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SPIROFORSKOLIN: STRUCTURE AND MODE OF FORMATION

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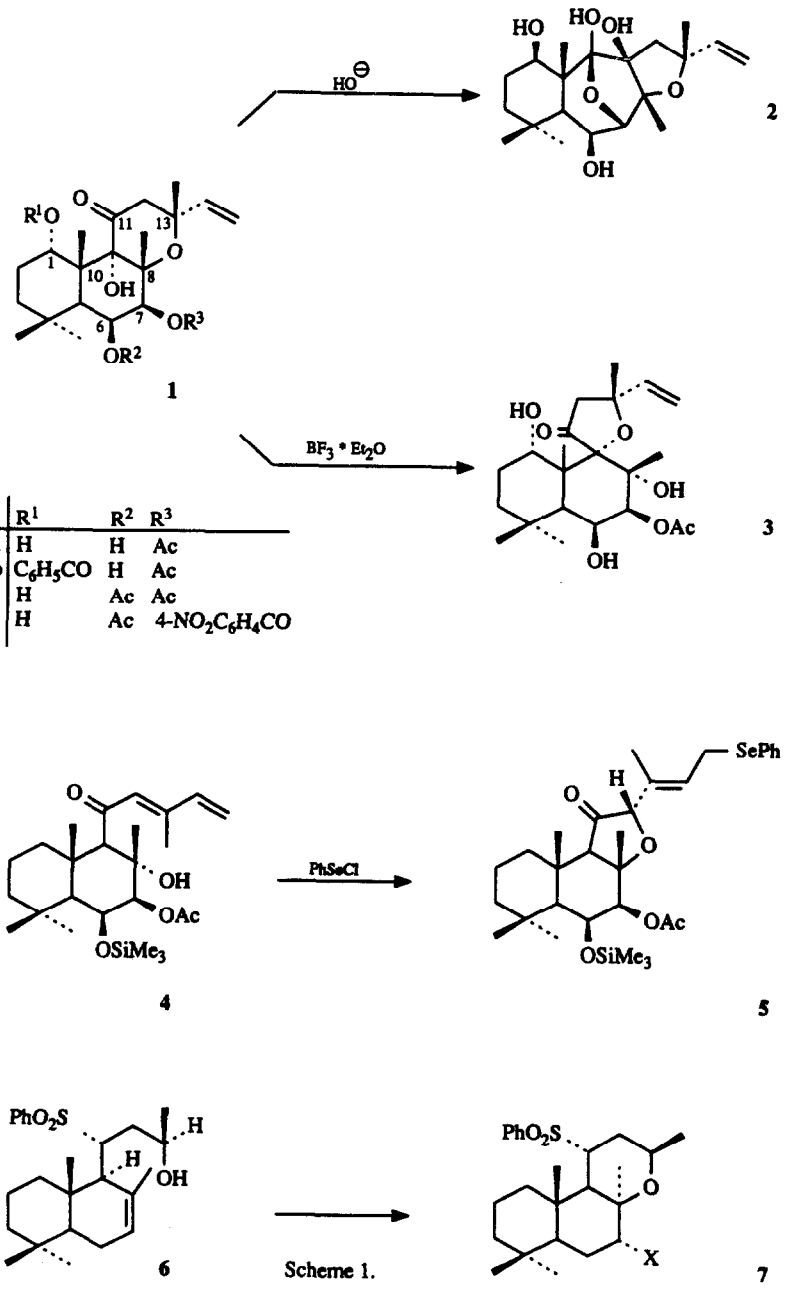
Abstract - Forskolin (**1a**) rearranges BF_3-mediated to the spiro compounds **12a** and **13**. It is assumed that **12a** and **13** are formed via cationic intermediate **18**.

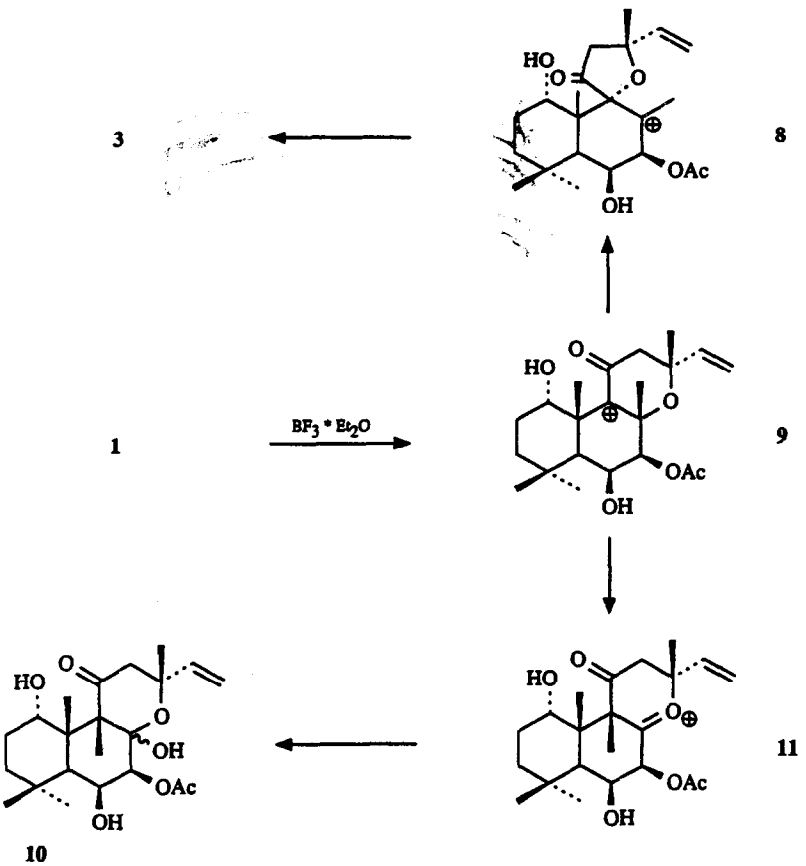
Introduction

The unexpected and highly interesting direct stimulation of the enzyme adenylate cyclase by forskolin (**1a**)[1] and the rather complex structure of this labdane diterpene have provoked great efforts directed at its synthesis.[2] In particular, for the construction of the AB part a multitude of elegant solutions have been invented. Less studies have been devoted to building up ring C but, nonetheless, three total synthesis, each with its own method of ring C formation have been published.[2] One may, therefore, arrive at the conclusion that forskolin ring C formation does not pose any special problems. This generalization would, however, be unjustified. Under a number of different conditions the system has been found to escape the pyranoid ring C or the correct configuration at C-8 (Scheme 1). Thus, base treatment of forskolin induced an α-ketol rearrangement which finally led to hemiacetal **2**.[3] On BF_3-mediated rearrangement forskolin was reported to give spiroforskolin (**3**).[4] Selenium-mediated cyclization of diene **4** furnished **5** rather than the desired derivative of 1,9-dideoxy forskolin.[5] Finally, both mercuric ion- and selenium-induced cyclization of bis-nor labdane derivative **6** led to the formation of **7** with the α-configuration of the methyl group at C-8. Related results were obtained on cyclizations of a diastereoisomer of **6**.[6] We have found other examples which will be reported in due course. In the present publication we wish to detail a study which is concerned with the rearrangement of forskolin (**1a**) to give **3**. Key steps of the mechanism as reported by Vishwakarma[4] (Scheme 2) are the formation of α-oxo carbocation **9** and migration of the ether bridge from the 8 to the 9 position to yield cation **8**. Vishwakarma's main structural arguments in favor of structure **3** were shifting of the IR CO absorption (KBr) from 1700 (in forskolin) to 1740 cm^{-1} and the appearance of a ^{13}C NMR


HO⊖
HO
HO
OH
O
O
OH
2
R¹O
O
11
13
1
O
10
OH
8
7
OR³
6
OR²
1
BF₃ • Et₂O
HO
O
O
OH
OAc
OH
3
1 | R¹ | R² | R³
a | H | H | Ac
b | C₆H₅CO | H | Ac
c | H | Ac | Ac
d | H | Ac | 4-NO₂C₆H₄CO
O
OH
OAc
OSiMe₃
4
PhSeCl
SePh
H
O
O
OAc
OSiMe₃
5
PhO₂S
H
H
OH
6
Scheme 1.
PhO₂S
O
X
7

signal at δ = 93.4 which, by comparison with griseofulvine, was taken as evidence for the presence of a spiro ether at C-9.

The paper of Vishwakarma contains a number of surprising assumptions. Thus, although α-oxo carbocations are well-established entities, their formation is usually markedly retarded when compared with ordinary carbocations.[7] One may ask, therefore, why the formation of ion **9** is preferred with regard to competing reaction paths. From a stereochemical point of view it is rather astonishing that all reactions (departure of the 9-OH group, migration of the ether bridge, and return of the hydroxyl group to C-8) proceed at the α-side of the molecule. The last steps could involve neighboring group participation of the acetoxy group. An alternative mechanism, in which removal of the 9α-OH group is accompanied



Scheme 2.

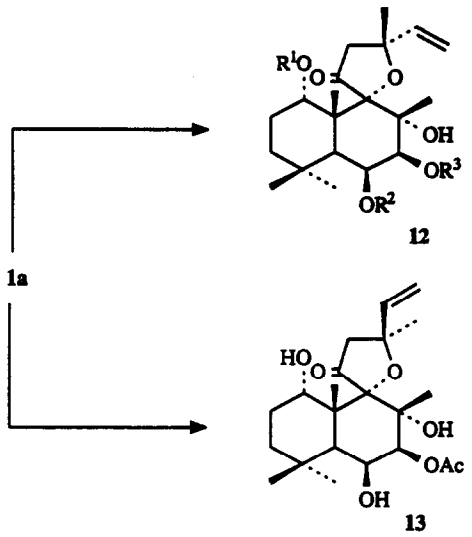
by migration of the methyl group from C-8 to C-9 would be stereochemically more reasonable and would lead to the very stable carbocation **11**. Trapping of this carbocation might give a hemiacetal such as **10** and this would probably also exhibit a ^{13}C NMR signal in the δ = 93 region. Furthermore, the reported IR argument (spectrum in KBr) is possibly not of too much significance. Forskolin itself exhibits only one band at 1700 cm^{-1} (in KBr) for the ester and the keto group.[8] In spiroforskolin the band at 1740 cm^{-1} is, of course, also a sum band.

In view of these ambiguities we decided to reinvestigate the BF_3-mediated rearrangement of forskolin.

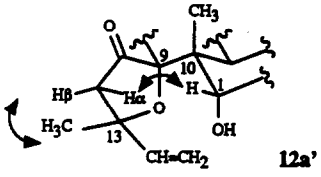
Results

The rearrangement of forskolin was performed by Vishwakarna[4] in dry benzene at 0 - 5°C. The yield of spiroforskolin was 45%. Polar forskolin has only a very limited solubility in benzene. On the other hand it is soluble in CH_2Cl_2. We thus performed, in addition to the heterogeneous reaction in benzene, a rearrangement in CH_2Cl_2 solution (at -10°C, homogeneous). Besides a rather polar compound (not further investigated) the formation of two products (both in benzene and in CH_2Cl_2 in a 6:1 ratio) with very similar ^{13}C and ^1H NMR spectra and almost identical R_f values was observed. The formation of two closely related products was originally taken as evidence for the hemiacetal hypothesis (see **1a** --> --> **10**). However, the findings that the two compounds could be separated by careful chromatography and could not be equilibrated with BF_3 ruled out the hemiacetal structures. All observations that will be presented below point to structures **12a** (= **3**) and **13** for the main and the minor rearrangement product, respectively.

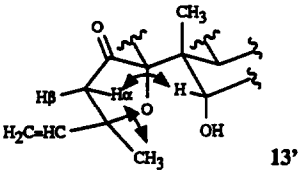
In contrast to the KBr spectrum, in $CHCl_3$ solution the IR spectrum of forskolin showed overlapping CO bands at 1710 (ketone) and 1740 cm^{-1} (ester). Both **12a** and **13** displayed a single CO band at 1740 cm^{-1} (ketone and ester) in agreement with the formation of a five-membered ring ketone. The NMR spectra of **12a** and **13** have been assigned by means of one- and two-dimensional techniques (see Experimental). Many of the signals in the ^1H NMR spectra were broad. The line-broadening was caused by a conformational change with a coalescence temperature close to 298K. Routinely, the spectra were therefore recorded at 320K where the exchange was fast on the NMR time scale. Recently, Kogler and Fehlhaber[9] have reinterpretated the NMR spectra of forskolin on the basis of a very careful analysis. Most of the ^{13}C and ^1H NMR features of **12a** and **13** correspond to those of forskolin. There are, however, characteristic differences. The ^{13}C



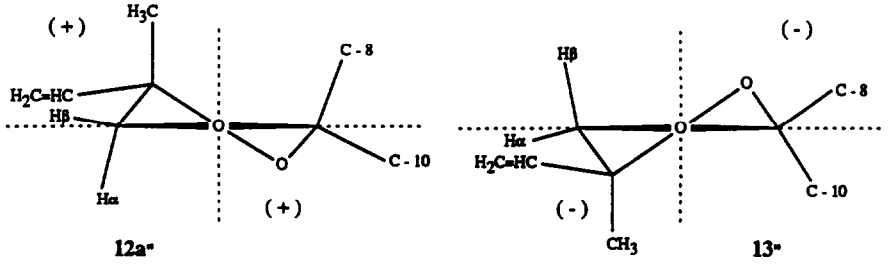
12	R^1	R^2	R^3
a=3	H	H	Ac
b	4-$NO_2C_6H_4CO$	H	Ac
c	3,5-$(NO_2)_2C_6H_3CO$	Ac	H
d	3,5-$(NO_2)_2C_6H_3CO$	H	Ac
e	3,5-$(NO_2)_2C_6H_3CO$	Ac	Ac
f	Ac	Ac	Ac



Hα : δ = 2.62
Hβ : δ = 3.01



Hα : δ = 2.62
Hβ : δ = 2.96

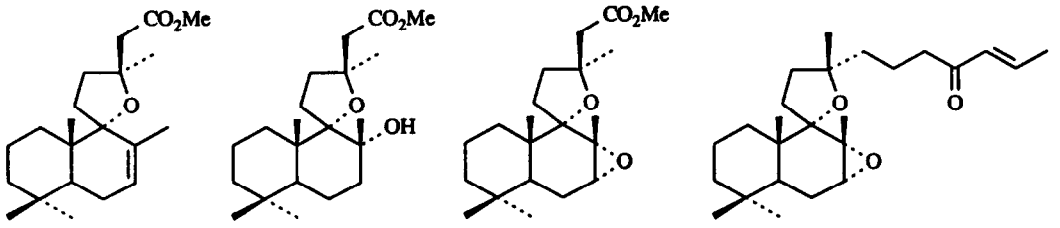


Scheme 3.

resonance of the C-11 carbon is shifted from δ = 205 (in **1a**) to δ = 211 (in **12a** and **13**). The same chemical shift difference is found when the CO signals of ordinary cyclohexanones and cyclopentanones are compared.[10] The C-9 signal of forskolin appears at δ = 82.65.[9] The shift of this signal to δ = 93.5 in **12a** (δ = 94.6 in **13**) indicated ether formation at C-9 (β effect). The 9,13-epoxy structure is found in a number of naturally occurring labdane derivatives such as grindelic acid (**14**) and erigerol (**17**). As Table 1 shows, the chemical shift of C-9 in all these compounds is in the δ = 90 region. The assignment of the C-13 chemical shift in **12a** rests on comparison with compounds **14** - **17** (see Table 1).

Table 1. Selected ^{13}C chemical shifts of forskolin, **12a** and some 9,13-epoxy labdane derivatives

	C-7	C-8	C-9	C-13.
1a[9]	76.58	81.41	82.65	75.01
12a	78.3	76.1	93.5	80.6
14[11]	126.5	134.8	90.5	81.5
15[11]	39.9	73.4	97.9	85.2
16[11]	61.5	59.0	88.3	81.4
17[12]	61.0-64.4	61.0-64.4	90.1	84.9



14 **15** **16** **17**

Scheme 4.

Highly informative is the large difference in the 1-H chemical shifts of **12a** (δ = 3.53) and forskolin (δ = 4.54). Models show that 1-H is in the shielding area of the 11-CO group in **12a** whereas it is probably deshielded in forskolin.[13] The 8-CH_3 chemical shift is also influenced by the anisotropy effect of the 11-carbonyl group (δ = 1.70 in forskolin and δ = 1.58 in **12a**). The minor isomer **13** must be very similar in structure to **12a**, since it exhibits almost identical spectroscopic properties. NOE experiments revealed that **12a** and **13** differ in their configuration at C-13. In **12a** NOE's between 1-H and 12α-H and 12β-H and 13-CH_3 show that the methyl group at C-13 is β-oriented (see Scheme 3, **12a'**). When the same NOE expe-

riments were performed with **13** NOE's between 1ß-H and 12α-H and 12α-H and the 13-CH_3 group are observed (Scheme 3, **13'**).

For many chiral cyclopentanones the sign of the Cotton effect in the n --> π* band is determined by the helicity of the ring.[14] If this also holds for **12a** the observed positive CD ($\Delta\epsilon$ = + 1.37) corresponds to a conformation of ring C as depicted in **12a''** in which the ring atoms C-13 and the ether oxygen are located in positive octants. In this conformation the methyl group at C-13 adopts a quasi axial and the vinyl group a quasi equatorial position. In agreement with this assumption stereoisomer **13** displays a negative CD around 300 nm ($\Delta\epsilon$ = - 1.01) corresponding to a ring conformation such as **13''** where C-13 and the ether oxygen are located in negative octants and again the 13-methyl group is quasi axial and the 13-vinyl group quasi equatorial. This conformational change would also explain the fact that in **12a** and **13** the C-12 protons differ considerably in chemical shift. In **12a** 12ß-H is more shielded than 12α-H. This is in agreement with a conformation such as **12a''** where 12ß-H is closer to the plane of the CO group than the quasi axial 12α-H. In **13** 12α-H has the smaller chemical shift in agreement with conformation **13''** where it is 12ß-H which adopts the quasi axial position.

With the aim of obtaining a crystalline derivative from amorphous **12a** for an X-ray analysis we prepared a number of esters from **12a**. Unfortunately, from neither of these compounds suitable crystals could be obtained. However, some of the esters displayed very interesting properties, and in addition, their spectra are in full agreement with structure **12a**. For comparison, the known esters **1b** and **1c** of forskolin are included in the discussion. Interestingly, reaction of **1a** with 3,5-dinitro benzoyl chloride under exactly the same conditions which lead from **1a** to **1b**,[15] the formation of a compound was observed which according to the ^1H NMR chemical shifts of 1-H, 6-H, 7-H (see Table 2) was 6,7-diacylated derivative **1d**. This would mean that in this case acylation is preceeded by the well-known migration of the acetyl group from the 7- to the 6-position.[16] It is conceivable that the reaction of the sterically more demanding substituted benzoyl chloride with the axial 1-OH group is retarded by steric hindrance.

Acylation of **12a** with 4-nitrobenzoyl chloride occurred in the 1-position to yield **12b** (downfield shift of the 1-H NMR signal, see Table 2).

When **12a** was esterified with 3,5-dinitrobenzoyl chloride, two compounds (**12c** and **12d**) were formed, both of them carrying a 3,5-dinitrobenzoate group in the 1-position. The two compounds equilibrated on standing in

solution by acetyl migration. In $CHCl_3$, not previously treated with alumina to remove HCl, the rearrangement was particularly fast.

In wet $CDCl_3$ the ^1H NMR spectrum of a mixture of **12c** and **12d** displayed doublets for the protons adjacent to the free OH group at C-6 and C-7, respectively, whereas in dry $CDCl_3$ solution these signals were doublets of doublets.

On treatment of a mixture of **12c** and **12d** with acetic anhydride only compound **12c** with the free 7-hydroxyl group reacted and gave triester **12e**. The characteristic NMR features are well in agreement (see Table 2) with those of triacetate **12f** obtained from **12a** on acetylation with acetic anhydride in the presence of Steglich's base.[17]

Inspection of the ^1H NMR spectra of the various esters revealed interesting differences. The coupling constant $J_{6,7}$ (7-8 Hz) was considerably larger in diesters **12b**, **12c**, and **12d** than in triesters **12e** and **12f**, and in **12a** (J = 4 Hz) indicating different conformations of ring B in the two groups of compounds. In triester **12e**, the signals of the olefinic protons (14-H, 15-H_{cis} und 15-H_{trans}) appear at considerably higher field than in **12f**. This is most probably due to the diamagnetic anisotropy of the aromatic ring. The shielding effect of the aromatic ring discussed for **12e** is absent in **12c** and **12d**, as well as in **12b**. This observation is in accord with the assumption derived above from the coupling constant $J_{6,7}$ that the conformation of rings A and B differ in the triesters and the diesters. The 1-H signals of triesters **12e** and **12f** are well-resolved doublets of doublets with coupling constants of 1.5/6.0 and 3.0/6.9 Hz, respectively, whereas in **12a** and in the diesters the 1-H signal is an unresolved multiplet. In the ^1H NMR spectra of the triesters **12e** and **12f** all signals were sharper than in the diesters and in **12a**. We assume that a rotation of the

Table 2. Selected ^1H NMR signals of compounds **1a-1d**, and **12a-12f**

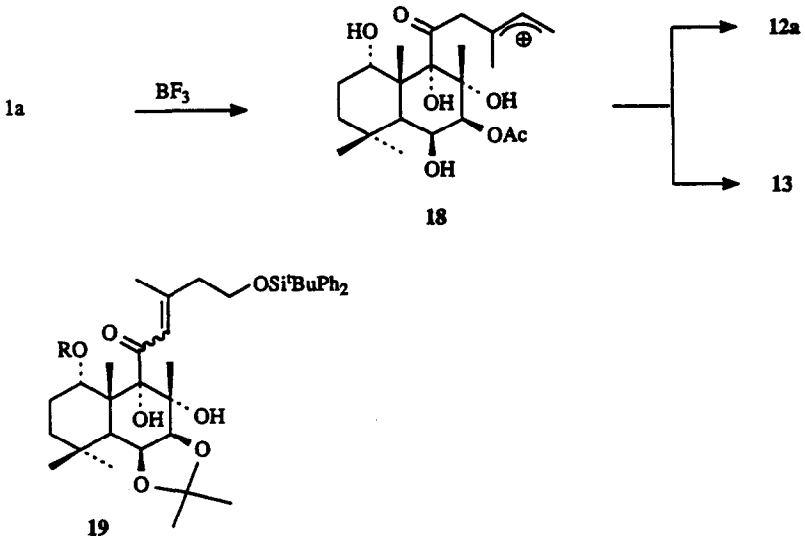
	1-H	6-H	7-H
1a[3]	4.54	4.44	5.46
1b[15]	5.83	4.52	5.50
1c[18]	5.00	6.15	6.15
1d	5.52	6.22	5.84
12a=3	3.54	4.26	5.14
12b	4.64	4.31	4.94
12c	5.23	5.89	3.82
12d	5.22	4.66	4.97
12e	5.01	5.83	5.26
12f	4.81	5.73	5.19

vinyl group causes the line-broadening discussed above and that this conformational change is frozen in the triesters.

Discussion

The formation of the two 13-epimers **12a** and **13** indicates that the rearrangement of forskolin (**1a**) is initiated by cleavage of ring C to give the allylic cation intermediate **18** which reacts with the 9-OH group to yield **12a** and **13**. The rearranged products are obviously thermodynamically more stable than **1a**. This view is supported by some force-field calculations (PCMODEL 4.0, Serena Software) indicating the spiro compound **12a** to be about 7 kcal/mol lower in energy than **1a** (flattened chair, torsional angle (12α-H-)C-12 - C-13(-13-CH_3): 152°). In keeping with this, no conditions could be found to effect a back reaction from **12a** to **1a**, in contrast to Vishwakarma's report.[4]

It is interesting to compare the results presented here with a key step in Ikegami's forskolin total synthesis, the selenium-mediated cyclization of **19**, which was found to furnish the desired tetrahydropyran.[19] In principle, this reaction could also lead to a (presumably thermodynamically more stable) tetrahydrofuran of the **12a/13** type. In this case, however, the reaction is probably governed by stereoelectronic control (Baldwin rules), the 5-endo process being disfavoured.[20]



Scheme 5.

EXPERIMENTAL

For instrumentations, general method and abbreviations, see ref.[21] All reactions were performed in an argon atmosphere.

BF_3-mediated rearrangement of forskolin

To a solution of forskolin (**1a**, 96.5 mg, 0.24 mmol) in CH_2Cl_2 (4 mL) at -10°C a precooled CH_2Cl_2 solution of $BF_3 \cdot Et_2O$ (0.262 mol/l, 1.5 eq) was slowly added. The reaction mixture was stirred at -10°C for 1.5 h, then ice-cold saturated aqueous $NaHCO_3$ was added. The organic phase was washed with aq. $NaHCO_3$ and brine. Drying, solvent evaporation, and LC (petrol - ethyl acetate 4:1) yielded a 6:1 mixture (^1H NMR) of **12a** (=3) and **13** (37.1 mg, 38%). The mixture could be separated by careful MPLC (n-hexane - acetone 5:1). Both the column and the solvent were cooled with ice-water.

7β-Acetoxy-9,13-epoxy-1α,6β,8-trihydroxy-labd-14-en-11-one (12a).

^1H NMR (400 MHz, $CDCl_3$, 320 K, NOE, C,H COSY): δ = 1.03 (s, 3H, CH_3-18), 1.06 (m, 1H, 3-H_{eq}), 1.25 (s, 3H, CH_3-19), 1.41-1.45 (m, 1H, 2-H_{eq}), 1.48 (s, 3H, CH_3-20), 1.58 (s, 6H, CH_3-16, CH_3-17), 1.79-1.88 (td, 1H, 3-H_{ax}), 1.94-2.01 (m, 2H, 2-H_{ax}, 5-H), 2.14 (s, 3H, 7-OAc), 2.62 (d, 1H, 12-H_β), 3.01 (d, 1H, 12-H_α), 3.51-3.56 (m, 1H, 1-H), 4.18-4.34 (m, 1H, 6-H), 5.14 (d, 1H, 7-H), 5.21 (d, 1H, 15-H_{cis}), 5.26 (d, 1H, 15-H_{trans}), 6.33 (dd, 1H, 14-H). $J_{2,3}$ = 3.9 Hz, $|J_{3,3}|$ = 13.9 Hz, $J_{6,7}$ = 4.0 Hz, $|J_{12,12}|$ = 18.9 Hz, $J_{14,15cis}$ = 10.7 Hz, $J_{14,15trans}$ = 17.2 Hz.- ^{13}C NMR (100.6 MHz, $CDCl_3$, 320K, DEPT): δ = 18.9 (CH_3, CH_3-20), 20.2 (CH_3, CH_3-16, CH_3-17), 21.0 (CH_3, CH_3-22), 25.2 (CH_3, CH_3-19), 26.6 (CH_2, C-2), 30.0 (CH_3, CH_3-16, CH_3-17), 33.3 (CH_3, CH_3-18), 34.8 (Cq, C-4), 36.0 (CH_2, C-3), 43.5 (CH, C-5), 46.8 (Cq, C-10), 49.4 (CH_2, C-12), 69.5 (CH, C-6), 73.8 (CH, C-1), 78.3 (CH, C-7), 80.6 (Cq, C-13), 93.5 (Cq, C-9), 113.9 (CH_2, C-15), 143.9 (CH, C-14), 171.2 (Cq, C-21), 210.9 (Cq, C-11). The C-8 signal was hidden by the $CDCl_3$ signals.- ^{13}C NMR (100.6 MHz, pyridine-d_5, 320K, DEPT): δ = 19.9 (CH_3, CH_3-20), 20.7 (CH_3, CH_3-16, CH_3-17), 22.2 (CH_3, CH_3-22), 26.4 (CH_3, CH_3-19), 27.8 (CH_2, C-2), 28.8 (CH_3, CH_3-16, CH_3-17), 33.0 (CH_3, CH_3-18), 34.3 (Cq, C-4), 37.3 (CH_2, C-3), 44.1 (CH, C-5), 48.2 (Cq, C-10), 50.3 (CH_2, C-12), 68.4 (CH, C-6), 75.6 (CH, C-1), 76.1 (Cq, C-8), 79.4 (CH, C-7), 80.0 (Cq, C-13), 92.9 (Cq, C-9), 111.7 (CH_2, C-15), 146.6 (CH, C-14), 170.7 (Cq, C-21), 213.9 (Cq, C-11).- IR ($CHCl_3$): 3432, 2931, 1740, 1636, 1457, 1374, 1250 cm^{-1}.- CD (CH_3CN): λmax (Δε) = 196.8 (-0.98), 302.8 (1.16), 314.4 (1.37), 327.0 nm (0,85).- $C_{22}H_{34}O_7$ (410.5), MS: m/z (%) = 410 (4) [M+], 392 (12), 224 (10), 182 (16), 165 (13), 109 (15), 95 (18), 81 (19), 69 (17), 55 (16), 43 (100), 29 (7).

(13S)-7β-Acetoxy-9,13-epoxy-1α,6β,8-trihydroxy-labd-14-en-11-one (13).

^1H NMR (400 MHz, $CDCl_3$, 320 K, NOE): δ = 1.05 (s, 3H, CH_3-18), 1.04-1.08 (m, 1H, 3-H_{eq}), 1.28 (s, 3H, CH_3-19), 1.38-1.45 (m, 1H, 2-H_{eq}), 1.47 (s, 3H, CH_3-20), 1.60 (s, 3H, CH_3-17), 1.66 (s, 3H, CH_3-16), 1.88-1.97 (td, 1H, 3-H_{ax}), 1.99 (d, 1H, 5-H), 1.87-2.02 (m, 1H, 2-H_{ax}), 2.12 (s, 3H, 7-OAc), 2.60 (brs, 1H, OH), 2.63 (d, 1H, 12-H_α), 2.97 (d, 1H, 12-H_β), 3.59 (brd, 1H, 1-H), 4.42 (t, 1H, 6-H), 5.16 (d, 1H, 7-H), 5.25 (d, 1H, 15-H_{cis}), 5.34 (d, 1H, 15-H_{trans}), 6.18 (dd, 1H, 14-H), $J_{1,2}$ = 3.2 Hz, $J_{2,3}$ = 3.0 Hz, $|J_{3,3}|$ = 13.0 Hz, $J_{5,6}$ = 2.7 Hz, $J_{6,7}$ = 3.8 Hz, $|J_{12,12}|$ = 18.5

Hz, $J_{14,15cis}$ = 11.0 Hz, $J_{14,15trans}$ = 17.7 Hz.- ^{13}C NMR (100.6 MHz, $CDCl_3$, 320K, DEPT): δ = 18.5 (CH_3, CH_3-20), 20.1 (CH_3, CH_3-16, CH_3-17), 21.2 (CH_3, CH_3-22), 25.9 (CH_3, CH_3-19), 26.6 (CH_2, C-2), 31.4 (CH_3, CH_3-16, CH_3-17), 33.5 (CH_3, CH_3-18), 35.0 (Cq, C-4), 36.2 (CH_2, C-3), 44.2 (CH, C-5), 46.8 (Cq, C-10), 49.8 (CH_2, C-12), 70.4 (CH, C-6), 74.3 (CH, C 1), 77.8 (CH, C-7), 81.1 (Cq, C-13), 94.6 (Cq, C-9), 114.7 (CH_2, C-15), 144.7 (CH, C-14), 171.2 (Cq, C-21). The C-8 signal was hidden by the $CDCl_3$ signals.- IR ($CHCl_3$): 3452, 2960, 2932, 1743, 1636, 1457, 1374, 1237 cm^{-1}.- CD (CH_3CN): λmax (Δε) = 195,8 (-1,56), 308,4 (-1,01), 319,4 (-1,01), 331,0 nm (-0,58).- $C_{22}H_{34}O_7$ (410.5), MS: m/z (%) = 410 (5) [M+], 392 (11), 191 (9), 165 (12), 109 (13), 95 (18), 95 (18), 81 (19), 69 (18), 55 (17), 43 (100).

1α,6β,7β-Triacetoxy-9,13-epoxy-8-hydroxy-labd-14-en-11-one (12f).

A mixture containing **12a** (10 mg, 0.025 mmol), CH_2Cl_2 (0.3 mL), pyridine (1 mL), acetic anhydride (1 mL), and a trace of 4-dimethylaminopyridine was left at 20° C for 3 d. After addition of water, freeze-drying, and LC (petrol - ethyl acetate 4:1) triacetate **12f** was obtained (4 mg, 33%).- ^1H NMR (400 MHz, $CDCl_3$, 320 K): δ = 1.02, 1.05, 1.25(3*s, 9H, CH_3-18, CH_3-19), 1.37-1.76 (mk, unexact integral, 3-H_{eq}, 2-H_{eq}, 3-H_{ax}, 2-H_{ax}), 1.48 (s, 3H, CH_3-20), 1.62, 1.68 (2*s, 6H, CH_3-16, CH_3-17), 1.97-2.32 (mk, unexact integral, 3-H_{eq}, 2-H_{eq}, 3-H_{ax}, 2-H_{ax}), 1.98, 2.03, 2.06 (3*s, 9H, OAc signals), 2.44 (d, 1H, 5-H), 2.62 (d, 1H, 12-$H_β$), 2.88 (d, 1H, 12-$H_α$), 4.81 (dd, 1H, 1-H), 4.98 (d, 1H, 15-H_{cis}), 5.04 (d, 1H, 15-H_{trans}), 5.19 (d, 1H, 7-H), 5.73 (dd, 1H, 6-H), 6.38 (dd, 1H, 14-H), $J_{1,2}$ = 3.0/6.9 Hz, $J_{5,6}$ = 2.2 Hz, $J_{6,7}$ = 4.7 Hz, $|J_{12,12}|$ = 18.8 Hz, $J_{14,15cis}$ = 11.1 Hz, $J_{14,15trans}$ = 17.6 Hz.- $C_{26}H_{38}O_9$ (494.6), MS: m/z (%) = 494 (3.6) [M+], 434 (3.4), 355 (3), 271 (3.5), 259 (8), 95 (21), 71 (19), 57 (28), 43 (100).

7β-Acetoxy-1α-(4-nitro)benzoyloxy-9,13-epoxy-6β,8-dihydroxy-labd-14-en-11-one (12b).

To a solution of **12** (10 mg, 0,025 mmol) in pyridine (0.5 mL) a 0.29 mol/l solution of 4-nitrobenzoyl chloride in pyridine (1.7 mL, 0.5 mmol) was added and the mixture was left at 20°C for 18 h. Work-up was performed by addition of water, freeze-drying, dissolving the residue in CH_2Cl_2 and washing with aq. $NaHCO_3$ and brine. LC (toluene - ethyl acetate 15:1) furnished pure **12b** (6,8 mg, 50%).- ^1H NMR (400 MHz, $CDCl_3$, 293 K): δ = 1.11 (s, 3H, CH_3-18), 1.20 (s, 3H, CH_3-19), 1.00-1.41 (m, no exact integral, 3-H_{eq}, 2-H_{eq}), 1.40, 1.56 (2*s, 9H, CH_3-16, CH_3-17, CH_3-20), 1.69-1.77, 1.93-2.33 (2*m, 2H, 2-H_{ax}, 3-H_{ax}), 2.09 (s, 3H, 7-OAc), 2.52 (d, 1H, 12-$H_β$), 2.53 (1H, 5-H), 2.72 (bs, 1H, OH), 3.04 (d, 1H, 12-$H_α$), 4.19-4.44 (m, 1H, 1-H, 6-H), 4.64 (t, 1H, 1-H, 6-H), 4.94 (d, 1H, 7-H), 5.05 (d, 1H, 15-H_{cis}), 5.15-5.32 (m, 2H, 15-H_{trans}, OH), 6.30-6.48 (m, 1H, 14-H), 8.22 (d, 2H, ArH), 8.34 (d, 2H, ArH), $J_{6,7}$ = 7 Hz, $|J_{12,12}|$ = 19.9 Hz, J_{ArH} = 9.0 Hz,- $C_{29}H_{35}NO_{10}$ (558.6), MS: m/z (%) = 559 (1) [M+], 541 (4), 499 (1,7), 443 (2,4), 318 (4), 224 (18), 182 (30), 150 (37), 95 (23), 43 (100).

Reaction of **12a** with 3,5-dinitrobenzoyl chloride

To a solution of **12a** (20 mg, 0.049 mmol) in pyridine (1 mL) a 0.75 mol/l solution of 3,5-dinitrobenzoyl chloride in pyridine (1.3 mL, 0.98 mmol) was added and the mixture was allowed to stand at 20°C for 1.5 h. Working up was performed as described for **12b**. LC (n-hexane - ethyl acetate 3:1) yielded **12d** (6.1 mg, 21%) and **12c** (9.2 mg, 31%).

7β-Acetoxy-1α-(3,5-dinitro)benzoyloxy-9,13-epoxy-6β,8-dihydroxy-labd-14-en-11-one (**12d**).

^{1}H NMR (400 MHz, $CDCl_3$ (treated with Al_2O_3, activity I, directly before being used), 320 K): δ = 1.14, 1.23, 1.37, 1.40, 1.42 (5*s, 15H, CH_3-16, CH_3-17, CH_3-18, CH_3-19, CH_3-20), 1.13-1.63, 1.71-1.80, 2.05-2.24 (3*m, 4H, 2-$H_{ax/eq}$, 3-$H_{ax/eq}$), 2.10 (s, 3H, 7-OAc), 2.37 (bs, 1H, 5-H), 2.53 (d u. bs, 2H, 12-H_β, OH), 3.00 (d, 1H, 12-H_α), 4.66 (dd, 1H, 6-H), 4.97 (d, 1H, 7-H), 5.04 (d, 1H, 15-H_{trans}), 5.12-5.31 (m, 2H, 15-H_{cis}, 1-H), 6.29-6.43 (m, 1H, 14-H), 9.15 (d, 2H, ArH), 9.20 (t, 1H, ArH), $J_{2,3}$ = 4.5 Hz, $|J_{3,3}|$ = 13.5 Hz, $J_{6,7}$ = 7.5 Hz, $|J_{12,12}|$ = 19.5 Hz, $J_{14,15trans}$ = 17.5 Hz, J_{ArH} = 2.0 Hz. The assignmenmt of the 1-H, 6-H, and 7-H signals rests on a H,H COSY spectrum of the **12c/12d** mixture.- $C_{29}H_{35}N_2O_{10}$ (603.6), no M^+ peak could be obtained.

6β-Acetoxy-1α-(3,5-dinitro)benzoyloxy-9,13-epoxy-7β,8-dihydroxy-labd-14-en-11-one (**12c**)

^{1}H NMR (400 MHz, $CDCl_3$ (treated with Al_2O_3, activity I, directly before being used), 320 K): δ = 1.03, 1.19, 1.21 (3*s, 9H, CH_3-18, CH_3-19, CH_3-20, 1.41, 1.43 (2*s, 6H, CH_3-16, CH_3-17), 1.13-1.42 (m, 2H, 3-H_{eq}, 2-H_{eq}), 1.56-1.65 (dt, 1H, 3-H_{ax}), 1.77-1.87, 2.04-2.15 (2*m, 2H, 2-H_{ax}, 5-H), 2.15 (s, 3H, 7-OAc), 2.51 (d, 1H, 12-H_β), 3.00 (m, 2H, 12-H_α, OH), 3.82 (dd, 1H, 7-H), 5.03 (d, 1H, 15-H_{trans}), 5.13-5.22, 5.27-5.33 (2*m, 2H, 1-H, 15-H_{cis}), 5.98 (dd, 1H, 6-H), 6.39 (m, 1H, 14-H), 9.17 (d, 2H, ArH), 9.21 (t, 1H, ArH), $J_{2,3}$ = 4.5 Hz, $|J_{3,3}|$ = 14.0 Hz, $J_{6,7}$ = 8.0 Hz, $|J_{12,12}|$ = 19.5 Hz, $J_{14,15trans}$ = 17.8 Hz, J_{ArH} = 2.1 Hz. The assignmenmt of the 1-H, 6-H, and 7-H signals rests on a H,H COSY spectrum of the **12c/12d** mixture.- $C_{29}H_{35}N_2O_{12}$ (603.6), no M^+ peak could be obtained.

6β,7β-Diacetoxy-1α-(3,5-dinitro)benzoyloxy-9,13-epoxy-8-hydroxy-labd-14-en-11-one (**12e**).

A solution of the **12c/12d** mixture (26.8 mg, 0.045 mmol) in pyridine (2 mL) and acetic anhydride (2 mL) was left at 20° C for 5 h. Under these conditions only the more polar 3,5-dinitrobenzoate (**12c**) was acetylated. Work-up was performed as described for **12f**. MPLC (pentane - tert-butyl methyl ether 2:1, solvent and column cooled with ice-water) provided **12e** (13 mg, 46%). The recovered starting material was again a **12c/12d** mixture, formed under the work-up conditions.- ^{1}H NMR (400 MHz, $CDCl_3$, 320K): δ = 1.08, 1.15, 1.21 (3*s, 9H, CH_3-18, CH_3-19, CH_3-20), 1.27-1.35 (m, 1H, 2-H_{eq}, 3-H_{eq}), 1.49 (s, 3H, CH_3-16, CH_3-17), 1.55-1.73 (m, 1H, 2-H_{eq}, 3-H_{eq}), 1.69 (s, 3H, CH_3-16, CH_3-17), 1.88-2.04 (m, 1H, 2-H_{ax}, 3-H_{ax}), 2.05, 2.13 (2*s, 6H, OAc groups), 2.19-2.34 (m, 1H, 2-H_{ax}, 3-H_{ax}), 2.56 (d, 1H, 5-H), 2.60 (d, 1H, 12-H_β), 2.73 (d, 1H, 12-H_α), 4.45 (d, 1H, 15-H_{cis}), 4.78 (d, 1H, 15-H_{trans}), 5.01 (dd, 1H, 1-H), 5.26 (d, 1H, 7-H), 5.83 (dd, 1H, 6-H),

6.04 (dd, 1H, 14-H), 9.09 (d, 2H, ArH), 9.21 (t, 1H, ArH), $J_{1,2}$ = 1.5/6.0 Hz, $J_{5,6}$ = 2.1 Hz, $J_{6,7}$ = 4.3 Hz, $|J_{12,12}|$ = 18.5 Hz, $J_{14,15cis}$ = 10.5 Hz, $J_{14,15trans}$ = 18.0 Hz, J_{ArH} = 2.1 Hz.- IR ($CHCl_3$): 3451, 3104, 2964, 2931, 1746, 1630, 1548, 1463, 1402, 1369, 1346, 1260 cm^{-1}.- $C_{31}H_{38}N_2O_{13}$ (646.6), MS: m/z (%) = 646 (1.5) [M+], 586 (6.6), 544 (4.2), 507 (1.7), 259 (2.3), 224 (2.8), 207 (8.5), 195 (6), 149 (9), 123 (7), 95 (18), 81 (8), 69 (13), 55 (9), 43 (100), 29 (3).

6ß-Acetoxy-7ß-(3,5-dinitro)benzoyloxy-8,13-epoxy-1α,9-dihydroxy-labd-14-en-11-one (**1d**)

A solution of **1a** (22.3 mg, 0.054 mmol) and 3,5-dinitrobenzoyl chloride (260.4 mg, 1.09 mmol) in pyridine (1,5 mL) was left at 20°C for 1.5 h. After addition of water work-up was performed as described for **12f**. LC (n-hexane - acetone 8:1) furnished **1d** (10.1 mg, 31%), 17 mg of impure forskolin were recovered.- ^1H NMR (400 MHz, $CDCl_3$, 20°C): δ = 1.28, 1.29 (2*s, 6H, CH_3-18, CH_3-19), 1.34 (s, 3H, CH_3-16), 1.40 (s, 3H, CH_3-20), 1.48-1.52 (m, 1H, 2-H_{eq}), 1.58 (s, 3H, CH_3-17), 1.61-1.67, 1.71-1.80 (2*m, 3H, 2-H_{ax}, 3-$H_{eq/ax}$), 2.14 (s, 3H, 7-OAc), 2.26-2.38 (m, 1H, 5-H), 2.54 (d, 1H, 12-$H_ß$), 2.88 (d, 1H, 12-$H_α$), 3.33 (bs, 1H, OH), 4.93 (dd, 1H, 15-H_{cis}), 5.24 (dd, 1H, 15-H_{trans}), 5.52 (dd, 1H, 6-H), 5.76 (dd, 1H, 14-H), 5.84 (d, 1H, 7-H), 6.22 (brt o. dd, 1H, 1-H), 9.08 (d, 2H, ArH), 9.18 (t, 1H, ArH), $J_{1,2}$ = 3.0 Hz, $J_{6,7}$ = 2.1 Hz, $|J_{12,12}|$ = 17.2 Hz, $J_{14,15cis}$ = 10.5 Hz, $J_{14,15trans}$ = 17.0 Hz, $|J_{15,15}|$ = 0.8 Hz, J_{ArH} = 2.1 Hz.- $C_{29}H_{35}N_2O_{12}$ (603.6), MS: m/z (% rel. Int.) = 586 (0.16) $[M-H_2O]^+$, 526 (0.8), 431 (26), 387 (2.7), 219 (28), 95 (21), 68 (35), 43 (100).

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