Asian Journal of Chemistry Vol. 19, No. 4 (2007), 2764-2772

Antimicrobial, Antioxidant and Cytotoxic Constitutents from Roots of *Rumex crispus* Linn†

CHIRRAVURI V. RAO, CHILLARA SIVARAMAKRISHNA, CHUNDI MALLIKHARJUNARAO, RAVI JAYALAKSHMI, DILSHAD B. SHAIK and GOTTUMUKKALA V. SUBBARAJU*
Laila Research Centre, Unit I, Phase III, Jawahar Autonagar
Vijayawada-520 007, India
Fax: (91)(866)2546216; Tel: (91)(866)2541303
E-mail: subbarajugottumukkala@hotmail.com

Anthraquinone derivatives (**1-5,7**) and stilbene derivative (**6**) were isolated from the roots of *Rumex crispus*. The extracts as well as pure compounds (**1-7**) have shown remarkable antimicrobial, antioxidant and cytotoxic activites. Compound **6** displayed significant antifungal activity and compound **3** exhibited potent brine shrimp lethality activity.

Key Words: *Rumex crispus*, Antimicrobial, Antifungal, Antioxidant, Brine shrimp lethality, *Artemia salina*.

INTRODUCTION

Rumex crispus Linn (Family: *Polygonaceae*), also known commonly as Yellow dock, is a perennial herb and widely distributed in many parts of the world[1-3]. Rumex species are rich source of anthraquinones and are reported to possess important medicinal properties. *Rumex crispus* has been reported to contain anthracene/anthraquinone derivatives, naphthalenes[4-11] and flavonoids[12,13]. Yellow dock root has been used in traditional medicine, as an astringent, purgative, laxative[14], diuretic[3,15] and as a treatment for variety of skin problems, burns and swellngs[16] for a long time. It is also used in homeopathy for dry cough and sore throat[17]. Although, a number of earlier reports have demonstrated that a wide range of biological activities to the extracts of yellow dock[18-20] but, none of them have identified the active ingredients. In this paper, the isolation, characterization and biological activity of the seven compounds isolated from yellow dock is reported.

†Laila Impex Communication # 56.

EXPERIMENTAL

General experimental procedures

Melting points (uncorrected) were recorded on a MEL temp melting point apparatus. Infrared spectra were recorded on a Perkin-Elmer model spectrum BX, FT-IR instrument using KBr disc. ^{1}H NMR spectra were recorded JOEL GSX 400 MHz spectrometer using DMSO-d_6 solvent and ^{13}C NMR spectra are recorded JOEL GSX 100 MHz spectrometer. Mass studies were performed on LC-MS system equipped with Agilent 1100 series, LC/ MSD detector and 1100 series Agilent HPLC pump. Analytical HPLC studies were done on a Shimadzu system equipped with LC10 ATVP pumps and SPD M10 AVP PDA detector and auto injector and loaded with Class-VP software using C_{18} Phenomenex Luna C_{18}, 5 Micron (4.6 × 250 mm) column and 0.1 % v/v H_3PO_4 in H_2O/CH_3CN with Gradient, (0.1 % v/v H_3PO_4 : Dilute 1 mL of orthophosphoric acid to 1000 mL with water), UV detector (225 nm). Normal phase silica gel ACME (60-120 mesh) was used for column chromatography. Silica gel precoated plates (Alugram Sil G/UV_{254}) were used for TLC using the solvent system $CHCl_3$/MeOH (9:1) and visualized by immersing the plate in vanillin sulfuric acid reagent followed by heating at 110ºC. Nitro blue tetrazolium (NBT), 1,1-diphenyl-2-picryl-hydrazyl (DPPH), were obtained from sigma chemicals (USA). Brine shrimp (*Artemia salina* cysts) eggs were obtained from Argent Chemical Laboratories, Redmond, (USA). The solvents and other chemicals are of AR grade and were procured from Qualigens Fine chemicals, Mumbai (India).

The bacterial cultures *Escherichia coli* (NCIM2065), *Pseudomonas aeruginosa* (NCIM5029), *Bacillus subtilis* (NCIM2549), *Staphylococcus epidermidis* (NCIM2493), *Aspergillus niger* (NCIM1025) and *Candida albicans* (NCIM3471) were obtained from National Collection of Industrial Microorganisms (NCIM), Pune.

Plant material: *Rumex crispus* roots were collected from the Tuticorn Market, India during December 2003 and were identified by Dr. K Hemadri. A voucher specimen is on deposit at the herbarium of Laila Research centre, Vijayawada, India.

Extraction and isolation: The air dired roots powder (300 g) was extracted, repeatedly, with 90 % MeOH (4 L × 4) under reflux for 2 h. The plant material was removed by filtration the combined extracts were concentration under vacuum to give dark brown residue (Fr I, 84.0 g). A part of Fraction **I** (42.0 g) was partitioned with EtOAc (3 × 4 L). Removal of solvent from the extract under reduced pressure gave Fraction **III** (21.0 g) as a dark brown residue.

The EtOAc soluble portion fraction **III**, (20.0 g) was chromatographed over silica gel column (600 g) using solvents of increasing polarity from

$CHCl_3$ to EtOAc. The fractions (250 mL, each) were collected and monitored by TLC. The initial fractions from $CHCl_3$ elution, which was found to contain one major compound and subjected to rechromatography over silica gel using hexane + EtOAc mixture (9:1) and the pure fractions after crystallization yileded chrysophanol (**2**, 1.3 g). The latter fractions eluted with $CHCl_3$ were rechromatographed over silica gel column using eluants hexane: EtOAc mixtures (8:2-7:3). Identical fractions were combined and recrystallized to afford three known compounds physcion (**1**, 0.05 g), emodin (**3**, 1.5 g) and aloe emodin (**5**, 0.45 g). The fractions eluted with 3 % EtOAc/$CHCl_3$ contained a major compound. Which on further purification over silica column using $CHCl_3$: MeOH (9:1) followed by recrystallization from $CHCl_3$: MeOH (9:1) yielded *trans*-3,5-dihydroxy-4'-O-methyl stilbene (**6**, 0.13 g). Finally, the fractions eluted with 20 % EtOAc/$CHCl_3$ on further chromatography over silica gel using $CHCl_3$: MeOH (9:1) as eluent followed by recrystallization from $CHCl_3$: MeOH mixture (8:2) gave 1,5-dihydroxyanthraquinone (**4**, 0.30 g) and citreorosein (**7**, 0.10 g).

The powderd raw material (300 g) was extracted 4 times with 60 % MeOH (4 L) under reflux for 2 h. Evoporation of the solvent under reduced pressure provided a dark brown residue (fraction **II**, 105 g).

The second part of fraction **I** (42 g) was refluxed with 2 % HCl in MeOH (200 mL) for 3 h. The reaction mixture was cooled at room temperature, neutralized with 5 % NaOH and evoparation of solvent under vacuum yielded fraction **IV** (35 g). It was suspended in 50 mL of H_2O and extracted with EtOAc (3 × 100 mL). The EtOAc layer was dried over Na_2SO_4 and concentration under reduced pressure to give a dark brown residue (fraction **V**, 20 g).

Physcion (**1**): m.p.: 204-206ºC; IR (KBr, ν_{max}): 3330, 3040, 2983, 2938, 1630, 1566, 1479, 1386, 1227, 1034, 759, 715 cm^{-1}; LC-MS (negative): 283 $(M-H)^-$.

Chrysophanol (**2**): m.p.: 194-97ºC; IR (KBr, ν_{max}): 3403, 2983, 2938, 1676, 1627, 1580, 1475, 1366, 1272, 1025, 771, 715 cm^{-1}; LC-MS (negative): 253 $(M-H)^-$.

Emodin (**3**) : m.p.: 254-256ºC; IR (KBr, ν_{max}): 3387, 3058, 2980, 2938, 1623, 1479, 1336, 1271, 1218, 1168, 1098, 1031, 762 cm^{-1}; LC-MS (negative): 269 $(M-H)^-$.

1,5-Dihydroxy anthraquinone (**4**): m.p.: 212-214ºC; IR (KBr, ν_{max}): 3483, 2980, 2926, 1629, 1570, 1419, 1372, 1261, 1197, 1086, 750 cm^{-1}; LC-MS (negative): 239 $(M-H)^-$.

Aloe emodin (**5**): m.p.: 220-222ºC; IR (KBr, ν_{max}): 3402, 3038, 2920, 1624, 1463, 1385, 1282, 1204, 750 cm^{-1}; LC-MS (negative): 269 $(M-H)^-$.

trans-3,5-Dihydroxy-4'-O-methyl stilbene (**6**): m.p. : 178-179ºC; IR (KBr, ν_{max}): 3356, 1603, 1510, 1460, 1350, 1299, 1266, 1175, 1031, 745 cm^{-1}; LC-MS (negative): 241 $(M-H)^-$.

Citreorosein (**7**): m.p. : 280-282ºC; IR (KBr, ν_{max}): 3456, 3050, 2973, 2921, 1680, 1626, 1590, 1476, 1398, 1260, 1172, 1098, 1031, 759 cm^{-1}; LC-MS (negative): 285 $(M-H)^-$.

Determination of antimicrobial and antifungal activity: The antimicrobial activity was determined by agar cup-plate (cup dia.: 8mm) method[21] against the organisms *E. coli* (Gram negative), *P. aeruginosa* (Gram negative), *B. subtilis* (Gram positive) and *S. epidermidis* (Gram positive) at different concentrations 500, 200, 100 50 µg/0.05 mL. Ciprofloxacin was used as standard. The antifungal activity was also determined by a similar procedure[21] against *Aspergillus niger* and *Candida albicans* using griseofulvin as standard.

Determination of superoxide radical scavenging activity: The superoxide radical scavenging activity of fractions **I-V** and pure compounds of *Rumex crispus* was determined by the method of McCord and Fridovich[22]. The assay mixture contained EDTA (6.0 mM) containing 3 µg NaCN, riboflavin (2 µM), NBT (50 µM), various concentrations of test substances and phosphate buffer (58 mM, pH 7.8) in a final volume of 3 mL. The tubes were mixed well and optical densities were measured at 560 nm. The tubes were uniformly illuminated with an incandescent lamp for 15 min and the optical densities were measured again at 560 nm. The percentage inhibition of superoxide radical generation was measured by comparing the absorbance values of control and those of the test substances. IC_{50} values were obtained from best-fit line drawn concentration (µg) *vs.* percentage inhibition.

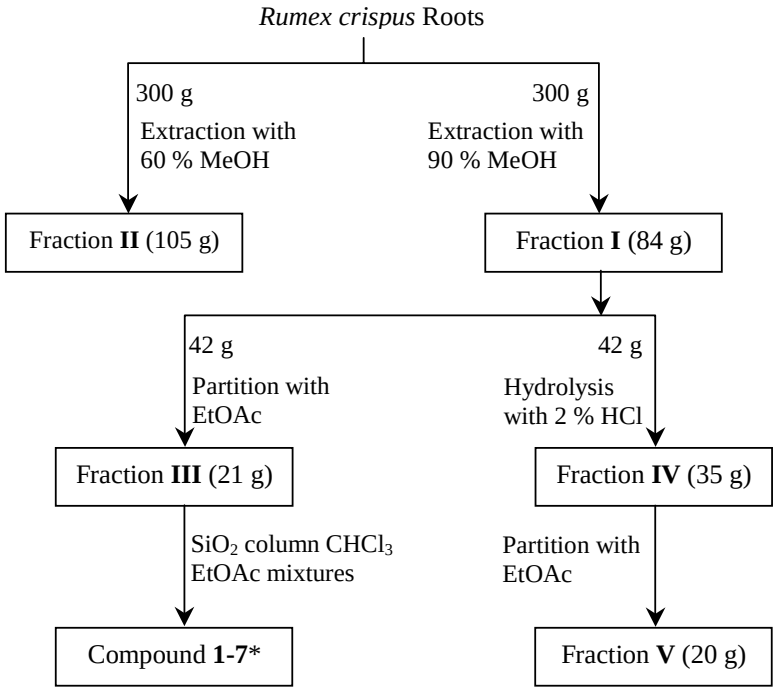
Determination of DPPH radical scavenging activity: DPPH radical scavenging activity was determined by the method of Lamaison *et al.*[23] based on the reduction of coloured methanolic solution of DPPH. Free radical scavenging ability of the test substances added to the methanolic solution of DPPH is inversely proportional to the difference in initial and final absorption of DPPH solution at 517 nm. Drug activity was expressed as the 50 % inhibitory concentration (IC_{50}). The reaction mixture contained 1×10^{-4} M methanolic solution of DPPH and various concentrations of the test substances. Percentage inhibition was determined by comparing the absorbance values of test and control tubes. IC_{50} values were obtained from the best-fit line drawn concentration (µg) *vs.* percentage inhibition.

Determination of brine shrimp lethality: The brine shrimp (*Artemia salina*) cysts were hatched in a cone shaped vessel (1 L) filled with sterile artificial sea water (prepared using sea salt 38 g/L and adjusted to pH 8.5 using 1 N NaOH) under constant aeration. After 48 h, 10 active nauplii were drawn through a glass capillary and placed in each vial containing 4.5 mL sterile artificial sea water and treated with known concentrations of test substances and the volume was finally made upto 5 mL using sterile

artificial sea water and maintained at 37ºC for 24 h under the light of incandescent lamps. The viability/mortality was obtained by counting the surviving larvae. LC_{50} values were calculated using Finney software for probit analysis. The LC_{50} values of *Rumex crispus* fractions (**I-V**) and pure compounds were summarized in Table-5.

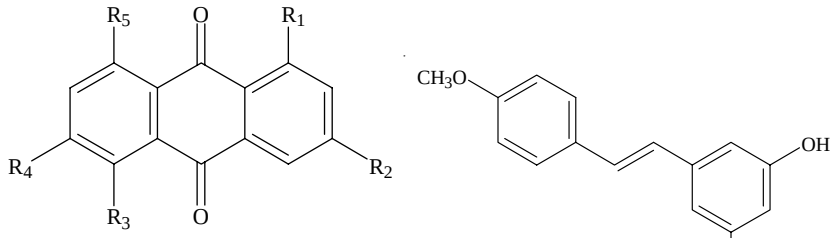
RESULTS AND DISCUSSION

Rumex crispus roots were extracted, separately, with 90 % MeOH (fraction **I**) and 60 % MeOH (fraction **II**). Further fractionation of fraction **I**, as shown in Fig. 1, resulted in fractions **III-V**. Fraction-**III** on extensive chromatography over a silica gel column yielded pure compounds **1-7**. These compounds have been identified as physcion (**1**)[24], chrysophanol (**2**)[24], emodin (**3**)[25], 1,5-dihydroxyanthraquinone (**4**)[11], aloe emodin (**5**)[26], *trans*-3,5 dihydroxy-4'-O-methyl stilbene (**6**)[27,28] and citreorosein (**7**)[29,30] (Fig. 2), by comparison of their physical and spectral data with those reported in the literature. Percentage concentration of compounds **1-7** in fractions **I-V** is incorporated in Table-1. Compounds **6** and **7** have been isolated for the first time from *Rumex crispus*.



***1** (0.05 g), **2** (1.30 g), **3** (1.50 g), **4** (0.30 g), **5** (0.45 g), **6** (0.13 g), **7** (0.10 g),

Fig. 1. Isolation of compounds **1-7**



1. $R_1=R_5=OH$; $R_2=OCH_3$;$R_3=H$;$R_4=CH_3$
2. $R_1=R_5=OH$; $R_2=CH_3$;$R_3=R_4=H$
3. $R_1=R_4=R_5=OH$; $R_2=CH_3$;$R_3=H$
4. $R_1=R_3=OH$; $R_2=R_4=R_5=H$
5. $R_1=R_5=OH$; $R_2=CH_2OH$;$R_3=R_4=H$
7. $R_1=R_4=R_5=OH$; $R_2=CH_2OH$;$R_3=H$

Fig. 2. Anthroquinones and stilbene from *Rumes crispus*

TABLE-1
PERCENTAGE OF COMPOUNDS **1-7** IN FRACTIONS **I-V**

Compound	Percentage composition				
	Fraction **I**	Fraction **II**	Fraction **III**	Fraction **IV**	Fraction **V**
1	0.37	0.14	1.17	0.63	1.33
2	2.48	0.79	13.60	8.49	9.56
3	3.06	1.39	8.24	4.52	9.58
4	1.13	1.03	2.96	1.21	2.88
5	0.57	0.42	1.57	0.81	1.33
6	0.87	0.65	1.86	0.60	1.55
7	0.62	0.33	1.43	0.59	1.36

The fractions **I-V** and the pure compounds **1-7** were tested for antimicrobial activity by Agar cup plate diffusion method[21] against *E. coli*, *P. aeruginosa*, *B. subtilis* and *S. epidermidis* and the data were compared against ciprofloxacin standard and the results are summarized in Table-2. The fractions **I-V** and compounds **4**, **6** and **7** exhibited significant activity against gram (-) positive organisms, *B. subtilis* and *S. epidermidis* while compounds **1** and **2** displayed weak activity. Moreover, compounds **1**, **2**, **3** and **5** showed significant antimicrobial activity against *E. coli* and *P. aerugenosa*.

The antifungal activity was also determined for compounds **1-7** using similar test procedure[21] against *Aspergillus niger* and *Candida albicans* and the results were compared with griseofulvin standared. The results are shown in Table-3. Compound **6** exhibited activity against *Candida albicans* and *Aspergillus niger*. The inhibihtion of *Candida albicans* by compound **6** is comparable to that of griseofulvin standard.

TABLE-2
MINIMUM INHIBITORY CONCENTRATIONS (MIC) OF FRACTIONS **I-V** AND COMPOUNDS **1-7**

Fraction/ Compound	MIC (µg/cup)			
	E. coli NCIM 2065	*P. aeruginosa* NCIM 5029	*B. subtilis* NCIM 2549	*S. epidermids* NCIM 2493
I	-	-	100	100
II	-	-	50	200
III	-	-	50	50
IV	-	-	50	50
V	-	-	50	50
1	10	10	-	10
2	10	10	-	-
3	10	10	-	-
4	-	-	25	-
5	10	10	-	-
6	-	-	25	25
7	-	-	25	-

Cup dia: 8mm; 0.05 µL; - No activity (diameter of the inhibitory zone less than 8 mm means absence of activity

TABLE-3
ANTI FUNGAL ACTIVITY OF COMPOUNDS **4** AND **6***

Compound	Conc. (µg/cup)	Antifungal activity[a]	
		Aspergillus niger	*Candida albicans*
4	500	10	-
	200	-	-
	50	-	-
6	500	16	15
	200	8	13
	50	-	11
Griseofulvin	50	11	12

*Fractions **I-V** and compounds **1**, **2**, **3**, **5** and **7** did not show any antifungal activity; [a]Zone of inhibition in mm

Antioxidant activity of pure compounds as well as the fractions **I-V** was determined using Superoxide radical scavenging activity[22] and DPPH (1,1-diphenyl-2-picryl-hydrazyl)[23] methods and the results are summarized in Table-4. Compounds **4**, **6** and **7** showed moderate antioxidant activity, in both the methods. The antioxidant activity of different fractions was found to be higher in comparison to the pure compounds. The results suggest that the higher antioxidant activity of the fractions may have been due to the presence of other unidentified compounds. Nevertheless, a synergistic interaction between the individual compounds **1-7** present in the extracts may also need to be considered.

TABLE-4
ANTIOXIDANT ACTIVITY OF FRACTIONS **I-V** AND COMPOUNDS **1-7**

Fraction/ compound	Superoxide radical scavenging activity IC_{50} µg/mL	DPPH radical scavenging activity IC_{50} µg/mL
I	16.0	12.0
II	16.0	13.0
III	14.5	12.5
IV	17.0	12.5
V	17.0	14.0
1	> 100.0	> 100.0
2	> 100.0	> 100.0
3	> 100.0	> 100.0
4	32.5	70.0
5	> 100.0	> 100.0
6	68.6	36.0
7	39.0	50.0
Vitamin C	160.0	3.5

The brine shrimp lethality has now been evaluated for various yellow dock fractions **I-V** and pure compounds and the results are given in Table-5. Brine shrimp lethality has been known to corroborative with the cytotoxity against 9 KB and 9 PS cells[31,32]. The data from the Table-5 reveals that the percentage of lethality was found to correlate anthraquinones concentration. It was also observed that compound **3** was found to be more potent compared to podophyllotoxin standard, while compound **7** displayed moderate activity. Some of the fractions have also shown moderate activity and the percentage of lethality seems to have correlation with their anthraquinones concentration (Table-1).

TABLE-5
BRINE SHRIMP LETHALITY OF FRACTIONS **I-V** AND COMPOUNDS **1-7**

Fraction	Brine shrimp lethality activity LC_{50} µg/mL	Compound	Brine shrimp lethality activity LC_{50} µg/mL
I	56.11	**1**	> 100.00
II	84.87	**2**	> 100.00
III	41.02	**3**	1.27
IV	23.62	**4**	> 100.00
V	9.86	**5**	> 100.00
–	–	**6**	> 100.00
Podophyllotoxin	2.46	**7**	8.25

ACKNOWLEDGEMENTS

The authors thank Sophisticated Analytical Instrument Facility (SAIF) of IIT Chennai, India for NMR spectral data and Sri G. Ganga Raju Chairman of Laila Group, Vijayawada, India for encouragement.

REFERENCES

1. R.N. Chopra, L. Nayar and I.C. Chopra, Glossary of Indian Medicinal Plants, CSIR, New Delhi, pp. 216-218 (1956).
2. J.A Duke, *Rumex crispus* L, Handbook of Medicinal Herbs, CRC press, Boca Raton, FL, pp. 414-415 (1985).
3. A. Chevallier, Encyclopedia of Medicinal Plants, Dorling Kindersley, London, p. 264 (2001).
4. A.A. Khan, *Can. J. Chem.*, **41**, 1622 (1963).
5. J.W. Fairbairn and F.J. EI-Muhtadi, *Phytochemistry*, **11**, 263 (1972).
6. E. Dabi-Lengyel, E. Jambor, B. Danos and P. Tetenyi, *Herba Hung.*, **30**, 91 (1991).
7. L.O. Demirezer, *Planta Med.*, **59(S)**, 630 (1993).
8. L.O. Demirezer, *Pharmazie*, **49**, 378 (1984).
9. L.O. Demirezer, *Z. Naturforsch., C: J. Biosci.*, **49**, 404 (1994).
10. J.O. Midiwo and G.M. Rukunga, *Phytochemistry*, **24**, 1390 (1985).
11. K. Gunaydin, G. Topcu and R.M. Ion, *Nat. Prod. Lett.*, **16**, 65 (2002).
12. H.A.E.I. Fattah, *Mansoura J. Pharm. Sci.*, **6**, 141 (1981).
13. J. Koukol and W.M. Jr. Dugger, *Plant Physiol.*, **2**, 1023 (1967).
14. Y.E. Tyler, The Honest Herbal, Haworth Press, Binghampton, New York, pp. 325-326 (1993).
15. D. Brown, Encyclopedia of Herbs and Their Uses, Dorling Kindarley, London, p. 344 (1995).
16. R.P. Steiner, Folk Medicine, American Chemical Society, Washincton DC, pp. 34-35 (1986).
17. M. Castro, The Complete Homeopathy Handbook, Macmillan, London, p. 333 (1990).
18. O. Demirezer and A. Kuruuzum, *Z. Naturforsch., C: J. Biosci.*, **50**, 461 (1995).
19. O. Demirezer and A. Kuruuzum, *Z. Naturforsch., C: J. Biosci.*, **52**, 665 (1997).
20. A. Yildirim, A. Mavi and A.A. Kara, *J. Agric. Food Chem.*, **49**, 4083 (2001).
21. K.E. Cooper and F. Kavanagh, *Anal. Microbiol.*, **2**, 13 (1972).
22. J.M. McCord and I. Fridovich, *J. Biol. Chem.*, **244**, 6049 (1969).
23. J.L. Lamaison, C. Ptitjean-Freytet and A. Carnet, *Pharm. Acta Helv.*, **66**, 185 (1991).
24. R.M. Munavu, L.O. Mudamba and J.A. Ogur, *Planta Med.*, **50**, 11 (1984).
25. A. Hasan, I. Ahmed, M. Jay and B. Voirin, *Phytochemistry*, **39**, 1211 (1995).
26. X. Zhiyan, C. Dihua, S. Jianyong, T. Guangzhony and M. Libin, *Acta Pharm. Sin.*, **32**, 120 (2000).
27. M. Chaudhari, G.K. Jain, J.P. Sarin and N.M. Khanna, *Indian J. Chem.*, **22B**, 1163 (1983).
28. M.J.T.G. Gonzalez, M.M.M. Pinto, A. Kijjoa, C. Anantachoke and W. Herz, *Phytochemistry*, **32**, 433 (1993).
29. A.M.R. Marinho, E. Rodrigues-Filho, L.R. Moitinho and L.S. Santos, *J. Braz. Chem. Soc.*, **16**, 280 (2005).
30. C.-K. Lee, P.-H. Lee and Y.-H. Kuo, *J. Chin. Chem. Soc.*, **48**, 1053 (2001).
31. J.L. McLaughlin, C.J. Chang and D.L. Smith, *Am. Chem. Soc. Sympos. Ser*, **534**, 114 (1992).
32. J.L. McLaughlin, L.L. Rogers and J.E. Anderson, *Drug Inform. J.*, **32**, 513 (1998).

(*Received*: 14 March 2006; *Accepted*: 27 December 2006) AJC-5318