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DISTRIBUTION OF UNSATURATED ALIPHATIC ACID AMIDES IN JAPANESE *ZANTHOXYLUM* SPECIES

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Key Word Index—*Zanthoxylum planispinum*; *Z. piperitum*; *Z. piperitum* f. *inerme*; *Z. piperitum* f. *brevispinosum*; *Z. beecheyanum*; *Z. ailanthoides*; *Z. inerme*; *Z. fauriei*; *Z. schinifolium*; Rutaceae; α-sanshoöl; hydroxy-α-sanshoöl; γ-sanshoöl; hydroxy-γ-sanshoöl; unsaturated aliphatic acid amide; pungent principle; ^{13}C NMR; chemotaxonomy; *Fagara*.

Abstract—Seven species and two varieties of *Zanthoxylum* in Japan were investigated for unsaturated aliphatic acid amides. In addition to the known amides α-sanshoöl, γ-sanshoöl and hydroxy-γ-sanshoöl, a new compound, hydroxy-α-sanshoöl, was isolated and established by chemical and spectroscopic evidence. The compounds, corresponding to hydroxyl derivatives of the amides in the barks, commonly existed in the pericarps of all collected materials. Japanese *Zanthoxylum* species were divided chemotaxonomically into two taxa. These taxa differ from the two assigned on the basis of botanical classification.

INTRODUCTION

The genus *Zanthoxylum* in Rutaceae can be divided into two taxa on the basis of the perianth morphology. Two classifications of this genus are described according to the different evaluations of the perianth structure; i.e. some authorities [1, 2] separate this genus into two genera, *Zanthoxylum* and *Fagara*, and others [3–5] regard *Fagara* as a subgenus of *Zanthoxylum* rather than as a distinct genus. As an aid to establishing the relationship of *Zanthoxylum* and *Fagara*, many investigators [6] have discussed the chemotaxonomy in these taxa. Ishii [7–11] has investigated alkaloids, coumarins, lignans, etc. of Japanese *Zanthoxylum* species.

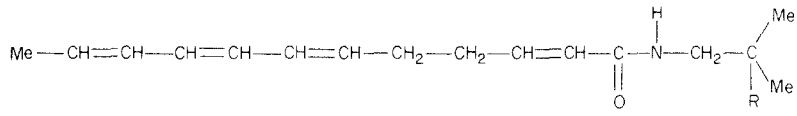
We have investigated the structures of unsaturated aliphatic acid amides in useful plants [12] and recently reported the isolation and structure elucidation of two new amides in the pericarps of *Z. ailanthoides* [13]. We now wish to describe the survey of distribution of unsaturated aliphatic acid amides among seven species and two varieties of Japanese *Zanthoxylum* and discuss the chemotaxonomy in these taxa.

RESULTS AND DISCUSSION

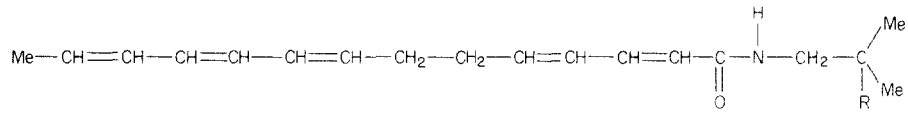
The fresh barks and the pericarps were used as the material of this investigation because the unsaturated aliphatic acid amides in the barks and the pericarps of *Z. piperitum* had been reported to differ in their components [14–16]. The geometric isomers of the unsaturated aliphatic acid amides were demonstrated to be identical in their chromatographic properties [12]. We isolated the amides (**1–4**) individually and measured their ^{13}C NMR spectra (Table 1) since this method is very effective for the structural elucidation of the amides [17]. Each sample was independently confirmed with the characteristic MS fragmentation on the basis of the molecular ion peak, the base peak and the prominent peaks.

α-Sanshoöl (**1**) has been isolated from the bark of *Z. piperitum* and its structure determined by Crombie and Tayler [18]. γ-Sanshoöl (**2**) and hydroxy-γ-sanshoöl (**3**), as previously reported, were isolated from the pericarps of *Z. ailanthoides* by us [13].

A new amide **4** was assumed to be a hydroxyl derivative of **1** from spectral and chemical evidence. In order to confirm the structure of **4**, the ^{13}C NMR spectra of **1**, **4** and their all-*trans* derivatives (**5** and **6**) were recorded (Table 1). **5** and **6** were individually prepared from **1** and **4** by UV irradiation in the presence of a little iodine [19]. The C-2′ carbon signals of **3** and **4** were shifted downfield in comparison with **1** and shown to be a singlet in the off-resonance ^{13}C NMR spectrum. It was apparent that the C-5 and C-8 carbon signals of **4** were shifted upfield by a *cis*-shielding effect [17] compared with those of **6**, and the other carbon signals of **4** appeared at almost the same chemical shifts in **6**. Since a similar effect was also observed in the carbon signals of **1** and **5**, the geometry of C-6 is *cis* and the other double bonds are *trans* in **4** as well as in **1**. Consequently, **4** is determined as (2*E*, 6*Z*, 8*E*, 10*E*)-2′-hydroxyl-*N*-isobutyl-2, 6, 8, 10-dodecatetraenamide and named as hydroxy-α-sanshoöl. All of these amides (**1–4**) are strong pungent principles.



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



2 R=H 2*E*,4*E*,8*Z*,10*E*,12*E*
3 R=OH 2*E*,4*E*,8*Z*,10*E*,12*E*

Table 1. ^{13}C NMR chemical shifts of **1–6***

Carbon	1	5	4	6	Carbon	2	3
C-1	166.2	166.1	167.3	167.2	C-1	166.6	167.8
C-2	124.4	124.4	124.1	124.0	C-2	122.5	122.2
C-3	143.4	143.5	144.3	144.4	C-3	141.0	141.5
C-4	32.1	31.9	32.2	32.0	C-4	129.0	128.9
C-5	26.6	31.5	26.6	31.5	C-5	141.7	142.2
C-6	129.7	129.4	129.6†	129.5	C-6	33.0	33.0
C-7	129.7	131.6‖	129.9†	131.7¶	C-7	27.1	27.2
C-8	125.4	131.7‖	125.4	131.8¶	C-8	129.6‡	129.7§
C-9	133.6	132.3	133.7	132.1	C-9	130.1‡	129.9§
C-10	131.9	131.9‖	132.0	131.6¶	C-10	125.5	125.4
C-11	130.2	130.3	130.3	130.2	C-11	133.5	133.5
C-12	18.3	18.3	18.3	18.3	C-12	132.0	132.0
					C-13	130.1	130.0
					C-14	18.3	18.2
C-1′	47.0	47.0	50.6	50.6	C-1′	47.0	50.7
C-2′	28.6	28.7	70.9	71.0	C-2′	28.7	71.0
C-3′	20.2	20.2	27.3	27.3	C-3′	20.1	27.2

*^{13}C NMR spectra were taken with a Varian NV-16 spectrometer (15.1 MHz) in $CDCl_3$ with TMS as an internal reference and are expressed in terms of ppm.
†,‡,§,‖,¶The assignments may be reversed.

The distribution of the amides and the taxonomic treatment of *Zanthoxylum* and *Fagara* are shown in Table 2. In the pericarps of *Z. planispinum*, **4** and a small amount of **1** were contained, while in the barks only **1** was identified (group I). In the pericarps of *Z. piperitum*, **1**, **2**, **3** and **4** were found. The main component was **4**, and **3** was found in small amounts. In the bark of this species and *Z. beecheyanum*, **1** was the main amide and **2** was found in smaller quantities (group II).

Aihara [14, 15] reported the isolation of two amides, named as sanshoöl I and II, from the barks of *Z. piperitum*, but Crombie and Tayler [18] indicated that sanshoöl I was an impure form of α-sanshoöl (**1**). Furthermore, they noted that sanshoöl II might be the all-*trans* isomer (**5**) of **1**, and named **5** as β-sanshoöl. However, we could not detect **5** but isolated **1** and **2** from the bark. Sanshoöl II may be **2**. Aihara [16] had also isolated 2′-hydroxyl-*N*-isobutyl-2, 4, 8, 10-dodecatetraenamide, named as sanshoamide, from the pericarps of the same species, but this we could not detect. Sanshoamide is probably a mixture of **4** and a small amount of **3**.

In the pericarps of *Z. ailanthoides*, **2** and **3** were found [13], and in the barks of this species and *Z. inerme*, only **2** was identified (group III). Neither in the pericarps of *Z. schinifolium* nor in the bark of this species and *Z. fauriei*, a hybrid of *Z. ailanthoides* and *Z. schinifolium* [4], were unsaturated aliphatic acid amides detected (group IV).

The composition of the amides in the pericarps and the bark of *Z. piperitum* f. *inerme* (*Z. piperitum* var.

Table 2. Distribution of the amides (1–4) in Japanese *Zanthoxylum* species*

Species	Parts	1	4	2	3	Group
Z. planispinum	Pericarp	0.16	1.33	—	—	Group I
	Bark	0.15	—	—	—	
Z. piperitum	Pericarp	0.32	1.89	0.21	0.08	Group II
	Bark	1.13	—	0.29	—	
Z. piperitum f. *inerme*	Pericarp	0.41	2.60	0.29	0.06	
(*Z. piperitum* var. *inerme*)	Bark	1.08	—	0.26	—	
Z. piperitum f. *brevispinosum*	Pericarp	0.28	1.52	0.22	+	
(*Z. piperitum* var. *brevispinosum*)	Bark	0.69	—	0.17	—	
Z. beecheyanum	Bark	1.75	—	0.26	—	
Z. ailanthoides	Pericarp	—	—	0.27	0.43	Group III
(*F. ailanthoides*)	Bark	—	—	0.20	—	
Z. inerme (*F. boninshimae*)	Bark	—	—	0.50	—	
Z. fauriei (*F. fauriei*)	Bark	—	—	—	—	Group IV
Z. schinifolium (*F. schinifolia*)	Pericarp	—	—	—	—	
	Bark	—	—	—	—	

*Data are shown as % of the dry weight. + indicates presence in trace in trace amounts. — signifies that the amides could not be detected with TLC and MS. Species were named according to Ohwi [5] and/or Hara [4].

inerme) and *Z. piperitum* f. *brevispinosum* (*Z. piperitum* var. *brevispinosum*) was similar to those of *Z. piperitum* (Table 2). Thus these chemotaxonomic observations between forms or varieties and mother species are in accord with botanical classification.

Various amides have been considered to occur randomly in both *Zanthoxylum* and *Fagara*, with the exception of the isobutyl cinnamic acid amide, which is confined to three species in Africa [6]. However, the isobutyl unsaturated aliphatic acid amides exist in both taxa in Japan. In addition, **1** may be identical with *neo*-herculin [20] isolated from *Z. clave-herculis* in America [18]. Thus the chemotaxonomy in these taxa may be discussed on the basis of these amides. Japanese *Zanthoxylum* species can be divided into four groups, group I containing **1** and **4**, group II containing **1**, **2**, **3** and **4**, group III contiaining **2** and **3**, and group IV containing none of them. From the view of classification of *Zanthoxylum* and *Fagara*, groups I and II belong to *Zanthoxylum* and groups III and IV to *Fagara*. However, the structures of **1** and **2** (or **3** and **4**) are similar. Their differences are only in the number of carbons and *trans*-double bonds in the unsaturated aliphatic acid functions. Further, the amides **2** and **3** in group III are also included in group II. It is rational that groups I, II and III are classified into one taxon. This chemotaxonomy is in disagreement with the traditional botanical treatment.

The compounds corresponding to hydroxyl derivatives of these amides identified in the barks commonly existed in the pericarps of all collected materials. Similarly, hydroxyl amides may be expected to occur in the pericarps of other *Zanthoxylum* species. When we examined the various parts of *Z. piperitum*, the amides identified in the barks existed also in the flowers and leaves as well as the pericarps. Although the hydroxyl amides occurred neither in the female nor in the male flowers, they were found in the pericarps (I. Yasuda *et al.*, unpublished results). The hydroxylation of the amides may be carried out in the pericarps.

EXPERIMENTAL

General procedures were the same as described earlier [12]. TLC was used, Kieselgel 60 F_{254}, pre-coated (Merck). Spots were detected by UV light (254 nm) and 10% H_2SO_4 (heating).

Plant material. Z. piperitum: Mount Tsukuba, Ibaragi (August 1979); *Z. piperitum* f. *inerme*: Nishiyoshino, Nara(August 1979); *Z. piperitum* f. *brevispinosum*: Mount Gozen, Tokyo (August 1980); *Z. planispinum* and *Z. fauriei*: Asakawa Experiment Forest, Forestry and Forest Products Research, Takao, Tokyo (September 1980); *Z. ailanthoides*: Tokyo Metropolitan Medicinal Plant Garden, Kodaira, Tokyo (October 1980); *Z. schinifolium*: Mount Yatsu, Yamanashi (October 1980); *Z. beecheyanum* and *Z. inerme*: Chichijima Island, Tokyo (December 1980).

Extraction and isolation. All of the bark (24–122 g) and the pericarps (37–150 g) of *Zanthoxylum* species, after being air-dried, were ground and extracted with $CHCl_3$. Each extract was chromatographed using the Si gel extract (20:1, w:w). Elution with $CHCl_3$–Et_2O (10:1) gave fraction A and elution with $CHCl_3$–Et_2O (1:2) gave fraction B. The two fractions were rechromatographed on prep. HPLC. CIG column system 22ϕ × 300 mm (Kusano Scientific Co. Tokyo) and stationary phase Wakogel LC-50 H (50 μ, silica gel, Wako Pure Chemical Industries, Tokyo) was used for prep. HPLC. **1** and **2** were isolated from fraction A using C_6H_6–EtOAc (25:2), and also **3** and **4** from fraction B using C_6H_6–EtOAc (5:4). **2** and **3** were identified as γ-sanshoöl and hydroxy-γ-sanshoöl as described previously [13].

1, unstable colourless needles, mp 69° (*n*-hexane), $C_{16}H_{25}NO$ (Found: C, 77.55; H, 10.19; N, 5.36; Calc. for $C_{16}H_{25}NO$: C, 77.68; H, 10.19; N, 5.66%). MS *m/z* (rel. int.): 247 (15)$[M]^+$, 141(72), 107(100), 98(12), 91(49), 79(99). ^1H NMR($CDCl_3$): δ 0.87(6H, *d*, J = 7 Hz, 3'-H), 1.74(4H, *m*, 12-and 2'-H), 2.25(4H, *m*, 4- and 5-H), 3.09(2H, *dd*, J = 7, 5.5 Hz, 1'-H), 5.37(1H, *dt*, J = 11, 7 Hz, 6-H), 5.71(1H, *dq*, J = 14, 6 Hz, 11-H), 5.79(1H, *d*, J = 15 Hz, 2-H), 5.80–6.36

(5H, *m*, 7-, 8-, 9-, 10-H and NH), 6.82(1H, *dt*, $J = 15$, 6.5 Hz, 3-H). UV and IR spectral data were in agreement with those of Crombie and Tayler [18].

4, unstable colourless oil, $C_{16}H_{25}NO_2$ (M^+ 263, Found: 263.1873; Calc.: 263.1884). IR $\nu_{max}^{CCl_4}$ cm^{-1}: 3430, 3310, 1680, 990. UV λ_{max}^{EtOH} nm (ϵ): 260 (22 400), 269(29 200), 280(23 200). ^1H NMR(CCl_4): δ 1.22 (6H, *s*, 3'-H), 1.78(3H, *d*, $J = 6$ Hz, 12-H), 2.31(4H, *m*, 4- and 5-H), 3.33(3H, *m*, 1'-H and OH), 5.37(1H, *dt*, $J = 11$, 7 Hz, 6-H), 5.71(1H, *dq*, $J = 14$, 6 Hz, 11-H), 5.84(1H, *d*, $J = 15$ Hz, 2-H), 5.85–6.50(5H, m, 7-, 8-, 9-,10-H and NH), 6.80 (1H, *dt*, $J = 15$, 6.5 Hz, 3-H). MS *m/z* (rel. int.): 263 (13)$[M]^+$, 157(35), 107(79), 98(18), 91(52), 79(100).

Degradation of **4**. Hydrogenation of **4** over PtO_2 in EtOH afforded, in almost quantitative yield, the octahydro-derivative (**7**); colourless oil, IR $\nu_{max}^{CCl_4}$ cm^{-1}: 3430, 3310, 2910, 2830, 1660. MS *m/z* (rel. int.): 213(65), 86(97), 73(100). **7** in pyridine at 0° was treated with $SOCl_2$ and then hydrogenated with PtO_2 as a catalyst to give a product, colourless needles, mp 53°(*n*-pentane); $C_{16}H_{33}NO$ (Found: C, 75.02; H, 12.84; N, 5.39; Calc. for $C_{16}H_{33}NO$: C, 75.29; H, 12.94; N, 5.49%). MS *m/z* (rel. int.): 255 (20) $[M]^+$ 200(29), 183(25), 128(29), 115(100). It was proved to be identical with *N*-isobutyldodecanamide by mixed fusion with an authentic sample [18].

Preparation of **5** *and* **6**. **5** was prepared according to the method of ref. [19] as follows. 500 mg **1** was dissolved in 50 ml *n*-hexane and irradiated in the presence of a little I_2 with a high pres. mercury lamp (400 W) for 1 hr. The reaction mixture was applied to a Si gel column and 190 mg **5**, colourless needles, mp 112°(*n*-hexane); MS *m/z* (rel. int.): 247 (18) $[M]^+$, 107 (100), was isolated. **6** was prepared as well as **5**. 500 mg of **4** in 100 ml C_6H_6 afforded 120 mg **6**, colourless oil; MS *m/z* (rel. int.): 263 (14) $[M]^+$, 79 (100).

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REFERENCES

1. Engler, A. (1931) *Die Naturlichen Pflanzenfamilien* (Engler, A. and Prantl, K., eds.) Vol. 19a, p. 214. Engelmann, Leipzig.
2. Brizicky, G. K. (1962) *J. Arnold Arbor. Harv. Univ.* **43**, 80.
3. Waterman, P. G. (1975) *Taxon* **24**, 361.
4. Hara, H. (1954) *A Bibliographic Enumeration of Flowering Plants*, Vol. 3, p. 12. Iwanamishoten, Tokyo.
5. Ohwi, J. (1975) *Flora of Japan* (*Phanerogamae*) p. 825. Shibundo, Tokyo.
6. Fish, F. and Waterman, P. G. (1973) *Taxon* **22**, 177.
7. Tomita, M. and Ishii, H. (1957) *Yakugaku Zasshi* **77**, 810.
8. Ishii, H. (1961) *Yakugaku Zasshi* **81**, 243.
9. Ishii, H. (1961) *Yakugaku Zasshi* **81**, 1633.
10. Ishii, H., Hosoya, K., Ishikawa, T. and Haginiwa, J. (1974) *Yakugaku Zasshi* **94**, 309.
11. Ishii, H., Murakami, K., Takeishi, K., Ishikawa, T. and Haginiwa, J. (1981) *Yakugaku Zasshi* **101**, 504.
12. Yasuda, I., Takeya, K. and Itokawa, H. (1981) *Chem. Pharm. Bull.* **29**, 564.
13. Yasuda, I., Takeya, K. and Itokawa, H. (1981) *Chem. Pharm. Bull.* **29**, 1791.
14. Aihara, T. (1950) *Yakugaku Zasshi* **70**, 405.
15. Aihara, T. (1950) *Yakugaku Zasshi* **70**, 409.
16. Aihara, T. (1951) *Yakagaku Zasshi* **71**, 1112.
17. Yasuda, I., Takeya, K. and Itokawa, H. (1980) *Chem. Pharm. Bull.* **28**, 2251.
18. Crombie, L. and Tayler, J. L. (1957) *J. Chem. Soc.* 2760.
19. Jacobson, M. (1954) *J. Am. Chem. Soc.* **76**, 4606.
20. Crombie, L. (1955) *J. Chem. Soc.* 995.