Antihypertensive Agents II: Synthesis and Hypotensive Activity of Certain 1,4,5-Trisubstituted Pyrazoles

V. P. ARYA, R. S. GREWAL, C. L. KAUL, S. P. GHATE, D. V. MEHTA and T. GEORGE

Abstract □ The preparation of a series of 1,4,5-trisubstituted pyrazoles is described. These compounds have been screened for their hypotensive and adrenolytic properties. The antihypertensive activity of one of the compounds, 1-[5-methyl-1-(2-quinoxalyl)-4-pyrazolyl]-3-[4-(*o*-tolyl)-piperazinyl]-1-propanone hydrochloride has been studied in detail.

Keyphrases □ Antihypertensive agents—synthesis, activity □ 1,4,5-trisubstituted pyrazoles—synthesis □ Hypotensive, adrenolytic activity—1,4,5-trisubstituted pyrazoles □ UV spectrophotometry—structure □ NMR spectroscopy—structure

Pyrazoles have been associated with various types of pharmacodynamic properties (1), *e.g.*, 3,5-dimethyl pyrazole is a carbonic anhydrase inhibitor (2, 3), 2-(*N*-methyl-4-piperidyl)-3-amino-5-(4-pyridyl)-pyrazole hydrochloride (4) is a good renal vasodilator, and pyrazolyl guanidines (5) are known to cause hypotension, sedation, and psychomotor stimulation. The outstanding antihypertensive activity (6, 7) of 1-[5-methyl-1-phenyl-4-pyrazolyl]-3-[4-(*o*-tolyl)-piperazinyl]-1-propanone hydrochloride[1] and excellent CNS depressant properties (8, 9) of some of the related compounds prompted the authors to investigate this class of compounds in detail. In this article, the synthesis, hypotensive, and adrenolytic activity of a number of 1,4,5-trisubstituted pyrazoles are reported.

DISCUSSION AND RESULTS

The compounds listed in Table I were prepared by the condensation of heterocyclic hydrazines with ethoxymethylene acetylacetone. The reaction usually occurred in the cold, but in two cases, *i.e.*, Compounds VII and XI, the pyrazoles could only be obtained by a two-step process. The first step led to the formation of the substituted hydrazine. This hydrazine in its tautomeric form then cyclizes to afford the resulting 4-acyl pyrazoles.

The NMR spectrum ($CDCl_3$) of Compound II (Table I) was in complete agreement with the proposed structure. A singlet representing one proton at δ 9.5 can be assigned to C_3 proton of the quinoxaline nucleus. A multiplet representing (5H) at 7.68–8.20 is attributed to aromatic protons in quinoxaline and pyrazole. Two singlets at δ 3.10 and δ 2.52 each representing three protons are attributable to the methyl group at C_5 of the pyrazole nucleus and methyl of the acetyl group, respectively.

Mannich condensations of Compounds I–XII were carried out in boiling ethanol using paraformaldehyde and traces of hydrochloric acid. The usual reaction time was 24 hr. When 4-acetyl-1-(2-quinoxalyl)-5-methyl pyrazole (II) was condensed in ethanol with *N*-(*o*-tolyl)-piperazine hydrochloride in the usual conditions, the corresponding Mannich product (XXIV; see Table II) was obtained in 55% yield. The NMR spectrum of the base obtained from XXIV is consistent with the structure assigned to this product. For instance, the singlets at δ 2.28 and δ 3.08 each representing three protons can be assigned to the methyl of the *o*-tolyl group and that attached to C_5 of the pyrazole nucleus. The multiplets at δ 2.5–3.05 representing 12 protons, at δ 6.9–7.20 representing four aromatic protons, and at δ 7.6–8.15 representing another five protons are attributed to the six $-CH_2-$ groups, aromatic protons of the *o*-tolyl, and the pyrazole nuclei and quinoxaline, respectively. The singlet representing one proton at δ 9.52 is due to the proton at C_3 of the quinoxaline nucleus.

Sodium borohydride reduces XXIV to give the corresponding alcohol. The spectrum of this alcohol shows the presence of a hydroxy group at 3,190 cm.$^{-1}$. The carbonyl band present in XXIV at 1,675 cm.$^{-1}$ is absent in this compound. The NMR spectrum ($CDCl_3$) of the alcohol (XLII) is in agreement with its structure. The singlet at δ 9.58 representing one proton is due to the proton at C_3 of the quinoxaline nucleus. The multiplet at δ 1.85–2.20 integrating for two protons can be ascribed to $-CH_2-$ attached to the carbon carrying the secondary alcohol group. The quartet (J 4 c.p.s.) centered at δ 5.0 is assigned to the proton on the carbon carrying the secondary alcohol group. The proton of the alcohol is observed as a singlet at δ 5.86. The complex multiplets at δ 2.50–3.10 (13H), at δ 6.9–7.25 (4H), and at δ 7.5–8.18 (5H) are attributable to a combination of the pyrazolyl methyl and $>N-CH_2$ groups, aromatic protons of *o*-tolyl group, and other remaining aromatic protons of the compound, respectively. The singlet at δ 2.28 is ascribed to the methyl of the *o*-tolyl group. When the NMR was recharted 17 hr. after the addition of D_2O to the above solution, the singlet at δ 5.86 disappeared.

The alcohol (XLII) was dehydrated by warming with concentrated sulfuric acid. The dehydrated product had no hydroxyl band in the IR spectrum. It showed, however, a band at 1,655 cm.$^{-1}$ indicating the presence of $-CH=CH-$ conjugated with pyrazole nucleus.

When Mannich condensation of I with *N*-benzyl piperazine dihydrochloride and paraformaldehyde in the presence of traces of hydrochloric acid was carried out, the expected product (XLIV) was isolated in good yield. On catalytic debenzylation of XLIV, concomitant reduction of the carbonyl group to the corresponding alcohol (XLV) also occurred.

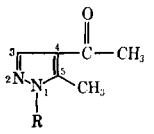
Mannich reaction of the pyrazole (VI) with *N*-carboethoxy piperazine hydrochloride gave the product (XLVI). However, when X was reacted with *N*-(*p*-fluorophenyl)-piperazine dihydrochloride, an unexpected product (XLVII), incorporating 2 moles of *N*-(*p*-fluorophenyl)-piperazine, was formed. The sequence of these reactions is described in Scheme I.

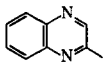
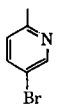
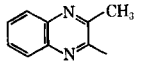
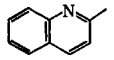
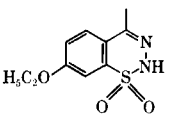
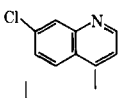
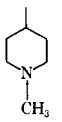
PHARMACOLOGICAL RESULTS

The hypotensive and adrenolytic activity of the various compounds investigated is shown in Table IV. The most active compound of the series was Compound XXIV. This compound was studied in greater detail. Compounds I–XII (Table I) and XLIV–XLVI (see *Experimental* section) were practically inactive. Compounds XX, XXXIV, XXXV, XXXIX, and XLVII showed moderate hypotensive response in experimental animals. The remaining compounds exhibited good hypoten-

[1] Ciba, 1002 Go.

Table I—4-Acetyl-1-heterocyclyl-5-methyl Pyrazoles



Compd.	R	Mol. Formula	Anal., % Calcd.	Anal., % Found	M.p.,[a] °C.	Yield,[b] %	IR Spectrum, cm.$^{-1}$, C=O
I[c]		$C_{11}H_{11}N_3O$	C, 65.67 H, 5.51 N, 20.88	C, 65.71 H, 5.59 N, 21.12	80	70[d]	1658
II[e]		$C_{14}H_{12}N_4O$	C, 67.02 H, 4.84 N, 22.11	C, 66.65 H, 4.79 N, 22.21	145	85[f]	1676
III[e]		$C_{11}H_{10}BrN_3O$	C, 47.16 H, 3.60 N, 15.00	C, 47.45 H, 3.61 N, 14.88	135	80[e]	1670
IV[g]		$C_{15}H_{14}N_4O$	C, 67.65 H, 5.30 N, 21.04	C, 67.51 H, 5.57 N, 21.41	125	80[f]	1662
V[g]		$C_{11}H_{11}N_3O$	C, 65.67 H, 5.51 N, 20.88	C, 65.53 H, 5.68 N, 20.89	70	55[d]	1660
VI[a]		$C_{15}H_{13}N_3O$	C, 71.69 H, 5.21 N, 16.72	C, 71.79 H, 5.32 N, 17.05	96	70[f]	1658
VII[a]		$C_{14}H_{12}N_4O$	C, 66.65 H, 4.79 N, 22.21	C, 66.61 H, 5.14 N, 22.08	185–186	55[f]	1660
VIII[h]		$C_{15}H_{13}N_3O$	C, 71.69 H, 5.21 N, 16.72	C, 71.36 H, 5.32 N, 16.50	134–138	80[e]	1668
IX[g]		$C_{15}H_{13}N_3O$	C, 71.69 H, 5.21 N, 16.72	C, 71.89 H, 5.32 N, 16.68	173	50[i]	1652
X[j]		$C_{15}H_{16}N_4O_4S$	C, 51.72 H, 4.63 N, 16.09	C, 51.78 H, 4.58 N, 16.68	172	87[k]	1655
XI[a]		$C_{15}H_{12}ClN_3O$	C, 63.05 H, 4.24 N, 14.71	C, 63.10 H, 3.87 N, 14.58	142–146	80[f]	1660
XII		$C_{11}H_{19}N_3O.HCl$	C, 55.91 H, 7.82 N, 16.30	C, 55.49 H, 7.88 N, 16.17	302 (dec.)	68[k]	1662

[a] Prepared by Method B. [b] Yields are of the products obtained from first crystallization. [c] Prepared by Method A using ether as solvent. [d] Recrystallized from hexane. [e] Recrystallized from ethanol. [f] Recrystallized from methanol. [g] Prepared by Method A using chloroform as solvent. [h] Prepared by Method A using tetrahydrofuran as solvent. [i] Recrystallized from methanol–isopropanol. [j] Prepared by Method A using dioxane as solvent. [k] Recrystallized from isopropanol–hexane.

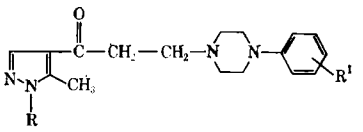
sive and adrenolytic activity. The adrenolytic activity of these compounds generally ran parallel to the hypotensive activity. Although most of the compounds showed adrenolytic properties as judged by the diminution or reversal of epinephrine pressor response, the effect on norepinephrine pressor response was small. The oral absorption of some of these compounds was good.

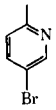
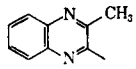
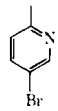
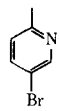
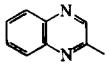
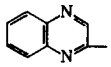
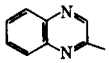
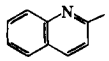
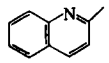
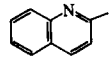
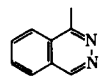
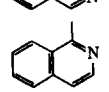
Compound XXIV produced a prolonged fall of blood pressure of 30–40 mm. Hg when given orally or intraintestinally at doses of 0.25–1 mg./kg. in pentobarbital-anesthetized cats and dogs. This compound, like many others, produced reversal of epinephrine response, blocked amphetamine pressor response, and produced no significant effect on the norepinephrine response. The reversal of epinephrine pressor response, without any marked effect on norepinephrine response, produced by this compound is of interest, as it shows that α-adrenergic block as judged by a diminution of norepinephrine pressor response, is not the sole factor involved in the phenomenon of epinephrine reversal. It is possible that sensitization of adrenergic β-receptors to epinephrine is involved. This has been shown in this laboratory in the case of a chemically related compound (10).

In chronic feeding experiments in dogs, when given at a dose of 7.5 mg./kg. p.o. for 12 days, the compound lowered the blood pressure by 30 mm. Hg. There was no significant effect on the responses of acetylcholine (5 mcg./kg.), angiotensin amide[a] (0.25 mcg./kg.), histamine (3 mcg./kg.), but amphetamine response was blocked and the heart rate was slightly reduced. In these experiments no change in the electrocardiogram was observed. At a dose of 1–3 mg./kg., this compound inhibited carotid occlusion pressor response. It also lowered the blood pressure of renal hypertensive rats by 45%

[a] Hypertensin, Ciba Pharmaceutical Co., Summit, N. J.

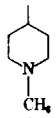
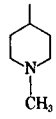
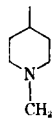
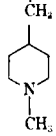
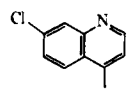
Table II—1-[5-Methyl-1-heterocyclyl-4-pyrazolyl]-3-[4-aryl-piperazinyl]-1-propanones



Compd.	R	R_1	Mol. Formula	Anal., % Calcd.	Anal., % Found	M.p.,[a] °C.	Yield,[b] %	IR Spectrum, cm.$^{-1}$, C=O
XIII		2-CH_3	$C_{23}H_{27}N_5O \cdot HCl$	C, 64.85 H, 6.62 N, 16.44	C, 64.94 H, 6.81 N 16.53	237	66[c]	1670
XIV		2-Cl	$C_{22}H_{24}ClN_5O \cdot HCl$	C, 59.19 H, 5.65 N, 15.69	C, 58.88 H, 5.43 N, 15.79	222	30[c]	1674
XV		4-F	$C_{22}H_{23}BrFN_5O \cdot HCl$	C, 51.93 H, 4.75 N, 13.76	C, 51.59 H, 5.18 N, 13.61	206	35[c]	1660
XVI		4-F	$C_{22}H_{24}FN_5O \cdot HCl \cdot 0.5H_2O$	C, 60.18 H, 6.20 N, 16.03	C, 60.51 H, 6.35 N, 15.78	215	33[d]	1662
XVII		4-F	$C_{26}H_{27}FN_6O \cdot 2HCl \cdot H_2O$	C, 56.82 H, 5.69 N, 15.29	C, 56.77 H, 5.82 N, 15.10	190	25	1665
XVIII		2-F	$C_{22}H_{24}FN_5O \cdot HCl$	C, 61.46 H, 5.86 N, 16.29	C, 61.29 H, 6.20 N, 16.18	220	56	1669
XIX		4-CH_3	$C_{23}H_{27}N_5O \cdot HCl$	C, 64.85 H, 6.62 N, 16.44	C, 64.43 H, 6.90 N, 16.12	206	56[c]	1671
XX		2-F	$C_{22}H_{23}BrFN_5O \cdot HCl$	C, 51.93 H, 4.75 N, 13.76	C, 51.89 H, 5.12 N, 13.40	230	55[e]	1662
XXI		3-Cl	$C_{22}H_{24}ClN_5O \cdot HCl$	C, 59.19 H, 5.65 N, 15.69	C, 59.49 H, 6.04 N, 15.42	208	45[c]	1663
XXII		2-OCH_3	$C_{23}H_{26}BrN_5O_2 \cdot 2HCl$	C, 49.56 H, 5.06 N, 12.57	C, 49.47 H, 5.20 N, 12.87	220	40[c]	1668
XXIII		2-CH_3	$C_{23}H_{27}N_5O \cdot 2HCl \cdot H_2O$	C, 57.70 H, 6.50 N, 14.57	C, 57.59 H, 6.66 N, 14.26	237	20[e]	1656
XXIV		2-CH_3	$C_{26}H_{28}N_6O \cdot HCl$	C, 65.45 H, 6.13 N, 17.61	C, 65.57 H, 6.23 N, 17.41	235	55[c]	1675
XXV		2-Cl	$C_{25}H_{25}ClN_6O \cdot HCl$	C, 60.36 H, 5.27 N, 16.80	C, 60.71 H, 5.48 N, 17.22	212	35[c]	1659
XXVI		4-F	$C_{25}H_{25}FN_6O \cdot HCl$	C, 62.42 H, 5.45 N, 17.47	C, 62.07 H, 5.66 N, 17.21	222	32[c]	1668
XXVII		2-OCH_3	$C_{27}H_{29}N_5O_2 \cdot HCl$	C, 65.89 H, 5.96 N, 14.23	C, 65.99 H, 5.96 N, 14.23	224–225	52[c]	1665
XXVIII		2-CH_3	$C_{27}H_{29}N_5O \cdot HCl$	C, 68.13 H, 6.35 N, 14.72	C, 68.51 H, 6.17 N, 14.35	233	51[c]	1665
XXIX		4-F	$C_{26}H_{26}FN_5O \cdot HCl$	C, 65.04 H, 5.67 N, 14.59	C, 64.97 H, 5.65 N, 14.33	225	36[c]	1657
XXX		2-CH_3	$C_{26}H_{28}N_6O \cdot HCl$	C, 65.45 H, 6.13 N, 17.62	C, 65.27 H, 6.29 N, 17.24	215	32[c]	1661
XXXI		2-CH_3	$C_{26}H_{28}N_6O \cdot HCl$	C, 65.89 H, 6.14 N, 14.23	C, 65.53 H, 5.90 N, 14.40	232	48[f]	1672

(*Continued on next page*)

Table II—(*Continued*)

Compd.	R	R_1	Mol. Formula	Anal., % Calcd.	Anal., % Found	M.p.,[a] °C.	Yield,[b] %	IR Spectrum, cm.$^{-1}$, C=O
XXXII		4-F	$C_{26}H_{26}FN_5O_2 \cdot HCl \cdot H_2O$	C, 62.70 H, 5.87 N, 14.06	C, 62.68 H, 6.06 N, 14.18	205	52[f]	1671
XXXIII		2-CH_3	$C_{27}H_{29}N_5O \cdot HCl$	C, 68.13 H, 6.35 N, 14.72	C, 67.67 H, 6.44 N, 14.38	237	45[c]	1673
XXXIV		2-CH_3	$C_{24}H_{35}N_5O \cdot 2HCl$	C, 59.74 H, 7.73 N, 14.52	C, 60.00 H, 7.48 N, 14.90	286	62[c]	1660
XXXV		4-F	$C_{23}H_{32}FN_5O \cdot 2HCl$	C, 56.79 H, 7.05 N, 14.40	C, 56.66 H, 6.95 N, 14.28	276	65[c]	1655
XXXVI		2-OCH_3	$C_{24}H_{35}N_5O_2 \cdot 2HCl$	C, 57.82 H, 7.48 N, 14.04	C, 57.39 H, 7.36 N, 14.02	263	66[c]	1652
XXXVII		2-Cl	$C_{23}H_{32}ClN_5O \cdot 2HCl$	C, 54.93 H, 6.81 N, 13.93	C, 54.47 H, 6.81 N, 13.49	282–283	65[c]	1652
XXXVIII		2-CH_3	$C_{27}H_{29}N_5O \cdot HCl \cdot 0.5H_2O$	C, 66.71 H, 6.44 N, 14.44	C, 66.62 H, 6.14 N, 14.60	222	32[e]	
XXXIX		2-CH_3	$C_{27}H_{29}ClN_5O \cdot HCl \cdot H_2O$	C, 62.30 H, 4.45 N, 13.46	C, 62.45 H, 4.89 N, 13.26	230	45[c]	

[a] All compounds melt with decomposition. [b] Yields are of the products from first crystallization. [c] Recrystallized from methanol. [d] Recrystallized from methanol–ethyl acetate. [e] Recrystallized from methanol–ether. [f] Recrystallized from chloroform.

when given at a dose of 30 mg./kg. p.o. for 10 days. The compound did not show a ganglion-blocking property. It produced powerful vasodilatation at a dose of 5–10 mcg./kg. when given intra-arterially, in perfused hind-limb preparation of the cat. These doses did not produce any effect on the systemic blood pressure.

Three hours after treatment (10 mg./kg. i.p.), a significant depletion of catecholamines from the rat heart (58%, $p < 0.001$) and brain (38%, $p < 0.05$) was observed. The compound produced an increase in heart rate, amplitude, and coronary flow in isolated perfused cat heart in doses of 10 to 100 mcg. The increase in the heart rate and amplitude was due to release of catecholamines from the stores as it was not seen in hearts from cats pretreated with reserpine. However, the coronary vasodilator effect was still present.

The compound showed antagonism to acetylcholine (ED_{50}, 0.99 mcg./ml.), histamine (ED_{50}, 0.33 mcg./ml.), and serotonin (ED_{50}, 1.46 mcg./ml.) in isolated guinea pig ileum.

EXPERIMENTAL[3]

Substituted Hydrazines—2-Hydrazino pyridine (11), 2-hydrazino-quinoxaline (12), 5-bromo-2-hydrazino pyridine (11), 2-hydrazino-3-methyl quinoxaline (12), 4-hydrazinopyridine (13), 2-hydrazinoquinoline (14), 1-hydrazino isoquinoline (15), 8-hydrazinoquinoline (16), 4-hydrazino-7-chloroquinoline (17), and 4-hydrazino-1-methyl-piperidine (18), were synthesized according to known methods.[4]

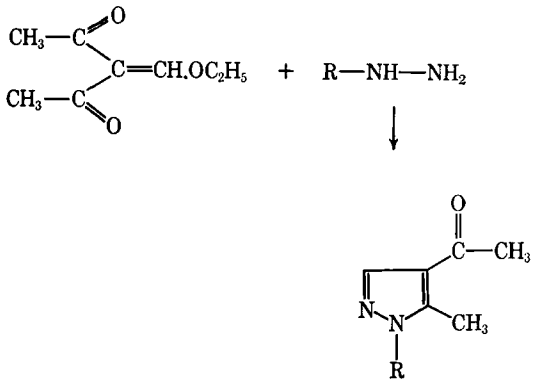
4-Acetyl-1-heterocyclyl-5-methyl Pyrazoles (I–XI, Table I) These compounds were synthesized by one of the two general methods.

Method A—A solution of ethoxymethylene acetylacetone (19) (0.05 mole) in an appropriate solvent (500 ml., see Table I) was cooled to 0° and a 5–10% solution of the appropriate hydrazine was added dropwise at 5° and was stirred at room temperature for 18 hr. The solvent was evaporated and the crystalline residue was recrystallized from a suitable solvent. This method is illustrated by the following example.

4-Acetyl-1-(2-quinoxalyl)-5-methyl pyrazole (II)—A solution of ethoxymethylene acetylacetone (7.8 g., 0.05 mole) *in chloroform* (50 ml.) was cooled to 0° and a solution of 2-hydrazinoquinoxaline (8 g., 0.05 mole) in chloroform (900 ml.) was added portionwise during 2 hr. at 5° and was stirred at room temperature for 18 hr. The solvent was evaporated off and the residue was crystallized from methanol to afford 10.6 g. II, m.p. 145°. UV, $\lambda_{max.}$ 234 mμ (log ε 4.33); 264 mμ (log ε 4.51); 330 mμ (log ε 4.14). NMR ($CDCl_3$) δ 2.52 (s,

[3] Melting points were determined in glass capillary tubes and are uncorrected. UV measurements were recorded on a Beckman DK-2 spectrophotometer using absolute ethanol as solvent. IR spectra were determined on a Perkin-Elmer model 421 spectrophotometer in mineral oil. NMR spectra were charted on a Varian A-60 using tetramethylsilane as the internal standard.

[4] 1-Hydrazinophthalazine hydrochloride and 1,2-dihydro-7-ethoxy-4-hydrazino-1,2,3-benzothiadiazine-1,1-dioxide were made available by the kind courtesy of Dr. Paul Schmidt, Ciba Ltd., Basle, Switzerland.



I-XII

I; R =

II; R =

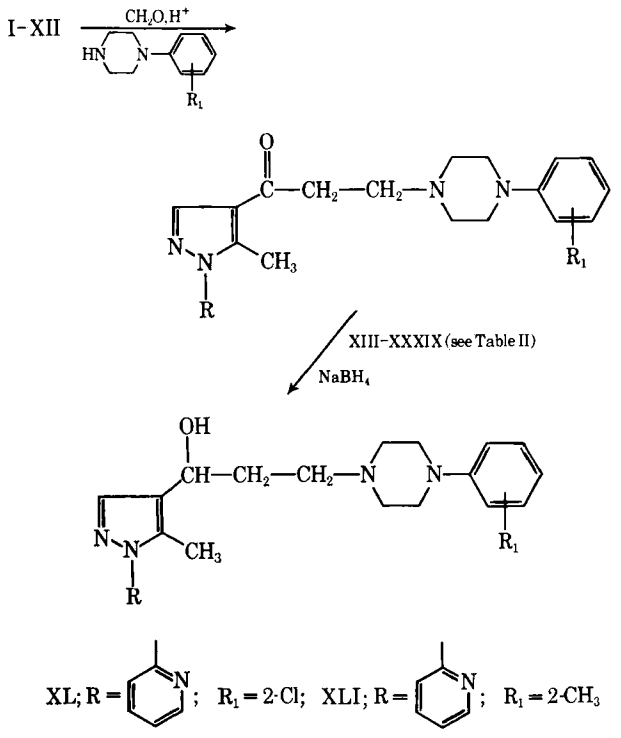
VI; R =

VII; R =

X; R =

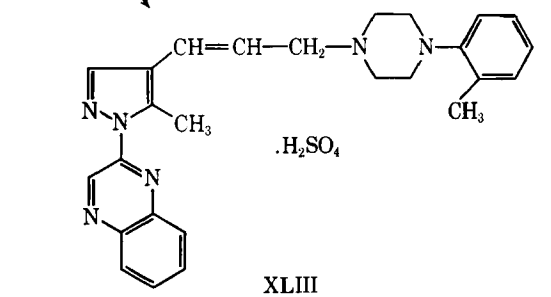
XII; R = .HCl

III-V; VIII-IX and XI; R = other heterocyclic substituents (see Table I).



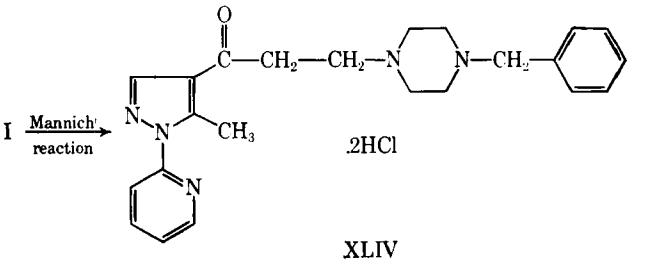
XL; R = ; R_1 = 2-Cl; XLI; R = ; R_1 = 2-CH_3

XLII; R = ; R_1 = 2-CH_3 (see Table III)



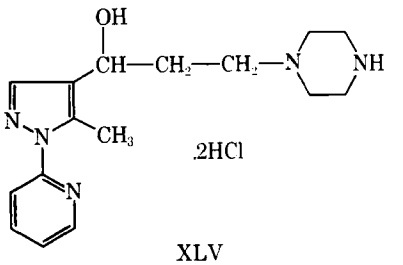
.H_2SO_4

XLIII



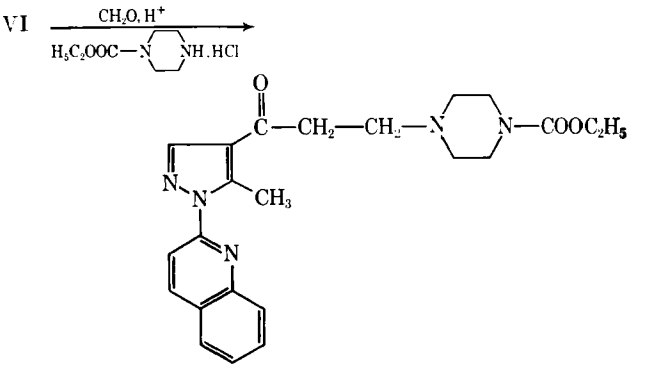
.2HCl

XLIV

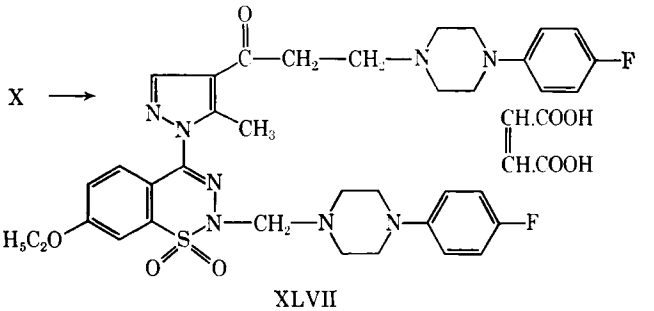


.2HCl

XLV



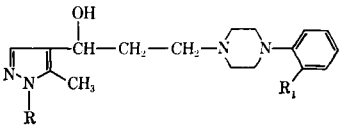
XLVI

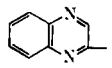


XLVII

Scheme 1

Table III—1-[5-Methyl-1-heterocyclyl-4-pyrazolyl]-3-[4-aryl-piperazinyl]-1-propanols



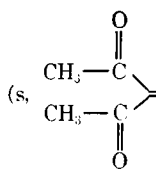
Compd.	R	R^1	Mol. Formula	Anal., % Calcd.	Anal., % Found	M.p.,[a] °C.	Yield,[a] %	IR spectrum, $cm.^{-1}$, OH
XL		Cl	$C_{22}H_{26}ClN_5O \cdot HCl$	C, 58.92 H, 6.07 N, 15.62	C, 58.57 H, 6.05 N, 15.69	210[b]	65[c]	3250
XLI		$-CH_3$	$C_{23}H_{29}N_5O \cdot HCl$	C, 64.55 H, 7.07 N, 16.37	C, 64.21 H, 6.68 N, 16.27	208[b]	65[c]	3200
XLII		$-CH_3$	$C_{26}H_{30}N_6O$	C, 70.56 H, 6.83 N, 18.99	C, 70.43 H, 6.93 N, 18.76	117–119	80[d]	3190

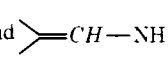
[a] Yields are of the products from the first crystallization. [b] The compound melts with decomposition. [c] Recrystallized from a mixture of methanol–ether. [d] Recrystallized from methylene chloride–hexane.

–C–CH_3, 3H), 3.10 (s, $\wedge CH_3$, 3H), δ 7.68–8.20 (m, aromatic, 5H), 9.50 (s, C_3 proton of quinoxaline, 1H).

Method B—This method is shown by the synthesis of the compound VII given below.

4-Acetyl-5-methyl-1-(1-phthalazinyl)-pyrazole (VII)—A solution of ethoxymethylene acetylacetone (27 g.) in dry tetrahydrofuran (200 ml.) is cooled to 0° and treated portionwise with a cooled solution of 1-hydrazinophthalazine (27 g.) in dry tetrahydrofuran (600 ml.). The addition of the latter was complete after 2 hr. and the reaction mixture was stirred for 18 hr. at room temperature. The yellow crystalline material formed was filtered off and recrystallized from chloroform to afford the resulting intermediate compound which melted at 183°, NMR (CF_3COOH), δ 2.87


(s, 6H), δ 8.5–9.06 (m, 4-aromatic protons and


, 5H), δ 10.43 (s, C_4-proton of phthalazine, 1H).

Anal.—Calcd. for $C_{14}H_{14}N_4O_2$: C, 62.21; H, 5.22; N, 20.73. Found: C, 62.46; H, 5.11; N, 20.71.

Ten grams of the above intermediate compound was heated to 190° under an atmosphere of nitrogen for 6 hr. On cooling to room temperature, the product was recrystallized from methanol to afford colorless needles of the compound (VII).

NMR ($CDCl_3$): o 2.57 (s, –C–CH_3, 3H) o 2.73

(s, CH_3, 3H), δ 7.90–8.26 (m, aromatic, 5H), 9.63 (s, C_4-proton of phthalazine, 1H).

4-Acetyl-1-(1-methyl-4-piperidyl)-5-methyl-pyrazole Hydrochloride (XII)—A solution of ethoxymethylene acetylacetone (16.2 g.) in dry dioxane (50 ml.) was treated dropwise with a solution of 1-methyl-4-hydrazino piperidine (13.2 g.) in dry dioxane (100 ml.) during 2 hr. at 5°. The reaction mixture was stirred for 18 hr. at room temperature. The solvent was evaporated *in vacuo* and the residual red oil was filtered through a column of neutral alumina (300 g.) using benzene as solvent. The eluate (18 g.) was dissolved in isopropanol (20 ml.) and treated with 5 *N* solution of dry hydrogen chloride in isopropanol to make pH 1 and ether (20 ml). A crystalline precipitate was formed. This was filtered and recrystallized from a mixture of methanol and isopropanol to afford the title compound, 16 g., m.p. 302° (dec.). UV $\lambda_{max.}$: 246 mμ (log ε 4.04).

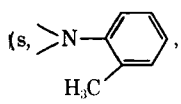
N-Arylpiperazines—*N*-(*o*-Tolyl)-piperazine hydrochloride, *N*-(*o*-methoxyphenyl)-piperazine hydrochloride, *N*-(*m*-chlorophenyl)-piperazine dihydrochloride, and *N*-(*p*-tolyl)-piperazine dihydrochloride were synthesized by known methods. *N*-(*o*-Fluorophenyl)-piperazine hydrochloride, m.p. 180°, was synthesized by the method of Mull *et al.* (20).

Anal.—Calcd. for $C_{10}H_{13}FN_2 \cdot HCl$: C, 55.42; H, 6.51; N, 12.93. Found: C, 55.33; H, 6.78; N, 12.91.

N-(*p*-Fluorophenyl)-piperazine dihydrochloride, m. p. 241° was also synthesized by the method of Mull *et al.* (20).

Anal.—Calcd. for $C_{10}H_{13}FN_2 \cdot 2HCl$: C, 46.33; H, 5.14; N, 11.04. Found: C, 46.30; H, 5.09; N, 11.01.

1-[5-Methyl-1-(2-quinoxalyl)-4-pyrazolyl]-3-[4-(o-tolyl)-piperazinyl]-1-propanone Hydrochloride (Table II; XXIV)—*General Procedure*—A mixture of 4-acetyl-5-methyl-1-(2-quinoxalyl)-pyrazole (5.04 g.; 0.02 mole) and paraformaldehyde (1.8 g.) in ethanol (70 ml.) was treated with *N*-(*o*-tolyl)-piperazine hydrochloride (5 g.) and 4 drops of concentrated hydrochloric acid and was boiled under reflux for 24 hr. At the end of this time, the contents were cooled in a refrigerator and the product thus separated was collected on a filter and recrystallized three times from methanol to afford XXIV, m.p. 235° (dec.). UV, $\lambda_{max.}$ 238 mμ (log ε 4.35); 267 mμ (log ε 4.44); 331 mμ (log ε 4.06). The base (XXIV*a*) was prepared as follows: a solution of XXIV (1 g.) in 50% aqueous methanol (100 ml.) was treated with a saturated solution of sodium bicarbonate (10 ml.). A crystalline precipitate was formed. This was filtered and recrystallized from a mixture of methylene chloride and *n*-hexane, m.p. 134–135°. UV, $\lambda_{max.}$: 238 mμ (log ε 4.37); 267 mμ (log ε 4.43); 333 mμ (log ε 4.11) IR: 1,678 $cm.^{-1}$; NMR($CDCl_3$): δ 2.28


(s, 3H); 2.5–3.05 (m, $-CH_2-$, 12H);

3.08
(s, CH_3, 3H), 6.9–7.20 [m, aromatic, (*o*-tolyl), 4H], δ 7.6–8.15 (m, aromatic, 5H), δ 9.52 (s, C_3 proton of quinoxaline).

Anal.—Calcd. for $C_{26}H_{28}N_6O$: C, 70.88; H, 6.41; N, 19.08. Found: C, 70.56; H, 6.34; N, 19.52.

The base (XXIV*a*) formed a maleate which was recrystallized from methanol, m.p. 176–177°.

Table IV—Hypotensive Activity of Certain 1,4,5-Trisubstituted Pyrazoles

Compd. No.	Hypotensive Activity[a] Cat	Dog	Effect on Pressor Response to Epinephrine	General Remarks
I	0[b]	−[c]	0	—
II	0	−	−	Lethal at 9 mg./kg. i.v.[d]
III	−	0	+	
IV	−	0	0	
V	0	−	0	
VI	0	−	−	Lethal at 9 mg./kg. i.v. due to respiratory failure.
VII	−	0	0	—
VIII[a]	0	−	−	Lethal at 9 mg./kg. p.o.
IX	−	0	Slight Potentiation	
X	0	−	0	
XI	−	0	Potentiated	
XII	−	+[e]	+	
XIII	+++[f]	+++	+++ (dog) +++ (reversed in cat)	Fair oral absorption. Respiratory depression in cats at 9 mg./kg. i.v.
XIV	+++	+++	+++ (dog) [g] ++ (reversed in cat)	Fair oral absorption. Respiratory depression in cats at 9 mg./kg. i.v.
XV	++	+++	++ (dog) ++ (reversed in cat)	Poor oral absorption.
XVI	+	+++	+++ (dog) ++ (reversed in cat)	Poor oral absorption.
XVII	+++	+++	+++ (dog) +++ (cat)	Good oral absorption.
XVIII	+++	−	+++ (reversed)	Marked tachyphylaxis with 3 and 9 mg./kg. i.v.
XIX	++	−	+ (reversed)	
XX	+	−	+	
XXI	+	+	+++ (dog) +++ (reversed in cats)	Good oral absorption.
XXII	++	−	++ (reversed)	
XXIII	+++	−	+++ (reversed)	Good oral absorption.
XXIV	++++[h]	++++	Reversed	Very good oral absorption.
XXV	++	+++	+++ (dog) ++ (reversed in cats)	Poor oral absorption.
XXVI	++	−	++	Lethal at 9 mg./kg. due to respiratory depression.[d] Slight sedation at 250 mg./kg. p.o. in mice.
XXVII	−	+++	+++	Tachyphylaxis observed at 3 and 9 mg./kg. Orally inactive. Sedation at 250 mg./kg. p.o. in mice.
XXVIII	−	++	+	Tachyphylaxis observed.
XXIX	−	++	++	Orally inactive. Sedation at 250 mg./kg. p.o.
XXX	+++	+++	+++	Poor oral absorption.
XXXI	+++	+++	+++ (dog) +++ (reversed in cat)	Poor oral absorption.
XXXII	−	++	++	
XXXIII	−	++	+	
XXXIV	+	+	+ (dog) + (reversed in cat)	
XXXV	+	+	+ (dog) + (reversed in cat)	
XXXVI	++	+	++ (dog) ++ (reversed in cat)	
XXXVII	++	−	++ (reversed)	
XXXVIII	++	−	+++ (reversed)	Poor oral absorption.
XXXIX	−	+	+	
XL	+++	+++	++ (reversed in cat)	Good oral absorption.
XLI	+++	+++	0 (dog) 0 (cat)	Lethal in one dog at 3 mg./kg.. Fall of blood pressure associated with cardiac slowing. Good oral absorption.
XLII	++	−	++ (reversed)	—

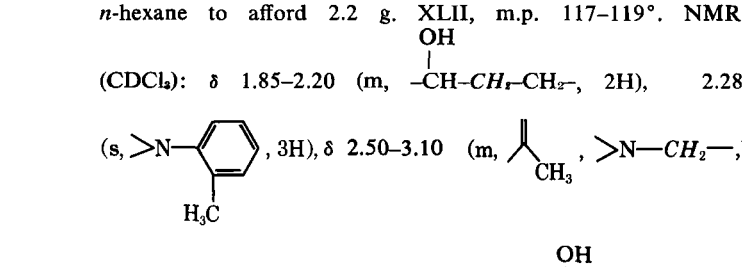
[a] By hypotensive activity is meant when the fall of blood pressure was more than 20 mm. Hg and for more than 15 min. [b] 0, no activity. [c] −, not done. [d] Lethal dose in anesthetized animal. [e] +, activity at 9 mg./kg. i.v. [f] +++, activity at 1 mg./kg. i.v. [g] ++, activity at 3 mg./kg. i.v. [h] ++++, activity at less than 1 mg./kg. i.v.

Anal.—Calcd. for $C_{26}H_{28}N_6O$. $C_4H_4O_4$: C, 64.73; H, 5.80; N, 15.10. Found: C, 64.44; H, 5.57; N, 15.26. The base (XXIV*a*) also formed a methanesulfonate monohydrate which was recrystallized from methanol, m.p. 205–206° (dec.).

Anal.—Calcd. for $C_{26}H_{28}N_6O.CH_3SO_3H.H_2O$: C, 58.47; H, 6.18; N, 15.15. Found: C, 58.74; H, 6.57; N, 15.09.

The compounds (XIII–XXXIX) listed in Table II were synthesized by the general method described for XXIV.

1-[5-Methyl-1-(2-quinoxalyl)-4-pyrazolyl]-3-[4-(o-tolyl)-piperazinyl]-1-propanol (XLII)—A solution of XXIV (3 g.) in 50% aqueous methanol (150 ml.) was added dropwise to a stirred solution of sodium borohydride (0.3 g.) in 50% aqueous methanol during 1 hr. The reaction mixture was stirred for another 1 hr. at room temperature and then at 70° for 4 hr. The solution was then concentrated and extracted with chloroform. The dried chloroform extract was evaporated and the

residue was crystallized from a mixture of chloroform and *n*-hexane to afford 2.2 g. XLII, m.p. 117–119°. NMR (): δ 1.85–2.20 (m, –CH–CH_2–CH_2–, 2H), 2.28 (s, >N–, 3H), δ 2.50–3.10 (m, , >N–CH_2–, 13H), quartet (J4 c.p.s.) centered at δ 5.0 (–CH–CH_2–, 1H), δ 5.86 (s, –CH–, 1H), δ 6.9–7.25 [m, aromatic, (*o*-tolyl), 4H], δ 7.5–8.18 (m, aromatic, 5H), δ 9.58 (s, C_3 proton of quinoxaline, 1H). Seventeen hours after addition of D_2O to the above solution, the singlet at δ 5.86 disappeared.

1-[5-Methyl-1-(2-quinoxalyl)-4-pyrazolyl]-3-[4-(o-tolyl)-piperazinyl]-prop-1-ene Sulfate (XLIII)—A solution of XLII (2 g.) in concentrated sulfuric acid (20 ml.) was warmed on a steam bath for 1 hr. and allowed to stand at room temperature for 3 hr. It was then poured on crushed ice (50 g.) when a crystalline precipitate was formed. This was filtered and recrystallized from methanol–ether to afford XLIII, m.p. 208°. UV, $\lambda_{max.}$ 252 mμ (log ε 4.49); 342 mμ (log ε 4.15); 351 mμ (log ε 4.14).

Anal.—Calcd. for $C_{28}H_{28}N_6 \cdot H_2SO_4$: C, 59.76; H, 5.79; N, 16.08. Found: C, 59.91; H, 5.86; N, 16.38.

1-[5-Methyl-1-(2-pyridyl)-4-pyrazolyl]-3-[4-benzyl piperazinyl]-1-propanone Dihydrochloride (XLIV)—A solution of 4-acetyl-5-methyl-1-(2-pyridyl)-pyrazole (I, 4.02 g., 0.02 mole), paraformaldehyde (3.2 g.) in absolute ethanol (75 ml.) was treated with *N*-benzyl piperazine dihydrochloride (5.0 g.) and concentrated hydrochloric acid (5 drops) and was boiled under reflux for 24 hr. It was allowed to stand at room temperature for 60 hr. when a crystalline product separated which was collected on a filter and recrystallized from methanol to give colorless needles of XLIV (6 g.), m.p. 255° (dec.). IR: 1,655 cm.$^{-1}$ (carbonyl).

Anal.—Calcd. for $C_{23}H_{27}N_5O.2HCl$: C, 59.73; H, 6.32; N, 15.17. Found: C, 59.51; H, 6.72; N, 15.08.

1-[5-Methyl-1-(2-pyridyl)-4-pyrazolyl]-3-[1-piperazinyl]-1-propanol Dihydrochloride (XLV)—A solution of XLIV (3.5 g.) in methanol (150 ml.) was hydrogenated over Adams platinum oxide catalyst (0.2 g.) in a Parr apparatus at 40° at 40 lb./in. pressure. After the theoretical uptake of hydrogen, the catalyst was filtered off and the filtrate was evaporated to dryness. The residue was crystallized from a mixture of isopropanol and ether to afford XLV (2 g.), m.p. 209–210° (dec.). IR: 3,400 cm.$^{-1}$ (hydroxyl).

Anal.—Calcd. for $C_{16}H_{19}N_5O.2HCl$: C, 53.34; H, 5.88; N, 19.45. Found: C, 53.52; H, 6.13; N, 18.98.

1-[5-Methyl-1-(2-quinolyl)-4-pyrazolyl]-3-[4-carboethoxypiperazinyl]-1-propanone Hydrochloride (XLVI)—A solution of 4-acetyl-5-methyl-1-(2-quinolyl)-pyrazole (7.53 g.; 0.03 mole) and paraformaldehyde (2.7 g.) in absolute ethanol (75 ml.) was treated with *N*-carboethoxypiperazine hydrochloride (5.85 g., 0.03 mole) and 5 drops of concentrated hydrochloric acid. The reaction mixture was boiled for 18 hr. and cooled to room temperature when a crystalline precipitate was formed. This was recrystallized from methanol to afford 7.21 g. (XLVI), m.p. 213–215°, IR: 1,700 cm.$^{-1}$ (ester carbonyl), 1,670 cm.$^{-1}$ (carbonyl).

Anal.—Calcd. for $C_{28}H_{27}N_5O_3.HCl$: C, 60.33; H, 6.16; N, 15.29. Found: C, 60.64; H, 6.06; N, 14.86.

1-{5-Methyl-[1,2-dihydro-1,1-dioxido-7-ethoxy-2-(4-p-fluorophenyl piperazino-methyl)-1,2,3-benzothiadiazin-4-yl]-4-pyrazolyl}-3-[4-(p-fluorophenyl)-piperazinyl]-1-propanone Maleate (XLVII)—A mixture of 4-acetyl-1-(1,2-dihydro-1,1-dioxido-7-ethoxy-1,2,3-benzothiadiazin-4-yl)-5-methyl pyrazole (2.56 g.) and paraformaldehyde (1.3 g.) in ethanol (50 ml.) was treated with *N*-(*p*-fluorophenyl)-piperazine dihydrochloride (5.1 g.) and 4 drops of concentrated hydrochloric acid and was boiled under reflux for 24 hr., then evaporated to dryness. The residue was dissolved in water. The aqueous solution was washed with ether and basified with a 10% solution of sodium carbonate. The liberated base was extracted with ether and dried over anhydrous sodium sulfate and evaporated to dryness. The residue was dissolved in ether and treated with an ether solution of maleic acid (1.2 g.). A crystalline precipitate was formed which was filtered off and recrystallized from a mixture of isopropanol and ether to yield XLVII as colorless plates (1.8 g.) m.p. 161° (dec.), IR: 1,650 cm.$^{-1}$ (carbonyl).

Anal.—Calcd. for $C_{37}H_{42}F_2N_6O_4S.C_4H_4O_4$: C, 57.99; H, 5.46; N, 14.31. Found: C, 58.02; H, 5.58; N, 14.78.

PHARMACOLOGICAL EXPERIMENTS

Effect on Blood Pressure—Dogs and cats of either sex were anesthetized with pentobarbitone, 35 mg./kg. (i.v.) and 45 mg./kg. (i.p.), respectively. The blood pressure was recorded from the carotid artery. The effects of various compounds were investigated by intravenous and intraintestinal routes. In the case of chronic normotensive dogs the animals were given 7.5 mg./kg. (p.o.)/day for 12 days. The ECG, blood pressure, and response to a number of drugs were investigated before and after treatment of the compound.

Renal Hypertensive Rats—These rats were prepared according to the method of Goldblatt (21). They were given 30 mg./kg. p.o. of the compound once a day for 10 days. The blood pressure was measured by plethysmographic method from the tail of a rat under light ether anesthesia.

Effect on Centrally Mediated Reflex—The effect on centrally mediated reflex (carotid occlusion) was observed in anesthetized cats.

Isolated Perfused Heart—Langendorf's method was used for the isolated perfused heart. The effect of the compound on heart rate, amplitude, and coronary flow was observed after treatment with various compounds. In some experiments reserpine was given 2.5 mg./kg. (i.p.) on two consecutive days and the hearts were perfused on the third day.

Isolated Perfused Hind Limbs of the Cat—Cats were perfused with a sigmamotor pump. The blood was taken from the abdominal aorta and pumped into the hind limb. Both the carotid blood pressure and perfusion pressure were recorded through a mercury manometer. The injections were made intra-arterially.

Effect on the Nictitating Membrane of the Cat—The contractions of the nictitating membrane were elicited by stimulation of the pre- and postganglionic fibers of the cervical sympathetic chain (3.2 v., 32 cycles/sec., 0.46 duration for 10 sec.).

Catecholamine Estimations—The tissues were extracted with 2% PCA adsorbed on acid-washed alumina and eluted with 0.2 *N* acetic acid as described by Crout *et al.* (22). The total catecholamine was estimated as norepinephrine on the blood pressure of a pithed rat.

Antiacetylcholine, Antihistamine, and Antiserotonin Activity—These were measured using isolated guinea pig ileum suspended in Tyrode's solution at 37°. Acetylcholine, serotonin, and histamine were used as agonist. Different concentrations of the antagonist were then added to get 20–80% inhibition. The ED_{50} was then calculated from the graph.

CONCLUSION

It has been found that 1,5-disubstituted 4-acetyl pyrazoles (Compounds I–XII, see Table I) are generally devoid of hypotensive or adrenolytic activity. Mannich bases derived from them as well as reduction products of these ketones are active. Thus, a three-carbon chain between the pyrazole and *N*-aryl piperazine is required for hypotensive and adrenolytic activity. In the *N*-aryl piperazine moiety, the replacement of *N*-aryl nucleus with a *N*-benzyl or *N*-carboethoxy group results in total loss of activity. The compound without the aryl substituent on the piperazine is also inactive.

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Inhibitory Effects of Detergents in Membrane Filters

S. A. ROSENBLUTH and G. W. CRIPPS

Abstract □ Aqueous filtrates from membrane filters contain material(s) with UV spectral characteristics quite similar to those of a nonionic isooctyl phenoxy polyethoxy ethanol (IPPE)-type detergent. A permeability test utilizing mammalian cell cultures was employed to evaluate the inhibitory properties of the extract and commercial IPPE. The cell culture test was sensitive to IPPE in concentrations as low as 0.006 percent and produced comparable results with extract concentrations having spectral absorbance (283 mμ) values equal to those of IPPE. Dose-response patterns were markedly similar. Spectral analyses of successive aqueous filtrates were used to study the extractability of the offending agent(s). Treatment of membrane filters with hot water prior to autoclaving rendered the filters safe for sterilization of solutions including cell culture media components.

Keyphrases □ Detergents in membrane filters □ Membrane filter extracts, effect—cell cultures □ Cell cultures, mammalian—filter extract inhibition □ UV spectrophotometry—identification

The presence of water-extractable detergents in membrane filters has concerned a number of investigators. Cahn noted persistent foams in solutions which were membrane-filtered and reported reductions in plating efficiency and degree of differentiation in cultured cells when medium was filtered through unwashed membranes (1). He further suggested that this type filter contains 2 to 3% by weight of water-soluble material(s) which may include an isooctyl phenoxy polyethoxy ethanol [1] (IPPE) or similar detergents. Such leached agents might not be detected when media contain solubilized protein (1), when large volumes of filtrate dilute the contaminant (2), or when relatively insensitive methods are employed. Conversely, problems may be anticipated with small volumes of filtered fluids, defined media, and/or sensitive biological and chemical tests.

Membrane filters are routinely used in the authors' laboratories for sterilization of various solutions including tissue culture media components. The purpose of this paper was to: (*a*) establish the patterns of extraction of detergent(s) by repeated filtration of volumes of hot and cold water; (*b*) quantitize certain inhibitory effects to mammalian cell cultures by aqueous

[1] Trademarked as Triton X-100, Rohm & Haas Co., Philadelphia, Pa. The material used in the present study bore the lot number 1984 and was obtained by courtesy of Mr. T. S. Rowland.