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Note

Isolation of quercetin, myricetin, and their respective dihydro-compounds by Sephadex LH-20 chromatography

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The direct synthesis of well characterised natural dimeric procyanidins from dihydroquercetin (see Fig. 1) and (+)-catechin[1] or (−)-epicatechin[2] requires the isolation of considerable amounts of these chemicals from natural sources. Whereas a good procedure has been published for the isolation of (+)-catechin and (−)-epicatechin from grapes by column chromatography[3], no such information is available on the isolation of dihydroquercetin.

The characterisation of trimeric proanthocyanidins from barley with mixed procyanidin–prodelphinidin stereochemistry[4–7] has prompted us to synthesise these compounds by a modification of existing procedures[1,2]. Therefore, we required dihydromyricetin (see Fig. 1) in addition to dihydroquercetin.

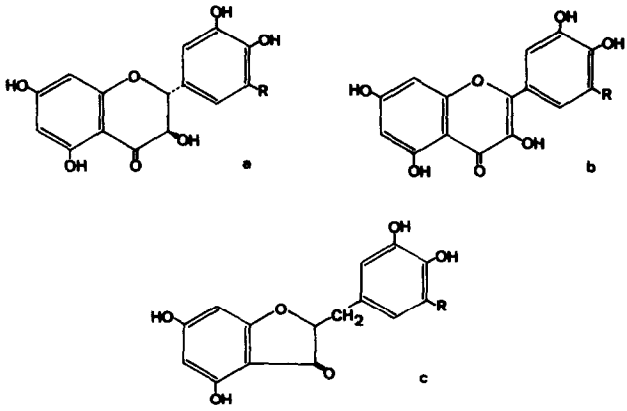

Fig. 1. Structural formulae of dihydroflavonols (a), flavonols (b) and tetra- (or penta-)hydroxy-2-benzylcoumaranones (c). For R = H: (a) dihydroquercetin; (b) quercetin; (c) tetrahydroxy-2-benzylcoumaranone. For R = OH: (a) dihydromyricetin; (b) myricetin; (c) pentahydroxy-2-benzylcoumaranone.

We describe in this paper the isolation of quercetin, myricetin, and their respective dihydro-compounds from the bark of *Pinus contorta* (lodgepole pine), as well as their subsequent purification by Sephadex LH-20 chromatography. It is generally accepted that Sephadex LH-20 is a useful adsorbent for the separation of proantho-

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cyanidins of different polymerisation degree, and we show here that it can be equally useful for the purification described.

Since quercetin can easily be reduced to dihydroquercetin in good yields with sodium dithionite[8–10], and since this method is also successful with myricetin[10], we were interested in the isolation of the two flavonols as well as of the two dihydroflavonols and in the Sephadex LH-20 separation of the dihydro-compounds, unreacted flavonols and tetra- (or penta-)hydroxy-2-benzylcoumaranones (see Fig. 1) formed by the sodium dithionite reduction of quercetin and myricetin[9].

EXPERIMENTAL

Isolation of flavonoids from Pinus contorta bark

A 36-year-old tree (*Pinus contorta*, 13.8 m) was removed from the arboretum of the Ministerie van Landbouw at Heverlee in Belgium. The bark (5.25 kg) was air-dried and subsequently dried in an oven overnight (105°C). After cooling to room temperature, the flakes were broken to small pieces and ground in a Retsch mill to pass a 2.0-mm sieve (total yield 3.97 kg).

Several procedures were tested for the extraction of flavonols and dihydroflavonols from the ground bark. Outtrup[11] described a simple method involving ethyl acetate Soxhlet extraction of the bark; after cooling of the ethyl acetate extract, removal of the precipitated waxes is accomplished by filtration. Concentration of the resulting solution yields a phenolic fraction that can be separated by preparative reversed-phase liquid chromatography. In our hands the cooling step to precipitate the waxes in the ethyl acetate extract proved to be insufficient.

Hergert[12] described the benzene extraction of an ether extract, the insoluble (in benzene) residue consisting of only myricetin (mainly) and quercetin. Extraction of dihydromyricetin and dihydroquercetin can be achieved, according to the same author, by treating the unextracted bark with boiling water.

Since we were interested in the isolation of flavonols as well as of dihydroflavonols, we adopted the following procedure. Oven-dried ground bark (400 g) was extracted in a Soxhlet extractor overnight with benzene. The benzene extract was discarded and the bark was further treated with ethyl acetate in the same manner. The ethyl acetate extract was concentrated to *ca.* 200 ml and cautiously poured into a beaker with boiling water (1 l). After evaporation of the ethyl acetate the waxes coagulated readily and were removed by suction (filtration) of the hot solution. The filtrate was allowed to cool and the flavonoids were adsorbed on four consecutive portions of Nylon 66 (10 g each). The Nylon 66 fractions were combined and stirred with a mixture of ethyl acetate (100 ml) and acetone (100 ml). Removal of the solvent by filtration and three more desorption with solvent mixture as above yielded a filtrate (*ca.* 800 ml) that was concentrated under reduced pressure to yield yellowish solids.

The solids were dissolved in methanol (20 ml) and chromatographed on Sephadex LH-20 (59 × 2.5 cm I.D.) in methanol–ethanol (1:1, v/v). Fractions (18.9 ml) were collected. The separation was monitored both by recording the E_{280} value of the eluates and by silica thin-layer chromatography on precoated Merck aluminium sheets (60PF_{254}, 0.25 mm) in toluene–chloroform–acetone–formic acid (8:5:7:3, v/v). Under these conditions the R_F values of dihydromyricetin, myricetin, dihydroquer-

cetin, and quercetin are 0.40, 0.50, 0.55, and 0.64, respectively. The compounds are easily visualised by spraying with sulphuric acid–formaldehyde (40:1, v/v) after development. In a typical separation, fractions 34–42 contained dihydroquercetin (245 mg), fractions 43–49 dihydromyricetin (165 mg), fractions 51–58 quercetin (160 mg), and fractions 50–76 myricetin (175 mg).

Reduction of flavonols to dihydroflavonols

The existing procedures[8–10] were modified as follows. To a mixture of 1.000 g of the flavonol, 6.62 mmoles (in the case of quercetin) or 6.29 mmoles (in the case of myricetin) of H_3BO_3 and 8.5 g of Na_2CO_3 under a nitrogen stream in a flask with a reflux condenser were added 100 ml of boiling water. The solution was heated to boiling, and 20 g of $Na_2S_2O_4$ was added. The mixture was kept at *ca.* 95°C for 25 min and then cooled in an ice-bath (20 min). The cooled solution was diluted to a volume of 200 ml, and then cautiously poured into a mixture of 200 ml of ethyl acetate and 13 ml of concentrated hydrochloric acid. The ethyl acetate layer was recovered and the mixture was extracted with more ethyl acetate (4 × 200 ml). The combined extracts were dried over Na_2SO_4 (100 g), and the solvent was removed on a rotary evaporator.

The crude phenolic mixtures obtained were separated on a Sephadex LH-20 column (58 × 2.5 cm I.D.) in methanol. The separation was monitored as above. Fractions (19 ml) were collected.

In the case of the reduction of quercetin the following fractions were recovered: 29–32, tetrahydroxy-2-benzylcoumaranone[9], R_F 0.50, 75 mg; 35–38, dihydroquercetin, 185 mg; and unchanged quercetin (295 mg), fractions 50–56.

The reduction of myricetin typically leads to the following products: pentahydroxy-2-benzylcoumaranone[9], R_F 0.36, 60 mg, fractions 30–33; dihydromyricetin, 260 mg, fractions 36–46; and myricetin, 420 mg, fractions 49–69.

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REFERENCES

1 J. A. Delcour, D. Ferreira and D. G. Roux, *J. Chem. Soc., Perkin Trans. I*, (1983) 1711.
2 J. A. Delcour, E. W. Serneels, D. Ferreira and D. G. Roux, *J. Chem. Soc., Perkin Trans. I*, (1985) in press.
3 J. D. Baranowski and C. W. Nagel, *J. Agr. Food Chem.*, 29 (1981) 63.
4 H. Outtrup, *Eur. Brew. Conv. Proc.*, (1981) 323.
5 H. Outtrup and K. Schaumburg, *Carlsberg Res. Commun.*, 46 (1981) 43.
6 P. Mulkay, R. Touillaux and J. Jerumanis, *J. Chromatogr.*, 208 (1981) 419.
7 L. J. Brandon, L. Y. Foo, L. J. Porter and P. Meredith, *Phytochemistry*, 21 (1982) 2953.
8 J. C. Pew, *J. Amer. Chem. Soc.*, 70 (1948) 3031.
9 T. A. Geismann and H. Lischner, *J. Amer. Chem. Soc.*, 74 (1952) 3001.
10 M. Shimizu and T. Yoshikawa, *J. Pharm. Soc. Japan*, 72 (1952) 331.
11 H. Outtrup, personal communication.
12 H. L. Hergert, *J. Org. Chem.*, 21 (1956) 534.