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"MICROBIAL TRANSFORMATIONS 18. REGIOSPECIFIC *para*-HYDROXYLATION OF AROMATIC CARBAMATES MEDIATED BY THE FUNGUS *Beauveria sulfurescens*"

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Summary : The bioconversion of various N-phenyl carbamates bearing alkyl groups of increasing size have been studied. These biotransformations, achieved by the fungus Beauveria sulfurescens, led to the highly regiospecific para-hydroxylation of most of the substrates. For compounds of higher lipophilic character, a double regio- and stereospecific hydroxylation process occurred, which included aromatic and alkyl hydroxylation.

Regiospecific *para* hydroxylation of aromatic rings is a very difficult task in organic synthesis (1-4). On the other hand, it is well known that enzymes (in particular cytochrome P-450 type enzymes) are able to perform this type of transformation, which belong to the few biological processes whose counterpart in organic synthesis are rarely seen. We have previously described results showing that the fungus *B. sulfurescens* is able to achieve regiospecific aromatic hydroxylations on simple substrates as Propham 3 (isopropyl-N-phenyl carbamate), its N-methylated derivative 4, as well as on cyclohexyl N-phenyl carbamate 5 (5,6). Since the aromatic nucleus of the homologous amides are not hydroxylated using the same experimental conditions (7,8), it appears that the presence of an urethane moiety is necessary in order to allow this reaction to be performed. However, no further information is available concerning the influence of various other factors -i.e. the nature of the alkyl moiety - the localization of the urethane function on the carbon framework, - the influence of some slight structural modifications. Therefore, we carried out a more systematic study in order to clarify these points. The substrates we have studied throughout this work are urethanes bearing alkyl groups of various "size". These have been chosen because of their increasing lipophilic characters as well as increasing bulkiness (leading to higher steric interactions in the enzymatic active site). Some additional structural modifications such as N-methylation or "inversion" of the carbamate function, have also been explored in some cases. We describe and analyze here the results obtained exploring the bioconversion of these different substrates.

RESULTS

Methyl N-phenyl-carbamate

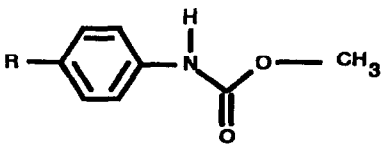
When submitted to a standard culture of the fungus *B. sulfurescens*, compound 1 (Scheme 1) is transformed into a single product whose structure has been determined as being 2*. Thus, regiospecific *para* hydroxylation occurred on the aromatic ring whereas no hydroxylation is observed at the methyl group.

Urethanes bearing an isopropyl group. The results obtained from 3 and 4 have been described previously (5). Two products 5 and 6 are obtained from 3 whereas 3, 5 and 6 are formed starting from the N-methylated urethane derivative 4. This strongly suggests that the first step in the bioconversion of the isopropyl N-methyl - N-phenyl carbamate 4 involves demethylation at the nitrogen atom, and that the increase of the lipophilic character of the substrate, due to N-methylation , allows easier bioconversion of this compound. Substitution of the aromatic *para* position of 3 with a methyl group (compound 7) is not sufficient to increase the conversion yield but leads to one single product 8. A deuterium retention as high as 72% has been observed starting from *para* deuteriated 3, showing an important NIH shift attributed to the transient formation of an arene oxide.

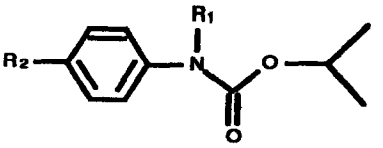
Urethanes bearing a cyclopentyl group. Cyclopentyl-N-phenyl carbamate 9 is an interesting compound since, being prochiral, a regio and stereospecific hydroxylation of one of the enantiotopic cyclopentane carbon atoms could lead to an optically pure alcohol. In spite of the higher lipophilic character of 9 as compared to that of isopropyl carbamates, no bioconversion occurs when 9 is submitted to a culture of *B. sulfurescens*. However its N-methyl derivative 10 is extensively transformed into two products 11 and 12. Interestingly both of these products result from hydroxylation of the cyclopentyl carbon atoms, whereas no hydroxylation has occurred on the aromatic ring. Unfortunately, it has not been possible to attribute unambiguously the relative stereochemistry of the cyclopentyl substituents by ^{1}H and ^{13}C NMR spectroscopy. This has been determined using X-Ray crystallography of the *para* bromobenzoate of 11, which indicates a trans configuration for the substituents (Scheme 2). Thus, the hydroxyl function has been introduced on the carbon framework via a highly stereoselective process. Interestingly also, both 11 and 12 are optically active, showing that the hydroxylation process has occurred preferentially on one of the two enantiotopic C(3) or C(4) carbon atoms. However, we have been unable to determine, using either classical analytical methods or X-Ray analysis, the enantiomeric excess of the product and/or the absolute configuration of the hydroxylated carbon atom.

(*) *The various yields and conversion ratios are indicated Table I*

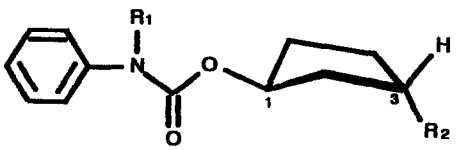
Scheme 1



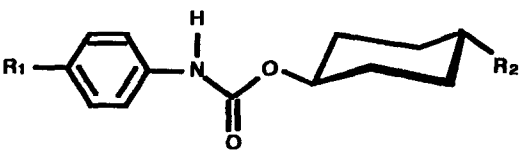
1 R = H
2 R = OH



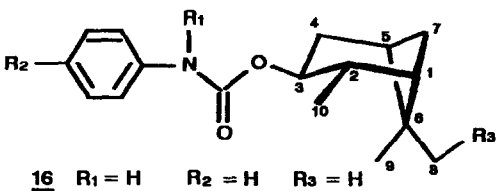
3 R_1 = H R_2 = H
4 R_1 = CH_3 R_2 = H
5 R_1 = H R_2 = OH
6 R_1 = H R_2 = O-Glu
7 R_1 = H R_2 = CH_3
8 R_1 = H R_2 = CH_2OH



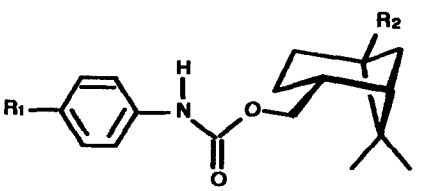
9 R_1 = H R_2 = H
10 R_1 = CH_3 R_2 = H
11 R_1 = H R_2 = OH
12 R_1 = CH_3 R_2 = OH



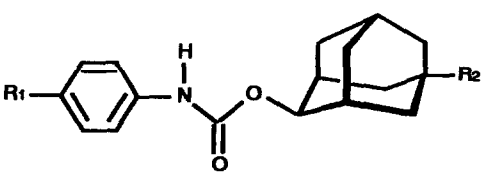
13 R_1 = H R_2 = H
14 R_1 = H R_2 = OH
15 R_1 = OH R_2 = OH



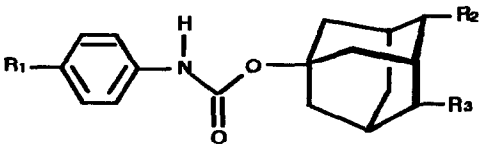
16 R_1 = H R_2 = H R_3 = H
17 R_1 = H R_2 = OH R_3 = OH
18 R_1 = CH_3 R_2 = H R_3 = H
19 R_1 = CH_3 R_2 = OH R_3 = OH



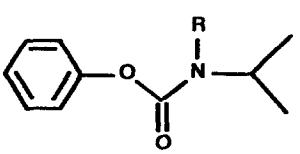
20 R_1 = H R_2 = H
21 R_1 = OH R_2 = OH



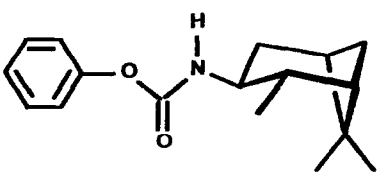
22 R_1 = H R_2 = H
23 R_1 = OH R_2 = OH



24 R_1 = H R_2 = H R_3 = H
25 R_1 = OH R_2 = OH R_3 = OH
26 R_1 = OH R_2 = OH R_3 = H
27 R_1 = O-Glu R_2 = OH R_3 = H

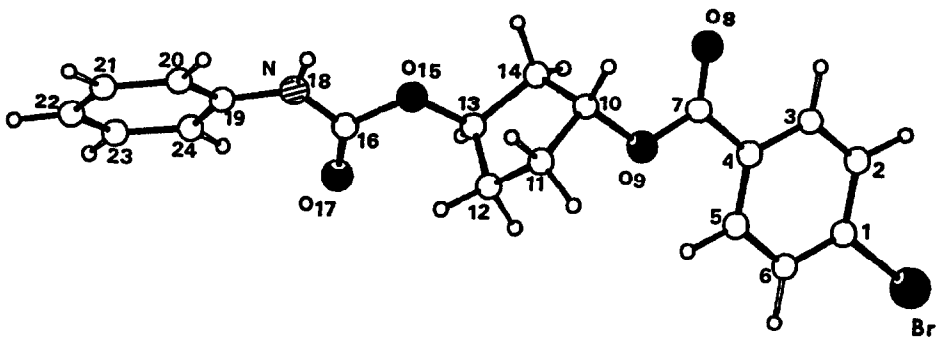


28 R = H
29 R = CH_3



30

Scheme 2. Perspective view of the *para*-bromobenzoate of 11, showing the atomic numerotation used in the crystallographic study.



Urethanes bearing a cyclohexyl ring. The results obtained from 13 have been described previously (6). Two main products 14 and 15 are obtained in this case and we have shown that alcohol 14 is very probably the precursor of 15, since it is itself converted into 15 by a culture of *B. sulfurescens*. It appears that, in this case, an important amount of substrate undergoes a double hydroxylation process, i.e. an alkyl hydroxylation and an aromatic ring hydroxylation. Both of these hydroxylation are highly regio and stereoselective processes.

Urethanes derived from pinanes. Similar to the results just described for the cyclohexyl derivative, the N-phenyl urethane 16 derived from racemic isopinocampheol undergoes a double hydroxylation process which leads to 17 as the sole product. In this case again, one hydroxylation occurs on the pinanyl moiety (interestingly on the chemically inaccessible C(8) carbon atom), the other one regiospecifically on the *para* position of the aromatic ring. This product is obtained in its racemic form, however, quite surprisingly, the racemic N-methylated derivative 18 leads to the optically active derivative 19 ($[\alpha]_D^{20} = -13°$) (c = 1.2, EtOH)) after 72 hr bioconversion. This indicates that a kinetic differentiation occurs between both enantiomers of the starting compound, similar to the results we have described previously on bi- or tricyclic amides (7,8). In fact, the same reaction conducted on the (1R) enantiomer of 18 leads to 19 showing an optical rotation of $[\alpha]_D^{20} = -17°$ (c = 1.1, EtOH). This indicate that the product obtained from racemic 18 is of (1R) absolute configuration and shows a 75% optical purity. Surprisingly enough, the C(3) endo epimer of this urethane, obtained from neoisopinocampheol, remains untransformed when placed in identical bioconversion conditions. However, its positional isomer 20, prepared from cis-myrtanol, is transformed into one single product showing that a double hydroxylation process has again occurred, leading to product 21 where the C(5) carbon atom of the pinanyl moiety has been hydroxylated instead of the primary C(8) methyl group, as observed in the case of 16 or 17.

Table I . Hydroxylation of aromatic carbamates mediated by the fungus *B. sulfurescens*

R	Substrate	Product	Yield %	Conversion ratio % #	Log P
Isopropyl (Ref. 4)	1	2	10	22	1.79
	3	5 6	27 22	48	2.73
	4	3 5 6	50 11 39	100	2.97
	7	8	12	30	3.23
Cyclopentyl	9	no product		58	3.44
	10	11 12	53 18	100	3.68
Cyclohexyl (Ref 5)	13	14 15	40 34	88	3.97
Pinanyl	16	17	43	--	5.35
	18	19	40	--	5.58
	20	21	23	--	4.94
Adamantyl	22	23	41	50	4.91
	24	25 26 27	40 26 18	86	5.12

Conversion ratio . % of non–recovered substrate

Urethanes bearing an adamantyl group. When submitted to a standard culture of *B. sulfurescens* the N-phenyl urethane 22 obtained from 2-adamantanol affords one single product 23. On the other hand substrate 24, prepared from 1-adamantanol, leads to a mixture of three products : 25 (a compound formed via a triple hydroxylation process); 26 and 27, these both products being formed via a double hydroxylation process.

"Inverse" isopropyl and pinanyl urethanes derivatives. We have observed previously that hydroxylations of non activated carbon atoms similar to the ones here described occur when the "activating" function is an amide moiety instead of an urethane function (7,8). In order to further explore the scope and limitations of these biohydroxylation processes, we checked the feasibility of these reactions starting from urethanes where the carbamate function has been "inverted", i.e. using substrates of structure 28 or 30. However, these substrates fail to be transformed by a culture of *B. sulfurescens*, a fact which remains unchanged for the N-methyl derivative 29. This is quite surprising in view of the good yields obtained from their homologous urethanes 3 and 16.

The structure and stereochemistry of the various products have been essentially attributed on the basis of their ^{1}H and ^{13}C NMR spectra analysis. This can be exemplified as follows in the case of the adamantyl derivatives (Table II). The various ^{13}C NMR signals can be attributed to the different carbon atoms of urethane 24 on the base of the previously described studies of 1-adamantanol (9). Using the increments introduced by an hydroxyl group located on an adamantyl structure (10), the theoretical values of the chemical shifts can be calculated for both syn or anti stereoisomers of 26. It appears that the observed values do almost perfectly fit with the ones calculated for the anti isomer 26, whereas they do not for the syn isomer. A similar approach allowed us to assign structure 25 to the trihydroxylated product, as well as structure 23 to the hydroxylation product of 22.

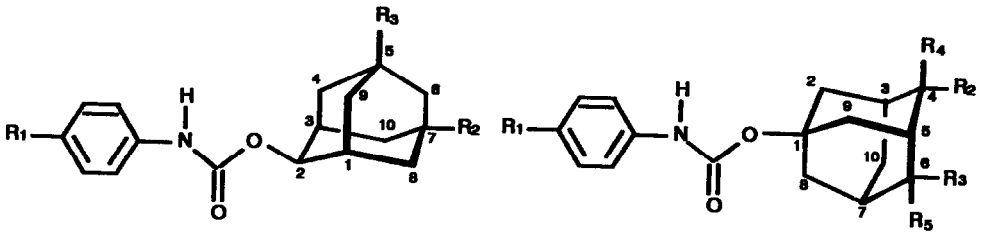
DISCUSSION

Two main conclusions and some interesting remarks can be drawn out of these results.

First, all substrates possessing an alkyl N-phenyl carbamate structure are metabolized by the fungus B. sulfurescens. On the other hand, we have observed that alkyl O-phenyl carbamates are not transformed using identical culture conditions. This is quite a puzzling and unexplained result. Apparently, an important conjugation must exist between the aromatic ring and the carbamate (or amide) function in order to allow these biotransformations to occur. This may partly be due to some stereoelectronic factors implied in the ES complex formation step, but also, eventually, to some membrane transport processes involved to get the substrate into the endoplasmic reticulum, where the majority of the cytochrome P-450 hydroxylating enzymes are known to be located.

Second, when the alkyl substituent is relatively "small" (R = methyl or isopropyl) the only observed hydroxylation occurs at the aromatic ring and in a highly regiospecific manner. This can be due to the relatively low lipophilic character (log P < 3.13)[11] (Table I) of these substrates but also to geometric factors, since it has been shown previously that the distance between the oxygen and/or the nitrogen atom and the hydroxylated carbon atom plays an important role for these biotransformations (12). This may explain that, going from the isopropyl to the cyclopentyl N-methyl carbamate 10, the hydroxylation locus switches from the aromatic ring to the alkyl substituent, no aromatic hydroxylation being any more observed. Finally, for substrates bearing highly lipophilic alkyl groups (log P > 3.9) hydroxylation occurs on both the alkyl part and the aromatic ring. Interestingly, we have shown previously in the case of the cyclohexyl derivative 13 (6) that hydroxylation first occurs on the alkyl group and that the alcohol 14 thus formed serves as a substrate

Table II . Attribution of the various ^{13}C-NMR signals for adamantyl structures



Product	C_1	C_2	C_3	C_4	C_5	C_6	C_7	C_8	C_9	C_{10}
22	32.1	78.0	32.1	31.9	27.3	37.4	27.0	36.4	31.9	36.4
$\Delta(C_5)$	+2.4	-1.4	+2.4	+7.8	+39.1	+7.8	+2.4	-1.4	+7.8	-1.4
R_1=R_3=OH R_2=H	34.5	76.6	34.5	39.7	66.4	45.2	29.4	35.0	39.7	35.0
$\Delta(C_7)$	+2.4	-1.4	+2.4	-1.4	+2.4	+7.8	+39.1	+7.8	-1.4	+7.8
R_1=R_2=OH R_3=H	34.5	76.6	34.5	30.5	29.7	45.2	66.1	44.2	30.5	44.2
23	34.7	76.5	34.7	31.2	30.5	46.0	66.9	44.2	31.2	44.2
24	80.4	41.7	30.9	36.2	30.9	36.2	30.9	41.7	41.7	36.2
Δ(syn-syn)	-1.4	-6.6	+5.0	+36.9	+12.4	+36.9	+5.0	-6.6	-13.2	-2.4
R_1=R_4=R_5=OH R_2=R_3=H	79.0	35.1	35.9	73.1	43.3	73.1	35.9	35.1	28.5	33.8
Δ(anti-anti)	-2.4	-1.1	+5.5	+36.9	+12.4	+36.9	+5.5	-1.1	-2.2	-13.2
R_1=R_2=R_3=OH R_4=R_5=H	78.0	40.6	36.4	73.1	43.3	73.1	36.4	40.6	39.5	23.0
25	77.7	40.6	37.5	75.4	40.5	75.4	37.5	40.6	39.2	24.8
24	80.4	41.7	30.9	36.2	30.9	36.2	30.9	41.7	41.7	36.2
Δ(syn)	-0.7	-6.6	+6.2	+36.9	+6.2	-1.1	-1.2	0.0	-6.6	-1.1
R_1=R_4=OH R_2=R_3=R_5=H	79.7	35.5	37.1	73.1	37.1	35.1	29.7	41.7	35.1	35.1
Δ(anti)	-1.2	-1.1	+6.2	+36.9	+6.2	-6.6	-0.7	0.0	-1.1	-6.6
R_1=R_2=OH R_3=R_4=R_5=H	79.2	40.6	37.1	73.1	37.1	29.6	30.2	41.7	40.6	29.6
26	78.7	40.9	37.5	73.1	37.5	30.9	31.2	42.7	40.9	30.9

Δ : Increment introduced by one (or two) hydroxyl group .
Attribution of the aromatic carbon atom signals are reported in the experimental part .

leading to the dihydroxylated product 15. The presence of a more lipophilic alkyl groups greatly enhances the rates of these reactions, which makes it impossible to isolate the monohydroxylated product in the cases of adamantyl derivatives. In addition, in the case of 24, a third hydroxylation takes place, leading to the trihydroxylated compound 25.

Examination of the results obtained in the case of pinanyl derivatives show that slight structural modifications may alter considerably the outcome of the reaction. Thus bioconversion of compound 16 and 20 (position isomers) show that a different localization of the urethane function on the carbon skeleton modifies the regioselectivity of the hydroxylation. Also, N-methylation of 16 leads to optically active products, whereas this is not the case for 16 itself.

Finally, it is interesting to emphasize the fact that some of the mono- or dihydroxylated products formed in the course of these bioconversions have been isolated in the form of their 4-O-methyl glucosides, a transformation which allows these products to become highly water soluble. Presumably, these bioconversions, which are quite common in plants, but are also sometimes observed with microbial cells (12-14), are part of the detoxication process of the fungus, similar to what is observed in mammalian liver.

CONCLUSION

In the course of this work, we have shown that highly regiospecific *para*-hydroxylation of various aromatic urethane derivatives can be achieved using the fungus *Beauveria sulfurescens*. Thus, several *para* substituted aromatic derivatives can be prepared in fair to good yields using this one step procedure.This appears to be an interesting synthetic process since such reactions are very difficult to perform using chemical transformations. We have observed that the higher the lipophilic character of the substrate, the easier the hydroxylation is achieved. For low lipophilic compounds, monohydroxylation occurs exclusively on the aromatic ring, whereas alkyl hydroxylation is the only observed transformation on cyclopentyl-N-methyl-N-phenyl carbamate, a substrate of medium lipophilic character. Higher alkyl groups lead to a double hydroxylation, where the aromatic ring as well as the alkyl moiety are hydroxylated.

EXPERIMENTAL PART

General : The ^{1}H and ^{13}C NMR spectra were realized on a Bruker AM 200 apparatus (Department of Pharmacology, Aix Marseille II University, Marseille, France). Chemical shifts (δ) are given in ppm relative to TMS as internal standard : Mass spectra were obtained on a Varian MAT 311. The optical rotation of the products was determined with a Perkin-Elmer-241C. IR spectra were recorded using a Beckman Acculab 4 spectrometer. Elemental analyses of C, H, N were performed by the Service Central d'Analyse du CNRS (Vernaison, France). Melting points were measured using a Büchi 510 apparatus and are not corrected. Gas chromatographic analyses were performed using a 25 m capillary column coated with OV17. High Performance Liquid Chromatography analyses were achieved using a 5 µm silicagel column (10x0.4 cm) (Merck), the preparative operations have been conducted with a column (25x0.9 cm) filed with 7 µm Merck silicagel.

Microorganism : the strain in the present work is *Beauveria sulfurescens* ATCC 7159 (originally purchased as *Sporotrichum sulfurescens*).

Medium composition : The medium used is constituted by 20 g of corn steep Oliquor (Roquette, France) and 10 g of glucose per liter (tap water, adjusted to pH 4.85 with sodium hydroxide).

General procedure : The sterilized medium is inoculated by a 48 h old vegetative culture and incubated with reciprocal shaking (80 tpm) at 28°C in 2 liter Erlenmeyer flasks filled with 0.5 L of medium. After 48 h growth, an ethanolic solution of substrate (10% w/v), is added to the culture (400 mg/L). After an additional 72 h period of incubation, the mycelium is separated by filtration, and washed with water. The filtrate is continuously extracted (24 h) with methylene chloride. This organic phase is dried ($MgSO_4$), and the solvent distilled off under vacuum. The crude residue is analyzed by TLC (Merck 60 F^{254}, 0.2 mm) using ether or ether-methanol (95-5) as eluants. The products are isolated by silica gel chromatography and recrystallized. For more difficult separations, preparative HPLC has been used.

Substrates synthesis : The substrates were obtained from phenyl isocyanate and the corresponding alcohols described in (5).

Biohydroxylation of cyclopentyl-N-methyl-N-phenyl carbamate 10.
Starting from 720 mg of 10 (200 mg/L of culture), 385 mg of 11 and 140 mg of 12 are isolated.

3-Hydroxycyclopentyl-N-phenyl carbamate 11.
m.p. 89°C $[\alpha]^{20}_{D}$ = +8.6° (c = 2.8, $CHCl_3$) . IR ($CHCl_3$)ν = 3400 (O-H) ; 1720 (C=O)cm^{-1}. ^{1}H-NMR ($CDCl_3$) : δ = 1.58 to 2.25 (m, 6H) ; 4.45 (m, 1H, H-C(3)) 5.27 (m, 1H, H-C(1)) 6.55 (s, 1H, N-H) ; 7.05 (t, 1H, ar) ; 7.20 to 7.40 (m, 4H, ar). ^{13}C-NMR ($CDCl_3$)δ = 31.0 (C5) ; 34.0 (C4) ; 42.5 (C2) ; 72.0 (C3) ; 77.0 (C1) ; 119.8, 123.8, 129.6, 140.5 (ar) ; 155.9 (C = O). Analysis for $C_{12}H_{15}NO_3$: found (calcd) : C, 65.00 (65.13) ; H, 6.71 (6.83) ; N, 6.21 (6.33). MS (70 ev) : 221 $M^{+\cdot}$, (40) ; 137 (92) ; 93 (100) ; 67 (57). Exact mass calcd for $C_{12}H_{15}NO_3$ 221.1051 ; found 221.1065

X-ray Analysis of 3-(4-bromobenzoate)-cyclopentyl-N-phenyl carbamate
Compound 11 (30 mg, 0.134 mmole) and 4-bromobenzoyl chloride (33 mg, 0.15 mmole) are mixed with dry CH_2Cl_2 and dry pyridine (6 drops) and stirred under argon for 24 hr. The reaction mixture is transferred to a separatory funnel with CH_2Cl_2 (25 mL). The organic layer, after washing successively with dilute hydrochloric acid (2%), water, saturated sodium

bicarbonate solution and water, is dried ($MgSO_4$). Silica-gel chromatography (hexane-ether) leads to 51 mg (92% yield) of product.
Crystal data : $C_{18}H_{18}NO_4Br$, Mr 404 ; monoclinic ; a = 10.538(2) Å, b = 17.597(3) Å, and c = 10.002(2) Å ; β = 99.37° ; space group P_{21}/n ; V = 1830 Å^3, Z = 4, dc = 1.467, λ = 1.5418 Å (CuKα). From the 3322 independent reflections, collected by the θ-2θ scan technique up to θ = 60°, 2360 were significant [I > 3σ(I)]. The reflections were corrected for Lorentz, polarization and absorption effects (16). The structure was solved by Patterson function (17). The atomic coordinates and anisotropic thermal parameters were refined by large blocks to a discrepancy factor of 9.7% for 2360 reflections. The hydrogen atoms were introduced in their theoretical position (C-H) = 1.08 Å) and assigned the equivalent isotropic thermal factor of the bonded atom. The highest residue on the final electronic density map was 0.55 e/Å^3. Atomic coordinates, anisotropic thermal parameters, bond lenghts, and angles with their estimated standard deviations are available from the Director of Cambridge Crystallographic Data Center, University Chemical Laboratory, Lensfield Road, Cambridge CB2 1EW. Any request should be accompanied by the full literature citation.

3-Hydroxycyclopentyl-N-methyl-N-phenyl carbamate 12.
$[\alpha]^{20}_D$ = +1.7° (c = 0.86, $CHCl_3$). IR ($CDCl_3$)ν = 3400 (O-H) ; 1680 (C=O)cm^{-1}. ^{1}H-NMR (d6-aceton) : δ = 1.5 to 2.3 (m, 6H) ; 3.25 (s, 3H, N-CH_3) ; 3.68 (s, 1H, OH) ; 4.29 (s, 1H, H-C(3)) ; 5.21 (m, 1H, H-C(1)) ; 7.2 to 7.4 (5H, ar). ^{13}C-NMR ($CDCl_3$) δ = 30.6 (C5) ; 33.6 (C4) ; 37.5 (N-CH_3) ; 42.7 (C2) ; 72.4 (C3) ; 77.2 (C1) ; 125.7, 125.9, 128.8, 143.5 (ar) ; 155.4 (C=O). Analysis for $C_{13}H_{17}NO_3$: found (calcd) : C, 65.67 (66.35) ; H, 7.33 (7.28) ; N, 5.76 (5.95). MS (70ev) : 235 M$^{+\cdot}$ (16) ; 151 (63) ; 107 (52) ; 67 (40) ; 28 (100). Exact mass calcd for $C_{13}H_{17}NO_3$ 235.1208 ; found 235.1208.

Biohydroxylation of exo-3-pinanyl-N-phenyl carbamate 16.
The biotransformation of 16 (840 mg) leads to one single metabolite 17 (405 mg) (viscous oil). IR (KBr)ν = 3420 (O-H) ; 1700 (C=O)cm^{-1}. ^{1}H-NMR (d6-DMSO) : δ = 1.05 (s, 3H, H-C(9)) ; 1.1 (d, 3H, H-C(10)) ; 1.70 to 2.6 (m, 7H) ; 3.55 (d, 2H, H-C(8)) ; 4.66 (dd, 1H, OH) ; 5.05 (m, 1H, H-(C3)) ; 6.75 (d, 2H, ar) ; 7.30 (d, 2H, ar) ; 9.17 (s, 1H, NH or OH) ; 9.35 (s, 1H, NH or OH). ^{13}C-NMR (d6-aceton) : 19.3 (C9 or C10) ; 20.89 (C9 or C10) ; 30.71 (C6)) ; 34.42 (C7) ; 36.94 (C4) ; 38.37 (C5) ; 44.54 (C1 or C2) ; 45.01 (C1 or C2) ; 69.78 (C8) ; 75.62 (C3) ; 121.98 ; 122.06, 133.0, 156.60 (ar) ; 154.46 (C=O). MS (70ev) : 305 M$^{+\cdot}$; 153(100) ; 109 (75) ; 95 (41) ; 58 (65). Exact mass calcd for $C_{17}H_{23}NO_4$ 305.162 ; found 305.1623.

Biohydroxylation of exo-3-pinanyl-N,N-methylphenyl carbamate 18
420 mg of 18 lead after 72 H of incubation to 210 mg of 19 (viscous oil). IR (KBr)ν = 3480 (O-H) ; 1680 (C=O)cm^{-1}. ^{1}H-NMR (d6-DMSO) : δ = 1.02 (s, 3H, H-C(9)) ; 1.50 (d, 3H, H-C(10)) ; 1.2 to 2.8 (m, 8H) ; 3.33 (s, 3H, N-CH_3) ; 3.64 (s, 2H, H-C(8)) ; 4.98 (m, 1H, H-C(3)) ; 6.85 (d, 2H, ar) ; 7.15 (d, 2H, ar) ; 8.5 (s, 1H, OH ar). Analysis for $C_{18}H_{25}NO_4$: found (calcd) : C, 67.20 (67.69) ; H 7.65 (7.89) ; 4.55 (4.38).

Biohydroxylation of 10-pinanyl-N-phenyl carbamate 20
The transformation of 20 (320 mg) leads, after usual work up to 82 mg of 21. m.p. 78-80°C. $[\alpha]^{20}_D$ = -8° (c = 0.93, EtOH). IR (KBr) : ν = 3250 (O-H) ; 1670 (C=O)cm^{-1}. ^{1}H-NMR (d6-aceton) : δ = 1.05 (s, 3H, CH_3) ; 1.12 (s, 3H, CH_3) ; 1.33 (d, 1H) ; 1.5 to 2.4 (m, 7H) ; 3.81 (s, 1H, OH) ; 4.02 (d, 2H, H-C(10)) ; 6.77 (d, 2H, ar) ; 7.36 (d, 2H, ar) ; 8.11 (s, 1H, OH or NH) ; 8.35 (s, 1H, OH or NH). ^{13}C-NMR (CD_3OD) : δ = 18.5 (C3) ; 23.16 (C9) ; 25.8 (C4) ; 27.82 (C8) ; 32.91 (C7) ; 38.48 (C6) ; 40.47 (C2) ; 41.24 (C5) ; 42.92 (C1) ; 69.53 (C10) ; 118.67, 123.16, 128.98, 138.08 (ar) ; 153.94 (C=O). MS (70ev) : 305 M$^{+\cdot}$; 153(94) ; 152(60) ; 137 (67) ; 109 (87) ; 95 (100) ; 83 (95) ; 62 (60) ; 53 (67). Analysis for $C_{17}H_{23}NO_4$: found (calcd) : C, 66.01 (66.86) ; H, 7.86 (7.59) ; N, 4.22 (4.59). Exact mass calcd for $C_{17}H_{23}NO_4$ 305.1626 ; found 305.1630.

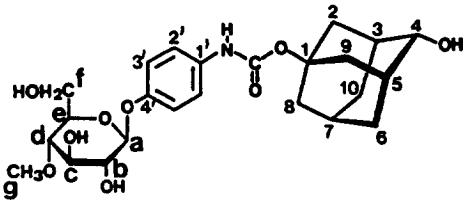
Biohydroxylation of 2-adamantyl-N-phenyl carbamate 22
The incubation experiment performed starting from 840 mg of 22 leads, after usual work up, to 362 mg of 23. M.p. 199°C (dec.). IR (KBr) : ν = 3400 (O-H) ; 1680 (C=O)cm^{-1}. ^{1}H-NMR (d6-aceton) : δ = 1.36 to 2.20 (m, 9H) ; 3.20 (m, 4H) ; 4.82 (m, 2H, OH, H-(C2)) ; 6.80 (d, 2H, ar) ; 7.38 (d, 2H, ar) ; 8.20 (s, 1H, NH or OH ar) ; 8.45 (s, 1H, NH or OH ar). ^{13}C-NMR (d6-aceton) : δ = 116.0 (C3', C5') ; 120.9 (C2', C6') ; 132.2 (C1') ; 153.8 (C4') ; 154.2 (C=O) (aliphatic part reported in table II). Analysis for $C_{17}H_{21}NO_4$: found (calcd) : C, 66.54 (67.30) ; H, 7.24 (6.99) ; N 4.38 (4.61). MS (70 ev) : 303 ($M^{+\cdot}$, 41) ; 259 (41) ; 151 (53) ; 109 (100) ; 93 (39) ; 91 (36). Exact mass calcd for $C_{17}H_{21}NO_4$ 303.1470 ; found 303.1492.

Biohydroxylation of adamantyl-N-phenyl carbamate 24
The transformation of 800 mg of 24 leads after silicagel chromatography to a mixture of three products 25 (380 mg) 26 (232 mg) and 27 (280 mg).

4,6-dihydroxyadamantanyl-N-para-hydroxyphenyl carbamate 25
m.p. 201°C. IR (KBr) : ν = 3400 (O-H) ; 1670 (C=O)cm^{-1}. ^{1}H-NMR (d6-aceton) : δ = 1.0 to 1.4 (m, 4H) ; 1.95 to 2.5 (m, 7H) ; 3.95 (s, 2H, H-C(4), H-C(6)) ; 4.93 (s broad, 2H, OH) ; 6.75 (d, 2H, ar) ; 7.34 (d, 2H, ar), 8.14 (s, 1H, NH or OH ar) ; 8.23 (s, 1H, NH or OH ar). ^{13}C-NMR (d6-aceton) : δ : 115.9 (C3', C5') ; 120.9 (C2', C6') ; 132.4 (C1') ; 153.7 (C4', C=O) (aliphatic part reported in table II). Analysis for $C_{17}H_{21}NO_5$: found (calcd) : C, 63.00 (63,93) ; H, 6.84 (6.63) ; N, 4.23 (4.38). MS (70ev) : 319 ($M^{+\cdot}$, 15) ; 149 (37) ; 148 (73) ; 135 (100) ; 95 (60). Exact mass calcd for $C_{17}H_{21}NO_5$ 319.1419 ; found 319.1419.

4-hydroxyadamantanyl-N-parahydroxyphenyl carbamate 26
m.p. 191°C. IR (KBr) : ν = 3380 (O-H) ; 1680 (C=O)cm^{-1}. ^{1}H-NMR (d6-aceton) : δ = 1.0 to 1.35 (m, 4H) ; 1.9 to 2.2 (m, 9H) ; 3.87 (m, 2H, OH, H-C(4)) ; 6.75 (d, 2H, ar) ; 7.35 (d, 2H, ar) ; 8.05 (s, 1H, NH or OH) ; 8.14 (s, 1H, NH or OH). ^{13}C-NMR (d6-aceton) : δ = 116.0 (C3', C5') ; 121.0 (C2', C6') ; 132.7 (C1') ; 153.7 (C4') (aliphatic part reported in table II). Analysis for $C_{17}H_{21}NO_4$: found (calcd) : C, 66.26 (67.30) ; H, 7.04 (6.99) ; 4.44 (4.61). MS (70ev) : 303 ($M^{+\cdot}$, 18) ; 259 (47) ; 151 (64) ; 133 (100) ; 91 (55). Exact mass calcd for $C_{17}H_{21}NO_4$ 303.1470 ; found 303.1469.

4-hydroxyadamantanyl N-para-4-O-methylglucoside-phenyl carbamate 27m.p. 189°C. IR (KBr) : ν = 3400 (O-H) ; 1690 (C=O)cm^{-1}. ^{1}H-NMR (d6-aceton) : δ = 1.2 to 1.6 (m, 4H) ; 2.0 to 2.2 (m, 9H) ; 2.8 to 4.0 (m, 12H) ; 4.42 (s, 1H) ; 4.7 (s, 1H) ; 4.84 (d, 1H) ; 6.98 (d, 2H, ar) ; 7.45 (d, 2H, ar) ; 8.32 (1H, s, NH). ^{13}C-NMR (d6-aceton) : δ = 30.9 (C6, C10) ; 31.3 (C7) ; 37.5 (C3, C5) ; 40.9 (C2, C9) ; 42.7 (C8) ; 60.4 (Cg) ; 62.2 (Cf) ; 73.0 (C4) ; 75.0, 77.1, 78.0 (Cb, Cd, Ce) ; 79.0 (C1) ; 80.3 (Cc) ; 102.5 (Ca) ; 118.0 (C3', C5') ; 120.4 (C2', C6') ; 135.1 (C1') ; 153.5 (C4') ; 154.3 (C=O). Analysis for $C_{24}H_{33}NO_9$: found (calcd) : C, 58.61 (60.11) ; H, 7.33 (6.94)) ; N, 2.83 (2.92).



BIBLIOGRAPHY

(1) March, *J. Advanced Organic Chemistry*; John Wiley; New York 1985, 498.
(2) Olah, G.A.; Ernst, T.D. *J. Org. Chem.* **1989**, *54*, 1204-1206.
(3) Komiyam, A. M. *J. Chem. Soc. Perkin Trans. I* **1989**, 2031-2034.
(4) Jacquesy, J.C.; Jouannetaud, M.P.; Morellet, G.; Vidal, Y. *Bull. Soc. Chim. Fr.* **1986**, *25*, 625.
(5) Vigne, B.; Archelas, A.; Fourneron, J.D.; Furstoss, R. *Tetrahedron* **1986**, *42*, 2451-2456.
(6) Vigne, B.; Archelas, A.; Fourneron, J.D.; Furstoss, R. *New J. of Chem.* **1987**, *11*, 297-298.
(7) Fourneron, J.D.; Archelas, A.; Vigne, B.; Furstoss, R. *Tetrahedron* **1987**, *43*, 2273-2284.
(8) Archelas, A.; Fourneron, J.D.; Furstoss, R.; Cesario, M.; Pascard, C. *J. Org. Chem.* **1988**, *53*, 1797-1799.
(9) Pehk, T.; Lippmaa, E. *Org. Mag. Res.* **1971**, *3*, 783-790.
(10) Duddek, H. *Org. Mag. Res.* **1975**, *7*, 151-153.
(11) Rekker, R. "The Hydrophobic Fragmental Constant" Pharmacochemistry Library, Ed. Nauta, W.T. and Rekker, R. Vol 1, Elsevier, Amsterdam, 1977.
(12) Furstoss, R.; Archelas, A.; Fourneron, J.D.; Vigne, B. "Enzymes as catalysts in Organic Chemistry" Nato Asi Series, **1986**, *178*, 361-370.
(13) Colas, C.; Quiclet-Sire, B.; Chleophax, J.; Delaumeny, J.M..; Sepulchre, A.M.; Gero, S.D. *J. Am. Chem. Soc.* **1980**, *102*, 857.
(14) El-Sharkawy, S.; Abul-Hajj, Y. *J. Nat. Prod.* **1986**, *50*, 520.
(15) Rosazza, J.P.N.; Sanchez, C.M.; Williamson, J., Van Orden, D.E.; Markham, A. *Dev. Ind. Microbiol.* **1989**, *30*, 173.
(16) Walker, N.; Stuart, D. *Acta Cryst.* **1983**, *A39*, 158-166.
(17) Sheldrick, G. **1976** SHELX76. A program for crystal structure determination, University of Cambridge, England.

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