tubular cells. The vacuoles in these cells consist of large and small vacuoles which are more numerous than that contained in cells of the adjacent non-perfused tubules (fig. 1). At higher magnification, it can be seen that vacuoles are membrane-lined and many in the apical cytoplasm are endocytotic vesicles containing serum protein. The newly formed small vacuoles are most likely dilated cisternae of rough-surfaced endoplasmic reticulum but a few are lined by smooth membrane (fig. 2). These small vesicles are clearly distinguished from endocytotic vesicles containing electron dense serum proteins which are present only in the apical cytoplasm. Other findings include occasional clubbing of the tip of the apical microvilli with loss of microfilamentous attachments and occasional extrusion of a portion of cytoplasm (fig. 1) and nuclei (fig. 3) into the tubular lumen. The extrusion of nuclei is reminiscent of the action of cytochalasin B, a drug known to depolymerize microfilaments in vitro. Although most tight junctions appear intact morphologically, it is apparent that some tight junctions have permitted passage of electron-dense proteinaceous material into the intercellular space. Mitochondria show a mild electron translucence of intracristal spaces (fig. 2). After 4 min, tubular cells became more vesiculated and the cytoplasmic architecture appears grossly distorted. Note that most of the vesicular structures are disrupted and distorted mitochondria. By this time, many endocytotic vacuoles containing serum proteins have traveled nearly to the basal cell membrane. The fine morphology of tubules perfused with heat-inactivated serum was not significantly different from that of non-perfused control tubules and are not shown.

Discussion. These alterations of ultrastructure observed after intraluminal perfusion of fresh autologous serum are comparable to or even more pronounced than those seen after 120 min of ischemia as reported by Glaumann et al.[8]. Since the proximal tubule cells lose membrane potential and luminal membrane resistance and take up luminally perfused trypan blue[3-5] after 2 min of luminal perfusion with serum, the observed ultrastructural alterations substantiate the previous thesis that the complement-mediated cell lysis may be involved as the mechanism for serum-induced inhibition of proximal tubular fluid absorption.

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Relationship between the structure of a series of carbamate derivatives of methomyl and their biological activity

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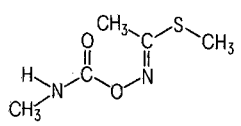
Summary. A series of 24 carbamates was obtained by structural modification of methomyl, or S-methyl-N[(methylcarbamoyl)oxy]thioacetimidate, an insecticide. The biological activity of this series and that of 7 other compounds was studied by measuring root growth of germinating corn plants with Texas and normal cytoplasm. The contribution of each substitution to phytotoxicity was determined and the sites on the molecule related to phytotoxicity and specificity localized.

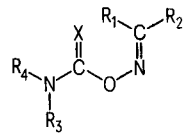
Corn (*Zea mays* L.) with a mitochondrial gene for male sterility (Texas male sterile cytoplasm, or T) is highly susceptible to corn pathogens like *Helminthosporium maydis* Nisikado and Myake race T or *Phyllosticta maydis* Arny and Nelson. The insecticide methomyl or S-methyl-N[(methylcarbamoyl)oxy]thioacetimidate was found to mimic the action of pathotoxins isolated and purified from *H. maydis* and *P. maydis* cultures[2-4] both in plants[5] and on isolated mitochondria[6,7]. The study of phytotoxicity and specificity of a series of carbamates derivatives of methomyl enabled an activity/structure relationship to be determined. In the present paper we report about the action of these compounds by measuring root growth of germinating corn plants with male sterile (T) and male fertile (N) cytoplasms. The series of carbamates was obtained by progressive substitution on methomyl **1** shown below (geometric E isomer), utilized as an insecticide. (The structure presented does not indicate a preferred geometrical isomer.)



1

[a]Control root growth was normalized to 100 mm, i.e. 100%. By convention compound active and specific corresponds to 0% on Texas and 100% on Normal. [b]The 1st number corresponds to a concentration of 0.5 mg/1 ml. The 2nd number corresponds to a concentration of 1 mg/ml. The measures for each concentration were repeated twice.

Relationships between carbamate structures and biological activity
(The structure presented does not indicate a preferred geometrical isomer).



Entry	X	R_1	R_2	R_3	R_4	Texas %[a,b]	Normal %[a,b]
1	O	CH_3	S–CH_3	CH_3	H	0	65
						0	50
2	O	CH_3	S–CH_3	CH_3	CH_3	56	52
						21	34
3	S	CH_3	S–CH_3	CH_3	H	7	100
						0	88
4	O	CH_3	S–CH_3	C_6H_5	H	84	93
						86	93
5	O	CH_3	S–CH_3	C_2H_5	H	0	86
						0	75
6	O	CH_3	CH_3	CH_3	H	34	89
						1	75
7	O	CH_3	C_2H_5	CH_3	H	12	47
						0	49
8	O	CH_3	C_2H_5	C_6H_5	H	36	38
						41	32
9	O	CH_3	C_2H_5	CH_3	CH_3	28	27
						13	24
10	S	CH_3	C_2H_5	CH_3	H	72	71
						47	63
11	O	CH_3	$(CH_3)_2CH$	CH_3	H	38	44
						30	36
12	O	CH_3	$(CH_2)_2$–CH_3	CH_3	H	0	41
						0	35
13	O	CH_3	$(CH_2)_4$–CH_3	CH_3	H	16	43
						0	25
14	O	CH_3	$(CH_2)_7$–CH_3	CH_3	H	0	38
						0	13
15	O	CH_3	$(CH_2)_7$–CH_3	CH_3	CH_3	1	0
						0	0
16	O	CH_3	$(CH_2)_7$–CH_3	C_2H_5	H	63	75
						30	53
17	O	CH_3	$(CH_2)_2COOC_2H_5$	CH_3	H	94	89
						90	77
18	O	CH_3	$CH(CH_3)COOC_2H_5$	CH_3	H	78	99
						65	78
19	O	CH_3	C_6H_5	CH_3	H	13	16
						2	8
20	O	C_2H_5	C_2H_5	CH_3	H	29	37
						12	26
21	O	C_2H_5	$(CH_2)_3$–CH_3	CH_3	H	56	42
						21	30
22	O	H_3C–$(CH_2)_2$	$(CH_2)_2$–CH_3	CH_3	H	51	42
						25	28
23	O	CH_3	$-CH_2-C(=N-O-C(=O)-N(H)-CH_3)-CH_3$	CH_3	H	100	100
						98	100
24	O	CH_3	CH_3	C_6H_5	H	40	24
						5	15
25	O	CH_3	CH_3	$C_{10}H_7$ (naphtyl)	H	38	63
						43	74
26	$H_3C-C(=N-O-C(=O)-CH_2-CH_3)-CH_2-CH_3$					83	82
						86	80
27	$H_3C-C(=N-O-C(=O)-CH_2-CH_3)-S-CH_3$					91	91
						76	84
28	Methylisocyanate [trimer, $(CH_3NCO)_3$]					40	60
						0	20
29	$CH_3NHCO-OCH_2CH_3$					100	100
						100	80
30	$CH_3NHCS-OCH_2CH_3$					100	100
						100	80
31	$CH_3NHCONHCH_3$					100	100
						100	80
32	$C_2H_5NHCONHC_2H_5$					100	100
						100	100

Materials and methods. The different carbamates were obtained by the action of methyl, ethyl, phenylisocyanates, methylisothiocyanate and N-dimethylcarbamylchloride with the corresponding oximes. The oximes were obtained with the usual methodology[8] from the appropriate ketones. The carbamates are either crystalline or liquid. The thiocarbamates are heat-labile and rapidly turn yellow in light.

NMR-spectrometry of ^{1}H and ^{13}C indicated the presence of 2 geometric isomers in most cases. TLC performed with the technique of successive elutions confirmed the presence of 1 or 2 of the isomers. The recovery of the 2 isomeric carbamates was unsatisfactory, since each geometric derivative yielded the equilibrium mixture E ⇆ Z in the solution, in which the tests were performed. Methomyl was isolated from 'lannate' the commercial solution, after removal of solvents in vacuo. It was crystallized from aqueous ethanol. The ^{1}H- and ^{13}C-spectra show only 1 geometric isomer[7].

Activity was measured with the standard germination test. The products tested were dissolved in methylene chloride at the concentrations of 0.5 and 1 mg/ml and were deposited on filter paper discs in 100 mm diameter Petri dishes. After allowing the CH_2Cl_2 to evaporate, the filter paper was moistened with 5 ml of distilled water. Corn seeds which had been germinated and which has a root about 10 mm long were then placed in each Petri dish (10/dish). 40 seeds were used for each condition and plates were placed in darkness at ambient temperature for 3 days. After this period, root growth was measured and compared to that of controls identically treated but with only distilled water in the Petri dishes.

Results. First, it is seen that phytotoxicity for Texas cytoplasm are not obligatorily related to the unique structure of methomyl **1** and thus to the functional group $R_2 = -SCH_3$ (table).

The replacement of the sulfur group by hydrocarbon chains of varying lengths (compounds 7, 12-14) did not modify biological activity. In comparison to compound 6 ($R_2 = CH_3$), these carbamates had a phytotoxicity comparable to that of the parent compound methomyl. Specificity nevertheless slightly decreased.

The modification of R_2 by introducing a branched chain (compound 11) or an aromatic ring (compound 19) seriously affected biological activity to a varying extent. Activity on root growth disappeared altogether when an ester function was introduced, regardless of chain branching (compounds 17, 18), or when the structural unit of methomyl is doubled (compound 23). Furthermore, the biological activity is strongly diminished when R_1 and R_2 were substituted especially by chain elongation (compounds 21 and 22).

Following the 1st nitrogen substitution, the effects were variable (compounds 4, 5, 8, 16, 24, 25), most probably as a result of stringent structural requirements. After the 2nd nitrogen substitution (compounds 2, 9, 15), specificity was strongly decreased and even disappeared.

The replacement of the amide carbonyl oxygen by a sulfur atom (compound 3) apparently reinforced specificity, even though phytotoxicity was strongly affected when R_2 was different from $-SCH_3$ (compound 10).

Propionic acid esters (compounds 26, 27) were inactive, although compound 28, methylisocyanate trimer, was toxic for Texas and Normal seeds. On the other hand, the initial oxime relative to methomyl, N-methylcarbamic acid esters (compounds 29, 30) and urea derivatives (compounds 31, 32) were inactive towards both seed types.

The results obtained with the carbamates in the table imply that all the compounds are biologically active. One can suppose that diffusion rates within the root may be different, but it is reasonable to admit that the imposed structural changes, which are occasionally minor, did not affect diffusion of compounds to the active site(s). Thus, although compound 14 had the longest R_2 chain, its toxicity was of the same order of magnitude as that of compound 8. In other cases, it was shown that the mobility of a compound, as well as its transmembrane diffusion, are increased by the length of a possible alkane chain[9]. This factor alone, however, is not sufficient to explain the diffusion of a substance in living tissue. Migration depends to a great extent on the hydrophilicity/hydrophobicity ratio.

Discussion and conclusions. The present results show that the phytotoxicity and specificity of the series of carbamates studied towards T and N corn seed germination are governed by narrow structural limits. Thus, both properties persist if R_2 is the only substituent changed. This suggests that the interactions between the molecule and active site are restrictive due to the topology. In particular, certain results suggest that specificity depends on a bond between the active site and the hydrogen bond to nitrogen; specificity disappears when the 2nd nitrogen is substituted. The introduction of a 2nd highly polarizable sulfur atom, does not lead to any spectacular effect; the specificity is only slightly enhanced in case of compound 3.

In conclusion, a few compounds were more efficient than methomyl but often without any selectivity. Only the carbamates 3 and 4 showed a greatest specific activity than the parent methomyl.

Finally, the results stress the importance of the electron conjugated system and suggest stringent structural requirements in the methomyl derivative for its efficiency.

The results presently obtained do not furnish evidence about the activity of carbamates against mitochondria[10,11]. Research on this question is currently in progress[12].

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