—Original—

Natural Medicines 59 (2), 76-84 (2005)

Chemical Constituents of *Capsicum annuum* L var. *angulosum*, and Anti *Helicobacter pylori* Activity

Toshimasa Ochi, Yoshihisa Takaishi*, Hirofumi Shibata, Tomihiko Higuti and Mai Kataoka

Graduate School of Pharmaceutical Sciences, University of Tokushima, Shomachi 1-78, Tokushima, 770-8505, Japan

(Received November 15, 2004)

One new and 47 known compounds were isolated from the fruits and the stems of *Capsicum annuum* var. *angulosum*. The structures of the isolated new and known compounds were determined by extensive NMR studies and compared with a reference value of the spectral data. Furthermore, antibacterial activity against *Helicobacter pylori* of the isolated 41 compounds was measured.

Keywords: *Capsicum annuum* var. *angulosum*; *Helicobacter pylori*; antibacterial activity

The *Capsicum* species, peppers, are important plants and have been used worldwide as food, spices, and medicines.[1] There are many reports on the constituents of the fruits of *Capsicum annuum*. Previously, we reported a new capsaicin derivative from *C. annuum*.[2] As a part of our continuing study of the chemical constituents of the *Capsicum* genus, we have examined the fruits and the stems of *C. annuum* L. var. *angulosum* which is known as a vegetable. The constituents of this plant have not been reported. In this report, we described the constituents of each part (stem, placenta and seed, peduncle, and pericarp) from *Capsicum annuum* var. *angulosum*, and their antibacterial activity against *Helicobacter pylori*.

RESULTS AND DISCUSSION

The dried stems of *C. annuum* var. *angulosum* were extracted with MeOH. The MeOH extracts were concentrated in vacuo to give a residue, which was suspended in H_2O and partitioned sequentially with EtOAC and *n*-BuOH. The EtOAc and the *n*-BuOH extracts were fractionated using column chromatography to give one new (**1**) and 15 known compounds (**2** - **16**). The known compounds were identified from spectral data analysis and comparison with the literature, respectively: isodihydrodehydrodiconiferyl alcohol (**2**), icariside E_5 (**3**)[3], dehydrodiconiferyl alcohol (**4**), 1, 2-bis

(4-hydroxy-3-methoxyphenyl)-1, 3-propanediol (**5**), *trans*-*N*-(4-hydroxyphenethyl)-ferulamide (**6**)[4], *trans*-*N*-feruloyl-tyramine (**7**)[5], *trans*-*N*-(*p*-coumaroyl) tyramine (**8**), *trans*-*N*-(*p*-coumaroyl) octopamine (**9**)[6], *trans*-*N*-feruloyloctopamine (**10**), *cis*-*N*-(4-hydroxyphenethyl) ferulamide (**11**)[4], 5, 6-epoxy-3-hydroxy-7-megastigmen-9-one (**12**), 9-β-D-xylopyranosyladenin (**13**)[7], uridine (**14**)[8], β-sitosterol (**15**)[9], β-sitosterol-β-D-gluco-pyranoside (**16**)[10] (Fig. 1). The isolation of compound **2** was the first time from a natural source.

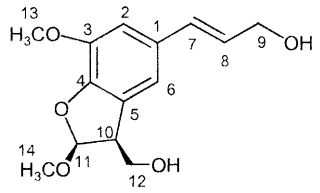
The wet placentas and seeds of *C. annuum* var. *angulosum* were extracted in the same way. The EtOAc and the *n*-BuOH extracts were fractionated using column chromatography to give 18 known compounds (**13** – **16**, **17** – **30**): 5-methoxy-2-pyrrolidinone (**17**), 5-(methoxycarbonyl)-2-pyrrolidinone (**18**), dihydrocapsaicin (**19**), 3β-hydroxystigmast-5-en-7-one (**20**)[11], β-amyrin (**21**)[12], methyl trans-*p*-coumarate (**22**)[13], methyl- *trans*-4-hydroxy-3-methoxy-cinnamate (**23**)[13], 1-O-(*E*)-feruloyl-β-D-glucopyranose (**24**), (*E*)-coniferin (**25**), benzoyl-β-D-glucopyranoside (**26**), 1-O-vanilloyl-β-D-glucopyranoside (**27**)[14], (*E*)-2-hexenyl-β-D-gluco-pyranoside (**28**), 1-hexyl-β-D-glucopyranoside (**29**), phenylmethyl-β-D-glucopyranoside (**30**) (Fig. 2).

The wet pericarps of *C. annuum* var. *angulosum* were extracted in the same way. The EtOAc and the *n*-BuOH extracts were fractionated using column chromatography to give 20 known compounds (**15**, **16**, **21**, **23**, **31** - **46**): α-tocopherol (**31**)[15], 6-hydroxy-2, 6, 8, 9-tetramethyl-2-phytyl-1-oxaspiro[4, 5]dec-8-ene-7, 10-dione (**32**)[16], capsidiol (**33**)[17], 13-hydroxy-capsidiol (**34**)[18], isocapsidienone (**35**)[19], α-amyrin (**36**)[20], lupeol (**37**)[21], friedelin (**38**)[22], 3-β-friedelinol (**39**)[23], D:B-*friedo*olean-5-en-3β-ol (**40**)[24], stigmast-5-en-3-one (**41**)[25], cafferic acid methyl ester (**42**)[13], 4-hydroxy-3-methoxybenzoic acid (**43**), 4-hydroxybenzoic acid (**44**), 1-(4-hydroxy -3-methoxyphenyl)-ethanone (**45**)[13], luteolin-7-O -β-D-glucopyranosidyl-(2 → 1)-D-apiofuranoside (**46**) (Fig. 3).

The dried peduncles of *C. annuum* var. *angulosum* were extracted in the same way. The EtOAc and the *n*-BuOH extracts were fractionated using column chromatography to give 6 known compounds (**3**, **15**, **16**, **46** - **48**): (−)-loliolide (**47**)[26], ergost-6, 22-dien-3β, 5α, 8α-triol (**48**)[27] (Fig. 4).

Compound **1** (capstemol) showed a hydroxy band at 3410 cm^{-1} in the IR spectrum. The ^{13}C NMR spectrum showed signals due to two methoxyl carbons (δ_C 56.8, 56.7), six aromatic carbons (δ_C 147.6, 145.8, 132.9, 130.4, 116.0, 112.2), two oxygenated methylene carbons (δ_C 63.8, 60.6), four methines (δ_C 132.0, 127.8, 110.1, 49.7). The ^1H NMR spectrum showed the presence of two methoxyl groups [δ_H 3.88 (3H, s), 3.53 (3H, s)], two olefinic methines [δ_H 6.54 (1H, d, J = 15.8), 6.23 (1H, dt, J = 15.8, 6.0)], two aromatic protons [δ_H 6.99 (1H, br. s), 6.92 (1H, br. s)]. The HREIMS of compound **1** gave a molecular ion peak at *m/z* 266.1132 $[M]^+$, suggesting a molecular formula of $C_{14}H_{18}O_5$. In the ^1H-^1H COSY spectrum of **1**, correlations were observed for δ_H 6.58 (H-11) with δ_H 3.61 (H-10), δ_H 3.61 (H-10) with δ_H 4.00 (H-12). From these data, it was assumed that **1** has a tetra-substituted benzene ring, *trans* double bond and the partial structure: -O-CH-CH-CH_2O-. In the HMBC spectrum of **1**, correlations of δ_H 6.54 (H-7) with δ_C 132.9 (C-1), 112.2 (C-2), 116.0 (C-6) and 63.8 (C-9), and δ_H 6.58 (H-11) with δ_C 147.6 (C-4), 130.4 (C-5) and 60.6 (C-12) were observed. Based on these data, the connections between C-1 and double the bond (C-7), C-5 and the center carbon (C-10) of the partial structure were suggested. The presence of a five membered ring was confirmed by the correlation of δ_H 6.58 (H-11) with δ_C 147.6 (C-4). The position of two methoxyl groups was determined to be C-3 and C-11 by HMBC. The relative configuration of compound **1** was determined by the

coupling constant ($J_{10\text{-}11}$ = 6.4Hz)[28] and the NOE correlation between H10 and H-11. Based on these and a molecular formula ($C_{14}H_{18}O_5$), the structure of **1** was determined as shown.



1

These compounds were the first isolation from *C. annuum* var. *angulosum*. The plants of *Capsicum* genus generally contain such compounds as **3**, **6-11**, **19**, **31**, **33-35**, and **46**.

As a result, it was found that the stems contained lignans (**2**, **3**), amide compounds (**6-11**) and steroids (**15**, **16**); the placentas and seeds contained capsaicinoid (**19**), many glycosides (**13**, **14**, **16**, **24-30**), phenyl propanoids (**23-25**), benzoic acid derivatives (**26**, **27**) and heptanol derivatives (**28**, **29**); the pericarps contained terpenoids (**21**, **33-40**), α-tocopherol derivatives (**31**, **32**) and phenyl propanoid (**42**); and the peduncles contained steroid derivatives (**15**, **16**, **48**).

The stems contained mainly lignans and amides; the placenta and seed contained mainly a capsaicinoid, phenyl propanoids and glycosides; and the pericarp contained mainly terpenoids.

Anti-*H. pylori* activity of 41 compounds is shown in Table 1. Some lignans (**2**, **3**), amides (**6**, **8**, **19**), phenyl propanoids (**22**, **23**, **42**), α-tocopherol derivatives (**31**, **32**), steroids (**20**, **48**), and sesquiterpenes (**33**, **35**) showed activity against *H. pylori*. Especially, dihydrocapsaicin (**19**) showed strong activity. Methyl trans-*p*-coumarate (**22**) and capsidiol (**33**) showed comparatively strong activity. New compound **1** showed slight activity.

Table 1 Antibacterial activity against *H. pylori* (ATCC43504 and SS1) based diffusion method

	Inhibition zone in diameter (mm)	
	H. pylori	*H. pylori*
compounds	ATCC43504	SS1
AMPC	29.0	-
1	8.0	7.5
2	7.0	±
3	±	8.0
4	8.5	8.0
5	8.0	7.5
6	8.0	7.5
8	8.5	9.0
9	±	7.5
13	-	-
14	±	7.5
15	-	8.0
16	-	8.5
17	-	8.0
18	-	7.5
19	11.0	26.5
20	8.0	9.0
21	±	±
22	13.0	20.0
23	10.0	15.0
24	-	7.5
25	-	7.5
26	±	7.5
27	-	-
28	-	7.5
29	7.0	7.5
30	-	±
31	8.0	10.0
32	9.0	8.5
33	19.0	11.0
34	±	7.5
35	9.0	9.0
38	±	8.0
39	-	7.5
40	-	7.5
41	±	±
42	10.0	14.0
43	-	8.0
45	-	7.5
46	7.5	7.5
47	-	9.0
48	12.0	11.0

AMPC: 250ng/disc (amoxicillin)
compounds: 100μg/disc
±: 6.0 < ~ < 7.0 -: non effect
disc size: 6mm (diameter)

EXPERIMENTAL METHOD

General Experimental Procedures Optical rotations were measured with a JASCO DIP-370 digital polarimeter. IR spectra were recorded on a JASCO Fourier transform infrared spectrometer (FT/ as internal stand) and were measured on a Bruker AM 400 spectrometer and MS spectra on a JEOL JMS D-300 instrument IR-420). NMR (400 MHz for ^{1}H NMR, 100MHz for ^{13}C NMR, both use TMS. Preparative thin layer chromatography (PTLC): silica gel $60F_{254}$ precoated TLC plates (Merck), Column chromatography: silica gel 60 (Merck, 63-210μm), Sephadex LH-20 (Pharmacia) and Toyopearl HW-40 (TOSHO); HPLC: silica gel (YMC-pack SIL-06 SH-043-5-06, 250×20 mm, Hibar RT 250-25 Si 60), Gel-Permition Chromatography (GPC): (Shodex H-2001, 2002, $CHCl_3$), (Asahipak GS-310 2G, MeOH).

Plant material The placentas and seeds, pericarps, and peduncles of *Capsicum annuum* var. *angulosum* (breed: Aoi shishitou) were purchased in Nangoku City, Japan. The stems of *Capsicum annuum* var. *angulosum* (Aoi shishitou) were provided by a farmer in Tokushima City, Japan. Herbarium speciments were deposited in the herbarium of the Graduate School of Pharmaceutical Sciences, University of Tokushima.

Extraction and isolation The dried stems of *C. annuum* var. *angulosum* (3.0kg) were extracted using MeOH at 70℃. The MeOH extracts (132g) were concentrated in vacuo to give a residue which was suspended in H_2O and partitioned sequentially with EtOAC and *n*-BuOH. The EtOAc extract (29g) was subjected to silica gel column chromatography using different solvents of increasing polarity (*n*-hexane－EtOAc; EtOAc－MeOH) to give 10 fractions (1－10). Fraction 3 (2.6g) was subjected to TOYOPEARL HW-40 ($CHCl_3$: MeOH = 2 : 1), SiO_2c.c. (*n*-Hexane : EtOAc = 96 : 4 ~ 0 : 100) and PTLC ($CHCl_3$: MeOH = 95 : 5) to give **15** (21mg). Fraction 4 (1.5g) was subjected to TOYOPEARL HW-40 ($CHCl_3$: MeOH = 2 : 1), SiO_2c.c. ($CHCl_3$: MeOH = 98 : 2), GPC (MeOH) and PTLC (*n*-Hexane : EtOAc = 1 : 3) to give **12** (2mg). Fraction 5 (447mg) was subjected to TOYOPEARL HW-40 ($CHCl_3$: MeOH = 1 : 2) and PTLC ($CHCl_3$: MeOH = 9 : 1) to give **6** (10mg), **8** (2mg) and **11** (2mg). Fraction 6 (618mg) was subjected to GPC (MeOH) to give fraction 6.1(25mg), 6.2 (45mg) and 6.3 (59mg). Fraction 6.1 was subjected to PTLC ($CHCl_3$: MeOH = 9 : 1) to give **1** (5mg), **2** (3mg) and **5** (7mg). Fraction 6.2 was subjected to PTLC ($CHCl_3$: MeOH = 9 : 1) to give **9** (15mg). Fraction 6.3 was subjected to PTLC ($CHCl_3$: MeOH = 9 : 1) to give **4** (4mg) and **7** (3mg). Fraction 7 (2.0g) was subjected to TOYOPEARL HW-40 (MeOH) to give fraction 7.1 (878mg) and **16** (392mg). Fraction 7.1 was subjected to SiO_2c.c. ($CHCl_3$: MeOH = 97 : 3 ~ 0 : 100), GPC (MeOH) and PTLC ($CHCl_3$: MeOH = 9 : 1) to give **10** (4mg). Fraction 9 (1.4g) was subjected to TOYOPEARL HW-40 ($CHCl_3$: MeOH = 1 : 1) and GPC (MeOH) to give **3** (26mg). The BuOH extract (35g) was SiO_2c.c. ($CHCl_3$: MeOH = 96 : 4 ~ 0 : 100), TOYOPEARL HW-40 ($CHCl_3$: MeOH = 1 : 1) and GPC (MeOH) to give fraction 11 (13mg) and **13** (13mg). Fraction 11 was subjected to PTLC ($CHCl_3$: MeOH = 7 : 3) to give **14** (8mg).

The wet placentas and seeds of *C. annuum* var. *angulosum* (8.3kg) were extracted using MeOH at 70℃. The MeOH extracts (415g) were concentrated in vacuo to give a residue, which was suspended in H_2O and partitioned sequentially with EtOAC and *n*-BuOH. The EtOAc extract (20g) was subjected to silica gel column chromatography using different solvents of increasing polarity (*n*-hexane－EtOAc; EtOAc－MeOH) to give 18 fractions (12－29). Fraction 19 (67mg) was subjected to

GPC ($CHCl_3$) to give **25** (5mg), **23** (12mg) and **22** (4mg). Fraction 24 (161mg) was subjected to SiO_2HPLC (*n*-Hexane : EtOAc = 1 : 1) to give **19** (4mg). Fraction 15 (482mg) was subjected to GPC ($CHCl_3$), SiO_2HPLC ($CHCl_3$) and PTLC ($CHCl_3$) to give **15** (3mg). Fraction 28 (1.2g) was subjected to TOYOPEARL HW-40 ($CHCl_3$: MeOH = 2 : 1) to give **16** (50mg). Fraction 16 (1.2g) was subjected to TOYOPEARL HW-40 ($CHCl_3$: MeOH = 2 : 1), SiO_2c.c. ($CHCl_3$: MeOH = 100 : 0 ~ 98 : 2) and SiO_2HPLC ($CHCl_3$: MeOH = 98 : 2) to give **20** (3mg). Fraction 14 (256mg) was subjected to GPC ($CHCl_3$) to give **21** (131mg). The *n*-BuOH extract (66g) was subjected to silica gel column chromatography using different solvents of increasing polarity (*n*-hexane－EtOAc; EtOAc－MeOH) to give 11 fractions (30－40). Fraction 34 (521mg) was subjected to TOYOPEARL HW-40 ($CHCl_3$: MeOH = 2 : 1) and GPC (MeOH) to give fraction 34.1 (11mg), **17** (6mg), **18** (18mg), **24** (61mg), **28** (10mg), **30** (13mg), **26** (8mg) and **27** (5mg). Fraction 34.1 was subjected to SiO_2HPLC ($CHCl_3$: MeOH = 9 : 1) to give **29** (4mg). Fraction 37 (604mg) was subjected to TOYOPEARL HW-40 ($CHCl_3$: MeOH = 1 : 1) and GPC (MeOH) to give **13** (125mg). Fraction 40 (26.5g) was subjected to SiO_2 c.c. ($CHCl_3$－MeOH) and Sephadex LH-20 (MeOH) to give **14** (15mg).

The wet pericarps of *C. annuum* var. *angulosum* (23kg) were extracted using MeOH at 70℃. The MeOH extracts (861g) were concentrated in vacuo to give a residue which was suspended in H_2O and partitioned sequentially with EtOAC and *n*-BuOH. The EtOAc extract (29g) was subjected to silica gel column chromatography using different solvents of increasing polalrity (*n*-hexane－EtOAc; EtOAc－MeOH) to give 9 fractions (41－49). Fraction 41 (4.0g) was subjected to SiO_2c.c. (*n*-Hexane : EtOAc = 95 : 5 ~ 0 : 100) to give 12 fraction (41.01 ~ 41.12). Fraction 41.04 (268mg) was subjected to GPC ($CHCl_3$) and SiO_2HPLC (*n*-Hexane : EtOAc = 95 : 5) to give **38** (2mg). Fraction 41.05 (292mg) was subjected to GPC ($CHCl_3$), SiO_2HPLC ($CHCl_3$) and PTLC ($CHCl_3$) to give **31** (30mg). Fraction 41.06 (152mg) was subjected to GPC ($CHCl_3$) to give fraction 41.06.01 (53mg), **35** (8mg), **39** (1mg) and **40** (2mg). Fraction 41.06.01 was subjected to SiO_2HPLC (*n*-Hexane : EtOAc = 95 : 5) and PTLC ($CHCl_3$) to give **32** (5mg). Fraction 41.07 (311mg) was subjected to GPC ($CHCl_3$) and SiO_2HPLC (*n*-Hexane : EtOAc = 9 : 1) to give **41** (6mg) and the 1 : 1 : 1 mixture (161mg) of **21**, **36** and **37**. Fraction 43 (3.0g) was subjected to SiO_2c.c. ($CHCl_3$: MeOH = 99.9 : 0.1 ~ 99.0 : 1.0), GPC ($CHCl_3$) and SiO_2HPLC ($CHCl_3$: MeOH = 99.5 : 0.5) to give **15** (25mg). Fraction 44 (1.4g) was subjected to TOYOPEARL HW-40 ($CHCl_3$: MeOH = 2 : 1), SiO_2c.c. ($CHCl_3$) and GPC ($CHCl_3$) to give fraction 44.1 (5mg) and **23** (15mg). Fraction 44.1 was subjected to PTLC ($CHCl_3$: MeOH : 99 : 1) to give **45** (3mg). Fraction 45 (931mg) was subjected to TOYOPEARL HW-40 ($CHCl_3$: MeOH = 2 : 1) and GPC ($CHCl_3$) to give fraction 45.1 (15mg), **43** (1mg) and **44** (1mg). Fraction 45.1 was subjected to SiO_2HPLC ($CHCl_3$: MeOH = 95 : 5) and GPC (MeOH) to give **42** (4mg). Fraction 46 (1.6g) was subjected to TOYOPEARL HW-40 ($CHCl_3$: MeOH = 2 : 1), GPC ($CHCl_3$), SiO_2HPLC ($CHCl_3$: MeOH = 95 : 5) and GPC (MeOH) to give **33** (5mg). Fraction 48 (4.4g) was subjected to TOYOPEARL HW-40 ($CHCl_3$: MeOH = 2 : 1) to give fraction 48.1 (37mg) and **16** (18mg). Fraction 48.1 was subjected to GPC ($CHCl_3$) and GPC (MeOH) to give **34** (12mg). Fraction 49 (9.1g) was subjected to TOYOPEARL HW-40 ($CHCl_3$: MeOH = 1 : 1) to give **46** (10mg).

The dried peduncles of *C. annuum* var. *angulosum* (415g) were extracted using MeOH at 70℃. The MeOH extracts (64g) were concentrated in vacuo to give a residue which was suspended in H_2O and partitioned sequentially with EtOAC and *n*-BuOH. The EtOAc extract (8.0g) was subjected to silica gel column chromatography using different solvents of increasing

polarity (*n*-hexane－EtOAc; EtOAc－MeOH) to give 11 fractions (50－60). Fraction 52 (136mg) was subjected to GPC ($CHCl_3$) and PTLC ($CHCl_3$: MeOH = 98 : 2) to give **15** (6mg). Fraction 54 (168mg) was subjected to GPC ($CHCl_3$) and SiO_2HPLC ($CHCl_3$: MeOH = 99.5 : 0.5) to give **48** (3mg). Fraction 57 (444mg) was subjected to GPC ($CHCl_3$) and SiO_2HPLC ($CHCl_3$: MeOH = 97 : 3) to give **47** (2mg). Fraction 59 (1026mg) was subjected to TOYOPEARL HW-40 ($CHCl_3$: MeOH = 1 : 1) to give **16** (20mg). The *n*-BuOH extract (14g) was subjected to silica gel column chromatography using different solvents of increasing polarity (*n*-hexane－EtOAc; EtOAc－MeOH) and TOYOPEARL (MeOH) to give fraction 61 (42mg) and **46** (20mg). Fraction 61 was subjected to SiO_2 c.c. ($CHCl_3$: MeOH = 95 : 5 ~ 0 : 100) and GPC (MeOH) to give **3** (7mg).

Capstemol (1): a colorless oil; $[\alpha]_D^{25}$ +2.7° (*c* 0.51, CH_3OH); IR (KBr) ν_{max} 3410, 1668, 1600 cm^{-1}; ^1H NMR (CD_3OD) δ_H 6.99 (1H, br. s, H-6), 6.92 (1H, br. s, H-2), 6.58 (1H, d, *J* = 6.4, H-11), 6.54 (1H, d, *J* = 15.8, H-7), 6.23 (1H, dt, *J* = 15.8, 6.0, H-8), 4.21 (2H, d, *J* = 6.0, H-9), 4.00 (1H, m, H-12), 3.92 (1H, m, H-12), 3.88 (3H, s, H-13), 3.61 (1H, m, H-10), 3.53 (3H, s, H-14); ^{13}C NMR (CD_3OD) δ_C 132.9 (C-1), 112.2 (C-2), 145.8 (C-3), 147.6 (C-4), 130.4 (C-5), 116.0 (C-6), 132.0 (C-7), 127.8 (C-8), 63.8 (C-9), 49.7 (C-10), 110.1 (C-11), 60.6 (C-12), 56.8 (C-13), 56.7 (C-14); HREIMS *m/z* 266.1132 $[M]^+$ (calcd for $C_{14}H_{18}O_5$, 266.1154).

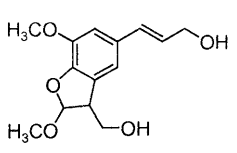
Bacterial strains and cultures

Helicobacter pylori ATCC43504 and SS1 were provided by Professor Dr. Keiji Oguma (Departments of Pathology, Bacteriology and Medicine, Okayama University Graduate School of Medicine and Dentistry, Okayama, Japan).

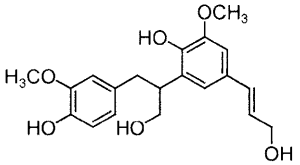
Antibacterial screening

The disc-diffusion method was used to screen the anti-*H. pylori* activity of the extracts. Sample solutions (10 mg/ml) were prepared by dissolving the extracts with dimethyl sulfoxide (Kanto Chemical Co., Inc., Tokyo, Japan). Positive control used the antibiotic amoxicillin (Sigma Chemical Co., St. Louis, MO, USA).

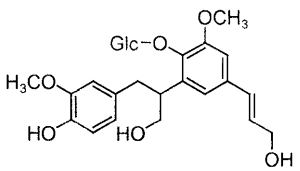
H. pylori was cultured for 4 days at 37℃ in Brucella broth containing 5% horse serum (Bio Whittaker, Walkersville, MD, USA) under the micro-aerophilic condition using a disposable O_2 absorbing and CO_2 generating agent, AnaeroPack Helico (Mitsubishi Gas Chemical Co., Inc., Tokyo, Japan), with humidity. The culture was then diluted and adjusted to about 1×10^7 CFU/ml with the fresh medium and was uniformly spread with a cotton swab onto the ISO-SENSI TEST AGAR (Oxoid Ltd., Basingstoke, Hampshire, England, UK) containing 10% horse blood (Nippon Biotest Laboratories Inc., Tokyo, Japan). Sterile blank disks (Whatman AA DISCS, 6mm, Whatman International Ltd., Maidstone, England, UK) were placed on the agar surface. Then, 10 μl of the sample solutions was transfused into the discs. After 4 days' incubation at 37℃ under the micro-aerophilic condition with humidity, the plates were screened for the growth inhibition zones.



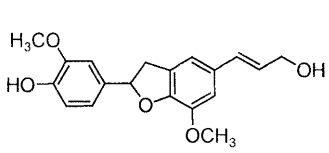
1



2



3



4

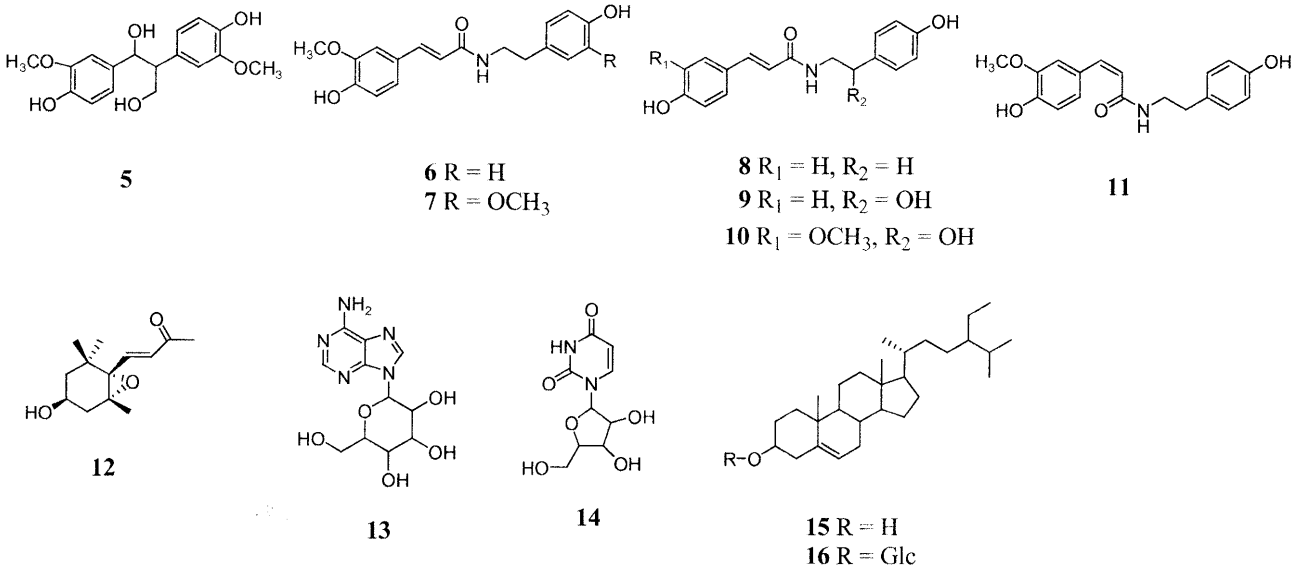


Fig. 1. Chemical constituents from the stem of *C. annuum* var. *angulosum*.

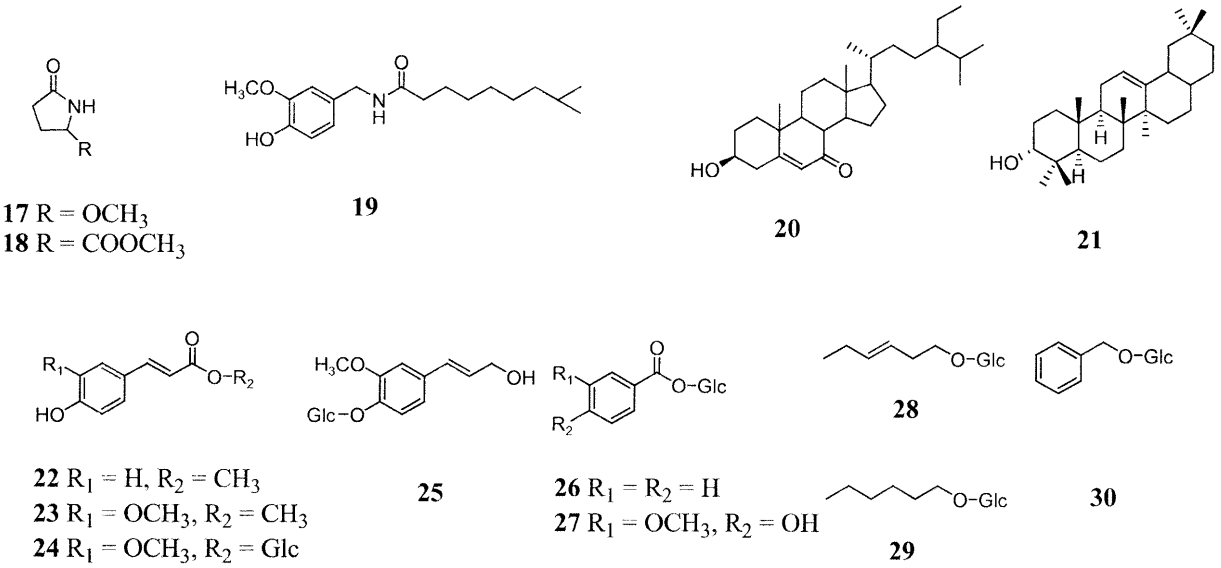
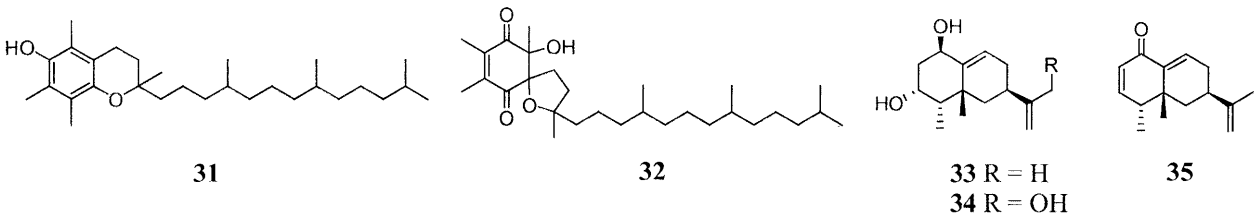
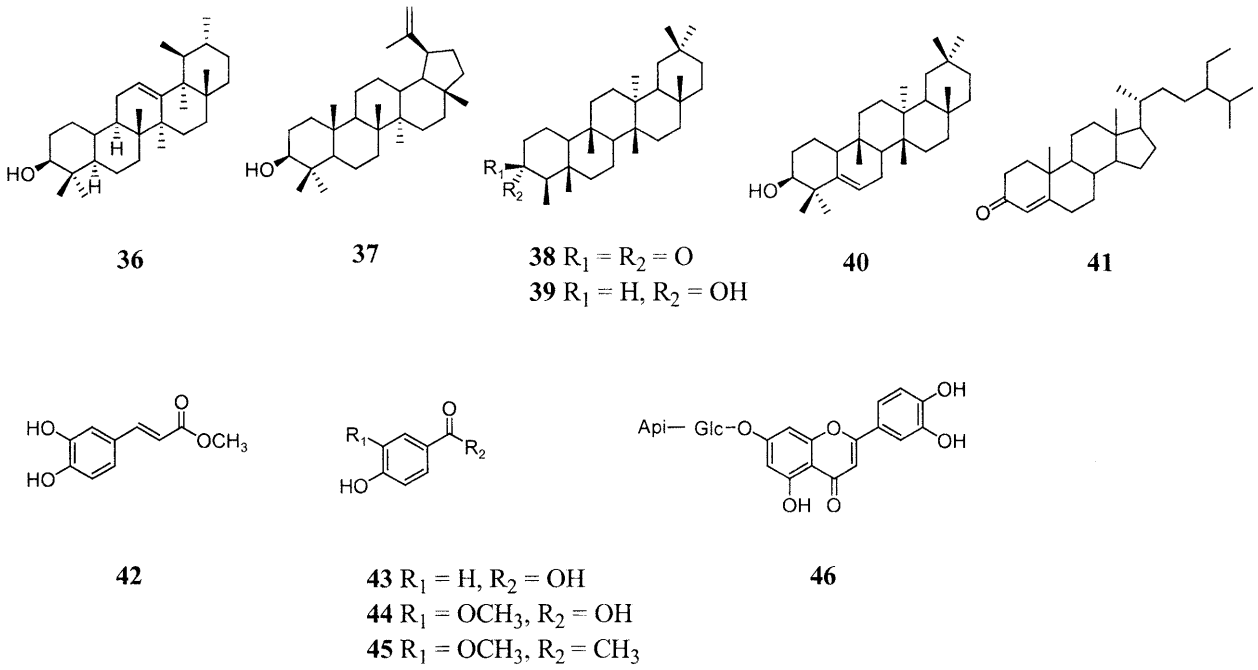


Fig. 2. Chemical constituents from the placenta and seed of *C. annuum* var. *angulosum*.
(**13**, **14**, **15**, **16** are contained)





36 **37** **38** $R_1 = R_2 = O$ **40** **41**

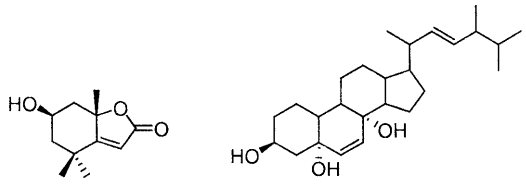
39 $R_1 = H$, $R_2 = OH$

42 **43** $R_1 = H$, $R_2 = OH$ **46**

44 $R_1 = OCH_3$, $R_2 = OH$

45 $R_1 = OCH_3$, $R_2 = CH_3$

Fig. 3. Chemical constituents from the pericarp of *C. annuum* var. *angulosum*.
(**15**, **16**, **21**, **23** are contained.)



47 **48**

Fig. 4. Chemical constituents from the peduncle of *C. annuum* var. *angulosum*.
(**3**, **15**, **16** are contained.)

REFERENCES

1) Kobata K., Todo T., Yazawa S., Iwai K., *J. Agric. Food. Chem.*, **46**, 1695-1697 (1998).
2) Ochi T.,Takaishi Y., Kogure K., Yamauchi I., *Journal of Natural Products*, **66** (8), 1094-1096 (2003).
3) Miyase T., Ueno A., Takizawa N., Kobayashi H., Oguchi H., *Phytochemistry*, **28**, 3483-3485 (1989).
4) Munoz O., Piovano M., Garbarino J., Hellwing V., Breitmaier E., *Phytochemistry*, **43**, 709-713 (1996).
5) Fukuda N., Yonemitsu M., Kimura T., *Chem. Pharm. Bull.*, **31**, 156-161 (1983).
6) Kaneda N., Nakanishi H., Kuraishi T., Katori T., *Yakugaku Zasshi*, **103**, 1133-1139 (1983).
7) Breitmaier E., Voelter W., *Tetrahedron*, **29**, 227-232 (1973).

8) Ching-jer C., Dennis J. A., Lih-Ju C., Jose D. G., Chi-Gen L., Pih W. M., Ramani N., *Organic Magnetic Resonance*, **22**, 671-675 (1984).

9) Koizumi N., Fujimoto Y., Takeshita T., Ikekawa N., *Chem. Pharm. Bull.*, **27**, 38-42 (1979).

10) Kojima H., Sato N., Hatano A., Ogura H., *Phytochemistry*, **29**, 2351-2355 (1990).

11) Kovganko N.V., Kashkan Zh. N., Borisov E. V., Batura E. V., *Chemistry of Natural Compounds*, **35**, 646-649 (1999).

12) Laxman R. K., Ramraj S. K., Subba R. T. V. P. R., Sundararamaiah T. *Phytochemistry*, **25**, 277-278 (1986).

13) Fujita M., Inoue T., Nagai M., *Yakugaku Zasshi*, **105**, 204-248 (1985).

14) Klick S., Herrmann K., *Phytochemistry*, **27**, 2177-2180 (1988).

15) Matsuo M., Urano S. *Tetrahedron*, **32**, 229-231 (1976).

16) Wei Y., Li Y., Zhong L. L., *Chinese Chemical Letters*, **9**, 823-829 (1998).

17) Stillman M. J., Stothers J. B., *Can. J. Chem.*, **59**, 2303-2305, (1981).

18) Ward E. W. B., Stoessl A., Stothers J. B., *Phytoshemistry*, **16**, 2024-2025 (1977).

19) George, I. B., Stoessl A., Grover S. H., Stothers J. B., *Can. J. Chem.*, **52**, 993-1005 (1974).

20) Seo S., Tomita Y., Tori K., *Tettrahedron Letters*, **1**, 17-10 (1975).

21) Mochammad S., Yamasaki K, Kasai R., Tanaka O., *Chem. Pharm. Bull.*, **28**, 1006-1008 (1980).

22) Ageta H., Arai Y., Suzuki H., Kiyotani T., Kitabayashi M., *Chem.Pharm. Bull.*, **43**, 198-203 (1995).

23) Salazar G. C. M., Silva G. D. F., Duarte L. P., Vieira F. S. A., Lula I. S., *Magnetic Resonance in Chemistry*, **38**, 977-980 (2000).

24) Carvalho L., Seita J., *Natural Product Letters*, **2**, 57-60 (1993).

25) Greca M. D., Monaco P., Previtera L., *Journal of Natural Products*, **53**, 1430-1435 (1990).

26) Ghosal S., Singh A. K., Chaudhuri R. K., *Journal of Pharmaceutical Sciences*, **65**, 1549-1551 (1976).

27) Hou Z. F., Shi Y. P., Li X. F., Li X. F., Li Y., *Indian Journal of Chemistry*, **36B**, 293-296 (1997).

28) Kagan H. B., *Stereochemistry: fundamentals and methods*, 89-92 (1977).

29) Yoshihara T., Yamaguchi K., Takamatsu S., Sakamura S. *Agric. Biol. Chem.*, **45** (11), 2593-2598 (1981).

30) Iwai K., Suzuki T., Lee K. R., Kobayashi M., Oka S., *Agric. Biol. Chem.*, **41** (10), 1877-1882 (1977).