Pergamon

Tetrahedron, Vol. 53, No. 30, pp. 10489-10502, 1997

0040-4020/97 $17.00 + 0.00

PII: S0040-4020(97)00659-5

Chemistry of Secologanin. Part3. Graph Analysis of the Acidic Deglycosylation of Secologanin Derivatives

Angéla Schwartz, László F. Szabó*, Benjamin Podányi**

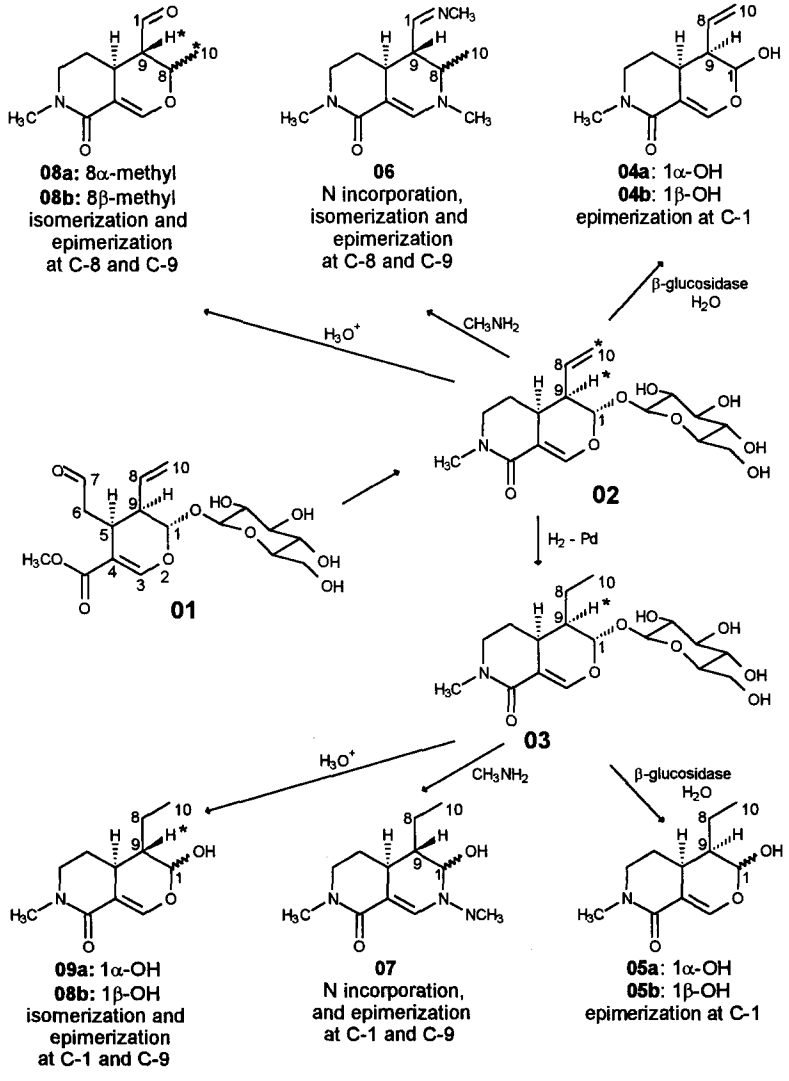
Institute of Organic Chemistry, Semmelweis University of Medicine, Hőgyes u. 7. H-1092 Budapest, Hungary
**Permanent address: Chinoin Pharmaceutical and Chemical Works, Ltd., Tó utca 1-5. H-1045 Budapest, Hungary

Abstract: Acidic deglycosylation of *N*-methylbakankosine **02** and its 8,10-dihydro derivative **03** gave a rearranged **08a-b** and an epimerized **09a-b** pair of aglycones, respectively. It could be demonstrated that the thermodynamically controlled complex reaction sequence involved in the "natural" series (**02**) 48 aglycones, 92 equilibria and 368 elementary steps, in the "dihydro" series (**03**) 24 aglycones, 40 equilibria and 160 elementary steps. However, the shortest and most probable reaction sequence could be prognosed by graph analysis. © 1997 Elsevier Science Ltd.

Secologanin **01**,[1] the precursor of more than 2200 indole and related alkaloids, is a monoterpenoid glycoside.[2] The compound is exceptionally rich in functional groups, and chemical reactions can be carried out at all carbon atoms of the aglycone skeleton. However, this multiple reactivity is partially blocked by the glycosidic linkage. As expected, a large number of transformations starts to run after deglycosylation. The β-D-glucopyranosyl unit can be removed by three methods (examples are given in Scheme 1): a) deglycosylation with β-glucosidase; b) reaction with amine under simultaneous incorporation of the amino unit; c) aqueous acidic deglycosylation. The enzymatic deglycosylation of secologanin itself was first carried out by R. T. Brown and C. L. Chapple.[3] In a series of papers, the same research group applied the enzymatic deglycosylation of such secologanin derivatives in which the formyl group had been blocked.[4-6] The reaction with amines involves a rather complicated reaction sequence which was recently analyzed in our laboratory,[7] and is still under further investigation. The simplest method for removal of the sugar unit is the acidic deglycosylation. In order to simplify the problem and to decrease the number of possible transformations and products, it was investigated by us on *N*-methylbakankosine **02** and its 8,10-dihydro derivative **03**. The lactamtype compound bakankosine itself is a natural product from *Strychnos Vacacoua* Baillon by E. Bourquelot and H. Herissey.[8] Its structure was investigated by R. Goutarel, V. Prelog *et al*,[9] and finally proved by L.-F. Tietze[10] and by H. Inouye's group.[11] Its preparation from secologanin and deglycosylation by b-glucosidase was carried out by Tietze.[10] During deglycosylation, partial epimerization on C-1 was observed. As the interpretation of possible individual steps

during acidic deglycosylation of **02** and **03** became increasingly more complicated, we decided to study the reaction matrix by graph analysis. Application of graphs in the investigation of structures, spectroscopic and quantum chemical properties of organic compounds is well known. Its use in the interpretation of reaction pathways and mechanisms is much less common. Early application of it was demonstrated by K. Mislow.[12,13] It proved to be particularly useful in cases when many possible structures and pathways should be considered systematically. That was the situation in our research in the field of the chemistry of secologanin, too.

Results and Discussion



Scheme 1. Preparation and Deglycosylation of Secologanin Derivatives

N-methylbakankosine **02** was prepared by treating secologanin with methylamine in ethanolic solution, followed by reduction with sodium tetrahydridoborate.[14] Catalytic hydrogenation of **02** gave 8,10-dihydro-*N*-methylbakankosine **03** (Scheme 1). Enzymatic deglycosylation of **02** and **03** was carried out with β-glucosidase in aqueous solution at pH 4. Both glycosides gave the appropriate aglycones **04a-b** (in 9:1 ratio) and **05a-b** (in 9:1 ration), respectively, by partial epimerization on C-1, only. Acidic deglycosylation of **02** and **03** were carried out in boiling 2 molar aqueous hydrochloric acid for 60 min. In both cases, epimer pairs **08a-b** (in 2:3 ration) and **09a-b** (in 4:1 ratio), respectively, were isolated as main products from the complex reaction mixture.

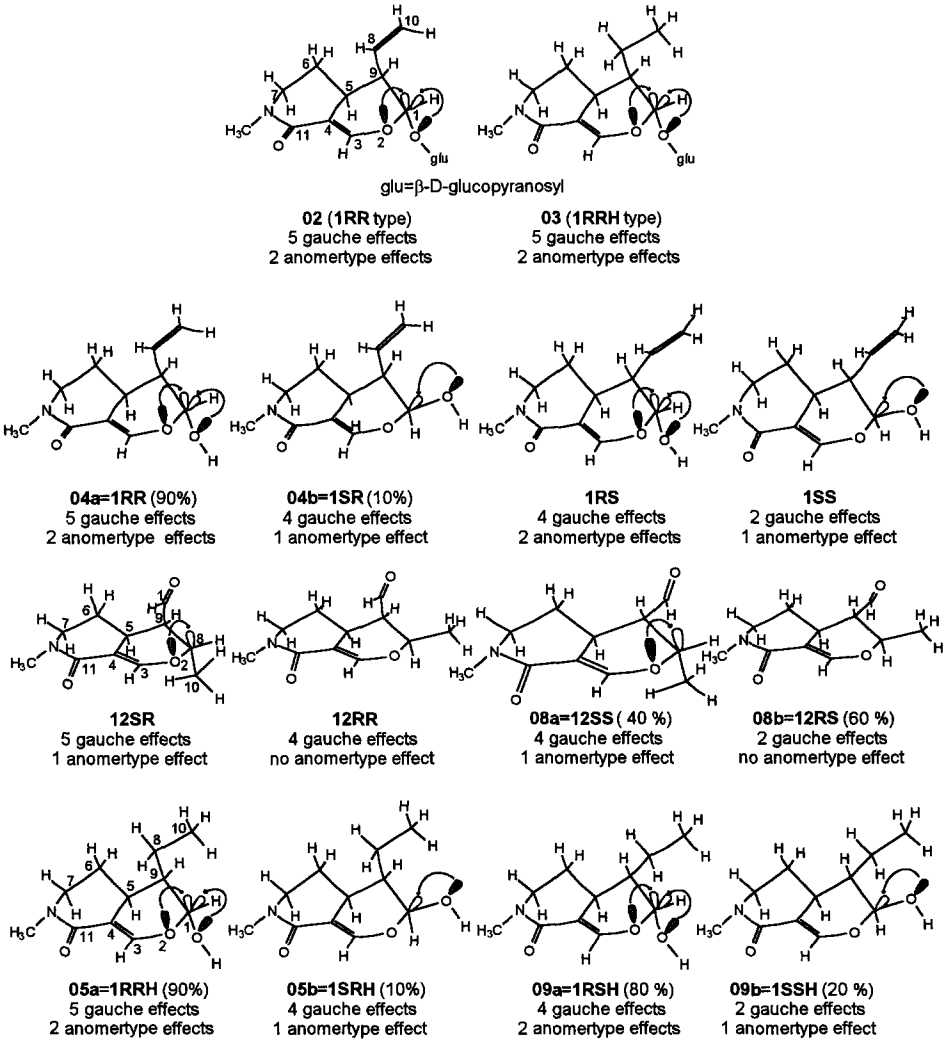
The ^{1}H- and ^{13}C-NMR spectra proved unequivocally the structures of all compounds. The formation of **02** was indicated by disappearance of the signal of the formyl and the methoxy protons, as well as by the appearance of the signal of the *N*-methyl group in the ^{1}H-NMR spectrum. Likewise, formation of **03** by catalytic hydrogenation of **02,** was proved by disappearance of the signals of the vinyl and appearance of the ethyl protons. Absence of the signals of the β-D-glucopyranosyl unit in the spectra of all aglycone samples demonstrated the deglycosylation. In the product samples, some (in the ^{1}H-NMR spectrum of **08a-b** nearly all) signals of protons and carbons appeared in pair which indicated the presence of two epimers. Absence of further more important changes in the NMR spectra of **04a**-b, **05a-b**, and **09a-b** excluded deeper structural changes during deglycosylation. However, in the spectrum of **08a-b,** the signals of a new formyl proton (at 9.82 ppm and 9.79 ppm) and of protons of a new methyl group (at 1.42 ppm and 1.20 ppm) were observed, with the simultaneous disappearance of the signals of the vinyl protons. These changes suggested the structural isomerization of the aglycone skeleton.

The stereochemical analysis based on the experimental fact that *N*-methyl-bakankosine **02** and its 8,10-dihydro derivative **03** were prepared from secologanin **1.** The stereochemistry of **1** (involving the *S* configuration of C-5 of the secologanin unit) was determined Hutchinson et al.[15] with X-ray diffraction analysis of *N*-4-(*p*-bromobenzyl)-*O,O,O,O*-tetraacetyl vincoside which was likewise obtained from secologanin. Our recent direct and detailed stereochemical analysis of strictosidine (also prepared from secologanin) supported it as well.[16]

The configuration of the centers of chirality could not be changed during the preparation of *N*-methyl-bakankosine **02** and its 8,10-dihydro derivative **03**. Molecular models and theoretical considerations suggested, that in the lactam type derivatives of secologanin (like **02** and **03** and their analogues) the dihydropyran ring was necessarily fixed in negative conformation and H-5 in α-axial position (stereostructures of the educts and products are given in Scheme 2). Consequently, the relatively small value of $J_{5,9}$ in **02**, **03**, **04a-b** and **05a-b** proved α-pseudo equatorial position of H-9 and the retention of configuration at C-9 during the enzymatic deglycosylation. As previous analysis[15,16] justified the trans relation of H-9 and H-1, the small value of $J_{1,9}$ in **02** (5.6 Hz and 1.8 Hz) and in **03** (1,8 Hz) involved trans-diequatorial position of them, and trans diaxial position of the substituents.

In the ^{13}C-NMR spectrum of **04a-b** and **05a-b**, the major epimer gave a signal for C-8 at higher ppm and for C-5 at lower ppm than the minor one. According to this γ effect, the hydroxy group was in α-axial position in the major (**04a=1RR** and **05a=1RRH**) and β-equatorial position in the minor (**04b=1SR** and **05b=1SRH**) epimer. The high value of $J_{5,9}$ in the ^{1}H-NMR spectrum of **08a**-b and **09a-b** proved the β-axial position of H-9 and necessarily epimerization of the configuration of C-9 in sample **09a-b**. In this latter sample, the α-axial and β-equatorial position of the hydroxy group was proved by a low $J_{1,9}$ value in the major (**09a=1RSH**) and by a high one in the minor epimer (**09b=1SSH**), respectively. In **08a-b** the $J_{8,9}$ had a low value (4.2 Hz) in the minor

isomer and a high one (10.2 Hz) in the major isomer which indicated their α-axial and β-equatorial position in **08a** (= **12SS**) and **08b** (= **12RS**), respectively.

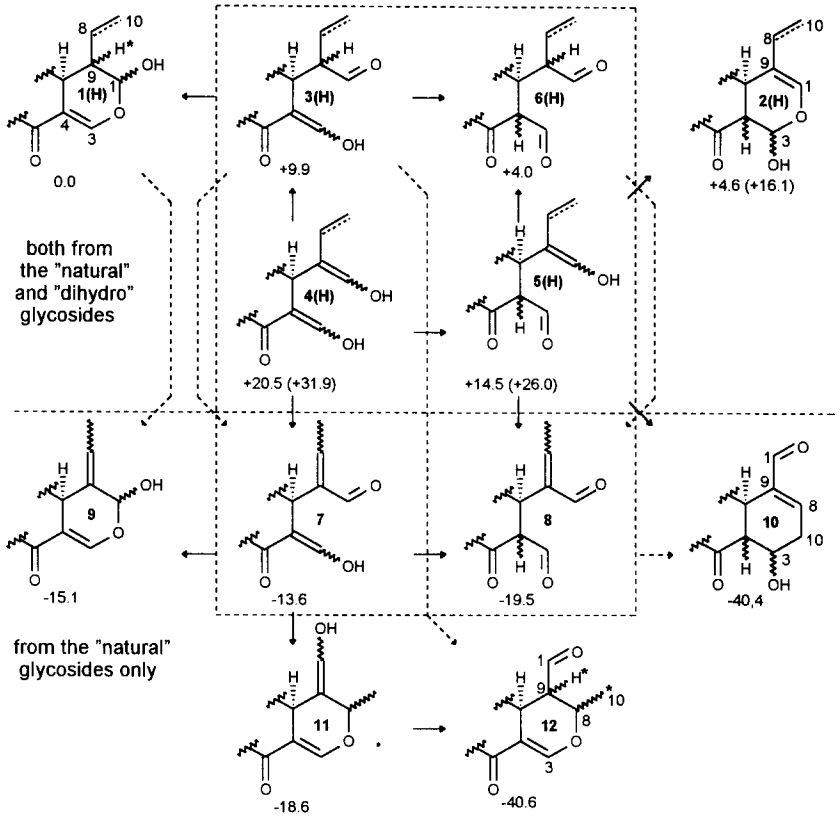


Scheme 2. Stereostructures of Educts and Products

In order to get a deeper insight into the events during acidic deglycosylation, both the educts **02** and **03** as well as the product samples **08a-b** (=**12**) and **09a-b** (=**1H**) were treated in deuterated water under the same reaction conditions as applied in the deglycosylation. H-D exchange was observed in both samples at H-9 and in the "natural" one at H-10, too (shown by asterisk in Scheme 1). Moreover, the epimer composition of the product samples isolated either directly from the deglycosylation reaction or after treatment mentioned above was the same.

The stereochemical changes and the H-D exchanges suggested that under circumstances of the acidic deglycosylation, the dihydropyran ring was temporarily cleaved, and simple and/or vinylogous tautomerizations

took place. Structural considerations proposed the possible formation of twelve structural isomers of the aglycone in the "natural" series and six in the "dihydro" series (shown in the energy graph, Scheme 3). The educts **02** and **03** and the products **04a-b, 05a-b** and **9a-b** correspond to structure **1(H)**, the product **8a-b** to structure **12**.

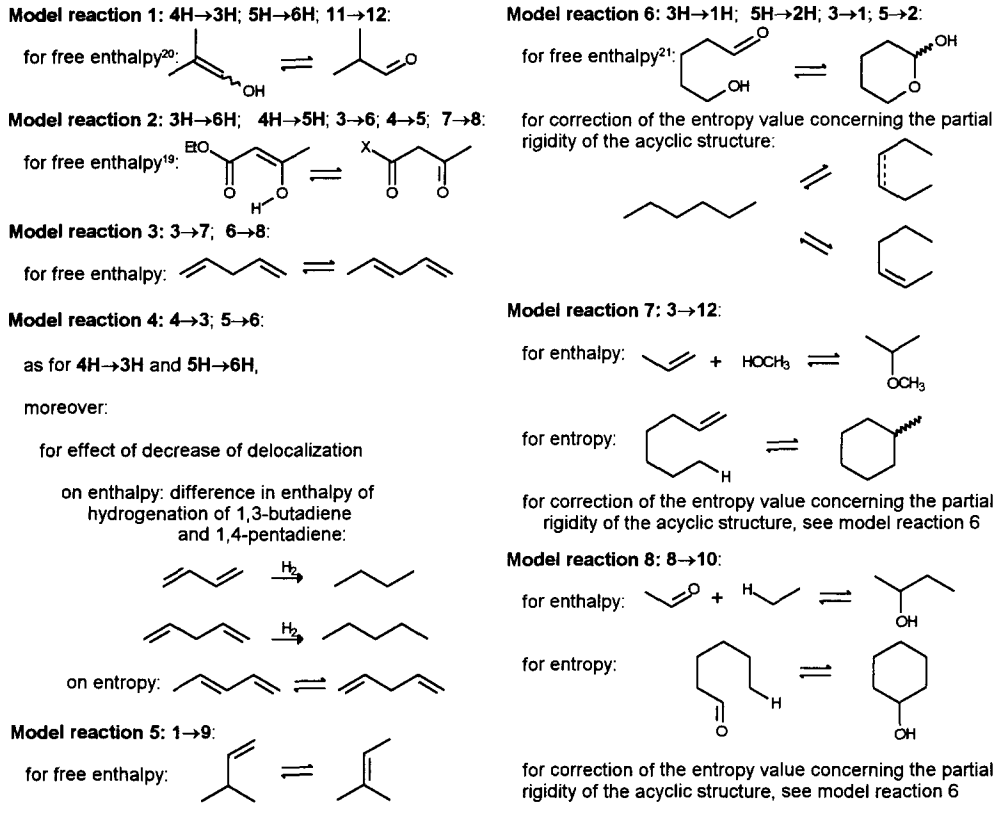


Scheme 3. The Energy Graph of the Possible Aglycones.
Estimated relative free enthalpies of formation in kJ/mol. Energy values in parentheses conc possible aglycones derived from 8,10-dihydro glycosides.

In Scheme 3, the arrows represent equilibria catalyzed by proton and indicate the direction toward the lower energy state. Normal line arrows show the most probable reactions, broken line arrows indicate transformations taken up for energy calculations, only (indirect reactions). Broken line in the appropriate formulae shows the position of the eventual double bond in aglycones derived from the "natural" glycoside. Structures in the upper part of the graph may be formed from both the "dihydro" and the "natural" educts, structures in the lower part from the "natural" one, only. Structures in the central part of the graph (shown in broken line frame) are pairwise enol-oxo tautomers, structures in the external and lower part are in chain-ring equilibrium with the appropriate partner.

The energy data in Scheme 3 are approximate free enthalpies of formation (at 298şC) related to the "primary" aglycone according to structure **1(H)**. The enthalpy and entropy values were estimated on model reactions shown in Scheme 4. Configurational and conformational isomerism could not be accounted at this

level. The values of enthalpy and entropy of formation of the model educts and products were taken from the literature.[17,18] In some cases, the free enthalpies of formation were directly calculated from equilibrium constants or composition.[19-21] When appropriate data were not available, values of more or less close derivatives were taken or extrapolated.



Scheme 4. Model Reactions for the Calculation of Relative Free Enthalpies of Formation

This necessarily involved compromises, which are described as follows. For model reaction 2, the free enthalpy was calculated from the equilibrium composition of enol and oxo form of ethyl acetoacetate, instead of the appropriate amide (the value probably contains the effect of the intramolecular hydrogen bond as well). In model reaction 3 and for correction of the entropy value of model reactions 4, 6 and 7, the values of the homoconjugate system were used instead of those of the heteroconjugate one. In model reaction 4, the value of free enthalpy used in model reaction 1 was corrected by the effect of the suppresssion of the conjugation. The difference in the enthalpy of hydrogenation of 1,3-butadiene and 1,4-pentadiene was taken as the effect of conjugation on the enthalpy value, and the difference in entropy of formation of 1,3- and 1,4-pentadiene as the effect on the entropy value. In model reactions 6, 7 and 8, the entropy value was corrected according to the partial rigidity of the acyclic compound. The entropy differences between pentane and *Z*-2- and -3-pentene

were used for that purpose. In model reaction 6 and 7, reaction of an alcoholic hydroxy group was considered instead of an enolic one. In model reaction 8, the reaction of nonactivated C-H bond was considered instead of a bond activated by a carbonyl group in vinylogous position. In reactions where no values could be found, the appropriate free enthalpy values were calculated indirectly (derived reactions in Scheme 4). The compromises could influence in some extent the free enthalpy values, however, it was thought they did not influence the order of values.

Some reaction runs were followed by NMR spectroscopy. In the ^{1}H-NMR spectrum of the reaction mixture of educt **02**, a dublet at 2.09 ppm (J=10 Hz) and a quartet at 6.87 ppm (J=10 Hz) ppm with an intensity ratio of 3:1, but with low intensity could be observed which suggested the temporary presence of an ethylidene group according to structures **7**, **8** and **9** (Scheme 3).

In the interpretations of the changes in acidic deglycosylation, it was considered, that H-D exchange and epimerization at C-9 should run through enol-oxo equilibrium involving C-1 and C-9 at the level of structure **4(H)** and/or **5(H)** which have the highest energy in the total scheme. Equilibrium should go back till this level. No direct proof could be found for an analogous tautomerization involving C-3 and C-4, as H-4 which could have indicated an eventual H-D exchange is absent in the product samples. However, this is strongly probable as H-4 in structures **5(H)**, **6(H)** and **8** should be more activated than H-9 or H-10 and the enol is stabilized by heteroconjugation (and even by intramolecular hydrogen bridge).

The structure of the products, the stereochemical changes, the H-D exchanges and the constancy of epimer composition of the product samples after equilibration suggested that the total reaction sequence of the acidic deglycosylation is thermodynamically controlled. As one can see from the energy graph (Scheme 3), compounds isolated from the reaction mixture have the lowest relative free enthalpy of formation both in the "dihydro" and "natural" series. Actually, in the "natural" series, the estimated free enthalpy value of structure **10** and **12** are nearly the same. Although it could not be isolated or demonstrated, a small amount of **10** can not be excluded to be formed during deglycosylation. It may also be supposed that some difference between the free enthalpies of **10** and **12** were covered by the approximate character of the procedure of energy estimation. However, it should be noted, that R. T. Brown and S. B. Pratt could isolated derivatives according to type **10** under slightly different conditions of enzymatic deglycosylation.[22] Moreover, this type of structure is well know in indole and related alkaloids.

As all possible aglycones in Scheme 3 have two stereogenic elements (asymmetrically substituted double bond and/or center of chirality) which could easily undergo stereomutation, each of formulae represents four diastereomers. Because of the lack of experimental data, it would be futile to estimate the energy levels of the individual stereoisomers. However, the energy differences of *E-Z* isomers in simple hydrocarbons, as well as the conformational energies of the simple substituents are known to be in the frame of the energy values estimated and given for interactions in Scheme 3. Moreover, studies on models generated by Alchemy 2 program suggested no special preference for *E-Z* or *R-S* isomers. Therefore it may be supposed that the equilibria of Scheme 3 are not radically influenced by the individual stereoisomers.

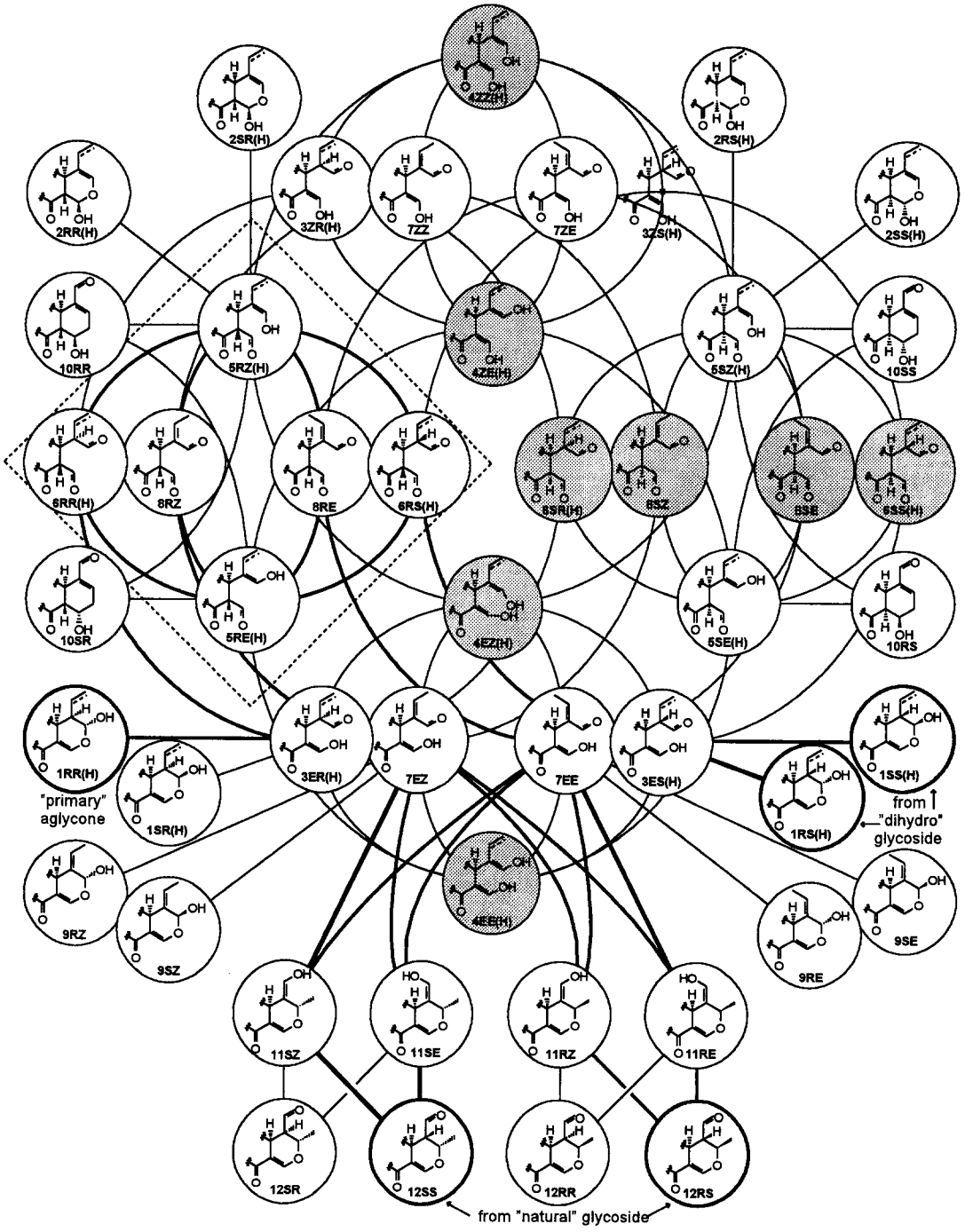
The 48 structural and stereoisomers are shown in the structure graph (Scheme 5). Aglycones indicated with **(H)** can be formed from both the "natural" and the "dihydro" glycosides, the others from the "natural" one, only. Aglycones having an intact dihydropyran or cyclohexene ring ("cyclized" isomers) were placed in the periphery of the graph, structures having cleaved this ring ("acyclic" isomers) in the central part. Curves in the central part and lines in low part of the periphery represent enol-oxo tautomeric equilibria, lines going out of the

central part toward the periphery indicate chain-ring isomerisation. The types of equilibria and the total number of elementary steps in the graph are shown in Table 1.

Table 1. Elementary Steps in the Acidic Deglycosylation of Secologanin Derivatives

type of transformation	in "natural" series		in "dihydro"series	
enol-oxo tautomerization cycle with *RS* isomerization at C-9 (medium sized curves in the central part)	4 x 4	16 equilibria	4 x 4	16 equilibria
enol-oxo tautomerization cycle with *RS* isomerization at C-4 (long curves in the central part)	4 x 4	16 equilibria	4 x 4	16 equilibria
enol-oxo tautomerization cycle with *RS* isomerization at C-4 in the "iso" compound (long curves in the central part)	2 x 4	8 equilibria		
enol-oxo tautomerization cycle with *ZE* isomerization at C-8 (short curves in the central part))	4 x 4	16 equilibria		
enol-oxo tautomerization cycle with *RS* isomerization at C-9 in the "iso" compound (short lines in the low part of the periphery)	2 x 4	8 equilibria		
ring-chain isomerization with formation of a lactol (straight lines toward the periphery)	3 x 4	12 equilibria	2 x 4	8 equilibria
ring-chain isomerization with formation of an oxacycle (straight lines toward the periphery)	2 x 4	8 equilibria		
ring-chain isomerization with formation of a carbocycle (straight lines toward the low part of the periphery)	2 x 4	8 equilibria		
total:		92 equilibria		40 equilibria
each equilibrium involves forward and backward reactions:	x 2	184 reactions	x 2	80 reactions
each step involves protonation and deprotonation	x 2	368 steps	x 2	160 steps

In the structure graph (Scheme 5), all possible transformations of the 48 isomers are indicated. As the estimated energy values for compounds on the highest energy level are only slightly higher than values for compounds which must be formed for H-D exchange, it can really be supposed, that any of the compounds of the structure graph may not be excluded from the equilibrium. However, some steps involving intermediates of higher energy level seem to run less probably than others. As the structures having two enolic groups and placed in the perpendicular diagonal of the central part (**4ZZ**, **4ZE**, **4EZ**, **4EE**, in gray background) have the highest energy, isomerization cycles containing them are less probable. Likewise dioxo compounds in the right part of the horizontal diagonal having H-4 and H-5 in cis relation (**6SR**, **8SZ**, **8SE**, **6SS**, in gray background) are less stable, than the trans isomers in the left part (**6RR**, **8RZ**, **8RE**, **6RS**) because the formers have one synclinal interaction more than the latters. Therefore, the C-6 *RS* and C-8 *EZ* isomerization cycles in broken line frame seem to be the most probable of the 16 tautomeric cycles in the central part of the graph. On the base of these



Scheme 5. Structure Graph of the Posssible Aglycones

"selection rules", the shortest pathway from the primary aglycone to the isolated epimer pair could be prognosed and are indicated by bold curves and lines in Scheme 5.

According to experimental data, the enzymatic deglycosylation is accompanied only by simple epimerization at C-1, which can easily be explained by cleavage of the primary aglycone **1RR(H)**, eventual tautomerization to **6RR(H)** and recyclization of the dihydropyran ring into the two epimers **1RR(H)** (**04a** or **05a**) and **1SR(H)** (**04b** or **05b**).

The events during the acidic deglycosylation of the "dihydro" glycoside **03** can likewise be interpreted. After hydrolytic removal of the β-D-glucopyranosyl unit, the dihydropyran ring of the "primary" aglycone **1RRH** is cleaved to **3ERH,** the compound tautomerized to **6RRH** which is already a member of the "privileged" C-6 *RS* isomerization cycle (medium sized curves in the dashed frame) where, through **5RZH** or **5REH,** the configuration of C-9 could be epimerized. Retautomerization of **6RSH** to **3ESH** and recyclization to **1RSH** (=**09a**) and **1SSH** (=**09b**) completes the reaction sequence.

In the acidic deglycosylation of the "natural" glycoside **02,** the interpretation is more complicated. The vinyl group of structure **3** can easily be isomerized through double tautomerization to the more stable ethylidene group of structure **07** (see the appropriate values of free enthalpy of formation in Scheme 3) in which new possibilities are opened for cyclization: 0-2 can be attached not only to C-1 (1,2-addition to structure **9**) but, after rotation around bond C-5–C-9, to C-8 (1,4-addition to structure **11**), too; moreover, a vinylogous aldol reaction can run between C-3 and C-10 (to structure **10**). As the energy values show, formation of **12,** after tautomerization of **11,** is the most favorable, being the most stable compound in Scheme 3. Therefore, the shortest and most probable way from the educt to the products can be interpreted as follows: After the hydrolytic removal of the β-D-glucopyranosyl unit, the dihydropyran ring of the "primary" aglycone **1RR** is cleaved to **3RH**, the compound tautomerized to **6RR** which, by tautomerization, enters the C-8 *ZE* isomerization cycle (short curves in the broken line frame) where the 8(10)-double bond could be isomerized through **5RZ** and **5RE** to **8RZ** and **8RE**, respectively. Retautomerization of these compounds to **7EZ** and **7EE** and recyclization of either of them to **11SZ**, **11SE**, **11RZ** and **11RE** gave, after a second tautomerization, the most stable final aglycones **12SS** (=**08a**)and **12RS** (=**08b**).

As it was shown above, the epimer ratio of the product components can approximately be measured from the ^{1}H-NMR spectrum. These ratios can easily be interpreted by considering stabilizing anomertype and destabilizing gauche interactions of the ligands of C-1 and C-9 in **09a-b** (=**1RSH** and **1SSH**) as well as of C-8 and C-9 in **08a-b** (= **12SS** and **12RS**). The perspective stereoformulae of the educts and the most important aglycones in Scheme 2 show, that in acidic deglycosylation of both the "natural" and "dihydro" glycoside, the aglycone epimers having the minimum number of gauche and maximum number of anomertype effects were formed and isolated. As expected, the anomertype effect involving the hydroxy group is stronger than that involving the methyl group which is a consequence of the higher electronegativity of oxygen over carbon.

In summary, the results presented in this paper show that, in spite of the enzymatic deglycosylation, in acidic hydrolysis the complicated reaction sequence involves in the "natural" series 48 aglycones, 92 equilibria and 368 elementary steps, in the "dihydro" series 24 aglycones, 40 equilibria and 160 elementary steps. Although any of these structures and transformation can be excluded, by graph analysis the shortest rational pathways could be found for the interpretation of the events. The results can be used for the investigation of more complicated cases in the bioorganic chemistry of indole and related alkaloids, too, as it will be shown in subsequent papers.

Experimental

^{1}H-NMR spectra were recorded at 400 MHz in $CDCl_3$, in acetone-d_6 and in pyridine-d_5 on a Bruker AC-400 or a Bruker DRX-400 instrument, using tetramethylsilane as chemical shift reference.. The assignment of the spectrum **08** was supported by a 2D TOCSY spectrum measured with 70 ms spin-lock time using the standard microprogram of the XWINNMR program. ^{13}C-NMR spectra were recorded at 100 MHz on the same instrument or at 50 Mhz an a Bruker AM-200 instrument. The assignments of the ^{13}C NMR spectra were suppported by a DEPT-135 spectrum.

***N*-methylbakankosine (02), (4β-ethenyl-3α-(β-D-glucopyranosyloxy)-3,4,4aα,5,6,7-hexahydro-7-methyl-8*H*-pyrano-[3,4-c]-pyridine-8-one).** Secologanin (0.388g 1 mmol) was dissolved in absolute methanol (1 mL) and 33 % ethanolic solution of methylamine (0.15 mL) was added to it. After stirring the mixture for 10 minutes at ambient temperature, the solvent was evaporated *in vacuo* . The residue was taken up in anhydrous methanol (2 mL) and sodium tetrahydridoborate (0.04 g 1.06 mmol) was added in portions. After stirring the mixture at 50°C for 1 hour it was cooled and the pH adjusted to 4 with diluted hydrochloric acid. Then the mixture was evaporated again. The residue was taken up in methanol (1 mL) and the inorganic salts were precipitated by addition of ethyl acetate (1 mL) . After evaporation of the filtrate a solid foam was obtained (0.35 g 95% R_f : 0.38 in chloroform:methanol 4:1). $C_{17}H_{25}NO_8$ (371.38): Calcd. C 54.98; H 6.78; N 3.77. Found: C 54.75; H 6.65; N 3.70. UV (EtOH), λ_{max} (log ε): 235 nm (3.13). R (KBr): 1657 cm^{-1} (νC=O), 1586 cm^{-1} (νC=C), 3387 cm^{-1} (νOH). ^{1}H-NMR(200 MHz, MeOD): δ 7.35 (d, H-3), 5.55 (dt ,H-8), 5.44 (d, H-1), 5.28 (dd, H_E-10), 5.22 (dd, H_Z-10), 4.65 (d, H-1′), 3.88 (dd, H_a-6′), 3.7-3.1 (H_b-6′, H-5′, H-4′, H-3′, H-2′, H_2-7), 3.04 (m, H-5), 2.96 (s, N-CH_3), 2.63 (ddd, H-9), 1.65 (m, H_2-6). $J_{3,5}$ = 2.6 Hz, $J_{8,10E}$ = 17.2 Hz, $J_{8,10Z}$ = $J_{8,9}$ = 9.7 Hz, $J_{1,9}$ = 1.8Hz, $J_{10E,10Z}$ = 2.7 Hz, $J_{1'2'}$ = 7.8 Hz, $J_{5,9}$ = 5.6 Hz. ^{13}C-NMR (200 MHz, MeOD):d 166.6 (s, C-11), 148.2 (d, C-3), 134.2 (d, C-8), 120.3 (d, C-10), 109.4 (s, C-4), 99.6 (d, C-1′), 97.3 (d, C-1), 78.3 (d, C-5′), 77.9 (d, C-3′), 74.8 (d, C-4′), 71.5 (d, C-2′), 62.7 (t, C-6′), 44.6 (q, N-CH_3), 35.3 (d, C-9), 29.0 (d, C-5), 25.9 (t, C-6).

8,10-dihydro-*N*-methylbakankosine (03), (4β-ethyl-3α-(β-D-glucopyranosyloxy)-3,4,4aα,5,6,7-hexahydro-7-methyl-8*H*-pyrano[3,4-c]pyridine-8-one). Palladium charcoal (0.06 g) was prehydroganated in methanol (10 mL), then the solution of *N*-methylbakankosine (0.371g 1 mmol) in methanol (35 mL) was added and the hydrogenation continued. Total uptake of dihydrogen : 23.4 mL. After filtration of the catalyst, the solution was evaporated to drynesss *in vacuo*. Colorless foam was obtained (0.309g 83% R_f : 0.33 in chloroform:methanol 4:1). $C_{17}H_{27}NO_8$ (374.44): Calcd. C 54.68; H 7.29; N 3.75. Found: C 54.85; H 7.35; N 3.82. UV (EtOH), λ_{max} (log ε: 240 nm (3.08). IR (KBr): 1649 cm^{-1} (νC=O), 3413 cm^{-1} (νOH). ^{1}H-NMR (200 MHz, MeOD): δ 7.35 (d, H-3), 5.58 (d, H-1), 4.65 (d, H-1′), 3.88 (dd, H_a-6′), 3.65 (dd, H_b-6′), 3.6-3.0 (H-5′, H-4′, H-3′, H-2′, H_2-7), 3.04 (m, H-5), 2.96 (s, N-CH_3), 1.9-1.7 (H-9, H_2-6), 1.45 (m, H_a-8), 1.12 (m, H_b-8), 0.99 (t, H_3-10). $J_{3,5}$ = 2.5Hz, $J_{1,9}$ = 1.8Hz, $J_{1',2'}$= 7.8 Hz. ^{13}C-NMR (200 MHz, MeOD): d 166.9 (s, C-11), 148.0 (d, C-3), 109.6 (s, C-4), 99.2 (d, C-1′), 96.2 (d, C-1), 78.2 (d, C-5′), 77.9 (d, C-3′), 74.7 (d, C-4′), 71.5 (d, C-2′), 62.6 (t, C-6′), 40.1 (d, C-9), 35.2 (q, N-CH_3), 30.1 (d, C-5), 25.4 (t, C-6), 18.9 (t, C-8), 12.5 (q, C-10).

***N*-methylbakankosine aglucone prepared by enzymatic deglucosylation (04a:04b, 3α-OH:3β-OH=9:1), (4β-ethenyl-3-hydroxy-3,4,4aα,5,6,7-hexahydro-7-methyl-8*H*-pyrano[3,4-c]pyridine-8-one).** *N*-methylbakankosine (0.369g 1 mmol) was dissolved in water (5 mL), the pH was adjusted to 4-5 with diluted

hydrochloric acid and β-glucosidase (0.05 g) was added to it. The reaction mixture was stirred for 24 hours at ambient temperature. The aqueous solution was extracted with chloroform (7 x 5 mL). The chloroformic layer was evaporated *in vacuo* to dryness. White solid material was obtained after treating of the crude oily material with ethyl acetate (0.12 g 57 % R_f: 0.79 in benzene : methanol = 4 : 1 mp 118 °C). $C_{11}H_{15}NO_3$ (209.25): Calcd. C 63.14; H 7.23; N 6.69. Found: C 62.95; H 7.28; N 6.59. UV λ_{max} (log ε): 240 nm (2.60, EtOH), 282 nm (2.78, NaOEt/EtOH). IR (KBr): 1650 cm^{-1} (νC=O), 3420 cm^{-1} (νOH). ^{1}H-NMR (400 MHz MeOD): δ 7.35 (d, H-3), 5.51 (dt, H-8), 5.23 (dd, H_Z-10), 5.21 (d, H-1), 5.18 (dd, H_E-10), 3.51 (td, H_a-7), 3.34 (ddd, H_e-7), 2.96 (s, N-CH_3), 2.89 (dtd, H-5), 2.53 (ddd, H-9), 1.72 (dtd, H_e-6), 1.60 (qd, H_a-6). $J_{3,5}$ = 2.4 Hz, $J_{8,10Z}$ = 17.2 Hz, $J_{8,10E} = J_{8,9}$ = 10.0 Hz, $J_{10E,10Z}$ = 2.2 Hz, $J_{1,9}$ = 1.8 Hz, $J_{7a,6a} = J_{7a,7e}$ = 12.5 Hz, $J_{7a,6e}$ = 1.8 Hz, $J_{7e,6a}$ = 4.9 Hz, $J_{7e,6e}$ = 2.2 Hz, $J_{5,6e}$ = 4.7 Hz, $J_{5,9}$ = 5.5 Hz, $J_{6e,6a}$ = 13.1 Hz. ^{13}C-NMR (100 MHz, MeOD) : d 166.69 (s, C-11), 150.92 (d, C-3, minor), 148.93 (d, C-3, major), 134.86 (d, C-8, major), 131.53 (d, C-8, minor), 121.60 (t, C-10, minor), 119.67 (t, C-10, major), 107.71 (s,C-4), 97.94 (d, C-1, minor), 96.22 (d, C-1, major), 49.98 (t, C-7), 47.09 (q, N-C), 35.26 (d, C-9), 35.22 (d, C-5, minor), 27.71 (d, C-5, major), 26.97 (t,C-6, minor), 25.89 (t, C-6, major).

8,10-dihydro-*N*-methylbakankosine aglucone prepared by enzymatic deglucosylation (05a:05b, 1α-OH:1β-OH=9:1), (4β-ethyl-3-hydroxy-3,4,4aα,5,6,7-hexahydro-7-methyl-8*H*-pyrano[3,4-c]pyri-dine-8-one). 8,10-dihydro-*N*-methylbakankosine (0.371 g 1 mmol) was dissolved in water (5 mL), the pH was adjusted to 4-5 with diluted hydrochloric acid and β-glucosidase (0.05 g) was added to it. The reaction mixture was stirred for 24 hours at ambient temperature. The aqueous solution was extracted with chloroform (7 x 5 mL). The chloroformic layer was evaporated *in vacuo* to dryness White. solid material was precipitated with ethyl acetate. (0.12 g 57 % R_f: .64 in ethyl acetate:isopropanol:water= 80:10:5 mp 138 °C). $C_{11}H_{17}NO_3$ (211.27): Calcd. C 62.54; H 8.11; N 6.63. Found: C 62.68; H 7.98; N 6.70. UV λ_{max}(log ε) : 239 nm (3.073, EtOH), 275 nm (2.97, NaOEt /EtOH). IR (KBr): 1650 cm^{-1} (νC=O), 3200 cm^{-1} (νOH). ^{1}H-NMR (200 MHz, pyridine-d_5): δ 7.97 (d, H-3), 5.78 (d, H-1, 0.9 H), 5.65 (d, 1-H, 0.1H), 3.25 (dddd, H-5), 3.19 (td, $H_α$-7), 3.07 (ddd, $H_α$-7), 1.86 (dtd, H-9), 1.61 (dtd, $H_β$-6), 1.50 (m, $H_α$-6), 1.35 (m, H_a-8), 1.16 (m, H_b-8), 1.07 (H_3-10, minor), 0.90 (t, H_3-10), $J_{3,5}$ = 2.5 Hz, $J_{1,9}$ = 1.8 Hz (major), $J_{1,9}$ = 2.0 Hz (minor), $J_{5,6β}$ = 13.0 Hz, $J_{5,9}$ = 5.3 Hz, $J_{5.6α}$ = 4.4 Hz, $J_{7α,7β}$ = $J_{6β,7α}$ =12.1 Hz, $J_{6α,7α}$ = 3.4 Hz, $J_{6\,β,7β}$ = 4.7 Hz, $J_{6α,7β}$ = 2.4 Hz, $J_{8b,9}$ = 9.3 Hz, $J_{8b,9}$ = 5.3 Hz, $J_{6a,6b}$ = 12.1 Hz, $J_{8,10}$ = 7.5 Hz. ^{13}C-NMR (200 MHz, pyridine-d_5): d 147.4 (C-3), 98.2 (C-1, minor), 94.3 (C-1, major), 49.2 (C-7, minor), 49.0 (C-7, major), 36.3 (C-9, minor), 34.6 (C-9, major), 34.3 (C-5, minor), 29.8 (C-5, major), 18,4 (C-8, major), 14,9 (C-8, minor), 14.9 (C-10, minor), 12.5 (C-10, major).

***N*-methylbakankosine aglucone prepared by acidic deglucosylation (08a:08b=12SS:12RS, 8α-methyl:8β-methyl=2:3), (3,7-dimethyl-4α-formyl-3,4,4aα,5,6,7-hexahydro-8*H*-pyrano[3,4-c]pyridine-8-one).** *N*-methylbakakosine (0.369g 1 mmol) was dissolved in 2M aqueous hydrochloric acid (20 mL) and stirred at 100°C for 1 hour. The reaction mixture was extracted with chloroform (5x20 mL), the chloroformic layer was evaporated *in vacuo* to dryness. Oily material was obtained (0.13g, 62% R_f: 0.44 in chloroform:acetone 4:1). $C_{11}H_{15}NO_3$ (209.25): Calcd. C 63.14; H 7.23; N 6.69. Found: C 63.25; H 7.12; N 6.62. UV λ_{max} (log ε): 238 nm (2.98, EtOH), 275 nm (3.01, NaOEt / EtOH). IR (KBr): 1650 cm^{-1} (νC=O), 1710 cm^{-1} (ν(H)C=O), 2725 cm^{-1}(νHC(=O)). Major isomer: ^{1}H-NMR (400 MHz, $CDCl_3$): δ 9.82 (H-1), 7.53 (H-3), 4.08 (H-8), 3.44 ($H_α$-7), 3.26 ($H_β$-7), 3.00 (s, N-CH_3), 2.84 (H-5), 2.29 (H-9), 1.98 ($H_α$-6), 1.49 ($H_β$-6), 1.42 (H_3-10). $J_{1,9}$ = 3.2 Hz, $J_{3,5}$ = 2.0 Hz, $J_{5,6a}$ = 3.8 Hz, $J_{5,6b}$ = 12.7 Hz, $J_{5,9}$ = 10.2 Hz, $J_{6a,6b}$ = 13.0 Hz, $J_{6a,7a}$ = 3.8 Hz, $J_{6a,7b}$ = 2.1 Hz, $J_{6b,7a}$ = 12.7 Hz, $J_{6b,7b}$ = 5.1 Hz, $J_{7a,7b}$ = 12.5 Hz, $J_{8,9}$ = 10.2 Hz, $J_{8,10}$ = 6.3 Hz. ^{13}C-NMR

(400 MHz, $CDCl_3$): d 201.3 (d, C-1), 164.0 (s, CON), 150.3 (d, C-3), 107.1 (s, C-4), 71.7 (d, C-8), 56.6 (d, C-9), 48.4 (t, C-7), 34.7 (d, N-CH_3), 31.8 (d, C-5), 27.3 (t, C-6), 19.3 (d, C-10). Minor isomer: ^{1}H-NMR (400 MHz, $CDCl_3$): δ 9.79 (H-1), 7.48 (H-3), 4.83 (H-8), 3.53 (H_a-7), 3.30 (H_b-7), 3.00 (s, N-CH_3), 2.84 (H-5), 2.65 (H-9), 2.19 (H_a-6), 1.48 (H_b-6), 1.20 (H_3-10). $J_{1,9}$=1.3 Hz, $J_{3,5}$ = 2.0 Hz, $J_{5,6a}$ = 3.8 Hz, $J_{5,6b}$ = 12.6 Hz, $J_{5,9}$ = 11.0 Hz, $J_{6a,6b}$ = 12.7 Hz, $J_{6a,7a}$ = 3.8 Hz, $J_{6a,7b}$ = 2.0 Hz, $J_{6b,7a}$ = 12.6 Hz, $J_{6b,7b}$ = 5.1Hz, $J_{7a,7b}$ = 12.6Hz, $J_{8,9}$ = 4.2 Hz, $J_{8,10}$ = 6.6 Hz. ^{13}C-NMR (400 MHz, $CDCl_3$): d 200.0 (d, C-1), 164.0 (CON), 148.7 (d, C-3), 106.3 (s, C-4), 69.2 (d, C-8), 53.8 (d, C-9), 48.5 (t,C-7), 34.7 (d, N-CH_3), 27.2 (t, C-6), 26.6 (d, C-5), 15.6 (d, C-10).

8,10-dihydro-N-methylbakankosine aglucon, prepared by acidic deglucosylation (09a:09b-=1RSH:-1SSH, 1α-hydroxy:1β-hydroxy=4:1), (4α-ethyl-3-hydroxy-3,4,4aα,5,6,7-hexahydro-7-methyl-8*H*-pyrano-[3,4-c]pyridine-8-one). 8,10-dihydo-*N*-methylbakankosine (0.371 g, 1 mmol) was dissolved in 2M aqueous hydrochloric acid (20 mL) and stirred at 100 °C for 1 hour. After cooling the reaction mixture was extracted with chloroform (5x20 mL). The chloroformic layer was evaporated *in vacuo* to dryness. Oily crude material was obtained. White solid material was precipitated with EtOAc. (0.12g, 60%. R_f:0.64 in EtOAc: iPrOH:H_2O, 80:10:5, mp. 169-170 °C). $C_{11}H_{17}NO_3$ (211.27): Calcd. C 62.54; H 8.11; N 6.63. Found: C 62.39; H 8.21; N 6.54. UV λ_{max} (log ε) : 239 nm (3.07, EtOH, 275 nm (2.97, NaOEt / EtOH). IR (KBr): 1650 cm^{-1} (nC=O), 3200 cm^{-1} (nOH). ^{1}H-NMR (200 MHz, $CDCl_3$): δ 7.43 (d, H-3), 5.47 (d,1-H, major, O.8 H), 5.06 (d, H-1, minor, 0.2 H) 3.44 (dt, H_{ax}-7), 3.32 (ddd, H_{eq}-7), 2.97 (s, N-CH_3), 2.35 (dt, H-5), 2.10 (ddd, H-9, 0.8 H), 1.8-1.2 (m, H-9, minor), H_2-8, H_2-6), 0.98 (t, H_3-10), $J_{1,9}$ = 2.2 Hz (major), $J_{1,9}$ = 9.0 Hz (minor), $J_{3,5}$ = 2.1Hz, $J_{5,6ax}$ = 10.2 Hz, $J_{5,9}$ = 12.0 Hz, $J_{7ax,6ax}$ = $J_{7ax,7eq}$= 12.5 Hz, $J_{7ax,6eq}$ =3.7 Hz. ^{13}C-NMR (200 MHz, MeOD): δ 165.3(s, C-11), 148.2 (d, C-3), 108.5 (s, C-4), 92.3 (d, C-1), 49.1 (t, C-7), 42.6 (d, C-9), 34.9 (q, N-CH_3), 30.5 (d, C-5), 26.8 (t, C-6), 21.2 (t, C-8), 11.4 (q, C-10).

Acknowledgement.

The financial support of this work by the National Scientific Research Foundation (OTKA) is greatly acknowledged. We gratefully thank Miklós Morvai Ph. D. student for the assistance in recording and evaluating the NMR spectra.

References and Notes

1. The cyclic skeletons were numbered according to the biogenetic numbering. However, in the experimental part, the IUPAC names of the compounds were given as well. In order to distinguish the formulae in the Scheme 1 and in Schemes 2-5, structures are indicated in Scheme 1 by figures **01**..., in Schemes 2 and 5 by figures **1**..., in Scheme 3 and 4 by figures **1RZ**... In the two latter cases, **R**, **S**, **Z** and **E** indicate configuration of the eventual centers of chirality at C-1, C-3, C-4, C-8 and C-9, and/or conformation of the eventual C=C bond at C-3, C-8 and C-9, respectively. Indication of the configuration of centre of chirality preceeds that of conformation of the C=C bond, and stereogenic element in the "lower" region (C-1, C-4) of the structure that in the "upper"region (C-1, C-8, C-9) of it. Letter **H** in Schemes 4 and 5 concerns the 8,10-dihydro derivatives.

2. Tietze, L.-F. Secologanin, a Biogenetic Key Compound. Synthesis and Biogenesis of the Iridoid and Secoiridoid Glycosides. *Angew. Chem. Int. Ed. Engl.* **1983**, *22*, 828-841.
3. Brown, R. T.; Chapple, C. L. *Tetrahedron Lett.* **1976**, 787-790.
4. Brown, R. T.; Chapple, C. L. *J. Chem. Soc. Chem. Commun.* **1973**, 886-888.
5. Brown, R. T.; Chapple, C. L.; Charalambides, A. A. *J. Chem. Soc. Chem. Commun.* **1974**, 756-757.
6. Brown, R. T.; Leonard, J. *Tetrahedron Lett.* **1978**, 1605-1608.
7. K. Szabó-Pusztay, L. F. Szabó, B. Podányi, *Acta. Chim. Hung. Models in Chemistry,* **1994**, 475-488. This paper should be considered as Part 1 of the present series.
8. Bourquelot, E.; Hérissey, H. *J. Pharm. Chim.* **1908**, *28*, 433.
9. Balenović, K.; Däniker, H. U.; Goutarel, R.; Janot, M. M.; Prelog, V. *Helv. Chim. Acta* **1952**, *35*, 2519-2540.
10. Tietze, L.-F. *Tetrehedron Lett.* **1976**, 535-538.
11. Inouye, H.; Tobita, H.; Moriguchi, M. *Chem. Pharm. Bull.* **1976**, *24*, 1406-1407.
12. Mislow, K. *Pure Appl. Chem.* **1971**, *25*, 549-562.
13. Gust, D.; Mislow, K. *J. Am. Chem. Soc.* **1973**, *95*, 1535-1547.
14. Kocsis, Á.; Pál, Z.; Patthy, Á.; Szabó, L.; Varga, J.; Tétényi, P. Hung. Teljes **HU 48,640** 28. Jun. 1989. C. A. **112**: P132478c (1990).
15. Mattes, K. L.; Hutchinson, C. R.; Springer, J. P.; Clardy, J. *J. Am. Chem. Soc.* **1975**, *97*, 6270-6271.
16. Patthy-Lukáts, A.; Károlyházy, L.; Szabó, L. F.; Podányi, B. *J. Nat. Prdocts,* **1997**, *60*, 69-75. This paper should be considered as Part 2 of the present series.
17. Stull, D. R.; Westrum, E. F. Jr.; Sinke, G. C. *The Chemical Thermodynamics of Organic Compounds*; J. Wiley, New York, 1969.
18 J. D. Cox, G. Pilcher: *Thermochemistry of Organic and Organometallic Compounds;* Academic Press, London, 1970.
19. Moriyasu, M.; Kato, A.; Hashimoto, Y. *J. Chem. Soc. Perkin Trans. 2* **1986**, 515.
20. Chiang, Y.; Kresge, A. J.; Walsh, P. A. *J. Am. Chem. Soc.* **1986**, *108*, 6314-6320.
21. Hurd, Ch. D.; Saunders, W. H. Jr. *J. Am. Chem. Soc.* **1952**, *74*, 5324-5329.
22. Brown, R. T.; Pratt, S. B. *J. Chem. Soc. Chem. Commun.* **1980**, 165-167.

(*Received in UK* 2 *April* 1997; *revised* 29 *May* 1997; *accepted* 5 *June* 1997)