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Transformation of Ascorbigen into 1-Deoxy-1-(indol-3-yl)-α-L-sorbopyranose and 1-Deoxy-1-(indol-3-yl)-α-L-tagatopyranose

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Abstract. Ascorbigen, 2-C-[(indol-3-yl)methyl]-α-L-xylo-3-hexulofuranosonic acid γ–lactone **1a** results from the interaction of 3-hydroxymethylindole and L-ascorbic acid in mild conditions. In alkaline media ascorbigen opens the lactone and furanose cycles and decarboxylates to yield a mixture of 1-deoxy-1-(indol-3-yl)-α-L-sorbopyranose **5a** and 1-deoxy-1-(indol-3-yl)-α-L-tagatopyranose **6a**. Formation of ascorbigen 3-O-methylfuranoside **1c** stabilizes furanose ring and prevents spontaneous decarboxylation. Diphenylmethyl esters of 2-C-[(indol-3-yl)methyl]-α-L-xylo-3-hexulofuranosonic acid and the corresponding 3-O-methylglycoside **4a** and **4c** were synthesized. A similar study was performed for N-methylascorbigen **1b**.

INTRODUCTION

In the last decade it was shown that a diet rich in cabbage or other vegetables of the cruciferous family demonstrates anticarcinogenic properties[1]. This discovery induced interest in minor dietary constituents from cabbage, especially in indole-derived compounds. The major indole-containing compound in cabbage is ascorbigen **1a**, which is formed in plant tissues from an indole alkaloid glucobrassicin (*via* 3-hydroxymethylindole or 3-thiocyanomethylindole) and L-ascorbic acid[2,3]. A human being gets with food about 30 -50 mg of **1a** every day[4]. Some important compounds are products of ascorbigen transformation in stomach (in acidic media), the process being accompanied by the release of L-ascorbic acid[5].

The products of natural ascorbigen **1a** degradation in alkaline media are of interest as they are accumulated in blood and tissues of humans and animals who get cabbage (fresh, cooked or fermented) with meals. Investigation of the properties of these compounds can help to understand the biological role of ascorbigen. Previously it was shown that ascorbigen and its N-alkyl derivatives in alkaline media open the lactone ring, undergo decarboxylation and produce indole-derived carbohydrates among which 1-deoxy-1-(indol-3-yl)-L-sorbopyranoses were identified as major components[6]. The minor products of ascorbigens decarboxylation were not investigated earlier.

RESULTS AND DISCUSSION

Under alkaline conditions (5% aqueous $NaHCO_3$ solution, 1.5 hours at 20°C), the lactone ring of ascorbigen **1a** opens to form an anion of 2-C-[(indol-3-yl)methyl]-α-L-xylo-3-hexulofuranosonic acid **2a**.

Table 1. Chemical shifts (ppm) and coupling constants values J (Hz) of the carbohydrate moiety in ^{1}H-NMR spectra of the compounds **4a,b,c,d** in $CDCl_3$

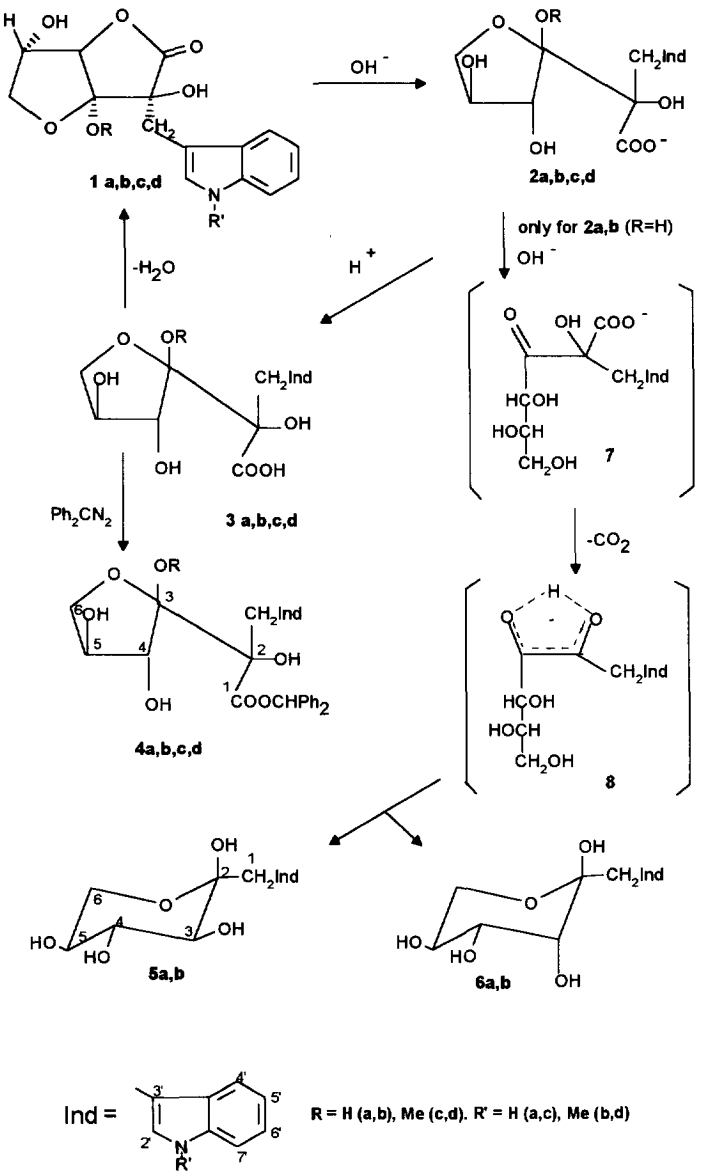
	Ascorbic acid moiety								
Compound	4-H	5-H	6-H_a	6-H_b	2-OH	3-OR	4-OH	5-OH	CH_2
4a	4.42	4.18	4.11 $J_{6a,6b}$= 9.6, $J_{6a,5}$= 3.9	3.76 $J_{6b,5}$= 1.9 $J_{6b,4}$= 1.1	3.60	4.92	3.05	2.5	3.53 3.23 J_{AB} 14.9
4b	4.49 $J_{4,5}$= 4.4	4.21 $J_{5,6a}$= 3.7	4.14d $J_{6a,6b}$= 9.5	3.80d $J_{6b,5}$= 2.8 $J_{6b,4}$= 1.1	3.60	4.97	3.12	2.40	3.55 3.22 J_{AB}= 14.9
4c	4.35	4.39	4.46 $J_{6a,6b}$= 9.5 $J_{6a,5}$= 5.9	3.95 $J_{6b,5}$= 4.0	3.66	3.36	3.89	2.40	3.51 3.63 J_{AB}= 14.7
4d	4.36 $J_{H,OH}$= 3.7 $J_{4,5}$= 3.4	4.38 $J_{5,6a}$= 5.7 $J_{5,6b}$= 4.2 $J_{5,OH}$ =6.0	4.47 $J_{6a,6b}$= 9.5	4.00	3.62	3.37	3.92 $J_{H,OH}$ = 3.7	2.40 $J_{H,OH}$ = 6.0	3.51 3.61 J_{AB}= 14.4

The attempts to isolate the individual acid **3a** failed as after careful acidification (to pH 4) and extraction with EtOAc **3a** easily transforms into the starting **1a** and the products of its transformation in acids, first of all 2'-skatylascorbigen[5] , accompanied with small amounts of the decarboxylation products. Fast extraction of the acid **3a** followed by immediate addition of a Ph_2CN_2 ether solution led to the diphenylmethyl ester of 2-C-[(indol-3-yl)methyl]-α-L-xylo-3-hexulofuranosonic acid **4a**, isolated by TLC in 36% yield. Similarly compound **4b** was obtained from **1b** in 50% yield.

Incubation of 3-O-methylascorbigen **1c** or its N-methyl analog **1d** in 0.05N NaOH solution for 2 h. led to the corresponding acids **3c,d**, which are more stable than **3a,b** and can be stored for 1 h., though corresponding lactones **1c,d** begin to form. No other products were detected. Acids **3c** and **3d** were transformed into the corresponding diphenylmethyl esters **4c** and **4d** in the 63 and 90% yields, respectively. The structures of esters **4a-d** were confirmed by mass-spectrometry and NMR methods (Table 1). The esters retained the α-furanoside structure of the starting ascorbigens similar to amides of acids **3a,b** that were previously obtained by the interaction of ascorbigen with primary amines or ammonia[7]. In the ^{13}C NMR spectrum of **4a** (see Experimental section) the signal of 4-C is shifted upfield in comparison with **1a**[8], indicating an opened lactone ring (detailed assignment of signals was performed by the 1D and 2D NMR techniques: selective INEPT, HETCOR, COSY).

Incubation of **1a** in sodium bicarbonate solution for several hours led to 1-deoxy-1-(indol-3-yl)-L-sorbopyranose **5a** with an admixture of minor component **6a** in a total yield about 30%. The decarboxylation products **5a+6a** or **5b+6b** were obtained in 90 and 83% yield respectively when **1a** or **Ib** was incubated in aqueous methanol in the presence of an equimolar amount of Et_3N (pH 7.5-8) for 1.5-2 h. at 40-50° C.

HPLC analysis of the **1a** decarboxylation products demonstrated the presence of two compounds **5a** and **6a** in a 58:40 ratio. For the decarboxylation products of **1b**, the ratio of **5b** and **6b** was 80:19.



^{1}H NMR data for the compound **6b** are similar to those for **6a**, which allowed us to propose a tagatopyranose structure for **6b**. Parameters of ^{1}H-NMR and ^{13}C-NMR spectra of **5a, b,** and **6a, 6b** are presented in Tables 2 and 3. Because of low content of the minor component **6b**, ^{13}C NMR data for this compound were not obtained.

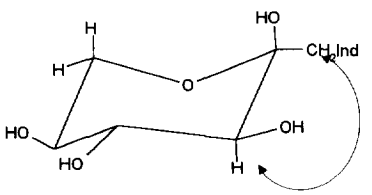
Methyl furanosides **1c,d** did not produce decarboxylation products. This is connected with the stability of full ketals **2c,d** in alkaline media and suggests that the easy decarboxylation of ascorbigen and its N-alkyl derivatives is determined by the possibility of an easy hemiketal cycle opening and formation of the corresponding α-ketoacids anions **7** and intermediate **8**.

The biological significance of the compounds **5a** and **6a**, which are formed in the bodies of humans and animals fed with cabbage and other cruciferous vegetables[2], remains to be investigated.

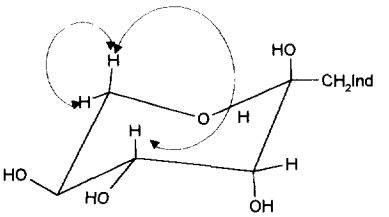
Table 2. ^{1}H NMR data for compounds **5a,b** and **6a,b** in Py-d_5 (L-ketopyranose moiety)

Compounds	H-1	H-3	H-4	H-5	H-6ax	H-6eq
5a	3.85 4.00 J_{AB}=14.3	4.18 $J_{3,4}$=9.1	4.65 $J_{4,5}$=9.5	4.11 $J_{5,6ax}$=10.3 $J_{5,6eq}$=5.4	4.49 $J_{ax,eq}$=10.6	4.16
5b	3.77 4.09 J_{AB}=14.7	4.09 $J_{3,4}$=8.8	4.61 $J_{4,5}$=8.8	4.10 $J_{5,6ax}$=10.7 $J_{5,6eq}$=5.6	4.44 $J_{ax,eq}$=10.7	4.14
6a	3.83 4.11 J_{AB}=14.4	4.62 $J_{3,4}$=3.0	4.80 $J_{4,5}$=9.1	4.85 $J_{5,6ax}$=10.1 $J_{5,6eq}$=5.1	4.48 $J_{ax,eq}$=10.3	4.31
6b	3.77 4.02 J_{AB}=13.0	4.54 $J_{3,4}$=3.1	4.76 $J_{4,5}$=9.1	4.79 $J_{5,6ax}$=10.0 $J_{5,6eq}$=5.2	4.43 $J_{ax,eq}$=10.3	4.27

NMR study showed that the major product of decarboxylation of the natural ascorbigen **1a** is 1-deoxy-1-(indol-3-yl)-L-sorbopyranose **5a**. Whereas trans-diaxial orientation of carbohydrate protons in the pyranose rings of this compound, built on the L-ascorbic acid backbone, clearly demonstrated the L-sorbopyranose structure, α-configuration at the anomeric 2-C-atom needed confirmation. We have measured the NOE-difference spectra of **5a**. An increase in the intensity of the CH_2 protons under selective saturation of the axial 2-H unambiguously demonstrated the α-configuration of the anomeric center.



5a



6a

NOE-effects

To determine the structure of the minor component **6a** in the **5a+6a** mixture, 1D and 2D NMR experiments (APT, DQCOSY and HETCOR) were used. The experiments have shown that **6a** has a structure isomeric to **5a.** A structure of L-tagatopyranose was ascribed to **6a** as the $J_{3,4}$ value (3.0 Hz) corresponds to the equatorial position of 3-H proton, whereas $J_{4,5}$, and $J_{5,6ax}$ (9.1 and 10.1 Hz respectively) correspond to the axial orientation of 4-H and 5-H protons. To determine the configuration at the anomeric center in **6a,** the NOE-difference experiment was performed. Due to equatorial position of 2-H proton, the difference spectra were recorded under saturation of 6-H_{ax} signal. In this case, an increase in the intensity of 6-H_{eq} and 4-H_{ax} was observed, demonstrating equatorial position of CH_2 group and hence the α-configuration of the anomeric center.

Table 3. Parameters of ^{13}C NMR spectra of the compounds **5a, 5b** and **6a** in CD_3OD (δ 49.00 ppm).

Chemical shifts (ppm)			
^{13}C-atoms	**5a**	**5b**	**6a**
L-Ketopyranose moiety			
1-C	34.95	34.80	34.30
2-C	99.90	99.61	100.63
3-C	74.29	74.31	74.99
4-C	76.51	76.50	73.31
5-C	71.61	71.63	68.32
6-C	63.47	63.49	64.10

Chemical shifts (ppm)			
Indole moiety			
2'-C	125.74	130.16	125.71
3'-C	110.14	109.60	109.90
3'a-C	130.00	130.52	129.97
4'-C	120.57	120.84	120.36
5'-C	119.52	119.56	119.52
6'-C	122.03	122.15	122.03
7'-C	111.90	109.80	111.93
7'a-C	137.86	138.37	137.85
N-CH_3	-	32.14	

EXPERIMENTAL SECTION

General. All NMR measurements were obtained on a Varian VXR-400 instrument operated at 400 MHz for ^1H, and at 100,6 MHz for ^{13}C. Optical rotations were measured on a Perkin-Elmer 241 instrument, IR (in KBr pellets) - using a SP-1100 (Pye Unicam, England) spectrometer; EI-mass-spectra were obtained on an SSQ 710 Finnegan instrument. HPLC was performed on a Shimadzu liquid chromatograph with a Zorbax C8 column in a linear gradient of acetonitrile - 0.01M H_3PO_4 (7 → 12%) during 20 min. Analytical TLC was carried out on Silufol plates UV-254 (Kavalier, Czechoslovakia) in chloroform-methanol, 10:1 (A) or precoated Merck Kieselgel F_{254} plates in chloroform-methanol (7:1) (B) or (4:1) (C). Preparative chromatography was performed on plates (20 x 20 cm, 0.5 mm) with Kieselgel 60 F_{254} (Merck) in the same systems. For the purification of esters **4a-d,** flash column chromatography on dry Kieselgel 60 (Merck) was used. Diphenyldiazomethane was prepared from benzophenone hydrazone by the method[9]. Ascorbigens **1a, 1b** and their 3-O-methyl derivatives (**1c, 1d**) were obtained as previously described[10,11].

2-C-[(Indol-3-yl)methyl]-α-L-xylo-3-hexulofuranosonic acid diphenylmethyl ester 4a. 50 mg (0.14 mmol) of **1a** was stirred at room temperature in a mixture of water (5 ml), methanol (5 ml) and 5% aqueous $NaHCO_3$ (2 ml); after 1.5 hr **1a** was completely transformed into **3a** (R_f 0 in A and B systems). The reaction mixture was acidified by 1 N HCl to pH 4, and acid **3a** was extracted with ethylacetate, dried over Na_2SO_4 for 15 min. and a solution of 50 mg (0.25 mmol) of diphenyldiazomethane in 2 mL of dry ether was added. After 2 h. the reaction mixture was evaporated *in vacuo,* and the product was isolated by TLC on silica gel plates to yield 27 mg of white amorphous powder of **4a** (36%), R_f 0.35 (B); IR: ν_{max} 1720 cm^{-1} ; $[\alpha]_{20}{}^{D}$+20.6 (C 1, EtOH). EI-Ms *m/z* 489 (M^+). Anal. Calcd for $C_{28}H_{27}NO_7$: C, 68.70; H, 5.56; N, 2.86. Found: C, 68.50; H, 5.60; N, 2.60.

2-C-[(1-Methylindol-3-yl)methyl]-α-L-xylo-3-hexulofuranosonic acid diphenylmethyl ester 4b was obtained similarly from **1b** in 47% yield. Rf 0.45 (B) ; IR: ν_{max} 1720 cm^{-1} . $[\alpha]_{20}{}^{D}$+7.5 (C 1, EtOH). EI- Ms: 503 (M^+). Anal. Calculated for $C_{29}H_{29}NO_7$: N, 2.78. Found: N, 2.95.

3-α-Methylglycoside of 2-C-[(indol-3-yl)methyl]-α-L-xylo-3-hexulofuranosonic acid diphenylmethyl ester 4 c. 50 mg (0.16 mmol) of **1c** were dissolved in 5 mL of H_2O and 5 mL of 0.1N NaOH and stirred at room temperature for 2 h. The reaction mixture was carefully acidified by 1N HCl to pH 4 and acid **3a** was extracted with ethyl actetate (R_f 0). The extract was dried over Na_2SO_4 during 30 min. and 56 mg (0.3 mmol) of diphenyldiazomethane in 5 mL of dry ether were added. The reaction mixture was stirred for 3 h., evaporated *in vacuo* and the product was purified from the admixture of diphenyldiazomethane on short

column with dry silica gel to yield 47 mg of **4c** (63%), powder with m.p.138-140° C, R_f 0.61 (A); IR: ν_{max} 1720 cm^{-1}. $[\alpha]_{20}{}^{D}$+51 (C 1, MeOH). Anal. Calcd for $C_{29}H_{29}NO_7$: N, 2.78. Found: N, 3.00.

3-O-Methylglycoside of 2-C-[(1-methylindol-3-yl)methyl]-α-L-xylo-3-hexulofuranosonic acid diphenylmethyl ester 4d was obtained similarly from **1d** in 90% yield as white powder, m.p. 145-148 C°. R_f 0.65 (A). IR: ν_{max} 1740 cm^{-1}. $[\alpha]_{20}{}^{D}$+48 (C 1, MeOH)., Anal. Calcd for $C_{30}H_{31}NO_7$: C, 69.63; H, 6.00; N, 2.70. Found: C, 69.64; H, 5.97; N, 2.42. ^{13}C NMR ($CDCl_3$): 172.57 (1-C), 139.47 (α-C), 136.64 (8'-C), 129.04 (9'-C), 128.52 (γ-C), 128.25 (β-C), 128.18 (2'-C), 126.94 (δ-C), 121.45 (6'-C), 119.51 (4'-C), 118.96 (5'-C), 109.67 (3-C), 108.96 (C-7'), 107.10 (3'-C), 84.12 (2-C), 82.87 (4-C), 78.81 (OCHPh), 77.02 (5-C), 75.09 (6-C), 50.32 (OMe), 32.47 (NMe), 29.18 (CH_2Ind).

A mixture of 1-deoxy-1-(indol-3-yl)-α-L-sorbopyranose and 1-deoxy-1-(indol-3-yl)-α-L-tagatopyranose 5a+6a. To a solution of 30 mg (0.094 mmol) of **1a** in 10 mL of methanol 134.2 mcl (9.6 mg. 0.094 mmol) of Et_3N and 1 mL of water were added. The reaction mixture was stirred at 40-60°C for 1h. 20 min. until TLC control showed the complete transformation of the acid **3a** into **5a+6a** mixture (R_f 0.50 , C system). After evaporation *in vacuo* the residue was dissolved in methanol and the compounds were purified chromatographically on plates with silica gel in B system to yield 25 mg (90%) of **5a+6a** mixture. R_f O.50 (C), 0.20 (B). HPLC: **5a** R_t 7.75 min. (58%), **6a** 6.16 min. (40%). Anal. Calcd for $C_{14}H_{17}NO_5$: C, 60.20; H, 6.13; N, 5.06. Found: C, 59.90; H, 5.97; N, 4.80

A mixture of 1-deoxy-1-(1-methylindol-3-yl)-α-L-sorbopyranose and 1-deoxy-1-(1-methylindol-3-yl)-α-L-tagatopyranose 5b+6b. Obtained similarly from **1b** in 83% yield. R_t of **5b** (80%) 15.45 min.; R_t of the minor **6b** (19%) 11.67 min. The mixture of **5b** and **6b** was boiled with BSTFA and after evaporation investigated by EI-Ms method. EI-Ms, *m/z* (relative intensity): 653 $[C_{30}H_{59}NO_5Si_5]^+$ (0.3); 581 $[C_{27}H_{51}NO_5Si_4]^+$ (0.5); 509 [$C_{24}H_{43}NO_9Si_3]^+$ (0.3), 437 $[C_{21}H_{35}NO_5Si_2]^+$ (0.2), 365 [$C_{18}H_{23}NO_5Si]^+$ (0.15), 346 [**11**]$^+$ (100), 274 [**12**]$^+$ (30), 144 [**13**]$^+$ (100).

REFERENCES

1 Wattenberg, L. W. *Cancer Res.(Suppl.)*, **1983,** *43*,.2448s.

2. Preobrazhenskaya, M.; Bukhman, V.; Korolev, A. ; Efimov, S. *Pharm. Ther.*, **1993,** *60*,. 301.

3. Gmelin, R. & Virtanen, A.I. *Ann. Acad. Sci. Fennicae, Ser. A II Chemica*, **1961,** *107*, 1.

4. Aleksandrova, L. G.; Korolev, A. M.; Preobrazhenskaya, M. N. *Food Chemistry,* **1992,** *45*, 61.

5. Korolev, A. M. ; Lazhko, E. I.; Yartseva, I. V.; Plikhtyak, I.L.; Aleksandrova, L. G. & Preobrazhenskaya, M. N. *Bioorg. Khim.* (Russ), **1991,** *17*, .981.

6. Plikhtyak, I. L.; Yartseva, I. V.; Kluev, N. A.;. Preobrazhenskaya, M. N. *Khim. Heterocycl. Soedinenii* (Russ), **1989,** 607.

7. Plikhtyak, I. L.; Yartseva, I. V.; Khan, Z. E.; Preobrazhenskaya, M. N.. *Bioorg. Khim.* (Russ), **1988,** *14*, 1437.

8. Lazhko, E.I.; Korolev, A.M.; Preobrazhenskaya, M.N.. *Khim. Heterocycl. Soedinenii* (Russ), **1993,** 352.

9. Eistert, B.; Regitz, M.; Heck, G & Schwall, H.. *Methoden zur Herstellung und Umwandlung von aliphatischen Diazoverbindungen. In* Methoden der Organischen Chemie (Houben Weyl), Vol. 10 (IV). Ed.., E. Muller, p. 569, Georg Thieme Verlag, 1968.

10. Kiss, G. & Neukom, H. *Helv. Chim. Acta* , **1966,** *49*, 989.

11. Mukhanov, V.I.; Yartseva, I.V.; Kikot, B.S.; Volodin, Y.Y.; Kustova, I.V.; Lesnaya, N.A.; Sof'ina, Z.P.; Ermakova, N.P. & Preobrazhenskaya, M.N.. *Bioorg. Khim.* (Russ), **1984,** *10*, 544.

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