SOME CONSTITUENTS OF THE STEMS OF *PIPER INTERRUPTUM* OPIZ

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ABSTRACT

From the stems of Piper interruptum *Opiz, crotepoxide (1) was isolated, together with eupomatene (2) and pipercallosine (3). The compounds were identified by physical methods and their ^{13}C NMR spectra were analyzed by using selective irradiation techniques.*

INTRODUCTION

Piper interruptum Opiz is a wild climber distributed in the eastern part of Thailand and used as an anti-flatulent. It has been reported that young leaves of the plant are warmed and applied as a poultice or as an emollient to the throat to relieve coughing.[1] Another source reports[2] that the leaves have a high oil content and suggests that it should be antiseptic. However, up to the present time nothing has been reported about the chemical composition of this species. The present investigation deals with the chemical constituents of *Piper interruptum* Opiz.

Extraction of the stems of *Piper interruptum* Opiz (Thai name, "Sa-Khan") with hexane gave crotepoxide **(1)**, a naturally occurring oxirane which has been shown to inhibit tumor formation.[3] Crotepoxide is found in the fruits of *Croton macrostachys* Hochst[4] (Euphorbiaceae), *Kaempferia rotunda* Linn[5] (Zingiberaceae), *Boesenbergia* sp.[6] (Zingiberaceae) and various members of Piperaceae.[7-9]

Besides crotepoxide **(1)**, eupomatene **(2)** and pipercallosine **(3)** were also isolated.

The ^1H NMR spectrum of **1** showed signals due to the methyl groups of the two acetate residues at δ 2.03 and 2.12. The two doublets of an AB quartet assigned to the C-7 geminal protons appeared between δ 4.23 and 4.58. The resonances of five methine protons were observed at δ 3.09, 3.45, 3.67, 5.01 and 5.73. Resonances for the five aromatic protons were observed between δ 7.46 - 8.04. Additional structural information was obtained from the attached proton test[10] and off-resonance decoupled ^{13}C NMR spectrum of **1**, which confirmed the presence of two methyl groups (q, δ 20.13 and 20.20), five tertiary carbons (d, δ 47.74, 52.31, 53.33, 69.36 and 70.12), one

methylene group (t, δ 62.06), one quaternary carbon (s, δ 59.27), monosubstituted benzene (s, δ 128.96; d, δ 128.18, 129.37 and 133.09) and three carbonyl groups (s, δ 168.28, 169.31 and 169.56).

The ^{13}C NMR assignments were aided by selective irradiation of protons (Table 1).

Eupomatene (**2**) was obtained as colourless crystals,[11] m.p. 155-157°, and has the molecular formula $C_{20}H_{18}O_4$ as indicated by its molecular ion peak at m/e 323 ($M^+ + 1$) and its elemental analysis. The IR spectrum of **2** showed a *trans* disubstituted double bond (960 cm^{-1}).

The ^1H NMR spectrum of **2** showed signals for three methyl groups at δ 1.90 (d), 2.36 (s) and 4.01 (s). The methylenedioxy protons were observed at δ 5.97 (s). The resonances of two *trans* olefinic protons were observed at δ 5.84 (dd, J = 6.0, 15.0 Hz) and 6.62 (broad d, J = 15.0 Hz) and those for the five aromatic protons between δ 6.89 - 7.51 (m).

The attached proton test[10] and off-resonance decoupled ^{13}C NMR spectrum of **2** confirmed the presence of three methyl groups (q, δ 9.59, 18.42 and 56.13), one methylene group (t, δ 101.21) and two olefinic carbon atoms (d, δ 108.47 and 131.50).

The ^{13}C NMR assignments were aided by selective irradiation of protons (Table 2).

Pipercallosine (**3**) was obtained as colourless crystals,[12] m.p. 114-115°. The presence of this constituent was not surprising, as it has been previously isolated from other *Piper sp.*[12]

The ^1H NMR spectrum of **3** showed a signal corresponding to two methyl groups at δ 0.90 and methylenedioxy protons at δ 5.89. The resonances of ten methylene protons were observed at δ 1.30-1.50, 2.13, 2.52 and 3.14. The four olefinic and amide protons' resonances appeared between δ 5.68-6.11 and the isobutyl methine proton at δ 1.70. Resonances for the three aromatic protons were observed at δ 6.64-7.10.

The off-resonance decoupled ^{13}C NMR spectrum of **3** confirmed the presence of two methyl groups (q, δ 20.10), one tertiary carbon (d, δ 28.61), six methylene groups (t, δ 28.23, 31.15, 32.73, 35.43, 46.97 and 100.67), four olefinic carbons (d, δ 120.98, 128.57, 140.65 and 142.11), trisubstituted benzene (s, δ 136.21, 145.47, 147.48 ; d, δ 108.04, 108.74 and 122.39) and one carbonyl carbon (s δ 166.55).

The ^{13}C NMR assignments were aided by selective irradiation of protons (Table 3).

Stems of *Piper interruptum* Opiz were collected in the eastern part of Thailand, Rayong Province, in 1984. A voucher specimen (Y 1248-83) of the plant material has been lodged at Botanical section, Department of Agriculture, Kasetsart University, Bangkok, Thailand.

Melting points were determined by a Fisher-Johns apparatus, and are uncorrected. The IR spectra were recorded with a Shimadzu Infrared Spectrophotometer Model IR-440 using KBr discs. ^{1}H NMR and $^{13}CNMR$ spectra were recorded on a Jeol JNM-FX 90 Q NMR spectrometer operating at 90 and 22.5 MHz respectively unless otherwise indicated. All chemical shifts are expressed in ppm from TMS as the internal standard. The Mass spectra were obtained with a Jeol JMS-DX 300/JMA 2000 Mass Spectrometer. Elemental analysis were carried out on a 240C Perkin-Elmer elemental Analyzer at the Science and Technology Research Equipment Center, Chulalongkorn University, Bangkok, Thailand.

The finely chopped stems of *Piper interruptum* Opiz (7 Kg) were extracted with hexane. The resulting extract was concentrated to give 110 g of crude syrup. The crude syrup was triturated with ether and filtered. The solid residue was recrystallized four times from methanol to give 1 (crotepoxide) (5.47 g.) as white crystalline meterials, m.p. 149-150° (lit[4] 149-150° ; R_f 0.46 (silica gel/$CHCl_3$) ; IR (KBr, cm^{-1}) 3050, 2980, 1770 (C=O), 1750 (C=O), 1730 (C=O), 1600, 1580, 1500, 1470, 1375, 1280, 1240, 1120, 720; MS : m/e 363 ($M^+ + 1$).

Anal. Found : C, 59.77; H, 4.99. Calcd. for $C_{18}H_{18}O_8$: C, 59.67; H, 5.01%

The filtrate was evaporated to give a crude syrup (103g). The crude syrup was chromatographed on an alumina column (1.40 Kg), and eluted with hexane containing a gradually increasing proportion of chloroform. Successive fractions, based on TLC behavior, were combined, concentrated and recrystallized from $CHCl_3$ - hexane to yield **2** and **3** successively.

The colourless crystals (**2**) (eupomatene) (1.16g) have m.p. 155-157° (lit[11] 154-156°); IR (KBr, cm^{-1}) 2980, 1605, 1504, 1490, 1450, 1390, 1380, 1309, 1250, 1230, 1150, 1090, 1060, 1035, 960, 920, 890, 815 ; MS : m/e 322 (M^+).

Anal. Found : C, 74.48 ; H, 5.67. Calcd. for $C_{20}H_{18}O_4$: C, 74.53 ; H, 5.63%.

The colourless crystals (**3**) (pipercallosine) (1.11g) have m.p. 114-115° (lit[12] 115-116°) ; IR (KBr, cm^{-1}) 3300 (s, N-H), 3100-2800, 1660 (C=O), 1630, 1610, 1550, 1500, 1490, 1440, 1370, 1340, 1250, 1040, 1000, 930, 800 ; MS : m/e 329 (M^+).

Anal. Found : C, 72.98 ; H, 8.29 ; N, 4.37. Calcd. for $C_{20}H_{27}NO_3$: C, 72.92 ; H, 8.26 ; N, 4.25%.

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บทคัดย่อ

จากการแยกสารประกอบจากต้นสะค้าน *(Piper interruptum* Opiz)ได้ crotepoxide, eupomatene และ pipercallosine การพิสูจน์โครงสร้างของสารเหล่านี้อาศัยวิธีทางฟิสิกส์และการวิเคราะห์ทาง ^{13}C NMR ด้วยเทคนิคการเลือก irradiation.

TABLE 1. ^{1}H and ^{13}CNMR spectral data of crotepoxide (1)*

H	δ	C	δ	C	δ
2	5.73 d, 1H, J = 9.0	1	59.27 s	10	128.18 d
3	5.01 dd, 1H, J = 9.0, 1.5	2	69.36 d	11	129.37 d
4	3.09 dd, 1H, J = 3.9, 1.5	3	70.12 d	12	133.09 d
5	3.45 dd, 1H, J = 3.9, 2.7	4	52.31 d	13	129.37 d
6	3.67 d, 1H, J = 2.7	5	47.74 d	14	128.18 d
7	4.23 and 4.58 AB, 2H, J = 12.0	6	53.33 d	15^{+}	169.31 s
10, 14	8.04 ddd, 2H, J = 7.0, 1.7, 1.5	7	62.06 t	16^{Δ}	20.13 q
11,13	7.46 ddd, 2H, J = 7.3, 7.0, 1.5	8	168.28 s	17^{+}	169.56 s
12	7.61 tt, 1H, J = 7.3, 1.5	9	128.96 s	18^{Δ}	20.20 q

* Recorded ($CDCl_3$) at 200 and 50.1 MHz respectively with TMS as internal standard, δ values in ppm and J in Hz.

The assignment of two pairs of resonances (marked + and △) is arbitrary and could be vice versa within each pair.

TABLE 2. ^{1}H and ^{13}C NMR spectral data of eupomatene (2)*

H	δ	C	δ	C	δ
5,7,11,14,15	6.89-7.51 m, 5H	2	142.11 s	12	147.86 s
16	5.84 dd, 1H, J = 6.0, 15.0	3	110.47 s	13	147.37 s
17	6.62 broad d, 1H, J = 15.0	4	125.38 s	14^{+}	107.28 d
18	1.90 d, 3H, J = 6.0	5	120.98 d	15	124.29 d
19	4.01 s, 3H	6	133.01 s	16	131.50 d
20	2.36 s, 3H	7^{+}	109.17 d	17	108.47 d
21	5.97 s, 2H	8	144.88 s	18	9.59 q
		9	151.11 s	19	56.13 q
		10	133.66 s	20	18.42 q
		11^{+}	104.73 d	21	101.21 t

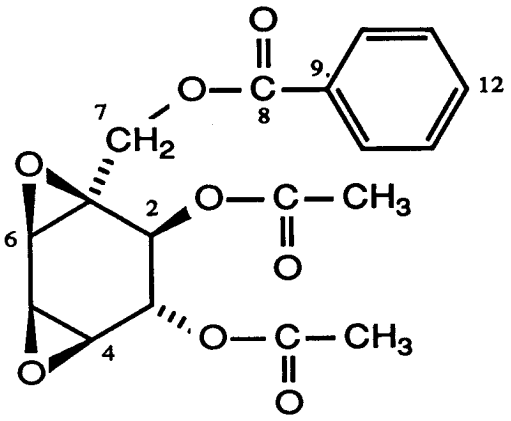
* Recorded in ($CDCl_3$) with TMS as internal standard, δ values in ppm and J in Hz.

The assignment of resonances marked + is arbitrary and could be vice versa.

TABLE 3. ^{1}H and ^{13}C NMR spectral data of pipercallosine **(3)***

H	δ	C	δ	C	δ
amide, 2,3,4,5	5.68,6.03-6.11 m, 5H	1	166.55 s	11^{+}	108.04 d
6	2.13 m, 2H	2	120.98 d	$12^{\triangle}$	147.48 s
7,8	1.30-1.50 m, 4H	3	142.11 d	$13^{\triangle}$	145.47 s
9	2.52 t, 2H, J= 7.3	4	128.57 d	14^{+}	108.74 d
11	7.10 s, 1H	5	140.65 d	15	122.39 d
14	6.67 d, 1H, J = 7.1	6	32.73 t	16	100.67 t
15	6.64 d, 1H, J = 7.1	7	28.23 t	17	46.97 t
16	5.89 s, 2H	8	31.15 t	18	28.61 d
17	3.14 t, 2H, J = 6.6	9	35.43 t	19	20.10 q
18	1.70 m, 1H	10	136.21 s	20	20.10 q
19,20	0.90 d, 6H, J = 6.6				

* Recorded in $CDCl_3$ with TMS as internal standard, δ values in ppn and J in Hz.
The assignment of two pairs of resonances (marked + and △) is arbitrary and could be vice versa within each pair.



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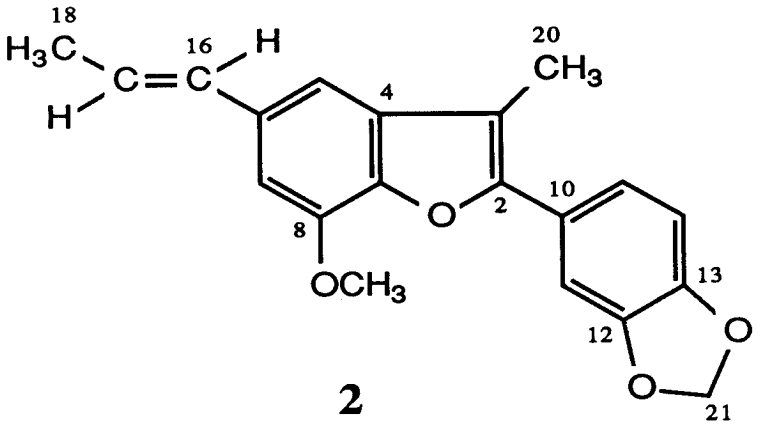

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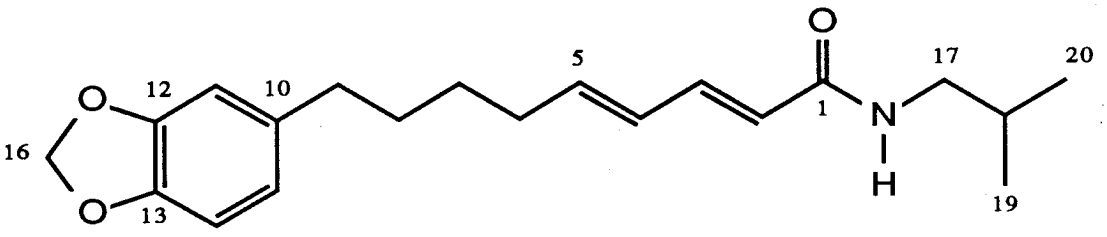

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