Phytochemistry, Vol. 29, No. 5, pp. 1671–1674, 1990.
Printed in Great Britain.

0031-9422/90 $3.00+0.00

THIOLYSIS OF BIRCH BARK PROCYANIDINS: STRUCTURAL DEPENDENCE IN FORMATION OF 2,3-*CIS*-3,4-*CIS*-FLAVAN-4-BENZYLTHIOETHERS FROM PROCYANIDINS

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(*Received in revised form* 6 *October* 1989)

Key Word Index—*Betula* sp.; Betulaceae; bark; procyanidins; thiolysis; all-*cis*-flavan-4-benzylthioethers.

Abstract—Thiolytic studies of the birch bark procyanidins, epicatechin-(4β→8)-catechin (B_1), epicatechin-(4β→6)-catechin (B_7) and epicatechin-(4β→8)-epicatechin-(4β→8)-catechin, provided, in addition to known β-thioethers, evidence for the presence of the first 2,3-*cis*-3,4-*cis*-flavan-4-benzylthioethers. Their formation is dependent on the 2,3-stereochemistry of the 'lower' terminal flavan unit and apparently on the relative lability of the interflavanoid linkage. By contrast, epicatechin-(4β→8)-epicatechin (B_2), epicatechin-(4β→6)-epicatechin (B_5) and epicatechin-(4β→8)-epicatechin-(4β→8)-epicatechin (C_1), afforded only 2,3-*cis*-3,4-*trans*-flavan-4-benzylthioethers.

INTRODUCTION

The recent structural examination of phenolic metabolites from medicinal birch bark (*Betula* sp.) has revealed the presence of a complex mixture of procyanidins [1]. Characterization of the oligomeric flavan-3-ols was provided by diagnostic ^{1}H NMR parameters available from high-temperature ^{1}H NMR spectroscopy. Previous investigations showed that procyanidins could be cleaved into their respective flavan units with retention of the stereochemistry at C-2 and C-3, using various sulphur nucleophiles [2]. Such chemical degradation techniques coupled with spectroscopic evidence are routinely used in the study of the structure of oligomeric procyanidins [3]. However, in reactions of 2,3-*cis*-procyanidins the products have hitherto been strictly limited to adducts of 3,4-*trans* stereochemistry, contrasting with the formation of two toluene-α-thioethers of (+)-catechin (**1** and **2**). This report discloses differences in the course of the reaction of birch bark procyanidins with toluene-α-thiol dependent on the 2,3-stereochemistry of the 'lower' terminal flavan unit.

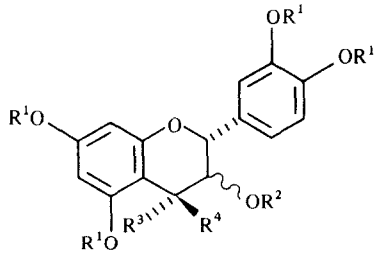
RESULTS AND DISCUSSION

The continued investigations of the natural procyanidins B_1 (**8**) and B_7 (**14**) isolated from birch bark [1] on the basis of thiolysis revealed indications of a novel reaction product, assumed to be a diastereomer of the known 4β-benzylthioepicatechin (**4**). Because it was produced in lowest yield during thiolysis, lack of material necessitated isolation of procyanidins in larger quantities in order to provide unequivocal proof for its structure and stereochemistry. Following extensive fractionation and enrichment procedures the procyanidins required were obtained in sufficient amounts from the plant material, thus permitting a more detailed study of the acid-catalysed toluene-α-thiol degradation of birch bark procyanidins. Treatment of **8** and **14** with toluene-α-thiol under conditions similar to those generally used [2] led to complete conversion into a mixture from which three cleavage products were obtained. These included (+)-catechin (**3**), derived from the 'lower' terminal unit, and two thioethers in the ratio of *ca* 35:1 identified as **4** and **6**, respectively, the latter representing the first all-*cis* procyanidin derivative. Noteworthy is that the (4→6) bond of **14** is cleaved at a slower rate than the (4→8) bond of **8**, consistent with similar recent observations [4, 5].

Initial identification of the epimeric sulphur-containing products was accomplished by analysis of ^{1}H NMR data of their peracetates, **5** and **7**, which revealed their close structural resemblance. Both displayed AMX-patterns (δ5.67, *d*, $J_{3,4}$=5.5 Hz, 3-H; δ5.12, *br s*, 2-H; δ4.34, *d*, $J_{3,4}$=5.5 Hz, 4-H for **7**; δ5.69, *br s*, 2-H; δ5.26, *dd*, $J_{2,3}$=0.8 and $J_{3,4}$=2.0 Hz, 3-H; δ4.12, *d*, J=2.0 Hz, 4-H for **5**) besides an isolated AB system (δ3.73 and 3.66, *d*, J=15.0 Hz for **7**; δ4.07 and 3.87, *d*, J=12.0 Hz for **5**), characteristic of the methylene functionality of $PhCH_2$ in the heterocyclic regions, as well as aromatic AB patterns (δ6.54 and 6.70 for **7**; δ6.54 and 6.65 for **5**) for their respective A rings.

The highfield position of 2-H (δ5.12) of **7** agrees fairly well with that of the unsubstituted (−)-epicatechin acetate (δ5.10), thus indicating relief from 1,3-*diaxial* interaction between the *axial* 2-H and the *quasi-axial* 4-benzylthio group as is reflected in analogue **5**. This observation is readily understandable in terms of a *quasi-equatorial* orientation of the 4-substituent. The relative configuration of **5** and **7** was evident from the ^{1}H NMR coupling constants of their heterocyclic protons ($J_{2,3}$ < 1.0 Hz; $J_{3,4}$=2.0 and 5.5 Hz, respectively) [6]. The respective positive and negative Cotton effect of **5** and **7** in the diagnostic 220–240 nm region of the CD spectra [7, 8], in conjunction with the above coupling constants of the heterocyclic ring protons, defined the absolute configuration of the epicatechin moiety of the thioethers **5** and **7** as 2*R*,3*S*,4*S* and 2*R*,3*S*,4*R*, respectively.

Independent support of the stereochemistry at C-4 was available from ^{13}C NMR data. The relative 3,4-*trans*



1 $\wr$ = ▲ $R^1 = R^2 = R^4 = H$; $R^3 = PhCH_2S$

2 $\wr$ = ▲ $R^1 = R^2 = R^3 = H$; $R^4 = PhCH_2S$

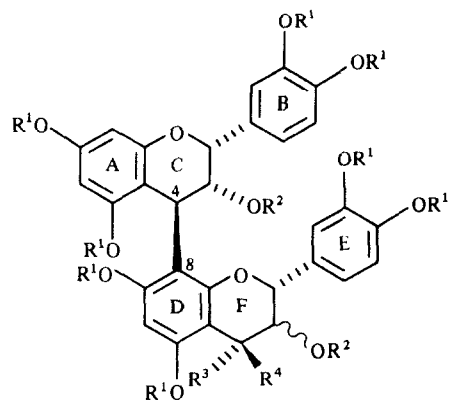
3 $\wr$ = ▲ $R^1 = R^2 = R^3 = R^4 = H$

4 $\wr$ = ⋮ $R^1 = R^2 = R^3 = H$; $R^4 = PhCH_2S$

5 $\wr$ = ⋮ $R^1 = R^2 = Ac$; $R^3 = H$; $R^4 = PhCH_2S$

6 $\wr$ = ⋮ $R^1 = R^2 = R^4 = H$; $R^3 = PhCH_2S$

7 $\wr$ = ⋮ $R^1 = R^2 = Ac$; $R^4 = H$; $R^3 = PhCH_2S$



8 $\wr$ = ▲ $R^1 = R^2 = R^3 = R^4 = H$

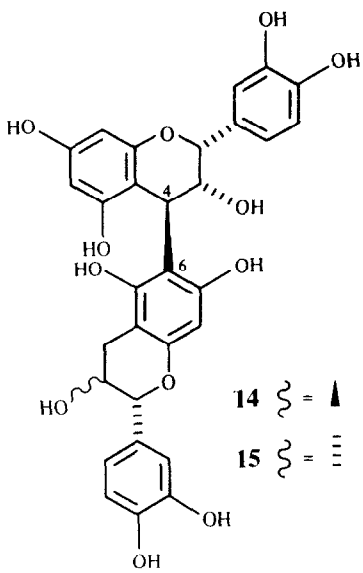
9 $\wr$ = ⋮ $R^1 = R^2 = R^3 = R^4 = H$

10 $\wr$ = ⋮ $R^1 = R^2 = R^3 = H$; $R^4 = PhCH_2S$

11 $\wr$ = ⋮ $R^1 = R^2 = Ac$; $R^3 = H$; $R^4 = PhCH_2S$

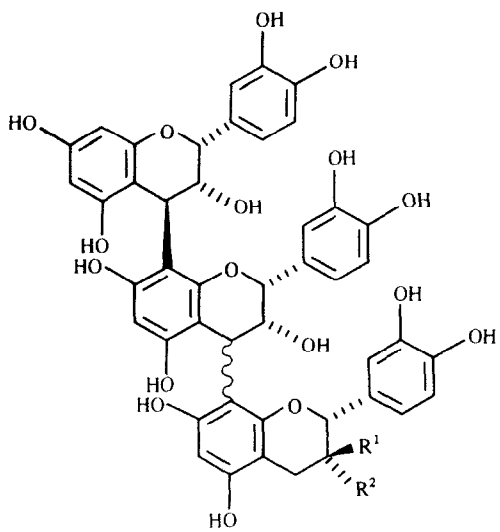
12 $\wr$ = ⋮ $R^1 = R^2 = R^4 = H$; $R^3 = PhCH_2S$

13 $\wr$ = ⋮ $R^1 = R^2 = Ac$; $R^3 = PhCH_2S$; $R^4 = H$



14 $\wr$ = ▲

15 $\wr$ = ⋮



16 $\wr$ = ▲ $R^1 = OH$; $R^2 = H$

17 $\wr$ = ▲ $R^1 = H$; $R^2 = OH$

configuration of **5** was confirmed by the chemical shift of C-2 at δ72.6 indicative of a *quasi-axial* orientation of the substituent at C-4 [9]. The analogous signal of the 3,4-*cis* isomer **7** at δ77.1 reflecting lack of 1,3-steric interaction was, therefore, consistent with a *quasi-equatorial* position.

With the above results in mind, similar studies were extended to procyanidins B_2 (**9**) and B_5 (**15**). However, these exclusively afforded the 4β-thioether **4**. The failure to provide similar proof for the generation of the isomeric thioethers **4** and **6** from procyanidins based exclusively on

epicatechin units prompted us to embark on a strategy of investigating analogous triflavanoids in order to clarify the obvious influence of the 2,3-stereochemistry of the respective next 'lower' epicatechin and catechin moiety linked to a (−)-epicatechin unit in the formation of one, **4**, and two thioethers, **4** and **6**, respectively.

Thus, treatment of epicatechin-(4β→8)-epicatechin-(4β→8)-catechin (**16**) occurring in the same plant source [1], with toluene-α-thiol–acetic acid yielded (+)-catechin **3** and two thioethers; the latter being characterized as the 4-benzylthioethers **4** and **6** of (−)-epicatechin by ^{1}H NMR analysis. Furthermore, characterization of the partial degradation products demonstrated, in addition to **4** and **6**, the presence of **8** and the dimeric thioethers epicatechin-(4β→8)-4β-benzylthioepicatechin (**10**) and epicatechin-(4β→8)-4α-benzylthioepicatechin (**12**). The present thiolytic study thus provides the first evidence for the formation of a dimeric flavan-4-thioether **12** which possesses the hitherto unique 2,3-*cis*-3,4-*trans*:2′,3′-*cis*-3′,4′-*cis* configuration. Differentiation between their acetates (**11**) and (**13**) was similarly possible by the high field position of 2-H (F) (δ *ca* 4.40; tentative assignment due to overlap of signals) in the ^{1}H NMR spectrum of **13** compared with that of **11** (δ4.66). Apart from this, the coupling constants of the heterocyclic protons of ring F of **11** and **13** ($J_{2,3}$ *ca* 1.0 Hz; $J_{3,4}$ = 2.0 and 6.0 Hz, respectively) are in agreement with those of the monomeric thioether **5** and **7**, as are the chemical shifts of 4-H (F) (δ4.15 and 4.33, respectively) and the methylene functionality of the benzylthio group (δ3.83 and 4.12, *d*, J = 14.0 Hz and δ3.68 and 3.74, *d*, J = 15.5 Hz, respectively).

The above results indicated that the 4α-benzylthioether **6**, obtained on complete thiolysis of **16**, was derived from the 'middle' epicatechin unit. This conclusion was supported by the stereospecific formation of the 4β-benzylthioethers **4** and **10** on partial degradation of epicatechin-(4β→8)-epicatechin-(4β→8)-epicatechin-(**17**).

These observations may presumably be rationalized in terms of the conformation of the 'lower' terminal flavan unit and the relative lability of the interflavanoid linkage. Owing to significant contributions by the non-preferred A-conformation of the terminal (+)-catechin unit [10] and hence steric restraints of the α-face, as shown by Dreiding models, initial protonation may occur from the less hindered 'upper' side. Subsequent nucleophilic attack by toluene-α-thiol on the incipient carbocation derived from (−)-epicatechin proceeding from the α-face necessarily leads to 2,3-*cis*-3,4-*cis* flavan-4-thioethers as evidenced by the formation of **6** and **12**. Once formed, approach by the nucleophile from the 'upper' side is favoured in view of neighbouring group participation by the 3-*axial* hydroxyl of the 2,3-*cis* carbocation [11]. This may explain the predominance of 2,3-*cis*-3,4-*trans* products. The low yields of the 3,4-*cis* thioethers may also partly result from their ready isomerization to the thermodynamically more stable 3,4-*trans* products.

By contrast, the stereospecificity of C-4 thiolysis of procyanidins based exclusively on (−)-epicatechin units, compounds **9** and **17**, is understandable in terms of initial addition of the proton to the α-face due to the predominant *E*-conformation of (−)-epicatechin [10] and hence approach by the nucleophile from the β-face on the incipient carbocation. Similarly, the generated 2,3-*cis* carbocation should, on the basis of previous argument, be exclusively susceptible to nucleophilic attack from the less hindered 'upper' side.

As cleavage of the interflavanoid bond of procyanidins is one of the most important reactions in condensed tannin chemistry, these results are highly significant in that degradative methods appear critical in structural conclusions of oligomers. It should be mentioned that synthetic and natural 3,4-*cis* procyanidins have recently been reported [12–14] but information is lacking on their acid-catalysed toluene-α-thiol degradation.

EXPERIMENTAL

NMR spectra were recorded in $CDCl_3$ with TMS as int. standard. CD data were obtained in MeOH. Analyses (C and H) were performed by the Department of Organic Chemistry, Westfälische-Wilhelms Universität, Münster. Prep. TLC plates (20 × 20 cm; Kieselgel PF_{254}, 0.5 mm) were air-dried and used without prior activation. Acetylations were performed in Ac_2O–pyridine at room temp.

Isolation of procyanidins from birch bark. The work-up procedure of the plant material followed that described in ref. [1].

Degradation of procyanidins with toluene-α-thiol: general analytical procedure. A sample of procyanidin (200 mg) was dissolved in EtOH (4 ml) and toluene-α-thiol (2 ml) and HOAc (1 ml) added. The mixt. was refluxed under N_2 and the products periodically sampled. The progress of the reaction was monitored by TLC on precoated plastic sheets (silica gel $60F_{254}$, 0.25 mm) in toluene–Me_2CO–HCO_2H (5:5:1) (solvent A). The oil obtained on subsequent removal of solvents was subjected to chromatography on Sephadex LH-20 with $CHCl_3$–hexane–HOPr (3:1:1); frs (10 ml) were collected.

Treatment of B_1 (**8**) with toluene-α-thiol for 2 hr yielded (+)-catechin (78 mg) from frs 137–170, which was identified by co-chromatography with an authentic sample.

(2R,3S,4R)-4-*Benzylthioflavan*-3,3′,4′,5,7-*pentaol* [*4α-benzylthioepicatechin*] (6). Frs 93–94 (3 mg) afforded the thioether **6** [R_f 0.70 (A)] Acetylation and subsequent purification by prep. TLC in C_6H_6–Me_2CO (4:1; R_f 0.66) yielded its peracetate (**7**). (Found C, 61.6; H, 5.0; S, 5.3. $C_{32}H_{30}O_{11}S$ requires C, 61.7; H, 4.9; S, 5.1%). ^{1}H NMR (300 MHz, $CDCl_3$): δ2.02 (*s*, aliph. OAc), 2.25, 2.28, 2.30, 2.31 (*s*, 4 × phenol OAc), 3.66 [*d*, J = 15.0 Hz, –CH_2S–], 3.73 [*d*, J = 15.0 Hz, –CH_2S–], 4.34 [*d*, J = 5.5 Hz, 4-H], 5.12 *br* [*s*, 2-H], 5.67 [*d*, J = 5.5 Hz, 3-H], 6.54 [*d*, J = 2.2 Hz, 6-H], 6.70 [*d*, J = 2.2 Hz, 8-H], 7.20–7.35 (*m*, 8 × H). ^{13}C NMR ($CDCl_3$): δ39.3 (–CH_2S–), 42.8 (C-4), 68.4 (C-3), 77.1 (C-2), 108.9 (C-8), 110.1 (C-6), 112.4 (C-4a). CD $[\theta]_{290}$ 0, $[\theta]_{276}$ −4220, $[\theta]_{244}$ −5215, $[\theta]_{237}$ −4160, $[\theta]_{215}$ −18 735, $[\theta]_{210}$ 0.

(2R,3S,4S)-4-*Benzylthioflavan*-3,3′,4′,5,7-*pentaol* [*4β-benzylthioepicatechin*] (**4**). The thioether **4** [R_f 0.68 (A)] was obtained from frs 95–120 (108 mg). Acetylation and subsequent prep. TLC purification in C_6H_6–Me_2CO (4:1; R_f 0.63) gave its pentaacetate (**5**), the physical data of which were identical with those of an authentic specimen [15].

Treatment of B_7 (**14**) with toluene-α-thiol for 24 hr yielded (+)-catechin (75 mg), 4α-benzylthioepicatechin (**6**) (2.5 mg), and **4** (118 mg) with physical data identical with those of the products described above.

Analogous treatment of B_2 (**9**) and B_5 (**15**), respectively, afforded (−)-epicatechin (*ca* 30–40% yields) and **4** (*ca* 60–70% yields).

Treatment of the trimer C_1 (**17**) with toluene-α-thiol yielded (−)-epicatechin, **9**, **4** and epicatechin-(4β→8)-4β-benzylthioepicatechin (**10**). The physical data of their acetates proved to be identical to those of the corresponding derivatives of those described above and authentic samples [15].

Treatment of epicatechin-(4β→8)-epicatechin-(4β→8)-catechin (**16**) with toluene-α-thiol for 6 hr afforded (+)-catechin (37 mg), **4** (72 mg), 4α-benzylthioepicatechin **6** (5 mg) and **10**

(28 mg). These were identified by comparison of the physical data of their acetates with those described in the previous section.

(2R,3R,4R)-2,3-cis-3,4-trans-3,3',4',5,7-*Pentahydroxy*-4-[(2R, 3S,4R)-2,3-cis-3,4-cis-3,3',4',5,7-*pentahydroxy*-4-*benzylthioflavan*-8-*yl*] *flavan* [*epicatechin*-(4β→8)-4α-*benzylthioepicatechin*] (**12**). Frs 132–142 (12 mg) contained a mixt. of **10** and **12**. Acetylation and subsequent purification by prep. TLC in C_6H_6–Me_2CO (4:1) afforded two frs at R_f 0.45 and 0.48. The former fr., (7 mg) yielded the acetate (**11**) with physical data identical with those of an authentic sample [15], while the latter, R_f (1.5 mg). gave the acetate (**13**) of the title compound. ^{1}H NMR (300 MHz, $CDCl_3$)*: δ1.75–2.30 (*m*, 10 × OAc), 3.68 [*d*, *J* = 15.5 Hz, –CH_2S–], 3.74 [*d*, *J* = 15.5 Hz, –CH_2S–], 4.30–5.55 [*m*, 6 × H (C and F)], 6.06 [*d*, *J* = 2.2 Hz, 6-H(A)], 6.23 [*d*, *J* = 2.2 Hz, 8-H (A)], 6.58 [*s*, 6-H (D)], 6.95–7.40 [*m*, 11 × H (B, E and –PhCH$_2$S–)].

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*The ^{1}H NMR spectrum is complicated by conformational isomerism.