# 3α-Hinokiol with Antitumor Activity from *Juniperus Przewalskii*

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## Abstract

Bioassay-guided fractionation of a methanol extract of the seeds of Juniperus przewalskii Komarov led to the isolation of one diterpene (3α-hinokiol). The structure was determined by means of 1D and 2D NMR spectroscopy, resulting in complete and unambiguous 1H and 13C NMR chemical shift assignments. This compound was evaluated for antiproliferative activities, and was demonstrated to exert significant cell growth inhibitory activity against human ovarian cancer (HO-8910) cells.

**Keywords:** *Juniperus przewalskii* • Diterpene • Antitumour activity

## About the Study

*Juniperus przewalskii* Komarov Is a perennial tree distributed in Sichuan, Gansu, and Qinghai provinces of China. As an endemic and dominant species to the Qinghai-Tibet Plateau, *J. przewalskii* plays a key role in the maintenance of ecological balance in this region [1]. It belongs to the genus *Juniperus* in the family Cupressaceae, and the trees can grow to a height of up to 12 m. The lifespan of *J. przewalskii* can more than 3,000 years, and it grows on sunny side at an elevation gradient (about 2,500 m-4,000 m). The flowering period of *J. przewalskii* occurs relatively late (from June to July) and last for a relatively long time (15-25 d), and the trees begin to bear fruit at the age of approximately 15 years [2]. The essential oils of *J. przewalskii* are widely applied in food, medicine, and agriculture and forestry [3]. Addition, *J. przewalskii* is a Chinese and Tibetan medicine for the treatment of acute uterine bleeding, nephritis, and arthritis [4].

*J. przewalskii* is rich in essential oils, and these volatile compounds belong to phenols, terpenoids, and flavonoids [5]. They mainly include D-limonene, myrcene, terpinolene, citronellol, γ-terpineol, (-)-4-terpineol, α-juniperol, 4(10)-arborvitae, 13-hydroxy labda-8(17), 14-diene-19-ald, 4-hydroxyperoxide-13-hydroxy-19-norla-bda-8(17), 14-diene, 4-epi-hydroxyperoxide-13-hydroxy-19-norlabda-8(17), 14-diene, 19-acetoxy-13-hydroxylabda-8(17), 14-diene, α-pinene, 1-methyl-4-(1-methylethyl)-1, 4-cyclohexadiene, 4-methyl-1-(1-methylet-hyl)-3-cycohexen-1-ol, (s-(E, E))-1-methy-l-5-methylene-8-(1-methylethyl)-1, 6-cyclodecadiene, (1S-cis)-1, 2, 3, 5, 6, 8a-hexah-y dro-4, 7-dimethyl-1-(1-methylethyl)-naphthalene, 1R-(1. alpha., 3. alpha., 4.beta.)-4-ethenyl, alpha,alpha-4-trimethyl-3-(1-methylethenyl)-cyclohexanemethanol, cedrol, β-phellandrene, thujopsene, (+) -a-muurolene, 4-methylene-1-(1-methylethyl)-bicyclo (3. 1. 0) hexane, caryophyllene, biocyclo (3. 1. 0) hex-2-ene, 4-methyl-1-(1-methylethyl)-, limonene, 1, 4-cyclohexadiene, 1-methyl-4-(1-methylethyl)-, cyclohexe-ne, 1-methyl-4-(1-methylethylidene)-, 1, 6-octadien-3-ol, 3, 7-dimethyl-, 3-cyclohex-en-1-ol, 4-methyl-1-(1-methylethy)-, cyclohexane, 1-ethenyl-1-methyl-2, 4-bis (1-m-ethylethenyl)-, (1S-(1. alpha., 2. beta., 4. beta.))-, D1, 6-cyclodecadiene, 1-methyl-5-methylene-8-(1-methylethyl)-, (s-(E, E))-, naphthalene, 3α-hinokiol, propoxy-8-ced-rane, alpha-funebrene, trans-totarol, naphthalene, ferruginol, sugiol, etc. In these volatile oils, one diterpenoid compound (3α-hinokiol) was found that it had strong anti-tumor activity against human ovarian cancer (HO-8910) cell lines [6].

### Structure and bioassay

3α-hinokiol of the complete $^{1}H$ NMR and $^{13}C$ NMR determination assignments as listed in Table 1, and the chemical structure was exhibited in Figure 1. Cytotoxic effect was measured *in vitro* on the HO-8910 (human ovarian cancer) cell lines by using the MTT colorimetric assay, and vincristine was used as a positive control. 3α-hinokiol and vincristine demonstrated the similar activity with $IC_{50}$ values of 63.1 μg/mL and 67.4 μg/mL on the HO-8910 cell lines, respectively [6]. The results showed that 3α-hinokiol had obvious inhibitory effect on this tumor cells.

**Table 1.** $^{1}H$ NMR and $^{13}C$ NMR of 3a-hinokiol.

| Order | $^{1}H$ (m) | $^{13}C$ (DEPT) |
|---|---|---|
| 1 | 1.05 (ddd, 11.3, 8.0, 5.0) 1.93 (dt, 11.3, 4.0) | 31.6 $CH_2$ |
| 2 | 2.09 (m) 1.86 (m) | 25.9 $CH_2$ |
| 3 | 3.49 (t, 2.8) | 75.8 CH |
| 4 | – | 37.5 C |
| 5 | 1.74 (dd, 11.0, 4.0) | 43.6 CH |
| 6 | 1.77 (m) 1.77 (m) | 18.8 $CH_2$ |
| 7 | 2.78 (ddd, 12.8, 10.3, 4.0) 2.85 (ddd, 12.8, 5.6, 1.5) | 29.5 $CH_2$ |
| 8 | – | 127.2 C |
| 9 | – | 148.3 C |
| 10 | – | 37.7 C |
| 11 | 6.63 (s) | 110.9 CH |
| 12 | – | 150.7 C |
| 13 | – | 131.5 C |
| 14 | 6.83 (s) | 126.6 CH |
| 15 | 3.10 (qq, 8.0) | 26.8 CH |
| 16 | 1.23 (d, 8.0) | 22.5 $CH_3$ |
| 17 | 1.23 (d, 8.0) | 22.8 $CH_3$ |
| 18 | 0.95 (s) | 22.1 $CH_3$ |
| 19 | 1.03 (s) | 28.1 $CH_3$ |
| 20 | 1.19 (s) | 24.6 $CH_3$ |

**Note:** 40 MHz $^{1}H$ NMR, 100 MHz $^{13}C$ NMR ($CDCl_3$, TMS, W)

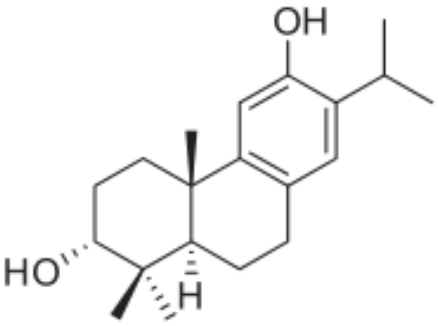


**Figure 1.** Structures of compound 3a-hinokiol.

## Conclusion

One diterpenoid was isolated from the seeds of *J. przewalskii* and its structure was identified by the spectroscopic data. Compound 3α-hinokiol showed *in vitro* cytotoxicity against human ovarian cancer (HO-8910) cell lines. This

detailed information is beneficial in the prevention and therapy of human tumors and other related diseases.

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