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Phenolic glycosides of forage legume *Onobrychis viciifolia*

Yinrong Lu[a,*], Yan Sun[a], L. Yeap Foo[a], W.C. McNabb[b], A.L. Molan[b]

[a]*Industrial Research, Gracefield Research Centre, PO Box 31-310, Lower Hutt, New Zealand*
[b]*AgResearch, Grasslands Research Centre, Private Bag 11008, Palmerston North, New Zealand*

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Abstract

A chemical examination of the extractives of the leaves of sainfoin was undertaken as part of a programme directed at understanding the factors which may contribute to its nutritive value as animal feed. Among the low molecular weight phenolic compounds characterized were seven cinnamic acid derivatives and nine flavonoid glycosides all of which were identified by NMR spectroscopy. Included among these compounds were two new natural hydroxycinnamic esters namely methyl 6-*O*-*p*-*trans*-coumaroyl-β-D-glucopyranoside and methyl 6-*O*-*p*-*cis*-coumaroyl-β-D-glucopyranoside and a novel flavonoid chrysoeriol-4′-*O*-(6″-*O*-acetyl)-β-D-glucopyranoside. © 2000 Elsevier Science Ltd. All rights reserved.

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1. Introduction

Sainfoin (*Onobrychis viciifolia* Scop.), sometimes also known as holy clover, is a perennial forage legume with a deep taproot often grown in conjunction with forage grasses to reduce bloat hazard as well as to improve soil fertility due to its nitrogen fixing ability. Another desirable trait is that sainfoin has an early growth habit, sprouting earlier than alfalfa in spring to give good forage yields. While the availability of early fresh forage for stock is appreciated by farmers it is the ability of the feed to reduce incidence of bloat and increase animal performance that provided the main incentive for its incorporation to farm management. Bloat is a major worldwide problem and frequently occurs in dairy cattle in New Zealand affecting up to 90% of the herds in a district causing many deaths (Clark and Reid, 1974).

The presence of the polyphenolic proanthocyanidins in feeds prevents bloat in cattle (Waghorn and Jones, 1989; Lees, 1992). At high level, proanthocyanidins exhibit anti-nutritional properties due to formation of complexes with protein (Singleton and Kratzer, 1973; Mitaru et al., 1984) but at low concentration in ruminant diets a number of beneficial effects have been reported such as reducing the effects of parasites in the gastrointestinal tract (Niezen et al., 1995) and minimizing microbial degradation of feed protein in the rumen (Waghorn et al., 1990). Sheep fed on fresh sainfoin have higher nitrogen retention than those fed on white clover or ryegrass, which contained little or no proanthocyanidins (Egan and Ulyatt, 1980; Waghorn et al., 1987; McNabb et al., 1993).

Other plant polyphenols such as the cinnamic acid derivatives and flavonoids have been shown to possess a range of biological activity which could be beneficial to animal health and potentially could improve flavour and shelf life of farm products (Simpson and Uri, 1956). An increased level of vitamin E in animal diets for instance has been linked to the increase of the vitamin in the tissue, which leads to a post-mortem protection against peroxidation and improving quality and storage stability of meat (Baker, 1999). These phenolic constituents in particular have been shown to be effective free radical scavengers and antioxidants which are central to the maintenance of homeostasis in biological systems (Torel et al., 1986; Ames et al., 1993). Cin-

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* Corresponding author. Tel.: +64-4-569-0000; fax: +64-4-569-0055.
E-mail address: y.lu@irl.cri.nz (Y. Lu).

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namic acids are also potent inhibitors of nitrosamine formation (Kikugawa et al., 1983) while proanthocyanidins and flavonoids have been reported to exhibit antimicrobial (Marwan and Nagel, 1986; Stavric, 1994) and anti-inflammatory activities (Kakegawa et al., 1985; Shoskes, 1998). As these phenolic compounds are increasingly being considered to contribute to animal health and productivity it is, therefore, important that considerations of the nutritive value of forages should include not only the proanthocyanidins but also other phenolics which are contained in them.

2. Results and discussion

The defatted 70% aqueous acetone extract of the fresh leaves of *O. viciifolia* Scop. was revealed by 2D TLC to contain a very complex mixture of phenolic compounds including those which were visible under UV and those which developed a red coloration on treatment with vanillin-HCl spray reagent. The freeze-dried extract (139 g) was stirred in water and the resulting suspension was centrifuged to yield a yellow solid (9.5 g) which was shown by HPLC to consist primarily of two flavonoid glycosides. Resolution of the mixture was effected by chromatographic treatment over Sephadex LH20 to yield quercetin-3-rutinoside (**8**) as the major and kaempferol-3-rutinoside (**12**) as the minor compound. The identity of both compounds was made from their NMR spectra as discussed below.

The water soluble extract was absorbed onto a Sephadex LH20 column which was developed first with water, which removed the sugars, then with aqueous methanol, which yielded three main fractions (a),

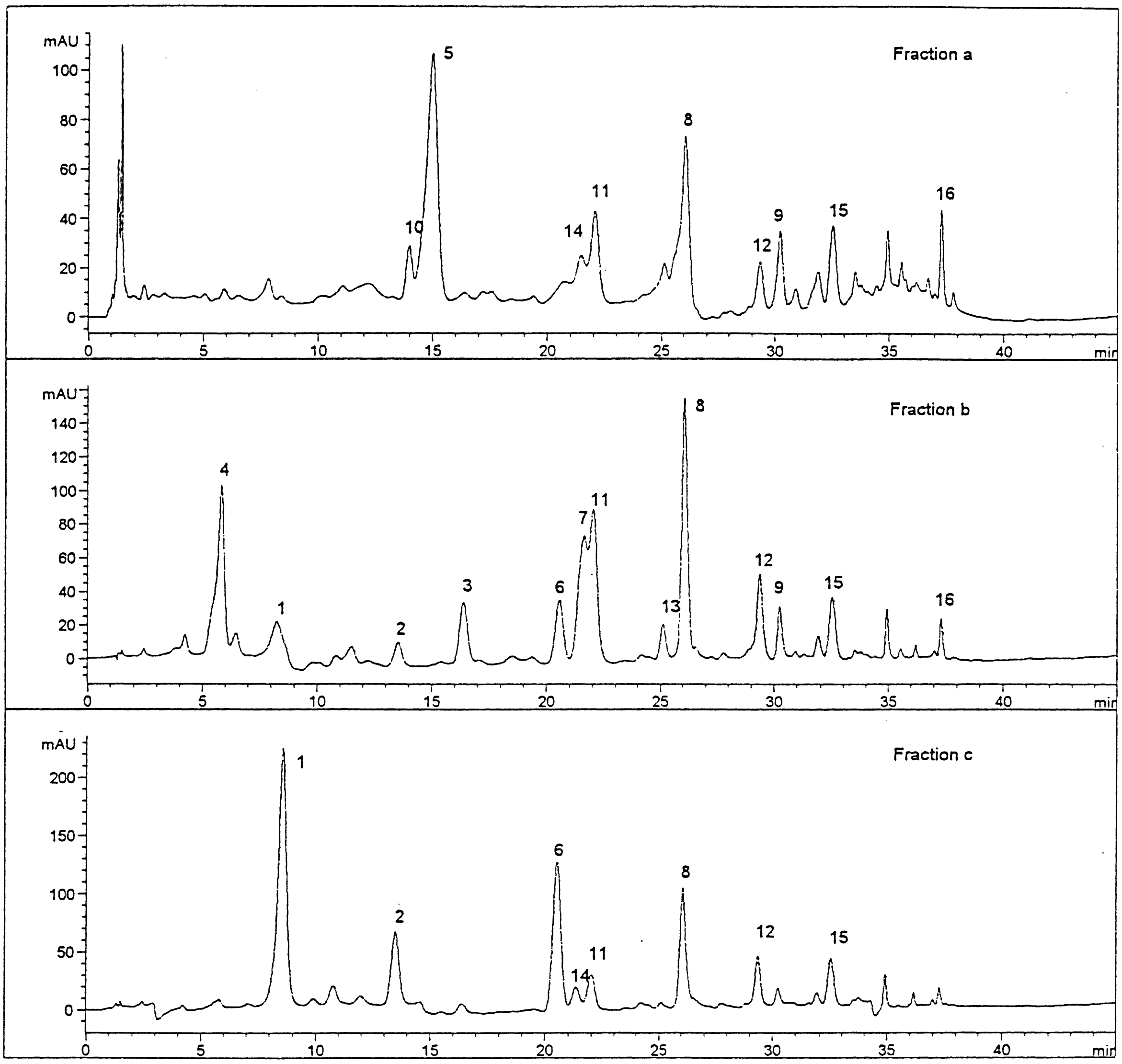


Fig. 1. HPLC of the three chromatographic fractions of sainfoin leaf extract. The numbering on HPLC peaks referred to compounds in the text.

(b) and (c). HPLC with UV detection at 280 nm showed each chromatographic fraction to consist of a complex mixture (see Fig. 1). Repeated column chromatography of the fractions separately using aqueous ethanol alternating between Sephadex LH20 and MCI HP20 led to the isolation of compounds **1–16**. Compound **1** was one of the more abundant constituents and was identified as chlorogenic acid by spectral and HPLC comparison with an authentic sample. Compound **2** had λ_{max} at 312 nm indicative of the presence of *p*-coumaric acid moiety as the sole chromophore. In addition, the observation of a pair of high field carbons (δ 38.6 and 39.1) in the ^{13}C-NMR together with three oxygenated carbon chemical shifts in the 70–75 ppm region suggested the presence of a quinic acid moiety. This was also corroborated by ^{1}H-NMR with proton chemical shifts and proton–proton coupling patterns consistent with a *p*-coumaroyl chemical structure and a quinic acid aliphatic ring system. Both ^{13}C- and ^{1}H-NMR of the quinic acid moiety agreed well with that in **1**, thus **2** was assigned to 5-(*p*-*trans*-coumaroyl)quinic acid. The *trans*-configuration was deduced from the J value (16.0 Hz) of the olefinic protons. Compound **3** was established as 5-(*p*-*cis*-coumaroyl)quinic acid first by the finding that during the course of workup it was relatively unstable and readily converted to the *trans* isomer **2** and further by NMR which showed that the olefinic protons had a smaller J value of 12.7 Hz compared with $J = 16.0$ Hz in **2**. A solution of purified **3** upon subjection to UV light for 2 h gave rise to about 1:1 mixture of *cis* and *trans* compounds.

The NMR spectra of compound **4** showed that it was a *p*-coumaric acid glucoside. This was corroborated by electrospray mass spectrometry (ES-MS) which gave $[M - H]^-$ peak at m/z 325 consistent with the elemental composition. The observation of the anomeric carbon signal at δ 100.2 was an indication that the sugar substituent was on the phenol rather than on the carboxyl. The anomeric proton (δ 5.10) had a J value of 7.2 Hz indicating that the glucose had a β-configuration. Compound **4** was finally identified by NMR comparison with published values (Cui et al., 1990) as *trans*-*p*-coumaric acid 4-*O*-β-D-glucopyranoside, a compound which reportedly occurs in many vegetables and berry fruits (Schuster et al., 1986).

Compound **5** was a more abundant constituent of the sainfoin extract and the NMR data indicated that it was a non-phenolic aromatic glycoside. The aromatic carbon chemical shifts (δ 128.9, 129.0, 130.2 and 134.1), of which the 129.0 and 130.2 contributed to two degenerate carbons each, was evidence of a phenyl moiety with no substituents. This was corroborated by the ^{1}H-NMR spectrum which showed two sets of multiplets at δ 7.36 and 7.76 which integrated for three and two protons, respectively and which were consistent with an unsubstituted phenyl ring. The presence of two unsaturated carbons (δ 120.1 and 147.4) was also evident indicating a cinnamic acid type structure. However, the carbonyl chemical shift (δ 166.0) was weak reflecting the effect of the dynamic changes surrounding the carbonyl carbon associated with the carboxylic acid group. Long range H, C coupling experiments (HMBC) showed a correlation between the singlet at δ 6.70 and the phenyl C2/C6 (δ 130.2). In addition, the anomeric proton (δ 5.04) of the glucose moiety was also long range coupled to the olefinic carbon at δ 147.4 thus confirming that the attachment of the sugar was indeed at this olefinic carbon. The ES-MS spectrum of **5** showed the $[M - H]^-$ peak at m/z 325 consistent with an elemental composition of $C_{15}H_{18}O_8$ of the proposed chemical structure. The configuration of the substituents on the double bond could not be established from the ^{1}H-NMR due to the absence of proton–proton coupling data as a result of substitution by the sugar on one of the olefinic carbons. However, NOE experiments showed spatial interactions between the anomeric proton (δ 5.04) of the sugar and H2/H6 (δ 7.76) of the phenyl ring showing that the substituents had to be in the Z orientation and compound **5** was, therefore, the (Z)-8-β-D-glucopyranosyloxycinnamic acid. Although phenylpyruvic acid serves as an intermediate in the biosynthesis of several amino acids, only recently was the enolic phenyl pyruvic acid glucoside reported to occur naturally in *Aspalathus linearis*, a plant used for the production of Rooibos Tea (Marais et al., 1996).

Compounds **6** and **7** were structurally similar as judged by their NMR chemical shifts. The existence of a *p*-coumaric acid moiety was apparent from the carbon chemical shift values which were comparable with those of **2** and **3**. In addition, there were six carbon signals at the higher field region which were attributable to a sugar residue and also a high field carbon signal (δ 57–58) with the associated proton shift (δ 3.5) which was diagnostic of a methoxyl group. The low-field position of the sugar anomeric carbon (δ ca. 105) suggested the presence of an *O*-alkyl substituent on the anomeric carbon and subsequently confirmed by HMBC experiments which showed long range interactions between the methoxy protons and the anomeric carbon. In **6** the large H–H couplings ($J = 16.0$ Hz) observed between the olefinic protons (δ 6.36 and 7.65) indicated that the substituent was in the *trans* orientation and hence the compound was methyl 6-*O*-*p*-*trans*-coumaroyl-β-D-glucopyranoside, a compound known only by synthesis (Helm et al., 1992). This chemical constitution was further corroborated by ES-MS which yielded the $[M - H]^-$ peak at m/z 339 which was fully consistent with the elemental composition for the molecular structure. Compound **7** by deduction should be the *cis* isomer of **6**. Examination of the magnitude of the couplings between the olefinic protons (δ 5.82 and 6.89) showed a *J* value of 13.0 Hz compared to 16 Hz for **6** thus confirming that **7** was methyl 6-*O*-*p*-*cis*-coumaroyl-β-D-glucopyranoside, which as far as can be established from a search of *Chemical Abstracts* was a new natural product.

The UV spectra of compounds **8**–**16** all showed a λ_{max} at 330–360 nm which was characteristic of a flavonoid chromophore. The most abundant flavonoid was **8** which was identified as quercetin-3-rutinoside or rutin from the NMR spectra and confirmed by comparison of its HPLC retention time and UV absorption with that of an authentic sample. Compounds **12** and **14** had very similar NMR spectra to rutin except for the B-ring resonances. In **12** the appearance of the aromatic carbon resonances at δ 115.4, 121.2, 131.2 and 160.2 was diagnostic of a 4-hydroxyphenyl B-ring which was corroborated by the observation in the ^{1}H-NMR spectrum of an AA′BB′ spin system (δ 6.88 and 7.98) with *ortho* proton coupling ($J = 8.8$ Hz), while a 3,4,5-trihydroxyphenyl B-ring in **14** was evident by the presence of three aromatic carbon chemical shifts (δ 137.1 and 145.7) in the relatively low field region with the more downfield signal (δ 145.7) accounting for two carbons and also from the ^{1}H-NMR spectrum which revealed only a two proton singlet at the low field (δ 7.16). Thus, **12** and **14** were kaempferol-3-rutinoside and myricetin-3-rutinoside, respectively. Their identities were confirmed by spectral comparison with published data (Slimestad et al., 1995).

The presence of a methoxy group in **9** was revealed by both ^{1}H (δ 3.84) and ^{13}C (δ 56.0) NMR spectra. Apart from this additional signal the rest of the spectra were very similar to rutin except that the C3′ oxygenated B-ring carbon (δ 149.8) had shifted downfield compared to that of the corresponding rutin (δ 145.1). This indicated that the hydroxyl at that position was methylated and **9** was, therefore, isorhamnetin-3-rutinoside. The structure was confirmed by comparison of the NMR data with published values (Markham and Chari, 1982). Isorhamnetin-3-rutinoside is apparently a fairly common secondary plant metabolite having being previously reported to occur also in *Iurtica dioica* (Chaurasia and Wichtl, 1987) and *Calendula officinalis* flowers (Videl-Ollivier et al., 1984).

The ^{13}C-NMR spectrum of **10** clearly showed that the compound was also related to rutin. However, there were six additional carbon signals in the sugar region and their proton chemical shift values and their proton coupling patterns indicated that this was a β-D-glucopyranoside moiety. Comparison of the carbon chemical shift values of the aglycon with those of rutin showed that the C7 had shifted upfield by about 1.0 ppm suggesting that the glucose was located on this carbon. Long range couplings using HMBC showed a correlation between the glucose anomeric proton (δ 5.07) and the C7 (δ 163.2) of the quercetin. Hence, compound **10** was a flavonoid triglycoside identified as quercetin-3-*O*-rutinoside-7-*O*-glucoside.

In the NMR spectra of **11** the chemical shifts associated with the structure of rutin were clearly discernible. However, the observation of two methyl carbon signals (δ 17.5 and 18.0) and the changes in chemical shifts and signal intensity of the oxygenated aliphatic carbons suggested the presence of an additional rhamnopyranoside moiety in the molecule. Examination of the sugar carbon resonances revealed a downfield shift of the glucose C2 (δ 77.5) indicating that the rhamnose was attached to this position. Confirmation of this linkage was made by HMBC which revealed the existence of a long range coupling between the anomeric proton (δ 5.07) of the rhamnose unit concerned and the glucose C2. Compound **11** was, therefore, quercetin-3-*O*-(2″,6″-di-*O*-α-L-rhamnopyranosyl)-β-D-glucopyranoside, a product which had been reported to be present also in the leaves of several other plant species including *Ribes rubrum* (Siewek et al., 1984) and *Ginkgo biloba* (Vantaelen and Vantaelen-Fastre, 1989).

The ^{1}H- and ^{13}C-NMR chemical shifts of compound **13** were very similar to those of **11** except for differences which could be attributed to those of the B-ring hydroxylation pattern. The nature of the ring symmetry and the presence of only one hydroxyl group in the B-ring, which gave rise to the chemical shifts similar to those observed in kaempferol-3-rutinoside (**12**), showed that the aglycon was kaempferol and **13** was, therefore, kaempferol-3-(2″,6″-di-*O*-α-L-rhamnosyl)-β-D-glucoside. Comparison of the proton NMR chemical shifts with those reported for the compound isolated from *G. biloba* (Vantealen and Vantaelen-Fastre, 1989) corroborated this structural assignment.

Compound **15** had UV absorption quite distinct from the other compounds discussed with λ_{max} at 338 and 268 nm suggesting that the flavonoid chromophore was different in some way. Examination of the ^{13}C-NMR spectrum showed that the C3 of the aglycon had shifted significantly upfield to δ 104.5 while the C4 carbonyl had moved downfield to δ 182.2 indicating that the aglycon was a flavone. The catechol B-ring oxidation pattern was apparent from the proton chemical shifts which showed an ABX spin system with both *ortho* and *meta* proton coupling pattern while the phloroglucinol A-ring was indicated by the presence of two mutually *meta* coupled doublets. The presence of a methoxy group was indicated by a three proton singlet at δ 3.89 and the corresponding carbon at δ 56.4. As deduced by the chemical shift changes on the aromatic ring, as compared with those of the catechol B-ring of rutin, both the methoxy group as well as a glucose unit were substituted at the B-ring hydroxyls. The marked downfield shift of C1′ (δ 124.4) compared to that of chrysoeriol (δ 121.7) indicated that the glucose was attached to C4′ and hence **15** was chrysoeriol-4′-*O*-β-D-glucopyranoside. This structural assignment was confirmed by comparison of the NMR data with published values (Fukanaga et al., 1989). Chrysoeriol-4′-*O*-β-D-glucopyranoside has also been reported to occur in several plant species including a Chinese medicinal herb (Qin et al., 1982) and in some Japanese mistletoes (Fukanaga et al., 1989).

The ^{13}C- and ^{1}H-NMR spectra of **16** were similar to those of **15** but contained two more carbon signals (δ 21.0 and 170.6) which were attributed to an acetyl group. Examination of the carbon chemical shifts of the sugar residue showed that the C6″ had shifted downfield by about 2.2 ppm to δ 63.1 compared to that in **15** suggesting that the C6″ hydroxyl was acetylated. Confirmation of this position was made by HMBC which revealed a long range correlation between the acetyl carbonyl (δ 170.6) and the two H6″ methylene protons (δ 4.10 and 4.28) of the glucose. In addition, the methoxy protons (δ 3.90) were also observed to be long range coupled to the C3′ (δ 149.5) while the anomeric proton (δ 5.11) was likewise coupled to the C4′ (δ 149.8) thus establishing **16** as the novel chrysoeriol-4′-*O*-(6″-*O*-acetyl)-β-D-glucopyranoside.

The range of phenolic compounds present in the leaves of sainfoin including the two new natural cinnamic acid derivatives namely methyl 6-*O*-*p*-*trans*-coumaroyl-β-D-glucopyranoside (**6**) and methyl 6-*O*-*cis*-*p*-coumaroyl-β-D-glucopyranoside (**7**) and the novel flavonoid chrysoeriol-4′-*O*-(6″-*O*-acetyl)-β-D-glucopyranoside (**16**) together with the rare (*Z*)-8-β-D-glucopyranosyloxy-cinnamic acid (**5**) would provide a valuable data base for chemotaxonomic investigation as well as for possible nutritional studies on the impact of diet on animal health and productivity. Recent studies have shown, many plant phenols possess a range of bioactives including antimutagenic and free radical scavenging activities (Hanasaki et al., 1994; Jovanovic et al., 1998) which although may not be very significant to ruminants per se due to their restricted life spans but could be important to consumers of farm products. In view of such properties it would be reasonable to suggest that ruminants fed on fodders rich in polyphenols may retain some of these desirable polyphenols in their milk or flesh which could benefit consumers (Vinson et al., 1998). In addition, the presence of such antioxidants and free radical scavengers could improve the quality and shelf life of farm products by their ability to inhibit lipid peroxidation.

3. Experimental

The ^{1}H- and ^{13}C-NMR spectra were recorded on a Bruker AC 300 instrument and chemical shifts (δ) were referenced to solvent signal. HPLC analysis was performed on a Hewlett Packard series 1100 equipped with a DAD detector and a LiChrospher® 100 RP-18 (5 μm) column (125 × 4 mm) held at 30° with the following solvent program: solvent A, 2% AcOH in H_2O; solvent B, 2% AcOH in CH_3CN; starting from 4% B up to 12% B in 20 min, to 20% B in 30 min and to 50% B in 45 min. Flow rate was set 1 ml/min and detection was made by UV set at 280 nm.

3.1. Isolation of polyphenols from sainfoin

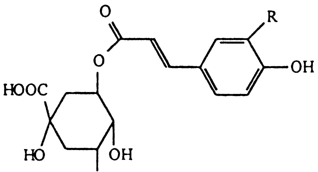
The defatted 70% aq. acetone extract (139 g) was suspended in H_2O and after filtering off the insoluble materials (9.5 g) the filtrate was chromatographed over a Sephadex LH20 column using aq. MeOH (0–100%). The fractions so obtained were chromatographed again on a MCI-HP 20 column eluting with aq. EtOH (up to 60%). Fractions were collected in 20 ml lots and were monitored by HPLC using UV detection set at 280 nm. When necessary, fractions were further treated until chromatographically pure compounds were obtained. Purified fractions were concentrated and freeze-dried. Yields: 116 mg of **1**, 18 mg of **2**, 27 mg of **3**, 40 mg of **4**, 1260 mg of **5**, 180 mg of **6**, 75 mg of **7**, 1005 mg of **8**, 180 mg of **9**, 300 mg of **10**, 548 mg of **11**, 210 mg of **12**, 170 mg of **13**, 110 mg of **14**, 322 mg of **15** and 190 mg of **16**.

3.2. 5-*p*-*trans*-Coumaroylquinic acid (**2**)

λ_{max} 234 and 312 nm. ^{1}H-NMR (300 MHz, CD_3OD) δ 2.01–2.29 (*m*, H-2, 6), 3.77 (*dd*, *J* 8.6, 3.0 Hz, H-4), 3.90 (*d*, *J* 3.1 Hz, H-3), 5.33 (*m*, H-5), 6.34 (*d*, *J* 16.0 Hz, H-8′), 6.84 (*d*, *J* 8.6 Hz, H-3′,5′), 7.48 (*d*, *J* 8.6 Hz, H-2′,6′), 7.65 (*d*, *J* 16.0 Hz, H-7′). ^{13}C-NMR (75 MHz, CD_3OD) δ 38.56 (C-2), 39.14 (C-6), 71.66 (C-4), 72.43 (C-3), 73.82 (C-5), 76.60 (C-1), 115.68 (C-8′), 117.31 (C-3′,5′), 127.70 (C-1′), 131.69 (C-2′,6′), 147.34 (C-7′), 161.48 (C-4′), 169.38 (C-9′), 177.64 (C-7).

3.3. 5-*p*-*cis*-Coumaroylquinic acid (**3**)

λ_{max} 232 and 306 nm. ^{1}H-NMR (300 MHz, CD_3OD) δ 1.99–2.33 (*m*, H-2, 6), 3.71 (*m*, H-4), 4.27 (*br s*, H-3), 5.37 (*br s*, H-5), 5.79 (*d*, *J* 12.7 Hz, H-8′), 6.76 (*d*, *J* 8.2 Hz, H-3′,5′), 6.83 (*d*, *J* 12.7 Hz, H-7′), 7.63 (*d*, *J* 8.2 Hz, H-2′,6′). ^{13}C-NMR (75 MHz, CD_3OD) δ 38.26 (C-2), 40.40 (C-6), 72.03 (C-4), 73.49 (C-3), 74.70 (C-5), 78.04 (C-1), 116.24 (C-3′,5′), 117.43 (C-8′), 128.09 (C-1′), 134.08 (C-2′,6′), 145.27 (C-7′), 160.38 (C-4′), 168.48 (C-9′), 180.45 (C-7).



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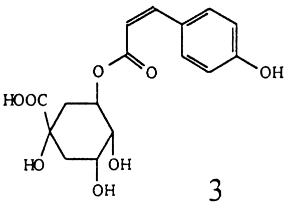
2 R=H



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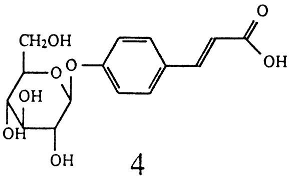

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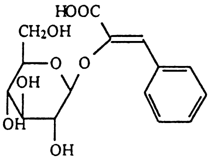

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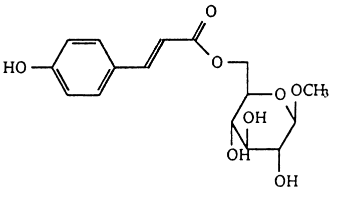

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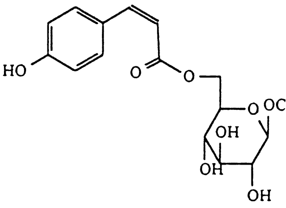

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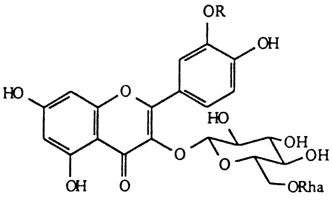

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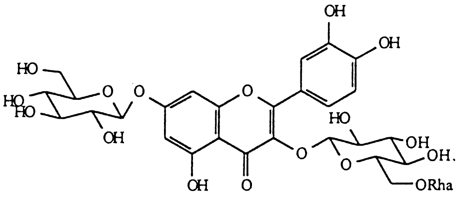
9 R = CH3



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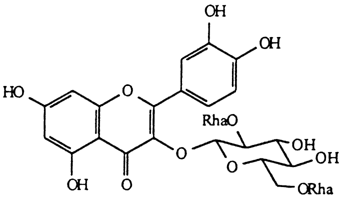

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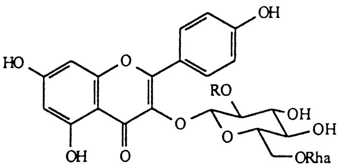

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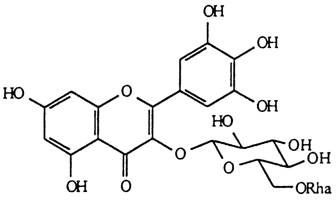

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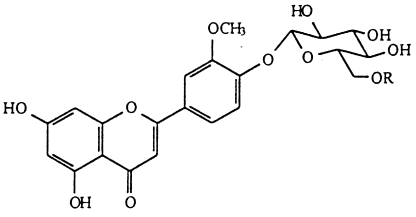

15 R = H
16 R = COCH3

3.4. 4-O-β-D-Glucopyranosyl-p-trans-coumaric acid (4)

λ_{max} 230 and 296 nm. ^{1}H-NMR (300 MHz, D_2O) δ 3.44–3.63 (*m*, sugar-H), 3.72 (*dd*, *J* 12.4, 5.6 Hz, H-6a′), 3.90 (*dd*, *J* 12.4, 1.8 Hz, H-6b′), 5.10 (*d*, *J* 7.2 Hz, H-1′), 6.36 (*d*, *J* 16.0 Hz, H-8), 7.09 (*d*, *J* 8.6 Hz, H-3,5), 7.43 (*d*, *J* 16.0 Hz, H-7), 7.54 (*d*, *J* 8.6 Hz, H-2,6). ^{13}C-NMR (75 MHz, D_2O) δ 60.96 (C-6′), 69.84 (C-4′), 73.32 (C-2′), 75.96 (C-3′), 76.56 (C-5′), 100.24 (C-1′), 117.13 (C-3,5), 120.49 (C-8), 130.09 (C-1,2,6), 142.84 (C-7), 158.23 (C-4), 172.17 (C-9).

3.5. (Z)-8-β-D-Glucopyranosyloxycinnamic acid (**5**)

λ_{max} 280 nm. ^{1}H-NMR (300 MHz, D_2O) δ 3.26 (*m*, H-5′), 3.34 (*d*, *J* 9.3 Hz, H-4′), 3.44 (*d*, *J* 9.0 Hz, H-3′), 3.48 (*d*, *J* 8.4 Hz, H-2′), 3.57 (*dd*, *J* 13.4, 5.4 Hz, H-6a′), 3.70 (*d*, *J* 12.7 Hz, H-6b′), 5.04 (d, J 7.0 Hz, H-1′), 6.72 (*s*, H-7), 7.33 (*t*, *J* 7.3 Hz, H-4), 7.40 (*t*, *J* 7.5 Hz, H-3,5), 7.76 (*d*, *J* 7.5 Hz, H-2,6). ^{13}C-NMR (75 MHz, D_2O) δ 60.93 (C-6′), 69.77 (C-4′), 74.12 (C-2′), 76.27 (C-3′), 76.76 (C-5′), 101.25 (C-1′), 120.11 (C-7), 128.87 (C-4), 129.01 (C-3,5), 130.18 (C-2,6), 134.10 (C-1), 147.40 (C-8), 165.96 (C-9).

3.6. Methyl 6-O-p-trans-coumaroyl-β-D-glucopyranoside (**6**)

λ_{max} 232 and 312 nm. ^{1}H-NMR (300 MHz, CD_3OD) δ 3.21–3.52 (*m*, H-2,3,4,5), 3.52 (*s*, OCH_3), 4.22 (*d*, *J* 7.7 Hz, H-1), 4.36 (*dd*, *J* 11.8, 5.6 Hz, H-6a), 4.52 (*d*, *J* 12.2 Hz, H-6b), 6.36 (*d*, *J* 16.0 Hz, H-8′), 6.82 (*d*, *J* 8.5 Hz, H-3′,5′), 7.60 (*d*, *J* 8.5 Hz, H-2′,6′), 7.65 (*d*, *J* 16.0 Hz, H-7′). ^{13}C-NMR (75 MHz, CD_3OD) δ 57.71 (OCH_3), 65.02 (C-6), 72.10 (C-4), 75.43 (C-2), 75.86 (C-5), 78.31 (C-3), 105.83 (C-1), 115.36 (C-8′), 117.26 (C-3′,5′), 127.57 (C-1′), 131.60 (C-2′,6′), 147.21 (C-7′), 161.69 (C-4′), 169.56 (C-9′).

3.7. Methyl 6-O-p-cis-coumaroyl-β-D-glucopyranoside (7)

λ_{max} 232 and 304 nm. ^{1}H-NMR (300 MHz, CD_3OD) δ 3.16–3.49 (*m*, H-2,3,4,5), 3.49 (*s*, OCH_3), 4.18 (*d*, *J* 7.7 Hz, H-1), 4.29 (*dd*, *J* 11.8, 5.6 Hz, H-6a), 4.49 (*d*, *J* 11.8 Hz, H-6b), 5.82 (*d*, *J* 13.0 Hz, H-8′), 6.82 (*d*, *J* 7.9 Hz, H-3′,5′), 6.89 (*d*, *J* 13.0 Hz, H-7′), 7.64 (*d*, *J* 8.5 Hz, H-2′,6′). ^{13}C-NMR (75 MHz, CD_3OD) δ 57.67 (OCH_3), 65.06 (C-6), 72.09 (C-4), 75.39 (C-2), 75.76 (C-5), 78.31 (C-3), 105.78 (C-1), 116.78 (C-3′,5′), 117.25 (C-8′), 128.03 (C-1′), 134.07 (C-2′,6′), 145.54 (C-7′), 160.46 (C-4′), 168.61 (C-9′).

3.8. Isorhamnetin 3-O-(6″-O-α-L-rhamnopyransoyl)-β-D-glucopyranoside or isorhamnetin 3-rutinoside (**9**)

λ_{max} 254 and 354 nm. ^{1}H-NMR (300 MHz, DMSO-d_6) δ 0.98 (*d*, *J* 6.0 Hz, H-6‴), 3.05–3.73 (*m*, sugar-H), 3.84 (*s*, OCH_3), 4.42 (*s*, H-1‴), 5.43 (*d*, *J* 6.1 Hz, H-1″), 6.20 (*d*, *J* 1.8 Hz, H-6), 6.41 (*d*, J 1.9 Hz, H-8), 6.91 (*d*, *J* 8.5 Hz, H-5′), 7.52 (*d*, *J* 8.4 Hz, H-6′),7.85 (*s*, H-2′). ^{13}C-NMR (75 MHz, DMSO-d_6) δ 18.04 (C-6‴), 56.03 (OCH_3), 67.21 (C-6″), 68.63 (C-5‴), 70.48 (C-3‴), 70.68 (C-2‴), 70.97 (C-4″), 72.18 (C-4‴), 74.65 (C-2″), 76.30 (C-3″), 76.77 (C-5″), 94.17 (C-8), 99.14 (C-6), 101.23 (C-1‴), 101.57 (C-1″), 104.29 (C-10), 113.66 (C-2′), 115.60 (C-5′), 121.41 (C-1′), 122.64 (C-6′), 133.38 (C-3), 147.25 (C-4′), 149.77 (C-3′), 156.84 (C-2,9), 161.54 (C-5), 164.71 (C-7), 177.67 (C-4).

3.9. Quercetin 3-O-(6″-O-α-L-rhamnopyransoyl)-β-D-glucopyranoside-7-O-β-D-glucopyranoside (**10**)

λ_{max} 256 and 354 nm. ^{1}H-NMR (300 MHz, DMSO-d_6) δ 0.99 (*d*, *J* 6.1 Hz, H-6‴), 3.05–3.76 (*m*, sugar-H), 4.39 (*s*, H-1‴), 5.07 (*d*, *J* 7.3 Hz, H-1⁗), 5.38 (*d*, *J* 6.9 Hz, H-1″), 6.44 (d, J 2.0 Hz, H-6), 6.72 (*d*, *J* 2.0 Hz, H-8), 6.85 (*d*, *J* 8.3 Hz, H-5′), 7.54 (*d*, *J* 8.3, H-6′), 7.56 (s, H-2′). ^{13}C-NMR (75 MHz, DMSO-d_6) δ 18.08 (C-6‴), 61.01 (C-6⁗), 67.33 (C-6″), 68.57 (C-5‴), 69.95 (C-4⁗), 70.38 (C-4″), 70.71 (C-2‴), 70.92 (C-3‴), 72.21 (C-4‴), 73.50 (C-2⁗), 74.42 (C-2″), 76.35 (C-3″), 76.64 (C-3⁗), 76.81 (C-5″), 77.51 (C-5⁗), 94.88 (C-8), 99.68 (C-6), 100.25 (C-1⁗), 101.07 (C-1‴), 101.40 (C-1″), 105.98 (C-10), 115.61 (C-2′), 116.85 (C-5′), 121.36 (C-6′), 122.01 (C-1′), 133.91 (C-3), 145.16 (C-3′), 149.01 (C-4′), 156.37 (C-2), 157.53 (C-9), 161.25 (C-5), 163.19 (C-7), 177.90 (C-4).

3.10. Quercetin 3-O-(2″,6″-di-O-α-L-rhamnopyransoyl)-β-D-glucopyranoside (**11**)

λ_{max} 256 and 354 nm. ^{1}H-NMR (300 MHz, DMSO-d_6) δ 0.80 (*d*, *J* 6.1 Hz, H-6‴), 0.97 (*d*, *J* 6.1 Hz, H-6⁗), 3.05–3.82 (*m*, sugar-H), 4.27 (*s*, H-1⁗), 5.07 (*s*, H-1‴), 5.53 (*d*, *J* 7.5 Hz, H-1″), 6.18 (*d*, *J* 1.8 Hz, H-6), 6.37 (*d*, *J* 1.8 Hz, H-8), 6.83 (*d*, *J* 8.3 Hz, H-5′), 7.49 (*d*, *J* 1.8 Hz, H-2′), 7.53 (*d*, *J* 8.3 Hz, H-6′). ^{13}C-NMR (75 MHz, DMSO-d_6) δ 17.55 (C-6‴) 18.02 (C-6⁗), 67.43 (C-6″), 68.59 (C-5‴,5⁗), 70.71 (C-2‴,2⁗), 70.90 (C-4″, C-3‴,3⁗), 72.21 (C-4‴,4⁗), 76.09 (C-3″), 77.55 (C-2″, C-5″), 93.88 (C-8), 98.96 (C-6, C-1‴), 100.81 (C-1⁗), 101.12 (C-1″), 104.34 (C-10), 115.48 (C-2′), 116.47 (C-5′), 121.58 (C-1′), 121.94 (C-6′), 133.05 (C-3), 145.11 (C-3′), 148.63 (C-4′), 156.71 (C-2), 157.02 (C-9), 161.59 (C-5), 164.32 (C-7), 177.57 (C-4).

3.11. Kaempferol 3-O-(2″,6″-di-O-α-L-rhamnopyransoyl)-β-D-glucopyranoside (**13**)

λ_{max} 266 and 346 nm. ^{1}H-NMR (300 MHz, DMSO-d_6) δ 0.81 (*d*, *J* 6.1 Hz, H-6‴), 0.96 (*d*, *J* 6.1 Hz, H-6⁗), 3.09–3.85 (*m*, sugar-H), 4.33 (*s*, H-1⁗), 5.06 (*s*, H-1‴), 5.49 (*d*, *J* 7.0 Hz, H-1″), 6.17 (*d*, *J* 2.0 Hz, H-6), 6.38 (*d*, *J* 2.0 Hz, H-8), 6.87 (*d*, *J* 8.9 Hz, H-3′,5′), 7.94 (*d*, *J* 8.8 Hz, H-2′,6′). ^{13}C-NMR (75 MHz, DMSO-d_6) δ 17.62 (C-6‴) 18.03 (C-6⁗), 67.21 (C-6″), 68.59 (C-5‴,5⁗), 70.67 (C-2‴,2⁗), 70.94 (C-4″, C-3‴,3⁗), 72.16 (C-4‴,4⁗), 75.93 (C-3″), 77.42 (C-5″), 77.69 (C-2″), 94.13 (C-8), 98.97 (C-6, C-1‴), 100.93 (C-1⁗), 101.12 (C-1″), 104.23 (C-10), 115.42 (C-3′,5′), 121.33 (C-1′), 131.04 (C-2′,6′), 132.90 (C-3), 156.82

(C-2), 157.15 (C-9), 160.12 (C-4′), 161.56 (C-5), 164.81 (C-7), 177.52 (C-4).

3.12. *Myricetin 3-O-(6″-O-α-L-rhamnopyransoyl)-β-D-glucopyranoside or myricetin-3-rutinoside (**14**)*

λ_{max} 230, 258 and 356 nm. ^{1}H-NMR (300 MHz, DMSO-d_6) δ 0.96 (*d*, *J* 6.1 Hz, C-6‴), 3.09–3.85 (*m*, sugar-H), 4.40 (*s*, H-1‴), 5.38 (*d*, *J* 7.5 Hz, H-1″), 6.17 (*d*, *J* 1.9 Hz, H-6), 6.33 (*d*, *J* 1.9 Hz, H-8), 7.16 (*s*, H-2′,6′). ^{13}C-NMR (75 MHz, DMSO-d_6) δ 18.03 (C-6‴), 67.45 (C-6″), 68.57 (C-5‴), 70.41 (C-3‴), 70.67 (C-2‴), 70.94 (C-4″), 72.16 (C-4‴), 74.29 (C-2″), 76.44 (C-5″), 76.90 (C-3″), 93.84 (C-8), 98.97 (C-6), 101.12 (C-1‴), 101.41 (C-1″), 104.23 (C-10), 108.91 (C-2′,6′), 120.39 (C-1′), 133.74 (C-3), 137.08 (C-4′), 145.72 (C-3′,5′), 157.15 (C-2,9), 161.56 (C-5), 164.81 (C-7), 177.52 (C-4).

3.13. *Chrysoeriol 4′-O-β-D-glucopyranoside (**15**)*

λ_{max} 232, 268 and 338 nm. ^{1}H-NMR (300 MHz, DMSO-d_6) δ 3.0–3.5 (*m*, sugar-H), 3.48 (*dd*, *J* 11.2, 5.6 Hz, H-6a″), 3.68 (*d*, *J* 10.5 Hz, H-6b″), 3.89 (*s*, OCH_3), 5.06 (*d*, *J* 7.3 Hz, H-1″), 6.20 (d, J 2.0 Hz, H-6), 6.53 (*d*, *J* 2.0 Hz, H-8), 6.97 (*s*, H-3), 7.23 (*d*, *J* 8.3 Hz, H-5′), 7.59 (*d*, *J* 2.2 Hz, H-2′), 7.63 (*dd*, *J* 8.3, 2.0 Hz, H-6′). ^{13}C-NMR (75 MHz, DMSO-d_6) δ 56.40 (OCH_3), 60.91 (C-6″), 69.96 (C-4″), 73.48 (C-2″), 77.18 (C-3″), 77.50 (C-5″), 94.52 (C-8), 99.30 (C-6), 99.92 (C-1″), 104.13 (C-10), 104.47 (C-3), 110.63 (C-2′), 115.45 (C-5′), 120.11 (C-6′), 124.43 (C-1′), 149.56 (C-3′), 150.10 (C-4′), 157.74 (C-9), 161.79 (C-5), 163.46 (C-2), 164.71 (C-7), 182.18 (C-4).

3.14. *Chrysoeriol 4′-O-(6″-O-acetyl)-β-D-glucopyranoside (**16**)*

λ_{max} 268 and 336 nm. ^{1}H-NMR (300 MHz, DMSO-d_6) δ 2.01 (*s*, $COCH_3$), 2.95–3.77 (*m*, sugar-H), 3.90 (*s*, OCH_3), 4.10 (*dd*, *J* 11.7, 6.7 Hz, H-6a″), 4.28 (*d*, *J* 10.2 Hz, H-6b″), 5.11 (*d*, *J* 7.2 Hz, H-1″), 6.22 (*d*, *J* 2.0 Hz, H-6), 6.55 (*d*, *J* 2.0 Hz, H-8), 6.99 (*s*, H-3), 7.21 (*d*, *J* 8.6 Hz, H-5′), 7.61 (*d*, *J* 2.0 Hz, H-2′), 7.64 (*dd*, *J* 8.6, 2.0 Hz, H-6′). ^{13}C-NMR (75 MHz, DMSO-d_6) δ 21.01 ($COCH_3$), 56.38 (OCH_3), 63.15 (C-6″), 70.09 (C-4″), 73.41 (C-2″), 74.11 (C-5″), 76.88 (C-3″), 94.54 (C-8), 99.31 (C-6), 99.70 (C-1″), 104.15 (C-10), 104.57 (C-3), 110.68 (C-2′), 115.45 (C-5′), 119.99 (C-6′), 124.65 (C-1′), 149.54 (C-3′), 149.81 (C-4′), 157.75 (C-9), 161.79 (C-5), 163.45 (C-2), 164.69 (C-7), 170.57 ($COCH_3$), 182.20 (C-4).

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