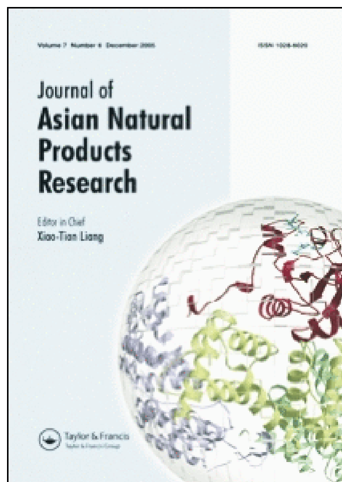
This article was downloaded by:
On: *22 January 2011*
Access details: *Access Details: Free Access*
Publisher *Taylor & Francis*
Informa Ltd Registered in England and Wales Registered Number: 1072954 Registered office: Mortimer House, 37-41 Mortimer Street, London W1T 3JH, UK



Journal of Asian Natural Products Research
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713454007

Xanthone glycosides from herbs of *Polygala hongkongensis* Hemsl and their antioxidant activities

Jian-Feng Wu[a]; Si-Bao Chen[bc]; Jing-Chun Gao[c]; Hao-Liang Song[b]; Li-Jun Wu[d]; Shi-Lin Chen[bc]; Peng-Fei Tu[de]
[a] Foshan University, Foshan, China [b] State Key Laboratory of Chinese Medicine and Molecular Pharmacology, Department of Applied Biology and Chemical Technology, The Hong Kong Polytechnic University, Shenzhen, China [c] Institute of Medicinal Plant Development, Chinese Academy of Medical Science, Peking Union Medical College, Beijing, China [d] Shenyang Pharmaceutical University, Shenyang, China [e] School of Pharmaceutical Sciences, Health Science Center, Peking University, Beijing, China

To cite this Article Wu, Jian-Feng , Chen, Si-Bao , Gao, Jing-Chun , Song, Hao-Liang , Wu, Li-Jun , Chen, Shi-Lin and Tu, Peng-Fei(2008) 'Xanthone glycosides from herbs of *Polygala hongkongensis* Hemsl and their antioxidant activities', Journal of Asian Natural Products Research, 10: 7, 665 — 670
To link to this Article: DOI: 10.1080/10286020801967201
URL: http://dx.doi.org/10.1080/10286020801967201

PLEASE SCROLL DOWN FOR ARTICLE

Full terms and conditions of use: http://www.informaworld.com/terms-and-conditions-of-access.pdf

This article may be used for research, teaching and private study purposes. Any substantial or systematic reproduction, re-distribution, re-selling, loan or sub-licensing, systematic supply or distribution in any form to anyone is expressly forbidden.

The publisher does not give any warranty express or implied or make any representation that the contents will be complete or accurate or up to date. The accuracy of any instructions, formulae and drug doses should be independently verified with primary sources. The publisher shall not be liable for any loss, actions, claims, proceedings, demand or costs or damages whatsoever or howsoever caused arising directly or indirectly in connection with or arising out of the use of this material.

Journal of Asian Natural Products Research
Vol. 10, No. 7, July 2008, 665–670

Taylor & Francis
Taylor & Francis Group

Xanthone glycosides from herbs of *Polygala hongkongensis* Hemsl and their antioxidant activities

Jian-Feng Wu[abcd], Si-Bao Chen[ce], Jing-Chun Gao[e], Hao-Liang Song[c], Li-Jun Wu[a], Shi-Lin Chen[ce]* and Peng-Fei Tu[ab]*

[a]*Shenyang Pharmaceutical University, Shenyang 110016, China;* [b]*School of Pharmaceutical Sciences, Health Science Center, Peking University, Beijing 100083, China;* [c]*State Key Laboratory of Chinese Medicine and Molecular Pharmacology, Department of Applied Biology and Chemical Technology, The Hong Kong Polytechnic University, Shenzhen 518057, China;* [d]*Foshan University, Foshan 528000, China;* [e]*Institute of Medicinal Plant Development, Chinese Academy of Medical Science, Peking Union Medical College, Beijing 100094, China*

(*Received 17 January 2007; final version received 31 May 2007*)

Downloaded At: 18:57 22 January 2011

Two new xanthone *O*-glycosides, polyhongkongenosides A (**1**) and B (**2**), together with four known xanthone glycosides, were isolated from the herbs of *Polygala hongkongensis* Hemsl. Their structures were elucidated on the basis of UV, IR, NMR, and MS spectral data. The antioxidant *in vitro* activities of **1**–**6** were determined by the scavenging activities against 1,1-diphenyl-2-picrylhydrazyl (DPPH) radicals and hydroxyl radicals and their reductive activities to Fe^{3+}. Mangiferin, one of the four known xanthone glycosides, showed potential scavenging effect on DPPH and hydroxy radicals and reductive activity to Fe^{3+} with IC_{50} values of 4.7, 13.9, 23.7 μM, respectively.

Keywords: *Polygala hongkongensis*; xanthone *O*-glycosides; polyhongkongenoside A; polyhongkongenoside B; antioxidant activities

1. Introduction

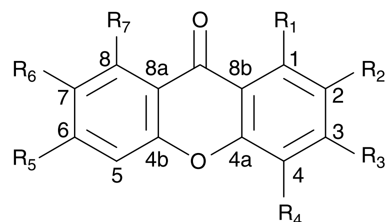
Recently, there has been increasing interest in the plant of genus *Polygala* due to their abundant biological activities and possibilities as a new source for unique natural products. Many chemical constituents such as saponins [1–3], xanthones [4–6], and oligosaccharide esters [7–9] have been isolated from *Polygala* plants. However, there is no chemical report on *Polygala hongkongensis* so far, which is widely spread in the south of China, and used for heat clearing, detoxicating and promoting blood flow, and expelling phlegm to arrest coughing agents in Chinese folk medicine [10]. In our search for bioactive materials, the *n*-butanol extract of the herbs of *P. hongkongensis* showed antioxidant activity. Subsequent chromatographic fractionation and purification led to the isolation of two new xanthone *O*-glycosides, polyhongkongenosides A (**1**) and B (**2**). In addition, four known xanthone glycosides, 7-*O*-methylmangiferin (**3**) [11], mangiferin (**4**) [12], polygalaxanthone III (**5**) [13], and polygalaxanthone V (**6**) [5], were also obtained (Figure 1). This paper deals with the isolation and the characterization of the two new xanthone *O*-glycosides. Evaluation of the antioxidant activities of six xanthone glycosides is also described.

2. Results and discussion

Compound **1** gave a molecular formula of $C_{25}H_{26}O_{16}$ established by HR-ESI-MS (at *m/z*

*Corresponding authors. Email: bcslchen@inet.polyu.edu.nk; pengfeitu@bjmu.edu.cn

ISSN 1028-6020 print/ISSN 1477-2213 online
© 2008 Taylor & Francis
DOI: 10.1080/10286020801967201
http://www.informaworld.com



1 R_1= R_6=OH, R_2= R_4= R_7=H, R_3=OCH_3, R_5=O-Glc-O-Rha

2 R_1=OH, R_2= R_4= R_5= H, R_3= R_6= OCH_3, R_7=O-Glc

3 R_1= R_3= R_5= OH, R_2= Glc, R_6= OCH_3, R_4= R_7=H

4 R_1= R_3= R_5= R_6=OH, R_2= Glc, R_4= R_7=H

5 R_1= R_3= R_5= OH, R_2= Glc-Api, R_4= R_7=H, R_6= OCH_3

6 R_1= R_3 = OH, R_2=R_4= R_7=H, R_5= O-Glc-Rha, R_6= OCH_3

Figure 1. Structures of compounds **1**–**6**.

Downloaded At: 18:57 22 January 2011

Table 1. ^{1}H (400 MHz) and ^{13}C NMR (100 MHz) spectral data of **1** and **2** (in DMSO-d_6).

	1		**2**	
Position	δ_H	δ_C	δ_H	δ_C
1		162.3		162.6
2	6.36 (d, $J = 2.1$ Hz)	96.7	6.60 (d, $J = 2.1$ Hz)	95.8
3		165.8		161.6
4	6.58 (d, $J = 2.1$ Hz)	92.3	6.72 (*d*, $J = 2.1$ Hz)	93.4
4a		157.3		157.9
4b		144.9		146.2
5	7.25 (s)	102.5	6.93 (d, $J = 9.2$ Hz)	103.7
6		152.2	7.44 (d, $J = 9.2$ Hz)	119.2
7		150.3		141.3
8	7.47 (s)	108.0		150.2
8a		113.5		107.3
8b		102.9		103.4
9		179.1		179.9
1-OH	13.04 (s)		13.23 (s)	
7-OH	9.87 (s)			
3-OCH_3	3.87 (s)	55.8	3.91 (s)	55.2
7-OCH_3			3.82 (s)	55.5
Glc-1	5.42 (d, $J = 7.6$ Hz)	97.4	5.20 (d, $J = 6.8$ Hz)	98.6
2	3.62 (m)	76.5	3.30 (m)	72.1
3	3.52 (m)	76.9	3.37 (m)	75.3
4	3.22 (m)	69.6	3.19 (m)	68.5
5	3.50 (m)	77.3	3.51 (m)	76.5
6	3.70 (m)	60.5	3.60 (m)	60.3
Rha-1	5.22 (s)	100.3		
2	3.24 (m)	70.4		
3	3.69 (m)	70.5		
4	3.18 (m)	72.0		
5	3.80 (m)	68.6		
6	1.12 (d, $J = 6.0$ Hz)	18.1		

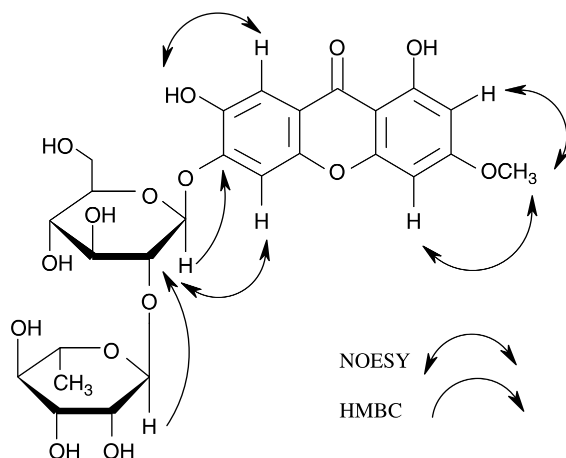


Figure 2. Key NOESY and HMBC correlations of **1**.

Downloaded At: 18:57 22 January 2011

583.1294 $[M + H]^+$). Its UV spectrum in MeOH (λ_{max} 235, 256, 308 and 365 nm) was similar to that of 1, 6-dihydroxy-3, 7-dimethoxyxanthone (λ_{max} 234, 255, 312, and 364 nm) [14], which suggested that **1** is a derivative of 1,3,6,7-tetraoxygenated xanthone. IR spectrum of **1** showed the presence of hydroxyl groups (3392 cm^{-1}), a carbonyl group (1654 cm^{-1}) and phenyl groups (1609, 1579, and 1492 cm^{-1}). The ^1H NMR spectrum showed two hydroxyl signals at δ 13.04 and 9.87, two aromatic proton singlets at δ 7.47 and 7.25, two meta-coupled aromatic protons at δ 6.58 (1H, d, $J = 2.1$ Hz), 6.36 (1H, d, $J = 2.1$ Hz), two anomeric proton signals at δ 5.42 (1H, d, $J = 7.6$ Hz) and 5.22 (1H, s), a methoxyl signal at δ 3.87 (3H, s), and a methyl signal at δ 1.12 (3H, d, $J = 6.0$ Hz) (Table 1). The ^{13}C NMR spectrum of **1** exhibited 12 aliphatic carbon signals due to two sugar moieties. After acid hydrolysis, **1** produced glucose and rhamnose. Therefore, **1** was suggested to be a 1,3,6,7-tetraoxygenated xanthone *O*-glycoside. The positions of the substituents were confirmed through NOESY experiments (Figure 2). In the NOESY spectrum of **1**, cross-peaks were observed between a methoxyl at δ 3.87 and two aromatic protons at δ 6.36 (H-2) and δ 6.58 (H-4). Hence, this methoxyl group was suggested to be located at C-3. The hydroxyl signal at δ 9.87 showed NOESY correlation with H-8 (δ 7.47) signal, which indicated that the hydroxyl group was at C-7. The correlation between the anomeric proton at δ 5.42 and H-5 at δ 7.25 indicated that the glucosyl moiety was linked to C-6 of the xanthone moiety. This location could be further proved by HMBC spectrum. In the HMBC spectrum, the anomeric proton of rhamnose moiety at δ 5.22 correlated with C-2 (δ 76.5) of the glucosyl residue, and the glucosyl anomeric proton at δ 5.42 correlated with C-6 of the aglycone (δ 152.2; Figure 2). The relative configurations of the anomeric carbons of glucosyl and rhamnosyl were deduced to be β and α, respectively, based on the coupling constants and comparison of the NMR spectra with those of known compounds [6]. Thus, compound **1** was determined to be 6-*O*-[α-L-rhamnopyranosyl-(1 → 2)-β-D-glucopyranosyl]-1,7-dihydroxy-3-methoxyxanthone and named polyhongkongenoside A.

Compound **2** gave a molecular formula of $C_{21}H_{22}O_{11}$ as established by HR-ESI-MS (at *m/z* 451.1247 $[M + H]^+$). The UV spectrum suggested that **2** has a hydroxyxanthone skeleton [15]. Its characteristic IR absorptions were similar to those of **1**. The ^1H NMR spectrum showed a hydroxyl signal at δ 13.23, two *ortho*-coupled aromatic proton

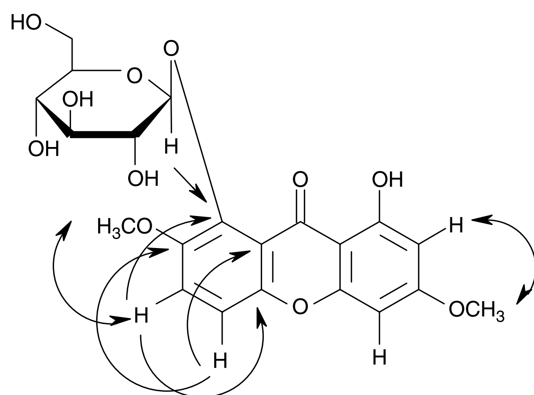


Figure 3. Key NOESY and HMBC correlations of **2**.

Downloaded At: 18:57 22 January 2011

signals at δ 7.44 (1H, d, $J = 9.2$ Hz) and 6.93 (1H, d, $J = 9.2$ Hz), two *meta*-coupled aromatic proton signals at δ 6.72 (1H, d, $J = 2.1$ Hz) and 6.60 (1H, d, $J = 2.1$ Hz), one anomeric proton signal at δ 5.20 (1H, d, $J = 6.8$ Hz), and two methoxyl signals at δ 3.91 (3H, s) and 3.82 (3H, s). The ^{13}C NMR spectrum of **2** exhibited six aliphatic carbon signals due to one sugar moiety. After acid hydrolysis, **2** produced glucose. Therefore, compound **2** was suggested to be a tetra-oxygenated xanthone *O*-glycoside. The NMR spectra showed the difference of compound **2** from **1** mostly in B ring. The positions of the two methoxyl groups were confirmed through NOESY experiments (Figure 3). In the NOESY spectrum of **2**, cross-peaks were observed between a methoxyl signal at δ 3.91 and an aromatic proton at δ 6.60 (H-2). So this methoxyl group was suggested to be located at C-3. Another methoxyl group at δ 3.82 showed NOESY correlation with this signal of H-6 (δ 7.44); hence, the methoxyl group was suggested to be located at C-7. In the HMBC spectrum, the *ortho*-coupled aromatic proton at δ 6.93 (H-5) was correlated to C-8a and C-7, and the proton at δ 7.44 (H-6) was correlated to C-8 and C-4b, which further indicated the location of 7-OCH_3. Moreover, the glucosyl anomeric proton at δ 5.20 correlated to C-8 of the aglycone (δ 150.2; Figure 3). The relative configuration of the anomeric carbon of glucosyl residue was deduced to be β, based on the coupling constant and comparison of the NMR spectra with those of known compounds [6]. Thus, compound **2** was determined to be 8-*O*-(β-D-glucopyranosyl)-1-hydroxy-3,7-dimethoxyxanthone and named polyhongkongenoside B.

All isolated compounds were tested for antioxidant activities, and the results (IC_{50} values) were summarized in Table 2. Among them, **4** exhibited potential scavenging activity against 1,1-diphenyl-2-picrylhydrazyl (DPPH) and hydroxy radicals, and reductive activity to Fe^{3+} with IC_{50} values of 4.7, 13.9, and 23.7 μM, respectively.

3. Experimental

3.1 General experimental procedures

Optical rotations were performed on a Perkin–Elmer digital polarimeter. Melting points were measured on a Fisher–Johns apparatus and are uncorrected. IR spectra were run on a Shimadzu FTIR 8400 infrared spectrometer as KBr patches. UV spectra were measured on Perkin–Elmer Lambda 35 UV/VIS spectrometer. NMR spectra were measured on a Bruker AV 400 spectrometer with TMS as an internal standard. ESI-MS spectra were obtained using a Micromass ZabSpec high-resolution mass spectrometer. Silica gel 60H (400–500 mesh) and silica gel GF_{254} sheets (0.20–0.25 mm; both from

Table 2. Antioxidant activities of compounds **1–6** (IC_{50}, μM[a]).

Compounds	DPPH radical scavenging activity	Hydroxy radical scavenging activity	Reductive activity to Fe^{3+}
1	98.3	N.T.[b]	N.T.
2	N.T.	N.T.	N.T.
3	10.2	27.8	36.2
4	4.7	13.9	23.7
5	76.1	83.5	54.9
6	N.T.	N.T.	89.4
BHT[c]	7.7	14.5	22.8

[a] IC_{50} (μM) values were calculated from regression lines using 12 different concentrations in triplicate.
[b] N.T.: 50% inhibition did not reach the concentrations of 100 μM.
[c] Butylated hydroxytoluene (BHT) was used as the positive control.

Downloaded At: 18:57 22 January 2011

Qingdao Haiyang Chemical Group Co., Shandong Province, China) were used for column chromatography and TLC, respectively. D101 resin was obtained from Tianjin Chemical Company.

3.2 Plant material

Polygala hongkongensis Hemsl. (Polygalaceae) were collected at Guangzhou, Guangdong Province, China, in May 2005, and identified by Professor Peng-fei Tu, School of Pharmaceutical Sciences, Peking University. A voucher specimen (PH200504) has been deposited in the Herbarium of Peking University Modern Research Center for TCM.

3.3 Extraction and isolation

The air-dried herbs of *P. hongkongensis* (10 kg) were pulverized and extracted with 70% MeOH under reflux (1.5 h × 3). The extract was combined and evaporated *in vacuum* to yield 4.1 kg of residue, 2 kg of which was suspended in water and successively partitioned with EtOAc and *n*-BuOH. Part of the *n*-BuOH extract (297.5 g) was subjected to a macroporous resin D101 column (11.0 × 80.0 cm). The absorbed material was eluted successively with H_2O, then with 30, 60 and 95% EtOH. The 30% EtOH eluate (76.5 g) was subjected to silica gel column chromatography using a gradient system of $CHCl_3$–MeOH (19:1, 9:1, 6:1, 4:1, 3:1, 1:1, 0:1) yielding 10 pooled fractions (1–10). Fraction 2 (3.6 g) was subjected to silica gel column chromatography, eluted with $CHCl_3$–MeOH (85:15) to produce 16 subfractions 1–16. Compound **3** (77.0 mg) was obtained from subfractions 7–11 by Sephadex LH-20 after repeated silica gel chromatography. Fraction 3 (40 g) was recrystallized in MeOH for three times to give compound **4** (20.0 g). Fraction 4 (2.2 g) was purified by Sephadex LH-20 column to give compound **2** (42.1 mg). Fraction 5 (4.5 g) was purified by Sephadex LH-20 column to give compound **1** (53.4 mg). Fraction 7 (5.3 g) was first subjected to reverse phase C18 column, and then purified by semi-preparative HPLC with MeOH–H_2O (5:5) as mobile phase to give compounds **5** (59.3 mg) and **6** (32.7 mg).

3.3.1 Polyhongkongenoside A

Yellow powder, mp 213–214°C, $[\alpha]_D^{26} - 62$ ($c = 0.1$, MeOH); UV (MeOH): λ_{max} nm (log ε) 235 (4.24), 256 (4.56), 308 (4.11) and 365 (3.82); IR (KBr) ν_{max} cm^{-1}: 3392, 2924, 1654, 1609, 1579 and 1492; ^1H and ^{13}C NMR spectral data (see Table 1); HR-ESI-MS *m/z*: 583.1294 $[M + H]^+$ (calcd for $C_{25}H_{27}O_{16}$, 583.1289). ESI-MS: 583 $[M + H]^+$, 437 $[M + H\text{-rha}]^+$ and 275 $[M + H\text{-rha-glc}]^+$.

3.3.2 Polyhongkongenoside B

Yellow powder. mp 173–175°C, $[\alpha]_D^{26} - 60.8$ ($c = 0.1$, MeOH); UV (MeOH): λ_{max} nm (log

ε): 240 (4.38), 257 (4.30), 318 (4.25) and 362 (4.10); IR (KBr) ν_{max} cm^{-1}: 3427, 2926, 1656, 1616, 1452 and 1275; ^1H and ^{13}C NMR spectral data (see Table 1); HR-ESI-MS *m/z*: 451.1247 $[M + H]^+$ (calcd for $C_{21}H_{23}O_{11}$, 451.1240). ESI-MS: 451 $[M + H]^+$ and 289 $[M + H\text{-Glc}]^+$.

3.4 Antioxidant activity assay in vitro

Compounds **1**–**6**, whose purities are all above 95% detected by HPLC, were tested for their scavenging effects on DPPH and hydroxy radicals, and reductive activity to Fe^{3+}, according to a slight modification of the procedure reported previously [15–17].

3.5 Acid hydrolysis of compounds 1–2

Compound **1** (5 mg) in 1 M HCl (10 ml) was refluxed at 80°C for 3 h. The reaction mixture was diluted with H_2O and then extracted with EtOAc; from the EtOAc layer the aglycone **1a** (2.3 mg) was obtained. In the aqueous phase D-glucose and L-rhamnose were identified by TLC comparison with authentic substances. By the same procedure, 4 mg of **2** was hydrolyzed to give aglycone **2a** (2.4 mg) and D-glucose.

Downloaded At: 18:57 22 January 2011

References

[1] M. Hamburger and K. Hostettmann, *Helv. Chim. Acta* **69**, 221 (1986).
[2] M.A. Ouyang, C.R. Yang, and H.Q. Wang, *Chin. Tradit. Herb. Drug*, **30**, 881 (1999).
[3] S. Sakuma and J. Shoji, *Chem. Pharm. Bull.* **29**, 2431 (1981).
[4] S. Ghosal, P.C. Basumatari, and S. Banerjee, *Phytochemistry* **20**, 489 (1981).
[5] Y. Jiang and P.F. Tu, *Phytochemistry* **60**, 813 (2002).
[6] T. Miyase, H. Noguchi, and X.M. Chen, *J. Nat. Prod.* **62**, 993 (1999).
[7] J.C. Li, M. Ono, and T. Nohara, *Chem. Pharm. Bull.* **48**, 1223 (2000).
[8] Y. Jiang and P.F. Tu, *J. Asian Nat. Prod. Res.* **5**, 279 (2003).
[9] S. Kobayash, T. Miyase, and H. Noguchi, *J. Nat. Prod.* **63**, 1066 (2000).
[10] Delectis Florae Reipublicae Popularis Sinicae, Agendae Academiae Sinicae Edits, *Flora Reipublicate Popularis Sinicae [M]*, (Science Press, Beijing, 1997), Tomus 43.
[11] Y. Jiang and P.F. Tu, *Chin. Tradit. Herb. Drugs*, **33**, 874 (2002).
[12] Y.I. Li, C.X. Ding, J.Q. Liu, and Z.X. Liao, *Chin. Tradit. Herb. Drugs*, **33**, 104 (2002).
[13] A. Bashir, M. Hamburger, and J. Msothi, *Phytochemistry* **31**, 309 (1992).
[14] Y. Ikeya, K. Sugama, M. Okada, and H. Mitsuhashi, *Phytochemistry* **30**, 2061 (1991a).
[15] T. Takao, F. Kitatani, N. Watanabe, A. Yagi, and K. Sakata, *Biosci. Biotech. Biochem.* **58**, 1780 (1994).
[16] S.R. Husain, J. Cillard, and P. Cillard, *Phytochemistry* **26**, 2489 (1984).
[17] Y.J. Suzuki, M. Tsuchiya, and L. Packer, *Free Radic. Res. Commun.* **15**, 255 (1991).