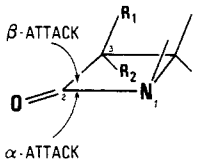
alosporins). Washkuhn and Robinson found that the reactivity of 3-monosubstituted β-lactams 1 correlates with the polar σ* values of the 3 substituents.[5] However, Holley and Holley found that disubstitution in isolated β-lactams (3,3-dimethyl) resulted in a 25-fold decrease in β-lactam reactivity relative to the monosubstituted (3-methyl) analog.[6] Thus, when both faces of a β-lactam are unhindered, monosubstitution affects reactivity in a manner predictable from linear free energy substituent values. In contrast, when one face of a β-lactam is already hindered, the introduction of a substituent on the unhindered face results in a β-lactam that is hindered on both faces to nucleophilic attack, and steric effects override polar effects. Penicillins are sterically similar to the case of the isolated β-lactam which has one face hindered. At the β face a penicillin β-lactam carbonyl is at an interatomic distance of only 2.79 Å from the C-3 proton (in the crystalline phase) and therefore is severely hindered to nucleophilic attack.[7] Thus, the addition of any α substituent results in a β-lactam hindered at both faces and lowers overall reactivity.§



1

In contrast, 7-α-methoxy substitution in cephalosporins has no pronounced effect upon the β-lactam reactivity (Table I). We believe that the difference in response to 7-α substitution in cephalosporins compared with the response to 6-α substitution in penicillins is the result of the availability of the β face of the cephalosporin to nucleophilic attack.= Cephalosporins are sterically similar to isolated β-lactams where *both* faces of the ring are unhindered. The addition of the 7-α-methoxy substituent therefore does not totally hinder the β-lactam and the polar substituent effect on the overall reactivity can be observed.

Thus, we have shown that the differences in antibacterial activity as a result of 6-α and 7-α substitution in penicillins and cephalosporins, respectively, are paralleled by differences in chemical reactivity of their corresponding β-lactams. We attribute these differences in chemical reactivity to steric factors resulting from α substitution.

Experimental Section

β-Lactams. The penicillins and cephalosporins used in this study were synthesized by colleagues at Lilly Research Laboratories. Synthetic procedures for all these compounds are referenced in Table I.

Kinetic Methods. The hydrolysis rates of the penicillins and cephalosporins were measured by constant pH titration and uv methods, respectively, as described in the accompanying paper; see ref 7. The pseudo-first-order rates of β-lactam hydrolysis at pH 10.0, 35°, are listed in Table I.

Acknowledgment. The authors acknowledge with thanks M. Gorman, P. P. K. Ho, G. A. Koppel, R. Nagarajan, and W. A. Spitzer for supplies of penicillins and cephalosporins and for helpful discussions.

§In apparent contradiction to this argument the hydrolysis of 6-α-acetyl-6-phenoxyacetamidopenicillanic acid methyl ester has been found to be 2.4 times as reactive as phenoxyacetamidopenicillanic acid methyl ester in aqueous glyme.[8]

= Reports of intramolecular nucleophilic attack of the α-amino moiety on the β-lactam of cephradine,[9] cephalexin esters, and cephaloglycin lactone (ref 7) demonstrate the availability of the β face of cephalosporins to nucleophilic attack.

References

(1) D. J. Tipper and J. L. Strominger, *J. Biol. Chem.*, **243**, 3169 (1968).
(2) R. Nagarajan, L. D. Boeck, M. Gorman, R. L. Hamill, C. E. Higgens, M. M. Hoehn, W. M. Stark, and J. G. Whitney, *J. Amer. Chem. Soc.*, **93**, 2308 (1971).
(3) P. P. K. Ho, R. D. Towner, J. M. Indelicato, W. A. Spitzer, and G. A. Koppel, *J. Antibiot.*, **25**, 627 (1972).
(4) P. P. K. Ho, R. D. Towner, J. M. Indelicato, W. L. Wilham, W. A. Spitzer, and G. A. Koppel, *J. Antibiot.*, **26**, 313 (1973).
(5) R. J. Washkuhn and J. R. Robinson, *J. Pharm. Sci.*, **60**, 1168 (1971).
(6) R. W. Holley and A. D. Holley, *J. Amer. Chem. Soc.*, **73**, 3172 (1951).
(7) J. M. Indelicato, T. T. Norvilas, R. R. Pfeiffer, W. J. Wheeler, and W. L. Wilham, *J. Med. Chem.*, 17, 523 (1974), footnote §.
(8) E. H. W. Bohme, H. E. Applegate, J. B. Ewing, P. T. Funke, M. S. Puar, and J. E. Dolfini, *J. Org. Chem.*, **38**, 230 (1973).
(9) A. I. Cohen, P. T. Funke, and M. S. Puar, *J. Pharm. Sci.*, **62**, 1559 (1973).

Derivatives of 3,4-Dihydrocarbostyril as β-Adrenergic Blocking Agents

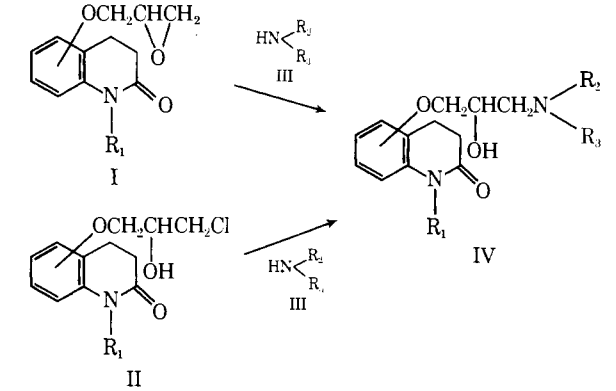
Kazuyuki Nakagawa,* Nanami Murakami, Shiro Yoshizaki, Michiaki Tominaga, Hideo Mori, Youichi Yabuuchi, and Shigeyuki Shintani

The First Research Laboratory, Tokushima Factory, Otsuka Pharmaceutical Company, Ltd., 463-10 Kagasuno, Kawauchi-cho, Tokushima-shi, Japan. *Received December 18, 1973*

In the last decade, numerous compounds have been synthesized in an effort to find a drug possessing more specific adrenergic β-receptor blocking potency or more significant activity without cardiac contraction. Such a compound is expected to have an aromatic or heterocyclic nucleus attached to a 1-hydroxy-2-substituted aminoethyl or 2-hydroxy-3-substituted aminopropoxy substituent.[1] The present paper deals with the effects of using 3,4-dihydrocarbostyril as nucleus and 2-hydroxy-3-substituted aminopropoxy as substituent.

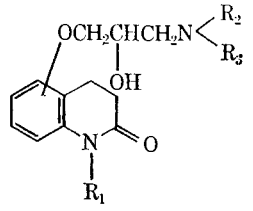
Chemistry. The compounds were prepared by reaction of derivatives of 5-, 6-, 7-, and 8-(2,3-epoxy)propoxy-3,4-dihydrocarbostyril (I) or 5-, 6-, 7-, and 8-(3-chloro-2-hydroxy)propoxy-3,4-dihydrocarbostyril (II) with the appropriate amine III in the usual manner (Scheme I). Formulas and physical properties of compounds IV are shown in Table I.

Scheme I



Pharmacology. Adrenergic β-receptor blocking activity was evaluated by inhibition of the depressor and the positive chronotropic responses to isoproterenol. It was gener-

Table I. (3-Substituted amino-2-hydroxy)propoxy-3,4-dihydrocarbostyrils



No.	Position[a]	R_1	R_2	R_3	Mp, °C	Crystn solvent	Formula[b]	Method[c]	Yield, %		Antagonistic act. against isoproterenol (% inhibn of changes in BP[d] and HR[e]) 5 min	10 min	20 min	30 min	60 min	90 min	120 min
1	5	H	H	Et	168–169	*i*-PrOH	$C_{14}H_{20}N_2O_3 \cdot C_4H_4O_4$[f]	A	55.3	BP	92.6	91.9	81.5	74.1	77.8	37.0	44.4
										HR	82.9	73.4	61.0	53.7	46.3	46.3	36.3
2	5	H	H	*i*-Pr	224–225	MeOH–Me_2CO	$C_{15}H_{22}N_2O_3 \cdot HCl$	B	59.0	BP	100	100	100	100	95.6	93.5	90.4
										HR	100	100	100	98.5	95.0	93.5	85.3
3	5	H	H	*n*-Bu	203–205	EtOH	$C_{16}H_{24}N_2O_3 \cdot HCl$	A	46.5	BP	21.1	21.1	21.1	21.1	10.5	10.5	5.3
										HR	35.7	33.3	23.8	16.7	9.5	9.5	4.8
4	5	H	H	*i*-Bu	194–196	EtOH	$C_{16}H_{24}N_2O_3 \cdot C_4H_4O_4$[f]	A	49.8	BP	15.8	15.8	10.5	5.3	0	0	0
										HR	18.4	15.3	15.8	10.5	4.3	0	0
5	5	H	H	*sec*-Bu	228–231	EtOH	$C_{16}H_{24}N_2O_3 \cdot HCl$	B	51.0	BP	81.0	77.1	52.4	47.6	47.6	42.9	47.6
										HR	92.0	92.0	84.0	78.0	68.0	58.2	60.3
6	5	H	H	*tert*-Bu	278	EtOH	$C_{16}H_{24}N_2O_3 \cdot HCl$	A	58.3	BP	100	100	100	100	100	100	100
										HR	100	100	100	100	100	100	100
7	5	H	H	Benzyl	210–212	MeOH	$C_{19}H_{22}N_2O_3 \cdot HCl$	A	51.4	BP	35.7	53.6	28.6	10.6	14.3	14.4	7.8
										HR	12.5	16.3	10.3	12.8	2.6	0	0
8	5	H	H	Cyclohexyl	251–253	EtOH	$C_{18}H_{26}N_2O_3 \cdot HCl$	A	68.3	BP	62.5	68.8	64.9	28.1	0	0	0
										HR	33.3	30.6	22.2	19.4	16.7	16.7	0
9	5	H	Piperidino		215–217	MeOH	$C_{17}H_{24}N_2O_3 \cdot HCl$	A	37.5	BP	35.5	47.0	47.5	17.6	0	0	0
										HR	12.5	16.4	16.4	16.4	5.5	0	0
10	5	H	Morpholino		252–254	MeOH	$C_{16}H_{22}N_2O_4 \cdot HCl$	A	42.7	BP	10.5	15.3	15.8	7.8	5.2	0	0
										HR	3.3	3.3	2.1	2.1	2.1	0	0
11	5	H	4-Methylpiperazino		256.5	MeOH	$C_{17}H_{25}N_3O_3 \cdot 2HCl$	A	34.2	BP	4.2	4.2	6.3	6.3	2.1	0	0
										HR	2.5	2.7	3.8	1.9	0	0	0
12	5	H	2-Methylpiperidino		123–125	MeOH	$C_{18}H_{26}N_2O_3 \cdot HCl$	A	30.8	BP	26.1	26.1	34.8	30.4	17.4	13.0	0
										HR	4.1	12.2	8.2	12.2	2.0	0	0
13	6	H	H	*i*-Pr	242–245	MeOH–Me_2CO	$C_{15}H_{22}N_2O_3 \cdot C_4H_4O_4$[g]	A	46.9	BP	13.1	5.6	0	0	0	0	0
										HR	2.3	2.5	2.3	0	0	0	0
14	6	H	H	*tert*-Bu	163–166	EtOH	$C_{16}H_{24}N_2O_3 \cdot C_4H_4O_4$[f]	A	40.3	BP	23.6	17.4	9.6	0	0	0	0
										HR	5.5	5.5	2.5	0	0	0	0
15	7	H	H	*i*-Pr	207–209	*i*-PrOH	$C_{15}H_{22}N_2O_3 \cdot HCl$	A	47.6	BP	17.2	17.2	7.6	0	0	0	0
										HR	5.8	5.8	3.4	0	0	0	0
16	7	H	H	*tert*-Bu	251–253	*i*-PrOH	$C_{16}H_{24}N_2O_3 \cdot HCl$	A	45.7	BP	36.9	35.5	28.7	24.3	10.8	5.4	0
										HR	14.1	20.8	19.3	26.1	16.6	4.2	0
17	8	H	H	*i*-Pr	230–232	*i*-PrOH	$C_{15}H_{22}N_2O_3 \cdot HCl$	B	30.5	BP	100	100	100	100	92.6	80.3	75.6
										HR	82.8	76.7	88.2	61.4	23.9	21.3	17.1
18	8	H	H	*tert*-Bu	236–238	*i*-PrOH	$C_{16}H_{24}N_2O_3 \cdot HCl$	A	44.1	BP	100	93.5	100	100	98.4	89.0	84.3
										HR	88.5	88.5	71.4	65.2	62.9	55.8	50.2
19	5	Me	H	*i*-Pr	157–159	Me_2CO	$C_{16}H_{24}N_2O_3 \cdot HCl$	A	66.8	BP	44.4	55.6	69.4	72.2	69.4	66.7	55.6
										HR	32.6	43.5	56.5	60.9	65.2	63.2	56.5
20	5	Me	H	*tert*-Bu	160–163	EtOH	$C_{17}H_{26}N_2O_3 \cdot C_4H_4O_4$[f]	A	52.0	BP	94.4	89.9	91.7	92.5	94.4	91.3	88.9
										HR	53.2	62.9	85.5	90.3	90.3	90.3	87.1

21	5	Me	H	*sec*-Bu	152–155	Me_2CO	$C_{17}H_{26}N_2O_3 \cdot C_4H_4O_4$[f]	A	50.3	BP	22.6	55.8	67.4	67.4	9.81	62.1	46.5
										HR	32.1	52.6	67.9	67.9	66.5	62.5	57.1
22	5	Me	H	Cyclo-hexyl	161–163	Me_2CO	$C_{19}H_{28}N_2O_3 \cdot C_4H_4O_4$[f]	A	57.0	BP	11.8	29.4	21.8	29.4	61.6	7.8	0
										HR	3.8	11.5	11.5	7.7	11.6	5.3	0
23	5	Et	H	*i*-Pr	207–209	EtOH	$C_{17}H_{26}N_2O_3 \cdot HCl$	A	61.3	BP	26.9	53.8	59.6	47.7	63.5	69.2	55.7
										HR	2.0	21.6	35.3	39.2	43.1	43.1	47.1
24	5	Et	H	*tert*-Bu	181–183	EtOH	$C_{18}H_{28}N_2O_3 \cdot HCl$	B	60.0	BP	75.6	82.4	88.2	88.2	87.5	85.3	76.3
										HR	55.6	80.0	88.9	91.1	93.3	91.1	88.9
25	5	Et	H	*n*-Bu	126–128	EtOH	$C_{18}H_{28}N_2O_3 \cdot HCl$	A	49.5	BP	11.5	11.5	11.5	5.3	0	0	0
										HR	5.8	13.2	7.8	13.4	3.3	0	0
26	5	Et	H	Benzyl	155–157	EtOH	$C_{21}H_{26}N_2O_3 \cdot HCl$	A	50.6	BP	5.4	5.4	8.7	12.2	5.4	0	0
										HR	8.2	10.5	5.3	14.2	7.9	0	0
27	5	Et	H	Cyclo-hexyl	185–186	EtOH	$C_{20}H_{30}N_2O_3 \cdot HCl$	A	60.3	BP	16.7	28.9	34.2	26.3	13.2	6.8	0
										HR	11.8	17.8	23.5	23.5	13.2	5.4	0
28	5	Benzyl	H	*i*-Pr	192–194	EtOH	$C_{22}H_{28}N_2O_3 \cdot HCl$	A	55.0	BP	2.7	24.3	18.9	16.9	10.8	18.9	13.5
										HR	2.1	9.1	12.7	16.3	18.2	20.0	16.4
29	5	Benzyl	H	*tert*-Bu	192–193	MeOH	$C_{23}H_{30}N_2O_3 \cdot HCl$	A	53.8	BP	46.8	44.3	51.1	61.7	59.6	69.8	74.0
										HR	25.6	27.9	34.9	41.9	58.1	67.4	67.4
30	5	Benzyl	H	*i*-Bu	156.5–158.5	MeOH	$C_{23}H_{30}N_2O_3 \cdot HCl$	A	51.4	BP	8.3	16.7	25.0	16.7	0	0	0
										HR	6.9	3.5	10.3	3.4	0	0	0
31	5	Benzyl	Piperidino		216.5–218.5	MeOH	$C_{24}H_{30}N_2O_3 \cdot HCl$	A	42.5	BP	13.3	36.7	20.0	20.0	13.3	13.3	5.6
										HR	10.0	20.0	20.0	22.5	15.0	10.4	3.8
32	5	Benzyl	4-Methyl-piperazino		247	MeOH	$C_{24}H_{31}N_3O_3 \cdot 2HCl$	A	30.1	BP	1.5	13.9	5.6	0	0	0	0
										HR	1.5	4.7	2.3	0	0	0	0
33	5	Allyl	H	*i*-Pr	192–194	EtOH	$C_{18}H_{26}N_2O_3 \cdot C_4H_4O_4$[g]	A	49.7	BP	40.0	56.0	56.0	62.0	58.5	66.3	56.6
										HR	20.4	51.4	61.2	65.3	67.3	63.3	63.3
34	5	Allyl	H	*tert*-Bu	209–210	EtOH	$C_{19}H_{28}N_2O_3 \cdot C_4H_4O_4$[g]	A	44.5	BP	65.9	77.6	81.8	81.8	81.5	80.3	81.5
										HR	67.5	85.0	92.5	92.5	92.3	92.3	90.0
35	5	Allyl	H	*i*-Bu	183–184.5	EtOH	$C_{19}H_{28}N_2O_3 \cdot C_4H_4O_4$[g]	A	42.4	BP	9.5	23.8	29.5	14.8	7.4	0	0
										HR	36.6	53.7	43.4	35.3	21.6	12.4	0
36	5	H	Me	Me	139–140	*i*-PrOH	$C_{14}H_{20}N_2O_3 \cdot C_4H_4O_4$[f]	A	45.0	BP	2.7	13.5	8.3	8.3	0	0	0
										HR	1.8	4.4	4.4	2.2	0	0	0
37	5	H	*n*-Pr	*n*-Pr	221–222	EtOH	$C_{18}H_{28}N_2O_3 \cdot HCl$	A	47.9	BP	8.9	20.0	26.2	20.0	15.5	15.6	13.8
										HR	9.3	18.5	22.2	24.1	24.1	20.4	16.2
38	5	H	*n*-Bu	*n*-Bu	218–220	EtOH	$C_{20}H_{32}N_2O_3 \cdot HCl$	A	38.5	BP	5.6	16.7	16.7	0	0	0	0
										HR	12.5	5.0	10.0	5.3	0	0	0
39	5	H	*i*-Pr	*i*-Pr	208–210	*i*-PrOH	$C_{18}H_{28}N_2O_3 \cdot HCl$	A	53.2	BP	29.2	45.8	45.8	45.5	25.3	16.7	12.5
										HR	12.9	22.6	29.0	29.0	35.5	29.3	25.5
40	5	H	*i*-Bu	*i*-Bu	190–192	Me_2CO	$C_{20}H_{32}N_2O_3 \cdot HCl$	A	44.0	BP	5.7	32.1	9.4	5.7	0	0	0
										HR	6.4	10.0	7.5	2.5	0	0	0
41	5	H	Me	Cyclo-hexyl	136–138	*i*-PrOH	$C_{19}H_{28}N_2O_3 \cdot C_4H_4O_4$[f]	A	51.5	BP	2.0	5.3	3.7	0	0	0	0
										HR	2.5	2.1	2.5	0	0	0	0
42	5	H	Me	*n*-Bu	121–123	*i*-PrOH	$C_{17}H_{26}N_2O_3 \cdot C_4H_4O_4$[f]	A	47.4	BP	7.3	13.4	26.4	11.7	5.8	0	0
										HR	5.6	11.2	20.3	13.8	5.6	0	0
43	5	H	Me	Benzyl	181–183	*i*-PrOH	$C_{20}H_{24}N_2O_3 \cdot C_4H_4O_4$[g]	A	39.6	BP	1.5	5.6	10.4	5.6	0	0	0
										HR	3.2	6.1	7.6	3.7	0	0	0
44	5	Et	Me	Benzyl	216–217	Me_2CO	$C_{22}H_{28}N_2O_3 \cdot HCl$	A	58.0	BP	3.8	12.3	12.3	8.1	2.5	0	0
										HR	1.5	7.4	7.4	4.5	0	0	0
45	5	Benzyl	Me	Cyclo-hexyl	128–131	EtOH	$C_{26}H_{34}N_2O_3 \cdot HCl$	A	43.1	BP	1.7	8.2	13.5	3.7	0	0	0
										HR	4.2	1.5	1.5	1.3	0	0	0

[a]Substituted position of propoxy group. [b]All compounds were analyzed for C, H, and N. [c]Methods refer to Experimental Section. [d]Blood pressure. [e]Heart rate. [f]Maleic acid. [g]Fumaric acid.

Table II. Antagonistic Activity against Isoproterenol (% Inhibition of Changes in BP[a] and HR[b])

Compd	Dose, μg/kg		Time, min 10	30	60	120	180	240	300	360	420	480
6	10	BP	83.3	76.3	70.0	61.5	55.9	50.8	45.2	46.5	40.9	31.0
			(4.6)	(5.8)	(4.0)	(4.6)	(3.9)	(3.2)	(5.1)	(6.8)	(7.1)	(9.6)
		HR	85.9	80.2	75.6	67.8	60.5	54.6	47.9	51.5	49.6	47.6
			(3.2)	(2.4)	(3.8)	(4.2)	(5.1)	(5.3)	(5.0)	(6.2)	(6.6)	(8.9)
Pindolol	10	BP	69.8	55.4	51.5	51.7	43.4	40.3	39.4	30.5	25.5	24.5
			(2.0)	(7.3)	(4.4)	(6.9)	(4.6)	(5.5)	(5.6)	(3.7)	(4.6)	(3.4)
		HR	65.5	55.5	46.3	41.0	39.0	34.2	35.9	27.8	17.5	11.6
			(4.2)	(1.1)	(2.3)	(4.9)	(5.4)	(2.4)	(2.9)	(6.0)	(5.1)	(5.2)
Propranolol	100	BP	80.1	63.7	57.9	44.3	31.1	10.4				
			(7.6)	(12.5)	(8.5)	(10.1)	(4.9)	(5.2)				
		HR	75.3	57.1	42.6	25.6	20.4	5.6				
			(4.2)	(8.1)	(10.9)	(11.2)	(8.9)	(3.9)				

[a]Blood pressure. [b]Heart rate. Standard error is given in parentheses.

ally observed that blood pressure (BP) and heart rate (HR) were in parallel with each other. This was indeed the case of any of the compounds tested, as is shown in Table I. The blocking potencies of the compounds obeyed the following order: **6** > **2** > **18** > **17** > **20** > **24** > **34**. **6** proved to be most effective. These compounds had significant adrenergic β-receptor blocking activity.

In Table II **6** was compared to pindolol and propranolol. The potency of **6** was almost the same as pindolol and approximately ten times that of propranolol. It is noteworthy that **6** was characterized not only by the most effective potency but also by the longest duration of activity.

Structure–Activity Relationships. The initial study of β-adrenergic blocking agents involved a systematic evaluation of the positional isomers in the 3,4-dihydrocarbostyril series. Results showed that when the side chain substitution was maintained as 3-*tert*-butylamino-2-hydroxypropoxy, the 5 isomer **6** exhibited the greatest potency as a β-adrenergic blocking agent, and the 8 isomer **18** was a little less active, while the 6 and 7 isomers **14** and **16** were much less active.

Therefore, most comparisons of the effects of substitution on the amino function and on the 1 position were made within the 5 isomers series. The observed potency order for the amino substituents was *tert*-Bu (**6**) > *i*-Pr (**2**) > *sec*-Bu (**5**) > Et (**1**) for the 5-(3-substituted amino-2-hydroxy)propoxy-3,4-dihydrocarbostyril series. Introduction of larger functional groups on N such as cyclohexyl (**8**), piperidino (**9**), morpholino (**10**), or aralkyls such as benzyl (**7**) or difunctional groups such as Me,Me (**36**), *i*-Pr,*i*-Pr (**39**) led only to weakly active or inactive compounds. And the 1-substituted 5-(3-*tert*-butylamino)propoxy-3,4-dihydrocarbostyril series (**20**, **24**, **29**, and **34**) was less active than the nonsubstituted isomer **6**.

Experimental Section

The general experimental methods A and B are representative for the compounds reported in Table I. All melting points were determined in an open capillary tube in a bath and are uncorrected.

5-(2,3-Epoxy)propoxy-3,4-dihydrocarbostyril. To a solution of 0.23 g (0.01 mol) of Na in 30 ml of MeOH were added 1.63 g (0.01 mol) of 5-hydroxy-3,4-dihydrocarbostyril[2] and 2.79 g (0.03 mol) of epichlorohydrin. The mixture was warmed with stirring at 50–55° for 4 hr and filtered to remove insoluble NaCl. The filtrate was evaporated to dryness *in vacuo*. Me_2CO (10 ml) was added to the residue and the mixture was allowed to stand at room temperature for 24 hr. The crystals were filtered and recrystallized (EtOH): yield 1.2 g (54.8%); mp 172–173°. *Anal.* ($C_{12}H_{13}NO_3$) C, H, N.

8-(3-Chloro-2-hydroxy)propoxy-3,4-dihydrocarbostyril. A mixture of 1.63 g (0.01 mol) of 8-hydroxy-3,4-dihydrocarbostyril,[3] 3.72 g (0.04 mol) of epichlorohydrin, and 3 drops of piperidine was heated with stirring at 95–100° for 5 hr and evaporated *in vacuo*. The residue was dissolved in 30 ml of Me_2CO and chromatographed over Al_2O_3 employing Me_2CO as eluent. The eluate was evaporated to dryness *in vacuo* and the residue was recrystallized (Me_2CO): yield 1.1 g (43.0%); mp 179.5–180.5°. *Anal.* ($C_{12}H_{14}ClNO_3$) C, H, N.

1-Methyl-5-(2,3-epoxy)propoxy-3,4-dihydrocarbostyril. To a solution of 0.65 g (0.028 mol) of Na in 50 ml of MeOH were added 5.0 g (0.028 mol) of 1-methyl-5-hydroxy-3,4-dihydrocarbostyril[4,†] and 9.5 g (0.1 mol) of epichlorohydrin. The mixture was warmed with stirring at 40–45° for 6 hr and filtered to remove insoluble NaCl. The filtrate was evaporated to dryness *in vacuo*. The residue was extracted with $CHCl_3$, washed with aqueous 5% NaOH and H_2O, dried (Na_2SO_4), and concentrated to dryness. The residue was dissolved in 30 ml of Me_2CO and chromatographed over Al_2O_3 employing Et_2O–Me_2CO–benzene (1:1:2) mixture as eluent. The eluate was evaporated to dryness *in vacuo* and the residue was recrystallized (CCl_4–hexane): yield 3.5 g (53.2%); mp 76–78°. *Anal.* ($C_{12}H_{15}NO_3$) C, H, N.

5-(3-*tert*-Butylamino-2-hydroxy)propoxy-3,4-dihydrocarbostyril Hydrochloride (6) (Method A). A mixture of 1.8 g (0.008 mol) of 5-(2,3-epoxy)propoxy-3,4-dihydrocarbostyril, 2.9 g (0.04 mol) of *tert*-butylamine, and 30 ml of MeOH was warmed with stirring at 55–60° for 6 hr. The reaction mixture was evaporated to dryness *in vacuo*. A solution of dry HCl gas in *i*-PrOH was added to a solution of the residue in Me_2CO. The crystals were filtered and recrystallized (EtOH): yield 1.5 g (58.3%); mp 278° dec. *Anal.* ($C_{16}H_{24}N_2O_3 \cdot HCl$) C, H, N.

8-(2-Hydroxy-3-isopropylamino)propoxy-3,4-dihydrocarbostyril Hydrochloride (17) (Method B). A mixture of 1.5 g (0.006 mol) of 8-(3-chloro-2-hydroxy)propoxy-3,4-dihydrocarbostyril, 1.5 g (0.025 mol) of isopropylamine, and 30 ml of MeOH was refluxed for 4 hr. The reaction mixture was evaporated to dryness *in vacuo*. A solution of dry HCl gas in Me_2CO was added to a solution of the residue in Me_2CO. The crystals were filtered and recrystallized (EtOH): yield 0.6 g (32.5%); mp 230–232°. *Anal.* ($C_{15}H_{22}N_2O_3 \cdot HCl$) C, H, N.

1-Methyl-5-(3-*tert*-butylamino-2-hydroxy)propoxy-3,4-dihydrocarbostyril Maleate (20) (Method A). A mixture of 1.7 g (0.007 mol) of 1-methyl-5-(2,3-epoxy)propoxy-3,4-dihydrocarbostyril, 3.0 g (0.04 mol) of *tert*-butylamine, and 40 ml of EtOH was refluxed for 4 hr. The reaction mixture was evaporated to dryness *in vacuo*. A solution of maleic acid in Me_2CO was added to a solution of the residue in Me_2CO. The crystals were filtered and recrystallized (EtOH): yield 1.6 g (52.0%); mp 160–163°. *Anal.* ($C_{17}H_{26}N_2O_3 \cdot C_4H_4O_4$) C, H, N.

Assay of Adrenergic β-Receptor Blocking Activity.[5] Male adult dogs, 8–15 kg, were anesthetized by intravenous administration of sodium pentobarbital (30 mg/kg). The blood pressure (BP) was measured at the right carotid artery with a pressure transducer and the heart rate (HR) was determined with a cardiotachograph triggered by the R wave of lead II of the electrocardiogram, both values being recorded on a two-pen oscillograph.

An aliquot of 1 ml/10 kg of a drug solution (1000 μg/ml) of isoproterenol, 0.3 μg/ml, was injected 15 min before (control) and *t* min (t = 5, 10, 20, etc) after the drug was injected. The per cent inhibition of BP and HR changes due to the administration of the drug was recorded as a measure of its adrenergic β-receptor blocking activity.

†Y. Tamura, M. Terahashi, Y. Higuchi, K. Ozaki, and K. Nakagawa, unpublished results.

Acknowledgment. The authors wish to thank Professor Tamura of the Faculty of Pharmaceutical Sciences, Osaka University, for his advice on methods of synthesis.

References

(1) (a) E. J. Ariens, *Ann. N. Y. Acad. Sci.*, 139, 606 (1967); (b) A. F. Crowther and L. H. Smith, *J. Med. Chem.*, 11, 1009 (1968); (c) A. F. Crowther, K. J. Lorghlin, L. H. Smith, R. W. Turner, and T. M. Wood, *ibid.*, 12, 638 (1969); (d) R. Howe, *ibid.*, 12, 642 (1969); (e) J. F. Giudicelli, H. Schmitt, and J. R. Boissier, *J. Pharmacol. Exp. Ther.*, 168, 116 (1969); (f) B. Levy and M. Wasserman, *Brit. J. Pharmacol.*, 39, 139 (1970); (g) R. P. Robson and H. R. Kaplan, *J. Pharmacol. Exp. Ther.*, 175, 157 (1970); (h) C. F. Schwender, S. Farber, C. Blaum, and J. Schavel, *J. Med. Chem.*, 13, 684 (1970); (i) M. Nakanishi, T. Muro, Y. Chihara, H. Inamura, and T. Naka, *ibid.*, 15, 45 (1972); (j) K. Murase, K. Niigata, T. Mase, and M. Murakami, *Yakugaku Zasshi*, 92, 1358 (1972); (k) Y. Kobayashi, T. Nakagaki, T. Oshima, S. Kumakura, K. Nakayama, and H. Koide, *Chem. Pharm. Bull.*, 20, 905 (1972).
(2) (a) Y. Tamura, M. Terashima, Y. Higuchi, and K. Ozaki, *Chem. Ind. (London)*, 1935, (1970); (b) M. Mano and Y. Tamura, Japan Patent 39,694 and 39,695 (1971).
(3) (a) *Chem. Abstr.*, 50, 4964e (1965); (b) F. Mayer, L. van Zutphen, and H. Philips, *Ber.*, 60, 858 (1927); (c) J. D. London and J. Ogg, *J. Chem. Soc.*, 739 (1955); (d) N. Shigematsu, *Chem. Pharm. Bull.*, 9, 970 (1961).
(4) K. Ozaki and Y. Tamura, Japan Patent 38,789 (1971).
(5) R. D. Robson and H. R. Kaplan, *J. Pharmacol. Exp. Ther.*, 175, 157 (1970).

Substructural Analysis. A Novel Approach to the Problem of Drug Design

Richard D. Cramer III,* George Redl, and Charles E. Berkoff

Technology Assessment, Smith Kline & French Laboratories, Philadelphia, Pennsylvania 19101. *Received September 10, 1973*

Of the many approaches to the problem of drug design, those of greatest current utility and application are the regression techniques commonly associated with the names of Hansch[1] and Free-Wilson.[2] A severe limitation shared by these methods is their restriction to structurally closely related series of compounds. Thus they are inappropriate for (a) correlation of data where compounds fall into many different structural series or into no series at all; (b) prediction of active compounds outside a structural class of established biological interest. A second major limitation of these methods is their weakness in accommodating data represented by inactive compounds. In essence, existing structure–activity correlation methodologies are useful only for optimizing a previously recognized "lead" structure and not in generating new "leads."

The continuing need for drug design techniques that would be applicable to a broader range of problems led us to consider the mental model on which the medicinal chemist bases his search for new lead structures. An evident truth forming the basis of this model is that the biological activity of a molecule, or for that matter any other of its properties, must be accounted for by a combination of contributions from its structural components (substructures) and their intra- and intermolecular interactions. The very large body of information generated by even the most modest of screening programs requires the medicinal chemist to make additional simplifying assumptions, such as (a) the probability of a given biological activity can be usefully approximated by a first-order analysis of substructural contributions (*i.e.*, one ignoring interactions); (b) the contribution of a given substructure to the probability of activity can be obtained from data on previously tested compounds containing that substructure. The specific question we sought to answer empirically was whether a significant correlation could be obtained by systematically organizing existing sets of biological and substructural data to correspond with this mental model. (A previous approach to this problem using the statistical technique of cluster analysis appeared to show promise.[3])

Existing schemes for the codification of substructures have been created solely in response to a need for selective retrieval of compounds from large files.[4] Most of the substructures that chemists habitually perceive are far more complex than the several-atom "fragments" of these codes. These limitations clearly applied even to the relatively rich "SK&F fragment code," which recognizes some 1200 fragments comprising functional groups, rings, chains, inorganic moieties, and 110 rather diffusely defined fragment combinations.[5] For our pilot study we nevertheless attempted to use this code for the analysis of the most structurally diverse testing experience available to us, consisting of 850 compounds examined for their antiarthritic–immunoregulatory effects in an adjuvant-induced rat model.[6] To remove inherent sample bias and to ensure that our analyses would not simply regenerate known information, compounds which were members of already recognized "lead" series were eliminated, leaving 770 compounds. Of these, 189 (24.5%) were active, producing a statistically significant reduction in hind paw volume during the secondary phase of the induced disease process. It should be noted that, since such activity is displayed by agents having quite varied pharmacological properties, neither the available biological data nor the SK&F fragment code were totally appropriate for our objective.

The first step was to prepare a substructure "experience table" summarizing the data. A "Substructure Activity Frequency" (SAF), defined for each substructure as (A/T), the ratio of the number of *active* compounds (A) containing that substructure to the number of *tested* compounds (T) containing the substructure, represents the contribution which that substructure can make to the probability of a compound being active. The experience table contained 492 SAF's corresponding to the complete set of 492 substructures (fragments) previously recognized and coded in the tested compounds.

We then computed for each compound a "Mean Substructure Activity Frequency" (MSAF), the arithmetic mean of the SAF values of the substructures present in that compound. A sample MSAF computation appears in Table I. The 770 compounds next were ranked by descending MSAF value. Since a meaningful correlation would be reflected in a tendency for compounds of higher MSAF value to be active more frequently, the 770 ranked compounds were partitioned into ten sets, each containing 77 compounds. Those sets with high MSAF values were indeed found to be active far more frequently than those with low MSAF values (Table II).

However, analysis of some individual MSAF computations showed that MSAF values could be strongly influenced by SAF values for substructures that were poorly represented within the total set of tested compounds. For example, the SAF for a unique fragment must take either of the extreme values of 1.0 or 0.0, depending on whether the compound in which it occurred was active or not, and the MSAF for that compound would thus be biased in a direction which would improve the apparent correlation. Thus, even though unique fragments contribute less than 1% of the quantity of information, their impact on the overall analysis is substantial. To remove this type of bias, and to estimate the predictive value of the method, we devised a novel computational approach.

Groups of ten compounds, selected at random, became