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A new mild PTSA-catalyzed method for sulfate ester hydrolysis and acid-catalyzed rearrangement of 12-acetyl-diene-11-ol tetracyclic triterpenoids involving an angular methyl migration

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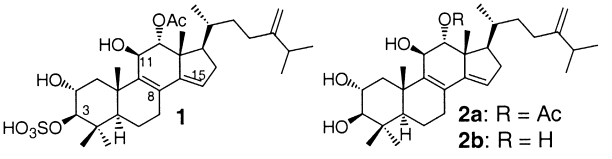
Abstract

Tetracyclic triterpenoids containing the 12-acetyl-$\Delta^{8,14}$-diene-11-ol moiety undergo a series of acid-catalyzed rearrangements. The rearrangement products have been characterized, plausible mechanisms for the rearrangement have been elucidated and conditions have been developed to give high yields of the rearrangement products. A new and general PTSA·H_2O and PPTS-catalyzed sulfate hydrolysis method has been developed.

The sulfated tetracyclic triterpenoid microbial metabolite **1** has been shown to possess a variety of biological activities. It was first discovered in our laboratories as an inhibitor of elastase ($IC_{50} = 2.4$ μM),[1] a protease which represents a potential therapeutic target for inflammatory and degenerative diseases. Subsequently, it was reported as an inhibitor of rhinovirus 3C protease ($IC_{50} = 80$ μM)[2] and cholesteryl ester transfer protein ($IC_{50} = 13.8$ μM).[3] The biological activities of compound **1** prompted us to investigate its chemistry for a structure–activity relationship (SAR). This required the conversion of compound **1** to compound **2a** by sulfate hydrolysis.



There are three main methods known for the hydrolysis of sulfate esters: (i) solvolysis by heating in dioxane;[4] (ii) mineral-acid-catalyzed hydrolysis;[5] and (iii) heating of the ester in a mixture of dioxane and pyridine.[6] The first method appeared attractive because of its simplicity and became the method of choice initially. In this method compound **1** was heated in dioxane at

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50–60°C for 30 min to give a good yield (~50–60%) of the desired compound **2a**.[1a] However, when the reaction was repeated it gave irreproducible results particularly on a larger scale and consistently afforded a mixture of rearrangement products **3**–**8**[7] in 2–5% yield. Sulfuric acid-catalyzed reaction also produced these compounds as the main products. Structures of these compounds (**3**–**8**) and plausible mechanisms for the rearrangements are shown in Figs. 1 and 2.

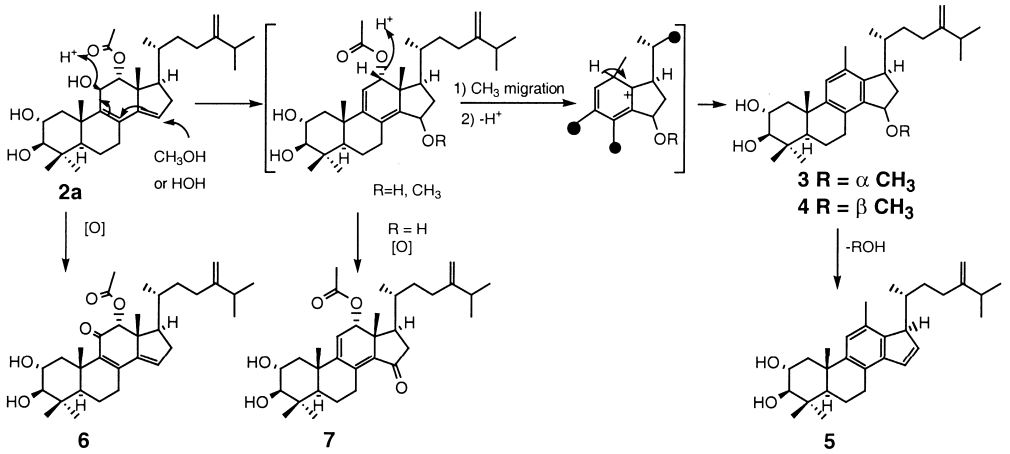


Figure 1. Structures and plausible mechanism of rearrangements

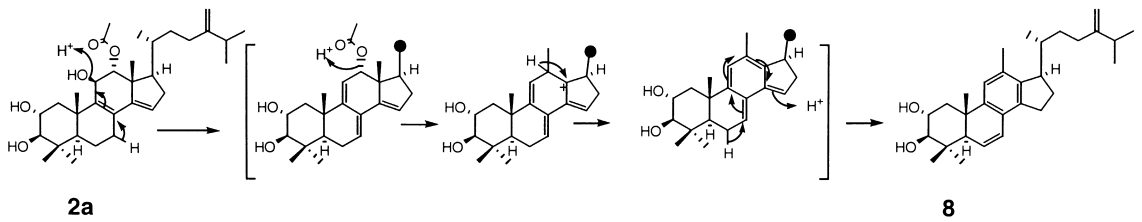


Figure 2. Structures and plausible mechanism of rearrangements

In the third hydrolysis method, heating of compound **1** in a 1:1 mixture of dioxane and pyridine gave a brownish reaction mixture mostly containing compound **2a** along with low levels of several uncharacterized products as detected by HPLC. However, none of the methods gave satisfactory results on a gram scale and a new method was urgently needed.

In order to probe for milder methods of hydrolysis and to find an efficient method for the preparation of the rearrangement products, a set of parallel experiments was designed where **1** or **2a** was reacted with either mineral acids (HCl or H_2SO_4) or organic acid (PTSA·H_2O) in various aprotic and protic solvents (dioxane, THF, CH_3CN, CH_3OH and H_2O) that led to the identification of the following conditions for both processes.

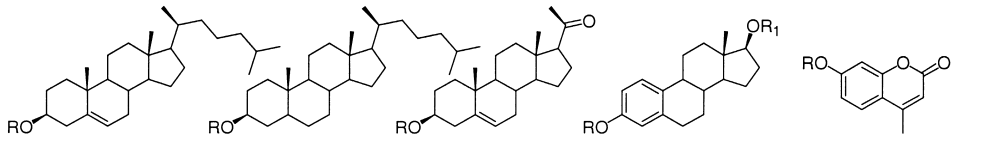
Controlled rearrangements: The reaction of either compound **1** or **2a** in neat methanol with H_2SO_4 or PTSA·H_2O at ambient temperature in less than 30 min produced compounds **3**, **4**, **5** and **6** in a ratio of 45, 45, 5, 5 (combined 90% yield), respectively. Addition of co-solvents (10 to 90%, v/v) such as dioxane and water in methanol did not have any detrimental effect on the H_2SO_4-catalyzed reaction other than slowing the rate of reaction. However, similar dilution had a significant impact on the reactivity of compound **2a** in the PTSA·H_2O-catalyzed reaction

including complete recovery of the starting substrate. Accordingly, the reaction of the 12-hydroxy compound **2b** with PTSA·H_2O in CH_3OH produced *exclusively* compounds **3** and **4** and therefore, these reactions provide a new method for efficient synthesis of C-ring aromatic compounds from this expanding series of natural products.

Efficient and decomposition free sulfate hydrolysis of compound 1: The aforementioned observation of the sulfate hydrolysis and stability of compound **2a** in dioxane containing PTSA·H_2O led to the development of the following efficient method for the conversion of compound **1** to **2a**. The reaction of **1** with 10–100 mol% of PTSA·H_2O in dioxane exclusively produced compound **2a** in 5–30 min (depending on the scale) without any rearrangement products.[8] The reaction is consistently reproducible and amenable to larger scale synthesis as demonstrated by the conversion of 29 g of **1** to 23 g of **2a** in 92% yield. During this study it was observed that the free acid form of compound **1** undergoes significant decomposition upon storage, which can be prevented by conversion to a sodium salt.

General sulfate hydrolysis: This PTSA·H_2O-catalyzed method of the sulfate hydrolysis is general as exemplified by the hydrolysis of the sulfates **9a**–**13a** (Fig. 3, Table 1) to their corresponding hydroxy derivatives **9b**–**13b**, respectively. The rate of the hydrolysis of these compounds varied due to the solubility of the sulfated salts in aprotic solvents. For example, the reactions of compounds **9a**–**11a** in dioxane with 50 mol% of PTSA·H_2O in less than 30 min gave over 90% yield of cholesterol (**9b**), cholestanol (**10b**) and pregenalone (**11b**), respectively. However, due to the lack of solubility of estradiol-potassium-bis-sulfate (**12a**) and 4-methyl-umbelliferone potassium sulfate (**13a**) in dioxane or THF, these substrates required the addition of water for better solubility. The hydrolysis of these sulfates then required longer reaction time and elevated temperature as exemplified below. The reaction of **12a** in 1:1 dioxane:water with 50 mol% of PTSA·H_2O required reaction for 72 h at ambient temperature to selectively produce the mono-sulfated estradiol **12b**, which, upon further heating at 60°C, afforded diol **12c**. Alternatively, the reaction in 1:1 methanol:water with heating at 80°C for 70 h also exclusively afforded **12b**. The hydrolysis of the second sulfate group in this reaction was difficult and required an additional equivalent of PTSA·H_2O followed by further heating. Likewise, the reaction of **13a** in 3:1 methanol:water with heating at 60°C for 16 h gave 4-methyl-umbelliferone **13b**. For a head-to-head comparison of different hydrolytic methods, sulfate hydrolysis reactions of compounds **10a** and **11a** were repeated using H_2SO_4 and solvolysis in dioxane. All three reactions gave identical results except that the rate of the hydrolysis in neat dioxane was slightly slower and required heating at 80°C. PPTS is also an effective hydrolytic agent but required heating.

In conclusion, a PTSA·H_2O-catalyzed versatile and mild method for the hydrolysis of sulfate esters, either as the free acid or salt form, has been developed.[9] In contrast to other methods, this method can be used for the hydrolysis of sulfates possessing *heat* and *mineral acids* sensitive



9a: R = SO_3Na **9b**: R = H
10a: R = SO_3Na **10b**: R = H
11a: R = SO_3Na **11b**: R = H
12a: R = R_1= SO_3K **12b**: R = H, R_1= SO_3K **12c**: R = R_1 = H
13a: R = SO_3Na **13b**: R = H

Figure 3. Structures of sulfates and hydrolyzed products

Table 1
PTSA·H_2O-catalyzed hydrolysis of sulfates

Substrate	Product	Solvent	Temperature	Time	Yield %
1	**2a**	Dioxane	Ambient	5 – 30 min	92
9a	**9b**	Dioxane	Ambient	5 – 15 min	93
10a	**10b**	Dioxane	Ambient	5 – 15 min	95
11a	**11b**	Dioxane	Ambient	5 – 15 min	97
12a	**12b**	Dioxane – H_2O	Ambient	72 h	80
12a	**12c**	Dioxane – H_2O	60 °C	16 h	90
12a	**12c**	CH_3OH – H_2O	80 °C	4 days	85
13a	**13b**	CH_3OH – H_2O	60 °C	16 h	96

molecular structures. In addition, the products of the acid-catalyzed rearrangement of compound **2a** have been characterized and several plausible mechanisms have been proposed. Efficient methods for the rearrangements have been developed that provide the possibility for the efficient synthesis of C-ring aromatic compounds of this expanding series of natural products.

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7. Purity of compounds was verified by reverse phase HPLC and their structures were fully elucidated by 1D and 2D NMR spectroscopy and mass spectrometry including MS/MS measurements.
8. In a typical experiment, to a solution of compound **1** (29 g, 0.047 mol) in dioxane (300 mL) was added PTSA·H_2O (2.9 g, 0.015 mol) and the solution was stirred at room temperature. After 30 min it was quenched by addition of a 10% aqueous solution of $NaHCO_3$, extracted with ethyl acetate (3×1.5 L), and the organic layer was separated, washed with water (2×1 L), dried (Na_2SO_4), concentrated and chromatographed on a silica gel column to give compound **2a** (23 g, 92%).
9. The sulfate group of a sulfated hexaglycoside was hydrolysed by this method without affecting the glycosidic linkages. Due to the interest of the biological activity of this new glycoside, the structure cannot be disclosed at this point and will be reported elsewhere in due course.