



AkiNik

Journal of Pharmacognosy and Phytochemistry

Available online at www.phytojournal.com

J
P
P

Journal
of
Pharmacognosy
and
Phytochemistry

ISSN 2278-4136

ISSN 2349-8234

JPP 2014; 2 (5): 95-97

© 2014 AkiNik Publications

Received: 09-12-2013

Accepted: 25-12-2013

Anil Kumar Sharma

Natural Products Laboratory,
Department of Chemistry, University
of Rajasthan, Jaipur-302004
(Rajasthan), India
Email: anilkumarsharma_008@yahoo.com

Kuldeep Kumar Sharma

Natural Products Laboratory,
Department of Chemistry, University
of Rajasthan, Jaipur-302004
(Rajasthan), India
Email: bhardwaja.kuldeep@gmail.com

Mahesh Chandra Sharma

Natural Products Laboratory,
Department of Chemistry, University
of Rajasthan, Jaipur-302004
(Rajasthan), India
Email: drmaheshin@uniraj.ernet.in

Mahaveer Prasad Dobhal

Natural Products Laboratory,
Department of Chemistry, University
of Rajasthan, Jaipur-302004
(Rajasthan), India
Email: mpdobhal@yahoo.com

Correspondence**Anil Kumar Sharma**

Natural Products Laboratory,
Department of Chemistry,
University of Rajasthan,
Jaipur-302004 (Rajasthan), India.
Email: anilkumarsharma_008@yahoo.com
Tel: +91-9571274892

Two Dibenzofuran identified as Heterocyclic Natural Compounds from Lichen *Parmelia perlata*

Anil Kumar Sharma, Kuldeep Kumar Sharma, Mahesh Chandra Sharma, Mahaveer Prasad Dobhal

Abstract

Two dibenzofuran, 2-acetyl-9b-carbomethoxy-7,9-dihydroxy-8-methyl-1,3(2H,9bH)-dibenzofurandione (1) and 2,6-diacetyl-7,9-dihydroxy-8,9b-dimethyl-1,3(2H,9bH)-dibenzofurandione (2) also known as (+)-Usnic acid, were identified as heterocyclic natural compounds from lichen *Parmelia perlata*. Their structures were elucidated on the basis of different spectroscopic techniques.

Keywords: *Parmelia perlata*, Lichen, Phytochemicals, Biological Activities.

1. Introduction

A considerable number of species of lichen forming fungi have wide geographical distribution and are used in the traditional system of medicine. Lichens are symbiotic combination of algae and fungi. They are well known to produce a variety of compounds with remarkable biological activities^[1-2]. The beautiful hills of Uttarakhand (In India) are the best source of lichens due to varied temperature difference in day and night. They have used in diarrhoea, dyspepsia, spermatorrhoea, amenorrhoea, dysentery and as wound healer^[3]. Lichens are used as traditional food by Rai and Limbu communities of east Nepal^[4]. Some of the species of the lichens are being used by cosmetic industries as skin lightening agent^[5]. *Parmelia perlata* (lichen) belongs to family Parmeliaceae and commonly known as Charila in India. *Parmelia perlata* is usually used as a spice to enhance the taste and flavor of the foods. It is also useful to treat sores, boils, inflammations, seminal weakness and amenorrhoea^[6] and it contains compounds like tridecyl myristate, 3-ketooleanane, icosan-1-ol, usnic acid^[7], parmolanostene permelabdone^[8], atranorin, lecanoric acid, orcin, erythrolein, azolitmin and spaniolitmin^[9]. *Parmelia perlata* has been used as light brown dye for wool as well as bio-indicator of air pollution of heavy metals such as zinc, lead, cadmium copper and mercury^[10]. *Parmelia perlata* contain acidic substance that has been used as an antibiotic in several countries as a topical antibacterial agent for human skin diseases^[10].

2. Materials and Methods**2.1 General Experimental Procedure**

Melting points were determined in soft glass capillaries in an electrothermal melting point apparatus. Qualitative TLC was conducted on aluminium sheet Kieselgel 60 F254 (E. Merck). Silica gel (E. Merck, 60-120 mesh, 550 gm) used for column (1.5 m × 4.0 cm) chromatography. The IR spectra were recorded on FTIR SHIMADZU 8400S spectrometer with KBr pellets. The ¹H and ¹³C NMR spectra were recorded in CDCl₃ at 300 MHz and 75 MHz on a Bruker NMR instrument, respectively, using TMS as internal standard. FAB mass spectra were recorded on JEOL SX 102 /DA-6000 mass spectrometer using Argon/Xenon as FAB gas.

2.2 Plant material

The plant material, *Parmelia perlata* was collected from the hills of Uttarakhand (India) and the authenticity was confirmed by Incharge of Herbarium, Department of Botany, University of Rajasthan, Jaipur (India).

A voucher specimen of the lichen was deposited at the Herbarium of the University (RUBL-3674).

2.3 Extraction and Isolation of the Constituents

The shade dried plant material (1.5 kg) was fine powdered and extracted with petroleum ether for 72 hrs on water bath. The extract was filtered hot and solvent was removed under reduced pressure where a semi-solid yellowish mass (7.0 gm) was obtained. The solvent free extract was chromatographed over silica gel column. For this purpose a column of 1.5 m in height with 4.0 cm in diameter was used and it was charged with 550 gm silica gel (60-120 mesh). The column was eluted with different solvents in order of increasing polarity where following two heterocyclic natural compounds were isolated, purified and characterized.

(a.) 2-acetyl-9b-carbomethoxy-7, 9-dihydroxy-8-methyl-1,3(2H,9bH)-dibenzofurandione(1)

Compound 1 was obtained when column was eluted with petroleum ether. After removal of solvent, a yellow solid mass was obtained which on crystallization with ethyl acetate yielded shining yellow crystals. It showed single spot on TLC examination ($R_f = 0.35$) in benzene as a mobile phase. The melting point of this compound was found to be 131 °C. IR (KBr, cm^{-1}), 3410 (O-H stretching), 1780 ($>\text{C}=\text{O}$, str.), 1625 ($>\text{C}=\text{C}<$ str.), 1055 (C-O str.) etc. ^1H NMR (δ ppm, CDCl_3), 2.50 (s, 3H, C-2, $-\text{COCH}_3$), 5.13 (s, 1H, C-4, -H), 6.20 (s, 1H, C-6, -H), 13.32 (s, 1H, C-7, -OH), 2.11 (s, 3H, C-8, $-\text{CH}_3$), 12.04 (s, 1H, C-9, -OH), 4.01 (s, 3H, C-9b, $-\text{COOCH}_3$), 18.84 (s, 1H, C-3, -OH). ^{13}C NMR (δ ppm, CDCl_3), 197.24 (C-1), 110.53 (C-2), 200.38 ($-\text{COCH}_3$ at C-2), 32.14 ($-\text{COCH}_3$ at C-2), 165.11 (C-3), 101.58 (C-4), 179.11 (C-4a), 140.16 (C-5a), 99.13 (C-6), 163.16 (C-7), 108.49 (C-8), 7.69 ($-\text{CH}_3$ at C-8), 158.02 (C-9), 105.14 (C-9a), 59.87 (C-9b), 172.62 ($-\text{COOCH}_3$ at C-9b), 51.84 ($-\text{COOCH}_3$ at C-9b). MS (m/z), 346 [M^+], 330, 260, 233, 165 etc. Molecular formula calculated as $\text{C}_{17}\text{H}_{14}\text{O}_8$.

(b.) 2,6-diacetyl-7,9-dihydroxy-8,9b-dimethyl-1,3(2H,9bH)-dibenzofurandione(2) also known as (+) Usnic acid

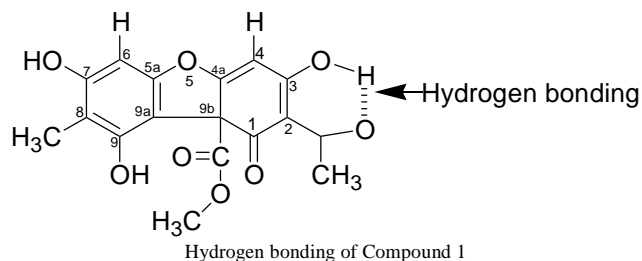
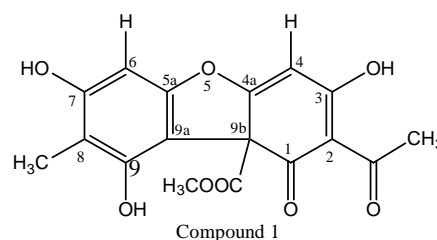
Compound 2 was isolated as yellow solid when column was eluting with petroleum ether and benzene (1:3). This mass was redissolved in acetone and acetone soluble part was purified and analysed. Its R_f value was 0.80 in chloroform and methanol (95% + 5%) system. The m.p. of this crystalline yellow compound was 204 °C. IR (KBr, cm^{-1}) 3150 (O-H stretching), 1800 (C=O, str.), 1550 (C=C, str.) etc. ^1H NMR (δ ppm, CDCl_3) 2.67 (s, 3H, $-\text{COCH}_3$ at C-2), 5.97 (s, 1H, C-4, -H), 2.66 (s, 3H, $-\text{COCH}_3$ at C-6), 13.30 (s, 1H, C-7, -OH), 2.11 (s, 3H, C-8, $-\text{CH}_3$), 11.01 (s, 1H, C-9, -OH), 1.76 (s, 3H, C-9b, $-\text{CH}_3$), 18.84 (s, 1H, C-3, -OH, enolic form) ^{13}C NMR (δ ppm, CDCl_3) 198.02 (C-1), 109.24 (C-2), 200.38 ($-\text{COCH}_3$ at C-2), 32.08 ($-\text{COCH}_3$ at C-2), 163.90 (C-3), 101.58 (C-4), 179.32 (C-4a), 155.31 (C-5a), 98.37 (C-6), 200.50 ($-\text{COCH}_3$ at C-6), 31.22 ($-\text{COCH}_3$ at C-6), 163.56 (C-7), 105.33 (C-8), 7.50 ($-\text{CH}_3$ at C-8), 157.38 (C-9), 104.03 (C-9a), 59.13 (C-9b), 27.83 ($-\text{CH}_3$ at C-9b). MS (m/z), 345 [M^+H], 344 [M^+], 261, 260, 234, 233, 232, 217, 215 etc. Molecular formula calculated as $\text{C}_{18}\text{H}_{16}\text{O}_7$.

3. Results and Discussion

(a.) 2-acetyl-9b-carbomethoxy-7,9-dihydroxy-8-methyl-1,3(2H,9bH)-dibenzofurandione(1)

In the mass spectrum of compound 1 prominent signal was observed at m/z 347 [$\text{M}+\text{H}$] and 346 [M^+]. Other prominent ions

were observed at m/z : 330, 260, 233, 165 etc. The ^1H NMR & ^{13}C NMR spectrum indicated that it contain fourteen protons and seventeen carbon atoms in the skeleton. On the basis of these observations the molecular formula of compound 1 was calculated as $\text{C}_{17}\text{H}_{14}\text{O}_8$. The IR spectrum (KBr, cm^{-1}) strong absorption at 3410 (br) indicated the presence of hydroxyl group. The absorption at 1625 confirmed the presence of olefinic ($>\text{C}=\text{C}<$, stretching) group whereas the absorption at 1055 was assigned for C-O stretching. In the ^1H NMR spectrum (δ ppm, CDCl_3) the presence of two hydroxy groups were observed at 12.04 (s, 1H, C-9) and 13.32 (s, 1H, C-7), both the hydroxy signals are comparable with usnic acid molecule [7]. The presence of two protons attached at C-4 and C-6 positions were observed as two singlets at 5.13 and 6.20 respectively. A singlet at 2.11 was assigned for methyl group at C-8 position and a sharp singlet for three protons was observed at 2.50 (s, 3H), which indicated the presence of one acetyl group and its possibility of attachment at C-2 position. The spectrum, showed a sharp singlet for three protons was observed at 4.01 which indicated the presence of carbomethoxy group at C-9b position which was further confirmed by its ^{13}C NMR spectrum by observing at 172.62 ($-\text{COOCH}_3$) and 51.84 ($-\text{COOCH}_3$) carbons. Due to intramolecular hydrogen bonding a sharp singlet was observed at 18.84 (s, 1H, C-3) for OH. Absorptions at 158.02, 163.16 and 165.11 in ^{13}C NMR spectrum (δ ppm, CDCl_3) also confirmed the presence of three phenolic groups and the attachment of these three hydroxyl groups were assigned at C-9, C-7 and C-3 positions respectively. The carbon atoms at C-4 and C-6 positions were confirmed by the absorption at 101.58 and 99.13 respectively. The absorption appearing at 197.24 indicated the presence of carbonyl group at position C-1. A signal at 172.62 indicated the presence of carbomethoxy group ($-\text{COOCH}_3$) and attachment of this group was established at C-9b position.



The absorption for methoxy group ($-\text{COOCH}_3$) was observed at 51.84. Other signals in ^{13}C NMR were obtained at 110.53 (C-2), 200.38 (C-2, $-\text{COCH}_3$), 32.14 (C-2, $-\text{COCH}_3$), 179.11 (C-4a), 140.16 (C-5a), 108.49 (C-8), 7.69 (C-8, $-\text{CH}_3$), 105.14 (C-9a) and 59.87 (C-9b) and their assignment have shown in parenthesis. On the basis of above observation and discussion compound 1 was identified as 6-deacetyl-9b-carbomethoxy-9b-demethylusnicacid. (IUPACName: 2-acetyl-9b-carbomethoxy-7,9-dihydroxy-8-methyl-1,3(2H,9bH)-dibenzofurandione). Isolation and characterization of compound 1 is being reported for the first time.

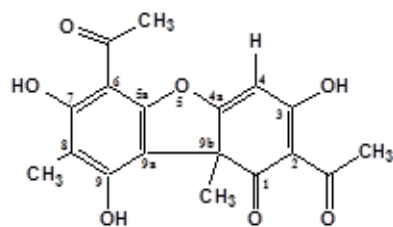
(b.) 2,6-diacetyl-7,9-dihydroxy-8,9b-dimethyl-1,3(2H,9bH)-dibenzofurandione(2) also known as (+)-Usnic acid

The mass spectrum of this compound showed a molecular ion peak at m/z 344 $[M^+]$. Other prominent ions were observed at m/z : 345 $[M^++H]$, 261, 260, 234, 233, 232, 217, 215 etc. The spectral analysis and molecular weight determination established its molecular formula as $C_{18}H_{16}O_7$. In the IR spectrum (KBr, cm^{-1}) the presence of phenolic (OH) group was observed at 3150 and the absorptions at 1800 confirmed the presence of carbonyl group whereas the absorption at 1550 was assigned for $>C=C<$ stretching.

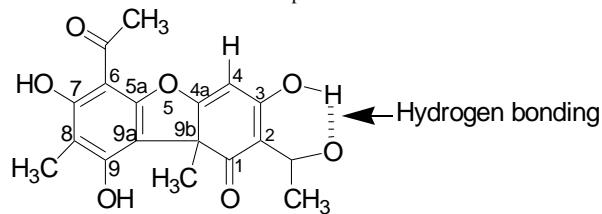
The 1H NMR spectrum (δ ppm, $CDCl_3$) showed two sharp singlets at 1.76 (s , 3H) and 2.11 (s , 3H) for two methyl groups and their positions were assigned as C-9b and C-8 respectively as reported [11]. The absorption at 2.66 (s , 3H) and 2.67 (s , 3H) as two sharp singlets confirmed the presence of two acetyl groups at C-6 and C-2 positions respectively. Two sharp singlets were observed at 11.01 (s , 1H) and 13.30 (s , 1H) for phenolic groups at position C-9 and C-7 correspondingly. Vinylic proton presents at C-4 position showed the absorption at 5.97 as a singlet for one proton.

In solution form this compound exists in two tautomeric forms i.e. ketonic and enolic. The enolic proton showed intense 1H NMR absorption at 18.84. The absorptions at 163.90, 163.56 and 157.38 in ^{13}C NMR spectrum (δ ppm, $CDCl_3$) also confirmed the presence of three phenolic groups linked at C-3, C-7 and C-9 positions respectively. Vinylic carbon atom was confirmed by the absorption at 101.58 which was assigned to C-4 carbon atom. The absorption appearing at 198.02 clearly indicated the presence of carbonyl group at C-1 position. The values of other carbon atoms in compound 2 were established as 109.24 (C-2), 200.38 ($-COCH_3$ at C-1), 32.08 ($-COCH_3$ at C-1), 179.32 (C-4a), 155.31 (C-5a), 98.37 (C-6), 200.50 ($-COCH_3$ at C-6), 31.22 ($-COCH_3$ at C-6), 105.33 (C-8), 7.50 ($-CH_3$ at C-8), 104.03 (C-9a), 59.13 (C-9b), and 27.83 ($-CH_3$ at C-9b).

On the basis of above spectral analysis compound 2 was identified as (+)-Usnic acid [11-18].



Compound 2



Hydrogen Bonding of Compound 2

4. Acknowledgement

The authors wish to thank Head, Department of Chemistry, University of Rajasthan for providing lab facilities.

5. References

- Huneck S. The significance of lichens and their metabolites. *Naturwissenschaften* 1999; 86:559-570.
- Takenata Y, Tanahashi T, Nagakura N, Itoh A, Hamada N. Three isocoumarins and a benzofuran from the cultured lichen mycobionts of *Pyrenula* sp. *Phytochemistry* 2004; 65:3119-3123.
- Nadkarni AK. *Indian Materia Medica*. Popular Prakashan, Mumbai, 1996, 922.
- Bhattarai T, Subba D, Subba R. Nutritional value of some edible lichens of east Nepal. *J Appl Bot* 1999; 73:11-14.
- H. Masako, K. Yasuhiro, M. Yasutaka and Y. Yoshikazu. Melamin inhibitor from lichens for manufacturing skin-lightening cosmetics. Japan Patent JP 04282319; 1991.
- Caccamese S, Toscano RM, Bellesia F, Pinetti A. Methyl β -Orcinolcarboxylate and Depsides from *Parmelia furfuracea*. *J Nat Prod* 1985; 48:157-158.
- Sharma AK, Sharma MC, Dobhal MP. Phytochemical investigation of therapeutic important lichen *Parmelia perlata*. *J Nat Prod Plant Resour* 2012; 2(1):101-106.
- Abdullah ST, Hamid H, Ali M, Ansari SH, Alam MS. Two new Terpenes from the lichen *Parmelia perlata*. *Indian J of Chemistry* 2007; 46B:173-176.
- Thippeswamy B, Sushma NR, Naveenkumar KJ. Antimicrobial property of bioactive factor isolated from *Parmelia perlata*. *Internati Multidiscipli Resear J* 2012; 2(2):01-05.
- Momoh MA, Adikwu MU. Evaluation of the effect of colloidal silver on the antibacterial activity of ethanolic extract of the lichen *Parmelia perlata*. *Afri J Pharm and Pharmacol* 2008; 2(6):106-109.
- Wat CK, Towers GHN. Isolation of vulpinic, pinastric and (+)-usnic acids from *Cetraria canadensis*. *Phytochemistry* 1972; 11:3540-3540.
- Takahashi K, Takani M, Wada Y. Usnic Acid XVI. Alkaline Degradation of Dihydrousnic Acid. *Chem Pharm Bull* 1980; 28(5):1590-1596.
- Francolini I, Norris P, Piozzi A, Donelli G, Stoodley P. Usnic Acid, a Natural Antimicrobial Agent Able To Inhibit Bacterial Biofilm Formation on Polymer Surfaces. *Antimicro. Agent And Chemoth* 2004; 48:4360-4365.
- Sassa T, Igarashi M. Structures of (-)-mycoucousnine, (+)-isomycousnine and (+)-oxymycousnine, new usnic acid derivatives from phytopathogenic mycosphaerella nawae. *Agric Biol Chem* 1990; 54:2231-2237.
- Takani M, Takahashi K. Usnic acid XVIII: the photolyses of usnic acid and its derivatives. *Chem Pharm Bull* 1985; 33:2772-2777.
- Yilmaz M, Aysen OT, Tay T, Kivanc M. The Antimicrobial Activity of Extracts of the Lichen *Cladoniafoliacea* and Its Usnic Acid, Atranorin, and Fumarprotocetraric Acid Constituents. *Z Naturforsch* 2004; 59C:249-254.
- Kutney JP, Sanchez H, Yee T. Studies in usnic acid series .4. base-catalyzed usnic acid isousnic acid rearrangement .2. improved synthesis of (+)-isousnic acid. *Can J Chem* 1977; 55:1073-1078.
- Kutney JP, Sanchez H. Studies in usnic acid series .5. base-catalyzed usnic acid isousnic acid rearrangement .3. (-)-usnic acid isomethoxide monoacetate. *Can J Chem* 1977; 55: 1079-1084.