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

Biological Activities in Seeds of *Hyoscyamus niger*

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The effect of certain thiophosphoric acid derivatives on germination and seedling growth of hyoscyamus seeds was observed. All of the compounds as 0.1% w/v suspensions or solutions inhibited the growth of the seedlings. Four ethylamine and two DL-methionine derivatives inhibited germination, whereas two esters stimulated germination. Inhibition and stimulation of germination and growth were observed with some of the 0.01% solutions. The 0.001% solutions had no effect on germination, but seven promoted the growth of the seedling. Structure-toxicity relationship is discussed.

THE SYNTHESIS of thiophosphoric acid derivatives of ethylamine, DL-methionine, and L-proline ethyl esters (1) and their biological activities in guppies (2) and fruit flies (3) have been reported recently.

These thiophosphoric acid derivatives have structures similar to known anticholinesterases.

$$\begin{array}{c} \quad\quad S \\ | \quad \| \\ -N-P(X)_2 \end{array}$$

Their general formula is —N—$P(X)_2$, 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 $(Cl)_2$. The observed biological responses of guppies and fruit flies which have been in contact with aqueous solutions of these compounds seem to indicate that these compounds are indeed acetylcholinesterase inhibitors.

There exists the possibility of these thiophosphoric acid derivatives inhibiting other enzymes since Aldridge has shown that organophosphorus compounds are not specific inhibitors for the cholinesterases but rather inhibitors for enzymes possessing carboxylic esterase activity (4). Seeds contain many enzymes playing a role in the synthesis and hydrolysis of carbohydrates, proteins, and lipids (5). The purpose of this investigation is to observe the effect of 0.1, 0.01, and 0.001% w/v aqueous suspensions or solutions of certain thiophosphoric acid derivatives on the germination and seedling growth of *Hyoscyamus niger* L. seeds.

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EXPERIMENTAL

Two milliliters of an aqueous suspension or solution (0.1, 0.01, and 0.001% w/v) of each compound in Table I was slowly introduced with a syringe on a filter paper containing 80 seeds (*Hyoscyamus niger*) in a 9-cm. Petri dish. For a control, two ml. of distilled water was used instead of the aqueous solution of the compound to be tested.

The Petri plates were covered and placed in the dark for 2 days. On the second day, two ml. of distilled water was introduced into each dish. After 4 days, the number of seedlings which had germinated was recorded and the length of each seedling was measured.

Table I shows the per cent germination, the average height of the seedling observed for each compound at three concentrations, and the standard deviation. The percentages of inhibition or stimulation of germination and seedling growth as compared to the control are presented in Tables II and III, respectively.

Germination.—Germination of the seeds was inhibited to various extents by 0.1% w/v aqueous suspensions or solutions of six compounds, four of which are in the ethylamine series and two in the DL-methionine series (Table II). The dichloro derivative in the ethylamine series is the strongest inhibitor (86%), whereas the diethyl ester (ethylamine series) and dihalo derivative of the methionine series are only half as effective. The ethoxychloro derivative (ethylamine series) is much less effective (16% inhibition), and the weakest inhibition is observed with the tertiary butyl ester (ethylamine series) and the diethyl ester in the methionine series (about 9% inhibition).

It was interesting to observe that the secondary butyl and phenyl esters in the ethylamine series stimulated germination to the extent of 10 and 5%, respectively.

The 0.01% solution of diethoxyethylamidothiophosphate is 17% inhibitory, whereas the diethyl ester in the methionine series is a weak inhibitor (6% inhibition). The normal butyl ester stimulated germination to the extent of 10%.

The 0.001% solutions of the compounds had no effect on the germination.

Growth.—All of the compounds as 0.1% suspensions or solutions inhibited the growth of the *Hyoscyamus niger* seedlings (Table III). Maximum

TABLE I.—EFFECT OF VARIOUS DERIVATIVES OF THIOPHOSPHORIC ACID ON GERMINATION AND SEEDLING GROWTH OF HYOSCYAMUS SEEDS

	Concentration, % w/v											
	0			0.001			0.01			0.1		
Compound[a]	Germination, %	Median Seedling Height, mm.	±S.D.[b]	Germination, %	Median Seedling Height, mm.	±S.D.[b]	Germination, %	Median Seedling Height, mm.	±S.D.[b]	Germination, %	Median Seedling Height, mm.	±S.D.[b]
$X(OC_2H_5)_2$	90.00	24.8	4.8	93.75	23.4	3.6	75.00	19.2	5.1	50.00	4.5	2.0
$X(OCH_2CH_2CH_3)_2$				87.50	25.8	5.1	93.75	23.3	3.4	87.50	4.9	1.1
$X(OCH(CH_3)_2)_2$				93.75	20.0	3.8	86.25	18.4	3.8	82.50	13.4	3.7
$X(OCH_2CH_2CH_2CH_3)_2$	88.75	15.9	4.0	90.00	19.7	4.1	97.50	15.3	3.3	96.25	7.9	1.9
$X(OCH(CH_3)CH_2CH_3)_2$				83.75	17.9	2.5	92.50	17.6	3.6	97.50	6.8	1.7
$X(OC(CH_3)_3)_2$				95.00	17.5	3.4	88.75	14.3	3.9	86.25	6.2	3.1
	95.00	17.6	4.0									
$X(OCH_2CH(CH_3)_2)_2$				91.25	17.5	4.7	97.50	15.1	2.5	96.25	7.3	2.1
$X(OC_6H_5)_2$	95.00	23.6	6.9	97.50	23.0	5.8	88.75	20.9	7.1	100.00	19.3	6.3
$X(OC_2H_5)Cl$	88.75	14.0	5.0	91.25	13.3	6.0	93.75	13.5	4.0	75.00	0.32	0.2
XCl_2				93.75	25.7	4.6	100.00	19.2	3.7	13.75	1.2	0.3
	97.50	23.1	5.1									
$(C_2H_5O)_2P(S)Cl$[c]				100.00	23.8	5.3	96.25	20.6	5.4	100.00	0.83	0.5
$Y(OC_2H_5)_2$				96.25	20.7	3.5	93.75	16.4	4.1	91.25	14.4	3.6
	100.00	17.3	3.3									
$Z(OC_2H_5)_2$				100.00	19.8	5.2	97.50	18.5	4.3	97.50	11.3	4.0
$Y(OC_2H_5)Cl$				98.75	24.0	4.4	97.50	24.1	4.6	96.25	18.0	2.9
	98.75	24.7	5.7									
YCl_2				98.75	23.7	4.9	98.75	21.7	3.4	58.75	0.73	0.3
$Z(OC_2H_5)Cl$				95.00	23.2	4.4	95.00	20.3	4.3	98.75	13.3	2.9
	95.00	22.3	5.7									
ZCl_2				95.00	24.0	4.2	95.00	19.5	5.3	98.75	3.5	3.1

[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). [b] S.D. = Standard deviation. [c] Reference standard.

TABLE II.—PERCENTAGES OF INHIBITION OR STIMULATION OF GERMINATION OF HYOSCYAMUS SEEDS

Compound[a]	Concentration, % w/v[b] 0.001	0.01	0.1
$X(OC_2H_5)_2$	(+)4.2	(−)16.7[c]	(−)44.4[d]
$X(OCH_2CH_2CH_3)_2$	(−)2.8	(+) 4.2	(−) 2.8
$X(OCH(CH_3)_2)_2$	(+)5.6	(−) 2.8	(−) 7.0
$X(OCH_2CH_2CH_2CH_3)_2$	(+)1.4	(+) 9.9[e]	(+) 8.5
$X(OCH(CH_3)CH_2CH_3)_2$	(−)5.6	(+) 4.2	(+) 9.9[e]
$X(OC(CH_3)_3)_2$	0.0	(−) 6.6	(−) 9.2[e]
$X(OCH_2CH(CH_3)_2)_2$	(−)4.0	(+) 2.6	(+) 1.3
$X(OC_6H_5)_2$	(+)2.6	(−) 6.6	(+) 5.3[e]
$X(OC_2H_5)Cl$	(+)2.8	(+) 5.6	(−)15.5[e]
XCl_2	(−)3.9	(+) 2.6	(−)85.9[d]
$Y(OC_2H_5)_2$	(−)3.8	(−) 6.3[e]	(−) 8.8[c]
$Y(OC_2H_5)Cl$	0.0	(−) 1.3	(−) 2.5
YCl_2	0.0	0.0	(−)40.5[d]
$Z(OC_2H_5)_2$	0.0	(−) 2.5	(−) 2.5
$Z(OC_2H_5)Cl$	0.0	0.0	(+) 4.0
ZCl_2	0.0	0.0	(+) 4.0
$(C_2H_5O)_2P(S)Cl$	(+)2.6	(−) 1.3	(+) 2.6

[a] X = $C_2H_5NHP(S)$; Y = DL-$CH_3SCH_2CH_2CH(CO_2C_2H_5)NHP(S)$; Z = L-$CH_2CH_2CH_2CH(CO_2C_2H_5)N—P(S)$. [b] (+) = % germination greater than control (% stimulation); (−) = % germination less than control (% inhibition). [c] Significant at the 1% level. [d] Significant at the 0.1% level. [e] Significant at the 5% level.

TABLE III.—PERCENTAGES OF INHIBITION OR STIMULATION OF GROWTH OF THE *Hyoscyamus niger* SEEDLING

Compound[a]	Concentration, % w/v[b] 0.001	0.01	0.1
$X(OC_2H_5)_2$	(−) 5.6[c]	(−)22.6[d]	(−)81.9[d]
$X(OCH_2CH_2CH_3)_2$	(+) 4.0	(−) 6.0[c]	(−)80.2[d]
$X(OCH(CH_3)_2)_2$	(+)25.8[d]	(+)15.7[d]	(−)15.7[d]
$X(OCH_2CH_2CH_2CH_3)_2$	(+)24.0[d]	(−) 3.8	(−)50.3[d]
$X(OCH(CH_3)CH_2CH_3)_2$	(+)12.6[d]	(+)10.7[c]	(−)57.2[d]
$X(OC(CH_3)_3)_2$	(−) 0.6	(−)18.8[d]	(−)64.8[d]
$X(OCH_2CH(CH_3)_2)_2$	(−) 0.6	(−)14.2[d]	(−)58.5[d]
$X(OC_6H_5)_2$	(−) 2.5	(−)11.4[c]	(−)18.2[d]
$X(OC_2H_5)Cl$	(−) 5.0	(−) 3.6	(−)97.9[d]
XCl_2	(+)11.3[d]	(−)16.9[d]	(−)94.8[d]
$Y(OC_2H_5)_2$	(+)19.7[d]	(−) 5.2	(−)16.8[d]
$Y(OC_2H_5)Cl$	(−) 2.8	(−) 2.4	(−)27.1[d]
YCl_2	(−) 4.0	(−)12.2[d]	(−)97.0[d]
$Z(OC_2H_5)_2$	(+)14.4[d]	(+) 6.9[c]	(−)34.7[d]
$Z(OC_2H_5)Cl$	(+) 4.0	(−) 9.0[c]	(−)40.4[d]
ZCl_2	(+) 7.6[c]	(−)12.6[d]	(−)84.3[d]
$(C_2H_5O)_2P(S)Cl$	(+) 3.0	(−)10.8[d]	(−)96.5[d]

[a] X = $C_2H_5NHP(S)$; Y = DL-$CH_3SCH_2CH_2CH(CO_2C_2H_5)NHP(S)$; Z = L-$CH_2CH_2CH_2CH(CO_2C_2H_5)N—P(S)$. [b] (+) = % growth greater than control (% stimulation); (−) = % growth less than control (% inhibition). [c] Significant at the 5% level. [d] Significant at the 0.1% level. [e] Significant at the 1% level.

growth inhibition (average of 94%) was caused by the ethoxychloro ester in the ethylamine series and all of the dihalo derivatives.

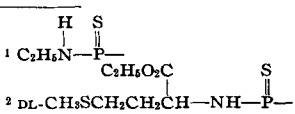
Almost all of the 0.01% solutions of compounds in the three series had an effect on seedling growth. Maximum inhibition (about 20%) was observed with derivatives in the ethylamine series, namely, the diethyl, tertiary butyl, and dichloro derivatives. Stimulation of seedling growth was caused by two esters in the ethylamine series, isopropyl (16%), secondary butyl (11%), and the diethyl ester (7%) in the proline series.

Seven of the 0.001% solutions promoted the growth of the seedling. In the ethylamine series, the isopropyl and normal butyl esters stimulated the seedling growth to the extent of 25%, and the secondary butyl and dichloro derivatives were 12% stimulatory. The diethyl esters in both amino acid series promoted growth to about 17%, whereas the dichloro derivative in the proline series was about 9% less stimulatory. Only one ester, diethoxyethylamidothiophosphate, inhibited seedling growth to the extent of 6%.

DISCUSSION

The effect of various derivatives of thiophosphoric acid on the germination and seedling growth of seeds of *Hyoscyamus niger* was primarily inhibition, however, some stimulation was also observed (Tables II and III).

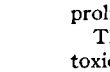
Structure-Toxicity Relationship.—The effects of the 0.1% suspensions or solutions on the germination of hyoscyamus seeds indicate that derivatives which have two chlorine atoms or two ethoxy groups attached to the ethylamine[1] or methionine[2] nucleus



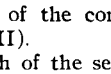
cause inhibition of germination. The ethylamine derivatives are more potent than the corresponding methionine derivatives. The ethoxychloro and tertiary butyl esters in the ethylamine series are weak inhibitors. It is interesting to note that the proline derivatives have no effect on germination.

The following relationships between structure and toxicity are based on the results obtained with the 0.1% suspensions or solutions of the compounds on the seedling growth (Table III).

Varying the Amine.[3]—Growth of the seedling is inhibited to the extent of about 92% irrespective of the particular amine that is attached to the

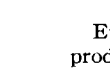


—$P(Cl)_2$ nucleus.



Ethylamine attached to the —$P(=S)(Cl)OC_2H_5$ nucleus produces a derivative which is 98% inhibitory to growth. When ethylamine is replaced by methionine or proline, much of the inhibitory effect of the derivative is lost since the two amino acid derivatives inhibit growth only to the extent of about 34%.

The amine imparting the most toxicity to the



—$P(OC_2H_5)_2$ nucleus is ethylamine (82% inhibition). A 47% decrease in inhibition of growth is observed when proline is substituted for ethylamine. The methionine derivative is 65% less inhibitory than the ethylamine derivative.

Varying the $(X)_2$ Groups[4]

C_2H_5N—P— **Nucleus.**—The order of decrease in the inhibition of growth when the X groups of this nucleus are varied is mono and dichloro (96%), ethyl, *n*-propyl (81%), tertiary (65%), secondary, isobutyl (58%), *n*-butyl (50%), phenyl, and isopropyl (17%).

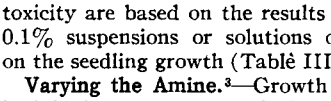
The halogen derivatives are the most potent growth inhibitors. Two and three carbon straight-chain esters also are very effective; however, the three carbon branched-chain ester has much less inhibitory effect on the growth of the seedling.

Unlike the three carbon esters, branching of the four carbon chain esters enhances the inhibitory effect since the branched-chain esters are more inhibitory to growth than the corresponding straight-chain ester.

Replacement of the two and three carbon straight-chains by a four carbon straight-chain results in an ester which is 31% less inhibitory.

A phenyl ring in place of a halogen or an alkyl group (with the exception of a three carbon branched-chain) greatly decreases the inhibitory action of the compound.

DL-$CH_3SCH_2CH_2C$—N—P— **Nucleus.**—In the methionine series, two chlorine atoms attached to this nucleus produces a derivative which inhibits seedling growth to the extent of 97%. The replacement of one chlorine atom with an ethoxy group results in a 70% loss in the inhibitory action, and when both chlorine atoms are replaced, 80% of this effect is lost.



L-$CH_2CH_2CH_2CHN$—P— **Nucleus.**—As was observed in the methionine series, two chlorine atoms on this nucleus result in a potent growth-inhibitory substance. The inhibitory effect is greatly diminished (by more than 40%) upon substitution of the halogen atoms with one or two ethoxy groups.

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

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

SUMMARY

Primarily, inhibition and, to some extent, stimulation were the effects of certain derivatives of thiophosphoric acid on the germination and seedling growth of the seeds of *Hyoscyamus niger*.

Seed germination was inhibited to various extents by the 0.1% suspensions of four ethylamine (dichloro, diethoxy, ethoxychloro, and tertiary butyl) and two methionine (dichloro and diethoxy) derivatives. In contrast, germination was stimulated by three esters, the 0.1% suspensions of the secondary butyl and phenyl esters, and the 0.01% solution of the normal butyl ester. The 0.01% solutions of the diethyl esters in the ethylamine and methionine series had an inhibitory effect. The 0.001% solutions of all the compounds had no effect on germination. Germination was unaffected by any of the solutions of the proline derivatives.

All of the compounds as 0.1% suspensions or solutions inhibited, to various extents, the growth of the seedling. Maximum growth inhibition was caused by the ethoxychloro ester in the ethylamine series and all of the dihalo derivatives. Ten of the 0.01% solutions had an inhibitory effect and three of the solutions had a stimulatory effect on seedling growth. Growth was promoted by seven of the 0.001% solutions.

The relationship between structure and toxicity is discussed.

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