Pergamon

PII: S0031-9422(96)00212-9

Phytochemistry, Vol. 43, No. 1, pp. 133–140, 1996

0031-9422/96 $15.00 + 0.00

CARBAZOLE ALKALOIDS FROM *CLAUSENA EXCAVATA* AND THEIR BIOLOGICAL ACTIVITY

TIAN-SHUNG WU,* SHIOW-CHYN HUANG, PEI-LIN WU and CHE-MING TENG†

Department of Chemistry, National Cheng Kung University, Tainan, Taiwan, R.O.C.; †Pharmacological Institute, College of Medicine, National Taiwan University, Taipei, Taiwan, R.O.C.

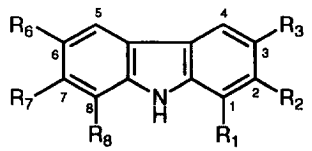
(*Received* 10 *October* 1995)

Key Word Index—*Clausena excavata*; Rutaceae; carbazole alkaloids; coumarins; clausines B, E, H, I and K; antiplatelet aggregation; vasorelaxing effect; Chinese medicine.

Abstract—Five new carbazole alkaloids, clausines B, E, H, I and K, as well as 22 known compounds, were isolated from the stem bark of *Clausena excavata*. The structures were established from spectral data and chemical transformation. These compounds showed significant inhibition of rabbit platelet aggregation and caused vasocontraction. The crude methanol extract, partitioned layers and chromatographic fractions revealed the presence of promotive and inhibitive constituents, simultaneously. These results might explain the philosophy of use in Chinese medicine, in that the dose and content variation in a prescription produced different, promotive or inhibitive, effects on therapy. Copyright © 1996 Elsevier Science Ltd

INTRODUCTION

Clausena excavata is used as a folk medicine for the treatment of snakebite, abdominal pain and as a detoxification agent [1]. We have reported the isolation and pharmacological evaluation of some carbazole alkaloids and carbazolequinone alkaloid obtained from this species [2–4]. Extensive investigation of the methanol extract of the stem bark of *C. excavata* resulted in the isolation of 27 compounds. We now describe the isolation and structural elucidation of five new carbazole alkaloids, clausines B (**1**), E (**2**), H (**3**), I (**4**) and K (**5**), and 22 known compounds, as well as their antiplatelet aggregating and vasorelaxing activity.

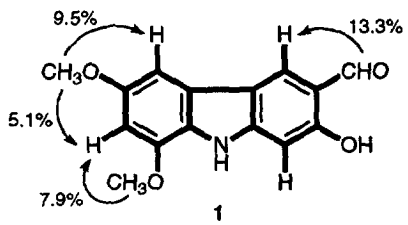


	R_1	R_2	R_3	R_6	R_7	R_8
1	H	OH	CHO	OMe	H	OMe
2	OH	H	CO_2Me	H	H	H
3	H	OMe	CO_2Me	H	OMe	H
4	OH	H	CHO	OMe	H	H
5	H	OMe	CO_2H	H	OMe	H
6	H	OH	CHO	H	H	H
7	OMe	H	CO_2Me	H	H	H
8	H	OMe	CHO	H	OMe	H
9	H	OH	CHO	OMe	H	H

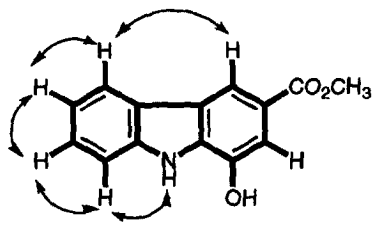
*Author to whom correspondence should be addressed.

RESULTS AND DISCUSSION

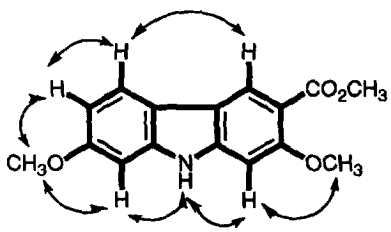
Clausine B (**1**) was isolated as yellow needles and determined to have the molecular formula $C_{15}H_{13}NO_4$ by high resolution mass spectrometry. The UV absorptions at 210, 217, 269 (*sh*), 278, 304 and 357 nm, as well as IR bands at 3400 (OH and NH), 1640 (C=O) cm^{-1} together with the ^1H NMR signals at δ 9.95 (*s*, CHO), 10.60 (*br s*, NH, D_2O-exchangeable), 11.36 (*s*, OH, D_2O-exchangeable) showed some similarity to those of mukonal (**6**), a 2-hydroxy-3-formylcarbazole alkaloid [5]. The downfield-shifted singlet at δ 8.38 in the ^1H NMR spectrum was assigned to H-4, which was deshielded not only by the ring current in carbazole but also by the aldehyde group. Hence, the other singlet at δ 6.89 belonged to H-1. Irradiation of the signal of CHO (δ 9.95) caused an increase of the signal at δ 8.38 (H-4) by 13.3%, which supported the partial structure of 2-hydroxyl-3-formyl ring-C (Fig. 1). The spectrum also showed the presence of two methoxyl singlets at δ 3.87 and 3.96 and two *meta*-coupled protons at δ 6.62 (*d*, J = 2.0 Hz) for H-7 and 7.24 (*d*, J = 2.0 Hz) for H-5. The 5.1 and 9.5% enhancements of the signals at δ 6.62 (H-7) and 7.24 (H-5), respectively, were observed by irradiation of the 6-OMe singlet (δ 3.96). In addition, a 7.9% increase of the signal for H-7 was also observed by irradiation of the other methoxyl (δ 3.87). These facts indicated a 6,8-dimethoxyl ring-A in this carbazole unit. The ^1H–^{13}C long-range correlation in the HMBC spectrum further confirmed the



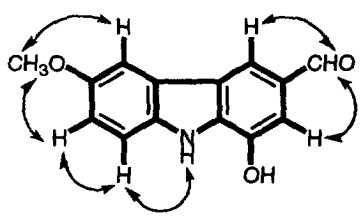
1



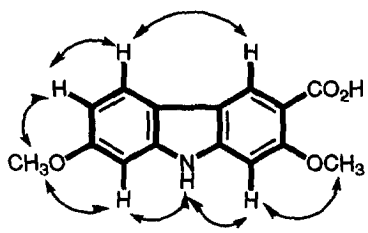
2



3

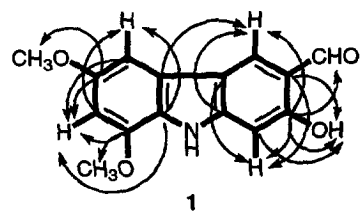


4

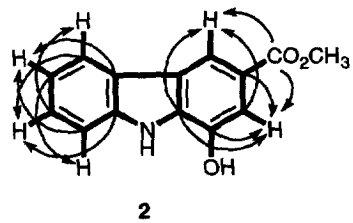


5

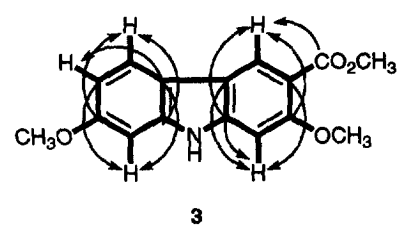
Fig. 1. NOE difference data for compound **1** and NOESY correlations for compounds **2**–**5**.



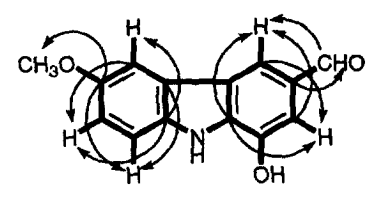
1



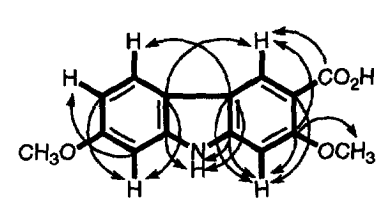
2



3



4



5

Fig. 2. ^{1}H–^{13}C long-range correlations for compounds **1**–**5**.

positions of these substituents (Fig. 2). Complete assignment of the ^{1}H and ^{13}C signals were achieved by NOE difference and HMBC experiments. On the basis of the above results, the structure of clausine B was deduced as **1**.

Clausine E (**2**) was obtained as white crystals with the molecular formula $C_{14}H_{11}NO_3$. The UV, IR and ^{1}H NMR spectra resembled those of mukonine (**7**) with regard to the type and position of substituents [6]. An unsubstituted ring A was established by the four mutually-coupled proton system at δ 7.22 (*td*, $J = 7.5$,

1.3 Hz, H-6), 7.43 (*td*, $J = 7.5$, 1.3 Hz, H-7), 7.61 (*dd*, $J = 7.5$, 1.3 Hz, H-8) and 8.17 (*dd*, $J = 7.5$, 1.3 Hz, H-5), whereas the existence of a *meta*-disubstituted ring-C was indicated by the two proton system at δ 7.57 (*d*, $J = 1.3$ Hz, H-2) and 8.37 (*d*, $J = 1.3$ Hz, H-4). Two other substituents were identified as a carbomethoxyl on C-3, from the signal at δ 3.88, also supported by mass fragment ion at *m/z* 182 $[M - CO_2Me]^+$ and a hydroxyl at δ 9.14 on C-1. The only difference between **2** and **7** was that the 1-OMe group in **7** was replaced by an OH in **2**. The $^1H-^{13}C$ long-range correlation (Fig. 2) between C = O (δ 167.8) and H-2 (δ 7.57), H-4 (δ 8.37), and C-1a (δ 133.5) and H-2 (δ 7.57), H-4 (δ 8.37), confirmed the structure of clausine E as **2**.

High resolution mass spectrometry established the molecular formula of clausine H (**3**) to be $C_{16}H_{15}NO_4$. The UV, IR and ^1H NMR spectra were similar to those of glycozolidal (**8**) having the 3-carbonyl-2,7-dimethoxycarbazole skeleton [7], *viz.*, a broad D_2O-exchangeable signal at δ 10.34 for NH, two methoxyl singlets at δ 3.85 and 3.90 for 7- and 2-OMe, respectively, two aromatic singlets at δ 7.10 and 8.40 for H-1 and H-4, and an ABX spin system of protons at δ 6.82 (*dd*, $J = 9.0$, 2.2 Hz), 7.02 (*d*, $J = 2.2$ Hz) and 7.94 (*d*, $J = 9.0$ Hz) for H-6, H-8 and H-5, respectively. The presence of an additional carbomethoxyl signal at δ 3.83 substituted for the aldehyde group in **8**. The assignment of the three methoxyls was determined by a NOESY experiment (Fig. 1). The methoxyl signal at δ 3.85, within NOE distance from the protons at H-6 (δ 6.82) and H-8 (δ 7.02), suggested that the methoxyl at δ 3.85 was attached to C-7. The NOE between the methoxyl signal at δ 3.90 and the proton at H-1 (δ 7.10) inferred that the methoxyl at δ 3.90 was at C-2. The $^1H-^{13}C$ long-range correlation between the carbonyl (δ 167.3) and H-4 (δ 8.40) confirmed that the carbomethoxyl group was also at C-3 (Fig. 2). Consequently, the above spectral data afforded the structure of clausine-H as **3**.

Clausine I (**4**) was isolated as a white powder, whose HR-mass spectrum indicated the molecular formula $C_{14}H_{11}NO_3$, an isomer of lansine (**9**) [8], which has the same formula as clausine E (**2**). The UV, IR and ^1H NMR spectra were also similar to those of **9**. The difference between **4** and **9** was only the position of hydroxyl substituent (δ 9.40) on C-1 in **4** instead of C-2 in **9**, which was confirmed by the *meta*-coupled signals at δ 7.40 (*d*, $J = 1.3$ Hz, H-2) and 8.26 (*d*, $J = 1.3$ Hz, H-4) in the ^1H NMR spectrum of **4** and from NOESY and HMBC experiments (Figs. 1 and 2). The remaining signals at δ 3.93 (*s*) for methoxyl and δ 7.11 (*dd*, $J = 9.0$, 2.5 Hz), 7.56 (*d*, $J = 9.0$ Hz) and 7.78 (*d*, $J = 2.5$ Hz) for H-7, H-8 and H-5, respectively, indicated a 6-methoxyl on ring A. Therefore, the above data was in accordance with structure **4** for clausine I.

Clausine K (**5**) was determined to have the molecular formula as $C_{15}H_{13}NO_4$, an isomer of clausine B (**1**). However, the UV, IR, ^1H NMR spectra were almost identical to those of clausine H (**3**). The carbomethoxyl group in **3** was hydrolysed into a carboxylic functionality in **5**. After methylation with diazomethane, acid **5** was converted back to ester **3** for spectral comparison of the methylation product **5a** with those of **3**. Further assignments of each signal were made by NOESY and HMBC experiments (Figs. 1 and 2). On the basis of the above spectral and chemical analyses, structure **5** was assigned for clausine K.

Twelve known carbazole alkaloids, mukonal (**6**) [5], mukonine (**7**) [6], lansine (**8**) [8], glycozolidal (**9**) [7], heptaphylline (**10**) [9], 3-methylcarbazole (**11**) [10], heptazoline (**12**) [11], methyl carbazole-3-carboxylate (**13**) [12], murrayanine (**14**) [13], 2-hydroxy-3-methylcarbazole (**15**) [14], clausine-D (**16**) [2] and clausine-F (**17**) [2], five coumarins, clausarin (**18**) [9], clausenidin (**19**) [9], xanthoxyletin (**20**) [9], nordentatin (**21**) [9] and scopoletin (**22**) [15], two phenols, methyl *p*-hydroxycinnamate (**23**) [16] and syringaldehyde (**24**) [17], two tetranortriterpenoids, zapoterin (**25**) [18] and *O*-methylclausenolide (**26**) [3], and one carbazoquinone, clausenaquinone-A (**27**) [4], were also isolated and characterized by the comparison of their spectroscopic data with literature values.

The crude MeOH extract, partitioned layers and chromatographic fractions, along with the isolated compounds **1–5**, **9**, **12**, **14**, **16–21**, **23**, **25** and **26**, from the stem bark of *C. excavata* were subjected to evaluation for antiplatelet aggregative activity and vasorelaxing effect. The results are summarized in Tables 1–5. Most of the isolated compounds from fractions 2–4 showed potent inhibitory activity on rabbit platelet aggregation induced by arachidonic acid (100 μM), collagen (10 $\mu g\,ml^{-1}$) and platelet activation factor (2ng ml^{-1}) (Tables 3 and 4). Especially, compound **2** showed 58.3% and 89.3% inhibition of rat aorta phasic and tonic contraction induced by NE (3 μM), together with 87.0% inhibition of tonic contraction induced by K^+ (80 mM) + Ca^{2+} (1.9 mM) (Table 5). Even though we only isolated one promotive constituent **26**, the crude MeOH extract, partitioned layers and chromatographic fractions displayed both inhibitive and promotive effects on platelet aggregation (Table 1). Therefore, the pharmacological activity, inhibition or promotion, of a Chinese medicine depends on the amount of the constituents. The presence of inhibitive and promotive components simultaneously in the plant indicated the possible philosophy of use in Chinese medicine, in that the dose and content variation in a prescription produced different, promotive or inhibitive, effects on therapy.

EXPERIMENTAL

General. Mps: uncorr. UV: MeOH. IR: KBr. ^1H and ^{13}C NMR: acetone-d_6 with TMS as int. ref. except where noted. MS: direct inlet.

Plant material. Clausena excavata was collected from Pin-Ton Hsien, Taiwan, in May 1989 and verified by Prof. C. S. Kuoh. A voucher specimen is deposited

Table 1. Effects of crude MeOH extract, partitioned layers and chromatographic fractions from stem bark of *C. excavata* on aggregation of washed rabbit platelets induced by adenosine diphosphate (ADP), arachidonic acid (AA), collagen (Col) and platelet activation factor (PAF)

	Inhibition (%)			
Component (mg ml^{-1})	ADP (20 μM)	AA (100 μM)	Col (10 μg ml^{-1})	PAF (2 ng ml^{-1})
MeOH extract (0.5)	A	A	23.5±2.8	N
H_2O layer (1.0)	17.2±1.8‡	3.67±1.1*	3.0±1.8	1.6±1.1
BuOH layer (1.0)	87.5±4.2‡	92.0±2.8‡	89.8±1.9‡	28.2±3.9
$CHCl_3$ layer (0.5)	A	A	34.2±3.4	N
Fr. 1 (0.2)	28.0±5.5	18.9±3.8	41.5±7.0	14.6±2.5
Fr.2 (0.2)	45.2±2.2‡	93.2±1.5‡	78.8±1.0‡	92.7±3.0‡
Fr. 3 (0.2)	46.3±3.6‡	92.2±1.7‡	75.1±2.7	69.2±6.5‡
Fr. 4 (0.2)	45.2±3.5	91.9±1.2‡	82.9±1.8‡	38.0±3.3‡
Fr. 5 (0.2)	A	A	A	A
Fr. 6 (0.2)	A	A	56.2±7.7	N
Fr. 7 (0.2)	A	A	28.5±3.4	N
Fr. 8 (0.2)	A	A	32.9±5.7	N
Fr. 9 (0.2)	A	A	13.4±2.6	N
Fr. 10 (0.2)	A	A	5.6±2.7	N
Fr. 11 (0.2)	A	A	55.9±5.7	N
Aspirin (0.02)	1.7±1.0	100.0±0.0‡	7.9±3.2	2.6±1.2

Platelets were preincubated with components or DMSO (0.5%, control) at 37° for 3 min, the inducer was then added. Values are means ±s.e.m. (n = 3–8).

A: Platelet aggregation promoted. N: no test.

*$P < 0.05$, ‡$P < 0.001$ as compared with respective control.

in the Herbarium of Cheng Kung University, Tainan, Taiwan.

Extraction and isolation. Dried stem bark (2.6 kg) was extracted with MeOH (31 × 6) and evapd to give a brown syrup (264 g). This syrup was partitioned between $CHCl_3$, *n*-BuOH and H_2O, successively. The $CHCl_3$ layer was dried (Na_2SO_4) and concd under red. pres. to leave a brown syrup (57.2 g) which was directly chromatographed on silica gel and eluted with a gradient of benzene and Me_2CO to afford 11 frs. Each

Table 2. Inhibition of KCl + $CaCl_2$ −, norepinephrine-induced contractions of rat aorta by crude MeOH extract, partitioned layers and chromatographic fractions from stem bark of *C. excavata*

	Inhibited contraction (%)		
	K^+(80 mM) + Ca^{2+}(1.9 mM)	NE (3 μM)	
Compound (100 μg ml^{-1})	Tonic	Phasic	Tonic
Control (0.5% DMSO)	0.0±2.5	0.0±7.1	0.0±10.0
MeOH extract (0.5)	N	N	N
H_2O layer (1.0)	17.3±2.6	12.3±10.0	57.7±6.2
BuOH layer (1.0)	29.2±3.7	26.8±4.2	47.9±9.1
$CHCl_3$ layer (0.5)	N	N	N
Fr. 1 (0.2)	50.2±8.1	8.4±6.5	19.2±11.0
Fr. 2 (0.2)	88.8±2.6‡	24.8±10.0	89.9±5.2‡
Fr. 3 (0.2)	98.0±2.3‡	58.4±5.8*	96.1±7.2‡
Fr. 4 (0.2)	97.1±1.3‡	93.3±5.1‡	97.1±5.0‡
Fr. 5 (0.2)	11.9±1.3	26.8±4.2	17.3±11.0
Fr. 6 (0.2)	18.6±8.2	−10.0±3.9	−4.5±5.2
Fr. 7 (0.2)	13.0±1.6	−37.0±12.0	−2.5±7.3
Fr. 8 (0.2)	17.6±6.3	12.5±8.0	9.5±7.2
Fr. 9 (0.2)	19.0±4.1	0.0±6.1	18.4±7.4
Fr. 10 (0.2)	17.7±7.9	4.2±5.0	−4.8±8.3
Fr. 11 (0.2)	3.9±1.5	−3.5±4.8	−1.9±5.3
Nifedipine (1)	100.0±0.0†	1.3±0.7	3.5±2.1

Rat aorta rings preincubated with components or DMSO (0.5%, control) at 37° for 15 min, then inducer added. Values are means ±s.e.m. (n = 3–8).

N: no test.

*$P < 0.05$, †$P < 0.01$, ‡$P < 0.001$ as compared with respective control.

Table 3. Effects of compounds from stem bark of *C. excavata* on aggregation of washed rabbit platelets induced by arachidonic acid (AA)

Compound	Inhibition (%) by AA (100 μM)								
	100 μg/ml	50	20	10	5	2	1	0.5	0.2
1	23.0±3.5‡	N	N	N	N	N	N	N	N
2	90.0±3.3‡	N	N	N	100.0±0.9‡	8.9±4.2	N	N	N
3	100.0±0.8‡	N	100.0±1.0‡	14.1±1.9‡	4.1±2.1	N	N	N	N
4	94.0±1.3‡	N	100.0±1.2‡	100.0±1.2‡	89.9±4.8‡	1.3±3.6	N	N	N
5	3.0±2.3	N	N	N	N	N	N	N	N
8	95.0±1.8‡	100.0±0.5‡	100.0±0.5‡	79.4±8.4‡	5.1±1.7	N	N	N	N
12	100.0±0.8‡	100.0±0.8‡	100.0±0.8‡	75.6±10.0‡	9.4±2.7*	4.4±1.0	N	N	N
14	100.0±0.8‡	97.0±1.9‡	40.0±12.0*	6.0±2.6	N	N	N	N	N
16	L	100.0±0.8‡	N	98.6±1.0‡	100.0±0.5‡	100.0±0.5‡	53.0±11.0*	26.7±1.0	0.2±1.0
17	L	N	96.0±0.9‡	97.5±1.4‡	97.7±1.4‡	68.2±9.4‡	37.4±9.6	7.5±2.8	N
18	100.0±0.8‡	N	100.0±0.8‡	10.8±2.6†	4.8±1.6	N	N	N	N
19	N	3.0±1.4	N	N	N	N	N	N	N
20	100.0±0.8‡	100.0±0.8‡	85.0±4.5‡	57.7±10.0†	14.2±3.3	N	N	N	N
21	100.0±0.5‡	57.3±8.7†	2.6±1.7	N	N	N	N	N	N
23	100.0±0.8‡	100.0±0.8‡	66.9±9.1‡	52.2±9.5*	34.4±8.7	N	N	N	N
25	2.0±2.3	N	N	N	N	N	N	N	N
26	A	N	N	N	N	N	N	N	N
Aspirin	—	—	100±0.0‡	53.4±2.6	2.1±0.5	—	—	—	—

Platelets preincubated with compounds or DMSO (0.5%, control) at 37° for 3 min, inducer was then added. Values are means ±s.e.m. ($n = 3$–6).

A: Platelet aggregation promoted. L: platelet lysis at 100 μg ml^{-1}. N: no test.

*$P < 0.05$.

†$P < 0.01$.

‡$P < 0.001$ as compared with respective control.

Table 4. Effects of compounds from stem bark of *C. excavata* on the aggregation of washed rabbit platelets induced by collagen (Col) and platelet activation factor (PAF)

	Inhibition (%)										
	Col (10 μg ml^{-1})							PAF (2 ng ml^{-1})			
Compound	100 μg/ml	50	20	10	5	2	1	100 μg/ml	50	20	10
1	16.0±2.0‡	N	N	N	N	N	N	19.0±1.9‡	N	N	N
2	92.0±1.7‡	94.4±2.4‡	74.7±3.2‡	59.0±6.4‡	22.2±6.1*	0.5±3.6	N	60.0±6.7‡	N	N	N
3	100.0±0.7‡	100.0±0.6‡	7.0±2.2	N	N	N	N	100.0±1.1‡	17.6±5.0	0.4±1.8	N
4	87.0±1.0‡	75.0±5.4‡	35.0±4.0‡	−0.4±5.8	N	N	N	17.1±6.1	N	N	N
5	5.0±1.7	N	N	N	N .	N	N	3.0±2.6*	N	N	N
9	88.0±2.9‡	89.0±1.0‡	64.5±4.6‡	22.3±6.4†	5.2±0.8*	N	N	43.0±5.5‡	N	N	N
12	100.0±0.4‡	89.2±2.0‡	50.9±9.5*	16.7±4.2	12.4±3.4	N	N	100.0±0.5‡	16.1±2.8*	3.3±1.0	N
14	62.0±5.2‡	45.0±1.9‡	31.5±5.9*	11.1±1.3‡	N	N	N	3.0±3.9	N	N	N
16	L	95.0±1.7‡	84.0±1.7‡	65.6±4.8‡	18.8±0.9‡	5.0±0.3‡	2.9±0.7	L	70.7±5.2‡	N	N
17	L	N	90.0±1.0‡	47.4±3.2‡	6.5±0.8‡	4.7±0.5	N	L	N	16.0±3.2†	N
18	60.5±1.0‡	N	N	N	N	N	N	100.0±0.5‡	N	11.4±2.1*	7.0±1.0‡
19	N	3.0±1.8	N	N	N	N	N	N	11.0±3.0	N	N
20	85.0±1.1‡	84.0±4.2‡	14.8±2.0‡	7.7±1.3†	N	N	N	50.0±4.3	N	N	N
21	90.3±1.6‡	9.3±0.9‡	2.5±0.5	N	N	N	N	79.0±5.9‡	N	N	N
23	82.4±3.9‡	49.6±6.7‡	5.8±0.7‡	N	N	N	N	5.0±2.1	N	N	N
25	3.0±2.0	N	N	N	N	N	N	2.0±2.4	N	N	N
26	37.0±2.5	N	N	N	N	N	N	N	N	N	N
Aspirin	N	N	7.9±3.2	N	N	N	N	N	N	2.6±1.2	N

Platelets preincubated with compounds or DMSO (0.5%, control) at 37° for 3 min, inducer was then added. Values are means ±s.e.m. ($n = 3$–6).
A: Platelet aggregation promoted. L: platelet lysis at 100 μg ml^{-1}. N: no test.
*$P < 0.05$.
†$P < 0.01$.
‡$P < 0.001$ as compared with respective control.

Table 5. Inhibition of KCl + $CaCl_2$-, norepinephrine-induced contractions of rat aorta by compounds **1**, **2**, **12**, **14**, **16–19**, **23** and **25**

	Inhibited contraction (%)		
	K^+(80 mM) + Ca^{2+}(1.9 mM)	NE (3 μM)	
Compound (100 μg ml^{-1})	Tonic	Phasic	Tonic
Control (0.5% DMSO)	0.0±5.9	0.0±2.6	0.0±1.9
1	−8.0±4.0	−25.0±25.0	−15.0±5.0
2	87.0	58.3	89.3
12	−28.5	30.0±3.3	24.0±4.0
14	−8.0	0.0±4.0	−6.0±5.0
16	21.5	−30.7	−8.5
17	60.0	N	N
18	10.5	15.0±10.0	24.8±5.0
19	77.3±0.0‡	0.0	8.0±5.0
23	19.3	24.0±7.0	15.0±4.0
25	0.0	0.0	8.0±5.0
Nifedipine (1)	100.0±0.0†	1.3±0.7	3.5±2.1

Rat aorta rings preincubated with components or DMSO (0.5%, control) at 37° for 15 min, then inducer added. Values are means ±s.e.m. (n = 3–8).

N: no test.

*$P < 0.05$, †$P < 0.01$, ‡$P < 0.001$ as compared with respective control.

fr. was rechromatographed on silica gel and prep. TLC or recrystallization. Pure compounds were obtained as follows. Fr. 1 gave **10** (3.9 mg) and **11** (5.8 mg). Fr. 2 gave **18** (163 mg), **12** (4.2 mg), **7** (14.6 mg), **6** (10.4 mg), unknown **a** (2.7 mg), **13** (1.0 mg), **23** (2.3 mg), **19** (260 mg), **21** (306 mg), **14** (31.7 mg), **1** (9.4 mg), **9** (6.4 mg), unknown **b** (8.1 mg) and unknown **c** (2 mg), successively. Fr 3 gave **8** (6.8 mg), **21** (48.7 mg), **15** (1.9 mg), **16** (10.8 mg), **2** (150 mg), **17** (18.8 mg) and unknown **d** (4.7 mg), successively. Fr. 4 gave **3** (29.4 mg) and **4** (65.9 mg). Fr. 5 gave **25** (712 mg) and **22** (0.5 mg). Fr. 6 gave **26** (44.6 mg), unknown **e** (1.5 mg) and **27** (0.3 mg). Frs 7 and 8 gave **24** (1.2 mg). Fr. 9 gave **5** (618 mg).

Clausine B (**1**). Yellow needles (Me_2CO), mp 228–229°. HRMS: calcd for $C_{15}H_{13}NO_4$, m/z 271.0845 $[M]^+$, found 271.0847. UV λ_{max} nm (log ε): 210 (4.28), 235 (4.30), 269 (4.24, *sh*), 278 (4.32), 304 (4.36), 357 (3.83). IR ν_{max} cm^{-1}: 3400, 1640, 1610, 1600. EIMS m/z (rel. int.): 271 ($[M]^+$, 100), 256 (46), 228 (15), 213 (34). ^{1}H NMR: δ 3.87 (3H, *s*, 8-OMe), 3.96 (3H, *s*, 6-OMe), 6.62 (1H, *d*, J = 2.0 Hz, H-7), 6.89 (1H, *s*, H = 1), 7.24 (1H, *d*, J = 2.0 Hz, H-5), 8.38 (1H, *s*, H-4), 9.95 (1H, *s*, CHO), 10.60 (1H, *br s*, NH), 11.36 (1H, *s*, 2-OH). ^{13}C NMR: δ 56.0 and 56.1 (*q*, 2 × OMe), 95.2 (*d*, C-5), 97.4 (*d*, C-1), 98.3 (*d*, C-7), 116.1 (*s*, C-3), 119.0 (*s*, C-4a), 124.9 (*s*, C-5a), 126.2 (*s*, C-8a), 129.0 (*d*, C-4), 146.8 (*s*, C-1a), 147.1 (*s*, C-6), 156.8 (*s*, C-8), 161.4 (*s*, C-2), 196.1 (*s*, CHO).

Clausine E (**2**). Powder (Me_2CO), mp 218–220°. HRMS: calcd for $C_{14}H_{11}NO_3$, m/z 241.0739 $[M]^+$, found 241.0734. UV λ_{max} nm (log ε): 222 (4.01, *sh*), 239 (4.14), 248 (4.08, *sh*), 269 (4.22, *sh*), 276 (4.23), 311 (3.65), 324 (3.64), 337 (3.50). IR ν_{max} cm^{-1}: 3360, 1660, 1630, 1600. EIMS m/z (rel. int.): 241 ($[M]^+$, 100), 210 (74), 182 (29), 154 (16). ^{1}H NMR: δ 3.88 (3H, *s*, CO_2Me), 7.22 (1H, *td*, J = 7.5, 1.3 Hz, H-6), 7.43 (1H, *td*, J = 7.5, 1.3 Hz, H-7), 7.57 (1H, *d*, J = 1.3 Hz, H-2), 7.61 (1H, *dd*, J = 7.5, 1.3 Hz, H-8), 8.17 (1H, *dd*, J = 7.5, 1.3 Hz, H-5), 8.37 (1H, *d*, J = 1.3 Hz, H-4), 9.14 (1H, *s*, 1-OH), 10.61 (1H, *br s*, NH). ^{13}C NMR: δ 51.7 (*q*, OMe), 111.3 (*d*, C-2), 112.2 (*d*, C-8), 115.2 (*d*, C-4), 120.3 (*d*, C-6), 121.0 (*d*, C-5), 122.3 (*s*, C-4a), 124.3 (*s*, C-3), 124.8 (*s*, C-5a), 126.8 (*d*, C-7), 133.5 (*s*, C-1a), 141.2 (*s*, C-8a), 143.2 (*s*, C-1), 167.8 (*s*, C = 0).

Clausine H (**3**). Grey needles (Me_2CO), mp 192–194°. HRMS: calcd for $C_{16}H_{15}NO_4$, m/z 285.1001 $[M]^+$, found 285.1002. UV λ_{max} nm (log ε): 225 (4.24, *sh*), 246 (4.44), 281 (4.39), 310 (3.86), 319 (3.86), 335 (3.65, *sh*). IR ν_{max} cm^{-1}: 3300, 1700, 1620. EIMS m/z (rel. int.): 285 ($[M]^+$, 100), 270 (48), 254 (26), 240 (10). ^{1}H NMR: δ 3.83 (3H, *s*, CO_2Me), 3.85 (3H, *s*, 7-OMe), 3.90 (3H, *s*, 2-OMe), 6.82 (1H, *dd*, J = 9.0, 2.2 Hz, H-6), 7.02 (1H, *d*, J = 2.2 Hz, H-8), 7.10 (1H, *s*, H-1), 7.94 (1H, *d*, J = 9.0 Hz, H-5), 8.40 (1H, *s*, H-4), 10.34 (1H, *br s*, NH). ^{13}C NMR: δ 55.6 (*q*, 2-OMe), 56.2 (*q*, 7-OMe), 51.4 (*q*, CO_2Me), 94.8 (*d*, C-1), 95.8 (*d*, C-8), 109.0 (*d*, C-6), 113.3 (*s*, C-3), 117.0 (*s*, C-4a), 117.5 (*s*, C-5a), 120.9 (*d*, C-5), 123.6 (*d*, C-4), 142.6 (*s*, C-8a), 144.5 (*s*, C-1a), 158.7 (*s*, C-2), 159.5 (*s*, C-7), 167.3 (*s*, C = 0).

Clausine I (**4**). Powder (Me_2CO), mp 222–224°. HRMS: calcd for $C_{14}H_{11}NO_3$, m/z 241.0739 $[M]^+$, found 241.0739. UV λ_{max} nm (log ε): 204 (4.24), 223 (4.14), 242 (4.14), 255 (4.09), 278 (4.23), 296 (4.17), 341 (3.85), 353 (3.86). IR ν_{max} cm^{-1}: 3400, 3358, 1668, 1640. EIMS m/z (rel. int.): 241 ($[M]^+$, 100), 226 (86), 198 (27), 141 (7). ^{1}H NMR: δ 3.93 (3H, *s*, 6-OMe), 7.11 (1H, *dd*, J = 9.0, 2.5 Hz, H-7), 7.40 (1H, *d*, J = 1.3 Hz, H-2), 7.56 (1H, *d*, J = 9.0 Hz, H-8), 7.78 (1H, *d*, J = 2.5 Hz, H-5), 8.26 (1H, *d*, J = 1.3 Hz, H-4), 9.40 (1H, *s*, 1-OH), 9.99 (1H, *s*, CHO), 10.68 (1H, *br s*, NH). ^{13}C NMR: δ 55.8 (*q*, 6-OMe), 103.5 (*d*, C-5),

107.8 (*d*, C-2), 113.2 (*d*, C-8), 116.4 (*d*, C-7), 119.2 (*d*, C-4), 124.8 (*s*, C-4a), 126.9 (*s*, C-5a), 130.5 (*s*, C-1), 135.2 (*s*, C-8a), 135.8 (*s*, C-1a), 144.1 (*s*, C-1), 155.2 (*s*, C-6), 191.5 (*s*, CHO).

Clausine K (**5**). Brownish powder (Me_2CO), mp 250–256°. HRMS: calcd for $C_{15}H_{13}NO_4$, *m/z* 271.0846 $[M]^+$, found 271.0846. UV λ_{max} nm (log ε): 224 (4.27, *sh*), 242 (4.51), 277 (4.35), 283 (4.33, *sh*), 310 (3.96), 320 (3.96). IR ν_{max} cm^{-1}: 3320, 1665, 1615. EIMS *m/z* (rel. int.): 271 ($[M]^+$, 100), 256 (28), 212 (15). ^1H NMR (DMSO-d_6): δ 3.83 (3H, *s*, 7-OMe), 3.89 (3H, *s*, 2-OMe), 6.77 (1H, *dd*, $J = 8.5$, 2.0 Hz, H-6), 6.97 (1H, *d*, $J = 2.0$ Hz, H-8), 7.03 (1H, *s*, H-1), 7.94 (1H, *d*, $J = 8.5$ Hz, H-5), 8.39 (1H, *s*, H-4), 11.27 (1H, *br s*, NH). ^{13}C NMR (DMSO-d_6): δ 55.3 (*q*, 2-OMe), 56.0 (*q*, 7-OMe), 94.0 (*d*, C-1), 95.1 (*d*, C-8), 108.1 (*d*, C-6), 112.3 (*s*, C-3), 115.8 (*s*, C-4a), 116.3 (*s*, C-5a), 120.4 (*d*, C-5), 123.1 (*d*, C-4), 141.6 (*s*, C-8a), 143.4 (*s*, C-1a), 157.4 (*s*, C-2), 158.1 (*s*, C-7), 167.5 (*s*, C = 0).

Methylation of clausine-K (**5**). Excess CH_2N_2-Et_2O was added to a soln of clausine-K (**5**) (2 mg) in MeOH-Et_2O. The reaction mixt. was allowed to stand overnight and concd under red. pres. The crude product was purified by prep. TLC using $CHCl_3$–MeOH (15:1) to afford a colourless powder **5a** which showed almost identical spectral data to those of **3**. Mp 190–192°. UV λ_{max} nm: 224, 246, 279, 309, 319, 333. IR ν_{max} cm^{-1}: 3450, 1715, 1690. EIMS *m/z* (rel. int.): 285 ($[M]^+$, 100), 270 (41), 254 (21). ^1H NMR: δ 3.84, 3.86 and 3.91 (3H each, *s*, 3 × OMe), 6.83 (1H, *dd*, $J = 8.6$, 2.3 Hz, H-6), 7.04 (1H, *d*, $J = 2.3$ Hz, H-8), 7.12 (1H, *s*, H-1), 7.95 (1H, *dd*, $J = 8.6$, 2.3 Hz, H-5), 8.42 (1H, *s*, H-4), 10.50 (1H, *br s*, NH).

Acknowledgement—We thank the National Science Council, R.O.C. (NSC 80-0420-B-006-07) for support of this research.

REFERENCES

1. Sasaki, S. (1924) *Khoyo Taiwan Minkan Yakyo Shokubutsu Shi*, p. 36. Khobunkan, Taipei.
2. Wu, T. S. and Huang, S. C. (1992) *Chem. Pharm. Bull.* **40**, 1069.
3. Wu, T. S., Huang, S. C. and Lai, J. S. (1992) *J. Chin. Chem. Soc.* **40**, 319.
4. Wu, T. S, Huang, S. C., Wu, P. L. and Lee, K. H. (1994) *Bioorg. Med. Chem. Letters* **4**, 2395.
5. Bhattacharyya, P. and Chakraborty, A. (1984) *Phytochemistry* **23**, 471.
6. Chowdhury, B. K. and Chakraborty, D. P. (1969) *Chem. and Ind.* 549.
7. Bhattacharyya, P. and Chowdhury, B. K. (1985) *J. Nat. Prod.* **48**, 465.
8. Prakash, D., Raj, K., Kapil, R. S. and Popli, S. P. (1980) *Indian J. Chem.* **19B**, 1075.
9. Wu, T. S. and Furukawa, H. (1982) *J. Nat. Prod.* **45**, 718.
10. Ray, S., Bhattacharyya, P. and Charkraborty, D. P. (1974) *Phytochemistry* **13**, 1017.
11. Chakraborty, D. P., Islam, A. and Ray, S. (1978) *Phytochemistry* **19**, 2043.
12. Li, W. S., McChesney, J. D. and El-Feraly, F. S. (1991) *Phytochemistry* **30**, 343.
13. Bhattacharyya, P. and Chakraborty, D. P. (1973) *Phytochemistry* **12**, 1813.
14. Bhattacharyya, P., Jash, S. S. and Chowdhury, B. K. (1986) *Chem. Ind.* 246.
15. Wu, T. S., Lin, C. N., Yang, L. K. and Lin, S. T. (1975) *J. Chin. Chem. Soc.* **22**, 167.
16. Reinners, W. (1964) *Naturwissenschaften* **51**, 193.
17. Pouchert, C. J. (1983) in *The Aldrich Library of NMR Spectra*, Vol. 2, p. 124. Aldrich Chemical Company, New York.
18. Ngadjui, B. T., Ayafor, J. F., Sondengamand, B. L. and Connolly, J. D. (1989) *J. Nat. Prod.* **52**, 832.