(1974).
(3) R. J. Alaimo and M. M. Goldenberg, U.S. Patent 3,763,174 (1973).
(4) M. M. Goldenberg and R. H. Burns, *Life Sci.*, **10**, 591 (1971).
(5) S. A. Komarov, H. Shay, M. Rayport, and S. S. Fels, *Gastroenterology*, **3**, 406 (1944).
(6) H. Shay, S. A. Komarov, S. S. Fels, D. Meranzi, M. Guienstein, and H. Siplet, *Gastroenterology*, **5**, 43 (1945).

Structure–Activity Relationships in Reactivators of Organophosphorus-Inhibited Acetylcholinesterase. 10. Hydroxyiminomethylarylethenylpyridine Methiodides†

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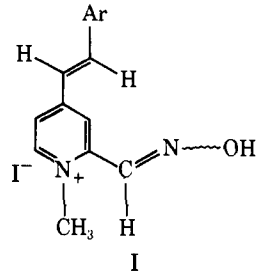
The synthesis of styrylpyridine methiodides where a hydrogen of the pyridyl moiety was replaced by the hydroxyiminomethyl group produced highly effective inhibitors of acetylcholinesterase. As starting materials 4-methylpyridine-2-aldoxime and 2-methylpyridine-4-aldoxime methiodides were prepared which, together with 4-imidazolylethenylpyridine-2-aldoxime methiodide, were the only substances for which some activity as reactivators of phosphorylated electric eel cholinesterase in vitro could also be found.

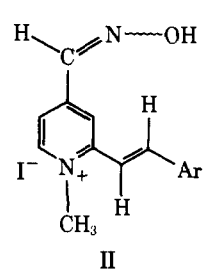
In a previous communication[1] we reported the synthesis of some heterocyclic acraldoximes methiodides, vinyl homologs of effective reactivators of acetylcholinesterase (AChE) inhibited by diisopropylphosphorofluoridate (DFP). The ethenyl group increased the binding to the enzyme and the compounds showed some inhibitory activity but still were fairly good reactivators.

Cavallito and coworkers[2–4] demonstrated a high inhibition of choline acetyltransferase (ChA) by *trans*-4-styrylpyridine methiodides and later Baker and coworkers[5,6] found this activity, although in lower degree, in nonquaternized stilbazoles; the authors agree on the importance of the trans vinyl bridge for binding to ChA either by direct interaction of the double bond with the enzyme or by maintenance of a coplanar molecule which can act both as donor and acceptor in a charge-transfer complex with the enzyme.

For our purpose it was very interesting that the abovementioned stilbazoles inhibited AChE only slightly or not at all. For this reason we decided to synthesize derivatives of 2-pyridinecarbaldoxime and 4-pyridinecarbaldoxime methiodides (2-PAM and 4-PAM) having arylethenyl residues in the 4 and 2 positions, respectively. Compounds of general formula I and II might have more or less affinity for AChE, depending on the kind of substituent on the aryl, but could theoretically have reactivating properties.



I



II

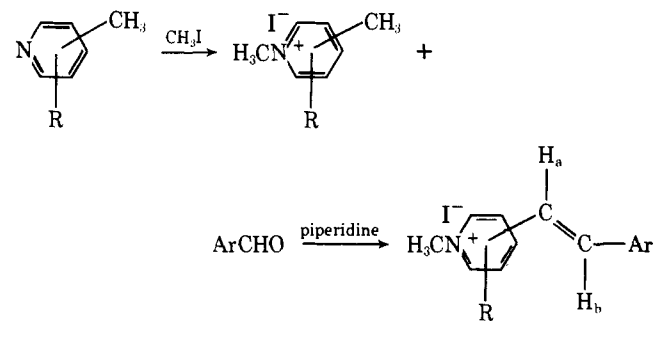
The substituents on Ar were chosen among those increasing the activity of the simple stilbazole as an inhibitor of ChA and not of AChE; imidazolyl groups in the place of Ar were taken into account for reasons given elsewhere.[7,8]

Chemistry. 2-Hydroxyiminomethyl-4-methylpyridine (**1**) was prepared by reaction of 4-methylpyridine-2-carboxaldehyde diacetate[9] with hydroxylamine hydrochloride. The *E* configuration was assigned to the oxime **1** since it gave a red-brown color with ferrous salts[10] and green copper chelate with cupric ions;[11] furthermore, the ir spectrum in $CHCl_3$ showed a sharp band at 3570 cm^{-1}, characteristic of a free OH group, and in the NMR spectrum the difference Δ [δ(OH) − δ(CH=N)] was 3.58 ppm.[12]

Forman's synthesis[13] was followed for the preparation of 4-hydroxyiminomethyl-2-methylpyridine (**15**) and the *Z* configuration of the oxime was confirmed by the signals at δ 12.20 (OH) and 7.40 (CH=N) of the NMR spectrum in Me_2SO-d_6 (Δ = 4.8 ppm).

In the reaction of **1** and **15** with methyl iodide the aldoxime group seems to retain its configuration since in the NMR spectra Δ is 4.35 ppm for **2** and 5.15 ppm for **16** (Scheme I).

Scheme I



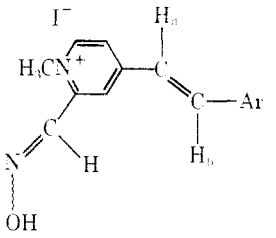
R = 2- or 4-CH=NOH, respectively, in the case of 4-methyl or 2-methyl derivatives

The quaternary salts **2** and **16** were condensed with aldehydes in methanol, using piperidine as catalyst.[14]

The trans configuration of the ethenyl group was proved by uv and NMR spectra; all compounds had λ_{max} between 352 and 400 nm and, when they were exposed in EtOH solution to sunlight, a rapid shift of the maxima to lower wavelengths occurred, showing photoisomerization from trans to cis configuration.[15] In the NMR spectra the protons H_a and H_b had a trans coupling constant ranging from 15.6 to 18 Hz.[16] The trans configuration of compounds **3, 4, 9,** and **17–19** was assigned by uv spectra only, because of the low solubility of **4, 18,** and **19** in Me_2SO-d_6 and of the

†Supported by the Italian National Research Council.

Table I. *N*-Methyl-2-hydroxyiminomethyl-4-arylethenylpyridinium Iodides



Compd	Ar	Mp, °C	Yield, %	Uv (EtOH) λ_{max}, nm	Uv (EtOH) ϵ	NMR (Me_2SO-d_6) chemical shifts (δ) and coupling constant (Hz) H_a	H_b	J_{ab}	Formula[a]
3	C_6H_5	220–221	93	364	26,106				$C_{15}H_{15}N_2OI$
4	α-Naphthyl	233–234	48	399	18,809				$C_{19}H_{17}N_2OI$
				286	15.725				
5	2-Cl-C_6H_4	209–210	57	352	24,273	7.60 (d)		15.6	$C_{15}H_{14}N_2OClI$
6	3-Cl-C_6H_4	212–213	32	354	24,122	7.55 (d)		18.0	$C_{15}H_{14}N_2OClI$
7	4-Cl-C_6H_4	222–223	43	366	25,134	7.55 (d)		18.0	$C_{15}H_{14}N_2OClI$
8	2-Br-C_6H_4	207–208	29	358	21,122	7.55 (d)		16.0	$C_{15}H_{14}N_2OBrI$
9	3-Br-C_6H_4	213–214	37	352	24,783				$C_{15}H_{14}N_2OBrI$
10	2-OCH_3-C_6H_4	222–223	91	388	22,219	7.70 (d)		16.0	$C_{16}H_{17}N_2O_2I$
11	3-OCH_3-C_6H_4	218–219	43	368	23,078	7.52 (d)		18.0	$C_{16}H_{17}N_2O_2I$
12	4-OCH_3-C_6H_4	220–221	22	400	31,052	7.60 (d)		18.0	$C_{16}H_{17}N_2O_2I$
13	*N*-Benzylimidazolyl-2	154–156	22	398	21,992	7.78 (d)	7.27 (d)	16.0	$C_{19}H_{19}N_4OI \cdot H_2O$
14	Imidazolyl-4	201–202	32	388	24,404	7.92 (d)	7.22 (d)	16.0	$C_{12}H_{13}N_4OI$

[a] All compounds were analyzed for C, H, and N.

complexity of the NMR spectra of **3**, **9**, and **17** which did not permit their interpretation.

Reaction of **2** with imidazole-2-carboxaldehyde gave only decomposition products, and attempts to debenzylate **13** were also unsuccessful, since *N*-methyl-2-hydroxyiminomethyl-4-(imidazolyl-2)ethenylpyridinium iodide was not obtained.

Results and Discussion

The results of enzymatic assays show that, with the exception of compounds **2**, **3**, and **14**, the products are of little value as in vitro reactivators of DFP-inhibited AChE. The stilbazoles obtained starting from 2-methyl-4-hydroxyiminomethylpyridine methiodide (**16**) appear still less active than the isomers; therefore, we prepared only a few representatives. Preliminary data (results obtained by Dr. L. Parente in the Pharmacological Institute of the Faculty of Pharmacy, University of Naples) obtained on 2-hydroxyiminomethyl-4-methylpyridine methiodide (**2**) indicate that it offers greater protection (1.33 times) from DFP in the mouse than does 2-PAM; better results can be expected from in vivo testing for the whole series, as the products should have a more favorable partition coefficient. In addition, a reactivator which is a good reversible inhibitor of AChE may serve a dual role in retarding further phosphorylation of the enzyme by DFP.

We wish to stress the increase of achE-inhibiting potency following the introduction of the hydroxyiminomethyl group in the pyridine moiety of the stilbazole methiodides, as appears in Table IV from the values obtained for the new compounds in comparison with *N*-methyl-4-(*m*-chlorophenylethenyl)pyridinium iodide (**21**) and *N*-methyl-4-(α-naphthylethenyl)pyridinium iodide (**22**).[4] The modifications accomplished at this time on the quaternary stilbazole relate mostly to the phenyl moiety, where electron-withdrawing substituents enhance AChE inhibition; as regards the pyridine ring, introduction of a 3-methyl substituent[4] or replacement of the whole pyridinium moiety with some aminopyrimidines[20] enhances ChA inhibition; in both cases specificity between the enzymes is claimed to be maintained.

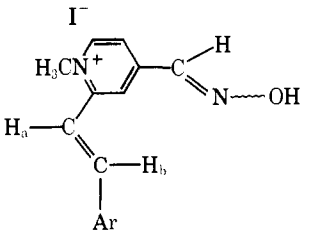
Experimental Section

Melting points were taken in capillary tubes on an Electrothermal apparatus and are uncorrected. The uv spectra were recorded on a Beckmann DB-GT spectrophotometer in EtOH solution; ir spectra were run on a Unicam SP 200 spectrophotometer; NMR spectra were recorded in Me_2SO-d_6 solution on a Perkin-Elmer R24 A spectrophotometer and chemical shifts are reported in parts per million (δ) from internal Me_4Si. Where analyses are indicated only by symbols of the elements, analytical results obtained for those elements were within 0.4% of the theoretical value. The following aldehydes were prepared according to the cited references: 1*H*-imidazole-4-carboxaldehyde,[17] 1-benzylimidazole-2-carboxaldehyde.[18]

(*E*)-2-Hydroxyiminomethyl-4-methylpyridine (**1**). A solution in 90 ml of 80% EtOH containing 18 g (0.26 mol) of NH_2OH and 30 g (0.13 mol) of 4-methylpyridine-2-carboxaldehyde diacetate,[9] purified by distillation [bp 120–122° (0.4 mm)], was refluxed for 4 hr. Evaporation of the solvent under reduced pressure left an oily residue which was dissolved in H_2O, the solution was made basic with saturated aqueous $NaHCO_3$, and the resulting precipitate was crystallized from EtOAc: mp 171–173°; yield 55%; uv max (EtOH) 248 nm (ε 11,059); ir ($CHCl_3$) 3570 cm^{-1}; NMR (Me_2SO-d_6) 11.62 (s, 1 H, OH), 8.35 (d, 1 H, J = 5 Hz, H-6), 8.1 (s, 1 H, CH=N), 7.55 (s, 1 H, H-3), 7.15 (d, 1 H, J = 5 Hz, H-5), 2.50 (s, 3 H, CH_3). Anal. ($C_7H_8N_2O$) C, H, N.

2-Hydroxyiminomethyl-4-methylpyridine Methiodide (**2**). To 1 g (0.007 mol) of **1** in 10 ml of dry EtOH 1.42 g (0.01 mol) of CH_3I was added; the solution, refluxed for 6 hr, afforded the quaternary salt which was crystallized from EtOH: mp 213–214°; yield 71%; uv max (EtOH) 298 nm (ε 12,989); NMR (Me_2SO-d_6) 12.9 (s, 1 H, OH), 8.85 (d, 1 H, J = 6.5 Hz, H-6), 8.62 (s, 1 H, H-3), 8.55 (s, 1 H, CH=N), 7.89 (d, 1 H, J = 6.5 Hz, H-5), 4.30 (s, 3 H, NCH_3).

Table II. *N*-Methyl-2-arylethenyl-4-hydroxyiminomethylpyridinium Iodides



Compd	Ar	Mp, °C	Yield, %	Uv (EtOH) λ_{max}, nm	Uv (EtOH) ϵ	NMR (Me_2SO-d_6) chemical shifts (δ) and coupling constant (Hz) H_a	H_b	J_{ab}	Formula[a]
17	C_6H_5	241–242	78	366	18,737				$C_{15}H_{15}N_2OI$
				288	19,357				
18	α-Naphthyl	252–253	90	395	10,823				$C_{19}H_{17}N_2OI$
				294	14,985				
19	3-Cl-C_6H_4	264–265	95	354	20,426				$C_{15}H_{14}N_2OClI$
				284	18,824				
20	Imidazolyl-4	222–224	66	387	19,934	7.95 (d)	7.30 (d)	16	$C_{12}H_{13}N_4OI$
				295	16,141				

[a] See footnote *a*, Table I.

Table III. pK_a and Activities on AChE and on Inhibited AChE

Compd	pK_a[a]	Rel potency as reactivator of the oximes, 1×10^{-3} *M* A[b]	Rel potency as reactivator of the oximes, 1×10^{-3} *M* B[c]	% inhibn by oximes, 1×10^{-3} *M*	I_{50}[d]
2-PAM	7.85	1	1	0	3.8×10^{-3}
4-PAM	8.50	0.32 ± 0.04[e]	0.32	0	4.0×10^{-3}
2	7.90	0.36 ± 0.05	0.71	49.5	1.01×10^{-3}
3	8.20	0.10 ± 0.01	1.00	90.1	1.50×10^{-4}
14	8.60	0.145 ± 0.02	1.74	91.6	1.60×10^{-4}
16	8.70	0.13 ± 0.02	0.19	32.0	3.46×10^{-3}
17	8.85	0.06 ± 0.007	0.14	57.2	7.00×10^{-4}
20	8.95	0.04 ± 0.006	0.12	66.9	4.00×10^{-4}

[a] pK_a values were obtained by potentiometric titration. [b] The *A* values are the mean of four experiments. [c] Corrected for anti-AChE activity at 1×10^{-3} *M*. [d] Concentration for 50% inhibition. [e] SD.

2.62 (s, 3 H, CH_3). Anal. ($C_8H_{11}N_2OI$) C, H, N.

2-Methyl-4-hydroxyiminomethylpyridine Methiodide (16). This was prepared from the oxime **15** by the same method as **2** and was crystallized from MeOH: mp 202–203°; yield 87%; uv max (EtOH) 285 nm (ε 14,081), 358 (2664); NMR (Me_2SO-d_6) 12.95 (s, 1 H, OH), 9.05 (d, 1 H, J = 6 Hz, H-6), 8.25–8.45 (m, 2 H, H-3 and H-5), 7.80 (s, 1 H, CH=N), 4.28 (s, 3 H, NCH_3), 2.82 (s, 3 H, CH_3). Anal. ($C_8H_{11}N_2OI$) C, H, N.

Hydroxyiminomethylarylethenylpyridine Methiodides (3–14 and 17–20). To 1 mol of **2** or **16** in the minimum amount of MeOH 2 mol of the proper aldehyde and 0.5 mol of piperidine were added. The solution, refluxed for 2.5 hr, left a crystalline product which was recrystallized from MeOH (Tables I and II).

Enzymatic Assays. The in vitro reactivating potency of the new products was determined on electric eel AChE (Sigma Chemical Co.) inhibited by DFP, according to the procedure previously described.[19] For most compounds the AChE inhibitory activity at the concentration used in the reactivation assay was too high to allow reactivation potency to be estimated; as is shown in Table IV, a notable inhibition was exerted by the products also at concentrations up to hundred times lower than that suitable for the reactivation assay. In Table III, therefore, we report only the results on products **2**, **3**, **14**, **16**, **17**, and **20**, related to the activity of 2-PAM determined in parallel experiments. The AChE-inhibiting activity of all compounds was determined by the same procedure as in the reactivation test, but without DFP. The I_{50} values are reported in

Table IV. pK_a and Activities on AChE

Compd	pK_a[a]	I_{50}[b]
4	8.10	8.70×10^{-6}
5	8.00	3.45×10^{-5}
6	8.20	2.45×10^{-5}
7	8.20	4.20×10^{-5}
8	7.85	1.60×10^{-5}
9	8.10	3.70×10^{-5}
10	8.29	3.80×10^{-5}
11	8.28	5.50×10^{-5}
12	7.98	3.80×10^{-5}
13	7.82	3.30×10^{-5}
18[c]		2.28×10^{-6}
19[c]		5.60×10^{-5}
21[d]		2.28×10^{-4}
22[d]		1.50×10^{-4}

[a] pK_a values were obtained by potentiometric titration. [b] Concentration for 50% inhibition. [c] Solubility was too low to allow determination of pK_a. [d] **21** = *N*-methyl-4-(*m*-chlorophenylethenyl)pyridinium iodide; **22** = *N*-methyl-4-(α-naphthylethenyl)pyridinium iodide.[4]

Tables III and IV and compared with the values obtained under the same experimental conditions for two of the compounds described by Cavallito and coworkers.[4]

References and Notes

(1) P. Franchetti, M. Grifantini, and S. Martelli, *J. Med. Chem.*, 18, 839 (1975) (paper 9).
(2) J. C. Smith, C. J. Cavallito, and F. F. Foldes, *Biochem. Pharmacol.*, **16**, 2438 (1967).
(3) C. J. Cavallito, H. S. Yun, J. C. Smith, and F. F. Foldes, *J. Med. Chem.*, **12**, 134 (1969).
(4) C. J. Cavallito, H. S. Yun, T. Kaplan, J. I. Smith, and F. F. Foldes, *J. Med. Chem.*, **13**, 221 (1970).
(5) B. R. Baker and R. E. Gibson, *J. Med. Chem.*, **14**, 315 (1971).
(6) B. R. Baker and R. E. Gibson, *J. Med. Chem.*, **15**, 639 (1972).
(7) T. Karlsson, K. E. Stensiö, and K. Wahlberg, *Acta Chem. Scand.*, **27**, 2244 (1973).
(8) P. Franchetti, M. Grifantini, S. Martelli, and M. L. Stein, *Farmaco, Ed. Sci.*, **29**, 309 (1974).
(9) N. Nischimoto and T. Nakashima, *Yakugaku Zasshi*, 81, 88 (1961); *Chem. Abstr.*, **55**, 134201 (1962).
(10) J. E. Blackwood, C. L. Gladys, K. L. Loening, A. E. Petrarca, and J. E. Rush, *J. Am. Chem. Soc.*, **90**, 509 (1968).
(11) Y. Ashani, H. Edery, J. Zahavy, W. Kunberg, and S. Cohen, *Isr. J. Chem.*, **3**, 133 (1965).
(12) G. G. Kleinspehn, J. A. Jung, and S. A. Studniarz, *J. Org. Chem.*, **32**, 460 (1967).
(13) S. E. Forman, *J. Org. Chem.*, **29**, 3323 (1964).
(14) A. P. Phillips, *J. Org. Chem.*, **12**, 333 (1947).
(15) J. L. R. Williams, S. K. Webster, and J. A. Van Allan, *J. Org. Chem.*, **26**, 4893 (1961).
(16) L. M. Jackman and S. Sternhell, "Application of Nuclear Magnetic Resonance Spectroscopy in Organic Chemistry", Pergamon, Oxford, 1969, p 301.
(17) F. L. Pyman, *J. Chem. Soc.*, **109**, 186 (1916).
(18) P. Fournari, P. de Cointet, and E. Laviron, *Bull. Soc. Chim. Fr.*, 2438 (1968).
(19) P. Franchetti, M. Grifantini, S. Martelli, and M. L. Stein, *J. Med. Chem.*, **17**, 18 (1974).
(20) R. E. Gibson and B. R. Baker, *J. Med. Chem.*, **17**, 1290 (1974).

Studies of Platinum Complex Inhibition of Leucine Aminopeptidase

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The inhibition of swine kidney leucine aminopeptidase by *N*-alkyl-substituted ethylenediamine liganded dihaloplatinum chelates has been investigated. The rate of this inhibition at 37° is considerably less than that for the platinum tetra- and hexahalo complexes and also the ethylenediamine dihalo chelates. Only mixed inhibition was observed in these studies. For the time studies used here little if any inhibition occurred at room temperature. L-Methionine and L-Ala-L-Met did not reverse the platinum chelate inhibition nor did they prevent it.

In earlier studies, Guthrie et al.[1] reported on the inhibition of swine kidney leucine aminopeptidase (E.C. 3.4.1.1) by halide complexes of platinum. The tetrabromoplatinate ion (5×10^{-3} *M*) inhibited the peptidase completely within 1 hr at 38° while the ethylenediamine dibromo complex required 50 hr of incubation at 38° and the same concentration of inhibitor to produce 80% inhibition of the peptidase. Melius et al.[2] presented evidence for the $PtBr_3(H_2O)^-$, aquoplatinum(II) complexes, actually acting as the enzyme inhibitor so that the rate of inhibition would depend on the rate of aquation of the Pt complexes used. Of course, if the Pt complex solutions are sufficiently aged, the rate of inhibition will depend then only on the rate of attack of nucleophilic groups in the protein on the aquoplatinum complex. The final extent of inhibition at equilibrium will depend on the K_i.

$$E + Pt(a_2NCH_2CH_2Nb_2)Cl_{x-1}\cdot H_2O \rightleftarrows E\cdot Pt(a_2NCH_2CH_2Nb_2)Cl_{x-1}\cdot(H_2O)$$

$$K_i = \frac{[E][Pt(a_2NCH_2CH_2Nb_2)Cl_{x-1}\cdot H_2O]}{[E\cdot Pt(a_2NCH_2CH_2Nb_2)Cl_{x-1}\cdot(H_2O)]}$$

Various studies by Melius,[2] Friedman et al.,[3] and Teggins and Friedman[4] indicate the haloplatinum and haloammine Pt complexes are potent general inhibitors of certain enzymes. As a continuation of these studies this report investigates the modification of the ammine ligand with various alkyl groups and the effect on inhibition of leucine aminopeptidase. Whereas many other laboratories have concentrated their efforts on the interaction of Pt complexes with nucleic acids, our emphasis has been on the Pt–protein (enzyme) interactions. Many platinum complexes have been evaluated as anticancer agents, examples of which are dichloro(1,2-diaminocyclohexane)platinum(II),[5] *cis*-dichlorodiammineplatinum(II),[6] and *cis*-dichlorobis(cyclopentylamine)platinum(II)[7] and have been found to be quite effective. Complexes **2**, **5**, and **6** reported here have been shown to be active against leukemia L1210.[8] The other complexes are currently being tested at the Chester Beattie Institute and the Imperial Cancer Research Fund.

From the various studies performed in this laboratory, it is well established that the platinum complexes react generally with proteins.[1-4,12] It is important that more specific platinum complex agents should be designed to reduce their toxic interactions with enzyme systems of the normal cells. Also the rate of reactions with proteins should be decreased so that the platinum complexes will be able to get to the appropriate tumor sites without producing toxic reactions in normal tissues.[9]

Experimental Section

The substituted ethylenediamine ligands were supplied by the Ames Chemical Co. and the platinum salts by Johnson Matthey Ltd.

Preparation of the Complexes. Platinum(II) Complexes. Potassium iodide (9.6 mmol) was added to potassium chloroplatinate(II) (2.4 mmol) in water (ca. 30 cm^3) and the solution was stirred. After 10–15 min the diamine (2.4 mmol) was added and stirring was continued for 24 hr. The product formed was filtered off and washed with water (15 cm^3). It was subsequently suspended in water (25 cm^3), silver nitrate (4.8 mmol) was added, and the mixture was stirred for 24 hr. The precipitate of AgI was filtered off after addition of 0.1 *M* HCl. Excess potassium chloride was