nerve stimulation was recorded on a polygraph. A dose of 0.1 mg/kg of each compound was injected into the cannulated femoral vein. Spasmolytic activity (percent inhibition of the contraction) was calculated from the following formula:

100 – (av contraction ht during 30-min period after drug injn/av contraction ht before drug injn) × 100

Measurement Method of Spasmolytic Activity on Gastric Contraction Induced by Vagus Nerve Stimulation. The assay was measured by the method described above. Mongrel dogs of either sex, weighing 8–15 kg, were anesthetized with sodium pentobarbital (30 mg/kg, iv). The left cervical vagus nerve was exposed through upper abdominal incisions and cut, and its peripheral end was stimulated every 3 min via platinum electrodes. The stimulation parameters were as follows: frequency, 20 Hz; duration, 1 ms; voltage, 8 V. A water-filled balloon connected to a low-pressure transducer was inserted into the stomach through the small incised corpus, and elevation in intraluminal pressure induced by the vagus nerve stimulation was recorded on a polygraph. A dose of 0.1 mg/kg of each compound was injected into the cannulated femoral vein. Spasmolytic activity (percent inhibition of the contraction) was calculated from the formula shown above.

Acknowledgment. The authors are indebted to Dr. G. Ohta, Director of this Institute, for his encouragement and helpful advice. We also thank to Y. Kasai for his excellent biological assistance and the members of the Analytical Section of this Institute for analytical service.

Synthesis and Comparison of Some Cardiovascular Properties of the Stereoisomers of Labetalol[1]

Elijah H. Gold,*[†] Wei Chang,[†] Marvin Cohen,[†] Thomas Baum,[‡] Stewart Ehrreich,[‡2] Gordon Johnson,[‡2] Nicholas Prioli,[‡] and Edmund J. Sybertz[‡]

Department of Medicinal Chemical Research and Department of Pharmacology, Research Division, Schering-Plough Corporation, Bloomfield, New Jersey 07003. *Received February 25, 1982*

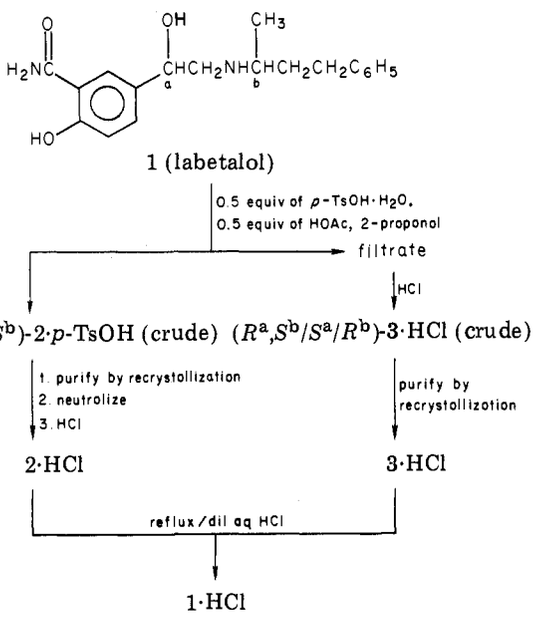
A useful method for the separation of labetalol into its two racemic diastereomers, as well as a stereoselective synthesis of its four stereoisomers, is described. The absolute stereochemistry of each isomer was determined by analysis of the CD spectra and confirmed by X-ray analysis. The α- and β_1-adrenergic blocking properties, as well as the relative antihypertensive activities, have been measured in rats. The *R,R* isomer, **2a** (SCH 19927), possesses virtually all of the β_1-blocking activity elicited by labetalol and displays little α-blocking activity. In contrast, the *S,R* isomer, **3a**, has most of the α-blocking activity. Of the four isomers, only **2a** has antihypertensive potency comparable to that of labetalol. These findings, coupled with published data showing that labetalol possesses β-adrenergic mediated peripheral vasodilating activity deriving essentially from its *R,R* isomer, lead to the following conclusion: The antihypertensive activity of labetalol can be ascribed to at least three identified complementary mechanisms, β-adrenergic blockade, β-adrenergic mediated vasodilatation, and α-adrenergic blockade, whereas the antihypertensive activity of **2a** derives from the first two mechanisms only.

The synthesis[3] and the pharmacological[4] and clinical[5] properties, as well as the metabolism,[6] of the new antihypertensive agent labetalol (**1**) are well documented. The novelty of this agent has been ascribed to its property of being both an α- and β-adrenergic receptor blocker, the ratio of α/β blockade, in a variety of animal and isolated tissue studies, being in the range of 1:4–16.[7] Recently several other arylethanolamines have also been shown to have combined α- and β-adrenergic blocking properties,[8] including an analogue of labetalol, the biology of which has been investigated in some detail.[8a,b] Labetalol consists of an approximately equicomponent mixture of its four optical isomers, and several recent reports have described some of their adrenoceptor properties. Thus, in isolated tissue, the α- and β-blocking properties have been shown to each derive from a different racemic diastereomer.[9] Other studies in anesthetized dogs[3b,10,26] ascribe these activities respectively to the *S,R* (**3a**) and *R,R* (**2a**) isomers. Finally, several of us have reported some comparative adrenoceptor properties of labetalol and its *R,R* isomer (**2a**, SCH 19927) in dogs and rats.[11]

This paper describes the synthesis and characterization of all four isomers and compares their relative blocking activity at adrenoceptors and their blood-pressure lowering properties in rats.[3b]

Separation of Labetalol into Its Racemic Diastereomers (2 and 3). After many attempts with various acid salts, including our inability to readily repeat the published procedure,[3] we easily effected the fractional crystallization

Scheme I



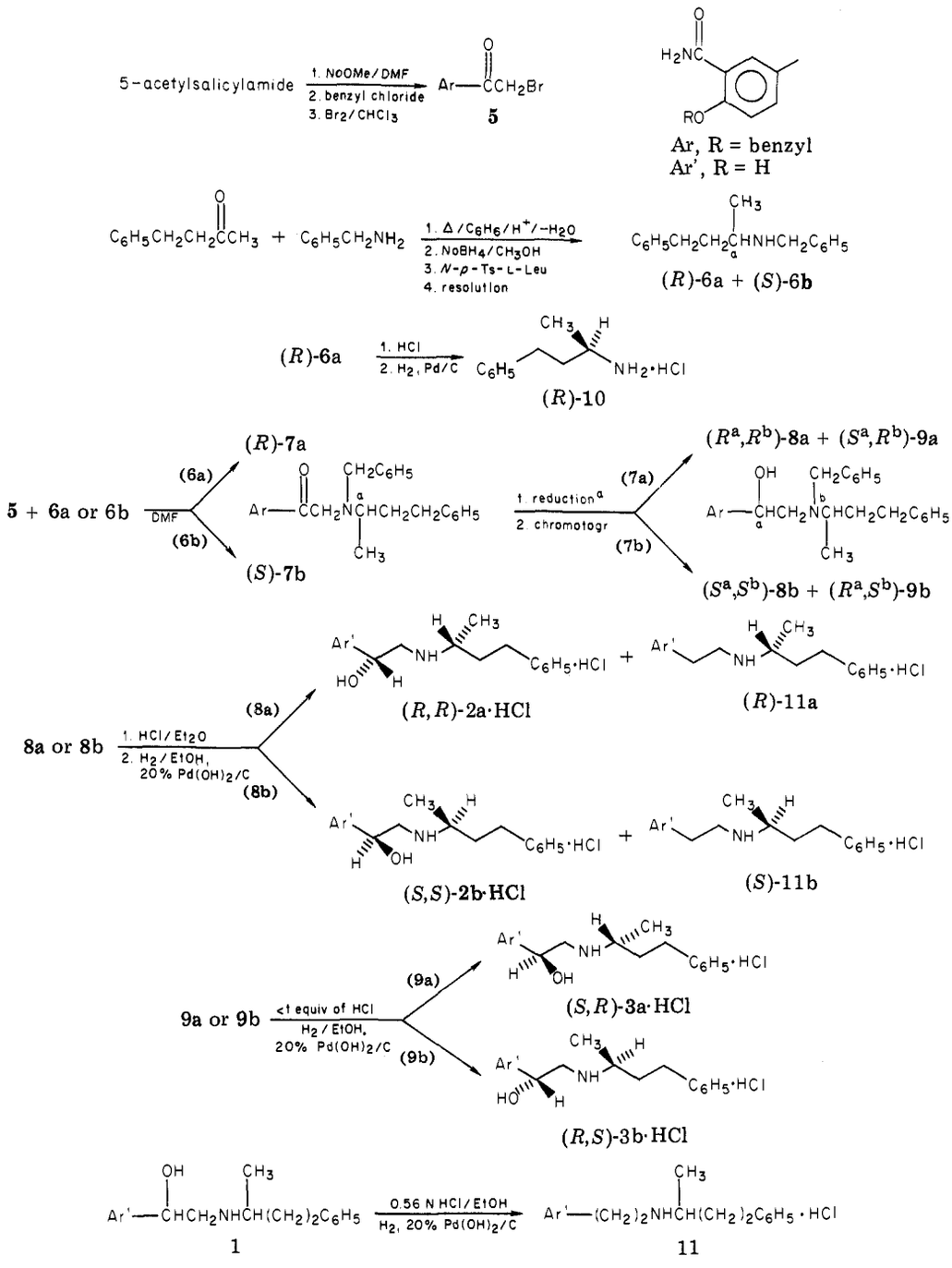
of **1** by taking advantage of the extreme insolubility of the *p*-TsOH salt of diastereomer **2** (*R,R/S,S*) and the solubility

[†] Department of Medicinal Chemical Research.
[‡] Department of Pharmacology.

(1) This paper has been presented, see E. H. Gold, T. Baum, W. Chang, M. Cohen, S. Ehrreich, G. Johnson, N. Prioli, and E. J. Sybertz, "Abstracts of Papers", 183rd National Meeting of the American Chemical Society, Las Vegas, NV, Mar 1982, American Chemical Society, Washington, DC, 1982, Abstr MEDI 36.

0022-2623/82/1825-1363$01.25/0

Scheme II



[a] Procedure A $\xrightarrow{NaBH_4/EtOH}$ 8/9 ≈ 85.15. Procedure B $\xrightarrow{LiBH_4,\ C_6H_6/THF\ (96:4)}$ 8/9 ≈ 65.35

of the acetate salt of diastereomer **3** (*R,S/S,R*) in 2-propanol. Thus, after filtering off **2**·*p*-TsOH, crude **3** was obtained by precipitation of its HCl salt. Both isomers were then readily purified by simple recrystallization from

(2) Present Address: Division of Cardiorenal Drug Products, U.S. Food and Drug Administration HFD-110, Rockville, MD 20852.
(3) (a) L. H. C. Lunts and D. T. Collin, U.S. Patent 4012444 (1977); (b) J. E. Clifton, I. Collins, P. Hallett, D. Hartley, L. H. C. Lunts, and P. D. Wicks, *J. Med. Chem.*, **25**, 670 (1982). This paper also describes the synthesis of the isomers.
(4) For reviews of the animal pharmacology, see (a) D. A. Richards, J. Tuckman, and B. N. C. Prichard, *Br. J. Pharmacol.*, **3**, 849 (1976); (b) R. T. Brittain and G. P. Levy, *Br. J. Clin. Pharmacol.*, **3**, 681 (1976).
(5) For a review of the clinical utility, see E. A. Rosei, R. Fraser, J. J. Morton, J. J. Brown, A. F. Lever, J. I. S. Robertson, and P. M. Trust, *Am. Heart. J.*, **93**, 124 (1977).
(6) (a) L. E. Martin, R. Hopkins, and R. Bland, *Br. J. Clin. Pharmacol.*, **3**, 695 (1976); (b) R. Hopkins, L. E. Martin, and R. Bland, *Biochem. Soc. Trans.*, **4**, 726 (1976).
(7) R. T. Brittain, "Abstracts of Papers", 2nd Joint Conference of the Chemical Institute of Canada and American Chemical Society, Montreal, Canada, May 1977, American Chemical Society, Washington, DC, 1977, p 32.
(8) (a) J. M. Grisar, G. P. Claxton, T. M. Bare, R. C. Dage, H. C. Cheng, and J. K. Woodward, *J. Med. Chem.*, **24**, 327 (1981); (b) H. C. Cheng, O. K. Reavis, Jr., J. M. Grisar, G. P. Claxton, D. L. Weiner, and J. K. Woodward, *Life Sci.*, **27**, 2529 (1980); (c) Japanese Patents, 80 53261 (1980) and 80 73610 (1980).
(9) Y. Nakagawa, N. Shimamoto, M. Nakazawa, and S. Imai, *Jpn. J. Pharmacol.*, **30**, 743 (1980).

ethanol (Scheme I). In addition, either **2** or **3** could be reconverted to **1** by simple aqueous acid-catalyzed equilibration.[12]

Although no TLC system was found that could separate **2** and **3**,[13] quantitative information could be obtained from the ^{13}C NMR spectrum of **1**.[14] GLC analysis of **2** and **3**, however, afforded the most precise and convenient method of assaying for purity (see Experimental Section).

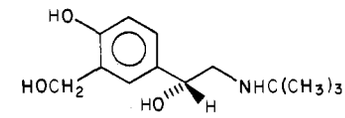
Synthesis of the Optical Isomers of Labetalol. Since several attempts to resolve **2** and **3** into their respective enantiomers by fractional crystallization of several chiral salts proved to be unsuccessful, the required compounds were prepared by direct synthesis (Scheme II).

The sodium salt of 5-acetylsalicylamide (**4**) was O-benzylated with benzyl chloride in DMF, followed by α-bromination of the ketone with Br_2 in $CHCl_3$, to afford **5**. α-Methyl-*N*-(phenylmethyl)benzenepropanamine (**6**) was readily obtained by $NaBH_4$ reduction of the imine formed during the dehydrative addition of benzylamine to benzylacetone. Amine **6** was resolved into its enantiomers by a fractional crystallization procedure (see Experimental Section). Their absolute stereochemistries were then determined by hydrogenolytic debenzylation of **6a**, which yielded amine **10** of known absolute configuration.[17a] The coupling of **5** and **6a** or **6b** at room temperature in DMF, followed by reduction of the crude intermediate ketones (**7a** and **7b**), afforded a mixture of optically active O,N-dibenzylated diastereomers **8a**/**9a** or **8b**/**9b**. Each pair of these benzylated precursors was separated by column chromatography, since, in contrast to racemic **1**, the two optically active pairs of final products (**2a**/**3a** and **2b**/**3b**) could not be separated via fractional crystallization of their *p*-TsOH salts. The fact that enantiomers often have different crystalline forms than their racemate can account for the difference in the results.

As might be expected, the ratio of the diastereomeric alcohols obtained upon reduction of the intermediate ketones **7a** or **7b** was dependent upon the nature of the reducing agent and the solvent polarity. Thus, reduction with $NaBH_4$ in ethanol (high polarity) gave mostly **8a** and **8b** (**8a**/**9a** and **8b**/**9b** = ca. 85:15), whereas $LiBH_4$ in a solvent of low polarity (C_6H_6/THF = 96:4) brought the proportions more closely in balance (**8a**/**9a** and **8b**/**9b** = ca. 65:35). Several experiments utilizing other reducing agents failed to further increase the proportions of **9a** and **9b**.[18] An analysis of this stereoselectivity has recently been reported.[22]

Finally, reductive debenzylation of the HCl salts of **8a,b** and **9a,b** afforded the corresponding HCl salts of the desired labetalol optical isomers **2a,b** and **3a,b**. Although the crude HCl salts of **8a** and **8b** (precipitated from ether) were once debenzylated to afford only **2a**·HCl and **2b**·HCl, respectively, repetition of the experiment resulted in the formation of a small amount (3–5%) of their respective dehydroxy analogues, **11a** and **11b**. This latter result was clearly due to the presence of excess HCl (despite considerable washing of the precipitate with ether to apparent neutrality), since "clean" debenzylation was always obtained when the HCl salts were prepared in situ by careful addition of slightly less than 1 equiv of hydrochloric acid to an ethanolic solution of the base (method used to prepare **3a** and **3b**). Hydrogenolysis of an ethanolic solution of a mixture of **8a** and **9a** as free bases resulted in the formation of only **2a** and **3a**. A similar hydrogenolysis under acid conditions, however, resulted also in the formation of a small quantity of **11a**. Taking advantage of this observation,[19] we cleanly synthesized "dehydroxylabetalol" (**11**) from labetalol (**1**) by hydrogenolysis in ethanolic HCl.

The absolute stereochemistry of the four isomers, subsequently substantiated by a single-crystal X-ray analysis of **2a**·HCl,[20] was determined as follows. Conversion of **6a** into **10**, of known absolute configuration, established chirality as *R* at the *N*-methine carbon. The absolute configuration at the benzylic carbinol was deduced from the CD spectra. The CD spectra were presumed to reflect only the chirality at the benzylic carbinol based on the fact that **10** and its enantiomer have been shown to be devoid of Cotton effects in the 230–300 nm region.[17b] Thus, comparison of the CD spectra of the HCl salts of **2a** and **3b** (λ_{max} ~300 nm) with that of the acetate salt of (–)-(*R*)-salbutamol (λ_{max} ~280 nm),[21] showed that they all had



(*R*)-salbutamol

clear negative Cotton effects, whereas their enantiomers all had clear positive Cotton effects. Assuming that the carboxamide substituent in **2** and **3** would cause only the expected bathochromic shift but would not qualitatively alter the sign of the Cotton effect (an assumption subsequently verified by the X-ray data), we deduced the absolute configurations of the benzylic carbinols of **2a** and **3b** as *R*, and those of **2b** and **3a** as *S*.

Pharmacological Methodology.[23] **β^1-Blockade** was measured by determining antagonism of the tachycardic responses to isoproterenol in anesthetized, normotensive

(10) R. T. Brittain, G. M. Drew, and G. P. Levy, *Br. J. Pharmacology*, **73**, 282 p (1981).
(11) (a) E. J. Sybertz, C. S. Sabin, K. K. Pula, G. V. Vliet, J. Glennon, E. H. Gold, and T. Baum, *J. Pharmacol. Exp. Ther.*, **218**, 435 (1981); (b) T. Baum, R. W. Watkins, E. J. Sybertz, S. Vemulapalli, K. K. Pula, E. Eynon, S. Nelson, G. V. Vliet, J. Glennon, and R. M. Moran, *ibid.*, **218**, 444 (1981); (c) T. Baum and E. J. Sybertz, *Fed. Proc., Fed. Am. Soc. Exp. Biol.*, in press.
(12) This enables one to convert **1** exclusively to either **2** or **3**. Thus, for example, should one desire only **3** it can easily be obtained as its hydrochloride of 96% purity in about 21% yield (ca. 42% based on **3** present) from **1**. At a purity level of 98–99%, the yield is about 16% (ca. 32% based on **3** present). Since crude **2**, obtained at the initial separation stage, can be recycled back to **1**, the process is repeatable until most of **1** has been converted into **3**.
(13) No one, to the best of our knowledge, has succeeded in finding a TLC system that separates the diastereomers.
(14) We are grateful to Dr. R. Brambilla for these measurements.
(15) Although a GLC assay is reported in the literature,[16] the method used in this work was developed by Messrs. B. Rosenkrantz and T. Mollitor. We are grateful to C. Taddei for most of the actual GLC data reported herein.
(16) G. Munro, J. H. Hunt, L. R. Rowe, and M. B. Evans, *J. Pharm. Pharmacol.*, **28**(Suppl), 27 (1976).
(17) (a) Y. Yamamoto, J. Oda, and Y. Inouye, *Bull. Chem. Soc. Jpn.*, **48**, 3744 (1975); (b) O. Cervinka, E. Kroupova, and O. Belovsky, *Collect. Czech. Chem. Commun.*, **33**, 3351 (1968).
(18) The experiments were: potassium tri-*sec*-butylborohydride in THF, lithium tri-*sec*-butylborohydride in THF, and lithium tri-*sec*-butylborohydride in benzene.
(19) Such dehydroxylations are, of course, precedented under strong acid conditions. See R. L. Augustine, "Catalytic Hydrogenation", Marcel Dekker, New York, 1965, p 135.
(20) Private communication: Professor A. T. McPhail, Department of Chemistry, Duke University, Durham, NC.
(21) D. Hartley and D. Middlemiss, *J. Med. Chem.*, 14, 895 (1971).
(22) D. Hartley, *Chem. Ind.*, 551 (1981).
(23) See ref 11 for methodological details.

Table I. α- and β_1-Adrenoceptor Blocking Activity of Labetalol and Its Stereoisomers in Rats

		β_1 blockade[a]			α blockade[a]		
compd[b]	absolute stereochem	n	dose range,[b] mg/kg	ID_{50},[c] mg/kg	n	dose range,[b] mg/kg	ID_{50},[d] mg/kg
1 (labetalol)		5	0.1-1	0.25 ± 0.01[e]	5	1-10	7.1 ± 0.5[e]
2a	*R,R*	8	0.03-0.3	0.07 ± 0.01[f]	6	3-30	35.0 ± 1.4[f,j,k]
3b	*R,S*	6	0.3-3	4.1 ± 0.6[e-g]	4	1-10	inactive[i]
3a	*S,R*	5	0.3-3	5.0 ± 0.9[e-h]	5	0.3-3	1.3 ± 0.4[e,f]
2b	*S,S*	3	0.3-3	inactive[i]	4	1-10	4.8 ± 2.0[e]

[a] Statistical analysis performed with analysis of variance; Duncan's multiple range statistic. [b] Compounds administered iv as HCl salts in three cumulative doses. [c] ID_{50} is the dose of antagonist (mean ± SEM) required to produce 50% inhibition of the tachycardic response to (±)-isoproterenol (0.1 mg/kg iv). [d] ID_{50} is the dose of antagonist (mean ± SEM) required to produce 50% inhibition of the pressor response to phenylephrine (10 mg/kg iv). [e] ID_{50} significantly different from that of 2a at $p < 0.05$. [f] ID_{50} significantly different from that of 1 at $p < 0.05$. [g] An *overestimate* of the β-blocking potency, since several animals tested did not show β blockade at the highest dose and were excluded from the ID_{50} determination (ID_{50} was extrapolated from beyond the range of doses tested). [h] The ID_{50} is fully attributable to contamination by ca. 0.6% 2a (see Experimental Section). [i] No inhibition in any animal at highest dose tested. Not subjected to analysis of variance. [j] An *overestimate* of the α-blocking potency, since two of the six animals tested showed no inhibition of the phenylephrine responses at the highest doses tested (ID_{50} was extrapolated from beyond the range of doses tested). [k] At least partially attributable to contamination by ca. 0.9% 3a.

Table II. Effects of Labetalol and Its Stereoisomers on Blood Pressure (BP)[a] and Heart Rate (HR)[a] in Conscious SH Rats

				change in BP (mmHg) or HR (beats/min) at the following times after dosing[b]			
drug[b]	n		BP prior to dosing	1 h	2 h	3 h	4 h
placebo	4	BP	160 ± 3	+12 ± 2	+11 ± 2	+13 ± 6	+10 ± 8
		HR	365 ± 8	+12 ± 11	+12 ± 18	+4 ± 15	+32 ± 14
1 (labetalol)	4	BP	160 ± 4	−29 ± 3[c]	−22 ± 2[c]	−20 ± 2[c]	−20 ± 2[c]
		HR	362 ± 6	−13 ± 9	−18 ± 7	−27 ± 7	−23 ± 7
2a	4	BP	160 ± 3	−26 ± 4[c]	−26 ± 1[c]	−29 ± 2[c]	−27 ± 1[c]
		HR	378 ± 5	−47 ± 12[c]	−41 ± 8	−55 ± 12[c]	−45 ± 7[c]
3b	4	BP	164 ± 4	−5 ± 4[c-e]	−2 ± 3[d,e]	+6 ± 4[d,e]	+8 ± 3[d,e]
		HR	362 ± 21	+7 ± 4	+5 ± 11	−8 ± 25	+6 ± 25
3a	4	BP	158 ± 7	−12 ± 4[c-e]	−5 ± 2[c-e]	−4 ± 6[c-e]	−3 ± 7[d,e]
		HR	359 ± 12	−9 ± 19	−16 ± 18	−34 ± 16	−39 ± 18[a]
2b	4	BP	159 ± 3	+4 ± 2[d,e]	+10 ± 4[d,e]	+11 ± 4[d,e]	+8 ± 4[d,e]
		HR	356 ± 16	+8 ± 5	+1 ± 14	−4 ± 13	−7 ± 6

[a] All values are means ± SEM. Statistical analysis performed with analysis of variance; Duncan's multiple range statistic. [b] Administered orally as HCl salts at a dose of 10 mg/kg. This dose is the midpoint on the dose-response curves for both 1 and 2a. [c] $p < 0.05$ vs. placebo at that time point. [d] $p < 0.05$ vs. 1 at that time point. [e] $p < 0.05$ vs. 2a at that time point.

Table III. Summary of Comparative Cardiovascular Effects of Labetalol and Its Stereoisomers

		relative potencies[a]				BP decrease (mm)/ duration (h)[d]
compd	absolute stereochem	β_1 block	α block[b]	β_1/α block	vasodilatation[c]	
1 (labetalol)		1	1	26-39	1	30-32/>4
2a (SCH 19927)	*R,R*	3.5	<0.2[e]	>500	7	30-32/>4
3b	*R,S*	<0.06	0			0
3a	*S,R*	<0.05[f]	5.1			12/~1
2b	*S,S*	0	1.5			0

[a] Relative potencies of the isomers are normalized to labetalol = 1. [b] β_1-Blockade and α-blockade are on different absolute scales (see Table I). [c] See ref 11b for data. [d] At a dose of 10 mg/kg, po. [e] See footnotes *j* and *k*, Table I. [f] See footnotes *g* and *h*, Table I.

rats. Thus, the *intravenously* administered doses of test compound necessary to reduce isoproterenol responses to 50% of control (ID_{50}) was determined.

α-Blockade was similarly measured by determining antagonism of the pressor response (ID_{50}, *intravenous* administration) to phenylephrine[24] in anesthetized, normotensive rats.

Antihypertensive activity was assessed by direct blood-pressure measurement in conscious, spontaneously hypertensive rats (SHR). Test compounds were administered *orally*.

Results and Discussion

The results of the experiments outlined in Tables I and II and summarized in Table III clearly demonstrate that the constituents of labetalol differ markedly in α-adrenergic and β-adrenergic blocking activity, as well as in their antihypertensive effects. Furthermore, the degree of adrenergic blockade displayed in rats is qualitatively similar to that seen in dogs.[3b,10,26] Several comments regarding

(24) Phenylephrine was used because of the reported "self-limiting" β blockade of norepinephrine vasopressor response. See (a) J. B. Farmer, I. Kennedy, G. P. Levy, and R. J. Marshall, *Br. J. Pharmacol.*, **45**, 660 (1972); (b) J. Kennedy and G. P. Levy, *ibid.*, **53**, 585 (1975).

(25) A subsequent batch of **2a** was found to contain ca. 0.9% **3a**. This latter material was used for the biological experiments described herein.

(26) We have also obtained results in dogs (unpublished) similar to those reported.[3b,10]

the effects of chirality on adrenergic activity are in order. At β receptors, activity of an isomer having the R configuration at the hydroxy-bearing carbon is consistent with the absolute configuration of all other known β-active arylethanolamines. In contrast, α-blockade is manifested exclusively by the two labetalol isomers having the S configuration at the hydroxy-bearing carbon. Of these two, the S,R isomer, **3a**, possesses most of the α-blocking activity elicited by labetalol. This is unexpected, since the α-receptor activity of known arylethanolamines (e.g., epinephrine and phenylephrine) have the opposite (R) configurational requirements at the carbinol site. A recent study[3b] of the adrenergic activities of a large series of labetalol analogues indicates that achievement of significant α blockade requires a methyl group vicinal to the basic nitrogen (as in labetalol). Both these results and those reported herein establish that the absolute configuration of the methyl-bearing carbon plays a key role in determining the activity at *both* α and β receptors. Thus, only the two isomers having the R configuration at the C-methyl center (**2a** and **3a**) are significantly active at these receptors.

In addition to adrenergic *blockade*, labetalol has been shown to possess β_2-adrenoceptor agonist activity on the rat and mouse uterine muscle.[27] We have recently established[11b] that labetalol possesses a β-mediated peripheral vasodilating activity (blocked only by a β blocker, i.e., propranolol) and that this activity is associated with the R,R isomer **2a** (SCH 19927). Evidence that the acute blood-pressure lowering effects of labetalol and **2a** are, at least in part, due to the observed vasodilatation comes from our observation that the antihypertensive effects of both of these agents in SH rats were inhibited by propranolol pretreatment.[11c] In contrast, we have also recently shown that **2a** is virtually devoid of cardiac β-sympathomimetic activity,[11a] as has been previously demonstrated for labetalol.[24a]

Thus, based on all available evidence, the antihypertensive activity of labetalol can be ascribed to at least three identified complementary mechanisms, β-adrenergic blockade, α-adrenergic blockade, and β-adrenergic mediated vasodilatation deriving mainly from the independent effects of its R,R and S,R component diastereomers. Of the four stereoisomers, only **2a** (currently undergoing clinical trials[28]) produces antihypertensive responses in SH rats comparable to labetalol. This is correlatable with its potent β_1-blocking activity (ca. four times labetalol), coupled with its β-mediated peripheral vasodilating effects (ca. seven times labetalol). In contrast, the α-blocking isomer, **3a**, shows only weak, short-acting blood-pressure lowering effects in SH rats at a dose that is highly effective for labetalol and **2a**.

Experimental Section

Methods and Materials. Melting points were determined in a capillary tube on a Thomas-Hoover apparatus (the melting point of the various isomers, however, is *not* a criterion for isomeric purity). Spectra were recorded as follows: IR spectra on a Perkin-Elmer Model 180 prism spectrophotometer, NMR spectra on either a Varian Model A-60A or a Varian Model CFT-20 spectrometer, CD spectra on a Cary Model 61 spectrophotometer, and mass spectra with a Varian MAT CH5 spectrometer. Optical rotations were measured on either a Bendix Model 1100 or a Rudolph Autopol III automatic polarimeter. Yields of **2** and **3** are expressed relative to 1·HCl. Purities of all batches were determined by GLC[15] and are expressed as percent of the isomer under discussion relative to its diastereomer. GLC determination utilized the methylboronic acid derivatives of **2** and **3** as follows: 1 mg of 1 (free base or salt) was dissolved in 0.7 mL of methylboronic acid reagent (prepared from 12 mg of GLC grade acid per milliliter of dry pyridine). After 20 min at room temperature, 2-μL aliquots were injected into a 4 ft × 2 m i.d. glass column (3% OV-17 on Gas Chrom Q 100/120 mesh) in a Hewlett Packard Model 5710A instrument with a flame-ionization detector. The oven was maintained at 265 °C (N_2 = 25 mL/min), while the detector (H_2 = 30 mL/min) and the injection port (air = 300 mL/min) were kept at 300 °C. The retention times were 6.9 (**2**) and 8.1 min (**3**). An unidentified peak (ca. 3–5%) was present in all assays. Since no extraneous impurities were detected by any other physical method (i.e., ^{1}H NMR, IR, MS, TLC), this peak is probably due to a "side product" arising from reaction of the substrate and methylboronic acid/pyridine reagent. The validity of this assay method was further corroborated by comparing GLC data with quantitative ^{13}C NMR data.[14] Thus, two pairs of resonances, 35.7 (**2**)/35.1 (**3**) ($C_6H_5CH_2CH_2$) and 15.9 (**2**)/16.4 (**3**) [$CH(CH_3)$] ppm, were readily measured, with signals resolved to internal MeOH and referred to Me_4Si: δ_c (Me_4Si) = δ_c (MeOH) + 49.8. On those samples of 1 compared, the data were comparable within 1%. Preparative chromatographic separations were carried out on the Chromatospac-Prep 100.[29] With the exception of melting points, all the above-noted measurements, as well as elemental analyses, were performed by the Physical and Analytical Department, Schering-Plough Corp. All preparative chromatography was run on silica gel G (type 60, E. Merck no. 7731) and TLC on 10 cm × 0.25 mm silica gel GF plates (Analtech Uniplate).

Separation of Labetalol (1) into Its Racemic Diastereomers (2 and 3). Neutralization of 1·HCl. Labetalol hydrochloride (598.5 g, 1.638 mol) was dissolved in 7 L of 0.52 N aqueous NaOH with cooling (ice bath) and stirring (ca. 1 h). A stream of CO_2 was passed in with vigorous stirring to pH 7, during which time precipitation began. The precipitate was filtered and washed with 2 L of H_2O. The filtrate was treated with more CO_2 to ensure complete precipitation, and the precipitate was dried at room temperature for 2 days and under a current of air for 24 h longer, affording 539.3 g (ca. 100%) of 1.

Separation of 2 and 3. Boiling 2-propanol (3.40 L) was added to a mixture of 532.70 g (1.625 mol) of 1, 154 g (0.813 mol) of p-TsOH·H_2O, and 48.70 g (0.813 mol) of HOAc, and heating on a stream bath was continued for 20 min to effect complete dissolution. The solution was cooled slowly to 30 °C, then seeded (preferable but not essential) with 0.5 g of **2**·p-TsOH, and allowed to crystallize at room temperature with occasional stirring. It then stood at room temperature overnight (ca. 19 h). To obtain a fair estimate of the purity and amount of precipitate in the flask at a given time, the mixture was vigorously stirred for 10 min to ensure a uniform suspension, a 10-mL sample was filtered, and the solid was weighed (756 mg; mp 176, 178–182 °C). After standing at room temperature for 3 h longer with occasional stirring, the mixture was filtered and washed with 2 × 200 mL of 2-propanol, yielding 365 g of crude **2**·p-TsOH, mp 178–182 °C. The filtrate contained the crude **3**·HOAc.

Purification of 3. Hydrochloride Salt. The volume of filtrate obtained in the above separation procedure was reduced to ca. 2 L and then slowly added to 470 mL of 1.9 N ethereal HCl with cooling and stirring. The precipitated salt was filtered, washed with ether, and air-dried at room temperature for 3 h to yield ca. 321 g, which was then recrystallized from 2.2 L of 90% EtOH to afford ca. 220 g, mp 204–208 °C dec. The solid was digested with 2 L of boiling EtOH for 5 min with stirring and then cooled and filtered to afford 174 g (29.2%, purity 90%) mp 212, 213–214 °C dec. We again recrystallized the salt slowly from 1.65 L of 90% EtOH by allowing the solution to stand at room temperature overnight. The solvent was decanted, stirred for 10 min with 500 mL of fresh 90% EtOH, and filtered and the filtrate

(27) (a) B. Carey and E. T. Whalley, *J. Pharm. Pharmacol.*, **31**, 791 (1979); (b) B. Carey and E. T. Whalley, *Br. J. Pharmacol.*, **67**, 13 (1979); (c) J. K. Woodward and H. C. Cheng, *J. Pharm. Pharmacol.*, **34**, 193 (1982).

(28) Preliminary clinical results (unpublished) have established both antihypertensive and β-adrenergic blocking activity for this drug in man.

(29) Jobin Yvon, 91160 Longjumeau, France, or J. Y. Optical Systems, Metuchen, NJ 08840.

was dried at room temperature to yield 123.80 g (20.8%, purity 96%), mp 213, 214–216 °C dec. The latter recrystallization process was repeated to afford 92.30 g [15.4% (based on 1·HCl), purity 98.5%], mp 212, 213–214 °C dec. The hydrochloride was recrystallized three more times[30] by this process (ca. 10 mL of 90% EtOH/g) to afford 57.0 g (9.5%, purity >99.5%), mp 215–217 °C dec (lit.[3a] 220 °C). Anal. ($C_{19}H_{25}ClN_2O_3$) C, H, N, Cl.

Free Base. We obtained this by dissolving 1.0 g (0.0027 mol) of **3**·HCl in 16 mL of 0.4 N aqueous NaOH and passing a stream of CO_2 into the solution until precipitation was complete. The product was filtered, washed with 3 mL of H_2O, and dried in vacuo at 80 °C for 2 h to yield 0.860 g (95%) of **3**, mp 164–165 °C. Recrystallization from 40 mL of EtOH raised the melting point to 166–166.5 °C. Anal. ($C_{19}H_{24}N_2O_3$) C, H, N.

Purification of 2. *p*-Toluenesulfonate Salt. The crude **2**·*p*-TsOH obtained in the above separation procedure was recrystallized from 4 L of EtOH when the solution was left to stand at room temperature for 68 h. After filtering and washing with EtOH, we obtained 241 g (29.3%), mp 183, 184–186 °C. This material was recrystallized from 1.32 L of 90% EtOH when the solution was allowed to stand at room temperature overnight. The solvent was decanted, stirred for 10 min with 400 mL of fresh 90% EtOH, and filtered, and the filtrate was dried at room temperature, yielding 173.6 g (21.1%, purity 95.5%), mp 186–187 °C. The salt (143 g) was recrystallized twice[30] more by this process (ca. 6 mL of 90% EtOH/g) to afford 112 g (13.7%, purity >99.5%), mp 184–185 °C. Anal. ($C_{26}H_{32}N_2O_6S$) C, H, N.

Free Base.[31] To 15.0 g (0.0330 mol) of **2**·*p*-TsOH dissolved in 30 mL of DMF, 0.717 g (0.0300 mol) of LiOH was added. The mixture was stirred for 30 min, poured into 400 mL of ice–H_2O, and stirred with scratching and seeding in an ice bath for 1 h. The solid was collected, washed with H_2O, and dried in vacuo at 35 °C overnight to afford 9.55 g (96.9%), mp 157–159 °C. Recrystallization from MeOH raised the melting point to 163.5–164.5 °C. Anal. ($C_{19}H_{24}N_2O_3$) C, H, N.

Hydrochloride Salt. We obtained this by adding dropwise 42 mL of 1 N ethereal HCl to a stirred suspension of 7.0 g (0.021 mol) of **2** in 50 mL of Et_2O and by stirring the mixture for another 1.5 h. The white solid was filtered, and the filtrate was washed well with Et_2O and recrystallized from 2-propanol to yield 3.46 g of the HCl salt as fine white needles, mp 171–173 °C dec (lit.[3a] mp 174 °C). Anal. ($C_{19}H_{25}ClN_2O_3$) C, H, N, Cl.

Conversion of the HCl Salt of 3 to the HCl Salt of 1. In a N_2 atmosphere, 1.50 g of **3**·HCl (GLC 96%) was heated under reflux in 75 mL of 0.0275 N HCl for 88 h. The solvent was removed in vacuo, and the residue was triturated with EtOH and filtered to yield 1.05 g (70.0%), mp 175–178 °C dec; isomer ratio **3**/**2** = 65.2:34.8. The mother liquor contained one major unidentified impurity, which, judging from its high polarity, is probably the salicyclic acid resulting from hydrolysis of the salicylamide.

Conversion of the HCl Salt of 2 to the HCl Salt of 1. In a N_2 atmosphere, 2.0 g of **2**·HCl (GLC 90%) was heated under reflux in 100 mL of 0.0275 N HCl for 65 h. The solvent was removed in vacuo, and the residue was triturated with EtOH and filtered to yield 0.70 g, mp 165–170 °C dec. The filtrate was evaporated to dryness, triturated with CH_3CN, and filtered to afford another 0.70 g; mp 158–161 °C dec; isomer ratio (total 1.4 g, 70.0%) **3**/**2** = 47.7:52.4. As in the conversion of **3** to **1**, the observed unidentified impurity was probably the corresponding salicylic acid.

5-(Bromoacetyl)-2-(phenylmethoxy)benzamide (5). To a solution of 115.4 (0.644 mol) of 5-acetylsalicylamide (**4**) in 1.2 L of DMF was added 33.1 g (0.613 mol) of $NaOCH_3$ in small portions, with cooling and stirring. A thick paste was formed, which was then heated on a steam bath and to which 75 mL (0.652 mol) of benzyl chloride was added dropwise. Heating and stirring was continued for 7 h. After stirring and cooling, the mixture was poured into 6 L of ice–H_2O containing 15 g of Na_2CO_3. The product was filtered, and the filtrate was washed well with H_2O, digested with 700 mL of EtOH, chilled, and refiltered to yield 127.2 g (84.5%) of analytically pure 5-acetyl-2-(phenylmethoxy)benzamide, mp 157–160 °C. Anal. ($C_{16}H_{15}NO_3$) C, H, N.

To a refluxing, stirred solution of 127.0 g (0.47 mol) of 5-acetyl-2-(phenylmethoxy)benzamide in 1.2 L of $CHCl_3$ was added a few milliliters of $Br_2/CHCl_3$ solution [76.5 g (0.49 mol) of Br_2 in 220 mL of $CHCl_3$] until the color was discharged (ca. 5–10 min). The solution was cooled to room temperature, and, with stirring, the remaining $Br_2/CHCl_3$ solution was added dropwise until precipitation began. The reaction mixture was then refluxed, and dropwise addition was continued. After refluxing for 10 min following completion of the addition, the solution was chilled in an ice bath and filtered, and the filtrate was then washed with cold $CHCl_3$. The crude solid was stirred for 20 min in 800 mL of ice-cold H_2O and filtered, and the filtrate was washed well with H_2O and dried. It was recrystallized from methyl ethyl ketone to afford two crops of crystals, mp 150–152 and 146–149 °C, neither of which were analytically pure but which were usable for the preparation of **7a** or **7b** (total yield 111 g, 61.5%).

α-Methyl-*N*-(phenylmethyl)benzenepropanamine (6). In an apparatus fitted with a Dean-Stark trap, a solution of 1.0 kg (6.75 mol) of benzylacetone, 725 g (6.75 mol) of benzylamine, and 5.0 g of *p*-TsOH·H_2O in 7 L of benzene was refluxed for 14 h. The solvent was removed in vacuo, and the residue was dissolved in 6.5 L of MeOH. With cooling and stirring, 125 g of $NaBH_4$ was carefully added, and the mixture was stirred for 16 h at room temperature. The MeOH was removed in vacuo, 2 L of H_2O and 4 L of benzene were added, and the product was extracted into the benzene. After the extract was dried ($MgSO_4$) and filtered, the product was distilled [bp 145–150 °C (0.5 mm)] to yield 1174 g (73%).

(+)-(*R*)-α-Methyl-*N*-(phenylmethyl)benzenepropanamine (6a). Racemic **6** (1028 g, 4.288 mol) and *N*-(*p*-toluenesulfonyl)-L-leucine (1230 g, 4.328 mol) were dissolved in 7.2 L of boiling EtOH and allowed to cool to room temperature without agitation. The precipitate was washed with a small amount of ice-cold EtOH and recrystallized twice from EtOH (4.8 L followed by 4.0 L; washed with ice-cold EtOH each time): ca. 670 g of product was obtained, mp 154–157 °C, that is highly enriched with the salt of the *S* enantiomer. We combined the mother liquors from the reaction mixture and the first recrystallization, removed the solvent, and recovered the free base by basifying with 500 mL of 20% aqueous NaOH and extracting with benzene. After drying ($MgSO_4$), filtering, asnd removing the benzene, we dissolved the residue (487 g, 2.04 mol) and *N*-acetyl-L-leucine (346 g, 2.06 mol) in 2.0 L of boiling EtOH, and the solution was allowed to cool to room temperature. The precipitate was filtered and recrystallized once from 1.8 L of EtOH, followed by recrystallization from 4.0 L of CH_3CN to yield ca. 370 g of product, mp 151–152 °C. We recovered pure **6a** (224 g, 21.8%) by basifying with 400 mL of aqueous 2.5 N NaOH, drying ($MgSO_4$), filtering, and removing the solvent in vacuo: $[\alpha]^{26}_D$ +4.5° (*c* 5.0, EtOH). Anal. ($C_{17}H_{21}N$) C, H, N.

(−)-(*S*)-α-Methyl-*N*-(phenylmethyl)benzenepropanamine (6b). Racemic **6** (2347 g, 9.83 mol)[32] and 2810 g (9.83 mol) of *N*-(*p*-toluenesulfonyl)-L-leucene were dissolved in 16.5 L of boiling EtOH and allowed to cool to room temperature without agitation. The precipitate was filtered and recrystallized 3 times from EtOH (10.9, 4.4, and 4.4 L) to yield 1114 g of the salt, mp 160.5–162 °C. The salt was stirred in a mixture of 9.2 L of Et_2O and 6.0 L of 1.3 N NaOH. The ether layer was separated, dried ($MgSO_4$), and filtered, and the solvent was removed in vacuo to afford 570 g (21.6%): $[\alpha]^{26}_D$ −4.4° (*c* 5, EtOH). Anal. ($C_{17}H_{21}N$) C, H, N.

(+)-(*R*)-α-Methylbenzenepropanamine Hydrochloride (10). Proof of the Absolute Configurations of 6a and 6b. A solution of 2.40 g (0.10 mol) of **6a** in 100 mL of EtOH, containing 10 mL of 1 N HCl and 0.20 g of 20% $Pd(OH)_2$ on carbon,[33] was reduced with H_2 (3 atm) in a Parr apparatus with shaking at room temperature for 6 h. After filtering off the catalyst and removing the solvent in vacuo, we triturated the residue with Et_2O to afford 1.50 g (77%) of the (+)-(*R*)-amine hydrochloride (**10**): mp 109–111

(30) The number of further crystallizations is determined by the level of isomeric purity desired.
(31) We thank Dr. R. Friary for carrying out this procedure.
(32) For clarity, resolution of a separate batch of racemic **6** (other than the one reported for the preparation of **6a**) is described.
(33) "Pearlman catalyst": W. H. Pearlman, *Tetrahedron Lett.*, 1663 (1967).

°C; $[\alpha]^{26}_D$ +7.6° (c 5.77, H_2O) [lit.,[17a] for (−)-(S)-amine hydrochloride, mp 111–113 °C, $[\alpha]^{26}_D$ −7.2° (c 5.77, H_2O)].

5-[[[(*R*)-1-Methyl-3-phenylpropyl](phenylmethyl)amino]acetyl]-2-(phenylmethoxy)benzamide (7a). A mixture of 224 g (0.94 mol) of **6a**, 372 g (ca. 1.07 mol) of **5**, and 372 g (2.7 mol) of K_2CO_3 in 1.6 L of DMF was stirred at room temperature for 4 h (reaction mildly exothermic). Water (8.7 L) was added, and the mixture was extracted twice with 2.2-L portions of Et_2O. The combined Et_2O extract was washed with H_2O, dried (Na_2SO_4), and filtered, and the Et_2O was removed in vacuo (30–40 °C) to yield 520 g (>100%) of crude product as a syrup.

5-[[[(*S*)-1-Methyl-3-phenylpropyl](phenylmethyl)amino]acetyl]-2-(phenylmethoxy)benzamide (7b). This experiment was conducted in the same manner as described for **7a**, with identical results; thus, crude **7b** was obtained from **6b**.

Mixture of 5-[(*R*)- and (*S*)-1-Hydroxy-2-[[(*R*)-1-methyl-3-phenylpropyl](phenylmethyl)amino]ethyl]-2-(phenylmethoxy)benzamide (8a and 9a). Procedure A. Reduction of 7a with $NaBH_4$ in EtOH (8a/9a ≈ 85:15). Crude **7a** (520 g, ca. 0.94 mol) was dissolved in 3.1 L of EtOH, and, with stirring and cooling, 35.5 g (0.94 mol) of $NaBH_4$ was added portionwise. The mixture was stirred at room temperature for 16 h, the solvent was removed in vacuo, 3.2 L of H_2O was added, and the mixture was heated for 30 min on a steam bath. The mixture was cooled, extracted with benzene, dried ($MgSO_4$), and filtered, and the solvent was removed in vacuo to afford 475 g (99.5%) of crude product as a syrup [ratio **8a/9a** ≈ 85:15, approximated by integration of the ^1H NMR ($CDCl_3$) methyl signals (**8**, δ 1.00; **9**, δ 1.11)].

Procedure B. Reduction of 7a with $LiBH_4$ in C_6H_6/THF, 96:4 (8a/9a ≈ 65:35). In a N_2 atmosphere, 63 g (0.124 mol) of **7a**, dissolved in 960 mL of benzene/40 mL of THF, was added to a stirred suspension of 4.20 g (0.193 mol) of $LiBH_4$ in 480 mL of benzene/20 mL of THF and cooled in an ice bath. The rate of addition was such that the reaction temperature did not rise above 10 °C. After the addition was complete, the reaction mixture was stirred for 2 h more (ice bath), and the excess $LiBH_4$ was decomposed by cautious addition of H_2O. The organic layer was separated, dried ($MgSO_4$), and filtered, and the solvent was removed in vacuo to yield 53.0 g (84%) of the crude product as a syrup (ratio of **8a/9a** ≈ 65:35).

Mixture of 5-[(*S*)- and (*R*)-1-Hydroxy-2-[[(*S*)-1-methyl-3-phenylpropyl](phenylmethyl)amino]ethyl]-2-(phenylmethoxy)benzamide (8b and 9b). Procedure A. Reduction of 7b with $NaBH_4$ in Ethanol (8b/9b ≈ 85:15). This experiment was conducted in the same manner as described for reduction of **7a**, with similar results; thus, a mixture of crude **8b** and **9b** (ca. 85:15) was obtained from crude **7b**.

Procedure B. Reduction of 7b with $LiBH_4$ in C_6H_6/THF, 96:4 (8b/9b ≈ 65:35). This experiment was conducted in the same manner as described for reduction of **7a**, with similar results; thus, a mixture of crude **8b** and **9b** (ca. 65:35) was obtained from crude **7b**.

Chromatographic Separation of 8a and 9a (or 8b and 9b). The following are typical preparative chromatographic procedures and results. A $CHCl_3$ (150 mL) solution of ca. 47 g of crude mixture [**8a/9a** (ca. 85:15)] was chromatographed on 1.5 kg of silica gel with 3:1 $CHCl_3$/EtOAc. Pure (TLC) **8a** (gum) was eluted first (ca. 22 g, 47%), followed by crude **9a** (ca. 3 g, 6%); the overlap fractions weighed ca. 15 g (32%). As isolated, **8a** was used directly for the preparation of **2a**; however, **9a** (ca. 26 g of pooled samples from nine runs) was rechromatographed on 1.5 kg of silica gel to yield 17.5 g of **9a** [(gum) one spot on TLC, but containing 0.6% of **8a** (GLC)], as well as 0.5 g of **8a** and 2.9 g of an **8a** and **9a** mixture.

As above, 150 mL of a $CHCl_3$ solution of ca. 49 g of crude mixture [**8b/9b** (ca. 65:35)] was chromatographed on 1.5 kg of silica gel with 3:1 $CHCl_3$/EtOAc to yield 15 g of crude **8b**, 9.5 g of crude **9b**, and 22 g of overlap fraction. Both **8b** and **9b** were rechromatographed on 1.5 kg of silica gel to yield 11 g of pure **8b** (gum) and 7 g of **9b** [(gum) one spot on TLC, but containing 0.8% **8b** (GLC)].

A TLC system that separates **8** and **9** is $CHCl_3$/EtOAc (3:1): **8** R_f 0.55; **9** R_f 0.45 (visualization by UV, H_2SO_4, charring, or I_2); mass spectra of **8a,b** and **9a,b**, *m/e* 508 (M^+). Difficulty in removing all entrained solvent precluded elemental analyses of **8a,b** and **9a,b**.

(+)-2-Hydroxy-5-[(*S*)-1-hydroxy-2-[(*S*)-(1-methyl-3-phenylpropyl)amino]ethyl]benzamide (2b). Hydrochloride Salt. Compound **8b** (10.0 g, 0.019 mol) in 50 mL of Et_2O was precipitated as an amorphous HCl salt with the dropwise addition of 2 N ethereal HCl, until no more precipitation occurred. The precipitate was filtered, washed well with Et_2O (to remove excess acid), and dissolved in 250 mL of EtOH. $Pd(OH)_2$ on carbon[33] (1.0 g, 20%) was added, and the salt was reduced with H_2 (3 atm) in a Parr apparatus with shaking at room temperature for 2 h. The solid product obtained after filtering off the catalyst and removing the solvent in vacuo was triturated with 50 mL of 2-propanol to yield 5.9 g of **2b**·HCl containing a small amount of **11b**·HCl (tlc R_f's, respectively, 0.34 and 0.53; $CHCl_3$/EtOH/NH_4OH, 50:10:1.25). This was dissolved in 35 mL of 1 N NaOH, the pH was adjusted to ca. 8, and the free bases were precipitated by bubbling in CO_2, collected, washed with H_2O, and dried in vacuo at 40 °C (5 g). This was chromatographed on 1.5 kg of silica gel with $CHCl_3$/EtOH/NH_4OH (50:10:1.25) to afford pure (TLC) **2b** (gum), which was dissolved in 50 mL of boiling CH_3CN. This solution was cooled and carefully acidified with 2 N ethereal HCl to ca. pH 2, whereupon the analytically pure **2b**·HCl (GLC >99.9%) that precipitated was filtered and washed with ether (4.6 g, 66%): mp 193–194 °C dec (lower melting crystalline form, mp 133–134 °C dec); $[\alpha]^{26}_D$ +30.4° (c 1.0, EtOH); CD (c 2.8×10^{-4}) $[\theta]_{260}$ 0, $[\theta]_{303}$ +36 892 (max), $[\theta]_{340}$ 0. Anal. ($C_{19}H_{25}ClN_2O_3$) C, H, N, Cl.

***p*-Toluenesulfonate Salt.** A solution of 2.50 g (0.00490 mol) of **8b** in 100 mL of EtOH, containing 0.75 g of 5% Pd on carbon, was reduced with H_2 (3 atm) in a Parr apparatus with shaking at room temperature for 17 h. After filtering off the catalyst and removing the solvent in vacuo, we dissolved the viscous residue in a boiling solution of 0.98 g (0.00517 mol) of *p*-TsOH·H_2O in 12 mL of EtOAc. The solution was cooled to ca. 0 °C, and the precipitated salt was filtered and dried to yield 1.35 g (53.8%), mp 120–123 °C. Digestion with 10 mL of boiling EtOAc for a few minutes gave 1.26 g (51.5%) of analytically pure product: mp 125–126 °C; $[\alpha]^{26}_D$ +19.3° (c 1.0, EtOH). Anal. ($C_{26}H_{32}N_2O_6S$) C, H, N.

(−)-(*S*)-2-Hydroxy-5-[2-[(1-methyl-3-phenylpropyl)amino]ethyl]benzamide Hydrochloride (11b). From the chromatography of **2b** (see above), 0.2 g (3%) of pure (TLC) **11b** (gum) was obtained, which was dissolved in 150 mL of Et_2O and acidified with 2 N ethereal HCl. The precipitate was filtered, and the filtrate was washed with Et_2O and digested for 10 min with 5 mL of refluxing CH_3CN. The crystalline solid was collected and recrystallized from EtOH to afford analytically pure **11b**·HCl ($^1/_3$ mol of H_2O): mp 169–173 °C dec; mass spectrum, *m/e* 313 (M^+); $[\alpha]^{26}_D$ −10.1° (c 0.3, EtOH). Anal. ($C_{19}H_{25}ClN_2O_2 \cdot {}^1/_3H_2O$) C, H, N, Cl.

(−)-2-Hydroxy-5-[(*R*)-1-hydroxy-2-[(*R*)-(1-methyl-3-phenylpropyl)amino]ethyl]benzamide (2a). Hydrochloride Salt. The reductive debenzylation of **8a** was conducted in the same manner as described for the preparation of **2b**. The liberated free bases (10.0 g, **2a** and **11a**) were chromatographed on 1.5 kg of silica gel, and the pure (TLC) **2a** (gum) was dissolved in 50 mL of boiling CH_3CN. The solution was cooled and carefully acidified with 2 N ethereal HCl to ca. pH 2, whereupon a gum precipitated, which was solidified by refluxing the mixture for 10 min. The solid was filtered, washed with Et_2O, and recrystallized from EtOH to afford analytically pure (GLC >99.9%[25]) **2a**·HCl: mp 192–193.5 °C dec (lower melting crystalline form, mp 133–134 °C dec), $[\alpha]^{26}_D$ −30.6° (c 1.0, EtOH), CD (c = 3.0×10^{-4}) $[\theta]_{260}$ 0, $[\theta]_{303}$ −43 956 (max), $[\theta]_{340}$ 0. Anal. ($C_{19}H_{25}ClN_2O_3$) C, H, N, Cl.

***p*-Toluenesulfonate Salt.** This experiment was conducted in the same manner as described for the preparation of **2b**·*p*-TsOH from **8a**. Thus, analytically pure **2a**·*p*-TsOH was obtained: mp 123–125 °C; $[\alpha]^{26}_D$ −19.5° (c 1.0, EtOH). Anal. ($C_{26}H_{32}N_2O_6S$) C, H, N.

(+)-(*R*)-2-Hydroxy-5-[2-[(1-methyl-3-phenylpropyl)amino]ethyl]benzamide Hydrochloride (11a). From the chromatography of **2a** (see above), 0.5 g (5%) of pure (TLC) **11a** (gum) was obtained, which was dissolved in 250 mL of Et_2O and acidified with 2 N ethereal HCl. The precipitate was filtered, and the filtrate was washed with Et_2O and digested for 10 min with 25 mL of refluxing CH_3CN. The analytically pure **11a**·HCl

was filtered, and the filtrate was washed with Et_2O: mp 172–175 °C dec; mass spectrum, m/e 313 (M^+); $[\alpha]^{26}_D$ +10.4° (c 0.3, EtOH). Anal. ($C_{19}H_{25}ClN_2O_2$) C, H, N, Cl.

(+)-2-Hydroxy-5-[(*S*)-1-hydroxy-2-[(*R*)-(1-methyl-3-phenylpropyl)amino]ethyl]benzamide Hydrochloride (3a). A solution of 3.0 g (0.0059 mol) of **9a** in 150 mL of EtOH, containing 5.85 mL of 1 N aqueous HCl (0.00585 mol) and 0.25 g of 20% $Pd(OH)_2$ on carbon,[33] was reduced with H_2 (3 atm) in a Parr apparatus with shaking at room temperature for 2 h. After filtering off the catalyst and removing the solvent in vacuo, we digested the residue with 50 mL of boiling CH_3CN to yield 1.50 g (70%) of **3a**·HCl (GLC 0.6% **2a**): mp 171–172 °C dec; $[\alpha]^{26}_D$ +27.8° (c 1.0, DMF); CD (c 3.0 × 10^{-4}) $[\theta]_{260}$ 0, $[\theta]_{304}$ +28960 (max), $[\theta]_{340}$ 0. Anal. ($C_{19}H_{25}ClN_2O_3$) C, H, N, Cl.

(−)-2-Hydroxy-5-[(*R*)-1-hydroxy-2-[(*S*)-(1-methyl-3-phenylpropyl)amino]ethyl]benzamide Hydrochloride (3b). This experiment was conducted in the same manner as described for the preparation of **3a** from **9a**, with similar results. Thus, from **9b**, **3b**·HCl (GLC 0.8% **2b**) was obtained: mp 167–168.5 °C dec; $[\alpha]^{26}_D$ −28.4° (c 1.0, DMF); CD (c 2.7 × 10^{-4}) $[\theta]_{260}$ 0, $[\theta]_{304}$ −30820 (max), $[\theta]_{340}$ 0. Anal. ($C_{19}H_{25}ClN_2O_3$) C, H, N, Cl.

Racemic 2 from 2a and 2b. The analytically pure p-TsOH salts of **2a** and **2b** (4.0 mg each) were dissolved in 0.5 mL of hot EtOH. This was cooled in an ice bath, the precipitate was filtered, and the filtrate was dried: mp 185–186 °C; mmp with authentic **2**·p-TsOH (GLC >99.5%) 185.5–186 °C.

Racemic 3 from 3a and 3b. The HCl salts of **3a** and **3b** (5.0 mg each) were dissolved in 1 mL of hot EtOH, which was then evaporated to dryness. The solid residue was triturated with 0.5 mL of cold EtOH and filtered, and the filtrate was dried: mp 212–213 °C dec; mmp with authentic **3**·HCl (GLC >99.5%) 213–214 °C dec.

Hydrogenolysis of a Mixture of 8a and 9a as Free Bases. A 1:1 mixture of **8a** and **9a** (1.0 g, 0.0020 mol) was dissolved in 25 mL of EtOH; 0.1 g of 20% $Pd(OH)_2$ on carbon[33] was added, and it was reduced with H_2 (3 atm) in a Parr apparatus with shaking at room temperature for 3 h. After filtering off the catalyst and removing the solvent in vacuo, we obtained 0.58 g (88%) of a gummy solid, which corresponded (^{1}H NMR, TLC) to a 1:1 mixture of **2a** and **3a**, with no evidence for the presence of **11a**.

Hydrogenolysis of a Mixture of 8a and 9a in the Presence of Excess HCl. A 1:1 mixture of **8a** and **9a** (1.0 g, 0.0020 mol) was dissolved in 25 mL of EtOH, containing 0.65 mL (0.00227 mol) of 3.50 N ethereal HCl; 0.1 g of 20% $Pd(OH)_2$ on carbon[33] was added, and it was reduced with H_2 (3 atm) in a Parr apparatus with shaking at room temperature for 3 h. A TLC analysis of the solution showed the presence of an intense spot corresponding to **2a**/**3a** and a weak spot corresponding to **11a**. The reduction was continued for 5 days; after filtering off the catalyst and removing the solvent in vacuo, we obtained 0.72 g of a gummy solid that corresponded (^{1}H NMR, TLC) to a 3:1 mixture of **11a**:**2a**/**3a**.

(*R*,*S*)-2-Hydroxy-5-[2-[(1-methyl-3-phenylpropyl)amino]ethyl]benzamide Hydrochloride (11). By Hydrogenolysis of 1. A solution of 20 g (0.055 mol) of 1·HCl in 420 mL of EtOH was acidified with 80 mL of 3.5 N ethereal HCl ("free" HCl concentrated in resulting solution = 0.56 N), 2.0 g of 20% $Pd(OH)_2$ on carbon[33] was added, and the mixture was reduced with H_2 (3 atm) in a Parr apparatus with shaking at room temperature for 4 weeks.[34] The gum (homogeneous by TLC R_f = R_f of authentic 11), obtained after the catalyst was filtered off and the solvent was removed in vacuo, was triturated with boiling CH_3CN and then recrystallized from 2-propanol to afford 9.0 g (47%) of 11, mp 160–163 °C.

From 11a and 11b. Compounds **11a** and **11b** (5.0 mg each) were dissolved in 1 mL of MeOH, which was then evaporated to dryness. The solid residue was triturated with Et_2O and filtered, and the filtrate was dried, mp 160–163 °C. Compound 11 was identical in all respects (TLC, ^{1}H NMR, IR, MS, mmp) with an authentic sample, kindly supplied to us by Dr. Geoffrey P. Levy, Allen & Hanburys Research, Ltd., Great Britain.

(34) An experiment under identical conditions, but at 60 °C overnight, resulted in an unidentified mixture of products. No attempt was made to shorten the reaction time by increasing the HCl concentration.

Structure–Activity Relationships of Some Technetium-99m Labeled [(Thioethyl)amino] Carboxylates

E. Chiotellis,* C. I. Stassinopoulou, A. Varvarigou, and H. Vavouraki

Isotopes Department and Biology Department, Radiopharmaceuticals Laboratory, Demokritos Nuclear Research Center, Athens Greece. *Received February 8, 1982*

The synthesis, NMR studies, radiochemical labeling with technetium-99m, and tissue-distribution characteristics of some [(thioethyl)amino] carboxylates are described. The ^{99m}Tc agents prepared were eliminated either by the urinary or the hepatobiliary system of mice. The excretion route of the ^{99m}Tc complexes was influenced by the structure and total charge of the ligands.

In the past years, several technetium-99m complexes have been introduced for diagnosis of renal diseases or for kidney function tests. The technetium-99m–iron ascorbate complex introduced by Harper and Lathrop[1] was first suggested for renal studies due to its significant localization in the kidneys. Since then, a great number of ^{99m}Tc complexes have been developed for this purpose. The ligands of these chelates were of various chemical structure, such as ethylenediamine or triamine acetates,[2-4] peptide derivatives,[5] gluconic acids,[6] or thio carboxylates.[7-10]

(1) P. V. Harper, K. A. Lathrop, G. M. Hinn, A. Gottschalk, F. Jimenez, and D. Paloyan, "Pharmaceuticals", G. A. Andrews, R. M. Kniseley, and H. N. Wagner, Eds., Symposium Series 6, Springfield, VA, National Bureau of Standards, 347-351 Department of Commerce, Washington, DC, 1966, pp 347–351, Abstr CONF-651111.

(2) R. F. Fleay, *Austas. Radiol.*, 12, 265 (1968).
(3) W. Hauser, H. L. Atkins, K. G. Nelson, and P. Richards, *Radiology (Easton, Pa.)*, 94, 679 (1970).
(4) W. Eckelman and P. Richard, *J. Nucl. Med.*, 11, 761 (1970).
(5) H. S. Winchell, M. S. Lin, B. Shipley, T. Sargent, and A. Katchalsky-Katzir, *J. Nucl. Med.*, 12, 678 (1971).
(6) R. W. Arnold, G. Subramanian, J. G. McAfee, R. J. Blair, and F. D. Thomas, *J. Nucl. Med.*, 16, 357 (1975).
(7) T. H. Lin, A. Khentigan, and H. S. Winchell, *J. Nucl. Med.*, 15, 34 (1974).
(8) P. L. Hagan, P. R. Ayres, S. E. Halpern, and M. C. Depew, *J. Nucl. Med.*, 16, 531–532 (1975).
(9) O. Inoue, J. Ikeda, and K. Kurata, *Radioisotopes*, 25, 25 (1976).

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