Characterisation of sainfoin (*Onobrychis viciifolia*) tannins by degradation studies, chromatography and calorimetry

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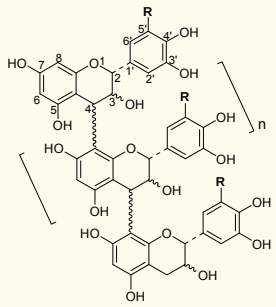
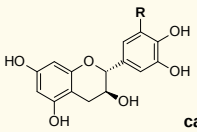
Introduction

Sainfoin (*Onobrychis viciifolia*) is an excellent fodder legume with very high voluntary intakes by cattle, sheep and horses. Ruminants utilise sainfoin **protein** much more **efficiently** than lucerne or soy protein. More efficient nutrient utilisation from sainfoin leads to **less emission of nitrogen, and methane**, which is one of the most damaging greenhouse gases. Research also suggests that sainfoin possesses natural **anthelmintic properties**. It could therefore serve as an ideal fodder legume in more sustainable livestock farming systems.[1]

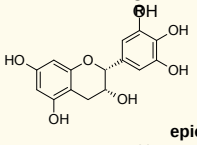
This interdisciplinary research examines the hypothesis that the **condensed tannins** are responsible for the above mentioned benefits of sainfoin. However, there are contradictory reports in the literature concerning the **structures and molecular weights** of sainfoin tannins.[2] Therefore, detailed chemical analysis is required to identify its active, beneficial tannins. This **structure-activity relationship** study will be of use in future plant breeding/selection studies.

Condensed tannin composition and structures





catechin (C) **R** = H
gallocatechin (GC) **R** = OH



epicatechin (EC) **R** = H
epigallocatechin (EGC) **R** = OH

Tannin extraction & fractionation

Procedure:

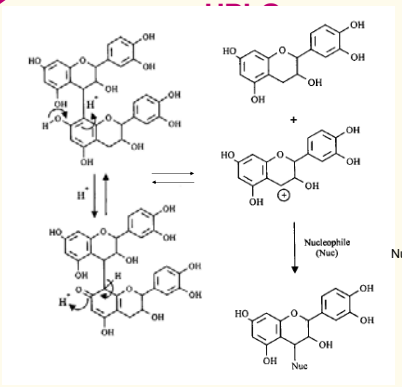
1. freeze dry *Onobrychis viciifolia* var. Cotswold Common (50% flowering, whole plants)
2. extract with acetone/H_2O-7:3
   yield = 21g/100g sainfoin (on dry matter basis)[3]
3. separate into 2 fractions (with Sephadex LH20) resp. 9 fractions (with Toyopearl HW50F;
   first 3 fractions were eluted with water and were not analysed)

column packing	MeOH/H_2O-1:1* (A)			acetone/H_2O-7:3 (B)		
Sephadex LH20[4]	13.1%			4.1%		
Toyopearl HW50F	0.9% (A1)	10.5% (A2)	3.6% (A3)	0.1% (B1)	2.3% (B2)	1.2% (B3)

% is expressed asg extract/100g sainfoin (on dry matter basis)
* may contain some sugars

Table 1

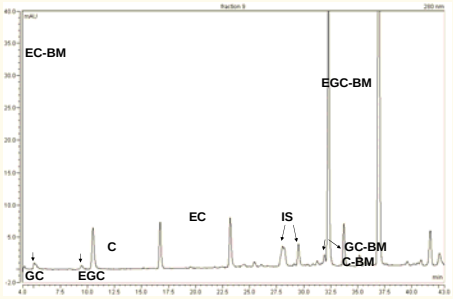
Tannin degradation & HPLC



Nuc = benzyl mercaptan (BM)

Degradation conditions:
1. tannin (1mg), 100 µl of 5% benzyl mercaptan/MeOH; 100 µl of 3.3% HCl/MeOH, 50 °C, 20 min
2. 40 mM NaOAc/H_2O, rt, 5 min

HPLC conditions: column: Intersil ODS-2, 250x4.6mm, 5µ; solvent A: 1%HOAc/H_2O; solvent B: MeOH; wavelength: 280 nm; internal standard = dihydroquercetin



fraction	mDP	PC: PD	*cis:trans*
A	8.2 (1.4)	35.0 (0.7) : 66.0 (0.7)	91.0 (0.2) : 9.0 (0.2)
B	14.2 (0.4)	40.0 (0.1) : 60.0 (0.1)	85.0 (0.2) : 15.0 (0.2)
B3	36.3 (5.7)	20.0 (1.4) : 80.0 (1.4)	92.0 (1.4) : 8.0 (1.4)

mDP = mean degree of polymerisation
PC:PD = ratio of procyanidin to prodelphinidin content in the tannins
cis:trans = ratio of *cis* and *trans* orientation of the benzyl group on C2 and the OH on C3; values are not corrected for C3-isomerisation
* value between brackets is the standard deviation (n = 2)

Table 3

Isothermal titration calorimetry

	sumac tannins (GT)	tara tannins (GT)	sainfoin tannins (CT)	chestnut tannins (ET)	myrabolan tannins (ET)
K_a (M^{-1})	$1.7x10^5$	$1.0x\ 10^4$	$6.6x10^3$	$9.0x10^2$	$7.0x10^2$
	binding affinity ←				
ΔH_{obs} (kJ/mol)	-30.3	-33.0	-21.82	-39.8	-58.1
$T\Delta S$ (kJ/mol)	-0.45	-10.17	0.03	-22.94	-41.87
ΔG (kJ/mol)	-29.85	-22.83	-21.79	-16.86	-16.23
	stability of tannin•BSA complex ←				
N	9.4	2.5	35.2	17.7	22.0

GT = gallotannins
CT = condensed tannins
ET = ellagitannins

K_a = equilibrium binding constant
N = tannin/protein binding stoichiometry

Table 2[5]

Experimental procedure:
A solution of 3mg/ml of **fraction B (Table1, entry 1)** in a 50mM citric acid buffer (pH 6) was titrated into a 10 µM BSA solution in 50 mM citric acid buffer (pH 6) at 298K.
A CSC Nano ITC Series III calorimeter (Calorimetry Sciences Corp., Lindon, UT) was used for all experiments.

Conclusions

1. Calorimetry shows that both the binding affinity and the stability of the sainfoin tannin-BSA complex falls between gallotannins and ellagitannins

2. Toyopearl enabled the isolation of a fraction containing high molecular weight tannins

3. The high molecular weight fraction has a higher prodelphinidin content, this confirms earlier studies

4. Sainfoin contains a highly complex mixture of condensed tannins in terms of PC:PD ratios and molecular weights. Comparisons between the tannins from sainfoin, *Lotus pedunculatus* and *Lotus corniculatus* are ongoing in order to determine if this complexity is the key to their beneficial effects of sainfoin

References & acknowledgement

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