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THE SAPOGENIN OF *CHAMAEDOREA ELEGANS*

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ABSTRACT.—From the saponin fraction of the extract of *Chamaedorea elegans*, a crystalline sapogenin was isolated and characterized as 5α, 14α, 25R-spirostane-1β-3α-diol, also known as cannigenin (**1**).

Chamaedorea elegans Mart. (Palmaceae), commonly known as parlor palm, is an ornamental palm and is much prized for its foliage in the florists' trade. It grows and is also heavily cultivated in the Central American region, especially in Guatemala. The plant has not been examined so far for its constituents. Our work was initiated as a result of an inquiry from a local florist concerning the possible economic value of this plant. We report here the isolation of a crystalline sapogenin and its characterization as the 5α, 25R-spirostane-1β,3α-diol.

The aqueous extract of the dried, ground plant gave strong evidence (foaming and hemolysis) for saponin components which were extractable into *n*-BuOH. The saponin fraction on hydrolysis and purification yielded a colorless crystalline solid **1**, $C_{27}H_{44}O_4$ (M^+ 432). Spectral data (ir 869, 905, 928, and 988 cm^{-1}; pmr δ3.40, 2H; δ4.36, 1H), generally considered suggestive of a spiroketal system, indicated that the compound might be a steroidal sapogenin (1). Mass spectral fragmentation, which gave, besides the molecular ion and the base peak at *m/z* 139, a series of peaks considered to be typical of steroidal sapogenins, supported this characterization (2). The presence of two hydroxyl groups was ascertained by acetylation to a crystalline diacetate **2**, $C_{31}H_{48}O_6$ (M^+ 516; pmr δ1.97, 3H; δ2.07 3H). Its mass-spectral fragmentation showed that the hydroxyls were not present in the spiroketal portion of the molecule. The molecular formula also suggeted that there was no unsaturation, although the compound reacted with Br_2 and underwent catalytic hydrogenation to give mixtures of products. Presumably, these reactions were affecting the spiroketal system (mass-spectral evidence). Oxidation of the sapogenin with chromic acid-Me_2CO gave two products: **3** and **4**, separated by adsorption chromatography. The faster-moving component (**3**), $C_{27}H_{40}O_4$ (M^+ 428) showed two carbonyl bands (1728 and 1695 cm^{-1}) and a uv spectrum (λ max 255 nm; shift with base to 285 nm) which was identical with that of 5,5-dimethylcyclohexane-1,3-dione, thereby showing a 1,3-relationship for the two hydroxyls in the sapogenin. The slower-moving component (**4**), $C_{27}H_{42}O_4$ (M^+ 430) was found to be a hydroxy ketone (3415, 1685 cm^{-1}). The ms of these compounds further confirmed that the hydroxyls were not located in the spiroketal system of the sapogenin. On the assumption that one of the hydroxyls is generally located at C-3, the other hydroxyl was placed at C-1.

The physical and spectral data of the *Chamaedorea* sapogenin were compared with the published data of the known spirostane-1,3-diols. They differed (see Table 1) from those of cannigenin (3), cordylagenin (3), brisbagenin (3), isorhodeasapogenin (4), and rhodeasapogenin (4). Further studies were therefore needed to establish the structure and stereochemistry in order to determine whether it was, indeed, a new compound.

Since cmr spectral data on spirostane-1,3-diols was not available, a comparison was made with the spectra of other, better known sapogenins to establish some of the basic stereochemical features (5-7). The chemical shifts of C_{13}-C_{27} were in agreement with those reported for tigogenin (5α, and 25R) and showed clearly that the *Chamaedorea* sapogenin had the same configuration at these points. Ir spectral evidence (ratio of in-

TABLE 1. Comparison of the 1,3-Dihydroxyspirostanes

Compound and Stereochemistry	Sapogenin		Diacetate		
	mp	[α]D	mp	[α]D	Ref.
Chamaedorea sapogenin	217-218°	−79°±1	195-196° (anhydrous) 115-116° (hydrate)	−31.8°	
Cannigenin 1β, 3α, 5α, 25R	215.5-217°	−58.03°	oil	−23.86°	3
Brisbagenin 1β, 3β, 5α, 25R	203-204°	−76°	164-165°	−50.9°	3
Cordylagenin 1β, 3α, 5α, 25S	216°	−50°	155°	−31.06°	3
Ishorhodeasapogenin 1β, 3β, 5β, 25R	241-243°	−71°	205°	−73°	4
Rhodeasapogenin 1β, 3β, 5β, 25S	293-295°	−72°	185-187°	−71°	4

tensity at 898 cm^{-1}: intensity at 920 $cm^{-1} > 1$) also indicated the 25R configuration, although this evidence is said to be not always reliable (1, 8). For further confirmation of these assignments, conversion of the sapogenin to a known spirostane-3-ol such as tigogenin was carried out by a procedure similar to the one described by Jewers *et al.* (9). The diacetate **2** was subjected to mild basic hydrolysis to yield the monoacetate **5**, which on oxidation by Jones reagent gave the 1-en-3-one **6** with the uv, ir, pmr, and cd-spectral data similar to those described for cholest-1-en-3-one (10) (see Table 2 for the cd data). The sign and magnitude of the Cotton effects of **6** clearly pointed to the 5α ring fusion as opposed to the 5β-(11, 12). This result also established that the *Chamaedorea* sapogenin was different from rhodeasapogenin and isorhodeasapogenin, both of which possess the 5β-configuration. In addition, the result supported the 1,3-dihydroxy substitution in ring A. Catalytic hydrogenation of **6** gave a monohydroxy spirostane, identical with tigogenin (12).

TABLE 2. Comparison of cd Data of **6** with Steroids of 5α-1-en-3-one Structure

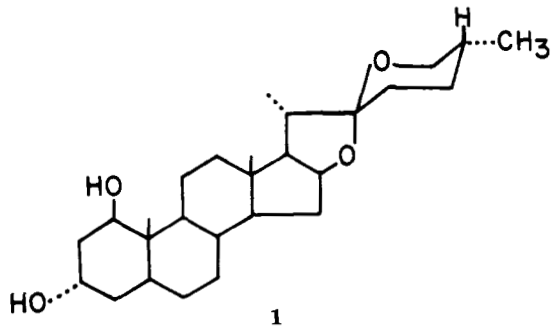
	λ[a]	Q[b]	λ	Q	λ	Q
Compound **6**	332	−3300	235	+34,650	205	−20,460
5α-Cholest-1-en-3-one	333	−3036	235	+25,311	202	−17,350
5α,25R-Spirost-1-en-3-one (from cannigenin)	336	−4290	235	+40,260	203	−32,109
5α,25S-Spirost-1-en-3-one (from cordylagenin)	340	−3069	234	+34,287	203	−28,677
5α,25R-Spriost-1-en-3-one (from brisbagenin)	336	−5180	235	+48,180	202	−43,560

[a]λ, wavelength.

[b]Q, molecular ellipticity given by 3300 × ΔE, when ΔE is the differential dichroic absorption (11).

With regard to the assignment of stereochemistry for the hydroxyls at C-1 and C-3, the pmr spectral data of the diacetate indicated that one has the α-(δ2.06) and the other a β-(δ1.96) orientation (3). A comparison of the chemical shifts and the shapes of the signals of protons at C-1 and C-3 of a series of derivatives of the sapogenin (see Figure 1) with published chemical shifts and shapes (13) clearly showed that the hydroxyl at C-1 has the β- and the one at C-3, the α- configuration. Additional evidence was obtained from the cmr spectrum in which the shift of the C-19 methyl signal pointed to the 1-β-

hydroxyl. The presence of one hydroxyl with α- and another with a β- orientation distinguishes the *Chamaedorea* sapogenin from brisbagenin which contains both hydroxyls in β-configuration.



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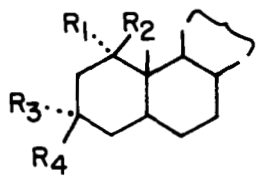
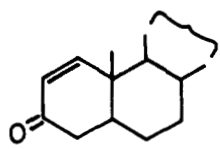
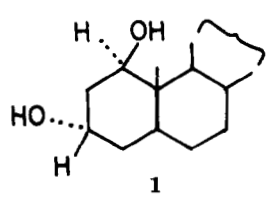




6

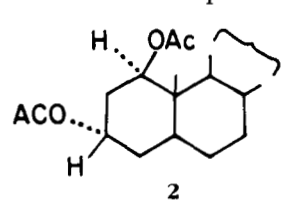
2 $R_1=R_4=H$; $R_2=R_3=OCOCH_3$
3 $R_1+R_2=O$; $R_3+R_4=O$
4 $R_1=H$; $R_2=OH$; $R_3+R_4=O$
5 $R_2=OCOCH_3$; $R_3=OH$; $R_1=R_4=H$
7 $R_1=H$; $R_2=OCOCH_3$; $R_3+R_4=O$
8 $R_1=R_3=H$; $R_2=R_4=OH$

The assignment of a 5α, 25R-spirostane-1β,3α- diol structure to the *Chamaedorea* sapogenin clearly pointed out that it must be identical with cannigenin. The discrepancies, however, remained in the reported physical properties of the sapogenins and their acetates. Repeated recrystallization of the *Chamaedorea* sapogenin gave a sample, homogeneous by gc, with a rotation of $-79°\pm1$ in contrast with the reported value of



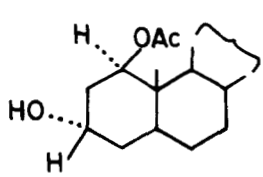
1

1α*H* 3.76
3β*H* 4.06



2

1α*H* 4.88 3β*H* 5.0
1βCH_3COO 1.97 3αCH_3COO 2.07



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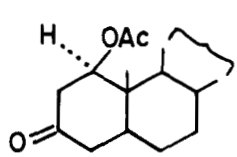
1α*H* 4.88
1βCH_3COO 1.97



7

1α*H* 4.88 3β*H* 4.06
1βCH_3COO 1.97

FIGURE 1. Chemical shifts (δ) of C_1 and C_3 protons.

−58.03. The diacetate of the *Chamaedorea* sapogenin crystallized as a monohydrate (mp 115-116°) or as an anhydrous sample (mp 195-196°) with a rotation of −31.8° as opposed to the cannigenin diacetate which was reported to be an oil with a rotation of −23,86°. However, the physical properties of the 1-monoacetate **5** and the 1-en-3-one **6** were in better agreement with the corresponding reported values. A sample of pure cannigenin was kindly supplied by Professor Gerald Blunden with a notation that the sample of cannigenin described in the literature might have some cordylagenin (25S isomer) as contaminant. Comparison of the *Chamaedorea* sapogenin with the reference cannigenin showed that they had identical gc-retention times and ir spectra.

In order to establish further the identity of the sapogenin with cannigenin, an experiment to convert the *Chamaedorea* sapogenin to the 1β,3β-diol, brisbagenin was undertaken. The 1-monacetate **5** was oxidized under mild conditions with Jones' reagent to yield the 3-keto-1β-acetate **7**. For the next step, catalytic hydrogenation was selected because this, as well as metal hydride reduction, was reported to produce the β-isomer predominantly (14). However, after basic hydrolysis, the product was found to be a near equal mixture of the *Chamaedorea* sapogenin and another compound with a very similar Rf value. By careful and repeated crystallization, this second compound was obtained pure, and its properties were found to agree with those of brisbagenin (**8**). Besides the elemental analysis and ms which established the molecular formula and spirostane diol structure, the pmr spectrum (300 Hz) showed clearly that the 3β-H was replaced by the 3α-H in the new compound (see Figure 2). The signal due to the 3β-H (δ 4.06) found in cannigenin was absent in the new diol and shifted to δ3.45, as part of the multiplet due to the 26α and 26β-protons. The chemical shift range reported for the 3α-H is 3.5-3.7 (13). The presence of the extra proton in the multiplet due to 26α and 26β protons is clearly shown by the integral which equaled 3H, while the corresponding multiplet of cannigenin equaled 2H. These studies establish the identity of the *Chamaedorea* sapogenin with cannigenin.

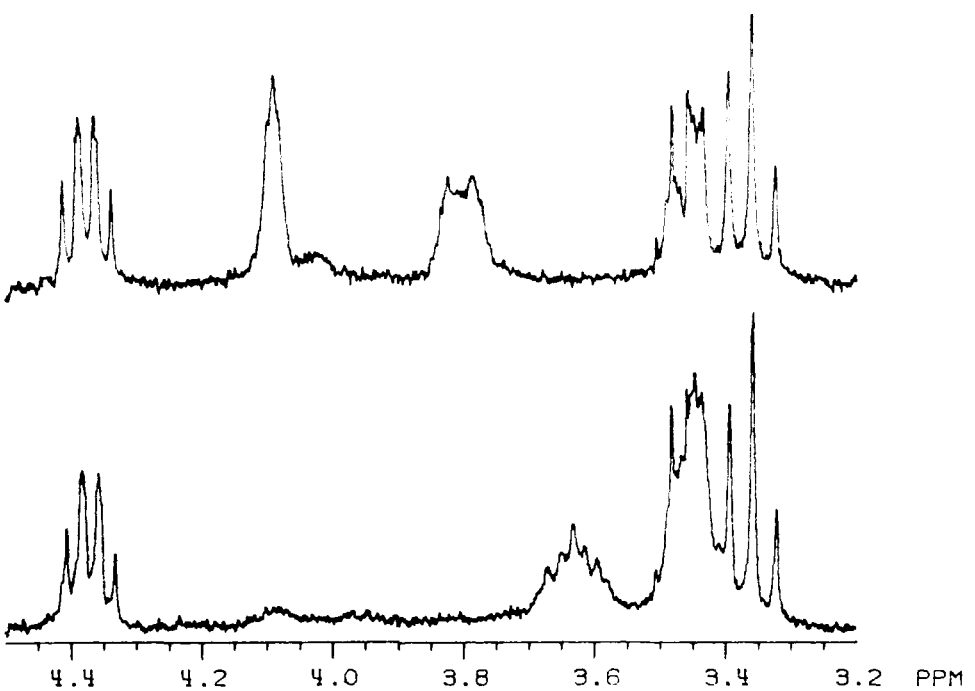


FIGURE 2. Portions of 300 MHz pmr spectra of cannigenin (top) and brisbagenin (bottom).

Spirostane-1,3-diols may serve as valuable raw materials for the synthesis of those steroidal drugs where the presence of the 1-hydroxyl function can confer some advantages. In this connection, *Chamaedorea* is a better source for cannigenin than *Cordylene cannifolia*, from which cannigenin was originally isolated. This is because, in *Chamaedorea*, cannigenin is not accompanied by cordylagenin and hence, is obtainable in higher purity. Secondly, although *Cordylene cannifolia* in its natural habitat produces 0.34-0.37% of the mixture of the two sapogenins, the cultivated variety only has 0.05-0.09% of the mixture at the time of harvest, after two years of growth (3). *Chamaedorea*, on the other hand, is a readily available, cultivated palm which yields 0.3-0.5% cannigenin.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Melting points were determined with a Fisher-Johns apparatus and were uncorrected. The spectra were obtained with the following instrumentation: uv, Beckman 25; ir, Beckman Acculab 3; pmr, Varian T60 and Nicolet NT-300 equipped with Nicolet 1280 computer with tetramethylsilane as internal standard; cmr, Jeol-JNM-FX100 spectrometer; mass spectra, Hitachi-Perkin-Elmer high resolution mass spectrometer, model RMU 6E and, circular dichroism, Jasco, J-500 spectropolarimeter.

Tlc was carried out with E. Merck Silica Gel HF 254+366. Column chromatography was performed with Merck silica gel (200-400 mesh) and gc with a Varian 2100 instrument equipped with a flame-ionization detector.

The plant material was received from Mr. Ben Graves, a florist from Tampa, Florida, and was authenticated at the Herbarium, University of Florida. The dried and coarsely ground leaves (~1 kg) were stirred in H_2O at 70-80° for 4 h and the extract drained. After two more such extractions, the combined extract was concentrated under reduced pressure to approximately 1 liter and shaken once with Et_2O (1 liter). The clear aqueous layer was then extracted with *n*-BuOH (400 ml) four times. The combined solvent layer was concentrated azeotropically, and the residual semisolid was heated with 4N H_2SO_4 (500 ml) under reflux for 4 h. After cooling, the solid was filtered, washed with H_2O, and extracted with hot $CHCl_3$ (3×200 ml). The combined extract was washed with aqueous NaOH (200 ml, 0.5 N), dried (Na_2SO_4), concentrated to approximately 20 ml, diluted with C_6H_6 (30 ml), and applied to a column of silica gel (250 g) in C_6H_6. The column was eluted first with C_6H_6 and then with 2-5% Me_2CO in C_6H_6. Concentration of the Me_2CO-C_6H_6 eluate gave a colorless crystalline solid which was recrystallized twice from Me_2CO, mp 217-218°; yield, 4.1 g; [α]D, −79° (c=1, $CHCl_3$); in ν 3500, 1080, 1055 (OH) 980, 920, 906, 870 cm^{-1} (spiroketal); pmr δ0.83, s, 6H, C_{13}/C_{10}-CH_3; 0.95, d, 3H, C_{25}-CH_3 3.45, d, 2H; 3.79, m, 1H; 4.06, m, 1H; 4.36, m, 1H; cmr 107.9, 79.9, 72.5, 65.8, 64.6, 62.2, 55.9, 54.7, 43.1, 41.6, 41.0, 40.1, 39.5, 37.6, 35.7, 35.3, 31.8, 31.4, 30.8, 29.5, 28.3, 27.8, 23.5, 16.6, 15.8, 14.1, 5.5 ppm; ms *m/z* 432, 412, 373, 363, 360, 318, 303, 298, 289, 139, 121, 119, 115.

Anal. calcd for $C_{27}H_{44}O_4$; C, 74.95: H, 10.25. Found: C, 75.12; H, 10.34.

The sample was homogeneous in tlc (5% MeOH in $CHCl_3$) with Rf 0.5 when visualized by 1% H_2SO_4 in HOAc spray, followed by heat to obtain a violet brown spot. In gc on a column of OV-17 at 250°, the silylated sample gave a single peak with R_T of 6 min.

ACETYLATION.—A mixture of **1**(0.2 g), Ac_2O (2 ml), and pyridine (0.5 ml) was heated at 100° for 30 min. It was cooled, diluted with H_2O, the solid filtered and crystallized from aqueous MeOH; yield, 0.2 g; mp 115-116°; [α]D, −31.2° (c=1, $CHCl_3$). Recrystallization from Et_2O-hexane gave a colorless crystalline solid; mp 195-196°, [α]D, −31.8°; ν 1735 cm^{-1}; pmr δ 0.75, s, 3H; δ 0.95, s, 3H δ 1.95, s, 3H; δ 2.08, s, 3H; δ 4.11, d, 2H; δ 4.37, m, 1H; δ 4.9, m, 1H; δ 5.06, m, 1H; ms *m/z* 516, 458, 447, 402, 373, 303, 139, 115.

Anal. calcd for $C_{31}H_{48}O_6$: C, 76.81, H; 9.98. Found: C, 76.95; H, 10.12.

OXIDATION.—A suspension of **1** (0.5 g) in Me_2CO (50 ml) was treated with chromic acid (1 M solution in 1 N H_2SO_4) added dropwise until the starting material was spent. After dilution with H_2O, the solid which separated was filtered, dissolved in C_6H_6 (20 ml), and applied to a column of silica gel. Elution was carried out with C_6H_6 and 2-5% Me_2CO-C_6H_6.

The first substance, which was eluted with 2% Me_2CO-C_6H_6, gave a colorless crystalline solid; yield, 0.15 g; mp 253-255°; in ν 1720, 1696 cm^{-1}; uv λ max 255 nm, log ε 3.93.

Anal. calcd for $C_{27}H_{40}O_4$: C, 75.66; H, 9.41. Found: C, 75.76; H, 9.58.

The second substance, which was eluted with 5% Me_2CO-C_6H_6, yielded a colorless crystalline solid; yield 0.19 g; mp 226-228°; in ν 3440, 1685 cm^{-1}.

Anal. calcd for $C_{27}H_{42}O_4$: C, 75.31; H, 9.83; Found: C, 75.18; H, 9.98.

HYDROLYSIS OF THE DIACETATE.—The diacetate **2** (0.5 g) was dissolved in 50 ml of 1 M methanolic KOH. After remaining 30 min at room temperature, the solution was neutralized with dilute H_2SO_4, and the solid **5** was filtered. It was crystallized from aqueous MeOH; yield 0.38 g; mp 243-245° [lit. 245-246.5° (9)]; $[\alpha]_D$ −56.5°.

OXIDATION.—The monoacetate **5** (0.1 g) was dissolved in Me_2CO (20 ml) and stirred with 25 ml of 3 N chromic acid in 1 N H_2SO_4 for 36-48 h. After dilution with H_2O and addition of $NaHSO_3$, the mixture was extracted with $CHCl_3$ and the solvent layer concentrated to dryness. The solid was purified by chromatography on silica gel in C_6H_6. The major band obtained with 2% Me_2CO-C_6H_6 was crystallized from MeOH; yield, 0.05 g; mp 204-205° [lit. 204.5-206° (9)]; $[\alpha]_D$ −33°.

REDUCTION OF **6**.—A solution of **6** (0.1 g) in HOAc (10 ml) was hydrogenated in a Parr apparatus in the presence of Adam's catalyst (0.02 g) for 16-20 H, and the reaction mixture processed by filtration, dilution of filtrate with H_2O, and refiltration of the solid. The solid was crystallized from MeOH; yield, 0.08 g; mp 207-208° [lit. 205-208 (12)]; $[\alpha]_D$ −63° [lit. −67° (12)]. It was identical with an authentic sample of tigogenin.

3-KETO-1-β-ACETATE **7**.—The monoacetate **5** (0.5 g) was dissolved in Me_2CO (30 ml) and stirred at 20° with 1 N chromic acid in 1 N H_2SO_4 (2-3 ml). When tlc showed the absence of the starting material, the mixture was diluted with H_2O and extracted with $CHCl_3$. Concentration of the $CHCl_3$ layer gave a crystalline solid which was recrystallized from Et_2O-hexane (1:1). The product separated as colorless needles mp 187-188°; $[\alpha]_D$ −27.50°; ν 1722 cm^{-1}.

Anal. calcd for $C_{29}H_{44}O_5$: C, 73.69; H, 9.38. Found: C, 73.60; H, 9.43.

BRISBAGENIN **8**.—A solution of **7** (0.3 g) in tetrahydrofuran (15 ml) was hydrogenated using Pd-C (5%) at 50 psi for 3 h. The filtered reaction mixture was treated with 5 ml of 2 N KOH and let stand at room temperature for 3 h, at which time, the hydrolysis was complete (tlc). Dilution with H_2O, extraction with $CHCl_3$, and concentration of the extract gave a crystalline solid which was recrystallized three times from MeOH to yield the product, mp 203-205°; pmr (300 MHz): δ 0.75; 0.77; 0.79; 0.85; 0.94; 0.96; 1.6, singlets; 3.35, t; 3.48, m; 3.63, m; 4.37, q; cmr: 80.68, 78.02, 68.06, 66.86, 62.33, 56.3, 54.79, 42.41, 41.67, 41.49, 40.43, 40.03, 38.20, 35.52, 32.18, 32.08, 31.40, 30.32, 28.82, 28.36, 24.32, 17.14, 16.36, 14.50, 6.8; ms *m/z* 432 (M^+), 360, 318, 289, 139, (base peak), 81, 69.

Anal. calcd for $C_{27}H_{44}O_4$: C, 74.95; H, 10.25. Found: C, 74.65; H, 10.33.

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