β-Adrenergic Blocking Agents: Substituted Phenylalkanolamines. Effect of Side-Chain Length on β-Blocking Potency in Vitro

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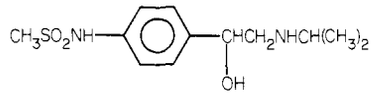
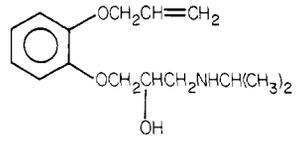
The synthesis of a group of potential β-blockers bearing a new 5-ethoxysalicylamide substituent on nitrogen is described. These compounds were tested for β-adrenergic blocking potency in vitro and compared with analogous compounds bearing a *tert*-butyl group on nitrogen. The new N-substituent increased the β-blocking potency substantially. In a series of five homologous compounds of the type $Ar(CH_2)_nCHOHCH_2NHR$ (R = 5-ethoxysalicylamide; n = 0–4), two maxima of β-blocking potency were found for n = 0 and 2. Moreover, the carbon isostere of the corresponding (aryloxy)propanolamine still proved to be a very potent β-blocker. The ether oxygen in the side chain is therefore not an absolute requirement for activity. Structure–activity relationships are discussed.

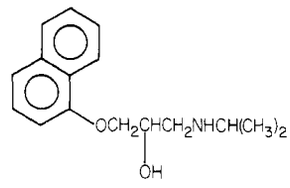
The β-adrenergic blocking agents in therapeutic use are either arylethanolamines [e.g., sotalol (I) or (aryloxy)-



I



II



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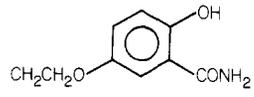
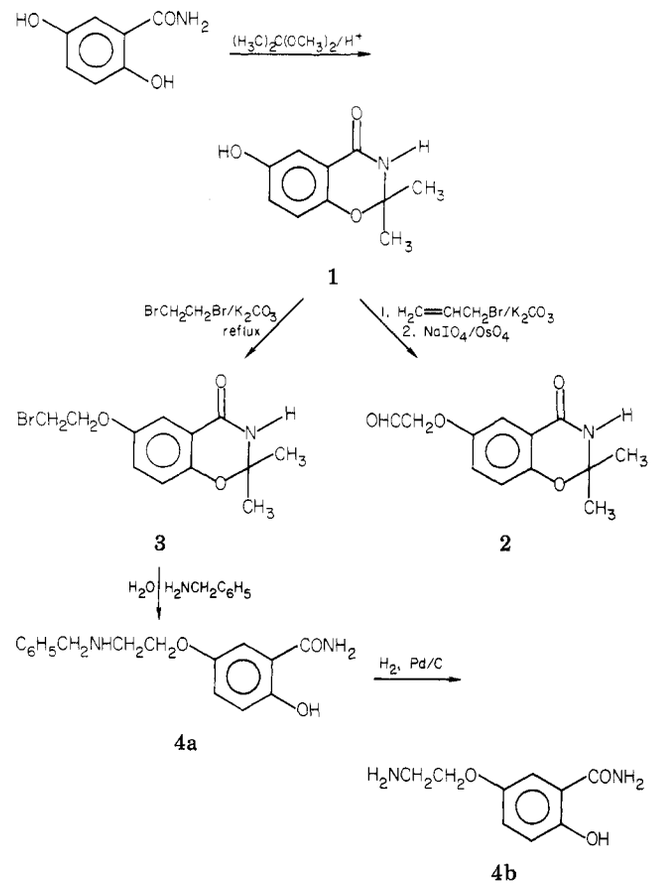


IV (see Scheme I)

propanolamines [oxprenolol (II) propranolol (III), etc.]. Only relatively few examples of arylpropanolamines and arylbutanolamines have been described in the literature and investigated as β-blocking agents.[1-6] All these compounds are much weaker β-receptor blockers than the corresponding (aryloxy)propanolamines, and it was therefore concluded that in addition to the optimal length of the side-chain, the ether oxygen was also an essential prerequisite for binding to the β-receptor. However, most of these studies were made with *N*-isopropyl or *N*-*tert*-butyl derivatives.

In the course of investigations of β-blocking agents in our laboratories, a new nitrogen substituent was developed: 5-ethoxysalicylamide (IV). It was found that incorporation of this substituent into suitable amino alcohols often resulted in a very substantial increase in β-blocking potency compared with the activity of the corresponding isopropyl or *tert*-butyl analogues. We therefore made a more systematic study of the influence of the length and character of the side chain on the activity of β-blockers bearing this 5-ethoxysalicylamide substituent. For this purpose, we prepared a series of five compounds in which the substituent on the aromatic ring was always an *o*-methoxy group, and the distance between the phenyl ring and the ethanolamine moiety was increased stepwise by insertion of one to four CH_2 groups (compounds **7**–**11**). The β-antagonistic activity of these compounds was determined in vitro in the Langendorff heart preparation. They were compared with the corresponding (aryloxy)propanolamine compounds incorporating the ethoxysalicylamide substituent (**18**) and also with the *tert*-butyl derivative **20**. Also included in this study were the carbon isostere of the latter compound (**19**) and an additional six compounds (**12**–**17**) in which the 2-methoxy group was varied.

Scheme I



Chemistry. The benzoxazinone **1**, a protected salicylamide, was a suitable starting material for the conventional synthesis of the aldehyde **2**, the bromide **3**, and the amines **4a,b**. They were prepared as depicted in Scheme I. It is worth noting that benzoxazinones of structure **2** or **3** are hydrolyzed to the corresponding salicylamide derivatives (e.g., **4a**) not only by aqueous acid but also by primary amines (e.g., phenylmethyl- or 1-methylethyl-

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(6) Löwgren, K.; Hedberg, A.; Nilson, J. L. G. *J. Med. Chem.* **1981**, *24*, 451.

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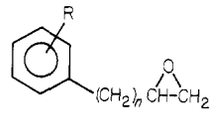
Table I



5

no.	R	n	method of prep	purification of 5: bp, °C	yield, %
5a	2-OCH_3	1	A[a]	*b*	
5b	2-OCH_3	2	A	*b*	85
5c	2-OCH_3	3	A	*b*	33
5d	2-OCH_3	4	A	*b*	17
5e	H	1	A[c]	*d*	
5f	H	2	B[e]	80–85 (0.3)	57
5g	H	4	B[e]	132–134 (11)	28
5h	2-CH_3	2	B	135–140 (0.5)	20
5i	4-OCH_3	2	B	117 (0.05)	26

[a] Prepared from commercial 2-methoxyallylbenzene.[8] [b] Product was purified by column chromatography. [c] Prepared from commercial allylbenzene.[9,10] [d] Crude product was used in next step. [e] Reference 9.

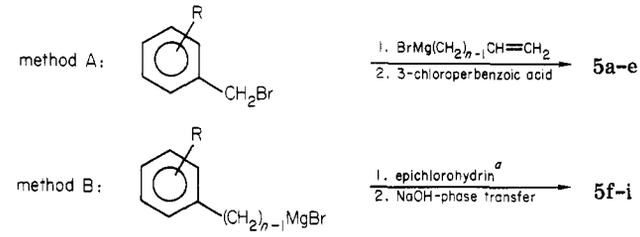
amine), possibly by removal of the acetonide protecting group and formation of a Schiff base. The epoxides **5** were prepared according to Scheme II, either by epoxidation of the appropriate phenylalkene with 3-chloroperbenzoic acid (method A) or by reaction of a suitable (phenylalkyl)magnesium halide with epichlorohydrin, followed by cyclization of the chlorohydrin with base under phase-transfer conditions (method B) (see Table I). The final products were obtained by one of the standard methods C, D, or E (Scheme III, Table II).

Pharmacology. The β-adrenoceptor blocking activity of the compounds was determined in vitro by measuring their capacity to antagonize isoproterenol-induced tachycardia in the Langendorff isolated, perfused guinea pig heart. The negative logarithms of the inhibitory concentrations of the compounds are given in Table III.

Results and Discussion

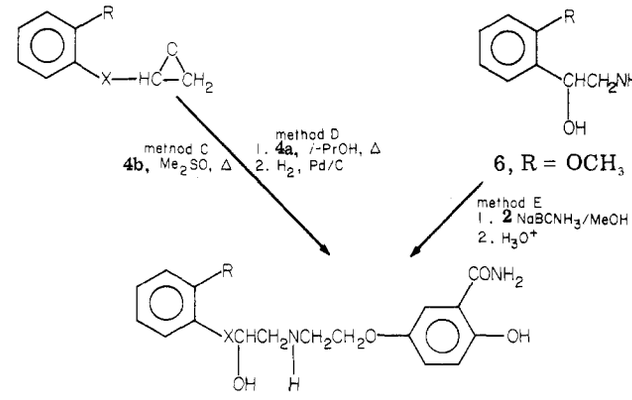
To the best of our knowledge, this paper presents the first description of a complete series of homologous β-blockers with the general formula $R_1Ar(CH_2)_nCHOHCH_2NHR_2$ (n = 0–4). Exploration of this series proved particularly interesting, because, by comparison with the conventional nitrogen substituents (e.g., *tert*-butyl), the new nitrogen substituent (R_2 = 5-ethoxysalicylamide) considerably augments the β-antagonistic activity. This enhancement of activity is clearly evident upon comparison of the two pairs **19** and **9**, and **20** and **18** (cf. Table III). Although compound **9** does not belong to the (aryloxy)propanolamine type, it is at least as potent in vitro as oxprenolol. Within the first series of homologues (compounds **7**–**11**; cf. Figure 1, plot A), two distinct maxima of β-antagonistic activity can be observed: the

Scheme II



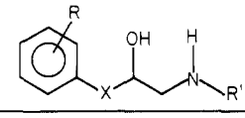
[a] See ref 7.

Scheme III



7-16 (see Table II)

Table II



no.	R	R′	X	formula[a]	recrystn solvent	mp,[b] °C	method	yield,[c] %
7	2-OCH_3	CH_2CH_2O-C_6H_3(3-$CONH_2$, 4-OH)		$C_{18}H_{22}N_2O_5$[d]	*i*-PrOH	167–168	E	23[e]
8	2-OCH_3	CH_2CH_2O-C_6H_3(3-$CONH_2$, 4-OH)	CH_2	$C_{19}H_{24}N_2O_5$·HCl	*i*-PrOH	153–155	D	38[f]
9	2-OCH_3	CH_2CH_2O-C_6H_3(3-$CONH_2$, 4-OH)	$(CH_2)_2$	$C_{20}H_{26}N_2O_5$·HCl	CH_3CN–Et_2O	124–126	D	51[f]
10	2-OCH_3	CH_2CH_2O-C_6H_3(3-$CONH_2$, 4-OH)	$(CH_2)_3$	$C_{21}H_{28}N_2O_5$·HCl	*i*-PrOH–CH_3CN–Et_2O	110–112	D	37[f]
11	2-OCH_3	CH_2CH_2O-C_6H_3(3-$CONH_2$, 4-OH)	$(CH_2)_4$	$C_{22}H_{30}N_2O_5$·HCl	*i*-PrOH–CH_3CN–Et_2O	117–120	D	32[f]
12	H	CH_2CH_2O-C_6H_3(3-$CONH_2$, 4-OH)		$C_{17}H_{20}N_2O_4$·CH_3SO_3H	MeOH	182–183	D	62[g]
13	H	CH_2CH_2O-C_6H_3(3-$CONH_2$, 4-OH)	CH_2	$C_{18}H_{22}N_2O_4$·HCl	*i*-PrOH–MeOH	180–181	C[b]	45[g]
14	H	CH_2CH_2O-C_6H_3(3-$CONH_2$, 4-OH)	$(CH_2)_2$	$C_{19}H_{24}N_2O_4$[d]	MeOH	159–160	C	30[g]
15	H	CH_2CH_2O-C_6H_3(3-$CONH_2$, 4-OH)	$(CH_2)_4$	$C_{21}H_{28}N_2O_4$[d]	MeOH	153–154	D	62[g]
16	2-CH_3	CH_2CH_2O-C_6H_3(3-$CONH_2$, 4-OH)	$(CH_2)_2$	$C_{20}H_{26}N_2O_4$·HCl	MeOH–*i*-PrOH	155–157	D	38[g]
17	4-OCH_3	CH_2CH_2O-C_6H_3(3-$CONH_2$, 4-OH)	$(CH_2)_2$	$C_{20}H_{26}N_2O_5$[d]	MeOH	164–165	D	63[g]
18	2-OCH_3	CH_2CH_2O-C_6H_3(3-$CONH_2$, 4-OH)	OCH_2	$C_{19}H_{24}N_2O_6$[d]	MeOH	125–126	D	51[g]
19	2-OCH_3	t-Bu	$(CH_2)_2$	$C_{15}H_{25}NO_2$·[1]/$_2C_4H_4O_4$[h]	*i*-PrOH	184–185	C[i]	48[f]
20[k]	2-OCH_3	*t*-Bu	OCH_2	$C_{14}H_{23}NO_3$·HCl	acetone–Et_2O	139–140	C[i]	78[f]

[a] All compounds showed statisfactory elemental analysis. [b] Melting points not corrected. [c] As analytically pure sample. [d] Free base. [e] Based on amino alcohol **6**. [f] Based on epoxide **5**. [g] Based on amine **4a** or **4b**. [h] Fumarate. [i] Using an excess of amine in *i*-PrOH. [j] Known compound: M 66′527 (ICI).[11]

Table III. Biological Results and Log P and pK Values

compd	β-adrenoceptor blocking potency in the isolated guinea pig heart: n[a]	−log of concn, mol/L, giving 50% inhibn of isoproterenol-induced tachycardia ($\bar{X} \pm S\bar{X}$)	rel to oxprenolol (= 1)	log P	pK
7	3	7.12 ± 0.13	0.33		
8	3	6.39 ± 0.10	0.061		
9	3	7.85 ± 0.02	1.8	1.56	8.9
10	3	6.60 ± 0.08	0.10		
11	3	6.43 ± 0.02	0.068		
12	3	(5.66 ± 0.11)[b]	(0.011)[b]	1.24	
13	3	5.84 ± 0.02	0.018		
14	3	6.90 ± 0.10	0.19		
15	4	5.85 ± 0.07	0.018		
16	4	7.08 ± 0.12	0.30		
17	3	6.07 ± 0.06	0.029		
18	4	8.45 ± 0.04	6.9	1.14	8.8
19	3	5.67 ± 0.08	0.012	−0.21	9.9
20	4	7.26 ± 0.05	0.45	−0.44	9.8
oxprenolol	6	7.60 ± 0.09	1.000	0.20	9.4

[a] Number of animals used. [b] This compound also exhibits marked β-adrenoceptor stimulating effects. As a consequence of this, the dose–response curve in this test is very flat, and these values are therefore not exactly comparable.

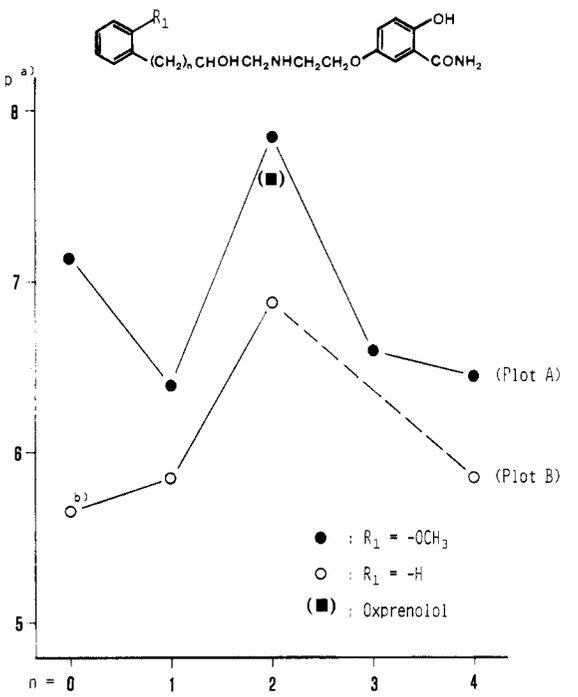


Figure 1. β-Blocking potency in vitro in relation to the length of the side chain. Footnote *a*: p = negative logarithm of concentrations of the compounds exerting 50% attenuation of isoproterenol-induced tachycardia. Footnote *b*: see corresponding footnote in Table III.

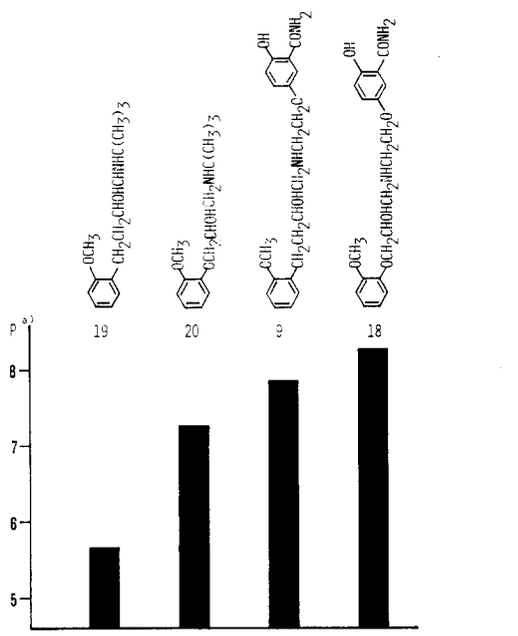


Figure 2. β-Blocking potency in vitro in relation to some characteristic structural parameters of the side chain. Footnote *a*: p = negative logarithm of concentrations of the compounds exerting 50% attenuation of isoproterenol-induced tachycardia.

first corresponds to $n = 0 \sim$ arylethanolamine, which resembles the natural agonists adrenaline and noradrenaline in structure. The second and more pronounced maximum corresponds to $n = 2 \sim$ arylbutanolamine, which resembles the (aryloxy)propanolamines in the distance between the ethanolamine group and the aromatic ring. The other members of the series are distinctly less active.

In the series of unsubstituted compounds (Figure 1, plot B), represented by only four homologues (**12–15**), a similar pattern is evident on a lower level of activity. Apart from the β-blockers of the arylethanolamine type, evidently only the $Ar(X)CH_2CHOHCH_2NH$ (X = O, CH_2) group allows a side-chain conformation ensuring an optimal fit of the molecule at the β-receptor. Various studies have been published dealing with the preferred conformations, in particular of the (aryloxy)propanolamines.[12] In most, a decisive role is attributed to the ether oxygen. The present

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(8) Fujimoto, Y.; Suzuki, Y.; Tanaka, Y.; Tominaga, T.; Takeda, H.; Sekine, H.; Morito, N.; Miyaoka, Y. *Heterocycles* **1977**, 1604.
(9) *Beilstein* **1931**, *17*(III/IV), 409. Lêvy, Sfinas *Bull. Soc. Chim. Fr.* **1931**, *49*, 1823.
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data demonstrate, however, that an ether oxygen in the β side chain is not an absolute prerequisite for potent β-blockade and might be replaced by an ether function in the ortho substituent, as in the very potent compound **9** (2-OCH_3). In principle, it is possible to form a hydrogen bond between the 2-methoxy group and the secondary OH group in the side chain. It is therefore conceivable that the 2-methoxy group in **9** could partially compensate for the function of the ether group in (aryloxy)propanolamines. By contrast, compounds **16** (2-CH_3) and **17** (4-OCH_3), in which no such intramolecular hydrogen bond can be formed, show considerably less activity.

As can be seen from Figure 2, in addition to optimal length, various other structural features of the side chain can contribute toward an increase in the potency of β-blockers. The ether oxygen does certainly augment the activity by comparison with the CH_2 group; the positive effect of the new nitrogen substituent, ethoxysalicylamide, by comparison with the *tert*-butyl group is, however, much more pronounced. It must be assumed that the ethoxysalicylamide group occupies an accessory binding site on the receptor.

Surprisingly enough, the measured log *P* values of some representatives of the series investigated revealed that, by comparison with the effect of the *tert*-butyl residue, the ethoxysalicylamide residue increases the lipophilicity of the derivatives by a factor of about 50 (e.g., **9** vs. **19**; **18** vs. **20**) (see Table III). This is much more than would be expected from calculations based on fragment constants. As evidenced by the measured p*K* values, however, the difference can largely be explained by the reduced basicity of the amine function.

Some of the compounds described, e.g., compounds **9** and **18**, were also tested for β-blocking activity in vivo in preliminary experiments on reserpine-pretreated, barbiturate-anesthetized cats (n = 2–3). The influence of ascending intravenous (iv) doses of the compounds on the tachycardia and vasodilative effects of isoproterenol was determined. The respective ED_{50} values of compound **9** for inhibition of isoproterenol-induced tachycardia and vasodilatation were 0.02 and 0.2 mg/kg iv, and those of compound **18** were 0.002 and 0.02 mg/kg iv. By comparison with oxprenolol, compound **9** was twice as potent and compound **18** was 20 times as potent in inhibiting isoproterenol-induced tachycardia. Their potency ratios in relation to oxprenolol in vivo were therefore similar to those found in vitro (cf. Table III). In addition, both compounds inhibited the cardiac effects of isoproterenol to a slightly greater extent than its vasodilative effect and displayed intrinsic sympathomimetic activity, as evidenced by the increase in basal heart rate. This last-mentioned effect was more pronounced than that of oxprenolol.

The pharmacological properties of various further β-blockers containing the new N-substituent will be reported on in more detail in subsequent publications.

Experimental Section

Chemical Methods. Melting points were determined on a Tottoli melting point apparatus or on a Koffler hot stage microscope and are uncorrected. All compounds have NMR spectra consistent with the assigned structure. Analytical samples were dried in vacuo and were free of significant impurities on TLC (Merck Darmstadt, silica gel plates with F254 indicator). Where analyses are indicated for C, H, and N, analytical values are within ±0.4% of calculated values.

2,3-Dihydro-2,2-dimethyl-6-hydroxy-4*H*-1,3-benzoxazin-4-one (1). A suspension of 2,5-dihydroxybenzamide (153 g, 1.0 mol) and 4-toluenesulfonic acid (14 g) in 2,2-dimethoxypropane (1.2 L) was stirred under reflux for 4–5 h. The suspension was cooled in ice and filtered with suction. The resulting crystals were successively washed with H_2O and *i*-PrOH and dried: yield 168 g (87%); mp 216–218 °C; IR (Nujol) 1684 (CONH); NMR (Me_2SO-d_6) δ 1.55 (s, 6, CH_3), 6.8 (m, 2, Ar H), 7.2 (d, J = 3 Hz, 1, Ar H), 8.5 (s, 1, OH), 9.3 (s, 1, NH). Anal. ($C_{10}H_{11}NO_3$) C, H, N.

[(2,3-Dihydro-2,2-dimethyl-4-oxo-4*H*-1,3-benzoxazin-6-yl)oxy]acetaldehyde (2). Compound **1** (96.5 g, 0.5 mol), allyl bromide (91.0 g, 0.75 mol), and anhydrous potassium carbonate (103 g, 0.75 mol) were suspended in 1.5 L of acetonitrile and heated under stirring to reflux for 2.5 h. Suction filtration of the hot suspension, followed by concentration of the filtrates and cooling to room temperature, afforded the crystalline allyloxy compound: mp 137–138 °C; yield 94%. This product (4.7 g, 0.02 mol) was dissolved in a mixture of 50 mL of dioxane and 10 mL of water. Under stirring, two crystals of osmium tetroxide were added. After 15 min, the solution turned dark, and 8.6 g (0.04 mol) of sodium periodate was added portionwise in the course of the next 35 min. A white precipitate formed and the temperature rose to 45 °C. The suspension was filtered, the filtrates were evaporated to dryness, and the residue was purified by column chromatography on silica gel/EtOAc. Recrystallization of the product from Et_2O gave 2.0 g (43%) of **2**: mp 163–166 °C; IR (CH_2Cl_2) 3410, 3200 (NH), 1750 (HCO), 1680 (CONH), 1480 (aromatic C=C) cm^{-1}; NMR ($CDCl_3$) δ 1.62 (s, 2 CH_3, 6), 4.71 (s, OCH_2, 2), 6.87 (d, J = 9 Hz, 1, Ar H), 7.11 (q, 1, Ar H), 7.35 (d, J = 3 Hz, 1, Ar H), 8.30 (br s, 1 NH), 9.80 (s, HCO, 1). Anal. ($C_{12}H_{13}NO_4$) C, H, N.

6-(2-Bromoethoxy)-2,3-dihydro-2,2-dimethyl-4*H*-1,3-benzoxazin-4-one (3). A mixture of **1** (70 g, 0.36 mol), potassium carbonate (75 g, 0.54 mol), and 1,2-dibromoethane (0.4 L) was heated at reflux for 14 h while stirring. The mixture was cooled and filtered with suction; the solid residue was stirred with H_2O (0.4 L) for 1 h, again filtered, and dried in vacuo at 80 °C: yield 89 g (82%) of crude **2**; mp 190–200 °C. A sample was recrystallized from MeOH: mp 205–208 °C; IR (CH_2Cl_2) 3410 (NH), 1678 (CONH); NMR ($CDCl_3$) δ 1.6 (s, 6, CH_3), 3.68 (t, J = 6 Hz, 2, CH_2Br), 4.31 (t, J = 6 Hz, 2, Ar OCH_2), 6.85 (d, J = 9 Hz, 1, Ar H_8), 7.05 (2 d, J = 3 and 9 Hz, 1, Ar H_7), 7.35 (d, J = 3 Hz, 1, Ar H_5), 8.35 (s, 1, NH). Anal. ($C_{12}H_{14}BrNO_3$) C, H, N, Br.

5-[2-[(Phenylmethyl)amino]ethoxy]salicylamide (4a). A suspension of crude **3** (60 g, 0.20 mol) in a mixture of H_2O (100 mL) and phenylmethanamine (87 mL) was heated at reflux for 0.5 h, when all the solid was dissolved. Heating at reflux was continued until all **3** had reacted (TLC, ca. 0.5 h). More H_2O was added (100 mL), and the reaction mixture was acidified with concentrated HCl (ice cooling). After the mixture was left standing for several hours, the crystals were filtered, successively washed with H_2O, *i*-PrOH, and EtOAc, and dried: yield 57 g (88%) **4a**·HCl; mp 213–217 °C. The base **4a** was liberated by the addition of aqueous NH_3 and extraction with EtOAc: mp 126–128 °C (from EtOAc–ether); IR (Nujol) 3414, 3310 ($CONH_2$), 1680 ($CONH_2$); NMR (Me_2SO-d_6) δ 2.73 (t, J = 6 Hz, 2, CH_2NH), 3.36 (s, 2, Ar $NHCH_2$), 4.0 (t, J = 6 Hz, 2, Ar OCH_2), 6.8 (d, J = 9 Hz, 1, Ar H_3), 7.05 (2 d, J = 3 and 9 Hz, 1, Ar H_4), 7.3 (s, 5, C_6H_5), 7.46 (d, J = 3 Hz, 1, Ar H_6), 7.8–8.4 (broad, 2, $CONH_2$). Anal. ($C_{16}H_{18}N_2O_3$) C, H, N.

5-(2-Aminoethoxy)salicylamide (4b). A solution of **4a** (25.0 g, 87 mmol) in MeOH (300 mL) was hydrogenated in the presence of Pd/C (10%, 5.2 g) at room temperature and atmospheric pressure until 1 equiv of H_2 was taken up. By concentration of the filtered solution, 13.3 g (78%) of crystals was obtained: mp 145–147 °C (*i*-PrOH); NMR (Me_2SO-d_6) δ 2.9 (t, J = 6 Hz, 2, CH_2NH_2), 3.9 (t, J = 6 Hz, 2, Ar OCH_2), 4.8–5.6 (broad, 4, NH), 6.8 (d, J = 9 Hz, 1, Ar H_3), 7.1 (2 d, J = 3 and 9 Hz, 1, Ar H_4), 7.45 (d, J = 3 Hz, 1, Ar H_6), 7.6–8.2 (broad, ~1, OH). Anal. ($C_9H_{12}N_2O_3$) C, H, N.

General Procedure for the Preparation of the Aryl and Alkyl Olefins. A solution of 0.4 mol of the appropriate alkenylmagnesium bromide in 400 mL of ether, prepared by a standard Grignard procedure, was stirred under nitrogen at 15 °C. Via a dropping funnel, a solution of 0.18 mol of the corresponding benzyl bromide in 60 mL of toluene was slowly added in 30 min. The reaction was slightly exothermic at the beginning. The mixture was further stirred overnight and then heated at reflux for 1 h. After the mixture was cooled in an ice bath, 20 mL of water was added dropwise. The resulting suspension was filtered through Celite, and the filtrates were washed successively three times with

0.1 N HCl and water. Drying of the organic phase on $MgSO_4$ and evaporation of the solvent gave a crude oil which was fractionated on high vacuum. The resulting olefins were pure on TLC (hexane) and showed correct NMR (20–85% yields).

General Epoxidation Procedure (Method A). To 50 mmol of the appropriate olefin dissolved in 50 mL of CH_2Cl_2, a solution of 55 mmol of *m*-chloroperbenzoic acid in 110 mL of CH_2Cl_2 was added dropwise at 20–25 °C under stirring. After 2 h an additional 4 mmol of *m*-chloroperbenzoic acid in 10 mL of CH_2Cl_2 was added to the resulting suspension. After stirring for an additional 2 h, the suspension was filtered, and the filtrates were washed successively with aqueous solutions of Na_2SO_3, $NaHCO_3$, and water and then dried over Na_2SO_4, and the solvent was removed on the rotovapor. The resulting products were almost pure on TLC (toluene) and showed NMR data in full agreement with the structures (80–95% yields). These products were used without further purification in the next steps.

1,2-Epoxy-4-(2-methylphenyl)butane (5h). Method B. To the Grignard compound, prepared from α-bromo-*o*-xylene (92.5 g, 0.50 mol) and magnesium turnings (12,15 g, 0.50 mol), in absolute ether (250 mL), was added, with stirring and cooling, a solution of epichlorohydrin (46.3 g, 0.50 mol) in benzene (175 mL). After heating at reflux for 2 h, the reaction mixture was cautiously decomposed by the addition of water and acidified with 2 N H_2SO_4, and the phases were separated. The organic layer was washed with brine, dried ($MgSO_4$), and evaporated to yield a yellow oil (98 g), representing crude 1-chloro-4-(2-methylphenyl)-2-butanol. This was dissolved in dichloromethane (500 mL), treated with 4 N NaOH (300 mL) and tetrabutylammonium hydrogen sulfate (15 g), and stirred for 7 to 10 h. The organic layer was separated, washed with H_2O, dried ($MgSO_4$), and evaporated. The oily residue (58 g) was distilled (Kugelrohr, 130–140 °C bath temperature, 0.5 mmHg). After a forerun of 1,2-bis(2-methylphenylethane, **5h** was obtained (15.8 g, 20% yield): NMR ($CDCl_3$) δ 1.7–1.9 (m, 2, CH_2), 2.3 (s, 3, CH_3), 2.45 (2 d, J = 3 and 5 Hz, 1, CH- CH_2), 2.7–2.8 (m, 2, Ar CH_2), 2.85 (s, 1, CH- CH_2), 2.8–3.0 (m, 1, CH- CH_2), 7.12 (s, 4, Ar H).

1,2-Epoxy-4-(4-methoxyphenyl)butane (5i) was prepared analogously to **5h** from the Grignard compound of anisyl chloride[16] and epichlorohydrin: bp 117 °C (0.05 mmHg); yield 26%; NMR ($CDCl_3$) δ 1.7–1.9 (m, 2, CH_2), 2.45 (q, J = 3 Hz, 1, CH- CH_2), 2.6–2.8 (m, 3, Ar CH_2, CH- CH_2), 2.8–3.0 (m, 1, CH- CH_2), 3.78 (s, 3, OCH_3), 6.8 (d, J = 9 Hz, 2, Ar H), 7.1 (d, J = 9 Hz, 2, Ar H).

General Procedure for Method C (Scheme III). A solution of 19.6 g (0.1 mol) of **4b** and 0.125 mol of the appropriate epoxide in 100 mL of Me_2SO was stirred for 1 h in a bath at 120 °C and then cooled and poured into 200 mL of ice–water. After the solution was extracted three times with EtOAc and the extracts were washed with water, dried over $MgSO_4$, and concentrated by evaporation, the crude product was obtained, which was purified by recrystallization from MeOH or by formation of the salt indicated in Table II.

General Procedure for Method D (Scheme III). A solution of the appropriate expoxide **5** (10 mmol) and **4a** (2.68 g, 10 mmol) in *i*-PrOH (40 mL) was heated at reflux for 24–80 h. The solvent was removed, and the oily residue debenzylated either directly or after purification by column chromatography (200 g of silica gel; $CHCl_3$/MeOH/concentrated NH_3, 350:50:1). The debenzylation was carried out as for the preparation of **4b**. Salts were prepared as indicated in Table II.

α-[[[2-(3-Carbamoyl-4-hydroxyphenoxy)ethyl]amino]-methyl]-2-methoxybenzyl Alcohol (7). Method E. α-(Aminomethyl)-2-methoxybenzyl alcohol (**6**; 4.15 g, 25 mmol)[14] and 5.6 g (23.8 mmol) of aldehyde **3** were dissolved in 250 mL of MeOH at 25 °C and 25 g of molecular sieves (3 Å) were added. The pH was adjusted to 8.5 by adding a MeOH/HCl solution. Under stirring, a solution of 594 mg (9.46 mmol) $NaBCNH_3$ in 60 mL of methanol was slowly added, and the suspension was further stirred at 25 °C overnight. After filtration, 50 mL of a MeOH/HCl solution (6 N) was added (**caution**: HCN evolution!), and the mixture was heated at reflux for 1 h. Evaporation of the solvent gave a residue, which was purified by column chromatography on silica gel with $CHCl_3$/MeOH/concentrated NH_3 (400:50:5). The crude product was dissolved in hot *i*-PrOH, and the solution was filtered with charcoal and cooled to 25 °C: 1.9 g (22%) of white crystals was isolated; mp 67–68 °C; IR (Nujol) 3420 (NH), 3420–2500 (br NH, OH), 1680, 1640, 1590 cm^{-1}; NMR (Me_2SO-d_6) 2.47–2.80 (m, CH_2N, 2), 2.90 (t, J = 6 Hz, 2, N-CH_2), 3.77 (s, OCH_3, 3), 3.98 (t, J = 6 Hz, 2, CH_2O), 4.98 (q, Ar CH, 1), 6.83–7.47 (m, aromatics, 7), 7.8–8.3 (3 br d, ArOH, $CONH_2$).

1-Octanol/pH 7.4 Phosphate Buffer Partition Coefficients. The compounds were dissolved in 0.067 M, pH 7.4 phosphate buffer saturated with 1-octanol to concentrations of about 10^{-4} M. Appropriate volume ratios of these solutions with 1-octanol, previously saturated with buffer solution, were shaken for 1 h at 25.0 °C. After centrifugation at 4000 rpm for 10 min, concentrations were determined spectrophotometrically on a Cary 118 spectrophotometer. The partition coefficients were determined in triplicate as the ratio of the concentrations in the 1-octanol and the pH 7.4 phosphate buffer phase.

Determination of pK_a Values. Acid dissociation constants were determined by spectrophotometric microfiltration. Dilute sample solutions were circulated through an invested glass electrode and a 1-cm cell in a Cary 118 spectrophotometer. Evaluation of the pH-dependent spectra and calculation of the pK_a's were performed according to the method described in ref 15.

Pharmacology Method. Isolated hearts [removed under anesthesia with urethane (1.5 g/kg intraperitoneally)] from male white Pirbright guinea pigs (Tif:DHP), weighing 330–640 g, were perfused according to the method of Langendorff with oxygenated (95% O_2/5% CO_2) Krebs–Henseleit solution supplemented with 20 μg/mL of ascorbic acid and 15 μg/mL of NaEDTA at a hydrostatic pressure of 63 cm of H_2O and a temperature of 36 °C. Heart rate, contractile force, and coronary flow were recorded continuously. After an initial control period of ~30 min, DL-isoproterenol sulfate (0.005 μg/mL) was added to the perfusion solution. Subsequently, the influence of increasing concentrations of the compounds on isoproterenol-induced tachycardia was determined, each concentration being allowed to act for 10 min. The concentrations of the test compounds (in moles per liter) producing 50% inhibition of isoproterenol-induced tachycardia (IC_{50}) were graphically interpolated. Potencies as β-receptor antagonists are given as the negative logarithms of the IC_{50} values.

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Registry No. 1, 74469-70-8; **2**, 76814-00-1; **3**, 74454-60-7; **4a**, 76813-69-9; **4a**·HCl, 74454-72-1; **4b**, 74454-87-8; **5a**, 62826-28-2; **5b**, 71813-53-1; **5c**, 89789-93-5; **5d**, 89789-94-6; **5e**, 4436-24-2; **5f**, 1126-76-7; **5g**, 86088-39-3; **5h**, 79407-27-5; **5i**, 56438-59-6; **6**, 54942-63-1; **7**, 79407-05-9; **8**, 89789-95-7; **8**·HCl, 79407-03-7; **9**, 89789-96-8; **9**·HCl, 79407-00-4; **10**, 84815-02-1; **10**·HCl, 84815-03-2; **11**, 89789-97-9; **11**·HCl, 89789-85-5; **12**·CH_3SO_3H, 89789-87-7; **13**, 79407-02-6; **13**·HCl, 89789-88-8; **14**, 79407-23-1; **15**, 89789-89-9; **16**, 79407-24-2; **16**·HCl, 79407-25-3; **17**, 89789-90-2; **18**, 76813-08-6; **19**·$C_4H_4O_4$, 89789-92-4; **20**, 37708-25-1; **20**·HCl, 15149-21-0; 2,5-dihydroxybenzamide, 52405-73-9; 2,2-dimethoxypropane, 77-76-9; 1,2-dibromoethane, 106-93-4; phenylmethanamine, 100-46-9; 4-bromo-1-butene, 5162-44-7; 5-bromo-1-pentene, 1119-51-3; 2-methoxybenzyl bromide, 52289-93-7; benzyl bromide, 100-39-0; α-bromo *o*-xylene, 89-92-9; 3-(2-methoxyphenyl)-1-propene, 3698-28-0; 4-(2-methoxyphenyl)-1-butene, 63667-83-4; 5-(2-

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methoxyphenyl)-1-pentene, 52093-69-3; 6-(2-methoxyphenyl)-1-hexene, 89789-98-0; allylbenzene, 300-57-2; 4-phenyl-1-butene, 768-56-9; 5-phenyl-1-pentene, 1075-74-7; 4-(4-methoxyphenyl)-1-butene, 20574-98-5; epichlorohydrin, 106-89-8; 1-chloro-4-(2-methylphenyl)-2-butanol, 79407-26-4; 1,2-bis(2-methylphenyl)-ethane, 952-80-7; *p*-anisyl chloride, 824-94-2; allyl bromide, 106-95-6; 2,3-dihydro-2,2-dimethyl-6-(allyloxy)-4*H*-1,3-benzoxazine, 76813-99-5.

A Photoaffinity Reagent To Label the Opiate Receptors of Guinea Pig Ileum and Mouse Vas Deferens

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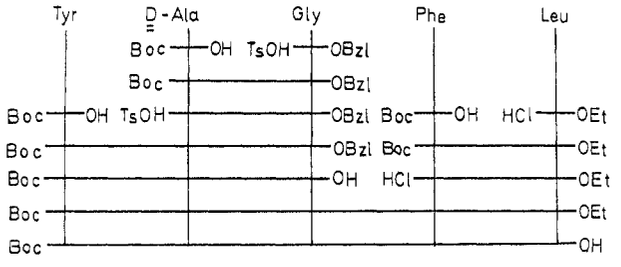
An enkephalin derivative, [D-Ala^2,Leu^5]enkephalin *N*-[(2-nitro-4-azidophenyl)amino]ethylamide, has been synthesized as a photoaffinity label for the opiate receptor. This compound retains the full biological activity of [D-Ala^2,Leu^5]enkephalin in guinea pig ileum and mouse vas deferens tests with IC_{50} values of 4.4 and 2.6 nM, respectively, and inhibits the binding of [^{3}H]naloxone to rat brain membrane preparation with an IC_{50} value of 2.5 nM. Photolysis of a muscle strip of the guinea pig ileum or of the mouse vas deferens in the presence of the peptide derivative caused irreversible inhibition of electrically stimulated contractions with high efficiencies (80 and 66%, respectively), while the inhibitory effect in the dark was fully reversed by washing. This irreversible inhibition during photolysis was completely prevented by the presence of [D-Ala^2,Leu^5]enkephalin. These results demonstrate that [D-Ala^2,Leu^5]-enkephalin *N*-[(2-nitro-4-azidophenyl)amino]ethylamide is a prominent candidate as a photoaffinity label for the opiate receptor.

The discovery of enkephalins[1] and endorphins,[2-4] endogenous peptide ligands with opiate properties, sparked the enormous research activity that has characterized the opiate field in recent years. Other new endogenous peptides have been discovered subsequently.[5-9] At present the opioid peptides can be classified into the following three groups: endorphins, enkephalins, and dynorphins/neo-endorphins; the occurrence of these three sets of opioids derived from three different genes is clearly established.[10-13] A number of reports on subcellular distributions of the opioid peptides and their receptors have been published.[14-17] The observations of heterogeneous binding of opioid ligands to receptors[18,19] raise some intriguing questions on whether multiple classes of opioid receptors (at least three types designated μ, δ, and κ[18]) correlate with the products of the three opioid peptide genes.

Little significant progress, however, has been made with respect to receptors at the molecular level. It is still unclear whether these various receptor forms represent different molecular entities or result from conformational changes of a single receptor species. Isolation and biochemical characterization of the cell-surface receptor are thus of prime importance. Several attempts along this line have been reported, such as solubilization of the receptor[20-23] and partial purification by affinity chromatography.[24]

Special difficulties in studying opiate receptors lie in the fact that the receptors are present in very small quantities (ca. 10 fmol/mg of guinea pig brain) and are membrane-bound proteins sensitive to protelytic enzymes and detergents. One promising approach is the use of affinity labels, particularly the photoaffinity labeling technique.[25,26] We have shown the usefulness of this technique in the labeling of an enzyme active site.[27] Several photoaffinity compounds for the opiate receptors, based on opiate drugs[28-30] or enkephalins,[31-34] have been synthesized and applied. However, for the wavelength used (254 nm) it has been shown recently that this short-wavelength UV light causes rapid destruction of the opiate binding activity of the receptor.[35] Accordingly, we have designed a pho-

Scheme I. A Synthetic Route for N^{α}-Boc-[D-Ala^2,Leu^5]enkephalin



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