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Scutellarein 4′-methyl ether glycosides as taxonomic markers in *Teucridium* and *Tripora* (Lamiaceae, Ajugoideae)

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Abstract

The flavonoid profiles of two monotypic genera, *Teucridium* and *Tripora*, have been studied by analytical methods. These genera were formerly placed in the Verbenaceae, but are now classified in the Lamiaceae, subfamily Ajugoideae. The major flavonoids of both genera were identified as glycosides of scutellarein 4′-methyl ether (5,6,7-trihydroxy-4′methoxyflavone) and acacetin (5,7-dihydroxy-4′-methoxyflavone). The new flavone glycoside, scutellarein 4′-methyl ether 7-*O*-rutinoside, was isolated from *Teucridium parvifolium* and the rare scutellarein 4′-methyl ether 7-*O*-glucuronide from *Tripora divaricata*. The latter compound has only been reported previously in the related genus *Clerodendron*. The potential of these flavonoids as taxonomic markers for the tribe Ajugoideae is discussed.

Keywords: *Teucridium parvifolium*; *Tripora divaricata*; Lamiaceae; Ajugoideae; Flavonoids; Scutellarein 4′-methyl ether 7-*O*-rutinoside; Scutellarein 4′-methyl ether 7-*O*-glucuronide; Chemosystematics

1. Introduction

The taxonomic treatment of *Teucridium parvifolium* Hook.f. and *Tripora divaricata* (Maxim.) P.D.Cantino (=*Caryopteris divaricata* Maxim.) has changed many times during the last decade. *Teucridium parvifolium* is one of only four species of Lamiaceae native to New Zealand, which are all endemic (Allan, 1961). It is a woody shrub up to 2 m tall, with interlacing branches, small leaves and very small flowers, and is the only species in the genus *Teucridium*. *Tripora* is also a monotypic genus with a name derived from the triporate pollen of *T. divaricata*, a unique character in the Lamiaceae. *Tripora*, a tall herb native to China, Japan and Korea, was recently removed from *Caryopteris*, because this genus was found to be paraphyletic (Cantino et al., 1999). *Teucridium* and *Caryopteris* including *Tripora* were formerly classified in the family Verbenaceae, but later transferred to the newly created Lamiaceae subfamily Teucrioideae, together with a number of other genera (Cantino et al., 1992). Recently all the genera of the Teucrioideae were transferred to the subfamily Ajugoideae (Cantino et al., 1997), which now comprises ca. 850 species in 24 genera. These include *Clerodendrum* (500 spp.), *Aegiphila* (150 spp.), *Teucrium* (100 spp.), *Ajuga* (50 spp.), *Oxera* (23 spp.), *Caryopteris* (7 spp.), *Faradaya* (3 spp.) and the monotypic *Huxleya*, *Oncinocalyx*, *Teucridium* and *Tripora*. Evidence from chloroplast DNA sequences suggests that *Teucridium* is sister to the Australian genus *Oncinocalyx* and that these form a clade with the cosmopolitan genus *Teucrium*. *Clerodendrum*, *Caryopteris, Tripora* and *Ajuga* form a sister group to this clade (Wagstaff et al., 1998). To the best of our knowledge *Teucridium parvifolium* and *Tripora divaricata* have not yet been investigated for their flavonoid constituents. This paper describes the isolation and identification of flavonoids from these species and discusses their use in the chemosystematics of the Ajugoideae.

2. Results and discussion

Aqueous methanol extracts of *Teucridium parvifolium* and *Tripora divaricata* were analyzed by 2-D PC, HPLC

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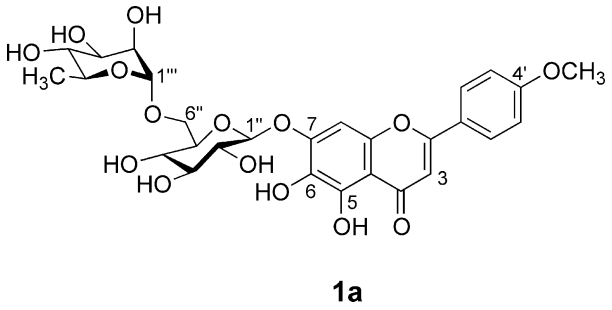
with diode array detection and LC–MS using atmospheric pressure chemical ionisation (APCI). The flavonoids from the extracts of *Teucridium parvifolium* (**1a**, **2a**, **3**, **4a**, **5**) and *Tripora divaricata* (**1b**, **2b**, **3**, **4b**, **6**, **7**) were isolated by means of preparative PC and semi-preparative HPLC. The short wavelength UV band of **1a** ($\lambda_{max}=285$ nm) was typical of that of a flavone with hydroxylation at C-6 (Harborne and Williams, 1971). Analysis of the ^{1}H NMR spectrum of **1a** revealed characteristic resonances of aromatic and glycosidic protons (including two anomeric protons) and one methoxyl group. The chemical shift values and coupling constant data for the aromatic proton resonances at δ 8.02 (2H, *d*, $J=9.2$ Hz, H-2′,6′), 7.18 (2H, *d*, $J=9.2$ Hz, H-3′,5′), 6.91 (1H, *s*, H-8) and 6.87 (1H, *s*, H-3) indicated that the aglycone was a derivative of apigenin (5,7,4′-trihydroxyflavone) (Markham et al., 1982; Markham and Geiger, 1994). Furthermore, the downfield shift of the H-3′,5′ proton resonances with respect to those of apigenin (δ 6.98) suggested that the 4′-hydroxyl group was substituted. A strong ROE connectivity detected between H-3′,5′ of **1a** and the methoxyl group at δ 3.87 confirmed that the substituent was a methyl group (4′-OCH_3). The singlet at δ 6.91 was assigned to H-8 of the flavone A-ring on the basis of its characteristic ^{13}C resonance at δ 94.0 (Markham et al., 1982). Site selective excitation of the anomeric proton resonance at δ 5.00 gave a strong ROE connectivity to H-8, indicating that the site of *O*-glycosylation was at C-7. The broad singlet observed at δ 12.70 was assigned to the 5-hydroxyl proton. These observations confirmed that the aglycone was 5,6,7-trihydroxy-4′-methoxyflavone (scutellarein 4′-methyl ether). This was consistent with the protonated aglycone $[A+H]^+$ ion at m/z 301 observed in first-order APCI–MS (positive mode) of **1a**.

Assignment of the primary sugar resonances was achieved by using the anomeric proton resonance at δ 5.00 as a starting point for the interpretation of DQF-COSY and HSQC datasets. Identification of the primary sugar as β-glucopyranose was supported by the $^3J_{H\text{-}1'',H\text{-}2''}$ coupling constant of 7.1 Hz and the ROE connectivities detected between H-1″ and both H-3″ and H-5″. The downfield and upfield shifts of the C-6″ (+5.5 ppm) and C-5″ (−1.4 ppm) Glc resonances, respectively, compared to scutellarein 7-*O*-β-glucopyranoside (Markham et al., 1982), indicated that the second sugar was attached at C-6″. This was identified as α-rhamnopyranose using the ^{1}H and ^{13}C NMR assignments obtained from DQF-COSY and HSQC data and the anomeric proton resonance at δ 4.61. Compound **1a** was therefore identified as the new flavone glycoside, scutellarein 4′-methyl ether 7-*O*-α-rhamnopyranosyl(1‴→6″)-β-glucopyranoside (Fig. 1). Its molecular formula of $C_{28}H_{32}O_{15}$ was confirmed by high-resolution MS.

The UV spectrum of compound **1b** was identical to that of **1a** (Table 1) and analysis of first- and second-order APCI–MS spectra indicated that both compounds were glycosides of the same aglycone. The ^{1}H NMR spectrum of compound **1b** was similar to that of **1a** with respect to aromatic and methoxyl resonances, but contained only one anomeric proton resonance. ROE connectivities were detected between resonances at δ 7.12 (H-3′,5′) and 3.85 (4′-OCH_3) and between δ 5.01 (H-1″) and 7.01 (H-8). These confirmed the presence of a methyl ether at C-4′ and a glycosylation site at C-7, as expected. The assignments of the ^{1}H and ^{13}C resonances of the sugar moiety were consistent with its identification as glucuronic acid, in particular the characteristic ^{13}C resonance at δ 171.3 corresponding to 6″-COOH and the ^{1}H resonance at δ 3.65 (*d*, $J=9.2$ Hz, H-5″). The protonated aglycone $[A+H]^+$ ion at m/z 301 observed in first-order APCI–MS (positive mode) of **1b** was equivalent to $[(M+H)-176]^+$, as expected (Table 1). The configuration of the GlcA residue was determined to be β from the $^3J_{H\text{-}1'',H\text{-}2''}$ coupling constant of 7.3 Hz. A molecular formula of $C_{22}H_{20}O_{12}$ was obtained for compound **1b** by high-resolution MS and was consistent with its identification as scutellarein 4′-methyl ether 7-*O*-β-glucuronopyranoside. This compound has only been found previously in *Clerodendrum trichotomum* (Morita et al., 1977) and was given the trivial name clerodendroside.



1a

Fig. 1. Structure of scutellarein 4′-methyl ether 7-*O*-α-rhamnopyranosyl(1‴→6″)-β-glucopyranoside.

The other major flavonoid from *Teucridium parvifolium* was identified as acacetin 7-*O*-rutinoside (**2a**) by UV spectral analysis (Mabry et al., 1970; Markham, 1982), acid hydrolysis to aglycone and sugars (Harborne, 1998), APCI-MS (Grayer et al., 2000) and comparison with an authentic standard. Similarly, compound **3** was identified as apigenin 6,8-di-*C*-glucoside (vicenin-2). The UV and APCI–MS spectra of **4a** and **5** allowed them to be identified as scutellarein 7-*O*-rutinoside and pectolinarigenin 7-*O*-rutinoside, respectively. Use of UV spectroscopy, acid hydrolysis and APCI–MS allowed the second most abundant flavonoid from *Tripora divaricata* to be identified as acacetin 7-*O*-glucuronide (**2b**), and the remaining flavonoids from this species as vicenin-2 (**3**), scutellarein 7-*O*-glucuronide (**4b**), luteolin 7-*O*-glucuronide (**6**), and the aglycone, scutellarein 4′-methyl ether (**7**).

Table 1
HPLC retention times, UV absorption maxima and APCI mass spectra of flavonoids identified in *Teucridium parvifolium* and *Tripora divaricata*

Compound	Identification	R_t (min)	UV λ_{max} (nm)	APCI–MS in positive mode (m/z)[a]			Species from which isolated
				$[M+H]^+$	$[I+H]^+$	$[A+H]^+$	
1a	Scutellarein 4′-methyl ether 7-*O*-rutinoside	16.5	285, 333	609	463	301	*Teucridium parvifolium*
1b	Scutellarein 4′-methyl ether 7-*O*-glucuronide	16.4	284, 333	477		301	*Tripora divaricata*
2a	Acacetin 7-*O*-rutinoside	17.6	266, 333	593	447	285	*Teucridium parvifolium*
2b	Acacetin 7-*O*-glucuronide	17.8	267, 332	461		285	*Tripora divaricata*
3	Vicenin-2	8.3	270, 336	595	577, 505, 475, 457, 385, 355		*Teucridium parvifolium* and *Tripora divaricata*
4a	Scutellarein 7-*O*-rutinoside	13.0	285, 338	595	449	287	*Teucridium parvifolium*
4b	Scutellarein 7-*O*-glucuronide	12.6	283, 335	463		287	*Tripora divaricata*
5	Pectolinarigenin 7-*O*-rutinoside	17.7	271, 333	623	477	315	*Teucridium parvifolium*
6	Luteolin 7-*O*-glucuronide	12.7	255, 267sh, 350	463		287	*Tripora divaricata*
7	Scutellarein 4′-methyl ether (aglycone)	20.0	285, 336	301		301	*Tripora divaricata*

[a] M = Molecular mass of the compound; I = mass of the intermediate(s); A = mass of the aglycone.

The occurrence of scutellarein 4′-methyl ether glycosides in *Tripora* and *Teucridium* is of taxonomic interest because they are relatively uncommon flavonoids in angiosperms and only a few examples have been reported in the family Lamiaceae. Tomás-Barberán et al. (1988) found that reports of scutellarein 4′-methyl ether glycosides in species of *Stachys* (subfamily Lamioideae) were erroneous, as these actually contain glycosides of isoscutellarein 4′-methyl ether, which yield scutellarein 4′-methyl ether as an aglycone on acid hydrolysis due to the Wessely-Moser rearrangement. Scutellarein 4′-methyl ether 7-*O*-glucuronide, a flavonoid with hypotensive activity, was isolated originally from *Clerodendrum trichotomum* (Lamiaceae, subfamily Ajugoideae) (Morita et al., 1977). We have now isolated the same compound (**1b**) from *Tripora divaricata*. The aglycone scutellarein 4′-methyl ether (**7**) has been reported from another *Clerodendrum* species, *C. inerme* (Vendantham et al., 1977), together with its 6-methyl ether, pectolinarigenin. It is possible that these flavones are also present as glycosides in this species. The occurrence of the uncommon scutellarein 4′-methyl ether derivatives in species of *Clerodendrum*, *Tripora* and *Teucridium* is evidence for a close relationship among these three genera in the Ajugoideae. The flavonoids of the monotypic Australian genus *Oncinocalyx*, which is thought to be the closest relative of *Teucridium* on the basis of DNA sequences, have not yet been studied. Although no scutellarein 4′-methyl ether glycosides have been reported from European species of the closely related genus *Teucrium*, other 6-hydroxylated flavone glycosides are common in these species (Harborne et al., 1986). It would be of interest to examine *Oncinocalyx* and Australian species of *Teucrium* to see whether they are more similar to *Teucridium* and contain glycosides of scutellarein 4′-methyl ether. Genera of the Ajugoideae that have already been surveyed for glycosides of 6-hydroxyflavones are *Oxera*, *Faradaya* and *Huxleya*. These compounds are present in *Huxleya* (de Kok et al., 2000), but completely absent from *Oxera* and *Faradaya* (Grayer and de Kok, 1998), so that the latter two genera are chemically distinct from the remainder of the Ajugoideae investigated to date. The present results suggest that an extended survey of the distribution of 6-hydroxylated flavone glycosides in genera of the Ajugoideae is likely to provide additional taxonomic characters for future phylogenetic studies of this subfamily of the Lamiaceae.

3. Experimental

3.1. General

^{1}H NMR (500 MHz) and ^{13}C NMR (125 and 67.8 MHz) spectra were recorded in DMSO-d_6 with the residual solvent resonances used as internal references at δ 2.50 and 39.5, respectively. ROE connectivities were measured using the 1D XSROESY pulse sequence (Gradwell et al., 1997). High-resolution ESI-MS (positive mode) were obtained on a Bruker Apex II instrument using internal calibrants. Positive ion first-order APCI-MS were obtained with a quadrupole ion-trap instrument (Finnigan LCQ) using a vaporiser temperature of 550 °C, sheath and auxiliary nitrogen gas pressures of 80 and 20 psi, a needle current of 5 μA, and a heated capillary temp. of 150 °C. Samples were introduced by direct infusion, or via an HPLC fitted with a Merck (Darmstadt, Germany) LiChrospher 100RP-18 (250×

4.0 mm i.d.; 5 μm particle size) column using a 20 min linear gradient of 25–100% MeOH in 1% aq. HOAc at 1 ml/min. The system for analytical and semipreparative HPLC consisted of a Waters LC600 pump and a 996 photodiode array detector. For analytical HPLC, the same column and gradient were used as for LC–MS described above, but the eluting solvents were 2% aq. HOAc and MeOH, HOAc, H_2O (18:1:1). An identical LiChrospher column but with 10 mm i.d. was used for semipreparative HPLC, and the eluting solvents consisted of H_2O and MeOH. The column temperature was 30 °C for both analytical and semipreparative HPLC.

3.2. Plant material

Plant material of *Teucridium parvifolium* was collected from a cultivated plant growing on the campus of the Victoria University of Wellington, New Zealand. The material was dried in a plant press. A herbarium voucher has been deposited in the Herbarium of the Royal Botanic Gardens, Kew. Plant material of *Tripora divaricata* was obtained from a cultivated specimen growing at Wakehurst Place (part of the Royal Botanic Gardens, Kew; garden accession no. 1982-8128). A herbarium voucher was prepared and the remainder of the leaf material was freeze-dried.

3.3. Extraction and isolation of flavonoids

For the extract of *T. parvifolium*, 22.8 g of dried leaves, stems and fruits were were broken up into small pieces and then ground with a pestle and mortar. For *T. divaricata* 20.0 g of freeze-dried ground leaves were used for extraction. Plant material of each species was boiled for 5 min in 300 ml of 80% aq. MeOH to deactivate enzymes, and then extracted further at room temperature for 24 h. The plant material was re-extracted with another 300 ml of 80% aq. MeOH and all extracts filtered, combined and concentrated by rotary evaporation at 40°. The concentrated extracts were resuspended in 80% aq. MeOH to a total volume of 10 ml. These solutions were centrifuged to remove precipitated chlorophyll and a small sample of each of the extracts was analysed for flavonoid content by HPLC with diode array detection. The extracts were separated by preparative PC into fractions, the flavonoids of which were monitored by analytical HPLC. Final purification of the flavonoids was carried out by means of semipreparative HPLC of flavonoid-rich fractions.

3.4. Scutellarein 4′-methyl ether 7-O-α-rhamnopyranosyl(1‴→6″)-β-glucopyranoside (**1a**)

Yellow solid (MeOH); UV (MeOH) λ_{max} nm: 285, 333; +NaOMe 271, 360 (decreased intensity); +$AlCl_3$ 299, 352; +$AlCl_3$ and HCl 298, 350; +NaOAc 283, 336; +NaOAc and H_3BO_3 293, 333. R_f on paper in BAW (*n*-BuOH, HOAc, H_2O = 4:1:5, top layer) 0.40; R_f on paper in 15% HOAc (HOAc, H_2O = 15:85) 0.38. ^{1}H NMR (DMSO-d_6, 500 MHz) δ 12.70 (1H, *br s*, 5-OH), 8.02 (2H, *d*, *J* = 9.2 Hz, H-2′,6′), 7.18 (2H, *d*, *J* = 9.2 Hz, H-3′,5′), 6.91 (1H, *s*, H-8), 6.87 (1H, *s*, H-3), 5.00 (1H, *d*, *J* = 7.3 Hz, H-1″), 4.61 (1H, *br s*, H-1‴), 3.92 (1H, *m*, H-6″), 3.87 (3H, *s*, 4′-OCH_3), 3.73 (1H, *m*, H-2‴), 3.65 (1H, *m*, H-5″), 3.52 (1H, *m*, H-3‴), 3.49 (1H, *m*, H-6″), 3.45 (1H, *m*, H-5‴), 3.38 (1H, *m*, H-2″), 3.35 (1H, *m*, H-3″), 3.19 (1H, *m*, H-4″), 3.18 (1H, *m*, H-4‴), 1.11 (3H, *d*, *J* = 6.1 Hz, 6‴-CH_3); ^{13}C NMR (DMSO-d_6, 125 MHz) (assignment of non-quaternary C atoms by HSQC) δ 128.3 (C-2′,6′), 114.8 (C-3′,5′), 103.3 (C-3), 101.0 (C-1″), 100.3 (C-1‴), 94.0 (C-8), 75.6 (C-5″), 75.3 (C-3″), 72.9 (C-2″), 71.8 (C-4‴), 70.6 (C-3‴), 70.3 (C-2‴), 69.5 (C-4″), 68.1 (C-5‴), 65.9 (C-6″), 55.2 (4′-OCH_3), 17.4 (6‴-CH_3); HRESIMS *m*/*z*: 609.1810 $[M+H]^+$ (calc. for $C_{28}H_{33}O_{15}$, 609.1814).

3.5. Scutellarein 4′-methyl ether 7-O-β-glucuronopyranoside (**1b**)

Yellow solid (MeOH); UV (MeOH) λ_{max} nm (and after addition of shift reagents): same data as for **1a**. R_f on paper in BAW 0.33; R_f on paper in 15% HOAc 0.17. ^{1}H NMR (DMSO-d_6, 500 MHz) δ 8.02 (2H, *d*, *J* = 8.6 Hz, H-2′,6′), 7.12 (2H, *d*, *J* = 8.6 Hz, H-3′,5′), 7.01 (1H, *s*, H-8), 6.86 (1H, *s*, H-3), 5.01 (1H, *d*, *J* = 7.3 Hz, H-1″), 3.85 (3H, *s*, 4′-OCH_3), 3.65 (1H, *d*, *J* = 9.2 Hz, H-5″), 3.37 (1H, *m*, H-2″), 3.33 (1H, *m*, H-3″), 3.26 (1H, *m*, H-4″); ^{13}C NMR (DMSO-d_6, 67.8 MHz) δ 182.3 (C-4), 171.3 (C-6″), 163.5 (C-2), 162.2 (C-4′), 151.5 (C-9), 148.9 (C-7), 146.7 (C-5), 130.7 (C-6), 128.2 (C-2′,6′), 122.9 (C-1′), 114.6 (C-3′,5′), 105.9 (C-10), 103.1 (C-3), 100.9 (C-1″), 94.3 (C-8), 75.8 (C-3″), 74.2 (C-5″), 72.9 (C-2″), 71.9 (C-4″), 55.5 (4′-OCH_3); HRESIMS *m*/*z*: 477.1029 $[M+H]^+$ (calc. for $C_{22}H_{21}O_{12}$, 477.1028).

3.6. Chromatographic data of the flavonoids isolated

Table 1 shows the retention times, UV spectra and APCI mass spectra of the compounds isolated from *Teucridium parvifolium* and *Tripora divaricata*.

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References

Allan, H.H., 1961. Flora of New Zealand, Vol. 1. Government Printer, Wellington.

Cantino, P.D., Harley, R.M., Wagstaff, S.J., 1992. Genera of Labiatae: status and classification. In: Harley, R.M., Reynolds, T. (Eds.), Advances in Labiate Science. Royal Botanic Gardens, Kew, pp. 511–522.

Cantino, P.D., Olmstead, R.G., Wagstaff, S.J., 1997. A comparison of phylogenetic nomenclature with the current system: a botanical case study. Systematic Biology 46, 313–331.

Cantino, P.D., Wagstaff, S.J., Olmstead, R.G., 1999. *Caryopteris* (Lamiaceae) and the conflict between phylogenetic and pragmatic considerations in botanical nomenclature. Systematic Botany 23, 369–386.

Gradwell, M.J., Kogelberg, H., Frenkiel, T.A., 1997. Applying excitation sculpting to construct singly and doubly selective 1D NMR experiments. Journal of Magnetic Resonance 124, 267–270.

Grayer, R.J., de Kok, R.P.J., 1998. Flavonoids and verbascoside as chemotaxonomic characters in the genera *Oxera* and *Faradaya*. Biochemical Systematics and Ecology 26, 729–741.

Grayer, R.J., Kite, G.C., Abou-Zaid, M., Archer, L.J., 2000. The application of atmospheric pressure chemical ionization liquid chromatography-mass spectrometry in the chemotaxonomic study of flavonoids: characterization of flavonoids from *Ocimum gratissimum* var. *gratissimum*. Phytochemical Analysis 11, 257–267.

Harborne, J.B., 1998. Phytochemical Methods, 3rd Ed. Chapman and Hall, London.

Harborne, J.B., Williams, C.A., 1971. 6-Hydroxyluteolin and scutellarein as phyletic markers in higher plants. Phytochemistry 10, 367–378.

Harborne, J.B., Tomás-Barberán, F.A., Williams, C.A., Gil, M.I., 1986. A chemotaxonomic study of flavonoids from European *Teucrium* species. Phytochemistry 25, 2811–2816.

de Kok, R.P.J., Grayer, R.J., Kite, G.C., 2000. Relationships of the endemic Australian genus *Huxleya* Ewart & Rees (Labiatae) based on fruit and flavonoid characters. Australian Systematic Botany 13, 425–428.

Mabry, T.J., Markham, K.R., Thomas, M.B., 1970. The Systematic Identification of Flavonoids. Springer Verlag, Berlin.

Markham, K.R., 1982. Techniques of Flavonoid Identification. Academic Press, London.

Markham, K.R., Chari, V.M., Mabry, T.J., 1982. Carbon-13 NMR Spectroscopy of Flavonoids. In: Harborne, J.B., Mabry, T.J. (Eds.), The Flavonoids. Advances in Research. Chapman and Hall, London, pp. 19–134.

Markham, K.R., Geiger, H., 1994. ^{1}H NMR spectroscopy of flavonoids and their glycosides in DMSO-d_6. In: Harborne, J.B. (Ed.), The Flavonoids. Advances in Research Since 1986. Chapman and Hall, London, pp. 441–497.

Morita, N., Arisawa, M., Ozawa, H., Chen, C.-S., Kan, W.-S., 1977. Clerodendroside, a new glycoside from the leaves of *Clerodendron trichotomum* Thunb. var. *fargesii* Rehd. (Verbenaceae). Yakugaku Zasshi 97, 976–979.

Tomás-Barberán, F.A., Grayer-Barkmeijer, R.J., Gil, M.I., Harborne, J.B., 1988. Distribution of 6-hydroxy-, 6-methoxy- and 8-hydroxyflavone glycosides in the Labiatae, the Scrophulariaceae and related families. Phytochemistry 27, 2631–2645.

Vendantham, T.N.C., Sankara Subramanian, S., Harborne, J.B., 1977. 4′-Methylscutellarein and pectolinarigenin from *Clerodendrum inerme*. Phytochemistry 16, 294.

Wagstaff, S.J., Hickerson, L., Spangler, R., Reeves, P.A., Olmstead, R.G., 1998. Phylogeny in *Labiatae* s.l., inferred from cpDNA sequences. Plant Systematics and Evolution 209, 265–274.