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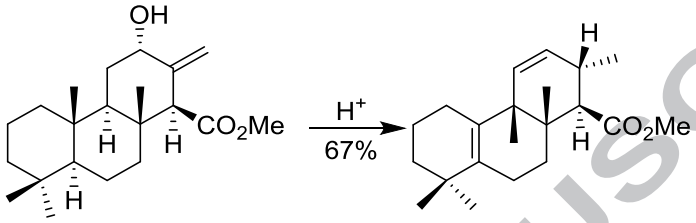

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ABSTRACT

The first biomimetic synthesis of methyl (8*S*,9*R*,13*R*,14*R*)-4,4,8,9,13-pentamethyl- 20(10→9)-abeo-*ent*-isocopal-5(10),11(12)-dien-15-oate – a diterpenoid with the *ent*-verrucosin A/B skeleton has been performed by electrophilic isomerization of methyl 12α-hydroxy-*ent*-isocopal-13(16)-en-15-oate. The structure and stereochemistry of the synthesized compound have been established on the basis of spectroscopic data.

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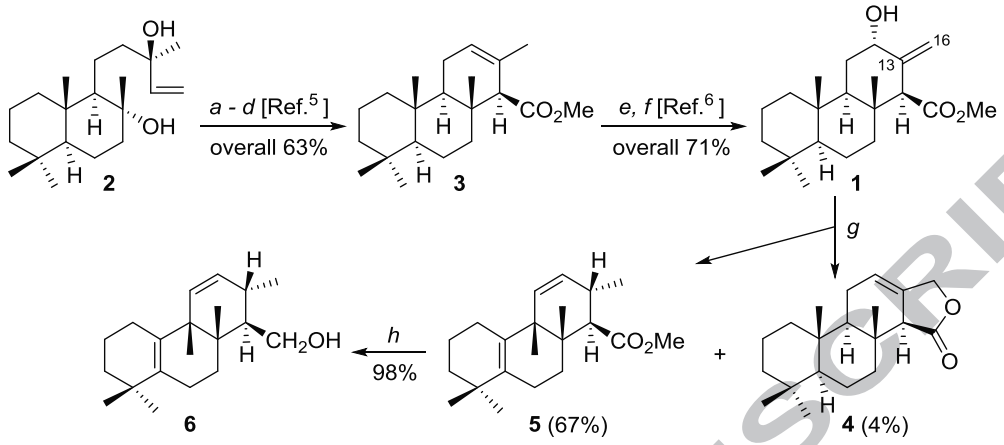
Terpenoids are one of the most numerous and important classes of natural compounds, their roles in living systems being extensively revealed over the past three decades. They are particularly widespread, *e.g.* in lower and higher plants,[1] microorganisms and aquatic animals.[2] An important chemical property of terpenoids is their isomerization under the influence of acidic reagents.[3] In this paper we report the isomerization of the methyl ester of 12α-hydroxy-*ent*-isocopal-13(16)-en-15-oic acid (**1**)[4] under the influence of *p*-toluenesulfonic acid.



Scheme 1. *Reagents and conditions*: (*a*) $KMnO_4$, Me_2CO, r.t., 12 h, 90%; (*b*) I_2, PhMe, reflux, 3 h, 78%; (*c*) $(MeO)_2P(O)CH_2CO_2Me$, PhH, MeONa, reflux, 2 h, 98%, (13*E*:13*Z* = 10:1); (*d*) FSO_3H (5 eq), *i*-$PrNO_2$, -78 °C, 15 min, then NEt_3, 92%; (*e*) *m*-CPBA, CH_2Cl_2, 0 °C, 12 h, 97%; (*f*) Al(O*i*-Pr)$_3$, PhMe, reflux, 24 h, 78%; (*g*) *p*-TsOH/$CHCl_3$, reflux, 3 h, 71%; (*h*) $LiAlH_4$, THF, reflux, 4 h, 98%.

(-)-Sclareol (**2**) served as a starting compound, which was converted into the methyl ester of *ent*-isocopalic acid (**3**), according to the described procedure.[5] The latter compound was in turn transformed into the methyl ester of 12α-hydroxy-*ent*-isocopal-13(16)-en-15-oic acid (**1**), according to the reported two-step sequence[6] (Scheme 1).

The reaction of a chloroform solution of ester (**1**) with *p*-toluenesulfonic acid at reflux for 4 h afforded a ~5:2 mixture of two compounds. The polar minor compound was identified by its spectroscopic data and other properties as the known diterpenic lactone (**4**).[4]

According to elemental analysis, IR and NMR spectra, the major compound (**5**)[7] was identified as a diterpenic diene ester with the molecular formula $C_{21}H_{32}O_2$. The NMR data of compound (**5**) were assigned (Table 1) on the basis of its 1D (^{1}H, ^{13}C, DEPT-135) and 2D homo- (^{1}H/^{1}H COSY-45, ^{1}H/^{1}H NOESY) and heteronuclear (^{1}H/^{13}C HSQC and ^{1}H/^{13}C HMBC) correlation spectra (Figure 1). Thus, the ^{1}H NMR spectrum displayed singlets of four tertiary methyl groups: at δ_H 0.98 (3H, H-17) and 0.994 (3H, H-20), geminal dimethyls at δ_H 0.986 and 0.988, (each 3H, H-18 and H-19), signals of one secondary methyl group at δ_H 0.88 (3H, H-16) that appears as a doublet of multiplets with J = 6.7 Hz due to splitting of H-13, H-12 and H-14 nuclei, a downfield singlet of the methyl ester group at δ_H 3.69 as well as deshielded signals of two sp^2 methines: δ_H 5.38 (*dd*, J = 10.1; 1.2 Hz, H-12) and 5.45 (*br. d*, J = 10.1, H-11). The assignment of signals that characterize methylene protons belonging both to ring A (complex splitting patterns H_2-1-H_2-2-H_2-3) and B (splitting patterns H_2-6-H_2-7) is depicted in Table 1, being to a certain degree impeded by couplings and signal overlapping.

Table 1. ^{1}H (400.13 MHz) and ^{13}C NMR (100.61 MHz) data of compound **5** in $CDCl_3$[a]

Position	δ_H	δ_C	Position	δ_H	δ_C
1_{ax}	1.90 (1H, m[b])	27.1, t	9	-	42.7, s
1_{eq}	2.00 (1H, m, 6.8)		10	-	133.4, s
2	1.58 (2H, m)	20.0, t	11	5.45 (1H, br.d, 10.1)	133.5, d
3_{ax}	1.36 (1H, td, 12.3, 3.6)	39.8, t	12	5.38 (1H, dd, 10.1, 1.2)	129.1, d
3_{eq}	1.44 (1H, dddd, 12.5, 4.9, 3.3, 1.5)		13	2.48 (1H, m[b])	31.5, d
			14	2.48 (1H, m[b])	51.3, d
4	-	33.9, s	15	-	175.9, s
5	-	131.5, s	16	0.88 (3H, m, 6.7)	19.9, q
6_{ax}	2.35 (1H, m)	20.8, t	17	0.97 (3H, s)	18.7, q
6_{eq}	1.90 (1H, m[b])		18	0.986 (3H, s)	27.9, q
7_{ax}	1.65 (1H, ddd, 14.0, 12.5, 6.3)	30.1, t	19	0.988 (3H, s)	28.8, q
7_{eq}	1.23 (1H, dd, 14.0, 6.3)		20	0.994 (3H, s)	22.3, q
8	-	36.8, s	O*Me*	3.69 (3H, s)	51.0, q

[a] chemical shifts (δ) are in ppm, coupling constants (J) are in Hz.

[b] overlapping signals.

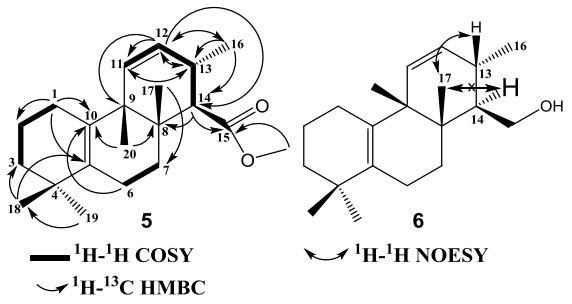
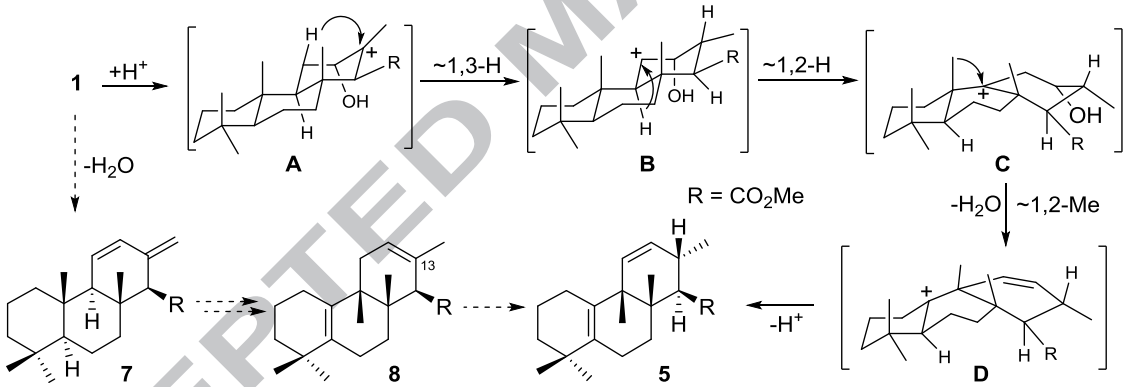


Figure 1. Key 2D NMR correlations for compounds (**5**) and (**6**).

The ^{13}C NMR data (Table 1) exhibited twenty one carbon signals, which were assigned by DEPT as six methyls, five sp^3 methylenes, two sp^3 and two sp^2 methines, three sp^3 and three sp^2, including one ester, quaternary carbons. The presence of two double bonds, one of which being disubstituted and another tetrasubstituted, was corroborated by the ^{13}C NMR data [δ_C 133.5 (C-11), 129.1 (C-12), 131.5 (C-5) and 133.4 (C-10)]. The rearranged carbon framework of compound (**5**) becomes obvious with detailed analysis of its HMBC spectrum. Thus, the observed correlations from both H-6 and H-1 to two sp^2 hybridized carbons (C-5, δ_C 131.5 and C-10, δ_C 133.4) were indicative of $\Delta^{5,10}$ localization, which was also supported by the correlations of H_3-19/C-5 and H_3-18/C-5. The migration of the H_3-20 methyl from the C-10 to C-9 position was ascertained by the H_3-20/C-10, H_3-20/C-9, H_3-20/C-11 and H_3-20/C-8 cross-peaks in the HMBC spectrum, while the presence of the $\Delta^{11,12}$ function was especially proven by long-range correlations between H_3-16/C-12, H_3-20/C-11, H-12/C-14, H-11 and H-12/C-9 and by mutual HMBC correlations as indicated in Figure 1.

During elucidation of the stereochemistry at the C-13 asymmetric center of compound (**5**) a problem emerged, caused by the overlapped signals of methines H-13 and H-14 that appeared in the ^{1}H NMR spectrum as a multiplet centered at δ_H 2.48. In light of this, interpretation of its ^{1}H-^{1}H NOESY spectrum only furnished inconclusive data for the identification of the configuration at C-13. Therefore, we decided to solve this problem by correlation of the NMR data of compound (**5**) with that of its reduction product, compound (**6**).[8] In the ^{1}H NMR spectrum of compound (**6**) signals of the H-13 and H-14 nuclei were well-separated: δ_H 1.39 (dt, J = 10.5, 3.6 Hz, 1H, H-14$_{ax}$) and 2.03 (m, J= 10.5, 7.0, 1.6, 1.3 Hz, 1H, H-13$_{ax}$). The large coupling constant (10.5 Hz) between the vicinal methine protons under discussion demonstrated their *trans*-diaxial interaction, which was confirmed by molecular modelling.[9] Finally, the *(R)* relative configuration at C-13 was deduced from the NOESY correlation between H_3-17/H-13 and the lack of H_3-17/H-14 correlation. Thus, compound (**5**) was definitively identified as methyl (8*S*,9*R*,13*R*,14*R*)-4,4,8,9,13-pentamethyl- 20(10→9)-abeo-*ent*-isocopal-5(10),11(12)-dien-15-oate.



Scheme 2. Proposed reaction mechanism for the acid-promoted isomerization of **1** into **5**.

Based on these data, it was possible to confirm that the acid-promoted isomerization of the methyl ester of 12α-hydroxy-*ent*-isocopal-13(16)-en-15-oic (**1**) acid furnished a diene ester with a rearranged framework (**5**). A plausible path for its formation involving the electrophilic isomerization of compound (**1**) is depicted in Scheme 2.

Thus, after protonation of ester **1** carbocation **A** is formed, which through a 1,3-hydride shift (H-11β moves to C-13) generates carbocation **B**. A second hydride shift forms carbocation **C**, which after angular methyl migration and dehydration forms carbocation **D**, which is stabilized by deprotonation to afford the methyl ester of (8*S*,9*R*,13*R*,14*R*)-4,4,8,9,13-pentamethyl-20(10→9)-abeo-*ent*-isocopal-5(10),11(12)-dien-15-oic acid (**5**).

The fact that this mechanistic explanation relies upon a rare 1,3-hydride shift[10] forced us to consider other alternatives. For example, an acid induced dehydration could compete with the formation of intermediate **A** and bring about formation of a diene **7**. However, subsequent re-protonation and rearrangement would lead to a trisubstituted compound **8**, the isomer of **5**. Its isomerisation to a less favored disubstituted olefin proceeding with total facial selectivity of the protonation at C-13 seems less probable.

There are also similar reports on terpene synthesis which have been shown to occur *via* such rearrangements. For example Urones and collaborators postulated formation of a carbonium ion very similar to **A** upon treatment of the related epoxide with a Lewis acid. Its subsequent conversion involved a skeletal rearrangement *via* ring contraction.[11]

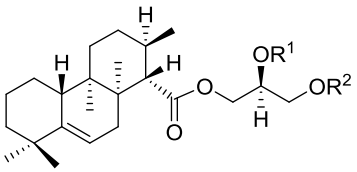


Figure 2. Natural verrucosins A and B.[12,13]

It is noteworthy that the carbon skeleton of diterpenoid (**5**) has *ent*-stereochemistry relatively to that of verrucosins A (**9**) and B (**10**) - acylglycerols isolated for the first time from *Doris verrucosa* mollusks collected in the Mediterranean Sea.[12,13]

Diterpenoids (**9**) and (**10**) are highly ichthyotoxic[12] and have demonstrated *in vivo* bioactivity as morphogens in the *Hydra* tentacle regeneration assay and their parallel function as activators of rat brain protein kinase C was also described.[14]

It should also be noted that the synthesis of terpenoids with the verrucosin A and B carbon skeleton has not yet been achieved. The biogenetic pathway leading to **9** and **10** has been previously suggested[15] and the present communication helps to confirm it with additional synthetic data.

In conclusion - the first biomimetic synthesis of methyl (8*S*,9*R*,13*R*,14*R*)-4,4, 8,9,13-pentamethyl- 20(10→9)-abeo-*ent*-isocopal-5(10),11(12)-dien-15-oate – a diterpenoid with the *ent*-verrucosin A/B skeleton has been performed by electrophilic isomerization of methyl 12α-hydroxy-*ent*-isocopal-13(16)-en-15-oate. The presented method opens the possibility of the synthesis of bioactive diterpenoids possessing this unique rearranged framework.

Acknowledgement

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7. **Methyl (8*S*,9*R*,13*R*,14*R*)-4,4,8,9,13-pentamethyl- 20(10→9)-abeo-*ent*-isocopal-5(10),11(12)-dien-15-oate (5).** To a solution of **1** (95 mg, 0.28 mmol) in $CHCl_3$ (35 mL) was added *p*-TsOH (145 mg, 0.84 mmol), which was heated at reflux for 4 h. Then, the mixture was washed twice with water, dried, and concentrated. The crude reaction product (94 mg) was purified by silica gel column chromatography (4 g, 1.5% AcOEt in petroleum ether) to give *methyl (8S,9R,13S,14R)-4,4,8,9,13-pentamethyl-20(10→9)-abeo-ent-isocopal-5(10),11(12)-dien-15β-oate* (**5**) (59 mg, 67%): white gum; $[\alpha]_D^{25}$ = 37.7 (*c* 1.33, $CHCl_3$). IR liquid film (ν, cm^{-1}): 1164, 1732. Anal. Calcd. for $C_{21}H_{32}O_2$: C 79.70, H 10.19; found: C 79.81, H 10.14. ^1H and ^{13}C NMR see Table 1.
8. **(8*S*,9*R*,13*S*,14*R*)-4,4,8,9,13-Pentamethyl-*20(10→9)*-abeo-*ent*-isocopal-5(10),11(12)-dien-15β-ol (6).** A solution of ester (**5**) (10 mg, 0.032 mmol) in anhydrous THF (5 mL) was treated with $LiAlH_4$ (15 mg, 0.40 mmol) under stirring. After 3 h at reflux, the reaction was cooled and quenched with ethyl acetate (0.1 mL). The reaction product was subsequently treated with a 10% H_2SO_4 solution (2 mL), extracted with diethyl ether (3x2 mL) and washed with brine, sat. $NaHCO_3$ solution and brine. The diethyl ether extract was dried over Na_2SO_4 for 2 h and then evaporated *in vacuo*. The crude product was purified by flash chromatography (silica gel (0.2 g, 1.5% AcOEt in petroleum ether) to give alcohol **6** (9 mg, 98%). Colorless viscous oil. $[\alpha]_D^{25}$ = 31.5 (*c* 0.61, $CHCl_3$). IR liquid film (ν, cm^{-1}): 1360, 1460, 2925. 3375. ^1H NMR (400 MHz, $CDCl_3$, δ_H): 0.75 (s, 3H, H-17), 0.86 (s, 3H, H-19), 0.907 (s, 3H, H-20), 0.91 (s, 3H, H-18), 0.99 (d, *J* = 7.0 Hz, 3H, H-16), 1.26 (td, *J* = 12.5, 3.3 Hz, 1H, H-3_{ax}), 1.36 (dddd, *J* = 12.5, 5.1, 3.3, 1.3 Hz, 1H, H-3_{eq}),1.39 (dt, *J* = 10.5, 3.6 Hz, 1H, H-14_{ax}), 1.43 (br. s, OH), 1.47 (m, 2H, H-2), 1.55 (m, *J* = 14.0, 12.6, 5.9 Hz, 1H, H-7_{ax}), 1.63 (ddd, *J* = 14.0, 5.9, 1.7 Hz, 1H, H-7_{eq}), 1.87 (m, 4H, H-1 and H-6), 2.03 (m, *J* = 10.5, 7.0, 1.6, 1.3 Hz, 1H, H-13), 3.66 (dd, *J* = 11.5, 3.3 Hz, 1H, H-15_A), 3.82 (dd, *J* = 11.5, 3.8 Hz, 1H, H-15_B), 5.31 (dd, *J* = 10.1, 1.6 Hz, 1H, H-12), 5.35 (dd, *J* = 10.1, 1.3 Hz, 1H, H-11). ^{13}C NMR (100 MHz, $CDCl_3$, δ_C): 19.6 (q, C-17), 20.0 (t, C-2), 20.2 (q, C-16), 21.2 (t, C-6), 22.5 (q, C-20), 27.2 (t, C-1), 27.8 (q, C-18), 28.6 (t, C-7), 28.9 (q, C-19), 31.1 (d, C-13), 33.9 (s, C-4), 36.4 (s, C-8), 39.8 (t, C-3), 43.3 (s, C-9), 45.0 (d, C-14), 62.9 (s, C-15), 130.6 (d, C-12), 131.2 (s, C-5), 133.3 (s, C-11), 134.6 (d, C-10).
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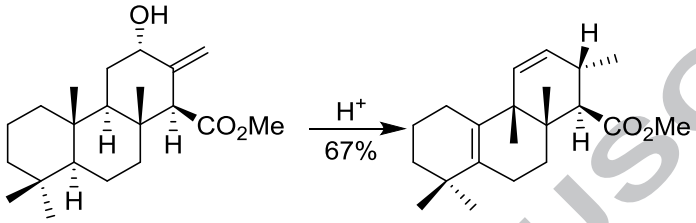

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Highlights

- The first biomimetic synthesis of a diterpenoid with the *ent*-verrucosin A/B skeleton has been performed.
- The method opens the possibility of synthesis of bioactive diterpenoids possessing this rearranged framework.
- The structure and stereochemistry of the synthesized compound have been established on the basis of spectral data.