First Direct and Detailed Stereochemical Analysis of Strictosidine

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Using the easy lactamization of vincoside (**4**), epimer-free strictosidine (**1**) was prepared from secologanin (**2**) and tryptamine (**3**). 2D NMR methods were used to determine unambiguously the ^{1}H- and ^{13}C-NMR chemical shifts, the ^{1}H−^{1}H and ^{13}C−^{1}H coupling constants, and the ^{1}H−^{1}H NOE interactions in strictosidine (**1**). A minimal number of spectroscopic parameters (11 coupling constants, 3 NOEs) and some theoretical considerations have made it possible to select the single species of the 648 selected stereoisomers and to confirm directly the *S* configuration at the newly formed C-3 chiral center, the *P* helicity of the dihydropyran and tetrahydropyridine rings, and the conformations around C-14 and the glycosidic bridge.

Strictosidine (**1**), a well-known monoterpene indole alkaloid glycoside,[1,2] is the precursor of the monoterpene indole and related alkaloids and was first isolated by G. N. Smith from *Rhazya stricta*.[3] It is constructed in vivo from secologanin (**2**) and tryptamine (**3**) by plant species,[1] as well as in vitro in the presence of the enzyme strictosidine synthase,[4] or under biomimetic conditions in aqueous solution at pH 4.5[5] (Scheme 1). In the coupling reaction, a new chiral center is formed with complete stereoselectivity in the presence of the enzyme, or together with vincoside (**4**) in a 1:1 ratio in the absence of the enzyme. For many years, there was much controversy about the configuration of the new center of chirality at C-3, as well as about the question of which stereoisomer is the precursor of the alkaloids mentioned above.[1,2,6] On the basis of chemical and optical correlations with ipecoside, Battersby *et al.* indicated a H-3β (corresponding to *R* configuration) at this center.[5] Later, however, it became clear that in the chemical correlation with (−)-protoemetine, the inversion of C-1 in ipecoside (analogous to C-3 in vincoside) under strongly acidic conditions at 100 °C had not been recognized. This conclusion was corrected by the X-ray diffraction analysis of *O*,*O*-dimethylipecoside, which unequivocally proved the H-1β (*R*) configuration in the ipecoside series.[7] Chemical and optical correlations carried out in the strictosidine−vincoside series suggested that the configuration of C-3 in strictosidine should likewise be corrected from H-3β to H-3α (i.e., from *R* to *S*).[8−10] However, these and similar papers were based on the instrumental limitations of their period, so the configurations were derived from compounds of "known stereochemistry" rather than proved by direct instrumental measurements (e.g., X-ray diffraction analysis or NMR spectroscopy). The correlations involved many steps (e.g., the chemical correlation with cinchonine by two ways through dihydroanthirine and corynantheine and dihydrocinchonamine in 19 and 17 steps, respectively[11−17]) and sometimes vigorous reaction conditions (e.g., treatment with strong base at a position α to an oxo group,[13] heating with KOH at 155 °C for 5−6 h,[17] reflux in strongly acidic solution for several hours,[18] etc.) were applied without establishing what had happened during the reaction at the center of chirality. Moreover, in the paper by De Silva *et al.*[8] the configuration of C-3 in dihydroanthirine [the reference compound "of known stereochemistry" for strictosidine (**1**)] was incorrectly given just at this delicate point, and some other problems were found in the same communication. Thus, in our view, the papers of this period cannot be considered unambiguous.

Relevant biosynthetic studies disclosed an analogous problem.[6] Originally it was vincoside that had been considered as the precursor of both the H-3α and H-3β series of the Iα-type indole alkaloids, which likewise had to be changed later.[19,20] Finally, the configuration of C-3 in the H-3β (*R)* (vincoside) series was established unequivocally by Hutchinson *et al.* by X-ray analysis of *N*-4-(*p*-bromobenzyl)-*O*,*O*,*O*,*O*-tetraacetyl vincoside.[21] A similarly strong proof was never presented for strictosidine (**1**). It could not be obtained in crystalline form, and no detailed NMR studies have been conducted as yet. Until now, most investigations were carried out on more or less remote derivatives, rather than on strictosidine (**1**) itself. The *S* configuration of C-3 was assigned by analogy—by supposing that the other product of the coupling reaction of secologanin and tryptamine could not be anything but the H-3α isomer. The principle *tertium non datur*, however, is not always a convincing argument in chemistry.

As strictosidine (**1**) is the building stone of nearly 2200 indole and related alkaloids, our aim was the direct and detailed investigation of its stereochemistry (configuration and conformation) as an introduction to its bioorganic chemistry. Even at the start of our work, the *S* configuration of strictosidine was not supposed by us to be incorrect, neither was the subsequent work by many others put into doubt. They were correct not by proof, but by analogy. We did not intend to correct but to confirm it by rigorous proof in the general frame of the stereochemical analysis. We were convinced that preparation of a sample of high purity and the new NMR techniques gave an opportunity to prove simultaneously both the configuration *and* the conformation of this alkaloid.

Results and Discussion

Our studies were carried out on strictosidine (**1**) prepared according to a method previously published[22] and recently modified. The NMR spectra were recorded

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Scheme 1. Coupling Reaction of Secologanin (**2**) with Tryptamine (**3**)

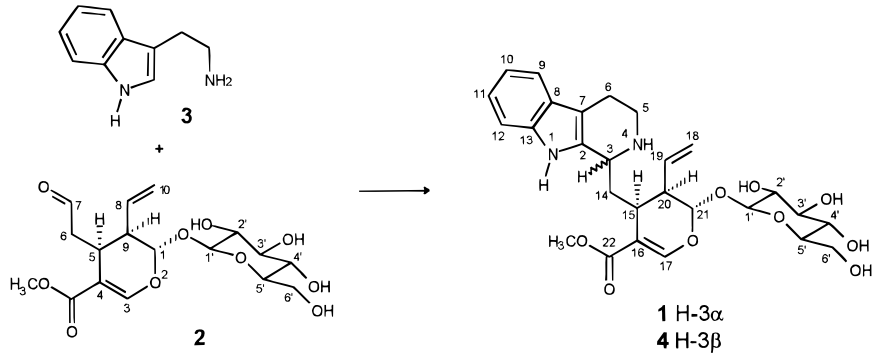

Table 1. Stereogenic Elements and Selected Stereoisomers of Strictosidine (**1**)

(1) staggered conformations around the bond C-15−C-14	3 ×
(2) staggered conformations around the bond C-14−C-3	3 ×
(3) possible configurations at C-3	2 ×
(4) conformations of the dihydropyran ring	2 ×
(5) conformations of the tetrahydropyridine ring	2 ×
(6) staggered conformations around the bond C-21−O bridge	3 ×
(7) staggered conformations around the bond O bridge−C-1′	3
Total number of selected stereoisomers isomers:	648

Table 2. ^{13}C- and ^{1}H-NMR Chemical Shifts of Strictosidine (**1**) in CD_3OD[a]

carbon	δ_C	δ_H
C-2	133.2	
C-3	52.4	4.30
C-5	42.9	α: 3.17; *β*: 3.50
C-6	21.0	α: 2.85; *β*: 2.95
C-7	107.7	
C-8	127.9	
C-9	118.8	7.40
C-10	120.1	6.98
C-11	122.7	7.07
C-12	112.0	7.29
C-13	137.9	
C-14	35.9	*proS*: 2.21, *proR*: 2.09
C-15	32.5	3.06
C-16	109.9	
C-17	156.1	7.74
C-18	119.5	*Z*: 5.33; *E*: 5.24
C-19	135.7	5.85
C-20	45.6	2.70
C-21	97.5	5.85
C-22	170.6	
OCH_3	52.4	3.77
C-1′	100.3	4.80
C-2′	78.6	3.45−3.20
C-3′	78.0	3.45−3.20
C-4′	74.6	3.45−3.20
C-5′	71.7	3.45−3.20
C-6′	62.9	3.97; 3.65

[a] Internal standard, TMS; $\delta_{TMS} = 0.00$.

in deuteriomethanol (Table 2). The coupling constants and the large chemical-shift differences of the diastereotopic methylene protons of the ^{1}H-NMR spectrum suggested that in MeOH solution the molecule exists predominantly in a single conformation. However, to select the correct structure from the 648 stereoisomers of Table 1, it was necessary to collect as much stereochemically relevant spectroscopic data as possible. Analysis of the ^{1}H-NMR spectrum yielded 13 vicinal coupling constants (Table 3). The NOESY spectrum

Table 3. Measured and Calculated ^{1}H-NMR Coupling Constants (in Hz) of Strictosidine (**1**)

assignment	measured	calculated[a]
$^{3}J_{3,14R}$	11.4	11.8
$^{3}J_{3,14S}$	3.0	3.2
$^{5}J_{3,6\alpha}$	1.3	
$^{2}J_{5\alpha,5\beta}$	12.3	
$^{3}J_{5\alpha,6\alpha}$	5.3	4.9
$^{3}J_{5\alpha,6\beta}$	8.9	12.2
$^{3}J_{5\beta,6\alpha}$	4.2	1.1
$^{3}J_{5\beta,6\beta}$	5.4	5.5
$^{2}J_{6\alpha,6\beta}$	15.8	
$^{3}J_{9,10}$	7.9	
$^{4}J_{9,11}$	1.2	
$^{5}J_{9,12}$	1.0	
$^{3}J_{10,11}$	7.1	
$^{4}J_{10,12}$	1.1	
$^{3}J_{11,12}$	8.1	
$^{2}J_{14R,14S}$	14.7	
$^{3}J_{14R,15}$	3.9	2.9
$^{3}J_{14S,15}$	11.5	12.3
$^{3}J_{15,20}$	4.8	3.7
$^{2}J_{18Z,18E}$	2.1	
$^{3}J_{18Z,19}$	17.4	
$^{4}J_{18Z,20}$	1.0	
$^{3}J_{18E,19}$	10.6	
$^{4}J_{18E,20}$	1.0	
$^{3}J_{19,20}$	7.6	
$^{3}J_{20,21}$	8.8	9.1
$^{3}J_{1',2'}$	7.9	
$^{3}J_{5',6'a}$	2.1	
$^{3}J_{5',6'b}$	6.7	
$^{2}J_{6'a,6'b}$	11.9	

[a] Values are for the structure shown in Figure 4.

provided information about the steric proximity of 14 atom pairs, in addition to the structurally defined close atom pairs (the geminal methylene protons, the *ortho*-related aromatic protons, and the vinyl moiety) (Table 4). Finally, from slices of the 2D-HSQC (heteronuclear single quantum coherence) NMR spectrum, 26 stereochemically relevant vicinal carbon−proton coupling constants were determined (Table 5). In such a manner, 54 structural parameters were collected in all, and it was anticipated that only one of the considered structures would fit all parameters.

The starting point of the analysis is the experimental fact that the sample of strictosidine was prepared from secologanin (**3**), whose stereochemistry at the chiral center C-5 (corresponding to C-15 in strictosidine) is *S*. This configuration was established by chemical correlation through asperuloside with *O*,*O*,*O*,*O*,*O*-pentaacetyl-4-bromo-3-methoxyloganin,[23−26] whose absolute configuration was determined by X-ray diffraction analysis.[27] The X-ray diffraction analysis by Hutchinson *et al.*[21] gave the same result concerning the stereochemistry of

Table 4. Through-Space Distances of Protons in Strictosidine (**1**)[a] and the Intensity of the Corresponding Cross Peaks from the NOESY Spectrum

hydrogen	neighboring protons, inter-proton distances (Å), and NOESY cross peaks intensity[b]
H-3	H-14*proS* 2.4 w; H-5α 2.6 w; H-14*proR* 3.1 n
H-5α	H-5*β* 1.8 s; H-6α 2.5 n[c]; H-3 2.6 w; H-6*β* 3.1 n
H-5*β*	H-5α 1.8 s; H-6*β* 2.5 n
H-6α	H-6*β* 1.8 s; H-5α 2.5 n
H-6*β*	H-6α 1.8 s; H-5*β* 2.5 n
H-9	H-10 2.6 s; H-6α 3.0 n
H-10	H-9 2.6 s; H-11 2.6 s; H-12 4.5 n
1H-1	H-10 2.6 s; H-12 2.6 s; H-9 4.5 n
H-12	H-11 2.6 s; H-10 4.5 n
H-14*proR*	H-14*proS* 1.8 s; H-19 2.4 w; H-15 2.5 w; H-3 3.1 n
H-14*proS*	H-14*proR* 1.8 s; H-21 2.2 s; H-3 2.4 w; H-15 3.1 n
H-15	H-20 2.4 s; H-14*proR* 2.5 w; H-14*proS* 3.1 n
H-18*Z*	H-18*E* 1.9 s; H-20 2.4 m; H-19 3.1 w; H-15 3.6 n
H-18*E*	H-18*Z* 1.9 s; H-19 2.4 m; H-20 3.7 n
H-19	H-14*proR* 2.4 s; H-18*E* 2.4 m; H-21 2.7 ?[d]; H-18*Z* 3.1 n
H-20	H-15 2.4 s; H-18*Z* 2.4 m; H-21 3.1 w; H-19 3.1 w; H-14*proR* 3.8 n
H-21	H-14*proS* 2.2 s; H-19 2.7 ?[c]; H-1′ 3.0 s; H-20 3.1 n
H-1′	H-5′ 2.5 s; H-3′ 2.6 s; H-21 3.0 s; H_a-6′ 4.2 n

[a] Corresponding to the structure shown in Figure 4. [b] Abbreviations—s: strong; m: medium; w: weak; n: cross peak not detected. [c] The cross peak was missing due to a coupling interaction between the two protons. [d] No cross peak could be observed because the two corresponding signals were overlapping.

Table 5. Measured and Calculated $^{13}C-^{1}H$ Coupling Constants of Strictosidine (**1**) in Hz

assignment	measured[a]	calculated[b]
$J_{C-2,H-14R}$	2	2.6
$J_{C-2,H-14S}$	3	1.9
$J_{C-3,H-15}$	2	2.3
$J_{C-14,H-20}$	6	6.7
$J_{C-15,H-21}$	2.7	1.9
$J_{C-16,H-14R}$	6.5	6.5
$J_{C-16,H-14S}$	<2	2.3
$J_{C-17,H-21}$	2	0.6
$J_{C-19,H-15}$	2.8	1.4
$J_{C-19,H-21}$	2	0
$J_{C-20,H-14R}$	2.5	3.0
$J_{C-20,H-14S}$	<2	1.7
$J_{C-21,H-15}$	8	8.0
$J_{C-21,H-17}$	7.0	6.7
$J_{C-21,H-1'}$	2.7	1.5
$J_{C-22,H-15}$	3	4.8
$J_{C-1',H-21}$	3.2	1.8

[a] Further $^{13}C-^{1}H$ coupling constants in Hz: $^{2}J_{C-2,H-3} = 6.5$, $^{1}J_{C-3,H-3} = 144$, $^{2}J_{C-3,H-14R} < 1$, $^{2}J_{C-3,H-14S} = 4.2$, $^{3}J_{C-7,H-9} = 3.4$, $^{3}J_{C-8,H-12} = 4.9$, $^{2}J_{C-8,H-9} = 3$, $^{1}J_{C-9,H} = 157.5$, $^{3}J_{C-10,H-12-} = 6.5$, $^{1}J_{C-12,H} = 58.6$, $^{2}J_{C-14,H-3} = 7$, $^{2}J_{C-15,H-14R} = 3$, $^{2}J_{C-15,H-14S} < 1$, $^{3}J_{C-15,H-17} = 5.8$, $^{2}J_{C-16,H-17} = 6.0$, $^{3}J_{C-17,H-15} = 4.0$, $^{1}J_{C-17,H-17} = 193.3$, $^{1}J_{C-18,H-18Z} = 155.0$, $^{1}J_{C-18,H-18E} = 158.2$, $^{3}J_{C-18,H-20} = 5.4$, $^{2}J_{C-19,H-18Z} = 3.9$, $^{2}J_{C-19,H-18E} = 2.6$, $^{1}J_{C-19,H-19} = 156$, $^{2}J_{C-19,H-20} = 6$, $^{2}J_{C-20,H-15} = 6.0$, $^{3}J_{C-20,H-18Z} = 3.1$, $^{3}J_{C-20,H-18E} = 11.8$, $^{2}J_{C-20,H-19} = 1.1$, $^{1}J_{C-20,H-20} = 133$, $^{2}J_{C-21,H-20} = 7.5$, $^{1}J_{C-21,H-21} = 172.9$, $^{3}J_{C-22,H-17} = 3.4$, $^{2}J_{C-22,OMe} = 3.9$, $^{1}J_{OMe,H} = 147.3$. [b] Values are for the structure shown in Figure 4.

the secologanin unit in *N*-4-(*p*-bromobenzyl)-*O*,*O*,*O*,*O*-tetraacetyl vincoside.

The stereogenic elements and the number of possible stereoisomers of strictosidine (**1**) are shown in Table 1. In our work only staggered conformations around C-14 and the glycosidic oxygen bridge, as well as the two half-chair conformations of the two partially saturated heterocycles, were considered. The less stable configuration of N-1 and N-4 and the rotational position of the methoxycarbonyl, vinyl, hydroxymethyl, and hydroxy groups to the rings were not investigated because of the lack of NMR data. According to these restrictions, two series of configurational isomers based on C-3 can be expected, each having 324 conformers.

Table 6. Calculated Coupling Constants in the Three Staggered Conformations Across the C-3−C-14, C-14−C-15, C-1−O, and O−C-1′ Bonds and the Measured Values for Strictosidine (**1**)

assignment	calculated coupling constant (Hz) $\varphi = 60°$	$\varphi = 180°$	$\varphi = 300°$	measured (Hz)
$J_{H-3,H-14S}$	3.4	11.8	2.9	3.2
$J_{H-3,H-14R}$	3.6	11.8	2.6	11.8
$J_{H-14S,H-15}$	3.2	12.4	3.0	12.3
$J_{H-14R,H-15}$	3.0	12.4	3.2	2.9
$J_{C-2,H-14S}$	2.1	8.5	2.1	3
$J_{C-2,H-14R}$	2.1	8.5	2.1	2
$J_{C-3,H-15}$	2.1	7.7	2.1	2
$J_{C-14,H-20}$	2.1	6.8	2.1	6
$J_{C-16,H-14S}$	0.1	6.5	0.1	<2
$J_{C-16,H-14R}$	0.1	6.5	0.1	6.5
$J_{C-20,H-14S}$	2.1	8.5	2.1	<2
$J_{C-20,H-14R}$	2.1	8.5	2.1	2.5
$J_{C-21,H-1'}$	1.6	6.8	1.6	2.7
$J_{C-1',H-21}$	1.6	6.8	1.6	3.2

The conformations around the single bonds were determined from vicinal proton−proton and carbon−proton coupling constants. The measured values were compared with calculated coupling constants of the three staggered conformations. The vicinal proton−proton coupling constants were calculated using the parametrized Karplus type of equation of Haasnoot *et al.*[28] The vicinal carbon−proton coupling constants of the $C_\alpha-C_\beta-C_\gamma-H$ fragments were calculated using the equation of Aydin and Günther.[29] In the calculation of torsion angles larger than 90°, these authors applied −0.9 and −1.7 Hz correctional terms for carbon substituents at *β*- and *γ*-carbons, respectively; no correction was needed for carbon substituents at the α-carbon. These calculations were supported also by those of van Beuzekom *et al.*[30] However, Parella *et al.*[31] reported a negative contribution of the carbonyl substituent on the α-carbon. Therefore, in the calculation of $J_{C-16,H-14S}$ and $J_{C-16,H-14R}$, we applied a correctional term of −2 Hz to the equation of Aydin and Günther.[28] The vicinal carbon−proton coupling constants of C−O−C−H fragments were calculated by the Karplus type of equation described by Mulloy *et al.*[32] The calculated coupling constants of the three staggered conformations across bonds C-3−C-14, C-14−C-15, C-21−O, and O−C-1′ are summarized in Table 6.

There were nine conformations resulting from the rotation around C-3−C-14 and C-14−C-15 bonds in each of the two configurational series. One of the protons at C-14 had a large coupling constant (11.4 Hz) at H-3 and a small one (3.9 Hz) at H-15, whereas the other proton had a large coupling constant (11.5 Hz) at H-15 and a small one (3.0 Hz) at H-3. According to the calculated coupling constants in Table 6, this means that, in the dominant conformation, one of the H-14 atoms is antiperiplanar ($\varphi = 180°$) to H-3 and the other to H-15. Such an arrangement can be found only in two conformations in each (H-3α and H-3*β*) series: **S11** and **S22** as well as **V11** and **V22** (shown in Figure 1). In these conformers neither of the big ligands of C-3 and C-15 (C-2, N-4, C-16, C-20) interfere each other. Consequently, 14 of the 18 structures in which such interferences exist may be disregarded. Moreover, the coupling

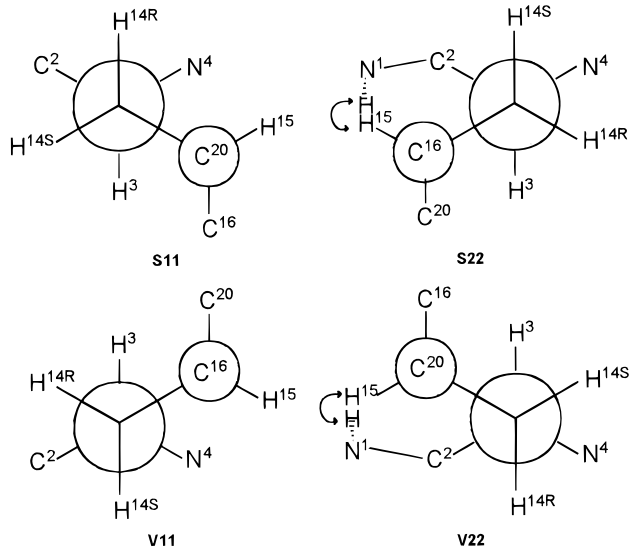

Figure 1. Conformations around C-14.

constants of C-2 with each of the diastereotopic hydrogens of C-14 proved to be small (2 and 3 Hz, respectively) which means, according to the calculated coupling constants of Table 6, that neither of the two diastereotopic hydrogens can be antiperiplanar to C-2 in the dominant conformation. Such an arrangement can be seen only in one structure of each selected pair (i.e., in **S11** and **V11**, respectively). Thus, the conformation around the bonds C-3−C-14 and C-14−C-15 was unequivocally established. **S22** and **V22** are destabilized by steric interference between H-1 and H-15 (indicated in Figure 1).

The difference between these two structures is that in **S11** one of the diastereotopic hydrogens of C-14 is antiperiplanar to C-16, while it is true for C-20 in structure **V11**. Because all other conformers were ruled out by the observed coupling constants described above, when we distinguish between **S11** and **V11** based on the coupling constants of C-16 and C-20 with the diastereotopic hydrogens of C-14 methylene group, we directly determine the configuration of the C-3 center of strictosidine (**1**). The coupling constant of C-16 and one of the hydrogens of the C-14 methylene group was 6 Hz, which means that these atoms are antiperiplanar. Moreover, C-20 has coupling constants of 2.5 Hz and less than 2 Hz with the H_2-14 protons, which indicates that neither of the H_2-14 protons are antiperiplanar to C-20 in the dominant conformation. These observations are consistent with structure **S11** and rule out structure **V11**. Therefore, the coupling-constant data unequivocally proved the *S* configuration of C-3 in strictosidine (**1**). The assignment of the H_2-14 protons can also be gained from structure **S11**. Because H-14*proR* is antiperiplanar to H-3, the signal at 2.09 ppm showing the 11.4 Hz coupling to H-3 belongs to H-14*proR*.

In principle, each of the 18 conformations around C-14 involves four possible conformers according to the *P* and *N* helicity of the half-chair conformations of the dihydropyran and tetrahydropyridine rings, respectively. Partial structures of the possible conformers of these heterocycles for the structure of **S11** are shown in Figure 2. The torsion angles around the C-20−C-21 bond in the dihydropyran and N-4−C-5 in the tetrahydropyridine rings may have negative (in the oxacycle of **S11NN** and **S11NP**, as well as in the azacycle of **S11NN** and **S11PN**) or positive (in the oxacycle of **S11PN** and **S11PP**, as well as in the azacycle of **S11NP** and **S11PP**) values, respectively. The molecular model of the two half-chair conformations of the dihydropyran ring was studied in detail. Here, and in the subsequent paragraphs, the structural formulas were generated and energy-minimized, and the torsion angles and through-space distances were estimated by the ALCHEMY molecular mechanics program.[33] The torsion angles between vicinal protons, as well as vicinal carbons and protons, were gained from these models (Table 7). The proton−proton and the carbon−proton coupling constants were calculated from these values using Karplus-type equations as described above for the data of Table 6. $J_{C-15,H-21}$ was calculated with the equation of Aydin and Günther;[29] however, for the antiperiplanar arrangement, the effect of oxygen on the γ-carbon was corrected by an additional term of −2 Hz according to the data of Parella *et al.*[31] The effect of a non-carbon substituent (X) on the α-carbon in a $X-C_\alpha-C_\beta-C_\gamma-H$ fragment was studied by van Beuzekom *et al.*[30] An increase of the coupling constant of about 2 Hz was reported for an oxygen substituent in a conformation with $\varphi = 180°$ and $\psi = 180°$ (where φ is the torsion angle of $C_\alpha-C_\beta-C_\gamma-H$ atoms and ψ is the torsion angle of $X-C_\alpha-C_\beta-C_\gamma$ atoms), while this effect was negligible in conformations where φ was about 60°. Therefore, a 2-Hz correction was applied in the calculation of $J_{C-21-H-15}$ in the positive conformation. In Table 7, we have summarized the calculated and measured data of those coupling constants that are characteristically different in the two half-chair conformations. These values show unambiguously that the conformational equilibrium is shifted strongly towards the positive conformation of the dihydropyran ring. The NOE between H-3 and the axial H-5α atoms indicates the closeness of these two atoms, which is possible only if in the tetrahydropyridine ring the torsion angle around the N-4−C-5 bond is positive. Were this angle negative, a NOE would be expected between H-*14proS* and the axial H-5*β* atoms, which was not observed, further supporting the positive torsion angle around the N-4−C-5 bond. Thus, both heterocycles in strictosidine have positive conformations (**S11PP**). According to the molecular models, this conformation is the least crowded one. The negative conformation of the dihydropyran ring (in **S11NN** and **S11NP**) would involve the pseudoequatorial position of the C-14 atom (having the *β*-carboline ring system) and a steric interference between the H-3 atom and the methoxycarbonyl group. The negative conformation of the tetrahydropyridine ring (in **S11NN** and **S11PN**) would force the C-14 atom with its likewise bulky substituent into a less favorable pseudoaxial position.

The nine Newman projections corresponding to the rotation around the bonds between C-21 and the glycosidic oxygen (O bridge), as well as between that and C-1′, are shown in Figure 3. Small values (2.7 and 3.2 Hz) for both three-bond carbon−proton coupling constants through the anomeric bonds (i.e., $J_{C-21,H-1'}$ and $J_{C-1',H-21}$) were measured, so that structures corresponding to **G13**, **G23**, **G31**, **G32**, and **G33** can be omitted, as the antiperiplanar arrangement of at least one of the above-mentioned carbons and protons should be present in these structures, and according to the data in Table 6, much higher coupling constants are expected

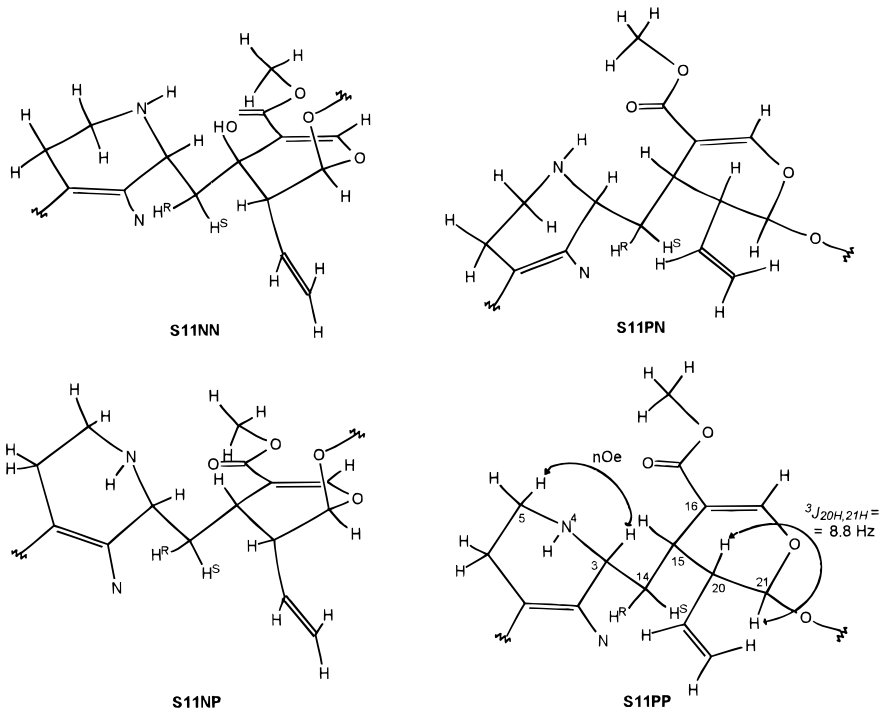

Figure 2. Conformations of the dihydropyran and tetrahydropyridine rings according to the partial structure of **S11**.

Table 7. Calculated Coupling Constants of the Two Half-chair Conformation of the Dihydropyrane Ring and the Measured Values

	positive conformation		negative conformation		
	ϕ (degree)	J calculated (Hz)	ϕ (degree)	J calculated (Hz)	J measured (Hz)
H-20−H-21	180	9.1	60	2.3	8.8
C-14−H-20	170	6.5	70	1.1	6
C-15−H-21	60	2.1	180	6.5	2.7
C-21−H-15	170	8.5	80	0.6	8
C-17−H-21	80	0.6	180	6.8	2
C-19−H-15	70	1.1	170	6.5	2.8

for these. The strong NOEs observed between H-21 and H-1′ also exclude these conformational arrangements, as the distances between these protons are more than 3.5 Å. Precisely in these conformations, the smallest ligands of C-21 and C-1′ (i.e., H atoms) are in synclinal position to both non-bonding electron pairs of the oxygen bridge; consequently, big ligands would interfere each other. Four conformations remained whose models were also studied. In **G22,** the hydrogen atoms of the vinyl group and the anomeric H-1′ of the β-D-glucopyranosyl unit would be closer than 3 Å in any rotational position around the C-19−C-20 bond. However, in the NOESY spectrum, no cross peaks according to these interactions were observed, so that the conformation **G22** can likewise be disregarded. For distinguishing between the three last conformers, **G11**, **G12**, and **G21**, no data can be expected from the NMR spectra. However, the through-space distance (obtained as described above) between C-19 and O-5′ (2.623 Å) in **G21**, as well as between the oxygen of the dihydropyran ring (O_p) and O-2′ (2.480 Å) in **G12** is shorter than the sum of the Van der Waals radii (3.0 Å and 2.8 Å, respectively). Thus, the preferred conformation should be **G11**, in which there are no short through-space distances between ligands. Moreover, in this conformation one of the non-bonding electron pairs of the O-bridge to bond C-21−O_p, the other to bond C-1′−O_g, is antiperiplanar. Consequently, the conditions for two stabilizing σ conjugations are fulfilled.

The molecular model containing all the above-described structural elements of strictosidine (**1**) was constructed and energy-minimized. The three-dimensional structure obtained is shown in Figure 4. To check the correctness of this structure, the torsion angles of the model were determined, and the expected coupling constants calculated by Karplus-type equations. Comparison of the measured and calculated coupling constants (Tables 3 and 5) show a reasonably good agreement. Moreover, measured NOE effects (Table 4) matched well all the short proton−proton distances of the calculated structure. Unfortunately, we could not obtain appropriate spectroscopic data for the determination of the preferred steric orientation of the vinyl group around the C-19−C-20 bond and of the methoxycarbonyl group around the C-16−C-22 bond. However, studies on the molecular models constructed by the ALCHEMY program suggest their position as shown in Figure 4.

This study has established the detailed stereochemistry of strictosidine (**1**) directly for the first time and also presents an argument for the substantial conformational preference for existence of a single species in MeOH from the 648 selected stereoisomers. The three-dimensional structure of strictosidine (**1**) determines its rich chemistry and bioorganic chemistry, which will be presented in future papers.

Experimental Section

Preparation of Strictosidine (1). Equimolar amounts of tryptamine (**2**) base (0.16 g, 0.1 mmol) and tryptaminium chloride (0.20 g, 0.1 mmol) were dissolved in a mixture of H_2O (10 mL) and glacial HOAc (0.5 mL); secologanin (**2**) (0.76 g, 0.2 mmol) was added, and the reaction mixture was stirred under N_2 in a glycerol bath at 100 °C for 6 h. After treatment in an ice bath for 30

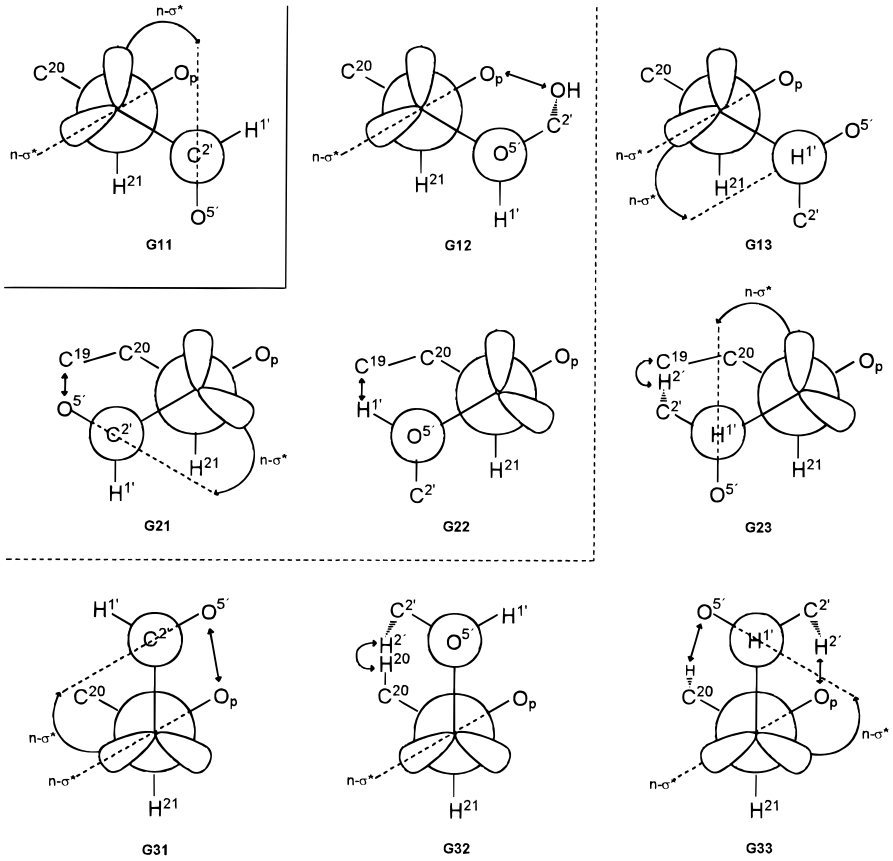

Figure 3. Conformations around the glycosidic bridge.

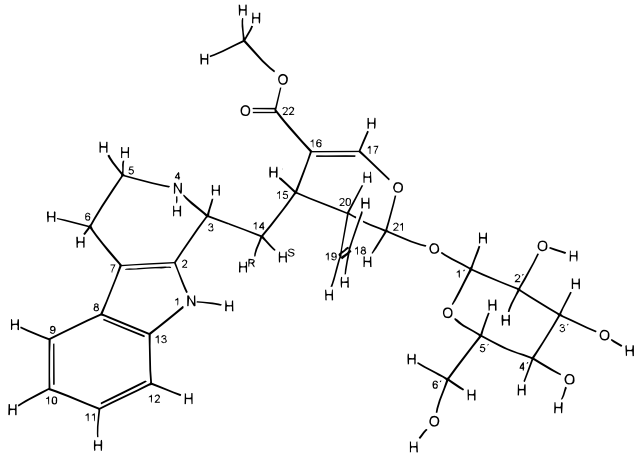

Figure 4. Perspective formula of strictosidine.

min, the vincosamide crystals were filtered out and the mother liquid extracted with EtOAc (3 times, each 30 mL). The organic phase was dried with anhydrous Na_2SO_4 and evaporated. The total amount of filtered and extracted vincosamide was 0.27 g (27%). The aqueous phase was evaporated *in vacuo*, and the crude strictosidine hydrochloride (0.67 g, 62%) was dissolved in a buffer solution of pH 6.5 (5.0 mL), extracted with EtOAc (3 times, each 10 mL) to eliminate traces of vincosamide, and lyophilized. The product was taken up in EtOH (2.0 mL), and the insoluble inorganic salts were filtered out. After evaporation of the solvent, the product was chromatographed on a Kieselgel 60 column (30 g; eluents: $CHCl_3$−MeOH, 4:1). Fractions 28−58 (each 1.0 mL) gave pure strictosidine (**1**) (0.39 g, 36%) after evaporation. Anal. Calcd for $C_{27}H_{34}N_2O_9$: C, 61.12; H, 6.45; N, 5.28. Found: C, 60.85; H, 6.12; N, 5.20.

NMR Measurements. NMR spectra were recorded on a Bruker AC-400 instrument in deuteriomethanol. Unambiguous assignments of the ^{1}H-NMR spectrum were based on COSY and NOESY 2D spectra. These assignments were transferred to the protonated carbons by a carbon-detected heteronuclear chemical shift correlation 2D experiment (HETCOR). The unambiguous assignments of all the other carbons were based on modified HSQC experiments,[34] and the carbon−proton coupling constants were determined from the slices of these measurements. The delay for polarization transfer was 30 and 45 ms in two HSQC experiments, respectively. A 1-ms purging pulse was applied in the HSQC experiments. The NOESY spectrum was recorded with a 0.8-s mixing time. The NMR spectral data of strictosidine (**1**) are given in Tables 2−5.

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References and Notes

(1) Atta-ur-Rahman; Basha, A. *Biosynthesis of Indole Alkaloids*; Clarendon Press: Oxford, 1983; pp 45−93.
(2) Kapil, R. S.; Brown, R. T. In *The Alkaloids*; Manske, R. H. F., Rodrigo, R., Eds.; Academic Press: New York, 1979; Vol. 18, pp 545−588.
(3) Smith, G. N. *Chem. Commun.* **1968**, 912.
(4) Stöckigt, J.; Zenk, M. H. *Chem. Commun.* **1977**, 646−648.
(5) Battersby, A. R.; Burnett, A. R.; Parsons, P. G. *J. Chem. Soc.* **1969**, 1193−1200.
(6) Cordell, G. A. *Lloydia* **1974**, *37*, 219−298.
(7) Kennard, O.; Roberts, P. J.; Isaacs, N. W.; Allen, F. H.; Motherwell, W. D. S.; Gibson, K. H.; Battersby, A. R. *Chem. Commun.* **1971**, 899−900.
(8) De Silva, K. T. D.; Smith, G. N.; Warren, K. E. H. *Chem. Commun.* **1971**, 905−907.
(9) De Silva, K. T. D.; King, D.; Smith, G. N. *Chem. Commun.* **1971**, 908−910.

(10) Blackstock, W. P.; Brown, R. T.; Lee, G. K. *Chem. Commun.* **1971**, 910−911.
(11) Johns, S. R.; Lamberton, J. A.; Occolowitz, J. L. *Aust. J. Chem.* **1967**, *20*, 1463−1471.
(12) Janot, M. M.; Goutarel, R. *Bull. Soc. Chim. France* **1951**, 588−602.
(13) Battersby, A. R.; Breuer, S. W.; Garratt, S. *J. Chem. Soc. (C)* **1968**, 2467−2471.
(14) Vamvacas, C.; von Philipsborn, W.; Schlittler, E.; Schmid, H.; Karrer, P. *Helv. Chim. Acta* **1957**, *40*, 1793−1808.
(15) Wenkert, E.; Bringi, N. V. *J. Am. Chem. Soc.* **1958**, *80*, 3484−3485.
(16) E. Wenkert, E.; Bringi, N. V. *J. Am. Chem. Soc.* **1959**, *81*, 1474−1481.
(17) Ochioai, E.; Ishikawa, M. *Chem. Pharm. Bull.* **1958**, *6*, 208−213; *Chem. Abstr.* **1959**, *53*, 6279c.
(18) Battersby, A. R.; Gregory, B.; Spencer, H.; Turner, J. C.; Janot, M.-M.; Potier, P.; Francois, P.; Levisalles, J. J. *Chem. Commun.* **1967**, 219−221.
(19) Scott, A. I.; Lee, S. L.; de Capite, P.; Culver, M. G.; Hutchinson, C. R. *Heterocycles* **1977**, *7*, 979−984.
(20) Mattes, K. L.; Hutchinson, C. R.; Springer, J. P.; Clardy, J. *J. Am. Chem. Soc.* **1975**, *97*, 6270−6271.
(21) Hutchinson, C. R.; Heckendorf, A. H.; Straugh, J. L.; Daddona, P. E.; Cane, D. E. *J. Am. Chem. Soc.* **1979**, *101*, 3358−3369.
(22) Kocsis, Á.; Pál, Z.; Szabó, L.; Tétényi, P.; Varga-Balázs, M. Eur. Pat. Appl. EP 156267, October 2, 1985; *Chem. Abstr.* **1986**, *104*, P 149345q.
(23) Inouye, H.; Yoshida, T.; Tobita, S.; Okigawa, M. *Tetrahedron* **1970**, 3905−3915.
(24) Inouye, H.; Yoshida, T.; Nakamura, Y.; Tobita, S. *Chem. Pharm. Bull.* **1970**, *18*, 1889−1899; *Chem. Abstr.* **1971**, *74*, 3837s.
(25) Souzu, I.; Mitsubashi, H. *Tetrahedron Lett.* **1970**, 191−192.
(26) Birch, A. J.; Grimshaw, J. *J. Chem. Soc.* **1961**, 1407−1708.
(27) Lentz, P. J., Jr.; Rossmann, M. G. *Chem. Commun.* **1969**, 1269.
(28) Haasnoot, C. A. G; de Leeuw, F. A. A. M.; Altona, C. *Tetrahedron* **1980**, *36*, 2783−2792.
(29) Aydin, R.; Günther, H. *Magn. Res. Chem.* **1990**, *28*, 448−457.
(30) van Beuzekom, A. A.; de Leeuw, F. A. A. M.; Altona, C. *Magn. Reson. Chem.* **1990**, *28*, 68−74.
(31) Parella, T.; Sanchez-Ferrando, F.; Virgili A. *Magn. Res. Chem.* **1994**, *32*, 657−664.
(32) Mulloy, B. Frenkiel, T. A.; Davies, D. B. *Carbohydr. Res.* **1988**, *184*, 39−44.
(33) ALCHEMY II molecular modeling system for the IBM PC. Tripos Associates, Inc., St. Louis, MO.
(34) Kövér, K. E.; Prakash, O.; Hruby, V. J. *Magn. Res. Chem.* **1993**, *31*, 231−237.

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