[Chem. Pharm. Bull. 33(8)3142—3152(1985)]

Tannins and Related Compounds. XXXI.[1] Isolation and Characterization of Proanthocyanidins in *Kandelia candel* (L.) DRUCE

FENG-LIN HSU, GEN-ICHIRO NONAKA, and ITSUO NISHIOKA*

Faculty of Pharmaceutical Sciences, Kyushu University 62, 3-1-1 Maidashi, Higashi-ku, Fukuoka 812, Japan

(Received November 12, 1984)

Together with propelargonidin dimers (**14**, **15** and **16**), and procyanidin trimers (**23**, **24** and **25**) of common types, two novel proanthocyanidin dimers, kandelins A-1 (**12**) and A-2 (**13**), and four trimers, kandelins B-1 (**19**), B-2 (**20**), B-3 (**21**) and B-4 (**22**), which all contain a phenylpropanoid substituent in the upper flavan unit, have been isolated from the bark of *Kandelia candel* (L.) DRUCE (Rhizophoraceae). Spectroscopic evidence combined with chemical studies involving acid-catalyzed thiolytic degradation permitted the assignment of their structures. The presence in this plant source of flavan-3-ols, (+)-afzelechin (**2**), (+)-catechin (**3**), (−)-epicatechin (**4**) and (+)-gallocatechin (**5**), known proanthocyanidins B-1 (**8**), B-2 (**9**), C-1 (**17**) and trimer (**18**), and cinchonains Ia (**6**), Ib (**7**), IIa (**10**) and IIb (**11**) was also demonstrated.

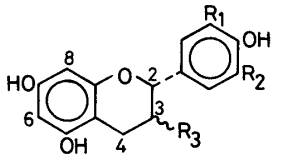
Keywords——*Kandelia candel*; Rhizophoraceae; kandelin; cinchonain; phenylpropanoid-substituted proanthocyanidin; propelargonidin; procyanidin; flavan-3-ol; condensed tannin; thiolytic cleavage

Proanthocyanidins, which occur widely in plants of a woody habit, consist almost entirely of 5,7,3′,4′-tetrahydroxy- and 5,7,3′,4′,5′-pentahydroxyflavan-3-ol units linked through carbon–carbon bonds at C(4)–C(8) or C(4)–C(6), and are of considerable importance because of their biological properties (astringency, enzyme inhibition, tanning of hides, *etc.*), which are all related to their ability to bind with proteins. Much of the chemistry of lower-molecular-weight proanthocyanidins, particularly that of singly linked dimers (B-type), has been elucidated during the past fifteen years.[2] However, owing to difficulties in isolating individual components from a complex mixture of proanthocyanidins, the structures of trimers and higher oligomers are not conclusively established except in a few cases.[3]

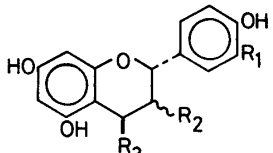
In previous papers, we reported on the isolation from red cinchona (the bark of *Cinchona succirubra* PAVON *et* KLOTZSCH) of a new class of dimeric proanthocyanidins, cinchonains IIa and IIb, in which a phenylpropanoid (C_6–C_3) moiety is attached to the A-ring of the upper flavan units,[4] and of accompanying monomeric flavan-3-ol (epicatechin) derivatives substituted in the A-ring with a similar C_6–C_3 moiety.[5] In further chemical studies on tannins and related compounds, we have now isolated a series of cinchonain-type proanthocyanidins from the bark of *Kandelia candel* (L.) DRUCE (Rhizophoraceae), which is a mangrove growing on tropical coasts and is regarded as a rich source of tannins. In addition, the concomitant isolation of proanthocyanidin dimers with a rare 5,7,4′-trihydroxyl substitution system, and trimers where the points of the interflavanoid linkages differ, has been achieved. This paper reports the details of the isolation and characterization of these compounds.

Earlier work showed that proanthocyanidins could be separated in their free forms by a combination of adsorption (Sephadex LH-20 dextran gel) and partition (high-porosity polystyrene gel: MCI-gel CHP-20P) chromatography. With various solvent systems (EtOH, H_2O–MeOH, acetone, EtOH–H_2O–acetone,[6] *etc.*), the former chromatography allows in principle the fractionation of proanthocyanidins according to their degree of

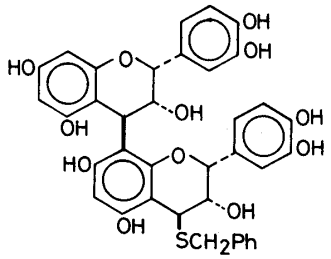
polymerization,[2b,6] while the latter permits the separation of structural isomers.[4,5] Similar Sephadex LH-20 chromatography of the bark polyphenols of *Kandelia candel* yielded from earlier fractions monomeric flavan-3-ol derivatives and simple phenolic compounds, which were successfully separated by MCI-gel chromatography to give chlorogenic acid (**1**), (+)-afzelechin (**2**), (+)-catechin (**3**), (−)-epicatechin (**4**), (+)-gallocatechin (**5**), and cinchonains



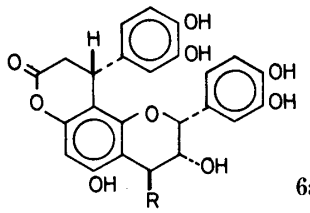
2 : R_1 =H, R_2=H, R_3=—OH
3 : R_1 =H, R_2=OH, R_3=—OH
4 : R_1 =H, R_2=OH, R_3=---OH
5 : R_1 =OH, R_2=OH, R_3=—OH



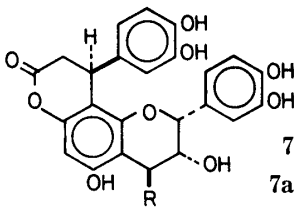
2a : R_1 =H, R_2=—OH, R_3=SCH_2Ph
4a : R_1 =OH, R_2=---OH, R_3=SCH_2Ph



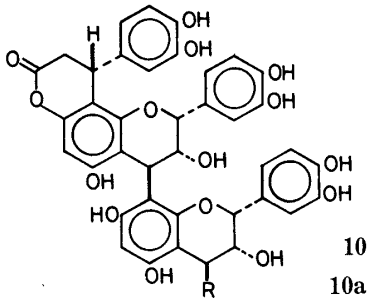
9a



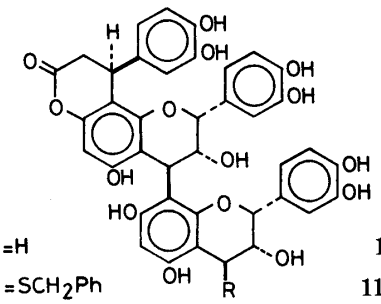
6 : R=H
6a : R=SCH_2Ph



7 : R=H
7a : R=SCH_2Ph



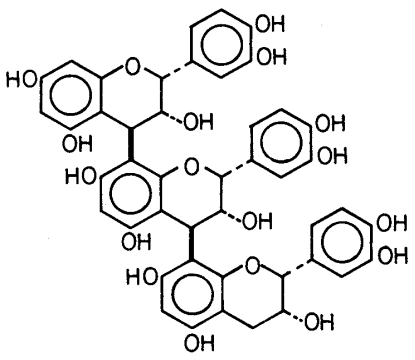
10 : R=H
10a : R=SCH_2Ph



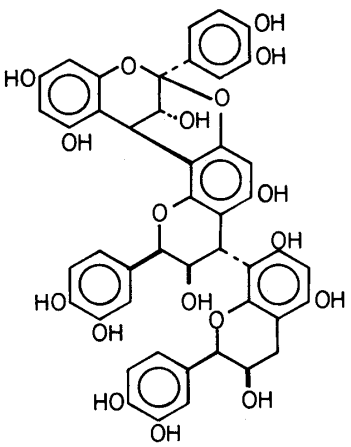
11 : R=H
11a : R=SCH_2Ph

Ia (**6**) and Ib (**7**).[5] A dimeric proanthocyanidin fraction was also obtained by repeated chromatography over Sephadex LH-20. This complex mixture was separated by combined chromatography over MCI-gel and LiChropreps RP-8 and CN to furnish nine compounds (**8**—**16**), of which four were found to be identical with procyanidins B-1 (**8**) and B-2 (**9**),[2b,d,5] and cinchonains IIa (**10**) and IIb (**11**)[4] by comparisons of their chromatographic and spectral data with those of authentic samples. Similar reverse-phase chromatography of trimeric flavanoids eluted later from Sephadex LH-20 yielded compounds **17**—**25**, and by comparisons of the chromatographic properties and ^{1}H-nuclear magnetic resonance (^{1}H-NMR) spectra, compounds **17** and **18** were identified as the C(4)–C(8) linked trimer, procyanidin C-1,[2b,4] and the trimer containing a doubly bonded proanthocyanidin A-2 moiety,[7] respectively.

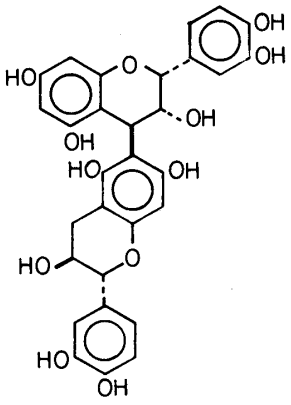
The ^{13}C-nuclear magnetic resonance (^{13}C-NMR) spectra of compounds **12** and **13** were almost indistinguishable from each other, and were closely related to those of **10** and **11**. The somewhat lowfield shift (δ 81.7, 82.9 in **12** and **13**; δ 78.9, 79.6 in **10** and **11**, respectively) of one of the two flavan C(2)-carbons in the spectra of **12** and **13** suggested the presence of a flavan-3-ol with catechin [C(2), C(3): *trans*] stereochemistry in each molecule.[8] The ^{1}H-NMR spectrum of **12** clearly differed from that of **13**. The broadness of each signal and a lowfield



17



18

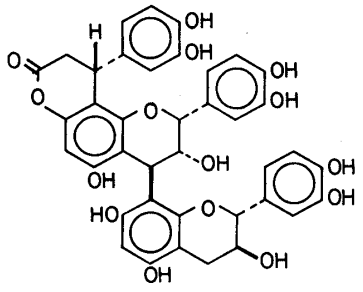


26

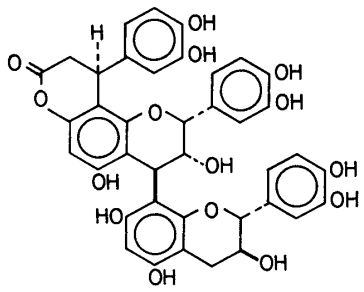
shift (δ 5.44, s) of the flavan C(2)-proton in the spectrum of **13**, consistent with those observed in **11**,[4] implied the occurrence of steric interaction between the aromatic ring in the phenylpropanoid moiety and flavan units. The ^{1}H-NMR spectrum of **12**, on the other hand, showed sharp signals, and was almost identical with that of **10** except for the presence of a doublet signal (δ 4.91, J=6 Hz) due to the flavan C(2)-proton in place of a singlet signal.

The component units of each compound were characterized by acid-catalyzed degradation with benzylmercaptan,[2b] which afforded (+)-catechin (**3**) and a benzylthioether (**6a** from **12** and **7a** from **13**) as the only products: the products **6a** and **7a** were found to be identical with the 4-benzylthioethers of cinchonains Ia and Ib, respectively, which were previously obtained by similar thiolytic degradation of cinchonains IIa (**10**) and IIb (**11**).[4]

As mentioned above, the ^{1}H-NMR spectra of **12** and **13** were closely related to those of **10** and **11**, respectively. In particular, the chemical shifts (Table I) of two A-ring protons were almost identical in each case, suggesting that the interflavanoid linkage in both compounds is C(4)–C(8). The lower-field shift (δ 4.91 in **12** and δ 4.70 in **13**) of the C(2)-proton in the catechin moiety than that (δ 4.55) of (+)-catechin (**3**) indicates that this proton is magnetically affected by the aromatic ring in the upper flavan unit. The chemical shift of this proton was consistent with that (δ 4.76) of procyanidin B-1 (**8**) rather than that (δ 4.52) of the alternative C(4)–C(6) linked dimer, procyanidin B-7 (**26**).[3a] Based on these observations, the points of the interflavanoid linkage was concluded to be C(4)–C(8). Thus, the structures **12** and **13** were assigned to these compounds (now designated as kandelins A-1 and A-2, respectively).



12



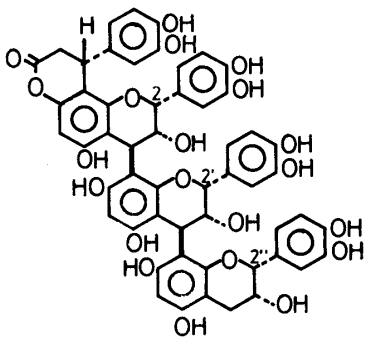
13

The triflavanoid constitution of the compounds **19**, **20** and **21** (named kandelins B-1, B-2 and B-3, respectively) was easily deduced from the appearance in their ^{1}H-NMR spectra of three lowfield signals due to the flavan C(2)-protons and of three aromatic resonances around 6 ppm arising from the A-ring. The presence of a pair of three aliphatic carbon resonances

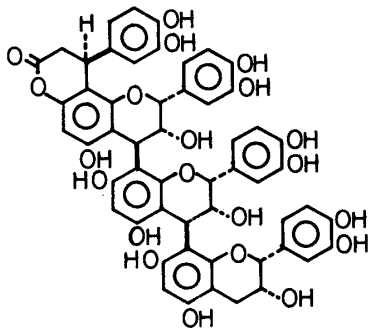
(δ 66.1—79.0) attributed to the flavan C(2) and C(3) also indicated their trimeric nature. The chemical shifts (δ 76.4—79.5) of the C(2)-carbons, as well as the coupling patterns of the C(2)-proton signals (singlets in each case), suggested that they all contain flavan-3-ol units with epicatechin [C(2), C(3): *cis*] stereochemistry.[8]

In contrast, the ^{1}H- and ^{13}C-NMR spectra of compound **22** (kandelin B-4) differed from those of **19**, **20** and **21** in the presence of a doublet (δ 4.52, J=7 Hz) due to the C(2)-proton instead of the singlet, and one C(2)-carbon resonance shifted relatively downfield (δ 82.3), suggesting the existence of a catechin moiety in **22**.

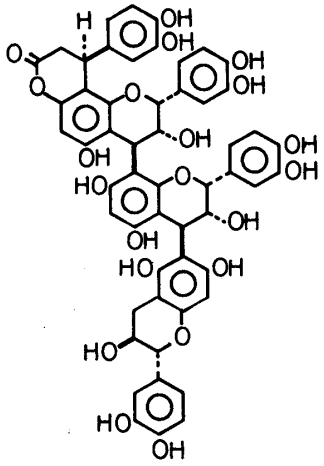
The presence of a phenylpropanoid moiety in each molecule was implied by ^{13}C-resonances [an ester carbon (δ 170.1), a methine carbon (δ 34.3) β to the carboxyl group, *etc.*] analogous to those found in cinchonains.[4,5] In **19**, **20** and **22**, its location was considered to be limited to the upper flavan unit since each ^{1}H-NMR spectrum showed singlets due to the A-



19



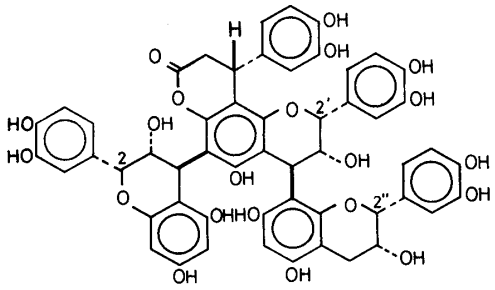
20



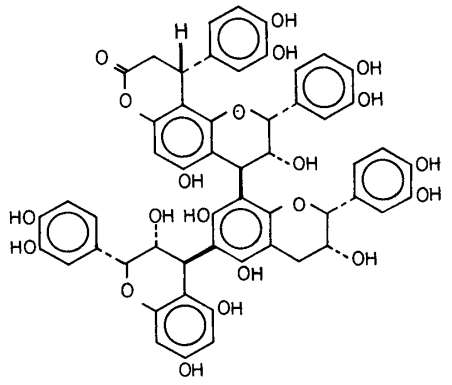
22

ring protons. On the other hand, the appearance in **21** of a pair of *meta*-coupled doublets (δ 6.03, 6.10, each d, J=2 Hz) suggested that the C_6–C_3 unit is present in the lower flavan units.

The constitution and the points of the interflavanoid linkages in each compound were established by acid-catalyzed degradation with benzylmercaptan. Complete degradation of **19** and **21** afforded (−)-epicatechin (**4**) and the benzyl sulfides (**6a**)[4] and (**4a**),[2b,d] establishing that they consist of cinchonain Ia and (−)-epicatechin units. Partial degradation of **19** gave, in addition to the above products, procyanidin B-2 (**9**)[2b,d] and a benzyl sulfide (**10a**), while that of **21** yielded **4a** and **10**. The structure of the benzyl sulfide (**10a**) was characterized by



21



21′

TABLE I. ^{1}H-NMR Data for Kandelins and Cinchonains[a)]

	12	13	19	20	21	22	6	7	10	11
α-H	3.04 (m)	2.2—3.2 (m)	3.1 (m)	2.4—3.1 (m)	3.1 (m)	2.4—3.1 (m)	2.85 (dd, J=2, 16), 3.12 (dd, J=6, 16)	2.72—3.18 (m)	2.6—3.2 (m)	2.5—3.0 (m)
β-H	4.68 (m)	3.90 (m)	4.74 (m)	4.30 (m)	4.76 (m)	4.28 (m)	4.54 (dd, J=2, 16)	4.47 (dd, J=2, 6)	4.63 (dd, J=2, 6)	4.04 (m)
2-H	5.26 (s)	5.44 (br s)	5.22 (br s)	5.66 (br s)	4.94 (br s)	5.68 (br s)	4.89 (s)	4.99 (s)	5.24 (s)	5.66 (br s)
3-H	4.04 (br s)	3.99 (br s)	4.10 (m)	3.75 (br s)	3.98 (br s)	3.80 (br s)	4.30 (m)	4.26 (m)	4.02 (m)	4.03 (m)
4-H	4.84 (br s)	4.70 (br s)	4.92 (br s)	4.56 (br s)	4.68 (br s)	4.58 (br s)	2.90 (m)	2.90 (m)	4.86 (br s)	4.36 (br s)
6-H	6.21 (s)	6.21 (s)	6.26 (s)	6.30 (s)	6.03 (d, J=2)	6.32 (s)	6.24 (s)	6.24 (s)	6.19 (s)	6.20 (s)
8-H					6.10 (d, J=2)					
2′-H	4.91 (d, J=6)	4.70 (m)	5.22 (br s)	4.94 (br s)	5.06 (br s)	4.72 (br s)			5.03 (br s)	4.72 (br s)
3′-H	4.12 (m)	3.90 (m)	4.10 (m)	4.16 (br s)	3.98 (br s)	4.16 (br s)			4.31 (m)	3.88 (m)
4′-H	2.70 (m)	2.2—3.2 (m)	4.82 (br s)	4.70 (br s)	4.62 (br s)	4.50 (br s)			2.6—3.2 (m)	2.5—3.0 (m)
6′-H	5.98 (s)	6.02 (s)	6.04 (s)	6.04 (s)		6.18 (s)			5.97 (s)	5.99 (s)
2″-H			5.06 (br s)	4.70 (br s)	4.94 (br s)	4.52 (d, J=7)				
3″-H			4.40 (m)	4.18 (m)	4.30 (m)	3.98 (m)				
4″-H			3.90 (m)	2.4—3.1 (m)	2.5—2.9 (m)	2.4—3.1 (m)				
6″-H			6.04 (s)	5.90 (s)	5.96 (s)	6.01 (s)				

a) Spectra were taken in acetone-d_6 + D_2O at 100 MHz, s, singlet; d, doublet; m, multiplet; br, broad. J-values are expressed in Hz (in parentheses).

desulfurization with Raney-nickel to yield **10**. Thus, the interflavanoid linkages in **19** were established unequivocally as C(4)–C(8). On the other hand, from the above chemical evidence two alternative structures (**21** and **21'**) were considered for compound **21**, and comparison of the chemical shift of the A-ring signals with those of cinchonains and compounds **12**, **13** and **19** permitted the assignment of the structure. In the ^{1}H-NMR spectrum of **21**, the A-ring signal appeared as a singlet at δ 5.96, together with the above-mentioned two *meta*-coupled signals, and the chemical shift of the singlet was in close agreement with those of the A-ring signals of the lower epicatechin moieties in **12**, **13** and **19** (Table I). In addition, the absence of a singlet due to the proton of the A-ring with the C_6–C_3 unit, which almost invariably appeared relatively downfield (Table I), confirmed the structure as **21**.

On similar partial degradation, **20** and **22** yielded the same benzyl sulfide (**11a**), which was characterized by desulfurization with Raney-nickel, giving **11**. The production of **9** from **20** established the lower interflavanoid linkage to be C(4)–C(8), while the formation of procyanidin B-7 (**26**) from **22** indicated a C(4)–C(6) linkage for the trimer. Based on these chemical findings, the structures of kandelins B-2 and B-4 were established as **20** and **22**, respectively.

The biflavanoid constitution of compounds **14**, **15** and **16** was confirmed by mass spectrometry (MS) of their methyl ethers (M^+: *m/z* 630 in **14a** and *m/z* 660 in **15a** and **16a**). The ^{1}H-NMR spectra of these compounds were complicated by rotational isomerism caused by steric interaction between the two flavan units. However, the signal patterns of the two flavan C-rings in each of **14** and **15** were similar to those observed in procyanidin B-3 (**27**),[2b,f] and the spectrum of **16**, measured at elevated temperature (150 °C), closely resembled that (taken at 150 °C) for procyanidin B-4 (**28**)[2b] except for the aromatic signals arising from the B-rings. The MS of **15a** and **16a** showed similar fragmentation patterns, with a substantial fragment ion (*m/z* 479) formed by a retro-Diels–Alder type cleavage of the lower flavan C-ring (Chart 1). A similar fragment peak was also observed at *m/z* 481 in the case of **14**. These

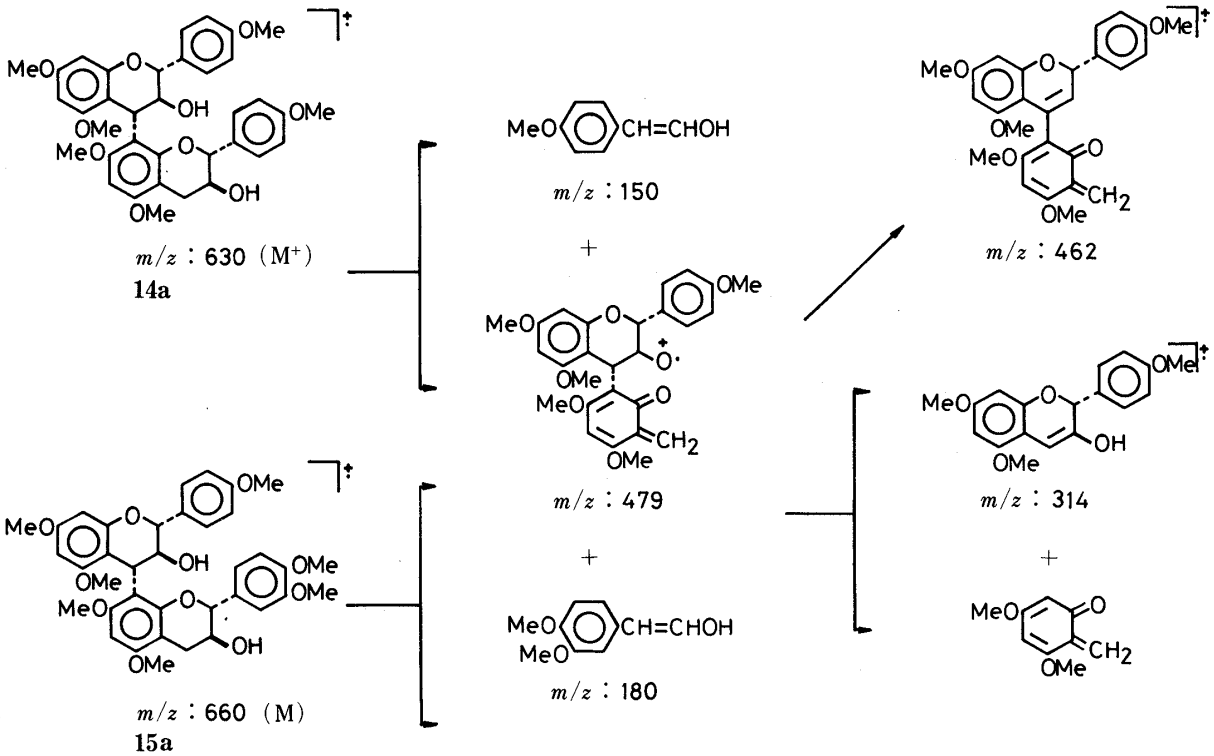


Chart 1

observations suggested that **14** and **15** are structurally related to **27**, but lack B-ring hydroxyl group(s), and that **16** possesses a procyanidin B-4 type structure in which a hydroxyl group in the upper B-ring is absent.

Unequivocal proofs for the structures of these compounds were obtained by specific cleavage of the interflavanoid bond with benzylmercaptan in the presence of acid. In each case, a benzyl sulfide (**2a**) characterized by converting it into (+)-afzelechin (**2**) with Raney-nickel was produced, thus establishing the upper flavan unit as **2**. In addition, the formation of **2**, **3** and **4** from the lower half led to the assignment of the structures of **14**, **15** and **16**, respectively.

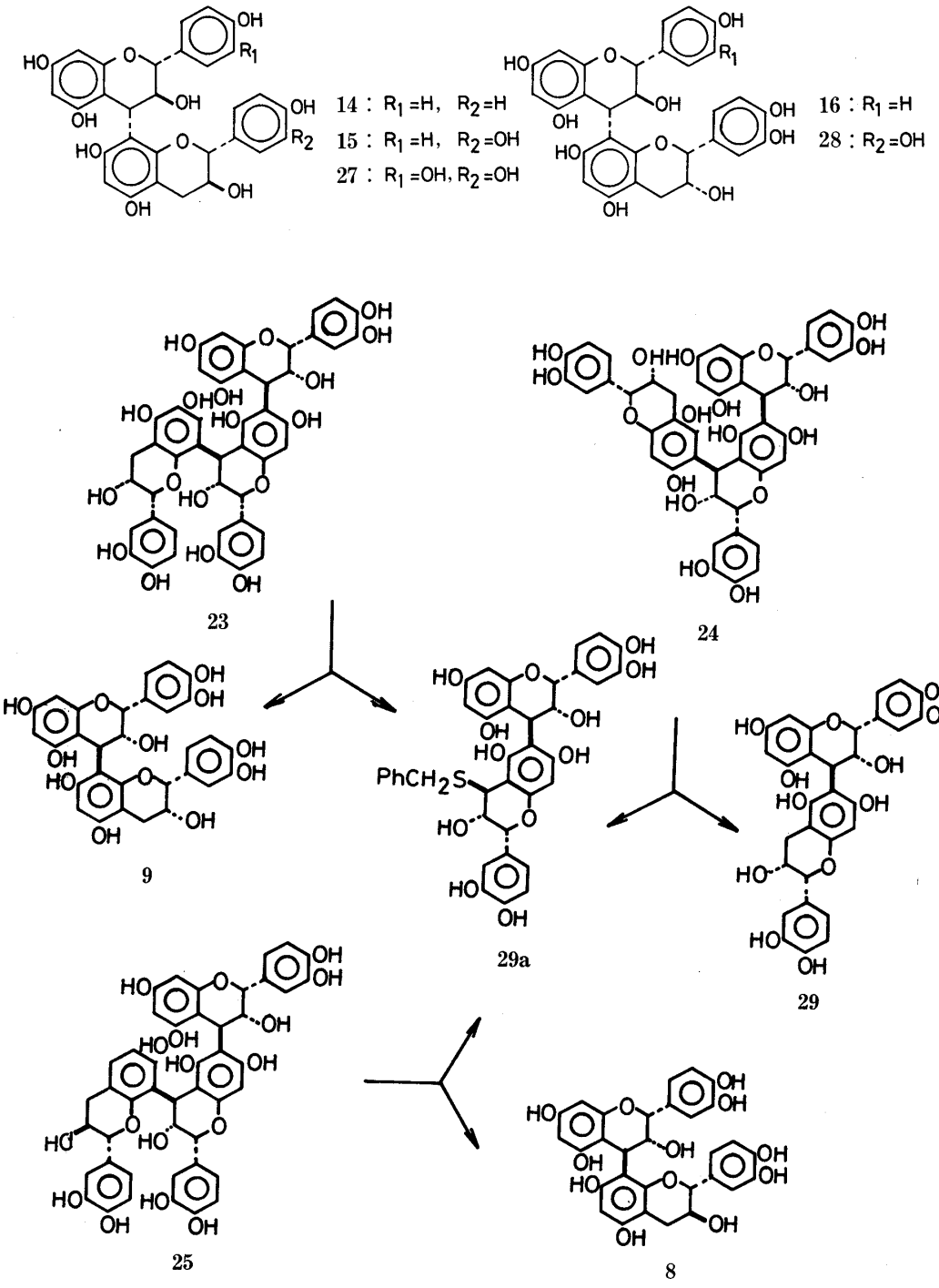


Chart 2

For the characterization of compounds **23**, **24** and **25**, similar spectroscopic and chemical degradation methods were used. In the ^{13}C-NMR spectra, the observation of a pair of three aliphatic methine signals due to flavan C(2)- and C(3)-carbons confirmed their triflavanoid constitution. The chemical shifts (δ 76.6—80.0) of the C(2)-carbons in **23** and **24** suggested that they consist entirely of epicatechin units, and a lowfield resonance (δ 81.8) in the spectrum of **25** implied the presence of a catechin moiety. Partial degradation of these trimers yielded dimeric procyanidin benzyl sulfides and procyanidin dimers (see Chart 2). The structures of the products were confirmed by direct comparisons with authentic samples, or by conversion into procyanidin B-5 (**29**) in the case of procyanidin B-5 4-benzylthioether (**29a**). On the basis of these observations, the structures were concluded to be epicatechin-(4β→6)-epicatechin-(4β→8)-epicatechin (**23**) epicatechin-(4β→6)-epicatechin-(4β→6)-epicatechin (**24**) and epicatechin-(4β→6)-epicatechin-(4β→8)-catechin (**25**).[9] Compound **25** is probably identical with the trimer isolated by Hemingway *et al.* from the phloem of loblolly pine (*Pinus taeda*).[3b]

The occurrence of dimeric and trimeric proanthocyanidins with a variety of component units linked randomly at C(4)–C(8) and/or C(4)–C(6) in the bark of *Kandelia candel* suggests that more complex composition and linkage isomerism exist in higher oligomeric and polymeric proanthocyanidins of this plant. We are currently examining several biological activities of the proanthocyanidins isolated in this study.

Experimental

Optical rotations were measured with a JASCO DIP-4 digital polarimeter. Infrared (IR) spectra were obtained with a JASCO DS-301 spectrometer, and MS with a JEOL spectrometer. ^{1}H- and ^{13}C-NMR spectra were taken with JEOL PS-100 and FX-100 spectrometers, respectively, using tetramethylsilane as an internal standard, and chemical shifts are given in δ (ppm). Thin-layer chromatography (TLC) was conducted on precoated Kieselgel 60 F_{254} plates (0.20 mm thick, Merck) with benzene–ethyl formate–formic acid (2:7:1 or 1:7:1), and spots were detected by spraying the plates with 2% ethanolic ferric chloride, 10% H_2SO_4 and anisaldehyde–sulfuric acid reagents. Column chromatography was carried out with Sephadex LH-20 (25—100 μ, Pharmacia Fine Chemical Co., Ltd.), MCI-gel CHP-20P (75—150 μ, Mitsubishi Chemical Industries Ltd.), and LiChropreps RP-8 and CN (40—63 μ, Merck). High-performance liquid chromatography (HPLC) was performed with a Toyo Soda apparatus equipped with an SP 8700 solvent delivery system, a UV-8 model II spectrometer and a TSK-410 column (4 mm i.d. × 300 mm).

Isolation——Dry bark (3.0 kg) of *Kandelia candel* (L.) DRUCE, collected in August at Taipei, Republic of China, was extracted six times with 70% aqueous acetone. The aqueous solution, after removal of the acetone by concentration under reduced pressure, was extracted with *n*-BuOH. The *n*-BuOH layer was separated, and mixed with Celite 545 (1.5 kg), then the solvent was evaporated off under reduced pressure. A brown powder (2.35 kg) thus obtained was packed in a glass column. Elution with acetone and evaporation gave a dark brown powder, which was chromatographed over Sephadex LH-20 with H_2O containing increasing amounts of MeOH to furnish five fractions. Fraction I consisted of compounds negative to the ferric chloride reagent (probably sugars, amino acids, proteins, waxes, *etc.*). Fraction II containing relatively non-polar phenolic compounds was rechromatographed over Sephadex LH-20 with EtOH to give chlorogenic acid (**1**) (*ca.* 10 g) and compound **14** (191 mg). Chromatography of fraction III (60 g) over Sephadex LH-20 with EtOH gave two fractions, which were separately rechromatographed over MCI-gel CHP-20P with a mixture of H_2O–MeOH (7:3) to yield (+)-catechin (**3**) (*ca.* 10 g), (−)-epicatechin (**4**) (*ca.* 8 g), (+)-afzelechin (**2**) (350 mg), and cinchonains Ia (**6**) (300 mg) and Ib (**7**) (125 mg) from the earlier fraction, and (+)-gallocatechin (**5**) (129 mg), procyanidins B-1 (**8**)[3a] (1.1 g) and B-2 (**9**)[2d] (4.5 g), cinchonain IIb (**10**)[4] (615 mg), compound **15** (150 mg) and procyanidin C-1 (**17**)[4] (200 mg) from the later fraction. Fraction IV gave, on chromatography over Sephadex LH-20 with EtOH, three further fractions (Frs. IV-a, IV-b and IV-c). Separation of fraction IV-a by repeated chromatography over Sephadex LH-20 (EtOH, 60% aqueous MeOH) and LiChroprep CN [H_2O–MeOH (9:1)] afforded cinchonains IIa (**10**) (122 mg) and IIb (**11**) (348 mg), and compounds **13** (89 mg) and **16** (63 mg). Fraction IV-b consisting of cinchonains was separated by MCI-gel CHP-20P (70% aqueous MeOH) and LiChroprep RP-8 (75% aqueous MeOH) chromatographies to give cinchonains IIa (**10**) (944 mg) and IIb (**11**) (31 mg), and compound **12** (50 mg). Fraction IV-c contained a complex mixture of proanthocyanidin trimers, and was separated by repeated chromatography over Sephadex LH-20 (EtOH, 60% aqueous MeOH), MCI-gel CHP-20P (70% aqueous MeOH) and LiChroprep CN (10% aqueous MeOH) to furnish compounds **17** (168 mg), **18**[7] (25 mg), **19** (336 mg), **20** (520 mg), **21** (50 mg), **22** (38 mg), **23** (280 mg), **24** (26 mg) and **25** (77 mg).

Kandelin A-1 (12)——An off-white amorphous powder, $[\alpha]_D^{20}$ −57.5° (c=0.6, acetone). *Anal.* Calcd for

TABLE II. ^{13}C-NMR Data for Kandelins and Cinchonains[e)]

	12	13	19	20	21	22	6	7	10	11
α-C	37.8	37.0	38.1	37.0	38.0	37.4	38.0	37.6	38.0	36.6
β-C	34.4	34.3	33.9	34.3	34.3	34.3	34.5	34.2	34.0	34.3
C-2	76.7	76.5	76.4	76.8	76.5[b)]	76.6[a)]	79.0	79.4	76.4	76.4
C-3	72.0	72.3	71.8[a)]	72.3	72.4[c)]	72.1	65.8	66.0	72.0	72.2
C-4	36.4	36.6	36.7	36.8	37.2[a)]	37.1	28.9	28.8	36.4	36.4
C-2′	81.7	82.9	76.4	77.3	76.8[b)]	77.3[a)]			78.9	79.6
C-3′	67.7	69.2	72.4[a)]	72.3	73.0[c)]	72.1			66.1	66.6
C-4′	29.8	30.0	36.7	36.8	37.6[a)]	37.1			28.8	—[d)]
C-2″			79.0	78.9	78.9	82.3				
C-3″			66.1	66.1	66.2	68.2				
C-4″			29.2	28.4	29.6	29.3				
–COO–	168.4	169.9	170.1	170.1	170.1	170.1	168.9	168.9	169.2	169.6

Assignments with the superscript *a*), *b*) or *c*) may be interchanged in each column. *d*) Overlapped with solvent signals. *e*) Spectra were measured in acetone-d_6+D_2O at 25.05 MHz.

$C_{39}H_{32}O_{15}\cdot 2H_2O$: C, 60.31; H, 4.67. Found: C, 60.50; H, 4.88. ^{1}H-NMR data are given in Table I. ^{13}C-NMR (acetone-d_6+D_2O): 95.4, 96.7 (C-6, C-6′), 100.0 (C-4a′), 104.6 (C-4a), 107.3, 107.8 (C-8, C-8′). Signals of flavan C-rings and a carboxyl carbon are listed in Table II.

Kandelin A-2 (13)——An off-white amorphous powder, $[\alpha]_D^{20}$ +9.3° (*c*=0.9, acetone). *Anal.* Calcd for $C_{39}H_{32}O_{15}\cdot H_2O$: C, 61.74; H, 4.49. Found: C, 61.73; H, 4.70. ^{1}H-NMR: Table I. ^{13}C-NMR (acetone-d_6+D_2O): 95.1, 96.5 (C-6, C-6′), 101.6 (C-4a′), 104.8 (C-4a), 108.7 (C-8, C-8′). Other signals are given in Table II.

Afzelechin-4(α→8)-afzelechin (14)——An off-white amorphous powder, $[\alpha]_D^{28}$ −226.7° (*c*=1.2, acetone). *Anal.* Calcd for $C_{30}H_{26}O_{10}\cdot 3/2H_2O$: C, 62.83; H, 5.06. Found: C, 62.77; H, 5.30. The ^{1}H- and ^{13}C-NMR spectra were complicated by rotational isomerism. The hexamethyl ether (**14a**), prepared by methylation with dimethyl sulfate and potassium carbonate in dry acetone, had the following properties; a white amorphous powder, $[\alpha]_D^{28}$ −155.7° (*c*=1.3, $CHCl_3$). *Anal.* Calcd for $C_{36}H_{38}O_{10}\cdot 1/2H_2O$: C, 67.61; H, 6.10. Found: C, 67.30; H, 5.80. MS *m/z*: 630 $(M)^+$, 481, 479, 462, 314, 165, 150.

Afzelechin-(4α→8)-catechin (15)——An off-white amorphous powder, $[\alpha]_D^{28}$ −189.6° (*c*=0.5, acetone). *Anal.* Calcd for $C_{30}H_{26}O_{11}\cdot 3/2H_2O$: C, 61.12; H, 4.92. Found: C, 61.32; H, 5.19. The ^{1}H- and ^{13}C-NMR spectra showed complex signal patterns due to rotational isomerism. The hexamethyl ether (**15a**): a white amorphous powder, $[\alpha]_D^{28}$ −163.0° (*c*=1.0, $CHCl_3$). *Anal.* Calcd for $C_{37}H_{40}O_{11}\cdot 1/2H_2O$: C, 66.36; H, 6.13. Found: C, 66.59; H, 5.89. MS *m/z*: 660 $(M)^+$, 479 (base peak), 462, 314, 180, 165.

Afzelechin-(4α→8)-epicatechin (16)——An off-white amorphous powder, $[\alpha]_D^{26}$ −164.2° (*c*=0.55, acetone). *Anal.* Calcd for $C_{30}H_{26}O_{11}\cdot 3/2H_2O$: C, 61.12; H, 4.92. Found: C, 60.84; H, 4.96. The assignments of the ^{1}H- and ^{13}C-NMR spectral signals could not be made owing to complicated signal patterns caused by non-bonded interaction between the two flavan units.

Kandelin B-1 (19)——An off-white amorphous powder, $[\alpha]_D^{28}$ +36.0° (*c*=1.0, acetone). *Anal.* Calcd for $C_{54}H_{44}O_{21}\cdot 2H_2O$: C, 60.90; H, 4.54. Found: C, 61.02; H, 4.76. ^{1}H-NMR: Table I. ^{13}C-NMR (acetone-d_6+D_2O): 95.8, 97.1, 97.4 (C-6, C-6′, C-6″), 100.4, 101.0 (C-4a′, C-4a″), 104.8 (C-4a), 106.9, 107.1 (C-8′, C-8″), 107.9 (C-8). Other signals are given in Table II.

Kandelin B-2 (20)——An off-white amorphous powder, $[\alpha]_D^{28}$ +163.6° (*c*=1.0, acetone). *Anal.* Calcd for $C_{54}H_{44}O_{21}\cdot 7/2H_2O$: C, 59.39; H, 4.67. Found: C, 59.07; H, 4.82. ^{1}H-NMR: Table I. ^{13}C-NMR (acetone-d_6+D_2O): 95.4, 96.8, 97.2 (C-6, C-6′, C-6″), 99.5 (C-4a′), 100.6 (C-4a″), 105.3 (C-4a), 106.7 (C-8″), 108.5 (C-8, C-8′). Other signals are listed in Table II.

Kandelin B-3 (21)——An off-white amorphous powder, $[\alpha]_D^{20}$ +65.0° (*c*=0.5, acetone). *Anal.* Calcd for $C_{54}H_{44}O_{21}\cdot 7/2H_2O$: C, 59.39; H, 4.67. Found: C, 59.08; H, 4.98. ^{1}H-NMR: Table I. ^{13}C-NMR (acetone-d_6+D_2O): 96.2, 96.7 (C-6′, C-6″, C-8), 100.2 (C-4a, C-4a″), 105.5 (C-4a′), 107.0 (C-6′, C-8″), 108.7 (C-8′). Other signals are given in Table II.

Kandelin B-4 (22)——An off-white amorphous powder, $[\alpha]_D^{20}$ +197.6° (*c*=0.5, acetone). *Anal.* Calcd for $C_{54}H_{44}O_{21}\cdot 7/2H_2O$: C, 59.39; H, 4.67. Found: C, 59.26; H, 4.96. ^{1}H-NMR: Table I. ^{13}C-NMR (acetone-d_6+D_2O): 95.3, 95.9, 96.6 (C-6, C-6′, C-8″), 98.5 (C-4a″), 101.2 (C-4a′), 105.2 (C-4a), 107.7 (C-6″), 108.5 (C-8, C-8′). Other signals are listed in Table II.

Epicatechin-(4β→6)-epicatechin-(4β→8)-epicatechin (23)——An off-white amorphous powder, $[\alpha]_D^{28}$ +138.0° (*c*=1.0, acetone). *Anal.* Calcd for $C_{45}H_{38}O_{18}\cdot 5/2H_2O$: C, 59.27; H, 4.72. Found: C, 59.23; H, 5.09. ^{1}H-NMR

(acetone-d_6+D_2O): 2.50—3.10 (2H, m, H-4″), 3.94 (2H, br s, H-3, H-3′), 4.32 (1H, m, H-3″), 4.60 (2H, br s, H-4, H-4′), 4.80—5.10 (3H in total, m, H-2, H-2′, H-2″), 5.84—6.30 (4H in total, H-6, H-8, H-8′, H-6″), 6.48—7.24 (9H in total, B-ring H). ^{13}C-NMR (acetone-d_6+D_2O): 29.2 (C-4″), 37.2 (C-4, C-4′), 66.1 (C-3″), 72.3, 72.7 (C-3, C-3′), 76.6 (C-2, C-2′), 79.0 (C-2″), 95.9, 96.5 (C-6, C-8, C-8′, C-6″), 99.9 (C-4a′), 100.5 (C-4a, C-4a″), 106.5, 107.0 (C-6′, C-8″).

Epicatechin-(4β→6)-epicatechin-(4β→6)-epicatechin (24)——An off-white amorphous powder, $[\alpha]_D^{20}$ +127.8° (c=0.5, acetone). *Anal.* Calcd for $C_{45}H_{38}O_{18}\cdot 3H_2O$: C, 58.59; H, 5.01. Found: C, 58.69; H, 4.78. ^{1}H-NMR (acetone-d_6+D_2O): 2.50—3.10 (2H, m, H-4″), 3.96 (2H, br s, H-3, H-3′), 4.32 (1H, m, H-3″), 4.46 (1H, br s, H-4′), 4.82 (1H, br s, H-4), 4.89 (2H, br s, H-2′, H-2″), 4.94 (1H, br s, H-2), 6.00—6.20 (4H in total, H-6, H-8, H-8′, H-8″), 6.44—7.24 (9H in total, B-ring H). ^{13}C-NMR (acetone-d_6+D_2O): 29.4 (C-4″), 37.2, 37.5 (C-4, C-4′), 66.1 (C-3″), 72.3, 72.7 (C-3, C-3′), 76.9, 77.2 (C-2, C-2′), 79.0 (C-2″), 95.9, 96.5, 96.8 (C-6, C-8, C-8′, C-8″), 98.5 (C-4a′, C-4a″), 101.3 (C-4a), 107.2, 107.7 (C-6′, C-6″).

Epicatechin-(4β→6)-epicatechin-(4β→8)-catechin (25)——An off-white amorphous powder, $[\alpha]_D^{21}$ +138.2° (c=0.5, acetone). ^{1}H-NMR (acetone-d_6+D_2O): 2.40—3.10 (2H, m, H-4″), 3.96 (2H, br s, H-3, H-3′), 4.28 (1H, m, H-3″), 4.42—4.76 (3H, m, H-2″, H-4, H-4′), 4.88, 4.96 (each 1H, br s, H-2, H-2′), 5.90—6.28 (4H, H-6, H-8, H-8′, H-6″), 6.50—7.20 (9H in total, B-ring H). ^{13}C-NMR (acetone-d_6+D_2O): 28.8 (C-4″), 37.2 (C-4, C-4′), 67.6 (C-3″), 72.3, 72.5 (C-3, C-3′), 76.7, 76.9 (C-2, C-2′), 81.8 (C-2″), 95.9, 96.6 (C-6, C-8, C-8′, C-6″), 98.9 (C-4a′), 101.0 (C-4a, C-4a″), 107.6 (C-6, C-8″).

General Procedure for Thiolytic Cleavage——a) Complete Degradation: A mixture of a sample (20—50 mg), benzylmercaptan (0.2 ml), acetic acid (0.1 ml) and EtOH (3 ml) was refluxed for 8 h with stirring. After removal of the solvent by evaporation under reduced pressure, the oily residue was applied to a Sephadex LH-20 column. Elution with $CHCl_3$–EtOH (4 : 1) afforded benzylthioether(s). Further elution with $CHCl_3$–EtOH (2 : 1) yielded a flavan-3-ol.

b) Partial Degradation: A mixture of a sample (100—200 mg), benzylmercaptan (0.3 ml), acetic acid (0.15 ml) and EtOH (4 ml) was heated under reflux for 3—5 h. The solution was concentrated under reduced pressure, and the residue was chromatographed over Sephadex LH-20 using $CHCl_3$–EtOH (4 : 1). Stepwise elution with an increasing amount of EtOH yielded monomeric benzylthioether(s), a dimeric benzylthioether and a proanthocyanidin.

The known benzylthioethers and flavan-3-ols (proanthocyanidins) were identified by direct comparisons of their physical and spectral data with those of authentic samples. The physical and spectroscopic properties of the unreported benzylthioethers are as follows.

(+)-Afzelechin 4-Benzylthioether (**2a**): An off-white amorphous powder, $[\alpha]_D^{20}$ +51.0° (c=1.0, acetone). *Anal.* Calcd for $C_{22}H_{20}O_5S\cdot 1/2H_2O$: C, 65.19; H, 5.19. Found: C, 65.54; H, 5.68. ^{1}H-NMR (acetone-d_6): 4.12 (2H, s, –SCH_2–), 4.40 (1H, d, J=4 Hz, H-4), 5.00 (1H, d, J=8 Hz, H-2), 5.83 (1H, d, J=2 Hz, H-6), 6.04 (1H, d, J=2 Hz, H-8), 6.85 (2H, d, J=8 Hz, H-2′, H-6′), 7.32 (2H, d, J=8 Hz, H-3′, H-5′), 7.19—7.49 (5H in total, aromatic H).

Cinchonain IIa 4′-Benzylthioether (**6a**): A tan amorphous powder, $[\alpha]_D^{28}$ −16.1° (c=0.5, acetone). *Anal.* Calcd for $C_{46}H_{38}O_{15}S\cdot 5/2H_2O$: C, 60.86; H, 4.74. Found: C, 60.78; H, 4.28. ^{1}H-NMR (acetone-d_6+D_2O): 3.07 (1H, m, α-H), 4.01 (2H, m, H-3, H-3′), 4.07 (2H, s, –SCH_2–), 4.18 (1H, d, J=2 Hz, H-4′), 4.68 (1H, m, β-H), 4.87 (1H, d, J=2 Hz, H-4), 5.22 (1H, br s, H-2), 5.42 (1H, br s, H-2′), 6.00 (1H, s, H-6′), 6.19 (1H, s, H-6), 6.55—7.44 (14H in total, aromatic H).

Cinchonain IIb 4′-Benzylthioether (**7a**): A tan amorphous powder, $[\alpha]_D^{28}$ +154.7° (c=1.2, acetone). *Anal.* Calcd for $C_{46}H_{38}O_{15}S\cdot 2H_2O$: C, 61.47; H, 4.68. Found: C, 61.30; H, 4.71. ^{1}H-NMR (acetone-d_6+D_2O): 2.40—2.76 (2H, m, α-H), 3.70 (1H, m, H-3), 3.90 (1H, m, H-3′), 3.96 (2H, br s, –SCH_2–), 4.08 (1H, br s, H-4′), 4.20 (1H, m, β-H), 4.68 (1H, br s, H-4), 4.84 (1H, br s, H-2′), 5.60 (1H, br s, H-2), 6.02 (1H, s, H-6′), 6.24 (1H, s, H-6), 6.40—7.06 (14H in total, aromatic H). ^{13}C-NMR (acetone-d_6+D_2O): 34.3 (β-C), 36.6 (–SCH_2–), 37.2 (α-C, C-4), 44.1 (C-4′), 70.9 (C-3′), 72.1 (C-3), 75.6 (C-2′), 76.5 (C-2), 95.1, 96.7 (C-6, C-6′), 99.8 (C-4a, C-4a′), 105.1 (C-8′), 108.2 (C-8), 169.8 (–COO–).

Procyanidin B-2 4′-benzylthioether (**9a**): A tan amorphous powder, $[\alpha]_D^{28}$ +69.4° (c=1.0, acetone). *Anal.* Calcd for $C_{37}H_{32}O_{12}S\cdot 2H_2O$: C, 60.03; H, 4.89. Found: C, 60.48; H, 4.99. ^{1}H-NMR (acetone-d_6+D_2O): 3.92 (1H, br s, H-3), 4.05 (2H, s, –SCH_2–), 4.02—4.12 (1H, m, H-3′), 4.18 (1H, d, J=2 Hz, H-4′), 4.70 (1H, br s, H-4), 5.08 (1H, br s, H-2), 5.13 (1H, br s, H-2′), 5.96—6.12 (3H, s, H-6, H-8, H-6′), 6.60—7.56 (11H in total, aromatic H). ^{13}C-NMR (acetone-d_6+D_2O): 36.8 (–SCH_2–), 37.4 (C-4), 44.1 (C-4′), 70.4 (C-3′), 72.7 (C-3), 75.2 (C-2), 76.7 (C-2′), 95.8, 96.3, 97.5 (C-6, C-8, C-6′), 100.2 (C-4a), 100.9 (C-4a′), 106.8 (C-8′).

Procyanidin B-5 4′-benzylthioether (**29a**): A tan amorphous powder, $[\alpha]_D^{28}$ +94.2° (c=1.0, acetone). *Anal.* Calcd for $C_{37}H_{32}O_{12}S\cdot 2H_2O$: C, 60.03; H, 4.89. Found: C, 60.04: H, 4.95. ^{1}H-NMR (acetone-d_6+D_2O): 3.90—4.13 (2H, m, H-3, H-3′), 3.95 (2H, s, –SCH_2–), 4.02 (1H, d, J=2 Hz, H-4′), 4.60 (1H, br s, H-4), 5.00 (1H, br s, H-2), 5.21 (1H, br s, H-2′), 6.05 (1H, s, H-8′), 6.09 (2H, s, H-6, H-8), 6.52—7.41 (11H in total, aromatic H). ^{13}C-NMR (acetone-d_6+D_2O): 37.2 (C-4, –SCH_2–), 43.9 (C-4′), 71.0 (C-3′), 72.1 (C-3), 75.1 (C-2), 77.0 (C-2′), 96.0, 96.2, 96.5 (C-6, C-8, C-8′), 99.0 (C-4a′), 100.2 (C-4a), 108.1 (C-6′).

General Procedure for Desulfurization—A benzylthioether (20—30 mg) in EtOH was treated at room temperature with an EtOH slurry of Raney-nickel (W-4) for 30 min. After filtration of the catalyst, the filtrate was concentrated under reduced pressure to dryness, and the residue was purified by Sephadex LH-20 chromatography with EtOH.

Acknowledgement The authors wish to thank Mr. Y. Tanaka, Miss K. Soeda and Mr. K. Isobe for measurements of ^{13}C- and ^{1}H-NMR spectra, and MS, respectively. This work was supported by a grant (No. 59570897) from the Ministry of Education, Science and Culture, Japan, which is gratefully acknowledged.

References and Notes

1) Part XXX: T. Tanaka, G. Nonaka, and I. Nishioka, *Phytochemistry*; "in press."
2) *a*) K. Weinges, K. Goritz, and F. Nader, *Justus Liebigs Ann. Chem.*, **715**, 164 (1968); *b*) R. S. Thompson, D. Jacques, E. Haslam, and R. J. N. Tanner, *J. Chem. Soc., Perkin Trans. 1*, **1972**, 1387; *c*) R. K. Gupta and E. Haslam, *ibid.*, **1981**, 1148; *d*) G. Nonaka, I. Nishioka, T. Nagasawa, and H. Oura, *Chem. Pharm. Bull.*, **29**, 2862 (1981); *e*) G. Nonaka, M. Muta, and I. Nishioka, *Phytochemistry*, **22**, 237 (1983); *f*) T. Tanaka, G. Nonaka, and I. Nishioka, *ibid.*, **22**, 2575 (1983); *g*) G. Nonaka, O. Kawahara, and I. Nishioka, *Chem. Pharm. Bull.*, **31**, 3906 (1983).
3) *a*) G. Nonaka, F.-L. Hsu, and I. Nishioka, *J. Chem. Soc., Chem. Commun.*, **1981**, 781; *b*) R. W. Hemingway, L. Y. Foo, and L. J. Porter, *J. Chem. Soc., Perkin Trans. 1*, **1982**, 1209; *c*) M. W. Barett, W. Klyne, P. M. Scopes, A. C. Fletcher, L. J. Porter, and E. Haslam, *ibid.*, **1979**, 2375.
4) G. Nonaka, O. Kawahara, and I. Nishioka, *Chem. Pharm. Bull.*, **30**, 4277 (1982).
5) G. Nonaka and I. Nishioka, *Chem. Pharm. Bull.*, **30**, 4268 (1982).
6) M. Nishizawa, T. Yamagishi, G. Nonaka, and I. Nishioka, *J. Chem. Soc., Perkin Trans. 1*, **1982**, 2963.
7) G. Nonaka, S. Morimoto, and I. Nishioka, *J. Chem. Soc., Perkin Trans. 1*, **1983**, 2139.
8) A. C. Fletcher, L. J. Porter, E. Haslam, and R. K. Gupta, *J. Chem. Soc., Perkin Trans. 1*, **1977**, 1628; L. J. Porter, R. H. Newman, L. Y. Foo, and H. Wong, *ibid.*, **1982**, 1217.
9) According to the proposed new system of nomenclature of proanthocyanidins.[3b)]