J.Natn.Sci.Foundation Sri Lanka 2014 42 (2): 193-196
DOI: http://dx.doi.org/10.4038/jnsfsr.v42i2.6988

SHORT COMMUNICATION

Enzyme inhibitory and immunomodulatory activities of the depsidone lobaric acid extracted from the lichen *Heterodermia* sp.

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Revised: 07 October 2013; Accepted: 11 December 2013

Abstract: This study evaluates the enzyme inhibitory and immunomodulatory activities of the lichen specific depsidone, lobaric acid. Lobaric acid was extracted with methanol from *Heterodermia* sp. found in Labukelle, Sri Lanka with a yield of 0.67 %. It was subjected to enzyme inhibition assays using acetyl and butyryl-cholinesterase, phosphodiesterase, β-glucuronidase, α-glucosidase and urease. In the β - glucuronidase inhibitory activity it showed an IC_{50} value of 3.28 ± 0.05 µM, which was significantly lower than that of the standard, D-saccharic acid 1, 4-lactone (IC_{50} = 48.4 ± 1.2 µM). Lobaric acid showed a significant inhibition of phosphodiesterase enzyme with an IC_{50} = 313.7 ± 2.2, when compared with the two standards EDTA (IC_{50} = 274.0 ± 0.1 µM) and cysteine (IC_{50} = 748.0 ± 0.1 µM). Lobaric acid showed a moderate actyl and butyryl -cholinesterase inhibitory activity while it showed no activity against the enzymes α-glucosidase and urease.

In the immunomodulatory assay, lobaric acid exhibited a potent oxidative burst inhibitory activity in human polymorphonuclear (PMN) cells. It suppressed both the myloperozidase dependant and myloperozidase independent reactive oxygen species (ROS) production of PMNs. The results indicate the pharmacological potential of lobaric acid as a lead compound for further studies.

Keywords: β-glucuronidase, enzyme inhibitory activity, *Heterodermia* sp., immunomodulatory activity, lobaric acid, phosphodiesterase.

INTRODUCTION

Lichens are a rich source of bioactive secondary metabolites (Mitrovi'c *et al.,* 2011). Previous studies have reported a number of compounds including the Ambewela amide A, B, sekikaic acid and lecanoric acid from Sri Lankan lichens such as *Parmotrema* sp., *Pyxine consocians* and *Heterodermia leucomelos* with anticancer, antioxidant, α-PLK1 inhibitory and insect herbivoury activities (Karunaratne *et al*., 2002, 2005, 2008; Kathirgamanathar *et al*., 2006; Thadhani *et al*., 2011; Williams *et al*., 2011). Of the major structural classes of lichen metabolites, depsidones comprise a tricyclic ring system with two benzene rings connected through ether and ester moieties and are relatively rare. The depsidone lobaric acid is one of the most biologically potent secondary metabolites reported from lichens such as *Stereocaulon sasakii*, *Stereocaulon alpinum* Laur and *Sterocaulon azoreum* (Morita *et al*., 2009).

Various biological activities of lobaric acid have been reported earlier including antitumor, antiproliferative (Burlando *et al*., 2009), anti-inflammatory, antioxidant (Thadhani *et al*., 2011), antimicrobial (Thadhani *et al.*, 2012) and selective serine threonine protein kinase (PLK1) inhibitory activity. Lobaric acid inhibits the 5-lipoxygenase pathway by inhibiting the formation of cysteinyl - leukotrines as determined by enzyme immunoassay (Gissurarson *et al*., 1997). The antimitotic activity of lobaric acid has also been studied and it has been shown to inhibit the polymerization of tubulin (Morita *et al*., 2009). Through interaction with a dynamic surface of the CBP/p300 GACKIX domain, lobaric acid demonstrated an enormous potential for targeting difficult protein-protein interactions (Majmudar *et al*., 2012; Stojanovic *et al*., 2012). Furthermore, it has

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been reported that lobaric acid elicit contact allergy in sensitive persons (Thune & Solberg, 1980).

In this study, the pharmacological potential of lobaric acid, particularly its immunomodulatory and inhibitory activities against β - glucuronidase, acetyl - and butyryl-cholinesterases, phosphodiesterase, α-glucosidase, and urease enzymes are reported.

METHODS AND MATERIALS

Manually cleaned, air-dried and crushed lichen specimens of a *Heterodermia* sp. collected from Labukelle, Sri Lanka were sequentially extracted with CH_2Cl_2 followed by MeOH. Lobaric acid was isolated with a yield of 0.67 % from the MeOH extracts when fractionated *via* silica gel column using hexane/CH_2Cl_2 to CH_2Cl_2/MeOH as solvents. The dentification of the compound was carried out by using spectral data and its comparison with the reported data (Gonzilez *et al*., 1992).

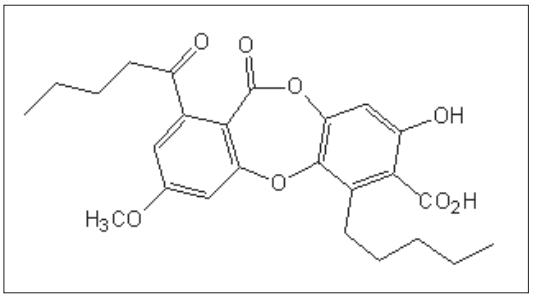

Figure 1: Structure of lobaric acid

To determine the inhibitory activity of lobaric acid on acetyl and butyryl-cholinesterases enzymes, electric-eel acetyl-cholinesterase (AChE, EC 3.1.1.7) and horse serum butyryl-cholinesterase (BChE, EC 3.1.1.8) were used. The inhibition was measured using the spectrophotometric method developed by Ellman *et al*. (1961).

β-Glucuronidase inhibitory activity was determined by measuring the absorbance of *p*-nitrophenol at 405 nm, formed from the substrate *p*-nitrophenyl-β-D-glucuronide on addition of β-glucuronidase in the presence of lobaric acid (Khan *et al*., 2002).

Phosphodiesterases enzyme inhibitory activity was measured by a spectrophotometric method using bis-(*p*-nitropheny1) phosphate as chromogenic substrate, which was added to a mixture containing a buffer, phosphodiesterase enzyme and the test compound lobaric acid. The release of *p*-nitrophenol was measured at 410 nm.

Lobaric acid was evaluated for its α-glucosidase inhibitory activity using the method of Oki *et al*. (2000) and against the urease enzyme using the indophenols method (Weatherburn, 1967).

Lobaric acid was screened over a range of concentrations (3.1 – 50 µg/mL) for its oxidative burst inhibitory potential. The measurement of chemiluminescence was employed to investigate the different kinds of reactive oxygen species (OH, O^{-2} and H_2O_2). Luminol-enhanced chemiluminescence assay was performed as described by Helfand *et al*. (1982). Briefly, whole blood neutrophils and polymorphonuclear leukocytes (PMNs) were suspended in Hank's balance salt solution (HBSS) with calcium and magnesium salts and incubated with lobaric acid for 30 min. To each well, serum opsonized zymosan (*Saccharomyces cerevisiae* origin) was added, followed by the addition of luminol (3-aminophthalhydrazide), and then HBSS to adjust the final volume to 0.2 mL. HBSS was used as a control. Chemiluminescence peaks were recorded with a luminometer.

RESULTS AND DISCUSSION

Cholinesterase inhibitors are used for the management of Alzheimer's disease. Lobaric acid showed moderate inhibition against acetyl-cholinesterase with an IC_{50} value of 26.86 ± 0.9 µM and butyryl-cholinesterase with an IC_{50} value of 36.76 ± 0.8 µM, when compared to the standard gallanthamine (AChE: 0.50 ± 0.01 µM and BChE: 8.50 ± 0.01 µM). It has been reported that acetylated derivatives of depsidones isolated from *Lobaria pulmonaria* (L.) Hoffm. (Lobariaceae) possess moderate acetyl-cholinesterase inhibitory activity (Mortia *et al*., 2009). This is the first report on AChE and BChE inhibitory activity of lobaric acid.

In certain diseases such as cancer, inflammatory joint disease, some hepatic diseases and AIDS, the activity of β-glucuronidase increases. An IC_{50} value of 3.28 ± 0.05 µM was observed, which is 12 fold more potent than the standard D-saccharic acid 1,4 - lactone (IC_{50} = 48.4 ± 1.25 µM).

Many β - glucuronidase inhibitors such as 8 - hydroxytricetin-7- glucuronide, isovitexin, trihydroxypipecolic acid and scoparic acid A and C, L - aspartic acid, tectorigenin and benzothiazoles have been isolated from different plants and some are used clinically (Khan *et al*., 2002). However, there are no reports of β - glucuronidase inhibitory activity of compounds isolated from lichens.

Table 1: Immunomodulatory activities of lobaric acid

Compound	Immunomodulatory activity IC_{50} ± SEM (µg)		
	Whole blood + luminol	PMN's + luminol	PMN's + lucigenin
Lobaric acid (1)	37.6 ± 0.9	< 3.1	< 3.1
Ibuprofen	11.8 ± 1.87	2.5 ± 0.6	-
Sodium diethyldithio carbamatetrihydrate	-	1.27 ± 0.23	8.16 ± 1.9

Phosphodiesterase is believed to be involved in a wide variety of processes, such as bone formation, insulin resistance and metastasis of cancer cells. The inhibitors of phosphodiesterase are used in the treatment of some forms of arthritis. Only a few inhibitors have been reported so far, majority of them being of synthetic origin (Ahmad *et al*., 2003) and none from lichen sources. Lobaric acid showed a significant inhibition of phosphodiesterase enzyme with an IC_{50} ± SEM (µM) of a 313.7 ± 2.2 when compared with the two standards, EDTA (IC_{50} = 274.0 ± 0.1 µM) and cysteine (IC_{50} =748.0 ± 0.1 µM).

Lobaric acid did not show α-glucosidase inhibitory activity or any significant inhibition against the urease enzyme.

Immunomodulators are substances capable of interacting with the immune system to up-regulate or down-regulate specific aspects of the host response. Due to the broad application of their action, immunomodulators are becoming very popular in the global natural product based health industry (Yeap *et al*., 2011). Various disease conditions such as infections, organ transplant rejection, cancer, rheumatoid arthritis, and systemic lupus erythematosus are currently treated with immunomodulating agents (Long *et al*., 2011). Different immunomodulatory agents have been screened from a variety of plants, including the lichen *Thamnolia vermicularis* var. *subuliformis* (Omarsdottir *et al*., 2007). Lobaric acid was screened over a range of concentrations (3.1–50 µg/mL) for its oxidative burst inhibitory potential. It suppressed both the myeloperoxidase dependant and myeloperoxidase independent ROS production with PMNs at the lowest concentration tested (3.1 µg/mL), when compared with standards ibuprofen and sodium diethyldithiocarbamatetrihydrate (Table 1).

CONCLUSION

Although acetyl and butyryl-cholinesterases inhibitory activities of acetylated and diacytylated depsidones have been reported (Pejin *et al*., 2012), this is the first report of these activities of lobaric acid. However, lobaric acid did not show any inhibitory potential against *α*-glucosidase or urease enzymes. In the *β*-glucuronidase enzyme inhibitory assay, lobaric acid has shown 12 times higher activity than the available standard D-saccharic acid 1,4-lactone further indicating the pharmacological potential of lobaric acid as a lead compound. Even though a few heteroglycans isolated from the lichen *Thamnolia vermicularis* var. *subuliformis* (Omarsdottir *et al*., 2007) have shown potential immunomodulatory activity, this is the first report of the promising immunomodulatory activity of a lichen depsidone. The results presented above make lobaric acid with highly diverse functional groups, such as three carbonyls in the form of keto, ester and acid, phenolic OH and an ether group, an excellent candidate for future studies with structural optimization.

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