(rel. int.): 398 $[M]^+$ (100), 383 $[M-Me]^+$ (81), 260 $[M-C_9H_{14}O]^+$ (82), 245 $[M-138-Me]^+$ (40).

Compounds **2**, **4** *and* **5**. From the Sephadex LH-20 column, 268 mg of a mixture of **4** and **5** were obtained as white needles, mp 164–167° (EtOAc). IR ν_{max}^{KBr} cm^{-1}: 3440 (OH), 1700 (ketone). EIMS (probe 70 eV) *m/z* (rel. int.): 470 $[M]^+$ (3), 452 $[M-H_2O]^+$ (11), 437 $[M-H_2O-Me]^+$ (15), 398 $[M-C_4H_8O]^+$ (21), 383 $[398-Me]^+$ (48), 260 $[398-C_9H_{14}O]^+$ (30), 245 $[260-Me]^+$ (28), $[43]^+$ (100). After the mixture was boiled in MeOH for 10 min the product was eluted through Sephadex LH-20 and compound **2** was obtained. After repeated crystallization from EtOAc and MeOH of the obtained material, 150 mg of white needles were obtained, mp 211–212°. IR ν_{max}^{KBr} cm^{-1}: 1700 (ketone), 1450, 1380, 1110, 1050, 1000, 910. EIMS (probe 70 eV) *m/z* (rel. int.): 484 $[M]^+$ (5), 469 $[M-Me]^+$ (4), 452 $[M-MeOH]^+$ (65), 437 $[452-Me]^+$ (41), 413 $[M-C_4H_7O]^+$ (100).

Oxidation of **4** *and* **5**. Compounds **4** and **5** (2 mg) were reacted with 10 mg HIO_4 in Et_2O for 30 min. TLC afforded 1.3 mg of **1**, identical with an authentic sample (^1H NMR and TLC).

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SPIROSTANOL GLYCOSIDES FROM *AGAVE CANTALA*

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Key Word Index—*Agave cantala*; Agavaceae; saponins; spirostanol glycosides; schistosomiasis; *Biomphalaria glabrata*.

Abstract—Two spirostanol glycosides, cantalasaponins -2 and -4 were isolated from the methanolic extract of the rhizomes of *Agave cantala* and were characterized. The first glycoside was found to be lethal against *Biomphalaria glabrata*, the snail vector of the disease schistosomiasis, at a concentration of 7 ppm.

INTRODUCTION

Agave species are reported to have anticancer [1] and piscicidal [2] properties. Steroidal sapogenins [3] and saponins [4–6] have been reported from the leaves and fruits of *A. cantala*. We have shown that the rhizomes of this plant contain sapogenins [7], a hongguanggenin diglucoside [8] and other steroidal saponins. This paper describes the isolation and characterization of two hecogenin glycosides (**1** and **3**) from the methanolic extract of the rhizomes. Compound **1** was found to be molluscicidal against *Biomphalaria glabrata* at a concentration of 7 ppm.

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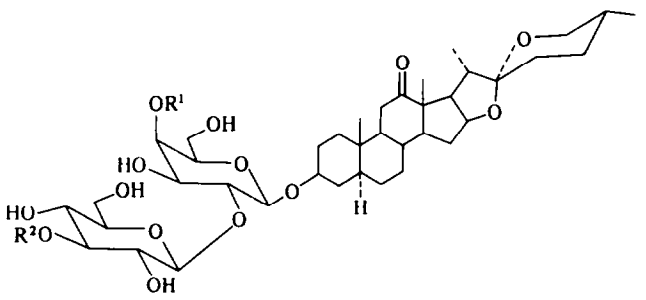
RESULTS AND DISCUSSION

Repeated column chromatography of the methanolic extract of the rhizomes gave cantalasaponins-2 (**1**), -3 (**2**), -4 (**3**) and -5 (**4**). Compounds **1** and **3** belonged to the 25*R* spirostane series (IR).

FD-mass spectrometry showed that the *M*,s of **1** and **3**



1 $R^1 = \beta$-D-xyl (pyr), $R^2 = \beta$-D-glu (pyr)
3 $R^1 = \beta$-D-xyl (pyr), $R^2 = \beta$-D-xyl (pyr) (1→4)-β-D-glu (pyr)
PS_2 $R^1 = R^2 = H$
PS_3 $R^1 = H$, $R^2 = \beta$-D-glu (pyr)

were 1048 (*m/z* 1087 $[M+K]^+$, 1071 $[M+Na]^+$ and 1049 $[M+H]^+$) and 1180 (*m/z* 1203 $[M+Na]^+$), respectively. The lower mass regions of the spectra were almost identical. Fragment ions at *m/z* 939 $[M+Na-132]^+$, 909 $[M+Na-162]^+$ and 754 $[M-132-162]^+$ in **1** and at 1071 $[M+Na-132]^+$ and 939 $[M+Na-2\times132]^+$ in **3** indicated the presence of a terminal pentose in both and a terminal hexose in the former.

Acidic hydrolysis of **1** and **3** gave hecogenin, and D-galactose, D-glucose and D-xylose in the ratio 1:2:1 and 1:2:2, respectively. Partial hydrolysis of **1** gave prosapogenins, PS_1, PS_2 and PS_3 whereas **3** additionally gave PS_4. The FD-MS of PS_3 contained a $[M+H]^+$ ion at *m/z* 917. PS_1 on hydrolysis gave galactose whereas PS_2 and PS_3 gave glucose also. Oxidation of PS_3 with sodium periodate followed by acidic hydrolysis gave glucose.

The permethylate of **1** (**1a**) prepared by Hakomori's method [9] on hydrolysis yielded 2,3,4-tri-*O*-methyl-D-xylose, 3,6-di-*O*-methyl-D-galactose (Wallenfel's [10] positive), 2,4,6-tri-*O*-methyl-D-glucose and 2,3,4,6-tetra-*O*-methyl-D-glucose. The permethylate of **3** (**3a**) gave the first three sugars and 2,3,6-tri-*O*-methyl-D-glucose. PS_2 permethylate gave 2,3,4,6-tetra-*O*-methyl-D-glucose and 3,4,6-tri-*O*-methyl-D-galactose whereas PS_3 permethylate additionally furnished 2,4,6-tri-*O*-methyl-D-glucose. PS_4 permethylate gave sugars identical to those given by **1a**. The identities of the methylated sugars were established by comparison with authentic samples [11–13].

The points of linkages in the glycone parts in PS_2, PS_3, **1** and **3** were further supported by the application of glycosylation shifts [14, 15]. ^{13}C chemical shifts of methyl pyranosides of β-D-galactose, β-D-glucose and β-D-xylose in pyridine-d_5 [14–16] and those of hecogenin [17] are available.

The δ values of C-3 and C-5 of the sugars in the ^{13}C NMR spectra of PS_3 and **1** confirmed the presence of β-linkages [14] at the anomeric carbons. β-Linkages in **1** and **3** were further supported by their ^1H NMR spectra and by the application of Klyne's rule [18].

Thus, **1** and **3** were characterized as 3-*O*-[{β-D-glucopyranosyl(1 → 3)-β-D-glucopyranosyl(1 → 2)} {β-D-xylopyranosyl(1 → 4)}-β-D-galactopyranosyl]-(25*R*)-5α-spirostan-12-one-3β-ol and 3-*O*-[{β-D-xylopyranosyl (1 → 4)-β-D-glucopyranosyl(1 → 3)-β-D-glucopyranosyl (1 → 2)} {β-D-xylopyranosyl(1 → 4)}-β-D-galactopyranosyl]-(25*R*)-5α-spirostan-12-one-3β-ol, respectively.

EXPERIMENTAL

Mps: uncorr.; FDMS: JEOL JMS DX-300/JMA-3500 system (E.H.C.: 22–23 mA; cathode voltage: −5 kV, accelerating voltage: 2 kV, ion multi. voltage: 2.5 kV); ^1H NMR (100 MHz) and ^{13}C NMR (25 MHz): C_5D_5N with TMS as an internal standard; CC: silica gel (BDH, 60–120 mesh) (i) and Lobar LiChroprep Rp-8 (Merck, size B) (ii); TLC: Kieselgel 60 G (Merck) (iii) and DC-Alufolien Kieselgel 60 F_{254} (Merck) (iv); HPTLC: Fertigplatten Rp-8 F_{254} (Merck) (v). The spots on TLC were visualized by spraying with 10% alcoholic H_2SO_4 followed by heating. PC: Whatman No. 1 paper using the descending mode and aniline hydrogen phthalate as the developer. Colorimetric estimations [19]. The following solvent systems were used: (A) $CHCl_3$–MeOH (9:1); (B) MeOH–H_2O (9:1); (C) MeOH–H_2O (4:1); (D) $CHCl_3$–MeOH–H_2O (13:4:2); (E) petrol (60–80°)–EtOAc (1:1); (F) C_6H_6–Me_2CO (4:1); (G) EtOAc–C_5H_5N–H_2O (10:4:3) and (H) *n*-BuOH–EtOH–H_2O (5:1:4).

Isolation of saponins. The saponin fraction (6.5 g) from a MeOH extract of the rhizomes of *A. cantala* (collected from Srinagar, U.P.) gave cantalasaponin 1 [8] and a complex mixture which on repeated CC [(i), solvent A] gave two fractions (1, 2.1 g and 2, 2.8 g), each of which crystallized as colourless flakes (aq. EtOH) and appeared homogeneous on TLC.

Fraction 1. FDMS *m/z* (rel. int.): 1071 (76.1), 1057 (60.5), 903 (100), 755 (60.5), 431 (19.2), 417 (9.6), 399 (20.1).

Fraction 2. FDMS *m/z* (rel. int.): 1203 (23.3), 1189 (26.2), 1057 (63.1), 916 (21.3), 903 (100), 431 (32.0), 417 (25.7), 399 (12.6).

CC of fraction 1 (1 g) [(ii), solvent B] afforded **1** and **2** and fraction 2 (1.25 g) gave **3** and **4** (homogeneity of **1**–**4** checked by HPLC (v), solvents B and C) (characterization of **2** and **4** is in progress).

Compound **1**. Colourless needles (372 mg) from aq. EtOH, mp 287–293°, $[\alpha]_D^{15-17}$ −30° (C_5H_5N; *c* 1.98). IR ν_{max}^{KBr} cm^{-1}: 3400 (OH), 1710 (>C=O), 989, 922, 902, 870 (intensity 902 > 922, 25*R*-spiroketal). FDMS *m/z* (rel. int.): 1087 $[M+K]^+$ (14.7), 1071 $[M+Na]^+$ (100), 1049 $[M+H]^+$ (16.0), 1048 $[M]^+$ (22.5), 939 $[M+Na-132]^+$ (43.6), 916 $[M-132]^+$ (12.4), 909 $[M+Na-162]^+$ (9.9), 886 $[M-162]^+$ (9.0), 754 $[M-132-162]^+$ (10.3), 592 $[M-132-(2\times162)]^+$ (5.5), 430 (1.8), 133 [xyl + H

$-H_2O]^+$ (17.8), 115 [xyl + H $-2H_2O]^+$ (6.2); ^{1}H NMR: δ0.67 (6H, *m*, 19-Me, 27-Me), 1.09 (3H, *s*, 18-Me), 1.36 (3H, *d*, 21-Me), 4.85 (1H, *d*, J = 7 Hz, H-1 of glu), 5.07 (2H, *m*, H-1 of glu and gal), 5.56 (1H, *d*, J = 7 Hz, H-1 of xyl); ^{13}C NMR: δaglycone: 36.6, 29.5, 79.6, 34.3, 44.3, 28.5, 31.7, 34.6, 55.5, 36.2, 37.9, 212.8, 55.3, 55.9, 31.7, 79.1, 54.2, 16.0, 11.6, 42.5, 13.9, 109.2, 31.7, 29.2, 30.5, 66.9, 17.3 (C-1–C-27); sugar moiety: 102.3, 81.2, 73.1, 79.6, 76.1, 60.6 (galactosyl C-1–C-6), 104.8, 70.7[a*], 86.8, 70.4, 78.5, 62.4[b] (glucosyl C-1–C-6), 104.8, 75.5, 78.5, 70.9[a], 78.5, 62.9[b] (glucosyl C-1′–C-6′), 104.8, 75.0, 77.5, 70.7, 67.1 (xylosyl C-1–C-5). (Found: C, 57.16; H, 7.84. $C_{50}H_{80}O_{23}$ requires C, 57.25; H, 7.63%.)

Compound **3**. Colourless needles (483 mg) from aq. EtOH, mp 268–271°, $[\alpha]_D^{15-17}$ −31.7° (C_5H_5N; *c* 2.20). IR ν_{max}^{KBr} cm^{-1}: 3450 (OH), 1712 (>C=O), 980, 920, 899, 860 (intensity 899 > 920, 25*R*-spiroketal); FDMS *m/z* (rel. int.): 1219 $[M+K]^+$ (12.2), 1203 $[M+Na]^+$ (92.3), 1071 $[M+Na-132]^+$ (100), 939 $[M+Na-2\times132]^+$ (44.0), 916 (17.3), 909 (25.8), 886 (15.6), 754 (14.8), 592 (10.0), 431 (19.7), 133 (22.2), 115 (15.9); ^{1}H NMR: δ0.67 (6H, *m*, 19-Me, 27-Me), 1.09 (3H, *s*, 18-Me), 1.36 (3H, *d*, 21-Me), 4.85 (1H, *d*, J = 7 Hz, H-1 of glu), 5.10 (3H, *m*, H-1 of glu, gal and xyl), 5.56 (1H, *d*, J = 7 Hz, H-1 of xyl); ^{13}C NMR: δsugar moiety: 102.4, 80.7, 73.1, 79.7, 75.4, 60.6 (galactosyl C-1–C-6), 103.9[a], 70.7, 86.8, 70.7, 78.3, 62.1[b] (glucosyl C-1–C-6), 104.8[a], 75.4, 77.7, 79.7, 77.2, 62.9[b] (glucosyl C-1′–C-6′), 104.8†, 75.1, 77.7, 70.7, 69.1 (xylosyl C-1–C-5), 106.1†, 75.1, 77.2, 70.7, 67.0 (xylosyl C-1′–C-5′). (Found: C, 55.69; H, 7.61. $C_{55}H_{88}O_{27}$ required C, 55.93; H, 7.45%.)

Acidic hydrolysis of **1** *and* **3**. Compounds **1** and **3** (20 mg each) were refluxed with 2 M HCl–EtOH (1:1, 10 ml) on a boiling water bath for 3.5 hr to afford the aglycone (hecogenin), mp 262–265° (MeOH) (lit. [7] mp 262–264°, $[\alpha]_D^{20}$ −3°). IR ν_{max}^{KBr} cm^{-1}: 3400 (OH), 1700 (>C=O), 990, 920, 904, 860 (intensity 904 > 920). EIMS: *m/z* 430 $[M]^+$. The neutralized (Ag_2CO_3) and conc. aq. hydrolysate showed the presence of D-galactose, D-glucose and D-xylose (PC, solvent G, R_f values 0.20, 0.23 and 0.37, respectively). Sugars were estimated at 420 nm.

Partial hydrolysis of **1**. Compound **1** (200 mg) in 1 M HCl–*n*-BuOH (1:1, 25 ml) was heated at 70° for 2.5 hr. The BuOH layer was washed with H_2O and evaporated to dryness *in vacuo*. The residue on CC [(i), solvent D] yielded hecogenin, PS_1 (15 mg), PS_2 (13.5 mg) and PS_3 (16.5 mg). PS_1, PS_2 and PS_3 (2 mg each) on acidic hydrolysis carried as above gave D-galactose. PS_2 and PS_3 hydrolysates also contained D-glucose (PC, solvent G).

PS_2. Colourless plates from aq. MeOH, mp 270–273° (decomp.). ^{13}C NMR: δsugar moiety: 102.5, 80.1, 73.4, 72.3, 76.9, 61.0 (galactosyl C-1–C-6), 107.1, 75.5, 78.7, 72.5, 78.5, 63.1 (glucosyl C-1–C-6).

PS_3. Colourless plates from aq. MeOH, mp 296–299° (decomp.), $[\alpha]_D^{20}$ −23.5° (C_5H_5N; *c* 0.9). FDMS *m/z* (rel. int.): 917 $[M+H]^+$ (100), 754 (72.1), 593 (23.2), 431 (25.4), 413 (79.3). ^{13}C NMR: δsugar moiety: 102.5, 81.0, 73.3, 70.4, 76.7, 60.6 (galactosyl C-1–C-6), 105.2, 71.8, 86.1, 70.4, 77.7[a], 61.7[b] (glucosyl C-1–C-6), 106.9, 75.6, 78.5, 71.8, 78.2[a], 63.2[b] (glucosyl C-1′–C-6′).

$NaIO_4$ oxidation of PS_2 and PS_3. PS_2 and PS_3 (3 mg each) were separately mixed with an aq. soln of $NaIO_4$ (10%, 1.5 ml) and left in the dark at room temp. for 5 days. Excess of the reagent was decomposed by adding ethylene glycol (0.5 ml). Each on usual work-up followed by acidic hydrolysis was checked by PC. The product from PS_3 showed D-glucose only.

Permethylation of **1**, **3**, *PS_1, PS_2 and PS_3*. Compounds **1** and **3** (80 mg each), PS_1, PS_2 and PS_3 (7 mg each) were separately permethylated by Hakomori's method. The products from **1** and **3** were purified by CC [(i), solvent E] to yield **1a** (20 mg) and **3a** (15 mg), respectively, while from PS_1, PS_2 and PS_3 by prep. TLC [(iii), solvent F; visualizing agent, H_2O].

Compound **1** *permethylate* (**1a**). Colourless syrup. IR ν_{max}^{KBr} cm^{-1}: no OH. (Found: C, 61.36; H, 7.91. $C_{62}H_{104}O_{23}$ requires C, 61.18; H, 8.55%.)

Hydrolysis of **1a** *and* **3a**. Compounds **1a** and **3a** (10 mg each) were separately refluxed with 1 M HCl–MeOH (1:1, 6 ml). The neutralized and conc. hydrolysate from **1a** contained (PC, solvent H) 2,3,4,6-tetra-*O*-methyl-D-glucose, 2,3,4-tri-*O*-methyl-D-xylose, 2,4,6-tri-*O*-methyl-D-glucose and Wallenfel's positive 3,6-di-*O*-methyl-D-galactose (R_G values 1.00, 0.94, 0.76 and 0.47, respectively). Hydrolysate from **3a** revealed methylated sugars as above except that at R_G 1.00. An additional spot 2,3,6-tri-*O*-methyl-D-glucose appeared at R_G 0.83.

Acidic hydrolysis of PS_1, PS_2 and PS_3 permethylates. Permethylates of PS_1, PS_2 and PS_3 (3 mg each) were hydrolysed as above and checked by PC (solvent H). The hydrolysate of PS_1 permethylate showed 2,3,4,6-tetra-*O*-methyl-D-galactose (R_G 0.88), the hydrolysate of PS_2 permethylate contained 2,3,4,6-tetra-*O*-methyl-D-glucose and Wallenfel's positive 3,4,6-tri-*O*-methyl-D-galactose (R_G 1.00 and 0.72, respectively) whereas the hydrolysate of PS_3 permethylate additionally carried 2,4,6-tri-*O*-methyl-D-glucose (R_G 0.76).

Partial hydrolysis of **3**. Partial hydrolysis of **3** (180 mg) as carried with **1** gave a mixture of prosapogenins and hecogenin which was purified by CC [(i), solvent A] to afford PS_1 (10 mg), PS_2 (10 mg), PS_3 (13.5 mg) and PS_4 (20 mg).

PS_4. Crystallized as colourless needles from aq. EtOH, mp 286–294°. IR ν_{max}^{KBr} cm^{-1}: 3400 (OH), 1711 (>C=O), 989, 922, 902, 870. It was found identical to **1** by mmp, co-TLC [solvent systems EtOAc–EtOH–H_2O (15:5:4) and $CHCl_3$–MeOH–H_2O (13:7:2)], superimposable IR and permethylation studies.

Molluscicidal test. Compound **1** was submitted to assay against *Biomphalaria glabrata* by the method of ref. [20].

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*^{13}C NMR data: [a,b]Assignments are interchangeable between carbons marked with similar sign in a compound.

†Assignments are interchangeable between two xyloses.

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NAPHTHOQUINONES FROM JUGLANDACEAE

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Key Word Index—*Pterocarya*; *Juglans*; Juglandaceae; juglone; 3,3′-bijuglone; cyclotrijuglone.

Abstract—The oligomeric juglones isolated from the barks of Juglandaceae plants, *Pterocarya* and *Juglans*, were shown to be 3,3′-bijuglone (8,8′-dihydroxy[2,2′-binaphthalene]-1,1′,4,4′-tetrone and unsymmetrical cyclotrijuglone (1,7,16-trihydroxy-5,6,11,12,17,18-trinaphthalenehexone).

INTRODUCTION

It is well known that juglone (5-hydroxy-1,4-naphthoquinone) and hydrojuglone glucoside (4,8-dihydroxy-1-naphthalenyl-β-D-glucopyranoside) occur in different parts of Juglandaceae [1]. Other monomeric naphthoquinones have also been found (e.g. 1,4-naphthoquinone [2, 3] and 5,8-dihydroxy-1,4-naphthoquinone [4] from *Juglans* species). The natural co-occurrence of a monomeric quinone with its oligomers (dimer, trimer and tetramer) has been widely studied, e.g. 7-methyljuglone (ramentacenone) and its oligomers in Ebenaceae and 2-methyljuglone (plumbagin) and its oligomers in Droseraceae [1]. However, the oligomers from the quinones which occur among Juglandaceae plants have received little attention. Juglone oligomers, 3,3′-bijuglone (**1**) [5] and symmetrical cyclotrijuglone (**2**) [6], have been reported to occur in root-bark of *J. regia* in one instance only. The present paper is concerned with the isolation and structure elucidation of juglone oligomers from *P. rhoifolia* Sieboldiana et Zucc. (wingnut, Japanese name Sawagurumi) and *J. mandshurica* Maxim. var. Sieboldiana Makino (walnut, Japanese name Onigurumi).

RESULTS AND DISCUSSION

The chloroform extracts of the fresh root-bark of *P. rhoifolia* was chromatographed on silica gel (benzene as eluant) to give in addition to juglone two polar light yellow compounds. The less polar of the two polar compounds was identified as bijuglone (**1**) by comparison with an authentic sample prepared by addition of juglone and 1,4,5-trihydroxynaphthalene [7]. The ^{1}H NMR spectrum ($CDCl_3$) of isolated **1** revealed two singlets of H-3 and H-3′ (δ7.04) and 8- and 8′-OH (δ11.75). Bijuglone (**1**) was distinguished from its isomers, 2,2′-bijuglone (**3**) and 2,3′-bijuglone (**4**), by ^{1}H NMR spectroscopy [8, 9]*. It gave a reddish violet colour with aqueous alkali and underwent reversible reduction with sodium dithionite like many other hydroxynaphthoquinones.

The second polar compound was unsymmetrical cyclotrijuglone (**5**), having hydroxyl groups at the unsymmetrical position. The structure followed from the ^{1}H NMR spectral data (1- and 16-OH singlet and 7-OH singlet at δ11.20 and 11.27, respectively, in $CDCl_3$). The structure of cyclotrijuglone from *J. regia* had previously been assigned as symmetrical **2** rather than unsymmetrical **5** [6]. More recently, synthetic cyclotrijuglone has been obtained from the addition of juglone to 1,4,5-trihydroxynaphthalene followed by treatment with chloranil. Its structure was assigned as **5** on the grounds that the ^{1}H NMR spectrum ($CDCl_3$) of its triacetate showed two singlet signals at δ2.51 (3H, OAc) and 2.48 (6H, 2OAc) [7]. The ^{1}H NMR spectrum ($CDCl_3$) of cyclotrijuglone from

*The chemical shifts ($CDCl_3$/TMS = 0) for vinyl and hydroxyl protons of **3** and **4**, prepared independently by the method of Laatsch [8, 9], are as follows. Compound **3**: δ7.05 (2H, *s*, H-3 and H-3′), 11.86 (2H, *s*, 5- and 5′-OH); **4**: δ7.02 (1H, *s*, H-3′), 7.06 (1H, *s*, H-3), 11.73 (1H, *s*, 8′-OH), 11.85 (1H, *s*, 5-OH).