

the presence or absence of agents in the aqueous phase, were recorded on a differential scanning calorimetric (Model DSC-2, Perkin Elmer) at a heating rate of 2.5 °C/min. A 10- $\mu$ L sample (1 mg of phospholipid) was applied and encapsulated in an aluminum sample pan. Both abscissa (temperature) and ordinate (transition energy) were calibrated with indium and lead as standards. The calorimetric enthalpy changes ( $\Delta H_{cal}$ ) were measured from the area under the peak. The standard van't Hoff enthalpy changes ( $\Delta H_{vH}$ ) were calculated by eq 7 where  $T_m$  is the

$$H_{vH} = 6.9 \frac{T_m^2}{\Delta T_{1/2}} \quad (7)$$

phase transition of the phospholipid and  $\Delta T_{1/2}$  is the half-width of the transition peak, and the cooperativity of the transition process of the phospholipid liposome was followed by the definition of Hinz and Sturtevant (eq 8).<sup>31</sup>

$$n = \frac{\Delta H_{vH}}{\Delta H_{cal}} \quad (8)$$

## $\beta$ -Adrenergic Blocking Agents. $\alpha$ - and $\gamma$ -Methyl(aryloxy)propranolamines

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A series of  $\gamma$ -methyl- (5) and  $\alpha$ -methyl(aryloxy)propranolamines (6) were synthesized and evaluated for  $\beta$ -adrenergic blocking action. The binding constant ( $K_D$ ) was determined for compounds 5a-j on cultured C6-2B astrocytoma cells. Compounds 5a-j and 6a-e were evaluated for  $\beta_1$ -antagonist action on guinea pig atria and  $\beta_2$ -antagonist action on guinea pig tracheal strips. Several  $\gamma$ -methyl(aryloxy)propranolamines showed affinity for  $\beta$  receptors and a possible preference for  $\beta_2$  receptors.

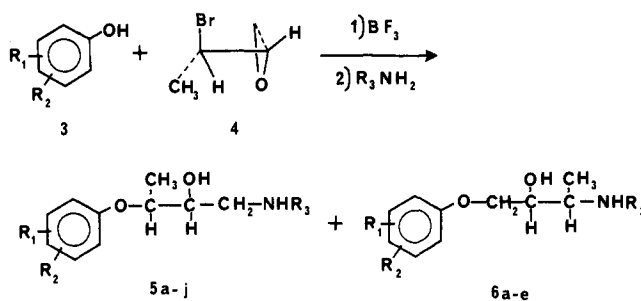
The realization that  $\beta$ -adrenoceptors are not homogeneous but instead can be subdivided into at least two subclasses ( $\beta_1$  and  $\beta_2$ ) has resulted in the publication of numerous articles dealing with  $\beta_1$ - and  $\beta_2$ -adrenoceptor agonists and antagonists. Several years ago we were asked to prepare a series of  $\alpha$ -methyl(aryloxy)propranolamines which were under active consideration as  $\beta_2$  selective antagonists.<sup>1</sup> Since then, studies have shown that introduction of a methyl group into the  $\alpha$  position of the non-selective  $\beta$ -adrenoceptor antagonist propranolol does enhance the selectivity of propranolol toward the  $\beta_2$ -adrenoceptors.<sup>2,3</sup> While investigating a general synthetic method for synthesis of the  $\alpha$ -methyl(aryloxy)propranolamines, we discovered a novel method for preparing  $\gamma$ -methyl(aryloxy)propranolamines.<sup>4</sup> The only  $\gamma$ -methyl(aryloxy)propranolamine that had been reported previous to this was the erythro/threo mixture of propranolol (5i) reported by Howe.<sup>5</sup> While Howe's study suggested that introduction of the methyl group into the side chain gave compounds which are less potent antagonists, the study only considered the effects of the compounds on the heart

**Circular Dichroism Measurements.** A Jasco Model ORD/CD5 with Sproul Scientific SS 10 modification was used for circular dichroism (CD) measurements. To minimize the scattering effect, the cell was positioned as close as possible to the photomultiplier.

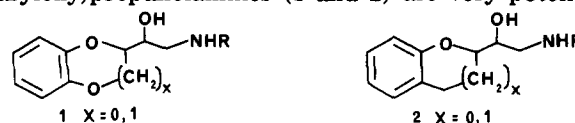
**Acknowledgment.** We thank Dr. T. R. Beattie and Ms. C. S. Tripp for initiating this DSC work, to Dr. W. Stoeckenius for providing purple membrane and laboratory facilities to carry out some experiments in this study, and to Drs. J. M. Sturtevant, P. Kroon, J. C. Robbins, and T. W. Doebber for helpful discussions.

- (31) H.-J. Hinz and J. M. Sturtevant, *J. Biol. Chem.*, **247**, 6071-6075 (1972).  
 (32) H. Lecuyer and D. G. Dervichian, *J. Mol. Biol.*, **45**, 39 (1969).  
 (33) H. A. Saber, Ed., "Handbook of Biochemistry, Selected Data for Molecular Biology", The Chemical Rubber Co., Cleveland, Ohio, 1968.

Scheme I



and, thus,  $\beta_1$  adrenoceptors. Subsequent studies by Howe and co-workers<sup>6,7</sup> have shown that semirigid  $\gamma$ -alkyl(aryloxy)propranolamines (1 and 2) are very potent  $\beta_1$ -





adrenoceptor antagonists. We now report our studies directed toward elucidating the structural relationship of

(1) Levy, B. *Br. J. Pharmacol.* **1973**, *49*, 514.  
 (2) Fitzgerald, J. D.; O'Donnell, S. R. *Clin. Exp. Pharmacol. Physiol.* **1978**, *5*, 579.  
 (3) O'Donnell, S. R.; Walduck, K.; Wanstall, J. C. *Br. J. Pharmacol.* **1980**, *68*, 705.  
 (4) Lemke, T. L.; Boblitt, R. L.; Capiton, G. A.; Cates, L. A.; Martin, G. E. *J. Org. Chem.* **1978**, *43*, 2079.  
 (5) Howe, R. *J. Med. Chem.* **1969**, *12*, 642.

(6) Howe, R.; Rao, B. S.; Chodnekar, M. S. *J. Med. Chem.* **1970**, *13*, 169.  
 (7) Chodnekar, M. S.; Crowther, A. F.; Hepworth, W.; Howe, R.; McLaughlin, B. J.; Mitchell, A.; Roa, B. S.; Slatcher, R. P.; Smith, L. H.; Stevens, M. A. *J. Med. Chem.* **1972**, *15*, 49.  
 (8) Terasaki, W. L.; Brooker, G. *J. Biol. Chem.* **1978**, *253*, 5418.

Table I.  $\gamma$ -Methyl- (5) and  $\alpha$ -Methyl(aryloxy)propanolamines (6)

| no. | R <sub>1</sub>  | R <sub>2</sub>                  | R <sub>3</sub>                    | mp, °C                     | yield, <sup>h</sup> % | formula   |
|-----|---|---------------------------------|-----------------------------------|----------------------------|-----------------------|---|
| 5a  | H   | H                               | C(CH <sub>3</sub> ) <sub>3</sub>  | 132-135 <sup>a,b</sup>     | 41                    |   |
| 5b  | 3-CH <sub>3</sub>   | 4-CH <sub>3</sub>               | CH(CH <sub>3</sub> ) <sub>2</sub> | 122.5-123.4 <sup>a,f</sup> | 20.6                  | C <sub>15</sub> H <sub>25</sub> NO <sub>2</sub> ·HCl  |
| 5c  | 3-CH <sub>3</sub>   | 4-CH <sub>3</sub>               | C(CH <sub>3</sub> ) <sub>3</sub>  | 154-157 <sup>a,b</sup>     | 14.4                  |   |
| 5d  | H   | 4-C <sub>2</sub> H <sub>5</sub> | CH(CH <sub>3</sub> ) <sub>2</sub> | 177-180 <sup>d,c</sup>     | 14.2                  | C <sub>15</sub> H <sub>25</sub> NO <sub>2</sub> ·C <sub>6</sub> H <sub>3</sub> N <sub>3</sub> O <sub>7</sub> <sup>i</sup> |
| 5e  | H   | 4-C <sub>2</sub> H <sub>5</sub> | C(CH <sub>3</sub> ) <sub>3</sub>  | 150-152 <sup>a,c</sup>     | 21.0                  |   |
| 5f  | 3-Cl  | 4-Cl                            | CH(CH <sub>3</sub> ) <sub>2</sub> | 134.5-136 <sup>a,b</sup>   | 81                    | C <sub>13</sub> H <sub>19</sub> Cl <sub>2</sub> NO <sub>2</sub> ·HCl  |
| 5g  | 2-CH <sub>2</sub> CH=CH <sub>2</sub>  | H                               | CH(CH <sub>3</sub> ) <sub>2</sub> | 134-135 <sup>d,e</sup>     | 28.5                  | C <sub>16</sub> H <sub>25</sub> NO <sub>2</sub> ·C <sub>6</sub> H <sub>3</sub> N <sub>3</sub> O <sub>7</sub> <sup>j</sup> |
| 5h  | 2-CH <sub>2</sub> CH=CH <sub>2</sub>  | H                               | C(CH <sub>3</sub> ) <sub>3</sub>  | 122-125 <sup>a,g</sup>     | 27                    | C <sub>17</sub> H <sub>27</sub> NO <sub>2</sub> ·HCl  |
| 5i  |  |                                 | CH(CH <sub>3</sub> ) <sub>2</sub> | 163-164 <sup>a,b</sup>     | 15.2                  | C <sub>17</sub> H <sub>23</sub> NO <sub>2</sub> ·HCl  |
| 5j  |  |                                 | C(CH <sub>3</sub> ) <sub>3</sub>  | 157-158 <sup>a,c</sup>     |                       | C <sub>18</sub> H <sub>25</sub> NO <sub>2</sub> ·HCl  |
| 6a  | H   | H                               | C(CH <sub>3</sub> ) <sub>3</sub>  | 135-139 <sup>a,b</sup>     | 7.0                   |   |
| 6b  | 3-CH <sub>3</sub>   | 4-CH <sub>3</sub>               | CH(CH <sub>3</sub> ) <sub>2</sub> | 134-135 <sup>a,b</sup>     | 9.2                   | C <sub>15</sub> H <sub>25</sub> NO <sub>2</sub> ·HCl  |
| 6c  | 3-CH <sub>3</sub>   | 4-CH <sub>3</sub>               | C(CH <sub>3</sub> ) <sub>3</sub>  | 159-161 <sup>a,b</sup>     | 8.1                   |   |
| 6d  | H   | 4-C <sub>2</sub> H <sub>5</sub> | CH(CH <sub>3</sub> ) <sub>2</sub> | 118-119.5 <sup>d,e</sup>   | 7.0                   | C <sub>15</sub> H <sub>25</sub> NO <sub>2</sub> ·C <sub>6</sub> H <sub>3</sub> N <sub>3</sub> O <sub>7</sub>              |
| 6e  | H   | 4-C <sub>2</sub> H <sub>5</sub> | C(CH <sub>3</sub> ) <sub>3</sub>  | 174-176 <sup>a,b</sup>     | 8.4                   |   |

<sup>a</sup> Hydrochloride salt. <sup>b</sup> From EtOAc. <sup>c</sup> From EtOAc-c-C<sub>6</sub>H<sub>12</sub>. <sup>d</sup> Picrate salt. <sup>e</sup> From c-C<sub>6</sub>H<sub>6</sub>. <sup>f</sup> From EtOAc-hexanes. <sup>g</sup> From c-C<sub>6</sub>H<sub>6</sub>-c-C<sub>6</sub>H<sub>12</sub>. <sup>h</sup> Yield was based on crude free base. <sup>i</sup> N: calcd, 11.66; found, 11.20. <sup>j</sup> C: calcd, 53.65; found, 54.16.

the  $\gamma$ -methyl substituent and  $\beta_1$ - and  $\beta_2$ -adrenoceptor blockade.

**Chemistry.** Synthesis of the  $\gamma$ -methyl(aryloxy)propanolamines (5) was accomplished, as previously reported,<sup>4</sup> by addition of substituted phenols (3) to *threo*-3-bromo-1,2-epoxybutane (4) using catalytic amounts of boron trifluoride etherate (Scheme I). When phenol, 3,4-dimethylphenol, or 4-ethylphenol was used, 5a-e were accompanied by the  $\alpha$ -methyl(aryloxy)propanolamines (6a-e), which were separated by column chromatography. When 2-allylphenol, 3,4-dichlorophenol, or 1-naphthol was used, only the rearrangement product 5f-j was obtained (Table I). With the introduction of the methyl group at C-3 or C-1 of the propanolamine side chain, erythro and *threo* forms of the compound are possible. There is no indication that 5 or 6 are mixtures, but rather it appears that they each consists of a single compound. With the previous benzodioxan and chroman analogues reported by Howe and co-workers<sup>5,6</sup> (1 and 2), where diastereomers were separated, a distinct difference in the chemical shift and coupling pattern was noted for the  $\gamma$  proton of the erythro and *threo* isomers. A chemical-shift difference of  $\delta$  0.1-0.6 was found. This would suggest that if compound 5 is a mixture of isomers, one should find a complex and varied chemical shift for the  $\gamma$  proton. To the contrary, we have found that 5a-e,g,h all show a five peak pattern (ABX<sub>3</sub>) for the  $\gamma$  proton in the region of  $\delta$  4.0-4.57 with a  $w_{1/2}$  of 14-16 Hz. Compounds 5i and 5j both have the  $\gamma$  proton appearing as a five-peak pattern at  $\delta$  4.23-4.73 with a  $w_{1/2}$  of 16 Hz. In 5f the  $\gamma$  proton overlapped the  $\beta$  proton and could not be distinguished. At the present time, the NMR suggests a single compound but does not distinguish the configuration of this compound.

## Results and Discussion

Two *in vitro* assays were used to assess the  $\beta$ -adrenoceptor antagonistic activity of compounds 5 and 6. Compounds 5a-j were tested on cultured C6-2B astrocytoma cells by studying their ability to prevent the radioiodinated  $\beta$ -adrenergic receptor probe [<sup>125</sup>I]iodohydroxybenzylpindolol ([<sup>125</sup>I]HYP) from binding to the cellular receptor sites. The results are reported in Table II as the concentration of test compound needed to displace 50% of the bound antagonist, [<sup>125</sup>I]HYP, and is reported as the

Table II. Displacement Constants for  $\gamma$ -Methyl(aryloxy)propanolamines (5) on [<sup>125</sup>I]Iodohydroxybenzylpindolol Binding Sites

| compd           | K <sub>D</sub> , mol   |
|-----------------|------------------------|
| 5a              | 3 × 10 <sup>-7</sup>   |
| 5b              | 2 × 10 <sup>-7</sup>   |
| 5c              | 2 × 10 <sup>-7</sup>   |
| 5d              | 2 × 10 <sup>-6</sup>   |
| 5e              | 2 × 10 <sup>-6</sup>   |
| 5f              | 5 × 10 <sup>-7</sup>   |
| 5g              | 5 × 10 <sup>-8</sup>   |
| 5h              | 1.5 × 10 <sup>-8</sup> |
| 5i              | 4 × 10 <sup>-8</sup>   |
| 5j              | 5 × 10 <sup>-9</sup>   |
| (-)-propranolol | 7 × 10 <sup>-10</sup>  |

apparent K<sub>D</sub>. The compounds 5a-j and 6a-e were also evaluated on the spontaneously beating guinea pig atrial preparations as an indication of  $\beta_1$ -receptor blocking activity and on guinea pig tracheal strips used to evaluate  $\beta_2$ -receptor blocking activity (Table III).<sup>9,10</sup>

The binding studies on the  $\gamma$ -methyl(aryloxy)propanolamines, 5, give results which parallel previous structure-activity relationships (SAR).<sup>11</sup> Hydrogen or alkyl substitution at the 3 and/or 4 position of the aromatic ring has not previously been reported to elicit strong  $\beta$ -antagonist activity. Therefore, it is not surprising that compounds 5a-e have low-binding affinity. Substitution of the 3,4 position of the aromatic ring with chloro, 5f (as in the phenylethanolamine antagonist), or 2-allyl, 5g-h, or replacement of the phenyl with 1-naphthyl, 5i-j, did result in agents with good affinity for the  $\beta$  receptor. It is especially encouraging since the K<sub>D</sub> for  $\gamma$ -methylalprenolol (5g), which is a racemate, is within one-tenth of the K<sub>D</sub> reported for isomerically pure (-)-alprenolol (3.4 × 10<sup>-9</sup>).<sup>11</sup> Increasing the size of alkyl or aralkyl substitution on the amine nitrogen would be expected to increase the

- Turner, R. A., "Screening Methods in Pharmacology"; Turner, R. A.; Hebborn, P., Eds.; Academic Press: New York, 1971; p 21.
- Timmerman, H; Scheffer, N. G. *J. Pharm. Pharmacol.* 1968, 20, 18.
- Mukherjee, C.; Caron, M. G.; Mullikin, D.; Lefkowitz, R. J. *Mol. Pharmacol.* 1976, 12, 16.

Table III. Effects of Methyl(aryloxy)propanolamines on Guinea Pig Atria and Tracheal Strips

| compd       | atria ( $\beta_1$ )             |                          |   | trachea ( $\beta_2$ )           |                          |   |
|-------------|---------------------------------|--------------------------|---|---------------------------------|--------------------------|---|
|             | EC <sub>50</sub> <sup>a,b</sup> | $\pm 1$ SEM <sup>c</sup> | test EC <sub>50</sub> /<br>control EC <sub>50</sub> | EC <sub>50</sub> <sup>a,b</sup> | $\pm 1$ SEM <sup>c</sup> | test EC <sub>50</sub> /<br>control EC <sub>50</sub> |
| control     | 158.6 (65)                      | 142.0-177.2              | 1.00  | 2 191 (60)                      | 2 009-2 389              | 1.00  |
| propranolol | 1911.6 (6)                      | 1427.7-2559.3            | 12.05   | 10 229 (4)                      | 6 453-16 214             | 4.67  |
| 5a          | 798.8 (3)                       | 695.2-917.8              | 5.04  | 13 023 (3)                      | 10 649-15 924            | 5.94  |
| 5b          | 676.0 (3)                       | 575.5-794.1              | 4.26  | 4 064 (3)                       | 2 675-6 174              | 1.85  |
| 5c          | 490.1 (4)                       | 261.0-920.1              | 3.09  | 10 689 (3)                      | 8 762-13 038             | 4.88  |
| 5d          | 504.8 (4)                       | 306.5-831.2              | 3.18  | 1 964 (6)                       | 1 715-2 250              | 0.90  |
| 5e          | 198.9 (3)                       | 150.3-263.4              | 1.25  | 1 918 (3)                       | 1 189-3 095              | 0.88  |
| 5f          | 259.3 (4)                       | 210.5-319.3              | 1.63  | 4 123 (3)                       | 3 859-4 405              | 1.88  |
| 5g          | 736.6 (4)                       | 627.1-893.0              | 4.64  | 6 176 (3)                       | 4 522-8 433              | 2.82  |
| 5h          | 615.9 (3)                       | 477.6-794.3              | 3.88  | 4 383 (3)                       | 1 583-12 134             | 2.00  |
| 5i          | 2278.9 (7)                      | 1725.4-3036.1            | 14.37   | 15 057 (6)                      | 12 698-17 853            | 6.87  |
| 5j          | 1815.8 (3)                      | 1528.5-2157.0            | 11.45   | 25 857 (3)                      | 18 383-36 365            | 11.80   |
| 6a          | 522.8 (4)                       | 479.4-570.1              | 3.30  | 4 406 (3)                       | 2 717-7 145              | 2.01  |
| 6b          | 996.8 (3)                       | 662.3-1500.2             | 6.28  | 2 962 (6)                       | 2 137-4 104              | 1.35  |
| 6c          | 206.4 (3)                       | 90.3-471.5               | 1.30  | 1 774 (3)                       | 1 419-2 216              | 0.81  |
| 6d          | 402.6 (4)                       | 161.7-1002.2             | 2.54  | 4 420 (6)                       | 3 800-5 140              | 2.02  |
| 6e          | 206.9 (5)                       | 132.3-323.6              | 1.30  | 2 127 (3)                       | 797-5 681                | 0.97  |

<sup>a</sup> Number in parentheses indicates number of replications, with each replication being done on a fresh preparation.

<sup>b</sup> EC<sub>50</sub> values represent micrograms of isoproterenol in a 40-mL bath required to elicit 50% of the maximal increase in atrial rate or 50% maximal tracheal relaxation in the presence of 1  $\mu$ g of propranolol or 10  $\mu$ g of test compounds. <sup>c</sup> Range of EC<sub>50</sub> values encompassed by  $\pm 1$  times standard error of the mean.

antagonism at the  $\beta$ -adrenergic receptor.<sup>12</sup> This was found to occur with 5h and 5j. In general, it would appear that the presence of the  $\gamma$ -methyl substituent caused a decrease in binding on the order of one-tenth compared with compounds of similar structure.

Results from the isolated atrial and tracheal strip preparations are not as easily interpreted. If one applies the term "active" to those compounds which at 10  $\mu$ g had an effect approximately equal to or exceeding that obtained with 1  $\mu$ g of propranolol, it is clear that none of the  $\alpha$ -methyl compounds, 6a-e, had significant activity. The unsubstituted phenyl, 5a, and the 3,4-dimethylphenyl, 5c, derivatives were markedly weaker than propranolol in the atrial system but were somewhat more effective than 1  $\mu$ g of propranolol in the tracheal system.  $\gamma$ -Methylpropranolol (5i) had substantial  $\beta$ -receptor blocking activity but was no more selective than propranolol itself. The *tert*-butyl derivative, 5j, which had substantial activity in both test systems, showed a distinctly stronger relative effect in the tracheal preparation. One might conclude that the addition of a  $\gamma$ -methyl group to the side chain of (aryloxy)propanolamines decreases activity of these  $\beta$  blockers but at the same time may confer a shift toward  $\beta_2$ -adrenoceptor antagonism and away from  $\beta_1$ -adrenoceptor action.

### Experimental Section

Melting points were obtained on a Thomas-Hoover capillary melting point apparatus and are uncorrected. Microanalyses were performed by Atlantic Microlab, Inc., Atlanta, Ga. Satisfactory IR and <sup>1</sup>H NMR spectra were obtained for all compounds reported. <sup>1</sup>H NMR spectra were recorded on a Varian EM-360 spectrometer using Me<sub>4</sub>Si as an internal standard.

1-(*tert*-Butylamino)-3-(2-allylphenoxy)butan-2-ol (5h). To a stirred solution of 2-allylphenol (32.2 g, 0.24 mol) and freshly distilled BF<sub>3</sub>-Et<sub>2</sub>O (0.7 g) in dry C<sub>6</sub>H<sub>6</sub> (220 mL) maintained at 0-5 °C was added 4' (9.2 g, 0.06 mol) in benzene (30 mL). The addition took 0.75 h and was followed by 1 h at 0 °C and 1-5 h at 25 °C. A few drops of H<sub>2</sub>O were added and the mixture was concentrated in vacuo, taken up in ether, washed with 10% NaOH, and dried (MgSO<sub>4</sub>). Removal of the solvent resulted in recovery of 14.0 g of crude bromohydrin. A solution of the bromohydrin and freshly distilled *tert*-butylamine (10.95 g, 0.15 mol) in EtOH

(50mL) was heated under reflux for 44 h. The reaction mixture was concentrated, taken up in ether, and extracted with 10% HCl. The aqueous phase was washed with ether, cooled in an ice bath, made basic with 20% NaOH, and extracted with CHCl<sub>3</sub>. Both the ether layer and CHCl<sub>3</sub> layer were found by TLC to contain product. The layers were separately dried (MgSO<sub>4</sub>), and the residues were individually chromatographed on silica gel (500 g) using EtOAc-MeOH-Et<sub>3</sub>N (94:3:3) as the eluting solvent. From the ether extract was recovered 3.3 g of 5h, while the CHCl<sub>3</sub> extract gave 1.2 g of 5h. The combined oil was converted to its HCl salt by addition of an ethereal solution of the amine to a saturated solution of anhydrous HCl in diethyl ether. The recrystallization solvent, yield, and melting point for this product and other compounds 5 and 6, which were prepared by a similar procedure, are given in Table I.

**Binding Assay.** The ability of each compound to bind to  $\beta$ -adrenergic receptors of C6-2B astrocytoma cells was tested essentially as previously described by Terasaki and Brooker.<sup>8</sup> A  $2 \times 10^{-2}$  M solution of each compound (5a-j) was prepared in 1 mL of a 1:1 solution of 5 mmol of HCl and 95% EtOH. Serial dilutions were then made in 5 mmol HCl and each compound was tested at a wide range of concentrations (10<sup>-6</sup> to 10<sup>-10</sup> mol). The C6-2B astrocytoma cells in growth medium (Ham's F-10, 1 mL/well) were added to the culture plates at 37 °C, and 50  $\mu$ L of blocker was added, followed by 50  $\mu$ L of [<sup>125</sup>I]HYP (6.5 fmol, approximately 20 000 cpm/well). The plates were incubated for 1.5 h. The reaction was terminated by replacing the medium with buffered salt solution (130 mmol of NaCl, 4 mmol of KCl, 0.6 mmol of MgSO<sub>4</sub>, 0.3 mmol of CaCl<sub>2</sub>, 5 mmol of H<sub>3</sub>PO<sub>4</sub>, adjusted to pH 7.5 with NaOH) containing 0.1 mmol of ( $\pm$ )-propranolol. The cells were washed 3 times at 21 °C with the buffered salt solution (containing propranolol) over a period of 0.3 h. The cells were dissolved in 1 mL of 0.2 N NaOH and quantitatively transferred to a glass tube and counted for 1 min in a Beckman Gamma 300 Counter.

**Guinea Pig Preparations.** On a few occasions, some of the test compounds appeared to be weak agonists immediately upon addition to the bath. However, such effects were not observed consistently with any of the compounds, and addition of isoproterenol to the bath was not begun until the preparation base line had returned to normal.

**Isolated Atrial Preparations.** The isolated spontaneously beating guinea pig atrial preparation was used for evaluation of  $\beta_1$  receptors blocking activity.<sup>9</sup> Following a 0.5-h equilibrium of the atria in Krebs-Ringer bicarbonate solution (40 mL, 37 °C, oxygenated with 95% O<sub>2</sub>-5% CO<sub>2</sub>), isoproterenol was added to the bath in a cumulative fashion. Individual doses of isoproterenol were added after the maximal response (increase in rate) was obtained to the preceding dose. After achieving maximal increase

(12) Mylecharane, E. J.; Raper, C. *Arch Int. Pharmacodyn. Ther.* 1973, 202, 163, and references cited therein.

in atrial rate, the preparation was washed, allowing to reequilibrate, and a second isoproterenol dose-response curve was obtained. Preliminary experiments revealed that the initial isoproterenol dose-response curve was displaced to the left relative to subsequent curves. The second curve was used as a control. After the preparation was washed and was allowed to reequilibrate, 1  $\mu\text{g}$  of ( $\pm$ )-propranolol and 10  $\mu\text{g}$  of 5a-j or 6a-e were individually added to the bath and, after 0.25 h, another isoproterenol dose-response curve was obtained. The extent to which this curve was displaced to the right of the control curve was taken as a measure of  $\beta_1$ -receptor antagonist activity. In all cases, curves obtained after addition of test compounds were parallel to and achieved the same maximum value as control curves.

**Isolated Tracheal Strips.** The isolated guinea pig tracheal strip was used for evaluation of  $\beta_2$ -receptor blocking activity.<sup>9,10</sup> Carbachol ( $10^{-5}$  mol) was added to the tissue bath to produce maximal contraction of tracheal smooth muscle 0.25 h prior to addition of the first aliquot of isoproterenol. The effect of carbachol was shown to persist for more than 1 h. Cumulative dose-response curves for isoproterenol, in the presence and ab-

sence of 1  $\mu\text{g}$  of ( $\pm$ )-propranolol or 10  $\mu\text{g}$  of compounds 5a-j or 6a-e were determined in a manner analogous to that described for isolated atria. In this case, the degree to which the antagonist blocked isoproterenol-induced relaxation of the tracheal smooth muscle was taken as a measure of  $\beta_2$ -receptor antagonist activity. In all cases, curves obtained after addition of test compounds were parallel to and achieved the same maximum value as control curves.

Results were plotted using linear regression (log dose vs. probit of percent of response) and the  $\text{EC}_{50}$  was determined from these plots.

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## Retinoic Acid Analogues with Ring Modifications. Synthesis and Pharmacological Activity

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Analogues of retinoic acid that have their major modifications in the 5,6 double bond and 4-methylene group regions of the  $\beta$ -cyclogeranylidene ring have been synthesized as potential agents for the treatment and prevention of epithelial cancer. These modifications were intended to reduce retinoid toxicity by lowering the effective treatment dose because the major metabolic deactivation pathway would be inhibited. Ethyl (*E*)-3,7-dimethyl-9-(*exo*-2-bicyclo[2.2.1]heptyl)-2,4,6,8-nonatetraenoate (7), ethyl (*E*)-3,7-dimethyl-9-(2,2,6-trimethylbicyclo[4.1.0]hept-1-yl)-2,4,6,8-nonatetraenoate (18), (*E*)-1-(4-carbomethoxyphenyl)-2-methyl-4-(2,2,6-trimethylbicyclo[4.1.0]hept-1-yl)-1,3-butadiene (28), (*E*)-retinoic acid-4,4,18,18,18-*d*<sub>5</sub> (39), and ethyl (*E*)-3,7-dimethyl-9-(3,3-ethano-2,6,6-trimethyl-1-cyclohexen-1-yl)-2,4,6,8-nonatetraenoate (47) displayed moderate to excellent activity in an assay for the inhibition of tumor promoter-induced mouse epidermal ornithine decarboxylase.

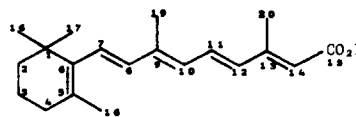
The reversal or prevention of the transformation of epithelial cells to a neoplastic state by both natural and synthetic retinoids is well established by both in vitro and in vivo experiments.<sup>1</sup> Unfortunately, the dose level of retinoids necessary for therapeutic effectiveness can also produce toxic side effects.<sup>2</sup> However, dose levels could be reduced if the metabolic deactivation of these compounds were reduced or eliminated. A major pathway for

metabolic deactivation is allylic oxidation at the 4<sub>R</sub> position<sup>3</sup> of the  $\beta$ -cyclogeranylidene ring of the retinoid skeleton.<sup>4</sup> Interference with this hydrogen-abstraction process may therefore produce less toxic and more effective retinoids. We have undertaken the design and synthesis of retinoid analogues that have their major modifications in the region of the 5,6<sub>R</sub> double bond to reduce the allylic nature of the 4<sub>R</sub> protons (7, 18, and 28) or that have substituents replacing the 4<sub>R</sub> protons (39 and 47).

The 5,6<sub>R</sub> bond of norbornyl analogue 7 is saturated. This compound was proposed as an analogue of the labile, but active, 2-norbornenylretinoid 9<sup>5</sup> and 5,6-dihydro-

- (1) (a) Sporn, M. B.; Dunlop, N. M.; Newton, D. L.; Smith, J. M. *Fed. Proc., Fed. Am. Soc. Exp. Biol.* 1976, 35, 1332. (b) Sporn, M. B.; Dunlop, N. M.; Newton, D. L.; Henderson, W. R. *Nature (London)* 1976, 263, 110. (c) Sporn, M. B.; Squire, R. A.; Brown, C. C.; Smith, J. M.; Wenk, M. L.; Springer, S. *Science* 1977, 195, 487. (d) Moon, R. C.; Grubbs, C. J.; Sporn, M. B.; Goodman, D. G. *Nature (London)* 1977, 267, 620. (e) Todaro, G. J.; De Larco, J. E.; Sporn, M. B. *Ibid.* 1978, 276, 272. (f) Becci, P. J.; Thompson, H. J.; Grubbs, C. J.; Squire, R. A.; Brown, C. C.; Sporn, M. B.; Moon, R. C. *Cancer Res.* 1978, 38, 4463. (g) Sporn, M. B.; Newton, D. L.; Smith, J. M.; Acton, N.; Jacobson, A. E.; Brossi, A. In "Carcinogens: Identification and Mechanism of Action"; Annual Symposium on Fundamental Cancer Research, 31st, M. D. Anderson Hospital and Tumor Research Institute, Houston, 1978, Griffin, A. C.; Shaw, C. R., Eds.; Raven Press: New York, 1979; pp 441-453. (h) Bollag, W. *Cancer Chemother. Pharmacol.* 1979, 3, 207. (i) Sporn, M. B.; Newton, D. L.; Roberts, A. B.; De Larco, J. E.; Todaro, G. J. In "Molecular Actions and Targets for Cancer Chemotherapeutic Agents"; Sartorelli, A. C.; Bertino, J. R.; Lazo, J. S., Eds.; Academic Press: New York, 1980.
- (2) (a) Bard, D. R.; Lasnitzki, I. *Br. J. Cancer* 1977, 35, 115. (b) Hixson, E. J.; Denine, E. P. *Toxicol. Appl. Pharmacol.*, 1978, 44, 29. (c) Hixson, E. J.; Burdeshaw, J. A.; Denine, E. P.; Harrison, S. D., Jr. *Ibid.* 1979, 47, 359.

- (3) For structural comparisons, standard retinoid numbering has been used:



Similar proton and carbon atoms in the analogues have been denoted by the subscript R. Aryl carbon atoms have been denoted in the spectral tabulations as 1' and 6'. The position bearing the polyene substituent is numbered 1' and the remaining positions are numbered in the direction of lowest numerical assignment to the other substituents. The norbornyl ring has also been numbered 1' to 7' following standard numbering.

- (4) (a) Roberts, A. B.; Nichols, N. D.; Newton, D. L.; Sporn, M. B. *J. Biol. Chem.* 1979, 254, 6296. (b) Roberts, A. B.; Lamb, L. C.; Sporn, M. B. *Arch. Biochem. Biophys.* 1980, 199, 374.
- (5) Dawson, M. I.; Hobbs, P. D.; Kuhlmann, K.; Fung, V. A.; Helmes, C. T.; Chao, W.-R. *J. Med. Chem.* 1980, 23, 1013.