THE TOXICITY OF ORGANIC SULPHIDES TO THE EGGS AND LARVAE OF THE GLASSHOUSE RED SPIDER MITE. I.—*SS'*-Disubstituted Alkane-$\alpha\omega$-dithiols

By R. F. BROOKES, J. E. CRANHAM, W. A. W. CUMMINGS, D. GREENWOOD, B. S. JACKSON and H. A. STEVENSON

A laboratory test method for assessing the activity of chemicals against the eggs and young mites of the glasshouse red spider (*Tetranychus telarius* L.) is described. The results given by a series of *SS'*-disubstituted alkane-$\alpha\omega$-dithiols using this test method are tabulated and discussed.

Introduction

Workers who have reviewed the problem of controlling red spider mites (Tetranychidae) have stressed the need for materials toxic to the eggs of the mites.[1] As part of a routine ' screening ' of chemicals for possible new insecticides a method was devised for testing chemicals in the laboratory for activity against the eggs of *Tetranychus telarius* L., the glasshouse red spider mite.

The initial discovery of ovicidal activity by this method, as noted by Cranham, Higgons & Stevenson,[2] was in bisphenylthiomethane and 1 : 2-bisphenylthioethane. Substituted derivatives of these two compounds were made and tested for ovicidal activity as also were a number of bisalkylthioalkanes, bis(heterocyclicthio)alkanes and the sulphoxides and sulphones of a number of the bisarylthioalkanes. Variations in the bridge between two benzene nuclei which led to the discovery of *p*-chlorobenzyl *p*-chlorophenyl sulphide (chlorbenside) will be described in a later communication.

As the present work was part of a larger programme involving further tests on those compounds selected as promisingly active, no attempt has been made to establish accurately the existence of comparatively small toxicity differences in laboratory tests.

This, usually, has little relevance to the practical usefulness of a compound. Furthermore, the degree of activity, or even the relative order of activity, against other tetranychid mites cannot be forecast from the results of tests against one species. This point has been well expressed by Elliot *et al.*[3] in relation to a wider range of test insects.

Experimental

Synthesis of compounds

The *SS'*-disubstituted alkane-$\alpha\omega$-dithiols were usually prepared by heating together the appropriate thiol and dibromoalkane in alcohol containing a slight excess of dissolved sodium (or sodium ethoxide or sodium hydroxide). In some cases the thiol was prepared (but not isolated) by refluxing the corresponding disulphide in xylene with a small excess of sodium. The resulting sodium mercaptide was separated off and heated with the appropriate dibromoalkane in alcohol. Generation of the thiol *in situ* was also effected by reducing the corresponding disulphide with sodium sulphide or glucose.

Some derivatives of methanedithiol were prepared by the action of formalin on the appropriate thiol in the presence of hydrogen chloride.

In the synthesis of the $\alpha\omega$-bisalkylthioalkanes the thiol was often liberated *in situ* from the *S*-alkyl*iso*thiuronium salt and reacted with the dibromoalkane.

The following examples illustrate these various modifications.

1 : 6-*Bisphenylthiohexane.*—Benzenethiol (12 g.) and 1 : 6-dibromohexane (12·2 g.) were refluxed for one hour in a solution of sodium ethoxide (7·5 g.) in alcohol (200 c.c.). Cooling and addition of water precipitated the product as large colourless plates of m.p. 82°. Recrystallization from alcohol yielded colourless plates (13·8 g. ; 92% yield) of the same melting point.

1 : 2-*Bisphenylthioethane.*—Sodium (2·5 g.) was melted under xylene (300 c.c.) and stirred vigorously as the mixture cooled in order to produce ' powdered ' sodium. Diphenyl disulphide (10 g.) was added and the mixture stirred at 100° in an atmosphere of nitrogen until the sodium

was replaced by sodium phenyl sulphide—a colourless powder. The reaction time was about 4 hours depending on the particle size of the sodium. After filtration and washing with a little dry ether, the sodium phenyl sulphide (12·9 g.) was dissolved in alcohol (120 c.c.) and refluxed with ethylene dibromide (5 c.c.) for 2 hours. The product (9·85 g. ; 87% yield) which separated after cooling and the addition of water was pure without recrystallization (m.p. 67-68°).

1 : 2-*Bis*-m-*nitrophenylthioethane.*—A solution of sodium sulphide (24 g.) and sodium hydroxide (16 g.) in the minimum amount of water (approx. 40 c.c.) was added slowly to a solution of bis-*m*-nitrophenyl disulphide (54 g.) in boiling alcohol (200 c.c.). After refluxing for 30 minutes the solution was diluted with hot water (1 l.), cooled, and the aqueous layer decanted from the oil which separated. The aqueous solution was refluxed for one hour with ethylene dibromide (40 g.), cooled, and the solid filtered off and washed with alcohol. One recrystallization from glacial acetic acid gave the *product* (22·8 g. ; 38·6% yield) as yellow crystals, m.p. 135–136°.

Bisphenylthiomethane.—Dry hydrogen chloride was passed into benzenethiol (11 c.c.) and formalin (4·3 c.c. ; 40% aqueous solution) in acetic acid (50 c.c.) for 2 hours. Addition to ice-cold sodium hydroxide solution and recrystallization of the precipitated solid from alcohol gave the product (7·7 g. ; 66% yield) as long, white needles, m.p. 40°.

Bishexylthiomethane.—Hexyl bromide (20 g.) and thiourea (9·5 g.) were refluxed in alcohol (50 c.c.) for 4 hours. After the addition of sodium hydroxide (14·6 g.) in water (20 c.c.) methylene dibromide (10·6 g.) was added slowly. Refluxing for 2 hours, cooling, the addition of an equal volume of water, and extraction with ether gave the *product* (7·5 g. ; 50% yield) as an oil, b.p. 136°/1 mm.

The aminophenyl derivatives were invariably prepared by reduction of the corresponding nitro compounds.

Stock solutions of the compounds for biological testing were prepared by dissolving them, if sufficiently soluble, in a mixture of Cellosolve (90 parts) and Empilan A (a proprietary non-ionic emulsifying agent) (10 parts) at a concentration of 5%. Compounds too sparingly soluble in this mixture were dispersed by wet-milling at a concentration of 5% in water containing 0·5% of Dispersol LN (a proprietary dispersing agent).

Rearing of mites

Tetranychus telarius L. was reared on dwarf French beans (*Phaseolus vulgaris*). This was carried out in a glasshouse with a minimum temperature of 60° F in winter. Artificial light was supplied by ' daylight ' fluorescent strips from October to March to provide a day-length of 14 hours or more. No difficulties were encountered under these conditions in rearing sufficient mites, or in the fecundity of the female mites.

The mites used to infest French beans initially were taken from natural infestations occurring on roses in our glasshouses at Nottingham. The majority of adult female mites which developed on French beans were red in colour throughout the year like those usually occurring on carnations. When mites were removed to strawberry, cucumber or rose as host plants the succeeding generations assumed the pale lemon to green coloration usual to the summer stages of this mite on most host plants.

Test method used

Young terminal leaves were selected from uninfested bean plants. These leaves were cut with about 2 inches of petiole or stem, so that they could be stood in flat-bottomed specimen tubes containing tap water. Corks with small centrally bored holes were used in the mouths of the specimen tubes, so that with the petiole passing through the hole and its cut end in water, the leaf was supported more or less horizontally.

Leaves of 1½–2 inches in length were chosen and uniformity of the different characteristics of the leaf was sought. The smaller the leaf, the more easily it could be examined under the microscope. Smaller French bean leaves than the above were unsuitable, however, since they did not have a flat surface, wilted badly when cut and were too easily damaged by chemicals. Larger leaves also wilted more, tended to rest on the tray and presented a larger area to examine.

The leaves after cutting and placing in water were allowed to recover in a room conditioned to 75° F and 60% R.H. where they were kept 12 inches under one ' daylight ' fluorescent strip. After recovery, the leaves were infested with adult female mites transferred by hand with fine (Sable 00) paint brushes from infested bean plants. Normally 7–8 female mites per leaf laid between 30–70 eggs within 24–32 hours and it was sometimes, but seldom, necessary to increase the number of mites to 10–12 in order to obtain this number of eggs. After egg-laying the female mites were removed with a fine suction pipette and leaves were examined under a microscope × 20. Those bearing less than 30 eggs were rejected, and eggs were removed from others if the number exceeded 70.

Eggs were laid almost entirely on the under surface of the leaves ; an occasional few on the upper surfaces were removed. Leaves were put back again in the conditioned room and used the following day when the eggs were approximately 1–2 days old. Dilutions of the stock solutions of the test chemical were made with a 0·01% solution of non-ionic proprietary wetting agent (Ethylan K).

Treatment was carried out by dipping each leaf for 10 seconds in the dispersion or emulsion of the test chemical. After dipping, leaves were suspended across two parallel lengths of string secured, one inch apart, so that the petioles rested on the double line and the leaves hung roughly vertically with apices downwards. This ensured roughly equal run-off of excess dispersion although deposits were heavier at the apical tips of the leaves. When dry, the leaves were replaced in their tubes and stored in the conditioned room for 7 days. Normally, four replicate leaves were used per dose per substance tested, but five or six were used for more precise tests.

A more precise method of treatment was also evolved by which the leaves were pinned lightly on cork sheets with the lower surfaces upwards, and sprayed with the diluted wash of test chemical using a tower of the type described by Potter.[4] This gave even distribution of deposit on the lower surface of the leaf only. The deposit used was standardized throughout all assays at 0·3 ± 0·02 g. of wet spray (determined by weighing) per 9-cm. diameter Petri dish receiving spray on the platform of the spraying tower. Weight of wet spray was kept constant and variations in dosage were obtained using different dilutions of test chemical. The degree of spray cover of the lower leaf surface was just short of complete, being an even distribution of fine droplets which did not coalesce to give a complete ' wet '. The leaves were allowed to dry while still pinned upside down on the cork sheets. When dry, leaves were replaced in their tubes.

This method of treatment by spraying was only used in this work for the more precise tests, the results of which are given in Tables II and III.

Under the conditions of storage, the hatch of eggs was complete on untreated leaves 5 days after treatment, that is, 6 to 7 days after laying. Seven days after treatment, all young mites had developed to the nymphal stage ; being a mixture of mostly protonymphs, a few deutonymphs, and the intermediate resting stages. Adult female mites developed by the 9th day after treatment (10–11 days after laying). Assessment of effect was made 7 days after treatment under a microscope × 20 and a count made of (1) unhatched eggs, (2) dead young mites, including larvae, shrivelled resting stages, and nymphs and (3) live young mites including resting stages healthy in appearance.

It was found from precounting on the number of eggs on certain leaves, that in practice very few young mites indeed left the leaves, nor did dead mites fall off, so that the total count of (1), (2) and (3) above corresponded very well with the number of eggs originally present. It did not prove necessary, therefore, to count the number of hatched eggs (egg shells) which is a more difficult task.

From the sums of (1), (2) and (3) above, taken singly and together for replicate leaves at each dose the following percentage effects were evaluated :

(*a*) Percentage ' ovicidal mortality ' $= \dfrac{\text{Total (1)}}{\text{Total (1 + 2 + 3)}}$

(*b*) Percentage kill of eggs plus young mites here called the ' total mortality '

$$= \frac{\text{Total (1 + 2)}}{\text{Total (1 + 2 + 3)}}$$

Design of experiments

Initial screening tests were carried out at 0·1% concentration of each test chemical, several compounds being included in each test with a ' formulation control ' of suspension-medium only, and a ' standard ' ovicide for comparison. Azobenzene was employed as a standard at first, but after the discovery of activity in the unsubstituted bisphenylthiomethane, this compound was employed throughout.

Where assays were carried out at three or more doses for each toxicant, probit analysis[5] could generally be applied to the results. Generally, however, dosage mortality lines were fitted only by eye. As the work progressed, active compounds were followed up by the synthesis and testing of related compounds. Thus, the grouping of compounds for initial tests was not systematic. This being so, and since conclusions drawn are based on relatively large differences, the results of statistical analyses are omitted from the tables.

Results

Table I gives the initial ' ovicidal mortality ' obtained at 0·1% concentration of each compound, and at 0·025% where carried out, corrected by the method of Finney[6] for mortality in the ' formulation controls '. With the compounds here described, negligible toxicity was exerted on larvae, so that the ' total mortality ' (kill of eggs and young mites) never exceeded the ' ovicidal mortality ' by more than 5%. For this reason ovicidal mortalities are generally omitted from the tables of results.

Table I

A. *αω-Bisarylthioalkanes*

$XC_6H_4{\cdot}S{\cdot}[CH_2]_n{\cdot}S{\cdot}C_6H_4X$

Ref. No.	X	*n*	% ' Total ' mortality 0·1%	% ' Total ' mortality 0·025%	M.p., ° c or b.p., ° c/mm.	Formula	Analysis Found % C	Analysis Found % H	Analysis Required % C	Analysis Required % H	Reference
1804	H	1	99	56	40	$C_{13}H_{12}S_2$	—		—		7
1805	H	2	99	49	70	$C_{14}H_{14}S_2$	—		—		8
2079	H	3	96	28	200/3·0	$C_{15}H_{16}S_2$	—		—		9
2080	H	4	26	0	86	$C_{16}H_{18}S_2$	—		—		10
2124	H	5	19	0	220/2·5	$C_{17}H_{20}S_2$	71·0	6·8	70·8	6·9	*
2197	H	6	0	—	82	$C_{18}H_{22}S_2$	71·0	7·05	71·5	7·3	*
1782	*o*-OCOMe	10	0	—	65–66	$C_{26}H_{34}O_4S_2$	66·1	7·25	65·8	7·2	11*
2354	*o*-Cl	1	88	32	69	$C_{13}H_{10}Cl_2S_2$	52·2	3·5	51·8	3·3	*
2355	*o*-Cl	2	56	0	83	$C_{14}H_{12}Cl_2S_2$	52·75	3·8	53·3	3·8	*
2494	*m*-Cl	1	99	69	190–200/2·5	$C_{13}H_{10}Cl_2S_2$	52·2	3·5	51·8	3·3	*
2356	*m*-Cl	2	85	22	63	$C_{14}H_{12}Cl_2S_2$	53·4	3·6	53·3	3·8	*
2055	*p*-Cl	1	99	78	44	$C_{13}H_{10}Cl_2S_2$	—		—		12
2054	*p*-Cl	2	52	0	94	$C_{14}H_{12}Cl_2S_2$	53·6	3·9	53·3	3·8	*
2455	*p*-F	1	100	44	144–147/1·1	$C_{13}H_{10}F_2S_2$	57·8	3·4	58·2	3·7	†
2429	*p*-F	2	96	63	70·5–71·5	$C_{14}H_{12}F_2S_2$	59·2	4·25	59·6	4·3	†
2424	2 : 4 : 5-Cl_3	1	0	—	143–144	$C_{13}H_6Cl_6S_2$	35·5	1·4	35·5	1·4	*
2428	2 : 4 : 5-Cl_3	2	5	—	119–120	$C_{14}H_8Cl_6S_2$	37·0	1·7	37·1	1·8	*
1920	*p*-Me	1	18	0	32	$C_{15}H_{16}S_2$	69·1	5·9	69·2	6·15	*
1779	*p*-Me	2	0	—	80	$C_{16}H_{18}S_2$	—		—		16
1695	*o*-OH	1	72	18	67–68	$C_{13}H_{12}O_2S_2$	59·05	4·2	59·1	4·5	11*
1694	*o*-OH	2	0	—	108	$C_{14}H_{14}O_2S_2$	60·7	5·1	60·4	5·0	11*
1894	*p*-OH	2	0	—	168	$C_{14}H_{14}O_2S_2$	60·05	5·1	60·4	5·0	11*
1867	*o*-OCOMe	1	0	—	52	$C_{17}H_{16}O_4S_2$	58·65	4·6	58·6	4·6	11*
1806	*o*-OCOMe	2	0	—	79	$C_{18}H_{18}O_4S_2$	60·15	4·75	59·7	5·0	11*
2130	*o*-CO_2H	1	0	—	286–288	$C_{15}H_{12}O_4S_2$	56·2	4·1	56·3	3·75	††
2357	*o*-$OCH_2{\cdot}CO_2H$	2	0	—	approx. 220°	$C_{18}H_{18}O_6S_2$	54·4	4·15	54·8	4·6	**

* Prepared by D. G.
† Prepared by Dr. N. G. Clark
** Prepared by R. F. B.
†† Prepared by W. A. W. C.

Table I (*contd.*)

Ref. No.	X	*n*	% 'Total' mortality 0·1%	0·025%	M.p., °c or b.p., °c/mm.	Formula	Analysis Found % N	Required % N	Reference
2181	*o*-NO_2	1	0	—	172–173	$C_{13}H_{10}O_4N_2S_2$	8·8	8·7	**
2084	*o*-NO_2	2	0	—	203–205	$C_{14}H_{12}O_4N_2S_2$	—	—	13
2179	*m*-NO_2	1	0	—	136–137	$C_{13}H_{10}O_4N_2S_2$	9·1	8·7	**
2182	*m*-NO_2	2	0	—	136–137	$C_{14}H_{12}O_4N_2S_2$	8·6	8·3	**
2147	*p*-NO_2	1	0	—	177–179	$C_{13}H_{10}O_4N_2S_2$	8·8	8·7	**
2083	*p*-NO_2	2	0	—	132·5–133·5	$C_{14}H_{12}O_4N_2S_2$	—	—	14
2178	2 : 4-$(NO_2)_2$	1	0	—	203–204	$C_{13}H_8O_8N_4S_2$	13·6	13·6	**
2209	*o*-NH_2	1	67	—	59·5–60·5	$C_{13}H_{14}N_2S_2$	10·8	10·7	**
2180	*o*-NH_2	2	90	28	76–77	$C_{14}H_{16}N_2S_2$	—	—	14
2210	*m*-NH_2	1	38	0	71·5–72·5	$C_{13}H_{14}N_2S_2$	10·9	10·7	**
2211	*m*-NH_2	2	56	0	98–99	$C_{14}H_{16}N_2S_2$	10·2	10·2	**
2212	*p*-NH_2	1	39	0	99·5–100	$C_{13}H_{14}N_2S_2$	10·8	10·7	**
2149	*p*-NH_2	2	39	0	110–111	$C_{14}H_{16}N_2S_2$	—	—	15

** Prepared by R. F. B.

B. *αω-Bisalkylthioalkanes*

$R{\cdot}S{\cdot}[CH_2]_n{\cdot}S{\cdot}R$

Ref. No.	R	*n*	% 'Total' mortality 0·1%	0·025%	M.p., °c or b.p., °c/mm.	Formula	Analysis Found % C	% H	Required % C	% H	Reference
2122	Bu^n	1	0	—	100/2·0	$C_9H_{20}S_2$	—		—		17
2095	Bu^n	2	69	0	116–118/2·0	$C_{10}H_{22}S_2$	—		—		18
2213	*n*-C_6H_{13}	1	0	—	136/1·0	$C_{13}H_{28}S_2$	62·95	11·4	62·9	11·2	***
2215	*n*-C_6H_{13}	2	45	0	160/2·0	$C_{14}H_{30}S_2$	63·6	11·4	64·1	11·5	***
2167	*n*-$C_{12}H_{25}$	2	0	—	52–54	$C_{26}H_{54}S_2$	72·2	12·4	72·6	12·6	***
2076	$CH_2{\cdot}CO_2H$	1	6	0	122·5–123	$C_5H_8O_4S_2$	—		—		19
2066	$CH_2{\cdot}CO_2H$	2	56	0	108–109	$C_6H_{10}O_4S_2$	—		—		20
2876	$CH_2{\cdot}CO_2Bu^n$	1	14	10	176–178/1·0	$C_{13}H_{24}O_4S_2$	50·2	7·9	50·6	7·8	††
2877	$CH_2{\cdot}CO_2Bu^n$	2	17	6	188–190/1·0	$C_{14}H_{26}O_4S_2$	52·7	8·3	52·2	8·1	††
2136	CH_2Ph	1	35	0	46	$C_{15}H_{16}S_2$	—		—		21
2101	CH_2Ph	2	38	0	38	$C_{16}H_{18}S_2$	—		—		14
2634	$CH_2{\cdot}C_6H_4Cl$-*p*	1	61	0	56–57	$C_{15}H_{14}Cl_2S_2$	54·4 [Cl 21·9	3·8	54·7 21·6]	4·3	***
2459	*cyclo*Hexyl	2	0	—	166/2·0	$C_{14}H_{26}S_2$	65·4	10·2	65·1	10·1	***
2387	Bu^n	3	6	0	106–108/1·0	$C_{11}H_{24}S_2$	60·3	10·5	60·0	10·9	***

*** Prepared by B. S. J. †† Prepared by W. A. W. C.

C. *αω-Bis(heterocyclicthio)alkanes*

Hetero·S·$[CH_2]_n$·S·Hetero

Ref. No.	Hetero	*n*	% 'Total' mortality 0·1%	0·025%	M.p., °c or b.p., °c/mm.	Formula	Analysis Found % N	Required % N	Reference
2233	Pyrid-2-yl	1	87	23	93·0–93·5	$C_{11}H_{10}N_2S_2$	12·0	12·25	††
2456	Pyrid-4-yl	1	31	0	146·5–147·5	$C_{11}H_{10}N_2S_2$	12·2	12·25	††
2457	Pyrid-4-yl	2	30	0	126–127	$C_{12}H_{12}N_2S_2$	11·3	11·3	††
2346	Quinol-2-yl	1	0	—	136–137	$C_{19}H_{14}N_2S_2$	8·4	8·3	††
3076	Benzoxazol-2-yl	1	7	7	127–128	$C_{15}H_{10}O_2N_2S_2$	9·2	8·9	††
3078	Benzoxazol-2-yl	2	2	6	141·5–142·5	$C_{16}H_{12}O_2N_2S_2$	8·4	8·5	††
2131	Benzthiazolin-2-yl	1	0	—	95–96·5	$C_{15}H_{10}N_2S_4$	8·3	8·1	††
2086	Benzthiazolin-2-yl	2	0	—	144–146	$C_{16}H_{12}N_2S_4$	8·1	7·8	**
3085	5-Chlorobenzthiazolin-2-yl	2	31	4	207·5–208·5	$C_{16}H_{10}N_2Cl_2S_4$	6·4	6·5	††
2625	4-Hydroxy-5-methylpyrimid-2-yl	1	0	—	268–270 (decomp.)	$C_{11}H_{12}O_2N_4S_2$	18·6	18·9	††

†† Prepared by W. A. W. C. ** Prepared by R. F. B.

Table I (*contd.*)

D. *Sulphoxides and sulphones*

$RC_6H_4 \cdot SO_x \cdot [CH_2]_n \cdot SO_x \cdot C_6H_4R$

Ref. No.	R	x	n	% 'Total' mortality 0·1%	% 'Total' mortality 0·025%	M.p., °C or b.p., °C/mm.	Formula	Analysis Found % C	Analysis Found % H	Analysis Required % C	Analysis Required % H	Reference
2194	H (racemic ?)	1	1	0	—	194	$C_{13}H_{12}O_2S_2$	—		—		22
2196	H (*meso* ?)	1	1	71	0	115–116	$C_{13}H_{12}O_2S_2$	58·9	4·6	59·1	4·55	*
1781	*p*-Me	1	2	0	0	166	$C_{16}H_{18}O_2S_2$	—		—		16
2235	H	2	1	0	0	116–117	$C_{13}H_{12}O_4S_2$	—		—		23
2123	H	2	2	0	0	181	$C_{14}H_{14}O_4S_2$	—		—		8
1780	*p*-Me	2	2	0	0	201	$C_{16}H_{18}O_4S_2$	—		—		16
1808	*o*-OCOMe	2	2	0	0	204–205	$C_{18}H_{18}O_8S_2$	50·8	4·2	50·7	4·2	11*

* Prepared by D. G.

The results of further tests on certain active compounds are summarized in Table II and the results of comparison with known active acaricides is given in Table III. 'Total' and ovicidal mortalities are given in this latter table, to illustrate the small differences involved.

Table II

The activity of αω-bisarylthioalkanes against eggs and young mites of red spider (T. telarius *L.*)

$XC_6H_4 \cdot S \cdot [CH_2]_n \cdot S \cdot C_6H_4X$

Test	Ref. No.	X	n	% 'Total' mortality at 0·1%	0·05%	0·025%	0·0125%
I	1804	H	1	99	71	35	9
	1805	H	2	99	75	29	12
	2079	H	3	94	62	24	6
II	1804	H	1	93	83	50	—
	2055	*p*-Cl	1	99	93	86	36
	2494	*m*-Cl	1	99	91	83	36
	2354	*o*-Cl	1	84	58	28	—
III	1804	H	1	98	83	44	—
	2356	*m*-Cl	2	82	55	17	—
	2355	*o*-Cl	2	54	57	6	—
IV	1804	H	1	95	79	32	—
	2055	*p*-Cl	1	98	88	55	—
	2054	*p*-Cl	2	49	17	5	—
	1695	*o*-OH	1	78	49	30	—
V	1804	H	1	—	69	28	5
	2455	*p*-F	1	—	82	48	11
	2429	*p*-F	2	—	99	68	29
	1805	H	2	—	72	31	11

All substances were formulated as stock solutions in a mixture of Cellosolve (90 parts) and Empilan A (10 parts).

Discussion of results

Examination of the experimental results revealed certain trends in the relationship between structure and biological activity.

Thus, in the unsubstituted αω-bisphenylthioalkanes there was little difference in activity in the first three members of the series but there was a very sharp fall in the activity of higher members.

The effect of introducing a chlorine atom into each benzene nucleus of bisphenylthiomethane was to increase the activity in the case of the *meta*- and *para*-isomers which were the two most active compounds in this series and which were not significantly different in activity. The *ortho*-isomer, however, was very much less active than the other two and slightly less active than the unsubstituted compound. Similar substitution by chlorine in 1 : 2-bisphenylthioethane gave compounds considerably less active than the corresponding bischlorophenylthiomethanes and all were less active even than the unsubstituted compound.

Table III

Known acaricides compared with bisphenylthiomethane

Test (i)	% ' Total ' mortality (ovicidal mortality in brackets) at			
	0·08%	0·04%	0·02%	0·01%
Bisphenylthiomethane	100 (99)	86 (86)	59 (57)	45 (42)
1 : 2-Bisphenylthioethane	97 (95)	79 (76)	41 (41)	20 (19)
Azobenzene	87 (87)	66 (62)	24 (22)	10 (10)
CPBS	100 (99)	99 (98)	99 (94)	95 (59)
CPCBS	100 (100)	100 (99)	99 (93)	94 (43)

Test (ii)	% ' Total ' mortality (ovicidal mortality in brackets) at		
	0·1%	0·05%	0·025%
Bisphenylthiomethane	93 (93)	83 (82)	50 (48)
Azobenzene	98 (96)	79 (79)	46 (46)
CPCBS	98 (98)	98 (98)	98 (94)

All substances were formulated as stock solutions in a mixture of xylene (90 parts) and Insem 108 (a proprietary emulsifying agent) (10 parts).

Bis-*p*-fluorophenylthiomethane and 1 : 2-bis-*p*-fluorophenylthioethane were both slightly less active than bis-*m*- or bis-*p*-chlorophenylthiomethane but it was noteworthy that 1 : 2-bis-*p*-fluorophenylthioethane was more active than its chlorine substituted analogue.

Both bis-2 : 4 : 5-trichlorophenylthiomethane and the ethane homologue were inactive, as were all of the nitro-substituted bisarylthioalkanes, whilst the activity in the corresponding amines was only slight.

Bis-*o*-hydroxyphenylthiomethane had a slight activity but no activity was shown by any other hydroxy- or acetoxy-substituted compound. Only small activity at the higher test concentration was to be found in a few of the bisalkylthioalkanes and bis(heterocyclicthio)alkanes.

There was complete lack of activity in the sulphoxides and sulphones of the bisarylthioalkanes with the notable exception of the lower melting isomer (*meso* ?) of bisphenylsulphinylmethane.

In connexion with the influence of physical properties on activity, it was noted that all those compounds which are only sparingly soluble (1% or less) in the usual organic solvents had little or no activity. The active compounds were readily soluble (10% or more) but, of course, all soluble compounds were not active.

Comparison of the activity of bisphenylthiomethane and 1 : 2-bisphenylthioethane with known acaricides such as azobenzene, CPBS (*p*-chlorophenyl benzenesulphonate), and CPCBS (*p*-chlorophenyl *p*-chlorobenzenesulphonate), showed them to be promising in these laboratory tests by comparison with azobenzene, but both CPBS and CPCBS gave much higher kills at the lower test concentrations.

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Research Department
Boots Pure Drug Co. Ltd.
Nottingham

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ANALYTICAL STUDIES ON THE CARBOHYDRATES OF GRASSES AND CLOVERS. VIII.*—Changes in Carbohydrate Composition during the Growth of Perennial Rye-grass

By D. J. MACKENZIE and CLARE B. WYLAM†

Analyses of the carbohydrates in the leaf and stem of perennial rye-grass were carried out during uninterrupted growth from May to November. Although there were individual variations in the amounts of glucose, fructose and sucrose present, no major seasonal trends in these sugars were evident. The plant accumulated in the stem a reserve of fructosan which reached a maximum of 21% of the dry matter before it diminished in the autumn. There was a gradual increase in the percentages of cell-wall polysaccharides. The relation between fructosan accumulation and synthesis of cell-wall material during the growth of the plant is discussed.

The autumn aftermath growth was analysed and its composition compared with that of the first growth.

Introduction

The importance of the water-soluble constituents of herbage has been recognized for a considerable time, both from the point of view of its energy value in fresh and preserved feeding stuffs, and on account of the advantage of a high sugar content in silage making. In the latter respect, an approximate knowledge of the fructosan content of a grass is particularly useful, since this polysaccharide undergoes a wide variation throughout the summer and is probably sufficient under certain conditions to contribute most of the sugar necessary for adequate lactic acid production.[1]

Production of fatty acids by fermentation of cellulose is well known as a source of energy in ruminants,[2, 3, 4] and the importance of hemicelluloses in the diet is now also being realized.[5] For instance Heald[6] has recently suggested that in 24 hours 60–80 g. of xylan may be fermented in the rumen of a sheep at pasture.

In view of the recognition of the importance of these constituents it is essential to obtain information about the variation of both the water-soluble and cell-wall carbohydrates throughout the growing season

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† Present address: Imperial Chemical Industries Ltd., Central Agricultural Control, Jealott's Hill, Bracknell, Berks.