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INVESTIGATION OF NEOASCORBIGEN

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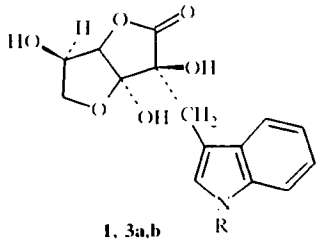
The synthesis of N-methoxyascorbigen (neoascorbigen) – a natural substance from plants of the Cruciferae family – and also N-ethoxyascorbigen is described. In an acidic media under drastic conditions N-alkoxyascorbigens undergo transformations with the release of ascorbic acid and the formation of oligomers of 1-alkoxy-3-methyleneindolenine or with opening of the lactone ring, decarboxylation, and dehydration and the formation of 2-hydroxy-3-(1-alkoxy-3-indolyl)-4-hydroxymethylcyclopent-2-enone. Amides of neoascorbigen, 3-O-methylglycoside of N-ethoxyascorbigen, and the product of the reduction of N-ethoxyascorbigen by sodium borohydride were obtained for the first time.

Keywords: 2-hydroxy-3-(1-alkoxy-3-indolyl)-4-hydroxymethylcyclopent-2-enones, neoascorbigen, N-ethoxyascorbigen.

The properties of ascorbigen – 2-C-(3-indolylmethyl)-α-L-xylo-3-hexulofuranosono-1,4-lactone (**1**) formed in plants of the *Cruciferae* family from 3-hydroxymethylindole (the product from disintegration of the alkaloid glucobrassicin) and L-ascorbic acid (**2**) [1-3] – have been studied fairly well. In an acidic medium it releases ascorbic acid and forms the products from oligomerization of 3-methyleneindolenine [4], of which the most important is 5H,11H-indolo[3,2-*b*]carbazole – a powerful activator of cytochrome-dependent oxidase P4301A1 and a modulator of cancerogenase [5, 6].

In addition to glucobrassicin, plants of the *Cruciferae* family include a series of other indole alkaloids (glucosinolates) [7, 8]. They include neoglucobrassicin (N-methoxyglucobrassicin) – a source of neoascorbigen (N-methoxyascorbigen **3a**). The chemical and biochemical characteristics of the latter have not been investigated before, and its biological significance has not been determined.



1, 3a,b R

1 R = H; **3a** R = OMe; **3b** R = OEt

The aim of the present work was to synthesize neoascorbigen **3a** and its homolog N-ethoxyascorbigen **3b** and to study the chemical properties of these compounds. The key compound in the synthesis of alkoxyascorbigens **3a,b** is 1-hydroxyindole (**4**). We used the two most convenient methods for its preparation, i.e., the condensation of *o*-nitrotoluene with dimethylformamide dimethyl acetal followed by the reduction of the dimethylaminovinyl derivative **5** with zinc in an aqueous solution of NH_4Cl [9, 10] or the oxidation of indoline with hydrogen peroxide in the presence of Na_2WO_4 [11].

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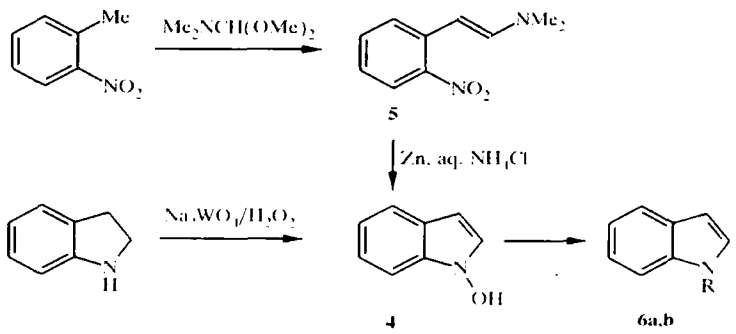
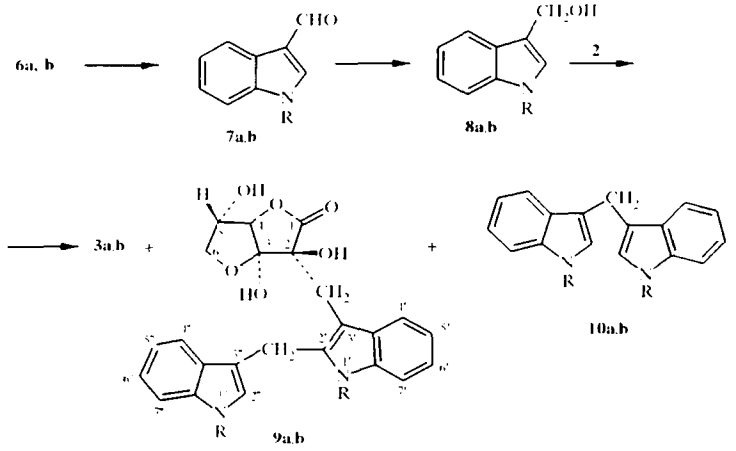


6 a R = OMe, b R = OEt

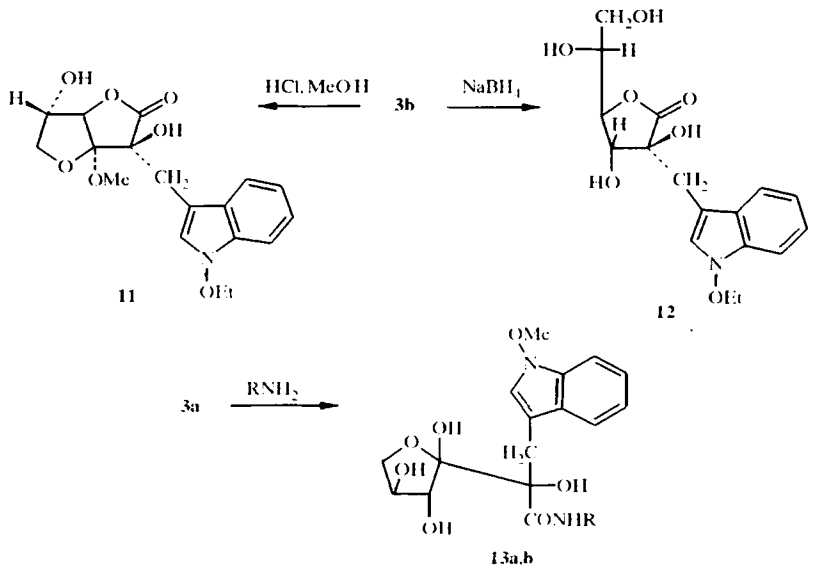
1-Hydroxyindole **4** is an unstable compound, and it was therefore used in the O-alkylation reaction without purification. During synthesis by the first method the total yield of 1-methoxyindole **6a** was 31% (calculated on *o*-nitrotoluene), and in the second method it was 44% while the yield of 1-ethoxyindole **6b** was 41% (calculated on indoline).

1-Alkoxyindoles **6a,b** were converted into the corresponding 3-formyl derivatives **7a,b**, which were then reduced with sodium borohydride in ethanol to 1-alkoxy-3-hydroxymethylindoles **8a,b**. The latter were immediately brought into condensation with L-ascorbic acid **2**. As a result the required products **3a,b** were obtained with yields of 70 and 65% (calculated on the respective 1-alkoxy-3-formylindole **7**). The synthesis of N-alkoxyascorbigens by this method has been described before [12]. However, we showed that apart from these compounds the reaction mixture contained a resinous mixture of side compounds, from which by preparative TLC 1'-methoxy-2'-(1''-methoxy-3''-indolylmethyl)ascorbigen (**9a**) or its 1'-ethoxy analog (**9b**) were isolated with yields of 4.2 and 2.5%. Di(1-methoxy-3-indolyl)methane (**10a**) or its 1-ethoxy analog (**10b**) were also isolated with yields of about 8.3 and 9.0%. In methanol solution of HCl N-ethoxyascorbigen **3b** forms 3-OMe glycoside **11** with a yield of 55%. By the action of sodium borohydride the hemiketal group in compound **3b** at position 3 is reduced, and lactone of [2-C-(1-ethoxy-3-indolyl)methyl]-L-gulonic acid (**12**) is formed with a yield of 60%. The structure of the latter was established by PMR using the Overhauser effect: since the intensity of the signals of 2-OH, 5-H, 5-OH, and 6-OH is reduced if the signal of the 3-H proton is suppressed while the intensity of the CH, group signal is unchanged, it can be concluded that the 3-H proton and the 2-OH group are in the relative *cis* position, i.e., compound **12** has the *R*-configuration at the $C_{(3)}$ atom and is a derivative of L-gulonic acid.



7–10 a R = OMe; b R = OEt

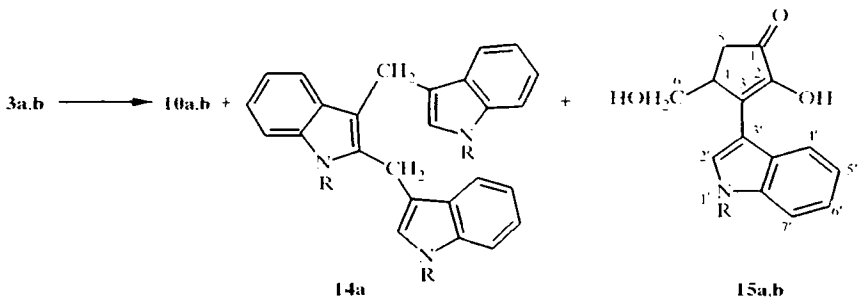
N-Methoxyascorbigen **3a** reacts with primary amines with opening of the lactone ring and the formation of benzylamide **13a** (yield 60%) or butylamide **13b** (55%) of 2-C-(3-indolylmethyl-1-methoxy-)-α-L-xylo-3-hexulofuranosonic acid.



13 a R = CH_2Ph, b R = Bu-*n*

In the presented examples the chemical behavior of N-alkoxyascorbigens **3a,b** is similar to the behavior of ascorbigen **1** and also its phenyl analogs [13-16], but in an alkaline or acidic medium the behavior of compounds **3a,b** and **1** differs. Thus, in an acidic medium alkoxyascorbigens are significantly more stable than ascorbigen **1**. In the table below the stabilities of compounds **1** and **3a** in water–methanol solution at pH < 1 (according to HPLC) are compared. In the investigated solution methoxyascorbigen **3a**, unlike the less stable ascorbigen **1**, disappears only after heating at 60°C for 4-5 h.

Under sufficiently drastic conditions N-alkoxyascorbigens **3a,b**, like ascorbigen, release ascorbic acid and give the products from oligomerization of derivatives of 1-alkoxy-3-methyleneindolenine, i.e., bis(1-alkoxy-3-indolyl)methanes **10a,b** (yield about 30%), which are also formed as side products during the synthesis of compounds **3a,b**. In the case of methoxyascorbigen **3a** it was possible to isolate 1-methoxy-2,3-di(1-methoxy-3-indolylmethyl)indole (**14a**), identified by mass spectrometry, with a yield of 1-2%.

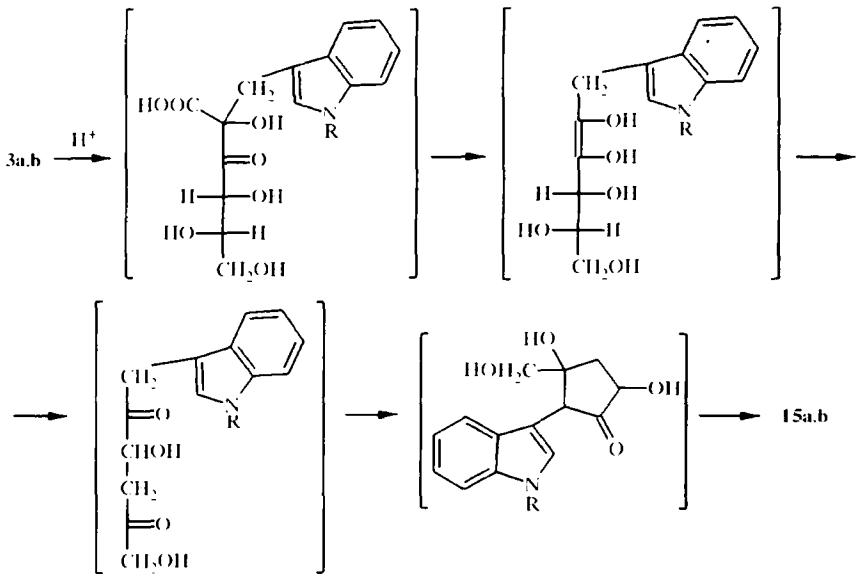


15 a R = OMe, b R = OEt

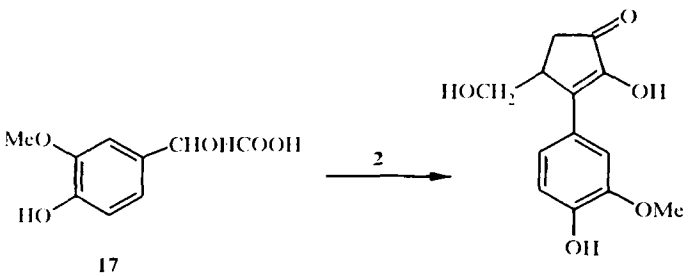
TABLE 1. The Stability of Ascorbigens in Acidic Medium (pH < 1, 37°C)

Compound	Initial compound, %	
	after 3 h	after 3 days
Ascorbigen 1	90	15
Neoascorbigen 3a	~98	85-90

Of greatest interest, however, is the fact that in acidic medium compounds **3a,b** also form their transformation products containing the ascorbic acid residue – 2-hydroxy-3-(1'-methoxy-3'-indolyl)- or 2-hydroxy-3-(1'-ethoxy-3'-indolyl)-4-hydroxymethylcyclopent-2-enone (**15a**) or (**15b**) respectively. After the heating of neoascorbigen **3a** for 3 days at 37°C (pH ~1.0) the amount of ketone **15a**, according to HPLC, was 9%, while 85% of the initial compound **3a** remained unchanged. After 4-5 h at 60°C almost all neoascorbigen has been consumed, while the amount of cyclopentenone **15a** has increased to 15-20%, although the main transformation product is di(1-methoxy-3-indolyl)methane (**10a**). Thus, it has been shown for the first time that in an acidic medium ascorbigens can undergo transformations in two independent directions, i.e., with the release of ascorbic acid or by a hypothetical scheme, involving opening of the lactone and furanose rings, decarboxylation, dehydration with the formation of diketone, aldol condensation, and rearrangement of the bonds with the formation of compounds **15a,b**. The stability of neoascorbigen **3a** in acids and the reduced nucleophilicity of the $C_{(2)}$ atom of the indole ring (as a result of the electronic effect and also the steric effect of the substituents at the nitrogen atom) impedes the elimination of ascorbic acid and the formation of oligomers of 1-alkoxymethyleneindolenines, and this leads to an increase in the yield of cyclopentenone derivatives **15a,b**. The latter exhibit strong fluorescence, which facilitates their isolation by chromatographic methods.



Earlier a derivative of cyclopentenone **16** was obtained by the condensation of vanillomandelic acid **17** with ascorbic acid [17]. The presence of the carboxyl function at the benzylic position in compound **17** was suggested to facilitate a cascade of reactions following one after the other with the formation of cyclopentenone derivative **16**.



17 16

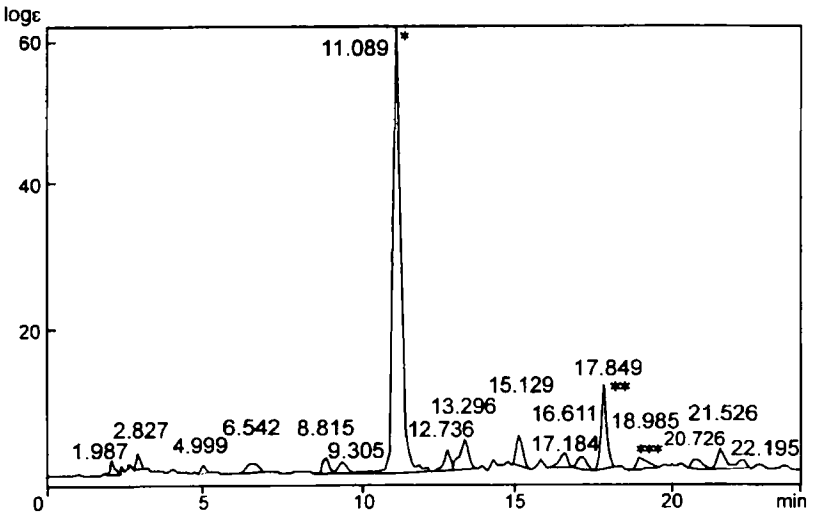


Fig. 1. HPLC of kohlrabi extract: *ascorbigen (**1**); **neoascorbigen (**3a**); ***3-hydroxymethylindole.

Chromatographic and mass-spectrometric analysis of the substances formed during the acid decomposition of neoascorbigen showed that derivatives of 5H,11H-indolo[3,2-*b*]carbazole are not formed here. It is interesting to note that N-alkoxyascorbigens are also considerably more stable than ascorbigen under alkaline conditions.

We used the compounds **1** and **3a** that we synthesized to determine the content of these natural substances in kohlrabi (*Brassica caulorapa*), rape (*Brassica rapa*), and radish (*Raphanus sativus*). Earlier it had been shown that the content of neoascorbigen in root crops of the *Cruciferae* family was higher than in leafy vegetables [7, 18]. Figure 1 shows the chromatogram obtained during HPLC of an extract of homogenized fresh stalk of kohlrabi. A kilogram of fresh kohlrabi contains 22.50 mg of ascorbigen and 3.50 mg of neoascorbigen, and fresh rape contains 15.94 and 14.56 mg respectively. There was hardly any neoascorbigen in radish.

The relatively low content of neoascorbigen compared with that of ascorbigen in vegetables, the stability of neoascorbigen in acidic media [as a result of which it is not a donor of ascorbic acid under biological conditions (under the influence of gastric juices)], and the absence of condensed heterocyclic systems of the indolocarbazole type among its transformation products make it possible to conclude that the biological role of neoascorbigen is less important or at any rate different from that of ascorbigen. At the same time cyclopentenone derivatives formed from alkoxyascorbigens under drastic conditions, which can be regarded as C-nucleosides of a new type, are of undoubted interest and merit further investigation.

EXPERIMENTAL

The PMR spectra were obtained on a Varian VXR-400 spectrometer at 400 MHz. The reactions and the purity of the compounds were monitored by TLC on Merck Kieselgel F_{254} plates. Preparative chromatography was conducted on 20×20 glass plates with 0.5-mm layer of silica gel (Kieselgel 60 F_{254}) in the chloroform–methanol 10:1 (A), 8:1 (B), 5:1 (C), or 12:1 (D) solvent systems or in chloroform (E). The electron-impact mass spectra were recorded on a Finnigan SSQ 710 spectrometer. HPLC was realized on a Shimadzu LC 10 liquid chromatograph with Diasorb C-16 column (4.0×250 mm, 7 μ, BioKhimMak, Russia) and a detector operating at 280 nm, the sample volume being of 10 μl. Mobile phases: 0.01 M H_3PO_4, pH 2.6 (A); acetonitrile (B) with linear gradient phase of B in phase A from 10 to 45% (30 min) and flow rate 1.3 ml/min.

1-Alkoxy-3-formylindoles **7a,b** were obtained by the method [12].

2-C-(1-Methoxy-3-indolylmethyl)-α-L-xylo-3-hexulofuranosono-1,4-lactone [Neoascorbigen (3a)], Di(1-methoxy-3-indolyl)methane (10a), and 2-C-[1'-Methoxy-2'-(1''-methoxy-3''-indolylmethyl)-3'-indolylmethyl]-α-L-xylo-3-hexulofuranosono-1,4-lactone (9a). Solution of 1-methoxy-3-formylindole **7a** (260 mg, 1.49 mmol) in ethanol (8 ml) was stirred and heated at 40°C with sodium borohydride (169 mg, 4.44 mmol). After 10 min the reaction mixture was filtered from the unreacted sodium borohydride, partly evaporated, and diluted with water. The product **3a** was extracted three times with ether, and the extract was washed with water saturated with sodium chloride, dried over sodium sulfate, and evaporated. The residue was dissolved in 3 ml of ethanol and added to solution of ascorbic acid (393 mg, 2.24 mmol) in citrate–phosphate buffer solution (10 ml), pH 4.2. The reaction mixture was stirred at room temperature for 16 h. The solution, which contained the product **3a**, was decanted and extracted twice with ethyl acetate. The extract was dried over sodium sulfate and evaporated until a cream-colored scum had formed. Neoascorbigen **3a** (350 mg, 70% on **7a**) was obtained. R_f 0.42 (A), R_t 17.85 min. EI mass spectrum, *m/z* (I_{rel}, %): 335 $[M]^+$ (20), 305 $[M\text{-}OCH_3]^+$ (15), 160 $[CH_2C_8H_5NOMe]^+$ (100). $[\alpha]_D^{20}$ +8° (C 0.05, methanol). ^{1}H NMR spectrum (tetradeuteromethanol), ppm: 7.60 (1H, d, $J_{4',5'}$ = 8.1, $J_{4',6'}$ = 0.9 Hz, 4'-H); 7.35 (1H, d, $J_{6',7'}$ = 8.1, $J_{5',7'}$ = 1.1 Hz, 7'-H); 7.33 (1H, s, 2'-H); 7.17 (1H, t, $J_{5',6'}$ = 7.1 Hz, 5'-H); 7.02 (1H, t, $J_{6',5'}$ = 7.1 Hz, 6'-H); 4.22 (1H, m, 5-H); 4.05 (3H, s, OCH_3); 4.13 (1H, dd, $J_{6a,6b}$ = 9.7, $J_{5,6a}$ = 3.4 Hz, 6-H_a); 3.99 (1H, dd, $J_{5,6b}$ = 5.7 Hz, 6-H_b); 3.87 (1H, s, 4-H); 3.33 (1H, d, J_{gem} = 15.0 Hz, $\underline{CH_2}$–C_8H_5NOMe); 3.19 (1H, d, J_{gem} = 15.0 Hz, $\underline{CH_2}$–C_8H_5NOMe). The residue in the flask after decanting the solution was dissolved in chloroform, and compound **10a** (19.0 mg; 8.3% on **7a**) was isolated from the solution by preparative TLC on glass plates; R_f 0.70 (E), R_t 24.10 min. EI mass spectrum, *m/z* (I_{rel}, %): 306 $[M]^+$ (100), 275 $[M\text{-}OMe]^+$ (72), 243 $[M\text{-}2OMe\text{-}H]^+$ (24). ^{1}H NMR spectrum (deuterochloroform), ppm: 7.66 (2H, d, $J_{4,5}$ = 8.0, $J_{4,6}$ = 1.0 Hz, 4-H); 7.41 (2H, d, $J_{6,7}$ = 8.1, $J_{5,7}$ = 0.9 Hz, 7-H); 7.23 (2H, s, 2-H); 7.19 (2H, t, $J_{5,6}$ = 7.2, $J_{4,5}$ = 8.0 Hz, 5-H); 7.03 (2H, t, $J_{5,6}$ = 7.2, $J_{6,7}$ = 8.1 Hz, 6-H); 4.15 (2H, br. s, CH_2); 4.01 (6H, s, OCH_3).

From the same plates after isolation of compound (**10a**) and repeated chromatography in the system A lactone **9a** (16 mg, 8.3% on **7a**) was obtained. R_f 0.55 (A); R_t 22.08 min. EI mass spectrum, *m/z* (I_{rel}, %): 494 $[M]^+$ (100). High-resolution EI mass spectrum, *m/z*: 494.5041. ($C_{30}H_{26}N_2O_8$, calculated: 494.5057). ^{1}H NMR spectrum (tetradeuteromethanol), ppm: 7.62 (1H, d, $J_{4'',5''}$ = 8.2 Hz, 4''H); 7.51 (1H, d, $J_{4',5'}$ = 7.5 Hz, 4'-H); 7.38 (1H, d, $J_{6'',7''}$ = 8.1 Hz, 7''-H); 7.32 (1H, d, $J_{6',7'}$ = 8.0 Hz, 7'-H); 7.17 (1H, t, $J_{5'',6''}$ = 7.2 Hz, 5''-H); 7.13 (1H, t, $J_{5',6'}$ = 1.1, $J_{5',6'}$ = 7.1 Hz, 5'-H); 7.09 (1H, s, 2''-H); 7.03 (1H, t, $J_{6'',5''}$ = 7.2 Hz, 6''-H); 6.99 (1H, t, $J_{6',5'}$ = 7.1 Hz, 6'-H); 4.31 (1H, m, 5-H); 4.10 (1H, dd, $J_{6a,6b}$ = 9.7, $J_{5,6a}$ = 3.4 Hz, 6-H_a); 4.08 (3H, s, OCH_3); 4.02 (1H, dd, $J_{5,6b}$ = 5.7 Hz, 6-H_b); 3.99 (3H, s, OCH_3); 3.77 (1H, s, 4-H); 3.30 (1H, d, J_{gem} = 15.0 Hz, $\underline{CH_2}$–C_8H_5NOMe); 3.20 (1H, d, $\underline{CH_2}$–C_8H_5NOMe).

2-C-(1'-Ethoxy-3'-indolylmethyl)-α-L-xylo-3-hexulofuranosono-1,4-lactone (3b), Di(1-ethoxy-3-indolyl)methane (10b), and 2-C-[1'-Ethoxy-2'-(1''-ethoxy-3''-indolylmethyl)-3'-indolylmethyl]-α-L-xylo-3-hexulofuranosono-1,4-lactone (9b). N-Ethoxyascorbigen **3b** (395 mg, 65%) was obtained similarly to N-methoxyascorbigen **3a** from 1-ethoxy-3-formylindole **8b** (330 mg, 1.74 mmol); R_f 0.41 (A), R_t 20.95 min, $[\alpha]_D^{20}$ +10.1° (C 0.85, ethyl acetate). EI mass spectrum, *m/z* (I_{rel}, %): 349 $[M]^+$ (20), 174 $[CH_2C_8H_5NOEt]^+$ (100). ^{1}H NMR spectrum (tetradeuteromethanol), ppm: 7.61 (1H, d, $J_{4',5'}$ = 7.8 Hz, 4'-H); 7.34 (1H, d, $J_{6',7'}$ = 8.1 Hz, $J_{5',7'}$ = 1.1 Hz, 7'-H); 7.31 (1H, s, 2'-H); 7.15 (1H, t, $J_{5',6'}$ = 7.2 Hz, 5'-H); 7.01 (1H, t, $J_{6',5'}$ = 7.1 Hz, 6'-H); 4.28 (2H, dd, J = 7.1 Hz, $\underline{CH_2}CH_3$); 4.23 (1H, m, 5-H); 4.13 (1H, dd, $J_{6a,6b}$ = 9.7, $J_{5,6a}$ = 3.4 Hz, 6-H_a); 3.99 (1H, dd, $J_{5,6b}$ = 5.7 Hz, 6-H_b); 3.88 (1H, s, 4-H); 3.34 (1H, d, J_{gem} = 15.0 Hz, $\underline{CH_2}$–C_8H_5NOMe); 3.18 (1H, d, J_{gem} = 15.0 Hz, $\underline{CH_2}$–C_8H_5NOMe); 1.37 (3H, t, J = 7.1 Hz, $CH_2\underline{CH_3}$).

Compound **10b** was isolated similarly to compound **10a**. Yield 26 mg (9% on **7b**); R_f 0.68 (E), R_t 24.53 min. EI mass spectrum, *m/z* (I_{rel}, %): 334 $[M]^+$ (15), 289 $[M\text{-}OEt]^+$ (100). ^{1}H NMR spectrum (deuterochloroform), ppm: 7.67 (2H, d, $J_{4,5}$ = 8.0, $J_{4,6}$ = 1.0 Hz, 4-H); 7.40 (2H, d, $J_{6,7}$ = 8.1 Hz, $J_{5,7}$ = 0.9 Hz, 7-H); 7.23 (2H, s, 2-H); 7.18 (2H, t, $J_{5,6}$ = 7.1, $J_{4,5}$ = 8.0 Hz, 5-H); 7.03 (2H, t, $J_{7,6}$ = 7.1, $J_{6,5}$ = 8.0 Hz, 6-H); 4.28 (4H, dd, J = 7.1 Hz, $\underline{CH_2}CH_3$); 4.15 (2H, br. s, CH_2); 1.36 (6H, t, J = 7.1 Hz, $CH_2\underline{CH_3}$).

Lactone **9b** was isolated similarly to compound **9a**. Yield 11.0 mg (2.5% on **7b**); R_f 0.53 (A), R_t 25.03 min. EI mass spectrum, *m/z* (I_{rel}, %): 522 $[M]^+$ (100). With the exception of the signals for the protons of the ethoxy groups the ^{1}H NMR spectrum was fully analogous to the spectrum of compound **9a**.

3-(1'-Methoxy-3'-indolyl)-2-hydroxy-4-hydroxymethylcyclopent-2-enone (15a) and 1-Methoxy-2,3-di(1'-methoxy-3'-indolylmethyl)indole (14a). Solution of compound **3a** (100 mg, 0.3 mmol) in methanol (6 ml) and 1N hydrochloric acid (6 ml) was stirred for 4-5 h at 60-65°C until the greater part of the initial neoascorbigen had disappeared (TLC control, system A). Methanol was evaporated from the reaction mixture, and the residue was diluted with water and extracted three times with ethyl acetate. The extract was washed with brine, dried over sodium sulfate, and evaporated. A strongly fluorescent fraction with R_f 0.72 and fraction with R_f 0.90 were isolated from the residue by preparative TLC (system A). Repeated purification of the fraction with R_f 0.72 in system A gave 9 mg (10%) of the product **15a**; R_f 0.60 (A), R_t 15.25 min. High-resolution EI mass spectrum, *m/z*: 242.0843. ($C_{15}H_{15}NO_4$-CH_2O, calculated 242.0830). Found 243.0894. $C_{15}H_{15}NO_4$-CH_2O. Calculated 243.0895. ^{1}H NMR spectrum (tetradeuteromethanol): 8.21 (1H, s, 2'-H); 7.97 (1H, d, $J_{4,5'}$ = 7.8 Hz, 4'-H); 7.51 (1H, d, $J_{6,7'}$ = 8.1, $J_{5,7'}$ = 1.1 Hz, 7'-H); 7.29 (1H, t, $J_{6,5'}$ = 7.1 Hz, 6'-H); 7.72 (1H, m, 4-H); 3.43 (1H, dd, J_{gem} = 10.7, $J_{4,6b}$ = 3.10 Hz, 6-H_b); 2.66 (1H, dd, J_{gem} = 18.61, $J_{5a,4}$ = 6.15 Hz, 5-H_a); 2.45 ppm (1H, dd, J_{gem} = 18.61, $J_{5b,4}$ = 0.8 Hz, 5-H_b).

From the fraction with R_f 0.9 (A) by preparative TLC in system E we isolated 1.5 mg (1%) of compound **14a**; R_f 0.57 (E). EI mass spectrum, *m/z* (I_{rel}, %): 465 [M]$^+$ (100), 434 [M-OMe]$^+$ (74), 403 [M-2OMe]$^+$ (30), 372 [M-3OMe]$^+$ (35), 287 [M-C_8H_7NOMe-OMe]$^+$ (28), 256 [M-C_8H_7NOMe-2OMe]$^+$ (42), 160 [$CH_2C_8H_7NO$Me]$^+$ (80). High-resolution EI mass spectrum, *m/z*: 465.5679. ($C_{28}H_{27}N_3O_4$, calculated 465.5684).

3-(1'-Ethoxy-3'-indolyl)-2-hydroxy-4-hydroxymethylcyclopent-2-enone (15b). The compound was obtained similarly to compound **15a** from compound **3b** (100 mg, 0.28 mmol). Yield 12 mg (15%); R_f 0.70 (A), R_t 15.24 min. High-resolution EI mass spectrum, *m/z*: 287.1169 [M]$^+$. ($C_{16}H_{17}NO_4$, calculated 287.1180). ^{1}H NMR spectrum (tetradeuteromethanol), ppm: 8.18 (1H, s, 2'-H); 7.93 (1H, d, $J_{4,5'}$ = 8.2 Hz, 4'-H); 7.47 (1H, d, $J_{6,7'}$ = 8.1, $J_{5,7'}$ = 1.1 Hz, 7'-H); 7.26 (1H, t, $J_{5,6'}$ = 7.1 Hz, 6'-H); 7.18 (1H, t, $J_{6,5'}$ = 7.2 Hz, 5'-H); 4.37 (2H, dd, J = 7.1 Hz, $\underline{CH_2}CH_3$); 4.06 (1H, dd, J_{gem} = 10.7, $J_{4,6a}$ = 7.6 Hz, 6-H_a); 3.70 (1H, m, 4-H); 3.41 (1H, dd, J_{gem} = 10.7, $J_{4,6b}$ = 3.0 Hz, 6-H_b); 2.65 (1H, dd, J_{gem} = 18.56, $J_{5a,4}$ = 6.10 Hz, 5-H_a); 2.47 (1H, d, J_{gem} = 18.61 Hz, 5-H_b); 1.40 (3H, t, J = 7.1 Hz, $CH_2\underline{CH_3}$).

3-O-Methylglycoside of 2-C-[(1'-Ethoxy-3'-indolyl)methyl]-α-xylo-3-hexulofuranosono-1,4-lactone (11). Solution of N-ethoxyascorbigen **3b** (40 mg, 0.115 mmol) in absolute methanol (1.5 ml), saturated with HCl, was boiled and stirred for 2 days. From the residue after evaporation of the reaction mixture by preparative TLC in system B we isolated 23 mg (55%) of the product **11**; R_f 0.25 (A); $[\alpha]_D^{20}$ +14.6° (C 0.49, methanol). EI mass spectrum, *m/z* (I_{rel}, %): 363 [M]$^+$ (20), 174 [$CH_2C_8H_7NO$Et]$^+$ (100). High-resolution EI mass spectrum, *m/z*: 363.3694. ($C_{18}H_{21}NO_5$, calculated 363.3706). ^{1}H NMR spectrum (tetradeuteromethanol), ppm: 7.59 (1H, d, $J_{4,5'}$ = 8.2 Hz, 4'-H); 7.35 (1H, d, $J_{5,7'}$ = 1.1 Hz, 7'-H); 7.28 (1H, s, 2'-H); 7.17 (1H, t, $J_{5,6'}$ = 7.1 Hz, 5'-H); 7.02 (1H, t, $J_{5,6'}$ = 7.2 Hz, 6'-H); 4.28 (1H, m, J_{gem} = 9.1, $J_{5,6a}$ = 3.3 Hz, 6-H_a); 4.27 (2H, dd, J = 7.1 Hz, $\underline{CH_2}CH_3$); 4.23 (1H, m, 5-H); 3.85 (1H, m, $J_{5,6b}$ = 6.3 Hz, 6-H_b); 3.84 (1H, s, 4-H); 3.58 (3H, s, OCH_3); 3.32 (1H, d, J_{gem} = 14.5 Hz, $\underline{CH_2}$–C_8H_7NOEt); 3.20 (1H, d, J_{gem} = 14.5 Hz, $\underline{CH_2}$–C_8H_7NOEt); 1.35 (3H, t, $J_{1,2}$ = 7.1 Hz, CH_2CH_3).

Lactone of 2-C-(1'-Ethoxy-3'-indolylmethyl)-L-gulonic Acid (12). To solution of ethoxyascorbigen **3b** (50 mg, 0.143 mmol) in methanol (4 ml) $NaBH_4$ (17 mg, 0.45 mmol) was added with stirring. After 15 min the reaction mixture was acidified to pH 2, diluted with water, and extracted twice with ethyl acetate. From the dry residue after preparative TLC in system C we obtained 30 mg (60%) of the product **12**; R_f 0.35 (C); $[\alpha]_D^{20}$ +18.6 (C 0.75, methanol). EI mass spectrum, *m/z* (I_{rel}, %): 351 [M]+ (60), 174 [$CH_2C_8H_7NO$Et]$^+$ (100). High-resolution EI mass spectrum, *m/z*: 351.3584. ($C_{17}H_{21}NO_5$, calculated 351.3594). ^{1}H NMR spectrum (acetone-d_6), ppm: 7.63 (1H, d, $J_{4,5'}$ = 8.1 Hz, 4'-H); 7.40 (1H, s, 2'-H); 7.37 (1H, d, $J_{6,7'}$ = 8.1 Hz, 7'-H); 7.15 (1H, t, $J_{5,6'}$ = 7.1 Hz, 5'-H); 7.02 (1H, t, $J_{5,6'}$ = 7.1 Hz, 6'-H); 5.17 (1H, d, $J_{3,3\text{-OH}}$ = 5.3 Hz, 3-OH); 4.89 (1H, s, 2-OH); 4.53 (1H, dd, $J_{3,4}$ = 7.2, $J_{3,3\text{-OH}}$ = 5.3 Hz, 3-H); 4.36 (1H, d, $J_{4,5\text{-OH}}$ = 6.7 Hz, 5-OH); 4.30 (2H, dd, J = 7.1 Hz, $\underline{CH_2}CH_3$); 4.09 (1H, dd, $J_{3,4}$ = 7.2, $J_{4,5}$ = 2.9 Hz, 4-H); 3.94 (1H, t, $J_{6,6\text{-OH}}$ = 4.3 Hz, 6-OH); 3.79 (1H, m, 5-H); 3.62 (1H, m, $J_{6a,b}$ = 11.1, $J_{5,6b}$ = 6.8 Hz, 6-H_a); 3.55 (1H, m, 6-H_b); 3.34 (1H, d, J_{gem} = 14.0 Hz, $\underline{CH_2}$–C_8H_7NOEt); 3.16 (1H, d, J_{gem} = 14 Hz, $\underline{CH_2}$–C_8H_7NOEt); 1.35 (3H, t, J = 7.1 Hz, $CH_2\underline{CH_3}$).

Benzylamide of 2-C-(1-Methoxy-3-indolyl)-α-L-xylo-3-hexulofuranosonic Acid (13a). To solution of neoascorbigen **3a** (20 mg, 0.060 mmol) in methanol (2 ml) benzylamine (19 mg, 0.18 mmol) was added, and the mixture was stirred for 16 h. From the residue after evaporation of the reaction mixture on two plates in system D we obtained 12 mg (47%) of the product **13a**; R_f 0.73 (A). EI mass spectrum, *m/z* (I_{rel}, %): 441 [M]$^+$ (5),

160 [$CH_2C_8H_5NOMe$]$^+$ (100), 146 [C_8H_5NOMe]$^+$ (35), 106 [$PhCH_2NH$]$^+$ (20), 91 [CH_2Ph]$^+$ (50). ^{1}H NMR spectrum (acetone-d_6), ppm: 7.72 (1H, s, NH); 7.11 (5H, m, Ph); 6.84 (2H, d, J_{orth} = 6.9 Hz, $\underline{CH_2}$Ph); 7.67 (1H, d, $J_{4',5'}$ = 8.2 Hz, 4'-H); 7.40 (1H, d, $J_{7',6'}$ = 8.2, $J_{5',7'}$ = 1.0 Hz, 7'-H); 7.27 (1H, s, 2'-H); 7.19 (1H, t, $J_{5',6'}$ = 7.2 Hz, 5'-H); 7.04 (1H, t, $J_{5',6'}$ = 7.1 Hz, 6'-H); 3.98 (3H, s, OCH_3); 4.35 (1H, m, 4-H); 4.25 (1H, m, 5-H); 4.04 (1H, dd, $J_{5,6a}$ = 5.1, $J_{6a,b}$ = 9.14 Hz, 6-H_a); 3.63 (1H, dd, $J_{5,6b}$ = 3.3, $J_{6a,b}$ = 9.14 Hz, 6-H_b); 3.32 (1H, d, J_{gem} = 14.8 Hz, $\underline{CH_2}C_8H_5NOMe$); 3.20 (1H, d, J_{gem} = 14.8 Hz, $\underline{CH_2}C_8H_5NOMe$).

Butylamide of 2-C-(1-Methoxy-3-indolyl)-α-L-xylo-3-hexulofuranosonic Acid (13b). Solution of neoascorbigen **3a** (25 mg, 0.074 mmol) in *n*-butylamine (0.4 ml) was stirred for 0.5 h. From the residue after evaporation of butylamine by preparative TLC in system A the product **13b** (16 mg, 55%) was isolated; R_f 0.83 (A). EI mass spectrum, *m/z*: 408 [M]$^+$ (20), 160 [$CH_2C_8H_5NOMe$]$^+$ (100). High-resolution EI mass spectrum, *m/z*: 408.4565. ($C_{20}H_{28}N_2O_7$, calculated 408.45536). ^{1}H NMR spectrum (tetradeuteromethanol), ppm: 7.63 (1H, d, $J_{4',5'}$ = 8.2 Hz, 4'-H); 7.35 (1H, d, $J_{7',6'}$ = 8.1, $J_{5',7'}$ = 1.0 Hz, 7'-H); 7.25 (1H, s, 2'-H); 7.15 (1H, t, $J_{5',6'}$ = 7.2 Hz, 5'-H); 7.03 (1H, t, J = 7.1 Hz, 6'-H); 4.05 (3H, s, OCH_3); 4.32 (1H, d, $J_{4,5}$ = 5.1 Hz, 4-H); 4.30 (1H, dd, 5-H); 4.10 (1H, dd, $J_{5,6a}$ = 6.0, $J_{6a,b}$ = 8.8 Hz, 6-H_a); 3.61 (1H, dd, $J_{5,6b}$ = 4.6, $J_{6a,b}$ = 8.8 Hz, 6-H_b); 3.45 (1H, d, J_{gem} = 14.8 Hz, $\underline{CH_2}C_8H_5NOMe$); 3.18 (1H, d, J_{gem} = 14.8 Hz, $\underline{CH_2}C_8H_5NOMe$); 2.95 (1H, m, CH_2N); 2.78 (1H, m, CH_2N); 0.95 (4H, m, $2CH_2$); 0.70 (3H, t, Me).

Determination of the Content of Ascorbigen and Neoascorbigen in Kohlrabi (*Brassica caulorapa*). Kohlrabi stem (500 g) was ground and acetone added. The mixture was kept at room temperature for 1 h, filtered, and rewashed with acetone. The combined extract was evaporated, and the aqueous residue was extracted with ethyl acetate (3 × 100 ml). The dry ethyl acetate extract was evaporated, the residue was dissolved in methanol, the solution was made up to 25 ml, and an aliquot portion was taken for determination of ascorbigen and neoascorbigen by HPLC with the respective synthetic samples as standards.

The ascorbigen and neoascorbigen contents in rape (*Brassica rapa*) were determined similarly.

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