both the 2-nitro-5-azidobenzoyl chromophore and peptide, which upon evaporation and lyophilization from a small volume of glacial acetic acid gave 13 mg (64% yield by weight) of **9**, which was 63% peptide by amino acid analysis; Asp 1.03, Pro 1.03, Val 0.88, Ile 0.98, Nle 0.83, Tyr 0.97, Phe 1,00, His 0.99, Arg 0.99; $R_{1,9}$ 0.40, $R_{f(B)}$ 0.60, $R_{f(C)}$ 0.75, $R_{f(D)}$ 0.75; ninhydrin negative, Pauly positive; free of angiotensin II by HVE and TLC.

Avidin-[Asp^1,Ile^5]angiotensin II (10). N^{α}-[(2-Nitro-5-azidobenzoyl)norleucyl]-[Asp^1,Ile^5]angiotensin II (**9**) (6.5 mg, 3000 nmol, of peptide) and 5.8 mg of avidin (Sigma, 40% protein by weight by amino acid analysis, 30 nmol) were dissolved in 0.4 mL of 0.1 M boric acid adjusted to pH 9 with NaOH. This mixture was photolyzed employing a Hanovia 450-W high-pressure mercury lamp (Ace Glass) within a Corning No. 3220 glass filter in a borosilicate immersion well[15] for 1.5 h and then chromatographed on a 1.2 × 60 cm column of Sephadex G-50 (fine) in 1% NH_4HCO_3. The protein peak in the void volume was analyzed for angiotensin content by ultraviolet spectroscopy of the photolyzed 2-nitro-5-azidobenzoyl chromophore and by amino acid analysis. By ultraviolet absorbance at 317 nm, 3 mol of angiotensin per mole of avidin was found: ϵ_{317} (compound **9**) 13 000, ϵ_{317} (photolyzed compound **9**) 6600, ϵ_{280} (avidin) 110 000. By amino acid analysis for norleucine, an average of 2 mol of angiotensin per mole of avidin was found, and this ratio was assumed to be the more accurate determination. The recovery of avidin was 90% by amino acid analysis. Avidin-angiotensin II retained 100% of the biotin binding ability of native avidin, assayed by the method of Green.[28] Gel filtration and analysis of this preparation after several months of storage at 4 °C again showed 2 mol of angiotensin per mole of avidin.

A second preparation of compound **10** by this method but with three successive additions of compound **9**, each followed by 1.5 h of photolysis, yielded avidin-[Asp^1,Ile^5]angiotensin II with 5 mol of angiotensin per mole of avidin, determined by amino acid analysis.

Acknowledgment. This work was supported by U.S. Public Health Service Membrane Center Grant P17-GM-21714 and Biomedical Research Support Grant RR-05358. The authors are grateful to Gary Davis and Dr. Paul L. Fletcher, Jr., for amino acid analyses.

References and Notes

(1) C. R. Sachatello, J. Sedwick, C. L. Moriarity, O. Grahl-Nielsen, and G. L. Tritsch, *Endocrinology*, **88**, 1300 (1971).
(2) G. Forget, S. Heisler, W. K. Park, P. Sirois, D. Gagnon, and D. Regoli, *J. Pharm. Pharmacol.*, **27**, 491 (1975).
(3) R. C. Nairn, "Fluorescent Protein Tracing", E. S. Livingston, Ltd., London, 1964, p 44.
(4) M. Weigele, S. DeBernardo, W. Leimgruber, R. Cleeland, and E. Grunberg, *Biochem. Biophys. Res. Commun.*, **54**, 899 (1973).
(5) T. B. Paiva and A. C. M. Paiva, *J. Med. Chem.*, **15**, 6 (1972).
(6) A. C. M. Paiva, V. L. A. Nouailetas, M. E. Miyamoto, G. B. Mendes, and T. B. Paiva, *J. Med. Chem.*, **16**, 6 (1973).
(7) R. J. Freer and J. M. Stewart, *J. Med. Chem.*, **15**, 1 (1972).
(8) J. Turk, P. Needleman, and G. R. Marshall, *J. Med. Chem.*, **18**, 1135 (1975).
(9) R. Walter, I. L. Schwartz, O. Hechter, T. Dousa, and P. L. Hoffman, *Endocrinology*, **91**, 39 (1972).
(10) D. H. Rich, P. D. Gesellchen, A. Tong, A. Cheung, and C. K. Buckner, *J. Med. Chem.*, **18**, 1004 (1975).
(11) D. Levey, *Biochim. Biophys. Acta*, **322**, 329 (1973).
(12) R. E. Galardy and J. D. Jamieson, *Mol. Pharmacol.*, **13**, 852 (1977).
(13) M. Das, T. Miyakawa, C. F. Fox, R. Pross, A. Aharanov, and H. R. Herschman, *Proc. Natl. Acad. Sci. U.S.A.*, **74**, 2790 (1977).
(14) J. R. Knowles, *Acc. Chem. Res.*, **5**, 155 (1972).
(15) R. E. Galardy, L. C. Craig, J. D. Jamieson, and M. P. Printz, *J. Biol. Chem.*, **249**, 3510 (1974).
(16) J. P. Kraehenbuhl, R. E. Galardy, and J. D. Jamieson, *J. Exp. Med.*, **139**, 208 (1974).
(17) H. Heitzman and F. M. Richards, *Proc. Natl. Acad. Sci. U.S.A.*, **71**, 3537 (1974).
(18) R. E. Galardy, H. E. Bleich, P. Ziegler, and L. C. Craig, *Biochemistry*, **15**, 2303 (1976).
(19) B. W. Erickson and R. B. Merrifield, *J. Am. Chem. Soc.*, **95**, 3750 (1973).
(20) D. Yamashiro and C. H. Li, *J. Am. Chem. Soc.*, **95**, 1310 (1973).
(21) D. Yamashiro and C. H. Li, *J. Org. Chem.*, **38**, 591 (1973).
(22) B. Riniker and R. Schwyzer, *Helv. Chim. Acta*, **47**, 2357 (1964).
(23) D. Regoli, W. K. Park, and F. Rioux, *Pharmacol. Rev.*, **26**, 69 (1974).
(24) H. E. Ives, R. E. Galardy, and J. D. Jamieson, *J. Cell Biol.*, **70**, 328a (1976).
(25) R. M. McKinney, J. J. Spillane, and G. W. Pearce, *Anal. Biochem.*, **7**, 74 (1964).
(26) M. Rozwadowski, *Acta Physiol. Pol.*, **20**, 1005 (1961).
(27) R. F. Furchgott and S. Bhadrakom, *J. Pharmacol. Exp. Ther.*, **108**, 129 (1952).
(28) N. M. Green, *Methods Enzymol.*, **18A**, 418 (1970).

Trichloroacetamidines, a New Class of Positive Inotropic Agents

Walfred S. Saari,* Mark B. Freedman, Joel R. Huff, Stella W. King, Andrew W. Raab, Susan J. Bergstrand, Edward L. Engelhardt,

Department of Medicinal Chemistry, Merck Sharp & Dohme Research Laboratories, West Point, Pennsylvania 19486

Alexander Scriabine, George Morgan, Audrey Morris, John M. Stavorski, Ronald M. Noll, and Daniel E. Duggan

Merck Institute for Therapeutic Research, West Point, Pennsylvania 19486. *Received June 1, 1978*

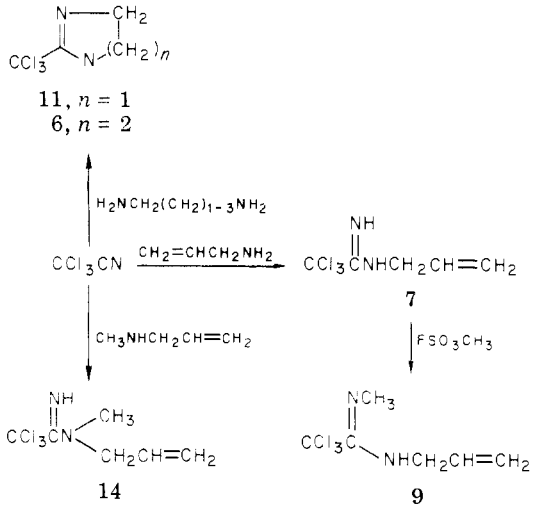
A series of trichloroacetamidine derivatives, obtained by addition of amines to trichloroacetonitrile, was evaluated for positive inotropic activity on isolated cat heart papillary muscles. Increased contractility, not antagonized by β-adrenergic blockade with sotalol or reserpine pretreatment, was observed in this assay with a variety of N-substituted trichloroacetamidine derivatives. More extensive pharmacological studies with the 3-indolylmethyl analogue **2** showed that this amidine in dogs, 5 mg/kg iv, produced a positive inotropic effect more pronounced than that of ouabain, 50 μg/kg iv. Several of the trichloroacetamidines were found to be inhibitors of guinea pig kidney and calf heart Na–K-dependent ATPase and to have a specificity for these enzymes different from that of ouabain. Bacterial mutagenic activity was observed with three members, **2**, **3**, and **12**, of the series.

The use of myocardial stimulants, such as the cardiac glycosides and sympathomimetic amines, currently employed in the treatment of heart failure is limited by undesirable effects of these agents upon the heart or peripheral circulation. During the course of a program aimed at developing a positive inotropic agent with a

0022-2623/78/1821-1283$01.00/0

greater margin of safety than the digitalis glycosides,[1] trichloroacetamidine (**5**) was found to increase the contractile force of heart papillary muscle, apparently by a nonadrenergic mechanism. This report describes the subsequent syntheses of some trichloroacetamidine derivatives and their evaluation as positive inotropic agents.

Chemistry. All of the N-substituted and N,N-disubstituted amidines of Tables I and II were prepared by addition of the corresponding amine to the halonitrile, preferably in C_6H_6, Me_2SO, or DMF at or below room temperature. Cyclic N,N'-disubstituted amidines **6** and **11** were obtained from reaction of CCl_3CN with 1 equiv



of the mono-*p*-toluenesulfonic acid salt of the diamine in MeOH. Addition of ethylenediamine to 2 equiv of CCl_3CN yielded the bisamidine **31**.

Formation of amidines through the uncatalyzed addition of aliphatic primary amines to CCl_3CN was first described by Dachlauer[2] and subsequently extended to other haloacetonitriles and amines by Oxley et al.,[3] Backer and Wanmaker,[4] and Grivas and Taurins.[5] Infrared studies[5b,c,6,7] indicate that *N*-alkyltrichloroacetamidines exist primarily in the imino form as represented in Table I.

Previously undescribed *N,N'*-dialkyl- or *N,N,N'*-trialkyltrichloroacetamidines **9**, **19**, and **22** were prepared easily by alkylation of the corresponding mono- or dialkylamidines with methyl fluorosulfonate in C_6H_6. Confirmation that alkylation of **7** occurred at the less hindered N to give **9** was obtained by the nonidentity (melting point and ^1H NMR) of the HCl salt of this alkylation product with the alternative product **14** prepared through addition of *N*-methylallylamine to CCl_3CN.

Biological Results and Discussion. Positive inotropic activity was observed in the cat papillary muscle assay with a large variety of N-substituted trichloroacetamidine derivatives (see Table I). The most potent member of the series proved to be the *N*-methyl derivative **1** which, at a concentration of 0.98 μg/mL, increased contractile force by 500 mg in this protocol. Comparison of **1** with ouabain showed that, in this assay, the amidine was nearly as potent as the highly active cardiac glycoside.

The cardiotonic potency of **1** was reduced by removal of the *N*-methyl group to give the unsubstituted amidine **5**, lengthening the alkyl group to propyl **3** or allyl **7**, or alkylation to the *N,N,N'*-trimethyl analogue **22**. Positive inotropic activity was observed with the cyclic derivatives **6** and **11** while the bisamidine **31** proved to be only weakly active. Cardiotonic activity of the indolylmethyl compound **2** was reduced significantly through acylation (**16**) or alkylation (**28**) of the indole N. The indolylethyl homologue **4** was slightly less effective than **2**.

Although the *N*-benzyl derivative **32** was found to have relatively weak, positive inotropic activity, introduction of methoxy (**10**, **13**, and **24**), methyl (**17**), and hydroxymethyl (**25**) substituents led to more potent compounds. Similarly, introduction of phenolic and side-chain hydroxyl functions to give the tyramine (**20**), dopamine (**26**), and epinine (**21**) amidines improved potency in the phenylethyl (**38**) series. Four phenoxypropanolamine derivatives, **12**, **27**, **29**, and **30**, also exhibited positive inotropic activity in this assay.

Presence of a trichloromethyl group appears to be an essential requirement for cardiotonic activity in this series. Replacement of the trichloromethyl function by a number of chemically similar groups yielded derivatives which were not significantly active at the highest cat papillary muscle screening concentration of 10 μg/mL (see Table II). The substantially reduced activities of the bromo and fluoro derivatives **44** and **45** were particularly surprising since these compounds are structurally very similar to the active trichloroacetamidine **13**. In addition, the pK_a values of **44** and **45**, 6.15 and 6.18, respectively, are not greatly different from those of the trichloro derivatives **2** (pK_a = 6.10) and **13** (pK_a = 6.00).

Positive inotropic effects of most of the trichloroacetamidines on cat papillary muscle were not blocked by the β-adrenergic blocking agent, sotalol, at a concentration of 10 μg/mL or by pretreatment of the animals with reserpine, 0.5 mg/kg ip. These compounds, therefore, appear to increase myocardial contractility by a mechanism not involving catecholamines. The cardiotonic activities of **1**, **6**, **7**, **22**, and **26** were partially antagonized by sotalol while that of **7** was partially antagonized by reserpine pretreatment, suggesting that an adrenergic mechanism may contribute to the observed positive inotropic effect of these derivatives.

More extensive pharmacological studies were performed with the indolylmethyl analogue **2**. This compound exhibited positive inotropic activity on isolated cat heart papillary muscles at 0.156-10 μg/mL. Its positive inotropic effect was not antagonized by β-adrenergic blockade with sotalol, 10 μg/mL, or pretreatment of animals with reserpine, 0.5 mg/kg ip. At 1.7 μg/mL **2** increased the contractile force of papillary muscles from 855 to 1375 mg (average value for four experiments). The maximal effect was reached in 10 min and the duration of action was 45 min. In similar experiments dobutamine, 0.8 μg/mL, increased the contractile force of papillary muscles from 1080 to 1640 mg, but the duration of action was only 5 min. In anesthetized dogs **2**, at 1.25 mg/kg iv, increased the left-ventricular contractile force from 76 to 125 g within 5 min after intravenous administration. Its duration of action exceeded 1 h. This increase in contractile force was associated with an increase in the left-ventricular dp/dt maximum and a slight increase in the left-ventricular pressure. Only a transient increase in heart rate was noted during the first 2 min after administration of **2** at 1.25 or 5 mg/kg iv. Mean arterial pressure was not significantly affected by the compound at 1.25 mg/kg iv and only transiently reduced at 5 mg/kg iv. The positive inotropic effect of **2**, 5 mg/kg iv, was consistently more pronounced than that of ouabain, 50 μg/kg iv.

Since inhibition of myocardial Na^+/K^+-ATPase is implicated in the cardiotonic effects of the cardiac glycosides,[12] several members of this series were tested as inhibitors of membrane ATPase enzymes (Table III). The trichloroacetamidines studied that exhibited potent positive inotropic activity on the cat papillary muscle, **1**,

2, 4, 12, and 13, were also inhibitors of membrane ATPase preparations, with a disproportionate activity with respect to the cardiac, as compared to the renal, enzyme. As with other classes of inhibitors, preincubation with enzyme resulted in enhancement of inhibition. However, a strict correlation of positive inotropic activity with ATPase inhibition was not obtained. The most active cardiotonic amidine, 1, was not the most potent enzyme inhibitor, while 44, which exhibited relatively potent enzyme inhibitory properties, was inactive in the cat papillary muscle assay.

Trichloroacetamidine derivatives constitute a new and novel class of positive inotropic agents which appear to act by a mechanism not involving catecholamines. Unfortunately, 2, 12, and the simple *N*-propyl derivative 3 were found to have bacterial mutagenic activity[13] which has discouraged further development of this series.

Experimental Section

All melting points were obtained on a Thomas-Hoover Unimelt capillary melting point apparatus using open capillaries and are corrected. Analytical results are indicated by atom symbols and are within ±0.4% of theoretical values unless otherwise indicated. ^{1}H NMR spectra were recorded for all intermediates and final products on a Varian T-60 spectrophotometer in either $CDCl_3$, Me_2SO-d_6, or D_2O and were consistent with assigned structures. TLC's were performed on fluorescent silica gel plates and spots detected by UV or exposure to I_2 vapor.

Preparation of Amidines. Method A. *N*-Allyl-2,2,2-trichloroethanimidamide Hydrochloride (7). A solution of 8.55 g (0.15 mol) of allylamine in 75 mL of C_6H_6 was added over 20 min to a stirred solution of 15 mL of CCl_3CN in 100 mL of C_6H_6 at 10–15 °C. After addition was complete, the solution was stirred at 20–25 °C for 18 h and then concentrated at 45 °C under reduced pressure. The residue was dissolved in a mixture of 50 mL of EtOH and 50 mL of EtOAc and treated with an excess of anhydrous EtOH–HCl. Addition of 400 mL of EtOAc precipitated 30.2 g (85%) of *N*-allyltrichloroacetamidine hydrochloride, mp 177.8–181.8 °C dec.

Method B. *N*-(1*H*-Indol-3-ylmethyl)-2,2,2-trichloroethanimidamide (2). A solution of 4.76 g (0.033 mol) of CCl_3CN in 15 mL of C_6H_6 was added over 15 min to a stirred solution of 3.18 g (0.022 mol) of 3-(aminomethyl)indole in 25 mL of dry Me_2SO at 20–25 °C. After being stirred at 20–25 °C for 20 h, the mixture was concentrated under reduced pressure to a viscous oil. The residue was extracted with two 100-mL portions and one 50-mL portion of warm $CHCl_3$. After being filtered, the combined $CHCl_3$ extracts were washed with two 100-mL portions of H_2O, followed by extraction with three portions of dilute HCl. The aqueous acid extracts were combined, extracted with two 100-mL portions of $CHCl_3$, and filtered. The clear aqueous filtrate was made basic with a saturated aqueous solution of $NaHCO_3$, and the product was extracted into three 200-mL portions of $CHCl_3$. The combined chloroform extracts were washed (H_2O), dried ($MgSO_4$), filtered, and concentrated. Three recrystallizations from C_6H_6–hexane, the first of which utilized decolorizing C, gave 2.34 g (37%) of analytically pure product, mp 138–141.5 °C.

Method C. *N*-[2-(Acetyloxy)-3-phenoxypropyl]-2,2,2-trichloroethanimidamide Hydrochloride (12). A total of 24.69 g (0.244 mol) of Et_3N was added in small portions over 10 min to a well-stirred mixture of 60 g (0.244 mol) of 2-acetoxy-3-phenoxypropylamine hydrochloride and 70 g (0.488 mol) of CCl_3CN in 200 mL of DMF at 5–10 °C. After being warmed to room temperature over 1.5 h, the reaction mixture was diluted with 2 L of H_2O and extracted with 600 mL of C_6H_6. The C_6H_6 extract was washed five times with H_2O, dried ($MgSO_4$), filtered, and concentrated. The residue was dissolved in C_6H_6 and treated with an excess of anhydrous EtOH–HCl to give 80.1 g (84%) of analytically pure product, mp 157.8–161.8 °C dec.

Method D. 2,2,2-Trichloroethanimidamide (5). Thirty grams of CCl_3CN was added over 10 min to 300 mL of liquid NH_3. After addition was complete, the mixture was stirred at reflux under a dry ice condenser for 7 h. The condenser was then removed and the NH_3 allowed to evaporate overnight. Benzene, 100 mL, was added to the residue, the mixture was filtered through diatomaceous earth, and the filtrate was concentrated under reduced pressure to an oil. Crystallization from hexane provided 17.5 g (52%) of the trichloroacetamidine base, mp 41.5–43.5 °C (lit. mp 44 °C,[21] 49–50 °C[22]). The HCl salt was prepared by adding excess anhydrous EtOH–HCl to a solution of the base in EtOAc. An analytical sample, mp 210–220 °C dec, was obtained by further recrystallization from EtOH–EtOAc.

Method E. 2-(Trichloromethyl)-1,4,5,6-tetrahydropyrimidine (6). A solution of 3.7 g (0.050 mol) of 1,3-diaminopropane and 9.5 g (0.050 mol) of *p*-toluenesulfonic acid hydrate in 50 mL of MeOH was stirred at 25 °C for 10 min, followed by the addition of 7.22 g (0.050 mol) of CCl_3CN. After being stirred at 25 °C for 5 days, the reaction mixture was filtered and the filtrate concentrated under reduced pressure. The residue was washed with H_2O and recrystallized from cyclohexane to give 6.0 g (60%) of product, mp 113.8–116.8 °C.

Method F. *N*-[2-(3,4-Dihydroxyphenyl)ethyl]-2,2,2-trichloroethanimidamide Hydrochloride Hydrate (26). A mixture of 4.75 g (0.025 mol) of dopamine hydrochloride, 2.75 g (0.027 mol) of Et_3N, and 7.20 g (0.050 mol) of CCl_3CN in 5 mL of DMF was stirred at 25 °C for 20 h. The mixture was filtered, the filtrate was concentrated at 70 °C (0.5 mm), and the residue was dissolved in EtOAc and washed with 5% Na_2CO_3, H_2O, and saturated NaCl solution. The residue was dried ($MgSO_4$) and filtered, and the filtrate was treated with excess anhydrous EtOH–HCl solution and concentrated under reduced pressure. The residue was washed with two 50-mL portions of EtOAc, dissolved in MeOH, and concentrated at 0.5 mm and 25 °C to give 7.0 g (82%) of *N*-(3,4-dihydroxyphenylethyl)trichloroacetamidine HCl·0.25H_2O.

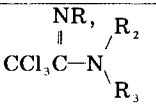
Method G. *N*-Allyl-*N'*-methyl-2,2,2-trichloroethanimidamide Hydrochloride (9). A mixture of 1.5 g (7.5 mmol) of *N*-allyltrichloroacetamidine and 0.86 g (7.5 mmol) of methyl fluorosulfonate in 30 mL of C_6H_6 was stirred at 20–25 °C for 18 h. The C_6H_6 solution was decanted from the insoluble oil which was then washed with more C_6H_6. After portioning the insoluble oil between C_6H_6 and saturated $NaHCO_3$ solution, the C_6H_6 extract was washed with a saturated NaCl solution, dried (Na_2SO_4), filtered, and treated with excess anhydrous EtOH–HCl. After being filtered and dried, 0.90 g (47%) of *N*-allyl-*N'*-methyltrichloroacetamidine hydrochloride, mp 83–87 °C dec, was obtained.

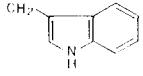
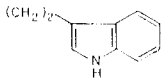
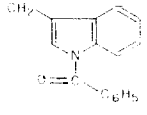
Method H. 2-(Trichloromethyl)-4,5-dihydro-1*H*-imidazole (11). A solution of 3.0 g (0.050 mol) of ethylenediamine and 9.5 g (0.050 mol) of *p*-toluenesulfonic acid hydrate in 50 mL of MeOH was stirred at 25 °C for 30 min, followed by the addition of 7.22 g (0.050 mol) of CCl_3CN. The mixture was stirred at 20–25 °C for 20 h and filtered, and the filtrate was concentrated under reduced pressure. The residue was washed with H_2O, dissolved in C_6H_6, and washed again with H_2O and a saturated NaCl solution. After drying ($MgSO_4$), the C_6H_6 extract was concentrated and the residue chromatographed on silica gel. A total of 1.2 g (13%) of product, mp 85 °C dec (lit. mp 97–98 °C dec,[3] 105 °C dec[23]), was eluted with 25% MeOH–75% $CHCl_3$.

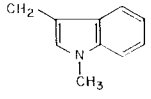
Method I. *N*-Allyl-*N*-methyl-2,2,2-trichloroethanimidamide Hydrogen Maleate (14). A solution of 3.6 g (0.0506 mol) of *N*-methylallylamine in 30 mL of C_6H_6 was added over 20 min to a stirred solution of 6 mL of CCl_3CN in 30 mL of C_6H_6 at 10–15 °C. After addition was complete, the solution was stirred at 20–25 °C for 20 h and then concentrated at 50 °C under reduced pressure. Distillation of the residue gave 6.2 g (57%) of *N*-allyl-*N*-methyltrichloroacetamidine, bp 66–70 °C (0.2 mm). To a solution of 5 g of the *N*-allyl-*N*-methyltrichloroacetamidine base in EtOH was added 2.5 g of maleic acid. Addition of EtOAc and cooling gave 4.9 g (36%) of *N*-allyl-*N*-methyltrichloroacetamidine hydrogen maleate, mp 101–107 °C. An analytical sample, mp 101–106 °C, was obtained upon recrystallization from EtOH–EtOAc.

Method J. *N*-[(4-Methoxyphenyl)methyl]-2,2,2-trichloroethanimidamide Hydrochloride (13). A solution of 11.05 g (0.077 mol) of CCl_3CN in 10 mL of C_6H_6 was added to a stirred solution of 10.0 g (0.073 mol) of *p*-methoxybenzylamine in 50 mL of C_6H_6 at 25 °C. After being stirred at 20–25 °C for 20 h, the solution was concentrated under reduced pressure at 30–40 °C. The residue was dissolved in $CHCl_3$ and passed through a column

Table I. Chemical Properties and Positive Inotropic Activity of Trichloroacetamidines



compd	R,	R_2	R_3	mp, °C	recrystn solvent	method	yield, %	formula	analyses	positive inotropic act. on cat papillary muscle at μg/mL[a] 10	2.5	0.63	μg/mL[b] (95% confidence limits)
ouabain										+++[c]	+++[d]	++[e]	0.68 (0.40-1.23)
1	H	H	CH_3	223-232 dec[f]	EtOH-Et_2O	A	21	$C_3H_5Cl_3N_2 \cdot HCl$	C, H, N	+++[g]	+++	++	0.98 (0.61-1.37)
2[h]	H	H		138-141.5	C_6H_6-hexane	B	37	$C_{11}H_{10}Cl_3N_3$	C, H, N, Cl	+++[i]	++	++[j]	1.27 (0.76-2.14)
3	H	H	n-C_3H_7	210.5-212.5 dec	EtOH-EtOAc	C	38	$C_5H_9Cl_3N_2 \cdot HCl$	C, H, N, Cl	+++	++	++[j]	2.22 (1.54-3.43)
4	H	H		141.0-143.5	EtOAc-hexane	B	39	$C_{12}H_{12}Cl_3N_3$	C, H, N	+++	++	++[j]	2.33 (0.98-7.45)
5	H	H	H	210-220 dec	EtOH-EtOAc	D	52	$C_2H_3Cl_3N_2 \cdot HCl$	C, H, N	+++[i]	++	+	2.68 (1.28-5.33)
6	$-CH_2CH_2CH_2-$		H	113.8-116.8	cyclohexane	E	60	$C_5H_7Cl_3N_2$	C, H, N, Cl	+++[g]	++	+[j]	2.77 (1.92-4.37)
7	H	H	$CH_2CH=CH_2$	177.8-181.8 dec	EtOH-EtOAc	A	85	$C_5H_7Cl_3N_2 \cdot HCl$	C, H, N, Cl	+++[g,k]	++	0	2.78 (1.42-4.68)
8	H	H	CH_2-c-C_3H_5	198-203 dec	EtOH-EtOAc	F	65	$C_6H_9Cl_3N_2 \cdot HCl$	C, H, N	++[i]	++	+	3.21 (2.36-4.64)
9	CH_3	H	$CH_2CH=CH_2$	83-87 dec		G	47	$C_6H_9Cl_3N_2 \cdot HCl$	H, N; C[l]	+++	++	0	3.89 (2.72-5.80)
10	H	H	$CH_2C_6H_4$-o-OCH_3	242-245 dec	EtOH	A	59	$C_{10}H_{11}Cl_3N_2O \cdot HCl$	C, H, N	++[i]	++	+	4.76 (2.91-9.73)
11	$-CH_2CH_2-$		H	85 dec		H	13	$C_4H_5Cl_3N_2$	C, H, N	+++	++	+	4.86 (2.97-10.75)
12	H	H	$CH_2CH(OAc)CH_2$-OC_6H_5	157.8-161.8 dec	EtOH-EtOAc-hexane	C	84	$C_{13}H_{15}Cl_3N_2O_3 \cdot HCl$	C, H, N	+++[i]	++	+	4.96 (3.58-7.69)
13[n]	H	H	$CH_2C_6H_4$-p-OCH_3	227.5-229.5 dec	EtOH-Et_2O	J	76	$C_{10}H_{11}Cl_3N_2O \cdot HCl$	C, H, N, Cl	+++[i]	++	+	4.99 (2.84-13.67)
14	H	CH_3	$CH_2CH=CH_2$	101-106	EtOH-EtOAc	I	36	$C_6H_9Cl_3N_2 \cdot C_4H_4O_4$[m]	C, H, N	+++	++	+	5.47 (3.83-7.88)
15	H	H	$(CH_2)_2OH$	74.5-76.5	EtOAc-hexane	K	52	$C_4H_7Cl_3N_2O$	C, H, N, Cl	+++	+	0	5.50 (3.83-9.22)
16	H	H		113.5-115.5	C_6H_6-pet. ether	B	54	$C_{18}H_{14}Cl_3N_3O$	C, H, N	+++[i]	++	+	6.20 (3.45-20.36)
17	H	H	$CH_2C_6H_4$-p-CH_3	241-243	EtOH	J	55	$C_{10}H_{11}Cl_3N_2 \cdot HCl$	C, N; H[o]	+++	+	+	8.43 (5.51-16.68)
18	H	CH_3	CH_3	191-194 dec	EtOH-EtOAc	L	49	$C_4H_7Cl_3N_2 \cdot HCl$	C, H, N	+++			

19	CH_3	H	CH_2-c-C_3H_5	60–65 dec		G	60	$C_7H_{11}Cl_3N_2$·HCl	C, H, N	+++			
20	H	H	$(CH_2)_2C_6H_4$-*p*-OH	94–95	C_6H_6–hexane	M	50	$C_{10}H_{11}Cl_3N_2O$	C, H, N	+++			
21	H	H	$CH_2CH(OH)C_6H_5$	104–106	C_6H_6–hexane	K	72	$C_{10}H_{11}Cl_3N_2O$	C, H, N	+++			
22	CH_3	CH_3	CH_3	103–107		N	83	$C_5H_9Cl_3N_2$·HFO_3S	C, H, N	++[g]			>10
23	H	H	$(CH_2)_3OH$	136 dec	EtOH–Et_2O	A	41	$C_5H_9Cl_3N_2O$·HCl	C, H, N	++	+		>10
24	H	H	$CH_2C_6H_4$-*m*-OCH_3	203–207 dec	EtOH–Et_2O	A	16	$C_{10}H_{11}Cl_3N_2O$·HCl	C, H, N	++			>10
25	H	H	$CH_2C_6H_4$-*o*-CH_2OH	110–112	cyclohexane	O	32	$C_{10}H_{11}Cl_3N_2O$	C, H, N	++			
26	H	H	$(CH_2)_2C_6H_3$-*m*,*p*-$(OH)_2$	*p*		F	82	$C_{10}H_{11}Cl_3N_2O_2$·HCl·0.25H_2O	C, N, Cl; H[q]	++[g]	+	0	>10
27	H	H	$CH_2CH(OH)CH_2OC_6H_5$	86–87	C_6H_6–hexane	K	78	$C_{11}H_{13}Cl_3N_2O_2$	C, H, N	++			
28	H	H		90–92.5	hexane	K	32	$C_{12}H_{12}Cl_3N_3$	C, H, N	++			
29	H	H	$CH_2CH(OCH_3)CH_2OC_6H_5$	212–215 dec	EtOH–C_6H_6–pet. ether	C	79	$C_{12}H_{15}Cl_3N_2O_2$·HCl	C, H, N	++			
30	H	H	$CH_2CH(OH)CH_2O$-C_6H_4-*p*-NHAc	124.5–131.5	EtOH–C_6H_6–hexane	P	37	$C_{13}H_{16}Cl_3N_3O_3$	H, N; C[r]	++			
31	$CCl_3C(=NH)NH(CH_2)_3NHC(=NH)CCl_3$			221–225 dec	EtOH–EtOAc	A	77	$C_7H_{10}Cl_6N_4$·2HCl·H_2O	C, H, N	+			>10
32	H	H	$CH_2C_6H_5$	81.5–84.0[s]	hexane	K	75	$C_9H_9Cl_3N_2$	C, H, N	+			
33	H	H	(*S*)[t]-CHC_6H_5 (CH$_3$)	83 dec	EtOH–Et_2O	J	17	$C_{10}H_{11}Cl_3N_2$·HCl	C, H, N	+			>10
34	H	H	(*R*)[t]-CHC_6H_5 (CH$_3$)	95 dec	EtOH–Et_2O	J	16	$C_{10}H_{11}Cl_3N_2$·HCl	C, H, N, Cl	0			
35	H	H	$C(=NH)CNH_2$	168.8–170.8 dec	C_6H_6	*u*	21	$C_3H_5Cl_3N_4$	C, H, N	0			
36	H	H	C_6H_5	101–102	hexane	*v*	21	$C_8H_7Cl_3N_2$	C, H, N	0			
37	H	$CH_2CH=CH_2$	$CH_2CH=CH_2$	178.8–183.8 dec	EtOH–EtOAc	A	31	$C_8H_{11}Cl_3N_2$·HCl	C, H, N	0			
38	H	H	$(CH_2)_2C_6H_5$	69.5–71.0	hexane	K	25	$C_{10}H_{11}Cl_3N_2$	C, H, N	0			
39	$CCl_3C(=O)NH(CH_2)_2OH$			50–51	C_6H_6–hexane	Q	63	$C_4H_6Cl_3NO_2$	C, H, N	0			

[a] +++ = >600 mg increase in contractile force, ++ = 201–600 mg increase, + = <200 mg increase, 0 = no effect. Positive inotropic activity was not antagonized by sotalol, 10 μg/mL, unless otherwise noted. [b] Concentration required to increase contractile force by 500 mg. [c] Concentration = 2 μg/mL. [d] Concentration = 1 μg/mL. [e] Concentration = 0.5 μg/mL. [f] Reported[5c] mp 229 °C dec. [g] Partially antagonized by sotalol, 10 μg/mL. [h] pK_a = 6.10 (30% EtOH–70% H_2O). [i] Not antagonized by pretreatment of animals with reserpine, 0.5 mg/kg ip. [j] + at 0.15 μg/mL. [k] Partially antagonized by pretreatment of animals with reserpine, 0.5 mg/kg ip. [l] C: calcd, 28.60; found, 27.98. [m] Hydrogen maleate. [n] pK_a = 6.00 (H_2O). [o] H: calcd, 4.01; found, 4.56. [p] Foam, melting point not taken. [q] H: calcd, 3.72; found, 4.24. [r] C: calcd, 42.35; found, 42.84. [s] Reported[5a] mp 83.5 °C. [t] Absolute configuration. [u] Prepared by the procedure of ref 4, reported mp 158 °C dec. [v] Prepared in EtOH by the procedure of ref 5a, reported mp 101–102 °C.[4,5a]

Table II. Chemical Properties of Amidines Tested for Inotropic Activity[a]

$R_1C(=NH)NHR_2$

compd	R_1	R_2	mp, °C	recrystn solvent	method	yield, %	formula	analyses
40	$CHCl_2$	CH_3	143–153 dec	EtOH–EtOAc	L	54	$C_3H_6Cl_2N_2 \cdot HCl \cdot 0.25H_2O$	C, H, N
41	CCl_2CH_3	CH_3	170.7–174.7 dec	EtOH–EtOAc	L	29	$C_4H_8Cl_2N_2 \cdot HCl$	C, H, N
42	$C(CH_3)_3$	H	191–192	EtOH–EtOAc	*b*	68	$C_5H_{12}N_2 \cdot HCl$	C, H, N
43	C_6H_4-*p*-NO_2	H	290.6–293.6 dec[c]	MeOH	*d*	34	$C_7H_7N_3O_3 \cdot HCl$	C, H, N
44[e]	$CBrCl_2$	$CH_2C_6H_4$-*p*-OCH_3	207.5–208.0 dec	EtOH–Et_2O	A	71	$C_{10}H_{11}BrCl_2N_2O \cdot HCl$	C, H, N
45[f]	$CFCl_2$	$CH_2C_6H_4$-*p*-OCH_3	66–67	hexane	A	70	$C_{10}H_{11}FCl_2N_2O$	C, H, N
46	CF_3	$CH_2C_6H_4$-*p*-OCH_3	86.5–87.5	hexane	R	22	$C_{10}H_{11}F_3N_2O$	C, H, N
47	CCl_2CCl_3	$CH_2C_6H_4$-*p*-OCH_3	105–106	hexane	D	6	$C_{11}H_{11}Cl_5N_2O$	C, H, N

[a] These amidines did not exhibit significant positive inotropic activity when tested in the cat papillary muscle at 10 μg/mL. [b] Prepared by the procedure of ref 9, reported mp 190–192.5 °C. [c] Reported mp 294–295 °C[10] and 285–287 °C.[11] [d] Prepared by procedure of ref 11. [e] $pK_a = 6.15$ (H_2O). [f] $pK_a = 6.18$ (H_2O).

Table III. Inhibition of Membrane ATPase by Trichloroacetamidines

	I_{50}, mol/L			
	guinea pig renal enzyme[a]		calf cardiac enzyme[b]	
compd	acute	30-min preincubation	acute	30-min preincubation
ouabain	5×10^{-7}		5×10^{-7}	2×10^{-7}
1	inactive		1×10^{-3}	
2			4.5×10^{-5}	2×10^{-5}
4	1.5×10^{-4}	4×10^{-6}	3.3×10^{-5}	
12			1.7×10^{-4}	7×10^{-5}
13	8×10^{-4}	1×10^{-4}	2×10^{-5}	5×10^{-6}
25	inactive		inactive	
45	1.5×10^{-5}	2×10^{-6}	2×10^{-5}	2×10^{-6}

[a] Preparation and assay according to ref 18. [b] Preparation and assay according to ref 19.

containing 100 g of silica gel. $CHCl_3$ fractions containing the product were combined and concentrated. The residue was dissolved in EtOH and treated with excess EtOH–HCl solution at 0 °C, and the HCl salt was precipitated with Et_2O. Recrystallization from EtOH–Et_2O gave 17.5 g (76%) of *N*-(4-methoxybenzyl)trichloroacetamidine hydrochloride, mp 227.5–229.5 °C dec.

Method K. *N*-(2-Hydroxyethyl)-2,2,2-trichloroethanimidamide (15). A solution of 18.3 g (0.30 mol) of ethanolamine and 57.6 g (0.40 mol) of CCl_3CN in 600 mL of C_6H_6 was stirred at 20–25 °C for 4 h. After being concentrated under reduced pressure, the residue was recrystallized twice from C_6H_6–hexane and once from EtOAc–hexane to give 31.8 g (52%) of *N*-(2-hydroxyethyl)trichloroacetamidine, mp 74.5–76.5 °C.

Method L. *N,N*-Dimethyl-2,2,2-trichloroethanimidamide Hydrochloride (18). A mixture of 8 mL of CCl_3CN and 15 mL of 25% aqueous Me_2NH was stirred vigorously with ice-bath cooling for 10 min and then allowed to warm to room temperature over 2 h. The bottom layer was separated, washed twice with 10 mL of H_2O, dissolved in 100 mL of EtOAc, and dried ($MgSO_4$). After being concentrated, the residue was dissolved in EtOAc and converted to the HCl with anhydrous EtOH–HCl. Recrystallization from EtOH–EtOAc gave a product with mp 191–194 °C dec (reported[5c] mp 175–176 °C dec).

Method M. *N*-[2-(4-Hydroxyphenyl)ethyl]-2,2,2-trichloroethanimidamide (20). To a stirred mixture of 10.41 g (0.060 mol) of tyramine hydrochloride and 60 mL of DMF at 0–5 °C was added 6.06 g (0.060 mol) of Et_3N, followed by 12 g (0.083 mol) of CCl_3CN. After being stirred at 0–5 °C for 1 h, the reaction mixture was stirred at 20–25 °C for an additional 3 h and then poured into 600 mL of H_2O. The crude product was extracted with C_6H_6, washed (H_2O), dried ($MgSO_4$), filtered, and concentrated under reduced pressure. The residue was dissolved in EtOAc and saturated with HCl gas. The gummy precipitate was separated by decantation, washed with EtOAc, and dissolved in H_2O. After adding excess saturated Na_2CO_3 solution, the product was extracted into C_6H_6. The C_6H_6 extract was washed (H_2O), dried ($MgSO_4$), filtered, and concentrated under reduced pressure. Recrystallization from C_6H_6–hexane gave 8.5 g (50%) of *N*-(4-hydroxyphenylethyl)trichloroacetamidine, mp 94–95 °C.

Method N. *N,N,N'*-Trimethyl-2,2,2-trichloroethanimidamide Fluorosulfonate (22). A solution of 1.7 g (0.015 mol) of methyl fluorosulfonate in 10 mL of C_6H_6 was added slowly to a well-stirred solution of 3.24 g (0.017 mol) of *N,N*-dimethyltrichloroacetamidine in 15 mL of C_6H_6 at 25 °C. The mixture was stirred at this temperature for 23 h, and *N,N,N'*-trimethyltrichloroacetamidine fluorosulfonate, 3.8 g (83%), mp 103–107 °C, was removed by filtration.

Method O. *N*-[[2-(Hydroxymethyl)phenyl]methyl]-2,2,2-trichloroethanimidamide (25). A solution of 3.0 g (0.022 mol) of 2-(aminomethyl)benzyl alcohol and 3.32 g (0.024 mol) of CCl_3CN in 12 mL of C_6H_6 was stirred at 20–25 °C for 3 days. The mixture was concentrated under reduced pressure, 100 mL of $CHCl_3$ was added, and the mixture was filtered and chromatographed over 200 g of silica gel. The $CHCl_3$ fractions containing the product were combined, concentrated, and recrystallized twice from cyclohexane to give 2.0 g (32%) of *N*-[2-(hydroxymethyl)benzyl]trichloroacetamidine, mp 110–112 °C.

Method P. *N*-[4-[2-Hydroxy-3-[(2,2,2-trichloro-1-iminoethyl)amino]propoxy]phenyl]acetamide (30). After combining 4.1 g (0.0157 mol) of 2-hydroxy-3-[4-(acetamido)phenoxy]-propylamine hydrochloride, 1.59 g (0.0158 mol) of Et_3N, and 4.7 g (0.0326 mol) of CCl_3CN in 20 mL of DMF at 0.5 °C, the mixture was stirred at room temperature for 30 min and then diluted with 400 mL of H_2O. The residue was extracted several times with $CHCl_3$ to give the crude product which, after recrystallization from EtOH–C_6H_6–hexane, led to 2.16 g (37%) of amidine, mp 124.5–131.5 °C.

Method Q. *N*-(2-Hydroxyethyl)-2,2,2-trichloroacetamide (39). Trichloroacetyl chloride, 18 g (0.10 mol), was added slowly to a well-stirred slurry of 12.2 g (0.20 mol) of ethanolamine and 150 mL of Et_2O with ice-bath cooling. After addition was complete, the mixture was stirred at room temperature for 5 h and filtered. After removing Et_2O under reduced pressure, the residue was recrystallized from C_6H_6–hexane to give 13 g (63%) of amide, mp 50–51 °C (reported[8] mp 66–69 °C).

Method R. *N*-[(4-Methoxyphenyl)methyl]-2,2,2-trifluoroethanimidamide (46). To approximately 9.50 g (0.010 mol) of CF_3CN cooled with a dry ice–Me_2CO bath was added, over 15 min, 6.85 g (0.050 mol) of *p*-methoxybenzylamine. The mixture was stirred and allowed to warm to 20–25 °C over 20–22 h. Two recrystallizations from hexane gave 5.08 g (22%) of *N*-(*p*-methoxybenzyl)trifluoroacetamidine, mp 83.5–86.5 °C. An analytical sample, mp 86.5–87.5 °C, was obtained upon further recrystallization from hexane.

Preparation of Amine Intermediates. 2-(1-Amino-3-phenoxypropyl) Acetate Hydrochloride. A solution of 113 g (0.555 mol) of 2-hydroxy-3-phenoxypropylamine[24] in 1.9 L of glacial HOAc was saturated with HCl gas. Acetyl chloride, 49 g (0.62 mol), was added and the solution stirred at 20–25 °C for 24 h. The solution was concentrated under reduced pressure, toluene was added, and the mixture was concentrated again under reduced pressure. Recrystallization from *i*-PrOH–hexane afforded 129.6 g (95%) of product: mp 117.5–119.5 °C; ^{1}H NMR (Me_2SO-d_6) δ 2.25 (s, 3 H, CH_3CO), 3.38 (br d, 2 H, CH_2N^+), 4.22 (d, J = 4 Hz, 2 H, CH_2O), 5.53 (quintet, 1 H, CH), 7.0–7.67 (m, 5 H, aromatic CH), 8.73 (br s, D_2O exchangeable, 3 H, NH_3^+).

3-(Aminomethyl)-1*H*-indole. Hydrazine hydrate, 5.41 g (0.108 mol), was added to a suspension of 25.0 g (0.090 mol) of *N*-(3-indolylmethyl)phthalimide[25] in 300 mL of absolute MeOH, and the mixture was stirred at 20–25 °C for 18 h. MeOH, 375 mL, and 3 N HCl, 250 mL, were added and, after being stirred for 2 h, the mixture was filtered and the solid washed with MeOH. The combined filtrate and washings were concentrated under reduced pressure below 60 °C. The residue was treated with 250 mL of warm H_2O and filtered, and the filtrate was rendered alkaline with 5 N KOH. The product was extracted with three 350-mL portions of $CHCl_3$, and the combined extracts were washed with H_2O, dried (Na_2SO_4), and concentrated under reduced pressure. The product, 7.99 g (61%), melted at 70–77 °C.

1,1-Dimethylethyl (1*H*-Indol-3-ylmethyl)carbamate. A mixture of 3.75 g (0.026 mol) of 3-(aminomethyl)indole, 7.62 g (0.026 mol) of *tert*-butyl 2,4,5-trichlorophenylcarbonate, 3.2 g (0.032 mol) of Et_3N, and 150 mL of $CHCl_3$ was stirred at reflux for 17 h. The cooled solution was washed with two 50-mL portions of 1 N NaOH, two 30-mL portions of 1 N HCl, two 50-mL portions of 1 N NaOH, and three 50-mL portions of H_2O. The $CHCl_3$ extract was dried ($MgSO_4$), filtered, and concentrated. The residue was recrystallized from Et_2O–petroleum ether to give 3.5 g (55%) of *tert*-butyl *N*-(3-indolylmethyl)carbamate, mp 102–103 °C.

1-Benzoyl-3-(aminomethyl)-1*H*-indole Hydrochloride. *tert*-Butyl *N*-(3-indolylmethyl)carbamate, 3.70 g (0.015 mol), was added to a stirred mixture of NaH, 0.72 g (0.015 mol), of a 50% NaH–mineral oil dispersion washed free of mineral oil with petroleum ether in 30 mL of DMF. The mixture was stirred at 50 °C for 20 min and cooled to 20–25 °C, and 2.1 g (0.015 mol) of benzoyl chloride was added. The reaction mixture was stirred at 20–25 °C for 2 h and then diluted with H_2O and Et_2O. The Et_2O layer was separated, washed (H_2O), dried ($MgSO_4$), filtered, and concentrated under reduced pressure. The residue was chromotographed over 250 g of silica gel and eluted with C_6H_6, 50% $CHCl_3$–C_6H_6, and $CHCl_3$ to give 2.2 g (42%) of *tert*-butyl *N*-(1-benzoyl-3-indolylmethyl)carbamate.

A solution of 2.1 g (6.0 mmol) of *tert*-butyl *N*-(1-benzoyl-3-indolylmethyl)carbamate in 50 mL of EtOAc was cooled in an ice bath while anhydrous HCl was passed through the solution for 10 min. After removing excess HCl gas by purging with N_2, Et_2O was added to precipitate 3-(aminomethyl)-1-benzoylindole hydrochloride, 1.27 g (74%), mp 247–253 °C. Anal. ($C_{16}H_{14}N_2O$·HCl) C, H, N.

***N*-[4-(3-Amino-2-hydroxypropoxy)phenyl]acetamide Hydrochloride.** A solution of 10.4 g (0.050 mol) of 4-(acetamido)phenoxyepoxypropane[26] and 5.4 g (0.050 mol) of benzylamine in 20 mL of DMF was stirred at 100 °C for 75 min. Trituration with hexane gave the crude product which was recrystallized from EtOAc to give 12.4 g (79%) of *N*-benzyl-2-hydroxy-3-[4-(acetamido)phenoxy]propylamine, mp 117–123 °C.

Hyrogenation of 5.5 g (0.0157 mol) of the HCl salt of the *N*-benzyl derivative in 100 mL of EtOH and 50 mL of H_2O over a 10% Pd/C catalyst at 50 °C and an initial pressure of 50 psi gave, after filtration, concentration, and crystallization from absolute EtOH, 3.26 g (80%) of product: mp 194–197 °C; ^{1}H NMR (Me_2SO-d_6) δ 2.13 (3 H, s, CH_3), 3.08 (2 H, m, CH_2), 4.07 (3 H, m, NH and CH_2), 5.85 (1 H, br m, CH), 7.0 (2 H, d, J = 9 Hz, aromatic CH), 7.58 (2 H, d, J = 9 Hz, aromatic CH), 8.33 (3 H, br s, $^+NH_3$, D_2O exchangeable), 10.08 (1 H, s, OH, D_2O exchangeable).

Isolated Cat Heart Papillary Muscle Assay. Papillary muscles from the right ventricle of cat hearts were isolated according to the technique of Cattell and Gold,[14] mounted in a holder described by Garb,[15] and placed in a 100-mL bath containing Krebs–Henseleit solution at 37 °C (solution C of Thorp and Cobbin[16]). The papillary muscles were stimulated electrically with square-wave shocks of 4–10 ms duration, 6-10 V, and one per second frequency. Contractile force was recorded through Statham UC3 universal transducing cells. All preparations were allowed to equilibrate for about 90 min before any drugs were added to the bath. Under these conditions the contractile force of the preparations declined rapidly and reached 25–30% of the original force within 1.5–2 h. At that time the compounds were introduced into the bath, and the contractile force was recorded at 5, 10, 20, and 30 min after drug addition. Thereafter, ouabain was added to the bath at 0.5 μg/mL without change of the bath fluid. Addition of ouabain serves two purposes: (1) to indicate the responsiveness of the preparation to a cardiotonic drug and (2) for detection of a specific digitalis antagonist. An increase in contractile force over 20% indicated the presence of positive inotropic activity, while a decrease of the contractile force by over 30% indicated negative inotropic activity. Compounds with suspected positive inotropic activity were reevaluated on three additional preparations at at least three concentrations.

Effects on Myocardial Contractility in the Dog. Beagles of either sex were anesthetized with sodium vinbarbital (50–60 mg/kg iv) and respired artificially with air using a respiratory pump, Model AR-2 (Electro-Med, Inc., Minneapolis, MN). The vagi were cut. The dog's chest was opened on the left side between the fourth and sixth ribs. The left-ventricular contractile force was recorded with a strain gauge[17] sutured on the left ventricle. To record left-ventricular pressure, the left-ventricular wall was punctured with a Teflon catheter of 2-mm diameter. Left-ventricular pressure was recorded through a Statham Model p23Db pressure transducer. The maximal rate of rise of the left-ventricular pressure (first derivative, LV dp/dt max) was obtained with a Sanborn Model 350-1500A preamplifier and low-level differentiator. Arterial blood pressure was recorded from the right femoral artery with a Sanborn Model 267B transducer. The heart rate was measured by using a Viso-cardiette record inserter at a paper speed of 25 mm/s. Drugs were administered either orally or intravenously. All variables were recorded on a Sanborn Model 964 recorder.

Inhibition of Membrane ATPase. Partially purified membrane ATPase preparations were derived from guinea pig renal cortex and from calf heart and assayed according to procedures described elsewhere.[18,19] In both systems, reactions were initiated by the final addition of enzyme ("acute") and by the addition of inhibitor plus enzyme preincubated for 30 min at 10 times their ultimate concentrations to the otherwise complete assay system ("pre-incubation"). Incubations were made at 37 °C, under air, with aliquots removed at 10 and 20 min for measurement of liberated phosphate according to Lowry and Lopez.[20]

For those compounds effecting an acute inhibition of >50% at 1×10^{-3} M, I_{50} values were determined from the logarithm of the inhibitor vs. percent inhibition lines based upon a minimum of three data points.

Acknowledgment. The authors are indebted to K. Streeter and Y. Lee for the microanalyses and pK_a determinations. We also wish to thank Dr. N. R. Bohidar for the statistical analyses of biological data and Dr. M. Hite, H. R. Skeggs, and Dr. H. E. Scribner for the bacterial mutagenicity results.

References and Notes

(1) Recent attempts to obtain an effective and safer alternative to digitalis therapy have been reviewed by R. Thomas, J. Boutagy, and A. Gelbart, *J. Pharm. Sci.*, **63**, 1649 (1974).
(2) K. Dachlauer, German Patent 671785 (1939); *Chem. Abstr.*, **33**, 6345 (1939).
(3) P. Oxley, M. W. Partridge, and F. W. Short, *J. Chem. Soc.*, 303 (1948).
(4) H. J. Backer and W. L. Wanmaker, *Recl. Trav. Chim. Pays-Bas*, **70**, 638 (1951).
(5) (a) J. C. Grivas and A. Taurins, *Can. J. Chem.*, **36**, 771 (1958); (b) *ibid.*, **37**, 795 (1959); (c) *ibid.*, **37**, 1260 (1959); (d) *ibid.*, **39**, 761 (1961).
(6) A. G. Moritz, *Spectrochim. Acta*, **20**, 1555 (1964).

(7) R. A. Cummins and A. G. Moritz, *Aust. J. Chem.*, 18, 1107 (1965).
(8) A. A. Patchett, D. R. Hoff, and C. S. Rooney, U.S. Patent 3608087; *Chem. Abstr.*, **76**, 23951u (1972).
(9) D. J. Brown and R. F. Evans, *J. Chem. Soc.*, 4039 (1962).
(10) P. E. Fanta and E. A. Hedman, *J. Am. Chem. Soc.*, **78**, 1434 (1956).
(11) F. C. Schaefer and G. A. Peters, *J. Org. Chem.*, **26**, 412 (1961).
(12) A. Schwartz, *Circ. Res.*, **39**, 2 (1976).
(13) M. Hite, H. R. Skeggs, and H. E. Scribner, private communication.
(14) M. Cattell and H. Gold, *J. Pharmacol. Exp. Ther.*, **62**, 116 (1938).
(15) S. Garb, *J. Pharmacol. Exp. Ther.*, **101**, 317 (1951).
(16) R. Thorp and W. Cobbin, "Cardiac Stimulant Substances", Academic Press, New York, N.Y., 1967, p 24.
(17) K. Boniface, O. Brodie, and R. P. Walton, *Proc. Soc. Exp. Biol, Med.*, **84**, 263 (1953).
(18) D. E. Duggan and R. M. Noll, *Arch. Biochem. Biophys.*, **109**, 388 (1965).
(19) H. Matsui and A. Schwartz, *Biochim. Biophys. Acta*, **128**, 380 (1966).
(20) O. H. Lowry and J. Lopez, *J. Biol. Chem.*, **162**, 421 (1946).
(21) H. Schroeder and C, Grundmann, *J. Am. Chem. Soc.*, **78**, 2447 (1956).
(22) A. Albert and B. Paal, *Chem. Ind. (London)*, 874 (1974).
(23) G. Holan, E. L. Samuel, B. C, Ennis, and R. W. Hinde, *J. Chem. Soc. C*, 20 (1967).
(24) H. J. Roth, *Arch. Pharm. Ber. Dtsch. Pharm. Ges.*, **292**, 194 (1959).
(25) R. O. Atkinson, *J. Chem. Soc.*, 1329 (1954).
(26) G. Shtacher, M. Erez, and S. Cohen, *J. Med. Chem.*, **16**, 516 (1973).

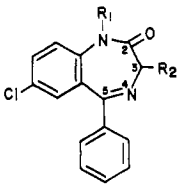
Pharmacology of Some Metabolites of Triazolam, Alprazolam, and Diazepam Prepared by a Simple, One-Step Oxidation of Benzodiazepines

Martin Gall,* B. V. Kamdar, and R. J. Collins

CNS Diseases Research, The Upjohn Company, Kalamazoo, Michigan 49001. *Received July 5, 1978*

A simple, one-step chemical oxidation of triazolam (**7**) to its 4-hydroxy analogue, **7a**, has been developed and applied to other triazolo- and imidazobenzodiazepines. The reaction may be used to convert diazepam to temazepam. 4-Hydroxytriazolo[4,3-*a*][1,4]benzodiazepines have low central nervous system sedative and anticonvulsant activity in sharp contrast to metabolites of diazepam which remain active. While **10**, an α-hydroxy metabolite of triazolam, retains much of the activity of **7**, **10a**, an α,4-dihydroxy metabolite of triazolam, is 250 times less potent than **7** on the nicotine-antagonism assay and over 300 times less potent on the traction assay.

Diazepam (**1**) is metabolized in man and the dog according to three major pathways: N-demethylation to demethyldiazepam (**2**), hydroxylation in the 3 position to temazepam (**3**), and N-demethylation plus 3-hydroxylation to oxazepam (**4**),[1] Other clinically useful benzodiazepines



1, $R_1 = CH_3$; $R_2 = H$ (diazepam)
2, $R_1 = H$; $R_2 = H$ (demethyldiazepam)
3, $R_1 = CH_3$; $R_2 = OH$ (temazepam)
4, $R_1 = H$; $R_2 = OH$ (oxazepam)
5, $R_1 = H$; $R_2 = H$ (4-*N*-oxide)
6, $R_1 = H$; $R = OCOCH_3$

are metabolized similarly in vivo.[2] In vitro hepatic microsomal preparations from mice enzymatically hydroxylate **1** and **2** at C-3 via the stereospecific removal of the pro-*S* hydrogen atom.[3] Moreover, various streptomyces stock cultures hydroxylate 1,4-benzodiazepin-2-ones in the 3 position; they do not produce 4-*N*-oxide derivatives (i.e., **5**) nor do they convert *N*-oxides to hydroxy derivatives (i.e., **5** $\not\rightarrow$ **4**).[4,5] These results suggest that **4** is produced directly from **2**,

By contrast, the standard chemical synthesis of 3-hydroxybenzodiazepine derivatives such as **4** requires either converting the 4-*N*-oxide, **5**, to the acetate, **6**, by brief heating in acetic anhydride, followed by careful hydrolysis with sodium hydroxide,[6] or treating **5** with Lewis acids in nitrile solvents to obtain **4** directly.[7] Similar methods have been used to prepare the 4-hydroxytriazolo and -imidazo analogues, **8a**[10a,c] and **13a**.[11] Both methods require the 4-*N*-oxide derivative, which is not always conveniently prepared from the parent benzodiazepine.[7b]

In this paper we describe a direct, one-step oxidation procedure which cleanly converts diazepam (**1**), triazolobenzodiazepines such as triazolam (**7**) and alprazolam (**8**), and imidazo analogue **13** to the corresponding hydroxy derivatives (**3**, **7a**, **8a**, and **13**). We also describe the animal pharmacology of the triazolobenzodiazepine metabolites whose structure–activity relationship differs considerably from the parent diazepam metabolites.

Chemistry. When oxygen was bubbled through a cold tetrahydrofuran (THF) solution of the green-black lithium anion generated from **13** and lithium diisopropylamide (procedure A), alcohol **13a** was isolated in only 11% yield.[8a] The major byproducts were partially characterized dimers of **13** (see Experimental Section).

In contrast, oxidation at −20 °C of the green-black potassium anion obtained from **13** and a slight excess of potassium *tert*-butoxide in a mixture of *tert*-butyl alcohol–DMF–THF–DME–$(EtO)_3P$ produced **13a** in 50% yield, unaccompanied by dimeric side products (procedure B).[8b] Keeping the reaction temperature cold and quenching the reaction mixture in ice-cold 5% acetic acid were essential; the 1,2-dimethoxyethane (DME) cosolvent could be replaced by THF. This procedure for oxidation of triazolam (**7**) to **7a** produced crude yields as high as 95%. Representative yields (not maximized) for the oxidation of various benzodiazepines by procedure B are summarized in Table I,

Oxidation of alcohol **11**, itself a metabolite of alprazolam, deserves further comment, With **11** as the substrate, procedure B yielded only the starting material. Since the characteristic green-black color associated with benzodiazepine anions was not observed, we conclude that the requisite anion did not form. However, the anion was

0022-2623/78/1821-1290$01.00/0