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ANTI-AIDS AGENTS, 9.[1] SUBEROSOL, A NEW C_{31} LANOSTANE-TYPE TRITERPENE AND ANTI-HIV PRINCIPLE FROM *POLYALTHIA SUBEROSA*

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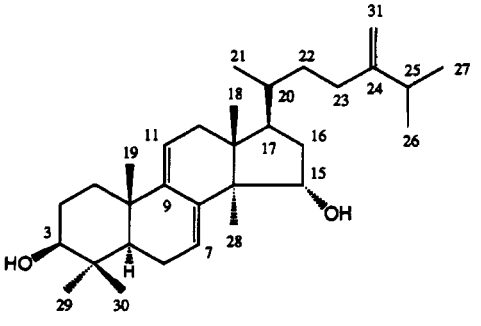
ABSTRACT.—A new C_{31} lanostane-type triterpene, assigned the trivial name suberosol [**1**], has been isolated from *Polyalthia suberosa* as an anti-HIV principle. The structure has been characterized as 24-methylenelanost-7,9(11)-diene-3β,15α-diol (suberosol) [**1**], based on spectroscopic evidence. Compound **1** was found to show anti-HIV replication activity in H9 lymphocyte cells with an EC_{50} of 3 μg/ml.

In the course of our continuing search for new anti-HIV agents from plant natural products, the EtOH extract of the stems and leaves of *Polyalthia suberosa* (Roxburgh) Thwaites (Annonaceae) was found to show significant anti-HIV activity. Subsequent bioactivity-directed fractionation and repeated chromatography on Si gel have led to the discovery of suberosol, a new triterpene, as an anti-HIV principle. We report here on the isolation and characterization of this compound and on its anti-HIV activity.

RESULTS AND DISCUSSION

Compound **1**, $[\alpha]^{20}D$ +107° ($CHCl_3$), was obtained as colorless needles from C_6H_6. The ir spectrum of **1** showed absorption bands at 3400 (hydroxyl) and 1640 (double bond) cm^{-1}. The uv spectrum revealed characteristic absorptions at 253, 243, and 237 nm, indicating the presence of a transoid heteroannular diene group in the molecule. In the eims, **1** gave a molecular ion peak at *m/z* 454, and its molecular formula was confirmed as $C_{31}H_{50}O_2$ by hrms. The ^{1}H-nmr spectrum exhibited the presence of five tertiary methyl groups [δ 0.62, 0.88, 0.95, 0.98, and 1.00 (each 3H, s)] and three secondary methyl groups [δ 0.90 (3H, d, J=7 Hz) and 1.02, 1.03 (each 3H, d, J=6.5



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[1]For Part 8, see Kilkuskie *et al.* (1).

Hz)], suggesting **1** to be a tetracyclic triterpene. The low-field region also showed two oxygen-bearing methine signals at δ 3.25 (1H, dd, J=4.5, 11.5 Hz) and 4.28 (1H, dd, J=6, 9.5 Hz), two one-proton singlets at δ 4.66 and 4.72, ascribable to an exomethylene group, and two olefinic signals at δ 5.31 (1H, d, J=6 Hz) and 5.85 (1H, d, J=6.5 Hz). The ^{13}C nmr exhibited thirty-one carbon resonances, showing the existence of eight methyl groups, two oxygen-bearing methine groups, two tri-substituted double bonds, and an exomethylene group. Taking into account the presence of a transoid heteroannular diene group in **1**, the comparison of the ^{13}C-nmr resonances with those of tetracyclic triterpenoids suggested that **1** was a $\Delta^{7,9(11)}$ lanostane-type triterpenoid. The ^{1}H-^{1}H COSY spectral examination of **1** established the proton connections of the following six segments: C-1–C-3, C-5–C-7, C-11–C-12, C-15–C-17, C-21–C-23, C-25–C-27. The assignments of the carbon resonances were achieved by the ^{1}H-^{13}C COSY spectroscopy of **1**, except for the quaternary carbons at δ 37.43, 38.67, 44.37, 51.98, 140.87, 146.14, and 156.55. Furthermore, the ^{1}H-^{13}C long-range COSY spectrum clearly indicated the correlations through a three- or a two-bond coupling, including those quaternary carbons with the tertiary methyl protons (Figure 1). These ^{1}H-^{13}C long-range correlations were consistent with the existence of a $\Delta^{7,9(11)}$ lanostane type skeleton in **1**.

The hydroxy-bearing methine proton signal at δ 3.24 in the ^{1}H-nmr spectrum was assigned to H-3, and the β configuration of the C-3 hydroxyl group was confirmed by its large coupling constant (dd, J=4.5, 11.5 Hz). The ^{1}H-^{1}H COSY spectrum, as well as the ^{1}H-^{13}C long-range correlation between 14-Me and the hydroxy-bearing methine carbon resonance at δ 74.71, indicated that the other hydroxyl group was present at C-15. Furthermore, observation of the nOe between 13-Me (δ 0.62) and H-15 (δ 4.27) confirmed the configuration of the hydroxyl group at C-15 to be α. The nOe was also observed between 13-Me and H-20 (δ 1.37); therefore the configuration of the side chain at C-17 is β. The position of the exomethylene group was established by the observation of the ^{1}H-^{13}C long-range correlation between the exomethylene proton signals [δ 4.66 and 4.72 (each s)] and the C-22 and C-25 carbon resonances (δ 31.21 and 33.81, respectively). Moreover, the ^{13}C resonances of **1** were in good accord with those of structurally related triterpenoids (2,3), which possess the same partial structure. On the basis of the spectroscopic evidence, the structure of **1** was characterized as 24-methylenelanost-7,9(11)-diene-3β,15α-diol (suberosol) [**1**]. The absolute configuration at C-20 still remains to be determined because of the small amount of the sample.

Compound **1** inhibited HIV replication in H9 lymphocyte cells with an EC_{50} value of 3 μg/ml, while it inhibited uninfected H9 cell growth with an IC_{50} value of 20 μg/ml.

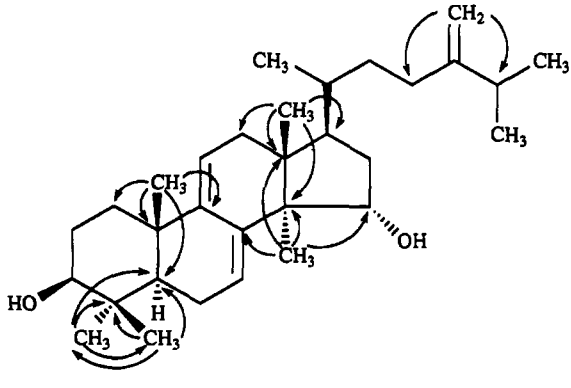

FIGURE 1. ^{1}H-^{13}C long-range correlations in suberosol [**1**].

Although a variety of C_{31} lanostanes have been isolated as metabolites of Basidiomycetes, such as *Polyporus* spp. (4–6), *Fomes* spp. (7–10), *Lentinus* spp. (9,10), *Daedalea* spp. (4,11), to our knowledge this is the first example of the isolation of a C_{31} lanostane from a dicotyledonous plant, and also the first report of a C_{31} lanostane possessing anti-HIV activity.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—The melting point was taken on a Fisher-Johns melting point apparatus and is uncorrected. ^{1}H-nmr, ^{13}C-nmr, and COSY spectra were obtained on Bruker AM-500 and AC-300 spectrometers in $CDCl_3$ with TMS as an internal standard. Eims and hreims measurements were carried out on a VG 70-250S mass spectrometer. The ir spectrum was run on a Perkin-Elmer 1320 spectrometer.

PLANT MATERIAL.—Stems and leaves of *Polyalthia suberosa* used in this investigation were from a collection made in September 1989 in Yunnan, China. A voucher specimen is kept in the Institute of Medicinal Plant Development, Chinese Academy of Medical Science, Beijing, China.

EXTRACTION AND ISOLATION OF SUBEROSOL.—The dried stems and leaves of *Polyalthia suberosa* (5 kg) were extracted with 95% EtOH at 50°. After removal of the solvent by evaporation, the residue (150 g) was dissolved in MeOH, mixed with Celite 545 (400 g), dried, and packed into a column. Successive elution with hexane (2.0 liters), $CHCl_3$ (2.5 liters), Me_2CO (1.5 liters), MeOH (2.0 liters), and 50% aqueous Me_2CO (1.5 liters) gave five fractions. The hexane-soluble fraction (22.9 g) was chromatographed on Si gel by stepwise elution with increasing percentages of EtOAc in hexane to furnish 10 fractions. The biologically active

TABLE 1. ^{1}H- and ^{13}C-nmr Resonances for Suberosol [**1**] (in $CDCl_3$).

Position	δ_C	δ_H
1	37.75	ca. 1.45, 2.0
2	27.79	ca. 1.7
3	78.88	3.25 (1H, dd, J=4.5, 11.5 Hz)
4	38.67	
5	48.98	1.10 (1H, dd, J=3.5, 12 Hz)
6	22.93	ca. 2.1
7	121.32	5.85 (1H, d, J=6 Hz)
8	140.87	
9	146.14	
10	37.43	
11	116.04	5.31 (1H, d, J=6 Hz)
12	38.52	2.06 (1H, dd, J=6, 17.5 Hz), 2.30 (1H, d, J=17.5 Hz)
13	44.37	
14	51.98	
15	74.71	4.28 (1H, dd, J=6, 9.5 Hz)
16	40.11	ca. 1.75, 1.95
17	48.87	ca. 1.65
18	15.92	0.62 (3H, s)
19	22.84	0.98 (3H, s)
20	35.99	1.37 (1H, m)
21	18.45	0.90 (3H, d, J=6.5 Hz)
22	34.89	1.14, 1.53 (each 1H, m)
23	31.21	1.89 (1H, ddd, J=5.5, 11, 14.5 Hz), ca. 2.1
24	156.55	
25	33.81	2.23 (1H, m)
26	21.98	1.02 (3H, d, J=7 Hz)
27	21.85	1.03 (3H, d, J=7 Hz)
28	17.14	0.95 (3H, s)
29	28.15	0.88 (3H, s)
30	15.79	1.00 (3H, s)
31	106.15	4.66, 4.72 (each 1H, s)

fraction 4 (590 mg) was further separated by preparative tlc (Si gel GF, 1 mm thick, Analtech Inc.) with $CHCl_3$-MeOH (24:1) (R_f 0.75) to yield suberosol [**1**] (17 mg).

Suberosol [**1**].—Colorless needles (C_6H_6): mp 179–182°; $[\alpha]^{20}D$ +107° (c=0.19, $CHCl_3$); uv λ max (MeOH) nm (log ε) 253 (3.91), 243 (4.03), 237 (3.98); ir ν max (KBr) cm^{-1} 3450 (OH), 1640 (diene); eims *m/z* $[M]^+$ 454, $[M-H_2O]^+$ 436, $[M-H_2O-Me]^+$ 421; hreims *m/z* $[M]^+$ 454.3845 ($C_{31}H_{50}O_2$, calcd 454.3811); ^1H and ^{13}C nmr ($CDCl_3$) see Table 1.

HIV GROWTH INHIBITION ASSAY.—HIV inhibition was measured as described previously (12,13). H9 lymphocytes (3.5×10^6 cells/ml) were incubated in the presence or absence of HIV-1 (HTLV-IIIB, 0.01–0.1 $TCID_{50}$/cell) for 1h at 37°. Cells were washed thoroughly to remove unadsorbed virions and resuspended at 4×10^5 cells/ml in culture medium. Aliquots (1 ml) were placed in wells of 24-well culture plates containing an equal volume of test compound (diluted in culture medium). After incubation for 3 days at 37°, cell density of uninfected cultures was determined by counting cells in the Coulter counter to assess toxicity of the test compound. A p24 antigen capture assay was used to determine the level of virus released in the medium of the HIV-infected cultures. The antigen capture assay uses a mouse monoclonal antibody as the capture antibody and rabbit serum specific for p24 as the detector antibody. In the culture medium, p24 was quantitated against a standard curve containing known amounts of p24. The effective (EC_{50}) and inhibitory (IC_{50}) concentrations (for anti-HIV activity and cytotoxicity, respectively) were estimated graphically. The percent inhibition (for infected and uninfected cultures) was plotted versus concentration and the 50% inhibition value read from the graph.

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