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9-HYDROXY SUBSTITUTED IRIDOIDS FROM *GELSEMIUM SEMPERVIRENS*

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Key Word Index—*Gelsemium sempervirens*; Loganiaceae; iridoids; gelsemide; gelsemide 7-glucoside; gelsemiol; gelsemiol 1-glucoside; gelsemiol 3-glucoside; semperoside; 9-hydroxysemperoside; brasoside; fabiatrin.

Abstract—Two chemotypes of *Gelsemium sempervirens* were investigated. One, cultivated in Copenhagen, contained six iridoids in appreciable amounts, namely gelsemide, gelsemide 7-glucoside, gelsemiol, gelsemiol 1- and 3-glucoside, and 9-hydroxysemperoside. The other sample, collected in the wild in North Carolina, U.S.A., contained semperoside and the known brasoside as well as the coumarin glycoside fabiatrin. The two iridoids were found only in trace amounts in the cultivated specimen. The structures of the seven new iridoids were established mainly by spectroscopic methods and that of gelsemide was confirmed by X-ray analysis. Two new structural features were noted: three of the compounds carried a hydroxyl group at the 9-position, while two contained the glucosyl moiety at C-3. All iridoids were lactones of the asperuloside type with or without a 3,4-double bond.

INTRODUCTION

Members of the genus *Gelsemium* have been investigated chemically several times (for a recent account see [1]), and a number of complex indole alkaloids have been reported from two of the three known species. During our investigation of the family Loganiaceae we have isolated a series of iridoids from *G. sempervirens*. Here we report the results.

RESULTS AND DISCUSSION

Two collections of *Gelsemium sempervirens* (L.) J. St. Hil. were available for investigation. One plant was cultivated in The Botanical Garden of Copenhagen and material was collected in 1975 and kept in the freezer until use. The second sample was collected in its natural habitat (North Carolina, U.S.A.) and dried before examination. Most of the iridoids were common for the two collections, but some occurred only in the cultivated plant.

Extraction of the cultivated plant material with ethanol followed by extensive chromatography (see Experimental) gave rise to two iridoid aglucones and six glucosides, only one of which was formerly known. The first compound (**1**) was obtained by extraction of the water solubles with ethyl acetate and was named gelsemide. Acetylation of **1** provided either a monoacetate (**1a**) or a diacetate (**1b**), depending on the conditions (see Experimental). The ^{1}H NMR spectrum was simple and led readily to the structure **1**, assuming the usual stereochemistry at C-5 and C-9 for iridoids. Thus the signal at δ 7.53 (*d*, J = 2.5 Hz) was assigned to H-3, and successive decoupling experiments identified the signals from H-5 to H-8 as well as 10-CH_3. No signal could be assigned to a 9-proton, and this was surprising, as no iridoids substituted at C-9 so far were known. This was supported by the presence of an AB-system at δ 4.25 and 3.95 (J = 12 Hz) without further couplings, which could be ascribed to a 1-CH_2 group. The ^{13}C NMR spectrum contained ten signals (Table 1) and was in complete agreement with the proposed structure (**1**). A singlet at 72.3 ppm could be assigned to C-9 with a hydroxyl group, and a triplet at 67.3 to the 1-oxymethylene group. The presence of a lactone ring was ascertained by the low-field position of the C-6 signal (80.3 ppm), while the large value of the one bond coupling constant $J_{C\text{-}6,H\text{-}6}$ (169 Hz) showed the presence of an acylated oxygen in the α-position [2]. [Corresponding values are found in the spectrum of asperuloside (**13**), namely δ86.7 (J = 166 Hz).] In order to determine the relative stereochemistry of **1**, a number of ^{1}H NOE experiments were performed (Table 2). The NOE effects between H-5, H-6, H-7 and 10-CH_3 as well as the absence of effects between H-5/H-6 and H-8 confirmed the location of the lactone moiety and further showed that the 7-OH group was in the α-position while the 10-CH_3 group was in the β-position.

The structural elucidation was eventually completed by a crystal structure determination, using single crystal X-ray diffraction methods. The molecular conformation in the crystal structure is shown in Fig. 1. The two 5-membered rings both adopt envelope conformations with the C(5) and the C(9) carbon atoms respectively, as apical atoms.

The 6-membered ring has an almost ideal half-boat conformation with the C(5) carbon atom as the apical atom, the other atoms of this ring deviate by less than 0.002 Å from a common best plane. Apart from conventional van der Waals forces, the molecules are held together in the crystalline state by two intermolecular

Table 1. ^{13}C NMR data

C	1	2	3	4	5	6	7	8	10	12
1	67.3 (*t*, 155)	67.2 (*t*, 152)	62.6 (*t*, 146)	70.5	62.2	60.7 (*t*, 144)	57.8 (*t*, 148)	95.3 (*t*, 177)	94.6 (*d*, 171)	57.9 *t*
3	154.0 (*d*, 199)	154.1 (*d*, 196)	61.2 (*t*, 142)	61.0	69.6	97.9 (*d*, 173)	98.1 (*d*, 173)	150.7 (*d*, 197)	55.2 (*t*, 149)	89.0 *d*
4	102.5 *s*	102.0 *s*	51.1 (*d*, 130)	51.0	49.0	44.6 (*d*, 135)	42.3 (*d*, 133)	104.1 *s*	37.5 (*d*, 134)	43.7 *d*
5	47.5 (*d*, 150)	47.7 (*d*, 149)	44.7 (*d*, 143)	44.8	44.8	46.9 (*d*, 144)	36.2 (*d*, 143)	38.6 (*d*, 146)	35.9 (*d*, 146)	46.0 *d*
6	80.3 (*d*, 169)	78.5 (*d*, 167)	86.4 (*d*, 161)	86.5	86.5	85.7 (*d*, 164)	87.2 (*d*, 164)	84.4 (*d*, 167)	86.7 (*d*, 165)	82.9 *d*
7	78.8 (*d*, 146)	84.1 (*d*, 146)	41.9 (*t*, 130)	41.6	41.6	38.5 (*t*, 133)	41.4 (*t*, 133)	41.8 (*t*, 132)	40.9 (*t*, 135)	37.4 *t*
8	42.1 (*d*, 125)	41.1 (*d*, 128)	33.5 (*d*, 124)	33.3	33.3	37.0 (*d*, 125)	33.4 (*d*, 120)	32.3 (*d*, 127)	34.5 (*d*, 130)	35.2 *d*
9	72.3 *s*	72.1 *s*	44.7 (*d*, 140 ×)	42.9	44.4	74.9 *s*	41.4 (*d*, 129)	45.5 (*d*, 138)	45.6 (*d*, 134)	72.7 *s*
10	9.7 (*q*, 129)	9.8 (*q*, 126)	17.4 (*q*, 125)	17.2	17.2	10.5 (*q*, 126)	16.5 (*q*, 126)	17.5 (*q*, 125)	16.8 (*q*, 126)	9.1 *q*
11	174.5 *s*	174.3 *s*	183.1 *s*	182.6	182.6	178.5 *s*	179.5 *s*	175.0 *s*	182.3 *s*	176.0 *s*
1′		102.0 (*d*, 162)		103.4	103.2	103.3 (*d*, 159)	103.1 (*d*, 160)	99.4 (*d*, 163)	98.0 (*d*, 163)	
2′		74.1		73.7	73.9	75.0	74.2	73.4	73.8	
3′		76.5		76.3	76.7	75.7	76.5	76.3	76.6	
4′		70.5		70.4	70.4	71.1	70.3	70.4	70.5	
5′		76.9		76.7	76.7	77.4	77.2	77.1	77.1	
6′		61.5		61.5	61.5	61.5	61.5	61.5	61.5	

*Spectra were recorded at 22.6 MHz, and spectra of glucosides and their acetates were aligned to C-6′ = 61.5 ppm, see ref. [2]. Solvents: Compounds **1**–**8**, **10**: D_2O; compound **12**: $CDCl_3$–CD_3OD; compounds **1a**, **1b**, **2a**, **3a**, **4a**, **5a**, **6a**, **6b**, **7a**, **8a**: $CDCl_3$.

hydrogen bonds (O . . . O distances 2.82 and 2.74 Å, respectively). These bonds are formed between the two hydroxyl groups at O(2) and O(3) of one molecule to the oxygen atoms O(3) and O(5) of two different neighbouring molecules.

Gelsemide 7-glucoside (**2**) was obtained from the aqueous extract. The ^1H NMR spectrum was very similar to that of **1**, except that signals from the β-glucopyranosyl moiety were present, indicating that **2** was a glucoside of **1**. The ^{13}C NMR spectrum confirmed this conclusion, as six signals, evidently arising from a β-glucopyranosyl moiety, were seen in addition to the 10 signals arising from the aglucone. Furthermore, in the spectrum of **2**, compared to that of **1**, a large downfield shift was seen for C-7, with smaller upfield shifts for C-6 and C-8 (Table 1) proving the position of glucosyloxylation at C-7. Acetylation under mild conditions provided a tetraacetate (**2a**), exhibiting NMR spectra in accordance with the assigned structure. An attempt to hydrolyse **2** with emulsin failed.

Table 2. ^1H NMR NOEs obtained for compound **1** (%)

Irradiated proton	Observed proton					
	3	5	6	7	8	10
5	2	–	7	n.d.	0	2
6	3	11	–	15	0	5
7	1	1	10	–	3	6
8	4	0	1	*ca* 3	–	5

The experiments were performed at 270 MHz ($CDCl_3$ without degassing) at 310 K in the difference mode.

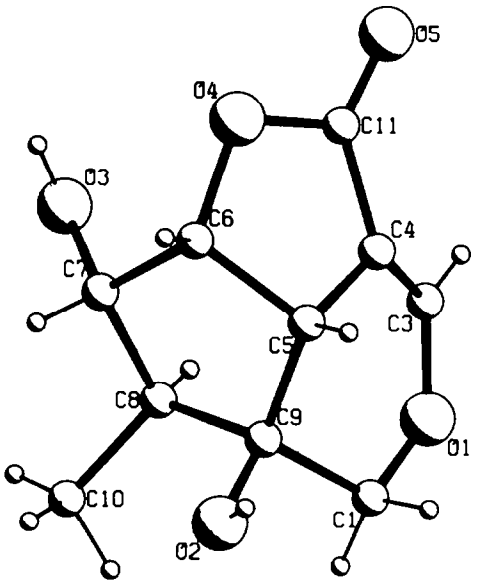


Fig. 1. The molecular conformation found for gelsemide and the atomic labels used.

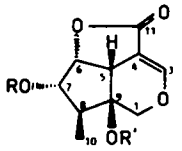
for iridoids and their acetates*

1a	1b	2a	3a	4a	5a	6a	6b	7a	8a
66.9	64.3 (*t*, 153)	66.6	63.6 (*t*, 152)	70.1	63.5	59.6 (*t*, 146)	59.3	55.7	93.0 (*d*, 175)
151.9	151.3 (*d*, 194)	151.1	62.7 (*t*, 147)	62.7	66.9	97.3 (*d*, 174)	97.0	97.4	147.3 (*d*, 175)
101.7	101.4 *s*	101.1	47.5 (*d*, 131)	47.3	48.4	43.2 (*d*, 133)	42.7	41.1	104.0 *s*
47.4	46.5 (*d*, 148)	47.1	44.0 (*d*, 135)	43.5	43.9	46.1 (*d*, 143)	43.2	35.3	37.8 (*d*, 149)
76.4	77.6 (*d*, 169)	75.5	82.7 (*d*, 159)	83.5	82.6	82.7 (*d*, 161)	82.6	83.5	80.8 (*d*, 162)
79.8	79.9 (*d*, 155)	82.4	41.4 (*t*, 130)	41.3	41.3	37.9 (*t*, 131)	38.4	41.1	41.8 (*t*, 131)
39.4	41.5 (*d*, 128)	40.1	33.1 (*d*, 124)	33.1	32.2	35.2 (*d*, 128)	36.8	31.9	31.2 (*d*, 130)
71.1	80.4 *s*	71.2	40.7 (*d*, 126)	42.1	40.4	73.5 *s*	83.8	40.7	44.6 (*d*, 131)
9.4	10.9 (*q*, 127)	8.6	16.9 (*q*, 126)	16.8	16.8	9.6 (*q*, 126)	11.0	16.1	17.5 (*q*, 126)
–	–	175.6	176.6 *s*	177.5	177.0	174.1 *s*	174.0	174.7	–
		98.8		101.3	100.0	99.7 (*d*, 160)	99.8	99.7	95.9 (*d*, 164)
		70.6		70.4	70.8	71.2	71.3	71.3	70.3
		72.2		72.0	72.4	72.2	72.4	72.3	72.0
		68.2		68.1	68.1	67.9	68.0	67.9	67.9
		70.8		71.6	71.6	72.0	72.0	72.0	72.0
		61.5		61.5	61.5	61.5	61.5	61.5	61.5

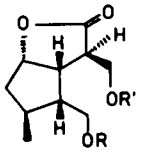
Besides **1**, another non-glucosidic iridoid, gelsemiol (**3**), was isolated from the cultivated plant specimen. Its ^{13}C NMR spectrum contained 10 signals, one arising from an ester carbonyl group (183 ppm). A signal at 86 ppm (*d*, J = 161 Hz) was consistent with the presence in **3** of a lactone ring, as in **1** and **2**, while two triplets at *ca* 63 ppm were assigned to two primary hydroxyl groups. The remaining six signals at high field could be attributed to four methine, one methylene, and one methyl carbon atoms. Assuming a biogenetic relationship between gelsemiol and **1**, the formula **3** could be drawn as a tentative structure. The 400 MHz ^1H NMR spectrum was well resolved and successive decoupling experiments (see Experimental) confirmed the gross structure **3**, consistent with an iridoid origin of **3** and thus suggestive of the usual β-H stereochemistry at C-5. As seen from a Dreiding model of **3**, the flatness of the 5-membered lactone ring demands a ^8V or a V_8 conformation of the cyclopentane ring resulting in a rather rigid bicyclic system. As fusion can only take place in the *cis*-fashion, the stereochemistry at C-6 becomes settled with H-5 and H-6 in the β-positions. In the ^1H NMR spectrum of **3**, the coupling constants between H-6 and the two protons at C-7 are *ca* 0 and 8 Hz. This is consistent only with the V_8 conformation of the cyclopentane ring in which the dihedral angles are $\angle H_6H_{7\alpha} \sim 90°$ and $\angle H_6H_{7\beta} \sim 30°$. In the ^8V conformation these angles would be *ca* 150° and 30°, respectively. Thus the 7β proton must be in a pseudo-axial position and the large coupling constants $J_{7\beta,8}$ and $J_{8,9}$ (12 Hz) both show that this is the case also for H-8 and H-9. It follows that the methyl group at C-8 and the hydroxymethyl group at C-9 are placed in β- and α-positions, respectively. The stereochemistry at the remaining centre (C-4) could be determined through the coupling constant $J_{4,5}$ = *ca* 5 Hz. Due to the rigidity of the bicyclic system this is consistent only with a *trans*-disposition of the involved protons placing the hydroxymethyl group (C-3) in a β-position. (In contrast, the $J_{4,5}$ coupling in the similar compounds **6**, **7** and **10**, having the opposite stereochemistry at C-4 (see below), is consistently larger than 10 Hz.) Acetylation of **3** yielded a diacetate (**3a**).

Two glucosides of **3**, namely **4** and **5**, were obtained as an inseparable mixture in the proportion *ca* 2:1, and were named gelsemiol 1-glucoside and gelsemiol 3-glucoside, respectively. Acetylation of the glucoside mixture provided a similarly inseparable mixture of the pentaacetates **4a** and **5a**. Whereas the ^1H NMR spectra of **4**/**5** and **4a**/**5a** were not very informative, the unequal amounts made the ^{13}C NMR spectra of the mixtures readily assignable, even more so because nine of the signals from each glycoside coincided in the two compounds (Table 1). When comparing the spectrum of the major component (**4**) with that of gelsemiol (**3**), almost complete coincidence was seen for all the signals arising from the iridoid moiety, except for those assigned to C-1 and C-9, showing a downfield shift of 7.9 ppm for C-1 and an upfield shift of 1.8 ppm for C-9, consistent with C-1 as the site of glucosylation. Likewise, when the spectrum of **5** was compared with that of **3**, a downfield shift of 8.4 ppm for C-3, and an upfield shift of 2.5 ppm for C-4, demonstrated that **5** was gelsemiol 3-glucoside.

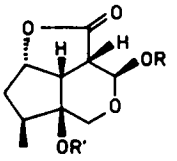
Similar trends were noted when comparing the spectra



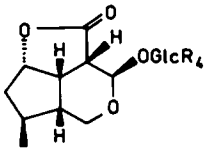
1: R = R' = H
1a: R = Ac; R' = H
1b: R = R' = Ac
2: R = Glc; R' = H
2a: R = GlcAc_4; R' = H



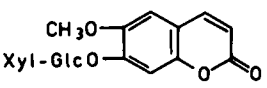
3: R = R' = H
3a: R = R' = Ac
4: R = Glc; R' = H
4a: R = GlcAc_4; R' = Ac
5: R = H; R' = Glc
5a: R = Ac; R' = GlcAc_4



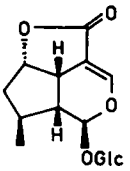
6: R = Glc; R' = H
6a: R = GlcAc_4; R' = H
6b: R = GlcAc_4; R' = Ac
12: R = R' = H
12a: R = Ac; R' = H



7: R = H
7a: R = Ac



9



8

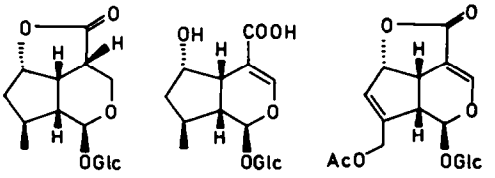
of **3a**, **4a** and **5a**, confirming the above conclusion. Finally, enzymatic cleavage of the **4**/**5** mixture with β-glucosidase provided glucose and gelsemiol as the sole products.

The last major compound from the cultivated plant was named 9-hydroxysemperoside, and the structure **6** was deduced for it. The NMR spectra clearly showed that a β-glucopyranose moiety was present in **6**. The ^{1}H NMR spectrum was completely resolved at 400 MHz and allowed all couplings and shifts to be elucidated. Several features were similar to those seen in the spectra of **1**, **2** and **3**, although the presence of a conjugated 3,4-double bond as in **1** and **2** could be excluded. The triplet at δ 5.21 was assigned to H-6 in a lactone moiety like those found in **1**–**5**. Successive decoupling experiments allowed the identification of on one hand the signals arising from H-5 and H-4, and on the other those from 7-CH_2, H-8 and 10-CH_3. Significantly, neither H-8 nor H-5 showed couplings additional to those demonstrated above, and therefore the presence of a 9-hydroxyl group was surmised. Furthermore, the presence in the spectrum of an AB-system at δ 3.63 and 3.99 (J = 12 Hz) without further couplings—similar to that seen in **1** and **2**—could be assigned to an oxymethylene group in position 1, adding more evidence for the presence of a 9-hydroxyl substitution. Finally, a singlet at δ 5.45, was assigned to H-3 situated at an acetalic centre carrying the glucopyranosyl moiety, leaving H-3 with a dihedral angle of 90° to H-4. Thus the initial analysis of the ^{1}H NMR spectrum led to the gross structure **6**, exhibiting the unusual iridoid features of a 9-hydroxyl group and a 3-glucopyranosyloxy group. The couplings observed between H-5, H-6, H-7α, H-7β, H-8 and the 10-CH_3 group were virtually the same as those seen in **3**, demonstrating the V_8 conformation of the cyclopentane ring with the 10-CH_3 group attached in the β-position. Examination of a Dreiding model of **6** showed that this was rather rigid and required a somewhat flattened $^5C_0$ conformation of the tetrahydropyrane ring. In order to exhibit the presumed 90° dihedral angle between H-4 and H-3 ($J_{3,4}$ = < 0.5 Hz) the glucopyranosyloxy moiety must occupy an axial position. The ^{13}C NMR spectrum of **6** fully supported the structure (Table 1). Mild acetylation of **6** provided a tetraacetate (**6a**) while forcing conditions gave a pentaacetate (**6b**), demonstrating the presence in **6** of a hindered hydroxyl group.

Two additional iridoid glucosides were isolated in minor amounts from the cultivated plant, namely semperoside, later assigned structure **7**, (see below) and brasoside (**8**), previously known from *Verbena brasiliensis* [3].

The plant sample collected in the wild differed chemically from the one analysed above. Thus, **1**, **3**, **4**, and **5** were absent from this plant while **2**, **6** and **8** were the main constituents, accompanied by smaller amounts of **7**. This compound, semperoside, was the last novel glucoside. Its ^{1}H NMR spectrum was similar to that of **6**, the main difference being a signal arising from an additional proton at high field in **7**. At 400 MHz the spectrum was well resolved and allowed all the coupling constants and chemical shifts to be measured. The coupling constants were virtually the same as those seen in the spectrum of **6**, except for those arising from the additional proton, demonstrating the same relative stereochemistry and the same conformation in **6** and **7**. Since the additional proton showed coupling to one H-1, to H-5 and to H-8, it could only be assigned as H-9, thus confirming the structure of **7** as the 9-deoxy derivative of **6**. The coupling pattern resulting from the presence of the proton at C-9 gave additional proof about the conformation deduced above (for **6**). Firstly, the coupling constant $J_{8,9}$ (12 Hz) confirmed the V_8 conformation of the cyclopentane ring and secondly, the very small (< 0.5 Hz) coupling constants $J_{1\alpha,9}$ and $J_{3,4}$ reflected the symmetry of the pairwise disposition of the involved protons and thus the rigid $^5C_0$ conformation of the tetrahydropyrane ring. The ^{13}C NMR spectrum was in keeping with the above analysis, namely that **7** was a deoxy-derivative of **6**. When comparing the spectra of **7** and **6**, we found a large downfield shift for C-9 (34 ppm), with smaller shifts in the same direction for C-1, C-5 and C-8 (5, 12 and 4 ppm, respectively) as would be expected upon substitution by a 9-hydroxyl group.

Besides the above compounds the 'normal' iridoid brasoside (**8**) was found in this plant sample as one of the major constituents. Brasoside has been correlated with cornin [4] and has the absolute configuration depicted in the figure (**8**). As the dihydro-derivative (**10**) of brasoside would be isomeric with semperoside (**7**), it seemed relevant to make a comparison between the physical data of these two compounds. Therefore, brasoside (**8**) was subjected to catalytic hydrogenation to give dihydrobrasoside (**10**) as the sole product. Considering the different positions of the sugar moieties in **7** and **10** a striking



10 11 13

similarity in shifts and particularly coupling constants in the ^{1}H NMR spectra could be seen. This demonstrated that the aglucone moieties in the two compounds took up very similar conformations. As could be expected, the ^{13}C NMR spectra were less similar, carbon shifts being more sensitive to structural changes. Of particular interest was the significantly higher C-1′ shift in **7** (and **6**), namely 103 ppm, when compared with that found in **10** (98 ppm).

In order to correlate **3** with **7** and/or **10**, the two latter compounds were subjected to cleavage with β-glucosidase. However, neither compound appeared to be susceptible to the enzyme (see Experimental). On the other hand, **6** readily underwent hydrolysis to give the aglucone **12**. Acetylation provided the crystalline monoacetate **12a**.

In addition to the iridoids so far described, we isolated from the wild collection of the plant the coumarin glycoside fabiatrin (**9**), first isolated from *Fabiana imbricata* (Solanaceae) [5]. The physical data obtained for **9** and the hexaacetate (**9a**) compared well with those reported [6] (see Experimental).

In iridoids **1**, **2** and **6** all carry a hydroxyl group at C-9 and the compounds **6** and **7** a glucopyranosyloxy moiety at the 3-position. Neither of these features have previously been encountered among the approximately 300 iridoids reported so far [7].

EXPERIMENTAL

Microanalyses were performed at NOVO Microanalytical Laboratory, Bagsvaerd, Denmark. Mps are corr. Prep. TLC (PLC) was performed on 1 mm silica gel and the compounds detected by UV or by charring with a glowing wire. A plant grown from seeds collected in Tallahassee, Florida in 1961 and cultivated in the Botanical Garden, The University of Copenhagen (voucher P 1963/596; authenticated by Dr. K. Rahn) provided fresh plant material in 1975–76; it was kept in the deep-freeze until use. Dry plant material was obtained from a collection in the wild near Duke University campus, Durham Co., North Carolina. The voucher (W. J. Kress 83–619) has been deposited in The University herbarium (DUKE).

Extraction of frozen sample. Foliage (280 g) was homogenized in EtOH, and the extract taken to dryness followed by partition in H_2O–Et_2O. The organic phase was again extracted with H_2O–MeOH (3:1) and the aq. fractions combined and again taken to dryness. Passage through neutral Al_2O_3 (400 g) in H_2O followed by washing with H_2O (1000 ml) gave, after evapn, a colourless syrup (12.2 g). TLC and HPLC at this point indicated the presence of compounds with widely differing polarity and therefore the mixture was dissolved in H_2O and extracted with EtOAc (3 × 50 ml), to give, after evapn, fraction A (11.4 g) and fraction B (810 mg), respectively. Fraction B was applied to a reversed phase column (Merck Lobar RP-8 (C)) and eluted with H_2O–MeOH (5:1 to 2:1); UV-detection at 254 and 206 nm. Carbohydrates were first eluted followed by gelsemide (**1**, 160 mg). Crystallization from EtOH gave the pure compound mp 179–180°; $[\alpha]_D^{20}$ −343° (MeOH, *c*, 0.9). ^{1}H NMR (270 MHz, D_2O): δ 7.53 (*d*, *J* = 2.5 Hz, H-3), 5.12 (*t*, *J* = 7.2 Hz, H-6), 4.25 and 3.95 (AB-system, *J* = 11.9 Hz, 1-CH_2), 4.14 (*dd*, *J* = 7.2 and 10.5 Hz, H-7), 3.44 (*dd*, *J* = 2.5 and 7.3 Hz, H-5), 1.98 (*dq*, *J* = 6.8 and 10.5 Hz, H-8), 1.11 (*d*, *J* = 6.8 Hz, 10-CH_3). (Found: C, 56.5; H, 5.7. $C_{10}H_{12}O_5$ requires: C, 56.6; H, 5.7%.) The last fraction consisted of almost pure gelsemiol (**3**) which was purified by PLC ($CHCl_3$–MeOH, 4:1) to give the pure compound (140 mg) mp 91–93°; $[\alpha]_D^{20}$ +13° (MeOH, *c* 0.5). ^{1}H NMR (400 MHz, D_2O): δ 5.09 (*dd*, *J* = 6 and 8 Hz, H-6) 3.94 (*dd*, *J* = 4.5 and 11.5 Hz, H-3x), 3.84 (*dd*, *J* = 4.5 and 11.5 Hz, H-1x), 3.82 (*dd*, *J* = 4 and 11.5 Hz, H-3y), 3.59 (*dd*, *J* = 9.5 and 11.5 Hz, H-1y), 3.11 (*dt*, *J* = 5.5 and 8.0 Hz, H-5), 2.95 (*q*(*br*), *J* = *ca* 4.5 Hz, H-4), 2.12 (*dd*, *J* = 6 and 14.5 Hz, H-7α), 1.86 (*dddd*, *J* = 4.5, 8, 9 and 12 Hz, H-9), 1.75 (*dtq*, *J* = 6, 12 and 6 Hz, H-8), 1.55 (*ddd*, *J* = 6.5, 12 and 14.5 Hz, H-7β), 1.00 (*d*, *J* = 6.1 Hz, 10-CH_3). (Found: C, 58.0; H, 8.0. $C_{10}H_{16}O_4$, 0.4 H_2O requires: C, 57.9; H, 8.2%.)

Fraction A from above was dissolved in H_2O–MeOH (2:1, 35 ml), mixed with silica gel (110 g) and taken to dryness. Elution with Me_2CO (1 l) in a column gave a mixture of glucosides (4.35 g) which was applied to a home-made low pressure reversed phase column loaded with 2 kg silica gel [8]. Elution was done with H_2O–MeOH (10:1, 5:1, and 3:1) to give fraction C (400 mg) and fraction D (1450 mg). PLC of the former (silica gel, $CHCl_3$–MeOH, 3:1) gave 2 UV-absorbing bands. The faster moving band was gelsemide (**1**, 190 mg; total 0.13%) while the slower moving band consisted of gelsemide 7-glucoside (**2**, 200 mg, 0.07%), isolated as a foam, $[\alpha]_D^{20}$ −199° (MeOH; *c* 0.6). ^{1}H NMR (90 MHz, D_2O): δ 7.51 (*d*, *J* = 2.5 Hz, H-3), 5.24 (*t*, *J* = 7.3 Hz, H-6), 4.57 (*d*, *J* = 7 Hz, H-1′), 4.36 (*dd*, *J* = 7 and 10 Hz, H-7), 4.24 and 3.91 (AB-system, *J* = 12 Hz, 1-CH_2), 2.07 (*m*, H-8), 1.12 (*d*, *J* = 7 Hz, 10-CH_3). (Found: C, 47.4; H, 6.2. $C_{16}H_{22}O_{10}$, 2 H_2O requires: C, 47.1; H, 5.9%.)

Fraction D was also further separated by prep. TLC ($CHCl_3$–MeOH, 4:1) to give 4 fractions. The fastest moving band gave gelsemiol (**3**, 79 mg; total 0.08%). The next band (58 mg) could be further separated into semperoside (**7**, 11 mg) and brasoside (**8**, 15 mg), see below. The third band (315 mg, 0.11%) was a mixture of gelsemiol 1-glucoside (**4**) and gelsemiol 3-glucoside (**5**). These two compounds could not be separated in any chromatographic systems tested, and were solely characterized by NMR, which showed **4** and **5** to be present in the proportion *ca* 2:1. ^{1}H NMR spectrum of **4** (270 MHz, D_2O): δ 5.16 (*t*, *J* = 7 Hz, H-6), 4.48 (*d*, *J* = 8 Hz, H-1′), 4.24 (*dd*, *J* = 4 and 10 Hz, H-1), 3.02 (*q*(*br*), *J* = 6.5 Hz, H-4), 1.08 (*d*, *J* = 6.2 Hz, 10-CH_3). ^{1}H NMR spectrum of **5**: 5.19 (*t*, *J* = 7 Hz, H-6), 4.50 (*d*, *J* = 8 Hz, H-1′), 4.20 (*dd*, *J* = 3.9 and 10.2 Hz, H-3), 3.14 (*t*(*br*), *J* = 6.7 Hz, H-4), 1.07 (*d*, *J* = 6 Hz, 10-CH_3). The slowest moving fraction consisted of pure 9-hydroxysemperoside (**6**, 720 mg, 0.26%), crystallized from EtOH, mp 132–135°, $[\alpha]_D^{20}$ +58° (MeOH, *c* 1.1). ^{1}H NMR (400 MHz, D_2O): δ 5.45(*s*, H-3), 5.21 (*dd*, *J* = 4.5 and 6 Hz, H-6), 4.72 (*d*, *J* = 8 Hz, H-1′), 3.98 and 3.64 (AB-system, *J* = 12.0 Hz, 1-CH_2), 3.41 (*d*, *J* = 11.4 Hz, H-4), 3.00 (*m*, H-5), 2.13 (*dd*, *J* = 6 and 14 Hz, H-7α), 1.97 (*m*, H-8), 1.84 (*dt*, *J* = 4.5 and 14 Hz, H-7β), 0.98 (*d*, *J* = 6.5 Hz; 10-CH_3). (Found: C, 49.5; H, 6.7. $C_{16}H_{24}O_{10}$, 0.5 H_2O requires: C, 49.9, H, 6.6%.)

Acetylation of gelsemide (**1**). Acetylation overnight (Ac_2O–C_5H_5N, room temp.) gave as the main product the 7-*O*-acetate, (**1a**) mp (toluene) 162–164°, $[\alpha]_D^{20}$ −399° ($CHCl_3$, *c* 0.7). ^{1}H NMR (90 MHz, $CDCl_3$): δ 7.28 (*d*, *J* = 2.5 Hz, H-3), 5.10 (*t*, *J* = 7 Hz, H-6), 4.72 (*dd*, *J* = 7 and 10 Hz, H-7), 4.00 and 3.76 (AB-system, *J* = 12 Hz, 1-CH_2), 3.30 (*dd*, *J* = 2.5 and 7 Hz, H-5), 2.1 (*m*, H-8), 2.00 (*s*, OAc), 0.98 (*d*, *J* = 7 Hz, 10-CH_3). (Found: C, 56.3; H, 5.6. $C_{12}H_{14}O_6$ requires: C, 56.7; H, 5.6%.) Using forcing conditions (addition of 4-dimethyl-aminopyridine, 60°, 48 hr),

the diacetate (**1b**) was obtained, mp (EtOH) 119–120°, $[\alpha]_D^{20}$ −288° ($CHCl_3$, *c* 0.6). ^1H NMR (90 MHz, $CDCl_3$): δ 7.37 (*d*, *J* = 2 Hz, H-3), 5.31 (*t*, *J* = 7 Hz, H-6), 4.97 (*dd*, *J* = 7 and 8 Hz, H-7), 4.16 (*s*, 1-CH_2), 3.76 (*dd*, *J* = 2 and 7 Hz, H-5), 2.36 (*m*, H-8), 2.08 and 2.06 (*s*'s, 2 × OAc), 1.04 (*d*, *J* = 7 Hz, 10-CH_3). (Found: C, 56.7; H, 5.5. $C_{14}H_{16}O_7$ requires: C, 56.8; H, 5.5%.)

Acetylation of gelsemide 7-O-glucoside (**2**). Acetylation (Ac_2O–C_5H_5N, 2 hr) gave the tetraacetate (**2a**), mp (toluene–EtOH, 19:1) 134–136°, $[\alpha]_D^{20}$ −179° ($CHCl_3$, *c* 0.5). ^1H NMR (90 MHz, $CDCl_3$): δ 7.28 (*d*, *J* = 2.5 Hz, H-3), 4.6 (*d*, *J* = 7 Hz, H-1'), 4.00 and 3.78 (AB-system, *J* = 11.5 Hz, 1-CH_2), 3.26 (*dd*, *J* = 2.5 and 7 Hz, H-5), 2.14, 2.07, 2.02, and 1.99 (*s*'s, 4 × OAc), 1.04 (*d*, *J* = 7 Hz, 10-CH_3). (Found: C, 51.5; H, 5.6. $C_{24}H_{30}O_{14}$, H_2O requires: C, 51.4; H, 5.8%.)

Acetylation of gelsemiol (**3**). This provided the diacetate (**3a**), a syrup, $[\alpha]_D^{20}$ +6° ($CHCl_3$, *c* 0.9). ^1H NMR (90 MHz, $CDCl_3$): δ 4.87 (*dd*, *J* = 6 and 7 Hz, H-6), 4.29 (*dd*, *J* = 4 and 12 Hz, H-1x), 4.28 (*d*, *J* = 4 Hz, 3-CH_2), 3.93 (*dd*, *J* = 8.5 and 12 Hz, H-1y), 2.9 (*m*, H-5), 2.75 (*m*, H-4), 2.02 and 2.01 (*s*'s, 2 × OAc), 2.25–1.35 (4H), 1.00 (*d*, *J* = 5.5 Hz, 10-CH_3). (Found: C, 58.7; H, 7.2. $C_{14}H_{20}O_6$ requires: C, 59.1; H, 7.1%.)

Acetylation of 9-hydroxysemperoside (**6**). Under mild conditions this gave the tetraacetate (**6a**), mp (EtOH) 206.5–207°, $[\alpha]_D^{20}$ +48° ($CHCl_3$, *c* 0.6). ^1H NMR (500 MHz, $CDCl_3$): δ 5.32 (*s* (*br*), H-3), 4.99 (*dd*, *J* = 4.7 and 6.4 Hz, H-6), 4.72 (*d*, *J* = 7.8 Hz, H-1'), 3.90 and 3.44 (AB-system, *J* = 12.0 Hz, 1-CH_2), 2.94 (*dd*, *J* = 6.5 and 11.3 Hz, H-5), 2.88 (*d*, *J* = 11.5 Hz, H-4), 2.08, 2.06, 2.02, and 2.00 (*s*'s, 4 × OAc), 1.85 (*dt*, *J* = 4.8 and 12.0 Hz, H-7β). (Found: C, 52.5; H, 5.9. $C_{24}H_{32}O_{14}$ requires: C, 52.9; H, 5.9%.) Forcing conditions (as above) gave the pentaacetate (**6b**), mp (EtOH) 147–148°, $[\alpha]_D^{20}$ +40° ($CHCl_3$, *c* 0.5). ^1H NMR (90 MHz, $CDCl_3$): δ 5.33 (*s*, H-3), 4.99 (*dd*, *J* = 4.5 and 6.5 Hz, H-6), 4.72 (*d*, *J* = 7 Hz, H-1'), 4.46 and 3.71 (AB-system, *J* = 12 Hz, 1-CH_2), 3.61 (*dd*, *J* = 6.5 and 12 Hz, H-5), 2.91 (*d*, *J* = 12 Hz, H-4), 2.08, 2.06, 2.02, 2.00 and 1.99 (*s*'s, 5 × OAc), 1.02 (*d*, *J* = 6 Hz, 10-CH_3). (Found: C, 53.3; H, 5.8. $C_{26}H_{34}O_{15}$ requires: C, 53.2; H, 5.9%.)

Extraction of dry sample. The dry foliage (223 g) was homogenized with EtOH (2 l) and left to stand for 1 week, after which it was worked up as above to give fraction A (13.1 g) and fraction B (2.1 g). Chromatography of fraction B on the Lobar-C column as above gave as the only iridoid brasoside (**8**, 340 mg), isolated as a foam $[\alpha]_D^{20}$ −283° (EtOH, *c* 1.4). Ref. [3]: $[\alpha]_D^{20}$ −170° (EtOH, *c* 0.97). ^1H NMR (500 MHz, D_2O): δ 7.45 (*d*, *J* = 2.6 Hz, H-3), 5.75 (*s*(*br*), H-1), 5.20 (*t*, *J* = 7.7 Hz, H-6), 4.91 (*d*, *J* = 8.0 Hz, H-1'), 3.50 (*dt*, *J* = 2.5 and 7.2 Hz, H-5), 2.18 (*ddd*, *J* = 0.8, 6.8 and 11.0 Hz, H-9), 2.08 (*dd*, *J* = 7.6 and 15.2 Hz, H-7α), 1.94 (*m*, H-8), 1.73 (*ddd*, *J* = 8.0, 11.4 and 15.1 Hz, H-7β), 1.03 (*d*, *J* = 7 Hz, 10-CH_3), except for $\delta_{H\text{-}1'}$ in good agreement with that reported [3].

Fraction A was chromatographed on the large reversed phase column as above to give four fractions, C–F. Fraction C consisted of almost pure gelsemide 7-glucoside (**2**, 229 mg; 0.1%). Fraction D was 9-hydroxysemperoside (**6**, 690 mg; 0.3%). Fraction E (1720 mg) was further separated by PLC (EtOAc–EtOH–PhMe, 4:2:1) to give as the faster moving band brasoside (**8**, 890 mg, total 0.55%) while the slower moving band provided semperoside (**7**, 114 mg, 0.05%), mp (EtOH) 179–181°, $[\alpha]_D^{20}$ +52° (MeOH, *c* 0.3). ^1H NMR (400 MHz, D_2O): δ 5.35 (*s*, H-3), 5.08 (*dd*, *J* = 4.5 and 5.5 Hz, H-6), 4.61 (*d*, *J* = 8 Hz, H-1'), 4.20 (*dd*, *J* = 4.1 and 12.7 Hz, H-1β), 3.59 (*d*(*br*), *J* = 12.7 Hz, H-1α), 3.23 (*m*, H-5), 3.08 (*d*, *J* = 10.5 Hz, H-4), 2.10 (*dd*, *J* = 5.3 and 14.2 Hz, H-7α), 1.74 (*m*, H-8), 1.44 (*ddd*, *J* = 4, 10 and 12 Hz, H-9), 1.36 (*ddd*, *J* = 4.3, 13.0 and 14.0 Hz, H-7β), 0.94 (*d*, *J* = 6.4 Hz, 10-CH_3). No correct analysis could be obtained for this compound. Fraction F consisted of fabiatrin (**9**, 360 mg, 0.16%), mp (H_2O) 240–242°, $[\alpha]_D^{20}$ −136° (H_2O *c* 0.6). Ref. [6]: mp 236–238°, $[\alpha]_D^{20}$ −140° (H_2O, *c* 0.5). ^1H NMR (90 MHz, D_2O): δ 7.65 (*d*, *J* = 9 Hz, H-4), 7.00 and 6.85 (*s*'s, H-5 and H-8), 6.20 (*d*, *J* = 9 Hz, H-3), 5.08 and 4.33 (*d*'s, *J* = *ca* 7 Hz, H-1' and H-1''), 3.81 (*s*, OMe).

Acetylation of brasoside (**8**). This gave the tetraacetate (**8a**), mp (MeOH) and mmp 188–189°, $[\alpha]_D^{20}$ −229° ($CHCl_3$, *c* 0.9). Ref. [4]: mp 185–187°. The specific rotation was not reported, but for an authentic sample we found $[\alpha]_D^{20}$ −225° ($CHCl_3$, *c* 0.3). ^1H NMR (90 MHz, $CDCl_3$): δ 7.25 (*d*, *J* = 2.5 Hz, H-3), 5.45 (*s*(*br*), H-1), 4.9 (*m*, partly obsc., H-6), 3.24 (*ddd*, *J* = 2.5, 4 and

Table 3. Experimental conditions for the crystal structure determination

Name	Gelsemide
Formula	$C_{10}H_{12}O_5$
Formula weight	212.20
Space group	$P2_1$
Unit cell dimensions	a = 6.256(2) b = 7.488(2) c = 10.391 (2) Å and β = 84.94 (2)°
V	484.9 (3) Å^3
Z	2
D_x	1.453 (1) g/cm^3
Radiation	MoKα
λ	0.71069 Å
μ	1.1 cm^{-1}
F(000)	224
T	293°K
R	0.028
Crystal shape	Prismatic
Crystal size	0.5 × 0.2 × 0.1 mm
Diffractometer	CAD 4
Determination of unit cell:	
Number of reflections used	15
θ-range of reflections used	11–15°
Intensity data collection:	
Max. of sin θ/λ	0.807 Å^{-1}
Range of h, k and l	0 to 10, 0 to 12 and −16 to 16
Standard refls	(142) and (115)
Intensity variation	$< 0.05 \cdot I_{net}$ ($< 4\sigma(I_{net})$)
No. of measured refls	2728
No. of unique refls	2250
No. of observed refls	935
Criterion for observed refls	$I_{net} > 5\sigma(I_{net})$
R_{int}	0.022
Absorption correction	
Linear absorption coefficient	1.1 cm^{-1}
Min. and max. transmission	0.97–0.99
Structure determination technique	Direct methods
Determination of H-atoms	$\Delta\rho$
Structure refinement:	
Minimization of	$\sum w(\Delta F)^2$
Anisotropic model for	C and O
Isotropic model for	H
Parameters fixed for	y of O(2) (origin)
Number of parameters	183
Weighting scheme	$1/(\sigma^2(F_o) + 0.0002\ F_o^2)$
Final R	0.028
Final wR	0.033
Max. final Δ/σ	0.2
Max. and min. $\Delta\rho$	0.2 and −0.2 e$^-$/Å^3

5 Hz, H-5), 2.09, 2.02, 1.99 and 1.97 (*s*'s, 4 × OAc), 1.03 (*d*, *J* = 5 Hz, 10-CH_3), in agreement with that reported [4].

Acetylation of semperoside (**7**). This provided the tetraacetate (**7a**), mp (EtOH) 188–190°, $[\alpha]_D^{20}$ + 52° ($CHCl_3$, *c* 0.6). ^{1}H NMR (400 MHz, $CDCl_3$): δ 5.34 (*s*(*br*), H-1), 4.96 (*dd*, *J* = 4.4 and 6.2 Hz, H-6), 4.73 (*d*, *J* = 8.1 Hz, H-1'), 4.22 (*dd*, *J* = 3.9 and 12.6 Hz, H-1β), 3.52 (*d*, *J* = 12.6 Hz, H-1α), 3.18 (*dt*, *J* = 6.5 and 10.4 Hz, H-5), 2.66 (*d*(*br*), *J* = 10.7 Hz, H-4), 2.23 (*dd*, *J* = 5.2 and 14.2 Hz, H-7α), 2.09, 2.08, 2.04 and 2.02 (*s*'s, 4 × OAc), 2.0 (*m*, H-8), 1.36 (*m*, H-9), 1.34 (*m*, H-7), 1.02 (*d*, *J* = 6.3 Hz, 10-CH_3). (Found: C, 54.6; H, 6.2. $C_{24}H_{32}O_{13}$ requires: C, 54.5; H, 6.1%.)

Acetylation of fabiatrin (**9**). This gave the syrupy hexaacetate (**9a**), solely characterized by NMR. ^{1}H NMR (90 MHz, $CDCl_3$): δ 7.61 (*d*, *J* = 9.5 Hz, H-4), 7.07 and 6.91 (*s*'s, H-5 and H-8), 6.30 (*d*, *J* = 9.5 Hz, H-3), 3.87 (*s*, OMe), *ca* 2 (6 × OAc). ^{13}C NMR (22.6 MHz, $CDCl_3$): δ 160.7 (C-2), 149.0 (C-7), 147.4 (C-6), 142.8 (C-4), 115.2 (C-3), 114.3 (C-4a), 109.8 (C-5), 100.1 and 99.6 (C-1' and C-1''), 73.8, 72.3, 70.9, 68.5 and 66.8 (C-2' to C-6'), 70.9, 70.1, 68.5 and 61.7 (C-2'' to C-5'').

Hydrogenation of brasoside (**8**). To Pd/C (10 mg, 5%) in EtOAc–MeOH (2:1, 5 ml) was led H_2 for 15 min. Brasoside (120 mg) and 0.1 ml Et_3N was added and the reaction was continued for 2.5 hr, when the theoretical amount of H_2 had been absorbed. After work-up dihydrobrasoside (**10**, 113 mg) was isolated as a syrup. ^{1}H NMR (500 MHz, D_2O): δ 5.25 (*s*(*br*), H-1), 5.11 (*dd*, *J* = 3.5 and 6 Hz, H-6), 4.64 (*d*, *J* = 8.0 Hz, H-1'), 4.16 (*dd*, *J* = 4.4 and 12.5 Hz, H-3β), 3.86 (*d*(*br*), *J* = 12.5 Hz, H-3α), 3.26 (*dt*, *J* = 6.0 and 10.2 Hz, H-5), 2.87 (*dd*, *J* = 4.2 and 10.4 Hz, H-4), 2.14 (*dd*, *J* = 5.2 and 14.2 Hz, H-7α), 1.73 (*nonet*-like, *J* = ca 6 Hz, H-8) 1.54 (*dd*, *J* = 10 and 12 Hz, H-9), 1.40 (*ddd*, *J* = 4.0, 12.5 and 14.2 Hz, H-7β), 1.00 (*d*, *J* = 6.2 Hz, 10-CH_3). (Found: C, 51.1; H, 6.9. $C_{16}H_{24}O_9$, H_2O requires: C, 50.8; H, 6.9%.)

Alkaline hydrolysis of brasoside (**8**). 20 mg with NaOH (2M, 2 ml) for 20 hr gave after work-up α-dihydrocorninic acid (**11**, 15 mg), solely characterized by the ^{1}H NMR spectrum (90 MHz, D_2O): δ 7.69 (*d*, *J* = 1 Hz, H-3), 5.21 (*d*, *J* = 8 Hz, H-1), 4.51 (*t*(*br*), *J* = 4 Hz, H-6), 3.00 (*ddd*, *J* = 1, 4 and 9 Hz, H-5), 1.17 (*d*, *J* = 6 Hz, 10-CH_3), superimposable with the spectrum of an authentic sample prepared from cornin [9].

Enzymatic hydrolysis of gelsemiol 1- and 3-glucoside mixture (**4** and **5**). 123 mg with β-glucosidase (50 mg) in H_2O (10 ml) for 0.5 hr gave as the sole products glucose and gelsemiol (**3**, 48 mg after PLC) as seen by ^{1}H NMR.

Enzymatic hydrolysis of 9-hydroxysemperoside (**6**). 183 mg with β-glucosidase (40 mg) showed no appreciable reaction after 2 hr. A further amount (160 mg) of the enzyme was added and cleavage was now effected after 24 hr. Extraction with EtOAc (3 × 20 ml) gave 9-hydroxysemperoside aglucone (**12**, 90 mg), solely characterized by NMR; ^{1}H NMR (90 MHz, $CDCl_3$): δ 5.52 (*s*(*br*), H-3), 5.06 (*q*-like, *J* = 4.5 Hz, H-6), 3.91 and 3.47 (AB-system, *J* = 13 Hz, 1-CH_2), 2.98 (*d*-like, *J* = 3 Hz, H-4 and H-5), 2.35–1.75 (3H, H-7, H-7 and H-8), 0.98 (*d*, *J* = 5.5 Hz, 10-CH_3). Acetylation (2 hr, 20°) provided the monoacetate, (**12a**) mp (EtOH) 150–151°; $[\alpha]_D^{20}$ + 52° ($CHCl_3$, *c* 0.5); ^{1}H NMR (500 MHz, $CDCl_3$): δ 6.43 (*s*(*br*), H-3), 5.04 (*dd*, *J* = 4.7 and 6.4 Hz, H-6), 3.71 and 3.60 (AB-system, *J* = 11.8 Hz, 1-CH_2), 3.01 (*dd*, *J* = 6.5 and 11.4 Hz, H-5), 2.96 (*d*, *J* = 11.4 Hz, H-4), *ca* 2.2 (2H, H-7α and H-8), 2.14 (*s*, OAc, 1.86 (*dt*, *J* = 4.5 and 15.2 Hz, H-7β), 1.00 (*d*, *J* = 6.0 Hz, 10-CH_3). (Found: C, 56.2; H, 6.4. $C_{12}H_{16}O_6$ requires: C, 56.2; H, 6.3%.)

Enzymatic hydrolysis of gelsemide 7-glucoside (**2**), *semperoside* (**7**) *and dihydrobrasoside* (**10**). This was attempted, with β-glucosidase, but no reaction took place.

Crystal structure determination of gelsemide. Single crystals of gelsemide are monoclinic, space group $P2_1$, with the unit cell parameters *a* = 6.256(2), *b* = 7.488(2), *c* = 10.391 (2) Å and β = 84.94 (2)° and with two gelsemide molecules in the unit cell. The structural model, derived by direct methods, was refined to an *R*-value of 0.028 for 935 significant reflections collected with CuKα radiation. The most relevant experimental details are listed in Table 3. Lists of 'atomic coordinates', 'thermal parameters', 'bond distances and angles' and 'observed and calculated structure factors' have been deposited at the Cambridge Crystallographic Data Centre.

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