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# Transformation of naturally-occurring 1,9-*trans*-9,5-*cis* sweroside to all *trans* sweroside during acetylation of sweroside aglycone

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**Abstract**

From the rootstock of *Scabiosa columbaria* L. loganin and sweroside were isolated. Sweroside showed moderate antibacterial activity. Pure sweroside was converted to the sweroside aglycone 1-acetoxy derivative (DABCO/$Ac_2O$) after hydrolysis of the glucose unit. X-ray crystallography of the monoacetate showed unambiguously that it had been transformed to a new seco-iridoid having the novel *trans* diaxial configuration for the protons on C-1, C-9 and C-5. The mechanism for the rearrangement is discussed. © 2001 Elsevier Science Ltd. All rights reserved.

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## 1. Introduction

*Scabiosa columbaria* L. is a perennial herb, which grows to a meter in height, flowers in midsummer, and has a large woody rootstock in the form of a tuber. Traditionally root decoctions are used for sterility. Ointments made from the charred tubers are also applied to venereal sores (Hutchings, 1996). Chemically almost nothing is known about the plant although the glycoside scabiosin has been isolated from it (Watt and Breyer-Brandwijk, 1962).

We started investigating the plant because it grows profusely in many parts of South Africa, and, despite its frequent use by the Zulu people, the chemistry of its constituents is almost unknown (Van Wyk, 1997).

## 2. Results and discussion

### 2.1. *Isolation of loganin and sweroside*

It was immediately clear from the preliminary examination of the extracts ($CHCl_3$)/EtOH) that *Scabiosa columbaria* contained several iridoids, mostly in the form of their glycosides. Fractionation of the extract yielded pure loganin **1a**. This compound was first isolated from the fruits of *Strychnos nux vomica* (Dunstan and Short, 1883). The full structure, stereochemistry and biosynthesis was only elucidated some 86 years later (Battersby and Hall, 1969).

Also present in the extract we found the seco-iridoid sweroside **2a** (Inouye et al., 1970) and this is the first isolation from the family Dipsaceae. It was accompanied by a complex mixture of other iridoids, all possessing an aldehyde functionality. This latter mixture and sweroside, **2a**, showed only moderate antibacterial activity when tested against a selection of gram-positive and gram-negative bacteria.

### 2.2. *Rearrangement of sweroside on acetylation and X-ray analysis of the resultant product*

Iridoids and seco-iridoids all possess the labile acetal moiety and are frequently subjected to varying degrees of acid or base treatment during work-up or subsequent manipulation. In this connection Bianco (1994) has published a review article which deals specifically with acid hydrolysis and the subsequent rearrangements which can occur.

We report here on the rearrangement which sweroside aglycone **2b** undergoes during acetylation in the presence

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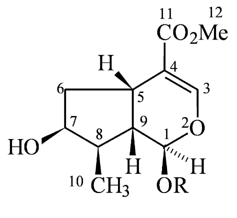
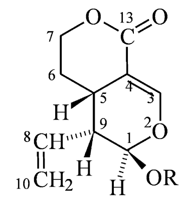
of the base diazabicyclooctane (DABCO). (DABCO is easier to remove at the end of the reaction than pyridine). The reaction afforded the highly crystalline 1-acetoxy sweroside aglycone, a new compound with novel stereochemistry. This seco-iridoid **3**, has the protons at C-1, C-9 and C-5 in the axial orientation, giving rise to an all *trans*-configuration at these three carbon atoms. This stands in contrast to the "normal" 1,9-*trans*-9,5-*cis* arrangement found in iridoids and seco-iridoids in which the protons on C-1, C-9 and C-5 assume *eq.eq.* and *ax* orientations respectively. Since **3** was obtained as colourless orthorhombic crystals, amenable to X-ray analysis, the relative stereochemistry could be established with certainty (Fig. 1). Comparison with the extensive published NMR data (Ma et al., 1994) confirmed the view that "our" parent sweroside indeed had the accepted 1,9-*trans*-9,5-*cis* stereochemistry. Subsequent formation of **3**, with its 1,9-*trans*-9,5-*trans*-configuration was, therefore, attainable only if rearrangement had occurred during acetylation.

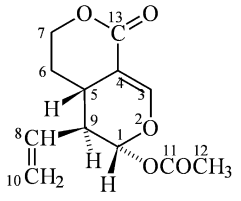
The seco-iridoid decentapicrin A, a compound for which X-ray data was available is shown in (**4**) (Van der Sluis and Labadie, 1981; Kojic-Prodic et al. 1985). This compound is identical in all respects to sweroside and differs only in the nature of the glycoside at C-1. A comparison of the magnitude of the coupling constants $J_{1,9}$ and $J_{5,9}$ respectively for **4** and **3** shows clearly a H-1/H-9 (*trans* di*equatorial*), H-5/H-9 (*cis axial*, *equatorial*) stereochemistry for **4**, compared with a *trans diaxial*, *trans diaxial*, arrangement for the same proton on **3**.



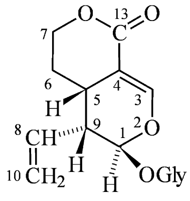
**1a** R = Glucose (Loganin)
**1b** R = H (aglucone)
**1c** R = $COCH_3$



**2a** R = glucose (sweroside)
**2b** R = H (aglucone)

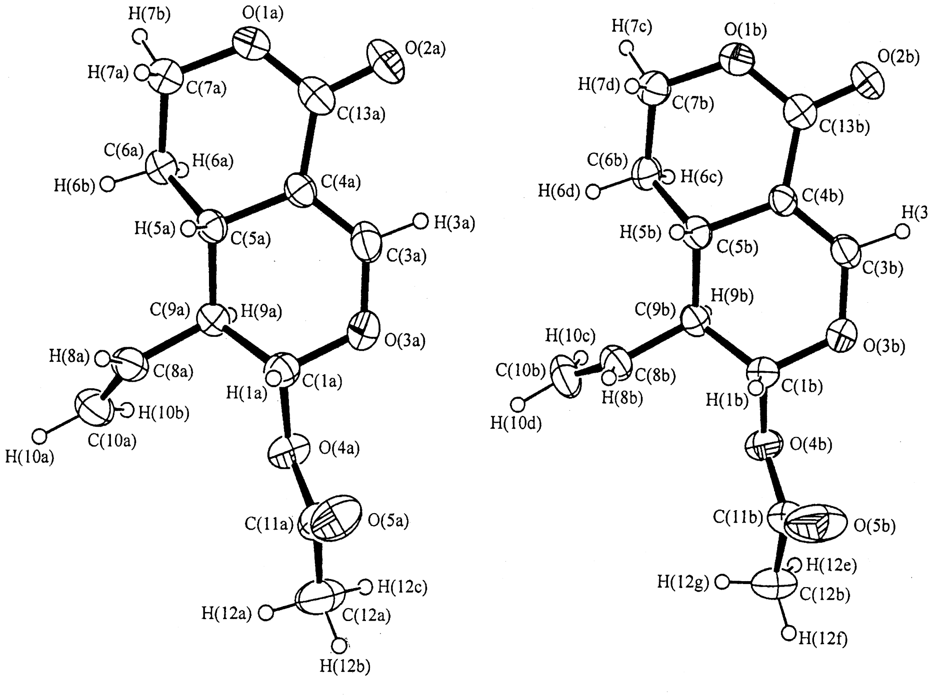


**3**



Decentapicrin **4**

We were reassured that the proposed rearrangement occurs *during* the acetylation procedure, and not earlier, by showing that the sweroside aglycone (**2b**) had a stereochemistry unchanged from that of the parent sweroside (**2a**). A comparison of $^1H$ NMR spectral data for sweroside (**2a**), sweroside aglycone (**2b**) (see Section 3), and the rearranged acetate (**3**) (Table 1) confirms the



**Molecule A** **Molecule B**

Fig. 1. Two crystallographically-distinct conformations of sweroside aglycone-1-acetate **3**.

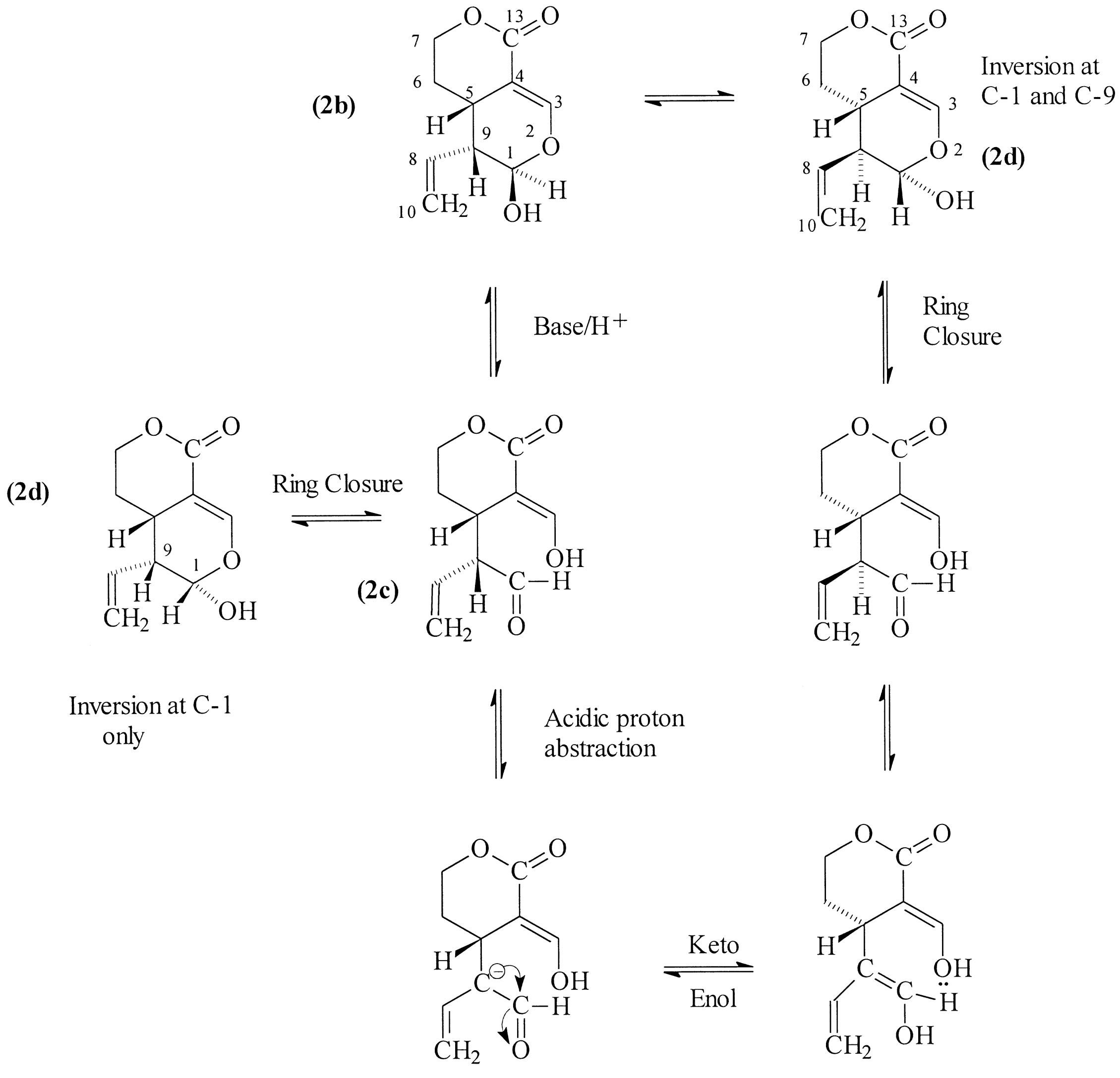
Table 1
NMR spectral data of rearranged acetate **3** ($CDCl_3$, $\delta$ ppm, $J$ = Hz)

| No. | $^1H$ | $^{13}C$ |
|---|---|---|
| 1 | 6.11 (1H, *d*, 9.3) | 93.1 |
| 3 | 7.64 (1H, *d*, 2.2) | 154.1 |
| 4 | – | 104.4 |
| 5 | 2.56 (1H, *ddd*, 4.5,2.3) | 34.0 |
| 6 | 6a 1.56 (1H, *m*) | |
| | 6b 2.06 (1H, *m*) | 27.2 |
| 7 | 7a 4.27 (1H, *ddd*, 24.1, 12.5, 2.4) | |
| | 7b 4.48 (1H, *ddd*, 11.5, 4.5, 2.0) | 68.1 |
| 8 | 5.52 (1H, *ddd*, 16.9, 10.3, 9.3) | 132.6 |
| 9 | 2.21 (1H, *ddd*, 9.2, 7.4, 1.6) | 46.2 |
| 10 | 10a 5.29 (1H, *ddd*, 16.9, 1.5, 0.7) | |
| | 10b 5.33 (1H, *ddd*, 10.4, 1.4) | 121.4 |
| $CO_2$ lactone | | 169.1 |
| Acetate CO | | 164.7 |
| $OCH_3$ | 2.13 (*s*) | 20.7 |

stereochemistry shown for (**2a**), (**2b**) and **3**. There is no doubt that the aglycone still retains the 1,9 *trans* d*ieq*. arrangement prior to acetylation. It is suggested that the rearrangement during acetylation proceeds as shown in Scheme 1.

1. The aglycone of sweroside, **2b**, under influence of the base (DABCO) ring opens to generate **2c**.
2. In a final step, prior to attachment of the acetyl moiety, **2c** loses an acidic proton at C-9, undergoes keto-enol tautomerism and ring-closes to **2d** (and also presumably the isomer of opposite configuration).

We suggest that a series of products, all in equilibrium with one another, are formed. On acetylation, and quite fortuitously, one of these products, **3**, crystallised out from the reaction mixture in reasonable yield. The product was amenable to X-ray and NMR analysis.



Scheme 1. Mechanism of inversion at C-1 and C-9.

Since acetylation usually occurs in the presence of pyridine (rather than DABCO/$Ac_2O$) the reaction on sweroside aglucone was also carried with this reagent. To our pleasant surprise the rearranged all *trans* sweroside **3** was obtained in 73% yield.

### 2.3. Rearrangement in the NMR tube

In order to study the effect of DABCO on sweroside aglycone **2b** (with intact stereochemistry at C-1, C-9 and C-5) the compound was dissolved in $CDCl_3$ in an NMR tube and DABCO only added. Progress of the reaction was monitored over 10 h. Small changes in the NMR spectrum were apparent within minutes. The H-1 signal disappeared rapidly, and after 40 min several new peaks appeared in the region $\delta$ 6.5–9.55. The most significant of these was a doublet at $\delta$ 9.4. ($J=3.82$ Hz). This peak gradually increased in amplitude and after 4.5 h a further peak was visible at $\delta$ 9.87. Both peaks are presumed to be aldehyde peaks and their presence is regarded as evidence that ring opening occurs (Scheme 1). The effect is in line with earlier proposals (Bianco, 1994).

### 2.4. Iridoids with an *all*-trans ring fusion in nature

The first naturally-occurring iridoid of the above nature was reported by Foderaro et al. (1992). These iridoids are rare and the authors make the observation that "there are no known instances of epimerisation at C-5 among the hundreds of iridoid isolations". In a subsequent report Foderaro and Stermitz (1992) present detailed spectral data to support their finding. They record a $J_{1,9}$ coupling constant of 9.9 Hz for the compound (5dH)-6-*epi*dihydrocornin. The X-ray structure of a suitable derivative revealed clearly the all *trans* diaxial nature of the protons on C-1, C-9 and C-5.

The seco-iridoid **3**, produced synthetically, thus joins a small group of naturally-occurring iridoid compounds with distinctive stereochemical features and hopefully will alert other researchers to stereochemical changes which can occur during manipulation of iridoids.

### 2.5. Attempts to ring-open loganin with DABCO

Loganin aglycone **1b** (from $\beta$-glucosidase hydrolysis of loganin) was subjected to the same acetylation conditions of DABCO/acetic anhydride as described for sweroside aglycone. No rearrangement occurred and the 1-acetoxy derivative was isolated in good yield and high purity. The $^1$H NMR spectrum differs very little from that of the parent aglucone apart from H-1 which showed the anticipated downfield shift from $\delta$ 4.98 to $\delta$ 6.07. The $J_{1,9}$ coupling constant is 3.5 Hz and in loganin itself it is 4.5 Hz (Boros and Stermitz, 1990). In this instance it is possible that the iridoid skeleton (as in loganin) is less susceptible to ring opening and subsequent rearrangement than the seco-iridoid system as in sweroside aglucone.

### 2.6. Biological activity tests

Since aq. root decoctions of Scabiosa columbaria find a traditional use in the treatment of venereal sores (Hutchings, 1996), some of the isolated components (sweroside, loganin and a semi-purified mixture) were tested for antibacterial activity. At concentrations of 0.001–0.50 mg/ml none of the compounds showed any activity. At a conc. of 1.0 mg/ml (minimum inhibitory concentration, MIC), sweroside and the "mixture" were active against *Bacillus cereus*, *B. pumulus*, *B. subtilis*, *Micrococcus kristinae*, *Staphylococcus aureus* (gram positive) and *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aerugenosa*, *Enterobacter cloacae* (gram negative). Neither inhibited the growth of Serratia marcescence. These activities can be regarded as "moderate" only.

## 3. Experimental

### 3.1. General

$^1$H and $^{13}$C NMR spectra were recorded on Varian-200 and Varian-500 spectrometers. High resolution mass spectra were recorded on a Kratos MS 80 RF double-focussing magnetic sector instrument at 70 eV. For optical rotations a Perkin Elmer 241 digital polarimeter was used.

### 3.2. Plant material

*Scabiosa columbaria* (rootstock) was collected in an open field in the Hayfields area of Pietermaritzburg during January 1999. It was identified by Dr Trevor Edwards, Curator of the Bews Herbarium at the University of Natal. A voucher specimen (SED No. 6) of the whole plant has been deposited in the Herbarium.

### 3.3. Extraction and isolation

Freshly-collected tubers (850 g) were milled and extracted with 95% EtOH–$CHCl_3$ (1:1) for 6 days at RT. The solution was filtered and the lower phase separated off and concentrated (16 g). A portion of this material (9 g) was fractionated on a dry silica gel (Merck Silica Gel 9385) column using $CH_2Cl_2$–MeOH (100: 0→85: 15) as eluent. Three fractions were collected: fraction (i) afforded loganin aglycone (**1b**) (35 mg) after purification on the chromatron [$CH_2Cl_2$–$Et_2O$(1: 1)]; fraction (ii) gave loganin (**1a**) (70 gm) following separation on the chromatotron [$CH_2Cl_2$–MeOH (93: 7)]; fraction (iii) gave a crude mixture

(726 mg) which on separation as above [$CH_2Cl_2$–MeOH (90:10→85:15)] afforded impure sweroside (**2a**) (140 mg) and a mixture of other compounds unidentified as yet (280 mg). Further separation on the chromatotron [$CH_2Cl_2$–MeOH (93:7→90:10)] gave pure sweroside (**2a**) (77 mg).

### *3.4. Studies on loganin*

The properties of loganin (above, mp 220°C) were identical in all aspects (NMR, mass spectra) to values published in the literature (Boros and Stermitz, 1990). The optical rotation, $[\alpha]_D^{25} = -62.4°$ ($c = 0.0004$, MeOH), is somewhat lower than the literature value of $-82.8°C$ (El-Naggar and Beal, 1980).

Loganin (**1a**) (230 mg) was suspended in water pH 6.8 (5 ml) and β-glucosidase (30 mg) added. The solution was stirred at 37°C for 5 h, then exhaustively extracted with ether to give impure loganin aglucone. The latter was purified on the chromatotron [($CH_2Cl_2$–$Et_2O$ (5:4)] to give pure loganin aglucone (**1b**) (98 mg), oil. $^1H$ NMR (200 MHz, $CDCl_3$): $\delta = 1.15$ (3H, *d*, $J = 6.6$ Hz, Me), 1.53 (1H, *m*, H-6a), 2.30 (1H, *m*, H-6b), 1.95 (1H, *m*, H-8), 1.95 (1H, *m*, H-9), 3.17 (1H, *m*, H-5), 3.72 (3H, s, $CO_2Me$), 4.13 (1H, *m*, H-7), 4.98 (1H, *d*, $J = 4.9$ Hz, H-1), 7.41 (1H, *d*, $J = 1.3$ Hz, H-3). $^{13}C$ NMR (50 MHz, $CDCl_3$): $\delta = 13.3$ (C-10), 31.5 (C-5), 41.4 (C-6), 42.0 (C-8), 46.2 (C-9), 51.4 (OMe), 74.5 (C-7), 95.6 (C-1), 115.5 (C-4), 151.7 (C-3), 168.2 (C-11).

Loganin aglycone-1-monoacetate **1**(**c**). Loganin aglycone (43 mg) was treated with DABCO (6 mg) and shaken to dissolve in THF (2.5 ml). $Ac_2O$ (0.2 ml) was added and the solution stirred at RT overnight. Removal of solvent, followed by separation on a small column (silica gel) eluted with $CHCl_3$, followed by $CH_2Cl_2$–$Et_2O$ (7:3) gave a pure oil, (35.4 mg). EI–MS *m*/*z* (rel. int.): 270 ($M^+$, 2.1), 210 (M-OAc, 19), 192 (M–OAc–$H_2O$, 22), 150 (21), 139 (100), 125 (24), 97 (19). $\delta$ H NMR (200 MHz, $CDCl_3$): $\delta = 1.15$ (3H, *d*, $J = 6.8$ Hz, Me), 1.80 (1H, *m*, H-6a), 2.11 (3H, *s*, OCOMe), 2.20 (2H, *m*, H-8, H-9), 2.32 (1H, *m*, H-6b), 3.18 (1H, *m*, H-5), 3.73 (3H, *s*, $CO_2Me$), 4.14 (1H, *m*, H-7), 6.07 (1H, *d*, $J = 3.5$ Hz, H-1), 7.32 (1H, *d*, $J = 0.8$ Hz, H-3).

### *3.5. Studies on sweroside **2a** and its derivatives*

Pure sweroside (**2a**) crystals softened at 82°C and melted (decomp.) at 120°C, (lit. Ma et al., 1994, 106–109°C), $[\alpha]_D^{25} = -164.6°$ ($c = 0.005$, MeOH), (lit. Ma et al., 1994, $-157.6°$). The $^1H$ NMR spectrum in $CD_3OD$ was identical to that recorded by Ma et al. (1994).

Sweroside (**2a**) (280 mg) was hydrolysed as described for loganin and afforded the aglycone (**2b**) (after separation on a column) (73 mg) as an unstable oil. $^1H$ NMR (500 MHz, $CDCl_3$): $\delta = 1.52$ (1H, *m*, H-6a), 1.96 (1H, *m*, H-6b), 2.02–2.11 (1H, *m*, H-9), 2.70 (1H, sym. *m*, H-5), 4.32 (1H, *m*, H-7a), 4.48 (1H, *m*, H-7b), 5.21–5.28 (2H, *m*, H-10), 5.44 (1H, broadened s, H-1), 5.73 (1H, *ddd*, $J = 17.3$, 10.3, 9.2 Hz, H-8), 7.63 (1H, *d*, $J = 2.1$ Hz, H-3). $^{13}C$ NMR (125 Hz, $CDCl_3$): $\delta = 26.7$ (C-6), 28.5 (C-5), 46.9 (C-9), 68.5 (C-7), 94.4 (C-1), 104.8 (C-4), 119.8 (C-10), 134.7 (C-8), 153.6 (C-3), 166.3 (COO-). EI–MS *m*/*z* (rel int.): 120 (.5), 85 (62), 83 (100).

### *3.6. Sweroside aglucone-1-acetate (**3**)*

Sweroside aglucone (**2b**) (above, 45 mg) was treated with DABCO (6.2 mg), THF (2.5 ml) and acetic anhydride as detailed for loganin aglycone to give the 1-acetory derivative (**3**) (31 mg), mp 112–113°C, (obtained only after several recrystallisations from 95% EtOH), $[\alpha]_D^{20} = +36.4°$ ($c = 0.112$, $CHCl_3$); IR (KBr): $\nu_{(mu)}$: 3160, 1765, 1701, 1618, 1404, 1284, 1201, 1189, 1060. EI–MS *m*/*z* (rel int.): 238 ($M^+$, 2.4), 196 (1.4). 178 (2.6), 160 (8.5), 127 (100), 112 (2.5), 97 (8.8). $^1H$ and $^{13}C$ NMR (500 and 125 MHz, $CDCl_3$) are collected in Table 1.

For the X-ray study on (**3**) diffraction data were collected on a Nonius CAD 4 diffractometer with graphite monochromated MoK radiation. The unit cell parameters were determined by a least squares fit. The structure was resolved by direct methods. Further details for 1-acetoxy sweroside aglucone (**3**) are: $C_{12}H_{14}O_5$. Final *R* value 0.0470, colourless orthorhombic crystals, cell parameters (A°) $a = 7.9637$ (7), $b = 10.2678$ (8), $c = 29.326$ (2), crystal dimensions (mm) 0.58×0.46×0.35, space group $P2_12_12_1$, $V(A^{\circ 3})$ 2398.0(3), $Z = 8$. (Fig. 1).

The dihedral angles H-1/C-1–C-9/H-9 and H-5/C-5–C-9/H-9 were 177.9 and 174.5° respectively, in good accord with the stereochemistry predicted by the $^1H$ NMR data.

## Acknowledgements

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## References

Battersby, A.R., Hall, E.S., Southgate, R., 1969. The structure, stereochemistry and biosynthesis of loganin. J. Chem. Soc. (C) 712–728.

Bianco, A., 1994. Recent developments in iridoids chemistry. Pure and Appl. Chem. 66, 2335–2338.

Boros, C.A., Stermitz, F.R., 1990. Iridoids. An updated review. J. Nat. Prod. 53, 1055–1147.

Dunstan, W.R., Short, F.W., 1883. Isolation of loganin from the fruit of *Strychnos nux-vomica*. Pharm. J. 14, 1025–1028.

El-Naggar, L.J., Beal, J.L., 1980. Iridoids. A review. J. Nat. Prod. 43, 649–707.

Foderaro, T.A., Stermitz, F.R., Hope, H., 1992. (5αH)-6-Epidihydrocornin, the first known iridoid glycoside with a *trans*-fused ring system. Tetrahedron Letters 33, 2953–2954.

Foderaro, T.A., Stermitz, F.R., 1992. Iridoid glycosides from Penstemon species: a C-5, C-9 *trans* iridoid and C-8 epimeric pairs. Phytochemistry 31, 4191–4195.

Hutchings, A., 1996. Zulu medicinal plants. University of Natal Press, Pietermaritzburg, South Africa.

Inouye, H., Ueda, S., Nakamura, Y., 1970. Secoiridoid-glucoside aus *Swertia japonica*. Isolierung von fünf secoiridoid glucosiden sowie die strukturaufklarung des swerosids, des swertiamarins und des gentiopicrosids. Chemical and Pharmaceutical Bulletin 18, 1856–1865.

Kojic-Prodic, B., Spek, A.L., Van der Sluis, W.G., Labadie, R.P., 1985. Structure of decentapicrin A. Acta Cryst. Sect. C-Cryst. Structure. Commun. C41, 379–383.

Ma, W.G., Fuggati, N., Wolfender, J.L., Hostettmann, K.H., Yang, C.R., 1994. Rhodentoside A, a new type of acylated secoiridoid glycoside from *Gentiana rhodentha*. Helv. Chim. Acta 77, 1660–1671.

Van der Sluis, W.G., Labadie, R.P., 1981. Secoiridoids and xanthones in the genus Centaurium. Planta Medica 41, 150–160.

Van Wyk, B.E., Van Oudshoorn, B., Gericke, N., 1997. Medicinal Plants of South Africa. Briza Publications, Pretoria, South Africa.

Watt, J.M., Breyer-Brandwijk, M.G., 1962. Medicinal and Poisonous Plants of Southern and East Africa. E and S Livingstone, London.