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24-EPI-PTEROSTERONE: A NOVEL PHYTOECDYSONE FROM THE ROOTS OF *ATHYRIUM YOKOSCENSE*

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Key Word Index—*Athyrium yokoscense*; Polypodiaceae; roots; ecdysteroid; phytoecdysone; 24-epi-pterosterone.

Abstract—A novel phytoecdysone, 24-epi-pterosterone, was isolated from a water extract of the roots of *Athyrium yokoscense* and the structure was determined to be (20*R*,22*R*,24*R*)-2β,3β,14α,20,22,24-hexahydroxy-5β-cholest-7-en-6-one by a combination of spectroscopic methods and single-crystal X-ray analysis. 24-Epi-pterosterone is the first example of an ecdysteroid possessing a 24*R*-hydroxyl group.

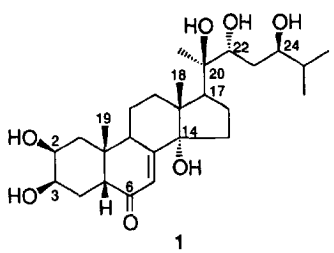
INTRODUCTION

Many ecdysteroids have been proved to have high biological activities, such as insect moulting hormone activity [1, 2]. In the course of our investigations on the biologically active compounds of ferns [3, 4], a novel phytoecdysone, named 24-epi-pterosterone (**1**), was isolated from a water extract of the roots of *Athyrium yokoscense* (Hebinonegoza in Japanese), in addition to ecdysone (**2**) [5] and ecdysterone (**3**) [6, 7]. In this paper, we report the elucidation of the structure of 24-epi-pterosterone (**1**) by a combination of spectroscopic methods and single-crystal X-ray analysis.

RESULTS AND DISCUSSION

Air-dried roots of *A. yokoscense* were extracted with distilled water to give a water-soluble fraction. This fraction was treated with chloroform to remove less polar materials. The chloroform insoluble fraction was suspended in methanol to give a methanol-soluble fraction. The methanol-soluble fraction was subjected to reversed-phase column chromatography (ODS) and then silica gel column chromatography to give a crude fraction containing compounds **1**–**3**. The reversed-phase HPLC (ODS) of the crude fraction gave pure 24-epi-pterosterone (**1**) as colourless prisms, in addition to ecdysone (**2**) and ecdysterone (**3**).

Compound **1** has a molecular formula, $C_{27}H_{44}O_7$, which was determined by high-resolution secondary ion mass spectrometry (HR-SIMS) (*m/z* 481.3172 $[M + H]^+$, $\Delta + 0.9$ mmu). The IR and UV spectral data of **1** showed the presence of hydroxyl groups (ν_{max} 3400 cm^{-1}) and α,β-unsaturated carbonyl group (ν_{max} 1660 cm^{-1} and λ_{max} 336 nm). The chemical shifts of ^1H and ^{13}C NMR signals (Table 1) assignable to C-1 to C-19 of **1** agreed with those of pterosterone (**4**) [1, 8]. ^1H and ^{13}C NMR, ^1H–^1H COSY, ^{13}C–^1H COSY and COLOC spectra of **1** indicated that **1** possesses a 20,22,24-trihydroxylated



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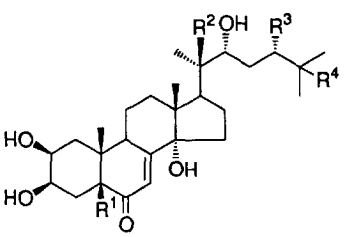

	R¹	R²	R³	R⁴
2	H	H	H	OH
3	H	OH	H	OH
4	H	OH	OH	H
5	H	OH	H	H
6	OH	OH	OH	H

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Table 1. 500 MHz ^{1}H NMR and 125 MHz ^{13}C NMR data of compound **1** in pyridine-d_5

C	δ_C	δ_H
1	38.0	1.97 *m*
		2.17 *m*
2	68.1	4.19 *br d* (11.9)
3	68.1	4.24 *br s*
4	32.4	1.80 *m*
		2.02 *m*
5	51.4	3.03 *m*
6	203.4	
7	121.7	6.25 *d* (1.8)
8	166.1	
9	34.4	3.60 *m*
10	38.7	
11	21.1	1.80 *m*
		1.90 *m*
12	32.0	2.10 *m*
		2.66 *m*
13	48.1	
14	84.2	
15	31.8	1.89 *m*
16	21.7	2.36 *m*
		2.50 *m*
17	50.0	3.08 *m*
18	17.9	1.25 *s*
19	24.4	1.09 *s*
20	76.8	
21	21.3	1.66 *s*
22	73.4	4.13 *br s*
23	37.5	1.87 *m*
		2.03 *m*
24	73.5	4.50 *br d* (10.1)
25	35.0	1.85 *m*
26	19.5	1.01 *d* (6.4)
27	18.6	1.10 *d* (6.4)

Coupling constants in Hz are shown in parentheses.

steroid side chain at C-17, which is similar to that of **4**. The relative configuration between C-20 and C-22 of **1** is the same as that of **4**, because of the similarity of chemical shifts of the carbon signals due to C-17 (δ50.0), C-20 (δ76.8) and C-21 (δ21.3) of **1** to those of **4**. However, the ^{13}C NMR signals due to C-22 (δ73.4) and C-24 (δ73.5) of **1** were shifted to upfield by 3–4 ppm with respect to those of the corresponding carbon atoms of **4**. These findings suggest that compound **1** is the 24-epimer of **4**.

The relative configuration of **1** was completely determined by single-crystal X-ray analysis. As shown in Fig. 1, the hydroxyl groups at C-2 and C-3 are equatorial and axial, respectively. Ring A has the chair conformation, and ring A is *cis*-fused with ring B [H-C5-C10-C19 = 51.6°]. Ring B is twisted into a half-chair form owing to the presence of the α,β-unsaturated carbonyl group, while ring C assumes a distorted chair conformation.

Having defined the complete relative stereochemistry of **1**, we next sought to determine the absolute configuration via the CD spectrum of compound **1**. Compound **1** exhibited the positive Cotton effect ($[\theta]_{337}$ + 4170) due to the $n \rightarrow \pi^*$ transition of the carbonyl group. This indicates that the absolute configuration of the steroid skeleton (C-1–C-19) of **1** is identical to that of many ecdysteroids, e.g. **3**, **4** and ponasterone A (**5**) [1, 9]. Thus, a combination of CD and X-ray analyses revealed the chirality of C-24 of **1** to be *R*.

Consequently, the structure of the novel phytoecdysone, 24-epi-pterosterone (**1**), was unambiguously defined as (20*R*,22*R*,24*R*)-2β,3β,14α,20,22,24-hexahydroxy-5β-cholest-7-en-6-one. The chirality of C-24 has been reported to be all *S* for ecdysteroids possessing a 24-hydroxyl group, e.g. **4** and ponasterone C (**6**) [9]. 24-Epi-pterosterone (**1**) is the first example of an ecdysteroid possessing a 24*R*-hydroxyl group.

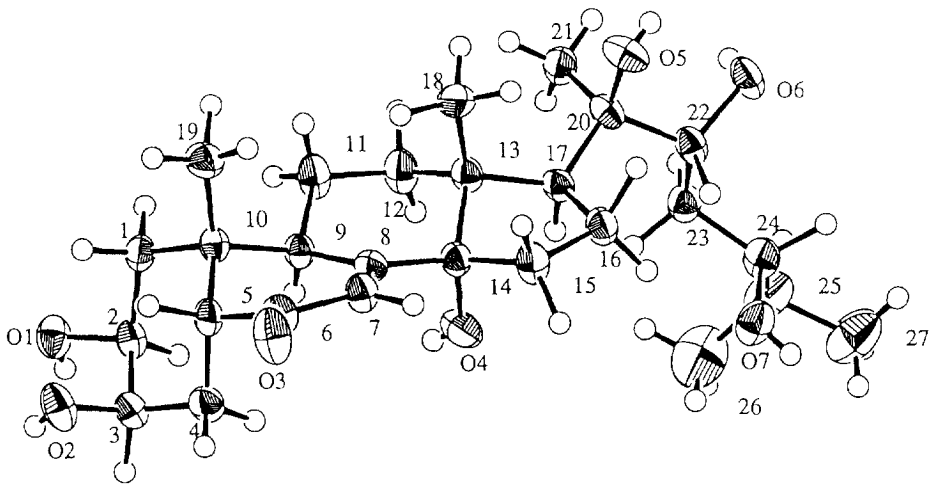

Fig. 1. ORTEP drawing of compound **1**, showing atom numbering.

EXPERIMENTAL

General. ^1H (500 MHz) and ^{13}C (125 MHz) NMR: pyridine-d_5 with TMS as int. standard; SIMS: glycerol as the matrix; TLC: silica gel (Merck 60 F_{254}).

Plant material. Plant material was collected in Mihara City, Hiroshima prefecture in June, 1993 and identified as *Athyrium yokoscense* by the authors (S.O. and Y.H.). The voucher specimen is kept in the laboratory of one of the authors (Y.H.).

Isolation. Air-dried roots (1.3 kg) were extracted with distilled H_2O (3.5 l) for 14 days. After filtration, the water-soluble fraction was treated with $CHCl_3$ to remove less polar materials. The $CHCl_3$-insoluble fr. (9.8 g) was suspended in MeOH to give a MeOH-soluble fr. (3.7 g). The MeOH-soluble fr. was subjected to reversed-phase CC (ODS) using H_2O–MeOH (MeOH: 0–100%) and then silica gel CC by MeOH–$CHCl_3$ (1:4) to give a crude fr. The reversed-phase HPLC (ODS) of the crude fr. using H_2O–MeOH (1:1) gave 24-epi-pterosterone (**1**) (7 mg), ecdysone (**2**) (4 mg) and ecdysterone (**3**) (16 mg).

24-*Epi-pterosterone* (**1**). Prisms, mp 151–152° (from H_2O–MeOH); $[\alpha]_D^{25}$ + 66° (MeOH; *c* 0.63); UV λ_{max}^{MeOH} nm: 243 (ε 10 600) and 336 (ε 100); IR ν_{max}^{kBr} cm^{-1}: 3400 (OH) and 1660 br (C=O and C=C); ^1H NMR and ^{13}C NMR: see Table 1; SIMS *m/z*: 481 $[M + H]^+$, 463 $[M - H_2O + H]^+$, 445 $[M - 2H_2O + H]^+$, 427 $[M - 3H_2O + H]^+$; CD (dioxane; 9.1×10^{-5} M): $[\theta]_{337}$ + 4170, $[\theta]_{246}$ − 12 400, $[\theta]_{220}$ + 11 000.

Crystal data of **1**. Single crystals of **1** crystallized from MeOH–H_2O were $C_{27}H_{44}O_7 \cdot CH_3OH \cdot 2H_2O$, M_r = 548.71, tetragonal, space group $P4_12_12$, a = 13.749 (3), c = 31.157 (8) Å, U = 5889 (2) Å^3, Z = 8, D_c = 1.24 g cm^{-3}. Intensity measurements were made with $2\theta \leqq 120.1°$ by using graphite monochromated Cu-Kα radiation at 20° on a Rigaku AFC7R diffractometer. A total of 2618 independent reflections were collected, of which 2365 were considered to be observed [$I > 3\sigma(I)$]. The structure was solved by direct methods and expanded using Fourier techniques. The nonhydrogen atoms were refined anisotropically by full-matrix least-squares refinement. Hydrogen atoms were included but not refined. The structure was finally refined to $R = 0.049$ ($R_w = 0.071$). Atomic coordinates, bond lengths and angles, and thermal parameters have been deposited at the Cambridge Crystallographic Data Centre, U.K.

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