Thiophosphoric Acid Derivatives of Ethylamine, DL-Methionine, and L-Proline Ethyl Esters III

Biological Activities in Fruit Flies

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Biological responses of the flies which eventually did not survive suggest that the compounds are cholinesterase inhibitors. Male fruit flies are the most susceptible in 0.01 and 0.001% w/v solutions. Concentration-toxicity and structure-toxicity relationships are discussed.

THE SYNTHESIS of thiophosphoric acid derivatives of ethylamine, DL-methionine, and L-proline ethyl esters (1) and their biological activities in guppies (2) have been published recently.

The purpose of this investigation was to observe the biological responses of fruit flies (*Drosophila melanogaster*) exposed to 0.1, 0.01, and 0.001% w/v aqueous solutions or suspensions of thiophosphoric acid derivatives which have structures similar to those of known acetylcholinesterase inhibitors. The derivatives have the general formula $-\overset{|}{N}-\overset{\overset{S}{\|}}{P}(X)_2$ where N represents the amine or amino acid ester moiety and $(X)_2$ = $(O\text{-alkyl})_2$, $(O\text{-}C_6H_5)_2$, $(C_2H_5O)Cl$, or $(Cl)_2$.

If these derivatives are anticholinesterases certain biological responses should be observed such as secretion of saliva, dizziness, evacuation of rectum, convulsions, and total paralysis (death).

EXPERIMENTAL

A cornmeal-molasses agar medium (3) was prepared, poured into Erlenmeyer flasks, and autoclaved after cotton stoppers were inserted. Several pairs of red eye and white eye female and male flies were introduced into each flask, and within 11 days many flies developed. One or two-day old flies were used in the investigation of the insecticidal activity of the compounds.

Aqueous suspensions or solutions (0.1, 0.01, and 0.001% w/v) of each compound listed in Table I were tested with 100 flies consisting of 50 males and 50 females in different test tubes. By separating the sexes the biological responses of each sex could be easily observed.

A 9-cm. filter paper was inserted into a 6-inch Pyrex test tube and firmly pressed against the glass surface about one inch from the top of the tube. Young flies were anesthetized with ether and transferred to the paper-lined tubes. Cotton stoppers were inserted and the flies were allowed to recover from the ether. The solution to be tested (0.9 ml.) was slowly introduced onto the filter paper by means of a syringe supplied with a long needle. The flies were periodically observed and the results at the end of 24 hours are presented in Table I. When distilled water was used instead of a suspension or solution of a compound under test it was observed for control purposes that both sexes survived after 24 hours.

Inhibition of cholinesterase is suggested from observations of the fruit flies which eventually did not survive in test tubes containing aqueous suspensions or solutions of the compounds. The flies were extremely agitated and frequently exhibited muscle contractions (of the proboscis and leg) and wing flutter (convulsions). Later they fell on their dorsal side and remained in this position for many hours due to dizziness and leg unsteadiness. All attempts to place them on their feet were unsuccessful.

Sex Differences.—Neither sex survived in 0.1% w/v aqueous suspensions or solutions of 10 of the 17 compounds in Table I.

Ethylamine Series.—Three of the esters which are extremely toxic to male fruit flies are not as toxic to the female flies. About 37% of the females survived after being in contact for 24 hours with 0.1% suspensions of the dichloro derivative or the tertiary ester, and 74% survived after being subjected to the isobutyl ester. The phenyl ester is somewhat toxic (32%) to males and has no effect on female fruit flies.

Amino Acid Series.—The diethyl esters in both amino acid series and the dichloro derivative in the methionine series are relatively less toxic to both sexes since about 45% of the males and about 87% of the female fruit flies survived.

The 0.01% aqueous solutions of all the compounds are more toxic to the male flies with the exception of the isopropyl ester (ethylamine series), ethoxychloro ester (methionine series), and dichloro derivative (proline series) which apparently are not toxic.

Received April 28, 1961, from the College of Pharmacy, University of Illinois, Chicago.

Acccepted for publication August 21, 1961.

This paper is abstracted in part from a dissertation submitted to the Graduate College at the Chicago Professional Colleges of the University of Illinois by Florence C. Klee in partial fulfillment of the requirements for the degree of Doctor of Philosophy in pharmaceutical chemistry.

Presented to the Scientific section A.PH.A., Chicago meeting, April 1961.

The authors wish to thank Dr. Katsuyuki Yokoyama for his suggestions concerning the culturing and handling of large numbers of fruit flies. The authors also wish to thank Dr. Halina Presley for Drosophila cultures consisting of two stocks, wild or red eye, and white eye.

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Table I.—Per Cent Expired of Fruit Flies in 24 Hours

		Concentration, % w/v								
		0.1			0.01			0.001		
No.	Compound[a]	Total	Male	Female	Total	Male	Female	Total	Male	Female
1	$X(OC_2H_5)_2$	100	100	100	15	28	2	7	14	0
2	$X(OCH_2CH_2CH_3)_2$	93	96	90	9	12	6	0	0	0
3	$X(OCH(CH_3)_2)_2$	100	100	100	2	4	0	0	0	0
4	$X(OCH_2CH_2CH_2CH_3)_2$	96	100	92	19	32	6	6	8	4
5	$X(OCH(CH_3)CH_2CH_3)_2$	100	100	100	47	88	6	4	4	4
6	$X(OC(CH_3)_3)_2$	83	100	66	37	54	20	18	26	10
7	$X(OCH_2CH(CH_3)_2)_2$	63	100	26	25	46	4	7	10	4
8	$X(OC_6H_5)_2$	18	32	4	17	30	4	11	20	2
9	$X(OC_2H_5)Cl$	100	100	100	8	14	2	1	2	0
10	XCl_2	75	90	60	5	10	0	0	0	0
11	$Y(OC_2H_5)_2$	34	58	10	9	10	8	3	4	2
12	$Y(OC_2H_5)Cl$	100	100	100	0	0	0	2	0	4
13	YCl_2	26	46	6	19	32	6	11	18	4
14	$Z(OC_2H_5)_2$	42	62	22	7	12	2	1	2	0
15	$Z(OC_2H_5)Cl$	100	100	100	14	26	2	0	0	0
16	ZCl_2	94	96	92	0	0	0	1	2	0
17	$(C_2H_5O)_2P(S)Cl$[b]	100	100	100	5	10	0	0	0	0

[a] X = $C_2H_5NHP(S)$; Y = DL-$CH_3SCH_2CH_2CH(CO_2C_2H_5)NHP(S)$; Z = L-$CH_2CH_2CH_2CH(CO_2C_2H_5)N$-P(S). For control purposes, distilled water was used instead of an aqueous suspension or solution of a compound under test; it was observed that both sexes survived after 24 hours. [b] Reference standard.

Both sexes are unaffected by 0.001% aqueous solutions of the compounds with the exception of five derivatives in the ethylamine series (phenyl, ethyl, normal, tertiary, and isobutyl esters) and the dichloro derivative in the methionine series which are somewhat toxic to the male flies.

DISCUSSION

The biological responses of the fruit flies indicate to the authors that these compounds are acetylcholinesterase inhibitors.

Concentration-Toxicity Relationship

Varying the Concentration of Compounds in the Ethylamine Series, $C_2H_5N(H)—P(=S)(X)_2$.[1]—The greatest decrease in toxicity after one tenfold dilution of the 0.1% w/v aqueous suspension or solution is observed with the two and three carbon chain esters and no change in toxicity is observed with the phenyl ester (Table I). The ethoxychloro, ethyl, and normal propyl esters are 87% as effective, whereas the isopropyl ester is no longer toxic to fruit flies. Following a second tenfold dilution, only the diethyl and phenyl esters are somewhat toxic (about 17%) to males.

The four carbon straight-chain ester (normal butyl) and the dihalo derivative are about 74 and 9% as effective after each tenfold dilution.

Only a 46% decrease in toxicity is observed upon one tenfold dilution of the 0.1% suspensions of the four carbon branched-chain esters. After the second tenfold dilution, the decrease in toxicity is 43% for the secondary butyl ester, and about 18% for the tertiary and isobutyl esters. The most toxic compound in 0.01% solution is the *sec*-butyl ester, and the *tert*-butyl ester is the most lethal in 0.001% solution.

Varying the Concentration of Compounds in the DL-Methionine Series, DL-$CH_3SCH_2CH_2CH(CO_2C_2H_5)—NH—P(=S)(X)_2$.[2]—The ethoxychloro derivative is not toxic to flies after one tenfold dilution of the 0.1% suspension.

The dihalo derivative exhibits a 7% decrease in toxicity for each tenfold dilution of the 0.1% suspension and is toxic to males in 0.01% and 0.001% solutions.

The diethoxy ester, which is slightly toxic to both sexes as a 0.01% solution, decreases in toxicity 25 and 6% per tenfold dilution of the 0.1% suspension.

Varying the Concentration of Compounds in the L-Proline Series L-$CH_2CH_2CH_2CH(CO_2C_2H_5)N—P(=S)(X)_2$ (ring).[2]—The order of decreasing toxicity upon one tenfold dilution is the dichloro derivative (94%), followed by the ethoxychloro (86%), and diethoxy (35%) esters. The latter two esters are toxic to males as 0.01% solutions, the ethoxychloro ester being twice as potent as the diethoxy ester.

These compounds are not toxic when a second tenfold dilution is made.

Structure-Toxicity Relationship

Varying X[1] Groups: Ethylamine Series $C_2H_5N(H)—P(=S)(X)_2$.—Observations of the results obtained with the 0.1% suspensions or solutions indicate that with the exception of the isobutyl and concentration %, w/v phenyl esters, all of the esters are more toxic than the dihalo derivative (Table II).

The compounds display a greater range of toxicity when tested as 0.01% solutions. Branching of the four carbon atom chain enhances toxicity to fruit flies since the four carbon branched-chain esters are more toxic than the corresponding straight-chain ester.

[1] $(X)_2$ = (O-Alkyl)$_2$, $(O\text{-}C_6H_5)_2$, $(C_2H_5O)Cl$, or $(Cl)_2$.

[2] $(X)_2$ = $(OC_2H_5)_2$, $(OC_2H_5)Cl$, or $(Cl)_2$.

TABLE II.—TOTAL PER CENT EXPIRED OF FRUIT FLIES IN 24 HOURS,[a] VARYING THE GROUPS[b] ATTACHED TO THE AMINE[c]-P(S) NUCLEUS

Concentration %, w/v: 0.1		0.01		0.001	
Compound[d]	Total	Compound[d]	Total	Compound[d]	Total
$X(OC_2H_5)_2$	100	$X(OCH(CH_3)CH_2CH_3)_2$	47	$X(OC(CH_3)_3)_2$	18
$X(OCH(CH_3)_2)_2$	100	$X(OC(CH_3)_3)_2$	37	$X(OC_6H_5)_2$	11
$X(OCH(CH_3)CH_2CH_3)_2$	100	$X(OCH_2CH(CH_3)_2)_2$	25	$X(OC_2H_5)_2$	7
$X(OC_2H_5)Cl$	100	$X(OCH_2CH_2CH_2CH_3)_2$	19	$X(OCH_2CH(CH_3)_2)_2$	7
$X(OCH_2CH_2CH_2CH_3)_2$	96	$X(OC_6H_5)_2$	17	$X(OCH_2CH_2CH_2CH_3)_2$	6
$X(OCH_2CH_2CH_3)_2$	93	$X(OC_2H_5)_2$	15	$X(OCH(CH_3)CH_2CH_3)_2$	4
$X(OC(CH_3)_3)_2$	83	$X(OCH_2CH_2CH_3)_2$	9	$X(OC_2H_5)Cl$	1
XCl_2	75	$X(OC_2H_5)Cl$	8	XCl_2	0
$X(OCH_2CH(CH_3)_2)_2$	63	XCl_2	5	$X(OCH_2CH_2CH_3)_2$	0
$X(OC_6H_5)_2$	18	$X(OCH(CH_3)_2)_2$	2	$X(OCH(CH_3)_2)_2$	0
$Y(OC_2H_5)Cl$	100	YCl_2	19	YCl_2	11
$Y(OC_2H_5)_2$	34	$Y(OC_2H_5)_2$	9	$Y(OC_2H_5)_2$	3
YCl_2	26	$Y(OC_2H_5)Cl$	0	$Y(OC_2H_5)Cl$	2
$Z(OC_2H_5)Cl$	100	$Z(OC_2H_5)Cl$	14	$Z(OC_2H_5)_2$	1
ZCl_2	94	$Z(OC_2H_5)_2$	7	ZCl_2	1
$Z(OC_2H_5)_2$	42	ZCl_2	0	$Z(OC_2H_5)Cl$	0
$(C_2H_5O)_2P(S)Cl$[e]	100	$(C_2H_5O)_2P(S)Cl$[e]	5	$(C_2H_5O)_2P(S)Cl$[e]	0

[a] The compounds at each dilution are arranged according to decreasing toxicity. [b] (O-Alkyl)$_2$, (O-C_6H_5)$_2$, (OC_2H_5)Cl, or (Cl)$_2$. [c] Ethylamine, DL-methionine, or L-proline ethyl esters. [d] X = $C_2H_5NHP(S)$; Y = DL-$CH_3SCH_2CH_2CH(CO_2C_2H_5)NHP(S)$; Z = L-$\overline{CH_2CH_2CH_2CH(CO_2C_2H_5)N}$-P(S). [e] Reference standard.

Apparently there is no differentiation as far as toxicity to male flies is concerned when ethoxy or phenoxy groups are substituted for the normal butoxy groups attached to the $C_2H_5N(H)—P(=S)—$ nucleus (Tables I and II). These esters are toxic to male fruit flies to the extent of 30%.

The normal propyl ester is somewhat toxic (12%) to male flies. Branching of this chain, unlike the butyl series, causes a decrease in toxicity and the resulting isopropyl ester is not toxic to flies.

The ethoxychloro and dichloro derivatives are as toxic to male flies as the normal propyl ester.

The tertiary butyl ester, representing maximum branching of a four carbon chain is the most toxic compound to both sexes in 0.001% solution, and the secondary butyl, halo, and three carbon chain derivatives are the least toxic since the latter have no effect on flies at this concentration.

The normal and isobutyl esters are about one-third as toxic and the phenyl and ethyl esters are about two-thirds as toxic to males as the tertiary butyl ester.

Varying X² Groups: DL-Methionine Series, DL-$CH_3SCH_2CH_2C(H)(CO_2C_2H_5)—N(H)—P(=S)(X)_2$.—The results obtained with the 0.1% suspensions of the compounds indicate that when C_2H_5O, Cl groups are attached to this nucleus, maximum toxicity to both sexes results since there are no survivors (Tables I and II). The replacement of the halogen atom by an ethoxy group causes a 90% loss in toxicity to the females and a 42% decrease in toxicity to males. Similarly, when two chlorine atoms are attached to this nucleus the compound is not toxic to females and there is a 54% decrease in toxicity to males. The dichloro derivative is toxic to males to the extent of 18% even after two tenfold dilutions.

Varying X² Groups: L-Proline Series, L-$\overline{CH_2CH_2CH_2CH(CO_2C_2H_5)N}—P(=S)(X)_2$.—In contrast to what is observed in the methionine series, equally potent derivatives are obtained when C_2H_5O, Cl groups or two Cl atoms are attached to this nucleus. There are practically no survivors of either sex. Two ethoxy groups on this nucleus produces a decrease in the toxicity to the males and females of about 40 and 80%, respectively. The toxicity of the dichloro derivative is lost upon further dilution. The diethoxy and ethoxychloro derivatives are no longer toxic to females upon one tenfold dilution, but weakly toxic to males.

Varying the Amine:[3] $—P(=S)—(OC_2H_5)_2$ Nucleus.—When the amine attached to this nucleus is ethylamine, an ester is obtained which is quite toxic to both male and female flies when tested in a 0.1% solution and somewhat toxic to males after two tenfold dilutions (Table III). Substituting methionine or proline for ethylamine results in approximately 40% loss in toxicity to males and about 84% loss in toxicity to females. The 0.01% solutions of the amino acid esters are somewhat toxic to male fruit flies but have no effect on flies after a tenfold dilution.

Apparently, substituting methionine for proline makes very little difference in the toxicity of the ester obtained, but substituting ethylamine for either amino acid enhances its toxicity.

Varying the Amine:[3] $—P(=S)(Cl)—OC_2H_5$ Nucleus.—All of the amines produce esters which are equally potent in 0.1% suspensions or solutions and non-toxic in 0.001% solutions.

[3] Ethylamine or the DL-methionine and L-proline ethyl esters.

TABLE III.—PER CENT EXPIRED OF FRUIT FLIES IN 24 HOURS VARYING THE AMINE[a] ATTACHED TO THE —$P(S)(X)_2$[b] NUCLEUS

	Concentration, % w/v								
	0.1			0.01			0.001		
Amine[a]	Total %	Male %	Female %	Total %	Male %	Female %	Total %	Male %	Female %
				$P(S)(OC_2H_5)_2$ Nucleus					
Ethylamine	100	100	100	15	28	2	7	14	0
Methionine	34	58	10	9	10	8	3	4	2
Proline	42	62	22	7	12	2	1	2	0
				$P(S)(OC_2H_5)Cl$ Nucleus					
Ethylamine	100	100	100	8	14	2	1	2	0
Methionine	100	100	100	0	0	0	2	0	4
Proline	100	100	100	14	26	2	0	0	0
				$P(S)Cl_2$ Nucleus					
Ethylamine	75	90	60	5	10	0	0	0	0
Methionine	26	46	6	19	32	6	11	18	4
Proline	94	96	92	0	0	0	1	2	0

[a] Ethylamine or the DL-methionine and L-proline ethyl esters. [b] $(X)_2 = (OC_2H_5)_2$, $(OC_2H_5)Cl$, or $(Cl)_2$.

A difference in toxicity can be noted only with the 0.01% solutions where L-proline produces a derivative more toxic than that of ethylamine or DL-methionine. The methionine derivative is not toxic at this concentration.

Varying the Amine:[3] —$P(=S)(Cl)_2$ **Nucleus.**—The order of decreasing toxicity in 0.1% suspensions or solutions is proline, followed by ethylamine, and then methionine. When proline is attached to this nucleus, a derivative results which is toxic to both sexes. Replacement of proline by ethylamine produces a derivative which retains the same toxicity to males but is about 30% less toxic to females. Methionine attached to this nucleus produces a derivative which is not toxic to females and, compared to the proline derivative, is 50% less toxic to males.

The order of decreasing toxicity for the 0.01% solutions is reversed, that is, methionine, followed by ethylamine, and then proline, because ethylamine is only weakly toxic to males and proline is no longer toxic to flies at this concentration.

It is interesting to observe that the methionine derivative in 0.001% solution retains its toxicity to males to the extent of 18%.

SUMMARY

Biological responses of the fruit flies indicate that the thiophosphoric acid derivatives are anticholinesterases.

Results on mature fruit flies show that neither sex survives in 0.1% w/v suspensions of most of the derivatives. The 0.01% solutions are more toxic to the male flies than to the females. Both sexes are unaffected by 0.001% solutions, with the exception of five esters of the ethylamine series (*n*-propyl, *n*-, *tert*-, isobutyl, and phenyl esters), and one methionine derivative (dichloro), which were somewhat toxic to the male flies.

Concentration-toxicity and structure-toxicity relationships are discussed.

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