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THE FIRST 2,3-*TRANS*-3,4-*CIS* PROCYANIDIN*

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Key Word Index—Biomimetic synthesis; procyanidins B-3 and B-6; 2,3-*trans*-3,4-*cis*:2,3-*trans* [4,8]-bi-[(+)-catechin].

Abstract—Condensation of (+)-leucocyanidin with (+)-catechin under acidic conditions afforded the novel 2,3-*trans*-3,4-*cis*:2,3-*trans* [4,8]-bi-[(+)-catechin] as the first representative of a 3,4-*cis* procyanidin unit.

INTRODUCTION

Direct biomimetic condensations of flavan-3,4-diols, as potential electrophiles, with nucleophilic phloroglucinol-derived flavan-3-ols to form bi- and triflavanoids have recently been demonstrated for procyanidins [1, 2]. Natural procyanidins and also their synthetic counterparts have hitherto been strictly limited to units of 2,3-*trans*-3,4-*trans* and 2,3-*cis*-3,4-*trans* stereochemistry [3, 4]. Stereospecificity involved in the formation of the former has been attributed to the stability-selectivity principle, considering the effective delocalization of the charge of the 4-carbeniumion resulting from the (+)-2,3-*trans*-leucocyanidin (flavan-3,4-diol) during *in vitro* synthesis [2].

RESULTS AND DISCUSSION

The present reinvestigation of the products of condensation of (+)-leucocyanidin with (+)-catechin resulted in the formation of the all-*trans* configurated procyanidins B-3, B-6, C-2, and the all-*trans* [4,8] linked tetraflavanoid analogue as previously described [1], but revealed in addition a new novel biflavanoid (**1**), the first related to procyanidins possessing a 3,4-*cis* stereochemistry.

In order to obtain confirmatory evidence of the structures the products have been converted into their respective fully acetylated derivatives. Subsequent prep. TLC separation of the biflavanoid fraction afforded the novel product (**2**) which was identified by NMR spectroscopy and CD measurements as the 2,3-*trans*-3,4-*cis*:2,3-*trans* [4,8]-bi-[(+)-catechin] ($J_{2,3} = 9.6$ Hz, $J_{3,4} = 6.5$ Hz) [5–7].

Irradiation of the methylene protons (*dd* at δ2.60 and 3.19) simplified the signal of H-3 (F) (δ5.11) indicating association with the heterocyclic system of the 'lower' unit (F-ring). Decoupling of the multiplet at δ5.11 had similar results in that the couplings exhibited by both the methylene protons and the doublet at δ4.38 collapsed, hence establishing the relationships between those three resonances. The heterocyclic protons of the 'upper' flavan unit (C-ring) were identified as the resonances at δ5.30 (*m*), 5.24 (*d*, $J_{2,3} = 9.6$ Hz) and 4.70 (*d*, $J_{3,4} = 6.5$ Hz) attributed to H-3, H-2 and H-4, respectively. Such diagnostic differentiation was confirmed by decoupling H-4 which caused collapse of coupling associated with H-3, while irradiation of the H-2 doublet selectively reduced the intensity of the multiplet assigned to H-3.

The relative 3,4-*cis* stereochemistry was supported by the position of the heterocyclic ring carbon resonances in the ^{13}C NMR spectrum, showing the chemical shift of C-2 (C-ring) at δ78.18 indicative of a *quasiaxial* orientation of the phenyl substituent at C-4 [4]. Circular dichroism confirmed the 4*R* configuration (C-ring) at the point of linkage as evident from positive Cotton effects between 200–220 nm [8–10].

Although **1** could not be obtained as the Me ether acetate defining unambiguously the bonding position [11] compound **1** was considered to represent the [4,8] biflavanoid based on the presence of one conformer in the ^{1}H NMR spectrum [4].

By contrast, at ambient temperatures ^{1}H NMR spectra of procyanidin acetates, particularly those which have (+)-catechin as the 'upper' flavan unit, exhibit both duplication of resonances and line-broadening due to rotational isomerism [3, 12]. Lack of these phenomena in the ^{1}H NMR spectrum of **2** at ambient temperatures may be attributed to the relatively higher energy barrier to 'slow' rotation about the interflavanoid linkage of 3,4-*cis* biflavanoids relative to those with 3,4-*trans* configuration [13]. This different behaviour in terms of free energies of activation $\Delta G^{\neq}_{\text{rot}}$ for the conformational interconversion, when taken in conjunction with the bulky acetyl groups, suggested that biflavanoid **2** adopted a 'static' conformation.

Evidence of the bonding position was therefore defined by the relative upfield position of H-2 (F) at δ4.38 which is consistent with the proposed [4,8] interflavanoid linkage rather than the rotamer population as cited above. This aspect has proved to be of diagnostic value for the differentiation between [4,8] and [4,6] linkages of profisetinidins [14] and procyanidins (unpublished data). The shielding effect of H-2 (F) is contributed mainly by the close proximity of the anisotropic carbonyl group of 3-acetoxy functions (C-ring), assuming half-chair conformations of the heterocyclic ring systems.

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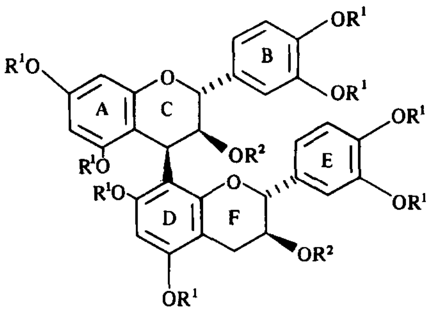
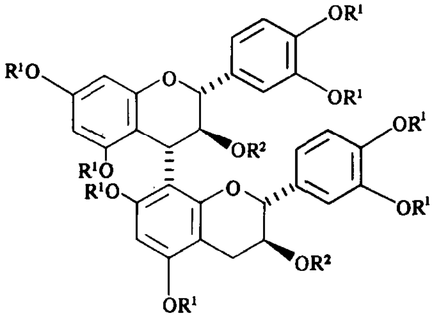
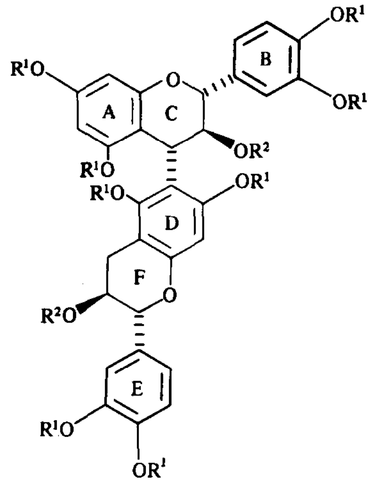
*Dedicated to Professor Dr. H. Friedrich, Münster, on the occasion of his 65th birthday, 15th February 1985.



1 $R^1 = R^2 = H$
2 $R^1 = R^2 = Ac$



3 $R^1 = R^2 = H$
4 $R^1 = R^2 = Ac$



5 $R^1 = R^2 = H$
6 $R^1 = R^2 = Ac$

Due to prolonged reaction times required for the condensation process under conditions close to neutrality (pH 5, 0.15 M HOAc) [1] this reaction was performed under more acidic conditions (0.1 M HCl) which rapidly proceeded in the formation of the expected products.

This novel 2,3-*trans*-3,4-*cis* : 2,3-*trans* biflavanoid **1** has, as yet, no counterpart in nature. The generation of **1** in significant yield approximating to the level of procyanidin B-6 acetate (**6**) obtained from the same reaction may indicate that 3,4-*cis* configurated condensed tannins may, in principle, exist in nature.

Structural confirmation of procyanidin B-3 (**3**) was derived from the analysis of the derivative (**4**) consistent with previously reported data [10, 15]. The ^1H NMR spectrum of B-6 acetate **6**, hitherto poorly characterized, differed from that of **4** by the presence of two overlapping sets of resonances due to rotameric forms. Complete NMR assignment was established by means of extensive spin-decoupling experiments. Temperature elevation of the equally-populated rotamers in DMSO-d_6 to 170° was required for coalescence and sharpening of all temperature-dependent resonances, in order to overcome the effects of dynamic rotational isomerism.

EXPERIMENTAL

NMR spectra were recorded at 300 MHz in $CDCl_3$ (unless stated otherwise) with TMS as int. standard. CD measurements were made in MeOH. Acetylations were performed in Ac_2O–pyridine at room temp. Preparative plates (Kieselgel PF_{254}, 0.5 mm) were used for sepn of derivatives.

Biomimetic synthesis of all-2,3-trans *procyanidins.* Condensation of (+)-dihydroquercetin (1 g) with (+)-catechin (1 g) was performed according to the procedure of ref. [1], but under more acidic conditions (0.1 M HCl). The solid obtained on evapn of solvent was resolved on Sephadex LH-20 (50 × 2 cm) using EtOH as eluant. Appropriate fractions (15 ml) were combined and grouped as follows: fractions 10–15 contained (+)-catechin (378 mg), while fractions 18–23 afforded unchanged (+)-dihydroquercetin (32 mg).

[4,8]-*All*-trans-*bi*-[(+)-*catechin*]. *Procyanidin* B-3 (**3**). Procyanidin B-3 (**3**) was obtained from fractions 28–43. Acetylation gave the deca-acetate (**4**) (113 mg) as a white solid after purification by prep. TLC (C_6H_6–Me_2CO, 4:1; R_f 0.37). $[\alpha]^{20}_{578} - 52.3°$ (*c* 0.44 in $CHCl_3$) lit. [16] $[\alpha]_{578} - 134°$ (*c* 1.0 in Me_2CO); $[M]^+$ m/z 998; ^1H NMR ($CDCl_3$): δ 1.66–2.34 [30 H, *m*, 10 × OAc], 2.66 [*dd*, $J_{3,4} = 7.5$ Hz and $J_{4ax,4eq} = 16.5$ Hz, H-

4_{ax}(F)], 2.94 [*dd*, $J_{3,4} = 5.5$ Hz and $J_{4eq,4ax} = 16.5$ Hz, H-4_{eq}(F)], 4.48 [*d*, $J = 9.6$ Hz, H-4 (C)], 4.76 [*d*, $J = 9.6$ Hz, H-2 (C)], 4.94 [*d*, $J = 8.0$ Hz, H-2 (F)], 5.12 [*m*, H-3 (F)], 5.62 [*t*, $\Sigma J = 19.2$ Hz, H-3 (C)], 6.48 [*d*, $J = 2.2$ Hz, H-6 (A)], 6.50 [*d*, $J = 2.2$ Hz, H-8 (A)], 6.62 [*s*, H-6 (D)], 7.00–7.12 [*m*, 6 × H (B and E)]; CD $[\theta]_{290}$ 0, $[\theta]_{278} - 14\,850$, $[\theta]_{252} - 3090$, $[\theta]_{234} - 22\,270$, $[\theta]_{208} - 193\,400$, $[\theta]_{200} - 131\,510$.

[4,6]-*All*-trans-*bi*-[(+)-*catechin*]. *Procyanidin* B-6 (**5**). Fractions 52–73 afforded procyanidin B-6 (5) which was acetylated, and subsequent prep. TLC sepn with C_6H_6–$(Me)_2CO$, 4:1 (R_f 0.38) yielded the deca-acetate (**6**) (21 mg). $[\alpha]_{578}^{20} - 3.5°$ (*c* 0.2 in $CHCl_3$) lit. [4] $[\alpha]_{578} - 19.9°$ (*c* 0.7 in $CHCl_3$). ^{1}H NMR ($CDCl_3$): δ1.62–2.30 [30 H, *m*, 10 × OAc], 2.38–2.58 and 2.82–2.96 [2 × *m*, 4 × H, H-4 (F)], 4.34 and 4.44 [2 × *d*, $J = 9.0$ Hz, 2 × H-4 (C)], 4.78 and 4.80 [2 × *d*, $J = 10.0$ Hz, 2 × H-2 (C)], 4.86 and 4.98 [2 × *d*, $J = 9.0$ Hz, 2 × H-2 (F)], 5.06 [*m*, 2 × H-3 (F)], 5.68 [*m*, 2 × H-3 (C)], 6.46 [*d*, $J = 2.4$ Hz, H-6 (A)], 6.49 [*d*, $J = 2.4$ Hz, H-8 (A)], 6.60 and 6.63 [2 × *s*, 2 × H-8 (D)], 6.64 [*dd*, $J = 2.5$ Hz, H-6 and H-8 (A)], 7.16–7.34 [*m*, 6 × H (B and E)]*. ^{1}H NMR (DMSO-d_6, 170°), δ1.62–2.22 [30H, *m*, 10 × OAc], 2.68 [*m*, 2 × H, H-4 (F)], 4.53 [*d*, $J = 8.5$ Hz, H-4 (C)], 5.00 [*d*, $J = 10.0$ Hz, H-2 (C)], 5.12 [*d*, $J = 10.0$ Hz, H-2 (F)], 5.15 [*m*, H-3 (F)], 5.53 [*m*, H-3 (C)], 6.58 [*d*, $J = 2.5$ Hz, H-6 (A)], 6.70 [*d*, $J = 2.5$ Hz, H-8 (A), 6.75 [*s*, H-8 (D)], 7.25–7.34 [*m*, 6 × H (B and E)]. ^{13}C NMR ($CDCl_3$) heterocyclic system C-ring: δ79.65 (C-2), 71.82 (C-3), 36.50 and 37.00 (C-4); F-ring: 77.72 (C-2), 68.46 (C-3), 29.65 (C-4). CD $[\theta]_{292}$ 0, $[\theta]_{269}$ 16 633, $[\theta]_{245} - 5822$, $[\theta]_{228} - 30\,772$, $[\theta]_{225} - 27\,445$, $[\theta]_{215} - 48\,237$, $[\theta]_{211} - 15\,802$, $[\theta]_{208} - 24\,950$, $[\theta]_{205}$ 0.

[4,8]-2,3-trans-3,4-cis : 2,3-trans-*bi*-[(+)-*catechin*] (**1**). Rechromatography of the biflavanoid fractions (28–43) gave two subfractions: tubes 31–45 contained procyanidin B-3 and from tubes 53–62 the product (**1**) was obtained (18 mg). Acetylation and subsequent prep. TLC sepn with C_6H_6–$(Me)_2CO$, 4:1 (R_f 0.47) yielded the deca-acetate (**2**) (24 mg). $[\alpha]_{578}^{20} + 51.8°$ (*c* 0.2 in $CHCl_3$): $[M]^+$ *m/z* 998. ^{1}H NMR ($CDCl_3$): δ1.78–2.30 [30 H, *m*, 10 × OAc], 2.60 [*dd*, $J_{3,4} = 6.5$ Hz and $J_{4ax,4eq} = 16.4$ Hz, H-4_{ax} (F)], 3.19 [*dd*, $J_{3,4} = 8.75$ Hz and $J_{4eq,4ax} = 16.4$ Hz, H-4_{eq} (F)], 4.38 [*d*, $J = 9.3$ Hz, H-2 (F)], 4.70 [*d*, $J = 6.5$ Hz, H-4 (C)], 5.11 [*m*, H-3 (F)], 5.24 [*d*, $J = 9.6$ Hz, H-2 (C)], 5.30 [*m*, H-3 (C)], 5.96 [*d*, $J = 2.4$ Hz, H-6 (A)], 6.26 [*d*, $J = 2.4$ Hz, H-8 (A)], 6.66 [*s*, H-8 (B)], 7.00–7.26 [*m*, 6 × H (B and E)]; CD $[\theta]_{290}$ 0, $[\theta]_{275} + 3220$, $[\theta]_{255}$ 0, $[\theta]_{234} + 36\,060$, $[\theta]_{218} + 69\,500$, $[\theta]_{210} + 19\,320$. ^{13}C NMR ($CDCl_3$) heterocyclic system C-ring: δ78.18 (C-2), 71.02 (C-3), 32.37 and 29.66 (C-4); F-ring: 77.20 (C-2), 68.37 (C-3), 27.07 (C-4).

REFERENCES

1. Delcour, J. A., Ferreira, D. and Roux, D. G. (1983) *J. Chem. Soc. Perkin Trans.* 1, 1711.
2. Kolodziej, H., Ferreira, D. and Roux, D. G. (1984) *J. Chem. Soc. Perkin Trans.* 1, 343.
3. Thompson, R. S., Jacques, D., Haslam, E. and Tanner, R. J. N. (1972) *J. Chem. Soc. Perkin Trans.* 1, 1387.
4. Fletcher, A. C., Porter, L. J., Haslam, E. and Gupta, R. K. (1977) *J. Chem. Soc. Perkin Trans.* 1, 1628.
5. Baig, M. I., Clark-Lewis, J. W. and Thompson, M. J. (1969) *Aust. J. Chem.* **22**, 2645.
6. Baig, M. I., Clark-Lewis, J. W., Jemison, R. W. and Thompson, M. J. (1969) *Chem. Commun.* 820.
7. Clark-Lewis, J. W., Jackman, L. M. and Spotswood (1964) *Aust. J. Chem.* **17**, 632.
8. Botha, J. J., Ferreira, D. and Roux, D. G. (1978) *J. Chem. Soc. Chem. Commun.* 698.
9. Botha, J. J., Young, D. A., Ferreira, D. and Roux, D. G. (1981) *J. Chem. Soc. Perkin Trans.* 1, 1213.
10. Barrett, M. W., Klyne, W., Scopes, P. M., Fletcher, A. C., Porter, L. J. and Haslam, E. (1979) *J. Chem. Perkin Trans.* 1, 2375.
11. Hundt, H. K. L. and Roux, D. G. (1981) *J. Chem. Soc. Perkin Trans.* 1, 1227.
12. Weinges, K., Marx, H.-D. and Göritz, K. (1970) *Chem. Ber.* **103**, 2336.
13. Kessler, H. (1970) *Angew. Chem. Int. Engl. Ed.* **9**, 219.
14. Viviers, P. M., Kolodziej, H., Young, D. A., Ferreira, D. and Roux, D. G. (1983) *J. Chem. Soc. Perkin Trans* 1, 2555.
15. Weinges, K., Göritz, K. and Nader, F. (1968) *Liebigs Ann. Chem.* **715**, 164.
16. Hemingway, R. W., Karchesy, J. J., McGraw, G. W. and Wielesek, R. A. (1983) *Phytochemistry* **22**, 275.

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*Duplication of signals is due to rotameric forms. Recently reported [*Phytochemistry* **23**, 1745 (1984)] chemical shifts of the AB-system (A-ring) of B-6 acetate (**6**) are in error with the novel biflavanoid derivative (**2**).