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Tetrahedron: *Asymmetry* 16 (2005) 3011–3023

A combined experimental and computational strategy in the assignment of absolute configurations of 4-methyl-5-oxo-tetrahydrofuran-3-carboxylic acids and their esters

Cristina Forzato,* Giada Furlan, Patrizia Nitti, Giuliana Pitacco, Domenico Marchesan, Sonia Coriani and Ennio Valentin*

Dipartimento di Scienze Chimiche, Università di Trieste, via Licio Giorgieri 1, I-34127 Trieste, Italy

Received 14 June 2005; revised 10 August 2005; accepted 11 August 2005

Abstract—Enantiopure *cis*- and *trans*-4-methylparaconic acids and their alkyl esters were synthesized by a procedure involving the kinetic enzymatic resolution of diastereomeric lactonic esters. Thus, ethyl *cis*- and *trans*-4-methyl-5-oxo-tetrahydrofuran-3-carboxylates were isolated, at high conversion values, with 99% ee from the hydrolyses with HLAP and α-CT, respectively. The corresponding enantiopure *cis*- and *trans*-lactonic acids were also obtained. The absolute configurations of the products were assigned by chemical correlation, by analysis of their circular dichroism spectra and by electronic structure calculations. All three methodologies lead to the same assignment for the species considered, another example of successful interplay between experiment and theory.

1. Introduction

The interest in the γ-lactone ring relies on the fact that it is part of many enantiopure natural products[1] showing biological activity as antimicrobials, antitumourals, immunomodulators, antifungal, plant growth inhibitors,[2] and as key flavours of aged alcoholic beverages.[3] It can also be found in ubiquitous products present in a variety of fruits and flowers,[4] in insect pheromones,[5] as well as in lignans, a wide class of natural compounds present in plants and their mammalian metabolites.[6] Paraconic acids constitute a small class of variously functionalized γ-lactones exhibiting antibiotic and antitumoural properties and characterized by the presence of a carboxylic group at the β-position.[7] In the context of our work aimed at the chemoenzymatic synthesis of some of these systems, we recently investigated the synthesis of paraconic acid (5-oxo-3-furancarboxylic acid) itself and of its γ-methyl and γ,γ-dimethyl derivatives in enantiomerically pure forms.[8] Herein, we report the chemoenzymatic syntheses and absolute configuration assignment of optically active α-methyl paraconic acids, **1**[9] and **2**,[9] which can be chemically correlated with two molecules, botryodiplodin **3**[10] and *epi*-botryodiplodin **4**,[11] respectively, of already known absolute configuration (Fig. 1).

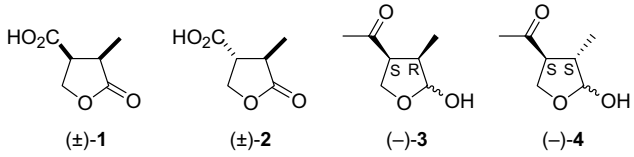


Figure 1. α-Methyl-paraconic acids **1** and **2**, botryodiplodin **3** and *epi*-botryodiplodin **4**.

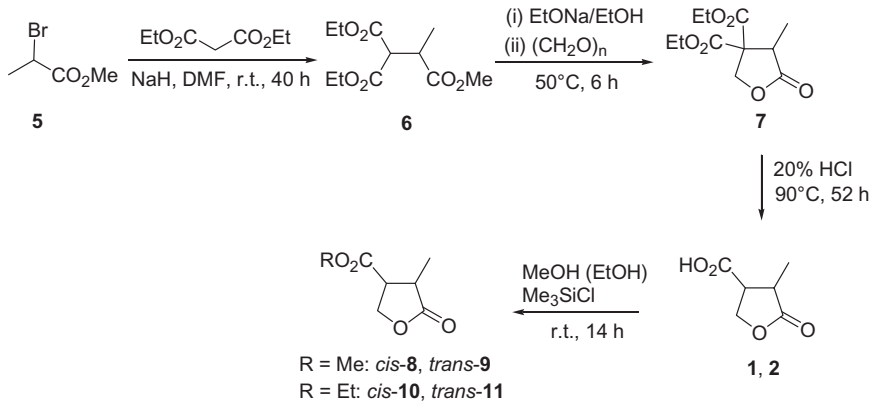
2. Results and discussion

2.1. Synthesis of substrates

Chiral racemic lactones **1** and **2** were synthesized according to a literature procedure[12] from methyl 2-bromopropionate **5** and diethyl malonate (Scheme 1). The resulting intermediate **6** was treated with paraformaldehyde to afford the corresponding lactone **7**, from which a mixture of diastereomeric *cis*- and *trans*-lactonic acids **1** and **2** was obtained by prolonged heating in acidic solution. Their esterification with methanol or ethanol in the presence of trimethylsilyl chloride[13] gave the corresponding mixtures of *cis*- and *trans*-lactonic esters

* Corresponding authors. Tel.: +39 040 5583917; fax: +39 040 5583903; e-mail: valentin@dsch.units.it

0957-4166/$ - see front matter © 2005 Published by Elsevier Ltd.
doi:10.1016/j.tetasy.2005.08.018



Scheme 1. Synthesis of substrates **8**–**11**.

8, **9**[12] and **10**, **11**[9] respectively. The overall yield was around 90% in both cases. The diastereomeric lactonic ester mixtures were separated on column chromatography, with each isomer characterized spectroscopically and then subjected to enzymatic resolution.

2.2. Enzymatic hydrolyses

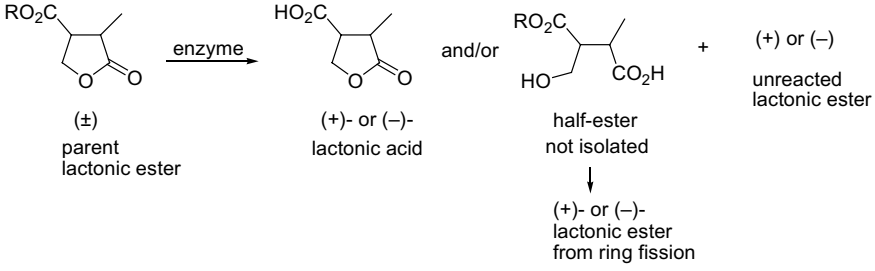
The enzymatic hydrolyses were performed on the lactonic esters **8**–**11** using a series of commercially available enzymes, namely porcine pancreatic lipase (PPL), lipase from *Pseudomonas* species (PS), lipase from *Pseudomonas fluorescens* (AK), *Candida cylindracea* lipase (CCL), *Aspergillus niger* (AP12), Lipase from *Candida rugosa* (AY), *Mucor miehei* lipase (MML), *Candida antarctica* lipase (CAL), porcine liver acetone powder (PLAP), horse liver acetone powder (HLAP), α-chymotrypsin (α-CT) and proteasis from *Bacillus subtilis*. The hydrolytic reactions were monitored by a pH-STAT instrument with the addition of 1 M NaOH. The main results are summarized in Table 1.

Unfortunately, the hydrolyses proved only regioselective in a few cases, while in general both alkoxycarbonyl and lactone groups were hydrolyzed simultaneously, leading to mixtures of products (Scheme 2). A further complication was the cyclization of the hydroxy half ester, resulting from the hydrolytic opening of the heterocycle, occurring during the acidic work-up, with the subsequent formation of the optically active parent molecule. Therefore, in order to have a thorough understanding of the outcome of the reaction, an accurate work-up had to

Table 1. Selected data for the enzymatic hydrolyses

Substrate		Enzyme	E[a]	Conv.[b]	Time (min)	Acid				Ester from ring fission				Unreacted ester		
							ee (%)	Abs. config.	Rel. yield		ee (%)	Abs. config.	Rel. yield		ee (%)	Abs. config.
cis	**8**	HLAP	—	20	6	(+)-**1**	51[c]	3*R*,4*S*	24	(+)-**8**	85	3*R*,4*S*	76	(−)-**8**	24	3*S*,4*R*
	10	HLAP	16	32	24	—	—	—	—	(+)-**10**	84	3*R*,4*S*	100	(−)-**10**	40	3*S*,4*R*
	10	HLAP	10	72	437	—	—	—	—	(+)-**10**	38	3*R*,4*S*	100	(−)-**10**	99	3*S*,4*R*
trans	**9**	α-CT	10	21	20	(−)-**2**	78[c]	3*S*,4*S*	100	—	—	—	—	(+)-**9**	21	3*R*,4*R*
	9	PPL	8	27	30	(−)-**2**	73[c]	3*S*,4*S*	100	—	—	—	—	(+)-**9**	27	3*R*,4*R*
	11	α-CT	27	24	27	(−)-**2**	91[d]	3*S*,4*S*	100	—	—	—	—	(+)-**11**	28	3*R*,4*R*
	11	α-CT	—	65	480	(−)-**2**	53[d]	3*S*,4*S*	92	(+)-**11**	67	3*R*,4*R*	8	(+)-**11**	99	3*R*,4*R*

[a] The E values are given only for those enzymes that reacted regioselectively.
[b] Calculated.[14]
[c] Determined by chiral HRGC on the ethyl ester derivative.
[d] Determined by chiral HRGC on the methyl ester derivative.



Scheme 2. Enzymatic resolution of lactonic esters.

be followed. Initially, the crude hydrolysis mixture was extracted at pH 7.4 to isolate the unreacted lactonic ester from the organic phase. Acidification of the aqueous phase to pH 2 and extraction allowed the isolation of the lactonic ester, derived from the hydrolytic ring fission, in an admixture with the lactonic acid. This latter compound was esterified with an alcohol different from that originally present, so as to distinguish the two esters by gas-chromatographic analysis.

For the *cis*-lactonic esters **8** and **10** the enzyme HLAP proved more effective, while for the *trans*-diastereomers **9** and **11** α-CT gave the best results, although both with low enantiomeric ratios. Interestingly, the methyl derivative **8** underwent hydrolytic ring fission preferentially over the hydrolysis of the methoxycarbonyl group (3:1), whereas the ethyl ester **10** was hydrolyzed at the ring only. At low conversion values, the esters (+)-**8** and (+)-**10** were obtained with 85% and 84% ee, respectively, while at high conversion value the unreacted ester (−)-**10** could be separated with 99% ee. In that manner, although the lactonic acid **1** could not be obtained directly in either satisfactory yield or enantiopurity, both enantiomers of its ethyl ester with good enantiomeric excesses could be separated.

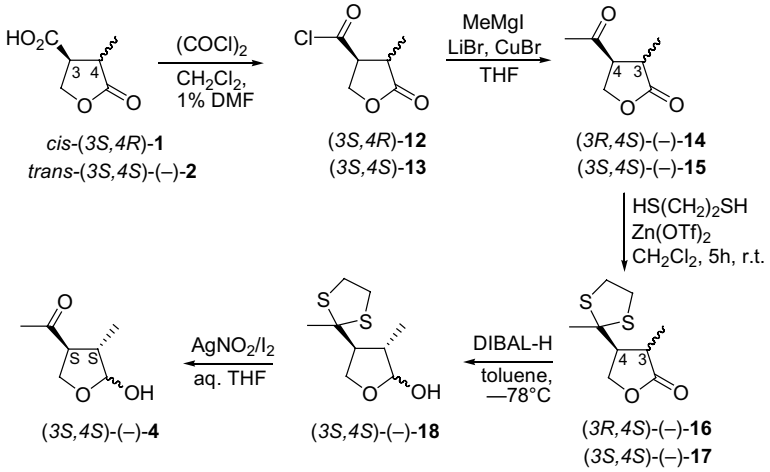
Conversely, at low conversion values the *trans*-diastereomers **9** and **11** were hydrolyzed by α-CT at the alkoxycarbonyl group exclusively, thus affording the corresponding acids (−)-**2** with 78% and 91% ee, respectively. At high conversion values, only ethyl ester (+)-**11** could be obtained in 99% ee. In that case, however, a partial hydrolysis of the lactone ring was observed. Finally, *trans*-isomer **9** was hydrolyzed successfully by PPL with the same enantiopreference as α-CT but in a slightly lower enantiomeric ratio. In fact, the resulting acid (−)-**2** was obtained with 73% ee.

2.3. Determination of the absolute configurations of the products

From an experimental point of view, the assignment of the absolute configuration to new enantiopure compounds is a problem that can be solved either by chemical correlation, circular dichroism empirical rules application, circular dichroism comparison with known compounds or by ^{1}H NMR in some cases. An alternative, or complementary, way to assign the absolute configuration is represented by theoretical calculations of the specific rotation and comparison with sign and magnitude of the corresponding experimental values.

2.3.1. Determination of the absolute configurations of the products by chemical correlation. The absolute configurations of the *trans*-products were assigned by the conversion of acid (−)-**2** into the already known *epi*-botryodiplodin (−)-**4**.[11] The acid (−)-**2** with 91% ee was converted into the corresponding unstable acyl chloride **13**, and immediately transformed into the corresponding 4-acetyl derivative (−)-**15** (numbering is reversed, owing to a different priority) using a literature procedure (Scheme 3).[15] Protection of the carbonyl group with 1,2-ethanedithiol to give (−)-**17** and reduction of the lactone carbonyl group with DIBAL-H furnished the protected lactol (−)-**18** as a diastereomeric mixture, owing to the double configuration of the acetal carbon atom. Deprotection of the carbonyl function with silver nitrite and iodine in aqueous THF[16] afforded *epi*-botryodiplodin (−)-**4**, whose absolute configuration is (3*S*,4*S*). As a consequence, the configuration of (−)-**2** is also (3*S*,4*S*) and those of its methyl and ethyl esters (+)-**9** and (+)-**11**, respectively, are (3*R*,4*R*).

The absolute configuration of the *cis*-diastereomers was determined starting from ethyl ester (−)-**10** with 99% ee. Chemical hydrolysis under acidic conditions afforded the corresponding acid **1** with 99% ee in an admixture with 20% of (−)-**2** with 70% ee (indicating an inversion at the α-carbon atom), to which the same procedure used above for (−)-**2** was applied, with the aim of arriving at the natural molecule (3*R*,4*S*)-(−)-botryodiplodin **3**.[17] However, after having obtained the unstable acyl chloride **12**, the subsequent reaction with methyl magnesium iodide afforded the corresponding 4-acetyl lactone (−)-**14**, in an admixture with 30% of its *trans*-diastereomer (+)-**15** with 48% ee. Compound (−)-**14** with 91% ee



Scheme 3. Synthesis of *epi*-botriodiplodin **4**.

was purified by flash-chromatography and characterized spectroscopically, while the *trans*-diastereomer **15** was identified by chiral HRGC as the enantiomer of the already known (−)-**15**. Since in the conversion of **1**[18] into (+)-**15**, an inversion of configuration occurred at the β-carbon atom, the absolute configuration of C-α is the same in both lactones, that is, *R*. As a consequence, the absolute configurations of **1** and (−)-**10** are (3*S*,4*R*). From the *cis*-lactone (−)-**14**, the protected compound (−)-**16** with 90% ee was obtained. However, the remaining steps applied so successfully to the *trans*-isomer with the eventual obtainment of (−)-*epi*-botryodiplodin **4** proved completely unsuccessful when applied to the *cis*-isomer with the aim of preparing (−)-botryodiplodin **3**. Different reaction conditions, different protecting groups and deprotecting strategies were also attempted, although with no result.

2.3.2. Determination of the absolute configurations of the products by circular dichroism. Circular dichroism is considered a reliable method for establishing the absolute configuration of γ-lactones by the application of empirical rules. Among the several rules present in the literature,[19] the Okuda rule[20] originally stated for α-hydroxy-γ-lactones also proved valid for α-methyl-γ-lactones.[21] In accordance with this rule, the sign of the Cotton effect associated to the $n \rightarrow \pi^*$ transition was determined by the absolute configuration of C-α and hence by the orientation of the α-substituent with respect to the ring average plane. The signs of the Cotton effects found for the above mentioned lactones support the assignments made for their absolute configurations. Thus, from the *cis*-diastereomers, compounds (−)-**10** and (−)-**14**, which exhibit a negative Cotton effect (Fig. 2), possess their α-carbon atom in an (*R*)-configuration. Therefore, the configurations of (−)-**10** and (−)-**14** are (3*S*,4*R*) and (3*R*,4*S*), respectively. Application of the rule to the *trans*-lactones (Fig. 3) indicates that the configuration of the α-carbon atom of compounds (−)-**2** and (−)-**15** is *S*, since their CD curves show a positive Cotton effect, while that of (+)-**11**, exhibiting a negative Cotton effect, is *R*. Therefore, the configurations of (−)-**2**, (+)-**11** and (−)-**15** are (3*S*,4*S*), (3*R*,4*R*) and (3*S*,4*S*), respectively.

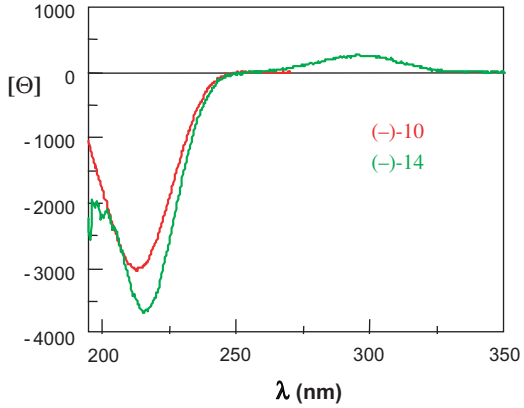


Figure 2. CD spectra of the *cis*-lactones (−)-**10** and (−)-**14** in methanol.

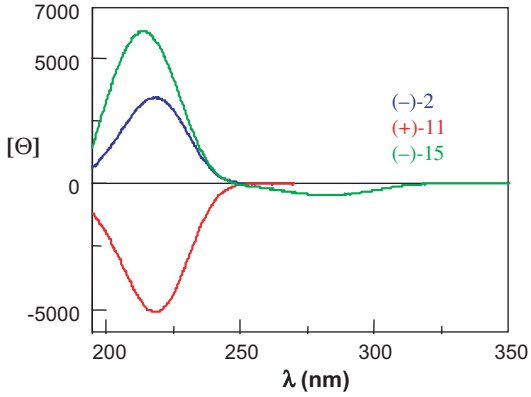


Figure 3. CD spectra of the *trans*-lactones (−)-**2**, (+)-**11** and (−)-**15** in methanol.

The protected lactones (−)-**16** and (−)-**17** exhibit CD curves complicated by the $n \rightarrow \sigma^*$ transition bands of the sulfur atoms, which overlap the $n \rightarrow \pi^*$ transition bands of the lactone function. In fact, in the protected γ-lactol (−)-**18** in which the lactone function is not present, the UV spectrum exhibits two bands at 214 and 242 nm.

In Table 2 are listed the most significant UV and CD spectroscopic data for compounds (−)-**2**, (−)-**10**, (+)-**11**, (−)-**14**, (−)-**15**, (−)-**16** and (−)-**17**, in two different solvents, together with their specific rotation values.

2.4. Theoretical calculations

Advances in the fields of time dependent-density functional theory (TD-DFT)[22] and solvated environment models[23] have provided the organic chemists with a quick and reliable tool for determining the absolute configuration of relatively large chiral molecules (see also Ref. 24 for a review). Calculated values of specific rotation can be directly related to experimental data, and the correct absolute configuration is obtained by simply comparing both the sign and magnitudes of the two values.[24] The amount of theoretical studies that have been published in the recent years elucidates the requirements to get accurate specific rotation values (within an error of ∼30 units of specific rotation): optimize the molecular geometry using DFT, along with the B3LYP functional and a basis set of at least 6-31G** quality; calculate the specific rotation value on this geometry using TD-DFT with the B3LYP functional and a diffused basis set (of aug-cc-pVDZ quality).[25] Using this recipe, the absolute configuration of a large number of (rigid) molecules has been assigned in a reliable way.[26]

For the molecules considered herein, further complications arise in comparison to most of the molecules that are being considered nowadays. First, our lactones have more than one stable conformation, so that the optimization and the specific rotation calculation have to be iterated for each conformer. The total specific rotation is then obtained by adding together the values of specific rotation for the various conformers, each of them weighted by the population fraction of the given

Table 2. Specific rotations, UV and CD data for compounds (−)-**2**, (−)-**10**, (+)-**11**, (−)-**14**, (−)-**15**, (−)-**16** and (−)-**17**

Compound	Abs. config.	ee (%)	$[\alpha]_D^{25}$ (c, g/100 mL)	UV data λ_{max} (ε_{max})	CD data $[\Theta]$ (λ)	Solvent
(−)-**2**	(3*S*,4*S*)	91	−66.2 (0.63)	213 (112) 223[a] (86)	+3439 (219)	MeOH
(−)-**2**	(3*S*,4*S*)	91	−70.7 (0.28)	199 (78) 214 (106) 223[a] (85)	+3697 (220)	MeCN
(−)-**10**	(3*S*,4*R*)	>99	−18.5 (0.33)	213 (335) 222[a] (261)	−3021 (213)	MeOH
(−)-**10**	(3*S*,4*R*)	>99	−15.7 (0.68)	209 (310) 213[a] (288)	−3616 (215)	MeCN
(+)-**11**	(3*R*,4*R*)	>99	+68.5 (0.87)	213 (308) 223[a] (216)	−5119 (218)	MeOH
(+)-**11**	(3*R*,4*R*)	>99	+63.0 (0.57)	195 (426) 209[a] (320)	−4613 (220)	MeCN
(−)-**14**	(3*R*,4*S*)	99	−8.8 (0.17)	213 (705) 244[a] (333) 279 (153)	−3678 (216) +271 (296)	MeOH
(−)-**14**	(3*R*,4*S*)	99	−6.7 (0.18)	198 (1387) 242 (479) 278 (183)	−4096 (217) +287 (295)	MeCN
(−)-**15**	(3*S*,4*S*)	90	−72.1 (0.58)	213 (130) 225[a] (90) 272 (23)	+6073 (214) −479 (281)	MeOH
(−)-**15**	(3*S*,4*S*)	90	−70.1 (0.60)	208 (149) 245[a] (43) 286 (30)	+6438 (216) −563 (286)	MeCN
(−)-**16**	(3*R*,4*S*)	95	−10.0 (0.18)	213 (962) 242 (320)	+5776 (200) −1427 (223) −1506 (241)	MeOH
(−)-**16**	(3*R*,4*S*)	95	−14.7 (0.36)	211 (398) 213 (855) 242 (315)	+5874 (197) −2115 (224) −1939 (239)	MeCN
(−)-**17**	(3*S*,4*S*)	90	−51.7 (0.70)	212 (824) 244 (189)	−5013 (206) −2285 (246)	MeOH
(−)-**17**	(3*S*,4*S*)	90	−54.4 (0.32)	196 (3836) 203[a] (3226) 243 (204)	−4600 (206) −2517 (245)	MeCN

[a] Shoulder.

conformer calculated according to Boltzmann's statistics based on the relative conformational (free) energies.[27] A second complication that can arise for both rigid and flexible molecules is the solute–solvent interaction. The experimental specific rotation is in most cases recorded in solution, and the effect of the solvent molecules on the solute can be very large[28] and can even change the sign of the specific rotation, which would result in a wrong absolute configuration assignment if not taken into account.[29] A very 'popular' method to treat the solute–solvent interaction in specific rotation is the polarizable continuum model[23] (PCM): the solvent is modelled as a polarizable continuum surrounding the molecule, the latter being placed in a molecule-shaped cavity formed by compenetrating spheres, each one of them centred on the position of a heavy atom.[30]

The absolute configuration of paraconic acid, the precursor of the molecules considered here, and those of the diastereomeric *cis*- and *trans*-γ-methyl paraconic acids and their esters have been successfully assigned using this methodology,[29,31] showing the possibility of employing the same approach for the molecules under investigation here.

2.4.1. Computational procedure. The specific rotation of molecule **2** could be measured directly in methanol solution, where specific rotation calculations on this molecule were therefore performed. Calculated and experimental values of the specific rotation of the ethyl ester **11** (from which **2** derives) were considered as an alternative source of information for the absolute configuration assignment of molecule **2**. Due to an isomerization process that takes place during the hydrolysis of

10 that yields molecule **1** in an admixture with **2**, it was not possible to isolate the sole molecule of **1** and measure its specific rotation. Therefore, in order to assign the absolute configuration of **1**, specific rotation calculations and measurements were performed on molecule **10**, from which the absolute configuration of **1** is directly derived.

To assign the absolute configuration by electronic structure calculations, we considered the three lactones (3*S*,4*S*)-**2**, (3*S*,4*R*)-**10** and (3*S*,4*S*)-**11**. Geometry optimizations at the B3LYP/6-31G** level were carried out both in vacuum and in methanol solution (as modelled by the PCM) for lactones (3*S*,4*S*)-**2** and (3*S*,4*R*)-**10**, whereas the geometry of species (3*S*,4*S*)-**11** was only optimized in vacuum. All geometry optimizations were performed with the GAUSSIAN 03 program.[32] For the *trans*-α-methyl paraconic acid **2**, we found a total of six conformational forms (labelled I, IV, VII, X, XIII and XVI in Fig. 4), derived from the carboxylic group torsional motion (three positions) and the five-member ring puckering (two positions). For molecules **10** and **11**, the number of optimized conformers reached the 14 and 18 forms, respectively, due to the addition of the ethyl group torsion. For all three lactones, specific rotation calculations were carried out at the sodium D line (589 nm), all of which were carried out with a development version of the DALTON program.[33] Moreover, for molecule (3*S*,4*R*)-**10**, calculations of specific rotation at 578, 546 and 436 nm were also performed for reasons that will be explained later. The specific rotation calculations on species (3*S*,4*R*)-**10** and (3*S*,4*S*)-**11** were carried out only for those conformers with population fraction larger than 3%—that is, conformers I–IX in the case of (3*S*,4*S*)-**11**, conformers IV–VI, X, XI, XV–XVIII for (3*S*,4*R*)-**10** in vacuum and conformers IV–VI, X, XI, XIII, XV–XVIII for (3*S*,4*R*)-**10** in methanol solution (Fig. 4).

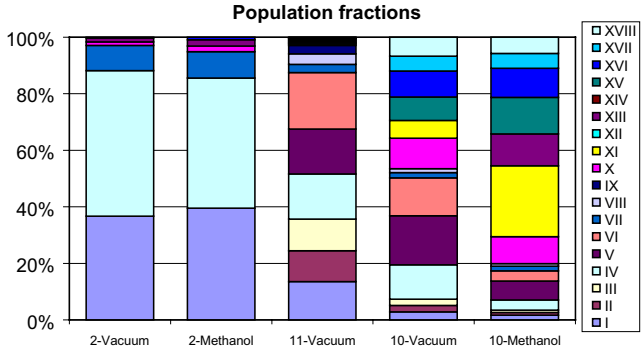


Figure 4. The population fraction of molecules **2**, **10** and **11**. In the case of molecule **2**, only the conformers I, IV, VII, X, XIII and XVI exist. For molecules **2** and **10**, both the vacuum and solvated cases are reported.

For consistency, the specific rotation calculations on the vacuum optimized structures were carried out in vacuum, whereas those for the solvent relaxed conformers were performed in solvent. London orbitals have been used to ensure gauge-origin independence of the results.

2.4.2. Discussion and results. We report in Figure 4 the population fractions of the various conformers of all three molecules, both for the vacuum and PCM solvated cases where available. According to the nomenclature introduced in our previous paper[29] on paraconic acid, conformers labelled I–IX represent the so called 'bottom-of-plane' forms, whereas those labelled X–XVIII are the 'top-of-plane' ones (see also Fig. 5 for an example of bottom–top conformers). In Figure 6, the weighted specific rotations of the same molecules are given while Figure 7 shows the trend of the calculated specific rotation values of molecule (3*S*,4*R*)-**10** at different wavelengths both in vacuum and in methanol, together with the experimental measures (in methanol).

In our previous studies on the specific rotation and absolute configuration of other members of the paraconic acid family,[29,31] we found that the main contribution of the solute–solvent interaction to the final specific rotation came from geometry relaxation effects, that is, it was due to changes in the relative stability of the different conformers in solution, in particular a stabilization of the 'top-of-plane' forms with respect to the 'bottom-of-plane' ones.

For molecule (3*S*,4*S*)-**2** (see the first and second columns in Fig. 4), the stabilization due to solvation is very small: the methyl group in *trans* apparently destabilizes all

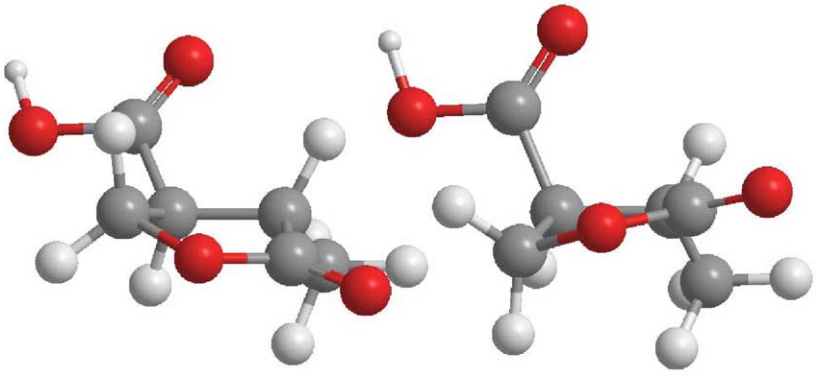


Figure 5. Two conformational forms of the *trans*-α-methylparaconic acid molecule **2**. On the left, the 'bottom-of-plane', and on the right the 'top-of-plane' (as reported in the text).

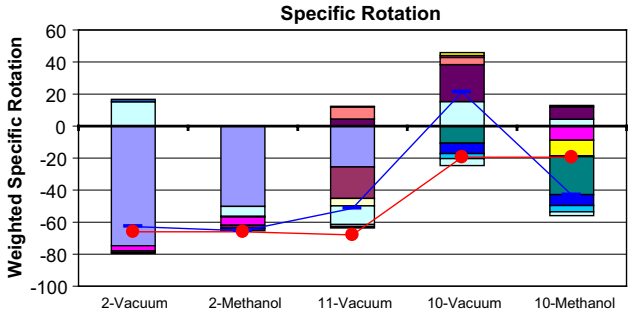


Figure 6. The calculated weighted specific rotation for the conformers of molecules (3*S*,4*S*)-**2**, (3*S*,4*R*)-**10** and (3*S*,4*S*)-**11**, the colours referring to the same conformers of Figure 4. The two lines represent: (▬) the total specific rotation value, (●) the experimental value. For compound **11** the specific rotation of (3*R*,4*R*)-(+)-**11** was measured {$[\alpha]_D = +68.5$ (*c* 0.87, methanol) ee >99%}.

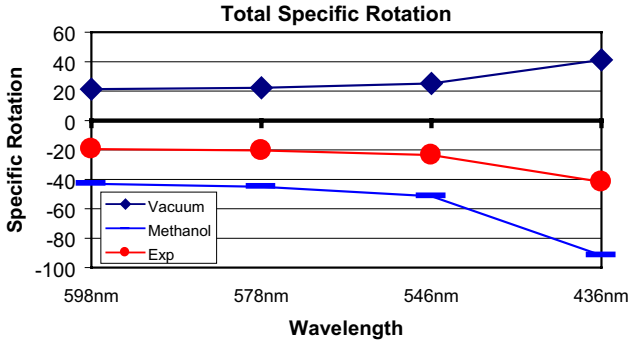


Figure 7. The total optical rotation calculated at different wavelengths for the molecule (3*S*,4*R*)-**10**, both in vacuum and for a methanol solution. The experimental values, also obtained in methanol, are reported for comparison.

'top-of-plane' forms, and the solute–solvent interaction is not strong enough to stabilize them. With the conformational space being almost identical in the two cases (vacuum and methanol solution), the solvent effect on the total specific rotation of molecule (3*S*,4*S*)-**2** is expected to be very small. This is confirmed by the results shown in Figure 6: the only changes that can be seen are a reduction in the value of the weighted specific rotation of conformer I and a change in sign in the specific rotation of conformers IV and VII. Despite these variations, the total specific rotation is −63 units in vacuum and −65 units in methanol solution; both values are in very good agreement with the experimental value of −66 units. Our assignment of the absolute configuration of molecule (3*S*,4*S*)-**2** is thus (3*S*,4*S*)-(−) and (3*R*,4*R*)-(+).

A similar situation was observed for molecule (3*S*,4*S*)-**11**. The sterical hindrance of the methyl group in *trans* leads again to a conformational space dominated by the 'bottom-of-plane' forms (accounting for more than 97% of the population, as shown in the third column of Fig. 4). Also in this case, the solvent effect was expected to be negligible, and the results of the calculation in vacuum can be trusted in order to assign the absolute configuration. The third column of Figure 6 shows that almost all the weighted specific rotation are negative in sign, and the total specific rotation is −51, in good agreement with the experimental value of −68, confirming the assignment (3*S*,4*S*)-(−) and (3*R*,4*R*)-(+) obtained from the corresponding acid case.

Molecule (3*S*,4*R*)-**10** is a far more complicated case than the two previous ones. The fourth column of Figure 4 clearly shows that there is no predominance of the 'bottom-of-plane' forms on the 'top-of-plane' ones, and as a consequence the solvent effect is expected to be strong, as also seen from the modification of the conformational space in methanol shown in the fifth column of Figure 4. The 'top-of-plane' forms account for 46% of the conformational space in the vacuum case, and more than 80% in the solvated one. We can therefore conclude that specific rotation results from vacuum calculations alone cannot be considered a reliable source of data to compare with the experimental data and assign the absolute configuration.

As it can be seen in column four of Figure 6, the calculated total specific rotation in vacuum is +21, whereas the experimental result is −19 in methanol solution. As the calculated value of +21 was obtained for the (3*S*,4*R*)-enantiomer, it would appear that the absolute configuration should be (3*R*,4*S*)-(−) and (3*S*,4*R*)-(+) (with a very small deviation from experiment of 2 units) if the results obtained in vacuum are used to assign the absolute configuration. However, as previously stated, the solvent effect is in this case expected to be fairly large: the last column in Figure 6 shows that the total specific rotation changes sign going from vacuum to a methanol solution, with a value of −43 and supporting a (3*S*,4*R*)-(−) and (3*R*,4*S*)-(+) absolute configuration's assignment. Also in our previous studies on variously substituted paraconic acids and esters, we found that the inclusion of solvent effects was mandatory for molecules having a conformational space similar to that of molecule **10**, and in all these cases that the specific rotation signs predicted including the solvent effect were always in agreement with experimental data.[29,31]

To put the above conclusion on a more firm footing, the total specific rotation of molecule (3*S*,4*R*)-**10** was calculated and measured at four different wavelengths, as reported in Figure 7. The (3*S*,4*R*)-(−) and (3*R*,4*S*)-(+) hypothesis drawn from the results obtained in solution is the only one capable of reproducing the experimental specific rotation trend over the entire range of wavelengths, opposite to what was observed for the vacuum calculation.[34]

Another piece of evidence in favour of the assignment based on the results of the solvent calculations is the positive experimental specific rotation value of ca. +4 units measured in cyclohexane solution. Assuming that the calculations in vacuum can be considered as reliable descriptors of this kind of apolar solution (as done for instance by Lattanzi et al.[35] for CCl_4 solutions), the comparison between calculated specific rotation in vacuum and measured specific rotation in cyclohexane solu-

tion again confirms the (3*S*,4*R*)-(−) and (3*R*,4*S*)-(+) assignment obtained before. Thus, the solvent treatment allowed by the PCM theory improved the calculated values of the specific rotation calculation, leading to the correct absolute configuration determination even for this quite difficult molecule.

3. Conclusions

Apart from the obvious consideration that kinetic enzymatic resolution is an important tool for the obtainment of enantiopure molecules, we have also demonstrated that, although chemical correlation is one of the most reliable methods in assigning the absolute configuration, provided either a natural or a synthetic molecule with known absolute configuration is available in the literature, also a careful application of empirical methods in the circular dichroism spectroscopy can be a satisfactory methodology. Thus, for example, the absolute configuration of the *trans*-α-methylparaconic acid (−)-**2** was assigned as (3*S*,4*S*), being correlated with the already known *epi*-botryodiplodin (−)-**4** by chemical steps of known stereochemistry. Its CD curve shows a positive Cotton effect which is consistent with the (*S*)-configuration of its α-carbon atom, in accordance with the Okuda rule. As to the *cis*-α-methylparaconic acid **1**, its absolute configuration was established as being (3*S*,4*R*), by an inversion of configuration occurring at the β-carbon atom of its 4-acetyl lactone derivative (−)-**14**, which furnished the *trans*-diastereomer (+)-**15** of known absolute configuration. This assignment was confirmed by the finding that the CD spectrum of the ethyl ester of the *cis*-α-methylparaconic acid (−)-**10** exhibited a negative Cotton effect, which can be related with the (*R*)-configuration of its α-carbon atom, in accordance with the Okuda rule. Finally, theoretical calculations at the DFT/B3LYP level of the optical rotatory power on the *trans*-α-methylparaconic acid (3*S*,4*S*)-**2**, the ethyl ester of the *trans*-α-methylparaconic acid (3*S*,4*S*)-**11** and that of the ethyl ester of the *cis*-α-methylparaconic acid (3*S*,4*R*)-**10** supported the correctness of the experimental assignments (3*S*,4*S*)-(−)-**2**, (3*S*,4*S*)-(−)-**11** and (3*S*,4*R*)-(−)-**10**—and thus from the latter the (3*S*,4*R*)-**1** one for the *cis*-α-methylparaconic acid. Due to the high flexibility of the three molecules considered, a thorough investigation of their conformational space was required. The molecular structures of all energetically relevant conformers were optimized and the total specific rotatory power was obtained from a Boltzmann average of the specific rotation of each conformer. For the ethyl ester of the *cis*-α-methylparaconic acid (3*S*,4*R*)-**10**, in particular, inclusion of the solvent effect during both the optimization process and the specific rotation calculation via the PCM model and, at the same time, an analysis of the specific rotation dispersion curve over a range of frequencies proved to be the key factors in the correct assignment of its absolute configuration. Thus, despite the floppiness of the molecules investigated the computational procedure adopted proved to be a promising and reliable method in predicting the absolute configuration of this class of compounds.

4. Experimental

4.1. General

IR spectra were recorded on a Jasco FT/IR 200 spectrophotometer. ^{1}H NMR and ^{13}C NMR spectra were run on a Jeol EX-400 spectrometer (400 MHz for proton, 100 MHz for carbon), and on a Jeol EX-270 spectrometer (270 MHz for proton, 68 MHz for carbon) using deuteriochloroform as a solvent and tetramethylsilane as the internal standard. Chemical shifts are expressed in parts per million (δ). Coupling constants are given in Hertz. Optical rotations were determined on a Perkin Elmer Model 241 polarimeter. CD spectra were obtained on a Jasco J-700A spectropolarimeter (0.1 cm cell). GLC analyses were run on a Carlo Erba GC 8000 instrument and on a Shimadzu GC-14B instrument, the capillary columns being OV 1701 (25 m × 0.32 mm) (carrier gas He, 40 kPa, split 1:50) and a Chiraldex™ type G-TA, trifluoroacetyl γ-cyclodextrin (40 m × 0.25 mm) (carrier gas He, 180 kPa, split 1:100) or DiMePe β-cyclodextrin (25 m × 0.25 mm) (carrier gas He, 110 kPa, split 1:50). Enzymatic hydrolyses were performed using a pH-stat Controller PHM290 Radiometer Copenhagen. Mass spectra were recorded on a VG 7070 (70 eV) spectrometer and on a ion trap instrument Finnigan GCQ (70 eV). TLC's were performed on Polygram® Sil G/UV_{254} silica gel pre-coated plastic sheets (eluant: light petroleum/ethyl acetate). Flash chromatography was run on silica gel for flash-chromatography (BDH). Light petroleum refers to the fraction with bp 40–70 °C and ether to diethyl ether. Diethyl malonate and methyl 2-bromopropionate **5** were purchased from Sigma–Aldrich.

4.2. Synthesis of substrates

4.2.1. 1,1-Diethyl, 2-methyl 1,1,2-propanetricarboxylate 6.[36] To a solution of 6.44 g (40 mmol) of diethyl malonate in 50 mL of anhydrous DMF, 1.60 g of 60% NaH was cautiously added at rt, under an argon atmosphere. A solution of 6.68 g (40 mmol) of methyl 2-bromopropionate **5** in 5.0 mL of DMF was then added and the mixture stirred at 0 °C for 30 min and at rt for 40 h. At the end of the reaction, the mixture was poured into water and extracted with ether. The organic phase was washed with water and dried over anhydrous Na_2SO_4. Evaporation of the solvent gave 8.95 g (91% yield) of **6**. ^{1}H NMR and ^{13}C NMR data are in accordance with those reported in the literature.[36]

4.2.2. Diethyl 4-methyl-5-oxo-tetrahydrofuran-3,3-dicarboxylic acid 7.[37] To a solution of **6** (8.15 g, 33.1 mmol) in 20 mL of anhydrous ethanol, polymeric aldehyde (2.00 g, 66.2 mmol) was added, under stirring and in an argon atmosphere at rt. The mixture was heated to 50 °C and sodium ethoxide (prepared from 20 mg of sodium and 3.0 mL of anhydrous ethanol) added. The mixture was stirred at 50 °C for 6 h. At the end of the reaction, the mixture was poured into cold water, neutralized with concd H_2SO_4 and extracted with ether. The organic phase was washed with water and dried over anhydrous Na_2SO_4. After evaporation of the

solvent, **7** was obtained in 89% yield (7.18 g). Colourless oil; IR (neat), cm^{-1}: 1789, 1735; ^1H NMR δ 4.73 (d, $J = 9.9$, 1H, H-2), 4.33 (d, $J = 9.9$, 1H, H-2), 4.28 (q, $J = 7.2$, 2H, OCH_2CH_3), 4.27 (q, $J = 7.2$, 2H, OCH_2CH_3), 3.26 (q, $J = 7.4$, 1H, $CHCH_3$), 1.31 (t, $J = 7.2$, 3H, CH_3CH_2O), 1.31 (d, $J = 7.4$, 3H, CH_3CH), 1.30 (t, $J = 7.2$, 3H, CH_3CH_2O); ^{13}C NMR δ 176.1 (s, C-5), 168.1 (s, COOEt), 167.7 (s, COOEt), 69.0 (t, C-2), 62.5 (t, OCH_2CH_3), 62.4 (t, OCH_2CH_3), 60.2 (s, $C(COOEt)_2$), 39.9 (d, $CHCH_3$), 14.0 (q, CH_3CH_2O), 13.9 (q, CH_3CH_2O), 11.2 (q, CH_3CH); MS, *m/z*: 244 ($M^{+\cdot}$, 8), 199 (37), 173 (66), 171 (100), 143 (66), 141 (62), 127 (40), 126 (46), 100 (50), 99 (88).

4.2.3. *cis*- and *trans*-4-Methyl-5-oxo-tetrahydrofuran-3-carboxylic acids 1 and 2.[9,38] Lactone **7** (4.40 g, 18 mmol) was added to 125 mL of 20% HCl and refluxed for 52 h. After evaporation of the solvent, a mixture of **1** and **2**, in the ratio of 23:77, respectively, was obtained (2.54 g, 98% yield). White solid, mp 58–70 °C (of the mixture); IR (Nujol) cm^{-1}: 3130, 1731. For the sake of clarity, the NMR spectra are given separately for the two isomers.

Compound **1**: ^1H NMR δ (D_2O) 4.54 (dd, $J_1 = 6.8$, $J_2 = 9.8$, 1H, H-2), 4.30 (dd, $J_1 = 6.8$, $J_2 = 9.8$, 1H, H-2), 3.41 (m, 1H, $CHCOOH$), 3.05 (dq, $J_1 = 7.3$, $J_2 = 8.8$, 1H, $CHCH_3$), 1.06 (d, $J = 7.3$, 3H, CH_3CH); ^{13}C NMR δ (D_2O) 181.7 (s, COOH), 175.2 (s, C-5), 68.7 (t, C-2), 45.0 (d, $CHCOOH$), 36.6 (d, $CHCH_3$), 10.0 (q, CH_3CH). ^1H NMR δ ($CDCl_3$) 9.99 (br s, 1H, OH), 4.56 (dd, $J_1 = 4.2$, $J_2 = 6.5$, 1H, CH_2O), 4.38 (dd, $J_1 = 6.5$, $J_2 = 9.3$, 1H, CH_2O), 3.51 (ddd, $J_1 = 4.2$, $J_2 = 7.0$, $J_3 = 9.3$, 1H, $CHCOOH$), 2.99 (dq, $J_1 = 7.3$, $J_2 = 9.2$, 1H, $CHCH_3$), 1.33 (d, $J = 7.7$, 3H, CH_3CH). Compound **2**: ^1H NMR δ (D_2O) 4.46 (dd, $J_1 = 8.8$, $J_2 = 9.2$, 1H, H-2), 4.25 (dd, $J_1 = 6.8$, $J_2 = 9.8$, 1H, H-2), 3.16 (q, $J = 9.2$, 1H, $CHCOOH$), 2.88 (dq, $J_1 = 7.2$, $J_2 = 9.9$, 1H, $CHCH_3$), 1.17 (d, $J = 7.2$, 3H, CH_3CH); ^{13}C NMR δ (D_2O) 181.8 (s, COOH), 174.5 (s, C-5), 68.0 (t, C-2), 47.1 (d, CHCOOH), 37.9 (d, $CHCH_3$), 13.5 (q, CH_3CH).

4.2.4. *cis*- and *trans*-Methyl 4-methyl-5-oxo-tetrahydrofuran-3-carboxylates 8 and 9.[12] To a solution of 3.60 g (24.9 mmol) of the 23:77 mixture of **1** and **2** in 150 mL of anhydrous methanol, 7.5 mL (56.2 mmol) of trimethyl silyl chloride was added and stirred overnight under argon atmosphere at rt. After evaporation of the solvent a 23:77 mixture of **8** and **9**, respectively, was obtained in 97% yield. Separation by flash-chromatography (petroleum ether/ethyl acetate 85:15) afforded 0.83 g of **8** and 2.27 g of **9** as pure compounds.

Compound **8**: Pale yellow oil, IR (neat), cm^{-1}: 1792, 1732; ^1H NMR δ 4.52 (dd, $J_1 = 4.2$, $J_2 = 9.5$, 1H, H-2), 4.35 (dd, $J_1 = 7.0$, $J_2 = 9.5$, 1H, H-2), 3.76 (s, 3H, OCH_3), 3.48 (ddd, $J_1 = 4.2$, $J_2 = 7.0$, $J_3 = 9.1$, 1H, $CHCOOMe$), 2.93 (dq, $J_1 = 7.5$, $J_2 = 9.1$, 1H, $CHCH_3$), 1.23 (d, $J = 7.5$, 3H, CH_3CH); ^{13}C NMR δ 177.5 (s, C-5), 171.0 (s, COOMe), 67.1 (t, C-2), 52.1 (q, CH_3O), 44.8 (d, CHCOOMe), 36.5 (d, $CHCH_3$), 11.2 (q, CH_3CH); MS, *m/z* 158 ($M^{+\cdot}$, 2), 127 (18), 144 (23), 99 (35), 83 (36), 82 (27), 71 (28), 69 (79), 59 (42), 56 (16), 55 (100), 54 (18); HRGC (γ-CDX): R_t 39.18 min for enantiomer (3*S*,4*R*) and R_t 42.82 for enantiomer (3*R*,4*S*) (100 °C 10 min, 3 °C/min, 150 °C). Compound **9**: Pale yellow oil, IR (neat), cm^{-1}: 1776, 1732; ^1H NMR δ 4.49 (dd, $J_1 = 8.8$, $J_2 = 9.2$, 1H, H-2), 4.27 (dd, $J_1 = 9.2$, $J_2 = 9.5$, 1H, H-2), 3.78 (s, 3H, OCH_3), 3.12 (m, 1H, $CHCOOMe$), 2.88 (dq, $J_1 = 7.1$, $J_2 = 10.6$, 1H, $CHCH_3$), 1.36 (d, $J = 7.1$, 3H, CH_3CH); ^{13}C NMR δ 177.4 (s, C-5), 170.9 (s, COOMe), 66.5 (t, C-2), 52.4 (q, CH_3O), 47.6 (d, CHCOOMe), 37.7 (d, $CHCH_3$), 14.4 (q, CH_3CH); MS, *m/z* 158 ($M^{+\cdot}$, 1), 127 (21), 144 (18), 101 (12), 99 (29), 83 (30), 82 (18), 71 (29), 69 (67), 59 (44), 56 (15), 55 (100), 54 (15); HRGC (γ-CDX): R_t 29.72 min for enantiomer (3*S*,4*S*) and R_t 30.23 for enantiomer (3*R*,4*R*) (100 °C 10 min, 3 °C/min, 150 °C).

4.2.5. *cis*- and *trans*-Ethyl 4-methyl-5-oxo-tetrahydrofuran-3-carboxylates 10 and 11.[9,39] To a solution of 2.16 g of the 23:77 mixture of **1** and **2** in 75 mL of anhydrous ethanol, 4.2 mL (33 mmol) of trimethyl silyl chloride was added and stirred in argon atmosphere at rt overnight. After evaporation of the solvent, a 23:77 mixture of **10** and **11**, respectively, was obtained in 95% yield. Separation by flash-chromatography (eluant: petroleum ether/ethyl acetate 85:15) afforded 0.42 g of **10** and 1.17 g of **11**. IR, ^1H NMR and MS data are in accordance with those reported in the literature.[39] Compound **10**: ^{13}C NMR δ 177.6 (s, C-5), 170.4 (s, COOEt), 67.1 (t, C-2), 61.3 (t, OCH_2CH_3), 44.8 (d, CHCOOEt), 36.5 (d, $CHCH_3$), 14.1 (q, CH_3CH_2O), 11.2 (q, CH_3CH); HRGC (γ-CDX): R_t 41.47 min for enantiomer (3*S*,4*R*) and R_t 45.90 for enantiomer (3*R*,4*S*) (100 °C 10 min, 3 °C/min, 150 °C); compound **11**: ^{13}C NMR δ 177.5 (s, C-5), 170.4 (s, COOEt), 66.6 (t, C-2), 61.5 (t, OCH_2CH_3), 47.8 (d, CHCOOEt), 37.7 (d, $CHCH_3$), 14.4 (q, CH_3CH), 14.0 (q, CH_3CH_2O); HRGC (γ-CDX): R_t 31.15 min for enantiomer (3*S*,4*S*) and R_t 31.48 for enantiomer (3*R*,4*R*) (100 °C 10 min, 3 °C/min, 150 °C).

4.3. Chemical hydrolysis

Lactones **10** and **11** were hydrolyzed separately under acidic conditions (6 M HCl, reflux) to give the corresponding lactonic acids **1** (in admixture with 20% of **2**) and **2** (as a pure compound) in quantitative yield.

Compound (±)-**2**: white solid, mp 92–98 °C; MS, *m/z* 145 (MH^+, 35), 100 (16), 99 (18), 85 (100), 82 (57), 71 (79), 69 (41), 68 (39).

4.4. Enzymatic hydrolyses

To the appropriate lactones **8**–**11** (1 mmol) in 0.1 M phosphate buffer (14 mL), at pH 7.4, were added the following amounts of enzymes: 0.150 g of porcine pancreatic lipase (PPL), 0.140 g of lipase from *Pseudomonas* species (Amano PS, 30,000 U/g), 0.052 g of lipase from *C. cylindracea* (CCL, 943 U/mg), 0.160 g of lipase from *A. niger* (Amano AP 12, 120,000 U/g), 0.400 g of lipase from *M. miehei* (MML, Lipozyme), 0.180 g of lipase from *C. antarctica* (Novozyme 435®, 7000 U/g), 0.230 g of porcine liver acetone powder (PLAP),

0.900 g of Horse liver acetone powder (HLAP), 0.019 g of α-chymotrypsin (α-CT, 51.8 U/mg) and 0.100 g of subtilisin (39.1 U/mg). The course of the reaction was monitored with a pH-STAT, with continuous addition of 1.0 N NaOH. At about 20% conversion, the reaction mixture was extracted with ether to separate the unreacted lactone. The mother liquors were acidified with 5% HCl to pH 2 and extracted with ether to obtain the corresponding lactonic acids **1**, **2** or the lactonic esters (**8**–**11**) derived from the hydroxy halfester intermediates. The organic phases were dried over anhydrous Na_2SO_4 and treated with diazomethane to esterify the carboxylic group before chiral HRGC analysis.

4.4.1. Ethyl (3*S*,4*R*)-(−)-4-methyl-5-oxo-tetrahydrofuran-3-carboxylate 10. Lactone (±)-**10** (0.43 g, 2.50 mmol) was hydrolyzed with HLAP (2.25 g) in 38 mL phosphate buffer following the general procedure. After 7 h and 17 min (72% conversion), the unreacted lactone (−)-**10** was recovered in 22% yield. $[\alpha]_D^{25} = -18.5$ (*c* 0.33, MeOH); >99% ee (determined by chiral HRGC on a γ-CDX column).

4.4.2. Ethyl (3*R*,4*R*)-(+)-4-methyl-5-oxo-tetrahydrofuran-3-carboxylate 11. Lactone (±)-**11** (0.50 g, 2.90 mmol) was hydrolyzed with α-CT (37 mg) in phosphate buffer (45 mL) following the general procedure. After 9 h and 10 min (65% conversion), the unreacted lactone (+)-**11** was recovered in 28% yield. $[\alpha]_D^{25} = +68.5$ (*c* 0.87, MeOH); >99% ee (determined by chiral HRGC on a γ-CDX column).

4.4.3. (3*S*,4*S*)-(−)-4-Methyl-5-oxo-tetrahydrofuran-3-carboxylic acid 2. Lactone (±)-**11** (0.73 g, 4.27 mmol) was hydrolyzed with α-CT (55.5 mg) in a phosphate buffer (45 mL) following the general procedure. After 27 min (24% conversion), the lactonic acid (−)-**2** was recovered in 14% yield. White solid, mp 98–100 °C; $[\alpha]_D^{25} = -66.2$ (*c* 0.63, MeOH); 91% ee (determined by chiral HRGC on a γ-CDX column of its methyl ester derivative).

4.4.4. *cis*-4-Methyl-5-oxo-tetrahydro-3-furanoyl chloride 12. To a solution of 0.60 g (4.2 mmol) of **1** [in an admixture with 20% of (−)-**2**], dichloromethane (11 mL) and 1% DMF (0.110 mL), a solution of oxalyl chloride (0.4 mL) in dichloromethane (5 mL) was added. The mixture was stirred for 2 h at rt. After evaporation of the solvent, the unstable compound **12** was obtained in quantitative yield. Yellow oil, IR (neat), cm^{-1}: 1776; ^1H NMR δ 4.58 (dd, $J_1 = 4.8$, $J_2 = 9.9$, 1H, H-2), 4.43 (dd, $J_1 = 7.0$, $J_2 = 9.9$, 1H, H-2), 3.96 (ddd, $J_1 = 4.8$, $J_2 = 7.0$, $J_3 = 8.8$, 1H, H-3), 3.07 (dq, $J_1 = 7.6$, $J_2 = 8.8$, 1H, H-4), 1.40 (d, $J = 7.3$, 3H, CH_3CH); ^{13}C NMR δ (68 MHz) 166.3 (s, C-5), 164.1 (s, COCl), 66.2 (t, C-2), 55.6 (d, C-3), 36.8 (d, C-4), 11.0 (q, CH_3).

4.4.5. *trans*-4-Methyl-5-oxo-tetrahydro-3-furanoyl chloride 13. To a solution of 0.83 g (5.8 mmol) of (−)-**2** in a mixture of dichloromethane (16 mL) and 1% DMF (0.160 mL), a solution of oxalyl chloride (0.5 mL) in dichloromethane (6 mL) was added. The mixture was stirred for 2 h at rt. After evaporation of the solvent, the unstable compound **13** was obtained in quantitative yield. Pale yellow oil. IR (neat), cm^{-1}: 1822, 1776; ^1H NMR δ 4.60 (dd, $J_1 = 8.8$, $J_2 = 9.2$, 1H, H-2), 4.37 (dd, $J_1 = 9.2$, $J_2 = 9.5$, 1H, H-2), 3.56 (ddd, $J_1 = 8.8$, $J_2 = 9.2$, $J_3 = 9.5$, 1H, H-3), 3.00 (dq, $J_1 = 7.3$, $J_2 = 9.9$, 1H, H-4), 1.45 (d, $J = 7.3$, 3H, CH_3CH); ^{13}C NMR δ (68 MHz) 175.9 (s, C-5), 171.7 (s, COCl), 65.6 (t, C-2), 58.2 (d, C-3), 37.7 (d, C-4), 14.6 (q, CH_3CH).

4.4.6. (3*R*,4*S*)-(−)-4-Acetyl-3-methyl-2(3*H*)-dihydrofuranone 14. To a stirred suspension of CuBr (0.81 g, 5.64 mmol) in anhydrous THF (7.4 mL), a solution of anhydrous LiBr (0.98 g, 11.2 mmol) in anhydrous THF (7.4 mL) was added. The mixture was stirred under argon atmosphere for 5 min at rt. Then, a solution of 3 M MeMgI (1.87 mL, 5.61 mmol) in ether was quickly added, followed by the addition of the optically active compound **12** (0.76 g, 4.7 mmol), in anhydrous THF (7 mL). The mixture was stirred for 1 h, the solution was quenched with a saturated aqueous solution of NH_4Cl and extracted with ethyl acetate. The organic extracts were dried over anhydrous Na_2SO_4 and, after evaporation of the solvent, (−)-**14** was obtained [in an admixture with 30% of the *trans*-isomer (+)-**15**] and purified by flash-chromatography (petroleum ether/ethyl acetate 85:15) (67% yield). Yellow oil; IR (neat), cm^{-1}: 1761, 1716; ^1H NMR δ (270 MHz) 4.53 (dd, $J_1 = 6.3$, $J_2 = 9.6$, 1H, H-5), 4.29 (dd, $J_1 = 6.9$, $J_2 = 9.6$, 1H, H-5), 3.60 (ddd, $J_1 = 6.3$, $J_2 = 6.9$, $J_3 = 8.9$, 1H, H-4), 2.95 (dq, $J_1 = 7.5$, $J_2 = 8.9$, 1H, H-3), 2.24 (s, 3H, CH_3CO), 1.22 (d, $J = 7.5$, 3H, CH_3CH); ^{13}C NMR δ (68 MHz) 205.3 (s, $COCH_3$), 177.5 (s, C-2), 66.7 (t, C-5), 51.1 (d, C-4), 36.5 (d, C-3), 30.5 (q, CH_3CO), 11.3 (q, CH_3CH); MS, *m*/*z* 143 (MH^+, 100), 125 (13), 100 (24), 98 (14), 87 (49), 85 (80), 71 (20), 69 (22); $[\alpha]_D^{25} = -8.8$ (*c* 0.17, CH_3OH); 99% ee. HRGC (γ-CDX): R_t 35.31 min for (3*R*,4*S*)-enantiomer and R_t 36.85 for (3*S*,4*R*)-enantiomer (150 °C isotherm).

4.4.7. (3*S*,4*S*)-(−)-4-Acetyl-3-methyl-2(3*H*)-dihydrofuranone 15. To a stirred suspension of CuBr (1.00 g, 6.99 mmol) in anhydrous THF (10 mL), a solution of anhydrous LiBr (1.22 g, 14.0 mmol) in anhydrous THF (10 mL) was added. The mixture was stirred under an argon atmosphere for 5 min at rt. Subsequently, a solution of 3 M MeMgI (2.33 mL, 6.99 mmol) in ether was quickly added followed by the addition of the optically active compound **13** (0.94 g, 5.82 mmol), in anhydrous THF (8 mL). The mixture was stirred for 35 min, quenched with a saturated aqueous solution of NH_4Cl and extracted with ethyl acetate. The organic extracts were dried over anhydrous Na_2SO_4 and, after evaporation of the solvent, (−)-**15** was obtained and purified by flash-chromatography (petroleum ether/ethyl acetate 85:15) (61% yield). Yellow oil; IR (neat), cm^{-1}: 1776, 1716; ^1H NMR δ 4.48 (t, $J = 8.8$, 1H, H-5), 4.20 (t, $J = 9.3$, 1H, H-5), 3.25 (q, $J = 9.4$, 1H, H-4), 2.85 (dq, $J_1 = 7.1$, $J_2 = 10.3$, 1H, H-3), 2.27 (s, 3H, CH_3CO), 1.36 (d, $J = 7.1$, 3H, CH_3CH); ^{13}C NMR δ 204.2 (s, $COCH_3$), 177.6 (s, C-2), 66.2 (t, C-5), 55.5 (d, C-4), 36.9 (d, C-3), 29.9 (q, CH_3CO), 14.8 (q, CH_3CH); MS, *m*/*z* 143 (MH^+, 100), 125 (17), 100 (30), 87 (64), 85 (74), 71 (16), 69 (21); $[\alpha]_D^{25} = -72.1$ (*c* 0.58, CH_3OH);

90% ee. HRGC (γ-CDX): R_t 20.00 min for enantiomer (3*S*,4*S*) and R_t 21.64 for enantiomer (3*R*,4*R*) (150 °C isotherm).

4.4.8. (3*R*,4*S*)-(−)-3-Methyl-4-(2-methyl-1,3-dithiolan-2-yl)-2(3*H*)-dihydrofuranone 16. To a solution of ethanedithiol (0.57 mL, 6.8 mmol) in anhydrous dichloromethane (7.0 mL), $Zn(OTf)_2$ (1.38 g, 3.8 mmol) was added. A solution of (−)-**14** (0.30 g, 2.1 mmol) in anhydrous dichloromethane (7.0 mL) was added under vigorous stirring. After 5 h of stirring at rt water (16 mL) was added. The mixture was extracted with a 1:1 mixture of ether/*n*-hexane, the combined extracts were washed with 3 M HCl and with a saturated aqueous solution of $NaHCO_3$. Evaporation of the solvent gave the product (−)-**16**, which was purified by flash-chromatography (62% yield). Pale yellow oil; IR (neat), cm^{-1}: 1776, 773; ^1H NMR δ 4.48 (dd, $J_1 = 7.0$, $J_2 = 9.6$, 1H, H-5), 4.43 (dd, $J_1 = 6.0$, $J_2 = 9.6$, 1H, H-5), 3.42–3.23 (m, 4H, CH_2S), 3.10 (dt, $J_1 = 6.4$, $J_2 = 8.1$, 1H, H-4), 2.80 (quintet, $J = 7.7$, 1H, H-3), 1.78 (s, 3H, CH_3C), 1.41 (d, $J = 7.3$, 3H, C*H*$_3$CH); ^{13}C NMR δ (68 MHz) 178.9 (s, C-2), 70.4 (t, C-5), 66.6 (s, *C*CH$_3$S), 51.2 (d, C-4), 40.1 (t, CH_2S), 39.5 (t, CH_2S), 38.4 (d, C-3), 31.5 (q, *C*H$_3$C), 12.0 (q, *C*H$_3$CH); MS, *m*/*z*: 221 (2), 220 (4), 219 (MH^+, 17), 218 ($M^{+\cdot}$, 5), [calcd for $C_9H_{15}O_2S_2^+$ and $C_9H_{14}O_2S_2$: 221 (2), 220 (2.4), 219 (MH^+, 17), 218 ($M^{+\cdot}$, 5)], 145 (12), 121 (16), 120 (12), 119 (100) [calcd for $C_4H_7S^+$: 121 (8.9), 120 (6), 119 (100)]; $[\alpha]_D^{25} = -10.0$ (*c* 0.18, CH_3OH); 95% ee. HRGC (β-CDX): R_t 174.43 min for enantiomer (3*S*,4*R*) and R_t 177.16 for enantiomer (3*R*,4*S*) (150 °C isotherm).

4.4.9. (3*S*,4*S*)-(−)-3-Methyl-4-(2-methyl-1,3-dithiolan-2-yl)-2(3*H*)-dihydrofuranone 17. To a solution of ethanedithiol (0.700 mL, 8.3 mmol) in dichloromethane (7.7 mL), $Zn(OTf)_2$ (1.7 g, 4.7 mmol) was added. A solution of (−)-**15** (0.37 g, 2.6 mmol) in dichloromethane (7.7 mL) was added under vigorous stirring. After 5 h of stirring at rt, water (30 mL) was added. The mixture was extracted with a 1:1 mixture of ether/*n*-hexane, the combined extracts were washed with 3 M HCl and a saturated solution of $NaHCO_3$. Evaporation of the solvent gave the product (−)-**17,** which was purified by flash-chromatography (80% yield). Pale yellow oil; IR (neat), cm^{-1}: 1776, 714; ^1H NMR δ 4.46 (dd, $J_1 = 8.1$, $J_2 = 9.5$, 1H, H-5), 4.14 (dd, $J_1 = 7.5$, $J_2 = 9.5$, 1H, H-5), 3.38 (m, 2H, CH_2S), 3.32 (m, 2H, CH_2S), 2.70 (q, $J = 7.7$, 1H, H-4), 2.63 (quintet, $J = 7.2$, 1H, H-3), 1.81 (s, 3H, CH_3C), 1.42 (d, $J = 7.0$, 3H, C*H*$_3$CH); ^{13}C NMR δ 179.5 (s, C-2), 69.4 (t, C-5), 68.2 (s, *C*CH$_3$S), 54.5 (d, C-4), 40.8 (d, C-3), 39.9 (t, CH_2S), 39.8 (t, CH_2S), 33.0 (q, *C*H$_3$C), 17.9 (q, *C*H$_3$CH); MS, *m*/*z* 221 (3), 220 (4), 219 (MH^+, 40), [calcd for $C_9H_{15}O_2S_2^+$: 221 (3.6), 220 (4.6), 219 (MH^+, 40)], 145 (8), 121 (9), 120 (6), 119 (100) [calcd for $C_4H_7S^+$: 121 (8.9), 120 (6), 119 (100)]; $[\alpha]_D^{25} = -51.7$ (*c* 0.70, CH_3OH); 90% ee. HRGC (β-CDX): R_t 129.06 min for (3*R*,4*R*)-enantiomer and R_t 134.40 for (3*S*,4*S*)-enantiomer (150 °C isotherm).

4.4.10. (2*R,3*S*,4*S*)-(−)-2-Hydroxy-3-methyl-4-(2-methyl-1,3-dithiolan-2-yl)-tetrahydrofuran 18.** To a solution of compound (−)-**17** (0.26 g, 1.2 mmol) in anhydrous toluene (21 mL) at −78 °C under argon atmosphere, DIBAL-H (1 M in THF) (1.8 mL) was added and the mixture was stirred for 1 h. After the addition of a solution of 2 M 2-propanol in toluene (12 mL), the temperature was allowed to rise to 0 °C and water (1.2 mL) was added. After addition of ethyl acetate (24 mL) the mixture was vigorously stirred and added with Kieselgur and anhydrous Na_2SO_4 (2.4 g). The solid was filtered off and washed with a 4:1 mixture of toluene/ethyl acetate. After evaporation of the solvent, the crude reaction mixture was purified by flash-chromatography (petroleum ether/ethyl acetate 75:25); a mixture of the two isomers of (−)-**18** was obtained (60% isomer **A**, 40% isomer **B**). Oil, IR (neat), cm^{-1}: 3408, 730; MS, *m*/*z* 222 (1), 221 (1), 220 ($M^{+\cdot}$, 4), 219 ($M-H^+$, 3), [calcd for $C_9H_{16}O_2S_2$ and $C_9H_{15}O_2S_2^+$: 222 (0.3), 221 (0.7), 220 ($M^{+\cdot}$, 4.3), 219 ($M-H^+$, 3)], 205 (6), 204 (7), 203 ($M-OH^+$, 53), [calcd for $C_9H_{15}OS_2^+$: 205 (4.7), 204 (6.1), 203 ($M-OH^+$, 53)], 121 (17), 120 (13), 119 (100), [calcd for $C_4H_7S^+$: 121 (8.9), 120 (6), 119 (100)]; $[\alpha]_D^{25} = -29.5$ (*c* 0.38, CH_3OH).

Isomer **A** (60%): ^1H NMR δ 5.02 (dd, $J_1 = 0.7$, $J_2 = 9.2$, 1H, H-2), 4.21 (t, $J = 9.1$, 1H, H-5), 3.82 (t, $J = 9.1$, 1H, H-5), 3.75 (d, $J = 9.2$, 1H, OH), 3.36 (m, 4H, CH_2S), 2.39 (dt, $J_1 = 4.5$, $J_2 = 8.6$, 1H, H-4), 2.13 (m, 1H, H-3), 1.81 (s, 3H, CH_3C), 1.19 (d, $J = 7.3$, 3H, C*H*$_3$CH); ^{13}C NMR δ 105.0 (d, C-2), 70.5 (t, C-5), 68.6 (s, *C*CH$_3$S), 58.2 (d, C-4), 46.7 (d, C-3), 40.2 (t, CH_2S), 40.0 (t, CH_2S), 34.2 (q, *C*H$_3$C), 20.8 (q, *C*H$_3$CH).

Isomer **B** (40%): ^1H NMR δ 5.39 (dd, $J_1 = 3.7$, $J_2 = 4.8$, 1H, H-2), 4.25 (dd, $J_1 = 2.6$, $J_2 = 8.8$, 1H, H-5), 3.83, (dd, $J_1 = 5.9$, $J_2 = 9.0$, 1H, H-5), 3.36 (m, 4H, CH_2S), 2.60 (dt, $J_1 = 6.0$, $J_2 = 8.2$, 1H, H-4), 2.50 (d, $J = 3.4$, 1H, OH), 2.14 (m, 1H, H-3), 1.79 (s, 3H, CH_3C), 1.20 (d, $J = 7.0$, 3H, C*H*$_3$CH); ^{13}C NMR δ 101.1 (d, C-2), 70.7 (C-5), 69.4 (s, *C*CH$_3$S), 55.1 (d, C-4), 43.0 (d, C-3), 39.7 (t, CH_2S), 39.5 (t, CH_2S), 32.2 (q, *C*H$_3$C), 14.5 (q, *C*H$_3$CH).

4.4.11. *epi*-Botryodiplodin (−)-4. To a suspension of $AgNO_2$ (28 mg, 0.18 mmol) and I_2 (23 mg, 0.09 mmol) in aqueous THF (1 mL), stirred for 30 min at rt, compound (−)-**18** (33 mg, 0.15 mmol) was added and the mixture stirred for a further 5 h. An aqueous solution of $Na_2S_2O_3{\cdot}5H_2O$ (3 mL) was added at 0 °C and the mixture was extracted with dichloromethane. The organic phase was washed with brine and dried over anhydrous Na_2SO_4. Evaporation of the solvent gave the desired compound (−)-**4,** which was purified by flash-chromatography (17% overall yield). IR, ^1H NMR and ^{13}C NMR were in accordance with those reported in the literature.[11] $[\alpha]_D^{25} = -65.9$ (*c* 0.22, $CHCl_3$) {lit. $[\alpha]_D^{25} = -83$ (*c* 0.69, $CHCl_3$)}.[40]

Acknowledgements

Financial supports by PRIN 2003 and the University of Trieste are gratefully acknowledged.

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