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^{13}C AND ^{1}H NMR SPECTROSCOPY AS A TOOL IN THE CONFIGURATIONAL ANALYSIS OF IRIDOID GLUCOSIDES

SØREN DAMTOFT, SØREN ROSENDAL JENSEN and BENT JUHL NIELSEN

Department of Organic Chemistry, The Technical University of Denmark, DK-2800 Lyngby, Denmark

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Key Word Index—Iridoid glucosides; epimers; ^{13}C and ^{1}H NMR spectra; simulated spectra; novel iridoids; revision of structure; tecomoside; ajugol; myoporoside; Horeau's method.

Abstract—The ^{13}C NMR data of 51 iridoid glucosides or glucoside acetates are tabulated. The collection includes 20 pairs of C-6, C-7 or C-8 epimers. Three parameters in using the data for the configurational assignment of 6-*O*-substituents are given. The chemical shift for C-9 in a range of substituted compounds is shown to be numerically related to the stereochemistry at C-8. This allows the determination of the configuration at this centre for most types of substitution patterns by calculation of the C-9 shift using increments for each substituent. Such increments are given for 25 substituents in three different solvents. A method for simulation of spectra of unknown iridoid glucosides is presented. By this method, the structures of five novel iridoid glucosides have been elucidated, and that of tecomoside has been revised. The methods used to assign the configurations to C-6 and C-8 epimeric iridoid glucosides by ^{1}H NMR spectroscopy are discussed and a table with selected data is presented. It is suggested that the structures in the literature for ajugol and myoporoside should be interchanged. Consequently, Horeau's method has failed in these instances. Finally, the differences in the ^{13}C NMR spectra of pairs of C-6 and C-8 epimeric iridoid glucosides have been interpreted as originating from *cis*/*trans*-interactions.

INTRODUCTION

The advent of NMR spectroscopy has made it progressively easier to determine the structure of natural products. Thus access to ^{1}H NMR and ^{13}C NMR spectra of iridoid glucosides has made it possible to determine their substitution patterns. However, establishing the relative configuration at the cyclopentane ring centres (C-6, C-7 and C-8) in substituted iridoids has been difficult by spectroscopy alone, mostly due to the flexibility of the ring. Coupling patterns in the ^{1}H NMR spectra have been used with some success, particularly when all coupling constants in the cyclopentane ring are considered [1, 2]. It has recently been demonstrated [3–5] that significant differences exist in the ^{13}C NMR spectra of 6-hydroxy or 8-hydroxy epimers. The differences are most conspicuous in positions β and γ to the epimeric centres. However, spectra for only a few such pairs have been published. We now wish to present data for a larger variety of substitution patterns. In addition, methods are provided for assigning relative configurations to C-6 and C-8 by spectroscopic means alone.

RESULTS AND DISCUSSION

1. *Notes on methodology*

Solvents. In the present work, D_2O has been used as the solvent for glucosides as it is inexpensive and easy to remove. Others have used d_4-methanol [4], d_5-pyridine [6] and d_6-dimethylsulfoxide [7]. However, when comparing spectra of compounds recorded in different solvents, one may expect rather large and irregular solvent shifts. We recommend that D_2O, whenever possible, is used as the standard solvent for glycosides. The spectra of acetates were recorded in $CDCl_3$.

NMR standards. Dioxane is the standard reference compound for ^{13}C NMR spectra recorded in D_2O, while TMS is used in organic solvents. Due to difficulties in interpreting proton-coupled spectra caused by overlapping dioxane signals, we now record spectra without this reference. We find that the glucopyranosyl signals, except for C-1′, in a large number of compounds are remarkably constant (see Table 1). In fact, the C-6′ signal at 61.5 ppm ($\pm$0.6 ppm) serves as a reliable internal reference, making the spectrum more independent of effects from solvents, concentration and temperature. In the present work, all spectra (Table 1) have been aligned by adjusting the C-6′ signal to 61.5 ppm. This approach is justified by the constancy of the adjusted methyl ester signals from compounds **1**–**20** which all fall within the narrow range of 52.7 $\pm$ 0.2 ppm for the glucosides and 51.2 $\pm$ 0.4 ppm for the acetylated derivatives.

Proton-coupled spectra. 'Off-resonance decoupling' has been used in assigning signals [4, 5], whereas fully coupled spectra have not been extensively used for iridoids. We have, however, in an earlier paper [8] shown that the signals from C-1 and C-1′ can be distinguished, the one bond coupling constant $J_{C\text{-}1,H\text{-}1}$ being consistently larger (4–15 Hz) than the constant $J_{C\text{-}1',H\text{-}1'}$. This is also seen in the present work (Table 1). From the table it also appears that the one bond coupling constant for C-8 is consistently smaller than those of C-5 and/or C-9: $J_{C\text{-}5,H\text{-}5}$: 133–140 Hz; $J_{C\text{-}8,H\text{-}8}$: 123–136 Hz; $J_{C\text{-}9,H\text{-}9}$: 130–140 Hz.

Assignment of spectra. Most signals in a ^{13}C NMR spectrum of an iridoid glucoside are readily assigned by

Table 1. ^{13}C NMR data of iridoid glucosides and acetates. Spectra of glucosides were recorded in D_2O (glucosides) or in $CDCl_3$ (acetates) at 22.6 MHz except for **5a**, **6a**, **7a**, **19a**, **20a**, **23**, **23a** (67.9 MHz). No internal standard was used (see text). obs, obscured

Carbon atom	**1**	**1b**	**1a**	**2***	**2b**	**2a**	**3**	**3a**	**4**	**4a**	**5**
1	96.5 *d* (172)	95.1	93.9 *d* (173)	101.3	100.0	98.4 *d* (173)	96.4 *d* (175)	94.0 *d* (176)	100.6 *d* (175x)	97.5 *d* (173)	96.0 *d* (176)
3	153.0 *d* (191)	150.2	150.8 *d* (193)	155.7	154.3	152.5 *d* (191)	153.8 *d* (195)	151.5 *d* (193)	156.2 *dd* (193/3)	153.0 *d* (193)	154.8 *dd* (194/5)
4	110.1 *s*	111.1	108.8 *s*	106.8	—	106.5 *s*	109.0 *s*	107.9 *s*	106.2 *t*(7)	105.6 *s*	112.2 *s*
5	41.8 *d* (139)	41.4	38.6 *d* (136)	41.9	41.3§	39.1 *d* (136x)	41.5§ *d* (139)	38.5 *d* (140)	42.8 *d* (134)	39.9 *d* (134)	73.3 *s*
6	77.7 *d* (148)	76.9	78.3 *d* (155x)	74.6	74.0	76.7 *d* (160)	76.4 *d* (145x)	77.4 *d* (154)	73.9 *d* (153x)	76.1 *d* (160)	76.2 *d* (148x)
7	41.8 *t* (128)	41.4	39.3 *t* (129)	41.9	41.7§	39.7 *t* (136x)	35.7 *t* (133)	33.2 *t* (133)	37.8 *t* (132)	35.5 *t* (131)	39.8 *t* (130)
8	34.0 *d* (132)	33.0	32.7 *d* (126x)	34.4	33.7	33.8 *d* (130x)	43.8 *d* (131)	42.4 *d* (136x)	45.0 *d* (133)	43.5 *d* (133)	30.8 *d* (133)
9	47.1 *d* (132)	46.6	46.6 *d* (135)	46.2	45.6	45.5 *d* (134)	42.8§ *d* (139)	42.7 *d* (136x)	42.1 *d* (139)	42.1 *d* (140)	54.8 *d* (134)
10	19.9 *q* (125)	18.8	*ca* 20	21.3	*ca* 20	*ca* 20 *q*	177.9 *s*	173.7 *s*	178.7 *s*	174.6 *s*	19.6 *q* (126)
11	170.5 *s*	—	166.5 *s*	170.8	—	167.5 *s*	170.2 *s*	165.7 *s*	170.6 *s*	166.7 *s*	169.4 *s*
OMe	52.7	51.2	51.1	52.5	51.1	51.0	53.6 52.8	52.0 51.2	53.6 52.8	52.1 51.3	52.8
1′	99.4 *d* (161)	95.9	95.6 *d* (163)	99.9	97.1	96.3 *d* (164)	99.4 *d* (163)	95.8 *d* (164)	100.2 *d* (161x)	96.5 *d* (163)	99.7 *d* (164)
2′	73.5	70.3	70.3	73.5	70.7	70.6	73.4	70.4	73.5	70.7	73.3
3′	76.5	72.0	72.0	76.6	71.7	71.7	76.4	72.2	76.6	72.0	76.2
4′	70.4	68.0	68.0	70.4	68.2	68.1	70.4	68.0	70.3	68.2	70.5
5′	77.2	72.2	72.2	77.1	72.5	72.2	77.2	72.3	77.1	72.5	77.3
6′	61.5	61.5	61.5	61.5	61.5	61.5	61.5	61.5	61.5	61.5	61.5

Table 1.—*Continued*

Carbon atom	**5a**	**6**	**6a**	**7a**	**8**	**8a**	**9**	**9a**	**10**	**10a**	**11**
1	95.7 *d* (175x)	101.3	99.8 *d* (175)	94.8	95.2 *d* (177)	93.7 *d* (176)	98.0 *d* (172)	95.1 *d* (174x)	96.3 *d* (175)	94.5 *d* (172)	98.6 *d* (168)
3	152.0 *d* (194)	156.7	154.3 *d* (191)	152.6	153.7 *d* (195)	151.8 *d* (194)	152.2 *d* (193)	149.2 *d* (192)	152.6 *d* (193)	150.0 *d* (193)	153.2 *d* (191)
4	111.7 *s*	110.7	109.4 *s*	112.3	110.2 *s*	108.1 *s*	113.2 *s*	112.9 *s*	113.8 *s*	113.0 *s*	111.8 *s*
5	73.6 *s*	80.7	79.4 *s*	73.7	40.4 *d* (141)	38.0 *d* (142)	33.6 *d* (133)	32.3 *d* (135)	32.6 *d* (137)	32.1 *d* (137x)	35.0 *d* (135x)
6	78.1 *d* (154x)	79.3	79.4 *d* (145x)	77.7	76.4 *d* (143x)	76.8 *d* (148x)	32.2 *t* (131)	30.8§ *t* (132x)	31.6§ *t* (130)	30.9§ *t* (131)	32.4 *t* (130x)
7	36.1 *t* (135x)	40.0	39.6 *t* (135x)	37.4	44.1 *t* (129)	42.4 *t* (129)	33.2 *t* (130x)	32.7§ *t* (130x)	33.1§ *t* (130x)	32.5§ *t* (130x)	27.8 *t* (129x)
8	30.7 *d* (130)	34.2	33.5 *d* (133x)	30.2	81.8 *s*	78.3 *s*	35.7 *d* (130)	34.6 *d* (126)	36.1 *d* (130x)	35.2 *d* (130x)	43.1 *d* (129)
9	52.9 *d* (135)	56.0	54.1 *d* (135)	47.5	44.6 *d* (132)	44.0 *d* (135)	48.4 *d* (131)	47.9 *d* (131)	43.4 *d* (134)	42.4 *d* (135)	43.7 *d* (134)
10	21.2 *q* (128)	21.1	20.8 *q*	16.2	69.1 *t* (143)	71.1 *t* (148x)	20.1 *q* (125)	19.3 *q* (126)	16.3 *q* (126)	15.8 *q* (125)	65.9 *t* (139)
11	167.1 *s*	169.7	167.8 *s*	166.3	170.9 *s*	166.3 *s*	170.1 *s*	167.2 *s*	171.2 *s*	167.1 *s*	170.7 *s*
OMe	51.4	52.7	51.4	51.6	52.8	51.4	52.6	50.8	52.6	50.9	52.6
C-1′	96.2 *d* (160x)	99.7	96.7 *d* (163)	95.8	99.2 *d* (162)	96.2 *d* (164)	99.6 *d* (163)	95.7 *d* (163x)	99.3 *d* (163)	95.5 *d* (164)	99.9 *d* (162)
2′	70.5	73.5	70.8	70.5	73.5	70.3	73.5	70.4	73.5	70.5	73.6
3′	72.1	76.5	71.9	72.1	76.4	72.2	76.6	71.8	76.6	71.9	76.6
4′	68.1	70.4	68.3	68.1	70.4	67.9	70.3	68.0	70.4	68.1	70.4
5′	72.5	77.2	72.4	72.1	77.1	72.2	77.1	72.2	77.1	72.4	77.1
6′	61.5	61.5	61.5	61.5	61.5	61.5	61.5	61.5	61.5	61.5	61.5

Table 1.—*Continued*

Carbon atom	**11a**	**12**	**12a**	**13**	**14**	**15**	**15a**	**16**	**16a**	**17a**	**18a**
1	95.3	96.3 *d* (172)	93.9 *d* (175)	97.5 *d* (172)	96.4 *d* (174)	96.0 *d* (173)	96.5 *d* (174)	95.2 *d* (173)	93.6 *d* (171)	94.2 *d* (175)	93.2 *d* (175)
3	150.2	152.9 *d* (194)	150.4 *d* (193)	151.7 *d* (192)	152.3 *d* (194)	153.0 *d* (193)	151.0 *d* (194)	152.5 *d* (195)	150.0 *d* (194)	150.0 *d* (195)	149.7 *d* (195)
4	111.4	112.7 *s*	112.4 *s*	113.8 *s*	113.0 *s*	112.2 *s*	111.5 *s*	112.4 *s*	112.6 *s*	111.5 *s*	112.4 *s*
5	33.6	34.4 *d* (134x)	33.2 *d* (137x)	30.6 *d* (135)	29.4 *d* (139)	33.6 *d* (136x)	35.3 *d* (136)	32.5 *d* (136)	32.0 *d* (obs)	29.8 *d* (140x)	27.6 *d* (138)
6	30.8	31.1 *t* (131x)	30.5 *t* (130x)	41.2 *t* (129)	39.6 *t* (130)	30.6 *t* (130x)	30.9 *t* (135)	29.4 *t* (132x)	29.0 *t* (obs)	36.0 *t* (134)	35.0 *t* (136)
7	26.9	27.0 *t* (129)	27.1 *t* (130)	74.8 *d* (148)	78.9 *d* (146)	36.0 *t* (133x)	36.9 *t* (133x)	34.3 *t* (131)	35.0 *t* (obs)	80.3 *d* (153)	74.9 *d* (obs)
8	38.8	44.4 *d* (129)	40.2 *d* (130)	40.9 *d* (123)	43.6 *d* (131)	82.9 *s*	80.5 *s*	84.7 *s*	81.0 *s*	80.8 *s*	78.3 *s*
9	43.9	42.2 *d* (137)	41.5 *d* (137)	45.7 *d* (134)	41.7 *d* (135)	45.7 *d* (134)	45.3 *d* (135x)	50.4 *d* (130)	50.1 *d* (135)	44.5 *d* (133)	43.3 *d* (134)
10	66.9	63.4 *t* (145)	65.0 *t* (143)	12.8 *q* (124)	13.9 *q* (126)	68.6 *t* (142)	70.6 *t* (149)	66.0 *t* (142)	68.3 *t* (152x)	66.4 *t* (150)	66.6 *t* (149)
11	166.8	171.1 *s*	167.2 *s*	170.5 *s*	170.4 *s*	170.4 *s*	167.2 *s*	171.2 *s*	167.2 *s*	166.6 *s*	166.4 *s*
OMe	51.0	52.6	51.1	52.5	52.6	52.6	51.3	52.7	51.5	51.1	51.2
1′	95.9	99.4 *d* (164)	95.9 *d* (165)	99.4 *d* (161)	99.1 *d* (162)	99.8 *d* (162)	98.3 *d* (164)	99.4 *d* (161)	96.1 *d* (165)	96.7 *d* (163)	96.2 *d* (163)
2′	70.5	73.5	70.6	73.6	73.4	73.5	70.3	73.5	70.7	70.4	70.4
3′	72.0	76.5	72.1	76.5	76.5	76.5	72.3	76.4	72.3	72.1	72.1
4′	68.1	70.3	68.1	70.4	70.2	70.3	68.1	70.3	68.0	68.0	68.0
5′	72.4	77.1	72.4	77.1	77.1	77.1	72.3	77.1	72.6	72.1	72.1
6′	61.5	61.5	61.5	61.5	61.5	61.5	61.5	61.5	61.5	61.5	61.5

Table 1.—*Continued*

Carbon atom	**19a**	**20a**	**21**	**22†**	**23**	**23a**	**24**	**24a**	**25**	**26**	**27**
1	92.4 *d* (177)	98.6 *d* (176)	98.9 *d* (176x)	97.5 *d*	97.7 *d* (178)	96.1	97.3 *d* (177)	95.8 *d* (obs)	98.2 *d* (176)	96.4 *d* (175)	95.5 *d* (177)
3	151.7 *d* (193)	152.5 *dd* (193/4)	164.7 *d* (192)	164.0 *d*	164.8 *dd* (191/5)	156.9	165.6 *dd* (191/4)	157.8 *dd* (187/4)	163.9 *d* (192)	164.2 *d* (191)	164.3 *dd* (193/5)
4	107.0 *d* (5)	104.6 *t* (7)	125.3 *d* (15x)	125.3 *s*	124.7 *d* (20)	125.5	123.5 *d* (20)	125.6 *d* (20)	125.8 *d* (20)	125.0 *d* (25x)	124.7 *d* (21)
5	31.8 *d* (140)	36.4 *d* (134)	31.3 *d* (137)	30.6 *d*	73.6 *s*	73.2	73.9 *s*	73.0 *s*	28.4 *d* (136)	28.6 *d* (137)	71.0 *s*
6	58.4§ *d* (188)	60.0§ *d* (190)	30.7 *t* (133)	30.3§ *t*	37.9 *t* (132)	37.3	38.3 *t* (133)	37.6 *t* (132)	40.0 *t* (131)	28.6 *t* (133)	36.8 *t* (134)
7	59.2§ *dd* (192/7)	56.1§ *d* (192)	33.2 *t* (128x)	32.9§ *t*	31.1 *t* (134)	30.8	32.3 *t* (132)	31.9 *t* (obs)	75.0 *d* (150)	40.4 *t* (133x)	39.3 *t* (132)
8	77.7 *s*	79.3 *s*	35.5 *d* (125x)	35.6 *d*	35.1 *d* (127)	34.6	34.2 *d* (130)	33.5 *d* (128)	40.5 *d* (125)	80.2 *s*	79.0 *s*
9	42.8 *d* (135)	39.4 *d* (135)	48.4 *d* (134)	42.9 *d*	56.8 *d* (134)	55.8	51.7 *d* (135)	50.7 *d* (137)	45.3 *d* (133)	51.1 *d* (133)	60.5 *d* (131)
10	67.9 *t* (149)	67.3 *t* (147)	19.9 *q* (125)	16.0 *q*	20.0 *q* (126)	19.8	16.0 *q* (126)	16.0 *q* (125)	12.6 *q* (125)	23.7 *q* (126)	23.1 *q* (126)
11	166.2 *s*	166.6 *s*	196.0 *d* (174)	194.2 *d*	195.0 *dd* (175/5)	190.2	194.6 *dd* (175/5)	190.1 *dd* (172/5)	195.8 *dd* (174/4)	195.4 *dd* (175/5)	194.5 *dd* (177/5)
OMe	51.4	51.4									
1′	95.1 *d* (162)	99.2 *d* (164)	99.7 *d* (164)	99.0 *d*	99.8 *d* (163)	96.2	99.4 *d* (162)	95.8 *d* (obs)	99.5 *d* (163)	99.2 *d* (163)	99.4 *d* (162)
2′	70.4	70.8	73.5	73.3	73.3	70.8	73.2	70.8	73.4	73.4	73.2
3′	72.2	72.4	76.5	76.3	76.2	71.9	76.1	72.0	76.4	76.4	76.1
4′	68.3	68.2	70.4	70.2	70.4	68.1	70.4	68.1	70.3	70.3	70.4
5′	72.2	72.4	77.2	76.7	77.3	72.1	77.1	72.0	77.1	77.1	77.1
6′	61.5	61.5	61.5	61.5	61.5	61.5	61.5	61.5	61.5	61.5	61.5

Table 1.—*Continued*

Carbon atom	**28**	**29‡**	**29a‡**	**30‡**	**31‡**	**31a‡**	**32‡**	**32a‡**	**33‡**	**33a‡**	**34**
1	97.0 *d* (178)	97.3 *d* (171)	93.7	97.4 *d* (172)	95.8 *d* (172)	93.0	93.2 *d* (172)	90.9 *d* (173)	93.5	90.8	96.7 *d* (171)
3	163.9 *dd* (191/5)	134.3 *d* (190)	133.6	138.6 *d* (190)	134.9 *d* (189)	135.0	140.1 *d* (194)	139.9 *d* (190)	139.9	139.7	139.4 *d* (193)
4	125.5 *d* (20)	115.8 *s*	112.3	117.9 *s*	112.4 *s*	108.7	114.9 *s*	111.7 *s*	115.7	112.2	109.0 *d* (161)
5	71.2 *s*	38.8 *d* (135)	36.6	35.0 *d* (134)	45.5 *d* (135x)	42.3	35.7 *d* (134x)	35.6 *d* (135)	33.4	32.7	33.5 *d* (135)
6	47.9 *t* (132)	30.1 *t* (129)	27.6	29.7 *t* (128)	75.7 *d* (148)	76.8	30.0 *t* (132x)	29.5 *t* (130x)	39.9	37.0	31.7 *t* (131)
7	73.2 *d* (148x)	27.7 *t* (131)	26.4	27.6 *t* (128)	35.8 *t* (131)	32.8	27.6 *t* (131x)	26.9 *t* (131)	72.3	73.5	27.4 *t* (131)
8	40.0 *d* (127)	43.2 *d* (129)	38.0	42.7 *d* (129)	38.0 *d* (130x)	36.8	42.6 *d* (128)	38.6 *d* (133)	48.1	42.7§	42.5 *d* (129)
9	53.7 *d* (137)	44.9 *d* (133)	45.0	44.7 *d* (132)	44.0 *d* (138x)	43.5	43.5 *d* (133)	43.3 *d* (135)	41.5	41.7§	44.9 *d* (131)
10	12.8 *q* (127)	66.1 *t* (141)	67.3	66.1 *t* (142)	69.2 *t* (147x)	67.6	65.8 *t* (142)	66.8 *t* (148)	61.5	62.0	66.1 *t* (144)
11	194.9 *dd* (176/5)	15.9 *q* (127)	15.4	61.5 *t* (144)	15.4 *q* (128)	15.1	69.8 *t* (146)	68.5 *t* (146x)	69.6	68.3	
1′	99.8 *d* (163)	99.4 *d* (163)	95.4	99.5 *d* (163)	99.1 *d* (162)	95.3	102.1 *d* (161)	98.8 *d* (162)	102.0	98.6	99.3 *d* (163)
2′	73.3	73.6	70.5	73.6	73.5	70.3	74.0	70.9	73.9	70.8	73.6
3′	76.2	76.5	71.7	76.5	76.4	71.7	76.6	71.4	76.6	71.5	76.5
4′	70.5	70.3	68.1	70.5	70.4	68.0	70.4	68.0	70.4	68.0	70.4
5′	77.3	76.9	72.3	77.0	76.9	72.2	76.6	72.5	76.6	72.5	77.0
6′	61.5	61.5	61.5	61.5	61.5	61.5	61.5	61.5	61.5	61.5	61.5

* Data from [5].
† Data from [17]; some signals were reassigned.
‡ Data from [14].
§ Values in same vertical column may be reversed.

comparison with one or a few model compounds. However, depending on the substitution pattern it may be difficult to distinguish between C-6 and C-7 on the one hand, and C-5, C-8 and C-9 on the other. Without an appropriate model compound this problem can sometimes be solved by selective proton decoupling, which in the present work has been used to assign the spectra of compounds **1**, **13** and **14**. Deuterium labelling is only occasionally useful with natural products. In the present work, deoxyloganin (**9**) was prepared synthetically; additionally, it was possible to prepare a 7,8,10,10-tetradeuterated analogue of **9**, which made a confident assignment of the spectrum possible. It has been pointed out [4] that in *vic*-dihydroxy derivatives the carbon signals in a *trans*-arrangement appear at lower field than those in the corresponding *cis*-isomer.

2. ^{13}C *NMR spectroscopy*

6-O-*Substituted epimeric pairs.* We have recorded spectra of the glucosides and/or acetates **1**–**6a** and **19a**/**20a**, all of known structure, together with compounds **7a** and **8**/**8a**, of undetermined configuration at C-6 and C-8. When going from a 6β-hydroxy to a 6α-hydroxy substituent, Chaudhuri *et al.* [4] noted shift changes of C-3 (*ca* +1.5 ppm) and C-4 (*ca* −3 ppm). We can confirm these trends, although a wider range is observed in our examples, some of which are acetates. An effect not noted by Chaudhuri *et al.* [4] but observed by Bianco and Passacantilli [5], is the consistent downfield shift (0.6–4.9 ppm) of C-1 when going from 6β-hydroxy to the corresponding 6α-hydroxy substituted compounds. Comparing the available data (Table 2), we find consistent and rather large shift differences (2–5.3 ppm) for C-1 in all compounds substituted at C-4. This feature may be useful in determining the configuration of new compounds. For compounds not substituted at C-4, this approach seems less promising. The other effects, namely the shift differences found for C-3 and C-4 (see above), can be used in combination, i.e. by calculating the shift difference between C-3 and C-4 for each compound. This difference is in all cases (Table 2) larger for the α-epimers than for the corresponding β-epimers (3.8–7.7 ppm).

Finally, the available $J_{C\text{-}6,H\text{-}6}$ coupling constants have been included in Table 2. Apparently, there is a systematic difference here, the α-epimers having coupling constants 5–10 Hz larger than those of the β-epimers. Unfortunately, this coupling constant is often difficult to measure with accuracy due to overlap with signals arising

Table 2. Selected ^{13}C NMR data for 6β- and 6α-*O*-substituted compounds

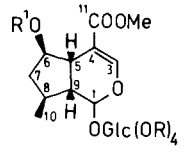
	6β-*O*-substituted				6α-*O*-substituted			
	δC-1 (ppm)*	δC-3–δC-4 (ppm)	$J_{C\text{-}6\text{–}H6}$ (Hz)†	Ref.	δC-1 (ppm)*	δC-3–δC-4 (ppm)	$J_{C\text{-}6H\text{-}6}$ (Hz)†	Ref.
Glucosides				solvent				
1/**2** (D_2O)	96.5	42.9	148	‡	101.3	48.9	—	[5]
3/**4** (D_2O)	96.4	44.8	145x	‡	100.6	50.0	153x	‡
5/**6** (D_2O)	96.0	42.6	148x	‡	101.3	50.3	—	‡
41 (D_2O)					93.4	45.0	166x	‡
35b/**36b** (MeOH)	96.1	43.0	—	[4]	100.4	47.3	—	[4]
35/**36** (MeOH)	97.1	43.1	—	[9]	99.1	47.1	—	[9]
(D_2O)					100.9	48.5	156	‡
44 (D_2O)	94.7	42.0	145x					
42/**43** (D_2O)	96.1	22.7	—	[5]	100.6	27.8	—	[5]
37/**38** (MeOH)	93.6	35.4	—	[4]	94.2	39.7	—	[4]
(D_2O)	94.9	36.1	140x		95.5	40.8	—	[10]
39 (MeOH)§	93.6	33.7	—	[4]				
Acetates ($CDCl_3$)								
1a/**2a**	93.9	42.0	155x	‡	98.4	46.0	160	‡
1b/**2b**	95.1	39.1	—	‡	100.0	—	—	‡
3a/**4a**	94.0	43.6	154	‡	97.5	47.4	160	‡
5a/**6a**	95.7	40.3	154x	‡	99.8	44.9	155x	‡
19a/**20a**	92.4	43.7	—	‡	98.6	47.9	—	‡
35a/**36a**	95.2	42.1	—	‡	100.0	46.3	158	‡
44a	93.7	43.7	154	‡				
37a/**38a**	94.2	34.4	—	[11]	94.0	39.8	—	[10]
39a	93.5	37.5	—	[11]				
Correlation limits								
Glucosides	<97	<45	<150		>99	>47	>150	
Acetates	<96	<44	<155		>98	>46	>155	

* The values have been corrected (δC-6′ = 61.5 ppm), see text.

† Coupling constants are ±2 Hz, except in cases marked with postscript 'x' (±5 Hz) when determination was difficult due to peak overlap.

‡ This work.

§ We suggest (see section 4) that ajugol has the 6β-OH configuration.



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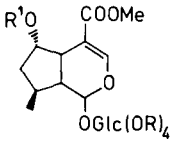
1b R = Ac; R¹ = H



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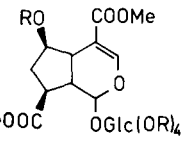
2a R = R¹ = Ac
2b R = Ac; R¹ = H



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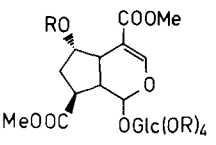

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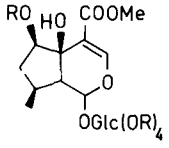

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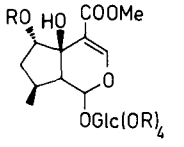

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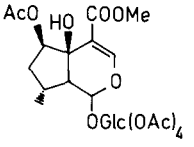
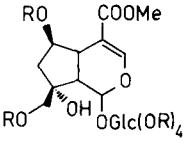


7a



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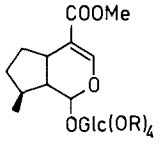
8a R = Ac



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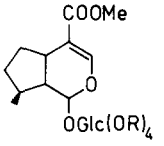

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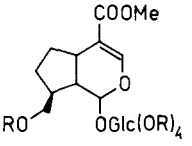

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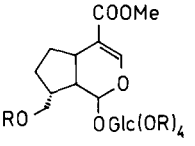

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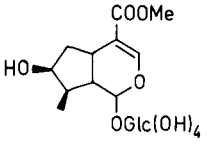
12a R = Ac



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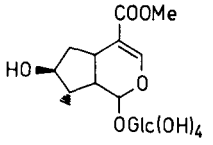

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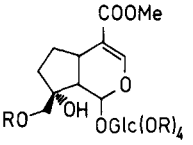

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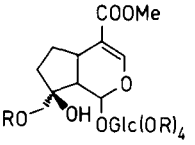


16 R = H
16a R = Ac

from the glucopyranosyl moiety, particularly in spectra recorded at low frequency (15–25 MHz), but usually a reliable value can be obtained on a high-frequency spectrometer. Using the above three parameters, it is possible to determine the configuration at C-6 in 4-methoxycarbonyl iridoids. For compounds with other substituents at C-4 the data are too few (see also comments in Section 4, below). To permit a conclusion, the compound (or its acetate) must agree with at least two of the three conditions set up for each series in Table 2. Asperuloside (**41**) agrees only with regard to the $J_{C\text{-}6,H\text{-}6}$ value, possibly due to the strain in the molecule caused by the lactone ring. Besides the compounds listed in Table 2, seven additional compounds with a 6β-*O*-substituent tally with all three conditions. No additional 6α-epimers were available.

Pedicularioside pentaacetate (**7a**) was isolated after acetylation of a fraction of glucosides originating from *Pedicularis palustris*. From ^{1}H NMR spectroscopy the structure **7a** was deduced, although with undetermined stereochemistry at C-6 and C-8. As **7a** was not identical with **5a** or **6a**, the difference must reside at C-8, demonstrating the α-configuration at this centre. The C-1 shift (94.8 ppm) together with the difference between the shifts of C-3 and C-4 (40.3 ppm) demonstrate the β-orientation of the 6-*O*-acetyl group. In fact, by comparison with the spectra of **5a** and **6a**, a close fit (< 1 ppm) is seen for all signals of the former, except for those of C-7, C-9 and C-10.

In Table 2, we have included the available data [4, 11] for ajugol, claimed to possess the structure **40**, and its acetate. When comparing the data for the glucoside with those for **37**/**38**, the best fit is seen with **37**. On the other hand, when comparing the data for the acetates the best fit is seen with **38a**. We will consider the question of the structure of ajugol in Section 4, below.

C-7-*Epimers*. Only one pair (**17a**/**18a**) was available. The notable shift differences are those of C-5 (−2.2 ppm), C-7 (−5.4 ppm) and C-8 (−2.5 ppm), when going from **17a** to **18a**. The changes observed for C-7 and C-8 are due to the change from a *trans*- to a *cis*-disposition of the oxygen substituents of these centres, and may thus be used to distinguish between C-7 epimers, if the configuration is known at C-8. Similar changes are seen for C-5 and C-6 (−5.8 and −1.3 ppm, respectively), when going from **6a** (*trans*) to **5a** (*cis*).

C-8-*Epimers not* O-*substituted at* C-7 *or* C-8. In pairs with a C-8 methyl group (**5**/**7**, **9**/**10**, **21**/**22**, **23**/**24**, including

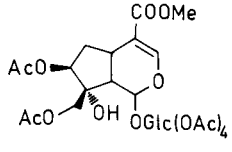
acetates), large upfield shifts for C-9 (−5.0 to −5.5 ppm) and for C-10 (−3.5 to −5.0 ppm) are observed on going from the 8β- to the 8α-epimers. This allows a distinction between the 8α-series (δ C-10 < 17 ppm) and the 8β-series (δ C-10 > 18 ppm). Besides the compounds in Table 1, cornin and hastatoside and their acetates [12], as well as montinioside and its acetate [13], and α- and β-dihydroverbenol (**42** and **43** [5]), all belonging to the β-series, conform with this rule (C-10: δ 18.7 to 21.1 ppm, 20 compounds).

If the methyl group (C-10) is substituted with oxygen (**11**/**12** and **11a**/**12a**), a similar but smaller effect is seen for C-9 and C-10 (*ca* −2 ppm for each) when going from the β- to the α-series. Including compounds **29**, **30** and **32** (all of known stereochemistry [14]) with different C-4 substituents, we find in the β-series that δ C-10 is very close to 66.0 ppm for the glucosides and 67.2 ppm for the acetates. The only available compounds in the α-series (**12**/**12a**) give values of 63.4 and 65.0 ppm, respectively, here only 2.5 and 2 ppm lower than in the β-series.

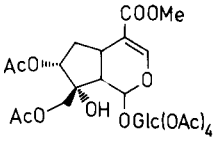
Capensioside, a compound isolated from *Retzia capensis* [15] was assigned the structure **34**, although with undecided configuration at C-8. The spectrum of **34** (Table 1) is very similar to those of **11**, **29** and **30** with regard to the signals from C-7 to C-10, and must thus belong to the β-series.

A glucoside from *Viburnum hupehense* was tentatively assigned the structure **31** [14]. This compound is a C-10 acetate, and consequently the fully acetylated derivative **31a** is chosen for comparison with **11a**/**12a**, **29a** and **32a**. Due to the effects from the C-6 substituent only the signal from C-10 (67.6 ppm) can be used directly, and this shows that **31**/**31a** belong to the 8β-series. Since **31**/**31a** contain a C-4-Me group, it is not attractive to determine the configuration at C-6 by direct comparison with the C-6 epimers above as these all carry a COOMe group at C-4. However, a set of increments accounting for the substituent effects can be calculated. Thus, by subtracting the shift value for each carbon atom in the spectrum of **9a** [S(**9a**)] from the corresponding shift value of S(**1a**) one obtains the increment set E (6β-OAc), constituting the isolated effect of a 6β-OAc substituent. By correcting the spectrum of **29a** with the increment set, one obtains a simulated spectrum [S(**A**)], of **31a**. The procedure can be expressed as follows: E(6β − OAc) = S(**1a**) − S(**9a**); S(**A**) = S(**29a**) + E(6β − OAc). This procedure is only permissible if the interactions (electronic or steric) between the iridoid moiety and the substituent are similar in the model compounds and in the unknown compound. In the present example, E(6β − OAc) and S(**A**) have been calculated (Table 3, entry 1). Comparison with S(**31a**) shows excellent agreement, all values corresponding within 1 ppm, except for C-6 with a deviation of 1.7 ppm. Likewise, E(6α − OAc) (entry 2) has been calculated, and from this, the simulated spectrum S(**B**) of the 6α-epimer of **31a**. Comparison of this with S(**31a**) shows much larger deviations (1.9–4.0 ppm) for the carbon atoms which are most sensitive to configurational change at C-6, namely C-1, C-3 and C-4. Thus we conclude that the structures of **31**/**31a** are as shown in the figure, compound **31** thus being 10-*O*-acetyl-6β-hydroxy-mongolioside.

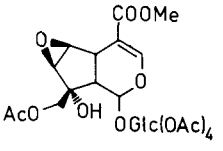
C-8-*Epimers O-substituted at* C-7, *but not at* C-8. Only one pair of compounds was available for analysis, namely **13**/**14**. In comparing S(**13**) with S(**14**), we find the same effects as in the foregoing examples, except that the C-10 signal shifts upfield only 1.1 ppm upon going from the β- to the α-epimer. The effects on C-9 seem to be similar to those in the preceding examples. Consequently, in the case



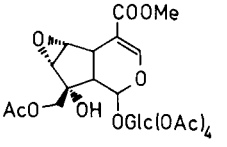
17a



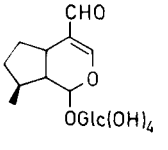
18a



19a



20a



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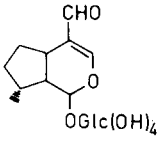

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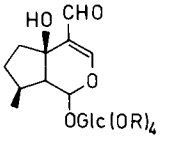

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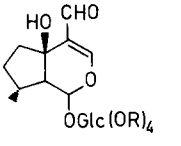

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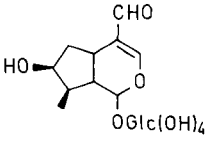

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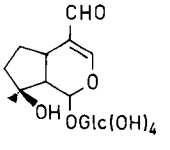

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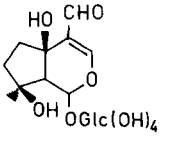

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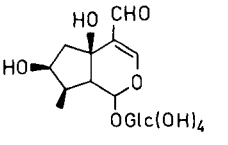
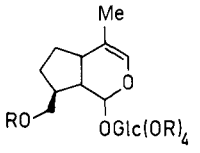


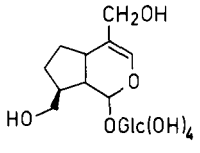
28

Table 3. Simulated ^{13}C NMR spectra

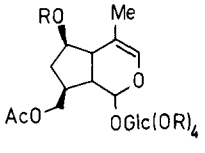
	C-1	C-3	C-4	C-5	C-6	C-7	C-8	C-9	C-10
1. E(6β–OAc) = S(**1a**) – S(**9a**)	−1.2	+1.6	−4.1	+6.3	+47.5	+6.6	−1.9	−1.3	+0.7
S(**A**) = E(6β–OAc) + S(**29a**)	92.5	135.2	108.2	42.9	75.1	33.0	36.1	43.7	68.0
S(**31a**) – S(**A**)	+0.5	−0.2	+0.5	−0.6	+1.7	−0.2	+0.7	−0.2	−0.4
2. E(6α–OAc) = S(**2a**) – S(**9a**)	+3.3	+3.3	−6.4	+6.8	+45.9	+7.0	−0.8	−2.4	+0.7
S(**B**) = E(6α–OAc) + S(**29a**)	97.0	136.9	105.9	43.4	73.5	33.4	37.2	42.6	68.0
S(**31a**) – S(**B**)	−4.0	−1.9	+2.8	−1.1	+3.3	−0.6	−0.4	+0.9	−0.4
3. E(6β–OH) = S(**1**) – S(**9**)	−1.5	+0.8	−3.1	+8.2	+45.5	+8.6	−1.7	−1.3	−0.2
S(**C**) = S(**15**) + E(6β–OH)	94.5	153.8	109.1	41.8	76.1	44.6	81.2	44.4	68.4
S(**8**) – S(**C**)	+0.7	−0.1	+1.1	−1.4	+0.3	−0.5	+0.6	+0.2	+0.7
4. S(**D**) = S(**16**) + E(6β–OH)	93.7	153.3	109.3	40.7	74.9	42.9	83.0	49.1	65.8
S(**8**) – S(**D**)	+1.5	+0.4	+0.9	−0.3	+1.5	+1.2	−1.2	−4.5	+3.3
5. Eβ(7β–OH) = S(**13**) – S(**9**)	−0.5	−0.5	+0.6	−3.0	+9.0	+41.6	+5.2	−2.7	−7.3
S(**E**) = S(**21**) + Eβ(7β–OH)	98.4	164.2	125.9	28.3	38.7	74.8	40.7	44.7	12.6
S(**25**) – S(**E**)	−0.2	−0.3	−0.1	+0.1	+0.3	+0.2	−0.2	+0.6	0
6. Eα(7β–OH) = S(**14**) – S(**10**)	+0.1	−0.3	−0.8	−3.2	+8.0	+45.8	+7.5	−1.7	−2.4
S(**F**) = S(**22**) + Eα(7β–OH)	97.0	165.0	121.8	28.7	38.1	78.7	43.4	40.7	13.5
S(**25**) – S(**F**)	−1.2	−1.1	+4.0	−0.3	+1.9	−3.7	−2.9	+4.6	−0.9
7. Eα(8β–OH) = S(**26**) – S(**22**)	−0.5	−1.1	+2.4	−3.3	−1.5	+7.5	+44.3	+8.2	+7.8
S(**G**) = S(**24**) + Eα(8β–OH)	96.8	164.5	125.9	70.6	36.8	39.8	78.5	59.9	23.8
S(**27**) – S(**G**)	−1.3	−0.2	−1.2	+0.4	0	−0.5	+0.5	+0.6	−0.7
8. S(**H**) = S(**24**) + Eα(7β–OH)	97.4	165.3	122.7	70.7	46.3	78.1	41.7	50.0	13.6
S(**28**) – S(**H**)	−0.4	−1.4	+2.8	+0.5	+1.6	−4.9	−1.7	+3.7	−0.8
9. S(**J**) = S(**23**) + Eβ(7β–OH)	97.2	164.3	125.3	73.3	48.9	72.7	40.3	54.1	12.7
S(**28**) – S(**J**)	−0.2	−0.4	+0.2	−2.1	−1.0	+0.5	−0.3	−0.4	+0.1



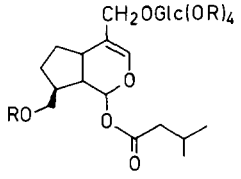
29 R=H
29a R=Ac



30



31 R=H
31a R=Ac



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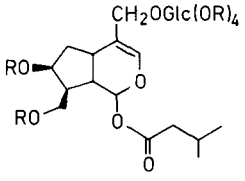
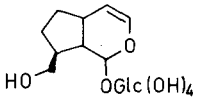
32a R=Ac



33 R=H
33a R=Ac



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of a 7β-*O* substituent being present, the C-10 shift does not permit determination of the configuration at C-8.

C-8-*Epimers* O-*substituted at* C-8. Chaudhuri *et al.* [3] found in the spectra of monotropein methyl ester (**45**) and gardenoside (**46**) a large downfield shift (7 ppm) of C-9 from **45** to **46**. The data from the present work, obtained from **15/16** and **15a/16a**, indicate that this effect is general. Notably, it is opposite to that seen for the epimeric pairs not substituted with an 8-hydroxyl group. Apparently, the effect from the 8-hydroxyl group overrides that of C-10.

6β-Hydroxy-splendoside from *Fouquieria splendens* [16] has been assigned the structure **8** by the following approach: the overall structure was determined by ^{1}H NMR and the β-configuration at C-6 by comparing its ^{13}C NMR data (95.2 ppm, 43.5 ppm and 143 Hz for the glucoside; 93.7 ppm, 43.7 ppm and 148 Hz for the acetate) with the correlation limits given in Table 2. Comparison of S(**8**) with the simulated spectra S(**C**) and S(**D**) shows clearly the best fit with S(**C**) (Table 3, entries 3 and 4) and strongly suggests the stereochemistry presented in **8**.

Further use of increment sets in simulation of spectra. As shown above, a set of increments representing the effects of a single substituent can be used to modify the spectrum of another compound producing a 'synthetic spectrum' of a new compound carrying this substituent. This additive procedure cannot be used for compounds with vicinal

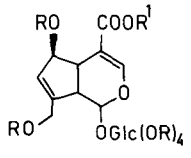
substituents, as the *cis/trans* interactions from these override the effects of the single substituents. Vicinal interactions also exist between a single substituent and the iridoid skeleton, but additivity is preserved because the use of appropriate model compounds give increment sets inherently containing these interactions. The above examples of simulated spectra are of compounds with 6,10- and 6,8,10-substituents. We shall proceed to demonstrate that the approach can be used also for 7-hydroxy, 5,7-di-hydroxy and 5,8-di-hydroxy substituted compounds, the aldehydes **21**–**28** serving as model compounds and examples.

The structures of boschnaloside (**22**) [17], plantarenaloside (**24**) [18] and ixoroside (**26**) [19] have all been established by chemical transformations, while that of tecomoside, (8α–**28**) was suggested on the basis of its chemical conversion and ^{1}H NMR data [20]. Except for **22**, we have had access to all these compounds. The ^{13}C NMR data for the pairs **21**/**22** (stanside/boschnaloside) and **23**/**24** (stansioside/plantarenaloside) make it possible to determine the structures of each of these compounds (assuming the usual absolute stereochemistry for C-1, C-5 and C-9) by comparison with the pair **9**/**10**. With regard to compound **25** (7β-hydroxystanside), no epimer is available, but comparison with the pair **13**/**14** shows that **25** resembles the former most, although the effects of the change from 4-carboxaldehyde to 4-methoxycarbonyl can be accounted for only by inclusion of more model compounds. In Table 3 (entries 5 and 6) the simulated spectra of **25** [S(**E**)] and its 8α-epimer [S(**F**)] are compiled. S(**E**) was calculated by adding the effects of the 7β-hydroxyl group [E(7β-OH) = S(**13**) − S(**9**)] to S(**21**), while S(**F**) was found analogously from S(**14**), S(**10**) and S(**22**). [Note that two different E(7β-OH) sets are used, one for the 8β-series, and one for the 8α-series and that these are quite different (Table 3).] Comparing S(**25**) with S(**E**) and S(**F**), we find almost coincidence with only the former. Theoretically, **25** could possess a 7α-group, giving rise to two additional isomers. However, the ^{1}H NMR data exclude this possibility, as the spectra of **13** and **25** are almost identical, allowing for the different functionalities at C-4. In conclusion, the structure of compound **25** is that presented in the figure.

We have calculated the 'synthetic spectrum' S(**G**) for the 5,8-di-hydroxy substituted compound **27** (euphroside), using S(**26**), S(**22**) and S(**24**) as models (Table 3, entry 7). Again a good correlation is seen with S(**27**), the largest deviation being 1.3 ppm. In this case **27** has also been correlated with ipolamiide [21] by chemical transformations. Compounds **21**, **23**, **25** and **28** were all found in *Euphrasia cuspidata*, and tentatively they might all be expected to have the same stereochemistry at C-8. Compound **28** is identical with tecomoside (see Experimental), which has been assigned the 8α-configuration [20]. To clarify this discrepancy we have simulated the spectra S(**H**) and S(**J**) (8α- and 8β-forms, respectively); comparison with S(**28**) (Table 3, entries 8 and 9) shows by far the best correlation with the latter, although in this case a difference for the C-5 shift of 2.1 ppm is seen. However, for the sensitive signals from C-7 to C-10, we find almost coincidence in the two spectra. Thus we conclude that the structure of tecomoside is as shown in the figure (**28**), and we will comment upon this below.

Calculation of C-9 *chemical shifts for determination of the stereochemistry at* C-8. As shown in the preceding sections, large or moderate shift changes could be seen for C-9 when comparing the available pairs of C-8-epimers. This indicated that calculation of the shift value of C-9 alone might be sufficient to decide the stereochemistry at C-8 of an unknown iridoid glucoside. The large range of C-9 shifts (39.4–60.5 ppm in Table 1) and the fact that no C-9-substituted iridoid has so far been described were promising prospects for such an attempt.

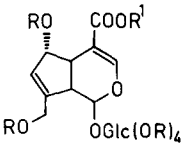
To isolate the effects arising from the C-8 centre, it was necessary to account for the effects caused by other substituents. By using the data from Table 1, together with other available spectra, we calculated the effects on the C-9 shifts caused by the substituents in singly substituted compounds; it appeared that the additivity was consistently satisfactory. Furthermore, it was obvious that the nature of the C-4 substituent had only a minor influence on the C-9 shift, as seen when comparing compound **11** with compounds **29**, **30**, **32** and **34**. Using a computer program the best 'least-squares estimate' was found for the substituent effects upon the C-9 shifts in the spectra of 113 compounds (glucosides and acetates) including published values for spectra recorded in D_2O or $CDCl_3$ [6, 9–14, 22]. The fit is within ±1.1 ppm for 108 compounds, leaving five compounds with a somewhat larger deviation (1.3–1.7 ppm). The latter compounds (**5a**, **6**, **7a**, aralidioside [12] and harpagide hexaacetate [11]) have the common feature of being 5,6-dioxygenated, but other compounds with this substitution pattern (i.e. **5** and **6a**) give a good fit. The increment values are given in Table 4; by using these, the C-9 shift for the two C-8 epimers of most iridoid glucosides can be calculated, thus allowing the decision of which epimer is at hand. Even Valeriana-compounds [22] and the 6-enol-acetates of cornin and griselinoside [12] are included. To demonstrate the potential of the method, assume that we have an iridoid glucoside (or its acetate) with known substitution pattern,



35 R = H; R¹ = Me

35a R = Ac; R¹ = Me

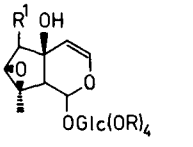
35b R = R¹ = H



36 R = H; R¹ = Me

36a R = Ac; R¹ = Me

36b R = R¹ = H

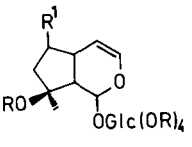


37 R = H; R¹ = β-OH

37a R = Ac; R¹ = β-OAc

38 R = H; R¹ = α-OAc

38a R = Ac; R¹ = α-OAc



39 R = H; R¹ = β-OH

39a R = Ac; R¹ = β-OAc

40 R = H; R¹ = α-OH

40a R = Ac; R¹ = α-OAc

but with unknown stereochemistry at C-8. The shift for C-9 of both the 8β- and 8α-alkyl substituted compounds are calculated using the values from Table 4.

With compound **8a** as an example, we find:

	ppm		ppm
Base-value (β-series)	48.5	Base-value (α-series)	43.4
Solvent $CDCl_3$	−0.8	Solvent $CDCl_3$	−0.8
8α-OH	+1.4	8β-OH	+8.5
10-OH (β-form)	−3.8	10-OH (α-form)	−1.2
6β-OH	−1.7	6β-OH	−1.7
Acyl in 6,10	+0.3	Acyl in 6,10	+0.3
Calc. for β-form:	43.9	Calc. for α-form:	48.5

Found (Table 1): 44.0 ppm

For patrinoside (**33**), we find:

	ppm		ppm
Base-value (β-series)	48.5	Base-value (α-series)	43.4
7-OH	−2.0	7-OH	−2.0
10-OH (β-series)	−3.8	10-OH (α-series)	−1.2
1-OAcyl	−0.6	1-OAcyl	−0.6
Calc. for β-form:	42.1	Calc. for α-form:	39.6

Found (Table 1): 41.5 ppm.

In both cases, we find the best fit with the value calculated for the β-form. However, while in the first example we find a difference of 4.6 ppm between the calculated values, in the second example a difference of only 2.5 ppm is noted, demanding a better fit to decide with certainty which epimer is at hand. An even more demanding situation arises when attempting to calculate the C-9 value for ixoroside (**26**). In this case we calculate the shifts 49.9 and 51.9 ppm for the β- and α-forms, respectively; the known value is 51.1 ppm (α-form). In this case, no decision can be made, and other methods must be used. As it is seen from these examples, care must be taken using this calculation in cases where either C-8 or C-10 (but not both) are substituted with oxygen, particularly in cases where the compounds are substituted also with oxygen in both the C-5 and C-6 positions. Harpagide hexaacetate [11] is such an example. However, in most cases, the method can be used with confidence, particularly if a good or a reasonably good fit is obtained with both the glucoside and the corresponding acetate.

We have tested the method on the 25 compounds used as examples in the paper of Chaudhuri *et al.* [4], in which all spectra were recorded in d_4-MeOH with TMS as the standard. First, the spectra were aligned to give 61.5 ppm for the C-6′ absorption; second, the solvent effect for MeOH was determined (−0.4 ppm for C-9) by comparing spectra of six glucosides recorded both in D_2O and in d_4-MeOH. Calculation of the C-9 values gave a fit within ±1.7 ppm for the glucosides in that study (including harpagide: −0.2 ppm), the only exception being acetylharpagide (+2.4 ppm).

3. ^{1}H *NMR spectroscopy*

Determination of configuration at C-6. This is often difficult when only one epimer is available, except if the coupling constant $J_{5,6}$ is smaller than 1 Hz [cf. 1] in which case the substituent occupies the β-position. If both epimers are available many systematic differences can be used in assigning structures. In such a case, the shift differences between the signals of H-1 [5, 23, 24 and Table 5], H-3 [23, 24] or H-6 [24] may be used. Also the coupling constant, $J_{1,9}$ [5, 23] (Table 5) is useful, the 6α-substituted compounds consistently showing the larger value. Apparently, the configuration can be assigned as 6α if $J_{1,9} \geqq 8$ Hz.

Table 4. Increments for calculation of C-9 chemical shifts

	ppm	
Base-values in D_2O:	48.5	(β-series)
	43.4	(α-series)
Increments:		
$CDCl_3$ as solvent	−0.8	
CD_3OD as solvent	−0.4	
5-OH	+8.0	(25)*
5-OAc	+4.6†	(3)
6β-OH,OAc	−1.7	(26)
6α/7β/7α-OH,OAc	−2.0	(9/21/2)
8β-OH	+8.5	(21)
8β-OAc	+7.0	(6)
8α-OH	+1.4	(22)
10-OH,OAc (in β-series)	−3.8	(36)
10-OH,OAc (in α-series)	−1.2	(9)
6-keto	−4.2	(12)
7-keto	−3.2†	(1)
8β-carboxyl (ester)	−3.9	(11)
8β-carbaldoxime	+1.6†	(3)
5,6-double bond	+4.2	(5)
6,7-double bond	−0.3	(11)
6.7β-epoxy	−2.7†	(1)
6.7α-epoxy	−6.1†	(1)
7,8β-epoxy	+2.2	(8)
8,10α-epoxy	−6.7†	(2)
1-OAcyl	−0.6	(9)
Acylation of 6/7/10-OH add for each	+0.15	
Standard:		
δ C-6′	61.5	

* Number of compounds on which the value is based.
† These values are only based on a few examples.

Determination of configuration at C-8. The chemical shifts of H-1 and H-9 in 8-*O*-acyl substituted iridoid glucosides appear at lower field in the α-series than in the β-series [25]. It has also been proposed [18, 25] that the magnitude of the $J_{1,9}$ coupling constant is significantly smaller in the α-series than in the β-series, and the structure of plantarenaloside (**24**) was determined [18] partly on this basis. However, in view of the small differences seen for the C-8 epimers in this work (Table 5), this appears to be a rather unreliable criterion, except if both epimers are available. In another study [6], the coupling constant $J_{8,9}$ has also been used to determine the structure of plantarenaloside (**24**; the name 'yuheinoside' is redundant). In this case, the compound was found together with boschnaloside (**22**) in a *Leucocarpus* sp. and the magnitude of $J_{8,9}$ (9 Hz) was taken to indicate that **24** belonged to the α-series. In another work [20], the $J_{8,9} = 12$ Hz of tecomoside (**28**) was likewise interpreted as a *cis*-coupling, and the 8α-configuration was therefore assigned to this compound. In Section 2, we have argued that tecomoside belongs to the 8β-series, and considering the large $J_{8,9}$ coupling constant, we find that this

Table 5. Selected ^{1}H NMR data*

Compound	$J_{1,9}$ (Hz)	$J_{8,9}$ (Hz)	δH-1 (ppm)	δH-10 (ppm)
1a	2.0	—	5.37	1.13
2a	8	—	5.06	1.13
5a	3	8.5	5.38	1.17
6a	8	—	5.22	1.13
7a	1.5	—	5.55	0.99
8a	2.5	—	5.74	4.25
9	4.5	—	5.33	1.06
10	3.2	8	5.44	1.02
11	6.0	—	5.24	3.58
12	5	—	5.50	—
13	3.5	10.2	5.41	1.09
14	2.9	8.4	5.57	1.04
15	4.0	—	5.54	3.60
16	5.5	—	5.46	3.73
17a	4.5	—	5.38	4.18
18a	3.5	—	5.51	4.08
19a	<1	—	5.57	4.26
20a	9.0	—	5.31	4.11
23	2.0	10	5.72	1.13
23a	2.5	—	5.42	1.12
24	1	—	5.87	0.91
24a	2.0	10†	5.56	0.94
35‡	5.0	—	5.35	—
36‡	8.3	—	5.06	—
37§	6.5	—	5.50	1.51
38§	8	—	5.36	1.49

* Spectra of glucosides were recorded in D_2O (DSS), acetates in $CDCl_3$ (TMS) at 90 MHz, except for **7a**, **8a**, **9/10**, **12**, **13/14**, **23a/24a** (270 MHz).

† This value was obtained using Eu(fod)$_3$ reagent.

‡ Data from [5].

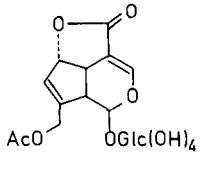
§ Data from [10].

represents a *trans*-diaxial rather than a *cis*-relationship of the protons. We have included a few $J_{8,9}$ coupling constants in Table 5, and as these are often of equal magnitude in the β- and α-series, they cannot be used alone for the determination of the configuration at C-8.

4. *Reassignment of the 6-epimeric pair ajugol/myoporoside*

The compound 'leonuride' hexaacetate was isolated after acetylation of a fraction of an extract of *Leonurus cardiaca* [27]. The compound was assigned the structure **39a**, of uncertain configuration at C-6 and C-8. Later ajugol was isolated from *Ajuga reptans*, *L. cardiaca* and *Melittis melissophyllum* and the compound was assigned structure **40** [28], the configuration at C-8 being determined by comparison (^{1}H NMR) with a number of model compounds while the 6α-hydroxy-substitution was determined by Horeau's method [29]. Finally, identity of ajugol and leonuride was claimed [28]. Myoporoside (**39**) was isolated from *Myoporum insulare* [30] and the compound was found to be the 6-epimer of ajugol, again configuration at C-6 was determined by Horeau's method.

When comparing the $J_{1,9}$ coupling constants for ajugol and myoporoside the values are 1.0 and 2.5 Hz [30], respectively, the supposed β-epimer having the larger value, and this is the opposite of what has consistently been observed for a large number of other 6-epimeric pairs of iridoid glucosides (see the preceding section). As the ^{13}C NMR data (Table 2) did not seem to correspond unambiguously with the proposed structure, we have made further comparisons. In Table 6 are compiled the published spectra of ajugol and its hexaacetate together with the spectra of shanzhiside methyl ester (**44**) and its hexaacetate (**44a**). The structure of shanzhiside has been determined unequivocally by chemical means [31]; moreover, shanzhiside (**44**) and the hexaacetate (**44a**) qualify as 6β-*O*-substituted compounds with regard to the limits set up in Table 2. As noted in Section 2 (see also [4, 5]) the C-4 substituent has a significant influence only on the ^{13}C NMR shift of C-3 and C-4, the remaining carbon signals being largely unaffected. In Table 6, the spectra of the pair of glucosides and the pair of acetates, respectively, are almost identical, except for the



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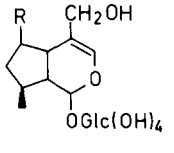
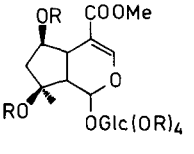


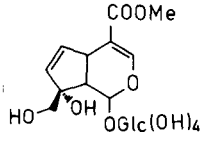
42 R = β-OH

43 R = α-OH



44 R = H

44a R = Ac



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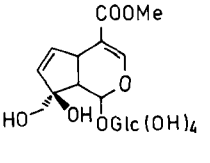

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Table 6. Comparison of ^{13}C NMR spectra of ajugol and shanzhiside and their acetates

Compound	Ref.	Solvent	C-1	C-3	C-4	C-5	C-6	C-7	C-8	C-9	C-10	C-11	C-1′
Ajugol (**39**)*	[4]	CD_3OD	93.6	139.4	105.7	39.6	76.9	49.0	79.1	50.4	24.9		98.7
Shanzhiside (**44**)		D_2O	94.7	152.7	110.0	39.7	76.2	48.6	78.8	50.5	24.2	170.4	99.1
Ajugol Ac_6 (**39a**)*	[11]	$CDCl_3$	93.5	139.9	102.4	37.5	77.5	44.8	86.6	47.6	21.6		95.3
Shanzhiside Ac_6 (**44a**)		$CDCl_3$	93.7	151.7	108.0	38.0	76.6	44.6	86.5	48.4	21.8	166.0	95.7

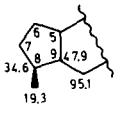
* Spectra have been corrected (δ C-6′ = 61.5 ppm).

differences due to the different substitution pattern at C-4. On account of this, and of the observed difference in the proton–proton coupling constant $J_{1,9}$, we suggest that ajugol is the 6β-hydroxy epimer (**39**) and consequently myoporoside the 6α-hydroxy epimer (**40**), assuming that these two compounds are indeed C-6 epimeric. A consequence of this is that Horeau's method must have failed in both cases.

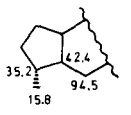
5. *Interpretation of the effects of epimerism*

Chaudhuri *et al.* [4] when discussing the differences between the spectra of the pair monotropein methyl ester/gardenoside (**45**/**46**) state that: "Comparison of the chemical shift values shows that an axial hydroxyl function (in the former) exerts a shielding of 5–7 ppm in respect to its equatorial partner". By examination of Dreiding models of these two compounds, both having a double bond in the five-ring, the terms 'axial' and 'equatorial' seem unfortunate in the usual sense of the words. To us it seems more useful to discuss the changes seen for the C-9 shift in C-8 epimeric pairs in the light of the difference in *trans*- and *cis*-interactions between the substituents at C-8 and C-9. Thus when comparing the pair **9a**/**10a** with *trans*-/*cis*-dimethylcyclopentane (**47**/**48** [32]) some similarities exist in the ^{13}C NMR spectra. In **9a** and **47** we find the same chemical shift for the methyl groups (*ca* 19.5 ppm) while in the epimeric **10a** and **48** (*cis*-forms) this signal appears at higher field (*ca* 16.0 ppm). Furthermore, the β-effect (seen for the C-2 signal) when going from S(**47**) to S(**48**) is virtually the same as that (seen for the C-9 signal) found when going from S(**9a**) to S(**10a**), namely -5.1 ppm, and -5.5 ppm, respectively. However, the changes expected for C-8 and C-1 when comparing the iridoids are almost negligible. Examining the pair **15a**/**16a**, we find the same effect for C-9 ($+4.8$ ppm) as when comparing the pair **49**/**50** [32] (*cis*/*trans*-forms; $+4.5$ ppm), again no significant change is seen for C-8. It thus appears that the β-effect from the hydroxyl group overrides that of the alkyl substituent, and consequently, in this connection **15a** should be viewed as a '*cis*'-form and **16a** as a '*trans*'-form. Similar effects can be seen in the 7,8-*trans*/*cis*-substituted compounds **19a**/**20a**, where a large shift change is seen for C-7 (-5.4 ppm) and a minor change for C-8 (-2.5 ppm).

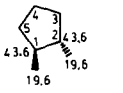
In attempts to explain the differences in the spectra of the 6-hydroxy substituted 6-epimeric compounds, it has also been assumed [4, 5] that the 6β-hydroxyl group is (pseudo)equatorial, while the 6α-hydroxyl group is (pseudo)axial, and that this should account for the differences. The substantial changes seen for C-1 when comparing the epimers have furthermore been claimed to be 'linked to configurational variations' [5], although these 'variations' were not specified. The difference seen for the one bond coupling constant $J_{C\text{-}6,H\text{-}6}$ in the two epimeric series (see Table 2) could very well be accounted for by the difference in spatial positions assumed above. Thus it has been claimed [33] that in pyranoses the equatorial proton associated with an axial substituent should give rise to a larger coupling constant than the corresponding axial proton, and this is perhaps what is seen in the two series here. However, another explanation may apply. The large difference seen for C-1 (Table 2) when comparing the two series, combined with the consistent change in the proton-proton coupling constant $J_{1,9}$ discussed above, indicates that more fundamental changes in the conformation of the whole molecule take place when going from the β- to the α-series. Particularly the change seen for $J_{1,9}$ may indicate that a 'flip-over' is made by the dihydropyrane ring. Thus, in the case when the coupling constant is small (the 6β-*O*-series), the sugar moiety is in an axial position, while in the other case it is probably in an equatorial position [26]. Such changes in the dihydropyrane ring could well account for the large



9a



10a



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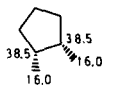

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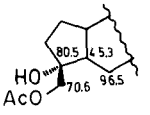
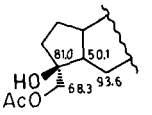
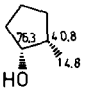


15a



16a



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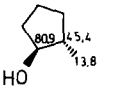

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differences for C-1, C-3 and C-4 seen in the ^{13}C NMR spectra when comparing C-6 epimers. Against such an explanation are the minimal changes seen for C-5 and C-9, the other members of the dihydropyrane ring.

Also in this case we find it useful to interpret the changes in the ^{13}C NMR spectra of the C-6 epimers in the light of *cis/trans*-interactions. As shown above, it appears that when two substituents are present on a five-ring carbon atom, the effects from a hydroxyl-substituent override those of an alkyl substituent. Thus **1** and **3** behave as 5,6-*trans*-substituted compounds and the epimers **2** and **4** as 5,6-*cis*-substituted compounds as seen (Table 1) from the C-6 chemical shifts (low and high field, respectively; the C-5 shifts do not conform to this view). On the other hand, when comparing compounds **5** and **6**, which are 5-hydroxy-substituted, we here find that **5** behaves as a *cis*-form, **6** as a *trans*-form, the former with the C-6 chemical shift at highest field The corresponding acetates (**1a** through **6a**) behave similarly.

EXPERIMENTAL

^{13}C NMR spectra were recorded essentially as described in ref. [8] (see Table 1). Conditions for ^1H NMR spectra are given in Table 5.

Compounds **1**, **1a**, **1b**, **2a** and **2b** have been described [23]. Reduction of griselinoside [12] with $NaBH_4$ in MeOH gave a 1:1 mixture of **3** and **4**, which were separated by reversed phase chromatography and characterized only by their NMR spectra (Tables 1 and 5). Acetylation of **3** gave **3a**, mp 149–50.5°; $[\alpha]_D$ −90.3° (*c* 0.9; $CHCl_3$). (Found: C, 52.16; H, 5.56. Calc. for $C_{28}H_{36}O_{17}$: C, 52.17; H, 5.63%.) Acetylation of **4** gave **4a**; $[\alpha]_D$ −58.5° (*c*, 0.8; $CHCl_3$). (Found: C, 52.30; H, 5.51. Calc. for $C_{28}H_{36}O_{17}$: as above.)

Reduction of hastatoside with $NaBH_4$ in MeOH (cf. [24]) gave **5** and **6** which were sepd by PLC (EtOAc–C_6H_6–EtOH, 4:1:1) giving as the faster moving band **6** followed by **5**. The ^1H NMR spectra were essentially as described in ref. [24]. Acetylation of either gave **5a**, mp (EtOH) 96–98°; $[\alpha]_D^{19}$ −93° (*c* 0.3; $CHCl_3$). (Found: C, 52.30; H, 5.88. Calc. for $C_{27}H_{36}O_{16}$: C, 52.60; H, 5.88.) or **6a**, mp (EtOH) 209–211° (dec); $[\alpha]_D^{19}$ −171° (*c* 0.2; $CHCl_3$). (Found: C, 52.78; H, 5.64. Calc. for $C_{27}H_{36}O_{16}$, as above.)

The glucoside (pedicularioside) corresponding to **7a** is a constituent of *Pedicularis palustris*, from which also plantarenaloside (**24**) [18] and euphroside (**27**) [34] were isolated (unpublished work).

6β-Hydroxy-splendoside (**8**), splendoside (**15**) and the glucosides corresponding to **17a** and **19a** are constituents of *Fouquieria splendens*, while **18a** and **20a** have been synthesized [16].

Compounds **9/9a**, **11/11a** and **12/12a** were prepared from geniposide or its pentaacetate as described [35, 36].

Compounds **10/10a** were prepared from geniposide pentaacetate by Pd catalysed elimination of HOAc using $Pd(OAc)_2$ and PPh_3 in toluene [37] to give 6,7-dehydro-7-deoxygardoside methyl ester tetraacetate [38]. Hydrogenation (Rh/C, H_2) gave **10a**, which was characterized only by NMR (Tables 1 and 5). Deacetylation provided **10**, mp (EtOAc) 150–152°; $[\alpha]_D^{21}$ −128° (*c* 0.4; MeOH).

8-Epiloganin (**14**) was isolated from *Melampyrum arvense*. The structure was proved by comparison with a sample prepared by hydrogenation of gardoside methylester [39] using Rh/C (5%) as the catalyst (unpublished work).

Compounds **16/16a** were prepared from gardenoside (**46**) as described [40].

Stanside (**21**) [41], stansioside (**23**) [42], 7β-hydroxy-stanside (**25**) and tecomoside (**28**) [20] were isolated from *Euphrasia cuspidata* collected in Alpi Carniche, Italy (unpublished work). The ^1H NMR spectrum of **28** was identical to that published [20], while the ^{13}C NMR spectrum of **28** was identical to that of tecomoside [Dr. A. Bianco, private communication]. The pentaacetate prepared from **28** had mp 118–120° (lit. [20] mp 124–125°); $[\alpha]_D^{21}$ −97° (*c* 0.6; $CHCl_3$).

Ixoroside (**26**) was isolated from *Euphrasia stricta* as a minor constituent (unpublished work).

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