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HETEROCYCLIC STILBENE AND BIBENZYL DERIVATIVES IN LIVERWORTS: DISTRIBUTION, STRUCTURES, TOTAL SYNTHESIS AND BIOLOGICAL ACTIVITY

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Dedicated to Professor Dr. Somsak Ruchirawat on the occasion of his 80th birthday

Abstract – Liverworts are a rich source of lipophilic terpenoids, and aromatic compounds especially bibenzyl and bis-bibenzyl derivatives. This review is concerned with the distribution of heterocyclic stilbenes and bibenzyls in liverworts, belonging to the Acrobolbaceae, Aytoniaceae, Frullaniaceae, Lejeuneaceae, Plagiochilaceae, and Radulaceae families. Some *Radula* species elaborate bibenzyl cannabinoids, which possess remarkably similar biological activity to that of the Δ^9-tetrahydrocannabinoids, the psycho- and anti-inflammatory active metabolites found in *Cannabis sativa*.

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1. INTRODUCTION

The 20,000 species of bryophyte are placed taxonomically between algae and pteridophyte. They are divided into three classes, the Bryophyta (mosses, 14,000 species), the Marchantiophyta (liverworts, 6,000), and the Anthocerotophyta (hornworts, 300). Liverworts are further divided into three orders, the Jungermanniales, Metzgeriales and Marchantiales. Among the bryophytes, almost all liverworts contain cellular oil bodies (Figure 1), which contain lipophilic terpenoids and aromatic compounds, many of which show interesting biological and pharmacological activities, such as cytotoxic, antimicrobial, antifungal, antiviral, insecticidal, muscle relaxant, antioxidant, NO production and tubulin polymerization inhibitory activities, etc.[1-11] The morphology of the oil body is one of the significant indicators for the taxonomy of liverworts. In addition to the morphological features of oil bodies, particular attention is paid to the chemical constituents, especially the terpenoids and aromatic compounds, which have been used as markers in chemosystematic studies.[2,3,7]

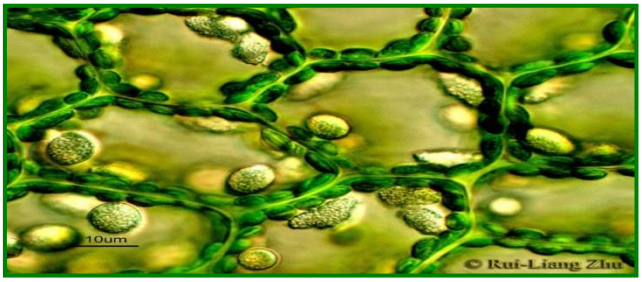


Figure 1. Typical cellular oil bodies of the liverwort, *Solenostoma truncatum*
(Permission obtained from Dr. R.-L. Zhu, Shanghai)

For 50 years this laboratory has continued to study the phytochemicals of liverworts and has isolated several hundred new metabolites and established their structures, including 60 new skeletons.[2,3,7] Among the Jungermanniales, the genus *Radula*, which belongs to the family Radulaceae is very isolated chemically from the other genera of the Jungermanniales. The genus *Radula* produces mainly simple bibenzyls, heterocyclic bibenzyls and bis-bibenzyls, and the presence of terpenoids is very rare although liverworts belonging to the other families elaborate lipophilic terpenoids predominantly.[2,3,7,10] Here the distribution of heterocyclic stilbenes and bibenzyl derivatives in 56 liverworts belonging to the different families, and their structures, total synthesis, and biological activity, and the story of discovery of bibenzyl cannabinoid, perrottetinene (PET) from some *Radula* species, are presented.

2. DISTRIBUTION OF HETEROCYCLIC STILBENE AND BIBENZYL DERIVATIVE IN LIVERWORTS

2-1. Radulaceae

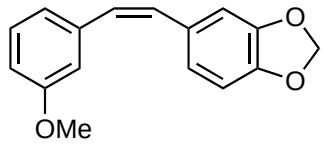
There are 350 species of the genus *Radula* in the world, and in Asia and Japan, 60 and 24 *Radula* species have been recognized, respectively. As seen in the Table 1, each *Radula* species produces several types of

Table 1. Distribution of heterocyclic bibenzyl derivatives among *Radula* species (Radulaceae)

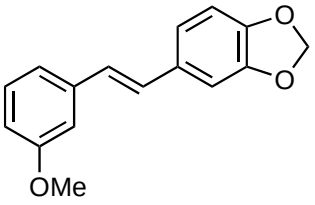
Species names	Compound
Radula amoena	**14***, **18**, **19**, **20**, **21**, **23**, **24**, **25**
R. appressa	**13**, **41**, **44**, **58***, **59**, **67**
R. boryana	**14**, **18**, **58**
R. brunnea	**14**, **18**, **19**, **26**, **58**
R. buccinifera	**13**, **14**, **18**, **34**, **41**, **42**, **43**, **58**, **59**
R. campanigera	**14**, **18**, **19**, **45**, **59**, **75**
R. carringtoni	**34**, **52**
R. chinensis	**14**, **58**, **75**
R. complanata	**14**, **15**, **26**, **27**, **36**, **41**, **42**, **43**, **45**, **46**, **47**, **52**, **53**, **58**
R. constricta	**15**, **26**, **31**, **32**, **38**, **39**, **40**, **41**, **44**, **45**, **47**, **55**, **57**, **59**, **61**, **62**, **63**, **64**
R. frondescens	**14**, **52**, **57**
R. grandis	**19**, **31**, **32**, **41**, **58, 68***
R. holti	**34**, **41**
R. japonica	**45**, **53**
R. javanica	**3**, **14**, **18**, **19**, **26**, **31**, **32**, **33**, **41**, **43**, **45**, **49**, **51**, **52**, **58**, **59**
R. kojana	**13**, **14**, **15**, **18**, **19**, **20**, **21**, **26**, **34**, **36**, **37**, **41**, **42**, **45**, **47**, **58**, **59**, **60**
R. laxiramea	**13**, **14**, **26**, **27**, **41**, **45**, **58**, **69**, **75**
R. lindenbergiana	**13**, **14**, **15**, **59**
R. marginata	**14**, **22**, **58**, **75**, **76**
R. obtusiloba	**14**, **18**, **31**, **32**, **34**, **35**, **58**, **59**
R. okamurana	**14**, **18**, **19**, **31**, **32**, **58**, **59**
R. oyamensis	**13**, **14**, **42**
R. perrottetii	**13**, **14**, **16**, **17**, **18**, **19**, **20**, **21**, **75**
R. sainsburiana	**14**
R. sumatrana	**31**, **32**, **33**, **41**, **48**, **49**, **54**, **62**, **63**, **64**, **65**, **66**
R. tokiensis	**14**, **26**, **31**, **32**, **33**, **34**, **41**, **43**, **44**, **50**, **51**, **58**, **59**
R. uvifera	**14**
R. voluta	**14**
R. wichurae	**45**
Radula sp. (unidentified)	**14**, **58**, **59**
Radula spp. (6 unidentified)	**58**
Radula spp. (9 unidentified)	**13**, **14**, **34**, **58**, **59**, **75**
Radula spp. (20 unidentified)	**13**, **14**, **34**

*Compounds **14**, **26, 58** and **68** are 3,5-dihydroxy-2-(3-methyl-2-butenyl)bibenzyl, 3,5-dihydroxy-4-(3-methyl-2-butenyl)bibenzyl, 3,5-dihydroxy-2-geranylbibenzyl and 3,5-dihydroxy-4-geranylbibenzyl, respectively.

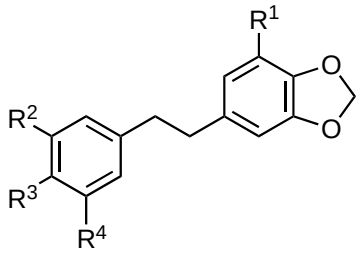
heterocyclic bibenzyl derivatives, having 3-, 5-, 6- and 7-membered rings as shown in Charts 1-6. 3,5-Dihydroxy-2-(3-methyl-2-butenyl)bibenzyl (**14**) and 3,5-dihydroxy-2-geranylbibenzyl (**58**), which were found in 23 and 15 *Radula* species, respectively, are the significant precursors of the heterocyclic bibenzyls



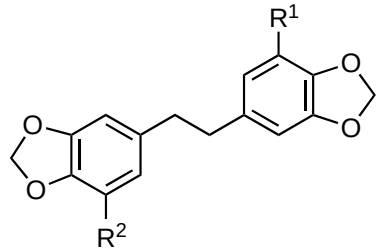
3-methoxy-3',4'-methylenedioxy-(*Z*)-stilbene (**1**)



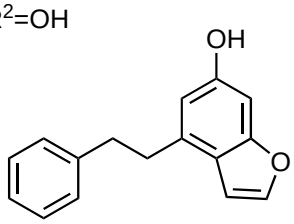
3-methoxy-3',4'-methylenedioxy-(*E*)-stilbene (**2**)



3-hydroxy-4,5-methylenedioxybibenzyl (**3**)
R^1=OH, R^2=R^3=R^4=H
3-methoxy-3',4'-methylenedioxybibenzyl (**4**)
R^1=R^3=R^4=H, R^2=OMe
3,3'-dihydroxy-4,5-methylenedioxybibenzyl (**5**)
R^1= R^2=OH, R^3=R^4=H,
3-hydroxy-4,5-methylenedioxy-3'-methoxybibenzyl (**6**)
R^1=OH, R^2=OMe, R^3=R^4=H,
3,3'-dimethoxy-4,5-methylenedioxybibenzyl (**7**)
R^1=R^2=OMe, R^3=R^4=H
3-methoxy-4,5-methylenedioxy-4'-hydroxybibenzyl (**8**)
R^1=OMe, R^2=R^4=H, , R^3=OH
3,3'-dimethoxy-4,5-dimethylenedioxy-4'-hydroxybibenzyl (**9**)
R^1=R^2=OMe, R^3=OH, R^4=H
3,3',4',5'-tetramethoxy-4,5-methylenedioxybibenzyl (=brittonin B) (**10**)
R^1=R^2=R^3=R^4=OMe



3,4;3',4'-dimethylenedioxybibenzyl (**11**)
R^1= R^2=H
3,3'-dihydroxy-4,5;4',5'-dimethylenedioxybibenzyl (**12**)
R^1= R^2=OH

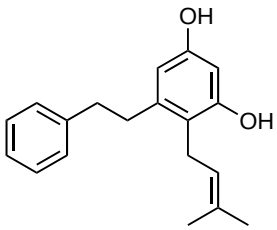


6-hydroxy-4-(2-phenylethyl)-benzofuran (**13**)

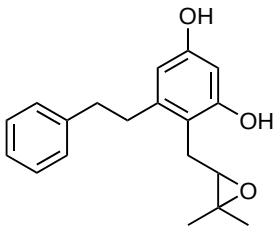
Chart 1. Heterocyclic stilbenes (**1** and **2**) and bibenzyls (**3-13**) found in several liverworts

(**15-25**) and **59-61**, respectively. 3,5-Dihydroxy-4-(3-methyl-2-butenyl)bibenzyl (**26**) and 3,5-dihydroxy-4-geranylbibenzyl (**68**) which were identified in five and one *Radula* species, respectively, are the precursors of heterocyclic bibenzyls (**41-57**) having a dihydro-oxepin structure and the chromene (**69)**. The two chromenes (**18** and **19**), radulanin A (**41**) and the chromene (**59**) are the most frequently found metabolites in *Radula* species, followed by the simple bibenzyl (**13**) with a dihydrobenzofuran unit, radulanin I (**31**), and J (**32**) having a cyclopropane ring, and radulanin H **(45**) with a dihydro-oxepin moiety were obtained as the second most prevalent heterocyclic bibenzyls.

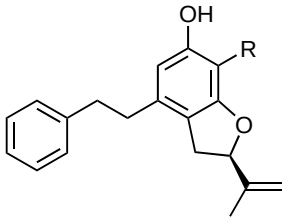
It is noteworthy that bibenzyls possessing a dihydro-oxepin structure might be biosynthesized from 3,5-dihydroxy-4-(3-methyl-2-butenyl)bibenzyl (**26**), however, possible derivatives from its isomer, 3,5-dihydroxy-2-(3-methyl-2-butenyl)bibenzyl (**14**) were not identified in any *Radula* species. This is one of the significant chemical features of the Radulaceae. *R. amoena*, *R. buccinifera*, *R. complanata*, *R. constricta*, *R. javanica*, *R. kojana*, *R. laxiramea*, *R. obtusiloba*, *R. perrottetii*, *R. sumatrana* and *R. tokiensis* are rich sources of heterocyclic bibenzyl derivatives. Among them, three Chinese *Radula* species, *R. amoena*,[12] *R.*



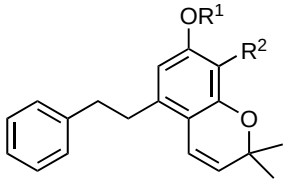
3,5-hydroxy-2-(3-methyl-2-butenyl)-bibenzyl (**14**)



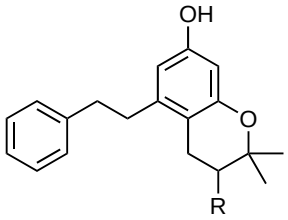
3,5-dihydroxy-2-(2,3-epoxy-3-methylbutyl)-bibenzyl (**15**)



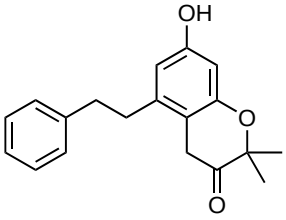
(2*R*)-isopropenyl-6-hydroxy-4-(2-phenylethyl)dihydrobenzofuran (**16**) R=H
perrottetin D (**17**) R=OH



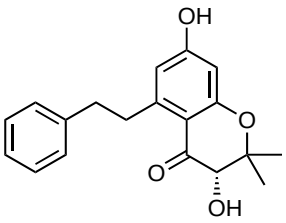
2,2-dimethyl-7-hydroxy-5-(2-phenyethyl)chromene (**18**) R^1=R^2=H
2,2-dimethyl-7-methoxy-5(2-phenylethyl)chromene(**19**) R^1=Me, R^2=H
2,2-dimethyl-7,8-dihydroxy-5-(2-phenylethyl)chromene (**20**) R^1=H, R^2=OH



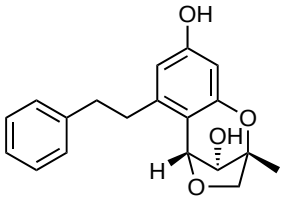
2,2-dimethyl-7-hydroxy-5-(2-phenylethyl)chromane (**21**) R=H
2,2-dimethyl-3,7-dihydroxy-5-(2-phenylethyl)chromane (**22**) R=OH



radulamoenin B (**23**)



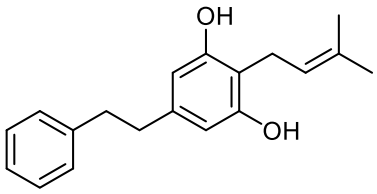
radulamoenin C (**24**)



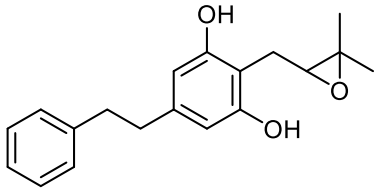
radulamoenin A (**25**)

Chart 2. Heterocyclic bibenzyls (**15-25**) found in *Radula* species

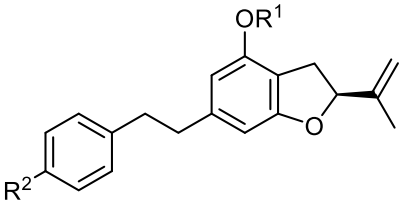
constricta,[13] and *R. sumatrana*[14] elaborate distinct types of heterocyclic bibenzyls from those found in other *Radula* species: three chromanes (**23-25**) from *R. amoena*, dihydrooxepin (**55**) and four prenyl chromanes (**61-64**) from *R. constricta*, and two dihydro-oxepins (**48**, **54**) and prenyl chromanes (**62-66**) from *R. sumatrana*, respectively. The Malagasy-originating *R. appressa* biosynthesizes the common dihydro-oxepins (**41** and **44**) and a cyclic chromane derivative, *o*-cannabicyclol (**67**),[15] which is the diastereomer of **62** found in *R. constricta* and *R. sumatrana*.



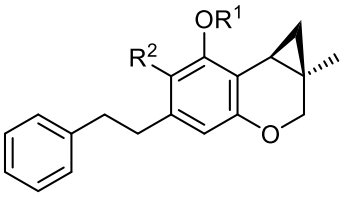
3,5-dihydroxy-4-(3-methyl-2-butenyl)bibenzyl (**26**)



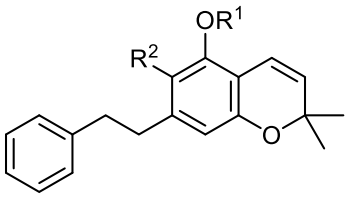
3,5-dihydroxy-4-(2,3-epoxy-3-methylbutyl)bibenzyl (**27**)



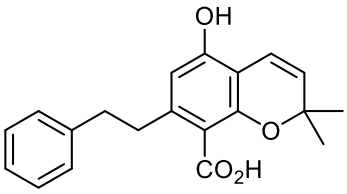
tylimanthin A (**28**)
R^1=Me, R^2=H
tylimanthin B (**29**)
R^1=R^2=H
tylimanthin C (**30**)
R^1=H, R^2=OMe



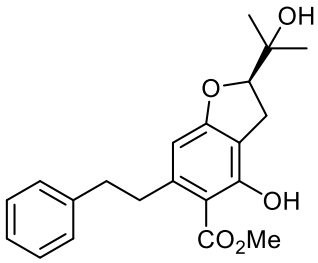
radulanin I (**31**) R^1=R^2=H
radulanin J (**32**) R^1=Me, R^2=H
radulanin K (**33**) R^1=H, R^2=CO_2H



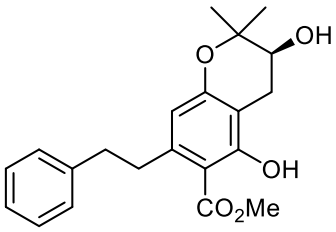
2,2-dimethyl-5-hydroxy-7-(2-phenylethyl)chromene (**34**)
R^1=R^2=H
2,2-dimethyl-5-methoxy-7-(2-phenylethyl)chromene (**35**)
R^1=Me, R^2=H
2,2-dimethyl-5-hydroxy-6-carboxy-7-(2-phenylethyl)chromene (**36**)
R^1=H, R^2=CO_2H



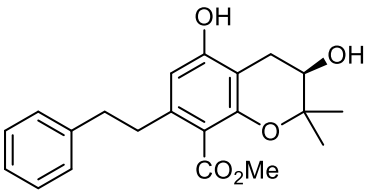
saccatene C (**37**)



radstrictin B (**38**)



radstrictin C (**39**)

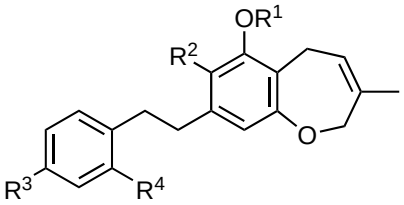


radstrictin D (**40**)

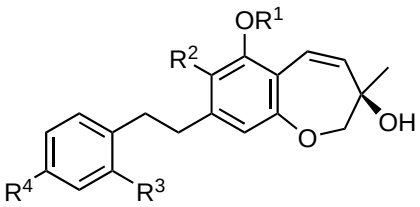
Chart 3. Heterocyclic bibenzyls (**26-40**) found in *Radula* species

R. sainsburiana, *R. uvifera*, and *R. voluta* were chemically simple, since they did not contain any heterocyclic bibenzyls, only 3,5-dihydroxy-2-(3-methyl-2-butenyl)bibenzyl (**14**).[10] The chemosystematics of the *Radula* genus was reported by our group using the similarities and differences of the chemical constituents among *Radula* species.[3,7,10] The Japanese *R. perrottetii* (Figure 2),[16] the New Zealand *R. marginata*,[17] and the Costa Rican *R. laxilamea*[18] biosynthesize bibenzyl cannabinoid, named (-)-*cis*-perrottetinene (PET) (**75**).

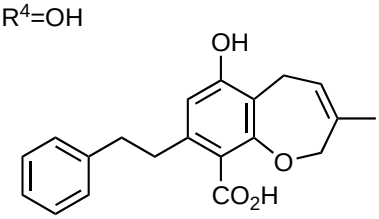
R. marginata also produces the precursor of PET, perrottetinenic acid (**76**),[17] which is the most characteristic of the metabolites of the Radulaceae. Further GC/MS analysis indicated that perrottetinene



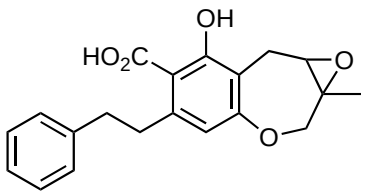
radulanin A (**41**) R^1=R^2=R^3=R^4=H
radulanin B (**42**) R^1=Me, R^2=R^3=R^4=H
radulanin C (**43**) R^1=R^2=R^4=H, R^3=OH
radulanin L (**44**) R^1=R^2=R^3=H, R^4=OH
radulanin H (**45**) R^1=R^3=R^4=H, R^2=CO_2H
4-hydroxyradulanin H (**46**) R^1=R^4=H, R^2=CO_2H, R^3=OH
2'-hydroxyradulanin H (**47**) R^1=R^3=H, R^2=CO_2H, R^4=OH



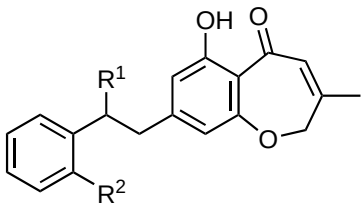
radulanin M (**48**) R^1= R^2=R^3=R^4=H
radulanin N (**49**) R^1= R^2=R^4=H, R^3=OH
radulanin D (**50**) R^1= R^2=R^3=H, R^4=OH
radulanin G (**51**) R^1= R^3=R^4=H, R^2=CO_2H



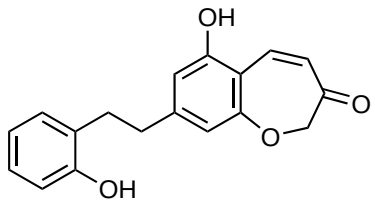
radulanin E (**52**)



radulanin F (**53**)



radulanin A-5-one (**54**) R^1=R^2=H
radstrictin H (**55**) R^1=H, R^2=OH
β-hydroxyradulanin A-5-one (**56**) R^1=OH, R^2=H



radstrictin I (**57**)

Chart 4. Heterocyclic bibenzyls (**41**-**57**) from several liverworts

(**75**) was present in *R. chinensis and R. campanigera,* and two unidentified Peruvian *Radula* specie.[10] The Japanese liverwort *Plagiochila ovalifolia* belonging to the Plagiochilaceae also contains perrottetinenic acid (**76**).[19]

The discovery of *cis*-perrottetinene (PET) (**75**) attracted organic chemists and pharmacologists because the structure of PET structurally resembles that of (-)-*trans*-Δ^9-tetrahydrocannabinoid (THC) (**78**) and its derivatives which are well-known psychoactive and anti-inflammatory drugs from the vascular plant, *Cannabis sativa*. In fact, Chicca and colleagues[20] reported that *cis*-PET and its diastereomeric, *trans*-isomer (**77**), which were synthesized through a stereodivergent catalytic reaction (see later), readily penetrated the brain and induced catalepsy, hypolocomotion, hypothermia, and analgesia in a CB1 receptor-dependent manner in mice. *cis*-PET (**75**) was profiled to act on major brain receptors, indicating a selective

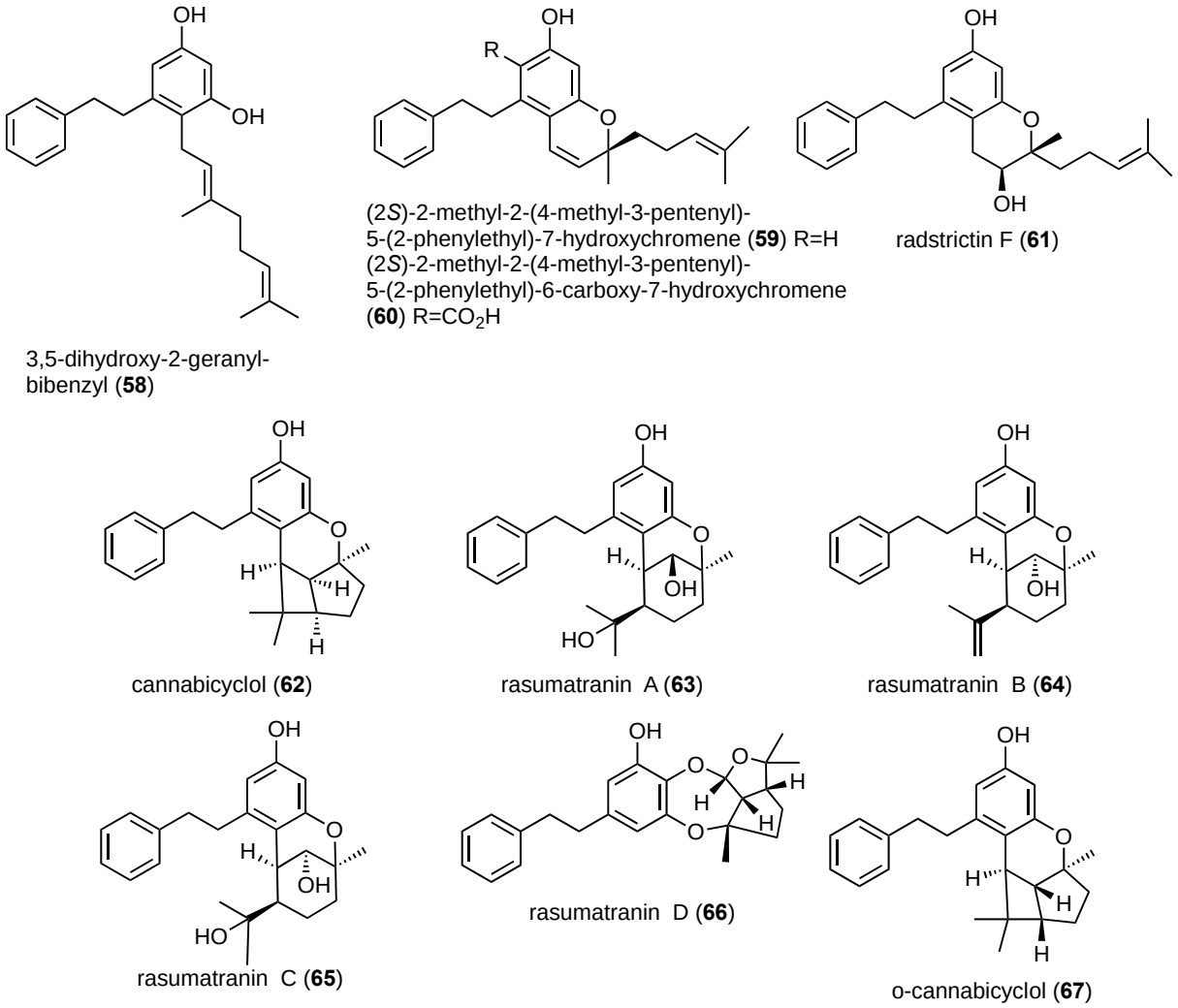
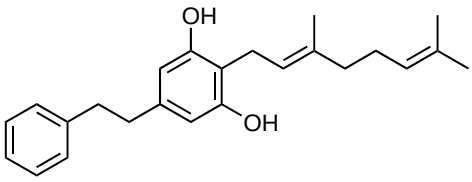
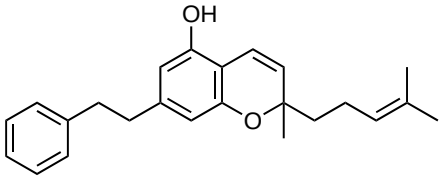


Chart 5. Heterocyclic bibenzyls (**58-67**) found in several liverworts

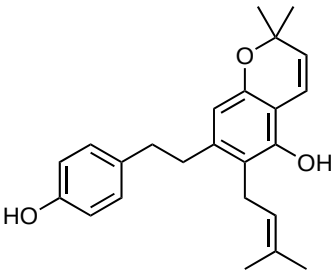
cannabinoid-like pharmacology. PET-containing *R. marginata* has been used by the Maori people in New Zealand for a long time as a medicinal plant.[10,20] However, there are no data that people used other *Radula* species as foods, food additives, beverages, or as medicinal agents. For 1,000 years, people believed that only *C. sativa* produced psychoactive compounds, such as THC and its derivatives, and are used as drugs. When in this laboratory PET was found in two *Radula* species, the Japanese *R. perrottetii*[16] in 1994 and in the New Zealand *R. marginata*[17] in 2004, biological and pharmacological tests were not conducted because the stereochemistry and the side chain between PET and THC are different Therefore, it was considered that PET might be inactive against mammals. The discovery of PET in *Radula* species, which are totally different taxonomically from *C. sativa*, will generate attention towards a potential drug candidate because the abuse potential of *cis*-PET (**75**) is probably low, and PET indicates lower psycho activity and stronger anti-inflammatory activity than THC in mammals and the side effects might be lower than *C. sativa*.[20] The distribution of *Radula* species is not limited to Asia. South America, especially Brazil, is a rich area of



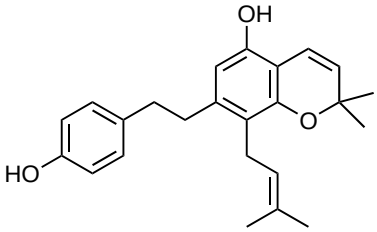
3,5-dihydroxy-4-geranylbibenzyl (**68**)



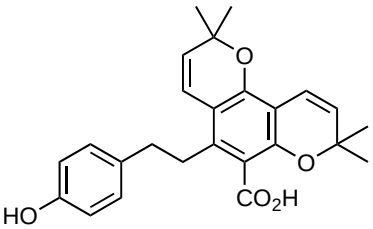
2-methyl-2-(4-methyl-3-pentenyl)-5-hydroxy-7-(2-phenylethyl)chromene (**69**)



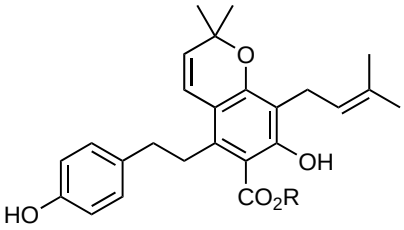
2,2-dimethyl-5-hydroxy-6-(3-methyl-2-butenyl-7-[2-(4'-hydroxyphenyl-ethyl)]chromene (**70**)



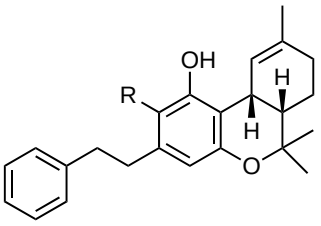
2,2-dimethyl-5-hydroxy-7-[2-(4'-hydroxyphenylethyl)]-8-(3-methyl-2-butenyl)chromene (**71**)



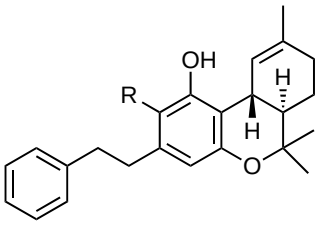
saccatene A (**72**)



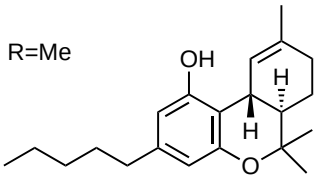
saccatene B (**73**) R=H
saccatene B methyl ester
2,2-dimethyl-5[2-(4'-phenylethyl)-6-carbomethoxy-7-hydroxy-8(3-methyl-2-butenyl)chromene (**74**) R=Me



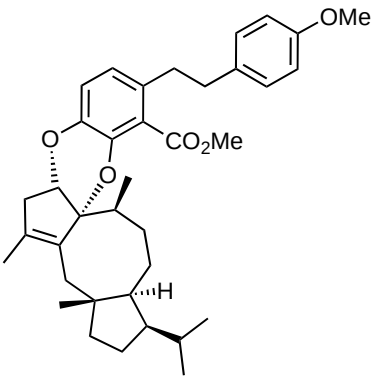
(-)-*cis*-perrottetinene (**75**) R=H
perrottetinenic acid (**76**) R=CO_2H



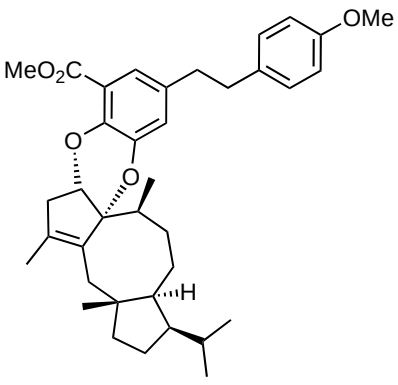
(-)-*trans*-perrottetinene (**77**)



(-)-*trans*-Δ^9-tetrahydrocannabinoide (**78**)



spinuloplagin A (**79**)



spinuloplagin B (**80**)

Chart 6. Heterocyclic bibenzyls (**70**-**76**, **79**, and **80**) found in several liverworts

Radula species. In the past 50 years, this laboratory has studied the chemistry of more than 1,000 species of bryophytes, however, PET was found only in Radulaceae species. Further investigation of other *Radula*

species, the *Radula* taxa containing PET could be found. Although *Radula* are morphologically small, a large amount of *R. perrottetii* is easily obtained through cultivation under LED light irradiation.[21]

Figure 2. *Radula perrottetii* (Radulaceae, Jungermanniales, Marchantiophyta)

2-2. Acrobolbaceae

The genera *Acrobolbus, Lethocolea, Marsupidium* and *Tylimanthus* belong to the Acrobolbaceae (Jungermanniales). As summarized in Table 2, each species in these families produces a different profile of heterocyclic bibenzyl derivatives. The East Malaysian *Acrobolbus saccatu*s produces two chromenes, **36**, and **37** named saccatene C, together with radulanin A-5-one (**54**), and the dichromene named saccatene A (**72**) and monochromene with a prenyl group named saccatene B (**73**).[22] *A. saccatus* is closely related chemically to *Marsupidium epiphyllum* (see later) since they produce the same bibenzyl (**54**) and the same bibenzyls as found in the latter species.[22,23] The Ecuadorian *Lethocolea grossophylla* elaborates two diprenylbibenzyls, 2-carboxy-3-methoxy-4,6-di-(3-methyl-2-butenyl)-5,4'-dihydroxybibenzyl, and 2,4-di-(3-methyl-2-butenyl)-3,5,4'-trihydroxybibenzyl and two new chromene derivatives, **70** and **71**, derived from the latter bibenzyl, and a novel bis-prenylated bis-bibenzyl named glossophyllin.[24] The New Zealand *M. epiphyllum* elaborates the seven membered bibenzyls, radulanin A-5-one (**54**) and β-hydroxyradulanin A-5-one (**56**) and saccatene B methyl ester (**74**).[23] *Marsupidium perpusillum* contains 3-methoxy-4'-hydroxybibenzyl and its dehydro derivative, 3-methoxy-4'-hydroxystilbene, and the 3-hydroxy- and 3-methoxybibenzyls.[25] However, the seven-membered bibenzyls, found in *M. epiphyllum* were not isolated from nor detected in *M. perpusillum*, and the former four bibenzyls and a stilbene derivative were not isolated from *M. epiphyllum*. The Chilean *Tylimanthus urvilleanus* produced three heterocyclic bibenzyls, tylimanthins A-C (**28**-**30**),[26] however, the New Zealand species *T. saccatus*, did not contain such bibenzyls, instead, two chromenes, 2,2-dimethyl-7-hydroxy-5-(2-phenylethyl)chromene (**18**) and its methyl ether (**19**) were isolated.[25]

2-3. Aytoniaceae

The Aytoniaceae family includes the genera, *Asterella*, *Cryptomitrium*, *Mannia*, *Plagiochasma,* and *Reboulia*. Except for the genus, *Cryptomitrium* the liverworts in these species, produce various bis-bibenzyl derivatives as the major components. Only *Reboulia hemisphaerica* elaborates 3-methoxy-3',4'-methylenedioxybibenzyl (**4**).[27]

Table 2. Distribution of heterocyclic stilbene and bibenzyl derivatives among liverworts

Family and species names	Compound
Acrobolbaceae	
Acrobolbus saccatus	**36**, **37**, **54**, **72**, **73**
Lethocolea grossophylla	**70**, **71**
Marsupidium epiphyllum	**54**, **56**, **74**
Tylimanthus saccatus	**18**, **19**
T. urvilleanus	**28**, **29**, **30**
Aytoniaceae	
Reboulia hemisphaerica	**4**
Frullaniaceae	
Frullania amplicrania	**3**, **4**
F. anomala	**1**, **2**, **4**
F. bonincola	**4**, **9**
F. brittoniae subsp. *truncatifolia*	**10**
F. ericoides	**4**, **7**
F. falciloba	**4**
F. incumbens	**4**
F. parvistipula	**4**, **7**, **11**, **12**
F. pycnantha	**4**, **10**
F. scandens	**4**, **6**
F. serrata	**7, 10**
F. spinifera	**4**, **11**
Lejeuneaceae	
Trocholejeunea sandvicensis	**4**
Plagiochilaceae	
Plagiochila chacabucensis	**11**, **12**
P. ovalifolia	**76**
P. permista var. *integerrima*	**18**
P. spinulosa	**79**, **80**

2-4. Frullaniaceae

Generally, members of the genus *Frullania* are very small epiphytic liverworts comprising about 300 species. Some species, such as *F. asagrayana*, *F. dilatata*, *F. nisquallensis* and *F. tamarisci* subsp. *tamarisci* cause strong allergenic contact dermatitis, which is due to some eudesmane and/or eremophilane sesquiterpenes possessing an α-methylene-γ-butyrolactone ring system.[3] *Frullania* species are classified into several chemotypes, the sesquiterpene lactones and bibenzyl (Type I), sesquiterpene lactones (Type

II), bibenzyls (Type III), monoterpenoids (Type IV), diterpenoids (Type V), pacifigorgianes (VI), and cyclocolorenone (VII).[3,7] In Types I and III, the most common bibenzyl is 3-methoxy-3',4'-methylenedioxybibenzyl (**4**) as seen in Table 2. Among these liverworts, the stilbene derivatives (**1** and **2**), which are exceedingly rare in liverworts, were found only in *Frullania anomala*. Compounds **1** and **2** may be the precursors of **4**, since this liverwort produces both the stilbenes (**1** and **2**) and the bibenzyl derivative (**4**).[28] *F. parvistipula* is chemically similar to the liverwort *Plagiochila chacabucensis* belonging to the Plagiochilaceae since both species produce the same dimethylenedioxybibenzyls (**11** and, **12**) although the species are morphologically quite different.[26,29] It is noteworthy that all of the *Frullania* species examined chemically do not produce the bibenzyls found in *Radula* species, and the latter species do not elaborate the bibenzyls found in the *Frullania* species, except for the presence of **4** in *R. javanica*.[10]

2-5. Lejeuneaceae

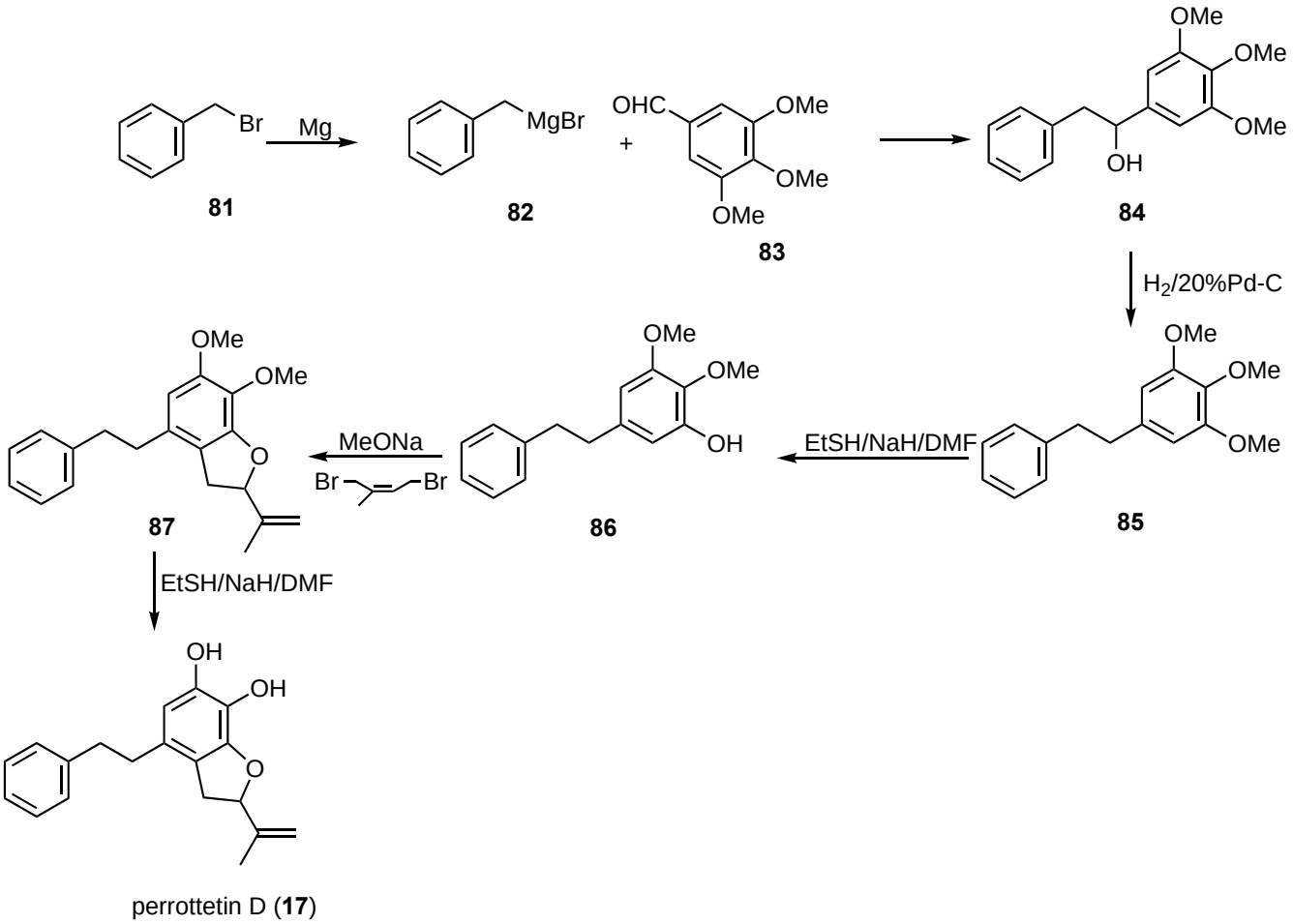
There are more than 1500 species belonging to the Lejeuneaceae and generally their morphologies are very small. Chemosystematic studies of several species were conducted in this laboratory and their metabolites profiles were extraordinarily complex.[3,7] Thus far only 3-methoxy-3',4'-methylenedioxybibenzyl (**4**) was identified in *Trocholejeunea sandvicensis*.[30] However, the presence of **4** in this species is doubtful, since *Frullania* species which contain **4** often intermingle in *T. sandvicensis*.

2-6. Plagiochilaceae

There are more than 1,600 *Plagiochila* species known which are chemically very complex, and are divided into at least 12 chemotypes.[3,7] The most common skeleton is the 2,3-secoaromadendrane sesquiterpene-having very hot taste (Type I), followed by the 2,3-secoaromadendrane-diterpenoids, (Type II), 2,3-secoaromadendrane-gymnomytranes (type III), diterpene-bis-bibenzyl (Type IV), diterpenoids (Type V), bibenzyls (Type VI), bibenzyl-diterpenoids (Type VII), and bibenzyl-bis-bibenzyls (Type VIII). The pungent *P. ovalifolia* (Type II) elaborates perrottetinenic acid (**76**) and the bis-bibenzyl, plagiochin D.[19] *P. chacabucensis* (Type VI) produces the bibenzyls (**11** and **12**).[26] The Scottish *Plagiochila spinulosa* (Type VII) is chemically different from other *Plagiochila* species since it produces the unusual spinuloplagins A (**79**) and B (**80**) which combine bibenzyl and fusicoccane diterpene scaffolds. Each moiety, the fusicoccane diterpene, anadensin and 1-carbomethoxy-2,3-dihydroxy-4'-methoxybibenzyl was isolated from the same species.[31] *P. permista* var. *integerrima* (Type VIII) produces (**18**) and four bis-bibenzyls, isoplagiochin A, isoplagiochins E and F, and 12-chloroisoplagiochin D.[32]

3. TOTAL SYNTHESIS OF HETEROCYCLIC BIBENZYL DERIVATIVES

2,2-Dimethyl-7-methoxy-5-(2-phenylethyl)chromene (**19**), 2,2-dimethyl-5-methoxy-7-(2-phenylethyl)chromene (**35**), and 7-methoxy-2-methyl-2-(4-methyl-3-pentyl)-5-(2-phenylethyl)chromene,

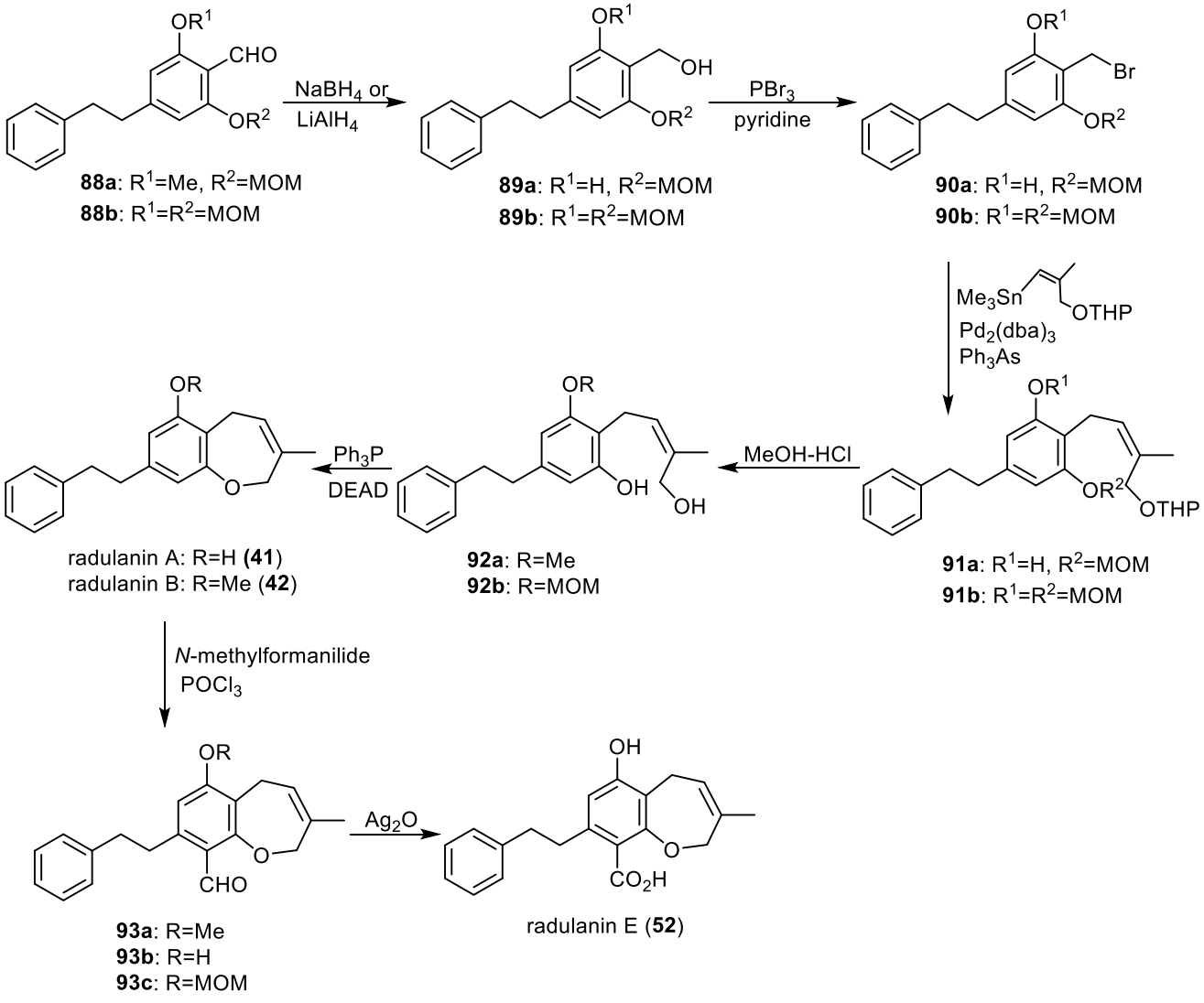


Scheme 1. Total synthesis of perrottetin D **(17)** (Asakawa, 1990)

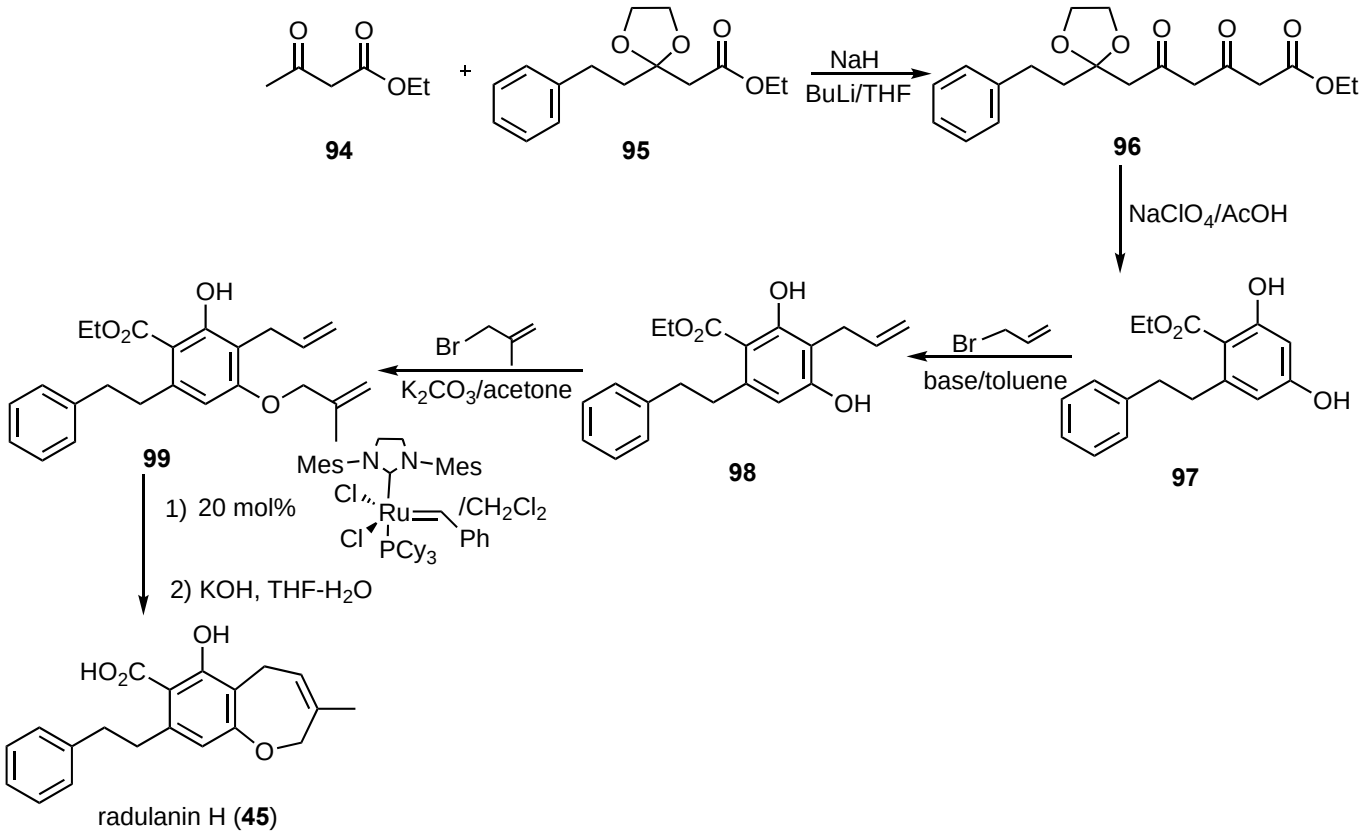
which is the methyl ether of the natural product (**59**) were synthesized from 3,5-dihydroxy-2-(3-methyl-2-butenyl)bibenzyl (**14**) and 3,5-dihydroxy-2-geranylbibenzyl (**58**),[33] the major prenylated bibenzyls in *Radula* species (Table 1). Perrottetin D (**17**) was synthesized through a Grignard reaction, followed by a Wittig reaction, hydrogenation, selective demethylation and coupling with 1,4-dibromo-2-methyl-2-butene,[34] as shown in Scheme 1.

Yamaguchi et al.[35] reported the total synthesis of radulanin A (**41**) and B (**42**) using the di-MOM-protected ether (**88b**) of 3,5-dihydroxy-4-formylbibenzyl by the five steps. Radulanin E (**52**) was also synthesized from **93b** which was obtained by Vilsmeier formylation of the 6-MOMoxy derivative (**93c**) of radulanin A (**41**), followed by oxidation to afford radulanin E (**52**) as indicated in Scheme 2. The total syntheses of radulanin H (**45**) and radulanin E (**52**) were accomplished by Yoshida et al. (2009)[36] as shown in Schemes

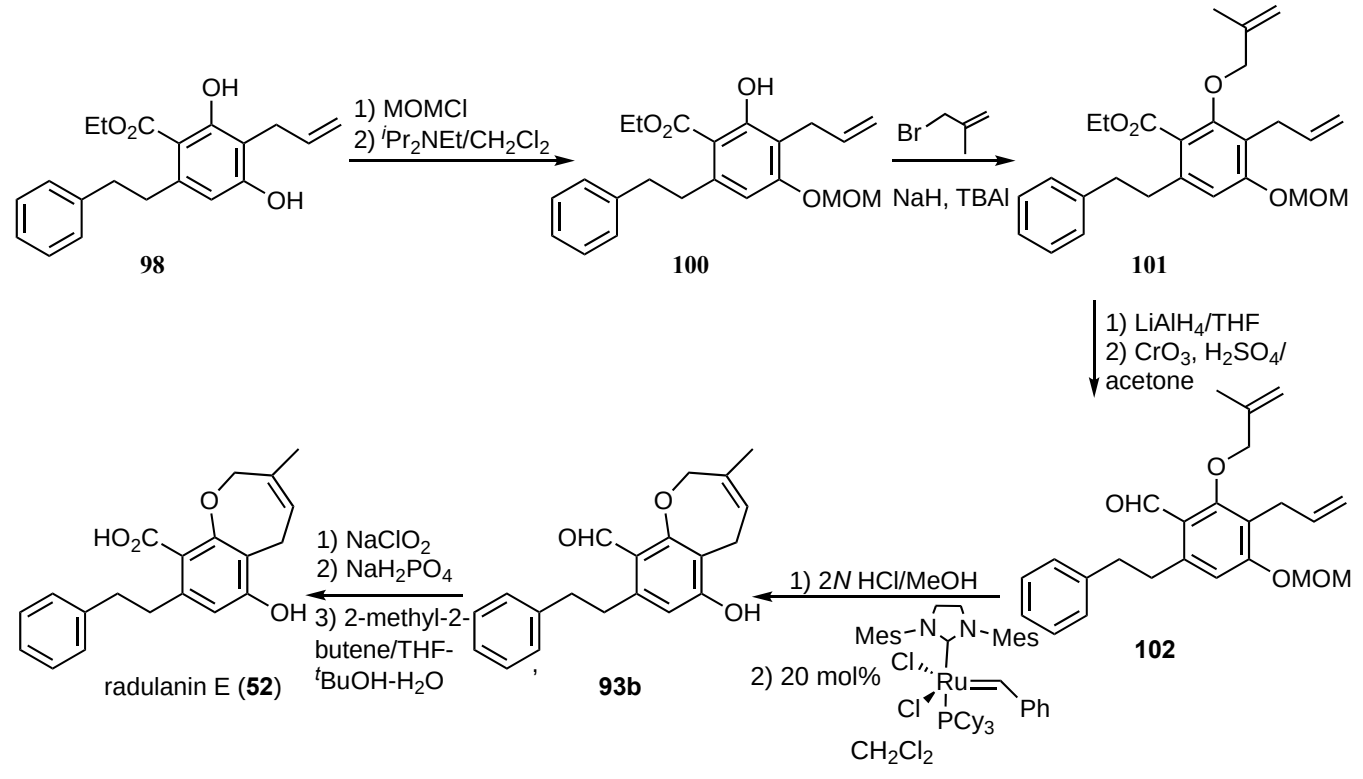
3 and 4. The key intermediate for the synthesis of **45** is 3,5-dihydroxy-4-(2-propenyl)-6-carboethoxybibenzyl (**98**), which was treated with β-methylallyl bromide, followed by ring closing metathesis using Grubbs catalyst, and hydrolysis of the ethyl ester to afford radulanin H (**45**). The key intermediate for the total synthesis of **52** is the propenyl ether (**101**) which was obtained from **100**. Reduction of **101**, followed by oxidation gave (**102**), MOM group of which was deprotected, then ring closure metathesis using Grubbs catalyst, followed by oxidation to furnish radulanin E (**52**). Radulanin A



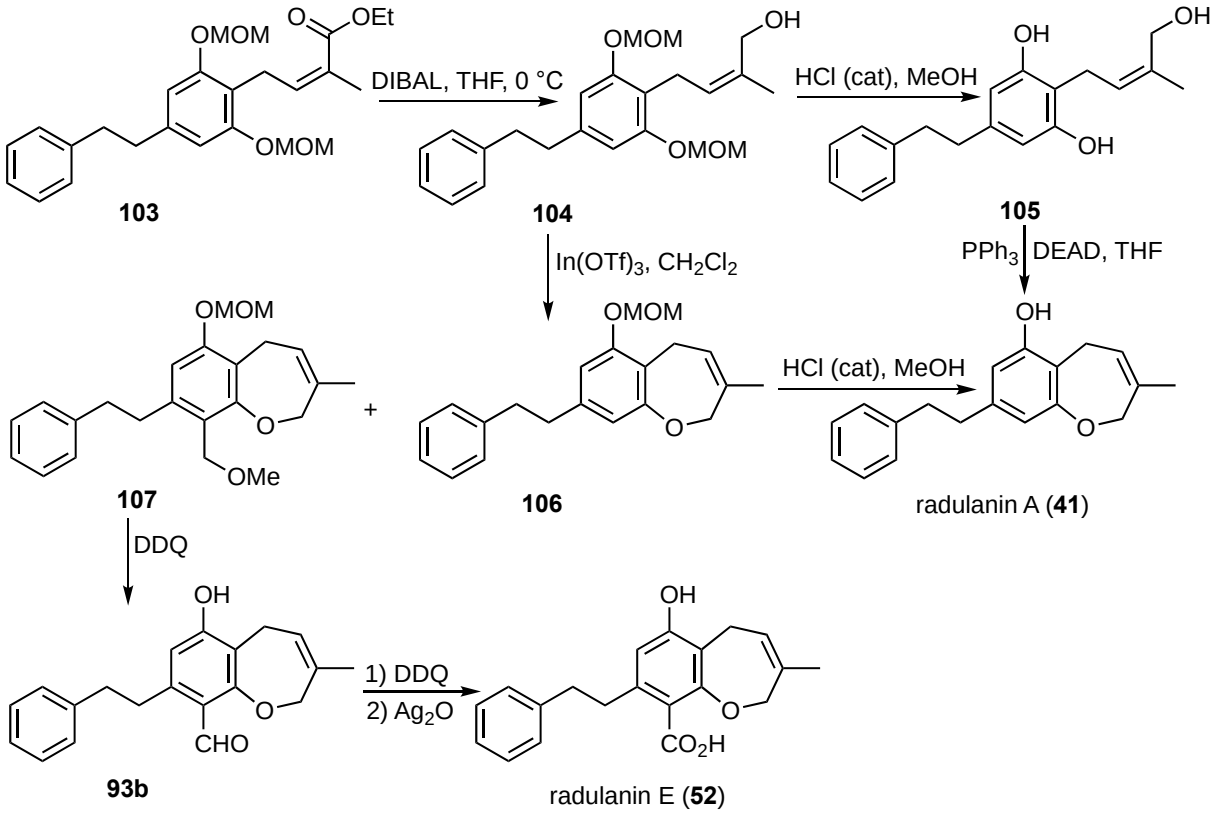
Scheme 2. Total synthesis of radulanins A (**41**), B (**42**), and E (**52**) (Yamaguchi et al. 2005)



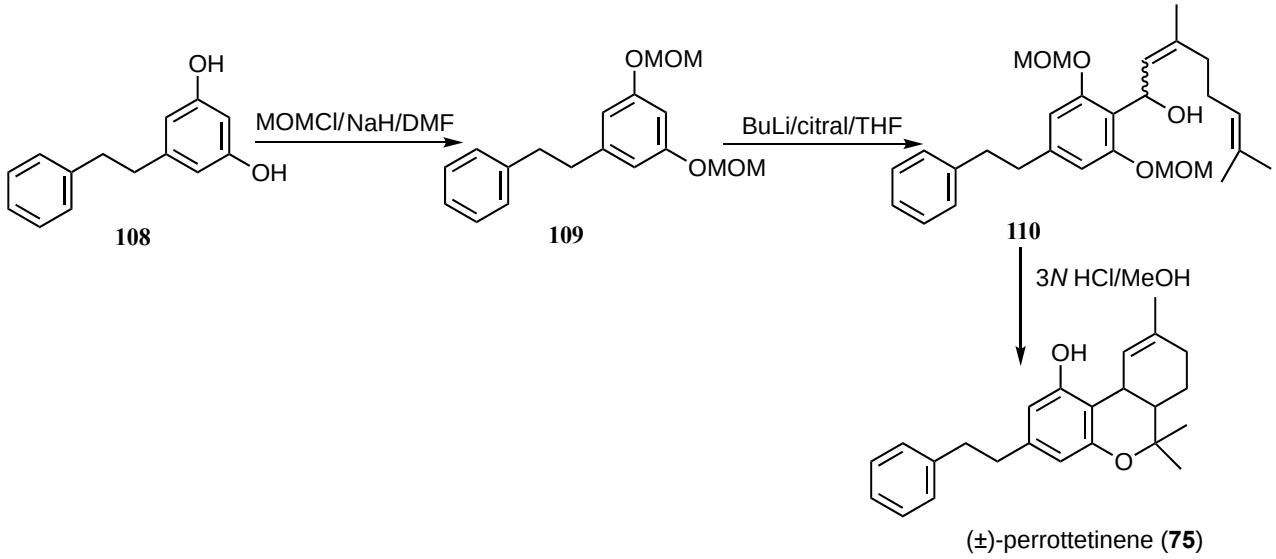
Scheme 3. Total synthesis of radulanin H (**41**) (Yoshida et al. 2009)



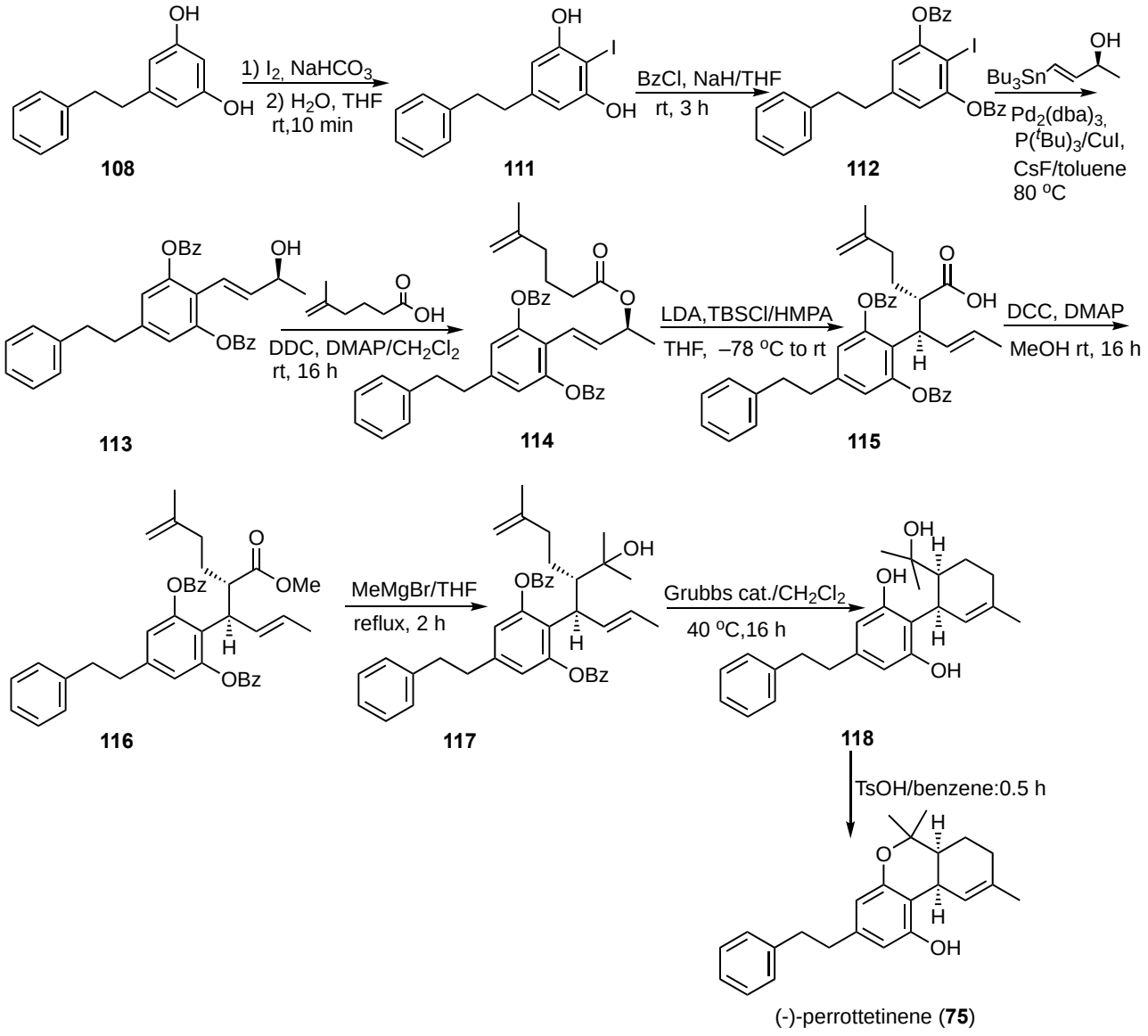
Scheme 4. Total synthesis of radulanin E (**52**) (Yoshida et al. 2009)



Scheme 5. Total synthesis of radulanin A (**41**) and radulanin E (**52**) (Richardson 2012)

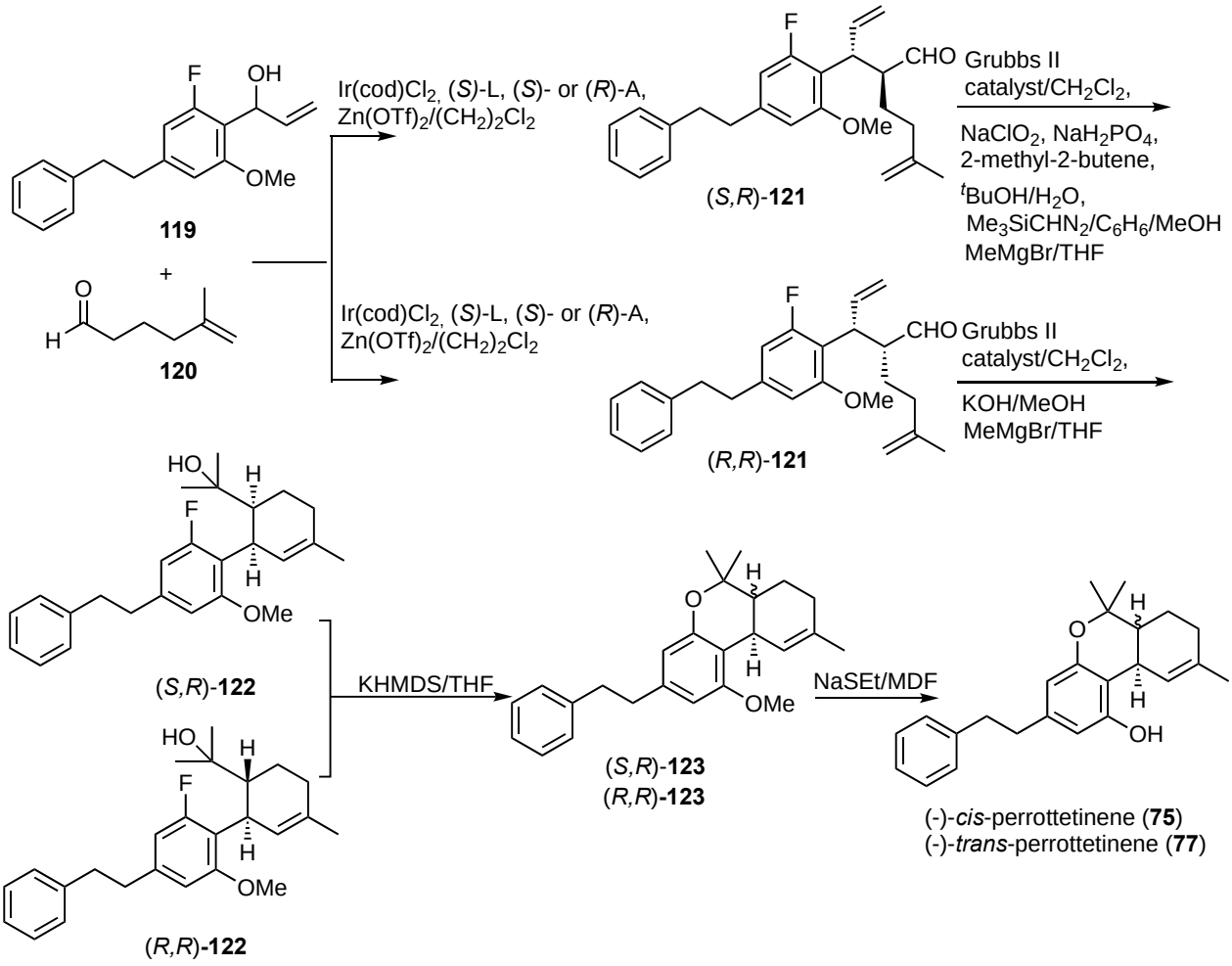


Scheme 6. Total synthesis of racemic perrottetinene (**75**) (Park and Lee, 2010)



Scheme 7. Total synthesis of (-)- perrottetinene (**75**) (Song et al. 2008)

(**41**) and radulanin E (**52**) were also synthesized by Richardson (2012)[37] using the di-MOM ether (**103**) of 3,4-dihydroxy-4-(2-carboethoxy-2-butenyl)bibenzyl as shown in Scheme 5, which implied and implicated the total synthesis of tylimanthin B (**29**) through this method. In 2010, Park and Lee[38] reported a concise total synthesis of racemic perrottetinene (**75**). Treatment of 3,5-dihydroxybibenzyl (**108**) with MOM/NaH produced the MOM-ether derivative **109**, which was treated with *n*-butyllithium, followed by the addition of citral to afford **110**. Cyclization of **110** with HCl in methanol gave racemic perrottetinene (**75**) as shown in Scheme 6. The stereoselective total synthesis of (-)-perrottetinene (**75**) was accomplished through formation of the Ireland-Claisen rearrangement substrate (**114**) from 3,5-dihydroxybibenzyl (**108**), followed by the formation of the rearranged product (**115**), and its methyl ester (**116**), which was treated with methyl magnesium bromide to give the tertiary alcohol (**117**), followed by treatment of **117** with



Scheme 8. Total synthesis of (-)- *cis*- (**75**) and (-)- *trans*-perrottetinenes (**77**) (Chicca et al. 2018)

Grubbs' catalyst produced **118**, which afforded with the α-terpineol moiety. Cyclization of **118** with a catalytic amount of TsOH afforded (-)-perrottetinene (**75**) [39] as shown in Scheme 7.

(-)-*cis*-Perrottetinene (**75**) and (-)-*trans*-diastereomer (**77**) were synthesized by Chicca and colleagues in 2018,[20] as indicated in Scheme 8. Stereodivergent coupling of 3-fluoro-4-(1-hydroxy-2-propene) (**119**) and 5-methyl-5-hexenal (**120**) using iridium and amine-catalyzed aldehyde allylation gave (*S,R*)-**121** and (*R,R*)-**121**. The S_NAr precursors (*S,R*)-**122** and (*R,R*)-**122** were prepared through ring-closing metathesis, oxidative esterification, and subsequent Grignard addition. Each product was treated with KHMDS resulting in the formation of (*S,R*)-**123** and (*R,R*)-**123**, followed by demethylation with sodium ethanethiolate in DMF which furnished (-)-*cis*-perrottetinene (**75**) and (-)-*trans*-perrottetinene (**77**), respectively.

4. BIOLOGICAL ACTIVITY OF HETEROCYCLIC BIBENZYL DERIVATIVES

Some heterocyclic bibenzyls demonstrated enzyme inhibitory activity. Two simple bibenzyls (**4** and **11**) and perrottetin D (**17**), two dihydrooxepin derivatives, radulanin A (**41**), radulanin H (**45**) and 4-hydroxyradulanin H (**46**), exhibited calmodulin inhibitory activity (LD_{50} 100, 100, 2.0, 95.0, 17.0 and 18.5 μg/mL, respectively).[34] Radulanin D (**17**) and radulanin H (**45**) indicated 5-lipoxygenase inhibitory activity (4.0 μM and 15 μM).[34] Radulanin K (**33**) showed moderate cyclooxygenase inhibitory activity with an with IC_{50} value of 39.7 μM.[40] 6-Hydroxy-4-(2-phenylethyl)benzofuran (**13**), radulanin A (**41**), and L (**44**), 3,5-dihydroxy-2-geranylbibenzyl (**58**) and *o*-cannabicyclol (**67**) isolated from *R. appressa* were tested for inhibition of NO production in cultured RAW 264.7 cells in response to lipopolysaccharide (LPS). All of the bibenzyls inhibited NO production with LD_{50} values 12.7, 20.0, 15.3, 4.5, 16.1 and 13.2 μM, respectively, with the best activity shown by the non-heterocyclic 3,5-dihydroxy-2-geranylbibenzyl (**58**).[15] Radulanin K (**33**) also inhibited superoxide anion radical release from guinea pig macrophage with IC_{50} 6 μg/mL.[41] Perrottetin D (**17**) showed selective and potent antitrypanosomal activity *in vitro* (IC_{50} 4.44 μg/mL) against *Trypanosoma brucei* GUTat 3.1. This activity was 3.6 and 6.2 times stronger than those of the standard drugs, suramin and eflonithine, respectively.[42] Bibenzyl derivatives isolated from *R. constricta* were evaluated for their cytotoxic activity against several cancer cell lines. Among them, *o*-cannabicyclol (**67**) induced cytoplasmic vacuolization in A549 and NCI-H1299 cell lines with IC_{50} 9.8 μM.[13] Wang et al. (2017)[14] evaluated all of heterocyclic bibenzyls isolated from Chinese *R. sumatrana* against various cancer cell lines. Among them, radulanin A-5-one (**54**) showed cytotoxic activity against MCF-7, PC-3, SMMC-7721 cells with IC_{50} values3.86, 6.60, and 3.53 μM, respectively, and induced MCF-7 cell death through a mitochondria-mediated apoptosis pathway. Radulanin A-5-one (**54**), saccatene A (**72**), and saccatene B (**73**) from *A. saccatus* showed antimicrobial activity against *Listeria monocytogenes*, *Salmonella enteritidis* and *Yersinia enterocolitica* with an MIC value of 25 μg/mL.[22]

5. CONCLUSION

There are about 6,000 species of liverworts in the world. Although they are very small photosynthesizing, terrestrial, spore-forming plants, they produce a vast number of different terpenoids, aromatic compounds and polyketides. Among the families of three orders, Jungermanniales, Metzgeriales and Marchantiales, belonging to the Marchantiophyta (liverworts), the species of the family Radulaceae are totally separated chemically from the other families, since they elaborate mainly bibenzyls, prenylated bibenzyls and the heterocyclic bibenzyls which are formed from the 2- and 4-prenylated bibenzyls, as well as the heterocyclic bis-bibenzyls. Some *Radula* species, such as the Japanese *R. perrottetii* and the New Zealand *R. marginata*, biosynthesize characteristic bibenzyl cannabinoids, such as perrottetinene (PET) and its carboxylic acid, perrottetinenic acid. The former bibenzyl shows the same psychoactive and potent anti-inflammatory effect

as those of Δ^9-tetrahydrocannabinoids (THC) obtained from *Cannabis sativa.* Several different liverwort families from the Radulaceae also produce various heterocyclic bibenzyls which are structurally simpler than those found in Radulaceae species.

At present, only 5-7% of the known liverworts have been analyzed chemically, and their collection sites are extremely limited, in New Zealand, Japan, China, Europe, and parts of South America. Further phytochemical studies of the liverworts in these localities will provide many different types of secondary metabolites, especially heterocyclic compounds with significant bio- and pharmacological activities, such as bibenzyl cannabinoids and heterocyclic bis-bibenzyls possessing muscle relaxant, tubulin polymerization inhibitory and antiviral activities.[11]

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