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New and scalable access to Karrikin (KAR_1) and evaluation of its potential application on corn germination

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Karrikin (KAR1) also named "karrikinolide" was isolated in 2004 from smoke water and identified as a very potent germination stimulant as well as controlling early seedling development for a large variety of plant species. We reported herein an unprecedented and scalable synthesis of Karrikin KAR1 based on an elegant and efficient 6-endo-dig cyclisation. Moreover we evaluated the effect of KAR_1 on corn seeds germination, we carried out additional studies on the uptake of this butenolide into corn seeds and the main soil chemiodynamic properties (i.e. persistence and mobility) were calculated or estimated. Finally we provided a rationalization of the experimental results by building a homology model of corn KAI2, using as template the X-ray of the KAI2 protein from *Arabidopsis thaliana.*

Keywords: Karrikin • KAR1 • corn germination • 6-endo-dig cyclisation • Soil Stability • Strigolactone

Introduction

Karrikin (KAR1) also named "karrikinolide" was isolated in 2004[i] from smoke water and identified as a potent germination stimulant as well as controlling early seedling development for a large variety of plant species (Figure 1)[ii]. As the well-known phytohormones strigolactones,[iii] Karrikins bear a butenolide ring and share similar signaling pathway (Figure 1).[iia] Indeed, whereas D14 is the strigolactone receptor, a very close paralog known as KARRIKIN INSENSITIVE 2 (KAI2) and also named D14-like has been proven to be involved in the Karrikin signaling and both pathways (strigolactone and Karrikin) interact with the same F-box protein named MAX2. Five other karrikin analogs have been so far detected and identified in smoke water named KAR_2-KAR_6 (structure variation based on

their methyl-substitution, Figure 1)[iv] and several synthetic analogs have been prepared and reported in the literature.[v] KAR_1 is by far the major component among the Karrikins contained in smoke water and usuallly the most potent seed germination inducer.[ii] Consequently, we decided to concentrate our study on KAR_1.

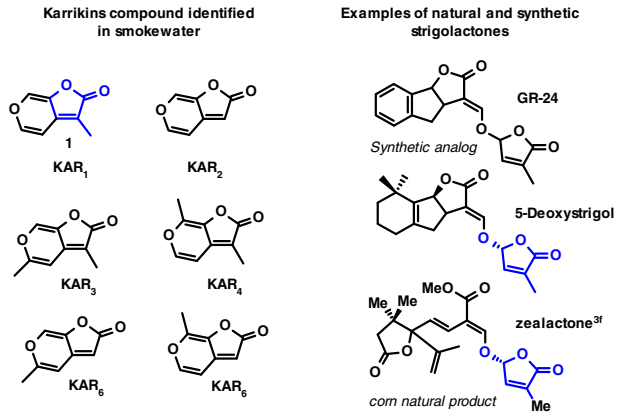


Figure 1. KAR_{1-6} and some strigolactones

Considering the promising biological effects reported in the literature and some chemical structure diversity within the family of Karrikins, we were interested to explore further the potential applications of Karrikins in agriculture for the production of healthier and more vigorous crops through mainly the promotion of germination and early seedling development. We used corn as the most important commercial crop. In fact previous investigations specifically disclosed the promotion effects of KAR_1 on corn.[iie,f]

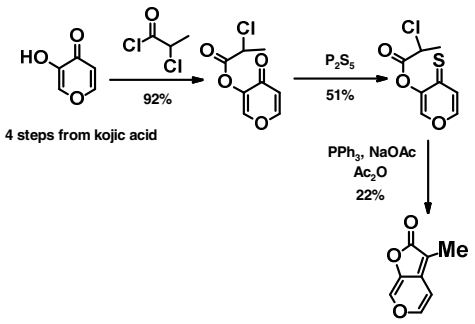
In pratical terms any potential use of Karrikins will be in soil either directly applied (in-furrow or sprayed) or as seed treatment. Thus, KAR_1 needs to be sufficiently bioavailable in soil to be effective under a broad range of agronomical conditions. Soil mobility and soil persistence are two key components to evaluate the bioavailable of chemicals in soil. Previous reports indicated that other butenolides derivatives such as simplified strigolactones or strigolactams were highly instable in soil.[iiic]

Alongside a favorable soil behaviour, Karrikins need to be taken up within the seed by passing across the seed coat to be effective. Despite seed uptake of exogenous chemicals is critical, limited studies attempted to understand what mechanisms are involved in the processes.[vi] Early reports disclosed simple and sufficiently precise methods to study the seed uptake of herbicides in different species.[vii] A similar methodology was used in this study to evaluate the seed uptake of KAR_1.

A careful examination of the literature revealed that no efficient synthesis of such molecule has been reported yet especially on large scale that would be needed for its biological evaluation on crop of interest such as corn. As depicted in Scheme 1, the first approach developed by Flematti *et al*. starts from kojic acid and use a thio-Wittig cyclisation as key steps.[viii] However, the synthesis suffers from low yields making this method not suitable for scale-up and access to large quantities (Scheme 1).

Others reported synthetic routes such as Goddard,[ix] Shindo,[x] Dupont[xi] and Tanabe[xii,xiii] either required multi-steps synthesis (approx. 10 steps)[ix,x] or also suffer from low yielding steps.[xi,xii,xiii]

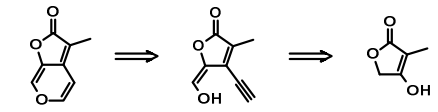
Hence, the main aims of the present study were: i) to present a new efficient and scalable synthesis of KAR_1; ii) to evaluate its biological effect on different commercial hybrids of corn considering different temperature conditions; iii) to provide new information about the soil bioavailablity of KAR_1 by evaluating its mobility and persistence in soil; iv) to compare the corn seed uptake of KAR_1 vs other compounds at two temperatures. Finally we studied the molecular interactions of KAR1 occuring at the receptor level by using an homology model of KAI2 in corn.



Scheme 1. Flematti's approach to KAR1

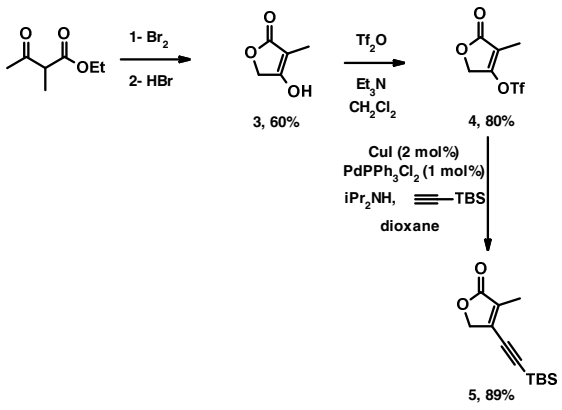
Results and Discussion

We started to investigate a new synthesis of KAR_1. In order to have a more reliable and efficient access, our retrosynthetic analysis of KAR_1 (**1**) would involve a key 6-endo-dig cyclisation to form the pyranone ring (Scheme 2). The enol precursor **2** used in the cyclisation would be then easily synthesized from tetronic acid **3** (**Scheme 2**).



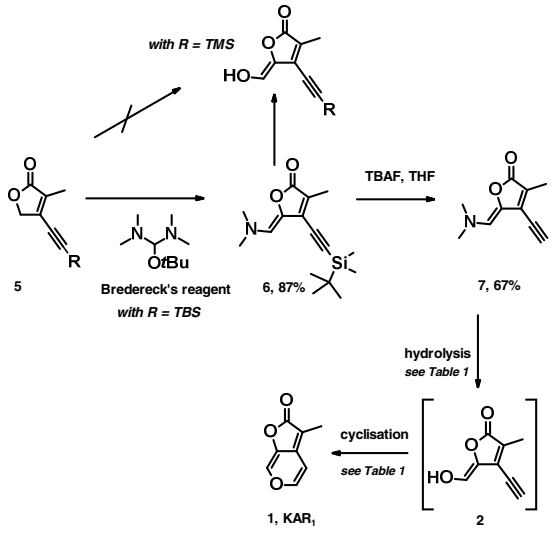
Scheme 2: Retrosynthetic analysis

We started our synthesis from ethyl-2-methyl acetoacetate which was easily converted to tetronic acid **3** in good yield (60%, Scheme 3) *via* an efficient bromination/acid-catalyzed cyclisation developed by Stolz.[xiv] The acetylene moiety was then introduced by a Sonogashira coupling between TMS or TBS acetylene and the corresponding triflate **4**, affording **5** in excellent yield (89%, Scheme 3).[xv,xvi]



Scheme 3: Preparation of the alkynyl-intermediate **5**

Attempts to formylate butenolide **5** via deprotonation and reaction with ethyl formate failed (attempt done with R = TMS, Scheme 4). These butenolides are indeed known to dimerize very quickly under basic conditions.[xvii] However, to our delight, stirring the compound **5** with the more robust TBS protecting group with Bredereck's reagent furnished dimethyl enamine **6** (Scheme 4) and **7** was finally efficiently obtained in only 6 steps and in high overall yield, even on large scale (Scheme 4).



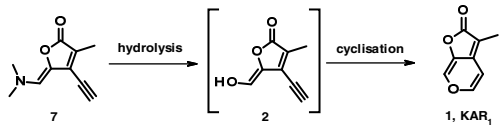
Scheme 4: Access to key intermediate **7**, direct precursor of Karrikin **1**.

We then tried to hydrolyze the enamine **7** to the corresponding enol. Although we had evidence that enol **2** was formed upon treatment with aqueous HCl, we could not isolate it due to its high instability. We decided then to try a one-pot hydrolysis/cyclisation cascade and first considered an acid catalyzed addition of the enol into the viniloguous acetylene ester. However, neither HCl nor TsOH were able to trigger the cyclisation (Entries 1 and 2, Table 1). We also considered a base catalyzed process in which the enolate would add on the activated acetylene but addition of potassium carbonate to the reaction mixture after the hydrolysis did not led to the formation of the expected Karrikin (KAR1) **1**.

Acid and base mediated 6-*endo*-dig cyclisation of enol and in particular phenol to acetylene have often been reported in the literature for the synthesis of chromones but the alkyne moiety from **7** is weakly activated and the enol too sensitive for these conditions. Metal-catalyzed activation of the acetylene was then considered and in particular gold (I) complexes, as already been intensively studied and used.[xviii] Gold triphenylphosphine triflimidate (10 mol %) was added to the reaction mixture after completion of the hydrolysis and to our delight, 17% of **1** were isolated. Introduction of the catalyst from the start of the hydrolysis led to a strong increase of the final yield (67%) which was further improved by replacing TsOH with MsOH (75%, entries 5, 6, Table 1). Control reaction showed that gold triphenylphosphine triflimidate alone was not enough to trigger the cyclisation in the absence of acid, suggesting that the latter is required at least for the hydrolysis of the enamine and possibly assisting the cyclisation step (entry 7, Table 2).

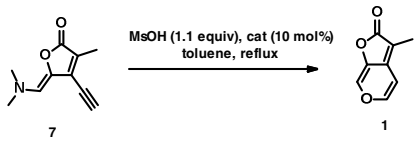
With this first results in hand, we then look at optimizing the conditions of the final metal-catalyzed cyclization step and in particular to find a cost-attractive catalyst. Different metals reported to successfully activate alkynes were investigated and the use of Cu(I), Cu(II), Ag, Pd, Pt or Fe did not allow to obtain KAR_1 **1** or only in traces (entries 1/2, 11-20, Table 2). Gold catalysts $MeAu(PPh_3)$ appeared to be the only suitable one for the reaction and gave by far the best result even with only 5 mol% (entry 6,table 2).

Table 1: Optimization of the hydrolysis/cyclisation sequence – Access to Karrikin (KAR_1) **1**



Entry	Hydrolysis	Cyclisation	Yield (Isolated)
1	Aq. HCl, THF	Aq. HCl	Decomposition
2	TsOH, aq.toluene	TsOH	Decomposition
3	Aq. HCl, THF	K_2CO_3	Decomposition
4	TsOH, aq.toluene	Then $AuNTf_2PPh_3$	17%
5	TsOH, $AuNTf_2PPh_3$, (10%), aq. toluene	*(One-pot)*	62%
6	MsOH, $AuNTf_2PPh_3$, (10%), aq. toluene	*(One-pot)*	75%
7	$AuNTf_2PPh_3$, (10%), aq. toluene	*(One-pot)*	-

Table 2: Optimization of the metal-catalyzed cyclisation to Karrikin (KAR_1) **1**



7 1

entries	Catalyst	t(h)	Yield (%)[a]	entries	Catalyst	Yield (%)[a]
1	$PtCl_2$	3.5	7[a]	11	CuI	-
2	$PtCl_4$	4	11[a]	12	CuOTf	10[a]
3	$[(\eta^2\text{-}C_2H_4)PtCl_2]_2$	4	6[a]	13	$CuCl_2$, $2H_2O$[b]	11[a]
4	AuCl[b]	22	Traces	14	$CuOTf_2$	16[a]
5	$MeAu(PPh_3)$	2	86%	15	$CuOTf_2$[e]	17[a]
6	$MeAu(PPh_3)$[c]	2	86%	16	$AgNO_3$[b]	5[a]
7	$MeAu(PPh_3)$[b,d]	3	35%[a]	17	$PdCl_2$	2[a]
8	$HAuCl_4$	3.5	traces	18	$Pd(OAc)_2$	4[a]
9	$AuCl_3$[b]	4.5	-	19	$FeCl_3$[b]	-
10	$AuPPh_3Cl$	2	traces	20	$FeCl_2$, $4H_2O$[b]	-

[a]NMR yield; [b]1.1 equiv of MsOH was added again after 1h; [c]5 mol% catalyst were used; [d]2 mol% catalyst were used; [e]20 mol % catalyst were used.

In order to evaluate the bioavailability of KAR_1 in soil we measured or calculated different parameters related to the mobility and persistence (Table 4). We estimated the soil organic carbon-water partitioning coefficient (Koc) starting from the Log P value and by using the Briggs' equation[iiid]. The Koc is useful in predicting the mobility of small organic molecules in soil and low Koc values indicate high mobility. The estimated Koc value for KAR_1 was very low less than 50 suggesting a very high mobility. The low Koc value was also consistent with the very high water solubility of KAR_1. Soil half-life is another important parameter to ensure that compound persists sufficiently long to delivery the desired biological effect. The half-life of KAR_1 under lab conditions at 25°C was 37 days which can be considered sufficiently long. These results together with the long hydrolytical half-life suggest that KAR_1 is relativly stable against the main biotic and abiotic mechanisms of degradation. It is worth to mention that conversly, other biologically active butenolides belonging to the class of strigolactones such as GR-28 or GR-24, displayed a very short soil persistence, in the range of a few hours.[iiic] This was hystorically considered one of the main limitations to use this other class of butenolides in agriculture. Thus, the combination of soil persistence and high mobility suggests that KAR_1 can be sufficiently bioavailable once is applied in soil directly or via seed treatment.

Table 4. Chemiodynamic properties of KAR_1.

Hydrolytic stability (T1/2)	Soil stability[a] (DT50)	Water Solubility	Log P	Koc (estimated)[b]
>100 h	37 days	1622 ppm	0.96	23

[a] Sand soil (pH 7.6); [b] Estimated using Log P.

To test the biological effect of KAR_1 we used corn as crop model and we studied the dose-response of KAR_1 on different commercial corn hybrids encompassing different maturity groups and tolerance to cold stresses. Since the corn seeds used in this study did not have any dormant phenotype we wanted to check whether KAR_1 had any benefit in supporting the germination under normal or slightly stressful condition represented by low temperature. In more details, we wanted to evaluate whether KAR_1 can alleviate any quiencensce physiological status caused by low temperature and we carried out dynamic analysis of corn seed populations by studying the kinetic of germination. The curve of germinated seeds over time allowed us to calculated the speed (T50) and uniformity of germination (slope).

Under our conditions we did not observed any consistent improvement of the kinetic of germination across hybrids both at 15 or 23 °C. No obvious dose-response were observed for KAR_1. As expected and already indicated from previous studies,[iiie] at highest concentrations we could observed an inhibitoring effect particulary for the speed of germination.

Our results differ from previous reports[iief] disclosing that KAR_1 or smoke water applied as liquid or aerosol indeed improved germination and vigor of corn seeds. This divergence in results could be linked to the different genetics and seed batches used in their experiments compared to our ones. Indeed in both studies they reported to have very low germinabiltiy of corn < 70% in the control treatment,[iief] which is not relevant with potential agronomic applications. In our experiments the germination was always higher than 95%.

In order to further understand the results of the germination assays we studied the uptake of KAR_1 alongside other two compounds chlorpropham (commercially use as anti germinating agent for potatoes) and GR-24, into corn seeds. For the experiment, corn seeds were incubated at different temperatures (T = 7 °C and 23 °C) in a solution containing the chemical and the decrease in concentration was monitored over a period of 96 h. By defining the uptake as

$$uptake = 100\% * \left(1 - \frac{[AI]_{seed}}{[AI]_{control}}\right) \quad \text{(eq.1)}$$

where $[AI]_{seed}$ is the concentration of an AI in a solution containing seed and $[AI]_{control}$ the concentration of an AI in a control solution where no seed is present, only the relative decrease in concentration is considered, allowing for compensation of processes other than related to seed, such as degradation processes and adsorption to glass. The uptake behaviour of the three chemicals differs significantly (Figures 2 and 3). While chlorpropham was taken up readily and nearly thoroughly, an observation in good accordance with the results reported by Rieder and collaborators,[viic] the uptake of GR-24 and KAR1 was less complete.

After 96h, only around 30% of KAR1 was taken up by the seed at both temperatures, a low value indicating that bioavailability of this substance might be a critical factor. The fact that uptake of all compounds was not only observed at 23 °C but also at 7°C, albeit slower as expected by thermodynamics, suggesting that a passive uptake mechanism might be the dominating one for all three compounds.

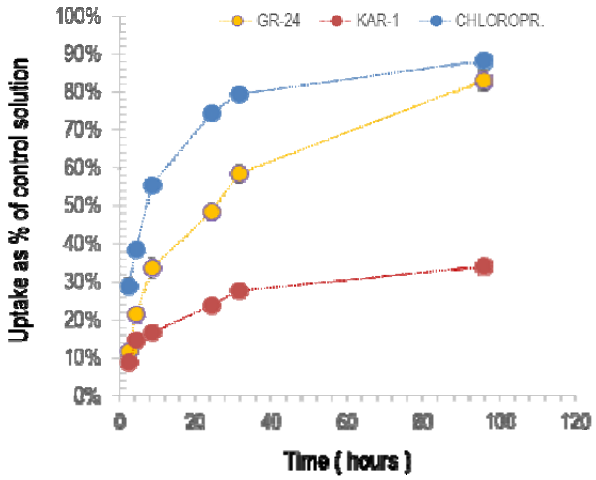


Figure 2. Uptake of GR-24, KAR1 and chloropropham into seed at 23 °C. The bars represent the standard errors of the mean.

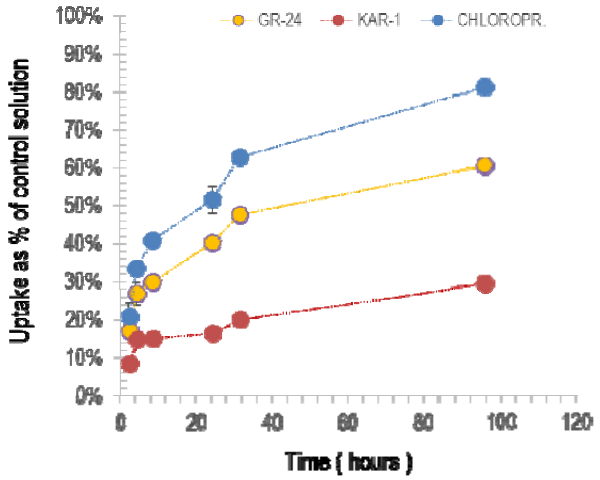


Figure 3. Uptake of GR-24, KAR1 and chloropropham into seed at 7 °C. The bars represent the standard errors of the mean.

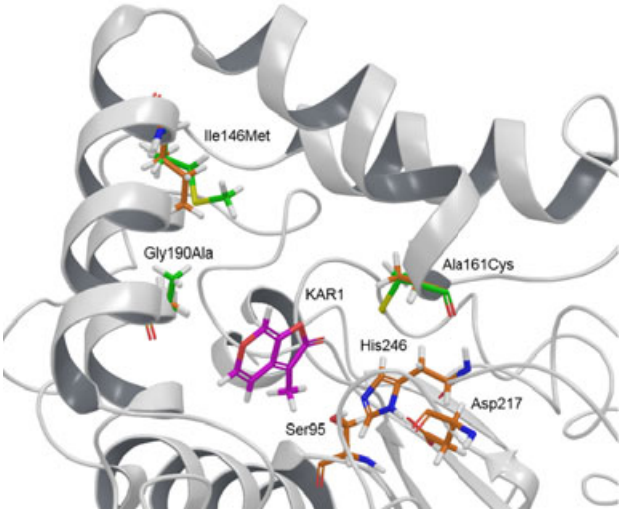


Figure 4. Active site of AtKAI2 (PDB 4JYM, orange carbons). The amino acids differing in ZmKAI2 are shown in green carbons.

We also tried to rationalize the experimental results by building a homology model of corn KAI2, using as template the X-ray of the KAI2 protein from *Arabidopsis thaliana* (PDB ID 4JYM,[xix] see Supporting Material), reported to positively respond to Karrikins in germination.[xx] The active site in corn differs by only three amino acids (Figure 4 and Table 2), only one of which (Ala161Cys) is located in proximity to the catalytic triad (Ser95/His246/Asp217) and the putative binding region of KAR1, increasing the polar character of the biding pocket in corn. On the other hand, Ile146Met and Gly190Ala are located at higher distances (ca. 13Å and 8Å, respectively) from KAR_1 and likely have no influence on the ligand binding.

Corn and *Arabidopsis* sequences were also compared to other KAI2 genes from parasitic weeds, which were reported to have different germination responses to karrikins, according to Conn *et al.*[xxi]Table 2).

Interestingly, the clade gene KAI2c in *Phelipanche aegyptiaca* (PaKAI2c), which is described to confer germination response to KAR_1, shows a 100% active site sequence identity compared to corn KAI2. This seemingly indicates that the same Ala161Cys, Ile146Met and Gly190Ala differences in PaKAI2c are not affecting KAR1 binding and would likewise allow karrikins to bind in the identical binding site in the corn receptor. Our result clearly suggest that KAR1 could bind to its KAI2 receptor in corn. However, the previously described induction of corn seeds germination[iief] has been performed with low germinating seeds which are not relevant for commercial applications. In contrast, we performed our study with three commercial corn seeds varieties demonstrating that KAR_1 does not improve their germination. This has very important consequences for the hypothetized value of KAR_1 and commercial use.

Table 2: Active site sequence alignment of KAI2 in corn (*Zea mais*, *ZmKAI2*) with *Arabidopsis thaliana* (*AtKAI2*), *Striga hermontica* (*ShKAI2*) and *Phelipanche aegyptiaca* (*PaKAI2*). Genes not conferring germination responses to KAR1 are shaded in grey. Amino acid differences between corn and Arabidopsis are highlighted in red. [a] Absence of response in the case of *ZmKAI2* might be due to genetics and seed batches quality (see discussion).

	Active site amino acids																								
	25	26	27	94	95	96	97	124	139	134	142	145	146	153	157	158	161	174	178	190	193	194	217	219	246	247
AtKAI2	G	F	G	H	S	V	S	Y	L	F	L	A	I	W	F	A	A	F	L	G	I	F	D	A	H	L
ZmKAI2[a]	G	F	G	H	S	V	S	Y	L	F	L	A	M	W	F	A	C	F	L	A	I	F	D	A	H	L
ShKAI2i	G	F	G	H	S	L	S	F	V	F	L	A	I	W	F	A	V	F	L	F	I	F	D	A	H	L
PaKAI2c	G	F	G	H	S	V	S	Y	L	F	L	A	M	W	F	A	C	F	L	A	I	F	D	A	H	L
ShKAI2d1	G	F	G	H	S	L	S	M	L	F	L	A	M	L	F	A	M	F	L	V	I	H	D	A	H	I
PaKAI2d3	G	F	G	H	S	L	S	S	L	F	L	A	M	L	M	A	A	F	L	V	I	H	D	V	H	L

Conclusion

We have developed an unprecedented and scalable synthesis of **1**–based on an elegant and efficient 6-endo-dig cyclisation. This represents an important and essential requirement to ensure that some promising chemicals can be synthetically produced for potential practical applications. We have also shown that conversely to other butenolides derivatives, KAR_1 is sufficiently stable and mobile in soil, suggesting that the compound should be bioavailable. We can expect a certain degree of flexiblility in the application and due the high water solubility and stabiltiy the compound can be used both for in-furrow or seed treatment applications.

Despite other studies[ii,iii] indicated that KAR_1 promotes the germination and/or subsequent development of young seedlings we could not

distinctly observed these effects in our studies. In several studies it is reported that KAR_1 is able to break primary or secondary dormancy in diverse species; in most of the cases it is about a wide range of weeds

or *A. thaliana* [iig.] A few papers reported the effects on corn cultivars. In these studies, the corn displayed a surprisingly low germination level which may indicate that low vigor seed batches were used. In high vigor and commercial corn hybrids we could not observed any effect with KAR_1 in a wide range of rates and at two temperatures. The high vigor of these seed batches might have masked any effect of KAR_1.

Surprisingly seed uptake of KAR_1 appeared suboptimal compared to other molecules. This might be an additional factor to explain the lack of biological effect of KAR_1 in corn.

Furtherer work to better understand the role of KAR1 in the development of corn or other field crops will be needed. Evaluating the potential benefit effect of KAR1 in low vigour crop seed batches could be technically very interesting, althought of dubious commercial applicability, untill when only the high vigour seed batches can be released and commercialized.

Table 3: Effect of **1** (Karrikin, KAR_1) on the germination of three corn seeds varieties at various concentrations and T°C.

Corn variety	T°C	[c]	Effect on the germination slope (% compared to control)[a]	Effect on the germination T50 (% compared to control)[b]
Falkone	23°C	2	+3.9	-0.7
		10	-1.2	+0.1
		50	+6.5	+0.1
		250	+4.1	-1.3
Falkone	15°C	2	-18.9	**+5.3**[c]
		10	-7.3	-0.7
		50	-10	+1.5
		250	-19.1	**+5.1**[c]
Famoso	15°C	2	+8.9	+1.1
		10	-0.6	-0.1
		50	+10.2	+0.9
		250	+10.0	**+8.0**[c]
Famoso	23°C	2	+28.2	-2.6
		10	-0.9	-1.3
		50	+7.3	-3.2
		250	+10.6	**+13.6**[c]
Multitop	15°C	2	+7.4	-0.1
		10	+5.2	0
		50	+5.1	+0.8
		250	+8.1	**+3.7**[c]
Multitop	23°C	2	+21.4	-1.0
		10	-12.3	**+6.3**[c]
		50	+9.2	**+4.5**[c]
		250	-5.5	**+7.8**[c]

[a] Positive values mean a more homogeneous germination and *vice versa* for negative values; [b] Positive values mean a slower germination and *vice versa* for negative values; [c] Data in bold are statistically validated.

Experimental Section

General Aspects and Materials

All reactions were carried out under an argon atmosphere with dry solvents unless otherwise noted. Reagents were purchased at highest available commercial quality and used without further purification unless otherwise stated. Preparative flash chromatography was performed using silica gel 60 (40–63 lm, E. Merck). NMR spectra were recorded on Brucker ultrashield 400 PLUS instrument. Chemical shifts are given in parts per million (δ) and calibrated using residual undeuterated solvent as an internal reference. The relative stereochemistry was determined by ROESY correlations. Compound

3 was prepared according to literature known procedure[xiv] and analytics were consistent with those reported.

3-methylfuro[2,3-c]pyran-2-one, 1 (KAR1): To a solution of enamine **7** (0.5g, 3 mmol) in toluene, was added MsOH (1.1 eq) and MeAu(PPh_3) (5 mol%, 0.067 g) and the resulting reaction mixture was stirred at reflux for 2 hours. After cooling to room temperature, the solution was filtered on a pad of Celite® and concentrated under reduce pressure. The resulting crude reaction product was purified by flash chromatography on SiO_2 (EtOAc/CyHexane) affording **1** (KAR1) as a pure yellowish solid in 86% yield (2.43 mmol, 0.42 g). Analytical data were consistent with those previously reported in the literature.[xxii]

(5Z)-5-(dimethylaminomethylene)-4-ethynyl-3-methyl-furan-2

one, 7: To a solution of 6 (10 g) in THF (100 mL) was added dropwise

tertrabutylammonium fluoride (TBAF 1M in THF, 38 mL) at room temperature and the resulting solution was stirred for 12 hours. Saturated NaClaq. solution was then added and the reaction mixture

diluted with ethyl acetate (200 mL). The phases were separated, the aqueous layer extracted with EtOAc (2 x 100 mL) and the combined organic fractions were dried over sodium sulfate and concentrated under vacuum. The resulting crude solid was washed with cyclohexane, dried and used without further purification in the gold-catalyzed cyclization step (67%, 4.1 g, 23 mmol). ^{1}H NMR (400MHz, $CDCl_3$) δ (ppm) 6.07 (s, 1H), 3.6 (s, 1H), 3.15 (s, 6H), 1.97 (s, 3H). ^{13}C NMR (101MHz, $CDCl_3$) δ (ppm) 170.6, 131.8, 127.8, 124.4, 116.3, 88.6, 73.1, 42.4, 9.0; ^{13}C (101MHz, $CDCl_3$) δ (ppm) 170.6, 131.8, 127.8, 124.4, 116.3, 88.6, 73.1, 42.4, 9.0; HRMS ($M+H^+$):178.0872 (*calc*. 178.0790 for $M+H^+$).

(5Z)-4-[2-[tert-butyl(dimethyl)silyl]ethynyl]-5-(dimethylamino-methylene)-3-methyl-furan-2-one, 6: To a solution of **5** (150 g, 636 mmol) in toluene (1.5 L) was added 221.2 g of Bredereck's reagent. The reaction mixture was then stirred for 12 hours at room temperature and quenched with 1L of water. The phases were then separated, the aqueous layer extracted with TBME (2 x 500 mL) and the combined organic fractions were dried over sodium sulfate, concentrated under vacuum and **6** was obtained as a viscous gum in 87% yield (161 g) which was used without further purification. ^{1}H NMR (400MHz, $CDCl_3$) δ (ppm) 6.04 (s, 1H), 3.14 (s, 6H), 1.97 (s, 3H), 1.00 (s, 9H), 0.20 (s, 6H). ^{13}C (101MHz, $CDCl_3$) δ (ppm) 171.0, 133.2, 128.1, 124.5, 115.7, 106.2, 94.5, 42.6, 25.9, 16.4, 9.3, -4.9 ; HRMS ($M+H^+$): 292.1759 (*calc.* 292.1654 for $M+H^+$).

3-[2-[tert-butyl(dimethyl)silyl]ethynyl]-4-methyl-2H-furan-5-one, 5: Compound **4** (225 g, 914 mmol) was dissolved in dioxane (2.25 L) and the resulting solution was degassed with nitrogen. Palladium catalyst ($PdPPh_3Cl_2$, 5 mol%, 32.4 g, 45.7 mmol) followed by CuI (5 mol%, 8.7 g, 45.7 mmol) and diisopropylamine (1.5 eq, 194 mL, 1371 mmol) were then added to the reaction mixture. To the resulting black solution was added dropwised at room temperature *tert*-butyl-ethynyl-dimethyl-silane (1.1 eq, 191 mL, 1005 mmol). The reaction mixture was stirred for 1 hour at room temperature, filtered through a pad of Celite® (filter cake washed with ethyl acetate) and the resulting solution was concentrated under vacuum. The resulting crude gum was then suspended in TBME and **5** was isolated as a brown solid (192 g, 615 mmol) in 89% yield. ^{1}H NMR (400MHz, $CDCl_3$) δ (ppm) 4.5 (m, 2H), 1.8 (m, 3H), 0.78 (s, 9H), 0.0 (s, 6H). ^{13}C (101MHz, $CDCl_3$) δ (ppm) 174.2, 139.4, 133.2, 111.3, 94.8, 71.1, 26.0, 16.5, 10.2, -4.9; HRMS ($M+H^+$): 237.1303 (*calc*. 237.1233 for $M+H^+$).

(4-methyl-5-oxo-2H-furan-3-yl) trifluoromethanesulfonate, 4: 3 (220 g, 1928 mmol) was solved in dichloromethane (1.1 L) and the resulting solution was cooled to -78°C. Triethylamine (1.5 eq, 403 mL, 2892 mmol) was then added, followed by triflic anhydride (1.2 eq, 391 mL, 2314 mmol) dropwise. After complete addition, the reaction mixture was slowly warmed to 0°C and stirred at this temperature for 1 hour. Water was added (1 L), the phases separated and the aqeous layer was extracted with dichlorormethane (3 x 500 mL). The combined organic extracts were washed with aq HCl solution (1M), dried over sodium sulfate and concentrated under vacuum. Compound **4** was isolated as a brown liquid in 80% yield (380 g, 1544 mmol). ^{1}H NMR (400MHz, $CDCl_3$) δ (ppm) 4.90 (m, 2H), 1.92 (t, 3H); ^{13}C (101MHz, $CDCl_3$) δ (ppm) 170.5, 160.6, 120.2, 117.4, 66.9, 7.8; HRMS ($M+H^+$): 246.9876 (*calc*. 246.9810 for $M+H^+$)

Supplementary Material

Supporting information for this article is available on the WWW under http://dx.doi.org/10.1002/MS-number.

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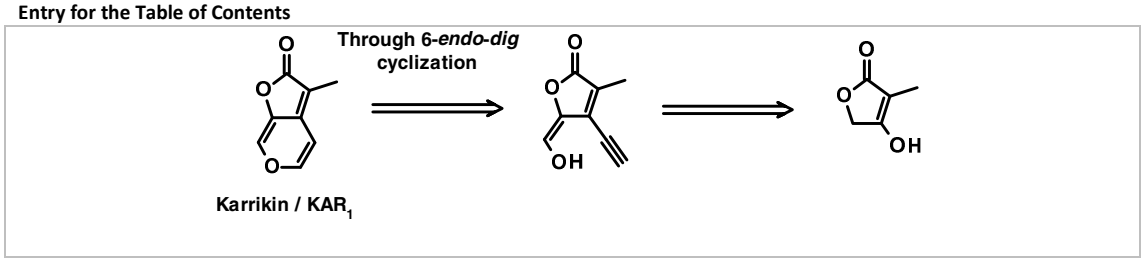

References

[i] G. R., Flematti, E. L. Ghisalberti, K.W. Dixon, R. D. Trengove *Science*, **2004**, *305*, 977.

[ii] (a) C. De Cuyper, S. Struk, Braem, L., K. Gevaert, G. De Jaeger, S.Goormachtig, *Plant, Cell and Environment* **2017**, *40*, 1691; (b) D. C. Nelson, J.-A. Riseborough, G. R. Flematti, J. Stevens, E. L. Ghisalberti, K. W.Dixon, S. M. Smith, *Plant Physiology* **2009**, *149*, 863; (c) W. Li, K. H. Nguyen, Chuc, H. D., Ha, C. V., Watanabe, Y., Osakabe, Y., M. A. Levva-González, M. Sato, K. Toyooka, L. Voges, M. Tanaka, M. G. Mostofa, M. Seki, M. Seo, S. Yamaguchi, D. C. Nelson, C. Tian, L. Herrera-Estrella, L.-S. Phan Tran *PLoS Genet* **2017**, *13*(11): e1007076; (d) S. D. S. Chiwocha, K. W. Dixon, G. R. Flematti, E. L. Ghisalberti, D. J. Merritt, J. C. Stevens, *Plant Science*, **2009**, *177*, 252. (e) J. van Staden, S. Sparg, M.G. Kulkarni, & M.E. Light, *Field Crops Res* **2006**, 98, 98. (f) S. G. Sparg, M. G. Kulkarni & J. van Standen. *Crop Sci*. **2006**, 46, 1336. (g) E. Villedieu-Percheron, M. Lachia, P. M. J. Jung, C. Screpanti, R. Fonné-Pfister, S. Wendeborn, D, Zurwerra, & A. De Mesmaeker. *CHIMIA*, **2014,** *68*, 654.

[iii] (a) C. Screpanti, R. Fonné-Pfister, A. Lumbroso, S. Rendine, M. Lachia, A. De Mesmaeker, *Bioorg. Med. Chem. Lett.* **2016**, *26*, 2392; (b) S. Al-Babili, H. J. Bouwmeester, *Annu. Rev. Plant Biol*. **2015**, *66*, 161; (c) A. Lumbroso, E. Villedieu-Percheron, D. Zurwerra, C. Screpanti, M. Lachia, P.Y. Dakas, & A. Beck, *Pest Manag. Sci.* **2016,** *72*, 2054; (d) G. G. Briggs, *J. Agric. Food Chem*. **1981**, *29*, 1050; (e) T. Kretzschmar, W. Kohlen, J. Sasse, L. Borghi, M. Schlegel, J. Bachelier, D. Reinhardt, R. Bours, H. Bouwmeester & E. Martinoia, *Nature* **2012,** *483*, 341; (f) T. V. Charnikova, K. Gaus, A. Lumbroso, M. Sanders, J.-P. Vincken, A. De Mesmaeker, C. P. Ruyter-Spira, C. Screpanti, H.J. Bouwmeester, *Phytochemistry* **2017**, *137*, 123.

[iv] G. R. Flematti, E. L. Ghisalberti, K. W. Dixon, R. D. Trengove, *J. Agric. Food Chem*. **2009**, *57*, 9475.

[v] For example of synthetic analogs, see G. R. Flematti, A. Scaffidi, E. D. Goddard-Borger, C. H. Heath, D. C. Nelson, L. E. Commander, R. V. Stick, K. W. Dixon, S. M. Smith, E. L. Ghisalberti, *J. Agric. Food Chem*. **2010**, *58*, 8612.

[vi] S. Niemann, M. Burghardt, C. Popp, M. Riederer, *Plant, cell & environment* **2013**, *36*, 1027.

[vii] (a) G. Rieder, K.P. Buchholtz, C.A. Kust, *Weed Science* **1970**, *18*, 101: (b) H. D. Scott, R. E. Phillips, *Weed Science* **1971**, 128.

[viii] G. R. Flematti, E. D. Goddard-Borger, D. J. Merritt, E. L. Ghisalberti, K. W. Dixon, R. D. Trengove, *J. Agric. Food Chem*. **2007**, *55*, 2189

[ix] E. D. Goddard-Borger, E. L. Ghisalberti, R. V. Stick, *Eur. J. Org. Chem*. **2007**, 3925.

[x] K. Matsuo, M. Shindo, *Tetrahedron* **2011**, *67*, 971.

[xi] K. Sun, Y. Chen, T. Wagerle, D. Linnstaedt, M. Currie, P. Chmura, Y. Song, M. Xu, *Tetrahedron Lett*. **2008**, *49*, 2922.

[xii] R. Nagase, M. Katayama,H. Mura,N. Matsuo, Y. Tanabe, *Tetrahedron Lett.* **2008**, *49*, 4509.

[xiii] Low yield was obtained when scaling-up Tanabe's approach in our lab.

[xiv] U. K. tambar, T. Kano, J. F. Zepernick, P. M. Stolz, *J. Org. Chem*. **2006**, 8357.

[xv] R. Grigg, P. Kennewell, V. Savic, *Tetrahedron* **1994**, *50*, 5489.

[xvi] R. H. Pouwer, H. Schill, C. M. Williams, P. V. Berhardt, *Eur. J. Org. Chem*. **2007**, 4699.

[xvii] S. I. Clarke, B. Kasum, R. H. Prager, A. D. Ward, *Aust. J. Chem.* **1993**, *36*, 3483.

[xviii] (a) R. Dorel, A. M. Echavarren *Chem. Rev*. **2015**, *115*, 9028; (b) C. Jiang, Z. Xiong, S. Jin, P. Gao, Y. Tang, Y. Wang, C. Du, X. Wang, Y. Liu, B. Lin, Y. Liu, M. Cheng, *Chem. Commun*. **2016**, *52*, 11516.

[xix] Y. Guo, Z. Zheng, J. J. La Clair, J. Chory, J. P. Noel, *Proceedings of the National Academy of Sciences* **2013**, *110*, 8284.

[xx] M.T. Waters, D.C. Nelson, A. Scaffidi, G.R Flematti, Y.K. Sun, K.W. Dixon, & S.M. Smith, *Development* **2012**, *139*, 1285.

[xxi] C. E. Conn, R.Bythell-Douglas, D. Neumann, S. Yoshida, B. Whittington, J. H. Westwood, K. Shirasu, C. S. Bond, K. A. Dyer, D. C. Nelson, *Science* **2015**, 349, 540.

[xxii] G. R. Flematti, E. L. Ghisalberti, K. W. Dixon, R. D. Trengove, *Tetrahedron Lett.* **2005**, *46*, 5719.