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THE FATE OF THE PHYTOALEXIN IPOMEAMARONE: FURANOTERPENES AND BUTENOLIDES FROM *CERATOCYSTIS FIMBRIATA*-INFECTED SWEET POTATOES

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Key Word Index—*Ipomoea batatas*; Convolvulaceae; sweet potato; *Ceratocystis fimbriata*; furanoterpene; ipomeamarone; butenolide; phytoalexin; stress metabolite; biosynthesis.

Abstract—Nine new sesquiterpenes related biosynthetically to ipomeamarone, the well-known sweet potato phytoalexin, have been isolated from *Ceratocystis fimbriata*-infected sweet potato root tissue and the structures determined. Their biosynthetic relationship with previously identified furanoterpenes is discussed.

INTRODUCTION

When infected with *Ceratocystis fimbriata* Ell. et Halst. and some other pathogenic fungi, the root tissue of the sweet potato *Ipomoea batatas* Lam. accumulates various kinds of sesquiterpenes such as ipomeamarone (**15**) [1–3] in the infected region. Treatment with $HgCl_2$ also induces the accumulation of sesquiterpenes in the injured region [4]. Since the finding and chemical characterization of **15** [1, 2, 4] more than ten sesquiterpenes have been isolated and characterized from fungal-infected or $HgCl_2$-injured tissue. The compounds previously isolated from *C. fimbriata*-infected tissue are **15**, ipomeamaronol (**22**) [5, 6], dehydroipomeamarone (**11**) [7], 7-hydroxymyoporone (**30**) [8], 4-hydroxymyoporone (**17**) [9], 4-hydroxydehydromyoporone (**14**) [10], 6-oxodendrolasin (**7**) [11], which is the same as component A_2 [12, 13], and component A_1 (**12**) [12, 13], which is now called ipomeabisfuran (see Scheme 1). Interestingly, $HgCl_2$-injured tissue has given rise to several other sesquiterpenes, namely 9-hydroxy farnesol (**2**) [11], 9-oxofarnesol (**5**) [11], 6-hydroxydendrolasin (**25**) [11], 6-myoporol (**26**) [14], 6-dihydro-7-hydroxymyoporone (**27**)‡ [15], 1-myoporol (**28**) [14], and myoporone (**29**) [14]. Possibly these stress metabolites are also present in *C. fimbriata*-infected tissue. Indeed, during the course of this work we have been able to isolate both **2** and **29** from *C. fimbriata* infected tissue. Finally, degraded sesquiterpenes such as ipomeanine (**18**) [9, 16, 17], 4-ipomeanol (**19**) [9, 17], 1-ipomeanol (**20**) [9] and 1,4-ipomeadiol (**21**) [9], are obtained from mould-damaged sweet potatoes as a result of the metabolism of such pathogens as *Fusarium solani* (Scheme 1).

It was shown that not only the crude extract from mouldy sweet potatoes, but also pure **15** were inhibitory to spore germination and growth of *C. fimbriata* [1, 18]. Furthermore, all spots showing positive reaction to Ehrlich's reagent on silica gel TLC plates were more or less antibiotic to the fungus [19], and hence these metabolites can be regarded as phytoalexins. A possible biogenesis of **15** from *trans*, *trans*-farnesol (**1**) has been proposed [11, 13, 20]§; however, a more detailed analysis of the biogenesis and biodecomposition of **15** would clearly contribute towards clarifying the poorly understood roles that the phytoalexins play in plant physiology and pathology. We have thus investigated minor stress metabolites present in the crude oily substance including the previously studied components B_1 and B_2 [21]. This has led to the characterization of nine new stress metabolites (denoted by asterisks in Scheme 1) and to a clearer biogenetic scheme.

RESULTS

Detailed investigation of the chloroform extract ('crude oily substance') [5] from *C. fimbriata*-infected sweet potato root tissue has led to the characterization of nine new stress metabolites; 9-hydroxyfarnesoic acid (**3a**), 6-oxodendrolasinolide (**4**), ipomeatetrahydrofuran (**6a**), (*Z*)- and (*E*)-1,6-dioxoisodendrolasin (**9** and **10**), 10-hydroxyipomeabisfuran (**13a**), ipomeamaronolide (**16**), 4-hydroxymyoporonol (**23a**) and 4-hydroxymyoporonol ketal (**24**). Of the five new furanoterpenes **13a** and **23a** were isolated and characterized as the corresponding acetates **13b** and **23b**.

9-Hydroxyfarnesoic acid (**3a**) was isolated from the extract as its methyl ester (**3b**) ($[\alpha]_D$ +2°; IR ν_{max} cm^{-1}: 1720 and 1650; UV λ_{max} nm: 231). The position of the hydroxyl group of **3b** was verified by decoupling experiments (Table 1) as follows: irradiation of the C-9 carbinol proton caused collapse of the δ5.15 resonance to a broad singlet, identifying it as H-10; irradiation at δ5.15 resulted in sharpening of both 11-methyl groups, while irradiation of the other unconjugated vinyl proton (H-6) sharpened

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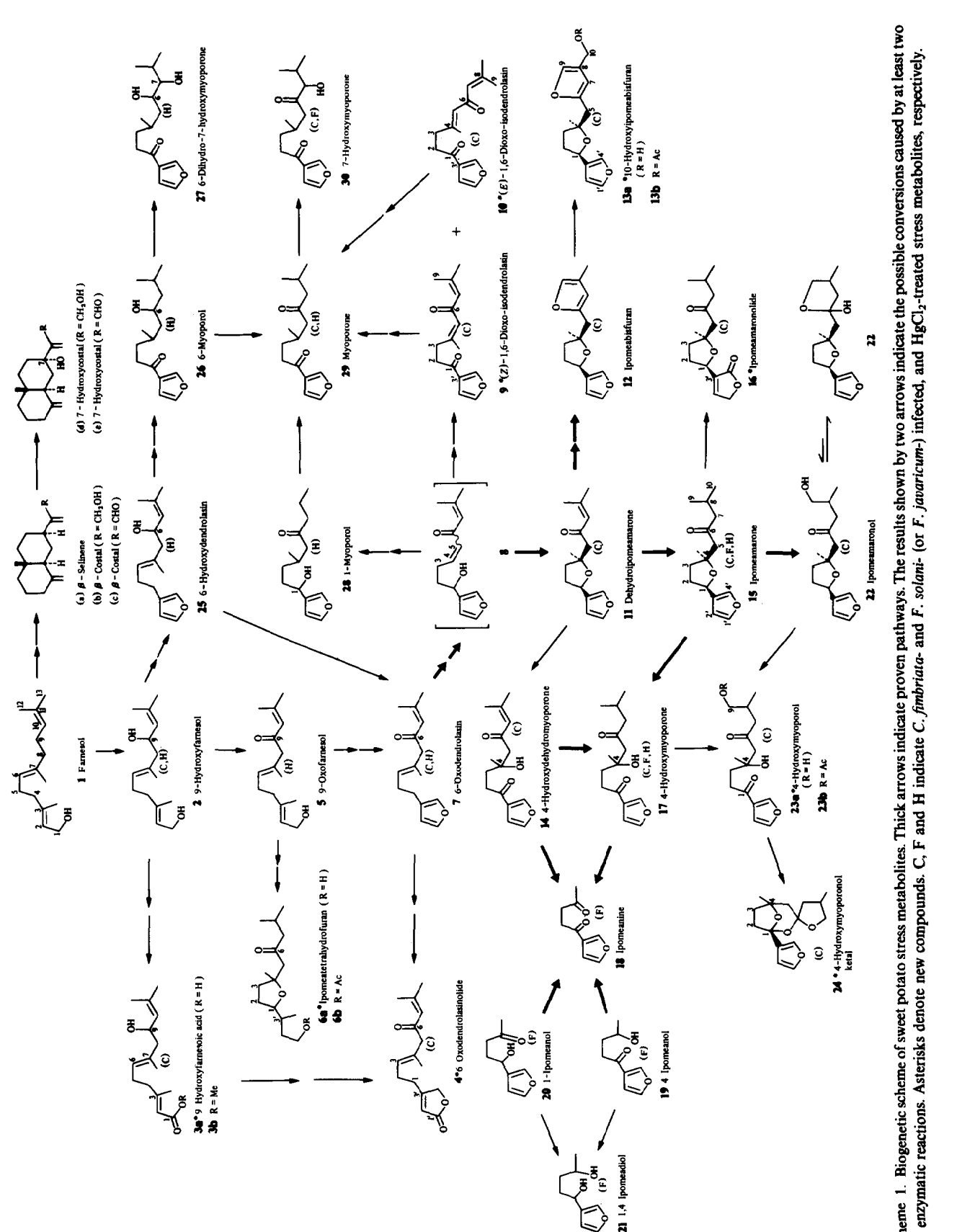
‡Since **27** and **30** are reportedly [15] enantiomeric at C-7 they are presumably not precursors of each other.

§The biogenesis of (−)-ngaione, the enantiomer of **15** and a normal constituent of *Myoporum deserti*, has also been discussed [Sutherland, M. D. and Park, R. J. (1969) in *Terpenoids in Plants* (Pridham, J. B., ed.) p. 147. Academic Press, London.]



Scheme 1. Biogenetic scheme of sweet potato stress metabolites. Thick arrows indicate proven pathways. The results shown by two arrows indicate the possible conversions caused by at least two enzymatic reactions. Asterisks denote new compounds. C, F and H indicate *C. fimbriata*- and *F. solani*- (or *F. javaricum*-) infected, and $HgCl_2$-treated stress metabolites, respectively.

Table 1. ^{1}H NMR data for **3b**, **4** and **6b**

H		**3b**	**4**	**6b**
1′	(1)†	—	—	4.20 m
2′	(2)	5.62 *s(br)*	5.85 *s(br)*	1.63 *m*
3′	(3)	—		1.37 *m*
4′	(3-Me)	2.16 *d* J = 1.5 Hz	4.73 *d* J = 2 Hz	0.94 *d*
1	(4)	2.19 *m*	2.49 *t(br)* J = 6.5 Hz	3.77 *dt* J = 6, 5.5 Hz
2	(5)	2.19 *m*	2.37 *q(br)* J = 6.5 Hz	1.92 *m**
3	(6)	5.20 *t(br)* J = 6 Hz	5.20 *t(br)* J = 6.5 Hz	1.78 *m**
5	(8)	2.19 *m*	3.06 *s(br)*	2.54 2.66 J_{AB} = 15 Hz
6	(9)	4.43 *t(br)* J = 7,6 Hz	—	—
7	(10)	5.15 *t(br)* J = 7,1.5 Hz	6.07 *s(br)*	2.36 *d* J = 7 Hz
8	(11)	—	—	2.11 *m*
9	(12)	1.68 *d* J = 1.0 Hz	2.14 *d* J = 0.7 Hz	0.91 *d* J = 7 Hz
10	(13)	1.72 *d* J = 1.0 Hz	1.88 *d* J = 0.7 Hz	0.91 *d* J = 7 Hz
4-Me	(7-Me)	1.66 *s(br)*	1.64 *s(br)*	1.23 *s*
OAc		—	—	2.04 *s*
OMe		3.68 *s*	—	—

*Assignments may be interchanged.

†The farnesol numbering system (shown in parentheses) is used for methyl 9-hydroxyfarnesoate (**3b**).

only one vinyl methyl group (Me-7). Hence, the secondary alcohol must be at C-9. In the mass spectrum, the $[M]^+$ was not apparent, but an $[M - H_2O]^+$ peak was observed at m/z 248. The base peak at m/z 114 was assigned to the 6-methyl crotonate cation radical. Diol **2**, which could be a common precursor to **3a**, **4** and **7**, has been isolated from both the *C. fimbriata* infected sweet potatoes (this study) as well as $HgCl_2$-treated sweet potatoes [10].

6-Oxodendrolasinolide (**4**), one of two new butenolides isolated from the extract, gave m/z 248 $[M]^+$ corresponding to $C_{15}H_{20}O_3$. Presence of the enone moiety was apparent from the UV absorption at 244 nm and IR bands at 1685 and 1620 cm^{-1}. The split carbonyl band at 1780 and 1750 cm^{-1} together with the 1635 cm^{-1} absorption is characteristic of an α,β-unsaturated γ-lactone bearing an α-proton [22]. The simplicity of the ^{1}H NMR spectrum (Table 1) i.e. three vinyl methyl groups at δ1.64, 1.88 and 2.14; two allylic methylene groups at δ2.49 [*t* (*br*), H-1] and 2.37 [*q* (*br*), H-2] and two allylically coupled methylene resonances at δ3.06 (H-5) and δ4.73 (H-4′), unequivocally led to structure **4**. Butenolide **4** may have arisen via enzymatic or nonenzymatic oxygen-dependent oxidation of **7**. Alternatively, it is possible that this substance is derived from **3a**.

Ipomeatetrahydrofuran (**6a**) was isolated and characterized as the acetate **6b** [m/z 298 ($C_{17}H_{30}O_4$); IR (neat): 1740 (acetate), 1710 cm^{-1} (ketone)]. As in ipomeamaronolide (**16**), a tetrahydrofuran ring and two methylene groups (C-5 and C-7) next to a carbonyl group were inferred from the ^{1}H NMR data (Table 1). In addition to these resonances and the isopropyl group, the NMR showed signals at δ0.94 (Me-3′, *d*), 1.37 (H-3′), 1.63 (H-2′) and 4.20 (–CH_2OAc). The data are readily accommodated by structure **6b**. Due to the lack of unsaturation of 2′/3′, this sesquiterpene may not play a direct role in the biosynthesis of **15**.

(*Z*) and (*E*)-1,6-dioxoisodendrolasins (**9** and **10**) exhibited similar mass (m/z 246, $C_{15}H_{18}O_3$) and IR (1675 and 1625 cm^{-1}) spectra. ^{1}H NMR analysis (Table 2) revealed a 3′-substituted furan conjugated with a carbonyl group (**9**: δ8.23, 7.39 and 6.79; **10**: δ8.02, 7.39 and 6.78). The C-2/C-3 ethylene group appeared as an allylically coupled singlet at δ2.82 in **9**. In **10**, these four protons appeared as multiplets centered at δ2.58 and δ2.86. Both isomers displayed broad two-proton singlets at δ6.02 for the C-5 and C-7 vinyl hydrogens. As anticipated, the (*E*)-isomer **10** had downfield shifted Me-4 and Me-8 singlets at δ2.07 and δ2.09 due to the deshielding effect of the carbonyl group. The (*Z*)-isomer **9**, on the other hand, displayed only one deshielded vinyl methyl group (δ2.07, Me-8).

10-Hydroxyipomeabisfuran (**13a**) was characterized as the acetate **13b** which exhibited IR frequencies character-

Table 2. ^{1}H NMR data for **9**, **10** and **13b**

H	**9**	**10**	**13b**
1′	8.23 *s(br)*	8.02 *s(br)*	7.37 *s(br)*
2′	6.79 *s(br)*	6.78 *s(br)*	6.32 *t* J = 1.5 Hz
4′	7.39 *s(br)*	7.39 *s(br)*	7.37 *s(br)*
1	—	—	4.92 *t* J = 7.5 Hz
2	2.82 *s(br)*	2.58 *m*	2.15 *m* 1.75 *m*
3	2.82 *s(br)*	2.86 *m*	2.15 *m* 1.75 *m*
5	6.02 *s(br)*	6.02 *s(br)*	2.83 2.87 J_{AB} = 14 Hz
7	6.02 *s(br)*	6.02 *s(br)*	6.15 *s(br)*
9	2.07 *d* J = 1 Hz	2.09* *d* J = 1 Hz	7.37 *s(br)*
10	1.83* *d* J = 1 Hz	1.85 *d* J = 1 Hz	4.93 *s*
4-Me	1.85* *d* J = 1 Hz	2.07* *d* J = 1 Hz	1.30 *s*
–OAc			2.07 *s*

*Assignments may be interchanged.

istic of a furan moiety at 1555, 1505 and 880 cm^{-1}. The ^1H NMR spectrum (Table 2) revealed five furanoid protons (δ7.37, 3H; 6.32, 1H; 6.15, 1H). An intact tetrahydrofuran moiety was characterized by a broad triplet at δ4.92 (H-1), multiplets at δ2.15 and 1.75 (H-2 and H-3), as well as a methyl singlet at δ1.30 (Me-4). In addition to the resonances associated with the primary acetoxyl group (δ2.07, OAc; δ4.93, H-10), an AB quartet (δ2.85, H-5) that was allylically coupled to the H-7 peak at δ6.15 was recognized. The mass spectrum showed a molecular ion at *m/z* 304 and a base peak at *m/z* 151, corresponding to the moiety **13**. The derived structure corresponds to the hydroxylated analogue of previously isolated ipomeabisfuran **12** [11, 12].

Ipomeamaronolide (**16**), $[\alpha]_D$ +31°, exhibited only one lactone band in the IR spectrum (1760 cm^{-1}), thus indicating α-substitution [22]. In addition, an absorption at 1710 cm^{-1} implied a saturated ketone. The molecular formula $C_{15}H_{22}O_4$ was determined from the mass spectrum (*m/z* 266). As in the case of **13b** the ^1H NMR spectrum (Table 3) was in accordance with the intact tetrahydrofuran moiety. Two methylene groups adjacent to the ketone were present at δ2.72/2.62 (H-5) and 2.30 (H-7). The isopropyl group was evident from resonances at δ2.10 (H-8) and two methyl doublets centered at δ0.87 and 0.89 (Me-8's). It is likely that **16** is generated by enzymatic or non-enzymatic oxidation of **15**. Interestingly, it appears to be a more potent anti-fungal agent than **15** in incubation experiments [19] with *C. fimbriata*. Together with **3a**, **4** and **13a**, ipomeamaronolide **16** was found in the B_2 containing region [21] of the crude oily substance.

4-Hydroxymyoporonol (**23a**) proved to be the 9-hydroxylated derivative of the known 4-hydroxymyoporone (**17**) [9]. The IR spectrum of **23b** exhibited absorptions at 3500 (hydroxyl), 1740 (acetate), 1710 (saturated ketone) and 1680 cm^{-1} (conjugated enone). The furan group was deduced from additional bands at 3130, 1560, 1505 and 870 cm^{-1}. A molecular ion at *m/z* 324 led to a molecular formula of $C_{17}H_{24}O_6$. As in **17**, AB quartet derived patterns were observed for all methylene groups (Table 3). The remaining aliphatic resonances consisted of the Me-4 singlet (δ1.22), an Me-8 doublet (δ0.06) and a multiplet for H-8 (δ2.39).

4-Hydroxymyoporonol ketal (**24**) (*m/z* 264, $C_{15}H_{20}O_4$) showed no hydroxyl or carbonyl stretching in the IR spectrum; hence all four oxygens are involved in ether linkages. Since ^1H NMR analysis (Table 3) revealed the presence of a furan ring (δ7.43, 7.32 and 6.41) and no vinyl protons, the remaining three unsaturations and three oxygens had to be accommodated by cyclic ethers. This requirement was satisfied by the spiro ketal moiety in **24**. This structure assignment is fully consistent with detailed decoupling studies carried out at 360 MHz. Simple dehydration of **23a** would account for the formation of **24**.

DISCUSSION

Since 1952 when Kubota and Matsuura determined the chemical structure of **15** [2], the first phytoalexin found in the plant kingdom [1], more than ten furanoterpenes have been isolated and characterized from *C. fimbriata*-infected and $HgCl_2$-treated sweet potato root tissues. *In vivo* and *in vitro* experiments in the *C. fimbriata* sweet potato system [12, 20] indicated that **15** was the most abundant metabolite, and that the others were related to **15** either as biosynthetic precursors or as further conversion products [12, 20]. Biosynthetic studies using $HgCl_2$-treated tissue have also been performed [11, 23] and these have led to the proposal of biogenetic pathways [11, 12, 20, 23]. However, details of the pathway have not yet been elucidated. One of the most important approaches in solving the subject is to clarify the unknown intermediates in the biosynthetic and biodecomposing pathway of **15**.

We have learned from past experiences that unidentified sesquiterpenes are contained in the crude oily substance. Hence, we have carried out the isolation and

Table 3. ^1H NMR data for **16**, **23b** and **24**

H	16	23b	24
1′	4.75 *d* J = 0.7 Hz	7.44 *dd*	7.43 *s(br)*
2′	6.77 *d* J = 0.7 Hz	6.77 *dd*	6.41 *s(br)*
4′	—	8.08 *dd*	7.32 *s(br)*
1	4.70 *t(br)* J = 8 Hz	—	—
2	2.38 *m*	2.89 *dd* J = 7.5, 6.5 J_{AB} = 16 Hz	2.75 *ddd* J = 12, 10, 5 Hz
	1.85 *m*	2.94 *dd* J = 7.5, 6.5	2.36 *m*
3	2.10 *m*	1.85 *dd* J = 7.5, 6.5 J_{AB} = 14 Hz	2.13 *m*
	1.85 *m*	1.95 *dd* J = 7.5, 6.5	1.76 *ddt* J = 12, 5, 2 Hz
5	2.72 J_{AB} = 15 Hz	2.61 J_{AB} = 16 Hz	1.81 *d* J = 13.5 Hz
	2.62	2.63	2.11 *d* J = 13.5 Hz
7	2.30 *d* J = 7 Hz	2.31 *d* J = 6	2.18 *dd* J = 12.5, 9.5 Hz
		2.55 *d* J = 8 J_{AB} = 17 Hz	1.38 *dd* J = 12.5, 9.5 Hz
8	2.10 *m*	239 *m*	2.57 *m*
9	0.89* *d* J = 6.5 Hz	3.96 *d* J = 6.5 J_{AB} = 11 Hz	4.13 *t* J = 8 Hz
		3.98 *d* J = 6	3.44 *dd* J = 8, 7 Hz
10	0.87* *d* J = 6.5 Hz	0.96 *d* J = 6.5 Hz	1.03 *d* J = 7 Hz
4-Me	1.28 *s*	1.22 *s*	1.39 *s*
OAc	—	2.05 *s*	

*Assignments may be interchanged.

identification of minor stress metabolites found in the extract from *C. fimbriata*-infected tissue. This has led to the identification of nine new sesquiterpenes with structures related to **15**, as well as other previously characterized stress metabolites found in *C. fimbriata*-infected or $HgCl_2$-treated tissue. Here, we should like to present a modified pathway incorporating past data and the present results. As shown in Scheme 1, most of the newly isolated sesquiterpenes are positioned rather near the terminals of the pathway, which constitutes the network system [20]. During the course of the experiments, Schneider and Nakanishi [24] have found several sesquiterpenes of the selinene class in the crude oily substance. The selinene type sesquiterpenes (a–e, Scheme 1) represent a new class of potent phytoalexins or stress metabolites of the sweet potato; it should be noted that the eudesmane skeleton follows a biosynthetic scheme different from that of the furanoterpenoids.

The two furanoterpenes **13a** and **23a** with primary alcohol functions are presumably produced in the tissue by oxygenation of the terminal methyl groups of **12** and **17**, respectively. This hydroxylation is not unexpected in view of the fact that **15** is converted to ipomeamaronol **22** (which was found to be mainly in the hemiketal form **22'** which displayed one pair of diasteromeric methyl doublets in the ^1H NMR) by ipomeamarone 9-hydroxylase involving cytochrome P450 localized in the microsomal membrane in the infected tissue [25, 26]. Recently it has been shown that all three oxygens of **15** are derived from molecular oxygen [27].

The two butenolides **4** and **16** appear to be intermediates in the biodecomposing pathways of **7** and **15**, respectively (Scheme 1). In response to infection, both biosynthetic and biodecomposing pathways are induced in the non-infected tissue adjacent to the infected region. Thus **15** and the other furanoterpenes are decomposed to some extent in the tissue [28]. Since the mechanism of enzymatic decomposition of the furan ring in the furanoterpenes is not known, the finding of butenolides **4** and **16** is informative in elucidating the biochemical basis of furan ring biodegradation. There is a possibility, however, that the butenolides may have been formed non-enzymatically during the course of extraction and fractionation. Butenolide **4** may also have been produced by enzymatic oxidation of **3a** (Scheme 1). As indicated in Scheme 1, **3a** and **6a** may be produced from **2** via bypaths rather than being involved in the biosynthesis of **15**. Ketal **24** represents a new terminus in one of the decomposing pathways of **15**.

Diketones **9** and **10** appeared as attractive intermediates in the biosynthesis of **15**, since reduction of the carbonyl group at C-1 would give the hydroxy-enone (**8**) which could undergo facile Michael addition to furnish **15** [11]. This possibility, however, has been excluded on the basis of the mode of 2-d_2-mevalonate incorporation into **15** [29]. Namely, it was shown that oxidation at C-1 involves the removal of only one of the C-1 protons. Furthermore, isomerization of the 3,4-double bond to the conjugated 4,5-position prior to cyclization was rigorously established. In view of these results [29], **8** is the most likely intermediate between **7** and **11**. Dienones **9** and **10** therefore are produced via a bypath and may be precursors to **29**.

Thus a total of 19 furanoterpenes and furanoterpene-related, as well as five selinene-type, stress metabolites (see Scheme 1) have been isolated from *C. fimbriata*-infected sweet potato root tissue, and many of them play a role in the defence reaction to the mycelial penetration. In order to clarify the defence mechanism of plants it would be useful to determine the time course of production of the respective stress metabolites in the tissue as well as their tissue and cellular localizations. The antibiotic activity of each compound to such pathogenic micro-organisms as the *C. fimbriata* sweet potato strain also merits further study.

EXPERIMENTAL

Materials and methods. Sweet potato roots (cv Norin 1) were vertically cut into slices 4 mm thick which were inoculated with a spore suspension of *C. fimbriata* (1×10^7/ml) on the cut surfaces, incubated for 3 days at 30°, then extracted with $CHCl_3$–MeOH (1:1). After adding H_2O to the soln, the $CHCl_3$ layer was evaporated *in vacuo* to a crude oily substance, yielding *ca* 2% [5, 7]. Flash chromatography [30] was carried out with Merck kieselgel 60 (with or without $AgNO_3$ impregnation), and the solvents were evaporated under red. pres. ^1H NMR spectra were recorded at 360 MHz or 100 MHz in $CDCl_3$ with $CHCl_3$ (δ 7.24) as the int. standard. IR spectra were measured neat with the Nicolet Series 7000 FT-IR. UV measurements were performed in MeCN or hexane utilizing a Shimadzu UV-210A spectrophotometer. Mass spectra were run on a Hitachi M-80 at 70 eV. Rotations were measured in CH_2Cl_2.

9-Hydroxyfarnesoic acid (**3a**). The polar fraction obtained after flash chromatography of 15 g infected sweet potato extract was extracted with 0.5 N NaOH. Acidification with satd KH_2PO_4, extraction with EtOAc, and filtration through Na_2SO_4 gave an acidic fraction which was coarsely chromatographed with EtOAc–*n*-hexane (1:1) to give a relatively non-polar acidic fraction. Ethereal CH_2N_2 was added to a THF soln of crude **3a**. Excess reagent was destroyed with AcOH. The mixture of methyl esters was chromatographed with EtOAc–*n*-hexane (1:1) to afford impure **3b**. Pure methyl ester was obtained by $AgNO_3$ chromatography with EtOAc–*n*-hexane (1:9) as the eluant. IR ν_{max} cm^{-1}: 3440, 1720, 1650, 1225 and 1150; MS *m/z*: 248 $[M - H_2O]^+$, 235, 182, 114 (100%) and 85; UV λ_{max}^{MeCN} nm (log ε): 231 (3.34); $[\alpha]_D = +2°$.

6-Oxodendrolasinolide (**4**). A minor fraction of R_f 0.35 in EtOAc–*n*-hexane (1:1) was flash chromatographed on $AgNO_3$ impregnated silica gel with EtOAc–*n*-hexane (1:4). After **13** and **16** were eluted, the column was flushed with EtOAc to give a fraction containing **4**. Chromatography of this fraction with EtOAc–*n*-hexane (3–5:7–5) gave crude butenolide. Final purification was achieved by chromatography with EtOAc–CH_2Cl_2 (3:47), giving **4** (11 mg) as an oil. IR ν_{max} cm^{-1}: 1780, 1750, 1685, 1635, 1620, 1445, 1130, 1035 and 885; MS *m/z*: 248 $[M]^+$, 149, 97, 83 (100%) and 55; UV λ_{max}^{MeCN} nm (log ε): 244 (3.83).

Ipomeatetrahydrofuran (**6a**). The same minor fraction was chromatographed on $AgNO_3$ impregnated silica gel with EtOAc–*n*-hexane (1:4) to give crude **6a**. After acetylation in the usual manner the reaction mixture was applied on a silica gel column and eluted with EtOAc–*n*-hexane (1:4) to afford 3 mg pure **6b**. IR ν_{max} cm^{-1}: 1740, 1710 and 1240; MS *m/z*: 298 $[M]^+$, 285, 255, 213 and 85; $[\alpha]_D$ +1°.

(Z)-1,6-Dioxoisodendrolasin (**9**). Flash chromatography of a non-polar fraction obtained from 12 g crude oily substance gave crude **9**. Purification was accomplished via Lobar chromatography followed by HPLC (EtOAc–*n*-hexane, 3:47). Approximately 4 mg of **9** as a clear colourless oil were obtained. IR ν_{max} cm^{-1}: 3150, 1675, 1625, 1510, and 872; MS *m/z* (rel. int.): 246 $[M]^+$ (17), 163 (60), 151 (28), 136 (87), 121 (25), 109 (55), 95 (100), 83 (64), 55 (20); UV λ_{max}^{hexane} nm (log ε): 261 (4.36), 201 (4.15).

(E)-1,6-*Dioxoisodendrolasin* (**10**). Flash chromatography of crude oily substance with EtOAc–*n*-hexane (1:9), followed by application on a Merck SI 60 Lobar column and eluting with EtOAc–*n*-hexane (3:47). Finally, silica gel HPLC gave 5.4 mg pure **10** from 12 g of crude oily substance. IR ν_{max} cm^{-1}: 3130, 2920, 1675, 1625, 1560 and 872; MS *m/z* (rel. int.): 246 $[M]^+$ (12) 163 (43), 151 (15), 136 (68), 121 (29), 190 (36), 95 (92), 83 (100), 55 (25); UV λ_{max}^{hexane} nm (log ε): 259 (4.60), 204 (4.31).

10-*Hydroxyipomeabisfuran* (**13a**). Chromatography of the fraction containing **4** on $AgNO_3$ impregnated silica gel gave crude **13a**. Acetylation and silica gel chromatography with EtOAc–*n*-hexane (1:4) gave 15 mg pure **13b**. IR ν_{max} cm^{-1}: 1740, 1555, 1505, 1230, 1030 and 880; MS *m/z*: 304 $[M]^+$, 276, 245, 153, 151, 109 and 67; UV λ_{max}^{MeCN} nm (log ε): 237 (3, 18); $[\alpha]_D \sim 0°$.

Ipomeamaronolide (**16**). The same fraction containing **13a** was chromatographed on $AgNO_3$ impregnated silica gel with EtOAc–*n*-hexane (1:4). Butenolide **16** was eluted just after **13a** which contaminated the desired compound. As described, **13a** was effectively removed by acetylation, followed by chromatography to give essentially pure **16**. Highly pure **16** could be obtained by chromatography with EtOAc–*n*-hexane (3:7) and selecting a core cut of the fractions (yield = 35 mg). IR ν_{max} cm^{-1}: 1760, 1710, 1660, 1450, 1365, 1150 and 1080; MS *m/z*: 266 $[M]^+$, 251, 224, 167 (100%), 148 and 85; UV λ_{max}^{MeCN} nm (log ε): 210 (3.73); $[\alpha]_D$ +31°.

4-*Hydroxymyoporonol* (**23a**). The most polar fraction R_f 0.05–0.25 in EtOAc–*n*-hexane (1:1) was chromatographed with EtOAc–*n*-hexane (1:1) to give a fraction containing **23a** and polymeric material, the bulk of which was removed by precipitation from a $CHCl_3$ soln with Me_2CO. The filtrate was rechromatographed with EtOAc–*n*-hexane (7:13) to give crude **23a**. Acetylation followed by silica gel chromatography with EtOAc–*n*-hexane (3:7) gave 21 mg pure **23b**. IR ν_{max} cm^{-1}: 3500, 3130, 1740, 1710, 1680, 1560, 1505, 1240 and 870; MS *m/z*: 324 $[M]^+$, 279, 201, 167 and 95 (100%); UV λ_{max}^{MeCN} nm (log ε): 248 (3.56); $[\alpha]_D$ = +3°.

4-*Hydroxymyoporonol ketal* (**24**). During the purification of **9** and **10** approximately 2.5 mg **24** was obtained. IR ν_{max} cm^{-1}: 2920, 1505, 1455, 1210, 943, 873; MS *m/z* (rel. int.): 264 $[M]^+$ (8), 193 (4), 164 (7), 152 (41), 148 (57), 137 (30), 95 (100), 85 (12).

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