J. Braz. Chem. Soc., Vol. 13, No. 1, 66-70, 2002.
Printed in Brazil - ©2002 Sociedade Brasileira de Química
0103 - 5053 $6.00+0.00

Article

Biological Activity of Quinoline Alkaloids from *Raulinoa echinata* and X-ray Structure of Flindersiamine

Maique W. Biavatti [a#], ***Paulo C. Vieira*** [a*], ***M. Fátima das G. F. da Silva*** [a], ***João B. Fernandes*** [a], ***Sandra R. Victor*** [b], ***Fernando C. Pagnocca*** [b], ***Sérgio Albuquerque*** [c], ***Ignez Caracelli*** [a] ***and Julio Zukerman-Schpector*** [a,d*]

[a]*Departamento de Química, Universidade Federal de São Carlos, CP 676, 13565-905, São Carlos - SP, Brazil*

[b]*Centro de Estudos de Insetos Sociais, Universidade Estadual Paulista, Rio Claro - SP, Brazil*

[c]*Faculdade de Ciências Farmacêuticas, Universidade de São Paulo, Av. do Café s/n, 14040-903, Ribeirão Preto - SP, Brazil*

[d]*Instituto de Química, Universidade de São Paulo, CP 26077, 05513-970, São Paulo - SP, Brazil*

A investigação fitoquímica dos extratos de caules e folhas da espécie endêmica *Raulinoa echinata* Cowan, Rutaceae, levou ao isolamento de alcalóides furoquinolínicos (esquimianina, kokusaginina, maculina e flindersiamina) e quinolônicos (1-metil-2-*n*-nonil-4-quinolona, 2-*n*-nonil-4-quinolona e 2-fenil-1-metil-4-quinolona). Os alcalóides isolados mostraram atividade antifúngica contra *Leucoagaricus gongylophorus*, o fungo simbionte de formigas cortadeiras (*Atta* spp). Quando testados *in vitro* contra formas tripomastigotas de *Trypanosoma cruzi*, os alcalóides foram inativos ou parcialmente ativos. Neste trabalho descrevem-se o isolamento dos alcalóides, a elucidação estrutural dos alcalóides quinolônicos e a estrutura obtida por difração de raio-X da flindersiamina, juntamente com os resultados dos bioensaios realizados.

Phytochemical survey of stems and leaves extracts of the South Brazilian endemic plant *Raulinoa echinata* Cowan, Rutaceae, led to the isolation of known furoquinoline alkaloids: the widespread skimmianine; kokusaginine, maculine, flindersiamine, and also quinolone derivatives: 1-methyl-2-*n*-nonyl-4-quinolone, 2-*n*-nonyl-4-quinolone and 1-methyl-2-phenyl-4-quinolone. These alkaloids showed antifungal activity against *Leucoagaricus gongylophorus*; the symbiotic fungus of leaf-cutting ants (*Atta sexdens*). They were inactive or displayed weak inhibitory activity when assayed *in vitro* against trypomastigote forms of *Trypanosoma cruzi*. In this paper, the isolation, structure elucidation and bioactivity results of these compounds are reported together with the X-ray structure of flindersiamine.

Keywords: *Raulinoa echinata*, furoquinoline and quinolone alkaloids, antifungal, *Trypanosoma cruzi*, X-ray structure determination

Introduction

Raulinoa is a monospecific genus and the species *Raulinoa echinata* Cowan (Rutaceae) is endemic in the Itajaí Valley, SC, Brazil.[1] This perennial woody shrub is characterized by the presence of spines and has only been found in a short interval (1000 m) on the frequently inundated banks of Itajaí river in an approximate altitude of 100 m, showing a high degree of adaptation to the environment.

We have recently reported the isolation and biological activities of some compounds of *R. echinata*.[2] In continuation of our search for trypanocidal compounds and natural products to be used in the control of leaf-cutting ants, we have investigated the methanol extracts of stems and leaves of *R. echinata* leading to the isolation of typical rutaceous furoquinoline alkaloids: the widespread skimmianine (**1**), kokusaginine (**2**), maculine (**3**), flindersiamine (**4**) and also the quinolone derivatives: 2-*n*-nonyl-4-quinolone (**5**), 1-methyl-2-*n*-nonyl-4-quinolone (**6**) and 1-methyl-2-phenyl-quinolone(**7**).

Leaf-cutting ants are one of the most serious agricultural pests in South American countries and the biological control

* e-mail: paulo@dq.ufscar.br (chemistry); * e-mail: julio@dq.ufscar.br (X-ray crystallography); # Permanent address: Universidade do Vale do Itajaí, Curso de Farmácia, CP 360, 88303-202, Itajaí - SC, Brazil

of these insects has been the aim of many studies. Their control can be achieved by inhibiting the growth of their symbiotic fungus.

Trypanocidal compounds are important for the treatment of Chagas´ disease which is responsible for a considerable number of deaths in Central and South American countries.

In this paper we describe the isolation, structural determination and evaluation of the activity of furoquinoline alkaloids as inhibitors of the growth of the symbiotic fungus *Leucoagaricus gongylophorus* of leaf-cutting ants. The alkaloids were also assayed *in vitro* against trypomastigote forms of *Trypanosoma cruzi*.

Experimental

General experimental procedures

NMR spectra: a Bruker DRX-400 spectrometer, operating at 400.13 MHz for ^{1}H and 100.62 MHz for ^{13}C was used. All spectra were run in $CDCl_3$ using TMS as internal standard. For GC-MS analysis was used a Shimadzu GC-17 A gas chromatograph fitted with a fused silica DB-1 (30 m x 0.25 mm i.d. x 0.25 μm film thickness) capillary column, with helium as the carrier gas at a flow rate of 2.6 mL.min^{-1}. The temperature was programmed initially at 70 °C for 1 min, then increased with a rate of 10 °C.min^{-1} to 280 °C and kept for 15 min. The injection volume was 1 μL in a split mode and temperatures of the detector/injector were 300 °C/280 °C respectively. The chromatograph was coupled to a Shimadzu QP5000 mass selective detector at 70 eV with scans from 50 to 500 amu.

X-ray crystallographic data were recorded on an Enraf-Nonuis CAD-4 diffractometer using graphite monochromated MoKα radiation (λ = 0.71073 Å) and $\omega/2\theta$ scan mode at room temperature (291 K). During data collection, the intensity of three standard reflections were monitored every 30 min of X-ray exposure time showing no significant decay. Direct Methods solved the structure. H atoms were placed in calculated positions with fixed C-H distances (0.93 Å for Csp2 and 0.96 Å for Csp3), each riding on a carrier atom with isotropic displacement parameters amounting to 1.5 (for methyl H atoms) or 1.2 (for the other H atoms) times the value of the equivalent isotropic displacement parameter of the carrier atom. The CAD-4 Software[3] was used for data collection; cell refinement was done on 25 centred reflections ($10.23<\theta<18.11^{o}$),[3] data reduction: XCAD4,[4] program used to solve the structure: SIR92;[5] program used to refine structure: SHELXL97;[6] software used to prepare material for publication and deposition: SHELXL97,[6] PARST95[7] and WinGX.[8]

Plant material

Stems and leaves of *R. echinata* were collected in Ressacada, Ibirama, SC, Brazil and identified by A. Reis (Universidade Federal de Santa Catarina) and J. R. Pirani (Universidade de São Paulo). Voucher specimens [A. Reis & M. Biavatti 2.570 (26/07/98)] were deposited in the Herbário Barbosa Rodrigues (HBR), Itajaí, Santa Catarina, Brazil.

Extraction and isolation of compounds

The dried and powdered stems (5 kg) and leaves (3 kg) were extracted with hexane and MeOH. Successively, half part (100.00 g) of the MeOH stems extract was fractionated over silica gel 60 (70-230 mesh) into three fractions: CH_2Cl_2, EtOAc and MeOH. The methanol fraction (89 g) was dissolved in H_2O-MeOH (3:7) and successively partitioned using solvents of increasing polarity (hexane, CH_2Cl_2 and EtOAc), affording the correspondent sub fractions. The CH_2Cl_2 sub fraction (2 g) was chromatographed over silica gel (230-400 mesh) using as eluent CH_2Cl_2-EtOAc (9:1 and gradient) to furnish the compounds (in eluting order): maculine (**3**) (36 mg), flindersiamine (**4**) (32 mg), kokusaginine (**2**) (42 mg), skimmianine (**1**) (29 mg), and the alkaloid **7** (10 mg).

The MeOH leaves extract (100 g) was also submitted to liquid partition with hexane, CH_2Cl_2, EtOAc and MeOH. Column chromatography (silica gel 230-400 mesh, hexane-acetone 7:3) of the leaves hexane fraction (10 g) yielded compounds **5** (600 mg) and **6** (300 mg). All the furoquinoline alkaloids described above (except flindersiamine) were also found in the leaves extract in considerable amounts.

Identification of the isolated compounds

The isolated alkaloids skimmianine[9] (**1**), kokusaginine[10] (**2**), maculine[9] (**3**), and flindersiamine[11] (**4**) presented spectral data in agreement with the literature.

Flindersiamine (**4**) Yellow needles, mp 206-208°. Crystallographic data: publication no. CCDC 151162.

2-n-Nonyl-4-quinolone[12] (**5**) Amorphous solid, mp 74-76°, EIMS *m/z* (rel. int. %) [M$^+$] 271 (10), 256 (3), 228 (5), 214 (4), 186 (11), 172 (60), 159 (100), 130 (30). ^{1}H and ^{13}C NMR Table 2.

1-Methyl-2-n-nonyl-4-quinolone[13] (**6**) Amorphous solid, mp 71-73°, EIMS *m/z* (rel. int. %) [M$^+$] 285 (10), 270 (3), 242 (5), 214 (4), 186 (60), 172 (100), 144 (50). ^{1}H and ^{13}C NMR: Table 2.

1-Methyl-2-phenyl-4-quinolone[14] (**7**) Amorphous solid, mp 118-120°, EIMS *m/z* (rel. int. %) [M$^+$] 235 (80),

207 (100), 191 (5), 178 (8), 165 (15), 130 (10), 102 (50), 89 (30), 77 (55), 51 (50).^{1}H and ^{13}C NMR Table 2.

Fungicidal assay

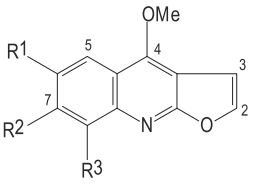
The fungus *Leucoagaricus gongylophorus* Singer (*Rozites gongylophora* Möller) was isolated from a *Atta sexdens* Forel nest, kept in culture media and the fungicidal activity was performed according to established protocols.[15]

Trypanocidal activity

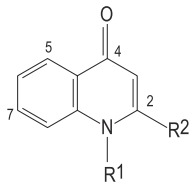
The *in vitro* trypomastigote forms of *T. cruzi* (Y strain) lethality assay was also performed according to established protocols.[16]

Results and Discussion

Compounds **1-4** were respectively identified as skimmianine, kokusaginine, maculine and flindersiamine, by comparison with the spectral data described in the literature.[9-11]



1 R^1=H, R^2=R^3=OMe
2 R^1=R^2=OMe, R^3=H
3 R^1=R^2=OCH_2O, R^3=H
4 R^1=R^2=OCH_2O, R^3=OMe



5 R^1=H, R^2=*n*-nonyl
6 R^1=Me, R^2=*n*-nonyl
7 R^1=Me, R^2=phenyl

Flindersiamine (**4**) has a methoxy group attached to C8, but spectroscopic data published could not furnish clear evidence of this moiety. In an attempt to confirm the extra methoxy group position on the furoquinoline nucleus, nOe experiments were performed. According to these experiments, by irradiation on the C4 methoxy group only the furan H3 signal was enhanced. Also, when the singlet corresponding to the quinoline hydrogen was irradiated, no enhancement was observed for the methoxy group as expected. These questionable irradiation results of **4** were verified by HMBC experiments. Unexpectedly, the C4 methoxy group showed correlation with the quinoline hydrogen. In order to solve these unclear findings, an X-ray analysis (Figure 1) was performed to confirm the C8 methoxy group position.

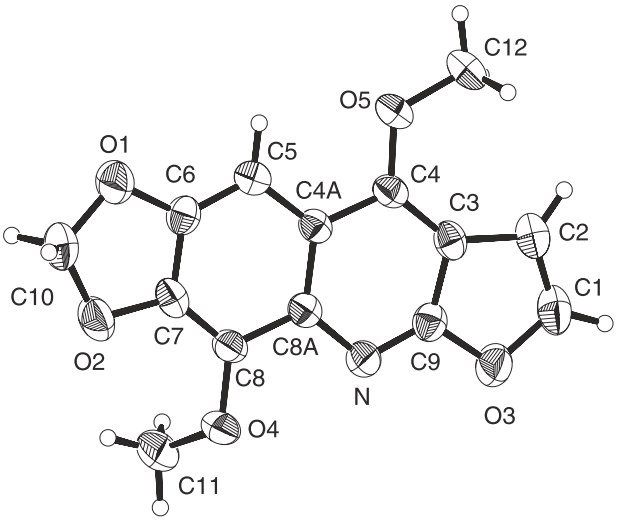


Figure 1. ZORTEP view of **4** showing crystallographic atom labelling. Ellipsoids of non-H atoms are drawn at 50% probability.

The molecule is planar except for C11, in fact the rms deviation for 16 non-H atoms fitted to the best least squares plane through them is of 0.026 and C11 being 1.069(3) Å out of that plane. A net of hydrogen bonds as shown in Table 3 joins the molecules in the crystal.

Special attention should be given to a C-H...π interaction and following Zukerman-Schpector *et al.*[17] this

Table 1. Evaluation of the growth inhibitory activity of crude extracts, fractions and compounds of *Raulinoa echinata*

Extract/fraction/ compound	% of growth inhibition of *L. gongylophorus*				
µg mL-1	50	100	250	500	1000
MSE[a]	-	-	-	-	80
Skimmianine(**1**)	60	80	NT[b]	NT	NT
Kokusagine (**2**)	20	100	NT	NT	NT
Maculine (**3**)	10	50	NT	NT	NT
Flindersiamine (**4**)	-	50	NT	NT	NT
MLE[c]	-	-	-	-	80
MLE (hexane fraction)				80	100
2-*n*-Nonyl-4-quinolone (**5**)	20	50	NT	NT	NT
1-Methyl-2-*n*-nonyl-4-quinolone (**6**)	-	-	-	-	-
1-methyl-2-phenyl-4-quinolone (**7**)	NT	NT	NT	NT	NT

[a]MSE: methanol stems extract, [b]NT: not tested, [c]MLE: methanol leaves extract

Table 2. ^{1}H and ^{13}C NMR chemical shift data for compounds **5**, **6** and **7**.

Position	δ_H			δ_C		
	5	**6**	**7**	**5**	**6**	**7**
2				155.1	154.7	154.8
3	6.23 s	6.22 s	6.31 s	108.2	109.8	112.7
4				178.9	177.8	177.6
5	8.36 dd (8.0; 1.2)	8.41 dd (8.0; 1.5)	8.51 dd (8.0; 1.4)	125.3	126.3	126.8
6	7.32 dt (8.0; 0.9)	7.35 dt (7.5; 1.0)	7.45 br t (1.0)	123.5	124.8	123.8
7	7.58 dt (8.0; 1.5)	7.63 dt (7.7; 1.6)	7.73 dt (7.8; 1.6)	131.7	133.2	132.3
8	7.72 d (8.0)	7.48 d (8.6)	7.57 d (8.5)	118.4	115.8	115.9
9				140.6	141.4	141.9
10				125.0	126.3	126.8
1’	2.68 t (7.8)	2.69 t (7.9)		34.4	31.8	135.9
2’	1.71 pent (7.8)	1.65 pent (7.3)	7.42 m	29.4	29.3	128.5
3’	1.17 br s	1.24 br s	7.52 m	31.8	29.2	128.8
4’	1.17 br s	1.24 br s	7.52 m	29.2	29.2	129.6
5’	1.17 br s	1.24 br s	7.52 m	29.2	29.2	128.8
6’	1.17 br s	1.24 br s	7.42 m	29.2	29.2	128.5
7’	1.17 br s	1.24 br s		29.2	28.6	
8’	1.17 br s	1.24 br s		22.6	22.6	
9’	0.83 t (7.1)	0.84 t (7.0)		14.1	14.1	
N-H	11.88 br s					
N-Me		3.71 s	3.62 s		35.2	37.3

interaction is characterized by three parameters, the H11C...Cg^{i} (Cg is the centroid of the O3-C1-C2-C3-C9 ring) distance of 3.39 Å, the C11-H11C...Cg^{i} angle of 128^{o} and the angle between the H...Cg^{i} vector and the plane of the ring of 92^{o}. (symmetry operation: i = 1-x, -0.5+y, 1.5-z).

Whether all the cited interactions are true hydrogen bonds is difficult to assert, because as pointed out by Cotton *et al.*,[18] “..the field is getting muddier and muddier as the definition of a hydrogen bond is relaxed.” In any case they are worth to be taken into account.

The crystallographic data obtained for the alkaloid **4** confirmed the previous HMBC spectroscopic attributions, but not the results obtained by nOe experiments.

The structures of compounds **5**, **6** and **7** were elucidated on the basis of ^{1}H NMR and EIMS spectra and carbon attributions were made by HMBC and HSQC correlation spectra. Data are presented on Table 2.

Crude extracts of leaves and stems as well as fractions of *R. echinata* showed good inhibitory activity on *L. gongylophorus* growth. Among the isolated compounds of these fractions, each of the alkaloids **1**-**5** maintained or showed higher inhibition of the fungal growth at 100 mg mL^{-1} (Table 1), compared with the original extracts. Contrary to the expectations, at a 50 μg mL^{-1} dose the inhibitory effect was quite strongly reduced. In general, furoquinoline alkaloids showed higher activity than quinolones. Probably, the activity demonstrated by the methanol extract of leaves (fraction hexane) is due to the high concentration of total alkaloids, both furoquinoline and quinolone derivatives.

Only the quinolinone alkaloids **5** and **6** displayed some moderate activity on trypomastigote forms (Y strain) of *T. cruzi* with IC_{50} of 134.9 and 100.9 μg mL^{-1} respectively.

Table 3. Hydrogen bonds parameters of crystalline compound **4**. Symmetry operations: i) 2-x, 0.5+y, 1.5-z; ii) x, -0.5-y, 0.5+z; iii) x, y, z; iv) x, -0.5-y -0.5+z; v) x, 0.5-y, 0.5+z.

D-H...A	H...A/Å	D...A/Å	D-H...A/^{o}
C1-H1...N^{i}	2.96	3.429(3)	147
C10-H10...N^{ii}	2.49	3.421(3)	160
C11-H11A...O2^{iii}	2.90	3.53(3)	124
C11-H11B...O1^{iv}	2.60	3.201(3)	121
C12-H12B...O3^{v}	2.89	3.680(3)	141

Fungicidal activities have been described for these alkaloids against plant pathogens *Cladosporium cucumerinum*[19] and *Phytophthora* spp.[20] 4-Quinolones containing long alkyl chains at C-2 were first obtained from microorganisms (from *Pseudomonas*, also called pseudans[21]) and have been also isolated from rutaceous genera, mainly *Evodia*,[22] *Boronia*,[23] *Ruta*[13] and *Esenbeckia*.[24] Species from the former genus are used in Chinese herbal medicine and the isolated alkaloids have demonstrated inhibitory activity against the bacteria *Helicobacter pilori*.[25,26]

Furoquinoline alkaloids have also demonstrated *in vitro* activity against *Leishmania* spp and *Plasmodium falciparum*.[27,28]

Acknowledgements

We wish to thank Prof. A. Reis for collecting and identifying and Prof. J. R. Pirani for identifying the plant

material, CAPES/PIDCT and UNIVALI for Ph.D. sponsorship of MWB and FAPESP, CNPq/PRONEX, MCT/FINEP for financial support. The X-ray facilities at Instituto de Química, USP was installed with a grant from FAPESP (94/2061-4).

Supplementary Information - Supplementary Crystallographic data have been deposited with the Cambridge Crystallographic Data Centre as supplementary publication no. CCDC 151162. Copies of available material can be obtained, free of charge on application to the Director, CCDC, 12 Union Road, Cambridge CH2 1EZ, UK (fax: +44-1223-336-033 or e-mail: deposit@ccdc.cam.ac.uk or http://www.ccdc.cam.ac.uk)

References

1. Kaastra, R.C.; *Flora Neotropica*, monograph no 33, 1982.
2. Biavatti, M.W.; Vieira, P.C.; Silva, M.F.G.F.da; Fernandes, J.B.; Albuquerque, S.; Magalhães, C.M.I.; Pagnocca, F.C.; *Phytomedicine* **2001**, *8*, 121.
3. CAD-4 Software; Version 5.0 Enraf-Nonius, Delft; The Netherlands, 1989.
4. Harms, K.; Wocadlo, S.; XCAD4; University of Marburg; Germany, 1995.
5. Altomare, A.; Cascarano, G.; Giacovazzo, C.; Guagliardi, A.; SIR92; *J. Appl. Cryst*. **1993**, *26*, 343.
6. Sheldrick, G.M.; SHELXL97; University of Göttingen; Germany, 1997.
7. Nardelli, M.; PARST95; *J. Appl. Cryst*. **1995**, *28*, 659.
8. Farrugia, L.J.; WinGX; University of Glasgow; Scotland, 1998.
9. Robertson, A.V.; *Aust. J. Chem*. **1963**, *16*, 451.
10. Khalid, S.A.; Waterman, P.G.; *Phytochemistry* **1981**, *20*, 2761.
11. Ayafor, J.F.; Sondengam, B.L.; Kimbu, S.F.; Tsamo, E.; Connolly, J.D.; *Phytochemistry* **1982**, *21*, 2602.
12. Kan-Fan, C.; Das, B. C.; Boiteau, P.; Potier, P.; *Phytochemistry* **1970**, *9*, 1283.
13. Grundon, M.F.; Okely, H.M.; *Phytochemistry* **1979**, *18*, 1768.
14. Akhmedzhanova, V.I.; Bessonova, I.A.; Yunusov, S.Y.; *Chem. Nat. Compd.* **1986**, *22*, 78.
15. Pagnocca, F.C.; Silva, O.A.; Hebling-Beraldo, M.J.; Bueno, O.C.; Fernandes, J.B.; Vieira, P.C.; *Bull. Entomol. Res.* **1990**, *80*, 349.
16. Alves, T.M.D.; Alves, R.D.; Romanha, A.J.; dos Santos, M.H.; Nagem, T.J.; Zani, C.L.; *J. Nat. Prod.* 1**999**, *62*, 369.
17. Zukerman-Schpector, J.; Camillo, R.L.; Comasseto, J.V.; Santos R.A.; Caracelli, I.; *Acta Crystallogr*. **1999**, *C55*, 1577.
18. Cotton, F.A.; Daniels, L.M.; Jordan, G.T.; Murillo, C.A.; *J. Chem. Soc. Chem. Commun*. **1997**, *17*, 1673.
19. Zhao, W.; Wolfender, J.L.; Hostettmann, K.; Xu, R.; Qin, G.; *Phytochemistry* **1998**, *47*, 7.
20. Moon, S.S.; Kang, P.M.; Park, K.S.; Kim, C.H.; *Phytochemistry* **1996**, *42*, 365.
21. Reisch, J.; Rózsa, Z.; Szendrei, K.; Körösi, J.; *Phytochemistry* **1975**, *14*, 840.
22. Tang, Y.Q.; Feng, X.Z.; Huang, L.; *Phytochemistry* **1996**, *43*, 719.
23. Sarker, S.D.; Armstrong, J.A.; Waterman, P.G.; *Phytochemistry* **1995**, *39*, 801.
24. Guilhon, G.M.S.P.; Baetas, A.C.S.; Maia, J.G.S.; Conserva, L.M.; *Phytochemistry* **1994**, *37*, 1193.
25. Rho, T.C.; Bae, E.A.; Kim, D.H.; Oh, W.K.; Kim, B.Y.; Ahn, J.S.; Lee, H.S.; *Biol. Pharm. Bull.* **1999**, *22*, 1141.
26. Hamasaki N.; Ishii, E.; Tominaga, K.; Tezuka, Y.; Nagaoka, T.; Kadota, S.; Kuroki, T.; Yano, I.; *Microbiol. Immun.* **2000**, *44*, 9.
27. Fournet, A.; Barrios, A.A.; Munoz, V.; Hocquemiller, R.; Cave, A.; Bruneton, J.; *Antimicrob. Agents Chemother*. **1993**, *37*, 859.
28. Basco, L.K.; Mitaku, S.; Skaltsounis, A.L.; Ravelomanantsoa, N., Tillequin, F., Koch, M., Le Bras, J.; *Antimicrob. Agents Chemother*. **1994**, *38*, 1169.

Received: November 23, 2000
Published on the web: November 8, 2001

FAPESP helped in meeting the publication costs of this article.