TABLE III
INHIBITORY ACTIVITY OF OTHER PEPTIDES

ID_{50}[a] *L. plantarum* Inhibitory dipeptides (all L)	*P. cerevisiae*	Noninhibitory peptides (all L)		Noninhibitory amino acids
Try-try 0.01,	≪0.02	Cyc-pro	Leu-cyc	Ornithine
Try-gly .025,	≪0.02	Cyc-ser	Cyc-cyc	Citrulline
Try-tyr .06,	≪0.02	Ser-cyc	Ala-cyc	ε-Aminocaproic acid
Gly-try .05,	0.03	Val-cyc	Phe-cyc	Diaminopropionic acid
Leu-try .02,	.02	Arg-glu	Glu-arg	3-Aminotyrosine
Arg-cyc .06,	.1	Arg-asp	Arg-ala	S-Ethylcysteine
His-cyc .1,	.1	Pro-arg	Ileu-aleu	Homocysteic acid
			Ileu-ileu	
Tyr-phe N,[b]	.06	N-Formyl-phenylalanyl-melphelan·OEt		α-Methylglutamic acid
Lys-gly Inhibits		Val-gly-melphelan-OEt·2HCl		Melphelan (L-sarcolysin)
Arg-val N,	.08	Cys-bis-(melphelan-OEt)·2HCl		*ortho*-DL-Merphelan
Arg-leu N,	.08	Ala-melphelan-OEt picrate		Medphelan
Meth-cyc Variable		Val-melphelan		N,N,Di-(2-chloroethyl)-*p*-aminophenylbutyric acid

[a] ID_{50} is the concentration in mg./ml. at which growth is reduced 50%. [b] N, noninhibitory.

Thomas-Hoover melting point apparatus, calibrated against U.S.P. reference standards.

Butanol–water–acetic acid, 90:30:10, and pyridine–isoamyl alcohol–water, 35:35:30, were used routinely for paper chromatography with detection by ninhydrin, NaOCl–KI–starch, or ultraviolet for Z-peptides. Peptides with an N-terminal cycloleucine do not give a ninhydrin spot. Several blocked tripeptides did not give spots on the NaOCl–KI–starch peptide bond test. In these systems, protected peptide esters move close to the solvent front. Changes in optical configuration do not give R_f changes sufficient to detect.

R. Free Peptides by Other Procedures.—Hydrolysis at 37 and 100° of ileu-ileu-ileu-methyl ester hydrochloride by dilute HCl to remove the methyl group was used as an approach to the desired peptides. Hydrolysis of both ester and peptide bonds occurred, determined chromatographically. Base saponification of the tripeptide ester as used for valine[14] gave some polymeric products. Hydrolysis of ester moiety of L-val-L-val-D-phe methyl ester with crystalline chymotrypsin[20] was very slow. The peptide bonds cleaved in this time. The method could not be used for preparation of free L-L-D peptides.

S. Inhibitory Activity of Other Peptides.—Other peptides were evaluated[2b] to determine other possible types of inhibitory peptides. These are listed in Table III. The inhibition indices for *L. plantarum* and *P. cerevisiae* are given in that order after each peptide.

Acknowledgment.—We gratefully acknowledge the assistance of H. A. Florsheim, V. Gold, F. Lew, T. Giudici, and Dr. J. Davis on portions of this project. Generous gifts of cyclic and linear peptides and peptides of sarcolysin were given by Professor R. Schwyzer, Dr. J. Rudinger, Dr. K. Lentz, Dr. E. Schroder, and Professor F. Bergel. Cycloleucine was kindly furnished by Dr. R. B. Ross, CCNSC, NIH, Bethesda, Maryland. C and H analyses were made by Drs. Weiler and Strauss, Oxford, England.

(20) E. Walton, J. P. Rodin, C. H. Stammer, and F. W. Holly, *J. Org. Chem.*, **27**, 2255 (1962).

A New Series of Substances which Block the Adrenergic β-Receptors

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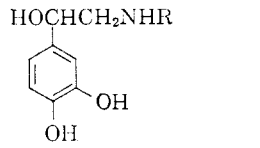
A series of phenylethanolamines and phenylpropanolamines with chlorine or alkyl substitution in the benzene nucleus has been synthesized which block adrenergic β-receptors in mammals. A clear relationship between structure and activity allowed us to synthesize potent β-receptor blockers with or without intrinsic sympathomimetic activity.

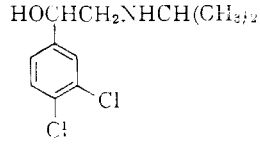
To explain the effects of catecholamines [norepinephrine (Ia), epinephrine (Ib), and isoproterenol (Ic)] on different organs, Ahlquist[1] proposed that responses to catecholamines might be elicited by stimulation of two different receptors. Relaxation of smooth muscle resulting in vasodilatation and bronchodilatation, the positive inotropic and chronotropic effect on the heart, as well as metabolic effects (*e.g.*, glycogenolysis) are due to stimulation of β-receptors, while other effects

(1) R. P. Ahlquist, *Am. J. Physiol.*, **153**, 586 (1948).

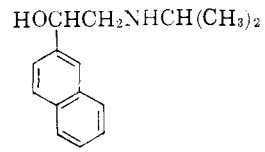
of catecholamines (piloerection, peripheral vasoconstriction, and contraction of the nictitating membrane) are mediated through stimulation of α-receptors.



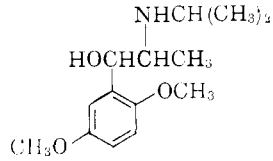
Ia, R = H
b, R = CH_3
c, R = $C_2H(CH_3)_2$



II (DCI)



III (nethalide)

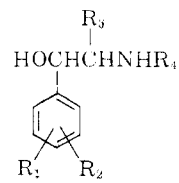


IV

Norepinephrine (Ia) stimulates mainly the α-receptors and isoproterenol (Ic) the β-receptors, whereas epinephrine (Ib) stimulates both types of receptors. While there are numerous agents which block α-receptors, there are relatively few compounds which have been reported to block the β-receptors. In this regard, DCI (dideoxydichloroisoproterenol (II),[2a,b] nethalide (III),[3] and N-isopropylmethoxamine[4a,b] (IV) have been described as β-receptor blocking agents. DCI has found a wide application in pharmacological work[5] but since it also produces cardiac stimulation, it is unsuitable as a blocking agent of cardiac β-receptors. Nethalide (III) has undergone clinical trials as a therapeutic agent in the treatment of arrhythmia and angina pectoris[6a,b,c]; N-isopropylmethoxamine (IV) on the other hand is a new compound which was published after completion of the present studies.

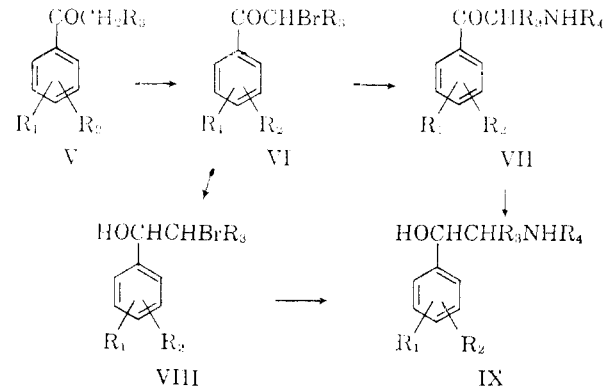
The present investigation is concerned with a new series of compounds which in some cases appear to produce rather specific β-receptor blockade. It is anticipated that these compounds will be of great value as pharmacologic tools and as therapeutic agents in the treatment of certain cardiac disorders.

Inspection of the structure of DCI (II) and isoproterenol (Ic) suggested that the isopropylaminoethanol side chain determines the affinity of the compound for the β-receptor while substitution in the nucleus determines whether the compound will stimulate or block the receptor. Furthermore, the question arose as to whether electronic or the space filling properties of the chlorine atoms in DCI are essential for blocking activity. For these reasons we synthesized and tested a series of compounds with the general formula



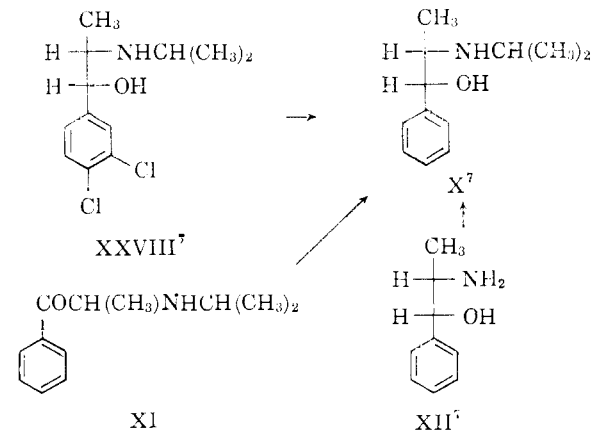
An alkyl substitution in R_3 was expected to make enzymatic inactivation of this type of substance by monoamine oxidase (MAO) more difficult.

Synthesis.—From the α-bromo ketones VI which were easily synthesized by bromination of the corresponding ketones V, the desired amino alcohols IX were formed in essentially two ways



(a) Reaction with amine R_4NH_2 to amino ketone VII and reduction with metal hydride or catalytic hydrogenation, or (b) reduction with sodium borohydride and reaction of the crude bromohydrins VIII with the appropriate amine R_4NH_2. Both methods gave essentially the same over-all yield; typical examples are described in the Experimental section. In Table I the intermediary ketones VII are listed and in Table II the corresponding reduced racemic compounds are depicted.

Stereochemistry.—In the case of R_3 = alkyl the amino alcohols can exist in the *erythro* or *threo* form; in XXVIII the *erythro* configuration of the side chain was proven by reduction to N-isopropylnorephedrine (X), which was identical in all respects with a sample prepared by reduction of α-isopropylaminopropiophenone (XI). X can also be prepared by reductive alkylation of norephedrine (XII) which is known to have *erythro* configuration.[8a-c] Since in all cases IX was prepared in the same manner as XXVIII and X, it can be assumed that all compounds with alkyl group in R_3 possess the *erythro* configuration in their side chain. Reduction with sodium borohydride or catalytic hydrogenation of an amino ketone VII gave iden-



(2) (a) J. Mills, U. S. Patent 2,938,921 (May 31, 1960); (b) C. E. Powell and I. H. Slater, *J. Pharmacol. Expti. Therap.*, **122**, 480 (1958).

(3) J. S. Stephenson, British Patent 909357 (October 31, 1962).

(4) (a) Wellcome Foundation, Belgian Patent 620521 (July 20, 1962); (b) C. H. Ellis and S. Gross, *Federation Proc.*, **22**, 247 (1963), *Abstr.* 522.

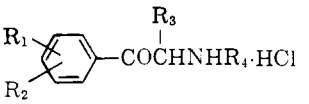
(5) *Cf.* E. E. Vogin and W. W. Baker, *Am. J. Pharm.*, **133**, 314 (1961).

(6) (a) I. W. Black and J. S. Stephenson, *Lancet*, **2**, 311 (1962); (b) A. C. Dornhorst and B. F. Robinson, *ibid.*, **2**, 314 (1962); (c) E. M. V. Williams and A. Sekiya, *ibid.*, **1**, 420 (1963).

(7) The formulas represent racemic forms.

(8) (a) K. Freudenberg, E. Schoeffel, and E. Braun, *J. Am. Chem. Soc.*, **54** 234 (1932); (b) W. Leithe, *Ber.*, **65**, 660 (1932); (c) K. Freudenberg and F. Nikolai, *Ann.*, **510**, 223 (1934).

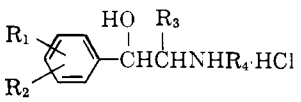
TABLE I



R_1	R_2	R_3	R_4	M.p., °C.	Formula	Ionized Cl— Calcd., %	Ionized Cl— Found, %
4-CH_3	H	H	$CH(CH_3)_2$	160	$C_{12}H_{18}ClNO$	15.6	15.6
4-CH_3	3-CH_3	H	$CH(CH_3)_2$	193–195	$C_{13}H_{20}ClNO$	14.6	14.6
4-C_2H_5	H	H	$CH(CH_3)_2$	163–165	$C_{14}H_{24}ClNO_2$[a]	13.0[a]	12.9
4-$(CH_2)_2CH_3$	H	H	$CH(CH_3)_2$	178–180	$C_{14}H_{22}ClNO$	13.9	13.6
4-C_2H_5	3-C_2H_5	H	$CH(CH_3)_2$	195–205	$C_{15}H_{24}ClNO$	13.1	13.2
4-$CH(CH_3)_2$	H	H	$CH(CH_3)_2$	182	$C_{14}H_{22}ClNO$	13.9	14.1
4-$C(CH_3)_3$	H	H	$CH(CH_3)_2$	166	$C_{15}H_{24}ClNO$	13.9	14.0
4-Cl	3-CH_3	H	$CH(CH_3)_2$	228–229	$C_{12}H_{17}Cl_2NO$	13.5	13.3
4-CH_3	3-Cl	H	$CH(CH_3)_2$	233	$C_{12}H_{17}Cl_2NO$	13.5	13.4
4-CH_3	H	CH_3	$CH(CH_3)_2$	231–232	$C_{13}H_{20}ClNO$	14.6	14.7
H	3-CH_3	CH_3	$CH(CH_3)_2$	230–232	$C_{13}H_{20}ClNO$	14.6	14.7
4-CH_3	3-CH_3	CH_3	$CH(CH_3)_2$	247–248	$C_{14}H_{22}ClNO$	13.9	13.8
4-CH_3	2-CH_3	CH_3	$CH(CH_3)_2$	204–206	$C_{14}H_{22}ClNO$	13.9	14.0
5-CH_3	2-CH_3	CH_3	$CH(CH_3)_2$	220–222	$C_{14}H_{22}ClNO$	13.9	13.9
4-C_2H_5	H	CH_3	$CH(CH_3)_2$	231–232	$C_{14}H_{22}ClNO$	13.9	13.9
4-Cl	3-Cl	CH_3	$CH(CH_3)_2$	252	$C_{12}H_{16}Cl_3NO$	35.9[b]	36.0[b]
4-Cl	3-Cl	C_2H_5	$CH(CH_3)_2$	248–249	$C_{13}H_{18}Cl_3NO$	11.4	11.4
4-CH_3	3-CH_3	H	$CHCH_2CH_2$	186–187	$C_{13}H_{18}ClNO$	14.8	14.7
4-CH_3	3-CH_3	CH_3	n-C_4H_9	218–220	$C_{15}H_{24}ClNO$	13.1	13.2
4-CH_3	3-CH_3	CH_3	$CH(C_2H_5)_2$	227–229	$C_{16}H_{26}ClNO$	12.5	12.4

[a] Contains 1 mole of methanol as solvent of crystallization. [b] Total Cl content.

TABLE II



	R_1	R_2	R_3	R_4	M.p., °C.	Formula	Ionized Cl— Calcd., %	Ionized Cl— Found, %
XIII	4-CH_3	H	H	$CH(CH_3)_2$	132–134	$C_{12}H_{20}ClNO$	15.4	15.4
XIV	4-CH_3	3-CH_3	H	$CH(CH_3)_2$	158–159	$C_{13}H_{22}ClNO$	14.5	14.6
XV	4-C_2H_5	H	H	$CH(CH_3)_2$	94	$C_{14}H_{26}ClNO_2$[a]	12.9[a]	13.1[a]
XVI	4-$(CH_2)_2CH_3$	H	H	$CH(CH_3)_2$	143–145	$C_{16}H_{25}NO_5$[b]		
XVII	4-C_2H_5	3-C_2H_5	H	$CH(CH_3)_2$	132–134	$C_{15}H_{26}ClNO$	13.1	13.0
XVIII	4-$CH(CH_3)_2$	H	H	$CH(CH_3)_2$	122	$C_{14}H_{24}ClNO$	13.8	13.8
XIX	4-$C(CH_3)_3$	H	H	$CH(CH_3)_2$	222	$C_{15}H_{26}ClNO$	13.1	13.0
XX	4-Cl	3-CH_3	H	$CH(CH_3)_2$	133–136	$C_{12}H_{19}Cl_2NO$	13.4	13.2
XXI	4-CH_3	3-Cl	H	$CH(CH_3)_2$	162	$C_{12}H_{19}Cl_2NO$	13.4	13.2
XXII	4-CH_3	H	CH_3	$CH(CH_3)_2$	202–204	$C_{15}H_{22}ClNO$	14.5	14.5
XXIII	H	3-CH_3	CH_3	$CH(CH_3)_2$	200–201	$C_{13}H_{22}ClNO$	14.5	14.5
XXIV	4-CH_3	3-CH_3	CH_3	$CH(CH_3)_2$	214	$C_{14}H_{24}ClNO$	13.8	13.7
XXV	4-CH_3	2-CH_3	CH_3	$CH(CH_3)_2$	243–245	$C_{14}H_{24}ClNO$	13.8	13.8
XXVI	5-CH_3	2-CH_3	CH_3	$CH(CH_3)_2$	238–239	$C_{14}H_{24}ClNO$	13.8	13.8
XXVII	4-C_2H_5	H	CH_3	$CH(CH_3)_2$	190–200	$C_{14}H_{24}ClNO$	13.8	13.8
XXVIII	4-Cl	3-Cl	CH_3	$CH(CH_3)_2$	220	$C_{12}H_{18}Cl_3NO$	35.6[c]	35.5[c]
XXIX	4-Cl	3-Cl	C_2H_5	$CH(CH_3)_2$	247–248	$C_{13}H_{20}Cl_3NO$	11.3	11.3
XXX	4-CH_3	3-CH_3	H	$CHCH_2CH_2$	166–168	$C_{13}H_{20}ClNO$	14.7	14.6
XXXI	4-CH_3	3-CH_3	CH_3	n-C_4H_9	203[d]	$C_{15}H_{26}ClNO$	13.1	13.1
XXXII	4-CH_3	3-CH_3	CH_3	$CH(C_2H_5)_2$	234	$C_{16}H_{28}ClNO$	12.4	12.4

[a] Contains 1 mole of methanol as solvent of crystallization. [b] Oxalate. [c] Total Cl content. [d] *Cf.* lit.[e] m.p. 203°. [e] *Cf.* M. Furukawa and S. Toyoshima, *Chem. Pharm. Bull.*, **11**, 518 (1963).

tical amino alcohols in excellent yield; therefore, it was not possible to obtain the *threo* form by changing the conditions for reduction.

Pharmacological Screening.—All potential β-receptor blocking compounds were tested on the rabbit isolated heart for intrinsic sympathomimetic activity and for any reduction in the response to isoproterenol.

The β-receptor blocking activity of the substances was estimated in papillary muscles isolated from the rabbit heart. The muscle was stimulated electrically and the blocking action of the substances was tested on the increase in contraction height produced by isoproterenol.

The antiarrhythmic properties were evaluated in anesthetized cats against arrhythmias induced by the combined action of epinephrine and vagal stimulation ("vagus-amine" test[9]).

(9) J. Roberts and R. Baer, *J. Pharmacol. Exptl. Therap.*, **129**, 36 (1960).

Methods

(a) **Langendorff Heart.**—Female rabbits were killed by a blow on the head and bled. The heart was removed and set up according to Langendorff. The perfusion pressure was adjusted and kept constant by means of a sigmamotor pump and the heart rate was recorded by a photoelectric device connected to an ordinate writer. The heart was perfused by oxygenated Krebs solution at 37°. Compounds were added to the perfusion fluid just before the fluid entered the heart. After a suitable response to isoproterenol (0.01–0.02 γ) was obtained, the β-receptor blocking drugs were added and the effect of isoproterenol was redetermined. An interval of 15 min. was allowed to wash out the substances.

(b) **Papillary Muscle.**—Female rabbits were killed as before, the heart was removed, and a papillary muscle was suspended in a bath containing Krebs solution at 37° through which 95% O_2 and 5% CO_2 was bubbled. To record contractions a force displacement transducer connected to an Offner dynograph was employed. The muscle was stimulated at a frequency of 1–2 pulses/sec. and at a voltage of 2–5 v. Isoproterenol in doses of 0.1–0.2 γ (0.1–0.2 ml.) was added to the bath (vol. 50 ml.) every 15 min. and remained in contact with the muscle for 3 min. before

washing out. Stimulation, which had commenced some minutes previously, was continued while isoproterenol was in the bath. β-Receptor blocking activity was indicated by a reduction in the stimulatory effect of isoproterenol.

(c) **Antiarrhythmic Activity.**—For details of the method see Roberts and Baer.[9] In the "vagus-amine" test, the sinus rate is slowed by vagal stimulation to a critical point; namely, a rate just short of that at which the A-V node or ventricle escapes ("critical sinus slowing"). In this circumstance ventricular ectopic beats are induced by agents which raise the rhythmicity of the A-V node or the ventricle.

Cats were anesthetized with diallyl barbiturate-urethane (Ciba, 0.5 to 0.7 ml./kg. i.p.) and the "critical sinus slowing" was produced by stimulating the vagus nerve with pulses of 5–12 v., for 0.5 msec. duration, at a frequency of 5–30/sec. The smallest or threshold dose of epinephrine which induces arrhythmia in the setting of "critical sinus slowing" was determined before and after the blocking drugs were administered. Antiarrhythmic activity was indicated by an increase in the epinephrine threshold dose.

(d). In a few experiments the influence of these substances on the vasodepressor effect of isoproterenol in cats anesthetized with pentobarbital sodium was investigated. As in the other test preparations, β-receptor blockade was produced. All compounds caused a transient moderate vasodepressor effect of unknown etiology in anesthetized cats on intravenous injection. In anesthetized mice the substances in varying doses blocked the positive chronotropic effect of isoproterenol.[10]

In all the screening procedures DCI (II) and nethalide (III) were used as reference substances. The results are summarized in Table III.

TABLE III

RESULTS OF SCREENING

Compound	Intrinsic activity Langendorff heart Kind of effect + or − Inotropic	dose[b]	β-Receptor blocking effect (pap. muscle) compared with DCI (= 1.0)	Antiarrhythmic potency[a]
DCI	+	10	1.0	[c]
Nethalide	[d]		1.2	1.0
XIV	+	5	1.2	0.1
XXIV	−	50	0.2	0.5
XXVIII	−	50	0.08	2
XXXI	−	50	0.1	0.5
XXXII	−	50	0.06	1.0
XVII	Not tested		0.08	1
XIII	+	10	1.2	0.5
XXIX	−	50	0.15	2
XXVI	−	100	0.06	0.5
XXVII	−	100	0.2	2.5
XXII	−	50	0.2	1
XXIII	−	100	0.05	2.5
XVIII	−	25	0.2	0.5
XX	+	10	1.0	
XV	−	25	0.15	
XXX	−	25	0.08	
XVI	−	50	0.1	
XXI	−	1	1.0	
XXV	−	100	0.03	0.5

[a] The smallest dose of the drug (mg./kg.) which causes a two-fold increase in the dose of epinephrine necessary to produce arrhythmia. [b] The smallest dose of the drug (γ/50 ml.) which causes an effect. [c] 1 mg./kg. enhances arrhythmia. [d] 25 γ/50 ml. did not produce a definite effect.

Discussion

The data show that α-alkyl substitution in DCI-(XXVIII, XXIX) not only abolished the positive inotropic effect of DCI but also lowered β-receptor blocking potency of the compound. A similar relationship was also shown with other compounds which have an α-methyl group in the side chain (*e.g.*, XIV → XXIV). Substitution in the nucleus with 3,4-dimethyl or 4-methyl groups resulted in compounds which exerted a positive inotropic action and which were the most potent β-blocking agents. Longer alkyl chains in the 4-position or dimethyl substitution other than in the 3,4-positions resulted in compounds which produced a negative inotropic action and weaker blocking activity in the rabbit heart preparation (XIII → XV). While dimethyl substitution other than in the 3,4-positions also resulted in less potent antiarrhythmic agents, longer alkyl chains in the 4-position with the exception of 4-ethyl had little effect on potency (XXVII → XXII; XVIII → XIII). N-Isopropyl derivatives seem to be optimal since other substitution on the nitrogen tended to lower blocking activity (*e.g.*, XXIV → XXXII). Most impressive is the loss of activity if the isopropyl group is replaced by a cyclopropyl group (*cf.* XIV → XXX).

There is a considerable difference in the intrinsic activity of DCI and XIV or XIII which is not evident from the experiments with isolated organs. In small doses (1 mg./kg.) DCI enhances epinephrine-induced arrhythmia in cats while XIV or XIII did not cause such an effect. Further work on pharmacology and on physicochemical relationships in this series of compounds will be published elsewhere.

Experimental[11]

DCI (II) and nethalide (III) were synthesized as described in the literature.[2a,3] The compounds listed in Tables I and II were synthesized in a similar manner; examples of the procedures employed are described.

α-Bromo Ketones (VI)

4-Methyl-α-bromoacetophenone (VI, R_1 = 4-CH_3, R_2 = R_3 = H).—A solution of 67 g. (0.5 mole) of 4-methylacetophenone in 200 ml. of benzene was treated with 80 g. (0.5 mole) of bromine for 60 min. After 1 hr. at 20° the solution was washed with water and sodium carbonate solution, dried over potassium carbonate, and evaporated *in vacuo*. The remaining oil was distilled, b.p. 105° (0.1 mm.), or used without purification. The other bromoketones were used without distillation.

α-Amino Ketones (VII)

4-Methyl-α-isopropylaminoacetophenone Hydrochloride [VII, R_1 = 4-CH_3, R_2 = R_3 = H, R_4 = $CH(CH_3)_2$].—4-Methyl-α-bromoacetophenone (113 g., 0.53 mole) dissolved in 540 ml. of absolute ethanol was mixed with 215 ml. (2.51 moles) of isopropylamine and 500 ml. of absolute ethanol and left at 20° overnight. The solution was evaporated to dryness and the remainder was dissolved in water and made alkaline with potassium carbonate. Extraction with ether, drying over potassium carbonate, and evaporation gave an oil which was dissolved in absolute ether and treated with hydrogen chloride. The precipitated hydrochloride was recrystallized from methanol–ethyl acetate, m.p. 160° dec. The chlorine analyses of the compounds are listed in Table I. Methanol–ethyl acetate was used as solvent for recrystallization of all compounds listed in Table I.

Bromohydrine (VIII)

1-(3,4-Dimethylphenyl)-2-bromoethanol [VIII, R_1 = 4-CH_3, R_2 = 3-CH_3, R_3 = H].—3, 4-Dimethyl-α-bromoacetophenone (25 g., 0.11 mole) dissolved in 30 ml. of methanol was reduced by addition of 5.2 g. (0.14 mole) of sodium borohydride at 15–25°. After 1 hr. the solution was treated with 200 ml. of ice-cold 2 *N* hydrochloric acid and the bromohydrine extracted with ether. After drying over sodium sulfate and after evaporation, the remainder was used without purification.

Amino Alcohols (IV)

(10) Experiments performed by Dr. S. Ross, AB Astra, Södertälje. Methods and results will be published elsewhere.

(11) The melting points are determined in the Kofler block and are corrected.

(a) From Amino Ketone VII. 1-(4-Methylphenyl)-2-isopropylaminoethanol Hydrochloride [R_1 = 4-CH_3, R_2 = H, R_3 = H, R_4 = $CH(CH_3)_2$].—4-Methyl-α-isopropylaminoacetophenone (16 g., 0.084 mole) dissolved in 50 ml. of methanol was reduced at 0° with 3.2 g. (0.085 mole) of sodium borohydride. After 1 hr. at 20°, 200 ml. of water was added and the base was extracted with ether. Drying over potassium carbonate and evaporation gave an oil which was converted to the hydrochloride. The hydrochloride was recrystallized from methanol–ethyl acetate, m.p. 132–134°. The chlorine analyses of the compounds are listed in Table II.

(b) From Amino Ketone VII by Catalytic Hydrogenation. 1-(3,4-Dimethylphenyl)-2-isopropylaminopropanol Hydrochloride [IX, R_1 = 4-CH_3, R_2 = 3-CH_3, R_3 = CH_3, R_4 = $CH(CH_3)_2$].—3,4-Dimethyl-α-isopropylaminopropiophenone hydrochloride (7.65 g., 0.035 mole) was reduced catalytically with 1 g. of 10% palladium on charcoal in 100 ml. of ethanol. After absorption of 0.035 mole of hydrogen the solution was filtered and evaporated *in vacuo*. The remainder was recrystallized from methanol–ethyl acetate, m.p. 214°.

Anal. Calcd. for $C_{14}H_{24}ClNO$: C, 65.22; H, 9.38; Cl, 13.75. Found: C, 65.11; H, 9.27; Cl, 13.65.

This product was identical with a sample prepared by reduction with sodium borohydride.

(c) From Bromohydrin VIII. 1-(3,4-Dimethylphenyl)-2-isopropylaminoethanol Hydrochloride [IX, R_1 = 4-CH_3, R_2 = 3-CH_3, R_3 = H, R_4 = $CH(CH)_2$].—1-(3,4-Dimethylphenyl)-2-bromoethanol (17.2 g., 0.075 mole) dissolved in 150 ml. of absolute ethanol and 13.2 g. (0.22 mole) of isopropylamine were refluxed for 12 hr. After evaporation, the remainder was dissolved in water and made alkaline with sodium hydroxide. Extraction with ether gave an oil which was converted to the hydrochloride. The latter was recrystallized from methanol–ethyl acetate, m.p. 158–159°.

Anal. Calcd. for $C_{13}H_{22}ClNO$: C, 64.05; H, 9.10; Cl, 14.54. Found: C, 64.06; H, 9.20; Cl, 14.61.

Methanol–ethyl acetate was used as solvent for recrystallization of all hydrochlorides listed in Table II.

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Occupancy of Adrenergic Receptors and Inhibition of Catechol O-Methyl Transferase by Tropolones[1,2]

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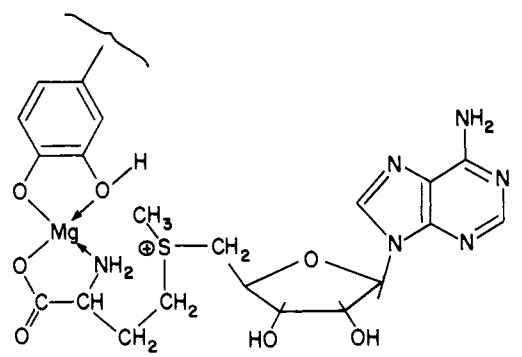
In the light of the mechanism of action of catechol-O-methyl transferase (COMT), it was conceived that tropolones should act as specific inhibitors of the enzyme. Confirmation of this postulate was obtained. A variety of tropolones were discovered to be effective inhibitors of COMT. The earlier claim that these inhibitors act noncompetitively has been withdrawn. Structure–activity relationships were also established. A new assay procedure for COMT was designed. A structure for the Michaelis complex between COMT and tropolones is proposed. It is shown that the suggested structure for the complex accounts for the behavior of tropolones toward COMT. The conclusion was reached that catechol and tropolone rings are biochemically isosteric. On the basis of preliminary pharmacological data it was shown that the catechol–tropolone isosterism also applies to adrenergic receptors. Depending on the dose of tropolone, *in vivo* and *in vitro* COMT inhibition as well as β-receptor "blockade" could be observed separately. It would appear that this is the first time that a group of substances that are not amines are shown to display affinity for adrenergic receptors.

The mechanisms whereby the catecholamine hormones are inactivated *in vivo* has been elucidated by Axelrod, *et al.*[3] It is now recognized that catechol-O-methyl transferase (COMT) is the enzyme primarily concerned in the inactivation of circulating catecholamines. The enzyme has been isolated and partially purified,[4] and the nature of its cofactor requirements elucidated. The substrate specificity and cation requirement for enzymic O-methylation led Senoh, *et al.*,[5] to propose the mechanism depicted in I as accounting for the methyl group transfer reaction from adenosylmethionine to the *meta*-phenolic group of substrates. From the pharmacological standpoint, the role of COMT acquires special significance because of its



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involvement in the termination of the physiological action of the circulating catecholamine hormones.[6,7] It is in this connection that accessibility to suitable inhibitors of the enzyme is critical since it is through

(1) Taken in part from the thesis submitted by J. Burba in partial fulfillment of the requirements for the Ph.D. degree, University of Ottawa, 1962.

(2) For a preliminary account of this work see B. Belleau and J. Burba, *Biochim. Biophys. Acta*, **54**, 195 (1961); see text for a correction.

(3) This subject has been reviewed by J. Axelrod, in "Adrenergic Mechanisms," A Ciba Foundation Symposium, J. R. Vane, G. E. W. Wolstenholme, and M. O'Connor, Editors, J. and A. Churchill Ltd., London, 1960, p. 28.

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