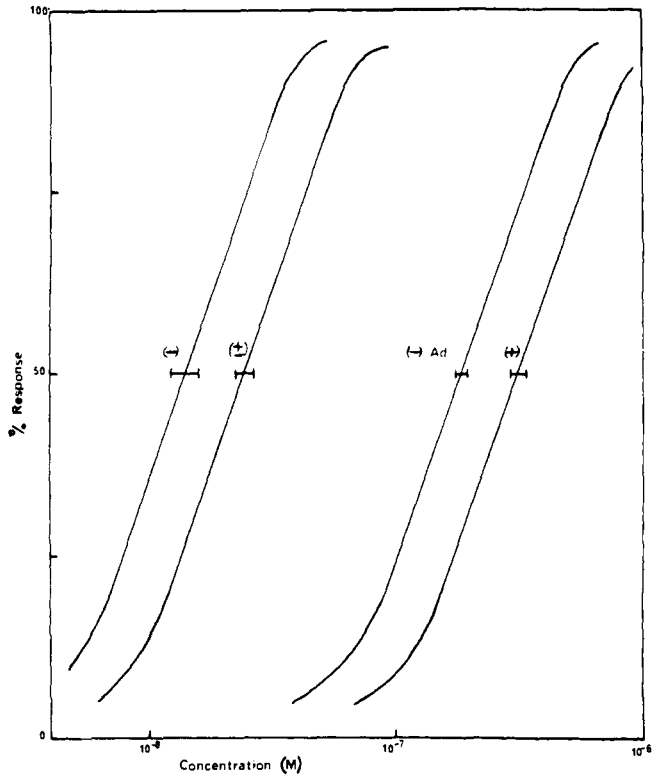

Figure 1. The mean log dose–response lines for (−), (±), and (+)-salbutamol and (−)-adrenaline. Doses are given as molar concentration in the tissue bath. The error bars represent the standard error of the mean log ED_{50}'s.

value due to contributions from the more active (−) isomer. These results, in contrast to those of Hartley and Middlemiss,[1] show that the (−) isomer is significantly more active than the racemic in agreement with the general finding that a racemic drug's activity lies between those of the two enantiomers.[3,4] From a comparison of the response curves for a set of tissues, it was found that (−)- and (±)-salbutamol are 12.6 and 5.6 times, respectively, more active than (−)-adrenaline.

The technique used in the present investigation, which is a standard method for studying the relaxation of smooth muscle,[5] differs from that used by Hartley and Middlemiss.[1] Their method, which was developed in their own laboratory,[6] is an intraluminal pressure technique. The two techniques and preparations might be expected to give small differences in the absolute values of the ED_{50}'s but the different techniques should not yield such large variations in the relative potencies of the isomers as observed.

Experimental Section

Melting points were observed on a Büchi oil-bath melting point apparatus and microchemical analyses were performed by the Australian Microanalytical Service, Melbourne, Australia. Optical rotations were measured with a Perkin-Elmer Model 141 automatic polarimeter in H_2O at 20°. The compounds gave satisfactory uv and ir spectral data obtained with a Cary 14 and a Perkin-Elmer 225 instrument, respectively.

Resolution of 2-*tert*-Butylamino-1-(4'-hydroxy-3'-hydroxymethylphenyl)ethanol (Salbutamol). To a warm solution of racemic salbutamol[§] (0.8 g, 0.0034 mol) in dilute H_2SO_4 (4 ml) was added $(+)_{546}$-$Ba[CoEDTA]_2 \cdot 4H_2O$ (0.76 g, 0.0008 mol), $[\alpha]^{20}_{546}$ +890° (*c* 0.05, H_2O),[#] which was prepared from the resolved potassium salt.[7] The precipitated $BaSO_4$ was filtered off and the diastereoisomer (1.2 g) obtained by the addition of EtOH and Et_2O to the solution while cooling in ice.

(−)-2-*tert*-Butylamino-1-(4'-hydroxy-3'-hydroxymethylphenyl)ethanol Hydrochloride Monohydrate. To the diastereoisomer (1.0 g) in H_2O (4 ml) was added $BaCl_2$ (0.22 g). The $(+)_{546}$-$Ba[CoEDTA]_2 \cdot 4H_2O$ was recovered by the addition of EtOH and Et_2O while cooling in ice. (−)-Salbutamol was precipitated as the HCl salt from the oil formed on evaporation of the filtrate at reduced pressure. The recrystallized product yielded 0.24 g, $[\alpha]^{20}D$ −32.2° (*c* 0.10, H_2O). The compound changed crystalline form at 175° and decomposed over the range 185–195°. *Anal.* ($C_{13}H_{24}NO_4Cl$) C, H, N.

(+)-2-*tert*-Butylamino-1-(4'-hydroxy-3'-hydroxymethylphenyl)ethanol Hydrochloride Monohydrate. A HCl salt of (+)-salbutamol was prepared from the oil obtained on reducing the volume of the filtrate remaining after diastereoisomer removal. The recrystallized product yielded 0.15 g, $[\alpha]^{20}D$ +30.8° (*c* 0.10, H_2O). The compound changed crystalline form at 175° and decomposed over the range 185–195°. *Anal.* ($C_{13}H_{24}NO_4Cl$) C, H, N.

Relaxation Studies. The drugs were tested by a cumulative dose method on guinea pig tracheal chain at a tension of 300 mg in Krebs physiological salt solution. Linear regression lines were obtained by a least-squares method. Mean log ED_{50} and the standard error of the mean were found for each drug and tested at the 10% significance level for differences between the drugs using a student's t test. The mean log dose–response curves were obtained from approximately 20 tissue experiments for each drug. The tissue responses were recorded on a Hewlett-Packard 680M recorder using a Sanborn FTA-1-1 microforce transducer with a Sanborn Model 311A amplifier.

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[§] Kindly supplied by Allen and Hanburys Ltd., England.

[#] EDTA is ethylenediaminetetraacetate.

A Synthesis of Noformycin

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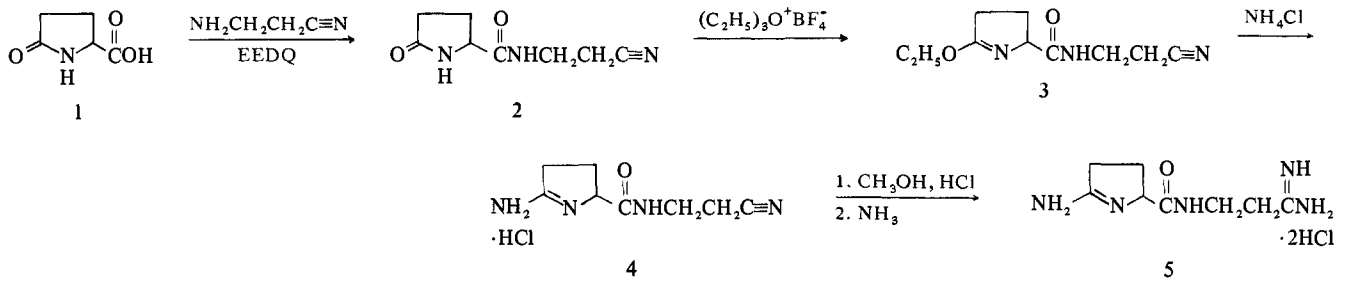
Noformycin (**5**) was isolated from a culture of *Nocardia formica* and was identified as the active constituent of this microorganism.[1-3] This material was unusual in that it exhibited a wide range of antimicrobial activity. Of particular interest was its *in vivo* activity in mice against swine influenza and SK poliomyelitis. Subsequent to its isolation and identification, noformycin was tested against a wide variety of plant and animal viruses[4-9] and found to possess very potent activity. However, this material appeared to possess considerable toxicity, which was confirmed in our laboratories.

In view of the broad spectrum of activity, we became interested in synthesizing homologs of noformycin with the expectation of reducing toxicity while retaining activity. Specifically, we were interested in developing a versatile synthesis which would adapt itself to a variety of transformations. A detailed synthesis of noformycin itself has not been published although it has been reported that the synthetic racemic material possesses half the activity of the isomer obtained from the culture.[2] Consequently, we wish to report a facile synthesis of both racemic and (+)-norformycin which



is applicable to homologation. (±)-Pyroglutamic acid, obtained by fusing (−)-glutamic acid,[10] was treated with β-aminopropionitrile in the presence of *N*-ethoxycarbonyl-2-ethoxy-1,2-dihydroquinoline (EEDQ) which gave the amide **2**. **2** was converted with triethyloxonium fluoroborate to the imino ester **3** which on treatment with ammonium chloride in methanol produced the nitrile **4**. Conversion of **4** to (±)-noformycin (**5**) was achieved *via* the Pinner synthesis without isolation of the intermediate imino ester. (+)-Noformycin was prepared in the same manner from (−)-pyroglutamic acid. Its melting point was 263–264° with an optical rotation in methanol of +8.8.

Biology. Antiviral effects of the compounds were evaluated in tissue culture systems of a serially propagated human amnion cell line (CATR, human rhinovirus type 2 and parainfluenza type 3; and monkey kidney cells, in the case of equine rhinovirus) grown in stationary tubes and infected with 100 $TCID_{50}$'s of the appropriate viruses per tube. The virus was added to 3-day-old cultures and permitted to adsorb for 1 hr at 35°. After virus adsorption, appropriate concentrations of the compounds were added to the tubes in maintenance medium of M-199 + 5% inactivated fetal calf serum. The cultures were then incubated at 32° for 5 days, and the presence or absence of viral CPE (cytopathic effect) was noted at the end of 5 days.

Toxicity of each compound to the cell systems was observed simultaneously in parallel tests. The cells were treated with identical concentrations of the compound in the absence of virus. Only those concentrations which produced no visible toxic effect upon microscopic examination at the end of 5 days were considered for evaluation.

The lowest concentration of the compounds which completely inhibited viral CPE was designated as MIC (minimum inhibitory concentration). A summary of the comparative *in vitro* antiviral activity of the (+)-and (±)-noformycin is shown in Table I. The three viruses used were equine rhino, human rhino type 2, and parainfluenza type 3. There appears to be no significant difference in activity between the (+) and (±) forms of noformycin.

Table I

	MIC, γ/ml		
	Equine rhinovirus	Human rhinovirus type 2	Parainfluenza type 3
(+)-Noformycin	1.5	3	12
(±)-Noformycin	1.5	6	6

Experimental Section†

(±)-*N*-Cyanoethyl-5-oxopyrrolidine-2-carboxamide (**2**). To a solution of 50 g (0.388 mol) of (−)-pyroglutamic acid in 600 ml of absolute EtOH was added 27.2 g (0.399 mol) of β-aminopropionitrile. To the mixture was added 99 g (0.4 mol) of EEDQ in 200 ml of absolute EtOH. On refluxing, a solution was obtained at which point heating was terminated and the solution allowed to cool to room temperature. After stirring for an additional 6 hr, the mixture which had formed was cooled in an ice bath and the solid collected and recrystallized from *i*-PrOH: 50 g (71%); mp 147–148°. *Anal.* ($C_8H_{11}N_3O_2$) C, H, N.

(±)-*N*-(2-Cyanoethyl)-2-ethoxy-1-pyrroline-5-carboxamide (**3**). To a solution of triethyloxonium fluoroborate, prepared from 10.89 g (0.0767 mol) of BF_3 etherate and 5.9 g (0.0633 mol) of epichlorohydrin, in 250 ml of CH_2Cl_2 was added 10 g (0.0533 mol) of **2**. The mixture was stirred at room temperature for 18 hr. During this period, the solid dissolved and an oil began to separate. To the suspension was added dropwise 10 g of 50% aqueous K_2CO_3. After the addition was complete, the solid was removed by filtration, the filtrate dried, and the solid obtained after removal of the solvent was recrystallized from EtOAc: 7.3 g (62%); mp 86–87°; mass spectrum *m/e* 209, abundant fragments at 112 (), 84 (), 41 (C_2H_5O–). *Anal.* ($C_{10}H_{15}N_3O_2$) C, H, N.

(±)-2-Amino-*N*-(2-cyanoethyl)-1-pyrroline-5-carboxamide Hydrochloride (**4**). To a solution of 5.83 g (0.0297 mol) of **3** in 80 ml of MeOH was added 1.493 g (0.0279 mol) of NH_4Cl and the mixture stirred overnight at room temperature. By this time, complete solution was achieved. The solution was then refluxed for 3 hr and the solvent removed *in vacuo*. The residual white solid was recrystallized from *i*-PrOH: 3.6 g (60%); mp 138.5–139.5°. *Anal.* ($C_8H_{12}N_4O \cdot HCl$) C, H, N, Cl.

(±)-2-Amino-*N*-(2-amidinoethyl)-1-pyrroline-5-carboxamide Dihydrochloride (**5**). A solution of 5.6 g (0.0267 mol) of **4** in 20 ml of MeOH saturated with HCl was kept at 5° for 17 hr. The solution was then poured into 200 ml of Et_2O, the supernatant liquid decanted from the gum, and the gum washed five times with Et_2O, dissolved in 50 ml of dry MeOH, and 150 ml of MeOH containing 10.6 g of NH_3 was added. The solution was kept at room temperature for 2 days. The solvent was removed and the residual white solid was recrystallized from MeOH: 3.5 g (60.5%); mp 252–254°. *Anal.* ($C_8H_{15}N_5O \cdot 2HCl$) C, H, N.

(−)-*N*-Cyanoethyl-5-oxopyrrolidine-2-carboxamide was prepared in the same manner as the racemic mixture: mp 114–115°; $[\alpha]^{25}D$ −13.7° (1% in MeOH). *Anal.* ($C_8H_{11}N_3O_2$) C, H, N.

(−)-*N*-Cyanoethyl-2-ethoxy-1-pyrroline-5-carboxamide: mp 105.5–106°; $[\alpha]^{25}D$ −41.1° (1% in MeOH). *Anal.* ($C_{10}H_{15}N_3O_2$) C, H, N.

(+)-2-Amino-*N*-cyanoethyl-1-pyrroline-5-carboxamide hydrochloride: mp 162–164°; $[\alpha]^{25}D$ +14.3° (1% in MeOH). *Anal.* ($C_8H_{12}N_4O \cdot HCl$) C, H, N, Cl.

(+)-2-Amino-*N*-(2-amidinoethyl)-1-pyrroline-5-carboxamide dihydrochloride (noformycin) was prepared in the same manner as the racemic mixture. The material was recrystallized from MeOH in 64% yield: mp 263–264°; $[\alpha]^{25}D$ +8.8° (1% in MeOH). *Anal.* $C_8H_{15}N_5O \cdot 2HCl$) C, H, N, Cl.

†All melting points were run according to the USP procedure and are uncorrected. Nmr and mass spectra were determined by R. K. Kullnig and S. Clemans. Analyses were performed by Gabraith and Swartzkopf Laboratories.

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Preparation and Cardiovascular Actions of a Group of Tetrahydroisoquinoline Derivatives†

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We have prepared a series of *N*-(alkylthioalkyl)tetrahydroisoquinolines and their sulfinyl and sulfonyl derivatives (Table I, compounds **1–13**) and have found particularly the ethyl-X-propyl analogs, where X = thio (**8**), sulfinyl (**9**), and sulfonyl (**10**), to produce distinctive and interesting hemodynamic effects apparently mediated by adrenergic mechanisms.[1,2,‡]

Experimental Section

Melting points were determined with a capillary melting point apparatus and are corrected for stem exposure. Microanalyses were performed by the Galbraith Laboratories, Knoxville, Tenn., and, unless indicated otherwise, are within ±0.4% of calculated values. Infrared spectra were determined with a Beckman Model IR-5 or IR-10 spectrophotometer and nmr spectra with a Varian Model A-60. Spectra were consistent with the assigned structures.

Most of the bases from which the salts listed in Table I were derived were characterized but, to save space and since the chemistry is unexceptional, the properties of only those obtained in the following illustrative procedures are indicated.

2-(Methylthiomethyl)-1,2,3,4-tetrahydroisoquinoline (1). Chloromethyl methyl sulfide (38.6 g, 0.4 mol) was added, dropwise with stirring, to a solution warmed to 35° of tetrahydroisoquinoline (106 g, 0.8 mol) in C_6H_6 (250 ml). A white precipitate formed during the addition. The reaction mixture was stirred at room temperature for 6 hr, tetrahydroisoquinoline hydrochloride (57.5 g, 85%) was separated, and the filtrate extracted with dilute HCl. The acid solution was made alkaline with NaOH and the precipitated oil dissolved in Et_2O. Drying and removal of the Et_2O left a yellow oil which was twice distilled to yield 29.8 g (39%) of 1: bp 120–122° (1.0 mm); n^{24}D 1.5819. *Anal.* ($C_{11}H_{15}NS$) S. The **hydrochloride salt** was recrystallized from EtOH–Et_2O. The **methanesulfonate salt**, recrystallized from EtOH–Et_2O, showed mp 116–118°. *Anal.* ($C_{12}H_{19}NO_3S_2$) S. Attempts to oxidize 1 to the sulfoxide were unsuccessful. Although salts of 1 were reasonably stable, they did decompose with release of MeSH on prolonged standing in aqueous solution.

2-(3-Chloropropyl)-1,2,3,4-tetrahydroisoquinoline (14). Tetrahydroisoquinoline (215 g, 1.6 mol) and trimethylene chlorobromide (126 g, 0.8 mol) in C_6H_6 (1300 ml) was stirred at room temperature for 100 hr. Tetrahydroisoquinoline hydrobromide (130 g, 75%) was filtered off. Work-up of the filtrate afforded 89 g (53%) of 14: bp 131–137° (3 mm); n^{24}D 1.5463. *Anal.* ($C_{12}H_{16}ClN$) N. The **hydrochloride salt**, recrystallized from *i*-PrOH–Et_2O, showed mp 187–187.5°. *Anal.* ($C_{12}H_{17}Cl_2N$) C, H, Cl.

2-(3-Ethylthiopropyl)-1,2,3,4-tetrahydroisoquinoline (8). To the solution obtained by dissolving Na metal (18.7 g, 0.81 g-atom) and ethanethiol (29.8 g, 0.48 mol) in EtOH (300 ml) was added, dropwise with stirring at room temperature, a solution of 14 hydrochloride (79.3 g, 0.32 mol) in MeOH (150 ml). The reaction mixture was stirred for 0.5 hr at room temperature followed by 1 hr at reflux. Work-up provided 61.5 g (81%) of 8: bp 150–156° (0.4 mm); n^{25}D 1.5521. *Anal.* ($C_{14}H_{21}NS$) N. The **hydrochloride** salt was recrystallized from *i*-PrOH.

2-(3-Ethylsulfinylpropyl)-1,2,3,4-tetrahydroisoquinoline (9). A solution of 19 g of commercial (FMC) 40% peracetic acid (0.1 mol) in MeCN (25 ml) was added, dropwise with stirring, to an ice-cold solution of 23.5 g (0.1 mol) of 8 and glacial AcOH (6 ml) in MeCN (50 ml). The reaction mixture was stirred for 1 hr at room temperature (higher yields of cleaner material were realized when the entire reaction was carried out at 5–10°), poured into H_2O (150 ml), and made basic with dilute aqueous NH_3 and the oil precipitate was dissolved in Et_2O. The Et_2O solution was washed, dried, and treated with ethereal HCl, and the resultant precipitate was recrystallized from *i*-PrOH to yield 13.4 g (47%) of 9 hydrochloride: mp 198–199°; ν_{max} (KBr) 1055 and 1018 cm^{-1} (sulfoxide).

2-(3-Ethylsulfonylpropyl)-1,2,3,4-tetrahydroisoquinoline (10). To a solution of 23.5 g (0.1 mol) of 8 in glacial AcOH (100 ml) was added, dropwise with stirring and maintenance of the temperature at about 30° by external cooling, 27.2 g of 50% H_2O_2 (0.4 mol). The solution was allowed to stand for 48 hr at room temperature, poured into H_2O (400 ml), made alkaline, and extracted with Et_2O. The Et_2O layer was washed, dried, and treated with ethereal HCl. Recrystallization of the precipitate from a mixture of *i*-PrOH and EtOH afforded 13.6 g (45%) of 10 hydrochloride: mp 226–228°; ν_{max} (KBr) 1303 and 1132 cm^{-1} (sulfone).

Discussion of Results

The compounds were screened pharmacologically in trained, unanesthetized, normotensive dogs using a tail cuff attachment to monitor blood pressure (see Table I for details). Tabulated results are relative effects on systolic blood pressure, from a significant decrease (more than 10% lasting for at least 1 hr) to a marked increase (at least a 30–40% increase with blood pressure remaining above normal for more than 3 hr). Compounds of interest from this screen, 2-(3-ethylsulfinylpropyl)-1,2,3,4-tetrahydroisoquinoline hydrochloride (**9**) in particular, were subjected to detailed pharmacological work-up.

The pharmacological profile of **9** is qualitatively illustrative of the compounds which significantly elevate systolic blood pressure. Its acute LD_{50} (iv, mice) is 77 mg/kg. **9**, at an iv dose of 1 mg/kg or an oral dose of 5 mg/kg administered to an unanesthetized, normotensive dog, increased systolic blood pressure a maximum of about 30% with blood pressure returning to normal in 3–5 hr, increased the heart rate a maximum of about 40%, but had little or no effect on diastolic blood pressure. Elevated systolic blood pressure could be maintained over a 5-day period in the unanesthetized dog given 1 mg/kg oral doses t.i.d.

In the anesthetized (pentobarbital) dog, at an iv dose of 1 mg/kg, **9** either had little effect or reduced mean arterial blood pressure, increased cardiac output (dye-dilution technique) a maximum of about 80% for a 45% maximum reduction in total peripheral resistance, and increased heart rate about 65% (maximum) and stroke volume about 30%. **9** increased the rate of blood flow through the femoral artery (Shipley-Wilson rotameter) at doses which had no effect on mean blood pressure.

Thus, the overall hemodynamic effects of **9** apparently are a resultant of a marked and prolonged increase in cardiac output coupled with vasodilatation and reduced peripheral resistance. These actions could be explicable in terms of α-adrenergic blockade coupled with β-adrenergic stimulation[3] and this view is supported by the finding that in the anesthetized dog in iv doses of 0.5–1 mg/kg, **9** blocked the α response and potentiated the β response to normally effective amounts of exogenous norepinephrine and epinephrine, respectively. The cardiac effects of **9** were blocked by the β-adrenergic blocking agent, propranolol. That, as seemed most probable, the β stimulation is indirect is indicated by the observation that **9** is ineffective as an agonist in reser-

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‡One of these compounds, 2-(3-ethylsulfinylpropyl)-1,2,3,4-tetrahydroisoquinoline (**9**), is currently undergoing clinical trial as an orally active agent against shock.