β-Adrenergic Antagonists with Multiple Pharmacophores: Persistent Blockade of Receptors

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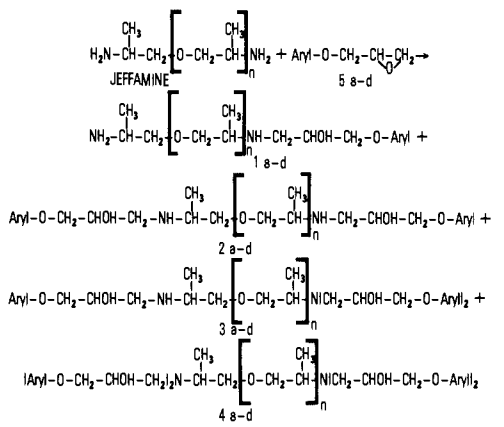
β-Adrenergic antagonists containing from one to four identical pharmacophores were prepared and studied. These compounds had the general structure $R_2NCH(CH_3)CH_2[-OCH_2CH(CH_3)-]_{2-8}NR_2$, where R is either H or an aryl-$OCH_2CHOHCH_2$ group. Synthesis was achieved by reaction of aryl glycidyl ethers with Jeffamines, which are primary diamines used in the manufacture of plastics. The following aryl groups were used: 2-allylphenyl, 4-(2-methoxyethyl)phenyl, 1-naphthyl, and 4-methoxyphenyl. The first three correspond to moieties of the established drugs alprenolol, metoprolol, and propranolol, respectively. The affinities of these compounds for β-adrenergic receptors of rat heart and lung were estimated by measuring their ability to compete with the specific binding of (-)-[^{3}H]-dihydroalprenolol. Compounds containing one pharmacophore bound to the receptors with affinities comparable to those of the parent drugs and the blockade of receptors could be dissociated by successive washes as easily as were those of the parent drugs. Compounds containing two or three pharmacophores had somewhat lower affinities for receptors, but the resulting blockade was persistent even after successive washing.

Introduction of two or more of the same pharmacophores into one molecule may produce bioeffects different from those expected on the basis of simple additivity. A potentiation of activity may be expected where a multifunctional drug is able to span several binding sites. Such bridging may easily be obtained where a nucleic acid is the binding site for the drug. Bifunctional intercalators[1,2] and polymeric intercalators[3] of nucleic acids show such increases. For drugs interacting with receptors located on the cell membrane, the derivatives that would be multifunctional and potent are more difficult to obtain; e.g., the difunctional organomercurials,[4] difunctional derivatives of opiates,[5] and polyfunctional catecholamines[6] were described.

Several compounds containing multiple pharmacophores of β-adrenergic antagonist activity were previously prepared and studied without obtaining remarkable potency.[7-10] In contrast to the design of those compounds, in the present series the pharmacophores were connected by chains that were chosen for their relative length, conformational flexibility, and amphiphilicity. Evaluation of the binding of this series to membrane preparations revealed that some of its members block β-adrenergic receptors persistently. Preliminary experiments indicate that similar persistency also may be obtained in vivo. This indicates that application of a similar design may lead to drugs with long-lasting effects. Prolongation of activity of β-adrenergic antagonists is of practical importance and has been intensively investigated. Nadolol, satolol, betaxolol, and FM24 are examples of β-adrenergic blockers with prolonged pharmacological activity that was achieved by other mechanisms.[11-13]

Chart I



Results

Chemistry. The general structure of β-adrenergic antagonists of the aromatic ether type is aryl-$OCH_2CHOHCH_2NH$-alkyl. While compounds with various aromatic residues were found active, the alkyl group has to be secondary or tertiary to obtain a fully active compound.[14]

Primary diamines, substituted by secondary alkyl groups and suitable for the intended syntheses, have been produced in industrial quantities and used as curing agents in the manufacture of plastics under the trade name Jeffamines (Chart I). These compounds are prepared by a catalytic exchange reaction of oligomeric propylene glycols with ammonia. Two different fractions of Jeffamine, D-230 and D-400, were used; these have an average n of 2.6 and 5.6, respectively (Chart I). Thin-layer chromatography resolves Jeffamine D-230 into two major fractions (n = 2 and 3) and Jeffamine D-400 into about five fractions (n = 4-8); these fractions are presumably mixtures of diastereomers.

(1) Chen, T. K.; Fico, R.; Canellakis, E. S. *J. Med. Chem.* **1978**, *21*, 868.
(2) LePeeg, J. B.; Lebret, M.; Barket, J.; Rogues, P. *Proc. Natl. Acad. Sci. U.S.A.* **1975**, *72*, 2915.
(3) Pitha, J. *Eur. J. Biochem.* **1978**, *82*, 285.
(4) Berger, N. A.; Kociolek, K.; Pitha, J. *Biochem. Biophys. Res. Commun.* **1979**, *86*, 1234.
(5) Hazum, E.; Chang, K.-J.; Leighton, H. J.; Lever, O. W.; Cuatrecasas, P. *Biochem. Biophys. Res. Commun.* **1982**, *104*, 347.
(6) Verlander, M. S.; Venter, J. C.; Goodman, M.; Kaplan, N. O.; Saks, B. *Proc. Natl. Acad. Sci. U.S.A.* **1976**, *73*, 1009.
(7) Ozdowska, Z. *Rocz. Chem.*, **1976**, *50*, 371.
(8) Krajewska, M.; Palanowski, R.; Quas de Penno, R.; Wolf, J. Polish Patent 53 677, 1967.
(9) Zaagsma, J.; Nauta, W. T. *J. Med. Chem.* **1977**, *20*, 527.
(10) Pitha, J.; Zjawiony, J.; Lefkowitz, R. J.; Caron, M. G. *Proc. Natl. Acad. Sci. U.S.A.* **1980**, *77*, 2219.
(11) Antonaccio, M. J.; Evans, D. *Drugs Expl. Clin. Res.* **1980**, *6*, 585.
(12) Bianchetti, G.; Blatrix, C.; Gomeni, R.; Kilborn, J. R.; Larribaud, J.; Lucker, P. W.; Thebault, J. J.; Trocherie, S.; Morselli, P. L. *Arzneim.-Forsch.* **1980**, *30*, 1912.
(13) Lucas, M.; Homburger, V.; Dolphin, A.; Bockaert, J. *Mol. Pharmacol.* **1979**, *15*, 588.
(14) Lednicer, D.; Mitscher, L. A. "The Organic Chemistry of Drug Synthesis"; Wiley: New York, 1980; Vol. 2, p 105.

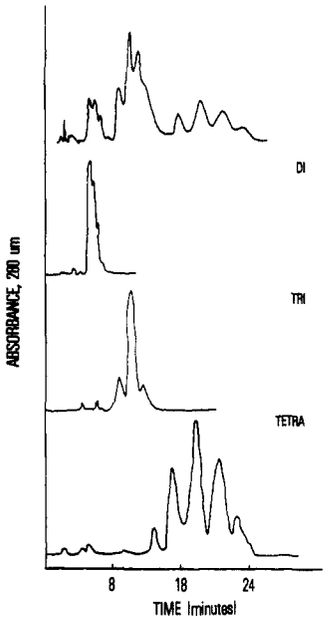


Figure 1. Profiles of high-pressure chromatographic separation of products of the reaction of **5a** with Jeffamine D-230 as monitored by UV absorption at 280 nm; conditions as described in text. At the top is a profile of the reaction mixture followed by di-, tri-, and tetrasubstituted products as isolated by column chromatography; the individual peaks there correspond to compounds with a different n (Chart I).

The Jeffamines were converted by reaction with aryl glycidyl ethers **5a–d** into substituted amines **1a–d** to **4a–d**; 2-propanol was used as a solvent. The corresponding moieties of established β-adrenergic antagonists were used as aryl groups in compounds **5a–d**. Thus, compound **5a** has the aromatic moiety of alprenolol, compound **5c** that of metoprolol, compound **5d** that of propranolol, and compound **5b** that of the previously described blocker.[15] In the reaction of Jeffamine with glycidyl ethers, five different compounds may be formed—one each of mono-, tri-, and tetrasubstituted (structures **1**, **3**, and **4** in Chart I, respectively) and two isomeric disubstituted compounds (structure **2** in Chart I is one of them). Previous studies of the reactions of diamines with epoxides established that pure mono- and tetrasubstituted products can be easily obtained with an excess of diamine or epoxide, respectively, whereas intermediate ratios of reactants led to mixtures that were very difficult to separate.[16] Using the same approach, we easily prepared pure monofunctional **1a** and tetrafunctional products (**4a–d**) (Table I). The mixtures of di- to tetrafunctional products, which were obtained from reaction of intermediate ratios of reactants, had to be separated by chromatography. Chromatography on silica gel with chloroform containing a small amount of methanol (1–10%) as the solvent readily separated tri- and tetrasubstituted derivatives, whereas separation of mono- and di-substituted derivatives was unsatisfactory; derivatives differing only by value of n (Chart I) were not resolved. For isolation of pure difunctional derivatives **2**, additional chromatography on Sephadex LH20 was used. Since these columns have low capacity, the separation was rather tedious even for small quantities of this material.

The degree of substitution found in the products was also influenced by the solvent used in the reaction of Jeffamine with glycidyl ether. Toluene favored the monofunctional derivative, whereas use of 2-propanol led to a higher proportion of di- to tetrafunctional derivatives. Probably the solvent effect has a kinetic foundation, since it is known that an increase in polarity of solvents increases the rate of reaction between amine and epoxide.[16]

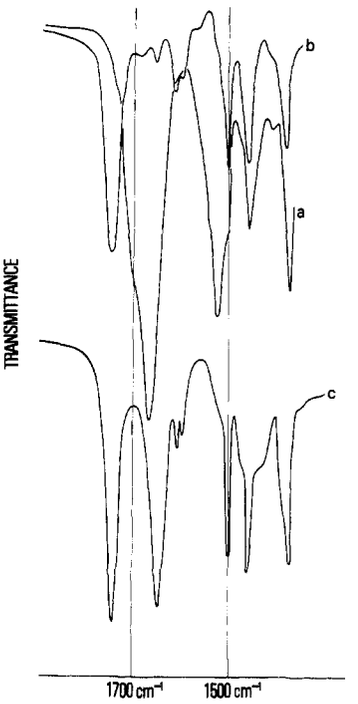


Figure 2. Infrared spectra of acetylated derivatives: curve a, Jeffamine D-230; curve b, compound **4a**; curve c, compound **2a**. The spectra were measured in a chloroform solution.

Eventually, it was found that analytical high-performance liquid chromatography, on μBondapak phenyl column (Waters Associates, Milford, MA) with methanol/chloroform/water (5:3:2 mixture) and 0.1% of 1-butylamine as eluent, led to further separation of the products, as illustrated in Figure 1. In this system, compounds were separated both on the basis of the degree of substitution and the value of n (Chart I), but even then each peak probably represents a mixture of diastereomers. In this manner, the purity of the fractions obtained from preparative chromatography was ascertained (Figure 1).

The products were identified, and the number of substituents per molecule was measured by NMR. Integration of the NMR spectra yielded a ratio of the number of aromatic protons located in the substituents to the number of methyl protons in Jeffamine (Table I), which then was compared with the calculated values. To confirm independently these results, we acetylated all compounds by acetic anhydride under conditions that led to full acetylation of hydroxy groups and to monoacetylation of all amines, except the tertiary amines. Thereafter, infrared spectroscopy was used to establish the number of *N*- and *O*-acetyl residues in these derivatives. *N*-Acetyl residues stem from the Jeffamine part of the molecule and absorb at 1645–1665 cm^{-1} (examples in Figure 2), and *O*-acetyl residues stem from the substituents and absorb at 1735–1740 cm^{-1}; thus, the degree of substitution of Jeffamine could be easily established. Spectra of compounds in chloroform solutions were measured (example in Figure 2), and relative absorbancies of the bands in question were found to be in agreement with the structures proposed.

Furthermore, the infrared spectra of acetyl derivatives were used to derive the structure of disubstituted derivatives (Figure 2). There are two possible structures, sym-

(15) Lovgren, K.; Hedberg, A.; Nilsson, J. L. G. *J. Med. Chem.* **1981**, *24*, 451.
(16) Hilgetag, G.; Martini, A., Eds. "Preparative Organic Chemistry"; Wiley: New York, 1972.

Table I. Preparation and Properties of Compounds Based on Jeffamine D-230 (n = 2.6 in Figure 1)[a]

starting materials		products						
Jeffamine concn, mmol	epoxide (concn, mmol)	no.	R_f[b]	purification[c]	formula	anal.	UV,[d] nm (ε)	NMR,[e] arom H's/molecule
60	5a (10)	1a	0.03	SG	$C_{23}H_{40}N_2O_5$	N	271 (1970) 278 (1790)	found, 3.6 [δ 6.65-7.35 (m)]; calcd, 4
6.1	5a (22.1)	2a	0.15	Seph	$C_{35}H_{54}N_2O_7$	N[f]	271 (3220) 278 (2920)	found, 7.5 [δ 6.6-7.4 (m)]; calcd, 8
6.3	5a (27.4)	3a	0.50	SG	$C_{47}H_{68}N_2O_9$	N	271 (5590) 278 (5160)	found, 11.5 [δ 6.6-7.4 (m)]; calcd, 12
1.16	5a (5.26)	4a	0.93	SG	$C_{59}H_{82}N_2O_{11}$	N	271 (8180) 278 (7360)	found, 15 [δ 6.6-7.4 (m)]; calcd, 16
12.6	5b (50)	3b	0.11	SG	$C_{41}H_{62}N_2O_{12}$	N	288 (7980)	found, 12 [δ 6.86 (m)]; calcd, 12
		4b	0.41	SG	$C_{51}H_{74}N_2O_{15}$	N	288 (10 400)	found, 16 46 [δ 6.80 (m)]; calcd, 16
12.6	5c (50)	3c	0.19	SG	$C_{47}H_{74}N_2O_{12}$	N	276 (4230) 282 (3560)	found, 11.3 [δ 6.80 (d, J = 9 Hz), 7.10 (d, J = 9 Hz)]; calcd, 12
		4c	0.40	SG	$C_{59}H_{90}N_2O_{15}$	N	276 (6410) 282 (5400)	found, 16 [δ 6.82 (d, J = 9 Hz), 7.13 (d, J = 9 Hz)]; calcd, 16
12.6	5d (50)	3d	0.32	SG	$C_{50}H_{62}N_2O_9$	N	293 (13 800) 320 (4870)	found, 20.4 [δ 6.55-7.00 (m), 7.10-7.65 (m), 7.65-7.95 (m), 7.65-7.95 (m)]; calcd, 21
		4d	0.58	SG	$C_{63}H_{74}N_2O_{11}$	N	293 (20 500) 320 (7290)	found, 26.3 [δ 6.53-6.93 (m), 7.10-7.55 (m), 7.55-7.95 (m), 8.00-8.46 (m)]; calcd, 28

[a] Compounds derived from Jeffamine D-400 were prepared analogously. [b] Thin-layer chromatography on silica gel with chloroform/methanol mixtures (9:1) for 1a-4a or (10:1) for the remaining compounds. [c] Column chromatography; from silica gel (sg) column, compounds were eluted with chloroform/methanol mixtures; from Sephadex LH20 (Seph) column, the mixture of chloroform/methanol/water (2:5.5:2.5) was used. [d] Measured in ethanol. [e] Spectra in deuteriochloroform; Me_4Si used for calibration. The number of aromatic protons observed in the products was related to the number of methyl protons of Jeffamine. Abbreviations used are: s, singlet; d, doublet; m, multiplet. [f] N: calcd, 4.56; found, 4.11.

metrical (structure **2** in Chart I) and unsymmetrical, which has one NH_2 group. The infrared spectra of the acetylated disubstituted fraction lacked absorptions attributable to amide NH bonds that would have had to be present in the unsymmetrical form. Thus, the isolated disubstituted derivatives have a symmetrical structure (**2** in Chart I). The validity of the procedure was checked by acetylation of the starting Jeffamine; expected absorptions of N–H amidic bond in that derivative were found at 3450 (stretching vibration), 3345 (stretching vibration of associated form), and 1525 (amide II band) cm^{-1}.

Pharmacology. The affinity of the compounds for β-adrenergic receptors was determined by competition with the specific binding of (–)-[^{3}H]dihydroalprenolol. Membranes isolated from rat hearts and lungs were used as a source of receptors; the former have 65% β_1- and 35% β_2-adrenergic receptors, whereas the latter have 20% β_1- and 80% β_2-adrenergic receptors.[17] After they were incubated with membranes for 20 min at 30 °C, we evaluated the compounds binding by measuring their ability to displace (–)-[^{3}H]dihydroalprenolol (5 nM), which was also present in the incubation mixture, from membranes; results are given in Table II. Compared to parent drugs, represented in Table I by (±)-alprenolol, binding of these Jeffamine derivatives is 5- to 10 333-fold weaker. The order of potency of derivatives of the lowest molecular weight Jeffamine fraction tested (n = 2.6) was: monosubstituted **1a** ≥ disubstituted **2a** > trisubstituted **3a** > tetrasubstituted **4a**. The variation of the aromatic moiety (**a**–**d** in Chart I) caused some changes in potency as well. Alprenolol analogues (**a**) were the most potent, followed by propranolol (**d**) and metoprolol analogues (**c**). There was no remarkable β_1-adrenergic receptor selectivity in any of the prepared compounds. Monosubstituted drugs derived from the higher molecular weight Jeffamine (n value of 5.6, Chart I) had lower potency than those with n values of 2.6.

(17) Nahorshi, S. R. *Trends Pharmacol. Sci.* **1981**, *2*, 95.

Table II. Dissociation Constants (K_D) of Jeffamine Derivatives for Rat β-Adrenergic Receptors[a]

compd (n)	heart membranes	lung membranes
dl-alprenolol	1.51×10^{-8}	1.70×10^{-8}
1a, n = 2.6	$4.57 \times 10^{-8} \pm 2.13 \times 10^{-8}$	$6.21 \times 10^{-8} \pm 1.05 \times 10^{-8}$
2a, n = 2.6	$1.37 \times 10^{-7} \pm 0.60 \times 10^{-7}$	$1.80 \times 10^{-7} \pm 0.48 \times 10^{-7}$
3a, n = 2.6	$6.01 \times 10^{-6} \pm 1.5 \times 10^{-6}$	$2.13 \times 10^{-6} \pm 0.68 \times 10^{-6}$
4a, n = 2.6	$>1 \times 10^{-4}$	$>1 \times 10^{-4}$
3b, n = 2.6	$4.01 \times 10^{-5} \pm 1.72 \times 10^{-5}$	$2.67 \times 10^{-5} \pm 2.67 \times 10^{-5}$
4b, n = 2.6	$>1 \times 10^{-4}$	$>1 \times 10^{-4}$
3c, n = 2.6	$2.56 \times 10^{-5} \pm 0.24 \times 10^{-5}$	$3.48 \times 10^{-5} \pm 0.88 \times 10^{-5}$
4c, n = 2.6	$>1 \times 10^{-4}$	$>1 \times 10^{-4}$
3d, n = 2.6	$4.28 \times 10^{-6} \pm 1.40 \times 10^{-6}$	$4.83 \times 10^{-6} \pm 1.20 \times 10^{-6}$
4d, n = 2.6	$3.57 \times 10^{-6} \pm 0.21 \times 10^{-6}$	$1.03 \times 10^{-5} \pm 0.27 \times 10^{-6}$
1a, n = 5.6	$7.95 \times 10^{-8} \pm 1.73 \times 10^{-8}$	$1.64 \times 10^{-7} \pm 0.65 \times 10^{-7}$
1b, n = 5.6	$2.85 \times 10^{-5} \pm 5 \times 10^{-8}$	$4.07 \times 10^{-5} \pm 1.13 \times 10^{-5}$
1c, n = 5.6	$5.29 \times 10^{-6} \pm 2.30 \times 10^{-6}$	$1.74 \times 10^{-5} \pm 0.80 \times 10^{-5}$
1d, n = 5.6	$2.63 \times 10^{-7} \pm 0.88 \times 10^{-8}$	$4.25 \times 10^{-7} \pm 0.95 \times 10^{-5}$

[a] Results are the average of at least two experiments done in triplicate plus or minus standard error of the mean.

The persistency of binding was determined as follows. Membranes were preincubated with compounds at concentrations approximately 10–50 times their K_D values for 1 h at 30 °C and then centrifuged. Then the drug was dissociated from membranes by five serial washes, each of which included resuspension of membranes in buffer and incubation of membranes for 10 min at 30 °C, followed by centrifugation. After these washes, the specific binding of (–)-[^{3}H]dihydroalprenolol was measured (20 min, 30 °C

Table III. Persistence of Specific Binding of β-Adrenergic Antagonists to Their Receptors[a]

compound (concn, M)	% of specific binding blocked by drug after serial washing: heart membranes	lung membranes
Jeffamine, n = 2.6 (1×10^{-5})	0	0
Jeffamine, n = 5.6 (1×10^{-5})	0	0
dl-alprenolol (1×10^{-5})	0	6.7
1a, n = 2.6 (1×10^{-6})	3.8	14
2a, n = 2.6 (1×10^{-6})	42.5	77
3a, n = 2.6 (1×10^{-5})	26	68
1a, n = 5.6 (1×10^{-6})	0	0
1a + *dl*-alprenolol[b]	1.7	20
2a + *dl*-alprenolol[b]	42	66
3a + *dl*-alprenolol[b]	20	66

[a] Membranes were preincubated for 1 h at 30 °C with the indicated concentration of compounds. The membranes were then washed five times as outlined under Experimental Section and assayed for specific [^{3}H]dihydroalprenolol binding. The results are expressed as the percent inhibition of binding at 5 nM (-)-[^{3}H]dihydroalprenolol. Each value is the average of at least two experiments assayed in triplicate. [b] Concentrations as in the above experiments.

incubation) and compared with untreated membranes that had undergone the serial washes; results are given in Table III. In membranes pretreated with (±)-alprenolol or monosubstituted derivative **1a**, specific binding was only slightly less than that of control membranes after this washing procedure. In contrast, membranes pretreated with di- and trisubstituted derivatives (**2a**, **3a**) exhibited specific binding that was reduced between 26 and 77% of control membranes.

Discussion

The present results may be summarized as follows: the monofunctional antagonists studied were bound efficiently to the receptors but their blocking action could be easily dissociated, whereas multifunctional antagonists were bound in a fashion that was of weaker affinity but the blockade achieved was very persistent. The most plausible explanation of this phenomenon is that, at first, a complex between one molecule of a multifunctional antagonist and a receptor is formed, which is then gradually converted into a complex of one antagonist molecule and several components of the membrane, of which at least one is a drug receptor; such a multipoint attachment complex can obviously resist attempts to dissociate it more effectively than a single point attachment complex.

In this context it may be noted that cell-surface receptors for peptide hormones have lateral mobility on the membrane surface, and hormone binding leads to a gradual aggregation of these receptors.[18] We feel that the observed persistent blockade is a specific phenomenon. This view is supported by the following facts: (a) Preincubation of membranes with unsubstituted Jeffamine or alprenolol did not result in any change in receptor binding (Table III). (b) Only di- and trisubstituted members of the series (2 and 3 in Chart I) showed this persistent blockade; mono- and tetrasubstituted members (1 and 4 in Chart I) were nonpersistent and inactive, respectively. The former derivative, being monosubstituted, is not capable of multipoint attachment, whereas the latter lacks a high-affinity pharmacophore unit (i.e., a secondary amino function). Since all nonspecific properties of the members in the series may be expected to change gradually, e.g., lipophilicity, the persistent blocking activity of middle members of the series has to have specific origin. (c) In preliminary in vivo experiments,[19] compound **3a** was injected into rats (30 mg/kg); membranes prepared from animals 16 h later had a considerable number of receptors blocked (59% heart, 84% lung). No such blockade was observed in control experiments, where alprenolol or unsubstituted Jeffamine was injected at similar doses.

In preliminary experiments, both in vitro and in vivo, the binding of compound **2a** was analyzed by linear rearrangement of saturation isotherms (i.e., Scatchard analysis). The results suggest a competitive type of inhibition and reduced dissociation of ligand.

Experimental Section

All compounds designated as pure showed a single spot on thin-layer chromatography on silica gel with chloroform and an admixture of methanol as solvent, when detected by UV light. Proton NMR spectra were obtained with a Varian A 60 spectrometer; deuteriochloroform was used as solvent with tetramethylsilane as internal reference. Ultraviolet spectra were obtained with a Cary Model 14 recording spectrophotometer and measured in ethanol. Infrared spectra were obtained with a Beckman IR-12 spectrophotometer and measured in carbon tetrachloride or in potassium bromide pellets.

Jeffamines were kindly donated by Dr. Howard P. Klein of Texaco Chemical Co., Bellaire, TX, and the compounds were used as received. The average values of n for the Jeffamines were measured by comparison of integrated NMR spectra and found to be in agreement with the supplied data. Compound **5c** was a gift from Dr. Enar Carlsson of Hässle Pharmaceutical Co., Sweden.

Preparation of Aryl Glycidyl Ethers 5a–d. To a solution of 1-chloro-2,3-epoxypropane (148 g, 1.6 mol) in methanol (480 mL) was added dropwise a solution of the corresponding phenol (0.4 mol) and NaOH (0.48 mol) in water (700 mL). After the solution was stirred overnight, the volatile organics were evaporated in vacuo with a water bath, and the product was extracted with ethyl acetate (300, 150, and 150 mL). Extracts were washed with NaOH solution (10%, 100 mL) and with H_2O and then dried with Na_2SO_4. After evaporation of the solvent, products were distilled in vacuo. Compound **5a**: bp 109–114 °C (0.8 mmHg; yield 73%. Compound **5d**: bp 145–149 °C (0.5 mmHg); yield 70% (compare references given in ref 11).

Reaction of Aryl Glycidyl Ethers 5a–d with Jeffamine. The solution of aryl glycidyl ether (50 mmol) and Jeffamine (quantity as given in Table I) in 2-propanol (30 mL) was refluxed for 5 h, organic volatiles were then removed by distillation in vacuo, and the residue was purified by column chromatography on silica gel or Sephadex LH20 as described in Table I. Fractionation was followed by thin-layer chromatography in the same system.

Binding Assay. Rat lung and heart membranes were prepared as described previously.[20] The binding of (-)-[^{3}H]dihydroalprenolol to membranes and the determination of K_D values were performed as follows. Substrate (5 nM final concentration, 35–54 Ci/mmol) was incubated with membranes (ca. 150 μg of protein for lung, ca. 500 μg of protein for heart) in a total volume of 250 μL of buffer (50 mM Tris, 10 mM $MgCl_2$, pH 7.4). Triplicate incubations were carried out at 30 °C for 20 min. The reactions were stopped by rapid dilution with 4 mL of ice-cold buffer and immediate filtration through Whatman GF/C filters. The reaction tubes and filters were washed an additional three times with 4 mL of buffer. The dried filters were then counted in a liquid scintillation counter. Specific binding is defined as the difference between binding in the absence and presence of 1×10^{-5} M (±)-aprenolol. The inhibitory potency of the Jeffamine derivatives was determined by adding aliquots of serial 1:10 dilutions of the

(18) Willingham, M. C.; Pastan, I. *Cell* **1980**, *21*, 67.

(19) Kusiak, J. W.; Pitha, J., unpublished results.

(20) Kusiak, J. W.; Pitha, J. *Biochem. Pharmacol.* **1982**, *31*, 2071.

derivatives to substrate prior to the addition of membranes. The specific binding was then measured, IC_{50} concentrations were determined graphically, and K_D values were calculated as described.[21]

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Registry No. **1a**, 83650-73-1; **2a**, 83650-74-2; **3a**, 83650-75-3; **3b**, 83650-77-5; **3c**, 83650-79-7; **3d**, 83650-81-1; **4a**, 83650-76-4; **4b**, 83650-78-6; **4c**, 83650-80-0; **4d**, 83681-19-0; **5a**, 4638-04-4; **5d**, 2461-42-9; 1-chloro-2,3-epoxypropane, 106-89-8; *o*-allylphenol, 1745-81-9; α-naphthol, 90-15-3; jeffamine, 9046-10-0.

(21) Cheng, Y.-C.; Prusoff, W. H. *Biochem. Pharmacol.* **1973**, *22*, 3099.

Adriamycin Analogues. Preparation and Biological Evaluation of Some Novel 14-Thiaadriamycins[1]

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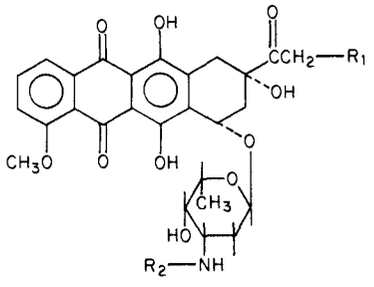
Condensation of 14-bromodaunorubicin with thiols in methanol, in the presence of potassium carbonate, resulted in the formation of 14-thia analogues of the antitumor antibiotic adriamycin. However, similar condensation of *N*-(trifluoroacetyl)-14-iododaunorubicin with thiols invariably led to a redox reaction, with the formation of *N*-(trifluoroacetyl)daunorubicin and disulfides. Accordingly, *N*-(trifluoroacetyl)-14-bromodaunorubicin was used for reaction with thiols to yield thia analogues of the clinically active but non-DNA-binding adriamycin analogue *N*-(trifluoroacetyl)adriamycin 14-valerate (AD 32). Reaction of 14-bromodaunorubicin with α,ω-alkanedithiols gave bis(thiaadriamycin) analogues as potential difunctional intercalating agents. The aforementioned products, plus two related phenylselena derivatives, were examined for in vitro growth inhibition, in vivo antitumor activity, and, where appropriate, DNA binding. A number of agents, most notably 14-(carbethoxymethyl)-14-thiaadriamycin and *N*-(trifluoroacetyl)-14-phenyl-14-selenaadriamycin, were active against murine L1210 leukemia in vivo. Several of the amino glycoside unsubstituted 14-thiaadriamycin analogues exhibited DNA-binding properties equivalent to those of adriamycin.

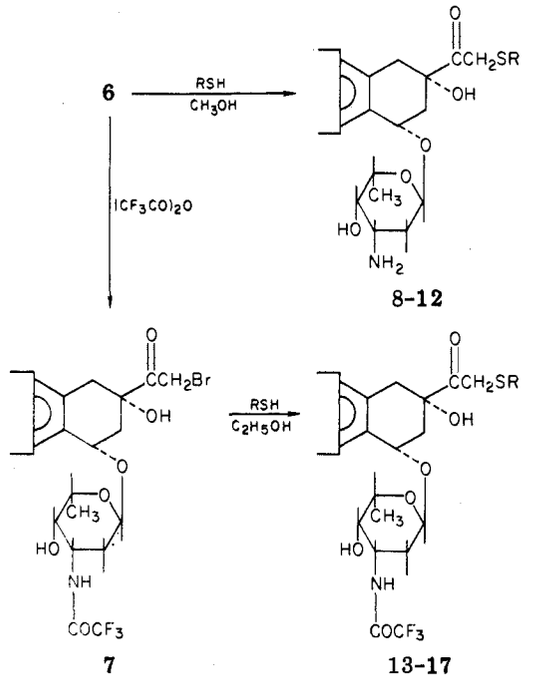
For some time these laboratories have been involved in the search for analogues of the clinically important antitumor antibiotics daunorubicin (**1**) and adriamycin (**2**) and



1, R_1 = H; R_2 = H (daunorubicin)
2, R_1 = OH; R_2 = H (adriamycin)
3, R_1 = $OCO(CH_2)_3CH_3$; R_2 = $COCF_3$
4, R_1 = I; R_2 = $COCF_3$
5, R_1 = H; R_2 = $COCF_3$
6, R_1 = Br; R_2 = H
7, R_1 = Br; R_2 = $COCF_3$

in the determination of the structure–activity correlates among this family of compounds. In connection with this program, we have now prepared a number of hitherto unknown 14-thia- and 14-selenaadriamycin derivatives and have evaluated these products for in vitro cell growth inhibitory activity, for in vivo antileukemic activity in a murine tumor model, and, where appropriate, for their ability to interact with DNA. Like the parent agents (**1** and **2**), some of the target compounds reported here possess an unsubstituted glycosidic amino function, a molecular feature claimed to be essential for DNA binding and resultant antitumor activity.[2-4] These products in-

Scheme I



(1) A preliminary report on this work has appeared: Seshadri, R.; Pegg, W. J.; Israel, M. "Abstracts of Papers", 175th National Meeting of the American Chemical Society, Anaheim, CA, Mar 1978; American Chemical Society: Washington, DC, 1978; Abstr MEDI 47.

(2) DiMarco A., & Lenaz, L. In "Cancer Medicine", Holland, J. F.; Frei III, E., Eds., Lea & Febiger: Philadelphia, 1973; pp 826–835.

(3) Skovsgaard, T.; Nissen, N. I. *Dan. Med. Bull.* **1975**, *22*, 62.

0022-2623/83/1826-0011$01.50/0