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Zeapyranolactone − A novel strigolactone from maize

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ABSTRACT

The structure of a new strigolactone present in the root exudate and root extract of maize hybrid cv NK Falkone plants was elucidated and characterized as zeapyranolactone: *Methyl* .(*E*)-3-((4-methyl-5-oxo-2,5-dihydrofuran-2-yl)oxy)-2-(4,4,5-trimethyl-2-oxo-2,3,4,6,7,7a-hexahydrocyclopenta[b]pyran-7-yl)acrylate. Unlike any other strigolactone published so far, it contains a 4,4-dimethyltetrahydropyran-2-one as A ring. The impact of the elucidation of this structure on the earlier postulated biosynthetic pathway of another maize strigolactone, zealactone, is discussed.

1. Introduction

The strigolactones are a new class of plant hormones that play a prominent role in the regulation of plant architecture, and are also exuded by plants into the rhizosphere where they induce hyphal branching in arbuscular mycorrhizal fungi and germination in root parasitic plant seeds (Screpanti et al., 2016). The existing collection of strigolactones was recently extended with the zealactones **1a/b**, compounds with a non-cannonical structure, i.e. not having the conventional A, B and C-rings (Charnikhova et al., 2017; Yoneyama et al., 2015). In maize root exudate six additional putative strigolactones were described (Charnikhova et al., 2017), but only the structures of zealactones **1a/b** were identified. Here, we reveal an additional structure for compound **2**, which we coined zeapyranolactone as a subclass of the zealactones published earlier. Together with the previously described zealactones it was isolated from the root exudates of maize NK Falkone plants on the basis of the characteristic MS/MS fragmentation of strigolactones. Its accurate mass is the same as for the zealactones, *m/z* 377.1602 Da, and it has the same chemical formula, $C_{20}H_{24}O_7$.

2. Results and discussion

The structure of zeapyranolactone **2** (Fig. 1) was elucidated using NMR spectroscopy. In addition to zeapyranolactone **2** a side product **3** (3-(3-methoxycarbonyl-5-methyl-4,6a-dihydro-3aH-cyclopenta[b] furan-6-yl)-3-methyl-butanoic acid) was observed in the spectra with a molar ratio of nearly 1:1 (52%, 48%). Table 1 shows the chemical shifts of zeapyranolactone **2**, side product **3** and zealactone **1b**. In Fig. 2, the ^{1}H and ^{13}C NMR spectra as well as the correlations observed in (^{1}H,^{13}C)-HMBC, (^{1}H,^{1}H)-ROESY and (^{1}H,^{1}H)-COSY for zeapyranolactone **2** and side product **3** are depicted. 2D NMR spectra are shown in Suppl. Figs. 1–4.

For zeapyranolactone **2,** the presence of the canonical strigolactone butenolide (D-ring) as well as a methyl ester and an enol ether function – as also observed in heliolactone and zealactones **1a/b** – were confirmed. However, the two olefinic signals for the *E*-configured double bond of heliolactone and zealactones **1a/b** were not observed. Instead, a CH (H4, δ_H 5.29, δ_C 40.2) shows HMBC correlations to the ethylene proton of the enol ether and the carboxylic carbon of the methyl ester. This H4 has a CH group as neighbour with a carbon chemical shift at 89.9 ppm, C4a. This low field shift indicates the presence of oxygen next to it. On the other side of H4 is a CH_2 group (H5, δ_H 2.35-2.44, 2.54-2.59; δ_C 42.4). In addition to the methyl ester and the methyl group in the D-ring, three methyl groups (11, 12 and 13) are observed. Two of these (H11, H12) show (^{1}H, ^{13}C)-HMBC correlations to each other and to a sp^2 hybridized quaternary carbon (C6a, δ_C 136.5), which is also observed for the methylene group 5. HMBC correlations show that the methyl group 13 is connected to an sp^2 carbon (C6, δ_C 136.4). In addition, the two methyl groups 11 and 12 show correlations to a CH_2 (H8, δ_H 2.18 and 2.47, δ_C 46.6), which, in turn, is next to a

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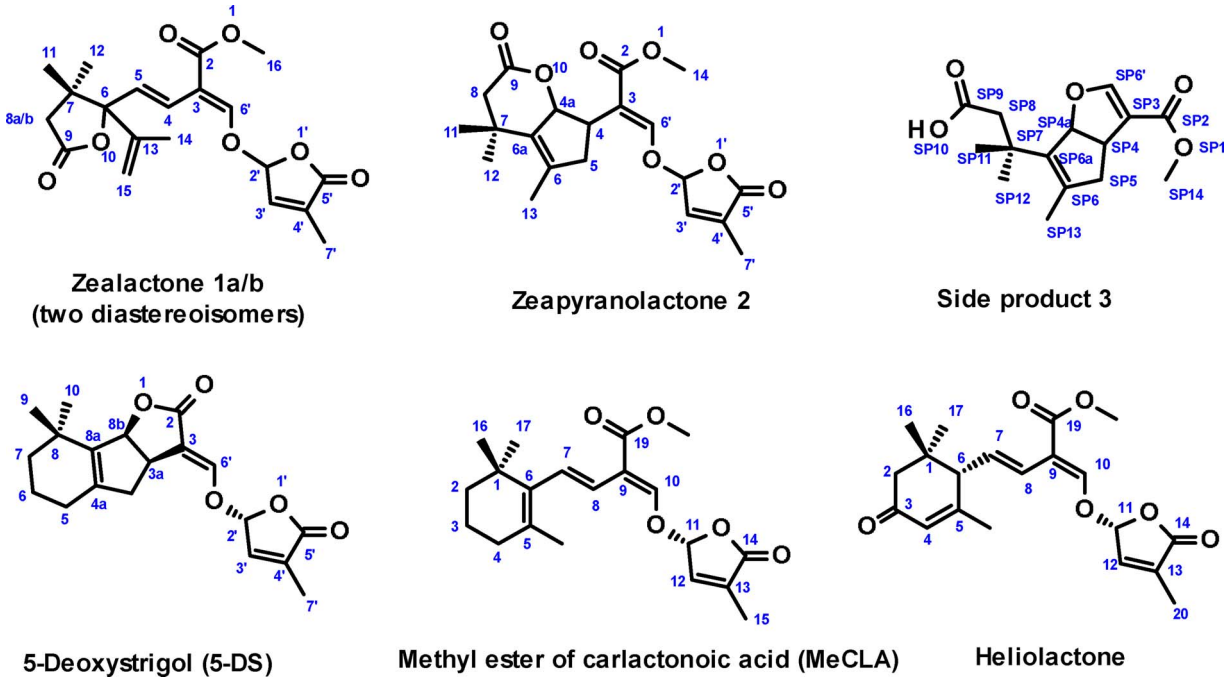


Fig. 1. Chemical structures of previously elucidated zealactones **1a/b** (Charnikhova et al., 2017), zeapyranolactone **2**, Side product **3**, heliolactone (Ueno et al., 2014), methyl ester of carlactonoic acid (MeCLA) (Abe et al., 2014), 5-deoxystrigol (5-DS) (Ueno et al., 2014).

carboxyl group (C9, δ_C 173.8), as proven by (^{1}H, ^{13}C)-HMBC and the low field chemical shift. These structural motifs are complemented by additional correlations in (^{1}H, ^{13}C)-HSQC, (^{1}H, ^{1}H)-COSY, and (^{1}H, ^{13}C)-HMBC, especially also by through-space vicinity information as obtained from (^{1}H, ^{1}H)-ROESY (Fig. 2C, D) and in their combination lead to the structure proposal for zeapyranolactone **2** as shown in Fig. 1.

We expect the biosynthetic pathway of zeapyranolactone **2** from β-carotene to comprise the same first steps as the biosynthesis of zealactones **1a/b** until the carboxylic acid **8** (Scheme 1). Further lactonization/cyclization of **8** can result in zealactone **1a/b** as described in our previous publication (Charnikhova et al., 2017). Alternatively, dehydration of **8** followed by cyclization can result in the two stereoisomers zealactones **1a/b** as we propose here. Nazarov cyclization of ion **9** can result in intermediate **10** and – upon lactonisation – in zeapyranolactone **2.**

In side product **3,** the typical canonical strigolactone D-ring is missing; it therefore seems to represent a degraded strigolactone as a consequence of hydrolysis of the enol-ether bridge. However, there is still a CH group on a double bond next to the oxygen (SP6′, δ_H 7.16, δ_C 156.1) and it still shows a (^{1}H, ^{1}H)-ROESY correlation to a methoxy group (SP14, δ_H 3.66, δ_C 51.3). The methoxy group 14 clearly belongs to the ester moiety previously observed, as it shows an (^{1}H, ^{13}C)-HMBC correlation to a carboxylic carbon (CSP2, δ_C 166.1). HSP6′ in addition shows (^{1}H, ^{13}C)-HMBC correlations to a quaternary carbon (CSP3, δ_C 113.8) and two CH (SP4, δ_H 3.53-3.59, δ_C 40.3 and SP4a, δ_H 5.90, δ_C 99.9). The sequence SP4a, SP4, SP5 (δ_H 2.35-2.44 and 2.74, δ_C 47.4) is confirmed by (^{1}H,^{1}H)-COSY correlations. That SP5 is from the original B-ring is confirmed by (^{1}H, ^{13}C)-HMBC correlations, amongst others from a methyl group (SP13 δ_H 1.85, δ_C 16.7) to CSP5. Two additional methyl groups (SP11 δ_H 1.38, δ_C 28.4 and SP12 δ_H 1.32, δ_C 28.9) are shifted as compared to zeapyranolactone **2**, but show a similar correlation pattern as the methyl groups 11 and 12 discussed previously. One of the correlations observed is a (^{1}H, ^{13}C)-HMBC correlation to a CH_2 group (SP8 δ_H 2.51–2.59 and 2.69, δ_C 46.3). It is next to a carboxylic carbon (CSP9 δ_C 175.3), and, as the latter, is also shifted in the spectra as compared to zeapyranolactone **2**. The chemical structure of side product **3** was further scrutinised by comparison of the MS/MS fragmentation spectra (Suppl. Fig. 5). The fragments *m/z* 231, 203, 189, 175 and 161 present in the MS/MS fragmentation spectra of side product **3** (M + *m/z* 281) correspond to degradation products due to the stepwise loss of small molecules such as water, acetic acid, CO and/or methanol (Charnikhova et al., 2017). These fragments are also present in the MS/MS fragmentation spectra of zeapyranolactone **2** obtained at the same conditions. The fragment *m/z* 97, however, is only present in the MS/MS spectrum of **2** and corresponds to the D-ring, which is absent in the MS/MS fragmentation spectrum of side product **3**. Combining all this information leads to the structure proposed for side product **3** as presented in Fig. 1.

Side product 3 has a quite different structure and polarity than zeapyranolactone 2, consequently it is very unlikely that they co-eluted during prep-HPLC–MS–MS purification. This is confirmed by Suppl. Fig. 6, which shows an LC–MS chromatogram of the zeapyranolactone **2** sample after NMR analysis. Zeapyranolactone **2** and side product **3** have a quite different retention time. The amount of **3** increased in the sample used for NMR from about 1% before to around 48% after storage of the sample and NMR analysis in CD_2Cl_2, possibly due to remaining traces of formic acid. Side product **3** is thus very likely a degradation product of zeapyranolactone **2** which could be formed by rearrangement of the cyclic systems after hydrolysis of the D-ring linkage as depicted in Scheme 2. To support this assumption, we checked the stability of zeapyranolactone **2** under aqueous acidic conditions using UPLC–MS–MS analysis. Treatment of zeapyranolactone **2** for 24 h in 1 or 10% formic acid at 20 °C resulted in about 25 or 75% hydrolysis. Unfortunately, side product **3** also appeared to be highly unstable under the aqueous acidic conditions and decomposition occurred even faster than for zeapyranolactone **2**.

3. Conclusion

An unprecedented structure of a strigolactone-like molecule, zeapyranolactone **2**, was elucidated after isolation from maize root exudate. The sample contained a side product **3** probably resulting from degradation of zeapyranolactone.

Table 1
^{1}H NMR and ^{13}C NMR spectral data for zeapyranolactone **2**, side product **3** and zealactone **1b** (Charnikhova et al., 2017).

Zeapyranolactone 2			Side product 3			Zealactone 1b		
Position	δC in CD_2Cl_2	δH in CD_2Cl_2	Position	δC in CD_2Cl_2	δH in CD_2Cl_2	Position	δC in CD_2Cl_2	δH in CD_2Cl_2
2	167.3 (C)		**SP2**	166.1 (C)		**2**	166.9 (C)	
3	114.0 (C)		**SP3**	113.8 (C)		**3**	111.9 (C)	
4	40.2 (CH)	3.41 (td, J = 8.9, 6.9 Hz, 1H)	**SP4**	40.3 (CH)	3.53–3.59 (m, 1H)	**4**	117.7 (CH)	6.33 (d, J = 16.1 Hz, 1H)
4a	89.9 (CH)	5.46 (dsxt,J = 6.6, 2.1 Hz, 1H)	**SP4a**	99.9 (CH)	5.90 (br d, J = 9.2 Hz, 1H, HSP4a)			
5a	42.4 (CH_2)	2.35–2.44 (m, 1H) (overlapped with HSP5a)	**SP5a**	47.4 (CH_2)	2.35–2.44 (m, 1H) (overlapped with H5a)	**5**	132.3 (CH)	6.87 (dd, J = 16.1, 0.4 Hz, 1H)
5b		2.51–2.59 (m, 1H) (overlapped with HSP8a)	**SP5b**		2.74 (dd, J = 17.5, 8.7 Hz, 1H)			
6	136.4 (C)		**SP6**	140.3 (C)		**6**	93.2 (C)	
6a	136.5 (C)		**SP6a**	137.6 (C)				
7	33.2 (C)		**SP7**	35.2 (C)		**7**	43.4 (C)	
8a	46.6 (CH_2)	2.18 (d, J = 14.4 Hz, 1H);	**SP8a**	46.3 (CH_2)	2.51–2.59 (m, 1H) (overlapped with H5b)	**8a**	44.2 (CH_2)	2.22 (d, J = 17.0 Hz, 1H)
8b		2.47 (d, J = 14.4 Hz, 1H)	**SP8b**		2.69 (d, J = 14.8 Hz, 1H)	**8b**		2.43 (d, J = 17.0 Hz, 1H)
9	173.8 (C)		**SP9**	175.3 (C)		**9**	175.6 (C)	
11	28.0 (CH_3)	1.19 (s, 3H)	**SP11**	28.4 (CH_3) (overlapped with C12)	1.38 (s, 3H)	**11**	24.1 (CH_3)	1.06 (s, 3H)
12	28.4 (CH_3) (overlapped with CSP11)	1.30 (s, 3H)	**SP12**	28.9 (CH_3)	1.32 (s, 3H)	**12**	24.7 (CH_3)	1.24 (s, 3H)
13	15.7 (CH_3)	1.81 (s, 3H)	**SP13**	16.7 (CH_3)	1.85 (q, J = 1.0 Hz, 3H)	**13**	143.4 (C)	
						14	21.2 (CH_3)	1.84 (dd, J = 1.4, 0.8 Hz, 3H)
						15a	112.3 (CH_2)	4.94 (quin, J = 1.4 Hz, 1H)
						15b		5.05–5.09 (br m, 1H)
2′	101.2 (CH)	6.15 (quin, J = 1.4 Hz, 1H)				**2′**	101.1 (CH)	6.16 (quin, J = 1.3 Hz, 1H)
3′	142.1 (CH)	7.02 (quin, J = 1.6 Hz, 1H)				**3′**	141.8 (CH)	6.99 (quin, J = 1.6 Hz, 1H)
4′	135.8 (C)					**4′**	136.2 (C)	
5′	171.2 (C)					**5′**	170.9 (C)	
6′	154.9 (CH)	7.65 (s, 1H)	**SP6′**	156.1 (CH)	7.16 (d, J = 1.5 Hz, 1H)	**6′**	153.7 (CH)	7.56 (s, 1H)
7′	11.0 (CH_3)	1.97 (t, J = 1.5 Hz, 3H)				**7′**	11.0 (CH_3)	1.99 (dd, J = 1.6, 1.4 Hz, 3H)
14	51.8 (CH_3)	3.71 (s, 3H)	**SP14**	51.3 (CH_3)	3.66 (s, 3H)	**16**	52.1 (CH_3)	3.73 (s, 3H)

*The numbering and the ring position are shown in Fig. 1. It is based on the nomenclature of carotenoids (IUPAC, 1975) and the biosynthetic pathway of carlactone proposed by (Alder et al., 2012).

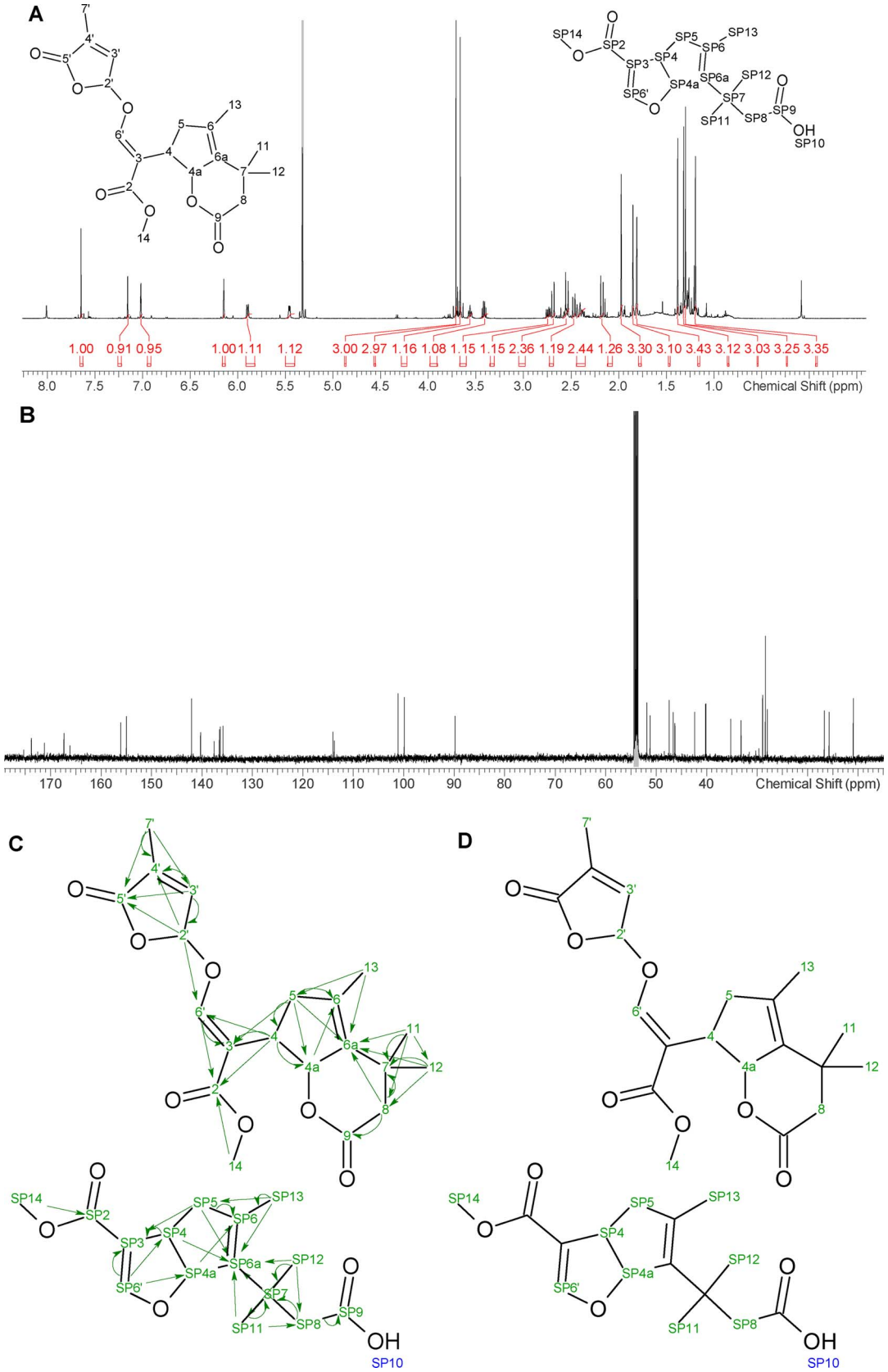


Fig. 2. NMR information for zeapyranolactone **2** and side product **3**. **A** 1H-spectra (600 MHz in CD_2Cl_2), **B** 13C-spectra (150 MHz in CD_2Cl_2), **C** (^1H,^{13}C)-HMBC correlations, **D** (^1H,^1H)-ROESY correlations, **E** (^1H,^1H)-COSY correlations.

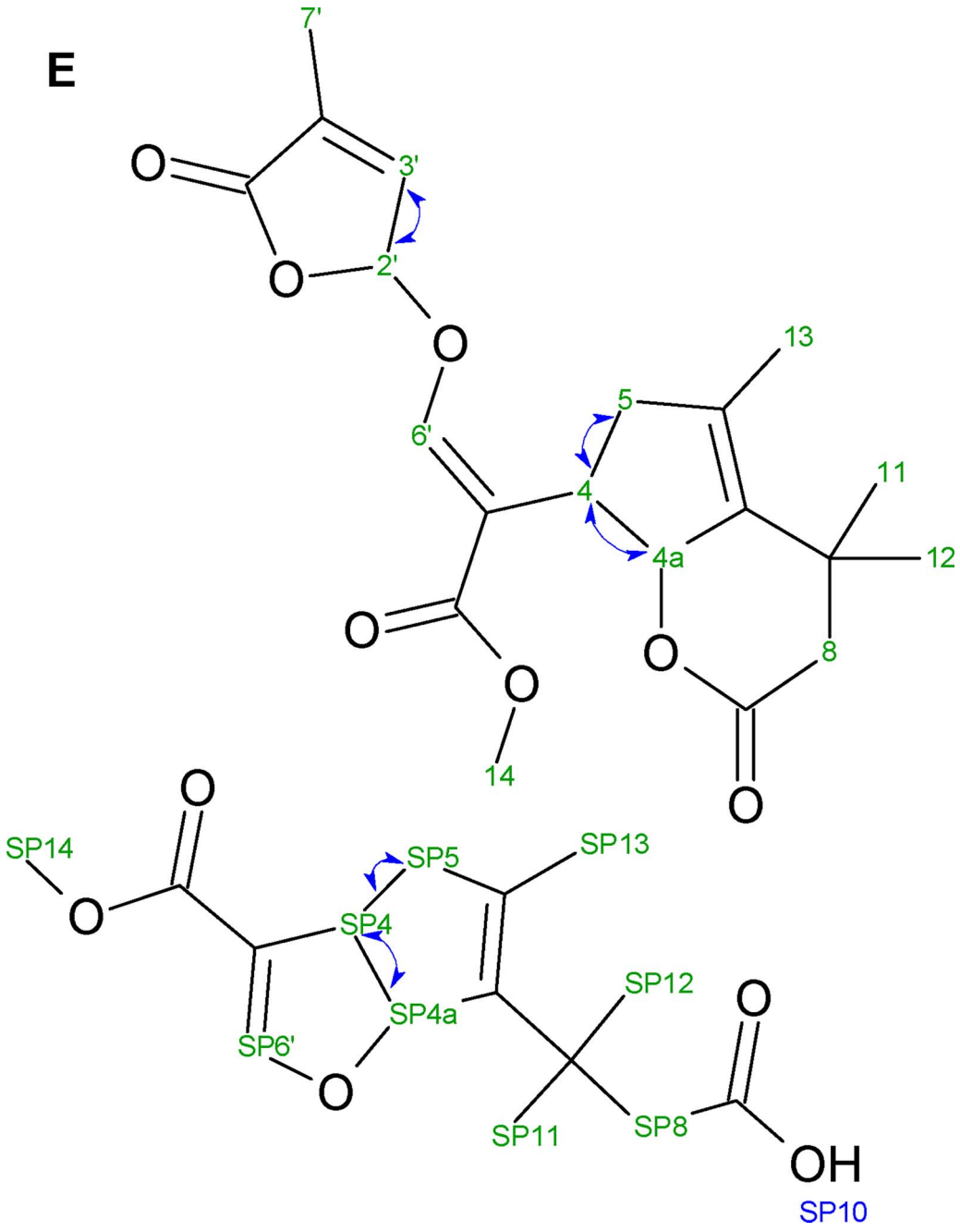


Fig. 2. (*continued*)

4. Experimental

NMR spectra were measured using a Bruker Avance III 600 MHz NMR spectrometer equipped with a Cryoprobe BBO Prodigy. A sample containing about 1.8 mg of the isolated zeapyranolactone was dissolved in 250 µL of CD_2Cl_2 and subjected to 1D, ^1H and ^{13}C NMR experiments as well as 2D ^1H-^1H-COSY, ^1H-^{13}C-HSQC (*Heteronuclear Single Quantum Coherence*), ^1H-^{13}C-HMBC (*Heteronuclear Multiple Bond Correlation*) and ^1H-^1H-ROESY (Rotating-Frame Overhauser Spectroscopy) NMR experiments using a microtube. Detailed acquisition and processing parameters can be found in the Supplementary information.

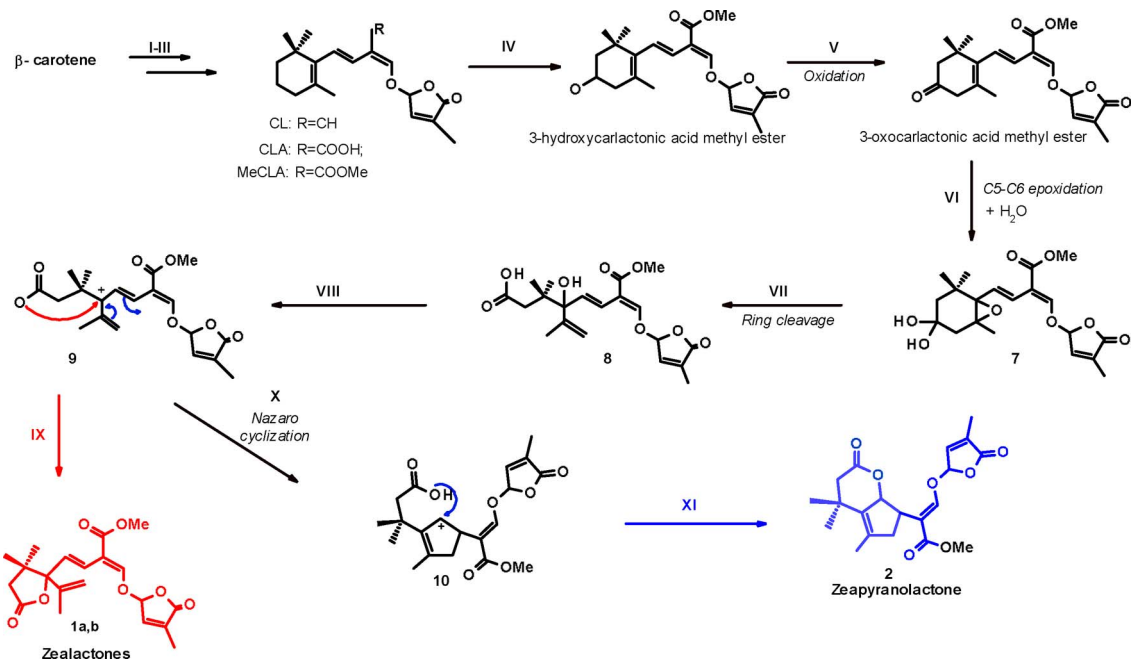
4.1. NMR spectroscopic data

The following abbreviations are used throughout this section: s = singlet; d = doublet; dd = doublet of doublets; t = triplet; quin = quintett; m = multiplet; br. = broad.

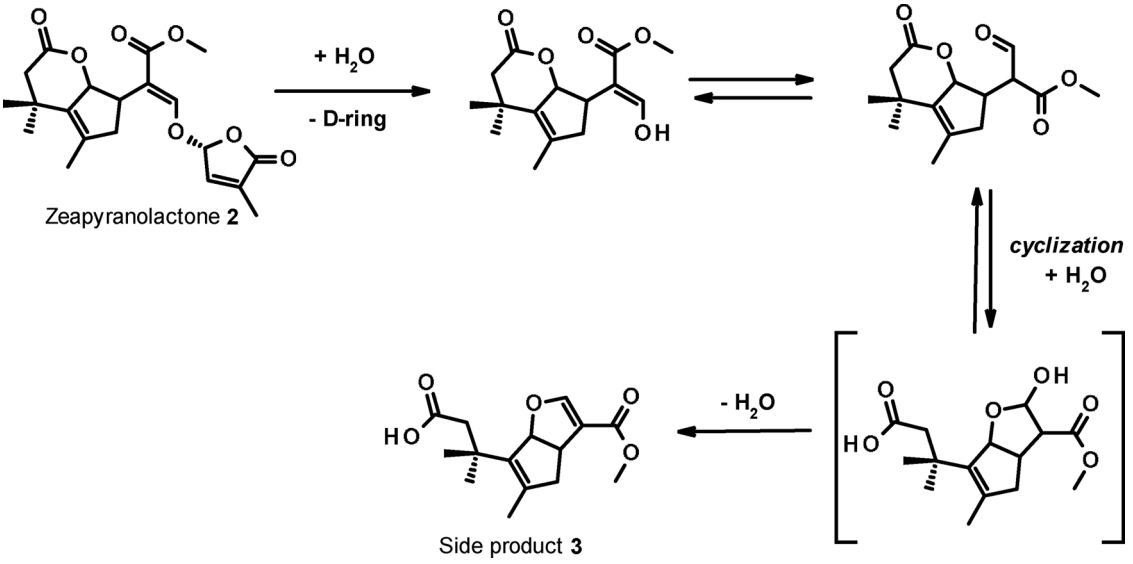
Methyl (E)-3-((4-methyl-5-oxo-2,5-dihydrofuran-2-yl)oxy)-2-(4,4,5-trimethyl-2-oxo-2,3,4,6,7,7a-hexahydrocyclopenta[b]pyran-7-yl)acrylate ***2*** and *3-[3-(Methoxycarbonyl)-5-methyl-4,6a-dihydro-3aH-cyclopenta[b]furan-6-yl]-3-methylbutanoic acid* **3.**

4.1.1. ^1H NMR (600 MHz, CD_2Cl_2)

δ ppm 1.19 (s, 3H, H11); 1.30 (s, 3H, H12); 1.32 (s, 3H, HSP12); 1.38 (s, 3H, HSP11); 1.81 (s, 3H, H13); 1.85 (q, J = 1.0 Hz, 3H, HSP13); 1.97 (t, J = 1.5 Hz, 3H, H7′); 2.18 (d, J = 14.4 Hz, 1H, H8a); 2.35–2.44 (m, 2H, H5a, HSP5a); 2.47 (d, J = 14.4 Hz, 1H, H8b); 2.51–2.59 (m, 2H, H5b, HSP8a); 2.69 (d, J = 14.8 Hz, 1H, HSP8b); 2.74 (dd, J = 17.5, 8.7 Hz, 1H, HSP5b); 3.41 (td, J = 8.9, 6.9 Hz, 1H, H4); 3.53–3.59 (m, 1H, HSP4); 3.66 (s, 3H, HSP14); 3.71 (s, 3H, H14); 5.46 (dsxt, J = 6.6, 2.1 Hz, 1H, H4a); 5.90 (br d, J = 9.2 Hz, 1H, HSP4a); 6.15 (quin, J = 1.4 Hz, 1H, H2′); 7.02 (quin, J = 1.6 Hz, 1H, H3′); 7.16



Scheme 1. Putative biosynthetic pathway for zealactones **1a/b** and zeapyranolactone **2** from β-carotene: Step I = biosynthesis of carlactone as described by (Alder et al., 2012); Step II-III = C-19-oxydation to carlactonoic acid and its methylation (Abe et al., 2014); Step IV = hydroxylation at C3 and step V = further oxidation of C3-hydroxyl to C3-carbonyl, 3-oxo-MeCLA. Step VI = epoxidation of the C5-C6 double bond resulting in intermediate **7**. Step VII = subsequent ring cleavage resulting in carboxylic acid **8** (Charnikhova et al., 2017). Partial ionization of **8** through dehydration (step VIII) and further cyclization (step IX) resulting in the two stereoisomers, zealactones **1a/b**. Step X = Nazarov cyclization of ion **9** resulting in intermediate **10**. Step XI = stabilization by deprotonation and lactone ring formation resulting in zeapyranolactone **2.**



Scheme 2. Putative degradation pathway from zeapyranolactone **2** to side product **3**. Hydrolysis of the D-ring linkage can be followed by tautomerisation to the aldehyde. Hydrolysis of the A-ring would cause the aldehyde to cyclise. Dehydration then leads to side product **3**.

(d, J = 1.5 Hz, 1H, HSP6′); 7.65 (s, 1H, H6′).

4.1.2. ^{13}C NMR (150 MHz, CD_2Cl_2)

δ ppm 11.0 (C7′); 15.7 (C13); 16.7 (CSP13); 28.0 (C11); 28.4 (C12, CSP11); 28.9 (CSP12); 33.2 (C7); 35.2 (CSP7); 40.2 (C4); 40.3 (CSP4); 42.4 (C5); 46.3 (CSP8); 46.6 (C8); 47.4 (CSP5); 51.3 (CSP14); 51.8 (C14); 89.8 (C4a); 99.9 (CSP4a); 101.2 (C2′); 113.8 (CSP3); 114.0 (C3); 135.8 (C4′); 136.4 (C6); 136.5 (C6a); 137.6 (CSP6a); 140.3 (CSP6); 142.1 (C3′); 154.9 (C6′); 156.1 (CSP6′); 166.1 (CSP2); 167.3 (C2); 171.2 (C5′); 173.8 (C9); 175.3 (CSP9).

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at https://doi.org/10.1016/j.phytol.2018.01.003.

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