International Journal of Biological Macromolecules 104 (2017) 261–273

Contents lists available at ScienceDirect

International Journal of Biological Macromolecules

journal homepage: www.elsevier.com/locate/ijbiomac

# Structural profiling of wax biopolymer from *Pinus roxburghii* Sarg. needles using spectroscopic methods

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ARTICLE INFO

*Article history:*
Received 29 January 2017
Received in revised form 7 May 2017
Accepted 3 June 2017

*Keywords:*
Wax
Biomacromolecule
*Pinus roxburghii*
ω-Hydroxy fatty acids
Polyesters

ABSTRACT

*Pinus roxburghii* Sarg. is the most abundant species in Himalayan region. The needles of the species largely contribute to the forest biomass and remain the major cause of forest fires leading to climate change, biodiversity loss, etc. Intriguingly, the layer of needles contains wax, a biomacromolecule with potential chemical functionalities for value addition. In the present study, a distinctive approach towards complete structural analysis of the isolated wax in its native state has been done using $^{1}H$, $^{13}C$, HSQC, HMBC, COSY, TOCSY along with GC–MS of the methyl esters of constituent fatty acids. The wax was isolated in a quantitative yield of 1.64% and analyses suggest that it is a polymer of linearly attached fatty acid esters which on hydrolysis yielded three types of ω-hydroxy fatty acids viz. 12-hydroxydodecanoic acid, 14-hydroxytetradecanoic acid and 16-hydroxyhexadecanoic acid in a ratio of 1:1:2 respectively. Complete assignments for a carbonyl group, α-, β- and other methylenes present in wax were achieved; corroborating the presence of polyester. In particular, identification of wax structure was accomplished through NMR; thereby providing a lead towards future structural analysis of waxes in their native form. The study would also be helpful to generate commercially important compounds derived from pine needle wax. This will offer an opportunity for utilisation of pine needle biomass: a root cause of Himalayan forest fires.



## 1. Introduction

The leaf surface of plants is virtually covered with a coating of different lipids. The upper layer commonly known as cuticle mainly consists of fatty acid cutin [1] providing a structural framework of a complex mixture of compounds known as waxes [2] for the surface protection of leaves. The composition of cuticular wax varies widely across the species [3] and it is also related to indigenity, plant age and environmental conditions. Earlier studies testify that the composition also differs between organs of the same species, between tissues of the same organ and sometimes between different sections of the cuticle layering the same tissue. Some studies also account for the presence of shorter chain of fatty acids or alcohol in the intracuticular wax and longer ones in the cuticular wax [4]. In most plant species, the major constituents of wax are long chain saturated aliphatic compounds with or without functional group. These include alkanes in the range of C-16 to C-33, primary or secondary alcohols, aldehydes, ketones, hydroxy-ketones, diols, wax esters, hydroxy fatty acids, triterpenoids, etc. [5–16].

In the present communication, structural profiling of the wax biopolymer from the leaves of *Pinus roxburghii* Sarg. has been investigated as a case study. *Pinus roxburghii* Sarg. commonly known as 'Chir Pine' contains needle-shaped leaves; generally referred as 'pine needles' and are one of the most abundantly available forest biomass in India. The huge availability of needles is due to the fact that the species is prevalent in Himalayan region extending from Pakistan to North East India, Bhutan, China (Tibet) and Nepal. In India, the area covered stretches between Assam, Himachal Pradesh, Jammu-Kashmir, Sikkim and Uttarakhand where it occupies an area of 677813 ha in Northern India alone. As per an estimate, more than one million tonnes of needles are available annually which are the major cause of forest fires [17]. Recurrence of forest fires due to needles leads to vast destruction of flora and fauna, thereby, impacting the ecosystem services with immense tangible and intangible losses. These services possess significant bearing with respect to mitigation as well as adaptation towards climate change, biodiversity conservation and sustainable development. Intriguingly, needles of pine are also an important resource possessing a unique type of wax with reasonable yield.

In a view to the literature available, it was found that studies on needle wax from Pinus species have attracted the attention

*Abbreviations:* COSY, proton correlation spectroscopy; HSQC, heteronuclear single quantum coherence; HMBC, heteronuclear multiple bond correlation; TOCSY, proton total correlation spectroscopy; eV electron Volt, EI electron ionization.

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http://dx.doi.org/10.1016/j.ijbiomac.2017.06.012
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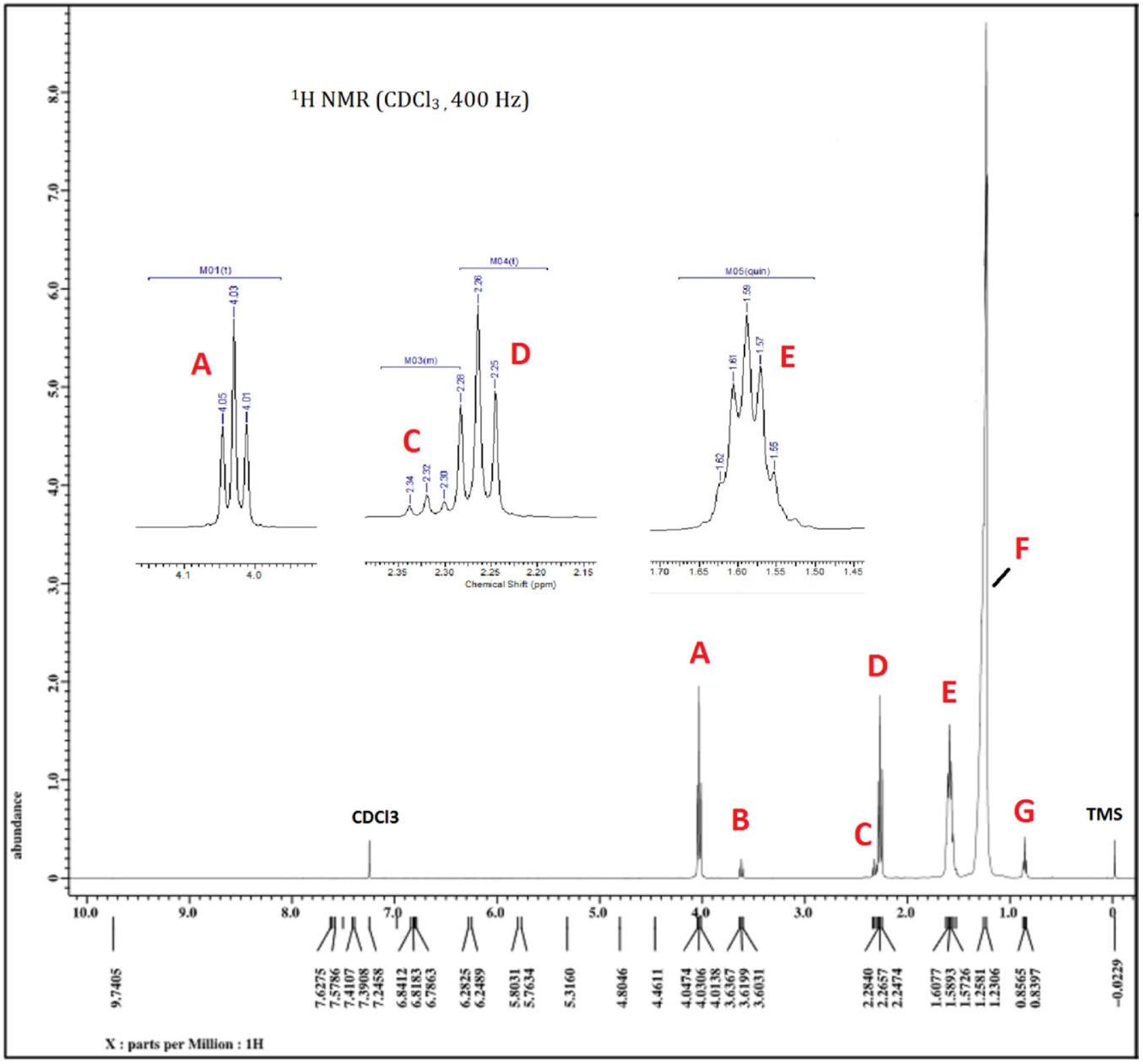


**Fig. 1.** [1]H NMR of the *Pinus roxburghii* needle wax with alphabetical denotations.

of chemists since a long period of time due to the vast abundance of needles and unique chemical constituents. These studies have been directed towards analysis of wax from pine species occurring in different parts of the world. The studies report phytochemicals like juniperic acid, and sabinic acid in *Pinus sylvestris*; sabinic acid, juniperic acid, 1,16-hexadecanediol, dodecane-1,12-diol and 1-triacontanol in *Pinus thunbergii*; alkyl esters (C24-C64), nonacosan-10-ol, heptacosane-5,10-diol, nonacosane-4,10-diol, nonacosane-5,10-diol, and nonacosane-10,13-diol, *n*-acids (C12-C32), diterpene acids and C29 diols in *Pinus radiata*; stearic acid and linolenic acids in *Pinus sylvestris*; *n*-alkanes, identified as C27, C23, C25 and C29 in *Pinus heldreichii* var. pancici; labdane acids and other components in *Pinus pumila* and ώ − *hydroxy* acids (C12, C14 and C16) in *Pinus roxburghii* Sarg. needles [5,17–27].

As evident from the above-mentioned reports, substantial work has been carried out on needle wax. However, the research work on plant wax analyses were mainly based on degradative and derivatization methods including Gas Chromatography–Mass Spectrometry. Though, GC–MS is a useful technique; but individually it is not sufficient to characterise the wax structure and thus necessitates use of other spectroscopic methods.

Surprisingly, no work has been carried out on characterisation of wax present in pine needles using NMR; which is nevertheless, an accurate and a non-degradative method for structural analysis. The targeted approach will most importantly contribute and support the existing knowledge of structural profiling of natural polymers in their native state. Further, chemical components like wax, exist in nature as complex mixtures and it is almost difficult to obtain them in their pure form.

It is worth mentioning here that, the only study on *P. roxburghii* needles wax also lacks the details on structural information like type of linkages, ratio of acids and the resultant wax structure, etc. Therefore, the present study fulfils the gap in structural profiling of *P. roxburghii* needles wax using spectroscopic methods viz. [1]H,