Available online at www.sciencedirect.com
SCIENCE DIRECT®
Bioorganic & Medicinal Chemistry Letters 14 (2004) 2983–2986
Bioorganic & Medicinal Chemistry Letters

Synthesis of dammarane-type triterpenoids with anti-inflammatory activity in vivo

Dieter Scholz,[a,*] Karl Baumann,[a] Max Grassberger,[a] Barbara Wolff-Winiski,[a] Grety Rihs,[b] Hansrudolf Walter[b] and Josef G. Meingassner[a]

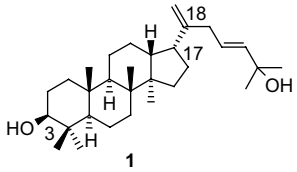
[a]*Novartis Institute for Biomedical Research, A-1230 Vienna, Austria*
[b]*Novartis Pharma AG, CH-4002 Basle, Switzerland*

Received 3 December 2003; revised 4 February 2004; accepted 23 February 2004

Abstract—The 17-α-substituted triterpene **1** [(17α)-23-(*E*)-dammara-20,23-diene-3β, 25-diol] showed promising activity in animal models of immunosuppression and inflammation. Using a mouse model for inflammatory skin diseases (oxazolone-induced allergic contact dermatitis, ACD) as the directing in vivo test system, Structure–activity-relationship studies with the aim to understand the necessary structural requirements for the biological activity of **1** were conducted. Furthermore, we anticipated to identify biologically active compounds with the 17β configuration, which are thermodynamically more stable and much easier to synthesize. This was achieved by identifying the 17-β substituted dammarane $\mathbf{5_B}$ and its analogues.

The novel triterpene **1** ([(17α)-23-(*E*)-dammara-20,23-diene-3β, 25-diol], Fig. 1) has been elucidated as the active principle[1] by tracking the observation that the flour of the palmyrah palm (*Borassus flabellifer*) induces immunosuppression.[2]

Compound **1** shows a very promising immunosuppressive profile in vitro and in vivo. It is active in assays for mixed lymphocyte reaction and murine delayed type hypersensitivity,[1] and efficacious in models of acute skin inflammation (oxazolone-induced allergic contact dermatitis, ACD) in mouse[3] and domestic pigs[3] upon topical application (Table 1). Furthermore, efficacy in the mouse model could also be demonstrated after oral administration (data not shown).



1

Figure 1. Structure of the immunosuppressant triterpene.

As **1** is inactive in the bis-pyridyl-N-oxide-disulfide (BPNOD)-induced edema model in the rat,[1] its potent anti-inflammatory effect in the ACD models occurs by a mechanism distinct from that of the structurally related glucocorticoids.[2] Based on the promising biological profile we initiated a medicinal chemistry program with the aim to identify new highly active anti-inflammatory triterpenoids starting with **1** as new lead. Compound **1** features the thermodynamically less stable α-configuration at C-17, which is unique for triterpenoids of the dammarane type Table 2.

Due to the very low concentration of **1** in its natural source (only 0.5 mg of **1** had been isolated from 50 kg of flour) an efficient synthetic access was mandatory. Starting from dipterocarpol **2**, the major constituent of commercially available Dammar resin, an 11-step synthesis via the 17β-substituted key intermediate **3** has been developed (Scheme 1).[1]

Compound **3**, which can be conveniently obtained from **2**, served as the common starting material for our program. Since all efforts to identify an in vitro marker predictive for the anti-inflammatory in vivo activity of **1** were unsuccessful, we used the results of the well

Keywords: Anti-inflammatory; Immunosuppressive; Allergic contact dermatitis; Triterpene; Triterpenoids; Dammar resin; Dammarane; Wittig.
* Corresponding author. Tel.: +43-1-86634-584; fax: +43-1-86634-354; e-mail: dieter.scholz@pharma.novartis.com

0960-894X/$ - see front matter © 2004 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2004.02.104

Table 1. In vivo activity of **1** in comparison to dexamethasone in the murine and porcine allergic contact dermatitis (ACD) models after topical application

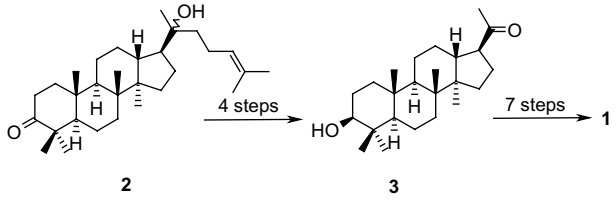
% Inhibition versus controls[a]					
ACD mouse			ACD pig		
Concn (μM)	**1**	Dexamethasone	Concn (μM)	**1**	Dexamethasone
40	$35 \pm 4.5^{**}$	$65 \pm 4.1^{**}$	400	$32 \pm 13.6^{**}$	NT
4	$44 \pm 3.5^{**}$	$37 \pm 3.7^{***}$	130	$35 \pm 6.7^{***}$	$43 \pm 5.3^{**}$
0.4	$41 \pm 3.3^{**}$	6 ± 2.9 ns	40	$20 \pm 9.6^{***}$	$28 \pm 7.4^{**}$
0.04	16 ± 3.1 ns	NT			

***: $p < 0.001$, **: $p < 0.01$, ns: not statistical significant ($p > 0.05$), ANOVA followed by Tukey post hoc test used; NT: not tested.
[a] Means ± SEM of $\geqslant 2$ experiments with eight animals per group.

Table 2. Yields for the preparation of **4a–f** from **3**

Entry	R1	Yield[a] of **4** (%)
a	$(CH_2)_3CH_3$	93
b	$CH_2C_6H_5$	83
c	2-Benzimidazolylmethyl	92
d	$CH_2CO_2C(CH_3)_3$	89
e	(4-Aminosulfonylphenyl)ethyl	31
f	2-(1-Imidazolyl)-2-phenylethyl	79

[a] Sum of both isomers [all isomers were obtained as 1:1 mixture with the exception of **4d** ($\mathbf{4d_A}$:$\mathbf{4d_B}$ = 2:1)].

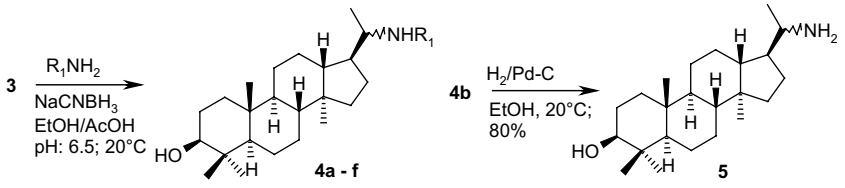


Scheme 1. Synthesis of **1**.

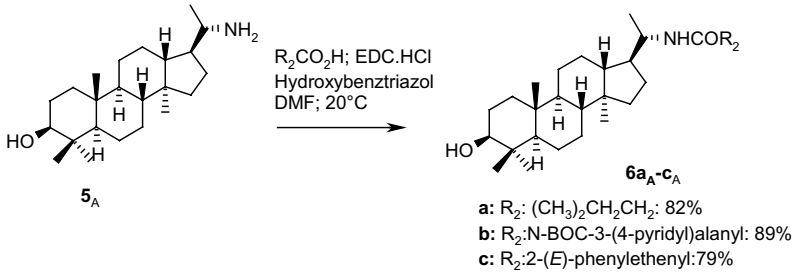
established ACD/mouse model[3] (topical application), for guidance in the design of synthetic analogues.

Anti-inflammatory active derivatives with the 17β configuration are synthetically attractive, because this configuration is thermodynamically more stable and therefore much easier accessible. Therefore, we investigated the influence of the configuration of the C-17 substituent on the biological activity together with side chain variations. Reductive amination reactions of **3** with a series of primary amines (**a–f**) in presence of $NaCNBH_3$ produced an array of different amines (**4a–f**, Scheme 2) and after acylation of amides (**6a–c**, Scheme 3) at C-18 (all with the 17β configuration), as their mixtures of diastereoisomers, which could be separated by conventional chromatography on silica gel. The absolute configurations of the newly created chiral center at C-18 could not be determined via NMR methods due to the unhindered rotation at the C-17 C-18 bond. We designated therefore the isomer with the higher R_f value with the letter A and the one with the lower R_f value with B (solvent systems: **4a**, **4f**, **5**, **13**, **15**: CH_2Cl_2/methanol: 9/1; **4a**: toluene/ethyl acetate: 1/2; **4d**: toluene/ethyl acetate: 2/1; **11**, **12**: toluene/ethyl acetate: 9/1).

Catalytic hydrogenation of **4b** gave $\mathbf{5_{AB}}$ in high yield, which were again separated via conventional chromatography. Crystals of the hydrochloride salt of $\mathbf{5_A}$



Scheme 2. Reductive aminations of **3** furnishing **4a–f**.



Scheme 3. Preparation of $\mathbf{6a_A}$–$\mathbf{c_A}$.

showing appropriate diffracting properties allowed us to determine its 3D structure by X-ray crystallography (Fig. 2).

The complete set of crystallographic data has been deposited in the Cambridge Crystallographic Data Base.[4] It is reasonable to assume, that compounds **6a**$_A$–**c**$_A$, which were obtained from **5**$_A$ have the same absolute stereochemistry.

Next we aimed at compounds more closely related to **1**. Compound **1** carries a methylene substituent and an aliphatic side chain at C-18. Scheme 4 shows our successful synthetic strategy using a Wittig approach to obtain analogues of **1** with –NH_2, –OH, –CH_3 and a methylene group at C-18 together with a phenethenyl side chain. This Wittig strategy, which will allow preparing an array of different side chains, made it necessary to monobrominate protected **3**, a reaction that proceeded in high yield.

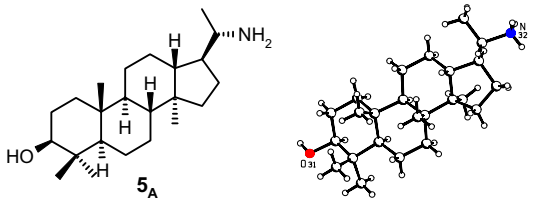
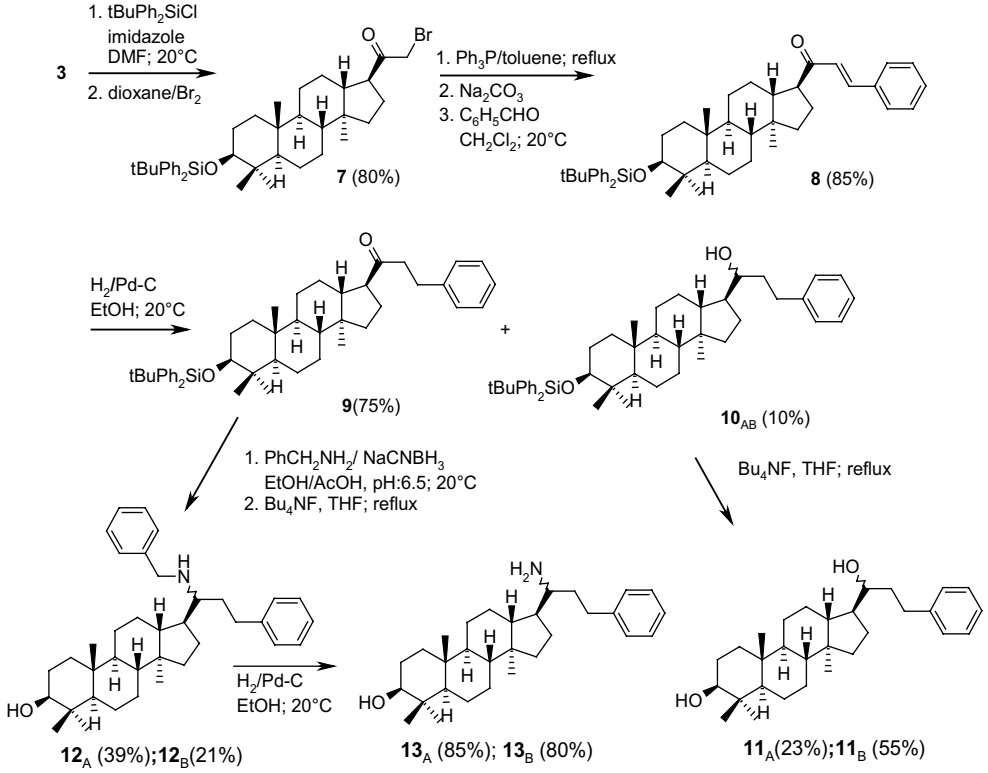


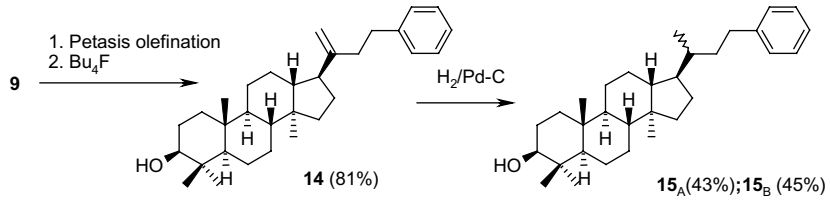
Figure 2. Structure and ORTEP plot of **5**$_A$.

Bromination of 3-*O*-*tert*-butyl diphenylsilyl (TBDPS) protected **3** with Br_2 in CCl_4 gave a mixture of starting material together with mono-, di- and tribrominated products. *N*-Bromosuccinimide did not lead to the desired **7** either. Finally we succeeded by applying dioxane dibromide[5] as the halogenating agent affording **7** in excellent yield. The yield of the side products (dibromide and tribromide) amounted to less than 3%. Straightforward treatment of **7** with triphenylphosphine gave the expected phosphonium salt, which was transformed into the stabilized Wittig ylide under alkaline conditions. Reaction with benzaldehyde produced smoothly the olefin **8**. Hydrogenation of **8** gave **9** together with a diastereoisomeric mixture of **10**. Removal of the TBDPS group from the unsaturated and the saturated ketones, **8** and **9**, with TBAF failed. However, deprotection of the alcohol **10** could be achieved leading to **11**. The two diastereomers again could be separated via chromatography (toluene/ethylacetate: 9/1). Reductive amination of **9** and removal of the TBDPS group furnished after separation the desired benzylamine derivatives **12**$_A$ and **12**$_B$. Hydrogenolytic cleavage of the *N*-benzyl residue resulted in **13**$_A$ and **13**$_B$. Compound **9** served also as suitable starting material for the introduction of a methyl group instead of an amino group.

Thus, as outlined in Scheme 5, Petasis olefination[6] of **9** followed by cleaving off the TBDPS group gave the *exo*-methylene compound **14** in 81% yield. Catalytic hydrogenation of **14** with H_2/Pd–C resulted in **15**$_A$ and **15**$_B$ as diastereomeric mixture. Chromatographic separation gave **15**$_A$ and **15**$_B$.



Scheme 4. Wittig strategy for the preparation of **10**–**13**.



Scheme 5. Petasis olefination and hydrogenation of **9**.

Table 3. Activity of analogues **3**–**15** of **1** in the inflammatory ACD/mouse model after topical application

Compd	R_1 (for **4** and **5**) or R_2 (**6**)	ACD mouse; % inhib.[a]
1	—	$42 \pm 3.7^{**}$
3	—	12 ± 7.5 ns
$\mathbf{4a}_A$	$(CH_2)_3CH_3$	$30 \pm 4.9^{**}$
$\mathbf{4a}_B$	$(CH_2)_3CH_3$	$28 \pm 4.3^{**}$
$\mathbf{4b}_A$	$CH_2C_6H_5$	$28 \pm 8.1^{*}$
$\mathbf{4b}_B$	$CH_2C_6H_5$	15 ± 6.5 ns
$\mathbf{4c}_{AB}$	2-Benzimidazolylmethyl	$27 \pm 8.9^{*}$
$\mathbf{4d}_A$	$CH_2CO_2C(CH_3)_3$	$31 \pm 6.3^{*}$
$\mathbf{4d}_B$	$CH_2CO_2C(CH_3)_3$	$21 \pm 3.8^{*}$
$\mathbf{4e}_A$	(4-Aminosulfonylphenyl)ethyl	16 ± 8.1 ns
$\mathbf{4f}_{AB}$	2-(1-Imidazolyl)-2-phenylethyl	13 ± 9.1 ns
$\mathbf{5}_A$	H	$38 \pm 4.3^{**}$
$\mathbf{5}_B$	H	$41 \pm 4.6^{**}$
$\mathbf{6a}_A$	$(CH_3)_2CH_2CH_2$	$33 \pm 6.6^{*}$
$\mathbf{6b}_A$	*N*-Boc-3-(4-pyridyl)alanyl	28 ± 7.7 ns
$\mathbf{6c}_A$	2-(*E*)-Phenylethenyl	11 ± 12.4 ns
$\mathbf{11}_A$	—	12 ± 15.7 ns
$\mathbf{11}_B$	—	14 ± 13.6 ns
$\mathbf{12}_A$	—	$22 \pm 6.9^{*}$
$\mathbf{12}_B$	—	15 ± 8.2 ns
$\mathbf{13}_A$	—	30 ± 9.1 ns
$\mathbf{13}_B$	—	6 ± 11.9 ns
14	—	$36 \pm 6.0^{**}$
$\mathbf{15}_A$	—	$30 \pm 7.4^{**}$
$\mathbf{15}_B$	—	$29 \pm 6.9^{**}$

**: $p < 0.01$, *: $p < 0.05$, ns: not statistical significant ($p > 0.05$), ANOVA followed by Tukey post hoc test used; for details see Ref. 2.

[a] One experiment; mean ± SEM values of eight mice per group; applied concn (0.1 M).

The biological activities of the compounds **3**–**15** are summarized in Table 3. Whereas the starting ketone **3** is inactive, we were able to obtain, as desired, anti-inflammatory active compounds with the 17β conformation. Interestingly, the stereochemistry at C-18 has nearly no influence on the biological activity. Alkylamino analogues (**4a**–**f**) are in general less active then the primary amine (**5**) and acylation of **5** abolishes the activity (**6a**–**c**). More bulky side chains are allowed, but in this case only methylene (**14**) and methyl (**15**) substituents at C-18 proved to be biologically active. The amino (**12**, **13**) or hydroxyl (**11**) analogues are inactive. The most active compound is $\mathbf{5}_B$. For details see Table 3.

In conclusion, starting from the 17-α-substituted triterpene lead **1** we have developed an efficient derivatization platform based on dammarane **3** as the common starting material. By this way, we identified compounds with the thermodynamically more stable and synthetically better accessible C-17β configuration, particularly $\mathbf{5}_B$, which exhibit promising in vivo activity in a well established animal model of skin inflammation.

Acknowledgements

We would like to thank Sabine Weber-Roth, Hannelore Schmidt for technical assistance, Gerhard Schulz and Ewald Haidl for Spectra recording and assistance in their interpretation and Trixi Wagner for depositing the X-ray data in the CCDC and Erwin Schreiner for fruitful discussions and critically reading the manuscript.

References and notes

1. Revesz, L.; Hiestand, P.; La Vecchia, L.; Naef, R.; Naegeli, H.-U.; Oberer, L.; Roth, H.-J. *Bioorg. Med. Lett.* **1999**, *9*, 1521.
2. Citations 1–3 in Ref. 1.
3. Meingassner, J. G.; Grassberger, M.; Farngruber, H.; Moore, H. D.; Schuurman, H.; Stütz, A. *Br. J. Dermatol.* **1997**, *137*, 568.
4. Crystallographic data (excluding structure factors) for this structure in the paper have been deposited with the Cambridge Crystallographic Data Centre as supplementary publication number CCDC212085. Copies of the data can be obtained, free of charge, on application to CCDC, 12 Union Road, Cambridge, CB2 1EZ, UK [fax: +44(0)1223 336033 or e-mail: deposit@ccdc.cam.ac.uk].
5. Pettit, G. R.; Green, B.; Dasgupta, A. K.; Whitehouse, P. A.; Yardley, J. P. *J. Org. Chem.* **1970**, *35*, 1381.
6. For a review concerning methenylation of C=O bonds with titanium reagents see: Hartley, R. C.; McKiernam, G. J. *J. Chem. Soc., Perkin Trans.* **2002**, 2763.