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Note

Proanthocyanidins of barley: separation and identification

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Because of the lack of adequate separation techniques, proanthocyanidins of barley, which are major factors in beer chill haze, have usually been considered as a whole[1–8]. The first identification of these proanthocyanidins and their suggested formulae appeared not to be completely correct[9]. Later, they were partially established by analogy with the compounds isolated from other vegetal materials[10,11].

In order to study the influence of each proanthocyanidin on the colloidal stability of beer, we separated and purified each one by high-performance liquid chromatography (HPLC) according to the Jerumanis method[12] and established their structural formulae by ^{1}H and ^{13}C NMR spectroscopy. The structural formulae of propelargonidin and other prodelphinidins are currently being studied and the results will be published in the near future.

EXPERIMENTAL

Barley polyphenols were extracted with 75% acetone solution in a mixer. After removing the acetone with benzene, the polyphenols were extracted from aqueous solution with ethyl acetate. They were then fractioned by HPLC on a Polyamid-6 (Macherey, Nagel & Co., Düren, G.F.R.) preparative column (50 × 0.9 cm I.D.) or on a Sephadex LH-20 column (80 × 2.5 cm I.D.). For the Polyamid-6 column, the elution gradient was obtained using water and methanol (0–80%). With the Sephadex LH-20 column, elution was effected isocratically with 100% methanol (60 ml · h^{-1})

The proanthocyanidins fractions were collected and evaporated to dryness under vacuum, and the residue was dissolved in methanol and the solution injected into a preparative column (50 × 0.9 cm I.D.) filled with a reversed phase (Sil C_{18} HL 30 μm, RSL, Eke, Belgium). The gradient was linear from 0 to 10% water–acetic acid and the flow-rate was 2 ml · min^{-1}. The purified proanthocyanidins are peracetylated with acetic anhydride in pyridine.

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The compounds were identified by ^1H and ^{13}C nuclear magnetic resonance (NMR) spectroscopy. The ^1H NMR spectrum of peracetylated procyanidin B_3 was recorded on a Varian XL 200 spectrometer under the following conditions: spectrum width, 2000 Hz; impulse time, 5 μsec; solvent, deuterochloroform. The ^{13}C NMR spectra were recorded on a Varian CFT 20 spectrometer under the following conditions: spectrum width, 4000 Hz; acquisition time, 0.511 sec; α, 25°; free decay induction stored in 8K data points; complete decoupling of protons; solvent, deuterochloroform; chemical shift in parts per million relative to tetramethylsilane.

RESULTS AND DISCUSSION

Structural formulae of procyanidin B_3 and prodelphinidin (dimers) are shown in Fig. 1, and that of procyanidin C_2 (trimer) in Fig. 2.

Fig. 3 shows a pattern for the separation of dimers and trimers of procyanidins, prodelphinidins and propelargonidin. Separation of the prodelphinidins oligomers is possible only on the Sil C_{18} HL 5 μm (RSL) column.

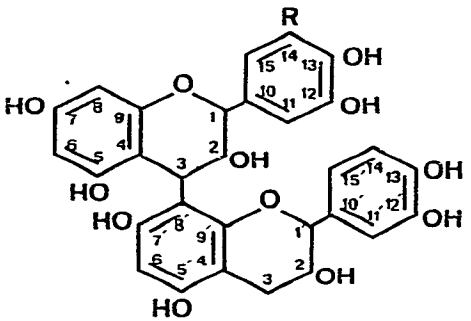

Fig 1. Structural formulae of dimers Procyanidin B_3, R = H; prodelphinidin (c), R = OH.

2 2′ 3
S S S

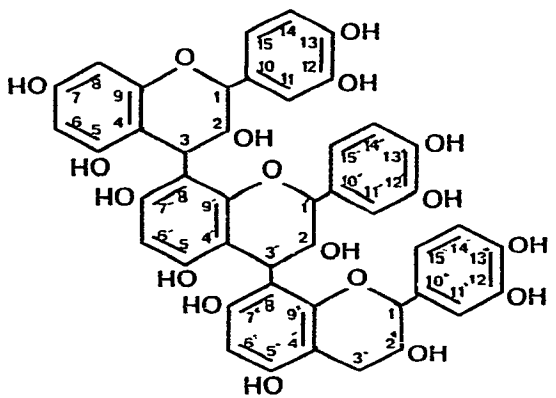

Fig. 2. Structural formula of trimer, procyanidin C_2

2 2′ 2″ 3 3
S S S S S

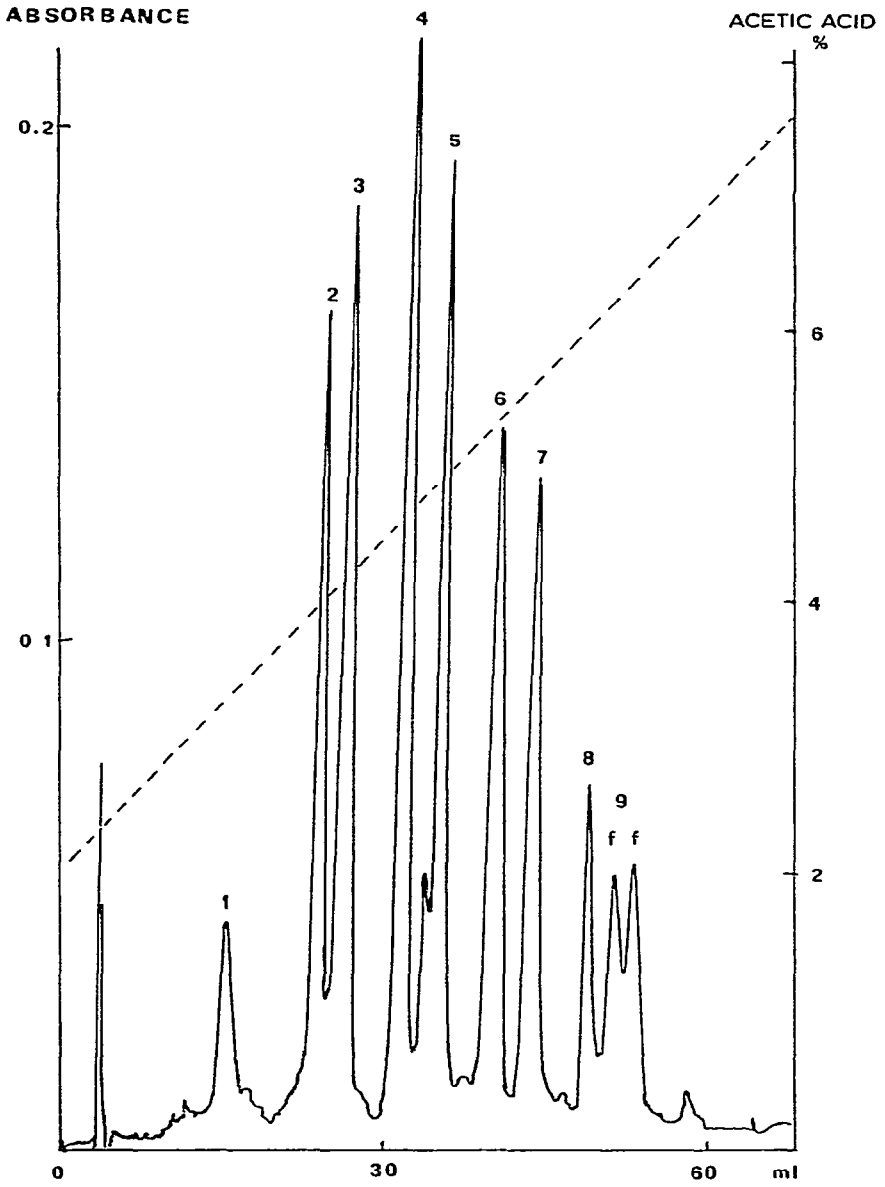

Fig. 3. Separation of proanthocyanidin dimers and trimers. Sil C_{18} 5 μm column; eluent, water–acetic acid; flow-rate, 1 ml min^{-1}; absorbance, 280 nm Peaks: 1 = prodelphinidin (a); 2 = prodelphinidin (b); 3 = prodelphinidin (c); 4 = prodelphinidin (d), 5 = prodelphinidin (e); 6 = procyanidin B_3; 7 = procyanidin C_2; 8 = propelargonidin; 9 = prodelphinidin (f) and (f')

The ^1H NMR spectrum of procyanidin B_3 (Table I) consists of two broad signals corresponding to 9 aromatic protons and 30 acetyl group protons. The protons H(1), H(2) and H(3) give a similar spectrum to procyanidin B_4[10]; the coupling constants, J[H(2) − H(1)] and J[H(2) − H(3)], both of 10 Hz, indicate that the proton H(2) is in an anti-configuration relative to the protons H(1) and H(3). The protons H(3′) correspond to two groups of four lines that can be analysed easily; finally, protons H(1′) and H(2) form the AB part of an ABPX system whose coupling constant J_{AB} {J[H(1′) − H(2′)] = 8.0 Hz} corresponds to an anti-configuration.

The ^{13}C NMR spectra of procyanidin B_3 (Table II) and prodelphinidin (Table III) have been interpreted on the basis of results obtained for reference compounds and of the fine line structure observed with "off-resonance" decoupling of protons. The only differences between the spectra of the compounds considered are on carbons 11, 14 and 15. In both instances, we observed three lines (121.6, 123.2, 124.4), ascribed to carbons 11′, 14′, 15′. Because of the replacement of one atom of hydrogen by a hydroxyl group, carbons 11 and 15 of prodelphinidin give only one line (119.3), whereas the carbon 14 gives a line in the region of =C–OH. Both lines (141.4, and 141.9) ascribed to carbons 12, 12′ and 13, 13′ of procyanidin become two lines (141.4 and 141.9) in the case of prodelphinidin, corresponding to carbons 12′ and 13′, plus one line (143.0) corresponding to carbons 12, 13 and 14.

The ^{13}C NMR spectrum of procyanidin C_2 (Table IV) has been interpreted by comparison with the ^{13}C NMR spectrum of procyanidin B_3; ^1H NMR spectra are being studied in order to check the stereochemistry of this compound.

TABLE I
^1H SPECTRUM OF PROCYANIDIN B_3 (DECAACETATE)
δ, chemical shift in ppm relative to tetramethylsilane. J = coupling constant in Hz. The numbering of the carbon atoms used in this paper differs from the ordinary pattern

6.50–7.40 (9 H, m), 5 66 {H(2), d d, J[H(2) − H(3)] = 9 2, J[H(2) − H(1)] = 10 0}; 5.06 {H(1′) + H(2), m, J[H(1′) − H(2′)] = 8 0}, 4 80 {H(1), d, J[H(1) − H(2)] = 10 0}, 4.52 {H(3), d, J[H(3) − H(2)] = 9 2}; 2 98 {H_a(3′), d d, J[H_a(3′) − H(2′)] = 5.2, J[H_a(3′) − H_b(3′)] = 16 0}; 2 70 {H_b(3′), d d, J[H_b(3′) − H(2′)] = 7 2}; 1 6–2.6 (30 H, m).

TABLE II
^{13}C SPECTRUM OF PROCYANIDIN B_3 (DECAACETATE)
The carbon-13 and the lines associated in a group have no selective designation

1,1′. 77.9, 78.5 2,2′: 68 2, 70 2. 3. 36 4. 3′. 25.5. 4,4′: 114.9, 116.7. 5,5′, 7,7′ 147.4, 147.7, 148.8, 149,4. 6,6′, 8,8′ 108 0, 109 3, 109 8, 111.3 9,9′· 152 6, 155 6 10,10′· 135 0 (2 lines) 11,11′, 14,14′, 15,15′ *121.7*, 122 5, *123 2* (2 lines), *124 4*, 124 7 12,12′, 13,13′ 141 4 (2 lines), 141.9 (2 lines) CH_3· 20.3 CO. ~168 5

TABLE III
^{13}C SPECTRUM OF PRODELPHINIDIN (c) (DECAACETATE)

1,1′: 77.7, 78.5. 2,2′· 68.1, 70 2 3. 36.4. 3′. 25.1. 4,4′. 114.9, 116.5. 5,5′, 7,7′: 147.4, 147.7, 148.8, 149 4. 6,6′, 8,8′. 108.0, 109.2, 109 9, 111.1 9,9′· 152.4, 155 5 10,10′ 134 8 (2 lines). 11,15: 119.3 (2 lines) 11′,14′,15′· *121 6, 123 2, 124 4.* 12,13,14· 143 0 (3 lines). 12′,13′ 141.4, 141.9. CH_3· 20.3 CO: ~168 5.

TABLE IV
^{13}C SPECTRUM OF PROCYANIDIN C_2 (PENTADECAACETATE)

1,1′,1″. 76 2, 78 5, 79 5, 2,2′,2″ 66 5, 70 7, 71.9, 3,3′. 36 5 (2 lines). 4,4′,4″,8′,8′ 116 5, 117.0, 118 6, 119 3 (2 lines). 5,5′,5″,7,7′,7″: 147 0, 147 1, 147 3, 147 8, 149 0, 149.8 6,6′,6″,8 108 1, 108 8, 109 6, 110 2 9,9′,9″· 151.0, 154.5, 156.1. 10,10′,10″: 134.8, 134 9, 136.4. 11,11′,11″,14,1r′,14″,15,15′,15″ 122 2, 122.3, 122 7, 123.0, 123.4 (2 lines) 124.4, 125 4. 12,12′,12″,13,13′,13″ 141 0, 141 3 (2 lines), 141 7, 142 1, 142 3 CH_3 20.3. CO: ~168 5

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