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Synthesis and Biological Evaluation of Fragmented Derivatives of Tetrahydroisoquinolines. 3. Trimetoquinol Studies

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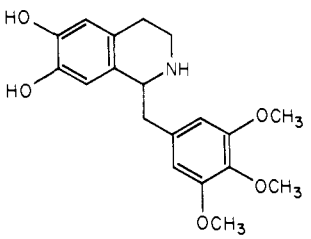
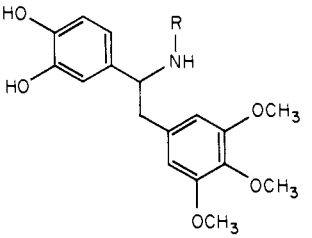
The synthesis of 1-(3,4-dihydroxyphenyl)-2-(3,4,5-trimethoxyphenyl)ethylamine along with the *N*-methyl, *N*-ethyl, and *N*-isopropyl derivatives is presented as fragmented derivatives of trimetoquinol (1). Comparative pharmacological effects of these compounds to 1 are presented and discussed.

In continuing our investigation of tetrahydroisoquinolines as agonists and antagonists in adrenoceptor systems,[1-5] we have initiated a program in determining the relationship of chemical structure to the production of biological actions. One portion of this program involves delineating the importance of an intact tetrahydroisoquinoline ring system for adrenergic activity with a goal toward the development of selective and/or potent β-adrenoceptor stimulants. This report is concerned with the modification of trimetoquinol (**1**, TMQ) which is known to possess potent β-adrenoceptor activity.[1-8] One of the analogues, **4**, prepared can be considered a fragmented 1 in which the bond between C_4 and the aromatic ring is broken. Other derivatives such as **2**, **3**, and **5** were also prepared and investigated for their profile of β-adrenoceptor activity.



1

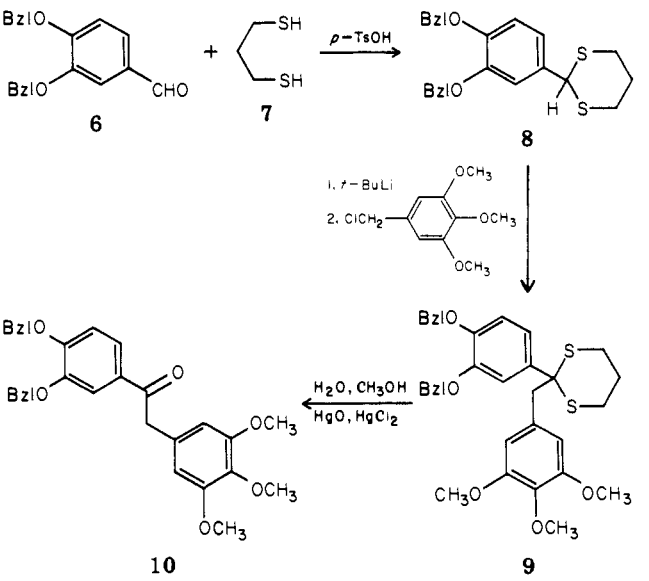


2, R = H
3, R = Me
4, R = Et
5, R = *i*-Pr

The synthesis of the analogues of trimetoquinol was accomplished through the key intermediate ketone, 2-(3',4',5'-trimethoxyphenyl)-1-(3',4'-dibenzyloxyphenyl)-ethanone (**10**, see Scheme I). The ketone was prepared by treating 3,4-dibenzyloxybenzaldehyde with 1,3-propanedithiol in refluxing benzene containing a trace of *p*-TsOH.[9] The resulting dithiane, **8**, was allowed to react with *tert*-butyllithium[10] followed by 3,4,5-trimethoxybenzyl chloride to give **9**. The alkylated dithiane **9** was then hydrolyzed to ketone **10** via the standard method using mercuric oxide and mercuric chloride in aqueous methanol.[11]

The fragmented trimetoquinol derivative **4** along with derivatives **3** and **5** was prepared by the following general reaction procedures (Scheme II). The ketone **10** was converted to the desired imines **11a-c** by allowing the appropriate alkylamine (methylamine, ethylamine, and isopropylamine) to react with **10** in the presence of titanium tetrachloride.[12] The resulting imines were then reduced to the amines **12a-c** using $NaBH_4$ or B_2H_6. The amine was then converted to a hydrochloride salt and the benzyl-protecting groups were removed via hydrogenolysis using 10% palladium on charcoal to give the desired catechols **3**·HCl, **4**·HCl, and **5**·HCl.

Scheme I

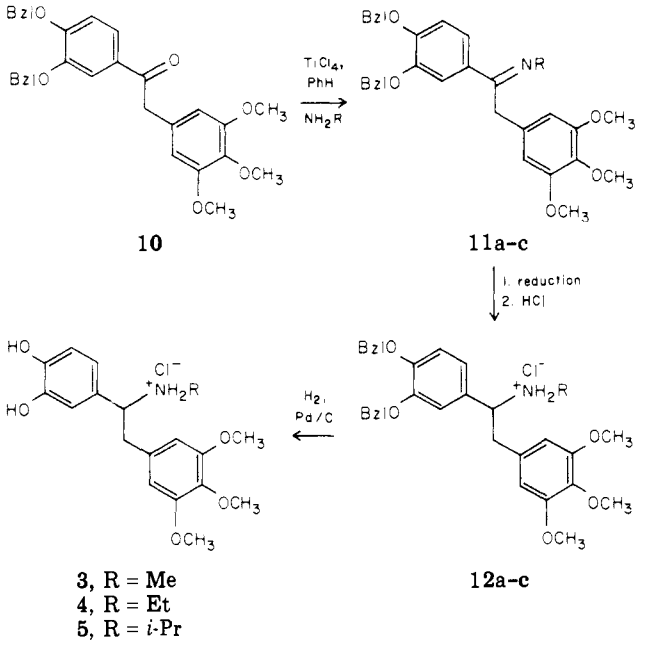


The primary amine derivative **2** was prepared by allowing ketone **10** to react with methoxyamine hydrochloride in pyridine and ethanol as shown in Scheme III.[13] The *O*-methyloxime **13** was then reduced using diborane and the resulting amine was converted to hydrochloride salt **14**. Hydrogenolysis of **14** using 10% palladium on charcoal afforded the desired catechol **2**·HCl.

Biological Activity. Pharmacological studies were carried out in the isolated β-adrenoceptor receptor systems of guinea pig trachea, guinea pig atria, and rat adipose tissue. All of the fragmented derivatives possessed an ability to produce tracheal relaxation, chronotropic effects on the heart, and lipolysis.

The relative abilities of each fragmented derivative to stimulate chronotropic response and tracheal relaxation are summarized in Figure 1. Data are presented in terms of the mean pD_2 value ± SEM obtained from cumulative dose–response curves. As indicated, all the analogues possessed pD_2 values which were smaller than the parent

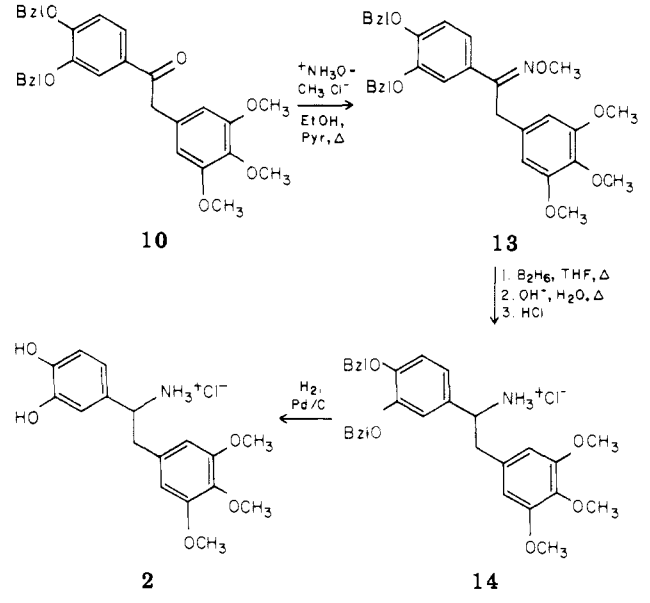
Scheme II



3, R = Me
4, R = Et
5, R = *i*-Pr

a, R = Me; b, R = Et; c, R = *i*-Pr

Scheme III



drug *dl*-TMQ in both β-adrenoceptor systems. Although each analogue and *dl*-TMQ possessed a greater pD_2 value on atria than on trachea, we were unable to observe any changes in relative selectivity for these receptor systems. It should be noted that analogue **3** was the most active of the synthesized analogues whereas compounds **2**, **4**, and **5** were nearly equivalent as weak stimulants of both β-adrenoceptor systems.

Data obtained for analogues **2**–**5** and *dl*-TMQ on the release of glycerol from isolated fat cells are presented in Figure 2. Clearly, *dl*-TMQ was more active than any of the analogues tested. Moreover, each of the analogues was unable to exhibit a maximal rate of glycerol release over the concentration range of 10^{-8}–10^{-4} M. Of these analogues, only **3** showed significant lipolytic activity at 10^{-6} M with the remaining analogues **2**, **4**, and **5** being only weakly active in this β-adrenoceptor system.

These studies indicate that the intact tetrahydroisoquinoline nucleus appears to be necessary for the potent

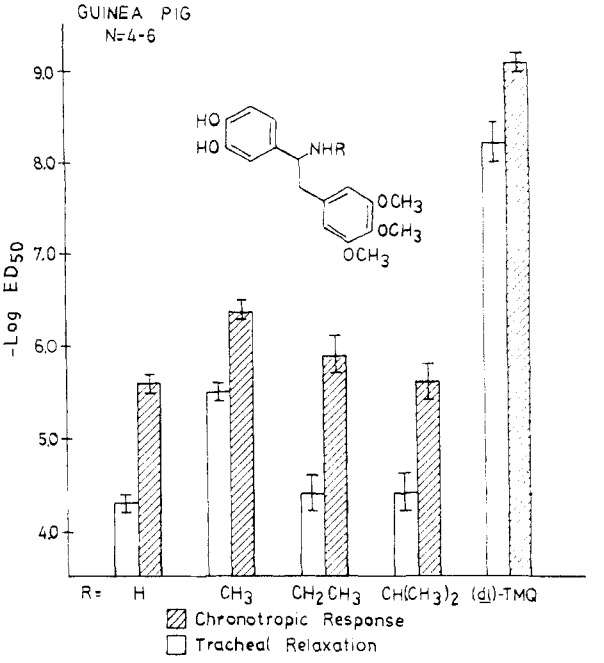


Figure 1. Comparison of trimetoquinol (*dl*-TMQ) and analogues in guinea pig atria and trachea. Values plotted represent the mean pD_2 value ($-\log ED_{50}$) of $N = 4$–6 ± SEM for each compound.

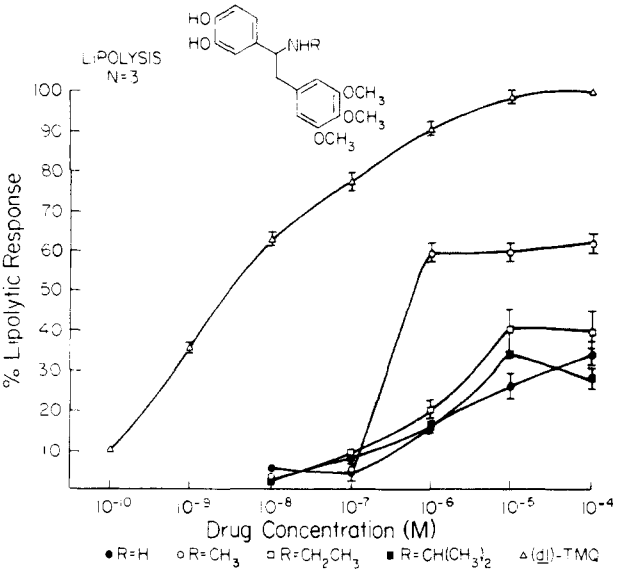


Figure 2. Dose-response curves for trimetoquinol (*dl*-TMQ) and analogues on the release of glycerol from rat epididymal fat cells. Values plotted represent the mean percent lipolytic response of $N = 3$ ± SEM as indicated by the vertical lines.

β-adrenoceptor action possessed by TMQ. Fragmentation of the intact tetrahydroisoquinoline ring between the C_4 position and aromatic ring leads to a considerable reduction in β-adrenoceptor activity (compare *dl*-TMQ with **4** in Figures 1 and 2). Of the synthesized analogues **3** showed the greatest activity, although its relative potency to *dl*-TMQ was at least 100-fold less in the β-adrenoceptor systems examined. In addition, none of the analogues exhibited any change in selectivity for the β-adrenoceptor systems over that observed with *dl*-TMQ.

Experimental Section

Melting points (uncorrected) were determined on a Thomas-Hoover melting point apparatus. Spectral data were obtained using a Perkin-Elmer 237 infrared spectrophotometer

Table I

Compd	Mp, °C	Solvent	% yield
2	180.5–181.5	Ether–ethanol	85
3	145–149	Ether–ethanol	80
4	186–187	Ether–ethanol	89
5	171.5–172.5	Ether–ethanol	89
12a	78–79[a]	Ether–ethanol	64[b]
12b	188–189	Ether–ethanol	48[b]
12c	164–166	Ether–methanol	43[b]
14	226–227	Ether–ethanol	73[b]

[a] Glassy material. [b] Overall yield from ketone 10.

and a Varian A-60A nuclear magnetic resonance spectrometer. Analyses were performed by Galbraith Laboratories, Inc., Knoxville, Tenn. Analytical results for C, H, and N were within ±0.4% of the theoretical values.

2-(3,4-Dibenzyloxyphenyl)-1,3-dithiane. To a 250-ml round-bottom flask equipped with a Dean-Stark trap were placed 14.65 g (45 mmol) of 3,4-dibenzyloxybenzaldehyde, 5.0 g (45 mmol) of 1,3-propanedithiol, a crystal of *p*-TsOH, and 150 ml of benzene. The mixture was refluxed until the appropriate amount of H_2O was removed. The benzene solution was then allowed to cool, washed with saturated solution of $NaHCO_3$ and H_2O, dried (Na_2SO_4), and evaporated to yield 17.7 g of 8 (96%) (mp 109–110°, acetone–hexane). Anal. ($C_{24}H_{24}O_2S_2$) C, H, N.

2-(3,4-Dibenzyloxyphenyl)-2-(3,4,5-trimethoxybenzyl)-ethanone (10). To a flame-dried 1-l. jacketed reaction flask equipped with a magnetic stirrer and under an argon atmosphere was added 30.00 g (73.5 mmol) of 2-(3,4-dibenzyloxyphenyl)-1,3-dithiane dissolved in 600 ml of freshly distilled THF. After cooling to −25 ± 5° an equimolar amount of *tert*-butyllithium (Ventron) was added to the stirred solution in 0.5 h forming a dark green solution. After 18 h 16.05 g (74 mmol) of 3,4,5-trimethoxybenzyl chloride dissolved in 125 ml of THF was added and stirring was continued for 12 h. Water was cautiously added and the reaction was allowed to come to room temperature. Et_2O was added to the solution and the ethereal solution was washed with 10% aqueous HCl. After drying ($MgSO_4$) the solvent was removed under vacuum. The oily residue was placed on a 2-kg silica gel column and eluted with benzene to give 9.33 g (22.9 mmol) of the starting dithiane 8 and 21.50 g (72.1% calcd from reacted material) of the desired alkylated product 9 as a golden oil.

To a 1-l. round-bottom flask equipped with a magnetic stirrer was added 10.00 g (16.9 mmol) of 1,3-dithiane (9), 9.5 g of red mercuric oxide, 21.0 g of mercuric chloride, 70 ml of H_2O, and 800 ml of MeOH. The stirred solution was refluxed for 5 h. After filtration the methanolic solvent was removed under vacuum. The residue was dissolved in chloroform, washed with saturated NH_4Cl, and dried ($MgSO_4$). After filtration the $CHCl_3$ was removed under vacuum to give a crude yellow product which crystallized from chloroform–ether to give 5.20 g (62%) of white crystals (mp 141.5–142.5°). Anal. ($C_{31}H_{30}O_6$) C, H.

General Procedure for the Preparation of 3, 4, and 5. To a solution of 1-(3,4-dibenzyloxyphenyl)-2-(3,4,5-trimethoxyphenyl)ethanone (10, 2 mmol) in 200 ml of benzene was added excess anhydrous amine (methylamine or ethylamine or isopropylamine). To this solution was added 0.35 g of titanium tetrachloride and the solution turned a reddish orange. The solution was stirred at room temperature until it turned yellow and the solvent was then removed in vacuo after filtration. The crude Schiff base obtained as an oil was dissolved in 150 ml of anhydrous THF and to this was added 10 ml of 0.9 M diborane. The mixture was refluxed for 10 h and cooled, 50 ml of 10% aqueous NaOH was added, and the solution was further refluxed for 10 h. After cooling, the product was extracted with ether, dried ($MgSO_4$), and filtered, and the solvent was removed in vacuo. The residue was dissolved in 10 ml of ethanol and ether saturated with HCl was added with stirring until no further precipitation occurred. Upon cooling, the hydrochloride salt was collected by filtration (see Table I). Anal. C, H, N.

The protected catecholamine hydrochloride salt **12a–c** (0.27 mmol) was placed in 100 ml of anhydrous EtOH along with 25 mg of 10% Pd/C and the mixture was hydrogenated for 8 h at 40 psi. After the addition of several drops of ether saturated with HCl, the solution was filtered and concentrated to 5 ml. To this solution was added ether to the cloud point and then the mixture was placed in a saturated ether chamber. The solid hydrochloride salt was then collected by filtration. The product gave a positive ferric chloride test (see Table I). Anal. C, H, N.

2-(3,4,5-Trimethoxyphenyl)-1-(3,4-dibenzyloxyphenyl)-ethylhydroxylimine Methyl Ether (13). To 1.56 g (3 mmol) of 1-(3,4-dibenzyloxyphenyl)-2-(3,4,5-trimethoxyphenyl)ethanone (10) and 0.27 g (3 mmol) of methoxyamine hydrochloride (Kodak) were added 10 ml of absolute ethanol and 10 ml of pyridine. After refluxing the mixture for 24 h the ethanol–pyridine was removed under vacuum to give a white solid. After recrystallization from ethanol 1.43 g (87%) of a white crystalline material, mp 91.5–92.5°, was obtained. Anal. ($C_{32}H_{33}O_6N$) C, H, N.

2-(3,4,5-Trimethoxyphenyl)-1-(3,4-dibenzyloxyphenyl)-ethylamine Hydrochloride (14). To 1.00 g (1.8 mmol) of 2-(3,4,5-trimethoxyphenyl)-1-(3,4-dibenzyloxyphenyl)ethylhydroxylimine methyl ester (13) in 50 ml of tetrahydrofuran was added 10 ml of 0.9 M diborane (Ventron). The mixture was refluxed for 12 h under argon atmosphere with stirring, then 20 ml of 20% aqueous potassium hydroxide was cautiously added to the cooled solution, and refluxing was continued for an additional 12 h. The amine was extracted with ether and dried ($MgSO_4$). Evaporation of the solvent under vacuum gave a golden oil. Addition of the oil to ethereal hydrochloric acid gave 0.85 g (84%) of the desired amine hydrochloride, mp 226–227° (recrystallized from ether–ethanol). Anal. ($C_{31}H_{34}NO_5Cl$) C, H, N.

1-(3,4-Dihydroxyphenyl)-2-(3,4,5-trimethoxyphenyl)-ethylamine Hydrochloride (2). A solution containing 300 mg (0.6 mmol) of 1-(3,4-dibenzyloxyphenyl)-2-(3,4,5-trimethoxyphenyl)ethylamine hydrochloride (14) in 150 ml of anhydrous EtOH and 30 mg of 10% Pd/C catalyst was hydrogenated for 8 h at 40 psi. After the addition of 2 ml of ethanolic hydrochloric acid the solution was gravity filtered and concentrated in vacuo to a volume of ca. 2 ml. After the addition of a small amount of ether (ca. 10 ml) the solution was placed in a saturated ether chamber. Upon slow crystallization, 178 mg (89.4%) of the catecholic amine 2, mp 186–187°, was obtained. The material gave a positive ferric chloride test. Anal. ($C_{17}H_{22}NO_5$) C, H, N.

Biological Testing. Isolated Tracheal Strip Preparation. Guinea pigs of either sex weighing 300–500 g were killed by a sharp blow on the head. The trachea of each animal was isolated and cleaned free of fatty tissue. From each guinea pig two spiral tracheal strips were prepared and mounted in a 12-ml jacketed muscle chamber containing a modified Kreb's solution (composition in millimoles: NaCl, 118; KCl, 4.7; $MgCl_2 \cdot 6H_2O$, 0.54; $CaCl_2 \cdot 2H_2O$, 2.5; NaH_2PO_4, 1.0; NaH_2PO_4, 1.0; $NaHCO_3$, 25; glucose, 11) maintained at 37° through which a mixture of 95% CO_2 was bubbled. Drug-induced effects were recorded on a Grass polygraph (Model 7C) via a force displacement transducer. Strips were allowed to equilibrate for 1–1.5 h before each experiment under a tension of 1 g. Carbachol (3×10^{-7} M) was used to increase the tone of each preparation and cumulative dose–response curves were obtained for each drug. Individual plots of tracheal relaxation, expressed as a percent of the maximum relaxation obtained with 10^{-5} M isoproterenol added at the end of each experiment vs. log molar concentration of each drug, were prepared and the ED_{50} values determined individually. In all biological experiments the ED_{50} values represent the concentration of each agonist required to produce a response equal to one-half of the maximal response in the appropriate system.

Isolated Right Atrial Preparation. Guinea pigs of either sex were killed by a sharp blow on the head. The atrium was dissected from extraneous tissue and placed in a 12-ml jacketed muscle bath. The atrium was allowed to equilibrate for a 1-h period in a modified Kreb's solution maintained at 37° through which a mixture of 95% O_2–5% CO_2 was bubbled. The increase in atrial rate was recorded on a Grass polygraph (Model 7C) via a force displacement transducer.

In each experiment, the atrium was exposed to a test dose of a drug and the atrial rate recorded during a 3-min period. Individual recordings were made at 1- and 3-min intervals. Cumulative dose–response curves were obtained for each analogue. The data were plotted on a log scale and the chronotropic responses expressed in terms of the maximum response obtained in the presence of 10^{-5} M isoproterenol added at the end of each

experiment. ED_{50} values were determined from individual plots.

Isolated Fat Cells. Epididymal fat tissue obtained from nonfasted male Sprague–Dawley rats weighing 200–250 g was used. Fat cells were isolated by the method of Rodbell[14] after digestion of adipose tissue with crude collagenase (Worthington) in a Krebs bicarbonate buffer containing 3% bovine serum albumin.

Incubation mixtures contained 0.2 ml of fat cell suspension, test drug, and Krebs bicarbonate–albumin solution in a total volume of 2.5 ml. Drugs were tested in the concentration range of 1×10^{-8}–3×10^{-4} M. Flasks were incubated in air at 37° for 1 h. All reactions were terminated by the addition of an equal volume of 10% TCA and the amount of glycerol released was measured by procedures described previously.[15,16]

In each experiment, a maximal release of glycerol was obtained in the presence of 10^{-6} M isoproterenol and this maximal figure was used to calculate the dose–response relationships obtained in this study.

Drugs. All drugs were prepared in normal saline containing 0.05% sodium metabisulfite.

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Synthesis and Some Properties and Antitumor Effects of the Actinomycin Lactam Analog, [Di(1'-L-α,β-diaminopropionic acid)]actinomycin D[1]

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A lactam analog of actinomycin D (AMD) has been synthesized as a potential antitumor chemotherapeutic agent. Both L-threonine residues were replaced by L-α,β-diaminopropionic acid. Starting with N^{α}-benzyloxycarbonyl-N^{β}-*tert*-butyloxycarbonyl-L-α,β-diaminopropionic acid methyl ester hydrochloride the linear intermediate N^{α}-benzyloxycarbonyl-N^{β}-(*tert*-butyloxycarbonylsarcosyl-L-*N*-methylvalyl)-L-α,β-diaminopropionyl-D-valyl-L-proline *p*-nitrophenyl ester was prepared by conventional methods of peptide synthesis in solution. Selective cleavage of the N^{β}-*tert*-butyloxycarbonyl group and lactam formation afforded the desired cyclic pentapeptide derivative. The chromophore precursor, N^{α}-(2-nitro-3-benzyloxy-4-methylbenzoyl) substituent, was introduced *via* its symmetric anhydride. Catalytic reduction followed by ferricyanide-mediated phenoxazinone formation provided the lactam analog, [di(1'-L-α,β-diaminopropionic acid)]actinomycin D ($[Dpr^1]_2$-AMD). Its binding to natural and synthetic DNA and that of an analogous L-*threo*-α,β-diaminobutyric acid containing lactam ($[Dbu^1]_2$-AMD) compared with the binding of AMD (in which the peptides are in lactone form) was studied by circular dichroic (CD) spectroscopy. The visible and uv CD spectra of free AMD differed from those of the free lactam analogs, indicating that the asymmetric environment of the pentapeptide rings in the region of the chromophore differs in free actinomycin lactone and lactams. In the presence of calf thymus DNA, PM2 DNA, and the synthetic d(A-T)-like copolymers containing 2,6-diaminopurine (DAP), poly[d(DAP-T)], and poly[d(DAP-A-T)], the rotational strengths of the optically active transitions in the visible region of the actinomycins increased, and the CD spectra in the presence of the various DNA duplexes were qualitatively similar. The CD spectra of bound actinomycin lactams resembled the spectrum of bound AMD. This suggests that the lactone and lactam actinomycins acquire a similar environment when bound to DNA. $[Dpr^1]_2$-AMD was less cytotoxic than AMD in antibacterial assays but exhibited somewhat higher toxicity in mice than AMD. At optimal dose levels the lactam analog had little or no antitumor activity in three murine tumor systems.

Actinomycin D (AMD), Figure 1 (a), is a clinically used chemotherapeutic agent capable of effecting cures in treatment of gestational choriocarcinoma[3] and Wilms' tumor.[4] Although being one of the few antitumor drugs that exhibit curative effects in two different tumors (of comparatively infrequent occurrence), the overall spectrum of actinomycin antitumor activity in man remains narrow.[5,6] At present, clinical use extends to only a few other cancers as trophoblastic tumors,[7] rhabdomyosarcoma,[8] and melanoma.[9] Moreover, its high host toxicity[10] and difficult administration have not encouraged wider application. Thus, the search for modified actinomycins of a broader range of activity and/or improved therapeutic index remains to be a worthwhile effort.[11]

The well-known importance of the two peptide lactone moieties in actinomycin molecules for the manifestation