Steroids 77 (2012) 602–608

Contents lists available at SciVerse ScienceDirect

Steroids

journal homepage: www.elsevier.com/locate/steroids

NMR assignment of the absolute configuration of C-25 in furostanol steroidal saponins

Victoria L. Challinor [a], Sonia Piacente [b], James J. De Voss [a,*]

[a] School of Chemistry and Molecular Biosciences, The University of Queensland, Brisbane, QLD 4072, Australia
[b] Dipartimento di Scienze Farmaceutiche e Biomediche, Università degli Studi di Salerno, Via Ponte Don Melillo, I-84084 Fisciano, Italy

ARTICLE INFO

Article history:
Received 21 December 2011
Received in revised form 2 February 2012
Accepted 4 February 2012
Available online 14 February 2012

Keywords:
Steroidal saponins
Furostanol
Stereochemistry
NMR spectroscopy
Structure elucidation

ABSTRACT

The chemical shifts of the geminal proton resonances of H_2-26 (δ_a and δ_b) are a widely used predictor of C-25 stereochemistry in furostanol steroidal saponins, being in general more resolved in 25*S* than 25*R* compounds. Unexpectedly, we found that application of this empirical rule in different solvents led to conflicting assignments of stereochemistry. An experimental survey revealed that, while the chemical shifts of H_2-26 exhibit a dependence on C-25 configuration, it is less pronounced in methanol-d_4 than pyridine-d_5 solvent, and thus the general rule derived for pyridine-d_5 fails when NMR spectra are acquired in methanol-d_4. We propose a modified empirical method for the direct assignment of C-25 stereochemistry in furostanol saponins in methanol-d_4 (Δ_{ab} = 0.45–0.48 ppm for 25*S*; Δ_{ab} = 0.33–0.35 ppm for 25*R*), and provide several detailed examples. In addition, the absolute configuration of compound **8**, a steroidal saponin isolated in previous work from *Ruscus colchicus*, is corrected from 25*R* to 25*S* stereochemistry.

1. Introduction

Steroidal saponins are a structurally diverse class of natural products that are abundant in terrestrial plants. Saponins are thought to be responsible for the therapeutic effect proposed for many medicinal herbs, as they exhibit a range of biological activities, including cytotoxic, anti-inflammatory, hemolytic, anti-fungal, and anti-bacterial properties [1]. Steroidal saponins consist of a C_{27} skeleton, typically an oxidized cholesterol derivative, bearing varying numbers of sugar residues at different positions. The majority of steroidal saponins isolated from plants fall into two structural subclasses: the pentacyclic furostanol saponins, which usually contain a hemiacetal moiety at C-22 and a glycosidic linkage (typically to a single β-D-glucose residue) at C-26, and the spirostanol saponins, which instead possess a bicyclic acetal at C-22. Pentacyclic "furostanol" saponins may be further divided into those that possess either a hydroxy or methoxy moiety at C-22 and those that posses $\Delta^{20(22)}$-unsaturation. In both furostanol and spirostanol saponins, C-25 is found naturally with either the *R* or *S* configuration. Further structural diversity is generated in this class of natural products by differences in the stereochemistry of C-22, *cis* or *trans* fusion of the steroid A and B rings, as well as differences in the steroid hydroxylation and glycosylation patterns. This structural variation may account for the wide range of bioactivities reported for steroidal saponins.

The structure and stereochemistry of steroidal saponins is typically elucidated using a combination of 1D and 2D NMR spectroscopy, multistage mass spectrometry (MS^n), and chemical degradation. In spirostanol saponins, the stereochemistry of C-25 may be determined via examination of the proton vicinal coupling constants for positions 24–26, as well as comparison of the ^1H and ^{13}C NMR chemical shifts of the steroid F ring with literature values [2,3]. Infrared spectroscopy has been used for distinguishing between spirostanol and furostanol saponins, as the former possess characteristic absorption bands at around 980, 920, 900, and 860 cm^{-1} [4–6]. The relative intensities of the 920 and 900 cm^{-1} bands are predictive of the C-25 configuration in spirostanols (920 > 900 in 25*S*; 900 > 920 in 25*R*) [4–6]. The determination of C-25 stereochemistry is more challenging in furostanol saponins, which do not posses the rigid bicyclic system present in their spirostanol analogues. There are reports in which the intensities of IR absorption bands have been used to assign the C-25 configuration in furostanols [7,8], as well as spirostanols, but there appears to be no basis for the former application [9,10]. The most reliable method for determination of C-25 configuration in furostanol saponins is conversion to the corresponding spirostanol form, either through enzymatic cleavage of the C-26 glucose residue specifically to effect ring-closure of the side chain [11], or complete hydrolysis to yield the free spirostanol aglycone. The stereochemistry of the resulting spirostanol can then be determined as discussed above.

* Corresponding author. Tel.: +61 7 3365 3825; fax: +61 7 3365 4299.
E-mail address: j.devoss@uq.edu.au (J.J. De Voss).

0039-128X/$ - see front matter © 2012 Elsevier Inc. All rights reserved.
doi:10.1016/j.steroids.2012.02.002

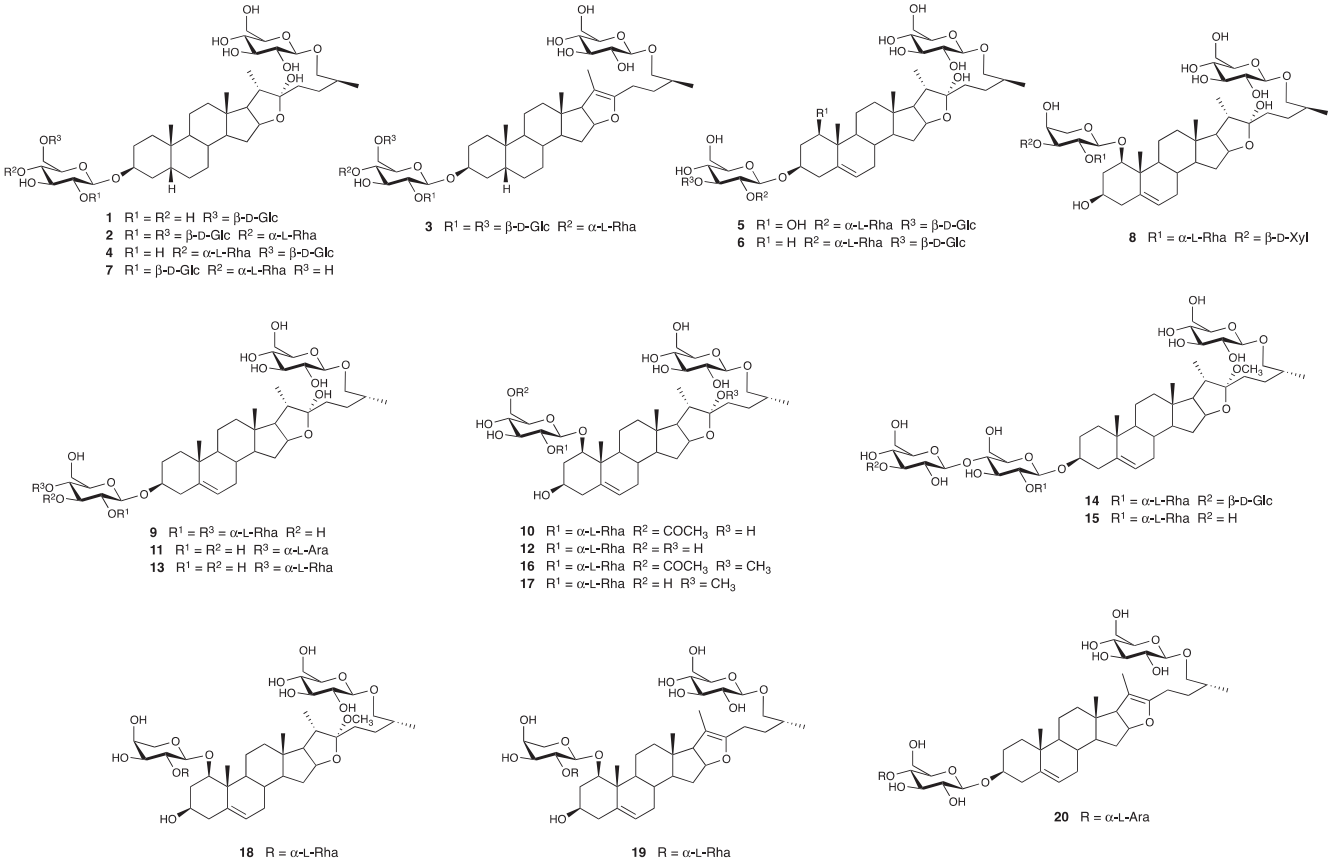

Fig. 1. Furostanol steroidal saponins studied in this work.

The absolute configuration of C-25 in furostanol saponins may also be assigned directly by NMR spectroscopy. Empirically, it has been found that both the chemical shifts and resolution of the geminal proton resonances of H_2-26 (δ_a and δ_b, $\Delta_{ab} = \delta_b - \delta_a$) are dependent on the configuration of C-25. The resolution of the H_2-26 resonances is normally used to assign C-25 stereochemistry, as these signals are more separated in 25*S* ($\Delta_{ab} \geqslant 0.57$ ppm) than 25*R* furostanols ($\Delta_{ab} \leqslant 0.48$ ppm) [9,10]. In addition, the chemical shift of H_3-27 occurs at slightly higher field in 25*R* (δ_H 0.98–1.03 ppm) as compared with 25*S* saponins (δ_H 1.01–1.05 ppm) [9,10]. The initial reports gave examples in both pyridine-d_5 and methanol-d_4 [9,10], and this predictor of C-25 stereochemistry has since been widely applied in both of these solvents [12–16].

Previously, we have reported the spectroscopic characterization of furostanol saponins **1**–**4** (Fig. 1) isolated from *Smilax* sp. in either pyridine-d_5 or pyridine-d_5/D_2O (~9:1) [12]. We subsequently investigated the assignment of **1**–**4** in methanol-d_4 because of significantly improved spectrum quality. We noted that, according to the originally reported empirical rule [9,10], the stereochemical assignment of C-25 in these compounds (**1**–**4**) appeared to vary with the NMR solvent used i.e. 25*S* assigned in pyridine-d_5, 25*R* assigned in methanol-d_4. This observation led to our discovery that, by relying on the chemical shifts of H_2-26 in methanol-d_4, we had incorrectly assigned the 25*R* configuration to furostanols **5** and **6** (Fig. 1) isolated from *Dioscorea* sp. [15,16]. We wished to investigate whether our incorrect assignment of 25*R* configuration in **5** and **6** was an isolated instance, or whether it reflected a general trend of direct NMR assignment of C-25 stereochemistry being unreliable in methanol-d_4. To examine this behavior, and compare the spectra of saponins in pyridine-d_5 and methanol-d_4 solvents, we undertook the complete ^1H and ^{13}C assignment of **1**–**3** (originally assigned in pyridine-d_5), and **5** and **6** (originally assigned in methanol-d_4), in the complementary solvents. Subsequently, another 15 saponins (**4**, **7**–**20**) were examined in methanol-d_4 and pyridine-d_5 in order to understand the relationship of the configuration at C-25 and the chemical shifts of H_2-26.

2. Experimental

2.1. General

Compounds **1**–**4** were isolated from the roots of *Smilax* sp. (sample provided as *Smilax ornata*) as described previously [12]. Compounds **5** and **6** were obtained from the rhizome of *Dioscorea* sp. (provided as *Dioscorea transversa*) as described previously [15,16]. Compound **7** was isolated from the roots of *Asparagus racemosus* as described previously [17,18]. Compounds **8** and **13** were isolated from *Ruscus colchicus* [19]. Compound **9** was isolated from *Tribulus terrestris* [20]. Compounds **10**–**12**, **16**, **17**, **19**, and **20** were isolated from *Ruscus ponticus* as described previously [21]. Compounds **14** and **15** were isolated from *Dioscorea villosa* in previous work [22]. Compound **18** was obtained from *Helleborus caucasicus* [23].

2.2. NMR Spectra

Spectra were recorded at 298 K on a Bruker AV500, AV750 or DRX600 spectrometer using 5 mm triple-resonance inverse probes equipped with *z*-gradients. ^1H NMR spectra were recorded at 500.13 MHz for **2**, at 749.15 MHz for **1** and **3**–**6**, at 749.02 MHz for **7** and **9**, and at 599.19 MHz for compounds **8**, **10**–**13**, **16**–**18**,