Studies of Active Substances in Herbs Used for Hair Treatment. IV. The Structure of the Hair Regrowth Substance, Polyporusterone A, from *Polyporus umbellatus* F.[1)]

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The crystal structure of polyporusterone A, from *Polyporus umbellatus* F. was determined by X-ray diffraction analysis. The crystals are orthorhombic, space group $P2_12_12_1$, $Z=4$, unit-cell dimension $a=17.968(2)$, $b=26.201(5)$, $c=6.227(1)$ Å. The structure, (+)-2β,3β,14α,(20*R*,22*R*)-pentahydroxy-(24*S*)-methyl-5β-cholest-7-en-6-one, was solved from diffractometric data by direct methods and refined by least-squares calculations to $R=0.053$ (2345 observed independent significant reflections ($I>3\sigma(I)$). All the hydroxyl groups are involved in a hydrogen-bonding network. The NMR data indicate that the side-chain has the same conformation in both the crystalline state and solution.

Key words *Polyporus umbellatus* F.; 24*S*-polyporusterone A; 20-hydroxyecdysone; hair regrowth; crystallographic analysis

In the course of studies to isolate hair regrowth substances from herbs which were selected on the basis of a literature survey,[1)] we have already isolated polyporusterones A and B as active substances from *Polyporus umbellatus* F.[2)] Their structures were identified as (+)-2β,3β,14α,(20*R*,22*R*)-pentahydroxy-24-methyl-5β-cholest-7-en-6-one and (+)-2β,3β,14α,(20*R*,22*R*)-pentahydroxy-5β-cholest-7,24(28)-dien-6-one by Osawa *et al*. on basis of physicochemical data, but stereochemistry at the C(24) of polyporusterone A remained unestablished.[3)]

It had been reported that polyporusterones A and B are cytotoxic to L1210 cells.[3)] We recently found that polyporusterones A and B and 20-hydroxyecdysone promote hair regrowth, and polyporusterone A is more effective than the latter two compounds in mammals.[2)]

The stereochemistry of polyporusterone A is of interest for pharmacological reasons. Because of the poor crystallization properties of ecdysteroids, the determination of their stereochemistry usually poses a problem. However, polyporusterone A gives good crystals under suitable conditions and so an investigation was conducted to determine its absolute configuration and stereochemistry at C(24) by X-ray crystallographic and NMR techniques. The results are presented here.

Experimental

The melting points were determined with a Yanaco micro-melting point apparatus and are uncorrected. The instruments used were as follows: mass spectrum (MS), JEOL DX-300 spectrometer; nuclear magnetic resonance (NMR) spectra, JEOL GSX-500 and Brucker DRX-500 instruments; optical rotation, a JASCO DIP-140 instrument.

Isolation of Polyporusterone A As reported previously,[2)] polyporusterone A was isolated from *Polyporus umbellatus* F.

X-Ray Crystallographic Analysis Polyporusterone A was carefully recrystallized from methanol to afford colorless plate-shaped crystals, mp 283 °C and $[D]_\alpha^{23}+53.1°$ ($c=0.60$, MeOH). A crystal with dimensions 0.1×0.1×0.3 mm was used to measure the cell constants and for data collection. The recrystallization solvent was readily lost in air, so all data for the crystallographic analysis were obtained from the crystal sealed in a glass capillary. The cell parameters, shown below, and orientation matrices of polyporusterone A were obtained by the least-squares procedure applied to 20 reflections carefully measured on a Rigaku four-circle diffractometer equipped with a rotating anode (graphite-monochromated Cu$K\alpha$ radiation ($\lambda=1.5418$ Å)). Intensities, on a relative scale, were measured by the ω–2θ scan technique on the same instrument over the range of $5<2\theta<140°$ (Cu$K\alpha$) (scan speed 4 min^{-1} in ω, scan range in ω ($1.3\pm0.14\tan\theta$). Three standards (monitored every 50 reflections) showed no significant change during data collection.

Finally, 2345 independent data with $I>3\sigma(I)$ that remained after averaging the 3160 symmetry-related reflections were used in the analysis. Lorentz and polarization, but not absorption, corrections were applied. The space group of the compound, $P2_12_12_1$ was determined from the systematic absences. The correctness of the choice of space group was confirmed by the successful structure determination.

Crystal data: $C_{28}H_{46}O_6\cdot CH_3OH\cdot H_2O$, M.W.=528.36, Orthorhombic, $a=17.968(2)$, $b=26.201(5)$, $c=6.227(1)$ Å, $V=2931.4$ (7) Å^3, $D_o=1.22$ g/cm^3, $D_c=1.19$ g/cm^3, $Z=4$, Space group $P2_12_12_1$.

Structure Determination and Refinement The structure of polyporusterone A was solved by direct methods using normalized structure factors with $E>1.2$, and refinement was carried out as follows.

The first E map, in the total of the 4 phase sets generated, gave the positions of 29 non-hydrogen (C, O) atoms in the structure. The successive difference electron density map located the remaining 8 non-hydrogen atoms ($R=0.128$). Further refinement of atomic coordinates with anisotropic thermal parameters reduced R to 0.088. At this stage, all the hydrogen atoms were located in the positions obtained from a subsequent difference map. Further full-matrix least-squares refinement of the position parameters and temperature factor coefficients, anisotropic for the non-hydrogen atoms and isotropic for the hydrogens, resulted in $R=0.053$ ($R\omega=0.056$). The final atomic parameters for polyporusterone A are given in Table 1.

For the structure solution, the programs MULTAN80[4)] and SAPI85[5)] were used with scattering factors from volume 4 of reference 6. The block-diagonal program employed for the refinement did not use a weigting system. The function minimized was $\Sigma w[(F_O)^2-(F_C)^2]^2$ with $w=1/[\sigma^2(F_O)+0.02(F_O)^2]$ and $\sigma(F_O)$ determined from counting statistics. The molecular structure was drawn with the aid of the ORTEP program.[7)] All calculations were made on PANAFACOM computers (A-70).

NMR Experiments ^{1}H–^{1}H correlation spectroscopy (COSY), ^{13}C–^{1}H-COSY, heteronuclear multibond connectivity (HMBC), two-dimensional nuclear Overhauser effect (NOE) spectroscopy (2D-NOESY) and distortionless enhancement by polarization transfer (DEPT) in C_5D_5N were carried out on the instruments above.

Results and Discussion

Stereoscopic views of the polyporusterone A molecule, with atomic numberings, are depicted in Fig. 1. In the side-chain, the H-atoms at C(17) and O(5) are in an antiperiplanar

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Table 1. Positional ($\times 10^4$) and Thermal Parameters of 24*S*-Polyporusterone A with Estimated Standard Deviations in Parentheses, Denoting the Least Significant Digits

Atom	*X*	*Y*	*Z*	B_{eq}
O(1)	3495(3)	703(2)	4553(8)	4.0(3)
O(2)	2407(3)	683(2)	1210(8)	3.8(3)
O(3)	2284(3)	2091(2)	−3463(10)	4.6(3)
O(4)	3390(3)	3231(2)	3270(10)	4.0(3)
O(5)	5668(3)	4127(2)	283(9)	3.8(3)
O(6)	5894(3)	4950(2)	2979(9)	3.5(2)
O(H)	1788(3)	9861(2)	3257(9)	3.8(3)
O(M)	8041(3)	7892(2)	1103(13)	6.1(4)
C(1)	3890(3)	1250(2)	1611(12)	2.7(2)
C(2)	3314(4)	1154(2)	3370(12)	3.0(2)
C(3)	2528(4)	1131(2)	2491(12)	3.2(3)
C(4)	2356(3)	1600(2)	1151(13)	3.3(2)
C(5)	2935(3)	1673(2)	−645(11)	2.7(2)
C(6)	2719(3)	2131(2)	−1965(12)	3.1(2)
C(7)	3011(4)	2627(2)	1287(13)	3.3(3)
C(8)	3507(3)	2677(2)	283(11)	2.6(2)
C(9)	3826(3)	2224(2)	1517(12)	2.5(2)
C(10)	3740(3)	1717(2)	220(12)	2.5(2)
C(11)	4634(4)	2315(2)	2256(14)	3.4(3)
C(12)	4809(4)	2856(2)	3088(13)	3.2(3)
C(13)	4569(3)	3257(2)	1472(11)	2.4(2)
C(14)	3716(3)	3197(2)	1153(11)	2.6(2)
C(15)	3496(4)	3663(2)	−162(15)	4.0(3)
C(16)	4014(4)	4091(2)	705(15)	3.7(3)
C(17)	4588(3)	3829(2)	2223(11)	2.5(2)
C(18)	4276(4)	1694(3)	−1707(13)	3.3(2)
C(19)	4963(4)	3177(3)	−678(13)	3.4(2)
C(20)	5335(3)	4114(2)	2375(11)	2.7(2)
C(21)	5881(4)	3871(3)	3956(17)	4.1(3)
C(22)	5186(4)	4685(2)	3022(12)	2.9(2)
C(23)	4801(5)	4776(3)	5151(13)	3.7(2)
C(24)	4524(5)	5328(3)	5409(14)	3.7(2)
C(25)	3813(5)	5426(3)	4114(18)	5.2(3)
C(26)	3687(7)	5990(5)	3603(29)	7.7(4)
C(27)	3117(7)	5206(5)	5122(31)	7.8(4)
C(28)	4446(7)	5461(4)	7784(18)	6.0(3)
C(M)	8731(7)	7872(5)	1808(29)	9.5(5)

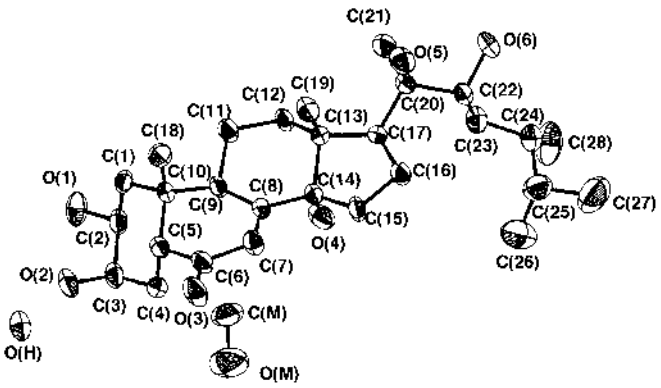

Fig. 1. ORTEP Drawing of 24*S*-Polyporusterone A with Atomic Numbering

Hydrogens not shown.

conformation, and O(5) is antiperiplanar with respect to the C(13)–C(17) bond, whereas C(22) is synclinal. O(5) is synclinal with respect to C(17)–C(20), whereas O(6) is antiperiplanar. The absolute configurations at the 20, 22 and 24-positions were assigned as *R*, *R* and *S* (stigmasterol configuration, as in carpesterol and makisterone A),[8,9] respectively. The

Table 2. Intramolecular Bond Lengths (Å) of 24*S*-Polyporusterone A with Estimated Standard Deviations in Parentheses

O(1)–C(2)	1.429(8)	O(2)–C(3)	1.435(8)	O(3)–C(6)	1.222(9)
O(4)–C(14)	1.445(9)	O(5)–C(20)	1.435(9)	O(6)–C(22)	1.449(8)
O(M)–C(M)	1.32(1)	C(1)–C(10)	1.522(9)	C(1)–C(2)	1.53(1)
C(2)–C(3)	1.515(9)	C(3)–C(4)	1.517(9)	C(4)–C(5)	1.54(1)
C(5)–C(6)	1.505(9)	C(5)–C(10)	1.548(8)	C(6)–C(7)	1.462(9)
C(7)–C(8)	1.33(1)	C(8)–C(14)	1.514(8)	C(8)–C(9)	1.524(8)
C(9)–C(11)	1.541(9)	C(9)–C(10)	1.564(8)	C(10)–C(18)	1.54(1)
C(11)–C(12)	1.542(9)	C(12)–C(13)	1.517(9)	C(13)–C(19)	1.53(1)
C(13)–C(14)	1.554(8)	C(13)–C(17)	1.570(8)	C(14)–C(15)	1.521(9)
C(15)–C(16)	1.55(1)	C(16)–C(17)	1.56(1)	C(17)–C(20)	1.540(8)
C(20)–C(21)	1.53(1)	C(20)–C(22)	1.573(8)	C(22)–C(23)	1.51(1)
C(23)–C(24)	1.54(1)	C(24)–C(25)	1.53(1)	C(24)–C(28)	1.53(1)
C(25)–C(26)	1.53(1)	C(25)–C(27)	1.51(2)		

Table 3. Intramolecular Bond Angles (°) of 24*S*-Polyporusterone A with Estimated Standard Deviations in Parentheses

C(10)–C(1)–C(2)	114.9(5)	O(1)–C(2)–C(3)	111.5(5)
O(1)–C(2)–C(1)	110.6(5)	C(3)–C(2)–C(1)	112.2(6)
O(2)–C(3)–C(2)	112.0(5)	O(2)–C(3)–C(4)	109.0(6)
C(2)–C(3)–C(4)	110.9(5)	C(3)–C(4)–C(5)	111.3(5)
C(6)–C(5)–C(4)	108.8(5)	C(6)–C(5)–C(10)	111.8(5)
C(4)–C(5)–C(10)	112.8(6)	O(3)–C(6)–C(7)	121.8(6)
O(3)–C(6)–C(5)	120.8(6)	C(7)–C(6)–C(5)	117.3(6)
C(8)–C(7)–C(6)	122.7(6)	C(7)–C(8)–C(14)	121.2(5)
C(7)–C(8)–C(9)	123.1(5)	C(14)–C(8)–C(9)	115.3(5)
C(8)–C(9)–C(11)	112.6(5)	C(8)–C(9)–C(10)	111.3(5)
C(11)–C(9)–C(10)	112.2(5)	C(1)–C(10)–C(18)	107.6(5)
C(1)–C(10)–C(5)	107.7(5)	C(1)–C(10)–C(9)	111.8(6)
C(18)–C(10)–C(5)	108.1(6)	C(18)–C(10)–C(9)	111.9(5
C(5)–C(10)–C(9)	109.6(4)	C(9)–C(11)–C(12)	115.7(5)
C(13)–C(12)–C(11)	110.9(6)	C(12)–C(13)–C(19)	110.7(5)
C(12)–C(13)–C(14)	107.2(5)	C(12)–C(13)–C(17)	117.2(5)
C(19)–C(13)–C(14)	109.4(5)	C(19)–C(13)–C(17)	112.5(5)
C(14)–C(13)–C(17)	98.9(4)	O(4)–C(14)–C(8)	106.4(5)
O(4)–C(14)–C(15)	109.7(5)	O(4)–C(14)–C(13)	106.0(5)
C(8)–C(14)–C(15)	117.7(6)	C(8)–C(14)–C(13)	112.4(5)
C(15)–C(14)–C(13)	104.1(5)	C(14)–C(15)–C(16)	103.7(6)
C(15)–C(16)–C(17)	106.8(5)	C(20)–C(17)–C(16)	113.6(5)
C(20)–C(17)–C(13)	120.0(5)	C(16)–C(17)–C(13)	103.0(5)
O(5)–C(20)–C(21)	109.1(5)	O(5)–C(20)–C(17)	108.7(5)
O(5)–C(20)–C(22)	106.3(5)	C(21)–C(20)–C(17)	113.4(5)
C(21)–C(20)–C(22)	109.9(6)	C(17)–C(20)–C(22)	109.2(5)
O(6)–C(22)–C(23)	110.0(6)	O(6)–C(22)–C(20)	107.6(5)
C(23)–C(22)–C(20)	116.8(6)	C(22)–C(23)–C(24)	112.7(6)
C(28)–C(24)–C(25)	113.3(8)	C(28)–C(24)–C(23)	110.2(7)
C(25)–C(24)–C(23)	111.9(7)	C(27)–C(25)–C(26)	109.5(8)
C(27)–C(25)–C(24)	114.0(10)	C(26)–C(25)–C(24)	113.2(8)

geometry of the rings is as expected, with the A/B-ring juncture *cis*, while the C/D-ring juncture is *trans*, as shown in Fig. 1. Rings A and C have normal chair conformations. Thus, the absolute structure is (+)-2β,3β,14α, (20*R*,22*R*)-pentahydroxy-(24*S*)-methyl-5β-cholest-7-en-6-one (24*S*-polyporusterone A).

The bond lengths and angles, given in Tables 2 and 3, in the asymmetric unit are normal. The geometry of the whole skeleton resembles that of ecdysone.[10] The C(6)–C(7) bond is similar in length to a C–C single bond adjacent to a C=O bond together with a C=C bond. The atoms C(5), C(6), O(3), C(7), C(8), C(9) and C(14) define a plane from which the maximum deviation is 0.13 Å and the average deviation is 0.07 Å.

The hydrogen bonds in the crystal are described in Table

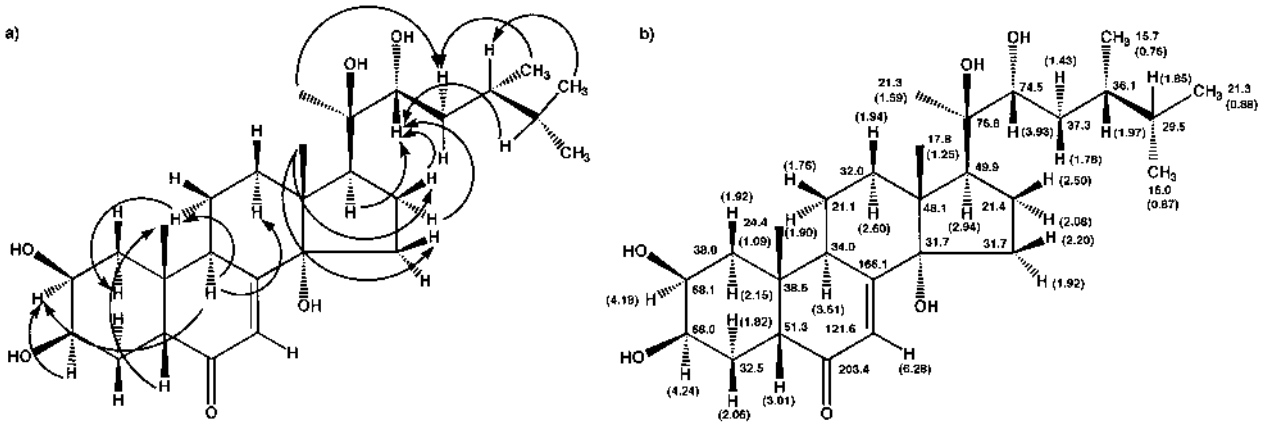

Fig. 2. Relative Stereochemistry Based on the Results Obtained from NMR Experiments on 24*S*-Polyporusterone A (a) and the Carbon and Proton Signal Assignments (b)

a) Arrows indicate NOE's, measured in NOE difference experiments. b) The attached numbers denote ^{13}C-NMR (^{1}H-NMR) chemical shifts, ppm in C_5D_5N.

Table 4. O–O Distances (Å) of the Hydrogen Bonds in 24*S*-Polyporusterone A

O(1)–O(6iii)	2.754(7)	O(2)–O(Hiv)	2.738(7)	O(3)–O(Mv)	2.730(9)
O(4)–O(Mi)	2.749(7)	O(5)–O(2ii)	3.296(7)	O(6)–O(7vi)	2.884(8)
O(H)–O(1iv)	2.787(7)	O(H)–O(2i)	2.738(7)		

Symmetry code: i) x, y, z; ii) $-x+3/2, -y+2, z-1/2$; iii) $-x+1, y+1/2, z+3/2$; iv) $-x+3/2, -y+2, z+1/2$; v) $x, y, z+1$; vi) $x-1/2, -y+3/2, -z+1$.

4. The molecular packings are characterized by an assembly of rows of molecules. In each row, molecules are linked by head-to-tail hydrogen bonds *via* O(1) and O(6). Rows are connected by hydrogen bonds *via* methanol and water molecules. All of the oxygen atoms are involved in intermolecular hydrogen bonding. With the exception of the oxygen at C(20), each external hydrogen bond distance O–O is *ca*. 2.8 Å.

The assignments of carbon atoms and protons and the NOE's are shown in Fig. 2. The stereochemistry and conformation of 24*S*-polyporusterone A are supported by the NMR data, especially the NOESY results. The NOESY data indicate that the side-chain has the same conformation in both the crystalline state and solution. Some of the chemical shifts, assigned to the 12, 16, 24, 25, 26 and 28 position protons and/or carbons of this compound in a previous report,[3] are corrected for the values in Fig. 2, based on the NMR experimental data. In these assignments, the significant high-field shift of the C-16 carbon, compared with those of C-12 and 15, is fully explained by the substituent effects (ppm) of the hydroxyls (β: +8, γ: −5) at C-14 and 20, of the methyls at C-13 and 21 (β: +8, γ: −2), and of the alkyl side-chain at C-17 (β: +8, γ: −2).

In summary, we have shown that the absolute structure of polyporusterone A from *Polyporus umbellatus* F. is (+)-2β,3β,14α,(20*R*,22*R*)-pentahydroxy-(24*S*)-methyl-5β-cholest-7-en-6-one on the basis of X-ray crystallographic analysis and NMR data.

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