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ALKYLAMIDES FROM PERICARPS OF *ZANTHOXYLUM BUNGEANUM*

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Key Word Index—*Zanthoxylum bungeanum*; Rutaceae; pericarps; unsaturated alkylamides; HPLC.

Abstract—Ten unsaturated alkylamides were isolated from the pericarps of *Zanthoxylum bungeanum*. Three of them identified as (2*E*, 4*E*)-2′-hydroxy-*N*-isobutyl-2,4-tetradecadienamide (named tetrahydrobungeanool), (2*E*, 4*E*, 8*Z*)-2′-hydroxy-*N*-isobutyl-2,4,8-tetradecatrienamide (named dihydrobungeanool) and (2*E*, 4*E*, 8*Z*, 10*E*, 12*E*)-1′-isopropenyl-*N*-(2′-bisobutenyl)-2,4,8,10,12-tetradecapentaenamide (named dehydro-γ-sanshool), are novel compounds. The content of each compound in crude drugs was determined by HPLC.

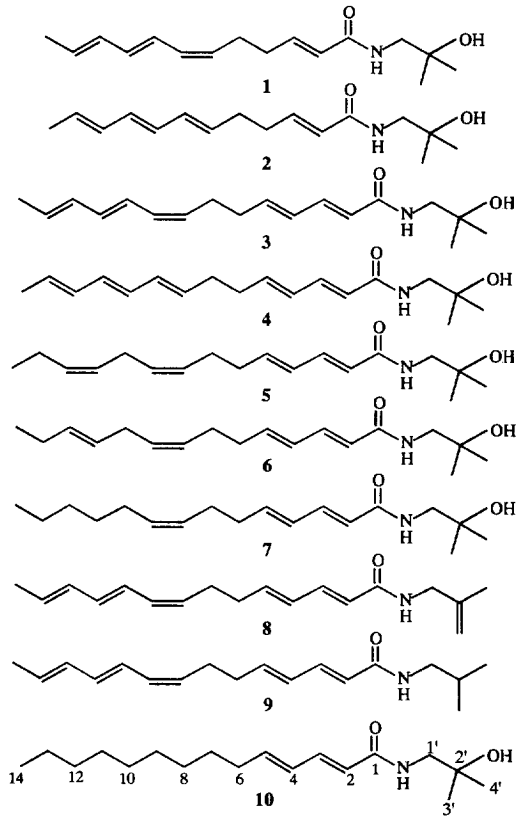
INTRODUCTION

In the preceding paper, we reported on the flavonol glucosides in pericarps of *Zanthoxylum bungeanum* [1]. In the present work, the unsaturated alkylamides, which are the pungent, anaesthetic [2] and insecticidal (Xiong, Q. B., personal communication) constituents of this species and which play an important role in the chemotaxonomy of the genus *Zanthoxylum* and the family Rutaceae [3–5], were isolated and identified.

RESULTS AND DISCUSSION

An ethanol extract of dried pericarps was chromatographed on a silica gel column, then rechromatographed on an ODS column, and finally purified on a Sephadex LH-20 column or a reverse phase HPLC column, affording 10 compounds (**1–10**). The infrared spectra of each compound showed absorption band attributable to a hydroxyl group (except compound **8** and **9**), an amide-carbonyl group and several double bonds.

By comparison of the ^{13}C and ^{1}H NMR spectra with those of the known amides [2, 6], **1–6** and **9** were identified as hydroxy-α-sanshool, hydroxy-β-sanshool, hydroxy-γ-sanshool, (2*E*, 4*E*, 8*E*, 10*E*, 12*E*)-2′-hydroxy-*N*-isobutyl-2,4,8,10,12-tetradecapenta enamide (named hydroxy-γ-isosanshool), (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 (named bungeanool and isobungeanool) and γ-sanshool, respectively. Yasuda *et al.* [7] reported the isolation of γ-sanshool



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from a commercial crude drug, but the botanical origin was not positively identified as *Z. bungeanum*. Here we can declare the reliable existence of γ-sanshool in the pericarps of this species.

Amide **10**, named tetrahydrobungeanool, was obtained as a white powder. The high resolution EI-mass spectrum indicated the formula $C_{18}H_{33}NO_2$. The ^1H NMR spectrum showed a 1,3-diene group and a hydroxy-isobutyl amine which had the same ^{13}C and ^1H NMR chemical shift values and H—H coupling constant as the amide moiety in compounds **3**–**7**. A ^{13}C DEPT spectrum displayed the existence of three —CH_3, nine >CH_2 and four >CH— groups. Thus, amide **10** was formulated as (2*E*,4*E*)-2′-hydroxy-*N*-isobutyl-2,4-tetradecadienamide, which was confirmed by H—H and C—H COSY. The prominent mass spectral fragments at *m/z* 59 and 237 were obviously formed by cleavage at C-1′—C-2′; the base peak *m/z* 110 was assumed to be $[CH_2(CH)_4CONH_2]^+$.

Amide **7**, named dihydrobungeanool, was obtained as a colourless oil. The ^1H NMR showed one more isolated double bond at C-8—C-9 which drew the allylic H-7 and H-10 into lower field. The position of this isolated double bond was confirmed by H—H COSY and C—H COSY. The HR-mass spectrum gave the molecular formula $C_{18}H_{31}NO_2$ and the EI-mass spectrum gave peaks *m/z* 235 and 110 which were considered to be from the same fragmentation pathway as described for compound **10**. These also supported the assignment of the position of the isolated double bond. However, the geometry of this double bond was difficult to determine from the ^1H NMR spectrum because of the overlaps of the proton signals but could be deduced from the difference in shielding of the allylic carbons in the ^{13}C NMR spectrum. Generally, the chemical shifts of allylic carbons of linear olefins of *Z*-isomers resonate at *ca* 5 ppm higher fields ($\delta < 29$) than those of *E*-isomers ($\delta > 31$) [2, 8]. With the chemical shifts of 26.5 ppm (C-7) and 27.2 ppm (C-10), the geometry of this isolated double bond was determined to be the *Z*-form. Moreover, the ^1H NMR and ^{13}C NMR data of the acid moiety were almost superimposable on that of (2*E*,4*E*,8*Z*)-*N*-isobutyltetradecatrienamide [9]. It follows that the structure of **7** was established as (2*E*,4*E*,8*Z*)-2-hydroxy-*N*-isobutyl-2,4,8-tetradecatrienamide.

Amide **8**, named dehydro-γ-sanshool, was also obtained as a colourless oil. The EI-mass spectrum gave the $[M]^+$ at *m/z* 271, corresponding with $C_{18}H_{25}NO$. In the ^1H NMR spectrum, the acid moiety gave a spectrum similar to those of compounds **3** and **9**, whereas a broad singlet signal of two vinyl protons appeared at 4.84 ppm and only one methyl group attached on double bond was observed at 1.75 ppm as a broad singlet. A methylene group was shifted down to 3.90 ppm; an NOE effect between NH and this methylene group as well as an NOE effect between NH and H-2 were observed when irradiating proton N—H. Therefore, the structure of compound **8** was unambiguously determined as (2*E*,4*E*,8*Z*,10*E*,12*E*)-1′-iso-propenyl-*N*-(2′-isobutenyl)-2,4,8,10,12-tetradecapentaen amide, which was also confirmed by H—H COSY, C—H COSY and ^{13}C DEPT spectra. In the EI-mass spectrum, the prominent peak *m/z* 165 and the base peak *m/z* 107 were due to cleavage at C-6—C-7.

Using amide **7** as the reference substance, the contents of amides **1**–**10** in the pericarps of *Z. bungeanum* were measured by HPLC as 4.17, 0.44, 1.09, 0.11, 0.06, 0.03, 0.03, 0.02, 0.13 and 0.02%, respectively.

Yasuda *et al.* [3] investigated the distribution of unsaturated alkylamides in seven species and two varieties of *Zanthoxylum* in Japan. Compared with these results, either the pericarps of *Z. piperitum* or its two varieties, *viz. Z. piperitum* var (forma) *inerme* and *Z. piperitum* var (forma) *brevispinosum*, has a more similar alkylamide content pattern to that of *Z. bungeanum* than other Japanese species. This may be one of the reasons why *Z. piperitum* and *Z. piperitum* var (forma) *inerme* are used as ersatzs of *Z. bungeanum* in Japan [10].

EXPERIMENTAL

General. NMR spectra were recorded on JEOL GX-270 or GX-400 spectrometers using TMS as an int. standard in $CDCl_3$. For CC, Kieselgel 60 (70–230 mesh, Merck), Sephadex LH-20 (Pharmacia) and ODS 60-C_{18} (250–350 Mesh, Nakarai Chemicals Ltd.) were used. HPLC analysis was performed on a Cosmosil 5C_{18} column (4.0 × 250 mm, Nakarai Tesque). Flow-rate: 0.5 ml min^{-1}, Eluent: linear gradient MeCN 35–70% in H_2O within 40 min. Detection: 254 nm. Temp. 40°. Prep. HPLC was carried out on a Unisil Q C 5 μm column (16.7 mm × 250 mm, Gasukuro Kogyo) and a UV detector at 254 nm. Temp.: 40°.

Extraction and isolation. Air-dried pericarps (1.5 kg, collected in September, 1991, Sichuan, China; voucher specimen, no. 910905, deposited at the herbarium of the School of Pharmacy, Shanghai Medical University) were extracted under reflux with 95% EtOH to give an extract (428 g). A 50 g portion of this was chromatographed on silica gel (1 kg) with $CHCl_3$–MeOH (10:1–5) to give 5 frs. Fr. 2 (16 g) was rechromatographed on silica gel (320 g) and eluted with benene–*n*-hexane–Me_2CO (5:2:1) to give 6 frs. Although fr. 3 (7.6 g) contained **1**–**4**, only fr. 2 (2.52 g) was subjected to ODS (50 g) CC and eluted with 80% MeOH, providing 4 frs. After prep. HPLC with 60, 65 and 75% MeOH as eluents, respectively, fr. 1 gave **1** (136 mg), **2** (28 mg), **3** (38 mg) and **4** (12 mg), fr. 2 gave **5** (10 mg) and **6** (7.5 mg), and fr. 3 gave **7** (7 mg). When extracted with *n*-hexane, a white powder was obtained from fr. 4. Using 70% MeOH as an eluent on prep. HPLC, **8** (6 mg) and **9** (52 mg) were obtained from this powder. **10** (35 mg) was obtained

Table 1. ^{13}C NMR data of compounds **1–10** in $CDCl_3$ (67.5 MHz)

Carbon	1*	2	3	4	5	6	7*	8*	9	10*
1	167.2	166.9	167.4	167.3	167.5	167.4	167.4	166.1	166.3	167.6
2	123.9	123.6	121.6	121.5	121.7	121.6	121.4	121.8	122.3	121.3
3	144.3	144.5	141.7	141.8	141.7	141.8	141.9	141.4	140.9	141.9
4	32.2	31.9	128.7	128.6	128.6	128.6	128.5	128.7	128.8	128.2
5	26.5	31.4	142.4	142.4	142.6	142.7	142.9	142.1	141.7	143.7
6	129.5†	132.0†	33.0	32.8	32.9	33.0	33.1	32.9	32.9	33.0
7	129.8†	131.6†	27.0	32.0	26.5	26.4	26.5	27.4	27.1	28.8
8	125.3	131.6†	129.5†	132.4	127.0	127.0	128.2	129.9†	129.5†	29.4†
9	133.6	131.4†	129.8†	131.4†	129.0†	128.9	131.0	129.5†	129.9†	29.3†
10	131.9	130.1†	125.3	131.3†	25.5	30.4	27.2	125.3	125.3	29.2†
11	130.3†	129.4†	133.4	130.2	132.0	132.6	29.3	133.4	133.3	29.5†
12	18.4	18.3	131.8	131.7†	128.5†	128.9	31.5	131.8	131.8	31.9
13			130.1	129.3	20.5	25.6	22.6	130.0†	130.0	22.7
14			18.3	18.3	14.3	13.8	14.1	18.3	18.3	14.1
1′	50.6	50.4	50.5	50.5	50.6	50.5	50.5	45.1	46.9	50.6
2′	70.9	71.0	71.1	71.1	70.9	71.0	71.1	142.1	28.6	71.0
3′	27.3	27.3	27.3	27.4	27.2	27.3	27.3	111.0	20.1	27.2
4′	27.3	27.3	27.3	27.4	27.2	27.3	27.3	20.4	20.1	27.2

* Measured at 100 MHz.

† Assignments may be reversed in each column.

by purifying the supernant on a Sephadex LH-20 column, eluted with MeOH.

(2E, 4E, 8Z)-2′-*hydroxy*-N-*isobutyl*-2,4,8-*tetradecatrienamide* (*dihydrobungeanool*, **7**). Unstable colourless oil. IR ν^{CHCl_3} cm^{-1} 3449, 1664, 1632, 1614, 1519. HR EIMS *m/z*: 293.2339 $[M]^+$ (Calcd. for $C_{18}H_{31}NO_2$: 293.2355). EIMS *m/z* (rel. int.): 275 $[M—H_2O]^+$ (16), 235 (7), 205 (22), 178 (8), 165 (33), 164 (26), 150 (33), 124 (13), 110 $[CH_2(CH)_4CONH_2]^+$ (29). ^1H NMR (400 MHz): δ 0.89 (3H, *t*, *J* = 6.8 Hz, H-14), 1.24 (6H, *s*, H-3′, 4′), 1.26–1.36 (6H, *m*, H-11, 12, 13), 1.98–2.05 (2H, *dt*, *J* = 7.0, 14.0 Hz, H-10), 2.16–2.22 (4H, *m*, H-6, 7), 3.35 (2H, *d*, *J* = 5.9 Hz, H-1′), 5.31–5.41 (2H, *m*, H-8, 9), 5.80 (1H, *d*, *J* = 14.7 Hz, H-2), 5.93 (1H, *br t*, N—H), 6.10 (1H, *dd*, *J* = 6.3, 15.1 Hz, H-5), 6.13 (1H, *dd*, *J* = 10.2, 15.1 Hz, H-4), 7.21 (1H, *dd*, *J* = 10.3, 14.7 Hz, H-3). ^{13}C NMR: Table 1.

(2E, 4E, 8Z, 10E, 12E)-1′-*isopropenyl*-N-(2′-*isobutenyl*)-2, 4, 8, 10, 12-*tetradecapentaenamide* (*dehydro-γ-sanshool*, **8**). Unstable colourless oil. EIMS *m/z* (rel. int.): 271 $[M]^+$ (13), 165 $[M\text{-}C_8H_{11}+H]^+$ (20), 107 $[C_8H_{11}]^+$ (100). ^1H NMR (400 MHz) δ: 1.75 (3H, *s*, H-4′), 1.78 (3H, *d*, *J* = 7.3 Hz, H-14), 2.24–2.33 (4H, *m*, H-6, 7), 3.90 (2H, *d*, *J* = 5.9 Hz, H-1′), 4.84 (2H, *m*, H-3′), 5.36 (1H, *dt*, *J* = 6.8, 10.7 Hz, H-8), 5.51 (1H, *br t*, NH), 5.70–5.75 (1H, *m*, H-13), 5.79 (1H, *d*, *J* = 15.1 Hz, H-2), 5.99–6.20 (5H, *m*, H-4, 11, 12, 5, 9), 6.34 (1H, *dd*, *J* = 11.7, 13.2 Hz, H-10), 7.2 (1H, *dd*, *J* = 10.5, 15.1 Hz, H-3). ^{13}C NMR: Table 1.

(2E, 4E)-2′-*hydroxy*-N-*isobutyl*-2,4-*tetradecadienamide* (*tetrahydrobungeanool*, **10**). Unstable white powder. IR ν^{CHCl_3} cm^{-1}: 3448, 1664, 1633, 1615, 1519. HRMS *m/z*: 295.2503 $[M]^+$ (Calcd. for $C_{18}H_{33}NO_2$: 295.2511). EIMS *m/z* (rel. int.): 296 $[M+1]^+$ (1), 237 $[M\text{-}COHMe_2+H]^+$ (29), 110 $[CH_2(CH)_4CONH_2]^+$ (100), 59 $[COHMe_2]^+$ (20). ^1H NMR (400 MHz) δ: 0.88 (3H, *t*, *J* = 6.8 Hz, H-14), 1.23 (6H, *s*, H-3′,4′), 1.26 (12H, *br s* like *m*, H-8, 9, 10, 11, 12, 13), 1.41 (2H, *m*, H-7), 2.13 (2H, *dt*, *J* = 6.8, 7.3 Hz, H-6), 3.33 (2H, *d*, *J* = 5.9 Hz, H-1′), 5.84 (1H, *d*, *J* = 15.1 Hz, H-2), 6.07 (1H, *dt*, *J* = 6.3, 15.1 Hz, H-5), 6.13 (1H, *dd*, *J* = 9.8, 15.1 Hz, H-4), 6.38 (1H, *br t*, NH), 7.22 (1H, *dd*, *J* = 9.8, 15.1 Hz, H-3). ^{13}C NMR: Table 1.

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