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(25*R*)-ISONUATIGENIN, AN UNUSUAL STEROIDAL SAPOGENIN FROM *VESTIA LYCIOIDES*

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Key Word Index—*Vestia lycioides*, Solanaceae, steroidal sapogenin; (25*R*)-isonuatigenin.

Abstract—(25*R*)-Isonuatigenin, a Δ^5-spirosten-3β,25-diol, was isolated from aerial parts of *Vestia lycioides*. Its structure was elucidated mainly by ^{1}H NMR and ^{13}C NMR spectroscopy. A mixture of (25*R*)-nuatigenin and (25*S*)-isonuatigenin was also characterized. This is the first report on the natural occurrence of the two (25*R*)-isomers.

INTRODUCTION

V. lycioides, a monotypic endemic genus of Chile, has been the subject of a number of reports on the chemistry of its secondary metabolites. Thus the isolation and structural elucidation of isoquercetrine and a new flavonol-glycoside, quercetine-3-α-(2-*O*-β-D-glucopyranosyl)-D-glucofuranoside, was reported [1, 2]. We published the first report of the isolation of an indole-type alkaloid in the Solanaceae, 1-acetyl-3-carboxymethyl-β-carboline [3], followed by the isolation of the corresponding 3-carboxy-acid, together with β-amyrin, oleanolic acid, fraxetine and diosgenin [4]. The isolation of a second steroidal sapogenin, designated G-2, and the preliminary analysis of its spectroscopic data was also described [4]. In this communication we describe the structure elucidation of a naturally occurring steroidal sapogenin, (25*R*)-isonuatigenin, and provide physical and spectroscopic evidence which indicate that G-2 is a mixture of (24*S*)-isonuatigenin and (25*R*)-nuatigenin.

RESULTS AND DISCUSSION

The mixture of aglycones obtained from the hydrolysate of saponins, was separated by repeated silica gel column chromatography. One of the compounds isolated, $C_{27}H_{42}O_4$ (M^+ at m/z 430) gave a mass spectrum very similar to that reported for nuatigenin and (25*S*)-isonuatigenin and other steroidal sapogenins with an hydroxyl group in ring F [5]. The mass spectrum showed a base peak at m/z 155, together with prominent signals at m/z 399, 342, 300, 282 and 271. A direct comparison (TLC, IR, NMR) with authentic samples clearly indicated that this product did not correspond to either nuatigenin or (25*S*)-isonuatigenin. The assignment of the hydroxyl group at C-25, its equatorial disposition, as well as the full stereochemistry of this compound, followed from the examination of the high frequency ^{1}H NMR and ^{13}C NMR spectroscopic data. The chemical shifts of the methyl groups corresponding to C-18, C-19 and C-21 were found to be very close to those corresponding to isonuatigenin [6], but the value for the protons of C-27 at δ1.29 (1.1 in isonuatigenin) clearly indicated the 25*R*-configuration [7]. This assignment was fully corroborated by the ^{13}C NMR data (see Experimental) which by comparison with that of closely related analogs also confirmed the complete stereochemistry. Thus, placement of a hydroxyl group at C-25 induced the expected shifts of the carbon atoms of ring F, as compared with the corresponding values reported for diosgenin [8] while the remaining signals were nearly identical. Furthermore, the small (-1.5 ppm) γ_g-effect displayed by C-23 is more consonant with an axial methyl group at C-25 instead of an axial hydroxyl group. The latter would show a larger γ_g-effect, e.g. -6.7 ppm, as is the case in (25*R*)-Δ^5-spirostan-25-ol [9]. Considering the above evidence, the complete structure and stereochemistry of this sapogenin was deduced to be (20*S*,22*S*,25*R*)-Δ^5-spirosten-3β,25-diol, that is (25*R*)-isonuatigenin.

A re-examination of the spectroscopic data of G-2 and comparison with the data obtained for (25*R*)-isonuatigenin, clearly indicated that it was a mixture of two closely related steroidal sapogenins. On the basis of the ^{1}H NMR spectrum (400 MHz) of this mixture and comparison with values reported in the literature [10] together with direct comparison (TLC) with authentic samples it was shown that G-2 was a mixture of (25*R*)-nuatigenin (major compound) and (25*S*)-isonuatigenin. Of particular diagnostic value were the signals corresponding to the H-27 and H-26 for both compounds (Table 1). This is the first report on the natural occurrence of (25*R*)-nuatigenin and its isomer (25*R*)-isonuatigenin. The latter compound had been previously obtained but poorly characterized) as a by-product in the synthesis of (25*S*)-isonuatigenin [11]; (25*R*)-nuatigenin was also known only as a synthetic product [10]. On the other hand (25*S*)-isonuatigenin, although not a common metabolite, has been already isolated from a number of *Solanum* species [5, 12].

It is generally accepted that the Δ^5-furosten-26-ols are the natural saponins which, during the acidic work-up, rearrange to the Δ^5-spirosten-25-ols, although recent work by Evans and co-workers [12] suggest that the reverse transformation might also occur in nature. In any event, our findings show that the 25*R*-isomers are natural

Table 1. ^{1}H NMR spectral data* of compounds 1, 2 and 3

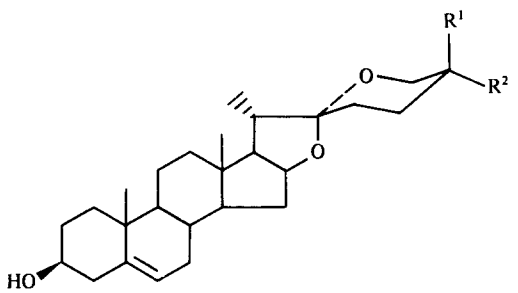
Compounds	H-19	H-18	H-21	H-27	H-26	H-16	H-3
1	1.02	0.79	0 98 *d* (7.0)	1 29	3.28 *dd* (10.3, 2.4) 3.60 *d* (10 3)	4.41 *ddd* (6 5, 6 5, 6 1)	3 54 *m*
2	1.02	0.79	1.03 *d* (6.3)	1 17	3 25 *dd* (11.0, 2.3) 3 74 *d* (11.0)	4.47 *m*	3.52 *m*
3	1 02	0.79	0.99 *d* (6.7)	1.31	3.34 *d* (11.0) 3.44 *d* (11 0)	4.40 *m*	3.52 *m*

*400 MHz, $CDCl_3$, TMS as int. standard. Values in parentheses are coupling constants in Hz

products since the configuration around C-25 does not change during the isomerization (hence, precluding a rearrangement of 25*S*-isonuatigenin into the 25*R*-isomers). Further work on the saponin content in unripe berries of *V. lycioides* is in progress.

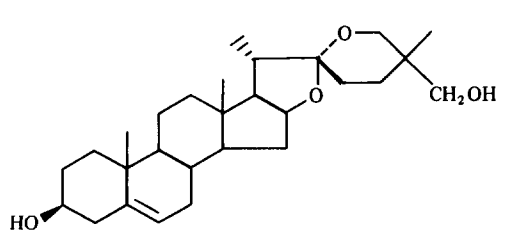
EXPERIMENTAL

^{1}H NMR and ^{13}C NMR spectra were recorded at 400 and 100 MHz, respectively. Mps (uncorr.) were determined on a Kofler hot stage apparatus. Analytical TLC was performed on silica gel 60-F (chromatoplates, Merck) and silica gel 60 (70–230) was used for CC. EIMS were recorded by direct inlet with 70 eV ionization. For experimental details on the isolation procedure see ref. [4]



1 R^1 = Me , R^2 = OH

2 R^1 = OH , R^2 = Me



3

(25R)-*Isonuatigenin* (**1**) (132 mg) was isolated from fractions 182–239 of the original CC [4] and further purified by CC (Al_2O_3, activity II) and elution with $CHCl_3$ Mp 260–262° (MeOH–Me_2CO) (lit. 215–218° [11]), R_f $CHCl_3$–MeOH (95:5) 0 25; IR ν_{max}^{KBr} cm^{-1}· 3350, 2925–2825, 1620, 1440, 1365, 1125, 1035, 1015, 910. EIMS *m/z* (rel. int)· 430 3064 $[M]^+$ (1), $C_{27}H_{42}O_4$ requires 430.3083, 399.3889 (27), calc. for $C_{26}H_{39}O_3$ 342.2554 (62), calc. for $C_{23}H_{34}O_2$ 342 2559; 300 2461 (11), calc for $C_{21}H_{32}O$ 300 2453; 282 2356 (16), calc for $C_{21}H_{30}$ 282.2348, 271 2053 (45), calc for $C_{19}H_{27}O$ 271 2062, 155 1070 (100), calc for $C_9H_{15}O_2$ 155.1072. ^{13}C NMR ($CDCl_3$, TMS as internal standard): 37.6 *t* (C-1), 31 6 *t* (C-2), 71.3 *d* (C-3), 42 3 *t* (C-4), 141.5 *s* (C-5), 121 5 *d* (C-6), 32.9 *t* (C-7), 31 8 *d* (C-8), 50 6 *d* (C-9), 37.0 *s* (C-10), 21 2 *t* (C-11), 40.1 *t* (C-12), 40 6 *s* (C-13), 56.9 *d* (C-14), 31.9 *t* (C-15), 81 6 *d* (C-16), 62.4 *d* (C-17), 16 4 *q* (C-18), 19 5 *q* (C-19), 41 4 *d* (C-20), 14.3 *q* (C-21), 109 5 *s* (C-22), 29 9 *t* (C-23), 34.6 *t* (C-24), 81 6 *s* (C-25), 69.2 *t* (C-26), 23 8 *q* (C-27)

(25S)-*Isonuatigenin* (**2**) *and* (25R)-*nuatigenin* (**3**) ^{1}H NMR (see Table 1). R_f $CHCl_3$–MeOH (95 5)· 0 43 and 0 49, respectively. IR and EIMS as in ref [4].

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