Sidisterone, a C_{24} Ecdysteroid from *Silene dioica* and *Silene otites*

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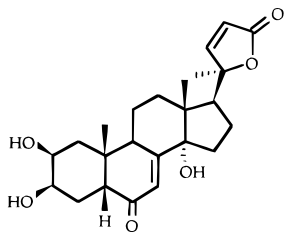
Sidisterone (**1**), the first C_{24} ecdysteroid, has been isolated from two species of the Caryophyllaceae in the genus *Silene (S. dioica* and *S. otites)* and identified through ^{1}H-, ^{13}C-, and multidimensional NMR and molecular modeling techniques.

Phytoecdysteroids represent a large family of plant steroids that are the same or similar to insect molting hormones.[1,2] Usually, they have retained the entire carbon skeleton of cholesterol (C_{27}) or phytosterols (C_{28} or C_{29}), but some of them have a reduced carbon number resulting from side-chain cleavage between C-20 and C-22 (C_{21} ecdysteroids) or between C-17 and C-20 (C_{19} ecdysteroids). Here we report on the isolation and identification of the first C_{24} ecdysteroid from *Silene dioica* (L.) Clairv. and *Silene otites* (L.) Wib. (Caryophyllaceae).

The isolated compound **1** had the following characteristics: the UV spectrum in MeOH gave an unusually broad peak with a λ_{max} at 240 nm. Resolution enhancement (fourth-order derivatization) of this spectrum indicated the presence of two chromophores absorbing, respectively, at 234 and 244 nm. The IR spectrum showed bands at 1643 cm^{-1} (cyclohexenone) and 1749 cm^{-1} ($\nu_{C=O}$ of an olefinic γ-lactone). The mass spectrum gave major ions at *m/z*, 434 $(M + H + NH_3)^+$, 417 $(M + H)^+$, and 399 $(M + H - H_2O)^+$, consistent with a molecular weight of 416. Such a molecular mass fits with the elementary formula $C_{24}H_{32}O_6$, and this was confirmed by HREIMS.



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From the analysis of its 1D and 2D ^{1}H-NMR data (Table 1), it is clear that the steroid nucleus of **1** is "classical" with respect to the presence of the 2β-OH, 3β-OH, and 14α-OH functionalities.[3] The side chain showed a single methyl signal at δ 1.52 and two ethylene signals at δ 6.01 (d, J = 5.4 Hz) and 7.76 (d, J = 5.4 Hz). The large differences of these chemical shifts and the coupling constants are in agreement only with structure **A** (Figure 1). On the basis of the ^{1}H-NMR data and the IR band at 1749 cm^{-1} and from the lowfield position of the methyl signal at δ 1.52 (s), structure **B** was therefore proposed (Figure 1).

Table 1. NMR Data for Sidisterone (**1**)[a]

δ_C		δ_H (mult, J (Hz))	
C-1	37.38	Ha-1	1.43 (t, 12)
		He-1	1.78
C-2	68.70	Ha-2	3.82 (ddd, 12, 3.5, 3)
C-3	68.49	He-3	3.94 (qb, $W_{1/2}$ = 9)
C-4	32.85	Ha-4	1.65
		He-4	1.75
C-5	51.80	H-5	2.38 (dd, 11.3, 5.4)
C-6[b]	206.2		
C-7	122.32	H-7	5.78 (d, 2.5)
C-8[b]	167.1		
C-9	35.07	Ha-9	3.14 (m, $W_{1/2}$ = 22)
C-10[b]	39.2		
C-11	21.46	Ha-11	1.65
		He-11	1.8
C-12	31.92	Ha-12	2.19 (dt, 13, 5)
		He-12	1.85
C-13[b]	48.2		
C-14[b]	85.0		
C-15	31.56	Ha-15	1.95
		Hb-15	1.65
C-16	22.56	Ha-16	1.8
		Hb-16	1.45
C-17	52.20	Ha-17	2.77 (t, 9.2)
C-18	18.16	CH_3-18	0.76 (s)
C-19	24.37	CH_3-19	0.95 (s)
C-20[b]	92.8		
C-21	25.17	CH_3-21	1.52 (s)
C-22	163.92	H-22	7.76 (d, 5.4)
C-23	120.05	H-23	6.01 (d, 5.4)
C-24[b]	175.5		

[a] Values were recorded in CD_3OD at 500 MHz for ^{1}H and 125 MHz for ^{13}C. CH, CH_2, CH_3 carbons were assigned using PFG-HMQC ^{1}H-^{13}C direct correlation[10] and 1D broad band decoupled spectrum. [b] Values were assigned and detected from PFG-HMBC ^{1}H-^{13}C long-range correlation.[10] $W_{1/2}$: width at half-height in hertz; δ in ppm (TMS as a reference); e = equatorial; a = axial.

Once the nature of the side chain was established, two problems of configuration remained, that is, the stereochemistry of the side chain at C-17 and the configuration of the C-20 substituent. The 17-β position could be assigned to the butenolide side chain because of the multiplicity of the ^{1}H-NMR signal of H-17, which appeared as a triplet (J = 9.2 Hz) because Hα-16 and Hβ-16 are not isochronous; consequently, the values $J_{17-16\alpha}$ and $J_{17-16\beta}$ were equal to 9.2 Hz. This finding is in agreement with 20-hydroxyecdysteroids in which the side chain is β and in which a triplet ($J \sim$ 9.2 Hz) is

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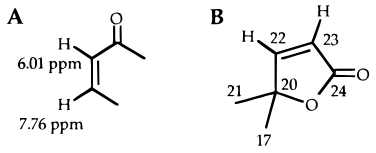

Figure 1. Side-chain structure of compound **1**.

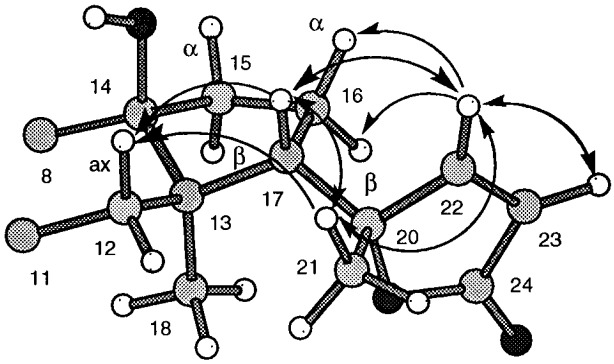

Figure 2. Different correlations observed with NOE experiments.

observed for the Hα-17 signal.[3−5] Such a signal is distorted when the difference between the chemical shifts of Hα-16 and Hβ-16 is smaller (second-order spectra).[3−5] These values are also in accordance with values for the torsion angles θ_1 (Hα-17−C-17−C-16−Hα-16) and θ_2 (Hα-17−C-17−C-16−Hβ-16) of 146° and 26°, respectively, calculated from a generalized Karplus equation.[6,7] Inspection of CPK and Dreiding molecular models and molecular modeling using Alchemy II software[8,9] showed that for the major conformation where the bulky 17-butenolide substituent is pseudoequatorial the values for these torsion angles are 143° and 23° for θ_1 and θ_2, respectively. A 17α-oriented side chain would lead to a torsion angle Hα-17−C-17−C-16−Hα-16 of ca. 90° and consequently to a value for the coupling constant $J_{17\beta-16\alpha}$ of ca. 0°.[5,6,10] Moreover, NOE experiments led to the observation of an NOE between H-17 and Hax-12 (δ 2.19), an α-oriented proton, but no NOE was observed on irradiation of CH_3-18.

The establishment of the *R* or *S* configuration of the two possible epimers at C-20 was more difficult because one had to consider the rotation around C-17−C-20. Inspection of the CPK model and molecular modeling using the Alchemy Software[8,9] showed that the main steric interactions are between CH_3-18 and CH_3-21 and/or C-20−C-22, leading to conformations where CH_3-21 and C-20−C-22 are directed to the α side (synclinal with respect to the C-17−H-17 bond).

Strong positive NOEs were observed (H-17 to H-22 (7%) and H-22 to H-17 (3%)) along with a small indirect negative NOE (H-17 to H-23), consistent with an almost linear arrangement of H-17−H-22−H-23.[11] All these NOEs confirmed the α position of both CH_3-21 and H-22 for the major conformation of **1** present in solution. Moreover, NOEs for H-22 to H-16 (δ 1.45) and CH_3-21 to Hax-12 and a small NOE for CH_3-18 to CH_3-21 were observed, in agreement only with the 20S configuration (Figure 2). This configuration corresponds to the same orientation of C-17, CH_3-21, and OH-20 as normally encountered in natural 20-hydroxyecdysteroids that are 20*R*. This inversion in the chiral label is the consequence of the inversion of the priority of the substituents around C-20 between the present ecdysteroid and 20-hydroxyecdysone.

All these structural conclusions for **1** were confirmed by the ^{13}C-NMR chemical shifts observed from the 1D broad-band decoupled spectrum, with direct pulse-field gradient-HMQC and long-range PFG-HMBC ^{1}H−^{13}C correlations.[10] This allowed the observation of the following ^{1}H−^{13}C long-range correlations: H-17 → C-20, C22; C-12, C-13, C-15, C-16; H-22 → C-20, C-23, C-24; H-23 → C-20, C-22, C-24; Me-18 → C-12, C-13 C-14, C-17; Me-21 → C-17, C-20, C-22. These correlations led to an unambiguous assignment of the ^{13}C-NMR signals of the side chain. The chemical shifts of C-22, C-23, and C-24 were in agreement with the presence of an α-β ethylenic lactone.[12]

The full structure of compound **1** was thus established, and the trivial name sidisterone is proposed for this new ecdysteroid; the systematic name is (2β,3β,-14α,20*R*,5β)-2,3,14,20-tetrahydroxy-6-oxocholesta-7,22-dien-24-oic acid γ-lactone. This compound is unusual in that it has only 24 carbon atoms, and it shares two common characteristics with bile acids, namely the presence of a COOH-24 substituent and of a 5β-H (cis A/B ring junction). It may be supposed that its biosynthesis takes place from a COOH-26 ecdysteroid (= ecdysonoic acid) (the latter compounds are frequent in plants at least as lactone derivatives[1,2]) through β-oxidation, as it proceeds for the biosynthesis of bile acids.[13] Alternatively, it could arise directly from the oxidation of 24-OH compounds (e.g., abutasterone or pteroserone). This compound has been isolated from two species of the *Silene* genus. It is worth mentioning that it was not observed routinely in *S. dioica*, as in several subsequent extractions (samples from various other locations in the Massif Central and the Alps, collected in the summer) it was present at best in minute amounts.

Experimental Section

General Experimental Procedures. UV spectra (in MeOH) were obtained with a Shimadzu UV160A spectrophotometer. IR spectrometry was performed (as a KBr film) with a Bruker FT-IR apparatus. Mass spectrometry (CI/D mode) was carried out on a Riber 10-10B instrument equipped with a direct-inlet probe, with NH_3 as the reagent gas. HRMS used a hybrid ZAB-HSQ (BEqQ geometry) mass spectrometer from Fisons Instruments. NMR spectra (in CD_3OD) were recorded on a 500 MHz Bruker apparatus.

Plant Material. The red campion, *S. dioica*, was collected in July 1986 (flowering stage), in the vicinity of Murat, Massif Central, France. A voucher specimen is deposited at the Departement de Biologie, Ecole Normale Supérieure, Paris. *S. otites* was collected in the vicinity of Ásotthalom, a village near Szeged, Hungary, in July 1991.

Extraction and Isolation. For *S. dioica*, air-dried plants (20 g dry wt) were milled and then extracted with 200 mL of MeOH under reflux for 20 min. The extract was filtered and evaporated to dryness. The residue (1.7 g) was dissolved in 5 mL of MeOH and then 5 mL of $(CH_3)_2CO$ was added, and the resulting precipitate was centrifuged and discarded. The supernatant was evaporated, and the residue (0.522 g) was purified by DCCC.[3] DCCC was performed using an Eyela DCC-A instrument (Tokyo Rikakikai Co., Ltd., Tokyo, Japan) with $CHCl_3$−MeOH−H_2O (65:20:20) in the descending mode.

Samples were dissolved in the upper phase (2 mL). The flow rate was 15 mL·h^{-1}, and 5-mL fractions were collected. Several ecdysteroid-containing fractions were obtained, among which a major one (fractions 173−185) that was further purified by normal-phase HPLC (solvent system, CH_2Cl_2−iPrOH−H_2O, 125:25:2) column, Zorbax-Sil 250 mm long, 9.4 mm i.d., flow rate 4 mL·min^{-1}, t_R 14.0 min), yielded ca. 1 mg of pure compound **1**.

For *S. otites*, the herb was cleaned, air-dried, and milled. The dry powder (3.8 kg) was percolated with 76 L of MeOH at room temperature. The MeOH extract was evaporated, and the dry residue (191 g) was dissolved in 350 mL of MeOH and adsorbed on 570 g of alumina by mixing and taking to dryness. A column was packed with 1 kg of alumina, and the alumina with the adsorbed extract was packed at the top of this column. Elution used a step-gradient of MeOH in CH_2Cl_2, and 42 fractions (500 mL each) were collected: fractions 1−5 (CH_2Cl_2), 6−13 (CH_2Cl_2−MeOH, 95:5), 14−29 (CH_2Cl_2−MeOH, 9:1), 30−39 (CH_2Cl_2−MeOH, 8:2), 40−42 (MeOH). Fractions 26−34 were combined and evaporated to dryness, and the residue (16.8 g) was adsorbed on 51 g of silica, mixed with CH_2Cl_2, and packed to the top of a silica column (510 g). Gradient elution was performed, and 200-mL fractions were collected: fractions 1−22 (CH_2Cl_2), 23−50 (CH_2Cl_2−96% EtOH, 94:6), 51−198 (CH_2Cl_2−96% EtOH, 9:1), 199−250 (CH_2Cl_2-96% EtOH, 8:2). Fractions 59−75 contained sidisterone (**1**) in addition to other ecdysteroids. These fractions were taken into dryness (0.7 g) and then further purified by DCCC as described above. Fractions containing sidisterone were again purified by preparative TLC (solvent EtOAc−MeOH−ammonia, 85:10:5), yielding two fractions (total wt 18 mg). Final purification was performed by normal-phase HPLC (solvent system cyclohexane−iPrOH−H_2O, 100:40:3, column Zorbax-Sil 250 mm long, 9.4 mm i.d., flow rate 4 mL·min^{-1}, t_R 26.0 min), yielding 5 mg of pure compound **1**.

Sidisterone (1): white powder; UV (MeOH) λ_{max} 240 nm (ϵ 13 300) and 209 nm (14 500); IR (KBr) 1749, 1643 cm^{-1}; ^{1}H- and ^{13}C-NMR data, see Table 1; CI/D MS *m/z* 434 $(M + H + NH_3)^+$, 417 $(MH)^+$, and 399 $(M + H - H_2O)^+$; HR-EIMS found *m/z* 416.219 784 5 $(M)^+$, $C_{24}H_{32}O_6$ requires 416.219 884.

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