

## Notes

ARABENOIC ACID, A NATURAL PRODUCT  
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In the course of screening for herbicides produced by microorganisms, we have isolated an  $\alpha,\beta$ -unsaturated pentenoic acid, arabenoic acid (**1**), which inhibits the growth of *Arabidopsis thaliana*, from the cultured broth of the unclassified fungus F6286.<sup>1)</sup> This report describes the production, isolation, physico-chemical properties, structure, and herbicidal activity of this novel natural product.

The fungal strain F6286 was isolated from a soil sample collected at LaRue Swamp, Illinois, U.S.A. Two 250-ml Erlenmeyer flasks each containing 50 ml of medium consisting of CZAPEK-Dox 3.5% and yeast extract 0.2% were inoculated and fermented at 30°C for 3 days on a rotary shaker at 250 rpm. Fifty ml of this inoculum broth was transferred to each of two 2-liter Erlenmeyer flasks containing 500 ml of production medium consisting of corn-starch 1.0%, glucose 0.25%, baker's yeast 0.5%, soybean flour 0.25%, KH<sub>2</sub>PO<sub>4</sub> 0.05%, and Amberlite XAD-7 2% (pH 6.5). The fermentation was carried out on a rotary shaker at 30°C and 250 rpm for 7 days. The herbicidal activity of the resulting broth was assayed by an agar diffusion method using *A. thaliana* as the test organism.

The herbicidal metabolite produced by F6286 was extracted from 700 ml of sterile culture filtrate by adsorption onto 10 g of neutral decolorizing carbon. Subsequent elution with acetone gave 0.28 g of crude, active material which was further purified by RP C18 flash chromatography using a stepwise gradient of pH 3 water (acidified with conc HCl) and methanol mixtures. The 3:1 pH 3 water-methanol eluate containing the majority of the

herbicidal activity was evaporated under reduced pressure to give 0.12 g of crude arabenoic acid. This material was further purified by Sephadex LH-20 chromatography with water-methanol (3:1) elution to yield 0.064 g of pure arabenoic acid.

Arabenoic acid is readily soluble in water, methanol, and acetone. The molecular formula was established as C<sub>6</sub>H<sub>10</sub>O<sub>4</sub> by HRFAB-MS ((M-H)<sup>-</sup> *m/z* 145.0501, Calcd: 145.0501). The UV spectrum of **1** in water showed an absorption maximum at  $\lambda_{\max}$  nm 230 ( $\epsilon$  38,832), consistent with the presence of an  $\alpha,\beta$ -unsaturated acid bearing one polar substituent. In addition, the IR spectrum (neat) displayed bands at 3200 (br), 1540 and 1401 cm<sup>-1</sup> indicative of a carboxylate salt, and at 1640 and 848 cm<sup>-1</sup> indicative of a trisubstituted double bond. The existence of this structural feature, accounting for all of the unsaturation in the molecule, was further confirmed by the observation of a one proton singlet at  $\delta$  5.08 in the 300 MHz <sup>1</sup>H NMR spectrum of **1** in D<sub>2</sub>O. Two deshielded methylene groups were observed at  $\delta$  3.65 (2H, t, *J* = 6.3 Hz) and  $\delta$  2.78 (2H, t, *J* = 6.3 Hz) and the presence of a methoxy group was suggested by a three proton singlet at  $\delta$  3.50, thus accounting for all of the carbon atoms of compound **1**. The two unobserved protons were attributed to carboxylic acid and hydroxy groups. All six carbons were observed in the 75 MHz <sup>13</sup>C NMR spectrum of **1** in D<sub>2</sub>O ( $\delta$  179.07, 169.16, 100.61, 62.10, 57.36, and 36.21).

The <sup>13</sup>C chemical shift of the quaternary olefinic carbon ( $\delta$  169.16) indicated that this atom was bonded directly to a methoxy or hydroxy group and was in the  $\beta$  position of the  $\alpha,\beta$ -unsaturated acid moiety. Signals observed in the <sup>1</sup>H and <sup>13</sup>C NMR spectra at  $\delta$  5.08 and  $\delta$  100.61, respectively, were consistent with the assignment of the  $\alpha$ -carbon bearing the lone olefinic proton. The coupled <sup>1</sup>H NMR signals observed at  $\delta$  3.65 and  $\delta$  2.78 were indicative of a CH<sub>2</sub>CH<sub>2</sub>OCH<sub>3</sub> or CH<sub>2</sub>CH<sub>2</sub>OH unit which must in turn be attached to the  $\beta$ -carbon of the double bond.

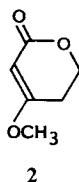
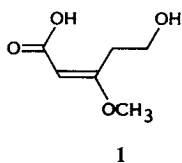
The remaining unresolved structural features of this molecule included the location of the methyl group and the geometry of the double bond. Utilizing NOE difference spectroscopy, a 40%

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enhancement was observed in the intensity of the olefinic proton resonance upon saturation of the methyl resonance. Consequently, the methoxy group must be placed on the double bond, *cis* to the olefinic proton. Thus, the structure of arabeoic acid was determined to be (*E*)-5-hydroxy-3-methoxy-2-pentenoic acid (**1**). An agar-based titration assay employing *A. thaliana* showed that treatment with **1** resulted in growth reduction at 50  $\mu\text{g}/\text{ml}$ , while higher rates resulted in complete inhibition of germination. When evaluated in post-emergence tests, arabeoic acid was active against velvetleaf, morning glory, cocklebur, and Indian mustard at 11.2 kg/ha.

Confirmation of the derived structure was provided by diazomethane derivatization. Under acidic conditions, arabeoic acid reacted with diazomethane to form the known *Penicillium italicum* mycotoxin, 5,6-dihydro-4-methoxy-2*H*-pyran-2-one (**2**), as identified by comparison of spectral data.<sup>2)</sup> The lactone **2** was inactive against *A. thaliana* at 50  $\mu\text{g}/\text{ml}$ .

The existence of the cyclic analog of arabeoic acid as a known natural product raised the question as to whether the free acid was a natural product or an artifact of the isolation procedure. The purification step most likely to cause ring opening was the pH 3 water-methanol RP C18 flash chromatography. In order to evaluate the stability of the lactone under these conditions, the diazomethane reaction product was dissolved in pH 3 water-methanol (3:1) for 2 hours. The <sup>1</sup>H NMR



spectrum of the resulting neutralized sample contained signals for the lactone only. Arabeoic acid could not be detected in this experiment. A later isolation of arabeoic acid from a separate fermentation of F6286 employed an alternative purification procedure (carbon, RP C18 (neutral water elution), Sephadex LH-20). Only compound **1** was obtained from this procedure thereby confirming that arabeoic acid was not an isolation artifact.

As part of an ongoing program to discover natural product herbicides from microorganisms, the novel pentenoic acid derivative, arabeoic acid (**1**), was identified as the herbicidal component produced by the unclassified fungus F6286. Although the relatively weak post-emergence whole plant activity exhibited by arabeoic acid precludes synthetic follow-up, the isolation of this compound from a fungal source nevertheless provides further demonstration of the value of microorganisms as a source of novel structures with agriculturally relevant activities.

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#### References

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