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STRUCTURE OF A NEW STEROID 24*S*-ERGOST-4-EN-3,6-DIONE FROM *Aconitum septentrionale*

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β-Sitosterol and the new phytosteroid 24S-ergost-4-en-3,6-dione were isolated from the industrial extraction waste of the alkaloid-bearing plant Aconitum septentrionale *Koelle. The structure of the isolated compound was determined by x-ray structure analysis and PMR, ^{13}C NMR, and IR spectroscopy. The crystal structure of the steroid contains a disordered terminal 24-methyl,24-isopropyl group that occupies two positions in a 0.55:0.45 ratio.*

Keywords: steroids, 24*S*-ergost-4-en-3,6-dione, absolute configuration, x-ray crystal structure.

Industrial wastes from targeted extraction of plant drugs may turn out to be useful alternate sources of natural steroids for scientific and practical purposes. In particular, a large quantity of the alkaloid-bearing plant *Aconitum septentrionale* Koelle is extracted at the pilot plant at the Institute of the Chemistry of Plant Substances in order to obtain the drug substance allapinin [1, 2]. The plant contains in addition to alkaloids [3, 4] organic acids [5], coumarins [6], and flavonoids [7]; the seeds, essential oils [8].

These wastes were separated by column chromatography using extraction benzine in order to isolate useful neutral substances. Separation of the benzine fraction over a column of silica gel isolated *β*-sitosterol, $C_{29}H_{50}O$, mp 135–137°C [9]. Further elution of the column isolated an ergostane-type diketosteroid **1**.

The IR spectrum of **1** had absorption bands characteristic of C–H (2962 cm^{-1}), CH_3 (2871), C=O (1688), and C=C (1603, 1463) groups.

The PMR spectrum (Table 1) showed at strong field two 3H singlets of angular methyl C-18 (0.68 ppm) and C-19 (1.03) in addition to four 3H doublets of secondary methyls C-21 (0.86, SSCC 6.4 Hz) and C-26 and C-27 (0.77, 6.8), and C-28 (0.87, 8.4). An olefinic proton coupled to a carbonyl was observed as a singlet at 5.96.

The ^{13}C NMR spectrum exhibited 28 resonances. Of these, two appeared at weak field characteristic of ketones. An analysis of all results using DEPT suggested that the isolated compound was a Δ^4-3,6-diketosteroid.

A diketosteroid of similar structure was described earlier from *Stephania cepharantha* [10] and from soy culture [11] based on GC–MS analysis. Of course, the configuration of the C-24 atom was not determined. Therefore, that compound could not be matched with the diketosteroid **1** isolated by us.

The structure of the isolated steroid was reliably established based on an x-ray crystal structure analysis (XSA). Figure 1 shows the molecular structure of **1** from the XSA. A rotational conformer (along the C-23–C-24 bond) was observed in the crystal structure because of disorder at the terminal 24-methyl and 24-isopropyl groups. Atoms C-25, C-26, C-27, and C-28 had large thermal parameters and were fixed in two positions in a 0.55:0.45 ratio. Figure 1 shows the disordered atoms with additional unshaded bonds.

Considering the (8*S*,9*S*,10*R*,13*R*,14*S*,17*R*)-stereochemistry of **1** from the given molecular structure, asymmetric C-20 and C-24 had the 20*R*,24*S* configurations. As expected, rings B/C and C/D were *trans*-fused. The C-10 and C-13 methyls were situated in *β*-axial positions.

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TABLE 1. PMR and ^{13}C NMR and DEPT Data for **1** [$(CCl_4 + C_6D_6) + DMSO\text{-}d_6$), δ, ppm, J/Hz]

C atom	δ_H CCl_4	δ_C $(CCl_4 + C_6D_6)$ + $DMSO\text{-}d_6$	DEPT	C atom	δ_H CCl_4	δ_C $(CCl_4 + C_6D_6)$ + $DMSO\text{-}d_6$	DEPT
1		36.63	CH_2	15		24.50	CH_2
2		34.46	CH_2	16		28.69	CH_2
3		198.16	C	17		56.98	CH
4	5.96 s	125.82	CH	18	0.63 s	12.54	CH_3
5		160.77	C	19	1.03 s	18.03	CH_3
6		200.98	C	20		36.63	CH
7		46.36	CH_2	21	0.86 d (J = 6.4)	19.39	CH_3
8		34.44	CH	22		35.97	CH_2
9		51.09	CH	23		26.69	CH_2
10		39.78	C	24		34.40	CH
11		23.65	CH_2	25		29.71	CH
12		40.07	CH_2	26	0.76 d (J = 6.8)	19.77	CH_3
13		43.07	C	27	0.77 d (J = 6.8)	20.53	CH_3
14		56.46	CH	28	0.80 d (J = 8.4)	21.41	CH_3

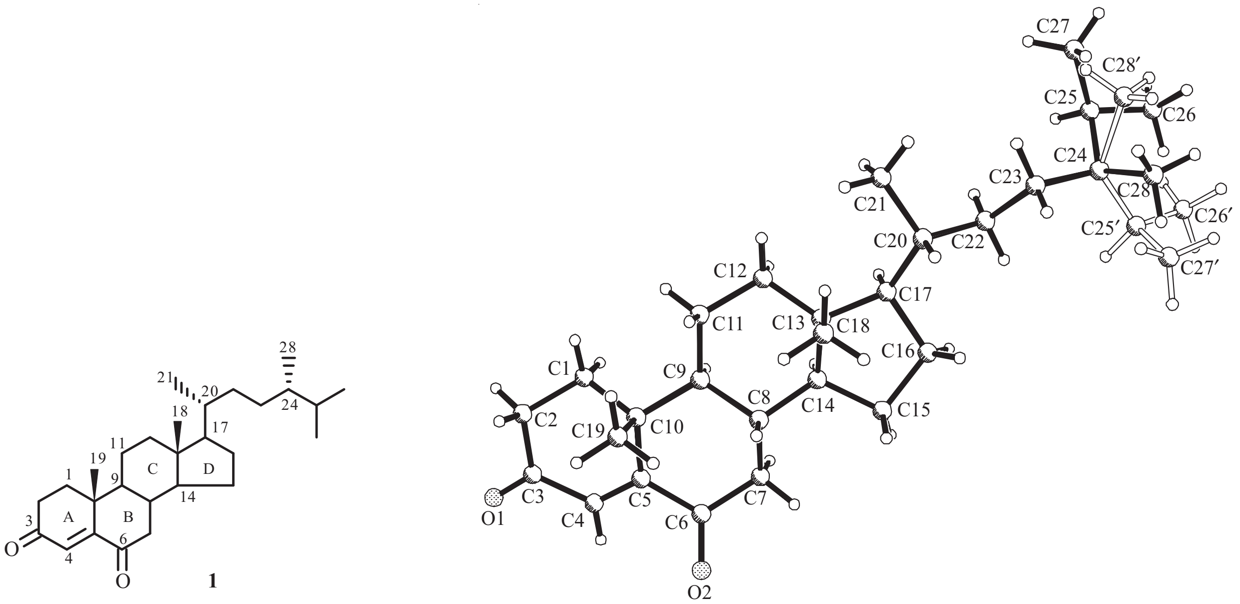

Fig. 1. Molecular structure and atomic numbering of **1** (atoms C-25, C-26, C-27, and C-28 with large thermal parameters were fixed in two positions in a 0.55:0.45 ratio; the disordered atoms are shown as additional unshaded bonds; the H atom on C-24 is not shown).

Ring A had a 1*α*-chair conformation because of the ketone [C-3=O-1, 1.208(4) Å] and the double bond [C-4=C-5, 1.322(4) Å] in the ring; ring B, a slightly distorted chair; ring C, an ideal chair; ring D, a twist with C_2 symmetry passing through C-16 and the middle of the C-13–C-14 bond.

The ketonic groups formed a conjugated system via the C-4=C-5 double bond although the C-6=O-2 bond was slightly groups elongated [1.281(4) Å] because of the conjugation and was twisted out of the conjugation plane [24.8(4)°].

Thus, **1** was isolated from industrial waste of *A. septentrionale* and had the structure 24*S*-ergost-4-en-3,6-dione.

EXPERIMENTAL

General Comments. IR spectra were taken on a Perkin–Elmer Model 2000 Fourier IR spectrometer; PMR spectra, in CCl_4 and $(CCl_4 + C_6D_6) + DMSO\text{-}d_6$ on a Unity (Varian) spectrometer at operating frequency 400 MHz using the δ scale. The internal standard was hexamethyldisiloxane (HMDS). Column chromatography was carried out over KSK SiO_2 (<0.25 mm). TLC used extraction benzine:EtOAc (2:1) and Silufol plates (Chemapol) with detection by vanillin solution (1%) in conc. H_2SO_4.

Isolation of Steroids. The thick syrupy industrial alcohol extract (1000 mL) (wastes from allapinin production) from the alkaloid-bearing plant *A. septentrionale* was diluted with water to 2.0 L. The aqueous alcohol extract was shaken successively with extraction benzine (8 × 0.7 L), EtOAc (6 × 0.7 L), and *n*-BuOH (6 × 0.7 L). Removal of the solvents afforded extraction-benzine (20 g), EtOAc (10.0 g), and *n*-BuOH (12.0 g) fractions.

The extraction-benzine extract (20 g) was chromatographed over a column (3.0 × 180 cm) of silica gel (500 g) using the systems extraction hydrocarbons (1) and extraction hydrocarbons:$CHCl_3$ (2, 2:1; 3, 1:1). Fractions of 500 mL were collected. Elution of the column by system 2 afforded β-sitosterol (0.25 g); by system 3, 24*S*-ergost-4-en-3,6-dione (**1**, 0.15 g), $C_{28}H_{44}O_2$, mp 174°C (MeOH).

IR spectrum (KBr, ν, cm^{-1}): 2962, 2871, 1688, 1603, 1463, 1415, 1381, 1330, 1270, 1245, 1222, 1199, 1172, 1126, 1085, 1024, 1002, 917, 922, 867, 815, 778, 748, 698, 665, 641, 590, 535, 506, 458, 427.

X-ray Crystal Structure Analysis. Crystals of **1** were grown from EtOH solution by slow evaporation at room temperature. Single crystals were transparent elongated prisms, $a = 7.7358(12)$, $b = 12.5461(15)$, $c = 27.1277(18)$ Å, $\beta = 90.464(7)°$, $V = 2643.9(6)$ Å^3, $\rho_{calc} = 1.041$ g/cm^3, space group *C*2, $Z = 4$. Unit-cell constants were determined and refined on an Xcalibur Ruby CCD diffractometer (Oxford diffraction) using Cu K_α-radiation (300 K, graphite monochromator). A three-dimensional data set of reflections was obtained on the same diffractometer. Absorption corrections were applied semi-empirically using the SADABS program [12].

The structure was solved by direct methods using the SHELXS-97 program set. The structure was refined using the SHELXL-97 program. All nonhydrogen atoms were refined by anisotropic full-matrix least-squares methods (over F^2). The positions of H atoms were found geometrically and refined with fixed isotropic thermal parameters $U_{iso} = nU_{eq}$, where n = 1.5 for methyls and 1.2 for others and U_{eq} is the equivalent isotropic thermal parameter of the corresponding C atom. During least-squares refinement it was noticed that the thermal parameters of the terminal isopropyl and methyl atoms (C-25–C-28) were very large. The difference electron-density synthesis showed additional peaks corresponding to their disordered positions. Further refinement of their occupancy factors using the FVAR instruction showed that these groups in the crystal were located in two positions in the ratio 0.55:0.45. The lengths of single Csp^3–Csp^3 bonds in this moiety were refined (DFIX instruction) with fixed lengths 1.54 Å. The final agreement factors (*R*) were 0.0555 for 3071 reflections [$I > 2\sigma(I)$] (w$R2 = 0.1529$) and 0.0689 over all 4136 reflections (w$R2 = 0.1701$). Results of the XSA were deposited in the Cambridge Crystallographic Data Centre (CCDC 791025).

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