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AMIDES OF THE FRUIT OF *ZANTHOXYLUM* SPP.

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Key Word Index—*Zanthoxylum* spp.; Rutaceae; Budo-Zanthoxylum fruit; unsaturated aliphatic acid amide; HPLC.

Abstract—Examination of the amide constituents in Budo-Zanthoxylum fruit, the most traded commercial Zanthoxylum fruit in the Japanese market, has led to the isolation of a new amide, along with α-, β-, γ-, hydroxy-α-, hydroxy-β- and hydroxy-γ-sanshools. The structure of the new amide was assigned as (2*E*, 4*E*, 8*E*, 10*E*, 12*E*)-*N*-isobutyl-2,4,8,10,12-tetradecapentaenamide by spectral examination. Copyright © 1997 Elsevier Science Ltd

INTRODUCTION

Zanthoxylum fruit, derived from mature pericarps of *Zanthoxylum* spp., have been used as an aromatic stomachic as well as a spice in Japan. There are several kinds of commercial Zanthoxylum fruit on the Japanese market. Among them, the fruit of *Z. piperitum* f. *inerme* is very popular [1]. However, the most traded one currently in the Japanese market is Budo-Zanthoxylum fruit (Japanese name: Budo-Zansho) (Ito, C. *et al.*, unpublished data). Budo-Zanthoxylum fruits are cultivated in a limited area in the Wakayama Prefecture, Japan [2, 3], and the origin of this fruit is not clear [2, 3]. Anatomical studies [4] and chemical examination of the essential oil [2] of Budo-Zanthoxylum fruit have been reported recently, whereas the amide constituents, which include pungent principles such as α-shanshool and hydroxy-α-shanshool have not been investigated in spite of the fact that the quality of Zanthoxylum fruit is evaluated for their fragrance and pungent taste. To clarify the amide constituents in Budo-Zanthoxylum fruit, we have examined the amides of this fruit; this has resulted in the isolation of a new amide (**7**), together with six known amides (**1**–**6**).

RESULTS AND DISCUSSION

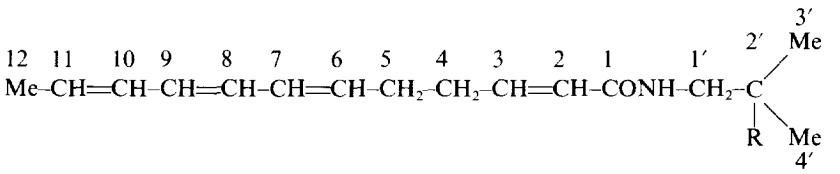
Preliminary HPLC examination of a chloroform extract of Budo-Zanthoxylum fruit according to the methods reported by Yasuda *et al.* [5] showed that it gave a similar chromatogram to that of *Z. piperitum* f. *inerme* reported in the literature. Subsequent large-scale extraction, followed by silica gel chromatography and semi-preparative scale HPLC led to the isolation of seven amides (**1**–**7**).

Amides **1**, **2** and **5** were identified as α-, β-, γ-sanshools, respectively, while the structures of amides **3**, **4**, and **6** were assigned as hydroxy-α-, hydroxy-β- and hydroxy-γ-sanshools, respectively, by comparison of their physical and spectral data with those described in the literature [6, 7].

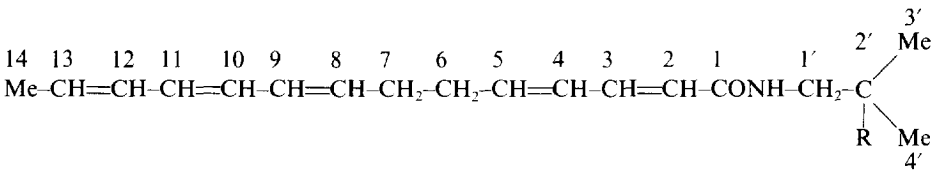
A new amide (**7**) was isolated as a powder. The EI-mass spectrum of **7** gave an $[M]^+$ ion peak at *m/z* 273, along with prominent peaks at *m/z* 107 $[C_8H_{11}]^+$ and 167 $[M-C_8H_{10}]^+$, which were similar to those found in γ-sanshool (**5**) [6]. The molecular formula of **7** was confirmed as $C_{18}H_{27}NO$ by high-resolution mass spectrometry. The ^{13}C NMR spectrum was similar to that of **5**, showing the presence of a carbonyl carbon (δ166.3), 10 olefinic methine carbons (δ122.2, 128.7, 129.2, 130.2, 131.3, 131.4, 131.7, 132.4, 141.1 and 141.9), three methylene carbons (δ32.1, 32.8 and 47.0), a methine carbon (δ28.6) and three methyl carbons [δ18.3 and 20.3 (2C)]. The ^1H–^1H COSY, HSQC, HMBC and ^{13}C NMR examinations revealed that **7** was the all-*trans* isomer of **5**. In addition, carbon resonances for C-1 through C-14 were in good accord with those found in the all-*trans* isomer of hydroxy-γ-sanshool (**8**) [8]. Based on the evidence described above, the structure of **7** was assigned as (2*E*, 4*E*, 8*E*, 10*E*, 12*E*)-*N*-isobutyl-2,4,8,10,12-tetradecapentaenamide.

EXPERIMENTAL

NMR: 400(^1H) and 100(^{13}C) MHz, $CDCl_3$, TMS as int. standard; EI-MS and HR-EI-MS: HITACHI



1 R = H 2*E*, 6*Z*, 8*E*, 10*E*
2 R = H 2*E*, 6*E*, 8*E*, 10*E*
3 R = OH 2*E*, 6*Z*, 8*E*, 10*E*
4 R = OH 2*E*, 6*E*, 8*E*, 10*E*



5 R = H 2*E*, 4*E*, 8*Z*, 10*E*, 12*E*
6 R = OH 2*E*, 4*E*, 8*Z*, 10*E*, 12*E*
7 R = H 2*E*, 4*E*, 8*E*, 10*E*, 12*E*
8 R = OH 2*E*, 4*E*, 8*E*, 10*E*, 12*E*

M-2500 spectrometer; CC: Kieselgel 60 PF_{254} (Merck); TLC: precoated Kieselgel 60 F_{254} plates (0.2 mm, Merck), and spots were detected under UV; HPLC: YMC-Pack SIL (YMC, Inc.) column (4 mm i.d. × 250 mm or 20 mm i.d. × 250 mm, for analyt. and semi-prep. scale, respectively); column temp. 40°; detector wavelength 275 nm.

Plant material. Fruits were collected in Wakayama Prefecture, Japan, and were confirmed to be Budo-Zanthoxylum by anatomical examination [4]. A voucher specimen has been deposited at the Herbarium of the Research Institute for Wakan-Yaku, Toyama Midical and Pharmaceutical University.

Extraction and isolation of amides. Fruits (100 g) were extracted (×3) with $CHCl_3$ (500 ml) at room temp. After removal of solvent by evapn, the extract (10.2 g) was subjected to CC on silica gel [$CHCl_3$–EtOAc (1:1 → 2:3)] to give 4 frs (I–IV). HPLC examination showed that fr. I contained 4 compounds. They were sepd by semi-prep. scale HPLC on YMC-Pack SIL with hexane–*iso*-PrOH (40:1) to yield **1** (20.5 mg), **2** (15.8 mg), **5** (10.3 mg) and **7** (2.9 mg). Fr. II consisted of **6** and **3**, which were sepd by recycled semi-prep. scale HPLC on YMC-Pack SIL with hexane–*iso*-PrOH (10:1) to furnish pure samples (**6**: 2.1 mg; **3**: 5.7 mg). Fr. III consisted mainly of **3** and was purified by HPLC to afford pure **3** (28.2 mg). Sepn of fr. IV, which contained **3** and **4**, by HPLC on YMC-Pack SIL with hexane–*iso*-PrOH (20:1), gave **4** (4.8 mg). Compounds **1**, **2** and **5** were identified as α-, β- and γ-sanshools, respectively, while **3**, **4** and **6** were assigned as hydroxy-α-, hydroxy-β- and hydroxy-γ-sanshools, respectively, by comparison of their physical and spectral data with lit. values [6, 7]

(2E,4E,8E,10E,12E)-N-*Isobutyl*-2,4,8,10,12-*tetradecapentaenamide* (**7**). Amorphous powder. IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 2950, 1665, 1635, 1616, 1517; EIMS (probe) 70 eV, *m/z* (rel. int.): 273 $[M]^+$ (32), 167 $[M - C_8H_{10}]^+$ (41), 121 (31), 107 $[C_8H_{11}]^+$ (100); HR EIMS *m/z*: 273.2097 $[M]^+$ calc. for $C_{18}H_{27}NO$: 273.2093; ^1H NMR (400 MHz, $CDCl_3$): δ 0.91 (6H, *d*, *J* = 6.5 Hz, H-3′, H-4′), 1.76 (3H, *d*, *J* = 7 Hz, H-14), 1.81 (1H, *m*, H-2′), 2.23 (4H, *m*, H-6 and -7), 3.16 (2H, *t*, *J* = 6.5 Hz, H-1′), 5.48 (1H, *dd*, *J* = 6.5, 15 Hz, H-13), 5.76 (1H, *d*, *J* = 15 Hz, H-2), 6.02–6.10 (5H, *m*, H-5, H-9, H-10, H-11, H-12), 6.16 (1H, *dd*, *J* = 11, 15 Hz, H-4), 7.16 (1H, *dd*, *J* = 11, 15 Hz, H-3); ^{13}C NMR (100 MHz, $CDCl_3$): 18.3 (C-14), 20.1 (C-3′, C-4′), 28.6 (C-2′), 32.1 (C-7), 32.8 (C-6), 47.0 (C-1′), 122.2 (C-2), 128.7 (C-4), 129.2 (C-13), 130.2, 131.3, 131.4, 131.7 (C-9, C-10, C-11, C-12), 132.4 (C-8), 141.1 (C-3), 141.9 (C-5), 166.3 (C-1).

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