[Chem. Pharm. Bull. 36(7)2362—2365(1988)]

Amides from Huajiao, Pericarps of *Zanthoxylum bungeanum* MAXIM.

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(Received January 5, 1988)

From Huajiao, the pericarps of *Zanthoxylum bungeanum* MAXIM. (Rutaceae), six unsaturated aliphatic acid amides (**1**—**6**) were isolated. Of these, three were identical with the known amides, hydroxy-α-, hydroxy-β- and hydroxy-γ-sanshools (**1**, **2** and **3**). The other three are new compounds, and the structures were established as (2*E*,4*E*,8*E*,10*E*,12*E*)-2′-hydroxy-*N*-isobutyl-2,4,8,10,12-tetradecapentaenamide, and (2*E*,4*E*,8*Z*,11*Z*)- and (2*E*,4*E*,8*Z*,11*E*)-2′-hydroxy-*N*-isobutyl-2,4,8,11-tetradecatetraenamide (**4**, **5** and **6**, respectively).

Keywords——Huajiao; *Zanthoxylum bungeanum*; pungent principle; Chinese folk medicine; hydroxy-sanshool; 2′-hydroxy-*N*-isobutyl-2,4,8,11-tetradecatetraenamide; 2′-hydroxy-*N*-isobutyl-2,4,8,10,12-tetradecapentaenamide

Huajiao (花椒), the pericarps of *Zanthoxylum bungeanum* MAXIM. (Rutaceae) which grows in Sichuan, China, has been utilized as a pungent foodstuff. This drug was listed in Ben cao gang mu (本草綱目) and has been used for treatment of vomiting, toothache, stomachache due to cold in the stomach, and abdominal pain due to roundworm.[1] As for the chemical constituents of this drug, the isolation of pungent amides and identification of essential oils were reported.[2]

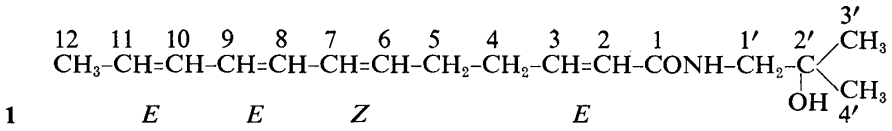
A chloroform extract of this drug was chromatographed on a silica gel column and further purified by high-performance liquid chromatography (HPLC) on a reversed-phase silica gel column, affording six compounds (**1**—**6**) in yields of 0.3%, 0.15%, 0.13%, 0.013%, 0.016% and 0.008%, respectively.

The infrared (IR) spectra of each compound (**1**—**6**) showed absorption bands attributable to an amide-carbonyl, a double bond and a hydroxyl group. By comparison of the carbon-13 and proton nuclear magnetic resonance (^{13}C- and ^{1}H-NMR) spectra with those of the known amides,[3,4] compounds **1**, **2** and **3** were identified as hydroxy-α-, hydroxy-β- and hydroxy-γ-sanshools, respectively. Yasuda *et al.*[4] prepared **2** from **1** but this is the first example of the identification of **2** in nature.

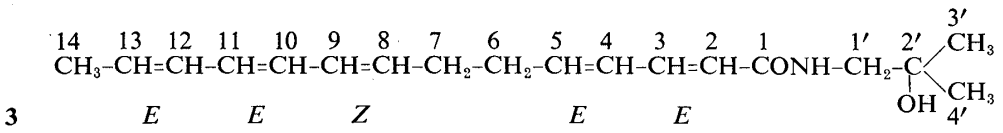
A new amide **4** was obtained as an unstable white powder. The high-resolution mass spectrum (Hi-MS) of **4** led to the same molecular formula as that of **3**, $C_{18}H_{27}NO_2$. Based on the MS fragmentation and the assignments of olefinic proton signals by proton–proton correlated spectroscopy (H–H COSY), **4** was identified as a geometrical isomer of **3** and the geometries of the double bonds at positions 2, 4, 8 and 12 were unambiguously established as

2*E*, 4*E*, 8*E* and 12*E*. Because of the overlapping of the signals due to H-9 through H-12, the geometry of the 10-double bond was determined not from the ^{1}H-NMR spectrum, but by means of ^{13}C-NMR. The carbon resonances of the amides were assigned by C–H COSY as shown in Table I. It was revealed that the carbon signals of C-7 through C-14 of **4** appeared at almost the same positions as those of C-5 through C-12 of **2**, respectively. This indicated the 8*E*, 10*E* and 12*E* geometries in **4**. Consequently, **4** was formulated as (2*E*,4*E*,8*E*,10*E*,12*E*)-2′-hydroxy-*N*-isobutyl-2,4,8,10,12-tetradecapentaenamide.

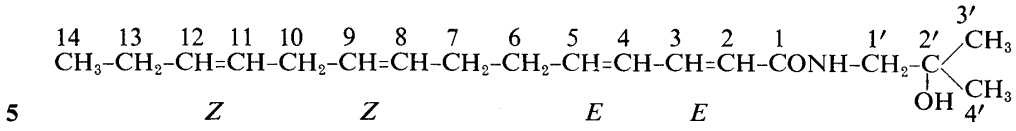
New amides **5** and **6** were obtained as unstable colorless syrups. The Hi-MS of both amides gave the same molecular formula, $C_{18}H_{29}NO_2$, and the same fragmentation pattern, suggesting the formulation as 2′-hydroxy-*N*-isobutyl-2,4,8,11-tetradecatetraenamide. As in the case of **4**, the geometries of the 2- and 4-double bonds were determined as *E* from the



1 *E E Z E*
2 *E E E E*



3 *E E Z E E*
4 *E E E E E*



5 *Z Z E E*
6 *E Z E E*

Chart 1

TABLE I. ^{13}C-NMR Chemical Shifts of Compounds **1**—**6** in $CDCl_3$

	1	**2**	**3**	**4**	**5**	**6**
C-1	167.0	167.0	167.4	167.4	167.6	167.4
C-2	123.8	123.7	121.7	121.7	121.8	121.6
C-3	144.0	144.4	141.6	141.6	141.6	141.8
C-4	32.0	31.9	128.7	128.6	128.7	128.6
C-5	26.4	31.4	142.2	142.3	142.6	142.6
C-6	129.3[a]	132.0	32.9	32.9	32.9	33.0
C-7	129.9[a]	131.6[a]	27.0	31.9	26.5	26.4
C-8	125.1	131.6[a]	129.5[a]	132.3	127.0[a]	127.0[a]
C-9	133.4	130.1[a]	129.8[a]	131.4[a]	129.1[a]	128.8[a]
C-10	131.7	131.5[a]	125.3	131.3[a]	25.6	30.4
C-11	130.0[a]	129.3	133.3	130.2[a]	132.0[a]	132.6[a]
C-12	18.2	18.3	131.8	131.6[a]	128.5[a]	128.9
C-13	—	—	130.0	129.2	20.6	25.5
C-14	—	—	18.3	18.2	14.3	13.9
C-1′	50.4	50.4	50.5	50.5	50.6	50.5
C-2′	71.0	71.0	71.0	71.0	71.0	71.0
C-3′	27.3	27.3	27.0	27.0	27.2	27.3
C-4′	27.3	27.3	27.0	27.0	27.2	27.3

a) These assignments may be reversed in each column. The assignments of compounds **1**—**3** were based on refs. 3 and 4, and those of compounds **4**—**6** were made by the C–H COSY method.

coupling constants of the olefinic proton signals. The geometries of the 8- and 11-double bonds were determined not from the ^{1}H-NMR but from the difference in shielding of allylic carbons[5] in the ^{13}C-NMR. In general, the chemical shifts of allylic carbons of linear olefins of *Z*-isomers resonate at higher field (about 5 ppm) than those of *E*-isomers. The C-7 signal of both **5** and **6** appeared at higher field by about 5 ppm than the allylic carbon (C-7) signal on in the case of the 8(*E*)-double bond of **4**, being observed at almost the same positions as that in the case of the 8(*Z*)-double bond of **3**. This indicated the 8*Z* geometry of **5** and **6**. In the same way, by comparison of the chemical shifts of C-10 and C-13 of **5** with those of **6**, the geometries of the 11-ene of **5** and **6** were assigned as *Z* and *E*, respectively. It follows that the structures of **5** and **6** were established as (2*E*,4*E*,8*Z*,11*Z*)- and (2*E*,4*E*,8*Z*,11*E*)-2′-hydroxy-*N*-isobutyl-2,4,8,11-tetradecatetraenamide.

It is noteworthy that the all-*trans* amides **2** and **4** are tasteless, while the amides **1**, **3**, **5** and **6** having a *cis* double bond are strongly pungent.

It was reported[1] that the 10% ethanolic extract of this drug exhibited more potent anesthetic action on swabbing than 0.5% procaine in guinea pigs, although its surface anesthetic activity on the cornea of rabbits was less than that of tetracaine. The amide mixture of the present study showed significant swabbing and surface anesthetic action. The details will be reported elsewhere.

Experimental

NMR spectra were recorded on JEOL GX-270 and GX-400 spectrometers using Me_4Si as an internal standard in $CDCl_3$. For column chromatography, Kieselgel 60 (70—230 mesh, Merck) and Diaion HP-20 (Mitsubishi Chem. Ind. Co., Ltd.) were used. HPLC was carried out with a Tosoh CCPM pump, with a Tosoh RI-8000 differential refractometer as a detector. All solvent systems for chromatography were homogeneous.

Extraction and Isolation——Fresh pericarps (450 g) collected in Sichuan, China, in 1986, were extracted with $CHCl_3$ to give the $CHCl_3$ extract (87 g). The $CHCl_3$ extract was chromatographed on a silica gel column with $CHCl_3$–MeOH (30 : 1) to give five fractions. Fraction 3 (13.4 g) was rechromatographed on silica gel with C_6H_6–Me_2CO (17 : 3) and further purified by HPLC on TSKgel (ODS-120T (21.5 × 300 mm), Tosoh Co., Ltd.) with 50% MeCN to give the amides **1**—**6** in yields of 0.3%, 0.15%, 0.13%, 0.013%, 0.016% and 0.008%, respectively.

Hydroxy-α-sanshool (1)——An unstable colorless syrup. IR (CCl_4): 3350, 1670, 1630 cm^{-1}. ^{1}H-NMR (in $CDCl_3$ at 270 MHz) δ: 1.22 (6H, s, H-3′ and -4′), 1.70 (3H, d, J=7 Hz, H-12), 2.29 (4H, m, H-4 and -5), 3.30 (2H, d, J=6 Hz, H-1′), 5.35 (1H, dt, J=11, 7 Hz, H-6), 5.73 (1H, dq, J=15, 7 Hz, H-11), 5.89 (1H, d, J=15 Hz, H-2), 6.01 (1H, dd, J=11, 11 Hz, H-7), 6.13 (1H, dd, J=11, 15 Hz, H-10), 6.19 (1H, dd, J=15, 11 Hz, H-9), 6.31 (1H, dd, J=11, 15 Hz, H-8), 6.33 (1H, t, J=7 Hz, NH), 6.84 (1H, dt, J=15, 7 Hz, H-3). ^{13}C-NMR ($CDCl_3$): Table I.

Hydroxy-β-sanshool (2)——An unstable white powder. IR (CCl_4): 3400, 1670, 1630 cm^{-1}. ^{1}H-NMR (in $CDCl_3$ at 270 MHz) δ: 1.23 (6H, s, H-3′ and -4′), 1.70 (3H, d, J=7 Hz, H-12), 2.27 (4H, m, H-4 and -5), 3.32 (2H, d, J=6 Hz, H-1′), 5.64 (1H, dt, J=15, 7 Hz, H-6), 5.69 (1H, dq, J=15, 7 Hz, H-11), 5.83 (1H, d, J=15 Hz, H-2), 6.00—6.12 (5H, m, H-7, -8, -9 and -10, and NH), 6.85 (1H, dt, J=15, 7 Hz, H-3). ^{13}C-NMR ($CDCl_3$): Table I.

Hydroxy-γ-sanshool (3)——An unstable colorless syrup. IR (CCl_4): 3400, 1660, 1630, 1610 cm^{-1}. ^{1}H-NMR (in $CDCl_3$ at 270 MHz) δ: 1.23 (6H, s, H-3′ and -4′), 1.78 (3H, d, J=7 Hz, H-14), 2.27 (4H, m, H-6 and -7), 3.34 (2H, d, J=6 Hz, H-1′), 5.37 (1H, dt, J=11, 7 Hz, H-8), 5.72 (1H, dq, J=15, 7 Hz, H-13), 5.83 (1H, d, J=15 Hz, H-2), 6.02 (1H, dd, J=11, 11 Hz, H-9), 6.05—6.15 (1H, m, H-5), 6.12 (1H, dd, J=11, 15 Hz, H-4), 6.15 (1H, dd, J=11, 15 Hz, H-12), 6.18 (1H, dd, J=15, 11 Hz, H-11), 6.33 (1H, dd, J=11, 15 Hz, H-10), 6.35 (1H, t, J=7 Hz, NH), 7.20 (1H, dd, J=15, 11 Hz, H-3). ^{13}C-NMR ($CDCl_3$): Table I.

(2*E*,4*E*,8*E*,10*E*,12*E*)-2′-Hydroxy-*N*-isobutyl-2,4,8,10,12-tetradecapentaenamide (4)——An unstable white powder. IR (CCl_4): 3400, 1650, 1630 cm^{-1}. UV λ_{max}^{EtOH} nm (ε): 258 (26300), 268 (28900), 279 (26010). EI-MS *m/z*: 289.2043 (M^+, Calcd for $C_{18}H_{29}NO_2$: 289.2044), 271 (M^+-H_2O), 256 ($M^+-H_2O-CH_3$), 230 ($M^+-H_2O-C_3H_5$), 204 ($M^+-H_2O-C_5H_7$), 164 ($M^+-H_2O-C_8H_{11}$), 150 ($M^+-H_2O-C_9H_{13}$), 107 (C_8H_{11}). ^{1}H-NMR (in $CDCl_3$ at 400 MHz) δ: 1.22 (6H, s, H-3′ and -4′), 1.78 (3H, d, J=7 Hz, H-14), 2.25 (4H, m, H-6 and -7), 3.34 (2H, d, J=6 Hz, H-1′), 5.61 (1H, dt, J=15, 7 Hz, H-8), 5.68 (1H, dq, J=15, 7 Hz, H-13), 5.82 (1H, d, J=15 Hz, H-2), 6.02—6.10 (5H, m, H-5, -9, -10, -11 and -12), 6.15 (1H, dd, J=11, 15 Hz, H-4), 6.17 (1H, br s, NH), 7.21 (1H, dd, J=15, 11 Hz, H-3). ^{13}C-NMR ($CDCl_3$): Table I.

(2*E*,4*E*,8*Z*,11*Z*)-2′-Hydroxy-*N*-isobutyl-2,4,8,11-tetradecatetraenamide (5)——An unstable colorless syrup. IR (CCl_4): 3400, 1650, 1550 cm^{-1}. UV λ_{max}^{EtOH} nm (ε): 268 (26190). EI-MS *m/z*: 291.2192 (M^+, Calcd for $C_{18}H_{29}NO_2$: 291.2189), 273 (M^+-H_2O), 258 ($M^+-H_2O-CH_3$), 244 ($M^+-H_2O-C_2H_5$), 218 ($M^+-H_2O-C_4H_7$), 204

($M^+ - H_2O - C_5H_9$), 150 ($M^+ - H_2O - C_8H_{13}$), 124 ($M^+ - H_2O - C_{10}H_{15}$). ^{1}H-NMR (in $CDCl_3$ at 400 MHz) δ: 0.97 (3H, t, J=7 Hz, H-14), 1.23 (6H, s, H-3′ and -4′), 2.07 (2H, dq, J=7, 7 Hz, H-13), 2.21 (4H, m, H-6 and -7), 2.77 (2H, dd, J=7, 7 Hz, H-10), 3.33 (2H, d, J=6 Hz, H-1′), 5.26—5.44 (4H, m, H-8, -9, -11 and -12), 5.85 (1H, d, J=15 Hz, H-2), 6.08 (1H, dt, J=15, 7 Hz, H-5), 6.16 (1H, dd, J=11, 15 Hz, H-4), 6.31 (1H, t, J=6 Hz, NH), 7.20 (1H, dd, J=15, 11 Hz, H-3). ^{13}C-NMR ($CDCl_3$): Table I.

(2*E*,4*E*,8*Z*,11*E*)-2′-Hydroxy-*N*-isobutyl-2,4,8,11-tetradecatetraenamide (6)——An unstable colorless syrup. IR (CCl_4): 3400, 1650, 1550 cm^{-1}. UV λ_{max}^{EtOH} nm (ε): 268 (27350). EI-MS m/z: 291.2187 (M^+, Calcd for $C_{18}H_{29}NO_2$: 291.2189); fragmentation nearly identical with that of **5**. ^{1}H-NMR (in $CDCl_3$ at 400 MHz) δ: 0.96 (3H, t, J=7 Hz, H-14), 1.24 (6H, s, H-3′ and -4′), 2.05 (2H, dq, J=7, 7 Hz, H-13), 2.20 (4H, m, H-6 and -7), 2.72 (2H, dd, J=7, 7 Hz, H-10), 3.34 (2H, d, J=6 Hz, H-1′), 5.34—5.45 (3H, m, H-8, -9 and -11), 5.48 (1H, dt, J=15, 7 Hz, H-12), 5.82 (1H, d, J=15 Hz, H-2), 6.05 (1H, br t, J=6 Hz, NH), 6.08 (1H, dt, J=15, 7 Hz, H-5), 6.16 (1H, dd, J=11, 15 Hz, H-4), 7.20 (1H, dd, J=15, 11 Hz, H-3). ^{13}C-NMR ($CDCl_3$): Table I.

Acknowledgements We thank Dr. H. Kanamori and Dr. I. Sakamoto of Hiroshima Prefectural Institute of Public Health, for MS measurements. Thanks are also due to Miss Y. Iwase of Fukuoka University, for NMR measurements.

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