3,5-Dialkyl-4-hydroxybenzylidenemalononitrile; A New Group of Fungicidal and Acaricidal Agents[†]

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Several 3,5-dialkyl-4-hydroxybenzylidenemalononitriles and related compounds were tested for their fungicidal and acaricidal activities. The influence of structural variation on biological activity was studied by preparing and using a total of 22 compounds of benzylidenemalononitrile analogues.

Amongst the compounds tested 3,5-di-*tert*-butyl and amyl-4-hydroxybenzylidenemalononitrile were most effective against fungus, *Piricularia oryzae* Car. and mite, *Tetranychus telarius* L.

3,5-Diiodo-4-hydroxybenzonitrile is known to have herbicidal activity. During the course of an investigation on the analogues of this compound in which iodine was substituted with the *tert*-butyl group, authors came upon compounds 3,5-dialkyl-4-hydroxybenzylidenemalononitrile, having an inhibitory activity on fungi and mites.

Substituted quinoxalines and 2,4-dinitrophenol are known to have both fungicidal and acaricidal activities. Since these benzylidenemalononitriles have a new chemical structure which is effective on fungi, especially rice blast disease and mites, it is interesting to prepare a variety of related compounds and to study the influence of structural variations on the biological activity.

Recently, the fungicidal property of nitro and chloro substituted benzylidenemalononitriles was studied by Edwards and Pianka,¹⁾ and

these compounds were shown to be inactive. An effective compound 1,4-bis($\beta\beta$ -dicyanovinyl)benzene was disclosed in a Japanese patent.²⁾

MATERIALS AND METHODS

Synthesis of chemicals

3,5-Di-*tert*-butyl-4-hydroxybenzylidenemalononitrile (VII). 3,5-Di-*tert*-butyl-4-hydroxybenzaldehyde (23.3 g), malononitrile (7.0 g), piperidine (10 drops), acetic acid (5 drops) and benzene (200 ml) were boiled in a flask fitted with a water separator until no more water formed (7 hr). After the reaction mixture was cooled, the solid was filtered, washed with ethanol and recrystallized.

Compound (IV)~(XII), (XIV)~(XXI) were synthesized according to the Knoevenagel reaction as described above in VII.

3,5-Di-*tert*-butyl-4-hydroxycinnamonitrile (XIII) was synthesized by the same method as described in VIII, but only piperidine was used as a catalyst.

3,5-Di-*tert*-butyl-4-hydroxybenzylalcohol(I). 3,5-Di-*tert*-butyl-4-hydroxybenzaldehyde (11.6 g) was suspended in methanol (120 ml). To this suspension was added sodium borohydride (1.4 g) during a period of 3 days

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1) J. D. Edwards and M. Pianka, *J. Sci. Food Agr.*, **14**, 55 (1963).

2) Japan Patent, 8030 (1968).

TABLE I. *p*-HYDROXYBENZALDEHYDE DERIVATIVES

No.	Compound	mp ^{a)} °C	Recryst. solvent	Formula	Anal. (%)					
					Calcd.			Found		
					C	H	N	C	H	N
I	DTBH ^{b)} benzylalcohol	141~2 ^{d)}	Pe. bz + Bz	C ₁₅ H ₂₄ O ₂	76.22	10.24		76.65	10.08	
II	DTBH benzaldehyde	188.5~9.5 ^{r)}	Toluene, EtOH	C ₁₆ H ₂₂ O ₂	76.88	9.46		76.97	9.51	
III	DTBH benzoic acid	206~7 ^{f)}	Bz	C ₁₆ H ₂₂ O ₃	71.97	8.86		72.13	8.72	
IV	3,5-Dimethyl-4-hydroxy BMN ^{e)}	165.5~6.5	EtOH	C ₁₂ H ₁₆ N ₂ O	72.71	5.09	14.13	72.50	5.15	13.95
V	3,5-Disopropyl-4-hydroxy BMN	154~5	aq. EtOH, Bz + Ligroin	C ₁₈ H ₂₈ N ₂ O	75.56	7.13	11.02	75.36	6.97	11.10
VI	3,5-Di- <i>sec</i> -butyl-4-hydroxy BMN	110.5~1.5	aq. EtOH	C ₁₈ H ₂₈ N ₂ O	76.56	7.85	9.92	76.59	7.77	9.97
VII	DTBH BMN	140~1 ^{g, h)}	EtOH, Bz	C ₁₈ H ₂₈ N ₂ O	76.56	7.85	9.92	76.78	7.76	9.65
VIII	3,5-Di- <i>tert</i> -amyl-4-hydroxy BMN	80	aq. EtOH	C ₃₀ H ₄₆ N ₂ O	77.38	8.44	9.03	77.49	8.31	8.96
IX	3- <i>tert</i> -Butyl-5-methyl-4-hydroxy BMN	225.5~6.5	EtOH	C ₁₅ H ₁₆ N ₂ O	74.97	6.71	11.66	74.77	6.94	11.44
X	3,5-Dichloro-4-hydroxy BMN	172~4	EtOH	C ₁₀ H ₄ Cl ₂ N ₂ O	50.24	1.69	11.72	49.98	1.64	11.71
XI	3,5-Dinitro-4-hydroxy BMN	148~9	Bz, EtOH	C ₁₀ H ₄ N ₄ O ₅	46.16	1.55	21.54	45.98	1.45	21.40
XII	DTBH cinnamionitrile	116.5~7.5	aq. EtOH	C ₁₇ H ₂₃ NO	79.33	9.01	5.44	79.86	8.91	5.39
XIII	DTBH benzylidene cyanoacetic acid	201~6 (decomp.)	aq. EtOH, Toluene	C ₁₈ H ₂₃ NO ₃	71.73	7.69	4.65	71.95	7.51	4.80
XIV	Methyl DTBH benzylidenecyanoacetate	103~4	EtOH, Pe. ether	C ₁₉ H ₂₅ NO ₃	72.35	7.99	4.44	72.60	7.86	4.60
XV	Ethyl DTBH benzylidenecyanoacetate	99.5~100.5 ⁱ⁾	EtOH, Pe. ether	C ₂₀ H ₂₇ NO ₃	72.92	8.26	4.25	72.86	8.12	4.36
XVI	DTBH benzylidenecyanoacetamide	202	EtOH	C ₁₈ H ₂₄ N ₂ O ₂	71.97	8.05	9.33	72.08	8.03	9.19
XVII	DTBH cinnamic acid	216~8 ^{j)} (decomp.)	Ligroin	C ₁₇ H ₂₄ O ₃	73.88	8.75		74.11	8.56	
XVIII	Diethyl DTBH benzylidenemalonate	115.5~6.5 ^{k)}	Ligroin	C ₂₂ H ₃₂ O ₅	70.18	8.57		70.38	8.56	
XIX	Ethyl DTBH benzylidenecyanoacetate	104.5~6.5	Ligroin, EtOH	C ₂₁ H ₃₀ O ₄	72.80	8.73		73.00	8.74	
XX	DTBH benzylidenecetylacetone	124~4.5	Ligroin, EtOH	C ₂₀ H ₂₈ O ₃	75.91	8.92		76.32	8.97	
XXI	3,5-Di- <i>tert</i> -butyl BMN	76~7	EtOH	C ₁₈ H ₂₈ N ₂	81.16	8.33	10.52	81.19	8.29	10.40
XXII	4-Acetoxy-3,5-di- <i>tert</i> -butyl BMN	107~8	EtOH	C ₂₀ H ₂₄ N ₂ O ₂	74.04	7.46	8.64	74.22	7.40	8.47

^{a)} Melting points are uncorrected.

^{b)} Di-*tert*-butyl-4-hydroxy.

^{c)} Benzylidenemalononitrile.

^{d)} Coppinger³⁾ reported mp 137.7~81°C.

^{e, f, j)} Coffield⁴⁾ reported, respectively, mp 189°C, 218~9°C, 218°C.

^{g, i, k)} Lauener⁵⁾ reported, respectively, mp 134~6°C, 98°C, 116~7°C.

^{h)} Müller⁶⁾ reported mp 131~2°C.

3) G. M. Coppinger and T. W. Campbell, *J. Am. Chem. Soc.*, **75**, 734 (1953).

4) T. H. Coffield, A. H. Filbey, G. G. Ecke and A. J. Kolka, *ibid.*, **79**, 5019 (1957).

5) D. Lauener, M. Coenen, M. Pestemer and G. Scheibe, *Zeit. Phys. Chem.*, **10**, 236 (1957).

6) E. Müller, R. Mayer, H. D. Spanagel and K. Sckeffler, *Ann.*, **645**, 53 (1961).

TABLE II. FUNGICIDAL AND ACARICIDAL ACTIVITIES

No.	Structure	<i>P. oryzae</i>				<i>T. telarius</i>
		Inhibition % of spore germination				LC ₅₀ Conc. μM
		400	40	4	0.4
	(A)					
I	R=CH ₂ OH	0	0	0	0	>500
II	=CHO	0	0	0	0	>500
III	=COOH	0	0	0	0	>500
	(B)					
IV	R=Me	100	100	0	0	>500
V	=Isopr	100	100	98	76	>500
VI	=sec-Bu	100	98	79	47	>500
VII	=tert-Bu	100	100	100	100	14
VIII	=tert-Am	100	100	100	66	13
IX	=Me	0	0	0	0	>500
X	=Cl	100	0	0	0	>500
XI	=NO ₂	100	0	0	0	>500
	(C)					
XII	R=H	0	0	0	0	>500
XIII	=COOH	100	70	0	0	>500
XIV	=COOMe	100	100	97	76	198
XV	=COOEt	100	100	0	0	>500
XVI	=CONH ₂	0	0	0	0	>500
	(D)					
XVII	R=COOH	0	0	0	0	>500
XVIII	=COOEt	0	0	0	0	>500
XIX	=COOEt	100	97	10	0	>500
XX	=COMe	0	0	0	0	>500
	(E)					
XXI	R=H	0	0	0	0	>500
XXII	=OAc	100	100	0	0	>500

in three portions. The clean solution was poured into water. The precipitates were collected and recrystallized.

3,5-Di-*tert*-butyl-4-hydroxybenzoic acid (III) was synthesized by the method of Cohen.⁷⁾

4-Acetoxy-3,5-di-*tert*-butylbenzylidenemalononitrile (XX-II). To a mixture of VII (18.1 g), acetyl chloride (6.3 g) and benzene (200 ml) was added pyridine (23.8 g). The reaction mixture was refluxed for 5 hr, cooled, washed with 5% HCl, 5% Na₂CO₃ and water, and dried (MgSO₄). The solvent was distilled *in vacuo*. The crystalline residue was dissolved in hot petroleum ether (500 ml) and the solid was filtered off. The filtrate was concentrated to give crude XXII.

Synthesis of substituted benzaldehydes. 3,5-Di-*tert*-butyl and amyl-4-hydroxybenzaldehydes were prepared from their corresponding *p*-cresols by a known method.⁸⁾ 3,5-Dimethyl, diisopropyl, di-*sec*-butyl and 3-*tert*-butyl-5-methyl-4-hydroxybenzaldehyde were prepared by the Duff reaction⁹⁾ from 2,6-substituted phenol. 3,5-Di-*tert*-butylbenzaldehyde¹⁰⁾ and 3,5-dichloro¹¹⁾ and 3,5-dinitro¹²⁾-4-hydroxybenzaldehyde were synthesized by known methods.

Table I shows the physical properties and analytical data of the compounds.

Determination of fungicidal activity. *P. oryzae* was incubated with the Misato medium on a test tube slant, and spores were taken from 14 days incubation. The spores were suspended in distilled water approximately at a concentration of 3000 spores per 1 ml by a hemacytometer. To obtain varying concentrations of the compounds, weighed amounts of the compounds were respectively dissolved in a small amount of DMF and emulsifier. These solutions were then diluted to desired concentrations with distilled water. One ml of a solution was added to an equi-

TABLE III. RESULTS OF FLOWER POT TEST ON RICE BLAST

Compound	Conc. (ppm)	Infected leaves (%)	Preventive value ^{a)} (%)
100	0	100
	20	0	100
	10	17	97
100	0	100
	20	69	91
10	100	76
Non-treatment		100	0

^{a)}
$$\frac{(\text{Number of spots in non-treated area} - \text{Number of spots in treated area}) \times 100}{\text{Number of spots in non-treated area}}$$

volume of the above spore suspension. The mixture was well shaken, dropped on slide glasses covered with collodion and kept at 25°C and 100% relative humidity. After 18 hr each slide was examined under a microscope and inhibition percentage of spore germination was recorded after observing and counting 150 spores.

Determination of acaricidal activity. A mottled kidney plant at the 2 leaf-stage, which had elapsed 20 days after sowing, was parasitized with a large number of carmine mite (*T. telarius*). The leaves of the treated plants were individually dipped for 1 min in each solution of the test compounds prepared by the same method as described in the fungicidal test. Subsequently, water was given so as not to wither the leaves. After 48 hr, the alive and dead mites were observed. From the mortality of mites, LC₅₀ values were calculated.

Flower pot test. To rice plants (Waseashi) of the 4 leaf-stage grown in flower pots of about 3 inches in diameter, solutions of each compound at a given concentration (a solution of 10 parts compound, 80 parts dioxane and 10 parts emulsifier were diluted with water) were sprayed in a proportion of 7 ml per pot. After 2 hr, the rice plants were sprayed with 20 ml of a spore-suspension of *P. oryzae* per 3 pots and kept at 26°C in a fungicabinet for 5 days. The number of spots generated on upper leaves was counted, and the result is presented in Table III.

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RESULTS AND DISCUSSION

Table II shows the results of the fungicidal and acaricidal tests. The compounds are classified into groups A-E according to structure resemblance for easy recognition of the correlation of structure to activity.

Group B indicates that bulky tertiary alkyl groups in 3,5-positions of the aromatic ring are required in order to exhibit activity. The activity increases with an increase in the Van der Waals radius of substituted alkyl groups. When 3,5-positions of the aromatic ring were substituted with such electron-withdrawing groups as nitro groups and chlorine atoms, the activity was almost lost.

Replacement of cyano groups in the β -position of the vinyl group by hydrogen atoms and carboalkoxy and acetyl groups reduced or eliminated the activity of the compounds (group C and D).

In group E, the free hydroxy group in the aromatic ring was found to be necessary for the activity.

In group A compound II generated by cleavage of the side chain double bond seemed to be the weakest and its derivatives were inactive.

From the above results, it was found that

slight changes in the structure, 3,5-di-*tert*-alkyl-4-hydroxybenzylidenemalononitrile, remarkably decreased the activity.

The flower pot test showed that compound VII completely inhibited rice blast. It is therefore suggested that the activity is not influenced even when the compound contacts with rice leaves. However, subsequent field tests were less successful apparently because of too rapid decomposition of the compound under a drastic field condition.

In order to evaluate the compounds as agricultural fungicides, pathogens of some other representative plant diseases were applied for a growth inhibition test. But all the compounds were nearly ineffective for the pathogens.

In preliminary tests compound VII showed high toxicity to warm-blood animals and fish and moderate phytotoxic action against some kinds of crop seedlings. The acute oral LD₅₀ to mouse was 29 mg/kg.

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