PERGAMON

Phytochemistry 59 (2002) 465–468

PHYTOCHEMISTRY

www.elsevier.com/locate/phytochem

Nigrumin-5-*p*-coumarate and nigrumin-5-ferulate, two unusual nitrile-containing metabolites from black currant (*Ribes nigrum*) seed

Yinrong Lu*, L. Yeap Foo, Herbert Wong

Industrial Research, PO Box 31310, Lower Hutt, New Zealand

Received 17 May 2001; received in revised form 11 October 2001

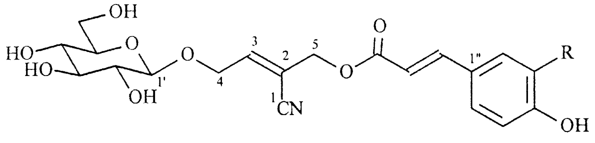
Abstract

Two novel nitrile-containing compounds, nigrumin-5-*p*-coumarate and nigrumin-5-ferulate, together with six known flavonoids, were isolated from the seed of black currant (*Ribes nigrum*). The chemical structures of nigrumin-5-*p*-coumarate and 5-ferulate were elucidated using NMR spectroscopy as 2-*trans*-*p*-coumaroyloxymethyl-4-β-D-glucopyranosyloxy-2(*E*)-butenenitrile and 2-*trans*-feruloyloxymethyl-4-β-D-glucopyranosyloxy-2(*E*)-butenenitrile, respectively. © 2002 Elsevier Science Ltd. All rights reserved.

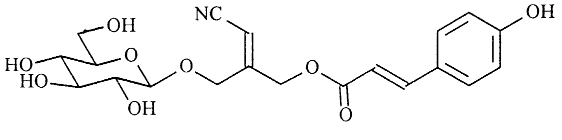
Keywords: Black currant (*Ribes nigrum*); Grossulariaceae; Seed; Nigrumin-5-*p*-coumarate; Nigrumin-5-ferulate; 2-*trans*-*p*-Coumaroyloxymethyl-4-β-D-glucopyranosyl-oxy-2(*E*)-butenenitrile; 2-*trans*-Feruloyloxymethyl-4-β-D-glucopyranosyloxy-2(*E*)-butenenitrile

1. Introduction

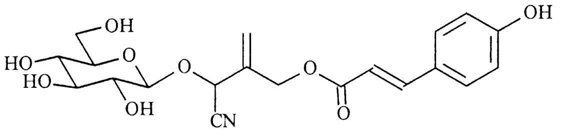
Although black currant (*Ribes nigrum*) berries are cultivated principally for juicing, the small seeds within also have commercial value due to their high content of nutritionally desirable polyunsaturated γ-linolenic acid (Traitler et al., 1984; Ucciani et al., 1995). The presence of this and other accompanying highly labile polyunsaturated fatty acids in the seeds aroused our interest as to the nature of the co-occurring compounds that may be important for understanding the chemical basis for their protection against oxidative degradation in the intact seeds. For this purpose the black currant seed residue from a commercial oil extraction operation using supercritical CO_2 was used for chemical investigation. In earlier communications we reported the presence of anthocyanins, which occurred principally as the glucosides and rutinosides of cyanidin and delphinidin, and their unusual addition products with acetone known as the pyranoanthocyanins (Lu et al., 2000; Lu and Foo, 2001). This paper describes the isolation and the unusual chemical features of two new nitrile-containing compounds nigrumin-5-*p*-coumarate (**1**) and nigrumin-5-ferulate (**2**) together with six known flavonoids.



1 nigrumin-5-*p*-coumarate (R = H)
2 nigrumin-5-ferulate (R = OCH_3)



3 sutherlandin-5-*p*-coumarate



4 cardiospermin-5-*p*-coumarate

---

* Corresponding author. Tel.: +64-4-569-0000: fax: +64-4-569-0055.

E-mail address: y.lu@irl.cri.nz (Y. Lu).

PII: S0031-9422(01)00441-1

2. Results and discussion

Column chromatography on polyamide of the black currant seed extracts yielded a flavonoid fraction, which was free of black currant pigments. Further repeated chromatographic treatment of the fraction on MCI–HP20 column using HPLC detection led to the resolution and isolation of the two nitrile-containing compounds **1** and **2**, and the six flavonoids. The flavonoids were identified by NMR spectral comparison with published data (Markham and Chari, 1982; Agrawal, 1989) as dihydroquercetin, myricetin 3-glucoside, myricetin 3-rutinoside, quercetin 3-glucoside, quercetin 3-rutinoside and kaempferol 3-glucoside, which had been identified before as black currant constituents (Koeppen and Herrmann, 1977; Calamita et al., 1983; Hakkinen and Auriola, 1998).

The HR–MS spectrum of compound **1** gave a $[M–H]^-$ peak at *m/z* 420.1304 corresponding to the molecular formula of $C_{20}H_{23}NO_9$ (calc. 420.1289). Its UV absorption with λ_{max} at 314 nm matched that of *p*-coumaric acid, thus suggesting that it possessed a *p*-coumaric acid chromophore. A *trans*-*p*-coumaroyl unit was apparent from the ^1H NMR (Table 1), which showed a pair of mutually coupled doublets at δ 7.69 and 6.37 with $J = 16$ Hz. Another pair of doublets that integrated for four protons at δ 7.49 and 6.82 with $J = 8.7$ Hz was consistent with the AA′BB′ spin system characteristic of a *para*-substituted phenyl ring. The ^{13}C NMR spectrum displayed nine carbon signals at δ 114.5, 117.3 (2C), 127.4, 131.8 (2C), 148.1, 161.9 and 168.5 consistent with the carbon resonances of an ester form of the *p*-coumaric acid. In addition, a set of six carbon signals characteristic of a glucopyranose moiety was also present. The H–C COSY spectrum showed that the anomeric carbon (δ 104.7) was correlated to the doublet at δ 4.36 ($J = 7.7$ Hz), suggesting that the sugar was β-glycosidically linked to an unknown alkyl group rather than the *p*-coumaroyl moiety. There were five other carbon signals in the ^{13}C NMR spectrum of **1** that remained unaccounted for. These included a methine carbon at δ 149.4, two quaternary carbons at 116.69 and 113.3 and two methylene carbons at 68.6 and 64.8 as established by DEPT. The carbon at δ 149.4 was correlated to the olefinic proton at δ 6.82, the CH_2 at δ 64.8 was correlated to a broad singlet (δ 4.82), while the other CH_2 at δ 68.6 was correlated to the two sets of protons (δ 4.66, *dd*, $J = 14.5$, 5.9 Hz; 4.54, *dd*, $J = 14.4$, 6.5 Hz), indicating that the latter was attached to the sugar moiety. The latter CH_2 was also found to be H–H coupled with the olefinic proton (δ 6.82). The quaternary carbon at δ 116.7 could be assigned to a cyano group (Kalinowski et al., 1984) in accordance with the chemical formula of **1**. These data suggested that the five carbon alkyl portion was best assigned to a 2-hydroxymethyl-4-hydroxy-2-butenenitrile moiety. This five carbon structural unit was also supported by HMBC data which showed that the olefinic proton H-3 (δ 6.82) was long range coupled to C-1 (δ 116.7) and C-5 (δ 64.8), the broad singlet H-5 (δ 4.82) to C-3 (δ149.4) and C-9″ (δ 168.5), the H-4 (δ 4.66/4.54) to the quaternary carbon C-2 (δ 113.3) and the sugar anomeric carbon (δ 104.7). These data enabled the unambiguous assignment of the sugar to the 4-CH_2OH and the *p*-coumaroyl unit to the 5-CH_2OH, respectively. The *E*-configuration of the central 2-butenenitrile was established by ROESY experiments that clearly showed positive interaction of the broad singlet of the 5-CH_2 with the olefinic proton H-3, but no interaction with the 4-CH_2. Thus, the chemical structure of **1** was established as the novel 2-*trans*-*p*-coumaroyloxymethyl-4-β-D-glucopyranosyloxy-2(*E*)-butenenitrile named here as nigrumin-5-*p*-coumarate. The assignment of nigrumin-5-*p*-coumarate (**1**) was also supported by the comparison with the NMR data of a related compound sutherlandin-5-*trans*-*p*-coumarate (**3**) found in *Sorbaria sorbifolia* var. *stellipila* (Kim and Zee, 2000). Compounds **1** and **3** gave very similar ^{13}C NMR data except for C-2 and C-3 (δ 113.3 and 149.4 for **1** compared with 97.6 and 159.8 for **3**). A corresponding difference was also observed in the olefinic proton H-3 (δ 6.82) of **1** and H-2 (δ 5.73) of **3**.

Table 1
^1H NMR (300 MHz, CD_3OD) chemical shift data for nigrumin-5-*p*-coumarate (**1**) and nigrumin-5-ferulate (**2**)

H	1	2
3	6.84 *dd* (6.5, 5.9)	6.85 *m*
4a	4.54 *dd* (14.4, 6.5)	4.55 *dd* (14.3, 6.4)
4b	4.66 *dd* (14.5, 5.9)	4.66 *dd* (14.3, 5.5)
5	4.82 *s*	4.82 *s*
1′	4.36 *d* (7.7)	4.36 *d* (7.6)
2′-5′	3.21–3.50 *m*	3.21–3.47 *m*
6′a	3.70 *dd* (11.9, 4.9)	3.70 *dd* (12.3, 5.6)
6′b	3.88 *m*	3.89 *m*
2″	7.49 *d* (8.7)[a]	7.22 *d* (2.1)
3″	6.82 *d* (8.7)[a]	–
5″	6.82 *d* (8.7)[a]	6.83 *d* (8.2)
6″	7.49 *d* (8.7)[a]	7.10 *dd* (8.0, 2.1)
7″	7.69 *d* (16.0)	7.68 *d* (15.9)
8″	6.37 *d* (16.0)	6.41 *d* (15.9)
OCH_3	–	3.91 *s*

[a] An AA′BB′ spin system in which the splitting in each sub-spectrum (AA′ and BB′ parts) corresponds to the sum of the couplings J(AB) and J(AB′).

The molecular formula $C_{21}H_{25}NO_{10}$ (calcd 450.1395) of compound **2** was deduced from its HR–MS spectrum which showed the $[M–H]^-$ peak at *m/z* 450.1435, indicating that **2** was also a nitrile-containing compound with an additional methoxyl group. The UV spectrum of **2** with λ_{max} at 328 was more characteristic of a caffeic acid derivative. The ^1H (Table 1) and ^{13}C NMR data of **2** showed resonances containing a β-D-glucopyranose and a five carbon alkyl moiety similar to those of **1** except for the signals in the aromatic region. These observations were consistent with **2** being a *trans*-feruloyl derivative as opposed to a *trans*-*p*-coumaroyl derivative found in **1**. This deduction was confirmed by 1D and 2D NMR experiments. The HMBC experiments showed that the methoxyl group (δ 3.91) was long range coupled to C-3$''$ (δ 151.3), which in turn was coupled to H-5$''$ (δ 6.83, *d*, $J = 8.2$ Hz). The HMBC experiments also showed long range couplings between C-1$'$ (δ 104.7) and H-4 (δ 4.55/4.66) and between C-9$''$ (δ 168.5) and H-5 (δ 4.82), respectively, revealing that the attachments of the sugar was at 4-CH_2 and the *trans*-feruloyl at 5-CH_2. Therefore compound **2** was 2-*trans*-feruloyloxymethyl-4-β-D-glucopyranosyloxy-2(*E*)-butenenitrile, or nigrumin-5-ferulate.

While cyanogenic compounds are common in nature (Jones, 1998), particularly in the seeds of Rosaceae occurring as cyanohydrin glycosides such as amygdalin (R-mandelonitrile-β-D-gentiobioside) and prunasin (R-mandelonitrile-β-D-glucopyranoside) (Nahrstedt, 1972, 1973) derived from phenylalanine, cardiospermin and its 5-*p*-hydroxybenzoate and 5-*p*-coumarate (**4**; Nahrstedt and Scheid, 1981) derived from leucine, non-cyanogenic nitrile-containing compounds are also known but are rare in nature. These include cyanolipids (Mikolajczak et al., 1970), sutherlandin (Swenson et al., 1987) and its 5-*p*-coumarate derivative (Kim and Zee, 2000). According to the finding of Braekman et al. (1982), sutherlandin and its derivatives could be formed from the corresponding cyanogenic cardiospermins by allylic rearrangement of the cyano group. However, nigrumin-5-*p*-coumarate and nigrumin-5-ferulate reported here could not by analogy be derived from leucine. Their biosynthetic pathway, whether derived from isoleucine, in black currant seed is unknown and needs further study.

3. Experimental

^{1}H and ^{13}C NMR were recorded on a Bruker Avance 300 instrument and chemical shift (δ) were referenced to the solvent signal. HPLC was performed as described previously (Lu and Foo, 1999).

3.1. Extraction and isolation

The black currant seed residue after supercritical CO_2 extraction (2000 g) was extracted with 70% aq. acetone (3×2000 ml) and the combined extracts conc. and freeze-dried to afford 64 g of solid reddish material. The extract (60 g) dissolved in water was applied to a Polyamide column and the black currant pigments were eluted with $CH_3OH/AcOH/H_2O$ (10:4:86) followed by 50% aq. methanol to yield a fraction (2.65 g) containing mainly flavonoids. This fraction was further chromatographed repeatedly on MCI–HP20 column using water with increasing proportions of methanol up to 50% until chromatographically pure compounds were obtained.

*3.2. Nigrumin-5-p-coumarate (2-trans-p-coumaroyloxy methyl-4-β-D-glucopyranosyloxy-2(E)-butenenitrile) (**1**)*

Freeze-dried powder (28 mg). HPLC R_t 30.2 min, on line UV λ_{max} 314, 234 nm. ^{1}H NMR: Table 1. ^{13}C NMR (75 MHz, CD_3OD) δ 63.00 (C-6$'$), 64.75 (C-5), 68.62 (C-4), 71.85 (C-4$'$), 75.36 (C-2$'$), 78.47 (C-3$'$, 5$'$), 104.72 (C-1$'$), 113.34 (C-2), 114.50 (C-8$''$), 116.69 (C-1), 117.30 (C-3$''$, 5$''$), 127.41 (C-1$''$), 131.79 (C-2$''$, 6$''$), 148.08 (C-7$''$), 149.42 (C-3), 161.91 (C-4$''$), 168.49 (C-9$''$).

*3.3. Nigrumin-5-ferulate (2-trans-feruloyloxymethyl-4-β-D-glucopyranosyloxy-2(E)-butenenitrile) (**2**)*

Freeze-dried powder (5 mg). HPLC R_t 31.6 min, on line UV λ_{max} 328, 236 nm. ^{1}H NMR: Table 1. ^{13}C NMR: (75 MHz, CD_3OD) δ 56.91 (OCH_3), 63.00 (C-6$'$), 64.76 (C-5), 68.62 (C-4), 71.86 (C-4$'$), 75.36 (C-2$'$), 78.41 (C-3$'$), 78.48(C-5$'$), 104.74 (C-1$'$), 112.27 (C-8$''$), 113.35 (C-2), 114.81 (C-5$''$), 116.59 (C-1), 116.93 (C-2$''$), 124.81 (C-6$''$), 127.96 (C-1$''$), 148.34 (C-7$''$), 149.43 (C-3), 149.83 (C-4$''$), 151.31 (C-3$''$), 168.46 (C-9$''$). ES-MS (-ve mode): *m*/*z* 450.5 ([M–H]$^-$, 100%).

Acknowledgements

The authors wish to thank Oleg Zubkov, Victoria University of Wellington, for the provision of HR–MS data and the NZ Foundation for Research, Science and Technology (FRST) for financial support (C0 8811-4).

References

Agrawal, P.K., 1989. Carbon-13 NMR of Flavonoids. Elsevier, Amsterdam, Oxford, New York, Tokyo.

Braekman, J.C., Daloze, D., Pasteels, J.M., 1982. Cyanogenic and other glucosides in the neo-guinean bug *Leptocoris isolata*: possible precursors in its host-plant. Biochemical Systematics and Ecology 10, 355–364.

Calamita, O., Malinowski, J., Strzelecka, H., 1983. Flavonoid compounds in black currant (*Ribes nigrum*) leaves. Acta Poloniae Pharmaceutica 40, 383–387.

Hakkinen, S., Auriola, S., 1998. High-performance liquid chromatography with electrospray ionization mass spectrometry and diode array ultraviolet detection in the identification of flavonol aglycones and glycosides in berries. Journal of Chromatography A 829, 91–100.

Jones, D.A., 1998. Why are so many food plants cyanogenic? Phytochemistry 47, 155–162.

Kalinowski, H.-O., Berger, S., Braun, S., 1988. Carbon-13 NMR spectroscopy. John Wiley, Chichester.

Kim, D.K., Zee, O.P., 2000. A new cyanogenic glycoside from *Sorbaria sorbifolia* var. *stellipila*. Chemical and Pharmaceutical Bulletin 48, 1766–1767.

Koeppen, B.H., Herrmann, K., 1977. Flavonoid glycosides and hydroxycinnamic acid esters of black currants (*Ribes nigrum*). Zeitschrift für Lebensmittel-Untersuchung und-Forschung 164, 263–268.

Lu, Y., Foo, L.Y., 1999. Rosmarinic acid derivatives from *Salvia officinalis*. Phytochemistry 51, 91–94.

Lu, Y., Foo, L.Y., 2001. Unusual anthocyanin reaction with acetone leading to pyranoanthocyanin formation. Tetrahedron Letters 42, 1371–1373.

Lu, Y., Sun, Y., Foo, L.Y., 2000. Novel pyranoanthocyanins from black currant seed. Tetrahedron Letters 41, 5975–5978.

Markham, K.R., Chari, V.M., 1982. Carbon-13 NMR spectroscopy of flavonoids. In: Markham, K.R., Mabry, T.J. (Eds.), The Flavonoids, Advances in Research. Chapman and Hall, New York.

Mikolajczak, K.L., Smith Jr., C.R., Tjarks, L.W., 1970. Cyanolipids of *Cardiospermum halicacabum* L. and other sapindaceous seed oils. Lipids 5, 812–817.

Nahrstedt, A., 1972. Zur Cyanogenese von *Prunus avium*. Phytochemistry 11, 3121–3126.

Nahrstedt, A., 1973. Cyanogenesis in Cotoneaster-Arten. Phytochemistry 12, 1539–1542.

Nahrstedt, A., Scheid, H., 1981. Leucine-derived cyanogenic glucosides in the Rosaceae-Spiraeoideae. Phytochemistry 20, 1309–1310.

Swenson, W.K., Dunn, J.E., Conn, E.E., 1987. Cyanogenesis in *Acacia sutherlandii*. Phytochemistry 26, 1835–1836.

Traitler, H., Winter, H., Richli, U., Ingenbleek, Y., 1984. Characterization of γ-linolenic acid in *Ribes* seed. Lipids 19, 923–928.

Ucciani, E., Costagliola, G., Gruber, M., 1995. Fatty acid composition of Saxifragaceae. Ol, Crops Gras, Lipides 2, 491–493.