Revised Structures of Flavidulols, Constituents of *Lactarius flavidulus* IMAI, and the Structure of Flavidulol D

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The structure 1a proposed for flavidulol A, which was isolated from the mushroom *Lactarius flavidulus* IMAI, was revised to 1b based upon comparison with a synthetic specimen, as well as a difference nuclear Overhauser effect (NOE) experiment. As a consequence, the structures of flavidulols B (2a) and C (3a) were also revised to 2b and 3b, respectively. Flavidulol D (4) was newly isolated from the same mushroom, and its structure was elucidated as flavidulol A stearate on the basis of spectroscopic data.

Keywords flavidulol; *Lactarius flavidulus*; mushroom; Basidiomycete

Flavidulol A was isolated as an antifungal metabolite from the mushroom (Basidiomycete) *Lactarius flavidulus* IMAI in 1988, and the structure **1a** containing two olefinic linkages with *E,E*-assignment was given to this compound on the basis of spectroscopic data.[2] Recently, we have received a personal communication from Dr. Arigoni concerning the structure of flavidulol A, in which a *Z, E* assignment was suggested for the two olefinic linkages in flavidulol A, judging from the stability of a synthetic specimen. In this note, we wish to describe the structure revision of flavidulols A, B and C as well as the structural elucidation of flavidulol D newly isolated from the same mushroom.

Flavidulols A, B and C were re-isolated from the fruiting bodies of *L. flavidulus* IMAI after chromatographies of the *n*-hexane–EtOAc extract. Their physico-chemical and spectral data were unequivocally identical with the precious ones. During this isolation, we have also isolated a related compound, named flavidulol D.

The structural re-invesigation of flavidulol A was primarily performed by means of nuclear Overhauser effect (NOE) difference spectroscopy. Namely, irradiation of the methyl proton signals (H-9) at δ 1.60 induced 10% NOE on the olefinic proton signal (H-3) at δ 5.13, which was not observed in the previous experiment, because we observed NOE only for H-9 on irradiation at δ 5.13 (H-3).[2] No enhancement of the methyl proton signal (H-10) was recognized on irradiating the olefinic proton signal (H-7). These findings, therefore, indicated that flavidulol A contains two olefinic linkages with 2*Z*, 6*E* configurations, not the previously assigned 2*E*, 6*E*. Furthermore, the ^{1}H-NMR spectrum of natural flavidulol A methyl ether was identical with that of the synthetic specimen.[3] Thus, the structure of flavidulol A (**1a**) was revised to **1b**, as shown in Chart 1.

The structures of flavidulols B (**2a**) and C (**3a**) were also revised to **2b** and **3b**, respectively, because the chemical correlation among A, B and C has already been established.[2]

Wigandol is a structurally related compound isolated from *Wigandia kunthii* CHOISY in 1980 and its structure has been reported to be **6a** containing two olefinic linkages with *E, E* configurations, as indicated from a Cope rearrangement product.[4] In view of its spectral similarity to **1b** and the acetate (**5**) as well as the results of Takeda *et al.*,[5] the structure may also have to be revised to **6b**.

Flavidulol D (**4**) was obtained as a waxy colorless solid with mp 40—41 °C. The molecular formula, $C_{35}H_{56}O_3$, of **4** was established by high-resolution electron impact (EI) MS exhibiting a molecular ion peak at *m/z* 524.4235 (M^+).

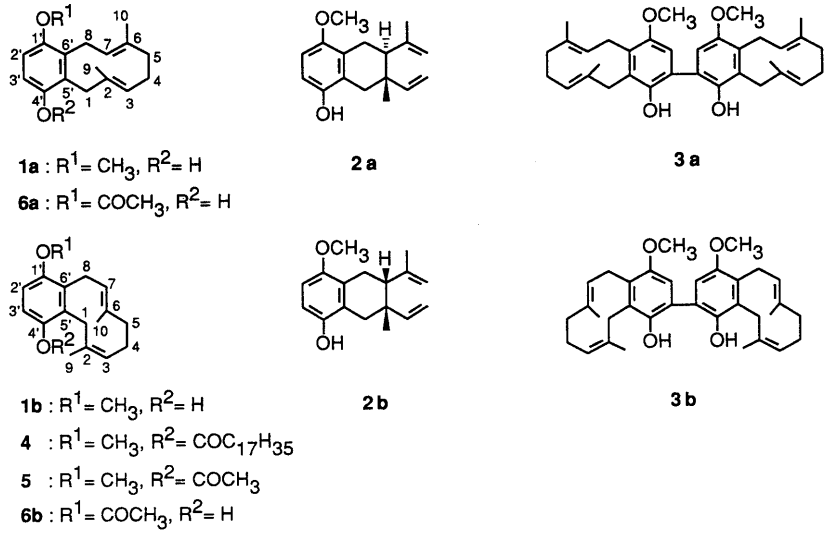

Chart 1

The IR spectrum of **4** was indicative of the presence of an ester carbonyl group (1720 cm^{-1}) and a benzene ring (1580 cm^{-1}). The ^{1}H-NMR spectrum of **4** closely paralleled that of flavidulol A acetate (**5**) except that the acetoxyl methyl signal at δ 2.27 was replaced by signals [δ 0.88 (3H, t, J=6.8), 1.26 (28H, br s), 1.74 (2H, quintet, J=7.3), 2.54 (2H, t, J=7.3)] assignable to a stearoyl group. Further, this assignment was supported by the EIMS fragment at m/z 258 corresponding to the loss of that group. The structure of flavidulol D was, thus, concluded to be **4** as shown in Chart 1.

Experimental

Melting points were determined on a Yanagimoto micro hot plate and are uncorrected. The spectroscopic data were measured with the following instruments: IR spectra, JASCO A-100S. Mass spectra, JEOL JMX DX-303. ^{1}H-NMR spectra, JEOL JNM GX-500. The ^{1}H-NMR spectra were recorded with tetramethylsilane (TMS) as an internal standard. Chemical shifts are shown in δ (ppm) and multiplicities are given as follows: singlet=s, doublet=d, triplet=t, multiplet=m, and broad=br. Coupling constants (J) are shown in hertz (Hz). TLC analyses were performed on Kieselgel 60 F_{254} (Merck) and spots were detected under UV irradiation and by heating on a hot plate after spraying with Ehrlich reagent.

Isolation Procedure The fruiting bodies (1.5 kg) of *L. flavidulus* IMAI, collected in Miyagi prefecture in September 1991, were extracted twice with *n*-hexane–EtOAc (1:1, 1.5 l) at room temperature. The combined extracts were washed with water, dried over anhydrous Na_2SO_4 and concentrated *in vacuo* to give a gummy syrup (8.9 g). A portion of the extract (7.0 g) was fractionated by column chromatography on silica gel (70 g: 3.5 cm i.d. × 24 cm) with *n*-hexane–EtOAc and $CHCl_3$–EtOAc as the eluants, and then the fractions containing flavidulols were re-chromatographed on silica gel to afford flavidulols A (**1**, 1.83 g), B (**2**, 25.7 mg), C (**3**, 520.0 mg), and D (**4**, 230.1 mg). The MS, IR and ^{1}H-NMR spectra of flavidulols A—C were in good agreement with those of authentic specimens.

Properties of Flavidulol D (4) *Rf* 0.47 (*n*-hexane : $CHCl_3$ = 1 : 1). EIMS m/z: 524 (M^+), 258, 243. High-resolution EIMS m/z: 524.4235 (M^+). Calcd for $C_{35}H_{56}O_3$: 524.4230. IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 2925, 1850, 1740 (ester C=O), 1580, 1460. ^{1}H-NMR ($CHCl_3$) δ: 0.88 (3H, t, J=6.8, $CH_3(CH_2)_{16}$), 1.26 (28H, br s, $CH_3(CH_2)_{14}CH_2$), 1.39 (1H, m, H-5), 1.55 (3H, s, H-9), 1.74 (2H, quintet, J=7.3, CH_2CH_2COO), 1.76 (3H, s, H-10), 1.99 (2H, m, H-4), 2.14 (1H, m, H-5), 2.54 (2H, t, J=7.3, CH_2COO), 2.89 (1H, d, J=14.2, H-1), 3.24 (1H, dd, J=17.1, 11.7, H-8), 3.35 (1H, d, J=14.2, H-1), 3.81 (3H, s, OCH_3), 3.92 (1H, br d, J=17.1, H-8), 5.11 (1H, dt, J=7.8, 1.0, H-3), 5.23 (1H, dd, J=11.7, 2.0, H-7), 6.76 (1H, d, J=8.8, H-2′), 6.86 (1H, d, J=8.8, H-3′).

Differential NOE Data for Flavidulol A NOE data for a 0.065 M solution of flavidulol A were taken at 23 °C, with 32 transients including 4.3 sec pulse separation. Acquisition time was 2.73 s. Observed NOE: 5% for H-9 (δ 1.60) on irradiation at δ 5.13 (H-3); 10% for H-3 on irradiation at H-9.

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References and Notes

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