(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.

Acknowledgments.—The authors are indebted to Mr. L. Boberg, Miss M. Ettles, Mrs. C. Burlin, Mr. G. Hallhagen, Mr. R. Svahn, and Mr. V. Tarkkanen for skillfull help in various phases of this work and to the co workers of Dr. Roberts: Dr. R. Ito, Mr. V. Cairoli, and Mr. R. W. Pritchard, who performed many of the antiarrhythmia experiments.

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

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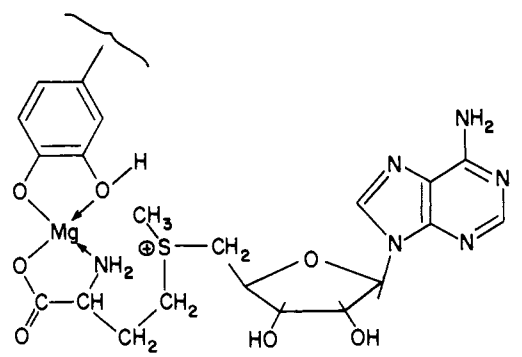
In the light of the mechanism of action of catechol-O-methyl transferase (COMT), it was conceived that tropolones should act as specific inhibitors of the enzyme. Confirmation of this postulate was obtained. A variety of tropolones were discovered to be effective inhibitors of COMT. The earlier claim 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



I

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.

(4) J. Axelrod and R. Tomchick, *J. Biol. Chem.*, **233**, 702 (1958).

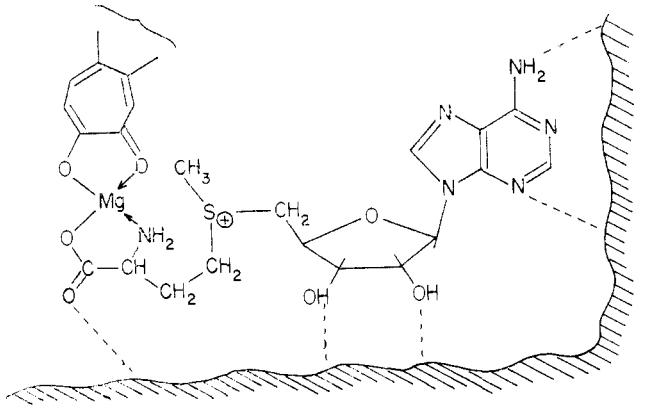
(5) S. Senoh, J. Daly, J. Axelrod, and B. Witkop, *J. Am. Chem. Soc.*, **81**, 6240 (1959).

(6) J. Axelrod and M.-J. Laroche, *Science*, **130**, 800 (1959).

(7) E. V. Evarts, L. Gillespie, T. C. Fleming, and A. Sjoerdsma, *Proc. Soc. Exptl. Biol. Med.*, **98**, 74 (1958).

their use that the prolongation of the physiological actions of catecholamines could be demonstrated *in vivo*.[3] Although pyrogallol[3,6] and quercetin[3] as well as a variety of other polyphenols[8] could be shown to dampen the rate of enzymatic methylation of catecholamines both *in vivo* and *in vitro*, they cannot be considered as true inhibitors of COMT as has been frequently implied, because their primary function is to act as substrates for the enzyme[9]; hence, the need for true and specific inhibitors of COMT. It is the purpose of this communication to describe such a class of novel inhibitors and to report briefly on their activity at the adrenergic receptor level.

Discussion of Results.—A consideration of the concept of isosterism led us to conceive that the tropolone ring[10] (III) should be ideally suited for complex formation with the COMT active sites. The prediction is permissible on the basis of the rational representations I and II, that tropolones should act as inhibitors because of their ability to form stable chelates with divalent metal ions,[11] a property which is shared by catechol rings.[12] However, the question of their specificity of action could not be predicted safely, since the possibility existed that they could simply act as metal ion scavengers as is the case, for instance, with ethylenediaminetetraacetic acid (EDTA).[13] If tropolones were to act through an EDTA-like mechanism, no applications to *in vivo* studies on the specific role of COMT could obviously be envisaged. The virtually perfect isosterism between catechol and tropolone rings suggests, on the other hand, that the analogous Michaelis complex II ought to form initially and, provided the coordinating power of tropolones toward magnesium does not exceed that of the enzyme coordinating sites, the resulting inhibition would be rather specific for COMT. If, indeed, the tropolone and catechol rings are biochemically isosteric as studies with COMT could conceivably establish, then the possibility would arise that tropolones could also display a specific affinity for adrenergic receptors and in this way allow a deeper insight into the biochemical nature of the latter. A preliminary account of such work has already been presented.[14]



II

The effect of a number of synthetic and naturally occurring tropolones on the COMT-catalyzed methylation of norepinephrine was initially examined. The first prototype used in our studies was β-methyltropolone (III), the synthesis of which was accomplished according to Haworth, *et al.*[15] Compounds V (β-thujaplicin) and VI (γ-thujaplicin) are naturally occurring and were kindly provided by Dr. Gardner[16]; stipitatic acid (IV),[17] colchiceine, and colchicine were also tested against COMT; β-acetamidomethyltropolone (XI) was synthesized according to Nozoe, *et al.*[18] The melting point behavior of our product XI was in excellent agreement with the reported value.[18] The tropolone analog IX of norepinephrine was synthesized from β-formyltropolone (VII), itself synthesized according to Tarbell, *et al.*[19] The reaction sequence involved condensation of VII with nitromethane to give VIII in high yield, followed by selective catalytic hydrogenation of the nitro group to give IX as an amorphous solid in moderate yield. The structure of IX was confirmed by n.m.r. spectroscopy (see Experimental). The troponimine amide XIII and thioamide XIV were generous gifts from Dr. W. R. Brasen.[20]

The enzyme (COMT) was prepared and purified from rat liver according to Axelrod and Tomchick.[1] The method of assay most generally employed relies on the spectrofluorimetric determination of O-methylated products. Although extremely sensitive, this method presented a number of technical problems.[21] An alternative method of assay of the O-methylated product was, therefore, designed which is based on the nitrosonaphthol method of Udenfriend and Cooper[22] for the determination of reactive phenols. Using norepinephrine as the substrate, it was established that tropolones and free catechols do not interfere with the assay of normetanephrine. Less reactive phenols such as *meta*-O-methyl-noradrenalone do not give a color with nitrosonaphthol. This assay procedure, although less expedient and less sensitive, proved to be adequately reproducible. The results of a typical run using norepinephrine as substrate and the nitrosonaphthol method for the assay of normetanephrine are illustrated in Fig. 1, where it can be seen that the observed rate of the methylation reaction is in good agreement with published results, using the spectrofluorimetric method of assay. A typical inhibited rate using 4-methyltropolone is also illustrated in Fig. 1. The various tropolones referred to before were then

(8) O. W. Wylie, S. Archer, and A. Arnold, *J. Pharmacol. Exptl. Therap.*, **130**, 239 (1960).

(9) S. Archer, A. Arnold, R. K. Kullnig, and O. W. Wylie, *Arch. Biochem. Biophys.*, **85**, 153 (1960).

(10) For a review of the chemistry of tropolones see T. Nozoe, in "Non-Benzenoid Aromatic Compounds," D. Ginsburg, Editor, Interscience Publishers Inc., New York, N. Y., 1959, p. 339.

(11) B. E. Bryant, W. C. Fernelius, and B. E. Douglas, *J. Am. Chem. Soc.*, **75**, 3784 (1953).

(12) G. L. Eichhorn, in "The Chemistry of Coordination Compounds," J. C. Bailar, Editor, Reinhold Publishing Corp., New York, N. Y., 1956, p. 698.

(13) S. Senoh, Y. Tokuyama, and B. Witkop, *J. Am. Chem. Soc.*, **84**, 1719 (1962).

(14) B. Belleau, Symposium on Structure–Activity Relationships, Vol. 7, First International Congress of Pharmacology, Stockholm, Sweden, August 1961; Pergamon Press, London, 1963, p. 75.

(15) R. D. Haworth and J. D. Hobson, *J. Chem. Soc.*, 561 (1951).

(16) Forest Products Laboratory, Vancouver, B.C.

(17) We are grateful to Dr. I. H. Hall, Smith Kline and French Laboratories, for a generous gift of this substance.

(18) T. Nozoe, Y. Kitahara, K. Doi, M. Funamizu, and T. Terasawa, *Chem. Pharm. Bull.*, **55**, 2589a (1954).

(19) D. S. Tarbell, K. I. H. Williams, and E. J. Sehm, *J. Am. Chem. Soc.*, **81**, 3443 (1959).

(20) Central Research Dept. Experimental Station, E. I. du Pont de Nemours and Company, Wilmington, Del.

(21) We have since found that the source of difficulties was largely elucidated when an Aminco-Bowman spectrofluorimeter was used.

(22) S. Udenfriend and J. Cooper, *J. Biol. Chem.*, **196**, 227 (1952).

tested for their inhibitory activity toward the COMT-catalyzed methylation of norepinephrine. The concentration of substrate was adjusted so that maximum reproducibility could be achieved with our method of assay. This necessitated substrate concentrations approaching maximum velocity of methylation. The inhibition constants K_i were calculated in the usual fashion.[23] The Michaelis constant for norepinephrine was estimated in the conventional manner to be 7×10^{-4} M, a value in good agreement with the published one.[24a] The values of K_i are assembled in Table I where it can be seen that the presence of ring substituents or their nature has only a marginal effect on the affinity of the tropolone ring for the enzyme. It is interesting to note that 4-methyltropolone (III) is the most active inhibitor of the series, its affinity for COMT being somewhat greater than that of pyrogallol. Both the amino-imino and the amino-thioxo analogs XIII and XV display significant inhibitory activity in agreement with the postulate that a chelation mechanism involving enzyme-bound magnesium is operative. The ability of these nitrogen and sulfur analogs of tropolone to form stable chelates with metal ions has been established.[24b] The chelation mechanism of inhibition is further substantiated by the observation that colchicine which has no free hydroxyl groups is totally inactive as an inhibitor. Furthermore, *meso*-inositol, which is known to chelate metal ions[25] but less strongly, also displays some inhibitory activity. Finally, the relative indifference of the enzyme toward substituents, including a norepinephrine side chain as in IX on the tropolone ring, offers strong support in favor of a chelation mechanism of inhibition.

TABLE I

INHIBITION CONSTANTS (in moles/l.) FOR VARIOUS SUBSTITUTED TROPOLONES TOWARD CATECHOL-O-METHYL TRANSFERASE. The K_m for norepinephrine as substrate was 7×10^{-4} M.

Compound	$K \times 10^{-5}$
4-Methyltropolone (III)	1.2
Stipitatic acid (IV)	1.6
Colchiceine	1.8
β-Thujaplicin (V)	2.3
γ-Thujaplicin (VI)	2.5
4-(2-Amino-1-hydroxy)ethyltropolone (IX)	4.5
4-Acetamidomethyltropolone (XI)	3.1
7-Hydroxy-4-isopropyltropolone (XII)	3.4
1-Methylamino-7-methylimino-1,3,5-cycloheptatriene (XIII)	4.2
1-Methylamino-7-thioxo-1,3,5-cycloheptatriene (XIV)	5.6
Colchicine	∞
Pyrogallol	1.7
meso-Inositol	20.0

It was of interest to attempt the determination of the inhibition mechanism and we initially reported[2] that tropolones act noncompetitively. We now wish to withdraw this claim in view of the impossibility to construct reliable Lineweaver–Burk plots[26] with the data at hand. Even though it appeared possible initially to construct plots which are typical of noncompetitive inhibition,[2] it was observed subsequently that the substrate concentrations which had to be used were too close to maximum velocity conditions, so that large experimental errors were inadvertently introduced. On the basis of preliminary pharmacological data (see following), *in vivo* COMT blockade by tropolones has the characteristics of competitive rather than noncompetitive inhibition. We have since been informed by Dr. D'Iorio[27] that new *in vitro* data have clearly confirmed a competitive type of inhibition in agreement with the pharmacological data.

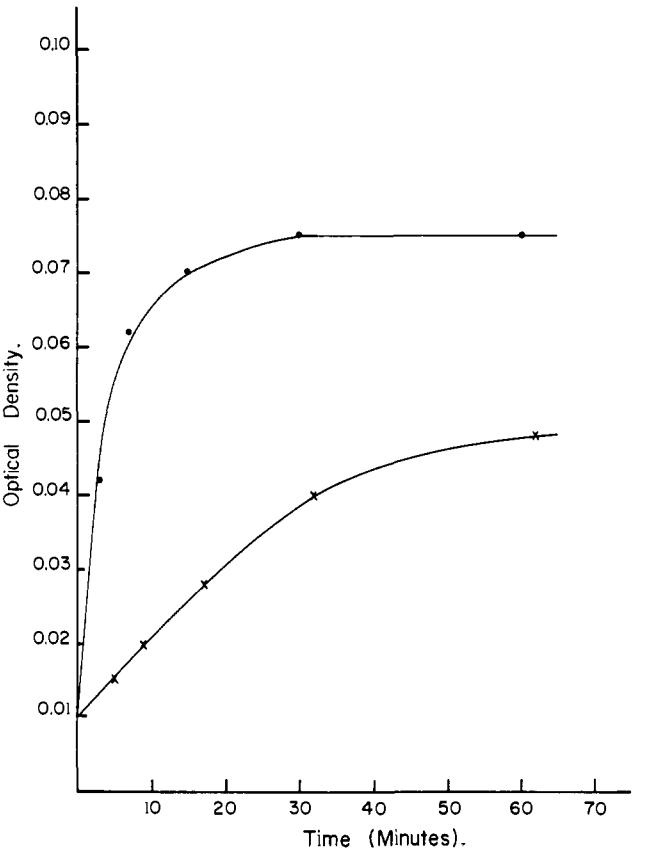


Fig. 1.—Typical uninhibited and inhibited (by 4-methyltropolone) rate curves for the COMT-catalyzed methylation of norepinephrine: ●, 1-noradrenaline-*d*-bitartrate 2×10^{-3} M; ×, 1-Noradrenaline-*d*-bitartrate 2×10^{-3} M plus 3×10^{-4} M 4-methyltropolone.

The possibility that tropolones could act by partially depriving COMT of its magnesium ions was examined next. To this end, the effect of magnesium concentration on COMT activity was first established and, in agreement with Axelrod's observations,[4] excess magnesium can be seen (Fig. 2) to produce a slight inhibitory effect on the rate of methylation. In the presence of 4-methyltropolone (III) a fivefold relative increase in magnesium concentration had no effect on the inhibited rate (Fig. 2). It seems clear therefore that magnesium does not reverse the inhibition, thus suggesting that tropolones form a complex with the enzyme active sites normally occupied by the substrate. An appreciable degree of specificity may therefore be expected from this novel class of inhibitors. That the active sites responsible for the binding of catechols

(23) K. J. Laidler, "The Chemical Kinetics of Enzyme Action," Oxford at the Clarendon Press, 1958, p. 81.

(24) (a) J. R. Crout, *Biochem. Pharmacol.*, **6**, 47 (1961); (b) W. R. Brasen, H. E. Holmquist, and R. E. Benson, *J. Am. Chem. Soc.*, **83**, 3125 (1961).

(25) A. Lindenbaum, M. R. White, and J. Schubert, *Arch. Biochem. Biophys.*, **52**, 110 (1954).

(26) H. Lineweaver and D. Burk, *J. Am. Chem. Soc.*, **56**, 658 (1934).

(27) Private communication; A. D'Iorio, C. Mavrides, and K. Missala, *Can. J. Biochem. Physiol.*, **41**, 1581 (1963). We are grateful to Dr. d'Iorio for having informed us of his result prior to publication.

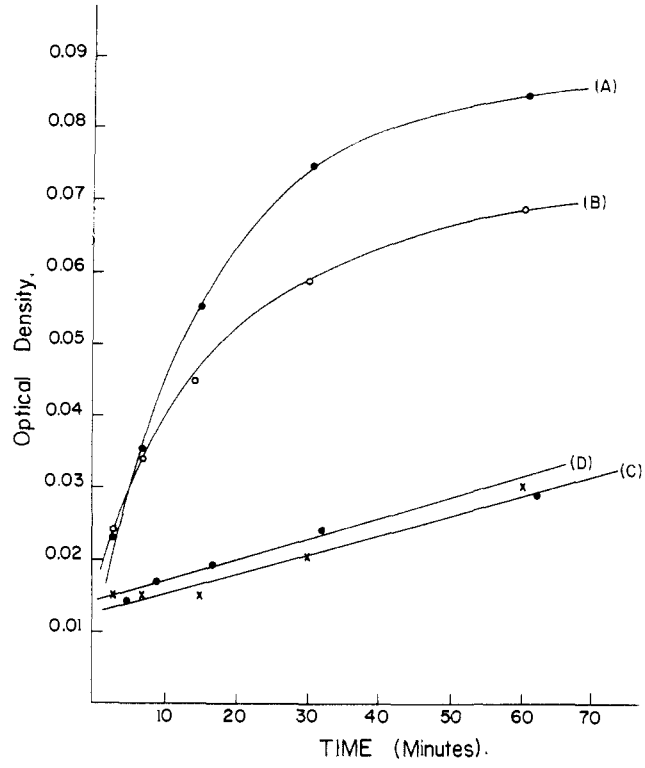
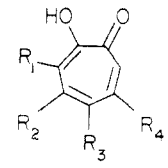


Fig. 2.—Curve A, l-norepinephrine D-bitartrate = 1×10^{-3} *M* and $MgCl_2 = 1 \times 10^{-2}$ *M*; curve B, same as A except 5×10^{-3} *M* $MgCl_2$; curve C, same as A except 0.5×10^{-2} *M* $MgCl_2$ and 0.5×10^{-4} *M* 4-methyltropolone; curve D, same as A except 5×10^{-3} *M* $MgCl_2$ and 1×10^{-4} *M* 4-methyltropolone.

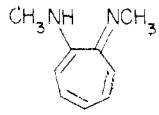
may be the same when tropolones are involved is evidenced by the observation that in both the substrate and inhibitor series, the presence of ring substituents produces parallel marginal variations on the affinity for COMT. It is not improbable therefore that a 1:1:1 complex is formed between COMT, magnesium, and inhibitor (II) as has been postulated for Michaelis complex formation with substrates I. The ability of tropolones to engage readily into charge-transfer types of complexes[28] suggests, on the other hand, that additional points of interaction with COMT may be created and this may partly account for the over-all affinity of tropolones for the enzyme. These *in vitro* studies with COMT and tropolones lead to the important conclusion that *catechol and tropolone rings are biochemically isosteric;* a new and potentially useful tool in the study of adrenergic receptor mechanisms may, therefore, be provided.

Some Pharmacological Characteristics of Tropolones.[29]—The *in vivo* blockade of COMT was initially demonstrated with compounds III, V, IX, and colchiceine. Cats were anesthetized and set up for recording nictitating membrane responses in the usual way. The compounds were administered intravenously as solutions in aqueous phosphate buffer (pH 7). Doses of 2 and 4 mg./kg. of inhibitor were administered followed 5 min. later by the injection of norepinephrine at doses of 1 to 5 μg.

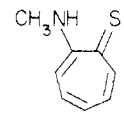
Compounds III, IX, and colchiceine produced a relatively small potentiation of the norepinephrine response



III, $R_1 = R_2 = R_3 = H$; $R_4 = CH_3$
IV, $R_1 = R_3 = H$; $R_2 = CO_2H$; $R_4 = OH$
V, $R_1 = R_2 = R_3 = H$; $R_4 = CH(CH_3)_2$
VI, $R_1 = R_2 = R_4 = H$; $R_3 = CH(CH_3)_2$
VII, $R_1 = R_2 = R_3 = H$; $R_4 = CHO$
VIII, $R_1 = R_2 = R_3 = H$; $R_4 = CHOHCH_2NO_2$
IX, $R_1 = R_2 = R_3 = H$; $R_4 = CHOHCH_2NH_2$
X, $R_1 = CO_2H$; $R_2 = CH_2CO_2H$; $R_3 = R_4 = H$
XI, $R_1 = R_3 = R_4 = H$; $R_2 = CH_2NHCOCH_3$
XII, $R_1 = OH$; $R_2 = R_4 = H$; $R_3 = CH(CH_3)_2$



XIII



XIV

at 2 mg./kg. but a readily detectable intensification and prolongation of the response at 4 mg./kg. The activity of β-thujaplicin (V) in this test was much smaller than would have been predicted on the basis of its *in vitro* activity. Colchicine was completely ineffective in the potentiation experiments, a result which parallels its total inactivity *in vitro*. This observation conflicts with the reports of du Chatelier[30,31] on the potentiating effect of colchicine on the blood pressure response of the dog to epinephrine. No interpretation was offered, however, for this observation. The possibility that colchicine may be demethylated to colchiceine in the dog but not in the cat is remote but not excluded. Possibly, du Chatelier's observations may be ascribed to the presence of colchiceine in the colchicine preparations that he used (crude colchicum extract being known to consist of a complex mixture of closely related analogs[32]) or else the potentiating effect is the consequence in the dog of an as yet unknown mechanism. The effect of the foregoing tropolones lasted approximately 2 hr. when 4-mg. doses were administered. This relatively short duration of action suggests a competitive inhibition type of mechanism *in vivo* (see the previous section).

Toxicity.—Preliminary toxicity data indicated that the nature of the substituents on the tropolone ring has a marked influence. For instance, in mice the LD_{50} (i.p.) for β-methyltropolone (III) and β-isopropyltropolone (V) was 535 mg./kg. and 85 mg./kg., respectively. This suggests that detoxification of the former may involve oxidation of the methyl substituent to a carboxyl group, a mechanism which has biochemical precedents.[33] Obviously, such an oxidative mechanism would hardly apply when an isopropyl substituent is involved. Insufficient quantities of the other tropolones precluded determination of their toxicity.

Some Effects of 4-Methyltropolone on Adrenergic Responses.—It has already been mentioned[14] that tropolones can act as β-receptor blocking agents. The

(28) W. von E. Doering and L. H. Knox, *J. Am. Chem. Soc.*, **73**, 828 (1951).

(29) Dr. Merle Pindell and his staff, Bristol Laboratories, Syracuse, N. Y., kindly performed some of the pharmacological tests.

(30) M. G. G. du Chatelier, *Therapie*, **15**, 1157 (1960).

(31) M. G. G. du Chatelier and A. Mugler, *ibid.*, **12**, 376 (1957).

(32) W. C. Wildman, "The Alkaloids," Vol. VI, edited by R. H. F. Manske, New York Academic Press 1960, Chapter 8.

(33) J. D. H. Slater, in "Progress in Medicinal Chemistry," G. P. Ellis and G. B. West, Editors, Butterworths, London 1961, p. 187.

activity spectrum of tropolones at that level is under study by Professor M. F. Murnaghan at the University of Ottawa and some of his results have been reported separately.[34] It may be pointed out at this time that the affinity of tropolones for adrenergic β-receptors is entirely consistent with the view that the interaction of catecholamines with the latter involves primarily the catechol ring rather than the cationic head of the molecule as was previously suggested.[35] *In vitro* studies of β-receptor blockade are complicated by a papaverine-like activity of 4-methyltropolone. It is pertinent to note that catechol itself shares this property[36] in agreement with the view that catechols and tropolones are pharmacologically isosteric.

Finally, attention may be drawn to the recent observations of Murnaghan and Mazurkiewicz,[34] who noted that high doses of 4-methyltropolone (50 mg./kg. or more) can protect mice for 2 hr. or more against lethal doses of epinephrine. Although this effect may be typical of α-receptor blockade,[37] it is too early to appraise the significance of these most interesting observations.

Experimental[38]

4-(2-Nitro-1-hydroxy)ethyltropolone (VIII).—Two grams of tropolone-4-carboxaldehyde[19] (VII) was dissolved in 50 ml. of methanol followed by the addition at 0° of 4 ml. of triethylamine and 10 ml. of nitromethane. After standing at 5° for 48 hr., the solution was acidified at 0° with 20% aqueous hydrochloric acid and evaporated to dryness *in vacuo* at room temperature. Trituration of the residue with cold water gave cream-colored crystals which when recrystallized from acetone–heptane had m.p. 140–141°, unchanged by further recrystallization; yield, 90%.

Anal. Calcd. for $C_9H_9NO_5$: C, 51.18; H, 4.26. Found: C, 51.20; H, 4.31.

4-(2-Amino-1-hydroxy)ethyltropolone (IX).—After numerous unsuccessful attempts at the selective reduction of the nitro group, the following procedure was ultimately found to be applicable.

A solution of 0.34 g. of the nitroalcohol VIII in 30 ml. of ethanol containing an equimolar amount of acetic acid was shaken in a hydrogen atmosphere at atmospheric pressure in the presence of 0.15 g. of 10% palladium-on-carbon. After 24 hr., hydrogen absorption had ceased; the mixture was worked up in the usual manner to yield an oil which was dissolved in methanol. Addition of dry ether caused the separation of a hygroscopic light yellow solid which was collected under dry nitrogen. The compound does not form well defined crystals. It had m.p. 145–155° dec., yield 0.250 g. Several attempts at the preparation of crystalline salts failed. The compound gave a deep blue color with ninhydrin at 100°. When chromatographed on paper (methanol–water–pyridine, 20:5:1), evidence for the presence of a small amount of an impurity was obtained. The ultraviolet spectrum (methanol) showed peaks at 350 and 245 mμ and was almost identical with that of 4-methyltropolone. In the infrared (liq. film) it showed ν_{max} 3200, 3450, 1600, and 1490 cm.$^{-1}$ In the n.m.r. (D_2O + DCl, acetonitrile as reference) it gave lines characteristic of aromatic protons at low field and a poorly resolved multiplet characteristic of the side chain protons at medium field. The areas under these regions of absorption were in the correct ratio of 4:3; in addition, lines characteristic of the presence of small amounts (~5%) of ethanol (of crystallization?) were observed.

Anal. Calcd. for $C_9H_{11}NO_3$: C, 59.66; H, 6.07; N, 7.73. Found: C, 59.06; H, 6.27; N, 7.45.

4-Acetamidomethyltropolone (XI).—The sequence described by Nozoe, *et al.*,[18] was applied using 3-carboxy-4-tropoloneacetic acid (X) as starting material. The product (XI) thus obtained had m.p. 146–147° (lit.[18] 146°); ν_{max}^{Nujol} 3300, 1630, and 1540 cm.$^{-1}$

Enzymatic Methods.—Catechol-O-methyl transferase (COMT) was prepared and purified as described by Axelrod and Tomchick.[4] For our specific purposes it was found unnecessary to carry the purification beyond the 6 hr. dialysis against phosphate buffer (pH 7).

Incubation Method.—The following technique was used throughout. A mixture of 5 ml. of the COMT preparation, 2 ml. of 0.5 *M* phosphate buffer, pH 7.9, 5 mg. of S-adenosylmethionine iodide, 400 mmoles of $MgCl_2 \cdot 6H_2O$, and 3 mmoles of the tropolone to be assayed was brought to a total volume of 11 ml. and the solution was pre-incubated at 37° in a Dubnoff metabolic shaker for 10 min. prior to the addition of 20 mmoles of norepinephrine D-bitartrate. Aliquots of 2 ml. were withdrawn at intervals and mixed with 1 ml. of 0.5 *M* borate buffer, pH 10. Controls were run and assayed simultaneously. The quenched aliquots were then processed for the assay of normetanephrine. The latter was first extracted as follows. Each quenched aliquot mixture was shaken 30 min. with 25 ml. of ethylene dichloride–isoamyl alcohol (98:2). After standing, 20 ml. of the clear organic phase was withdrawn and extracted for 33 min. with 3 ml. of 0.1 *N* hydrochloric acid. The acid extract was then assayed for normetanephrine by the nitrosonaphthol method of Udenfriend and Cooper.[22] It was established that tropolones, norepinephrine, and vanillin do not give a color by this method.

The same techniques were applied in the study of the effect of magnesium ion concentration on the inhibited rates.

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(38) Melting points were determined on a Kofler hot stage and are corrected. Microanalyses were by Miss E. Busk, Department of Chemistry, University of Ottawa. N.m.r. spectra were measured at 60 Mc./sec. with a Varian V-4302 spectrometer. Infrared spectra were determined with a Perkin-Elmer Infracord instrument; only the most characteric absorption bands are given.