NATURAL PRODUCT RESEARCH, 2017
VOL. 31, NO. 6, 615–625
http://dx.doi.org/10.1080/14786419.2016.1209670

Taylor & Francis
Taylor & Francis Group

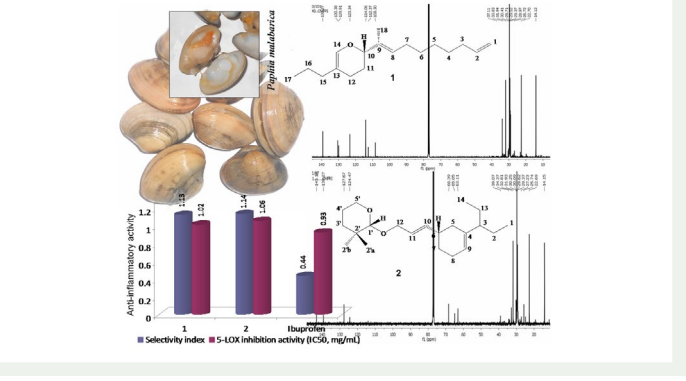
First report of two new antioxidative meroterpeno 2*H*-pyranoids from short-necked yellow-foot clam *Paphia malabarica* (family: Veneridae) with bioactivity against pro-inflammatory cyclooxygenases and lipoxygenase

Minju Joy and Kajal Chakraborty

Marine Bioprospecting Section of Marine Biotechnology Division, Central Marine Fisheries Research Institute, Cochin, India

ABSTRACT

Two new meroterpeno 2*H*-pyranoids were isolated from the EtOAc:MeOH extract of yellow-foot clam *Paphia malabarica*. The structures of these newly reported compounds were elucidated based on spectroscopic interpretations. This is the first report of biogenic 2*H*-pyrans bearing decadienyl and allyloxy-(isopentanyl)-cyclohexene skeletons from marine biota. The extended C_{18} sesquiterpenoid with prenylated irregular farnesene framework was characterised as 2-((*E*)-deca-1,8-dien-10-yl)-11,12-dihydro-13-propyl-2*H*-pyran (**1**). The compound **2**, 1′-((10*E*)-10-(10-(pentan-4-yl)-cyclohex-4-enyl)-allyloxy)-tetrahydro-2′,2′-dimethyl-2*H*-pyran represents the first example of naturally occurring C_{21} prenylated bisabolene-type meroterpenoid, whereas tetrahydro-2′,2′-dimethyl-2*H*-pyran remains attached at C-2′ position of rearranged bisabolene framework formed by allyloxy linkage. The antioxidant activities (DPPH/ABTS^{+}) of **1** and **2** were comparable (IC_{50} < 1.0 mg/mL) with *α*-tocopherol. In addition, these compounds exhibited greater activity against cyclooxygenase-2 than COX-1, and the selectivity indices were significantly lesser (~1.1). No significant differences in anti-5-lipoxygenase activity of **1** and **2** (IC_{50} 1.02–1.06 mg/mL) than ibuprofen (IC_{50} 0.93 mg/mL) indicated the potential anti-inflammatory properties of title compounds.



ARTICLE HISTORY
Received 8 April 2016
Accepted 21 June 2016

KEYWORDS
Paphia malabarica; meroterpeno 2*H*-pyranoids; antioxidative; anti-inflammatory; cyclooxygenases; lipoxygenase

CONTACT Kajal Chakraborty kajal.chakraborty@icar.gov.in
Supplemental data for this article can be accessed here at http://dx.doi.org/10.1080/14786419.2016.1209670.

1. Introduction

Pyran derivatives, an important category of organic compounds, which proved to constitute an important class of heterocycle, were found to occur in marine organisms, and attracted a great deal of interest due to their pharmacological potentials (Cueto et al. 1998a; Ciavatta et al. 2011). The occurrence of pyranoids in biological system and their role as precursors in the production of pharmacologically active metabolites as anti-fungal, anti-coagulant, anti-inflammatory, antimicrobial and anti-cancer compounds were reported from natural and synthetic origin (Arora & Mathur 1963; Goel & Ram 2009; Nemouchi et al. 2012; Rajguru et al. 2013). The pyranoid cladiellane diterpenes were isolated from marine mollusk, *Tritoniopsis elegans* (Ciavatta et al. 2011) and naturally occurring 1-(6-butyl-3, 4-dihydro-2*H*-pyran-2yl)-pentanone was reported from a marine invertebrate, *Neosadocus maximus* as their self-defence agents (Rocha et al. 2011). The bioactive pyranoid diterpenes were isolated from soft coral, *Lobophytum pauciflorum* (Govindam et al. 2012). Polyoxygenated marine monoterpenes pantopyranoids A–C, and pantoisofuranoids A–C, oxane derivative monoterpenes and monoterpenes with tetrahydrofuran ring have been isolated from the Antarctic marine alga *Pantoneura plocamioides* (Cueto & Darias 1996; Cueto et al. 1998b). 6-pentyl-2*H*-pyran-2-one and its analogs showed antibacterial properties (Parker et al. 1997) and furanoid monoterpenes, furoplocamioids A–C from the marine red alga *Plocamium cartilagineum* were earlier reported (Darias et al. 2001).

The short-necked yellow-foot clam *Paphia malabarica* (family, Veneridea) is a benthic filter feeding bivalve, and is distributed mainly in the estuarine habitats on the southern coasts of India. As part of our ongoing programme, we aimed at the isolation of biologically active compounds from crude EtOAc:MeOH extract (1:1 v/v) of *P. malabarica*, collected from the south-west coast of Arabian Sea, showed promising antioxidative and anti-inflammatory activities by various *in vitro* assays. Its chemical constituents have not been reported previously, suggesting *P. malabarica* would be an attractive source for chemical investigation. In view of this, *P. malabarica* was chosen as a research target, and two unprecedented meroterpeno 2*H*-pyranoids were obtained, 2-((*E*)-deca-1,8-dien-10-yl)-11,12-dihydro-13-propyl-2*H*-pyran (**1**) and 1′-((10*E*)-10-(10-(pentan-4-yl)-cyclohex-4-enyl)-allyloxy)-tetrahydro-2′, 2′-dimethyl-2*H*-pyran (**2**). The structures were elucidated with the help of detailed spectroscopic analysis and the isolated compounds were tested against several pro-inflammatory enzymes (COX-1, 2 and 5-LOX) as well as antioxidant activities (DPPH/$ABTS^+$ radical scavenging assays) to evaluate their potential biological activities, and the results obtained are described herein. It is also noteworthy that the compounds **1**–**2** represent the first example of naturally occurring meroterpeno 2*H*-pyranoids featuring unique decadienyl and allyloxy-(isopentanyl)-cyclohexene skeletons possessing rare C_{18} and C_{21} prenylated irregular bisabolene frameworks.

2. Results and discussion

2.1. General

Two (**1**–**2**) new meroterpeno 2*H*-pyranoids characterised as 2-((*E*)-deca-1,8-dien-10-yl)-11,12-dihydro-13-propyl-2*H*-pyran (**1**) and 1′-((10*E*)-10-(10-(pentan-4-yl)-cyclohex-4-enyl)-allyloxy)-tetrahydro-2′,2′-dimethyl-2*H*-pyran (**2**) have been isolated from the EtOAc:MeOH (1:1, v/v) extract of the freeze dried powder of the edible part separated from *P. malabarica*. These

compounds are new marine natural products that possess a hitherto unknown pyranyl regiochemistry, with C_{18} sesquiterpenoid with prenylated irregular farnesene framework as in compound **1**. The compound **2** incorporate unusual naturally occurring C_{21} prenylated rearranged bisabolene type meroterpenoid with the allyloxy linkage coordinating between the C_{14} meroterpene and substituted terahydropyran network.

2.2. Spectral analyses of compounds from P. malabarica

The compound **1**, 2-((*E*)-deca-1,8-dien-10-yl)-11,12-dihydro-13-propyl-*2H*-pyran, a new C_{18} sesquiterpenoid with prenylated irregular framework, was isolated as yellow amorphous powder upon repeated chromatographic purifications over silica gel columns and preparatory TLC plates. The detailed ^1H, ^{13}C NMR and mass spectral analysis confirmed the structure (Table S1, Figures S1–S2). The title compound exhibited a molecular ion peak at *m/e* 262 (HRESIMS *m/e* 262.1604 ($[M]^+$), along with ^1H and ^{13}C NMR spectra, signifying the elemental composition as $C_{18}H_{30}O$ with 4° of unsaturation associated with three double bonds and one ring system. The ^1H–^1H COSY couplings from δ_H 4.68 (assigned to H-10)/δ_H 1.58, 1.60 (H-11)/δ_H 2.02, 2.00 (H-12) were apparent (Table S1, Figures 1(A) and S4). The downfield methine (–CH) at δ_H 4.68 was found to be a triplet, HSQC with δ_C 108.3 was attributed to the part of a pyran ring system (Figure S5). The quaternary carbon at C-13 (δ_C 112.37) recognised as the junction point of pyran ring system and the propyl moiety. However, the lower chemical shift value of quaternary carbon predicted that it might be adjacent to highly electron withdrawing group with high downfield shift at δ_C 123.34/δ_H 6.96. The higher chemical shift of this alkenic proton was due to the neighboring oxygen atom and found to be a singlet proton, but not related to an aromatic ring. The HMBC relation from δ_H 6.96 (assigned as H-14) to δ_C 108.30 (C-10), 112.37 (C-13); δ_H 4.68 (H-10) to δ_C 112.37 (C-13); δ_H 1.58 (H-11) to δ_C 29.71 (C-12); and δ_H 2.02 (H-12) to δ_C 108.30 (C-10) established the 2*H*-pyran ring system (Table S1, Figure S6). The signal at δ_C 112.37 (assigned to C-13) attached to a propyl side chain was evident from the HMBC relations from the protons of propyl moiety to the pyran ring (Figure 1(A)). The two methylenes at δ_C 37.11 (C-5a), δ_C 22.7 (C-5b) and methyl at δ_C

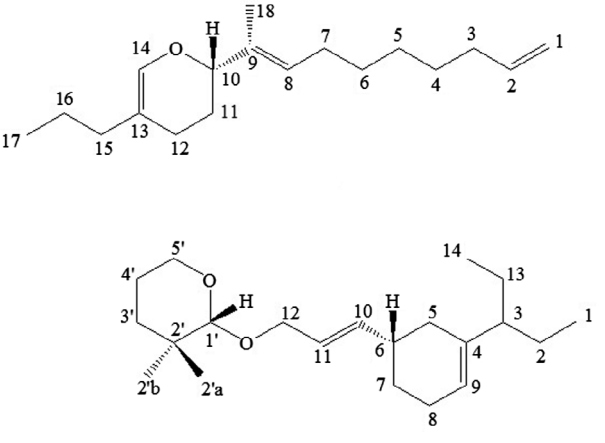


Figure 1. Structures of **(A)** 2-((*E*)-deca-1, 8-dien-10-yl)-11, 12-dihydro-13-propyl-2*H*-pyran **(1)** and **(B)** 1′-((10*E*)-10-(10-(pentan-4-yl)-cyclohex-4-enyl)-allyloxy)-tetrahydro-2′, 2′-dimethyl-2*H*-pyran **(2).**

14.12 (C-5c) were in good agreement with the reported 2*H*-pyranoid compound, 1-(6-butyl-3, 4-dihydro-2*H*-pyran-2-yl)-pentanone (Rocha et al. 2011). The long range couplings from δ_H 2.02 (H-12) to δ_C 37.11 (C-15)/δ_C 22.70 (C-16); δ_H 1.37 (H-16) to δ_C 29.71 (C-12); and δ_H 6.96 (H-14) to δ_C 37.11 (C-15) confirmed the attachment to pyran moiety and ^{1}H–^{1}H COSY correlations between δ_H 2.03 (H-15)/δ_H 1.37 (H-16)/δ_H 0.88 (H-17) established the propyl side chain. The proton at δ_H 4.68 (H-10) exhibited HMBC relations with δ_C 129.91 (C-9) implied the attachment to the alkenic quaternary carbon of deca-1,8-dien-10-yl side chain. The signal at δ_C 129.91 was found to be due to the quaternary carbon, and was attached to an alkenic proton, δ_H 5.35 (corresponding to the ^{13}C NMR signal at δ_C 130.38 at C-8 position) (Wang et al. 2010). The singlet proton at δ_H 1.44 (H-18) was attached to δ_C 30.41 exhibiting HMBC relations to δ_C 129.91 (C-9), δ_C 130.38 (C-8), δ_C 31.94 (C-7) and δ_C 108.30 (C-10). It was found that the group of carbons from C-1 to C-9 were linearly aligned as assigned by the ^{1}H–^{1}H COSY correlation analyses. The ^{1}H–^{1}H COSY correlations from δ_H 4.94/δ_H 5.10 (H-1)/δ_H 5.81 (H-2)/δ_H 2.06 (H-3)/δ_H 1.36 (H-4)/δ_H 1.26 (H-5)/δ_H 1.30 (H-6)/δ_H 1.97 (H-7)/δ_H 5.35 (H-8) and long range HBMC correlations from δ_H 1.97 (H-7) to δ_C 130.38 (C-8)/δ_C 129.91 (C-9)/δ_C 108.30 (C-10); δ_H 1.26 (H-5) to δ_C 33.83 (C-3)/δ_C 29.37 (C-4)/δ_C 28.97 (C-6); δ_H 2.06 (H-3) to δ_C 139.27 (C-2)/δ_C 114.06 (C-1); δ_H 5.81 (H-2) to δ_C 114.06 (C-3), and δ_H 5.01/4.94 (H-1) to δ_C 139.27 (C-2)/δ_C 33.83 (C-3) unambiguously confirmed the presence of side straight chain of **1** (Figure 1(A)). The >CH_2 group at δ_H 5.01 and δ_H 4.94 were found to be considerably downfield due to its terminal position and the presence of highly downfielded alkene (–CH=, δ_H 5.81). The geometrical arrangement of these alkenic protons was confirmed from their *J* values, which were 5.81 (*J* = 10.21, 6.99 Hz), 5.01 (*J* = 14.52 Hz) and 4.94 (*J* = 10.21 Hz), thus established the *trans* (*E*) configuration comparable with a related compound, lobatriene, identified from soft coral, (Govindam et al. 2012). The ^{13}C NMR spectrum of this compound in combination with DEPT indicated the presence of a total of 18 carbons, which enclosed two CH_3, ten CH_2, and four CH groups. The relative stereochemistries of the chiral centre of **1**, particularly that of C-10 carrying the methine proton, δ 4.68 (1H, t) was deduced from the NOESY spectrum (Figure S7) and their *J*-values. NOE correlations between the protons, δ 1.60 (H-11)/δ 4.98 (H-1)/δ 5.81 (H-2)/δ 2.00 (H-12)/δ 4.68 (H-10), δ 5.35 (H-8) indicated the close proximity of these groups and their α-disposition (Figure S17). Further NOE couplings were observed between the protons at δ 1.58 (H-11), δ 2.02 (H-12)/δ_H 5.01 (H-1)/δ_H 1.43 (H-18)/δ_H 6.96 (H-14) which indicated that these groups are on the same side of the plane of the molecule, and disposed in β-orientation. Notably, the geometric isomerism of the olefinic protons δ 4.94 and δ 5.01 (H-1) had a large coupling constant (*J* = 10.2 and 14.5 Hz, respectively) which showed the *E* configuration of the olefinic bond. Additionally, the large coupling constant of 10.2 Hz (each) between the pertinent olefinic protons at 5.81 (related to H-2$^\alpha$) and δ 5.35 (assigned to H-8$^\alpha$, bearing C8–C9 double bond) revealed that they are disposed in the same plane of geometry; all these effects are in accord with the *J* values observed and indicate the stereochemistry. The protons at δ_H 1.44 (CH_3, s, C-18) was found to be situated at β position because of NOE relationship with δ_H 6.96 (H$^\beta$-14, s), and has no NOE associations with the protons at δ_H 4.94 (H$^\alpha$-1) and δ_H 4.68 (H$^\alpha$-10) which suggested the *cis*-orientation for the methyl groups at C-18 and olefinic proton at C-14, and that these groups must be disposed on the β-side. The H-10 should be trans-orientation with the methyl protons at C-18 and olefinic proton at C-14 since there was no cross peak could be detected between H-14 and H-10 in NOESY experiment. The IR spectrum revealed the presence of olefinic (C=C) and alkyl (C–H) bending vibrations that were represented by the 1661 and 1455/965 cm^{-1} absorption

bands, respectively. The absorption bands at 2932/2860 cm^{-1} indicated C–H stretching vibration, whereas those at 1372, 1242, 1187, 1108 and 1042 cm^{-1} showed the C–O stretch, thereby substantiated the structure of prenylated sesquiterpeno pyranoid framework. The mass spectrum exhibited the molecular ion peak at *m/e* 262, which appeared to undergo elimination of methyl radical to yield a radical ion fragment at *m/e* 247 (**1a**, attributed to dihydro-2-(nona-1,8-dienyl)-13-propyl-2*H*-pyran). The latter appeared to undergo fragmentation by eliminating $^{\cdot}CH_3$ and $^{\cdot}C_2H_3$ radicals to obtain fragment peaks at *m/e* 233 (**1d**, 13-ethyl-dihydro-2-(nona-1, 8-dienyl)-2*H*-pyran) and *m/e* 220 (**1b**, 2-(hept-1-enyl)-9,10-dihydro-11-propyl-2*H*-pyran), respectively. Fragmentation of the ion at *m/e* 220 (**1b**) was perceived to be accompanied by the loss of a C-3 fragment resulting in an ion at *m/e* 179 (**1c**), which on subsequent rearrangement yielded the fragments at *m/e* 110 (**1f**, 5-methylhex-5-en-2-ol) and 97 (**1g**, dihydro-5-methyl-2*H*-pyran). The latter eliminated CH_2O radical to yield *m/e* 66 (**1j**, penta-1,3-diene). The fragment peak at *m/e* 85 (assigned to tetrahydro-2*H*-pyran) was found to be the base peak (Figure S8).

The compound **2**, 1′-((10*E*)-10-(10-(pentan-4-yl)-cyclohex-4-enyl)-allyloxy)-tetrahydro-2′,2′-dimethyl-2*H*-pyran, a naturally occurring C_{21} prenylated bisabolene type meroterpenoid, was isolated as a amorphous powder on repeated chromatographic purifications over silica gel. The molecular ion peak was recorded at HRESIMS *m/e* 320.2715 ($[M]^+$) and ^1H–^{13}C spectral details established the elemental composition as $C_{21}H_{36}O_2$ with four double bond equivalence enclosing two double bonds and two cyclic system. The singlet at δ_H 4.59 (assigned to C-1′) attributed to the methine (–CH) group, in which the upfield shift of the corresponding carbon (δ_C 68.39) was due to the electronegative –O–C moiety (Table S1, Figures S3, S9 and S10). This carbon (C-1′) was also attached to an oxygenated side chain as apparent from the HMBC correlations. The long range couplings from δ_H 4.59 (H-1′) to δ_C 25.74 (C-4′), δ_C 39.07 (C-2′), δ_C 30.25 (C-2′a) and δ_C 30.09 (C-2′b) proved that it was a part of pyran moiety. The ^1H–^1H COSY revealed that the presence of four spin systems, H-3′ to H-6′ {(δ_H 1.63, 1.66 (H-3′)/δ_H 1.55, 1.56 (H-4′)/δ_H 3.64 (H-5′)} in the pyran ring, H-11 to H-12 {(δ_H 4.12, 4.14 (H-12)/δ_H 5.35 (H-11)} in the allyloxy chain, H-5 to H-9 {δ_H 2.02 (H-5)/δ_H 2.35 (H-6)/δ_H 1.57, 1.59 (H-7)/δ_H 1.99, 2.00 (H-8)/δ_H 5.34 (H-9)} including H-6 to H-10 {δ_H 2.35 (H-6)/δ_H 5.38 (H-10)} in the cyclohexenyl ring and from H-1 to H-3 consisting of H-3/H-13 to H-14 {(δ_H 0.89 (H-13)/δ 1.43 (H-14), δ 0.87 (H-1)/δ 1.42 (2-H)/δ 2.31 (H-13)} in the isopentanyl side chain (Table S1, Figures 1(B) and S12). Two intense singlet protons due to methyl (–CH_3) at δ_H 1.48 and δ_H 1.46 showed HSQC correlation with δ_C 30.25 (C-2′a) and δ_C 30.09 (C-2′b), respectively, which were attached to a quaternary carbon at δ_C 39.07 (Figure S13). This was apparent from the HMBC correlations, δ_H 1.46/1.48 (C-2′a/2′b) to δ_C 63.11 (C-5′), δ_C 39.07 (C-2′), which proved the attachment of dimethyl groups to the pyran ring (Table S1, Figure S14). The HMBC relations between δ_H 1.55 (H-4′) to δ_C 29.62 (C-3′), δ_C 30.25 (C-2′a), δ_C 30.09 (C-2′b) and δ_H 3.64 (H-5′) to δ_C 25.74 (C-4′) established the pyran moiety (Table S1, Figure 1(B)). The attachment of carbon atom in the allyloxy side chain to pyran network was evident. The methine (–CH) groups, δ_H 5.38/δ_C 139.27 and δ_H 5.35/δ_C 127.67 at H-10 and H-11, respectively with large coupling constants (J = 9.18 and 8.58, respectively) revealed its *trans* (*E*) geometry (assigned to C10=C11). The cyclohexenyl ring system enclosed –C=CH moiety in which the olefinic quaternary carbon (–C=) registered higher chemical shift of δ 143.18 (C-4) compared to the methine (=CH; δ 124.47) and a pentanyl group attached to the quaternary carbon attributed to C-12. This cyclic system exhibited HMBCs from δ_H 5.38 (H-10) to δ_C 31.93 (C-5), δ_H 2.35 (H-6) to δ_C 29.37 (C-7). The spectral data for quarternary olefinic carbon at δ_C 143.18 (C-4) and the methine

at δ_C 68.39 (C-1′) were comparable with 1-(6-butyl-3, 4-dihydro-2*H*-pyran-2yl)-pentanone (Rocha et al. 2011). The HMBC couplings from δ_H 0.89 (H-14) to δ_C 22.69 (C-13), δ_H 1.43 (H-13) to δ_C 34.38 (C-3)/δ_C 143.18 (C-4)/δ_C 31.93 (C-5), δ_H 1.42 (H-2) to δ_C 34.38 (C-3)/δ_C 143.18 (C-4), δ_H 0.87 (H-1) to δ_C 34.38 (C-3)/δ_C 22.69 (C-2) confirmed the attachment of isopentanyl moiety to the cyclohexenyl ring system (Figure 1(B)). The ^{13}C NMR and DEPT spectra identified the presence of 21 carbon atoms, in which four CH_3, nine CH_2, six CH were accounted for in the compound (Table S1, Figures S10 and S11). The relative stereochemistries of compound **2**, mainly at C-1′ and C-6 protons, δ_H 4.59 (1H) and δ_H 2.35 (1H) were confirmed from the NOESY spectrum (Figure S17). The NOE couplings between δ_H 1.55 (H-4′)/δ_H 4.59 (H-1′), δ_H 2.39 (H-6), δ_H 2.00 (H-8) along with δ_H 2.35 (H-6)/δ_H 4.12 (H-12) demonstrated that these protons are in the same plane of geometry and *α*-disposed (Figure S15). Further the NOE relations among the protons H-8 (δ_H 1.99), H-3′ (δ_H 1.66), H-12 (δ_H 4.14) and H-2′b (δ_H 1.46, CH_3) showed that they are disposed in the identical plane of geometry, and are disposed at *β*-orientation. The methyl protons at δ_H H-2′b (δ_H 1.46) was found to be *β* disposed with the reference plane due to the NOE correlations with δ_H 4.14 (H^{β}-12), and not with the protons at δ_H 4.59 (H^{α}-1′) and δ_H 2.39 (H^{α}-6). This further explained the trans-orientation of the methine and methyl groups at C-6 and C-2′b positions of **2**. The distinctive IR absorptions at 2932 and 2860 cm^{-1} were due to the C–H alkane stretching, whereas the olefinic (C=C) stretching and =C–H bending were represented by the 1661 and 965 cm^{-1} absorption bands, respectively. The distinctive absorption at 1456 cm^{-1} indicated bending vibration due to C–H groups. The fourier-transform infrared (FTIR) absorption bands at 1372, 1242, 1187, 1108 and 1042 cm^{-1} substantiated the C–O stretching vibration. The molecular ion peak at *m/e* 320 ($[M]^+$) appeared to undergo elimination of one $-C_2H_5$ group to yield 1′-(10-(3-propylcyclohexyl) propoxy)-tetrahydro-2′,2′-dimethyl-2*H*-pyran (**2a**, *m/e* 296). The elimination of $-C_3H_7$ group from the fragment ion at *m/e* 296 yielded the fragment with *m/e* 253 (**2b**, attributed to 1′-(3-cyclohexylpropoxy)-tetrahydro-2′,2′-dimethyl-2*H*-pyran) and *m/e* 254 (**2c**, 1′-(4-methyloctyloxy)-tetrahydro-2′,2′-dimethyl-2*H*-pyran). The elimination of one $-CH_3$ group from the fragment ion at *m/e* 254 yielded the fragments with *m/e* 238 (**2d**, attributed to 1′-(4-methylenehept-1-enyloxy)-tetrahydro-2′,2′-dimethyl-2*H*-pyran). The elimination of $-C_3H_5$ group from the fragment ion at *m/e* 238 yielded the fragments with *m/e* 198 (**2e**, attributed to 1′-(pent-2-enyloxy)-tetrahydro-2′,2′-dimethyl-2*H*-pyran) and *m/e* 184 (**2f**, attributed to 1′-(butoxy)-tetrahydro-2′,2′-dimethyl-2*H*-pyran), which on subsequent rearrangement yielded the fragments at *m/e* 142 (**2h**, 1′-(allyloxy)-tetrahydro-2*H*-pyran), 102 (**2j**, tetrahydro-2*H*-pyran-2-ol), 86 (pentan-1-ol) and 73 (**2l**, but-3-en-1-ol). The fragment peak at *m/e* 85 (**2k**, assigned to tetrahydro-2*H*-pyran) appeared as base peak of **2** (Figure S16).

2.3. Antioxidant and anti-inflammatory activities

The antioxidant activities of **1** and **2** as determined by *in vitro* DPPH (IC_{50} 0.76–0.78 mg/mL) and $ABTS^+$ radical scavenging properties (IC_{50} 0.92–0.96 mg/mL) were comparable to that of *α*-tocopherol (0.6–0.7 mg/mL) used in various commercial preparations. In addition, it was found that compounds exhibited greater activity against cyclooxygenase-2 (COX-2) when compared to COX-1, and therefore, their selectivity indices (anti-COX-1 IC_{50}/anti-COX-2 IC_{50}) were significantly lesser (~1.1) than the synthetic anti-inflammatory drug ibuprofen (0.44) (Table 1). No significant differences in anti-5-lipoxidase (5-LOX) activity of **1** and **2** (IC_{50} 1.02–1.06 mg/mL) than ibuprofen (IC_{50} 0.93 mg/mL) indicated the potentially selective

Table 1. Antioxidant and anti-inflammatory activities of meroterpeno 2*H*-pyranoids (**1** and **2**) isolated from *Paphia malabarica* vis-à-vis the commercially available ingredients.

	IC_{50} values (mg/mL)		
Antioxidant activities	Compound 1	Compound 2	*α*-tocopherol
[c]DPPH scavenging activity	$0.78^{a} \pm 0.02$	$0.76^{a} \pm 0.09$	$0.63^{b} \pm 0.04$
[c]$ABTS^{+}$ scavenging activity	$0.92^{a} \pm 0.06$	$0.96^{a} \pm 0.02$	$0.73^{b} \pm 0.05$
Anti-inflammatory activities	Compound 1	Compound 2	Ibuprofen
[c]COX-1 inhibition activity	$1.07^{a} \pm 0.07$	$1.05^{a} \pm 0.03$	$0.04^{b} \pm 0.00$
[c]COX-2 inhibition activity	$0.95^{a} \pm 0.03$	$0.92^{a} \pm 0.08$	$0.09^{b} \pm 0.02$
[d]Selectivity index	$1.13^{a} \pm 0.06$	$1.14^{a} \pm 0.03$	$0.44^{b} \pm 0.03$
[c]5-LOX inhibition activity	$1.02^{a} \pm 0.07$	$1.06^{a} \pm 0.01$	$0.93^{b} \pm 0.11$

[c]The bioactivities were expressed as IC_{50} values (mg/mL).
[d]Selectivity index has been calculated as the ratio of anti-COX-1 (IC_{50}) and anti-COX-2 (IC_{50}).
Notes: The samples were analyzed in triplicate ($n = 3$) and expressed as mean ± standard deviation. Means followed by the different superscripts (a and b) within the same row indicate significant differences ($p < 0.05$).

anti-inflammatory properties of the title compounds without any adverse side effects. It is apparent that the synthetic non-steroidal anti-inflammatory drugs, such as ibuprofen has greater inhibitory effect towards the COX-1 isoform of cyclooxygenase, which is considered to be a constitutive enzyme, and is a vital component of various metabolic functions. Notably, ibuprofen, a commonly used anti-inflammatory and painkiller, recorded greater inhibitory properties towards COX-1 (IC_{50} 0.04 mg/mL) than COX-2 isoform (IC_{50} 0.09 mg/mL) resulting in lesser selectivity index (Table 1). The title compounds purified from *P. malabarica* can be used as potential selective inhibitors of COX-2 with significantly lesser side effect profiles, such as renal and gastric damage than the present therapies by using non-steroidal anti-inflammatory drugs used to combat inflammatory disorders.

To the best of our knowledge, 2-((*E*)-deca-1,8-dien-10-yl)-11,12-dihydro-13-propyl-2*H*-pyran (**1**) and 1′-((10*E*)-10-(10-(pentan-4-yl)-cyclohex-4-enyl)-allyloxy)-tetrahydro-2′,2′-dimethyl-2*H*-pyran (**2**) represent the first description of meroterpeno 2*H*-pyranoids possessing the C_{18} sesquiterpenoid with prenylated irregular farnesene framework and C_{21} prenylated bisabolene-type meroterpenoid with the allyloxy linkage coordinating between the C_{14} meroterpene and substituted terahydropyran system from a natural source. These unprecedented meroterpeno 2*H*-pyranoids from *P. malabrica* has bioactive potential as natural antioxidant and anti-inflammatory pharmacophore.

3. Experimental

3.1. Chemicals and instrumentation

All compounds, reagents and solvents were of spectroscopic/chromatographic/analytical grade and were obtained from Merck (Darmstadt, Germany). FTIR spectra (KBr) recorded in a Perkin-Elmer Series 2000 FTIR spectrophotometer scanning between 4000 and 400 cm^{-1}. GC-MS analyses were performed in electronic impact ionisation mode in a Perkin-Elmer Clarus 680 GC-MS fitted with a Elite 5 MS non-polar, bonded phase capillary column (50 m × 0.22 mm i.d. × 0.25 µm film thicknesses). Helium (He) was used as the carrier gas, and the flow rate used was 1 mL/min. The injection volume was 1 µL in a split mode and temperature of the injector was 280 °C. Electron ionisation energy was set at 70 eV. The scan rates were set as 10 spectra and ion source temperature was maintained at 200 °C. ESI-MS

spectra were acquired on a liquid chromatography-mass spectrometry system (Applied Biosystems QTrap 2000, Applied Biosystems, Darmstadt, Germany). 1D and 2D NMR spectra were recorded on a Bruker Avance DPX 500 (500 MHz) spectrometer in $CDCl_3$ solvent at ambient temperature with TMS as the internal standard (δ 0 ppm). Standard pulse sequences were used for DEPT, ^1H–^1H COSY, NOESY, HSQC and HMBC experiments. All the reagents and solvents used in this study were of analytical grade and purchased from E-Merck.

3.2. Sample collection and preparation of crude extracts

The clam samples, *P. malabarica* (10 kg) (voucher specimen No. CMFRI/MoES/DFS/AC 368) were freshly collected from Ashtamudi Lake (8°59′ N and 76°36′ E) situated along the south-west coast of India. The edible portion (6 kg) was separated from the cleaned shell-on samples were homogenised and freeze-dried by lyophilisation (Martin Christ alpha 1-4 LD Plus freeze drier, Germany). The dried powder (1.2 kg, yield 20%) was extracted with EtOAc–MeOH (1:1, v/v, 500 mL × 3) at 40 °C followed by shaking (8 h) and sonication (8 h). The extracts were filtered over anhydrous Na_2SO_4 (100 g), before being evaporated *in vacuo* using a rotary vacuum concentrator (50 °C) (Heidolf, Germany) to afford a dark brown viscous residue, which was considered as the crude extract of *P. malabarica* (55.0 g, yield on dry basis 4.58%).

3.3. Chromatographic purification and spectral analysis of pure compounds from P. malabarica

The EtOAc:MeOH crude extract of *P. malabarica* (45.0 g) was slurried with silica gel (4 g, 60–120 mesh), and packed into a column (1000 mm × 40 mm) containing silica (60–120 mesh). The column was initiated by increasing the polarity from 100% *n*-hexane followed by ethyl acetate and methanol to attain 16 fractions of 25 mL each, which were pooled six different factions (PM_1–PM_6) after TLC analysis. The fraction PM_4 (eluted at 70% EtOAc:*n*-hexane) was selected for further purification to afford pure bioactive metabolites since the percentage yield (3.53 g, yield 7.84%) and bioactive potentials (antioxidant and anti-inflammatory) were significantly higher for PM_4 fraction compared to other sub-fractions. This fraction was flash chromatographed (Biotage AB SP1-B1A, Uppsala, Sweden) on a silica gel column (Biotage, 230–400 mesh, 12 g, Biotage No. 25 + M) at a collection UV wavelength of 258 nm and a monitor wavelength of 264 nm with a step gradient elution of *n*-hexane/EtOAc/MeOH to afford 29 fractions (10 mL each), which were pooled to seven fractions (PM_{4-1}–PM_{4-7}) based upon analytical TLC. The fractions PM_{4-1} and PM_{4-3} were found to exhibit higher in antioxidant activity against DPPH radical and higher yield compared to other fractions. The fraction PM_{4-1} was further fractionated over PTLC on silica gel GF_{254} using *n*-hexane:EtOAc (49:1, v/v) to afford the compound **1** (90 mg; 94% purity by C_{18} $HPLC_{RP}$, MeOH:ACN, 3:2 v/v) as major component, which was homogenous by TLC (Si gel GF_{254} 15 mm; 100% *n*-hexane, R_f: 0.75). The fraction PM_{4-3} was fractionated over preparatory TLC over silica GF_{254} using *n*-hexane:EtOAc (22:3, v/v) to afford the compound **2** (118 mg; 97% purity by C_{18} $HPLC_{RP}$, MeOH: ACN, 3:2 v/v) as the main component. Evaporation of solvents from the fractions followed by TLC (silica GF_{254} using 5% EtOAc:*n*-hexane, R_f: 0.43) supported the purity.

3.3.1. 2-((E)-deca-1,8-dien-10-yl)-11,12-dihydro-13-propyl-2H-pyran (1)

Amorphous yellow; m.p. 169.6 °C (decom.); UV (MeOH) λ_{max} (log ε): 265 nm (3.37); TLC (Si gel GF_{254} 15 mm; 100% *n*-hexane) R_f: 0.75; R_t (HPLC, MeOH:ACN 3:2 v/v): 20.43 min.; IR ν_{max} (KBr) cm^{-1} (v = stretching, δ = bending, ρ = rocking vibrations): 2955.83, 2921.24, 2852.45 (C–H_v), 1641.60 (C=C_v), 1462.91 (C–H_δ), 1376.87, 1259.93, 1200.56, 1098.43, 1034.57(C–O_v), 992.42, 908.77 (=C–H_δ), 802.87, 721.66, 636.88 (C–H_ρ). ^{1}H NMR (500 MHz, $CDCl_3$): δ 6.96 (1H, s), 5.81 (1H, m, J = 10.21, 6.99 Hz), 5.35 (1H, t, J = 10.68, 5.52 Hz), 5.01 (1H, m, J = 14.52 Hz), 4.94 (1H, m, J = 10.21 Hz), 4.68 (1H, t), 2.06 (2H, t), 2.03 (2H, t), 2.02 (2H, t), 1.97 (2H, t), 1.58 (2H, m), 1.44 (3H, s), 1.37 (2H, m), 1.36 (2H, m), 1.30 (2H, m), 1.26 (2H, m), 0.88 (3H, t); ^{13}C NMR (125 MHz, $CDCl_3$): δ 139.27, 130.38, 129.91, 123.34, 114.06, 112.37, 108.30, 37.11, 33.83, 31.94, 30.41, 29.71, 29.52, 29.37, 28.97, 26.72, 22.70, 14.12. ^{1}H–^{1}H COSY, and HMBC data, see Table S1; HRESIMS *m/e* Calcd for $C_{18}H_{30}ONa$ 262.1604, found 262.1612 ([M^+]).

3.3.2. 1′-((10E)-10-(10-(pentan-4-yl)-cyclohex-4-enyl)-allyloxy)-tetrahydro-2′, 2′-dimethyl-2H-pyran (2)

Amorphous light green; m.p. 148.7 °C (decom.); UV (MeOH) λ_{max} (log ε): 260 nm (3.46); TLC (Si gel GF_{254} 15 mm; 5% EtOAc:*n*-hexane) R_f: 0.43; R_t (HPLC, MeOH:ACN 3:2 v/v): 22.92 min.; IR ν_{max} (KBr) cm^{-1} (v = stretching, δ = bending, ρ = rocking vibrations): 2932.51, 2860.14 (C–H_v), 1661.31 (C=C_v), 1455.51 (C–H_δ), 1372.35, 1241.84, 1187.08, 1108.18, 1042.27 (C–O_v), 965.02 (=C–H_δ), 803.72 (C–H_ρ). ^{1}H NMR (500 MHz, $CDCl_3$) δ 5.38 (1H, d, J = 8.58), 5.35 (1H, t, J = 9.18), 5.34 (1H, t, J = 9.18), 4.59 (1H, s), 4.14 (1H, d), 4.12 (1H, d), 3.64 (2H, t),2.35 (1H, t), 2.31 (2H, m), 2.02 (2H, d), 1.99 (2H, t), 1.63 (2H, m), 1.57 (2H, m), 1.55 (2H, m), 1.48 (3H, s), 1.46 (3H, s), 1.43 (2H, m), 1.42 (2H, m), 0.89 (3H, t), 0.87 (3H, t); ^{13}C NMR (125 MHz, $CDCl_3$): δ 143.18, 139.27, 127.67, 124.47, 68.39, 65.05, 63.11, 39.07, 34.37, 32.84, 31.93, 30.25, 30.09, 29.62, 29.37, 27.23, 25.74, 22.69, 22.69, 14.15, 14.15. ^{1}H–^{1}H COSY, and HMBC data, see Table S1; HRESIMS *m/e* Calcd for $C_{21}H_{36}O_2$ 320.2715, found 320.2722 ([M^+]).

3.4. Antioxidant and anti-inflammatory activities

The antioxidant properties were evaluated by the stable 1, 1-diphenyl-2-picrylhydrazyl (DPPH) (Lim et al. 2008) and 2, 2′-azino-bis-3-ethylbenzothiozoline-6-sulfonic acid diammonium salt ($ABTS^+$) radical scavenging assays (Vijayabaskar & Shiyamala 2012). *In vitro* anti-inflammatory properties were determined by COX-2 (Larsen et al. 1996) and 5-lipoxygenase (5-LOX) enzyme inhibition assay (Baylac & Racine 2003). The plot of scavenging and pro-inflammatory enzyme inhibitory activities were recorded, and the results were expressed as IC_{50} (the concentration of samples at which it inhibits/scavenge 50% of enzyme/radical activities and expressed in mg/mL) values.

3.5. Statistical analysis

Statistical evaluation was carried out with the Statistical Program for Social Sciences 13.0 (SPSS Inc, Chicago, USA, ver. 13.0). Analyses were carried out in triplicate, and the means of all parameters were examined for significance by analysis of variance. The level of significance for all analysis was $p \leq 0.05$.

4. Conclusions

Bioassay guided chromatographic fractionation of the EtOAc–MeOH extract of *P. malabarica* afforded two unprecedented meroterpeno 2*H*-pyranoids characterised as 2-((*E*)-deca-1,8-dien-10-yl)-11,12-dihydro-13-propyl-2*H*-pyran (**1**) and 1′-((10*E*)-10-(10-(pentan-4-yl)-cyclohex-4-enyl)-allyloxy)-tetrahydro-2′,2′-dimethyl-2*H*-pyran (**2**) with potential antioxidative and anti-inflammatory activities. The antioxidant activities of the title compounds were comparable to that of *α*-tocopherol used in various commercial preparations. Additionally, the compounds exhibited greater activity against COX-2 than COX-1 isoform, and therefore, exhibited greater selectivity than the synthetic non-steroidal anti-inflammatory drugs. These meroterpeno 2*H*-pyranoids isolated from *P. malabrica* can potentially be used as new generation antioxidant and anti-inflammatory lead pharmacophore for use against oxidative stress and inflammation.

Acknowledgements

The authors thank the Director, Central Marine Fisheries Research Institute, for his guidance and support. Thanks are due to the Head, Marine Biotechnology Division of Central Marine Fisheries Research Institute for facilitating the research works. One of us, M.J. acknowledges Ministry of Earth Science for the award of a scholarship.

Disclosure statement

No conflict of interest was reported by the authors.

Funding

This work was supported by the Ministry of Earth Science, New Delhi, India for funding under the project "Drugs from the sea" [grant number MoES-2/DS/6/2007 PC-IV]; Indian Council of Agricultural Research, New Delhi, India for providing the necessary facilities.

References

Arora RB, Mathur CN. 1963. Relationship between structure and anticoagulant activity of coumarin derivatives. Br J Pharmacol Chemother. 20:29–35.

Baylac S, Racine P. 2003. Inhibition of 5-lipoxygenase by essential oils and other natural fragment extracts. Int J Aromather. 13:138–142.

Ciavatta ML, Manzo E, Mollo E, Mattia CA, Tedesco C, Irace C, Guo YW, Li XB, Cimino G, Gavagnin M. 2011. Tritoniopsins A–D, cladiellane-based diterpenes from the South China sea nudibranch *Tritoniopsis elegans* and its prey *Cladiella krempfi*. J Nat Prod. 74:1902–1907.

Cueto M, Darias J. 1996. Uncommon tetrahydrofuran monoterpenes from Antarctic *Pantoneura plocamioides*. Tetrahedron. 52:5899–5906.

Cueto M, Darias J, Rovirosa J, San Martin A. 1998a. Tetrahydropyran monoterpenes from *Plocamium cartilagineum* and *Pantoneura plocamioides*. J Nat Prod. 61:1466–1468.

Cueto M, Darias J, Rovirosa J, San Martin A. 1998b. Unusual polyoxygenated monoterpenes from the Antarctic alga *Pantoneura plocamioides*. J Nat Prod. 61:17–21.

Darias J, Rovirosa J, San Martin A, Díaz AR, Dorta E, Cueto M. 2001. Furoplocamioids A–C, novel polyhalogenated furanoid monoterpenes from *Plocamium cartilagineum*. J Nat Prod. 64:1383–1387.

Goel A, Ram VJ. 2009. Natural and synthetic 2*H*-pyran-2-ones and their versatility in organic synthesis. Tetrahedron. 65:7865–7913.

Govindam SVS, Yoshioka Y, Kanamoto A, Fujiwara T, Okamoto T, Ojika M. 2012. Cyclolobatriene, a novel prenylated germacrene diterpene, from the soft coral *Lobophytum pauciflorum*. Bioorg Med Chem. 20:687–692.

Larsen LN, Dahl E, Bremer J. 1996. Peroxidative oxidation of leuco-dichloroluorescein by prostaglandin-H synthase in prostaglandin biosynthesis from polyunsaturated fatty acids. *Biochim Biophys Acta Lipids Lipid Metab*. 1299:47–53.

Lim YY, Chew YL, Omar M, Khoo KS. 2008. Antioxidant activity of three edible seaweeds from two areas in South East Asia. LWT-Food Sci Technol. 41:1067–1072.

Nemouchi S, Boulcina R, Carboni B, Debache A. 2012. Phenyl-boronic acid as an efficient and convenient catalyst for a three-component synthesis of tetrahydrobenzo-(b)-pyrans. C R Chim. 15:394–397.

Parker SR, Cutler HG, Jacyno JM, Hill RA. 1997. Biological activity of 6-pentyl-2*H*-pyran-2-one and its analogs. J Agric Food Chem. 45:2774–2776.

Rajguru D, Keshwal BS, Jain S. 2013. Solvent-free, green and efficient synthesis of pyrano-(4, 3-b)-pyrans by grinding and their biological evaluation as antitumor and antioxidant agents. Med Chem Res. 22:5934–5939.

Rocha DFO, Hamilton K, Goncalves CCS, Machado G, Marsaioli AJ. 2011. 6-Alkyl-3, 4-dihydro-2*H*-pyrans: chemical secretion compounds in neotropical harvestmen. J Nat Prod. 74:658–663.

Vijayabaskar P, Shiyamala V. 2012. Antioxidant properties of seaweed polyphenol from *Turbinaria ornata* (Turner) J. Agardh, 1848. Asian Pac J Trop Biomed. 2:S90–S98.

Wang F, Zhu T, Zhang M, Lin A, Zhua W, Gu Q. 2010. Structural determination of aspericins A–C, new furan and pyran derivates from the marine-derived fungus *Rhizopus* sp. 2-PDA-61, by 1D and 2D NMR spectroscopy. Magn Reson Chem. 48:155–158.