Pure & Appl. Chem., Vol. 62, No. 7, pp. 1347–1352, 1990.
Printed in Great Britain.

Secondary metabolites related to host selection by plant pathogenic fungi

Tamio Ueno

Pesticide Research Institute, Faculty of Agriculture, Kyoto University.
Kitashirakawa, Sakyo-ku, 606 Kyoto, Japan.

Abstract - Role of host-selective toxins and phytoalexins in host-parasite interaction was investigated from chemical as well as phytopathological point of view. As the results of phytopathological studies on host selection by Alternaria mali and A. kikuchiana, the causal agents of Alternaria leaf spot disease of apple and black spot disease of Japanese pear respectively, AM-toxin I, II and III, and AK-toxin I and II were isolated as the primary determinants of these diseases , chemical structure of which were elucidated to be (1a), (1b), (1c), (2a) and (2b) respectively, and the proposed structures were confirmed by total syntheses. The isolated crystalline AM- and AK-toxins showed not only extremely potent host-selective toxicity, but dilute solutions of these toxins also induced the same necrotic symptoms only on their host plant tissues as those observed on inoculation with the spores of the causal fungi. Structural features of the toxins that express the host selective toxicity were also analyzed using synthetic analogs of AM- and AK-toxins.
On the other hand, in the course of the studies on the host-selection mechanism by obligate parasites, specific accumulation of the phytoalexins , avenalumin I, II and III, were confirmed in oat leaves infected with incompatible strains of crown rust fungus, Puccinia coronata f. sp. avenae and their structures were characterized as (6a), (6b) and (6c). These structures were also confirmed by total synthesis.

INTRODUCTION

The complex processes of infectious plant diseases caused by some fungal pathogens are summarized schematically in Fig. 1. Various types of biochemical response between host and parasite in plant diseases have been postulated by plant pathologists. It is widely known that small organic molecules play impotant roles not only in the infection process but also in disease development: that is, in some cases toxic substances arise from positive offensive reaction by fungus itself, and other cases antifungal substances such as prohibitin, inhibitin, post inhibitin and phytoalexin are released in the host tissue as defensive substances(ref. 1). However, at present, the most intriguing problem in plant disease is "host-selectivity of the pathogens"; in plant infectious disease fixed combinations are observed between pathogens and host plants. Here the we present chemical approach made in our laboratory to analyze the host selection by plant pathogenic fungi.

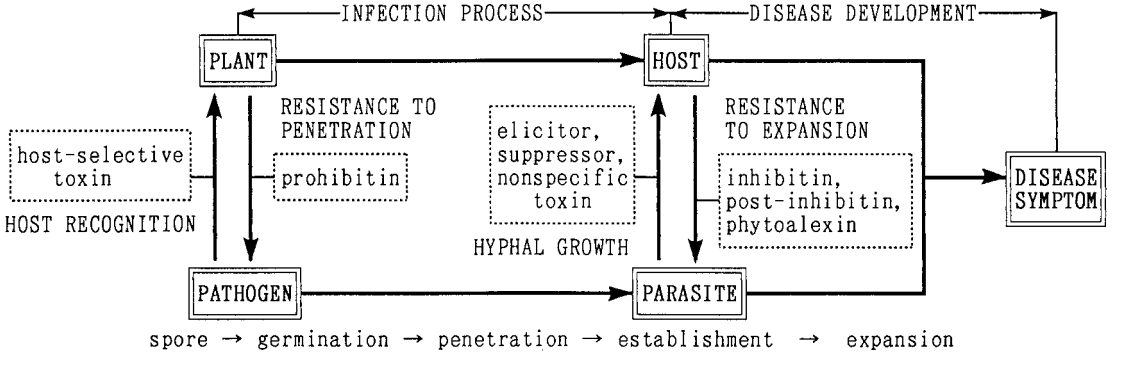


Fig.1 Scheme for the host-parasite interaction in plant diseases and chemical substances which concern in it(enclosed by ·····).

HOST SELECTIVE TOXINS FROM *ALTERNARIA* SPECIES

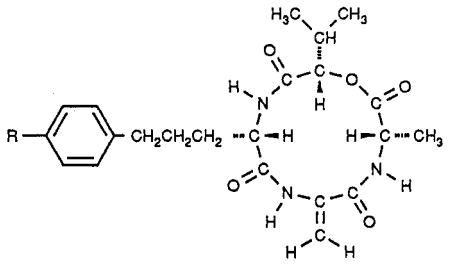
In order to explain the host-selective(or specifc) interaction observed in some diseases caused by Alternaria and Helminthosporium species, "host-selective toxin" or pathotoxin was proposed as descriptive words for the fungal metabolites which have selective toxicity only to the host plant and were considered to be major determinants of pathogenicity (ref. 2, 3 and 4).

Alternaria mali and A. kikuchiana cause leaf spot disease of apples and black spot disease of Japanese pears respectively, and produce necrotic blotches especially on the leaves, shoots and fruits of susceptible cultivars. However, great differences in tolerances to these diseases are observed among apple and Japanese pear cultivars (see TABLE 1). As the results of detailed plant pathological experiments on these diseases, Sawamura and Tanaka observed that the culture filtrates of these fungi induced the same necrotic symptoms as did the spore inoculations and they suggested the presense of host-selective toxins in the culture media (see TABLE 1) (ref. 5 and 6). These two fungi can not be discriminated from their morphological features. In order to explicate the chemical basis of the host selection by the two species of Alternaria, we started on the chemical studies on the host selective toxins.

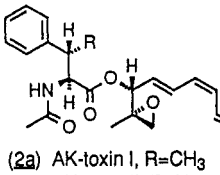
TABLE 1. Pathogenicity of A. mali, A. kikuchiana and A. fragariae and host-selective toxicity of AM-, AK- and AF-toxins

pathogenic fungi		apple		Japanese pear		strawberry	
		Indo	Jonathan	Nijisseiki	Chojuro	Morioka-16	Hokowase
name of fungi	assay methods	(s.)	(r.)	(s.)	(r.)	(s.)	(r.)
Alternaria mali	spore inoculation	+++	+	+	-	-	n.t.
	culture filtrate	+++	+	+	-	-	n.t.
	AM-toxin I(10^{-5}M)	+++	+	+	-	-	n.t.
	AM-toxin I(10^{-8}M)	+	-	-	-	-	n.t.
Alternaria kikuchiana	spore inoculation	-	-	+++	-	-	-
	culture filtrate	-	-	+++	-	-	-
	AK-toxin I	-	-	+++	-	-	-
	AK-toxin II	-	-	+++	-	-	-
Alternaria fragariae	spore inoculation	-	-	++	-	+++	-
	culture filtrate	-	-	++	-	+++	-
	AF-toxin I	-	-	++	-	+++	-
	AF-toxin II	-	-	++	-	-	-
	AF-toxin III	-	-	±	-	+++	-

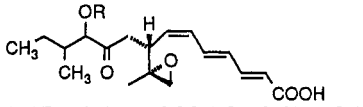
+ and - Marks indicate positive and negative response respectively. Induced necrosis rating scale is as follows: +++, very severe; ++, severe; +, moderate; and -, no necrosis.
s. and r. are abbreviation of "susceptible" and "resistant" respectively.
n.t. means "not tested".



(1a) AM-toxin I, R=OCH_3
(1b) AM-toxin II, R=H
(1c) AM-toxin III, R=OH



(2a) AK-toxin I, R=CH_3
(2b) AK-toxin II, R=H



(3a-a) AF-toxin Ia, R=$COCH(OH)C(CH_3)_2OH$
(3b-a) AF-toxin IIa, R=H
(3c-a) AF-toxin IIIa, R=$COCH(OH)CH(CH_3)_2$

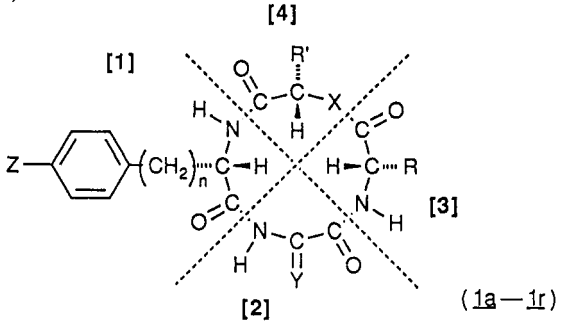
AM-TOXINS, HOST-SELECTIVE TOXINS PRODUCED BY *A. MALI*

A. mali produces host-selective AM-toxin I (alternariolide) (1a), II (1b) and III (1c) which are analogous cyclic depsipeptides containing unusual amino acids. Chemical studies of them has been extensively developed by two groups in Japan. Both of them proposed the same structure for (1a) and our group reported further isolation and structure elucidation of (1b) and (1c) (ref. 7, 8, 9 and 10). The proposed structures were confirmed by total syntheses (ref. 11, 12 and 13).

After total syntheses, Izumiya et al. have developed the synthetic work on AM-toxin analogs

in order to investigate the requisite structural features to express phytotoxicity (ref 14, 15, 16, 17, 18, 19 20 and 21). As the result, the following conclusion was obtained from the results shown in TABLE 2. 1) Cyclic peptide structure is necessary, because linear peptides obtained by selective metanolyses of the lactone linkage were no longer active. 2) The presence of the bulky and hydrophobic side chain in [1] is impotant for exhibiting biological activity, because (1i), (1j) and (1k) composed of shorter and longer methylene group indicated still high activity. 3) Hydrogenation of the exomethylene in [2] gave (1d) and (1e), the latter of which still retained activity. However, the activity disappeared by replacement of a hydrogen of the exomethylene to methyl (1f). These facts indicate that shape and bulkiness of this part in [2] are closely related to toxicity. 4) Replacement of the methyl group in [3] by bulky ethyl, isopropyl and benzyl groups, (1n), (1o) and (1p), did not affect the toxicity remarkably. 5) Lactam analogs containing L-valine in place of L-2-hydroxy-3-methylbutyric acid in [4], (1l) and (1m), still retained activity. This fact indicates that the lactone linkage can be replaced by amide bonding. 6) The bulkiness of alkyl group of the side chain of the part [4] in (1g) and (1r) affected a slight change in activity.

TABLE 2. Structures and biological activity of AM-toxins and their synthesized analogs inducing necrosis on the leaves of a susceptible cultivar Indo (see ref. 7 - 10 and 14 - 21)



(1a—1r)

No. AM-toxins, synthesized analogs	X	Y	Z	n	R	R'	Activity*
(1a) AM-toxin I	O	$=CH_2$	OCH_3	3	CH_3	$CH(CH_3)_2$	10^{-8}M
(1b) AM-toxin II	O	$=CH_2$	H	3	CH_3	$CH(CH_3)_2$	10^{-6}M
(1c) AM-toxin III	O	$=CH_2$	OH	3	CH_3	$CH(CH_3)_2$	10^{-7}M
(1d) [L-Ala^2]AM-toxin I	O	$-CH_3$,⋯H	OCH_3	3	CH_3	$CH(CH_3)_2$	$>10^{-3}$M
(1e) [D-Ala^2]AM-toxin I	O	⋯CH_3,-H	OCH_3	3	CH_3	$CH(CH_3)_2$	10^{-4}M
(1f) [ΔAbu,^{2}AM-toxin I	O	$=CH-CH_3$	OCH_3	3	CH_3	$CH(CH_3)_2$	$>10^{-3}$M
(1g) [L-Tyr(Me)^1,L-Ala^2]AM-toxin I	O	$-CH_3$,⋯H	OCH_3	1	CH_3	$CH(CH_3)_2$	$>10^{-3}$M
(1h) [L-Tyr(Me)^1,D-Ala^1]AM-toxin I	O	⋯CH_3,-H	OCH_3	1	CH_3	$CH(CH_3)_2$	$>10^{-3}$M
(1i) [L-Tyr(Me)^1]AM-toxin I	O	$=CH_2$	OCH_3	1	CH_3	$CH(CH_3)_2$	10^{-4}M
(1j) [L-Amb^1]AM-toxin I	O	$=CH_2$	OCH_3	2	CH_3	$CH(CH_3)_2$	10^{-6}M
(1k) [L-Amh^1]AM-toxin I	O	$=CH_2$	OCH_3	4	CH_3	$CH(CH_3)_2$	10^{-4}M
(1l) [L-Val^4]AM-toxin I	NH	$=CH_2$	OCH_3	3	CH_3	$CH(CH_3)_2$	10^{-5}M
(1m) [L-Tyr(Me)^1,L-Val^4]AM-toxin I	NH	$=CH_2$	OCH_3	1	CH_3	$CH(CH_3)_2$	10^{-5}M
(1n) [L-Abu^3]AM-toxin I	O	$=CH_2$	OCH_3	3	CH_2CH_3	$CH(CH_3)_2$	10^{-5}M
(1o) [L-Leu^3]AM-toxin I	O	$=CH_2$	OCH_3	3	$CH_2CH(CH_3)_2$	$CH(CH_3)_2$	10^{-6}M
(1p) [L-Phe^3]AM-toxin I	O	$=CH_2$	OCH_3	3	CH_3Ph	$CH(CH_3)_2$	10^{-7}M
(1q) [L-Lac^4]AM-toxin I	O	$=CH_2$	OCH_3	3	CH_3	CH_3	10^{-3}M
(1r) [L-Hmp^4]AM-toxin I	O	$=CH_2$	OCH_3	3	CH_3	$CH_2CH(CH_3)_2$	10^{-3}M

* The values indicate threshold concentrations inducing necrosis after 18hrs when a droplet of the sample solution was placed on the bottom of a young fresh leaf of cultivar Indo.

AK-TOXINS, HOST-SELECTIVE TOXINS PRODUCED BY *A. KIKUCHIANA*

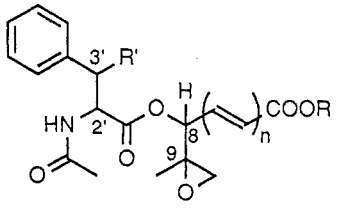
For more than 50 years, black spot disease of Japanese pear (Pyrus serotina Rehd.) caused by A. kikuchiana has been one of the most destructive disease in pear orchard in Japan. The most popular cultivar, Nijisseiki, is highly susceptible. The report on this disease by Tanaka (ref. 6), which suggested the presense of host-selective toxin(s) in the culture medium, enticed natural product chemists in Japan to try the isolation of the active principle(s), and as the results several phytotoxic metabolites were isolated from the culture broth of A. kikuchiana, but none of them showed host-selective toxicity which is characteristic of the culture filtrate. However, isolation of the host-selective active principles, named AK-toxin I and II, was performed by our group in 1983 (ref. 22, 23 and 24). The chemical structure of AK-toxin I was characterized as 8-[(2'S,3'S)-2'-acetylamino-3'-methyl-3'-phenylpropionyloxy]-(8R,9S)-9,10-epoxy-9-methyl-deca-(2E,4Z,6E)-trienoic acid (2a) from

chemical, spectral and X-ray crystallographic data. The structure of AK-toxin II was also shown to be 3'-demethyl derivative of AK-toxin I (2b), by comparison of the spectral data with those of AK-toxin I.

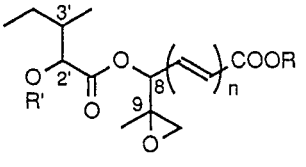
Another example of Alternaria host-selective toxin in Japan was recently reported in the case of black spot disease of strawberry (ref. 25). The causal fungus A. fragariae, produces host selective AF-toxin I(3a), II(3b) and III(3c), all of which also contain (8R,9S)-9,10-epoxy-8-hydroxy-9-methyl-decatrienoic acid as one of the structural moieties. The difference between AK- and AF-toxins is observed in the geometry at the 2, 4 and 6 positions of the decatrienoic acid as well as another moiety. AF-toxins contain 2-hydroxy-3-methyl pentanoic acid in which the 2-hydroxy group is free in AF-toxin II and esreified with 2,3-dihyroxy-2-methyl-butyric acid and 2-hydroxy-2-methyl-butyric acid in AF-toxin I and III respectively. In spite of the difference of the geometry on the trienoic acid moiety, both AK-toxins and AF-toxin I and II exhbit selective toxicity against Nijisseiki (see TABLE 1).

In order to investigate the structural features of these compounds showing the host-selective toxicity, we performed stereo-selective synthesis of AK- and AF-toxins starting with Vitamine C as a chiral synton (scheme 1) (ref. 26 and 27). The following conclusion is obtained from the results of bioassay in TABLE 3 using the natural and synthetic analogs of AK- and AF-toxins. 1) The absolute configuration at C-2' of N-acetyl phenylalanine in AK-toxin II does not affect the toxicity. 2) The absolute configurations at C-8(R) and C-9(S) in the epoxy-decatrienoic acid are indispensable to the toxicity in both AK- and AF-toxins. 3) The geometrical arrangements and the free carboxyl group on the conjugate trienoic acid are not indispensable, but the length of the conjugate system affects expression of the toxicity. 4) All of the biologically active compounds indicated the host selective toxicity. Total synthesis of AK-toxin II was also performed by other chemists (see ref. 28, 29 and 30)

TABLE 3. Structures and biological activity of AK- and AF-toxins and their synthesized analogs inducing necrosis on the leaves of a susceptible cultivar Nijisseiki.



AK-toxin analogs (2a — 2n)

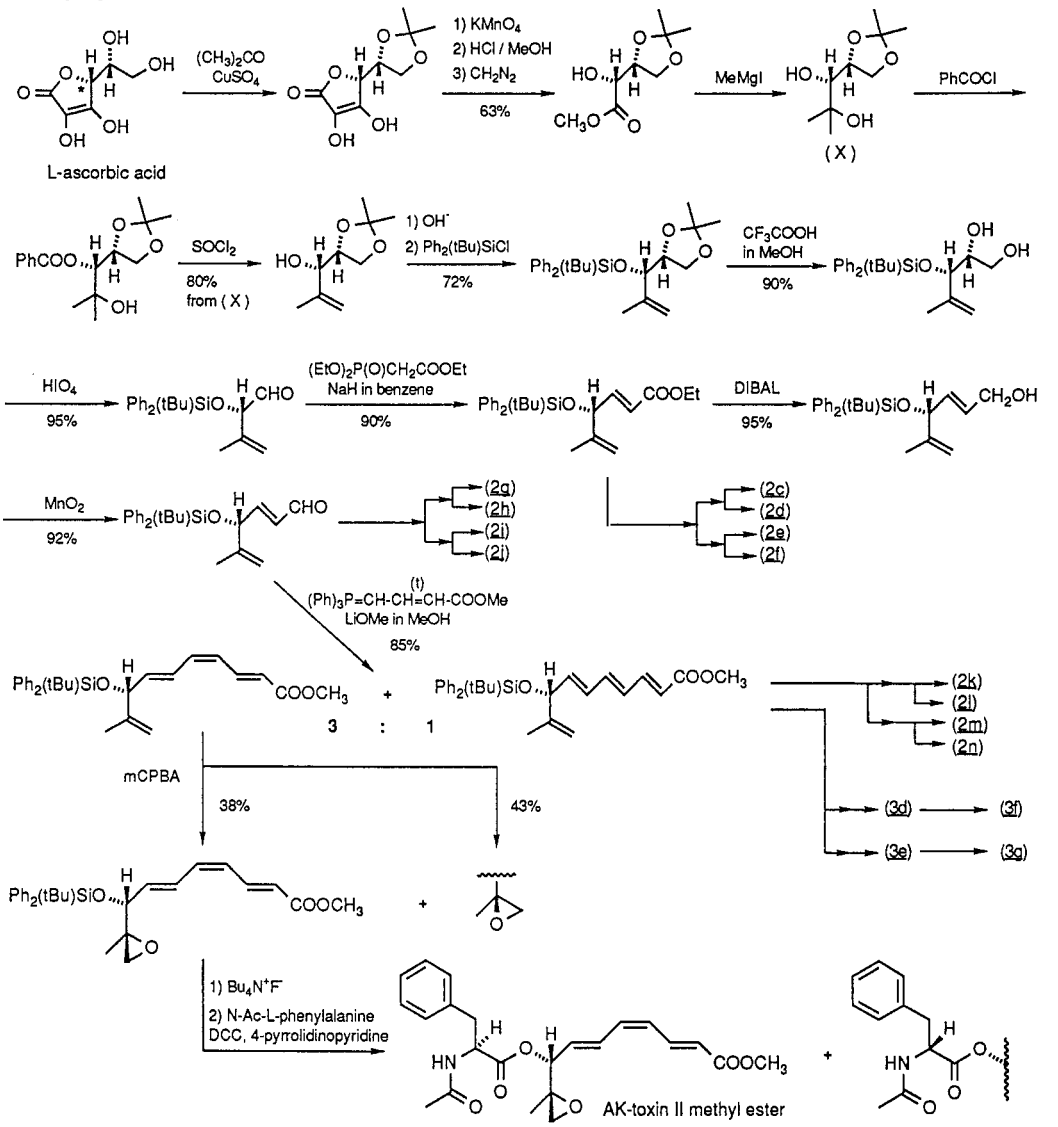


AF-toxin analogs (3a-a — 3g)

No. AK- and AF-toxin synthesized analogs	abs. configurations C-2'	C-3'	C-8	C-9	R'	length and geometries n	C-2	C-4	C-6	R	Activity (M)
(2a) AK-toxin I	S	S	R	S	CH_3	3	E	Z	E	H	10^{-9}
(2b) AK-toxin II	S	-	R	S	H	3	E	Z	E	H	10^{-7}
(2c)	S	-	R	S	H	1	E			CH_3	10^{-3}
(2d)	R	-	R	S	H	1	E			CH_3	10^{-3}
(2e)	S	-	R	R	H	1	E			CH_3	none
(2f)	R	-	R	R	H	1	E			CH_3	none
(2g)	S	-	R	S	H	2	E	E		CH_3	10^{-4}
(2h)	R	-	R	S	H	2	E	E		CH_3	10^{-4}
(2i)	S	-	R	R	H	2	E	E		CH_3	none
(2j)	R	-	R	R	H	2	E	E		CH_3	none
(2k)	S	-	R	S	H	3	E	E	E	CH_3	$<10^{-4}$
(2l)	R	-	R	S	H	3	E	E	E	CH_3	$<10^{-4}$
(2m)	S	-	R	R	H	3	E	E	E	CH_3	none
(2n)	R	-	R	R	H	3	E	E	E	CH_3	none
(3a-a) AF-toxin Ia					$COCH(OH)C(CH_3)_2OH$	3	Z	E	E	H	$<10^{-4}$
(3b-a) AF-toxin IIa					H	3	Z	E	E	H	$<10^{-4}$
(3b-b) AF-toxin IIb		unknown			H	3	E	Z	E	H	$<10^{-4}$
(3b-c) AF-toxin IIc					H	3	E	E	E	H	$<10^{-4}$
(3c-a) AF-toxin IIIa					$COCH(OH)CH(CH_3)_2$	3	Z	E	E	H	10^{-2}
(3d)	S	S	R	R	H	3	E	E	E	CH_3	none
(3e)	S	S	R	S	H	3	E	E	E	CH_3	$<10^{-4}$
(3f)	S	S	R	R	H	3	E	E	E	H	none
(3g)	S	S	R	S	H	3	E	E	E	H	$<10^{-4}$

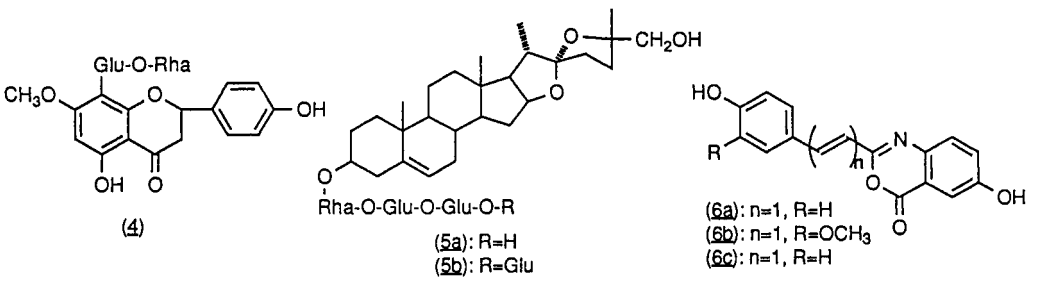
* The values indicate threshold concentrations inducing necrosis after 18hrs when a droplet of the sample solution was placed on a young fresh leaf of cultivar Nijisseiki.

Scheme 1



PHYTOALEXINS, AVENALUMINS, ACCUMULATED IN OAT

Relationship between phytoalexin production and specific expression of resistance by host plants was demonstrated in crown rust disease in oat (Avena sativa) to the causal fungus Puccinia coronata f. sp. avenae. As the results of experiments, we isolated and identified three antifungal compounds, (4), (5a) and (5b). One of them (4) should be classified as a prohibitin and two of them (5a) and (5b) as post-inhibitin according to the Ingham's proposal (ref. 1). In addition, we obtained analogous three antifungal compounds, named avenalumin I (6a), II (6b) and III (6c), which demonstrate the property of phytoalexins (ref. 31 and 32). One antifungal flavonoid was isolated in the same quantity from both healthy and infected primary leaves of oat seedling (cv. Shokan) and identified to be O''-rhamnosyl-isoswerticin (4). The two steroid saponins were isolated from injured oat leaves and they were identified as 26-desgluco-avenacoside A (5a) and B (5b). These compounds are easily converted from non-antifungal precursors avenacoside A and B present in the healthy leaves by enzymatic activation of β-glucosidases. However, specific accumulation of avenalumins (6a), (6b) and (6c) was observed only in the case of incompatible combination of oat cultivars and P. coronata. These compounds were not also isolated from uninoculated healthy oat leaves as well as the leaves infected with compatible race of the fungus. Their structures were determined to be analogous 2-substituted 6-hydroxy-4H-3,1-benzoxazin-4-one, (6a), (6b) and (6c) respectively. These structures were confirmed by total syntheses and phytopathological significance in the rust infection were investigated (ref. 33). As the results, production of avenalumins is involved in the specific resistance of oat cultivars against in compatible crown rust races.



REFERENCES

1. J.L. Ingham, Phytopathol. Z., 28, 314-335 (1973)
2. R.B. Pringle and R.P. Scheffer, Annu. Rev. Plant Pathol., 2, 133-156 (1964)
3. S. Nishimura and K. Kohmoto, Annu. Rev. Plant Pathol., 21, 87-116 (1983)
4. H. Wheeler and H.H. Luke, Annu. Rev. Microbiol., 17, 223-242 (1963)
5. K. Sawamura, Bull. Hort. Res. Stan. Japan, C4, 43-59 (1966)
6. S. Tanaka, Mem. Coll. Agr. Kyoto Imp. Univ., 28, 1-31 (1933)
7. T. Okuno, Y. Ishita, K. Sawai and T. Matsumoto, Chem. Lett., 1974, 635
8. T. Okuno, Y. Ishita, A. Sugahara, A. Mori, K. Sawai and T. Matsumoto, Tetrahedron Lett., 1975, 335
9. T. Ueno, T. Nakashima, Y. Hayashi and H. Fukami, Agr. Biol. Chem., 39, 1115-1122 (1975)
10. T. Ueno, T. Nakashima, Y. Hayashi and H. Fukami, Agr. Biol. Chem., 39, 2081-2082 (1975)
11. S. Lee, H. Aoyagi, Y. Shimohigashi, N. Izumiya, T. Ueno and H. Fukami, Tetrahedron Lett., 1976, 843-846
12. Y. Shimohigashi, S. Lee, T. Kato, N. Izumiya, T. Ueno and H. Fukami, Chemistry Lett., 1411-1414 (1977)
13. T. Kanmera, H. Aoyagi, M. Waki, T. Kato, N. Izumiya, K. Noda and T. Ueno, Tetrahedron Lett., 22, 3625-3628 (1981)
14. Y. Shimohigashi, S. Lee, T Kato, N Izumiya, T. Ueno and H. Fukami, Agric. Biol. Chem., 41, 1533-1544 (1977)
15. T. Kozono, T. Kanmera, T. Kato, T. Ueno and N. Izumiya, Agric. Biol. Chem., 47, 2631-2632(1983)
16. H. Mihara, H. Aoyagi, T. Kato, T. Ueno and N. Izumiya, Chemistry Lett., 811-814 (1983)
17. H. Aoyagi, H. Mihara, S. Lee, T. Kato T. Ueno and N. Izumiya, Int. J. Peptide Protein Res., 25, 144-148 (1985)
18. H. Mihara, K. Ikesue, S. Lee, H. Aoyagi, T. Kato, T. Ueno and N. Izumiya, Int. J. Peptide Protein Res., 28, 141-145 (1986)
19. H. Mihara, H. Aoyagi, T. Ueno, T. Kato and N. Izumiya, Bull. Chem. Soc. Japan, 59, 2041-2043 (1986)
20. H. Mihara, H. Aoyagi, T. Ueno, T. Kato and N. Izumiya, Bull. Chem. Soc. Japan, 59, 2651-2653 (1986)
21. H. Aoyagi, H. Mihara, T. Kato, M. Yamada and T. Ueno, Agric. Biol. Chem., 51, 1707-1709 (1987)
22. T. Nakashima, T. Ueno and H. Fukami, Tetrahedron Lett., 23, 4469-4472 (1982)
23. H. Otani, K. Kohmoto, S. Nishimura, T. Nakashima, T. Ueno and H. Fukami, Ann. Phytopath. Soc. Japan, 51, 285-293 (1985)
24. T. Nakashima, T. Ueno, H. Fukami, T. Taga, H. Masuda, K. Osaki, H. Otani, K. Kohmoto and S. Nishimura, Agric. Biol. Chem., 49, 807-815 (1985)
25. S. Nakatsuka, K. Ueda, T. Goto, M.Yamamoto, S. Nishimura and K. Kohmoto, Terahedron Lett., 27, 2753-2756 (1986)
26. H. Irie, J. Igarashi, K. Matsumoto, Y. Yanagawa, T. Nakashima, T. Ueno and H. Fukami, Chem. Pharm. Bull., 33, 1313-1315 (1985)
27. H. Irie, K. Matsumoto, T. Kitagawa, Y. Zhang, T. Ueno, T. Nakashima and H. Fukami, Chem. Pharm. Bull., 35, 2598-2601 (1987)
28. K. Ando, T. Yamada, Y. Takaishi and M. Shibuya, Heterocycles, 29, 1023-1027 (1989)
29. L. Crombie, M.A. Horsham and S.R. Jarrett, Terahedron Lett., 30, 4299-4032 (1989)
30. L. Crombie, S.R. and Jarrett, Terahedron Lett., 30, 4303-4306 (1989)
31. S. Mayama, T. Tani, T. Ueno, K. Hirabayashi, T. Nakashima, H. Fukami, Y. Mizuno and H. Irie, Terahedron Lett., 22, 2103-2106 (1981)
32. S. Mayama, T. Tani, Y. Matsuura, T. Ueno, K. Hirabayashi, H. Fukami, Y. Mizuno and H. Irie, Nippon Kagaku Zasshi, 697-704 (1981) (in Japanese)
33. S. Mayama, T. Tani, Y. Matsuura, T. Ueno and H. Fukami, Physiol. Plant Pathol., 19, 217-226 (1981)