CHEMISTRY JOURNAL OF MOLDOVA. General, Industrial and Ecological Chemistry. 2021, 16(1), 99-104
ISSN (p) 1857-1727 ISSN (e) 2345-1688
http://cjm.asm.md
http://dx.doi.org/10.19261/cjm.2021.820

ONE - STEP SELECTIVE SYNTHESIS OF 13-*EPI*-MANOYL OXIDE

Olga Morarescu [a], Marionela Traistari [b], Alic Barba [a], Gheorghe Duca [a], Nicon Ungur [a], Veaceslav Kulcițki [a*]

[a]*Institute of Chemistry, 3, Academiei str., Chisinau MD-2028, Republic of Moldova*
[b]*Moldova State University, 60, Mateevici str., Chisinau MD-2009, Republic of Moldova*
[*]*e-mail: kulcitki@yahoo.com; veaceslav.kulcitki@ichem.md; phone/fax (+373 22) 73 97 75*

Abstract. The selective one-step synthesis of 13-*epi*-manoyl oxide is reported based on a low-temperature superacidic cyclization of sclareol. The reaction conditions have been finely tuned in order to achieve a 9:1 ratio between epimeric oxides in favour of the desired 13-*epi*-oxide. The structures were confirmed by ^{1}H and ^{13}C NMR, and composition of the crude reaction products determined by GC-MS. These results have been interpreted by a hypothetical S_N2 mechanism which occurs with inversion of configuration around the C-13 chiral center of the starting substrate. The preparative value of the elaborated procedure is demonstrated on a gram-scale experiment.

Keywords: sclareol, cyclization, superacid, ether, labdane.

Received: 01 March 2021/ Revised final: 11 May 2021/ Accepted: 14 May 2021

Introduction

Labdane diterpenoids are widespread in nature and many relevant representatives play important roles in the terrestrial and marine ecosystems [1]. Besides, an impressive number of labdanes show relevant biological activities [2], including anti-inflamatory [3] and citotoxic properties [4]. These secondary metabolites are formed biogenetically, following an enzymatic cyclization of the geranylgeranyl diphosphate oligomer, which is suspended at two carbocycles, having one isoprene residue pendant [2]. The following late-stage functionalization provides a broad structural diversity that includes many additional functional groups attached to different positions of the bicyclic backbone and lateral chain. The biological activity of such compounds is tightly connected to their degree of "decoration" with heteroatoms, which represent the main tools for interaction with biomolecules in the living cells. Such interactions lead to relevant properties for mediation of a whole array of biochemical processes [5].

A perspective pathway for the exploration of labdane compounds represents the *in vitro* late-stage functionalization of representatives which can be easily assembled from the readily available substrates. In particular, manoyl oxide (**1**) and 13-*epi*-manoyl oxide (**2**) (Figure 1) have been extensively investigated in this context and their broad microbiological transformations have been reported, basically on the use of fungi [6].

The interest towards oxides **1** and **2** is triggered by their tricyclic backbone that is identical to the well-known forskolin (**3**) – a non-selective activator of the adenylate cyclase enzyme [7].

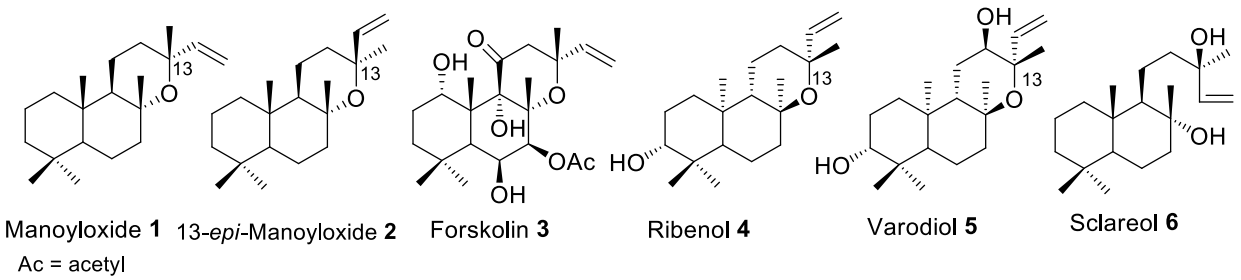


Figure 1. Selected representatives of natural labdanes.

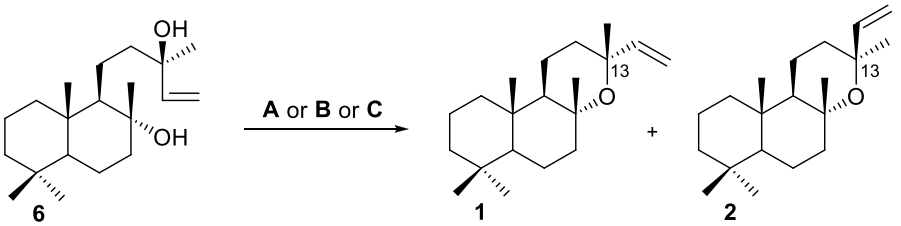
Other relevant natural compounds having the 13-*epi*-manoyl oxide skeleton and belonging to the *ent*-series are ribenol (**4**) and varodiol (**5**). Both **4** and **5** have been reported in structure-activity relationship (SAR) studies, providing derivatives with antimicrobial properties [8,9]. A free-radical procedure for structural modification of both forskolin and manoyl oxides has been demonstrated recently [10] and an unusual reactivity, leading to a distal functionalization of diterpenic framework of *epi*-oxide **2** has been noticed. In order to explore the full potential of such late-stage functionalization, one needs reliable sources of 13-*epi*-manoyl oxide, which, ideally, is made available *via* selective synthesis and the current paper aims to fulfil this goal.

Results and discussion

The tricyclic manoyl oxides **1** and **2** can be prepared *via* several synthetic protocols on the cyclization of the readily available (-)-sclareol (**6**) (Scheme 1). All published direct transformations of **6** into **1** report only minor quantities of 13-*epi*-manoyl oxide (**1**), which is usually very difficult to separate. In particular, the cerium-ammonium nitrate (CAN) mediated cyclization of **6** (Scheme 1, procedure **A**) provides a 95% conversion into a mixture of **1** and **2**, where **1** predominates (**1**:**2**= 5:2) [11]. An older contribution of some of us (Scheme 1, procedure **B**) demonstrated superacidic cyclization of **6** leading to 60% of an equimolar mixture of **1** and **2** [12]. To the best of our knowledge, there was a single paper reporting a synthesis of 13-*epi*-manoyl oxide (**2**) with an excellent 91% yield [13], but its preparative application is discouraged by the lengthy four-step synthetic sequence and the use of toxic phosphine reagents (Scheme 1, procedure **C**). Therefore, it was decided to elaborate an alternative selective procedure for cyclization of (-)-sclareol (**6**) into **2**, making use of the known methodology of low temperature superacidic cyclization [12].

Optimization experiments based on the published cyclization conditions (5 molar excess of FSO_3H at -78ºC), showed a specific dependence of the reaction outcome on experimental conditions, including reagent addition procedure, reaction temperature and the amount of cyclization agent (Table 1). Estimation of the reaction course relied upon the GC-MS analysis of the worked-up reaction mixtures and separate integration of peaks, providing a relative content of oxides **1** and **2**, starting diol **6** and all other reaction by-products taken together. No internal standard has been applied. The chromatograms corresponding to each experiment shown in Table 1 are provided in the supplementary material file. Minor amounts of polymeric material that is usually formed during such reactions have been neglected in the optimization studies, having in mind that its yield is lowered while lowering the reaction temperature below -78ºC.

The known [12] cyclization procedure involves addition of the solid substrate to the chilled solution of superacid in 2-nitropropane ($^{i}PrNO_2$) to provide a 1:1 ratio of **1** to **2**. An alternative addition procedure consisting of dropwise addition of a substrate solution in a 2:1 mixture of dichloromethane (DCM) – $^{i}PrNO_2$ to the solution of FSO_3H in $^{i}PrNO_2$ at -85ºC resulted in a slight excess of epimer **2** (Table 1, entry 1). This positive trend towards higher content of oxide **2** has been encouraging and it was decided to investigate the effect of cyclization agent amount on both reaction yield and selectivity. Decreasing the quantity of the superacid from 5 to 3 mol equiv. resulted in the selectivity switch back to manoyl oxide (**1**) (Table 1, entry 2). The experiment with a 1.5 mol equiv. of superacid showed a similar selectivity towards oxide **1**, accompanied by a significant drop of substrate conversion (Table 1, entry 3).



Reagents and conditions: **A**. CAN, CH_3CN, 10 hours at r.t.; **B**. FSO_3H, 15 min at -78ºC, then 30% aq. KOH; **C**. *i*) *m*-CPBA, $CHCl_3$, 2 hours at r.t., *ii*) 0.5 N aq. NaOH, *t*-BuOH, 3 hours at r.t., *iii*) TFA, CCl_4, 0ºC to r.t. in 2 hours, *iv*) Ph_2PCl, imidazole, Ph-Me, 10 min at 80ºC, then I_2 and 2 hours of reflux, followed by Zn and additional 2 hours of reflux.

Scheme 1. Cyclization of sclareol (6) into manoyl oxides 1 and 2.

It was clear at this point that 3 mol equiv. of superacid ensure quantitative substrate conversion and three different reagent addition procedures have been applied with this reagent excess. They included either slow addition of dissolved substrate onto the reaction flask internal walls in order to effect pre-cooling (Table 1, entry 4), addition of either solid substrate in one batch (Table 1, entry 5) or dissolved substrate added in one batch (Table 1, entry 6). All these experiments resulted in total consumption of starting material and trace amounts of byproducts, which represent hydrocarbons, resulting from dehydration of starting material under superacidic treatment [12]. But the ratio of manoyl oxides was favourable for undesired epimer **1**. Therefore, we decided to come back to the reaction conditions with a higher reagent excess that proved to be efficient for selective formation of *epi*-oxide **2**, but with the following modifications of addition procedures: slow addition of the dissolved substrate onto the reaction flask internal walls (Table 1, entry 7), addition of the cyclization agent solution to the solution of the substrate (Table 1, entry 8) or addition of the dissolved substrate in one batch (Table 1, entry 9). As it was expected, the higher yield of *epi*-oxide **2** was observed in the last experiment and the selectivity was matching exactly the results of the substrate solution dropwise addition. We concluded that dropwise addition was not critical for the reaction selectivity and proceeded further with single batch addition of the substrate at lower temperatures (Table 1, entries 10-14). The reaction outcome tendency is clearly demonstrated by the data in the table: first of all, the selectivity of cyclization to 13-*epi*-manoyl oxide increases on lowering the reaction temperature and reaches a 93:7 maximum ratio **2** to **1** at -105ºC over 15 min reaction time. Unfortunately, the yield of by-products also increases on lowering the temperature, affecting detrimentally the cyclization yield.

The optimal conversion-selectivity reaction conditions are achieved at -95ºC (Table 1, entry 11). A parallel preparative experiment was performed and included isolation of reaction products *via* column chromatography. The mixture of oxides **1** and **2** was obtained with an acceptable 60% yield and the prevalence of the desired oxide **2** was exactly the same as determined in the optimization experiment (GC-MS and NMR data, see supplementary material for details). Further purification of 13-*epi*-manoyl oxide (**2**) to ~100% purity was achieved by recrystallization. All spectral data of the purified material matched those published [13].

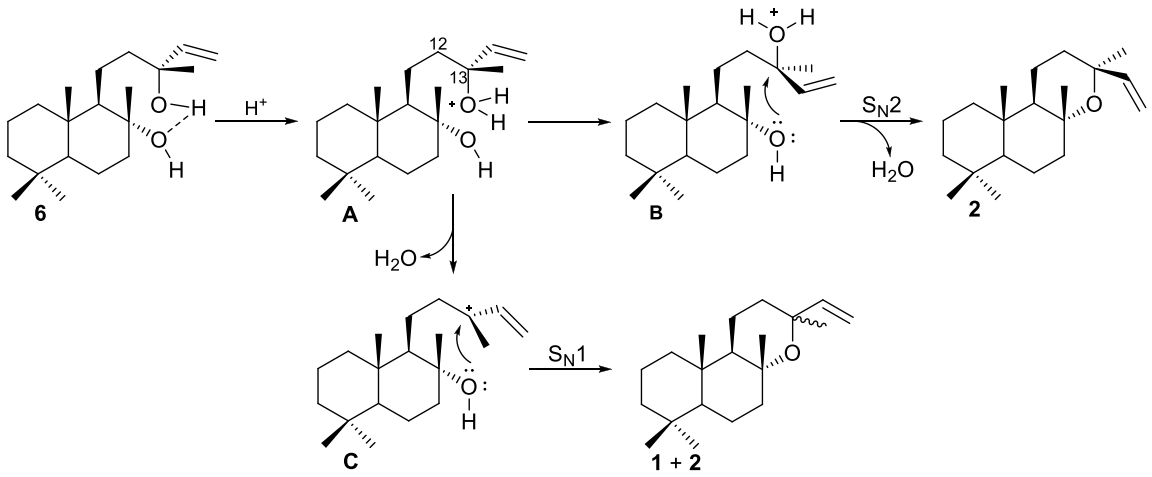
The mechanistic proposal (Scheme 2) is based on a hypothetical S_N2 mechanism that involves protonation of the hydroxyl group in the lateral chain that is more likely to be stabilized by the allylic effect of the adjacent olefinic bond. After –OH protonation and its conversion into a better leaving group, the water molecule is expelled by the hydroxyl group from the C-8 position.

Table 1

Results of optimization experiments for selective synthesis of 13-*epi*-manoyl oxide (2) from (-)-sclareol (6).

No	*Procedure**	*Reaction temperature*	*FSO_3H, mol equiv.*	*Reaction products, % by GC-MS*				***2/1***
				*Recovered **6***	*Secondary products*	***1***	***2***	
1	P1	-85	5	-	4	37	59	1.59
2	P1	-85	3	-	2	52	46	0.88
3	P1	-85	1.5	27	3	42	28	0.67
4	P2	-85	3	-	1	59	40	0.68
5	P3	-85	3	1	1	56	42	0.75
6	P5	-85	3	-	1	56	43	0.77
7	P2	-85	5	-	1	53	46	0.87
8	P4	-85	5	-	1	50	49	0.98
9	P5	-85	5	-	2	38	60	1.58
10	P5	-90	5	-	6	19	75	3.95
11	P5	-95	5	-	17	8	75	9.38
12	P5	-100	5	-	27	7	66	9.43
13	P5	-105	5	-	31	6	63	10.5
14	P5	-110	5	-	45	6	49	8.17

**For the description of procedures P1-P5 see experimental part.*



Scheme 2. Mechanistic proposal on the selective synthesis of 2.

Inversion of the configuration occurs at the C-13 chiral carbon on the rotation of the C-12 – C-13 (step **A** to **B** in Scheme 2) bond, leading to the *epi*-oxide **2**. In our opinion, the reaction temperature affects significantly the reaction path and higher temperatures promote water elimination before this conformational change occurs (step **A** to **C** in Scheme 2). As a result, the substitution is directed in parallel *via* a monomolecular substitution leading to mixtures of **1** and **2**. On the contrary, at lower temperatures, water elimination is not facile and it occurs only after a sequential conformational change leading to conformation **B**, followed by a bimolecular substitution process to yield selectively the *epi*-oxide **2**.

Conclusions

A careful optimization of reaction conditions for low temperature cyclization of (-)-sclareol allowed a highly selective synthesis of 13-*epi*-manoyl oxide – a labdane compound with a carbon backbone similar to different natural products and derivatives with relevant biological activity.

The optimized procedure opens the path for a broader investigation of similar compounds in SAR studies basing on commercially available starting materials.

Experimental

Generalities

^{1}H and ^{13}C NMR spectra were recorded in $CDCl_3$ on Bruker 400 Avance III spectrometer (400.13 and 100.61 MHz); chemical shifts are given in ppm and are referenced to chloroform ($CHCl_3$) as internal standard (δ_H= 7.26 ppm for proton and δ_C= 77.0 for carbon). Commercial Merck silica gel 60 (70-230 mesh ASTM) was used for flash chromatography and Merck pre-coated SiO_2 plates were used for TLC. The chromatograms were sprayed with 0.1% solution of cerium(IV) sulfate in 2N sulfuric acid and heated at 80°C for 5 min to detect the spots. *GC-MS analysis* were recorded on Agilent 7890A chromatograph, equipped with quadrupole MS detector MSD 5975C and HP-5 ms capillary column (30 m/0.25 mm). The isopropanol – liquid nitrogen system was used for low-temperature cooling bath preparation. Workup of reaction mixtures included extraction with Et_2O, washing of the extract with brine up to neutral, drying over anhydrous Na_2SO_4, and solvent removal in vacuum.

Cyclization of (-)-sclareol (6)

Procedure 1. (-)-Sclareol (**6**) (1 mmol) was dissolved in 5 mL of DCM-$^{i}PrNO_2$ mixture (2:1) and the resulting solution was added dropwise to the stirred solution (4 M) of the specified amount of FSO_3H (Table 1) in $^{i}PrNO_2$, preliminary chilled at the required temperature. After 15 min of stirring at this temperature, the reaction mixture was quenched with 5 mL of 30% NaOH aqueous solution. Following workup provided the crude reaction product.

Procedure 2. (-)-Sclareol (**6**) (1 mmol) was dissolved in 5 mL of DCM-$^{i}PrNO_2$ mixture (2:1) and the resulting solution was slowly added onto the internal walls of reaction flask, containing the stirred solution (4 M) of the specified amount of FSO_3H (Table 1) in $^{i}PrNO_2$, preliminary chilled at the required temperature. After 15 min of stirring at this temperature, the reaction mixture was quenched with 5 mL of 30% NaOH aqueous solution. Following workup provided the crude reaction product.

Procedure 3. (-)-Sclareol (**6**) (1 mmol) was added carefully to the vigorously stirred solution (0.33 M) of the specified amount of FSO_3H (Table 1) in $^{i}PrNO_2$, preliminary chilled at the required temperature. After 15 min of stirring at this temperature, the reaction mixture was quenched with 5 mL of 30% NaOH aqueous solution. Following workup provided the crude reaction product.

Procedure 4. (-)-Sclareol (**6**) (1 mmol) was dissolved in 5 mL of DCM-$^{i}PrNO_2$ mixture (2:1) and the resulting solution was chilled at the required temperature (Table 1) on stirring. The specified amount of FSO_3H (Table 1) dissolved in $^{i}PrNO_2$ (4 M) was added dropwise to the sclareol solution and stirring continued for 15 min at the same temperature. The reaction mixture was quenched with 5 mL of 30% NaOH aqueous solution. Following workup provided the crude reaction product.

Procedure 5. (-)-Sclareol (**6**) (1 mmol) was dissolved in 5 mL of DCM-$^{i}PrNO_2$ mixture (2:1) and the resulting solution was poured out to the stirred solution (4 M) of the specified amount of FSO_3H (Table 1) in $^{i}PrNO_2$, preliminary chilled at the required temperature. After 15 min of stirring at this temperature, the reaction mixture was quenched with 5 mL of 30% NaOH aqueous solution. Following workup provided the crude reaction product.

Cyclization of (-)-sclareol (6). Preparative experiment

(-)-Sclareol (**6**) (1 g, 3.24 mmol) was dissolved in 15 mL of DCM-$^{i}PrNO_2$ mixture (2:1) and the resulting solution was added in one batch to the stirred solution of 1.62 g (16.2 mmol) FSO_3H in 3.5 mL $^{i}PrNO_2$, preliminary chilled at -95ºC. After 15 min of stirring at this temperature, the reaction mixture was quenched with 20 mL of 30% NaOH aqueous solution. Following workup provided 920 mg of crude reaction product, which was submitted to column chromatography. Gradient elution with petroleum ether-ethylacetate mixtures of increasing polarities provided 180 mg of nonpolar fraction of byproducts, followed by 563 mg (1.94 mmol, 60%) of manoyl oxides, containing 90% 13-*epi*-manoyl oxide (**2**) and 10% manoyl oxide (**1**) (GC-MS data).

Funding

This work was funded by the National Agency for Research and Development (ANCD) of the Republic of Moldova: projects "New products with preventive and therapeutic potential basing on natural products of vegetal origin and modern methods of organic synthesis", no. 20.80009.8007.03 (O.M., M.S., N.U. and V.K.) and "Physico-chemical mechanisms of redox processes with electron transfer involved in vital, technological and environmental systems", no. 20.80009.5007.27 (A.B. and G.D.).

Supplementary information

Supplementary data are available free of charge at http://cjm.asm.md as PDF file.

References

1. Frija, L.M.T.; Frade, R.F.M.; Afonso, C.A.M. Isolation, chemical, and biotransformation routes of labdane-type diterpenes. Chemical Reviews, 2011, 111(8), pp. 4418-4452. DOI: https://doi.org/10.1021/cr100258k
2. Chinou, I. Labdanes of natural origin-biological activities (1981-2004). Current Medicinal Chemistry, 2005, 12(11), pp. 1295-1317. DOI: https://doi.org/10.2174/0929867054020990
3. Tran, Q.T.N.; Wong, W.S.F.; Chai, C.L.L. Labdane diterpenoids as potential anti-inflammatory agents. Pharmacological research, 2017, 124, pp. 43-63. DOI: https://doi.org/10.1016/j.phrs.2017.07.019
4. Könst, Z.A.; Szklarski, A.R.; Pellegrino, S.; Michalak, S.E.; Meyer, M.; Zanette, C.; Cencic, R.; Nam, S.; Voora, V.K.; Horne, D.A.; Pelletier, J.; Mobley, D.L.; Yusupova, G.; Yusupov, M.; Vanderwal, C.D. Synthesis facilitates an understanding of the structural basis for translation inhibition by the lissoclimides. Nature Chemistry, 2017, 9(11), pp. 1140-1149. DOI: https://doi.org/10.1038/nchem.2800
5. Dewick, P.M. Medicinal natural products: A biosynthetic approach. 3rd ed., Wiley: West Sussex, 2009, 539 p. DOI: 10.1002/9780470742761
6. Garcia-Granados, A.; Martinez, A.; Parra, A.; Rivas, F. Manoyl-oxide biotransformations with filamentous fungi. Current Organic Chemistry, 2007, 11(8), pp. 679-692. DOI: https://doi.org/10.2174/138527207780598774
7. Sengupta, S.; Mehta, G. Natural products as modulators of the cyclic-AMP pathway: evaluation and synthesis of lead compounds. Organic & Biomolecular Chemistry, 2018, 16(35), pp. 6372-6390. DOI: https://doi.org/10.1039/C8OB01388H
8. Kalpoutzakis, E.; Aligiannis, N.; Mitaku, S.; Chinou, I.; Charvala, C.; Skaltsounis, A.L. New hemisynthetic manoyl oxide derivatives with antimicrobial activity. Chemical and Pharmaceutical Bulletin, 2001, 49(7), pp. 814-817. DOI: https://doi.org/10.1248/cpb.49.814
9. Garcia-Granados, A.; Fernández, A.; Gutierrez, M.C.; Martınez, A.; Quirós, R.; Rivas, F.; Arias, J.M. Biotransformation of *ent*-13-epi-manoyl oxides difunctionalized at C-3 and C-12 by filamentous fungi. Phytochemistry, 2004, 65(1), pp. 107-115. DOI: https://doi.org/10.1016/j.phytochem.2003.09.017

10. Pruteanu, E.; Tappin, N.D.C.; Gîrbu, V.; Morarescu, O.; Dénès, F.; Kulciţki, V.; Renaud, P. Forskolin editing *via* radical iodo-and hydroalkylation. Synthesis, 2021, 53(07), pp. 1247-1261. DOI: 10.1055/s-0040-1706003
11. Alvarez-Manzaneda, E.J.; Chaboun, R.; Alvarez, E.; Cabrera, E.; Alvarez-Manzaneda, R.; Haidour, A.; Ramos, J.M. Cerium(IV) ammonium nitrate (CAN): a very efficient reagent for the synthesis of tertiary ethers. Synlett, 2006, 12, pp. 1829-1834. DOI: 10.1055/s-2006-947356
12. Vlad, P.F.; Ungur, N.D.; Barba, A.N.; Korchagina, D.V.; Bagryanskaya, I.Yu.; Gatilov, Yu.V.; Gatilova, V.P.; Barkhash, V.A. Cyclization of some labdane alcohols with a hydroxy group at C-13 by a superacid. Chemistry of Natural Compounds, 1988, 24, pp. 166-170. DOI: https://doi.org/10.1007/BF00596743
13. Moulines, J.; Lamidey, A.M.; Bats, J.P.; Morisson, V. A short and efficient synthesis of (+)-13-epimanoyl oxide from sclareol. Synthetic Communications, 1993, 23(21), pp. 2991-2998. DOI: https://doi.org/10.1080/00397919308011142