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https://doi.org/10.5109/23702

出版情報：九州大学大学院農学研究院紀要. 24 (2/3), pp.125-132, 1979-11. Kyushu University
バージョン：
権利関係：

J. Fac. Agr., Kyushu Univ., 24 (2 • 3) 125-132 (1979).

Synthesis and Biological Activity of Oxadiazolo [3, 2-a] pyrimidine and Related Compounds

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(Received July 24, 1979)

Oxadiazolo[3,2-*a*]pyrimidin-5-ones were prepared from 2-amino-5-substituted-1,3,4-oxadiazoles and ethyl acetoacetate or dimethyl acetylenedicarboxylate. 2-Phenyl-5-methyl-oxadiazolo[3,2-*a*]pyrimidin-7-one was obtained by the reaction with diketene followed by dehydrating cyclization in conc. sulfuric acid. Referred to the reaction of 2-aminooxadiazole with ethyl cyanoacetate and acetylacetone, the intermediary N-cyanoacetylated products *D* and acetylacetonides C resisted to cyclization to oxadiazolopyrimidines. The synthesized oxadiazolopyrimidines and their intermediates were subjected to biological tests for plant growth regulation, antitumor and antimicrobial activities.

In previous papers (Okabe *et al., 1972,* 1973a, b, 1975a, b; Suiko ***et al.,*** 1979 a, b) the authors reported the synthesis of purine analogues such as triazolopyrimdines and thiadiazolopyrimidines having biological activities. Another analogues series of purine base, oxadiazolopyrimidine is interesting compound to investigate the biological activities.

Starting compounds, 2-amino-5-substituted-1,3,4-oxadiazoles *I* possessing alkyl or aryl substituent were synthesized according to Swain's method (Swain, 1959). The compounds *I* reacted with diketene, ethyl acetoacetate or dimethyl acetylenedicarboxylate to yield the corresponding oxadiazolo[3,2-*a*]pyrimidine derivatives in A(2) and *B*(3-6) series, respectively. However, the products from the reactions of I with ethyl cyanoacetate or acetylacetone were such intermediates as C (7, 8, 8') or ***D*** (9-11) series and the following ring-closure reaction of them hardly gave oxadiazolopyrimidines (Fig. 1).

2-Amino-5-phenyl-oxadiazole *I* (R=phenyl) was treated with diketene to produce the acetoacetylaminooxadiazole 1, which was cyclized to give 7-oxo-isomer 2 in conc. sulfuric acid. By heating the oxadiazole with ethyl acetoacetate, 2-substituted-7-methyl-5*H*-1,3,4-oxadiazolo[3, 2-*a*]pyrimidin-5-one derivative 3 was obtained in a low yield (Gehlen 1970). The 7-oxo-isomer shows the amide I absorption band below 1680 cm^{-1}, while 5-oxo-isomers above 1680 cm^{-1} (Fig. 2). This is consistent with the result shown in IR spectra of 1,3,4-thiadiazolopyrimidines (Okabe *et al.*, 1975a) and s-triazolopyrimidines. Oxadiazoles *I* and dimethyl acetylenedicarboxylate in tetrahydrofuran (THF)

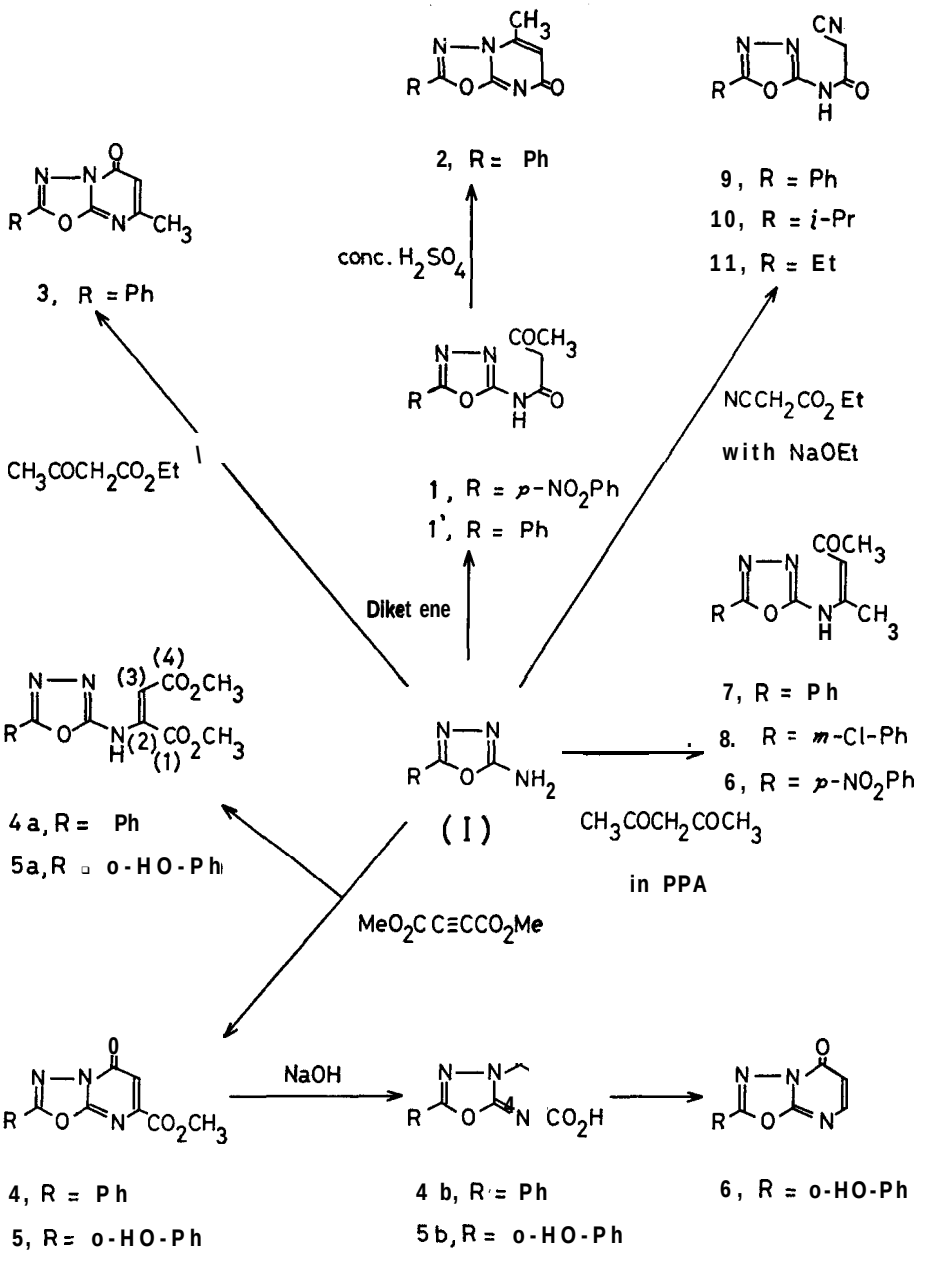

Fig. 1. Preparation of oxadiazolopyrimidines

gave 7-methoxycarbonyl-5 *H*-1,3,4-oxadiazolo[3,2-*a*]pyrimidin-5-one derivatives 4, 5 and by-products 4a, 5a. The compound 5 was hydrolyzed in an ethanolic sodium hydroxide solution and followed by decarboxylation in dimethylformamide (DMF) to afford the compound 6. The compound was identified

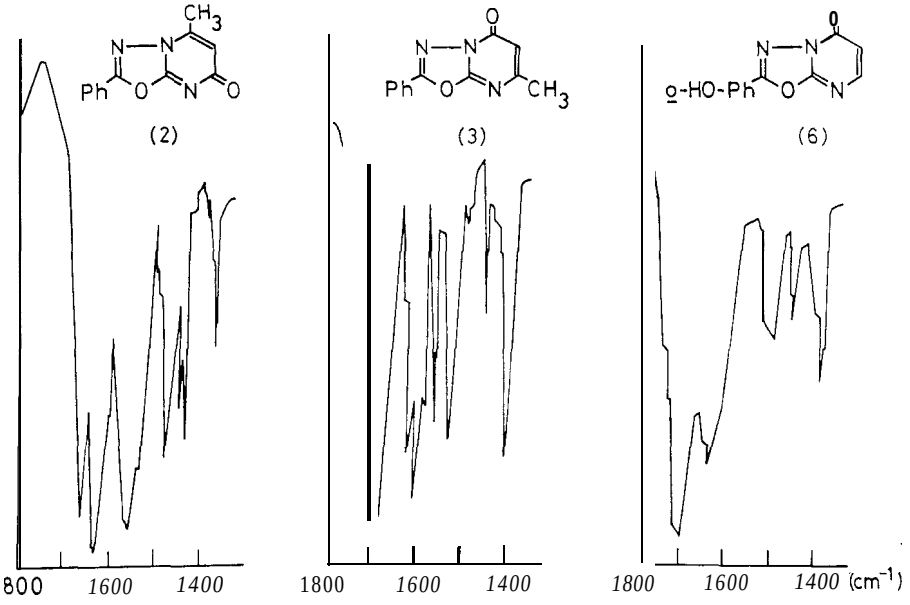

Fig. 2. IR spectra of 1, 3, 4-oxadiazolopyrimidin-7-one (2) and -5-ones (3), (6). (KBr)

as 5-oxo-isomer based on the IR spectral data (Henklein *et al.*, 1973; Shafiee and Lalezari, 1975). Oxadiazole *I* reacted with ethyl cyanoacetate in boiling ethanol for 24 hr in the presence of sodium ethoxide to give the products *D* (Okabe *et al., 1972;* Wamhoff and Wehling, 1975). A mixture of oxadiazole *I* and acetylacetone in polyphosphoric acid (PPA) was heated for 2-3 hr to yield the compounds *C*. For the compounds *C* and *D*, iminolactam structures resulting from electrophilic attack on to 3-N were denied by the absence of the broad band between 1720 and 1705 cm^{-1} as discussed by Gehlen (Gehlen and Simon, 1970).

Physical properties and analytical data of the compounds synthesized were enumerated in Table 1.

These compounds were examined in regard to (a) phytotoxic effect on rice, radish and barnyard grass, (b) antimicrobial activity on *Escherichia coli* and *Aspergillus niger,* and (c) cytotoxic activity on Ehrlich ascites tumor cells (EATC). The results are shown in Table 2 and 3.

It was found that the compounds exhibited more inhibitory effect on the growth of barnyard grass than on rice; the compounds 2, 3 and 10 inhibited the root growth of barnyard grass above 50 % at a level of 100 ppm. The growth of radish roots was affected by the substituent on C-7 of series *B* (COOMe<COOH<CH_3<H). Any inhibition on the EATC growth was not observed at a concentration of 70ppm. Against *E. coli,* only the cyanoacetamide 9 was inhibitory at a level of 500 μg.

It was interesting that an oxadiazolo[3,2-*a*]pyrimidin-5-one 3 strongly inhibited the growth of *Asp. niger.* but the 7-oxo-isomer 2 did not. However,

Table 1. Oxadiazolo [3,2-*a*] pyrimidines

Group	No	R	a) Solvent for recrystn.	Yield (%)	M. p. (°C)	UV spectra λ_{max}^{EtOH} (ε_{max} x 10^{-3})	IR (cm^{-1}) C=O	NMR (δ: ppm)
	2	Ph	e	57	220	205(16.4) 230(13.2) 330(12.6)		
A[b]	3	Ph	c	43	218-9	209(12.7) 230(5.9)	1670	2.62, 6.38, 7.88-8.36
B		Ph	c	13	177	288(12.3) 249(18.4) 209(17.3)	1700	(CDCl$_3$)
〃	4	Ph	c	30	240 dec.	285(20.2) 295(20.9)	1750, 1600	
〃	4b	Ph	c	63	220	274(68.6) 208(23. a)		
〃	5	*o*-HOPh	c	38	220	212(23.0) 260(11.2) 328(14.9)	1735, 1600	4.03, 6.82, 7-8, 10.2-11.6
〃	6	*o*-HOPh	c	40	260	211(21.0) 254(13.1) 300(4.5)	1695-1720	(DMSO-d_6)
C	7	Ph	c	30	217	210(12.8) 220(14.0) 305(6.6)	1640-1690	2.32, 2.40, 6.32, 11.6,
〃	8	*m*-ClPh	d	57	197	210(11.9) 303(7.2)	1690-1600	7.2-7.92(CDCl$_3$)
D	9	Ph	c	71	.a3 dec.	210(6.0) 270(16.9)	1730	4.24, 7.94-8.28
〃	10	*i*-Pr	c	33	178	226(11.3)	1727	1.4(doublet), 3.3 (multiplet)
〃	11	Et	c	25	77 dec.	224(6.1)		4.18(d_6-Acetone)

a) Solvent for recrystallization, c: methanol, d : methanol-ether, e : DMF-water
b) See reference (Gehlen and Simon, 1970).

Table 2. Inhibition of plant growth

Compd. No.	Radish		Rice		Barnyard grass	
	Root	Stalk	Root	Stalk	Root	Stalk
1	3	2	0	0	1	2
2	3	3	0		3	2
3	2	1	0		3	2
4	0	0	2		2	1
4b	1	1	2		2	2
5	0	0	1	0	2	2
6	3	3	0	0	2	2
7	0	0	0	0	2	2
a	3	2	0	0	1	2
9	2	1	0	0	2	2
10	2	1	0	0	3	2
11	3	1	0	0		

Concentration : 100 ppm
0: no inhibition
1: below 20 % inhibition
2 : 20-50 % inhibition
3 : 50-80 % inhibition
4: above 80 % inhibition

the ring-opening compound 1 was inhibitory.

Table 3. Inhibitory effect on the growth of *E. coli*, *Asp. niger* and EATC

Compd. No.	*E. coli* (500 μg)	*Asp. niger* (500 μg)	EATC (70 ppm)
1	—	*11 mm	—
2	—	+	—
3	—	15 mm	—
4	—		—
4b	—	+	—
5			—
6		6mm	—
7	—	6 mm	—
8	—	+	—
9	*8 mm	6 mm	—
10		—	—
11			

—: no inhibition
+: spore formation on the disk paper
*: diameter of inhibitory zone

EXPERIMENTALS

Chemicals

2-Amino-5-(m-chlorophenyl)-1,3,4-oxadiazole (I, R=m-chlorophenyl)

Cyanogen bromide (8.7 g) was added to m-chlorophenylhydrazide which was prepared from *m*-chlorobenzoyl chloride and 'hydrazine in EtOH. The mixture was refluxed for 3 hr. After being cooled, the mixture was neutralized with ammonia gas. The precipitate was collected and recrystallized from MeOH. yield 69 %.

2-Acetoacetylamino-5-phenyl-1,3,4-oxadiazole (1', R=phenyl)

A suspension of 1.2 g of diketene and 2g of oxadiazole (1, R=phenyl) in dried benzene was refluxed in an oil bath. After refluxing for 10 hr, the solvent was evaporated *in vacuo* in a steam bath and the residue was recrystallized from MeOH to give 2 g of 2-acetoacetylamino-5-phenyl-1,3,4-oxadiazole. Mp. 131°C, yield 66.7 %, IR_{max}^{KBr} cm^{-1}: 1730 (C=O), 1700 (C=O).

2-Acetoacetylamino-5-(*p*-nitrophenyl)-1,3,4-oxadiazole (1, R=*p*-nitrophenyl) was prepared similarly adopting 2-amino-5-(*p*-nitrophenyl)-1,3,4-oxadiazole. Mp. 220°C, yield 57 %.

5-Methyl-2-phenyl-7 H-1,3,4-oxadiazolo[3,2-a]pyrimidin-7-one (2, R=phenyl)

One gram of 2-acetoacetylamino-5-phenyl-1,3,4-oxadiazole was dissolved in 10 ml of conc. sulfuric acid and warmed in a steam bath for 10 hr at 50–60°C. The mixture was poured into ice-water and neutralized with sodium hydroxide. The resulting crystals were recrystallized from MeOH-Et_2O *to* give 0.4 g of the title compound. Mp. 218–219°C, yield 43 %.

7-Methoxycarbonyl-2-(phenyl or o-hydroxyphenyl)-5 H-1,3,4-oxadiazolo[3,2-a]pyrimidin-5-one (4, R=phenyl; 5, R=o-hydroxyphenyl)

2-Amino-5-pheny-1,3,4-oxadiazole (*I*, R=phenyl) (7.6 g) was refluxed with dimethyl acetylenedicarboxylate (6. 8 g) in THF (100 ml) for 1 day. White

crystals appeared in the course of the reaction. The precipitate was collected by filtration and recrystallized from MeOH to give 3.9 g of transparent needle crystals. Mp. 240°C (decomp.), yield 31 %. The structure was assigned to dimethyl-N-(5-phenyl-1,3,4-oxadiazol-2-yl)-aminomaleate (4*a*, R=phenyl). In the NMR spectrum of compound 4*a*, chemical shifts of protons were assigned as follows. δ=3.72 ppm (3H, singlet), CH_3 of C_4; δ=3.94 ppm (3H, singlet), CH, of C_2; δ=6.90 ppm (1H, singlet), CH of C_3; δ=7.60 and 7.80 ppm (6H, multiplet), protons of phenyl and NH. In the IR spectrum, compound 4a shows two streching absorption bands at 1750 and 1700 cm^{-1}. Anal. Found: C, 55.30 ; H, 4.27 ; N, 13.80 %, Calcd. for $C_{14}H_{13}O_5N_3$: C, 55.44 ; H, 4.32 ; N, 13.86 %.

2- (o-Hydroxyphenyl) -7-methoxycarbonyl-5 *H*-1,3,4-oxadiazolo[3,2-*a*]pyrimidin-5-one was prepared from 2-amino-5-(*o*-hydroxyphenyl)-1,3,4-oxadiazole. Mp. 220°C,yield 38 %. The column chromatography (silica gel, ether: benzene= 1 : 20)afforded 0.8 g of by-product 5a. Mp. 169°C, yield 10 %. Anal. Found: C, 52.71; H, 4.07 ; N, 12.77%, Calcd. for $C_{14}H_{13}O_6N_3$: C, 52.66 : H, 4.10 ; N, 13.16 %.

7-Carbometlioxy-2-(phenyl or o-hydroxyphenyl) ***-5*** *H-1,3,4-oxadiazolo[3,2-a]pyrimidin-5-one (4b, R=phenyl; 5b, R=o-hydroxyphenyl)*

To ***20*** ml of 5 % methanolic sodium hydroxide solution was added 0.5 g of 2- (phenyl or o-hydroxyphenyl) -7-methoxycarbonyl-5 *H*-1,3,4-oxadiazolo[3,2-*a*] pyrimidin-5-one (4, R=phenyl; 5, R=o-hydroxyphenyl). The mixture was refluxed for 100 minutes. Then the solvent was evaporated in *vacuo* and the residue was dissolved in distilled water and neutralized with diluted hydrochloric acid. The crude product was recrystallized from MeOH. The compound 4*b*, mp. 220°C, yield 63 %. The crude product of the compound ***5b*** was used for the following reaction.

2-(o-Hydroxyphenyl) ***-5*** *H-1,3,4-oxadiazolo* ***[3,2-a]*** *pyrimidin-5-one* ***(6,*** *R=o-hydroxyphenyl).*

The crude product 5 *b* was heated in 20 ml of DMF for 5 hr at 120°C in an oil bath. After being cooled, distilled water was added to the mixture. The precipitate was recrystallized from MeOH. Mp. 260°C, yield 40% from 5.

2-(2′-Acetyl-1′-methyl)-vinylamino-5-(p-nitrophenyl)-1,3,4-oxadiazole(8′,R=p-nitrophenyl)

To 5 g of 2-amino-5-(*p*-nitrophenyl)-1,3,4-oxadiazole (*I*, *R*=*p*-nitrophenyl) was added 2g of acetylacetone. The mixture was heated for 6-7 hr at 100–110°C in an oil bath. After being cooled, 30 ml of distilled water was added to the viscous mixture. The undissolved substance was removed and the filtrate was neutralized with sodium hydroxide. The resulting crystals were recrystallized from EtOH-Et_2O. Mp. 204°C (dec.), yield 56 %. Other compounds (7, 8) were obtained similarly.

2-Cyanoacetylamino-5-phenyl-1,3,4-oxadiazole (9,R=phenyl)

One gram of 2-amino-5-phenyl-1,3,4-oxadiazole (1, R=phenyl) was added to the mixture containing 0.7 g of ethyl cyanoacetate and 0.2 g of sodium in 30ml of abs. ethanol. The mixture was refluxed until the spot of oxadiazole disappeared on a TLC. Then the solvent was evaporated ***in*** *vacuo* and distilled water was added to dissolve the oily residue. The mixture was neutralized

with conc. hydrochloric acid. The resulting precipitate was recrystallized from MeOH to give 0.25 g (71 %) of white crystals. Mp. 183°C (dec.). Other compounds 10 and 11 were prepared similarly.

Biological Activity

Phytotoxicity

In each Petri-dish, a filter paper was placed. A compound (1 mg) was dissolved in 1 ml of acetone and poured into the dishes. After the acetone was evaporated, 10 ml of distilled water was added. Then 20 seeds of test plant were sowed in the solution and cultured at 27°C under light. After 6 days for radish and after 10 days for rice and barnyard grass, the lengths of roots and stalks of the seedlings were measured. The growth values were expressed in percents of the tested lot against controlled lot.

Antimicrobial activity

Antimicrobial activity tests on *Asp. niger* and *E. coli* were carried out as follows: a test compound was dissolved in a small portion of acetone or methanol and the solution containing 0. 5mg of sample was applied on a disk paper (6mm in diameter). After complete evaporation of the solvent the test paper was put on the surface of agar medium in a Petri-dish. In each Petri-dish four test papers and one control paper were arranged and then the medium plates were sprayed by a suspension of *E. coli* or spores of *Asp. niger*. In the case of *E. coli* after incubation for 24 hr at 37°C and in the case of *Asp. niger* after incubation for 48 hr at 30°C, the diameters of inhibitory zones were measured.

Cytotoxicity

The adopted cell lines were Ehrlich ascites tumor cells (EATC). The medium used in the test was Eagle's MEM, which was supplemented with calf serum of up to 20 %. The pH of the medium was adjusted to 7.2. The number of cells in an EATC suspension was adjusted to ca. 1×10^5 cells/ml. After 2 days culture, a test compound dissolved in dimethyl sulfoxide-phosphate buffer saline (1 : 1) was added to the culture medium in a concentration of 70 ppm. In order to promote the cell growth, the medium was refed every 2 days. After 6 days at 37°C, the cells in each tube were stained with crystal violet and the dead cells were counted under a microscope (Suiko and Maekawa 1977).

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