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15-ACETOXYCOSTUNOLIDE FROM *MAGNOLIA SIEBOLDII*

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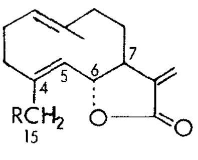
Key Word Index—*Magnolia sieboldii*; Magnoliaceae; sesquiterpenes; costunolide; 15-acetoxycostunolide.

Abstract—A new germacranolide isolated from *M. sieboldii* was shown to be 15-acetoxycostunolide by spectroscopic and chemical methods. ^{1}H NMR spin decoupling and NOE experiments in the presence of a lanthanide shift reagent were used for structure elucidation.

Many germacranolides (costunolide type) have been isolated from Magnoliaceae plants, e.g. parthenolide [1], lanuginolide [2], tulipinolide [3], costunolide, etc. We now wish to report a new germacranolide from this family.

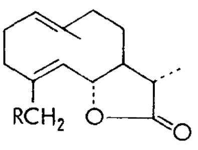
The ether extract of the root bark or the trunk bark of *M. seiboldii* K. Koch afforded two germacranolides, the major one of which was identified as costunolide (**1**). The second sesquiterpene lactone, 15-acetoxycostunolide (**2**), m.p. 100°, $C_{17}H_{22}O_4$ (M^+ 290), $[\alpha]_D^{24}$ +82.3°, was obtained as a crystalline substance which was labile even at 0°. Its IR spectrum ($CHCl_3$) contained bands assignable to a lactone (1760 cm^{-1}), an acetoxyl group (1736 cm^{-1}), and a double bond (1662 cm^{-1}). The ^{1}H NMR spectrum ($CDCl_3$) closely resembled that of costunolide **1**, except for the presence of signals due to an acetoxy methyl group in place of one of the methyl signals of **1**. Signals due to H-1, H-5, H-6, and H-15 were overlapping around δ 5.05–4.40 and were completely separated when a lanthanide shift reagent (0.4 mol equiv. Eu(fod)$_3$-d_{27}) was added to the solution: (100 MHz, $CDCl_3$) δ 2.03 (*d*, J = 1.0 Hz, H-14), 3.95 (*m*, H-7), 5.00 (OAc), 5.56 (*dm*, J = 11.0 Hz, H-1), 6.31 (*d(br)*, J = 10.0 Hz, H-5), 6.45 (*d*, J = 3.5 Hz, H-13c). Double irradiation at δ 2.03 8.5 Hz, H-6), 8.00 and 8.17 (AB*q*, J = 13.0, H-15), 8.48 (*d*, J = 3.5 Hz, H-13c). Double irradiation at δ 2.03 (vinyl methyl) caused the double multiplet signal at δ 5.56 (H-1) to change into a double doublet. Irradiation at δ 6.31 (H-5) changed the H-6 double doublet into a doublet and sharpened the H-15 signal (AB quartet). These observations implied that the acetoxyl group was bound to C-15, and that the lactone ring was situated at C-6 and C-7. The CD spectra (MeOH) of **2** ($[\theta]_{227}$ +69 700, $[\theta]_{263}$ −5520) and of **1** ($[\theta]_{222}$ +107 400, $[\theta]_{263}$ −7730) suggested that both compounds have the same absolute configuration around the lactone group and the same orientation of the two transannular double bonds.

Reduction of **2** with $NaBH_4$ afforded a dihydro compound (**3**) as a sole product: m.p. 127–130°, $C_{17}H_{24}O_4$ (M^+ 292). The configuration of the secondary methyl group was confirmed as being pseudo-equatorial by comparison of the solvent shift [4, 5] [Δδ ($CDCl_3$–C_6D_6): +0.17 ppm] with those of the known compounds dihydrocostunolide (**4**) (+0.20) and dihydroparthenolide (**5**) (+0.19). Again, the CD spectra of **3** ($[\theta]_{225}$ +88 300) and its desacetyl derivative (**6**) ($[\theta]_{223}$ +91 300) suggested that the transannular double bonds were cross-oriented [6], as in **4** ($[\theta]_{221}$ +93 200). When **3** was heated at 180° for 3 min, Cope rearrangement occurred to give **7**, the spectral features of which were very similar to those of saussurea lactone (**8**) [7] derived from **4**, suggesting that both Cope products **7** and **8** had the same configuration. The $J_{5,6}$ (12.0 Hz) and $J_{6,7}$ (9.0 Hz) values in **7**



1 R = H

2 R = OAc

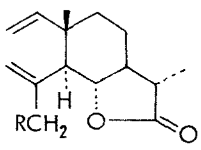


3 R = OAc

4 R = H

5 R = H (4α,5β epoxy)

6 R = OH



7 R = OAc

8 R = H

and the presence of nuclear Overhauser effects (NOEs) between the H-14 and H-6 signals (13%) indicated that the methyl group (C-14), H-5, H-6, and H-7 were *trans*-diaxially disposed.

The conformation of the 10-membered ring of **2** was determined by NOE experiments in $CDCl_3$ in the presence of a 0.4 mol equiv. Eu(fod)$_3$-d_{27} (Table 1). The vinyl methyl group (C-14), the acetoxymethyl group (C-15), and H-6 were all found to be situated above the plane of the 10-membered ring, and the two double bonds had a crossed orientation. This conformation was very similar to that determined for **1** [8].

Table 1. The results of NOE measurements on **2***

Irradiated signal	Observed signal	NOE (increases in integrated intensities, ±2% or less)† Eu(fod)$_3$-d_{27} 0 mol eq. (%)	0.4 mol eq. (%)
H-13*t*	H-13*c*	46	24
H-13*c*	H-13*t*	35	30
H-15	H-6	‡	18
H-14	H-6	10§	5
H-7	H-5	‡	10
H-5	H-7	‡	6

*For experimental procedures and the influence of the paramagnetic shift reagent on NOEs, see ref. [8].

†Only positive NOE values are recorded.

‡Undeterminable.

§Determined approximately by the signal height enhancement.

EXPERIMENTAL

Voucher specimens were deposited in the Herbarium of the Biological Institute, Tohoku University, Sendai Japan: TUS 39530–39549.

Isolation of costunolide **1** *and* 15-*acetoxycostunolide* (**2**). From the conc Et_2O extract of the root bark of *M. sieboldii* K. Koch (270 g), **1** (3.8 g, m.p. 102–105°) was precipitated on standing. After evaporation of Et_2O from the filtrate, the residual oil (16 g) was chromatographed on Si gel (150 g, deactivated with additional 10% H_2O). The eluate from the EtOAc–C_6H_6 (1 : 49) fractions was triturated with *iso*-PrOH to yield more of **1** (2.8 g; total 6.6 g, 2.6%). From the EtOAc–C_6H_6 (1 : 9) fractions, **2** (0.5 g, 0.2%) was obtained by trituration with *iso*-PrOH. **1** and **2** were very labile compounds, which deteriorated even in a fridge. Recrystallization of crude **2** from Et_2O–*iso*-PrOH gave a pure compound, m.p. 100°, $[\alpha]_D^{24}$ + 82.3° (MeOH, *c* 1.0). From the trunk bark (480 g), the yields of **1** and **2** were 11.9 g (2.5%) and 0.65 g (0.13%), respectively.

$NaBH_4$ *reduction of* **2**. To a soln of **2** (450 mg) in MeOH (30 ml), $NaBH_4$ (110 mg) was added at 0°. After stirring for 20 min, H_2O containing a small amount of NH_4Cl was added and the mixture extracted with Et_2O. Evaporation of the Et_2O gave a solid (420 mg, TLC 1 spot), which was crystallized from CH_2Cl_2–Et_2O to give pure 15-acetoxydihydrocostunolide (**3**), m.p. 127–130°, $[\alpha]_D^{22}$ +87.3° (MeOH, *c* 1.0). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 1766 (lactone), 1734 (OAc), 1662 (C=C); ^{1}H NMR (60 MHz, $CDCl_3$): δ 1.25 (*d*, J = 7.0 Hz, H-13), 1.37 (*d*, J = 1.5 Hz, H-14), 4.3–5.1 (*m*, H-1, H-5, H-6, and H-15). Since the ^{1}H NMR spectrum of the pure **3** and that of the crude **3** were superimposable, **3** must be the sole product.

Hydrolysis of **3**. **3** (125 mg) was dissolved in 15 ml 3% KOH in 80% MeOH–H_2O and stirred 30 min at room temp. Acidification and extraction with Et_2O afforded the product (110 mg), which on recrystallization from CH_2Cl_2–Et_2O gave **4** (70 mg), m.p. 138–141°, $[\alpha]_D^{24}$ + 80.2° (MeOH, *c* 1.0).

Cope rearrangement of **3**. **3** (146 mg) was heated in a pre-heated oil bath at 180° for 3 min. The reaction mixture was separated by prep. TLC plate to give the Cope product **7**, oil, 84 mg, $[\alpha]_D^{22}$ + 45.4° (MeOH, *c* 1.0). CD (MeOH), $[\theta]_{224}$ + 5450; IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 1770 (lactone), 1733 (OAc), 3090, 1647, 918 (C=C); ^{1}H NMR (60 MHz, $CDCl_3$): δ 1.11 (*s*, H-14), 1.23 (*d*, J = 7.0 Hz, H-13), 2.30 (*d*, J = 12.0 Hz, H-5), 4.15 (*dd*, $J_{6,5}$ = 12.0 Hz, $J_{6,7}$ = 9.0 Hz, H-6), 4.52 (*s*(*br*), H-15), 5.03, 5.38 (*s*(*br*), H-3), 4.97, 5.02, 5.83 (ABX, H-1 and H-2).

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