Structure-Activity Relations in Organophosphorus Inhibited Acetylcholinesterase Reactivators I: Methiodides of New Mono- and Dioximes with Pyridine Nucleus

MARIO GRIFANTINI, SANTE MARTELLI, and MARIA L. STEIN

Abstract □ A series of methiodides deriving from *syn* β-pyridyl-α,-β-dihydroxyimino-propioanilides, from β-pyridyl-β-oxo-α-hydroxyimino-propioanilides showing the hydroxyimino group in the two possible configurations and from *anti* β-pyridyl-β-hydroxyimino-propioanilides was prepared. The *in vitro* acetylcholinesterase-reactivating activity of the new compounds was evaluated. The results show that the β-hydroxyimino group contributes very little to the reactivation, probably because it is held by the amide group through a hydrogen bond; in fact, the β-monoximes are nearly inactive. Therefore the activity of the dioximes should be ascribed to the α-hydroxyimino group.

Keyphrases □ Acetylcholinesterase reactivators—organo-phosphorus inhibited □ Methiodides of mono- and dioximes—synthesis □ Structure-activity relationships—acetylcholinesterase reactivators □ UV spectrophotometer—structure □ IR spectrophotometry—structure

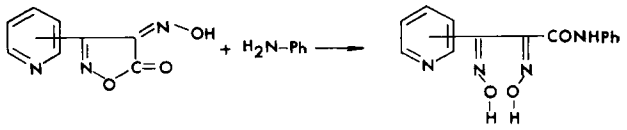
Several quaternary salts of pyridyl-oximes have been synthesized in the last few years in order to obtain nucleophilic agents suitable for restoring the activity of phosphoric ester-inhibited cholinesterase (1–3). The substances prepared up to now with the aim of studying the structure-activity relations in this class of drugs, generally contain the hydroxyimino group in the side chain in the position α to the pyridine cycle. Little has been done on compounds containing two hydroxyimino groups in the side chain or one of such groups in the β-position. For instance, Pitman and Sadler (4) described dimethiodides of two isomeric 2,2′-pyridyl dioximes, which, however, are to be considered bis oximes α to the cycle. Their reactivating properties were found to be similar to those of the salts of 2-hydroxyiminomethyl-1-methyl-pyridinium (2-PAM). Wilson (5) reported that the methiodides of nicotinoyl and isonicotinoyl-formaldoximes are 30 times less active than 2-PAM.

It appeared interesting to investigate further the reactivating properties of quaternary derivatives of the pyridine series containing in the side chain two *vic* hydroxyimino groups or one β-hydroxyimino group.

CHEMISTRY

A method for obtaining easily the β-substituted α,β-dihydroxyimino-propionamides starting from 3-substituted 4-hydroxyimino-5-isoxazolinones was recently developed in the authors' laboratory (6, 7). By applying this method to the hydroxyimino-isoxazolinones of the pyridine series it was possible, through the action of aniline, to obtain the corresponding β-pyridyl-α,β-dihydroxyimino-propioanilides (Scheme I).

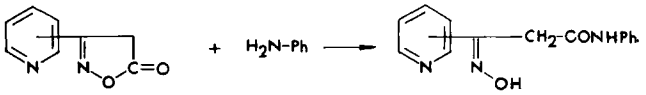
On the basis of the analogy of chemical behavior with the corresponding benzene derivatives (7, 8), the *syn* configuration can be



Scheme I

ascribed to these dioximes. In fact, they give no precipitate with nickel salts and undergo fission by the action of bases to the corresponding cyanopyridine and formylamidoxime (7); moreover, the product with the chain in 2-position of the pyridine nucleus gives a red color with ferrous salts typical of a hydroxyimino group in the *anti*-pyridyl position (9).

In an attempt to obtain some indication of the contribution of each hydroxyimino group in these molecules, β-pyridylpropioanilides containing only one hydroxyimino group in the α- or β-position, whose steric configuration would be identical with that of the corresponding group present in the dioxime were synthesized. The *anti* β-pyridyl-β-hydroxyimino-propioanilides were synthesized from the 3-pyridyl-isoxazolin-5-ones. Like their 4-hydroxyimino derivatives, these undergo a nucleophilic attachment to carbon 5 with aniline at room temperature, followed by opening of the cycle and retention of the configuration of the hydroxyimino group (Scheme II).

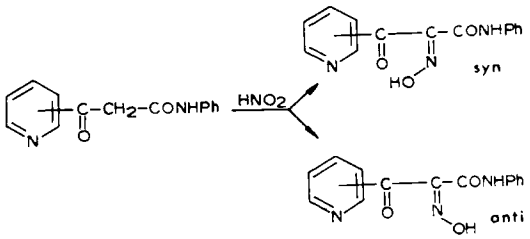


Scheme II

The *anti*-pyridyl configuration was confirmed by the fact that the β-(2-pyridyl)-β-hydroxyimino-propioanilide gives a red-colored complex with ferrous sulfate (8). The same configuration was ascribed to the β-(3-pyridyl)- and β-(4-pyridyl)- derivatives, both because of the resemblance of the UV spectra ($\lambda_{max.}^{EOH}$ 244 mμ, ϵ = 22,000) and because the preparation method was identical.

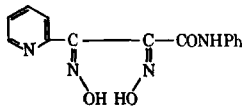
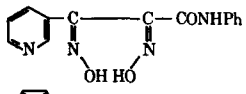
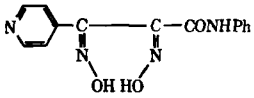
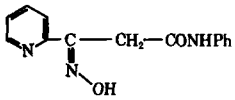
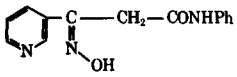
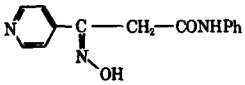
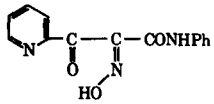
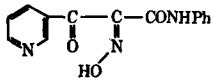
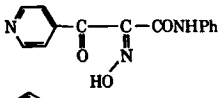
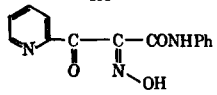
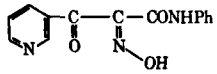
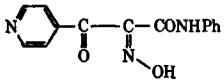
In order to obtain derivatives containing a hydroxyimino group in the α-position to the amide group, β-pyridyl-β-oxo-propioanilides were nitrosated; these compounds containing a keto group in the β-position were chosen in order to synthesize a type of molecule whose electronic structure would be comparable to that of the dioximes. The treatment with nitrous acid gives in every case two isomeric oximes separable by chromatography on silica gel, eluted with ethyl acetate (Scheme III).

A comparison of UV spectra was used to ascribe the structure to the compounds obtained. The *anti* products give an absorption



Scheme III

Table I—Mono- and Bis-Hydroxyimino-β-Pyridyl-Propioanilides

No.	Compd.	Crystn. Solvent	M.p., °C.	Formula	Analysis, % Calcd.	Found
I		EtOH	174–175		(See *ref.* 6)	
III		EtOH	200–201	$C_{14}H_{12}N_4O_3$	C, 59.15 H, 4.26 N, 19.71	C, 59.47 H, 4.23 N, 19.46
IV		EtOH–H_2O	221–222	$C_{14}H_{12}N_4O_3$	C, 59.15 H, 4.26 N, 19.71	C, 59.09 H, 4.33 N, 19.41
V		EtOH–H_2O	125–127	$C_{14}H_{13}N_3O_2$	C, 65.87 H, 5.13 N, 16.46	C, 65.87 H, 5.04 N, 16.57
VI		EtOH–H_2O	150–152	$C_{14}H_{13}N_3O_2$	C, 65.87 H, 5.13 N, 16.46	C, 66.11 H, 5.17 N, 16.31
VII		EtOH	205–206	$C_{14}H_{13}N_3O_2$	C, 65.87 H, 5.13 N, 16.46	C, 65.97 H, 5.39 N, 16.76
IX		EtOAc–petroleum ether	200–202	$C_{14}H_{11}N_3O_3$	C 62.45 H, 4.12 N, 15.61	C, 62.50 H, 4.13 N, 15.57
XI		EtOH–H_2O	185–186	$C_{14}H_{11}N_3O_3$	C, 62.45 H, 4.12 N, 15.61	C, 62.73 H, 4.22 N, 15.86
XIII		EtOAc–petroleum ether	194–195	$C_{14}H_{11}N_3O_3$	C, 62.45 H, 4.12 N, 15.61	C, 62.68 H, 4.19 N, 15.56
X		EtOAc–cyclohexane	173–175	$C_{14}H_{11}N_3O_3$	C, 62.45 H, 4.12 N, 15.61	C, 61.97 H, 4.30 N, 15.36
XII		EtOH	180–181	$C_{14}H_{11}N_3O_3$	C, 62.45 H, 4.12 N, 15.61	C, 62.22 H, 4.05 N, 15.26
XIV		EtOAc–cyclohexane	185–186	$C_{14}H_{11}N_3O_3$	C, 62.45 H, 4.12 N, 15.61	C, 62.18 H, 4.01 N, 15.25

maximum in ethanol at 243–245 mμ, which by addition of dilute NaOH undergoes a shift towards greater wavelengths with a Δλ of 30–38 mμ; this has been assumed in the literature as an index of an *anti* structure of α-hydroxyiminoketones (10). The structures of the products were further confirmed by the fact that only the *anti* derivatives give precipitates with nickel salts (11). The *syn* series products easily isomerize in acidic or basic medium, originating the *anti* series products; it should be noted that the 2-pyridyl *anti* derivative is very unstable since the isomerization reaction is reversible. For this reason it was not possible to obtain a pure methiodide of Compound X. In ethanol the *anti* derivatives have an UV spectrum with an absorption maximum at 223 mμ.

The series of oximes obtained is listed in Table I. By reaction with methyl iodide, in suitable solvents, the quaternary salts reported in Table II were synthesized. All the pyridinium compounds were subjected to biological assay.

BIOLOGICAL ASSAY

The biological assay was performed by measuring the *in vitro* reactivating velocity of acetylcholinesterase inhibited by DFP and TEPP, according to the technique described by Ashani *et al.* (12). Table III shows the results obtained.

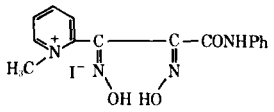
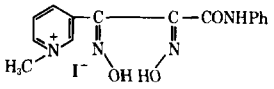
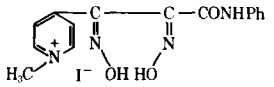
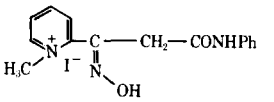
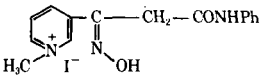
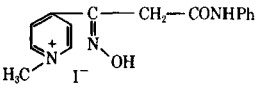
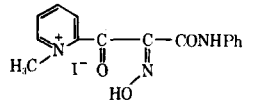
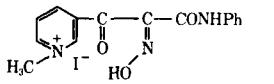
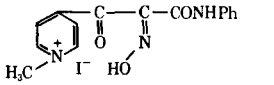
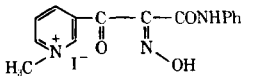
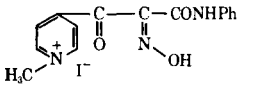
EXPERIMENTAL[1]

3-(γ-Pyridyl)-4-hydroxyiminoisoxazolin-5-one (II)—To a solution of 1 g. of 3-(γ-pyridyl)-2-isoxazolin-5-one (14) in 20 ml. of 2 *N* NaOH was added 0.5 g. of $NaNO_2$ and enough 2 *N* HCl to make the pH slightly acid; a violet solid precipitated which was purified by washing with H_2O. The product, which could not be crystallized because it decomposed when hot, melted at 170–172° (dec.): $\lambda_{max.}^{EtOH}$ mμ (ε × 10^{-3}): 258 (17.2), inflection 335 (4.04); ν (cm.$^{-1}$): 1,770 (C=O), 3,100 (OH).

Anal.—Calcd. for $C_8H_5N_3O_3$: C, 50.26; H, 2.64; N, 21.99. Found: C, 49.96; H, 2.59; N, 21.60.

[1] Melting points are uncorrected. UV spectra were recorded on a UNICAM model SP 800 spectrophotometer and IR spectra on a UNICAM model SP 200. Microanalyses were performed by Dr. G. Valentini.

Table II—Methiodides of Oximes of Table I

No.	Compd.	Crystn. Solvent	M.p., °C.	Methods[a]	Formula	Analysis, % Calcd.	Analysis, % Found
XV		Me_2CO–Et_2O	164–165	A	$C_{15}H_{15}IN_4O_3$	C, 42.26 H, 3.54 N, 13.14	C, 42.50 H, 3.80 N, 12.85
XVI		H_2O	162–164	B	$C_{15}H_{15}IN_4O_3$	C, 42.26 H, 3.54 N, 13.14	C, 42.18 H, 3.46 N, 13.30
XVII		H_2O	157–159	B	$C_{15}H_{15}IN_4O_3$	C, 42.26 H, 3.54 N, 13.14	C, 42.30 H, 3.64 N, 13.31
XVIII		Me_2CO–EtOAc	202–204	C	$C_{15}H_{16}IN_3O_2$	C, 45.69 H, 4.06 N, 10.57	C, 45.27 H, 4.32 N, 10.33
XIX		Me_2CO	164–166	D	$C_{15}H_{16}IN_3O_2$	C, 45.69 H, 4.06 N, 10.57	C, 45.96 H, 3.98 N, 10.16
XX		Me_2CO–EtOAc	151–153	C	$C_{15}H_{16}IN_3O_2$	C, 45.69 H, 4.06 N, 10.57	C, 45.97 H, 3.84 N, 10.89
XXI		EtOAc–Et_2O	170–171	A	$C_{15}H_{14}IN_3O_3$	C, 43.81 H, 3.43 N, 10.22	C, 44.16 H, 3.27 N, 10.40
XXII		Me_2CO	196–198	A	$C_{15}H_{14}IN_3O_3$	C, 43.81 H, 3.43 N, 10.22	C, 43.94 H, 3.62 N, 10.28
XXIII		EtOH	210–211	A	$C_{15}H_{14}IN_3O_3$	C, 43.81 H, 3.43 N, 10.22	C, 44.11 H, 3.53 N, 9.93
XXIV		Me_2CO–EtOAc	193–195	A	$C_{15}H_{14}IN_3O_3$	C, 43.81 H, 3.43 N, 10.22	C, 43.73 H, 3.68 N, 10.16
XXV		Me_2CO–EtOAc	178–180	A	$C_{15}H_{14}IN_3O_3$	C, 43.81 H, 3.43 N, 10.22	C, 44.09 H, 3.53 N, 10.18

[a] See *Experimental* section.

syn α,β-Dihydroxyimino-β-(3-pyridyl)-propioanilide (III)—To a suspension of 0.5 g. of 3-(β-pyridyl)-4-hydroxyimino-2-isoxazolin-5-one (15) in 20 ml. of EtOH was added 0.4 g. of aniline; it was heated to the boiling point until the solid had passed completely into solution and then for a further 15 min. By evaporation of the solvent, a residue was obtained which was crystallized from a little EtOH. It gave no precipitate with nickel salts. $\lambda_{max.}^{EtOH}$ mμ ($\epsilon \times 10^{-3}$): 252 (29), inflection at 275 (24); ν (cm.$^{-1}$): 1,665 (C=O), 3,150–3,300 (NH, OH).

See Table I for additional data on this as well as Compounds IV–VII and IX–XIV.

syn α,β-Dihydroxyimino-β-(4-pyridyl)-propioanilide (IV)—To a suspension of 1 g. of II in 30 ml. of EtOH was added 0.8 g. of aniline; it was heated with refluxing for 3 hours, then the ethanol was evaporated to a small volume. Addition of water precipitated a product which was collected and recrystallized from EtOH–H_2O. $\lambda_{max.}^{EtOH}$ mμ ($\epsilon \times 10^{-3}$): 258 (31.5); ν (cm.$^{-1}$): 1,665 (C=O), 3,200–3,350 (NH, OH).

anti β-(2-Pyridyl)-β-hydroxyimino-propioanilide (V)—To 1 g. of 3-(2-pyridyl)-isoxazolin-5-one (7) suspended in 30 ml. of EtOH, was added 1 ml. of aniline; after 2 days the ethanol was evaporated leaving a residue that solidified by treatment with ethyl acetate; it crystallized from EtOH–H_2O. $\lambda_{max.}^{EtOH}$ mμ ($\epsilon \times 10^{-3}$): 243 (20.7); ν (cm.$^{-1}$): 1,670 (C=O), 3,230–3,340 (NH, OH).

anti β-(3-Pyridyl)-β-hydroxyimino-propioanilide (VI)—Prepared starting from 3-(3-pyridyl)-isoxazolin-5-one (14) according to the method described for Product V. After 1 day it was filtered from the isoxazolinone that had not reacted; from the filtrate, on standing, a white solid was obtained which was crystallized from ethanol. $\lambda_{max.}^{EtOH}$ mμ ($\epsilon \times 10^{-3}$): 243 (23.9); ν (cm.$^{-1}$): 1,680 (C=O), 3,300 (NH).

anti β-(4-Pyridyl)-β-hydroxyimino-propioanilide (VII)—Prepared starting from II according to the method described for the preparation of V. After 2 days the isoxazolinone that had not reacted was filtered; after 2 days more the ethanol was concentrated to half volume and by cooling crystals were obtained which were recrystallized from ethanol. $\lambda_{max.}^{EtOH}$ mμ ($\epsilon \times 10^{-3}$): 243 (22.4); ν (cm.$^{-1}$): 1,660 (C=O), 3,180–3,250 (NH, OH).

β-(2-Pyridyl)-β-oxo-propioanilide (VIII)—The product was prepared by refluxing equimolecular quantities of ethyl picolinoylacetate and aniline for 12 hr. in xylene. By cooling and addition of petroleum ether, a yellow solid was obtained which, filtered and crystallized from methylene chloride, melted at 127–128°. ν (cm.$^{-1}$): carbonyl bands at 1,660–1,680, 3,150 (NH).

Table III—Reactivation of Inhibited Bovine Erythrocyte Acetylcholinesterase by Means of Oximes XV–XXV at pH 7.4 and 25°C.

Oximes (iodides) (5×10^{-3} *M*)	pKa_1[a]	pKa_2	Inhibiting Group: Diethyl Phosphoryl $K_{obsd.}$[b]	Diethyl Phosphoryl Relative Rate Constant	Diisopropyl Phosphoryl $K_{obsd.}$	Diisopropyl Phosphoryl Relative Rate Constant
2-PAM	7.7	—	1.1×10^{-2}	1	1.98×10^{-3}	1
XV	6.8	9.6	9.45×10^{-5}	8.5×10^{-3}	1.56×10^{-6}	7.8×10^{-4}
XVI	7.1	9.5	1.12×10^{-4}	1.0×10^{-2}	1.53×10^{-5}	7.7×10^{-3}
XVII	6.4	8.5	5.78×10^{-5}	5.2×10^{-3}	6.20×10^{-6}	3.1×10^{-3}
XVIII	9.1	—	1.50×10^{-5}	1.3×10^{-3}	2.10×10^{-6}	1.0×10^{-3}
XIX	9.2	—	9.74×10^{-6}	8.8×10^{-4}	1.70×10^{-6}	8.6×10^{-4}
XX	8.5	—	8.12×10^{-6}	7.3×10^{-4}	1.60×10^{-6}	8.1×10^{-4}
XXI	5.7	—	1.06×10^{-4}	9.6×10^{-3}	4.42×10^{-6}	2.2×10^{-3}
XXII	7.6[c]	—	6.77×10^{-5}	6.1×10^{-3}	none	—
XXIII	5.9	—	6.90×10^{-5}	6.2×10^{-3}	none	—
XXIV	6.4	—	1.57×10^{-5}	1.4×10^{-3}	none	—
XXV	5.5	—	9.34×10^{-5}	8.5×10^{-3}	3.20×10^{-6}	1.6×10^{-3}

[a] pKa values were obtained by potentiometric titration and, for overlapping values, by application of the calculation method of Noyes, as given by Albert and Serjeant (13). [b] $K_{obsd.}$ is in min.$^{-1}$. [c] See *Experimental* section.

Anal.—Calcd. for $C_{14}H_{12}N_2O_2$: C, 69.99; H, 5.03; N, 11.66. Found: C, 70.13; H, 5.31; N, 11.83.

syn β-(2-Pyridyl)-β-oxo-α-hydroxyimino-propioanilide (IX)—To a solution of 1 g. of VIII in 30 ml. of EtOH and 5 ml. of 2 *N* HCl was added 0.5 g. of $NaNO_2$; by neutralization with dilute acetic acid a yellow product was obtained which was chromatographed on a column of silica gel, eluting with a 50:50 ethyl acetate–benzene mixture. From the first fractions the product was obtained which was crystallized from ethyl acetate–petroleum ether. $\lambda_{max.}^{EtOH}$ mμ ($\epsilon \times 10^{-3}$): 222 (16.5), 273 (12.6); ν (cm.$^{-1}$): 1,650–1,690 (C=O), 3,180–3,280 (NH, OH).

anti β-(2-Pyridyl)-β-oxo-α-hydroxyimino-propioanilide (X)—The product was obtained by isomerizing Product IX in EtOH with HCl gas. After 30 min. the solution was alkalinized with Na_2CO_3, and was neutralized with diluted acetic acid; by adding water, a precipitate was obtained which was crystallized from ethyl acetate–cyclohexane. It gave a precipitate with nickel salts. $\lambda_{max.}^{EtOH}$ mμ ($\epsilon \times 10^{-3}$): 243 (9.6), 268 (7.9); ν (cm.$^{-1}$): 1,690 (C=O), 3,200–3,300 (NH, OH).

syn β-(3-Pyridyl)-β-oxo-α-hydroxyimino-propioanilide (XI), and anti β-(3-Pyridyl)-β-oxo-α-hydroxyimino-propioanilide (XII)—β-(3-Pyridyl)-β-oxo-propionanilide (16) (1 g.) was dissolved in 30 ml. of EtOH and 5 ml. of 2 *N* HCl; to the solution 0.5 g. of $NaNO_2$ was added, dissolved in the minimum quantity of water. After neutralizing with Na_2CO_3, the addition of water precipitated a mixture of two products which were separated by chromatography on a silica gel column, eluting with ethyl acetate. From the first eluates, the *syn* isomer (XI) was obtained, which was crystallized from ethanol–water. $\lambda_{max.}^{EtOH}$ mμ ($\epsilon \times 10^{-3}$): 223 (15.5), 267 (9.8). ν (cm.$^{-1}$): 1,680 (C=O), 3,370 (NH, OH). From the second eluates, the *anti* isomer (XII) was obtained, which was crystallized by ethanol. It gave a precipitate with nickel salts. $\lambda_{max.}^{EtOH}$ mμ ($\epsilon \times 10^{-3}$): 245 (20.5); ν (cm.$^{-1}$): 1,640–1,660 (C=O), 3,100–3,200 (NH, OH).

The *anti* isomer can also be obtained by isomerizing the *syn* isomer with HCl gas in ethanol.

syn β-(4-Pyridyl)-β-oxo-α-hydroxyimino-propioanilide (XIII) and anti β-(4-Pyridyl)-β-oxo-α-hydroxyimino-propioanilide (XIV)—β-(4-Pyridyl)-β-oxo-propioanilide (1.5 g.) (17) was dissolved in ethanol containing 6 ml. of 2 *N* HCl; 0.75 g. of $NaNO_2$ was added to the solution. By neutralizing with Na_2CO_3 up to pH 4–5 and adding water, the *syn* isomer (XIII) was obtained, which was crystallized from ethanol. $\lambda_{max.}^{EtOH}$ mμ ($\epsilon \times 10^{-3}$): 224 (17.8), inflection at 251 (10.5). ν (cm.$^{-1}$): 1,665–1,685 (C=O), 3300 (NH, OH).

By further alkalinization of the reaction mother liquors and then neutralization with dilute acetic acid, a solid was obtained which consisted of a mixture of two isomers which were separated by chromatography on silica gel, eluting with ethyl acetate. The *syn* isomer was obtained from the first eluates; eluting further, the *anti* isomer (XIV) was obtained which was crystallized from ethyl acetate–cyclohexane. It gave a precipitate with nickel salts. $\lambda_{max.}^{EtOH}$ mμ ($\epsilon \times 10^{-3}$): 243 (16.05). ν (cm.$^{-1}$): 1,660–1,690 (C=O), 3,200–3,300 (NH, OH).

By treating the *syn* isomer with HCl gas in ethanol, a partial isomerization to the *anti* isomer takes place.

Preparation of the Methiodides—*Method A*—To 1 g. of pyridine derivative, dissolved in 50 ml. of anhydrous ethanol, 1 ml. of CH_3I was added; the mixture was heated for 2–3 days at 60° in a sealed vessel. By evaporating the solvent, a residue was obtained which was washed with ethyl acetate to remove the starting product. The quaternary salt was then crystallized from the most suitable solvent.

Method B—A solution of 1 g. of the starting product and 1 ml. of CH_3I in 50 ml. of an ethanol–acetone mixture was heated in a sealed tube for 4 days at 60°. By evaporating the solvent, a residue was obtained which was treated with 25 ml. of water. The solution was filtered and concentrated to half volume; by cooling, crystals were obtained which were collected and recrystallized from water.

Method C—The pyridine derivative (0.5 g.) and 0.5 ml. of CH_3I in 50 ml. of acetone were heated in a sealed tube for 2 days at 50°. By evaporating the solvent, a solid residue was obtained which was crystallized from acetone–ethyl acetate.

Method D—A solution of 0.5 g. of the product and 0.4 ml. of CH_3I in 40 ml. of dioxane was left to stand for a few days. On standing a product crystallized which was collected and recrystallized from acetone.

UV Spectra Data of Iodides—$\lambda_{max.}^{EtOH}$ mμ ($\epsilon \times 10^{-3}$). XV: 220 (18.7); 290 (5.96). XVI: 220 (43.4); 262 (24.7); inflection at 285 (17.5). XVII: 222 (34.1); 282 (38). XVIII: 222 (23.4). XIX: 225 (23.6); 235 (24.2). XX: 222 (21.5); inflection at 237 (18.9); 285 (11.7). XXI: 221 (20); 242 (17.6). XXII: 227 (26.3); 290 (12.3). XXIII: 220 (27); inflection at 242. XXIV: 218 (18.1); 285 (11.1). XXV: 225 (24.0); 238 (24.4).

IR Spectra Data of Iodides—ν (cm.$^{-1}$), mineral oil. XV: 1,685 (C=O); 3,150 (NH, OH). XVI: 1,695 (C=O); 3,150–3,380 (NH, OH). XVII: 1,665 (C=O); 3,200–3,350 (NH, OH). XVIII: 1,695 (C=O); 3,150–3,350 (NH, OH). XIX: 1,685 (C=O); 3,150–3,250 (NH, OH). XX: 1,695 (C=O); weak absorption at 3,150–3,250 (NH, OH). XXI: carbonyl bands at 1,658 and 1,680; 3,140–3,280 (NH, OH). XXII: carbonyl bands at 1,650–1,680; 3,150–3,280 (NH, OH). XXIII: carbonyl band at 1,680; weak bands at 3,150–3,350 (NH, OH). XXIV: carbonyl bands at 1,660–1,720; 3,100–3,400 (NH, OH). XXV: carbonyl band at 1,680; NH and OH are strongly bonded.

pKa Values—It must be noted that the value obtained for Product XXII is probably altered by the fact that during the potentiometric titration it precipitates an orange-colored product.

RESULTS AND DISCUSSION

From the hydrolysis velocity measurements it may be seen that the dioximes assayed are in general weak reactivators of acetylcholinesterase inhibited by TEPP or DFP; the most active of them, the α,β-dihydroxyimino-β-(3-pyridyl)-propioanilide methiodide, is about 100 times less active than 2-PAM. As regards the difference in activity in the series of dioximes, it may be observed that toward the enzyme inhibited by TEPP, it varies as follows: -(3-pyridyl) ≃ -(2-pyridyl) > -(4-pyridyl); in regard to the enzyme inhibited by DFP, the activity of the nicotinic isomer is about 10 times higher than that of the picolinic isomer, whereas that of the isonicotinic

isomer is intermediate between the two. A comparison of the activities of the dioximes XV–XVII with that of the monooximes XVIII–XX and XXI–XXIII, in the case of the enzyme inhibited by TEPP, shows that the α-hydroxyiminoketones XXI–XXIII have an activity like that of the dioximes, while the β-pyridyl-β-hydroxyimino-propioanilides XVIII–XX are about 10 times less active. This fall of activity may be due to the existence of a hydrogen bond between the hydroxyimino group in the β position and the amide group, as occurs in the *syn* α,β-dihydroxyimino-butyranilide and the *syn* β-phenyl-α,β-dihydroxyimino-propioanilide (6–8). No sound conclusion can be drawn from a comparison of the mono- and dioximes activities with the values of their dissociation constants; however, the fact that the reactivating properties of the oximes depends both on their degree of dissociation and the good nucleophilicity of the dissociated form appears to be confirmed.

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Studies on Mechanism of Action of Salicylates V: Effect of Salicylic Acid on Enzymes Involved in Mucopolysaccharides Synthesis

K. H. LEE and MICHAEL R. SPENCER*

Abstract □ Salicylic acid (SA) and acetylsalicylic acid (ASA), unlike cortisone, promote the release of lysosomal enzymes rather than protecting rat liver lysosomal membrane. Salicylic acid inhibits the oxidation of uridine-5-diphosphoglucose (UDPG) competitively with nicotinamide adenosine dinucleotide (NAD) and noncompetitively with UDPG. It also competitively inhibits the transferring of glucuronyl group of uridine-5-phosphoglucuronic acid (UDPGA) to the phenolic acceptor. The wound-healing retardation action of salicylates is probably due mainly to its inhibitory action on mucopolysaccharide synthesis.

Keyphrases □ Salicylates—action mechanism □ Mucopolysaccharides synthesis—salicylate effect □ Lysosome stability—salicylate effect □ UV spectrophotometry—analysis

Recently the authors have shown that aspirin retards skin wound healing in rats (1). Inflammation and acid mucopolysaccharide synthesis are two essential features in the early stage of wound healing (2). Aspirin inhibits both features. Two possible mechanisms of action have been proposed (3).

Lysosomal enzymes have been suggested to be involved in inflammation (4). Since aspirin has been reported by Miller and Smith (5) to protect lysosomal membrane, it was proposed that aspirin probably retards healing by a mechanism that prevents the release of lysosomal enzymes (1). However, this laboratory was not able to confirm the results reported by Miller and Smith. Acetylsalicylic acid and salicylic acid, in this laboratory, did not protect rat liver lysosomal membrane; instead, they accelerated the release of lysosomal enzymes. These findings were verified by using purified rat liver lysosome. The results are reported and discussed in this paper.

Mucopolysaccharide formation in the early phase of wound healing was noticed in many laboratories (6). The role of mucopolysaccharide in the formation of connective tissue or collagen has been of interest for many years (7, 8). Salicylates inhibit the biosynthesis of acid mucopolysaccharide and sulfate uptake for chondroitin sulfate synthesis by rat rib cartilage (9). It was found that aspirin also inhibits mucopoly-