

A NEW REARRANGED LANOSTANOID, MARIESIIC ACID A,
FROM THE SEED OF ABIES MARIESII

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An antimicrobial triterpenoic acid, named mariesiic acid A, has been isolated from the seed of Abies mariesii. The structure with a rearranged lanostane-type skeleton has been elucidated by spectral and X-ray diffraction analyses.

Our examination of the seed extract of Abies mariesii Mast. (Pinaceae) led to the isolation of several new triterpenoic acids showing antimicrobial activity against some Gram-positive bacteria. The major substance of them, named mariesiic acid A (1a), mp 197-200 °C (fine needles from ethyl acetate), $[\alpha]_D^{+116}$ (c 1.73, acetone), has a molecular formula of $C_{30}H_{46}O_4$ [m/z 470.3411 (M^+ , calcd: 470.3398)] and shows IR (KBr) absorption bands at 2625, 1685, 1230 (COOH), 3400, 1055 (OH), 1640 (C=C), 980, 905, 800, and 750 cm^{-1} and a UV absorption maximum at 215.5 nm (ϵ 17700, EtOH).

On treatment with diazomethane, 1a gave a monomethyl ester (1b), which on acetylation afforded a diacetate (1c) indicating that 1a included one carboxylic and two hydroxyl groups. The 1H NMR spectrum (400 MHz) of 1b shows the presence of a secondary methyl [δ 0.94 (d, $J=7.0$ Hz)], a vinylic methyl [δ 1.87 (d, $J=1.4$ Hz)], and a methoxycarbonyl [δ 3.76 (s)] groups, five tertiary methyl groups [δ 0.86, 0.93, 0.94, 0.97, and 1.00 (each s)], two hydroxylmethine protons [δ 3.47 (br. s, $W_{1/2}=7.0$ Hz) and 4.57 (ddd, $J=2.6, 8.0, 10.5$ Hz)], and three olefinic protons [δ 5.18 (br. s, $W_{1/2}=7.0$ Hz), 5.57 (br. d, $J=4.2$ Hz), and 6.71 (dd, $J=1.4, 8.0$ Hz)].

The above spectral features and co-occurrence of abieslactone (2)^{1,2)} in the bark and leaves of the same plant suggest that mariesiic acid A is a dehydro derivative of (24E)-3 α ,23-dihydroxy-lanosta-24-en-26-oic acid or its methyl-rearranged isomers, and then its two double bonds in the ring are trisubstituted since the olefinic proton signals (δ 5.18 and 5.57) are not mutually coupled, which is confirmed by the 1H shift-correlated 2D-NMR spectrum of 1b.

Except for the absolute configuration, the gross structure of mariesiic acid A was established by X-ray diffraction analysis. The crystals were grown in an ethanol solution as colorless thick plates, mp 153-155 °C, solvated with ethanol. A small specimen of approximate dimensions 0.2 mm x 0.4 mm x 0.5 mm was cut from

the crystal and used for X-ray diffraction study. The specimen was coated with epoxy resin to prevent evaporation of solvent of crystallization.

The cell dimensions and intensity data were obtained from the measurement on a Philips PW1100 diffractometer using Cu K α radiation monochromated with graphite plate.

Crystal data of mariesiic acid A ethanol solvate, C₃₀H₄₆O₄·C₂H₅OH, are as follows: FW=516.8, monoclinic, space group P2₁, a=14.265(8), b=10.150(6), c=11.030(8) Å, β =98.21(5)°, V=1581 Å³. z=2, D_c=1.086 g cm⁻³, μ for Cu K α =5.3 cm⁻¹.

Intensities of 3203 reflections out of 3520 theoretically possible ones were measured as above the 2 σ (I) level in 2 θ range 6° through 156°.

The crystal structure was determined by the direct method based on MULTAN³⁾ procedure and refined by the block-diagonal least-squares method. The terminal methyl carbon atom of ethanol molecule was found to be disordered due to the rotation and distributed mainly to the two sites (shown in Fig. 1 by C2 and C2', bonded to O1-C1): the occupancy factor for each site was estimated to be 0.5. All 46 hydrogen atoms except those belonging to ethanol molecule were located on the difference electron-density map and refined with isotropic temperature factors. The final R value was 0.059.⁴⁾ The molecular structure is illustrated in Fig. 1 drawn by PLUTO⁵⁾ program. The solvent ethanol molecule is hydrogen bonded to O1 [O1(S)···O1 2.727(6) Å] and O2 [O1(S)···O2 2.691(7), O1(S)···HO2 1.79(6) Å]. Another intermolecular hydrogen bond to be of note is that between O1 and O3 [O1···O3 2.766(6), HO1···O3 1.79(7) Å].

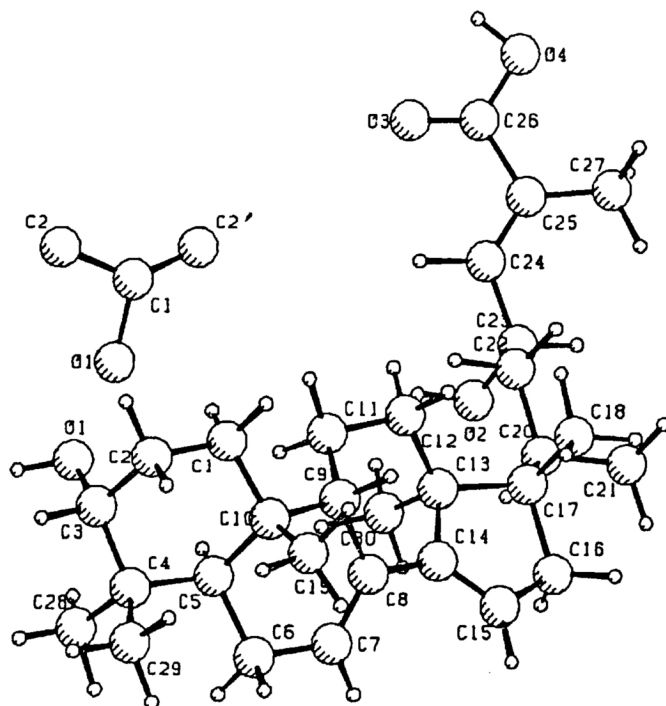
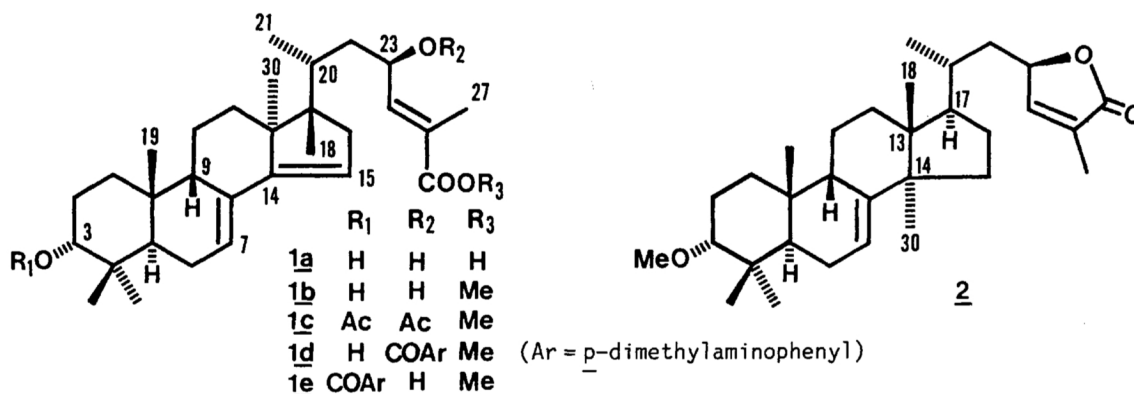


Fig. 1. Molecular structure of mariesiic acid A.



The absolute configuration was determined by the CD measurement for *p*-dimethylaminobenzoate (**1d**) of **1b** according to the exciton chirality method for allyl benzoate.⁶⁾ The benzoate **1d** was prepared by the following manner. On treatment with *p*-dimethylaminobenzoyl chloride in pyridine (105 °C, 2h), **1b** gave a chromatographically unseparable mixture of monobenzoates (**1d** and **1e**) and a dibenzoate. The desired benzoate, **1d**, was isolated from the mixture by the conversion of **1e** into its 23-oxo derivative with activated manganese dioxide followed by silica gel chromatography. [**1d**: a gum, $\lambda_{\max}$ (EtOH) 210 (ϵ 29000), 223 (20000), 311.5 nm (22000); ^1H NMR (CDCl_3): δ 7.86, 6.65 (each 2H, d, $J=10$ Hz, aromatic protons), 6.70 (1H, br. d, $J=9$ Hz, 24-H), 5.84 (1H, br. t, $J=9$ Hz, 23-H), 3.72 (3H, s, -COOMe), 3.48 (1H, br. s, 3-H), 3.04 (6H, s, -NMe₂)].

The coupling constant, 9 Hz, between 23-H and 24-H in the ^1H NMR spectrum of **1d** and a negative first Cotton effect at 307 nm ($\Delta\epsilon$ -4.75) in the CD spectrum suggested that the configuration of C-23 was R as shown in Fig. 2. Thus, the structure of mariesiic acid A is represented by formula **1a**.

Mariesiic acid A is a new triterpene having a methyl-rearranged skeleton of lanostane and might be produced biogenetically by enzymic dehydrogenation of 17-H of a lanostane-type skeleton followed by successive rearrangements of methyl groups at C-13 and C-14.

Upon antimicrobial test using paper disc method, mariesiic acid A indicated activity against Gram-positive bacteria and actinomycetes as shown in Table 1.

We acknowledge Mr. Hideaki Kondo, Taisho Pharmaceutical Co., LTD., for the measurement of the 2D-NMR spectrum of **1b**.

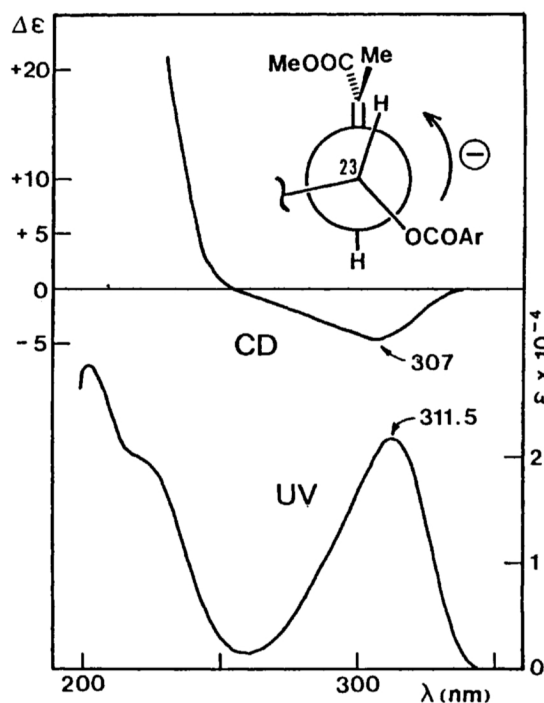


Fig. 2. CD and UV spectra of *p*-dimethylaminobenzoate (**1d**) of mariesiic acid A in EtOH.

Table 1. Antimicrobial spectra of mariesiic acid A⁷⁾

Test organism	Diameter of clear zone/mm		
	30 µg/disc	15 µg/disc	7.5 µg/disc
<i>Bacillus subtilis</i>	14	12	9
<i>Micrococcus luteus</i>	15	13	9
<i>Escherichia coli</i>	-	-	-
<i>Pseudomonas aeruginosa</i>	-	-	-
<i>Nocardia carollina</i>	11	10	9
<i>Sacchromyces cerevisiae</i>	-	-	-
<i>Candida utilis</i>	-	-	-

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