PART I

BIOACTIVE CONSTITUENTS OF THE ROOTS OF *MAYTENUS MEKONGENSIS*

CHAPTER 1

INTRODUCTION

Introduction

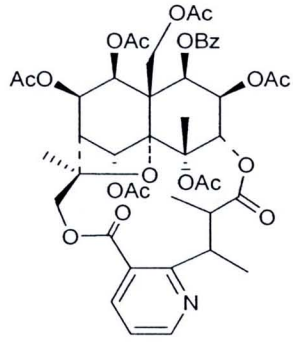
The Celastraceae family contains many plant genus that have been studied extensively due to their use in folkloric medicine. Plants of the genus *Maytenus*, a widely distributed member of the Celastraceae family, have been used to treat cancer in Africa, as an insecticide in Asia, and by shepherd on the Conary Islands to battle fatigue.[1] *Maytenus* plants have been reported to process numerous biological activities including cytotoxic,[2] antibiotic,[3] antifeedant,[4-5] anti-leukemic.[6] Many sesquiterpene alkaliods, triterpenes, and sesquiterpene polyesters, ansa macrolides and flavoniods have isolated.

In Thailand, There are *Maytenus mekongensis*, *Maytenus marcanii* and *Maytenus curtisii* found. Since no prior works on chemical constituent of *Maytenus mekongensis* have been reported, this plant which is known in Thai as "Naam Kaan Chaang" (หนามก้านจาง)[7] is therefore our target plant.

Since number of the compounds have been isolated from genus *Maytenus*, the majority of the biological activities virtually came from the compounds of type sesquiterpene pyridine alkaloids, and triterpenes, so literature search has been emphasized on compounds of these two types and they will be dealt with here in.

Sesquiterpene Alkaloids from Plants in the Genus *Maytenus*

In 1986, De Sousa *et al.*[8] investigated the chemical constituents of the roots of *M. guinensis* and reported the isolation of new compounds mayteine (**1**).



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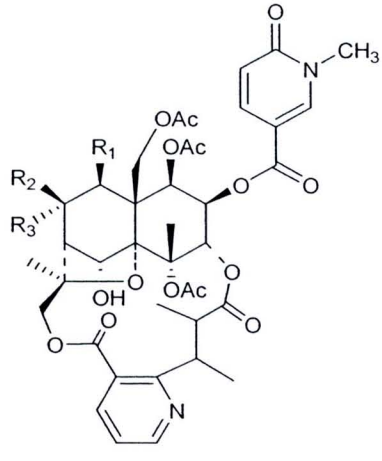
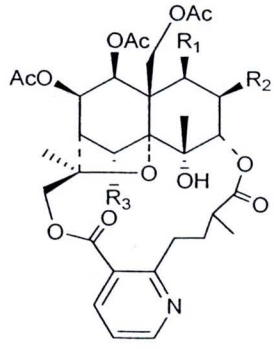
In 1990, Kuo *et al.*[9] reported the isolation of a new compound, emarginatine B (**2**), along with emarginatine A (**3**) from the stems of *M. emarginata*. Emarginatine B (**2**) and A (**3**) were found to be active against KB cell, with ED_{50} of 0.4 and 4.0 μg/mL, respectively.



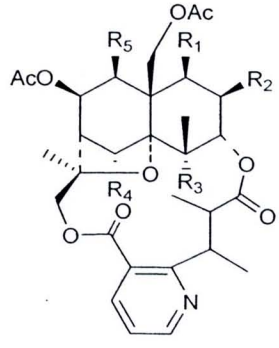
	R_1	R_2	R_3
2	OBz	H	OAc
3	OAc	OAc	H

In 1993, Itokawa *et al.*[10] isolated seven alkaloids, ebenifoline W-I (**4**), W-II (**5**), ebenifoline E-I (**6**), E-II (**7**), E-III (**8**), E-IV (**9**), E-V (**10**) together with

five known compounds, euojaponine C (**11**), F (**12**), euonine (**13**) mayteine (**1**), euonynine (**14**) from dry bark of *M. ebenifolia.*

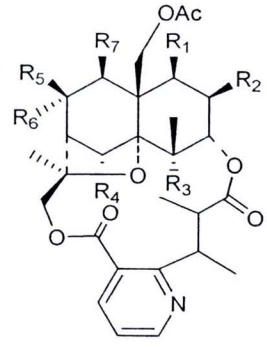


	R_1	R_2	R_3
4	OBz	OBz	OAc
5	OBz	OBz	OH
6	OBz	OAc	OAc
7	OAc	OAc	OAc



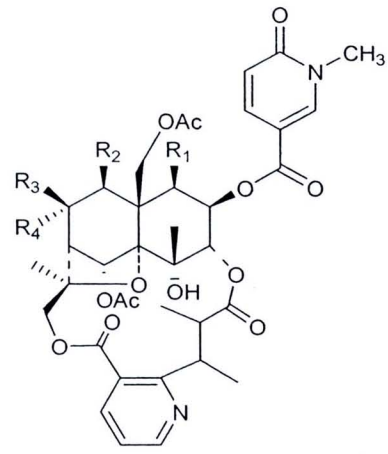
	R_1	R_2	R_3	R_4	R_5
8	OBz	OH	OH	OAc	OAc
9	OBz	OAc	OH	OBz	OAc
10	OBz	OAc	OH	OAc	OBz
11	OBz	OH	OH	OBz	OAc
12	OBz	OAc	H	OAc	OAc
13	OBz	OAc	OH	OH	OBz
14	OAc	OAc	OH	OAc	OAc

In 1994, Shitota *et al.*[11] reported the separation of seven sesquiterpene alkaloids, chuchuhuanines E-I (**15**), E-II (**16**), E-III (**17**), E-IV (**18**), E-V (**19**), W-I (**20**) and 4- deoxyeuonymine (**21**), which were isolated along with seven known alkaloids, ebenifolines E-I (**6**), E-II (**7**), E-III (**8**), E-IV (**9**), E-V (**10**), mayteine (**1**), and euonymine (**14**) from barks of *M. chuchuhuasca.*



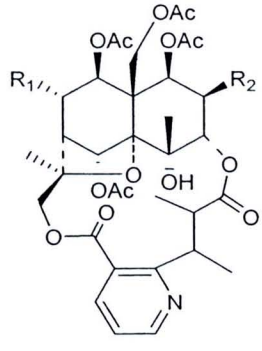
	R_1	R_2	R_3	R_4	R_5	R_6	R_7
15	OAc	OAc	H	OAc	H	OAc	OAc
16	OBz	OAc	H	OAc	H	OAc	OAc
17	OAc	OBz	H	OAc	H	OAc	OAc
18	OAc	OAc	H	OH	H	OAc	OAc
19	OAc	OH	H	OAc	H	OAc	OAc
20	OAc	OAc	H	OAc	H	OAc	OAc
21	OBz	OH	OH	OAc	OAc	H	OAc

Kuo *et al.*[12-14] investigated the stem bark of *M. emarginata*, seven new sesquiterpenes, emarginatine (**22**), C (**23**), D (**24**), E (**25**), F (**26**), G (**27**), and H (**28**) were isolated in 1994 and 1995. Compound **22** and **25** showed cytotoxicity against human KB cells with ED_{50} values of 2.5 and 2.1 μg/mL, respectively. Compound **26** found to be active against six cancer cell lines, namely, KB, A-549, HCT-8, P-388, RPMI-7951, and TE-671 with ED_{50} of 0.51, 5.50, 1.29, 0.69, <0.1 and 0.21 μg/mL, respectively.



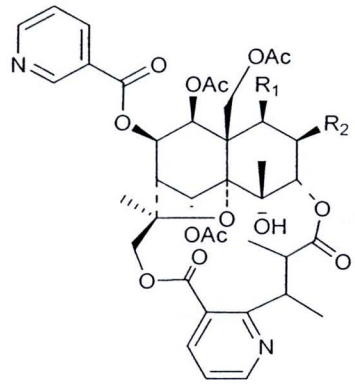
	R_1	R_2	R_3	R_4
22	OAc	OAc	OAc	H
23	OAc	OH	OAc	H
24	OH	OAc	OAc	H
25	OH	OH	H	OAc
26	OBz	OAc	H	OH
27	$CH_3CH{=}C(CH_3)COO$	OAc	OAc	H
28	OAc	OAc	OAc	H

In 1998, Corsino *et al.*[15-16] reported four new sesquiterpene evoninate alkaloids, aquifoliunines E-I (**29**), E-II (**30**), E-III (**31**) and E-IV (**32**) from the methanol extract of the roots bark of *M. aquifolium*.



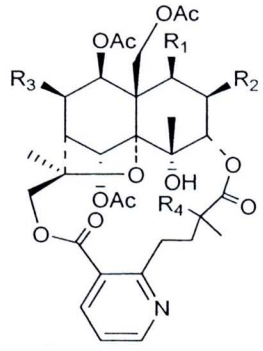
	R_1	R_2
29	OBz	OAc
30	OH	OH
31	H	OAc
32	Nic	OAc

In 2001, Schaneberg *et al.*[17] reported the isolation of putterines A (**33**), B (**34**) and mayteine (**1**) from the roots of *M. putterlickoides*.

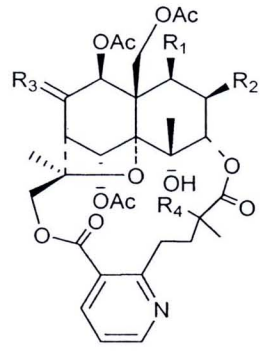


	R_1	R_2
33	CH_3COO	CH_3COO
34	CH_3COO	$(CH_3)_2CHCOO$

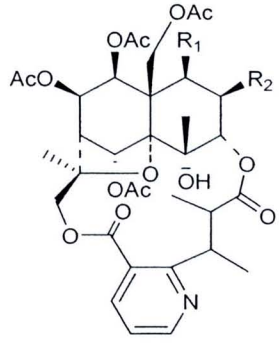
In 2004, Núñez *et al.*[4] reported the isolation of chiapenines ES-I (**35**), ES-II (**36**), ES-III (**37**), and ES-IV (**38**) in addition to ten known alkaloids, wilfordine (**39**), alatamine (**40**), wilforidine (**41**), alatusinine (**42**), euonine (**12**), euonymine (**14**), ebenifoline E-I (**6**), forrestine (**43**), mayteine (**1**), and 4-hydroxy-7-*epi*-chuchuhuanine E-V (**44**) from the leaves of *M. chiapensis*. Compounds **35-42** and **12** exhibited strong antifeedant activity against *Spoduptea littoralis*.



	R_1	R_2	R_3	R_4
35	OBz	OBz	OAc	OH
39	OAc	OBz	OAc	OH
41	OAc	OH	OAc	OH
42	OAc	OAc	OAc	OH

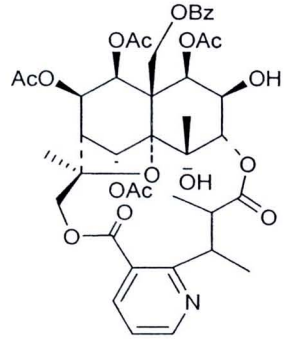


	R_1	R_2	R_3	R_4
36	OBz	OBz	O	OH
37	OBz	OH	O	OH
38	OAc	OH	O	OH
40	OAc	OBz	O	OH



	R_1	R_2
43	OAc	OBz
44	OAc	OH

In the same year, Nakawa *et al.*[18] isolated 7-(acetyloxy)-11-*O*-benzoyl-2,11-*O*-deacetyl-7-deoxoevonine (**45**) from methanol extract of the bark of the Colombian medicinal plant *M. laevis*.



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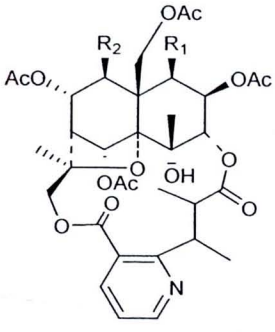
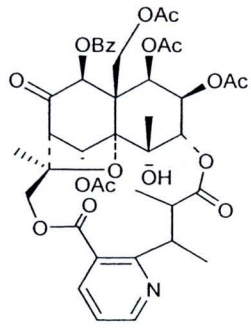
In 2010, De Almeida *et al.*[19] reported the isolation of 8β-acetoxy-1-*O*-benzoyl-1-*O*-deacetyl-8-deoxoevonine (**46**), 9-*O*-benzoyl-9-*O*-deacetylevonine (**47**) together with four known compounds, euonine (**12**), horridine (**48**), cangorinine E-I (**49**) and ebenifoline E-II (**7**) from dried roots of *M. spinosa*.



	R_1	R_2
48	Ac	Bz
46	Bz	Ac



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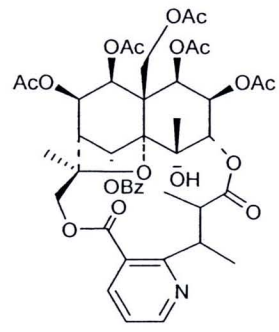

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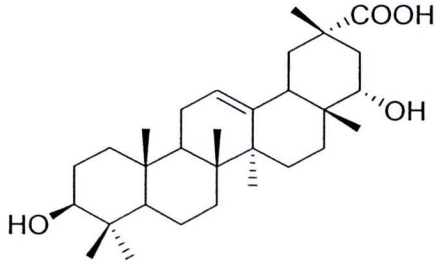
Triterpenes from Plants in the Genus *Maytenus*

In 1982, Nozaki *et al.*[20] studied the chemical constituents of *M. diversifolia* and revealed the presence of two triterpenes, maytenfolic (**50**) and maytenfoliol (**51**) which showed significant antileukemic activity.



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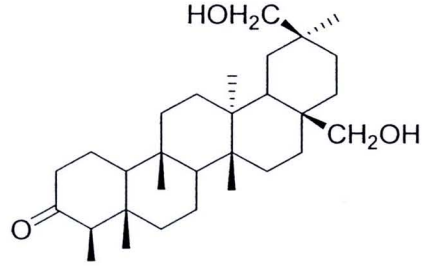
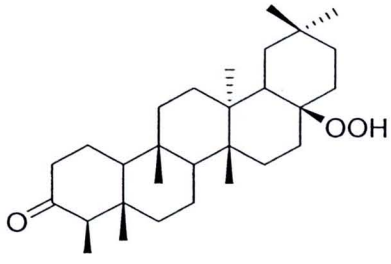


51

In 1984, Lee and Nozaki[21] reported the isolation of a hydroperoxy triterpene, maytensifolin A (**52**) from a methanol extract of *M. diversifolia*. Later in 1995, a new triterpene lactone, maytenfolone (**53**) was isolated from the dried leaves of *M. diversifolia* by Kuo *et al*.[14]



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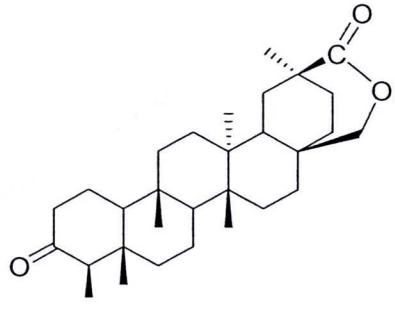
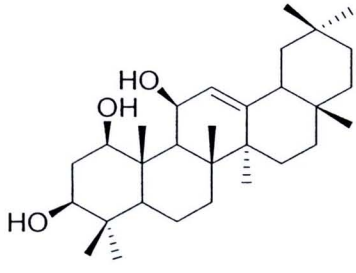


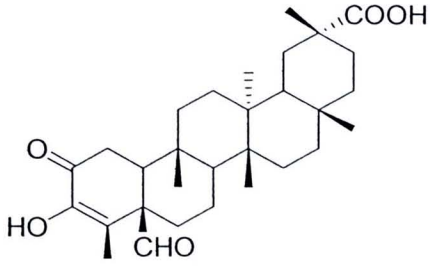
53

In 1987, González *et al.*[22] isolated a new triterpene, 1β,3β,11α-trihydroxy-olean-12-en (**54**) from the root bark of *M. horrida*.



54

In 1991, Itokawa *et al.*[23] reported the isolation of cangoronine (**55**) and ilicifoline (**56**) from the methanol extract of *M. ilicifolia*.



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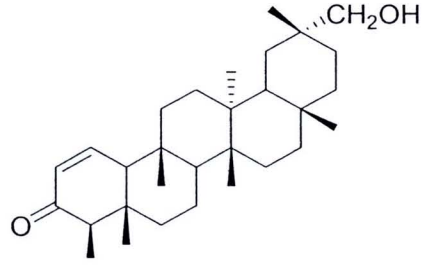
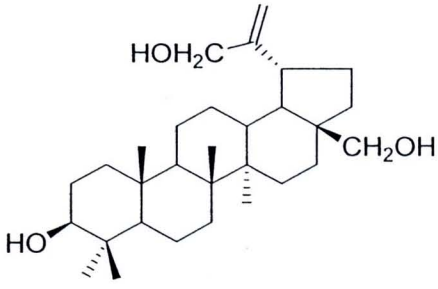


56

In 1992, González *et al.*[24] isolated 3β,28,30-lup-20(29)ene-triol (**57**) and 28,30-dithydroxy-lup-20(29)-en-3-one (**58**) from *M. canariensis*.



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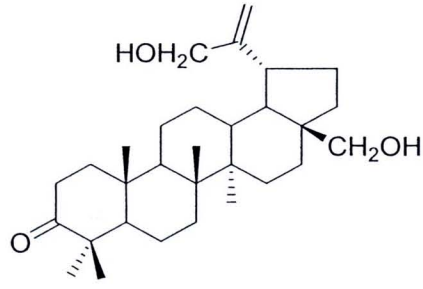
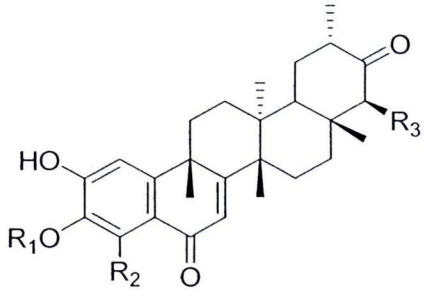
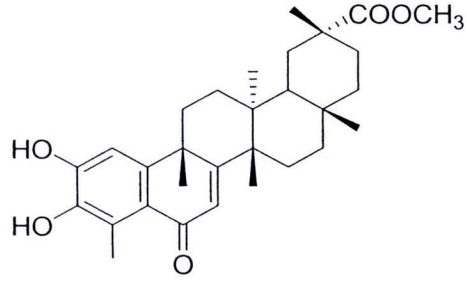


58

In 1994, Shirota *et al.*[25] reported the isolation of four cytotoxic aromatic triterpenes, 6-oxotingenol (**59**), 6-oxopristimerol (**60**), 3-methyl-6-oxotingenol (**61**), 3-methyl-22β,23-dihydroxy-6-oxotingenol (**62**) together with three known quinoid triterpenes, pristimerin (**63**), tingenone (**64**) and 22β-hydroxytingenone (**65**) from *M. ilicifolia* and *M. chuchuhuasca*.



	R_1	R_2	R_3
59	H	CH_3	H
61	CH_3	CH_3	H
62	CH_3	CH_2OH	OH



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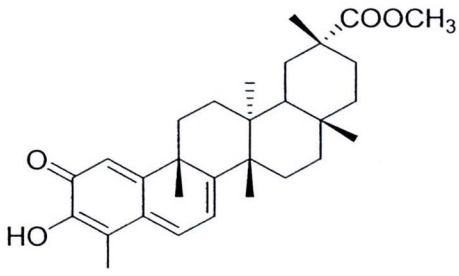

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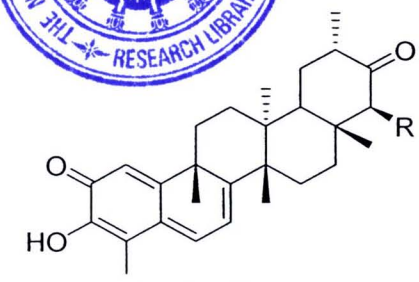
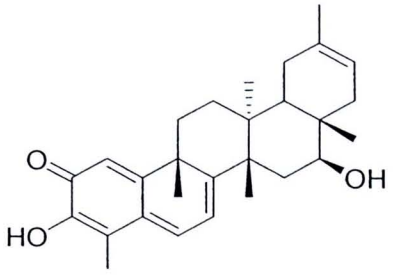


64 R = H
65 R = OH

In 1995, González *et al.*[26] reported the isolation of the nortriterpene quinine methide, 16β-hydroxyiguesterin (**66**) and 2α-hydroxy-3-oxofriedolean-30-oic acid (**67**) from the root bark of *M. canariensis*. A year later, González *et al.*[27] isolated 6-oxo-tingenol (**68**), 3-*O*-methyl-6-oxo-tingenol (**69**) and 6-oxo-iguesterol (**70**) from *M. canariensis*. Compounds **68-70** showed antibiotic activity against *B. subtilis*, with MIC of 12-14, 35-39 and 25 μg/mL, respectively. Compound **68** was also active against *S. aur*eus with a MIC of 40-50 μg/mL.



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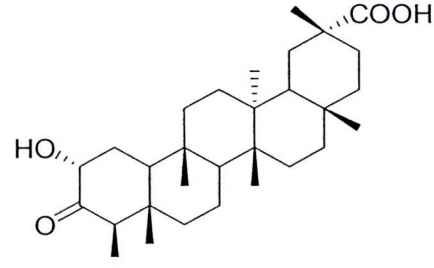

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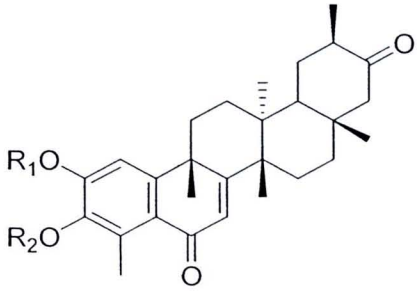
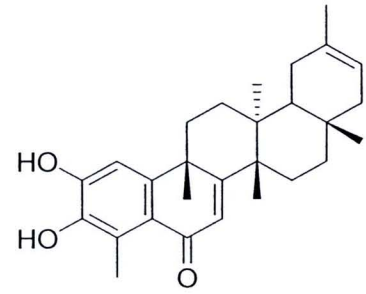
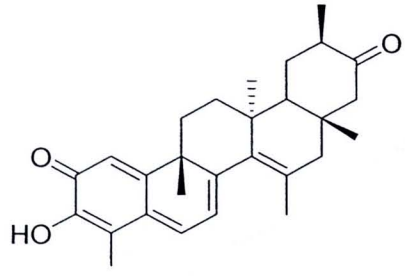


	R_1	R_2
68	H	H
69	H	CH_3



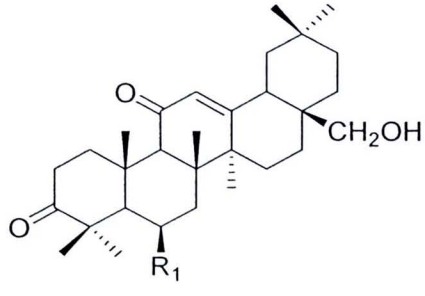
70

In 1996, scutione (**71**) was isolated from the root bark of *M. scutioides* by González *et al.*[28] Scutione (**71**) showed antibiotic activity against Gram-positive bacteria and modest cytotoxic activity against HeLa, Hep-2 and Vero cell lines.



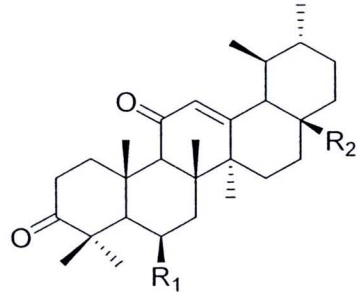
71

In 1996, Shirota *et al.*[29] investigated the methanol extract of the stem bark of *M. krukovii* and five new triterpenes, krukovines A (**72**), B (**73**), C (**74**), D (**75**), and E (**76**) were isolated in the same year.



72 R_1 = H

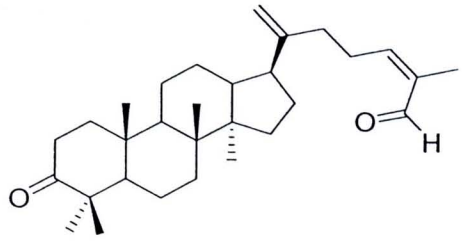
74 R_1 = OH



	R_1	R_2
73	H	CH_2OH
75	OH	CH_2OH
76	H	OH

In 1997 and 1988, Chávez *et al*[30] reported the isolation of the eight new dammarane triterpenes, 24-(*Z*)-3-oxo-dammara-20,24-dien-26-al (**77**), 24-(*E*)-

3-oxo-dammara-20,24-dien-26-ol (**78**), 24-(*E*)-3-oxo-dammara-23-α-hydroxy-20,24-dien-26-al (**79**), 24-(*E*)-3-oxo-dammara-23-β-hydroxy-20,24-dien-26-al (**80**), 24-(*E*)-3-oxo-dammara-6-β-hydroxy-20,24-dien-26-al (**81**), 24-(*E*)-3-oxo-dammara-6-β-hydroxy-20,24-dien-26-ol (**82**), 23-(*Z*)-3,25-dioxo-25-nor-dammara-20,24-diene (**83**), and 24-(*E*)-3-oxo-23-methylene-dammara-20,24-dien-26-oico (**84**) from the stem bark exudates of *M. macrocarpa*. A year later, a new friedelan triterpene, 28-hydroxyfriedelane-1,3-dione (**85**) was also isolated from *M. macrocarpa* by Chávez *et al.*[31]



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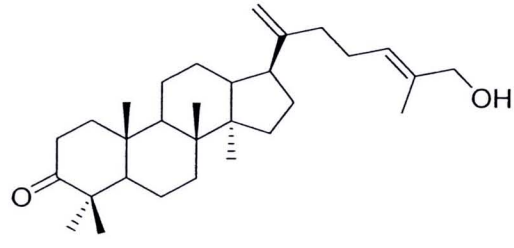

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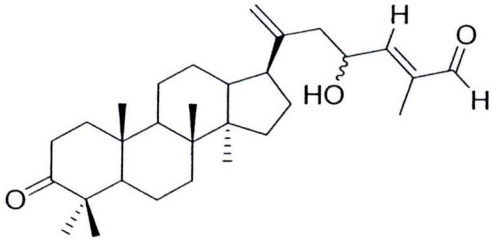
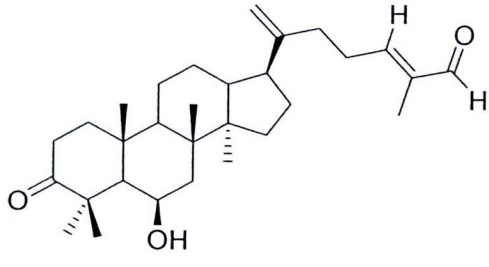


79, 80



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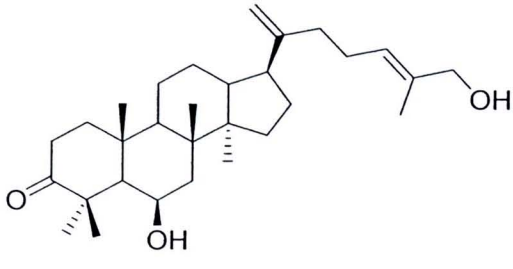

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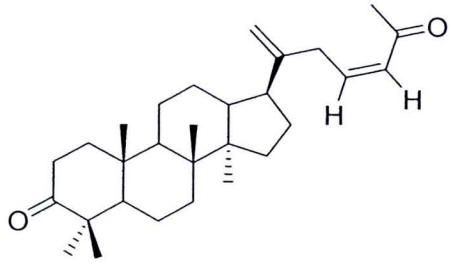

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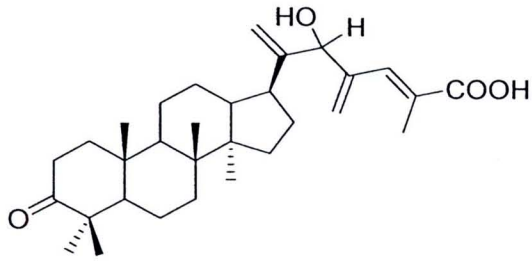

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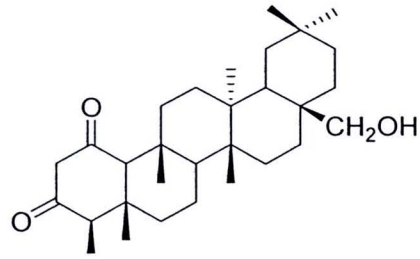
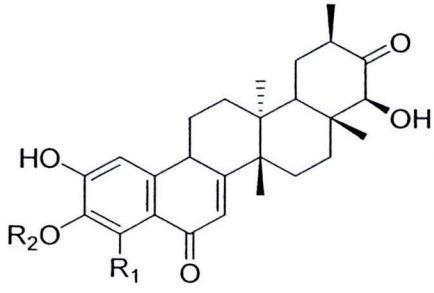
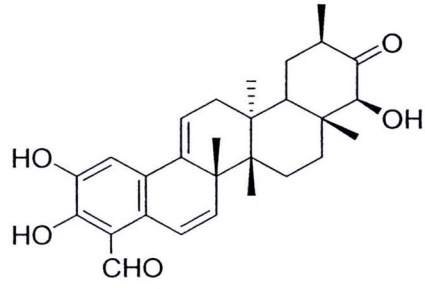


85

In 1998, Chávez *et al.*[32] investigated the n-hexane-diethyl ether extract of the root bark of *M.amazonica* and 2,3,22β-trihydroxy-24,29-dinor-1,3,5(10),-7-friedelatetraene-6,21-dione-23-al (**86**), 2,3,22β-trihydroxy-24,29-dinor-25 (9→8)-1,3,5(10),7-friedelatetraene-21-one-23-al (**87**), 2,22β-dihydroxy-3-methoxy-24,29-dinor-1,3,5(10),7-friedelatetraene-6, 21-dione (**88**), 2,3,22β-trihydroxy-23,24,29-trinor-1,3, 5(10),7-friedelatetraene-6,21-dione (**89**), 2,22β-dihydroxy-3-methoxy-24,29-dinor-1,3,5(10),7-friedelatetraene-6,21-dione (**90**), 2,3,22β-trihydroxy-24, 29-dinor-1,3,5(10)-friedelatriene-6,21-dione (**91**), 2,15α, 22β-trihydroxy-3-methoxy-24,29-dinor-1,3,5(l0)-friedelatriene-21-one (**92**), 3,22β-dihydroxy-24,29-dinor-l(10)3,5-friedelatriene-2,7,21-trione (**93**), and 3,22β-dihydroxy-24,29-dinor- l(10),3,5-friedelatriene-21-one (**94**) were isolated. Some of them showed cytotoxic activities. Later in 1999, two nortriterpene methylene quinones, amazoquinone (**95**), and (*7S,8S*)-7-hydroxy-7,8-dihydro-tingenone (**96**), and three norphenolic triterpenes 7,8-dihydro-6-oxo-tingenol (**97**), 23-*nor*-6-oxo-tingenol (**98**), and 23-oxo-iso-tingenone (**99**) were also isolated from *M. amazonica* by Chávez *et al.*[33] Compounds **95-97** and **99** showed low antitumor activities against four cancer cell lines.



	R_1	R_2
86	CHO	H
88	CH_3	H
89	H	H
90	CH_3	CH_3



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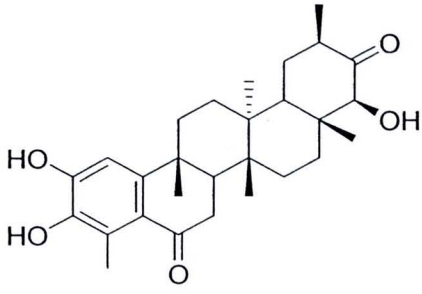
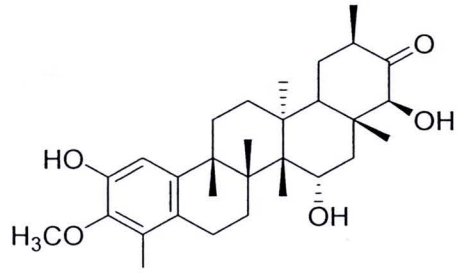


91



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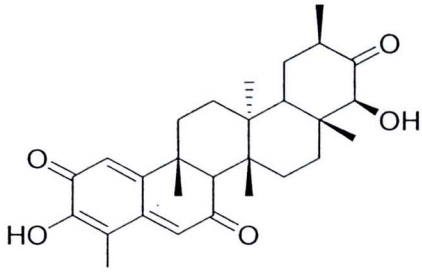

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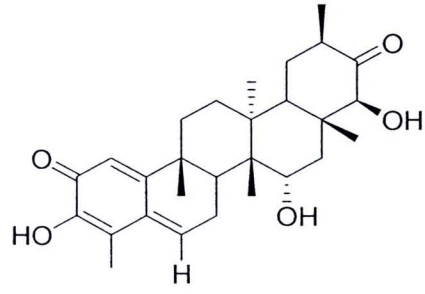

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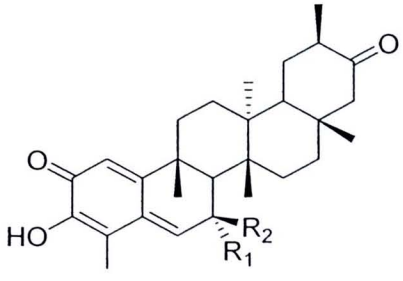
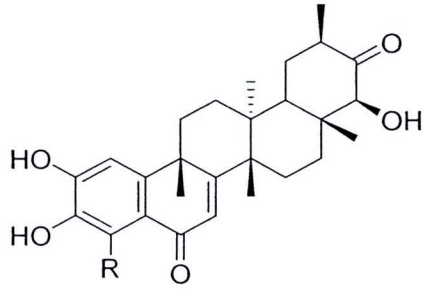


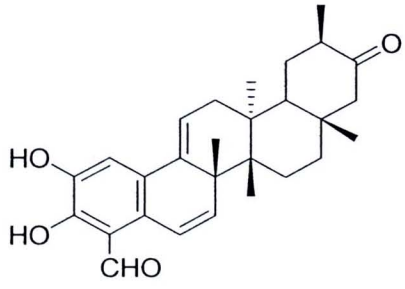
95 $R_1 + R_2 = O$

96 $R_1 = OH, R_2 = H$



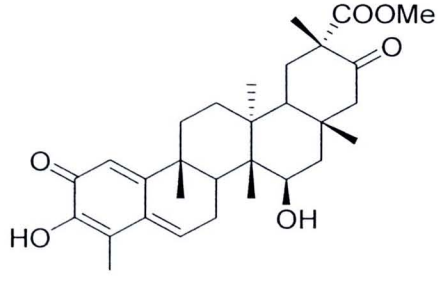
97 $R = CH_3$; 7,8-dihydro

98 R = H



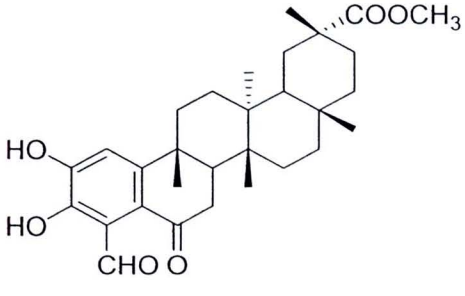
99

In 1999, Alvarenga *et al.*[3] reported the isolation of 15α-hydroxy-21-keto-pristimerine (**100**) from the root bark of *M. catingarum*. Compound **100** showed antibiotic activity against Gram-positive bacteria.

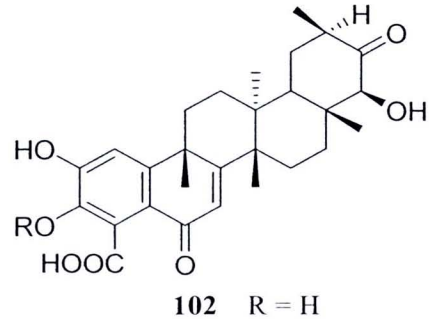


100

In 1998, Chávez *et al.*[34] reported the isolation of macrocarpins A (**101**), B (**102**), C (**103**), and D (**104**) from the roots of *M. macrocarpa*. Compounds **101**, **102** and **104** showed cytotoxicity against four tumoral cell lines (P-388, A-549, HT-28 and MEL-28) with IC_{50} values ranging between 0.4 and 5.2 μg/mL.

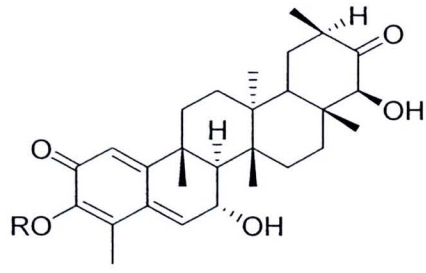


101



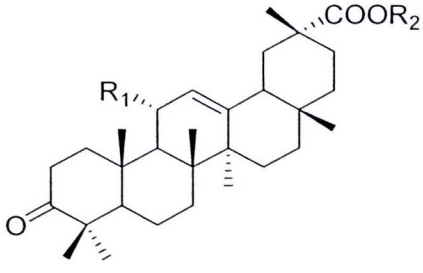
102 R = H

103 R = CH_3

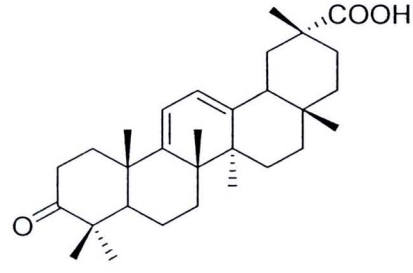


104

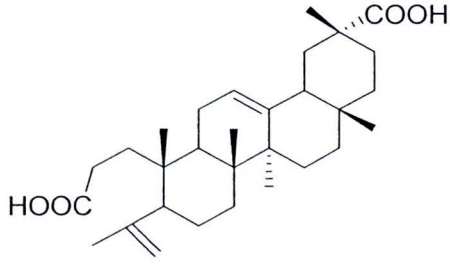
In 2000, Muhammad *et al.*[35] investigated the ethanol extract of the aerial parts of *M. undata*, 12-oleanene and 3,4-seco-12-oleanene triterpene acids, namely, 3-oxo-11α-methoxyolean-12-ene-30-oic acid (**105**), 3-oxo-11α-hydroxyolean-12-ene-30-oic acid (**106**), 3-oxo-olean-9(11),12-diene-30-oic acid (**107**), and 3,4-seco-olean-4(23),12-diene-3,29-dioic acid (**108**) were isolated. Compound **108** potently inhibited rat neonatal brain microglia phorbol ester-stimulated thromboxane B2 (with IC_{50} value = 0.5 μM) and superoxide anion generation (with IC_{50} value = 1.9 μM).



	R_1	R_2
105	OCH_3	H
106	OH	H

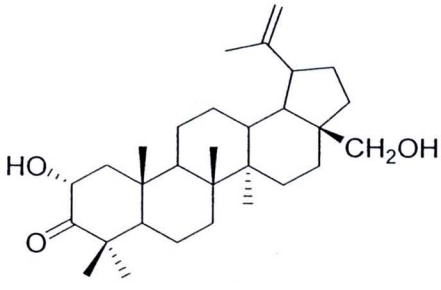


107

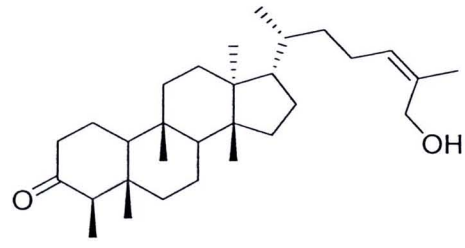


108

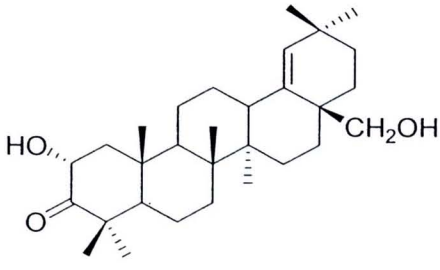
In 2004, Ohsaki *et al.*[36] investigated the leaves of *M. ilicifolia*, maytefolins A (**109**), B (**110**), C (**111**) and uvaol-3-caffeate (**112**) were isolated.



109



111



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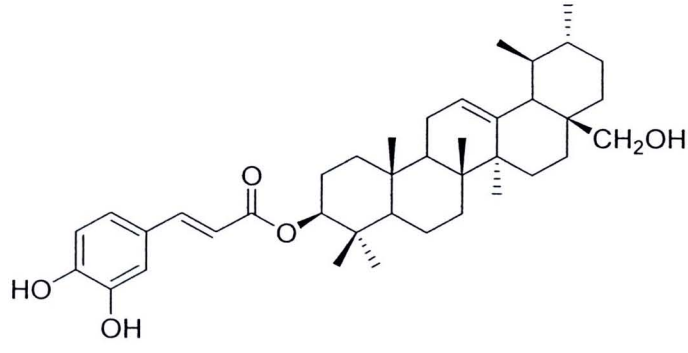
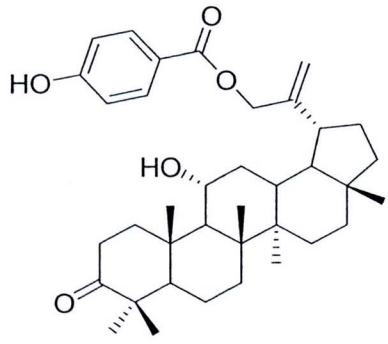


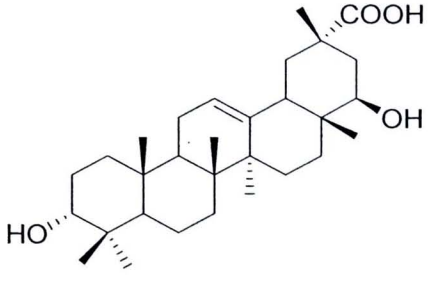
112

In 2004, Feng *et al.*[37] reported the isolation of 30-(4′-hydroxy benzoyloxy)-11α-hydroxylupane-20(29)-en-3-one (**113**) which exhibited DNA polymerase β lyase inhibitory activity with IC_{50} values of 62.8 μM from the methyl ethyl ketone extract of *M. putterlickoides*.



113

Nakagawa *et al.*[18] reported the isolation of 3α,22β-dihydroxyolean-12-en-29-oic acid (**114**) and 28-hydroxy-12-ursene-3β-yl-caffeate, (uvaol-3-yl-caffeate) (**115**) from methanol extract of the bark of a Colombian medicinal plant *M. laevis*.



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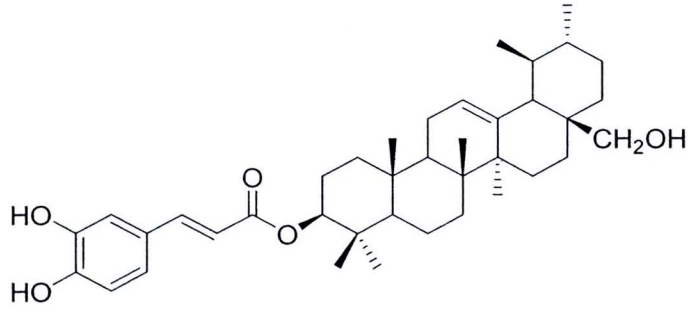
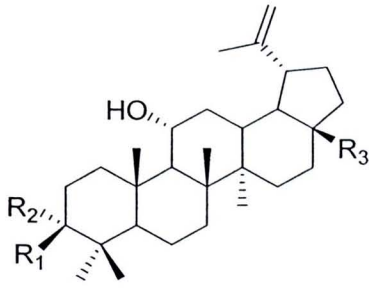
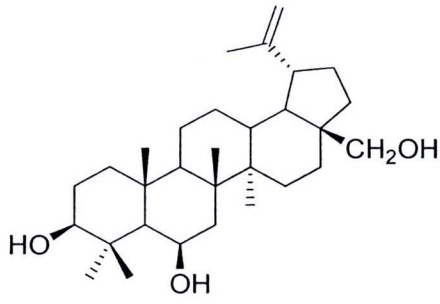


115

In 2005, Núñez *et al*[38] studied the chemical constituents of *M. cuzcoina* and *M. chiapensis* and identified five new lupane triterpenes, 11α-hydroxy-*epi*-betulin (**116**), 6β-hydroxybetulin (**117**), 24- hydroxybetulone (**118**), rigidenol-28-aldehyde (**119**) and 28-hydroxyglochidone (**120**) from the CH_2Cl_2 extract of the leaves of *M. chiapensis* and the n-hexane-Et_2O (1:1) extract of the root bark of *M. cuzcoina*.



116 $R_1 = H, R_2 = OH, R_3 = CH_2OH$
119 $R_1 + R_2 = O, R_3 = CHO$



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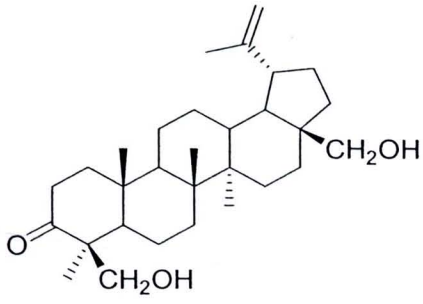
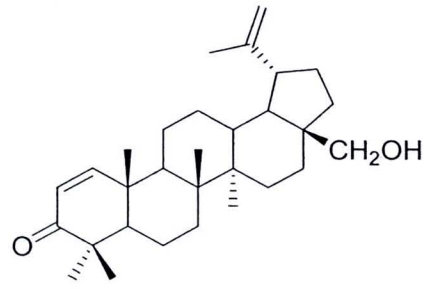
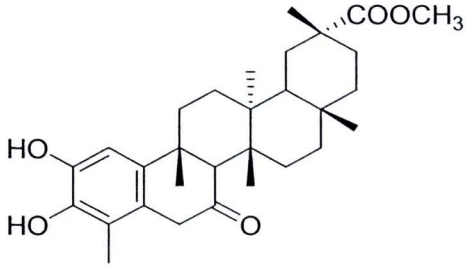


118



120

Four new phenolic triterpenes with a 24-nor-D:A-friedoleane skeleton, isoblepharodol (**121**), 7-oxoblepharodol (**122**), blepharotriol (**123**) and 6-deoxoblepharodol (**124**) were isolated from *M. bleharodes* by Rodríguez *et al.*[39] The cytotoxic activity assay showed that compounds **122-124** were slightly active against HeLa cell.



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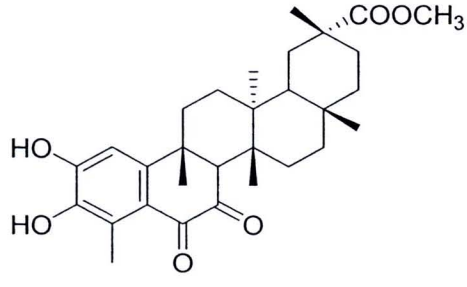

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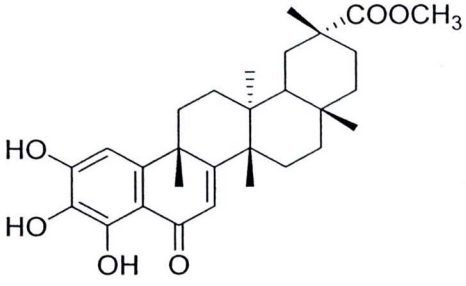

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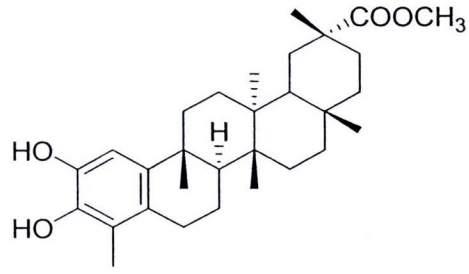
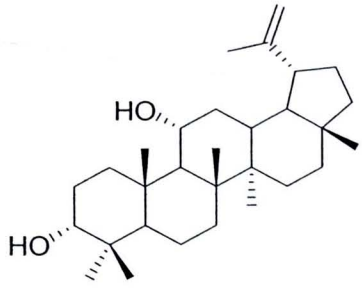


124

In 2006, Reyes *et al.*[40] reported the isolation of three new lupane triterpenes, 3-*epi*nepeticin (**125**), 3-*epi*calenduladiol (**126**) and 11α- hydroxyl glochidone (**127**) from the root bark of *M. cuzcoina* and the leaves of *M. chiapensis*.



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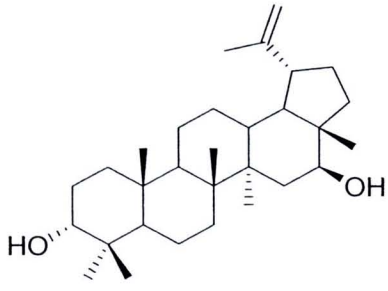

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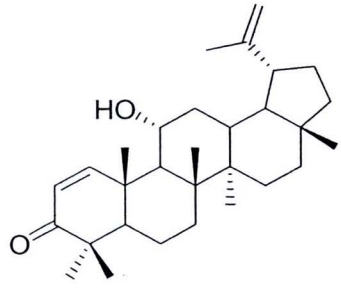
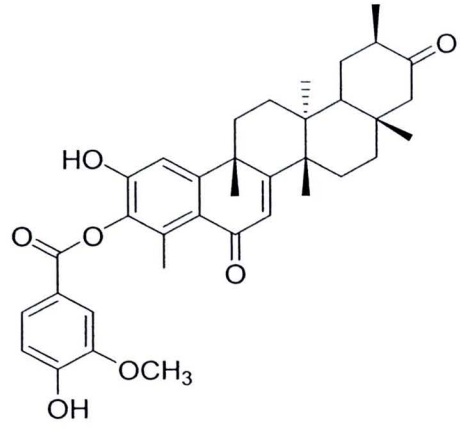


127

In 2007, Gutiérrez *et al.*[41] reported the isolation of milicifolines A (**128**), B (**129**), C (**130**) and D (**131**) from the root bark of *M. ilcifolia*.



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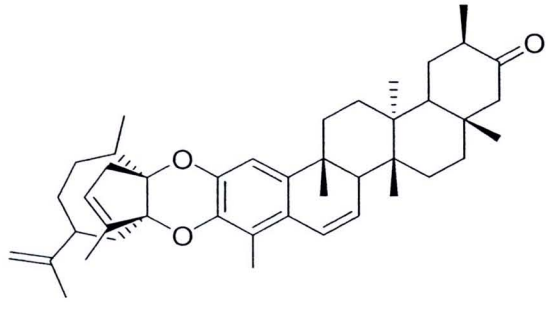

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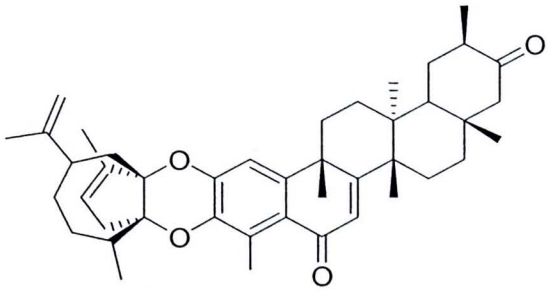

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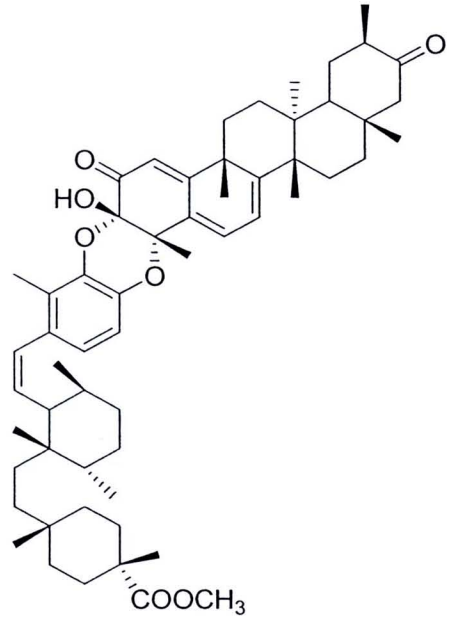
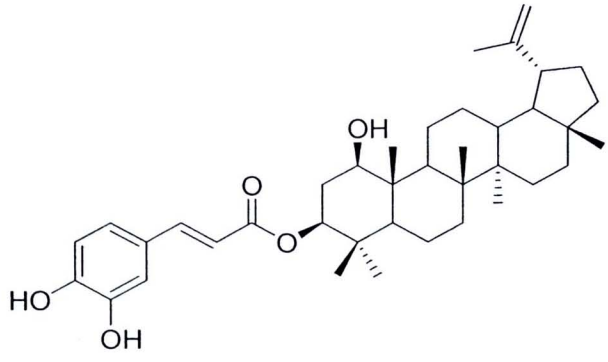


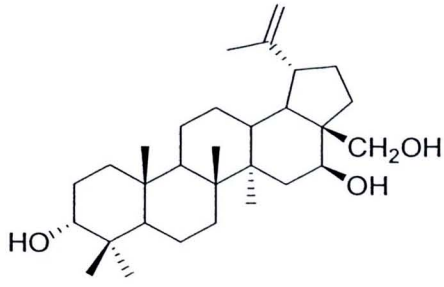
131

In 2008, Delgado-Méndez *et al.*[42] reported the isolation of 1β-hydroxy-3β-caffeatelup-20(29)-ene (**132**) from the roots of *M. apurimacensis*.

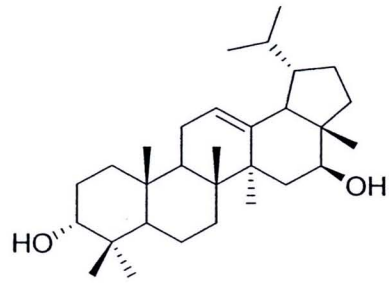


132

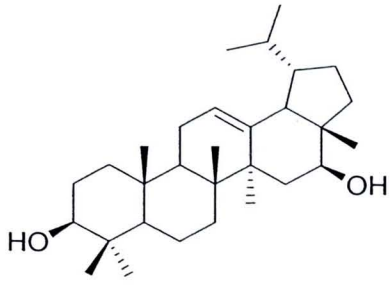
Later in 2009, Vazdekis *et al.*[43] reported the isolation of 3α,16β,28-trihydroxylup-20(29)-ene (**133**), 3α,16β-dihydroxylup-12-ene (**134**), 3β,16β-dihydroxylup-12-ene (**135**), 16β-3,4-secolup-20(29)-en-3-oic acid (**136**) and 3β-peroxy-7β,25-epoxy-D:B-friedoolean-5-ene (**137**) from the ethanol extract of *M. apurimacensis*.



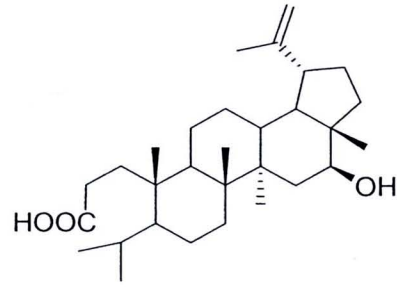
133



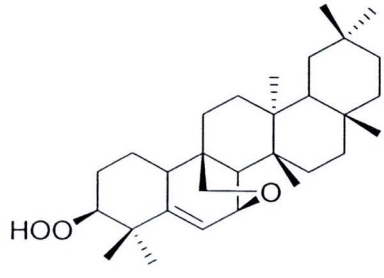
134



135

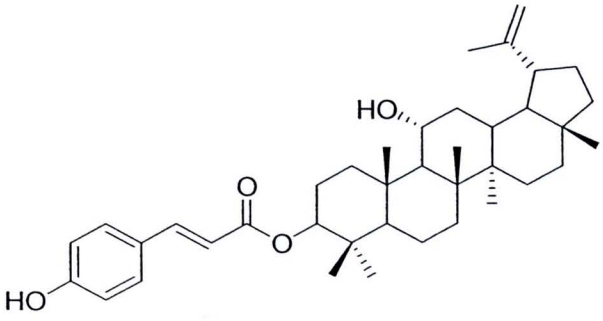


136

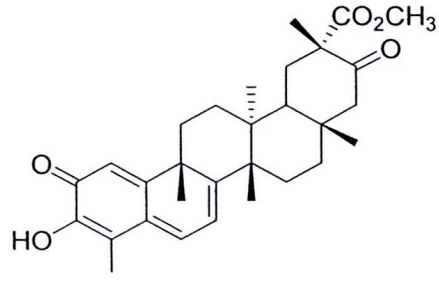


137

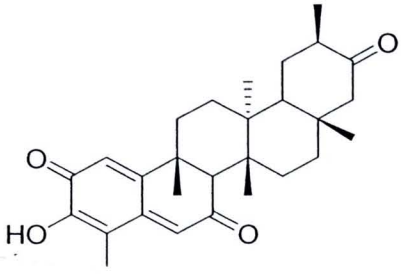
In 2010, Oramas-Royo *et al.*[44] reported the isolation of seven new triterpenes, 3β-(*E*)-*p*-coumaroylnepeticin (**138**), 21-oxopristimerine (**139**), 7-oxo-7,8-dihydroscutione (**140**), 3-methoxy-6-oxotingenol-23-oic acid (**141**), 6,23-dioxo-7,8-dihydropristimerol-23-oic acid (**142**), 23-*nor*-blepharodol (**143**) and retusonine (**144**) from the root bark of *M. retura*. Compound **139** showed cytotoxicity against HL60 and MCF7 with IC_{50} values ranging between 1.4 and 10 μM.



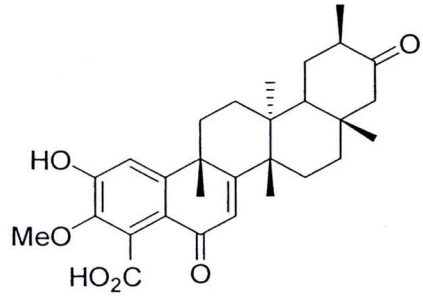
138



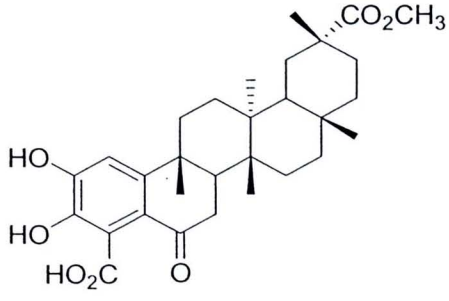
139



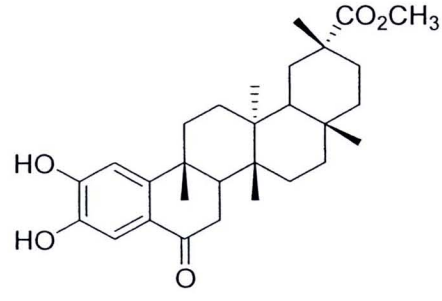
140



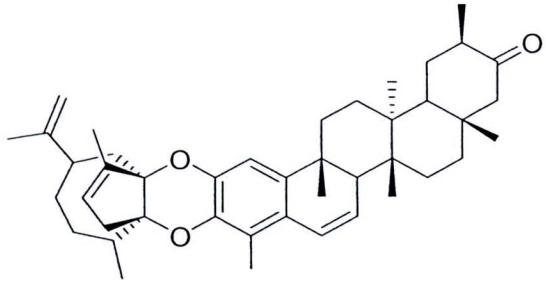
141



142



143



144

In our ongoing search for biologically active compounds from *Maytenus* plants, we studied the chemical constituents of the roots of *M. mekongensis*. Thorough investigation of the CH_2Cl_2 extract led to the isolation of several pure compounds. Details are given in the next section.