Phytochemistry, Vol. 21, No. 12, pp. 2871–2874, 1982.
Printed in Great Britain.

0031-9422/82/122871-04$03.00/0

ELLAGITANNINS OF THE CASUARINACEAE, STACHYURACEAE AND MYRTACEAE*

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(*Received* 13 *April* 1982)

Key Word Index—*Casuarina stricta*; Casuarinaceae; *Stachyurus praecox*; Stachyuraceae; *Psidium*; *Syzygium*; *Feijoa sellowiana*; *Eucalyptus viminalis*; Myrtaceae; tannin.

Abstract—Eight ellagitannins and related polyphenols, found in *Casuarina stricta* and *Stachyurus praecox*, were detected in the leaf of *Psidium* species. Five of them and a new polyphenol, named isostrictinin, were isolated from *Psidium guajava*. Most of these compounds were detected in several species of Myrtaceae, and 2, 3-hexahydroxydiphenoylglucose and 4, 6-hexahydroxydiphenoylglucose were found in some.

INTRODUCTION

Recent investigation of the tannins of *Casuarina stricta* Ait. (Casuarinaceae) and *Stachyurus praecox* Sieb. et Zucc. (Stachyuraceae) revealed the occurrence of several identical tannins [1, 2]. Succeeding studies of the leaf of *Psidium guajava* L. (Myrtaceae), used as an astringent medicine in Asian countries [3], showed identity of its tannins with those in the above two species.

Since Myrtaceae is known to be rich in phenolics [4], we extended our investigation to other species of this family. *Eucalyptus* species contain several ellagitannins [5] and isolation of polyphenols related to ellagitannins from *P. guajava* has been reported [6]. Our present investigation includes HPLC and TLC of leaf extracts of *P. guajava*, *P. cattleianum* Sabine, *Syzygium jambos* Alst., *S. aromaticum* Merr. et Perry, *S. samarangense* Merr. et Perry, *S. aqueum* Alst., *Feijoa sellowiana* Berg. and *Eucalyptus viminalis* Labill. Fractionation of the leaf extract of *P. guajava* and detection of tannins in *C. stricta* and *S. praecox* by HPLC and TLC were also carried out.

RESULTS AND DISCUSSION

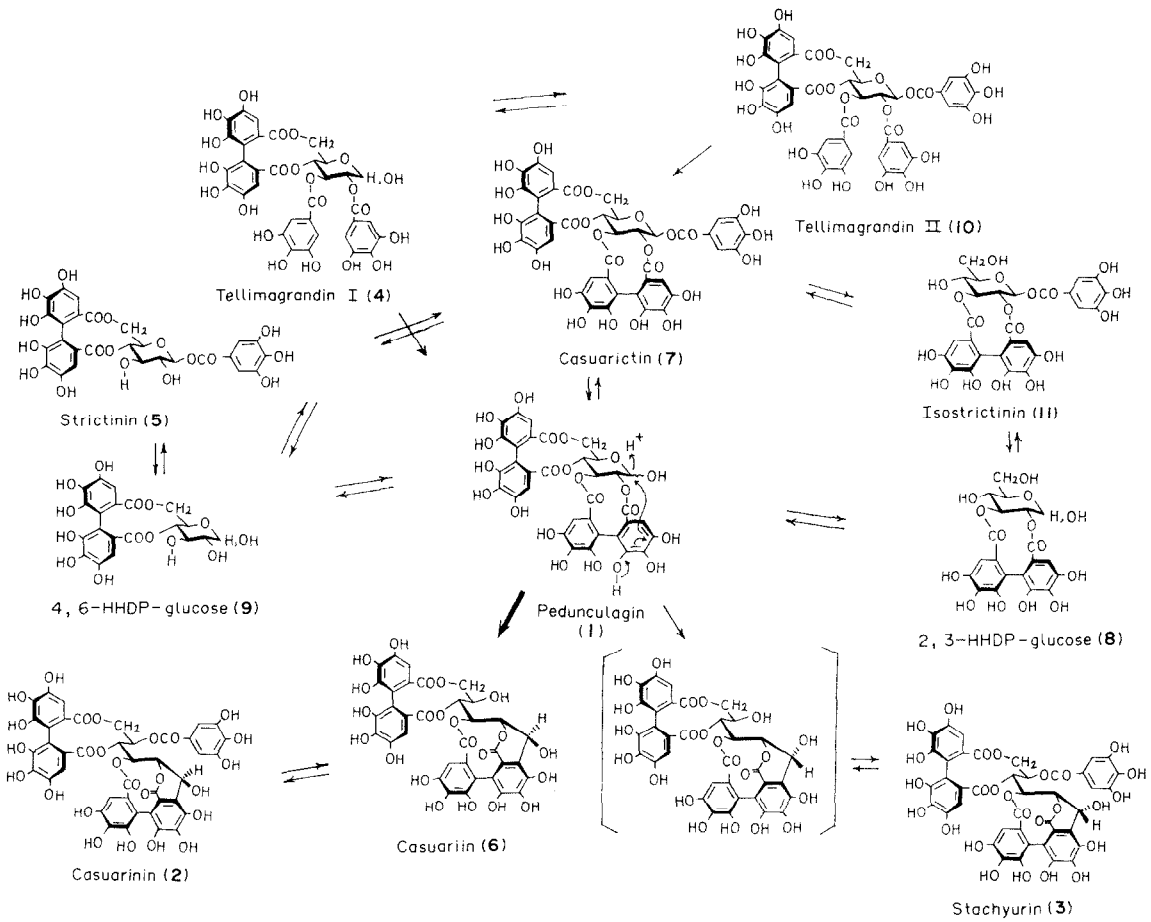
All eight ellagitannins including structurally related polyphenols, which were found by HPLC and TLC in *P. guajava*, were detected in *C. stricta* and *S. praecox* (Table 1). The presence of five, namely pedunculagin (**1**) [1, 7], casuarinin (**2**) [1], stachyurin (**3**) [2], tellimagrandin I (**4**) [1, 8] and strictinin (**5**) [2], was confirmed by direct comparisons.

Casuariin (**6**) [2], casuarictin (**7**) [1] and 2, 3-hexahydroxydiphenoylglucose (**8**) [7], which had been isolated from *C. stricta* and *S. praecox* [1, 2], were detected by chromatography in *P. guajava*. 4, 6-Hexahydroxydiphenoylglucose (**9**) [9] and tellimagrandin II (**10**) [1, 8] were detected in some species of Myrtaceae in the present chromatographic study. The sixth isolated tannin of *P. guajava*, named isostrictinin (**11**) was determined as (*S*) - 1 - *O* - galloyl - 2, 3 - *O* - hexahydroxydiphenoyl - β - D - glucose by spectral analysis and degradation with tannase.

It is remarkable that all eight compounds co-occur in three species belonging to Casuarinaceae, Stachyuraceae and Myrtaceae, and that most of these compounds also occur in other species of Myrtaceae. The biogenetic relationships between these tannins are shown in Scheme 1. The *C*-glycosidic ellagitannins, **2**, **3** and **6** are regarded as the products of C–C bond formation between C-1 of glucose and a carbon in the biphenyl ring in **1**. Since **7** is also regarded as a product from **1**, galloylated at O-1 of the glucose core, **1** could be the key compound which yields **2**, **3**, **6** and **7**, and also may be regarded as a product formed by oxidative coupling between the galloyl groups in **4**, as proposed by Schmidt [10] for the biogenesis of ellagitannins. The hexahydroxydiphenoylglucoses, **8** and **9**, are interconvertible with **1** by partial hydrolysis or esterification and their galloylations give **11** and **5**. The detection of **1** in all examined species, and its occurrence as the most abundant constituent in some support the biogenetic route shown in Scheme 1.

The systematic distribution of ellagitannins in relation to the phylogeny and classification of the angiosperms has been presented elsewhere [11]. Since the Casuarinales, Violales and Myrtales, to which orders the species of the present investigation belong, are not close to each other in plant classification, further identifications of this series of tannins in other related plants will be of interest.

*Part 1 in the series "Tannins and Related Compounds in Myrtaceae".



Scheme 1.

Table 1. Ellagitannins in *C. stricta*, *S. praecox* and several species of Myrtaceae

	1	2	3	4	5	6	7	8	9	10
Casuarina stricta‡	+*†	+*†	+*	+*	+*	+*	+*	+	—	—
Stachyurus praecox‡	+*†	+*†	+*	+*	+*	+	+*	+	—	—
Psidium guajava	+*†	+*	+*	+*	+*	+	+	+	—	—
Psidium cattleianum	+†	+†	+	—	+	±	+	+	—	+
Syzygium jambos	+†	+	±	+	+	±	+	+	—	trace
Syzygium aromaticum	+	—	—	+	+	—	+	+	—	+†
Syzygium samarangense	+	+	+	—	+	+	+†	—	+	—
Syzygium aqueum	+	+†	—				+†		+	—
Feijoa sellowiana	+†	+	+	+	+	+	—	+	+	—
Eucalyptus viminalis	+	+	trace	+	+	+	+	trace	+	±

For key to tannins, see Table 2.
*Isolated tannins.
†Main component.
‡Isolation is reported in refs. [1, 2].

Table 2. Retention times and R_f values of ellagitannins

Compound	HPLC		TLC
	RR_t*(reversed-phase)	RR_t† (normal-phase)	R_f§
Pedunculagin (1)	0.28	1.15	0.63
Casuarinin (2)	0.43	1.23	0.26
Stachyurin (3)	0.40	1.31	0.41
Tellimagrandin I (4)	0.44, 0.72	1.02, 1.09	0.57
Strictinin (5)	0.36	0.91	0.50
Casuariin (6)	0.20	1.14	0.44
Casuarictin (7)	1.4	1.28	0.54
2,3-HHDP-glucose‡ (8)	0.20	0.81	0.76
4, 6-HHDP-glucose (9)	0.20	0.86	0.76
Tellimagrandin II (10)	3.0	1.28	0.36
Isostrictinin (11)	0.32	0.95	0.56

*R_t rel. to rutin.
†R_f rel. to geraniin [12].
‡HHDP: hexahydroxydiphenoyl.
§Solvent:7% HOAc.

EXPERIMENTAL

Preparation of the plant extracts for HPLC. Casuarina stricta, Stachyurus praecox, Psidium guajava, P. cattleianum, Syzygium jambos, S. samarangense and *S. aqueum* were obtained at the Herbal Garden, Okayama University, and *Syzygium aromaticum, Feijoa sellowiana* and *Eucalyptus viminalis* were from the Kyoto Herbal Garden of Takeda Chemical Industries, Ltd. Fresh leaves (20 g) of each plant were homogenized in Me_2CO–H_2O (7:3) × 3, and centrifuged. The combined supernatants were concd to 20 ml *in vacuo* at room temp. The solns were extracted with Et_2O × 5 and the residual aq. layers were then extracted with EtOAc × 10, and the combined EtOAc layers were evaporated *in vacuo*. The dried extracts were dissolved in MeOH (1–2 mg/ml). Vols. of 1–5 μl of the solns were injected on the column of HPLC.

HPLC and TLC. Reversed-phase HPLC was run on a column of Merck LiChrosorb RP-18 (10 μm), 4 × 300 mm, developing with 0.1 M H_3PO_4–0.1 M KH_2PO_4–EtOH–EtOAc (50:50:2:5, by vol.). Normal-phase HPLC was performed on a column of Nomura Develosil 60–5, 4 × 150 mm, developing with *n*-hexane–MeOH–THF–HCOOH (55:33:11:1, by vol.), containing oxalic acid (450 mg/l.). Detection was effected by the UV absorption at 280 nm. TLC was developed on a cellulose plate with *n*-BuOH–AcOH–H_2O (4:1:5, upper), and also with 7% HOAc, and visualized by spraying with 50% HOAc and 4% $NaNO_2$ successively. Retention times of HPLC and R_f values on TLC of each tannin are shown in Table 2.

Fractionation of tannins of P. guajava. Dried leaves (400 g) of *P. guajava* were treated analogously, to give the EtOAc extract (11.8 g). The extract (7 g) was chromatographed on a column of Sephadex LH-20 satd with EtOH and 11 ml/fraction were collected. The column was eluted with EtOH (fractions 1–269), EtOH–MeOH (1:1) (270–399) and then with MeOH. Combined fractions 73–110 were purified by droplet counter-current chromatography (DCCC) (*n*-BuOH–*n*-PrOH–H_2O, 2:1:3, ascending method, 120 cm × 3.2 mm column × 100, 12 g/fraction). Combined fractions 37–50 of DCCC were further purified by Sephadex LH-20 column 15 × 1.1 cm, EtOH) and gave chromatographically pure isostrictinin (**11**). Fractions 111–184 of LH-20 CC were purified by DCCC with the same solvent system as above to give strictinin (**5**). Fractions 240–289 from the column gave tellimagrandin I (**4**). Fractions 300–345 afforded pedunculagin (**1**), casuarinin (**2**) and stachyurin (**3**) on DCCC followed by cellulose CC. Yield of each tannin from the EtOAc extract (7 g): **1**, 240 mg; **2**, 83 mg; **3**, 6 mg; **4**, 95 mg; **5**, 25 mg; **11**, 12 mg.

Isostrictinin (**11**). Off-white amorphous powder, $[\alpha]_D^{17}$ −11.5° (MeOH; *c* 0.4), IR ν_{max}^{KBr} cm^{-1}: 3400, 1725, 1615, 1515, 1445, 1350, 1235–1190, 1065–1040. UV λ_{max}^{MeOH} nm: 218, 275. ^1H NMR (90 MHz, CD_3COCD_3): δ 7.16 (2H, *s*, galloyl), 6.72, 6.43 (1H each, *s*, hexahydroxydiphenoyl), 6.13 (1H, *d*, *J* = 9 Hz, glucose H-1), 5.21 (1H, *t*, *J* = 9 Hz, glucose H-3), 5.00 (1H, *t*, *J* = 9 Hz, glucose H-2), 4.10–3.50 (4H, *m*, glucose H-4–H-6). (Found: C, 48.6; H, 4.2. $C_{27}H_{22}O_{18} \cdot 2H_2O$ requires: C, 48.4; H, 3.9%.)

Acknowledgements—We wish to thank Dr. M. Goto and Mr. Y. Nagao of Kyoto Herbal Garden, Takeda Chemical Industries, Ltd. for supplying plant material of some species.

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