The Structure and Antibiotic Activities of Hydroxy Acid of Lanostenol Compound in *Daedalea dickinsii*

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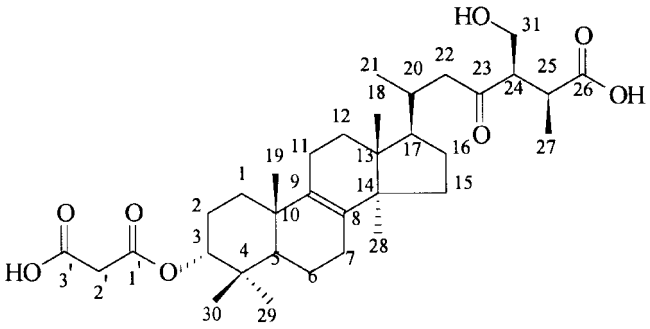
31-Hydroxycarboxyacetylquercinic acid, a lanostenoid hydroxy acid has been isolated from *D. dickinsii* by solvent extraction, silica gel column chromatography and recrystallization. The structure of this compound has been determined to be 31-hydroxycarboxyacetylquercinic acid by a combination of spectral data and by HMBC. This compound showed antimicrobial activities against human pathogenic fungi and bacteria.

Introduction

D. dickinsii belonging to Polyporaceae family is known as an annual red-root fungi. The triterpene acids in fungi were investigated mostly about G*anoderma licidum* and many hydroxy derivatives have been reported.[1-3] Trametoside, dehydrotumulosic acid, carbomethoxyacetylquercinic acid methylester, quercinic acid methylester, carbomethoxyacetylquercinic acid, carboxyacetylquercinic acid and polyporenic acid C as bioactive substances in this mushroom have been reported[4-6] but 31-hydroxycarboxyacetylquercinic acid was not reported.

In order to develop the bioactive substances, we purified the carboxyacetylquercinic acid from the fruit body of *D. dickinsii* as antibiotics and anti-tumour components. In this study, we report the method of isolation, structure elucidation and antimicrobial activities of 31-hydroxycarboxyacetylquercinic acid.



Results and Discussion

This compound was yellowish amorphous solid and deduced to be a derivative of triterpenoid or steroid by the results of coloring reactions showing positive reactions against sulfuric acid, I_2-vapor, anisaldehyde-sulfuric acid, ferric chloride, antimony chloride(III)-acetic acid, picric acid-perchloric acid, tungstophosphoric acid and α-naphthol-sulfuric acid reagent but negative reactions against ninhydrin and dragendroff's reagent. The compound showed strong absorption at 206 nm (ε=5,410) in its UV spectrum and infrared (IR) absorption bands were exhibited to be hydroxyl (3,406 cm^{-1}), carbonyls (1,734, 1,716) and double bond (1,680 cm^{-1}).

The proton nuclear magnetic resonance (^{1}H-NMR) spectrum at δ 3.45 and δ 3.52 due to a oxygenated methylene, δ 4.69 due to a oxygenated methine, δ 2.61 and δ 3.13 due to methine protons along with two sec-methyl and five *tert*-methyl signals. Thus, this compound estimated to be a derivative of lanostene containing the hydroxyl group. The ^{13}C-NMR spectrum showed 34 carbon signals which are corresponded to four carbonyl carbons, six quaternary carbons, six methine carbons, eleven methylene carbons and seven methyl carbons. Two quaternary carbon signals at δ 134.8 and δ 135.5 indicated to exist a double bond between C-8 and C-9. These physicochemical properties of this compound resembled that of 24S, 25S, 3α-carboxyacetylquercinic acid, which were purified and elucidated its structure in our laboratory.[7] However, it showed only one difference of a signal due to a hydroxymethylene group instead of a methyl group in carboxyacetylquercinic acid.

All of carbons and major protons were matched by the Heteronuclear Multiple Quantum Correlation (HMQC) experiments. The long-range C-H couplings were observed by Heteronuclear Multiple Bond Coherence (HMBC) experiments as shown in Figure 1 and 2. The carbon signals at δ 169.0, δ 46.1 and δ 37.5 showed correlations with methine proton at δ 4.69 (1H, dd, J = 2.6, 2.6) and the carbon signals at δ 46.1, δ 37.5 and δ 28.0 showed correlations with methyl protons at δ 0.91 (3H, s). From these results, it revealed connection of carboxyacetyl group at C-3. The carbon signals at δ 135.5, δ 46.1, δ 37.6 and δ 31.5 correlated with a proton at 0.95 (3H, s) and the carbon signals at δ 134.8, δ 50.5 and δ 45.4 correlated with a proton at δ 0.95 (3H, s). Thus, we defined the environments around C-8 and C-9. The carbon signals at δ 45.4 and δ 31.6 showed correlations with a proton at δ 0.78 (3H, s), the carbon signals at δ 51.0, δ 50.8, δ 50.4, δ 33.7 and δ 16.2 showed correlations with a proton at 0.94 (3H, d, J = 6.3) and the carbon signal at δ 39.7 showed correlations with a proton at δ 2.06 (1H, m), respectively. Thus, we defined the environments around C-18 and C-21.

The C-17 side chain was also determined by combined spectral methods. The binding position of hydroxymethylene group was determined by ^{1}H-^{1}H COSY experiments. H-27 (δ 1.28) was spin coupled with H-25 (δ 2.61), H-24 (δ 3.13) was spin coupled with H-25, two protons of H-31 (δ 3.45 and δ 3.52) long-range spin coupled with H-24. Thus,

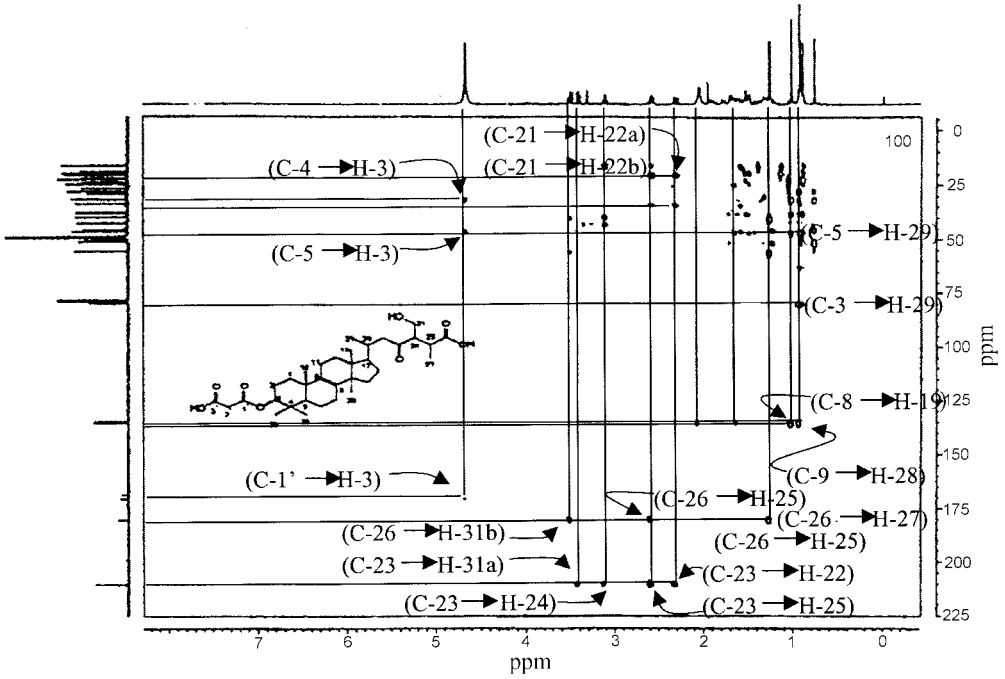


Figure 1. HMBC spectrum 31-hydroxycarboxyacetylquercinic acid purified from *Daedalea dickinsii* dissolved in a mixed solutions of $CDCl_3$ and CD_3 OD.

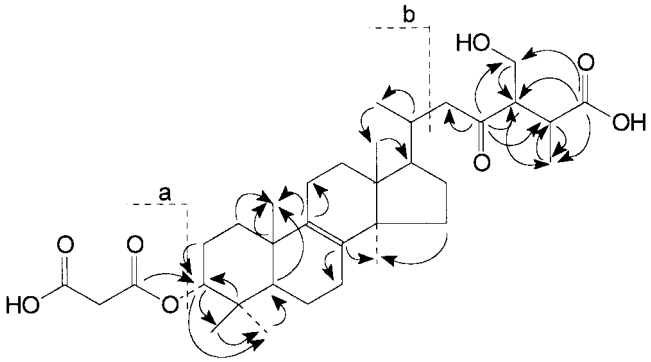


Figure 2. HMBC key correlations (CH) of 31-hydroxycarboxyacetylquercinic acid purified from *Daedalea dickinsii*.

the partial spin system was determined to be -CH(CH_3)-CH(CH_2OH)- and confirmed by HMBC experiments.

From these results, we confirmed that the structure of this compound was a 31-hydroxycarboxyacetylquercinic acid along with molecular modeling.

The mass spectrum gave the ions *m/z* 525, 465, 450, 354 and 309 represent succesive loses of M-H_2O-COOH, M-H_2O-a, M-H_2O-CH_3-a, M-H_2O-COOH-a and M-H_2O-CH_3-a-b from molecular ion *m/z* 588, so we reconfirmed above structure.

Chairul *et al.* (1990)[8] only reported this compound from Indonesian *Ganoderma* species and to have anti-tumour promoting activity. However, there is no informations available from other fungi and a living thing.

A biological activity of this compound as antibiotics was determined using the disk diffusion method[9] (Table 1). When we examined that it showed antimicrobial activities against *Candida albicans* causing candidasis, *Cyrptococcus neoformans* causing meningitis and *Microsporum canis* causing dermatophytosis as a fungi, *Bacillus licheniformis*, *Bacillus subtilis* causing pneumonitis and conjunctivitis, *Staphylococcus epidermidis* causing endocarditis, *Pseudomonas aeruginosa* causing septicemia and *Providencia rettigeri* causing urinary infection[10] as a bacteria.

Table 1. Antimicrobial activities of 31-hydroxycarboxyacetylquercinic acid purified from *Daedalea dickinsii*

Strain	Inhibition (mm)
Microsporium canis	12
Cyrptococcus neoformans	10
Candida albicans	12
Bacillus licheniformis	10
Bacillus subtilis	9
Staphylococcus epidermidis	12
Staphylococcus aureus	11
Pseudomonas aeruginosa	14
Serratia marcescens	10
Providencia rettigeri	12

Disc diameter: 8 mm, Loading sample: 500 μg/disk.

Experimental Section

General. NMR spectra were taken on a Varian Unit 500 (500 MHz) spectrometer in $CDCl_3$ and CD_3OD solutions, chemical shift values were recorded with respect to Me_4Si. Proton and carbon NMR were measured at 500 and 125 MHz, respectively. UV spectrum was taken on HP8452 spectrophotometer in ethanol. Optical roration was measured in ethanol on a JASCO DIP-4 automatic polarimeter at

20 °C. IR spectrum was recorded on a Bomem 100 spectrophotometer mixed with KBr. Mass were obtained VG 70-VSEQ (ionization voltage 70 eV) using a direct inlet system. Melting point was measured on a Melt Temp. II apparatus and is reported uncorrected. All solvents were used spectral grade or were distilled prior to use and media of sabouraud and meuller-hinton were purchased Difco co.

Collection and identification of mushroom. Materials of *D. dickinsii* were collected Taebaek mountain (Kangwon Do, Korea) in August, 1998. The genus of the mushroom was identified by Ji-Yul Lee (Institute for Genetic Engineering, Konkuk University, Korea).

Extraction and purification of 31-Hydroxycarboxyacetylquercinic acid. Airdried and powdered fruit body of *D. dickinsii* (1 Kg) was extracted with 16 L of 80% ethanol and vacuum evaporated under 40 °C. This crude solid extract was dissolved in 25% methanol and fractionated by *n*-hexane and butanol subsequently. The fraction of butanol was isolated by silica gel column chromatography (5 × 60 cm) with *n*-hexane : ethyl acetate : methanol : ammonia water (30 : 14 : 4 : 1, v/v). The fraction II of obtained six fractions was rechromatographied above same conditions and recrystallized with acetonitrile to yield 30 mg of 31-hydroxycarboxyacetylquercinic acid. The identification of purity were accomplished with one and two dimensional silica thin layer chromatography.

24R,25S,31-Hydroxycarboxyacetylquercinic acid. Pale yellow amorphous solid, mp 163-166 °C; UV (EtOH) λ_{max}: 206 nm (molar extinction coefficient: 5,410); $[\alpha]_D^{20} = -2.4$ (c 0.1); IR (KBr) cm^{-1}: 3,406, 3,199, 2,961, 2,942, 2,871, 1,734, 1,718, 1,680, 1,385, 1,145; EI-MS (70 eV) *m/z* (relative intensity): 525 (31), 510 (8), 465 (10), 450 (100), 354 (6), 328 (5), 309 (16), 227 (6), 187 (25), 173 (6), 121 (23); ^{13}C NMR ($CDCl_3$ + CD_3OD): 31.5 (C-1), 23.8 (C-2), 79.7 (C-3), 37.5 (C-4), 46.1 (C-5), 18.7 (C-6), 26.6 (C-7), 134.8 (C-8), 135.5 (C-9), 37.6 (C-10), 21.6 (C-11), 31.6 (C-12), 45.4 (C-13), 50.8 (C-14), 31.4 (C-15), 29.4 (C-16), 51.0 (C-17), 16.2 (C-18), 19.4 (C-19), 33.7 (C-20), 24.6 (C-21), 50.4 (C-22), 210.4 (C-23), 55.5 (C-24), 39.7 (C-25), 180.2 (C-26), 16.1 (C-27), 22.3 (C-28), 28.0 (C-29), 20.3 (C-30), 42.6 (C-31), 169.0 (C-1'), 45.4 (C-2'), 170.3 (C-3'); ^1H NMR ($CDCl_3$ + CD_3OD): 0.78 (H-18, 3H, s), 1.06 (H-19, 3H, s), 0.94 (H-21, d, J = 6.3), 1.27 (H-27, 3H, d, J = 7.2) 0.95 (H-28, 3H, s), 0.91 (H-29, 3H, s), 0.95 (H-30, 3H, s), 3.45 (H-31_a, 1H, dd, J = 9.9, 7.3), 3.52 (H-31_b, 1H, dd, J = 9.9, 8.8), 4.69 (H-3, dd, J = 2.6, 2.6), 1.58 (H-17, 1H, m), 2.06 (H-20, 1H, m), 3.13 (H-24, 1H, dq, J = 8.6, 7.3), 2.61 (H-25, 1H, dt, J = 8.6, 7.2), 3.34 (H-2', 2H, s).

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