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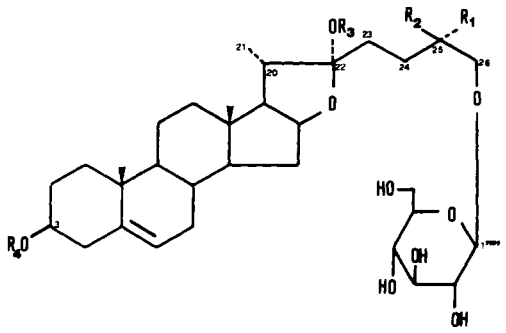
FUROSTANOL OLIGOSIDES FROM *TAMUS COMMUNIS*

RITA AQUINO, ISABELLA BEHAR, FRANCESCO DE SIMONE, MARIO D'AGOSTINO, and COSIMO PIZZA

Dipartimento di Chimica delle Sostanze Naturali, Facoltà di Farmacia, Università di Napoli, via L. Rodinò, 22, 80138 Napoli, Italy

Some *Tamus* species (Dioscoreaceae) have already been reported as a source of steroidal sapogenins (1). Attempting to isolate the parent glycosides of these sapogenins, we recently have isolated two spirostane triglycosides, dioscin and gracillin, from the rhizomes of *Tamus communis* L. (2). Continuing with our systematic research of the biologically active metabolites (3-5) from *T. communis*, we now report the isolation of methylprotogracillin (**3**) and a successful example of the separation of the (25*R*)-furostanol oligoside, methylprotodioscin (**2**) (6), from its (25*S*)-epimer, methylprotoneodioscin (**1**). Structures were determined by chemical and spectral studies; solvent effects on ^{1}H-nmr and ^{13}C-nmr spectra and fabms spectra proved useful in the structural analysis. Furthermore, in the course of this work we have also assigned certain ^{1}H-nmr



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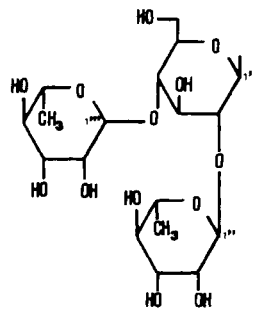

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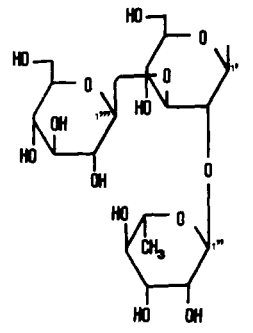
3 R_1=H, R_2=CH_3, R_3=CH_3, R_4=



4 R_1=H, R_2=CH_3, R_3=H, R_4=

data for 22-methoxy and 22-hydroxy-furostanosides.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—The following instruments were used: nmr, Brüker MW-250 Spectrospin; ms, Kratos MS 902 mass spectrometer equipped with Kratos fab source; hplc, Waters Model 6000A pump equipped with a U6K injector and a model 401 refractive index detector; glc, Perkin-Elmer Sigma 115 Instrument; optical rotation, NPL THORN Type 204 polarimeter.

The fabms spectra were obtained by dissolving the samples in a glycerol matrix and placing them on a copper probe tip prior to bombardment with Ar atoms of energy 2-6 KV. The dept experiments were performed using polarization transfer pulses of 90° and 135°, respectively, to obtain in the first case only CH groups and in the other case positive signals for CH and CH_3 and negative ones for the CH_2 groups. Polarization transfer delays were adjusted to an average CH coupling of 135 Hz.

EXTRACTION AND ISOLATION.—The plant material and the extraction procedure have been described earlier (2). The MeOH dried extract (96 g) was redissolved in H_2O and passed through an Amberlite XAD-2 (1 kg) column. This column was washed with H_2O, the adsorbate was eluted with MeOH, and the dried eluate (18 g) was rechromatographed on a Sephadex LH-20 column (80×4 cm). Fractions of 20 ml were eluted using MeOH as solvent. Fractions 8-11 were checked by tlc-SiO_2 with BuOH-HOAc-H_2O (60:15:25) and shown to contain a mixture of furostanol glycosides (400 mg) which was submitted to hplc on a C_{18} μ-Bondapak column (30 cm×7.8 mm) with H_2O-MeOH (38:62) (flow rate: 3.5 ml/min) to give three main fractions. The fraction collected after 16.5 min from injection contained the 25*S* epimer of methylprotodioscin (**1**, 26 mg), $[\alpha]^{18}D=-88.7°$ (c=0.80, pyridine), (*Anal.* calcd for $C_{52}H_{86}O_{22}$: C, 57.76; H, 8.20. Found: c, 57.93; H, 8.32), fabms *m/z* 1085 $(M+Na)^+$. The fraction collected after 21 min contained methylprotodioscin (**2**, 132 mg), fabms *m/z* 1085 $(M+Na)^+$. The fraction collected after 23.5 min contained methylprotogracillin (**3**, 72 mg), $[\alpha]^{18}D=-76.7°$ (c=0.71, pyridine), (*Anal.* calcd for $C_{52}H_{86}O_{23}$: C, 57.41; H, 8.67. Found: C, 57.50; H, 8.60), fabms *m/z* 1101 $(M+Na)^+$. The ^1H- and ^{13}C-nmr spectra of **1**, **2**, **3** are in Table 1 and 2; fabms spectra in Table 3.

Methanolysis.—A solution of each glycoside (0.5-1 mg) in anhydrous 2M HCl/MeOH was heated at 80° in a stoppered reaction vial for 8 h. After being cooled, the reaction mixture was neutralized with Ag_2CO_3, centrifuged, and the supernatant evaporated to dryness under N_2. The residue was dissolved in TRISIL Z (0.1 ml, *N*-trimethylsilylimidazole in pyridine, Pierce Chemical Co.), left at room temperature for 15 min and analyzed by glc (140°, SE-30, 20 m).

Demethylation of **3**.—A solution of **3** (20 mg) in a mixture of Me_2CO-H_2O (7:3 v/v, 20 ml) was refluxed for 10 h. The reaction mixture was evaporated to dryness in vacuo to afford 12 mg of protogracillin (**4**).

Methylation of **4**.—A solution of **4** (10 mg) in absolute MeOH (10 ml) was heated at reflux for 15 h. The MeOH was concentrated to a small volume and diluted with Me_2CO (2 ml). The resulting precipitate was filtered off and dried to afford **3**.

Enzymatic hydrolysis of **3** *and* **2**.—Solutions of **3** and **2** (20 mg, each) in H_2O (2 ml) were separately incubated with emulsin (10 mg) at 37° for 48 h. The precipitate was collected by filtration, dried, and subjected to hplc (C-18 μ-Bondapak column 30 cm×7.8 mm i.d., MeOH-H_2O, 75:25) to give gracillin from the glycoside **3** and dioscin from the glycoside **2** identified by direct comparison (hplc, ^1H nmr, and fabms spectra) with the authentic samples isolated from *T. communis* (2).

Acid hydrolysis of **1** *and* **2**.—Solutions of **1** and **2** (20 mg, each) were separately hydrolyzed by refluxing with 2 M HCl-50% dioxane (6 ml) for 3 h. The reaction mixture was diluted with H_2O and extracted with $CHCl_3$. The $CHCl_3$ layer was washed with H_2O and dried over Na_2SO_4. The $CHCl_3$ solution was filtered, and the filtrate was chromatographed by preparative tlc on silica gel using 15% EtOAc/C_6H_6 to afford the aglycone (5 mg).

The aglycone of glycoside **1** was identified as yamogenin: ^1H nmr ($CDCl_3$) δ 1.09 (Me-27, d, *J*=7 Hz), 1.03 (Me-19, s), 1.01 (Me-21, d, *J*=6,7 Hz), 0.79 (Me-18, s); Rf=0.258. The aglycone of glycoside **2** was identified as diosgenin: ^1H nmr ($CDCl_3$) δ 0.79 (Me-18, s and Me-27, d, *J*=7 Hz), 0.97 (Me-21, d, *J*=6,6 Hz), 1.03 (Me-19, s); Rf=0.262. These genins were also identified by comparing ^{13}C-nmr data with those of the literature (7).

RESULTS AND DISCUSSION

The saponin mixture obtained from the rhizomes of *T. communis* afforded two new compounds, methylprotoneodioscin (**1**) and methylprotogracillin (**3**), and a known compound, methylprotodioscin (**2**).

METHYLPROTOGRACILLIN (**3**).—On acid methanolysis, methylproto-

TABLE 1. ^{13}C-nmr Data for **1**, **2**, **3**, and **4**[a]

Carbon No. Aglycone	Compound						Carbon No. Sugars	Compound		
	1	2	3			4		3		
	δ C(CD_3OD)	δ C(CD_3OD)	δ C(CD_3OD)	dept	δ C(C_5D_5N)	δ C(C_5D_5N)		δ C(CD_3OD)	dept	δ C(C_5D_5N)
1	38.6	38.6	38.6	CH_2	37.7	37.6	G-1′	100.7	CH	100.3
2	30.8	30.8	30.8	CH_2	30.2	30.3	G-2′	82.5	CH	81.5
3	78.1	78.1	78.3	CH	78.6	78.6	G-3′	88.8	CH	89.4
4	40.9	40.9	41.0	CH_2	40.0	40.1	G-4′	70.3	CH	69.9
5	142.1	142.1	142.1	C	141.1	141.2	G-5′	78.5	CH	78.3
6	122.6	122.5	122.5	CH	121.8	121.8	G-6′	62.9	CH_2	62.8
7	32.8	32.8	32.8	CH_2	32.8	32.5				
8	32.8	32.8	32.8	CH	31.9	31.8	RHA-1″	102.3	CH	102.1
9	51.8	51.8	51.8	CH	50.6	50.6	RHA-2″	72.2[b]	CH	72.4[c]
10	38.1	38.1	38.1	C	37.3	37.2	RHA-3″	72.5[b]	CH	72.8[c]
11	22.0	22.0	22.0	CH_2	21.2	21.2	RHA-4″	74.1	CH	74.3
12	39.5	39.6	39.6	CH_2	38.9	39.1	RHA-5″	69.9	CH	69.9
13	41.9	41.9	41.2	C	41.0	41.0	RHA-6″	18.0	CH_3	18.6
14	57.8	57.8	57.8	CH	56.8	56.8				
15	31.5	31.5	31.4	CH_2	32.5	32.5	G-1‴	104.5	CH	104.5
16	80.5	80.6	79.4	CH	81.5	81.2	G-2‴	75.2	CH	75.1
17	65.2	65.1	65.1	CH	64.3	64.0	G-3‴	77.9	CH	77.8
18	16.3	16.7	16.7	CH_3	16.3	16.5	G-4‴	71.7	CH	71.7
19	19.9	19.8	19.8	CH_3	19.5	19.4	G-5‴	78.5	CH	78.2
20	41.2	41.2	41.2	CH	40.6	40.8	G-6‴	62.7	CH_2	62.7
21	16.2	15.9	15.9	CH_3	16.1	16.3				
22	114.1	114.1	114.1	C	112.8	110.8	G-1⁗	104.6	CH	104.9
23	33.2	33.2	33.2	CH_2	31.0	37.3	G-2⁗	75.2	CH	75.2
24	29.0	29.0	29.0	CH_2	28.4	28.1	G-3⁗	77.3	CH	77.2
25	35.1	35.0	35.0	CH	34.4	34.3	G-4⁗	71.9	CH	72.1
26	75.9	76.0	76.0	CH_2	75.2	75.2	G-5⁗	78.3	CH	78.1
27	17.4	17.3	17.3	CH_3	17.2	17.5	G-6⁗	63.0	CH_2	63.2
$-OCH_3$	=	=	=	CH_3	47.3	=				

[a]The signals for the trisaccharide carbon atoms of compounds **1** and **2** were virtually identical to that of dioscin (2); the signals of the sugar moiety of **4** were identical to that of **3**, both in CD_3OD and pyridine.

[b,c]Assignments can be interchanged in each vertical column.

TABLE 2. Partial ^{1}H-nmr Data for **3**,**4**,**2**,**1** in CD_3OD

Proton No.	Compounds			
	3	**4**[a]	**1**	**2**
H-18	0.87 s (0.79)[b]	(0.86) s	0.88 s (0.80)	0.87 s (0.79)
H-19	1.07 s (1.03)	(1.02) s	1.08 s (1.03)	1.08 s (1.03)
H-21	1.03 d, J=6 Hz (1.17)	(1.30) d, J=6 Hz	1.01 d (1.15)	1.03 d (1.17)
H-27	0.98 d, J=6 Hz (0.98)	(0.95) d, J=6 Hz	1.00 d (1.00)	0.98 d (0.98)
-OCH_3 . . .	3.17 s (3.25)		3.19 s (3.23)	3.17 s (3.25)
H-1	4.50 d, J=7 Hz		4.53 d, J=7 Hz	4.53 d
H-1′	5.32 d, J=1.5 Hz		5.23 d, J=1.5 Hz	5.23 d
H-1″	4.55 d, J=7 Hz		4.87 d, J=1.5 Hz	4.87 d
H-1‴	4.26 d, J=7 Hz		4.27 d, J=7 Hz	4.27 d
H-6	5.40 m		5.41 m	5.41 m
RHA-CH_3 .	1.26 d, J=6.2 Hz	(1.60) d, J=6 Hz	1.27 d, J=6.2 Hz (1.60)	1.26 d, J=6.2 Hz
RHA-CH_3			1.29 d, J=6.2 Hz (1.74)	1.28 d, J=6.2 Hz
H-20	2.20 m (2.23)	(2.24) m		

[a]Unambiguous assignment of the other signals appearing at δ 4.86, 5.01, 5.36, 6.21 was not possible.

[b]Data in parentheses are for spectra in C_5D_5N at room temperature.

gracillin gave methylglucoside and methylrhamnoside in the ratio 3:1. The molecular formula $C_{52}H_{86}O_{23}$ was determined by dept ^{13}C nmr (Table 1), elemental analysis, and the fabms spectrum which showed a molecular ion species at *m/z* 1101 $(M+Na)^+$ and a base peak at 1047 $[(M+Na)-OCH_3]^+$. The ion peaks at *m/z* 885 and 723 clearly showed the loss of two units of glucose (162 m.u.) from the peak at *m/z* 1047. The ion peak at *m/z* 577 confirmed the further loss of one unit of rhamnose (146 m.u.) while one glucose molecule remained attached to the aglycone. The peak at *m/z* 415 is ascribable to the mass of the protonated aglycone. In addition to characteristic furostanol carbon signals (8) and four anomeric carbons for the sugar moieties, the ^{13}C-nmr spectrum (Table 1) also indicated the points of attachment of sugar residues in the aglycone: the signal at 76.0 ppm (CD_3OD) (CH_2 by dept pulse sequence) was assigned to glycosidated C-26; comparison of the chemical shifts of the signals assigned to C-2 (CH_2), C-3 (CH), and C-4 (CH_2) with those of the corresponding signals for a spirostane (7) showed shifts which would be expected from glycosidation at C-3 (9).

The ^{1}H-nmr spectrum in CD_3OD (Table 2) was in agreement with a furostanol structure (6) and showed four doublets at δ 4.26 (1H, J=7 Hz), 4.50 (1H, J=7 Hz), 4.55 (1H, J=7 Hz), and 5.32 (1H, J=1.5 Hz) for the anomeric protons. The anomeric configurations were deduced from the δ and J values of the anomeric protons and carbons that suggested the presence of three β-glucose units and one α-rhamnose unit. A detailed comparison of ^{1}H- and ^{13}C-nmr data for compound **3** and gracillin (2) showed that the trisaccharide chain is identical in both compounds. On refluxing in aqueous Me_2CO, **3** was converted to **4**, while **4** was converted back to **3** on

TABLE 3. Fabms Spectral Data for Compounds **3**, **2**, and **1**.

Compounds			
m/z	**3**	*m/z*	**2** and **1**
1117	$[M+K]^+$	1101	$[M+K]^+$
1101	$[M+Na]^+$	1085	$[M+Na]^+$
1079	$[M+H]^+$	1063	$[M+H]^+$
1047	$[M+H\text{-}OCH_3]^+$	1031	$[M+H\text{-}OCH_3]^+$
901	$[(M+H\text{-}OCH_3)\text{-}146]^+$[a]		
885	$[(M+H\text{-}OCH_3)\text{-}162]^+$	869	$[(M+H\text{-}OCH_3)\text{-}162]^+$[a]
739	$[(M+H\text{-}OCH_3)\text{-}308]^+$		
723	$[(M+H\text{-}OCH_3)\text{-}324]^+$	723	$[(M+H\text{-}OCH_3)\text{-}308]^+$
577	$[(M+H\text{-}OCH_3)\text{-}470]^+$	577	$[(M+H\text{-}OCH_3)\text{-}454]^+$
415	[577-162]	415	[577-162]
397	[415-18]	397	[415-18

[a]The mass units lost corresponded to the following fragments: *m/z* 162, glucose; *m/z* 146, rhamnose; *m/z* 308, rhamnose+glucose; *m/z* 454, two rhamnose+glucose; *m/z* 470, rhamnose+two glucose.

refluxing in MeOH. In addition to the lack of the signals due to the methoxyl group found at δ 3.17 (CD_3OD) or 3.25 (C_5D_5N) in **3**, the ^1H-nmr spectrum of **4** also showed differences for signals due to the protons at C-18, C-21, and C-27. In contrast, it has been reported (8,10) that 22-OH and 22-OCH_3 furostanosides are different only by a methoxy signal in the ^1H-nmr spectrum. Furthermore, we have discriminated H-21 and H-27 signals by ^1H selective decoupling experiments: irradiation at δ 2.23 (H-20 m) in deuteropyridine and δ 2.20 (H-20 m) in deuteromethanol led to the decoupling of the H-21 doublet (δ 1.17 or 1.03, respectively).

Comparison of the chemical shifts of the signals assigned to C-23, C-22, and C-24 of **3** with those of the corresponding signals of **4** (C_5D_5N, Table 1) showed that in **4** C-23 was deshielded (7.3 ppm) and C-22 and C-24 were shielded (2 and 0.3 ppm), as would be expected from a demethylation effect. This observation indicated that 22-OCH_3 became a 22-OH group; such chemical transformations are known in oligofurostanosides (9,10). In the light of the easy methylation and demethylation of these compounds, it is probable that **3** as well as **2** and **1** are artifacts produced from the corresponding 22-hydroxyfurostanosides during the extraction of the plant material with MeOH (11). Enzymatic hydrolysis of **3** with β-glucosidase afforded a spirostanol glycoside, gracillin, a compound of known configuration at C-25 of the aglycone diosgenin, and provided further evidence for the configuration of **3** at this asymmetric center.

These data indicated that **3** is methylprotogracillin or 3-*O*-[α-L-rhamnopyranosyl-(1→2)[β-D-glucopyranosyl-(1→3)-β-D-glucopyranosyl]-26-*O*-(β-D-glucopyranosyl)-22 α-methoxy-(25*R*)-furost-5-en-3β,26 diol.

METHYLPROTODIOSCIN (**2**) (6).—The chemical shifts of the signals due to the aglycone protons and carbons were superimposable on those of **3**, while the chemical shifts of the signals due to the trisaccharide sequence are superimposable on those of dioscin (2,8). On acid hydrolysis, **2** gave diosgenin; on enzymatic hydrolysis, as with all furostanolic saponins, **2**, undergoes elimination of the glucose molecule at C-26 and ring closure to form the spirostanolic compound (12) dioscin. The fabms (Table 2) spectrum confirmed the identification as 3-*O*-[α-L-rhamnopyranosyl-(1→4)[α-L-

rhamnopyranosyl-(1↦2)]-β-D-glucopyranosyl]-26-*O*-(β-D-glucopyranosyl)-22-α-methoxy-(25*R*)-furost-5-en-3β, 26 diol.

METHYLPROTONEODIOSCIN (**1**).—On acid methanolysis, methylprotoneodioscin (**1**) gave methylglucoside and methylrhamnoside in the ratio 1:1. The fabms spectra of **1** and **2** are identical (Table 2). That the molecular weight of **1** was 1062 was clear from the peaks at 1085 $(M+Na)^+$ and 1063 $(M+H)^+$, and from the base peak at 1031 $[(M+H)-OCH_3]^+$. The 869 peak corresponded to a loss of a hexose (glucose) unit, whereas the peaks at 723 and 577 corresponded to further losses of a 2-deoxyhexose (rhamnose). The 415 peak was ascribable to the mass of the protonated aglycone.

The ^{1}H-nmr spectra of **1** in CD_3OD and pyridine-d_5 showed small but significant changes with respect to **2**; the H-21 methyl protons were shifted by 0.02 ppm upfield, and the H-27 and H-18 methyl protons were shifted by 0.02 and 0.01 ppm downfield (Table 2). These shifts seemed to correspond to those due to a 25*S* epimer (13). The chemical shifts of the other protons were similar for furostanol of the 25-*R* and 25-*S* series and also for the sugar moieties; no significant differences are present in the ^{13}C-nmr spectra (Table 1) for carbons neighboring C-25. Further evidence for the 25-*S* configuration of **1** was provided by acidic hydrolysis that afforded yamogenin, a compound with known 25-*S* stereochemistry. The most significant differences in the ^{1}H-nmr spectra between yamogenin (from **1**) and diosgenin (from **2**) reside in the chemical shifts of 27-Me and 21-Me groups: 1.09 and 1.01 ppm, respectively, for yamogenin and 0.79 and 0.97 for diosgenin.

These data indicated that **1** is 3-*O*-[α-L-rhamnopyranosyl-(1↦4)[α-L-rhamnopyranosyl-(1↦2)]-β-D-glucopyranosyl]-26-*O*-(β-D-glucopyranosyl)-22-α-methoxy-(25*S*)-furost-5-en-3β,26 diol.

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