Phytochemistry, 1974, Vol 13, pp 1477 to 1478 Pergamon Press Printed in England

[^{14}C]EPICATECHIN AND [^{14}C]PROCYANIDINS FROM SEED SHELLS OF *AESCULUS HIPPOCASTANUM*

RICHARD EASTMOND and RONALD JOHN GARDNER

Brewing Industry Research Foundation, Nutfield, Redhill, Surrey, England

(*Received* 30 *November* 1973 *Accepted* 18 *January* 1974)

Key Word Index—*Aesculus hippocastanum*, Hippocastanaceae, horse chestnut, flavonoid biosynthesis, labelled polyphenols, epicatechin, procyanidin

Abstract—Direct injection of sodium-[1-^{14}C]acetate into growing fruits of horse chestnut provides a convenient route to [^{14}C]labelled epicatechin and procyanidins

INTRODUCTION AND DISCUSSION

RECENTLY we required substantial quantities of [^{14}C] labelled polyphenols for use in investigations concerning haze formation in beer.[1] A synthetic route to [^{14}C]epicatechin would necessitate a multi-stage synthesis of cyanidin chloride[2] followed by reduction to give epicatechin.[3,4] However, epicatechin and a number of procyanidins have been isolated in good yield from seed shells of *Aesculus hippocastanum*.[5–7] Therefore, feeding a [^{14}C] flavonoid precursor to the growing fruit of *A. hippocastanum* seemed a possible route to the desired compounds.

We now report the isolation of [^{14}C]epicatechin and various [^{14}C]procyanidins from seed shells of *A. hippocastanum* fed with sodium [1-^{14}C]acetate.

Two methods of feeding were examined and one of these gave a useful level of incorporation of [^{14}C] into the phenolic fraction. In this more useful procedure, aqueous sodium [1-^{14}C]acetate was injected into the fruit whilst still on the tree. The fruits were allowed to grow for a further 2 weeks and then, when almost mature, harvested at the end of August. An appreciably lower incorporation was obtained by an alternative procedure in which aq. sodium-[1-^{14}C]acetate was slowly fed, via a burette, into a hole drilled in a fruit bearing branch.[8] The [^{14}C]procyanidins were isolated from the seed shells by a procedure similar to that described by Haslam *et al.*[5] Separation of the crude phenolic extract on a column of Sephadex LH20 gave six fractions, one of which (fraction 3; see Table 1) was rich in (-)-epicatechin. It was found that (-)-epicatechin in methanol is not retained by Sephadex QAE in the chloride form, in contrast to dimeric and polymeric procyanidins.

[1] EASTMOND, R and GARDNER, R J. (1974) *J Inst Brewing*, **80**, 192
[2] ROBERTSON, A and ROBINSON, R (1928) *J. Chem Soc* 1526
[3] FREUDENBERG, K, FIKENTSCHER, H, HARDER, M and SCHMIDT, O (1925) *Annalen* **444**, 135
[4] REYNOLDS, G A and VANALLAN, J. A., (1967), *J Org Chem* **32**, 3616
[5] THOMPSON, R S, JACQUES, D., HASLAM, E. and TANNER, R. J N (1972) *J. Chem Soc Perkin* **1**, 1387.
[6] WEINGES, K, KALTENHAUSER, W, MARX, H.-D, NADER, E, NADER, F, PERNER, J and SEILER, D (1968) *Annalen* **711**, 184.
[7] MAYER, W, GOLL, L., ARNDT, E M, VON, and MANNSCHRECK, A (1966) *Tetrahedron Letters* 429.
[8] HOWARD, G A and WRIGHT, D, (1961) *J. Inst Brewing* **67**, 236

Therefore, fraction 3 was chromatographed on a short column of Sephadex QAE and, after evaporation of the eluate, the brown–white solid was recrystallized from water to give pure [^{14}C]epicatechin (1·6 μCi/mmol, 0·4% incorporation) [^{14}C]Procyanidin A2[9] (1 1 μCi/mmol) obtained from fraction 5 (Table 1) without the use of Sephadex QAE, was directly recrystallized from methanol/water (1:3). Paper chromatography and autoradiography demonstrated that other fractions also contained [^{14}C]procyanidins In particular, fraction 4 was rich in [^{14}C]procyanidins B1 and B2

TABLE 1 FRACTIONATION OF A [^{14}C]PHENOLIC EXTRACT FROM *Aesculus hippocastanum* ON SEPHADEX LH20

Fraction	Tube nos *	Weight† (mg)	Activity × 10^{6}‡ (dpm/g)	Phenolic components
1	20–27	15	23	–
2	28 37	23	8 0	– –
3	38–49	126	9 4	epicatechin
4	50–63	42	6 4	procyanidins B1 & B2
5	64–75	50	6 9	procyanidin A2
6	76–100	17	6 6	

* The methanol eluent was monitored at 280 nm and collected in 50 ml tubes (100 fractions)

† The phenolic extract (0 5 g) was obtained from 62 g of seed shells labelled by method 2

‡ By liquid scintillation (Nuclear Enterprises, NE250 scintillator) on a Beckman LS100 counter

EXPERIMENTAL

Feeding of [^{14}C]acetate to Aesculus hippocastanum Method 1 A soln of sodium [1-^{14}C]acetate (2 mCi, 56 mCi/mmol) in H_2O (10 ml) was allowed to flow through a stainless steel tube into a fruit bearing branch (*ca* 1 m dia) of a mature specimen of *A hippocastanum*, during a period of 2 weeks in mid-August At the end of this time the seed shells (750 g) were harvested and the crude phenolic extract (8 g, 6 0 × 10^{6} dpm/g) obtained as described below *Method* 2 A solution of sodium [1-^{14}C]acetate (1 mCi) in H_2O (5 ml) was used to inject fruits of *A hippocastanum* growing on a mature tree in mid-August The fruits (42) were injected just below the outer skin with samples (100 μl, 20 μCi) of the above solution The fruits were protected by perforated plastic bags and the injections were repeated 1 week later with a further 20 μCi of sodium [1-^{14}C]acetate per fruit After a further week's growth, the seed shells (1300 g, 42 fruits) were harvested and the procyanidins extracted

Extraction of procyanidins from fruits The seed shells (1300 g, labelled by method 2) were macerated with methanol through a short column (6 × 1 cm) of Sephadex QAE (chloride form) The crude [^{14}C]epicatechin obtained washed with petrol (b p 60–80, 4 × 1 l) diluted with H_2O (2 l), and then extracted with EtOAC (5 × 1 l) Evaporation of the dried EtOAC extract gave a crude phenolic extract (10 5 g 1 8 × 10^{7} dpm/g) The crude phenolic extract (0 5 g portions) was chromatographed on a column (50 × 800 mm) of Sephadex LH20[9] using methanol as eluant to give five major fractions (see Table 1) Examination by PC (Whatman No 1 paper, *t*-BuOH HOAc–H_2O (14 1 5) and 6% HOAc, spray 0 2% aq $FeCl_3$ $K_3Fe(CN)_6$) demonstrated the phenolic composition of each fraction Dimeric procyanidins and colouring matter were removed from fraction 3 (150 mg) by passage in methanol through a short column (6 × 1 cm) of Sephadex QAE (chloride form) The crude [^{14}C]epicatechin obtained (96 mg) was purified by recrystallization, with charcoal, from H_2O The identity of the [^{14}C]epicatechin was confirmed by comparison with authentic (-)-epicatechin (mp mixed mp, UV, IR, PC and GLC of the trimethylsilyl derivative) [^{14}C]Epicatechin (1 5 g) was obtained from the seed shells (1300 g) with an activity of 1 6 μCi/mmol representing 0 4% incorporation Direct recrystallization of fraction 5 from methanol/H_2O (1 3) with charcoal, gave [^{14}C]procyanidin A2 (630 mg, from 1300 g seed shells) with an activity of 1 1 μCi/mmol (0 1% incorporation) [^{14}C]Procyanidins of the B type were obtained in lower yield from fraction 4

Acknowledgement—We thank Dr J Jones of the University of Surrey for the use of the scintillation counter

[9] BEDFORD, G R , GREATBANKS D , HASLAM, E , and JACQUES. D (1973) *Chem Commun* 518