From Forest Nursery Notes, Winter 2010

127. © Identification of alkyl substituted 2H-Furo[2,3-c]pyran-2-ones as germination stimulants present in smoke. Flematti, G. R., Ghisalberti, E. L., Dixon, K. W., and Trengove, R. D. Journal of Agricultural and Food Chemistry 57:9475-9480. 2009.

JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY
ARTICLE

J. Agric. Food Chem. 2009, 57, 9475–9480 9475
DOI:10.1021/jf9028128

Identification of Alkyl Substituted 2*H*-Furo[2,3-*c*]pyran-2-ones as Germination Stimulants Present in Smoke

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The butenolide, 3-methyl-2*H*-furo[2,3-*c*]pyran-2-one (**1**), is a major compound in smoke responsible for promoting the seed germination of a wide range of plant species. We now report the structure of five alkyl substituted variants of **1** that are also present in smoke. The concentrations of these analogues, as well as that of **1**, in a typical smoke−water solution have been determined using high-performance liquid chromatography (HPLC) purification followed by gas chromatography−mass spectrometry (GC-MS) analysis. The analogue, 3,5-dimethyl-2*H*-furo[2,3-*c*]pyran-2-one (**3**), was identified at levels that indicate that it is a contributor to the overall germination-promoting activity of crude smoke extracts.

KEYWORDS: Butenolide; karrikinolide; karrikin; seed germination; seed dormancy; smoke; germination stimulant

INTRODUCTION

The butenolide, 3-methyl-2*H*-furo[2,3-*c*]pyran-2-one (**1**) has been identified as the compound in cellulose- and plant-derived smoke responsible for promoting seed germination (*1*, *2*). The germination-promoting activity of **1** was demonstrated with the key indicator species *Lactuca sativa* L. cv. Grand Rapids (Asteraceae) as well as a broader range of well-known smoke-responding species from Australia, South Africa, and North America. Subsequent studies have confirmed **1** as a potent germination cue for many species including various weed and crop species (*3*−*9*). While **1** has been established as the major germination stimulant present in smoke (*10*, *11*), it is not known whether smoke contains additional compounds that also promote seed germination.

Smoke is known to contain a complex array of volatile compounds with over 4400 compounds known to be present in cigarette smoke alone (*12*). At least a similar number of compounds would be expected to be derived from the burning of plant material. Given this complexity, it is feasible that more than one compound may contribute to the germination-promoting activity of all smoke-responsive species. Other researchers have previously reported that smoke contains more than one germination-promoting compound. A study by Baldwin et al. (*13*) suggested that at least three compounds in smoke were responsible for promoting the germination of *Nicotiana attenuata* Torr. ex Wats. (Solanaceae). Van Staden et al. (*14*) also showed that at least two different fractions of plant-derived smoke were active toward Grand Rapids lettuce seed (*L. sativa*). During our own work that led to the isolation of **1** using bioassay-guided fractionation (*1*), the presence of other germination-promoting compounds similar to **1** also became evident.

A number of synthetic analogues of **1** have been prepared in our laboratory, and most of these show germination-promoting activity (*15*). We therefore investigated whether any of these analogues were present in plant-derived smoke. We now report that at least five analogues of **1** are present in smoke and that some of these are likely to contribute to the overall germination-promoting activity of smoke extracts. A rationale for the formation of **1** and these naturally occurring analogues from cellulose-derived pyrolysis products is also given.

MATERIALS AND METHODS

General Experimental Procedures. High-performance liquid chromatography (HPLC) was conducted using a Hewlett-Packard 1050 HPLC system equipped with a multiple wavelength detector (MWD). Gas chromatography−mass spectrometry (GC-MS) was performed using a Shimadzu GCMS-QP2010 instrument operating in the electron impact (EI, 70 eV) mode. Analogues **2**−**7** were prepared as previously described (*15*).

Extraction of Smoke−Water (SW). Smoke−water (SW) was prepared from burning straw as previously described (*5*). SW (1 L) was extracted with dichloromethane (3 × 200 mL), and the combined organic extract was washed with 1 M NaOH (3 × 100 mL) to remove the acidic compounds. The resulting organic extract was washed with brine, dried (Na_2SO_4), filtered, and evaporated in vacuo to give the neutral fraction (630 mg).

HPLC Separation of SW Neutral Fraction. Separation was achieved using a 250 × 10 mm i.d., 5 μm, Apollo C_{18} reversed-phase column (Grace- Davison) with a 33 mm × 7 mm guard column of the same material. The column was eluted at 4 mL/min with 10% acetonitrile/water,

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Published on Web 09/28/2009