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Microbiological Transformations. 25. Enantioselective Baeyer-Villiger Oxidation as a Tool for the Synthesis of Enantiopure Bicyclic Furofuran and Pyrofuran Chirons.

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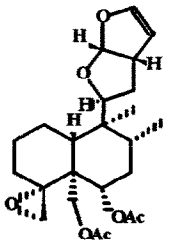
Abstract : Several bicyclic [n.2.0] ketones were submitted to enzymatic Baeyer-Villiger oxidation and provided the corresponding regioisomeric lactones showing high enantiopurities. These products could be used as interesting chirons for the synthesis of various natural compounds. The absolute configurations of the obtained lactones were assigned using circular dichroism measurements.

Introduction

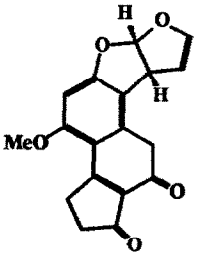
The ability of several microbial strains to catalyze the enantioselective Baeyer-Villiger oxidation of various substrates has been recently recognized by several authors and appears to be a new emerging type of useful bioconversion applied to fine organic synthesis[1]. These microbiological transformations appear to be, at the present time, the only way to achieve asymmetric Baeyer-Villiger oxidations, since no counterpart of such a reaction is known using conventional chemistry. We have previously described the first example of such a biotransformation which, applied to racemic bicyclic ketones, allows enantioselective - combined to regioselective - oxidation, thus leading to a mixture of two regioisomeric lactones showing high enantiomeric purity[2]. In this context, we have proposed a temptative model for the (hypothetically unique) active site of this enzyme, based on this surprising observation.

We here describe further results we have obtained studying substrates structurally similar to the ones used previously, but bearing an additional oxygen atom in their carbon skeleton. This choice was made for two reasons. First it has been described previously[1b,1g] that the presence of an additional oxygenated function in the starting substrate may strongly influence the outcome of the reaction (loss of enantioselectivity), presumably because of the formation of an hydrogen bond in the enzymatic active site, thus perturbing the positioning of the substrate. Therefore, studying such oxygenated substrates could help to gather some more structural informations in order to refine our active site model. Second, these oxygenated bicyclic ketones appear to be interesting precursors of enantiopure bicyclic lactones which could be of interest as key building blocks for the synthesis of various natural products showing important biological activities. For instance, clerodine **A** (Figure 1), as well as a variety of structurally similar compounds showing very challenging structures, have attracted intense attention because of their potent antifeedant activity against various insects and larvae[3]. All

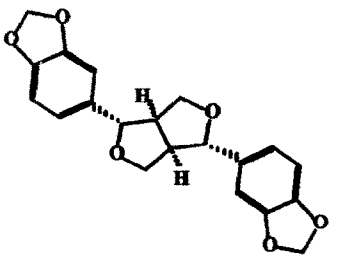
these compounds contain a dihydro or a perhydro-furo[2,3b]furan moiety. Synthesis of such building blocks in racemic form has been recently described by Lallemand and coll.[4], Aede De Groot and coll.[5], as well as by Kato and coll [6]. However, to our best knowledge, no method is available up to now allowing access to the enantiopure forms of these building blocks. The same furo[2,3b]furan framework is also found in aflatoxines **B**[7] which are extremely toxic and carcinogenic fungal metabolites possessing profound biological activity. Similarly, numerous lignan compounds like **C**, which exhibit a wide range of physiological activities, possess a [3,7] furofuran bicyclic ring system[8]. Finally, it is well known that the bicyclo[3.3.0]lactones are key building blocks widely used for prostaglandin and prostacyclin synthesis[9]. Thus, our products could also allow easy preparation of enantiopure oxo-derivatives of these interesting molecules.



Clerodin **A**



Aflatoxin B_2 **B**



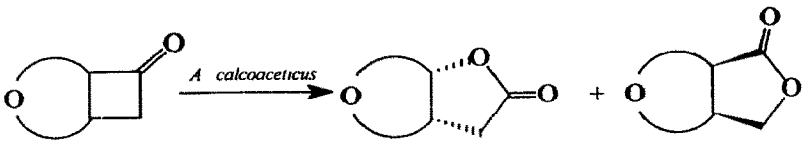
Sesamin **C**

Figure 1 : Examples of natural products bearing bicyclic furofuran rings.

Results and Discussion

The starting substrates were synthesized following two different routes. Thus, ketones **1** and **4** (See Table I) were prepared following a procedure described by Ghosez and coll.[9a] and by Snider and coll.[10] involving an intramolecular [2+2] cycloaddition of a ketene or a keteniminium salt. Ketones **2**, **3** and **5** were obtained *via* the classical intermolecular cycloaddition of dichloroketene with the corresponding dihydrofuran or dihydropyran[11], followed by reduction of the dichloroketone either with zinc in acetic acid[12] to obtain **3**, or with tributyltin hydride[13] to obtain **2** or **5**[14]. All these substrates were tested for chemical Baeyer-Villiger oxidation using *meta*-chloroperbenzoic acid and led predominantly to the corresponding racemic lactones **a**[15].

When submitted to a culture of *A. calcoaceticus* (NCIB 9871), substrates **1** to **5** were completely transformed into a mixture of two regioisomeric lactones as shown on Figure 2.

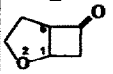
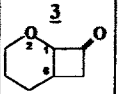
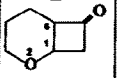


Ketone Lactone **a** Lactone **b**

*Figure 2 : General scheme for the enzymatic Baeyer-Villiger oxidation of **1** to **5**.*

As in the case of the previously studied substrates[2], the structures of these products are of two types : one type of lactone (1a to 5a) corresponds to the product expected for a "normal" Baeyer-Villiger type oxygen insertion (between the carbonyl group and the more substituted carbon atom). The second type of lactone (1b to 5b) arises from the "abnormal" insertion of the oxygen atom into the less substituted bond, thus leading to the corresponding regioisomer. With the exception of the case of ketone 5, both these lactones are formed in approximately a 1:1 ratio and with almost quantitative yields as can be seen on Table I. Their enantiomeric purities (determined using chiral g.c. analysis) depend primarily on the structure of the starting substrate, but are generally excellent. This is in particular true for all "abnormal" lactones which all show ees higher than 98%, as well as for the "normal" products bearing an adjacent five membered ring. The case of substrates 4 and 5, which bear six membered pyran-type rings, is more peculiar. Indeed, in these two cases, the "abnormal" lactones still show ees > 98%, but the "normal" ones show ees of respectively 70 and 33% depending on the structure of the substrate (i.e. on the localization of the oxygen in the pyran ring). Obviously the greater flexibility of the six membered ring allows structural changes which lead to several positioning possibilities into the enzymatic active site. In the case of 5, the relative proportions of these products are 3:1 respectively, indicating a clear disproportion in the reactivity of each one of the enantiomers of this particular molecule. Figure 3 shows the time course of the bioconversion of 5. It indicates that the 3:1 ratio is independent of time and of the extent of conversion and also that the lactones are not further metabolized by the bacteria after their formation. Similar results have been observed previously with the corresponding cyclohexane derivative[2].

Table I : Bioconversion of ketones 1 to 5 with Acinetobacter NCIB 9871.

Ketone	react. time	Lactone a				Lactone b			
	(h)	Yield [a] (%)	$[\alpha]_D^{25}$	ee [b] (%)	abs. conf.[c]	Yield [a] (%)	$[\alpha]_D^{25}$	ee [b] (%)	abs. conf [c]
 1	2.5	35	- 40.8 c = 0.51 $CHCl_3$	90	1R,5S	32	- 103.2 c = 0.5 $CHCl_3$	> 98	1R,5S
 2	3	35	- 37 c = 0.5 $CHCl_3$	97	1R,5R	35	- 101.2 c = 0.5 $CHCl_3$	> 98	1S,5R
 3	4 5	33	- 67.3 c = 0.639 $CHCl_3$	> 98	1S,5S	41	- 113.3 c = 0.6 $CHCl_3$	> 98	1R,5S
 4	2 5	33	- 4 c = 0.55 $CHCl_3$	70	1R,6S	33	- 105.1 c = 0.69 $CHCl_3$	> 98	1R,6S
 5	3 5	60	- 24 c = 0 5 $CHCl_3$	33	1S,6S	18	- 26 5 c = 0 577 $CHCl_3$	> 98	1S,6R

(a) Yield of isolated product (b) ee's measured by chiral gas chromatography using a capillary column coated with heptakis (6-O-methyl-2,3-di-O-Pentyl)-β-Cyclodextrin (c) All absolute configurations were assigned using circular dichroism measurements

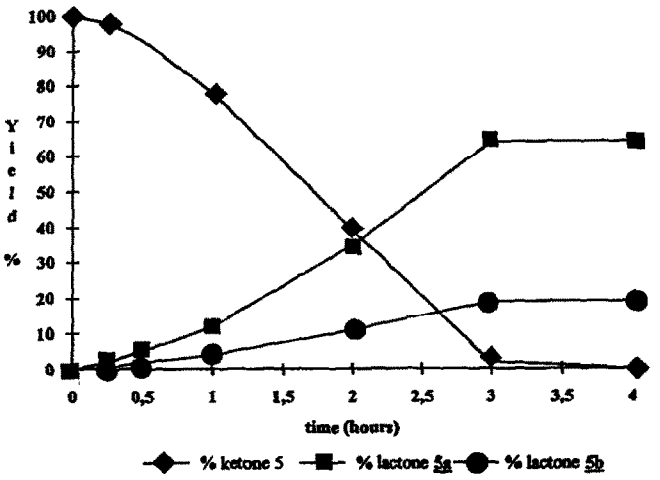
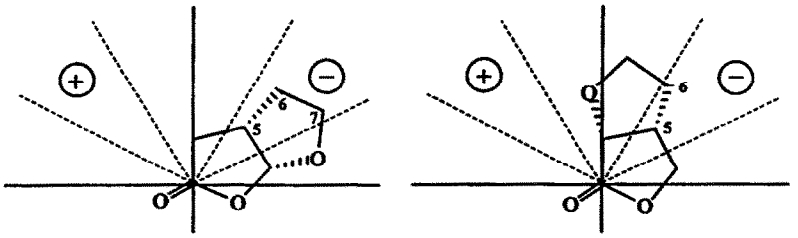


Figure 3 : Time course of the bioconversion of ketone 5.

Interestingly, all lactones of one particular type are formed from the same enantiomer of the starting ketone. Thus, the enantiomer of 1 bearing a (S) configuration at the bridgehead carbon atom α to the carbonyl group leads to the "normal" lactone **1a**, whereas the one of (R) configuration affords the "abnormal" lactones **1b**.

To our best knowledge, neither of the lactones we have obtained in the course of this work have been described previously in optically active form, in spite of their interest as chiral synthons. This emphasizes the high potential of these bioconversions. Moreover these bioconversions can be conveniently and efficiently achieved on a several-gram scale. Thus, in a typical experiment, 5 g of 1 were totally transformed, within 2 h, to a mixture of **1a** and **1b** using a 5 L culture. These were readily separable using careful bench-top flash chromatography, thus leading to 2.7 g (47% isolated yield) of (1R,5S) **1a** (ee = 86%) and to 2.3 g of (1R,5S) **1b** showing an ee of about 100% (40% isolated yield).

Since no information was available in the literature in order to determine the absolute configuration of these products, this was achieved using circular dichroism spectroscopy which, in spite of being a semi-empirical method, affords valuable informations in this respect. Klyne et al.[16] have previously described a sector rule (similar to the octant rule for ketones), which specifically applies to lactones. This rule enables the rotatory dispersion of lactones to be predicted from considerations on the asymmetric surroundings of the chromophore, and was established after measurements for an extensive series of lactones. The various space sectors are shown on Figure 4 where lactones **1a** and **1b** formed from 1 are positioned as proposed by these authors. Thus, both these lactones are positioned into the sector rule with the lactone carbonyl function towards the left side This leads in both cases the carbon skeleton to be behind the plane (The signs of the sectors indicated on this figure are therefore those of the back sectors.) In these particular cases, the optical rotatory dispersion (o.r.d.) curves both show a negative Cotton effect in the region of 200-230 nm. This led us to assign the (1R,5S) absolute configuration to lactone **1a**, as well as to lactone **1b**. Interestingly, the negative Cotton effect observed for the corresponding cyclopentane compounds obtained previously[2], which absolute configurations are known from



1a 1b

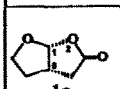
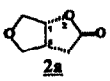
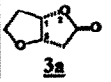
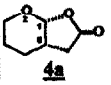
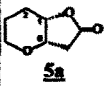
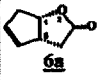
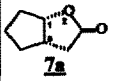
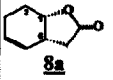
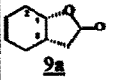
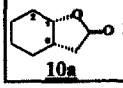
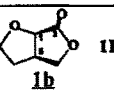
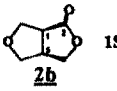
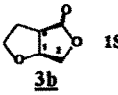
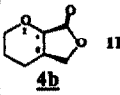
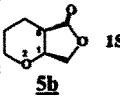
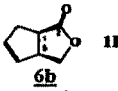
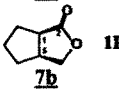
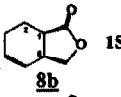
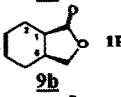
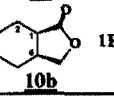
Figure 4 : Sector rule applied to lactones 1a and 1b.

the literature, are in complete agreement with the predictions one can make using this lactone sector rule. The results of the o.r.d. measurements of all the obtained lactones, as well as of those achieved on their carbon-ring analogs, are recorded on Table II. It appears that all butyrolactones bearing an adjacent five membered ring (except **6a**) show a negative Cotton effect in agreement with the sector rule prediction. The positive Cotton effect observed for **6a** is probably due to electronic disturbance by the double bond. Indeed, hydrogenation of **6a** led to (1S, 5R)-**7a** showing again a negative Cotton effect.

Similarly, butyrolactones bearing an adjacent six membered ring show generally also a negative Cotton effect, as predicted by the sector rule. However this is not the case for **5a** and for **10a** which both exhibit a positive Cotton effect. We presume that this is not due to an opposite absolute configuration but rather to the flexibility of the pyran or cyclohexane ring which could lead to various conformers, and thus to a poor understanding of their positioning in the proposed lactone sector rule. This is confirmed by the fact that the "abnormal" lactones **5b** and **10b** (obtained from the antipodal starting ketone) show, as all the other ones, a negative Cotton effect. Since this leads to assign respectively the (1S, 6R) and (1R, 6S) absolute configurations to these compounds, it is obvious that the "normal" lactones must have been formed from the other ketone enantiomer.

We have previously proposed a temptative model which allows to understand, and therefore also to predict, the stereoselectivity of these reactions[2a]. This was based on bicyclic models bearing either cyclopentane of cyclohexane rings adjacent to the cyclobutanone moiety. Since introduction of an oxygen atom into these rings does not considerably alter the structure of the substrates, our model can be used without problem in these cases. It thus appears that the predictions related to the absolute configuration of the formed lactones, which can be drawn using this model, are in total agreement with the observed results. This model has been based on the hypothesis that one single enzyme is operative in these reactions previously proposed by Walsh and coll.[17]. This hypothesis seems to have been confirmed recently in the course of an elegant work performed by Knowles and coll [1d] which, as we described previously, have confirmed that the purified cyclohexanone oxygenase (from *A. calcoaceticus*) indeed catalyses parallel conversion of the bicyclo [3.2.0] hept-2-ene-6-one into the two regioisomeric lactones. Nevertheless, one can consider that there is still no *absolute* answer to the question whether this very surprizing selectivity is due to one single enzyme or to two very similar isoenzymes.

Table II : Circular dichroism measurement of optically active lactones **1a** to **10a** and **1b** to **10b**.

Lactone	Abs. Conf.	Sign of Cotton effect (Predict.)	Sign of Cotton effect (found)	λ max (nm)	[Φ]
 **1a**	1R,5S	–	–	215	- 1346
 **2a**	1R,5R	–	–	212.5	- 2032
 **3a**	1S,5S	–	–	213	- 2602
 **4a**	1R,6S	–	–	214.5	- 910
 **5a**	1S,6S	–	+	210	+ 175
 **6a**	1S,5S	–	+	205.5	+ 582
 **7a**	1S,5R	–	–	212	- 582
 **8a**	1S,6S	a	a	a	a
 **9a**	1S,6S	–	–	213	- 890
 **10a**	1S,6S	–	+	208	+ 175
 **1b**	1R,5S	–	–	227	- 4140
 **2b**	1S,5R	–	–	211	- 1013
 **3b**	1S,5R	–	–	214	- 2182
 **4b**	1R,6S	–	–	225	- 5558
 **5b**	1S,6R	–	–	215.5	- 2499
 **6b**	1R,5S	–	–	211	- 2946
 **7b**	1R,5S	–	–	214 5	- 2487
 **8b**	1S,6R	–	–	212	- 2836
 **9b**	1R,6S	–	–	213	- 3232
 **10b**	1R,6S	–	–	215.5	- 1187

(a) Not measured

As a conclusion, the results described in this work illustrate the high potential of the microbiologically mediated Baeyer-Villiger oxidation as a new tool for asymmetric synthesis. Also, they indicate that the presence of an oxygen atom in the ring adjacent to the cyclobutanone does not alter the course of the reaction, thus leading to conclude that no perturbing hydrogen bond does occur inside the active site These bioconversions allow straightforward preparation of various chiral synthons furofurans and furopyrans which can thus be obtained in high enantiomeric purity. These constitute highly valuable building blocks for further synthesis of compounds showing interesting biological properties. Work is in progress in our laboratory in order to continue the study of this interesting microbiological biotransformation.

Experimental Part

^1H NMR spectra were recorded in $CDCl_3$ or C_6D_6 at 80 or 200 MHz with tetramethylsilane as internal reference. ^{13}C NMR spectra were recorded at 200 MHz. Abreviations employed are as follow : s (singlet) ; t (triplet) ; q (quadruplet) ; Q (quintuplet) ; d (doublet) ; dd (doublet of doublet)... Each reaction was monitored by TLC, except for Baeyer-Villiger reactions which were monitored by gas chromatography using a capillary column (OV-1701, 25 m). Separations by flash chromatography were performed using Merck silica gel 60H. Ether, benzene, petroleum ether, pentane and tetrahydrofuran were dried by distillation over sodium.

Acinetobacter NCIB 9871 was a generous gift from Prof. C. T. Walsh. Stock cultures were grown on nutrient agar at 30 °C and stored at 4 °C.

A - Synthesis of the ketones 1 to 5

(±)2-oxabicyclo[3,2,0]heptan-7-one 1 : According to the procedure described by Snider and Hui[10], 3-buten-1-ol was converted in two steps to ketone **1**.

(±) 3-oxabicyclo[3,2,0]heptane-7-one 2 : A 250 ml round bottom flask containing a mixture of 4 ml (54 mmol) of 2,5-dihydrofuran and 7.1 g (2 eq.) of zinc powder in 150 ml of anhydrous ether, placed under argon, was partially submerged in a sonicator water bath according to the procedure described by Mehta and Rao[11]. Trichloracetyl chloride (9.15 ml, 1.5 eq.) in 90 ml of ether was added within 3 h to generate *in situ* the dichloroketene. After an additional hour, the crude mixture was filtered over celite, washed with water and saturated $NaHCO_3$ solution and dried over $MgSO_4$. Flash chromatography with hexane/ether afforded 2.54 g of 6,6-dichloro-3-oxabicyclo[3,2,0]heptane-7-one as an odorant oil (26%). I.R. (cm^{-1}, neat) : 2860 ; 1805 ; 820 ^1H NMR (200 MHz, $CDCl_3$, ppm) : 4.55 (d, H_{4endo}) ; 4.42 (d, H_{2endo}) ; 4.23 (dd, H_1) ; 3.77 (dd, H_{4exo}) ; 3.60 (dd, H_5) ; 3.56 (dd, H_{2exo}). To 3 g (17 mmol) of this dichloroketone in 20 ml of acetic acid were added 5.6 g (5 eq.) of zinc powder with stirring[12] After 15 min the mixture was heated to 80 °C for 2 h, cooled, filtered over celite and diluted with 100 ml of CH_2Cl_2 and 100 ml of water. The organic layer was neutralized with saturated $NaHCO_3$ solution and dried over $MgSO_4$. Flash chromatography (ether/hexane) gave 1.65 g (90%) of pure cyclobutanone **2**. I.R. (cm^{-1}, neat) : 2850 , 1775 ; 905. ^1H NMR (200 MHz, C_6D_6, ppm) : 4.09 (d, H_{2endo}) ; 3.57 (d, H_{4endo}) ; 3.11 (dd, H_{4exo}) ; 3.00 (m, H_1) ; 2.95 (dd, H_{2exo}) ; 2.58 (ddd, H_{6exo}) ; 2.27 (ddd, H_{6endo}) ; 2.12 (m, H_5). ^{13}C NMR ($CDCl_3$, ppm) : 208.28 (C_7) ; 73.68 ($^*C_2$) ; 70.19 ($^*C_4$) ; 65.16 (C_1) ; 51.41 (C_6) ; 29.87 (C_5).

(±) 2-oxabicyclo[3,2,0]heptane-6-one 3 : To 17.5 ml (233 mmol) of 1,2-dihydrofuran and 8.28 ml (0.37 eq.) of dichloracetyl chloride in 35 ml of dry petroleum ether were added, within 3 h, 12 ml (0.37 eq.) of triethylamine in 75 ml of petroleum ether following the procedure described by Ghosez and al.[13]. After an additionnal hour, water was added to dissolve all the salts. The organic layer was worked up as described above to give 9 g (58%) of 7,7-dichloro-2-oxabicyclo[3,2,0]heptane-6-one after flash chromatography purification. I.R. (cm^{-1}, neat) : 2980 ; 1805 ; 815. ^1H NMR (200 MHz, C_6D_6, ppm) : 4.43 (d, H_1) ; 3.49 (td, H_{3exo}) ; 3.37 (ddd, H_{3endo}) ; 3.20 (dd, H_5) ; 1.52 (dd, H_{4endo}) ; 1.00 (dddd, H_{4exo}). Bu_3SnH (40.6 g 138 mmol) and 100 mg of AIBN were dissolved in 180 ml of dry cyclohexane under argon. The solution was refluxed and 8.3 g (46 mmol) of the dichloroketone in 130 ml of cyclohexane were added dropwise. After 2 h, a rapid flash chromatography of the crude solution allowed to eliminate most of the tributyltin salts. A second careful flash chromatography afforded 3.84 g (75%) of cyclobutanone **3**. I.R. (cm^{-1}, neat) : 2970 ; 1775 ; 950. ^1H NMR

(200 MHz, $CDCl_3$, ppm) : 4.95 (td, H_1) ; 4.14 (ddd, H_{3exo}) ; 3.91 (td, H_{3endo}) ; 3.84 (m, H_5) ; 3.25 (td, H_{7exo}) ; 2.91 (td, H_{7endo}) ; 2.21 (tdd, H_{4endo}) ; 1.96 (tdd, H_{4exo}). ^{13}C NMR ($CDCl_3$, ppm) : 209.51 (C_6) ; 70.02 (C_1) ; 68.24 (C_3) , 65.41 (C_5) , 52.72 (C_7) ; 29.00 (C_4).

(±) 2-oxabicyclo[4,2,0]octan-8-one **4**[10] : Treatment of 6 g (70 mmol) of 4-penten-1-ol by 4.62 g (2.2 eq.) of sodium hydride (80% in paraffin) in 150 ml of THF was followed 20 min after, by cautious addition of bromoacetic acid (9.7 g, 1 eq.) to the solution and the mixture was refluxed overnight. Water was added to dissolve all the salts and the solution was brought to pH = 10 with saturated Na_2CO_3 solution. It was then washed by ether and acidified (pH = 1) with 10N HCl solution. Extraction with 3 x 100 ml of ether, drying and removal of the solvent afforded 6.2 g of pure 3-pentenyloxy-acetic acid (62%). I.R. (cm^{-1}, neat) : 3100 ; 2940 ; 1720. ^1H NMR (80 MHz, $CDCl_3$, ppm) : 9.39 (s, 1H) ; 5.77 (m, 1H) ; 4.75 (m, 2H) ; 4.08 (s, 2H) ; 3.52 (t, 2H) ; 2.12 (q, 2H) ; 1.65 (q, 2H). 6.2 g (44 mmol) of 3-pentenyloxy-acetic acid and 10 eq. of oxalyl chloride were refluxed within 2 h in 60 ml of benzene to afford the corresponding acyl chloride. The excess of oxalyl chloride was removed by evaporation in vacuo I.R. (cm^{-1}, neat) : 2930 ; 1795 ; 930. ^1H NMR (80 MHz, $CDCl_3$, ppm) : 5.80 (m, 1H) ; 4.97 (t, 2H) ; 4.35 (s, 2H) ; 3.55 (t, 2H) ; 2.10 (q, 2H) ; 1.7 (q, 2H). The crude mixture was immediately dissolved in 800 ml of dry benzene under argon. Then 6.8 ml (1.4 eq.) of triethylamine in 75 ml of benzene were added dropwise as slow as possible to the refluxing solution. After an additional 6 h reflux, the solution was filtered and washed with 2 x 50 ml of a 5% HCl solution. Flash chromatography (ether/hexane) afforded 0.45 g of pure cyclobutanone **4** (8.3% for the 2 steps). I.R. (cm^{-1}, neat) : 2940 ; 1780 ; 910. ^1H NMR (200 MHz, $CDCl_3$, ppm) : 4.80 (d, H_1) ; 3.83 (m, $H_{3eq.}$) ; 3.56 (td, $H_{3ax.}$) ; 2.85 (ddd, H_{7exo}) ; 2.56 (Qd, H_6) ; 2.41 (m, $^*H_{5eq.}$) ; 2.28 (td, H_{7endo}) ; 1.51 (m, $H_{4ax.}$ and $H_{4eq.}$) ; 1.26 (m, $^*H_{5ax.}$). ^{13}C NMR ($CDCl_3$, ppm) : 205.83 (C_8) ; 83.33 (C_1) ; 64.79 (C_3) ; 46.35 (C_7) ; 26.73 (C_4) ; 22.73 (C_6) ; 22.45 (C_5).

(±) 2-oxabicyclo[4,2,0]octane-7-one **5** : Cyclisation of 2.5 ml (27 mmol) of 1,2-dihydropyran under ultrasonic activation[11] and usual work-up of the reaction gave 2.73 g (51%) of 8,8-dichloro-2-oxabicyclo[4,2,0]octan-7-one as a white odorant solid. M.p. : 69 °C (lit.[13] : 68-69.5 °C). I.R. (cm^{-1}, $CHCl_3$) : 2940 ; 1815 ; 810. ^1H NMR (80 MHz, $CDCl_3$, ppm) : 4.4 (d, 1H) ; 3.9 (m, 2H) ; 3.4 (dd, 1H) ; 2.2 (m, 1H) ; 1.6 (m, 3H). 3.11 g (16 mmol) of this dichloroketone were reduced using tributyltin hydride[13] and afforded 1.65 g (82%) of cyclobutanone **5**. I.R. (cm^{-1}, neat) : 2940 ; 1780 ; 900. ^1H NMR (200 MHz, $CDCl_3$, ppm) : 4.46 (t, H_1) ; 3.83 (d, $H_{3eq.}$) ; 3.28 (m, $H_{3ax.}$ and H_6) ; 3.23 (dd, H_{8exo}) ; 2.72 (d, H_{8endo}) ; 2.09 (d, $H_{5eq.}$) ; 1.55 (m, $H_{4ax.}$, $H_{4eq.}$ and $H_{5ax.}$). ^{13}C NMR ($CDCl_3$, ppm) : 206.87 (C_7) ; 64 98 (C_3) ; 63.94 (C_1) ; 57.11 (C_6) ; 53.34 (C_8) ; 22.01 (C_4) ; 18.67 (C_5)

B - Synthesis of racemic lactones

As a general approach, these lactones were prepared using the mCPBA oxidation of the corresponding ketones.

General procedure for Baeyer-Villiger oxidations

To 4.5 mmol of ketone and 1.13 g (3 eq.) of $NaHCO_3$ in 70 ml of CH_2Cl_2 were added 1.41 g (1 eq.) of m-CPBA (55% w/w). After 1.5 to 3 h, the mixture was treated by 50 ml of a 10% $NaHSO_3$ solution and 50 ml of saturated $NaHCO_3$ solution. Purification by flash chromatography (pentane/ethyl acetate) afforded the corresponding lactones.

Oxidation of ketone 1 : synthesis of lactone 1a. 150 mg (1.3 mmol) of **1** led to 160 mg (95%) of (±)-2.8-dioxabicyclo[3,3,0]octane-3-one **1a**. I.R. (cm^{-1}, neat) : 2980 ; 1755 ; 970. ^1H NMR (200 MHz, $CDCl_3$, ppm) : 6.10 (d, H_1) ; 4.11 (td, H_{7exo}) ; 3.96 (td, H_{7exo}) ; 3.18 (m, H_5) ; 2.90 (dd, H_{4exo}) ; 2.45 (dd, H_{4endo}) ; 2.22 (dddd, H_{6exo}) ; 1.80 (tdd, H_{6endo}). ^{13}C NMR ($CDCl_3$, ppm) : 174.97 (C_3) ; 108.43 (C_1) ; 67.35 (C_7) ; 38.58 (C_5) ; 35.08 (C_4) ; 32.37 (C_6). Anal. Calcd for $C_6H_8O_3$: C, 56.24 ; H, 6.29. Found : C, 56.57 ; H, 6.34.

Oxidation of ketone 2 : synthesis of lactones 2a and 2b. 500 mg (4.46 mmol) of **2** led to 420 mg (74%) of (±)-2,7-dioxabicyclo[3,3,0]octane-3-one **2a** and 75 mg (13%) of (±)-3,7-dioxabicyclo[3,3,0]octane-2-one **2b**. As **2a** and **2b** were unseparable by gas chromatography, HPLC (Spherisorb 5 m.m, CH_2Cl_2/Hexane (50/50) as eluent, 3 ml/min) was used to monitor the flash chromatography. **2a** : I.R. (cm^{-1}, neat) : 2970 ; 1760 ; 910. ^1H NMR (200 MHz, $CDCl_3$, ppm) : 5.12 (dd, H_1) ; 4.13 (d, H_{8endo}) ; 3.80 (m, H_{6endo} and H_{6exo}) ; 3.65 (dd, H_{8exo}) ; 3.13 (m, H_5) ; 2.88 (dd, H_{4exo}) ; 2.46 (dd, H_{4endo}). ^{13}C NMR ($CDCl_3$, ppm) : 176.52 (C_3) ; 83.86 (C_1) ; 74.70 (C_8) ; 73.58 (C_6) ; 38.46 (C_5) ; 34.43 (C_4). Anal. Calcd for $C_6H_8O_3$: C, 56.24 ; H, 6.29. Found : C, 56.40 ; H, 6.33. **2b** : I.R. (cm^{-1}, neat) : 2970 ; 1765 ; 910. ^1H NMR (200 MHz, C_6D_6, ppm) : 4.14 (dd, H_{8endo}) ; 3.57 (dd, H_{4exo}) ; 3.33 (dd, H_{4endo}) ; 3.20 (dd, H_{6endo}) ; 3.17 (dd, H_{8exo}) ; 3.02 (dd, H_{6exo}) ; 2.38 (ddd, H_1) ; 1.92 (m, H_5). ^{13}C NMR ($CDCl_3$, ppm) : 178.61 (C_2) ; 74.92 (*C_8) ; 72.95 (*C_4) ; 71.67 (C_6) ; 45.51 (C_1) ; 39.65 (C_5). Anal. Calcd for $C_6H_8O_3$: C, 56.24 ; H, 6.29. Found : C, 55.83 ; H, 6.35.

Oxidation of ketone 3 : synthesis of lactones 3a and 3b. 1 g (8.9 mmol) of **3** afforded 600 mg (53%) of (±)-2,6-dioxabicyclo[3,3,0]octane-3-one **3a** and 115 mg (10%) of (±)-3,8-dioxabicyclo[3,3,0]octane-4-one **3b**. **3a** : I.R. (cm^{-1}, neat) : 2990 ; 1775 ; 1180. ^1H NMR (200 MHz, C_6D_6, ppm) : 4.17 (t, H_1) ; 3.81 (t, H_5) ; 3.48 (td, H_{7endo}) ; 3.36 (td, H_{7exo}) ; 2.33 (d, H_{4endo}) ; 1.97 (dd, H_{4exo}) ; 1.68 (ddd, H_{8endo}) ; 1.27 (dddd, H_{8exo}). ^{13}C NMR ($CDCl_3$, ppm) : 175.33 (C_3) ; 84.18 (C_1) ; 78.11 (C_5) ; 66.97 (C_7) ; 36.25 (C_4) ; 33.11 (C_8). Anal. Calcd for $C_6H_8O_3$: C, 56.24 ; H, 6.29. Found : C, 55.95 ; H, 6.41. **3b** : I.R. (cm^{-1}, neat) : 2960 ; 1770. ^1H NMR (200 MHz, C_6D_6, ppm) : 4.41 (d, H_{2endo}) ; 4.23 (dd, H_1) ; 3.99 (dd, H_{2exo}) ; 3.88 (td, H_{7endo}) ; 3.77 (td, H_{7exo}) ; 2.79 (ddd, H_5) ; 2.46 (dddd, H_{6endo}) ; 1.99 (tdd, H_{6exo}). ^{13}C NMR ($CDCl_3$, ppm) : 178.13 (C_4) ; 78.62 (C_1) ; 73.28 (C_2) ; 68.14 (C_7) ; 44.75 (C_5) ; 30.16 (C_6). Anal. Calcd for $C_6H_8O_3$: C, 56.24 ; H, 6.29. Found : C, 55.93 ; H, 6.31.

Oxidation of ketone 4 : synthesis of lactone 4a. 150 mg (1.2 mmol) of **4** afforded 160 mg (95%) of (±)-2,9-dioxabicyclo[4,3,0]nonan-8-one **4a**. I.R. (cm^{-1}, neat) : 2930 ; 1780. ^1H NMR (200 MHz, $CDCl_3$, ppm) : 5.65 (d, H_1) ; 3.79 (tdd, $H_{3ax.}$ and $H_{3eq.}$) ; 2.61 (dd, H_{7exo}) ; 2.51 (m, H_6) ; 2.39 (dd, H_{7endo}) ; 1.92 (m, $H_{5ax.}$) ; 1.66 (m, 3H : $H_{4ax.}$, $H_{4eq.}$ and $H_{5eq.}$). ^{13}C NMR ($CDCl_3$, ppm) : 174.31 (C_8) ; 101.02 (C_1) ; 62.41 (C_3) ; 34.96 (C_7) ; 33.62 (C_6) ; 24.07 (C_4) ; 21.41 (C_5). Anal. Calcd for $C_7H_{10}O_3$: C, 59.14 ; H, 7.09. Found : C, 59.10 ; H, 7.15.

Oxidation of ketone 5 : synthesis of lactones 5a and 5b. 1 g (8.1 mmol) of **5** led to 1.2 g (87%) of solid (±)-5,9-dioxabicyclo[4,3,0]nonan-8-one **5a** and to 3% of lactone **5b** that was not isolated. **5a** : m.p. : 78 °C (lit.[19] : 77-79 °C). I.R. (cm^{-1}, $CHCl_3$) : 3000 ; 1775. ^1H NMR (200 MHz, $CDCl_3$, ppm) : 4.36 (d, H_6) ; 4.19 (t, H_1) ; 3.89 (d, $H_{3eq.}$) ; 3.41 (td, $H_{3ax.}$) ; 2.69 (dd, H_{9exo}) ; 2.50 (d, H_{9endo}) ; 2.26 (d, $H_{5eq.}$) ; 1.82 (m, $H_{4ax.}$ and $H_{4eq.}$) ; 1.46 (d, H_{5exo}). ^{13}C NMR ($CDCl_3$, ppm) : 175.92 (C_8) ; 76.79 (C_6) ; 72.87 (C_1) ; 65.83 (C_3) ;

39.00 (C_9) ; 24.86 (*C_5) ; 19.34 (*C_4). Anal. Calcd for $C_7H_{10}O_3$: C, 59.14 ; H, 7.09. Found : C, 58.89 ; H, 7 00.

*Synthesis of lactone (±)-**1b***. According to the procedure described by Korte and Machleidt[20], 1.5 g (65.3 mmol) of sodium pieces were introduced in 20 ml of anhydrous ether with one drop of absolute methanol. After 2 h, a mixture of 7.72 g (1 eq.) of dimethyloxalate and 5.63 g (1 eq.) of γ-butyrolactone in 30 ml of ether was added dropwise. The reaction was stirred for 2 days. The yellow mixture was poured on ice and acidified with 50% H_2SO_4 solution. A solid precipitated and was recrystallized in benzene/petroleum ether (50/50) to give 1.1 g of white needles (m. p. 140 °C ; lit.[19] : 138.5-141 °C). The aqueous layer was extracted sixteen times with 50 ml of ether and usual work-up gave a white solid that was recrystallized in benzene to afford an additional 1.7 g amount of white prisms (m. p. 104 °C ; lit.[19] : 106 °C). Thus a total amount of 2.8 g (25%) of α-methoxalyl-γ-butyrolactone was obtained. I.R. (cm^{-1}, $CHCl_3$) : 2940, 1725, 1660. ^1H NMR : 4.45 (t, 2H) ; 3.90 (s, 3H) ; 3.25 (t, 1H) ; 2.30 (q, 2H). 1.9 g of methoxalyllactone in 15 ml of HCl/MeOH 1N were refluxed for 14 h and, after cooling, stirred for another 3 days at room temperature. Work-up of the reaction mixture led to 1.5 g (60%) of a diastereoisomeric mixture of *cis* and *trans* 2-methoxy-tetrahydrofuran-dicarboxylic-(2,3)-dimethylester. The crude mixture was treated with one drop of a mixture of H_3PO_4/H_2SO_4 (50/50) and submitted to slow bulb-to-bulb distillation. Complete elimination of MeOH afforded 780 mg (60%) of pure 4,5-dihydrofuran-dicarboxylic-(2,3)-dimethylester. I.R. (cm^{-1}, neat) 2940, 1745, 1700, 1630. ^1H NMR : 4.55 (t, 2H) ; 3.85 (s, 3H) ; 3.70 (s, 3H) ; 2.98 (t, 2H). Hydrogenation (Pd/C in 50 ml of ethanol) of 500 mg of the diester afforded 460 mg (91%) of tetrahydrofuran-*cis*-dicarboxylic-(2,3)-dimethylester. I. R. (cm^{-1}, neat) : 2940, 1735, 1210. ^1H NMR : 4.60 (d, 1H) ; 4.11 (m, 2H) ; 3.70 (s, 3H) ; 3.63 (s, 3H) ; 3.38 (q, 1H) ; 2.27 (m, 2H). 450 mg of this diester were then selectively saponified using 140 mg (1 eq.) of KOH in 3.5 ml of water. After one day, the mixture was acidified using 10N HCl solution and extracted continuously with CH_2Cl_2 to afford 400 mg of crude tetrahydrofuran-dicarboxylic-(2,3)-3-monomethylester. ^1H NMR : 7.10 (s, 1H) ; 4.60 (d, 1H) ; 4.10 (m, 2H) ; 3.70 (s, 3H) ; 3.35 (q, 1H) ; 2.27 (m, 2H). This was treated by 150 mg (3 eq.) of $LiBH_4$ in 2.5 ml of THF at 50 °C with stirring. After 1 h, the mixture was poured on iced water, and acidified with 10N HCl solution. Continuous extraction with CH_2Cl_2 and purification by flash chromatography (pentane, ethyl acetate) afforded 25 mg (8.5% for the 2 steps) of (±)-3,8-dioxabicyclo[3,3,0]octane-2-one **1b** : I R. (cm^{-1}, neat) : 2980, 1770, 1180. ^1H NMR (200MHz, $CDCl_3$, ppm) : 4.66 (d, 1H) ; 4.53 (dd, H_{4exo}) ; 4.17 (dd, H_{4endo}) ; 4.05 (ddd, H_{7exo}) ; 3.84 (td, H_{7endo}) ; 3.22 (m, H_5) ; 2.30 (qd, H_{6exo}) ; 1.91 (tdd, H_{6endo}). ^{13}C NMR ($CDCl_3$, ppm) : 174.93 (C_2) ; 77.92 (C_1) ; 71.35 (C_4) ; 68.71 (C_7) ; 38.36 (C_5) ; 32.81 (C_6). Anal. Calcd for $C_6H_8O_3$: C, 56.24 ; H, 6.29. Found : C, 56.16 ; H, 6.33.

*Synthesis of lactone (±)-**4b***. All the procedures were the same as described above for (±)-**1b**. Thus, 8.47 g (84.7 mmol) of δ-valerolactone afforded 4 g (25%) of liquid α-methoxalyl-δ-valerolactone. I.R. (cm^{-1}, neat) : 2940, 1730, 1640, 1250. ^1H NMR : 4.38 (t, 2H) ; 3.85 (s, 3H) ; 2.82 (t, 1H) ; 1.90 (m, 4H). 4 g of methoxalyllactone were rearranged to a diastereoisomeric mixture of *cis* and *trans* 2-methoxy-tetrahydropyran-dicarboxylic-(2,3)-dimethylester which was immediatly treated by H_2SO_4/H_3PO_4 to afford 1.7 g (40% for the 2 steps) of 5,6-dihydropyran-dicarboxylic-(2,3)-dimethylester. I.R. (cm^{-1}, neat) : 2940, 1740, 1705, 1625. ^1H NMR : 4.12 (t, 2H) ; 3.80 (s, 3H) ; 3.70 (s, 3H) ; 2.35 (t, 2H) ; 1.93 (q, 2H). Hydrogenation of 500 mg of this unsaturated diester afforded 400 mg (80%) of *cis*-tetrahydropyran-dicarboxylic-(2,3)-dimethylester. I.R. (cm^{-1}, neat) : 2940, 1730. ^1H NMR : 4.21 (d, 1H) ; 4.02 (m, 2H) ; 3.75 (s, 3H) ; 3.65 (s, 3H) ; 3.05 (q, 1H) ; 2.25 (m, 1H) ; 1.60 (m, 3H). 400 mg of this diester were selectively saponified to afford 300 mg of tetrahydropyran-dicarboxylic-(2,3)-3-monomethylester as a crude mixture. ^1H NMR : 8.35 (s, 1H) ; 4.1 (m, 3H) ; 3.65 (s, 3H) ;

3.15 (q, 1H) ; 2.35 (m, 1H) ; 1.7 (m, 3H). Treatment of this mixture with $LiBH_4$ afforded 20 mg (7%) of (±)-2,8-dioxabicyclo[4,3,0]nonan-9-one **4b** : I.R. (cm^{-1}, neat) : 2960, 1770, 1180. ^1H NMR (200MHz, $CDCl_3$, ppm) : 4.50 (d, 1H) ; 4.27 (dd, H_{7exo}) ; 4.04 (dd, H_{7endo}) ; 3.81 (tdd, $H_{3eq.}$) ; 3.54 (dd, $H_{3ax.}$) ; 2.55 (m, H_6) ; 2.00 (m, $H_{5ax.}$) ; 1.61 (m, $H_{4ax.}$, $H_{4eq.}$, $H_{5eq.}$). ^{13}C NMR ($CDCl_3$, ppm) : 173.36 (C_8) ; 72.44 (C_1) ; 69.09 (C_7) ; 64.44 (C_3) ; 33.13 (C_6) ; 23.17 (*C_4) ; 22.65 (*C_5). Anal. Calcd for $C_7H_{10}O_3$: C, 59.14 ; H, 7.09. Found : C, 59.29 ; H, 7.12.

Bioconversion of ketones 1 to 5

Typical Biotransformation Experiment. In a 2 L fermentor containing the following medium : 4 g of Na_2HPO_4, 2 g of KH_2PO_4, 3 g of $(NH_4)_2SO_4$, 0.2 g of Yeast Extract, 0.1 g of $CaCl_2$, 0.5 g of $MgSO_4$, $7H_2O$, 0.01 g of Fe_2SO_4, and 2 g of 1,2-cyclohexanediol (mixture of *cis* and *trans*) as only source of carbon, cells were grown for 15 h at 30 °C with vigorous aeration (16 v/v/m) and stirring at 400 rpm. At the end of the growth period, the temperature was lowered to 25°C and the pH was adjusted at 7. Cyclohexanediol (0.5 g) was added followed by 1 g of ketone dissolved in 5 ml of ethanol. The bioconversion was monitored by G.C. using an internal standard (dodecane or tetradecane). After completion of the reaction the medium was acidified with 10N HCl solution, saturated with NaCl and extracted with CH_2Cl_2 for two days. Products were then purified using two (at least) flash chromatographies. All the lactones **1a** to **5a** and **1b** to **4b** were identified by comparison of their I.R. and ^1H and ^{13}C NMR spectra with those of the already prepared racemic compounds.

Bioconversion of ketone 1. Bioconversion of 1 g (8.93 mmol) of **1** afforded 390 mg (35%) of **(-)-1a** and 350 mg (32%) of solid **(-)-1b** (m.p. : 53-54 °C).

Bioconversion of ketone 2. 1 g of **2** led to 400 mg (35%) of solid **(-)-2a** (m.p. : 62-63 °C) and 400 mg (35%) of solid **(-)-2b** (m.p. : 48 °C).

Bioconversion of ketone 3. 1 g of **3** gave 380 mg (33%) of **(-)-3a** and 480 mg (41%) of **(-)-3b**.

Bioconversion of ketone 4. 1 g (7.94 mmol) of **4** was oxidized to 365 mg (33%) of **(-)-4a** and 365 mg (33%) of **(-)-4b**.

Bioconversion of ketone 5. 1 g of **5** led to 700 mg (60%) of **(-)-5a** and 200 mg (18%) of **(-)-5b**. **(-)-5b** : I.R. (cm^{-1}, neat) : 2960, 1765, 1140. ^1H NMR (200MHz, $CDCl_3$, ppm) : 4.35 (dd, 1H) ; 4.24 (d, H_{9endo} and H_{9exo}) ; 3.90 (td, $H_{3eq.}$) ; 3.37 (td, $H_{3ax.}$) ; 2.59 (t, H_6) ; 2.28 (d, $H_{5eq.}$) ; 1.85 (m, $H_{5ax.}$) ; 1.54 (m, $H_{4ax.}$, and $H_{4eq.}$). ^{13}C NMR ($CDCl_3$, ppm) : 176.37 (C_7) ; 77.63 (C_1) ; 72.28 (C_9) ; 66.07 (C_3) ; 39.47 (C_6) ; 22.12 (C_4) ; 20.15 (C_5). Anal. Calcd for $C_7H_{10}O_3$: C, 59.14 ; H, 7.09. Found : C, 59.19 ; H, 7.07.

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