Isolation, Structure Elucidation, and Absolute Configuration of 26-Deoxyactein from *Cimicifuga racemosa* and Clarification of Nomenclature Associated with 27-Deoxyactein

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A new triterpene glycoside, 26-deoxyactein (**1**), along with two known compounds, 23-epi-26-deoxyactein (**2**), previously designated as "27-deoxyactein", and actein (**3**), were isolated from the roots/rhizomes of *Cimicifuga racemosa*. The structures and absolute stereochemistry of **1** and **2** were established by spectroscopic methods (FABMS, ^{1}H and ^{13}C NMR) and single-crystal X-ray data analysis.

The roots/rhizomes of *Cimicifuga racemosa* (L.) Nutt., or according to more recent taxonomic nomenclature[1,2] *Actaea racemosa* L. (commonly known as black cohosh), have a long and diverse history of medicinal use dating back to Native North American indigenous groups.[3,4] This plant is presently used primarily as a "hormone-free" phytomedicine in the treatment of climacteric symptoms related to menopause,[5] with a clinical history spanning over the last 40 years.[6−11] Therapeutically, it is used to reduce the frequency and intensity of hot flashes.[3−5,12−14] It has been reported that there is an improvement in psychic complaints among users.[3−5] In the United States, extracts of *C. racemosa* are available for sale as a dietary supplement used for the treatment of menopausal and postmenopausal symptoms.

According to published reports, two main classes of compounds have been isolated from the roots/rhizomes of *C. racemosa*: 9,19-cycloartane highly oxygenated triterpene glycosides[17−21] and aromatic acids[22,23] and their derivatives. To date, more than 20 of these triterpene glycosides have been isolated from this species, with 23-epi-26-deoxyactein (**2**) being one of the major constituents. This compound, formerly known as "27-deoxyactein", is commonly used as a marker or standard compound for calculating the total triterpene glycoside content in commercially available preparations of black cohosh.[24] The structure of this compound was reported as 27-deoxyactein in 1988, without the benefit of NMR data,[21] and the stereochemistry of C-23 was assigned as *R* instead of the *S* configuration exhibited by the compound actein. Its ^{1}H and ^{13}C NMR data were published in 1995, but the authors did not elaborate on the stereochemistry nor the nomenclature of this compound.[25] Thus, it has not been clear whether the stereochemistry of C-23 in 23-epi-26-deoxyactein is the same as found in actein.[25]

In the present investigation on the roots/rhizomes of *C. racemosa*, two isomeric 26-deoxyacteins (**1**, **2**) were isolated along with the known triterpene, actein (**3**). Of the two 26-deoxyacteins, **1** is a minor component which was isolated at 1/30 the quantity of **2**, the major triterpene constituent. Although their NMR data were virtually identical, their retention times (t_R) on analytical RP-HPLC were significantly different. The t_R of the 26-deoxyactein isomer (**1**) was 39.3 min, whereas the t_R of the major isomer (**2**) was 32.0 min, thus confirming the presence of two distinct compounds. On the basis of a comparison of its NMR data to those reported in the literature, compound **2** was identified as the triterpene glycoside previously known as 27-deoxyactein;[25] thus, **1** and **2** are isomers differing only in the stereochemistry at C-23. In this paper, we report on the isolation, structural determination, and absolute configurational assignment of these isomers on the basis of spectroscopic data (FABMS, ^{1}H and ^{13}C NMR) and single-crystal X-ray data analysis and clarification of the nomenclature for compound **2**.

26-Deoxyactein (**1**) and 23-epi-26-deoxyactein (**2**) were isolated by repeated chromatography on silica gel (SiO_2) and ODS (RP-18) of an EtOAc-soluble fraction prepared from the roots/rhizomes of *C. racemosa*. 26-Deoxyactein (**1**) was obtained as colorless needles; the molecular formula was determined to be $C_{37}H_{56}O_{10}$ on the basis of a positive HRFABMS ion *m/z* 683.3775 (calcd 683.3771 for $C_{37}H_{56}O_{10}Na$). 23-Epi-26-deoxyactein (**2**) was obtained as colorless needles; the molecular formula of $C_{37}H_{56}O_{10}$ was based on a positive HRFABMS ion *m/z* 683.3779 (calcd 683.3771 for $C_{37}H_{56}O_{10}Na$).

In the ^{1}H NMR spectrum (Table 1) the cyclopropane methylene signals of **1** were observed at δ 0.25 and 0.60 (each 1H, d, $J = 4.0$ Hz). The spectrum also showed seven methyl groups at δ 0.86 (s), 0.96 (d, $J = 5.7$ Hz), 1.02 (s), 1.35 (s), 1.37 (s), 1.47 (s), and 2.15 (s) and an anomeric proton at δ 4.86 (d, $J = 7.6$ Hz). The ^{13}C and DEPT NMR spectra of **1** showed signals (Table 2) ascribable to four oxygen-bearing methine carbons at δ 88.1 (C-3), 77.1 (C-12), 73.0 (C-16), and 63.6 (C-24), one oxygen-bearing methylene carbon at δ 68.8 (C-26), and two oxygen-bearing quaternary carbons at δ 105.9 (C-23) and 63.3 (C-25). The spectra also showed five oxygenated carbons assignable to the β-D-xylopyranose moiety [δ 107.5 (C-1′), 75.6 (C-2′), 78.7 (C-3′), 71.3 (C-4′), 67.1 (C-5′)] and an acetyl group [δ 170.6,

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Table 1. ^{1}H NMR Data (δ) of 26-deoxyactein (**1**) and 23-Epi-26-deoxyactein (**2**)

proton	**1**[a]	**2**
1	1.15	1.11
	1.52	1.52
2	1.88	1.84
	2.28	2.28
3	3.48 dd (4.3, 11.7)	3.44, dd (3.4, 10.8)
5	1.26 brd (12.4)	1.22 brd (10.1)
6	0.69 brd (12.7)	0.60 brdd (13.6, 11.1)
	1.44	1.40
7	0.95	0.91
	1.30	1.21
8	*1.64* dd (4.7, 14.4)	1.59 brd (12.4)
11	1.20 dd (3.7, 15.9)	1.17 brd (15.5)
	2.74 dd (7.1, 15.9)	2.70 dd (8.6, 15.5)
12	5.11 dd (3.6, 8.8)	5.10 brd. (5.3 Hz)
15	*1.68* dd (6.6, 12.6)	1.78
	1.91 dd (8.0, 12.6)	1.89 dd (6.7, 11.8)
16	*4.53* brdd, (7.2, 14.2)	4.23 brd, (6.7 Hz)
17	1.80 (overlapped)	1.76 (overlapped)
18	1.35 s	1.40 s
19	0.25 d (4.0)	0.19 d (3.8)
	0.60 d (4.0)	0.52 d (3.8)
20	1.79	2.23
21	0.96 d (5.7)	1.00 d (6.7)
22	1.60	1.44 brd (12.4)
	2.19 brd (12.4)	1.59 brd (12.4)
24	*3.71* s	4.04 s
26	*3.95* d (10.0)	3.62 d (10.4)
	3.88, d (10.0)	4.05 d (10.4)
27	1.47 s	1.45 s
28	0.86 s	0.83 s
29	1.37 s	1.29 s
30	1.02 s	0.99 s
1′	4.86, d (7.6)	4.83 d (7.2)
2′	4.05 t (7.8)	4.01 t (7.8)
3′	4.18 t (8.7)	4.14 t (8.6)
4′	4.24 m	4.21 m
5′	3.77 t (10.0)	3.73 t (10.2)
	4.38 dd (11.2, 5.1)	4.35 dd (10.5, 4.2)
OAc	2.15 s	2.12 s

[a] Recorded at 500 MHz. Coupling constants (J in Hz) are in parentheses.

Table 2. ^{13}C NMR Data (δ) of 26-Deoxyactein (**1**) and 23-Epi-26-deoxyactein (**2**)

C	**1**	**2**	C	**1**	**2**
1	32.0 t	32.0 t	19	29.6 t	29.5 t
2	29.9 t	30.0 t	20	*26.0* d	23.3 d
3	88.1 d	88.1 d	21	21.0 q	21.7 q
4	41.2 s	41.2 s	22	*36.7* t	37.6 t
5	47.0 d	47.0 d	23	105.9 s	105.9 s
6	20.5 t	20.4 t	24	*63.3* d	62.5 d
7	25.7 t	25.7 t	25	*63.3* s	62.3 s
8	45.8 d	45.6 d	26	*68.8* t	67.1 t
9	20. 2 s	20. 2 s	27	*13.8* q	14.3 q
10	26.8 s	26.8 s	28	19.6 q	19.7 q
11	36.7 t	36.7 t	29	25.7 q	25.7 q
12	77.1 d	77.1 d	30	15.4 q	15.3 q
13	48.8 s	48.8 s	1′	107.5 d	107.5 d
14	48.0 s	47.9 s	2′	75.6 d	75.6 d
15	*43.7* t	44.2 t	3′	78.7 d	78.7 d
16	*73.0* d	74.5 d	4′	71.3 d	71.3 d
17	56.5 d	56.2 d	5′	67.1 t	67.2 t
18	13.5 q	13.5 q	OAc	170.6, 20.7	170.7, 21.4

20.7 for OAc]. The ^{1}H−^{1}H COSY and HMQC spectra disclosed that the xyloside **1** has partial structures −CH_2-CH_2CH− (due to C_1 to C_3), −$CHCH_2CH_2$CH− (for C_5 to C_8), −CH_2CH(OAc)− (for C_{11} to C_{12}), and −CH_2CHCHCH−(CH_3)CH_2− (for C_{15} to C_{17}, C_{20} to C_{22}) and two pairs of geminal signals for CH_2-19 and CH_2-26 that were compatible for rings A, B, C, D, and part of E of a 9,19-cycloartane triterpene skeleton. On the basis of these data, **1** was concluded to be a highly oxygenated 9,19-cycloartane triterpene monoglycoside with an acetyl substituent.

The HMBC spectrum showed correlations between H-12 (δ_H 5.11) and the acyl signal at δ_C 170.6 (OAc), the methine signal at δ_C 56.5 (C-17) and the methyl signal at δ_C 13.5 (C-18), and the quaternary carbon signal at δ_C 48.8 (C-13). There are correlations between H-22 (δ_H 1.60 and δ_H 2.19) and the quaternary carbon C-23 at δ_C 105.9, the C-24 methine carbon at δ_C 63.3, and the C-17 methine carbon at δ_C 56.5. There are also correlations between H-26 at δ_H 3.95 and δ_H 3.88 and C-23 (δ_C 105.9) and a quaternary carbon at C-25 (δ_C 63.3). Thus, ring F is determined as shown in **1**, and the acetyl group should be assigned to C-12 of ring C. Although no correlations between H-16 and C-23 were observed in the HMBC spectrum of **1**, the HRFABMS showed that there are 10 double bond equivalents in **1**. To accommodate the correlations and double bond equivalents, an epoxy ring between C-16 and C-23 must exist, which would lead compound **1** to be structurally related, if not identical, to 23-epi-26-deoxyactein (**2**).

Comparative analysis of the ^{1}H and ^{13}C NMR spectra of **1** and those of **2** showed them to be identical except for the signals at C/H-16 and C/H-20. The ^{13}C NMR signal at C-16 [δ_C 74.5 (d)] in **2** is shifted upfield to δ_C 73.0 (d) in **1**. Meanwhile, the C-20 in **2** is shifted downfield from δ_C 23.3 (d) to δ_C 26.0 (d) in **1**. Moreover, the ^{1}H NMR of H-16 in 26-deoxyactein is shifted downfield from 4.23 to 4.53 in **1**, while H-20 in **2** is shifted upfield from δ_H 2.23 to δ_H 1.79 in **1**, respectively. Because the radius and negative substituent effects of oxygen are larger than those of carbon, these chemical shifts can be explained as being due to the γ-effect of axial oxygen between C-23 and C-26 in **2** instead of an equatorial direction in **1**.[26] Therefore, the configuration of epoxy between C-23 and C-26 is α-oriented. The configurations of C-24 and C-25 were not assigned because there were not any correlation signals observed in a standard NOESY experiment. According to the chemical shift changes of the ^{13}C and ^{1}H spectra at C/H-16 and C/H-20 in **1**, we determined the structure of **1** should be 26-deoxyactein, which has the same stereochemistry at C-23 as actein (**3**).

To confirm the structures proposed for 26-deoxyactein (**1**) and 23-epi-26-deoxyactein (**2**), X-ray analyses were carried out, and the structural information is summarized in Table 3. The intramolecular bond lengths and angles are all normal and unremarkable (Supporting Information, Table S7). Most notably, the torsion angles around atom C-33 (Table 4) are different, revealing the difference in stereochemistry at that chiral center (see Pluton Figures 2 and 3), which confirmed the structures and absolute configurations proposed for 26-deoxyactein (**1**) and 23-epi-26-deoxyactein (**2**).

With the structure of **2** firmly established as being a 26-deoxyactein congener of actein (**3**) and the NMR data obtained for this isolate being identical to those reported for 27-deoxyactein, the nomenclatural difference was examined.

The registered IUPAC and ACS name for the compound known as 27-deoxyactein is designated as β-D-xylopyranoside, (3β,12β,16β,23*S*,24*R*,25*R*)-12-(acetyloxy)-16,23:23,26:24,25-triepoxy-9,19-cyclolanostan-3-yl, whereas the designation for actein (**3**) is β-D-xylopyranoside, (3β,12β,16β,-23*R*,24*R*,25*S*,26*S*)-12-(acetyloxy)-16,23:23,26:24,25-triepoxy-26-hydroxy-9,19-cyclolanostan-3-yl.[27] The hydroxyl group in actein (**3**) is located at C-26; thus, the corresponding reduced derivative must also be at the same position. Hence, 27-deoxyactein is an incorrect name. Additionally,

Table 3. Summary of Structural Information for 26-Deoxyactein (**1**) and 23-Epi-26-deoxyactein (**2**)

	1	**2**
chemical formula	$C_{37}H_{56}O_{10}\cdot H_2O$	$C_{37}H_{56}O_{10}$
fw	678.83	660.82
unit cell dimens	a = 9.3413(19) Å	a = 27.463(6) Å
	b = 11.859(2) Å	b = 6.1806(13) Å
	c = 16.155(3) Å	c = 22.404(5) Å
	β = 103.60(3)°	β = 116.140(4)°
unit cell volume	1739.4(6) Å^3	3413.9(13) Å^3
Z	2	4
space group	$P2_1$ (No. 4, P 1 21 1)	$C2$ (No. 5, C 1 2 1)
temperature	100(1) K	100(1) K
density (calcd)	1.296 Mg m^{-3}	1.286 Mg m^{-3}
F_{000}	736	1432
abs coeff (calcd)	0.094 mm^{-1}	0.092 mm^{-1}
wavelength	0.71073 Å	0.71073 Å
data limits h, k, l	−8→11,−14→14, −19→17	-32→32,−7→7, −26→26
θ(max)	25.0°	25.0°
total no. of obsns	8866	21 268
R(int)	0.0335	0.0508
$R(\sigma)$	0.0782	0.0464
no. of averaged obsns	5786	6044
total, greater than threshold 2$\sigma(I)$	5335	5382
$R(F)$, all data	0.0885	0.0795
$R(F)$, with threshold	0.0814	0.0670
$wR(F^2)$, all data	0.1703	0.1517
goodness-of-fit S, all data	1.192	1.112

Table 4. Selected Torsion Angles for 26-Deoxyactein (**1**) and 23-Epi-26-deoxyactein (**2**)[a]

	1	**2**
C29 C28 O28 C33	176.3(4)	−176.6(2)
C27 C28 O28 C33	−65.6(5)	−59.2(3)
C31 C30 C32 C33	169.4(4)	170.3(2)
C27 C30 C32 C33	−66.3(5)	−65.6(2)
C28 O28 C33 O33	−81.3(5)	141.8(2)*
C28 O28 C33 C34	166.5(4)	−105.8(3)*
C28 O28 C33 C32	39.4(5)	22.8(3)
C30 C32 C33 O33	148.7(4)	−75.9(2)*
C30 C32 C33 O28	25.7(6)	39.9(3)
C30 C32 C33 C34	−94.6(5)	170.6(2)*
O33 C33 C34 O34	52.5(5)	46.1(3)
O28 C33 C34 O34	170.2(4)	−67.0(3)*
C32 C33 C34 O34	−66.3(5)	163.8(2)*
O33 C33 C34 C35	−13.3(5)	−18.7(4)
O28 C33 C34 C35	104.3(4)	−131.8(3)*
C32 C33 C34 C35	−132.2(5)	98.9(3)*
C33 C34 O34 C35	−101.1(5)	−97.4(3)
C33 C34 C35 O34	103.5(4)	109.6(4)
O34 C34 C35 C36	100.1(6)	104.5(4)
C33 C34 C35 C36	−156.4(5)	−145.9(3)
O34 C34 C35 C37	−105.9(4)	−105.4(3)
C33 C34 C35 C37	−2.4(5)	4.2(4)
C34 O34 C35 C36	−121.4(5)	−118.1(4)
C34 O34 C35 C37	95.7(4)	−105.4(3)*
C34 C35 C37 O33	17.2(4)	12.0(4)
O34 C35 C37 O33	−45.1(4)	−49.9(3)
C36 C35 C37 O33	173.9(4)	164.7(3)
O28 C33 O33 C37	−87.6(4)	147.3(2)*
C34 C33 O33 C37	25.1(4)	26.6(3)
C32 C33 O33 C37	149.9(3)	−92.0(2)*
C35 C37 O33 C33	−26.9(3)	−25.0(3)

[a] Substantial differences in the torsion angles are noted by an asterisk.

designation of the name 26-deoxyactein to compound **2** would imply the same stereochemistry as actein, which would be incorrect. Compound **1**, a new structure, in fact, has the same stereochemistry as actein. The names of these compounds (**1** and **2**) should henceforth be known as 26-deoxyactein and 23-epi-26-deoxyactein, respectively.

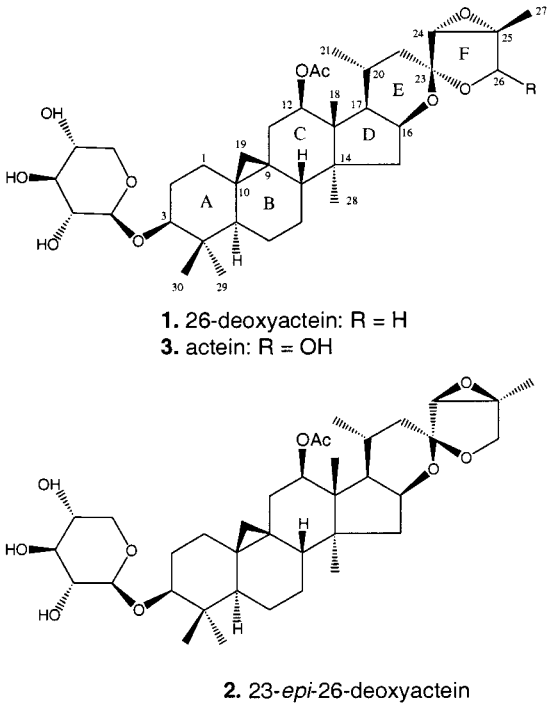

Figure 1. Structures and correct IUPAC numbering of 26-deoxyactein (**1**), 23-epi-26-deoxyactein (**2**), and actein (**3**).

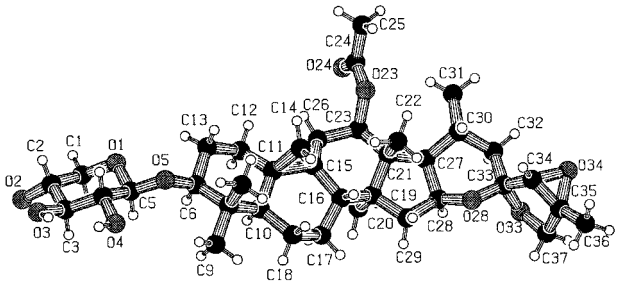

Figure 2. PLUTON drawing of **1**. (Note: crystal structure numbering does not follow IUPAC numbering.)

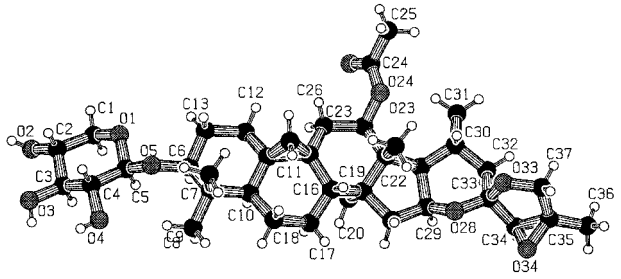

Figure 3. PLUTON drawing of **2**.

Experimental Section

General Experimental Procedures. Melting points were determined on a Fisher-Johns melting point apparatus. Optical rotations were obtained with a Perkin-Elmer 241 polarimeter. ^{1}H and ^{13}C NMR were measured on a Bruker Avance 500 MHz or a 300 MHz instrument using TMS as internal standard for chemical shifts. Chemical shifts (δ) were expressed in ppm with reference to TMS signals. HRFABMS data were recorded on a VG70E-HF system. Thin-layer chromatography was performed on E. Merck TLC plates (250 μm thickness, K6F Si gel 60 and K6F RP-18 Si gel 60) with compounds visualized by spraying the dried plates with 5% H_2SO_4 in nBuOH followed by heating. Semipreparative HPLC was carried out on a Waters 996 system equipped with a photodiode array detector on a Watrex GROM-Sil 120 ODS-4 HE semiprepara-

tive column (5 μm, 20 × 300 mm) with a flow rate of 6 mL/min. Reversed-phase low-pressure column chromatography was carried out on a Merck Lobar Lichroprep RP_{18} column, aided by a fluid pump and an autocollector. Silica gel (230−400 mesh) was used for column chromatography.

Analytical HPLC. Analysis was performed on a Waters 2690 Alliance HPLC system equipped with a Sedex 75 evaporative light scattering detector (ELSD) employing a Waters YMC ODS-AQ RP-18 column (5 μm, 120 Å 4.6 × 250 mm) with a Waters Delta-Pak RP-18 guard column. Samples of **1**, **2**, and **3** were dissolved with MeOH in concentrations of 0.200, 1.138, and 0.115 mg/mL, respectively. Chromatographic elution was accomplished by a gradient solvent system consisting of water containing 0.05% TFA (A), acetonitrile (B), and water (C). Gradient conditions were 80% A and 20% B; 8−15 min, changed to 32% B and 68% C; 15−55 min, to 64% B and 36% C; 55−65 min, to 95% B and 5% C, 65−70 min, to 95% B and 5% C; 70−85 min, to 80% A and 20% B. The flow rate was 1.6 mL/min. The retentive times of **1** and **2** were 39.3 and 32.0 min, respectively. The retentive times of actein (**3**) were 29.1 min (26*R*) and 31.8 min (26*S*).

X-ray Crystallographic Studies. Suitable crystals of **1** and **2** were grown from ethyl acetate and acetone, respectively. A summary of crystal data is given in Table 3. For each structure, the intensity data were collected at 100 K on a Bruker APEX CCD area detector with a Mo sealed tube and graphite monochromator at the University of Chicago,[28] and the data reduced with Bruker software.[29] The structures were solved by direct methods with SIR-92[30] and refined by full-matrix least-squares on F^2 with SHELX[31] using WinGX.[32] The non-hydrogen atoms were refined with anisotropic Gaussian displacement parameters (AGDP). Repeated cycling between refinement and difference Fourier maps yielded the remaining atom coordinates. All hydrogen atoms were modeled by riding on the attached atom with restrained isotropic displacement parameters. The crystal structure of **1** includes a water molecule, which hydrogen bonds with the molecule. There is some hydrogen atom disorder in each structure: on atoms O-2 (hydroxyl) and C-9 (methyl) in **1** and on atoms O-2 and C-25 (methyl) in **2**; the large AGDP on atom O-34 suggests substantial vibrational motion as well. A summary of refinement information is also included in Table 3. Final drawings (Pluton Figures 2 and 3) with labels were done using PLUTON[33] or ORTEP.[34]

Plant Material. *Cimicifuga racemosa* (L.) Nutt. roots/rhizomes were collected in Rockbridge County, Virginia (June 1999), GPS coordinates 37 48.27 N × 79 18.67 W, identified by Dr. G. Ramsey, Department of Biology, Lynchburg College, Lynchburg, VA. Voucher specimens have been deposited at the Ramsey-Freer Herbarium at Lynchburg College, Lynchburg, VA, and at the Field Museum of Natural History Herbarium, Chicago, IL. The dried, milled roots/rhizomes of *C. racemosa* (8 kg) were exhaustively extracted with MeOH. The MeOH extract was evaporated to yield 1250 g of a syrup residue. A sample (625 g) of the residue was suspended in water−MeOH (9:1, 1500 mL) and fractionated by successive partitions with EtOAc (2000 mL × 3) and *n*-BuOH (2000 mL × 3) to give EtOAc-soluble (262 g) and *n*-BuOH-soluble fractions (100 g).

A portion (250 g) of the EtOAc-soluble fraction was subjected to column chromatography on silica gel (2 kg). Elution with $CHCl_3$, $CHCl_3$−MeOH (10%), $CHCl_3$−MeOH (20%), $CHCl_3$−MeOH (30%), $CHCl_3$−MeOH (40%), $CHCl_3$−MeOH (50%), $CHCl_3$−MeOH (75%), and MeOH (100%) gave eight fractions: I (oil), II (3.0 g), III (1.2 g), IV (2.3 g), V (23 g), VI, VII (60 g), VIII (120 g).

Fraction V (23.0 g) was subjected to a normal-phase silica gel column eluted by a gradient of increasing polarity with a solvent system of petroleum ether−ethyl acetate (EtOAc) to afford 10 subfractions (CRSF-I to CRSF-X). 26-Deoxyactein (**2**, 210 mg) was obtained by direct crystallization from subfraction CRSF-V. The mother liquor was sequentially subjected to RP-18 chromatographic column separation eluted by ACN−H_2O followed by normal-phase silica gel separation eluted by petroleum ether−EtOAc−MeOH (10:7:0.5) to give 40 mg of actein (*R*,*S*) (**3**). Subfraction CRSF-III was subjected to a RP-18 column separation eluted by ACN−H_2O and MeOH−H_2O followed by silica gel column separation eluted with petroleum ether−EtOAc−MeOH (10:5:0.5) to yield 14 mg of compound **1**.

26-Deoxyactein (1): colorless needles, mp 253−254 °C, $[\alpha]^{20}_D$ −52.17° (*c* 0.025, $CHCl_3$); ^1H, ^{13}C NMR, Tables 1 and 2; FABMS *m*/*z* (relative intensity %) 661.4 (6), 451.3 (25), 307.1 (100), 289.1 (58), 219 (15), 154.1 (100), 136.1 (80), 107.9 (60), 78.9 (60); HRFABMS *m*/*z* 683.3775 (calcd 683.3771 for $C_{37}H_{56}O_{10}Na$).

23-Epi-26-deoxyactein (2): colorless needles, mp 251−253 °C, $[\alpha]_D^{20}$ −61.18° (*c* 0.255, CH_3Cl−MeOH, 1:1); ^1H, ^{13}C NMR, Tables 1 and 2 (data consistent with literature values for "27-deoxyactein");[25] FABMS *m*/*z* (relative intensity %) 661.4 (0.75), 613.2 (2.0), 460.1 (11), 307.1 (100), 289.1 (43), 219.2 (9), 154.1 (100), 136.1 (100), 107.9 (75), 78.9 (35), 66.3 (27); HRFABMS *m*/*z* 683.3779 (calcd 683.3771 for $C_{37}H_{56}O_{10}Na$).

Compounds **1** and **2** have been deposited in the Cambridge Crystallographic Data Center as CCDC 170703 and 170702, respectively.

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Supporting Information Available: X-ray crystallographic data (Tables S1−S9) for compounds **1** and **2**. This material is available free of charge via the Internet at http://pubs.acs.org.

References and Notes

(1) Compton, J. A.; Culham A.; Gibbings, J. G.; Jury, S. L. *Biochem. Syst. Ecol.* **1998**, *26*, 185−197.
(2) Compton, J. A.; Culham, A.; Jury, S. L. *Taxon* **1998**, *47*, 593−634.
(3) McKenna, D.; Jones, K.; Humphrey, S.; Hughes, K. *Altern. Ther.* **2001**, *7*, 93−100.
(4) Foster, S. *Herbalgram* **1999**, *45*, 35−49.
(5) Lieberman, S. *J. Women's Health* **1998**, *7*, 525−529.
(6) Kramer, H.; Geisenhofer, H. *Ther. Gegenwart* **1958**, *6*, 238.
(7) Kesselkaul, O. *Med. Mschr.* **1957**, *11*, 87−88.
(8) Foldes, J. *Arztl. Forsch.* **1959**, *13*, 623−624.
(9) Gorlich, N. *Arztl. Prax.* **1962**, *34*, 1742−1743.
(10) Schotten, E. W. *Landarzt* **1958**, *11*, 353−354.
(11) Stefan, H. Ringelh *Biol. Umsch.* **1959**, *10*, 149−152.
(12) Stolze, H. *Gyne* **1982**, *3*, 14−16.
(13) Warnecke, G. *Die Meizinische Welt* **1985**, *36*, 871−874.
(14) Lehmann-Willenbrock, E.; Riedel, H. *Zentbl. Gynakol.* **1988**, *110*, 611−618.
(15) Liske, E.; Wustenberg, P. *Menopause* **1998**, *5*, 250.
(16) Corsano, S.; Linde, H.; Piancatelli, G.; Panizzi, L. *Chimia* **1967**, *21*, 130−131.
(17) Bedir, E.; Khan, I. *Chem. Pharm. Bull.* **2000**, *48*, 425−427.
(18) Shao, Y.; Harris, A.; Wang, M.; Zhang, H.; Cordell, G.; Bowman, M.; Lemmo, E. *J. Nat. Prod.* **2000**, *63*, 905−910.
(19) Hamburger, M.; Wagner, C.; Benthin, B. *Pharm. Pharmacol. Lett.* **2001**, *1*, 15−17.
(20) Wende, K.; Mugge, C.; Thurow, K.; Schopke, T.; Lindequist, U. *J. Nat. Prod.* **2001**, *64*, 986−989.
(21) Berger, S.; Junior, P.; Kopanski, L. *Planta Med.* **1988**, *54*, 579−580.
(22) Kruse, S.; Lohning, A.; Pauli, G.; Wintergoff, H.; Nahrstedt, A. *Planta Med.* **1999**, *65*, 763−764.
(23) Hagels, H.; Baumert-Krauss, J.; Freudenstein, J. *48th Annual Meeting of the Society of Medicinal Plant Research. Zurich*; Switzerland Sept 3−7, 2000.
(24) He, K.; Zheng, B.; Kim, C. H.; Rogers, L.; Zheng Q. *Planta Med.* **2000**, *66*, 635−640.
(25) Koeda, M.; Aoki, Y.; Sakurai, N.; Nagai, M. *Chem. Pharm. Bull.* **1995**, *43*, 771−776.
(26) Breitmaier, E. *Structure Elucidation by NMR in Organic Chemistry: A Practical Guide*; John Wiley & Sons: New York, 1993; p 49.
(27) Scifinder Database, ACS Publications, through UIC library.
(28) The instrument is located in the X-ray Laboratory, 274 Searle Chemistry Building, Department of Chemistry, University of Chicago, and is operated by Dr. Ian M. Steele, 773-702-8109, steele@geosci.uchicago.edu, 5735 South Ellis Ave., Chicago, IL 60637.

(29) *SMART and SAINT-Plus*, Versions 5.0, Data Collection and Processing Software for the SMART System; Bruker AXS Inc.: Madison, WI, 1998.
(30) SIR92, A program for crystal structure solution: Altomare, A.; Cascarano, G.; Giacovazzo, C.; Guagliardi A. *J. Appl. Crystallogr.* **1993**, *26*, 343−350.
(31) SHELX: Sheldrick, G. M. In *Crystallographic Computing*; Moras, D., Podjarny, A. D., Thierry, J. C., Eds.; International Union of Crystallography and Oxford University Press: Oxford, U.K., 1992; pp 145−157.
(32) WinGX (version 1.64.01): Farrugia, L. J. *J. Appl. Crystallogr.* **1999**, *32*, 837−838.
(33) PLATON/PLUTON: (a) Spek, A. L. *Acta Crystallogr.* **1990**, *A46*, C34. (b) Spek, A. L. *PLATON, A Multipurpose Crystallographic Tool*; Utrecht University: Utrecht, The Netherlands, 1998.
(34) ORTEP. Johnson, C. K. *ORTEP-I*; Report ORNL-3794; Oak Ridge National Laboratory: Oak Ridge, TN, 1965. ORTEP3 for Windows: Farrugia, L. J. *J. Appl. Crystallogr.* **1997**, *30*, 565.

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