Naphthoquinone Esters from the Root of *Rhinacanthus nasutus*

Tian-Shung WU,* Hua-Chun HSU, Pei-Lin WU, Yann-Lii LEU, Yu-Yi CHAN, Ching-Yuh CHERN, Mou-Yung YEH, and Hsien-Ju TIEN

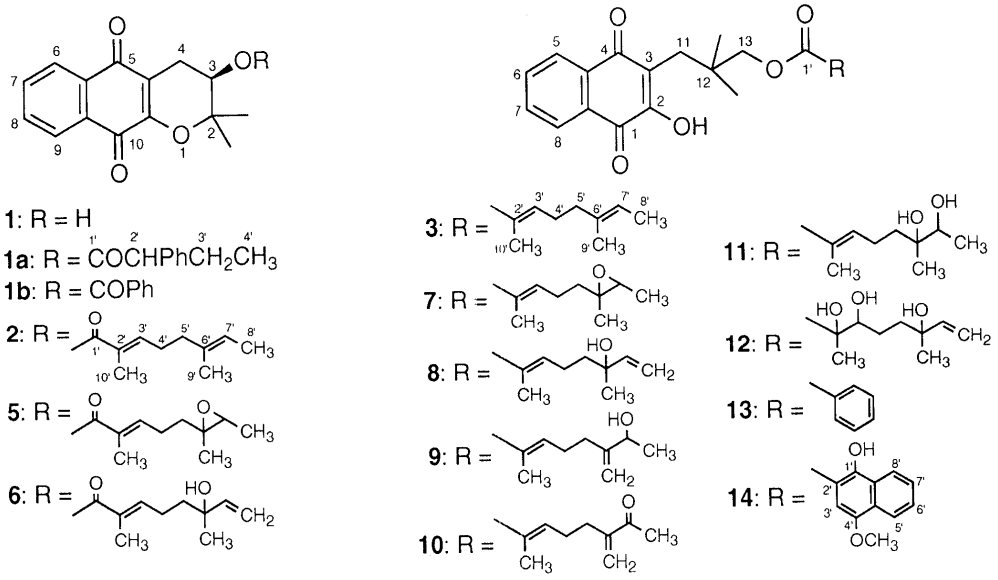
Department of Chemistry, National Cheng Kung University, Tainan, Taiwan 701, R.O.C.
Received September 11, 1997; accepted November 12, 1997

Reinvestigation of the root of *Rhinacanthus nasutus* afforded, in addition to rhinacanthin-A to -D reported previously, two new dimethyldihydropyranonaphthoquinone esters (5, 6) and eight new 2-hydroxy-1,4-naphthoquinone esters (7—14) were isolated. The stereochemistry of rhinacanthin-A was determined as the *R* configuration. Compounds rhinacanthin-G to -N, belong to a class of 2-hydroxy-3-(3-hydroxy-2,2-dimethylpropyl)-1,4-naphthoquinone esters, and so far have been isolated only in this plant. Their biosynthesis is also discussed.

Key words *Rhinacanthus nasutus*; Acanthaceae; naphthoquinone ester

Rhinacanthus nasutus (L.) KURZ (Acanthaceae), a shrub widely distributed in South China and India is now cultivated and used for the treatment of hepatitis, diabetes, hypertension and skin disease in Taiwan. We have reported previously that the methanolic extract of the root of *R. nasutus* showed significant cytotoxicity in the human KB tissue culture assay.[1] Four novel naphthoquinones, rhinacanthin-A (**1**),[1] -B (**2**),[1] -C (**3**)[2] and -D (**4**),[2] have been isolated from *R. nasutus*. Among them, rhinacanthin-B (**2**), -C (**3**) and -D (**4**) was found to be the cytotoxic principle whereas rhinacanthin-A (**1**) exhibited a lack of cytotoxicity indicating the important contribution of lipophilicity to the enhanced cytotoxicity.[1,2] In our continuing investigations of new medicinal sources, the methanolic extract from the root of *Rhinacanthus nasutus* was partitioned between $CHCl_3$ and H_2O. The aqueous solution was extracted with *n*-BuOH. Each organic layer was repeatedly chromatographed to afford ten new naphthoquinone esters: two dimethyldihydropyrano-1,4-naphthoquinones, rhinacanthin-O (**5**) and -P (**6**) and eight 2-hydroxy-1,4-naphthoquinone esters, rhinacanthin-G (**7**), -H (**8**), -I (**9**), -J (**10**), -K (**11**), -L (**12**), -M (**13**), -N (**14**), were isolated. Fourteen known compounds, six naphthoquinone esters: rhinacanthin-A (**1**), -B (**2**), -C (**3**), -D (**4**), rhinacanthone (**15**)[3-5] and dehydro-α-lapachone (**16**)[6]; three benzenoids: *p*-hydroxy-benzaldehyde (**17**),[7] methylvanillate (**18**)[8] and syringaldehyde (**19**)[9] one triterpenoid: lupeol (**20**)[10] two flavonoids: wogonin (**21**)[11] and oroxylin A (**22**)[11]; one coumarin: (+)-praeruptorin (**23**)[12]; one amide: allantoin (**24**),[13] were also found in this part. Their structures were determined by spectroscopic analyses or comparison with authentic samples. Herein we report the isolation, structural elucidation and biosynthesis of these new compounds. The absolute configuration of **1** is also discussed.

Although the structure of rhinacanthin-A (**1**) has been reported by our group,[1] attempts to obtain a single crystal for X-ray crystallography have been unsuccessful. However the absolute configuration at C-3 was assigned in two ways, Horeau's method[14,15] and the excitation chirality method.[16] The Horeau method, by reaction between **1** and (±)-α-phenylbutyric anhydride, gave α-phenylbutyrate (**1a**) and α-phenylbutyric acid with $[\alpha]_D = +26.5°$. By comparison of the specific rotation of isolated α-phenylbutyric acid with that of the known specific rotation for (+)-(*S*)-α-phenylbutyric acid ($[\alpha]_D = +92°$), we concluded that (+)-(*S*)-α-phenylbutyric acid was excess in the reaction mixture. This indicated that the absolute configuration of **1** at C-3 would be *R*. Application of the excitation chirality method to the benzoate (**1b**) show-



* To whom correspondence should be addressed.

Table 1. ^{1}H-NMR Spectral Data of Compounds **1**, **1a**, **1b**, **2**, **5** and **6** ($CDCl_3$, δ, multi, J, Hz)

	1	1a	1b	2	5	6
2-Me	1.41, 1.48 (s)	1.20, 1.24 (s)	1.46, 1.56 (s)	1.42, 1.48 (s)	1.42, 1.48 (s)	1.41, 1.47 (s)
H-3	3.90 (t, 5.1)	5.01 (t, 4.6)	5.35 (dd, 4.8, 4.2)	5.13 (t, 4.6)	5.14 (t, 4.8)	5.13 (t, 4.4)
H-4	2.68, 2.85 (dd, 18.6, 5.1)	2.69, 2.86 (dd, 14.6, 4.6)	2.91 (dd, 19.6, 4.2) 3.02 (dd, 19.6, 4.8)	2.77, 2.91 (dd, 19.5, 4.6)	2.78, 2.90 (dd, 19.6, 4.8)	2.77, 2.90 (dd, 19.6, 4.4)
H-6	8.00 (dd, 7.0, 2.0)	8.10 (m)	8.12 (m)	8.07 (dd, 7.9, 1.8)	8.07 (dd, 7.9, 1.8)	8.08 (dd, 7.6, 1.6)
H-7	7.64 (td, 7.0, 2.0)	7.71 (m)	7.68 (td, 7.0, 2.0)	7.72 (td, 7.9, 1.8)	7.72 (td, 7.9, 1.8)	7.72 (td, 7.6, 1.6)
H-8	7.68 (td, 7.0, 2.0)	7.71 (m)	7.73 (td, 7.0, 2.0)	7.69 (td, 7.9, 1.8)	7.68 (td, 7.9, 1.8)	7.69 (td, 7.6, 1.6)
H-9	8.04 (dd, 7.0, 2.0)	8.10 (m)	8.12 (m)	8.13 (dd, 7.9, 1.8)	8.11 (dd, 7.9, 1.8)	8.12 (dd, 7.6, 1.6)
H-2′	—	5.01 (dd, 7.8, 7.6)	—	—	—	—
H-3′	—	1.81, 2.04 (ddq, 7.8, 7.6, 7.4)	—	6.73 (tq, 7.6, 0.8)	6.71 (tq, 7.6, 1.2)	6.73 (tq, 7.6, 1.2)
H-4′	—	0.87 (t, 7.4)	—	2.24 (q, 7.6)	2.25 (q, 7.6)	2.1—2.2 (m)
H-5′	—	—	—	2.06 (t, 7.6)	1.5—1.8 (m)	1.6—1.7 (m)
H-7′	—	—	—	5.18 (qq, 6.5, 1.2)	2.81 (q, 4.0)	5.88 (dd, 17.2, 10.8)
H-8′	—	—	—	1.57 (d, 1.2)	1.27 (d, 4.0)	5.07 (dd, 10.8, 1.2) 5.20 (dd, 17.2, 1.2)
H-9′	—	—	—	1.57 (d, 1.2)	1.24 (s)	1.23 (s)
H-10′	—	—	7.98 (dd, 7.0, 1.4)	1.80 (d, 0.8)	1.82 (d, 1.2)	1.80 (d, 1.2)
Ph	—	7.22 (m)	7.42 (2H, dd, 8.8, 1.6) 7.47 (1H, m) 7.98 (2H, dd, 7.0, 1.4)	—	—	—

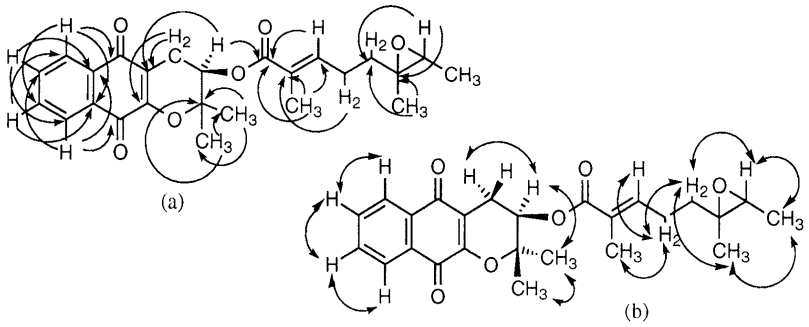


Fig. 1. HMBC Correlations (a) and NOESY Correlations (b) for Rhinacanthin-O (**5**)

ed that the benzoate group at C-3 produced a negative first Cotton effect ($\Delta\varepsilon_{252} = -61.0$) and a positive second Cotton effect ($\Delta\varepsilon_{213} = +31.7$) also suggesting a β orientation (R configuration) at C-3.

High resolution mass spectroscopic measurements showed that the molecular formulae of both **5** and **6** were $C_{25}H_{28}O_6$. Isomeric compounds **5** and **6** were optically active yellow oils and shown to be analogs of **2** in a 2,2-dimethyldihydropyrano-1,4-naphthoquinone skeleton with a ten-carbon ester side-chain. The new compounds differed from **2** only in the side-chain which was esterified by an alkenoic acid. The ^{1}H- and ^{13}C-NMR data of **1** and **2** provided an important reference point for the elucidation of the structures of **5** and **6**. The ^{13}C-NMR spectrum showed twenty-five carbon signals, fifteen of which almost exactly matched those of **1**. The ^{1}H-NMR and ^{1}H–^{1}H correlation spectroscopy (COSY) exhibited two mutually coupled spin systems consisting of H-3 and H-4; H-6, H-7, H-8 and H-9 as well as two germinal methyls at C-2 which were present in **1** and **2** and could also be found in **5** and **6** (Table 1). These allowed the assignment of a 3-hydroxy-2,2-dimethyldihydropyrano-naphthoquinone to be made as the alcohol part of esters **5** and **6**.

The differences in the ^{1}H-NMR spectrum of **5** compared with that of **2** were the upfield shift of signals of H-5′ (δ 1.5—1.8, m), H-7′ (δ 2.81, q, J=4.0 Hz), H-10′ (δ 1.24, s) and H-8′ (δ 1.27, d, J=4.0 Hz) together with the abnormally smaller ^3J coupling constant (4.0 Hz) between H-7′ and H-8′ which represented the characteristic property of a three-membered ring (Table 1). In addition, two ^{13}C signals at δ 58.8 and 59.9 for carbons adjacent to a heteroatom, O, replaced the alkenyl carbons in **2**. These results indicated that the acidic side-chain moiety should be assigned as 6′,7′-epoxy-2′,6′-dimethyl-2′-octenoic acid. Full ^{1}H- and ^{13}C-NMR assignments in **5** were provided by nuclear overhauser effect (NOE) spectroscopy and heteronuclear multiple-bond correlation (HMBC) spectroscopy (Fig. 1). The finding that the vinyl methyl H-10′ (δ 1.82) gave a strong NOE cross peak to H-4′ (δ 2.25), and H-8′ (δ 1.27) gave an NOE correlation with H-10′ (δ 1.24) established the side-chain double bond and epoxide geometry as E. Based on above spectral analyses, the structure of rhinacanthin-O was inferred as **5**.

For compound **6**, a broad IR band at 3404 cm^{-1} showed the presence of a hydroxyl group. The ^{1}H-NMR signals for the epoxide in **5** were replaced by a vinyl moiety at δ 5.07 (dd, J=10.8, 1.2 Hz, H-8′), 5.20 (dd, J=17.2, 1.2 Hz, H-8′) and 5.88 (dd, J=17.2, 10.8 Hz, H-7′) in **6** (Table 1). Consequently, a 6′-hydroxy-2′,6′-dimethyl-2′,7′-octadienoic acid was assigned for the acid part of **6**. This represented isomerization of epoxide **5** into an allylic al-

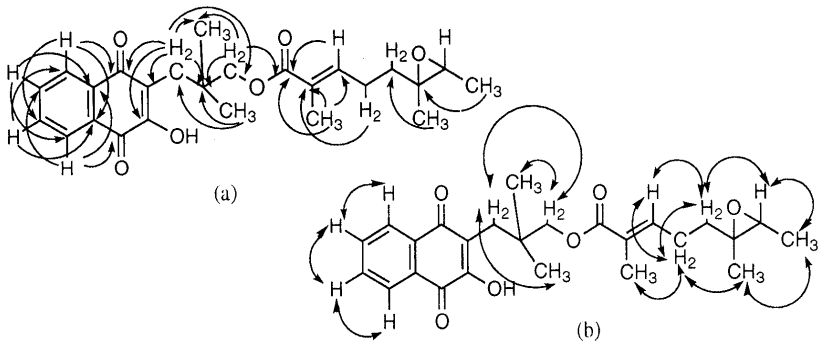


Fig. 2. HMBC Correlations (a) and NOESY Correlations (b) for Rhinacanthin-G (**7**)

Table 2. ^{1}H-NMR Spectral Data of Compounds **7**—**14** ($CDCl_3$, δ, multi, J, Hz)

	7	8	9	10	11	12	13	14
H-5	8.10 (dd, 7.6, 1.2)	8.03 (dd, 7.6, 1.2)	8.02 (dd, 7.6, 1.2)	8.11 (d, 7.2)	8.05 (d, 7.6)	8.12 (d, 7.6)	8.05 (d, 7.6)	8.00 (dd, 7.6, 1.2)
H-6	7.75 td (7.6, 1.2)	7.68 (td, 7.6, 1.2)	7.67 (td, 7.6, 1.2)	7.75 (t, 7.2)	7.68 (t, 7.6)	7.76 (t, 7.6)	7.66 (t, 7.6)	7.57 (td, 7.6, 1.2)
H-7	7.70 td (7.6, 1.2)	7.61 (td, 7.6, 1.2)	7.60 (td, 7.6, 1.2)	7.67 (t, 7.2)	7.61 (t, 7.6)	7.69 (t, 7.6)	7.71 (t, 7.6)	7.52 (td, 7.6, 1.2)
H-8	8.06 (dd, 7.6, 1.2)	8.00 (dd, 7.6, 1.2)	7.98 (dd, 7.6, 1.2)	8.08 (d, 7.2)	8.00 (d, 7.6)	8.09 (d, 7.6)	8.07 (d, 7.6)	7.97 (dd, 7.6, 1.2)
H-11	2.70 (s)	2.63 (s)	2.62 (s)	2.67 (s)	2.63 (s)	2.71 (s)	2.78 (s)	2.80 (s)
12-Me	1.02 (s)	0.94 (s)	0.93 (s)	1.01 (s)	0.95 (s)	1.00, 1.03 (s)	1.08 (s)	1.15 (s)
H-13	3.92 (s)	3.83 (s)	3.82 (s)	3.90 (s)	3.86 (s)	3.89, 4.02 (d, 10.7)	4.11 (s)	4.18 (s)
1′-OH	—	—	—	—	—	—	—	11.54 (br s)
H-2′	—	—	—	—	—	—	7.99 (dd, 7.6, 1.6)	—
H-3′	6.70 (tq, 7.6, 1.2)	6.64 (tq, 7.6, 1.6)	6.67 (tq, 7.2, 1.2)	6.68 (t, 6.5)	6.69 (t, 7.6)	4.25 (dd, 10.8, 2.2)	7.35 (t, 7.6)	7.00 (s)
H-4′ or 4′-OMe	2.21 (q, 7.6)	2.0—2.3 (m)	2.2—2.3 (m)	2.3—2.4 (m)	2.1—2.4 (m)	1.9—2.2 (m)	7.50 (tt, 7.6, 1.6)	3.86 (s)
H-5′	1.5—1.7 (m)	1.5—1.8 (m)	2.03, 2.11 (dt, 15.2, 7.6)	2.24 (t, 6.7)	1.2—1.7 (m)	1.5—1.9 (m)	7.35 (t, 7.6)	8.13 (br d, 8.4)
H-6′	—	—	—	—	—	—	7.99 (dd, 7.6, 1.6)	7.61 (ddd, 8.4, 6.8, 1.6)
H-7′	2.86 (q, 4.0)	5.82 (dd, 17.2, 10.8)	4.20 (q, 6.4)	—	3.60 (q, 6.0)	6.07 (dd, 17.7, 11.0)	—	7.55 (ddd, 8.4, 6.8, 1.6)
H-8′	1.30 (d, 4.0)	5.02 (dd, 10.8, 1.2) 5.16 (dd, 17.2, 1.2)	1.22 (d, 6.4)	2.33 (s)	1.19 (d, 6.0)	5.18 (d, 11.0) 5.25 (d, 17.7)	—	8.35 (br d, 8.4)
H-9′	1.27 (s)	1.23 (s)	4.71, 5.02 (s)	5.78, 6.03 (s)	1.19 (s)	1.20 (s)	—	—
H-10′	1.82 (d, 1.2)	1.72 (d, 1.6)	1.73 (d, 1.2)	1.77 (s)	1.75 (s)	1.35 (s)	—	—

cohol **6**. Therefore, rhinacanthin-P possessed the structure of **6**.

An orange oil rhinacanthin-G (**7**) has the molecular formula $C_{25}H_{30}O_6$ as shown by high resolution mass spectrometry. The UV absorption at 214, 239, 246, 251, 277 and 328 nm as well as the IR bands at 1663 and 1647 cm^{-1} are consistent with a 1,4-naphthoquinone moiety.[1,2] The IR absorption at 1705 cm^{-1} suggests an additional ester functional group. The IR broad band at 3304 cm^{-1}, the downfield ^{13}C signal at δ 154.4 (C-2), and four mutually coupled proton signals at δ 7.70 (td, J=7.6, 1.2 Hz, H-7), 7.75 (td, J=7.6, 1.2 Hz, H-6), 8.06 (dd, J=7.6, 1.2 Hz, H-8) and 8.10 (dd, J=7.6, 1.2 Hz, H-5) indicate the existence of the partial structure of a 3-substituted-2-hydroxy-1,4-naphthoquinone. Furthermore, apart from the 1,4-naphthoquinone skeleton, the γ-oxygenated-β,β-dimethylpropyl unit was attached at C-3, inferred by two singlet methylenes at δ 2.70 (H-11) and 3.92 (H-13) separated by a quaternary sp^3 carbon bearing geminal methyls at δ 1.02 (2×12-Me). The deshielded H-13 was attributed to an oxygen heteroatom on the same carbon. Hence, a 2-hydroxy-3-(13-oxygenated-12,12-dimethylpropyl)-1,4-naphthoquinone for the alcohol part of this ester **7** was established by comparison of the spectral data with that of **3**.[2] The acid moiety, 2′,6′-dimethyl-6′,7′-epoxy-2′-octanoic acid, was the same as that in **5** from the ^{1}H- and ^{13}C-NMR spectra (Tables 1, 2). All the connectivities were further confirmed by HMBC and NOESY experiments and are summarized in Fig. 2. In order to determine the geometry of the double bond in the acid-side chain, the NOESY spectrum was examined. The presence of NOE between H-10′ and H-4′ suggests the *E* configuration. Therefore, the structure of rhinacanthin-G was deduced as **7**.

Actually, rhinacanthin-G to -L (**7**—**12**) exhibited very similar UV absorption and IR bands. The ^{1}H-NMR signals showed the presence of the ester of 2-hydroxy-3-(13-oxygenated-12,12-dimethylpropyl)-1,4-naphthoquinone with a different acid (Table 2).

Also, two compounds (**8**, **9**) have the same molecular formula as **7**. The major difference between them was at the terminal of the acid side-chain. Compound **8**, a red-brown oil, possessed the same acid part, 6′-hydroxy-2′,6′-dimethyl-2′,7′-octadienoic acid, as **6** from the ^{1}H- and ^{13}C-NMR spectra (Tables 1, 2). However, compound **9**, a red-brown oil too, possessed a vinylidene group due to two singlet vinylidene protons at δ 4.71 and 5.02 (H-9′) and a methyl doublet at δ 1.22 (J=6.4 Hz, H-8′) coupled with a downfield methine proton at δ 4.20 (q, J=6.4 Hz, H-7′), this was further supported by the additional

secondary carbon signal at δ 109.1 by a distortionless enhancement by polarization transfer (DEPT) experiment. On the basis of the above analyses, rhinacanthin-H and -I were assigned the structures of **8** and **9**, respectively.

The molecular formula for compound **10**, an orange oil, is $C_{25}H_{28}O_6$, one degree of unsaturation more than that of **9**. The lack of a methine signal and a downfield shifted methyl signal for H-8′ (δ 2.33, s) indicate that the hydroxyl group in **9** is oxidized to a carbonyl group in **10** (Table 2). Consequently, the structure of rhinacanthin-J was suggested to be **10**.

Compound **11**, molecular formula $C_{25}H_{32}O_7$, is a red oil. It has two hydrogen and one oxygen atoms more than **7**. In the ^{1}H-NMR spectrum of **11**, the downfield vinyl proton at δ 6.69 (t, J=7.6 Hz) was assigned as H-3′ as in compounds **2**—**10** (Tables 1, 2). The conventional chemical shift for H-7′ at δ 3.60 (q, J=6.0 Hz) and the normal ^3J coupling constant between H-7′ and H-8′ together with a methyl singlet at δ 1.19 suggest that there are two hydroxyl groups substituted vicinally on C-6′ and C-7′. A fragment peak in the MS spectrum at m/z 399 ($M^+ - CH_3CHOH$) further supported the ready cleavage between C-6′ and C-7′ in this diol. The epoxide group in **7** was opened to give a hydrolyzed diol **11**. Therefore, the acid part of the ester **11** was proposed as 6′,7′-dihydroxy-2′,6′-dimethyl-2′-octenoic acid. Based on this information, the structure of rhinacanthin-K was confirmed as **11**.

Rhinacanthin-L (**12**) was isolated as a red oil and has the molecular formula $C_{25}H_{32}O_8$. By comparison of the ^{1}H-NMR spectrum of **12** with that of **8**, a vinyl proton H-3′ in **8** is replaced by a methine double doublet at δ 4.25 (J=10.8, 2.2 Hz, H-3′) which couples with the diasterotropic methylene protons at δ 1.9—2.2 (m, H-4′) in **11** (Table 2). The diagnostic MS peak at m/z 332 ($M^+ - CH_2{=}CHCCH_3OHCH_2CH_2CHOH + H$) indicates two hydroxyls located at C-2′ and C-3′ vicinally as in **11**. This asymmetric C-2′ also affects the other methylene protons on C-13 which being non-equivalence couple to each other to give signals at δ 3.89 and 4.02 (J=10.7 Hz, H-13). 2′,3′,6′-trihydroxy-2′,6′-dimethyl-7′-octenoic acid was deduced for the acid side-chain. Consequently, rhinacanthin-L has the structure as indicated by **12**.

Rhinacanthin-M (**13**), an orange oil, essentially has the same alcohol part, 2-hydroxy-3-(13-hydroxy-12,12-dimethylpropyl)-1,4-naphthoquinone, as **7**—**12**. The molecular formula was determined as $C_{22}H_{20}O_5$. In the aromatic region of the ^{1}H-NMR spectrum, a set of five additional mutually coupled protons at δ 7.35 (2H, t, J=7.6 Hz, H3′ and H-5′), 7.50 (1H, tt, J=7.6, 1.6 Hz, H-4′) and 7.99 (2H, t, J=7.6 Hz, H-2′, H-6′) suggests for a nonsubstituted phenyl side-chain. The relative low field of H-2′ and H-6′ established a benzoic acid part which is further supported by the ^{1}H–^{13}C long-range coupling between H-2′ or H-6′ (δ 7.99) and C=O (δ 166.5) in the HMBC spectrum. These results led us to assign the structure **13** to rhinacanthin-M.

Rhinacanthin-N (**14**) was isolated as orange needles and found to have the molecular formula $C_{27}H_{24}O_7$. Examination of the ^{1}H- and ^{13}C-NMR spectra suggests that **14** possessed the same alcohol part as **5**—**13**. On the other hand, an aromatic singlet at δ 7.00 (H-3′) and four mutually coupled proton signals at δ 7.55 (ddd, J=8.4, 6.8, 1.6 Hz, H-7′), 7.61 (ddd, J=8.4, 6.8, 1.6 Hz, H-6′), 8.13 (br d, J=8.4 Hz, H-5′) and 8.35 (br d, J=8.4 Hz, H-8′) together with ten aromatic ^{13}C signals including five quaternary carbons at δ 104.6 (C-2′), 125.5 (C-9′), 129.7 (C-10′), 147.5 (C-4′), 155.4 (C-1′) and five tertiary carbons at δ 100.3 (C-3′), 121.8 (C-5′), 123.7 (C-8′), 126.3 (C-7′), 128.9 (C-6′) infer the presence of a 1′,2′,4′-trisubstituted naphthalene moiety. The presence of intramolecular hydrogen bonding between a hydroxyl and a carbonyl group was supported by the bathochromic (red) shift of the UV spectrum in MeOH after the addition of $AlCl_3$. Thus, a 1-hydroxynaphthoyl structure was proposed. The last substituent had a signal at δ 3.86 (3H, s) which was assigned to a methoxyl at C-4′. The locations of these substituents were confirmed by HMBC and NOESY experiments (Fig. 3). The above evidence led to the assignment of structure **14** for rhinacanthin-N. The ester linkage between 1,4-naphthoquinone and the β-naphthoic acid of rhinacanthin-N (**14**) is the first time such a structure has been found in nature.

From a biogenetic point of view, an isoprenyl unit on a hydroxynaphthoquinone can be converted into an epoxide followed by cyclization *via* an ether linkage to give a 3-hydroxy-2,2-dimethyldihydropyranonaphthoquinone **1** or transformation *via* an enzymatic pinacol-pinacolone rearrangement to give an aldehyde **25**[17] which can be further reduced to 2-hydroxy-3-(13-hydroxy-12,12-dimethylpropyl)-1,4-naphthoquinone **26** (Chart 1). Compounds **1** and **26** are the alcohol part of esters **2**, **5**, **6** and **3**, **4**, **7**—**14**, respectively.

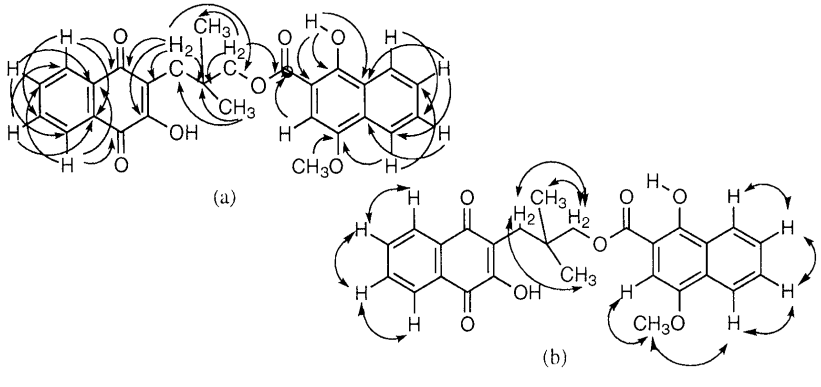


Fig. 3. HMBC Correlations (a) and NOESY Correlations (b) for Rhinacanthin-N (**14**)

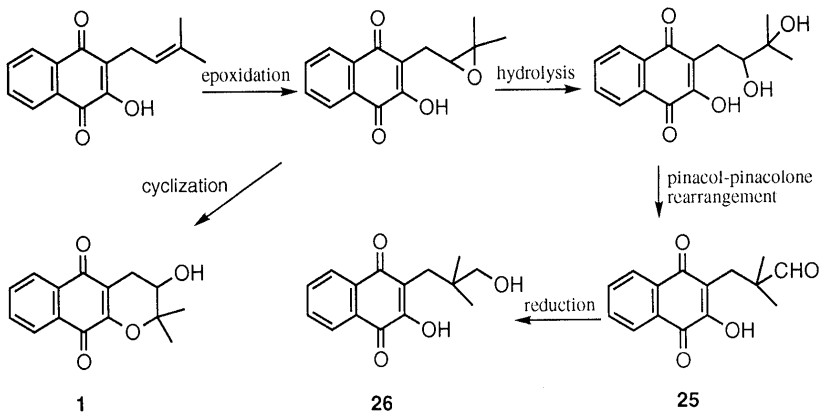


Chart 1

Experimental

Melting points (Yanagimoto apparatus) are uncorrected. Optical rotations were recorded on a Jasco DIP-370 digital polarimeter. UV spectra of MeOH solutions were obtained on a Hitachi UV-3210 spectrophotometer. IR spectra on KBr discs were recorded on a Jasco IR Report-100 spectrophotometer. Mass and high resolution mass spectra were measured on a VG-70-250S spectrometer having a direct inlet system. ^{1}H-NMR and ^{13}C-NMR spectra were determined on Bruker AC-200 and AMX-400 spectrometers. Chemical shifts are shown in δ values (ppm) with tetramethylsilane (TMS) as internal standard.

Plant Material The roots of *Rhinacanthus nasutus* used in this investigation were collected in Tainan, Taiwan and identified by Prof. C. S. Kuoh. A specimen of the plant has been deposited at the herbarium of the National Cheng Kung University, Tainan, Taiwan.

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 purified by column chromatography over silica-gel and eluted with a gradient of hexane and EtOAc to give eight fractions. Repeated column chromatography of fraction 3 over silica-gel, eluting with a gradient of hexane and EtOAc, gave **20** (1.20 g). In a similar separation, fraction 4 afforded **3** (1.65 g), **7** (67 mg), **10** (22 mg), **8** (40 mg), **9** (54 mg), **12** (3 mg) and **11** (25 mg); fraction 5 yield **2** (250 mg), **1** (71 mg), **5** (5 mg), **16** (54 mg), **14** (17 mg) and **13** (53 mg), successively. However, fraction 6 was subjected to column chromatography on silica-gel eluting with a gradient of benzene and $(CH_3)_2CO$ obtained **15** (3 mg), **4** (37 mg) and **17** (1 mg). Similarly, fraction 7 gave **6** (1 mg), **18** (1 mg), **21** (5 mg), **22** (3 mg), **23** (2 mg). Finally, fraction 8 was chromatographed using a gradient of hexane and EtOAc to give **19** (2 mg). The aqueous solution was extracted with *n*-BuOH and the *n*-BuOH layer was crystallized after standing to furnish **24** (2.50 g). In addition, a large amount of inorganic salt, KNO_3 (4.50 g), was found in the aqueous layer.

Acylation of 1 Compound **1** (6 mg) was dissolved in pyridine (2 ml) and excess (±)-α-phenylbutyric anhydride or benzoic anhydride (7 mg) added. After stirring at room temperature for 5 h, the crude product was subjected to column chromatography eluting with hexane–EtOAc (5 : 1) to give pure **1a** (8.3 mg) and **1b** (6.5 mg), respectively. In the former case, the other product, α-phenylbutyric acid (1.5 mg), was isolated with $[\alpha]_D = +26.5°$ (c=0.065, $CHCl_3$) and referred to as (+)-(*S*)-α-phenylbutyric acid by comparison with standard (+)-(*S*)-α-phenylbutyric acid which showed $[\alpha]_D = +92°$ (c=0.9, $C_6H_5CH_3$).

1a: Yellowish powder, mp 136—137 °C. $[\alpha]_D$ −17.1° (c=0.066, $CHCl_3$). CD (c=0.0016, MeOH): $[\theta]_{213}$ +162.2, $[\theta]_{267}$ −60.7, $[\theta]_{304}$ +382.1, $[\theta]_{361}$ +1083. HR-MS *m/z* : 404.1628 (Calcd for $C_{25}H_{24}O_5$: 404.1624). UV λ_{max} nm: 245, 251, 282, 334. IR ν_{max} cm^{-1}: 1732, 1664, 1624. EI-MS *m/z* (rel. int. %): 404 (M^+, 1), 258 (7), 241 (14), 240 (70), 225 (100), 212 (25), 197 (17).

1b: Yellow oil. $[\alpha]_D$ −29.1° (c=0.076, $CHCl_3$). CD (c=0.0022, MeOH): $[\theta]_{213}$ +31.7, $[\theta]_{252}$ −61.0, $[\theta]_{357}$ +404.1. UV λ_{max} nm: 223 (sh), 231 (sh), 244, 251, 274 (sh), 282, 333. IR ν_{max} cm^{-1}: 1689. FAB-MS *m/z* (rel. int. %): 363 (M^++1, 7), 257 (7), 241 (13), 165 (23), 121 (16), 115 (30), 105 (85).

Rhinacanthin-O (**5**): Yellow oil. $[\alpha]_D$ −30.95° (c=0.105, $CHCl_3$). HR-MS *m/z*: 424.1890 (Calcd for $C_{25}H_{28}O_6$: 424.1886). UV λ_{max} nm: 220 (sh), 245, 251, 274, 281, 331. IR ν_{max} cm^{-1}: 1711, 1679, 1646, 1620, 1596, 1581. EI-MS *m/z* (rel. int. %): 424 (M^+, 2), 258 (24), 240 (55), 225 (100), 212 (25), 197 (22), 95 (28), 69 (21), 55 (26). ^{13}C-NMR ($CDCl_3$) δ 12.2 (C-10′), 13.9 (C-8′), 16.1 (C-9′), 22.9 (2-Me), 23.0 (C-4), 24.2 (C-4′), 24.5 (2-Me), 36.9 (C-5′), 58.8 (C-7′), 59.9 (C-6′), 69.1 (C-3), 78.9 (C-2), 117.7 (C-4a), 125.9 (C-6), 126.3 (C-9), 127.4 (C-2′), 131.0 (C-9a), 131.9 (C-5a), 133.0 (C-8), 133.9 (C-7), 142.5 (C-3′), 153.5 (C-10a), 166.7 (C-1′), 179.2 (C-10), 183.9 (C-5).

Rhinacanthin-P (**6**): Yellow oil. $[\alpha]_D$ −60.00° (c=0.10, $CHCl_3$). HR-MS *m/z*: 424.1883 (Calcd for $C_{25}H_{28}O_6$: 424.1886). UV λ_{max} nm: 215 (sh), 245, 251, 277, 282, 327. IR ν_{max} cm^{-1}: 3404, 1712, 1693, 1666, 1644, 1594. EI-MS *m/z* (rel. int. %): 424 (M^+, 7), 368 (16), 258 (34), 241 (38), 225 (100), 197 (29), 167 (15), 149 (33), 139 (15), 121 (25), 111 (59), 104 (55), 95 (44), 83 (50), 71 (83), 69 (62), 57 (75), 55 (72).

Rhinacanthin-G (**7**): Yellow oil. $[\alpha]_D$ −1.95° (c=0.28, $CHCl_3$). HR-MS *m/z* : 426.2039 (Calcd for $C_{25}H_{30}O_6$: 426.2044). UV λ_{max} nm: 214, 239 (sh), 246 (sh), 251, 277, 328. IR ν_{max} cm^{-1}: 3304, 1705, 1663, 1647, 1593, 1579. EI-MS *m/z* (rel. int. %): 426 (M^+, 3), 243 (100), 200 (24), 187 (51), 166 (25), 159 (26), 139 (43), 123 (24), 95 (61), 67 (23), 55 (35). ^{13}C-NMR ($CDCl_3$) δ 12.2 (C-10′), 13.9 (C-8′), 16.1 (C-9′), 24.1 (C-4′), 25.1 (2×12-Me), 32.0 (C-11), 36.9 (C-12), 37.0 (C-5′), 58.9 (C-7′), 60.1 (C-6′), 72.7 (C-13), 121.6 (C-3), 125.8 (C-8), 126.8 (C-5), 128.1 (C-2′), 129.3 (C-9), 132.7 (C-7), 132.8 (C-10), 134.7 (C-6), 140.8 (C-3′), 154.4 (C-2), 167.8 (C-1′), 181.0 (C-4), 184.7 (C-1).

Rhinacanthin-H (**8**): Red-brown oil. $[\alpha]_D$ −1.84° (c=0.27, $CHCl_3$). HR-MS *m/z*: 426.2045 (Calcd for $C_{25}H_{30}O_6$: 426.2044). UV λ_{max} nm: 215 (sh), 246 (sh), 252, 282, 331. IR ν_{max} cm^{-1}: 3318, 1709, 1666, 1646, 1592. EI-MS *m/z* (rel. int. %): 426 (M^+, 1), 243 (100), 187 (36), 159 (16), 148 (19), 121 (24), 93 (21), 71 (23), 55 (22). ^{13}C-NMR ($CDCl_3$) δ 12.2 (C-10′), 23.3 (C-4′), 25.2 (2×12-Me), 27.9 (C-9′), 32.1 (C-11), 37.0 (C-12), 40.5 (C-5′), 72.8 (C-6′), 73.0 (C-13), 112.2 (C-8′), 121.7 (C-3), 126.0 (C-8), 126.9 (C-5), 127.8 (C-2′), 129.4 (C-9), 132.8 (C-7 and C-10), 134.9 (C-6), 142.0 (C-3′), 144.4 (C-7′), 155.0 (C-2), 168.1 (C-1′), 181.6 (C-1), 184.8 (C-4).

Rhinacanthin-I (**9**): Red-brown oil. $[\alpha]_D$ −1.04° (c=0.54, $CHCl_3$). HR-MS *m/z*: 426.2043 (Calcd for $C_{25}H_{30}O_6$: 426.2044). UV λ_{max} nm: 215 (sh), 247 (sh), 251, 282, 329. IR ν_{max} cm^{-1}: 3322, 1703, 1666, 1646, 1593. EI-MS *m/z* (rel. int. %): 426 (M^+, 4), 243 (100), 200 (20), 187 (48), 167 (9), 159 (26), 149 (24), 138 (24), 121 (50), 105 (22), 95 (31), 69 (27), 67 (16), 57 (28), 55 (38). ^{13}C-NMR ($CDCl_3$) δ 12.3 (C-10′), 22.1 (C-8′), 25.1 (2×12-Me), 26.9 (C-4′), 30.0 (C-5′), 31.9 (C-11), 37.0 (C-12), 70.8 (C-7′), 72.8 (C-13), 109.1 (C-9′), 121.8 (C-3), 126.0 (C-8), 126.9 (C-5), 128.0 (C-2′), 129.4 (C-9), 132.9 (C-7, C-10), 134.9 (C-6), 141.5 (C-3′), 151.8 (C-6′), 154.5 (C-2), 168.1 (C-1′), 181.2 (C-1), 184.9 (C-4).

Rhinacanthin-J (**10**): Orange oil. HR-MS *m/z*: 424.1884 (Calcd for $C_{25}H_{28}O_6$: 424.1886). UV λ_{max} nm: 212 (sh), 239 (sh), 250 (sh), 276, 325. IR ν_{max} cm^{-1}: 3318, 1710, 1666, 1647, 1592. EI-MS *m/z* (rel. int. %): 424 (M^+, 8), 244 (100), 187 (36), 164 (30), 159 (20), 137 (69), 121 (24), 55 (22).

Rhinacanthin-K (**11**): Red oil. $[\alpha]_D$ −2.16° (c=0.25, $CHCl_3$). HR-MS *m/z*: 444.2148 (Calcd for $C_{25}H_{32}O_7$: 444.2148). UV λ_{max} nm: 211, 246 (sh), 252, 277, 283 (sh), 327. IR ν_{max} cm^{-1}: 3362, 1711, 1666, 1644, 1594. EI-MS *m/z* (rel. int. %): 444 (M^+, 3), 399 (10), 243 (100), 187 (18), 159 (8), 139 (33), 111 (20), 95 (17).

Rhinacanthin-L (**12**): Red oil. $[\alpha]_D$ −32.36° (c=0.016, $CHCl_3$). UV

λ_{max} nm: 247 (sh), 252, 276, 285 (sh), 323. IR ν_{max} cm^{-1}: 3508, 3356, 1734, 1665, 1647, 1591. FAB-MS *m/z* (rel. int. %): 461 (MH^+, 18), 332 (11), 243 (100), 187 (27), 159 (11), 115 (77), 93 (27).

Rhinacanthin-M (**13**). Orange oil. HR-MS *m/z*: 364.1313 (Calcd for $C_{22}H_{20}O_5$: 364.1311). UV λ_{max} nm: 227 (sh), 250 (sh), 269 (sh), 275, 322. IR ν_{max} cm^{-1}: 3304, 1714, 1666, 1650, 1644, 1592. EI-MS *m/z* (rel. int. %): 364 (M^+, 21), 242 (14), 187 (11), 177 (18), 159 (7), 105 (100), 77 (25). ^{13}C-NMR ($CDCl_3$) δ 25.3 (2 × 12-Me), 32.3 (C-11), 37.1 (C-12), 73.2 (C-13), 121.7 (C-2), 126.0 (C-5), 127.0 (C-8), 128.2 (C-3′, C-5′), 129.3 (C-10), 129.5 (C-2′, C-6′), 130.4 (C-1′), 132.7 (C-4′), 132.8 (C-6), 133.0 (C-9), 134.9 (C-7), 154.2 (C-3), 166.5 (OC=O), 181.2 (C-4), 184.9 (C-1).

Rhinacanthin-N (**14**): Orange needles (acetone), mp 123—124 °C. HR-MS *m/z*: 460.1519 (Calcd for $C_{27}H_{24}O_7$: 460.1522). UV λ_{max} nm: 215, 254, 264, 275 (sh), 321 (sh), 359, 370 (sh), 451. IR ν_{max} cm^{-1}: 3330, 1712, 1661, 1645, 1596. EI-MS *m/z* (rel. int. %): 460 (M^+, 48), 243 (99), 218 (57), 200 (100), 187 (42), 159 (19), 129 (22). ^{13}C-NMR ($CDCl_3$) δ 25.4 (2 × 12-Me), 32.3 (C-11), 37.1 (C-12), 55.4 (4′-OMe), 73.3 (C-13), 100.3 (C-3′), 104.6 (C-2′), 121.5 (C-2), 121.8 (C-5′), 123.7 (C-8′), 125.5 (C-9′), 125.9 (C-8), 126.3 (C-7′), 126.8 (C-5), 128.9 (C-6′), 129.2 (C-9), 129.7 (C-10′), 132.8 (C-7, C-10), 134.8 (C-6), 147.5 (C-4′), 154.2 (C-2), 155.4 (C-1′), 170.6 (OC=O), 181.2 (C-1), 184.8 (C-4).

Acknowledgments We thank National Science Council, R. O. C. (NSC 82-0420-B006-014-M13) for supporting this research.

References and Notes

1) Wu T. S., Tien H. J., Yeh M. Y., Lee K. H., *Phytochemistry*, **27**, 3787—3788 (1988).
2) Sendl A., Chen J. L., Jolad S. D., Stoddart C., Rozhon E., Kernan M., Nanakorn W., Balick M., J. *Nat. Prod.*, **59**, 808—811 (1996).
3) Kuwahara S., Awai N., Kodama O., Howie R. A., Thomson R. H., *J. Nat. Prod.*, **58**, 1455—1458 (1995).
4) Kodama O., Ichikawa H., Akatsuka T., Santisopasri V., Kato A., Hayashi Y., *J. Nat. Prod.*, **56**, 292—294 (1993).
5) Kuwahara S., Nemoto A., Hiramatsu A., *Agric. Biol. Chem.*, **55**, 2909—2911 (1991).
6) Thomson R. H., "Naturally Occurring Quinones," 2nd ed., Academic Press Inc., London, LTD., 1971, p. 209.
7) The spectral data were in accord with authentic sample purchased from Aldrich Chemical Company Inc.
8) Wu T. S., Ou L. F., Teng C. M., *Phytochemistry*, **36**, 1063—1068 (1994).
9) Pouchert C. J., "The Aldrich Library of NMR Spectra," Vol. 2, Aldrich Chemical Company, New York, 1983, p. 124.
10) Appleton R. A., Enzell C. R., *Phytochemistry*, **10**, 447—449 (1971).
11) Lin Y. L., Ou J. C., Chen C. F., Kuo Y. H., *J. Chin. Chem. Soc.*, **38**, 619—623 (1991).
12) Duh C. Y., Wang S. K., Wu Y. C., *Phytochemistry*, **30**, 2812—2814 (1991).
13) Lou F. C., Ding L. S., Wu M. Y., *Yaoxue Xuebao*, **18**, 684—688 (1983).
14) Horeau A., Nouaille A., *Tetrahedron Lett.*, **1971**, 1939—1942.
15) Brooks C. J. W., Gilbert J. D., *J. Chem. Soc., Chem. Comm.*, **1973**, 194—195.
16) Harada N., Ohashi M., Nakanishi K., *J. Am. Chem. Soc.*, **90**, 7349—7351 (1968).
17) Dreyer D. L., *Tetrahedron*, **23**, 4613—4622 (1967).