Note

Labdane-type Diterpene Dialdehyde, Pungent Principle of Myoga, Zingiber mioga Roscoe

Masako Abe,1 Yoshio Ozawa,1,† Yasushi Uda,2 Yoichi Yamada,3 Yasujiro Morimitsu,4 Yoshimasa Nakamura,5 and Toshihiko Osa wa5

1Department of Health and Nutrition, Takasaki University of Health and Welfare, Takasaki 370-0033, Japan
2Department of Bioproductive Sciences, Utsunomiya University, Utsunomiya 321-8505, Japan
3Department of Chemistry, Faculty of Education, Utsunomiya University, Utsunomiya 321-8505, Japan
4Laboratory of Food Chemistry, Ochanomizu University, 2-1-1 Ohtsuka, Bunkyo-ku, Tokyo 112-8610, Japan
5Laboratory of Food and Biodynamics, Nagoya University Graduate School of Bioagricultural Sciences, Nagoya 464-8601, Japan

Received May 21, 2002; Accepted August 23, 2002

The pungent principle of myoga (Zingiber mioga Roscoe) was identified as (E)-8β(17)-epoxylabd-12-ene-15,16-dial (miogadial) on the basis of its physical and spectroscopic properties (MS, NMR, IR, and UV). Galanal A and B, isolated as well as miogadial, had no hot taste. Reduced miogadial also was tasteless. The pungency of miogadial depended on the presence of αβ-unsaturated-1,4-dialdehyde group.

Key words: Zingiber mioga Roscoe; pungent principle; labdane-type diterpene; miogadial

Myoga (Zingiber mioga Roscoe) is a perennial herb with pungent aromatic flower buds native to Eastern Asia. In Japan, the flower buds of myoga have usually been eaten as spice or pickles from ancient times, and are now being consumed in amounts exceeding 6,000 tons per year. The volatile constituents of fresh flower buds of myoga have been studied; 2-isopropyl-3-methoxy pyrazine, 2-sec-butyl-3-methoxy pyrazine, and 2-isobutyl-3-methoxy pyrazine were found to be the aroma compounds by GC-MS.1 Some of the biological activities of pungent principles occurring in the Zingiberaceae, such as ginger2,3 and alpinia galanga4,5 have been studied in recent years, but structural analysis of the pungent principle of myoga remains to be done. We report here the identification of the main pungent principle of flower buds of myoga.

TLC (Whatman K6) was done with ethyl acetate-benzene-chloroform (1:4:6). HPLC was done with a Shimadzu LC-10A instrument equipped with a TOSOH silica-60 column (7.8 × 300 mm) and a UV detector (233 nm). UV spectra were recorded with a Shimadzu UV-160 spectrophotometer. Melting points were measured on a Mitamura Riken melting-point apparatus. IR spectra were recorded with a JASCO FTIR-420 spectrophotometer in CHCl3 on NaCl. 1H (400 MHz) and 13C (100 MHz)-NMR spectra were obtained with a JEOL EX-400 NMR apparatus and Bruker ARX-400 spectrometer in CDCl3 with tetramethylsilane as the internal standard. MS were recorded with a JEOL JMS 700 (MStation) mass spectrometer.

Sensory evaluation of isolated compounds was done as follows. Paper discs 0.5 cm in diameter were soaked in an ethanol solution with different amounts of each sample (1 nmol being the smallest), air dried, and then placed on the tip of the tongue of five panelists. The amount was increased until pungency was perceived. Informal sensory screening was done through isolation and identification procedures.

Fresh flower buds of myoga were purchased at local market. Six hundred grams of fresh flower buds was sliced and pulped with ethyl acetate in a blender. After the mixture was left for 3 h at room temperature, the solvent was removed under reduced pressure to give a brown syrup (385 mg). The residue was fractionated into fractions 1 (53.3 mg), 2 (143.5 mg), and 3 (52.0 mg) by flash chromatography (silica gel, 2 × 40 cm) with ethyl acetate-benzene-chloroform (1:4:6). Fraction 2 was most pungent. TLC of the fractions showed that the pungent principles of myoga were in fraction 2 and 3. Both fractions were separated by HPLC (mobile phase, 2.5% and 5% acetone in hexane; flow rate, 4.0 ml/min) to give C-1 (134.0 mg, tR = 17.7 min, 5% acetone in hexane), C-2 (15.8 mg, tR = 36.8 min, 5% acetone in hexane) and C-3 (17.4 mg, tR = 49.5 min, 5% acetone in hexane). The purity of the compounds was measured by TLC and HPLC. Sensory evaluation of these compounds showed that C-1 was responsible for the hot taste of myoga. C-2 and C-3 had no hot taste, but weakly stimulated the throat. These compounds were ana-

† To whom correspondence should be addressed. Fax: +81-27-353-2055; E-mail: yozawa@takasaki-u.ac.jp
C-1: Colorless needles; m.p. 89-90°C; HRMS m/z (M+): calcld. for C_{60}H_{34}O_{4}, 318.2193; found, 318.2152; EI-MS m/z (%): 318 (10, M+), 303 (18), 289 (12), 179 (42), 137 (55), 123 (46), 109 (50), 95 (75), 81 (81), 69 (100); IR ν_{max} (CHCl_{3}, on NaCl) cm⁻¹: 1728 (C=O), 1683 (C=O), 1638 (C=C); UV_{max} λ(μm) (ε): 233 (15200); ¹H-NMR (CDCl₃) δ: 0.88 (3H, s, 19-H), 0.92 (3H, s, 18-H), 0.94 (3H, s, H-20), 2.32 (1H, d, J=4.8 Hz, 17-H), 2.42 (1H, d, J=4.8 Hz, 17-H), 3.37 (1H, br, 14-H), 3.44 (1H, br, 14-H), 6.68 (1H, t, J=6.6 Hz, 12-H), 9.41 (1H, s, 15-H), 9.65 (1H, s, 16-H); ¹³C-NMR δ: 39.4 (C-1), 18.4 (C-2), 41.8 (C-3), 33.5 (C-4), 52.7 (C-5), 20.0 (C-6), 39.3 (C-7), 57.5 (C-8), 55.2 (C-9), 39.8 (C-10), 22.3 (C-11), 160.3 (C-12), 135.1 (C-13), 35.8 (C-14), 196.8 (C-15), 193.2 (C-16), 48.4 (C-17), 33.6 (C-18), 21.6 (C-19), 14.8 (C-20).

These data were completely coincident with those of (E)-8β (17)-epoxylabd-12-ene-15,16-diol, isolated from seeds of the plant Afromomum danielli (Zingiberaceae) growing in many regions of Cameroon. Thus, C-1 was isolated for the first time from the fresh flower buds of myoga as the principal principle of myoga, and the compound is named miogadial here. Miogadial has antifungal activity, and inhibits cholesterol biosynthesis and human platelet aggregation.²⁻⁹

**Fig. 1. Chemical Structures of C-1, -2, -3, and -4.**
The taste of miogadiol was compared with that of the structurally related compound polygodial and the reduction product, C-4. The pungency of miogadiol was less than that of polygodial, and the reduction product, C-4 which had a diol group instead of the corresponding dialdial structure, had no hot taste (Table 1). As mentioned above, both galanal A and galanal B, which have no Ω-d-unsaturated 1,4-dialdehyde group, had no pungent taste. There have been studies of the structure-activity relationship of dialdehyde terpenes for their pungent sensation on the human tongue. For example, polygodial, warburganal, isovelleral, isovelleral, cinnamodial, and isocopalendial, have each with an unsaturated 1,4-dialdehyde group, have a hot taste on the human tongue, but drimenol and cinnamomolide, which lack the unsaturated 1,4-dialdehyde group, have no hot taste.\(^{12,13}\) In this study, we confirmed that an Ω-d-unsaturated 1,4-dialdehyde group is essential for the pungent taste of miogadiol.

### References