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Chemistry of Larixol I- Degradation of the Side-Chain and Microbial Hydroxylation

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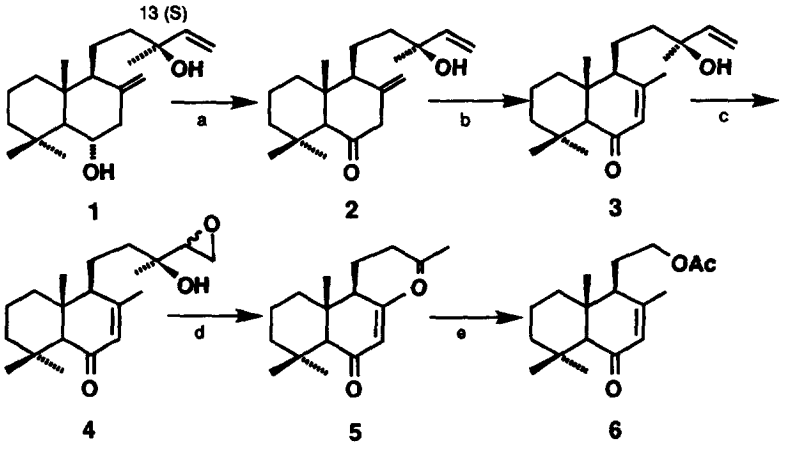
Abstract: Degradation of the side-chain of larixol **1**, isolated from the turpentine oil of *Larix sp.*, led to ester **6**, useful intermediate for the synthesis of polyhydroxylated labdane diterpenes. Microbial hydroxylation of larixol and derivatives led to 2α-hydroxylated compounds which could be used for the hemisynthesis of forskolin type compounds.

In previous papers, we reported the total syntheses of racemic polyoxygenated diterpenes, galanolactone,[1] crotomachlin,[2] possessing attractive biological activities. Hence, we needed a more efficient access to these compounds, in enantiomerically pure form, for pharmacological evaluation. Hemisynthesis from an easily available precursor might be a solution to this problem.

Due to its structure and its availability, larixol **1** looks to be an excellent candidate as a starting material for this purpose. It is a diterpene of the labdane series, which has been isolated, as its 6-acetate, from the turpentine oil of *Larix deciduaj, L. europea and L. sibirica*.[3] Its structure has been determined by Norin et al.[4] and the absolute configuration of the side-chain was defined as 13-(*S*).[5]

In this paper, we report a degradative study of the side-chain of larixol to obtain chiral intermediates for the synthesis of various diterpenes. The microbial hydroxylation of the A ring of larixol or derivatives is also described, leading to compounds potentially useful for the synthesis of forskolin[6] or related compounds.

Oxidation of larixol with Dess-Martin periodinane[7] led to ketone **2** which was transformed into the conjugated ketone **3** with methanolic sodium methoxide (93 % for two steps). The 14-15 double bond was selectively epoxidized with t-butyl hydroperoxide in the presence of $VO(acac)_2$ according to Sharpless' procedure.[8] A mixture of diastereomeric epoxides **4** was obtained (93 %) in a 7/3 ratio.[9] Epoxidation with mCPBA furnished a 1/1 mixture of diastereomers and some epoxidation of the 7-8 double bond occurred. Periodic acid oxidation of epoxides **4** gave diketone **5**[10] in 60 % yield. This diketone submitted to regioselective Baeyer-Villiger oxidation (mCPBA, 1.5 eq., $BF_3.OEt_2$ 1.5 eq., rt, 48 h) furnished the acetate **6**[11] in a moderate 30% yield, besides unreacted starting material (ca. 50%) (scheme 1). Various conditions were tried (CF_3CO_3H, mCPBA, mCPBA in the presence of CF_3CO_2H[12]) without improvement of this yield. The ester **6** is an important intermediate for the total synthesis of polyoxygenated diterpenes. We had prepared the corresponding t-butyldimethylsilyl ether, in racemic form, from β-ionone for the total synthesis of crotomachlin and 8-epi-crotomachlin.[2]



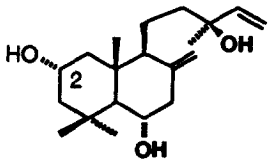
Scheme 1

a) Dess-Martin periodinane, 1 eq. CH_2Cl_2, 1h. rt, then ether, aqueous NaOH, 1h, 95 % ; b) 1N methanolic NaOMe, 1h. rt 98 % ; c) t-BuOOH, $VO(acac)_2$, lutidine, 38°C, 12h, 93 % ; d) IO_5H_6, 1 eq., THF, H_2O, rt, 3h, 60 % ; e) mCPBA, 1.5eq., $BF_3.OEt_2$ 1.5 eq., CH_2Cl_2, 48h, 30%.

Highly selective biotransformations of readily available natural compounds are of considerable value for the partial syntheses of bio-active compounds. Hydroxylations are well-known examples of these transformations and many papers reported such hydroxylations of diterpenes by fungi.[13] The presence of a hydroxyl at C(1) in forskolin, led many authors to study microbial hydroxylation of related compounds either to transform inactive diterpenes from *Coleus forskolii* into forskolin[14] or to have access to synthetic chiral intermediates.[15] Larixol derivatives, already functionalized on carbons 6, 7 and 8 of the B ring, might provide interesting derivatives for the hemisynthesis of forskolin or analogs after appropriate functionalization of the A ring.

Mucor plumbeus LCM was selected in our laboratory as a high activity hydroxylating fungus. Hydroxylation of the 3β position of sclareol by a strain of *Mucor plumbeus* has been recently published.[16] We studied the microbial oxidation of larixol **1** and derived compounds, ketones **3**, **4** and **5**, by this microorganism.[15] The major products we obtained from **1**, **3** and **5**, resulted in the introduction of a 2α-hydroxyl. The signal of the 2β-H in the ^1H nmr spectra of compounds **7** (60 % yield),[17] **8** (37 % yield)[18] and **9** (35 % yield)[19] as triplet of triplet (J=12 Hz, J'=4 Hz) led unambigously to this assignment, this position being the only one giving a proton with this multiplicity, two axial-axial coupling constants and two axial-equatorial coupling constants. No other transformation products could be characterized. Regioselective hydroxylations at C(2), 2α and/or 2β, have been described with various deoxy-forskolin derivatives in 2 up 24 % yield according the microorganisms which have been used.[14] The yields of the biotransformations we described herein are quite higher. With our strain of *Mucor plumbeus*, sclareol gave as a major product the 3-ketone **10** (47 % yield), the structure of which was demonstrated by $NaBH_4$ reduction to 3β-hydroxy derivative previously described[16] and the 6α and 18-hydroxyl compounds (1/1 mixture, 48 % yield).[15] Under similar conditions, the keto-epoxides **4**

led to the 14,15-dihydroxy derivatives **11** (50 % yield), in the same ratio (7/3) as in the starting mixture of epoxides, without further transformation.



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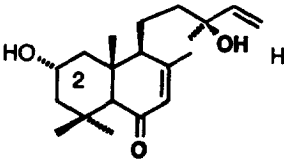

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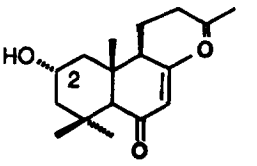

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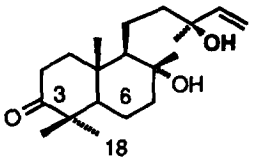

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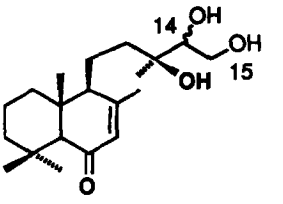

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In conclusion, in this preliminary communication, we presented results showing the potential interest of larixol for the hemisynthesis of various labdane diterpenes. Applications in this area will be published subsequently.

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9 The 14(*R*) configuration was attributed to the major isomer. Epoxidation in the presence of either D or L-diethyltartrate did not improve the diastereoselection. Details of these results will be published in a next paper.

10 5- crystals, mp 59°C (heptane), $[\alpha]_D$ = +43 ($CHCl_3$, c = 1.15), Anal. $C_{18}H_{28}O_2$, calc. % C 78.21, H 10.21, O 11.58 ; found % C 78.04, H 10.33, O 11.33 ; EIMS : M^+ 276, m/z 261, 219 ; IR ν cm^{-1} : 1715 and 1669 (C=O), 1629 (C=C) ; ^1H NMR, $CDCl_3$, 250 MHz, δ ppm : 0.85 (3H, s, CH_3), 1.11 (3H, s,

CH_3), 1.15 (3H, s, CH_3), 1.89 (3H, s, C-17H_3), 2.04 (1H, s, C-5H), 2.16 (3H, s, $COCH_3$), 2.52 and 2.70 (2H, 2m, C-12H_2), 5.77 (1H, broad s, C-7H) ; ^{13}C NMR $CDCl_3$, δ ppm : 14.46 (CH_3), 17.99 (CH_2), 20.43 (CH_2), 21.36 (CH_3), 21.93 (CH_3), 29.82 (CH_3), 32.11 (C), 33.30 (CH_3), 38.74 (CH_2), 42.95 (CH_2), 43.16 (C), 45.13 (CH_2), 55.44 (C-9H), 62.41 (C-5H), 128.70 (C-7H), 157.55 (C-8), 199.55 (C-6, C=O), 207.31 (C-13, C=O).

11 **6**- oil, $[\alpha]_D$ = +15 ($CHCl_3$, c = 1), HR EIMS : M^+ 292.2017 (292.2038 for $C_{18}H_{28}O_3$), m/z 277, 232 ; ^1H NMR, $CDCl_3$, 250 MHz, δ ppm : 0.80 (3H, s, CH_3), 1.10 (3H, s, CH_3), 1.11 (3H, s, CH_3), 1.88 (3H, s, C-17H_3), 2.03 (3H, s, $COCH_3$), 2.11 (1H, s, C-5H), 4.05 and 4.23 (2H, 2m, C-12H_2), 5.73 (1H, broad s, C-7H) ; ^{13}C NMR $CDCl_3$, δ ppm : 14.48 (CH_3), 18.03 (CH_2), 20.86 (CH_3), 21.40 (CH_3), 21.93 (CH_3), 26.04 (CH_2), 32.16 (C), 33.32 (CH_3), 38.59 (CH_2), 42.72 (C), 42.89 (CH_2), 52.33 (C-9H), 63.30 (C-5H), 84.82 (C-12H_2), 128.81 (C-7H), 157.17 (C-8), 170.72 (C=O ester), 199.50 (C-6, C=O).

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16 The microorganism was maintened on agar slants (Diagnostic Pasteur, Paris). Spores of *mucor plumbeus* were used to inoculate the liquid medium containing KH_2PO_4 1 g, K_2HPO_4 0.5 g, corn steep liquor 10 g , $MgSO_4$ 0.5 g, $NaNO_3$ 2 g,KCl 0.5 g, $FeSO_4$ 0.02 g and glucose 30 g per liter of distillated water. Cultures were grown for three days in erlenmeyer flasks at 27°C on a rotatory shaker (200 rpm). The biomass was then recovered by filtration and reincubated in the presence of the substrate (product concentration was 100 mg per liter; biomass concentration was 36 g wet weight per liter). Samples of the incubation medium were analized by TLC and the reaction stopped by removing the biomass and extracting the medium with methylene chloride or ethyl acetate.

17 **7**- mp 154°C (acetone), $[\alpha]_D$ = +64 ($CHCl_3$, c = 1.06), Anal. $C_{20}H_{34}O_3$, calc;% C 74.49, H 10.63, O 14.88; found % C 74.23, H 10.46 O 15.05 ; CIMS : MH^+ 323, m/z 305, 287, 269 ; ^1H NMR $CDCl_3$, 250 MHz, δ ppm : 0.73 (3H, s, CH_3), 1.06 (3H, s, CH_3), 1.24 (3H, s, CH_3), 1.28 (3H, s, C-16H_3), 2.10 (1H, t, J=12 C-7Hax), 2.67 (1H, dd, J=12, J'=4, C-7Heq), 3.81 (1H, td, J=12, J'=4, C-6H), 3.85 (1H, tt, J=12, J'=4, C-2βH), 4.64 (1H, s, C-17H), 4.96 (1H, s, C-17H), 5.10 (1H, d, J=10, C-15H*cis*), 5.22 (1H, d, J=17, C-15H*trans*) 5.93 (1H, dd, J=10, J'=17, C-14H) ; ^{13}C NMR, CD_3OD, δ ppm : 17.03 (CH_3), 19.19 (CH_2), 19.15 (CH_2), 22.35 (CH_3), 27.51 (CH_3), 36.46 (C), 37.36 (CH_3), 41.83 (C), 42.33 (CH_2), 43.80 (CH_2), 49.17 (CH_2), 57.64 (CH), 60.70 (CH), 65.40 (C-2H), 71.50 (C-6H), 73.49 (C-13), 109.20 (CH_2), 111.96 (CH_2), 146.24 (C-15H), 146.64 (C-8).

18 **8**- oil, $[\alpha]_D$ = +40 ($CHCl_3$, c = 0.93),Anal. $C_{20}H_{32}O_3$, calc.% C 74.96, H 10.06 ; found % C 74.74, H 9.86.; CIMS : MH^+ 321, 303; ^1H NMR, $CDCl_3$, 250 MHz, δ ppm : 0.89 (3H, s, CH_3), 1.19 (3H, s, CH_3), 1.21 (3H, s, CH_3), 1.32 (3H, s, C-16H_3), 1.94 (3H, s, C-17H_3), 2.05 (1H, s, C-5H), 2.08 (1H, m, C-9H), 2.10 (1H, m, H of CH_2), 3.90 (1H, tt, J=12, J'=4, C-2βH), 5.12 (1H, dd, J=10 J'=1, C-15H*cis*), 5.25 (1H, dd, J=17, J'=1, C-15H*trans*), 5.77 (1H, broad s, C-7H), 5.91 (1H, dd, J=17, J'=10, C-14H) ; ^{13}C NMR $CDCl_3$, δ ppm : 15.78 (CH_3), 21.68 (CH_2), 22.31 (CH_3), 22.73 (CH_3), 27.79 (CH_3), 33.69 (CH_3), 34.39 (C), 44.66 (CH_2), 45.02 (C), 47.68 (CH_2), 52.30 (CH_2), 56.79 (C-9H), 62.91 (C-5H), 64.26 (C-2H), 73.47 (C-13), 112.47 (C-15H_2), 128.70 (C-7H),145.09 (C-14H), 159.59 (C-8), 199.82 (C=O).

19 **9**- crystals, mp 115°C (AcOEt-heptane), $[\alpha]_D$ = +31 ($CHCl_3$, c = 0.63) ; Anal. $C_{18}H_{28}O_3$, 0.5 H_2O calc. % C 71.72, H 9.69, O 18.58 ; found % C 71.92, H 9.46, O 18,48 ; IR ν^{cm-1} : 3500 (OH), 1709 and 1668 (C=O), 1629 (C=C) ; CIMS : MH^+ 293, m/z 275 ; ^1H NMR, $CDCl_3$, 300 MHz, δ ppm : 0.91 (3H, s, CH_3), 1.18 (3H, s, CH_3), 1.21 (3H, s, CH_3), 1.92 (3H, s, C-17H_3), 2.04 (1H, s, C-5H), 2.18 (3H, s, $COCH_3$), 2.62 (2H, m, C-12H_2), 3.91 (1H, tt, J = 12, J'= 4, C2β-H), 5.78 (1H, broad s, C-7H) ; ^{13}C NMR $CDCl_3$, δ ppm : 15.93 (CH_3), 21.06 (CH_2), 22.47 (C-17H_3), 22.88 ($COCH_3$), 30.43 (CH_3), 33.80 (CH_3), 34.58 (C), 45.13 (C), 45.64 (C-12H_2), 48.24.(CH_2), 52.46 (CH_2), 56.01 (C-9H), 63.11 (C-5H), 64.49 (C-2H), 129.47.(C-7H), 158.21 (C-8), 199.49 (C-6, C=O), 207.95 (C-13, C=O).

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