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MICROBIAL TRANSFORMATIONS . PART 7 (1)
BIOHYDROXYLATION OF BORNYLAMIDE DERIVATIVES BY THE FUNGUS BEAUVERIA SULFURESCENS

by Jean-Dominique Fourneron, Alain Archelas, Bernard Vigne and Roland Furstoss *
Laboratoire de Chimie Organique et Bioorganique, Faculté des Sciences , Case 901
70 Route Léon Lachamp - 13288 MARSEILLE CEDEX 9 (FRANCE)

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SUMMARY : *The biohydroxylation of various amide derivatives of norbornane and of camphane have been studied, in order to explore the possible influence of the amide group configuration and substitution upon the regio, the stereo and the enantioselectivity of the process. The results obtained indicate that, for two cases out of the three, the variation of configuration of the amide group has no influence on the hydroxylation selectivity. However, it appears that, in the case of the endo N-methylated amide derived from camphane, the results are completely different. In particular, we observe partial resolution of the starting racemic compound, due to different regioselectivities occuring in the hydroxylation of each of the two enantiomers.*

INTRODUCTION : Because of the ease and the high selectivities shown by microbial enzymes which are able to perform hydroxylation of non activated carbon atoms, biohydroxylation constitues a highly powerfull tool in organic chemistry. However, the rules of the game are not yet clear, and some fundamental studies are still needed in order to allow a wider use of this technique in organic chemistry. Previous results have shown that the fungus Beauveria sulfurescens (ATCC 7159) is able to achieve such transformations on amide-type substrates, and it has been suggested that this function plays an anchoring role in the enzyme-substrate complex formation step (2).

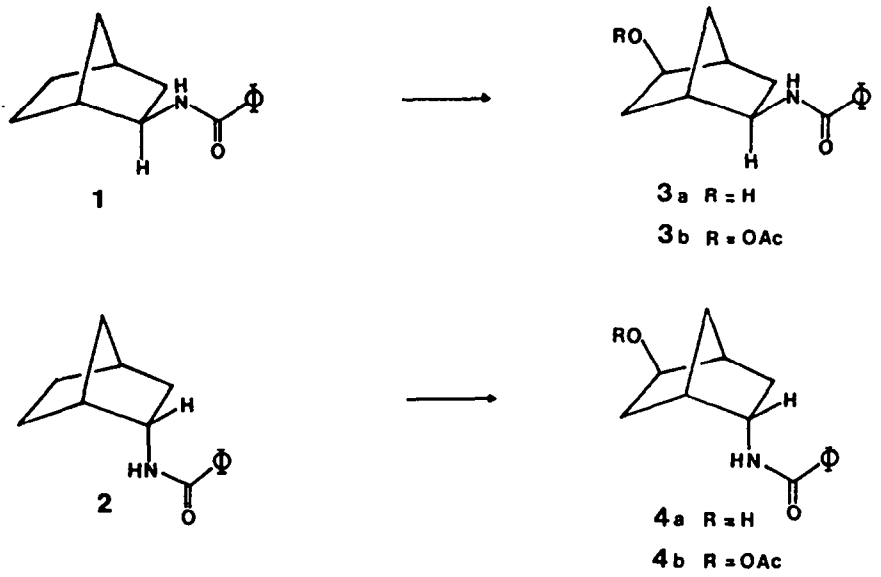
Therefore, it was of interest to us to achieve comparative studies on various compounds where the amide moiety would be differently located. Thus, in a previous work we have studied, using various amide and lactam models, the influence of the carbonyl oxygen atom localization (3) on the regioselectivity of the process.

We also wanted to check whether the amide group configuration could influence the hydroxylation selectivity, and did therefore perform detailled studies on several pinane derivatives (1). Surprisingly enough, these results did indicate that variation of the amide group configuration does not alter the localization of the hydroxylation process. However, as this result could have been due to the remaining flexibility of the model used, it was important to us to further study compounds of completely rigid structure. We do describe in this work the hydroxylation of substrates derived from norbornane and camphane, where this requirement is fullfilled.

RESULTS : The epimeric amides 1 and 2 have been prepared starting from the commercially available racemic amines and have been submitted to a two days old culture of the fungus Beauveria sulfurescens. After 72 hours culture, amide 1 has been transformed into one single compound 3a with a 35% yield. Acetylation (Ac_2O, pyr.) of the crude product affords the corresponding acetate whose structure has been determinated as being 3b (See below) . Surprisingly enough, using the same culture conditions, amide 2 fails to be hydroxylated. However, adjunction of 2‰ dodecylbenzene sulfonate, as previously described by Fonken and coworkers (2), allows hydroxylation to occur. This again leads to one single hydroxylated compound (30% yield). Acetylation of this product affords the corresponding acetate, identified as being amidoester (4b). The structures of these

products (see Figure 1) , as well as the stereochemistry of the acetyl groups, have been determined on the basis of their ^{1}H and ^{13}C-NMR spectra, using previously described results on similar compounds (4,5) (see below). At this point, one has to emphasize the fact that both 3 and 4, formed from racemic amides 1 and 2, prove to be optically inactive, which indicates that hydroxylation is not enantioselective . As will be seen, this is, however, no longer true for amides 5 and 6 having the camphane skeleton.

Figure 1 : Hydroxylation of norbornane derivatives.

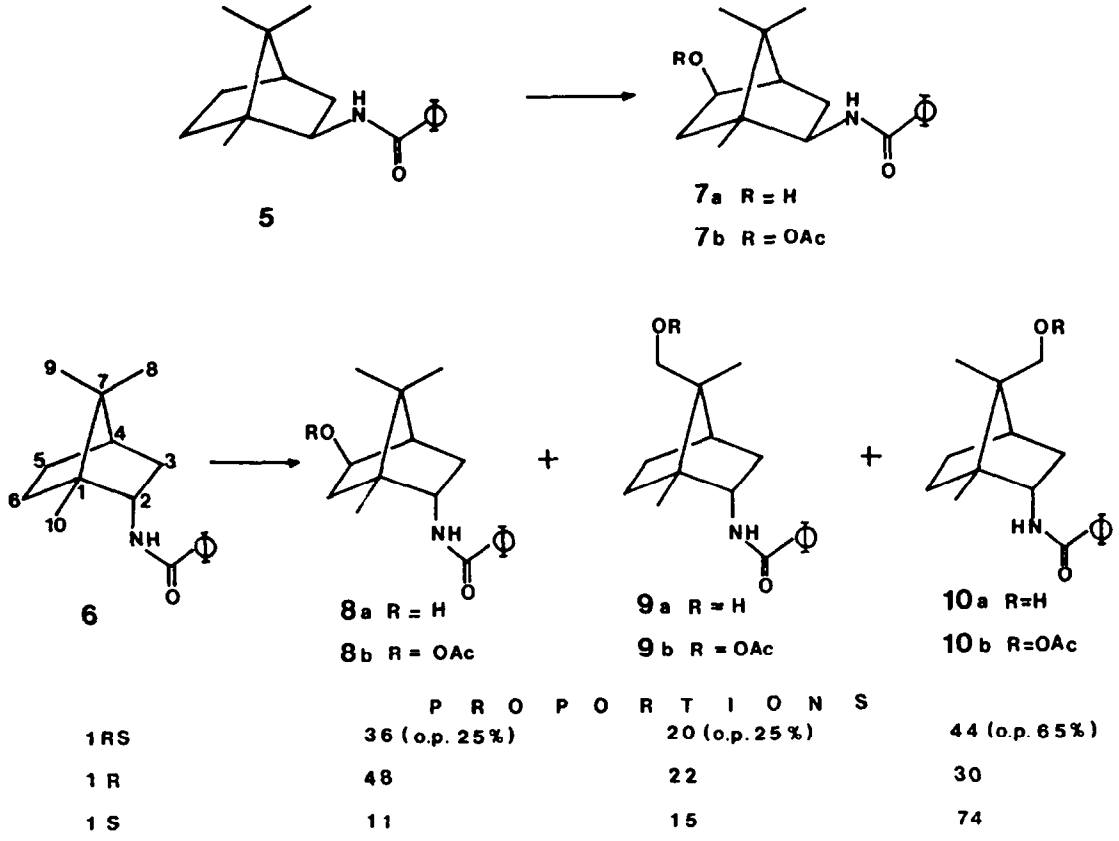


Racemic isobornylamide 5 has been prepared starting from racemic commercial isoborneol, using the Ritter reaction with benzonitrile (6). The enantiomer 5 (1R) has been prepared from the commercial (1R)-isobornylamine hydrochloride. The bornylamide derivatives 6, racemic or with (1R) or (1S) absolute configuration, have been obtained from, respectively , racemic , (1R) or (1S) camphor using a previously described procedure (7).

For these two compounds, we observe a largely different behaviour between the two epimeric amides upon microbial hydroxylation . Indeed, as in the case of 1 and 2, the racemic exo isomer 5 leads to one single racemic alcohol 7a. Of course, the 5 (1R) enantiomer affords the 7a (1R) optically active alcohol (with a 50%-60% yield) , which can be directly transformed into its acetate 7b. Surprisingly enough, however, the racemic bornylamide derivative 6, submitted to a culture of Beauveria sulfurescens, does afford a mixture of three compounds, which all prove to be optically active. The relative proportions of these compounds are indicated on Figure 2, and their yields, based on starting amide, are respectively of 18, 10 and 22%. These products, which are difficult to separate even using preparative HPLC, have been transformed by acetylation of the crude mixture to the corresponding acetates. Their structures have been established as being 8b, 9b and 10b on the basis of their ^{1}H and ^{13}C-NMR spectra, and confirmed using chemical correlations with compounds of known structure (see below).

In order to check whether these optically active compounds have been formed because of a difference in the hydroxylation rates of each of the two antipodes of the starting material, the remaining starting amide has been isolated and checked for optical activity. It proves to be optically inactive. This result strongly suggests that a) the two enantiomers are hydroxylated at the same apparent rate and b) that each of the enantiomers are being hydroxylated preferentially, although not exclusively, at different positions, each of these processes showing different rates.

Figure 2 : Hydroxylation of secondary amides derived from camphor.



	P R O P O R T I O N S		
1RS	36 (o.p. 25%)	20 (o.p. 25%)	44 (o.p. 65%)
1 R	48	22	30
1 S	11	15	74

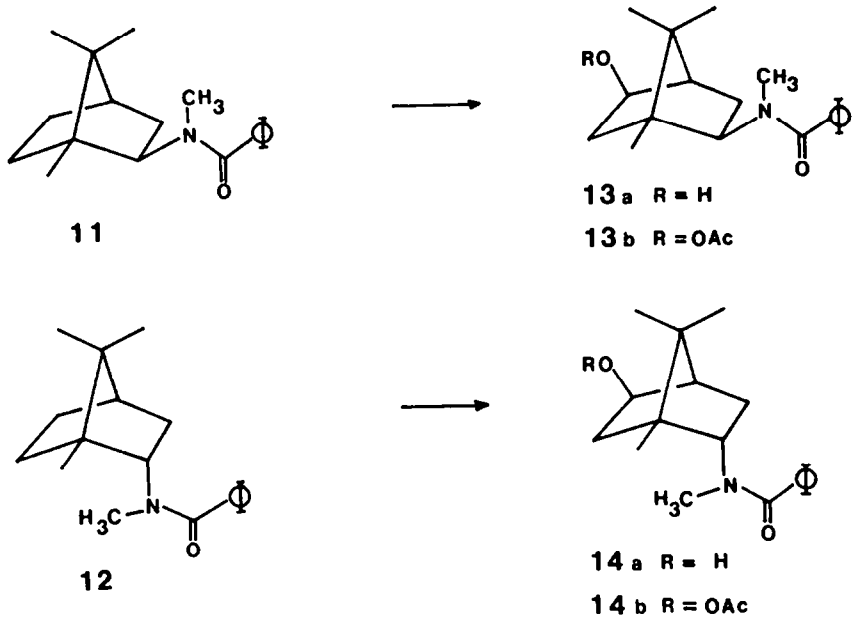
In order to check this hypothesis, we decided to repeat this biohydroxylation studies starting from either the (1R) or the (1S) optically pure amide 6. Thus, the aim of these experiments was a) to elucidate the behaviour of each of the enantiomers of 6, b) to determine the absolute configuration of each of the obtained compounds, c) and , finally, to establish the optical purity of the products obtained from racemic amide 6. The results obtained, shown on Figure 2, indicate that enantiomer 6 (1R) leads predominantly to hydroxylation at C(5), whereas its optical antipode is essentially hydroxylated at C(8). The absolute configurations of each of these compounds are directly deductible from those of the starting amides. The optical purity of their acetates can be calculated assuming that the starting compounds , prepared from optically pure camphor, are also optically pure. The thus obtained data, (Figure 2) show that these hydroxylation processes are only moderately enantioselective, a fact which is still quite interesting since, to our best knowledge, only few examples of enantioselective hydroxylation processes - i.e. leading to partial resolution of a racemic mixture, have been described previously (8).

One explanation to the fact that epimer 6 affords three products, whereas 5 only leads to hydroxylation at C(5), could have been that, because of rotation around the C(2)-N bond, various rotamers of 6 would be differently positionned on the enzymatic site involved in these transformations, thus leading to a different regioselectivity for each rotamer. Therefore, we decided to study the bioconversion of the N-methylated derivatives of 5 and 6, i.e. amides 11 and 12, where this rotation is disfavoured. These compounds are obtained in high yield by N-methylation of 5 and 6 (9).

When submitted to a culture of Beauveria sulfurescens, each of these substrates do lead to one single compound. Using the same procedure as described previously, the crude products could be directly converted into their acetates, which structures have been determined as being 13b and

14b. Thus, it appears that methylation of the nitrogen atom of 6 allows to highly increase the regioselectivity of the hydroxylation process . Interestingly enough, both of these products 13 and 14 are optically inactive, showing that, here again, no resolution has occured at all.

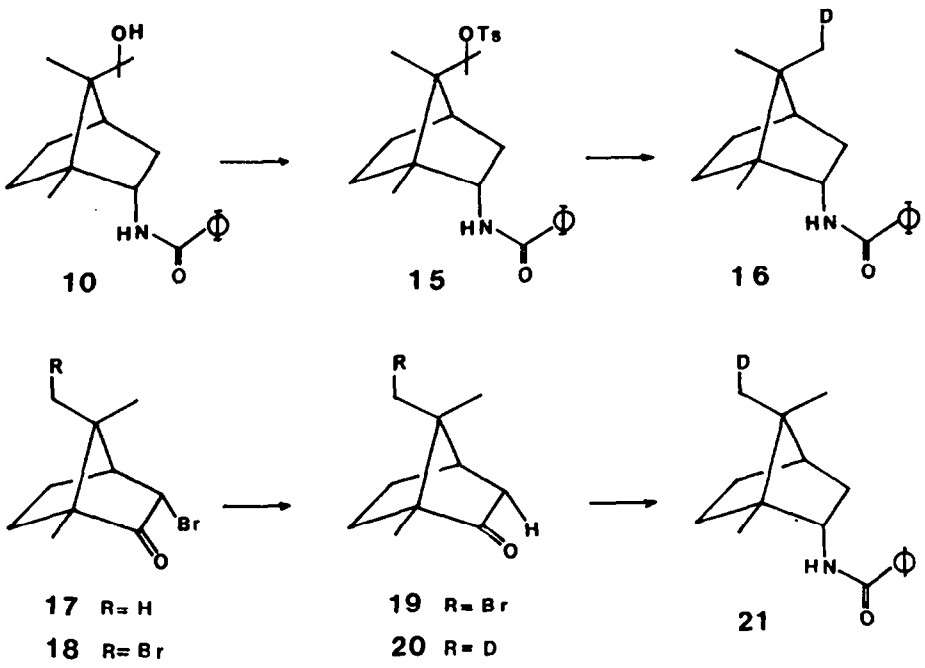
Figure 3 : Hydroxylation of the N-methylated amides derived from camphor.



As noted previously, the structures of the obtained products have been determined using chemical correlation with known compounds and/or by carefull analysis of their ^{1}H and ^{13}C NMR spectra. In particular, it has been impossible to determine unambiguously, using NMR spectroscopy, the exact location of the hydroxyl group on carbon atoms C(8) or C(9) for compounds 9 and 10. Therefore, we established these structures using the chemical correlation described on Figure 4. Thus, the alcohol 10a has been converted into its tosyl derivative 15 which is further reduced to the related deuterated derivative 16. On the other hand, commercial bromocamphor 17 has been brominated into its dibromo derivative 18 (10), which is further transformed into 9-bromo camphor 19 by reduction with zinc in an acetic acid/ether mixture. This is further reduced to 9-deutero camphor 20 (11) , and finally transformed into the 9-deuterated bornylamide 21 derivative as previously described (7). Comparison of the ^{13}C-NMR spectra of these two compounds show that they are different, thus leading to the conclusion that the alcohol leading to 16 bears the hydroxyl group on the C(8) methyl group, i.e. corresponds to structure 10, whereas the other hydroxylated compound has structure 9.

The ^{13}C NMR spectra of all starting and hydroxylated compounds, as well as of their acetates, are described on Table I. These have been used in order to attribute their structures to the various hydroxylation products. In particular, the exo configuration of all C(5) hydroxylated compounds has been deduced from their ^{13}C NMR spectra. Indeed, the introduction of the acetate moiety leads, in all cases , to a upfield shift of about 10 ppm according to previous work (12). These exo configurations of the acetate groups is also confirmed by analysis of the geminated hydrogen signal using 200 MHz ^{1}H-NMR spectroscopy. In all cases, it appears that the coupling constant of this hydrogen with H-C(4) is very small (less than 0,1 ppm), a fact which is only consistent with this exo configuration of the oxygenated group. In that case, indeed, the dihedral angle between H-C(4) and H endo-C(5) is about 90°, leading to a very small coupling constant between these two hydrogen atoms.

Figure 4 : Structure determination of amidoalcohol 10 .



Finally, these exo configurations are also confirmed by the fact that oxydation of alcohols 7a and 8a to the corresponding ketones (13), followed by reduction of these ketones with sodium borohydride in aqueous methanol, lead back to the starting alcohols. This does ascertain the exo stereochemistry of these alcohols, since it is well known that borohydride reduction of camphor (or epicamphor) essentially affords the exo alcohols (14).

DISCUSSION : The main result of this study is the fact that, except in one case, variation of the amide group configuration allows to alter neither the regio nor the stereo selectivity of the hydroxylation process. This is quite a surprising fact, since one could have expected that each of the substrates possessing an identical carbon framework would have been positioned differently on the enzymatic active site, because of the different configuration of the anchoring amide function. For instance, for amides 1 and 2, this should have resulted in hydroxylation of C(7) for amide 1, this carbon atom being quite near the location occupied by carbon atom C(5) of 2, when the amide functions, as well as the carbon frameworks, are identically positionned as shown in Figure 5.

Figure 5 : Hypothetical positionning of amides 1 and 2 on the enzymatic active site.

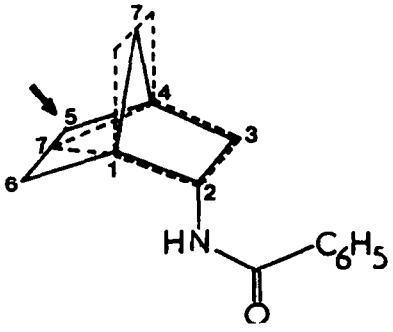


TABLE I

^{13}C NMR spectra of the various amides (δ ppm) ($CDCl_3$)

	C_1	C_2	C_3	C_4	C_5	C_6	C_7	C_8	C_9	C_{10}
1	42.5	53.4	40.5	35.8	28.2	26.6	35.7			
2	40.5	51.5	38.3	38.3	30	21.8	36.9			
4a	41.8	50.2	32.8	39.6	76.1	32.2	35			
4b	41.46	52.2	36.4	41	76.1	32.5	35.7			
5	48.8*	57.1	39.2	44.9	27.0	35.9	47.1*	20.3	11.8	
6	49.7	54.2	37.7	45	28.5*	28.2*	48.2	18.7[b]	19.8[b]	13.0
7a	50.0	55.9	35.2	52.6	74.8	46.8	47.8	21.3*	21.2*	11.4
7b	49.9	55.6	35.6	50.2	77.9	44.6	47.1	21.0*	20.1*	11.3
8b	50.7*	52.8	34.9	50.1*	77.2	38.3	48.2	20.6*	19.4*	13.06
9b	51.8*	50.7*	37.6	42.6	28.7	28.6	54.1	67.1	14.9*	14.6*
10b	50.1*	51.4*	37.8	42.3	28.3*	28.0*	54.2	14.3*	67.6	13.9*
11	50.9	62.3[a]	38.2	45	32.7	33.5	46.4	21.4	21.4	11.4
12	51.6	60.5[a]	33.8[a]	44.8	30.3	29.3	48.2	18.1*	19.3*	14.1
13a	53.1	60.9[a]	33.5[a]	52.6	75.2	46.9	45.8	21.8*	22.2*	11.0
13b	52.2	60.9[a]	34.0[a]	50.4	76.5	46.0	46.0	21.8*	21.2*	10.9
14b	52.4	56.4[a]	33.4[a]	49.9	77.2	38.9	46.0	19.3*	20.3*	13.5

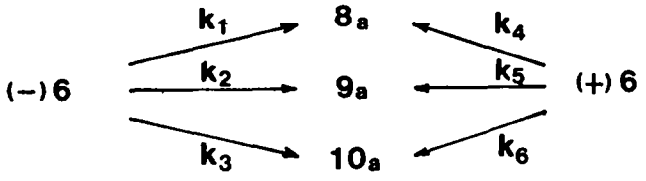
* The attribution of these signals may be inverted
[a] These signals are perturbed by the presence of the two amide rotamers
[b] These signals have been attributed by specific deuteration of C(9).

A second point of interest is the fact that all hydroxylations observed at the C(5) carbon atom of the various amides do exclusively lead to exo compounds , whereas one could have presumed that inversion of configuration of the anchoring amide group could have induced formation of the alcohol of opposite stereochemistry. One explanation to these facts could be that the key step of the hydroxylation process, instead of involving direct oxygen insertion in a C-H bond via an oxenoïd specie (15), would rather implie a two step homolytic mechanism (16-18), with formation of a radical intermediate. This could be formed either by abstraction of H endo-C(5) or of H exo-C(5) dependent upon the configuration of the amide function and lead, after inversion of configuration, to the exo alcohols (19).This type of mechanism has been previously proposed for instance in the case of the hydroxylation of camphor by the cyctochrome P_{450} of Pseudomonas putida (16).

The third interesting fact we have observed in the course of this work is the crucial importance of the nitrogen atom substitution, as exemplified by the hydroxylation of bornylamide derivative 6, which leads to three optically active alcohols, one of them showing about 65% optical purity, whereas the corresponding N-methylated compound 12 only affords one hydroxylation product, optically inactive. Thus, it is clear that even small steric factors, implied in the substrate - enzyme site interaction, may considerably alter the regioselectivity of the reaction.

Finally, we would like to emphasize the fact that, in the case of the bornylamide derivative 6, we do observe partial optical resolution, which is quite noteworthy since only rare examples of this type have been previously described (8). This phenomenon may be explained by the fact that each of the enantiomers of 6 was being hydroxylated on various carbon atoms with different comparative rates (as shown on Figure 7) i.e $k_1 \neq k_2$, $k_3 \neq k_4$, $k_5 \neq k_6$.The fact that the recovered starting material shows to be optically inactive does however indicate that $k_1 + k_3 + k_5 = k_2 + k_4 + k_6$

Figure 6



CONCLUSION : As a conclusion, we would like to emphasize the facts : a) that this fungus is able to perform in fair yields the hydroxylation of non activated carbon atoms on the various substrates we have described b) that these hydroxylations occur in a highly regio and stereoselective way c) that the regioselectivity of these processes may be greatly influenced by steric factors resulting from substitution of the amide nitrogen atom and d) that in certain cases , the hydroxylation may lead, because of a different regioselectivity on each of the two enantiomers of a racemic mixture, to a partial resolution process.

These hydroxylations may also be quite usefull from a synthetic point of vue, since they can compare very favourably with chemical or even enzymatic and microbiological oxidations of similar substrates, which generally do lead to low yield mixtures of isomeric products (20-26).

EXPERIMENTAL PART

General : The ^1H and ^{13}C NMR spectra have been realized on a Bruker AM 200 apparatus using deuterochloroform as solvent. Chemical shifts (δ) are given in ppm relative to TMS as internal standard. The coupling constants are indicated in Herz. IR spectra were recorded using a Beckman Acculab 4 spectrometer using chloroform as solvent. Elemental analyses of C, H, N were performed by the Service Central d' Analyse du CNRS (Vernaison, France). Meltings points have been measured using a Büchi 510 apparatus and are not corrected. Gaz chromatographic analysis has been performed using a 25m capillary column coated with OV17. High Performance Liquid Chromatography analyses has been 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.

Culture conditions : the strain used 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 liquor and 10 g of glucose per liter (tap water, adjusted to pH 4.85 with natrium hydroxide).

General procedure : The sterilized medium is inoculated by a 48 hour old vegetative culture and incubated with reciprocal shaking (80 cpm) at 28°C in 2 liter erlenmeyer flasks filled with 0.5 l of medium. . After 48 hours growth, an ethanolic solution of substrate (10% w/v), is added to the culture (400 mg/l) . After an additional 72 hours period of incubation, the mycelium is separated by filtration, and washed with water. The filtrate is continuously extracted (24h) with methylene chloride. This organic phase is dried over $MgSO_4$, and the solvent distilled off under vacuum. The crude residue is analysed by TLC (Merck 60 F^{254}, 0,2 mm) using ether or ether-methanol (95-5) as eluants . The products are isolated by silicagel chromatography and recristallized. For more difficult separations, preparative HPLC has been used.

2-exo-benzoylamino, norbornane 1 :
To a solution of commercial exo-norbornylamine (3.33 g, 30 mmol) in 50 ml of ether, cooled at 0°C, one adds 5,6 ml (40 mmol) of triethylamine, and 3.83 ml (33 mmol) of benzoylchlorid. After

one nigth, water is added, and the organic phase is separated and dried over $MgSO_4$. The solution is concentrated. Addition of hexane leads to formation of white crystals of 1 (5.16 g, 80%). m.p. 153-154°C (hexane-ether) ; IR : ν = 3340 (N-H) ; 1650 ; 1510 (amide) cm^{-1}. ^{1}H-NMR : δ = 1.0 to 1.7 (m, 8H) ; 1.84 (d, d, d, 1H, H endo-C(6), J H endo-C(6) - H exo-C(6) = 13 , J H endo-C(6) - H endo-C(5) = 8 ,J H endo-C(6) - H endo-C(5) = 2,5) ; 2.39 (br.s, 2H, H-C(1) and H-C(4)) ; 3.88 (t, d, 1H, H-C(2), J H-C(2) - H-N = 7.3, J H-C(2) - H endo-C(3) = 7.3, J H-C(2) - H endo-C(3) = 3.8) ; 6.25 (d, 1H, H-N, J H-N - H-C(2) = 7.3) ; 7.3 to 7.55 (m, 3H, aromatic) ; 7.68 to 7.8 (m, 2H aromatic).

2-endo-benzoylamino, norbornane 2 :

To a suspension of commercial endo-2-aminonorbornane hydrochloride (2.95 g, 20 mmol) in ether (50 ml) one adds 30 ml of a NaOH (1N) solution. When the solid is dissolved, benzoylchloride (26 ml, 22 mmol) is added . After one night, stirring at room temperature, the organic layer is separated and treated as previously . The benzamide 2 is obtained (3.1 g, 72%) as colorless crystals, m.p. 177-178°C (hexane-ether) ; IR : ν = 3340 (N-H) ; 1650 ; 1610 (amide) cm^{-1}. ^{1}H-NMR : δ = 0.83 (d, d, d, 1H, H endo-C(3), J H endo-C(3) - H exo-C(3) = 12.8, J H endo-C(3) - H-C(2) = 5, J H endo-C(3) - H-C(4) = 2.9) ; 1.2 to 1.8 (m, 6H) ; 2.15 (d, d, d, 1H, H endo-C(6), J H endo-C(6) - H exo -C(6) = 11.4 , J H endo-C(6) - H endo-C(5) = 4,6 , J H endo-C(6) - H exo-C(5) = 2.9) ; 2.25 (br.d, 1H, H-C(1)) ; 2.27 (br.s, 1H, H-C(4)) ; 4,31 (m,1H, H-C(2)) ; 6.17 (br.d, 1H, H-N) ; 7.38 to 7.6 (m, 3H, aromatic) ; 7.7 to 7.8 (m, 2H, aromatic).

5-exo-acetoxy,2- exo-benzoylamino, norbornane 3b

The incubation experiment performed starting from 2g of 1 leads, after usual work up, to 732 mg (35% yield) of very insoluble material. m.p. 155-156°C. Analysis : found (calcd) : C, 72.54 (72.72) ; H, 7.33 (7.35) ; N, 5.96 (6.06). This product (500 mg), on treatment with dry pyridine (3 ml) and acetic anhydride, gives the acetate 3b (530 mg, 90% yield), as colorless crystals, m.p. 208-209°C (hexane) ; IR : ν 3440 (N-H) ; 1735 (ester) ; 1650 ; 1610 (amide) cm^{-1}. ^{1}H-NMR : δ = 1.22 to 1.74 (m, 4H) ; 1.77 to 2 (m, 2H) ; 2.05 (s, 3H, Ac) ; 2.44 (br.s, 2H, H-C(1) and H-C(4)) ; 3.9 (m, 1H, H-C(2)) ; 4.66 (d, 1H, H-C(5), J H-C(5) - H endo-C(6) = 7) ; 6.14 (d,1H, H-N, J H-N - H-C(2) = 6) ; 7.4 to 7.66 (m, 3H, aromatic) ; 7.74 to 7.88 (m, 2H, aromatic). Analysis for $C_{16}H_{19}NO_3$: % found (calcd) : C, 70.05 (70.32) ; H, 7.14 (6.95) ; N, 4.97 (5.12).

5-exo-acetoxy, 2-endo-benzoylamino, norbornane 4b .

If the incubation is performed as above, starting from 2, it fails to lead to hydroxylated products. The only recovered material is starting compound . However addition of sodium dodecyl benzenesulfonate to the medium (0.2 g/l) just before the substrate is added allows hydroxylated product to be obtained. The crude product is acetylated as above, without purification of the amido-alcohol. Silica-gel chromatography leads to the acetate 4b (760 mg, 30% yield) as colorless crystals, m.p. 141-142°C (hexane). IR : ν = 3420 (N-H) ; 1735 (ester) ; 1640 ; 1500 (amide) cm^{-1}. ^{1}H-NMR : δ = 0.83 (d, d, d, 1H, H endo-C(3) - H exo-C(3) = 12.5, J H endo-C(3) - H exo-C(2) = 5 ; J H exo-C(3) - H-C(4) = 2.9) ; 1.27 to 1.5 (m, 2H) ; 1.67 to 1.79 (m, 2H) ; 2.01 (s, 3H, Ac) ; 2.05 to 2.26 (m, 2H) ; 2.32 (br.t, 1H, H-C(1)) ; 2.65 (br.s, 1H, H-C(4)) ; 4.25 (m, 1H, H-C(2)) ; 4.68 (d, 1H, H-C(5), J H-C(5) - H endo-C(6) = 6.7) ; 6.25 (d, 1H, H-N, J H-N - H-C(2) = 5.7) ; 7.37 to 7.5 (m, 3H, aromatic) ; 7.70 to 7.77 (m, 2H, aromatic). Analysis for $C_{16}H_{19}NO_3$: % found (calcd) : C, 70.12 (70.32) ; H, 7.14 (6.95) ; N, 5.03 (5.12).

2-exo-benzoylamino, bornane 5 and its enantiomer 5(1R)

Concentrated sulfuric acid (20g, 0.2 mol) is slowly added to a solution of isoborneol (15.6 g, 0.1 mol) in 50 ml of benzonitrile, cooled to 0°C. The temperature is allowed to raise at 20°C, one adds 100 ml of water and neutralizes with NaOH (1N). The excess of benzonitrile is steam distilled and the white solid which precipitates is filtered off, washed with water, and dried under vacuum. Recristallization in hexane afford the benzamide 5 (21 g, 80% yield), as white needles, m.p. 131°C (litt. 130°C) (27). IR : ν = 3330 (N-H) ; 1630 ; 1520 (amide) cm^{-1}. ^{1}H-NMR : δ = 0.87 ; 0.92 ; 1.01 (3s, 3x3H, H-C(8), H-C(9), H-C(10), non-attributed) ; 4.1 (t, d, 1H, H-C(2), J H-C(2) - H-N = 8.5, J H-C(2) - H endo-C(3) = 8.5, J H-C(2) - H exo-C(3) = 4) ; 6.1 (d, 1H, H-N, J H-N - H-C(2) = 8.5), 7.35 to 7.6 (m, 3H, aromatic) ; 7.7 to 7.77 (m, 2H, aromatic).

The (1R) enantiomer 5(1R) is obtained from commercialy available (1R)-isobornylamine hydrochloride following the procedure described for 2. The benzamide 5(1R) has the same physical properties than the racemic mixture and $[\alpha]_D^{20°}$ = - 47.3° (c = 1.1, chloroform).

2-exo-benzoylamino, 5-exo-hydroxy, bornane 7a, and its (1R) enantiomer.

Starting from 1.2 g of racemic isobornylamide 5, one obtains 700 mg (55% yield) of 7a, after purification by silicagel chromatography and recristallization in benzene-hexane mixture, as white crystals, m.p. 169-179°C. IR : ν = 3450 (O-H) ; 3380 (N-H) ; 1633 ; 1520 (amide) cm^{-1}. ^{1}H-NMR : δ = 0.94 ; 1.01 ; 1.14 (3s , 3x3H , H-C(8), H-C(9), H-C(10), non-attributed) ; 3.92 (d, d, 1H, H endo-C(5), J H endo-C(5) - H endo-C(6) = 7.6, J H endo-C(5) - H exo-C(6) = 3.8, J H endo-C(5) - H-C(4) = 0) ; 4,0 (t, d, 1H, H-C(2), J H-C(2) - H-N = 8.5 , J H-C(2) - H exo-C(3) = 8.5, J H-C(2) - H exo-C(3) = 4) ; 6.1 (d, 1H, H-N, J H-N - H-C(2) = 8.5) ; 7.35 to 7.65 (m, 3H, aromatic) ; 7.7 to 7.8 (m, 2H, aromatic). Analysis for $C_{17}H_{23}NO_2$: % found (calcd) : C, 74.81 (74.70) ; H, 8.05 (8.42) ; N, 5.08 (5.12).

Using pure enantiomer 5(1R), the amido-alcohol 7a(1R), obtained has $[\alpha]_D^{20}$ = -71° (c = 1, chloroform).

5-exo-acetoxy, 2-exo-benzoylamino, bornane 7b.

Acetylation of 7b (200 mg), carried out in anhydrous pyridin (1 ml) and acetic anhydride (1 ml), for one night at room temperature leads, after silicagel chromatography and recristallization to 190 mg (82%) of 7b, as white crystals, m.p. 129-130°C ; IR : ν = 3310 (N-H) ; 1735 (ester) ; 1625 ; 1530 (amide) cm^{-1}. ^{1}H-NMR : δ = 0.96 ; 1.04 ; 1.05 (3s, 3x3H, H-C(8), H-C(9), H-C(10), non attributed) ; 4.0 (t, d, 1H, H-C(2), J H-C(2) - H-N = 8.5, J H-C(2) - H endo-C(3) = 8.5 , J H-C(2) - H exo-C(3) = 4) ; 4.65 (d, d, 1H, H endo-C(5) , H endo-C(5) - H endo-C(6) = 8 , J H endo-C(5) - H exo-C(6) = 4 , J H endo-C(5) - H-C(4) = 0) ; 6.1 (d, 1H, H-N, J H-N - H-C(2) = 8.5) ; 7.4 to 7.6 (m, 3H, aromatic) ; 7.71 to 7.8 (m, 2H, aromatic). Analysisfor $C_{19}H_{25}NO_3$: found (calcd) : C, 72.49 (72.38) ; H, 8.06 (7.93) ; N: 4.33 (4.44).

2-exo-benzoylamino, bornane 6, and its enantiomers 6(1R) and 6(1S)

The corresponding amines are obtained from racemic, (+) and (-) camphor according the procedure describe in (7). Benzoylation are performed in ether using benzoylchloride and triethylamine as acid scavenger.

The benzamides are obtained as white crystals (heptane) m.p. 139-140°C (Litt. 139°) (27). IR: ν = 3300 (N-H) ; 1625 ; 1530 (amide) cm^{-1}. ^{1}H-NMR : δ = 0.89 ; 0.90 (2s, 2x3H, H-C(8), H-C(10) unattributed) ; 1.0 (s, 3H, H-C(9), attributed from deuteriated product) ; 2.4 (d, d, d, d, 1H, H exo-C(3), J H exo-C(3) - H endo-C(3) = 14, J H exo-C(3) - H exo-C(2) = 10.5, J H exo-C(3) - H-C(4) = 5.2, J H exo-C(3) - H exo-C(5) = 2) ; 4.7 (d, d, d, d, 1H, H exo-C(2), J H exo-C(2) - H-N = 8.2, J H exo-C(2) - H exo-C(3) = 10.5, J H exo-C(2) - H endo-C(3) = 4.4, J H exo-C(2) - H exo-C(6) = 2) ; 6.18 (d, 1H, H-N, J H-N - H exo-C(2) = 8.2) ; 7.4 to 7.52 (m, 3H, aromatic) ; 7.7 to 7.76 (m, 2H, aromatic).

The (1R) enantiomer has $[\alpha]_D^{20}$ = -4.9° (c = 2.9, chloroform) and -22.9° (c = 2.1, ethanol)(Litt. -22.93° (ethanol)(27)). The optical rotation of the (1S) enantiomer is $[\alpha]_D^{20}$ = + 4.75° (C = 1.7, chloroform).

Hydroxylation of the racemic benzamide 6

The incubation experiment is performed with 2 g of the benzamide 6. After usual work up, silicagel chromatography of the residue (3.4 g) leads to a mixture of 8a, and 9a (382 mg, 26% yield) and of compound 10a (190 mg, 13% yield) as white crystals.

10a : m.p. 227-228° (ethyl acetate) ; IR (KBr) : ν = 3450 (OH) ; 3300 (N-H) ; 1630 ; 1510 (amide) cm^{-1}. Analysis for $C_{17}H_{23}NO_2$: found (calcd) : C, 74.62 (74.7) ; H, 8.15 (8.42) ; N : 5.07 (5.12). This very insoluble product (150 mg) is acetylated by acetic anhydride (2 ml) in dry pyridine (2 ml) yielding, after silicagel chromatography, the corresponding acetate 10b (90% yield). m.p. 103-104° (aqueous ethanol).$[\alpha]_D^{20}$ = 4.4 (c = 2.8, chloroform) ; IR :ν= 3330 (NH) ; 1740 (ester) ; 1630 ; 1510 (amide) cm^{-1}. ^{1}H-NMR : δ = 0.98 (s, 3H, H-C(10)) ; 1.0 (s, 3H, H-C(9)) ; 2.05 (s, 3H, Ac) ; 2.45 (d, d, d, d, 1H, H exo-C(3), J H exo-C(3) - H endo-C(3) = 14, J H exo-C(3) - H exo-C(2) = 10.5, J H exo-C(3) - H-C(4) = 5.2 , J H exo-C(3) - H exo-C(5) = 2) ; 4.06 (d, 1H, H-C(8), J H-C(8) - H-C(8) = 11.4 ; 4.29 (d, 1H, H-C(8) , J H-C(8) - H-C(8) = 11.4) ; 4.51 (d, d, d, d, 1H, H-C(2), J H-C(2) - H-N = 8.2, J H-C(2) - H exo-C(3) = 8 , J H-C(2) - H endo-C(3) = 4.4, J H-C(2) - H exo-C(6) = 2) ; 6.2 (d, 1H, H-N, J H-N - H-C(2) = 8.2) ; 7.4 to 7.52 (m, 3H, aromatic) ; 7.7 to 7.76 (m, 2H, aromatic). Analysis for $C_{19}H_{25}NO_3$: found (calcd) : C, 72.20 (72.38) ; H, 7.88 (7.93) ; N, 4.52 (4.44).

The mixture of 8a and 9a (200 mg) acetylated as before, affords (90% yield) a mixture of the acetates 8b and 9b, which are separated by HPLC (hexane /ethylacetate 60/40). They are obtained as white crystals.

8b : m.p. 157-158°C (aqueous ethanol) ;$[\alpha]_D^{20}$ = -12° (c = 1.6, chloroform) ; IR :ν = 3330 (NH) ; 1735 (ester) ; 1635 ; 1515 (amide) cm^{-1}. ^{1}H-NMR : δ = 0.84 (d,d,1H, H endo-C(3), J H endo-C(3) - H exo-C(3) = 14.1, J H endo-C(3) - H-C(2) = 4.4) ; 0.94 ; 1.01 ; 1.06 (3s ; 3x3H ; H-C(8), H-C(9), H-C(10) non attributed) ; 1.66 (d, d, d, 1H, H exo-C(6), J H exo-C(6) - H endo-C(6) = 14.6, J H exo-C(6) - H endo-C(5) = 3.7 , J H exo-C(6) - H exo-C(2) = 2) ; 1.94 (d, 1H, H-C(4), J H-C(4) - H exo-C(3) = 5.2) ; 2.02 (s, 3H, Ac) ; 2.15 (d, d, 1H, H endo-C(6), J H endo-C(6) - H exo-C(6) = 14.6, J H endo-C(6) - H endo-C(5) = 8) ; 2.48 (d, d, d, 1H, H exo-C(3), J H exo-C(3) - H endo-C(3) = 14.1, J H exo-C(3) - H exo-C(2) = 8 , J H exo-C(3) - H-C(4) = 5.2) ; 4.43 (d, d, d, d, 1H, H-C(2) , J H-C(2) - H-N = 8.2, J H-C(2) - H exo-C(3) = 8, J H-C(2) - H endo C(3) = 4.4, J H-C(2) - H exo-C(6) = 2) ; 4.68 (d, d, 1H, H endo-C(5) , J H endo C(5) - H endo-C(6) = 7.8, J H endo-C(5) - H exo-C(6) = 3.6, J H endo-C(5) - H-C(4) = 0) ; 6.2 (d, 1H, H-N, J H-N - H-C(2) = 8.2) ; 7.4 to 7.55 (m, 3H, aromatic) ; 7.7 to 7.8 (m, 2H, aromatic).

9 : m.p. 157-158°C (aqueous ethanol) .$[\alpha]_D^{20}$ = + 0.8° (c = 1.5, chloroform) ; IR : ν = 3330 (N-H) 1740 (ester) ; 1630 ; 1510 (amide) cm^{-1}. ^{1}H-NMR :δ = 0.95 (s, 3H, H-C(10)) ; 1.11 (s, 3H, H-C(8)) 2.45 (d, d, d, d, 1H, H exo-C(3), J H exo-C(3) - H endo-C(3) = 14, J H exo-C(3)- H exo-C(2) = 8, J H exo-C(3) - H-C(4) = 5.2, J H exo-C(3) - H exo-C(5) = 2) ;3.95 (d, 1H, H-C(9), J H-C(9) - H-C(9) = 11) ; 4.14 (d, 1H, H-C(9), J H-C(9) - H-C(9) = 11) ; 4.51 (d, d, d, d, 1H, H-C(2), J H-C(2) - H-N = 8.2 , J H-C(2) - H exo-C(3) = 8 , J H-C(2) - H endo-C(3) = 4.4, J H-C(2) - H exo-C(6) = 2) ; 6,2 (d, 1H, H-N, J H-N - H-C(2) = 8.2), 7.4 to 7.55 (m, 3H, aromatic) ; 7.7 to 7.8 (m, 2H, aromatic). Analysis for $C_{19}H_{25}NO_3$: found (calcd) : C, 72.6 (72.38) ; H, 7.92 (7.93) ; N, 4.41 (4.44).

Hydroxylation of enantiomeric benzamides 6(1R) and 6(1S).
This incubations are performed starting from 1 g of pure enantiomer 6(1R) or 6(1S). The crude mixture is first analysed by HPLC (hexane/ethylacetate , 60/40), for quantitative determination of the percentage of hydroxylated products 8a, 9a and 10a. The mixture is then acetylated and the three acetates 8b (1R and 1S), 10b are separated by preparative HPLC, using the same eluent; 8b(1R) : m.p. 160-161°C ; $[\alpha]_D^{20}$ = -49.5° (c = 2.7, chloroform) ; 8b(1S), m.p. 159-160°C ; $[\alpha]_D^{20}$ = + 47.4 (c = 2.1, chloroform) ; 9b(1R), m.p. 163-164°C ; $[\alpha]_D^{20}$ = + 3.4 (c = 1.6, chloroform) ; 9b(1S), m.p. 162-163°C, $[\alpha]_D^{20}$ = -3.5 (c = 1.7, chloroform) ; 10b(1R), m.p. 110-111°C ; $[\alpha]_D^{20}$ = - 6.7° (c = 2.2, chloroform) ; 10b(1S), m.p. 110°C ; $[\alpha]_D^{20}$ = + 6.4° (c = 2, chloroform).

2-exo-benzoylamino, N-methyl, bornane 11
To a suspension of KOH (2.24 g, 40 mmol) in 20 ml of freshly distilled DMSO, is added a solution of racemic isobornylamide 5 (2.57g, 10 mmol) in 20 ml of DMSO at room temperature. To the resulting mixture one adds an excess of methyl iodide. After one hour, at room temperature, water is added and the mixture is extracted with ethylacetate. The organic layer is washed with water, and dried over $MgSO_4$. The solvent is stripped off, and the crude product is passed through silica gel yealding 2.43 g (90% yield) of N-methylated isobornylamide 11 ; m.p. 93-94°C (hexane). IR : ν = 1660 cm^{-1} (amide). The ^{1}H-NMR spectra performed at ambiant temperature shows a coalescence of several pics, due to the presence of the two rotamers of the amide. At -60°C, the two species can be observed, and an attribution can be made : δ = 0.81 ; 0.87 ; 0.90 ; 0.93 ; 1.03 (5s, methyl group of both rotamers) ; 1.15 to 2.2 (m, 7H) ; 2.94 (s, 2.25 H, H-C(11), major rotamer 75%) ; 3.11 (s, 0.75 H, H-C(11), minor rotamer 25%) ; 4.01 (t, 0.25 H, H-C(2) minor rotamer, J H-C(2) - H endo-C(3) = J H-C(2) - H exo-C(3) = 8.6) ; 4.76 (t, 0.75 H, H-C(2), major rotamer, J H-C(2) - H endo-C(3) = J H-C(2) - H exo-C(3) = 8.6) ; 7.42 (br ,s, 5H, aromatic). Analysis for $C_{18}H_{25}NO$: found (calcd) : C, 79.41 (79.70) ; H, 9.26 (9.22) ; N, 5.2 (5.16).

2-endo-benzoylamino, N-methyl, bornane 12.
This product is obtained as the preceding one, starting from racemic bornylamide 6. Colorless crystals, m.p. 99-100°C. IR : ν = 1660 cm^{-1} (amide). ^{1}H-NMR (-60°C): δ = 0.5 ; 0.52 ; 0.74 ; 0.88 ; 0.93 ; 1.05 (6s, methyl groups of both rotamers) ; 1.1 to 2.2 (m, 7H) ; 2.97 (s, 1.3 H, H-C(11), minor rotamer 44%) ; 3.18 (s, 1.7 H, H-C(11) major rotamer 56%) ; 4.22 (d, d, 0.56 H, H-C(2), J H-C(2) - H exo-C(3) = 11.3, J H-C(2) - H endo-C(3) = 5) ; 5.02 (d, d, 0.43 H, H-C(2), J H-C(2) - H exo-C(3) = 11.3 , J H-C(2) - H endo-C(3) = 5) ; 7.4 (m, 5H, aromatic). Analysis for $C_{18}H_{25}NO$: found (calcd) : C, 79.61 (79.70) ; H, 9.24 (9.22) ; N, 5.12 (5.16).

2-exo-benzoylamino, 5-exo-hydroxy, N-methyl, bornane 13a and its acetate 13b.
Starting from 2 g of 11, we obtain, after incubation and usual work up, 840 mg of the alcohol 13 a (40%), as white crystals. m.p. 173-174°C . IR : ν = 3450 (OH) ; 1650 (amide) cm^{-1}. ^{1}H-NMR : δ = 0.93 ; 0.98 ; 1.16 (3s, 3x3H, H-C(8), H-C(9), H-C(10), non attributed) ; 2.96 (br.s, 3H, H-C(11)) ; 3.93 (br.s, 1H, H endo-C(5)) ; 4.53 (br.s, 1H, H-C(2)). Analysis for $C_{18}H_{25}NO_2$: found (calcd) : C, 75.45 (75.26) ; 8.77 (8.71) ; N, 4.84 (4.87).
The acetate 13b, is obtained from 13a, (90% yield) as white crystals, m.p. 99-100°C.
IR : ν = 1740 (ester) ; 1650 (amide) cm^{-1}. ^{1}H-NMR : δ = 0.96 ; 1.0 ; 1.07 (3s, 3x3H, H-C(8) ; H-C(9), H-C(10) not attributed) ; 2.04 (s, 3H, Ac) ; 3.0 (br.s, 1H, H-C(11)) ; 4.75 (br.s, 2H, H-C(2) and H endo-C(5)). Analysis $C_{20}H_{27}NO_3$: found (calcd) : C, 72.75 (72.94) ; H, 8.16 (8.20) ; N, 4.33 (4.25).

2-endo benzoylamino, 5-exo-hydroxy, N-methyl, bornane 14a and its acetate 14b.
As previously the benzamide 12 is converted (with 35% yield), into the very insoluble amido-alcohol 14a. Colorless crystals, m.p. 206-207°C. IR (KBr) : ν = 3450 (OH) ; 1650 (amide) cm^{-1}. Analysis for $C_{18}H_{25}NO_2$: found (calcd) : C, 75.18 (75.26) ; H, 8.76 (8.71) ; N, 4.88 (4.87). The acetylation leads to the well soluble oily acetate 14b. IR : ν = 1740 (ester) ; 1650 (amide) . ^{1}H-NMR : δ = 0.98 ; 0.83 (br.s, 9H (coalescence)) ; 2.05 (s, 3H, CH_3CO-) ; 3.0 (br.s, 1H, H-C(11)) ; 4.6 (br.s, 1H, H-C(2)) ; 4.75 (d, d, 1H, H endo-C(5), J H endo-C(5) - H endo-C(6) = 8, J H endo-C(5) - H exo-C(6) = 4). Analysis for $C_{20}H_{27}NO_3$: found (calcd) : C, 73.10 (72.94) ; H, 8.25 (8.20) ; N, 4.26 (4.25).

Oxidation of the amidoalcohols 7a and 8a. 2-exo benzoylamino, 5-oxo, bornane 7c, and its 2-endo epimer 8c.
The oxidation of 7a, (150 mg) performed according to the biphasic Brown's procedure, furnished 130 mg of ketone 7c : m.p. 151-152°C (ether). IR : ν = 3330 (NH) ; 1735 (ketone) ; 1635 ; 1515 (amide) cm^{-1}. ^{1}H-NMR : δ = 1 ; 1.11 ; 1.16 (3s, 3x3H, H-C(8), H-C(9), H-C(10) not attributed) ; 1.92 (t, d, 1H, H exo-C(3), J H exo-C(3) - H endo-C(3) = 14, J H exo-C(3) - H endo-C(2) = 4.8, J H exo-C(3) - H-C(4) = 4.8) ; 1.98 (d, 1H, H exo-C(6), J H exo-C(6) - H endo-C(6) = 19) ; 2.24 (d, d, 1H, H endo-C(3), J H endo-C(C3) - H exo-C(3) = 14, J H endo-C(3) - H endo-C(2) = 8.8) ; 2.3 (d, 1H, H endo-C(6), J H endo-C(6) - H exo-C(6) = 19) ; 2.31 (d, 1H, H-C(4), J H-C(4) - H exo-C(3) = 4.8) ; 4.18 (t, d, 1H, H endo-C(2) , J H endo-C(2) - H-N = 8.5, J H endo-C(2) - H endo-C(3) = 8.5, J H endo-C(2) - H exo-C(3) = 4) ; 6.1 (d, 1H, H-N, J H-N - H endo-C(2) = 8.5);

7.35 to 7.65 (m, 3H, aromatic) ; 7.7 to 7.8 (m, 2H, aromatic). Analysis for $C_{17}H_{21}NO_2$: found (calcd) : C, 73.39 (75.27) ; H, 7.8 (7.74) ; N, 5.12 (5.16).
The ketone 8c is obtained by oxidation of the mixture containing 8a and 9a (the product derived from 9a is not identified) after silicagel chromatography of the crude product. m.p. 178-179°C (hexane-ether). IR : ν = 3340 (NH) ; 1735 (ketone) ; 1640 ; 1520 (amide) cm^{-1}. ^{1}H-NMR : δ = 0.99 ; 1.07 ; 1.13 (3s, 3x3H, H-C(8), H-C(9), H-C(10) non attributed) ; 1.27 (d, d, 1H, H endo-C(3), J H endo-C(3) - H exo-C(3) = 14.2, J H endo-C(3) - H exo-C(2) = 4.6) ; 2.1 (d, d, 1H, H exo-C(6), J H exo-C(6) - H endo-C(6) = 19, H exo-C(6) - H exo-C(2) = 2 ; 2.3 (d, 1H, H endo-C(6), J H endo-C(6) - H exo-C(6) = 19) ; 2.4 (d, 1H, H-C(4), J H-C(4) - H exo-C(3) = 5.4) ; 2.65 (d, d, d, 1H, H exo-C(3), J H exo-C(3) - H endo-C(3) = 14.2, J H exo-C(3) - H exo-C(2) = 19, J H exo-C(3) - H-C(4) = 5.4) ; 4.75 (d, d, d, d, 1H, H exo-C(2), J H exo-C(2) - H-N = 8.5, J H exo-C(2) - H exo-C(3) = 10.9, J H exo-C(2) - H endo-C(3) = 4.6, J H exo-C(2) - H exo-C(6) = 2) ; 6.2 (d, 1H, H-N, J H-N - H exo-C(2) = 8.5) ; 7.35 to 7.65 (m, 3H, aromatic) ; 7.7 to 7.8 (m, 2H, aromatic). Analysis for $C_{17}H_{21}NO_2$ found (calcd) : C, 75.17 (75.27) ; H, 7.72 (7.74) ; N, 5.12 (5.16).

Reduction of ketones 7c and 8c.
The reaction is carried out on 5 to 10 mg of ketones, in aqueous methanol (0.5 ml) using an excess of $NaBH_4$. Analysis of the crude product shows the presence of one compound (>90%) in each case, respectively identified as being 7a and 8b by TLC (ether), HPLC (hexane /ethyl acetate, 60/40 and hexane/ethanol 90/10) and GPG (capillary column OV 17, 25 m, 200°C)

2-endo-benzoylamino, 9-deutero bornane 20.
The dibromocamphor 18, is obtained from 3-bromocamphor by bromosulfonation, according to (10). Reduction of C(3)-bromine is performed using zinc in acetic acid/ether at 0°C, according to (11). Reduction of the other bromine atom is achieved under more drastic conditions using zinc in deuteroacetic acid at 60°C as described in (11). Transformation of 9-deutero camphor into 9-deutero benzamide, is realized as previously described, leading to the 9-deutero bornylamide 20. m.p. = 138-139°C. The physical properties of this deuterated product are the same as those of the non-deuterated one, except for the ^{13}C and ^{1}H-NMR spectra.

2-endo-benzoylamino, 8-deutero bornane 16.
The amido alcohol 10 (100 mg, 0.36 mmol) previously isolated, is added to a solution of paratoluenesulfochloride (95 mg, 0.5 mmol) in dry pyridine cooled at 0°C. After one night, one adds a solution of HCl (10%) and extracts with ether. The organic layer is washed with HCl (10%), then with NaOH (1N), with water and dried over $MgSO_4$. The crude tosylate 15 (138 mg, 90% yield) appears homogenous by TLC (hexane/ether, 50/50), and no starting material can be detected.

To a solution of the above crude tosylate in 5 ml of anhydrous THF, cooled to 0°C, and maintained under nitrogen atmosphere, are added, using a hypodermic syringe, 1.3 ml of commercially available (1M) solution of deutero triethyl borohydride (superdeuteride, Aldrich) . After 1h, at 0°C, water (5 ml) is added, and the THF is stripped off under vacuum. The resulting phase is extracted and 8-deutero benzamide 16 (74 mg, 95% yield) is obtained as white crystals . m.p. 139-140°C (heptane).

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