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CHARACTERIZATION OF A MINOR COMPOUND, WHICH ACCOMPANIES THE USUAL 22α(R)-O,25β(S)-SPIROSTANOL GLYCOSIDE, AS A NOVEL TYPE OF 22β(S)-O,25α(S) ANALOG

Keiko Kudo, Kazumoto Miyahara,[1] Nobuhiro Marubayashi and Toshio Kawasaki*,[1]
Faculty of Pharmaceutical Sciences, Kyushu University,
Maidashi 3-1-1, Higashi-ku, Fukuoka 812, Japan

The structure of one of the minor compounds, which coexists only with a 25β(S)-spirostanol glycoside (a steroid saponin) such as I, II, XI and XV, was determined to be the corresponding 22β(S)-O,25α(S) analog Ia, IIa, XIa and XVa, respectively.

Since it was believed that all the natural spirostanol glycosides have the 22α(R)-O configuration, these compounds are worthy of note as the first glycosides of 22β(S)-O-spirostanol so far isolated.

KEYWORDS —— steroid saponin; 25β(S)-spirostanol glycoside; rhodeasapogenin; sarsasapogenin; neotokorogenin; 22β(S)-O,25α(S)-spirostanol glycoside; IR; NMR; molecular rotation difference

Two glycosides R_3, 1-O-α-L-rhamnopyranosyl-(1-2)-β-D-xylopyranoside (rha-xyl) (I), and R_8, 3-O-β-D-glucopyranoside (glc) (II), of 5β,25β(S)-spirostane-1β,3β-diol (rhodeasapogenin) (III), which had been isolated from the underground parts of Rhodea japonica and regarded to be homogeneous, were found by high performance liquid chromatography (HPLC) to consist of four glycosides Ia∿d and IIa∿d. They were successfully separated in preparative scale, and the major component Id and IId were identified as pure I and II, while the aglycones of Ic and IIc were identified as 25α(R)-epimer (isorhodeasapogenin) (IV) and those of Ib and IIb as 25(27)-dehydro derivative (convallamarogenin) (V) of III. Ib∿d and IIb∿d have the same sugar moieties.[2]

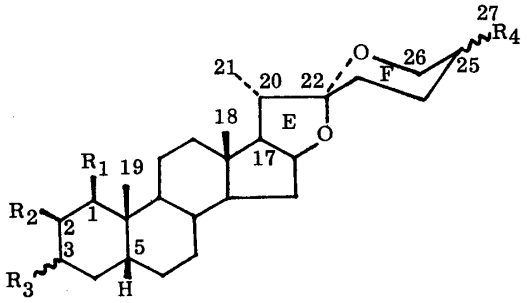
This paper deals with characterization of the fourth minor compounds Ia and IIa, and with examination of timosaponin A-III[3] and neotokoronin,[4] both of which have been known also as 5β,25β(S)-spirostanol glycosides.

Ia, prisms[5] (Me_2CO-MeOH-water), mp 275-277°C,[6] $[\alpha]_D$ -51.4°,[7] showed the $[M+H]^+$ ion at m/z 711 and $[M]^+$ at 710 in the field desorption(FD) - MS.[8] Taking the elemental analytical data into account, Ia has the same molecular formula, $C_{38}H_{62}O_{12}\cdot H_2O$, as Ic,d. In the ^{13}C-NMR[9] spectrum of Ia, only the signals assignable to C-20 ∿ C-27 and C-21 ∿ C-24 as well as C-26 of the aglycone were different from the respective signals of Id and Ic (Table I).[10] Ia showed an IR spectrum[11] somewhat unlike the spectra of Ic and Id in the characteristic absorptions of the spiroketal side chain,[12] but the relative intensity, 916 < 985 cm^{-1}, was more like that, 919 < 900, in Ic rather than the 919 > 894 in Id (Table II), suggesting a probable presence of the 25α(eq)-CH_3 group.[12] When Ia was hydrolyzed with 2N-H_2SO_4 in 50% EtOH with refluxing for 1.5 h, rhamnose and xylose together with III were yielded. But on treatment with 1N-HCl in 50% EtOH at 0°C for 2.5 h, Ia was transformed nealy quantitatively into Id. Thus, it was assumed that the aglycone of Ia might be different in stereo-structure from those of Ic and Id with respect to the E ∿ F ring moiety. The possibility of a β-configuration of the 20-CH_3 group could be excluded by comparing the ^1H-NMR[9] (Table III)[13] and ^{13}C-NMR data (Table I) of Ia with those of Ic, Id, cyclopseudo-diosgenin (VI) and -sarsasapogenin (VII).[14,15]

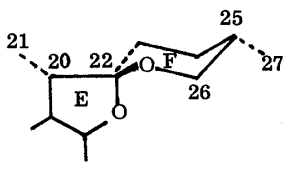
Therefore, the aglycone of Ia is regarded as the 22β-O,25α(eq)-CH_3 isomer of III. The configuration of the 25α(eq)-CH_3 group, suggested by the IR spectrum, was endorsed by the similarity of the signals of 26-H_2 and 27-H_3 in the ^{1}H-NMR spectrum and C-25 and C-27 in the ^{13}C-NMR spectrum with those of Ic (Tables I and III), and the unusual 22β-O-configuration was corroborated by the large molecular rotation difference (+379°) between Ia and Id.[17] Ia is now considered to be 1-O-rha-xyl of 5β,22β(S)-O,25α(S)-spirostane-1β,3β-diol (VIII), conventionally named 22-epirhodeasapogenin.

IIa, needles (dil.MeOH), mp 257-259°C, $[\alpha]_D$ -31,2°, has the same molecular formula, $C_{33}H_{54}O_9$ (FD-MS: $[M+H]^+$, m/z 595; elemental analytical data), as IIc,d. The ^{13}C-NMR and IR spectral data Tables I and II) suggested, as in the case of Ia, that IIa has the 25α(eq)-CH_3 group, but that the stereo-structure of the E ∿ F-ring moiety is different from either IIc or IId. In the ^{1}H-NMR spectrum of IIa in comparison with that of Ia (Table III), the signal of 19-H_3 was shifted to an upper field by 0.06 ppm, but others, 18-, 21- and 27-H_3 as well as 26-H_2, showed the δ-values and the coupling patterns identical to those of Ia. This implys that IIa has the same aglycone as Ia, but that the sugar moiety is combined with the hydroxyl group at C-3 and not C-1. The fact that acid treatment of IIa at 0°C in the same way as for Ia afforded IId, and the molecular rotation difference (+198°) between IIa and IId supported the 22β-O configuration of the aglycone of IIa.

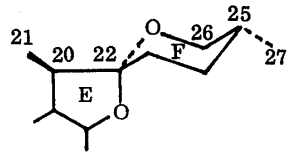
Consequently, IIa is 3-O-glc of 22-epirhodeasapogenin (VIII).



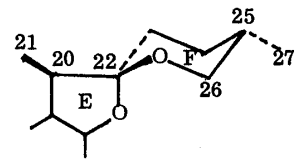
Ib: R_1= rha-xyl-O-; R_2= H; R_3= β-OH; R_4= =CH_2 (aglycone= V)
Ic: R_1, R_2, R_3= same as above; R_4= α(eq)-CH_3 (aglycone=IV)
Id: " R_4= β(ax)-CH_3 (aglycone=III)
IIb: R_1= OH; R_2= H; R_3= glc-O-(β); R_4= =CH_2 (aglycone= V)
IIc: R_1, R_2, R_3= same as above; R_4= α-CH_3 (aglycone=IV)
IId: " ; R_4= β-CH_3 (aglycone=III)
XIc: R_1=R_2= H; R_3= glc-gal-O-(β); R_4= α-CH_3 (aglycone=XIII)
XId: R_1, R_2, R_3= same as above; R_4= β-CH_3 (aglycone=XII)
XVc: R_1=ara-O-; R_2= OH; R_3= α-OH; R_4= α-CH_3
XVd: R_1, R_2, R_3= same as above; R_4= β-CH_3



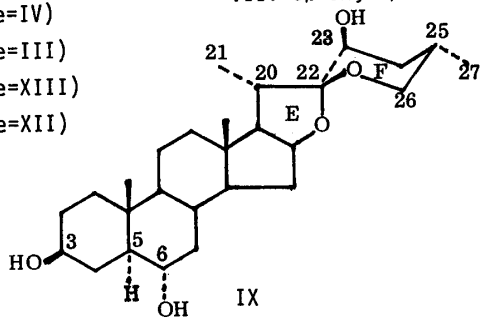
Ia: R_1= rha-xyl-O-; R_2= H; R_3= β-OH (aglycone=VIII)
IIa: R_1= OH; R_2= H; R_3= glc-O-(β) (aglycone=VIII)
XIa: R_1= R_2= H; R_3= glc-gal-O-(β) (aglycone=XIV)
XVa: R_1= ara-O-; R_2= OH; R_3= α-OH (aglycone=XVI)



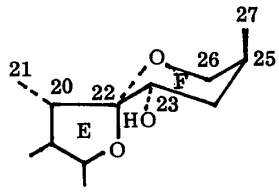
VI: 3β-OH, Δ^5



VII: 3β-OH, 5β-H



IX



X

The stereochemistry of the 17-side chain of the spirostane nucleus was extensively investigated,[10,12~18] but the studies were based on the fact that only two stereoisomers due to an asymmetric carbon atom in the E ~ F-ring moiety had so far been found in nature. It has been believed that both the configurations at C-20 and -22 are R (20α-CH_3 and 22α-O), and that the two natural isomers have either the 25(R)(α(eq)-CH_3) (more stable, iso-type) or the 25(S)(β(ax)-CH_3) (normal- or neo-type) configuration.

Lately, Chakravarty et al[19] obtained, by Smith degradation of the crude glycoside of Solanum hispidum, a novel type of spirostanol, hispigenin (IX), which has an unusual 22β-O- configuration, along with the usual (22α-O) isomer, 5α,25β(S)-spirostane-3β,6α,23α-triol (paniculogenin) (X).

Ia and IIa are noteworthy as the first naturally occurring 22β(S)-O-spirostanol glycoside ever isolated.

In contrast to the findings that Ia, IIa and IX accompany their 22α(R)-O,25β(S) analogs, the glycosides of 22α(R)-O,25α(R)-spirostanols such as diosgenin, gitogenin and digitogenin are, as reported previously,[2] coexistent only with their 25(S)-epimers. In order to verify the generality of co-occurrence of the glycosides of 22α(R)-O,25β(S)-spirostanols with those of the corresponding 22-epimers, two additional 25β(S)-spirostanol glycosides which had been regarded as homogeneous were examined.

Timosaponin A-III (XI),[3] 3-O-β-D-glucopyranosyl-(1-2)-β-D-galactopyranoside (glc-gal) of 5β, 25β(S)-spirostan-3β-ol (sarsasapogenin) (XII), prisms (70% MeOH), mp > 300°C, $[\alpha]_D$ -41.6°, was shown by HPLC[2] to consist of three compounds. They were separated to give XIa,c,d. XId (major), needles (MeOH-$CHCl_3$), mp > 300°C, $[\alpha]_D$ -47.5°, and XIc, needles (MeOH-$CHCl_3$), mp > 300°C, $[\alpha]_D$ -35.3°, were respectively identified as pure XI and its 25α(R)-epimer (3-O-glc-gal of smilagenin (XIII)).

XIa, prisms (MeOH-$CHCl_3$), mp 295-300°C, $[\alpha]_D$ -0.1°, has the same molecular formula, $C_{39}H_{64}O_{13}$ (FD-MS: $[M+H]^+$, m/z 741; $[M+Na]^+$, 763. elemental analytical data), and its IR (Table II), ^{13}C- and ^1H-NMR data (Tables I and III) indicated that XIa is presumably the 22-epimer of XId. The large molecular rotation difference (+338°) between XIa and XId substantiated the above presumption.

Thus, XIa is defined as 3-O-glc-gal of 5β,22β(S)-O,25α(S)-spirostan-3β-ol (22-episarsasapogenin) (XIV).

Table I. ^{13}C-NMR Data[9,10]

	C-20	C-21	C-22	C-23	C-24	C-25	C-26	C-27
Ia	42.1	17.0	110.6	28.3*	28.1*	30.7	69.6	17.3
Ic	42.0	15.0	109.2	31.7	29.2	30.6	66.9	17.3
Id	42.5	14.9	109.7	26.3	26.3	27.4	65.1	16.3
IIa	42.1	17.0	110.3	28.2*	28.1*	30.6	69.4	17.3
IIc	41.9	15.0	109.0	31,8	29.2	30.5	66.7	17.3
IId	42.4	14.8	109.5	26.1	26.1	27.4	64.9	16.2
VI**	46.4	11.4	108.4	31.8	28.5	37.3	68.1	17.2
VII**	45.9	9.9	106.8	34.1	29.1	29.9	67.5	17.1
XIa	42.2	17.0	110.5	28.2	28.2	30.9	69.6	17.4
XIc	42.0	15.1	109.2	31.8	29.3	30.6	66.9	17.4
XId	42.5	14.9	109.7	26.4	26.4	27.5	65.1	16.3
XVa	42.1	17.0	110.5	28.3*	28.4*	30.7	69.5	17.3
XVc	42.0	15.1	109.2	31.9	29.2	30.6	66.9	17.3
XVd	42.5	14.9	109.7	26.4	26.2	27.6	65.2	16.3

* may be reversed in horizontal column.
** taken in $CDCl_3$.

Table II. IR Data[11,12]

					(cm^{-1})
Ia	983	946	916 < 895	872	
Ic	981	945	919 < 900	863	
Id	986	945	919 > 894	850	
IIa	987	946	917 < 895	832	
IIc	980	945	918 < 900	864	
IId	988	945	920 > 898	850	
XIa	994	925	910 < 896	850	
XIc	981	949	919 < 899	861	
XId	986	948	919 > 898	850	

A glycoside, mp 277-278°C, obtained from the mother liquor of recrystallization (MeOH) of neotokoronin, 5β,25β(S)-spirostane-1β,2β,3α-triol 1-O-α-L-arabinopyranoside (ara) (XV),[4] was also a mixture of three compounds XVa,c,d as shown by HPLC,[2] and they were isolated in pure state. XVd, needles (dil.MeOH), mp 284-286°C, $[\alpha]_D$ -17.2°, and XVc, needles (dil.MeOH), mp 275-277°C, $[\alpha]_D$ -13.0°,

Table III. ^{1}H-NMR Data[9,13]

	19-H_3	18-H_3	21-H_3	27-H_3	26-H_2
Ia	1.30 s	0.97 s	1.00 d (J=6 Hz)	0.69 m	3.67 m
Ic	1.33 s	0.85 s	1.13 d (J=6 Hz)	0.69 d (J=6 Hz)	3.57 m
Id	1.32 s	0.83 s	1.13 d (J=6 Hz)	1.07 d (J=6 Hz)	3.35 d (J=11 Hz)*
VI**	1.01 s	0.95 s	1.13 d (J=8 Hz)	0.77 d (J=6 Hz)	3.45 m
VII**	0.96 s	1.01 s	1.09 d (J=7 Hz)	0.76 d (J=6 Hz)	3.47 m
IIa	1.24 s	0.97 s	1.01 d (J=7 Hz)	0.69 m	3.68 m
XIa	0.96 s	0.96 s	1.02 d (J=7 Hz)	0.70 m	3.70 m

* 26β(eq)-H. ** taken in $CDCl_3$.

were respectively identified as pure XV and tokoronin (25α(R)).[20] XVa, prisms (dil.MeOH), mp 287-290°C, $[\alpha]_D$ +19.4°, has the same molecular formula, $C_{32}H_{52}O_9$, as XVc,d. In the same manner as for Ia, IIa and XIa (^{13}C-NMR data (Table I); molecular rotation difference (+214°) between XVa and XVd), it was determined to be 1-O-ara of 22-epitokorogenin (22β(S)-O,25α(S)) (XVI).

It is very likely that, regardless of the structure of the A ∿ B ring moiety of aglycone, the usual 22α(R)-O,25β(S)-spirostanol glycoside is generally accompanied by a minor amount of the corresponding glycosides of the 22-epimer (22β(S)-O,25α(S)). Absence of the glycoside of 25(27)-dehydro derivative in XI and XV is also noted.

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5) All crystals described in this paper were colorless.
6) Melting points were determined on a micro melting point apparatus MP-S_3 (Yanagimoto) and are uncorrected. In all cases reported herein, melting was accompanied by decomposition.
7) Optical rotations were taken with a JASCO DIP-SL automatic polarimeter (cell= 1 dm) at 18∿28°C, in a pyridine solution (c= 0.2 ∿ 1.4).
8) FD-MS spectra were recorded on a JEOL JMS-DX-300/JMA 3500.
9) ^{13}C- and ^{1}H-NMR spectra were taken with a JEOL FX-100 (25 and 100 MHz, respectively) in a C_5D_5N solution unless otherwise specified. Chemical shifts are given in δ values (ppm) using TMS as an internal standard.
10) cf. K. Tori, S. Seo, Y. Terui, J. Nishikawa and F. Yasuda, Tetrahedron Lett., 22, 2405 (1981).
11) IR spectra were recorded on a JASCO IR-G Spectrometer in KBr disk.
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15) Prepared from diosgenin and sarsasapogenin, respectively, according to the reported methods.[16]
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