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Sulfation of β-resorcylic acid esters—first synthesis of zearalenone-14-sulfate

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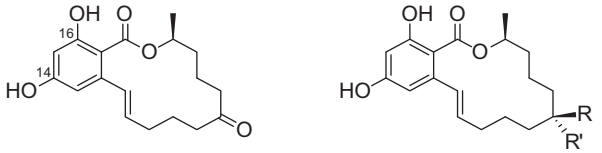
ABSTRACT

The chemical sulfation of β-resorcylic acid esters was investigated by applying state of the art procedures for the synthesis and deprotection of 2,2,2-trichloroethyl protected sulfates as appropriate intermediates. The selectivity of monosulfation was studied and reaction optimization was performed considering the effect of the solvent, different bases as well as the sulfation reagent itself. Finally the obtained protocols were applied for the first synthesis of zearalenone-14-sulfate (ammonium salt), an important conjugated (masked) mycotoxin, as reference material for further investigations in the field of bioanalytics as well as toxicology.

Zearalenone (ZEN, **1**, Fig. 1) is a mycotoxin produced by several plant pathogenic *Fusarium* species, including *Fusarium graminearum*, *Fusarium culmorum*, and *Fusarium cerealis*. This mycotoxin is common in maize, but also barley, oats, wheat, and rice are susceptible to contamination with ZEN.[1] *Fusarium* species are probably the most prevalent toxin-producing fungi of the northern temperate regions and are commonly found in cereals grown in America, Europe, and Asia, but also in Africa.[2,3] ZEN and its phase I metabolites possess estrogenic activity in mammals, including pigs, cattle, and sheep.[4] Problems of the reproductive tract as well as impaired fertility and abnormal fetal development in farm animals can be caused by ZEN.[5] Furthermore ZEN and its metabolites α-zearalenol (α-ZEL, **2a**) and β-ZEL (**2b**, Fig. 1) can interfere with various enzymes involved in steroid metabolism, which was recently investigated.[6–8] Therefore ZEN is of significant importance from an agricultural, economic, and health perspective.[9]

Additionally, conjugated mycotoxins, for example, glucosides and sulfates can emerge after metabolization by living plants.[10] The occurrence of ZEN-14-β,D-glucoside (**3**, Fig. 2) in wheat was shown by Schneweis et al.[11] and preparation of this conjugate to obtain reasonable amounts of reference material for further investigations has been first reported by Grabley et al. by applying an optimized Königs–Knorr procedure under phase transfer conditions.[12] Basically there are two sites for glycosylation present in



Zearalenone (**1**)

α-Zearalenol (**2a**): R = H, R' = OH
β-Zearalenol (**2b**): R = OH, R' = H

Figure 1. Structures of zearalenone (ZEN) and its main phase I metabolites α-zearalenol (α-ZEL) and β-ZEL.

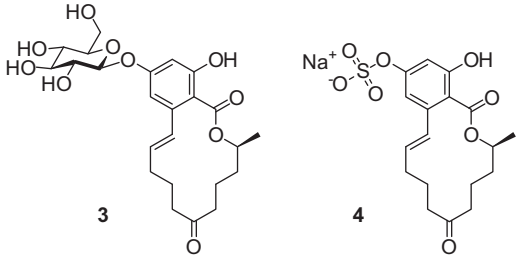


Figure 2. Structures of conjugated zearalenone (ZEN) metabolites: ZEN-14-β,D-glucoside (**3**) and ZEN-14-sulfate, sodium salt (**4**).

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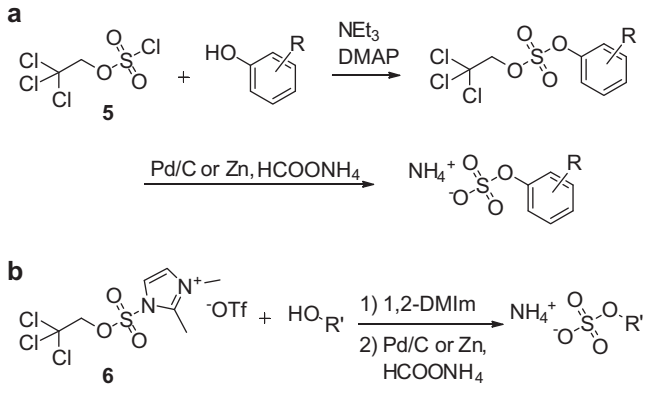
ZEN, but conjugation in position 14 (ring system numbering following the scheme proposed by Metzler[13]) is strongly favored compared to position 16.[14] ZEN-14-sulfate (formerly known as ZEN-4-sulfate) was first isolated by Plasencia and Mirocha from rice inoculated with *F. graminearum*.[15] El-Sharkawy et al. reported the conversion of ZEN into ZEN-14-sulfate (**4**, Fig. 2) by various microorganisms[16] and Berthiller et al. identified **4** as phase II metabolite of ZEN in the model plant *Arabidopsis thaliana*.[17] The conjugate most likely retains biological properties of the mycotoxin, since the sulfate moiety is easily cleaved under acidic conditions and in rats.[16] Therefore, ZEN-14-sulfate was included in analytical methods for the determination of free and masked mycotoxins during the last years.[18–21] Vendl et al. identified **4** as the most abundant analyte in 84 cereal based food products by LC–MS/MS measurements.[22]

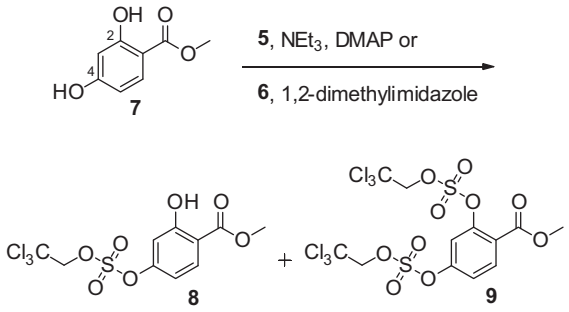
Nevertheless, reference material of **4** is still produced by time-consuming *F. graminearum* inoculation of rice. Due to its low stability the obtained sulfate is furthermore not suitable for long term storage.

Although alkyl and aryl sulfates as well as resorcylic acid lactones (cyclic 2,4-dihydroxybenzoates) such as ZEN are widespread in biological systems,[23–28] to the best of our knowledge there is still no procedure reported for the synthesis of sulfated resorcylic acid lactones or esters, which may be formed during plant or human metabolism (phase II detoxification). In general several synthetic methods were developed for the preparation of aryl sulfates, mainly by applying commercially available sulfur trioxide amine and amide complexes.[29] Since these methods are limited in terms of chemical modifications following installation of the sulfate group as well as regioselectivity, yield, and reproducibility, protecting groups for chemical sulfation were investigated during the last decade. Simpson and Widlanski introduced neopentyl and isobutyl chlorosulfates for chemical sulfation of phenols,[30] whereas the 2,2,2-trichloroethyl (TCE) group was used by Taylor and co-workers.[31] Application of the TCE group allows for efficient preparation (applying sulfuryl chloride **5** as reagent) and good stability of protected aryl sulfates. Catalytic transfer hydrogenation using Pd/C, ammonium formate ($HCOONH_4$) as well as cleavage by reaction with Zn/$HCOONH_4$ was reported for the deprotection of the TCE group under mild reductive conditions yielding arylsulfate ammonium salts (Scheme 1a). Additionally sulfuryl imidazolium salt **6** was introduced as reagent for incorporating trichloroethyl protected sulfate esters to carbohydrates (Scheme 1b).[32]

Considering different methods for the synthesis of aryl sulfates, we have started carrying out the chemical sulfation of β-resorcylic acid methyl ester (**7**) as a mimic for ZEN[14,33] by applying several



Scheme 1. (a) Synthesis of aryl sulfates by applying chlorosulfuric acid 2,2,2-trichloroethyl ester (**5**),[31] (b) sulfuryl imidazolium salt **6** for the preparation of alkyl sulfates (e.g. carbohydrates),[32] DMAP = 4-(dimethylamino)pyridine, and 1,2-DMIm = 1,2-dimethylimidazole.



Scheme 2. Chemical sulfation of β-resorcylic acid methyl ester (**7**), by applying sulfuryl chloride **5** or imidazolium triflate **6** as reagent.

sulfur trioxide complexes ($SO_3{\cdot}NMe_3$, $SO_3{\cdot}$Pyr, and $SO_3{\cdot}$DMF) all leading to complex product mixtures or low conversion of **7** as indicated by HPLC. Therefore, chlorosulfuric acid 2,2,2-trichloroethyl ester (**5**)[31] was used as the sulfation reagent for further investigations. In a first attempt reaction of **7** and **5** (1.2 equiv) in the presence of 4-(dimethylamino)pyridine (DMAP, 1.0 equiv) and NEt_3 (1.2 equiv), according to the original procedure of Taylor and co-workers[31] led to a conversion of 68% yielding the desired monosulfated product **8** (44%), but also the disulfate **9** (24%, Scheme 2). In contrast to glycosylation,[14,12] acetylation[34], silylation[35], and benzylation[36] no significant selectivity was observed for monosulfation in position 4 of compound **7** by applying this procedure. Since we have reasoned this outcome with the higher reactivity of sulfuryl chlorides compared to glycosyl donors as well as common acylation, silylation, and benzylation reagents, an optimization study was performed considering the effect of the solvent, different bases as well as the sulfation reagent itself (Table 1).

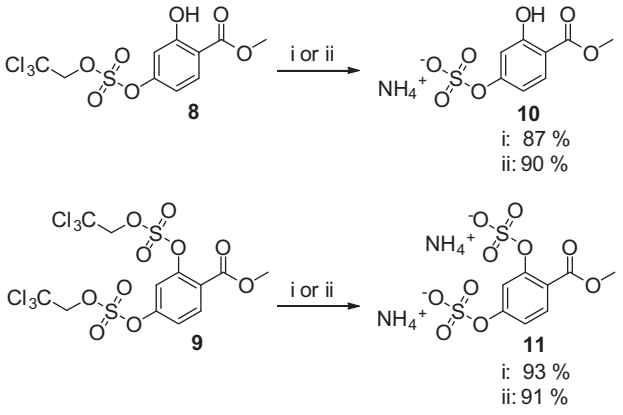
Starting by changing the solvent from tetrahydrofuran (THF) to dichloromethane (DCM) the selectivity of monosulfation by reaction of **7** with sulfuryl chloride **5** was increased to a **8**:**9** ratio of 3.5:1 using 0.5 M equiv of DMAP (Table 1, entry 5), whereas the application of pyridine instead of DMAP basically led to no (DCM) or only poor (THF) conversion of compound **7**. Interestingly sulfation of the monosulfate **8** (to form the disulfate **9**) was observed to occur faster than the sulfation of the ZEN mimic **7** when using an excess of DMAP instead of NEt_3/DMAP (entry 6). Basically higher selectivity was observed in DCM compared to reactions carried out in THF. Enhanced selectivity was achieved by applying imidazolium salt **6** for sulfation of **7**. This reaction was studied in terms of varying the amount of the sulfating reagent starting from 1.05 to 2.0 M equiv leading to **8**:**9** ratios between 8:1 and 1:3. In particular using 1.05 equiv of **6** and 1.5 equiv of 1,2-dimethylimidazole (1,2-DMIm), sulfation at 20 °C afforded 80% of the monosulfated product **8** (entry 7). Applying these conditions at −10 °C (entry 11) or using exactly one equivalent of the reagent (entry 12) did not lead to a significant improvement in terms of selectivity and conversion. Therefore conditions according to entry 7 were applied for the sulfation of **7** affording compounds **8** and **9** in reasonable isolated yields (75% and 10%, respectively). These TCE protected sulfates were readily separated and purified by silica gel chromatography revealing an important advantage of this strategy compared to common methods directly leading to nonprotected sulfate salts.

Deprotection of TCE protected sulfates was carried out by applying both strategies known from the literature. Catalytic transfer hydrogenation (Pd/C, $HCOONH_4$) as well as cleavage by reaction with zinc/ammonium formate ($HCOONH_4$) yielded the desired monosulfate **10** as well as the disulfate **11** as ammonium salts in excellent yields after purification by simple filtration over a pad of silica gel eluting with DCM/MeOH/NH_4OH (10:4:1) (Scheme 3). Both compounds were observed to be stable in solid form as well

Table 1
Optimization study on the sulfation of β-resorcylic acid methyl ester (**7**) via Scheme 1

Entry	Reagent (equiv)	Conditions[a]	**8** (%)[b]	**9** (%)[b]	Unreacted **7** (%)[b]
1	**5** (1.20)	NEt_3 (1.2 equiv), DMAP (1.0 equiv), THF, 20 °C	44	24	32
2	**5** (1.20)	NEt_3 (1.2 equiv), DMAP (1.0 equiv), DCM, 20 °C	67	20	13
3	**5** (1.20)	NEt_3 (1.2 equiv), Pyridine (1.0 equiv), THF, 20 °C	4	0	96
4	**5** (1.20)	NEt_3 (1.2 equiv), Pyridine (1.0 equiv), DCM, 20 °C	No conversion		100
5	**5** (1.20)	NEt_3 (1.2 equiv), DMAP (0.5 equiv), DCM, 20 °C	73	21	6
6	**5** (1.20)	DMAP (2.0 equiv), DCM, 20 °C	17	48	35
7	**6** (1.05)	1,2-DMIm (1.5 equiv), DCM, 20 °C	80	11	9
8	**6** (1.25)	1,2-DMIm (1.5 equiv), DCM, 20 °C	71	24	5
9	**6** (1.50)	1,2-DMIm (1.5 equiv), DCM, 20 °C	52	47	1
10	**6** (2.00)	1,2-DMIm (1.5 equiv), DCM, 20 °C	23	77	0
11	**6** (1.05)	1,2-DMIm (1.5 equiv), DCM, -10 °C	64	18	18
12	**6** (1.00)	1,2-DMIm (1.5 equiv), DCM, 20 °C	80	8	12

[a] Addition of reagent **5** or **6** at −10 °C to the reaction mixture and stirring over-night at the indicated temperature (DMAP = 4-(dimethylamino)pyridine, 1,2-DMIm = 1,2-dimethylimidazole).
[b] Determined by ^1H NMR after aqueous work-up (integration of H-6 signals; see Supplementary data for detailed information).



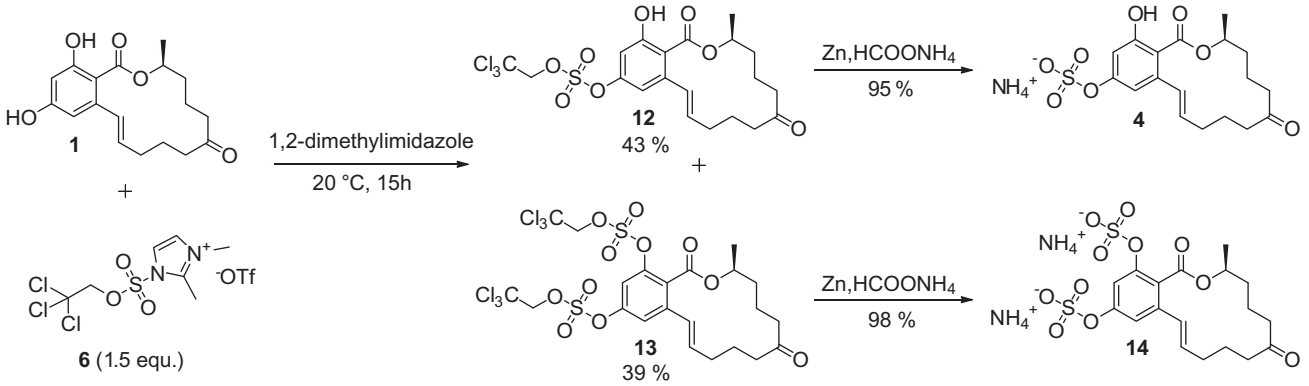
Scheme 3. Deprotection of TCE sulfates **8** and **9** ((i) Pd/C, $HCOONH_4$; (ii) Zn, $HCOONH_4$).

as dissolved in methanol at −20 °C and even room temperature (RT) for several weeks and days, respectively. This outcome was quite surprising, since these compounds were originally suspected to be unstable in protic solvents. An NMR sample of the disulfate **11** (in MeOH) showed slight degradation to the monosulfate **10** after several days at RT indicating lower stability of the sulfate moiety in position 2 compared to the 4-sulfate.

Based on these results and by applying the obtained optimized procedures, ZEN-14-sulfate (**4**) as well as ZEN-14,16-disulfate (**14**) were prepared in high overall yields. Due to the unexpected noticeable stability of compound **11** we also got interested in the preparation of disulfated ZEN (**14**) which is why reaction conditions according to entry 9 (see Table 1) were applied yielding both protected sulfates, **12** and **13** in an approximate ratio of 1:1. To avoid undesired hydrogenation of the conjugated double bond present in ZEN, Zn/$HCOONH_4$ was applied for reductive cleavage of TCE groups (Scheme 4).

The protected ZEN sulfates **12** and **13** were separated and purified by silica gel chromatography. Similar to compounds **10** and **11**, both ZEN sulfates, **4** and **14** were obtained as ammonium salts in pure form after short filtration over a pad of silica gel eluting with DCM/MeOH/NH_4OH (10:4:1). The obtained ZEN-14-sulfate was identical (NMR, ESI-MS) to natural occurring material that was previously isolated and characterized.[15,37]

In conclusion, the chemical sulfation of β-resorcylic acid esters by applying TCE protection was investigated leading to improved procedures for selective monosulfation as well as for simultaneous preparation of mono- and disulfates. Sulfuryl imidazolium salt **6**, which can be easily prepared in large scale starting from 2,2,2-trichloroethanol,[32] was shown to be an appropriate reagent for controlled synthesis of aryl sulfates and reductive conditions were applied for efficient deprotection of the TCE group after simple purification of the intermediates by silica gel chromatography. These protocols were used for the first chemical synthesis of zearalenone-14-sulfate (**4**). This fast and efficient procedure can easily be reproduced in other labs to obtain the masked/conjugated mycotoxin **4** in reasonable amounts and in short time for further investigations, for example, as reference material for bioanalytical studies or for toxicological testing. Additionally, the stability of ammonium salts of these sulfates was reported to be adequate for long term storage in solid or dissolved form.



Scheme 4. Preparation of ZEN-14-sulfate (**4**) and ZEN-14,16-disulfate (**14**) using sulfuryl imidazolium salt **6** and an optimized procedure for the sulfation of ZEN. Reductive conditions (Zn, $HCOONH_4$) were applied for the cleavage of the 2,2,2-trichloroethyl group.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.tetlet.2013.04.059.

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