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Studies on the Constituents of the Crude Drug "Piperis Longi Fructus." On the Alkaloids of Fruits of *Piper longum* L.

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Two new piperidine alkaloids, pipernonaline and piperundecalidine, were isolated from fruits of *Piper longum* L. (Piperaceae). Their structures were determined to be (2*E*, 8*E*)-*N*-[9-(3,4-methylenedioxyphenyl)-2,8-nonadienoyl]piperidine and (2*E*, 4*E*, 10*E*)-*N*-[11-(3,4-methylenedioxyphenyl)-2,4,10-undecatrienoyl]piperidine, respectively, on the basis of spectral data.

Keywords——piperidine alkaloid; *Piper longum* L.; pipernonaline; piperundecalidine; ^{13}C-NMR

Piperaceae plants were recently studied to determine their chemical components and biological properties.[1)] The crude drug "Piperis Longi Fructus" is widely used as an anodyne and a treatment for stomach disease in China. We now wish to report the isolation and the structure elucidation of two new alkaloids isolated together with eight known compounds from *Piper longum* L.

The ethanol extract of the medicinal fruits gave a crystalline compound, piperine (**1**),[2)] as a main component, which had a pungent effect. The residue after the separation of **1** was saponified with 15% KOH solution to give a precipitate, which was chromatographed over alumina gel to afford three fractions (I—III). Compounds **2**—**7** were isolated from fraction II by chromatography on a silica gel column and on a Lobar column. Compound **4** was also isolated from fraction III. Compounds **8**—**10** were isolated from fraction I. Compounds **2** and **3**, named pipernonaline and piperundecalidine, respectively, are new piperidine alkaloids.

Pipernonaline (**2**), $C_{21}H_{27}NO_3$, mp 54.0—55.5 °C, showed the following properties. The proton and carbon-13 nuclear magnetic resonance (^{1}H- and ^{13}C-NMR) spectra indicated the presence of 3,4-methylenedioxyphenyl and piperidine ring groups as in **1**.[3)] One of two double bonds was assigned to be conjugated to the aromatic ring since the ^{1}H-NMR spectrum was very similar to that of isosafrol.[4)] The presence of the conjugated system was also supported by the ultraviolet (UV) spectrum, which showed an absorption maximum at 305 nm, indicating the presence of a 3,4-methylenedioxy-*trans*-styryl moiety in the molecule.[5)] The other was an α,β-conjugated amide group from its ^{1}H-NMR and infrared (IR) spectra. The signals of the latter olefinic protons were seen at δ 5.90 (d, J=15.0 Hz) and δ 6.80 ppm (dt, J=15.0, 4.0 Hz), supporting the presence of a *trans* double bond. Moreover, the ^{1}H-NMR spectrum showed two allylic methylene groups at δ 2.00—2.36 ppm and two aliphatic methylenes at δ 1.28—1.74, overlapping methylene protons of the piperidine ring. The number of carbon atoms in the chain of **2** was concluded to be nine, including two double bonds, four methylenes, and one carbonyl carbon. Therefore, compound **2** was identified as (2*E*, 8*E*)-*N*-[9-(3,4-methylenedioxyphenyl)-2,8-nonadienoyl]piperidine.

Piperundecalidine (**3**), $C_{23}H_{29}NO_3$, mp 64.5—65.5 °C, showed the following properties. The ^{1}H- and ^{13}C-NMR spectra of **3** were similar to those of **6**, reported as pipercide,[1a)] except for the signals of the *N*-alkyl group, and showed the presence of a methylenedioxyphenyl moiety and three double bonds. One of the three double bonds was assigned to

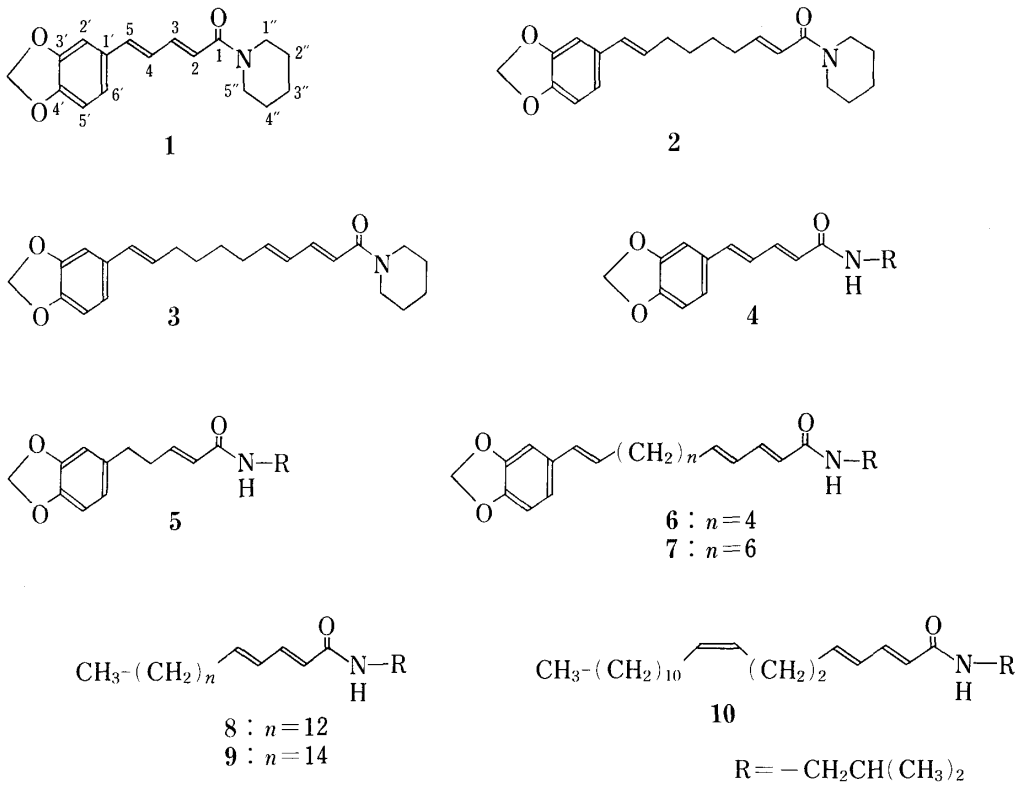

Chart 1

be conjugated to the aromatic ring as in **2**, and the other were assigned to the α,β,γ,δ-conjugated system of a 2*E*,4*E*-dienamide as in **4**, **6**—**10** on the basis of an absorption maximum at 262 nm in the UV spectrum.[6] The double bond geometry was assigned to be all-*trans* from the NMR and UV spectra, and this was confirmed by the ^{13}C-NMR spectrum, which showed no signal due to *cis* shielding.[7]

Compound **4** was one of the main components, as well as **1**. The spectral and physical data of **4** were identical with those of piperlonguminine.[8] Compound **5** was identical with dihydropiperlonguminine[9] in the terms of spectral data. Compounds **6** and **7** were identical with pipercide,[1a] an insecticidal amide, and guineensine,[5,10] respectively, in terms of spectral data.

Compounds **8**, **9**, and **10** were known compounds, (2*E*, 4*E*)-*N*-isobutyl-octadeca-2,4-dienamide,[5,6b] (2*E*, 4*E*)-*N*-isobutyl-eicosa-2,4-dienamide,[6c] and (2*E*, 4*E*, 8*Z*)-*N*-isobutyl-eicosa-2,4,8-trienamide,[5,11] respectively. The amide (**10**) was oxidized with potassium permanganate and sodium periodate, followed by treatment with diazomethane to give methyl laurate. The geometry of the double bond at C-8 was confirmed by the ^{13}C-NMR spectrum, in which two signals assiged to C-7 and 10 were shifted up-field to 26.9 and 27.2 ppm due to *cis* shielding.[7]

This medicinal fruit has a pungent effect; since piperine (**1**) was the only compound exhibiting such an effect, it appears to be the active principle.

Experimental

The fruits were obtained commercially in Peking, China. All melting points are uncorrected. IR spectra were taken with a JASCO IRA-2 spectrometer. UV spectra were measured in EtOH solution with a Shimadzu D-300 spectrometer. NMR spectra were measured in $CDCl_3$ solution with a JEOL FX-100 spectrometer using tetramethylsilane as an internal standard, and the following abbreviations are used: s = singlet, d = doublet, dd = double doublet, dt = double triplet, m = multiplet. Mass spectra (MS) and gas chromatography-mass spectra (GC-MS) were measured

Table I. ^{13}C-NMR Chemical Shifts

Carbon	2	3	6	7
1	165.2	165.5	166.3	166.4
2	120.5	118.7	121.9	121.9
3	145.2	142.0	141.0	141.0
4	32.2*	128.9	128.4	128.3
5	27.9**	142.6	142.6	142.7
6	28.9**	32.6*	32.6*	32.8
7	32.6*	28.8**	28.3**	28.7*
8	128.6	28.9**	28.9**	28.9
9	129.5	32.7*	32.7*	28.9
10		129.0	128.9	29.3*
11		129.5	129.5	32.8
12				128.3
13				129.2
1′	132.2	132.3	132.3	132.4
2′	105.2	105.3	105.3	105.3
3′	146.5	146.5	146.5	146.4
4′	147.9	147.8	147.8	147.8
5′	108.0	108.1	108.2	108.1
6′	120.1	120.1	120.2	120.1
$-OCH_2O-$	100.8	100.8	100.8	100.8
1″	42.9	43.2		
2″	26.5	26.6		
3″	24.6	24.5		
4″	25.6	25.0		
5″	46.6	46.8		

δ (ppm), solv.: $CDCl_3$.
Except for *N*-isobutyl group.
Assignments with the same type of asterisk may be interchanged in each column.

Table II. ^{13}C-NMR Chemical Shifts

Carbon	8	9	10	Carbon	8	9	10
1	166.3	166.2	166.5	$-(CH_2)_n-$	28.6	28.6	28.7
2	121.9	121.7	121.9		29.6	29.6	29.8
3	141.0	141.1	141.1	16	31.9		
4	128.2	128.2	128.3	17	22.7		
5	142.8	143.0	142.9	18	14.1	31.9	32.0
6	32.9	32.9	33.0	19		22.7	22.4
7			26.9	20		14.1	14.0
8			129.8	1′	46.9	46.9	47.0
9			128.9	2′	28.8	28.8	28.8
10			27.2	3′	20.1	20.1	20.2

δ (ppm), solv.: $CDCl_3$.

with a Shimadzu LKB-9000B spectrometer. column chromatography was performed on Silica gel 60 (0.063—0.200 mm, Merck) and alumina gel manufactured in Peking, China. Liquid chromatography was performed on a Lobar column RP-8 (Merck).

Isolation Procedure——Dried ground fruits (6.5 kg) were extracted with EtOH (16 l). The EtOH extract (500 g) was allowed to stand to give crystals, which were recrystallized to yield piperine (**1**, 120 g). The remaining solution was saponified with 15% KOH (1000 ml) to give a precipitate (230 g). The precipitate (55 g) was chromatographed over alumina gel (500 g) and eluted with petr. ether (fraction I, 28 g) and petr. ether–$CHCl_3$ (9 : 1) (fraction II, 21 g and III, 2.1 g). Fraction I was chromatographed over silica gel and eluted with a 9 : 1 mixture of hexane–$CHCl_3$

saturated with 28% NH_4OH to afford a mixture of unsaturated acid amides (14 g). The mixture (3.0 g) was chromatographed on a Lobar column using 95% MeOH to afford three fractions I-1, I-2, and I-3. Fractions I-1, I-2, and I-3 were crystallized from ethyl acetate to give octadecadienamide (**8**, 220 mg), eicosadienamide (**9**, 150 mg), and eicosatrienamide (10, 75 mg), respectively. Fraction II was chromatographed over silica gel with a 9 : 1 mixture of hexane–$CHCl_3$ saturated with 28% NH_4OH to afford three fractions, II-1 (5.8 g), II-2 (4.0 g), and II-3 (6.8 g). Fraction II-2 was crystallized from EtOH to give piperine (**1**, 1.2 g). The mother liquor was crystallized from ethyl acetate–hexane to afford piperlonguminine (**4**, 750 mg). The residue was chromatographed on a Lobar column with 80% MeOH to give dihydropiperlonguminine (**5**, 120 mg) and pipercide (**6**, 35 mg). Fraction II-3 was chromatographed over sillica gel with a 9 : 1 mixture of hexane–$CHCl_3$ saturated with 28% NH_4OH to afford three fractions, II-3-1, II-3-2, and II-3-3. Fraction II-3-1 was chromatographed on a Lobar column using 75% MeOH to give pipernonaline (**2**, 55 mg) and piperundecalidine (**3**, 75 mg). Fraction II-3-2 was crystallized from ethyl acetate to afford guineensine (**7**, 280 mg). Fraction III was crystallized from hexane–$CHCl_3$ to afford piperlonguminine (**4**, 1.5 g).

Compound 2——mp 54.0—55.5 °C (ethyl acetate–hexane). *Anal.* Calcd for $C_{21}H_{27}NO_3$: C, 73.87; H, 7.97; N, 4.10. Found: C, 74.01; H, 7.93; N, 4.16. UV λ_{max}^{EtOH} nm (log ε): 259 (4.21), 305 (3.79). IR ν_{max}^{KBr} cm^{-1}: 1650, 1600, 1235, 1020, 970, 915. NMR δ: 1.28—1.74 (10H, br m), 2.00—2.36 (4H, br m), 3.50 (4H, br m), 5.90 (1H, d, J=15.0 Hz, C_2–H), 5.92 (2H, s, –O–CH_2–O–), 6.01—6.38 (2H, m), 6.73 (2H, br s, $C_{5',6'}$–H), 6.80 (1H, dt, J=15.0, 4.0 Hz, C_3–H), 6.88 (1H, s, $C_{2'}$–H). MS *m/z*: 341 (M^+), 206, 166 (base peak), 135, 112.

Compound 3——mp 64.5—65.5 °C (ethyl acetate–hexane). *Anal.* Calcd for $C_{23}H_{29}NO_3$: C, 75.17; H, 7.95; N, 3.91. Found: C, 74.91; H, 8.24; N, 3.68. UV λ_{max}^{EtOH} nm (log ε): 262 (4.53), 309 (3.84). IR ν_{max}^{KBr} cm^{-1}: 1650, 1625, 1595, 1290, 1050, 955, 930. NMR δ:1.24—1.72 (10H, br m), 2.00—2.30 (4H, br m), 3.54 (4H, br m), 5.92 (2H, s, –O–CH_2–O–), 6.02—6.38 (5H, m, $C_{2,4,5,10,11}$–H), 6.72 (2H, br s, $C_{5',6'}$–H), 6.88 (1H, s, $C_{2'}$–H), 7.24 (2H, dd, J=15.0, 10.0 Hz, C_3–H), MS *m/z*: 367 (M^+), 232, 135, 112 (base peak).

Oxidation of Compound 10 with Potassium Permanganate and Sodium Periodate—— The compound (10 mg) was oxidized by a conventional method and methylated with diazomethane. The resulting residue was subjected to GC-MS; the retention time and the mass spectrum of the product were identical with those of authentic methyl laurate. Retention time, 2.1 min; column, 1.5% OV-17 on Shimalite-W (80—100 mesh) (3m × 3.0 mm); column temp., 180—230 °C (3 °C/min); flow rate of carrier gas, 30 ml/min.

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