The Occurrence of Tricin and Its Derivatives in Plants

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Green Chemistry

REVIEW

The Occurrence of Tricin and Its Derivatives in Plants

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Our understanding of the structure and biosynthetic pathway of lignin, a phenylpropanoid heteropolymer, continues to evolve especially with the discovery of new lignin monomers/structural moieties such as monolignol acetate, hydroxycinnamyl aldehyde/alcohol, and *p*-hydroxybenzoate in the past decades. Recently, tricin has been reported as a component incorporated into monocot lignin. As a falvonoid compound widely distributed in herbaceous plants, tricin has been extensively studied due to its biological significance in plant growth as well as potential for pharmaceutical importance. Tricin is biosynthesized as a constituent of plant secondary metabolites through a combination of phenylpropanoid and polyketide pathways. Tricin occurs in plants in either free or conjugated forms such as tricin-glycosides, tricin-lignans, and tricin-lignan-glycosides.The emergence of tricin covalently incorporated with lignin biopolymer implies the possible association of lignification and tricin biosynthesis. This review summarizes the occurrence of tricin and its derivatives in plants. In addition, synthesis, potential application, and characterization of tricin are also discussed.

Introduction

Lignocellulosic biomass is now well acknowledged as a viable sustainable resource for the production of bioenergy, biofuels, and biobased chemicals. U.S.A. governmental policy has outlined goals to produce 20% of its transportation fuels and 25% of its petroleum based chemical commodities from biomass over the next two decades.[1] Terrestrial plants are mainly composed of 35-45% cellulose, 15-30% hemicellulose, and 15-35% lignin, and to-date most developed biorefinery technologies have focused on the utilization of plant polysaccharides into fuels and chemicals. Whereas lignin, the second most abundant terrestrial polymer, is usually underutilized, and frequently used as a resource to generate power by combustion.[2] For this reason, valorization of lignin can be a solution for effective utilization of total biomass.[1, 2] Lignin is a racemic aromatic heteropolymer derived mainly from three traditional monolignols (i.e., *p*-coumaryl, coniferyl, and sinapyl alcohol). The monolignols are synthesized through the phenylpropanoid pathway followed by monolignol-specific pathways.[3] Subsequent lignin polymerization occurs via an oxidative radicalization and combinatorial radical coupling of monolignols.[4] Although lignin has been studied for decades, its structure and biosynthesis is still not completely understood. Substantial refinements about lignin especially in the biosynthetic pathway have been achieved over the last two decades due, in part, to the discovery of new lignin monomers and structural moieties such as dihydroconiferyl alcohol, monolignol acetate, coniferyl aldehyde, ferulic acid, *p*-hydroxybenzoate, and *p*-coumarate ester in normal, transgenic, or mutant plants.[5] The flexibility of lignin structures has been further extended by the recent discovery of C-lignin (catechyl lignin) derived from a new lignin monomer—hydroxycinnamyl alcohol (caffeyl alcohol).[6-8] More recently, the incorporation of tricin into lignin has also been reported.[9, 10]

Tricin (IUPAC, 5,7-dihydroxy-2-(4-hydroxy-3,5-dimethoxyphenyl)-4H-chromen-4-one), a flavonoid type compound, belongs to the flavanones subclass.[11] It is structurally comprised of two phenyl rings and one heterocyclic ring (**Fig. 1**): a benzoyl system (ring-A), a

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cinnamoyl system (ring-B), and a heterocyclic system (ring-C), which features the flavanone backbone.[12] Like other flavonoids, tricin is originated from the plant secondary metabolic pathways.[12, 13] The biosynthesis of tricin includes two stages: the flavanone backbone is formed by coupling compounds from polyketide and phenylpropanoid pathways followed by mediation with chalcone synthase and isomerase; the flavone synthesis and sequential methylation leading to tricin occur at a later stage.[14] Tricin is typically accumulated in the leaves and stems in herbaceous and cereal plants with sporadic amounts[15, 16] and is frequently isolated by solvent extraction from plant tissue.[14, 17, 18] The reported forms of the isolated tricin include free tricin (or tricin aglycone) and tricin derivatives (e.g., tricin-glycosides, tricin-lignans, and tricin-lignan-glycosides) (**Fig. 1**). Recently, tricin has also been observed as a moiety occurring in lignin isolated from a few monocots, such as perennials,[19, 20] rice straw,[21] wheat straw,[22] maize plant,[10, 23, 24] bamboo,[25] sugarcane,[26] and even brewer's spent grain.[27] The incorporation of tricin into lignin was further confirmed by biomimetic coupling reactions of tricin with monolignols, and the results indicated that tricin could act as an initiative lignification site and that the polyketide pathway for tricin biosynthesis was possibly associated with lignification.[10] In this review, advances in tricin related studies including the occurrence and identification of tricin and its derivatives, tricin biosynthesis, and its potential for biological and pharmaceutical applications are discussed. In particular, the recent development in our understanding of tricin incorporation into lignin is highlighted.

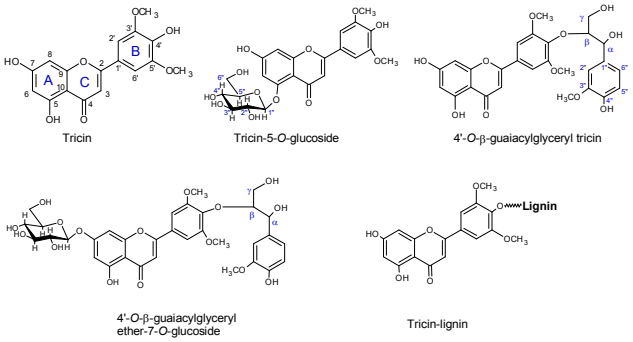


Fig. 1 Exemplary structure of tricin,[14] tricin-glycosides,[28] tricin-lignans,[29] tricin-lignan-glycosides,[30] and tricin-lignin.[10]

Occurrence of tricin and its derivatives

Tricin, in the free form, was first isolated from rusted wheat leaves dating back to 1930[31] and later was also observed in many monocotyledonous plants such as the family of Gramineae[32, 33] and Cyperaceae.[34, 35] The isolated tricin from plants usually contains tricin derivatives including tricin-glycosides, tricin-lignans, and tricin-lignan-glycosides. In tricin-glycoside, tricin is attached with carbohydrates units while tricin-lignan refers to tricin derivatives in which tricin is acylated with monolignols (**Fig. 1**). Tricin-lignan-glycosides consists of both carbohydrate and monolignol moieties. The detection of tricin and tricin conjugates from many new plant species indicates that tricin is more widespread than it was previously thought.[14]

Free tricin

The natural presence of free tricin occurs in a number of sources of plants and tissues, and its content varies from a few to hundred milligrams per kilogram of plants on a basis of reported isolation yields (**Table 1**). Tricin has been mostly isolated from leaves of cereal plants such as wheat, rice, and barley. It has also been reported in bamboo, palms, sugarcane, and even in the grains, seed,[36] and juice[37] of plants. It also exists as different forms such as 3-*O*-acetyl tricin[38] and 4'-*O*-methyl tricin.[39] Due to the plant protection function of tricin, the leaves of pest-resistant rice contained higher tricin than the susceptible controls.[40] Similarly, accumulation of tricin in wheat was enhanced when the wheat seedlings were treated with herbicide safener.[41] The seeds of *Orobanche ramosa*[42] and rice bran of *Oryza sativa* L.[43, 44] contain relatively high content of tricin. The cold-acclimated wheat and wheat husks also showed higher quantity of tricin when compared to the wheat under non-acclimated conditions and other parts of the plant (e.g. leaves and brans).[45, 46] Tricin was recently observed in two species of evergreen shrubs in Okinawan: *M. bontioides* A.[47, 48] and *Gynerium sagittatum.*[49] Other than in plants, tricin and its derivatives were also discovered as pigments in insects.[50] The occurrence of tricin in plants was previously overlooked because the difficulty to distinguish it from other methylated compounds via traditional characterization and separation techniques.[15] In addition, the presence of tricin in free form is also questioned due to the possibility of enzymatic hydrolysis during the extraction process.[51]

Table 1 Occurrence of free tricin in plants

Plant	Species	Part of plant	Isolation yield (mg/Kg)
Arundo grass	*Arundo donax* L.[52]	Aerial part	-
Bamboo	*Indocalamus herklotsii* & *pedalis*[53]	Leaves	10-20
	Neosinocalamus affinis[54]	Leaves	7.6
	Phyllostachys glauca[55]	Leaves	26500*
	Phyllostachys nigra[56, 57]	Leaves	618*[38]
	Pleioblastus amarus[58]	Leaves	1.2
	Sasa albo-marginata[59-61]	Leaves	0.2
	Sasa borealis[62]	Whole plants	10
	Sasa senanensis (Rehder)[63]	Leaves	46
	Sasa veitchii (Carr.)[64]	Leaves	1.4
Barley	*Hordeumv ulgare*[65]	Grains	10.4
	Hordeum vulgare[66]	Leaves	17.4
Barnyard millet	*Echinochloa utilis*[67]	Grains	36
Barrenwort	*Epimedium brevicornum*[68]	Aerial parts	6.4
	Epimedium hunanense[69]	Aerial parts	2
Blue grass	*Poa ampla*[70]	Stromata, seeds, Spikelet, leaves	6
Bristlegrass	*Setaria viridis*[71]	Aerial part	128
Buttercup	*Ranunculus sieboldii* Miq.	Whole plant	1.2
	Ranunculus sceleratus L.[72]		
Copacopa	*Artemisia copa* Phil.[73]	Aerial parts	5
Fenugreek	*Trigonella foenumgraecum* L.[74]	Seeds	-
Fescue	*Festuca spp.*[75]	Leaves	33
Halfa	*Desmostachia bipinnata*[76]	Aerial parts	172.5
Himalayan poppy	*Meconopsis horridula*[77]	Aerial parts	4
Jungle rice	*Echinochloa colona* L.[78]	Shoots	-
Malagasy	*Agelae pentagyna*[38]	Leaves	67
Oat plants	*Avena sativa* L.[79]	Brans	2.4
Palm	*Hyphaene thebaica* L.[80]	Leaves	1.9
Pearl millet	*Pennisetum glaucum*[81]	Fruit	-
Johnsongrass	*Sorghum halepense*[82]	Aerial parts	63.8
Corn stover	*Zea mays* L.[83]	Stems	0.5
Pyrethrum	*Pyrethrum tatsienense*[84]	Whole plant	99
Rice plant	-(pest resistant rice plant)[85]	Leaves, stems	18.4
	Oryza sativa L.[86]	Aerial parts	-
	Oryza sativa L.[40, 87-89]	Leaves	7060[89]
	Oryza sativa L.[90]	Rice hulls	-

	Oryza sativa L.[43, 91]	Rice brans	240[43]
	Oryza sativa[92]	Different tissues	-
	Zizania latifolia[93]	Aerial parts	4.2
Rice seeds	Transgenic[44]	Seeds	110
Rattan palm	*Calamus quiquesetinervius*[94]	Stem	1.1
Sandspurry	*Spergularia diandra* & *marina*[95]	Aerial parts	4 & 16.4
Sorghum	*Sorghum bicolor*[96]	Stem	10.9
Stoneshrub	*Lycopodium japonicum*[39]	Whole plant	0.5
Stoneshrub	*Myoporum bontioides*[47, 48]	Leaves	9.747
Stonecrop	*Sedum sarmentosum*[97]	Whole plant	-
Sugarcane	*Saccharum officinarum* L.[98]	Culms, syrups	3, 35, 132
	Saccharum spp. Hybrids*[76]*	Stem	-
	-	Sludge[99]	3.2
Tumbleweed	*Salsola collina* Pall.[100, 101]	Whole plant	23[100]
Wheat	*Triticum aestivum*[46]	Husks, brans, leaves	770
Wildcane	*Gynerium sagittatum*[49]	Roots	18.4
Blue grass	*Poa ampla*[70]	Stromata, seeds, Leaves	2; 8	12; -
Bristlegrass	*Setaria viridis*[71]	Aerial part	2	10.3
Buttercup	*Ranunculus sieboldii* Miq.	Whole plant	2	1.9
	Ranunculus sceleratus L.[72]			
Deviltree	*Alstonia macrophylla*[104]	Leaves	4	-
Fenugreek	*Trigonella foenumgraecum* L.[74]	Seeds	2	-
Halfa	*Desmostachia bipinnata*[76]	Aerial part	2	268
Hedgenettle	*Stachys officinalis*	Leaves	2; 5	-
	Stachys alopecuros			
	Stachys scardica[110]			
Honeysuckle	*Lonicerae japonica*[116]	Flower	8	-
Liverworts	*Metzgeria conjugata*[103]	Whole	13; 14	-
	Metzgeria leptoneura[103]	Whole	12	-
Oat plants	*Avena sativa* L.[105]	Leaves, stems, inflorescences	3; 4; 8	-
	Avena sativa L.[79]	Brans	2	3.3
Palm	*Hyphaene thebaica L.*[80]	Leaves	1	5.6
	Phoenix hanceana[117]	Leaves	2; 8	1.2; 0.5
Princess tree	*Paulownia tomentosa*[118]	Flower	2	-
Rice plant	-(pest resistant plant)[85, 119]	Leaves, stems	1;2	29.2; 33.5
	Oryza sativa L.[88, 120]	Leaves	1; 2	25; 42
	Oryza sativa[92]	Different tissues	1; 2	429; 150
	Zizania latifolia[121]	Aerial parts	2	6.4
Regional poppy	*Meconopsis horridula*[77]	Aerial parts	2	0.1
Stoneshrub	*Myoporum bontioides*[48]	Leaves	5	-
Stonecrop	*Sedum sarmentosum*[97]	Whole plant	2	-
Sugarcane	*Saccharum officinarum* L.[122]	Bagasse, leaves	10	-
	Saccharum officinarum L.[111]	Bagasse, leaves, juice	2; 8	-
	Saccharum officinarum L.[123]	Juice	2	3*
	Saccharum officinarum L.[124]	Leaves	2; 8	-
	Saccharum spp. Hybrids[125]	Bagasse	2; 8	-
	-	Juice[126]	2; 8	-
Tumbleweed	*Salsola collina* Pall.[100]	Whole plant	2	5
Wildcane	*Gynerium sagittatum*[49]	Roots	1	14.2

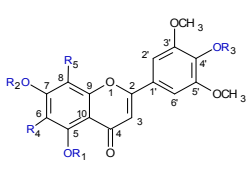
-: not reported; * based on leaves extract.

Glc: glucose; Ara: arabinose; Xyl: xylose; Rha: rhamnose; Boi: boivinose; Glur: glucuronic acid; Galur: galacturonic acid; Me: methyl; -: not reported; * mg/L.

Tricin-glycosides

Tricin-glycosides occur in plants in two forms (i.e., tricin-*O* and tricin-*C*-glycoside) in which the carbohydrate unit is attached to the hydroxyl group and carbon on the benzene rings (ring-A and ring-B), respectively.[102] Like the free form of tricin, tricin-glycosides are also widely distributed in herbaceous plants (**Table 2)**. Tricin-*O*-glycoside is dominated by tricin 5-*O*- and 7-*O*-glucoside with the glucose unit glycosylating to the 5-OH and 7-OH on ring-A, respectively. In contrast, tricin-*C*-glycoside is rarely found in plants.[54, 103] Although tricin-glucoside mainly entails glucose coupled tricin, other carbohydrate units such as xylose,[103] arabinose,[104] rhamnose,[105] and boivinose[54] have also been found in a conjugation with tricin. Units such as glucuronic acid and diglucuronic acid coupled with tricin have been isolated from alfalfa,[106-109] stoneshrub,[48] barrel medic,[108] and hedgenettle.[110] Tricin-diglycoside (e.g., rhamnosyl-hexoside) was detected in some plants such as blue grass,[70] sugarcane,[111] and liverworts.[103] A few other forms of tricin-glycosides including salt form of tricin-7-*O*-glucoside,[112] *C*-glycosyl tricin,[54] 7-*O*-methylated tricin-glucoside,[58] 4'-*O*-methtylated tricin-glucoside,[110] and tricin-malonylhexoside[92] were also reported. Alfalfa,[109] halfa,[76] and the subspecies *indica* of rice plant (leaves)[92] have shown relatively high content of tricin-glycosides (around 1600-4700, 300, and 400 mg/Kg plants isolation yields, respectively). Research on barley showed that the amount of total flavone glycosides including tricin-7-*O*-glucoside in the leaves decreased with increased rate of fertilizing.[113]

Table 2 Occurrence of tricin-glycosides in plants



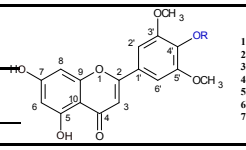
	R_1	R_2	R_3	R_4	R_5	Compound
1	-Glc	-H	-H	-H	-H	Tricin-5-*O*-glucoside
2	-H	-Glc	-H	-H	-H	Tricin-7-*O*-glucoside
3	-H	-H	-Glc	-H	-H	Tricin-4'-*O*-glucoside
4	-H	-H	-Ara	-H	-H	Tricin-4'-*O*-arabinoside
5	-H	-Glur	-H	-H	-H	Tricin-7-*O*-glucuronide
6	-Glc	-Me	-H	-H	-H	Tricin-7-methyl-5-*O*-glucoside
7	- H	-Me	-Glc	-H	-H	Tricin-7-methyl-4'-*O*-glucoside
8	-H	-Glc-Rha	-H	-H	-H	Tricin-7-*O*-neohesperidoside
9	-H	-Glur-Glur	-H	-H	-H	Tricin-7-*O*-Glur-glucuronide
10	-H	-Galur-Rha	-H	-H	-H	Tricin-7-*O*-Rha-galactoronide
11	-H	-H	-H	-Boi	-Glc	Tricin-6-C-Boi-8-C-glucoside
12	-H	-H	-H	-Xyl	-Glc	Tricin-6-C-Xyl-8-C-glucoside
13	-H	-H	-H	-Ara	-Glc	Tricin-6-C-Ara-8-C-glucoside
14	-H	-H	-H	-Glc	-Ara	Tricin-6-C-Glc-8-C-arabinoside

Plant	Plant species	Part of plant	Compound	Isolation yield (mg/Kg)
Alfalfa	*Medicago sativa* L.[106-109]	Aerial parts	5; 9	140; 81[107] 1600; 4700[109]
Bamboo	*Fargesia robusta*[28]	Leaves	1	62.5
	Neosinocalamus affinis[54]	Leaves	2; 11	2.2; 1
	Pleioblastus amarus[58]	Leaves	2; 6; 7	1.2-2.5
	Sasa borealis[114]	Leaves	2	45
	Sasa kurilensis[115]	Leaves	1; 3	0.7; <0.1
Barley	*Hordeum vulgare*[113]	Leaves	2	16
Barrel medic	*Medicago truncatula*[108]	Aerial parts	5; 9	-

Tricin-lignans

Tricin-lignans belong to flavonolignans in which the flavone backbone is acylated with another phenylpropanoid unit, usually at ring-B.[127] They are usually present along with tricin and tricin-glycosides in plants such as rice, oat, palm, alfalfa, and sugarcane (**Table 3**). The tricin-lignan was reported to occur in *Aegilops ovata* L. in the form of *p*-coumaryl alcohol acylated tricin,[128] namely aegicin. Salcolin is another type of tricin-lignans existing in *Salsola collina* with an additional methoxy group attached on the *p*-coumaryl unit.[101] The reported tricin-lignans are primarily in a form of tricin guaiacylglyceryl ether and its derivatives. Tricin coumarylglycerl ether was observed in rattan palm.[94] Some other guaiacylglyceryl units etherified with methyl, ethyl, acetyl groups, and acylated with coumaroyl unit have also been reported in conjugation with tricin.[64, 93, 129, 130] The contents of tricin-lignans (isolation yield usually less than 30 mg/Kg plant) are relatively lower than tricin and tricin-glycosides. Mohanlal et al. found that rice bran possessed relatively high content of tricin guaiacylglyceryl ether (61 mg/Kg).[29, 43]

Table 3 Occurrence of tricin-lignans in plants



	R	Compound
1	-guaiacylglyceryl	4'-*O*-β-guaiacylglyceryl tricin
2	-coumarylglyceryl	4'-*O*-β-*p*-hydroxyphenylglyceryl tricin
3	-(Ac)-guaiacylglyceryl	4'-*O*-β-(γ-*O*-acetyl)-guaiacylglyceryl tricin
4	-(Et)-guaiacylglyceryl	4'-*O*-β-(α-*O*-ethyl)-guaiacylglyceryl tricin
5	-(Me)-guaiacylglyceryl	4'-*O*-β-(α-*O*-methyl)-guaiacylglyceryl tricin
6	-(Me) (Ac)-guaiacylglyceryl	4'-*O*-β-(α-*O*-Me)(γ-*O*-Ac)-guaiacylglyceryl tricin
7	-(coumaroyl)-guaiacylglyceryl	4'-*O*-β-(γ-*O*-*p*-coumaroyl)-guaiacylglyceryl tricin

Plant	Plant species	Part of plant	Compound	Isolation yield (mg/Kg)
Corn stover	*Zea mays* L.[83]	Stems	1	1
Johnsongrass	*Sorghum halepense*[82]	Aerial parts	1	8.7
Kumazasa	*Sasa veitchii* (Carr.) [64]	Leaves	1; 3; 6; 7	1-4
Oat plants	*Avena sativa* L.[131]	Whole plant	1	0.9
Rattan palm	*Calamus quiquesetinervius*[94]	Stem	1; 2	0.3; 0.6

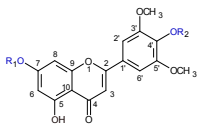
	Calamus quiquesetinervius[129]	Stem	4	0.2
Rice	*Oryza sativa* L.[29, 43]	Rice bran	1	61
	Oryza sativa[92]	Different tissues	1	-
	Oryza sativa L.[130]	Aerial parts	1; 5	0.9; 0.8
	Zizania latifolia[121]	Aerial parts	1	15.4
	Zizania latifolia[93]	Aerial parts	1; 5	11.8; 14.6
Sugarcane	*Saccharum officinarum* L.[122]	Bagasse, leaves	1	-
	Saccharum officinarum L.[111]	Bagasse, leaves, juice	1	-
	Saccharum spp. Hybrids[125]	Bagasse	1	-
Thatching grass	*Hyparrhenia hirta* L.[30]	Leaves	1	30
Tumbleweed	*Salsola collina*[101]	Epigeal part	1	1.1
Vetivergrass	*Vetiveria zizanioides*[132]	Aerial parts	1	28.9

-: not reported.

Tricin-lignan-glycosides

Compared to tricin-glycosides and tricin-lignans, tricin-lignan-glycosides have only been reported in a few plants such as alfalfa, rice plant, and sugarcane (**Table 4**). In tricin-lignan-gylcosides, the phenylpropanoid unit usually links to the 4'-OH of tricin, while the carbohydrate unit attaches to 7-OH separately. However, the phenylpropanoid unit (*p*-methoxycinnamate) has been reported to link with tricin through glucose moiety in tricin-lignan-glycoside occurring in sugarcane juice.[18] The guaiacylglyceryl ether and glucose glycosylation are the two main moieties reported in tricin-lignan-glycosides. Another group of tricin-lignan-glycosides, in which the ferulic, coumaric, and sinapic acids unit was coupled with tricin through glucuronic acid at the 7-OH, were also observed in alfalfa and barrel medic from species of *Medicago*.[106-108]

Table 4 Occurrence of tricin-lignan-glycosides in plants



	R_1	R_2	Compound
1	-Glc	-guaiacylglyceryl	T-7-*O*-(4'-*O*-β-guaiacylglyceryl ether)-glucoside
2	-H	-(Glc)-guaiacylglyceryl	T-4'-*O*-β-(α-*O*-glucoside)-guaiacylglyceryl ether
3	-H	-(Glc)-guaiacylglyceryl	T-4'-*O*-β-(γ-*O*-glucoside)-guaiacylglyceryl ether
4	-H	-Coumaroyl-Glc	Tricin 4'-*O*-(6"-*O*-*p*-Coumaroyl)-glucoside
5	-(Me)-Coumaroyl-Glc	-H	Tricin 7-*O*-(6"-*O*-*p*-methoxycoumaroyl)-glucoside
6	-Feruloyl-Glur-Glur	-H	Tricin 7-*O*-[2'-*O*-Feruloyl-Glur-(1-2)]-glucuronide
7	-Sinapoyl-Glur-Glur	-H	Tricin 7-*O*-[2'-*O*-Sinapoyl-Glur-(1-2)]-glucuronide
8	-Coumaroyl-Glur-Glur	-H	Tricin 7-*O*-[2'-*O*-Coumaroyl-Glur-(1-2)]-glucuronide
9	-Feruloyl-Glur-Glur-Glur	-H	Tricin 7-*O*-[2'-*O*-Feruloyl-Glur-(1-2)-Glur-(1-3)]-glucuronide
10	-Coumaroyl-Glur-Glur-Glur	-H	Tricin 7-*O*-[2'-*O*-Coumaroyl-Glur-(1-2)-Glur-(1-3)]-glucuronide
11	-Feruloyl-Glc-Glur	-H	Tricin 7-*O*-[2'-*O*-Feruloyl-Glucono-(1-2)]-glucuronide

Plant	Plant species	Compound	Isolation yield (mg/Kg)
Alfalfa	*Medicago sativa* L.[106-109]	6; 7; 8; 9	2-30[106] 100-160[107] 500-2,000[109]
Barrel medic	*Medicago truncatula*[108]	6; 7; 8; 9; 10; 11	-
Gum arabic tree	*Acacia nilotica* Linn.[133]	4	82.5
Rice	*Oryza sativa* L.[134]	3	1.3
	Zizania latifolia[121]	1; 2	10.6
Sugarcane	*Saccharum officinarum* L.[122]	1	-
	Saccharum officinarum L.[111]	1	-
	Saccharum spp. Hybrids[125]	1	-
	Saccharum spp. Hybrids[18]	5	13
Thatching grass	*Hyparrhenia hirta* L.[30]	1	<0.7

-: not reported.

Tricin-lignin

The presence of tricin in lignin was not determined until a recent report by Del Río et al. in 2012.[9] Later on, tricin was also found in lignin isolated from a few more monocot plants such as rice straw, coconut coir fibres, bamboo, corn stover (maize stover), and brewer's spent grain (**Table 5**). Both the whole cell wall and the isolated lignin of wheat straw,[9] corn stover,[10, 24] and sugarcane bagasse[26] showed the presence of tricin. In dioxane lignin (DL) of wheat straw without ball-milling, tricin constituted 15% of the sum of H, S, and G units; however, this amount decreased down to 8% when the biomass milled with extended time (i.e., 16 h).[135] Tricin in wheat straw lignin was reduced from % to 2% per hundred C6-C3 units after steam explosion pretreatment.[22] The presence of tricin was not detectable when the milled wood lignin (MWL) of bamboo was acetylated.[25] It appears that the detectable content of tricin in lignin is affected by the lignin isolation process as well as by pretreatments. In addition, the occurrence of tricin component in lignin varies on the tissue of plant sampled. For example, tricin was detected in the milled wood lignin and alkaline extracted lignin from foliage but not the stem of arundo grass.[19] The alkaline extracted lignin from rice straw rather than rice husk showed the presence of tricin.[21] Wen et al. reported that tricin was present in the milled wood lignin from bamboo stem but not in the pith.[25]

Table 5 Occurrence of tricin-lignin in plants

Plant	Plant species	Type of lignin
Arundo grass	*Arundo donax* Linn.[19]	MWL and alkaline lignin
Bamboo	*Phyllostachys pubescens*[25]	MWL of stem
Brewer's spent grain	*Hordeum vulgare* L.[27]	MWL, DL, CEL
Coconut coir	*Cocos nucifera*[136]	MWL
Corn stover	*Zea mays*[10, 24]	MWL, whole cell
Maize plant	Transgenic[23]	MWL
Rice straw	-	Alkaline lignin[21]
Sugarcane	*Saccharum spp.*hybrids[26]	MWL, whole cell
Wheat straw	*Tritium aestivum* L.[135]	DL and AL
	Triticum durum C.[9]	MWL, whole cell
	Triticum sativum[137, 138]	MWL and CEL
	-	EMAL[22]
Wula sedge	*Carex meyeriana* Kunth[20]	MWL

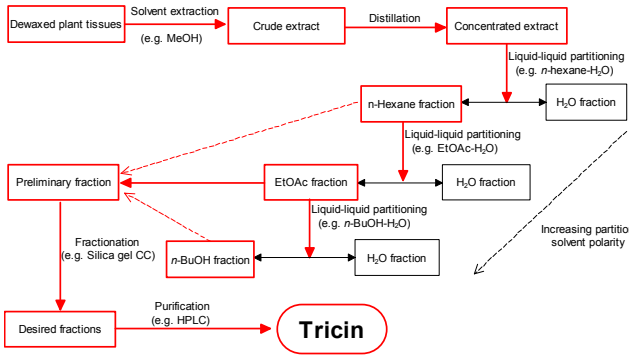
MWL: milled wood lignin; DL: dioxane lignin; CEL: cellulolytic enzyme lignin; AL: acidolysis lignin; EMAL: enzymatic mild acidolysis lignin; -: not reported.

Preparation and isolation

Solvent extraction from plant

Tricin can be isolated from plants by a combination of solvent extraction, liquid-liquid partitioning, and chromatography separation and purification (**Scheme 1**).[139] In general, plant tissue is cut and dewaxed and defatted by petroleum ether (PE) or hexane to remove lipids and chlorophyll pigments prior to solvent extraction. Tricin and its derivatives are usually extracted by aqueous methanol (MeOH) or ethanol (EtOH). It was reported that MeOH had better extraction efficiency than EtOH, and 80% MeOH was superior to pure and 50% MeOH for extracting tricin from Pyrethrum.[84] Other solvents such as hot-water,[57, 59, 60, 115] dichloromethane (CH_2Cl_2),[73, 131] and acetonitrile (CH_3CN)[113, 116] have also been used to obtain crude tricin extract. After concentration, the condensed extract (oily residue) is then partitioned by liquid-liquid extraction with solvent such as *n*-hexane, diethyl ether (Et_2O), ethyl acetate (EtOAc), chloroform ($CHCl_3$), CH_2Cl_2 and butanol (*n*-BuOH) with increasing polarity. A preliminary fraction containing tricin is obtained from the layer with lower polarity (e. g. ethyl acetate).

The separation and purification are the key steps to achieve desired fraction for identification and quantification of tricin. The available techniques for tricin separation and purification include thin-layer chromatography (TLC), column chromatography (CC), semi-preparative high-performance liquid chromatography (HPLC), preparative HPLC, and analytical HPLC. A list of separation techniques and columns used for tricin and its derivatives are summarized in **Table 6**.



Scheme 1. Isolation procedure for tricin and its derivatives from natural plants. Adapted from [140]

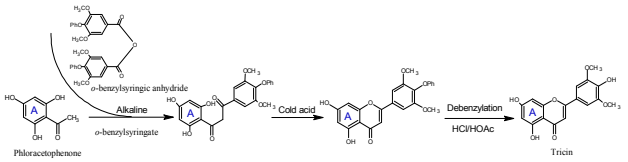
A repeated CC separation technique using appropriate stationary and mobile phases systems is often employed to get the target fractions and compounds. Silica gel CC was primarily employed to obtain the desired fractions.[78, 93] Further purification was conducted with diverse columns filled with dextran (e.g. Sephadex LH 20), C_{18}, octadecylsilyl (ODS), and polyamide. A simple purification method of tricin from wheat leaves was accomplished by injecting filtered MeOH extract directly into reversed-phase HPLC coupling with C_{18} and semi-preparative column.[84, 141] Recently, preparative HPLC[56] and resin CC followed by dialysis[99] were proposed to separate tricin from the extract of bamboo leaves and sugarcane, respectively. Other methods such as high performance thin layer chromatography (HPTLC)[53, 95] and high-speed counter-current chromatography (HSCCC) with two-phase solvent system[123] have been developed to isolate and purify tricin. In addition, capillary electrophoretic (CE)[116] and high-performance capillary electrophoresis (HPCE)[57, 131] have all been used for qualitative and quantitative analysis of tricin-glycosides and tricin-lignans.

Table 6 Isolation and purification techniques for tricin and its derivatives

Chromatography type	Columns
Normal-phase	Silica gel[30, 38, 39, 43, 47, 59, 60, 68-70, 76-78, 83, 90, 93, 94, 96, 121] Celite 535[28, 94]
Reversed-phase	Acquity BEH C_{18}[125] Alltima C_{18}[28] Bondapak C_{18}[49, 56] Cosmosil 5C_{18}[67, 94] Cosmosil 5 Ph[85] Eurospher 80 C_{18}[109] Eurospher PD 82[107] Kromasil C_{18}[84] LiChrosorb RP-18[30, 69, 90, 106, 110, 113, 131] Luna C_{18}[56] Macroporous absorption resin (AB-8)[54, 56, 123] MCI gel CHP-20P[38] Nucleosil 120 C-18[141] ODS[43, 47, 48, 64, 65, 67, 71, 78, 83, 85, 113-115] Omnisphere C_{18}[28] Polyamide[68, 69, 80] Sep-Pak C_{18}[109, 141] Shield RP18[111, 122, 124] Shim-pack C_{18}[123] Supersphere C_{18}[41] Synergi polar column[41] Varian Polaris 5-C_{18}[46] Vertex Eurospher RP-18[106] Zorbax SB-C_{18}[120] Zorbax SB-Aq[118]
Size-exclusion	Sephadex LH-20[28, 43, 48, 49, 58, 67, 73, 77, 80, 83, 96, 131, 132]
Ion-exclusion	Diaion HP-20[47, 64, 79, 115]

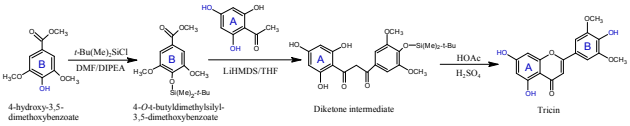
Organic and chemical synthesis

Chemical synthesis of tricin has been conducted using Baker-Venkataraman (BV) transformation as the base for flavone backbone synthesis.[142, 143] The key step of BV transformation involves the etherification of hydroxylacetophenone (e.g. phloracetophenone) with benzyl group (e.g. benzylsyringic anhydride) catalyzed under alkaline condition (**Scheme 2**). The flavone backbone is formed by cyclodehydration with cold acid. The resulting compound is debenzylated by hydrochloric acid (HCl) in acetic acid leading to tricin. Instead of using benzylsyringic anhydride, acyl chloride from oxalyl chloride and acetylsyringic acid can also be introduced to phloracetophenone to generate flavone backbone by BV reaction.[38] However, a selective protection and de-protection of phenolic hydroxyl groups is required to produce tricin.



Scheme 2. Baker-Venkataraman transformation for tricin synthesis.[142]

To protect the phenolic hydroxyl groups on ring-A, lithium bis(trimethylsilyl)amide (LiHMDS) has been introduced to deprotonate phenolic hydroxyl groups on phloracetophenone in a form of lithium polyanion.[144] In combination with *t*-butyldimethylsilyl protection of the phenolic hydroxyl on ring-B, this method yielded 82% tricin (**Scheme 3**). Based on this methodology, tricin has recently been synthesized via reaction of 4-*O*-*tert*-butyldimethylsilyl-3,5-dimethoxybenzoate with LiHMDS and 2',4'-*O*-bis(*tert*-butyldimethylsilyl)-6'-hydroxyacetophenone with a yield of 68%.[60, 145, 146] To simplify the extensive protections and de-protections of hydroxyl groups, BV transformation has been modified to a three-step reaction to generate flavone backbone.[147]



Scheme 3. Synthesis of tricin using LiHMDS protection of phenolic hydroxyl groups.[144]

A few other methods have also been used for tricin synthesis, such as demethylation of 3',4',5'-trimethyltricetin by sulfuric acid[148] and condensation of phloracetophenone with 4-hydroxy-3,5-dimethybenzaldehyde in the presence of boric acid (H_3BO_3), followed by double bond generation between C2 and C3.[149] An unexpected tricin isomer (i.e., aurone) was synthesized through aldol condensation of α-chloro-2-hydroxy acetophenone with aromatic aldehyde in aqueous alcoholic solution containing 5–10% sodium hydroxide at room temperature.[150] Recently, the flavone backbone was synthesized through Claisen-Schmidt condensation, in which a

double bond was formed between the diprotected triol and benzyl-protected syringaldehyde and tricin was formed following cyclodehydration.[10]

Biological functions and potential applications

Like most flavonoid compounds, tricin plays an important role in plant growth.[14, 151, 152] Tricin and its derivatives were reported to function as an antioxidant against organisms diseases in rice plant[91] and palm.[129] Moreover, tricin has been found to possess antibacterial, antifungal, anti-insect activity,[70] and anti-plant-hoppers in deviltree,[104] arundo grass[52] and rice plant.[40, 85-87, 150] The antifungal activity (related with antioxidant activity) of tested flavonoids was enhanced due to the increased number of hydroxyl groups per molecule compound.[153] Other reported functional activities of tricin derivatives include anti-weeds,[78, 120] anti-herbicide,[41] special genes inducer,[88] biotic and abiotic stress protection.[154] The released tricin from the root of allelopathic rice plant interferes with weeds and microbes in paddy soil.[120]

Tricin and its derivatives are also reported potentially applicable in pharmaceutical due to its preventive efficacy, low toxicity, and reasonable bioavailability.[57, 155] The bioavailability of tricin can be enhanced by modifications such as glycosylation[156] and coupling with amino acid as prodrugs.[145] **Table 7** summarizes the potential pharmaceutical applications of tricin related compounds. Tricin exhibits higher radical scavenging activity (e.g. EC50 values) in comparison with commonly used compounds such as quercetin, myricetin, and catechin.[157] Its potential as an anti-inflammatory agent has been suggested due to the antioxidant ability of tricin and its derivatives.[73, 83, 93, 129, 130, 158, 159] Tricin also showed inhibitory activity toward exocytosis from antigen-stimulated rat leukemia basophils[38] and hepatitis B virus[72]. Recently, tricin derived extracts were considered as a potential chemoprevention candidate against cancer due to its intestinal carcinogenesis interference and inhibition of breast cancer cells.[29, 43, 75] The dihydrotricin extracted from palm was reported to exhibit significant blood vessels widening potencies at 100 μM with 80.3% relaxation.[94] Other functions such as proliferation inhibition,[160] anti-influenza virus activity in *vivo*,[146] anti-human cytomegalovirus (HCMV) activity,[60, 61] intestinal adenomas reduction,[161] superior gastro-intestinal availability,[162] and tyrosinase inhibitor,[163] have also been reported which could expand its potential pharmaceutical applications.

Table 7 Pharmaceutical functions of tricin and its derivatives

Compound	Functions
Tricin	Anti-allergy activities[38, 93]
	Anti-HIV activity[63]
	Anti-inflammatory activity[43, 73, 83, 93, 158, 159, 164]
	Antioxidant[43, 63, 67, 91, 157]
	Anti-tumor activity[39, 46, 62, 75, 155, 160-162, 165-170]
	Anti-ulcerogenic activity[76]
	Anti-virus activity[59-61, 72, 145, 146]
	Potential diabetes suppression[78, 100]
	Pigmentation inhibition[66, 163]
Tricin-glycosides	Antioxidant[43, 71, 80]
	Anti-ulcerogenic activity[76]
	Anti-virus activity[72]
	Immunomodulatory[58, 69]
	Neuroprotective effects[117, 171]
Tricin-lignans	Anti-allergy activities[93]
	Anti-inflammatory activity[43, 83, 129, 172]
	Antioxidant[94]
	Antiplatelet aggregation activities[129]
	Antitumor activities[29, 39]
	Cardiovascular protection[94]
Tricin-lignan-glucosides	Antioxidant[18, 108]
	Antitumor activity[18, 129]

Structure identification

Ultraviolet (UV)-visible spectroscopic analysis

UV spectroscopy has been broadly used for tricin identification. With a backbone of 4',5,7-trihydroxy flavone, tricin is characterized by two strong adsorptions in the region 240-400 nm: band I at 300-380 nm corresponding to the cinnamoyl system of ring-B and band II at 240-280 nm corresponding to the benzoyl system of ring-A.[173] In methanol, band I of tricin peaks around 348 nm, and band II peaks near 244 and 269 nm. Reagents such as sodium methoxide (NaOMe), aluminium chloride ($AlCl_3$), $AlCl_3$/HCl, sodium acetate (NaOAc), and NaOAc/H_3BO_3 have been used and added to methanol resulting in a diagnostic shift of UV spectra of tricin.[50, 71, 141] The absorption peaks were observed to vary on chemical changes that occurred on the flavone backbone.[173] The typical absorption peaks of tricin and its derivatives detected by UV are summarized in **Table 8**.

Table 8 Absorption bands of tricin and its derivatives in UV spectra

Compounds	UV (λ/nm) in reagents					
	MeOH	NaOMe	$AlCl_3$	NaOAc	$AlCl_3$/HCl	NaOAc/H_3BO_3
Tricin	269, 346-349[56, 63, 70, 72, 81, 88, 89, 141]	264, 275sh, 418sh[71, 96, 141]	259sh, 277, 303, 370, 394[141]	275, 321sh, 362[71, 96]	259sh, 278, 303, 362, 387[141]	271, 343[71, 96, 141]
Tricin-7-*O*-glucoside	253, 269, 341[70, 71, 111]	260, 294, 427*[71]	248, 341, 380[71]	253, 269, 341[71, 111]	-	-
Tricin-5-*O*-glucosides[120]	297, 318†	-	-	-	-	-
Tricin-7-*O*-glucuronide[48]	247, 268sh, 351	247sh, 261, 399	272, 303sh, 364sh, 403	258, 422	257sh, 275, 300, 363, 385sh	267sh, 360, 385sh
Tricin-disaccharides[111, 122]	265-270, 351	-	265-270, 383	265-270, 340sh, 351	-	-
Tricin-lignan	271, 288sh,305sh, 335[30, 131, 132]	279, 298sh,367[30]	280, 303, 351, 393sh[30]	279, 312sh, 367[30]	280, 303, 345, 393sh[30]	272, 334[30]
Tricin-lignan-glycosides	272, 345[18]	272, 398[18]	270, 382[30, 122]	272, 348, 430[18]	-265, 350[133]	-
T-*C*-glycoside[54]	270, 350	-	-	-	-	-

Note: *NaOH used as diagnostic shift reagent; † EtOH; sh: shoulder; - data not reported.

Infrared (IR) spectroscopic analysis

The characteristics of functional groups in tricin and its derivatives including hydroxyl, aromatic rings, conjugated ketone group, double bond, C-O bond, and aromatic hydrogen, can be identified by IR (**Table 9**). The strong and broad absorbance in the region 3300-4000 cm^{-1} is assigned to hydroxyl group (O-H). The bands at 1653, 1247, and 1055 cm^{-1} were assigned to the conjugated carbonyl group, double bond, and C-O in ring-C, respectively.[96, 134] The region of 1490-1510 cm^{-1} was reported to correspond to aromatic hydrogens, while absorbance band around 1160 cm^{-1} was assigned to the aromatic ether linkage.[18, 64, 70] A broad band at 1128 was attributed to *O*-glycosylation.[133]

Liquid chromatography-mass spectrometric (LC-MS) analysis

Tricin and its derivatives display characteristic mass to charge ratio (*m/z*) using LC-MS analysis (**Table 10)**. Considering both the protonated and deprotonated capability of tricin and its derivatives, MS in either positive or negative mode has been used to study the fragmented ions.[174, 175] The *m/z* of 331 (at positive mode) and 329 (at negative mode) has been usually used to identify tricin. Fragments at *m/z* $[M+H]^+$ 153 and 178 correspond to the ring-A and ring-B fragment, respectively.[104, 133] Signals at *m/z* 315 and 300 are suggestive of cleavage of one and two molecular methyl groups[18, 133] from tricin. The fragmentation of tricin-glycosides and tricin-lignans gives rise to a substituted carbohydrate and phenylpropanoid moiety besides tricin ion.[70, 94, 106] The characteristic fragments from tricin derivatives are glucosyl (-162 u),[120] rhamnosyl (-146 u),[70] glucuronyl (-176 u),[106] sinapic acid (-206 u),[106] ferulic acid (-176 u), coumaric acid (-146 u), and methyl (-15 u).[18] Recently, LC coupled with tandem mass spectrometry (LC-MS/MS) has been used to strengthen the qualitative analysis of tricin derivatives by providing further structure characterization information.[92]

Nuclear magnetic resonance (NMR) spectroscopic analysis

NMR spectroscopy is another powerful tool employed to investigate the structure of tricin and its derivatives. The signal assignments in ^{1}H-NMR and ^{13}C NMR spectra of tricin are summarized in **Table 11** and **12**, respectively. A characteristic peak around δ13.0 was assigned to hydroxyl proton (5-OH).[86, 96] The presence of glucose moiety at C7 leads to a downfield shift of 0.36 and 0.31 ppm for H6 and H8, respectively.[70]

^{13}C NMR analysis provides characteristic differences between free tricin and the aglycone of tricin derivatives (**Table 12**). For example, the tricin-5-*O*-glucoside had a 4.5 ppm shift on the carbonyl carbon (C4)[28, 85] as a result of loss of hydrogen bond between H5 and O4, which was not observed on C7 from tricin-7-*O*-glucoside.[88] Notable chemical shifts of C1', 3', and 5' (δ125.4, 153.1, and 153.1 ppm)[101] toward downfield were observed on 4'-*O*-guaiacylglyceryl tricin as compared to free tricin (δ120.7, 148.6, and 148.6 ppm).[72] A similar difference has also been detected on 4'-*O*-hydroxyphenylglyceryl tricin, 4'-*O*-methylguaiacylglyceryl tricin, and 4'-*O*-coumaroyl-guaiacylglyceryl tricin (**Table 12**).

Table 9 IR absorbance bands of tricin and its derivatives

Wavelength (cm^{-1})	Assignment/functional group
3300-4000	O-H[59, 64, 79, 83, 90, 120, 121, 129]
2926	C-H[99]
2917	-OCH_3[54]
1643-1687	Conjugated C=O[71, 90, 130, 132]
1605-1615	C=C[18, 89, 94, 104]
1430-1598	Aromatic C=C[18, 54, 64, 70, 72, 89, 94, 132, 133]
1368	Aromatic C=C[99]
1247	Aromatic C=C[134]
1152, 1128	C-O[99, 133]
1055-1060	C-O[71, 96, 133]

Table 10 The common moieties and their characteristic product ions of tricin and its derivatives in LC-MS

Assignment	Characteristic fragments	Ref
Tricin	$[M + H]^+$ at *m/z* 331	46, 67, 71, 90, 120
	$[M – H]^-$ at *m/z* 329	18, 40, 55, 56, 83, 90, 125
	$[M]^+$ at *m/z* 330	41, 70, 72, 86-90
	$[M + Na]^+$ at *m/z* 353	65, 79
Tricin-glucoside	$[M + H]^+$ at *m/z* 493	71, 72, 85, 110, 114, 119, 124
	$[M – H]^-$ at *m/z* 491	79, 85, 119, 121, 123
	$[M – H]^-$ at *m/z* 507*	80
Tricin-glucuronide	$[M + H]^+$ at *m/z* 507	110
	$[M – H]^-$ at *m/z* 505	48
Tricin-diglycoside	$[M + H]^+$ at *m/z* 639	111, 124
	$[M – H]^-$ at *m/z* 637	126
Hydroxyphenylglyceryl tricin	$[M – H]^-$ at *m/z* 495	94
Guaiacylglyceryl tricin	$[M – H]^-$ at *m/z* 525	83, 93, 130-132
	$[M + H]^+$ at *m/z* 527	82, 176
	$[M – H]^-$ at *m/z* 553	129
	$[M + H]^+$ at *m/z* 569, 583, 695	64
	$[M]^+$ at *m/z* 540	93, 130
	$[M]^+$ at *m/z* 638	133
Tricin-lignan-glucoside	$[M + H]^+$ at *m/z* 689	134, 176
	$[M – H]^-$ at *m/z* 687	121
Methyl	$[M – H – CH_3]^-$: –15 u	18
Methoxy	$[M + H – methoxy]^+$: –30 u	124
Glucosyl	$[M + H – hexose]^+$: –162 u	72, 111, 118, 120, 124
Rhamnosyl	$[M + H – rhamnose]^+$: –146 u	70, 104, 124
Glucuronyl	$[M – H – Glucuronic\ acid]^-$: –176 u	48, 106
Feruloyl	$[M – H – ferulic\ acid]^-$: –176 u	106
Sinapoyl	$[M – H – sinapic\ acid]^-$: –206 u	106
Coumaroyl	$[M – H – coumaric\ acid]^-$: –146 u	106

Note: * an extra –OH was present in the 3C of tricin.

Table 11 ^{1}H NMR chemical shift assignment and coupling constant data for tricin

Solvent	^{1}H-NMR (δ)	Assignment	Ref
DMSO-d_6	6.93-6.98 (s, 1)	H3	56, 67, 96, 128
	6.19-6.21 (d, 1, J=2 Hz)	H6	
	6.54-6.56 (d, 1, J=2 Hz)	H8	
	7.30-7.33 (s, 2)	H2', H6'	
	3.87-3.90 (s, 6)	MeO3', MeO5'	
	12.96 (s, 1)	5-OH	
Acetone-d_6	6.729 (s, 1)	H3	86
	6.253 (d, 1, J=2 Hz)	H6	
	6.551 (d, 1, J=2 Hz)	H8	
	7.382 (s, 2)	H2', H6'	
	3.972 (s, 6)	MeO3', MeO5'	
	13.006 (s, 1)	5-OH	

The presence of a carbohydrate moiety can be deduced from the anomeric signal in the ^{1}H-NMR (δ5.27 ppm) and ^{13}C NMR spectra (δ98.2 ppm) together with heteronuclear multiple bond correlation (HMBC) analysis.[18, 58] The 4'-*O*-β linkage between C4' of tricin and C-β of phenylpropanoid was identified by HMBC and ^{1}H-^{1}H rotating frame Overhauser effect spectroscopy (ROESY).[30] In addition, the ether linkage between tricin flavone backbone and phenylpropanoid was also confirmed by HMBC and nuclear Overhauser effect spectroscopy (NOESY).[94, 129] Moreover, a new tricin-lignan was reported in stoneshrub by using HMBC and ^{1}H-^{1}H correlated spectroscopic (COSY) spectra suggesting that tricin was etherified to the α-position of the phenylpropanoid unit.[39]

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Table 12. Chemical shift of ^{13}C NMR signals of tricin and its derivatives

	Tricin		Tricin-glycosides					Tricin-lignans						Tricin-lignan-glycosides			
	T	T	T7	T5	T4'	T7Glur	T7neo	T4'G			T4'H	T4'mG	T4'CG	a	b	c	d
Solvent	D	A	D	D	D	D	D	M	D	P	M	M	M	D	M	D	P
C2	164.1	165.1	164.1	161.7	163.2	164.2	166.5	165.2	164.4	164.2	165	162.9	165	164.1	165.2	165	165
C3	103.9	104.7	105.9	107	104.8	103.9	105.7	105.9	104	105.9	105.7	105.5	105.4	103.8	105.9	101.5	107
C4	182.7	183.1	182	177.8	182	182.1	184	183.8	182	182.7	183.6	183.4	183.7	182	183.8	182.8	183.9
C5	161.8	163.4	160.8	159.3	161.1	161.2	163	163.3	161.5	163.6	163.2	162.9	163.2	161.2	163.3	161.8	164.3
C6	99.1	99.7	99.5	105.2	98.1	99.3	102	100.7	99.1	100.3	100.6	100.1	100.4	99.2	100.7	100.2	101.3
C7	163.6	164.9	163	161.7	165.3	162.6	164.7	167.4	163.2	166.5	167.8	165.9	166.5	162.2	167.4	163.3	167.3
C8	94.4	94.9	95.4	99.3	92.9	95.1	96.4	95.5	94.4	95.2	95.5	95.1	95.2	95.2	95.5	95.3	96.3
C9	157.8	158.8	156.9	159.1	157.4	156.9	158.9	159.6	157.5	158.9	159.5	159.1	159.4	156.8	159.6	157.6	159.7
C10	103.9	105.3	105.4	108.5	104.8	105.5	104.9	105.2	104.9	105.3	105	105.3	105.7	105.5	105.2	105.2	106.3
C1'	120.7	122.4	120.2	121.1	125.6	120.2	122.5	128	125.4	127.2	127.9	127.3	126.7	120.3	128	120.9	127.9
C2'	104.6	105.3	104.5	104.7	105	104.6	104.9	105.2	104.4	105.2	105	104.7	105.0	104.5	105.2	104.7	105.9
C3'	148.6	149.1	148.2	148.9	152.9	148.2	149.7	154.9	153.1	154.3	154.8	154.5	154.8	148.2	154.9	149	155.1
C4'	140.2	141	139.9	140	137.7	140.1	141	140.7	140	140.9	140.4	141.4	141.4	140	140.7	140.9	141.3
C5'	148.6	149.1	148.2	148.9	152.9	148.2	149.7	154.9	153.1	154.3	154.8	154.5	154.8	148.2	154.9	149	155.1
C6'	104.6	105.3	104.5	104.7	105	104.6	105.7	105.2	104.4	105.2	105	104.7	105.0	104.5	105.2	104.7	105.9
2*OCH3	56.4	57	56.4	57	56.8	56.4	57.2	57	56.6	56.6	56.9	57.1	56.9	56.4	57	57.1	57.2
Ref	72	94	88	28	58, 115	106	70	30	101	131	94	130	64	106	30	18	121

T: tricin; T7: tricin-7-*O*-glycoside; T5: tricin-5-*O*-glycoside; T7Glur: Tricin-7-*O*-glucuronide; T7neo: tricin-7-*O*-neohesperidoside; T4': tricin-4'-*O*-glycoside; T4'G: 4'-*O*-guaiacylglyceryl tricin; T4'H: 4'-*O*-hydroxyphenylglyceryl tricin; T4'mG: 4'-*O*-methylguaiacylglyceryl tricin; T4'CG: 4'-*O*-coumaroyl-guaiacylglyceryl tricin; a:tricin-7-*O*-[2'-*O*-sinapoyl-glucuro(1-2)-*O*-glucuronide]; b: tricin-7-*O*-(4'-guaiacylglyceryl)-glucoside; c: tricin 7-*O*-(6''-methoxycinnamic)-glucoside; d: tricin-4'-*O*-(α-glucono)-guaiacylglyceryl ether.
Solvent: D: DMSO-d_6; A: acetone-d_6; M: CD_3OD; P: pyridine-d_5

Table 13 ^{13}C–^{1}H HSQC correlation of chemical shift of tricin moiety in lignin

Lignin source	Chemical shift of assigned position (δC/δH)				Ref
	T2', T6'	T3	T8	T6	
Arundo grass	103.9/7.3	104.7/7.03	94.4/6.64	98.9/6.28	19
Bamboo stem	103.9/7.34	106.2/7.07	94.2/6.6	98.9/6.23	25
Barley	103.9/7.3	104.5/7.03	94.1/6.56	98.7/6.22	27
Coconut coir fibers	N/D	N/D	94.1/6.56	98.8/6.2	136
Corn stover	104.3/7.31	104.9/7.05	94.4/6.57	99/6.21	10
Maize plant	103.9/7.30	104.5/7.03	94/6.56	98.7/6.22	23
Rice straw	103.8/7.29	104.2/7.04	93.8/6.58	98.8/6.26	21
Sugarcane	103.9/7.30	104.5/7.03	94.0/6.56	98.7/6.22	26
Wheat straw	103.9/7.31	104.5/7.04	94.1/6.56	98.8/6.2	9
	104.04/7.3	104.65/7.03	94.1/6.56	98.8/6.22	138
	104/7.3	104/7.1	94/6.6	98/6.2	135
	104.04/7.3	104.65/7.03	94.1/6.56	98.8/6.22	137
	103.9/7.29	104.5/7.02	94.1/6.56	98.7/6.21	22
Wula sedge	N/D	104.1/7.31	94.5/6.56	98.8/6.23	20

N/D: not detected.

Recently, Del Río et al. have documented that tricin is covalently incorporated into lignin by using heteronuclear single-quantum correlation (HSQC) NMR analysis.[9] The HSQC correlation data of the tricin component in lignin are listed in **Table 13**. Two strong and well-resolved C/H signals at the 6 and 8 position of tricin were readily observed at δ98.8/6.20 and δ94.1/6.56 ppm in the aromatic region, respectively (**Fig. 2**). The two phenolic hydroxyl groups at C5 and C7 showed proton signals at δ12.86 and 10.88 ppm which were correlated with C5 and C7 by HMBC. The structure of tricin etherified with lignin through guaiacyl (G) unit has also been elucidated by HMBC analysis.[9] The etherified position on C4' was also confirmed by phosphorylation followed by ^{31}P NMR analysis with significantly reduced signal at 4'-OH compared to 5 and 7-OH positions.[22] Using HSQC and HMBC spectroscopic analysis, Lan et al. has observed the coupling of tricin and monolignols.[10] It should be noted that tricin was reported to link with syringyl (S) unit in rice straw[21] and corn stover[10] lignin, while other reports indicated a linkage with G-unit.

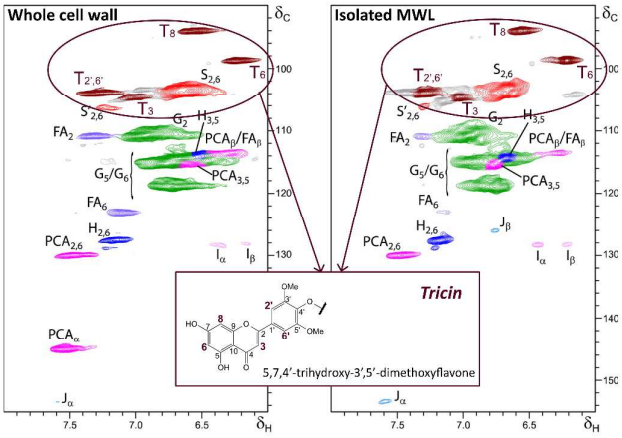

Fig. 2 2D HSQC NMR spectra of wheat straw cell wall (left) and of the isolated MWL (right). Reprinted (adapted) with permission from Del Río, J.C., et al.[9] Copyright (2012) American Chemical Society.

Biosynthesis of tricin

Although the major flavonoid biosynthetic pathway has been extensively studied, there is still a lack of information about enzymatic engineering of flavones.[151] The precursors for tricin biosynthesis have been reported to be *p*-coumaroyl-CoA derived from general phenylpropanoid pathway and malonyl-CoA derived from carbohydrate metabolism-polyketide pathway (**Fig. 3**).[177] The initial step of tricin biosynthesis is catalyzed by chalcone synthase (CHS) to yield tetrahydroxylchalcone.[178] Naringenin (a flavanone compound) is then rapidly formed by chalcone isomerase (CHI).[151] The subsequent hydroxylation in C3 by flavanone 3-hydroxylase (F3H) leads to the formation of dihydrokaempferol, followed by generation of dihydroquercetin or dihydromyricetin through adding hydroxyl group by flavanone 3'-hydroxylase (F3'H) and flavanone 3',5'-hydroxylase (F3'5'H).[151] Presumably, a double bond between C2 and C3 in dihydromyricetin is formed by the action of flavone synthase (FNS) and this reaction gives rise to tricetin ultimately.[14] Tricin is then produced following a sequential *O*-methylation of tricetin through flavone *O*-methyltransferase (FOMT).[14] However, Lam et al. have proposed a reconstructed biosynthesis pathway of tricin from naringenin in rice without formation of tricetin. The authors suggested that tricin is formed in the order of desaturation between C2 and C3, hydroxylation on C3', methylation on C3', hydroxylation on C5', and methylation on C5'.[179]

In addition to the biosynthesis of tricin from *p*-coumaroyl-CoA, a type of monolignols (i.e., lignin precursors) are biosynthesized through a series of side modifications, ring hydroxylations, and *O*-methylations (**Fig. 3**).[5, 180] The *p*-coumaryl, coniferyl, 5-hydroxyconiferyl, and sinapyl alcohol are formed from *p*-coumaroyl-CoA either via subsequent enzyme catalysis of *p*-coumaraldehyde or via caffeoyl-CoA.[5] The aromatic lignin polymers were then generally believed to form in plants resulting from oxidative combinatorial coupling of these monolignols.[4] Recent studies, especially in transgenic and mutant plants, have indicated that lignin can be derived from several new monomers such as hydroxycinnamyl alcohols,[6-8, 181-183] hydroxycinnamyl aldehyde,[184] hydroxycinnamic acid,[185, 186] 4-hydroxycinnamyl acetates,[181] and others.[5] These newly reported lignin monomers as well as genetic perturbation of lignification indicate the flexibility of lignification in plants. Although the biosynthesis of tricin was not considered to involve in the process of lignification, the incorporation of tricin into lignin through β-*O*-4 linkage has been recently reported in biomimetic coupling reactions.[10] The results suggested that tricin could act as a nucleation or initiation site for lignification, through which other monolignols cross-coupled to form lignin polymer. The evidence of tricin covalently incorporating into lignin imply a potential association between biosynthetic pathway of tricin and lignin, which could help redefine the lignin related phenylpropanoid metabolism inducing from the regulation of lignin biosynthesis genes.[5]

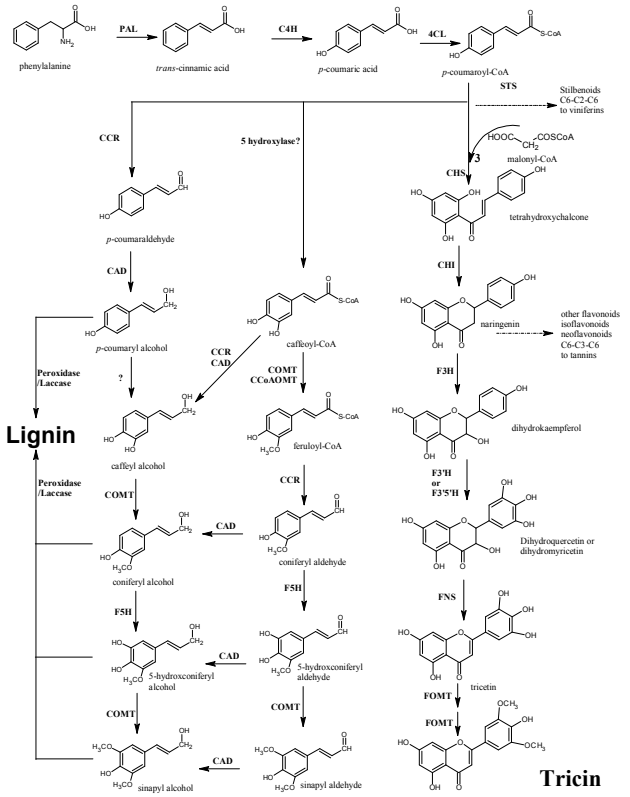

Fig. 3 Schematic overview of biosynthesis pathway for tricin and lignin. Adapted from previous literatures.[5, 13] PAL: Phe ammonia-lyase; C4H: cinnamate-4-hydroxylase; 4CL: *p*-coumaroyl:CoA-ligase; STS: stilbene synthase; CHS: chalcone synthase; CHI: chalcone isomerase; F3H: flavanone 3-hydroxylase; F3'H: flavonoid 3' hydroxylase; F3'5'H: flavonoid 3',5'-hydroxylase; CCR: cinnamoyl-CoA reductase; CAD: cinnamyl alcohol dehydrogenase; COMT: caffeic acid *O*-methyltransferase; CCoAOMT: caffeoyl-CoA *O*-methyltransferase; F5H: ferulate 5-hydroxylase; FOMT: flavone *O*-methyltransferase.

Conclusion and perspectives

Tricin, a flavonoid type compound from the secondary metabolic pathways in plants, possesses significant importance to plant growth by defending against disease, weeds, and microbes. It is widely distributed in herbaceous and cereal plants, and exists as free tricin and its derivatives such as tricin-glycosides, tricin-lignans, and tricin-lignan-glycosides. Tricin and its derivatives show potential pharmaceutical applications due to low toxicity, antioxidative activity, and cancer preventive activity. The structures of tricin and its derivatives conjugating with carbohydrates and phenylpropanoid were identified by various analytical methods. In particular, the incorporation of tricin into lignin was evidenced by NMR spectra. The presence of tricin in lignin in certain plants suggests a possible association between biosynthetic tricin and lignification and can provide a new insight into lignin biosynthesis.

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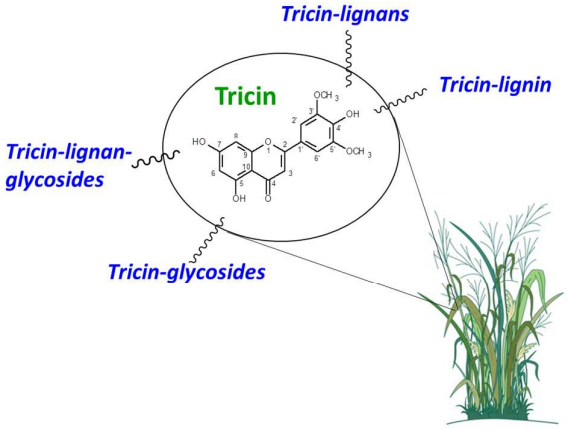
Notes and references

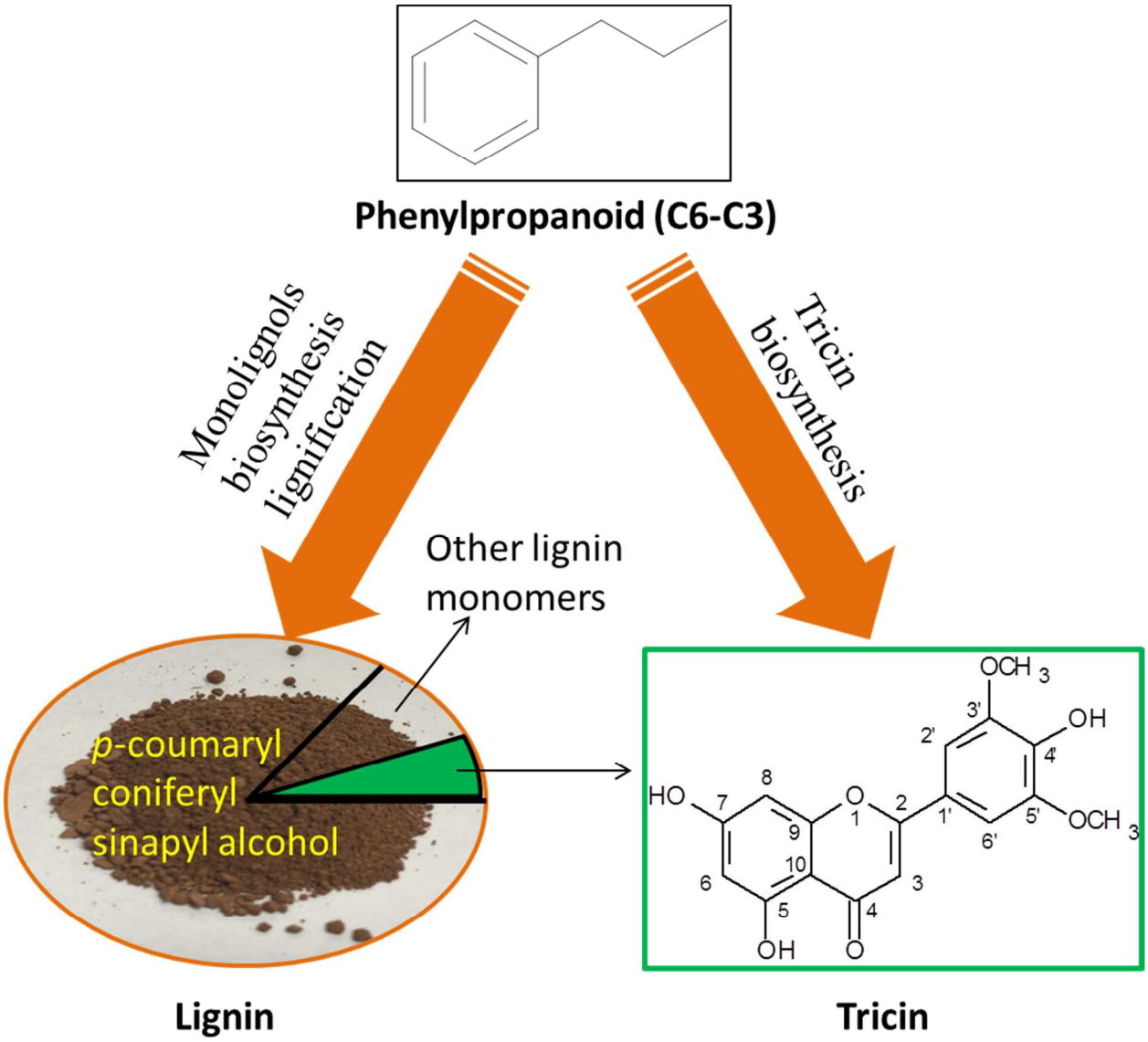
1. J. Zakzeski, P. C. A. Bruijnincx, A. L. Jongerius and B. M. Weckhuysen, *Chem. Rev.*, 2010, **110**, 3552-3599.
2. A. J. Ragauskas, G. T. Beckham, M. J. Biddy, R. Chandra, F. Chen, M. F. Davis, B. H. Davison, R. A. Dixon, P. Gilna and M. Keller, *Science*, 2014, **344**, 1246843.
3. R. Vanholme, B. Demedts, K. Morreel, J. Ralph and W. Boerjan, *Plant Physiol.*, 2010, **153**, 895-905.
4. J. Ralph, K. Lundquist, G. Brunow, F. Lu, H. Kim, P. F. Schatz, J. M. Marita, R. D. Hatfield, S. A. Ralph and J. H. Christensen, *Phytochem. Rev.*, 2004, **3**, 29-60.
5. W. Boerjan, J. Ralph and M. Baucher, *Annu. Rev. Plant Biol.*, 2003, **54**, 519-546.
6. F. Chen, Y. Tobimatsu, L. Jackson, J. Nakashima, J. Ralph and R. A. Dixon, *The Plant Journal*, 2013, **73**, 201-211.
7. F. Chen, Y. Tobimatsu, D. Havkin-Frenkel, R. A. Dixon and J. Ralph, *Proc. Natl. Acad. Sci.*, 2012, **109**, 1772-1777.
8. A. Wagner, Y. Tobimatsu, L. Phillips, H. Flint, K. Torr, L. Donaldson, L. Pears and J. Ralph, *The Plant Journal*, 2011, **67**, 119-129.
9. J. C. Del Río, J. Rencoret, P. Prinsen, A. n. T. Martínez, J. Ralph and A. Gutiérrez, *J. Agric. Food. Chem.*, 2012, **60**, 5922-5935.
10. W. Lan, F. Lu, M. Regner, Y. Zhu, J. Rencoret, S. A. Ralph, U. I. Zakai, K. Morreel, W. Boerjan and J. Ralph, *Plant Physiol.*, 2015, **167**, 1284-1295.
11. J. B. Harborne and H. Baxter, *The Handbook of Natural Flavonoids.* , John Wiley & Sons, 1999.
12. S. Martens and A. Mithöfer, *Phytochemistry*, 2005, **66**, 2399-2407.
13. B. Winkel-Shirley, *Plant Physiol.*, 2001, **126**, 485-493.
14. J.-M. Zhou and R. K. Ibrahim, *Phytochem. Rev.*, 2010, **9**, 413-424.
15. E. Wollenweber and M. Doerr, *Nat. Prod. Commun.*, 2008, **3**, 1293-1298.
16. C. A. Williams, J. B. Harborne and H. T. Clifford, *Phytochemistry*, 1973, **12**, 2417-2430.
17. J. Ø. Moskaug, H. Carlsen, M. C. W. Myhrstad and R. Blomhoff, *Am. J. Clin. Nutr.*, 2005, **81**, 277S-283S.
18. J. M. Duarte-Almeida, G. Negri, A. Salatino, J. E. de Carvalho and F. M. Lajolo, *Phytochemistry*, 2007, **68**, 1165-1171.
19. T.-T. You, J.-Z. Mao, T.-Q. Yuan, J.-L. Wen and F. Xu, *J. Agric. Food. Chem.*, 2013, **61**, 5361-5370.
20. J.-Z. Mao, X. Zhang, M.-F. Li and F. Xu, *BioResources*, 2013, **8**, 3869-3883.
21. M. Wu, J. Pang, F. Lu, X. Zhang, L. Che, F. Xu and R. Sun, *Ind. Crop Prod.*, 2013, **50**, 887-895.
22. H. Heikkinen, T. Elder, H. Maaheimo, S. Rovio, J. Rahikainen, K. Kruus and T. Tamminen, *J. Agric. Food. Chem.*, 2014, **62**, 10437-10444.
23. S. Fornalé, J. Rencoret, L. Garcia-Calvo, M. Capellades, A. Encina, R. Santiago, J. Rigau, A. Gutiérrez, J.-C. del Río and D. Caparros-Ruiz, *Plant Sci.*, 2015, **236**, 272-282.
24. C. G. Yoo, H. Kim, F. Lu, A. Azarpira, X. Pan, K. K. Oh, J. S. Kim, J. Ralph and T. H. Kim, *BioEnergy Research*, 2015, 1-10.
25. J.-L. Wen, S.-L. Sun, B.-L. Xue and R.-C. Sun, *Holzforschung*, 2013, **67**, 613-627.
26. C. José, A. G. Lino, J. L. Colodette, C. F. Lima, A. Gutiérrez, Á. T. Martínez, F. Lu, J. Ralph and J. Rencoret, *Biomass Bioenergy*, 2015, **81**, 322-338.
27. J. Rencoret, P. Prinsen, A. Gutierrez, A. T. Martinez and J. C. Del Rio, *J. Agric. Food. Chem.*, 2015.
28. L. Van Hoyweghen, I. Karalic, S. Van Calenbergh, D. Deforce and A. Heyerick, *J. Nat. Prod.*, 2010, **73**, 1573-1577.
29. S. Mohanlal, S. K. Maney, T. R. Santhoshkumar and A. Jayalekshmy, *J. Nat. Med.*, 2013, **67**, 528-533.
30. M. Bouaziz, N. C. Veitch, R. J. Grayer, M. S. J. Simmonds and M. Damak, *Phytochemistry*, 2002, **60**, 515-520.
31. J. A. Anderson and A. G. Perkin, *J. Chem. Soc. (Resumed)*, 1931, 2624-2625.
32. J. B. Harborne and C. A. Williams, *Biochem. Syst. Ecol.*, 1976, **4**, 267-280.
33. N. A. M. Saleh, C. Nozzolillo and I. Altosaar, *Biochem. Syst. Ecol.*, 1988, **16**, 597-599.
34. J. B. Harborne, C. A. Williams and K. L. Wilson, *Phytochemistry*, 1985, **24**, 751-766.
35. I. El-Habashy, R. M. A. Mansour, M. A. Zahran, M. N. El-Hadidi and N. A. M. Saleh, *Biochem. Syst. Ecol.*, 1989, **17**, 191-195.
36. J. B. Harborne and E. Hall, *Phytochemistry*, 1964, **3**, 421-428.
37. W. S. Ferguson, d. B. Ashworth and R. A. Terry, *Nature*, 1949, **163**, 606-606.
38. H. Kuwabara, K. Mouri, H. Otsuka, R. Kasai and K. Yamasaki, *J. Nat. Prod.*, 2003, **66**, 1273-1275.
39. J. Yan, L. Sun, X. Zhang and M. Qiu, *Heterocycles*, 2005, **65**, 661-666.
40. Z. Zhang, B. Cui and Y. Zhang, *Entomol. Exp. Appl.*, 2015.
41. I. Cummins, M. Brazier-Hicks, M. Stobiecki, R. Frańska and R. Edwards, *Phytochemistry*, 2006, **67**, 1722-1730.
42. J. B. Harborne, *Comparative biochemistry of the flavonoids*, Academic Press, London, New York, 1967.
43. S. Mohanlal, R. Parvathy, V. Shalini, A. Helen and A. Jayalekshmy, *Plant Food Hum. Nutr.*, 2011, **66**, 91-96.
44. Y. Ogo, K. Ozawa, T. Ishimaru, T. Murayama and F. Takaiwa, *Plant Biotechnol. J.*, 2013, **11**, 734-746.
45. A. Moheb, PhD, Concordia University, 2012.
46. A. Moheb, M. Grondin, R. K. Ibrahim, R. Roy and F. Sarhan, *Food Chem.*, 2013, **138**, 931-937.
47. M. Kanemoto, K. Matsunami, H. Otsuka, T. Shinzato, C. Ishigaki and Y. Takeda, *Phytochemistry*, 2008, **69**, 2517-2522.
48. T. Iwashina and G. Kokubugata, *Bul.l Natl. Mus. Nat. Sci. Ser. B*, 2010, **36**, 117-125.
49. A. Benavides, C. Bassarello, P. Montoro, W. Vilegas, S. Piacente and C. Pizza, *Phytochemistry*, 2007, **68**, 1277-1284.

50. A. Wilson, *Phytochemistry*, 1985, **24**, 1685-1691.
51. T. W. Goodwin, *Chemistry and biochemistry of plant pigments*, London, New York, Academic Press, 1965.
52. D. H. Miles, K. Tunsuwan, V. Chittawong, U. Kokpol, M. I. Choudhary and J. Clardy, *Phytochemistry*, 1993, **34**, 1277-1279.
53. J. Cui, Y. Yue, F. Tang and J. Wang, *JPC J. Planar Chromatogr. - Mod. TLC*, 2011, **24**, 394-399.
54. J. Sun, Y.-D. Yue, F. Tang, X.-F. Guo, J. Wang and X. Yao, *Chem. Nat. Compd.*, 2013, **49**, 822-825.
55. X. F. Guo, Y. D. Yue, F. Tang, J. Wang, X. Yao and J. Sun, *J. Food Biochem.*, 2013, **37**, 748-757.
56. J. Jiao, Y. Zhang, C. Liu, J. e. Liu, X. Wu and Y. Zhang, *J. Agric. Food. Chem.*, 2007, **55**, 10086-10092.
57. B. Lu, X. Wu, X. Tie, Y. Zhang and Y. Zhang, *Food Chem. Toxicol.*, 2005, **43**, 783-792.
58. H.-B. Wang, H. Yao, G.-H. Bao, H.-P. Zhang and G.-W. Qin, *Phytochemistry*, 2004, **65**, 969-974.
59. A. Sakai, K. Watanabe, M. Koketsu, K. Akuzawa, R. Yamada, Z. Li, H. Sadanari, K. Matsubara and T. Murayama, *Antiviral Chem. Chemother.*, 2008, **19**, 125-132.
60. K. Akuzawa, R. Yamada, Z. Li, Y. Li, H. Sadanari, K. Matsubara, K. Watanabe, M. Koketsu, Y. Tuchida and T. Murayama, *Antiviral Res.*, 2011, **91**, 296-303.
61. T. Murayama, Y. Li, T. Takahashi, R. Yamada, K. Matsubara, Y. Tuchida, Z. Li and H. Sadanari, *Microb. Infect.*, 2012, **14**, 1086-1092.
62. Y. H. Jeong, S. Y. Chung, A. R. Han, M. K. Sung, D. S. Jang, J. Lee, Y. Kwon, H. J. Lee and E. K. Seo, *Chem. Biodivers.*, 2007, **4**, 12-16.
63. T. Matsuta, H. Sakagami, K. Satoh, T. Kanamoto, S. Terakubo, H. Nakashima, M. Kitajima, H. Oizumi and T. Oizumi, *In Vivo*, 2011, **25**, 757-762.
64. Y. Nakajima, Y. S. Yun and A. Kunugi, *Tetrahedron*, 2003, **59**, 8011-8015.
65. H. Nakano, N. Kawada, M. Yoshida, H. Ono, R. Iwaura and T. Tonooka, *J. Agric. Food. Chem.*, 2011, **59**, 9581-9587.
66. T. X. Meng, N. Irino and R. Kondo, *J. Nat. Med.*, 2015, 1-5.
67. M. Watanabe, *J. Agric. Food. Chem.*, 1999, **47**, 4500-4505.
68. B.-L. Guo, W.-K. Li, J.-G. Yu and P.-G. Xiao, *Phytochemistry*, 1996, **41**, 991-992.
69. H. R. Liang, P. Vuorela, H. Vuorela and R. Hiltunen, *Planta Med.*, 1997, **63**, 316-319.
70. Y. Ju, J. N. Sacalis and C. C. Still, *J. Agric. Food. Chem.*, 1998, **46**, 3785-3788.
71. Y. S. Kwon, E. Y. Kim, W. J. Kim, W. K. Kim and C. M. Kim, *Arch. Pharmacal Res.*, 2002, **25**, 300-305.
72. H. Li, C. Zhou, Y. Pan, X. Gao, X. Wu, H. Bai, L. Zhou, Z. Chen, S. Zhang and S. Shi, *Planta Med.*, 2005, **71**, 1128-1133.
73. V. Moscatelli, O. Hnatyszyn, C. Acevedo, J. Megías, M. J. Alcaraz and G. Ferraro, *Planta Med.*, 2006, **72**, 72-74.
74. M. Shang, S. Cai, J. Han, J. Li, Y. Zhao, J. Zheng, T. Namba, S. Kadota, Y. Tezuka and W. Fan, *China Journal of Chinese Materia Medica*, 1998, **23**, 614-616.
75. E. A. Hudson, P. A. Dinh, T. Kokubun, M. S. J. Simmonds and A. Gescher, *Cancer Epidemiol. Biomarkers Prev.*, 2000, **9**, 1163-1170.
76. A. S. Awaad, N. H. Mohamed, D. J. Maitland and G. A. Soliman, *Rec Nat Prod*, 2008, **2**, 76-82.
77. Z. Guo, Q. Guo, S. Zhang, C. Li, X. Chai and P. Tu, *Biochem. Syst. Ecol.*, 2014, **55**, 329-332.
78. M. M. Hegab, H. Abdelgawad, M. S. Abdelhamed, O. Hammouda, R. P. V. Kumar and G. Zinta, *Allelopathy J.*, 2013, **31**, 345-354.
79. W.-K. Zhang, X. U. Jie-Kun, L. Zhang and D. U. Guan-Hua, *Chin. J. Nat. Med.*, 2012, **10**, 110-114.
80. O. A. Eldahshan, N. A. Ayoub, A.-N. B. Singab and M. M. Al-Azizi, *Afr. J. Pharm. Pharmacol.*, 2009, **3**, 158-164.
81. V. S. Nambiar, N. Sareen, M. Daniel and E. B. Gallego, *Functional Foods in Health and Disease*, 2012, **2**, 251-264.
82. H. Huang, Y. Liu, Q. Meng, S. Wei, H. Cui and C. Zhang, *Biochem. Syst. Ecol.*, 2010, **38**, 656-658.
83. Y.-J. Jung, J.-H. Park, J.-G. Cho, K.-H. Seo, D.-S. Lee, Y.-C. Kim, H.-C. Kang, M.-C. Song and N.-I. Baek, *Arch. Pharmacal Res.*, 2014, **38**, 178-185.
84. X. Liu, A. M. Yang and Y. P. Shi, *J. Anal. Chem.*, 2008, **63**, 472-476.
85. F. Adjei-Afriyie, C.-S. Kim, M. Takemura, M. Ishikawa and M. Horiike, *Biosci., Biotechnol., Biochem.*, 2000, **64**, 443-446.
86. L. Bing, D. Hongxia, Z. Maoxin, X. Di and W. Jingshu, *Acta Ecologica Sinica*, 2007, **27**, 1300-1306.
87. C. Kong, X. Xu, B. Zhou, F. Hu, C. Zhang and M. Zhang, *Phytochemistry*, 2004, **65**, 1123-1128.
88. D. Xu, B. Li, Y. Liu, Z. Huang and L. Gu, *Sci. Sinica Vitae*, 1996, **39**, 8-16.
89. C. Kong, W. Liang, X. Xu, F. Hu, P. Wang and Y. Jiang, *J. Agric. Food. Chem.*, 2004, **52**, 2861-2865.
90. I.-M. Chung, S.-J. Hahn and A. Ahmad, *J. Chem. Ecol.*, 2005, **31**, 1339-1352.
91. R. R. Devi, A. Jayalekshmy and C. Arumughan, *Food Chem.*, 2007, **104**, 658-664.
92. X. Dong, W. Chen, W. Wang, H. Zhang, X. Liu and J. Luo, *J. Integr. Plant Biol.*, 2014, **56**, 876-886.
93. S.-S. Lee, Y.-S. Baek, C.-S. Eun, M.-H. Yu, N.-I. Baek, D.-k. Chung, M.-H. Bang and S.-A. Yang, *Biosci., Biotechnol., Biochem.*, 2015, **79**, 700-706.
94. C.-L. Chang, G.-J. Wang, L.-J. Zhang, W.-J. Tsai, R.-Y. Chen, Y.-C. Wu and Y.-H. Kuo, *Phytochemistry*, 2010, **71**, 271-279.
95. O. G. El-Dien, E. Shawky, A. H. Aly, R. M. Abdallah and N. A. Abdel-Salam, *Am. J. Anal. Chem.*, 2013, **2013**.
96. Y. S. Kwon and C. M. Kim, *Arch. Pharmacal Res.*, 2003, **26**, 535-539.
97. H. Aimin and W. Mingshi, *Chinese Traditional and Herbal Drugs*, 1997, **9**, 001.
98. J. M. Duarte-Almeida, A. Salatino, M. I. Genovese and F. M. Lajolo, *Food Chem.*, 2011, **125**, 660-664.
99. P. Karambelkar, V. M. Jadhav and V. Kadam, *Indo American Journal of Pharmaceutical Research*, 2014, **4**, 2620-2627.
100. Y.-S. Jin, J.-L. Du, Y. Yang, L. Jin, Y. Song, W. Zhang and H.-S. Chen, *Chem. Nat. Compd.*, 2011, **47**, 257-260.
101. A. I. Syrchina, A. G. Gorshkov, V. V. Shcherbakov, S. V. Zinchenko, A. L. Vereshchagin, K. L. Zaikov and A. A. Semenov, *Chem. Nat. Compd.*, 1992, **28**, 155-158.
102. F. Ferreres, A. Gil-Izquierdo, P. B. Andrade, P. Valentão and F. A. Tomás-Barberán, *J. Chromatogr. A*, 2007, **1161**, 214-223.
103. R. Theodor, H. D. Zinsmeister, R. Mues and K. R. Markham, *Phytochemistry*, 1981, **20**, 1851-1852.

104. M. Parveen, Z. Khanam, A. Ali and S. M. Ahmad, *Chin. Chem. Lett.*, 2010, **21**, 593-595.
105. G. Popovici, G. Weissenböck, M.-L. Bouillant, G. Dellamonica and J. Chopin, *Z. Pflanzenphysiologie*, 1977, **85**, 103-115.
106. A. Stochmal, A. M. Simonet, F. A. Macias and W. Oleszek, *J. Agric. Food. Chem.*, 2001, **49**, 5310-5314.
107. S. Goławska, I. Łukasik, I. Kapusta and B. Janda, *Pol J Environ Stud*, 2012, **21**, 1613-1619.
108. Ł. Cieśla, I. Kowalska, W. Oleszek and A. Stochmal, *Phytochem. Anal*, 2013, **24**, 47-52.
109. A. Stochmal and W. Oleszek, *J. Food Agric. Environ.*, 2007, **5**, 170.
110. P. D. Marin, R. J. Grayer, S. Grujic-Jovanovic, G. C. Kite and N. C. Veitch, *Phytochemistry*, 2004, **65**, 1247-1253.
111. R. Colombo, J. H. Yariwake, E. F. Queiroz, K. Ndjoko and K. Hostettmann, *Phytochem. Anal*, 2006, **17**, 337-343.
112. C. A. Williams, J. B. Harborne and P. Smith, *Phytochemistry*, 1974, **13**, 1141-1149.
113. R. Nørbæk, D. B. F. Aaboer, I. S. Bleeg, B. T. Christensen, T. Kondo and K. Brandt, *J. Agric. Food. Chem.*, 2003, **51**, 809-813.
114. H.-S. Park, J. H. Lim, H. J. Kim, H. J. Choi and I.-S. Lee, *Arch. Pharmacal Res.*, 2007, **30**, 161-166.
115. T. Hasegawa, A. Tanaka, A. Hosoda, F. Takano and T. Ohta, *Phytochemistry*, 2008, **69**, 1419-1424.
116. J. Chen, S. L. Li, P. Li, Y. Song, X. Y. Chai and D. Y. Ma, *J. Sep. Sci.*, 2005, **28**, 365-372.
117. Y.-P. Lin, T.-Y. Chen, H.-W. Tseng, M.-H. Lee and S.-T. Chen, *Phytochemistry*, 2009, **70**, 1173-1181.
118. B. Dai, Z. Hu, H. Li, C. Yan and L. Zhang, *J. Chromatogr. B*, 2015, **978**, 54-61.
119. F. Adjei-Afriyie, C.-S. Kim, M. Takemura, M. Ishikawa, S.-i. Tebayashi and M. Horiike, *Z. Naturforsch., C: Biosci.*, 2000, **55**, 1038-1044.
120. C. H. Kong, H. Zhao, X. H. Xu, P. Wang and Y. Gu, *J. Agric. Food. Chem.*, 2007, **55**, 6007-6012.
121. S.-S. Lee, N.-I. Baek, Y.-S. Baek, D.-K. Chung, M.-C. Song and M.-H. Bang, *Molecules*, 2015, **20**, 5616-5624.
122. R. Colombo, J. H. Yariwake, E. F. Queiroz, K. Ndjoko and K. Hostettmann, *J. Chromatogr. A*, 2005, **1082**, 51-59.
123. L. Zhu, Z. Zhao, S. Yu and S. Zhu, *Int. Sugar J.*, 2010, **112**, 24.
124. R. Colombo, J. H. Yariwake and M. McCullagh, *Journal of the Brazilian Chemical Society*, 2008, **19**, 483-490.
125. A. Bottcher, I. Cesarino, A. B. dos Santos, R. Vicentini, J. L. S. Mayer, R. Vanholme, K. Morreel, G. Goeminne, J. C. M. S. Moura and P. M. Nobile, *Plant Physiol.*, 2013, **163**, 1539-1557.
126. Y. Zhao, L. Zhu, S. Yu and Z. Zhao, *Sugar Ind.-Zuckerindustrie*, 2013, **138**, 525-531.
127. S. A. Begum, M. Sahai and A. B. Ray, in *Fortschritte der Chemie organischer Naturstoffe/Progress in the Chemistry of Organic Natural Products, Vol. 93*, Springer, 2010, pp. 1-70.
128. R. Cooper, H. E. Gottlieb and D. Lavie, *Isr. J. Chem.*, 1977, **16**, 12-15.
129. C.-L. Chang, L.-J. Zhang, R.-Y. Chen, C.-C. Wu, H.-C. Huang, M. C. Roy, J.-P. Huang, Y.-C. Wu and Y.-H. Kuo, *Biorg. Med. Chem.*, 2010, **18**, 518-525.
130. R.-H. Jeong, D.-Y. Lee, J.-G. Cho, S.-M. Lee, H.-C. Kang, W.-D. Seo, H.-W. Kang, J.-Y. Kim and N.-I. Baek, *J. Korean Soc. Appl. Bi.*, 2011, **54**, 865-870.
131. E. Wenzig, O. Kunert, D. Ferreira, M. Schmid, W. Schühly, R. Bauer and A. Hiermann, *J. Nat. Prod.*, 2005, **68**, 289-292.
132. G.-c. Gao, Z.-x. Lu, H.-x. Xu, X.-s. Zheng and Y.-j. Yang, *Chem. Nat. Compd.*, 2012, **48**, 128-129.
133. Z. Khanam, F. Adam, O. Singh and J. Ahmad, *BioResources*, 2011, **6**, 2932-2940.
134. R.-H. Jeong, D.-Y. Lee, J.-G. Cho, K.-H. Seo, J.-W. Lee, M.-H. Lee, W.-D. Seo, H.-C. Kang, G.-S. Kim and H.-J. Noh, *Chem. Nat. Compd.*, 2014, **49**, 1003-1005.
135. F. Zikeli, T. Ters, K. Fackler, E. Srebotnik and J. Li, *Ind. Crop Prod.*, 2014, **61**, 249-257.
136. J. Rencoret, J. Ralph, G. Marques, A. Gutiérrez, A. n. T. Martínez and J. C. del Río, *J. Agric. Food. Chem.*, 2013, **61**, 2434-2445.
137. J. Zeng, D. Singh, D. Gao and S. Chen, *Biotechnology for Biofuels*, 2014, **7**, 161.
138. J. Zeng, G. L. Helms, X. Gao and S. Chen, *J. Agric. Food. Chem.*, 2013, **61**, 10848-10857.
139. I. Molnár-Perl and Z. Füzfai, *J. Chromatogr. A*, 2005, **1073**, 201-227.
140. S. S. Ebada, R. A. Edrada, W. Lin and P. Proksch, *Nature Protocols*, 2008, **3**, 1820-1831.
141. M. Estiarte, J. Peñuelas, S. Cañigueral and I. Casals, *Cereal Chem.*, 1997, **74**, 495-496.
142. K. C. Gulati and K. Venkataraman, *J. Chem. Soc. (Resumed)*, 1933, 942-943.
143. W. Baker, *J. Chem. Soc. (Resumed)*, 1933, 1381-1389.
144. D. Nagarathnam and M. Cushman, *The Journal of Organic Chemistry*, 1991, **56**, 4884-4887.
145. M. Ninomiya, K. Tanaka, Y. Tsuchida, Y. Muto, M. Koketsu and K. Watanabe, *J. Med. Chem.*, 2011, **54**, 1529-1536.
146. K. Yazawa, M. Kurokawa, M. Obuchi, Y. Li, R. Yamada, H. Sadanari, K. Matsubara, K. Watanabe, M. Koketsu and Y. Tuchida, *Antiviral Chem. Chemother.*, 2011, **22**, 1-11.
147. N. Pandurangan, *Lett. Org. Chem.*, 2014, **11**, 225-229.
148. J. A. Anderson, *Can. J. Res.*, 1933, **9**, 80-83.
149. W. L. Chan, Y. C. Lin, W. H. Zhang, P. L. Tang and Y. S. Szeto, *Heterocycles*, 1996, **43**, 551-554.
150. C. H. Kong, X. H. Xu, M. Zhang and S. Z. Zhang, *Pest Manage. Sci.*, 2010, **66**, 1018-1024.
151. E. G. W. M. Schijlen, C. H. R. De Vos, A. J. van Tunen and A. G. Bovy, *Phytochemistry*, 2004, **65**, 2631-2648.
152. M. S. J. Simmonds, *Phytochemistry*, 2003, **64**, 21-30.
153. P. E. Laks and M. S. Pruner, *Phytochemistry*, 1989, **28**, 87-91.
154. A. Moheb, Z. Agharbaoui, F. Kanapathy, R. K. Ibrahim, R. Roy and F. Sarhan, *Plant Sci.*, 2013, **201**, 115-120.
155. R. D. Verschoyle, P. Greaves, H. Cai, A. Borkhardt, M. Broggini, M. D'Incalci, E. Riccio, R. Doppalapudi, I. M. Kapetanovic and W. P. Steward, *Cancer Chemo. Pharm.*, 2006, **57**, 1-6.
156. H. Cai, S. Sale, R. G. Britton, K. Brown, W. P. Steward and A. J. Gescher, *Cancer Chemo. Pharm.*, 2011, **67**, 255-263.
157. M. J. Ajitha, S. Mohanlal, C. H. Suresh and A. Jayalekshmy, *J. Agric. Food. Chem.*, 2012, **60**, 3693-3699.
158. V. Shalini, A. Jayalekshmi and A. Helen, *Mol. Immunol.*, 2015, **66**, 229-239.

159. T. Oyama, Y. Yasui, S. Sugie, M. Koketsu, K. Watanabe and T. Tanaka, *Cancer Prev. Res.*, 2009, **2**, 1031-1038.
160. M. Ninomiya, K. Nishida, K. Tanaka, K. Watanabe and M. Koketsu, *J. Nat. Med.*, 2013, **67**, 460-467.
161. H. Cai, M. Al-Fayez, R. G. Tunstall, S. Platton, P. Greaves, W. P. Steward and A. J. Gescher, *Mol. Cancer Ther.*, 2005, **4**, 1287-1292.
162. H. Cai, D. J. Boocock, W. P. Steward and A. J. Gescher, *Cancer Chemo. Pharm.*, 2007, **60**, 257-266.
163. Y. Mu, L. Li and S.-Q. Hu, *Spectrochim. Acta, Pt. A: Mol. Biomol. Spectrosc.*, 2013, **107**, 235-240.
164. V. Shalini, S. Bhaskar, K. S. Kumar, S. Mohanlal, A. Jayalekshmy and A. Helen, *Int. Immunopharmacol.*, 2012, **14**, 32-38.
165. H. Cai, W. P. Steward and A. J. Gescher, *Biomed. Chromatogr.*, 2005, **19**, 518-522.
166. M. Al-Fayez, H. Cai, R. Tunstall, W. P. Steward and A. J. Gescher, *Cancer Chemo. Pharm.*, 2006, **58**, 816-825.
167. H. Cai, S. Sale, R. Schmid, R. G. Britton, K. Brown, W. P. Steward and A. J. Gescher, *Cancer Prev. Res.*, 2009, **2**, 743-750.
168. H. Cai, E. A. Hudson, P. Mann, R. D. Verschoyle, P. Greaves, M. M. Manson, W. P. Steward and A. J. Gescher, *Br. J. Cancer*, 2004, **91**, 1364-1371.
169. K. Plochmann, G. Korte, E. Koutsilieri, E. Richling, P. Riederer, A. Rethwilm, P. Schreier and C. Scheller, *Arch. Biochem. Biophys.*, 2007, **460**, 1-9.
170. N. Seki, U. Toh, K. Kawaguchi, M. Ninomiya, M. Koketsu, K. Watanabe, M. Aoki, T. Fujii, A. Nakamura and Y. Akagi, *J. Cell. Biochem.*, 2012, **113**, 2346-2355.
171. W.-L. Jiang, Y. Xu, S.-P. Zhang, H.-B. Zhu and J. Hou, *Eur. J. Pharm. Sci.*, 2012, **45**, 50-57.
172. Y.-S. Jung, D. H. Kim, J. Y. Hwang, N. Y. Yun, Y.-H. Lee, S. B. Han, B. Y. Hwang, M. S. Lee, H.-S. Jeong and J. T. Hong, *Toxicol. Appl. Pharmacol.*, 2014, **277**, 67-76.
173. T. J. Mabry, K. R. Markham and M. B. Thomas, *The Systematic Identification of Flavonoids* Springer, Berlin, 1970.
174. M. Stobiecki, *Phytochemistry*, 2000, **54**, 237-256.
175. F. Cuyckens and M. Claeys, *J. Mass Spectrom.*, 2004, **39**, 1-15.
176. E. F. Queiroz, J. R. Ioset, K. Ndjoko, A. Guntern, C. M. Foggin and K. Hostettmann, *Phytochem. Anal*, 2005, **16**, 166-174.
177. D. Barton and O. Meth-Cohn, *Comprehensive natural products chemistry*, Newnes, 1999.
178. T. A. Holton and E. C. Cornish, *The Plant Cell*, 1995, **7**, 1071.
179. P. Y. Lam, H. Liu and C. Lo, *Plant Physiol.*, 2015, pp-00566.
180. C. M. Fraser and C. Chapple, *The Arabidopsis Book/American Society of Plant Biologists*, 2011, **9**.
181. J. Ralph, *J. Nat. Prod.*, 1996, **59**, 341-342.
182. J. Ralph, C. Lapierre, F. Lu, J. M. Marita, G. Pilate, J. Van Doorsselaere, W. Boerjan and L. Jouanin, *J. Agric. Food. Chem.*, 2001, **49**, 86-91.
183. L. Jouanin, T. Goujon, V. de Nadai M.-T. Martin, I. Mila, C. Vallet, B. Pollet, A. Yoshinaga, B. Chabbert and M. Petit-Conil, *Plant Physiol.*, 2000, **123**, 1363-1374.
184. J. Ralph, C. Lapierre, J. M. Marita, H. Kim, F. Lu, R. D. Hatfield, S. Ralph, C. Chapple, R. Franke and M. R. Hemm, *Phytochemistry*, 2001, **57**, 993-1003.
185. J. Ralph, R. D. Hatfield, S. Quideau, R. F. Helm, J. H. Grabber and H.-J. G. Jung, *J. Am. Chem. Soc.*, 1994, **116**, 9448-9456.
186. J. Ralph, J. H. Grabber and R. D. Hatfield, *Carbohydr. Res.*, 1995, **275**, 167-178.




Phenylpropanoid (C6-C3)
Monolignols biosynthesis lignification
Tricin biosynthesis
Other lignin monomers
p-coumaryl
coniferyl
sinapyl alcohol
Lignin
Tricin