Journal of Medicinal Chemistry, 1972, Vol. 15, No. 9 891

Synthesis and Pharmacological Properties of 3-(10,11-Dihydro-5*H*-dibenzo[*a,d*]cyclohepten-5-ylidene)-1,2-dialkylpyrrolidine Derivatives

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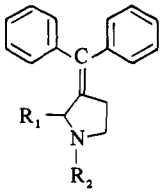
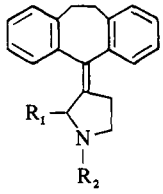
A series of 1,2-disubstituted 3-(10,11-dihydro-5*H*-dibenzo[*a,d*]cyclohepten-5-ylidene)pyrrolidines was prepared from 10,11-dihydro-5*H*-dibenzo[*a,d*]cyclohepten-5-one in four steps. The antiacetylcholine activity of these compounds in the isolated guinea pig ileum and the preventive effect against tremorine-induced tremor and salivation were investigated. Some of them had potent antiacetylcholine activity *in vitro* and prevented tremorine-induced temor and salivation in mice. These compounds were comparable to atropine and trihexyphenidyl in antitremor activity, but less effective in antisalivation.

The authors previously reported the antiacetylcholine activity of a series of 3-diphenylmethylenepyrrolidine derivatives (I),[1] which had been synthesized by Ohki, *et al.*,[2] and subsequently prifinium bromide was introduced as a spasmolytic.[3-5] We have extended this work and investigated derivatives represented by II.

In the present paper, the authors deal with the synthesis and pharmacological properties of 1,2-disubstituted derivatives of 3-(10,11-dihydro-5*H*-dibenzo[*a,d*]cyclohepten-5-ylidene)pyrrolidine (II).[6]† Some of these compounds were found to have remarkable pharmacological activities, represented by a potent antagonism against acetylcholine-induced contractions of the isolated guinea pig ileum and against tremorine-induced tremor in mice. The latter antagonism serves as a clue to the screening of antiparkinson drugs in experimental animals.



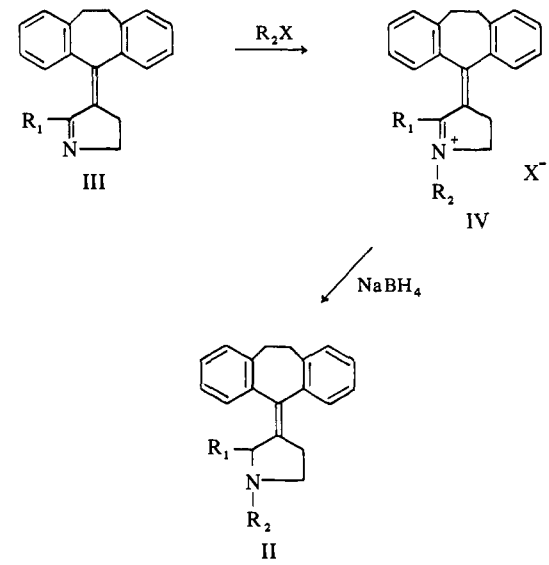
I



II

Synthesis. 3-(10,11-Dihydro-5*H*-dibenzo[*a,d*]cyclohepten-5-ylidene)-1,2-dialkylpyrrolidine (II) was prepared by alkylation of the related 1-pyrroline (III) with an alkyl halide, followed by $NaBH_4$ reduction of the resulting pyrrolinium halide (IV) in the usual way (Scheme I).[2,7] III, as a key intermediate for preparing II, was synthesized by the two following alternative methods. One was that of Ohki, *et al.*,[2] in which 3-diphenylmethylene-2-methyl-1-pyrroline was prepared by treating 4,4-diphenyl-3-butenyl bromide or its precursors[2],‡ with acetonitrile in the presence of stannic chloride. Another was that of Sugasawa,[8] in which *N*-(4-phenyl-3-butenyl)acetamide was cyclized to give 3-benzylidene-2-methyl-1-pyrroline under conditions of the Bischler–Napieralsky reaction. Three different starting materials for these reactions, *i. e.*, 5-(3-hydroxypropyl)-10,11-dihydro-5*H*-dibenzo[*a,d*]cyclohepten-5-ol (VI),[9] 5-cyclopropyl-10,11-dihydro-5*H*-dibenzo[*a,d*]cyclohepten-5-ol (VII),[10,11] and 5-(3-benzyloxypropylidene)-10,11-dihydro-5*H*-dibenzo[*a,d*]cycloheptene (VIII),[12] were prepared by the reaction of 10,11-dihydro-5*H*-dibenzo[*a,d*]cyclohepten-5-one (V) with

Scheme I



III IV II

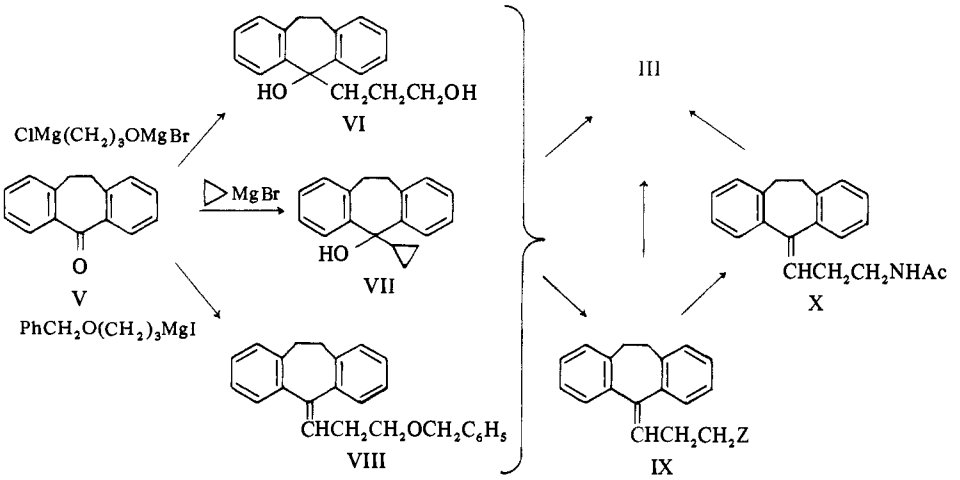
appropriate Grignard reagents as shown in Scheme II in *ca.* 100%, *ca.* 100%, and 76% yields, respectively. When these products were allowed to react with appropriate acids, (10,11-dihydro-5*H*-dibenzo[*a,d*]cyclohepten-5-ylidene)propyl halides or related alcohols (IX, Z = halogen or OH) were obtained.[9-11] VI had already been prepared from V and propargyl alcohol *via* two steps,[9] but was more conveniently obtained by an improved Grignard reaction using trimethylene chlorohydrin.[13] VIII was prepared by a modified Grignard reaction between V and benzyloxypropylmagnesium iodide.[14],§ *N*-[3-(10,11-Dihydro-5*H*-dibenzo[*a,d*]cyclohepten-5-ylidene)propyl]acetamide (X)[15] was prepared by the Gabriel reaction of IX (Z = Br) followed by acetylation of the resulting amine.[10] VI, VII, VIII, IX (Z = Br), and IX (Z = OH), each obtained this way, were all allowed to react with appropriate nitriles under the conditions described by Ohki, *et al.*,[2] and gave III in the yields listed in Table I. X was also readily cyclized to give III (R_1 = Me) in 51% yield. Of these varying synthetic routes for the preparation of III from V, those through VI and VII proved to be more advantageous than other routes because of their simplicity and better yields. Compounds in Tables I, II, and III were all synthesized in the manner described above. In the case of 3-chloro-10,11-dihydro-5*H*-dibenzo[*a,d*]cyclohepten-5-yli-

†The crystal structure of one of their salts, 3-(10,11-dihydro-5*H*-dibenzo[*a,d*]cyclohepten-5-ylidene)-1-ethyl-2-methylpyrrolidine hydrobromide, was determined by X-ray analysis.[5]

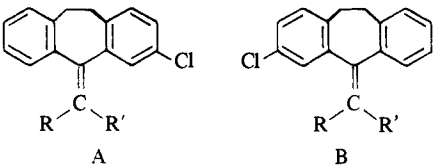
‡Precursors of 4,4-diphenyl-3-butenyl bromide are 1,1-diphenyl-1,4-butanediol and 2,2-diphenyltetrahydrofuran.

§After digestion of Mg, addition of V gave little reaction product, presumably due to the instability of the Grignard reagent prepared from γ-halo ether.

Scheme II



dene derivatives, which correspond to III, IV, and II, the presence of two diastereoisomers A and B are possible, though no attempt was made to separate and confirm them.



Pharmacology. Antagonism by this series of derivatives to acetylcholine and histamine was investigated in the isolated guinea pig ileum, and their antitremorine effect was investigated in mice.

Methods. (a) *In Vitro* **Test with the Isolated Ileum of Guinea Pigs.** A strip of guinea pig ileum was suspended in a Tyrode's bath maintained at 27° and saturated with a mixture of 95% oxygen and 5% carbon dioxide. Contractions of the ileal strip were recorded with a strain gauge. Spasmogens employed were acetylcholine (0.2 μg/ml) and histamine (0.1 μg/ml). Test compounds were added to the bath 1 min before adding each spasmogen. The EC_{50}, *i.e.*, a concentration causing a 50% reduction in size of the ileal contraction, was calculated.

(b) Tremorine Test in Mice. Male ICR-JCl mice weighing 20–30 g were used in groups of 10 animals. Animals were subcutaneously injected with graded doses of the test compounds. Thirty minutes later, 10 mg/kg of tremorine was injected intraperitoneally, and then the presence or absence of tremor and salivation was observed macroscopically. The number of animals showing no tremor and/or salivation was recorded. This number served as the calculation of the ED_{50} values on tremor and salivation.

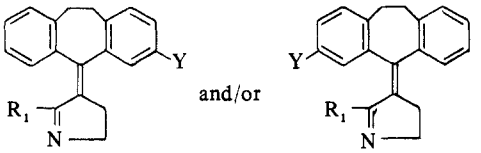
Results

The results are shown in Table III.

(a) Antiacetylcholine and Antihistamine Activities *in Vitro.* All the compounds tested in this experiment inhibited acetylcholine-induced contractions of the isolated guinea pig ileum. The compounds had varying substituents at the R_1 position. Of these, compounds **1–6**, each having CH_3 at R_1, were more potent than compounds **8–11**, which had C_2H_5. The most potent were compounds **2** and **3**, which had a methyl and an ethyl group, respectively, at R_2. These two compounds were almost as effective as atropine.

Most of these compounds also inhibited the histamine-induced ileal contractions at dose levels that inhibited acetylcholine-induced contractions. The antihistamine activity of these compounds was 0.1–0.01 as potent as that of promethazine or diphenhydramine with little variation between members of the series.

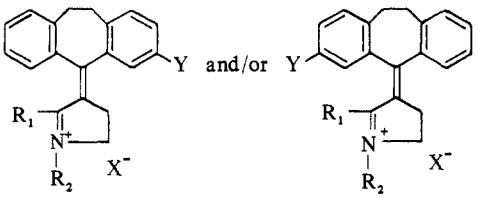
Table I. 2-Alkyl-1-pyrrolines (III)



Y	R_1	Starting material	Yield, %
H	Me	VI	88
H	Me	VII	58
H	Me	VIII	34
H	Me	IX (Z = OH)	31
H	Me	IX (Z = Br)	64
H	Me	X	51
H	Et	IX (Z = Br)	54
Cl	Me	VI	45
H[a]	Me[a]		
H[b]	Et[b]		
Cl[c]	Me[c]		

[a] See Experimental Section. [b] Mp of free base 96–99°. [c] Mp of hydrochloride 256°. *Anal.* ($C_{20}H_{19}Cl_2N$) C, H, Cl, N.

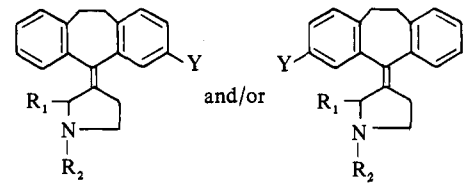
Table II. 1,2-Dialkyl-1-pyrrolinium salts (IV)



Y	R_1	R_2		Yield, %	Mp, °C	Formula[a]
H	Me	Me	I	85	275	$C_{21}H_{22}IN$
H	Me	Et	I	93	227	$C_{22}H_{24}IN$
H	Me	$CH_2C_6H_5$	Br		232	$C_{29}H_{26}BrN \cdot 0.5H_2O$
H	Et	Me	I	95	227	
H	Et	Et	I	70	218	$C_{23}H_{26}IN$
H	Et	CH_2CH_2OH	Br	39	193	$C_{23}H_{23}CBrN$[b]
Cl	Me	Et	I	71	276	$C_{22}H_{23}ClIN$

[a] All compounds were analyzed for C, H, N. All analyses were within ±0.4% of theoretical. [b] Also analyzed for Br.

Table III. 1,2-Dialkylpyrrolidines (II)



Compd	Y	R_1	R_2	Mp,[a] °C	Yield,[b] %	Antiacetylcholine EC_{50}, g/ml	Antihistamine EC_{50}, g/ml	Antitremorine: Tremor ED_{50}, mg/kg	Antitremorine: Salivation ED_{50}, mg/kg
1	H	Me	H	223		1.1×10^{-6}	1.2×10^{-6}	9.2	58.5
2	H	Me	Me	267	96	2.9×10^{-8}	1.5×10^{-7}	0.8	8.6
3	H	Me	Et	253	92	2.7×10^{-8}	1.2×10^{-7}	0.5	3.3
4	H	Me	CH_2Ph	218		$>1.6 \times 10^{-4}$	4.4×10^{-5}		
5	H	Me	CH_2CH_2OH	208	90	1.2×10^{-7}	1.5×10^{-7}	0.4	6.6
6	H	Me	$CH_2CH(OH)CH_3$	172	42	3.3×10^{-6}	1.1×10^{-6}	3.2	17.0
7	Cl	Me	Et	259	94	3.6×10^{-7}	2.3×10^{-7}	1.5	23.2
8	H	Et	H	197	81	5.2×10^{-6}	2.0×10^{-6}	>64	>64
9	H	Et	Me	212	91	6.0×10^{-7}	2.3×10^{-7}	8.5	>64
10	H	Et	Et	200	77	4.0×10^{-7}	8.6×10^{-7}	3.5	>64
11	H	Et	CH_2CH_2OH	200	92	1.8×10^{-7}	1.8×10^{-6}	2.4	41.0
Atropine·H_2SO_4						1.5×10^{-8}	4.3×10^{-6}	1.2	1.8
Diphenhydramine·HCl						1.3×10^{-6}	5.0×10^{-8}		
Trihexyphenidyl·HCl						5.0×10^{-8}	1.1×10^{-5}	2.3	4.7
Promethazine·HCl						8.1×10^{-8}	1.6×10^{-8}	2.0	57

[a] All melting points are expressed as those of hydrochloride except for compd 6, which is expressed as the maleate. Salts of compds 5, 7, 10, and 11 were analyzed for C, H, Cl, and N, and the others, only for C, H, and N. These analytical values did not differ by more than ±0.4% of the calculated values. [b] Compd 6 was prepared from II (R_1 = Me; R_2 = H), and the others from related IV. Their yields are each based on related starting materials.

The antiacetylcholine activity of compounds **2** and **3** was predominant over their antihistamine activity. This fact indicates that the antiacetylcholine effect of these two compounds is more selective than that of the other compounds.

(b) Antagonism to Tremor and Salivation Induced by Tremorine. Most of the tested compounds had potent inhibitory activity against tremorine-induced tremor. The effects of these compounds were of the same order as those of atropine and trihexyphenidyl. Compounds **2**, **3**, and **5** were more effective than any other member of this series and 1.5–3 times as effective as atropine. Potency of the antitremor effect of these compounds was parallel with their antiacetylcholine effect.

These compounds also inhibited tremorine-induced salivation. The effective doses, however, were at least 5 times as great as those required for inhibiting tremor.

In atropine and trihexyphenidyl, in contrast to the test compounds, the antitremor and antisalivary effect were nearly of the same degree.

In brief, the test compounds had a potent central anticholinergic activity comparable to that of atropine or trihexyphenidyl, an antiparkinson drug used clinically, while their peripheral anticholinergic activity was rather less pronounced.

Experimental Section#

3-Benzyloxypropyl Iodide. A suspension contg 314 g (1.74 moles) of 3-benzyloxypropyl chloride and 380 g (2.53 moles) of NaI in acetone (3.8 l.) was refluxed for 75 hr. The ppt formed was filtered and washed with acetone. The combined filtrate and washing were concd and distd to give 410 g (87%) of a liquid, bp 123–130° (7–10 mm).

5-(3-Benzyloxypropylidene)-10,11-dihydro-5*H*-dibenzo[*a*,*d*]cycloheptene (VIII). To anhyd Et_2O (3 ml) were added MeI (0.2 ml for activation) and Mg (3 g, 0.125 g-atom), and then dropwise an anhyd Et_2O soln (3-benzyloxypropyl iodide, 37 g (0.13 mole), V, 20 g (0.096 mole), and anhyd Et_2O, 150 ml) with stirring. The mixt was warmed and cautiously refluxed for 5 hr. After hydrolysis of the reaction mixt was satd NH_4Cl, the Et_2O soln was sepd, dried, and concentrated. Distn of the residue afforded 25 g (76%) of a liquid of bp 210–215° (0.5 mm); λ_{max} 238 mμ (log ε 3.80).

5-(3-Bromopropylidene)-10,11-dihydro-5*H*-dibenzo[*a*,*d*]cycloheptene (IX) (Z = Br).[9,10] A mixt of VIII (22.3 g, 0.066 mole) and 47% HBr (149 ml) was refluxed for 15 hr. After cooling, the mixt was dild with H_2O (200 ml) and extd with Et_2O. This Et_2O ext was washed (satd aqueous $NaHCO_3$ and then H_2O), dried, concd, and distd to give 15.7 g (77%) of a yellow liquid, bp 165–185° (0.5 mm).

***N*-[3-(10,11-Dihydro-5*H*-dibenzo[*a*,*d*]cyclohepten-5-ylidene)-propyl]acetamide (X).** This was prepd from 3-(10,11-dihydro-5*H*-dibenzo[*a*,*d*]cyclohepten-5-ylidene)propylamine (2 g, 0.008 mole) and Ac_2O (1.5 g, 0.015 mole) in the usual way (85%), mp 127° (recrystd from isopropyl ether). *Anal.* ($C_{20}H_{21}NO$) C, H, N.

General Procedures. (a) Preparation of 1-Pyrrolines having a General Structure III from VI, VII, VIII, and IX. To stirred, ice-cold $POCl_3$ (60 ml) were gradually added VI (or related compounds; VII, VIII, and IX, 0.1 mole), nitrile (0.4 mole), and anhyd $SnCl_4$ (0.3 mole) step by step. The mixt was gradually warmed and then refluxed for 7 hr. The reaction mixt was evapd to dryness under reduced pressure. The residue was made alkaline with 20% NaOH. The mixt was extd (Et_2O), and the Et_2O ext was further extd with 20% HCl. Then the acid ext was made alkaline with 20% NaOH and extd with Et_2O. This ext was dried and concd to give a crude product, which was either generally employed for further reactions or purified by distn under reduced pressure.

3-(10,11-Dihydro-5*H*-dibenzo[*a*,*d*]cyclohepten-5-ylidene)-2-methyl-1-pyrroline (III) (R_1 = Me) from X. A soln contg 2.1 g (0.0072 mole) of amide X and $POCl_3$ (16 ml) in anhyd benzene (16 ml) was refluxed for 3 hr and evapd under reduced pressure. The residue was worked up as described for general procedure a to give 1.0 g (51%) of a yellow oil which crytd on standing and melted at 105° after recrystn from Et_2O. The free base showed λ_{max} 268 mμ (log ε 4.20). The hydrochloride, mp 283–285°, showed uv γ_{max} 255, 302.5 mμ (log ε 3.89, 4.06). *Anal.* ($C_{20}H_{20}ClN$) C, H, N.

(b) 1-Alkyl-1-pyrrolinium Halide of General Structure IV. A soln contg III and excess alkyl halide in MeOH was heated at 90° for 2 hr in a sealed tube. Evapn of MeOH and the unreacted alkyl halide gave a crude solid, which was recrystd from MeOH–Et_2O. The

#Melting points and boiling points are uncorrected. Uv spectra were all measured in 95% EtOH.

crude product was usually employed for the following reactions without purification.

(c) 1-Alkylpyrrolidine of General Structure II from IV. IV was reduced with $NaBH_4$ in MeOH in the usual way.[1,6]

3-(10,11-Dihydro-5*H*-dibenzo[*a,d*]cycloheptен-5-ylidene)-2-methylpyrrolidine (II) ($R_1 = CH_3$; $R_2 = H$). This was prepd directly from related 1-pyrroline by redn ($NaBH_4$) of III ($R_1 = CH_3$), hydrochloride mp 223°.

2-[3-(10,11-Dihydro-5*H*-dibenzo[*a,d*]cycloheptен-5-ylidene)-2-methyl-1-pyrrolidinyl]ethanol (II) ($R_1 = CH_3$; $R_2 = CH_2CH_2OH$). A suspension of II ($R_1 = CH_3$; $R_2 = H$) (4.7 g, 0.017 mole), $BrCH_2CH_2Cl$ (1.9 g, 0.013 mole), and powd K_2CO_3 (3.1 g, 0.022 mole) in DMF (25 ml) was warmed stepwise with stirring, at 60 (3.5 hr), 70 (3.5 hr), and 80° (16 hr). The mixt was poured into 150 g of ice water, and the ppt produced was extd (Et_2O). This Et_2O ext was washed, dried, and concd. The residue was purified by alumina chromatography (AcOEt) and converted to the hydrochloride in the usual way. This compd was also prepd from IV.

β-[3-(10,11-Dihydro-5*H*-dibenzo[*a,d*]cycloheptен-5-ylidene)-2-methyl-1-pyrrolidinyl]isopropyl Alcohol (II) ($R_1 = CH_3$; $R_2 = CH_2CH(OH)CH_3$). A soln of II ($R_1 = CH_3$; $R_2 = H$) (2.8 g, 0.01 mole) and propylene oxide (0.6 g, 0.01 mole) in MeOH (25 ml) was refluxed for 8 hr. The soln was evapd and the residual oil (1.4 g, 42%) was converted into the maleate.

Acknowledgment. We are indebted to Professor Sadao Ohki and to Drs. Masao Ohara, Hiroshi Nakano, and Shigenobu Kumada for discussions and suggestions.

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[^{3}H]Vincristine. Preparation and Preliminary Pharmacology

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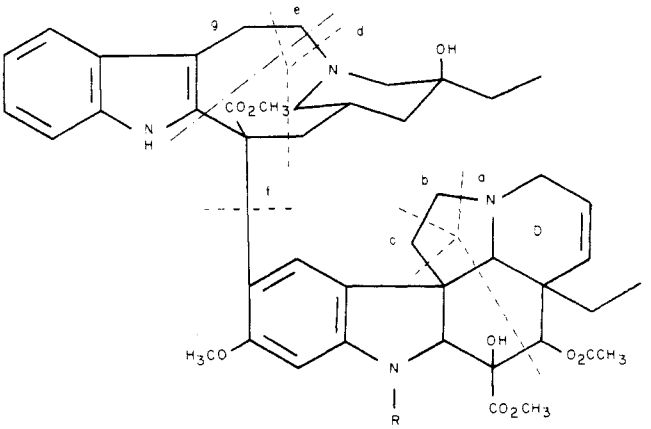
Received January 20, 1972

Vincristine (VCR) was radiolabeled by exchange with [^{3}H]trifluoracetic acid using a special platinum catalyst, giving 94% pure material with a specific activity of 304 mCi/mmole. When [^{3}H]VCR at 1.0 mg/kg was given iv to rats, the blood radioactivity initially decreased rapidly, then after 30 min followed first-order kinetics with a $t_{1/2}$ of 70 min. From 25 to 35% of the administered dose was excreted in the bile in the first 4 hr, in high concentration (10–50 times blood level). Examination of the bile by tlc revealed that from 50 to 65% of the radioactivity cochromatogrammed with authentic VCR. Those organs with high specific acitivity (20–70 times blood level) were the spleen, thyroid, adrenal, and large and small intentine. Moderate levels (7–20 times blood level) were found in the lung, kidney, liver, and marrow, while low levels (0.2–1.0 times blood level) were found in the fat, eye, and brain.

Vincristine (VCR) (I), a naturally occurring dimeric alkaloid derived from the plant *Catharanthus roseus*, is widely employed in the treatment of various neoplasms, particularly the leukemias and lymphomas.† Its utility is most commonly limited by cumulative neurotoxicity and less often by bone marrow depression. The mode of action of this compound has been deduced from a number of studies to be either a blockade of RNA and/or DNA synthesis, or a binding to and an inactivation of a specific class of protein derived from microtubules.[2] A major drawback to the further pharmacologic studies of VCR has been the lack of radiolabeled drug. We were able to prepare tritium-labeled VCR (94% purity, specific activity of 304 mCi/mmole) by an exchange with tritium-labeled trifluoracetic acid ([^{3}H]TFAA) in the presence of a special platinum catalyst, and to complete some selected pharmacologic studies.

Chemical Studies. An attempt to prepare labeled [^{3}H]VCR by the Wilzbach procedure was unsuccessful.‡ Exchange with tritium-labeled H_2SO_4 in our laboratory destroyed the VCR, yielding a variety of unidentified products. Moreover, deacetylation and reacetylation of VCR with tritium-labeled acetic anhydride, in a manner analogous to the preparation of tritium-labeled vinblastine (VLB) (II)[3] was unsuccessful in our hands. Several products were obtained from attempted deacetylation, one of which could have been deacetyl-VCR, by mass spectral analysis,



I, VCR, R = CHO
II, VLB, R = CH_3

†For a general review of VCR, its usefullness and problems, see the Symposium on Vincristine in ref 1.

‡Private communication from Dr. Robert Engle, National Cancer Institute.