15-HYDROXYPINUSOLIDIC ACID , A NEW DITERPENE FROM THE PERICARP OF PLATYCLADUS ORIENTALIS FRANCO

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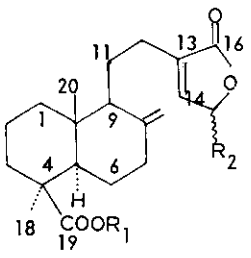
Abstract —— A new labdane-type diterpene, 15-hydroxypinusolidic acid, together with α-cedrol, β-sitosterol, and 5-hydroxy-7,4'-dimethoxyflavone was isolated from the pericarp of Platycladus orientalis . The structure of new diterpene has been elucidated by spectroscopic and chemical methods.

The studies of chemical constituents from Platycladus orientalis Franco (= Biota orientalis Endl.) are extensive. Sesquiterpenoids and diterpenoids from its heartwood,[1-5] flavonoids from its leaves ,[6-7] three labdane-type diterpenoids (15,16-bisnor- and 14,15,16-trisnor-labdane-type diterpenoids) from its seeds,[8] and two monolignol derivatives from its pollens [9] were reported by many chemists. In connection with our interests in labdane-type diterpenoids and this species as an important herb in chinese medicine (for hemostatic, expectorant, and cough remedy), we have investigated the terpenoid components of the pericarp of this species, from which we have isolated and identified four diterpenoids, pinusolide (1a) , trans-communic acid, isopimaric acid, and sandaracopimaric acid, and a monoterpene, platydiol.[10]

Now we have reinvestigated the acetone extract from the same source and did the detailed separation. A new pinusolidic acid derivative, 15-hydroxypinusolidic acid(1b), in addition to α-cedrol,[11] β-sitosterol ,[11] and 5-hydroxy-7,4'-dimethoxyflavone[12] was isolated, and this paper deals with the the structural elucidation of 15-hydroxypinusolidic acid(1b).

15-Hydroxypinusolidic acid(1b), mp 74-75°C, $[\alpha]_D^{25}$ + 30.5° (c 1.00 in $CHCl_3$), λ_{max}^{MeOH} 225 nm(log ε 4.21) , had the molecular formula $C_{20}H_{28}O_5$ on the basis of elemental analysis and its ir spectrum showed the presence of hydroxyl group(3419 cm^{-1}), carboxylic acid (3200-2600 and 1690 cm^{-1}), terminal methylene (3060, 1645 and 885 cm^{-1}), and butenolide(1775 cm^{-1}). The nmr spectrum of 15-hydroxypinusolidic acid (1b) exhibited signals for two methyl groups[^{1}H nmr($CDCl_3$) δ 0.58 and 1.22],two terminal olefinic protons [4.54 and 4.81(each 1H, br s)] , one hemiacetal lactone proton [6.06(d, J=1.4Hz)], and one conjugated olefinic proton [6.82(d, J=1.4Hz)].



1a R_1=Me, R_2=H
b R_1=H, R_2=OH
c R_1=H, R_2=H
d R_1=H, R_2=OAc
e R_1=Me, R_2=OAc

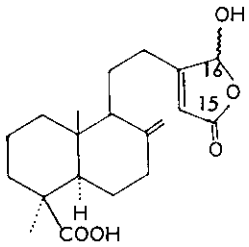


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Table 1. ^{13}C nmr data (δ -value) for(1b), (1c), and (2) (75 MHz, $CDCl_3$, TMS as internal standard)

C	1b	1c	2
1	39.1 t	39.1 t	39.2 t
2	19.8 t	19.8 t	21.0 t
3	38.5 t	38.6 t	38.6 t
4	44.1 s	44.2 s	44.5 s
5	56.2 d	56.2 d	56.6 d
6	25.9 t	25.9 t	26.7 t
7	37.8 t	37.8 t	37.8 t
8	147.2 s	147.3 s	147.2 s
9	55.6 d	55.6 d	56.3 d
10	40.5 s	40.4 s	41.1 s
11	21.7 t	21.8 t	21.1 t
12	24.4 t	28.9 t	26.8 t
13	136.6 s	134.8 s	170.2 s
14	142.9 d	143.9 d	117.1 d
15	96.6 d	70.1 t	170.2 s
16	175.0 s	174.4 s	99.3 d
17	106.6 t	106.8 t	106.8 t
18	28.9 q	28.9 q	28.9 q
19	183.4 s	183.8 s	183.1 s
20	12.7 q	12.7 q	12.7 q

Assignment established by DEPT method.

By comparison of the ^{1}H nmr spectra between 15-hydroxypinusolidic acid (1b) and pinusolidic acid (1c), [13-15] it suggested that 15-hydroxypinusolidic acid (1b) possessed of same carbon skeleton as pinusolidic acid (1c) with the addition of one hydroxyl group at C-15. The electron impact mass spectrum (EIMS) of (1b) exhibited the M^{+} peak at m/z (%) 348 (5) and peaks at 312 (25), 284 (44), 188 (32), 167 (17), 161 (34), and 121 (100) which further supported the structure.[14] The nmr spectra of (1b) is different from that of 16-hydroxylabda-8(17), 13-diene-15, 19-dioic acid butenolide (2), [16] a diterpenoid from Calocedrus formosana. The ^{13}C nmr data of (1b), (1c) and (2)[16] (Table 1) also confirmed the structure of (1b). The reaction of Ac_2O with (1b) in pyridine at room temperature overnight gave a monoacetate (1d) [mp 208-210°C; ^{1}H nmr ($CDCl_3$)δ 2.13 (3H, s)] and the signal of H-15 shifts downfield to δ6.85. The monoacetate (1d) subsequently reacted with diazomethane to yield methyl ester (1e) [^{1}H nmr ($CDCl_3$)δ 3.61 (3H, s)]. When (1b) was reduced by sodium borohydride in the presence of sodium hydroxide [17] and afforded a product (mp 130-132°C) which was identical with (1c) by the comparison of their physical data.

EXPERIMENTAL

Melting points were determined on a Yanagimoto micro melting point apparatus and are uncorrected. Optical rotations were measured with a JASCO DIP-4 digital polarimeter at room temperature. Ir spectra were recorded on a JASCO A-102 spectrometer. ^{1}H- And ^{13}C nmr spectra run on a Bruker AM 300 in $CDCl_3$ solution with tetramethylsilane (TMS) as internal standard. Chemical shifts are given in δ-values and coupling constants (J) are given in hertz (Hz). EI-MS and uv spectra were taken on a JEOL-JMS-100 and Hitachi RMS-4 spectrometer, respectively.

Extraction and Isolation

The air dried pericarps of Platycladus orientalis Franco (4.2 Kg) were extracted at room temperature with acetone (16 l) four times (three days for every time). The combined extracts were evaporated in vacuo to give brown viscous residue (170 g). Components were separated by column chromatography on silica gel and elution with hexane + ethyl acetate gradients. In addition to pinusolide (1a)(1g), trans-communic acid (50 mg), isopimaric acid (94.8 g), sandaracopimaric acid (4.7 g), and platidiol (53 mg), [10] four crystals, α-cedrol (10 g), β-sitosterol (200 mg), 5-hydroxy-7,4'-dimethoxyflavone (20 mg), and 15-hydroxypinusolidic acid (1b) (23 mg) were isolated.

15-Hydroxypinusolidic acid (1b): mp 74-75°C; ir(KBr)(ν_{cm}-1) 3419, 3200-2600, 3060, 1775, 1690, 1645, 1211, 1200, 1021, 991, 885, and 808; Anal. Calcd for $C_{20}H_{28}O_5$: C, 68.94; H, 8.10. Found C, 68.81; H, 8.02.

Acetylation of 15-hydroxypinusolidic Acid (1b)

A solution of 15-hydroxypinusolidic acid (1b) (10 mg) in pyridine (0.3 ml) and acetic anhydride (0.3 ml) was left overnight at room temperature. The reaction mixture was treated by the usual method and purified on silica gel chromatography (10 % EtOAc in hexane as eluent) to gave a monoacetate (1d) (mp 208-210°C) (from MeOH) (10 mg) [ir(KBr)($\nu_{cm^{-1}}$) 3200-2500, 1773, 1735, 1690, 1217, 1204, 1090, 1022, 989, 965, 797, and 739; ^{1}H nmr ($CDCl_3$)δ 0.59, 1.23, and 2.13 (each 3H, s), 4.54 and 4.88 (each 1H, br s), and 6.85 (2H, m, H-14, H-15)].

Methylation of Monoacetate (1d) with Diazomethane

Excess of diazomethane in ether was poured into a solution of monoacetate (1d) (8 mg) in 3 ml of methanol and was left to stand overnight. After purification on silica gel chromatography, it gave methyl ester (1e) (6 mg) [amorphous; ir(KBr)($\nu_{cm^{-1}}$) 3080, 1778, 1722, 1642, 1213, 1155, 1022, 992, and 886; ^{1}H nmr ($CDCl_3$)δ 0.51, 1.19, 2.15, and 3.61 (each 3H, s), 4.48 and 4.90 (each 1H, br s), and 6.87 (2H, m, H-14, H-15)].

Reduction of 15-hydroxypinusolidic Acid (1b)

NaOH (20 mg) was added to a solution of 15-hydroxypinusolidic acid (1b) (10 mg) in MeOH (5 ml). After stirring for 15 min, sodium borohydride (8 mg) was added and the mixture was refluxed for 1h under nitrogen. The mixture was cooled, acidified (pH 2) with 1N HCl and extracted with $CHCl_3$. The combined extracts were dried over Na_2SO_4, filtered, concentrated, and purified on silica gel (20 % EtOAc in hexane as eluent) to afford pinusolidic acid (1c) (6 mg) (mp 130-132°C).[15]

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