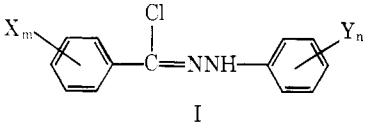
Miticidal Activity of Benzoyl Chloride Phenylhydrazones

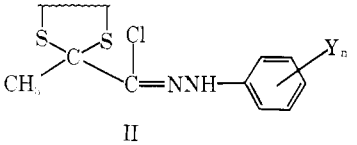
Girts Kaugars,* Edwin G. Gemrich, II, and Victor L. Rizzo

The synthesis, miticidal activity, and repellency of 70 benzoyl chloride phenylhydrazones are described. The miticidal activity and repellency can be significantly changed by the nature, position, and number of substituents in either or both aromatic rings. Replacement of the acid chloride function by other groups reduces or eliminates miticidal activity.

For the past several years we have been interested in the chemistry and biological activity of a variety of phenylhydrazones and related compounds. Our first report in the series (Kaugars and Gemrich, 1969) was a preliminary communication on the synthesis and miticidal activity of the benzoyl chloride phenylhydrazones (I). Our subsequent reports have described thioketal adducts of pyruvoyl chloride phenylhydrazones (II) and related compounds (Gemrich *et al.*, 1971; Moon *et al.*, 1972b). In an accompanying paper the herbicidal activity of alkanoyl chloride phenylhydrazones and related compounds is described (Moon *et al.*, 1972a). Other workers have described the insecticidal and miticidal activities of additional phenylhydrazones (Buchel *et al.*, 1968, 1969; Draber *et al.*, 1969; Urbschat and Unterstenhofer, 1966). We now wish to present a detailed report of the synthesis, miticidal activity, and repellency of our first steries—the benzoyl chloride phenylhydrazones. One of the compounds from this series, benzoyl chloride (2,4,6-trichlorophenyl)hydrazone, is being developed for the control of mites attacking citrus.



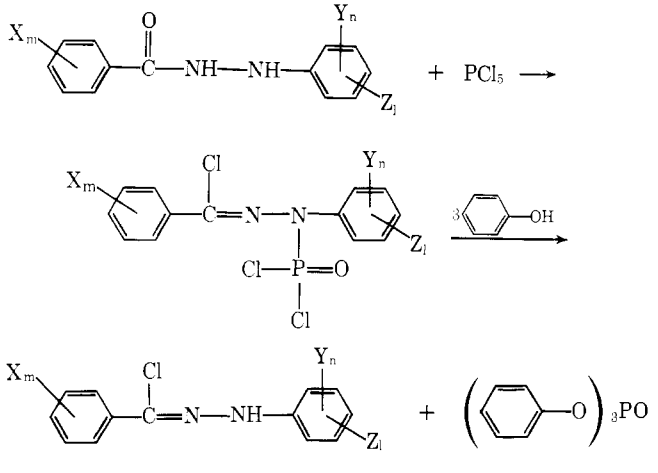
I



II

SYNTHESIS OF COMPOUNDS

The benzoyl chloride phenylhydrazones listed in Tables I–III were prepared by two generally applicable methods.



Agricultural Research Laboratories of The Upjohn Company, Kalamazoo, Michigan 49001.

Method 1. This consists of treatment of a benzoic acid phenylhydrazide with 1 equiv of phosphorus pentachloride followed by 3 equiv of phenol (Pechmann and Seeberger, 1894). The method is very general. However, yields are fairly low when Y_n and Z_1 are groups in the 2 and 6 positions.

Method 2. This is chlorination of a benzaldehyde phenylhydrazone with chlorine (Humphries *et al.*, 1925). This method is also of general applicability. However, the phenylhydrazone ring is readily chlorinated in any open ortho or para position unless a strong deactivating group such as nitro is present in the ring. In dilute solutions the chlorination can be stopped at the 2,4-dichloro stage. For convenience, most of the syntheses of benzoyl chloride (2,4,6-trichlorophenyl)hydrazones were done by monochlorination of the corresponding benzaldehyde trichlorophenylhydrazones.

Compound **46** was prepared by bromination of benzoyl chloride phenylhydrazone (**1**) in carbon tetrachloride.

Compounds **72** through **78** were prepared from **1** and the appropriate nucleophile (for a recent review see Butler and Scott (1970) and references cited therein). Compound **79** was made from **1** and trimethylphosphite under the usual Arbuzov reaction conditions, **80** was prepared from benzenediazonium chloride and ethyl diazoacetate (Huisgen and Koch, 1955), **81** from the hydrazide and phosphorus pentachloride, **82** by bromination of **51**, and **71** and **83** were obtained by condensation of benzaldehyde and the appropriate phenylhydrazine.

BIOLOGICAL METHODS

The activity of benzoyl chloride phenylhydrazones as toxicants and repellents of two-spotted spider mites was evaluated in the laboratory according to the procedure of Moon *et al.* (1972b).

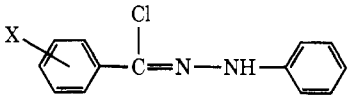
RESULTS AND DISCUSSION

For discussion purposes the benzoyl chloride phenylhydrazones have been divided in three series: substituents in the benzoyl chloride ring only (Table I), substituents in the phenylhydrazone ring only (Table II), and substituents in both rings (Table III).

The LC_{50} values of the 19 singly substituted benzoyl chloride phenylhydrazones listed in Table I vary from 10 to >1000 ppm. The variation is not directly related to substituent size (E_s) or Hammet's σ. Thus, the following compounds, which are arranged in the order of decreasing E_s values (Taft, 1956), show a scattered order of LC_{50}'s: **1** (40 ppm), **14** (>500), **5** (50), **4** (16), **6** (20), **9** (77), **7** (27), **20** (>500), and **17** (>1000). Similarly, the following compounds arranged in the order of decreasing σ values (Taft, 1956) also show a scattered order of activities: **20** (>500 ppm), **13** (>500), **19** (62), **11** (21), **3** (10), **7** (27), **6** (20), **4** (16), **5** (50), **17** (>1000), **1** (40), **8** (74), **9** (77), and **14** (>500).

The best activities were shown by the halogenated compounds 3-chloro-(**3**), 3,4-dichloro-(**25**), 4-chloro-(**4**), and 4-bromo-(**6**) benzoyl chloride phenylhydrazones, all of which have LC_{50} values ≤20 ppm. In the monochloro series the 3-chloro compound (**3**) is slightly better than the

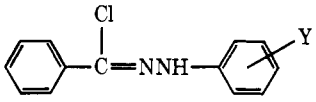
Table I. Chemical Data[a] and Miticidal Activities of Benzoyl Chloride Phenylhydrazones Substituted in the Acid Chloride Ring



Compd no.	X	Miticidal activity: LC_{50}, ppm	Repel, ppm	Method	Mp, °C
1	H	40	50	1	129.5–130
2	2-Cl	>100	>100	1	Liquid
3	3-Cl	10	12	1	80–81.5
4	4-Cl	16	25	1	148–149.5
5	4-F	50	50	1	118–120
6	4-Br	20	12	1	151.5–153
7	4-I	27	50	1	164–165
8	3-CH_3	74	50	1	66–67
9	4-CH_3	77	100	1	133–134.5
10	4-$CH(CH_3)_2$	26	50	1	100.5–102
11	3-CF_3	21	12	1	53.5–55.5
12	4-SCH_3	74	100	1	138.5–142
13	4-SO_2CH_3	>500		1	175–176
14	4-OCH_3	>500		1	129.5–131
15	4-$OCH_2CH_2CH_2CH_3$	78	50	1	110.5–111.5
16	4-$OC(=O)CH_3$	~100	100	1	124.5–126
17	4- 	>1000		1	202–204
18	4-C-(=O)OCH_3	>500		1	174–175.5
19	4-CN	62	100	1	148–150
20	4-NO_2	>500	100	1	157–158.5
21	2,3-Cl_2	65	50	1	84.5–85.5
22	2,4-Cl_2	100–200	100	1	88.5–89.5
23	2,5-Cl_2	52	25	1	Liquid
24	2,6-Cl_2	100–200	100	1	95.5–96.5
25	3,4-Cl_3	15	12	1	121–122
26	3,5-Cl_2	26	12	1	93–94
27	3,4-OCH_2O-	89	100	1	100.5–101.5
28	2-OCH_3, 5-Cl	>500		1	72–73
29	3-CH_3, 4-NO_2	>500		1	146–147.5
30	2-Cl, 4-NO_2	100–500	100	1	124–126
31	3,4,5-$(OCH_3)_3$	100–500	100	1	82.5–84
32	2,3,4,5,6-F_5	73	50	1	117–118

[a] Satisfactory elemental analyses were obtained for all compounds.

Table II. Chemical Data[a] and Miticidal Activities of Benzoyl Chloride Phenylhydrazones Substituted in the Phenylhydrazone Ring



Compd no.	Y	Miticidal activity: LC_{50}, ppm	Repel, ppm	Method	Mp, °C
33	2-Cl	15	12	1	84–85
34	3-Cl	51	25	1	100–101
35	4-Cl	34	50	1	107–108.5
36	2-CH_3	64	100	1	64.5–66
37	3-CF_3	12	6	1	95–96.5
38	4-SCH_3	59	100	1	94–95
39	4-NO_2	>1000		1	195–196
40	2,3-Cl_2	15	25	1	97–98
41	2,4-Cl_2	22	12	2	89–90.5
42	2,5-Cl_2	19	25	1	84.5–86
43	2,6-Cl_2	40	50	1	69.5–70.5
44	3,4-Cl_2	~100	100	1	148.5–149.5
45	3,5-Cl_2	32	25	1	123.5–124.5
46	2,4-Br_2	50	50		106–107
47	2-CH_3, 4-Cl	26	25	1	61–62
48	2,4-$(NO_2)_2$	>1000		1	228–230
49	2-Cl, 5-CF_3	23	100	2	147.5–149
50	2,4-Cl_2,5-CF_3	~100	100	2	85–86
51	2,4,6-Cl_3	62	50	2	96–97
52	2,3,4-Cl_3	32	100	1	138–139
53	2,4,5-Cl_3	100–200	>100	2	140.5–141.5
54	2,3,4,6-Cl_4	>500		2	76.5–77.5 and 94–95
55	2,3,4,5,6-Cl_5	500	100	2	161.5–162.5
56	2,3,4,5,6-F_5	>500		2	130.5–131.5

[a] Satisfactory elemental analyses were obtained for all compounds.

4-chloro (**4**), but the 2-chloro (**2**) is severalfold less active. The 2-chloro substituent is detrimental to activity also in the dichloro series, where the order of activities is 3,4-Cl_2 (**25**) >3,5-Cl_2 (**26**) >2,5-Cl_2 (**23**) >2,3-Cl_2 (**21**) >2,4-Cl_2 (**22**) ~2,6-Cl_2 (**24**).

A direct relationship between the miticidal activity and repellency was found in the benzoyl chloride phenylhydrazones; a similar relationship was reported for the thioketal acid chloride phenylhydrazones (Moon *et al.*, 1972b).

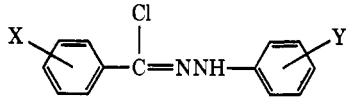
A comparison between the activity *vs.* the substitution pattern in the phenylhydrazone ring (Table II) with that in the benzoyl ring shows that some substitutents have a very similar effect on activity when placed in either ring; *e.g.*, compare the 3-CF_3 (**11** @ 21 ppm *vs.* **37** @ 12 ppm); the 4-SCH_3 (**12** @ 74 ppm *vs.* **38** @ 59 ppm); and the 4-NO_2 (**20** @ >500 ppm *vs.* **39** @ >1000 ppm). However, in the chlorine-substituted compounds, the order of activity was altered: 2-Cl (**33**) >4-Cl (**35**) >3-Cl (**34**). Whereas the 2-chloro substituent was detrimental to activity in the benzoyl ring, it appears to be beneficial for activity not only in the monochloro- but also in the dichlorophenylhydrazone ring substituted compounds: 2,3-Cl_2 (**40**) >2,5-Cl_2 (**42**) >2,4-Cl_2 (**41**) >3,5-Cl_2 (**45**) >2,6-Cl_2 (**43**) ≫3,4-Cl_2 (**44**).

In Table III are listed the compounds with substituents in both rings. Compounds **57** and **58**, with one substituent in each ring, are both excellent miticides. The other compounds all have a total of at least four substituents, three of which comprise the 2,4,6-trichlorophenylhydrazone group. Although the 2,4,6-trichlorophenylhydrazone group appears to slightly diminish the activity of the unsubstituted (**51** @ 62 ppm *vs.* **1** @ 40 ppm) and the 4-methyl (**63** @ ~100 ppm *vs.* **9** @ 77 ppm) benzoyl chloride compounds, the activity of these two 2,4,6-trichloro compounds is approximately equal to their corresponding unsubstituted analogs on a molecular basis (**51** @ 1.9×10^{-4} *M* *vs.* **1** @ 1.7×10^{-4} *M* and **63** @ ~2.9×10^{-4} *M* *vs.* **9** @ 3.1×10^{-4} *M*). However, the 2,4,6-trichloro group lowers activity significantly when at least one halogen is present in the benzoyl ring (**59** *vs.* **3**, **60** *vs.* **4**, **61** *vs.* **6**, **67** *vs.* **22**, **68** *vs.* **24**, **69** *vs.* **25**).

Replacement of the chlorine attached to C=N by other groups greatly diminishes the activity (Table IV). Only benzaldehyde phenylhydrazone (**71**), a known miticide (Urbschat and Unterstenhofer, 1966), and the SCH_3 derivative (**74**) show appreciable miticidal activity; however, both compounds are less active than the corresponding benzoyl chloride phenylhydrazone (**1**). The reduction is equally pronounced in the trichlorophenylhydrazone series (**51** *vs.* **82** and **83**, Table V).

Replacement of the hydrazone NH group by N-CH_3 also diminishes activity (**1** *vs.* **81**). Similarly, replacement

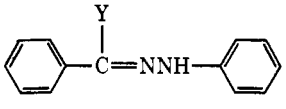
Table III. Chemical Data[a] and Miticidal Activities of Benzoyl Chloride Phenylhydrazones with Both Rings Substituted

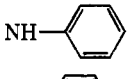
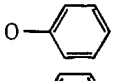
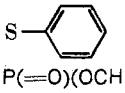


Compd no.	X	Y	Miticidal activity		Method	Mp, °C
			LC_{50}, ppm	Repel, ppm		
57	4-Cl	2-Cl	12	12	1	104–105
58	4-Cl	4-Br	22	25	1	142–143.5
59	3-Cl	2,4,6-Cl_3	100–500	>100	2	127–128
60	4-Cl	2,4,6-Cl_3	>500	>100	2	123–124
61	4-Br	2,4,6-Cl_3	>500		2	143.5–144.5
62	2-CH_3	2,4,6-Cl_3	100–500		2	70–71
63	4-CH_3	2,4,6-Cl_3	~100		2	80–81
64	3-OC(=O)NHCH$_3$	2,4,6-Cl_3	>1000		2	186.5–188
65	4-OC(=O)OCH$_2$CH$_3$	2,4,6-Cl_3	>500		2	121–121.5
66	4-CN	2,4,6-Cl_3	>500		2	183.5–185
67	2,4-Cl_2	2,4,6-Cl_3	>500		2	135–136
68	2,6-Cl_2	2,4,6-Cl_3	>500		2	142–143
69	3,4-Cl_2	2,4,6-Cl_3	>500		2	153–154
70	3,5-Cl_2, 4-OH	2,4,6-Cl_3	>500		2	193–194

[a] Satisfactory elemental analyses were obtained for all compounds.

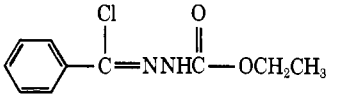
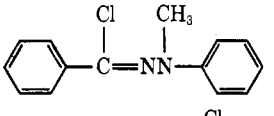
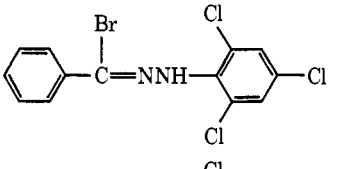
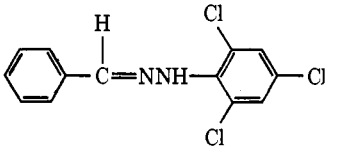
Table IV. Chemical Data[a] and Miticidal Activities of Related Phenylhydrazones



Compd no.	Y	Miticidal activity, LC_{50}, ppm	Mp, °C
71	H	100–200	157.5–158.5
72	CN	>1000	148.5–150
73	NH_2(picrate)	~1000	195.5–197.5
74	SCH_3	~100	Liquid
75		~500	133.5–135.5
76		500–1000	174.5–177
77		>1000	152–153
78		>1000	73–77.5
79	P(=O)(OCH$_3$)$_2$	>1000	110–111

[a] Satisfactory elemental analyses were obtained for all compounds.

Table V. Chemical Data[a] and Miticidal Activities of Miscellaneous Hydrazones

Compd no.	Structure	Miticidal activity, LC_{50}, ppm	Mp, °C
80		>500	55.5–56.5
81		>1000	Liquid
82		>500	88–89
83		>1000	85–87

[a] Satisfactory elemental analyses were obtained for all compounds.

of the aryl group of the phenylhydrazone by a carboethoxy reduces miticidal activity (80).

However, as discussed in the introduction, the aryl ring of the benzoyl chloride group can be replaced by other functions without loss of miticidal activity.

On the basis of the above data, the necessary structural feature for good miticidal activity in the present series is the RCCl=NNH-aryl group, although weaker activity can be retained when the chlorine atom is replaced by other selected functional groups.

ACKNOWLEDGMENT

The authors thank John H. Mazurek and Sydna D. Boyer for assistance with the biological evaluation and the Physical and Analytical Chemistry Department of The Upjohn Company for analytical data. We also thank Alan R. Friedman for a sample of compound **41**.

LITERATURE CITED

Buchel, K. H., Draber, W., Hammann, I., Netherlands Patent 6,900,033 (Apr 7, 1969).
Buchel, K. H., Draber, W., Hammann, I., Unterstenhofer, G., Netherlands Patent 6,813,353 (Mar 28, 1969).
Butler, R. N., Scott, F. L., *Chem. Ind.* 1216 1970).
Draber, W., Buchel, K. H., Hammann, I., Unterstenhofer, G., South African Patent 693,968 (July 4, 1969).
Gemrich, E. G., Kaugars, G., Moon, M. W., presented at the meeting of the Entomological Society of America, Los Angeles, Calif., Dec 1971.

Huisgen, R., Koch, H. J., *Justus Liebigs Ann. Chem.* **591,** 200 (1955).
Humphries, J. E., Humble, H., Evans, R., *J. Chem. Soc.* 1304 (1925).
Kaugars, G., Gemrich, E. G., Abstracts, 158th National Meeting of the American Chemical Society, New York, N. Y., Sept 1969, AGFD No. 45.
Moon, M. W., Friedman, A. R., Steinhards, A., *J. Agr. Food Chem.* **20,** 1187 (1972a).
Moon, M. W., Gemrich, E. G., Kaugars, G., *J. Agr. Food Chem.* **20,** 888 (1972b).
Pechmann, H. v., Seeberger, L., *Chem. Ber.* **27,** 2121 (1894).
Taft, R. W., in "Steric Effects in Organic Chemistry," Chapter 13, Newman, M. S., Ed., Wiley, New York, N. Y., 1956.
Urbschat, E., Unterstenhofer, G., U.S. Patent 3,234,447 (Feb 15, 1966).

Received for review January 8, 1973. Accepted March 20, 1973.

Sensitive Procedure for Aflatoxin Detection in Peanuts, Peanut Butter, Peanut Meal, and Other Commodities

Charles E. Holaday* and Phillip C. Barnes, Jr.

The contamination of peanuts and many other commodities by aflatoxin continues to be a serious worldwide problem. A rapid and reliable screening method is urgently needed for this dangerous toxin. A technique is described which has a sensitivity of about 3–5 ppb and which can be completed in approximately 15 min. The procedure is based on millicolumn chromatography and can be used on a number of commodities. Proximate quantitation of the aflatoxin is also possible.

Aflatoxin contamination in peanuts and other commodities continues to be a serious problem for the food industry. Many crops are subject to contamination by fungal metabolites, such as aflatoxin, in this and other countries. A rapid and inexpensive method is needed for screening susceptible commodities for this dangerous toxin.

Holaday (1968) developed a rapid screening method for detecting aflatoxin in raw peanuts based on a technique called "millicolumn chromatography." Although this method was simple and had a sensitivity of *ca.* 5 ppb, other fluorescent compounds occasionally were extracted along with the aflatoxin, which considerably reduced the sensitivity of detection. Velasco (1972) proposed a method for detecting aflatoxin in cottonseed based on a technique similar to the millicolumn procedure. This method also had a sensitivity of about 10 ppb, but required about 20–30 min to complete. More recently, Cucullu *et al.* (1972) adapted Holaday's millicolumn method for detecting aflatoxin in cottonseed and other commodities and reported a sensitivity of about 10 ppb; the time required varies from 15 to 20 min, depending upon the commodity. Dickens and Welty (1969) proposed a visual method for detecting *A. flavus* spores on peanut kernels. This method was helpful in removing large quantities of contaminated peanuts from the marketing channels. Most of the present quantitative methods for aflatoxin are based on thin-layer chromatography. These procedures are time consuming and require trained technicians for making the analyses.

This paper describes an improved millicolumn procedure for detecting total aflatoxin content ($B_1 + B_2 + G_1 + G_2$), with a sensitivity of 3–5 ppb, that can be completed in about 15 min. This is faster than the original millicolumn method and the problem of interference from other fluorescent compounds has been eliminated. The millicolumns are similar to those described earlier by Holaday (1968) but are twice as long (9 cm) and twice the diameter (6 mm).

National Peanut Research Laboratory, United States Department of Agriculture, Agricultural Research Service, Southern Region, Peanut Quality Investigations, Dawson, Georgia 31742.

EXPERIMENTAL SECTION

Detection of Aflatoxin in Peanuts, Peanut Meal, Peanut Butter, and Corn. Equipment and reagents required for the test are as follows: chromatovue chamber—equipped with long-wave uv, Ultraviolet Products, Inc., San Gabriel, Calif.; millicolumns—packed with column-type silica gel 60–200 mesh, American Society of Testing Materials (MCB-Grade 950); plugs to hold silica gel inside glass tube are made from ashfree filter pulp (S & S No. 289). The silica gel column is 90 mm long. The glass tubing is 6 mm i.d. and 200 mm long. Prepared columns may be purchased from the Tudor Scientific Glass Co., Belvedere, S. C.; extracting solution—95 parts toluene, 5 parts acetonitrile (v/v); developing solution—97 parts chloroform–2 parts methanol–1 part acetone (v/v/v). All reagents are ACS grade.

Weigh a well-mixed 50-g sample into a 1-qt blender jar fitted with a screw cap, add *ca.* 10 g of filter aid and 100 ml of hexane, and blend at high speed for 1 min. Because of the highly flammable nature of hexane, it is recommended that the extraction be carried out under a well ventilated hood. Vacuum filter through a 90-mm glass fiber filter disk placed on the Buchner funnel into a 500-ml sideneck flask attached to an aspirator. Wash the jar twice with *ca.* 25 ml of hexane. Add these washings to the Buchner funnel. After the hexane has filtered through, place the homogenate, together with the filter disk, back in the blender jar, add 100 ml of the toluene–acetonitrile solution, and blend for 1 min. Acetonitrile is a poisonous substance and should not be inhaled. It is recommended that it be handled under a hood. Filter through a 90-mm glass fiber filter disk placed in the Buchner funnel into the 500-ml sideneck filter flask from which the hexane extract has been removed. Collect *ca.* 10–20 ml of the filtrate. Tap a millicolumn several times to pack and insert lower end into a one-hole rubber stopper placed in the neck of a 1000-ml filter flask. The sideneck of the filter flask is attached to an aspirator. Transfer 1.0 ml of the extract to the top of the millicolumn with a disposable pipet. The vacuum will pull the extract through the column rapidly; the aflatoxin, however, remains at the top of the column. Add *ca.* 1 ml of hexane to the top of the column and continue pulling the vacuum until all of the sol-