accurate measurements to show the presence of any residue at this extremely low concentration level—i.e., below 0.05 p.p.m.—in crops receiving treatment would require considerable modification of the method and perhaps an entirely different approach to the analysis.

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RODENT REPELLENTS

Preparation and Properties of Thiouronium Compounds and Cyclic Imides

ERVIN BELLACK and JAMES B. DeWITT
Fish and Wildlife Service, U. S. Department of the Interior, Laurel, Md.

Syntheses and bioassays of cyclic imides and thiouronium compounds were carried out as part of a search for materials capable of preventing rodent damage to packaged commodities. Previous studies had shown that repellent activity was associated with functional groups containing nitrogen and sulfur, and was enhanced by the presence of ionic linkages. Twenty-seven thiouronium compounds and 40 imides, including 10 compounds not described previously, were prepared for these tests. Ten imides and 26 thiouronium compounds were repellent under the conditions of test. Information obtained in these studies will be utilized in the development and selection of more effective materials for prevention of rodent damage to foods and other commodities.

IN STUDIES OF RODENT DAMAGE to packaged articles and other commodities, the Fish and Wildlife Service has examined more than 5000 chemicals for repellency to rats and mice. Test procedures have been divided into three phases: a preliminary screening operation in which candidate compounds were incorporated in diets fed laboratory rats (*3*); more advanced laboratory studies in which paperboard panels were treated with promising materials for determination of relative resistance to gnawing attacks (*4*); and simulated warehouse studies in which cartons were treated with candidate repellents and exposed to gnawing attacks by wild rodents (*20*).

The preliminary screening (food acceptance) tests have been utilized as a means of selecting promising materials for the more laborious and time-consuming barrier and warehouse studies, and have furnished valuable information on possible relationships between chemical composition and repellent activity. Classification of candidate materials according to structure and composition has shown that repellency may be correlated with certain functional groups, such as $-NH_2$ and $-NO_2$, attached to alkyl, aryl, or heterocyclic nuclei (*5*). Activity of any group may be enhanced or negated by introduction of other substituents, or by changes in molecular weight, spatial configuration, or unsaturation in the nucleus.

The majority of active repellents contain nitrogen, sulfur, or halogen; amines and their derivatives form one of the most active classes. Some free amines are repellent, but activity seems to be enhanced by formation of salts, complexes, or quaternary halides. This enhanced activity appears to be a function of ionic or other linkages:

$$(R—N—)^+(X)^- \text{ or } (R—N—)—(R—X)$$

In addition to the quaternary ammonium and pyridinium compounds, many sulfonium, arsonium, boronium, and phosphonium compounds were found to be active repellents. These materials all contain ionic linkages similar to those of the quaternary ammonium halides, and it appeared that other materials having ionic structures should be investigated. One of the most promising groups in this category was the thiouronium, or isothiourea salts, whose structure can be written:

$$[(H_2NC(SR)=NH)]^+ (X)^-$$

A number of thiouronium compounds were prepared and tested by the food acceptance technique to investigate possible relationships between structure and repellent activity. The list of materials includes some compounds not previously described in the literature, in addition to several compounds developed by other investigators.

Preparation of Thiouronium Compounds

One-tenth mole of thiourea and 0.10 mole of alkyl halide were dissolved in 10 to 25 ml. of ethyl alcohol and refluxed until the solution no longer gave a positive test for =S when treated with ammoniacal silver nitrate. The lower (volatile) halides were obtained by removal of the alcohol and traces of alkyl halide in vacuo, followed by cooling of the residual oil. The higher halides were prepared by precipitation with ether and recrystallization from alcohol-ether. Substituted benzyl thiouronium salts

were prepared by adding an aqueous solution of the sodium salt of the acid to a slight excess of benzyl thiouronium chloride in hot alcohol, followed by cooling of the reaction mixture.

Preparation of Imides

Several methods for preparing cyclic imides have been described in the literature, and were adapted for the synthesis of the compounds listed in Table I. These procedures include distillation of the mono- or diammonium salt, heating of the diamide, or fusion of the monoamide. In addition, some imides can be prepared by indirect synthesis from an unrelated heterocyclic compound. *N*-Alkyl and *N*-aryl imides can be prepared by analogous means: distillation of the mono- or diamine salt, heating of the *N*-substituted diamide, fusion of the *N*-substituted monoamide, or indirect synthesis. Specialized methods are also available for preparing substituted imides from the unsubstituted imide or directly from some other form of the dibasic acid.

The best known cyclic imide, that of phthalic acid, is classically prepared by action of ammonia on warm phthalic anhydride, and the *N*-alkyl phthalamides have been isolated as intermediate products in Gabriel's synthesis of amines (*7*). A much simpler and more generally applicable method employed in these studies is that of Vanags (*19*). In this procedure, phthalic anhydride was heated with the appropriate amine until the theoretical amount of water was evolved, and the residue was recrystallized or distilled.

Concurrent studies were directed toward preparation and evaluation of imides. Interest in this group followed the finding that β-[2-(3,5-dimethyl-2-oxocyclohexyl)-2-hydroxyethyl] glutaramide was far more repellent than any other material tested (*18*). Although this by-product of the manufacture of streptomycin is too toxic and too expensive for direct commercial application, it appeared to furnish a clue to a highly repellent structure. As other ketones had been found relatively inactive, it was assumed that repellency was associated with the imide portion of the molecule. Support for this hypothesis was obtained when it was found that *N*-(*n*-butyl) phthalimide was effective in deterring gnawing attacks upon treated articles. Other members of this series were synthesized in efforts to obtain cheaper, less toxic, or more readily available repellents.

Itaconimide was prepared by heating the diamide until evolution of ammonia ceased, then distilling the residue in vacuo (*21*). Glutarimide was prepared by heating the diammonium salt (*6*, *15*, *17*), and the *N*-alkyl glutarimides were derived similarly by distilling the amine salts. *N*-Aryl glutarimides were prepared by distillation of the dianilides.

The most satisfactory method for preparation of citraconimide was distillation of the diammonium salt in vacuo. The *N*-phenyl citraconimide was obtained by heating citraconic anhydride with aniline above the melting point of the monoanilide (*13*), and the *N*-*p*-tolyl imide was prepared by boiling the mono-*p*-toluidide in water (*1*).

Succinimide has been prepared in various ways, including heating the mono- or diamide or the ammonium salt. *N*-Alkyl succinimides were prepared by distilling the appropriate amine salt (*10*), and *N*-aryl succinimides resulted from heating the aryl amine

Table I. Properties of Imides

Compound	Yield, %	M.P., °C.	B.P., °C.	Repellent Activity, K
Phthalimide, *N*-methyl-	90	133–134	285	62.2
Phthalimide, *N*-ethyl-	75	78–79	285	53.8
Phthalimide, *N*-propyl-	95	66	...	71.4
Phthalimide, *N*-isopropyl-	75	85	286	77.2
Phthalimide, *N*-isobutyl-	98	93	...	97.3
Phthalimide, *N*-amyl-	35	23	303	95.4
Phthalimide, *N*-hexyl-[a]	92	35	325–330	90.4
Phthalimide, *N*-octyl-	85	48–49	...	64.6
Phthalimide, *N*-decyl-[a]	70	50	...	83.0
Phthalimide, *N*-dodecyl-	95	64.5	...	−17.0
Phthalimide, *N*-*o*-tolyl-	85	182	...	50.0
Phthalimide, *N*-*m*-tolyl-	90	172	...	−277
Phthalimide, *N*-*p*-tolyl-	98	204	...	−9.3
Phthalimide, *N*-allyl-	75	71	295	93.5
Itaconimide	15	103	...	84.7
Glutarimide	50	152	...	20.0
Glutarimide, *N*-ethyl-	75	...	255–260	72.1
Glutarimide, *N*-phenyl-	40	144–145	...	63.2
Citraconimide	15	103–105	...	97.7
Citraconimide, *N*-phenyl-	60	98–99	172–172/12 mm.	93.8
Citraconimide, *N*-*p*-tolyl-	25	114–115	...	97.1
Succinimide, *N*-methyl-	50	66	234	−68.0
Succinimide, *N*-ethyl-	90	26	234–236	0.2
Succinimide, *N*-propyl-	75	15–16	247–248	35.2
Succinimide, *N*-isopropyl-	40	61	230	−2.5
Succinimide, *N*-isobutyl-	80	28	247–248	14.0
Succinimide, *N*-hexyl-[a]	95	...	278–280	48.2
Succinimide, *N*-*p*-tolyl-	75	153–155	...	10.7
Succinimide, *N*-allyl-	85	...	249–250	71.3
Maleimide	12	92–93	...	99.0
Maleimide, *N*-methyl-	40	90–93	...	100
Maleimide, *N*-ethyl-	25	45.5	...	78.6
Maleimide, *N*-dodecyl-	30	56–57	...	86.8
Maleimide, *N*-*p*-tolyl-	40	149–150	...	51.0
Methylsuccinnic imide	30	66	...	49.0
Methylsuccinnic *N*-methyl-	75	...	222–223	17.8
Methylsuccinnic *N*-ethyl-	80	...	222–223	51.5
Methylsuccinnic *N*-propyl-	25	...	233–236	23.0
Methylsuccinnic *N*-phenyl-	75	105–107	325–330	26.7
Methylsuccinnic *N*-*p*-tolyl-	55	107–108	335–340	44.5

[a] Compounds not previously described in literature.

Table II. Properties of Thiouronium Compounds

Compound	Yield, %	M.P., °C.	Repellency Index, K	Acute Oral Toxicity, Mg./Kg.
Methyl thiouronium iodide	90	115–117	80.8	>500
Methyl thiouronium sulfate	92	236 (d.)	85.1	250
Ethyl thiouronium bromide	100	88	85.0	..
Ethyl thiouronium iodide	30	67–68	91.8	..
Propyl thiouronium bromide	70	58–60	85.0	..
Propyl thiouronium iodide[a]	65	65	95.0	..
Isopropyl thiouronium bromide	65	76–78	96.0	..
Butyl thiouronium bromide	50	80–82	83.0	>500
Butyl thiouronium iodide[a]	100	100–103	94.5	>500
Isobutyl thiouronium bromide	95	96	92.3	>500
Isobutyl thiouronium iodide[a]	100	80	85.1	>500
sec-Butyl thiouronium iodide[a]	70	...	97.0	..
Amyl thiouronium bromide[a]	85	90	97.0	>500
Heptyl thiouronium bromide[a]	100	92–94	90.0	250
Dodecyl thiouronium chloride	100	132–135	94.5	>500
Dodecyl thiouronium bromide[a]	15	...	85.0	>500
Hexadecyl thiouronium iodide	60	95–97	73.0	>500
Benzyl thiouronium chloride	90	146–148	93.7	<100
	85	176–177	99.5	<100
Benzyl thiouronium acetate	55	134	98.7	..
Benzyl thiouronium propionate	60	152–153	96.8	..
Benzyl thiouronium myristate	85	139	85.0	>500
Benzyl thiouronium succinate	75	151–152	80.0	250
Benzyl thiouronium crotonate	95	164–165	92.0	>500
Benzyl thiouronium *p*-toluate	95	189–190	96.3	..
Benzyl thiouronium picrate	80	186–188	92.8	250
Amidoethyl thiouronium chloride	55	222 (d.)	39.0	..
Thiourea hydrochloride	15	135–136	80.0	..

[a] Compounds not previously described in literature.

with succinic acid until the theoretical amount of water was distilled. The crude product was then purified by recrystallization.

Preparation of maleimide was a little more difficult. Among the syntheses suggested were methods for deammonolysis of the diamide with phosphorus pentoxide or zinc chloride (*14*), but appreciably better yields were obtained from the oxidation of pyrrole with chromic acid (*11*, *12*). The lower alkyl-substituted maleimides were prepared by distilling the monoamides in vacuo, while the higher alkyl (such as the dodecyl) imides and the aryl imides were prepared by dehydrating the monoamides with acetic anhydride (*16*).

Methyl succinic imide (pyrotartaric imide) was obtained by the distillation of the diammonium salt in vacuo (*2*), and the *N*-alkyl and *N*-aryl imides resulted from distillation of the appropriate amine salt at ordinary pressures (*8*, *9*).

Repellency Tests

Individually caged laboratory rats, weighing between 150 and 250 grams, were given two food cups, one containing 20 grams of ground laboratory ration, and the other containing 20 grams of a 2% mixture of the test compound in similar food. Food consumption was determined daily during the 4-day assay period, and the repellent activity, expressed as the index number K, was calculated by the formula (*3*):

$$K = 100 - 1/100W(8T_1 + 4T_2 + 2T_3 + T_4)(U_1 + U_2 + 2U_3 + 4U_4 + 8X)$$

with W representing body weights (in kilograms) of the experimental animals; $T_1 \ldots T_4$, grams of treated food consumed on each day of test; $U_1 \ldots U_4$, daily consumption (grams) of untreated food, and X, grams of untreated food remaining at the end of the test period. An index of 85 or higher indicates that the compound was sufficiently active to warrant testing by barrier technique.

Toxicity Tests

Compounds were dissolved in water or propylene glycol, or suspended in aqueous gum acacia solutions, and administered by stomach tube to laboratory rats. Doses were computed in terms of milligrams per kilogram body weight, and results are expressed as the minimum quantity (M.L.D.) producing death within 7 days.

Results and Discussion

Data on chemical properties and biological activity of some thiouronium compounds are presented in Table II. With one exception, all compounds gave high repellency indices, thus confirming the theory as to their effectiveness as rodent repellents. The compound having a low K value possessed an additional functional group which may have detracted from the activity of the thiouronium configuration. Although an insufficient number of samples were tested to give definite proof, it appears that alkyl thiouronium iodides are more repellent than the corresponding bromides. In the series of benzyl thiouronium compounds, the highest repellency indices were obtained with acids of lower weight.

Ten imides gave repellency indices of 85 or higher, while four other imides had only slightly lower activity. The results give additional indication of the repellent activity of the imide configuration, while the low K values of the remaining compounds show the extent to which activity of a functional group may be affected by changes in other portions of the molecule.

The most consistently active phthalimide, the *N*-allyl derivative, has the unsaturation characteristic of many active compounds. The *N*-tolyl derivatives, while valueless as repellents, exhibit the importance of ortho, meta, and para arrangements. As in many other cases, the ortho compound is most active and the meta, the least. Although the *N*-isobutyl phthalimide failed to maintain its activity through the repeat tests, *N*-butyl phthalimide, a compound obtained from other sources, remained active at the lower levels. This compound has been tested in field applications on boxboard, and has proved to be an effective repellent.

The activity of the citraconimide and maleimide derivatives can again be attributed to unsaturation in the molecule. In the maleimides, activity seems

to decrease with increasing molecular weight of the *N*-substituent, with the unsubstituted or *N*-methyl maleimide being the most active. The addition of a methyl radical to the succinimide nucleus has little effect, as the methylsuccinic imides are as inactive as the succinimides.

In contrast to Actidione, a substituted glutarimide, glutarimide itself is inactive as a repellent, and addition of an *N*-substituent seems to have little beneficiating effect.

Summary

Synthesis of thiouronium compounds and cyclic imides was undertaken to determine efficacy of these classes of compounds as rodent repellents. Nearly all thiouronium compounds were highly repellent, but the addition of another functional group appeared to reduce biological activity. Ten cyclic imides effectively decreased rodent consumption of treated food, and the repellent activity is attributed to the imide configuration. Activity of the imide group is affected by changes in molecular weight or spatial configuration of other portions of the molecule.

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CEREALS IN NUTRITION

Nutritive Value of Rice Germ

MARINUS C. KIK

Department of Agricultural Chemistry, University of Arkansas, Fayetteville, Ark.

Because little is known of the nutritive value of rice germ, a study was made, using the albino rat as the experimental animal. Data were obtained on the protein efficiency of rice germ in comparison with milled rice, the value of the proteins of rice germ supplementing those of milled rice, and the amino acid, vitamin, and mineral content of rice germ. The high nutritive value of rice germ merits its introduction for human foods and for animal feeds.

THE NUTRITIVE VALUE of wheat germ and corn germ has been well investigated. Both germs are good sources of high quality protein, ranging from 18 to 35%, depending on treatment before milling, type of milling process, and the variety of wheat and corn. Wheat germ is equal to the proteins of skim milk and boiled egg white, and wheat germ protein and casein are equally effective in supplementing diets low in protein (*2*). Similar values can be expected from germs or embryos from other cereals like rice, and an investigation was started to establish the nutritional value of rice germ.

The rice grain is made up of the hull, the seed coat (pericarp), the starchy endosperm, and the embryo or germ (*15*). The rice germ is situated at one end of the kernel and consists of five different parts—epiblast, coleorhiza, plumule, radicle, and scutellum. The scutellum is the most important from the vitamin standpoint.

By means of suitable milling equipment rough rice is separated into milled rice, hulls, bran, and polish. The milled or white rice is usually marketed for human consumption; rice bran and rice polish are used in the feeding of livestock, and the hulls are used as abrasives, as conditioners for commercial fertilizers, in the manufacture of furfural, and in Europe and the Orient for fuel to run the mills. One of the first products obtained in milling is so-called first-break bran, which is composed mainly of embryo and outer layers of the rice kernel. It is rich in members of the vitamin B complex, especially thiamine, riboflavin, and niacin, and in protein. The inner seed coat layers along with some starchy material compose rice polish.

Earlier studies on rice germ were mainly concerned with the protein, fat, ash, and fiber content (*5*). Studies on enzymes, sugar, and phosphorus compounds have also been reported (*9, 10*). Altson and Simpson (*1*) first drew attention to the fact that about 50% of the thiamine in the rice grain is concentrated in the embryo, which comprises only 2% of the whole kernel. Detailed studies by Hinton (*6*) confirmed this observation. He dissected out the various parts, analyzed them, and found that 44 to 50% of the total thiamine of the rice grain was located in the scutellum, which contained only 2% of the nicotinic acid (*7*). The germ as a whole contained 50% of the thiamine and 3% of the niacin; 81% of the niacin was found in the pericarp and aleurone portion. It is apparent, then, that the distribution of niacin in the rice grain is different from that of thiamine.