Pergamon

*Phytochemistry* Vol. 49, No. 7, pp. 2001–2003, 1998

Printed in Great Britain
0031-9422/98/$ - see front matter

PII: S0031-9422(98)00425-7

# RHINACANTHIN-Q, A NAPHTHOQUINONE FROM *RHINACANTHUS NASUTUS* AND ITS BIOLOGICAL ACTIVITY

TIAN-SHUNG WU,†* HUA-CHUN HSU,† PEI-LIN WU,† CHE-MING TENG‡ and YANG-CHANG WU§

†Department of Chemistry, National Cheng Kung University, Tainan 701, Taiwan, R.O.China, ‡Pharmacological Institute, College of Medicine, National Taiwan University, Taipei, Taiwan, R.O.China and §Department of Pharmacy, Kaoshing Medical College, Kaoshing, Taiwan, R.O.China

(*Received in revised form* 1 *April* 1998)

**Key Word Index**—*Rhinacanthus nasutus*; Acanthaceae; 1,4-naphthoquinone; rhinacanthin-Q; cytotoxicity; antiplatelet aggregation.

**Abstract**—The continuing investigation of the root of *Rhinacanthus nasutus* afforded a 1,4-naphthoquinone ester, rhinacanthin-Q, accompanied by twenty-four known compounds. The structure was elucidated on the basis of spectroscopic analyses. The cytotoxicity and antiplatelet effect of this compound was also discussed. 

## INTRODUCTION

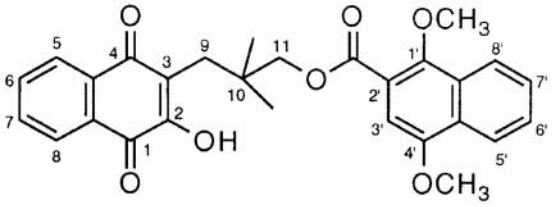
We have reported previously that the methanolic extract from the root of *R. nasutus* gave twenty-four compounds, rhinacanthin-A (**2**), -B (**3**), -C (**4**), -D (**5**), -G (**6**), -H (**7**), -I (**8**), -J (**9**), -K (**10**), -L (**11**), -M (**12**), -N (**13**), -O (**14**), -P (**15**), rhinacanthone (**16**), dehydro-α-lapachone (**17**), *p*-hydroxybenzaldehyde (**18**), methyl vanillate (**19**), syringaldehyde (**20**), lupeol (**21**), wogonin (**22**) oroxylin A (**23**), (+)-praeruptorin (**24**) and allantoin (**25**) [1, 2]. In our continuing interest in looking for new medicinals, a new naphthoquinone ester, rhinacanthin-Q (**1**), was obtained. Herein we reported the isolation, structural elucidation of this new compound. The pharmacological evaluation of this compound was also reported.

## RESULTS AND DISCUSSION

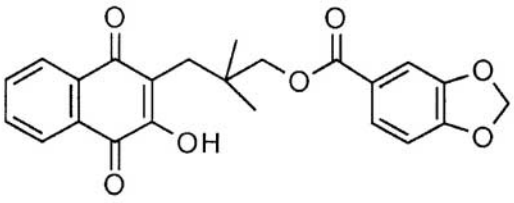
Rhinacanthin-Q (**1**) was isolated as orange needles and found to have molecular formula as $C_{28}H_{26}O_7$ by high resolution mass spectrometry. Examination of the $^1H$ and $^{13}C$ NMR spectra supported the ester structure **1**, the alcohol moiety being the same as in **5**, namely 2-hydroxy-3-(11-hydroxy-10,10-dimethylpropyl)-1,4-naphthoquinone. On the other hand, an aromatic singlet at $\delta$ 7.14 (H-3′) and four mutually coupled proton signals at $\delta$ 7.5–8.2 for H-5′, H-6′, H-7′ and H-8′ together with ten aromatic $^{13}C$ signals including five quaternary carbons at $\delta$ 118.8 (C-2′), 128.9 (C-9′), 129.0 (C-10′), 151.1 (C-4′), 151.8 (C-1′) and five tertiary carbons at $\delta$ 103.6 (C-3′), 122.1 (C-5′), 123.4 (C-8′), 126.8(C-6′), 127.5 (C-7′) implied the presence of a 1′,2′,4′-trisubstituted naphthalene moiety. The existence of two methoxyl signals at $\delta$ 3.95 and 3.96 suggested that the acid component of the ester was 1,4-dimethoxynaphthalene-2-carboxylic acid. The ester linkage between the 1,4-naphthoquinone and the β-naphthoic acid was proved by the $^3J$ long range correlation of $^1H$ and $^{13}C$ signals between O–C=O ($\delta$ 166.1) and H-3′ ($\delta$ 7.14), H-11 ($\delta$ 4.16). The location of these substituents was confirmed by HMBC and NOESY experiments. On the basis of this result, rhinacanthin-Q may be represented by structure **1**.

Rhinacanthin-Q (**1**), A (**2**), -B (**3**), -C (**4**), -D (**5**), -G (**6**), -H (**7**), -I (**8**), -K (**10**), -M (**12**), -N (**13**) as well as wogonin (**22**) were subjected to cytotoxic evaluation (Table 1). Most showed significant cytotoxicity in the P-388, A-549, HT-29 and HL-60 test systems. On the other hand, **1**–**4**, **6**–**8**, **10**, **12**, and **22** were also evaluated for their antiplatelet aggregation activity (Table 2). All test compounds demonstrated 36–100% inhibition of the rabbit pla-

*Author to whom correspondence should be addressed.



**1**



**5**

Table 1. The cytotoxicity of compounds **1**–**8**, **10**, **12**, **13**, **22**

| Compound | Cell lines $ED_{50}$ ($\mu$g/mL) | | | | |
|---|---|---|---|---|---|
| | KB | P-388 | A-549 | HT-29 | HL-60 |
| **1** | > 50 | 0.61 | 3.61 | 7.60 | 8.90 |
| **2** | 6.75 | 0.72 | 3.06 | 2.17 | 1.16 |
| **3** | 8.01 | 0.35 | 6.50 | 3.01 | 2.57 |
| **4** | 6.26 | 0.26 | 0.35 | 0.68 | 0.68 |
| **5** | 25.0 | 3.79 | 8.26 | 8.89 | 11.8 |
| **6** | 4.45 | 0.14 | 0.75 | 0.57 | 1.14 |
| **7** | 23.8 | 6.43 | 9.97 | 11.5 | 8.87 |
| **8** | 13.2 | 4.88 | 7.18 | 6.30 | 5.12 |
| **10** | 17.3 | 3.17 | 16.4 | 7.75 | 6.81 |
| **12** | 19.2 | 3.95 | 8.90 | 10.1 | 19.9 |
| **13** | 4.80 | 0.71 | 1.97 | 2.67 | 1.38 |
| **22** | 4.46 | 1.70 | 4.14 | 3.35 | 4.66 |

telet aggregation induced by arachidonic acid (100 mM). Compounds **2**–**4** and **22** also showed 72–100% inhibition of the rabbit platelet aggregation induced by collagen (10 $\mu$g $mL^{-1}$). Only **3** exhibited antiplatelet aggregation induced by platelet activation factor (2 ng $mL^{-1}$).

## EXPERIMENTAL

Melting points were uncorrected. UV spectra were obtained in methanolic solution. IR spectra were recorded on KBr disc. Mass and high resolution mass spectra were measured by a direct inlet system. $^1H$ NMR and $^{13}C$ NMR spectra were determined using tetramethylsilane (TMS) as internal standard.

### *Plant material*

The root of *Rhinacanthus nasutus* used in this investigation was collected in Tainan, Taiwan and identified by Professor Kuoh. A specimen of the plant has been deposited at the herbarium of the National Cheng Kung University, Tainan.

### *Extraction and separation*

The dried root (1.45 kg) of *R. nasutus* was extracted (×3) with methanol at room temperature. The combined methanol extracts were concentrated under reduced pressure to give a yellow–brown syrup which was partitioned between $CHCl_3$ and $H_2O$. The $CHCl_3$ layer was chromatographed over silica gel eluted with a gradient of hexane and

Table 2. The effects of compounds **1**–**4**, **6**–**8**, **10**, **12**, **22** on the aggregation of washed rabbit platelets induced by arachidonic acid (AA), collagen (Col), thrombin (Thr) and platelet activation factor (PAF)

| Induced inhibition (%) Compound ($\mu$g/mL) | Thr (0.1 U/mL) | AA (100 $\mu$M) | Col (10 $\mu$g/mL) | PAF (2 g/mL) |
|---|---|---|---|---|
| **1** (100) | –0.02 ± 2.3 | 54.6 ± 11* | 20.4 ± 3.7† | 6.88 ± 2.3 |
| **2** (100) | 2.30 ± 2.2 | 100 ± 1.1 | 100 ± 0.5‡ | 13.1 ± 3.3 |
| (50) | – | 12.5 ± 2.9 | 100 ± 0.5‡ | – |
| (20) | – | 2.80 ± 2.8 | 29.0 ± 2.4‡ | – |
| (10) | – | – | 2.30 ± 1.6 | – |
| **3** (100) | 0.88 ± 1.6 | 7.45 ± 5.6‡ | 100 ± 0.5‡ | 63.1 ± 8.5‡ |
| (50) | – | 22.7 ± 4.7† | 87.8 ± 4.8‡ | – |
| (20) | – | 0.24 ± 1.9 | 0.92 ± 1.4‡ | – |
| **4** (100) | 1.75 ± 1.2 | 100 ± 1.1 | 75.2 ± 7.3‡ | 8.50 ± 2.2* |
| **6** (100) | 0.22 ± 1.4 | 42.6 ± 8.9* | 13.8 ± 2.6† | 10.7 ± 2.1† |
| **7** (100) | 0.11 ± 1.3 | 54.8 ± 4.4‡ | 31.0 ± 3.9‡ | 11.4 ± 2.1† |
| **8** (100) | –0.66 ± 1.5 | 54.9 ± 8.2† | 10.8 ± 1.8† | 22.2 ± 3.9† |
| **10** (100) | 0.44 ± 1.7 | 36.8 ± 8.9* | 17.0 ± 1.6† | 12.0 ± 2.2† |
| **12** (100) | –0.55 ± 2.4 | 100 ± 1.1‡ | 5.40 ± 1.3* | 9.40 ± 2.7* |
| **22** (100) | 0.66 ± 2.3 | 100 ± 1.1 | 72.5 ± 3.9 | 8.60 ± 4.0 |

Platelets were preincubated with compounds or DMSO (0.5%, control) at 37°C for 3 min; the inducer was added. Values are means ± s.e.m. ($n$ = 3–4).

*$P < 0.05$.

†$P < 0.01$.

‡$P < 0.001$ were compared with the respective control.

EtOAc to give eight fractions. Repeatedly chromatography of each fraction in a similar way afforded **21** (1.20 g), **4** (1.65 g), **6** (67 mg), **9** (22 mg), **7** (40 mg), **8** (54 mg), **11** (3 mg), **10** (25 mg), **3** (250 mg), **2** (71 mg), **14** (5 mg), **17** (54 mg), **13** (7 mg), **12** (53 mg), **1** (3 mg), **5** (37 mg), **18** (1 mg), **15** (1 mg), **19** (1 mg), **22** (5 mg), **23** (3 mg), **24** (2 mg), **20** (2 mg) and **16** (5 mg), successively. The aqueous solution was extracted with *n*-BuOH and the *n*-BuOH layer was crystallized after standing to furnish **25** (2.50 g).

*Rhinacanthin-Q* (**1**)

Orange needles ($Me_2CO$), mp 116–117°C. HRMS: calcd for $C_{28}H_{26}O_7$, *m/z* 474.1679 $[M]^+$, found 474.1684. UV $\lambda$ nm: 217, 251, 277, 283 (sh), 336. IR $\upsilon$ $cm^{-1}$: 3370, 1703, 1649, 1594. EIMS *m/z* (rel. int.): 474 ($M^+$, 100), 232 (47), 215 (71), 187 (15), 159 (11), 129 (11). $^1H$ NMR ($CDCl_3$) $\delta$ 1.16 (6H, *s*, 2 × 10-Me), 2.79 (2H, *s*, H-9), 3.95 (3H, *s*, 1′-OMe), 3.96 (3H, *s*, 4′-OMe), 4.16 (2H, *s*, H-11), 7.14 (1H, *s*, H-3′), 7.39 (1H, *td*, *J* = 7.6, 1.2 Hz, H-7), 7.48 (1H, *td*, *J* = 7.6, 1.2 Hz, H-6), 7.5–7.6 (2H, *m*, H-6′ and H-7′), 7.84 (1H, *dd*, *J* = 7.6, 1.2 Hz, H-8), 7.90 (1H, *dd*, *J* = 7.6, 1.2 Hz, H-5), 8.0–8.1 (1H, *m*, H-8′), 8.1–8.2 (1H, *m*, H-5′). $^{13}C$ NMR ($CDCl_3$) $\delta$ 25.5 (2 × 12-Me), 32.4 (C-9), 37.0 (C-10), 55.5 (4′-OMe), 63.3 (1′-OMe), 73.7 (C-11), 103.6 (C-3′), 118.8 (C-2′), 121.8 (C-3), 122.1 (C-5′), 123.4 (C-8′), 125.6 (C-8), 126.4 (C-5), 126.8 (C-6′), 127.5 (C-7′), 128.5 (C-9), 128.9 (C-9′), 129.0 (C-10′), 132.3 (C-7), 132.6 (C-10), 134.3 (C-6), 151.1 (C-4′), 151.8 (C-1′), 154.0 (C-2), 166.1 (OC=O), 181.1 (C-1), 184.9 (C-4).

*Cytotoxicity assays*

The *in vitro* KB cytotoxicity assay was carried out according to procedures described by Geran *et al.* and Ferguson *et al.* [3,4]. The assay against P-388, A-549, HT-29 and HL-60 tumor cells was based on a method reported by Lee *et al.* [5].

*Antiplatelet aggregation assays*

The antiplatelet aggregation assays were based on a method reported by Teng *et al.* [6].

*Acknowledgements*—We thank National Science Council, R.O.C. (NSC 82-0420-B006-014-M13) for support of this research.

## REFERENCES

1. Wu, T. S., Yang, C. C., Wu, P. L. and Liu, L. K., *Phytochemistry*, 1995, **40,** 1247.
2. Wu, T. S., Hsu, H. C., Wu, P. L., Leu, Y. L., Chan, Y. Y., Yeh, M. Y. and Tien, H. J., *Chem. Pharm. Bull.*, 1998, **46,** 413.
3. Geran, R. I., Greenberg, N. H., MacDonald, M. M., Schumacher, A. M. and Abbott, B. J., *Cancer Chemother. Rep.*, 1972, **3,** 1.
4. Ferguson, P. J., Fisher, M. H., Stephenson, J., Li, D. H., Zhou, B. S. and Cheng, Y. C., *Cancer Res.*, 1988, **48,** 5956.
5. Lee, K. H., Lin, Y. M., Chang, J. J., Wu, R. Y. and Yang, T. H., *Planta Med.*, 1988, **54,** 308.
6. Yu, S. M., Ko, F. N., Su, M. J., Wu, T. S., Wang, M. L., Huang, T. F. and Teng, C. M., *Arch. Pharmacol.*, 1992, **345,** 349.