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Structures of Amides from *Asiasarum heterotropoides* Maek. var. *mandshuricum* Maek.

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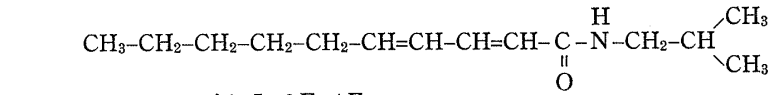
The structures of three unstable amides from the roots of *Asiasarum heterotropoides* Maek. var. *mandshuricum* Maek. (Aristolochiaceae) were established as (2*E*,4*E*)-N-isobutyl-2,4-decadienamide (pellitorine), (2*E*,4*E*,8*Z*,10*E*)-N-isobutyl-2,4,8,10-dodecatetraenamide and (2*E*,4*E*,8*Z*,10*Z*)-N-isobutyl-2,4,8,10-dodecatetraenamide by spectroscopic investigation and chemical transformation studies. (2*E*,4*E*,8*Z*,10*E*)-N-Isobutyl-2,4,8,10-dodecatetraenamide and (2*E*,4*E*,8*Z*,10*Z*)-N-isobutyl-2,4,8,10-dodecatetraenamide were identical in their chromatographic properties, but they could be identified in a mixture of both compounds by our carbon-13 nuclear magnetic resonance method, by observing the *cis* double bond shielding effect in comparison with the *all*-trans isomer.

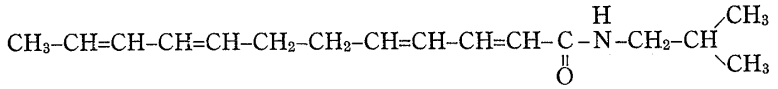
Keywords——*Asiasarum heterotropoides* var. *mandshuricum*; Aristolochiaceae; pellitorine; (2*E*,4*E*,8*Z*,10*E*)-N-isobutyl-2,4,8,10-dodecatetraenamide; (2*E*,4*E*,8*Z*,10*Z*)-N-isobutyl-2,4,8,10-dodecatetraenamide; (2*E*,4*E*,8*E*,10*E*)-N-isobutyl-2,4,8,10-dodecatetraenamide; ^{13}C-NMR spectra

Asiasari radix (Saishin in Japanese, J.P.IX) prepared from *Asiasarum sieboldi* Maek. or *A. heterotropoides* Maek. var. *mandshuricum* Maek. (Aristolochiaceae) is one of the most important crude drugs in Chinese medicine, and has been used from ancient times as an antitussive, expectorant or anodyne. We now wish to report on the structures of unsaturated aliphatic acid amides contained in the roots of *A. heterotropoides* var. *mandshuricum*, since no detailed study has yet appeared on the location and geometry of double bonds of N-isobutyldodecatetraenamide, which is one of the antitussive principles.[1]

We carefully investigated three unstable amides I—III isolated from the roots. Amide I was identified as (2*E*, 4*E*)-N-isobutyl-2,4-decadienamide (pellitorine) by direct comparison of its spectral data with those of an authentic sample.[2] Amides II and III were identical in their chromatographic properties under various conditions, and were inseparable by chromatography. On hydrogenation with PtO_2 as a catalyst, the mixture of amides II and III gave a single product, mp 53°, which was identified as N-isobutyldodecanamide by direct comparison with an authentic sample.[3] On the other hand, amide IV was obtained as a single product by *trans*-isomerization[4] of the mixture of amides II and III. Amide IV corresponded to $C_{16}H_{25}NO$ with mp 104—105° and showed an ultraviolet (UV) absorption maximum at 260 nm, indicating the presence of a conjugated system in sorbamide.[5,6] The infrared (IR) spectrum also showed characteristic bands suggesting the presence of a 2*E*,4*E*-dienamide derivative at 3300, 1625, 998 and 987 cm^{-1}.[6] Its mass spectrum (MS) exhibited characteristic fragments at *m/z* 247(M^+), 167, 166, 152, 100, 81 (base peak) and 79. These results indicated the structure of amide IV to be (2*E*,4*E*,8*E*,10*E*)-N-isobutyl-2,4,8,10-dodecatetraenamide.[7] Therefore, amides II and III were assumed to be geometric isomers of N-isobutyl-2,4,8,10-dodecatetraenamide. Furthermore, they were judged to be 2*E*,4*E*-dienamide derivatives by comparing the UV spectrum of the mixture of amides II and III with that of amide IV. In order to determine the location and geometry of double bonds in amides II and III, the carbon-13 nuclear magnetic resonance (^{13}C-NMR) spectra of amides I—IV were recorded in $CDCl_3$. Initially, signal assignments were performed by means of chemical shift rules[8]



amide I: 2*E*, 4*E*



amide II: 2*E*, 4*E*, 8*Z*, 10*E*
amide III: 2*E*, 4*E*, 8*Z*, 10*Z*
amide IV: 2*E*, 4*E*, 8*E*, 10*E*

Chart 1

TABLE I. ^{13}C-NMR Spectral Data for Amides I—IV

Carbon No.	Amide I	Amides II and III[a]		Amide IV
C-1	166.7	166.7		166.6
C-2	122.1	122.8		122.5
C-3	140.7	140.9		141.0
C-4	128.4	129.0		128.9
C-5	141.3	141.7		141.9
C-6	33.0	33.1[b]	32.9[b]	32.9
C-7	28.6	27.0[c]	26.9[c]	31.9
C-8	31.4	128.0	126.8	127.6
C-9	22.5	129.5	124.4[d]	131.3[e]
C-10	14.0	127.0	124.5[d]	131.6[e]
C-11		129.9	130.1	130.0
C-12		18.3	13.1	18.0
C-1′	47.1	47.1		47.1
C-2′	28.7	28.7		28.7
C-3′	20.2	20.2		20.2

The measurements were made on 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 maximum experimental error of the chemical shift was within 0.1 ppm. The FT conditions were as follows: spectra with 3 KHz, number of data points 8192, pulse repeat time 1.3 sec, number of pulses 5000—10000 and flipping angle 30°.
a) Amides II and III were measured in a mixture of both compounds.
b—*e*) The assignments may be reversed.

and single frequency off-resonance proton decoupling experiments. Furthermore, assignments were aided by comparison with known chemical shifts of unsaturated aliphatic acid amides[7] and especially by comparing the relative intensity of each signal in the spectrum of the mixture of amides II and III. The results are shown in Table I. In the assignments of amides II—IV, the chemical shifts of the carbon signals of C-7 to C-12 differed. From the careful observation of these spectral data, it was apparent that the C-7 carbon signal at δ 31.9 of amide IV was shifted upfield to δ 26.9 and 27.0 by *cis* shielding in the mixture of amides II and III, and that the C-12 carbon signal at δ 18.0 of amide IV appeared at almost the same chemical shift in one compound of the mixture, while in the other the C-12 carbon signal was shifted upfield to δ 13.1 due to *cis* shielding. That is to say, the geometry of C-8 is *cis* in both amides II and III, while C-10 is *trans* in one of them and *cis* in the other. Consequently, it is clear that the mixture of amides II and III consists of (2*E*,4*E*,8*Z*,10*E*)-N-isobutyl-2,4,8,10-dodecatetraenamide and (2*E*,4*E*,8*Z*,10*Z*)-N-isobutyl-2,4,8,10-dodecatetraenamide. From the relative intensities of the assigned signals, amides II and III were present in a ratio of approximately 1: 1.

Recently, amide II was isolated from *Spilanthes alba* (Compositae) and its structure was elucidated by 270 MHz proton nuclear magnetic resonance (^{1}H-NMR).[9] However, the structural elucidation of these unsaturated aliphatic acid amides by ^{1}H-NMR spectroscopy is often difficult due to the overlap of signals. Therefore, our ^{13}C-NMR method of observing the *cis* shielding influence of double bonds on the basis of comparison with the all-*trans* deriva-

tive, which is readily obtained by *trans*-isomerization,[4] is very useful for these structural elucidations.

Experimental

All melting points are uncorrected. IR and UV spectra were obtained with Hitachi EPI-G3 and Hitachi EPS-3T spectrometers, respectively. ^{1}H-NMR spectra were measured at 100 MHz on a JEOL PS-100 spectrometer and ^{13}C-NMR spectra at 15.1 MHz on a Varian NV-16 spectrometer. Chemical shifts are given as δ (ppm) with tetramethylsilane as an internal standard. MS were recorded on a Shimadzu LKB-9000. Silica gel column chromatography was carried out on Kieselgel 60 (230—400 mesh, Merck).

Extraction and Isolation——Dry roots (1.1 kg) of *Asiasarum heterotropoides* MAEK. var. *mandshuricum* MAEK., which were imported from North Korea in Novemver 1976, were exhaustively extracted with *n*-hexane at room temperature. The extract was subjected to column chromatography on silica gel. Elution with *n*-hexane–ether (1: 1) gave a crude reddish-brown liquid. Further chromatography on a column of silica gel impregnated with 2% $AgNO_3$ was carried out. Elution with C_6H_6–AcOEt (5: 1) gave fraction 1 (0.6 g) and elution with C_6H_6–AcOEt (4: 1) gave fraction 2 (1.6 g). Recrystallization of fraction 1 from *n*-pentane gave amide I (0.4 g) and its physical data were as follows: colorless needles, mp 88°, *Rf* value 0.50 in TLC analysis on silica gel G impregnated with 3% $AgNO_3$ using the solvent system of C_6H_6–AcOEt (1: 1), *Anal.* Calcd for $C_{14}H_{25}NO$: C, 75.28; H, 11.28; N, 6.27. Found: C, 75.26; H, 11.14; N, 6.26, MS *m/z*: 223 (M^+), 151 (base peak). UV λ_{max}^{EtOH} nm (ε): 259 (22500). IR ν_{max}^{KBr} cm^{-1}: 3300, 1625, 997. ^{1}H-NMR $\delta_{ppm}^{CCl_4}$: 3.07 (2H, t, J=6.5 Hz), 5.80 (1H, d, J=15.5 Hz), 5.90—6.22 (2H, m), 6.34—6.58 (1H, m), 7.03 (1H, dd, J=15.5, 10.0 Hz). It was identified as pellitorine by direct comparison of the MS and IR spectral data with those of an authentic sample.[2] The ^{13}C-NMR spectral data are shown in Table I. Recrystallization of fraction 2 from *n*-hexane gave a mixture of amides II and III, and its physical data were as follows: colorless needles, mp 69°, *Rf* value 0.34 in TLC analysis on silica gel G impregnated with 3% $AgNO_3$ using the solvent system of C_6H_6-AcOEt (1: 1), UV λ_{max}^{EtOH} nm (ε): 259 (35500), 237 (29300). IR ν_{max}^{KBr} cm^{-1}: 3300, 1625, 997, 945, Found: C, 77.43; H, 10.19; N, 5.36. The ^{13}C-NMR spectral data are shown in Table I.

Hydrogenation of the Mixture of Amides II and III——The mixture (250 mg) in MeOH (50 ml) was hydrogenated with PtO_2 as a catalyst (100 mg) at room temperature; almost exactly 4 mol of H_2 were absorbed during 1 hr. The reaction mixture was filtered and the filtrate was evaporated to dryness under reduced pressure to yield a pale yellow crystalline solid (mp 45—48°). Recrystallization of the solid from *n*-pentane gave colorless needles (180 mg), mp 53°, *Anal.* Calcd for $C_{16}H_{33}NO$: C, 75.29; H, 12.94; N, 5.49. Found: C, 75.02; H, 12.84; N, 5.39. MS *m/z*: 255 (M^+). It was proved to be identical with N-isobutyldodecanamide by mixed fusion with an authentic sample.[3]

***trans*-Isomerization of the Mixture of Amides II and III**——The mixture of amides II and III was isomerized to the all-*trans* derivative according to the method of Jacobson,[4] as follows. The mixture (400 mg) was dissolved in 80 ml of *n*-hexane, a small amount of iodine was added and the reaction mixture was exposed to the direct light of a high pressure mercury lamp (400 W) for 1 hr, while the reaction apparatus was cooled with an electric fan to prevent evaporation. By the end of this period, the mixture had crystallized to a solid mass. It was subjected to column chromatography on silica gel, and recrystallization from *n*-hexane of the fraction eluted with *n*-hexane–ether (1: 1) gave amide IV (280 mg). Its physical data were as follows: colorless needles, mp 104—105°, *Rf* value 0.34 in TLC analysis on silica gel G impregnated with 3% $AgNO_3$ using C_6H_6–AcOEt (1: 1). *Anal.* Calcd for $C_{16}H_{25}NO$: C, 77.68; H, 10.19; N, 5.66. Found: C, 77.53; H, 10.14; N, 5.80. MS *m/z*: 247 (M^+), 167, 166, 152, 100, 81 (base peak) and 79. UV λ_{max}^{EtOH} nm (ε): 260 (34700), 232 (29200). IR ν_{max}^{KBr} cm^{-1}: 3300, 1625, 998, 987. ^{1}H-NMR $\delta_{ppm}^{CCl_4}$: 0.90 (6H, d, J=6.5 Hz), 1.68—1.92 (4H, m), 2.20 (4H, br.s), 3.14 (2H, t, J=6 Hz), 5.32—6.36 (8H, m), 7.04—7.32 (1H, m). The ^{13}C-NMR spectral data are shown in Table I.

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