Ringer solution at room temperature in an organ bath of 30-mL capacity. The tissue was aerated with a gaseous mixture of 95% O_2 and 5% CO_2. The force of contraction and the rate of contraction were recorded on a two-channel Hellige recorder through a strain gauge. An initial tension of 0.5 to 1 g was given to the preparation. Stabilization time for the preparation was 30 min. Forskolin and its derivatives were dissolved in propylene glycol (concentration 1 mg/mL). Further dilutions were made in distilled water. After taking a basal response, the test compounds were added to the organ bath at doses mentioned below. The volume of the bath was always kept constant. A contact time of 10 min for each compound was given. Inotropic activity was calculated as percent increase over the initial value. EC_{50} values were calculated according to the method of Litchfield and Wilcoxon[19] from four to six dose levels with six atrium preparations per dose. A dose–response relationship for forskolin was achieved with 5, 10, 30, 100, 300, and 600 ng/mL dose levels. The forskolin derivatives were assessed at a dose of 100 ng/mL. Isoprenaline sulfate was used as a standard drug. Its EC_{50} value was 1.15×10^{-9} M (6.6×10^{-10} to 1.94×10^{-9} M).

Acknowledgment. We thank Dr. P. K. Inamdar for analytical and spectroscopic data and V. Shah for the supply of plant material. The skilful technical assistance provided by S. L. Kattige, R. Rajgopalan, R. D. Gupte, V. A. Aroskar, and G. Moraes is gratefully acknowledged.

(19) J. T. Litchfield and F. Wilcoxon, *J. Pharmacol. Exp. Ther.*, **96**, 99 (1949).

Registry No. 1, 66575-29-9; **2**, 64657-30-3; **3**, 81873-11-2; 4, 64657-22-3; **5**, 84048-17-9; **6**, 84048-18-0; **7**, 84010-19-5; **8**, 81873-10-1; **9**, 81873-08-7; **10**, 64657-20-1; 11, 84048-19-1; **12**, 81873-09-8; **13**, 84049-21-8; 14, 84010-20-8; **15**, 84048-20-4; **16**, 84010-21-9; **17**, 84010-22-0; **18**, 84010-23-1; **19**, 84048-28-2; **20**, 84048-29-3; 21, 84010-24-2; **22**, 64657-24-5; **23**, 84010-25-3; **24**, 81826-89-3; **25**, 81010-26-4; **26**, 84010-27-5; **27**, 81873-07-6; **28**, 81873-12-3; **29**, 75879-73-1; **30**, 84010-28-6; **31**, 84048-21-5; **32**, 84048-22-6; **33**, 64657-18-7; **34**, 81826-81-5; **35**, 64657-23-4; **36**, 84010-29-7; **37**, 64657-21-2; **38**, 84048-23-7; **39**, 84048-24-8; **40**, 84048-25-9; 41, 84048-26-0; **42**, 81826-82-6; **43**, 81873-13-4; **44**, 81873-14-5; **45**, 84010-30-0; **45** (free base), 84010-31-1; **46**, 84010-32-2; **47**, 84010-33-3; **47** (free base), 84048-27-1; **48**, 84010-34-4; **49**, 81873-15-6; **50**, 81873-17-8; **51**, 81826-83-7; **52**, 84010-35-5; **53**, 81873-16-7; **54**, 84010-36-6; benzoyl chloride, 98-88-4; phenyl isocyanate, 931-54-4; trimethylchlorosilane, 75-77-4; *m*-chlorobenzoic acid, 535-80-8; isopropylamine, 75-31-0; 1-deoxy-1-oxo-14,15-dihydroforskolin, 64657-25-6; hydroxyamine hydrochloride, 5470-11-1; 1-deoxy-1-oximino-14,15-dihydroforskolin, 84010-37-7; 1,7-dideoxy-1,7-dioximino-6-acetyl-14,15-dihydroforskolin, 84010-38-8; 2-(diethylamino)ethyl chloride, 100-35-6.

Conjugates of Catecholamines. 1. *N*-Alkyl-Functionalized Carboxylic Acid Congeners and Amides Related to Isoproterenol

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A series of functionalized catecholamines (congeners) has been synthesized in which, formalistically, the *N*-isopropyl group of isoproterenol has been extended by a linear alkyl chain of varying length, terminated by a carboxy group or a substituted amide. The compounds were prepared generally via the reductive amination of norepinephrine with a keto acid or a preformed keto amide. An alternate synthesis of the model amide derivatives, involving activation of the carboxylic acid congeners and coupling with amines, was complicated in the case of short-chain derivatives by facile cyclization to lactams. In vitro evaluation of these compounds as potential β-adrenergic agonists has shown that, while the carboxylic acid congeners have relatively low potencies, the model amide derivatives have potencies that are highly dependent on both the length of the alkyl chain and also the nature of the substituent on the amide. In general, aromatic amides are the most potent, although the nature and position of substituents on the aromatic group dramatically influences their potency. The implications of these studies, in terms of general β-adrenergic drug design and also the attachment of the carboxylic acid congeners to carriers, are discussed.

The use of natural or synthetic polymers to modify the activity of biologically active molecules has been the subject of increasing attention in recent years. In particular, the possibility of increasing the therapeutic index of drugs through covalent attachment to polymers offers exciting potential.[1-3] To date, a wide variety of drugs have been attached to polymeric carriers with promising results in many cases, though, as yet, no commercial product has resulted from these studies. This may, in part, be attributed to the difficulty in characterizing completely a conjugate formed between a small drug molecule and a polymeric carrier, which is usually high molecular weight and polydisperse.

Catecholamines and related compounds have been attached to both insoluble carriers, such as porous glass[4] and Sepharose,[5,6] and soluble carriers, such as polypeptides[6,7] and proteins.[6] Although many of these derivatives have shown interesting and reproducible in vitro and in vivo biological activity, none of the systems studied lends itself to a systematic investigation aimed at determining the role of the carrier in affecting the activity of the drug to which it is attached in the conjugate. For example, attempts to characterize previously prepared conjugates of random copolypeptides with isoproterenol have proven to be difficult.[8]

† University of California, San Diego.
‡ Stanford University Medical Center.

(1) H. Ringsdorf, *J. Polym. Sci., Polym. Symp.*, **no. 51**, 135–153 (1975).
(2) L. G. Donaruma, *Prog. Polym. Sci.*, 4, 1–25 (1975).
(3) H.-G. Batz, *Adv. Polym. Sci.*, **23**, 25–53 (1977).
(4) J. C. Venter, J. E. Dixon, P. R. Maroko, and N. O. Kaplan, *Proc. Natl. Acad. Sci. U.S.A.*, **69**, 1141–1145 (1972).
(5) M. S. Yong and J. B. Richardson, *Can. J. Physiol. Pharmacol.*, **53**, 616–628 (1975).
(6) K. L. Melmon, Y. Weinstein, B. M. Shearer, H. R. Bourne, and S. Bauminger, *J. Clin. Invest.*, **53**, 22–30 (1974).
(7) M. S. Verlander, J. C. Venter, M. Goodman, N. O. Kaplan, and B. Saks, *Proc. Natl. Acad. Sci. U.S.A.*, **73**, 1009–1012 (1976).

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Table I. Synthesis of Carboxylic Acid Congeners of Isoproterenol

3,4-$(HO)_2C_6H_3$–$CH(OH)CH_2\overset{+}{N}H_2CH(CH_3)(CH_2)_nCOO^-$

no.	*n*	yield,[a] %
6	2	51
7	3	50
8	4	65
9	5	*b*

[a] Prior to purification by HPLC. [b] Nearly quantitative.

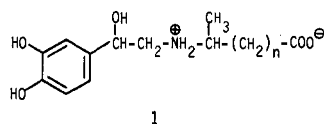
We therefore initiated a detailed investigation of methods by which the catecholamine molecule could be functionalized to form congener molecules that could be attached subsequently to a carrier molecule to form a conjugate. Initial studies focused on the catechol ring,[9,10] but subsequent evaluation of these derivatives in vitro revealed that none had sufficiently high activity to warrant further investigation.

Our attention, therefore, turned to the ethanolamine side chain of the molecule. While the benzylic alcohol function is considered essential for activity, substituents on the amine function may be varied substantially with maintenance of activity.[11] The selectivity (α- vs. β-adrenergic activity) of the molecule may be affected by varying the N substituent, but the biological activity is rarely destroyed, unless the amine is acylated.

In this paper, we describe the synthesis of a series of catecholamine derivatives in which the *N*-isopropyl group of isoproterenol has been derivatized by a methylene chain terminated by a carboxy group or substituted amide. These carboxylic acid congeners were synthesized for attachment to peptide carriers to form conjugates, the synthesis and biological activity of which will be reported elsewhere. While the carboxylic acid derivatives have relatively low potency, a series of substituted amides prepared as models for peptide conjugates showed interesting and unique biological activity when tested in both in vitro and in vivo test systems.

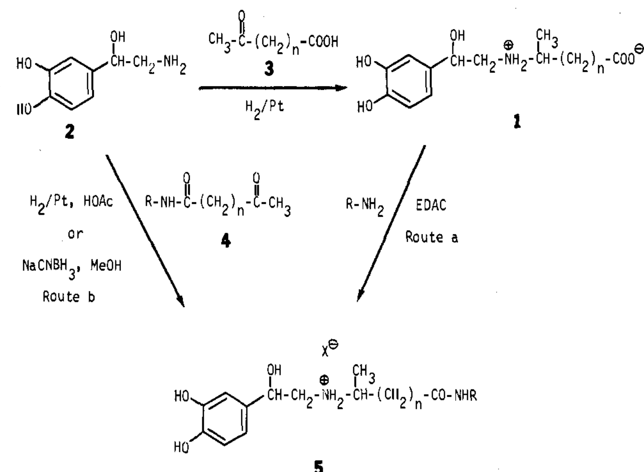
Results and Discussion

Carboxylic Acid Congeners. The catecholamine derivatives (1) contain a carboxy-functionalized methylene



chain of varying length, which was designed specifically as a "handle" for covalent attachment to peptides or other carriers through, for example, formation of an amide bond. Related compounds have been prepared previously, including carboxylic acid derivatives with other aliphatic configurations[12] and also ester derivatives.[13]

Scheme I



The carboxylic acid congeners 1 (Table I) were prepared by the reductive amination of norepinephrine (free base) (2) with an excess of the appropriate keto acid 3[14,15] over Adam's catalyst [platinum dioxide (Scheme I)] in methanol–acetic acid mixtures. Racemic norepinephrine was used in all cases. Since a second, optically active center is generated during the reductive amination, the products were actually mixtures of diastereomers. No attempt was made to separate these mixtures. Congeners in which *n* is 4 or 5, compounds 8 and 9, tended to precipitate from water or methanol. Separation of the catalyst after reductive amination then became a problem. For this reason, the hydrogenation was carried out in glacial acetic acid in which compounds 8 and 9 were soluble.

Model Amides of Carboxylic Acid Congeners. Amide derivatives (5, Scheme I) of the carboxylic acid congeners were synthesized as model compounds for their attachment to carriers. The congeners were linked to small aliphatic or aromatic amines (RNH_2) through their carboxylic acid groups. Scheme I outlines two alternate routes that were used to synthesize these amide model compounds: (a) reaction of the congener with the amine RNH_2 or (b) reductive amination of the appropriate, preformed keto amide 4 with norepinephrine. Although the former route might seem more efficient, i.e., requires fewer synthetic steps for a series of amides derived from the same congener, in practice, the latter route was experimentally more successful, mainly because of the potential of congeners with two and three methylenes to cyclize to lactams (see below). The route with reductive amination as the final step, i.e., route b, was therefore used most frequently for the synthesis of amide model compounds.

The keto amides (4, Scheme I) were prepared by standard carbodiimide[16,17] or mixed anhydride[18] methods (Table II). The reductive amination was carried out with either platinum dioxide[19] in acetic acid or trifluoroethanol (no reaction occurred in methanol) or with sodium cyanoborohydride[20] in methanol as solvent. Racemic nor-

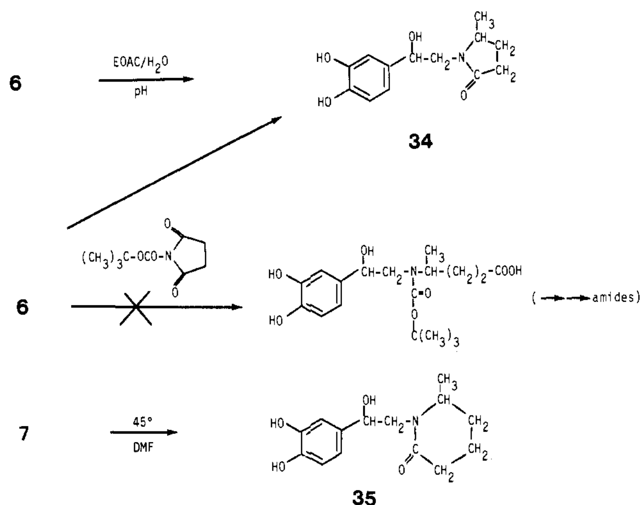
(8) M. Goodman, M. S. Verlander, K. L. Melmon, K. A. Jacobson, A. B. Reitz, J. P. Taulane, M. A. Avery, and N. O. Kaplan, unpublished results.
(9) M. A. Avery, M. S. Verlander, and M. Goodman, *J. Org. Chem.*, **45**, 2750–2753 (1980); *ibid.*, **46**, 5454 (1981).
(10) A. B. Reitz, M. A. Avery, M. S. Verlander, and M. Goodman, *J. Org. Chem.*, **46**, 4859–4863 (1981).
(11) D. J. Triggle, in "Medicinal Chemistry", A. Burger, Ed., Wiley-Interscience, New York, 1970, pp 1236–1295.
(12) H. Smith and B. Cantello (Beecham Group Ltd.), British Patent 1360457; *Chem. Abstr.*, 81, 120231*v* (1974).
(13) N. V. Philips Gloelampenfabriken, British Patent 927896; *Chem. Abstr.*, **59**, 13880*a* (1963).
(14) J. R. Schaeffer and A. O. Snoddy, *Org. Synth.*, 31, 3–5 (1951).
(15) C. R. Hauser, F. W. Swamer, and B. I. Ringler, *J. Am. Chem. Soc.*, **70**, 4023–4026 (1948).
(16) D. G. Hoare and D. E. Koshland, Jr., *J. Am. Chem. Soc.*, **88**, 2057–2058 (1966).
(17) J. C. Sheehan and G. P. Hess, Jr., *J. Am. Chem. Soc.*, **77**, 1067–1068 (1955).
(18) G. W. Anderson, J. E. Zimmerman, and F. M. Callahan, *J. Am. Chem. Soc.*, **89**, 5012–5017 (1967).
(19) W. S. Emerson, *Org. React.*, 4, 174–255 (1948).

Table II. Synthesis of Keto Amides[a] of the General Formula $CH_3CO(CH_2)_nCONRR'$

no.	*n*	R	R'	name	method[b]	yield, %	mp, °C	anal.
10	2	H	p-C_6H_4-CH_3	4-oxo-*n*-pentano-*p*-toluidide	C	63	103–104[c]	C, H, N
11	3	H	p-C_6H_4-CH_3	5-oxo-*n*-hexano-*p*-toluidide	B	82	121.5–122	C, H, N
12	4	H	p-C_6H_4-CH_3	6-oxo-*n*-heptano-*p*-toluidide	A	70	106–107.5	C, H, N
13	4	CH_3	p-C_6H_4-CH_3	*N*-methyl-6-oxo-*n*-heptano-*p*-toluidide	C	96	oil	C, H, N
14	4	H	p-C_6H_4-$(CH_2)_3CH_3$	4'-*n*-butyl-6-oxo-*n*-heptano-*p*-anilide	C	68	85–86	C, H, N
15	4	H	o-C_6H_4-CF_3	2'-(trifluoromethyl)-6-oxo-*n*-heptano-*o*-anilide	C[d]	60	78–80	C, H, N
16	4	H	m-C_6H_4-CF_3	3'-(trifluoromethyl)-6-oxo-*n*-heptano-*m*-anilide	C	80	101.5–103	C, H, N
17	4	H	p-C_6H_4-CF_3	4'-(trifluoromethyl)-6-oxo-*n*-heptano-*p*-anilide	C	57	139–140	C, H, N
18	4	H	p-C_6H_4-OCH_3	4'-methoxy-6-oxo-*n*-heptano-*p*-anilide	A	47	111–113	C, H, N
19	4	H	$(CH_2)_3CH_3$	*N*-6-oxo-*n*-heptanamide	C	71	49–50	C, H, N
20	4	H	c-C_6H_{11}	*N*-cyclohexyl-6-oxo-*n*-heptanamide	C	83	109–110	C, H, N

[a] Proton NMR spectra were obtained for each compound.[26] [b] Methods: A, dicyclohexylcarbodiimide in THF; B, EDAC in H_2O; C, mixed anhydride in THF. [c] Literature[32] mp 108–109 °C. [d] 1 equiv of HOBt and 1.8 equiv of base added; reflux (THF) 3 days.

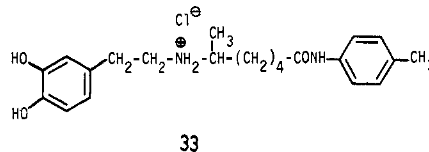
Scheme II



epinephrine was used in all cases.

Because of the maximal biological activity of the *p*-toluidide **23**, containing four methylenes (see below), our comparative studies of different substituent groups, R, in the model amides **5** concentrated on derivatives containing four methylenes (Table III). *p*-Toluidine was used as the model amine for the side chain of *p*-aminophenylalanine in a peptide carrier. Similarly, *n*-butylamine was used as a model for the side chain of lysine. A number of other amide derivatives were also synthesized in order to explore the effect of electron-donating and electron-withdrawing substituents and their position on the aromatic ring (Table III, **26–30**), as well as the steric effect and hydrophobicity of alkyl-substituted amides (compound **32**).

It is known that benzylic alcohols are reduced under some conditions of catalytic hydrogenation.[21] It was therefore important to prove that the catecholamines isolated, among both the carboxylic acid congeners and the model compounds, still contained the benzylic alcohol grouping, since NMR evidence was inconclusive. Compound **33**, the dopamine analogue of compound **23**, was



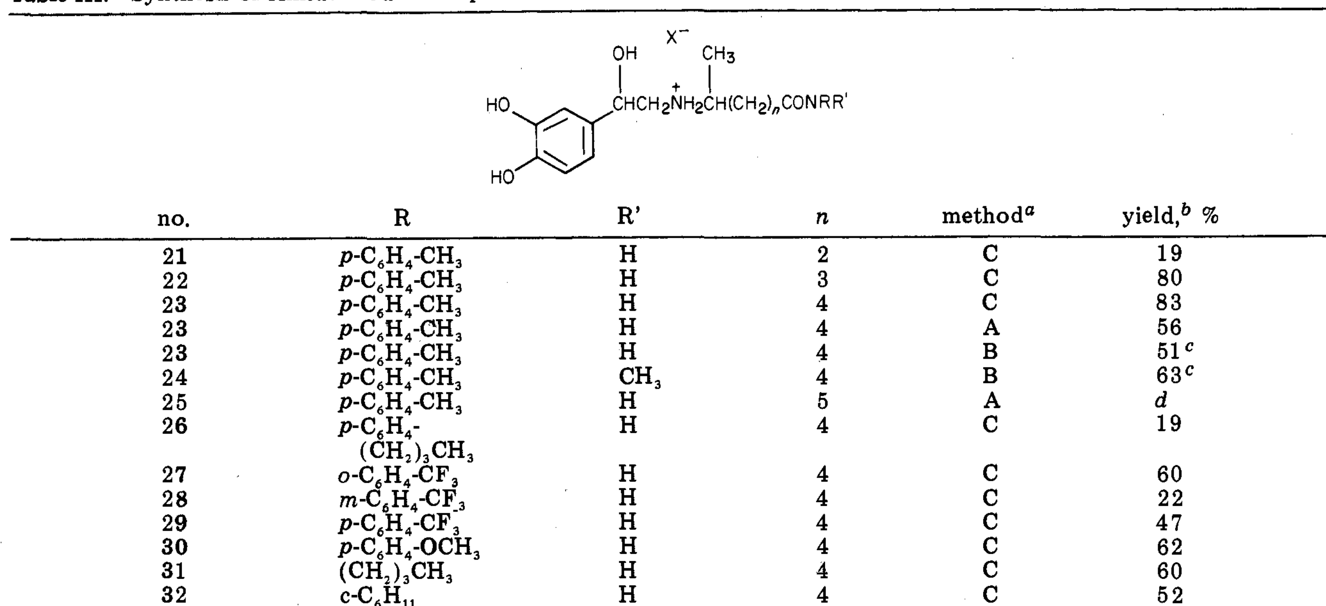
prepared by reductive amination of the keto amide **12** with dopamine. This dopamine derivative was not detected as a side product in the synthesis of compound **23**.

The route of direct coupling of the congener to an amine (route a) succeeded only with those congeners having four or five methylenes between the carboxy and the carbon α to the amine. Either of these congeners, compounds **8** and **9**, could be coupled selectively to *p*-toluidine hydrochloride with the water-soluble carbodiimide, 1-ethyl-3-[3-(dimethylamino)propyl]carbodiimide hydrochloride (EDAC),[16] at pH 5. The yields were comparable to those from the route in which reductive amination was the final step.

When congeners with fewer methylenes (two or three) were activated for direct coupling to an amine, facile cyclization to the lactams **34** and **35** (Scheme II) was the major reaction. Attempts to protect the secondary amine

(20) R. Borch, M. Bernstein, and H. Durst, *J. Am. Chem. Soc.*, **93**, 2897–2904 (1971).
(21) M. Freifelder, "Catalytic Hydrogenation in Organic Synthesis Procedures and Commentary", Wiley, New York, 1978.

Table III. Synthesis of Amide Model Compounds



no.	R	R'	n	method[a]	yield,[b] %
21	p-C_6H_4-CH_3	H	2	C	19
22	p-C_6H_4-CH_3	H	3	C	80
23	p-C_6H_4-CH_3	H	4	C	83
23	p-C_6H_4-CH_3	H	4	A	56
23	p-C_6H_4-CH_3	H	4	B	51[c]
24	p-C_6H_4-CH_3	CH_3	4	B	63[c]
25	p-C_6H_4-CH_3	H	5	A	d
26	p-C_6H_4-$(CH_2)_3CH_3$	H	4	C	19
27	o-C_6H_4-CF_3	H	4	C	60
28	m-C_6H_4-CF_3	H	4	C	22
29	p-C_6H_4-CF_3	H	4	C	47
30	p-C_6H_4-OCH_3	H	4	C	62
31	$(CH_2)_3CH_3$	H	4	C	60
32	c-C_6H_{11}	H	4	C	52

[a] A = EDAC in H_2O; B = DCC in pyridine/H_2O; C = reductive amination as a final step. [b] Prior to purification by HPLC. [c] Isolated as the acetate salt. [d] Nearly quantitative.

with N-(*tert*-butyloxycarbonyl)succinimide[22] resulted only in cyclization on heating the congener **6** in the presence of this reagent to 45–50 °C. Cyclization could also be effected, without activation, by heating to 45 °C in DMF. Reductive amination (route b) as the final step therefore proved to be the more versatile of the two general methods for the synthesis of amide model compounds.

After preliminary purification of reaction products by extraction techniques or ion-exchange chromatography, the products were purified rigorously by semipreparative high-performance liquid chromatography (HPLC)[23,24] or by flash chromatography.[25] HPLC purification utilized C-18 reverse-phase columns and an isocratic mobile phase, which was a mixture of methanol (0–50%, v/v) and aqueous phosphate buffer (0.05–0.2 M), pH 4.5. Diastereoisomers could not be separated under these conditions. Products could be obtained in moderate quantities (up to 50 mg) by repetitive injections on analytical or semipreparative columns. After lyophilization, these products were desalted by extraction into methanol. Large quantities of products were obtained by flash chromatography by using silica gel and a stepwise gradient of methanol (8–30%) in chloroform/acetic acid (10:1, v/v).

In all cases, the final products were characterized carefully by analytical HPLC and shown to be free of potential, biologically active contaminants such as the precursor, norepinephrine. Since the products were mixtures of diastereomers, they were amorphous solids in all cases. Attempted crystallization did not provide sharp-melting compounds; for this reason, melting points are not reported. However, the products were characterized carefully by 220-MHz proton NMR[26] in each case, and in a limited number of cases, elemental analyses were obtained.

Table IV. In Vitro Biological Activity of Isoproterenol Congeners and Model Compounds

no.	act. rel to isoproterenol[a]
6	1.6×10^{-4}
7	6.8×10^{-4}
8	9.7×10^{-4}
9	8.3×10^{-4}
21	7.1×10^{-1}
22	3.6×10^{-1}
23	5.4×10^{1}
24	7.3×10^{1}
25	1.1×10^{-1}
26	3.2×10^{3}
27	2.5×10^{-4}
28	9.8×10^{-1}
29	1.1×10^{4}
30	3.1
31	2.5×10^{-1}
32	1.7×10^{-1}

[a] Biological activity was measured by cyclic AMP accumulation in S49 cells.[27] Relative activity is expressed as the ratio of K_A for isoproterenol to K_A for the compound. Each K_A was the average of a minimum of three separate determinations, measured in triplicate. The ratio of the K_A of isoproterenol to that of the compound showed no statistically significant variation ($p < 0.05$).

Pharmacological Effects of Products. The catecholamine derivatives (congeners and model compounds) were screened for biological activity by using the response of wild-type S49 cells in production of cyclic AMP.[27] These cells are grown in suspension and remain generally constant in their response to isoproterenol. This response has been well characterized as being dependent on an intact β-adrenergic receptor linked to adenylate cyclase and is expressed in part by production of cyclic AMP. The activity of each compound was measured as a K_A (concentration of drug for half-maximal activity) in molarity units and an E_{max} (maximum response or efficacy) in units of picomoles of cyclic AMP found in 10^7 cells. All compounds with β-adrenergic-like effects had about the same efficacy as isoproterenol, which was routinely tested si-

(22) M. Frankel, D. Ladkany, C. Gilon, and Y. Woman, *Tetrahedron Lett.*, 4765–4768 (1966).

(23) G. A. Scratchley, A. N. Masoud, S. J. Stohs, and D. W. Wingard, *Chromatographia*, 11, 425–431 (1979).

(24) A. M. Krstulovic, *Adv. Chromatogr.*, 17, 279–309 (1979).

(25) W. C. Still, M. Kahn, and A. Mitra, *J. Org. Chem.*, **43**, 2923–2925 (1978).

(26) K. A. Jacobson, Ph.D. Thesis, University of California, San Diego, 1981.

(27) A. G. Gilman, *Proc. Natl. Acad. Sci. U.S.A.*, **67**, 305–312 (1970).

multaneously for comparison. The ratio of the K_A for isoproterenol to that of the test compound, as shown in Table IV, is considered an indicator of relative in vitro potency. All discussion of in vitro potencies to follow will be based on the relative values.

A number of striking effects are evident from the results presented in Table IV. First, the carboxylic acid congeners, compounds **6–9**, have relatively low potencies (three to four orders of magnitude less than isoproterenol). This is presumably due to their existence as zwitterions, with a net charge of zero, at physiological pH. Through amide formation, the net charge was restored to +1, and potencies were generally increased.

Within a particular series of amides, such as the *p*-toluidides (compounds **21–23** and **25**, Table IV) there was generally a marked dependence of biological activity on the length of the handle. Maximal potency was observed for compound **23**, with four methylenes adjacent to the carbonyl group, which was approximately one to two orders of magnitude more potent than isoproterenol. When the amide function of compound **23** was N-methylated (compound **24**), the in vitro potency and efficacy were not significantly altered.

Structural modification of the substituent on the amide function also resulted in relatively large variations in potencies. Alkyl amide derivatives (compounds **31** and **32**, Table IV) generally exhibited lower potencies than aryl amides, suggesting the possibility of an interaction between the aryl substituent on the amide and the catechol ring, leading to enhanced binding to the receptor. Within the series of aryl amides, the effect of different substituents on the aromatic ring and their orientation relative to the amide substituent was the most dramatic. When the *p*-methyl substituent in **23** was replaced by a *p*-*n*-butyl substituent, the potency of the derivative (compound **26**) was increased by two orders of magnitude. Replacement of the *p*-methyl substituent by an electron-donating *p*-methoxy substituent (compound **30**) reduced the potency by more than one order of magnitude. When an electron-withdrawing substituent, *p*-trifluoromethyl, was used (compound **29**), the potency was increased by more than two orders of magnitude to four orders of magnitude greater than isoproterenol. Surprisingly, when the trifluoromethyl substituent was in the meta position (compound **28**), the activity was decreased substantially to approximately the same level as isoproterenol. The *o*-trifluoromethyl derivative (compound **27**) was much less potent (approximately four orders of magnitude less than isoproterenol). The enhanced biological activity of the *p*-trifluoromethyl derivative, compound **29**, therefore, was not a simple function of the electron-withdrawing substituent on the aromatic ring and may also be related to steric effects.

The β-antagonist propranolol competitively blocked the ability of all compounds that stimulated accumulation of intracellular cyclic AMP in the S49 cell line. Despite their relative potency, the effects of each compound could be completely inhibited by appropriate concentrations of propranolol, which were proportional to the relative in vitro potencies of the drugs as agonists. For example, a 10^{-8} M concentration of propranolol was required to block 50% of a 10^{-8} M concentration [90% E_{max} (ED_{90})] of compound **23**, while the same 10^{-8} M concentration of propranolol was required to block 50% of a 10^{-10} M concentration (ED_{90}) of compound **29** and a 10^{-7} M concentration of isoproterenol. These observations suggest that the activity was caused by a specific interaction with the β receptor. Binding studies carried out by Insel and co-workers[28] in which the radiolabeled antagonist, [^{125}I]iodohydroxybenzylpindolol, was displaced from the β receptor by the drug derivative indicated that compound **23** bound to the receptor more strongly than isoproterenol by a factor of 14. Similar binding studies with [^{3}H]dihydroalprenolol showed that compound **23** bound more strongly by a factor of 60.[28] These studies strongly suggest that the changes in potency of this model amide derivative compared with isoproterenol can be fully explained on the basis of altered affinity for the β-adrenergic receptors on the S49 cell surface. The fact that the maximum efficacy of all compounds (isoproterenol, congeners, and model amides) was the same indicates that there is very little likelihood that any congeners or model amides are acting in part by affecting the cyclase enzyme or metabolism of cyclic nucleotides. Since the displacement studies with one compound (compound **23**) gave results that are consistent with the observed changes in potency, we did not extend these assays. Rather, we simply looked for appropriate changes in competitive inhibition of cyclic AMP accumulation by propranolol with each compound screened.

Conclusions

We have uncovered a novel principle for defining the structure–activity relationship of catecholamine derivatives. The model amide compounds of the congeners exhibit biological potencies that are very sensitive to structural features far removed from the biologically active portion of the molecule. As we have shown, the toluidides and other amides are much more active than the congeners containing a free carboxylic acid group. Even within a given class of derivatives of the carboxylic acid congeners, such as the aromatic amides, there are substantial variations in biological activity. Thus, we can see that a *p*-*n*-butyl or -trifluoromethyl group leads to higher potencies than a *p*-methyl group. Furthermore, the *p*-trifluoromethyl derivative is much more active than the *m*-trifluoromethyl derivative, which in turn is much more active than the *o*-trifluoromethyl-substituted aryl amide molecule. This dramatic dependence of biological activity on structural modifications distant from the catecholamine moiety suggests exciting potential for a flexible approach to drug design through modifications at a point distant from the pharmacophore.

The effect of such structural modifications on in vitro potency indicates that within the amide class, these modifications enhance or inhibit binding to the receptor. A possible mechanism for enhanced binding to the receptor may involve folding of the molecule, which leads to an interaction (stacking) between the aryl amide substituent and the catechol ring. This interaction would likely be sensitive to both steric and electronic effects on the aryl amide. However, the fact that the affinity of these isoproterenol congeners and model amides is so sensitive to structural modifications at a point distal to the pharmacophore suggests that further studies may lead to a clearer understanding of the mechanism of binding to the β receptor. Such studies, in turn, almost surely will result in a new, general approach to drug design for catecholamine derivatives.

We are currently extending our studies to the synthesis of oligopeptide carriers and the covalent attachment of these congener molecules to the carriers to form conjugates. We are also preparing related functionalized catecholamine derivatives containing different functional groups, for

(28) P. Insel and L. M. Stoolman, *Mol. Pharmacol.*, 14, 549–561 (1978).

which reductive amination appears to be a generally useful route, to determine whether altered potencies can be generalized for this class of drug. Our preliminary studies suggest that some interesting and therapeutically useful new drugs may result from these studies.

Experimental Procedures

Melting points were taken with Thomas-Hoover capillary melting point apparatus and are uncorrected. All solvents were reagent grade or distilled where indicated. Mass spectra were determined with an LKB-9000A mass spectrometer. IR spectra were recorded on a Perkin-Elmer 180 spectrophotometer.

High-resolution proton NMR spectra were obtained in the Fourier transform mode with a Varian HR-220 spectrometer, equipped with a Nicolet 1080 computer. Chemical shifts are reported in parts per million downfield from Me_4Si or $Me_3Si(CD_2)_3SO_3Na$. Elemental analyses were performed by Galbraith Laboratories, Knoxville, TN.

High-pressure liquid chromatography was carried out with a Waters Associates System comprising two Model M45 pumps equipped with a Model 660 solvent programmer and a Schoeffel Model 770 detector. Chromatograms were routinely monitored at 254 nm. Since compounds were purified in mobile phases that contained phosphate buffer, the final salt form was invariably the phosphate.

Isoproterenol Carboxylic Acid Congeners. The catecholamine derivatives of this series (Table I) were synthesized as described below for compound 8. The compounds were purified by semipreparative reversed-phase HPLC prior to biological testing.

Synthesis of Keto Acids. The two lower members of the series (levulinic acid and 4-acetylbutyric acid) were purchased from Aldrich Chemical Co.

6-Oxo-*n*-heptanoic Acid. The title compound was prepared from 2-methylcyclohexanol (Aldrich Chemical Co.; 122 mL, 1 mol) by oxidation with chromic/sulfuric acid according to Schaeffer and Snoddy[14] (for δ-acetyl-*n*-valeric acid): yield 39.5 g (24%) of a hygroscopic solid; mp (sealed tube) 30.5–33.0 °C (lit.[14] mp 34–35 °C).

7-Oxo-*n*-octanoic Acid. The title compound was prepared by refluxing 2-acetylcyclohexanone (Aldrich Chemical Co.; 23.6 g, 0.168 mol) in aqueous sodium hydroxide according to Hauser et al.:[15] yield 15.1 g (51.6%) of a hygroscopic solid; mp 27.5–28.5 °C (lit.[29] mp 27–29 °C).

DL-Norepinephrine (2). The procedure used for converting norepinephrine hydrochloride to the free base was essentially the same as in Tullar.[30] The salt (22.97 g, 0.112 mol) was dissolved in 230 mL of water containing a trace of sodium metabisulfite at 10 °C. One equivalent of concentrated aqueous ammonia was added dropwise with stirring. The cooling was continued for an additional 15 min. The crop of nearly white crystals was collected and washed with ice-cold water, MeOH, and Et_2O: yield 18.36 g (97% recovery); mp (sealed tube) 189–190 °C dec.

6-[[β-(3,4-Dihydroxyphenyl)-β-hydroxyethyl]amino]-*n*-heptanoic Acid (8). DL-Norepinephrine (free base, compound 2, 3.91 g, 23 mmol) and 6-oxo-*n*-heptanoic acid (7.50 g, 46 mmol) were dissolved in a 20% solution (75 mL) of acetic acid in methanol. Hydrogenation was performed overnight at room temperature and atmospheric pressure over 0.1 g of PtO_2 catalyst. After filtration and, if necessary, washing with acetic acid until only Platinum black remained on the filter (compounds 8 and 9 tended to precipitate during the reduction), the combined filtrates were evaporated. The resulting thick oil was dissolved in methanol and added slowly to ethyl ether with stirring. The whitish precipitate was filtered, washed with ether, and dried in vacuo to give 5.06 g of an amorphous solid (compound 8, 74%), which was homogeneous by TLC (chloroform/methanol/acetic acid, 50:50:5).

Compound 6 prepared in this manner gave the following elemental analysis prior to HPLC purification: Anal. ($C_{13}H_{19}NO_5 \cdot 0.75H_2O$) C, H, N.

(29) J. L. Charlish, W. H. Davies, and J. D. Rose, *J. Chem. Soc.*, 232–234 (1948).
(30) B. F. Tullar, *J. Am. Chem. Soc.*, **70**, 2067–2068 (1948).

The crude material was dissolved in water or methanol at a concentration of approximately 50 mg/mL, filtered on a MF-1 microfilter (Bioanalytical Systems), and purified with an analytical reverse-phase column (Waters μ-Bondapak C_{18} or Unimetrics Lichrosorb RP-18). An isocratic solvent mixture, consisting of methanol (0–50%) in aqueous monosodium phosphate (0.05–0.2 M), which had been filtered through a 0.5-μm Millipore filter, was used. Up to 2 mg of compound could be applied with each injection. Up to 8 mg could be injected on a larger semipreparative column (Whatman Partisil ODS-3 Magnum 9). The product peak was collected, and the methanol was evaporated in vacuo. After lyophilization, the product was removed from inorganic salts by extraction into methanol. It was assumed that $H_2PO_4^-$ was the counterion on the catecholamine (verified by phosphorus analysis of compound 23).

Synthesis of Keto Amides. Each of the compounds in this series was prepared by one of the three methods described below. Yields and characterization data for the keto amides are summarized in Table II.

Method A. Nonaqueous Carbodiimide Coupling. 6-Oxo-*n*-heptano-*p*-toluidide (12). 6-Oxo-*n*-heptanoic acid (1.2 g, 8.3 mmol) and *p*-toluidine (1.0 g, 9.3 mmol) were dissolved in tetrahydrofuran (30 mL). Dicyclohexylcarbodiimide (MCB; 1.9 g, 9.1 mmol) was added, and the solution was stirred overnight. The solvent was removed, and the residue was suspended in chloroform and filtered. After washing with 0.1 N HCl, saturated sodium bicarbonate, and water, the organic layer was dried ($MgSO_4$). The solution was filtered and evaporated. Recrystallization of the residue from chloroform/hexanes gave 1.36 g (70%) of white crystals, mp 106–107.5 °C.

Method B. Aqueous Carbodiimide Coupling. 5-Oxo-*n*-hexano-*p*-toluidide (11). *p*-Toluidine hydrochloride was prepared by slowly adding 1 equiv of 4 N HCl/dioxane (Pierce Chemical Co.) to a solution of the free base in dry ether. The crystals were collected by filtration, washed with ether, and dried in vacuo.

p-Toluidine hydrochloride (0.127 g, 0.88 mmol) and γ-acetyl-*n*-butyric acid (Aldrich Chemical Co.; 0.114 g, 0.88 mmol) were dissolved in 0.1 M sodium phosphate, monobasic (20 mL). The pH was adjusted to 5.0 with dilute sodium hydroxide. 1-Ethyl-3-[3-(dimethylamino)propyl]carbodiimide hydrochloride (Sigma Chemical Co.; 0.33 g, 1.72 mmol) was added, and within minutes a crystalline precipitate appeared. The product (0.158 g, 82%) was collected by filtration and washed with water; mp 121.5–122 °C.

Method C. Mixed Anhydride Coupling. 4′-*n*-Butyl-6-oxo-*n*-heptano-*p*-anilide (14). 6-Oxo-*n*-heptanoic acid (0.86 g, 6 mmol) was dissolved in tetrahydrofuran (20 mL), cooled to −15 °C, and treated with isobutyl chloroformate (0.77 mL, 6 mmol) and *N*-methylmorpholine (0.66 mL, 6 mmol). After 15 min, *p*-*n*-butylaniline (Aldrich Chemical Co.; 0.94 mL, 6 mmol) was added, and the mixture allowed to warm to room temperature and stirred overnight. The solvent was removed, and the residue was redissolved in chloroform. After washing with 0.1 N HCl, saturated sodium bicarbonate, and water, the organic layer was dried ($MgSO_4$). The solution was filtered and evaporated, and the residue was recrystallized from chloroform/hexanes to give 1.12 g (68%) of white crystals, mp 85–86 °C.

Isoproterenol Amide Model Compounds. Each of the catecholamine derivatives of this series (Table III) was synthesized according to one of the three methods described below for compound 23. All of the compounds were purified by semipreparative reverse-phase HPLC.

6-[[β-(3,4-Dihydroxyphenyl)-β-hydroxyethyl]amino]-*n*-heptano-*p*-toluidide (23). Method A. Water-Soluble Carbodiimide Coupling as Final Step. The catecholamine carboxylic acid congener 8 (2.97 g, 10 mmol) and *p*-toluidine hydrochloride (1.86 g, 13 mmol) were added to 0.05 M monosodium phosphate (400 mL) under an inert atmosphere. 1-Ethyl-3-[3-(dimethylamino)propyl]carbodiimide hydrochloride (Sigma Chemical Co.; 3.3 g, 17 mmol) was added to the solution. After 1 day the solution was extracted with chloroform (4×), the combined extracts were discarded, and then the solution was again extracted with 1-butanol (3×), and the combined 1-butanol extracts were evaporated under reduced pressure at 40 °C. The residual oil was redissolved in 1-butanol and filtered, and the

filtrates were evaporated in vacuo without delay. This oil was transformed into a white amorphous solid by stirring under ether. The solid was filtered, washed with ether and dried in vacuo to give 1.73 g (41% yield) of the hydrochloride salt of the title compound. A portion was then purified by semipreparative HPLC: UV λ_{max} 279 nm (ϵ 4.1 × 10^3), 240 (ϵ 1.6 × 10^4). Anal. ($C_{22}H_{33}N_2O_8P\cdot0.8H_2O$): H, N; C: Calcd, 52.96; found, 53.46; P: Calcd, 6.21; found, 5.34.

Method B. Dicyclohexylcarbodiimide Coupling as a Final Step. Compound 8 (0.102 g, 0.34 mmol) and *p*-toluidine hydrochloride (25 mg, 0.17 mmol) were dissolved in 5 mL of pyridine/water (4:1, v/v). Dicyclohexylcarbodiimide (88 mg, 0.43 mmol) was added, and the solution was stirred under nitrogen overnight. The solvent was evaporated under reduced pressure at 40 °C until a white precipitate formed. Water (15 mL) was added, and the urea was removed by filtration through Celite.

The solution was passed over a bed of Bio-Rex 70 cation-exchange resin (Bio-Rad, 2 g dry weight) in the H^+ form. After the bed was washed thoroughly with H_2O, the product was removed from the resin with 5% acetic acid. The eluate was lyophilized to give 40 mg (51% yield) of a white solid, the acetate salt of the title compound (Table III, X = CH_3COO). This compound was shown by TLC (chloroform/methanol/acetic acid 50:50:15) to be identical with the product prepared by method A and free from starting materials.

Method C. Catalytic Reductive Amination. DL-Norepinephrine (free base, compound 2; 30 mg, 0.16 mmol) and the keto amide 12 (75 mg, 0.32 mmol) were dissolved in acetic acid (1 mL). Adam's catalyst (PtO_2, 10 mg) was added, and the mixture was hydrogenated at atmospheric pressure overnight. The solution was decanted from the catalyst and added to 0.01 N hydrochloric acid, after which extractions with chloroform (to remove excess ketone) and 1-butanol were performed. The chloroform extracts were discarded, and the combined 1-butanol extracts were washed with saline until neutral and then evaporated. The residue was redissolved in 1-butanol and filtered through glass wool, and the fitrate was evaporated. The resulting oil was dried in vacuo to give 62 mg (90%) of the HCl salt of the title compound.

Method D. Reductive Amination with Sodium Cyanoborohydride.[31] Norepinephrine hydrochloride salt (3.65 g, 15.7 mmol) and the keto amide (12; 3.21 g, 15.7 mmol) were dissolved in a mixture of 8 mL of 10% acetic acid in MeOH and 50 mL MeOH and stirred for 30 min at room temperature. A solution of sodium cyanoborohydride (1.97 g, 31.4 mmol) in MeOH (20 mL) was added portionwise over 1 h. After the addition, the solution was stirred for 1 h at room temperature and then refluxed for 2 h. When reaction was complete, aqueous 3 N HCl was added until no more gas was evolved. The solution was evaporated, and 80 mL of 0.1 N HCl was added. The HCl solution was washed with $CHCl_3$ (2 × 40 mL, discarded) and 1-butanol (2 × 40 mL). The combined 1-butanol extracts were evaporated to an oil, which solidified on addition of anhydrous ether. The resulting white precipitate was collected by removal of the ether by decantation (twice). Evaporation of the ether in vacuo gave 4.84 g (65%) of a white solid (yield calculated as the HCl salt). The product was purified by flash chromatography[25] on silica by using a stepwise gradient of methanol (8–30%) in chloroform/acetic acid (10:1). Anal. ($C_{22}H_{31}N_2O_4Cl\cdot1.5H_2O$) C, H, N.

6-[[β-(3,4-Dihydroxyphenyl)ethyl]amino]-*n*-heptano-*p*-toluidide (33). Dopamine free base (82.3 mg, 0.54 mmol; prepared as described for compound 2) and the keto amide 12 (125 mg, 0.54 mmol) were subjected to catalytic reductive amination as described above for compound 23 (method C). After workup, the resulting oil was solidified by trituration with ether to give 131 mg (60%) of the title compound. A portion was purified by semipreparative HPLC to provide a sample that was free from detectable contamination by dopamine: NMR (D_2O) δ 7.25 (dd, 2 H), 7.20 (dd, 2 H), 6.9–6.7 (m, 3 H), 3.23 (m, 1 H), 3.20 (m, 2 H), 2.84 (dd, 2 H), 2.39 (m, 2 H), 2.28 (s, 3 H), 1.67 (m, 2 H), 1.5 (m, 4 H), 1.24 (dd, 3 H, *J* = 2.5 and 6.6 Hz).

1-[β-(3,4-Dihydroxyphenyl)-β-hydroxyethyl]-5-methyl-2-pyrrolidinone (34). The congener 6 (0.19 g, 0.70 mmol) was treated with *p*-toluidine hydrochloride (0.10 g, 0.70 mmol) and 1-ethyl-3-[3-(dimethylamino)propyl]carbodiimide hydrochloride (0.34 g, 1.8 mmol) in the manner described in method A for compound 23. An oil identified as the title compound (21 mg, 12%) was isolated. An analytical sample was isolated by preparative TLC (silica; chloroform/methanol/acetic acid, 50:50:5): IR showed a strong absorbance at 1655 cm^{-1} (KBr); NMR (D_2O) δ 6.91 (s, 1 H, aromatic C_2 H), 6.89 (dd, 1 H, aromatic CH), 6.81 (dd, 1 H, *J* = 7.9 Hz, aromatic CH), 4.8 (benzylic CH); each of the following four resonances is dd, 0.5 H; CH_2 α to N, coupled to δ 4.8, 3.80 (coupled to δ 3.11), 3.62 (*J* = 6.3 and 13.7 Hz, coupled to δ 3.30), 3.30 (*J* = 7.2 and 14 Hz), 3.11 (*J* = 5.5 and 14 Hz); 3.8 (0.5 H, methine α to N, coupled to δ 1.16), 3.48 (0.5 H, *J* = 6.4 Hz, methine α to N, coupled to δ 1.13), 2.37 (m, 2 H, CH_2 α to C=O), 2.1 and 1.6 (each: m, 1 H, CH_2 β to C=O), 2.03 (s, 9 H, CH_3CO_2H), 1.16 (d, 1.5 H, CH_3), 1.13 (d, 1.5 H, CH_3). Anal. ($C_{13}H_{17}NO_4\cdot3HOAc$) C, H, N.

1-[β-(3,4-Dihydroxyphenyl)-β-hydroxyethyl]-6-methyl-2-piperidinone (35). The congener 7 (69 mg, 0.24 mmol) was dissolved in dimethylformamide (3 mL) and heated at 45 °C for 36 h. The solvent was evaporated, and the residue was extracted with 1-butanol (2 ×) from an aqueous solution (10 mL). The 1-butanol was evaporated, leaving 48 mg (76%) of the lactam as an oil. An analytical sample was prepared as with compound 34. Anal. ($C_{14}H_{19}NO_4\cdot HOAc$) C, H, N.

Pharmacological Methods. S-49 Mouse Lymphoma Cell Assay.[27] S-49 mouse lymphoma cells were centrifuged and resuspended at 2–2.5 × 10^6 per mL in DME (13.3 g/L) and 20 mM Hepes (pH 7.4) plus 0.1% bovine serum albumin. They were next incubated at 37 °C for 10 min without drug and were then added to tubes with or without test compounds for an additional 6 min. The reaction was stopped by putting the tubes on ice. After centrifugation, the pellets of cells were resuspended and boiled. Aliquots were then used for the competitive binding assay described by Gilman.[27] Increased levels of cAMP were plotted as a function of the log of the concentration of the drug derivatives. Thus, K_A (an activity constant in molarity units) and E_{max} (maximum response in units of picomoles of cAMP per 10^7 cells) values were obtained for each test compound. The ratio of the K_A for isoproterenol to that of the compound tested at the same time is considered an indicator of relative in vitro potency.

S-49 Mouse Lymphoma β-Blocking Assay. This assay was carried out in the same manner as the agonist assay, with only one difference: the test compound was administered at a concentration equivalent to the effective dose that produced 90% E_{max} response (ED_{90}) in production of cAMP, and the antagonist propranolol was administered simultaneously at concentrations ranging from 10^{-14} to 10^{-4} M. The concentration of propranolol required to block 50% of the ED_{90} of the test compound was then calculated.

[^{125}I]Iodohydroxybenzylpindolol Binding Assay.[28] S-49 cells at a density of 1–2 × 10^{-6}/mL were incubated at 37 °C in 3 mL of assay buffer containing the β-adrenergic antagonist [^{125}I]iodohydroxybenzylpindolol. Binding reactions were terminated by the addition of a 0.5-mL aliquot of incubation mixture of 2.5 mL of a 37 °C solution containing 5 mM potassium phosphate (pH 7.0), 1 mM $MgSO_4$, and 0.01 mM (−)-propranolol and overlaying a glass-fiber filter (Gelman A/E). The samples were filtered at a rate of 50–60 mL/min by applying vacuum, and the filters were then washed with 30 mL of the same buffer used to terminate reactions, except that it lacked propranolol. Filters were dried by increasing the vacuum and were then counted in a liquid scintillation system (efficiency 60%). All samples were run in duplicate to quadruplicate. Duplicate determinations generally agreed within 10%. Aliquots of cells were maintained in ice prior to addition to the other reagents, which had been warmed to 37 °C. In binding experiments performed for less than 30 min, cells were incubated at 37 °C for at least 5 min prior to addition to other reagents.

[^{3}H]Dihydroalprenolol Binding Assay.[28] β-Adrenergic receptors were assayed by adding 4–8 × 10^6 cells (generally in 100-μL aliquots) to tubes containing DMEH medium. Incubations were

(31) Compound 29, prepared and purified by the same procedure, gave the following analysis: Anal. Calcd for $C_{22}H_{28}N_2O_4F_3Cl\cdot H_2O$: C, 52.43; H, 6.20; N, 5.56. Found: C, 52.46; H, 6.27; N, 5.27.

(32) R. Lukes and V. Prelog, *Collect. Trav. Chim. Tchec.*, 1, 282–287 (1929).

performed in duplicate. The results generally differed from each other by less than 5%. Nonspecific binding was determined by incubating samples with 100 and 300 nM nonradioactive (–)-alprenolol at 40 and 37 °C, respectively, and was subtracted from the total amount of $[^3H]$dihydroalpronolol bound in order to obtain the amounts of $[^3H]$dihydroalprenolol specifically bound. Radioactivity associated with cells was separated from unbound $[^3H]$dihydroalprenolol by filtering and washing of samples over glass-fiber filters with ice-cold buffer. Radioactivity bound to the filters was counted in PCS scintillation fluid (Amersham Searle) in an ambient temperature scintillation counter at 50% efficiency.

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Registry No. (±)-**2**, 138-65-8; (±)-(R^*,R^*)-**6**, 84417-30-1; (±)-(R^*,R^*)-**6**, 84418-14-4; (±)-(R^*,R^*)-**6**·H_3PO_4, 84417-61-8; (±)-(R^*,S^*)-**6**·H_3PO_4, 84418-29-1; (±)-(R^*,R^*)-**7**, 84417-31-2; (±)-(R^*,S^*)-**7**, 84418-15-5; (±)-(R^*,R^*)-**7**·H_3PO_4, 84417-62-9; (±)-(R^*,S^*)-**7**·H_3PO_4, 84418-30-4; (±)-(R^*,S^*)-**8**, 84417-32-3; (±)-(R^*,S^*)-**8**, 84417-86-7; (±)-(R^*,R^*)-**8**·H_3PO_4, 84417-63-0; (±)-(R^*,S^*)-**8**·H_3PO_4, 84417-87-8; (±)-(R^*,R^*)-**9**, 84417-33-4; (±)-(R^*,S^*)-**9**, 84417-88-9; (±)-(R^*,R^*)-**9**·H_3PO_4, 84417-64-1; (±)-(R^*,S^*)-**9**·H_3PO_4, 84417-89-0; **10**, 84417-34-5; **11**, 84417-35-6; **12**, 83086-06-0; **13**, 84417-36-7; **14**, 84417-37-8; **15**, 84417-38-9; **16**, 84417-39-0; **17**, 84417-40-3; **18**, 84417-41-4; **19**, 84417-42-5; **20**, 84417-43-6; (±)-(R^*,R^*)-**21**·HCl, 84417-44-7; (±)-(R^*,S^*)-**21**·HCl, 84418-16-6; (±-)(R^*,R^*)-**21**·H_3PO_4, 84417-66-3; (±)-(R^*,S^*)-**21**·H_3PO_4, 84417-91-4; (±)-(R^*,R^*)-**22**·HCl, 84417-45-8; (±)-(R^*,S^*)-**22**·HCl, 84418-17-7; (±)-(R^*,R^*)-**22**·H_3PO_4, 84417-68-5; (±)-(R^*,S^*)-**22**·H_3PO_4, 84417-93-6; (±)-(R^*,R^*)-**23**·HCl, 84417-46-9; (±)-(R^*,S^*)-**23**·HCl, 84418-18-8; (±)-(R^*,R^*)-**23**·HOAc, 84417-57-2; (±)-(R^*,R^*)-**23**·H_3PO_4, 84417-69-6; (±)-(R^*,S^*)-**23**·H_3PO_4, 84417-95-8; (±)-(R^*,S^*)-**23**·HOAc, 84418-28-0; (±)-(R^*,R^*)-**24**·HCl, 84417-47-0; (±)-(R^*,S^*)-**24**·HCl, 84418-19-9; (±)-(R^*,R^*)-**24**·H_3PO_4, 84417-71-0; (±)-(R^*,S^*)-**24**·H_3PO_4, 84417-97-0; (±)-(R^*,R^*)-**25**·HCl, 84417-48-1; (±)-(R^*,S^*)-**25**·HCl, 84418-20-2; (±)-(R^*,R^*)-**25**·H_3PO_4, 84432-82-6; (±)-(R^*,S^*)-**25**·H_3PO_4, 84417-99-2; (±)-(R^*,R^*)-**26**·HCl, 84417-49-2; (±)-(R^*,S^*)-**26**·HCl, 84418-21-3; (±)-(R^*,R^*)-**26**·H_3PO_4, 84417-73-2; (±)-(R^*,S^*)-**26**·H_3PO_4, 84418-01-9; (±)-(R^*,R^*)-**27**·HCl, 84417-50-5; (±)-(R^*,S^*)-**27**·HCl, 84418-22-4; (±)-(R^*,R^*)-**27**·H_3PO_4, 84417-75-4; (±)-(R^*,S^*)-**27**·H_3PO_4, 84418-03-1; (±)-(R^*,R^*)-**28**·HCl, 84417-51-6; (±)-(R^*,S^*)-**28**·HCl, 84418-23-5; (±)-(R^*,R^*)-**28**·H_3PO_4, 84417-77-6; (±)-(R^*,S^*)-**28**·H_3PO_4, 84418-05-3; (±)-(R^*,R^*)-**29**·HCl, 84417-52-7; (±)-(R^*,S^*)-**29**·HCl, 84418-24-6; (±)-(R^*,R^*)-**29**·H_3PO_4, 84417-79-8; (±)-(R^*,S^*)-**29**·H_3PO_4, 84418-07-5; (±)-(R^*,R^*)-**30**·HCl, 84417-53-8; (±)-(R^*,S^*)-**30**·HCl, 84418-25-7; (±)-(R^*,R^*)-**30**·H_3PO_4, 84417-81-2; (±)-(R^*,S^*)-**30**·H_3PO_4, 84418-09-7; (±)-(R*,R*)-**31**·HCl, 84417-54-9; (±)-(R^*,S^*)-**31**·HCl, 84418-26-8; (±)-(R^*,R^*)-**31**·H_3PO_4, 84417-83-4; (±)-(R^*,S^*)-**31**·H_3PO_4, 84418-11-1; (±)-(R^*,R^*)-**32**·HCl, 84417-55-0; (±)-(R^*,S^*)-**32**·HCl, 84418-27-9; (±)-(R^*,R^*)-**32**·H_3PO_4, 84417-85-6; (±)-(R^*,S^*)-**32**·H_3PO_4, 84418-13-3; (±)-**33**, 84417-58-3; **34**, 84417-59-4; **35**, 84417-60-7; levulinic acid, 123-76-2; 4-acetylbutyric acid, 3128-06-1; 6-oxoheptanoic acid, 3128-07-2; 2-methylcyclohexanol, 583-59-5; 7-oxooctanoic acid, 14112-98-2; 2-acetylcyclohexanone, 874-23-7; *p*-toluidine, 106-49-0; *p*-butylaniline, 104-13-2; *N*-methyl-*p*-toluidine, 623-08-5; *o*-trifluoromethylaniline, 88-17-5; *m*-trifluoromethylaniline, 98-16-8; *p*-trifluoromethylaniline, 455-14-1; *p*-methoxyaniline, 104-94-9; butylamine, 109-73-9; cyclohexylamine, 108-91-8; dopamine, 51-61-6.

β-Carbolines as Benzodiazepine Receptor Ligands. 1. Synthesis and Benzodiazepine Receptor Interaction of Esters of β-Carboline-3-carboxylic Acid

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Several esters of β-carboline-3-carboxylic acid were synthesized and tested in respect to their affinity for the benzodiazepine receptor in bovine cortex membranes. Out of these derivatives, the methyl, ethyl, and *n*-propyl ester were clearly the most potent, while the *n*-butyl, benzyl, and 3-pyridylmethyl ester were considerably less active. Moreover, several β-carboline-3-carboxylates with ethanol derivatives as ester alcohol components were all less active than the ethyl or *n*-propyl ester themselves. It is concluded that the affinity of β-carboline-3-carboxylates to the benzodiazepine receptor is profoundly dependent on molecular size, as well as hydrophobic and electronic parameters of the ester alcohol component.

Today, a variety of evidence suggest that the pharmacological effects of the benzodiazepines are mediated through CNS specific binding sites.[1–4] These "benzodiazepine receptors" exhibit a very high specificity for pharmacologically and clinically active benzodiazepines, and only a small number of compounds chemically different from the benzodiazepines bind to these receptors with high affinity.[1–4] A case in point is the group of β-carbolines, where some derivatives have been related to the endogenous ligand of the benzodiazepine receptor.[5–9] Furthermore, quite remarkable benzodiazepine receptor affinities have been reported for some β-carboline-3-carboxylates (low nanomolar range) that are about three orders of magnitude higher than that of the parent compound β-carboline-3-carboxylic acid,[9] making these β-

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(1) C. Braestrup and M. Nielsen, *Arzneim.-Forsch.*, **30**, 852 (1980).
(2) H. Möhler and T. Okada, *Br. J. Psychiatry*, **133**, 261 (1978).
(3) J. F. Tallmann, S. M. Paul, P. Skolnik, and D. W. Gallager, *Science*, **207**, 274 (1980).
(4) W. E. Müller, *Pharmacology*, **22**, 153 (1981).
(5) H. Rommelspacher, C. Nanz, H. O. Borbe, K. J. Fehske, W. E. Müller, and U. Wollert, *Naunyn-Schmiedeberg's Arch. Pharmacol.*, **314**, 97 (1980).
(6) H. Rommelspacher, C. Nanz, H. O. Borbe, K. J. Fehske, W. E. Müller, and U. Wollert, *Eur. J. Pharmacol.*, **70**, 409 (1981).
(7) W. E. Müller, K. J. Fehske, H. O. Borbe, U. Wollert, C. Nanz, and H. Rommelspacher, *Pharmacol. Biochem. Behav.*, 14, 693 (1981).
(8) H. A. Robertson, G. B. Baker, R. T. Coutts, A. Benderly, R. A. Locock, and I. L. Martin, *Eur. J. Pharmacol.*, **76**, 281 (1981).

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