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Studies on the Constituents of Philippine *Piper betle* Leaves

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Abstract□Fourteen volatile components including eight allypyrocatechol analogs were isolated and identified from the essential oil and ether soluble fraction of Philippine *Piper betle* leaves (Piperaceae). The major constituents of Philippine *Piper betle* oil were chavibetol and chavibetol acetate. Capillary GC analysis of the oil showed chavibetol (53.1%), chavibetol acetate (15.5%), caryophyllene (3.79%), allypyrocatechol diacetate (0.71%), campene (0.48%), chavibetol methyl ether (= methyl eugenol, 0.48%), eugenol (0.32%), α-pinene (0.21%), ß-pinene (0.21%), α-limonene (0,14%), safrole (0.11%), 1,8-cineol (0.04%), and allylpyrocatechol monoacetate. The major component of the ether soluble fraction was allylpyrocatechol (2.38% of the leaves).

Keywords□*Piper betle*, Piperaceae, Essential oil, Chavibetol, Chavibetol acetate, Caryophyllene, Allypyrocatechol diacetate, Campene, Chavibetol methyl ether, Eugenol, α-Pinene, ß-Pinene, α-Limonene, Safrole, 1,8-Cineol, Allypyrocatchol monoacetate, Allypyrocatechol.

Piper betle Linne (Piperaceae), commonly known in the Philippines as Ikmo, is a dioecious vine which thrives best under tropical forest conditions having a cool shade, considerable humidity, and a good supply of soil moisture. It is cultivated for its leaves. The leaf mixed with lime and areca nut is being used as a stimulant masticatory in India and the East Indies.[1,2] In the Philippines, the leaves together with lime and areca nut also constitute a masticatory in general use among the Filipinos who consider it a preservative of the teeth and a prophylactic against certain complaints of the stomach. The Filipinos use the fresh crushed leaves externally as an antiseptic for cuts and wounds and as a poultice for boils. The leaves when greased with lard or sesame oil are much used by Filipinos as an application to the abdomens of children suffering from flatulence. The juice of the leaves is regarded as a valuable stomachic.[3]

Scientific studies on the pharmacological activity of *Piper betle* showed hypotensive, cardiac and respiratory depressant effects, smooth and skeletal muscles relaxant actions, antimicrobial, fungicidal and nematocidal activity.[4, 5, 6]

The important constituents which determine the value of the leaf for chewing are the essential oil and the sugars. The oil consists of phenols and terpenes, their relative proportions varying with the origin of the leaves. Chavibetol is considered to be the characteristic constituent, however, betel oils of the Indian types contain eugenol as the predominant phenolic constituent (30-90%).[1,7,8,9]

Differences in findings on the constituents of *Piper betle* oil triggered a check on the constituents of the Philippine variety of *Piper betle*. In our study we could identify and determine 14 volatile components including eight allylpyrocatechol analogs (Fig. 3) in the essential oil and ether soluble fraction of the *Piper betle* leaves. Unlike Indian *Piper betle* oil, the major constituents of Philippine *Piper betle* oil were chavibetol (53.1%) and chavibetol acetate (15.5%). The major component of the leaf was allylpyrocatechol (2.38% isolated yield).

EXPERIMENTAL METHODS

Materials

The leaves of *Piper betle* were collected from the U.P. College of Pharmacy, pharmacognosy garden, U.P. Diliman, Quezon City, Philippines. Only leaves numbers 2 to 7 were harvested, the numbering starting from the first expanded leaf from the tip of the stem. The leaves were air-dried for two days.

Standard eugenol, eugenol acetate and safrole were purchased from Tokyo Kasei Co., Ltd., Japan. Caryophyllene, campene, α-pinene, ß-pinene, d-limonene

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and 1,8-cineol were generous gift from Pacific Chemical Co. Inc., Seoul, Korea.

Instruments

^{1}H-NMR measurements were obtained in $CDCl_3$ solution with Varian Model FT-80A NMR spectrometer (80MHz). GC/MS spectrum was recorded on Hewlett-Packard Model 5985-B GC/MS system. IR spectrum was taken on a Perkin-Elmer model 281-B. Preparative LC was carried out on Waters model Prep-LC-500A using PrepPAK-500/C_{18} column. Gas chromatographic analysis was carried out on Hewlett-Packard model 5840A GC using Carbowax 20M fused silica capillary column (0.2mm i.d. x 25 m).

Isolation of the constituents

Dried leaves of *Piper betle* (370g) were used for the steam distillation (3.4ml oil), and the residue from the steam distillation was extracted exhaustively with hot ethanol and the ethanol extract was concentrated in vacuo to give alcoholic extract (172g). The alcoholic extract was fractionated between ether and water.

The steam distilled volatile oil was subjected to flash column chromatography on silica gel (Merck Art. 7734) using the solvent system Haxane/Ethylacetate, in a stepwise-gradient elution (100/1, 40/1, 20/1, 10/1, 2/1). Six fractions were collected after TLC examination. Isolation of the constituents was done by preparative TLC using the solvent system Hexane: Chloroform: Methanol: Acetic acid (15:5:1:0.2, 7:5:1:0.2 or 5:5:1:0.2).

The ether extract was concentrated and subjected to flash column chromatography on silica gel (Merck Art 7734) using the solvent system Hexane/Ethylacetate

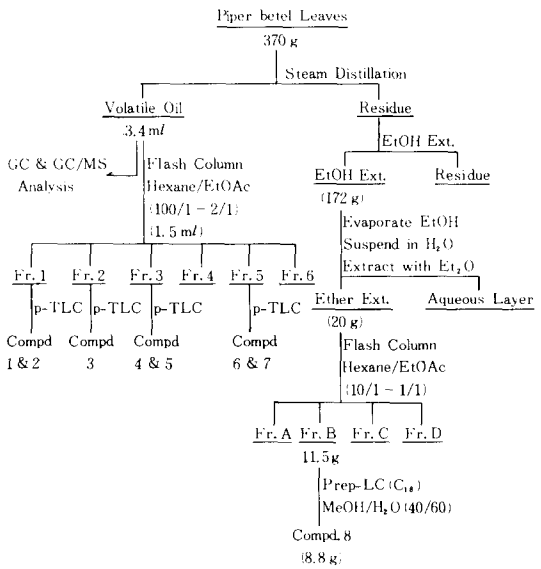

Fig. 1. Scheme for the Isolation of the Constituents of the Leaves of *Piper betle* Linne (Piperaceae).

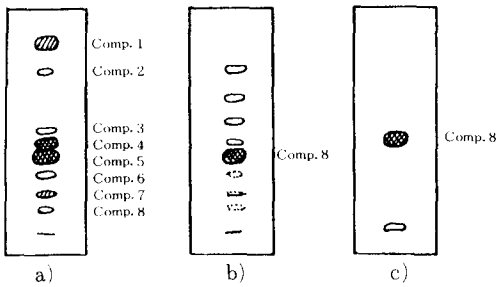

Fig. 2. TLC pattern of *Piper betle* oil and ether-soluble fraction (H_2SO_4 spray).

(a) *P. betle* oil, silica gel, Hexane/EtOAc = 4:1

(b) Ether-soluble fraction, Silica gel, Hexane/$CHCl_3$/MeOH/AcOH = 5:5:1:0.2

(c) Fraction B, HPTLC (RP-18), MeOH/H_2O = 60:40

(10/1, 8/1, 6/1, 4/1, 1/1). Four fractions were collected after TLC examination. (Fig. 1)

RESULTS AND DISCUSSION

The TLC of Piper betle oil showed at least eight spots, each labelled compound I to VIII in the order of decreasing Rf values as shown in Fig. 2. Of these, seven compounds were isolated. These were oily liquids having characteristic odors except compound VIII which was obtained as crystal. Compounds IV and V predominated on the TLC. These were identified to correspond to peaks No. 48 and 50, respectively, in the gas chromatogram (Fig. 4). Compound VIII was the minor component in the essential oil fraction, however, it was the most predominant component in the ether-soluble fraction.

Compound V (Chavibetol):

Compound V was an oily liquid having a characteristic odor. It was UV-positive, gave a red spot with Pauly's reagent, a green spot with $FeCl_3$ spray, suggesting phenolic compound, and a red-violet spot when heated after H_2SO_4 spray.

PMR ($CDCl_3$): δppm, 3.20 (2H, d, J = 7Hz, $-CH_2-$), 3.75 (3H, s, $-OCH_3$), 4.8-5.1 (2H, m, $C=CH_2$), 5.6-6.1 (1H, m, $-CH=C$), 6.5-6.8 (3H, m, aromatic-H), 5.5 (s, OH).

IR: 3600-3300 cm^{-1} (OH)

Mass: m/z (%), 164 (100, M^+), 149 (49, $M^+ - CH_3$), 137 (12, M-$CH=CH_2$), 131 (18), 121 (19), 103 (23). 91 (18), 77 (12).

Mass spectrum of this compound was almost the same with that of standard eugenol, but aromatic region in PMR and retention time in GC were different. Methylation of this compound with diazomethane gave the same product with methylated eugenol on NMR, IR, Mass and GC (Fig. 3). Compound V was identified as chavibetol, and isomer of eugenol.

Compound IV (chavibetol acetate)

Compound IV was an amber yellow oily liquid with a characteristic odor. It was positive with UV and gave a red-violet spot with H_2SO_4 spray. Its mass spectrum showed molecular ion peak at m/z 206 (9%) and base peak at m/z 149 (M^+-CH_3-42). NMR spectrum was similar to that of chavibetol (compound V), except for the appearance of the peak at δ2.24 (3H, s, -$COCH_3$) instead of the peak due to the hydroxyl proton. Acetylated product of chavibetol gave superimposable NMR and Mass spectra with those of compound IV. Compound IV was identified as chavibetol acetate.

Compound III (Methyl chavibetol)

Mass spectrum of this compound showed base peak at m/z 178 (100, M^+), and 163 (38, M-CH_3), 147 (32). NMR spectrum was also similar to that of chavibetol, but six protons were recorded for the methoxy proton at δ 3.85 and hydroxyl proton was disappeared. Methylation of chavibetol and eugenol gave superimposable NMR and Mas spectra with those of compound III (Fig. 3). Compound III proved to be methylchavibetol (= methyl eugenol).

Compound II (Safrole):

Mass spectrum showd m/z 162 (100), 135 (22), 131 (32) and 104 (37). NMR spectrum showed three aromatic protons, five allyl protons and two-proton singlet at δ 6.01 suggesting methylene dioxy moiety. Spectral data, as well as GC co-injected with the standard safrole, proved it to be safrole.

Compound VI (Allypyrocatechol diacetate):

Compound VI was also an oily liquid, UV-positive, and gave a red spot with H_2SO_4 spray. Its mass spectrum showed a molecular ion peak at m/z 234 (3%), and two other dominant peaks at 192 (18%, M^+-42) and 150 (100%, M^+-84) which suggested the removal of one and two acetyl groups, respectively. NMR spectrum was almost the same as that of chavibetol acetate, but without a shift for methoxyl proton. The integrator recorded six protons for the acetyl proton at δ2.20, instead. Compound VI gave the structure of allypyrocatechol diacetate and which was further confirmed by comparison of spectral and chromatographic data with acetylated compound VIII.

Compound VII (Allypyrocatechol monoacetate):

Compound VII was positive with UV, gave a red spot with H_2SO_4 spray, and a red-orange spot with Pauly's reagent, which indicated a phenolic compound. Its mass spectrum showed a molecular ion peak at m/z 192 (19%), base peak at m/z 150, which arose from the removal of one acetyl group, and other peaks at m/z 131 (32%), 132 (30%) and 104 (23%). It showed almost the same spectrum as that of allylpyrocatechol diacetate except for the absence of the peak at m/z 234. D_2O-treatment[10] of compound VIII shifted molecular peak to 193 and base peak to 151, which suggested one hydroxyl group. This compound, however, was decomposed after mass spectral analysis thus NMR analysis cannot be used as a diagnostic tool for its structural elucidation. Available data of compound VII strongly suggested it to be allylpyrocatechol monoacetate.

Compound VIII (Allylpyrocatechol):

Compound VIII, isolated from ether-soluble ethanol fraction by preparative LC using reverse phase column (Pre PAK-500/C_{18}, Waters Associates, U.S.A), occurred in white needles (in benzene/petroleum ether, in refrigerator), mp. 45-46°C (reference[11] 48-48.5°C), gave positive reactions with Pauly's and $FeCl_3$ reagents. Its mass spectrum gave a molecular ion peak at m/z 150 (100%) and other peaks at m/z 131 (48), 123 (42), 104 (38), 103 (45) and 77 (36). Molecular ion peak was shifted to m/z 152 upon D_2O treatment[10], suggesting two hydroxy groups. Its NMR spectrum showed three aromatic protons, allylic protons pattern, and a broad singlet at δ 6.0, which disappeared upon D_2O treatment.

Methylation of this compound gave a superimposable NMR and Mass spectra with standard methyl eugenol, and acetylation also gave a superimposable spectra with compound VI (Fig. 3). Compound VIII was proven to be allylpyrocatechol.

Compound I:

While this so-called "Compound I" appeared to be a single spot in the TLC, it was found to be a mixture of several compounds having molecular weights 136 and 204. Seven compounds were identified and determined by comparing GC and GC/MS data with standard compounds.

These were α-pinene, ß-pinene, camphene, d-limonene, 1,8-cineole, p-cymene, and caryophyllene (Table I and Fig. 2)

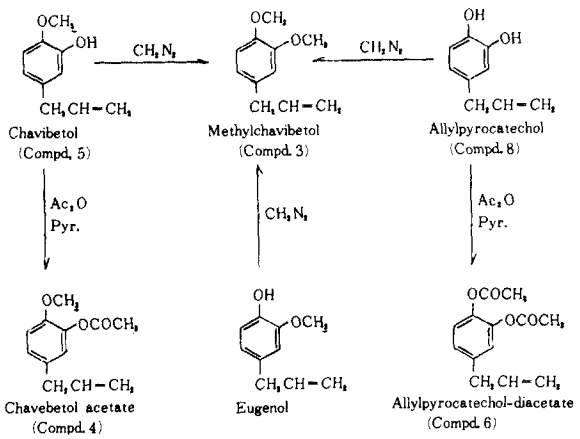

Fig. 3. Chemical Derivatization and Interrelationship of Allylpyrocatechol-derived Constituents of the Leaves of *Piper betle* Line (Piperaceae).

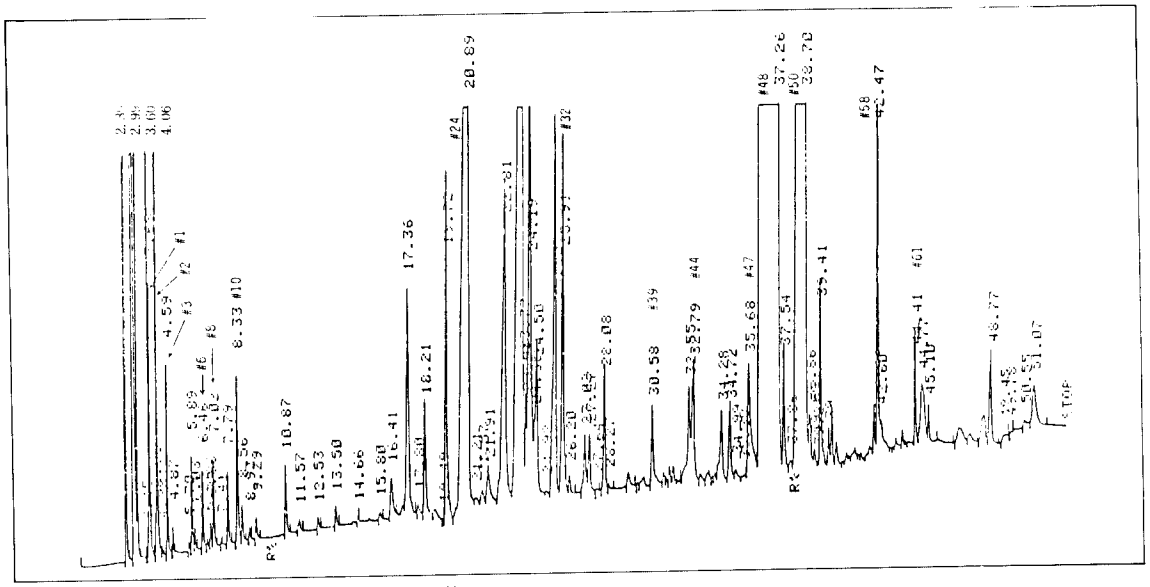

Fig. 4. Gas Chromatogram of *Piper betle* oil.
Column: Carbowax 20M Fused Silica Capillary Column (0.2*mm* i.d. × 25*m*).
Temperature Programme from 50°C (4 min), Rate 2°C/min until 10 min, 4°C/min after 10 min, 8°C/min after 38 min until 220°C. Carrier gas: Helium ($\bar{\mu}$=17.4 cm/sec). Inj. Temp.: 240°C. FID Temp.: 280°C.

Table I. Identified Constituents of *Piper betle*

Peak No.*	Retention time	Compound Name	Formula	MW	Content** (%)
1	3.68	*alpha*-Pinene	$C_{10}H_{16}$	136	0.21
2	4.06	Camphene	$C_{10}H_{16}$	136	0.48
3	4.59	*beta*-Pinene	$C_{10}H_{16}$	136	0.21
6	6.46	*d*-Limonene	$C_{10}H_{16}$	136	0.13
8	7.02	1,8-Cineole	$C_{10}H_{18}O$	154	0.03
10	8.33	*para*-Cymene	$C_{10}H_{14}$	134	0.08
24	20.89	Caryophyllene	$C_{15}H_{24}$	204	3.79
39	30.58	Safrole	$C_{10}H_{10}O_2$	162	0.11
44	32.79	Methyl chavibetol	$C_{10}H_{14}O_2$	178	0.72
47	35.68	Eugenol	$C_{10}H_{12}O_2$	164	0.42
48	37.26	Chavibetol	$C_{10}H_{12}O_2$	164	53.10
50	38.70	Chavibetol acetate	$C_{12}H_{14}O_3$	206	15.50
58	42.47	Allylpyrocatechol diacetate	$C_{13}H_{14}O_4$	234	0.61
61	44.41	Allypyrocatechol monoacetate	$C_{11}H_{12}O_3$	192	
		Allylpyrocatechol	$C_9H_{10}O_2$	150	2.38***

*Refer to Fig. 4
**Content in *Piper betle* oil
***Content in dried leaves

GC analysis of *Piper betle oil*

The results of gas chromatographic analysis of *Piper betle* oil are shown in Fig. 4 and Table I.

Identification of the peaks was done by co-injection of standard compounds with the volatile oil and by comparison of GC/MS spectral data.

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