Chem. Pharm. Bull. 25(5)1026—1030(1977)

UDC 547.91.02 : 581.192

The Constituents of *Clitoria macrophylla* Wall. Cat., a Thai Medicinal Plant. The Structure of a New Rotenoid, Clitoriacetal

Heihachiro Taguchi,[1a] Panida Kanchanapee, and Thanomwang Amatayakul[1b]

Tsumura Laboratory[1a] and Department of Medical Sciences[1b]

(Received August 11, 1976)

A new rotenoid, clitoriacetal (I), has been isolated from the crude drug named "Non-tai-yak," which was identified as the root of *Clitoria macrophylla* Wall. Cat. (syn. *C. hanceana* Hemsl., Leguminosae) by the comparison with the authentic specimen, together with a known rotenoid, stemonacetal (II).

The structure of clitoriacetal was elucidated to be I by chemical and spectral analysis.

Keywords——*Clitoria macrophylla* Wall. Cat.; Leguminosae; rotenoid; clitoriacetal; stemonacetal

"Non-tai-yak" (หนอนตายยาก) is one of the crude drugs traditionally used for the treatment of skin diseases in Thailand. *Stemona tuberosa* Lour., *S. collinsae* Craib. and *S. burkillii* Prain. (Stemonaceae), which contain the alkaloids such as stemonine,[2] have been known as the original plants of "Non-tai-yak" and also there is a root of Leguminosae plant sold under the same name in the market. The crude drug obtained in Bangkok (1971) under the name of "Non-tai-yak", which did not contain any alkaloid, was identified to be the root of *Clitoria macrophylla* Wall. Cat. (syn. *C. hanceana* Hemsl., Leguminosae) by the pharmacognostical study comparing with the authentic specimen collected at the botanical garden, which belongs to Department of Medical Sciences in Thailand, in Chanthaburi province.[3a,b]

This paper concerns with the structure of a new rotenoid, named clitoriacetal (I) (yield, 0.8%), isolated together with a known rotenoid, stemonacetal (II) (yield, 0.037%),[4] from the chloroform extract of this crude drug.

Clitoriacetal (I), $C_{19}H_{18}O_9$, $[\alpha]_D^{28}$ +259.5°, which has been reported to possess significant antiinflammatory and antipyretic activity,[3a,c] was obtained as an amorphous powder and gives a brown colour with ferric chloride and a slightly orange colour with magnesium and hydrochloric acid after standing for 48 hr. The ultraviolet (UV) spectrum of I showed the absorptions at 230 (log ε, 4.44), 293 (4.32) and 320 (sh, 3.88) nm and the infrared (IR) spectrum showed the absorptions at 3400 (OH) and 1640 cm^{-1} (chelated >C=O). The proton magnetic resonance (PMR) spectrum (Table I) revealed the presence of three methoxyl groups, four aromatic protons, a hydrogen bonded hydroxyl group and two alcoholic hydroxyl groups.

On acetylation with acetic anhydride and pyridine, I afforded a triacetate (III), $C_{25}H_{24}O_{12}$, mp 123—126°, whose PMR spectrum revealed the presence of a phenolic acetoxyl group

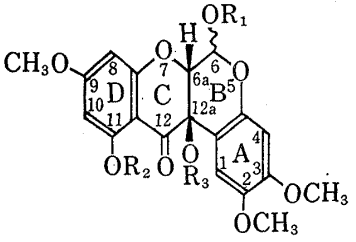
1) Location: *a) 1421, Izumi, Komae-shi, Tokyo, Japan; b) Yod-se, Bangkok, Thailand.*

2) R.H.F. Manske, "The Alkaloids," Vol. V, Academic Press, New-York, London, p. 322; *ibid.*, Vol. IX, p. 545.

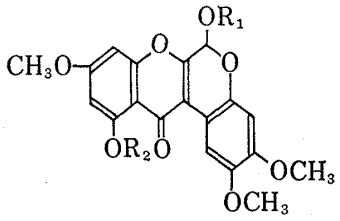
3) *a)* Thai Medicinal Plant Research Project, Division of Medical Research in Department of Medical Sciences, "The Bulletin of The Department of Medical Sciences," Vol. 14, No. 1, 1972, p. 1; *b)* H. Taguchi and Panida Kanchanapee, "Reports of The Thai Medicinal Plants Research Project, 1971—1973," Department of Medical Sciences, Ministry of Public Health, Bangkok, Thailand, 1974, p. 88; *c)* K. Watanabe, W. Ngarmwathana, K. Sawasdimongkol, P. Satravaha, and U. Permphiphat, *ibid.*, p. 9.

4) D. Shiengthong, T. Donavanik, V. Uaprasert, S. Roengsumran, and R.A. Massy-Westropp, *Tetrahedron Letters*, 1974, 2015.

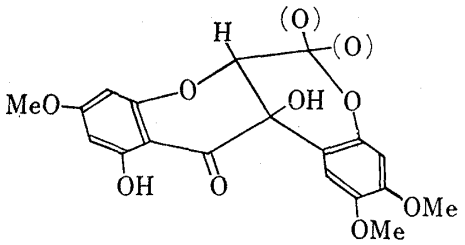
(δ 2.40) and two alcoholic acetoxyl groups (δ 2.16 and 2.28). Methylation of I with dimethyl sulfate and potassium carbonate gave a dimethyl ether (IV), $C_{21}H_{22}O_9$ (amorphous powder), $[\alpha]_D^{28}$ +235°, while the methylation with methyl iodide and silver oxide gave a trimethyl ether (V), $C_{22}H_{24}O_9$ (amorphous powder), $[\alpha]_D^{28}$ +115.4°.



I : R_1, R_2, R_3=H
III : R_1, R_2, R_3=$-COCH_3$
IV : R_1, R_2=CH_3 R_3=H
V : R_1, R_2, R_3=CH_3



II : R_1=CH_2CH_3 R_2=H
VI : R_1=CH_3 R_2=H
VII : R_1=CH_3 R_2=$-COCH_3$
VIII : R_1, R_2=CH_3
IX : R_1=CH_2CH_3 R_2=$-COCH_3$



1'

Fig. 1

TABLE I. PMR Spectral Data of I—V (in $CDCl_3$) and VI (in $CDCl_3+C_6D_6$=1:1) (δ Value)

	C_1–H	C_4–H	C_6–H	C_{6a}–H	C_8, C_{10}–H	OCH_3, s	OH	OEt or OAc
I	6.60, s 6.63, s (each 0.5H)	6.43, s 6.46, s (each 0.5H)	5.54, d [a] (0.5H, J=1) 5.65, d [a] (0.5H, J=2)	4.65, d (0.5H, J=1) 4.50, d (0.5H, J=2)	5.89, d (J=2) 6.00, d (J=2)	3.70, 3.73 3.79—3.77	11.75	—
II	8.45, s	6.70, s	5.58, s	—	6.45, s (2H)	3.93, 3.99 4.00	12.43	1.25(3H, t, J=7, -OEt)
III	6.70, s	6.60, s	6.50, d (J=1)	5.59, d (J=1)	6.30, d (J=2) 6.40, d (J=2)	3.80(3H) 3.85(6H)	—	2.16, s 2.28, s 2.40, s (3× -OAc)
IV	6.60, s	6.46, s	5.35, d (J=2)	4.65, d (J=2)	5.97, s (2H)	3.56, 3.70 3.72, 3.77 3.83		—
V	6.99, s	6.47, s	5.29, d (J=3.5)	4.85, d (J=3.5)	6.05, s [b] (2H)	3.40, 3.60 3.79, 3.88 3.83(6H)	—	—
VI	8.56, s	6.60, s	5.56, s	—	6.35, d (J=2) 6.21, d (J=2)	3.39, 3.41 3.66, 3.90	13.66	—

J value are given in Hz measured 60 MHz.
d=doublet, s=singlet, t=triplet
a) observed after the addition of D_2O
b) (in $CDCl_3+C_6D_6$=1:1), 5.90 (d, J=1 Hz) and 5.97 (d, J=1 Hz)

On the basis of the mass spectral and PMR spectral analysis of I and its derivatives (III—V) as mentioned below, I was assumed to be a rotenoid having two hydroxyl groups at C_6 and C_{12a} positions. As shown in Fig. 2 and Table II, the mass spectra of the compounds (I, III—V) showed the strong peaks corresponding with the fragments a, b and c, which are characteristic fragments in rotenoids.[5-7] The appearance of two doublet signals attributable to C_6 and C_{6a} protons in their PMR spectra also suggest the presence of two hydroxyl groups at C_6 and C_{12a} positions.

5) L. Crombie, "Fortschritte der Chemie Organischer Naturstoffe," Vol. 21, Wien. Springer-Verlag. New York., 1963, p. 275; E. Wong, *ibid.*, Vol. 28, 1970, p. 22.
6) R.I. Reed and J.M. Wilson, *J. Chem. Soc.*, **1963**, 5949.
7) W.D. Ollis, C.A. Rhodes, and I.O. Sutherland, *Tetrahedron*, **23**, 4741 (1967).

Furthermore, on treatment with *p*-toluenesulfonic acid in methanol, I afforded a crystalline substance (VI), $C_{20}H_{18}O_8$ [*m/e*, 386 (49%, M^+), 355 (100%, M^+-OCH_3)], mp 215.5 217°, which gave a monoacetate (VII), $C_{22}H_{20}O_9$, mp 155—157° and 185—187° (double melting point) [*m/e*, 428 (56%, M^+), 397 (28%, M^+-OCH_3)] by acetylation with acetic anhydride and pyridine. In the view of the ease of dehydration of C_{12a}-hydroxyrotenoids,[5,7] the formation of VI also supports the above assumption.

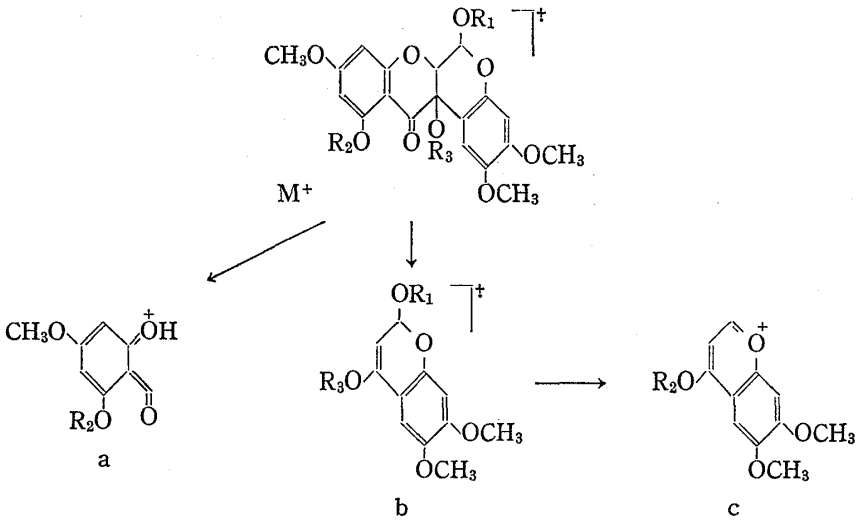


Fig. 2

Table II. Mass Fragments of I, III, IV and V

Compound	M^+	M^+-H_2O	a	b	c	b-CH_2CO	c-CH_2CO
I	390.095 (41%)	372 (7%)	167.032 (78%)	224.069 (100%)	207.065 (32%)	—	—
III	516 (35%)	—	167(a-42) 15%	308 (47%)	249 (43%)	266 (9%)	207 (100%)
IV	418.1197 (12%)	400 (4%)	181.0456 (94%)	238.0820 (100%)	207.0632 (94%)	—	—
V	432.1398 (4%)	—	181 (4%)	252.0946 (56%)	221.0791 (100%)	—	—

The signal at δ 8.56 (s) in the PMR spectrum of VI (in $CDCl_3+C_6D_6$,1:1) was assigned to C_1-proton, deshielded by C_{12} carbonyl group, and the signal at δ 6.60 (s) was assigned to C_4 proton. As a hydrogen bonded hydroxyl group was assumed to be attached to C_{11} position, two other aromatic proton signals at δ 6.21 and 6.35 (each 1H,d, J=2 Hz, meta coupling) were due to the C_8 and C_{10} protons. Thus, it was indicated that three methoxyl groups in I are attached to C_2, C_3 and C_9 positions and one hydroxyl group is attached to C_{11} position.

It has been known that C_1 proton in the PMR spectra of natural rotenoids having the *cis*-B/C ring fusion appears in the region δ 6.3—6.8, while in the case of rotenoids having the *trans*-B/C ring fusion, C_1 proton appears near δ 8.00.[8-10] The C_1 proton signals in the PMR spectra of I and its derivatives (III—V) appear in the region δ 6.60—6.99, indicating the *cis*-B/C ring fusion.

8) L. Crombie and J.W. Lown, *J. Chem. Soc.*, **1962**, 775.
9) G. Büchi, L. Crombie, P.J. Godin, J.S. Kaltenbronn, K.S. Siddalingaiah, and D.A. Whiting, *J. Chem. Soc.*, **1961**, 2843.
10) D.G. Carlson, D. Weisleder, and W.H. Tallent, *Tetrahedron*, **29**, 2731 (1973).

On the other hand, in the PMR spectrum of I, the signals of C_{6a} and C_6 protons appear as doublet pairs at δ 4.50 (0.5 H, d, J=2 Hz), 4.65 (0.5 H, d, J=1 Hz) and δ 5.54 (0.5 H, d, J=1 Hz), 5.65(0.5 H, d, J=2 Hz) respectively, and one methoxyl signal appeared in the lowest field and two aromatic proton signals at C_1 and C_4 positions are also slightly splitted. These observations suggest that I exists as a mixture of two C_6-epimers in almost equal amount.[11] The structure of clitoriacetal was thus formulated as I and the conformational structure was assumed to be I′ on the basis of the J values of C_6 and C_{6a} protons.

Stemonacetal (II), $C_{21}H_{20}O_8$ [*m/e*, 400 (45%, M^+), 355 (100%, $M^+-OCH_2CH_3$)], mp 206—208°, was obtained as pale yellow needles and its physical constants and spectral data are closely resemble with those of stemonacetal, reported as the constituent of *Stemona collinsae* Creib.[4] Thus, II was identified by the direct comparison of IR, PMR spectra and the mixed melting point with the authentic sample derived from I by the treatment with *p*-toluenesulfonic acid in ethanol.

Experimental

All melting points were determined on a Yanagimoto Micro Melting Point Apparatus and uncorrected. UV spectra were measured with a Hitachi Digital Spectrophotometer Model 624. IR spectra were recorded on a Hitachi Model EPI-G2. PMR spectra were recorded on a Varian Model T-60 Spectrometer. Specific rotations were measured with a JASCO Model DIP-SL. Silica gel (Kiesel gel HF_{254}, Merck) were used for thin-layer chromatography (TLC) and preparative TLC (p-TLC).

Extraction——The crude drug (1 kg) collected under the name of "Non-tai-yak" in Bangkok (1971) were extracted with $CHCl_3$ for 3 times. The combined extract was concentrated under reduced pressure. The residue was dissolved in MeOH, filtered and the filtrate was concentrated under reduced pressure to dryness. The residue (27 g) was chromatographed on silica gel (500 g) using a mixture of $CHCl_3$ and *n*-hexane. The fractions eluted with $CHCl_3$–*n*-hexane (3: 1) were combined and concentrated to give a crude mixture of stemonacetal and clitoriacetal (15 g). The mixture was rechromatographed on silica gel (300 g) using the same solvent to give stemonacetal (II) (0.37 g) as pale yellow needles and clitoriacetal (I) (8.0 g) as amorphous form. Clitoriacetal was further purified by p-TLC using a mixture of benzene and ether (1: 1) as the solvent.

Clitoriacetal (I): $C_{19}H_{18}O_9$ (M^+ Calcd. *m/e*, 390.095. Observed, 390.095), $[\alpha]_D^{28}$ +259.5° (c=0.275, $CHCl_3$), UV λ_{max}^{EtOH} nm (log ε): 230 (4.44), 293 (4.32), 320 (sh, 3.88), UV $\lambda_{max}^{EtOH+AlCl_3}$ nm (log ε), 220 (4.98), 295 (4.05), 310 (sh, 4.02). IR ν_{max}^{KBr} cm^{-1}: 3400 (OH), 1655 (sh), 1645 (sh), 1640 (>C=O), 1615, 1570, 1510 (aromatic). $FeCl_3$: brown. Mg–HCl: slightly orange (after 48 hr). Stemonacetal (II), *Anal.* Calcd. for $C_{21}H_{20}O_8$: C, 62.99; H, 5.04. Found: C, 62.75; H, 4.94. mp 206—208°. $[\alpha]_D^{28} \simeq 0°$ (c=0.60, $CHCl_3$). UV λ_{max}^{EtOH} nm (log ε): 225 (4.54), 249 (4.22), 275 (4.52), 320 (4.16). IR ν_{max}^{KBr} cm^{-1}: 3400 (OH), 1660 (>C=O), 1650 (>C=C<), 1610, 1590, 1510 (aromatic).

Clitoriacetal Triacetate (III)——A mixture of I (1 g) in pyridine (3 ml) and acetic anhydride (3 ml) was heated at 90° for 3 hr and then poured into a large amount of ice-water. The precipitates were filtered, dried and chromatographed on silica gel (50 g) using a mixture of benzene and ether [50: 1 (2550 ml) and 20: 1 (4250 ml), each fraction 85 ml]. The fractions eluted with benzene–ether (20: 1) (fraction No. 34—59) were combined and concentrated under reduced pressure to give crude acetate, which was rechromatographed on silica gel (50 g) using the same solvent. The fractions eluted with benzene–ether (50: 1) were combined and concentrated. The residue was recrystallized from MeOH to give pale yellow needles, mp 123—126°. *Anal.* Calcd. for $C_{25}H_{24}O_{12}$: C, 58.14; H, 4.68. Found: C, 58.00; H, 4.77. UV λ_{max}^{EtOH} nm (log ε): 230 (4.27), 235 (sh, 4.24), 281 (4.29). IR ν_{max}^{KBr} cm^{-1}: 1770 (sh), 1765, 1750 (sh) (–OCOC$\underline{H}_3$), 1690 (>C=O), 1610, 1570, 1510 (aromatic). $FeCl_3$: negative.

Clitoriacetal Dimethyl Ether (IV)——To a solution of I (50 mg) in acetone (10 ml) were added dimethyl sulfate (0.5 ml) and K_2CO_3 (1 g) and refluxed for 3 hr. The reaction mixture was filtered and the filtrate was concentrated under reduced pressure. The residue was stirred with 2N NaOH for 3 hr and extracted with ether. The ethereal extract was concentrated and purified by the p-TLC (solvent: $CHCl_3$: MeOH, 30: 1) to give IV as an amorphous form. *Anal.* Calcd. for $C_{21}H_{22}O_9$: M^+, *m/e* 418.1266. Found: *m/e* 418.1197. $[\alpha]_D^{28}$ +235° (c=0.22, $CHCl_3$). UV λ_{max}^{EtOH} nm (log ε): 225 (sh, 4.31), 289 (4.30). IR ν_{max}^{KBr} cm^{-1}: 3400 (OH), 1670 (>C=O), 1610, 1570, 1507 (aromatic). $FeCl_3$: negative.

Clitoriacetal Trimethyl Ether (V)——To a solution of I (100 mg) in dimethylformamide (2 ml) were added CH_3I (1 ml) and Ag_2O (2 g), and the reaction mixture was stirred for 60 hr at room temperature. After filtration, the reaction mixture was poured into a large amount of water and extracted with $CHCl_3$ for several times. The chloroform extracts were combined, washed with water and dried over Na_2SO_4. The solvent

11) H. Inouye, S. Tobita, Y. Akiyama, K. Ito, and T. Shingu, *Chem. Pharm. Bull.* (Tokyo), **21**, 846 (1973).

was removed under reduced pressure to give a yellow oil, which was purified by p-TLC (solvent; benzene–ether, 1: 1.5) to give V (59 mg) (*Rf*, 0.33) and VIII (4 mg) (*Rf*, 0.36). V: *Anal.* Calcd. for $C_{22}H_{24}O_9$: M+, *m/e*: 432.1422. Found: *m/e*: 432.1398. amorphous powder, $[\alpha]_D^{28}$ +115.4° (*c*=0.87, $CHCl_3$), UV λ_{max}^{EtOH} nm (log ε): 225 (sh, 4.28), 289 (4.25). IR ν_{max}^{KBr} cm^{-1}: 1680 (>C=O), 1610, 1590, 1510 (aromatic). $FeCl_3$: negative. VIII: *Anal.* Calcd. for $C_{21}H_{20}O_8$ [*m/e*: 400 (56%, M+), 369 (100%, M+−OCH_3)]. pale yellow prisms, mp 207°. IR ν_{max}^{KBr} cm^{-1}: 1655 (sh), 1645, 1605, 1565, 1505. PMR (δ in $CDCl_3$): 3.59 (3H, s), 3.90 (6H, s), 3.94 (3H, s), 3.99 (3H, s) (5×OCH_3), 5.75 (1H, s, C_6–H), 6.39 (1H, d, *J*=2 Hz), 6.48 (1H, d, *J*=2 Hz) (C_8,C_{10}–H), 6.65 (1H, s, C_4–H), 8.66 (C_1–H).

Treatment of I with *p*-Toluenesulfonic Acid in Methanol (Preparation of VI)——To a solution of I (100 mg) in MeOH (4 ml) was added *p*-toluenesulfonic acid (100 mg) and the mixture was refluxed for 3 hr. The yellow precipitates were filtered and recrystallized from a mixture of $CHCl_3$ and MeOH to give pale yellow needles (VI) (53 mg). *Anal.* Calcd. for $C_{20}H_{18}O_8$: C, 62.17; H, 4.70. Found: C, 61.97; H, 4.62. mp 215.5—217°. UV λ_{max}^{EtOH} nm (log ε): 220 (sh, 4.38), 248 (4.23), 275 (4.54). IR ν_{max}^{KBr} cm^{-1}: 3400 (OH), 1660 (>C=O), 1620 (>C=C<), 1610, 1580, 1509, 1500 (aromatic). Mass Spectrum *m/e*: 386 (49%, M+), 355 (100%, M+−OCH_3).

VI (42 mg) was acetylated with acetic anhydride and pyridine at 60° for 3 hr to give monoacetate (VII) as pale yellow needles. *Anal.* Calcd. for $C_{22}H_{20}O_9$: C, 61.68; H, 4.71. Found: C, 61.31; H, 4.78. mp 155—157° and 185—187° (double melting point). IR ν_{max}^{KBr} cm^{-1}: 1770 (OAc), 1655 (>C=O), 1620, 1565, 1510 (aromatic). PMR (δ in $CDCl_3$): 2.46 (3H, s, –OCOC$\underline{H}_3$), 3.58 (3H, s), 3.88 (6H, s), 3.94 (3H, s) (4×OCH_3), 5.73 (1H, s, C_6–H), 6.61 (1H, d, *J*=2 Hz), 6.81 (1H, d, *J*=2 Hz) (C_8,C_{10}–H), 6.65 (1H, s, C_4–H), 8.51 (1H, s, C_1–H). Mass Spectrum *m/e*: 428 (56%, M+), 397 (28%, M+–OCH_3), 355 (100%, 397-CH_2CO).

Treatment of I with *p*-Toluenesulfonic Acid in Ethanol (Preparation of II)——To a solution of I (76 mg) in EtOH (1.5 ml) was added *p*-toluenesulfonic acid (59 mg) and the reaction mixture was refluxed for 1 hr. The yellow precipitates were filtered and recrystallized from a mixture of $CHCl_3$ and MeOH to give pale yellow needles (34 mg). *Anal.* Calcd. for $C_{21}H_{20}O_8$: C, 62.99; H, 5.04. Found: C, 62.71; H, 4.90. mp 206—207°. $[\alpha]_D^{28} \simeq 0$ (*c*=0.39, $CHCl_3$), which was identified by the mixed melting point and the comparison of IR and PMR spectra with stemonacetal (II). II was also prepared from I by the treatment with citric acid in EtOH.

Stemonacetal Monoacetate (IX)——A mixture of II (40 mg) in pyridine (1 ml) and acetic anhydride (0.5 ml) was heated at 80° for 3 hr and then poured into ice-water. The precipitates were filtered, dried and recrystallized from a mixture of $CHCl_3$ and MeOH to give pale yellow prisms. *Anal.* Calcd. for $C_{23}H_{22}O_9$: C, 62.44; H, 4.97. Found: C, 62.19; H, 4.99. PMR (δ in $CDCl_3$): 1.21 (3H, t, *J*=7 Hz, –$CH_2C\underline{H}_3$), 2.45 (3H, s, –OCOC$\underline{H}_3$), 3.86 (6H, s), 3.91 (3H, s) (3×OCH_3), 5.81 (1H, s, C_6–H), 6.58 (1H, d, *J*=2.5 Hz), 6.79 (1H, d, *J*=2.5 Hz) (C_8,C_{10}–H), 6.00 (1H, s, C_4–H), 8.49 (1H, s, C_1–H).

Acknowledgement The authors express their gratitude to Dr. S. Natori, Dr. K. Nishimoto, National Institute of Hygienic Sciences of Japan, Dr. Komal Pengsritong, Dr. Prakorb Tuchinda, Ministry of Public Health of Thailand, Dr. T. Kariyone and Dr. I. Yosioka, Tsumura Laboratory, for their valuable discussions during this work. Thanks are also due to Prof. S. Fukushima, Shizuoka College of Pharmacy and Prof. Y. Yamada, Tokyo College of Pharmacy, for mass spectral measurements and to Mr Daroon Pecharaply, Department of Medical Sciences, for supplying the plant materials.