British Journal of Pharmaceutical Research
15(4): 1-6, 2017; Article no.BJPR.32881
ISSN: 2231-2919, NLM ID: 101631759

SCIENCEDOMAIN *international*
www.sciencedomain.org

Anti-*Mycobacterium* Metabolite from Marine Sponge *Iricinia fusca*

Srinu Meesala[1*], Ruby Singh[1] and Milind G. Watve[1]

[1]Department of Biology, Indian Institute of Science Education and Research, Dr. Homi Bhabha Road, Pune-411008, India.

Authors' contributions

This work was carried out in collaboration between all authors. Author SM designed the study, performed all experiments, wrote the protocol and wrote the first draft of the manuscript. Author RS did the bioactivity studies and author MGW corrected the manuscript and literature searches. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/BJPR/2017/32881
Editor(s):
(1) Syed A. A. Rizvi, Department of Pharmaceutical Sciences, College of Pharmacy, Nova Southeastern University, USA.
Reviewers:
(1) P. Shamsher Kumar, GITAM institute of science, GITAM University, India.
(2) Fu Xianjun, Shandong University of Traditional Chinese Medicine, China.
(3) V. Vasanthabharathi, Annamlai University, India.
Complete Peer review History: http://www.sciencedomain.org/review-history/18534

Short Communication

Received 21st March 2017
Accepted 2nd April 2017
Published 6th April 2017

ABSTRACT

Chemical investigation of the marine sponge *Ircinia fusca* leads to isolation of a new pyrrole derivative (**1**) and two known compounds namely, 3 methyl decanoic acid (**2**) and Benzenedicarboxylic acid (**3**). The structures of compounds were established by 1D and 2D NMR and MS data. Compound **1** exhibited strong antibacterial activity against *Mycobacterium smegmatis* at MIC 116 µM. Compounds 1-3 showed neither antifungal nor cytotoxic activity.

Keywords: Sponge; NMR and mass spectrometry; natural products; antimicrobial and anticancer activity.

1. INTRODUCTION

Marine invertebrates are a rich source of new metabolites as reported in marine libraries [1,2]. Till date majority of these compounds have been identified from marine invertebrates sources predominantly sponges [3]. Marine sponges are widely distributed from intertidal zones to

**Corresponding author: E-mail: drsrinumeesala@gmail.com, srinu.meesala@iiserpune.ac.in;*

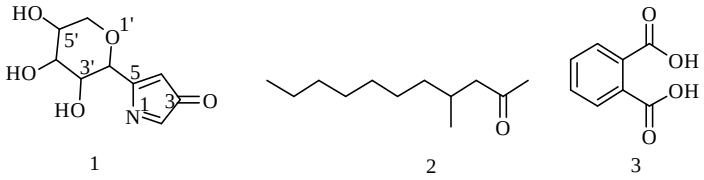

Fig. 1. Chemical structures of isolated compounds 1-3 from *Ircinia fusca*

thousands of meters deep in the ocean [4]. The demosponge, *Ircinia fusca* (Carter 1880) is a commonly found on the intertidal rocky shores of the Arabian sea, India. Sponges of the genus *Iricina* have been proven to be a rich source of diverse secondary metabolites like Cheilanthane sesterterpenoids [5], Irciniastatins [6], quinones [7] and Ircinialactams [8]. Some of the metabolites from *Iricinia sp* exhibited antifouling, anti-inflammatory and antimicrobial activities [9]. In addition to this, a number of cytotoxic compounds have also been reported from *Iricinia* genus [10,11,12].

During the search for bioactive compounds from marine sponges, we have collected *Iricnia fusca* from the Arabian Sea, West coast of Maharashtra, India. The organic DCM extract of the sponge exhibited broad-spectrum activity against bacteria and fungi in the preliminary studies. Repeated chromatography over silica gel (gradient elution, hexane-EtOAc), followed by Sephadex LH_{20} leads to the known compounds 2 & 3. On further purification by using C18 semi-preparative reversed phase HPLC in Fig. S1, a single new compound (**1**) was isolated as shown in above Fig. 1.

2. MATERIALS AND METHODS

2.1 General Experimental Details

Optical rotations were determined on a Rudolph Research Analytical (AUTOPOL V) polarimeter at a wavelength of 589 nm (sodium D line) using a 1.0-decimeter cell with a total volume of 1.0 mL. The UV spectra were measured on an Agilent technologies carry series UV-VIS spectrophotometer and Infrared spectra on Bruker ALPHA. All solvents were of analytical grade. Column chromatography was performed on Merck silica gel (120-200 mesh) and Sephadex LH-20 (Sigma-Aldrich Chemie GmbH). Thin layer chromatography was carried out with silica gel GF254 plates, Merck, USA. The ^{1}H and ^{13}C, DEPT-135, COSY, TOCSY, HSQC, HMBC, ROESY and 400 MHz (or 100 MHz for ^{13}C) at Bruker 400 MHz (Internal standard: TMS). The chemical shifts (δ) are reported in ppm and coupling constants (J) in Hz. The positive ion HR-ESI-MS spectra recorded on a Mass Q-TOF-LC-MS spectrometer (Bruker Daltonics).

2.2 Collection of Sponge

The sponge *Ircinia fusca* (Carter, 1880) was collected from Vayngani (N 16^{0}55.827, E 73^{0}16.973), West coast of Maharashtra, INDIA in Feb 2016, pre-monsoon season at low tide period by using sample grabbers. The sponge was identified by Dr. Satish S.Mokashe, Associate Professor, Department of Zoology, Dr. Babasaheb Ambedkar Marathwada University, India.

2.3 Extraction and Isolation

In the laboratory, the sponge was washed with distilled water to remove surface salts, sand, and epiphytes. The sponge was dabbed with tissue paper to remove excess water, cut into small pieces and placed in a lyophilizer to completely dry. The dried material was (2 gm dried weight) reduced to small pieces and extracted with MeOH (0.86 g). Desalting of sponge methanolic extract with acetone. The methanolic extract was concentrated under vacuum using a rotavapor at 40℃ followed by partition between hexane, DCM, water. All the partition layers were subjected to preliminary bioactivity studies (antibacterial & antifungal) by disc diffusion method [13].

2.4 Antimicrobial Activity

The isolated compounds 1–3 were tested against antibacterial i.e., *Escherichia coli* (NCIM 2065), *Salmonella typhimurium (*NCIM 2501), *Bacillus subtilis* (NCIM 2063), *Staphylococcus aureus* (NCIM 2079), *Mycobacterium smegmatis* (NCIM 5138) and antifungal strains *Aspergillus niger* (NCIM 1207), *Penicillium chrysogenum* (NCIM 1315), *Alternaria sp* (NCIM 900), and *Fusarium*

sp (NCIM 1372). The crude extracts were dissolved in DMSO at a concentration of 1 mg/mL, the zone of inhibition was showed in Fig. 2. The discs were loaded with different concentrations (10- 500 µg/disk) of the pure compound, to find out the inhibitory potential as showed in Table 1. The diameters of the inhibition zones generated around the discs were measured (Ø in mm). The diameters of the halos of inhibition can be rationalized on a qualitative basis as follows: Ø < 7 mm: inactive, 7 mm ≤ Ø < 8 mm: slightly active, 8 mm ≤ Ø < 9 mm: significantly active, Ø ≥ 9 mm: very active. The compound which showed ≥ 9 mm was selected for MIC studies.

Table 1. Antimicrobial activity of pure compounds

Microorganisms	Zone of inhibition in mm (Pure compounds)		
	1	2	3
E. coli	6	-	-
S. typhimurium	3	-	-
B. subtilis	6	-	-
S. aureus	-	9	9
M. smegmatis	10	-	-
A. niger	-	-	-
P. chrysogenum	-	-	-
Alternaria sp	-	-	-
Fusarium sp	-	-	-

2.5 Minimum Inhibitory Concentration

The bacteria was streaked on nutrient agar and incubated at 37℃ for 24 h. One single colony was then transferred to fresh nutrient broth (15 mL) and the cell density was adjusted to 10^4 -10^5 CFU/mL by adjusting the OD600 nm to 0.1. The compounds to be tested were dissolved in DMSO and diluted with H_2O to 460 µM stock solutions (10% DMSO). The stock solutions were then serially diluted with 10% DMSO to final concentrations of 400 µM to 7.25 µM in 1% DMSO. An aliquot (20 µL) of each dilution was transferred to a 96-well microtiter plate and freshly prepared bacteria containing broth (180 µL) was added to each well. The plates were incubated at 37℃ for 24 h and the optical density of each well was measured spectrophotometrically at 600 nm (POLAR star Omega plate; BMG LABTECH, Offenburg, Germany). The IC_{50} value was calculated as the concentration of the compound or antibiotic required for 50% inhibition of the bacterial cells using Prism 5.0 from GraphPad Software Inc. (La Jolla, CA) [14].

2.6 Cytotoxic Assay

Cytotoxicity assays were performed on HeLa, SiHa (cervical cancer cells) and MDA-MB-231 (Breast cancer cell line) lines as described [15].

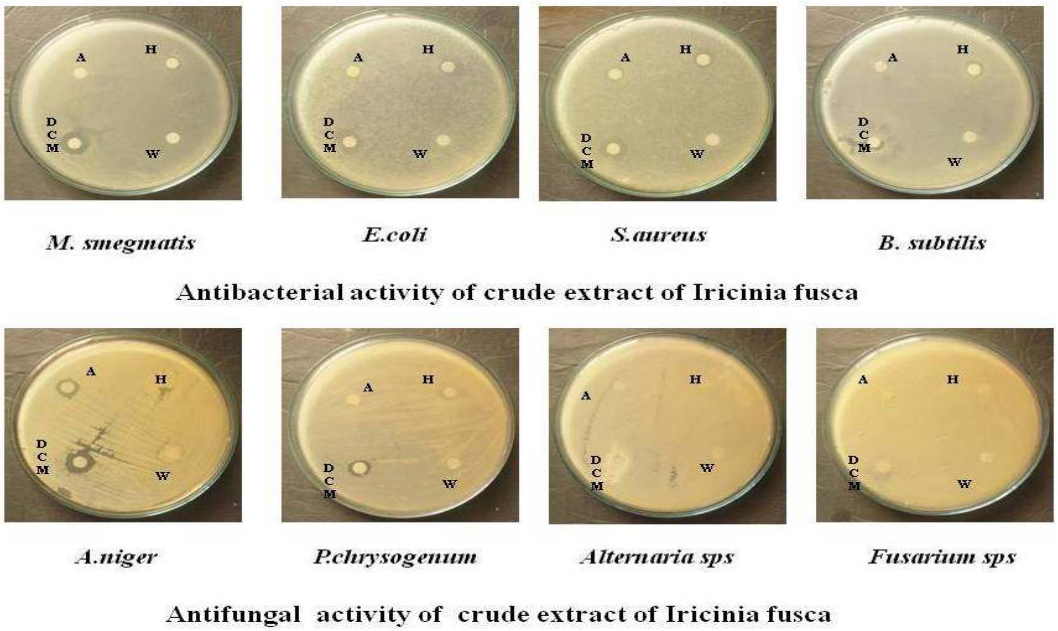

Fig. 2. Antimicrobial activity of crude extracts (DCM: Dichloromethane, A: Acetone, H: Hexane, W: Water)

An aliquot of 100 µL of each sub-confluent cell lines (cell density: $1x10^5$ cells ml^{-1}) was seeded in 96-well flat bottom microtiter plate. The plates were incubated at 37℃ in an atmosphere of 5% CO_2 and 95% relative humidity within a CO_2 incubator. After 24 h of incubation, the cells were treated with serial dilutions of compounds. The cells were washed with phosphate-buffered saline (PBS) followed by the addition of 10 µl of MTT solution (5 mg ml^{-1}, Sigma Chemicals, USA) in each well. After 4 h of incubation at 37℃, the formazan product was solubilized by the addition of 200 µl of acidified isopropanol and absorbance was measured on an SPECTRAmax PLUS 384 plate reader (Molecular Devices Inc, USA), at 570 nm. Percentage of cell viability was calculated with respect to untreated cells considered as 100% grown. All experiments were performed in triplicates, and the quantitative measurement was expressed as the average ± standard deviation.

3. RESULTS AND DISCUSSION

Compound 1, M.P. = 101–115℃, $[\alpha]_{25}^{D}$ = 0.0 (c 1.0, CH_3OH) was obtained as a white colorless substance. The strong IR absorptions band at 3348 (OH), 1454 & 1399 (CH_2), 1119 & 1025 (C-O), 974 (C=C) cm^{-1}. The ESI-MS-QTOF exhibited characteristic molecular ion peak at m/z 236.94 $[M+Na]^+$ (Fig. S2) corresponding to the molecular formula $C_9H_{11}NO_5Na$, indicating five degrees of unsaturation.

The ^{1}H NMR spectrum of 1 (Table 2) showed the presence of two olefinic methine protons at δ_H 8.54 (1H, s, H-2) and 8.33 (1H, s, H-4); four oxy methines at δ_H 5.92 (1H, d, *J* = 5.8 Hz, H-2'), 4.55 (1H, m, H-3'), 4.15 (1H, dd, *J* = 5.8, 3.5 Hz, H-4'), 3.97 (1H, dd, *J* = 7.1, 3.5 Hz, H-5') and oxygenated mehtylene group at 3.65& 3.70 (2H, m, H-6'). The ^{13}C NMR spectrum of 1 exhibited a total of 9 carbon signals which were categorized, based on DEPTs and HSQC spectroscopic data (Table 2), as a carbonyl signal at δ_C 153.6 (C-3), quaternary carbon at δ_C 118.6 (C-5), methine carbons at δ_C 141.1 (C-2), 148.7 (C-4); oxy methines at δ_C 87.9 (C-2'), 73.9 (C-3'), 70.4 (C-4'), 85.9 (C-5'); a mehtylene at δ_C 61.3 (C-6').

The ^{1}H NMR of 1 showed two singlet's δ_H 8.54 (H-2) & 8.33 (H-4); HMBC correlations of H-2 with C-5 and C-4 and from H-4 to C-3 revealed the presence of pyrrole-3-one (Fig. 3). The correlations of H-2'/H-3', H-3'/H-4', H-4'/H-5', H-5'/H-6' observed in the COSY spectrum, as well as HMBC correlations (Fig. 3), ^{1}H NMR chemical shifts revealed the presence of tetrahydropyran ring. Further HMBC correlation of H-2' to C-2, C-4 confirmed pyrrole ring was fused with tetrahydropyran ring, The NOE correlations of H-3'/ H-2' to H-2 supports above confirmation in Fig. 3. Based on these spectral data, the structure of compound **1** was established. Thus, the planar structure of **1** was elucidated as 5-(3,4,5-trihydroxytetrahydro-2H-pyran-2-yl)-3H-pyrrol-3-one named as Iricinopyrrole 'A'.

The isolated compounds 2 & 3 showed activity against only *S. aureus*, but the compound **1** was found to have selective activity against *M. smegmatis*, with MIC value of 116µM as shown in Fig. 4, and bactericidal activity was observed. No antifungal activity was recorded for all the compounds. Compounds 1-3 were also tested for their cytotoxicity, none of them exhibited significant inhibitory activity at the concentration up to 500 µg/mL.

Table 2. NMR data of compounds 1 (400 MHz, DMSO-d_6)

Position	δ_H/ppm, multi(J/Hz)	δ_C/ppm
1	-	-
2	8.54, s	141.0, CH
3	-	153.2, CO
4	8.33, s	148.7, CH
5	-	118.6, C
1'	-	-
2'	5.92, d, *J* = 5.8 Hz	87.9, CH
3'	4.55, m	73.9, CH
4'	4.15, dd, *J* = 5.8, 3.5 Hz	70.4, CH
5'	3.97, dd, *J* =7.1, 3.5 Hz	85.9, CH
6'	3.57, 3.68, m	61.3, CH_2

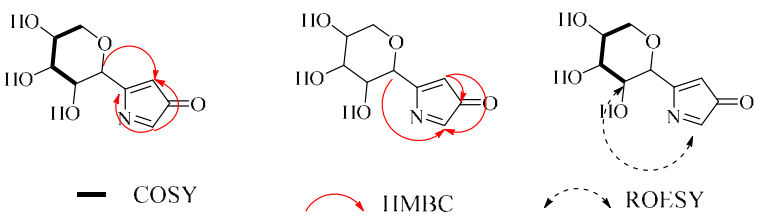

Fig. 3. Key COSY, HMBC, ROESY correlations of compound 1

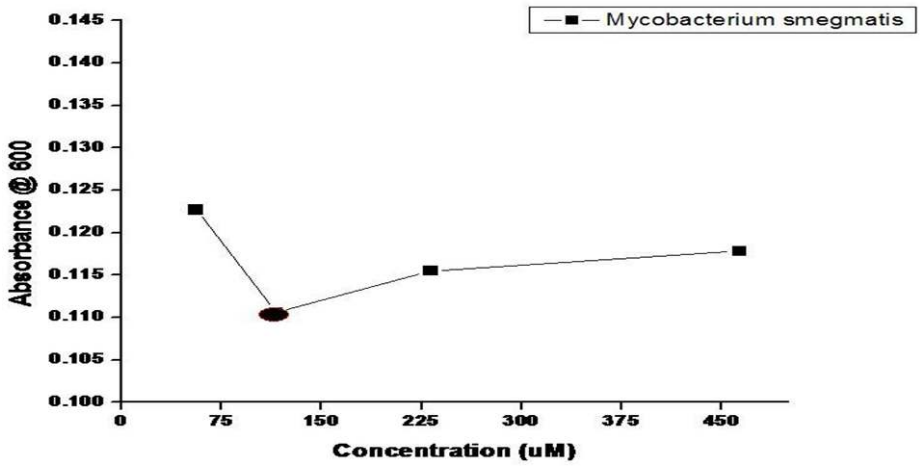

Fig. 4. MIC determination of compound 1 against *Mycobacterium smegmatis*

Mycobacterium sp affect millions annually, over the past few years, the incidence of tuberculosis has increased rapidly. According to the WHO (World Health Organization), it is estimated that one-third of the world's population is infected with *Mycobacterium* [16]. Therefore, new lead compounds, which are effective against *Mycobacterium sp* is need of the hour.

A few compounds from *Trichoderma* sp [17], *Neamphius* sp [18] have shown anti-mycobacterial properties.

In this paper, we report the isolation and characterization of one new pyrrole derivative from the marine sponge *Iricinia fusca*. This species also exhibits anti-mycobacterial activity. This study shows that *Iricinia fusca* proved to be a rich source of anti-mycobacterial compounds.

4. CONCLUSION

Compound 1 has been reported as a new pyrrole derivative from *I.fusca* and its structure was elucidated by NMR and mass spectroscopic analysis. The compound **1** exhibited selective toxicity against *M. smegmatis* at MIC 116µM and also bacteriocidic property was observed. Further studies are underway to isolate other metabolites from this sponge species. Also, efforts have been directed towards the synthesis of 1 to develop a definite SAR for this group of molecules.

Compound 1: ^{1}H NMR (400 MHz, DMSO) δ 8.54 (s, 1H), 8.33 (s, 1H), 5.92 (d, *J* = 5.8 Hz, 1H), 4.58 – 4.53 (m, 1H), 4.15 (dd, *J* = 5.8, 3.5 Hz, 1H), 3.97 (dd, *J* = 7.1, 3.5 Hz, 1H), 3.70 – 3.65 (m, 2H).

Compound 2: The ESI-MS-QTOF exhibited a pseudo molecular ion peak at m/z 187.295 $[M+H]^+$ corresponding to the molecular formula of $C_{11}H_{22}O_2$. Pub Chem CID: 143696. This compound is known, and commercially available with Sigma with CAS Number 60308-82-9.

Compound 3: The ESI-MS-QTOF exhibited a pseudo molecular ion peak at m/z 167.132 $[M]^+$ corresponding to the molecular formula of $C_8H_6O_4$. Pub Chem CID: 1017. This compound is known, and commercially available with Sigma with CAS Number 88-99-3.

SUPPLEMENTARY INFORMATION

Supplementary data associated with this article can be found in the online version.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

ACKNOWLEDGEMENTS

This work was financially supported (RGSTC/File-2007/DPP-054/CR-28) by the Rajiv Gandhi Science and Technology Commission, Maharashtra, India. We are thankful to NMR Research Centre at IISC, Bangalore for providing NMR facility and IISER Pune for Mass facility. My special thanks to NMR technicians

Chinmay Lowalekar and Deepali Jadhav, Sandeep C. Kanade for NMR and mass services.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Jha RK, Zi-Rong X. Biomedical compounds from marine organisms. Mar. Drugs. 2004; 2:123–146.
2. Blunt JW, Copp BR, Hu WP, Munro MHG, Northcote PT, Prinsep MR. Marine natural products. Nat. Prod. Rep. 2008;25:35–94.
3. Lie J, Zhou J. A marine natural product database. J. Chem. Inform. Model. 2002; 42:742–748.
4. Chairman K, Ranjit Singh AJA, Ramesh M. Screening twelve species of sponges for biomedical activity in gulf of Mannar Tuticorin Coast. Int. J. of Marine Science. 2012;2:43-50.
5. Malcolm SB, Annette E, Gordon K, Jacqueline W, Ronald JQ. Cheilanthane sesterterpenes, protein kinase inhibitors, from a marine sponge of the genus *Ircinia*. J. Nat. Prod. 2001;64:300-303.
6. George RP, Jun-Ping X, Jean-Charles C, Robin KP, Larry PT, Dennis LD, John NAH, Jean MS. Antineoplastic agents. 520. Isolation and structure of Irciniastatins A and B from the indo-pacific marine sponge *Ircinia ramosa*. J. Med. Chem. 2004;47:1149–1152.
7. Maria T, Claire H, Constantinos V, Catherine H, and Vassilios R. Chemical defense and antifouling activity of three Mediterranean sponges of the genus *Ircinia*. Z. Naturforsch. 2002;57c:161D171.
8. Walter B, Robiul I, Frank F, Andrew MP, Hua Z, Timothy IW, Daniel FG, Joseph WL, Robert JC. Ircinialactams: Subunit-selective glycine receptor modulators from Australian sponges of the family Irciniidae. Bioorg. Med. Chem. 2010;18:2912–2919.
9. Sharad S, Satish M, Gupta SG, Vijay L, Dinesh K. Antimicrobial potential of the marine intertidal sponge, *Ircinia fusca* from the west coast of India. Indian J. Pharm. Biol. Res. 2015;3:12-14.
10. Rashid MA, Gustafson KR, Boyd MR. New chondropsin macrolide lactams from marine sponges in the genus *Ircinia*. Tetrahedron Lett. 2001;42:1623-1627.
11. Yan S, Zhang G, Su J, Zeng L. A rare long conjugated diterpene ketene from the marine sponge *Ircinia selaginea* (Lamark). Gaodeng Xuexiao Huaxue Xuebao. 2001. 22:949-951.
12. Kokubo S, Yogi K, Udin M J, Inuzuka T, Suenaga K, Veda K, Uemura D. Kohamaic acids A and B novel cytotoxic sesterterpenic acids, from the marine sponge *Ircinia* sp. Chem. Lett. 2001;2: 176-177.
13. Lippert H, Brinkmeye R, Mulhaupt T, Iken K. Antimicrobial activity in sub-Arctic marine invertebrates. Polar Biol. 2003;26: 591-600.
14. Andrews JM. Determination of minimum inhibitory concentrations. J. Antimicrobial. Chemotherapy. 2001;48:5-16.
15. Visconti A, Minervini F, Lucivero G, Gambatesa V. Cytotoxic and immunotoxic effects of *Fusarium mycotoxins* using a rapid colorimetric bioassay. Mycopathologia. 1991;113:181-186.
16. Boucher HW, Talbot GH, Bradley JS, Edwards JE, Gilbert D, Rice LB, Scheld M, Spellberg B, Bartlett J. Bad bugs, no drugs: no ESKAPE! An update from the Infectious Diseases Society of America. J. Clin. Infect. Dis. 2009;48:1-12.
17. Pruksakorn P, Arai M, Kotoku N, Vilchèze C, Baughn AD, Moodley P, Jacobs WR Jr., Kobayashi M. Trichoderins, novel amino lipopeptides from a marine sponge-derived *Trichoderma* sp., are active against dormant mycobacteria. Bioorg. Med. Chem. Lett. 2010;20:3658-3663.
18. Yamano Y, Arai M, Kobayashi M. Neamphamide B, new cyclic depsipeptide, as an anti-dormant mycobacterial substance from a Japanese marine sponge of *Neamphius* sp. Bioorg. Med. Chem. Lett. 2012;22:4877-4881.

Peer-review history:
The peer review history for this paper can be accessed here:
http://sciencedomain.org/review-history/18534