Synthesis of Some 1′-(Substituted phenyl)spiro[indole-3,4′-azetidine]-2(3*H*),2′-diones as Potential Fungicides

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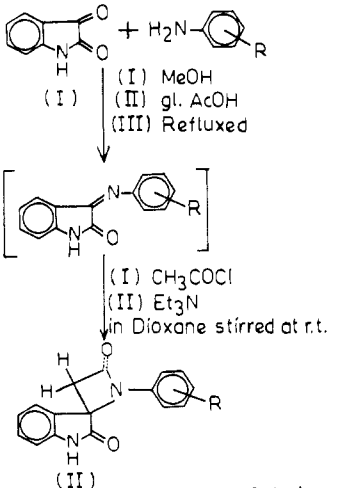
Several 1′-(substituted phenyl)spiro[indole-3,4′-azetidine]-2(3*H*),2′-diones have been synthesized by annulation of acid chloride on isatin anils. These compounds have been tested against three species of fungi, based on screening structure–activity relationships.

Encouraged by earlier records that spiro compounds exhibit significant biological properties (Wolf and Mascitti, 1970; Kobayashi et al., 1977; Agrawal et al., 1978; Rovnyak et al., 1978), we considered it of interest to undertake a systematic investigation on more biologically active spiro compounds. In this context, the 1′-(substituted phenyl)-spiro[indole-3,4′-azetidine]-2(3*H*),2′-diones described in this investigation were prepared to study their fungicidal activities. It was presumed that these molecules would have enhanced biological properties because they incorporate both indole and azetidinone moieties. The pesticidal value of the former is already known (Hedrich and Neighbors, 1984; Mueller et al., 1982), and the biocidal versatility of the latter has been amply demonstrated (Mukherji and Singh, 1978; Osman and Hasian, 1978; Mazid et al., 1979; Hannah et al., 1982). The investigation further appeared interesting because the fungicidal data on 2-azetidinones are scanty in the literature.

The title compounds have been prepared by annulation of acetyl chloride on isatin anils in the presence of Et_3N. The isatin anils were prepared by simple condensation of isatin with substituted amines in methanol with glacial acetic acid as catalyst. Alternatively, the same compounds have been prepared without isolating the isatin anils. Thus, condensation of isatin with aromatic amines in toluene offered isatin 3-anils I, which in situ were cyclized with acetyl chloride to give the title compounds. This last method was found to be superior to the first, and hence all the compounds have been synthesized by this method.



a. -Cl(p) d. -2,6-$(CH_3)_2$ g. -Cl(m)
b. -CH_3(m) e. -2,3-$(CH_3)_2$ h. -H
c. -Cl (o) f. -CH_3(o) i. -3,5-Cl_2

All the compounds have been screened for their antifungal activity against *Aspergillus niger*, *Helminthosporium oryzae*, and *Aspergillus flavus* at three different concentrations, viz. 1000, 100, and 10 ppm by agar growth technique. The results have been compared with the activity of the commercial fungicides Dithane M-45 and carbendazim tested under similar conditions. Some of these compounds showed fungitoxicity of the order of commercial fungicides and are supposed to be promising fungicides.

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EXPERIMENTAL SECTION

Melting points were taken in open capillaries and are uncorrected. The IR spectrum was recorded on a Perkin-Elmer spectrometer and ^1H NMR on a Varian EM 360 spectrometer at 60 MHz.

Isatin 3-Phenylanil. This compound was prepared by refluxing a mixture of isatin (0.01 mol) and aniline (0.01 mol) in methanol (50 mL) in the presence of glacial acetic acid (0.2 mL) as catalyst for 2 h. On cooling, fine crystals were obtained: mp 200 °C; yield 84%. Anal. Found: C, 81.08; H, 4.65; N, 13.13. Calcd: C, 81.55; H, 4.85; N, 13.59. Other compounds thus prepared have been used for further reaction.

1′-Phenylspiro[indole-3,4′-azetidine]-2(3*H*),2′-diones. *Method A.* A solution of acetyl chloride (1.5 mL) in dry benzene was added dropwise to a well-stirred solution of isatin 3-phenylanil (3.0 g) and triethylamine (4.0 mL) in anhydrous benzene (50 mL). After the addition was complete, the solution was stirred overnight. The resulting solution was filtered, and benzene was removed. The residue was poured into water. A precipitate of 1′-phenylspiro[indole-3,4′-azetidine]-2(3*H*),2′-dione was obtained, which was purified from chloroform and hexane (40:60): mp 205 °C; yield 75.5%; IR (ν_{max}, cm^{-1}; KBr disk) 1660 ($COCH_2$), 1620 (-C-ONH); ^1H NMR (ppm) 3.2 (2 H, CH_2CO), 6.50–7.55 (m, 11 H, Ar H), 10.05 (s, 1 H, OH); MS, 264 (M^+). Anal. Found: C, 72.60; H, 4.47; N, 10.40. Calcd: C, 72.72; H, 4.54; N, 10.60.

The other compounds thus prepared are recorded in Table I.

Method B. A mixture of indole-2,3-dione (0.01 M) and appropriate aromatic amine (0.01 M) was refluxed in toluene (50 mL) for 2 h, and a theoretical amount of water was collected azeotropically. On cooling of the mixture, acetyl chloride (0.01 M) was added and the resultant mixture refluxed further for 2 h. After cooling, the supernatant liquid was decanted off. The solid thus obtained on recrystallization from methanol offered the pure compound. The compounds thus prepared are recorded in Table I.

FUNGICIDAL TEST

The fungicidal activity was evaluated by the agar growth technique at three different concentrations. A fair number of controls were used. The number of replications was 3 in each case.

The technique in brief involves the mixing of toxicants with agar medium and allowing the planted fungus to grow on such poisoned food

$$\% \text{ inhibn} = \frac{C - T}{C} \times 100$$

where C = diameter of fungus colony (mm) in the control plate and T = diameter of fungus colony (mm) in the treated plate. Inhibition in fungus growth was determined

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Table I. Characterization Data of Various 1′-(Substituted phenyl)spiro[indole-3,4′-azetidine]-2(3*H*),2′-diones

compd	mp, °C	yield, %	mol formula	anal. found (calcd) C	H	N	spectral data[a]
IIa	138–40	63	$C_{16}H_{11}O_2N_2Cl$	64.00 (64.32)	3.58 (3.68)	9.35 (9.38)	IR (KBr): 1680 ($C(O)CH_2$), 1620 (C(O)NH) ^1H NMR ($CDCl_3$ + DMSO): 2.2 (s, 2 H, CH_2), 4.8 (s, 1 H, NH), 6.75–7.95 (m, 10 H, Ar H) MS: 298 (M^+), 300 (M + 2)
IIb	150–52	70	$C_{17}H_{14}O_2N_2$	73.60 (73.38)	5.00 (5.03)	10.01 (10.07)	IR (KBr): 1680 ($C(O)CH_2$), 1640 (C(O)NH) ^1H NMR ($CDCl_3$ + DMSO): 2.00 (s, 3 H, CH_3), 3.20 (s, 2 H, CH_2), 6.80–7.60 (10 H, Ar H), 10.65 (s, 1 H, OH) MS: 298 (M^+), 200 (M + 2)
IIc	80	60	$C_{16}H_{11}O_2N_2Cl$	63.95 (64.32)	3.55 (3.68)	9.25 (9.38)	IR (KBr): 1680 ($C(O)NH_2$), 1620 (C(O)NH) ^1H NMR (DMSO): 3.2 (s, 2 H, CH_2), 4.8 (s, 1 H, NH), 6.75–780 (m, 10 H, Ar H)
IId	215	67.4	$C_{18}H_{16}O_2N_2$	73.30 (73.97)	5.75 (5.47)	9.45 (9.58)	IR (KBr): 1760 ($C(O)CH_2$), 1680 (C(O)NH) ^1H NMR ($CDCl_3$): 2.05 and 2.25 (d, 6 H, 2 CH_3), 3.25 (s, 2 H, CH_2C), 6.50–7.45 (m, 9 H, Ar H) MS: 293 (M^+)
IIe	208	74.4	$C_{18}H_{16}N_2O_2$	74.60 (73.47)	5.32 (5.47)	9.60 (9.58)	IR (KB): 1680 ($C(O)CH_2$), 1640 (C(O)NH) ^1H NMR ($CDCl_3$): 2.05 and 2.20 (d, 6 H, 2 CH_3), 3.30 (s, 2 H, CH_2), 6.50–4.50 (m, 9 H, Ar H) MS: 289 (M^+)
IIf	168	73.8	$C_{17}H_{14}O_2N$	73.10 (73.38)	4.95 (5.03)	9.95 (10.07)	IR (KBr): 1760 ($C(O)CH_2$), 1680 (C(O)NH) ^1H NMR ($CDCl_3$ + DMSO): 2.00 (s, 3 H, CH_3), 3.65 (s, 2 H, CH_2), 6.20–7.25 (m, 10 H, Ar H)
IIg	208	65	$C_{16}H_{11}O_2N_2Cl$	64.00 (64.32)	3.45 (3.68)	9.28 (9.38)	IR (KBr): 1680 ($C(O)CH_2$), 1620 (C(O)NH) ^1H NMR ($CDCl_3$): 3.20 (s, 2 H, CH_2), 6.70–8.00 (m, 10 H, Ar H) MS: 298 (M^+), 300 (M + 2)
IIh	205	75.5	$C_{16}H_{12}O_2N_2$	72.60	4.47	10.48	IR (KBr): 1660 ($C(O)CH_2$), 1620 (C(O)NH) ^1H NMR ($CDCl_3$): 6.50–7.55 (m, 11 H, Ar H) MS: 264 (M^+)
IIi	210	81.5	$C_{16}H_{10}O_2N_2Cl_2$	57.48 (57.65)	3.18 (3.00)	8.31 (8.40)	IR (KBr): 1720 ($C(O)CH_2$), 1620 (C(O)NH) ^1H NMR ($CDCl_3$ + DMSO): 3.20 (s, 2 H, CH_2), 7.20–8.10 (m, 9 H, Ar H)

[a] Units: IR, cm^{-1}; ^1H NMR, ppm; MS, m/z.

Table II. Fungicidal Activities

compd	av % inhibn after 168 h *A. niger* 1000	*A. niger* 100	*A. niger* 10	*A. flavus* 1000	*A. flavus* 100	*A. flavus* 10	*H. oryzae* 1000	*H. oryzae* 1000	*H. oryzae* 10
IIa	70.2	60.8	50.6	69.3	45.5	40.5	77.2	67.0	58.9
IIb	5.0	4.2	0.0	43.9	29.0	10.8	44.2	31.3	14.4
IIc	80.6	66.5	60.4	82.6	67.8	63.8	83.7	75.6	70.5
IId	65.4	45.8	34.5	67.8	45.7	34.0	66.2	46.3	38.6
IIe	68.8	50.0	35.0	68.5	42.6	37.0	69.3	55.0	41.0
IIf	9.6	4.3	0.0	10.5	5.7	0.0	12.4	7.2	0.0
IIg	70.6	62.8	53.5	75.0	50.3	41.5	77.6	67.2	59.0
IIh	69.7	57.5	35.5	65.1	58.5	32.5	74.2	68.8	61.2
IIi	72.4	66.0	50.9	67.0	36.5	17.0	80.4	73.2	64.3
carbendazim	90.5	87.5	76.3	89.5	78.3	70.3	89.8	79.0	71.2
dithane M-45	93.5	88.6	78.8	93.0	88.8	78.7	94.1	80.2	78.9

as the difference in diameter of fungus colony between control plates and those treated with inhibitor (Giri et al., 1978). The fungicidal data are recorded in Table II.

RESULTS AND DISCUSSION

Perusal of fungicidal data reveals that all the compounds of this series are fungitoxic against the three species of fungi except **a** and **f**, but their toxicity decreases considerably with dilution. It is also notable that all the compounds are more active on *H. oryzae* than *A. niger*. The activity on *A. flavus* is in between the two. Introduction of chloro substituents in the phenyl ring at ortho, para, and meta positions increases the toxicity (**a**, **c**, **g**, **i**).

Compounds **c** and **i** are more active than **a** and **g**. This indicates that the presence of a chloro group at position 2 or position 3 imparts more fungitoxicity than presence at position 4.

The presence of methyl groups at either position of the phenyl ring do not seem to impart much activity. However, the presence of two methyl groups increases the activity (**d**, and **e**).

It is also to be noted that compound **c** has toxicity very close to that of both commercial fungicides tested under similar conditions. Further screening of this compound on a wider range of fungi as well as under more dilute conditions is desirable.

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Registry No. IIa, 120666-70-8; IIb, 120666-71-9; IIc, 120666-72-0; IId, 120666-73-1; IIe, 120666-74-2; IIf, 120666-75-3; IIg, 120666-76-4; IIh, 120666-77-5; IIi, 120666-78-6; isatin 3-phenylanil, 33828-98-7; isatin, 91-56-5; aniline, 62-53-3; indole-2,3-dione, 91-56-5; 4-chloroaniline, 106-47-8; 3-methylaniline, 108-44-1; 2-chloroaniline, 95-51-2; 2,6-dimethylaniline, 87-62-7; 2,3-dimethylaniline, 87-59-2; 2-methylaniline, 95-53-4; 3-chloroaniline, 108-42-9; 3,5-dichloroaniline, 626-43-7.

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Metabolism of Dibutyltin Dichloride in Male Rats

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When dibutyltin dichloride was administered intraperitoneally to male rats, butyl(3-hydroxybutyl)tin dichloride, butyl(4-hydroxybutyl)tin dichloride, and butyltin trichloride were detected as acid-stable metabolites. The major acid-stable metabolite, butyl(3-hydroxybutyl)tin dichloride, showed a tendency to accumulate in the kidney. Butyl(4-hydroxybutyl)tin dichloride was detected only in urine extracts. Dibutyltin dichloride, butyl(3-hydroxybutyl)tin dichloride, and butyltin trichloride were found in the brain in spite of differences in their relative polarities.

Butyltin compounds have been widely used as stabilizers for chlorinated polymers, catalysts for a variety of chemical reactions, and biocides for boat paints or fishing nets. Recently, pollution of the environment and foods, caused by these compounds, has become the object of public concern (Mueller, 1984; Maguire and Tkacz, 1985; Takami et al., 1987), and much attention has been focused on the biological effects of butyltin compounds (WHO, 1980; Wada et al., 1982). Informed discussion of these effects, however, requires the clarification of the metabolic fate of these compounds and the elucidation of the biological effects of each individual metabolite.

Some studies have been conducted on the metabolism of these compounds in microsomal monooxygenase systems (Fish et al., 1976; Kimmel et al., 1977). Dibutyltin compounds have been identified as the main metabolic intermediate of tributyltin chloride (Kimmel et al., 1977) and also as a contaminant in reared and natural fish (Sasaki et al., 1988a,b). Information on butyltin compounds metabolism in vivo, accumulations in organs, and their excretion is, however, lacking.

The aim of this study was to clarify the reasons for tributyltin chloride toxicity in animals including man. This paper describes identification of the metabolites of dibutyltin dichloride in vivo and their distribution in organs after intraperitoneal administration to male rat.

MATERIALS AND METHODS

Chromatography. Column chromatography was carried out with silica gel (Kieselgel 60, Art. 7734, E. Merck; Wakogel C-100; Wako Pure Chemical Industries Ltd.) and Florisil (100–200 mesh; Yoneyama Chemical Industries Ltd.). Silica gel (Kieselgel 60) was activated at 130 °C. Silica gel (Wakogel C-100) was made 50% (v/w) with hydrochloric acid (HCl, 36%), equilibrated overnight, and activated for ca. 4 h at 120 °C (Hatorri et al., 1984). Florisil was activated at 130 °C and used without further modification.

High-performance liquid chromatography (HPLC) was performed with use of two systems, a postcolumn Morin reagent modified fluorescence detection system (Yu and Arakawa, 1983; method I) and a fluorescence detection system with Morin in eluent (Langseth, 1984; method II).

Method I. A Shimadzu LC-6A for mobile phase and a Hitachi 635 for Morin reagent were used as the pumps for the solvents. A stainless-steel column (25 cm × 4.6 mm (i.d.), packed with Unisil Q CN (5 μm, cyanopropyl-bonded phase; Gasukuro Kogyo), coupled with the precolumn (5 cm × 4.6 mm (i.d.)) with the same packing, were used. The mobile phases of *n*-hexane–ethyl acetate (EtOAc)–acetic acid (80:20:5) for quantification and 95:5:5 for identification were used at a flow rate of 1.2 mL/min. Detections were carried out by postcolumn Morin reagent modification (0.005% Morin in ethanol at a flow rate of 0.5 mL/min), followed by monitoring the fluorescence (excitation 420 nm, emission 500 nm; Shimadzu RF-535).

Method II. A Shimadzu LC-6A was used as the solvent pump. The analytical column and the precolumn were the same as for method I. The mobile phase of toluene–acetic acid–methanol (95:3:2) containing 0.0015% Morin was used at a flow rate of 1.0 mL/min. The detector and operating conditions were the same as for method I.

Gas Chromatography/Mass Spectrometry (GC/MS). GC/MS spectra were obtained by a JEOL JMS-DX 300 in the electron-impact (EI) mode. Operating conditions were as follows: GC column, CBP 10 (12 m × 0.53 mm (i.d.), fused silica capillary column, OV-1701 equivalent; Shimadzu); helium gas flow, 15 mL/min; injection temperature, 240 °C; column temperature, 90 °C (0 min) to 230 °C (2 min) at 16 °C/min; separator temperature,

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