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CATECHIN-3-*O*-RHAMNOSIDE CHAIN EXTENDER UNITS IN POLYMERIC PROCYANIDINS FROM MANGROVE BARK

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Key Word Index—*Bruguiera gymnorrhiza*; Rhizophoraceae; mangrove; bark; condensed tannins; proanthocyanidins; 3-*O*-α-L-rhamnopyranosyl-(+)-catechin-(4α→2)-phloroglucinol; flavan-3-ol glycosides.

Abstract—Acid-catalysed cleavage of 'purified' condensed tannin isolates from *Bruguiera gymnorrhiza* (tancang) bark in the presence of phloroglucinol as a capture nucleophile gave, in addition to the expected procyanidin- and prodelphinidin-phloroglucinol adducts, 3-*O*-α-L-rhamnopyranosyl-(+)-catechin-(4α→2)-phloroglucinol, thus providing evidence for covalently bonded glycoside moieties in the chain extender units of mangrove bark tannins.

INTRODUCTION

The bark of *Bruguiera gymnorrhiza* (tancang), a commercially important mangrove species in the estuaries of Indonesia, contains a mixture of 3,5,7,3′,4′-pentahydroxy-flavan and 3,5,7,3′,4′,5′-hexahydroxy-flavan polymers [1]. In addition to the flavan-3-ols epicatechin and catechin, the dimeric procyanidins epicatechin-(4β→8)-epicatechin, the rare catechin-(4α→8)-epicatechin, and other oligomers not as yet identified are present. ^{13}C NMR spectra of the water-soluble polymer indicated a mixture of procyanidins and prodelphinidins mainly of 2,3-*cis* stereochemistry and the presence of bound carbohydrates of which a C-6-deoxy-glycoside was a major component as shown by the persistent signal at δ 17–18 [1].

Neilson and coworkers [2], after repeated attempts to separate proanthocyanidins from glycans in extracts isolated from *Rhizophora stylosa* leaves, proposed that a covalently bound procyanidin-glycoside was present. Porter and coworkers [3] suggested that proanthocyanidin glycosides might be natural metabolites of plants, especially where flavan-3-*O*-glycosides occurred as chain terminating units of polymers. After the isolation of a number of flavan-glycosides by Nishioka and coworkers [4–6] and Foo and Karchesy [7], it is now recognized that glycosylation of proanthocyanidins in plants is not exceptionally rare, even though proanthocyanidin glycosides are not commonly found in most plants.

The recent isolation of 3-*O*-β-D-glucosyl-catechin-(4α→8)-catechin from blackjack oak (*Quercus marilandica*) bark [8] and previous isolation of two 3-*O*-α-L-rhamnoside derivatives of catechin-(4α-8)-catechin from *Quercus miyagii* [5] and *Erythroxylum novogranatense* [9] prompted us to pursue the question of whether or not glycosidic linkages to the chain extender units of polymeric proanthocyanidins occur. To our knowledge, only oligomeric procyanidin glycosides with sugar moieties at the A-ring hydroxyls of the chain extender units such as the proluteolinidins found in sorghum [10] have been fully described. Proof of flavan-3-*O*-glycosides as the chain extender unit of proanthocyanidin polymers is lacking.

RESULTS AND DISCUSSION

The crude acetone extract of *Bruguiera gymnorrhiza* bark was repeatedly extracted with ethyl acetate to remove oligomeric proanthocyanidins (at least up to the tetramer level). The remaining water-soluble residue was applied to an LH-20 Sephadex column that was eluted with methanol–water (1:1) to remove carbohydrates but retain oligomers containing a high proportion of proanthocyanidin polymers [11]. ^{13}C NMR spectra showed the presence of glycosides (especially rich in rhamnose) in the polymeric proanthocyanidins retained on the column when eluting with methanol–water but that were elutable with acetone–water (1:1). Repeated attempts to separate the proanthocyanidin polymer from the carbohydrate by chromatography on LH-20 Sephadex failed.

The ^{13}C NMR spectrum of the acetone–water eluted fraction showed a mixture of proanthocyanidins with catechol (δ116–117 and 120) and pyrogallol (δ108–109) B-rings, phloroglucinol (δ96–98) A-rings, and predominantly 2,3-*cis* (δ76–79 and 71–73) C-ring stereochemistry

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in the chain extender units and 2,3-*trans* (δ84 and 68) stereochemistry in the terminal units [11, 12]. Smaller but significant resonances at δ102, 76–70 and 17 suggested the presence of rhamnose substituted to the flavan units [5].

The acetone–water eluted polymer was reacted with phloroglucinol as a capture nucleophile in the presence of catalytic amounts of acetic acid [13], and the reaction product was separated on LH-20 Sephadex using ethanol as the solvent to isolate a series of oligomeric proanthocyanidin-4-phloroglucinol adducts. Epicatechin-(4β→2)-phloroglucinol and epicatechin-(4β→8)-epicatechin-(4β→2)-phloroglucinol were major products of this reaction, but other (as yet unidentified) products are also present. In the course of study of these products, a compound appearing at R_f 0.45 and 0.67 on cellulose TLC plates developed with *t*-butanol–acetic acid–water (3:1:1) and 6% acetic acid, respectively, gave a red coloration after spraying with vanillin–HCl. This compound was isolated in chromatographically pure form by subsequent chromatography on LH-20 Sephadex using ethanol–water (7:3) as an eluting solvent.

The negative ion FAB-mass spectrum of the free phenol showed a significant parent ion at *m/z* 599 and the high resolution FAB-mass spectrum showed a M_r of 599.148, consistent with a molecular formula of $C_{27}H_{27}O_{13}$ for $[M-H]^-$ as calculated for a rhamnoside of catechin-(4→2)-phloroglucinol.

The basic structure of this compound (**1**) is evident from the following interpretation of NMR spectral data. The ^{13}C and ^1H NMR spectra (with assignments verified from a C–H HETCOR experiment) clearly showed catechin-(4α→2)-phloroglucinol with rhamnose in the molecule (Table 1). A 3-*O*-linkage of rhamnose to the flavan is evident from the C-3 resonance at δ78.9 [compare C-3 resonances at 67.9 for (+)-catechin [12]; at δ72.9 for (+)-catechin-(4α→2)-phloroglucinol [12] (a downfield shift, $\Delta\delta = 5.0$ ppm), and at δ74.5 for the 3-*O*-α-L-rhamnoside of (+)-catechin [5] (a downfield shift, $\Delta\delta = 6.6$ ppm)] showing reasonable agreement with additivity rules on the effects of substituents at C-4 and C-3. A 3-*O*-α-linkage of a rhamnopyranosyl to catechin is evident from the small coupling constant (<2 Hz) of the anomeric proton at δ3.71 (correlated with the carbon resonance at δ101) together with the rhamnose methyl at

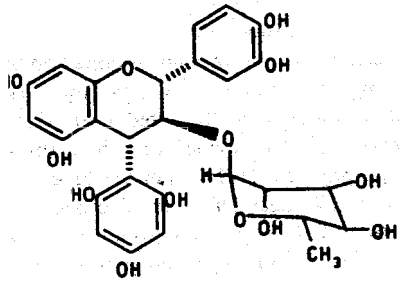

Table 1. ^{13}C and ^1H NMR spectral data for 3-*O*-rhamnosyl-(+)-catechin-(4α→2)-phloroglucinol (**6**)

C or H	^{13}C	^1H
Catechin		
2	84.0	4.33 *d* (9.9)
3	78.9	4.73 *dd* (7.7, 9.9)
4	38.1	4.44 *d* (7.7)
5	156–158	
6	97.2	
7	156–158	
8	98.0	
9	156–158	
10	116.5	
11	131.0	
12	116.5	
13	144–145	
14	144–145	
15	116.8	
16	121.1	
Rhamnose		
	101.0	3.71 <2.0 Hz
2	72.0	3.40 *dd* (≈2 and 3)
3	72.5	3.54 *dd* (≈3.0, 8)
4	74.2	3.10 *t* (8, 8)
5	69.8	3.20 *dd* (6.5, 8.0)
6	17.9	0.73 *d* (6.5)
Phloroglucinol		
1	108.8	
2	156–158	
3	98.2	
4	156–158	
5	98.2	

Coupling constants (J in Hz) in parentheses.

δ0.73 ($J = 6.5$ Hz) as well as the ^{13}C NMR chemical shifts observed for C-3 and C-5 of the rhamnose unit [14].

The C-ring C-3 was correlated with a double doublet ($J = 9.9$ and 7.7 Hz) at δ4.73. The C-2 at δ84.0 was correlated with the doublet ($J = 9.9$ Hz) at δ4.33 and C-4 at δ38.1 with the doublet ($J = 7.7$ Hz) at δ4.44. The $J_{2,3}$ coupling of 9.9 Hz together with the chemical shift of C-2 at δ84.0 shows that this compound is of 2,3-*trans* stereochemistry. Assignment of the stereochemistry at C-4 is not so straightforward. Procyanidin derivatives of 2,3-*trans*-3,4-*trans* stereochemistry typically show $J_{3,4}$ coupling of 9–10 Hz. The small $J_{3,4}$ coupling of 7.7 Hz in this compound is reminiscent of the data found for the 2,3-*trans*-3,4-*trans*-3-β-D-*O*-glucosyl-(+)-catechin-(4α→8)-catechin isolated from blackjack oak that had heterocyclic ring proton coupling constants of $J_{2,3} = 10$ and $J_{3,4} = 7$ Hz [7]. The C-2 resonance at δ84.0 suggests 2,3-*trans*-3,4-*trans* stereochemistry (downfield due to the equatorial substituent at C-4), and the $J_{2,3}$ coupling of 9.9 Hz indicates a 4α linkage to phloroglucinol. If the phloroglucinol unit at C-4 had been axial (4β-linkage), the C-2 resonance would have been expected to be shifted upfield to about δ78 because of the γ-gauche effect. As was indicated in the conformation of the dimer glycoside

isolated from blackjack oak, the small $J_{3,4}$ can be accounted for by a distortion to a C-2 sofa conformation.

The isolation of 1 from acid-catalysed cleavage of the polymeric proanthocyanidins with phloroglucinol as a capture nucleophile provides definitive proof that flavan-glycosides do form the chain extender units in some polymeric proanthocyanidins. It seems unusual that the compound isolated is the 2,3-*trans* isomer given the predominance of 2,3-*cis* stereochemistry in the chain extender units of the polymer. Additional unidentified products are present so it remains to be seen if the corresponding 2,3-*cis* isomer is also present.

EXPERIMENTAL

Acetone–water extracts were taken from the bark of *Bruguiera gymnorrhiza* collected in Cilacap estuary, Indonesia. These crude extracts were fractionated into EtOAc and H_2O-soluble fractions with a separatory funnel. The water-soluble fraction (2.1 g) was reacted with phloroglucinol (2.1 g) in EtOH (20 ml) in the presence of HOAc (5 ml) catalyst at 105° for 12 hr in a sealed ampule. After cooling, the product was diluted with H_2O and extracted with EtOAc (× 5 with equal volumes) to recover the EtOAc-soluble fraction (2.77 g) and H_2O-soluble residue (0.93 g). Column chromatography of the EtOAc-soluble fraction on LH-20 Sephadex with EtOH as the solvent gave 1 together with the expected series of procyanidin- and prodelphinidin-4-phloroglucinol adducts. Mass spectral determinations were made at the Midwest Center for Mass Spectrometry with partial support by the National Science Foundation, Biology Division (Grant No. DIR9017262).

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REFERENCES

1. Darmawan, A., Sayhbirin, G., Achmadi, S., Edwards, D., Hemingway, R. W. and Choong, E. T. (1992) *Progress Report on NSF Grant INT*-9023743.
2. Neilson, M. J., Painter, T. J. and Richards, G. N. (1986) *Carbohydr. Res.* **147**, 315.
3. Porter, L. J., Foo, L. Y and Furneaux R. H. (1985) *Phytochemistry* **24**, 567.
4. Kashiwada, Y., Nonaka, G.-I, and Nishioka, I. (1986) *Chem. Pharm. Bull.* **34**, 3208.
5. Ishimaru, K., Nonaka, G.-I. and Nishioka, I. (1987) *Phytochemistry* **26**, 1167.
6. Zhang, B., Nonaka, G.-I. and Nishioka, I. (1988) *Phytochemistry* **27**, 3277.
7. Foo, L. Y. and Karchesy, J. J. (1989) *Phytochemistry* **28**, 1237.
8. Bae, Y. S., Burger, J. F. W., Steynberg, J. P., Ferreira, D. and Hemingway, R. W. (1994) *Phytochemistry* (in press).
9. Kolodziej, H., Bonefeld, M., Burger, J. F. W., Brandt, E. V. and Ferreira, D. (1991) *Phytochemistry* **30**, 1255.
10. Gujer, R., Magnolato, D. and Self, R. (1986) *Phytochemistry* **25**, 1431.
11. Czochanska, Z., Foo, L. Y., Newman, R. H. and Porter, L. J. (1980) *J. Chem. Soc., Perkin Trans I* 2278.
12. Porter, L. J., Newman, R. H., Foo, L. J., Wong, H. and Hemingway, R. W. (1982) *J. Chem. Soc., Perkin Trans I* 1217.
13. Hemingway, R. W. (1989) In *Chemistry and Significance of Condensed Tannins*. (Hemingway, R.W. and Karchesy, J. J., eds), pp. 265–283. Plenum Press, New York.
14. Agrawal, P. K. and Bansal, M. C. (1989) In *Carbon*-13 *NMR of Flavanoids* (Agrawal, P. K., ed.), pp. 283–364. Elsevier, Amsterdam.