Journal of Molecular Catalysis B: Enzymatic 56 (2009) 46–54

Contents lists available at ScienceDirect

Journal of Molecular Catalysis B: Enzymatic

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

Regio-selective acylation of biologically important iridoid glycosides by *Candida antarctica* lipase

Parvinder Pal Singh, Naveed Ahmed Qazi, Syed Shafi, D. Mahendhar Reddy, Abid H. Banday, P. Bhaskar Reddy, K.A. Suri, B.D. Gupta, N.K. Satti, Basant P. Wakhloo, H.M. Sampath Kumar*, G.N. Qazi

Indian Institute of Integrative Medicine, Jammu-Tawi, 180 001, India

ARTICLE INFO

Article history:
Received 26 August 2007
Received in revised form 13 March 2008
Accepted 15 April 2008
Available online 25 April 2008

Keywords:
Trans-esterification
Lipase
Iridoid glycoside
p-Nitrophenyl alkanoate

ABSTRACT

Lipase catalyzed regio-selective acylation of five iridoid glycosides *viz*., picroside I&II, catalpol, agnuside and negundoside in the presence of various acyl donors such as vinyl acetate and *p*-nitrophenyl alkanoates was studied. The regio-selectivity of enzymatic acylation and yields were found to vary amongst different substrates. Monoacylated products were isolated with all the substrates under scrutiny indicating high regio-selective nature of such transformations. A series of acyl esters of picroside-I, picroside-II, catalpol, agnuside and negundoside have been synthesized by this enzymatic *trans*-esterification methodology.

1. Introduction

Iridoid glycosides [1] constitute an important class of compounds among natural products, employed for medicinal purpose from time immemorial to relieve various ailments and possess wide spectrum of biological activities *viz*. immunomodulatory [2], anti-asthamatic [3], hepatoprotective [4], choleretic [5], hypoglycemic and hypolipidemic [6], anti-inflammatory [7], antispasmodic [8], etc. Iridoid glycosides such as picrosides and catalpol were isolated from *Picrorhiza kurroa*, a high altitude Himalayan perennial herb. A crude preparation of *P. kurroa* [9], has been used in the Ayurvedic system of traditional medicine to treat disorders of the liver and upper respiratory tract, fevers, dyspepsia, chronic diarrhea, scorpion sting and various other immune related diseases. Similarly crude preparations of plant *Vitex negundo* that contains iridoid glycosides such as agnusides and negundoside have been used extensively in Chinese herbal medicine to cure ailments like chronic bronchitis, rheumatic difficulties, bacterial dysentery, cough, cold, burns and scalds and gonorrhea [10].

These iridoid glycosides are polyhydroxylated compounds. Acylation of these polyhydroxylated natural compounds not only increase the structural diversity, but also changes their physical and chemical properties, which may result in improved pharmacological and pharmacokinetic properties. While exploring the novel plant-based immunoadjuvants [11a,b], it was found that many glycoconjugates such as picroside-I, picroside-II, catalpol, agnuside and negundoside possess promising dose dependant immune potentiation ability as indicated by B and T cell proliferation. This prompted us to explore the possibility to develop these molecules as alternate plant-based immune adjuvants for vaccines. However, in spite of all their immunological traits [11c] our detailed evaluation revealed several drawbacks in these molecules *viz*., lack of immune memory and depot formation. Even though several iridoid glycosides in their native form are often acylated at specific hydroxyl groups of their sugar or aglycon moiety, we envisaged the need for improved structures derived from these iridoid glycosides with proper hydrophilic–lipophilic balance (HLB) that can be readily fine tuned through regio-selective acylation with acyl groups of varying chain length and topology. However, regio-selective acylation of iridoid glycosides using chemical acylation methods is cumbersome due to non-availability of suitable reagents and protocols to discriminate among several primary and secondary specific hydroxyl groups of the same molecule. Thus use of such conventional protocols invariably leads to a mixture of all possible mono-, di- and poly-acylated products rendering the purification process more difficult. Moreover, iridoid glycosides are susceptible to decomposition under acidic and thermal conditions, which makes them unsuitable for conventional acid mediated

* Corresponding author. Fax: +91 191 2569333.
E-mail address: hmskumar@yahoo.com (H.M.S. Kumar).

1381-1177/$ – see front matter © 2008 Elsevier B.V. All rights reserved.
doi:10.1016/j.molcatb.2008.04.005

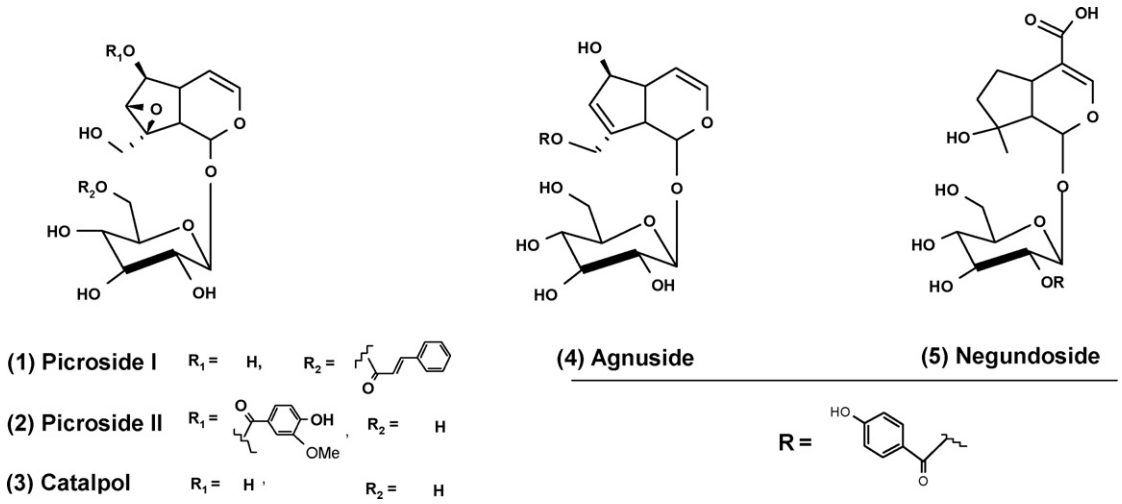


Fig. 1. Iridoid glycosides from *Picrorhiza kurroa* and *Vitex negundo*.

diol protection–deprotection strategies. Thus, the regio-selective acylation of such acid sensitive polyhydroxylated compounds is a challenging problem that limits the choice of reagents needed to accomplish a selective acylation. The use of lipases as biocatalysts for regio-selective acylation of iridoid glycosides would be an attractive alternate, which results in many valuable products such as amphiphiles and biosurfactant mimics. Lipases are superior catalysts that accept a wide array of complex molecules as substrates and catalyze reactions with high enantio- and regio-selectivities [12]. Lipases have been extensively used for the regio-selective acylation of polyhydroxylated natural products such as flavaonoids, saponins and other polyphenolic compounds [13,14].

In this context, together with our continued interest in lipase catalyzed selective *trans*-esterification reactions [15], we initiated a research programme directed towards the development of novel amphiphiles and biosurfactant mimics of iridoid glycosides. We have chosen picrosides-I/II, catalpol, agnusides and negundoside (Fig. 1) for lipase catalyzed regio-selective acylation studies using *p*-nitrophenyl alkanoate derived from various carboxylic acids of varying chain lengths as acyl donors. Regio-selective acylation of these iridoid glycosides was studied using a panel of commercially available lipases and a focused library of acylated compounds were generated employing immobilized *Candida antarctica* lipase B which gave optimum selectivity and good yields.

2. Results and discussion

Semi-synthetic modification of these natural iridoid glycosides through the introduction of lipophilic chains, glyceric acid and triethyleneglycol-based moieties selectively on some of the hydroxyl groups, greatly enhance the bioactivity of these molecules. In view of this, we synthesized novel analogues of picroside-I/II, catalpol, agnuside and negundoside through regio-selective *trans*-esterification using various labile esters.

2.1. Enzymatic acylation

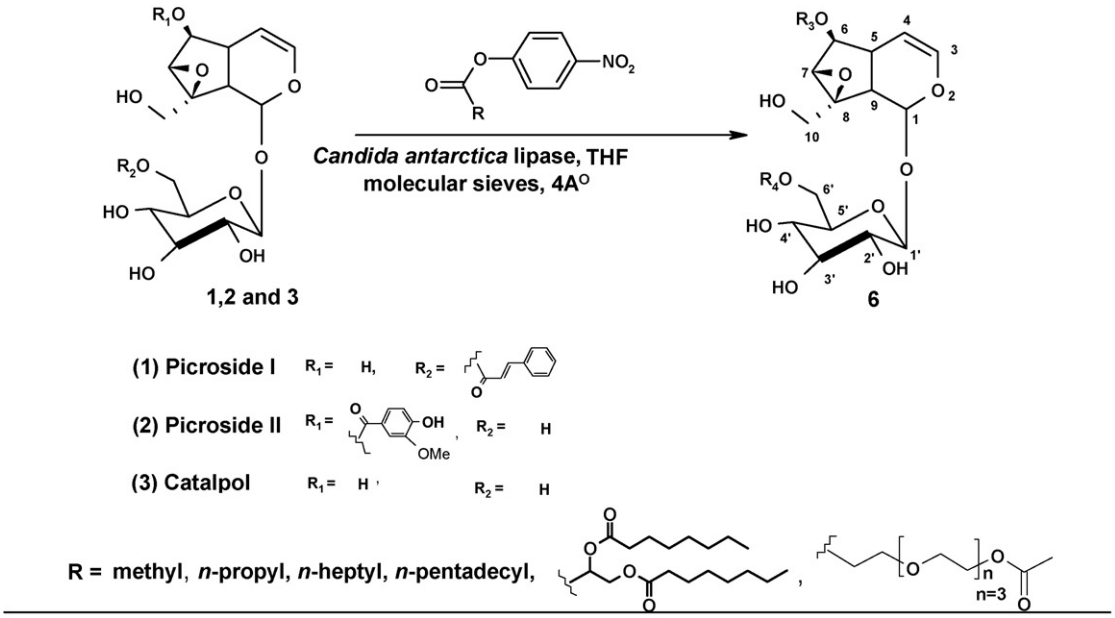
Five commercial lipases viz., *Candida rogusa* lipase, lipase acrylic resin from *C. antarctica* (lipase B), *C. antarctica* recombinant from *Aspergillus oryzae* (lipase B), *Candida cylinderacia* lipase and *Porcine pancreatic* lipase were used to study the regio-selective acylation of various lipophilic moieties of varying chain lengths, on picroside-I/II, catalpol, agnuside and negundoside using various labile esters such as *p*-nitrophenyl alkanoate and vinyl acetate in dry organic solvent (THF/DMF). The selection of enzyme being one of the most important parameters for enzyme-catalyzed reactions, in order to choose the more efficient enzyme for the *trans*-esterification of iridoid glycosides, both soluble lipases and immobilized lipases on macroporous resin were screened for their *trans*-esterification potential in terms of selectivity and yields using *p*-nitrophenyl alkanoate as acyl donor in dry THF at room temperature. The results were compared and presented in Table 1 which clearly shows that resin bound lipase exhibits its unique advantage to catalyze the *trans*-esterification reaction, while the soluble lipases reveal the lower catalytic activity. Furthermore, immobilized *C. antarctica* lipase B gave moderate yields of esters ranging from 23 to 55% with different substrates *viz.*, picroside-I, catalpol, agnuside and negundoside.

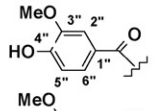
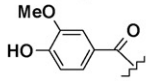
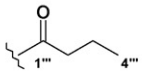
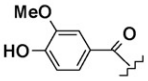
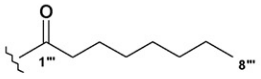
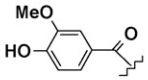
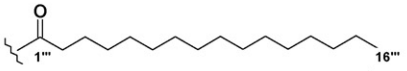
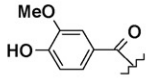
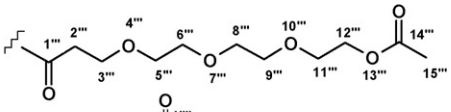
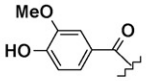
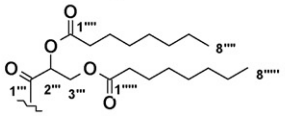
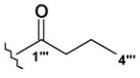
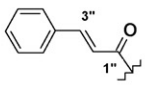
In all substrates studied, only one product was formed as examined by TLC, indicating high regio-selective nature of the lipases-catalyzed *trans*-esterification with iridoids. These commercial lipases were screened for the regio-selective acylation using several solvents such as diethyl ether, dichloromethane, *n*-hexane, diisopropyether, DMF and THF. Owing to the poor solubility of substrates in less polar solvents, polar solvents *viz.*, DMF and THF were found to be suitable for such study. Optimal conversions were obtained with resin bound immobilized *C. antarctica* lipase B/THF combinations. Result of screening revealed that resin bound immobilized *C. antarctica* lipase B was the enzyme of choice when used along with *p*-nitrophenyl alkanoate as acyl donor and this combination was chosen for all further studies. Various acylated products thus obtained were purified by flash chromatography and their structure determined by mass, $^{1}H/^{13}C$ NMR spectra and by comparison with spectra of their respective substrates. As anticipated

Table 1
Enzymatic trans-esterification of picroside-II (**2**) by commercial lipases and immobilized lipases on macroporous resin

Entry	Enzyme	Substrate	Yield (%)[a]
i	*C. rogusa*	PK-II (2)	15
ii	Lipase acrylic resin from *C. antarctica* (lipase B)	PK-II (2)	50
iii	*C. antarctica* recombinant from *A. oryzae* (lipase B)	PK-II (2)	21
iv	*C. cylinderacia*	PK-II (2)	15
v	*P. pancreatic* lipase	PK-II (2)	12

[a] Experimental conditions: 0.1 mmol PK-II (2); 0.2 mmol *p*-nitrophenyl alkanoate; 20% weight equivalent enzyme; dry THF; rt.



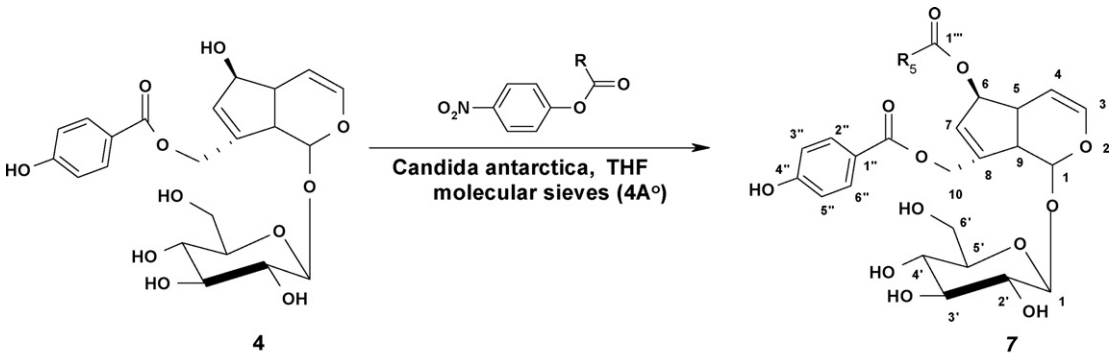
Entry	Product (6) R_3	R_4	Reaction time (h)	Isolated Yield (%)
a			30	52
b			30	50
c			48	38
d			50	33
e			45	30
f			50	23
g			60	30
h	H		40	23

Scheme 1. Regio-selective acylation of picroside-I/II and catalpol.

the enzymatic *trans*-esterifications were clean and the un-reacted starting compounds were recovered during chromatographic separations.

2.2. Acylation of picroside-I/II and catalpol

In case of picroside-II and catalpol, regio-selective acylation of primary hydroxyl of sugar moiety was affected by resin bound immobilized *C. antarctica* lipase B in dry solvent. Whereas, in case of picroside-I, enzymatic *trans*-esterification occurred regio-selectively on secondary hydroxyl group of aglycon moiety. The cinnamyl ester group of picroside-I might be offering considerable steric hindrance thereby preventing the primary hydroxyl of aglycon from participating in esterification. Hence the acylation might be possibly taking place at the secondary hydroxyl of aglycon through their proper projection and recognition in the binding and catalytic domain of the enzyme. The yield of acylated product varied for substrates and type of acyl donor employed. In gen-



Entry	Product (7) R_5	Reaction time (h)	Isolated Yield (%)
a	*n*-propyl	48	55
b	*n*-heptyl	48	43
c	*n*-pentadecyl	50	38

Scheme 2. Regio-selective acylation of agnuside.

eral, moderate yields were obtained in case of small acyl groups whereas the yields decreased with the increase in chain length. Picroside-II gave moderate yield with all types of acyl groups such as butanoyl, octanoyl, palmitoyl and micellar structure (glyceric acid, triethylene glycol) derived *p*-nitrophenyl alkanoates. In the case of picroside-I, lower homologues of acyl group such as acetyl- and butanoyl-derived acyl donors gave moderate yields (30%) whereas for higher homologues like palmitoyl, the yields of acylated product were low (<5%). Overall, the yield of acylated product of picroside-II was found to be higher than that of picroside-I or catalpol. In contrast, the acylation of catalpol was successful with vinylacetate when *C. antarctica* lipase B was employed in dry THF, whereas no traces of acylation products were detected in the reaction of catalpol with various other long chain *p*-nitrophenyl esters as acyl donors. Comprehensive mass, ^{1}H and ^{13}C NMR assignments have been listed in the experimental section and the key diagnostic signals leading to the structural assignments are summarized below.

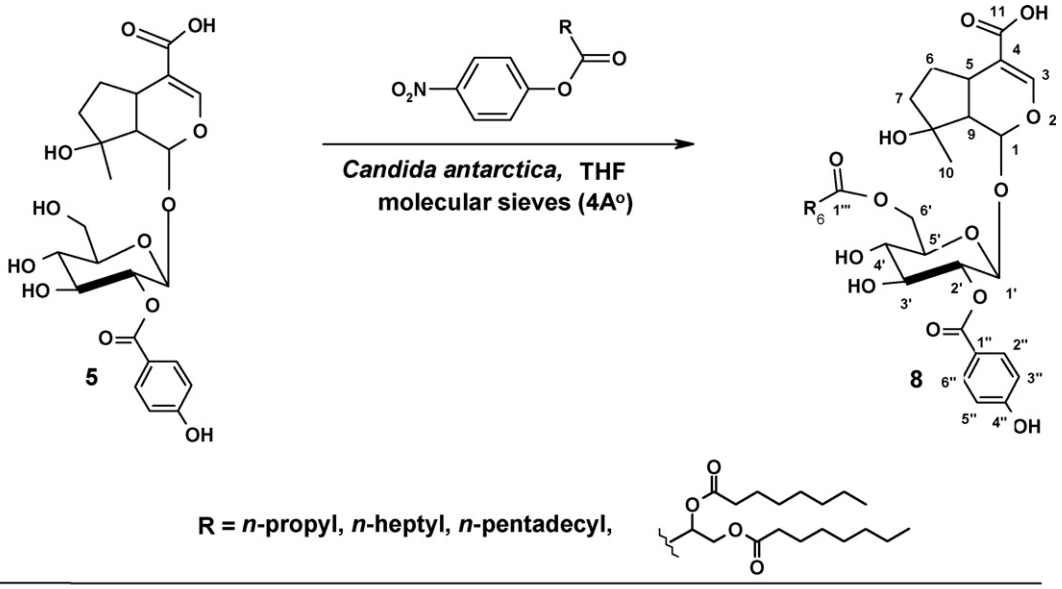
In case of picroside-II butanoate ester, ^{1}H NMR signal at δ 4.43 (J = 11.9, 1.9) and 4.31 (J = 11.9, 6.2) assigned to H-6′ (a′ and b′) of the glucose moiety appeared at a down field (0.50 and 0.66 ppm) compared to that of the picroside-II molecule (δ 3.93, J = 11.9, 1.9 and 3.65, J = 11.9, 6.7). Moreover, in the ^{13}C NMR the signal at δ 62.8 was assigned to the C-6′ of the glucose moiety in picroside II butanoate molecule. Downfield shift of this signal by 1.4 ppm in comparison with that of the picroside-II molecule (δ 61.4 suggested the presence of an ester bond on the C-6′ of the sugar moiety of picroside-II. Similar, structural assignments were made for other regio-selective esterification products of picroside obtained with various other acyl donors (Scheme 1). As in case of picroside-II, ^{1}H NMR, ^{13}C NMR of catalpol showed significant down field shift of the methylene signals of primary hydroxyl group of the sugar moiety which suggested that catalpol was regio-selectively acylated only at C-6′ of sugar moiety.

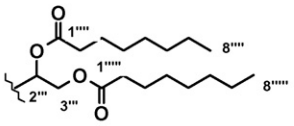
In the *trans*-esterification reaction of picroside-I, the acylation occurred at the secondary hydroxyl (C-6) of the aglycon moiety, the position of acylation was confirmed by ^{1}H NMR and ^{13}CNMR spectrum. In ^{1}H NMR spectra the signals of 6-H of aglycon moiety shifted downfield by 1.48 ppm (from 3.78 to 5.26) as compared to picroside-I. Furthermore, in ^{13}C NMR the signal of C-6 of aglycon moiety of picroside-I was shifted by 1.6 ppm. These results proved the presence of an ester bond on the C-6 of the aglycon moiety of picroside-I.

2.3. Acylation of agnuside and negundoside

Regio-selective acylation of agnuside was studied using various *p*-nitrophenyl alkanoates of varying chain lengths as acyl donor for enzyme catalyzed *trans*-esterification in the presence of *C. antarctica* lipase B. TLC monitoring revealed that agnuside was acylated to single product, which was purified on silica gel column and characterized as mono acylated product (6-*O*-butanoyl agnuside). ^{1}H NMR spectra revealed that esterification occurred at the secondary hydroxyl of aglycon moiety, as evidenced by the downfield shift of signal of H-6 of aglycon moiety of compound **7a**, to δ 5.26 (J = 2.0) as compared to agnuside (δ 4.48, J = 2.1). Moreover, in ^{13}C NMR compared with agnuside **4**, C-6 of the compound **7a** shifted downfield to δ 83.5. All these results suggested that agnuside **4** was regioselectively acylated only at C-6 of aglycon moiety without acylating the sugar moiety although there is a primary –OH group in agnuside **4** which is generally more reactive in other glycosides such as picroside-II and negundoside. Further, various acyl donors of varying chain length also afford single acylated product (Scheme 2).

It is noteworthy that the *acylation* of agnuside occurred preferentially on the secondary –OH at the C-6 of the aglycon moiety even though this molecule consists of a free primary hydroxyl group on the sugar moiety which may be attributed to molecular overcrowding between *p*-hydroxybenzoate entity of aglycon ester and the primary hydroxyl of sugar unit, which might be creating hindrance to the binding of substrate to the binding domain of lipase and their subsequent interaction with the catalytic site to enable effective *trans*-esterification at this site. This is further confirmed by the facile lipase mediated *trans*-esterification of negundoside



Entry	Product (8) R_6	Reaction time (h)	Isolated Yield (%)
a	*n*-propyl	30	45
b	*n*-heptyl	48	42
c	*n*-pentadecyl	50	35
d		45	28

Scheme 3. Regio-selective acylation of negundoside.

at the primary hydroxyl of sugar, which is devoid of such steric overcrowding and the results are discussed below. In case of negundoside, esterification occurred at the primary hydroxyl group of sugar. Negundoside also gave moderate yields irrespective of type of acyl groups.

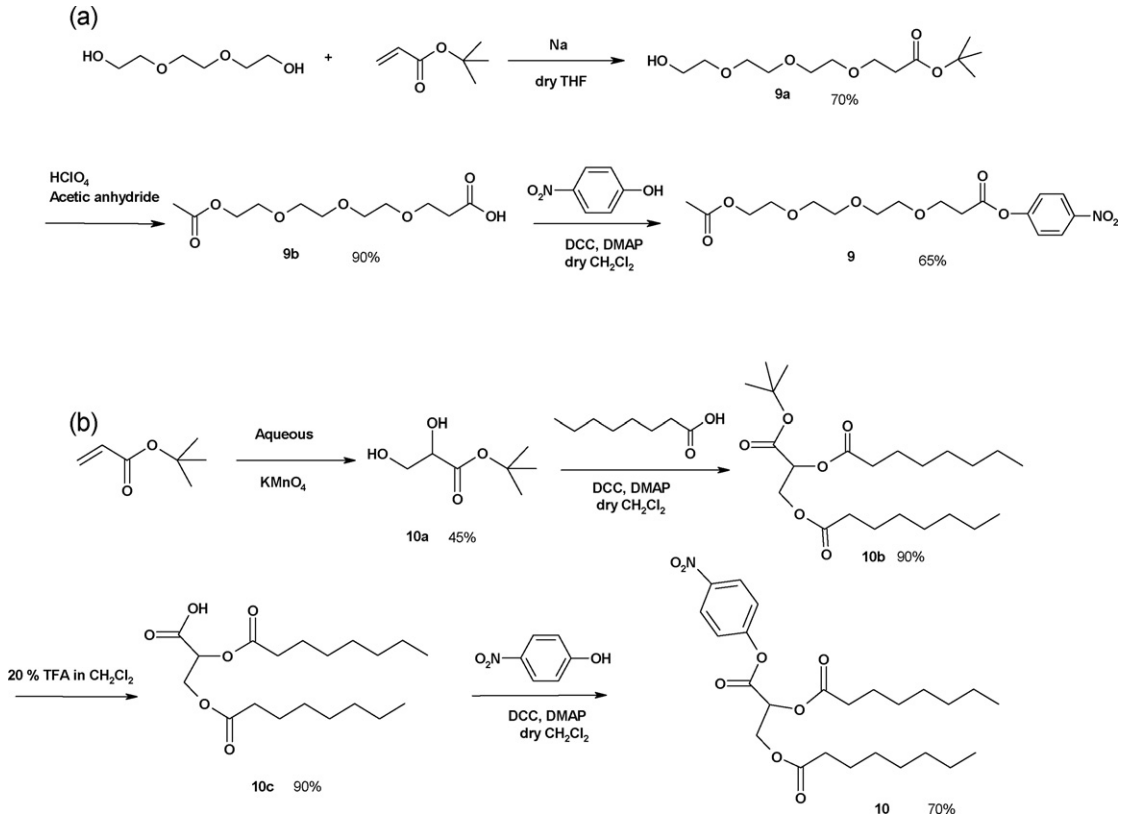
As in case of picroside-II, ^{1}H NMR, ^{13}C NMR of negundoside showed significant down field shift of the methylene signals of primary hydroxyl group of the sugar moiety which suggested that compound **5** was regio-selectively acylated only at C-6′ of sugar moiety. Acylation using various other nitrophenyl alkanoates of varying chain lengths gave the product without any deviation in the regio-selectivity (Scheme 3).

3. Conclusion

Regio-selective acylation of these polyhydroxylated iridoid glycosides could be achieved using resin bound immobilized *C. antarctica* lipase B in the presence of various acyl donors. In case of iridoid glycoside with free primary hydroxyl group at sugar moiety except agnuside, the acylation occurred only at the primary hydroxyl group of the sugar moiety. Thus, lipase catalyzed acylations of picroside-II, agnuside and negundoside proceeded in good yields with various acyl donors. In contrast, catalpol and picroside-I were readily acylated at the primary hydroxyl of sugar and secondary hydroxyl of aglycon moiety respectively by using acyl donors bearing lower homologues like acetyl and butanoyl groups. From these results, it can be concluded that lipophilic derivatives of iridoid glycosides can be easily synthesized through *trans*-esterification using labile esters in the presence of resin bound immobilized *C. antarctica* lipase B under mild reaction conditions. Resin bound immobilized *C. antarctica* lipase B is selective towards both primary and secondary hydroxyl groups of sugar or aglycon moieties respectively, depending on the structure of the iridoid glycosides enabling the synthesis of regio-selectively lipidated iridoids which are otherwise difficult to access by conventional methods. Further investigation on the adjuvant activity of these novel derivatives is currently in progress.

4. Experimental

Lipase from *C. rugosa* (1410 U/mg solid), *porcine pancreas type II* (100–400 units/mg protein, using olive oil), lipase acrylic resin



Scheme 4. (a) Synthesis of triethylene glycol-based acyl donor. (b) Synthesis of glyceric acid-based acyl donor.

from *C. antarctica* (lipase B, ≥10,000 U/g, recombinant) and lipase B *C. antarctica*, recombinant from *A. oryzae* (~9 unit/mg) were purchased from Sigma and *C. cylindracea* (2.8 U/mg) from Fluka. Iridoid glycoside *viz*. picroside-I/II, catalpol, agnuside and negundoside were isolated from their natural source (*P. kuroa* and *V. negundo*) by Natural Product Division, IIIM- Jammu as per literature known procedure [17,18]. All the commercial enzymes were used as such. Enzymatic reactions were carried out on IKA digital shaker at room temperature and 120 rpm. Melting points were recorded on Buchi Melting point apparatus D-545; IR spectra (KBr discs) were recorded on Bruker Vector 22 instrument. NMR spectra were recorded at 200 MHz and 500 MHz on Bruker DPX200 instrument in $CD_3OD/DMSO/CDCl_3$ with TMS as internal standard for protons and solvent signals as internal standard for carbon spectra. Chemical shift values were mentioned in δ (ppm) and coupling constants were given in Hz. Mass spectra were recorded on ESI-esquire 3000 Bruker Daltonics instrument. The progress of all reactions was monitored by TLC on 2 cm × 5 cm pre-coated silica gel 60 F254 plates of thickness of 0.25 mm (Merck). The chromatograms were visualized under UV 254-366 nm and iodine.

4.1. Synthesis of triethylene glycol-based donor (**9**)

Compound **9a** (Scheme 4a) was synthesized by a known procedure by Seitz et al. [19]. The reaction of **9a** (1 g, 7 mmol) with perchloric acid/acetic anhydride gave an intermediate **9b** (0.85 g, 90%) which on coupling with *p*-nitrophenol (0.42 g, 3 mmol, 1 equiv. to **9b**) in the presence of DCC (1.2 g, 6 mmol, 2 equiv. to **9b**) and DMAP (10 mg, catalytic amount) under dry conditions [16] gave the crude product. The crude product on purification by column chromatography (silica gel, 60–120 mesh, eluent; *n*-hexane/EtOAc gradient) gave compound **9** as a yellow liquid (0.79 g, 65%).

4.2. Synthesis of glyceric acid-based donor (**10**)

For the synthesis of glyceric acid-based donor **10** (Scheme 4b), *ter*-butyl acrylate (2 g, 15 mmol) was treated with aqueous $KMnO_4$ which resulted in the formation of 2,3-dihydroxy-propionic acid *ter*-butyl ester **10a** (1.02 g, 45%). Compound **10a** (1.02, 6 mmol) obtained as above, on reaction with octonoic acid (2 g, 14 mmol) in the presence of DCC (5 g, 24 mmol, 4 equiv. to **10a**), DMAP (10 mg, catalytic amount) under dry conditions gave diacylated product **10b** (2.34 g, 90%). Compound **10b** (2.34 g, 6 mmol) on deprotection using TFA gave compound **10c** (1.81 g, 90%), which on coupling with *p*-nitrophenol (0.7 g, 5 mmol, 1 equiv. to **10c**) using the same procedure as described above [16], gave crude product. Column chromatographic purification of the crude product (silica gel, 60–120 mesh, eluent; *n*-hexane/EtOAc gradient) gave compound **10** as a yellowish semi-solid (1.68 g, 70%).

4.3. General procedure

Substrate (iridoid glycoside, 0.1 mmol) was dissolved in dry organic solvent (THF/DMF) in the presence of the lipase (*C. antarctica* lipase B, 20% weight equivalent), an acyl donor (*p*-nitrophenyl alkanoate, 0.2 mmol), pre-activated molecular sieves (4 Å) and left for shaking on digital shaker at 120 rpm. The reaction was monitored by TLC and terminated when the highest conversion was achieved. The acylated product was purified by Flash chromatography and the structure was determined by MS and NMR spectra (^1H, ^{13}C, and by comparison with spectra of their respective substrate).

5. Compound characterization

5.1. 6′-O-Acetyl-picroside-II (**6a**)

As a syrupy liquid; $[\alpha]_D^{25}$ −106.0 (c 0.1, CH_3OH); IR (KBr, cm^{-1}): 1015, 1038, 1070, 1104, 1221, 1289, 1629, 1655, 1707, 2924; ^1H NMR (500 MHz, CD_3OD): δ 2.01 (s, 3H, H-2‴), 2.66 (m, 2H, H-5, H-9), 3.30 (m, 1H, signal obscured with solvent peak, H-4′), 3.34 (d, 1H, *J* = 9.5, H-2′), 3.41 (d, 1H, *J* = 9.0, H-3′), 3.53 (m, 1H, H-5′), 3.71 (br s, 1H, H-7), 3.77 (d, 1H, *J* = 13.1, H-10), 3.89 (s, 3H), 4.18 (d, 1H, *J* = 13.1, H-10), 4.32 (dd, 1H, *J* = 11.9, 6.2, H-6′), 4.41 (dd, 1H, *J* = 11.7, 1.9, H-6′), 4.78 (d, 1H, *J* = 7.9, H-1′), 4.91 (d, 1H, signal obscured in solvent peak, H-1), 5.00 (dd, 1H, *J* = 5.7, 2.2, H-4), 5.04 (d, 1H, *J* = 6.9, H-6), 6.39 (d, 1H, *J* = 5.6, H-3), 6.87 (d, 1H, *J* = 8.3, H-5″), 7.57 (br s, 1H, H-2″), 7.59 (dd, 1H, *J* = 8.3, 1.9, H-6″); ^{13}C NMR (125 MHz, CD_3OD): δ 19.4, 35.3, 41.7, 55.0, 58.7, 60.0, 63.5, 65.3, 70.2, 73.3, 74.5, 76.1, 80.3, 93.8, 98.4, 101.6, 112.2, 114.6, 120.6, 123.9, 141.0, 147.4, 151.8, 166.5, 173.8; MS (ESI): (M^++Na) 577. Elemental analysis calcd. for $C_{25}H_{30}O_{14}$, C = 54.15%, H = 5.45%. Found C = 54.01%, H = 5.62%.

5.2. 6′-O-Butanoyl-picroside-II (**6b**)

As a syrupy liquid; $[\alpha]_D^{25}$ −93.0 (c 0.8, CH_3OH); IR (KBr, cm^{-1}): 1017, 1036, 1068, 1104, 1221, 1287, 1631, 1655, 1707, 2856, 2924; ^1H NMR (500 MHz, CD_3OD): δ 0.89 (t, 3H, *J* = 7.4, H-4‴), 1.57 (m, 2H, H-3‴), 2.25 (t, 2H, *J* = 7.3, H-2‴), 2.57 (m, 2H, H-5, H-9), 3.21 (m, 1H, Signal obscured with solvent peak, H-4′), 3.26 (d, 1H, *J* = 9.5, H-2′), 3.31 (d, 1H, *J* = 9.0, H-3′), 3.45 (m, 1H, H-5′), 3.63 (br s, 1H, H-7), 3.67 (d, 1H, *J* = 13.1, H-10), 3.81 (s, 3H), 4.08 (d, 1H, *J* = 13.1, H-10), 4.31 (dd, 1H, *J* = 11.9, 6.2, H-6′), 4.43 (dd, 1H, *J* = 11.9, 1.9, H-6′), 4.72 (d, 1H, *J* = 7.9, H-1′), 4.82 (d, 1H, Signal obscured with solvent peak, H-1), 4.92 (dd, 1H, *J* = 5.7, 2.2, H-4), 5.09 (d, 1H, *J* = 6.9, H-6), 6.28 (d, 1H, *J* = 5.6, H-3), 6.76 (d, 1H, *J* = 8.3, H-5″), 7.47 (br s, 1H, H-2″), 7.49 (dd, 1H, *J* = 8.3, 1.9, H-6″); ^{13}C NMR (125 MHz, CD_3OD): δ 12.6, 18.2, 35.3, 35.6, 41.7, 55.0, 58.7, 60.0, 62.8, 65.3, 70.2, 73.3, 74.5, 76.1, 80.3, 93.8, 98.4, 101.6, 112.2, 114.6, 120.6, 123.9, 141.0, 147.4, 151.8, 166.5, 173.8; MS (ESI): (M^++Na) 605. Elemental analysis calcd. for $C_{27}H_{34}O_{14}$, C = 55.67%, H = 5.88%. Found C = 55.56%, H = 5.83%.

5.3. 6′-O-Octanoyl-picroside-II (**6c**)

As a syrupy liquid; $[\alpha]_D^{25}$ −80.0 (c 1.0, CH_3OH); IR (KBr, cm^{-1}): 1027, 1036, 1068, 1106, 1220, 1284, 1631, 1655, 1707, 2856, 2924; ^1H NMR (500 MHz, CD_3OD): δ 0.85 (t, 3H, *J* = 6.8, H-8‴), 1.29 (m, 8H, H-7‴ to H-4‴), 1.63 (m, 2H, H-3‴), 2.36 (t, 2H, *J* = 7.5, H-2‴), 2.67 (m, 2H, H-5, H-9), 3.31 (m, 1H, signal obscured with solvent peak, H-4′), 3.36 (d, 1H, *J* = 9.4, H-2′), 3.42 (d, 1H, *J* = 9.4, H-3′), 3.51 (m, 1H, H-5′), 3.74 (br s, 1H, H-7), 3.78 (d, 1H, *J* = 13.1, H-10), 3.91 (s, 3H), 4.19 (d, 1H, *J* = 13.1, H-10), 4.31 (dd, 1H, *J* = 11.9, 6.1, H-6′), 4.43 (dd, 1H, *J* = 11.7, 1.8, H-6′), 4.79 (d, 1H, *J* = 7.9, H-1′), 4.92 (d, 1H, signal obscured with solvent peak, H-1), 5.02 (dd, 1H, *J* = 5.8, 3.8, H-4), 5.05 (d, 1H, *J* = 6.9, H-6), 6.39 (d, 1H, *J* = 5.7, H-3), 6.87 (d, 1H, *J* = 8.3, H-5″), 7.57 (br s, 1H, H-2″), 7.59 (dd, 1H, *J* = 9.2, 1.6, H-6″); ^{13}C NMR (125 MHz, CD_3OD): δ 14.4, 23.6, 26.2, 30.1, 30.2, 32.8, 35.1, 36.7, 43.1, 56.5, 60.1, 61.4, 64.2, 66.8, 71.6, 74.7, 75.9, 77.5, 81.7, 95.3, 99.8, 103.1, 113.6, 116.0, 122.0, 125.3, 142.5, 148.8, 153.2, 167.8, 175.4; MS (ESI); (M^++Na) 661. Elemental analysis calcd. for $C_{31}H_{42}O_{14}$, C = 58.30%, H = 6.63%. Found C = 58.16%, H = 6.56%.

5.4. 6′-O-Palmitoyl-picroside-II (**6d**)

As a colorless solid; mp 160 °C; $[\alpha]_D^{25}$ −89.0 (c 1.0, CH_3OH); IR (KBr, cm^{-1}): 1015, 1037, 1068, 1107, 1221, 1287, 1631, 1655, 1707, 2856, 2924; ^1H NMR (500 MHz, CD_3OD): δ 0.90 (t, 3H, *J* = 6.7, H-16‴), 1.25 (m, 24H, H-15‴ to H-4‴), 1.62 (m, 2H, H-3‴), 2.38 (m, 2H, H-2‴), 2.70 (m, 2H, H-5, H-9), 3.31 (m, 1H, signal obscured with solvent peak, H-4′), 3.36 (d, 1H, *J* = 9.4, H-2′), 3.42 (d, 1H, *J* = 9.4, H-3′), 3.51 (m, 1H, H-5′), 3.74 (br s, 1H, H-7), 3.78 (d, 1H, *J* = 13.1, H-10), 3.91 (s, 3H), 4.19 (d, 1H, *J* = 13.1, H-10), 4.31 (dd, 1H, *J* = 11.9, 6.1, H-6′), 4.43 (dd, 1H, *J* = 11.7, 1.8, H-6′), 4.79 (d, 1H, *J* = 7.9, H-1′), 4.92 (d, 1H, signal obscured with solvent peak, H-1), 5.02 (dd, 1H, *J* = 5.8, 3.8, H-4), 5.05 (d, 1H, *J* = 6.9, H-6), 6.41 (d, 1H, *J* = 5.9, H-3), 6.88 (d, 1H, *J* = 8.1, H-5″), 7.61(br s, 1H, H-2″), 7.63 (dd, 1H, J = 8.3, 1.6, H-6″); ^{13}C NMR (125 MHz, CD_3OD): 14.9, 24.1, 26.6, 30.7, 30.8, 31.0, 31.2, 33.5, 35.6, 37.1, 43.5, 55.2, 60.5, 61.9, 64.7, 67.2, 72.1, 75.1, 76.3, 77.9, 82.1, 95.7, 100.2, 103.5, 114.1, 116.4, 122.4, 125.8, 142.9, 149.2, 153.6, 168.2, 175.8; MS (ESI): (M^++Na) 773. Elemental analysis calcd. for $C_{39}H_{58}O_{14}$, C = 62.38%, H = 7.79%. Found C = 62.24%, H = 7.71%.

5.5. 6′-O-[3-{2-[2-(2-Acetoxy-ethoxy)-ethoxy]-ethoxy}-propanoyl]-picroside-II (**6e**)

As a pale yellow liquid; $[\alpha]_D^{25}$ −78.0 (c 0.5, CH_3OH); IR (KBr, cm^{-1}): 1007, 1035, 1104, 1221, 1631, 1657, 1707, 2856, 2924; ^1H NMR (200 MHz, CD_3OD): δ 2.00 (s, 3H, H-15‴), 2.58 (m, 4H, H-5, H-9, H-12‴), 3.31 (m, 1H, signal obscured with solvent peak, H-4′), 3.36 (d, 1H, *J* = 9.4, H-2′), 3.42 (d, 1H, *J* = 9.4, H-3′), 3.51 (m, 1H, H-5′), 3.74 (m, 11H, H-7, H-5‴, H-6‴, H-8‴, H-9‴, H-11‴), 3.78 (d, 1H, *J* = 13.1, H-10), 3.91 (s, 3H), 3.99 (t, 2H, *J* = 6.2, H-2‴) 4.19 (m, 3H, H-10, H-3‴), 4.31 (dd, 1H, *J* = 11.9, 6.1, H-6′), 4.43 (dd, 1H, *J* = 11.7, 1.8, H-6′), 4.79 (d, 1H, *J* = 7.9, H-1′), 4.92 (d, 1H, signal obscured with solvent peak, H-1), 5.00 (dd, 1H, *J* = 5.8, 3.8, H-4), 5.03 (d, 1H, *J* = 6.9, H-6), 6.27 (d, 1H, *J* = 5.9, H-3), 6.83 (d, 1H, *J* = 8.3, H-5″), 7.47 (s, 1H, H-2″), 7.57 (dd, 1H, *J* = 8.3, 1.6, H-6″); MS (ESI): (M^++Na) 781. Elemental analysis calcd. for $C_{34}H_{46}O_{19}$, C = 53.82%, H = 6.11%. Found C = 53.58%, H = 6.08%.

5.6. 6′-O-(2‴,3‴-Di-octanoylpropanoyl)-picroside-II (**6f**)

As a syrupy liquid; $[\alpha]_D^{25}$ −100.8 (c 1.0, CH_3OH); IR (KBr, cm^{-1}): 1017, 1036, 1068, 1104, 1221, 1287, 1631, 1655, 1707, 2856, 2924; ^1H NMR (200 MHz, CD_3OD): δ 0.77 (t, 6H, *J* = 4.4, H-8⁗, H-8⁗′), 1.20 (m, 16H, H-7⁗ to H-4⁗, H-7⁗′ to H-4⁗′), 1.52 (m, 4H, H-3⁗, H-3⁗′), 2.26 (m, 4H, H-2⁗, H-2⁗′), 2.57 (m, 2H, H-5, H-9), 3.21 (m, 1H, signal obscured with solvent peak, H-4′), 3.26 (d, 1H, *J* = 9.5, H-2′), 3.31 (d, 1H, *J* = 9.0, H-3′), 3.45 (m, 1H, H-5′), 3.63 (br s, 1H), 3.67 (d, 1H, *J* = 13.1, H-10), 3.81 (s, 3H), 4.08 (d, 1H, *J* = 13.1, H-10), 4.20 (d, 2H, *J* = 5.3, H-3‴), 4.23 (dd, 1H, *J* = 11.9, 6.2, H-6′), 4.35 (dd, 1H, *J* = 11.7, 1.9, H-6′), 4.72 (d, 1H, *J* = 7.9, H-1′), 4.82 (d, 1H, signal obscured with solvent peak, H-1), 4.92 (dd, 1H, *J* = 5.7, 2.2, H-4), 5.21 (d, 1H, *J* = 6.9, H-6), 5.23 (m, 1H, H-2‴), 6.29 (d, 1H, *J* = 5.7, H-3), 6.76 (d, 1H, *J* = 8.07, H-5″), 7.48 (s, 1H, H-2″), 7.52 (dd, 1H, J = 8.0, 1.6, H-6″); ^{13}C NMR (125 MHz, $CDCl_3$); δ 13.0, 22.2, 23.5, 24.6, 24.7, 28.6, 28.8, 31.4, 33.6, 35.3, 43.4, 55.1, 59.3, 61.6, 63.5, 65.7, 67.2, 73.1, 75.0, 76.4, 78.1, 80.0, 82.8, 93.9, 99.5, 103.0, 113.5, 117.0, 121.0, 124.1, 141.0, 148.3, 152.4, 166.1, 174.1, 176.2; MS (ESI): (M^++Na) 875. Elemental analysis calcd. for $C_{42}H_{60}O_{18}$, C = 59.14%, H = 7.09%. Found C = 59.08%, H = 6.98%.

5.7. 6-O-Butanoyl-picroside-I (**6g**)

As a yellow syrupy liquid; $[\alpha]_D^{25}$ −45.1 (c 1.0, CH_3OH); IR (KBr, cm^{-1}): 799, 1019, 1088, 1173, 1260, 1713, 2854, 2925, 2962; ^1H NMR (500 MHz, $CDCl_3$): δ 0.89 (t, 3H, *J* = 7.4, H-4‴), 1.63 (m, 2H, H-3‴), 2.29 (t, 2H, *J* = 7.8, H-2‴), 2.51 (m, 1H, H-5), 2.71 (t, 1H, *J* = 8.3, H-9), 3.30 (m, 2H, H-2′, H-3′), 3.43 (m, 2H, H-4′, H-7), 3.58 (m, 1H, H-5′), 3.65 (d, 1H, *J* = 13.0, H-10), 4.14 (d, 1H, 13.0, H-10), 4.51 (dd, 1H, *J* = 11.9, 6.0, H-6′), 4.69 (dd, 1H, *J* = 16.0, H-6′), 4.82 (d, 1H, *J* = 7.8, H-1′), 4.90 (m, 1H, signal obscured with solvent peak, H-1), 5.01 (d,

1H, *J* = 4.7, H-4), 5.26 (m, 1H, H-6), 6.29 (d, 1H, *J* = 5.0, H-3), 6.47 (d, 1H, *J* = 16.0, H-2″), 7.33 (m, 3H, ArH), 7.51 (m, 2H, ArH), 7.69 (d, 1H, *J* = 15.9, H-3″); ^{13}C NMR (50 MHz, $CDCl_3$): δ 13.5, 13.8, 18.3, 18.8, 35.1, 35.9, 41.5, 59.2, 61.7, 63.1, 65.2, 69.9, 73.1, 74.5, 75.8, 79.3, 94.3, 97.8, 102.4, 166.7, 128.3, 128.8, 130.4, 134.5, 141.1, 145.6, 167.2, 173.4; MS (ESI): (M^++Na) 585. Elemental analysis calcd. for $C_{28}H_{34}O_{12}$, C = 59.78%, H = 6.09%. Found C = 59.66%, H = 6.13%.

5.8. 6′-O-Acetyl-catalpol (**6h**)

As a yellow syrupy liquid; $[\alpha]_D^{25}$ −15.0 (c 0.8, CH_3OH); IR (KBr, cm^{-1}): 794, 1015, 1028, 1183, 1262, 1717, 2854, 2929, 2963; ^{1}H NMR (200 MHz, $CDCl_3$): δ 1.91 (s, 3H, H-2‴), 2.31 (m, 1H, H-5), 2.49 (m, 1H, H-9), 3.31 (m, 2H, signal obscured with solvent peak, H-2′, H-3′), 3.42 (m, 2H, H-4′, H-5′), 3.58 (m, 1H, H-7), 3.65 (d, 1H, *J* = 13.0, H-10), 3.89 (m, 1H, H-6), 4.01 (d, 1H, *J* = 13.0, H-10), 4.31 (dd, 1H, *J* = 11.9, 6.2, H-6′), 4.45 (dd, 1H, *J* = 11.9, 1.8, H-6′), 4.89 (d, 1H, *J* = 7.8, H-1′), 4.92 (m, 1H, signal obscured with solvent peak, H-1), 5.01 (m, 1H, H-4), 6.41 (d, 1H, *J* = 5.4, H-3); ^{13}C NMR (125 MHz, $CDCl_3$): δ 19.72, 29.11, 35.08, 36.60, 43.53, 60.0, 60.92, 61.28, 66.35, 72.14, 75.47, 76.75, 77.80, 91.91, 95.27, 100.88, 140.11; MS (ESI): (M^++Na) 427. Elemental analysis calcd. for $C_{17}H_{24}O_{11}$, C = 50.50%, H = 5.98%. Found C = 50.38%, H = 5.93%.

5.9. 6-O-Butanoyl-agnuside (**7a**)

As a colorless solid; mp 148 °C; $[\alpha]_D^{25}$ −82.0 (c 0.5, CH_3OH); IR (KBr, cm^{-1}): 620, 1113, 1275, 1607, 1659, 1709, 2853, 2924, 3350; ^{1}H NMR (200 MHz, CD_3OD): δ 0.81 (t, 3H, *J* = 7.4, H-4‴), 1.50 (m, 2H, H-3‴), 2.20 (t, 2H, *J* = 7.3, H-2‴), 2.81 (m, 1H, H-5), 2.98 (m, 1H, H-9), 3.24 (d, 1H, *J* = 8.3, H-2′), 3.29 (m, 2H, signal obscured with solvent peak, H-4′, H-5′), 3.36 (d, 1H, *J* = 8.9, H-3′), 3.54 (dd, 1H, *J* = 11.9, 5.6, H-6′), 3.76 (dd, 1H, *J* = 11.9, 1.9, H-6′), 4.59 (d, 1H, *J* = 7.8, H-1′), 4.92 (m, 2H, signal obscured with solvent peak, H-10), 4.98 (m, 1H, H-1), 5.04 (d, 1H, *J* = 6.3, H-4), 5.26 (d, 1H, *J* = 2.0, H-6), 5.74 (m, 1H, H-7), 6.23 (dd, 1H, *J* = 6.1, 1.8, H-3), 6.74 (d, 2H, *J* = 8.7, H-3‴, H-5″), 7.81 (d, 2H, *J* = 8.7, H-2″, H-6″); ^{13}C NMR (125 MHz, CD_3OD): δ 12.4, 18.0, 35.6, 41.1, 49.1, 61.3, 61.9, 70.0, 73.4, 76.5, 76.8, 83.5, 95.6, 98.6, 103.4, 114.9, 120.5, 126.6, 131.5, 140.6, 144.9, 162.3, 166.3, 173.9; MS (ESI): (M^++Na) 559. Elemental analysis calcd. for $C_{26}H_{32}O_{12}$, C = 58.20%, H = 6.01%. Found C = 58.14%, H = 5.96%.

5.10. 6-O-Octanoyl-agnuside (**7b**)

As a syrupy liquid; $[\alpha]_D^{25}$ −80.0 (c 0.5, CH_3OH); IR (KBr, cm^{-1}): 618, 1113, 1275, 1607, 1659, 1707, 2853, 2924, 3353; ^{1}H NMR (200 MHz, CD_3OD): δ 0.86 (t, 3H, *J* = 6.7, H-8‴), 1.25 (m, 8H, H-7‴ to H-4‴), 1.57 (m, 2H, H-3‴), 2.27 (t, 2H, *J* = 7.1, H-2‴), 2.94 (m, 1H, H-5), 3.09 (m, 1H, H-9), 3.18 (d, 1H, *J* = 8.8, H-2′), 3. 29 (m, 2H, signal obscured with signal peak, H-4′, H-5′), 3.36 (d, 1H, *J* = 8.9, H-3′), 3.64 (dd, 1H, *J* = 11.9, 5.9, H-6′), 3.85 (dd, 1H, *J* = 11.8, 1.9, H-6′), 4.68 (d, 1H, *J* = 8.0, H-1′), 4.92 (m, 2H, signal obscured with solvent peak, H-10), 5.00 (m, 1H, H-1), 5.11 (d, 1H, *J* = 6.3, H-4), 5.36 (d, 1H, *J* = 2.0, H-6), 5.83 (m, 1H, H-7), 6.33 (dd, 1H, *J* = 6.0, 1.8, H-3), 6.83 (d, 2H, *J* = 8.7, H-3″, H-5″), 7.91 (d, 2H, *J* = 8.7, H-2″, H-6″); ^{13}C NMR (125 MHz, CD_3OD): δ 14.7, 20.1, 22.51, 26.4, 29.3, 31.7, 35.3, 41.8, 49.1, 61.8, 62.2, 71.1, 74.3, 76.2, 76.8, 83.6, 96.7, 98.5, 103.3, 114.8, 120.5, 126.4, 132.4, 141.2, 144.7, 162.2, 167.2, 174.0; MS (ESI): (M^++Na) 615. Elemental analysis calcd. for $C_{30}H_{40}O_{12}$, C = 60.80%, H = 6.80%. Found C = 60.72%, H = 6.76%.

5.11. 6-O-Palmitoyl-agnuside (**7c**)

As a syrupy liquid; $[\alpha]_D^{25}$ −90.0 (c 1.0, CH_3OH); IR (KBr, cm^{-1}): 619, 1100, 1277, 1609, 1659, 1707, 2853, 2923, 3355; ^{1}H NMR (200 MHz, CD_3OD): δ 0.90 (t, 3H, *J* = 6.6, H-16‴), 1.28 (m, 24H, H-15‴ to H-4‴), 1.58 (m 2H, H-3‴), 2.31 (t, 2H, *J* = 7.3, H-2‴), 2.92–3.01 (m, 2H, H-5, H-9), 3.19 (d, 1H, *J* = 9.0, H-2′), 3.29 (m, 2H, signal obscured with solvent peak, H-4′, H-5′), 3.43 (d, 1H, *J* = 8.8, H-3′), 3.64 (dd, 1H, *J* = 11.9, 5.6, H-6′), 3.85 (dd, 1H, *J* = 11.9, 1.9, H-6′), 4.67 (d, 1H, 7.9, H-1′) 4.93 (m, 2H, signal obscured with solvent peak, H-10), 5.00 (m, 1H, H-1), 5.09 (d, 1H, *J* = 6.2, H-4), 5.37 (d, 1H, *J* = 1.9, H-6), 5.84 (m, 1H, H-7), 6.34 (dd, 1H, *J* = 6.0, 1.8, H-3), 6.84 (d, 2H, *J* = 8.7, H-3″, H-5″), 7.92 (d, 2H, *J* = 8.7, H-2″, H-6″); ^{13}C NMR (125 MHz, $CDCl_3$): δ 14.9, 24.1, 26.6, 30.7, 30.8, 31.0, 31.2, 33.5, 35.6, 37.1, 41.1, 49.8, 61.4, 61.9, 70.2, 73.4, 76.5, 77.1, 83.4, 95.6, 98.6, 103.4, 114.9, 120.5, 126.6, 131.5, 140.6, 144.9, 162.3, 166.3, 173.9: MS (ESI): (M^++Na) 727. Elemental analysis calcd. for $C_{38}H_{56}O_{12}$, C = 64.75%, H = 8.01%. Found C = 64.58%, H = 7.96%.

5.12. 6′-O-Butanoyl-negundoside (**8a**)

As a colorless solid; mp 138 °C; $[\alpha]_D^{25}$ −100.0 (c 0.5, CH_3OH); IR (KBr, cm^{-1}): 802, 1079, 1169, 1262, 1609, 1712, 2964; ^{1}H NMR (500 MHz, CD_3OD): δ 0.86 (t, 3H, *J* = 7.4, H-4‴), 1.15 (s, 3H, H-10), 1.21 (m, 1H, H-6), 1.49 (m, 1H, H-6), 1.55 (m, 3H, H-3‴, H-7), 2.05 (m, 2H, H-9, H-7), 2.25 (t, 2H, *J* = 7.3, H-2‴), 2.90 (m, 1H, H-5), 3.38 (t, 1H, *J* = 9.4, H-3′), 3.53 (m, 1H, H-5′), 3.62 (t, 1H, *J* = 8.9, H-4′), 4.17 (dd, 1H, *J* = 11.9, 5.5, H-6′), 4.39 (dd, 1H, *J* = 11.9, 1.9, H-6′), 4.89 (d, 1H, *J* = 8.1, H-1′), 4.92 (d, 1H, signal obscured with solvent peak, H-2′) 5.21 (d, 1H, *J* = 3.7, H-1), 6.72 (dd, 2H, *J* = 9.6, 2.6, H-3″, H-5″), 7.01 (s, 1H, H-3), 7.76 (dd, 2H, *J* = 11.4, 1.0, H-2″, H-6″); ^{13}C NMR (125 MHz, CD_3OD): δ 14.1, 19.5, 24.6, 30.7, 31.7, 36.9, 40.9, 52.2, 64.2, 71.6, 74.8, 75.7, 75.8, 80.2, 95.3, 97.8, 113.8, 116.2, 122.1, 133.0, 151.2, 163.7, 167.3, 170.2, 175.3: MS (ESI): (M^+) 566. Elemental analysis calcd. for $C_{27}H_{34}O_{13}$, C = 57.24%, H = 6.05%. Found C = 57.13%, H = 5.94%.

5.13. 6′-O-Octanoyl-negundoside (**8b**)

As a colorless solid; mp 144 °C; $[\alpha]_D^{25}$ −97.0 (c 1.0, CH_3OH); IR (KBr, cm^{-1}): 800, 1077, 1169, 1262, 1609, 1715, 2960; ^{1}H NMR (500 MHz, CD_3OD): δ 0.88 (m, 3H, H-8‴), 1.25 (m, 11H, H-10, H-7‴ to H-4‴), 1.29 (m, 1H, H-6), 1.47 (m, 1H, H-6), 1.63 (m, 3H, H-7, H-3‴), 2.04 (m, 2H, H-7, H-9), 2.23 (t, 2H, *J* = 7.3, H-2‴), 2.89 (m, 1H, H-5), 3.30 (t, 1H, *J* = 9.5, H-3′), 3.56 (m, 1H, H-5′), 3.62 (t, 1H, *J* = 8.9, H-4′), 4.26 (dd, 1H, *J* = 11.8, 5.7, H-6′), 4.42 (dd, 1H, *J* = 11.9, 1.9, H-6′), 4.88 (d, 1H, *J* = 8.0, H-1′), 4.92 (d, 1H, signal obscured with solvent peak, H-2′), 5.20 (d, 1H, *J* = 3.7, H-1), 6.78 (dd, 2H, *J* = 7.6, 1.8, H-3″, H-5″), 7.07 (s, 1H, H-3), 7.83 (dd, 2H, *J* = 7.2, 1.0, H-2″, H-6″); ^{13}C NMR (50 MHz, CD_3OD): δ 13.0, 19.1, 22.0, 22.4, 25.5, 29.4, 29.6 29.4, 30.7, 36.1, 40.1, 50.0, 63.1, 69.3, 72.4, 74.9, 75.2, 79.2, 92.5, 96.6, 113.5, 115.6, 122.0, 130.9, 148.0, 161.8, 166.0, 167.9, 176.1; MS (ESI): (M^+) 622. Elemental analysis calcd. for $C_{31}H_{42}O_{13}$, C = 59.80%, H = 6.80%. Found C = 59.86%, H = 6.68%.

5.14. 6′-O-Palmitoyl-negundoside (**8c**)

As a colorless solid; mp 158 °C; $[\alpha]_D^{25}$ −86.0 (c 0.8, CH_3OH); IR (KBr, cm^{-1}): 803, 1075, 1168, 1262, 1610, 1715, 2960, 2970, 2982; ^{1}H NMR (200 MHz, CD_3OD): δ 0.78 (t, 3H, *J* = 6.74, H-16‴), 1.21 (m, 27H, H-10, H-15‴ to H-4‴), 1.47 (m, 2H, H-6), 1.63 (m, 3H, H-7, H-3‴), 2.04 (m, 2H, H-7, H-9), 2.23 (t, 2H, *J* = 7.3, H-2‴), 2.89 (m, 1H, H-5), 3.20 (t, 1H, *J* = 9.5, H-3′), 3.56 (m, 1H, H-5′), 3.62 (t, 1H, *J* = 8.9, H-4′), 4.26 (dd, 1H, *J* = 11.8, 5.7, H-6′), 4.42 (dd, 1H, *J* = 11.9, 1.9, H-6′), 4.88 (d, 1H, *J* = 8.0, H-1′), 4.92 (d, 1H, signal obscured with solvent peak, H-2′), 5.17 (d, 1H, *J* = 3.7, H-1), 6.70 (dd, 2H, *J* = 8.6, 1.5, H-3″, H-5″), 7.01 (s, 1H, H-3), 7.75 (dd, 2H, *J* = 8.6, 1.6, H-2″, H-6″); ^{13}C NMR (125 MHz, CD_3OD): δ 13.1, 22.2, 23.2, 25.0, 29.3, 29.4, 32.5, 33.6, 35.0, 39.9, 51.1, 63.9, 70.9, 74.4, 75.0, 79.9, 89.0, 94.5, 96.7, 113.1,

115.0, 121.2, 132.5, 149.5, 162.5, 166.1, 168.1, 174.9; MS (ESI): (M^+) 734. Elemental analysis calcd. for $C_{39}H_{58}O_{13}$, C = 63.74%, H = 7.96%. Found C = 63.58%, H = 7.37%.

5.15. 6′-O-(2‴,3‴-Di-octanoylpropanoyl)-negundoside (8d)

As a syrupy liquid; $[\alpha]_D^{25}$ −42.5 (c 1.0, CH_3OH); IR (KBr, cm^{-1}): 805, 1077, 1169, 1262, 1609, 1715, 2960, 2970, 2982; ^1H NMR (200 MHz, CD_3OD): δ 0.78 (m, 6H, H-8⁗, H-8⁗′), 1.20 (m, 19H, H-10, H-7⁗ to H-4⁗, H-7⁗′ to H-4⁗′), 1.29 (m, 1H, H-6), 1.46 (m, 1H, H-6), 1.63 (m, 5H, H-7, H-3⁗, H-3⁗′), 2.04 (m, 2H, H-7, H-9), 2.30 (m, 4H, H-2⁗, H-2⁗′), 2.93 (m, 1H, H-5), 3.21 (t, 1H, J = 9.5, H-3′), 3.45 (m, 1H, H-5′), 3.56 (t, 1H, J = 8.9, H-4′), 4.20 (d, 2H, J = 5.5, H-3‴), 4.26 (dd, 1H, J = 11.9, 5.8, H-6′), 4.42 (dd, 1H, J = 11.9, 1.8, H-6′) 4.88 (d, 1H, J = 8.0, H-1′), 5.91 (d, 1H, signal obscured with solvent peak, H-2′), 5.21 (m, 1H, H-2‴), 5.26 (d, 1H, J = 3.7, H-1), 6.70 (dd, 2H, J = 8.5, 1.6, H-3″, H-5″), 7.01 (s, 1H, H-3), 7.75 (dd, 2H, J = 8.6, 1.6, H-2″, H-6″); ^{13}C NMR (125 MHz, $CDCl_3$): δ 12.8, 13.0, 22.2, 24.5, 24.6, 28.7, 29.3, 31.4, 33.5, 33.6, 42.0, 50.0, 62.7, 64.1, 72.1, 73.8, 74.9, 75.2, 80.0, 82.7, 95.4, 98.9, 113.1, 117.9, 121.9, 133.2, 149.3, 162.1, 166.1, 168.5, 173.5, 173.7, 175.1; MS (ESI): (M^+) 836. Elemental analysis calcd. for $C_{42}H_{60}O_{17}$, C = 60.28%, H = 7.23%. Found C = 60.16%, H = 7.08%.

Acknowledgements

The authors thank Director, IIIM-Jammu for his interest and encouragement. PPS, PBR, SS, DMR and AHB thank CSIR-New Delhi for the award of fellowship.

References[1]

[1] P. Junior, Planta Medica 56 (1990) 1, and reference cited therein.
[2] (a) A. Puri, R.P. Saxena, P.Y. Guru, D.K. Kulshreshtha, K.C. Sexena, B.N. Dhawan, Planta Med. 58 (1992) 528–532;
(b) Z.K. Khan, A. Manglani, P.K. Shukla, A. Puri, R. Saxena, J.S. Tandon, Int. J. Pharmacogn. 33 (1995) 297–304;
(c) M. Yoshikawa, T. Ueda, H. Matsuda, J. Yamahara, N. Murakami, Chem. Pharm. Bull. 42 (1994) 1691–1693;
(d) H.S. Garg, S.P.S. Bhandari, S.C. Tripathi, G.K. Patnaik, A. Puri, R.P. Saxena, Phytother. Res. 8 (1994) 224–228.
[3] W. Dorsch, H. Stuppner, H. Wagner, M. Gropp, S. Demoulin, J. Ring, Int. Arch. Allergy Appl. Immunol. 95 (1991) 128–133.
[4] (a) B.H. Dhawan, Med. Chem. Res. 5 (1995) 595–605;
(b) R.A. Ansari, Indian J. Med. Res. 87 (1998) 401;
(c) Y. Dwivedi, R. Rastogi, N.K. Garg, B.N. Dhawan, Acta Pharm. Sinica 13 (1992) 197–200;
(d) G.L. Floersheim, A. Bieri, R. Koenig, A. Pletscher, Agenta Actions 29 (1990) 386–387;
(e) P. Morazzani, E. Bombardelli, Fitoterapia 56 (1995) 3–42.
[5] (a) Y. Mizoguchi, C. Sagasami, Y. Ichikawa, S. Kobbayashi, Planta Med. 58 (1992) 394–397;
(b) S. Takeda, T. Endo, M. Aburada, J. Pharmacobio-Dyn. 4 (1981) 612–623.
[6] (a) A. Trovato, A.M. Forestieri, L. Iauk, R. Barbera, M.T. Momtforte, M.E. Galati, Plant. Med. Phytother. 26 (1993) 300–308;
(b) T. Mirra, Y. Nishiama, M. Ichimaru, M. Moriyasu, A. Kato, Biol. Pharm. Bull. 19 (1996) 160–161;
(c) A.K. Khanna, R. Chander, N.K. Kapoor, B.N. Dhawan, Phytother. Res. 8 (1994) 403–407.
[7] (a) N. Ivanovska, T. Iossifova, I. Kostova, Phytother. Res. 34 (1996) 2375–2379;
(b) G.B. Singh, S. Bani, S. Singh, A. Khajuria, M.L. Sharma, B.D. Gupta, S.K. Banerjee, Phytother. Res. 1 (1993) 402–407.
[8] A.V. Ortiz de urbina, M.L. Martin, B. Fernandez, L. San Roman, L. Cubillo, Planta Med. 60 (1994) 512–515.
[9] (a) K.R. Kirtikar, B.D. Basu, Indian Medicinal Plants, vol. III, Lalit Mohan Basu, Allahabad, 1935, pp. 1825–1826;
(b) A.C. Dey, S. Bhishen, P.S.J. Mahendra, Res. Indian Med. (1980) 81;
(c) G.N. Chaturvedi, R.H. Singh, J. Res. Indian Med. 1 (1966) 1–14;
(d) S.S. Handa, A. Sharma, K.K. Chakraborti, Fitoterapia 58 (1986) 307–351;
(e) W. Dorsch, H. Stuppner, H. Wagner, M. Gropp, S. Demoulin, J. Ring, Int. Arch. Allergy Appl. Immunol. 95 (1991) 128–133.
[10] (a) R. Hansal, C. Leuckert, H. Rimpler, K.D. Schaaf, Phytochemistry 4 (1965) 19;
(b) Sehgal, Photochemistry 21 (1982) 363;
(c) A. Prabhakar, B.D. Gupta, K.A. Suri, N.K. Satti, S. Malhotra, R.K. Johri, B.S. Jaggi, B.K. Chandan, A.K. Sharma, D.K. Gupta, B.K. Kapahi, K.L. Bedi, O. Suri, US Patent 20040029816 (2004) and references cited therein.
[11] (a) M.L. Sharma, C.S. Rao, P.L. Duda, J. Ethnopharmacol. 41 (1994) 185–192;
(b) A. Gupta, A. Khajuria, J. Singh, K.L. Bedi, N.K. Satti, Prabhu Dutt, K.A. Suri, O.P. Suri, G.N. Qazi, International Immunopharm. 6 (2006) 1543–1549;
(c) A. Khajuria, A. Gupta, S. Singh, F. Malik, J. Singh, K.A. Suri, N. Satti, G.N. Qazi, V.K. Srinivas, Vaccine 25 (2007) 2706–2715.
[12] (a) F. Bjorkling, S.E. Godtfredsen, O. Kirk, Trends Biotechnol. 9 (1991) 360;
(b) J. Crosby, Tetrahedron 47 (1991) 4789;
(c) B.D. Martin, S. Ampofo, R.J. Linhardt, J.S. Dordick, Macromolecules 25 (1992) 7081;
(d) X. Chen, A. Johnson, J.S. Dordick, D.G. Rethwisch, Macromol. Chem. Phys. 195 (1994) 3567;
(e) R.T. Otto, U.T. Bornscheuer, H. Scheib, J. Pleiss, C. Syldatk, R.D. Schmid, Biotechnol. Lett. 20 (1998) 1091.
[13] (a) S. Riva, J. Mol. Catal. B: Enzymatic 19–20 (2002) 43–54;
(b) A. Kontogianni, V. Skouridou, H. Sereti, H. Stamatis, F.N. Kolisis, Eur. J. Lipid Sci. Technol. 103 (2001) 655–660.
[14] (a) A. Intra, A. Bava, G. Nasini, S. Riva, J. Mol. Catal. B: Enzymatic 29 (2004) 95–98;
(b) N. Wang, Q. Wu, B.K. Liu, Y. Ca, X.F. Lin, J. Mol. Catal. B: Enzymatic 27 (2004) 97–102.
[15] H.M. Sampath Kumar, S. Joyswal, M.S. Rao, J.S. Yadav, Tetrahedron Asymmetry 15 (2004) 127–130.
[16] B. Neises, W. Steiglich, Org. Synth. Coll. 7 (1990) 93.
[17] (a) K. Weinges, K. Künstler, Liebigs Ann. Chem. (1977) 1053–1057;
(b) K. Weinges, P. Kloss, W.D. Henkels, Liebigs Ann. Chem. 759 (1972) 173–182;
(c) I. Kitagawa, K. Hino, T. Nishimura, E. Iwata, I. Yosioka, Chem. Pharm. Bull. 19 (1971) 2534–2544;
(d) D.Q. Wang, Z.D. He, B.S. Feng, C.R. Yang, Acta Bot. Yunnan. 15 (1993) 83–88.
[18] (a) C.K. Sehgal, S.C. Taneja, K.L. Dhar, C.K. Atal, Phytochemistry 21 (1982) 363–366;
(b) P.K. Dutta, U.S. Chowdhury, A.K. Chakravarty, B. Achari, S.C. Pakrashi, Tetrahedran 39 (1983) 3067–3072.
[19] (a) H. Kunz, O. Seitz, H. Waldmann, Angew Chem. Int. Ed. Engl. 23 (4) (1984) 71;
(b) O. Seitz, H. Kunz, J. Org. Chem. 62 (4) (1996) 813–826.

[1] IIIM communication SCL07/9.