Sterilizing Activity of Homologous Bis(1-aziridinyl) alkylphosphinic Amides in Japanese Beetles (*Popillia japonica* Newman). Thyril L. Ladd, Jr.* and Alexey Bořkovec** (United States Department of Agriculture, *Japanese Beetle Research Laboratory, Agricultural Research Service, Wooster, Ohio 44691. **Insect Chemosterilants Laboratory, Agricultural Research Service, Beltsville, Maryland 20705) Received June 13, 1977. *Botu-Kagaku*, 41, 149, 1977.

22. マメコガネに対する Bis(1-aziridinyl) alkylphosphinic Amide 同族体の不妊化活性

Thyril L. Ladd, Jr. and Alexey Bořkovec (United States Department of Agriculture)

52. 6. 13 受理

雄のマメコガネに対し，tepa に類似した構造の bis(1-aziridinyl) alkylphosphinic amide 同族体5化合物を 0.5～32μg の範囲の量で経皮処理した．そのうち効力の高かったのは，*p*, *p*-bis (1-aziridinyl)-*N*-methylphosphinic amide で tepa の1.8 倍の不妊化作用があった．同族体の中では，アルキル基の炭素数の増加と共に活性の低下がみられた．

Bořkovec *et al.*[1] reported the synthesis of a series of aziridinylphosphine oxide insect chemosterilants related to tepa [tris (1-aziridinyl) phosphine oxide]. Subsequently it was found that the replacement of one aziridinyl group in tepa with alkylamino groups yielded a series of bisaziridinyl compounds active against several species of insects[2~15]. Also, quantitative studies of the structure-activity relationship in a homologous series of *P*, *P*-bis(1-aziridinyl)-*N*-alkyl phosphinic amides injected into male house flies, *Musca domestica* L., showed a progressive decrease in sterilizing activity with increasing length of the normal alkyl chain[6,7]. Although similar studies with pupae of a mosquito, *Anopheles albimanus* Wiedmann, did not indicate such a clear relationship between structure and activity[13], the pupae in these studies were treated by dipping; thus actual uptake of each compound into the hemolymph depended not only on its concentration in the dipping solution but also on the ease with which it was absorbed by and carried across the cuticle barrier. Initial tests with several *P*, *P*-bis (1-aziridinyl)-*N*-alkylphosphinic amides showed that male Japanese beetles, *Popillia japonica* Newman (Coleoptera ; Scarabaeidae), were highly susceptible to topical treatment though the data did not reveal the relative activities of individual compounds[12]. The present quantitative study with the same insect was conducted to examine the effectiveness of the topical application technique in elucidating structure-activity relationships in a series of 5 homologous *P*, *P*-bis (1-aziridinyl)-*N*-alkylphosphinic amides.

Materials and Methods

The chemosterilants used in the study were *P*, *P*-bis (1-aziridinyl)-*N*-methylphosphinic amide (A13-51254), *P*, *P*-bis (1-aziridinyl)-*N*-ethylphosphinic amide (A13-50767), *P*, *P*-bis (1-aziridinyl)-*N*-propylphosphinic amide (A13-51253), *P*, *P*-bis (1-aziridinyl)-*N*-isopropylphosphinic amide (A13-51256), and *P*, *P*-bis (1-aziridinyl)-*N*-butylphosphinic amide (A13-51023).[1] All compounds were at least 99% pure when used. The doses tested (6 levels each of from 0.5 to 16 μg of the methyl, ethyl, and isopropyl amides and of from 1 to 32 μg for the propyl and butyl amides) were topically applied with a micro-injector in 3 μl of ethanol to the coxasterna of field-collected male beetles of unknown ages. Four replicates of 7 males each were treated at each level and confined with 5 females, also collected in the field and presumably mated, the day after treatment in small glass jars (8 cm diam.) containing moist soil to a depth of 5 cm and pieces of fresh apple for food. Food and soil were replaced as needed. Ova were sifted from the soil twice each week with a fine screen and incubated in moist filter paper wells; viability was determined as previously reported[16]. Probit analyses were performed on the data using the maximum likelihood method of Finney[17] and the computer

Table 1. Sterilizing effects of structually related bis(1-aziridinyl)alkylphosphinic amides (C_1-C_4) applied topically to male Japanese beetles.

Alkyl moeity	Regression equation (probit on log dose)	Sterilizing dose ($\mu g/\sigma^{\prime}$)[a] SD_{50}	SD_{90}	Sterilizing efficiency[b] SD_{50}	SD_{90}	% decrease in effectiveness (SD_{90}) w/increasing C-chain length[c]
1 CH_3	$Y=3.78+3.39\times$	2.29(1.92-2.65)	5.47(4.60-6.90)	35	178	—
2 C_2H_5	$Y=3.50+3.16\times$	2.98(2.50-3.56)	7.59(5.92-11.14)	27	123	23
3 C_3H_7	$Y=3.03+3.07\times$	4.40(3.61-5.18)	11.53(9.52-15.07)	18	84	53
4 iso-C_3H_7	$Y=4.45+1.76\times$	2.05(1.56-2.59)	10.99(8.17-16.61)	39	88	50
5 C_4H_9	$Y=2.93+2.19\times$	8.80(7.15-10.55)	33.87(26.03-49.64)	9	29	84

[a] Figures in parentheses are fiducial limits at 95% probability level.
[b] Tepa ($SD_{50}=8\mu g$, $SD_{90}=9.7\mu g$)=100 (Ladd 1966).
[c] Single C chain=100%.

program developed by Daum[18]. Since earlier studies showed that the most recent matings of female Japanese beetles determine the fertility of ova that follow[16], the levels of sterility reported herein are assumed to reflect matings following treatment with chemosterilants.

Results and Discussion

Sterilizing doses at the 50 and 90% levels (SD_{50}, SD_{90}), sterilizing efficiencies, and changes in activity with increasing chain length are presented in Table 1 together with log-probit regression equations for each material tested. The extent to which the increasing lengths of the carbon chains of the alkyl moieties affect sterility was demonstrated by the increasing amounts of chemosterilant required to induce sterility at each level. With the straight-chain derivatives (C_1-C_4), calculated regression lines were sufficiently parallel to preserve these relationships at SD_{50} through SD_{90}. However, the slope of the dose-response line of the isopropyl derivative was less steep: compared with the other homologs, the compound was highly effective at SD_{50} but had only average effectiveness at SD_{90}. Quantitative reductions in the sterilizing activity of the straight-chain derivatives generally occurred in proportion to the increasing number of carbon atoms in the alkyl groups. At SD_{50}, for example, reductions in activity of 23, 25, and 27% occurred incrementally with the addition of single carbon atoms (C_1-C_4). At SD_{90}, the corresponding changes, though somewhat larger, were of a similar magnitude: 23, 25, and 31%.

The outstanding effectiveness of the methyl compound was also noted in the house fly[7], mosquito[15], and in other insects. Compared to its homologs and analogs, this compound has an exceptionally low partition coefficient[15] and its other physical properties also indicate a polarity exceeding that of tepa. Nevertheless, its broad sterilizing effectiveness suggests that it penetrates the cuticle of many insects quite successfully. The tobacco budworm, *Heliothis virescens* (F.), is a notable exception to the relatively high activity of the methyl compound: a topical appli cation of tepa to adult male budworms was 4 times as effective in inducing sterility as a similar treatment with the methyl compound[9]. Apparently, structure-activity relationships are influenced not only by the mode of administration of the agent but also by specific susceptibilities of individual insect species.

Summary

Male Japanese beetles were topically treated with 0.5-32 μg of 5 homologous bis(1-aziridinyl) alkylphosphinic amide chemosterilants closely related to tepa[tris(1-aziridinyl) phosphine oxide]. The most active, *P*, *P*-bis (1-aziridinyl)-*N*-methylphosphinic amide, was 1.8 times as effective a sterilant as tepa. Activity of the series declined with increasing lengths of the carbon chains of the alkyl portions of the molecules. These reductions appeared to be incrementally associated with increases in the numbers

of carbon atoms in the substituted moiety.

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Effects of Hempa on the Sterility and Mortality of *Drosophila melanogaster* (Meign). A. N. Chattoraj and B. B. L. Srivastava* (Entomological Research Laboratory, Department of Zoology, University of Allahabad, Allahabad, 211002, India) Received Jul. 25, 1977. *Botyu-Kagaku*, 42, 151, 1977.

23. Hempa によるショウジョウバエの不妊化率と死亡率 A. N. Chattoraj and B. B. L. Srivastava (University of Allahabad) 52. 7. 25 受理

ショウジョウバエの成虫，幼虫の餌に hempa を混入してその効力を検べた．幼虫時に処理した雌成虫は，正常雄と交尾すると産卵は増加するが，同時にその卵の孵化率も低下した．同様に処理した雄と正常雌では，産卵は少ないが，孵化率は処理雌の時より高かった．従って幼虫時の処理は雄よりも雌に行なう方が効果的である．0.5% hempa の混入で 100% の不妊化率がみられた．

成虫の餌では 2%混入して 100% の不妊化率であった．しかも，この時は雄成虫に処理した方が効果的であった．これは幼虫の場合とは，効果が逆転しているという結果になった．

The autocidal method of insect control has attracted much attention of the entomologists during the past decade and a large number of chemicals liable to induce sexual sterility in dipterans have been evaluated for this purpose (La Brecque 1960 & 1961)[14,15]. Chang *et al* (1961)[4] found that the non alkylating agent hempa could produce sterility in *M. domestica*, Fye *et al* (1966)[7] obtained 100% sterility in house flies when hempa was given in the food of the adult at a concentr ation of 0.05%. Similar results have also been reported by La Brecque *et al* (1966) when hempa was applied to pupae and adults of house flies. Davis and Eddy (1966)[6] found that hempa was more effective when given in food than in residual treatment in *Fannia canicularis* (L.) and that it affected males more than the females. McFadden and Rubio (1966) reported that when they fed 8 day old Mexican fruit fly larvae with food containing 0.5% hempa no emergence of adult flies was noted. They also found that pupae dipped in 5.0% solution of hempa for 60 seconds resulted in 62% of adult emergence with 100% sterility and that when adult flies were fed for 3

* Present address: Entomology Department, K.A. Degree College, Allahabad.