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STEROLS AND STERYL GLYCOSIDES FROM *URTICA DIOICA*

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ABSTRACT.—In addition to sitosterol and sitosterol-β-D-glucoside, six steryl derivatives have been isolated from the roots of *Urtica dioica* by a combination of different chromatographic methods and have been identified by spectroscopic analysis and hydrolytic reactions on tlc. The structures were determined at 7β-hydroxysitosterol, 7α-hydroxysitosterol, (6′-*O*-palmitoyl)-sitosterol-3-*O*-β-D-glucoside, and, so far unknown from nature, 7β-hydroxysitosterol-β-D-glucoside, 7α-hydroxysitosterol-β-D-glucoside, and 24*R*-ethyl-5α-cholestane-3β,6α-diol. The ^{1}H- and ^{13}C-nmr spectra indicated that the sterols occurred only with the 24*R*-ethyl substitution.

Urtica dioica L. (Urticaceae), stinging nettle, is a widespread, common, medical plant often used in folk medicine against various diseases. The aqueous methanolic extracts from roots are recommended for the treatment of prostate adenoma (1). Previously, we reported the isolation and characterization of scopoletin, steryl derivatives, phenylpropanes, lignan glucosides, and flavonol glycosides from *U. dioica* roots and flowers (2-4).

In continuing our pharmacognostic studies we have isolated six steroids closely related to sitosterol [**1**] and have determined their structures by spectroscopic methods as (6′-*O*-palmitoyl)-sitosterol-3-*O*-β-D-glucoside [**3**], 24*R*-ethyl-5α-cholestane-3β,6α-diol [**4**], 7β-hydroxysitosterol [**5**], 7α-hydroxy-sitosterol [**6**], 7β-hydroxysitosterol-3-*O*-β-D-glucoside (**7**), and 7α-hydroxysitosterol-3-*O*-β-D-glucoside [**8**]. The compounds **5-8** are known as oxidation products of sitosterol and sitosterol-3-*O*-β-D-glucoside [**2**] (5,6). The structures were further confirmed by hydrolytic and enzymatic cleavages on tlc (12).

RESULTS AND DISCUSSION

Tlc of a crude methanolic extract of *U. dioica* ($CHCl_3$-MeOH, 9:1) showed spots that gave reddish purple (**1-4**) and royal blue (**5-8**) coloration on spraying with the vanillin-phosphorous acid reagent (7). Repeated chromatography led to the separation of its components without acetylation or methylation of the mixture and to isolation of six compounds that are now described according to their increasing polarity.

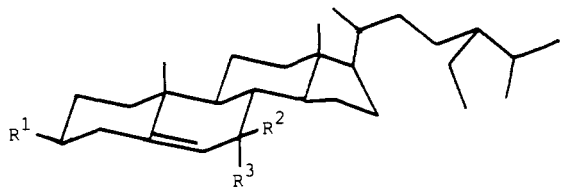
Compounds **5** and **6** had Rf values of 0.58 and 0.56, respectively, and gave a royal blue color with vanillin-phosphorous acid reagent; the spots also became blue on spraying with 50% H_2SO_4, which is typical for 7β- and 7α-hydroxycholesterol (8). The mobility value of **5** and **6** relative to sitosterol indicated the presence of 7β- and 7α-hydroxysitosterol.

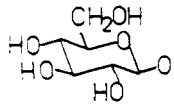
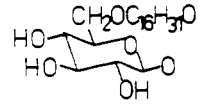
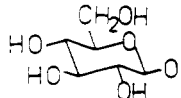
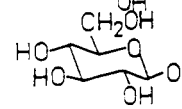
The ^{1}H-nmr spectra of **5** and **6** showed a complex aliphatic part very similar to that of sitosterol (9). The signals due to the C-6 methine proton at 5.58 ppm and 5.28 ppm were deshielded in **5** and shielded in **6**, respectively.

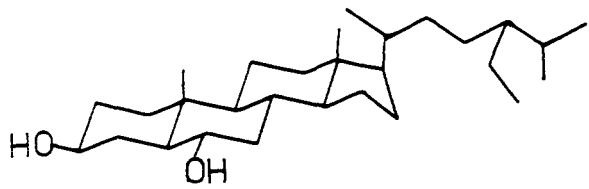
Two unresolved multiplets at 3.53 ppm/3.57 ppm and 3.83 ppm/3.83 ppm indicated the presence of two hydroxy functions in each molecule.

The molecular formulae, $C_{29}H_{50}O_2$, of **5** and **6** are based on the fdms (eims), the number of signals in the PND (complete decoupling) ^{13}C nmr, and their multiplicities in the SFORD and integration of the ^{1}H-nmr spectra.

Comparing the ^{13}C-nmr spectra of **5** and **6** with data of 5α-cholestane-7β-ol, 5α-cholestane-7α-ol, and sitosterol (10,11), it was confirmed that **5** and **6** were 7β- and 7α-hydroxysitosterol, respectively.



	R^1	R^2	R^3
1	OH	H	H
2		H	H
3		H	H
5	OH	OH	H
6	OH	H	OH
7		OH	H
8		H	OH



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If a 7α-hydroxy group is substituted into sitosterol, γ-gauche effects would be expected at carbon 9 and 14. C-9 and C-14 of **6** appeared upfield (−7.83 ppm and −7.33 ppm) corresponding to their signals in sitosterol (see Table 1). The effect for the 7β isomer at C-9 and C-14 was less because a γ-gauche orientation is not possible.

Compound **3** had an Rf value of 0.50 ($CHCl_3$-MeOH, 9:1). Its ir spectra and ^{1}H-nmr spectra suggested a fatty acid ester of a sterol glycoside. Deacylation of **3** with NH_3 vapor on a tlc plate (24 h) gave a steroidal glycoside with an Rf value identical to sitosterol-3-*O*-β-D-glucoside [**2**], also isolated from *U. dioica* roots (2).

Further hydrolysis with Luizym® (mixture of α- and β-glucosidase, Luitpold) afforded sitosterol and glucose, identified by tlc.

The compound was fragmented by fdms, showing a quasi molecular ion peak at 837 $[M+Na]^+$ and at 815 $[M+H]^+$ and peaks for sitosterol at 414 [aglycone]$^+$ and 396 [aglycone-H_2O]$^+$.

In the ^{13}C-nmr spectra six distinct signals were assigned to glucose, indicating its

TABLE 1. Carbon Shifts of Sterins **1**, **5-8**[a]

Carbon	Compounds				
	1	**5**	**6**	**7**	**8**
1	37,24t	37,02t	37,40t	36,71	36,73
2	31,61t	31,58t	31,38t	29,51	29,12
3	71,78d	71,43d	71,34d	78,64	78,30
4	42,28t	41,74t	42,15t	37,95	38,26
5	140,71s	143,47s	146,25s	142,62	145,24
6	121,68d	125,47d	123,87d	125,83	123,87
7	31,89t	73,36d	65,35d	73,32	64,82
8	31,89d	39,57d	37,02d	39,35	37,26
9	50,11d	48,29d	42,98d	48,19	41,90
10	36,48s	36,45s	37,54s	36,39	37,26
11	21,07t	21,88t	20,72t	20,89	20,46
12	39,77t	42,94t	39,19t	42,70	38,96
13	42,28s	40,93s	42,20s	40,23	41,91
14	56,75d	55,75d	49,43d	55,85	50,06
15	24,28t	26,38t	24,30t	26.06	23,93
16	28,24t	28,54t	28,27t	28,34	28,08
17	56,04d	55,41d	55,98d	55,20	55,60
18	11,85q	11,82q	11,63q	11,56	11,36
19	19,40q	19,14q	18,23q	18,77	17,91
20	36,12d	36,10d	36,11d	35,92	35,93
21	19,02q	19,04q	19,04q	18,75	18,73
22	33,92t	33,94t	33,99t	33,75	33,71
23	29,13t	29,17t	29,18t	28,93	28,89
24	45,80d	45,86d	45,87d	45,65	45,61
25	26,06d	26,16d	25,99d	25,85	25,77
26	18,77q	18,84q	18,80q	18,60	18,53
27	19,80q	19,79q	19,78q	19,55	19,52
28	22,04t	22,55t	22,67t	22,48	22,82
29	11,97q	11,98q	11,99q	11,61	11,68
1′				100,99	100,82
2′				73,32	73,34
3′				76,02	76,17
4′				69,87	69,68
5′				76,18	76,38
6′				61,60	61,60

[a]δ-values, ppm, **1**, **5**, and **6** in $CDCl_3$; **7** and **8** in $CDCl_3$-CD_3OD (9:1); external standard $CDCl_3$.

β-configuration and pyranose form (14). The downfield shift of C-6′ (β-effect, +1.62 ppm) and upfield shift of C-5′ (γ-effect, −2.33 ppm) confirmed the attachment of the palmitoyl residue at C-6′.

Compound **4** (Rf 0.48) gave a ^{13}C-nmr spectrum in which there was evidence of two hydroxyl groups (71.26 ppm, 69.52 ppm) but in which the methine signals, C-5 and C-6, of sitosterol were missing.

The ^{1}H-nmr spectrum showed typical signals for sitosterol (9), except for the signal due to the methyl group of C-19 which was shifted upfield (0.79 ppm, $\Delta = -0.2$ ppm). This observed shielding effect and the upfield shift of C-19 in the ^{13}C-nmr spectrum (13.44 ppm in **4** compared with 19.40 ppm in **1**) could be explained with one more γ-gauche interaction from an axial hydrogen at C-6. The γ-gauche effect caused by substitution of C-6 with an α-hydroxy group (equatorial orientation) is also in evidence. The calculated spectrum of 3β,6α-dihydroxy-5α-androstane (10) and the ^{13}C-nmr

spectrum of **4** showed an average deviation of less than 1 ppm. Thus, **4** was identified as 3β,6α-dihydroxy-24*R*-ethyl-5α-cholestane by fdms and nmr.

Compounds **7** and **8** had Rf values of 0.19 and 0.17, respectively. Except for the signals of carbons 2, 3, and 4, the ^{13}C-nmr spectra of **7** and **8** exhibited 29 aglycone signals at the same positions as those of **5** and **6**, respectively (see Table 1), including C-24 (9,13).

Placement of the glucosyl residue at C-3 of the aglycone follows from the observed downfield shift of the C-3 signals (+6.97 ppm for **8** and +7.21 ppm for **7**) and the upfield shift of the C-2/C-4 signals (−2.07 ppm −2.25 ppm/ −3.78 ppm, −3.88 ppm) relative to their corresponding values in **5** and **6**.

Careful acid and enzymatic hydrolysis yielded D-glucose, **5**, and **6**. The β-configuration of the glucose moiety was confirmed with large ^{1}H coupling constants of $J_{1,2}$=7.63 Hz and $J_{1,2}$=7.82 Hz and the ^{13}C absorption of 100.99 ppm and 100.82 ppm for the anomeric carbons of **7** and **8**, respectively (14).

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Mps were determined on a Mikroskopeiztisch 350 (Leitz) and are uncorrected. Ir spectra were recorded on a Perkin Elmer 700 as KBr pellets (1 mg in 200 mg). The 400 MHz ^{1}H-nmr and 100 MHz ^{13}C-nmr spectra were obtained with a Jeol GX 400 spectrometer using $CDCl_3$ or pyridine-d_5 as external standards, while fdms were done with a Varian MAT 711. The liquid-liquid partition chromatography was carried out on a droplet countercurrent chromatography apparatus (300 glass columns 2 mm ID, 40 mm length) or a rotation locular countercurrent chromatograph in descending mode, both from EYELYA® Tokyo Rikakikai C. Ltd., Tokyo, Japan. For preparative and analytical tlc, Si gel plates (Merck) were used, and detection was made by spraying with vanillin-phosphorous acid reagent or 50% H_2SO_4 in EtOH and heating for a few minutes (105°). Roots of *U. dioica* are a gift of Finzelberg/Nachf., Andernach, and identical with clutivated plant material from the Botanical Garden, Marburg. A voucher specimen is preserved at the Institut für Pharmazeutische Biologie, Philipps-Universität, Marburg.

EXTRACTION AND ISOLATION.—Ground, dried roots (4,5 kg) were extracted by maceration with 15 liters MeOH over 5 days (ambient temperature). After solvent evaporation, 80 g of crude extract was obtained and was chromatographed on a Si gel column (1 kg, 70-230 mesh ASTM, Merck). Elution was begun with CH_2Cl_2, and the polarity was increased by stepwise addition of MeOH. A total of 650 fractions were collected and then partially recombined according to their composition.

COMPOUNDS **5** AND **6**.—Fractions 85-212 (2550 mg, 0.25% MeOH) were combined and chromatographed by dccc ($CHCl_3$-toluene-MeOH-H_2O, 8:5:8:2, 8 ml/40 min/fraction). Repeated cc of dccc fractions 15-19 (1220 mg) on Si gel with a $CHCl_3$/MeOH gradient and preparative tlc, first developed with $CHCl_3$-MeOH (93:7) and then with Et_2O, afforded 11 mg of **5** and 13 mg of **6**.

COMPOUND **3**.—Cc of fractions 399-410 (383 mg, 2% MeOH) with $CHCl_3$/MeOH followed by preparative tlc ($CHCl_3$-MeOH, 95:5) yielded 25 mg of **3**.

COMPOUND **4**.—Fractions 411-427 (401 mg, 3% MeOH) afforded 15 mg of **4** by cc (EtOAc) and preparative tlc ($CHCl_3$-MeOH, 93:7).

COMPOUNDS **7** AND **8**.—From fractions 496-498 (346 mg, 5% MeOH) and 500-502 (238 mg, 5% MeOH) crude fractions of **7** and **8** were obtained, respectively, by rlcc I and II ($CHCl_3$-toluene-MeOH-H_2O, 13:1:7:4, 8 ml/30 min/fraction). Cc of fractions 12-20 (80 mg) of rlcc I and of fractions 14-22 (50 mg) of rlcc II with $CHCl_3$-MeOH (9:1) and preparative tlc ($CHCl_3$-MeOH, 85:15) yielded 12 mg of **7** and 9 mg of **8**, respectively.

SPECTRAL DATA.—*Compound* **3**.—mps 165-170°; ir 3410, 2985-2845, 1725, 1460-1450, 1380, 1055 cm^{-1}; ^{1}H nmr ($CDCl_3$, *J* in Hz) 5.34 H-6 (m, 1H), 4.40 H_a-6′ (dd, 5.0/12.2, 1H), 4.36 H-1′ (d, 7.7, 1H), 4.28 H_b-6′ (dd, 2.2/12.2, 1H), 2.33 H-2″ (t, 7.6, 2H), 1.25 methylenes (brs, 26H), 1.002 H-19 (s, 3H), 0.918 H-21 (d, 6.41, 3H), 0.869 H-26 (d, 6.71, 3H), 0.842 H-29 (t, 7.22, 3H), 0.811 H-27 (d, 6.87, 3H), 0.677 ppm H-18 (s, 3H); ^{13}C nmr ($CDCl_3$) 37.28 t C-1, 29.56 t C-2, 79.59 d C-3, 38.91 t C-4, 140.31 s C-5, 122.14 d C-6, 31.94 t C-7, 31.94 d C-8, 50.20 d C-9, 36.15 s C-10, 21.08 t C-11, 39.78 t C-12, 42.34 s C-13, 56.78 d C-14, 24.30 t C-15, 28.23 t C-16, 56.13 d C-17, 11.84 q C-18, 19.35 q C-19, 36.73 d C-20, 19.04 q C-21, 33.97 t C-22, 29.19 t C-23, 45.87 d C-24, 26.16 d C-25, 18.79 q C-26, 19.80 q C-27, 23.10 t C-28, 11.98 q C-29, 101.22 d C-1′, 73.59 d C-2′, 76.60 d C-

3′, 70.16 d C-4′, 73.95 d C-5′, 63.22 t C-6′, 174.60 s carbonyl, 34.25 t C-2″, 24.97 t C-3″, 22.68 t C-15″, 29.9 m methylenes, 14.10 ppm q C-16″; fdms *m/z* 837 $[M+Na]^+$ 22.38%, 815 $[M+H]^+$ 64.8%, 814 M^+ 8.03%, 796 $[M-H_2O]$ 40.28%, 396 $[aglycone-H_2O]^+$ 31.68%.

Compound **4**.—mps 102-104°; ir 3350, 2990-2860, 1450, 1375 cm^{-1}; ^1H nmr ($CDCl_3$, *J* in Hz) 3.56 H-3 (m, 1H), 3.40 H-6 (dt, 4.43/10.68, 1H), 0.882 H-21 (d, 6.71, 3H), 0.819 H-29 (t, 7.48, 3H), 0.809 H-26 (d, 6.87, 3H), 0.791 H-19 (s, 3H), 0.786 H-27 (d, 6.87, 3H), 0.627 ppm H-18 (s, 3H); ^{13}C nmr ($CDCl_3$) 37.24 C-1, 30.99 C-2, 71.26 C-3, 32.21 C-4, 51.65 C-5, 69.52 C-6, 41.65 C-7, 34.27 C-8, 53.67 C-9, 36.26 C-10, 21.14 C-11, 39.79 C-12, 42.57 C-13, 56.07 C-14, 24.19 C-15, 28.14 C-16, 56.74 C-17, 12.02 C-18, 13.44 C-19, 36.70 C-20, 18.71 C-21, 34.27 C-22, 26.07 C-23, 45.81 C-24, 29.12 C-25, 19.01 C-26, 19.80 C-27, 23.05 C-28, 12.02 ppm C-29; fdms *m/z* 432 M^+ 100%.

Compound **5**.—^1H nmr ($CDCl_3$, *J* in Hz) 5.59 H-6 (dd-like, 1.37/5.19, 1H), 3.83 H-7 (br s, 1H), 3.57 H-3 (m, 1H), 0.975 H-19 (s, 3H), 0.911 H-21 (d, 6.72, 3H), 0.825 H-29 (t, 7.42, 3H), 0.813 H-26 (d, 6.86, 3H), 0.792 H-27 (d, 6.87, 3H), 0.666 ppm H-18 (s, 3H); fdms *m/z* 430 M^+ 100%, 395 $[M-H_2O-OH]^+$ 55.9%.

Compound **6**.—^1H nmr ($CDCl_3$, *J* in Hz) 5.27 H-6 (br s, 1H), 3.83 H-7 (m, 1H), 3.539 H-3 (m, 1H), 1.039 H-19 (s, 3H), 0.907 H-21 (d, 6.56, 3H), 0.842 H-29 (t, 7.48, 3H), 0.835 H-26 (d, 6.84, 3H), 0.793 H-27 (d, 6.87, 3H), 0.674 ppm H-18 (s, 3H); eims (70 eV, *m/z*) 430 M^+ 0.74%, 412 $[M-H_2O]^+$ 20.64%, 396 $[M-20H^+]$ 10.03%.

Compound **7**.—^1H nmr ($CDCl_3$-CD_3OD, 9:1, *J* in Hz) 5.45 H-6 (dd-like, 5.87, 1H), 4.38 H-1′ (d, 7.71, 1H), 4.10 H-6′ (m, 2/5/11, 2H), 3.79 H-7 (m, 1H), H-3 overlapped, 3.2-3.7 sugar protons (m, 5H), 0.867 H-19 (s, 3H), 0.809 H-21 (d, 6.41, 3H), 0.754 H-29 (t, 7.40, 3H), 0.728 H-26 (d, 6.87, 3H), 0.688 H-27 (d, 6.87, 3H), 0.561 ppm H-18 (s, 3H); fdms *m/z* 592 M^+ 34.14%, 574 $[M-H_2O]^+$ 21.64%, 429 [aglycone-H] 32.6%.

Compound **8**.—^1H nmr ($CDCl_3$-CD_3OD, 9:1, *J* in Hz) 5.28 H-6 (br s, 1H), 4.16 H-1′ (d, 7.63, 1H), 4.05 H-6′ (m, 2H), 3.847 H-7 (m, 1H), 3.79 H-3 (m, 1H), 0.872 H-19 (s, 3H), 0.815 H-21 (d, 6.41, 3H), 0.768 H-29 (t, 7.40, 3H), 0.751 H-26 (d, 7.02, 3H), 0.734 H-27 (d, 7.1, 3H), 0.583 ppm H-18 (s, 3H); fdms *m/z* 616 $[M+Na+1]^+$ 10.5%, 592 M^+ 35.5%, 574 $[M-H_2O]^+$ 23.5%.

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