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DISTRIBUTION OF IRIDOIDS IN DIFFERENT POPULATIONS OF *PHYSOSTEGIA VIRGINIANA* AND SOME REMARKS ON IRIDOIDS FROM *AVICENNIA OFFICINALIS* AND *SCROPHULARIA NINGPOENSIS*

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Key Word Index—*Physostegia virginiana*; Lamiaceae; subspecies; varieties; populations; GC; iridoids; stegioside I, II, III; avicennioside; ningpogenin; acetylmyoporoside.

Abstract—Two subspecies of *Physostegia virginiana* from seven different origins were investigated for iridoid glycosides. The plant material included the authentic sources of 'deoxyloganic acid', isolated in 1970 by one of the authors. This substance has now been identified as a 2.6:1 mixture of 7-deoxy-8-epiloganic acid and 10-deoxygeniposidic acid. Additionally, we isolated 14 known compounds and three new iridoids: stegioside I from *P. virginiana* ssp. *virginiana* (origin Freiburg) and stegiosides II and III from *P. virginiana* ssp. *paemorsa* (origin Chicago). The distribution of 16 out of 20 iridoids, previously reported or now isolated from *P. virginiana*, was determined in all populations. The iridoid patterns were significantly different between the two subspecies. The differences within ssp. *virginiana* were substantially smaller. The structure of 'avicennioside', previously isolated from *Avicennia officinalis*, is revised to linarioside. Ningpogenin, previously isolated from *Scrophularia ningpoensis*, is identical to a compound obtained by partial synthesis from aucubin.

INTRODUCTION

Kooiman isolated three compounds, only characterized by R_f values on paper chromatograms and specific optical rotations, from *Physostegia virginiana* (L.) Benth. without referring to subspecific taxa [1]. In 1970, Rimpler and von Lehmann investigated two varieties of *P. virginiana*, which had been classified as var. *virginiana* and var. *speciosa* (Sweet) Gray. They isolated two iridoid acids, one of which was thought to be 7-deoxyloganic acid (bisdeoxydihydromonotropein) (**1**) [2]. The stereochemistry of this compound was determined by comparison (melting point optical rotation and IR) of the methyl ester and its acetate with a semi-synthetic substance obtained from Inouye *et al.* [3]. The structure of a second iridoid acid seemed to be a monohydroxybisdeoxydihydromonotropein [4]. In 1989, Jensen *et al.* investigated two varieties of *P. virginiana*, also classified as var. *virginiana* and var. *speciosa*. These workers isolated nine neutral iridoids together with 8-epiloganic acid, but could not detect 7-deoxyloganic acid. For biosynthetic reasons, they doubted the occurrence of derivatives of loganic acid in *P. virginiana* [5]. Loganic acid and 8-epiloganic acid are formed via different biosynthetic pathways [6] and, therefore, the exact determination of the configuration at C-8 is of considerable taxonomic significance.

To resolve these seemingly contradictory results, we have now analysed the original plant material of [1] and [5] as well as three additional populations of *P. virginiana* for their iridoid patterns. We also determined or redetermined all of the seven different populations down to subspecies and variety levels. There was a good correlation between the iridoid patterns and the infraspecific classification of *P. virginiana*.

RESULTS

Boivin recognized eight varieties of *P. virginiana* [7], whereas Cantino accepts only two infraspecific taxa, ssp. *virginiana* and ssp. *praemorsa* (Shinners) Cantino [8]. On the basis of these two reports, we determined the specimens of the different origins of *P. virginiana*. The classification of specimen 'Berlin A' as var. *virginiana* [2] was confirmed. However, specimen 'Berlin B', previously classified as 'var. *speciosa*', also turned out to be var. *virginiana*. The two specimens Copenhagen B and Copenhagen A, previously classified as var. *speciosa* and var. *virginiana* respectively [5], did not differ significantly from each other, but were distinguishable from Berlin A and Berlin B. They were both identified as var. *elongata* Boivin. Three further populations were examined and determined as *P. virginiana* var. *speciosa*, *P. virginiana* var. *formosior* (Lunell) Boivin and *P. virginiana* var.

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Table 1. GC determination of the iridoid content in different populations of *P. virginiana*

	*virginiana**								*praemorsa**	
	speciosa†	*formosior*†		*virginiana*†			*elongata*†		*reducta*†	
	Freiburg‡	Göttingen‡		Berlin A‡		Berlin B‡	Copenh A‡	Copenh B‡	Chicago‡	
Iridoid	AP§ (%)	AP§ (%)	R§ (%)	AP§ (%)	R§ (%)	R§ (%)	AP§ (%)	AP§ (%)	AP§ (%)	R§ (%)
Daunoside (**6**)	0.03 (± 0.001)	d	–	–		–	–		–	–
Myoporoside	0.05 (± 0.003)	0.19 (± 0.02)	0.06 (± 0.005)				0.53 (± 0.07)	1.12 (± 0.26)	–	–
Antirrhinoside	0.03 (± 0.008)	0.22 (± 0.09)	0.39 (± 0.16)	0.01 (± 0.002)	0.02 (± 0.004)	0.05 (± 0.01)	0.06 (± 0.02)	0.05 (± 0.01)		
Galiridoside	0.34 (± 0.04)	0.75 (± 0.09)	0.24 (± 0.03)	0.16 (± 0.01)	0.05 (± 0.001)	0.10 (± 0.004)	0.03 (± 0.001)	0.03 (± 0.001)	0.01 (± 0.001)	0.20 (± 0.01)
Stegioside I (**7**)	0.05 (± 0.002)	0.08 (± 0.004)	d		–	–		–		
Ac-harpagide		0.17 (± 0.02)	0.08 (± 0.01)	0.20 (± 0.02)	0.18 (± 0.02)	0.72 (± 0.15)	–	–	–	
Ac-myoporoside	0.11 (± 0.006)	0.29 (± 0.04)	0.08 (± 0.004)	0.01 (± 0.001)	0.02 (± 0.001)	0.01 (± 0.004)	0.03 (± 0.001)	0.06 (± 0.002)		–
Ajugoside	0.07 (± 0.004)	0.16 (± 0.02)	0.07 (± 0.004)	0.23 (± 0.02)	0.13 (± 0.01)	0.15 (± 0.01)	–	0.01 (± 0.001)	–	–
8-Epiloganic acid (**2**)	0.03 (± 0.002)	0.02 (± 0.003)		–	–	0.01 (± 0.001)	–	–	0.01 (± 0.001)	–

Gardoside (**3**)	0.01 (± 0.001)	0.02 (± 0.003)	0.02 (± 0.01)	–	–	–	–	–	–	–
Geniposidic acid (**4**)	0.02 (± 0.001)	0.01 (± 0.001)	–	–	–	–	–	–	–	–
Deox.epilog.acid (**14**)	–	–	–	0.17 (± 0.02)	0.03 (± 0.001)	–	–	–	–	–
Deox.genip.acid (**15**)	–	–	–	0.07 (± 0.01)	d	–	–	–	–	–
Harpagide (**10**)	–	–	–	0.05 (± 0.009)	0.05 (± 0.006)	0.15 (± 0.12)	–	–	–	–
Virginioside	–	–	–	0.12 (± 0.03)	0.19 (± 0.03)	0.20 (± 0.12)	0.01 (± 0.001)	0.79 (± 0.24)	–	–
Glc-antirrhinoside	–	n	n	n	+	+	n	n	n	n
Ajugol (**11**)	–	n	n	n	+	+	n	n	n	n
Stegioside II (**12**)	–	n	n	n	n	n	n	n	+	+
Stegioside III (**13**)	–	–	–	–	–	–	–	–	0.10 (± 0.02)	1.03 (± 0.13)
Total	0.74 (± 0.07)	1.91 (± 0.29)	0.94 (± 0.22)	1.02 (± 0.12)	0.62 (± 0.07)	1.39 (± 0.42)	0.66 (± 0.09)	2.06 (± 0.51)	0.12 (± 0.02)	1.11 (± 0.14)

*Subspecies.
†Variety.
‡Origin.
§Plant parts: AP = aerial parts; R = roots.
+ : Substance isolated from the corresponding source, amount too small for GC comparison.
– : Not detectable (detection limit 0.001%).
d: Detectable, but amount lower than 0.01%.
n: GC identification was not possible because pure reference sample not available.
( ): Standard error of the corresponding amount.

reducta Boivin. According to Cantino [8], the latter variety is *P. virginiana* ssp. *praemorsa*, whereas all other mentioned varieties belong to *P. virginiana* ssp. *virginiana*.

P. virginiana *ssp.* virginiana *var.* speciosa (*origin Freiburg*)

Column chromatography (CC) of the ethanol–water extract on Celite and subsequent flash chromatography (FC) on RP-18 provided several fractions, which contained the neutral iridoids, and one fraction, which contained the iridoid acids. Repeated CC of the latter fraction under acidic [5] and neutral conditions afforded a mixture of crude iridoid acids. HPLC of this mixture on RP-18 under acidic conditions afforded 8-epiloganic acid (**2**), gardoside (**3**) and a fraction containing geniposidic acid (**4**), together with one unidentified iridoid acid. This fraction after methylation with diazomethane and subsequent HPLC gave geniposide (**5**). From the neutral fractions obtained by CC and FC of the ethanol–water extract, we isolated, after repeated chromatography, the known iridoids myoporoside, antirrhinoside, galiridoside, acetylmyoporoside, ajugoside and daunoside (**6**) as well as a new iridoid glucoside, stegioside I (**7**).

The ESI mass spectrum of **7** exhibited characteristic peak patterns at *m/z* 405 $[M + Na]^+$ and 407 (isotope peak) as well as at *m/z* 421 $[M + K]^+$ and 423 (isotope peak). The ratios of the quasimolecular peaks and isotope peaks were *ca* 3:1, indicating a chlorine atom in the molecule. The relative isotope abundances of *m/z* 405 (100.0%), 406 (18.5%), 407 (37.9%) and 408 (7.4%) were consistent with the molecular formula $C_{15}H_{23}O_9Cl$. The ^1H and ^{13}C NMR spectra were very similar to those of **6**. Only the signals of H_3-10, H_2-6 and H-7 showed downfield shifts of 0.14–0.3 ppm, and the signal of C-7 was shifted upfield by 12.1 ppm. Similar shifts for C-7 can be observed for other iridoids on changing a hydroxyl substituent to a chlorine atom [9]. For those reasons, we concluded that the chlorine atom was attached to position 7. The remaining signals in the ^1H and ^{13}C NMR spectra were consistent with a structure such as **7**. After addition of deuterated NaOH to the NMR solution [10] and heating in a water bath for 5 min, the NMR spectrum was identical with that of galiridoside. This conversion supported structure **7** and indicated the usual stereochemistry at C-1, C-5 and C-9. The relative configurations at C-7 and C-8 were proven by NOE experiments: irradiation of H-9 strongly enhanced the signals for H-1 and H-7, but not the signal of H_3-10, indicating a β position of H-7 and an α position of the methyl group at C-8. Therefore, the structure of **7** can be formulated as shown.

P. virginiana *ssp.* virginiana *var.* virginiana (*origins Berlin A and Berlin B*)

The structure of 'deoxyloganic acid' isolated from this material [2] was re-examined. High resolution ^1H and ^{13}C NMR of the authentic methyl ester clearly showed the presence of two iridoids. These compounds were identified as 8-epideoxyloganin (**8**) and 10-deoxygeniposide (**9**) by comparison of their NMR data with those of the literature [11–13]. The ratio of **8** to **9** was determined as 2.6:1 through the intensities of the corresponding H_3-10 signals. The FAB mass spectrum exhibited signals at *m/z* 373 $[M(\mathbf{8}) - H]^-$ and 371 $[M(\mathbf{9}) - H]^-$ and confirmed the identifications. The $[\alpha]_D^{25}$ values of **8** and **9** were reported to be $-115.13°$ (methanol; *c* 0.72) and $-5.50°$ (MeOH; *c* 1.00), respectively [14]. Therefore, a 2.6:1 mixture of **8** and **9** would result in a specific optical rotation of *ca* $-85°$, in good agreement with the experimental value of $-90°$ [4].

In 1970, the structure of 'deoxyloganic acid' had been elucidated by ^1H NMR (60 MHz) and mass spectroscopy (MS) of the corresponding methyl ester and its acetate [2]. Under these conditions, the minor component was overlooked. The relative configuration at C-8 has been determined by comparison of the specific optical rotation with that of an authentic sample of 7-deoxyloganin [3]. The values have been shown to be $[\alpha]_D^{20} - 90°$ (ethanol; *c* 0.295) for the methyl ester of 'deoxyloganic acid' and $[\alpha]_D^{20} - 88.7°$ (ethanol; *c* 0.34) for authentic deoxyloganin [4]. The similarity of the specific optical rotation of the 'dexoyloganin' sample, which was thought to be a pure compound, explains the erroneous assignment of the configuration at C-8.

We also determined the iridoid patterns of the roots and of the aerial parts of the two samples named 'var. *virginiana*' (origin Berlin A) and 'var. *speciosa*' (origin Berlin B), respectively, by TLC and capillary GC (Table 1). The roots of Berlin A and Berlin B showed very similar iridoid patterns. Since we had only small amounts of plant material, we then combined the corresponding fractions of the roots of both samples. From this material, 5-*O*-β-glucosyl-antirrhinoside, virginioside, harpagide (**10**) and ajugol (**11**) were isolated.

The reported 'monohydroxylated bisdeoxydihydromonotropein' [4] was probably 8-epiloganic acid, since we could identify this compound in the authentic plant material of Berlin B by capillary GC (Table 1).

P. virginiana *spp.* virginiana *var.* formosior (*origin Göttingen*)

The iridoid pattern was determined by capillary GC (Table 1)

P. virginiana *ssp.* virginiana *var.* elongata (*origins Copenhagen A and Copenhagen B*)

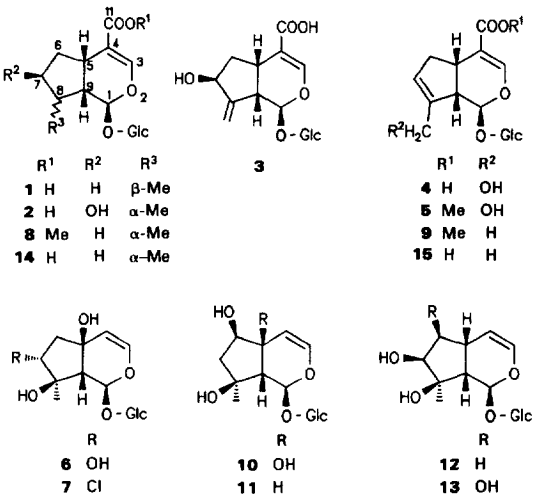
The iridoid patterns of both samples, which consisted of one leaf from each of the original vouchers [5], were investigated by capillary GC (Table 1). Our results agreed with those of Jensen *et al.* [5] with two exceptions: Jensen *et al.* reported the isolation of acetylharpagide (0.01%) and 8-epiloganic acid (0.01%), but we could detect neither 8-epiloganic acid nor acetylharpagide in either of the samples. Since investigations have shown that the iridoid amount of leaves of a single plant can

vary to a great extent [15], the observed divergences may be caused by quantitative differences between different plant parts. The very high amount of myoporoside in our sample compared with the results of Jensen *et al.* [5] supports this suggestion.

P. virginiana *ssp.* praemorsa (*origin Chicago*)

After FC on RP-18, CC on silica gel and HPLC on RP-18 of the ethanol–water extract, we isolated two new iridoid glucosides stegioside II (**12**) and stegioside III (**13**). The ESI mass spectrum of compound **12** showed quasimolecular peaks at *m/z* 371 $[M + Na]^+$ and 387 $[M + K]^+$. The relative isotope abundances of *m/z* 371 (100.0%), 372 (17.5%) and 373 (4.3%) indicated a molecular formula of $C_{15}H_{24}O_9$. The ^{13}C NMR spectrum exhibited the typical signals of a β-glucopyranosyl moiety. Furthermore, three signals (δ 93.9, 137.6, 109.3) could be assigned to C-1, C-3 and C-4 of an iridoid glucoside bearing no substituent at C-4. Additonal signals at δ78.3 and 78.9 indicated the presence of two hydroxylated carbons in the cyclopentane ring. The ^1H NMR spectrum showed the signals of H-3 (δ6.05 *dd*, J = 6.45, 1.8 Hz), H-4 (δ4.83, *dd*, J = 6.45, 3.0 Hz) and H-1 (δ5.37, *d*, J = 1.5 Hz). The remaining signals were assigned by H–H COSY: The signal of H-1 was correlated with the broad doublet of H-9 at δ2.30. H-3 and H-4 showed cross-peaks with a multiplet at δ2.67, which was assigned to H-5. The latter signal showed additional cross-peaks with H_2-6 (δ1.84 and 1.62, respectively). Due to intensive cross peaks of both signals of H_2-6 with a triplet at δ3.66, the latter signal was assigned to H-7. The chemical shift of H-7 indicated a hydroxylation of C-7. The methyl protons at δ1.15 showed no correlation signals and were assigned to H_3-10. As mentioned above, the cyclopentane ring bears two hydroxyl groups, one of them located on C-7. The only location which remained for the second hydroxyl group was on C-8. The relative configurations at C-7 and C-8 as well as the assignments of H-6α and H-6β were determined by NOE experiments: Irradiation of H-4 enhanced one signal of H_2-6 at δ1.62, but not the other one at δ1.84. Conversely, irradiation of H-5 enhanced the signal of H_2-6 at δ1.84, but not the one at δ1.62. Thus, the signals at δ1.84 and 1.62 could be assigned to H-6β and H-6α, respectively. When the signal of H_3-10 was irradiated, positive NOEs were seen for H-1, H-7, H-3 and H-6α, whereas H-9 showed no enhancement. Furthermore, H-7 showed a positive NOE to H-6α, but not to H-6β. Therefore, H_3-10 and H-7 must be located on the alpha-face of the molecule.

The ESI mass spectrum of compound **13** showed quasimolecular peaks at *m/z* 387 $[M + Na]^+$ and 403 $[M + K]^+$. The relative isotope abundances of *m/z* 387 (100.0%), 388 (17.2%) and 389 (4.2%) were consistent with the molecular formula $C_{15}H_{24}O_{10}$. The ^{13}C NMR spectrum showed 15 signals, six of which could be assigned to a β-glucopyranosyl moiety. Additional signals at δ93.5, 138.9 and 105.8 were assigned to C-1, C-3 and C-4 of an iridoid bearing no substituent at C-4. The chemical shifts of the signals at δ77.0, 77.7 and 78.4



indicated the presence of three hydroxylated carbons in the cyclopentane ring. The ^1H NMR spectrum showed the signals of H-1 (δ5.40, s), H-3 (δ6.10, *br d*, J = 6.0 Hz) and H-4 (δ4.93, *dt*, J = 6.0, 1.8 Hz). The broad singlet at δ2.53 was attributable to H-5 and H-9. The latter assignments were confirmed by a spin decoupling experiment: Irradiation at δ2.53 simplified the doublet of triplets of H-4 to a doublet. The three hydroxylated carbons mentioned above must then be located in at positions 6, 7 and 8. Two proton signals at δ3.83 (*dd*, J = 3.75, 1.65 Hz) and 3.60 (*d*, J = 3.75 Hz) were assigned to H-6 and H-7, respectively. The singlet at δ1.14 was assignable to H_3-10. NOE experiments confirmed the assignments and proved the relative configurations of **13**: irradiation at Me-10 had no effect on H-9, but enhanced the signals of H-1, H-6 and H-7. Thus, Me-10, H-6 and H-7 must be located on the alpha-face of the molecule, assuming the usual stereochemistry at C-1, C-5 and C-9. Strong positive NOE effects between H-6 and H-7 indicated a *cis* relationship between these protons.

In 1987, König *et al.* reported the isolation of avicennioside from *Avicennia officinalis* L. (Verbenaceae) [16]. Re-examination of the spectroscopic data of 'avicennioside' (UV, IR, ^1H NMR, H–H COSY, NOE and ^{13}C NMR), its diacetonide and peracetylated diacetonide (^1H NMR and NOE) revealed a comparatively low chemical shift for C-7 of 'avicennioside' (δ72.9) and a relatively low intensity of the acetyl signals (10.2 protons) in the ^1H NMR spectrum of the peracetylated diacetonide. The FAB-MS of an authentic sample of 'avicennioside' showed a characteristic peak pattern of *m/z* 397 $[M - H]^-$ and 399 (isotope peak) in the ratio of *ca* 3:1. This indicated a chlorine atom in position 7 of 'avicennioside' instead of a hydroxyl group. The resulting structure is identical to that of linarioside. The identity was confirmed by the good agreement between the NMR data for our component [16] and those reported for linarioside [17, 18]. This result has been reported as a private communication [10].

Qian *et al.* reported on the isolation and structure elucidation of the presumedly new iridoid-related aglycone ningpogenin as well as two new ningpogenin glycosides from fermented roots of *Scrophularia ningpoensis* Hemsl [19]. The isolated ningpogenin is identical to 6,7-bis(hydroxymethyl)-*cis*-2-oxabicyclo-[3.3.0]oct-7-ene, a semi-synthetic compound, that Bonini *et al.* had previously prepared by a three-step conversion of aucubin [20]. It is noteworthy that the proposed biogenetic formation of ningpogenin via reduction of the carbonyl groups at C-1 and C-3 of aucubigenin and subsequent ring closure between C-3 and O-6 [19] strictly follows the synthetic route of Bonini *et al.*

DISCUSSION

Boivin classified *P. virginiana* (L.) Benth. into eight varieties [7]. Cantino noticed a considerable morphological overlap and evident intergradations between these varieties and, therefore, recognized only two subspecies: ssp. *virginiana* and ssp. *praemorsa* [8]. Cantino's classification is supported by our results (Table 1): The iridoid pattern of *P. virginiana* ssp. *praemorsa* significantly differed from all populations of ssp. *virginiana*. Only four iridoids could be detected, one of which, stegioside III, seems to be characteristic for this taxon. It was by far the main component in aerial parts and roots and it did not occur in any taxon of ssp. *virginiana*. Stegioside III is the 6β-epimer of physoside (6α-OH) which was isolated from *P. virginiana* ssp. *virginiana* var. *elongata* by Jensen and co-workers [5]. *P. virginiana* ssp. *praemorsa* seems not to accumulate 6α-hydroxy-iridoids, although the presence of smaller amounts of physoside can not be excluded with certainty. On the other hand, all samples of *P. virginiana* ssp. *virginiana* accumulate the 6α-hydroxy-iridoid acetylmyoporoside together with the 6β-hydroxy-iridoids antirrhinoside and ajugoside.

The distribution of the remaining iridoids in the latter taxon showed a marked variability which was not dependent on the plant parts examined. Although these chemical variations were also distinctive, they were substantially smaller than the differences between the two ssp: var. *speciosa* and var. *formosior* showed very similar iridoid patterns. The lack of acetylharpagide in var. *speciosa* was the only qualitative character separating these two varieties. On the other hand, the presence of daunoside, gardoside and geniposidic acid differentiated these two varieties from the remaining taxa. The occurrence of virginioside, together with the remarkably high amount of myoporoside, separated var. *elongata* from the other varieties. Harpagide, 7-deoxy-8-epiloganic acid and 10-deoxygeniposidic acid were exclusively found in *P. virginiana* var. *virginiana*. The amount of the two latter iridoids was six times higher in the aerial parts of var. *virginiana* (origin Berlin A) than in the corresponding roots. Thus, the lack of both compounds in the roots of Berlin B may be explainable by the essentially lower amount of iridoid acids in this plant part. The largest differences within a variety were detected in var. *elongata*: the two origins Copenhagen A and Copenhagen B strongly differed in the amount of virginioside as well as in the total iridoid percentage. However, in this case, only one leaf of each origin was investigated by us, which may explain the observed differences.

The iridoid distribution thus confirms the classification of *P. virginiana* into ssp. *virginiana* and ssp. *praemorsa*, as proposed by Cantino [8]. The minor differences in iridoid distribution within ssp. *virginiana* did correlate with the morphological classification into varieties, as proposed by Boivin [7]. However, the small number of samples investigated chemically does not allow any conclusions as to whether the observed variability is continuous or discontinuous.

EXPERIMENTAL

Plant material. The plant material was identified by E.-M. Schmidt, R. Naß and H. Rimpler, Institut für Pharmazeutische Biologie, Freiburg. All populations were harvested in flowering states. The material of var. *virginiana* as well as the two investigated vouchers of var. *elongata* were air-dried, while the plant material of the remaining varieties was lyophilized. *P. virginiana* ssp. *virginiana* var. *speciosa* (voucher no. 2148) was purchased from a market-garden near Freiburg. The seeds of *P. virginiana* ssp. *virginiana* var. *formosior* (voucher no. 87.027.01) originated from a wild collection in Canada and were cultivated in the greenhouse of the Botanical Garden of Göttingen in 1984. The two populations of *P. virginiana* var. *virginiana* (Berlin A: voucher no. 26, Berlin B: no. 27) were cultivated in the Botanical Garden of Berlin and harvested in 1966. The seeds had been obtained from Meisert Co. (Hannover). *P. virginiana* ssp. *praemorsa* (voucher no. 2698) was cultivated in the greenhouse of the Botanical Garden in Freiburg and harvested in 1994. The seeds originated from the Chicago Botanic Garden (Glencoe, IL, U.S.A.). The two voucher specimens of *P. virginiana* ssp. *virginiana* var. *elongata* [Copenhagen A: Kultur nr 3641-ID (IOK 2-89); Copenhagen B: Kultur nr S 1877-0959 (IOK 1-89)] were obtained from Dr B. Hansen, Copenhagen. The two latter vouchers are deposited at the Herbarium of the Botanical Museum, Copenhagen, whereas all other vouchers have been deposited in the Herbarium of the Institut für Pharmazeutische Biologie, Freiburg.

General. NMR: 400 MHz (^{1}H) or 100 MHz (^{13}C), chemical shifts as δ values (ppm); FAB-MS: negative mode; glycerol (matrix), Cs (ionization), acceleration with 15 keV; ESI-MS: direct loop injection with flow rate of 5 μl min^{-1}; N_2 sheath gas, temp. of transfer capillary 200°; TLC: Silica gel 60 F_{254}, CH_2Cl_2–MeOH–H_2O (40:10:1, 70:30:3 and 15:10:1), spray reagent: vanillin (3%) and H_2SO_4 (1%) in EtOH followed by heating at 110° for 5–10 min; CC: Silica gel 60, 63–200 μm (Merck) and MCI gel CHP-20P, 75–150 μm (Mitsubishi); FC: red. pres. (600–800 mbar), prepacked columns (silica gel, RP-18, each 25–40 μm) of two sizes were used: size 1 (10 g, column vol. = 60 ml), size 2 (1 g, column vol. = 6 ml); MPLC: home-packed Europrep C-18 (25-40 μm;

500 × 26 mm) and Lichroprep C-18 (15–25 μm; 539 × 37 mm), frs were monitored by TLC; HPLC: Lichrosorb C-18 (10 μm; 250 × 16 mm) = column A, Nucleosil C-18 (5 μm, 250 × 4 mm) = column B, Nucleosil C-18 (7 μm; 250 × 10 mm) = column C, Eurospher C-18 (7 μm; 250 × 8 mm) = column D, Serva Si 100 polyol phenyl (5 μm, 250 × 9.5 mm) = column E; detection: UV 205 nm (C-9 iridoids) and 235 nm (C-10 iridoids). GC: WCOT Rt_x 200 (trifluoropropylmethylpolysiloxane) (30 m × 0.32 mm), film thickness 0.25 μm. Guard column: 5 m × 0.32 mm. Carrier gas: He, column head pres. 13 psi, linear velocity 22 cm s^{-1} (1.06 ml min^{-1}) set at 200°, split mode, split ratios between 70:1 and 85:1. Detector: FID, 250°. Operating conditions: 200° for 15 min, then 3° min^{-1} to 230°, injections of 1 μl. Sepn of galiridoside and virginioside and confirmation analysis: Column: WCOT Rt_x 5 (95% dimethyl-, 5% diphenyl-polysiloxane), same specifications and same guard column as above. Carrier gas: He, column head pres. 13 psi, linear velocity 20.5 cm sec^{-1} (1.03 ml min^{-1}) set at 260°. Injector: 290°, same split ratios as above. Detector: FID, 300°. Operating conditions: 260° isothermal, injections of 1 μl. The iridoids were identified by comparison of their R_fs with those of the corresponding pure compounds. Frs which contained iridoid acids were methylated and compared with the pure methyl esters. The distribution of ajugol, 5-*O*-β-glucosyl-antirrhinoside, stegioside II and physoside could not be determined owing to insufficient amounts of reference samples.

Extraction and isolation. P. virginiana ssp. *virginiana* var. *speciosa*: 802 g lyophilized powdered aerial parts were extracted by refluxing for 30 min successively with 96, 80 and 70% EtOH. The combined extracts were evapd *in vacuo*, and the residue adsorbed onto Celite and sepd on a Celite column. Elution with *n*-hexane–CH_2Cl_2 (1:1) afforded a lipophilic fr. which was discarded. Subsequent elution with CH_2Cl_2–MeOH (1:1) yielded a hydrophilic fr. which was sepd by FC on RP-18 with the following mixts of MeOH in H_2O: 0, 5, 10, 15, 20, 25, 30, 50 and 100%. Frs. were combined as follows: Fr. I (eluted with H_2O), fr. II (5, 10 and 15% MeOH), fr. III (20, 25 and 30% MeOH) and fr. IV (50 and 100% MeOH).

Fr. I was acidified to pH 3.8 with dil. HOAc [5], chromatographed once more by FC on RP-18 and eluted with dil. HOAc (pH 3.8), H_2O and MeOH. The two latter eluates were combined, neutralized with 0.1M NaOH and sepd by FC on RP-18 with H_2O. The first two frs were combined and after removal of solvent further purified on MCI gel with 5% MeOH. This afforded a soln. of crude iridoid acids which was concd and mixed with TFA (final pH of mixt. = 2.8). Subsequent HPLC on column A with MeCN–H_2O (1:9) (4.0 ml min^{-1}, 1200 psi) afforded gardoside (**3**; 37 mg, 0.005%), a mixt. of 2 additional iridoid acids (fr. Ia) and 8-epiloganic acid (**2**; 72 mg, 0.009%). Methylation of fr. Ia with CH_2N_2 and subsequent HPLC on column D with MeOH–MeCN–H_2O (6.5:43.5:50) (3.0 ml min^{-1}, 1300 psi) yielded geniposide (**5**; 4.9 mg, 0.001%) and an unidentified iridoid (3.2 mg).

MPLC of fr. II on Europrep RP-18 with MeOH–MeCN–THF–H_2O (4.5:0.25:0.25:95) (8.5 ml min^{-1} 170 psi) gave the 3 main frs IIA, IIB, IIC. Frs IIA and IIB were each purified by HPLC on column B with MeOH–MeCN–THF–H_2O (2:0.25:0.25:97.5) (0.8 ml min^{-1}, 1000 psi) and yielded daunoside (**6**; 115 mg, 0.01%), myoporoside (180 mg, 0.02%) and antirrhinoside (119 mg, 0.01%), resp. HPLC of fr. IIC on column C with MeOH–H_2O 1:4 (2.5 ml min^{-1}, 1100 psi) afforded galiridoside (800 mg, 0.10%). Fr. III was sepd by FC on silica gel with CH_2Cl_2–MeOH–H_2O (40:10:1). The first 160 ml were collected, concd *in vacuo* and sepd by HPLC on column A (solvent X: MeCN–H_2O, 3:17, solvent Y: MeOH; 0–20 min 100% X; 20–30 min 60% X; 30–60 min 30% X) (3.0 ml min^{-1}). This afforded the 3 main frs. IIIA, IIIB, IIIC. HPLC of fr. IIIA on column E with MeCN–H_2O (2:23) (3.0 ml min^{-1}, 1800 psi) yielded acetylmyoporoside (336 mg, 0.04%). Fr. IIIB was finally purified by HPLC on column D with MeOH–H_2O (3:17) (3.0 ml min^{-1}, 1300 psi) to yield stegioside I (**7**; 18 mg, 0.002%). CC of fr. IIIC on silica gel with CH_2Cl_2–MeOH–H_2O (40:10:1) afforded 2 main frs, the first of which was purified by HPLC on column A with MeOH–H_2O (3:7) (3.0 ml min^{-1}, 2000 psi) to yield ajugoside (146 mg, 0.02%).

P. virginiana ssp. *virginiana* var. *virginiana* (origins Berlin A, Berlin B). 4.03 and 2.88 g air-dried, powdered roots of the origins Berlin A ('var. *virginiana* [2]) and Berlin B ('var. *speciosa*' [2]), resp., were separately extracted by refluxing for 20 min successively with 96, 80 and 70% EtOH. The EtOH was removed and the aq. soln washed 3 × with EtOAc. The H_2O-layers were concd and fractionated by FC on RP-18 with the following H_2O–MeOH mixts: H_2O, 10, 30, 50 and 100% MeOH. The eluates with 10% MeOH of both origins were very similar according to TLC. Since only a small amount of plant material was available, the 2 frs were then combined and further purified by MPLC on Lichroprep 15–25 μm with a step gradient of MeOH–H_2O (11.3 ml min^{-1}, 0–55 min 1:49; 56–144 min 3.9:96.1; 145–227 min 4.9:95.1; 228–307 min 5.9:94.1; 308–367 min 2:23) to afford the 3 main frs I, II and III. HPLC of fr. I on column D with MeCN–H_2O (3:97) (3.5 ml min^{-1}, 1100 psi) yielded virginioside (10 mg, 0.15%). Fr. II mainly consisted of 5-*O*-β-glucosyl-antirrhinoside (1.4 mg, 0.02%). HPLC of fr. III on column D with MeCN–H_2O (3:97) (3.0 ml min^{-1}, 1000 psi) afforded harpagide (**10**; 4.7 mg, 0.07%) and ajugol (**11**; 2.3 mg, 0.03%).

P. virginiana ssp. *praemorsa*. 2.74 g lyophilized and powdered roots and 188 mg lyophilized and crushed flowers were separately extracted, washed with EtOAc and purified by FC under the same conditions as *P. virginiana* var. *virginiana*. The 10% MeOH eluates of FC from roots and flowers were very similar according to TLC. Since only a small amount of plant material was available, the two frs were combined. After removal of the solvent, further purification by CC on silica gel with CH_2Cl_2–MeOH–H_2O (70:30:3) yielded 2 main frs I and II. HPLC of fr. I on column D with MeOH–H_2O (1:9) (3.0 ml min^{-1}, 1800 psi) afforded stegioside II (**12**; 1 mg, 0.07%) and galiridoside (1 mg, 0.07%). Fr. II was purified

by HPLC on column A with MeOH–H_2O (7:93) (3.6 ml min^{-1}, 1100 psi) and yielded stegioside III (**13**; 7.3 mg, 0.5%).

Sample prepn for GC analysis. The dried and powdered plant material (188 mg–4.5 g) of the different samples (except *P. virginiana* ssp. *virginiana* origins Copenhagen A and Copenhagen B) was refluxed for 20 min successively with 96, 80 and 70% EtOH. The combined extracts were evapd and the residues partitioned between EtOAc–H_2O. The aq. layers were cond and fractionated by FC on RP-18, with H_2O (fr. I), 10% MeOH (fr. II), 30% MeOH (fr. III), 50% MeOH (fr. IV) and 100% MeOH (fr. V). Frs which contained iridoid acids were methylated with CH_2N_2. Aliquots of the lyophilized frs (0.4–4 mg) were trimethylsilylated [*N,O*-bis(trimethylsilyl)acetamide–trimethylchlorosilane–trimethylsilylimidazole (3:2:3)] at room temp. for at least 1 hr. Aucubin was added as int. standard. Work-up for *P. virginiana* ssp. *virginiana* var. *elongata* origin Copenhagen A ('var. *virginiana*', IOK 2-89 [5]) and origin Copenhagen B ('var. *speciosa*', IOK 1-89 [5]): one leaf of each voucher (75 and 60 mg, resp.) was crushed, suspended in 96% EtOH and sonicated with a Branson 250 sonifier (4 min, ice bath) followed by refluxing for 15 min successively with 96, 80 and 70% EtOH. The combined extracts were concd. *in vacuo* and fractionated by FC on RP-18. Elution and further work-up the same as for the other plant material.

Quantitation. Each iridoid glycoside (*ca* 2 mg) was dissolved in H_2O to give 10 ml stock solns. Five mixts were prepd, each containing 200 μl of every soln: Thereto aucubin (2.032 mg in 10.00 ml) was added in increasing amounts (50, 100, 200, 500, 1000 μl). After lyophilization and trimethylsilylation, three GC runs of each mixt. were performed. Linear regression equations, and the corresponding standard errors and correlation coefficients (r) were determined by plotting the ratio of aucubin area to component area against the ratio of aucubin amount to component amount using the computer program MS-Excel 5.0. All r values were higher than 0.997, except for the following 2 components: Antirrhinoside ($r = 0.983$) and acetylharpagide ($r = 0.972$). The standard errors of the amounts are given in Table 1. Compounds **8** and **9** were only obtainable as a mixt. For the determination of the regression equations, the ratio of **8** to **9** was determjined as 2.59:1 by the ^{1}H NMR integrals of the corresponding H_3-10 protons. Due to the scarcity of gardoside methyl ester and stegioside III no stock solns could be prepd. Therefore, the amounts of gardoside and stegioside III were calculated with the regression equations of 8-epiloganin and daunoside, resp.

Stegioside I (**7**) (6t-*chloro*-1c-β-D-*glucopyranosyloxy*-7t-*methyl*-(4*ar*, 7*ac*)-1,4*a*,5,6,7,7*a*-*hexahydro-cyclopenta*[c]*pyran*-4*a*,7*c*-*diol*). $[\alpha]_D^{25}$ −179.4° (MeOH; *c* 0.32 *g* 100 ml^{-1}); ESI-MS: *m/z* 423 $[M(^{37}Cl) + K]^+$, 421 $[M(^{35}Cl) + K]^+$, 407 $[M(^{37}Cl) + Na]^+$, 405 $[M(^{35}Cl) + Na]^+$, rel. isotope abundances of $[M + Na]^+$: *m/z* 405, 100.0%; 406, 18.5%; 407, 37.9%; 408, 7.4%; 409, 2.6% $C_{15}H_{23}O_9ClNa$ requires: *m/z* 405, 100.0%; 406, 17.5%; 407, 35.2%; 408, 6.0%; 409, 1.1%; ^{1}H NMR (CD_3OD, standard: $CD_2HOD = 3.30$ ppm): δ1.18 (3H, *s*, H-10), 1.88 (1H, *dd*, $J = 14.7, 12.0$ Hz, H-6α), 2.40 (1H, *dd*, $J = 14.25, 8.7$ Hz, H-6β), 2.42 (1H, *br s*, H-9), 3.20 (1H, *dd*, $J = 9.75, 7.9$ Hz, H-2′), 3.28 (2H, partly covered by CD_2HOD-signal, H-4′, H-5′), 3.38 (1H, *t*, $J = 9.0$ Hz, H-3′), 3.66 (1H, *dd*, $J = 12.75, 6.0$ Hz, H-6′B), 3.88 (1H, *dd*, $J = 12.75, 2.7$ Hz, H-6′A), 4.32 (1H, *dd*, $J = 12.0, 8.25$ Hz, H-7), 4.58 (1H, *d*, $J = 8.25$ Hz, H-1′), 5.09 (1H, *dd*, $J = 6.6, 1.5$ Hz, H-4), 5.67 (1H, *s*, H-1), 6.20 (1H, *d*, $J = 6.75$ Hz, H-3); ^{13}C NMR: (CD_3OD, standard: $CD_3OD = 49.0$ ppm): δC-1 (92.1), C-3 (139.5), C-4 (110.9), C-5 (66.4), C-6 (48.7), C-7 (66.1), C-8 (80.2), C-9 (59.3), C-10 (17.2), C-1′ (99.0), C-2′ (74.6), C-3′ (77.6), C-4′ (71.8), C-5′ (78.3), C-6′ (62.8).

Conversion of stegioside I into galiridoside. Stegioside I (1.5 mg) was dissolved in 400 μl D_2O in an NMR tube and the ^{1}H NMR spectrum was recorded. After addition of 50 μl NaOD (40% w/v in D_2O) and heating in a water bath for 5 min, the spectrum was recorded again, showing only the presence of galiridoside (*c/f* [10]).

Stegioside II (**12**) (1c-β-D-glucopyranosyloxy-7t-*methyl*-(4*ar*,7*ac*)-1,4*a*,5,6,7,7*a*-*hexahydro-cyclopenta*[c]*pyran*-6*c*,7*c*-*diol*). ESI-MS: *m/z* 387 $[M + K]^+$, 371 $[M + Na]^+$, rel. isotope abundances of $[M + Na]^+$. *m/z* 371, 100.0%; 372, 17.5%; 373, 4.3%; $C_{15}H_{24}O_9Na$ requires: *m/z* 371, 100.0%; 372, 17.5%; 373, 3.2%; ^{1}H NMR (D_2O, standard: $CH_3CN = 1.93$ ppm): δ1.15 (3H, *s*, H-10), 1.62 (1H, *dt*, $J = 13.5, 4.8$ Hz, H-6α), 1.84 (1H, *ddd*, $J = 13.5, 9.0, 5.25$ Hz, H-6β), 2.30 (1H, *br d*, $J = 10.2$ Hz, H-9), 2.67 (1H, *m*, H-5), 3.18 (1H, *dd*, $J = 9.0, 8.25$ Hz, H-2′), 3.28 (1H, *t*, $J = 9.0$ Hz, H-4′), 3.36 (1H, *m*, H-5′), 3.38 (1H, *t*, $J = 9.0$ Hz, H-3′), 3.59 (1H, *dd*, $J = 12.75, 6.0$ Hz, H-6′B), 3.66 (1H, *t*, $J = 5.25$ Hz, H-7), 3.80 (1H, *dd*, $J = 12.75, 2.25$ Hz, H-6′A), 4.62 (1H, partly covered by HDO signal, H-1′), 4.83 (1H, *dd*, $J = 6.45, 3.0$ Hz, H-4), 5.37 (1H, *d*, $J = 1.5$ Hz, H-1), 6.05 (1H, *dd*, $J = 6.45, 1.8$ Hz, H-3); ^{13}C NMR (D_2O, standard: $CH_3CN = 1.30$ ppm): δ C-1 (93.9), C-3 (137.6), C-4 (109.3), C-5 (25.3), C-6 (36.3), C-7 (78.3), C-8 (78.9), C-9 (48.0), C-10 (21.4), C-1′ (98.4), C-2′ (73.2), C-3′ (76.1), C-4′ (70.1), C-5′ (76.7), C-6′ (61.2).

Stegioside III (**13**) (1c-β-D-glucopyranosyloxy-7t-*methyl*-(4*ar*, 7*ac*)-1,4a,5,6,7,7a-*hexahydro-cyclopenta*[c]*pyran*-5*c*,6*c*,7*c*-*triol*). $[\alpha]_D^{25}$ −157.1° (H_2O; *c* 0.19 *g* 100 ml^{-1}); ESI-MS: *m/z* 403 $[M + K]^+$, 387 $[M + Na]^+$, rel. isotope abundances of $[M + Na]^+$. *m/z* 387, 100.0%; 388, 17.2%; 389, 4.2%; $C_{15}H_{24}O_{10}Na$ requires: *m/z* 387, 100.0%; 388, 17.6%; 389, 3.5%; ^{1}H NMR (D_2O, standard: $CH_3CN = 1.93$ ppm): δ1.14 (3H, *s*, H-10), 2.53 (2H, *br s*, H-5, H-9), 3.16 (1H, *dd*, $J = 9.0, 8.25$ Hz, H-2′), 3.26 (1H, *t*, $J = 9.0$ Hz, H-4′), 3.36 (1H, *ddd*, $J = 9.75, 6.0, 2.25$ Hz, H-5′), 3.38 (1H, *t*, $J = 9.0$ Hz, H-3′), 3.58 (1H, *dd*, $J = 12.75, 6.0$ Hz, H-6′B), 3.60 (1H, *d*, $J = 3.75$ Hz, H-7), 3.79 (1H, *dd*, $J = 12.75, 2.25$ Hz, H-6′A), 3.83 (1H, *dd*, $J = 3.75, 1.65$ Hz, H-6), 4.63 (1H, *d*, $J = 7.8$ Hz, H-1′), 4.93 (1H, *dt*, $J = 6.0, 1.8$ Hz, H-4), 5.40 (1H, *s*, H-1), 6.10 (1H, *br d*, $J = 6.0$ Hz, H-3); ^{13}C NMR (D_2O, standard: $CH_3CN = 1.30$ ppm): δ C-1 (93.5), C-3

(138.9), C-4 (105.8), C-5 (34.9), C-6* (77.0), C-7* (78.4), C-8 (77.7), C-9 (47.9), C-10 (21.8), C-1′ (98.4), C-2′ (73.1), C-3′ (76.1), C-4′ (70.1), C-5′ (76.6), C-6′ (61.2).

Mixture of 7-deoxy-8-epiloganin (**8**) *and 10-deoxygeniposide* (**9**). FAB-MS: *m/z* 373 **8**: $[M-H]^-$, 371 **9**: $[M-H]^-$, 211 **8**: $[M-\text{glucose}-H]^-$, 209 **9**: $[M-\text{glucose}-H]^-$[1], ^{1}H NMR (D_2O, standard: external TSPNaD_4 in a capillary = 0.00 ppm) δ1.02 (3H, *d*, *J* = 7.2 Hz, **8**: H-10), 1.31 (1H, *m*, **8**: H-7), 1.58 (1H, *m*, **8**: H-6), 1.80 (1H, *m*, **8**: H-7), 1.81 (3H, *br s*, **9**: H-10), 2.03 (1H, *dq*, *J* = 13.5, 7.5 Hz, **8**: H-6), 2.13 (1H, *m*, *J* = 16.5 Hz, **9**: H-6), 2.33 (1H, *br q*, *J* = 7.5 Hz, **8**: H-8), 2.40 (1H, *td*, *J* = 8.25, 3.0 Hz, **8**: H-9), 2.75 (1H, *m*, *J* = 16.5, 8.25, 2.25 Hz, **9**: H-6), 2.88 (1H, *m*, **9**: H-9), 2.94 (1H, *td*, *J* = 8.25, 4.8 Hz, **8**: H-5), 3.21 (1H, *td*, *J* = 8.25, 4.5 Hz, **9**: H-5), 3.29–3.55 (8H, **8**, **9**: H-2′-H-5′), 3.75 (2H, **8**, **9**: H-6′B), 3.76 (6H, *s*, **8**, **9**: $COOCH_3$), 3.94 (2H, **8**, **9**: H-6′A), 4.81 (1H, *d*, *J* = 8.25 Hz, **8**: H-1′), 4.84 (1H, *d*, *J* = 8.25 Hz, **9**: H-1′), 5.48 (1H, *br d*, *J* = 4.8 Hz, **9**: H-1), 5.54 (1H, *d*, *J* = 3.6 Hz, **8**: H-1), 5.57 (1H, *m*, **9**: H-7), 7.50 (1H, *s*, **8**: H-3), 7.54 (1H, *s*, **9**: H-3); in good agreement with data for pure **9** [11] and consistent with structure **8**.

^{13}C NMR data for the mixt. of **8** and **9** were in good accordance with those for the corresponding pure components [12, 13]. 8-Epiloganic acid (**2**) and gardoside (**3**) were methylated with CH_2N_2. ^{1}H and ^{13}C NMR data for 8-epiloganin and gardoside methyl ester were identical to lit. values [21–23]. Daunoside (**6**) was compared (^{1}H NMR, TLC) with a authentic sample prepd by base catalysed transformation of galiridoside [24]. 8-*O*-Acetylharpagide was compared (TLC, GC) with an authentic sample [25]. The ^{1}H and ^{13}C NMR data for acetylmyoporoside were identical to lit. values [26] except for H-3. Re-evaluation of the authentic spectrum [26] showed that the chemical shift of H-3 must be corrected from δ6.32 to δ6.41, in agreement with the results of this paper. The spectral data for the following known iridoids were identical to those published in the lit.: Harpagide (**10**) (^{1}H, ^{13}C NMR, [27]), virginioside (^{1}H, ^{13}C NMR, [5]), 5-*O*-β-glucosyl-antirrhinoside (^{1}H NMR, [28]), ajugol (**11**) (^{1}H NMR, [29]), galiridoside (^{1}H, ^{13}C NMR, [24]), *antirrhinoside* (^{1}H NMR [30]), *myoporoside* (^{1}H NMR [5]), *ajugoside* (^{1}H, ^{13}C NMR [31]), *geniposide* (**5**) (^{1}H NMR [32]).

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*Assignments may be interchanged.

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