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# Studies on Ether-Soluble Neutral Compounds of *Peperomia pellucida*

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**Abstract** □ From ether-soluble neutral fraction of the whole plant of *Peperomia pellucida* (L) HBK (Piperaceae), 4,7-dimethoxy-5-(2-propenyl)-1, 3-benzodioxole or apiol, in a liquid state, 2,4,5-trimethoxy styrene, mp 138°, and three phytosterols, campesterol, stigmasterol and β-sitosterol, were isolated and characterized by spectral data.

**Keywords** □ *Peperomia pellucida*, Apiol, 2,4,5-Trimethoxystyrene, Phytosterols.

*Peperomia pellucida* (Linne') HBK, is one of the members of the Family Piperaceae which is of both pharmaceutical and medicinal interest. It is a wild growing species found in Northern Luzon to Mindanao in damp and humid places at low and medium altitudes. It is believed to be a native of Tropical America, and to have been introduced in the Philippines in earlier times[1].

Olasiman-bato, as it is commonly known in the Philippines, is used as medicine for a number of ailments[2]. The whole plant is used as a warm poultice for abscesses and boils[1], while in Tropical West Africa it is used also as an ingredient in medicinal infusions for the treatment of convulsions[1].

Ordinarily, Peperomia is eaten raw like lettuce, it may be a substitute for vegetables as a source of vitamins with the advantage of being easily available. It is always highly valued in the rural areas as a nourishing food and a cure for such ailments as indigestion and muscular aches[1].

*Peperomia pellucida* has been investigated for the presence of essential oils[3]. In the screening of plants for antibacterial activity initiated at the Medical Research Center, National Institute of Science and Technology, Masilungan[4] reported that the petroleum ether extract showed significant antibacterial activity. No other study, however, on the other constituent of the species has been reported yet. For this reason, we attempted to isolate some of the neutral ether-soluble components.

*Peperomia pellucida* (Fig. 1), is an annual, very succulent, erect, branched herb, 5 to 40cm,

**Fig. 1**: Habit of *Peperomia pellucida* (L.) HBK. taken at the College of Agriculture, los Banos, Laguna. It thrives best at damp places.

high, the stems are rounds, oten 5mm thick, pale green. Leaves are ovate, acute or obtuse, base broads, cordate, pale green, pellucid, shinning, 1 to 3cm long. They are alternate, opposite or whorled, entire and are without stipules. Spikes green, erect, slender, 1 to 6cm long, the fruits globose, brownish, less than 1mm thick. Very common in damp shaded places, on damp walls etc. and it is found almost throughout the year.

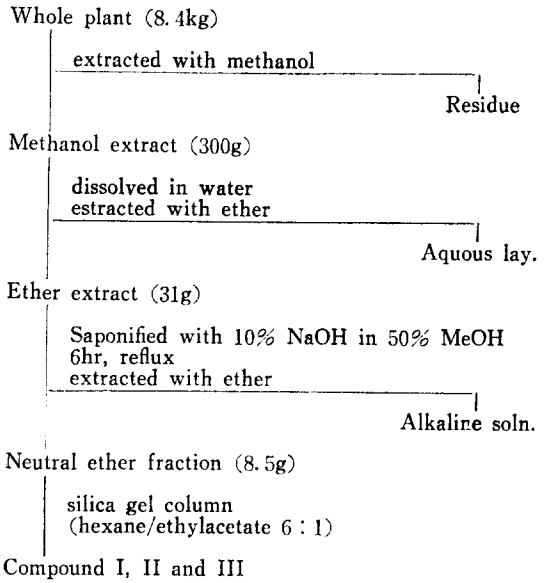
## EXPERIMENTAL METHODS

### *Fractionation of Neutral Ether Soluble Compounds*

The plant Peperomia was collected from the University of the Philippines compound, in the College of Agriculture, U.P. Los Banos in Laguna and within the vicinities of Metro Manila. When the plant was fully matured, it was harvested, washed, dried, cut into small pieces and weighed. The whole plant (8.4kg) were extracted with methanol for 48 hrs at room temperature, and concentrated in vacuo to give a syrupy extract (300gm). The methanol extract was fractionated in order to separating the neutral ether-soluble fraction as shown in Scheme I.

### *Isolation of Compound* I, II *and* III

Separation and isolation of the neutral ether-soluble compounds were done by silica gel column chromatography using the solvent system of hexane-ethylacetate (6 : 1). The eluates were examined by thin-layer chromatography on precoated silica gel $GF_{254}$ (E. Merck) plates, using hexane-ethylacetate (4 : 1) as the developing solvent system. Visualization of the chromatograms was done UV light and by 10% sulfuric acid spray reagent. By these separation procedures, three compounds designated I, II and III were isolated.




**Scheme I:** Extraction and isolation of three compounds.

### *Instrumental Analysis*

All melting points were taken on a Mitamura heat block apparatus and given uncorrected values. A recording spectrophotometer, Gilford type 2600 was used for the measurements of UV-visible absorption spectra. PMR spectra were obtained in $CDCl_3$ solution using TMS as internal standard on Perkin-Elmer NMR spectrometer (90HMz) and recorded by $\delta$ppm. IR spectra were determined in KBr pellets on Perkin-Elmer type 283 B spectrophotometer. Gas-liquid chromatograms were obtained on a Pye-Unicam type 104 chromatograph. Mass spectra were obtained on a Hewlett Packard GC/Mass spectrometer (type 5985B) using an electron impact method.

## RESULTS AND DISCUSSION

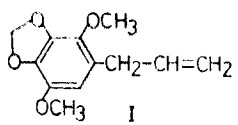
Thin-layer chromatogram of the neutral ether fraction showed the presence of at least five components. Three of these were isolated by

silica gel column chromatography. The first eluted compound I purified was obtained as a liquid state (parsley oder) and its R*f* value was 0.48. The second isolated compound II, mp 138°, had an R*f* value of 0.29 and the third compound III, mp 144°, had an R*f* value of 0.14.

*Identification of Compound* I

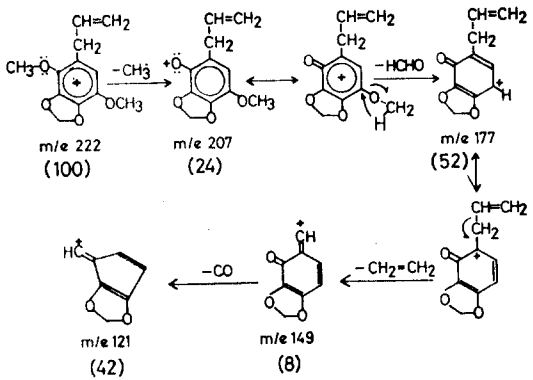
Compound I showed a purple color by Liebermann-Buchard reagent and did not react with Pauli's reagent. UV adsorption maximum of I was at 288.5 nm ($E_{1\%}$ 124) and was not shifted at alkaline phase. These suggest that I is aromatic but dose not have a phenolic hydroxyl group.

Mass spectrum of I showed a molecular ion (base peak) at m/e 222, giving a possible molecular formula $C_{12}H_{14}O_4$. IR spectrum of I showed absorption bands at 3070, 1638, 1623, 1618, 1500cm$^{-1}$ (phenyl ring); 995, 912cm$^{-1}$ (vinyl group); 1460, 1415, 1080cm$^{-1}$ (methoxyl group attached to benzene ring); 2780, 1480, 1240, 1050, 920cm$^{-1}$ (methylene dioxy) and 880cm$^{-1}$ (isolated hydrogen of benzene ring). In PMR spectrum I displays fourteen hydrogens $\delta_{ppm}^{CDCl_3}$ ;3.27 (2H, multi-d, $-CH_2-C=C$); 3.75, 4.0 ($2\times3$H, s, $-(OCH_3)\times2$); 4.95, 5.10, 5.70—6.15 (3H, ABC system, $H_C>C=C<^{H_A}_{H_B}$); 5.86 (2H, s, methylene dioxy) and 6.35 (1H, s, aromatic isolated hydrogen). These properties of I may give the following structure.



I

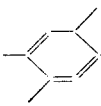
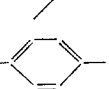
The possible mass fragmentation pattern of I is shown in Scheme II. The mass spectrum of I was idential with that of 4,7-dimethoxy-5 (2-propenyl)-1,3-benzodioxole or apiol referred to the library of the mass spectrometer.

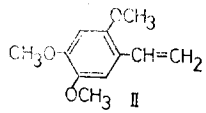



**Scheme II:** Possible mass fragmentation of apoil, 4, 7-dimethoxy-5 (2-propenyl)-1, 3-benzodioxole.

*Identification of Compound* II

Compound II gave a red to yellowish color by Liebermann-Buchard reagent and did not react with Pauli's reagent. UV absorbtion maxima of I were at 259.5nm ($E_{1\%}$ 690) and 314nm ($E_{1\%}$ 540) and was not shifted at alkaline phase. These suggest that II is aromatic but does not have a phenolic hydroxyl group,

Mass spectrum of II showed a molecular ion (base peak) at m/e 194, giving a possible formula $C_{11}H_{14}O_3$. IR spectrum of II showed the presences of a symmetic tetra-substituted benzene ring (3085, 1620, 1510cm$^{-1}$; benzene ring, 1800, 1720, 855, 840, 815cm$^{-1}$, —  —), a vinyl group (990, 895cm$^{-1}$) and methoxyl groups (1335, 1215, 1030cm$^{-1}$). Proton NMR gave fourteen hydrogens $\delta_{ppm}^{CDCl_3}$ ;3.86 and 3.88 ($3\times$ 3H, s,  $-(OCH_3)\times3$), 5.15, 5.58 and 6.83 to 7.17 (3H, ABX system, $H_X-C=C<^{H_B}_{H_A}$), 6.52 and 7.03 ($2\times1$H, s, H—  —H). From these results the chemical structure




II

of compound II is proved to be 2, 4, 5, -trimethoxy styrene.

*Characterization of Compound* III

Compound III, mp 144°, showed a red to bue color by Liebermann-Buchard reagent, and gave the typical pattern of phytosterols in IR spectrum. Further characterization of compound III was performed by gas-liquid chromatography under the conditions as shown in Fig. 2, comparing to the authentic phytosterol samples. Compound III is turned to be a mixture of three phytosterols, campesterol, stigmasterol and $\beta$-sitosterol.

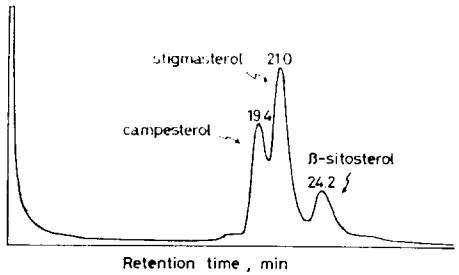



**Fig. 2**: Gas chromatogram of compound III. column, OV-1 (4mm×1.5m); temperature, column, 240°, FID detector 260°; flow rate, $N_2$ 40m*l*/min.

## ACKNOWLEDGEMENT

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