PEPEROMINS A, B AND C, NOVEL SECOLIGNANS FROM *PEPEROMIA JAPONICA*[1]

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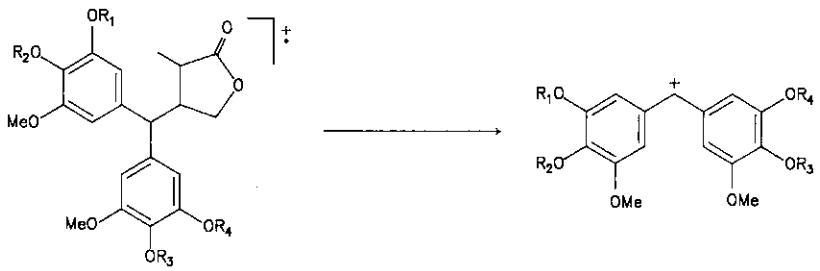
Abstract - *Three novel lignans having unusual seco structure, peperomins* A, B *and* C were isolated from the fresh whole plant of *Peperomia japonica* Makino (Piperaceae). The structures of peperomins A, B and C were established as I, II and III, respectively, on the basis of spectroscopic and crystallographic evidence.

Peperomia japonica Makino (Piperaceae) is a fleshy, perennial herb growing on wet rocks and trees from low altitudes up to 1500 m in Taiwan.[2] The aqueous and alcoholic decoctions of the whole herbs are used as a folk medicine for the treatment of malignant tumors.[3] Apiol, 2,4,5-trimethoxystyrene and phytosterols had been previously isolated from a species of *P. pellucida*,[4] but this species had not been so far investigated from a chemical point of view. As part of our continuing phytochemical study of the Formosan antitumor plants, we here describe the isolation of three novel lignans having unusual *seco* structure from this plant.

The methanol extract of the fresh whole herbs of *P. japonica* was fractionated on a charcoal column by eluting with methanol, *30% chloroform in methanol and chloroform, sequentially*. The concentrated methanol fraction was partitioned with water-ethyl acetate. Thereafter, the ethyl acetate layer was concentrated, dried *in vacuo* and chromatographed on a silica gel column with chloroform/methanol (15:1) as an eluent to afford three new compounds designated as peperomins A, B and C.

Peperomin A (I), colorless prisms (from methanol-water), mp 143-145°C, $[\alpha]_D^{27}$+20.6° (c=0.136 in $CHCl_3$), $C_{22}H_{22}O_8$ (m/z M^+ found 414.1314, calcd 414.1315) showed ir absorption bands due to aromatic ring (1630, 1510 and 1460 cm^{-1}) and a γ-butyrolactone (1770 cm^{-1}). The uv spectrum showed absorption maxima at 250 (log ε=3.96), 277 (3.51) and 285sh (3.41) nm, which indicated the presence of substituted benzene chromophore. The ^{1}H-nmr spectrum (400 MHz, in $CDCl_3$) exhibited the characteristic signals of two methylenedioxy groups attached on aromatic rings (δ 5.94 and 5.93, each 2H, s), two methoxy groups (δ 3.90 and 3.88, each 3H, s), one secondary methyl group (δ 0.95, 3H, d, J=7.2 Hz) and substituted aromatic rings (δ 6.30-6.50, 4H, m). Moreover, signals at δ 4.31 (1H, dd, J=9.5, 7.4 Hz) and δ 3.81 (1H, dd, J=9.5, 7.6 Hz) due to a methylene group of γ-butyrolactone moiety, and at δ 3.58 (1H, d, J=11.4 Hz, Ph_2-C<u>H</u>-CH-, H-5), 2.85 (1H, m, -CH-C<u>H</u>-CH_2O-, H-3) and δ 2.35 (1H, m,

-CH-CH-CH_3, H-2) ascribed to three methine groups were observed. All assignments of ^{1}H-nmr data were supported by appropriate spin decoupling experiments. In agreement with these assignments, the ^{13}C-nmr spectrum confirmed the presence of 22 carbons, and its chemical shifts are given in Table 1. In the mass spectrum, characteristic fragments were observed at m/z 414 (M^+), 315 and 285. The base peak at m/z 315 (M^+-99) due to the loss of an α-methyl-γ-butyrolactone moiety, resulted from benzylic cleavage as shown in Scheme 1.



I	R_1+R_2=CH_2, R_3+R_4=CH_2,	m/z 414 (M^+)	m/z 315
II	R_1=R_2=Me, R_3+R_4=CH_2,	m/z 430 (M^+)	m/z 331
III	R_1=R_2=R_3=R_4=Me,	m/z 446 (M^+)	m/z 347

Scheme 1

It was clearly confirmed that peperomin A (I) contains a methyl group at C-2 position and a bis-(3', 4'-methylenedioxy-5'-methoxyphenyl)methyl unit at C-3 position on a butyrolactone skeleton. However, due to the flexible nature of butyrolactone ring[5] and free rotation around the C-3/C-5 single bond, spectroscopic arguments are not reliable guides for the stereochemistry of these chiral centers.

Peperomin B (II), colorless prisms (from methanol-water), mp 143-145°C, $[\alpha]_D^{27}$+28.9° (c=0.444 in $CHCl_3$), $C_{23}H_{26}O_8$ (m/z M^+ found 430.1626, calcd 430.1627) showed following spectral data: uv λ_{max}^{MeOH}: 244 (log ε=3.99), 271 (3.41) and 285sh (3.23) nm; ir ν_{max}^{KBr}: 1770, 1600, 1510 and 1465 cm^{-1}; ^{1}H-nmr (400 MHz, in $CDCl_3$): δ 0.95 (3H, d, J=7.3 Hz, CH_3, H-6), 2.35 (1H, m, H-2), 2.90 (1H, m, H-3), 3.61 (1H, d, J=11.3 Hz, H-5), 3.83 (1H, dd, J=9.5, 7.9 Hz, H-4), 4.31 (1H, dd, J=9.5, 7.6 Hz, H-4), 3.80 (3H, s, OCH_3), 3.91 (9H, s, OCH_3x3), 5.94 (2H, s, OCH_2O), 6.40-6.50 (4H, m, H-2', 2", 6', 6"). The ^{13}C-nmr spectrum confirmed the presence of 23 carbons and its chemical shifts are given in Table 1. On account of the above data, peperomin B (II) showed the similar spectral properties as peperomin A (I), except that two methoxy groups of II appeared instead of the one methylenedioxy of I. These observations led to the formulation of structure II, which was further supported by the presence of a base peak at m/z 331 in the mass spectrum corresponding to formation of a doubly benzylic cation (Scheme 1).

Peperomin C (III), colorless needles (from methanol), mp 158-160°C, $[\alpha]_D^{27}$+42.7° (c=0.059 in $CHCl_3$), $C_{24}H_{30}O_8$ (m/z M^+ found 446.1939, calcd 446.1940) showed following spectral data: uv λ_{max}^{MeOH}: 241sh

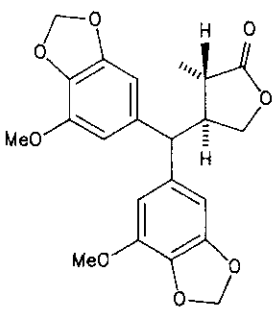
(log ε=4.09), 270 (3.30) and 280sh (3.12) nm; ir ν_{max}^{KBr}: 1775, 1600, 1515 and 1460 cm^{-1}; ^{1}H-nmr (400 MHz, in $CDCl_3$): δ 0.95 (3H, d, J=7.2 Hz, H-6), 2.37 (1H, m, H-2), 2.89 (1H, m, H-3), 3.53 (1H, d, J=11.3 Hz, H-5), 3.83 (1H, m, H-4), 4.31 (1H, dd, J=9.5, 7.5 Hz, H-4), 3.85, 3.86, 3.92 (each 6H, s, OCH_3x6) and 6.49 (4H, s, aromatic). Peperomin C (III) showed the similar spectral properties as peperomins A (I) and B (II) except that III possessed six methoxy groups and no methylenedioxy group. The ^{13}C-nmr spectrum confirmed the presence of 24 carbons and its chemical shifts are given in Table 1. The mass spectrum showed the base peak at m/z 347 (M^+-99), which resulted from benzylic cleavage (Scheme 1). Therefore, the structure III was reasonably assigned to peperomin C.

Final proof for the structure and stereochemistry of peperomin B (II) was obtained by X-ray crystallographic analysis. The crystal structure of peperomin B (II) was solved by direct methods. Blocks least-squares refinement of atomic positional and thermal (anisotropic C, N, O; isotropic H) parameters converged to R(F)=0.036 over 2191 reflections [I>3σ(I)] collected on an Enraf-Nonius CAD-4 four angles automated diffractometer under room temperature [MoK_α radiation, graphite monochromator, λ=0.7107 Å, ω/2θ scan, $2\theta_{max}$=60°]. All hydrogen atoms were located from Fourier difference syntheses map. Crystal data: $C_{23}H_{26}O_8$, M=430.46, monoclinic system, space group $P2_1$, a=11.044(1), b=9.500(2), c=11.696(2) Å, β=112.39(1)°, Z=2, V=1135(2) Å^3, D_{calc}=0.7585 g cm^{-3}. A computer perspective drawing of peperomin B (II) is shown in Figure 1.

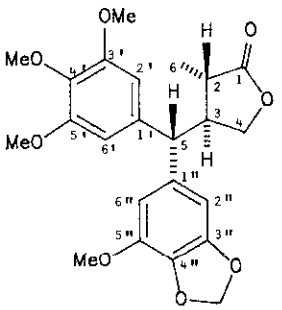
The cd spectra of peperomins A (I), B (II) and C (III) showed the positive Cotton effect at 275 ([θ]+11290), 277 ([θ]+5816) and 270 ([θ]+4460) nm, and the negative effect around 245-253 nm,[6] indicating I, II and III to have the 2*S*,3*S*-, 2*S*,3*S*,5*S*- and 2*S*,3*S*- configurations,[7] respectively.

In conclusion, the chemical structures including absolute configurations of peperomins A, B and C were established as (2*S*,3*S*)2-methyl-3-[bis(3',4'-methylenedioxy-5'-methoxyphenyl)methyl]butyrolactone (I), (2*S*,3*S*,5*S*)2-methyl-3-[5-(3',4',5'-trimethoxyphenyl)-5-(3",4"-methylenedioxy-5"-methoxyphenyl)methyl]butyrolactone (II) and (2*S*,3*S*)2-methyl-3-[bis(3',4',5'-trimethoxyphenyl)methyl]butyrolactone (III), respectively.

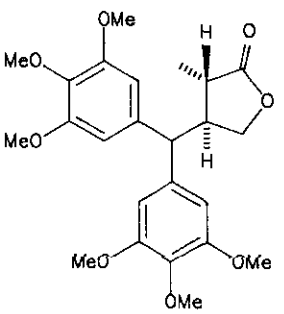
Peperomins A, B and C are rare examples of the naturally occurring secolignans.[8] Biogenetically, the occurrence of these secolignans in plant is quite significant.



I



II



III

Table 1. ^{13}C-Nmr Chemical Shifts and Assignments for Peperomins A (I), B (II) and C (III). (ppm)

C	I	II	III
1	179.15	179.23	179.22
2	46.90	46.99	47.17
3	40.04	40.08	40.17
4	70.10	70.14	70.23
5	55.86	56.07	56.50
6	15.63	15.70	15.76
1'	135.81	135.82	136.77
1"	135.81	133.95	136.77
2'	100.86	104.47	104.42[a)]
2"	100.86	100.90	104.54[a)]
3'	148.97	153.05	153.10
3"	148.97	149.06	153.10
4'	136.39	137.34	137.21
4"	136.39	136.87	137.21
5'	143.12	153.05	153.10
5"	143.12	143.26	153.10
6'	107.47	104.47	104.42[a)]
6"	107.47	107.63	104.54[a)]
OCH_2O	101.11(x2)	101.19	
OCH_3	56.50(x2)	56.07(x2)	56.12(x4)
OCH_3		56.78	60.69(x2)
OCH_3		60.64	

Solvent: $CDCl_3$. a) Assignments may be reversed.

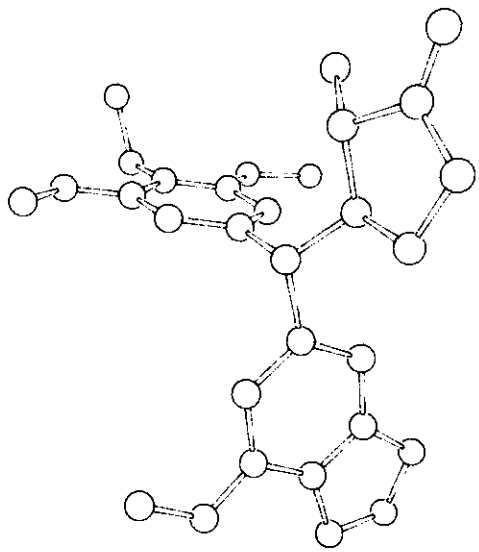


Figure 1. Perspective View of Peperomin B (II).

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REFERENCES AND NOTES

1. Part II "Constituents of Formosan Antitumor Folk Medicine". Part I: M.-T. Chen and C.-M. Chen, *Heterocycles*, 1985, **23**, 2543. A part of this work was presented at the 15th IUPAC International Symposium on the Chemistry of Natural Products, 17-22 August, 1986, The Hague, The Netherlands.
2. H. L. Li, T. S. Liu, T. S. Huang, T. Koyama, and C. E. DeVol, ed., "Flora of Taiwan", Vol. II, Epoch Publishing Co. Ltd., Taipei, 1976, p. 557.
3. Kiangsu New Medical College, ed., "Dictionary of Chinese Herbal Drugs", Shanghai Science and Technology Press, Shanghai, 1978, p. 622.
4. J. B. Manalo, B. H. Han, Y. N. Han, M. H. Park, and F. E. Anzaldo, *Arch. Pharm. Res.*, 1983, **6**, 133.
5. S. A. M. T. Hussain, W. D. Ollis, C. Smith, and J. F. Stoddart, *J. Chem. Soc., Perkin Trans. I*, 1975, 1480.
6. The cd spectra of I, II and III showed the negative effect at 248 ([θ]-4156), 253 ([θ]-415) and 245 ([θ]-1561) nm, respectively.
7. R. S. Burden, L. Crombie, and D. A. Whiting, *J. Chem. Soc. (C)*, 1969, 693.
8. P. Satyanarayana, P. Subrahmanyam, and K. N. Viswanatham, *J. Nat. Prod.*, 1988, **51**, 44.

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