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Phytochemistry of *Camptotheca* Decaisne

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Abstract: To date, chemical investigations of the genus *Camptotheca* have been primarily focused on *C. acuminata*. Total 78 compounds have been isolated from the species, including alkaloids (**1-28**), ellagic acids (**29-40**), flavonoids (**41-46**), sterols (**47-48**), terpenes (**49-55**), tannins (**56-71**), polyphenols and fatty acids (**65-72**), iridoid (**73**), lignan (**74**), polyols (**75** and **76**), amide (**77**), and saccharide (**78**). The contents of camptothecin (CPT, **1**), the major active alkaloid varies significantly with *Camptotheca* species and varieties, tissue and tree age and seasonal changes. Among all taxa of *Camptotheca, C. acuminata* var. *acuminata* has the lowest CPT contents (0.2249-0.3162% in young leaves, and 0.0392-0.0572% in older leaves). *C. lowreyana* "Hicksii" has the highest CPT contents in both young and old leaves, approximately 1.5-2 folds higher than those in *C. acuminata* var. *acuminata*. Young leaves and mature fruits have high CPT contents than other tissues in *Camptotheca*. In young tissues of *C. acuminata* var. *acuminata*, the lowest CPT levels were found in March and April (0.074% and 0.081%, respectively) and highest in June (0.265%).

Keywords: Alkaloids, *Camptotheca*, *Camptothecin* variations, *Camptothecins* (CPTs), Ellagic acids, Flavonoids, sterols, Terpenes.

CHEMICAL CONSTITUENTS

The report of chemical constituents of *Camptotheca* has been restricted to *C. acuminata*. To date, 78 compounds have been isolated from *C. acuminata*, including alkaloids (**1-28**), ellagic acids (**29-40**), flavonoids (**41-46**), sterols (**47-48**), terpenes (**49-55**), tannins (**56-71**), polyphenols and fatty acids (**65-72**), iridoid (**73**), lignan (**74**), polyols (**75** and **76**), amide (**77**), and saccharide (**78**) (Fig. **1** and Table **1**).

Alkaloids

Camptothecin (CPT, **1**), a pyrrolo[3,4-b]-quinoline alkaloid, was first identified from the stem wood of *C. acuminata* in 1966 by Dr. Monroe E. Wall and Dr. Mansukh C. Wani of Research Triangle Institute in systematic screening of natural products for anticancer drugs [1]. In 1969, Dr. Wani and Dr. Wall had also first isolated two minor and related components 10-hydroxycamptothecin (10-HCPT, **2**) and 10-methoxycamptothecin (**4**) by further fractionation of the stem wood of *C. acuminata*. Compounds **1** and **2** were also found to occur in other plant parts, including leave [2], stem bark [2], fruit [2, 3], and root [2, 4]. Since then *C. acuminata* is considered to be a rich plant source of the potent camptothecinoids. This class is characterized by a pentacyclic ring structure embodying pyrrolo[3,4-b]-quinoline moiety (rings A, B and C), conjugated pyridone moiety (ring D) and a chiral centre within a six membered α-hydroxy lactone (ring E) [5]. This planar pentacyclic ring structure is thought to be one of the most important factors in inhibition of DNA topoisomerase I. Oxygenation is also usually observed at C-10 and C-11 and often at C-18 and C-20. The oxygenated positions may be present either as hydroxyl and methoxy groups or occasionally ester or glucose (Table **1**). So far 10 quinoline camptothecinoids (**3**, **5-13**) have been reported by scientists in the United States, China, and Japan. 10-hydroxy-20-deoxycamptothecin (**3**) was isolated by Lin and coworkers in 1989 from the fruit of *C. acuminata* and possessed a strong cytotoxicity against P388 leukemia cell [6]. Two new camptothecinoids 10-methoxy-20-*O*-acetylcamptothecin (**5**) and 20-*β*-D-glucopyranosyl 18-hydroxycamptothecin (**13**), together 10-methoxycamptothecin (**4**), 18-hydroxycamptothecin (**8**), 20-*O*-acetylcamptothecin (**10**) and 20-deoxycamptothecin (**12**) were isolated from root bark and leaf of *C. acuminata* by Zhang *et al.* in 2004 [2]. Hsu *et al.* in 1977 [3], and Lin *et al.,* in 1978, 1979 and 1982 [7-9] isolated 11-hydroxycamptothecin (**6**), 11-methoxycamptothecin (**7**), respectively, together with 10-methoxycamptothecin (**4**) and 20-deoxycamptothecin (**12**) from the fruit of *C. acuminata*. 18-hydroxycamptothecin (**8**) was also found from the fruit of *C. acuminata* [10]. Two minor camptothecinoids 20-hexanoylcamptothecin (**9**), 20-hexanoyl-10-methoxycamptothecin (**11**) with one known compound **12** were identified from stem bark of *C. acuminata* in 1979 [11]. Compound 20-deoxycamptothecin (**12**) was reported as a minor camptothecinoid from the fruit of *C. acuminata* [12]. 20-formylbenz [6,7] indolizino[1,2-b]quinoline-11 (13H)-one (**14**), 22-hydroxyacuminatine (**15**), and 19-hydroxymappicine (**18**) are three minor quinoline alkaloids isolated from the root of *C. acuminata* [2] and the fruit of *C. acuminata* [13].

Five minor indole alkaloids were reported, including angustoline (**16**) from the stem and the fruit of *C. acuminata* [15, 16], 19-*O*-methylangustoline (**17**) from the fruit of *C. acuminata* [17], camptacumotine (**19**), camptacumanine (**20**), and nauclefícine (**21**) isolated from the fruit of

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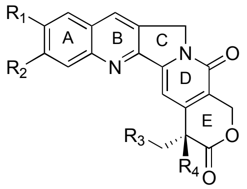
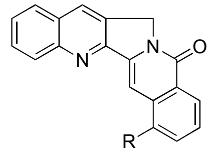


1 R_1 = H, R_2 = H, R_3 = CH_3, R_4 = OH
2 R_1 = OH, R_2 = H, R_3 = CH_3, R_4 = OH
3 R_1 = OH, R_2 = H, R_3 = CH_3, R_4 = H
4 R_1 = OCH_3, R_2 = H, R_3 = CH_3, R_4 = OH
5 R_1 = OCH_3, R_2 = H, R_3 = CH_3, R_4 = OAc
6 R_1 = H, R_2 = OH, R_3 = CH_3, R_4 = OH
7 R_1 = H, R_2 = OCH_3, R_3 = CH_3, R_4 = OH
8 R_1 = H, R_2 = H, R_3 = CH_2OH, R_4 = OH
9 R_1 = H, R_2 = H, R_3 = CH_3, R_4 = $OCO(CH_2)_4CH_3$
10 R_1 = H, R_2 = H, R_3 = CH_3, R_4 = OAc
11 R_1 = OCH_3, R_2 = H, R_3 = CH_3, R_4 = $OCO(CH_2)_4CH_3$
12 R_1 = H, R_2 = H, R_3 = CH_3, R_4 = H
13 R_1 = H, R_2 = H, R_3 = CH_2OH, R_4 =β-D-glucopyranosyl



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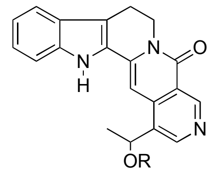
15 R=CH_2OH



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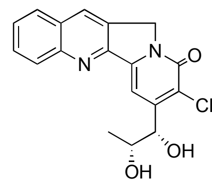
17 R=CH_3



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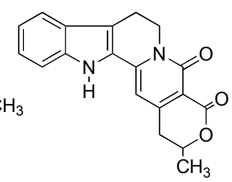

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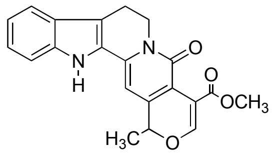

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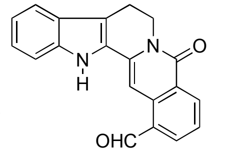

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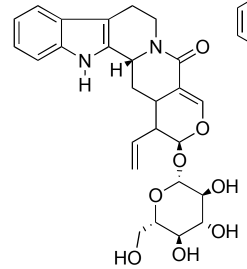

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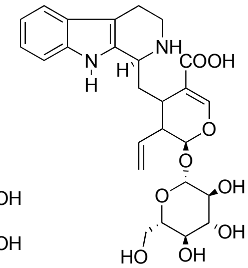

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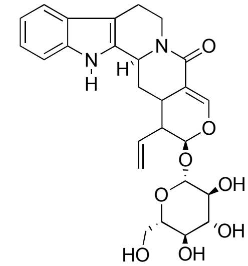

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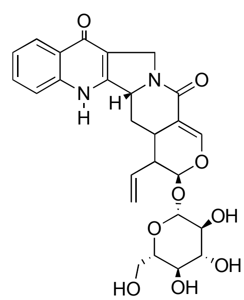

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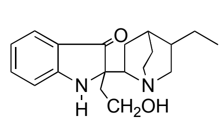

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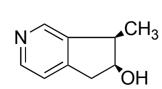

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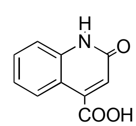

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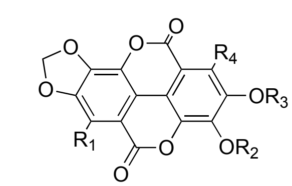
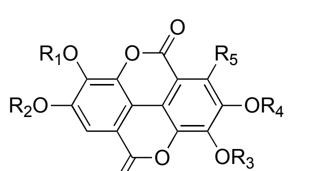
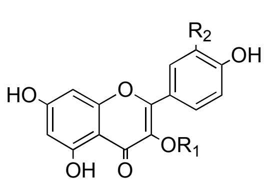


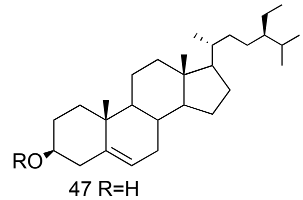
29 R_1=H, R_2=CH_3, R_3=H, R_4=OH
30 R_1=H, R_2=CH_3, R_3=β-D-glucopyranosyl, R_4=H
31 R_1=H, R_2=CH_3, R_3=CH_3, R_4=OCH_3
32 R_1=H, R_2=CH3, R_3=CH3, R_4=OH
33 R_1=OCH_3, R_2=CH_3, R_3=CH_3, R_4=OCH_3
34 R_1=H, R_2=H, R_3=H, R_4=H
35 R_1=H, R_2=CH_3, R_3=H, R_4=H
36 R_1=H, R_2=CH_3, R_3=CH_3, R_4=H



37 R_1=CH_3, R_2=CH_3, R_3=CH_3, R_4=H, R_5=H
38 R_1=CH_3, R_2=CH_3, R_3=CH_3, R_4=β-D-glucopyranosyl, R_5=H
39 R_1=CH_3, R_2=H, R_3=H, R_4=CH_3, R_5=H
40 R_1=CH_3, R_2=CH_3, R_3=CH_3, R_4=CH_3, R_5=OCH_3

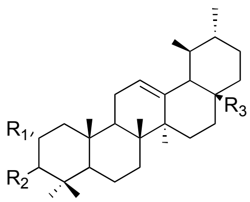


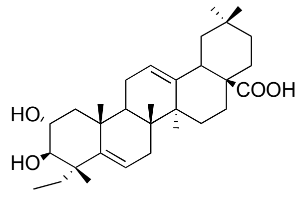
41 R_1=H, R_2=OH
42 R_1=β-D-galactopyranosyl, R_2=OH
43 R_1=β-D-glucopyranosyl, R_2=OH
44 R_1=β-D-galactopyranosyl, R_2=H
45 R_1=β-D-glucopyranosyl, R_2=H
46 R_1=H, R_2=H



47 R=H
48 R=β-D-glucopyranosyl

Fig. (1) contd….





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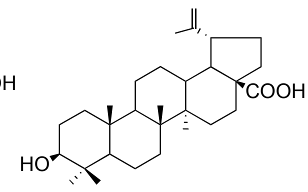

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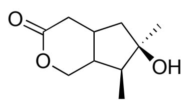
49 R_1=H, R_2=β-OH, R_3=$COOCH_3$
50 R_1=H, R_2=β-OH, R_3=CH_3
51 R_1=OH, R_2=α-OH, R_3=COOH



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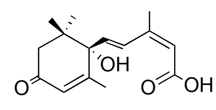

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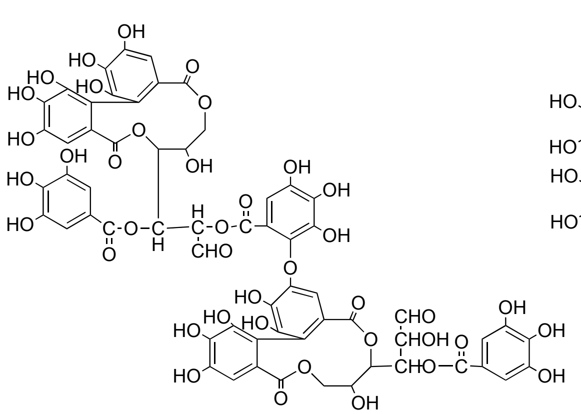

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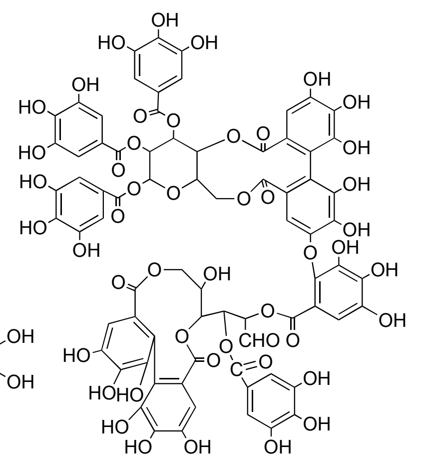

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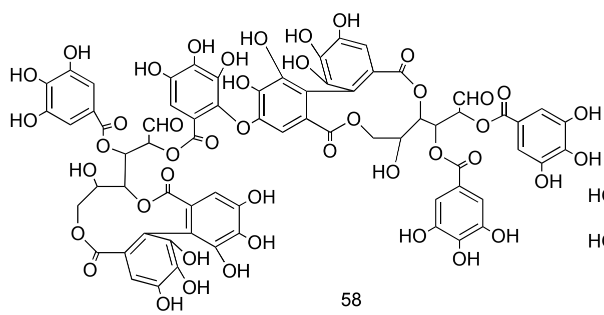

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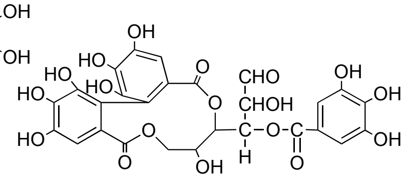

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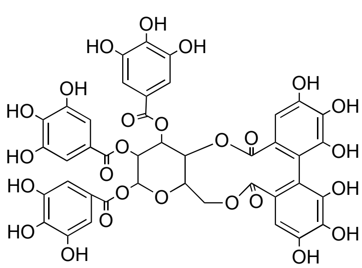

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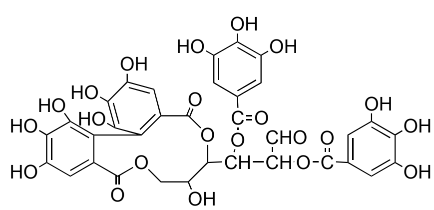

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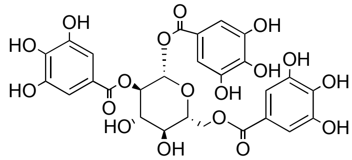

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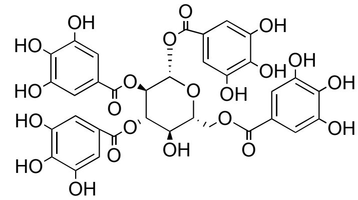

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Fig. (1) contd….

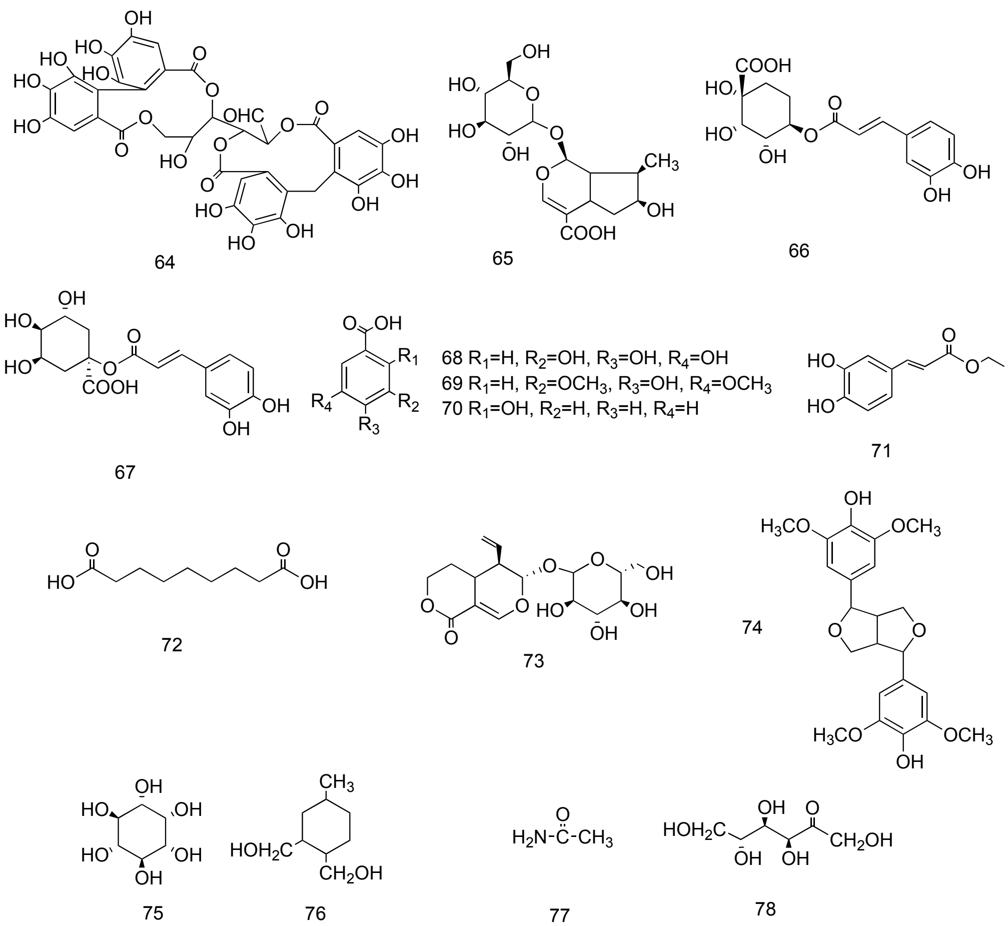

Fig. (1). Compounds isolated from *Camptotheca acuminata*.

C. acuminata [16]. In additional, four indole alkaloid glycosides (**22**-**25**) were also identified from *C. acuminata*, which are related to the camptothecin biosynthetic pathway (Fig **2**). Such as, strictosamide (**24**) isolated from the leave, stem bark, fruit, and root of *C. acuminata* [2, 18], and pumiloside (**25**) from the stem and fruit of *C. acuminata* [2, 15], might be plausible biogenetic precursors of camptothecin. However, vincosamide (**22**) [2, 3, 18] and strictosidinic acid (**23**) [2, 18] are structurally related alkaloids with **24** and **25** (Fig. **2**). Three simple alkaloids, dihydrosoquinamine (**26**) [16], venoterpine (**27**) [3, 4], 1,2-dihydro-2-oxoquinoline-4-carboxylic acid (**28**) [18], were also isolated from the fruit and the root of *C. acuminata*.

Ellagic Acids

Ellagic acid is a natural phenol antioxidant found in numerous fruits and vegetables. Its antiproliferative and antioxidant properties have showed potential health benefits. There are 12 ellagic acids (**29**-**40**) reported from various parts of *C. acuminata*. Most ellagic acid were identified from the fruits, including 3, 4-methylenedioxy-3'-*O*-methyl-4'-*O*-β-D-glucopyranosylellagic acid (**30**) [18], 3, 4-methylenedioxy-3', 4'-*O*-dimethyl-5'-methoxyellagic acid (**31**), 3, 4-methylenedioxy-3', 4'-*O*-dimethyl-5'-hydroxyellagic acid (**32**), 3, 4-*O*,*O*-methyleneellagic acid (**34**), 3'-*O*-methyl-3,4-*O*,*O*-methyleneellagic acid (**35**), 3',4'-*O*-dimethyl-3,4-*O*,*O*-methyleneellagic acid (**36**), 3, 3', 4-*O*-trimethylellagic acid (**37**), 3, 4'-*O*-dimethylellagic acid (**39**), and 3,3',4, 4'-*O*-tetramethyl-5'-methoxyellagic acid (**40**) [8, 19]. Compound **38**, 3,3',4-*O*-trimethyl-4'-*O*-β-D-glucopyranosylellagic acid, was isolated from the root and stem bark [2]. 3, 4-methylenedioxy-3'-*O*-methyl-5'-hydroxyellagic acid (**29**) was identified from the root of *C. acuminata* [2]. 3, 4-methylenedioxy-3', 4'-*O*-dimethyl-5, 5'-dimethoxylellagic acid (**33**) was found from the leave of *C. acuminata* [2]. Compounds **30**, **31**, and **37** were also reported from the root, leave or stem bark of *C. acuminata* [2, 4].

Flavonoids

Six flavonoids were reported, so far, from the leave of *C. acuminata* [2, 20] and the stem bark of *C. acuminata* [2]. It can be observed that four flavonol glycosides, including hyperoside (**42**), isoquercitrin (**43**), trifolin (**44**), and astragalin (**45**), are quercetin (**41**) and kaempferol (**46**) derivatives.

Sterols

Although the sterols are widely distributed in plant, there are only β-sitosterol (**47**) and its glycoside (**48**) isolated from the fruit of *C. acuminata* [9] and the root of *C. acuminata* [4, 21].

Terpenes

There are five pentacyclic triterpenes (**49**-**53**) were isolated from the fruit of *C. acuminata* (**49** and **53**) [3, 22] and

Table 1. Compounds isolated from *Camptotheca acuminata.*

No.	Compound Name	Leave	Stem Bark	Stem Wood	Fruit	Root
	Alkaloids					
1	camptothecin	[2]	[2]	[1]	[2, 3]	[2, 4]
2	10-hydroxycamptothecin	[2]	[2, 14]	[14]	[2, 3]	[2]
3	10-hydroxy-20-deoxycamptothecin				[6]	
4	10-methoxycamptothecin			[14]	[8, 9]	[2]
5	10-methoxy-20-*O*-acetylcamptothecin					[2]
6	11-hydroxycamptothecin				[3, 7-9]	
7	11-methoxycamptothecin				[7]	
8	18-hydroxycamptothecin				[10]	[2]
9	20-hexanoylcamptothecin		[11]			
10	20-*O*-acetylcamptothecin	[2]				[2]
11	20-hexanoyl-10-methoxycamptothecin		[11]			
12	20-deoxycamptothecin		[11]		[3, 12]	[2]
13	20-β-D-glucopyranosyl 18-hydroxycamptothecin					[2]
14	20-formylbenz [6,7] indolizino [1,2-b] quinoline-11 (13H)-one					[2]
15	22-hydroxyacuminatine				[13]	
16	angustoline		[15]		[16]	
17	19-*O*-methylangustoline				[17]	
18	19-hydroxymappicine				[13]	
19	camptacumotine				[16]	
20	camptacumanine				[16]	
21	naucleficine				[16]	
22	vincosamide				[2, 3, 18]	[2]
23	strictosidinic acid				[2, 18]	
24	strictosamide	[2]	[2]		[18]	[2]
25	pumiloside		[15]		[2]	
26	dihydrosoquinamine				[16]	
27	venoterpine				[3]	[4]
28	1,2-dihydro-2-oxoquinoline-4-carboxylic acid				[18]	
	Ellagic Acids					
29	3, 4-methylenedioxy-3'-*O*-methyl-5'-hydroxyellagic acid					[2]
30	3, 4-methylenedioxy-3'-*O*-methyl-4'-*O*-β-D-glucopyranosylellagic acid		[2]		[18]	[2]
31	3, 4-methylenedioxy-3', 4'-*O*-dimethyl-5'-methoxyellagic acid	[2]			[8, 19]	[2]
32	3, 4-methylenedioxy-3', 4'-*O*-dimethyl-5'-hydroxyellagic acid				[8, 19]	
33	3, 4-methylenedioxy-3', 4'-*O*-dimethyl-5, 5'-dimethoxylellagic acid	[2]				

(Table 1) contd….

No.	Compound Name	Leave	Stem Bark	Stem Wood	Fruit	Root
34	3, 4-*O,O*-methyleneellagic acid				[8, 19]	
35	3'-*O*-methyl-3,4-*O,O*-methyleneellagic acid				[19]	
36	3',4'-*O*-dimethyl-3,4-*O,O*-methyleneellagic acid				[8, 19]	
37	3, 3', 4-*O*-trimethylellagic acid	[2]			[19]	[2, 4]
38	3, 3', 4-*O*-trimethyl-4'-*O*-*β*-D-glucopyranosylellagic acid		[2]			[2]
39	3, 4'-*O*-dimethylellagic acid				[19]	
40	3,3',4, 4'-*O*-tetramethyl-5'-methoxyellagic acid				[19]	
	Flavonoids					
41	quercetin	[2]				
42	quercetin-3-*O*-*β*-D-galactopyranoside (hyperoside)	[2]				
43	quercetin-3-*O*-*β*-D-glucopyranoside (isoquercitrin)	[2]				
44	kaempferol-3-*O*-*β*-D-galactopyranoside (trifolin)	[2]	[2]			
45	kaempferol-3-*O*-*β*-D-glucopyranoside (astragalin)	[2]				
46	kaemferol	[20]				
	Sterols					
47	*β*-sitosterol				[9]	[4]
48	*β*-sitosterol-3-*O*-*β*-D-glucopyranoside					[21]
	Terpenes					
49	ursolic acid				[22]	
50	amyrin	[Supporting Data]				
51	2,3-dihydroxy-12-ursen-28-oic acid	[Supporting Data]				
52	bassic acid	[Supporting Data]				
53	betulic acid				[3, 22]	
54	strychnolactone				[10]	
55	(+)-abscisic acid				[9]	
	Tannins					
56	camptothin A	[23]				
57	camptothin B	[23]				
58	cornusiin A	[23]				
59	gemin D	[23]				
60	tellimagrandin II	[23]				
61	tellimagrandin I	[23]				
62	1,2,6-tri-*O*-galloyl-*β*-D-glucose	[23]				
63	1,2,3,6-tetra-*O*-galloyl-*β*-D-glucose	[23]				
64	pedunculagin				[23]	

(Table 1) contd….

No.	Compound Name	Leave	Stem Bark	Stem Wood	Fruit	Root
	Polyphenols and Fatty Acids					
65	loganic acid				[18]	
66	chlorogenic acid				[18]	
67	1-caffeoylquinic acid				[18]	
68	gallic acid	[23]				
69	syringic acid				[19]	
70	salicylic acid				[10]	
71	ethyl caffeate				[22]	
72	nonandioic acid				[10]	
	Iridoid					
73	sweroside				[18]	
	Lignan					
74	syringaresinol				[9]	
	Polyols					
75	inositol				[18, 22]	
76	4-methyl-1,2-cyclonexanedimethanol				[18]	
	Amide					
77	acetamide				[18]	
	Saccharide					
78	fructose		[2]			

from the leave of *C. acuminata* (**50**-**52**). Amyrin (**50**), 2,3-dihydroxy-12-ursen-28-oic acid (**51**), and bassic acid (**52**) were identified by Dr. Li' research team [Supporting Data]. One monoterpene (strychnolactone, **54**) [10], and one sesquiterpene (abscisic acid **55**) [9] were also reported from the fruit of *C. acuminata.*

Tannins

From the leaf of *C. acuminata*, camptothin A (**56**) and camptothin B (**57**) were isolated along with cornusiin A (**58**), gemin D (**59**), tellimagrandin II (**60**), tellimagrandin I (**61**), 1,2,6-tri-*O*-galloyl-*β*-D-glucose (**62**), and 1,2,3,6-tetra-*O*-galloyl-*β*-D-glucose (**63**) [23]. Pedunculagin (**64**) was isolated from the fruit of *C. acuminata* [23].

Polyphenols and Fatty Acids

Seven aromatic compounds (**65**-**71**) and one long chain fatty acid (**72**) were isolated from *C. acuminata*. Loganic acid (**65**) [18], chlorogenic acid (**66**) [18], 1-caffeoylquinic acid (**67**) [18], syringic acid (**69**) [19], salicylic acid (**70**) [10] , ethyl caffeate (**71**) [22], and nonandioic acid (**72**) [10] were isolated or identified from fruits. Gallic acid (**68**) was isolated from the leaves [23].

Miscellaneous Compounds

Five other classes of compounds (**73**-**78**) were found from *C. acuminata*. Two polyols (**75** and **76**) [18, 22], one iridoid (**73**) [18], one lignan (**74**) [9], one amide (**77**) [18] were isolated from the fruit of *C. acuminata*. Compound **78** was identified from the stem bark of *C. acuminata* [2].

VARIATIONS OF CPT CONTENTS

Presently, the bioactive nature of secondary metabolites in plants, such as alkaloids, phenolics and terpenoids has made them medicinally important. CPT is the most well-known example, which has shown a broad range of anticancer activity in animal models and continued to serve as a very attractive and challenging lead structure for the development of new anticancer drugs. To data, the extraction from *C. acuminata* is still considered as the main approach to obtain CPT compared with total synthesis. It becomes very important to understand content variations of CPT within and among plants, and with time. This review has also focused on presenting CPT variation among *Camptotheca* species and varieties, tissue and tree age, seasonal changes, and providing useful information for maximum yield of CPT and the discovery of novel camptothecinoids.

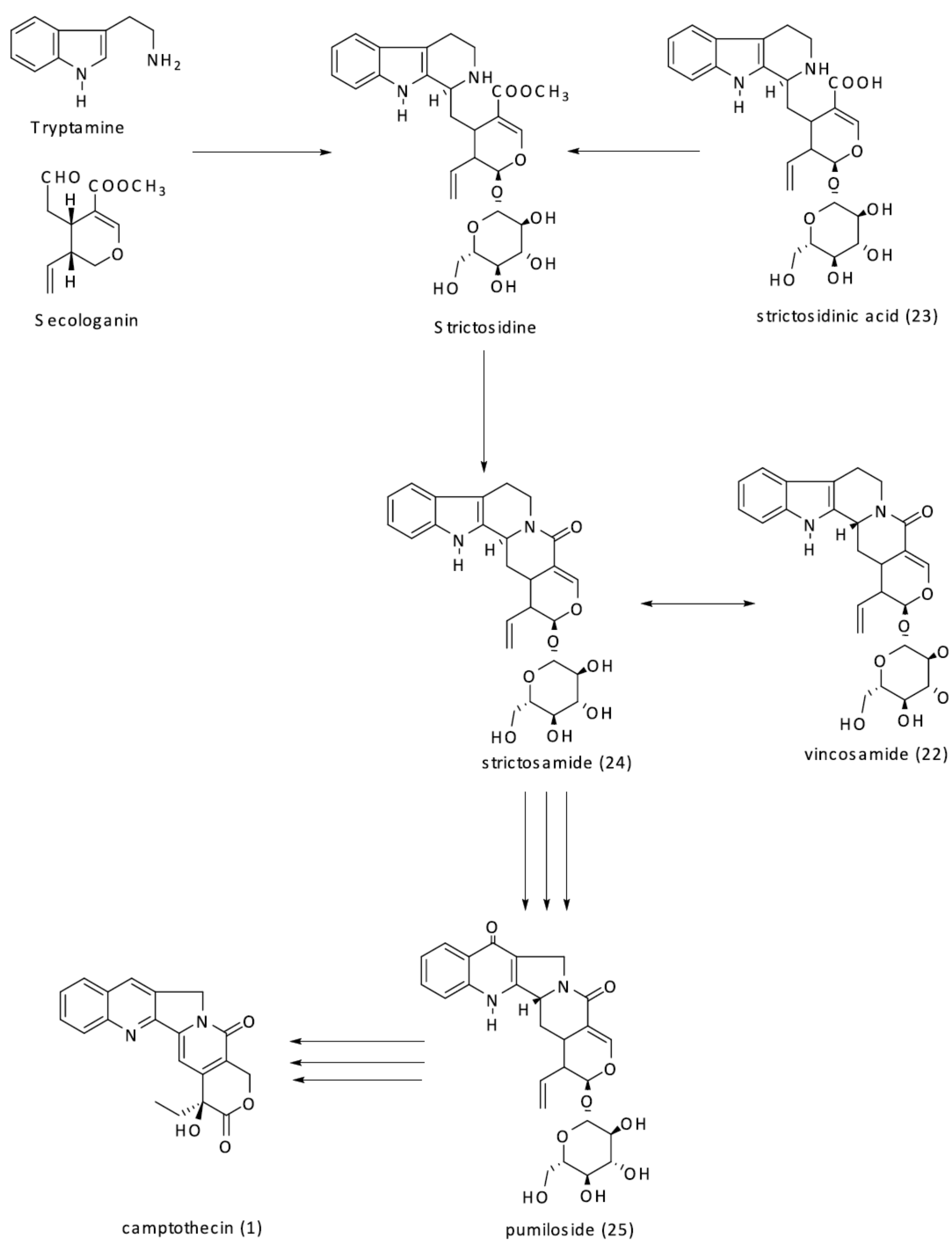

Fig. (2). Hypothetical biogenetic pathway of camptothecin (CPT) (**1**).

CPT Variations Among Species and Varieties

Among all *Camptotheca* taxa*, C. acuminata* var. *acuminata* has the lowest CPT contents, from a maximum of 0.3162% to minimum of 0.2249% in young leaves, and from a maximum of 0.0572% to minimum of 0.0392% in older leaves. *C. lowreyana* "Hicksii" has the highest CPT contents in both young and old leaves, approximately 1.5-2 folds higher than those in *C. acuminata* var. *acuminata* (Table **2**). *C. lowreyana* var. *lowreyana*, *C. yunnanensis*, and *C. acuminata* var. tenuifolia have not showed significant variation in young leaf CPT contents. *C. lowreyana* "Hicksii" and "Katie" was considered as the major management germplasm for CPT source by showing desirable features in both biomass and CPT concentration [24].

CPT Variations within Plants

These was evidenced early in 1977 that the CPT contents vary significantly within different tissues, although all parts of the *C. acuminata* plant contain some CPT (Table **3**) [3, 4]. In our previous studies, the highest concentrations of CPT were found in the young leaves and mature fruit [24]. Young leaves (< 1 weeks old) contained a mean CPT concentration of 0.2313%, which was 26-fold higher than that in young root (<4 weeks old), followed by young fruits (< 1 weeks old) and young stems (<4 weeks old) with a mean CPT concentration of 0.0842% and 0.0718% of dry weight, respectively. For old tissues, CPT content in fruit was 12-fold, 6-fold, and 4-fold higher than in stems, roots, and leaves, respectively. Valletta *et al.* reported that the CPT concentration

Table 2. CPT contents in young leaves (< 1 week old) and old leaves (8-10 weeks) of *Camptotheca* Decaisne (%, w/w, dry wt.) [24].

Species/Variety	CPT Content in Young Leaves Average (Rang)	CPT Content in Old Leaves Average (Rang)
C. acuminata Decaisne		
var. *acuminata*	0.2560 (0.2249-0.3162)	0.0482 (0.0392-0.0572)
var. *tenuifolia* Fang et Soong	0.3492 (0.3247-0.3737)	N/A
C. lowreyana Li		
'Hicksii'	0.5537 (0.5337-0.5737)	0.1184 (0.1058-0.1310)
'Katie'	0.4778 (0.4482-0.5074)	0.0977 (0.0900-0.1054)
'CT168'	0.5890	
var. *lowreyana*	0.3913 (0.373-0.4096)	0.0909 (0.0765-0.1053)
C. yunnanensis Dode	0.3452 (0.2262-0.4797)	0.0590 (0.0491-0.0689)

Table 3. CPT and 10-HCPT contents in different tissues of *C. acuminata* var. *acuminata* (%, w/w, dry wt.).

Tissue Type	Young Tissue		Intermediate Tissue		Old Tissue		Reference
	CPT	10-CPT	CPT	10-CPT	CPT	10-CPT	
Flower	0.23-0.25	0.141	UD	UD	UD	UD	[26]
Leaf	0.2145-0.432	N/A	0.1068-0.1138	N/A	0.04-0.07	N/A	[24, 25, 28, 29]
Stem	0.0648-0.0788	N/A	0.0349-0.0377	N/A	0.0181-0.048	0.02-0.03	[24, 26]
Stem Bark	0.0493-0.0611	N/A	0.0663-0.0685	N/A	0.0285-0.16	N/A	[24, 29]
Stem Wood	0.0134-0.0138	N/A	0.0079-0.0081	N/A	0.0116-0.0122	N/A	[24]
Stem Pith	0.106-0.1104	N/A	UD	N/A	UD	N/A	[24]
Fruit	0.0768-0.0916	N/A	0.106-0.1104	N/A	0.2234-0.28	0.025-0.032	[24-26, 29]
Root	0.0074-0.25	N/A	UD	N/A	0.0297-0.075	UD	[24-26]

Notes: N/A-no data available; UD-Undetectable.

in the mature fruit was 0.28%, which is similar to the 0.2312% in our study [25]. The authors described also CPT variations in the young leaves (0.011%-0.0432%) and very young roots (0.015-0.025%). Pi *et al.* investigated the contents of CPT and 10-HCPT in different tissues including roots, stems, leaves, young flower buds, opening flowers, fading flowers and seeds from *C. acuminata* [26]. The young flower buds had also found having the highest alkaloid concentrations with a mean CPT concentration of 0.246% and 10-HCPT of 0.141% of dry weight.

Of particular interest was the finding that CPT content is obviously related to tissue age rather than its location on the plant and tree age [24]. Against this background, although no detailed descriptions about leaf materials were given, it is likely that young tissue was used in the CPT analysis in some previous studies [1, 20, 27-30], while some negative results may had been caused by the use of relatively old tissue in the analyses [26, 28, 31, 32]. So, in conclusion, only fruits and young leaves of *Camptotheca* are currently suitable for CPT extract.

CPT Variations with Seasons

There was significant seasonal variation in CPT concentration in *C. acuminata* var. *acuminata* over the course of the growing season. Liu *et al.* found a steady decline of 11% per month in leaf CPT concentration from a maximum of 0.051% in April to a minimum of 0.017% in October. Branches showed a similar seasonal decline in CPT concentration to leaves from a maximum of 0.018% in April to a minimum of 0.011% in October. However, the rate of decline was threefold greater in leaves than in branches [28]. Our research results are very different from these statements [24]. In intact young tissues, significant seasonal change were showed with the lowest CPT contents in March and April (0.074% and 0.081%, respectively) and highest in June (0.265%) in *C. acuminata* var. *acuminata,* and no significant difference among May and July to October (0.117-0.171%). It was reported that CPT contents in mature fruits (usually in September to October) are 2-3 times of those in young fruits or flowers (in May to June). In additional, the seasonal changes of CPT in intact young tissues and fruits have confirmed indirectly the studies on CPT variations with tissue age.

CONFLICT OF INTEREST

The authors confirm that this article content has no conflict of interest.

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PATIENT'S CONSENT

Declared none.

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