

Sodium hydroxide enhances extractability and analysis of proanthocyanidins in ensiled sainfoin (onobrychis viciifolia)

Article

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SUPPORTING INFORMATION

Table S1. Analysis of variance for proanthocyanidin parameters using reaction times of 5 to 60 min and 0.5 to 4.0 M NaOH concentrations.

Sources	df	F-value	P
PA			
Time	3	21.1	0.001
NaOH	4	4.7	0.008
NaOH x Time	12	0.6	0.796
mDP			
Time	3	37.2	< 0.001
NaOH	4	24.6	< 0.001
NaOH x Time	12	5.7	< 0.001
PD %			
Time	3	10.6	< 0.001
NaOH	4	5.9	0.002
NaOH x Time	12	0.9	0.494
Cis %			
Time	3	59.8	< 0.001
NaOH	4	16.2	< 0.001
NaOH x Time	12	1.2	0.364

PA: proanthocyanidin content; mDP: mean degree of polymerization; PD %: molar percentage of prodelphinidins; *cis* %: molar percentage of *cis*-flavan-3-ols).

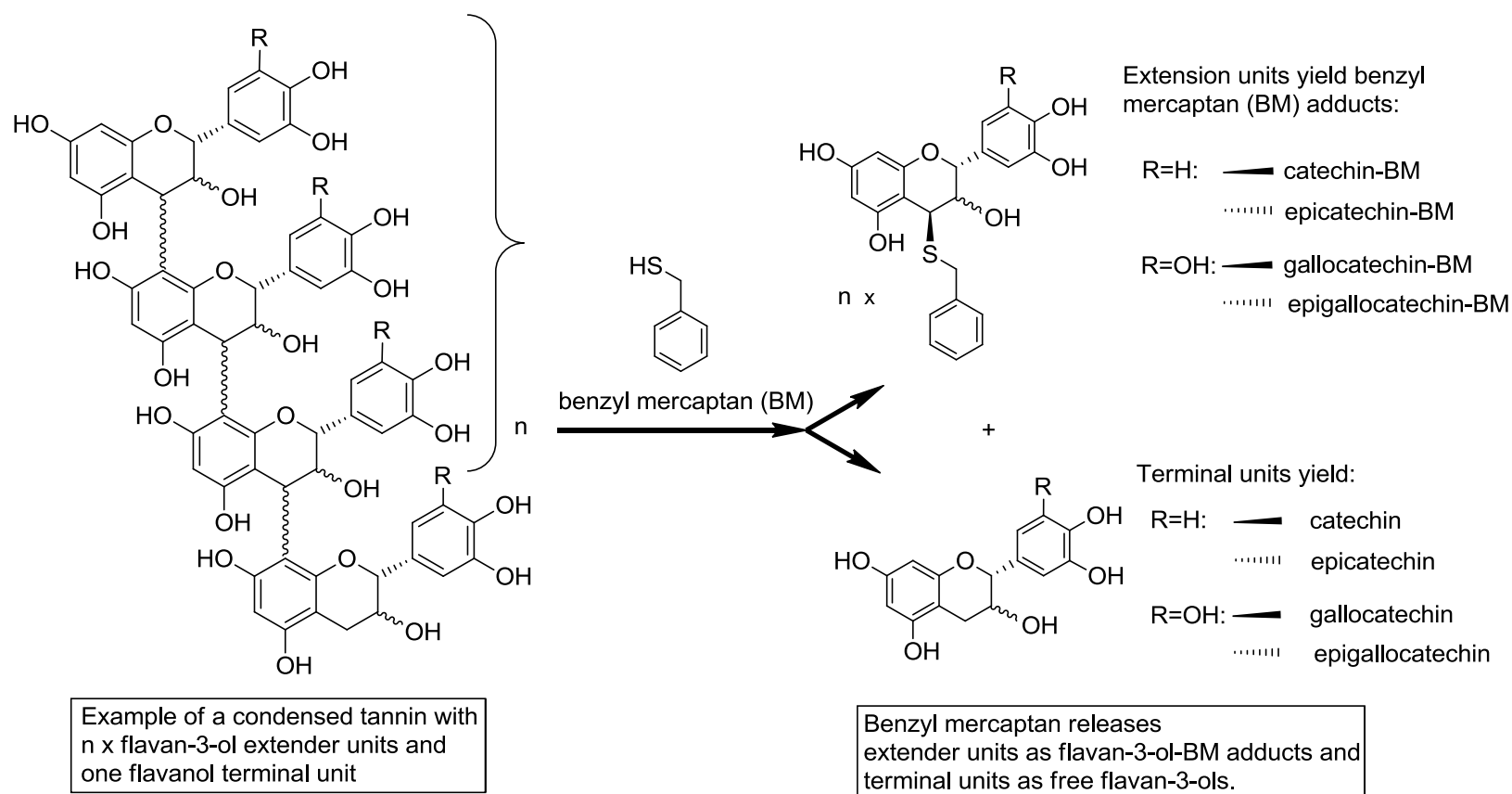


Figure S1. Thiolytic degradation of proanthocyanidins with benzyl mercaptan (Note: extension units of catechin and gallocatechin are released as the 3,4-*cis* and *trans* adducts).¹⁰

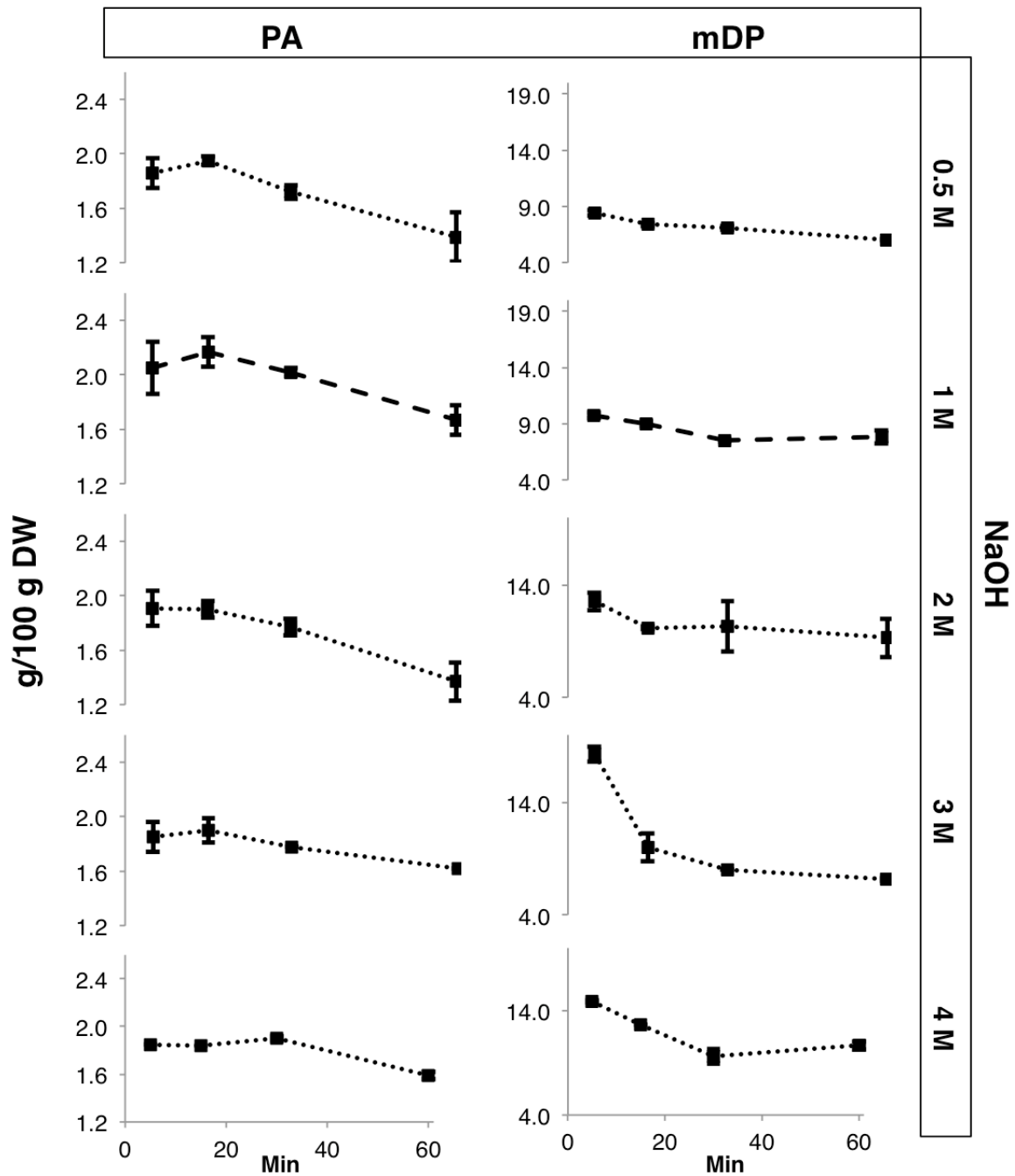


Figure S2. Changes in proanthocyanidin (PA) contents and mean degrees of polymerization (mDP) in sainfoin silage treated with different NaOH concentrations over a 60 minutes period at 40 °C followed by thiolysis with benzyl mercaptan and LC-MS analysis. Dashed lines include the chosen parameters (1 M NaOH, 15 minute reaction time).

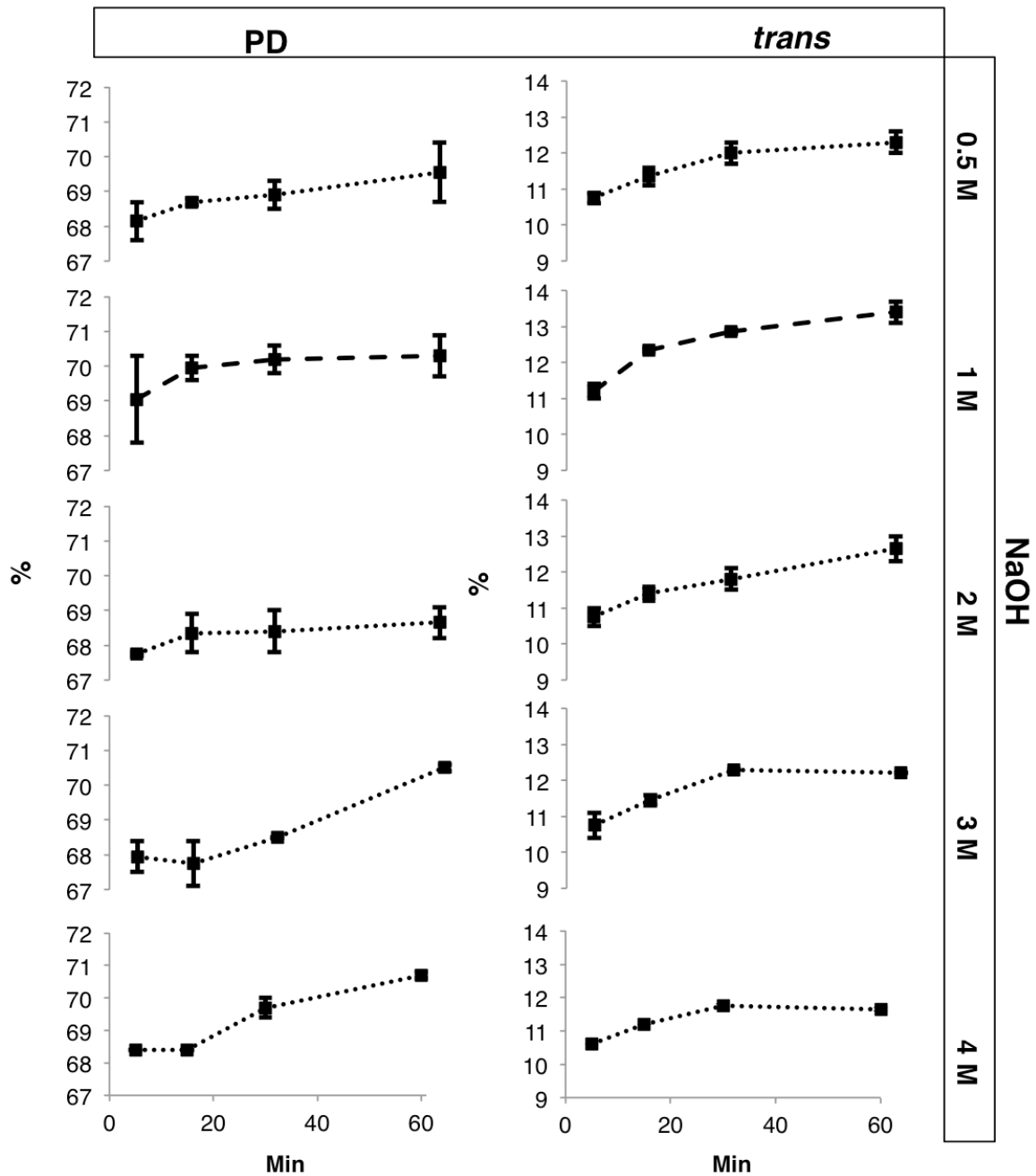


Figure S3. Changes in molar percentage of prodelfphinidins (PD) and *trans* flavan-3-ols (*trans*) in sainfoin silage with different NaOH concentrations over a 60 minute time period (dashed lines include the chosen parameters of 1 M NaOH and a 15 minute reaction time).

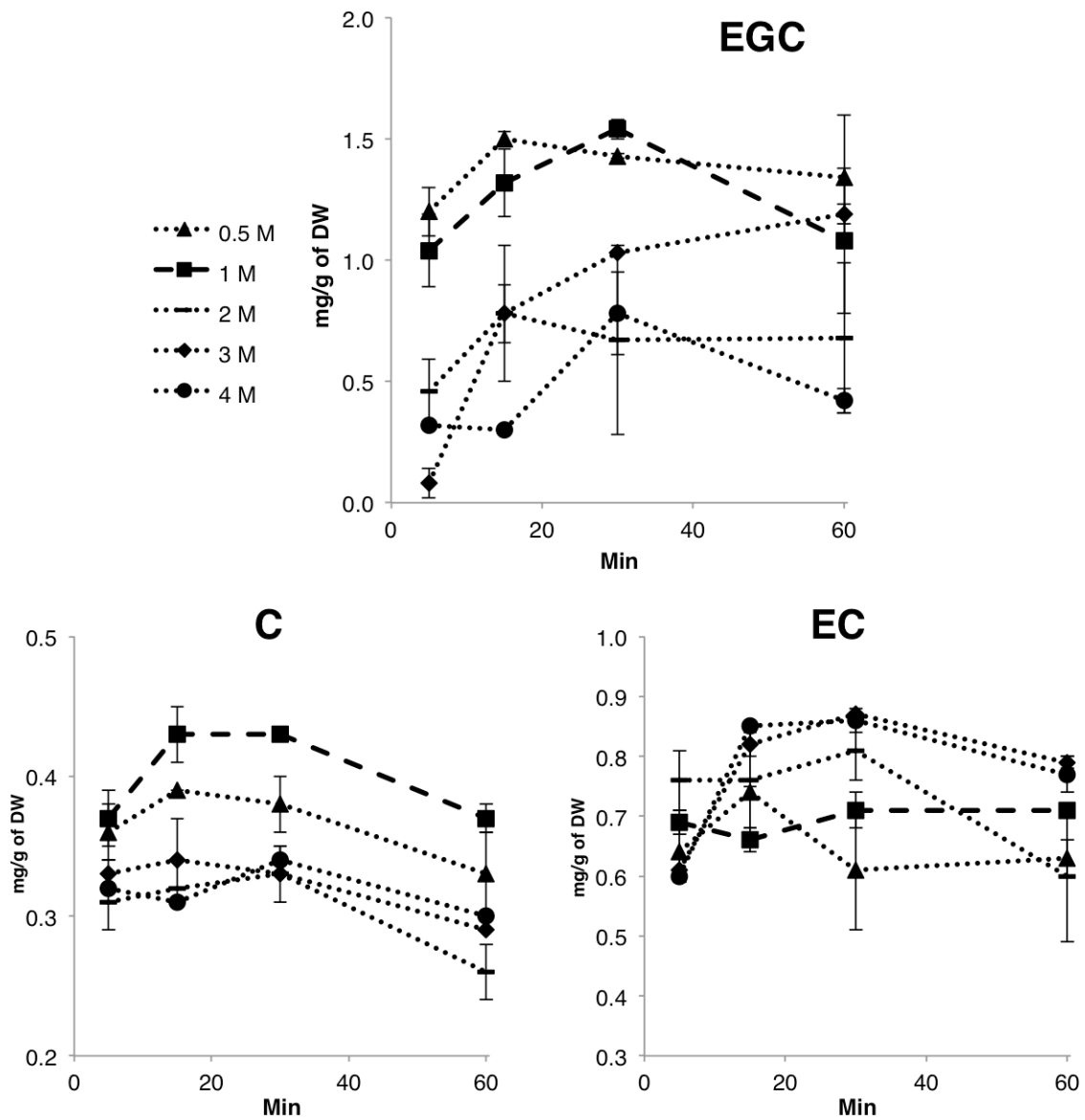


Figure S4. Changes in flavan-3-ol terminal units (mg/g of dry weight) in sainfoin silage. C: catechin; EC: epicatechin; EGC: epigallocatechin.

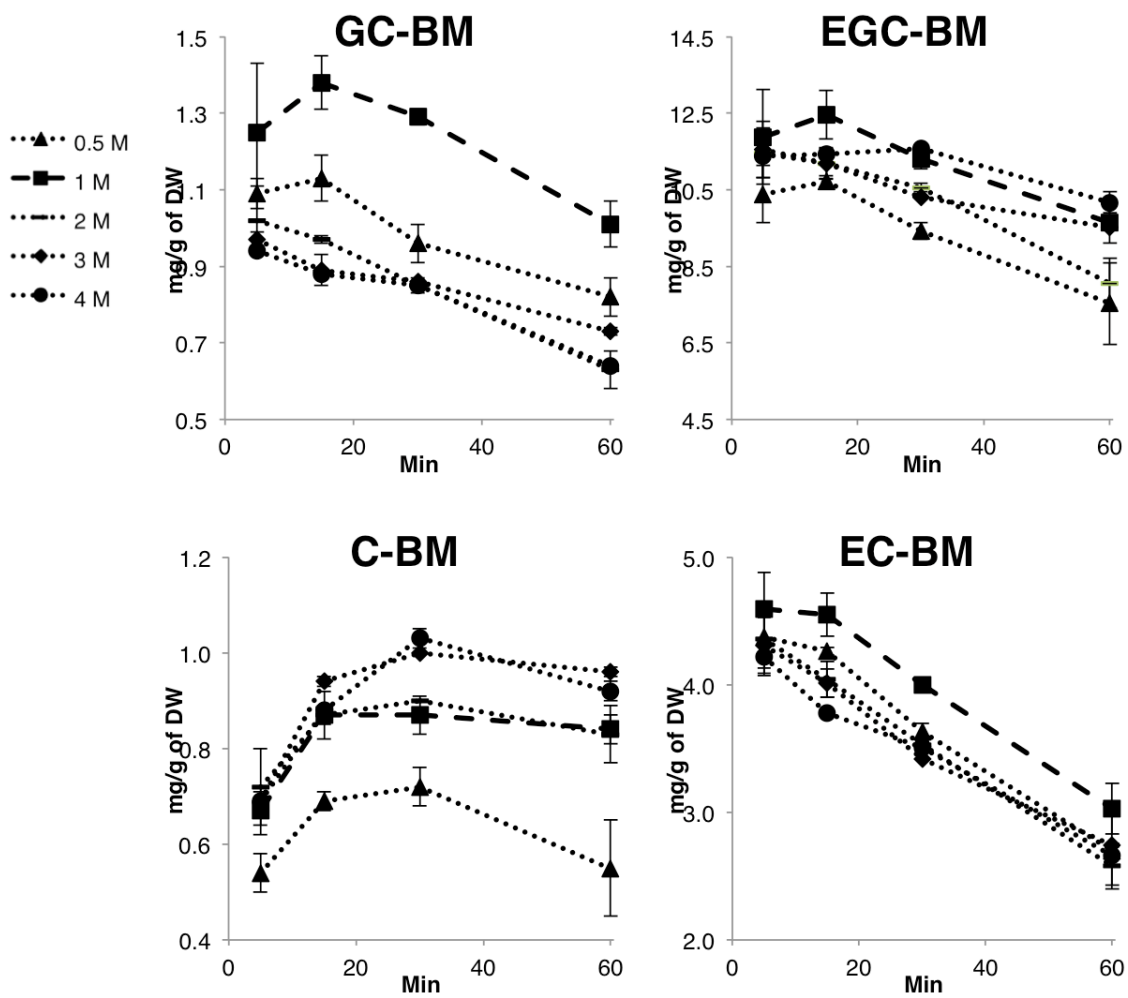


Figure S5. Changes in flavan-3-ol extension units (mg/g of dry weight) in sainfoin silage. BM: benzyl mercaptan adduct; C: catechin; EC: epicatechin; GC: galocatechin; EGC: epigallocatechin.

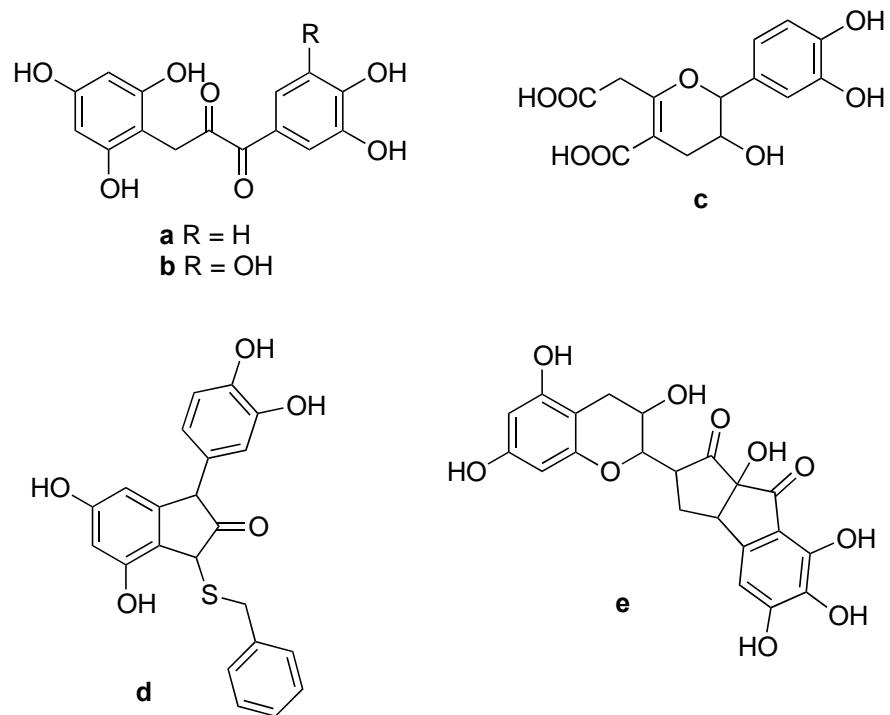


Figure S6. Tentative assignments of some reaction products obtained after NaOH pre-treatment and thiolysis of a pure proanthocyanidin fraction from sainfoin (see Figure 2 for peaks labeled a to e).