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Isolation, absolute configuration and bioactivities of megastigmanes or C_{13} isonorterpinoides

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A B S T R A C T

Megastigmanes or C_{13} isonorterpenoids a terpene class of compounds, specially the title compounds are related to sesquiterpenoides but due to C_{13} skeleton also it is called as norterpenoides. Most of the megastimane compounds have been isolated from the plant source such as *Cucumis sativus, Crotalaria zanzibarica, staphylea bumalda, Juniperus communis* and *Excoecaria cohinchinensis* etc. Few reports were showing the megastimane compounds are showing potencial activity against scavenging radical (DPPH) and hepatoprotective activities. The classification of C_{13} norisoprenoides, are assumed to be apocarotenoides that is formed from the degradation of carotenoides by the action of carotenoides cleavage dioxygenases which have been isolated from various plants. The majority of the evidences for this assumption is the similarly between most megastigmanes and the terminal component of plant carotenoides. Because such compounds are generally not oxygenated at C_7 but commonly oxygenated at C_9 presumably as a result of the oxidative cleavage of the acyclic portion of caritenoides, the key step in megastigmanes formation has been thought to be transportion of oxygen from C_9 to C_7. Hence, based on the above information the C_9 position and its absolute configuration is very important to get potential activity against various diseases, in this progression a lot of literature reports were showing the importance and absolute configuration at C_9 position in megastigmanes or C_{13} norisoprenoides. In this review, we are reporting the chemistry, absolute configuration and bioactivities of megastigmanes or C_{13} norisoterpenoides. To our knowledge, this is the first review on megastimanes and its absolute configuration.

Capsule Summary: Megastigmanes are having unique structural features containing C13 moiety called as norisoterpenoides with potential activities such as scavenging radical (DPPH) and hepatoprotective etc.

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INTRODUCTION

In yester years, the chemistry of natural products always attracts a very vivacious interest (Noble lecture, 1972). New substances, more or less complicated, useful, have bear constantly discovered and investigated. In order to determine the structure and architecture of the molecule, we have very powerful tools today, which are mostly borrowed from Physical Chemistry. The organic chemists of the year

1900 could have been greatly amazed had they heard of the systems now at hand. However, one cannot say that the work is easier; the steadily improving methods make it possible to solve much more difficult problems and the ability of nature to build up complicated substances, as it seems.

The chemistry of natural products and its stereochemistry/absolute configuration to the construction of molecule form isolated compounds sesquiterpenes/norsesquiterpenes and related compounds need extensive spectroscopic techniques. The title compounds are classified as terpene, nor-sesquiterpene subclass, which are trivially named as megastigmanes (Noble lecture, 1992) The megastigmanes are biosynthetically proved; the biosynthetic precursor for megastigmanes is isoprene unit has C_5 carbon skeleton origin ahed from the Mevalonic acid (C-6 unit) isolated from plant sources. The title/Nor-sesquiterpene (C_{13}) compounds are biosynthesized through Mevalonic acid path way and number of megastigmanes or nor-sesquiterpenes have been isolated from different plant sources (Kawakami et al., 2011). Structurally, megastigmanes are C_{13}-cabon skeleton such compounds which are commonly classified as C_{13} norisoprenoids, also assumed to be apocarotenoides, ie., formed from the degradation of Carotenoides by the action of carotenoid cleavage dioxygenages which have been isolated from various plants, much of the evidences for this assumption is the similarities between most megastigmanes and the terminal components of plant carotenoides (Sefton et al., 1989). Because such compounds which are not generally oxygenated at C-7, but commonly oxygenated at C-9 presumably as a result of the oxidative cleavage of the acyclic portion of carotenoides, based on above assumption the most of isolated megastimanes or C_{13} norisoprenoides do have their hydroxyl group mainly at C-6 and C-9 (Baumes et al., 2002). The stereochemistry/absolute configurations of existing group/groups on megastigmanes are essential for various aspects. Moreover, to confirm stereochemistry at C-6 and C-9 extended studies are required, and such studies are discussed here. The current chapter deals with the isolation, absolute configuration and bioactivities of megastigmanes or C_{13} isonorterpinoides.

Structural features of megastigmanes

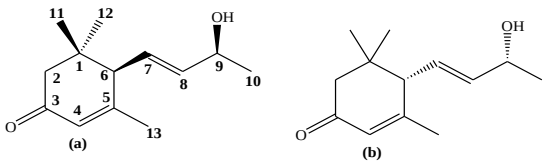


Fig. **1**: Basic skelton of megastigmanes

Megastigmanes a & b (Fig. 1) have unique basic skeleton related to norisoprenoides/nor sesquiterpenoides class of compounds, with a six membered ring with double bond within the ring system at $\Delta^4{}_5$, followed by a methyl substitution at C-5 position. The dimethyl substitution at C-1 position of the methyl's are arranged opposite to each other. In addition a four membered chain is attached at C-6 position with $\Delta^7{}_8$ double bond in a trans mode. Further, hydroxyl (for most of the compounds) group is present adjacent to double bond of side chain. This structure was made up of with support of complete structural analysis by ^{1}H, ^{13}C, DEPT, 2D, IR, UV mass and biosynthetic evidence (Takeda et al., 2004). The most important stereochemical aspects of Megestigmanes at C-6 and C-9 elucidated by the available advanced techniques such as NOESY, Mosher's acid, CD and others (Pabst et al., 1992).

Biosynthesis of megastigmanes

Megastigmanes are related to terpene class of compounds, terpenoides form a large and structurally diverse family of

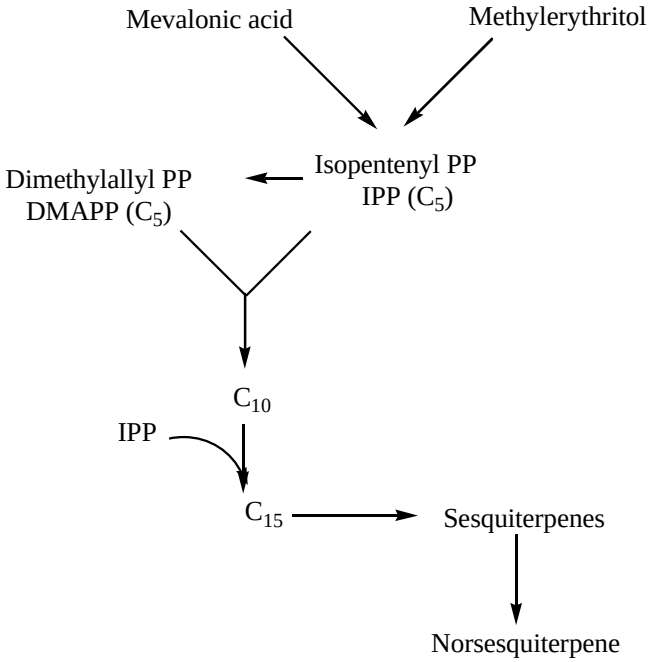
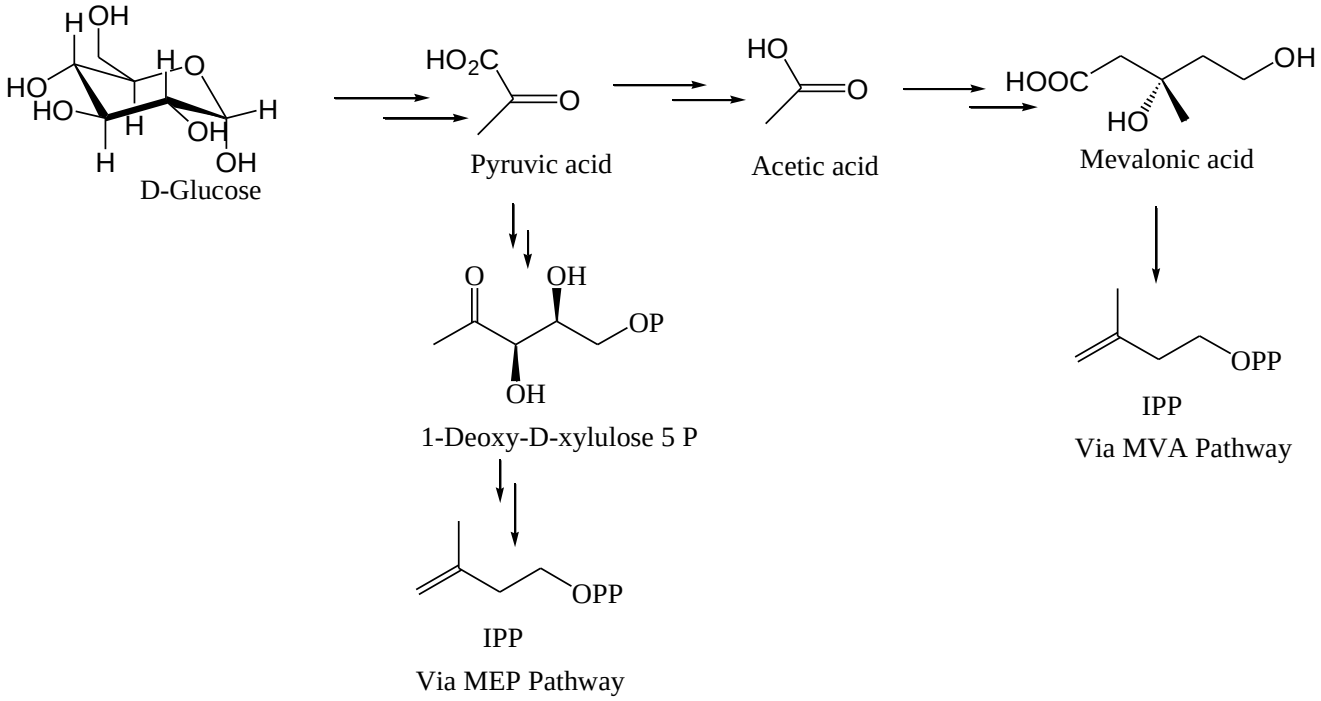
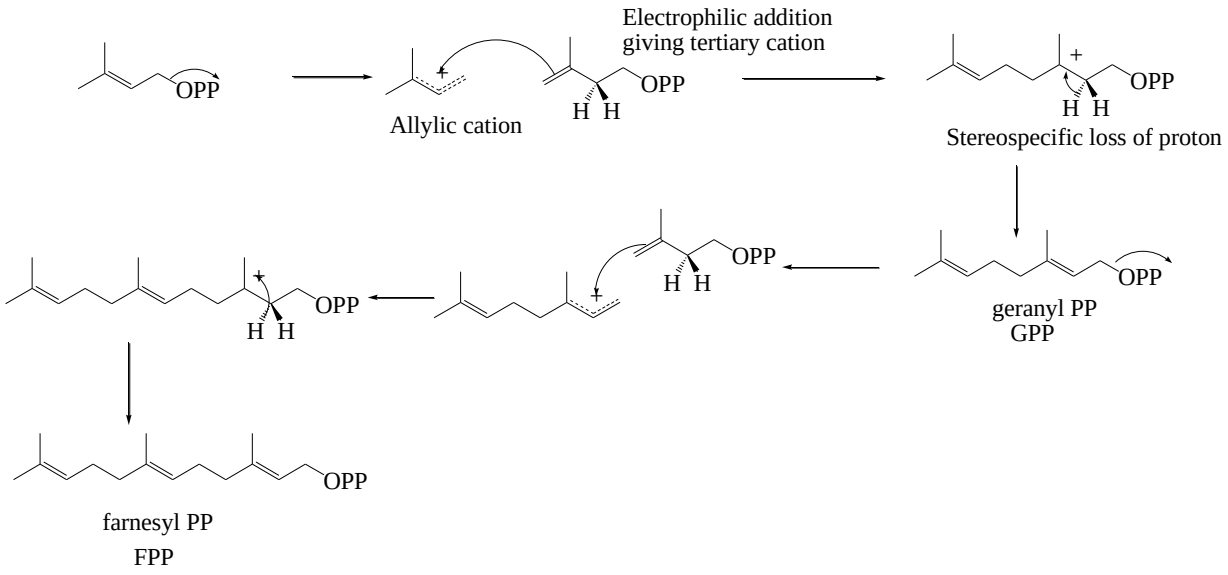


Fig. 2: Biosynthesis overview

natural products which are derived from C_5 isoprene units joined in a head-to-tail fashion (Bouvier et al., 2005). Biosynthesis has been suggested as the fundamental building block of these compounds, referred as isoprenoids (IPP or DMAPP). Isoprene is produced naturally, but which is not actively involved in the formation of such compounds. The isoprene units which were subsequently identified as the dimethylallyl diphosphate (DMAPP) and Isopentenyl diphosphate (IPP). Mevalonic acid is one of the active ingredients to get isoprene unit, it is constructed through biosynthetic path called as mevalonic acid pathway (Eisenreich et al., 2004). The entire biosynthetical approach represented in two ways i) In an overview and ii) Complete biosynthesis in step I-III (Fig. 2).



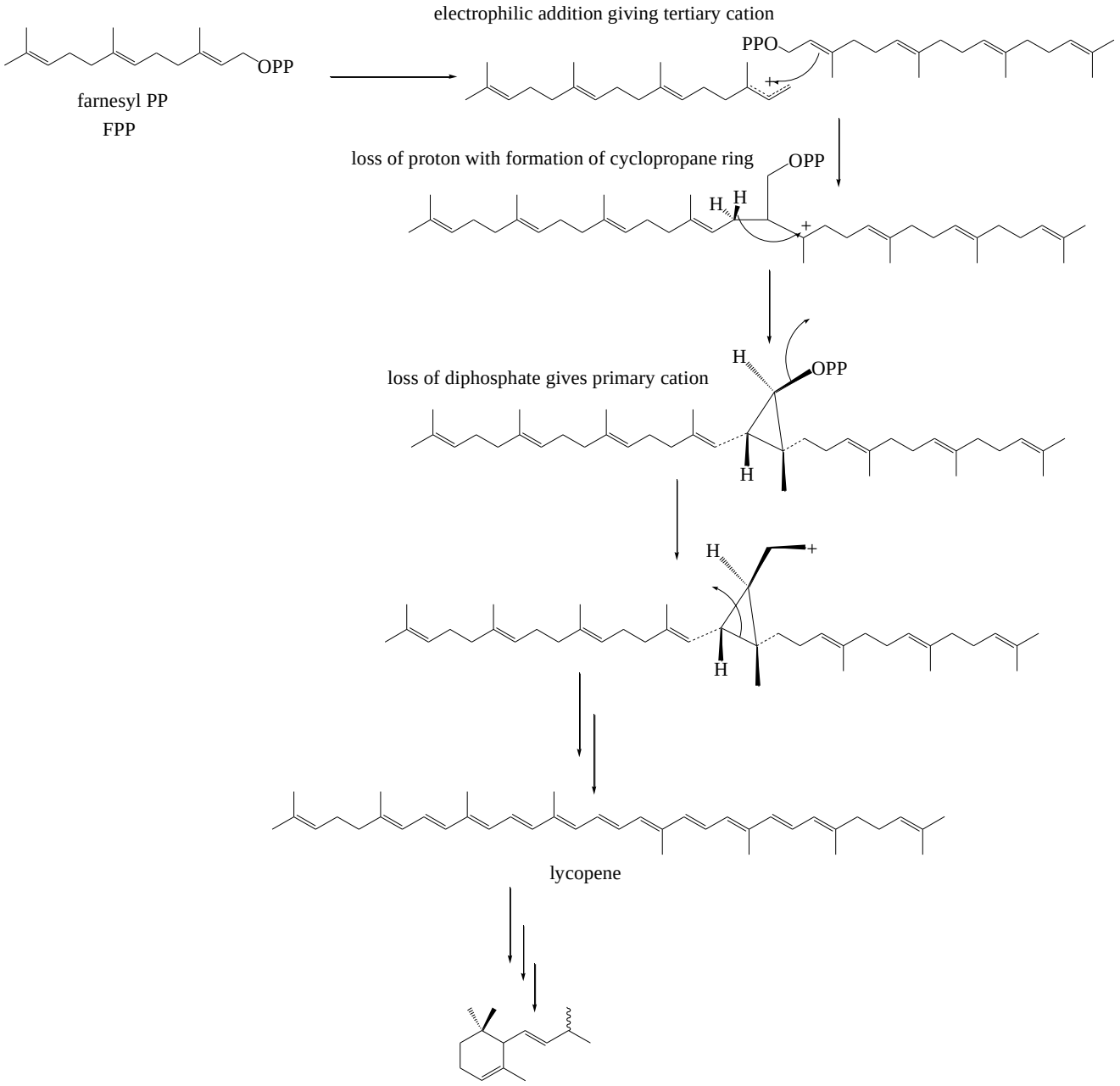
Step I: Formation of isoprene unit



Step II: Formation of farnesyl pyrophosphate

Fig. 2: Continue...

The tetraterpenes are responsible for the biosynthesis of sesquiterpenes and its related class of compounds such as megastigmanes and its glycosides. The tetraterpenes are represented by single calss of compounds, formation through tail-to-tail coupling of two molecules of geranylgeranyl pyrophosphate (GGPP). Ohloff et al., (1973) and Isoe et al., (1973) suggested that such a transposition could take place with allene intermediates. Ohloff et al hypothesized that damascenone (Ohloff et al., 1973) could be formed from an allenic triol (Skouroumounis et al., 2000) which derive from the known ketone (Sefton et al.,1989) which could in turn be derived directly by enzymatic cleavage of neoxanthin (Daniel et al.,2008). Damascenone basic structure is related to megastigmane, the only difference being at keto group present at C-7. In $\Delta^{7}{}_{8}$ position gets converted to keto position. The biosynthetic approach of megastigmane and its related



Step III: Biosynthesis of megastigmanes

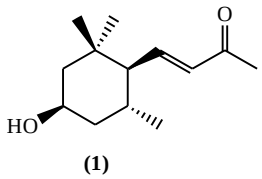
Fig. 2: Continue...

compounds is similar to that of damascenone (Market al., 2011).

MATERIAL AND METHODS

General procedures

^{1}H- (400 MHz), ^{13}C- (100 MHz) and 2D-NMR spectra were recorded using the residual solvent signal as internal standard on a Varian AS 400 spectrometer. IR spectra were measured on a Bruker Tensor 27 FT-IR spectrometer. UV spectra were obtained on a Varian Cary 50 Bio UV-visible spectrophotometer. Optical rotations were obtained at the sodium D line at ambient temperature on a Rudolph Research Analytical Autopol IV automatic polarimeter. CD spectra were measured on a JASCO J-715 spectrometer.



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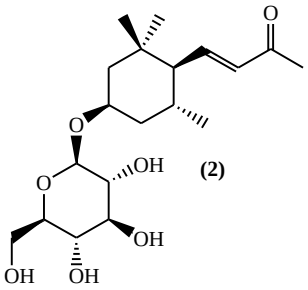

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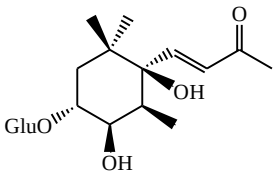

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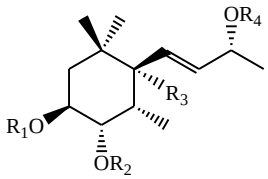


(4): R_1= Glu, R_2 = R_4 = H, R_3 = OH

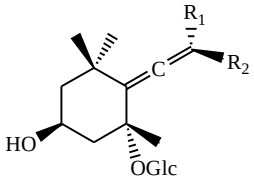
(5): R_1= R_2 = R_3 = H, R_4 = Glu

(6): R1= Glu, R_2 = R_3 = R_4 = H

(7): R_1= R_3 = R_4 = H, R_2 = Glu

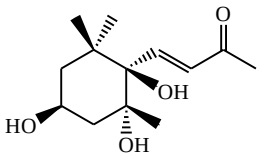
(8): R_1= R_2 = R_3= R_4 = H

(9): R_1= R2 = R_4 = H, R_3 = OH



(10): R_1= H, R_2 = Ac

(11): R_1= Ac, R_2 = H



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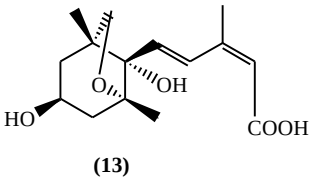

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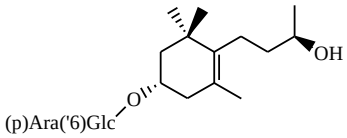

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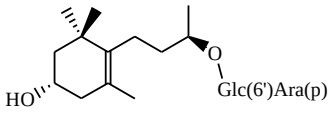

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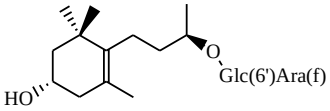

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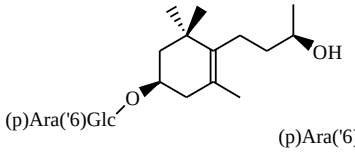

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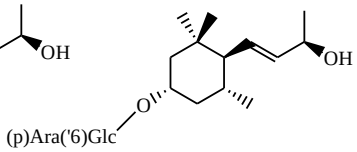

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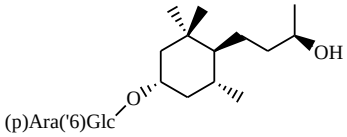

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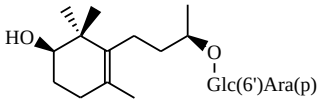

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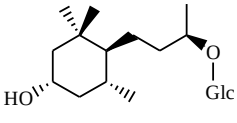

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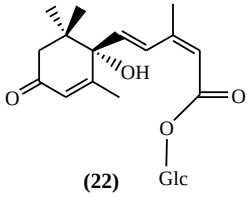

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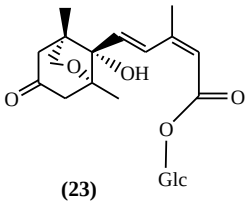

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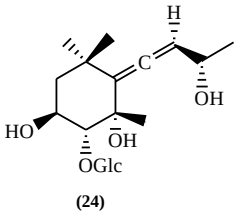

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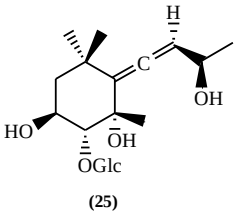

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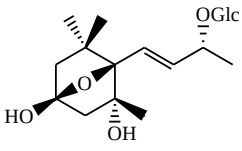

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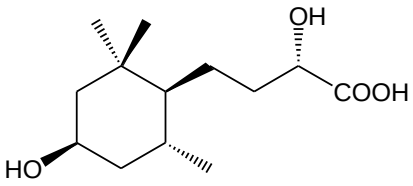

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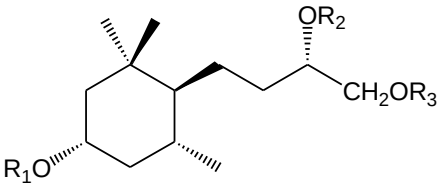
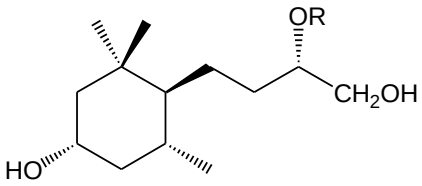


(28): $R^1 = R^2 = R^3 = H$

(29): R^1 =Glc, $R^2 = R^3 = H$

(30): $R^1 = R^3 = H$, R^2 = Glc

(31): $R^1 = R^2 = H$, R^3 = Glc



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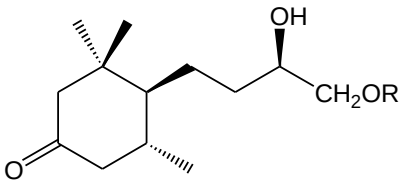
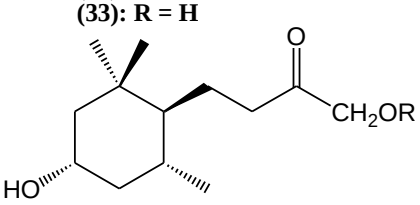
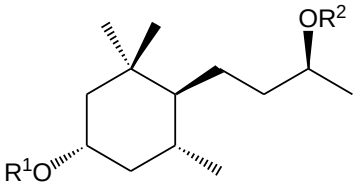
(33): R = H



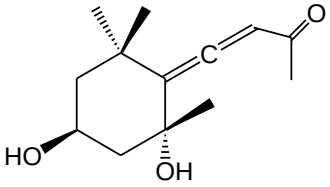
(34): R = Glc
(35): R =H



(36): R =Glc
(37): R =H



(38): $R^1 = R^2 = H$
(39): $R^1 = H$, R^2 = Glc
(40): R^1 = Glc, $R^2 = H$
(41): $R^1 = R^2$ = Glc



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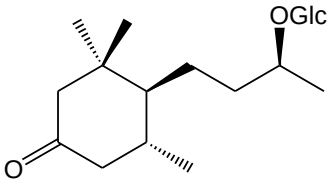

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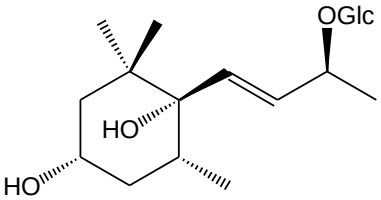

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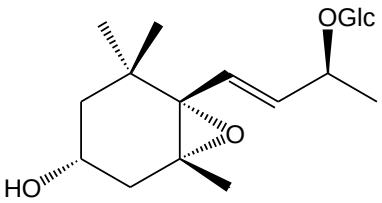

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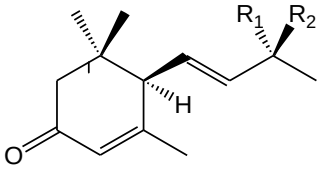
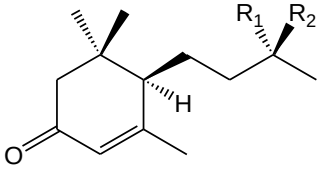


(46): R^1 = -O-Glc(2′-1″) Api; $R^2 = H$

(47): R^1 =H, R^2; -O-Glc(2′-1″) Api

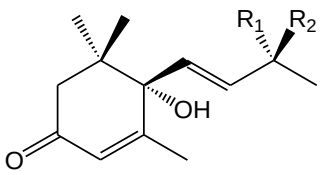
(48): R^1 = -O-Glc; $R^2 = H$

(49): R^1 =H, R^2= -O-Glc



(50): R1 = H, -O-Glc(2′-1″) Api

(51): R1 = -O-Glc; R2 = H



(52): R1 = -O-Glc; R2 = H

(53): R1 = H, R^2 = -O-Glc

Glc: β-D-Glucopyranosyl
Api: β-D-Apiofuranosyl

HRESIMS were obtained on an Agilent Series 1100 SL mass spectrometer. GC-MS analysis was carried out on a HP 6890 series GC, equipped with a split/splitless capillary injector, a HP 6890 Series injector autosampler, and an Agilent DB-5ms column (30 m × 0.25 mm × 0.25 μm). The GC was interfaced to a HP 5973 quadrupole mass selective detector through a transfer line set at 280°C. The injector temperature was 250°C, and 1 μL injections were performed in the split (1:10) mode. Column flow was set at a constant pressure of 20 psi, giving an initial flow of 2.2 mL/min, using helium as carrier gas. The oven temperature was raised from 70 to 300°C (hold 8.5 min) at a rate of 20°C/min, for a total run time of 20 min. The filament was operated at 70 eV, with an emission current of 35 μA. The multiplier voltage was automatically set to 2247 V. The ion source and quadrupole temperatures were 230 and 150°C. The acquisition range was m/z 30-800 at 1.95 scans per second, starting 3.5 min after injection. TLC was carried out on aluminum-backed plates precoated with silica gel F_{254} (20 × 20 cm, 200 μm, 60 Å, Merck). Visualization was accomplished by spraying with *p*-anisaldehyde [0.5 mL in glacial acetic acid (50 mL) and sulfuric acid (97%, 1 mL)] spray reagent followed by heating. Flash silica gel (60-120 μm, 60 Å, SiliCycle), SiliaBond C18 silica gel (40-63 μm, 60 Å, 17% carbon loading, SiliCycle) and Sephadex LH-20 (25-100 μm, lipophilic, Sigma-Aldrich) were used for column chromatography.

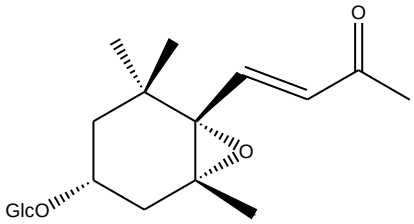
Extraction and isolation

Powdered plant materials are being extracted through sonication in Ethanol/Methanol and for cold extraction method the plant material was soaked at room temperature for several days, while hot extraction through Soxhlet apparatus was performed with various solvents like hexane, $CHCl_3$ and acetone to get better extraction (Zhang et al., 2010). The combined extracts have been filtered and the solvent was evaporated to obtain crude extract. The powder was partitioned between H_2O and EtOAc followed by vacuum liquid chromatography of the EtOAc fraction over C18 silica [H_2O (100%), H_2O/MeOH (2:8, 4:6, 6:4, 8:2), MeOH (100%)] yielding several fractions were further purified by silica chromatography

Isolation of megastigmane and its glycosides isolated from different plants

Boronia megastigma (Nees) Family; Rutaceae is a woody understory shrub that is endemic to the southwest of Western Australia. A group complex volatile compounds have been isolated and identified as 3-hydroxy-5, 6-dihydro-*β*-ionone (1) whose 3-hydroxy-5, 6-dihydro-*β*-ionone-*β*-D-glucopyranoside (2) (Cooper et al., 2011) whose structures were further confirmed through extensive spectroscopic methods like ^1H, ^{13}C, DEPT, 2D NMR, UV, IR and Mass. *Gynostemma pentaphyllum* important medicinal plant belongs to Cucurbitaceae Family, named Jiao-Gu-Lan in Chinese which is used for a popular Traditional Chinese Medicine (TCM) used as a cough suppressant, diuretic, antipyretic and tonic. The variety of megastigmane compounds were isolated were, (3*R*, 4*R*, 5*S*, 6*S*, 7*E*)-3, 4, 6-trihydroxymegastigmane-7-en-9-one-3-O-*β*-D-glucopyranoside (gynostemoside A, 3), (3*S*, 4*S*, 5*R*, 6*R*, 7*E*, 9*R*)-3, 4, 6, 9-tetrahydroxymegastigmane-7-en-3-O-*β*-D-glucopyranoside (gynostemoside B, 4), (3*S*, 4*S*, 5*S*, 6*S*, 7*E*, 9*R*)-3, 4, 9-trihydroxymegastigmane-7-en-9-O-*β*-D-glucopyranoside (gynostemoside C, 5), (3*S*, 4*S*, 5*S*, 6*S*, 7*E*, 9*R*)-3, 4, 9-trihydroxymegastigmane-7-en-3-O-*β*-D-glucopyranoside (gynostemoside D, 6), and (3*S*, 4*S*, 5*S*, 6*S*, 7*E*, 9*R*)-3, 4, 9-trihydroxymegastigmane-7-en-4-O-*β*-D-glucopyranoside (gynostemoside E, 7), (3S, 4S, 5S, 6S, 9R)-3,4-dihyroxy-5,6-dihydroxy-*β*-ionol (8), (3*S*, 4*S*, 5*R*, 6*R*)-3, 4, 6-trihydroxy-5, 6-dihydro-*β*-ionol (9), citroside A (10), Citroside B (11), (E)-4-(r-1′,t-2′,c-4′-trihydroxy-2′,6′,6′-trimethyl cyclohexyl) but-3-en-2-one (12), and 4′-dihydrophaseic acid (13) (Zhang et al., 2010; Cooper et al., 2011; Jiangsu et al., 1977). *Salacia chinensis* is rich in isolation of bioactive compounds like megastigmane glycosides which were isolated and characterized through different chemical and spectrochemical studies such as Foliasalaciosides E_1 (14), Foliasalaciosides E_2 (15), Foliasalaciosides E_3 (16), Foliasalaciosides F (17), Foliasalaciosides G (18), Foliasalaciosides H (19), Foliasalaciosides I (20), Myrsinionoside D (21), (+) Abscisyl-*β*-D-glucopyranoside (22), and 1-[5-(8-Hydroxy-1, 5-dimethyl-3-oxo-6-oxabicyclo(3, 2, 1) oct-8-yl)-3-methyl-2, 4-pentadienoate], [1R-[1α, 5α, 8S (2*Z*, 4*E*)]]-*β*-D glucopyranose (23) (Zang et al., 2008) . The *Crotalaria usaramoensis* Bentham *Syn*. *C. usaramoensis* belongs to Leguminosae family is a perennial shrubby herb which was introduced to be in the Okinawa Islands from Eastern Africa as a green manure crop. The Phytochemical investigation results shows that the isolation of three megastigmane glycosides which are crotalionoside A (24), crotalionoside B (25) and crotalionoside C (26) (Shitamoto et al., 2010).

The plant *Sedum sarmentosum* (Crassulaceae) which is a perennial herb widely distributed on the mountain slopes of China (e.g., Anhui, Hebei, Jiangxi, and Jiangsu Provinces). The entire plant of *S. sarmentosum* has been used for the treatment of chronic viral hepatitis in Chinese and Korean traditional medicines. 2, 3 sarmentoic acid (27), sarmentol A (28), sedumoside A1 (29), sedumoside A2 (30), sedumoside A3 (31), sedumoside B (32), sedumoside C (33), and sedumoside D (34), (3*S*, 5*R*, 6*S*, 9*R*)-megastigmane-3, 9-diol (35), staphylionoside D (36), myrsinionoside A (37) and myrsinionoside D (38), alangioside A (39), alangioside J (40), 3-hydroxy-5,6-epoxy-*β*-ionol 9-*O*-*β*-D-glucopyranoside (41), and staphylionoside D (42), alangioside B (43), myrsinionoside E (44) and sedumoside E (45) were isolated from the whole plant of *S. sarmentosum* (Yoshikawa et al., 2007). Leaves of *Salvia nemorosa* have been used in Turkish medicine, externally applied to stop bleeding.



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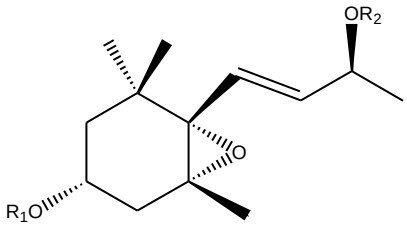
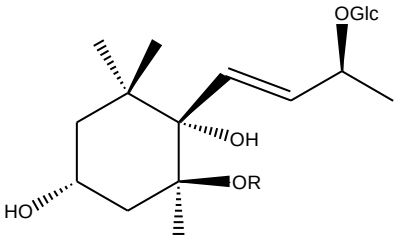
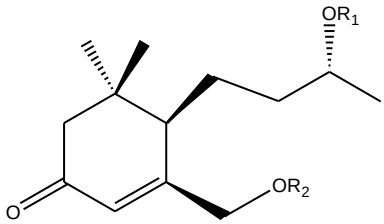


	R_1	R_2
(55)	**Glc**	**H**
(56)	**H**	**Glc**

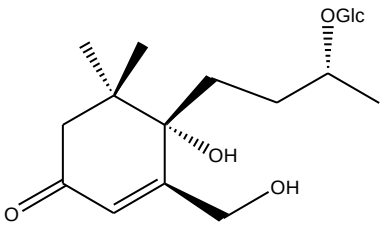


(57) R = H

(58) R = CH_3



	R_1	R_2
(59)	**Glc**	**H**
(60)	**H**	**Glc**



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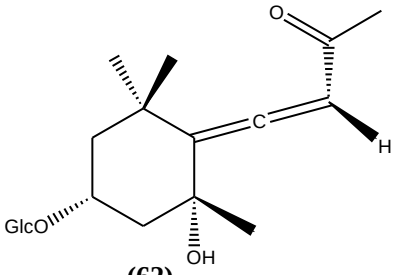

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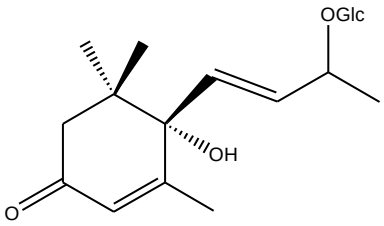

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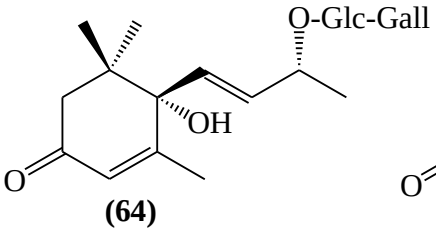

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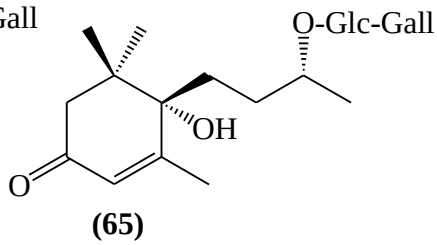

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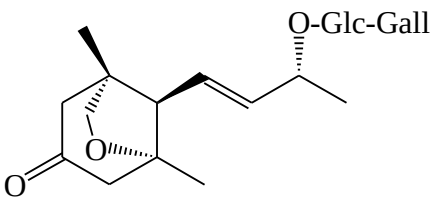

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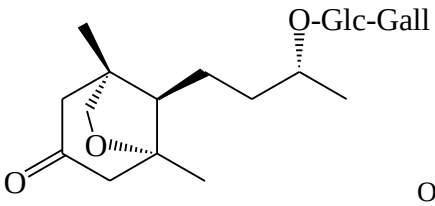

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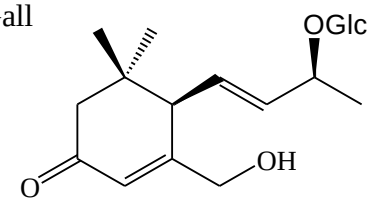

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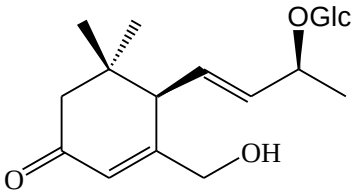

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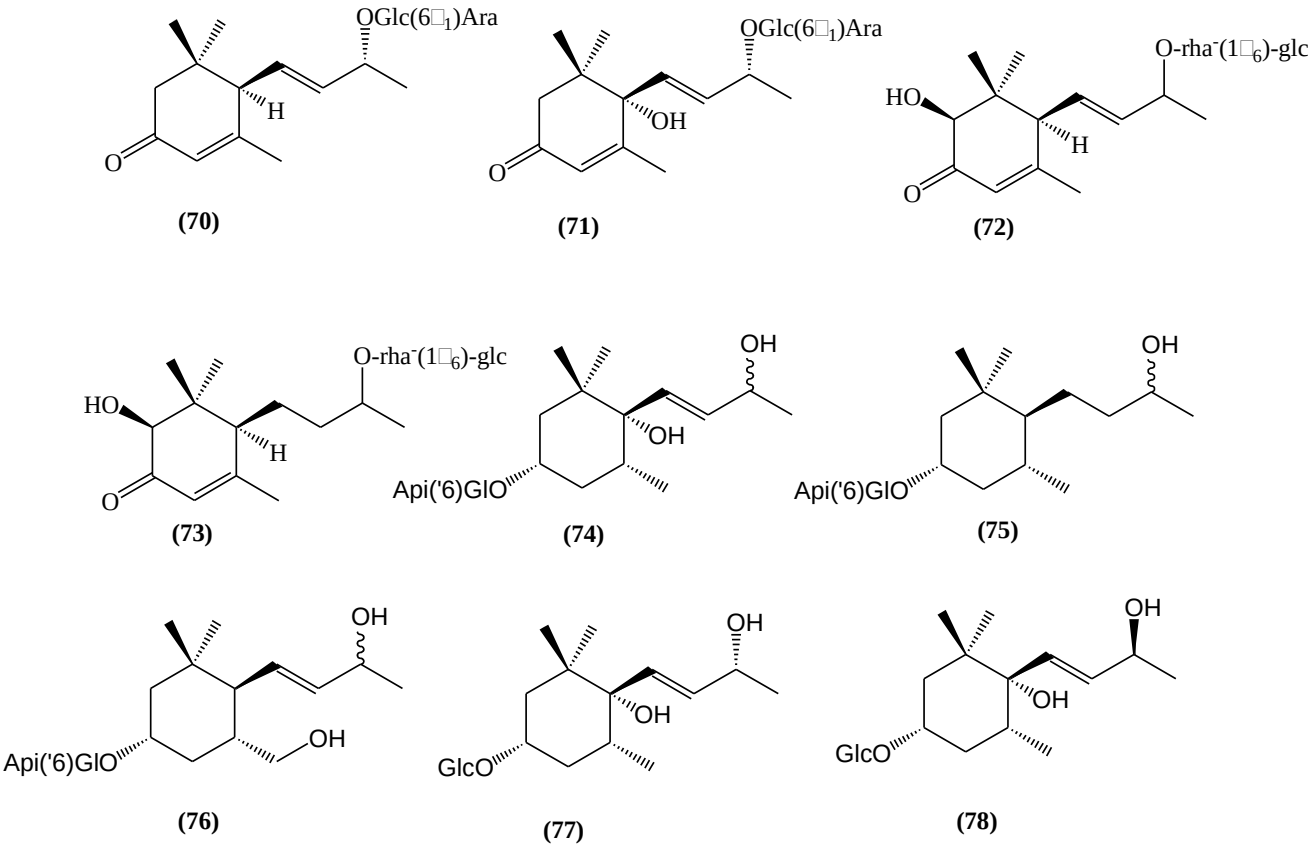
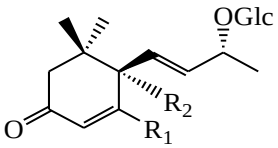
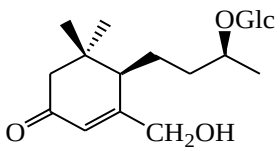


Phytochemical investigation of leaves of the same plant resulting eight megastigmane compounds listed below. The names of isolated compounds Salvionoside A (46), Salvionoside B (47), Salvionoside C (48), 6*R*, 9*R*-3-oxo-*α*-ionol-glucoside (49), 6*R*, 9*S*-3-oxo-*α*-ionol-glucoside (50), blumeol C glucoside (51), 6*S*, 9*R* reseoside (52) and 6*S*, 9*S* reseoside (53) were isolated (Takeda et al., 1997) . The fruits of *Evodia rutaecaepa* (Rutaceae) are a well-known crude drug which is included in the Japanese Pharmacopoeia XV. The genus name Evodia is not listed under the Rutaceae. Instead, many Euodia species appear in the databases as Rutaceous plants, from a 1-BuOH-soluble fraction of the MeOH extract of leaves of *E. meliaefolia*, ten megastigmane glucosides (54-63) were isolated Euodionoside A (54), Euodionosides B (55), Euodionosides C (56), Euodionoside D (57), Euodionoside E (58), Euodionosides F (59), euodionoside G (60), spinoside A (61), staphylionoside D (62) and corchoionoside C (63) (Yamamoto et al., 2008). The leaves of *Macaranga tanariuts* L. Mull.-Arg belong to Euphorbiaceae family. This is well known as a pioneer tree and also an ant-plant. This plant can be found throughout Eastern and Southern Asia, especially in south China, Korea and Japan. In China, root and bark of this plant which are used for haemopiosis and dysentery. Phytochemical investigation of *M. tanariuts* led to the isolation of six megastigmane compounds, such as mallophenol B (64), macarangioside A (65), macarangioside B (66), macarangioside C (67), macarangioside D (68), lauroside E (69) (Matsunami et al., 2006).

The leaves of *Loniceru gracillipes* var. *glandulosa,* isolation and structural elucidation of two megastigmane glucosides (6*R*, 7*E*, 9*R*)-9-hydroxy-4, 7 megastigmadiene-3-one-9-O-[*α*-L-arabinopyranosyl-(1→6)-*β*-D-glucopyranoside (70) and (6*R*, 7*E*, 9*R*)-6, 9-dihydroxy-4,7 megastigmadiene-3-one-9-O-[*α*-L-arabinopyranosyl]-(1→6)-*β*-D-glucopyranoside (71) (Matsuda et al., 1997). This particular genus *Caralluma* belongs to the family Asclepiadaceae, which comprises some 200 genera and 2500 species. Plants belonging to this genus are naturally rich inesterified polyhydroxypregnane glycosides, some of which shown antitumor activity and others were postulated as precursors of cardenolides. The genus is also characterized by the presence of flavone glycosides. (9*R*)-2-*β*,9-dihydroxymegastigma-4, 7-dien-3-one-9-O-*α*-l-rhamnopyranosyl-(1→6)-*β*-D-glucopyranoside (72), 2-*β*, 9-dihydroxymegastigma-4-en-3-one 9-O-*α*-l-rhamnopyranosyl-(1→6)-*β*-D-glucopyranoside (73) (Bader et al., 2003). *Croton oblongifolius Roxburgh* (Euphorbiaceae) which is a deciduous tree of about eight m in height that grows wild in Asian pantropical areas, such as Thailand, Myanmar and Indonesia. *C. oblongifoius* is a traditional folk medicine in Thailand, its flowers are being used as an anthelmintic, its fruit, macerated with 28–40% alcohol, as an oxytocic for post-labor, and its stem bark or leaves as an antidiarrheal and as a blood tonic etc.



	R_1	R_2
(79)	CH_3	H
(80)	CH_3	OH
(81)	CH_2OH	H
(82)	CH_2OH	OH



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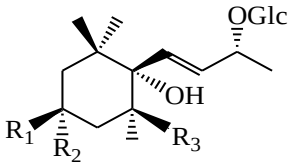
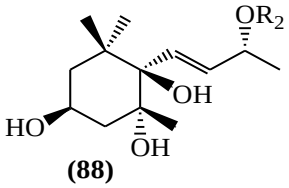


	R_1	R_2	R_3
(84)	H	OH	H
(85)	H	OH	OH
(86)	OH	H	OH
(87)	O	O	H



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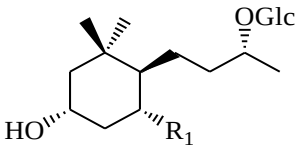
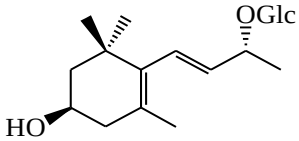


	R_1	R_2
(89)	CH_2OH	Glc
(90)	COOGlc	H



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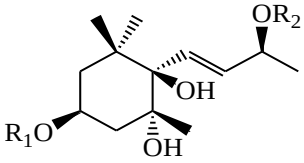
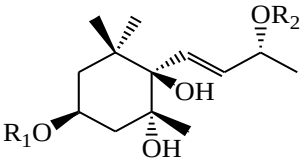
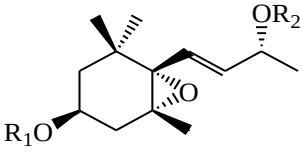


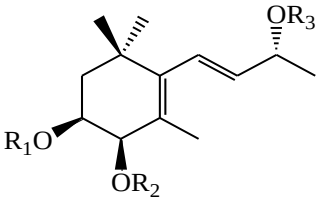
	R1	R2
(92)	Glc	H
(93)	H	H
(94)	H	Glc



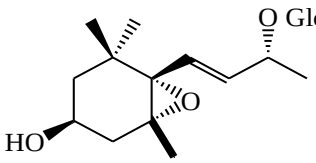
	R1	R2
(95)	Glc	H
(96)	H	H
(97)	H	Glc



	R1	R2
(98)	Glc	H
(99)	H	H



	R_1	R_2	R_3
(100)	Glc	H	H
(101)	H	Glc	H
(102)	H	H	Glc (2'-1") Api
(103)	H	H	H



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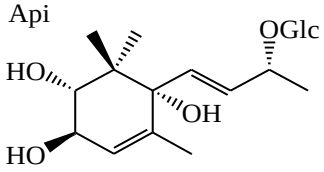

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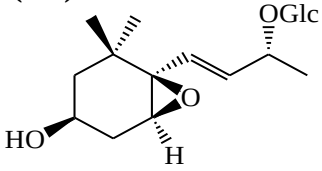

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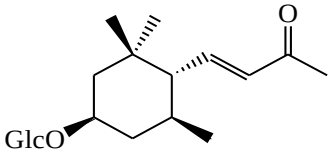

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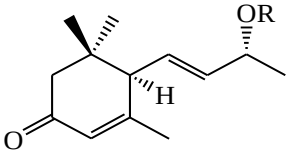
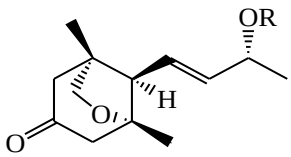
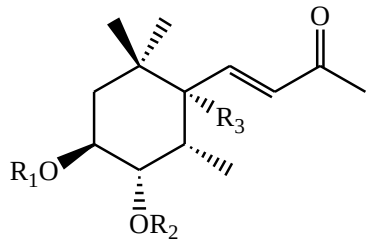


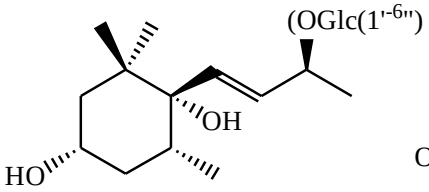
	R
(108)	Glc(6')Gall
(109)	Glc



	R
(110)	Glc
(111)	H



	R_1	R_2	R_3
(112)	**H**	**Glc**	**OH**
(113)	**Glc**	**H**	**H**
(114)	**H**	**H**	**Glc**



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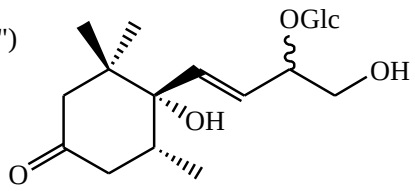

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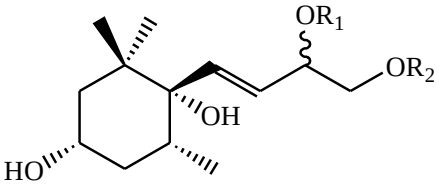
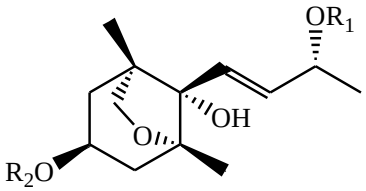
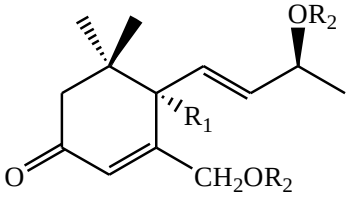


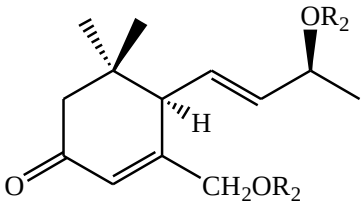
	R1	R2
(117)	**Glc**	**H**
(118)	**H**	**Glc**



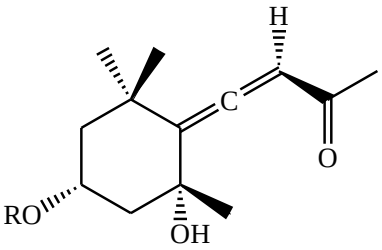
	R_1	R_2
(119)	**Glc**	**H**
(120)	**H**	**Glc**



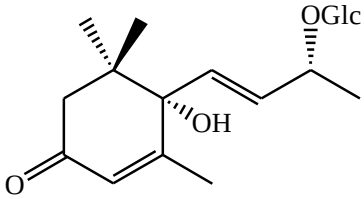
	R_1	R_2	R_3
(121)	**OH**	**Glc**	**H**
(122)	**OH**	**H**	**H**
(123)	**H**	**Glc**	**H**
(124)	**H**	**H**	**H**



	R_1	R_2
(125)	**Glc**	**H**
(126)	**H**	**H**



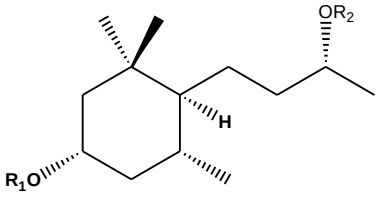
	R
(127)	**Glc**
(128)	**H**
(129)	**p-Bromobenzoyl**



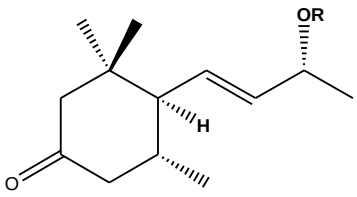
(130)

The previous phytochemical studies on the leaves of *C. oblongifolius* afforded three pairs of megastigmane glycosides, named oblongionosides A (74), oblongionosides B (75), oblongionosides C (76), dendranthemoside A (77), glochidionioside D (78) (Takeshige et al., 2012).

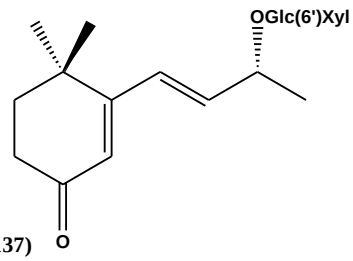
Bridelia glauca Bl. *F. balansae* (Tucht.) *Hatusima* (Euphorbiaceae) is an evergreen tree that grows to a height of about ten m, and is distributed in Okinawa, Taiwan, Southern China, Indochina and Philippines. Previous phytochemical studies of the leaves of the same plant species, collected in Okinawa, led towards the isolation of thirteen megastigmane glucosides to which bridelionosides A–F which are being assigned as trivial names and other megastigmane glucosides such as, 3-oxo-a-ionol glucoside (79), roseoside (80), inamoside (81), bridelionoside (82), megastigman-4-en-3-on-9,13-diol 9-O-*β*-glucopyranoside (83), alangionoside A (84), (3R,5S,6S,7E,9R)-megastigman-7-ene-3,5,6,9-tetrol-9-O-β-D-glucopyranoside (85), (3S,5S,6S,7E,9R)-megastigman-7-ene-3,5,6,9-tetrol-9-O-β-D-glucopyranoside (86), ampelopsisionoside (87), and actinidioionoside (88), (3S,5R,6S,9R)-megastigmane-3,9,13-triol (89), *β*-D-glucopuranosyl 3,9-dihydroxy-(3S,5R,6R,9R)-megastigmane-13-oate (90), (3R,4S,9R,5Z,7E)-megastigmane-5,7-diene-3,4,9-triol-9-O- *β*-D-glucopyranoside (91)(Sueyoshi et al., 2006). The previous phytochemical investigation of leaves of *Glochidion zeylanicum* afforded two new Megastigmane glucosides including six known compounds are being isolated (92-99). Which are listed below, (3*S*, 5*R*, 6*R*, 7*E*, 9*S*)-Megastigman-7-ene-3, 5, 6, 9-tetrol 3-O-*β*-D-glucopyranoside (92), Aglycon of compound-1 (93), 9-O-*β*-D-glucopyranosides of (3*S*, 5*R*, 6*R*, 7*E*, 9*S*)-megastigman-7-ene-3, 5, 6, 9-tetrol (94), kiwiionol



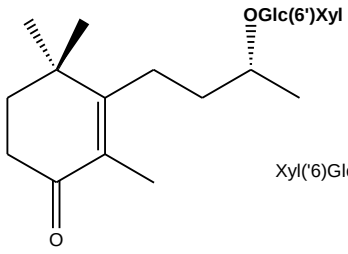
	R_1	R_2
(131)	Glc	Glc
(132)	Glc(6')Xyl	H
(133)	Glc	Glc
(134)	H	Glc



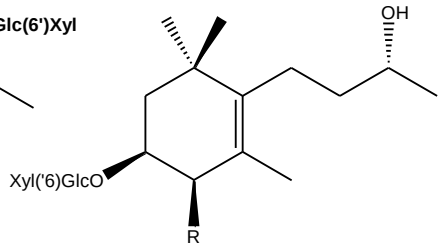
	R
(135)	Glc(6")Xyl
(136)	Glc



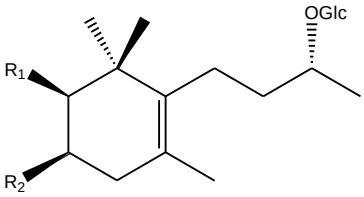
(137)



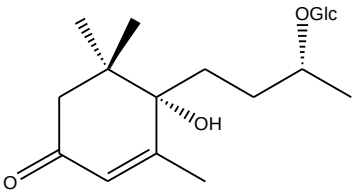
(138)



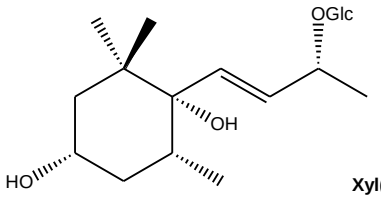
	R
(139)	H
(140)	OH



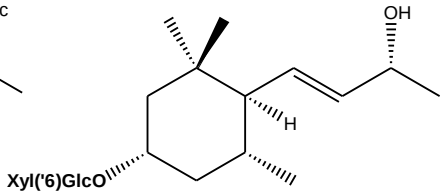
	R1	R2
(141)	OH	H
(142)	H	OGlc



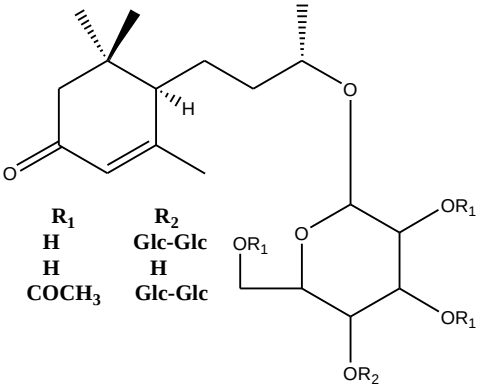
(52)



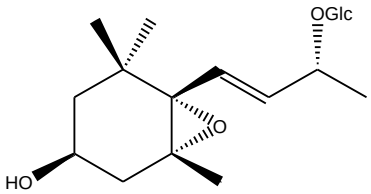
(143)



(144)

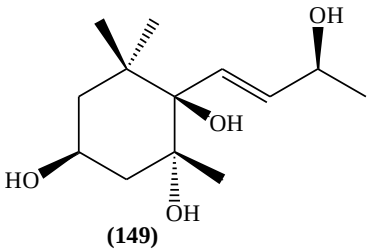


	R_1	R_2
(145)	H	Glc-Glc
(146)	H	H
(147)	$COCH_3$	Glc-Glc

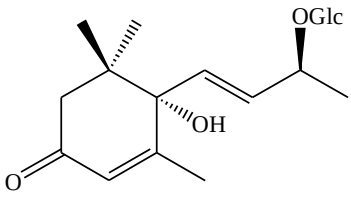


(148)

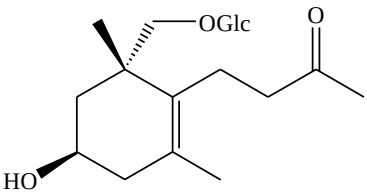
(95) kiwiionoside (96), actinidioionoside (97) Alangionosides E (98), Alangionosides O (99)(Otsuka et al., 2003). Thai medicinal plants in the genus Acanthus, *Acanthus ebracteatus* Vahl (Acanthaceae, Thai name: Ngueak-Pla-Mo, Nam-Mo). *A. ebracteatus* is aspiny herb has got which is widely distributed in the mangroves of southern Thailand. In Thai traditional medicine, the plant is widely used as a purgative and an anti-inflammatory, as well as the leaves dispensed with pepper (*Piper nigrum* L.) as tonic pills for longevity. In the preliminary studies, antimutagenicities of the organic extracts have been reported. Phytochemical results show the isolation of plucheoside B (100), alangionoside C (101), ebracteatoside A (102), ebracteatoside B (103) and ebracteatoside C (104) (Kanchanapooma et al., 2001). *Sauropus androgynus* Merr. (Euphorbiaceae, Thai name: Pak-Waan-Bann) is a shrub which is widely distributed in South and Southeast Asia. The leaves of this plant are a common with high nutricious values



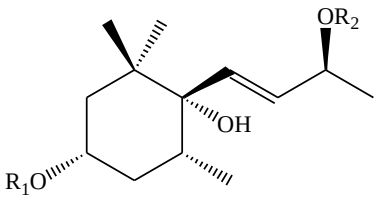
(149)



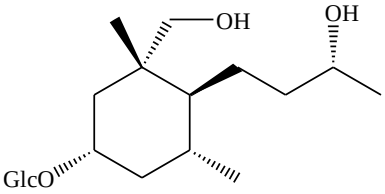
(150)



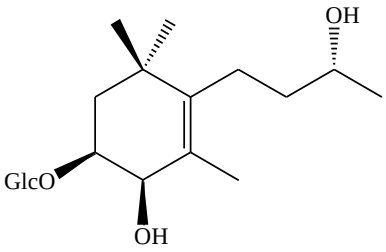
(151)



	R_1	R_2
(152)	**Glc**	**H**
(153)	**H**	**Glc**



(154)



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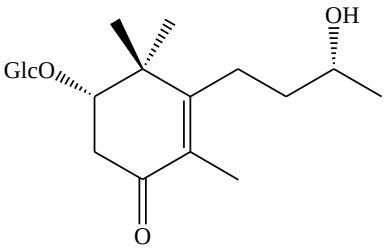
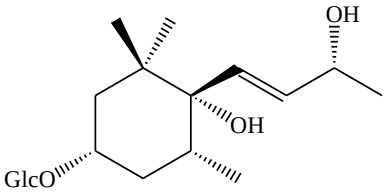


(156)



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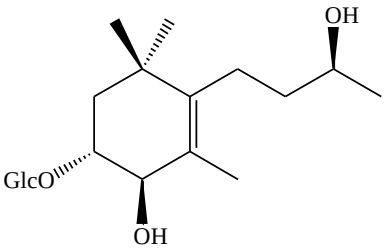

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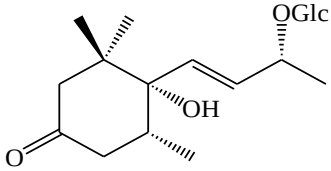
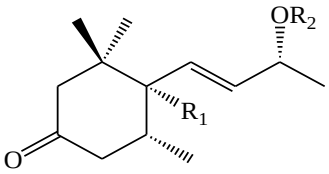
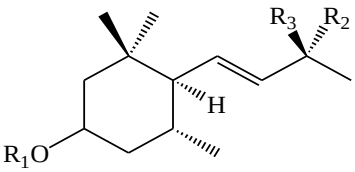


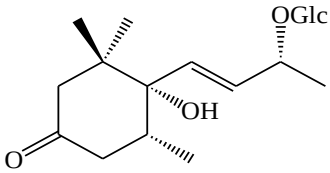
(159)



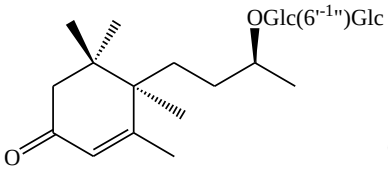
	R_1	R_2
(160)	**H**	**Glc**
(161)	**H**	**Glc(OAc)$_2$**
(162)	**OH**	**Glc**



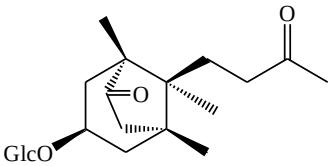
	R_1	R_2	R_3
(163)	**Glc**	**H**	**OH**
(133)	**Glc**	**OH**	**H**
(164)	**H**	**OH**	**H**
(165)	**H**	**OGlc**	**H**



	R_1	R_2
(166)	**Glc**	**H**
(167)	**Glc(6)Api**	**H**
(168)	**H**	**H**



(169)



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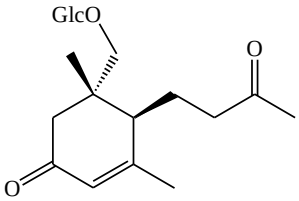
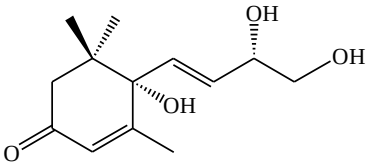


(171)



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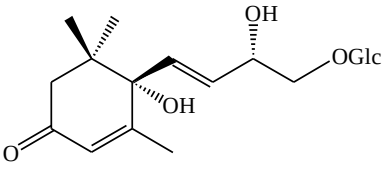

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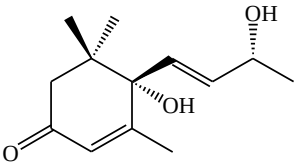

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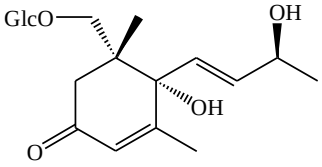
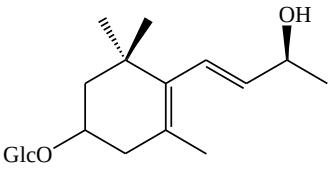
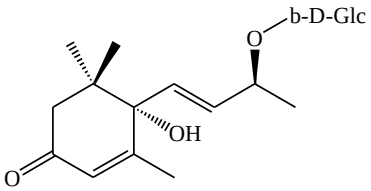


(175)



R
(176) H
(177) a-L-arabinofuranosyl



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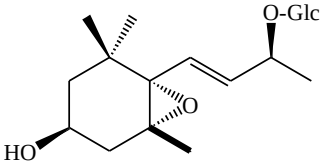

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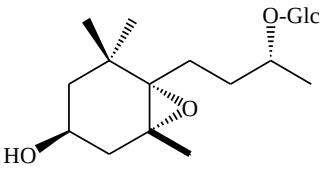

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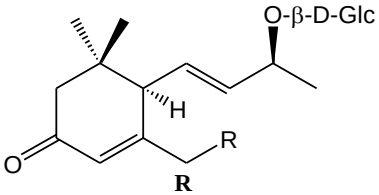
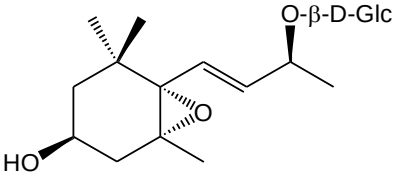
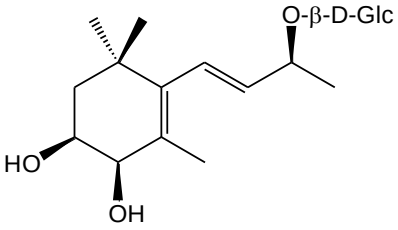


R
(181) OH
(182) H



	R_1	R_2
(183)	**β-D-Glc**	**H**
(184)	**β-D-Glc**	**β-D-Glc**
(185)	**H**	**β-D-Glc**



(186)

vegetable in Thailand. In Thai traditional medicine, the roots are being used as an anti-fever preparation, as an antidote for food poisoning, and an antiseptic agent, the previous investigation results showing the isolation of Megastigmane Glucoside Sauroposide (105) (Kanchanapooma et al., 2003). *Phlomis aurea* Decne (Lamiaceae) is a wild plant grow in Sinai region especially in Gebel Mousa and Saint Katharine mountains. In Egyptian folk medicine, the plant is being used as antidiabetic. Phytchemical investigation led towards isolation a megastigmane, (3*S*, 5*S*, 6*R*, 9*R*)-3-hydroxy-5, 6-

epoxy-*β*-ionol-9-O-*β*-glucopyranoside [phlomuroside] (106) (Mohamed et al., 2000) (Q. Z. Laggera (Astereae, Trib. Plucheae Cass.) which is a small genus of about 20 species, mainly found in tropical Africa and Southeast Asia. *Laggera alata* and *Laggera pterodonta* are the only two *Laggera* species found in China. Both are existed as traditional herbal medicines due to their anti-inflammatory and anti-bacterial activities. Chemical investigation results showing the isolation o a megastigmane compound name it is as Alatoside E [*β*-O-(*β*-D-glucopyranosyl) megastigmane-9-one (107) (Zheng et al., 2003). The plant leaves of *Macaranga tanarius* (L.) Müll.-Arg. (Euphorbiaceae), It is well known as a pioneer tree that grows first ahead of other plants on impoverished soil, and also known as an ant-plant defended by ants against herbivores by producing the ant-attracting food body. It is a small evergreen tree of about 4–5 m in the height found in the bush layer throughout eastern and southern Asia, Especially in Southern China, Korea and Okinawa, Japan. In China, the root and bark of this plant are being used as a folk medicine for hemoptysis and dysentery, the previous results showing the isolation of megastigmane glycosides, named macarangiosides A–D (108-111) (Matsumani et al., 2009).

Lasianthus fordii Hance (Rubiaceae) the glycosidic constituents of the leaves of *L. fordii* and isolated three megastigmane glucosides are lasianthionoside A (112), lasianthionoside B (113) and lasianthionoside C (114) (Takedda et al., 2004). *Breynia officinalis* belongs to Euphorbiaceae family which is poisonous perennial shrub grows in Okinawa, Taiwan and Southern China. This is used as a remedy for healing wounds and edema as an ointment, for syphilis and related problems. The previous phytochemical investigation results showed that the isolation of six secondary metabolites related to megastigmanes which are Turpinionoside B (115), Breyniaionoside A (116), Breyniaionoside B (117), Breyniaionoside C (118), Breyniaionoside D (119) and Cleposide C (120) (Morikawa et al., 2004). *Glochidion zeylanicum* belongs to Euphorbiaceae family and the results shows that the isolation of ten megastigmanes which are Blumenol C glucoside [(6*S*,9*R*)-megastigman-3-on-4-en-9-ol-9-O-*β*-D-glucopyranoside)] (130), Glochidionionoside A (121), Glochidionionol A (122), Glochidionionoside B (125), Glochidionionol B (126), Glochidionionoside C (123), Glochidionionol C (124), Glochidionionoside D (127), aglycon of Glochidionionoside D (128) and p-bromobenzyl ester (129) (Otsuka et al., 2003).

Alangum premnifolium groups in most of the Southern part and Northern to Southern parts of Japan. Phytochemical investigation on the title plant affords fifteen megastigmanes, plantanionoside D (131), Alangionoside K (132), alangionoside J (133), dihydroalangionoside I (134), plantanionoside E (135), myrsinionoside A (136), plantanionoside F (137), plantanionoside G (138), plantanionoside H (139), plantanionoside I (140), plantanionoside J (141), linarionoside A (142), 6S,9R-roscoside (52), alangionoside A (143), alangionoside H (144) (Otsuka et al., 2002). *Piper elongatum* VAHL. Which belongs to Piperaceaefamily and it is a small tree commonly found in Lowlands of the Amazon and it's leaves are being used as a folk treatment of dermatosis in South America. Phytochemical investigation led towards isolation of three megastigmane glycosides as pipeloside A [(6S,9S)-9-hydroxy-4-megastigmane-9-O-*β*-D-glucopyranosyl-(1→4)-O-*β*-D-glucopyranosyl-(1→4)-*β*-D-glucopyranoside (145), pipelol A (146) and byzantionoside B (147) (Masuoka et al., 2002). *Veronica hederifolia* belongs to veronica family, which is widely distributed in Europe and Asia, especially in the mediterranean arean. The previous phytochemical investigation results strongly show the isolation of a megastigmane glycoside such as 3-hydroxy-5, 6-epoxy-*β*-ionol-9-O- *β*-D-glucopyranoside (148) (Harput et al., 2002). *Turpinia ternate* NAKAI which belongs to Staphylaceae family and the results show the isolation of ten megastigmanes, 3S,5R,6R,9S-tetrahydroxy megastigmane (149), corchoionoside C (150), icariside B_4 (151), turpinionoside A (152), turpinionoside B (153), turpinionoside C (154), turpinionoside D (155), turpinionoside E (156), dendranthemoside A (157), and plucheoside B (158) (Yu et al., 2002) The leaves of *Myrsine seguinii* Lev. belong to Myrsinaceae which are distributed in temperate and subtropical areas and Japan, useful for the treatment of anthelmintics. The phytochemical results show the isolation of Eleven megastigmanes which are ampelopsisionoside (159), myrsinionoside A (160), myrsinionoside tetraacetate (161), myrsinionoside B (162), myrsinionoside C (163), alangionoside J (133), aglycon of alangionoside J (164), alangionoside D (165), linarionoside A (166), myrsinionoside E (167), aglycon of myrsinionoside E (168) (Otsuka et al., 2001). *Tricalysia dubia* (LINDL) *Syn*. *Canthium dubium* (LINDL), which belongs to Rubiaceae are used as folk medicine in Africa and widely spred in the South of China. The previous phytochemical investigation results show the isolation of a megastigmane glycoside tricalysionoside A (169) (Shitamoto et al., 2011). *Euphorbia supine* Rafinesque, belongs to Euphorbiaceae family, Isolated of megastigmanes are supinaionoside A (170), and supinaionoside B (171) (Wen-Hu et al., 2009). *Cucumis sativus* L. belongs to Cucurbitaceae family. The fruit of *C. sativus* (cucumber) is used not only as food, but also in folklore medicine and folk cosmetics. The phytochemical investigation studies reports show megastigmanes are cucumegastigmane I (172), cucumegastigmane II (173), and (+) dehydrovomifoliol (174) (Kai et al., 2007). *Juniperus communis* Var. *depressa*, which is belongs to Cupressaceae. Phytochemical investigation results shows that the isolation of four megastigmanes, as (1*R*, 6*R*, 9*S*)-6, 9, 11-trihydroxy-4, 7-megastigmadien-3-one-11-O-*β*-O-glucopyranoside (175), (3*R*, 9*S*)-megastigman-5-en-3, 9-diol-3-O-*β*-O-blucopyranoside (176), (3*R*, 9*S*)-megastigman-5-en-3, 9-diol-3-O-[*α*-L-arabinofuranoses-(1→6)]-*β*-D-glucopyranoside (177), and corchoinoside C (178) (Nakanishi et al., 2005) *Clerodendrum inerme* Gaertn. (Verbenaceae, Thai name: Sam-ma-nga) collected in the Botanical gardens, *C. inerme* is a shrub distributed in South and South-east Asia, Australia and Pacific islands. In Thai traditional medicine, the fresh leaves are externally used for

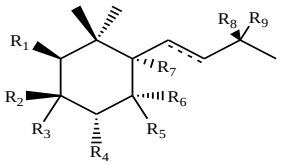
treating skin diseases. Phytochemical study deals with the isolation and structural elucidations of two new megastigmane glucosides (179-180) (Kanchanapoom et al., 2001) *Equisetum debile* and *E. diffusa*, which belongs to Equisetaceae which is useful to cure variety of biologically activities. Previous phytochemical studies show the isolation of megastigamnes as macarangioside D (181), 6*R* ,9*S*-oxo-*α*-ionol-9-O-*β*-glucopyranoside (182), sammangaoside A (183), (3*S*, 5*R*, 6*S*, 7*E*, 9*S*)-megastigman-7-ene-5, 6-epoxy-3, 9-diol 3, 9-O-*β*-D-diglucopyranoside (184), (3*S*, 5*R*, 6*S*, 7*E*, 9*S*)-megastigman-7-ene-5, 6-epoxy-3, 9-diol 3-O-*β*-D-glucopyranoside (185), and debiloside B (186) (Kanchanapoom et al., 2007). Leaves of *Staphylea bumarda* DC, which is belongs to Staphyleacea found throughout the China. Phytochemical results shows that the majority of megastigmanes as icariside B_2 (187), (3*S*, 5*R*, 6*R*, 9*S*, 7*E*)-megastigman-7-ene-3, 5, 6, 9-tetrol-9-O-*β*-D-glucopyranoside (188), staphylionoside (189), staphylionoside A (190), staphylionoside B (191), staphylionoside C (192), staphylionoside D (193), staphylionoside E (194), (3*S*, 4*R*, 9*S*, 5*Z*, 7*E*)-megastgma-5,7-diene-3, 4, 9-triol-9-O-*β*-D-glucopyranoside (195), staphylionoside G (196), staphylionoside H (197), staphylionoside I (198), and staphylionoside J (199) (Feng et al., 2013). The plant *Cinnamonum wilsonii*, belongs to Lauraceae family are distributed in tropical and subtropical regions, mostly in southern Asia and America. The previous reports showed the isolation of nineteen megastigmane and its glycosides. Wilsonols (200-212), (3S,9S)-megastigman-5-ene-3,9-diol (213), (3S,4R,9R)-3,4,9-hydroxymegastigmane-5-ene (214), (3S,4S,5S,6S,9S)-3,4-ihydroxy-5,6-dihydro-*β*-ionol (215), lasianthionoside A (216) (3S,5S,6S,9R)-3,6-dihydroxy-5,6-dihydroxy-5,6-dihydro-*β*-ionol (217), apocynol A (218), and (+)-(6S7E,9Z)-abscisic ester (219) (Shu et al., 2013) . *Laurus nobilis* is an aromatic ever green plant belongs to Lauraceae family. The previous phytochemical investigation studies showed the presence of a megastigmane glycoside named as Lauroside B (220). The roots of *Rehmannia glutinosa* belongs to Rehmanniae family and has intrusting biological activities. The previous phytochemical studies show that the isolation of a megastigmane named as rehmamegastigmane (221) (Feng et al., 2013). LLex paraguariensis St. Hil belongs to Aquifoliaceae family and widely available South America region. The previous phytochemical studies revealed two acylated Megastigmane glycosides named as Matenoside A & B (222 and 223) (Guang-Hua Xu et al., 2010).

Cissus, a genus of approximately 350 species of woody climber (family: Vitaceae) which includes *Cissus quadrangularis* Linn. (veldt grape, winged treebine, wouloudioloco, bone setter), one of the most frequently used medicinal plants found in warmer parts of India (known as "Harsankar" in Hindi and "Asthisanghara" in Sanskrit), Ceylon, East Africa, Malaysia and Thailand. The plant contains a high amount of Vitamin C, carotene A, anabolic sterodal substance and calcium (Sen et al., 1966) Phytochemical analysis has identified several classes of compounds as *C. quadrangularis* constituents, (6*R*, 7*Z*)-9, 10-dihydroxy-4, 7-megastigmadien-3-one (224), (6*S*, 7*E*)-6, 9, 10-trihydroxy-4, 7-megastigmadien-3-one 6-*O*-*β*-D-glucopyranoside (225) and (5*R*, 6*S*, 7*E*)-6, 9, 10-trihydroxy-7-megastigmane-3-one 9-*O*-*β*-D-glucopyranoside (226), (6*R*,7*E*)-6,9-dihydroxy-7-megastigmane-3-one 9-*O*-*β*-D-glucopyranoside (227), (6*S*, 7*E*, 9*S*)-6,9-dihydroxy-4, 7-megastigmane-3-one [(+)-corchoionol C] (228), (6*S*, 7*E*, 9*S*)-6, 9, 10-trihydroxy-7-megastigmane-3-one 9-*O*-*β*-D-glucopyranoside [(+)-corchoionoside C] (229), from *C. quadrangularis* (Sridhar rao et al., 2013)

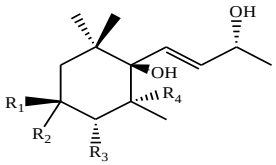
Absolute configuration at 6-position for megatigmanes by using various techniques like circular dichroism (CD) and other techniques

The absolute configuration at C-6 can be determined by comparison of specific rotation {$[\alpha]^{25}_{D}$ +53.0 (*c* 0.5, MeOH)} and electronic circular dichroism (CD) data [positive CE at 265 nm ($\pi\rightarrow\pi^*$), negative CE at 320 nm ($n\rightarrow\pi^*$)] with literature values for similar megastigmane analogs and by application of the chiroptical enone helicity and Snatzke's sector rules. Examination of the experimental CD data for 224 indicates positive helicity and therefore 6*R* absolute configuration (Fig. 3). Snatzke's sector rule, based on the octant rule but with opposite signs for the back octants, also indicates 6*R* absolute configuration due to the C-1 perturber in the negative bottom-right octant in conjunction with the negative $n\rightarrow\pi^*$ Cotton effect for 224 (Fig. 3). These chirality rules confirm the published data for megastigmane derivatives. In one experiment, racemic 9-hydroxy-4, 7-megastigmadien-3-one (3-oxo-α-ionol) was derivatized with (-)-(*R*)- α-phenylpropionic acid, followed by HPLC isolation of the four diastereoisomeric esters. ^{1}H-NMR analysis identified two esters as 9*S* and two as 9*R* isomers. Hydrolysis of the esters provided the four ionols, while CD analysis indicated 6*R* configuration for two of the ionols based on a positive Cotton effect at 243 nm ($\pi\rightarrow\pi^*$ transition) and comparison to authentic (+)-(*R*)- 3-oxo-α-ionone. The remaining two ionols displayed negative Cotton effects at 243 nm. In another experiment, optically active 6, 9-dihydroxy-4,7-megastigmadien-3-one (vomifoliol) and its glucosides (roseosides) were synthesized and the CD spectra of the four diastereoisomers recorded. The CD data followed the same pattern as discussed above, with positive $\pi\rightarrow\pi^*$ and negative $n\rightarrow\pi^*$ Cotton effects indicating 6*S* absolute configuration. The stereo chemical indicator changes from *R* to *S* due a change in Cahn–Ingold–Prelog priorities when replacing the C-6 hydrogen with a hydroxy group, but configuration remains the same.

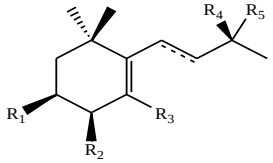
The CD data for the glucosides were almost identical to those of the aglycones. Therefore, the positive CE at 266 nm and negative CE at 317 nm indicates 6*R* absolute configuration for 224. The absolute configuration of the oxymethine chiral center of terminal 1, 2-diols (vicinal diols) cannot be determined by direct application of the Mosher ester method and therefore the C-9 absolute configuration could not be determined.



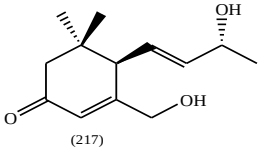
	R_1	R_2	R_3	R_4	R_5	R_6	R_7	R_8	R_9	Δ
(200)	H	OH	H	OH	H	CH3	OH	OH	H	Δ^7
(201)	H	OH	H	OH	H	CH3	OH	H	OH	Δ^7
(202)	H	OH	H	OH	H	CH3	OH	=O		Δ^7
(203)	H	H	OH	H	H	CH2OH	OH	OH	H	Δ^7
(204)	H	OH	H	OH	H	CH3	H	H	OH	Δ^7
(205)	H	OH	H	OH	H	CH3	H	OH	H	Δ^7
(206)	H	H	OH	H	H	CH3	OH	H	OH	Δ^7
(207)	OH	H	H	OH	OH	CH3	H	H	OH	Δ^7
(215)	H	OH	H	O-β-D-glc	H	CH3	OH	=O		Δ^7



	R1	R2	R3	R4
(208)	OH	H	OH	OH
(216)	H	OH	H	H



	R1	R2	R3	R4	R5	
(209)	OH	H	CH2OH	OH	H	
(210)	OH	H	CH2OH	H	OH	
(211)	OH	H	CH2OH	OH	H	D^7
(212)	OH	H	CH2OH	H	OH	D^7
(213)	O-β-D-glc		CH3	OH	H	
(214)	OH	H	CH3	H	OH	



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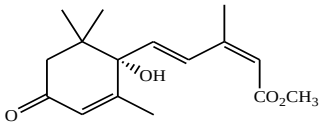

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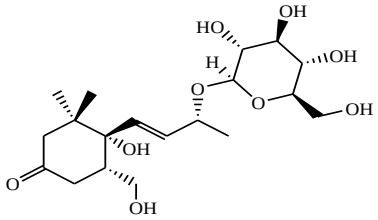

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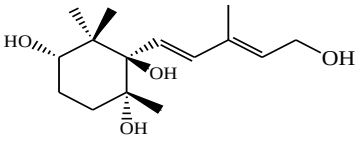

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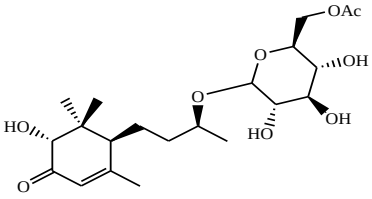

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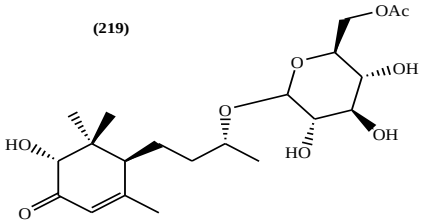

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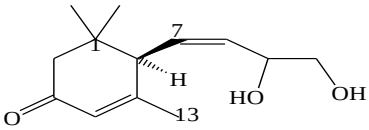

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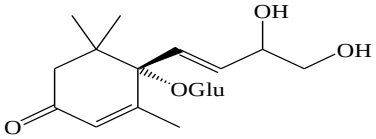

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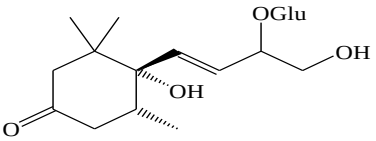

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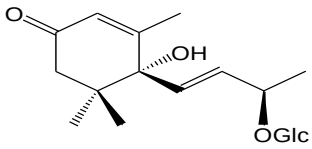

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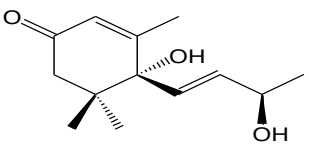

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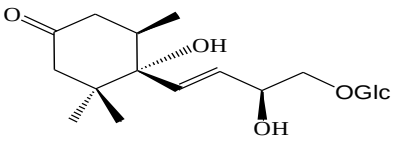

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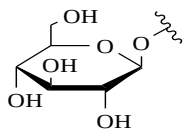


Glu = β-D-glucopyranoside

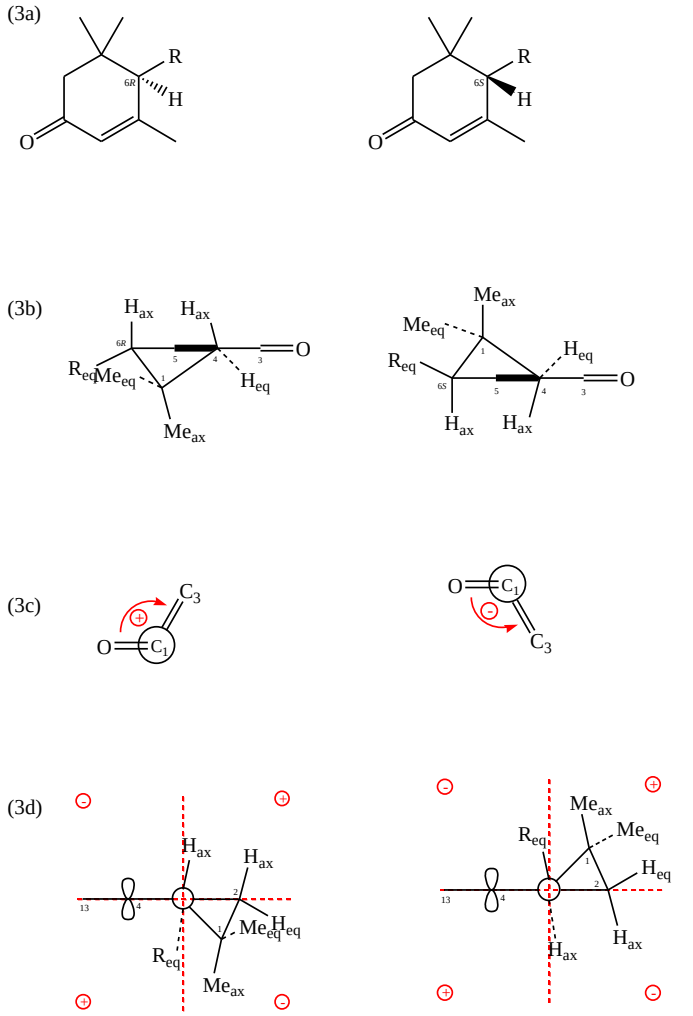


Fig. 3a-3d: CD analysis of 200 (a) 4,7-Megastigmadien-3-one skeleton. (b) Lowest energy 6*R* and 6*S* absolute configurations. (c) Enone helicity rule. (d) Snatzke's sector rule

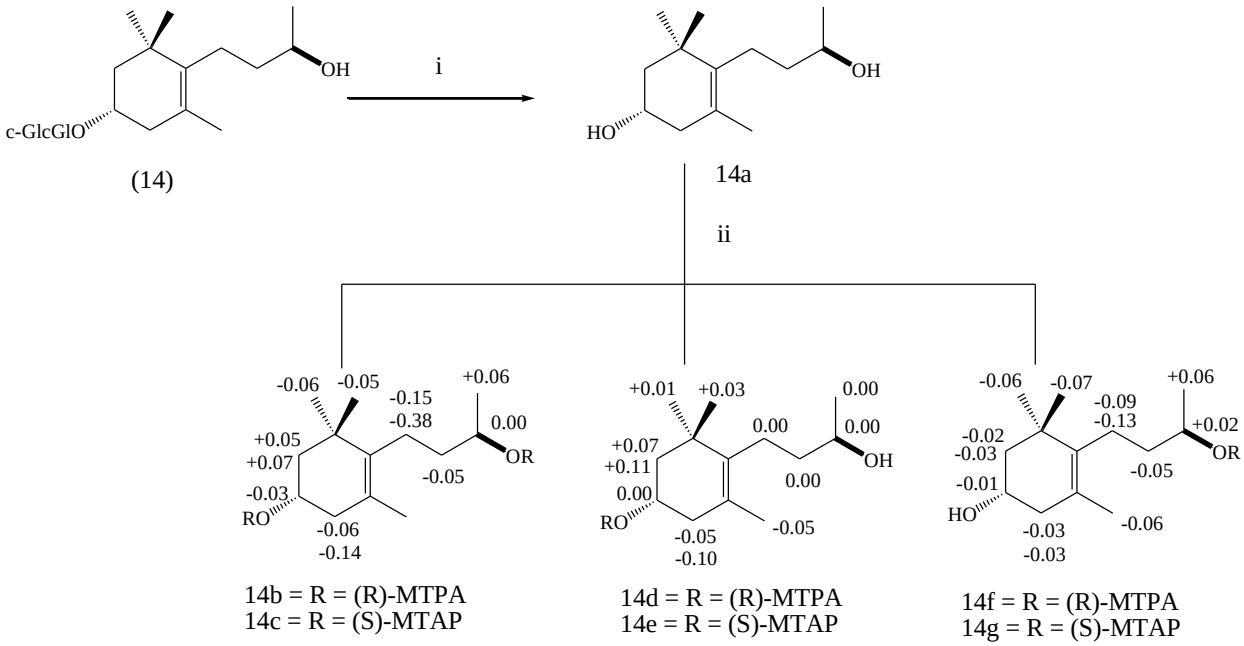
The *trans*-isomer and the C-9 glucoside of the *trans*-isomer (**225**) have been reported (Kapoor et al., 2001; Pramila et al., 2006).

Absolute Configuration at 9-position for Megastigmane compounds by using various techniques like Mosher's Acid other techniques

The relative stereochemistry of Foliasalaciosides E_1 (14), except the 9-position, was characterized in a nuclear Overhauser enhancement spectroscopy (NOESY) experiment, which showed NOE correlations between the following proton pairs: H-2α and H_3-12; H-2β and H_3-11; H-3β and H_3-11, H-2β, H-4β; H_2-7 and H_3-11. Finally, the absolute configuration of the title compound was characterized by the application of the modified Mosher's method. The enzymatic hydrolysis of title compound gave an aglycon; the name of aglycon is that foliasalaciol E (14a). Three pairs of (*R*)- and (*S*)-2-methoxy-2-trifluoromethylphenylacetic acid (MTPA) esters [3, 9-MTPA diester (1c, 1d), 3-MTPA ester (1e, 1f), 9-MTPA ester (1h, 1i) were derived from 14a upon reaction (R)- and (*S*)-MTPA in the presence of 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC) and 4-(dimethylamino) pyridine (4-DMAP). As shown in (Fig. 4), the protons at the 2- and 10- positions of the 3, 9-(*S*)-MTPA diester (14c) resonated at lower fields than those of the 3,9-(*R*)-MTPA diester (14b) (Δδ: positive), while the prorons at the 4-, 7-, 8-, 11-, 12-, and 13-positions of 14c were observed at higher fields compared with those of 14b (Δδ: negative). In addition, the protons at the 2-, 11-, and 12-positions of the 3-(*S*)-MTPA ester (14e) resonated at lower fields than those of the 3-(*R*)-MTPA ester (14d) (Δδ: positive), while the protons at the 4-, and 6-positions of 14e were observed at higher fields compared with those of 14d (Δδ: negative). Furthermore, the protons of the 9-(*S*)-MTPA ester (14g), except for that at the 10-position, were observed at higher fields than those of the 9-(*R*)-MTPA ester (14f) (Δδ: negative). Thus the absolute configuration at the 3- and 9-positions in 14 were elucidated to be 3*S* and 9*R*. Consequently, the structure of 14 was clarified to be (3*S*, 9*R*)-3, 9-dihydroxymegastigman-5-en 3-O-α-L-arabinopyranosyl (1→6)-β-D-glucopyranoside, trivially named as Foliasalaciosides E_1 (Zang et al., 2008; Rao et al., 2013). Mosher acid esterification process is shown in Fig. 4

Total synthesis of aripuanin amegastigmane isolated from *Ficus aripuanensis*

A new natural product aripuanin (230), the norsesqueterpene (3*S*,5*R*,6*R*,7*E*,9*E*)-megastigmane-7-ene-3,5,6,9-tetrol was isolated from the leaves of *Ficus aripuanensis* which belongs to Moraceae family (Nascimento et al., 1999). The synthesis of aripuanin started from isophorone (i), which by an application of the classic method of Kharash and Tawney (Kharasch et al., 1941) afforded the 3-cyclohexenone (ii) in 78-82% yield, recemic mixture (iii) was obtained by reduction with LAH. Treatment of alcohol (iii) with acetic anhydride in triethylamine and 4-N, N-dimethylaminopyridine (DMAP) afforded the acetate (iv), oxidation of (iv) with potassium permanganate in neutral aqueous medium produced the keto-alcohol (v) as a mixture of diastereomers which were separated by preparative HPLC for analysis. Hydrolysis of the acetate group of (v) was accomplished with a K_2CO_3 solution (10%) in methanol to afford a 1:1 mixture of the diastereomeric keto-diols (vi/vi'), which were separated by column chromatography. The protection of the two hydroxyl groups of (vii/vii') through reaction with methoxymethyl chloride and N, N-diisopropylethylamine (DPEA) furnished compound viii. Reaction of the lithium derivative of compound ix with ketone ix at temparature -78^{0}C afforded the alkyne x as a 1:1 mixture of diastereomers that may not be separated. Subsequent reduction of xa with Red-Al furnished the 7*E* isomer of compound xb, treatment of xb under acidic conditions (HCl/MeOH) cleave the protective groups and resulted in a complex mixture of products.



Reagents and Conditions

i) Compound/ 0.2 M acetate buffer (pH 3.8) 40^{O}C, 48 h
ii) (R) or (S)-MTPA, EDC.HCl, 4-DMAP/$CHCl_3$, 60^{O}C, 6h

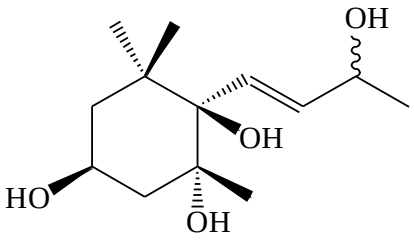
Fig. **4:** Mosher acid esterification analysis

On the other hand, the reaction of xb with 2 equivalents of pyridinium p-toluenesulfonate (PPTS) in 2-propanol at 50°C for 48 h afforded a mixture of final compounds (TM) 53% in a 1:1 ratio. The above mentioned method is absolute appropriate to synthesis of megastigmanes and its related compounds (Nascimento et al., 2012).

BIOACTIVITIES OF MEGESTIGMANES

Experimental procedures for biological activities

Free-radical scavenging activity: Free-radical (DPPH) scavenging activity assay procedure was adopted from the previous report (Rao et al., 2007). In a 96-well micro plates, 25-_L test sample (1 mg/mL DMSO), 125_L of 0.1M Tris–HCl buffer (pH 7.4) and 125 _L of 0.5mM DPPH (1, 1-diphenyl-2-picrylhydrazyl, Sigma Chemicals, USA, which were dissolved in absolute ethyl alcohol) were mixed and shaken well. After incubating 20 min in dark, the rate of the absorbance was recorded spectrophotometrically (SPECTRAMAX PLUS384, Molecular Devices, USA) at 517 nm. The free radical scavenging potential was determined as the percent decolourisation of DPPH due to the test samples and calculated as $(1–B/A)\times100$, where A is absorbance of DPPH control with solvent, and B is absorbance of decolorized DPPH in the presence of test compound. This complete analysis was made in duplicate; trolox was taken as reference compound. Several dilutions of primary solution (1 mg/mL DMSO) were made and assayed accordingly to obtain exact concentration of the sample which required scavenging 50% (SC50) of DPPH for applying suitable regression analysis.



(230)

α-Glucosidase inhibitory activity: Estimation of intestinal α-glucosidase inhibitory activity was carried out as reported earlier [63]. Rat intestinal acetone powder (Sigma Chemicals, USA) was sonicated properly in normal saline (100:1, w/v) and after centrifugation at 3000 rpm×30 min the supernatant was treated as crude intestinal α-glucosidase. Ten microlitres of test samples dissolved in DMSO (5 mg/ml solution) were mixed and incubated with 50 L of enzyme in a 96-well microplate for 5 min. Reaction mixture was further incubated

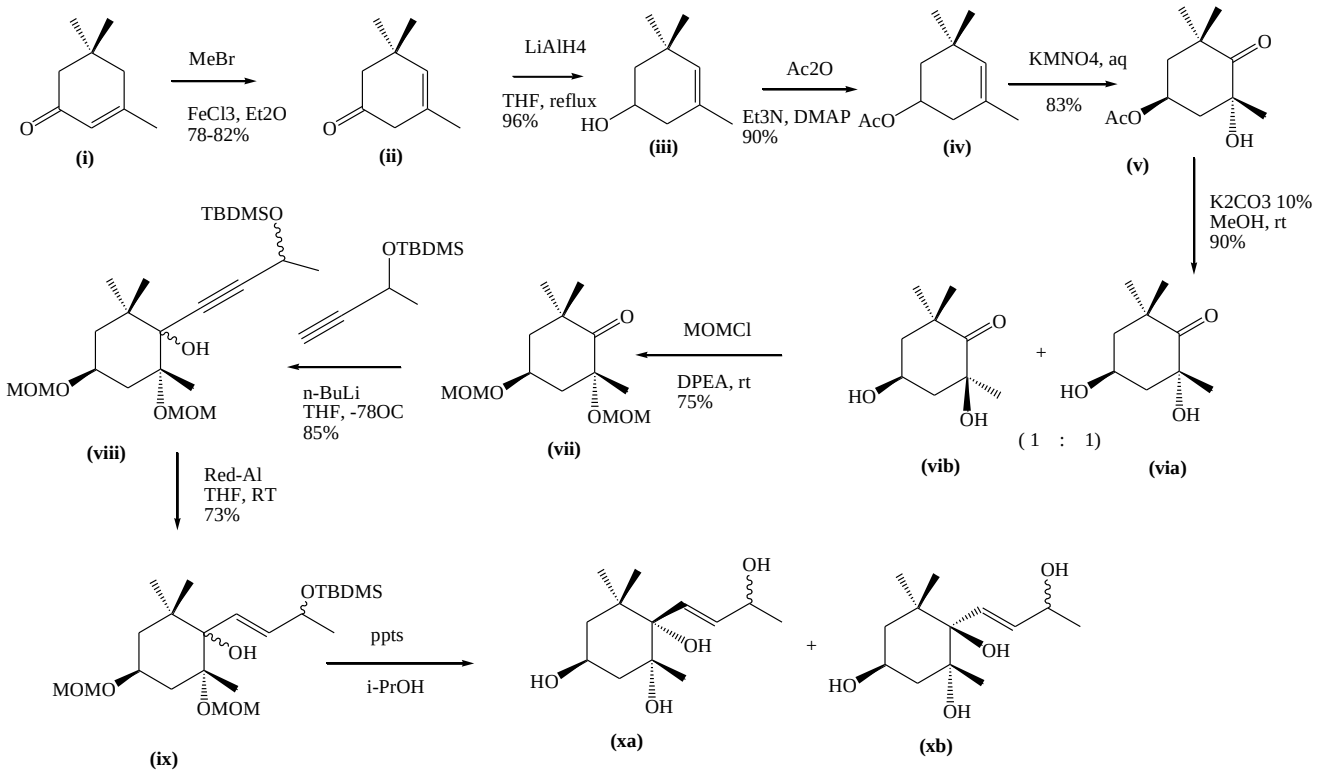


Fig. 5: The Total synthetic of Aripuanin (230)

for another 10 min with 50L substrate [5 mm, *p*-nitrophenyl-α-D-glucopyranoside, prepared in 100mM phosphate buffer (pH 6.8)] and release of nitro phenol was read at 405 nm spectrophotometrically (SPECTRAMAX PLUS384, Molecular Devices, USA). Percent α-glucosidase inhibition was calculated as (1-*B*/*A*) ×100, where A was the absorbance of reactants without test compound B was the absorbance of reactants with test samples. All the samples were running in triplicate and acarbose was taken as standard reference compound. Several dilutions of primary solution (5 mg/mL DMSO) were made and assayed accordingly to obtain exact concentration of the test sample which is required to inhibit 50% activity (IC50) of the enzyme for applying suitable regression analysis.

Hepatoprotective activity: Male Wistar rats were divided into five group's with six animals in each. Group I received normal saline and was kept as the control group. Groups II, III, IV and V received 0.125 ml of CCl4 in liquid paraffin (1:1) per 100 g body weight intraperitoncally. Group II received only CCl4 treatment. Group III was administered silymarin at a dose of 10 mg/kg *p.o* (20.7, µmol/kg). Groups IV and V were treated with 250 and compounds. Drug treatment was started 5 d prior to CCl4 administration and continued till the end of the experiment. After 48 h, following CCl4 administration the biochemical parameters: serum glutamate oxaloacetate transaminase (SGOT), serum glutamate pyruvate transaminase (SGPT), alkaline phosphate (ALP) and total bilirubin were measured using diagnostic strips (Reflotron, ROCHE) and were read on a Reflotron Plus Instrument (ROCHE). The livers of the treated animals were immediately removed and a small piece was fixed in 10% formation for histopathological assessment.

The free radical scavenging activity of compounds (64-69) was next evaluated by its ability to quench the stable radical DPPH. Trolox was used as a reference compound and the Trolox was used as determined for each compounds. Especially the compounds (64-67) had more radical-scavenging activity than the well-known antioxidants like quercetin glycosides. The megastigmane glucosides, named macarangiosides A (108) were galloylated on glucose and possessed potent DPPH radical-scavenging activity. The galloylated moiety is responsible for the activity but double bonds are not positioned at various positions like 4 and 7 and also hydroxyl is placed at different positions. Eventually the isolated megastigmanes acted as potential anti-oxidant agents based structural moiety, regarding to hepatoprotective activity; the methanol soluble portion was found to show hepatoprotective activities. The inhibitory effects of megastigmanes constituents examined, sedumoside A_1 (29), myrisinionosides A (37), myrisinionosides D (38) and sarmenol A (28) were found to show hepatoprotective

activity, which is equivalent to that of silybin. The more polar megastigmanes acted as hepatoprotective active compounds than the less polar as compounds. Finally, the megastigmane compounds showed a moderate activity against different assays.

CONCLUSIONS

The title compound is related to terpene class sub classed by sesquiterpene, trivially named as Megastigmanes. The actual class of megastigmanes is nor-sesquiterpenes having C_{13} skeleton with different stereo centers at C-6 and C-9. The megastigmanes and its glycosides are currently an expanding class of compounds, even with only thirteen carbon atoms in the basic skeleton of megastigmanes, several oxidation steps and glycosylation afforded many kinds of megastigmane derivatives and their glycosidic forms. The stereo chemistry/Absolute configuration of the title compounds to give a better understanding of various aspects of and megastigmane chemistry with the use of CD, Mosher's acid analysis and NOESY etc. Finally, the present chapter deals the Isolation, stereochemistry/absolute configuration, synthesis, biosynthetic approach and bioactivities of various megastigmanes by its glycosides isolated from different plant sources with available literature.

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