Occurrence of Progesterone and Related Animal Steroids in Two Higher Plants[#,△]

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Previously, the presence of a wide variety of chemically diverse steroids has been identified in both flora and fauna. Despite the relatively small differences in chemical structures and large differences in physiological function of steroids, new discoveries indicate that plants and animals are more closely related than previously thought. In this regard, the present study gathers supporting evidence for shared phylogenetic roots of structurally similar steroids produced by these two eukaryotic taxa. Definitive proof for the presence of progesterone (**3**) in a vascular plant, *Juglans regia,* is provided. Additional evidence is gleaned from the characterization of five new plant steroids from *Adonis aleppica*: three 3-*O*-sulfated pregnenolones (**6a/b**, **7**), a sulfated H-5β cardenolide, strophanthidin-3-*O*-sulfate (**8**), and spirophanthigenin (**10**), a novel C-18 oxygenated spirocyclic derivative of strophanthidin. The *ab initio* isolation and structure elucidation (NMR, MS) of these genuine minor plant steroids offers information on preparative metabolomic profiling at the ppm level and provides striking evidence for the conserved structural space of pregnanes and its congeners across the phylogenetic tree.

The present study elucidates cross-linkages between mammals and higher plants based on the structural similarity of their pregnane metabolite pool, which can be interpreted as biochemical congruence in steroid metabolism. In fact, linkages between vastly different species have already been studied by the late John W. Daly over the breadth of his illustrious scientific career. For example, the discovery of batrachotoxin (**1**) as the active principle of the Columbian arrow poison frog in 1965 led him to pursue the origin of this pregnane derivative.[1–3] The quest for the origin of **1** in *Phyllobates bicolor* has subsequently led to birds (genus *Pitohui*), beetles (family Meyridae), and unknown symbionts as sources of the compound. A phytochemical connection is also very possible: related bufadienolides such as bufalin (**2**) are found in various plants such as *Urginea maritima*, *Drimia robusta*, *Kalanchoe pinnata*, and *Helleborus niger*.[4] Indeed, the steroid structure itself appears to be especially amenable not only to biosynthesis in practically every known organism but also to being shared between species, genera, families, classes, orders, phyla, and kingdoms.

Pregnane steroidal hormones (C_{21}) play a key role in mammals by both having their own activity on the female reproductive tract[5] and serving as biosynthetic precursors of gestagens (C_{21}), androgens (C_{19}), estrogens (C_{18}), and other steroid hormones. On the other hand, there is comparably very little mention of pregnane steroids in the phytochemical literature. In contrast to the steroid investigations begun with animals in the 1920s, the discovery of progesterone (**3**) in plants would be an excellent illustration of how a well-known compound can be identified in a natural source, where it is present in small amounts, without ever being isolated as a compound. As a result, a crucial piece in the discovery of **3** in plants has yet to be reported: the isolation, identification, and spectroscopic characterization of **3** from a plant source. The biochemical significance of the mammalian corpus-luteum hormone (**3**) had already been recognized in 1927/28,[6,7] before it was chemically defined by Butenandt.[8–11] From that time, knowledge about pregnanes developed rapidly in order to elucidate the fundamental biological function, the therapeutic potency, and the medicinal usage of human steroids.[12,13] The wealth of diversity of pregnane biochemistry in higher plants and their structural similarity with animal hormone steroids were not recognized before the late 1950s, when **3** was proposed as a possible biosynthetic precursor of the holaphylline-type 3-amino pregnanes,[14,15] suggesting that the biogenetic sequence leading to **3** is identical in plants and animals.[15] Since that time, the presence of **3** in plants has been claimed through various methods, many of which have relatively low specificity, but not through actual isolation (S1, Supporting Information). Thus, previous reports remain putative, as they recognize the presence of **3** by a physicochemical method or hyphenated (co)chromatography without purification that lacks the spectroscopic characterization required for definitive structural assignments.

In addition, biochemical methods have been employed abundantly to claim the *de facto* presence of **3** in plants. In 1965, radiolabeled **3** was isolated from the leaves of *Holarrhena floribunda* after feeding radiolabeled pregnenolone.[15] More recently, enzymes involved in the biosynthetic pathway from cholesterol to cardenolides have been isolated from plants. The transformation of pregn-5-en-3β-ol-20-one (pregnenolone, **4**) to its 4-ene-3,20-dione analogue, **3**, is a step in that pathway.[16] Concerning the elucidation of biochemical pathways and evolutionary relationships, it must be realized that the ability of an organism to produce **3**, or any other given molecule, through metabolic conversion of a structurally related compound does create the likelihood, but is not absolute evidence, for the genuine presence of the observed product in that organism. Despite the immense progress made in biosynthesis research (S2, Supporting Information), such studies do not necessarily identify the endogenous ligands of the studied enzymatic metabolic systems. Overall, the proof of the presence of a steroid by isolation and unequivocal structure elucidation plays a vital role in making definitive conclusions. Thus, structural identification of

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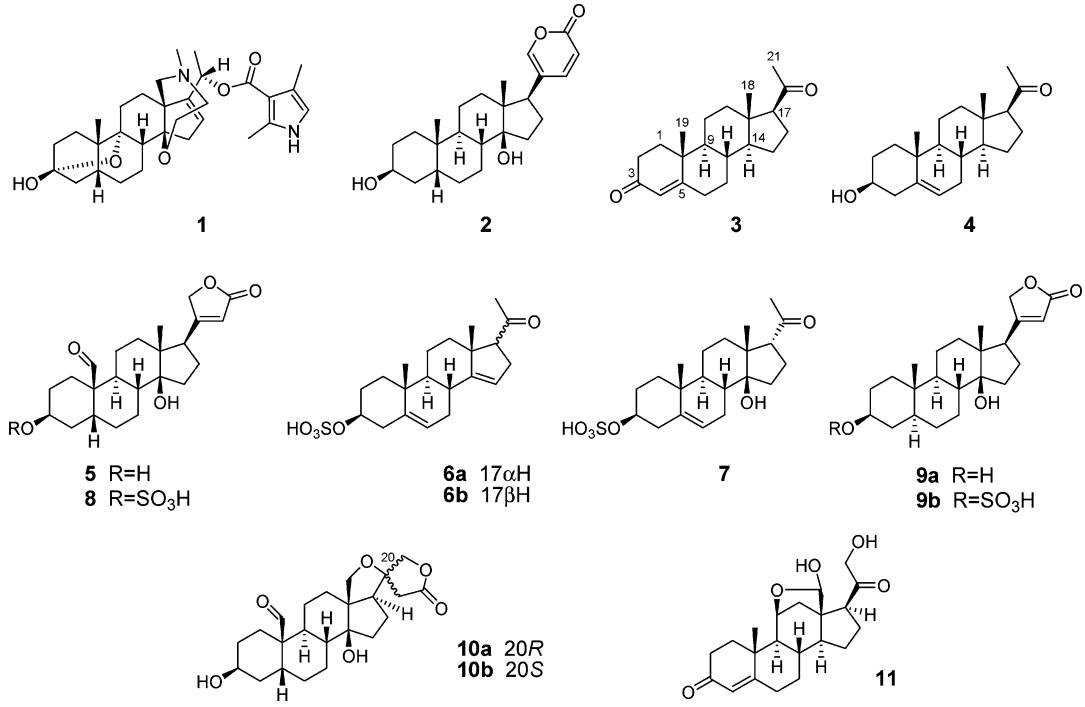
Chart 1



a key steroid such as **3** should be independent from published data (*ab initio*) and include full interpretation of NMR spectra.

Strophanthidin (**5**) steroids are considered to be downstream metabolites of **3**. Representative prototypes of 5*β*-cardenolides, **5**, and its congeneric cardioactive toxins were first isolated from the dried ripe seeds of *Strophanthus kombé*[17] and subsequently from phylogenetically diverse genera of higher plants (S1, Supporting Information). The biomedical importance of **5** lies in its action as an arrow poison, cardiotonic, and potential cytotoxic anticancer agent.

The interconnectivity of physicochemical and phylogenetic studies accentuates the challenge that confronts metabolomics studies. It is incomplete to consider the metabolome as simply the collection of compounds present in an organism at any given time in their life cycle. Not only are congeneric clusters of compounds expected to be present in the metabolome, but their unambiguous identification and characterization presents a major challenge in metabolomics research.[18,19] This particularly applies to studies dealing with *secondary* metabolites, which typically represent very rare chemicals, are chemically more complex, and are usually present at lower abundance compared to primary metabolites.

Results and Discussion

The present isolation of the five secondary steroidal metabolites (**3**, **6a/6b**, **7**, **8**, and **10**) from two higher plants is an important observation with regard to their occurrence in the producing organism and structural novelty (**6a/6b**, **7**, **8**, and **10**). This is because one of these steroids is the mammalian gestagen progesterone (**3**) from *Juglans regia* (Juglandaceae). The others are the polar inorganic/sulfate conjugates of two epimeric pregnanes, pregna-5,14-dienolone-3-*O*-sulfate (**6a**) and *allo*-pregna-5,14-dienolone-3-*O*-sulfate (**6b**), the pregnane sulfate, OH-14*β*-pregn-5-enolone-3-*O*-sulfate (**7**), the H-5*β* cardiac steroid strophanthidin-3-*O*-sulfate (**8**), and the C_{18}-oxygenated spirocyclic derivative of the H-5*β* cardenolide **5**, spirophanthigenin (**10**), possessing a novel skeleton. Compounds **6**−**8** and **10** are new natural products and were isolated from *Adonis aleppica* (Ranunculaceae).

Identification of Progesterone (3). The structure proof of isolate **3** followed the same workflow as for unknown compounds, with no assumptions of compound class. In addition to MS and basic spectroscopic characterization, full ^{1}H and ^{13}C NMR assignments were made including the relative configuration to unequivocally deduce the structure of **3**. The spectroscopic data were analyzed using the *ab initio* approach previously proposed for H-5α and $\Delta^{5,6}$ pregnane derivatives,[20] which takes advantage of the plethora of information contained in the heavily coupled yet overlapped ^{1}H NMR resonances. NOE contacts and the complete H,H-coupling pattern yielded the averaged solution conformation of **3**. The identification of **3** (S3 and Table S1, Supporting Information) as progesterone was an unexpected result.

Phytochemistry of *Adonis aleppica*. The herb *A. aleppica* (Ranunculaceae) is native to Mesopotamia and represents an annual relative of the perennial European *A. vernalis* and other Asian *A.* species, which have been used pharmaceutically and as ethnomedical remedies to treat congestive heart failure. Previous phytochemical investigations of *A. aleppica*, focused on its complex cardiac glycoside pattern, led to the elucidation of the alepposides as highly oligomeric glycosides of **5**,[21,22] containing up to 6 sugar moieties. In addition, an unusual monoterpene-triole-glycoside,[23] aleppotriole, and other polyphenolic glycosides[24] were characterized. Subsequently, the sugar portions of the oligoglycosidic alepposides, which contain the rare 2,6-dideoxy sugars typically found in cardenolides and Asclepiadaceaeous pregnane glycosides, were also determined to be present as structurally unique free oligosaccharides ($n = 3-5$), termed adoligoses.[25] The phytochemical fractionation was generally guided by the Kedde reaction for cardenolides as well as the vanillin−H_2SO_4 (VAN) reagent.[21] During the extensive fractionation pathway in this study, non-cardenolide secondary metabolites were noticed that either reacted weakly Kedde positive or yielded similar VAN color reaction products (various shades of blue-gray/blue/green-blue/green), or both. DCI-MS and ^{1}H NMR analysis of numerous isolates from this group confirmed the steroidal nature of these compounds and led to the prioritization of four fractions (subsequent structures **6**−**9**) for structural analysis.

Structure Elucidation of Pregna-5,14-dienolone-3-*O*-sulfate (6a) and *allo*-Pregna-5,14-dienolone-3-*O*-sulfate (6b). On the basis of ^{1}H and ^{13}C/APT NMR as well as DCI-MS analysis with NH_3 as reactant gas and the close similarity of its NMR spectra with **3**, isolate **6** was identified as a C_{21} pregnane steroid. The very polar solubility characteristics and TLC behavior, as well as the

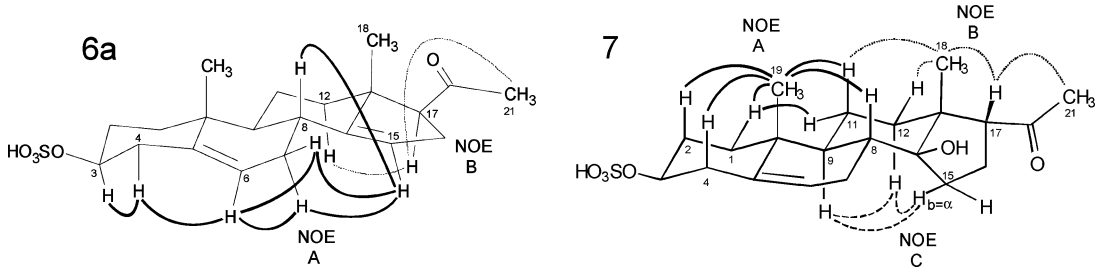


Figure 1. Relative stereochemistry of **6a** and **7**. In **6a**, one series of 2D NOESY contacts (NOE A) connects the α- and "front"-facing protons H-3/4/6/7ax/7eq/15, while the olefinic H-15 is also close to H-8ax=β. On the "rear"-face, H-17α correlates with H-21 and H-12ax (NOE B). Because **7** is an *allo* steroid, the NOE contact pathway starting at H-21 leads to the β-face protons (H-17β, H-18, H-12eq, H-11ax; NOE B). NOE A of H-19 in **7** allowed assignment of the A/B/C β-face, while the α-face protons were accessed through the NOE C correlations of H-12ax=β.

Table 1. NMR Assignments of **6a**, **6b** and **7** (CD_3OD, 600/400 MHz; Verified by COSY, HMQC, HMBC, and 1D Selective TOCSY)

	6a		**6b**		**7**	
pos.	δ_C[a]	δ_H (mult.; *J* in Hz)[a]	δ_C[a]	δ_H (mult.; *J* in Hz)[a]	δ_C[a]	δ_H (mult.; *J* in Hz)[a]
1	38.20	1.966 (br ddd; 13.6, 3.7, 3.6, <0.5)	38.10	1.900 (br dddd; 13.6, 3.7, 3.6, <0.5)	38.33	1.923 (br ddd; 13.7, 3.7, 3.6, <0.5)
	38.20	1.130 (ddd; 13.6, 13.5, 3.7)	38.10	1.081 (ddd; 13.6, 13.5, 3.7)	38.33	1.136 (ddd; 13.7, 13.5, 3.7)
2	29.87	2.078[b] (dddd; 13.0, 3.7, 3.6, 2.6)	29.87	2.051[b] (ddddd;, 13.0, 4.8, 3.7, 3.6, 2.6)	29.60	2.075 (ddddd; 13.0, 4.8, 3.7, 3.6, 2.6)
	29.87	1.669 (dddd; 13.5, 13.0, 11.6, 3.6)	29.87	1.625 (dddd; 13.5, 13.0, 11.6, 3.6)	29.60	1.636 (dddd; 13.5, 13.0, 11.6, 3.6)
3	79.74	4.160 (dddd/tt; 11.6, 11.6, 4.8, 4.8)	79.74	4.146 (dddd/tt; 11.6, 11.6, 4.8, 4.8)	79.96	4.148 (dddd/tt; 11.6, 11.6, 4.8, 4.8)
4	40.21	2.557 (ddd; 13.3, 4.8, 2.6)	40.21	2.552 (ddd; 13.3, 4.8, 2.6)	40.16	2.545 (ddd; 13.0, 4.8, 2.3)
		2.357 (ddddd; 13.3, 11.6, 2.8, 2.5, 2.1)	40.21	2.354 (ddddd; 13.3, 11.6, 2.8, ~2, ~2)	40.16	2.348 (ddddd; 13.0, 11.6, 2.8, 2.5, 2.1)
5	140.83		140.81		140.31	
6	123.01	5.450 (br ddd; 4.6, 2.8, 2.3, <0.5)	123.07	5.454 (br ddd; 4.6, 2.8, 2.7, <0.5)	123.66	5.432 (br ddd; 4.6, 2.8, 2.3, <0.5)
7	30.89	2.216[c] (dddd; 16.9, 5.5, 4.6, 2.1)	30.77	2.181[b] (dddd; 16.9, 5.5, 4.6, 2.1)	28.37	2.248 (ND)
		~2.10[b] (ND)	30.77	~2.10[b] (ND)	28.37	1.851[b] (ND)
8	32.15	~2.10[b] (ND)	32.34	~2.10[b] (ND)	37.81	1.710 (ddd; 11.5, 10.8, 5.5)
9	51.68	1.034 (ddd; 12.2, 11.6, 3.7)	52.67	0.940 (ddd; 12.2, 11.6, 3.7)	47.36	1.224 (ddd; 12.2, 11.5, 4.7)
10	38.06		38.06		38.08	
11	22.72	1.717 (dddd; 12.2, 6.1, 3.7, 1.5)	22.27	~1.71[b] (ND)	21.77	~1.55[c] (ND)
		~1.62[b] (ND)	22.27	~1.62[b] (ND)		~1.46[c] (ND)
12	42.36	~2.21[b] (ND)	35.51	~2.21[b] (ND)	39.61	~1.55[c] (ND)
		~1.55[b] (ND)	35.51	1.231 (ddd; 17.1, 12.3, 7.2)		~1.46[c] (ND)
13	49.32		51.44		50.03	
14	152.47		151.99		86.88	
15	119.17	5.210 (br ddd; 2.3, 2.2, 1.8, <0.5)	118.88	5.172 (ddd; 2.3, 2.3, 2.2)	35.11	2.132 (ddd; 13.2, 10.6, 9.0)
						1.740 (ddd; 13.2, 10.6, 2.3)
16	32.12	2.724 (dddd; 16.0, 10.1, 4.3, 1.8) [H-16β]	32.97	2.643 (dddd; 16.6, 5.4, 2.5, 2.3) [H-16β]	25.28	2.026 (dddd; 13.0, 10.6, 10.6, 9.4)
		2.181 (dddd; ND) [H-16α][d]	32.97	2.378 (dddd, 16.6, 8.8, 3.6, 2.2)		~1.88[b] (dddd; 13.0, 9.0, 4.6, 2.3)
17	66.23	3.013 (br dd/m; 10.1, 8.2, <0.5)	63.21	3.147 (dd, 8.8, 5.4)	63.89	2.971 (dd; 9.4, 4.6)
18	18.61	0.864 (s)	26.19	1.345 (s)	15.49	0.981 (s)
19	19.45	1.053 ([br]s)	19.52	1.070 ([br]s)	19.83	1.026 ([br]s)
20	212.20		213.18		219.73	
21	31.58	2.164 (s)	31.77	2.160 (s)	32.66	2.247 (s)

[a] Referenced to solvent signals at 3.300 and 49.00 ppm, respectively. ND, not determined. [b] Signals belonging to sets of isochronic nuclei and/or strongly overlapping signals. [c] Methylene protons 2H-11 and 2H-12 in **7** form pairs of isochronic signals. [d] Multiplicity of the overlapping signal of H-16b was assigned through 1D SelTOCSY experiments.

formation of desulfated molecular ions $[M - H_2SO_4 + NH_4]^+$ and $[M - H_2SO_4 + H]^+$ in the DCI-NH_3-MS, and the typical symmetric ^{13}C substituent chemical shift (scs; *syn* $\Delta\delta$) effects (+7.95 at C-3, −2.60 at C-2 and C-4) centered at the secondary alcohol C-3 (δ_C 79.73) of a sulfation in the A ring[21,26] established the sulfuric acid hemiester moiety of **6**. As has been previously established and discussed in detail,[21,26] steroid sulfates under positive DCI-NR_3-MS conditions do not form molecular ions, but yield two major DCI reaction products: molecular species of the type $[M - H_2SO_4 + NR_3H]^+$ and amine-sulfate species of the type $[(NR_3)_2\text{-}SO_4]^+$, both of which are characteristic for the amine used as CI reagent. This behavior had previously been verified by DCI-MS with NMe_3 and NEt_3.[21,26] Compound **6** showed this characteristic DCI-NH_3-MS behavior of a sulfate, and the observation of $[M - H_2SO_4 + NH_4]^+$ for **6** as well as the lack of dehydrated species provided proof of the absence of additional hydroxy functions beside the 3-*O*-SO_3H group. Isolate **6** exhibited two sets of NMR signals, which according to qHNMR analysis were due to a 2.1:1 mixture of two isomers, **6a** and **6b**. Based on the *J*-pattern and multiplicity of the C-17 methine protons resonating at δ 3.013 and 3.147, the two compounds **6a** and **6b** represented C-17 epimers: because **6b** exhibited one small H,H-coupling with H-16a, H-17 was β-configured and, thus, **6b** belonged to the *allo*-series of steroids. This assignment was supported by a 2D NOESY experiment, which allowed the full assignment of the relative stereochemistry in the major epimer **6a** (Figure 1), as well as key assignments (H-17, Me-18/19) in **6b**. Accordingly, the olefinic proton H-15 experienced different scs effects from the neighboring epimerism, i.e., the acyl side chain attached to C-17: while almost identical in signal multiplicity (br ddd), H-15 in the H-17α-configured **6a** was more deshielded (δ 5.210) than in **6b** (δ 5.172). The ^1H and ^{13}C NMR resonances of both epimers **6a** and **6b** were almost completely assigned (Table 1) by utilizing 2D COSY, LR-COSY, HMQC, and HMBC NMR experiments. Furthermore, 1D selective NOE experiments using selective excitation of H-6 and the protons H-15, H-3, H-17, H-16b, and H-4ax of both epimers (**6a**/**6b**) led to the identification of the multiplicity of otherwise strongly overlapped signals in the δ 3.1 to 1.0 region. Thus, the structure of **6a** was

Table 2. ^{1}H and ^{13}C NMR Data of **8**, **10a** (20*R*), and **10b** (20*S*) (CD_3OD, 400 MHz) and Comparison with Underivatized **5**[21] to Calculate ^{13}C scs Effects ($\Delta\delta$) of 3-*O* Sulfation in **8**, as Well as Formation of the Spirocyclic Cardanolide Moiety and C-20 Epimerism in **10**

	8			**10a**		**10b**		scs effects				**5**
pos.	δ_C	δ_H (mult.; *J* in Hz)	$\Delta\delta^a$ **8**−**5**	δ_C	δ_H (mult.; *J* in Hz)	δ_C	δ_H (mult.; *J* in Hz)	$\Delta\delta^a$ **5**−**10a**	$\Delta\delta^a$ **5**−**10b**	C-20 epi	rel. to C-20	δ_C
1	18.87		+0.25	18.57	2.077 (m) 1.633 (m)	18.57	2.077 (m) 1.633 (m)	Ø	Ø	Ø		18.52
2	25.35		−2.19	27.65		27.65		Ø	Ø	Ø		27.54
3	76.30	4.731 (dddd/m; 3.0, 3.5, 4.0, 4.0)	+8.32	67.98	4.107 (dddd/m; 3.1, 3.1, 6.2, 6.2)	67.98	4.074 (dddd/m; 3.1, 3.1, 6.2, 6.2)	Ø	Ø	Ø		67.98
4	37.14		−1.34	38.54		38.54		Ø	Ø	Ø		38.52
5	74.62		Ø	76.01		76.01		Ø	Ø	Ø		75.80
6	37.74		+0.29	37.33		37.33		Ø	Ø	Ø		37.45
7	25.24		Ø	23.99		24.07		1.17	1.09	Ø		25.16
8	42.54		Ø	45.06		45.03		−2.46	−2.43	Ø		42.60
9	40.15		Ø	40.47		40.47		Ø	Ø	Ø		40.33
10	55.85		−0.34	56.14		56.14		Ø	Ø	Ø		56.19
11	23.16		Ø	25.35	0.795 (ddd/dt; 10.0, 10.0, 13.0)	24.86	1.053 (m)	−2.12	−1.63	0.49		23.23
12	40.56		Ø	35.42	1.70 (a), 1.78 (b)	35.70	2.070 (m)	5.13	4.85	−0.28	δ	40.55
13	50.70		Ø	58.66		60.73		−7.75	−9.82	−2.07	$\beta+\gamma$	50.91
14	85.95		Ø	84.08		84.22		1.86	1.72	Ø	δ	85.94
15	32.44		Ø	38.52		38.38		−6.09	−5.95	Ø	γ	32.43
16	27.92		Ø	25.75		25.59		2.20	2.36	Ø	β	27.95
17	51.75	2.823 (dd/m; 9.1, 5.4)	Ø	56.70	2.383 (dd/m; 7.4, 9.7)	60.74	2.366 (dd/m; 7.4, 9.7)	−5.13	−9.17	−4.04	α	51.57
18	16.14	0.843 (s)	Ø	72.43	4.111 (d; 9.4) 3.417 (d; 9.4)	72.66	4.158 (d; 9.8) 3.466 (dd; 9.4, 0.7)	−56.26	−56.15	Ø	β	16.17
19	209.83	10.044 (s)	−0.31	209.87	10.013 (s)	209.87	10.013 (s)	0.27	0.27	Ø		210.14
20	178.38		Ø	88.67		90.07		89.50	88.10	−1.40		178.17
21	75.32	5.021 (dd; 18.4, 1.7) 4.903 (dd/t; 1.7, 1.7)	Ø	75.70	4.427 (d; 10.3) 4.359 (dd; 10.3, 0.9)	77.97	4.261 (dd; 9.7, 1.5) 4.099 (d; 9.7)	−0.39	−2.66	−2.27	α	75.31
22	117.88	5.891 (t; 1.7)	Ø	41.62	2.67 (dd; ∼2, 17.4) 2.563 (dd; 17.4, 0.9)	38.08	2.797 (dd; ∼1, 17.5) 2.517 (dd; 17.5, 1.5)	76.30	79.84	3.54	α	117.92
23	177.38		Ø	178.90		178.24		−1.72	−1.06	0.66	$\beta+\gamma$	177.18

[a] Ø = |scs| < 0.22 ppm.

consistent with pregna-5,14-dienolone-3-*O*-sulfate (the sulfate ester of the Δ^{14} derivative of **3**), while structure **6b** was assigned as *allo*-pregna-5,14-dienolone-3-*O*-sulfate. While the co-occurrence of both C-17 epimers, i.e., *allo*-forms, of steroids has precedence in the pregnane and cardenolides literature, the presence of both epimers has not been observed with any of the previously isolated steroids from *A. aleppica*. Moreover, the finding of **6b** represents the first *allo*-steroid isolated from the genus *Adonis*.

Structure Elucidation of 14β-Hydroxypregn-5-enolone-3-*O*-sulfate (7). Another steroid isolated from *A. aleppica*, **7**, was characterized as a pregnane through its DCI-NH_3-MS and 1D/2D NMR data and showed a very close resemblance to **6**. In fact, the ^{1}H and ^{13}C NMR resonances of rings A, B, and C/H-11 were nearly identical. Considering the higher mass of **7** (+18 amu compared to **6**), this evidence pointed to the structural difference in ring D that was caused by formal hydration of the $\Delta^{14,15}$ double bond. On the basis of the prominent ^{13}C NMR chemical shift of the tertiary carbon C-14 (δ 86.88), which is found typically in the C-14β-hydroxylated cardenolides,[21] **7** was identified as belonging to the same C-14β-OH series of steroids. Further evidence of the structure of **7** came from (a) long-range C,H-couplings identified from an HMBC map; (b) the long-range H,H-coupling pathway across $\Delta^{5,6}$, which connects both H-7eq and H-7ax with H-4ax (^5J = 2.5 Hz each), and the *W*-coupling (^4J = 2.3 Hz) involving the equatorial protons at C-2 and C-4; (c) the intensity of the cross-peaks in the COSY and LR-COSY maps of **6** and **7**, which confirmed the H-17α configuration in **7**; (d) a NOESY experiment that established the relative stereochemistry of **7** (Figure 1); (e) homodecoupling experiments for H-3 that confirmed its axial position; and (f) the full assignment of the NMR resonances summarized in Table 1. Altogether, the structure of **7** was consistent with 14β-hydroxy-pregn-5-enolone-3-*O*-sulfate, namely, the sulfate ester of the C-14 hydroxy derivative of **3**.

Structure Elucidation of Strophanthidin-3-*O*-sulfate (8). Considering the relative abundance of **5** as aglycone in the cardenolide metabolome of *A. aleppica*, and taking into account the extensive previous investigations of alepposides[22] and other glycosides[21] of **5**, the similarity of isolate **8** was apparent on the initial inspection of its ^{1}H NMR spectrum. However, in addition to its much higher solvent and TLC polarity, there was one striking difference in the ^{1}H NMR spectrum: compared to **5**, the typical dddd resonance of H-3eq was shifted downfield by +0.60 ppm. The analogous observation had previously been made with the major, highly polar cardenolide uzarigenin-3-*O*-sulfate (**9b**), isolated from *A. aleppica* and characterized by X-ray crystallography.[26] While the ^{1}H NMR spectra of underivatized uzarigenin (**9a**) and its 3-*O*-sulfate differed markedly in the resonances of H-3α ($\Delta\delta$ 0.77 ppm), the spectra were otherwise virtually identical. On the other hand, their ^{13}C NMR showed the typical scs of sulfation: +7.95 at C-3, −2.60 at C-2 and C-4, −0.07 at C-1, and −0.19 at C-10.[21] As opposed to the 3-*O*-glycosidation shifts, which are diastereotopic due to the nature of sugars and affect C/H-2 and C/H-4 differently (asymmetric scs), sulfation leads to a stronger deshielding of the glycosidation site and has a much reduced diastereotopism. Thus, it leads to almost symmetric scs effects in A/B-*trans*(*oid*) steroids (5α or Δ^5) such as **9a**, **6a**, **6b**, and **7**. When comparing the ^{13}C NMR resonances of **8** and **5**, analogous scs effects were observed, with slightly less deshielding effects on C-2 and C-4 (Table 2). This can be interpreted as being due to the C-19 aldehyde group and the A/B-*cis* linkage, which provides a slightly different chemical environment for interactions between the sulfate group and A/B ring substituents. In addition, **8** exhibited the typical DCI-NH_3-MS behavior of steroid sulfates (see above and detailed discussion in ref 21). Key ^{1}H NMR signals of the H-5β cardenolide **8** could be assigned as follows: H-3eq (δ 4.731, dddd), H17α (δ 2.823, dd/m), Me-18 (δ 0.843, s), aldehyde H-19 (δ 10.044, s),

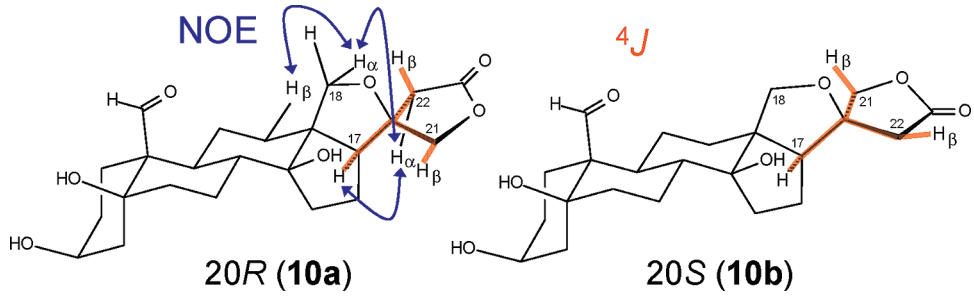


Figure 2. Relative configuration of the spirophanthigenins **10a** and **10b** derived from NOE contacts (arrows shown for **10a**) and characteristic *W*-type H,H-couplings (^4J) that provided proof for the planar arrangements along the bond pathways (red/bold marking).

2H-21 (δ 5.021 and 4.903, dd each), and H-22 (δ 5.891, t). All NMR and MS spectroscopic evidence was consistent with the structure strophanthidin-3-*O*-sulfate for **8**.

The high-field portion of the ^{1}H NMR spectrum of **8** was highly overlapped and much less dispersed than those of the A/B-*trans*(*oid*) steroids **6a**, **6b**, and **7**, consistent with previous observations. Additional spectroscopic overlap resulted from a ca. 30% impurity of a congeneric cardenolide sulfate contained in the sample of **8**.

Structure Elucidation of Spirophanthigenin (10). This isolate exhibited a characteristic emerald color during TLC detection with VAN reagent. The subtle but clearly noticeable deviation from the pure green reaction product of **5** (see summary of VAN color reaction in ref 21), in combination with ^{1}H and ^{13}C NMR evidence of **10** being a C-19 aldehyde, suggested that **10** is a close derivative of **5**. The DCI-NH_3-MS confirmed the presence of one additional oxygen in **10** ($C_{23}H_{32}O_7$) compared to **5** ($C_{23}H_{32}O_6$), while maintaining the degree of unsaturation. The lack of butenolide ^{1}H/^{13}C NMR resonances, which are typical for cardenolides, the absence of olefinic signals that would indicate acyclic unsaturation, and the occurrence of additional oxygenated methylene and methine ^{1}H NMR signals in the δ 3.0−4.5 region hinted at the presence of an *O*-heterocyclic partial structure. Comparison of the ^{13}C NMR chemical shifts of **10** with **5**, in the fashion of an scs map (Table 2), confirmed that both compounds possess identical A, B, and C rings, as indicated by identical shifts of most carbons in rings A and B ($\Delta\delta < 0.22$) and relatively small δ deviations ($\Delta\delta < 2.50$) for C-7/8/11. The lack of a C-18 methyl group and the additional hydroxymethylene signal at δ 72.43 provided further evidence of the involvement of carbon C-18 and the E ring in the formation of an *O*-heterocycle. Further interpretation of the 1D NMR spectra utilized 2D COSY, LR-COSY, HMQC, HMBC, and NOESY experiments. The inverse direct and long-range H,C-correlations provided definitive proof for the C-20 spirocyclic structure of **10** as follows: (a) the prominent bridgehead C-14 (δ 84.08) exhibited a $^3J_{H,C}$ with the C/H-18 oxymethylene protons (δ_H 4.111 and 3.417), which in turn correlated with the quaternary spirocyclic center, C-20 (δ 88.67); (b) HMBC correlations connected C-20 via ^2J with the butanolide hydroxymethylene (δ_H 4.427 [H-21a=α] and 4.359 [H-21b=β]) and α-ketone methylene protons (δ_H 2.670 [H-22a=α] and 2.563 [H-22b=β]); (c) the butanolide methylene protons were coupled to their carbon counterparts via ^3J in the HMBC and to their direct carbons ^1J in the HMQC maps; (d) both C-21 and C-22 had HMBC correlations with H-17α (δ_H 2.383, dd/m), which closely resembled the H-17α protons of cardenolides such as **5**; (e) H-17α in turn was coupled via $^3J_{H,C}$ with C-14. The observations (a) to (e) established a cross-walk along the spirocyclic arrangement of a 18,20-oxido-20,22-dihydrocardenolide.

The ^{1}H and ^{13}C NMR spectra also indicated that **10** is a mixture of two isomers, **10a** and **10b** (Table 2). Their C-20 epimeric relationship was established using NOE measurements (2D NOESY) and observations of a conclusive long-range H,H-coupling pattern as follows (Figure 2): (a) In the major epimer, **10a**, H-17α exhibited a significant ^4J coupling (2.5 Hz) with H-22a=α, indicating a planar *W*-type arrangement along the bonds H-17/C-17/C-20/C-22/H-22a=α; (b) in the minor epimer, **10b**, an analogous ^4J coupling (1.7 Hz) connected H-17α with H-21b=α, which also indicated *W*-type planarity, albeit less pronounced compared to **10a**, and provided proof for the α/β assignment of the cardanolide methylene protons; (c) in epimer **10b**, H-22b=β and H-22a=β of the cardanolide shared ^4J/*W*-couplings; (d) in an 800 ms NOESY map, strong NOE contacts were recognized between H-22a and H17α, H-22b and H-18b, and H-18a and H-12eq, all consistent with ∼2.3 Å interproton distances, as determined in a molecular model. The aforementioned ^4J couplings were also confirmed in the LR-COSY experiment. Altogether, working under the assumption that **10** does not represent an exception from all natural steroids isolated so far (i.e., C/D *trans* ring fusion, 8*R* configuration for **5**/**10**), this established the relative stereochemistry of **10a** and **10b** as 20*R* and 20*S*, respectively (Figure 2). In summary, **10** was identified as the C-20 epimeric pair of the spirocyclic cardanolide derived from strophanthidin (**5**). Therefore, **10** was named spirophanthigenin, consisting of 20*R*-spirophanthigenin (**10a**) and 20*S*-spirophanthigenin (**10b**), and establishes a new group of plant steroids, the cardaspiranes. Utilizing the characteristic ^{1}H NMR fingerprint of the cardaspirane spirolactone/-tetrahydrofurane moiety (Table 2), we have strong preliminary evidence for the presence of cardaspirane oligoglycosides in *A. aleppica* that bear sugar moieties that are closely related to those of the alepposides.[21,22]

Characterization of Trace Steroids. The co-occurrence of these endogenous eukaryotic metabolites seems much more common in nature than previously considered.[27] One such important finding is the discovery of the endogenous digitalis-like factors (EDLFs) in human plasma and urine as well as other mammalian tissue.[28–32] These compounds were found to be similar to the cardenolides and bufadienolides that are well-known constituents of green plants.[28,33–36] In both cases, steroid hormones and EDLF, the very low concentration of the metabolites dictates the research methodology required to detect their presence and gain access to *ab initio* structures. While digitaloids are accumulated in living plant tissue at levels of up 10^{-4} M (100 000 ppm), their counterpart in mammalian serum does not exceed ca. 10^{-9} M (1 ppm).[37,38]

Steroid Sulfates and Oxygenated Steroids. Sulfated steroids and other sulfated triterpenes are relatively common in marine organisms (sponges, corals, and starfish)[39,40] and mammalian bile acids, but only occasionally reported in higher plants.[41] In mammals, sulfotransferases are implicated in steroid hormone activity and metabolism (conjugation).[42,43] The same metabolic processes apparently occur in plants, as indicated by plant-soluble sulfotransferases catalyzing the *O*-sulfonation of brassinosteroids.[44–46] Evidently, *A. aleppica* is capable of performing the same conjugation reaction with both steroid cores (C_{21} pregnanes and C_{23} cardenolides) to form the 3-*O*-sulfates **8** and **9b**. In fact, even the new class of cardaspiranes are sulfated by *A. aleppica*, as is evident from the minor constituent of the previously described corotoxigenin-3-*O*-sulfate (ref 21 and unpublished data).

Several well-known steroidal saponin spiroketals are produced by a series of oxygenation reactions at the C-16, C-22, and C-26

positions (diosgenin, yamogenin, smilagenin, sarsasapogenin, and hecogenin).[47] The cardaspirane, **10**, reported herein may be generated as a result of the hydroxylation of C-18, presumably by a process similar to the biotransformation that is involved in the formation of aldosterone (**11**).[48,49] Therefore, the discovery of related C-18-ox(id)o derivatives marks another important parallel between the secondary metabolism of a higher plant and animals. In **10**, the C-18 primary alcohol evidently cyclized with the α,β-unsaturated butenolide ring of the precursor molecule in a Michael-type addition to form the cardaspirane. This compound class also suggests biogenetic similarity between plant pregnanes and mammalian corticosteroids.

Mammalian and Animal Steroids in Vascular Plants. To our best knowledge, **3** has not been isolated and unambiguously characterized from any higher plant previously (S4, Supporting Information). Therefore, the present report marks the first definitive evidence for **3** as being a genuine plant constituent. As pointed out earlier, **3** was thought previously to be an animal steroid exclusively. The significance of the unequivocal identification of **3** from a higher plant cannot be overstated. While the biological role of **3** has been extensively studied in mammals, the reason for its presence in plants is less apparent. Possibly **3** has endogenous biological activity in plants such as the regulation of seedling growth.[50] Potentially, **3** represents an evolutionary artifact, the biosynthesis of which is directed by genes that no longer serve a viable function for the organism. Finally, **3** may serve as an intermediate for the biosynthesis of downstream secondary natural products in much the same way that **3** is already believed to be a biosynthetic precursor for cardenolides, such as **2** and **5** in plants.[47,51–53] Interestingly, the enzymology of pregnane $\Delta^{4,5}$ reduction is multifunctional[16] and was found to be different in plants and animals.[54]

Global Steroid Occurrence and Function. Independent of their function, the presence of steroids in practically every organism suggests that they have a powerful role in chemosystematics.[27,55–62] Despite the plethora of publications, difficulties in chemosystematics arise from the lack of consistent secondary metabolite features available at the family level.[61] In addition, chemosystematic data of phylogenetically distant organisms remain scarce despite the advances made in the chemical ecology of the *Plantae*−*Animalia* relationships.[63,64] Considering the proven existence of pheromonal communication within and between members of the two taxa, steroids could potentially fill an analogous hormonal function where organisms actually share an unexpectedly large pool of secondary metabolites that are "understood" by both the effector and the receptor organism.[65–68] Thus, pursuing the elucidation of steroidal metabolomes is worthwhile, as they share the same gonane skeleton, which appears to be universal in nature.[69]

Finding "animal-like steroids" in higher plants supports the argument of viewing the steroid nucleus as a primordial molecule. This proposal was raised in 1979, stating that "*steroids are very ancient bioregulators, which evolved prior to the appearance of eucaryotes or were even possibly synthesized abiotically.*"[69] The idea of the universality of steroids was reinforced with respect to the mediation of steroidal action via analogous receptors.[70] In line with previous studies, the present work supports two key conclusions. First, when comparing plants and animals, it is more helpful to emphasize the similarities rather than the differences.[71] Second, the limitations of the role of animal steroidal glands should be viewed with caution, because not all steroidal functions are yet known. Highly valid biochemical information about the distribution of steroidal species in living organisms is needed to confirm that steroids are primordial molecules.[69] This represents an extraordinary task for metabolome research. Approaches in which steroids are isolated and their structure unequivocally elucidated will, thus, allow significant contributions to the evolutionary and biochemical role of steroids in the living world.

Experimental Section

General Experimental Procedures. Optical rotation, UV, and IR data were acquired on Perkin-Elmer 241, Perkin-Elmer 207, and Beckman DB-G instruments, respectively. DCI-MS were run on Finnigan INCOS 50 (emitter heating rate 10 mA s^{-1}, FC43 calibration) and Finnigan GC-Q spectrometers. MPLC/HPLC separations were carried out on self-built glass columns (300 to 200 × 10 to 16 mm i.d.) with Knauer and Waters 600 HPLC pumps and DuPont and Knauer UV detectors at 210 nm. TLC monitoring used silica 60 precoated Al sheets No. 10554 from Merck (Darmstadt, Germany). Preparative chromatographic fractionation and monitoring methods have been previously described in detail.[21–25] Molecular mechanics calculations (minimizations, molecular dynamics (MD); MM+ force field; constant energy and H_2O solvent MD) of **3** were performed using the Hyperchem 5.0 program from Hypercube Inc. (Gainesville, FL) for conformational searches. The solvents used for isolation of the compounds were of analytical grade and purchased from Merck (Darmstadt, Germany) or were triple-distilled from technical grade solvents.

NMR Spectroscopy. NMR spectra were recorded on Bruker AM 360 (5 mm dual probe), Bruker AM 400 (5 mm dual probe), and Varian Unity 600 (5 mm multi nuclear probe) instruments operating at 360/400/600 MHz for ^1H and 90/100/150 MHz for ^{13}C, respectively. Chemical shifts are reported in ppm on the δ scale with the residual solvent peak (CD_2HOD) as internal standard (3.300 and 49.00 ppm, respectively); the coupling constants (*J*) are given in Hz. Offline data processing and spin simulation for the refinement of coupling constants were performed with Nuts software, Acorn NMR, Inc., Livermore, CA. In general, the spectra were obtained under the following conditions: ^1H 30−0.90° pulse, delay after acquisition (D1) 1.5 s, processed with 0.2 Hz line broadening (LB) or with Lorentz−Gauss resolution enhancement and zero-filling. Acquisition using 16K (SI) (32K at 600 MHz) data points in a spectral window of ca. 6 ppm yielded a digital resolution better than 0.2 Hz (or 0.0004 ppm) for ^1H NMR spectra. Digital resolution was better than 0.008 ppm for ^{13}C. Water peak suppression was achieved by presaturation setting the HDO signal on resonance (O1). $^{13}C(^1H)$-decoupled SW 250 ppm, 30° pulse, D1 0.1 s, SI 32 K, Waltz decoupling, processing with LB 1.0 Hz; ^{13}C DEPT 90 and 135 conditions were similar except that D1 was 1.5 s. ^1H 2D COSY, LR-COSY (2.5 Hz), ROESY, and NOESY SW 8 ppm, D1 1.5 s, 1K × 512 or 256 increments, 90° shifted sinebell-squared apodization, zero-filled in t_1 dimension during processing, mixing times 200 to 300 ms (ROESY and NOESY); ROESY spin lock field 1500 Hz. HSQC and HMBC correlations were acquired at 400/100 and 600/150 MHz, as 1K × 512 word matrices with phase cycling and as 2K × 512 word matrices in pulsed field gradient (PFG) mode, respectively, and all relevant delays were optimized for $J_{C,H} = 145$ and $J_{C,H} = 8.5$ Hz, respectively. The 1D selective TOCSY experiments were performed at 600 MHz using the eburp1/25 selective pulse shape program (pws = 200, trimpwr = 54) and acquiring 16K data points (sw = 4400 Hz, aq = 2.00 s).

Plant Material. *Adonis aleppica* Boiss. was collected in April 1990 near Urfa, Turkey (37°21′15.17″ N, 38°49′05.47″ E), as described previously[21,25] and identified by Drs. I. Calis and A. Basaran, Hacettepe University, Ankara (Turkey), as well as G.F.P. *Juglans regia* L. leaves were harvested in August 1998 in Roxel, Münster (Germany; 51°57′14.59″ N, 7°32′34.78″ E) and identified by G.F.P. Voucher specimens are deposited at the Heinrich-Heine University of Düsseldorf (90-01) and the Westfälische Wilhelms-University Münster (MS-PB-039), respectively.

Extraction and Isolation. ***Juglans regia.*** After removal of the leaf stalks, fresh leaves (3530 g) were powdered in liquid N_2 and exhaustively extracted 5× with 6 L of MeOH plus 4 × 6 L of CH_2Cl_2 by maceration in an Ultra-Turax apparatus. Then, 700 g of the resulting dried extract (831.8 g) was mounted on 925 g of microcrystalline cellulose (Avicel, Fluka), and the dried mixture was loaded on a VLC column dry-packed with the same material (1520 g, *l* = 240 mm, 165 mm i.d.). Fraction control was generally performed by TLC using $CHCl_3$−MeOH−H_2O (80:19:1) (SS1). A gradient consisting of *n*-hexanes, EtOAc, EtOH, MeOH, and H_2O was applied in 1000 mL steps. Compound **3** was contained in combined fractions 4−34 (JR4−34), corresponding to an eluant composition *n*-hexanes−EtOAc−EtOH between 86:14:0 and 0:90:10. An additional four fractionation steps (VLC, GPCC, HSCCC, and DCCC) were employed as follows: (i) VLC (2200 g, *l* = 225 mm, i.d. = 165 mm) separating 84.04 g of JR4−34

on 30.0 g of silica (ICN silica 32−64 μm, 60 Å), using gradient eluted with *n*-hexanes, Et_2O, MeOH, and H_2O to yield 51 fractions of 1000 mL each. Compound **3** was contained in combined fraction 26−39 (JR4−34.36−39, 15.0 g), corresponding to an eluant composition *n*-hexanes−Et_2O−MeOH between 56:41:3 and 0:70:30: (ii) gel permeation column chromatography GPCC (l = 60 mm, i.d. = 1000 mm) on 800 g of Sephadex LH-20 (Fluka), using CH_2Cl_2−MeOH (1:1) and collecting 338 fractions of 20 mL each; (iii) further enrichment of **3** from combined fraction JR4−34.36−39.106−122 (7000 mg) by three successive HSCCC runs (run A = 1300 mg, B = 2700 mg, C = 2300 mg; remainder 617 mg) using a P.C. Inc. model HSCCC with preparative 300 mL triple-coil and *n*-heptanes−CH_2Cl_2−MeCN (10:3:7) as mobile upper and stationary lower phase, a flow rate of 1.3 mL/min, and collecting 5 min fractions. The resulting combined fraction JR4−34.36−39.106−122.4 (304 mg) was assembled according to TLC monitoring of **3** in fraction A/244−284 ($CHCl_3$−MeOH, 90:10, R_f 0.50): (iv) DCCC in descending mode using benzene−MeOH−$CHCl_3$−H_2O (5:7:5:2). The sample (304 mg) was dissolved in the lower phase and 103 fractions of 20 min each were collected at a flow rate of 0.6 mL/h. Compound **3** was primarily contained in fraction JR4−34.36−39.106−122.4.16−19 (17.7 mg). Final isolation was accomplished by means of MPLC using a self-built glass column (22.0 g of ICN silica 60, l = 620 mm, i.d. 10 mm) and by applying gradient elution with *n*-hexanes−*i*-PrOH (1 mL/min, 10 mL fractions). Under these conditions, **3** was eluted at 90% *i*-PrOH in fr. JR4−34.36−39.106−122.4.16−19.18−25, which was divided as follows: 18−20 (2.3 mg, traces of **3**), 21−22 (3 mg, mainly **3** by TLC, R_f 0.80 in $CHCl_3$−MeOH, 96:4, orange with anisaldehyde−H_2SO_4 reagent; one main impurity [~20%, R_f 0.55] plus four very minor bands), 23−25 (3.5 mg, mainly **3**). Fraction JR4−34.36−39.106−122.4.16−19.21−22 was sufficiently pure for structural analysis.

Progesterone (3): colorless solid (3.0 mg, yield 0.00010% w/w); $[\alpha]_D$ +180 (*c* 0.03, MeOH); UV (MeOH) λ_{max} 207 nm (4.0); IR (KBr) ν_{max} 3460, 2930, 1740, 1615 cm^{-1}; ^1H and ^{13}C NMR, see Table S1, Supporting Information; DCI-NH_3-MS *m/z* 332 (100) $[M + NH_4]^+$; EIMS *m/z* 314 (24) $M^{\cdot+}$.

Adonis aleppica. A fractionation tree starting from the combined $CHCl_3$−MeOH and MeOH−H_2O extracts of 3500 g of plant material has previously been described.[21] Isolate **6** was obtained from *i*-PrOH−$CHCl_3$ partition/primary VLC fraction A2CARD1P[21] in five steps as follows: silica VLC, fr. 6−11, 4270 mg; DCCC in 5−6−4 system, fr. 9, 312 mg; four times repeated MPLC RP-18 using aqueous MeOH, *i*-PrOH−THF, and MeCN gradients, yielding fr. 6 [112 mg], fr. 6.2 [51 mg], and fr. 6.2.2 [**6**, 9.0 mg]. Isolate **7** was obtained from *i*-PrOH−$CHCl_3$ partition/primary VLC fraction A2CARD1P[21] in six steps as follows: silica VLC, fr. 6−11, 4270 mg; DCCC in 5−6−4 system, fr. 9, 312 mg; four times repeated MPLC RP-18 using aqueous MeOH, *i*-PrOH−THF, and MeCN gradients, yielding fr. 5 [233 mg], fr. 5.2 [175 mg], fr. 5.2.3 [28 mg], and fr. 5.2.3.2 [**7**, 21.8 mg]. Isolate **8** was obtained from the *n*-BuOH partition/primary VLC fraction A2CARD3[21] in six steps as follows: silica VLC, fr. 4, 11018 mg; silica VLC, fr. 4, 2135 mg; Sephadex LH-20, fr. 3, 542 mg; MPLC RP-18, fr. 1, 22 mg; MPLC RP-18 and prep silica TLC yielding fr. 1 + 2 [20.8 mg] and fr. 1 [**8**, 5.3 mg]. Isolate **9** was obtained from *i*-PrOH−$CHCl_3$ partition/primary VLC fraction A2CARD2[21] in six steps as follows: silica VLC, fr. 1−7, 1382 mg; DCCC in 9−12−1−8 system, fr. 2, 393 mg; Sephadex LH-20, fr. 1, 263 mg; HPLC RP-18, fr. 4, 59 mg; repeated MPLC RP-18, yielding fr. 4 [15.5 mg] and fr. 12−13 [**9**, 9.6 mg].

Pregna-5,14-dienolone-3-*O*-sulfate (6a) and *allo*-Pregna-5,14-dienolone-3-*O*-sulfate (6b): colorless solid (9.0 mg, yield 0.00026% w/w), inseparable 70:30 mixture of the two epimers; UV (MeOH) λ_{max} <210 nm (4.0); IR (KBr) ν_{max} 3440, 1760, 1230, 820 cm^{-1}; ^1H and ^{13}C NMR data, see Table 1; DCINH_3MS *m/z* 331 $[M - H_2SO_4 + NH_3 + NH_4]^+$, 314 $[M - H_2SO_4 + NH_4]^+$, 297 $[M - H_2SO_4 + H]^+$.

14β-Hydroxypregn-5-enolone-3-*O*-sulfate (7): colorless solid (21.8 mg, 0.00062% w/w); $[\alpha]_D$ +104 (*c* 0.24, MeOH); UV (MeOH) λ_{max} <210 nm (4.0); IR (KBr) ν_{max} 3440, 1760, 1230, 820 cm^{-1}; ^1H and ^{13}C NMR data, see Table 1; DCINH_3MS *m/z* 332 $[M - H_2SO_4 + NH_4]^+$, 314 $[M - H_2SO_4 - H_2O + NH_4]^+$, 297 $[M - H_2SO_4 - H_2O + H]^+$.

Strophanthidin-3-*O*-sulfate (8): colorless solid (5.3 mg, 0.00015% w/w); $[\alpha]_D$ +40 (*c* 0.06, MeOH); UV (MeOH) λ_{max} 217 nm (4.0); IR (KBr) ν_{max} 3450, 1740, 1630, 1230, 820 cm^{-1}; ^1H and ^{13}C NMR data, see Table 2; DCINH_3MS *m/z* 438 $[MNH_4]^+$, 455 $[MNH_3NH_4]^+$.

Spirophanthigenin (10): colorless solid (9.6 mg, yield 0.00027% w/w); UV (MeOH) λ_{max} <210 nm; IR (KBr) ν_{max} 3410, 1750 cm^{-1}; ^1H and ^{13}C NMR data, see Table 2; DCINH_3MS *m/z* 404 $[M - H_2SO_4 + NH_4]^+$, 386 $[M - H_2SO_4 - H_2O + NH_4]^+$, 368 $[M - H_2SO_4 - 2H_2O + NH_4]^+$.

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Supporting Information Available: The structural analysis of **3**, NMR spectra of new compounds, and additional information related to this publication are available free of charge via the Internet at http://pubs.acs.org.

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