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INCREASE OF AUXIN-ANTAGONISTIC ACTION IN 3-PHOSPHORYLOXYCARBOSTYRILS

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Key Word Index—*Oryza sativa*; Gramineae; rice; synthesis; 3-phosphoryloxycarbostyrils; auxin-antagonism.

Abstract—Introduction of phosphoryloxy group at C-3 in carbostyrils has been found to increase auxin-antagonistic action. 3-Phosphoryloxycarbostyrils were synthesised from 3-hydroxycarbostyril and 3-hydroxy-4-phenylcarbostyril (viridicatin). The phosphates at very low concentrations markedly stimulated the root growth of rice seedlings, and competitively inhibited the IAA-induced lamina inclination in rice explants.

INTRODUCTION

Previously we have shown that although carbostyril itself has no physiological activity, carbostyrils bearing an oxygen function at C-3 are auxin-antagonists [1]. Since masking of the NH group in these derivatives destroys activity, it is presumably a requirement for biological activity. Modification of substituents at C-3 also alters this activity, and, the carbostyril having an $-OCH_2COOH$ group at C-3 shows the highest activity [1]. In connection with auxin-antagonistic action, the so called "two point attachment theory" [2,3] has been postulated. Accordingly, the negative charge or electron density of substituents at C-3 may play an important role in antagonistic action. From these points of view, it is of interest to introduce a phosphoryloxy group at C-3 in carbostyrils. Furthermore, introduction of phosphoryloxy group would increase the solubility of carbostyrils in water, which would facilitate the investigation of their biological activities.

RESULTS

Synthesis of 3-phosphoryloxycarbostyrils

Phosphorylation of 3-hydroxycarbostyrils was carried out according to the method of Kenner *et al.* [4]: 3-hydroxycarbostyrils (**1**) and (**4**) were converted into dibenzylphosphates (**2**) and (**5**), respectively, which were then debenzylated by means of hydrogenolysis into (**3**) and (**6**), respectively.

Growth effect of the phosphates on rice seedlings

As shown in Fig. 1, both (**3**) and (**6**) markedly stimulated the root growth and the effects were maximized at a concentration range, 1–3 ppm, respectively, while the effects of (**7**) and (**8**) were maximized at concentration ranges, 10–30 and 30–100 ppm, respectively. Also, though the root elongation caused by (**6**) was almost the same as that by (**8**), the effect of (**3**) was much greater than that of (**7**). The effects of (**3**) and (**6**) on shoot growth were rather repressive.

Combined effects of the phosphates with IAA on growth of rice seedlings

As shown in Table 1, when 1 ppm each of (**3**) and (**6**) was combined with 1 ppm of IAA, root growth overcame the inhibitory effect of IAA. The effect of (**3**) was especially remarkable. On the other hand, IAA restored the repression of shoot growth by (**3**) or (**6**), while IAA alone had no effect on it. It is evident that (**3**) and (**6**) possess auxin-antagonistic activity.

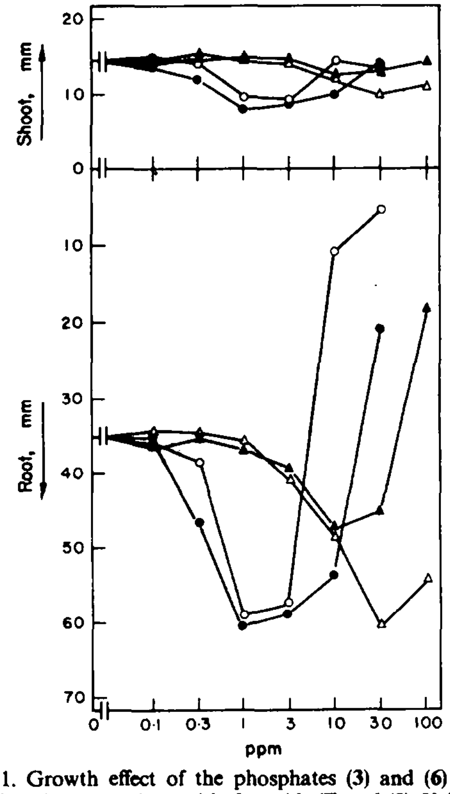
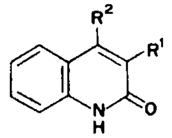


Fig. 1. Growth effect of the phosphates (**3**) and (**6**) on rice seedlings in comparison with the acids (**7**) and (**8**). Values represent average of 30 seedlings grown for 4 days. ●——● (3); ○——○ (6); ▲——▲ (7); △——△ (8).



(1) R^1 = OH; R^2 = H
(2) R^1 = $OP(O)(OCH_2C_6H_5)_2$; R^2 = H
(3) R^1 = $OP(O)(OH)_2$; R^2 = H
(4) R^1 = OH; R^2 = C_6H_5 (viridicatin [5])
(5) R^1 = $OP(O)(OCH_2C_6H_5)_2$; R^2 = C_6H_5
(6) R^1 = $OP(O)(OH)_2$; R^2 = C_6H_5
(7) R^1 = OCH_2COOH; R^2 = H
(8) R^1 = OCH_2COOH; R^2 = C_6H_5

Competition on lamina inclination between carbostyrils and IAA

Auxin-antagonistic action of (3) and (6) was further investigated on lamina inclination, and the activity was compared with (7) and (8). As shown in Table 2, IAA markedly increased the lamina inclination at 1 and 10 ppm, respectively, but the carbostyrils alone had no effect at 1 ppm. Compounds (6) and (8) rather inhibited the lamina inclination at 100 ppm. When these carbostyrils were respectively combined with 1 ppm of IAA, (3) only inhibited at 0·3 ppm the effect of IAA and (6) just inhibited at 1 ppm. Compounds (7) and (8) at 1 ppm could not compete with IAA, but (8) competed at 10 ppm. Compound (7) had only a slight competitive effect even at 10 ppm. Accordingly, the conversion of (7) to (3) caused much increased auxin-antagonistic action compared to converting (8) to (6). When (3) and (6) were both combined with 10 ppm of IAA, they required a higher concentration than 0·3–1 ppm for competition with IAA.

DISCUSSION

As shown above, both 3-phosphoryloxycarbostyrils (3) and (6) markedly stimulate the root growth of rice seedlings, and the effect overcomes the inhibitory effect of IAA on root growth. There is also a close relationship with auxin-antagonistic action on lamina inclination in the rice explants. Compound (3) exhibits a greater effect than (6). This may be due to the difference in stability of these phosphates and toxity of their hydrolysates, i.e. (1) and (4). Since (6), when dissolved in water, is more easily decomposed by heat than (3), (6) may be unstable *in vivo*, too. Also, (4) has higher antimicrobial activity and is more toxic on plant growth than (1). The hydroxyl groups of these compounds form metal complexes, as previously reported [1,5]. Masking of the hydroxyl group may have a significant effect on the root growth, because 3-methoxycarbostyril stimulates the root growth as well as (7), without the auxin-antagonistic action on lamina inclination, as previously reported [1]. There may be competition for active sites between the NH groups of carbostyrils and of IAA. On the other hand, the auxin-antagonistic action of the 3-phosphoryloxycarbostyrils indicates that the groups at C-3 are also important. The phosphate group may have a higher affinity than the carboxyl group for attachment to the IAA site in the cells, which in turn may affect the binding of the NH group too. Phosphatase activity may also be involved in the activity of 3-phosphoryloxycarbostyrils and for the further elucidation of the physiological activity of these phosphates, investigations on the formation and specificity of phosphatases in plants would be necessary.

Table 1. Combined effect of the phosphates (3) and (6) with IAA on growth of rice seedlings

Compound*	Growth† (mm) Shoot	Root
None	14·2	35·2‡
IAA	14·0	15·0
(3) + IAA	14·0	51·0
(3) alone	8·0	62·0
(6) + IAA	13·8	24·8
(6) alone	9·6	59·5

* Each 1 ppm. † Growth for 4 days. ‡ Figures represent average of 30 seedlings.

Table 2. Competition on lamina inclination between carbostyrils and IAA

Carbostyril	ppm	IAA (ppm) 0	1	10
None		100	157	163*
(3)	0·3	101	115	157
	1	105	101	126
	3	102	102	113
	10	96	99	101
(6)	0·3	104	158	163
	1	98	105	157
	3	96	104	130
	10	75	76	78
(7)	1	103	153	
	10	94	139	
(8)	1	98	159	
	10	76	98	

* Figures represent average inclination of 18 explants as % of control.

EXPERIMENTAL

The NMR spectra were recorded at 100 MHz using TMS as an internal standard, IR spectra as Nujol mulls.

0,0-*Dibenzyl* 3-[2(1*H*)-*quinolinonyl*] *phosphate*, (2). To dibenzylphosphorochloridate sol [4] prepared from dibenzylphosphite (1·048 g; 4 mmol) and *N*-chlorosuccinimide (534 mg; 4 mmol) in 30 ml dry C_6H_6, was added 3-hydroxycarbostyril [1,6] (483 mg; 3 mmol) and 0·45 ml of NEt_3 under N_2. After stirring for 2 hr at room temp. solvent was removed and residue treated with H_2O and $CHCl_3$. The organic layer was washed, dried and evaporated, giving a crystalline mass (1·196 g). Recrystallization from MeOH gave a dibenzylphosphate (2) as needles (980 mg; 77%), mp 163 6 (Found C, 65·19; H, 4·87; N, 3·38. $C_{23}H_{20}O_5NP$ requires: C, 65·34; H, 5·10; N, 3·31%); ν_{max} 1660, 1580, 1495, 1270, 1020, 755 and 740 cm^{-1}; δ (DMSO-d_6) 5·17 (*s*), 7·15–7·70 (*m*).

3-[2(1*H*)-*Quinolinonyl*] *phosphate*, (3). Dibenzyl phosphate (2; 550 mg) was dissolved in 250 ml EtOH, and hydrogenated at atm pres with Pd/C (55 mg; 30%). Catalyst was removed by filtration and filtrate was evaporated to give a crystalline mass. Recrystallization from MeOH and $CHCl_3$ gave 3-phosphoryloxycarbostyrils as needles (287 mg; 91%), mp 140° (sintered), *ca* 184° (dec.). (Found: C, 41·76; H, 3·72; N, 5·16. $C_9H_8O_5NP.H_2O$ requires: C, 41·75; H, 3·88; N, 5·38%); ν_{max} 3200–3050, 1650, 1620, 1580, 1290, 1230, 1005, 920 and 760 cm^{-1}; λ_{max}^{EtOH} 221 (log ϵ 4·55), 266 (log ϵ 3·76), 274 (log ϵ 3·86), 283 (log ϵ 3·80), 312 (log ϵ 3·86), 322 (log ϵ 3·95) and 335 nm (log ϵ 3·80).

O,O-Dibenzyl 3-[4-phenyl-2(1H)-quinolinonyl] phosphate (**5**). The dibenzyl phosphate of viridicatin [5] was prepared by a similar method as above. Crude product was recrystallized from MeOH to give needles (yield 30%), mp 171–171·5° (Found: C, 69·75; H, 4·98; N, 2·65. $C_{29}H_{24}O_5NP$ requires: C, 70·02; H, 4·86; N, 2·81%); ν_{max} 1660, 1570, 1500, 1270, 1020, 1030 and 760 cm^{-1}; δ ($CDCl_3$-DMSO-d_6) 4·78 (*s*), 4·85 (*s*) 7·08–7·40 (*m*).

3-[*4-Phenyl-2(1H)-quinolinonyl*] *phosphate*, (**6**). Debenzylation of (**5**) by a similar method as above gave the phosphate (**6**) as needles (yield 85%), mp 160° (sintered), 222–223° (dec.) (from MeOH). (Found: C, 56·23; H, 4·07; N, 4·00. $C_{15}H_{12}O_5NP$ requires: C, 56·64; H, 4·06; N, 4·40%); ν_{max} 3200–3060, 2400, 1650, 1575, 1510, 1285, 1230, 1010, 825 and 760 cm^{-1}; λ_{max}^{EtOH} 224 (log ϵ 4·51), 285 (log ϵ 3·85) and 321 nm (log ϵ 3·91).

3-*Carbostyriloxyacetic acid*, (**7**). This compound was prepared from 3-hydroxycarbostyril and monochloroacetic acid as reported previously [1].

4-Phenyl-3-carbostyriloxyacetic acid, (**8**). This compound was prepared from viridicatin and monochloroacetic acid as reported previously [5].

Growth test on rice seedlings. Rice (*Oryza sativa* L. cv. Shinkinmaze) seedlings were grown on sea sand in a Petri dish for 4 days as previously reported [7].

Lamina inclination test. A method of Maeda [8] was used with slight modification, employing the rice (*Oryza sativa* L. cv. Shinkinmaze) explants as described in a previous paper [1].

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