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Sorbifuranones A–C, sorbicillinoid metabolites from *Penicillium* strains isolated from Mediterranean sponges

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ABSTRACT

Three novel natural products, sorbifuranones A–C (**4**–**6**), were isolated from a *Penicillium chrysogenum* fungus isolated from the marine sponge *Ircinia fasciculata*. Sorbifuranones B (**5**) and C (**6**) and 2′,3′-dihydrosorbicillin, a putative precursor to sorbifuranone B, were also found in the culture of another *Penicillium* strain, which was isolated from the sponge *Tethya aurantium*. Their constitutions were elucidated mainly by 2D NMR. NOE correlations in combination with quantum chemical calculations and comparison of experimental and calculated electronic circular dichroism (CD) spectra permitted assignment of the absolute configuration of sorbifuranone C. The structures hint at a two-step cleavage-cyclization sequence of sorbifuranone A (**4**) leading to the spiro compound sorbifuranone C, while sorbifuranone B is likely to be the respective cleavage product of a putative 2′,3′-dihydrosorbifuranone A, which cannot cyclize further.

1. Introduction

Sorbicillin (**1**) is a twofold *C*-methylated fungal hexaketide. It was discovered as an impurity during early penicillin production[1] and soon turned out to be the key representative of the sorbicillinoids, a structurally diverse family of fungal metabolites originating from the highly reactive precursor sorbicillinol (**2**).[2–4] These polyketide-derived compounds are produced by a variety of fungal strains of *Penicillium*,[5] *Trichoderma*,[2] *Verticillium*,[6] and *Acremonium* species.[7]

Sorbicillinoids can be classified into three structural groups: monomeric sorbicillinol derivatives, bisvertinoids, and products of mixed origin. Starting from sorbicillinol (**2**), reactive oxygenated products are formed, among them epoxysorbicillinol[8] and oxosorbicillinol.[9] Reactions of two molecules of **2**—mainly cycloadditions, Michael additions, or hemiketalizations—lead to the structurally diverse group of bisvertinoids like, e.g., bisvertinol,[6] bisorbicillinol,[10] and trichodimerol.[11] The third class of sorbicillinol-derived natural products originates from sorbicillinol and a non-sorbicillinoid reaction partner; examples of this group are the rezishanones,[12] JBIR-59,[13] and sorbicillactone A (**3**),[5] the only nitrogen-containing sorbicillinol derivative.

The three new fungal metabolites, sorbifuranones A–C (**4**–**6**) belong to this third group of sorbicillinol derivatives of mixed origin. In this paper we describe their isolation from marine sponge-derived fungi and their structural assignment (Fig. 1).

2. Results and discussion

2.1. Isolation and structural elucidation of sorbifuranones A–C (4–6)

The culture extract of the sponge-derived *Penicillium chrysogenum* strain E01-10/3, which had previously been shown to produce sorbicillactone A (**3**),[5] was investigated by HPLC-UV and -MS. Besides **3**, the known alkaloids meleagrin[14] and roquefortin[15] were found, as well as three unidentified compounds, **4**–**6**. These metabolites were not only isolated from *P. chrysogenum* strain E01-10/3, but also from strain R03-8/4, to provide enough material for detailed structural elucidation work.

For compound **4** ESIMS suggested a molecular formula of $C_{24}H_{28}O_8$ (*m*/*z* 445.1852 $[M+H]^+$, 445.1862 calcd for $C_{24}H_{29}O_8^+$), which was in agreement with the number of carbons detected in the ^{13}C NMR experiment. Comparison of the NMR data (Table 1) with those of sorbicillactone A (**3**)[5] indicated the presence of a sorbicillinol moiety, yet with a different 'southeastern' portion as compared to **3**. By COSY, HMQC, and HMBC experiments two

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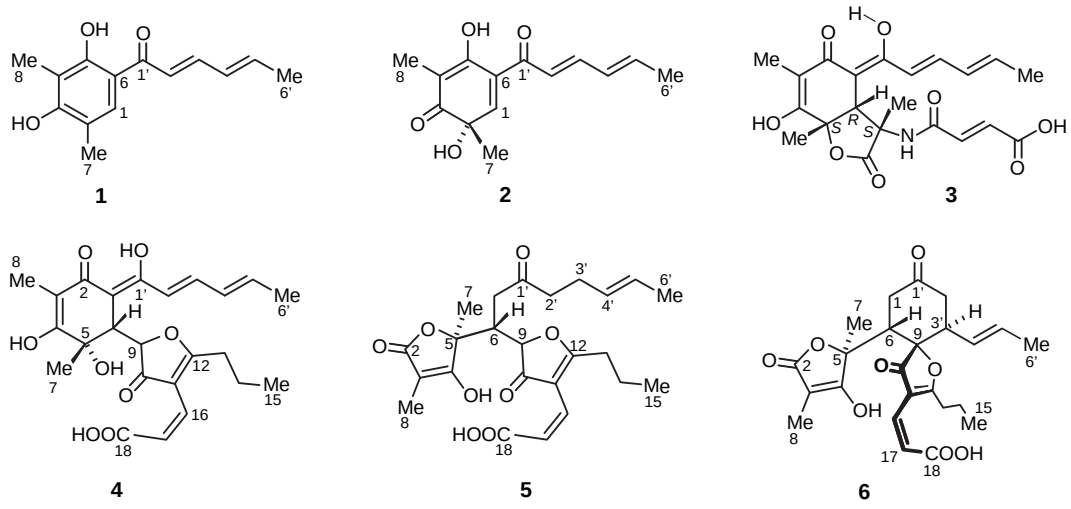


Fig. 1. Structures of sorbicillin (**1**), sorbicillinol (**2**), sorbicillactone A (**3**), and the new, related metabolites, the sorbifuranones A (**4**), B (**5**), and C (**6**).

Table 1
NMR data of sorbifuranone A (**4**) in MeOH-d_4

Position	δ_C	δ_H, mult.	HMBC	NOESY	COSY [J_{HH} (Hz)]
1	102.5				
2	194.0				
3	109.2				
4	172.0				
5	71.9				
6	50.6	3.38 d	1, 2, 4, 5, 7, 10, 1′	7, 9, 2′	9 (1.5)
7	28.4	1.47 s	4, 5, 6	6	
8	7.9	1.73 s	2, 3, 4		
9	84.7	5.27 d	1, 5, 6, 10, 12	6	6, 13 (<1)
10	203.8				
11	115.6				
12	193.6				
13	33.6	2.42 t	11, 12, 14, 15	16	9, 14 (7.8)
14	20.3	1.50 m	12, 13, 15		13, 15 (7.3)
15	14.3	0.88 t	13, 14		14
16	130.6	6.30 d	10, 11, 12, 18	13	17 (11.9)
17	124.4	5.92 d	11, 18		16
18	169.4				
1′	169.1				
2′	121.9	6.16 d	1, 1′, 3′, 4′	6	3′ (14.7)
3′	139.9	7.00 dd	1′, 4′, 5′	5′	2′, 4′(10.9)
4′	132.7	6.24 m	5′, 6′	6′	3′, 5′, 6′ (1.4)
5′	137.4	6.02 m	3′, 4′, 6′	3′	4′, 6′ (6.8)
6′	18.8	1.84 dd	2′, 4′, 5′	4′	4′, 5′

additional substructures were identified: an *n*-propyl residue and an α,β-unsaturated carboxylic acid. Its *Z*-configuration was deduced from the $^3J_{HH}$ coupling constant of 11.9 Hz (Fig. 2). The remaining ^{13}C NMR signals were attributed to an oxygen-substituted methine carbon C-9 at 84.7 ppm with a proton signal at 5.27 ppm and to three quaternary carbons C-10, C-11, and C-12 with chemical shifts of 203.8, 115.6, and 193.6 ppm, respectively. The three-bond

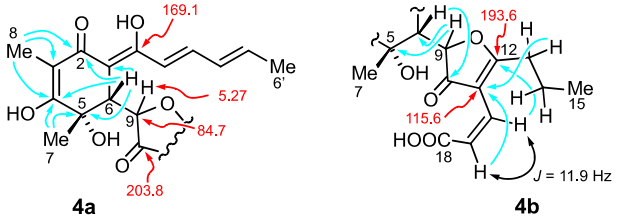


Fig. 2. Selected HMBC correlations (blue arrows) of the northwestern (**4a**) and of the southeastern (**4b**) partial structures for sorbifuranone A (**4**) (in red: selected ^1H and ^{13}C NMR shifts in ppm).

coupling of H-17 to C-11 suggested the unsaturated carboxylic acid fragment to be bound to C-11. Several other HMBC correlations (Fig. 2) showed that C-9 was attached to C-6 of the sorbicillinol moiety. The combined coupling and chemical shift data pointed at the 3-oxo-2,3-dihydrofuranone structure **4**. Due to the furanone structural element and its apparent biosynthetic relationship to sorbicillinol (**2**), this new compound was named sorbifuranone A. The structural hypothesis was supported by comparison of the chemical shifts with those published for (5-butyl-3-oxo-2,3-dihydrofuranone-2-yl) acetic acid, a natural plant product isolated from the eastern daisy fleabane, *Erigeron annuus*.[16] While the cis-configuration of H-6 and the methyl group C-7 at the cyclohexanone ring was deduced from the NOESY correlation between H-6 and H-7, no information about the stereochemical array at C-9 was obtained from the spectroscopic data. The shown (*S*)-configuration of C-5 is based on biosynthetic considerations and on the absolute configuration of sorbifuranone C (6) established below.

By ESIMS the molecular formula of sorbifuranone B (**5**) was established as $C_{24}H_{30}O_8$ (*m*/*z* 445.1856 $[M-H]^-$, 445.1862 calcd for $C_{24}H_{29}O_8^-$). The NMR data revealed the presence of the same dihydrofuranone substructure as in **4**, but with a 2′,3′-dihydrosorbyl residue instead of a sorbyl side chain as in **4**. The chemical shift of the carbonyl carbon of this side chain (C-1′, 207.0 ppm) is significantly higher than that of the corresponding atom (169.1 ppm) in sorbifuranone A (**3**), indicating that the respective carbonyl function is not enolized (Table 2), which is a consequence of the absence of a carbonyl group in β-position of C-1′. Analysis of the long-range H,C-correlations, starting from the methylene protons H-1_a and H-1_b as well as the methyl groups H-7 and H-8, indicated the presence of a 2,4-dimethyl-3-hydroxybutenolide system. Such structures are known to originate from a rearrangement of the central 6-ring of sorbicillinoids like, e.g., in bisorbibutenolide, which is formed by a similar cleavage reaction of bisorbicillinol.[10,17]

HMBC and NOESY correlations (Fig. 3) permitted to assemble the identified partial structures to give the overall constitution of sorbifuranone B (**5**). A determination of the relative configuration of the two stereocenters by NOESY methods was not possible because of the freely rotating bond between C-5 and C-6.

By HRESIMS, sorbifuranone C (**6**) was shown to have the same molecular formula as **4** (*m*/*z* 443.1715 $[M-H]^-$, 443.1706, calcd for $C_{24}H_{27}O_8^-$). The NMR data indicated the presence of a butenolide residue identical to that in **5**, again with a dihydrofuranone moiety, albeit with chemical shifts slightly different from those in **4** and **5**,

Table 2
NMR data of sorbifuranone B (**5**) in acetone-d_6

Position	δ_C	δ_H, mult.	HMBC	NOESY	COSY [J_{HH} (Hz)]
1	36.8	α 2.71 dd	5, 6, 1′	1β, 6, 7, 2′	1β (18.3), 6 (7.6)
		β 2.28 dd	5, 6, 1′	1α, 6, 2′	1α, 6 (3.2)
2	173.3				
3	97.1				
4	178.0				
5	82.9				
6	42.0	3.25 dd	1, 4, 5, 7, 10, 1′	1α, 1β, 7, 2′	1α, 1β
7	22.0	1.50 s	4, 5, 6	1α, 6	
8	6.4	1.65 s	2, 3, 4, 5, 7		
9	83.9	4.68	1, 3, 6		
10	198.9				
11	115.0				
12	190.7				
13	32.4	2.60 m	11, 12, 14, 15	14, 15, 16	14
14	20.1	1.75 m	12, 13, 15	13, 15, 16	13, 15 (7.4)
15	14.1	1.00 t	13, 14	13, 14, 16	14
16	130.2	6.49 d	10, 11,12, 17, 18	13, 14, 15	17 (11.7)
17	123.8	5.97 d	11, 18	16	16
18	167.1				
1′	207.0				
2′	42.7	2.39 t	1′, 3′, 4′	1α, 1β, 6, 3′, 5′	3′ (7.6)
3′	27.4	2.12 m	4′, 5′	2′, 5′	2′, 4′, 5′
4′	131.0	5.38 m	6′	6′	3′, 6′
5′	126.0	5.43 m	3′, 6′	2′, 3′, 4′	3′, 6′
6′	18.1	1.58 dm	4′, 5′	4′, 5′	4′, 5′

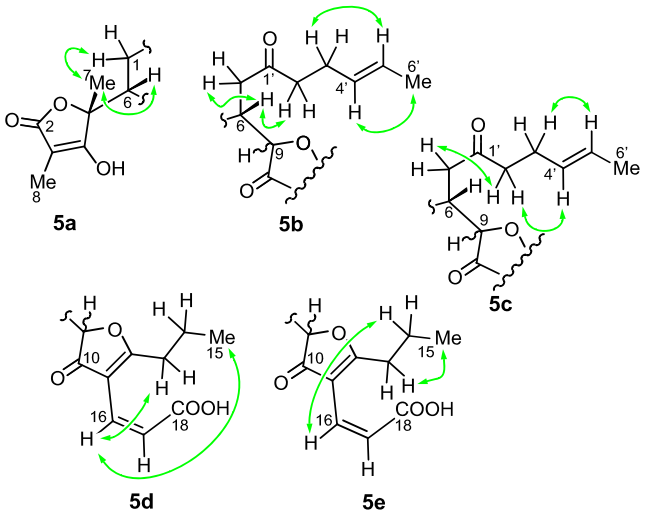


Fig. 3. NOESY (green double arrows) correlations of partial structures **5a-e** for sorbifuranone B (**5**).

which could be attributed to C-9 being quaternary in **6**. As in **5**, a 2′,3′-dihydrosorbyl moiety was detected, but with C-3′ being a tertiary C-atom. The HMBC correlations H-3′ to C-9 and H-3′ to C-10 clearly indicated a bond between C-3′ and C-9. Further evidence for this ring closure was the chemical shift difference of the diastereotopic protons at C-1, which was significantly larger than in the open-chain compound **5** (Table 1). Therefore, the structure of **6** had to correspond to a cyclic analog of **5** with an additional bond between C-3′ and C-9. The relative configuration of C-6 versus C-3′, i.e., of two of the three stereocenters in the cyclohexanone system, were assigned as trans (i.e., as 6*S*,3′*S* or 6*R*,3′*R*) based on the NOE interactions detailed in Fig. 4.

2.2. Relative configuration of sorbifuranone C (6)

From the above-discussed NMR investigations, only the relative configuration at C-6 versus C-3′ of sorbifuranone C (**6**) could be established, but not that of the spiro center C-9. For the assignment of this missing piece of stereoinformation an in-depth analysis of the conformational behavior by quantum chemical calculations was performed, in combination with the NMR results. These investigations showed that the initially neglected weak NOE correlations from H-16 to other parts of the molecule and those between H-14 and other protons were decisive for the determination of the relative configuration of the spiro center at C-9 (Fig. 4).

The proton H-14 is located in a quite flexible side chain, which made the interpretation of its NOESY/ROESY correlations difficult. The interaction of H-16 with H-7 was tricky, too, since it does not only depend on the unknown configuration of the molecule but also on its conformational behavior. Therefore, the NOE correlation between H-16 and H-4′ seemed to be the most promising one to determine the configuration of the spiro center C-9 relative to the stereogenic center at C-3′ in the six-membered ring (Fig. 4, structure **6f**, red arrow). It thus had to be investigated by conformational analysis in which of the four remaining diastereomers this interaction was possible—(5*R*,6*S*,9*S*,3′*S*), (5*S*,6*S*,9*S*,3′*S*), (5*R*,6*S*,9*R*,3′*S*), or (5*S*,6*S*,9*R*,3′*S*) (and the corresponding enantiomers). These computational investigations were initially done by using the B3LYP/SVP[18,19] method.

Surprisingly, for all considered diastereomers none of the conformers identified within a range of 12 kJ/mol above the global minimum permitted any NOE correlation between H-16 and H-4′. In all cases the distance between these protons was much larger than the usual maximum distance for NOE correlation of ca. 5 Å.[20] Single-point energy calculations including solvent effects[21] by the COSMO[22] model substantially changed the relative energies of all found conformers, thus making an NOE correlation between H-16 and H-4′ imaginable, for which the most probable configurations were (*S*,*S*,*S*,*S*) and (*S*,*S*,*R*,*S*)[23] and their enantiomers (for details, see Table 4). Only these stereoisomers were found to have main conformers with distances between the mentioned protons that were sufficiently short for at least weak NOE effects. Although in the case of (*R*,*S*,*S*,*S*) and (*R*,*S*,*R*,*S*) some conformers were found whose protons at C-16 and C-4′ were close enough for a weak correlation, it should be hardly possible to find any significant NOE, because of the low calculated population of these conformers. These results showed that the investigated NOE correlation was more probable in the (*S*,*S*,*S*,*S*)- or the (*S*,*S*,*R*,*S*)-configuration.

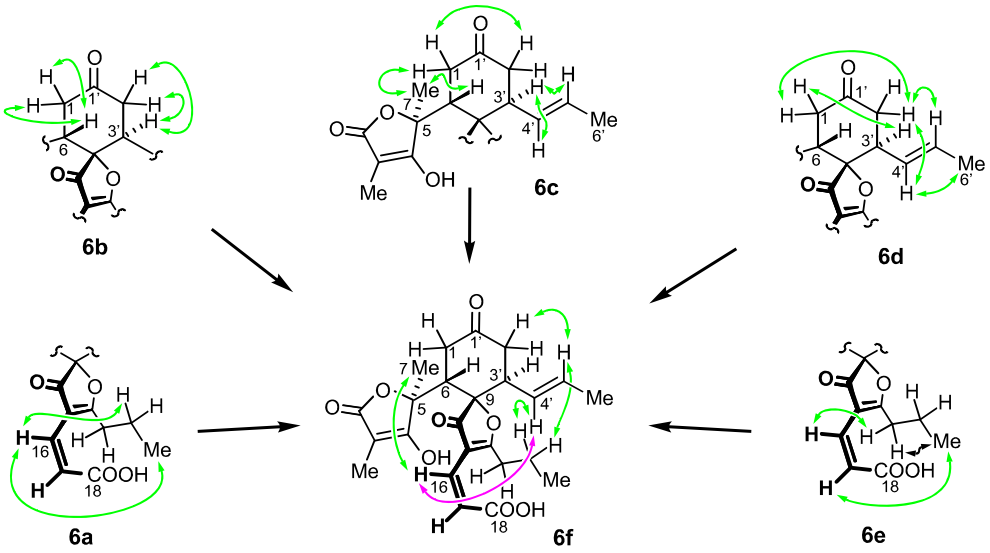


Fig. 4. Partial structures **6a**–**e** assembled to the gross structure **6f** (constitution and, in part, relative configuration) by NOESY correlations (green double arrows, magenta arrow: NOE decisive for the configuration at C-9 relative to C-3′).

Table 3
NMR data of sorbifuranone C (**6**) in acetone-d_6

Position	δ_C	δ_H, mult.	HMBC	COSY [J_{HH} (Hz)]	NOESY	ROESY
1	37.6	α 2.23 dd	5, 6, 9, 1′, 2′	1β (16.1), 6 (6.0)	1β, 6, 7, 2′α	1β, 2′α
		β 2.94 dd	5, 6, 9, 1′, 2′	1α, 6 (5.5),	1α, 6, 2′α, 2′β, 3′	1α,
2	173.0					
3	97.0					
4	176.5					
5	84.8					
6	43.8	2.71 t	1, 4, 5, 9, 10, 1′, 3′	1α, 1β	1α, 1β, 7	7
7	24.5	1.58 s	4, 5, 6		1α, 6, 13β, 16, 17, 3′	6, 3′
8	6.4	1.65 s	2, 3, 4, 5			
9	90.0					
10	200.3					
11	114.3					
12	188.5					
13	32.2	α 2.61 m	11, 12, 14, 15	13α, 14	13β, 14, 15, 16	14
		β 2.72 m	11, 12, 14, 15	13β, 14	7, 13α, 14, 15, 16	14
14	20.3	1.76 m	12, 13,15	13α, 13β, 15 (7.4)	13α, 13β, 15, 16, 5′	13α, 13β, 15
15	14.1	1.01 t	13, 14	14	13α, 13β, 14, 16, 17, 4′, 5′	14
16	129.5	6.54 d	10, 11, 12, 17, 18	17 (11.7)	7, 13α, 13β, 14, 15, 17, 4′	17
17	123.7	6.00 d	10, 11, 18	16	7, 15, 16	
18	167.2					
1′	205.8					
2′	41.9	α 2.39 dd	9, 1′, 3′, 4′	2′β (16.1), 3′(5.8) 2′α, 3′	1β, 2′β, 3′, 4′, 5′	2′β, 3′
		β 2.67 dd	9, 1′, 3′, 4′		1β, 2′α, 3′	2′α, 3′
3′	44.6	3.35 m	6, 9, 10, 1′, 2′, 4′, 5′	2′α, 2′β, 4′ (8.4), 5′ (0.9)	1β, 7, 2′α, 2′β, 4′, 5′	7, 2′α, 2′β, 4′, 5′
4′	128.2	5.28 ddd	2′, 3′, 6′	3′, 5′ (15.1), 6′ (1.7)	15, 16, 2′α, 2′β, 3′, 5′, 6′	2′α, 3′, 5′, 6′
5′	130.3	5.60 m	9, 3′, 6′	3′, 4′ 6′ (6.5)	14, 15, 2′α, 2′β, 3′, 4′, 6′	2′α, 3′, 4′, 6′
6′	18.2	1.60 dd	9, 4′, 5′	4′, 5′	4′, 5′	

2.3. Absolute configuration of sorbifuranone C (6)

These remaining possible isomers of **6**, (*S*,*S*,*S*,*S*)-**6** and (*S*,*S*,*R*,*S*)-**6**, were further investigated by CD calculations. Unfortunately time dependent DFT calculations using the B3LYP hybrid functional gave only ambiguous results. None of the calculated spectra matched with the experimental CD spectra (not shown) and thus higher-level methods had to be applied.

Comparison of the CD spectra of (*S*,*S*,*S*,*S*)-**6** and of (*S*,*S*,*R*,*S*)-**6** (and of their enantiomers) calculated by MRCI/SV(P),[19,24] with the experimental CD curve of sorbifuranone C (**6**) (Fig. 5) revealed that only the spectrum calculated for (*S*,*S*,*S*,*S*) showed a good agreement, with a first negative Cotton effect (CE) at 300 nm, a positive one at 250 nm, and a negative one at 220 nm. While the CD curve computed for (*R*,*R*,*R*,*R*) was fully mirror-imaged as compared to the experimental one, the spectra quantum chemically predicted for (*S*,*S*,*R*,*S*) and (*R*,*R*,*S*,*R*) did not fit, at least for parts of the curves. From the conformational analysis in combination with the NOE correlations and from the calculated CD spectra the absolute configuration of sorbifuranone C (**6**) was determined as (5*S*,6*S*,9*S*,3′*S*).

To further exclude that the natural product possessed an (*R*,*S*,*S*,*S*)- or an (*R*,*S*,*R*,*S*)-configuration (or the respective enantiomeric array), the CD spectra calculated for these isomers were compared with the experimental data, too (Fig. 6). As expected, none of the computed curves matched with the experimental one, unequivocally excluding these alternative configurations for the structure of the isolated sorbifuranone C (**6**).

Table 4
Energies, distances between H-16 and H-4′, and Boltzmann factors B_f of the conformers of the possible diastereomers of sorbifuranone C (**6**) optimized by B3LYP/SVP. Energies are taken from single-point energy calculations with COSMO

Conformer	Relative energy (kJ/mol)	Distance H-16–H-4′ (Å)	B_f (%)
(*S*,*S*,*S*,*S*) and (*R*,*R*,*R*,*R*):			
Conf_1	0.00	4.36	45
Conf_2	0.88	6.91	32
Conf_3	3.10	4.69	13
Conf_4	3.18	7.16	10
(*R*,*S*,*S*,*S*) and (*S*,*R*,*R*,*R*):			
Conf_5	0.00	7.16	35
Conf_6	0.33	5.25	31
Conf_7	1.80	4.61	17
Conf_8	2.51	7.20	13
Conf_9	6.87	7.27	2
Conf_10	8.62	5.24	1
Conf_11	9.34	4.57	1
(*S*,*S*,*R*,*S*) and (*R*,*R*,*S*,*R*):			
Conf_12	0.00	4.36	43
Conf_13	0.92	4.54	30
Conf_14	1.13	5.47	27
(*R*,*S*,*R*,*S*) and (*S*,*R*,*S*,*R*):			
Conf_15	0.00	7.32	41
Conf_16	0.80	6.95	30
Conf_17	3.39	4.66	10
Conf_18	3.60	5.50	10
Conf_19	3.58	4.71	9

2.4. Proposed biosynthetic origin of sorbifuranones A–C (4–6)

A common structural portion of all of the sorbifuranones A–C (**4**–**6**) is the dihydrofuranone moiety, which has so far not been reported as part of sorbicillinoid compounds. Biosynthetically the molecular frameworks of **4**–**6** could originate from an oxidative ring cleavage of the pentaketidic butyrophenone **7a**, with subsequent reduction of the resulting aldehyde **7b** and ring closure by enolether formation of the primary alcohol thus formed with the keto group at C-1′ to give the furanone **7c**. A similar pathway is known from the biosynthesis of the mycotoxin patulin in *Penicillium patulum*.[25] Michael addition of the nucleophilic enolate of **7c** to sorbicillinol (**2**) would eventually lead to sorbifuranone A (**4**) (Scheme 1).

Sorbifuranone B (**5**) can be considered as the rearrangement product of a hypothetical 2′,3′-dihydro product **8** of sorbifuranone A (**4**) (Scheme 2), resulting from a nucleophilic attack of the hydroxy function at C-5 of **8** to the carbonyl at C-2 with cleavage of the C-2–C-1 bond. Such rearrangement reactions have already been reported earlier for other sorbicillinoid compounds.[17]

The formation of sorbifuranone C (**6**) (Scheme 2) is closely similar to that of **5** and results in the hypothetical 2′,3′-didehydrosorbifuranone B (**9**), which, different from **5** itself, can undergo an intramolecular Michael reaction of C-9 of the enolized form of **9** to C-3′ of the sorbyl chain, thus leading to the cyclohexanone system observed in **6**. It is as yet unknown at what time of the biosynthesis the hydrogenation of the α,β-double bond (C-2′=C-3′) occurs, which precludes a similar ring closure for sorbifuranone B (**5**) itself.

3. Conclusions

Sorbifuranones A–C (**4**–**6**) constitute a novel class of stereochemically intriguing sorbicillinol-derived metabolites. While it has so far been impossible to elucidate the stereostructures of the sorbifuranones A (**4**) and B (**5**), an assignment of the relative and of the absolute configuration succeeded for the structure of the more rigid, since further cyclized sorbifuranone C (**6**), by an efficient combination of NMR and CD measurements and quantum chemical calculations.

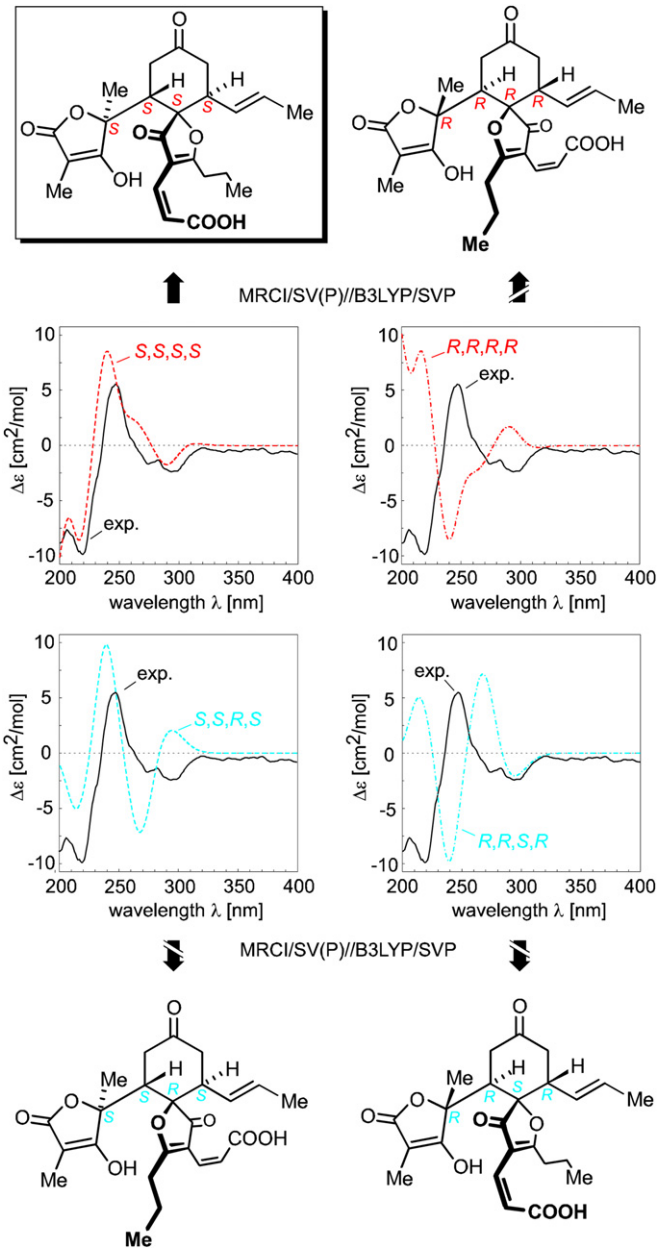


Fig. 5. Elucidation of the absolute configuration of sorbifuranone C (**6**) by comparison of the measured CD spectrum with the quantum chemically calculated ones. Only the CD spectrum predicted for (*S*,*S*,*S*,*S*) matches with the experimental curve.

4. Experimental

4.1. General experimental procedure

Melting points are uncorrected. NMR spectra were recorded on a 600 MHz spectrometer. For calibration of ^{13}C and ^{1}H chemical shifts the carbon signals and the residual proton signals of the solvents were used (CH_3OD: δ_H=3.31 and δ_C=49.15; acetone: δ_H=2.05 and δ_C=29.8), respectively. Proton-detected, heteronuclear correlations were measured using HMQC (optimized for $^{1}J_{HC}$=145 Hz) and HMBC (optimized for $^{n}J_{HC}$=7 Hz or 3.5 Hz) pulse sequences. ROESY experiments were performed by applying pulse sequences from the standard Bruker library. In HPLC separations, Symmetry C_{18} columns were used (Waters, 2.1×150 mm); the eluents were water and acetonitrile with 0.05% TFA each. All solvents used were of analytical grade.

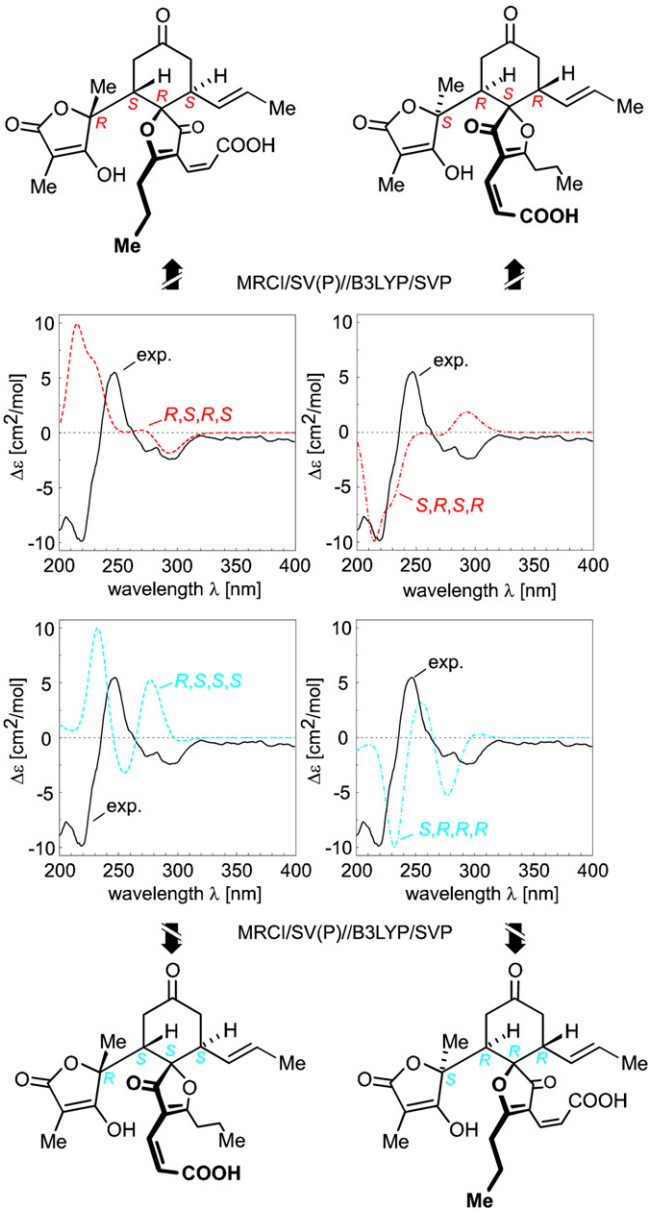
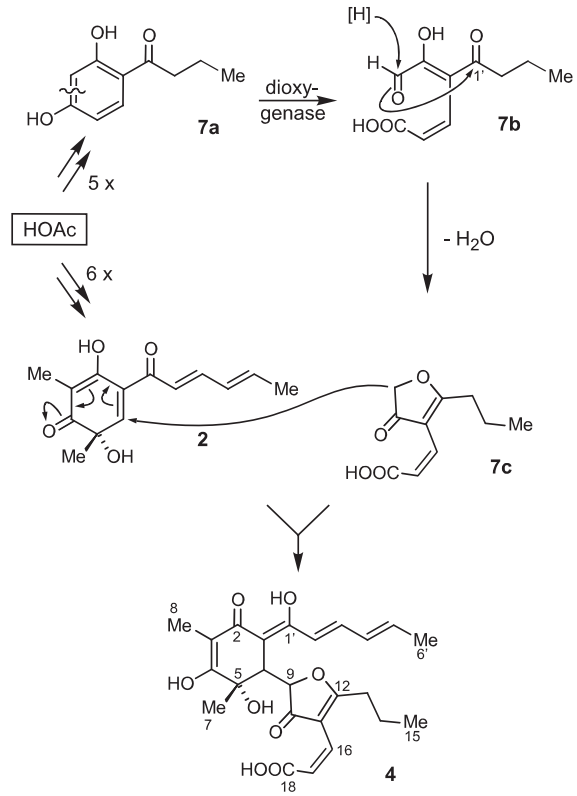


Fig. 6. Comparison of the CD spectrum of isolated sorbifuranone C (**6**) with the ones calculated for (*R*,*S*,*R*,*S*)-**6**, for (*R*,*S*,*S*,*S*)-**6**, and for their enantiomers: none of the computed spectra fits with the experimental CD curve.



Scheme 1. Postulated biosynthetic pathway of sorbifuranone A (**4**).

4.2. Computational methods

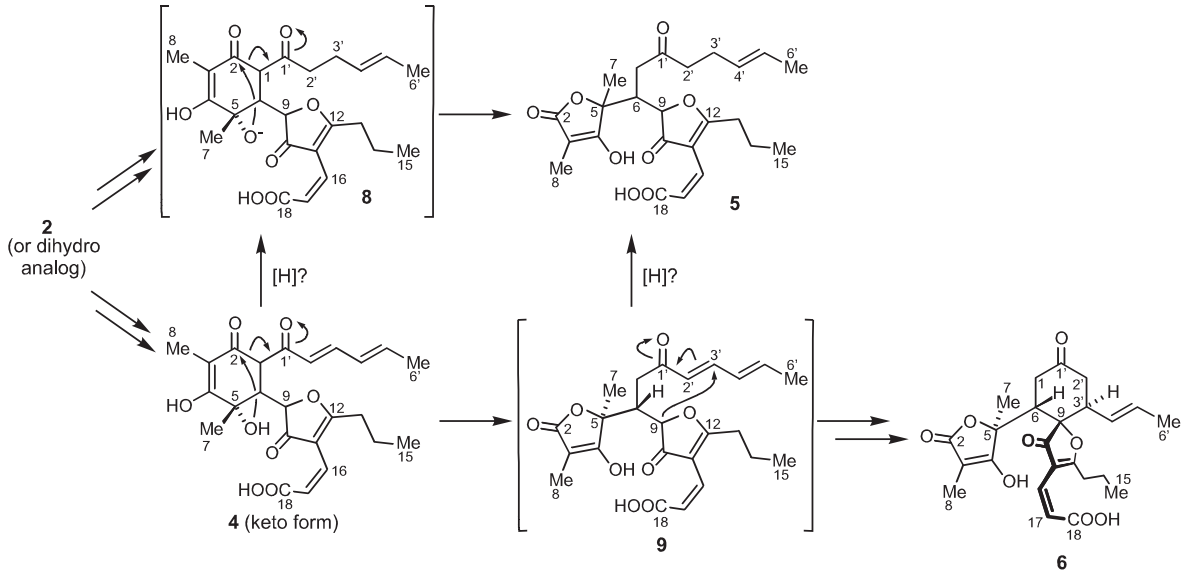
The conformational analyses were initially done with the semiempirical PM3[26] method. All conformers found were further optimized at the B3LYP/SVP[18,19] level. Both optimization procedures were performed with Gaussian 03.[27] Single-point calculations with B3LYP/SVP in combination with the COSMO[23] model (solvent: acetonitrile) yielded the energies that were used for the Boltzmann statistical weighting. These calculations as well as the MRCI[24] ones that provided the single UV and CD spectra of the conformers of sorbifuranone C were done with ORCA.[28] For the MRCI computations the conformers within an energetical range of 6 kJ/mol were used, as the higher-lying conformers do not significantly contribute to the overall UV and CD spectra of the substance.[29] The reference wavefunction was created using the B3LYP functional in combination with the SV(P) basis set. The complete active space (CAS) was defined as (12,12), meaning that excitations from the six highest occupied orbitals to the six lowest unoccupied ones were considered. For every conformer the first 30 single excitations were calculated, using a t_{sel} factor of 10^{-5} Eh. Gauss curve generation, the Boltzmann statistical weighting to yield the overall spectra of each configuration, and the comparison with the experimental spectra was done with SpecDis.[30] A UV correction of −55 nm for each diastereomer was determined and the computed CD spectra were UV shifted[29] by this value and compared with the experimental results.

4.3. Organisms

The fungi were isolated from different specimens of Mediterranean sponges. The strain E01-10/3 was obtained from *Ircinia fasciculata*, collected by scuba diving in the bight Fetovaia (Elba, Italy).[5] Additional 19 strains were derived from eight different sponges from the Limski Fjord (Rovinj, Croatia) and were examined for the production of sorbifuranones. By morphological criteria, 18S rDNA and ITS1/ITS4 sequence data, the fungi were identified as strains of *P. chrysogenum*. The HPLC chromatograms of these strains revealed R03-8/4 from *Tethya aurantium* to be the most potent producer of compounds **4**–**6**, which was, thus, selected for further fermentation.

4.4. Fermentation

Strains E01-10/3 and R03-8/4 were grown in static cultures in 2-L Erlenmeyer flasks containing 700 mL Wickerham Medium[31] (3 g yeast extract, 3 g malt extract, 5 g peptone, 10 g glucose dissolved in 1 L natural seawater). Spore material was taken from a well grown culture on agar plates and used for inoculation. Incubation was carried out for 14–20 d at 26 °C in the dark.



Scheme 2. Postulated biosynthetical pathway of sorbifuranones B (**5**) and C (**6**). Compounds in brackets are presumed intermediates.

4.5. Isolation

The culture broth of the static cultures was extracted with ethyl acetate. After evaporation of the solvents, the extracts obtained from the fungi of the different sponges were separated by preparative HPLC, using a linear solvent gradient from 30 to 100% acetonitrile in 30 min (flow rate 10.0 mL/min) in the case of strain R03-8/4 obtained from *T. aurantium* (extract A) and 30–100% acetonitrile in 25 min (flow rate 11.0 mL/min) for the strain E01-10/3 isolated from *I. fasciculata* (extract B). The sorbifuranones were obtained as follows:

Extract A: sorbifuranone B (**5**, 4.2 mg, t_R 25.6 min), sorbifuranone C (**6**, 6.7 mg, t_R 23.1 min).

Extract B: sorbifuranone A (**4**, 1.3 mg, t_R 16.1 min), sorbifuranone B (**5**, 0.7 mg, t_R 13.5 min), and sorbifuranone C (**6**, 2.4 mg, t_R 11.9 min).

*4.5.1. Sorbifuranone A (**4**).* Yellow amorphous solid (MeOH/H_2O); mp 107–110 °C; $[\alpha]_D^{20}$ −10° (*c* 0.1, MeOH); CD (*c* 0.1, MeOH) $\Delta\varepsilon_{212}$ −1.8, $\Delta\varepsilon_{250}$ +3.2, $\Delta\varepsilon_{288}$ −2.9, $\Delta\varepsilon_{325}$ +2.0; IR (KBr) ν_{max} 3407, 2967, 2937, 2876, 1708, 1635, 1443, 1382, 1342, 1195, 1078 cm^{-1}; NMR data see Table 1; ESIMS (positive) 445.1859 ([M+H^+], 445.1862 calcd for $C_{24}H_{29}O_8^+$).

*4.5.2. Sorbifuranone B (**5**).* Yellow amorphous solid (MeOH/H_2O); mp 78–83 °C; $[\alpha]_D^{20}$ +19.3 (*c* 0.1, MeCN); CD (*c* 0.1, MeCN) $\Delta\varepsilon_{220}$+0.0, $\Delta\varepsilon_{270}$+0.7, $\Delta\varepsilon_{308}$ −0.2, $\Delta\varepsilon_{332}$ +0.2; IR (KBr) ν_{max} 3405, 2967, 2942, 1715, 1659, 1579, 1441, 1385, 1316, 1252, 1166, 1061, 823 cm^{-1}; NMR data see Table 2; ESIMS (negative) 445.1856 ([M−H]$^-$, 445.1862 calcd for $C_{24}H_{29}O_8^-$).

*4.5.3. Sorbifuranone C (**6**).* Yellow amorphous solid (MeOH/H_2O); mp 100–101 °C; $[\alpha]_D^{20}$ −4.42 (*c* 0.1, MeCN); CD (*c* 0.1, MeCN) $\Delta\varepsilon_{202}$ −4.2, $\Delta\varepsilon_{208}$ −2.7, $\Delta\varepsilon_{213}$ −4.9, $\Delta\varepsilon_{216}$ −4.5, $\Delta\varepsilon_{220}$ −6.1, $\Delta\varepsilon_{249}$ +9.5, $\Delta\varepsilon_{267}$ +1.7, $\Delta\varepsilon_{281}$ +2.2, $\Delta\varepsilon_{298}$ +0.7, $\Delta\varepsilon_{321}$ +2.7, $\Delta\varepsilon_{337}$ +2.1; IR (KBr) ν_{max} 3373, 3175, 2967, 2939, 1699, 1654, 1440, 1384, 1260, 1176, 1047 cm^{-1}; NMR data see Table 3; ESIMS (negative) 443.1715 ([M−H]$^-$, 443.1706 calcd for $C_{24}H_{27}O_8^-$).

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Supplementary data

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.tet.2010.10.057.

References and notes

1. Cram, J.; Tishler, M. *J. Am. Chem. Soc.* **1948**, *70*, 4238–4239.
2. Abe, N.; Sugimoto, O.; Tanji, K.; Hirota, A. *J. Am. Chem. Soc.* **2000**, *122*, 12606–12607.
3. Abe, N.; Arakawa, T.; Hirota, A. *Chem. Commun.* **2002**, 204–205.
4. Sugaya, K.; Koshino, H.; Hongo, Y.; Yasunaga, K.; Onose, J.-i.; Yoshikawa, K.; Abe, N. *Tetrahedron Lett.* **2008**, *49*, 654–657.
5. Bringmann, G.; Lang, G.; Gulder, T. A. M.; Tsuruta, H.; Mühlbacher, J.; Maksimenka, K.; Steffens, S.; Schaumann, K.; Stöhr, R.; Wiese, J.; Imhoff, J. F.; Perović-Ottstadt, S.; Boreiko, O.; Müller, W. E. G. *Tetrahedron* **2005**, *61*, 7252–7265.
6. Trifonov, L. S.; Hilpert, H.; Floersheim, P.; Dreiding, A. S.; Rast, D. M.; Skrivanova, R.; Hoesch, L. *Tetrahedron* **1986**, *42*, 3157–3179.
7. Kontani, M.; Sakagami, Y.; Marumo, S. *Tetrahedron Lett.* **1994**, *35*, 2577–2580.
8. Sperry, S.; Samuels, G. J.; Crews, P. J. *J. Org. Chem.* **1998**, *63*, 10011–10014.
9. Abe, N.; Yamamoto, K.; Hirota, A. *Biosci. Biotechnol. Biochem.* **2000**, *64*, 620–622.
10. Abe, N.; Murata, T.; Hirota, A. *Biosci. Biotechnol. Biochem.* **1998**, *62*, 661–666.
11. Andrade, R.; Ayer, W. A.; Mebe, P. P. *Can. J. Chem.* **1992**, *70*, 2526–2535.
12. Maskey, R. P.; Grün-Wollny, I.; Laatsch, H. *J. Nat. Prod.* **2005**, *68*, 865–870.
13. Ueda, J.; Hashimoto, J.; Inaba, S.; Takagi, M.; Shin-ya, K. *J. Antibiot.* **2010**, *63*, 203–205.
14. Nozawa, K.; Nakajima, S. *J. Nat. Prod.* **1979**, *42*, 374–377.
15. Scott, P. M.; Merrien, M. A.; Polonsky, J. *Experientia* **1976**, *32*, 140–142.
16. Oh, H.; Lee, S.; Lee, H.-S.; Lee, D.-H.; Lee, S. Y.; Chung, H.-T.; Kim, T. S.; Kwon, T.-O. *Phytochemistry* **2002**, *61*, 175–179.
17. Abe, N.; Yamamoto, K.; Arakawa, T.; Hirota, A. *Chem. Commun.* **2001**, 23–24.
18. (a) Becke, A. D. *Phys. Rev. A* **1988**, *38*, 3098–3100; (b) Lee, C.; Yang, W.; Parr, R. G. *Phys. Rev. B* **1988**, *37*, 785–789.
19. Schäfer, A.; Horn, H.; Ahlrichs, R. *J. Chem. Phys.* **1992**, *97*, 2571–2577.
20. In general NOE correlation can be observed with distances up to 5 Å between the protons: gaps of 2–3 Å give strong correlations, 3–4 Å normal interactions, and 4–5 Å weak effects, see Neuhaus, D.; Williamson, M. P. *The Nuclear Overhauser Effect in Structural and Conformational Analysis*, 2nd ed.; Wiley: New York, NY, 2000.

21. In these calculations, acetonitrile was used as a solvent, because the CD measurements were performed in it and the difference between the energies calculated in acetone versus those in acetonitrile were found to be negligible.
22. Klamt, A. *COSMO-RS: From Quantum Chemistry to Fluid Phase Thermodynamics and Drug Design*; Elsevier: Amsterdam, 2005.
23. For simplicity the atom numbers of the stereocenters have been omitted in the following, thus, as an example (5*R*,6*S*,9*S*,3′*S*) will be named (*R*,*S*,*S*,*S*).
24. Neese, F. *J. Inorg. Biochem.* **2006**, *100*, 716–726.
25. (a) Tanenbaum, S. W.; Basset, E. W. *J. Biol. Chem.* **1959**, *234*, 1861–1866; (b) Priest, J. W.; Light, R. J. *Biochemistry* **1989**, *28*, 9192–9200.
26. (a) Stewart, J. J. P. *J. Comput. Chem.* **1989**, *10*, 209–220; (b) Stewart, J. J. P. *J. Comput. Chem.* **1989**, *10*, 221–264.
27. Frisch, M.J.; Trucks, G.W.; Schlegel, H.B.; Scuseria, G.E.; Robb, M.A.; Cheeseman, J.R.; Montgomery, J.A., Jr.; Vreven, T.; Kudin, K.N.; Burant, J.C.; Millam, J.M.; Iyengar, S.S.; Tomasi, J.; Barone, V.; Mennucci, B.; Cossi, M.; Scalmani, G.; Rega, N.; Petersson, G.A.; Nakatsuji, H.; Hada, M.; Ehara, M.; Toyota, K.; Fukuda, R.; Hasegawa, J.; Ishida, M.; Nakajima, T.; Honda, Y.; Kitao, O.; Nakai, H.; Klene, M.; Li, X.; Knox, J.E.; Hratchian, H.P.; Cross, J.B.; Bakken, V.; Adamo, C.; Jaramillo, J.; Gomperts, R.; Stratmann, R.E.; Yazyev, O.; Austin, A.J.; Cammi, R.; Pomelli, C.; Ochterski, J.W.; Ayala, P.Y.; Morokuma, K.; Voth, G.A.; Salvador, P.; Dannenberg, J.J.; Zakrzewski, V.G.; Dapprich, S.; Daniels, A.D.; Strain, M.C.; Farkas, O.; Malick, D.K.; Rabuck, A.D.; Raghavachari, K.; Foresman, J.B.; Ortiz, J.V.; Cui, Q.; Baboul, A.G.; Clifford, S.; Cioslowski, J.; Stefanov, B.B.; Liu, G.; Liashenko, A.; Piskorz, P.; Komaromi, I.; Martin, R.L.; Fox, D.J.; Keith, T.; Al-Laham, M.A.; Peng, C.Y.; Nanayakkara, A.; Challacombe, M.; Gill, P.M.W.; Johnson, B.; Chen, W.; Wong, M. W.; Gonzalez, C.; Pople, J.A.; Gaussian: Wallingford CT, 2004.
28. Neese, F. *Orca*; University of Bonn: Bonn, Germany, 2008.
29. Bringmann, G.; Bruhn, T.; Maksimenka, K.; Hemberger, Y. *Eur. J. Org. Chem.* **2009**, 2717–2727.
30. Bruhn, T.; Hemberger, Y.; Schaumlöffel, A.; Bringmann, G. *SpecDis 1.50*; University of Würzburg: Würzburg, Germany, 2010.
31. Wickerham, L. J. *Tech. Bull.*; U.S. Dept. Agric: Wash. D.C, 1951; 1029; 1–56.