Phytochemistry, Vol. 27, No. 12, pp. 3787–3788, 1988.
Printed in Great Britain.

0031 9422/88 $3.00 + 0.00

ISOLATION AND CYTOTOXICITY OF RHINACANTHIN-A AND -B, TWO NAPHTHOQUINONES, FROM *RHINACANTHUS NASUTUS*

TIAN-SHUNG WU, HSIEN-JU TIEN*, MOU-YUNG YEH* and KUO-HSIUNG LEE†

Department of Applied Chemistry, Providence College of Arts and Sciences, Shalu 43309, Taichung Hsien, Taiwan, R.O.C.; *Department of Chemistry, National Cheng Kung University, Tainan, Taiwan 70101, R.O.C.; †Natural Products Laboratory, Division of Medicinal Chemistry and Natural Products, School of Pharmacy, University of North Carolina, Chapel Hill, North Carolina 27599, U.S.A.

(*Received* 7 *April* 1988)

Key Word Index—*Rhinacanthus nasutus*; Acanthaceae; naphthoquinones; rhinacanthin-A and -B; lupeol; β-sitosterol; stigmasterol; glucosides of β-sitosterol and stigmasterol; cytotoxicity.

Abstract—Rhinacanthin-A and -B, two new naphthoquinones, and the known lupeol, β-sitosterol, stigmasterol, as well as the glucosides of β-sitosterol and stigmasterol were isolated from the roots of *Rhinacanthus nasutus*. The structures of rhinacanthin-A and -B were elucidated on the basis of spectral analyses. Rhinacanthin-B demonstrated significant cytotoxicity ($ED_{50} = 3.0\ \mu g/ml$) in the KB tissue culture cell.

INTRODUCTION

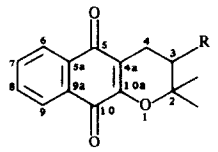
Rhinacanthus nasutus (L.) Kurz (Chinese name: Bái Hè Ling Zhi) (*Rhinacanthus communis* Nees; *Justicia nasuta* L.) is a shrub which is widely distributed in South China and India. The leaves and stems of this plant are often used to cure cutaneous eruptions due to ringworm (*Herpes miliaris*), eczema, pulmonary tuberculosis and neurodermatitis, and as an aphrodisiac and aplexipharmic [1–4]. In Taiwan this plant is now cultivated and used as a folk medicine for the treatment of hepatitis, diabetes and hypertension. Subramanian and Nagarajan [5] as well as Ho and Ho [6] have reported the isolation of flavonoids and anthraquinones from the leaves and flowers of this plant. As a result of our continuing search for novel bioactive natural products, the methanolic extract of the root of *R. nasutus* was found to show significant cytotoxicity in the human KB tissue culture assay ($ED_{50} < 20\ \mu g/ml$). Bioassay–directed fractionation of the active fraction led to the isolation and characterization of two new naphthoquinones, rhinacanthin-A (**1**) and -B (**2**) with **2** being the cytotoxic principle. In addition, lupeol, β-sitosterol, stigmasterol as well as the glucosides of β-sitosterol and stigmasterol were also isolated from this extract.

RESULTS AND DISCUSSION

Rhinacanthin-A (**1**) is optically active. The molecular formula was found by elemental and mass spectral analyses to be $C_{15}H_{14}O_4$. Its UV spectrum was consistent with that of a naphtho-1,4-quinone chromophore and resembled that of α-lapachone (**3**) [7]. This was confirmed by IR absorption bands at 1670 and 1650 cm^{-1} (1,4-quinone carbonyl) and the appearance of two carbonyl carbon signals at δ 179.42 and 184.35 in the ^{13}C NMR spectrum. The presence of an alcoholic hydroxyl group in the molecule was indicated by an IR absorption band at 3550 cm^{-1} and the absence of a red shift in the UV spectrum upon addition of NaOH. This was substantiated by a broad signal at δ 2.40 which disappeared upon addition of D_2O in the ^1H NMR spectrum. In the ^1H NMR spectrum of **1** the following characteristic signals were observed: a *gem*-dimethyl group at δ 1.41 and 1.48 (each 3H, *s*); an ABX system at δ 2.68 and 2.85 (each 1H, *dd*, $J = 5.1$ and 18.6 Hz, H-4) and 3.90 (*t*, $J = 5.1$ Hz, H-3); and four mutually coupling aromatic protons at δ 7.64 (*dt*, $J = 2.0$ and 7.0 Hz, H-7), 7.68 (*dt*, $J = 2.0$ and 7.0 Hz, H-8), 8.00 (*dd*, $J = 2.0$ and 7.0 Hz, H-6) and 8.04 (*dd*, $J = 2.0$ and 7.0 Hz, H-9). On the basis of these spectral data, structure **1** was assigned to rhinacanthin-A.

Rhinacanthin-B (**2**) was isolated as pale yellow needles. The molecular formula was determined as $C_{25}H_{28}O_5$ by HRMS. The UV spectrum of **2** showed a close resemblance to that of **1**, suggesting a dihydropyranonaphthoquinone structure for **2**. The IR spectrum of **2** revealed the presence of carbonyl groups at 1710 (ester),1675 and 1645 (*p*-quinone) cm^{-1} and lack of an absorption band for hydroxyl group. The presence of a 2,6-dimethyl-1,2-octadienoic acid ester in **2** was shown by mass spectral fragmentation ions at *m/z* 240 $[M-168]^+$, 151 $[C_{10}H_{15}O]^+$ and 123 $[C_9H_{15}]^+$ and by analysis of its ^1H NMR spectrum. The latter spectrum showed signals for methyl protons at δ 1.52 (3H, *d*, $J = 6.5$ Hz, 8′-Me), 1.57 (3H, *d*, $J = 1.2$ Hz, 10′-Me) and 1.80 (3H, *d*, $J = 0.8$ Hz, 9′-Me), and for two methylene protons at δ 2.06 (2H, *t*, $J = 7.6$ Hz, 5′-H) and 2.24 (2H, *dt*, $J = 7.6$ Hz, 4′-H). It contained a triple quartet signal for the C-3′ vinyl proton at δ 6.73 and the C-7′ vinyl proton at δ 5.18 appeared as a quartile quartet. The other signals of **2** appeared at δ 8.13 and 8.07 (each 1H, *dd*, $J = 1.8$ and 7.9 Hz, H-9 and H-6), 7.72 and 7.69 (each 1H, *dt*, $J = 1.8$ and 7.9 Hz, H-8 and H-7), 5.13 (1H, *t*, $J = 4.6$ Hz, H-3), 2.91 and 2.77 (each 1H, *dd*, $J = 4.6$ and 19.5 Hz, H-4), and 1.48 and 1.42 (each 3H, *s*, 2-Me).

A comparison of the spectral data with that of **1** suggested that **2** was a 2,2-dimethyldihydropyrano-



1 R = OH
2 R = O—CO—C(Me)=CH—CH$_2$—CH$_2$—C(Me)=CH(Me)
3 R = H

naphthoquinone containing a 10-carbon unit ester side chain in ring C instead of a hydroxyl group as seen in **1**. The above evidence led to the assignment of structure **2** for rhinacanthin-B.

Rhinacanthin-B exhibited significant ($ED_{50} \leqslant 4.0\,\mu g/ml$) cytotoxicity at $ED_{50} = 3.0\,\mu g/ml$ in the KB tissue culture assay [8]. The lack of significant cytotoxicity of **1** indicates the important contribution of lipophilicity to the enhanced cytotoxicity. This is also observed in the case of psychorubrin, a cytotoxic naphthoquinone isolated from *Psychotria rubra* [9].

EXPERIMENTAL

Mps: uncorr.; ^{1}H NMR (400 MHz) and ^{13}C NMR (100 MHz): $CDCl_3$ with TMS as internal standard; MS: direct inlet.

Plant material. The root of *Rhinacanthus nasutus* L. was collected in Tainan, Taiwan and identified by Prof. C. S. Kuoh. The voucher specimen is deposited in the Herbarum of National Cheng Kung University, Tainan, Taiwan, R.O.C.

Extraction and separation. The MeOH extract of the root of *R. nasutus* (1.8 kg) was treated with $CHCl_3$ and H_2O. The $CHCl_3$ extract, which showed significant cytotoxicity, was evapd to dryness to afford a brown syrup (16.5 g). This syrup was subjected to silica gel CC and eluted with $CHCl_3$–Me_2CO (30:1) to give 10 fractions. Fraction 2 was rechromatographed on a silica gel column with *n*-hexane–EtOAc (9:1) as eluant to furnish unknown compounds **a** (2 mg) and **b** (6 mg). The cytotoxic fraction 3 was treated similarly to yield unknown **c** (1 mg) and rhinacanthin-B (**2**, 105 mg). Fractions 4 and 5 were combined and also treated similarly to obtain a mixture of β-sitosterol and stigmasterol (350 mg) as well as lupeol (215 mg), respectively. Fraction 7 was subjected to CC on silica gel and eluted with *n*-hexane–EtOAc (3:1) to afford rhinacanthin-A (**1**, 60 mg), a colourless crystalline long chain acid (125 mg) and a mixture of the glucosides of β-sitosterol and stigmasterol (46 mg) were separated from fractions 9 and 10, respectively. The identity of lupeol, β-sitosterol and stigmasterol as well as the glucosides of β-sitosterol and stigmasterol were established by direct comparison with authentic samples. The aq. extract was extracted with EtOAc (3.6 g) and *n*-BuOH (10.2 g), and is currently under investigation.

Rhinacanthin-A (**1**). Orange needles from Me_2CO; mp 186.5–187° (calc. for $C_{15}H_{14}O_4$: C, 69.75; H, 5.46. Found: C, 69.56; H, 5.54%); $[\alpha]_D$ −12.9° ($CHCl_3$; *c* 0.25); UV λ_{max}^{MeOH} nm (log ε): 245.5 (sh, 4.59), 250.4 (4.63), 280.8 (4.35) and 332.1 (3.70); IR ν_{max}^{KBr} cm^{-1}: 3550, 1670, 1655, 1620 and 1600; EIMS *m/z* (rel. int.): 258 [M]$^+$ (100), 243 (18), 240 (11), 225 (29), 200 (14), 197 (18), 189 (18), 188 (29), 187 (25), 172 (18), 171 (18), 160 (18), 159 (29), 115 (32), 105 (25), 104 (21) and 72 (82); ^{13}C NMR: δ 184.35 (*s*, C-5), 179.42 (*s*, C-10), 157.73 (*s*, C-10a), 133.93 (*d*, C-7), 133.08 (*d*, C-8), 131.97 (*s*, C-5a), 131.02 (*s*, C-9a), 126.36 (*d*, C-9), 125.96 (*d*, C-6), 118.17 (*s*, C-4a), 80.48 (*s*, C-2), 68.29 (*d*, C-3), 25.73 (*t*, C-4), 24.68 (*q*, C_2-Me) and 21.76 (*q*, C_2-Me).

Rhinacanthin-B (**2**). Pale yellow needles (*n*-hexane); mp 78.80°; $[\alpha]_D$ −47.91° ($CHCl_3$; *c* 0.24) (calc. for $C_{25}H_{28}O_5$: C, 73.51; H, 6.91. Found: C, 73.05; H, 6.09 %); HRMS calc. for $C_{25}H_{28}O_5$: [M]$^+$ 408.1934; Found: 408.1960; UV λ_{max}^{MeOH} nm (log ε): 213.1 (sh, 4.19), 244.9 (4.27), 250.2 (4.26), 275 (sh, 3.95), 280.6 (3.97) and 331.5 (3.29); IR ν_{max}^{neat} cm^{-1}: 1710, 1675, 1645, 1620, 1595 and 1580; EIMS *m/z* (rel. int.): 408 [M]$^+$ (84), 340 (1.4), 258 (14), 243 (83), 214 (51), 240 (24), 227 (48), 226 (26), 225 (100), 212 (26), 197 (27), 151 (12), 150 (14), 123 (17), , 82 (47), and 69 (53); ^{13}C NMR: δ 184.04 (*s*, C-5), 179.35 (*s*, C-10), 166.95 (*s*, C-1′), 153.64 (*s*, C-10a), 143.59 (*d*, C-3′), 134.42 (*s*, C-6′), 133.97 (*d*, C-7), 133.11 (*d*, C-8), 132.06 (*s*, C-5a), 131.13 (*s*, C-9a), 127.01 (*s*, C-2′), 126.42 (*d*, C-9), 126.06 (*d*, C-6), 119.38 (*d*, C-7′), 117.86 (*s*, C-4a), 79.01 (*s*, C-2), 69.11 (*d*, C-3), 38.03 (*t*, C-4′), 27.31 (*t*, C-5′), 24.61 (*q*, C_2-Me), 23.16 (*t*, C-4), 22.94 (*q*, C_2-Me), 15.51 (*q*, C-10′), 13.24 (*q*, C-8′) and 12.30 (*q*, C-9′).

Acknowledgements—We thank Prof. I. S. Chen (Kaohsiung Medical College, Taiwan) for an authentic sample of lupeol, Dr M. Niwa (Meijo University, Japan) for HRMS and Dr Y. C. Cheng and Mr M. Fisher (University of North Carolina at Chapel Hill) for bioassay. This research was supported by grants from the National Science Council of Republic of China (NSC 74-0208-M006-11 to H. J. Tien) and the National Cancer Institute (CA 17625 to K. H. Lee).

REFERENCES

1. Curtar, M. and Crescent, G. (1796) *Bot. Mag. X.T.* 325
2. (1838) *Flora Med*, 500. John Lindley.
3. Jiang Su Medical College (1978) *Zhong Yao Da Tzu Dian*, Vol. I, p. 750. Shanghai Scientific and Technical, Shanghai.
4. Chuang, C. H. and Lee, L. H. (1981) *Chinese Medicinal Herbs of Hong Kong*, Vol. 2, p. 142. Shang-Wu, Hong Kong.
5. Subramanian, N. S. and Nagarajan, S. (1981) *J. Indian Chem. Soc.* **58**, 926
6. Ho, D. A. and Ho, D. K. (1979) *Duoc Hoc.* **3**, 12; *Chem. Abstr.* **93**, 37562f.
7. Inouye, H., Okuda, T. and Hayashi, T. (1975) *Chem. Pharm. Bull.* **23**, 384.
8. Geran, R. I., Greenberg, N. H., MacDonald, M. M., Schumacher, A. M. and Abbott, B. J. (1972) *Cancer Chemother. Rep.* 3. **3**, 1.
9. Hayashi, T., Smith, F. T. and Lee, K. H. (1987) *J. Med. Chem.* **30**, 2005.