Several Synthetic Hydroxy-acids as Plant Growth Regulators

Sir:

In 1965 Tamura and Chang¹⁾ isolated L- β -phenyllactic acid as a root growth promoter for rice and lettuce seedlings from culture filtrates of *Exobasidium symploci-japonicae* which is a fungus pathogenic for *Symplocos japonica*. Subsequently, we noticed that a few hydroxy-acids produced by a fungus of *Alternaria* species markedly accelerated root elongation.²⁾ These observations led us to synthesize various kinds of hydroxy-acids to test their biological activities. As the result, several compounds were found to exhibit marked root growth promotion which is preliminarily reported in this paper.

All of the hydroxy-acids tested here were known compounds and, unless commercially available, they were prepared according to the following methods:

- a) Treatment of amino acids with nitrous acid.
- b) Reformatskii reaction of carbonyl compounds followed by alkali hydrolysis.
- c) Addition of hydrogen cyanide to carbonyl compounds followed by acid hydrolysis.
- d) Treatment of long-chain unsaturated fatty acids with potassium permanganate or perbenzoic acid followed by acid hydrolysis resulting in the formation of *cis*- or *trans*-dihydroxy-acids.

The crude products thus obtained were purified by distillation under reduced pressure, silicic acid adsorption chromatography or recrystallization.

Biological activities were examined at first

on the root growth of lettuce seedlings by the method of Frankland and Wareing.³⁾ Subsequently, the compounds which exhibited marked promotion were subjected to the "Azukia rooting test" as well as the *Avena* coleoptile straight-growth test. In the *Azukia* test devised by Mitsuhashi *et al.*,⁴⁾ we used epicotyl cuttings without disbudding which were dipped in test solutions for the first 36 hr. Numbers of adventitious roots protruding from cuttings were counted seven days after preparation. A typical feature of rooting is shown in Fig. 1.

FIG. 1. Promotion on Root Formation of *Azukia* Cuttings by α -Hydroxy- β -methylvaleric Acid (**22**) and β -Hydroxy-isocaproic Acid (**25**).

Left, control; middle, **25**, 300 mg/liter; right, **22**, 300 mg/liter.

Effects of aromatic hydroxy-acids including β -phenyllactic acid (**4**) on lettuce seedling roots are illustrated in Table I. The activities of 4-hydroxy-3-methoxymandelic acid (**3**) and α -hydroxy- γ -phenylbutyric acid (**6**) were almost equal to that of **4**. Introduction of

1) S. Tamura and C. F. Chang, *Agr. Biol. Chem.*, **29**, 255 (1965).

2) The structures of these acids are under investigation.

3) B. Frankland and P. E. Wareing, *Nature*, **185**, 255 (1960).

4) M. Mitsuhashi, H. Shibaoka and M. Shimokoriyama, *Plant and cell Physiol.*, **10**, 715 (1969).

TABLE I. EFFECTS OF AROMATIC HYDROXY-ACIDS ON THE ROOT GROWTH OF LETTUCE SEEDLINGS

Control = 100; 20.4 mm

Compound ^{a)}	Method ^{b)}	Concentration (mg/liter)				
		10	30	100	300	600
1 Mandelic acid		113	118	91	27	0
2 <i>p</i> -Hydroxymandelic acid		108	106	137	127	96
3 4-Hydroxy-3-methoxymandelic acid		109	125	164	170	144
4 β -Phenyllactic acid	a	92	117	124	158	132
5 β -Hydroxy- β -phenylpropionic acid	b	103	92	65	55	0
6 α -Hydroxy- γ -phenylbutyric acid	c	102	136	188	101	13

^{a)} Purity of each compound was verified with gas chromatography or spectrometry. The behavior of the hydroxy-acids in the both analyses will be disclosed afterwards.

^{b)} See the text.

TABLE II. EFFECTS OF ALIPHATIC HYDROXY-ACIDS ON THE ROOT GROWTH OF LETTUCE SEEDLINGS

Control = 100; 20.5 mm

Compound ^{a)}	Method ^{b)}	Concentration (mg/liter)				
		10	30	100	300	600
7 Glycolic acid		98	78	69	18	0
8 β -Hydroxypropionic acid		110	101	97	61	18
9 Tartronic acid		69	69	68	57	44
10 α -Hydroxy- <i>n</i> -butyric acid	a	102	95	91	78	30
11 α -Hydroxy-isobutyric acid		103	111	113	100	20
12 β -Hydroxy- <i>n</i> -butyric acid	b	103	104	125	104	20
13 α -Hydroxy- <i>n</i> -valeric acid	a	125	148	177	111	105
14 α -Hydroxy- β -methylbutyric acid	a	100	100	106	105	63
15 β -Hydroxy- <i>n</i> -valeric acid	b	97	107	124	159	81
16 β -Hydroxy-isovaleric acid	b	94	98	86	32	0
17 α -Hydroxy- α -methylbutyric acid	c	104	100	84	38	0
18 L- α , δ -Dihydroxyvaleric acid	a	106	110	141	147	17
19 α -Hydroxy- <i>n</i> -caproic acid	a	157	169	121	83	0
20 α -Hydroxy-isocaproic acid	a	93	118	109	90	11
21 α -Hydroxy- α -methylvaleric acid	c	98	98	92	93	67
22 α -Hydroxy- β -methylvaleric acid	a	116	141	138	87	3
23 α -Hydroxy- α -methyl-isovaleric acid	c	101	108	117	94	45
24 β -Hydroxy- <i>n</i> -caproic acid	b	116	103	135	139	27
25 β -Hydroxy-isocaproic acid	b	112	108	147	100	14
26 β -Hydroxy- α -methylvaleric acid	b	106	127	204	168	34
27 β -Hydroxy- β -methylvaleric acid	b	105	108	119	35	5
28 L-2,6-Dihydroxycaproic acid	a	118	118	148	156	71
29 2-Isopropylmalic acid	c	136	146	195	121	81
30 α -Hydroxycaprylic acid	a	104	114	126	135	127
31 β -Hydroxymyristic acid		92	85	85	74	67
32 12-Hydroxystearic acid		99	94	86	78	72
33 Ricinoleic acid		104	100	101	105	96
34 <i>cis</i> -9,10-Dihydroxystearic acid	d	93	96	101	102	116
35 <i>trans</i> -9,10-Dihydroxystearic acid	d	101	98	98	102	112

^{a)} and ^{b)} cf. Table I.

hydroxy or/and methoxy groups into the benzene ring of mandelic acid (1) distinctly increased the efficacy.

As shown in Table II, several aliphatic hydroxy-acids (13, 15, 18, 19, 25, 26, 28 and 29) caused remarkable root elongation of lettuce seedlings indicating that the presence of benzene ring may not be required for the activity. On the other hand, some hydroxy-acids (7, 9, 16, 17, 31 and 32) listed in Table II exhibited suppression.

The activities of aliphatic and aromatic hydroxy-acids to promote the rooting of *Azukia* cuttings are shown in Table III. Several

TABLE III. EFFECTS OF HYDROXY-ACIDS ON THE ROOTING OF *Azukia* CUTTINGS

Control=100; Average 4.4 adventitious roots/cutting

Com-pound	Concentration (mg/liter)				
	10	30	100	300	600
1	104	98	231	238	186
2	88	94	98	137	133
3	98	116	111	151	90
4	93	116	127	153	24
5	102	99	114	112	140
6	113	120	211	242	170
12	79	98	95	111	133
14	125	145	149	131	130
15	120	128	126	169	135
18	77	73	113	154	220
19	84	193	193	120	102
20	93	98	100	123	113
22	82	86	144	276	348
24	135	140	173	271	114
25	133	207	233	405	451
26	96	140	319	120	120
27	96	100	163	333	412
28	90	93	105	166	163
29	105	126	190	203	157
33	71	95	101	131	119

hydroxy-acids (1, 6, 22, 24, 25, 26, 27 and 29) showed remarkable promotion of adventitious root formation. Among these, only 6, 25, 26 and 29 were effective in root elongation of lettuce seedlings as described above.

Contrary to the expectation, no hydroxy-

TABLE IV. EFFECTS OF HYDROXY-ACIDS ON THE ELONGATION OF *Avena* COLEOPTILE SEGMENTS

Control=100; 6.24 mm

Com-pound	Concentration (mg/liter)				
	10	30	100	300	600
4	98	101	100	98	86
6	102	104	103	99	97
14	108	111	112	111	113
15	113	110	101	110	105
18	104	104	102	107	103
19	102	100	102	96	80
22	107	133	113	104	81
24	111	114	107	110	103
25	104	110	113	110	105
26	113	110	104	110	103
29	109	107	110	111	108
33	105	118	110	112	97
IAA	146 (0.1 mg/liter)				

acids revealed activity in the *Avena* straight-growth test as shown in Table IV. This suggests that the action mechanism of the hydroxy-acids on the roots may differ from that of auxins.

From the results so far obtained, it is impossible to propose definite conclusion on structure-activity relationships, but this paper would be the first one to report the comparative activities of synthetic hydroxy-carboxylic acids as root growth promoters.

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