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PRENYLATED FLAVAN-3-OLS AND PROCYANIDINS FROM *ILLICIUM ANISATUM**

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Key Word Index—*Illicium anisatum*; Illiciaceae; prenylated flavan-3-ols; flavan-3-ols; procyanidins; tannins.

Abstract—Two prenylated flavan-3-ols were isolated from *Illicium anisatum* and their structures characterized by chemical and spectroscopic means as 8-(3,3-dimethylallyl)-(+)-catechin and 6-(3,3-dimethylallyl)-(+)-catechin. In addition, a new proanthocyanidin was isolated, together with several known compounds. The structure of the procyanidin was established as catechin-(4α→8)-epicatechin-(4β→8)-catechin.

INTRODUCTION

Illicium anisatum L., which is distributed in the south-western parts of Japan, is known to produce shikimic acid, anisatin, essential oils, etc. [1, 2]. In addition, the occurrence of tannins was recognized by the astrigency of the bark, although they had not been characterized. As a part of chemical studies on tannins and related compounds, we have investigated the bark of *I. anisatum*, and isolated two prenylated flavan-3-ols and a new trimeric procyanidin, along with (+)-catechin and several known procyanidins. We now report their isolation and structural elucidation.

RESULTS AND DISCUSSION

Repeated Sephadex LH-20 and reverse-phase chromatography of the 80% aqueous acetone extract of the bark of *I. anisatum* afforded compounds **1**–**3**, in addition to (+)-catechin (**4**), procyanidins B-1 (**5**), B-7 (**6**) and A-1 (**7**), epicatechin-(4β→8)-epicatechin-(4β→8)-catechin (**8**), epicatechin-(4β→8)-epicatechin-(4β→6)-catechin (**9**) [3] and epicatechin-(4β→6)-epicatechin-(4β→8)-catechin (**10**) [4], which were identified by comparison with authentic samples.

Compound **1** [FABMS *m/z*: 359 $(M+H)^+$] gave an orange coloration with the anisaldehyde–sulphuric acid reagent. The ^{1}H NMR spectrum of **1** showed a flavan-3-ol skeleton, and the coupling constant (δ 4.56; $J=8$ Hz) of the flavan H-2 proton signal indicated a catechin with 2,3-*trans* stereochemistry. In addition, the presence of a 3,3-dimethylallyl group was deduced from signals due to two methyl groups (δ 1.58; 6H, *s*), a methylene (δ 3.25; 2H, *d*, $J=8$ Hz) and an olefinic proton (δ 5.18; 1H, *t*, $J=8$ Hz). This was further supported by examination of the ^{13}C NMR spectrum (Table 1). The 3,3-dimethylallyl group was shown to be located at the C-8 or C-6 position of the catechin moiety, since in the ^{1}H NMR spectrum the signal (δ 6.10) due to the A-ring proton appeared as a singlet instead of two *meta*-coupled doublets. The position of the 3,3-dimethylallyl group was determined as follows. We reported previously the position of the substituent in the A-ring in 5,7,3′,4′-tetrahydroxyflavan-3-ol derivatives could be determined by comparison of the C-6, 8, and 4a chemical shifts in their methyl ethers [4–7]. In the ^{13}C NMR spectrum of the tetramethyl ether of **1** (**1a**) the chemical shifts (δ 88.6, 109.9 and 101.8) of the flavan C-6, 8 and 4a signals were consistent with those of C-8 substituted compounds [(e.g. gambiriin A_1 nonamethyl ether: C-6 (δ 88.6), C-8 (δ 112.2) and C-4a (δ 102.5)]. Thus it was concluded that the 3,3-dimethylallyl group was located at the C-8 position. Accordingly, **1** was characterized as 8-(3,3-dimethylallyl)-catechin. The absolute stereochemistry of the catechin moiety is described later.

Compound **2** gave the same $[M+H]^+$ ion peak at *m/z* 359 in the FABMS as that of **1**. The ^{13}C and ^{1}H NMR spectra were closely correlated with those of **1**, showing the presence of a 3,3-dimethylallyl group and a catechin moiety. Furthermore, the appearance of a singlet signal assignable to the A-ring proton indicated that the 3,3-dimethylallyl group is located on the A-ring. Similar ^{13}C NMR analysis of the tetramethyl ether **2a** led to confirmation of the position of the 3,3-dimethylallyl group. The ^{13}C NMR spectrum of **2a** showed signals at δ 116.4, 95.7 and 106.0 attributable to the flavan C-6, 8 and 4a, and the chemical shifts of these signals were in good agreement with those of C-6, 8 and 4a in C-6 substituted compounds [e.g. gambiriin A_3 nonamethyl ether, δ 117.5 (C-6), 96.1 (C-8) and 106.6 (C-4a)], thus indicating that the 3,3-dimethylallyl group was at the C-6 position.

In order to confirm the absolute stereochemistry of the catechin moiety in each compound an attempt was made to prepare **1** and **2** from a compound with known stereochemistry. Treatment of (+)-catechin and prenol in the presence of *p*-toluenesulphonic acid afforded several products, of which two major compounds were isolated and their physical and spectral data were identical with those of **1** and **2**. Accordingly, **1** and **2** were determined

*Part 62 in the series 'Tannins and Related Compounds'. For part 61 see Morimoto, S., Nonaka, G., Chen, R.-F. and Nishioka, I., *Chem. Pharm. Bull.* (submitted for publication).

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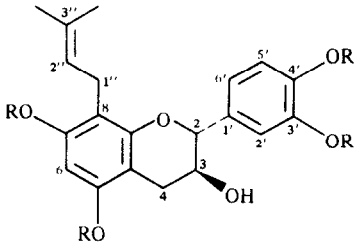
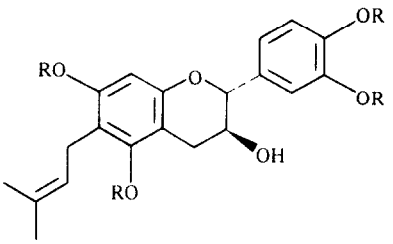


1 R = H
1a R = Me



2 R = H
2a R = Me

Table 1. ^{13}C NMR spectral data for **1**, **1a**, **2**, **2a**, and **4** (25.05 MHz)

C	1	1a	2	2a	4
2	82.4	81.4	82.4	81.9	82.3
3	68.3	68.4	68.4	68.4	68.0
4	29.1	27.7	29.1	28.3	27.5
4a	100.4	101.8	100.9	106.0	100.2
5	154.2[a]	156.3[a]	154.4[a]	153.3[a]	156.3[a]
6	96.0	88.6	108.5	116.4	95.6
7	154.6[a]	156.8[a]	155.2[a]	157.6[a]	156.7[a]
8	107.2	109.9	95.8	95.7	95.9
8a	154.6[a]	156.8[a]	155.2[a]	157.6[a]	157.1[a]
1′	129.9	130.6	130.2	130.2	131.5
2′	115.2[b]	109.8[b]	115.2[b]	110.0[b]	115.0
3′	145.5	149.0	145.7	149.5	145.2
4′	145.5	149.0	145.7	149.5	145.2
5′	115.6[b]	111.0[b]	115.9[b]	111.3[b]	111.5
6′	119.7	119.6	120.2	120.1	119.6
1″	22.6	22.0	22.8	22.7	
2″	125.0	123.4	124.2	123.6	
3″	132.1	131.0	130.9	130.2	
3″-Me_2	17.8	17.7	17.9	17.8	
	25.9	25.9	25.9	25.7	
MeO		55.5		55.6	
		55.9		55.9	

All spectra were measured in Me_2CO-d_6 except for **1a** and **2a** in $CDCl_3$.
[a,b] Signals in each column are interchangeable.

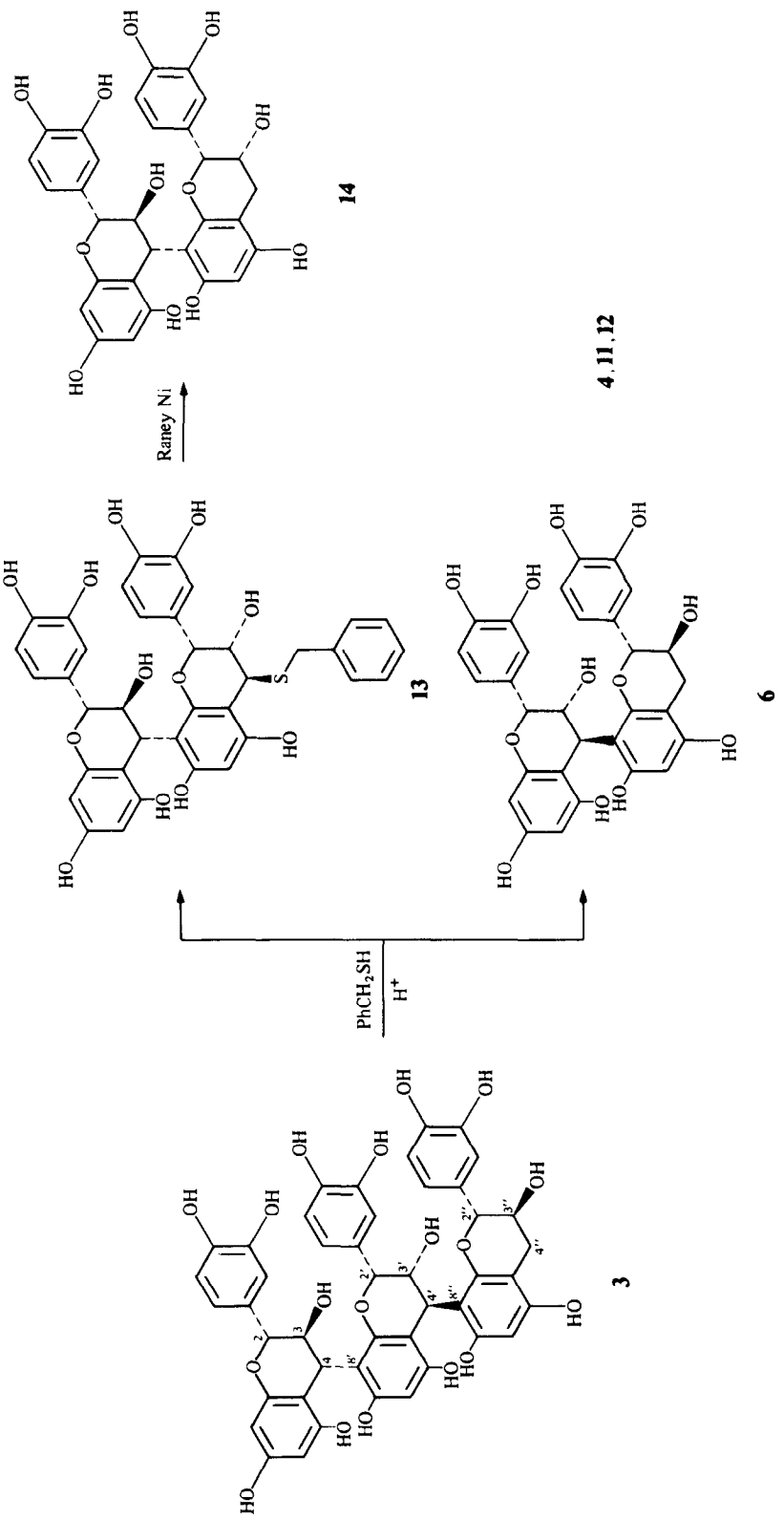
to be 8-(3,3-dimethylallyl)-(+)-catechin and 6-(3,3-dimethylallyl)-(+)-catechin, respectively.

Compound **3** was shown to possess a triflavanoid constitution by analysis of its FABMS ($[M+H]^+$ *m/z* 867). The ^{13}C NMR spectrum of **3** showed signals at δ 83.6, 82.9 and 78.1 ascribable to the flavan C-2 carbons, the former two signals being attributable to those of catechin units and the remaining to that of an epicatechin moiety. Treatment of **3** with benzylmercaptan in the presence of acid [8] furnished three degradation products, which were identified as (+)- catechin 4-benzylthioether (**11**), (−)-epicatechin 4-benzylthioether (**12**) and (+)-catechin (**4**). Thus it was concluded that **3** consisted of two (+)-catechin and one (−)-epicatechin units. The points and the configurations of the interflavanoid linkages were confirmed by partial thiolytic degradation of 3. This afforded, along with the above degradation products, procyanidin B-1 (**6**) and a thioether (**13**), which was characterized as procyanidin B-4 4′-benzylthioether (**13**) by ^1H NMR analysis and its conversion with Raney nickel to procyanidin B-4 (**14**). This formation of procyanidin B-1 (**6**) and B-4 4′-benzylthioether (**13**) established the structure of **3** as catechin-(4α→8)-epicatechin-(4β→8)-catechin.

In contrast to the relatively wide distribution of prenylated flavones, prenylated flavan-3-ols, are not often found in nature. Only three prenylated flavan-3-ols, namely, broussinol [9] and two prenylated 5-*O*-methyl afzelechins [10], have so far been isolated.

EXPERIMENTAL

Mps: uncorr. ^1H and ^{13}C NMR spectra were measured at 100 and 25.05 MHz, respectively, with TMS as ref. TLC was on silica gel with C_6H_6–HCO_2Et–HCO_2H (3:6:1 or 2:7:1), and spots were detected under UV or by spraying ethanolic $FeCl_3$ and


Raney Ni
PhCH2SH
H+
14
13
4, 11, 12
6
3

H_2SO_4. CC was performed on Sephadex LH-20 (25–100 μ, Pharmacia), MCI-gel CHP 20P (75–100 μ, Mitsubishi) and Bondapak C_{18}/porasil B (37–75 μ, waters).

Plant material. I. anisatum was collected at Mt Kusenbu, Saga Pref., Japan. A voucher specimen has been deposited in the Herbarium of the Faculty of Pharmaceutical Sciences, Kyushu University.

Isolation. Fresh bark of *I. anisatum* was extracted × 3 with 80% Me_2CO and the concd extract applied to a Sephadex LH-20 column. Elution with increasing amounts of MeOH in H_2O yielded four fractions (frs 1–4). Fr. 1 was negative to $FeCl_3$ and anisaldehyde–H_2SO_4 reagents, and this fraction was not examined further. Rechromatography of fr. 2, followed by cryst., afforded (+)-catechin (**4**; 400 mg). Fr. 3, containing prenylated flavan-3-ols, was separated by Sephadex LH-20 (EtOH) and Bondapak C_{18} (30% aq. MeOH) to afford **1** (90 mg) and **2** (45 mg). Subsequent separation of fr. 3 gave two fractions (fr. 3-1 and fr. 3-2). CC of fraction 3-1 on MCI-gel with 30% MeOH and on Sephadex LH-20 with 60% MeOH afforded procyanidins B-1 (**5**; 2.5 g), B-7 (**6**; 207 mg) and A-1 (**7**; 93 mg), while fr. 3-2 was repeatedly chromatographed over Sephadex LH-20 (60% MeOH), MCI-gel (30% MeOH) and Bondapak C_{18} (15% MeOH) to give **3** (125 mg), **8** (23 mg), **9** (32 mg) and **10** (125 mg).

8-(3,3-*Dimethylallyl*)-(+)-*catechin* (**1**). Colourless needles (water), mp 232–234°, $[\alpha]_D^{25}$ −47.6° (MeOH; c0.52), FABMS *m/z*: 359 $(M+H)^+$. ^1H NMR (Me_2CO-d_6): δ 1.58 (6H, *s*, Me × 2), 2.40–3.15 (2H, *m*, H-4), 3.25 (2H, *d*, *J* = 8 Hz, H-1″), 4.00 (1H, *m*, H-3), 4.56 (1H, *d*, *J* = 8 Hz, H-2), 5.10 (1H, *t*, H-2″), 6.10 (1H, *s*, H-6), 6.80 (1H, *d*, *J* = 8 Hz, H-3′), 6.87 (1H, *d*, *J* = 2 Hz, H-6′), 6.93 (1H, *dd*, *J* = 2, 8 Hz, H-2′). (Found: C, 63.47; H, 6.37. $C_{20}H_{22}O_6 \cdot H_2O$ require: C, 63.88; H, 6.54).

Methylation of **1**. A mixture of **1** (45 mg), Me_2SO_4 (0.5 ml) and K_2CO_3 (1.5 g) in dry Me_2CO (20 ml) was refluxed for 2 hr. After filtration the concd soln was purified by CC on silica gel. Elution with C_6H_6–EtOAc (17:3) furnished the tetramethyl ether (**1a**; 42 mg) as a white amorphous powder, $[\alpha]_D^{23}$ −66.4° ($CHCl_3$; *c* 0.9). EIMS *m/z*: 414 $(M)^+$. ^1H NMR ($CDCl_3$): δ 1.64 (6H, *s*, Me × 2), 2.90 (2H, *m*, H-4), 3.26 (2H, *d*, *J* = 8 Hz, H-1″), 3.80–3.90 (12H in total, MeO × 4), 4.02 (1H, *m*, H-3), 4.70 (1H, *d*, *J* = 8 Hz, H-2), 5.12–5.29 (1H, *m*, H-2″), 6.15 (1H, s, H-6), 6.86 (1H, *d*, J = 8 Hz, H-3′), 6.91 (1H, *d*, *J* = 2 Hz, H-6′), 6.97 (1H, *dd*, *J* = 2, 8 Hz, H-2′). (Found: C, 69.40; H, 7.25. $C_{24}H_{30}O_6$ require: C, 69.62; H, 7.25).

6-(3,3-*Dimethylallyl*)-(+)-*catechin* (**2**). Colourless needles (water), mp 213–215°, $[\alpha]_D^{20}$ −5.3° (MeOH; *c* 0.72), FABMS *m/z*: 359 $(M+H)^+$. ^1H NMR (Me_2CO-d_6): δ 1.63 (3H, *s*, Me), 1.74 (3H, *s*, Me), 2.44–3.07 (2H, *m*, H-4), 3.30 (2H, *d*, *J* = 8 Hz, H-1″), 4.00 (1H, *m*, H-3), 4.51 (1H, *d*, *J* = 8 Hz, H-2), 5.28 (1H, *t*, H-2″), 6.00 (1H, s, H-6), 6.75 (1H, *d*, *J* = 8 Hz, H-3′), 6.78 (1H, *d*, *J* = 2 Hz, H-6′), 6.90 (1H, *dd*, *J* = 2, 8 Hz, H-2′). (Found: C, 63.40; H, 6.45. $C_{20}H_{22}O_6 \cdot H_2O$ require: C, 63.88; H, 6.38).

Methylation of **2**. **2** (20 mg) was methylated with Me_2SO_4 (0.5 ml) and K_2CO_3 (0.8 g) in dry Me_2CO (20 ml). The reaction mixture was worked-up as above to afford the tetramethyl ether (**2a**; 15 mg) as a white amorphous powder, $[\alpha]_D^{20}$ −66.4° ($CHCl_3$; *c* 0.9). EIMS *m/z*: 414 $(M)^+$. ^1H NMR ($CDCl_3$): δ 1.61, 1.78 (each 3H, *s*, Me × 2), 2.95 (2H, *m*, H-4), 3.26 (2H, *d*, *J* = 8 Hz, H-1″), 3.76–3.98 (12H in total, MeO × 4), 4.05 (1H, *m*, H-3), 4.66 (1H, *d*, *J* = 8 Hz, H-2), 5.11–5.25 (1H, *m*, H-2″), 6.32 (1H, *s*, H-6), 6.90 (1H, *d*, *J* = 8 Hz, H-3′), 6.99 (1H, *d*, *J* = 2 Hz, H-6′), 7.07 (1H, *dd*, *J* = 2, 8 Hz, H-2′). (Found: C, 68.56; H, 7.39. $C_{24}H_{30}O_6 \cdot 1/2$ H_2O require: C, 68.14; H, 7.57).

Catechin-(4α-8)-epicatechin-(4β-8)-catechin (**3**). A white amorphous powder, $[\alpha]_D^{18}$ −102.5° (Me_2CO, *c* 1.1). FABMS *m/z*: 867 $(M+H)^+$. ^{13}C NMR (Me_2CO-d_6): δ 37.5, 38.5 (C-4, 4′), 70.3 (C-3″), 73.2 (C-3, 3′), 78.1 (C-2′), 82.9, 83.6 (C-2, 2″). (Found: C, 61.23; H, 4.56. $C_{45}H_{28}O_{18} \cdot H_2O$ require: C, 61.09; H, 4.33).

Complete thiolytic degradation of **3**. **3** (50 mg) was heated for 12 hr under reflux in EtOH (15 ml) containing benzylmercaptan (2 ml) and HOAc (2 ml). Removal of EtOH under red. pres. gave an oily residue, which was purified by Sephadex LH-20 CC. Elution with 60% MeOH afforded (+)-catechin 4-benzylthioether (**11**; 5 mg), (−)-epicatechin 4-benzylthioether (**12**; 8 mg) and (+)-catechin (**4**; 15 mg).

Partial thiolytic degradation of **3**. 3 (150 mg) was refluxed for 7 hr in EtOH (20 ml) containing benzylmercaptan (4 ml) and HOAc (2 ml). The EtOH was evap. off to give an oily residue, which was repeatedly chromatographed over Sephadex LH-20 with EtOH and MCI-gel with 40% MeOH to furnish **11** (4 mg) and **12** (14 mg), (+)-catechin (**4**; 11 mg), procyanidin B-1 (**5**; 25 mg) and a thioether (**13**; 9mg) as a white amorphous powder, $[\alpha]_D^{20}$ −152.5° (Me_2CO, *c* 1.1). ^1H NMR (Me_2CO-d_6): δ 4.00 (2H, *s*, $-SCH_2-$), 4.00–5.00 (6H in total, *m*, H-2, 2′, 3, 3′, 4, 4′), 5.80–6.20 (3H in total, *m*, A-ring H), 6.60–7.50 (11H in total, *m*, B-ring H, aromatic H).

Desulphurization of **13**. A solution of **13** (8 mg) in EtOH was treated with Raney Ni (W-4) at room temp. for 1 hr. Removal of the catalyst by filtration, followed by Sephadex LH-20 CC with EtOH, furnished procyanidin B-4 (**14**; 3 mg).

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