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Studies on the Terpenoids and Related Alicyclic Compounds. XXXVI.[1] Chemical and Microbiological Transformations of *l*-α-Santonin into 8-Epiartemisin

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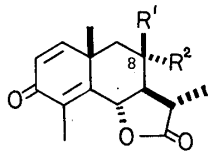
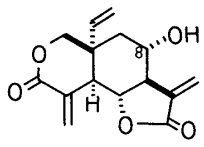
Introduction of a hydroxyl group at the C-8β position of *l*-α-santonin (**1**) was achieved by chemical and microbiological methods. The chemical transformation of **1** was accomplished *via* the 8β-hydroxy compound (**5b**) derived from 3,8-dioxoeudesm-1,4,6-triene (**4**). The enol acetate **7** of the 8β,12-olide (**6**) derived from **5b** was treated with hydrogen peroxide in formic acid, giving the 6α-hydroxy-8β,12-olide (**8**). Compound **8** was converted into 8-epiartemisin (**2b**) by hydrolysis of the ester group followed by lactonization.

The microbiological transformation of **1** into **2b** was performed by the use of *Aspergillus* sp. MIL 5024.

Keywords——*l*-α-santonin; 8-epiartemisin; hydroxylation; chemical transformation; microbiological oxidation; *Aspergillus* sp.

We have reported[2] the chemical transformation of *l*-α-santonin (**1**) into several related sesquiterpenoid lactones. There are various 8-hydroxy sesquiterpene lactones such as artemisin[3] (**2a**) and vernolepin[4] (**3**) having an 8α-hydroxyl group in eudesmane-type and elemane-type skeletons, respectively. For the purpose of introducing a hydroxyl group at the C-8 position of **1**, several microbiological methods have been examined. However, such attempts to achieve the transformation have not been successful.[5]





1 : $R^1=R^2=H$
2a: $R^1=H$, $R^2=OH$
2b: $R^1=OH$, $R^2=H$

3

Fig. 1

In this paper, the authors wish to report a chemical transformation (in Yamakawa's laboratory) and a microbiological transformation (in Iida's laboratory) of **1** into 8-epiartemisin (**2b**). These transformations involve the introduction of the hydroxyl group at the C-8β position of **1**.

Chemical Transformation of *l*-α-Santonin (1) into 8-epiartemisin (2b)

In the previous paper,[2g] we reported that an allylic oxidation of methyl 3-oxoeudesm-1,4,6-trienoate, which was derived starting from **1**, gave the corresponding 8-oxo compound (**4**). In order to prepare the 8α-hydroxy derivative (**5a**), **4** was reduced under various

conditions to afford an 8β-hydroxy compound, methyl 3-oxo-8β-hydroxyeudesm-4-enoate (**5b**), but not the 8α-hydroxy derivative (**5a**). Therefore, a transformation of **5b** into 8-epiartemisin (**2b**) was investigated.

Lactonization of **5b** gave 3-oxo-7α, 11α (*H*)-eudesm-4-en-8β,12-olide[2g] (**6**), which was treated with isopropenyl acetate in the presence of sulfuric acid to give an enol acetate (**7**) in good yield. Treatment of **7** with hydrogen peroxide in aqueous formic acid afforded a 53% yield of the 6-hydroxy enone (**8**) together with a 16% yield of the 3α,4α-epoxide (**9**).

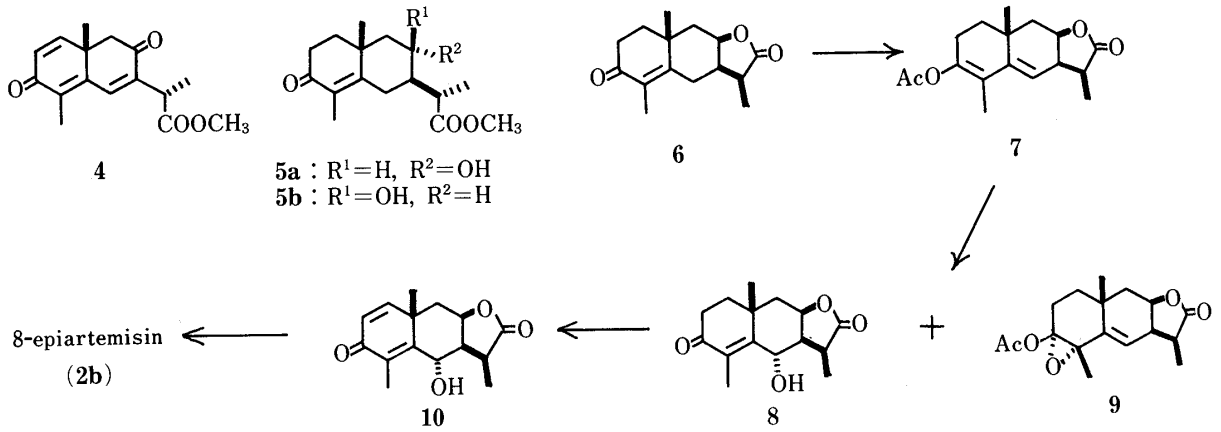


Chart 1

The structure of the enone (**8**) was determined from the infrared (IR) [ν 3510 (OH) and 1745, 1672 (CO) cm^{-1}] and the proton nuclear magnetic resonance (^{1}H-NMR) [2H, 6 and 8-H at δ 4.5—4.9] spectra. The high-resolution mass spectrum (MS) of the minor product (**9**) showed a molecular ion at *m/z* 306 corresponding to $C_{17}H_{22}O_5$. The IR (ν 1725 cm^{-1}) and the ^{1}H-NMR (3H, s, at δ 2.07) spectra showed the presence of an acetoxyl group. The ^{1}H-NMR spectrum exhibited an olefinic proton signal at δ 5.66 coupled with the C-7 proton (J= 4 Hz), and a singlet signal at δ 1.54 due to the C-4 methyl protons, suggesting the presence of a 3,4-epoxy-5-ene moiety. The configuration of the epoxy group was not determined, but the epoxidation reaction should occur at the less hindered α face of the C 3(4) double bond of **7**. Therefore the epoxide (**9**) was considered to have 3α,4α-configuration.

Dehydrogenation of **8** with 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ) in dioxane quantitatively yielded the dienone (**10**), which showed ultraviolet (UV) absorption at 240 nm (ε 9900), suggesting the presence of a cross-conjugated dienone system.[6] The configurations of the C-6 and C-8 protons were deduced from the ^{1}H-NMR spectrum: the C-6 proton signal appeared at δ 4.88 as a broad doublet (J= 10 Hz) and the C-8 proton signal at δ 4.57 as a multiplet ($W_{1/2}$ = 10 Hz), indicating them to be β and α, respectively.

Treatment of **10** with 3 N sodium hydroxide followed by hydrochloric acid afforded 8-epiartemisin (**2b**), mp 232—233 °C, in 44% yield together with the starting material. The signals assigned to the protons at C-1, C-2 and C-6, and to the methyl group at C-4 in the ^{1}H-NMR spcetrum of **2b** are similar to the corresponding resonances in the reported spectrum of (−)-artemisin[7] (**2a**) with regard to both chemical shifts and multiplicity. However, the singlet at δ 1.54 assigned to the C-10 methyl protons in the spectrum of **2b** was significantly different from the corresponding signal in the spectrum of **1** (δ 1.34)[7] or **2a** (δ 1.35).[7] This downfield shift can be rationalized in terms of the existence of the C-8β (axial) hydroxyl substituent.[8] The multiplicity of the C-8 proton signal (q, J= 3 Hz) at δ 4.35 in the spectrum of **2b** was different from that of the corresponding signal in the spectrum of **2a** (dt, J= 4.5, 10.5 Hz at δ 4.43 in pyridine). This suggested the presence of a β hydroxyl group at C-8 in **2b**. Compound **2b** was consequently concluded to be 8-epiartemisin.

Microbiological Transformation of *l*-α-Santonin (1) into 8-Epiartemisin (2b)

The chemical transformation described above required many steps and its yield was low. Therefore, we investigated a one-step transformation of **1** into (−)-artemisin (**2a**) by means of microbiological oxidation.

While some microbiological transformations of *l*-α-santonin (**1**) with the culture broths of microorganisms (*Cuninghamella blakesleena*,[5a] *Streptmyces aureofaciens*,[5a,c] *Aspergillus ochracens*,[5b] *Fusarium nivale*,[5b] *F. solani*[5b] and *S. roeochromogenes*,[5c,d]) have been reported, these microorganisms could not accomplished the selective hydroxylation of **1**. Among the various strains of fungi, we have screened, *Aspergillus* sp. MIL 5024 was found to be especially suitable for the introduction of the 8-hydroxy group.

The fermentation of **1** with the MIL 5024 at 27 °C for 8 d afforded several products, which were subjected to silica gel thin-layer chromatography (TLC). A transformed product (*Rf* 0.14) was isolated to afford colorless plates. The ^{1}H-NMR and IR spectra of the product were in good agreement with those of the synthetic **2b**.

Experimental

All melting points were measured with a Yanaco hot-stage micro melting point apparatus and are uncorrected. ^{1}H-NMR spectra were recorded on a JEOL FX-100 NMR spectrometer at 100 MHz. Spectra were recorded for solutions in $CDCl_3$ with tetramethylsilane (TMS) as an internal standard. IR spectra were run on a Hitachi 215 and a Hitachi-Perkin Elmer 225 grating spectrophotometers as KBr disks. UV spectra were obtained on a Hitachi 200-10 spectrophotometer using ethanol as a solvent. MS were recorded at 70 eV on a Hitachi RMU-7M double-focusing mass spectrometer (low-resolution) or a Hitachi M-80 double-focusing spectrometer (high-resolution) using a direct inlet system. Optical rotations were measured with a JASCO DIP-SL digital polarimeter using $CHCl_3$ as a solvent. Reverse-phase high performance liquid chromatography (HPLC) was performed by using a Hitachi 635A equipment with a Hitachi gel 3010 (2.6 mm i.d., 50 cm) column. Wako Silica gel C-200 (200 mesh) containing 2% fluorescence indicator F_{254} was used for column chromatography with a quartz column. Preparative TLC was carried out using Merck Silica gel 60 HF_{254}.

3-Acetoxy-7α,11α(*H*)-eudesm-3,5-dien-8β,12-olide (7)——A solution of 3-oxo-7α,11α(*H*)-eudesm-4-en-8β,12-olide[2g] (**6**, 220 mg) in isopropenyl acetate (5 ml) containing concd. H_2SO_4 (30 mg) was heated at 70 °C for 1.5 h. The isopropenyl acetate was removed under reduced pressure. The residue was purified on a silica gel column. Elution with hexane–ethyl acetate (5 : 1) afforded **7** (197 mg, 77%) as colorless plates, mp 152—154 °C (from hexane–ethyl acetate). High-resolution MS for $C_{17}H_{22}O_4$: M_r 290.1517. Observed: M^+ 290.1531. $[\alpha]_D^{23}$ −123.8° (c=0.46). UV λ_{max} (ε): 238 nm (21600). IR ν cm^{-1}: 1760, 1745, 1660, 1215, 1185. ^{1}H-NMR δ: 1.15 (3H, s, 10-CH_3), 1.28 (3H, d, J=7 Hz, 11-CH_3), 1.67 (3H, br s, 4-CH_3), 2.19 (3H, s, $OCOCH_3$), 4.80 (1H, q, J=3 Hz, 8-H), 5.41 (1H, d, J=3 Hz, 6-H). MS m/z (% rel. int.): 290 (M^+, 6), 248 (100), 192 (35). *Anal.* Calcd for $C_{17}H_{22}O_4$: C, 70.32, H, 7.64. Found: C, 70.25; H, 7.19.

Oxidation of the Enol Acetate (7)——A solution of 35% hydrogen peroxide (0.12 ml) was added to a solution of **7** (140 mg) in 80% formic acid (4 ml). The mixture was stirred at room temperature for 1.5 h, and then diluted with chilled water. The whole was neutralized with Na_2CO_3 and extracted with ethyl acetate. The extracts were washed with brine, dried and concentrated. The crude solid was recrystallized from hexane–ethyl acetate to give 3-oxo-6α-hydroxy-7α,11α(*H*)-eudesm-4-en-8β,12-olide (**8**) (55 mg, 43%) as colorless prisms, mp 185—186 °C. High-resolution MS for $C_{15}H_{20}O_4$: M_r 264.1360. Observed: M^+ 264.1360. $[\alpha]_D^{23}$ +14.7° (c=0.16). UV λ_{max} (ε): 248 nm (14700). IR ν cm^{-1}: 3510, 1745, 1672, 1630. ^{1}H-NMR δ: 1.30 (3H, s, 10-CH_3), 1.48 (3H, d, J=7 Hz, 11-CH_3), 2.06 (3H, d, J<2 Hz, 4-CH_3). 4.5—4.9 (2H, m, 6 and 8-H). MS m/z (% rel. int.): 264 (M^+, 12), 236 (8), 124 (100), 123 (68). *Anal.* Calcd for $C_{15}H_{20}O_4$: C, 68.16; H, 7.63. Found: C, 68.37; H, 7.58.

The residue from the mother liquor was subjected to reverse-phase HPLC using methanol as an eluant. The first fraction gave **8** (12 mg, 10%). The second fraction gave 3β-acetoxy-3α,4α-epoxy-7α,11α(*H*)-eudesm-5-en-8β,12-olide (**9**) (23 mg, 16%) as colorless plates, mp 170—172 °C (from hexane–ethyl acetate). High-resolution MS for $C_{17}H_{22}O_5$: M_r 306.1466. Observed: M^+ 306.1478. $[\alpha]_D^{23}$ +16.1° (c=0.23). UV λ_{max} (ε): 213 (2100), 291 nm (80). IR ν cm^{-1}: 1768, 1745, 1725, 1255. ^{1}H-NMR δ: 1.18 (3H, d, J=7 Hz, 11-CH_3), 1.32 (3H, s, 10-CH_3), 1.54 (3H, s, 4-CH_3), 2.07 (3H, s, $OCOCH_3$), 4.84 (1H, m, J=3 Hz, 8-H), 5.66 (1H, d, J=4 Hz, 6-H). MS m/z (% rel. int.): 306 (M^+, 3), 264 (62), 221 (100).

3-Oxo-6α-hydroxy-7α,11α(*H*)-eudesm-1,4-dien-8β,12-olide (10)——DDQ (80 mg) was added to a solution of **8** (55 mg) in dioxane (5 ml), and the mixture was heated to reflux for 27 h, then cooled, and the resulting precipitate was removed by filtration. The filtrate was concentrated and purified by preparative TLC with hexane–ethyl acetate (1 : 1) to afford **10** (52 mg, 95%) as colorless needles, mp 183—184 °C (from hexane–ethyl acetate). High-resolution MS for

$C_{15}H_{18}O_4$: M_r 262.1204. Observed: M^+ 262.1214. $[\alpha]_D^{23}$ −108.1° (c=0.31). UV λ_{max} (ε): 240 (9900), 263 nm (shoulder). IR ν cm^{-1}: 3480, 1765, 1655, 1610, 1600. ^{1}H-NMR :1.36 (3H, s, 10-CH_3), 1.50 (3H, d, J=7 Hz, 11-CH_3), 2.28 (3H, d, J<2 Hz, 4-CH_3), 4.57 (1H, m, $W_{1/2}$=10 Hz, 8-H), 4.88 (1H, br d, J=10 Hz, 6-H), 6.25 (1H, d, J=10 Hz, 2-H), 6.75 (1H, d, J=10 Hz, 1-H). MS m/z (% rel. int.): 262 (M^+, 16), 244 (8), 234 (17), 171 (26), 135 (100). *Anal.* Calcd for $C_{15}H_{18}O_4$: C, 68.68; H, 6.92. Found: C, 69.08; H, 7.02.

8-Epiartemisin (2b)——A solution of **10** (53 mg) in tetrahydrofuran (THF) (3 ml) containing 3 N NaOH (1.5 ml) was stirred at room temperature for 1 h. After addition of 3 N HCl (3 ml), the whole was stirred for 70 min and extracted with ethyl acetate. The extracts were washed with brine, dried and concentrated. The crude product was chromatographed on preparative TLC plates with hexane–ethyl acetate (2 : 3). A less polar band gave an oil (17 mg) which mainly consisted of the starting material (**10**) as evidenced by its ^{1}H-NMR spectrum. A polar band gave 8-epiartemisin (**2b**) (24 mg, 44%) as colorless columns mp 232—233 °C (from hexane–ethyl acetate). High-resolution MS for $C_{15}H_{18}O_4$: M_r 262.1204. Observed: M^+ 262.1205. $[\alpha]_D^{23}$ −231.8° (c=0.13). UV λ_{max} (ε): 241 (11400), 266 nm (soulder). IR ν cm^{-1}: 3360, 1780, 1660, 1625, 1608. ^{1}H-NMR δ: 1.29 (3H, d, J=7 Hz, 11-CH_3), 1.54 (3H, s, 10-CH_3), 2.17 (3H, d, J=2 Hz, 4-CH_3), 2.86 (1H, dq, J=12, 7 Hz, 11-H), 4.35 (1H, q, J=3 Hz, 8-H), 5.38 (1H, br d, J=12 Hz, 6-H), 6.22 (1H, d, J=10 Hz, 2-H), 6.70 (1H, d, J=10 Hz, 1-H). MS (% rel. int.): 262 (M^+, 67), 244 (15), 229 (22), 201 (28), 171 (100).

Fermentation of *l*-α-Santonin (1) with *Aspergillus* sp. MIL 5024——Strain MIL 5024 was grown in 5 l of the fermentation medium [composition (% w/v): glucose, 3; KH_2PO_4, 0.1; corn steep liquor, 1; $MgSO_4$, 0.05; $NaNO_3$, 0.2; K_2HPO_4, 0.2. pH, 6.0] in five 5 l Erlenmeyer flasks at 27 °C on a rotary shaker (220 rpm). After incubation 48 h, 50 mg of **1** dissolved in 5 ml of ethanol was added to each flask and the fermentation was continued for 8 d. The culture medium was filtered through double layers of gauze to remove the fungal mycelia. The filtrate was extracted three times with an equal volume of ethyl acetate, and the extracts were combined, dried and concentrated *in vacuo* to give 1.3 g of oily residue. The residue was chromatographed on a column of silica gel using first hexane and then ethyl acetate–hexane (3 : 2) as eluants. Fractions containing the products (43.2 mg) were subjected to TLC on silica gel (Merck, GF-254, 0.25 mm) with ethyl acetate–hexane (3 : 2). The compound of *Rf* 0.14 was eluted with $CHCl_3$–methanol (3 : 1). After removal of the solvent, the residue (12 mg) was crystallized from methanol to afford colorless plates (5.5 mg). The homogeneity of the sample was verified by gas-liquid chromatography. The physical and spectral properties of the crystals were in good agreement with those of 8-epiartemisin (**2b**) synthesized chemically as described above.

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