# Enzyme-Catalysed Transformations of *ent*-Kaurane Diterpenoids

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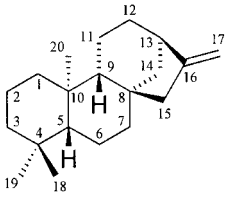
Several acetyl derivatives of linearol, atractyligenin and atractylitriol were obtained through enzyme-catalysed acetylation and deacetylation reactions. In most reactions lipases showed regio- and stereoselective behaviour, allowing a family of novel compounds to be prepared.



## Introduction

A large number of *ent*-kaurane diterpenoids,[1] biogenetically derived from (–)-kaur-16-ene (**1**), have been isolated from plants belonging to several genus of different families (Euphorbiaceae, Apiaceae, Rutaceae, Lamiaceae and Compositae). In the last few years, a considerable number of studies on their interesting biological properties have been published. In fact, antimicrobial activity has been reported for kauren-19-oic acid,[2–4] kaur-15-en-19-oic acid[5] and foliol (**2**),[6] the last isolated from some species of *Sideritis* (Lamiaceae) used in Spanish folk medicine. Some products have shown good cytotoxic,[7–11] anti-HIV,[12,13] hypotensive[14] and antiinflammatory[15,16] activities. Recently we reported on the anti-HIV[17] and the antifeedant[18] activities of linearol (**3**), a common metabolite of genus *Sideritis*,[19,20] and of several semisynthetic derivatives.



**1**

The presence of two free hydroxy groups in linearol (**3**) allowed us to synthesise a large number of C-3 mono- and C-3,C-7 diesters. A comparison of the activities of these derivatives suggested that the position of the ester group dramatically influences the activity. We tried to synthesise C-7 monoesters and also to perform selective esterifications on foliol (**2**). Unfortunately, we were not able to achieve significant results by conventional chemical methods.

In previous works we reported the regioselective acylation of natural products as polyhydroxy compounds by treatment with alkyl carboxylates or carboxylic acids with catalysis by enzymes in anhydrous organic solvents.[21–23] We have also performed enzyme-catalysed deacylation reactions on various steroids such as cholestanes, androstanes and pregnanes, which demonstrated the ability of enzymes to discriminate between acetoxy substituents in these substrates, furnishing deacetylation reactions at different positions in the steroid skeleton. While *Candida rugosa* lipase removed acetyl groups situated in ring A, *Candida antarctica* lipase was preferentially active on substituents located in ring D.[24,25]

With the aims of extending the biocatalytic approach to other natural products and of preparing a family of derivatives difficult to obtain through traditional chemical methods, in order to study the structure/activity relationship, we decided to apply an enzymatic approach to diterpenoids such as *ent*-kauranes. We found that lipases and esterases from several sources were able to catalyse regioselective acetylation and alcoholysis reactions.

## Results and Discussion

In this report we describe the results obtained by enzyme-catalysed acetylation and deacetylation reactions performed on three related *ent*-kauranes: linearol (**3**), atractyligenin (**8**) and atractylitriol (**11**).

### Reactions with Linearol (3)

#### *Enzymatic Acetylation*

The presence of the two hydroxy groups in linearol (**3**) makes this compound an interesting model for enzymatic

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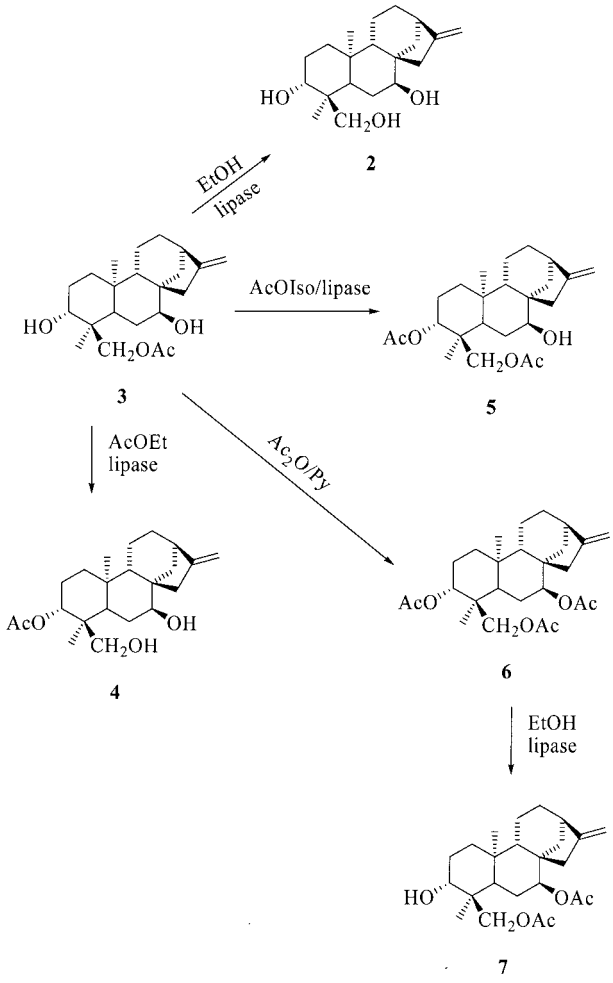
transformation, so we began investigating the behaviour of lipases from several sources in the acetylation of this *ent*-kaurane. We used lipases from yeasts [*Candida antarctica* lipase A (CAL A) and B (CAL B), *Candida rugosa* lipase (CRL)], from fungi [Lipozyme (LIP)], from mammals [porcine pancreatic lipase (PPL)], and from bacteria [*Pseudomonas sp.* lipase (PSL)].

As we had obtained good results with ethyl acetate as acylating agent in previous work,[21,26] we also tried it in the enzymatic acetylation of linearol (**3**). Accordingly, linearol (**3**) was dissolved in ethyl acetate, serving both as acylating agent and as solvent. The enzyme was then added and the suspension was shaken at 30 °C for 24 h (Scheme 1).

The six lipases from various sources (Table 1, Entries 1 to 6) afforded the same product, which had an $R_f$ value lower than linearol's when analysed by TLC. This product was fully identified by spectroscopic methods as sidol (**4**), a monoacetylated isomeric compound of linearol produced by the migration of the acetyl group from carbon 18 to carbon 3, as clearly shown by the downfield shifts of H-3β ($\delta$ = 4.91, dd) and C-3 ($\delta$ = 74.56) and the upfield shifts of the H-18 protons ($\delta$ = 3.31, d and 2.98, d) and of C-18 ($\delta$ = 64.20) in the $^1$H NMR and $^{13}$C NMR spectra (Table 5 and Table 7).

The reaction was not complete. Actually, linearol was present even at longer reaction times, and by flash chromatography separation of the products we observed a sidol/linearol ratio of 2:1. The migration was also produced in the absence of ethyl acetate and with use of other solvents such as acetonitrile or tetrahydrofuran (Entries 8 and 9), so the presence of an acetylating agent such as ethyl acetate did not seem to be necessary. On the other hand, no migration product was detected without the enzymatic participation (Entry 7).

Afterwards, when sidol (**4**) was treated under the same enzymatic conditions as linearol, we also obtained the sidol/linearol mixture in the same 2:1 ratio, suggesting that the two compounds reach an equilibrium situation, though one only achieved through enzyme-catalysed interconversion.




Scheme 1.

In order to explain the role of lipases in this migration, we decided to perform a molecular modelling analysis of the conformations of linearol and sidol. This should let us make an estimation of the activation energy involved in the intramolecular migration.

Table 1. Lipase-catalysed acetylation of linearol (**3**).

| Entry | Enzyme | Acylating agent | Solvent | *T* (°C) | Time (h) | Product yields (%) | |
|---|---|---|---|---|---|---|---|
| | | | | | | **4** | **5** |
| 1 | CAL B | ethyl acetate | AcOEt | 33 | 24 | 67 | – |
| 2 | CAL A | ethyl acetate | AcOEt | 33 | 24 | 66 | – |
| 3 | PSL | ethyl acetate | AcOEt | 33 | 24 | 65 | – |
| 4 | CRL | ethyl acetate | AcOEt | 33 | 24 | 66 | – |
| 5 | PPL | ethyl acetate | AcOEt | 33 | 24 | 64 | – |
| 6 | LIP | ethyl acetate | AcOEt | 33 | 24 | 65 | – |
| 7 | – | ethyl acetate | AcOEt | 33 | 168 | – | – |
| 8 | CAL B | – | $CH_3CN$ | 33 | 72 | 66 | – |
| 9 | CAL B | – | THF | 33 | 96 | 67 | – |
| 10 | CAL B | vinyl acetate | THF | 33 | 168 | 62 | – |
| 11 | CAL B | vinyl acetate | THF | 55 | 168 | 64 | – |
| 12 | CAL B | isopropenyl acetate | THF | 33 | 72 | 33 | 43 |
| 13 | CAL B | isopropenyl acetate | THF | 55 | 72 | 35 | 55 |
| 14 | CAL B | isopropenyl acetate | $CH_3CN$ | 55 | 72 | 58 | 28 |
| 15 | CAL B | isopropenyl acetate | *t*BuOH | 55 | 72 | 59 | 24 |
| 16 | CAL A | isopropenyl acetate | THF | 55 | 72 | 62 | – |

Our goal was to verify whether the acetyl migration from carbon 18 to carbon 3 could happen spontaneously without assistance of the enzyme.

Conformational searches on compounds **3** and **4** were performed by use of the semiempirical method AM1 approach as implemented in Hyperchem 7.5.

To begin with, molecular dynamic experiments were performed on the substrates to estimate local minima. Relative distances between reactive groups were monitored during these experiments. The conformations obtained by the above method were then minimised and stored as probable structures for the compound. The global minimum for linearol showed rings A and B in a chair conformation, as reported in the literature.[27] It can be also observed that in this case, the acetyl group at the 18-position adopts an extended conformation relative to the structure of the molecule and the distance between the acetyl group carbonyl carbon at 18 and the hydroxy group at carbon 3 is 4.99 Å. Similarly, the distance between the hydroxy group at carbon 18 of sidol and the acetyl substituent at carbon 3 is 3.64 Å (Figure 1 and Figure 2). Moreover, the analysis of the relative distances in molecular dynamics experiments showed no interaction between the groups.

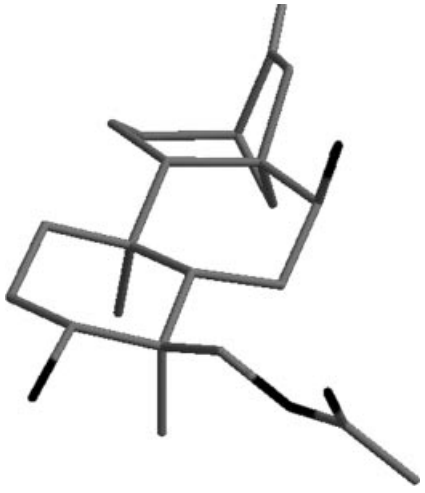


Figure 1. Conformational energy minimum of linearol (**3**)

Figure 2. Conformational energy minimum of sidol (**4**)

The synchronous transit algorithm was used to estimate the transition state based on the most probable structures of linearol and sidol. The energy of activation for the intramolecular migration was estimated to be 73 kcal mol$^{-1}$. These calculations correlate well with our experimental results and suggest that the thermal energy of the system is not enough to favour the migration. This fact could explain the absence of migration without enzyme.

The enzyme-catalysed migration could occur if it were possible to generate an acyl–enzyme complex between linearol or sidol and the active site of the lipase. It would be necessary that each of these substrates could be accommodated into the catalytic pocket. From the reported analysis of the structures of lipase-binding sites carried out by Pleiss,[28] it is known that lipases have large hydrophobic acyl binding pockets, their lengths varying considerably (between 7.8 Å and 22 Å). The sizes of the acyl binding sites of all the lipases used in this paper would allow both substrates **3** and **4** to fit to accomplish the migration of the acetyl group from one to the other. As one example, the CAL B binding site lies in a pocket some 9.5 Å deep and 4.5 Å wide, with Ser105 of the catalytic triad at its base.[29]

In conclusion, it could be assumed that the conformational change produced by the anchorage of substrate to the active site of the enzyme could help in the acetyl group migration between linearol and sidol.

Since ethyl acetate was not a good acylating agent of linearol, we tried the enzymatic acetylation with vinyl acetate and isopropenyl acetate (Table 1, Entries 10–16). Migration of the linearol acetyl group was slower in tetrahydrofuran, so it was used as solvent with these two acylating agents. Although treatment with vinyl acetate only produced sidol (**4**), we did obtain a monoacetylation product when using isopropenyl acetate in THF. Its $^{1}$H and $^{13}$C NMR spectra (Table 5 and Table 7) showed downfield shifts of H-3β ($\delta$ = 4.83, dd) and C-3 ($\delta$ = 74.3) and upfield shifts of C-2 ($\delta$ = 23.0) and C-4 ($\delta$ = 40.5), allowing us to assign the structure of 3-acetyl-linearol (**5**) to this compound. The best results were obtained with CAL B as biocatalyst and at 55 °C (Entry 13). Sidol was the side product in every case, but the acetylation reaction was highly regioselective and 3-acetyl-linearol (**5**) was obtained as the only diacetyl compound.

Considering the possibility that **5** could also arise from acetylation of sidol we used CAL A, which appears to acetylate primary alcohols, to increase the yield in **5**, but CAL A was inactive in this system (Entry 16).

### *Enzymatic Alcoholysis*

We next studied the lipase-catalysed alcoholysis reaction of linearol by treating it with alcohols in the presence of CAL A, CAL B and PSL.

In view of our previous work on enzymatic deacetylation of steroids,[24,25] we began using octanol as nucleophile, but there was no transformation in this case (Table 2, Entries 1–3). Only ethanol gave satisfactory results in THF as solvent, and we obtained foliol (**2**) in moderate yield with CAL B. It is remarkable that we did not detect any migration of the acetyl group of linearol to give sidol in the presence of alcohols. In the case of octanol, linearol remained unaltered.

Table 2. Lipase-catalysed alcoholysis of linearol (**3**) and triacetyl-foliol (**6**).

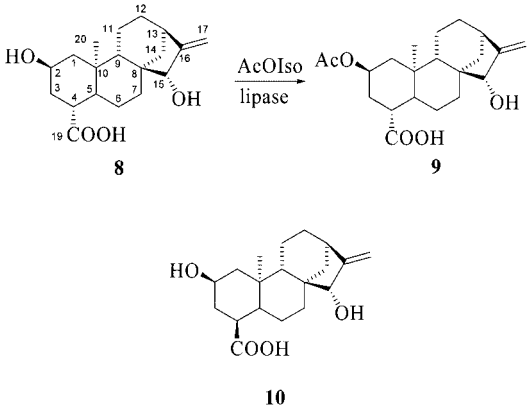
| Entry | Enzyme | Substrate | Nucleophile | Solvent | *T* (°C) | Time (h) | Product yield (%) | |
|---|---|---|---|---|---|---|---|---|
| | | | | | | | **2** | **7** |
| 1 | CAL A | **3** | *n*-octanol | $CH_3CN$ | 33 | 168 | – | – |
| 2 | CAL B | **3** | *n*-octanol | $CH_3CN$ | 33 | 168 | – | – |
| 3 | PSL | **3** | *n*-octanol | $CH_3CN$ | 33 | 168 | – | – |
| 4 | CAL B | **3** | EtOH | EtOH | 55 | 72 | 36 | – |
| 5 | CAL B | **3** | EtOH | THF | 33 | 72 | 43 | – |
| 6 | CAL B | **3** | EtOH | THF | 55 | 48 | 68 | – |
| 7 | CAL B | **3** | EtOH | $CH_3CN$ | 55 | 48 | 55 | – |
| 8 | CAL A | **6** | EtOH | THF | 33 | 168 | – | 5 |
| 9 | CAL A | **6** | EtOH | THF | 55 | 168 | – | 4 |
| 10 | CAL B | **6** | EtOH | THF | 33 | 168 | – | 12 |
| 11 | CAL B | **6** | EtOH | THF | 55 | 48 | – | 32 |

With the aim of extending the study of the enzymatic alcoholysis reaction we prepared the triacetyl-foliol (**6**), applying traditional chemical methodology, by treatment of **3** with an excess of acetic anhydride and pyridine. The triacetyl-foliol (**6**) was then subjected to the enzymatic alcoholysis with CAL A and CAL B as biocatalysts, two temperatures and various solvents (Entries 4–7). CAL A showed low activity, even at 55 °C (Entries 8 and 9). The best result was obtained with CAL B and THF as solvent at 55 °C (Entry 11). The enzyme catalysed alcoholysis of the acetyl group at C-3 in triacetyl-foliol, so it was possible to obtain 7-acetyl-linearol (**7**), the complementary diacetylated product of (**4**) (Scheme 1). It seems that the deacetylation reaction stopped at this point, since we did not detect any other product even at longer reaction times.

This result is in agreement with our previous work with pyridoxine as substrate,[21] in which CAL B was active at the same position both in the alcoholysis and the acetylation reactions. The site of alcoholysis in every case was unambiguously established by NMR spectroscopic analysis (Table 5 and Table 7). In fact compound **7** showed downfield shifts, with respect to linearol (**3**), of H-7α ($\delta$ = 4.77, t) and C-7 ($\delta$ = 79.53) and upfield shifts of C-6 ($\delta$ = 24.41) and C-8 ($\delta$, 46.72), consistently with the β effect of acetylation. Furthermore, the unexpected upfield shift of H-3β ($\delta$ = 3.32, dd) and the enlargement of the AB system of the H-18 protons ($\delta$ = 4.30, d and 3.56, d) was in full agreement with the $^1$H NMR spectrum of 7,18-diacetyl-leucanthol,[30] which has an identical substitution pattern at the decalin moiety.

**Enzymatic Acetylation on Atractyligenin (8)**

In view of the previous results on linearol we tested four lipases (PPL, CRL, CAL B and PSL) in the acetylation reaction of the 18-*nor*-*ent*-kaurane atractyligenin (**8**) (Scheme 2).




**8** **9** **10**

Scheme 2.

The reaction was performed with isopropenyl acetate as acylating agent and tetrahydrofuran as solvent (Table 3).

CAL B and PSL gave the best results at 33 °C and 55 °C, affording only one product. Its $^1$H and $^{13}$C NMR spectra (Table 6 and Table 8) showed downfield shifts of H-2β ($\delta$ = 5.27, dddd) and C-2 ($\delta$ = 69.9) and upfield shifts of C-1 ($\delta$ = 46.6) and C-3 ($\delta$ = 34.9) with respect to atractyligenin (**8**), allowing us to assign the structure of 2-acetyl-atractyligenin (**9**) to this compound.

Table 3. Lipase-catalysed acetylation of atractyligenin (**8**) and 4-*epi*-atractyligenin (**10**).

| Entry | Enzyme | Substrate | Solvent | *T* (°C) | Time (h) | Yield (%) |
|---|---|---|---|---|---|---|
| 1 | CRL | **8** | THF | 33 | 168 | – |
| 2 | CAL B | **8** | THF | 33 | 72 | 38 |
| 3 | PSL | **8** | THF | 33 | 72 | 12 |
| 4 | PPL | **8** | THF | 55 | 168 | – |
| 5 | PSL | **8** | THF | 55 | 48 | 23 |
| 6 | CAL B | **8** | THF | 55 | 48 | 57 |
| 7 | CAL B | **8** | $CH_3CN$ | 33 | 48 | 34 |
| 8 | CAL B | **8** | $CH_3CN$ | 55 | 48 | 46 |
| 9 | PSL | **10** | THF | 55 | 168 | – |
| 10 | CAL B | **10** | THF | 55 | 168 | – |
| 11 | CAL B | **10** | $CH_3CN$ | 55 | 168 | – |

CAL B showed high regioselectivity in this reaction, with the α-hydroxy group at carbon 15 remaining unaltered. No acetylation product was obtained when the carbon 4 epimer of atractyligenin – 4-*epi*-atractylgenin (**10**) – was treated with CAL B in the same solvent and acylating agent, even at 55 °C. This result shows that CAL B is also highly stereoselective in the acetylation of this type of substrates.[31]

### Reactions with Atractylitriol (11)

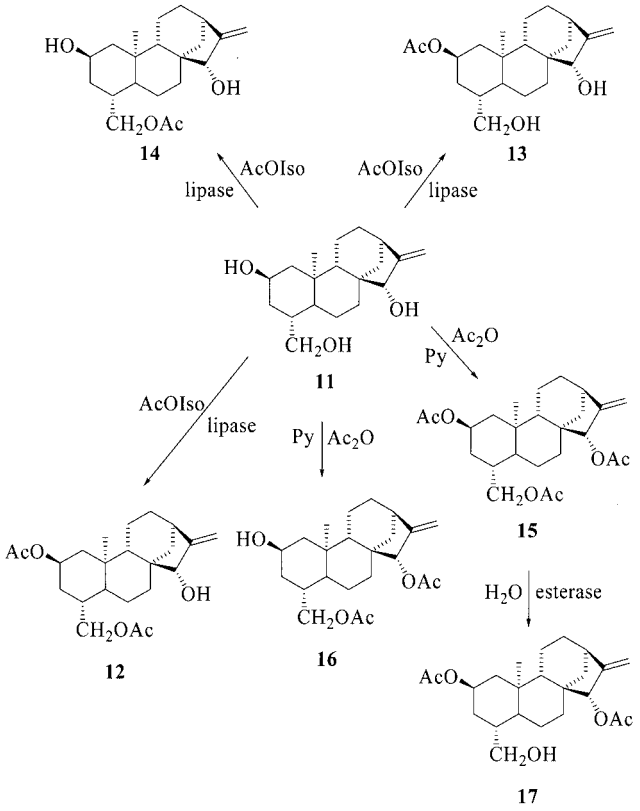
#### *Preparation and Enzymatic Acetylation*

To find out more about the catalytic activity of enzymes on this kind of substrate, we decided to synthesize atractylitriol (**11**), which contains three hydroxy groups. The synthetic route involves the reduction of the corresponding alkyl esters. As lipases are very efficient in esterification reactions,[26,32] we tested the enzymatic esterification of atractyligenin (**8**) and 4-*epi*-atractyligenin (**10**) with ethanol, butanol and octanol in the presence of CAL B. Unfortunately, we did not obtain any satisfactory result.

We then obtained atractylitriol (**11**) by the previously reported procedure.[33]

We attempted the enzymatic acetylation of **11** with isopropenyl acetate in THF. A mixture of acetyl derivatives (Scheme 3) was obtained in different proportions depending on the lipase used and the reaction conditions (Table 4).

Atractylitriol was soluble at concentrations appropriate for the enzymatic acetylation only in THF and ethanol. As ethanol would compete with the substrate, we chose THF. At 55 °C and after 24 hours of reaction, both CAL B and PSL had afforded the diacetyl derivative at carbons 2 and 19 (**12**) and a mixture of monoacetyl derivatives of atractylitriol in positions 2 (**13**) and 19 (**14**) (Entries 2 and 3). Only CAL A showed regioselectivity, and we were able to obtain **14** as the only product, but in low yield (Entry 1). At shorter periods of time – 4 hours reaction time with PSL, for example – the regioselectivity was no better. With the aim of improving regioselectivity we performed the reactions with the three enzymes at lower temperature – 33 °C




Scheme 3.

for 24 hours (Entries 5, 6 and 8) – but no reaction products were detected with CAL A under these conditions, while CAL B and PSL afforded the mixture of the isomers **12**, **13** and **14**. Finally, at shorter reaction times (4 hours), we were able to avoid the formation of diacetylated compound (**12**) with CAL B and PSL. A **13/14** ratio of about 1:1 was maintained under all reaction conditions. By TLC the **13/14** mixture seemed to be a single product, but it was possible to isolate them by column chromatography and they were identified by spectroscopic methods.

By comparison with the $^{1}H$ and $^{13}C$ NMR spectra (Table 6 and Table 8) of triacetyl derivative (**15**) (see below),

Table 4. Enzyme-catalysed acetylation of atractylitriol (**11**) and deacetylation of triacetyl-actractylitriol (**15**).

| Entry | Enzyme[a] | Substrate | *T* (°C) | Time (h) | Product yield (%) | | | |
|---|---|---|---|---|---|---|---|---|
| | | | | | **12** | **13** | **14** | **17** |
| 1 | CAL A | **11** | 55 | 24 | – | – | 12 | – |
| 2 | CAL B | **11** | 55 | 24 | 20 | 32 | 34 | – |
| 3 | PSL | **11** | 55 | 24 | 24 | 33 | 37 | – |
| 4 | PSL | **11** | 55 | 4 | 18 | 29 | 32 | – |
| 5 | CAL A | **11** | 33 | 24 | – | – | – | – |
| 6 | CAL B | **11** | 33 | 24 | 15 | 26 | 27 | – |
| 7 | CAL B | **11** | 33 | 4 | – | 41 | 44 | – |
| 8 | PSL | **11** | 33 | 24 | 22 | 23 | 25 | – |
| 9 | PSL | **11** | 33 | 4 | – | 43 | 48 | – |
| 10 | PLE[b,c] | **15** | 28 | 24 | – | – | – | – |
| 11 | PLE[d] | **15** | 28 | 24 | | | | 16 |
| 12 | PLE[e] | **15** | 28 | 2 | | | | 28 |
| 13 | PLE[e] | **15** | 28 | 24 | | | | 30 |

[a] Solvent: THF with lipases. [b] No product was obtained by lipase-catalysed alcoholysis of **15** at 55 °C over 168 h with CAL A, CAL B and PSL. [c] Solvent: acetone/water. [d] Solvent: phosphate buffer. [e] Solvent: acetone/phosphate buffer.

run in the same solvent, we were able to assign structures to compounds **12**–**14**. The $^1$H NMR spectrum of compound **12** showed an upfield shift of H-15 ($\delta$ = 3.83, br. s) and although the chemical shift of C-15 was not changed in the $^{13}$C NMR spectrum, an usual downfield shift (β effect) was observed for C-16 ($\delta$ = 160.0). Apart from the same shifts observed for compound **12**, the $^1$H NMR spectra of compounds **13** and **14** showed additional upfield shifts for Hs-19 ($\delta$ = 3.64, d) and for H-2 ($\delta$ = 3.97, dddd). The shifts observed in the $^{13}$C NMR spectra of compounds **13** and **14** (with respect to compound **15**) were in perfect agreement with the proposed structure.

### *Chemical Acetylation of Atractylitriol (11)*

By treatment of **11** with an excess of acetic anhydride and pyridine at room temperature, we prepared the triacetylatractylitriol **15**.

When **11** was treated with acetic anhydride in pyridine at low temperature (–30 °C) a mixture of derivatives was obtained. The main product was the 19-acetyl-atractylitriol (**14**), with minor quantities of **12**, **15** and of another diacetyl derivative (**16**), the structure of which was determined by analysis of spectroscopic data. The $^1$H NMR and $^{13}$C NMR spectra of compound **16** showed (with respect to compound **15**) upfield shifts of H-2 ($\delta$ = 3.96, ddd) and C-2 ($\delta$ = 64.2) and downfield shifts of C-1 ($\delta$ = 49.1) and C-3 ($\delta$ = 37.6).

### Enzymatic Deacetylation of Triacetyl-atractylitriol (15)

Lipase-catalysed alcoholysis of triacetyl-atractylitriol **(15)** with PSL, CAL A and CAL B was unsuccessful, but the hydrolysis of **15** in the 19-position with hog liver esterase (PLE) supported on Eupergit C® in phosphate buffer/acetone allowed us to obtain 2,15-diacetyl-atracytilitriol (**17**), as clearly indicated by the upfield shifts of Hs-19 ($\delta$ = 3.67, d) and C-19 ($\delta$ = 61.9) in the $^1$H and $^{13}$C NMR spectra with respect to the spectra of compound **15** (Table 6 and Table 8).

## Conclusions

This work describes application of enzymes to the preparation of specifically acylated derivatives of *ent*-kauranes. Lipases from different sources and hog liver esterase exhibited good performance as catalysts both in alcoholysis and acylation reactions. *Candida antartica* B lipase and *Pseudomnas sp.* lipase gave the best results in both reactions. By enzymatic acetylation and deacetylation reactions, various mono- and diacetylated derivatives of linearol, atractytligenin and atractylitriol have been regioselectively obtained; some of these products had not been reported earlier in the literature. Lipases have also catalysed acetyl migration in linearol to produce sidol.

Alcoholysis reactions afforded good results even in the presence of short-chain alcohols such as ethanol and allowed us to obtain complementary derivatives of some *ent*-kauranes. With regard to the current results showed by CAL B it could be assumed that, in the case of linearol and activated acylating agents, the active site of the enzyme acts in the same position of the *ent*-kaurane skeleton both in acylation and in alcoholysis reaction. Conventional acylating agents based on chemical methodology are not highly regioselective, and polyacylated compounds are usually obtained. These enzymatic reactions were always performed under mild conditions and allowed us to obtain a family of novel *ent*-kauranes with potential biological activity.

## Experimental Section

**General Remarks:** All solvents and reagents were reagent grade and were used without purification. Lipase from *Candida rugosa* CRL (905 U mg$^{-1}$ solid), and type II crude from porcine pancreas (190 U mg$^{-1}$ protein) were purchased from Sigma Chemical Co.; *Candida antarctica* lipase A: Chirazyme L-5, c.-f. lyo (400 U g$^{-1}$) was purchased from Roche Diagnostics GmbH; *Candida antarctica* lipase B: Novozym 435 CAL (7400 PLU g$^{-1}$) and Lipozyme RM (1 M, 7800 U g$^{-1}$) were generous gifts from Novozymes Latinoamerica Ltda and Novozymes A/S; *Pseudomonas* lipase: Lipase PS Amano PSL (33,200 U g$^{-1}$) was purchased from Amano Pharmaceutical Co. Hog liver esterase on Eupergit C® 450 U mg$^{-1}$ was supplied by Fluka. All enzymes were used "straight from the bottle".

**Enzymatic Reactions:** Enzymatic reactions were carried out with an Innova 4000 digital incubator shaker (New Brunswick Scientific Co.) at 33 °C and 55 °C and 200 rpm. Enzymatic transesterifications were followed by TLC on Merck silica gel 60F-254 aluminium sheets (0.2 mm thickness). For column chromatography, Merck silica gel 60 (70–230 mesh) was used. IR spectra were obtained on a Shimadzu FTIR-8300 spectrophotometer. $^1$H NMR and $^{13}$C NMR spectra were recorded at 250 and 500 MHz with Bruker AC 250 and Bruker AM 500 spectrometers, respectively. DEPT experiments were acquired on the same apparata. Chemical shifts are reported in $\delta$ units relative to tetramethylsilane (TMS) set at 0 $\delta$, and coupling constants are given in Hz. Solvents are indicated. EI-MS were obtained at 70 eV with TRIO-2 VG Masslab Shimadzu QP-5000 and Finnigan TSQ70 mass spectrometers, in *m*/*z* (%). Elemental analysis was carried out with a Perkin–Elmer 240 apparatus. Optical purities of products was determined by specific rotation with Perkin–Elmer 343 and Jasco P-1010 polarimeters. Solvents are indicated. GC-MS analyses were performed on a Shimadzu GCMS-QP5050A gas chromatograph–mass spectrometer, on an ULTRA-1 column (30 m × 0.25 mm × 0.25 μm). The following temperature program was employed: 80 °C (1 min)/15 °C min$^{-1}$/250 °C (1 min)/5 °C min$^{-1}$/270 °C (5 min).

**Isolation of Linearol (3) and Atractyligenin (8)**

**Linearol (3):** was isolated from several species of *Sideritis* (Lamiaceae) and the purification procedures have been previously reported (NMR: Tables 5 and 7.[19,20]

**Atractyligenin (8):** Isolated from *Atractylis gummifera* (Compositae) and the purification procedures have been previously reported (NMR: Tables 6 and 8).[33]

**4-*epi*-Atractyligenin (10) and Atractylitriol (11)**

The syntheses of 4-*epi*-atractyligenin (**10**) and atractylitriol (**11**) were performed by the previously reported procedure.[33]

**Atractylitriol (11):** M.p. 217 °C (from benzene/EtOH). $[\alpha]_D^{25}$ = –116.9 (*c* = 0.73, MeOH). $^1$H NMR ([D$_5$]pyridine, 250 MHz): see Table 6. $^{13}$C NMR ([D$_5$]pyridine, 62.7 MHz): see Table 8. IR (film)

$\tilde{\nu}_{max}$ = 3350, 1650, 1435, 1120, 1010, 903 $cm^{-1}$. EIMS: *m/z* (%) = $[M]^+$ (absent), 288 (33) $[M - H_2O]^+$, 273 (45), 257 (78), 239 (67), 189 (38), 132 (54), 105 (97), 91 (100). $C_{19}H_{30}O_3$ (306.44): calcd. C 74.47, H 9.87; found C 74.51, H 9.86.

**Enzymatic Acetylation of Linearol (3)**

Linearol (**3**, 40 mg, 0.11 mmol) was dissolved in the indicated solvent (3 mL). The acylating agent (0.25 mL) and an amount equiva-

Table 5. $^1$H NMR spectroscopic data of compounds **3**–**7** in $CDCl_3$.

| H | 3 | 4 | 5 | 6 | 7 |
|---|---|---|---|---|---|
| 3β | 3.58 dd | 4.91 dd | 4.83 dd | 4.74 dd | 3.32 dd |
| 7α | 3.56 t | 3.62 t | 3.60 t | 4.77 t | 4.79 t |
| 13 | 2.68 s | 2.68 br. s | 2.68 br. s | 2.69 br. s | 2.70 br. s |
| 15a, 15b | 2.27 br. s (2 H) | 2.27 br. s (2 H) | 2.26 br. s (2 H) | 2.17 br. d (15a-H)<br>2.13 br. d (15b-H) | 2.27 br. s (2 H) |
| 17a | 4.82 br. s | 4.82 br. s | 4.82 br. s | 4.78 s | 4.82 br. s |
| 17b | 4.79 br. s | 4.81 br. s | 4.80 br. s | 4.74 s | 4.77 br. s |
| 18a | 4.03 d | 3.31 d | 4.13 d | 3.92 d | 4.32 d |
| 18b | 3.92 d | 2.98 d | 3.70 d | 3.53 d | 3.56 d |
| Me-19 | 0.76 | 0.68 s | 0.85 s | 0.83 s | 0.74 s |
| Me-20 | 1.08 | 1.08 s | 1.09 s | 1.09 s | 1.08 s |
| Ac | 2.09 s | 2.07 s | 2.06 s | 2.02 s | 2.08 s |
| Ac | | | 2.03 s | 2.02 s | 2.06 s |
| Ac | | | | 2.00 s | |
| *J* (Hz) | | | | | |
| 2α,3β | 11.3 | 11.3 | 11.2 | 11.2 | 11.2 |
| 2β,3β | 5.1 | 5.1 | 5.2 | 5.2 | 5.2 |
| 6α,7β | 2.7 | 2.7 | 3.8 | 3.8 | 3.5 |
| 6β,7β | 2.7 | 2.7 | 3.8 | 3.8 | 3.5 |
| 15a,15b | – | – | – | 17.0 | – |
| 18a,18b | 11.3 | 12.3 | 11.6 | 11.8 | 12.0 |

Table 6. $^1$H NMR spectroscopic data of compounds **8**, **9** and **11**–**17**.

| H | 8[a] | 9[a] | 11[b] | 12[c] | 13[c] | 14[c] | 15[b] | 15[c] | 16[c] | 17[c] |
|---|---|---|---|---|---|---|---|---|---|---|
| 1α | 2.12 dd | 2.15 dd | 2.48 dd | 2.12 dd | 2.19 dd | 2.15 dd | 2.25 dd | 2.10 dd | 2.10 dd | 2.17 dd |
| 1β | 0.64 dd | 0.69 dd | 1.05 dd | 0.80 dd | 0.83 dd | 0.73 dd | 0.76 dd | 0.79 dd | 0.75 dd | 0.83 dd |
| 2β | 4.11 dddd | 5.27 dddd | 4.36 dddd | 5.07 dddd | 5.08 dddd | 3.97 dddd | 5.32 dddd | 5.03 dddd | 3.96 dddd | 5.06 dddd |
| 3α | 2.32 m | 2.25 m | 2.98 ddd | 2.20 ddd | 2.26 ddd | 2.24 ddd | 2.30 ddd | 2.17 ddd | 2.22 ddd | 2.25 ddd |
| 4β | 2.60 m | 2.61 m | 2.25 m | | | | | | | |
| 13 | 2.65 br. s | 2.61 m | 2.73 br. s | 2.76 br. s | 2.77 br. s | 2.77 br. s | 2.69 br. s | 2.77 br. s | 2.81 br. s | 2.80 br. s |
| 15 | 3.71 br. s | 3.66 br. s | 4.09 br. s | 3.83 br. s | 3.83 br. s | 3.83 br. s | 5.30 br. s | 5.08 br. s | 5.11 br. s | 5.10 br. s |
| 17a | 5.13 br. s | 5.09 br. s | 5.45 br. s | 5.23 br. s | 5.22 br. s | 5.22 br. s | 5.46 br. s | 5.24 br. s | 5.28 br. s | 5.27 br. s |
| 17b | 5.02 br. s | 4.98 br. s | 5.16 br. s | 5.10 br. s | 5.09 br. s | 5.09 br. s | 5.12 br. s | 5.09 br. s | 5.13 br. s | 5.12 br. s |
| 19a, 19b | | | 3.95 d (2H) | 4.19 dd (19a-H)<br>4.03 dd (19b-H) | 3.64 d (2H) | 4.08 d (2H) | 4.26 dd (19a-H)<br>4.18 dd (19b-H) | 4.13 dd (19a-H)<br>3.99 dd (19b-H) | 4.07 d (2H) | 3.67 d (2H) |
| 20 | 0.95 s | 0.96 s | 0.99 s | 1.05 s | 0.99 s | 0.99 s | 0.89 s | 1.01 s | 0.99 s | 0.99 s |
| Ac | | 1.89 s | | 2.06 s | 2.04 s | 2.07 s | 2.09 s | 2.04 s | 2.08 s | 2.07 s |
| Ac | | | | 2.04 s | | | 2.04 s | 2.02 s | 2.07 s | 2.03 s |
| Ac | | | | | | | 2.03 s | 2.00 s | | |
| *J* (Hz) | | | | | | | | | | |
| 1α,1β | 11.7 | 11.7 | 11.6 | 11.6 | 11.6 | 11.6 | 11.6 | 11.6 | 11.4 | 11.6 |
| 1α,2α | 1.5 | 1.5 | 1.5 | 1.5 | 1.5 | 1.5 | 1.5 | 1.5 | 1.5 | 1.5 |
| 1β,2α | 11.7 | 11.7 | 11.6 | 11.6 | 11.6 | 11.6 | 11.6 | 11.6 | 11.4 | 11.6 |
| 2α,3α | 1.5 | 1.5 | 1.5 | 1.5 | 1.5 | 1.5 | 1.5 | 1.5 | 1.5 | 1.5 |
| 2α,3β | 11.7 | 11.7 | 11.6 | 11.6 | 11.6 | 11.6 | 11.6 | 11.6 | 11.6 | 11.6 |
| 3α,3β | | | 11.0 | 11.0 | 11.0 | 11.0 | 11.0 | 11.0 | 11.0 | 11.0 |
| 3α,4β | | | 1.5 | 1.5 | 1.5 | 1.5 | 1.5 | 1.5 | 1.5 | 1.5 |
| 4β,19 | | | 6.6 | – | 7.0 | 7.2 | – | – | 7.3 | 7.3 |
| 4β,19a | | | – | 2.9 | – | – | 2.7 | 2.7 | – | |
| 4β,19b | | | – | 10.8 | – | – | 10.8 | 10.8 | – | |
| 19a,19b | | | – | 10.8 | – | – | 10.8 | 10.8 | – | |

[a] In $CD_3OD$ solution. [b] In $[D_5]$pyridine solution. [c] In $CDCl_3$ solution.

Table 7. $^{13}$CNMR spectroscopic data of compounds **3**–**7** in $CDCl_3$.

| C | 3 | 4 | 5 | 6 | 7 |
|---|---|---|---|---|---|
| 1 | 38.4 t | 38.5 t | 38.4 t | 37.7 t | 38.3 t |
| 2 | 26.5 t | 23.5 t | 23.0 t | 23.0 t | 25.8 t |
| 3 | 72.3 d | 74.6 d | 74.3 d | 73.8 d | 71.8 d |
| 4 | 41.9 s | 41.7 s | 40.5 s | 40.2 s | 42.0 s |
| 5 | 38.2 d | 37.4 d | 38.2 d | 39.6 d | 39.5 d |
| 6 | 27.4 t | 26.7 t | 27.1 t | 24.2 t | 24.4 t |
| 7 | 76.9 d | 76.9 d | 76.7 d | 79.2 d | 79.5 d |
| 8 | 48.0 s | 48.2 s | 48.0 s | 46.7 s | 46.7 s |
| 9 | 50.3 d | 50.3 d | 50.1 d | 50.8 d | 51.1 d |
| 10 | 38.8 s | 38.5 s | 38.7 s | 38.6 s | 38.7 s |
| 11 | 17.9 t | 17.9 t | 17.9 t | 18.0 t | 18.1 t |
| 12 | 33.6 t | 33.6 t | 33.5 t | 33.2 t | 33.3 t |
| 13 | 43.7 d | 43.7 d | 43.6 d | 43.4 d | 43.5 d |
| 14 | 38.3 t | 38.3 t | 37.8 t | 38.0 t | 38.1 t |
| 15 | 45.1 t | 45.0 t | 45.0 t | 44.9 t | 45.0 t |
| 16 | 155.0 s | 155.0 s | 154.8 s | 154.0 s | 154.1 s |
| 17 | 103.6 t | 103.7 t | 103.7 t | 103. t | 103.8 t |
| 18 | 66.1 t | 64.2 t | 64.6 t | 64.8 t | 66.3 t |
| 19 | 11.9 q | 12.8 q | 13.0 q | 12.8 q | 11.7 q |
| 20 | 18.0 q | 17.9 q | 17.9 q | 17.7 q | 17.7 q |
| Ac | 171.8 s | 171.9 s | 171.5 s | 170.6 s | 171.6 s |
| Ac | | | 171.1 s | 170.5 s | 170.3 s |
| Ac | | | | 170.2 s | |
| Ac | 21.2 q | 21.2 q | 21.2 q | 21.2 q | 21.3 q |
| Ac | | | 21.1 q | 21.1 q | 21.0 q |
| Ac | | | | 20.9 q | |

Table 8. $^{13}$C NMR spectroscopic data of compounds **8**, **9** and **11**–**17**.

| C | 8[a] | 9[a] | 11[b] | 12[c] | 13[c] | 14[c] | 15[b] | 15[c] | 16[c] | 17[c] |
|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 50.4 t | 46.6 t | 50.5 t | 45.1 t | 45.2 t | 49.2 t | 44.7 t | 44.9 t | 49.1 t | 45.1 t |
| 2 | 65.1 d | 69.9 d | 63.7 d | 67.9 d | 68.4 d | 64.2 d | 67.6 d | 67.6 d | 64.2 d | 68.1 d |
| 3 | 38.3 t | 34.9 t | 38.5 t | 33.6 t | 33.1 t | 37.6 t | 33.5 t | 33.4 t | 37.6 t | 33.2 t |
| 4 | 44.9 d | 44.8 d | 45.8 d | 40.0 d | 44.2 d | 40.3 d | 40.1 d | 39.8 d | 40.3 d | 44.1 d |
| 5 | 50.4 d | 50.2 d | 49.6 d | 48.7 d | 48.8 d | 48.6 d | 47.9 d | 48.2 d | 48.4 d | 48.6 d |
| 6 | 26.7 t | 26.6 t | 24.9 t | 24.0 t | 24.0 t | 24.0 t | 23.6 t | 23.7 t | 23.9 t | 24.0 t |
| 7 | 36.2 t | 36.1 t | 35.9 t | 36.2 t | 36.3 t | 36.3 t | 36.9 t | 37.1 t | 37.3 t | 37.2 t |
| 8 | 48.9 s | 48.9 s | 48.1 s | 47.5 s | 47.5 s | 47.5 s | 47.0 s | 47.1 s | 47.2 s | 47.2 s |
| 9 | 54.5 d | 54.3 d | 53.8 d | 52.9 d | 52.9 d | 52.9 d | 52.2 d | 52.3 d | 52.5 d | 52.5 d |
| 10 | 41.8 s | 41.9 s | 40.5 s | 40.1 s | 40.1 s | 40.1 s | 39.9 s | 40.0 s | 40.2 s | 40.2 s |
| 11 | 19.2 t | 19.2 t | 18.4 t | 18.0 t | 18.0 t | 18.0 t | 17.9 t | 18.0 t | 18.1 t | 18.2 t |
| 12 | 33.6 t | 33.5 t | 32.9 t | 32.3 t | 32.3 t | 32.4 t | 32.2 t | 32.2 t | 32.5 t | 32.4 t |
| 13 | 43.7 d | 43.6 d | 42.7 d | 42.4 d | 42.2 d | 42.2 t | 42.3 d | 42.2 d | 42.4 t | 42.4 t |
| 14 | 37.2 t | 37.2 t | 36.7 t | 34.6 t | 34.7 t | 34.7 t | 33.9 t | 34.0 t | 34.2 t | 34.2 t |
| 15 | 83.6 d | 83.5 d | 82.7 d | 82.7 d | 82.7 d | 82.7 d | 82.6 d | 82.8 d | 83.0 d | 83.0 d |
| 16 | 160.3 s | 160.2 s | 160.9 s | 160.0 s | 160.0 s | 160.0 s | 155.4 s | 154.6 s | 155.0 s | 155.0 s |
| 17 | 109.1 t | 109.1 t | 107.8 t | 108.6 t | 108.6 t | 108.6 t | 109.9 t | 110.2 t | 110.3 t | 110.3 t |
| 19 | 178.8 s | 178.6 s | 61.6 t | 64.0 t | 61.6 t | 64.4 t | 63.7 t | 63.9 t | 64.3 t | 61.9 t |
| 20 | 17.3 q | 17.0 q | 19.0 q | 18.1 q | 18.5 q | 18.8 q | 17.9 q | 18.4 q | 18.7 q | 18.5 q |
| Ac | | 172.4 s | | 171.1 s | 170.8 s | 171.3 s | 170.4 s | 171.0 s | 171.1 s | 171.0 s |
| Ac | | | | 170.5 s | | | 170.3 s | 170.9 s | 170.5 s | 170.7 s |
| Ac | | | | | | | 169.9 s | 170.4 s | | |
| Ac | | 21.3 q | | 21.4 q | 21.5 q | 21.1 q | 20.9 q | 21.3 q | 21.2 q | 21.5 q |
| Ac | | | | 21.1 q | | | 20.7 q | 21.2 q | 21.0 q | 21.3 q |
| Ac | | | | | | | 20.5 q | 20.9 q | | |

[a] In $CD_3OD$ solution. [b] In [$D_5$]pyridine solution. [c] In $CDCl_3$ solution.

lent to 1500 units of the indicated lipase were added. The suspension was shaken (200 rpm) at 33 °C or 55 °C and the progress of the reaction was monitored by TLC ($CH_2Cl_2$/AcOEt, 3:2). After the indicated time, the enzyme was filtered off, the solvent was evaporated, and the crude residue was purified by flash chromatography (eluent hexane/AcOEt), yielding sidol (**4**) and 3-acetyl-linearol (**5**) (see Table 1).

**Sidol (4):** The spectroscopic and physical data were in perfect agreement with those reported in the literature.[34] $^1$H NMR ($CDCl_3$, 500 MHz): see Table 5. $^{13}$C NMR ($CDCl_3$, 62.7 MHz): see Table 7.

**3-Acetyl-linearol (5):** Amorphous solid. $[\alpha]_D^{25}$ = –60.3 (*c* = 0.55, EtOH). $^1$H NMR ($CDCl_3$, 500 MHz): see Table 5. $^{13}$C NMR ($CDCl_3$, 62.7 MHz): see Table 7. IR (film) $\tilde{\nu}_{max}$ = 3442, 2936, 1721,

1658, 1459, 1425, 1367, 1235, 1185, 1019 $cm^{-1}$. EIMS: *m/z* (%) = 404 (1) $[M]^+$, 386 (1), 344 (1), 326 (19), 266 (28), 251 (12), 149 (28), 121 (38), 93 (32), 43 (100).$C_{24}H_{36}O_5$ (404.54): calcd. C 71.26, H 8.97; found C 71.30, H 8.93.

**Synthesis of Triacetyl-foliol (6)**

Linearol (**3**, 300 mg,0.83 mmol) was dissolved in a mixture (5 mL) of acetic anhydride and pyridine (1:1) and allowed to stand at room temperature for 24 h to give, after the usual workup, triacetyl-foliol (**6**, 340 mg, 92%).

**Triacetyl-foliol (6):** The spectroscopic and physical data were in perfect agreement with those reported in the literature.[20] $^1H$ NMR ($CDCl_3$, 500 MHz): see Table 5. $^{13}C$ NMR ($CDCl_3$, 62.7 MHz): see Table 7.

**Enzymatic Alcoholysis of Linearol (3) and Triacetyl-foliol (6):** An amount equivalent to 1500 units of the indicated lipase was added to a solution of compound **3** or **6** (40 mg) in the indicated solvent (10 mL) containing the indicated alcohol (5 mol equiv.). The suspension was shaken (200 rpm) at 33 °C or 55 °C and the progress of the reaction was monitored by TLC ($CH_2Cl_2$/AcOEt, 1:1). After the indicated time, the enzyme was filtered off, the solvent was evaporated, and the crude residue was purified by flash chromatography (eluent hexane/AcOEt), yielding foliol (**2**) and 7-acetyl-linearol (**7**) (see Table 2).

**Foliol (2):** The spectroscopic and physical data were in perfect agreement with those reported in the literature.[34]

**7-Acetyl-linearol (7):** Amorphous solid. $[\alpha]_D^{25}$ = –46.2 (*c* = 0.68, EtOH). $^1H$ NMR ($CDCl_3$, 500 MHz): see Table 5. $^{13}C$ NMR ($CDCl_3$, 62.7 MHz): see Table 7. IR (film) $\tilde{\nu}_{max}$ = 3423, 2934, 1718, 1657, 1462, 1425, 1368, 1230, 1190, 1020 $cm^{-1}$. EIMS: *m/z* (%) = 404 (1) $[M]^+$, 386 (2), 344 (2), 326 (28), 284 (9), 266 (38), 251 (18), 149 (30), 121 (35), 93 (37), 43 (100). $C_{24}H_{36}O_5$ (404.54): calcd. C 71.26, H 8.97; found C 71.33, H 8.94.

**Enzymatic Acetylation of Atractyligenin (8)**

As described for **3**.

**2-Acetyl-atractyligenin (9):** Amorphous solid. $[\alpha]_D^{25}$ = –53.3 (*c* = 0.80, EtOH). $^1H$ NMR ($CD_3OD$, 500 MHz): see Table 6. $^{13}C$ NMR ($CD_3OD$, 62.7 MHz): see Table 8. IR (film) $\tilde{\nu}_{max}$ = 3380, 3320, 2910, 1730, 1695, 1655, 1450, 1350, 1195, 1045, 990 $cm^{-1}$. EIMS: *m/z* (%) = 362 (1) $[M]^+$, 302 (23), 284 (10), 239 (3), 185 (5), 145 (10), 105 (22), 91 (35), 43 (100). $C_{21}H_{30}O_5$ (362.46): calcd. C 69.59, H 8.34; found C 69.53, H 8.37.

**Enzymatic Acetylation of Atractylitriol (11)**

As described for (**3**).

**2,19-Diacetyl-atractylitriol (12):** Amorphous solid. $[\alpha]_D^{25}$ = –29.7 (*c* = 0.21, EtOH). $^1H$ NMR ($CDCl_3$, 500 MHz): see Table 6. $^{13}C$ NMR ($CDCl_3$, 62.7 MHz): see Table 8. IR (film) $\tilde{\nu}_{max}$ = 3356, 2933, 1736, 1365, 1241, 1026, 911 $cm^{-1}$. EIMS: *m/z* (%) = $[M]^+$ (absent), 330 (10), 315 (8), 270 (26), 255 (19), 212 (17), 183 (6), 143 (13), 105 (24), 91 (33), 43 (100). $C_{23}H_{34}O_5$ (390.51): calcd. C 70.74, H 8.78; found C 70.70, H 8.82.

**2-Acetyl-atractylitriol (13):** Amorphous solid. $[\alpha]_D^{25}$ = +40.6 (*c* = 0.32, EtOH). $^1H$ NMR ($CDCl_3$, 500 MHz): see Table 6. $^{13}C$ NMR ($CDCl_3$, 62.7 MHz): see Table 8. IR (film) $\tilde{\nu}_{max}$ = 3352, 2933, 1732, 1360, 1243, 1026, 911 $cm^{-1}$. EIMS: *m/z* (%) = $[M]^+$ (absent), 330 (7) $[M - H_2O]^+$, 315 (5), 270 (8), 255 (5), 183 (3), 143 (7), 119 (11), 105 (9), 91 (26), 43 (100). $C_{21}H_{32}O_4$ (348.48): calcd. C 72.38, H 9.27; found C 72.34, H 9.24.

**19-Acetyl-atractylitriol (14):** Amorphous solid. $[\alpha]_D^{25}$ = –72.7 (*c* = 2.01, EtOH). $^1H$ NMR ($CDCl_3$, 500 MHz): see Table 6. $^{13}C$ NMR ($CDCl_3$, 62.7 MHz): see Table 8. IR (film) $\tilde{\nu}_{max}$ = 3350, 2930, 1735, 1358, 1243, 1026, 911 $cm^{-1}$. EIMS: *m/z* (%) = $[M]^+$ (absent), 330 (2) $[M - H_2O]^+$, 315 (1), 270 (10), 255 (6), 183 (5), 143 (11), 119 (16), 105 (8), 91 (31), 43 (100). $C_{21}H_{32}O_4$ (348.48): calcd. C 72.38, H 9.27; found C 72.40, H 9.30.

**Low Temperature Acetylation of Atractylitriol (11)**

Acetic anhydride (0.054 mL, 0.57 mmol) was added to a solution of atractylitriol (**11**, 35 mg, 0.11 mmol) in dry pyridine (3 mL) at *T* = –30 °C whilst stirring. After 1 h, the mixture was left standing at 0 °C for 3 h,ethyl acetate (10 mL) was then added,and the system was washed in turn with cold HCl solution (5%) and $H_2O$. The organic layer was separated, dried ($Na_2SO_4$), concentrated and chromatographed on a column (silica gel not deactivated, petroleum ether/ethyl acetate 4:1) to provide triacetyl-atractylitriol (**15**, 2 mg, 4%), 2,19-diacetyl-atractylitriol (**12**, 5 mg, 11%), 15,19-diacetyl-atractylitriol (**16**, 3 mg, 7%), 19-acetyl-atractylitriol (**14**, 23 mg, 58%) and starting material (**11**, 7 mg, 20%).

**Triacetyl-atractylitriol (15):** Amorphous solid. $[\alpha]_D^{25}$ = –19.7 (*c* = 1.44, $CHCl_3$). $^1H$ NMR ($CDCl_3$, 250 MHz): see Table 6. $^1H$ NMR ([$D_5$]pyridine, 250 MHz): see Table 6. $^{13}C$ NMR ($CDCl_3$, 62.7 MHz): see Table 8. $^{13}C$ NMR ([$D_5$]pyridine, 62.7 MHz): see Table 8. IR (film) $\tilde{\nu}_{max}$ = 2931, 1732, 1369, 1238, 1028, 908 $cm^{-1}$. EIMS: *m/z* (%) = $[M]^+$ (absent), 372 (7) $[M - AcOH]^+$, 357 (2), 330 (7), 312 (18), 297 (12), 270 (14), 252 (34), 237 (27), 195 (10), 105 (18), 91 (22), 43 (100). $C_{25}H_{36}O_6$ (432.55): calcd. C 69.42, H 8.39; found C 69.45, H 8.41.

**15,19-Diacetyl-atractylitriol (16):** Amorphous solid. $[\alpha]_D^{25}$ = –27.6 (*c* = 0.24, $CHCl_3$). $^1H$ NMR ($CDCl_3$, 250 MHz): see Table 6. $^{13}C$ NMR ($CDCl_3$, 62.7 MHz): see Table 8. IR (film) $\tilde{\nu}_{max}$ = 3353, 2930, 1732, 1360, 1243, 1020, 908 $cm^{-1}$. EIMS: *m/z* (%) = $[M]^+$ (absent), 375 (1), 330 (3), 312 (11), 270 (14), 255 (14), 252 (29), 237 (25), 195 (9), 143 (12), 119 (10), 105 (17), 91 (29), 43 (100). $C_{23}H_{34}O_5$ (390.51): calcd. C 70.74, H 8.78; found C 70.78, H 8.75.

**Synthesis of Triacetyl-atractylitriol (15)**

Atractylitriol (**11**, 300 mg,0.98 mmol) was dissolved in a mixture (5 mL) of acetic anhydride and pyridine (1:1) and allowed to stand at room temperature for 24 h to give, after the usual workup, triacetyl-atractylitriol (**15**, 395 mg, 93%).

**Preparation of 2,15-Diacetyl-atractylitriol (17)**

Esterase from hog liver (160 mg, immobilized on Eupergit C® 450 U $g^{-1}$) was added to a solution of triacetyl derivatives (**15**, 80 mg, 0.18 mmol) in acetone (0.2 mL)and phosphate buffer (pH = 8.0, 2 mL, 20 mM). The mixture was stirred at 28 °C for 2 h and $H_2O$ (10 mL) was then added. The mixture was extracted three times with $CH_2Cl_2$ (10 mL). The combined organic layers were dried ($Na_2SO_4$), concentrated and chromatographed by CC (silica gel, petroleum ether/ethyl acetate 1:1) to give **17** (20 mg, 28%).

**2,15-Diacetyl-atractylitriol (17):** Amorphous solid. $[\alpha]_D^{25}$ = +11.0 (*c* = 0.25, EtOH). $^1H$ NMR ($CDCl_3$, 250 MHz): see Table 6. $^{13}C$ NMR ($CDCl_3$, 62.7 MHz): see Table 8. IR (film) $\tilde{\nu}_{max}$ = 3352, 2935, 1734, 1361, 1248, 1031, 907 $cm^{-1}$. EIMS: *m/z* (%) = $[M]^+$ (absent), 375 (1), 372 (4), 330 (6), 312 (15), 255 (9), 252 (25), 237 (12), 195 (14), 143 (11), 119 (15), 105 (11), 43 (100). $C_{23}H_{34}O_5$ (390.51): calcd. C 70.74, H 8.78; found C 70.71, H 8.81.

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