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Secogalioside, an Iridoid Glucoside from *Galium album* Mill. and ^{13}C NMR Spectra of some Seco-iridoid Glucosides

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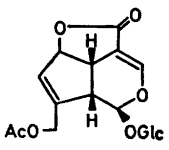
The known glucosides asperuloside and arbutin have been isolated from *Galium album*, in addition to a new iridoid glucoside, named secogalioside. The structure and the absolute configuration have been determined by spectroscopic methods, and by relating secogalioside to the glucoside sweroside, of known absolute configuration. Three other species of *Galium* and a hybrid, *G. verum* × *album*, were found to be devoid of the new compound.

^{13}C NMR spectra of some seco-iridoid glucosides and glucoside acetates have been recorded. Assignments of signals have been made by using general principles, as well as coupled and selectively decoupled spectra.

The genus *Galium* (Rubiaceae) is a known source of iridoid glucosides.[1–3] Asperuloside (*1*), monotropein (*2*) and "Galium glucoside" (*3*) have been reported from the genus. We have found that *Galium album* Mill. (= *G. mollugo* auct) contains asperuloside (*1*) and arbutin (*4*). A third compound, present in amounts equal to those of *1* and *4*, is a novel seco-iridoid glucoside ($C_{17}H_{24}O_{12}$), isolated as an amorphous foam. It is shown that the compound, for which we propose the name secogalioside, has the structure *5*.



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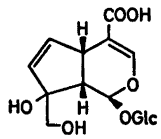

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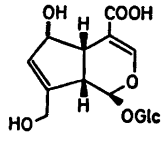

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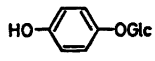

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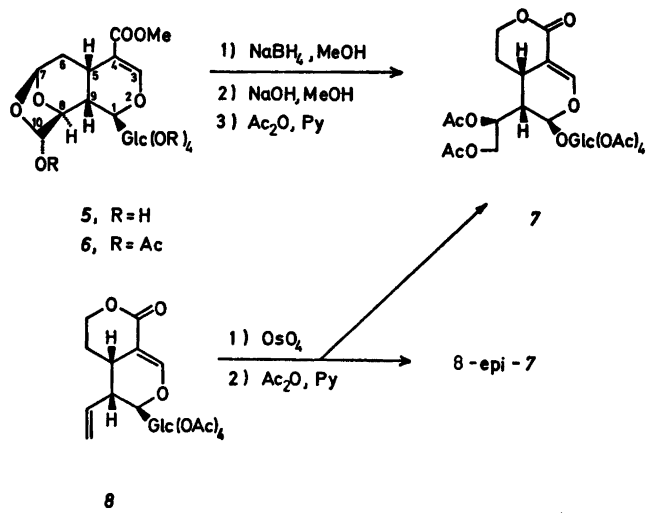
The ^1H NMR spectrum of *5* (Table 1) exhibited absorptions typical of iridoid glucosides. Signals at δ 7.58, 5.59 and 3.73 could be assigned to H-3, H-1 and COOMe, respectively. H-1 appeared as a doublet ($J_{1,9}$ 8 Hz), and successive decoupling experiments led to assignments of H-9, H-5, H-6ax, H-6eq, H-7 and H-8 (see Experimental). A singlet at δ 5.65 could not be assigned at this stage. However, a structure resembling that of 7-β-morroniside [4–6] (*9a*) was likely.

The ^1H NMR spectrum of the crystalline pentaacetate *6* resembled that of *5*; downfield shifts were observed only for the glucose protons (2′ – 6′) and for the unassigned proton (δ 0.64), indicating a position of the latter on a hemiacetalic centre.

The ^{13}C NMR spectra of *5* and *6* (see Table 2) showed four absorptions in the region 90 – 105 ppm, probably arising from as many acetalic (or hemiacetalic) centres in the molecules, *viz.* C-1, C-7, C-1′ and the unassigned carbon atom (see the discussion).

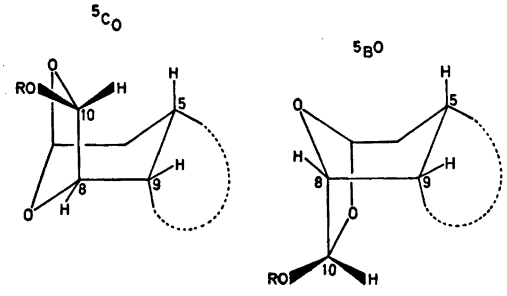
The information obtained seemed best to be accommodated in a structure such as *5*. The absolute configurations at C-1, C-5, C-9 and the presence of a β-D-glucopyranose moiety were established by relating *5* to sweroside tetraacetate (*8*), of known absolute configuration,[7] in the following way: Treatment of *5* with sodium borohydride in methanol converted the masked aldehyde functions at C-7 and C-10 to the corresponding primary alcohols. Saponification followed by neutralization



induced lactone formation, and the crude mixture was acetylated to give, after separation, the crystalline hexaacetate *7*.

Sweroside tetraacetate (*8*) by treatment with osmium tetroxide, yielded an inseparable mixture of the two epimeric diols. However, separation was achieved by chromatography of the acetylated product and two crystalline hexaacetates were obtained. One of these was indistinguishable from *7* derived from *5*.

Only the configurations at C-8 and C-10 now remained to be established. Models showed that two different structures were equally compatible with the information obtained from ^{1}H NMR spectra. The tetrahydropyrane ring could occupy either the ^5C_O or the $^5B^O$ conformation (see formulas below), having C-10 above or below the ring, respectively. These two possibilities imply different absolute configurations at C-8 and because of $J_{8,10}=0$ Hz, also at C-10. By inspection of the two possible



structures, it could be seen that in the ^5C_O conformation H-10 was close in space to H-5 and far removed from H-1, and *vice versa* with $^5B^O$. A ^{1}H NOE experiment carried out on *6*, showed that by irradiation of the H-5 signal an enhancement (15 %, using H-3 and H-7 as references) of the signal intensity of H-10 was obtained. On irradiation of H-1 no enhancement of the H-10 signal was observed.

Thus, the conformation of the tetrahydropyrane ring must be ^5C_O, and consequently the absolute configuration of secogalioside and its derivatives is that shown in *5*, *6* and *7*.

In solution morroniside is a mixture of 7-anomers (*9a* and *9b*, see below). Secogalioside, with a hemiacetalic centre at C-10, should have a similar possibility of anomerization. Nevertheless, the NMR spectra of *5* show the presence of only one anomer and only one acetate is obtained upon acetylation.

Three other *Galium* species and a hybrid have been investigated for the presence of secogalioside, using ^{1}H NMR spectra (Table 1). Only *G. album* (from two locations) contains the compound.

^{13}C NMR SPECTRA

Data of this type have been published only for a few acetates of cyclopentanoid iridoid glucosides.[8] We here report data for the secoiridoid glucosides *5*, *9*, *11*, and *13*, together

Table 1. Examination of *Galium* species for asperuloside (A) and secogalioside (S).

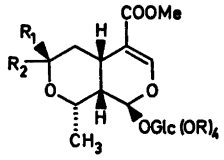
Plant	Location [a]	Voucher No.	S	A
G. album L.	Lundtofte	IOK 18/73	+ [b]	+
(=G. mollugo auct.*)*	Odden	IOK 20/75	+	+
G. verum L.	Odden	IOK 42a/73	− [c]	+
G. verum × *album*	Odden	IOK 43/73	−	+
G. aparine L.	Odden	IOK 15/73	−	+
G. palustre L.	Odden	IOK 46/73	−	+
G. uliginosum L.	Klint	IOK 45/73	−	−

[a] At Sjælland, Denmark. [b] +: present. [c] −: not detected.

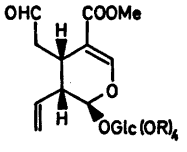
with the (partly corresponding) acetates *6*, *10a*, *10b*, *12*, and *14*. The results are shown in Table 2.

Assignments have been made using principles described by Shilling *et al.*[8] Extensive use has been made of ^1H coupled spectra together with selective decoupling experiments in order to confirm the assignments.

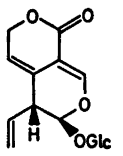
C-1 of the glucoside absorbs between 98.5 and 96.2 ppm; somewhat lower in the acetates. The chemical shifts of C-1′ are found at 100.4 – 99.5 ppm, and 2 – 4 ppm lower in the acetates. No safe assignments of these atoms can thus be made using shift values only. However, C-1′ of β-glucosides consistently shows $^1J_{C,H}$ values within 161 ± 1 Hz.[9] By using this observation we have been able to distinguish between C-1 ($^1J_{C,H}$ = 168 – 177 Hz) and C-1′ ($^1J_{C,H}$ = 161 – 164 Hz). Secogalioside (*5*) and its acetate (*6*) show four absorptions between 95 and 104 ppm; *i.e.* from carbons 1, 7, 10, and 1′. The assignments in *6* were based on selective proton decouplings. Shift values and coupling constants were then used to assign the signals of *5* in analogy to *6*. In aqueous solution morroniside is a mixture of the anomeric compounds *9a* and *9b*, in the proportion of 1:3. Only the B-ring carbon atoms of *9a* and *9b* show different chemical shifts and intensities; thus it is possible to distinguish between (i) the C-7 absorptions *versus* C-1 and C-1′, and (ii) the signals from each of the two anomers.



9a: R = R1 = H, R2 = OH
9b: R = R2 = H; R1 = OH
10a: R = Ac; R1 = H; R2 = OAc
10b: R = Ac; R1 = OAc; R2 = H



11: R = H
12: R = Ac



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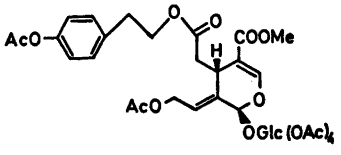

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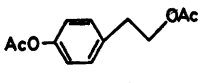

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Table 2. ^{13}C NMR chemical shifts [a] and $^{1}J_{C,H}$ coupling constants [b] for seco-iridoid glucosides and acetates.

C-atom	Compound Secologanin		α-Morroniside		β-Morroniside		Secogalioside		Gentiopicroside	10-OH-Ligstroside Ac_5
	11	*12*	*9a*	*10a*	*9b*	*10b*	*5*	*6*	*13*	*14*
1	97.6 (174)	95.4	96.2 (170x)	94.5 (173)	96.2 (170x)	94.2 (175)	96.3 (174x)	95.7 (168x)	98.5 (177)	92.5 (168)
3	154.0 (194)	150.7	154.9 (192)	151.9 (192)	154.9 (192)	151.9 (192)	151.1 (192)	152.2 (192)	150.4 (196)	152.5 (192)
4	109.6	109.1	110.2	109.9	110.9	110.7	109.8	109.7	104.4	108.1
5	27.5 (134x)	25.1	31.1 (130x)	30.0 (130)	26.8 (130x)	26.1	25.2 (132)	24.6 (132x)	125.2	30.8 (135x)
6	44.6	43.5 [c]	36.1 (130x)	32.9 (130x)	33.4 (130x)	31.4 (130x)	34.6 (130x)	34.4 (130x)	117.8 (169)	39.8
7	206.8 (177)	199.7	95.9 (160x)	93.5 (160x)	91.6 (170)	91.1 (172)	103.3 (174)	103.8 (174)	71.2 (154)	*ca* 170
8	133.8 (156)	131.8	73.8 [c]	73.3 (145x)	65.9	67.1	78.9 (160)	77.2 (162x)	133.9 (161)	124.0 (162)
9	44.6 (132x)	43.1 [c]	38.7 (130)	38.8 (130x)	39.3 (130)	39.2 (129)	37.2 (130x)	36.8 (132x)	45.4 (133)	130.9
10	121.6 (161)	120.6	19.6 (127)	18.8 (127)	19.6 (127)	18.8 (127)	96.7 (176x)	95.1 (178)	119.5 (160)	60.4 (148)
C=O	169.8	166.1	169.8	165.8	169.8	165.9	169.5	166.6	167.5	165.9
OMe	52.6 (147)	51.1	52.6 (147)	51.2 (146)	52.6 (147)	51.1 (146)	52.7 (147)	51.3 (147)	—	51.4 (147)
1′	99.6 (161)	95.4	99.5 (162)	96.5 (163)	99.5 (162)	96.6 (163)	100.4 (162)	98.4 (162)	99.6 (162)	96.8 (164)
2′	73.5	70.4	73.6	70.8	73.6	70.9	73.6	71.0	73.3	70.6
3′	76.6 [c]	72.0	76.8 [c]	72.4	76.8 [c]	72.5	76.7 [c]	72.7	76.5 [c]	72.3
4′	70.5	67.9	70.5	68.3	70.5	68.4	70.4	68.4	70.3	68.0
5′	77.2 [c]	72.0	77.1 [c]	71.8	77.1 [c]	72.0	77.1 [c]	72.1	77.1 [c]	72.1
6′	61.6	61.4	61.6	61.6	61.6	61.5	61.6	61.9	61.6	61.5

[a] In ppm from TMS; ± 0.1. [b] In Hz; ± 2 Hz; if the value is followed by an "x": ± 5 Hz. [c] Interchangeable, in the same vertical column.

With the aid of selective proton decoupling we have assigned C-9 and C-5 of *6* and found the former to absorb at the lowest field, in agreement with the results (two examples) of Shilling *et al.*[8] This criterion was then used for the corresponding assignments of the remaining compounds. Among the high field absorptions, that of C-6 is easily recognized, since this carbon atom is the only one which is coupled to two protons. An exception is *14*, which contains a *p*-acetoxyphenyl-ethyl moiety. The absorptions arising from this part

of the molecule were assigned by comparison with the spectrum of tyrosol diacetate (*15*).* The remaining absorptions arising from methylene carbons, *viz.* 60.4 ppm and 39.8 ppm were assigned to C-10 and C-6, respectively. Due to conjugation with the carboxymethyl group, C-3 and C-4 here absorb at lower field than in decarboxylated compounds.[8]

Except for C-1′, the absorptions arising from the β-D-glucopyranose moiety in both the glucosides and the acetates are consistently very close to published values [8,9] for analogous compounds and apparently not seriously affected by the nature of the aglucone. ^{13}C NMR thus appears to be a complementary way to identify the sugar part of simple iridoid glucosides.

EXPERIMENTAL

Melting points are corrected and determined in a capillary tube in a heated bath. ^1H NMR spectra were recorded at 90 MHz on a Bruker HX-90E instrument in D_2O or $CDCl_3$ with DSS or TMS, respectively, as internal references. ^{13}C NMR spectra were obtained on a Bruker WH-90 instrument at 22.63 MHz using D_2O or $CDCl_3$ with dioxan (5 %, $\delta = 67.4$) or TMS (1 %), respectively, as internal references. Samples were prepared as *ca.* 25 % solutions in 10 mm tubes. A pulse width of 20 μs (90° flip angle), 1 s repetition time, spectral width 5000 Hz, and 8K data points were generally used, resulting in a digital resolution of 1.22 Hz/pt. Broad band noise decoupling was used for the decoupled spectra, and for the selective decoupling experiments a decoupling power giving $\gamma H_2/2\pi = ca.$ 1000 Hz was used. Coupled spectra were recorded in the gated mode using a decoupling time of 1.1 times the sampling time. The ^1H NOE experiment was made on the same instrument at 90 MHz in the FT mode using gated technique as described by Rowan *et al.*[10] 50 Scans (90° pulses – 8 μs) were accumulated with the homodecoupler positioned at the H-5 resonance; a reference spectrum was obtained after moving the homodecoupling frequency 200 Hz upfield. Subtraction of the two spectra indicated the proton signal enhanced by the NOE and showed a very good cancellation of the other signals, except for the irradiated proton. A quantitative measure of the enhancement was obtained from the computer output.

*	α	β	1″	2″	3″	4″
15:	64.1	33.9	135.0	129.2	121.0	148.9
14:	64.8	34.2	134.9	129.5	121.3	149.1

Analyses were performed at Bernhardt, Mikroanalytisches Laboratorium, West Germany.

Isolation of glucosides. Fresh plant material (510 g, collected near the laboratory in July 1973) was homogenized in EtOH and worked up as previously described.[11] The resulting Me_2CO eluate (3.0 g) was crystallized from EtOH to give *asperuloside* (*1*, 925 mg, m.p. 126–129 °C) identified by its ^1H NMR spectrum. The mother liquors were passed through activated carbon to remove a fluorescent compound (if this is omitted, difficulties arise in the following separations). TLC (SiO_2, EtOAc:Bz:EtOH, 4:1:1) gave as the faster moving fraction *arbutin* (*4*, 755 mg, 0.15 %) identified by its ^1H NMR spectrum. The slower band appeared to be a mixture of two compounds and was chromatographed as above ($CHCl_3$:MeOH, 3:1) to give a further amount of *1* (205 mg, total 0.2 %) as the faster moving fraction, together with *secogalioside* (*5*, 650 mg, 0.13 %). Rechromatography, followed by passing a solution of the compound in MeOH through activated carbon, gave the analytical specimen as a colourless syrup $[\alpha]_D^{21} -82°$ (*c* 0.2, EtOH); UV [abs. EtOH (log ε)] : 238 (4.05). ^1H NMR spectrum: δ 7.58 (s, H-3), 5.80 (dd, *J* 2 and 0.5 Hz, H-7), 5.65 (s, H-10), 5.59 (d, *J* 8 Hz, H-1), 4.60 (d, *J* 1.5 Hz, H-8), 3.73 (s, OMe), 2.97 (m, *J* 5, 5 and 12 Hz, H-5), *ca.* 2.1 ($H\text{-}6_{eq}$ and H-9), 1.49 (m, *J* 0.5, 12 and 14 Hz, $H\text{-}6_{ax}$). Anal. $C_{17}H_{24}O_{12}$: C, H.

Secogalioside pentaacetate (*6*). Acetylation of *5* (170 mg) with Py (1 ml) and Ac_2O (0.5 ml) for 2.5 h gave crude *6*. Chromatography (TLC, SiO_2; EtOAc:$CHCl_3$, 1:1) followed by crystallization from EtOH (19 ml) gave the pure, crystalline compound (130 mg) m.p. 171–172.5 °C, $[\alpha]_D^{24} -73°$ (*c* 0.3, $CHCl_3$); UV [abs. MeOH (log ε)]: 236 (4.04). ^1H NMR spectrum: δ 7.46 (s, H-3), 6.29 (s, H-10), 5.77 (dd, *J* 0.5 and 1.5 Hz, H-7), 5.44 (d, *J* 9 Hz, H-1), 4.68 (d, *J* 2 Hz, H-8), 3.73 (s, OMe), 3.09 (m, *J* 5, 6 and 11.5 Hz, H-5), 2.26 (m, *J* 1.5, 6 and 14 Hz, $H\text{-}6_{eq}$), 1.88 (m, *J* 2, 5 and 9 Hz, H-9), 1.37 (m, *J* 0.5, 11.5 and 14 Hz, $H\text{-}6_{ax}$). Anal. $C_{27}H_{34}O_{17}$: C, H.

Conversion of secogalioside to 7. *5* (144 mg) was dissolved in MeOH (10 ml) and $NaBH_4$ (40 mg) was added. After 5 min TLC showed that the reaction was not complete, and additional $NaBH_4$ (20 mg) was added after 30 min. When no more *5* was present (TLC, 45 min), water (5 ml) and KOH (100 mg) was added. Stirring was continued for 10 min, whereafter the mixture was neutralized with AcOH, and taken to dryness. Acetylation of the crude mixture gave, after chromatography, the lactone hexaacetate *7* (37 mg). *7* was crystallized from Et_2O: m.p. 171–172 °C; $[\alpha]_D^{24} -110°$ (*c* 0.4, $CHCl_3$); UV [abs. MeOH (log ε)] : 243 (3.92). ^1H NMR spectrum: δ 7.54 (d, *J* 2.5 Hz, H-3), 5.56 (d, *J* 1.5 Hz, H-1), *ca.* 3.0 (m, H-5), 2.50 (dt, *J* 6.5 and 1.5

Hz, H-9) and 1.97–2.12 (6×AcO); the remaining signals were in accord with those expected from *7*. Anal. $C_{28}H_{36}O_{17}$: C, H.

OsO_4 treatment of sweroside tetraacetate (8). *8* (180 mg) was partly dissolved in ether (50 ml) and Py (0.2 ml). OsO_4 (86 mg) in ether was then added at room temperature during 30 min with stirring. After stirring for 5 days a brown precipitate had formed, but solid *8* was apparently still present. EtOH (10 ml) was added, and the osmate cleaved by bubbling H_2S through the mixture for 1.5 h to give a black precipitate. After filtration, the solvents were removed *in vacuo*. The residue (153 mg) was chromatographed (EtOAc) to give unreacted *8* (31 mg) and a slower moving fraction (69 mg). According to the ^1H NMR spectrum this fraction contained 2 iridoids (peaks at 7.60 and 7.50, H-3) in a proportion of *ca.* 3:2, but it proved impossible to separate them. However, after acetylation complete separation was achieved by chromatography using 4 elutions with Et_2O. The faster moving band (19 mg) was crystallized from Et_2O and was identical (m.p., m.m.p. and ^1H NMR) with *7* derived from secogalioside. The other fraction (35 mg) gave: 8-epi-*7*, m.p. 133.5–134.5 °C; $[\alpha]_D^{24}$ −97° (*c* 0.2, $CHCl_3$); UV [abs. MeOH (log ε)]: 244 (3.87); ^1H NMR spectrum: δ 7.54 (d, *J* 2.5 Hz, H-3), 5.65 (d, *J* 1.5 Hz, H-1), *ca.* 3.0 (m, H-5), 2.34 (dt, *J* 6 and 1.5 Hz, H-9) and 1.95–2.14 (6×AcO). Anal. $C_{28}H_{36}O_{17}$: C, H.

Qualitative examinations of crude plant extracts were done by ^1H NMR spectroscopy. An absorption at 7.58 (in D_2O) indicated the presence of secogalioside, one at 7.46 the presence of asperuloside. The results are shown in Table 3.

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