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2-(2-Aminoethylamino)-1,2-diphenylethanol Derivatives, a New Class of Topical Antiinflammatory Agents

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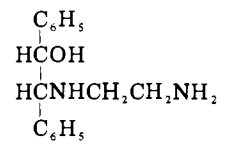
A number of analogues and derivatives of the title compound were synthesized and evaluated in a new test procedure used to detect topical antiinflammatory activity. Some general comments regarding observation on the structure–activity relationship of these compounds are made.

In connection with some unrelated work, one of us reported the synthesis of two novel isomeric diphenylethanol derivatives (compounds 1 and 2) which were prepared by treatment of *trans*- and *cis*-stilbene oxide, respectively, with ethylenediamine.[1] Since it was known that the reaction of stilbene oxides with amines generally proceeds by a trans addition,[2] the erythro configuration was assigned to compound 1 and the threo configuration for compound 2.



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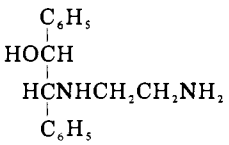

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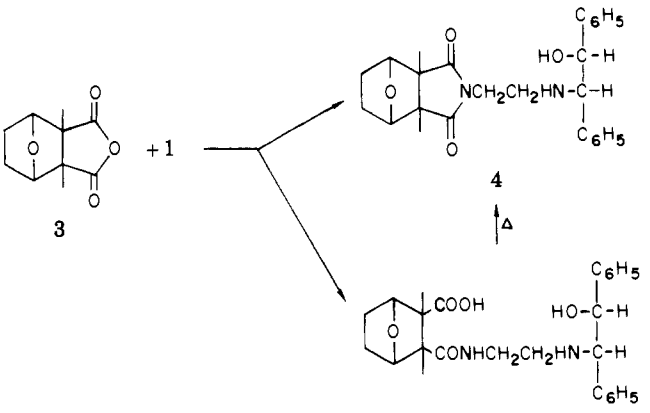
Both of these compounds were found to be active in a new topical antiinflammatory test developed in these laboratories.[3] While the topical activity parallels the activity of corticosteroids in this screen, these compounds do not show any systemic antiinflammatory activity and do not elicit any response against prostaglandin synthetase.

Discussion and Results

It was realized that the new topical antiinflammatory test procedures involved the admixture of the test compound with cantharidin 3 and acetone. Since both cantharidin and acetone are capable of reacting with compound 1, perhaps casting doubt on the interpretation of the test results, the three possible reaction products from 1, 3, and acetone were prepared as shown below and tested for topical antiinflammatory activity.



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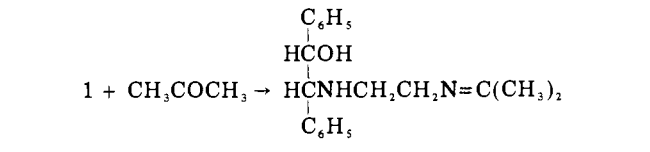

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Testing results indicated that the imide 4 had no activity while the amide 5 was active. The imine 6 had an activity comparable to compound 1 (Table I). The possibility that the activity observed for the diphenylethanols was, in fact,

Table I. Percent Inhibition of Inflammation

Compd	Topical dose, μg 50 (0.05%)	100 (0.1%)	200 (0.2%)	400 (0.4%)	800 (0.8%)
1	19[d]	19	46[c]	72[c]	88[c]
2		7	9	56[c]	88[c]
4	NT[e]	NT	NT	NT	0[f]
5	NT	NT	NT	NT	67[g]
6	14	13	10	59[c]	87[c]
7	14	0	17	5	0
8a	7	1	19[a]	51[b]	87[c]
8b	7	2	0	29[a]	96[c]
8c	0	0	8	0	0
8d	20[d]	23	35[a]	64[c]	90[c]
8e	3	0	12	35[b]	89[c]
8f	0	8	21	29[a]	51[b]
8g	0	11	9	0	40[b]
8h	0	3	0	3	5
8i	6	13	7	13	0
8j	16	10	16	15	17
8k	12	9	5	53[c]	68[c]
8m	9	0	16	9	32[a]
8n	10	5	37[a]	54[b]	87[c]
8p	15	20	20	6	26[a]
8q	0	8	1	11	41[b]

Dexamethasone	Topical dose, μg 5	10	20	40	80
% inhibn of inflammation	17	25[d]	30[a]	53[b]	76[c]

[a] $p < 0.05$. [b] $p < 0.01$. [c] $p < 0.001$. [d] $p < 0.05$ (Fieller's theorem). [e] NT = not tested. [f] Tested at 888 μg. [g] Tested at 924 μg.

Table II. Cotton Pellet Granuloma Test

Compd	Dose, μg/pellet	% redn of granuloma
Dexamethasone	10	16[a]
	20	28[c]
	40	34[c]
	80	49[c]
1	1000	19[a]
	2000	28[b]
	4000	44[c]
	8000	49[c]

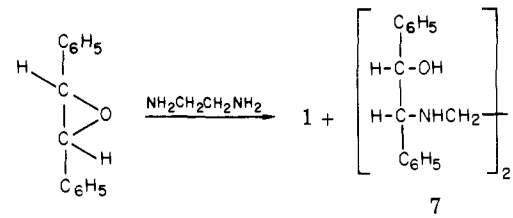
[a] $p < 0.05$. [b] $p < 0.01$. [c] $p < 0.001$.

due to the formation of imines related to **6** was resolved by demonstrating that compound **1** was active in a cotton pellet granuloma test system in which neither cantharidin nor acetone was used (Table II).

In order to determine if both of the optical antipodes of **1** were active, resolution was carried out by treating the racemate with (−)-diacetone-2-keto-L-gulonic acid hydrate (DAG). The resulting (−)(−) salt crystallized and gave, after purification and hydrolysis, the pure (−) enantiomer **8a**. By hydrolysis of the mother liquors and treatment of the enriched (+) enantiomer with *d*-tartaric acid, the pure (+) form **8b** was obtained after purification and hydrolysis of the salt. At higher doses both enantiomers showed activity comparable to that of the racemate. Thus, it can be seen that topical antiinflammatory activity resides in both enantiomers of compound **1** as well as in both diastereomers **1** and **2**.

During the course of a larger scale (100 g) synthesis of compound **1**, the dimer **7** was isolated and characterized. This compound was found to be devoid of worthwhile topical activity.

In terms of structural–activity relationships it has been shown for structure **8** (below) that while these compounds



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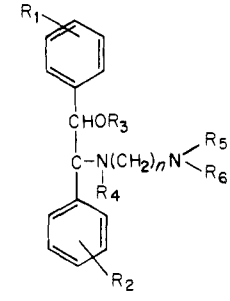
are most active when $n = 2$, good activity has also been found when $n = 3$ or 4. Alkylation of the amine functions decreases activity; thus **8**, $R_5 = R_6 = CH_3$, $R_4 = H$, is inactive, while **8**, $R_4 = CH_3$, $R_5 = R_6 = H$, and **8**, $R_4 = R_6 = H$, $R_5 = CH_3$, were weakly active.



8

With one exception, the *p,p*-dichloro-substituted compound **8j**, substituents on the aromatic rings seemed to have little if any effect on the activity (**8d,e,j,k** vs. **1**). Not only were the cantharidin acyl adducts **4** and **5** less active than **1**, but also simple N-acetylation of **1** (compounds **8g–i**) reduced the activity.

Chemistry. All of the analogues and homologues of **1** (structure **8**) were synthesized by the known method of addition of an amine to a stilbene oxide.[1,2] Subsequent modifications of the resulting product were also carried out by standard chemical techniques. With the exception of compounds **4–7**, all of the analogues and homologues are listed in Table III.

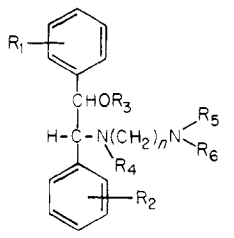
Experimental Section

Melting points were determined in a capillary melting point apparatus. Thus UV spectra were measured in *i*-PrOH on a Cary Model 14 spectrophotometer. NMR spectra were recorded with Varian T-60 instrument with Me_4Si as internal standard. IR spectra were determined on a Beckman IR-9 spectrometer. Spectra were taken for all intermediates and end products and were compatible with assigned structures. Silica gel Merck (70–325 mesh) was used for chromatography and anhydrous Na_2SO_4 for drying.

3,6-Epoxy-1,2,3,4,5,6-hexahydro-1,2-dimethyl-*N*-[(2-hydroxy-1-phenethyl)aminoethyl]phthalimide (4). A solution of 0.52 g (0.00204 mol) of **1**[1] in 15 mL of Me_2CO, 30 mL of EtOH, and 45 mL of Et_2O was treated with 0.4 g (0.00204 mol) of cantharidin (**3**) and after 18 h at room temperature the reaction mixture was heated at reflux for 5 h. An additional 0.52 g (0.00204 mol) of **1** was added since TLC showed that some of **1** had condensed with Me_2CO. The reaction mixture was refluxed for 1 h and evaporated to dryness. The residue was dissolved in CH_2Cl_2 and filtered through 50 g of Florisil in a sintered glass funnel. The Florisil was eluted with 100 mL of CH_2Cl_2 and 300 mL of a 50/50 (v/v) mixture of CH_2Cl_2 and Et_2O. The eluents were combined and evaporated. The residue was crystallized from a mixture of MeOH and H_2O to give 0.8 g (90%) of **4** as white needles, mp 130–134 °C. Anal. ($C_{26}H_{30}N_2O_4$) C, H, N.

2-Carboxy-3,6-epoxy-1,2-dimethyl-*N*-[2-(2-hydroxy-1-phenylphenethyl)aminoethyl]cyclohexanecarboxamide (5). A solution of 0.65 g (0.00254 mol) of **1** in 30 mL of $EtCl_2$ was treated with 0.5 g (0.00255 mol) of cantharidin, and the reaction was allowed to stand for 18 h at room temperature. Solvent was evaporated and the residue was crystallized from a mixture of MeOH and H_2O and then recrystallized from a mixture of CH_2Cl_2 and C_6H_{12} to give 0.4 g (35%) of **5** as white prisms, mp 105–110

Table III. 2-(2-Aminoalkylamino)-1,2-diphenylethanols



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Compd	R_1	R_2	R_3	R_4	R_5	R_6	n	Confign
1	H	H	H	H	H	H	2	Erythro *dl*
2	H	H	H	H	H	H	2	Threo *dl*
8a	H	H	H	H	H	H	2	Erythro (−)
8b	H	H	H	H	H	H	2	Erythro (+)
8c	H	H	H	H	CH_3	CH_3	2	Erythro *dl*
8d	Cl	H	H	H	H	H	2	Erythro *dl*
8e	H	Cl	H	H	H	H	2	Erythro *dl*
8f	H	H	H	H	H	H	3	Erythro *dl*
8g	H	H	H	$COCH_3$	$COCH_3$	H	2	Erythro *dl*
8h	H	H	H	H	$COCH_3$	H	2	Erythro *dl*
8i	H	H	$COCH_3$	$COCH_3$	$COCH_3$	H	2	Erythro *dl*
8j	Cl	Cl	H	H	H	H	2	Erythro *dl*
8k	CH_3	H	H	H	H	H	2	Erythro *dl*
8m	H	H	H	H	CH_3	CH_3	3	Erythro *dl*
8n	H	H	H	H	H	H	4	Erythro *dl*
8p	H	H	H	H	CH_3	H	2	Erythro *dl*
8q	H	H	H	CH_3	H	H	2	Erythro *dl*

°C. Anal. ($C_{26}H_{32}N_2O_5$) C, H, N.

Repeated recrystallization of the product from MeOH and H_2O caused the product to cyclize to compound **4** [0.6 g (55%)]: white needles; mp and mmp (with an authentic sample) 130–134 °C.

2-(2-Isopropylideneaminoethylamino)-1,2-diphenylethanol (6). A solution of 10 g (0.039 mol) of **1** in 500 mL of Me_2CO was heated under reflux for 5 h and then evaporated to dryness. The residual oil was dissolved in 100 mL of CH_2Cl_2 and filtered over 250 g of Florisil. Elution with CH_2Cl_2 and then Et_2O gave, after removal of solvents, 6 g of an oil. Using MeOH as the eluent, an additional 4 g of oil was obtained after evaporation. The MeOH fraction was crystallized from a mixture of Me_2CO and petroleum ether (bp 30–60 °C) to give 2.5 g of **6** as off-white rods, mp 129–137 °C. An additional 3 g (48% total yield) of product was obtained by stirring a solution of the 6 g of oil obtained from the CH_2Cl_2 and Et_2O fractions in 100 mL of CH_2Cl_2 and 50 g of Florisil for 1 h at 30 °C. The solution was filtered and the Florisil was washed with MeOH. The solvents were evaporated and the residue was crystallized from a mixture of Me_2CO and Et_2O to give pure **6** as white rods, mp 129–137 °C. Anal. ($C_{19}H_{24}N_2O$) C, H, N.

An additional experiment was performed in which a solution of **1** in Me_2CO was heated for 5 min and evaporated and the residue was crystallized from a mixture of Me_2CO and Et_2O to give an almost quantitative yield of **6** as white rods, mp 129–137 °C.

It is apparent that prolonged heating, as described in the first experiment, forms an unknown intermediate which is decomposed on Florisil to afford **6**.

***N,N*-Bis[(2-hydroxy-1,2-diphenyl)ethyl]ethylenediamine (7).** A solution of 300 g (1.53 mol) of *trans*-stilbene oxide in 900 mL of ethylenediamine was heated under reflux and stirred for 20 h. The mixture was cooled and poured into 3 L of ice and H_2O. The precipitate was filtered, washed with 150 mL of H_2O, and then stirred with 2.7 L of 1 N HCl for 90 min. The soluble hydrochloride of **1** was removed by filtration. The precipitate was washed with H_2O (2 × 150 mL) and then dissolved in 400 mL of CH_2Cl_2. The solution was washed with 200 mL of 1 N NaOH, dried, and evaporated to dryness. The residue was dissolved in MeOH, a 10% excess of ethanolic HCl was added, and the solution was evaporated. The residual hydrochloride salt was recrystallized from MeOH three times to give 23 g (4.8%) of white rods melting at 225–230 °C. Some of the salt was dissolved in a mixture of CH_2Cl_2 and dilute NH_4OH. After separating the layers, the organic layer was dried and evaporated. The residual free base crystallized from a mixture of THF and C_6H_{12} to give **7** as white prisms, mp 97–101 °C. Anal. ($C_{30}H_{32}N_2O_2$) H, N; C: calcd, 79.62; found, 79.20.

(−)-*erythro*-2-(2-Aminoethylamino)-1,2-diphenylethanol (8a) and (+)-*erythro*-2-(2-Aminoethylamino)-1,2-diphenylethanol (8b). To a hot solution of 15 g of *rac*-**1** in 100 mL of MeOH was added 18 g of (−)-diacetone-2-ketogulonic acid hydrate and the solution left at room temperature overnight. A total of 13 g of crystalline (−)-*erythro*-2-(2-aminoethylamino)-1,2-diphenylethanol diacetone-2-keto-L-gulonate was separated by filtration. This salt was recrystallized from MeOH until a constant melting point of 192–193 °C was obtained. The salt was suspended in CH_2Cl_2 and shaken with ice-cold dilute NaOH. The organic layer was separated, dried, and concentrated in vacuo to dryness. The residue after crystallization from a THF–Et_2O mixture gave 6.6 g of **8a**. The pure product formed colorless needles: mp 144–145 °C; $[\alpha]^{25}_D$ −9.45°. Anal. ($C_{16}H_{20}N_2O$) C, H, N.

The original filtrate from the DAG salt was concentrated in vacuo to dryness and the base was liberated as above. The enriched (+) base (4.2 g) melted at 134 °C.

A mixture of 2.15 g of enriched (+) base and 1.5 g of *d*-tartaric acid in 60 mL of MeOH was heated to boiling and then left at room temperature overnight. The crude (+)-*erythro*-2-(2-aminoethylamino)-1,2- diphenylethanol tartrate (4 g) was separated by filtration. The tartrate was recrystallized from 400 mL of boiling H_2O and gave 3.4 g of pure salt melting at 224–225 °C. The base was liberated as above. After recrystallization from a THF–Et_2O mixture, the pure **8b** (1.2 g) formed colorless needles: mp 144–145 °C; $[\alpha]^{25}_D$ +9.29°. Anal. ($C_{16}H_{20}N_{20}$) C, H, N.

***erythro*-2-(2,2-Dimethylaminoethylamino)-1,2-diphenylethanol (8c).** A solution of 20 g of *trans*-stilbene oxide in 40 mL of unsymmetrical dimethylethylenediamine was refluxed for 24 h. The reaction mixture was poured into H_2O (0 °C) and extracted with CH_2Cl_2. The organic extract was washed with H_2O, separated, dried, and concentrated in vacuo to dryness. The residue was crystallized from a mixture of THF–Et_2O–petroleum ether to give 24.5 g of crude product. After recrystallization from the same solvent mixture, the pure product formed colorless needles, mp 132–133 °C. Anal. ($C_{18}H_{24}N_2O$) C, H, N.

***erythro*-2-(2-Aminoethylamino)-1-(4-chlorophenyl)-2-phenylethanol (8d) and *erythro*-2-(2-Aminoethylamino)-2-(4-chlorophenyl)-1-phenylethanol (8e).** A solution of 29 g of *trans*-4 chlorostilbene oxide[5] in 200 mL of ethylenediamine

was refluxed for 20 h. The reaction mixture was cooled and poured into ice-cold dilute NaOH and extracted with CH_2Cl_2. The organic layer was separated, dried, and concentrated in vacuo to dryness. The residue (32 g) was dissolved in a small amount of MeOH and an excess of ethanolic HCl was added. The precipitated salt (20 g) was separated by filtration. A second crop of 7 g was obtained from the filtrate. After several recrystallizations of the first crop from MeOH a pure hydrochloride (3.7 g), mp 276–279 °C, was obtained. A solution of this hydrochloride in ice H_2O was treated with an excess of NH_4OH and the free base was extracted into Et_2O. The Et_2O extract was dried, filtered, and concentrated to a small volume. The crystalline **8d** (1.9 g), mp 108–109 °C, was separated by filtration. Anal. ($C_{16}H_{19}ClN_2O$) C, H, N.

The second crop of hydrochloride from the above reaction, mp 248–252 °C, was purified and treated in the same manner as the first crop to give 2 g of **8e** as colorless needles, mp 91–92 °C. Anal. ($C_{16}H_{19}ClN_2O$) C, H, N.

***erythro*-2-(3-Aminopropylamino)-1,2-diphenylethanol (8f).** A solution of 20 g of *trans*-stilbene oxide in 60 mL of 1,3-propanediamine was refluxed for 24 h. The reaction mixture was poured into ice H_2O and extracted with CH_2Cl_2. The organic extract was washed with H_2O, dried, and concentrated in vacuo to dryness. The residue (24 g) was crystallized from a mixture of THF and Et_2O to give 17 g of product, mp 99–100 °C. After recrystallization from the same solvent mixture the pure product formed colorless needles, mp 99–100 °C. Anal. ($C_{17}H_{22}N_2O$) C, H, N.

***erythro*-2-[*N*-(2-Acetamidoethyl)acetamido]-1,2-diphenylethanol (8g).** To a stirred, ice-cooled solution of 22.8 g of **1** in 600 mL of dioxane was added 12 mL of AcCl and then 22 mL of Et_3N. The reaction mixture was stirred at room temperature for 1.5 h and the insoluble Et_3NHCl was separated by filtration. The filtrate was concentrated in vacuo to dryness. The residue was crystallized from Me_2CO to give 18 g of product.

After recrystallization from Me_2CO, the pure product formed colorless rods, mp 118–122 °C. Anal. ($C_{20}H_{24}N_2O_3$) C, H, N.

***erythro*-2-(2-Acetamidoethylamino)-1,2-diphenylethanol (8h).** A stirred solution of 22.8 g of **1** in 600 mL of dioxane was treated with 6 mL of AcCl at 15 °C. The reaction mixture was stirred at the same temperature for 15 min and the precipitated crude HCl salt (31.5 g) was separated by filtration. The salt was dissolved in H_2O (0 °C), and excess of dilute NaOH was added, and the mixture was extracted with CH_2Cl_2. The CH_2Cl_2 extract was dried and concentrated in vacuo to dryness. The residue was crystallized from Et_2O to give 20.5 g of product melting at 120–122 °C. After recrystallization from Me_2CO the pure product formed colorless prisms, mp 122–124 °C. Anal. ($C_{18}H_{22}N_2O_2$) C, H, N.

***N*-[2-(Acetamido)ethyl]-*N*-[(β-acetoxy-α-phenyl)-phenylethyl]acetamide (8i).** To a stirred suspension of 20 g of **1** in 100 mL of pyridine was added 100 mL of Ac_2O. A solution was obtained which was left at room temperature for 23 h. The reaction mixture was concentrated in vacuo to dryness. The residue was crystallized from Et_2O and gave 23.4 g of crude product melting at 132–135 °C. After recrystallization from Me_2CO, the pure product formed colorless needles, mp 135–137 °C. Anal. ($C_{22}H_{26}N_2O_4$) C, H, N.

2-(2-Aminoethylamino)-1,2-bis(*p*-chlorophenyl)ethanol (8j). A solution of 11.8 g of *trans*-4,4-dichlorostilbene oxide[4] in 150 mL of ethylenediamine was refluxed for 24 h. The reaction mixture was poured into ice-cold dilute NaOH and extracted with CH_2Cl_2. The organic layer was separated, dried, and concentrated in vacuo to dryness. The residue was crystallized from a Et_2O–petroleum ether mixture and gave 10.8 g of crude product melting at 109–115 °C. After recrystallization from Et_2O the pure product formed colorless needles, mp 112–113 °C. Anal. ($C_{16}H_{18}Cl_2N_2O$) C, H, N.

***erythro*-2-(2-Aminoethylamino)-2-phenyl-1-(*p*-tolyl)-ethanol (8k).** A solution of 16.6 g of *trans*-4-methylstilbene oxide[5] in 190 mL of ethylenediamine was refluxed for 20 h. The reaction mixture was cooled, poured into ice-cold dilute NaOH, and extracted with Et_2O. The organic layer was separated, dried, and concentrated in vacuo to dryness. The residue was dissolved in a small amount of MeOH and an excess of ethanolic HCl was added. The precipitated hydrochloride was separated by filtration. After several recrystallizations from MeOH a pure hydrochloride was obtained, mp 267–268 °C. A solution of the salt in H_2O was treated with an excess of dilute NaOH and the crystalline base was separated. After recrystallization from Et_2O the pure product formed colorless needles, mp 132–133 °C. Anal. ($C_{17}H_{22}N_2O$) C, H, N.

***erythro*-2-(3,3-Dimethylaminopropylamino)-1,2-diphenylethanol (8m).** A solution of 20 g of *trans*-stilbene oxide in 60 mL of dimethylaminopropylamine was refluxed for 24 h. The reaction mixture was poured into H_2O (0 °C) and extracted with CH_2Cl_2. The organic extract was washed with H_2O, separated, dried, and concentrated in vacuo to dryness. The residue was crystallized from a mixture of THF–Et_2O–petroleum ether to give 21 g of crude product. After recrystallization from Et_2O the pure product formed colorless needles, mp 108–110 °C. Anal. ($C_{19}H_{26}N_2O$) C, H, N.

***erythro*-2-(4-Aminobutylamino)-1,2-diphenylethanol (8n).** A mixture of 10 g of *trans*-stilbene oxide, 20 mL of 1,4-diaminobutane, and 20 mL of toluene was refluxed for 20 h. The reaction mixture was poured into dilute ice-cold HCl and extracted with Et_2O. The aqueous acid layer was separated, made basic with dilute NaOH, and extracted with CH_2Cl_2. The CH_2Cl_2 extract was dried and concentrated in vacuo to dryness. The residue was crystallized from a mixture of THF–Et_2O–petroleum ether to give 7.5 g of product. After recrystallization from Et_2O, the pure product formed colorless needles, mp 108–110 °C. Anal. ($C_{18}H_{24}N_2O$) C, H, N.

***erythro*-2-(2-Methylaminoethylamino)-1,2-diphenylethanol (8p) and *erythro*-2-[*N*-Methyl-*N*-(2-aminoethyl)-amino]-1,2-diphenylethanol (8q).** A solution of 20 g of *trans*-stilbene oxide in 35 mL of *N*-methylethylenediamine was refluxed for 16 h. The reaction mixture was poured into H_2O (0 °C) and extracted with CH_2Cl_2. The organic extract was washed with H_2O, separated, dried, and concentrated in vacuo to dryness. The residue was dissolved in a small amount of MeOH and an excess of ethanolic HCl was added. The precipitated hydrochloride (12.2 g) was separated by filtration, mp 247–250 °C dec. After concentrating the filtrate in vacuo to a smaller volume and diluting it with 2-propanol, a second crop (15.3 g) of hydrochloride was obtained, mp 209–212 °C.

The first crop of hydrochloride was purified by recrystallization from MeOH until the melting point was 263–264 °C dec. A solution of this hydrochloride (6 g) in H_2O (0 °C) was made alkaline with NH_4OH and the mixture was extracted with CH_2Cl_2. The organic extract was separated, dried, and concentrated in vacuo to dryness. The residue (4.3 g) was crystallized from THF to give 3.7 g of **8p**. After recrystallization from the same solvent, the pure product formed colorless needles, mp 135–136 °C. Anal. ($C_{17}H_{22}N_2O$) C, H, N.

The base from the second crop of the original hydrochloride was liberated as above. The residue was crystallized from Et_2O and gave 7.9 g of **8q**. After recrystallization from Et_2O the pure product formed colorless needles, mp 118–119 °C. Anal. ($C_{17}H_{22}N_2O$) C, H, N.

Biological Testing Methods. Topical antiinflammatory activity was determined by testing the ability of the compounds to inhibit cantharidin-induced inflammation.[3] Compounds were applied topically to the outer surface of the ears of groups of 8–10 immature rats (50–60 g body weight) concurrently with cantharidin. A control group was treated with vehicle alone, and another group of rats received only the cantharidin solution. After 72 h a uniform section was punched from the treated area and weighed. The weights were averaged for the control group (C), irritant alone group (I), and test compound group (T), and the percent inhibition was computed by the following formula

$$\% \text{ inhibition} = 100(I - T)/(I - C)$$

Significance of the differences between T groups and I groups was determined by Student's t test with (a) $p < 0.05$, (b) $p < 0.01$, and (c) $p < 0.001$, or by Fieller's theorem (d) $p < 0.05$.

Cotton Pellet Granuloma Test. Compounds were dissolved in ethanol and applied to cotton pellets which were allowed to dry at room temperature. Two pellets were implanted sc in rats and 72 h later, pellets were removed at autopsy. Reduction in the dry granuloma tissue surrounding the pellets served as a measure of antiinflammatory activity (ten rats per dosage group with two pellets per rat). Since compound **1** was not active when given sc or po, the effect observed probably reflects "local" activity.

Higher levels of drug were required in the cotton pellet granuloma test than in the direct application to skin procedure. This could possibly be due to rapid metabolism of compound 1.

Significance of the differences between test group (T) and control group (C) was determined as above.

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3-N-Substituted Aminomethyl Derivatives of Rifamycin SV. A Convenient Method of Synthesis, Cyclization of Certain Derivatives, and Anticellular and Antiviral Activities of Several Derivatives

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A new synthesis of Mannich bases of rifamycin SV using the Borch[2] procedure with rifaldehyde is described. This new synthesis offers two advantages over the previously published method.[3] It provides a route to monoalkylaminomethylrifamycins (1e–h) and to unsubstituted aminomethylrifamycins that were not accessible by the old procedure. The new method also offers a preparative route to Mannich bases **1a** and **1b** which were needed in multigram quantities for biological testing. In addition, the cyclization of certain of the monoalkylaminomethylrifamycins to the novel *N*,15-didehydro-15-deoxo-3,15-epi[methano(alkylimino)]rifamycin SV derivatives (**2**) is described. The anticellular and antiviral effects of representatives of both series of compounds against cultured mouse cells and murine oncornavirus are discussed.

3-Dialkylaminomethylrifamycin SV derivatives are active antibacterial agents[3] and certain of these derivatives (**1a–c**) interrupt oncornavirus replication in infected 3T3 cells.[4] Because of the antiviral activity of rifamycins **1a–c** larger quantities of these materials and compound **1d** were needed for in vivo testing.[5] The route available to these compounds[3] uses rifamycin S (quinone form of 1 where R = H), which is treated under classical Mannich reaction conditions with an excess of the appropriate secondary amine and aqueous formaldehyde. Unfortunately, the reported[3] yields for both **1a** and **1b** are 5%. It was also of interest to us to prepare monoalkylaminomethylrifamycins (e.g., **1e–h**) and the unsubstituted Mannich base **1i**. However, there was no published method available for obtaining these types of rifamycins. Therefore, it was imperative for us to develop a new more general procedure for the preparation of Mannich base rifamycins.

Chemistry. The synthesis of the desired 3-N-substituted aminomethyl derivatives of rifamycin SV (**1a–i**) (Chart I) was achieved by using the reductive amination procedure of Borch.[2] Rifaldehyde (1, R = CH=O) was treated with a mixture of the desired amine, its hydrochloride (which can be prepared in situ), and Borch's reagent[2] (sodium cyanoborohydride). This results in a mildly exothermic reaction which proceeds to completion within a few hours. For the two rifamycins (**1a** and **1b**) that we needed in multigram quantities and were difficult to obtain in even small quantities, the new procedure provided the compounds in a much improved yield. Mannich base **1a** was isolated as an orange crystalline solid in 47% yield. Likewise, rifamycin **1b** was isolated as a crystalline solid from acetonitrile–ether in 38% yield. The other two rifamycins that were needed, the dimethyl (**1d**) and diethyl (**1c**) derivatives, could be prepared by the Borch procedure in good and comparable yields to those reported by the Mannich base procedure.[3] In addition, the previously unavailable monoalkylaminomethylrifamycins **1e–h** and the unsubstituted aminomethylrifamycin **1i** were readily prepared by the new procedure.

Chart I. 3-Alkylaminomethylrifamycin SV Derivatives