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Total Synthesis and Biological Evaluation of the Tetramic Acid Based Natural Product Harzianic Acid and Its Stereoisomers

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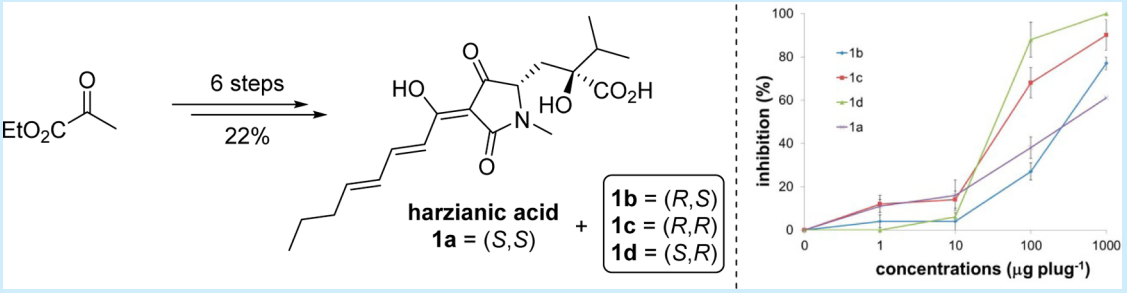
Supporting Information



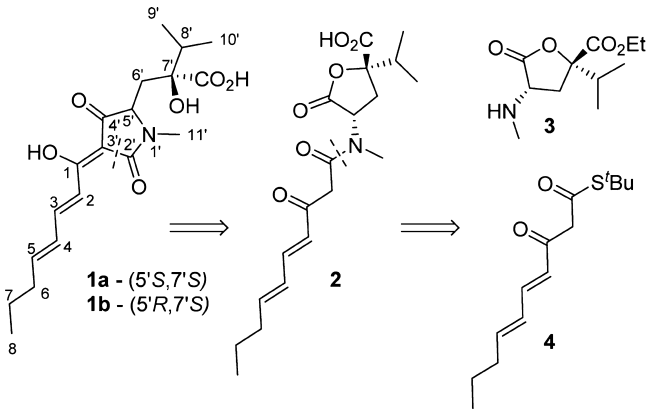
ABSTRACT: The bioactive natural product harzianic acid was prepared for the first time in just six steps (longest linear sequence) with an overall yield of 22%. The identification of conditions to telescope amide bond formation and a Lacey−Dieckmann reaction into one pot proved important. The three stereoisomers of harzianic acid were also prepared, providing material for comparison of their biological activity. While all of the isomers promoted root growth, improved antifungal activity was unexpectedly associated with isomers in the enantiomeric series opposite that of harzianic acid.

Harzianic acid (**1a**) is a member of a subfamily of tetramic acid containing natural products that is defined by the presence of an unnatural 4,4-disubstituted glutamic acid unit. Despite the unique and potent biological activities displayed by members of this subfamily,[1] there has been little synthetic activity until our recently reported total synthesis of JBIR-22.[5]

As a means of exploring further our synthetic approach to members of this subfamily, we next looked to prepare harzianic acid (**1a**), a secondary metabolite isolated from *Trichoderma harzianum*.[2] This filamentous fungus is used as a biopesticide and biofertilizer due to its growth-promoting and antifungal properties, and the production of **1a** by the fungus has been implicated in this biological activity.[3,4] The relative and absolute stereochemistry of **1a** was assigned by Vinale et al. using small-molecule X-ray crystallography (CCDC 745241) after an initial report by Sawa et al.[2,6] More recently, the C5′-isomer of **1a**, referred to as isoharzianic acid (**1b**), has also been isolated from the same fungus.[7]

Here, we report the first total synthesis of **1a** and its three stereoisomers, including isoharzianic acid (**1b**). To the best of our knowledge, no synthetic routes to **1a** and **1b** have been reported to date. All of the stereoisomers were assessed for their antifungal and plant growth-promoting activities. Our synthetic approach used the masked 4,4,-disubstitued glutamic acid **3** as a core fragment that could be combined with the appropriate polyene fragment **4** (Scheme 1). A late-stage

Scheme 1. Retrosynthetic Analysis of Harzianic Acid (1a)



Lacey−Dieckmann condensation of **2** to form the tetramic acid core in **1a** would limit the requirement for protecting groups and remove any issues associated with carrying the tetramic acid core through the synthesis, as this unit is both base labile and a powerful chelator of metals.[8,9] The synthetic route began with the synthesis of the masked 4,4-disubstituted glutamic acid **3** using our recently reported methodology

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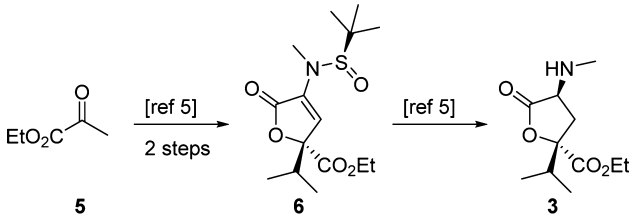
(summarized in Scheme 2).[5] A highly diastereoselective aldol condensation of the *tert*-butanesulfinamide imine derived from

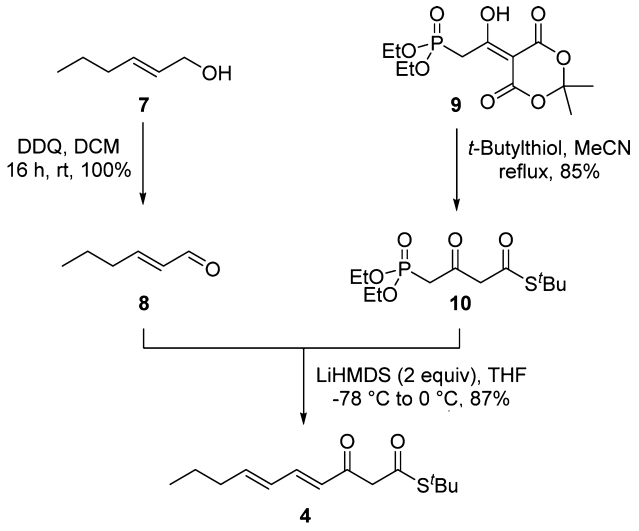
Scheme 2. Synthesis of Masked 4,4-Disubstituted Glutamic Acid Fragment 3[5]



ethyl pyruvate **5** and ethyldimethyl pyruvate followed by a subsequent *N*-methylation gave lactone **6**. A one-pot diastereoselective reduction and cleavage of the *N*-sulfinyl group then provided **3**, the stereochemistry of which was assigned on the basis of X-ray crystallographic analysis of **6**[10] and NOE studies on **3**.[5] This methodology has proved very robust with access to **3** being achieved rapidly on multigram scale.

As **3** was obtained in sufficiently high purity from **6**, it was decided to trap crude **3** directly with the required side chain **4** (Scheme 1) after a simple neutralization step. Assembly of the polyene side chain **4** began with DDQ oxidation of commercially available (*E*)-2-hexenol (**7**) to the corresponding known aldehyde **8** (Scheme 3). In tandem, Meldrum's

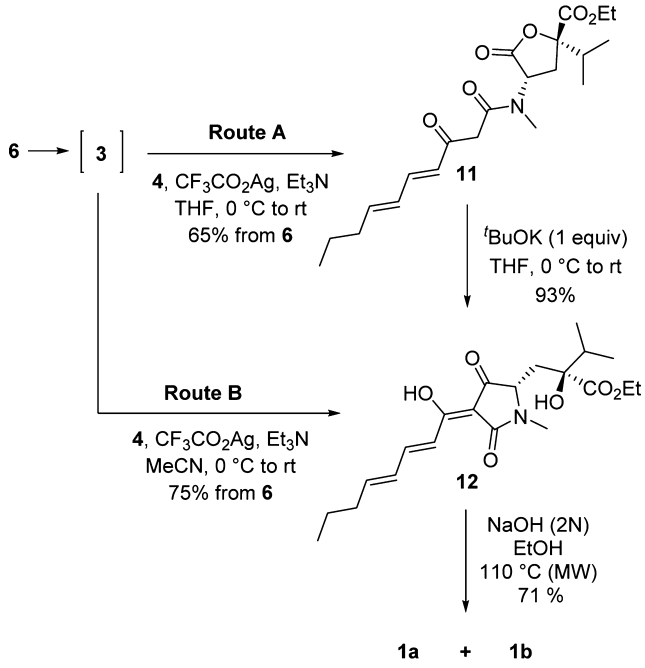
Scheme 3. Synthesis of β-Ketothioester Fragment 4



acid was condensed with diethylphosphonoacetic acid to give **9**, which was refluxed with *tert*-butyl thiol in acetonitrile to yield **10**.[11] Finally, a Horner–Wadsworth–Emmons reaction of aldehyde **8** with the dianion generated from **10** provided the required β-ketothioester **4** in good yield (Scheme 3).[12]

The synthesis of harzianic acid (**1a**) was completed via modification of the silver trifluoroacetate mediated coupling of **4** and crude **3** following the protocol developed by the Ley group.[13,14] This approach initially gave the desired β-keto amide **11** in good yield. Compound **11** was then cyclized using ^{t}BuOK to provide harzianic acid ethyl ester **12** as a single diastereomer (Scheme 4, route A). However, it was also observed that a small quantity (<10%) of **12** was formed

Scheme 4. Total Synthesis of Harzianic Acid (1a) and 5′-Isoharzianic Acid (1b)



during the initial coupling of **3** and **4**. Optimization of this reaction by changing the solvent from THF to acetonitrile resulted in the isolation of harzianic acid ethyl ester **12** in good yield (75% from lactone **6**) in just two steps (Scheme 4, route B). This highly efficient procedure enables the synthesis of harzianic acid ethyl ester **12** from lactone **6** in just 1 day.

Finally, hydrolysis of harzianic acid ethyl ester **12** with aqueous NaOH (2N) using microwave irradiation provided a readily separable 3:1 mixture of harzianic acid (**1a**) and 5′-isoharzianic acid (**1b**), which resulted from partial epimerization at the C5′ position, a common issue observed with tetramic acids.[9,15] Harzianic acid (**1a**) was synthesized in just six steps (longest linear route from ethyl pyruvate) with an overall yield of 22%. The NMR spectral data, HRMS, and the specific rotation obtained for the synthetic harzianic acid (**1a**) were in excellent agreement with the published data for the natural sample (Figure 1A and Table S1, Supporting Information).[2] We next turned our attention to the synthesis of the (*R*,*R*) enantiomer of harzianic acid **1c** and its epimer (*S*,*R*)-5′isoharzianic acid (**1d**). The described synthetic sequence was repeated using (*S*)-*tert*-butanesulfinamide to provide the (*R*,*R*) enantiomer of our key intermediate **3**, which was converted in an analogous manner to provide **1c** and **1d** in high purity and quantities (Scheme S1 and Table S2, Supporting Information).

Harzianic acid (**1a**) and isoharzianic acid (**1b**) have both been reported to display antifungal activity and to increase seed germination and shoot and root growth in canola and tomato seedlings.[6,7,16] The plant growth promoting activity of harzianic acid (**1a**) is believed to be linked to its potent siderophoric properties.[16] Microbial siderophores are iron-chelating agents involved in iron solubilization, which is a crucial mechanism in plant nutrient regulation.[17−19] Consistent with this, harzianic acid (**1a**) has been shown to increase seedling growth even under iron-deficient conditions and also increased iron concentration in the plants.[16] The antifungal and plant growth promoting activities of harzianic

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(A)

no.	(*S*,*S*)-harzianic acid (**1a**)[2] (isolated)	(*S*,*S*)-harzianic acid (**1a**) (synthetic)		(*R*,*S*)-*iso*harzianic acid (**1b**) (synthetic)	
	δc (ppm)[a]	δc (ppm)[b]	Δ (ppm)	δc (ppm)[b]	Δ (ppm)
2′	173.9	173.9	0	176.4	+2.5
3′	100.9	100.9	0	102.3	+1.4
4′	198.9	198.9	0	196.3	-2.6
5′	65.5	65.5	0	66.0	+0.5
6′	36.4	36.4	0	37.0	+0.6

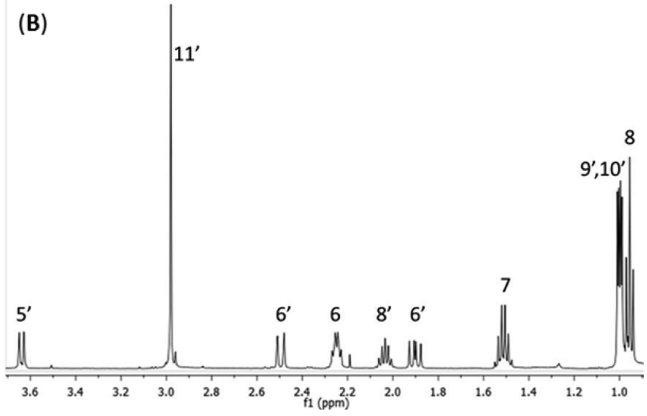

Figure 1. (A) Comparison of selected ^{13}C NMR signals of isolated harzianic acid (**1a**)[2] and synthetic **1a** and **1b** (see Scheme 1 for numbering and Table S1 for a complete comparison of the ^{1}H and ^{13}C NMR spectral data). (a) 400 MHz, CD_3OD; (b) 500 MHz, CD_3OD. (B) Section of the ^{1}H NMR spectrum of synthetic **1a** in $CDCl_3$ (see the Supporting Information for the full spectrum).

acid (**1a**) have highlighted it as a promising bioactive compound which could be used as an alternative to living antagonists. With samples of all four stereoisomers **1a**–**d** in hand, we decided to assess their relative biological activity in a series of assays.

When tested in an assay to assess the effect of the compounds on the root length of tomato seedlings, all of the stereoisomers significantly promoted root length at concentrations above 0.001 mM (Figure S2, Supporting Information). As it seems likely that **1a** and **1b** can interconvert under typical assay conditions (hence complicating interpretation[9,15]), results which demonstrate significant differences in behavior between the two enantiomeric series were also sought. In this context, assessment of the activity of stereoisomers **1a**–**d** against the pathogens *Sclerotinium rolfsii* and *Pythium ultimum* showed that while all the stereoisomers were able to inhibit the pathogens, the two isomers in the enantiomeric series *R*,*R*-**1c** and *S*,*R*-**1d** were significantly more active than *S*,*S*-**1a** and *R*,*S*-**1b** (Figure 2).

In summary, we have completed the first total synthesis of harzianic acid **1a** and its stereoisomers **1b**–**d** via a short, stereoselective route involving the key masked 4,4-disubstituted glutamic acid **3**. Variation of the aldehyde **8** and the pyruvate starting material would facilitate the synthesis of a wide range of analogues through this convergent route. The antifungal activity of the two isomers in the enantiomeric series (*R*,*R*-**1c** and *S*,*R*-**1d**) were significantly more active than the natural harzianic acid (**1a**) and isoharzianic acid (**1b**).

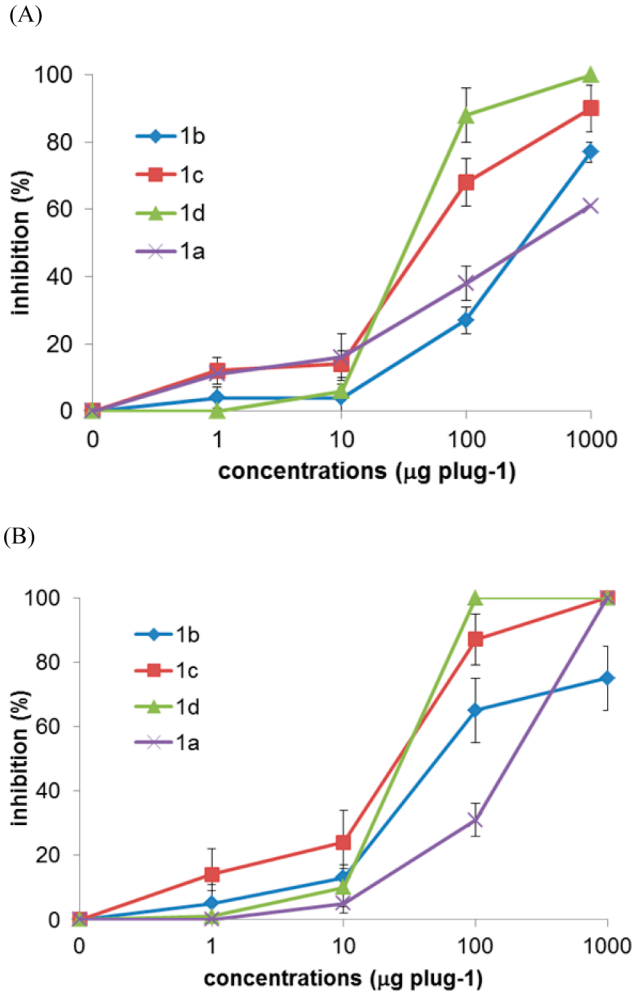

Figure 2. (A) Antibiotic activity of **1a**–**d** against *S. rolfsii* and (B) *P. ultimum*. Concentrations ranged from 1 to 1000 μg plug^{-1}.

■ ASSOCIATED CONTENT

Supporting Information

Detailed chemical and biological experimental procedures, spectroscopic data, and copies of ^{1}H and ^{13}C NMR spectra for all new compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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(10) Deposition no. CCDC-1034467 for compound **6**; see ref 5.

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