Phytochemistry, Vol. 23, No. 4, pp. 803–807, 1984.
Printed in Great Britain.

0031–9422/84 $3.00 + 0.00
Pergamon Press Ltd.

IRIDOID AGLYCONES FROM *DEUTZIA SCABRA*

PAOLA ESPOSITO, CARLO IAVARONE, ALINA SEN and CORRADO TROGOLO*

Centro di Studio per la Chimica delle Sostanze Organiche Naturali del C.N.R., Roma, Dipartimento di Chimica dell'Università di Roma, 'La Sapienza', P. le Aldo Moro, 2–00185 Roma, Italy

(*Revised received* 4 *August* 1983)

Key Word Index—*Deutzia scabra*; Saxifragaceae; scabrogenin; deutziogenin; natural iridoid aglycones.

Abstract—Scabrogenin and deutziogenin, the stable aglycones of known iridoid glucosides scabroside and deutzioside, have been isolated from *Deutzia scabra*. Their structures and mutarotational equilibria were investigated by detailed analysis of ^{1}H NMR and ^{13}C NMR data.

INTRODUCTION

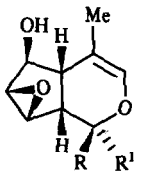
The present paper deals with the isolation from *Deutzia scabra* (Saxifragaceae) of deutziogenin (**1**) and scabrogenin (**2**), the stable aglycones of iridoid glucosides deutzioside (**3**) [1] and scabroside (**4**) [2], respectively, both co-occurring in the plant. The probable presence of **1** in *Mentzelia decapetala* was suggested by Danielson *et al.* [3]. As far as we know, this is the second case of isolation of free iridoid aglycones from natural sources after the well known genipin [4]. In fact the other reports published on this topic refer to the isolation of 3,4-dihydroiridoid aglycones neomatatabiol and isoneomatatabiol [5] and of silvestroside III [6], the aglycone of a bis-iridoid diglycoside. All other known iridoid aglycones, including compound **1**, have been obtained by enzymatic hydrolysis of parent glucosides [1, 7].

RESULTS AND DISCUSSION

The occurrence in the leaves of *Deutzia scabra* of **1** and **2**, the free aglycones of **3** and **4**, respectively, was first suggested by paper chromatography of an ethanolic extract of fresh leaves prepared immediately after their collection. Two spots were observed which were identical in colour (vanillin reagent) with those of **3** and **4** but with higher R_f values (**1**, 0.75, pink; **2**, 0.66, grey–brown; **3**, 0.40, pink; **4**, 0.21, grey–brown).

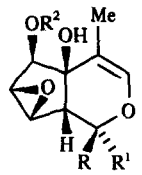
The ethanolic extract was carefully concentrated *in vacuo*, at low temperature and in neutral conditions, to an aqueous suspension which was continuously extracted with petrol to remove chlorophyll and apolar compounds. The residue from the aqueous fraction gave, after repeated chromatographies on cellulose and silica gel, pure compounds **1** and **2**.

*To whom correspondence should be addressed.



1a R = H, R^1 = OH

1b R = OH, R^1 = H

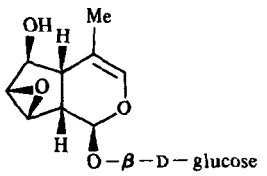


2a R = R^2 = H, R^1 = OH

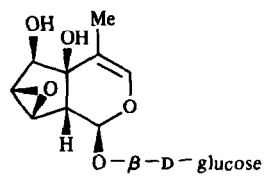
2b R^1 = R^2 = H, R = OH

5a R = H, R^1 = OAc, R^2 = Ac

5b R = OAc, R^1 = H, R^2 = Ac



3



4

From the examination of the ^1H NMR and ^{13}C NMR spectra (Tables 1 and 2) it was evident that **1** and **2** were the aglycones of the parent iridoid glucosides **3** and **4**. Both compounds were present as a mixture of the β- and α-epimers at C-1 in a ratio of about 2:1, as shown by the integral trace of the corresponding signal multiplicities in their ^1H NMR spectra and by the similar intensity ratio observed in the ^{13}C NMR spectra (PND) for each pair of signals relative to different carbons.

The assignment of the set of more intense ^1H NMR signals to the protons of the β-form was achieved by the correct identification of their $J_{1,9}$ values relative to the α and β H-1 protons in the pairs **1/3** (**1**, $J_{1\alpha,9}$ = 10.0 Hz, $J_{1\beta,9}$ = 3,5 Hz; **3**, $J_{1\alpha,9}$ = 10.0 Hz) and **2/4** (**2**, $J_{1\alpha,9}$ = 10.5 Hz, $J_{1\beta,9}$ = 3.8 Hz; **4**, $J_{1\alpha,9}$ = 9.2 Hz).

Analogous differentiation of the α- and β-epimeric forms by the ^{13}C NMR spectral approach based only upon the analysis of direct $J_{C\text{-}1,H\text{-}1}$ coupling constant values of the hemiacetalic C-1 carbons was not so reliable, in agreement with a previous report of Tietze *et al.* [8] on C-1 epimeric iridoid aglycone methyl ethers. In fact the 'gated decoupled' spectrum of **2** in D_2O showed a $J_{C\text{-}1,H\text{-}1}$ value of 165 Hz for the more intense C-1 resonance which was attributed to the β-form (**2b**). This value was different to that ($J_{C\text{-}1,H\text{-}1\alpha}$ = 172 Hz) of the parent glucoside (**4**) which has the *O*-glucosyl unit at C-1 in the same β-configuration. The less intense C-1 resonance in the spectrum of **2**, due to α-epimer (**2a**), had a $J_{C\text{-}1,H\text{-}1}$ value of 172 Hz. Also, in scabrogenin acetate (**5**) the lowest $J_{C\text{-}1,H\text{-}1}$ value (165 Hz) was linked to the more intense C-1 doublet (β-epimer, **5b**) and the highest value (174 Hz) to the less intense doublet of the α-epimer (**5a**).

The possibility of an inversion due to mutarotational equilibration occurring during the long period the sample remained in the probe during the accumulation time required for the measurement of the 'gated decoupled' spectrum and thus leading to a preponderance of the α-form, may be excluded because of the identical ^1H NMR spectra of **2** registered on the same sample immediately before and after taking the 'gated decoupled' spectrum.

An analogous comparison of $J_{C\text{-}1,H\text{-}1}$ values of the corresponding pair **1** and **3** was hindered by the very low solubility of **3** in D_2O which did not allow the recording of a satisfactory 'gated decoupled' spectrum of the parent glucoside **3**. However, there was a perfect agreement of the 'gated spectrum' of **1** with that of **2**, showing a $J_{C\text{-}1,H\text{-}1}$ of 165 Hz for the more intense C-1 signal (β-epimer) and a $J_{C\text{-}1,H\text{-}1}$ of 172 Hz for the less intense one (α-epimer) (Table 2).

In an attempt to overcome the solubility problem of compounds in D_2O a second set of 'gated decoupled' spectra were recorded for compounds **1**–**4** in DMSO-d_6. However, the results (Table 2) were rather confusing since the $J_{C\text{-}1,H\text{-}1}$ values of the parent glucosides **3** (166 Hz) and **4** (165 Hz) were both intermediate with respect to the values measured for the pairs of epimeric C-1 doublets of the corresponding aglycones **1** (162 and 171 Hz) and **2** (162 and 172 Hz), respectively.

Thus, the criteria for distinguishing α- and β-epimeric forms of iridoid aglycones (e.g. **1** and **2**) using their ^1H NMR spectra by comparing their $J_{1,9}$ values with those of the parent iridoids seems straightforward and can be diagnostic and is not solvent dependent (Table 1). However, the analysis of these compounds using their ^{13}C NMR spectra appears to be misleading and valueless showing, either in D_2O or in DMSO-d_6, rather different $J_{C\text{-}1,H\text{-}1}$ values between the β-aglycone and its parent iridoid having the same β-configuration at C-1. The appreciable shielding effect (*ca* 3–4 ppm) observable in both solvents for the C-1 of α-aglycones with respect to the β-ones is worthy of note. The deshielding of the aglycone C-1 carbon due to the glucosidation of hydroxyl-1 ('the glucosidation shift') may be calculated as $\Delta\delta_A = \delta$(R glucoside) − (β-aglycone, RH); in the pairs **1b/3** and **2b/4** its values are 3.32 and 2.34 ppm in D_2O and1.87 and 1.61 ppm in DMSO-d_6, respectively.

The possibility that **1** and **2** may be artefacts is very unlikely owing to the mild conditions used for the extraction (ethanol at room temperature) and to their constant presence in extracts prepared and investigated immediately after the collection of plant material. The extractions, repeated several times on different sets of samples and in different conditions (pH and evaporation temperature) always led to the same yields in aglycones.

Confirmation of the good stability of these aglycones was provided by the recovery of **1** and **2** chromatographically unaffected after the measurement of the ^{13}C NMR spectra: their D_2O solutions were only slightly coloured after 40 hr standing at a probe temperature of 36°. In addition **1** and **2** proved to be very stable at low temperature even after several months.

The unexpected and misleading decrease of the $J_{C\text{-}1,H\text{-}1\alpha}$ observed in the comparison of scabroside (**4**) with the β-form of its aglycone (**2b**) requires an investigation of the behaviour of other selected glucoside/β-aglycone pairs for a possible rationalization of this conformational effect.

EXPERIMENTAL

General techniques were as described earlier [11].

Isolation of the iridoid fraction. Paper chromatography (PC) of various EtOH extracts of fresh leaves of *Deutzia scabra*, obtained under different conditions (time, temp, pH) immediately after their harvest, showed the presence in constant amount of two sparingly polar compounds behaving like the aglycones of iridoid glucosides, namely deutzioside (**3**) and scabroside (**4**). In fact a PC, developed with *n*-BuOH–HOAc–H_2O (63:10:27) and visualized with vanillin reagent, showed at least five spots two of which were identical in colour to the iridoid glucosides present in the plant but with higher R_f values: 0.75 (**1**, pink, deutziogenin), 0.66 (**2**, grey–brown, scabrogenin), 0.40 (**3**, pink, deutzioside), 0.34 (pink, deutziol) [12], 0.21 (**4**, grey-brown, scabroside).

Fresh aerial part of *Deutzia scabra* (6.4 kg) were collected in July 1980 in the Botanical Garden of the University of Rome (a reference specimen A 28-45 has been deposited there) and extracted with 90% EtOH (15 l.) for 1 day at room temp and pH 7. The extract was concd at low temp to an aq. suspension and then continuously extracted with petrol 40–70° (2 l.). The residue was chromatographed on cellulose (350 g) in *n*-BuOH satd with H_2O and afforded the following fractions: *A* **1** and **2** (~ 1:2), 1.5 g; *B* **1** (small amounts) and **2**, 1.8 g; *C* compounds with lower R_f value (deutziol, **3**, **4**), 10 g.

Isolation of α- and β-deutziogenin (**1**). Fraction *A* (1.5 g) chromatographed over 150 g of silica gel in $CHCl_3$–MeOH (95:5) afforded crude **1** (410 mg), still contaminated by **2**, which was rechromatographed on silica gel (40 g) in Et_2O–EtOAc (95:5) and gave pure **1** (60 mg) as an amorphous mixture of β- and α-epimers which crystallized very slowly. For analytical purposes only, a sample was crystallized from EtOAc, mp 145–153° (dec). (Found: C, 58.80; H, 6.63. $C_9H_{12}O_4$ requires C, 58.69; H, 6.57%.)

Isolation of α- and β-scabrogenin (**2**). Fraction *B* (1.8 g) chromatographed over 180 g of silica gel in $CHCl_3$–MeOH (95:5)

Table 1. ^{1}H NMR spectra (90 MHz) of iridoid aglycones and glucosides of *Deutzia scabra*

	1a		**1b**		**3**		**2a**		**2b**		**4**	
	D_2O	DMSO-d_6	D_2O	DMSO-d_6	D_2O‡	DMSO-d_6†	D_2O	DMSO-d_6	D_2O	DMSO-d_6	D_2O‖	DMSO-d_6
H-1	5.41 *d* $J_{1,9}$ = 3.5	5.26 *d* $J_{1,9}$ = 3.6	4.60 *d* $J_{1,9}$ = 10.0	4.40 *d* $J_{1,9}$ = 9.5	4.80 *d* $J_{1,9}$ = 10.0	4.68 *d* $J_{1,9}$ = 10.0	5.54 *d* $J_{1,9}$ = 3.8	5.25 *d* $J_{1,9}$ = 4.0	4.82 *d* $J_{1,9}$ = 10.5	4.54 *d* $J_{1,9}$ = 10.0	5.12 *d* $J_{1,9}$ = 9.2	4.82 *d* $J_{1,9}$ = 10.0
H-3	5.98 *sfs*	5.92 *sfs*	6.10 *sfs*	6.05 *sfs*	6.15 *m*	6.13 *m* $J_{3,5}$ = 0.5 $J_{3,Me}$ = 0.5	6.13 *sfs*	5.93 *sfs*	6.20 *sfs*	6.02 *sfs*	6.25 *sfs*	6.08 *sfs*
H-5	2.1–2.7	1.82¶	1.96 *bt* $J_{5,6}$ = 7.5 $J_{5,9}$ = 7.7	1.82 *t* $J_{5,6}$ = 7.4 $J_{5,9}$ = 7.4	2.07 *t* $J_{5,6}$ = 7.5 $J_{5,9}$ = 7.5	1.91 *m* $J_{5,6}$ = 8.0 $J_{5,9}$ = 7.5 $J_{3,Me}$ = 0.5						
H-6	4.18 *d* $J_{5,6}$ = 7.5	4.03 *d* $J_{5,6}$ = 7.7	4.05 *dd* $J_{5,6}$ = 7.5 $J_{6,7}$ = 1.5	3.81 *d* $J_{5,6}$ = 8.4	4.12 *dd* $J_{5,6}$ = 7.5 $J_{6,7}$ = 1.5	3.89 *dd* $J_{5,6}$ = 8.0 $J_{6,7}$ = 1.5	4.30§	4.00 *bs*	4.34§ *d* $J_{6,7}$ = 1.5	4.00 *bs*	4.33 *d* $J_{6,7}$ = 1.5	4.01**
H-7	3.7–3.5	3.5–3.2*	3.7–3.5	3.5–3.2*	3.62 *dd* $J_{6,7}$ = 1.5 $J_{7,8}$ = 2.5	3.39 *m*	3.8–3.6	3.8–3.1	3.8–3.6	3.8–3.1	3.9–3.7	3.8–3.2
H-8	3.7–3.5	3.5–3.2*	3.7–3.5	3.5–3.2*	3.73 *d* $J_{7,8}$ = 2.5	3.50 *d* $J_{7,8}$ = 2.7	3.8–3.6	3.8–3.1	3.8–3.6	3.8–3.1	3.9–3.7	3.8–3.2
H-9	2.72 *dd* $J_{1,9}$ = 3.5 $J_{5,9}$ = 8.3	2.45 *dd* $J_{1,9}$ = 3.6 $J_{5,9}$ = nm	2.40 *dd* $J_{1,9}$ = 10.0 $J_{5,9}$ = 7.7	2.19 *dd* $J_{1,9}$ = 9.5 $J_{5,9}$ = 7.4	2.55 *dd* $J_{1,9}$ = 10.0 $J_{5,9}$ = 7.5	2.31 *dd* $J_{1,9}$ = 10.0 $J_{5,9}$ = 7.5	2.78 *d* $J_{1,9}$ = 3.8	2.41 *d* $J_{1,9}$ = 4.0	2.52 *d* $J_{1,9}$ = 10.5	2.24 *d* $J_{1,9}$ = 10.0	2.62 *d* $J_{1,9}$ = 9.2	2.31 *d* $J_{1,9}$ = 10.0
3H-11	1.56 *sfs*	1.57 *sfs*	1.56 *sfs*	1.57 *sfs*	1.64 *s*	1.60 *d* $J_{3,Me}$ = 0.5	1.60 *sfs*	1.49 *sfs*	1.60 *sfs*	1.49 *sfs*	1.62 *sfs*	1.50 *sfs*

sfs = Singlet with fine structure; J in Hz.
*Covered by HDO signal present in DMSO-d_6.
†Taken from ref. [7].
‡Taken from ref. [9].
§These signals are mutually overlapped.
‖Taken from ref. [2].
¶Overlapped by the H-5 signal of the β-form.
**Tentative assignment.

Table 2. ^{13}C NMR spectra (20 MHz) of iridoid aglycones and glucosides of *Deutzia scabra**

	1a		1b		3		2a		2b		4		5a	5b
	D_2O	DMSO-d_6	D_2O	DMSO-d_6	D_2O	DMSO-d_6	D_2O	DMSO-d_6	D_2O	DMSO-d_6	D_2O§	DMSO-d_6	$CDCl_3$	$CDCl_3$
C-1	89.39 *d* (172)	88.62 *d* (171)	93.47 *d* (165)	92.95 *d* (162)	96.79 *d*	94.82 *d* (166)	91.40 *d* (172)	90.49 *d* (172)	94.54 *d* (165)	94.18 *d* (162)	96.88 *d* (172)	95.79 *d* (165)	88.75 *d* (174)	90.62 *d* (165)
C-3	133.03 *d* (190)	132.83 *d* (190)	135.95 *d* (190)	135.74 *d* (188)	135.75 *d*	135.07 *d* (189)	136.43 *d* (192)	135.16 *d* (187)	138.18 *d* (191)	136.58 *d* (187)	137.89 *d* (193)	135.94 *d* (189)	136.30 *d* (190)	137.96 *d* (192)
C-4	112.99 *s*	109.78 *s*	112.99 *s*	110.75 *s*	113.48 *s*	111.42 *s*	113.88 *s*	112.59 *s*	115.25 *s*	114.70 *s*	115.65 *s*	115.43 *s*	113.18 *s*	113.18 *s*
C-5	38.29 *d* (132)	nm	41.13 *d* (135)	40.39 *d* †	41.12 *d*	40.12 *d* †	72.18 *s*	71.14 *s*	74.13 *s*	72.11 *s*	74.52 *s*	72.17 *s*	nm	73.33 *s*
C-6	77.95 *d* (142)	76.51 *d* (142)	78.53 *d* (142)	77.61 *d* (141)	78.58 *d*	77.06 *d* (141)	77.34 *d* (142)	76.12 *d* (142)	77.65 *d* (142)	76.96 *d* (142)	77.06 *d* (142)	76.55 *d* (141)	nm	78.22 *d* nm
C-7	61.06 *d* (191)	59.10 *d* (188)	59.50 *d* (192)	57.48 *d* (189)	59.68 *d*	57.52 *d* (190)	60.93 *d* (191)	59.42 *d* (190)	59.58 *d* (191)	58.80 *d* (191)	59.78 *d* (192)	57.91 *d* (190)	57.30 *d* (nm)	56.44 *d* (194)
C-8	57.63 *d* (192)	54.96 *d* (186)	56.66 *d* (191)	54.18 *d* (189)	56.24 *d*	53.81 *d* †	56.44 *d* (193)	55.02 *d* (190)	55.96 *d* (193)	54.44 *d* (190)	56.07 *d* (192)	54.00 *d* (189)	54.57‡ *d*	54.57 *d* (193)
C-9	41.64 *d* (nm)	nm	44.15 *d* (140)	43.24 *d* †	42.68 *d*	41.60 *d* †	50.00 *d* (138)	49.45 *d* (138)	52.34 *d* (136)	51.53 *d* (138)	50.79 *d* (140)	49.90 *d* (nm)	47.84 *d* (nm)	49.29 *d* (142)
C-11	16.61 *q* (129)	16.70 *q* (128)	16.11 *q* (129)	16.05 *q* (128)	16.01 *q*	15.91 *q* (128)	11.84 *q* (130)	11.97 *q* (130)	11.52 *q* (130)	11.52 *q* (130)	11.53 *q* (129)	11.34 *q* (130)	11.79 *q* (128)	11.43 *q* (128)

*Chemical shifts are expressed on the TMS scale according to the following equation $\delta_{TMS} = \delta_{dioxane} + 67.4$ ppm. Values in parentheses are coupling constants, which have been quoted to the nearest ± 1 Hz.

†Overlapped by DMSO-d_6 signals.

‡Tentative assignment.

§Taken from ref. [11].

nm, Not measurable.

gave pure **2** (0.6 g) as an amorphous mixture (2:1) of β- and α-epimers. For analytical purposes a sample was rechromatographed in the same conditions. (Found: C, 53.85; H.6.10. $C_9H_{12}O_5$ requires C, 53.99; H, 6.04%.)

Di-O-acetylscabrogenin (**5**). Compound **2** (α- and β-epimers, 80 mg) was acetylated with pyridine and Ac_2O for 1 hr at room temp. After addition of MeOH the soln was left for 20 min and then evaporated to dryness. The residue, chromatographed on silica gel (7 g) in Et_2O–C_6H_6 (1:1) afforded pure **5** (60 mg) as an inseparable mixture of α- and β-epimers.

REFERENCES

1. Bonadies, F., Esposito, P. and Guiso, M. (1974) *Gazz. Chim. Ital.* **104**, 17.
2. Esposito, P. and Guiso, M. (1973) *Gazz. Chim. Ital.* **103**, 517.
3. Danielson, T. J., Hawes, E. M. and Bliss, C. A. (1975) *J. Chromatogr.* **103**, 216.
4. Djerassi, C., Nakano, T., James, A. N., Zalkov, L. H., Eisenbraun, E. J. and Shoolery, J. N. (1961) *J. Org. Chem.* **26**, 1192.
5. Hyeon, S. B., Isoe, S. and Sakan, T. (1968) *Tetrahedron Letters* 5325.
6. Jensen, S. R., Lyse-Petersen, S. E. and Nielsen, B. J. (1979) *Phytochemistry* **18**, 273.
7. Danielson, T. J., Hawes, E. M. and Bliss, C. A. (1973) *Can. J. Chem.* **51**, 760.
8. Tietze, L. F., Niemeyer, U., Marx, P. and Glüsenkamp, K. H. (1980) *Tetrahedron* **36**, 1231.
9. Jensen, S. R., Mikkelsen, C. B. and Nielsen, B. J. (1981) *Phytochemistry* **20**, 71.
10. Bianco, A., Caciola, P., Guiso, M., Iavarone, C. and Trogolo, C. (1981) *Gazz. Chim. Ital.* **111**, 201.
11. Bonini, C., Davini, E., Iavarone, C. and Trogolo, C. (1981) *Phytochemistry* **20**, 1587.
12. Esposito, P., Guiso, M., Nicoletti, M. and De Luca, C. (1976) *Gazz. Chim. Ital.* **106**, 57.