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Preparation of 6-Amino-6-deoxy Derivatives of Ascorbic and iso-Ascorbic Acid

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6-Amino-6-deoxy derivatives of ascorbic and iso-ascorbic acid have been prepared by a series of nucleophilic substitutions on the C_6 atom. In the case of iso-ascorbic acid the nitrogen in position 6 was introduced via the 5,6-epoxy derivative of iso-ascorbic acid.

The C_6-derivatives of asorbic acid have been of particular interest since the possibility of non-oxydative transformation of ascorbic acid was demonstrated.[1] The first step in this transformation is the formation of a bicyclic compound by intramolecular dehydration of the C_6 and C_3 hydroxyl groups. Substitution of the C_6 hydroxyl group by another substituent would prevent this reaction.

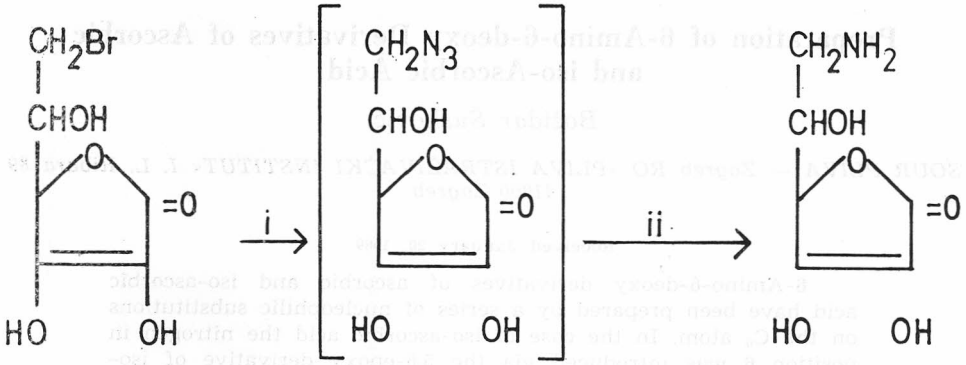
In continuation of our work[2] on the chemistry of halogen derivatives of ascorbic acid, we prepared 6-amino-6-deoxy derivatives of ascorbic (*I*) and iso-ascorbic acid (*II*).

The preparation of the 6-amino derivative of iso-ascorbic acid has not been reported in literature. However, two methods have been described for the preparation of the 6-amino derivative of ascorbic acid. Andrews[3] prepared *I via* the unstable 5,6-epoxy derivative, obtained from 6-bromo-6-deoxy-ascorbic acid (*III*). This intermediate in reaction with NaN_3 gave the 6-azido derivative which, subjected to catalytic hydrogenation, gave *I*. The crucial point in the sequence of the reactions was the opening of the epoxyde ring, although the autor showed that in the course of the reaction no inversion of configuration at C_5 atom occurred.

On the other hand, Von Dallacker *et al.*[4] reported the preparation of the amino derivative *I* by a series of selective protection and deprotection reactions of the hydroxyl groups of ascorbic acid and 2,3-O-dibenzyl ascorbic acid as key intermediate. By tosylation of the primary hydroxy group, followed by substitution with azide, and, finally, catalytic hydrogenation of the azido group, the 6-amino derivative *I* was obtained.

The method we used for preparation of the 6-amino derivative of ascorbic acid *I* was shown to ensure the stability of other chiral centres in the molecule and was based on nucleophilic substitution on C_6 atom (Scheme 1).

SCHEME 1



III IV I

i = NaN_3

ii = H_2, Pd/C

This method included 6-bromo-6-deoxy derivative *III* as the most favourable starting compound for preparation of the azido derivative *IV*. The reaction of *III* with NaN_3 was carried out in different solvents and at various temperatures. The reaction product was analyzed by TLC and by titration of bromide ions formed in the reaction. The results are shown in Figure 1—4.

The reaction proceeded in water and in a mixture of water and cellosolve (1 : 1), while in pure cellosolve and in water-cellosolve (1 : 2) a suspension was formed and the reaction stopped. No reaction took place in boiling acetone; under higher pressure and temperature a mixture of products was obtained. The reaction was faster in water-cellosolve (1 : 1) than in water alone. The influence of tetrabutylammonium bromide as a phase transpher catalyst was also examined. In both media addition of phase transpher catalyst did not affect the reaction rate. The crude product was isolated by evaporation of the solvent. It contained 18—19% bromide ions (titration by $AgNO_3$) and 30—31% azido derivative *IV* (titration by iodine). The product was purified by column chromatography and showed, in the IR spectrum, the characteristc band of the azido group at 2115 cm^{-1}. Since isolation of azido derivative *IV* was not possible, the crude reaction mixture was hydrogenated (5% Pd/C). During isolation of amino derivative *I*, special attention was given to the

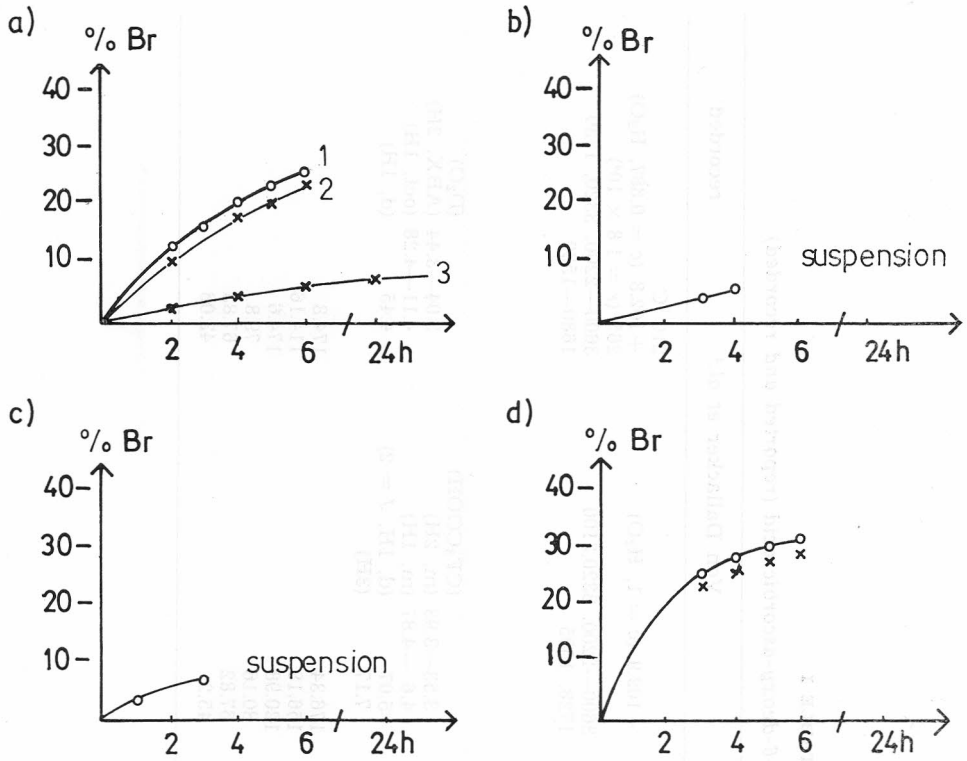


Figure 1. Formation of free Br^- in reaction of *III* with NaN_3. a) in water (1 = 100 °C, 2 = 80 °C, 3 = 45 °C), b) in cellosolve (80 °C), c) in water-cellosolve (1 : 1, 80 °C), d) in water-cellosolve (1 : 2, 80 °C). —O— with *t*-Bu_4NBr; —X— without *t*-Bu_4NBr.

pH of the medium. The isolated compound *I* was purified by crystallization from water. Physical constants and spectral characteristics were compared with literature data and are given in Table I.

There is evident discrepancy of these data. Some of the disagreement can be attributed to pH dependency. However, it can be seen that there is no correlation between ^1H signals measured at pD = 1 (Andrews G. C.[3]), and those measured in trifluoroacetic acid (Von Dallacker *et al.*[4]), although in both cases the sample has to be in the form an ammonium ion. Our interpretation of the ^{13}C NMR spectrum based on the data given for ascorbic acid[5] differs from Von Dallacker *et al.*[4] The values are given in Table II.

From the data in Table II, it may be noticed that the chemical shift of C-6 of ascorbic acid is independent of the pD atoms. The remarkable shift of C_6 of *I* must be caused by the amino group. Since at pD = 7.2 the sodium salt of ascorbic acid is involved, we assume that our product in solution is a »zwitter ion« or a bimolecular ammonium ascorbate.

For the preparation of the 6-amino derivative of iso-ascorbic acid *II* the same method as used for the preparation of *I* was attempted, but without success. The bromo derivative of iso-ascorbic acid *VI* was prepared by the reaction of iso-ascorbic acid *V* with the CBr_4—Ph_3P reagent. According to literature data[6], the 6-bromo derivative *VI* was obtained from *V* with

TABLE I

Physical and spectral data of 6-amino-6-deoxy-ascorbic acid (reported and recorded)

		Andrews[3]	Von Dallacker *et al.*[4]	recorded[+]
M. p.		210 °C		210 °C
$[\alpha]_D^{23-24}$		+92.8 (c = 0.687, H_2O)	+109.9 (*c* = 1, H_2O)	+102.8 (*c* = 0.687, H_2O)
UV λ_{max} (nm)		258 ($\varepsilon = 11.2 \times 10^4$)		265 ($\varepsilon = 1.8 \times 10^4$)
IR (KBr, cm^{-1})		1735, 1600	3600—2200, 3320,3100 1728, 1605	3600—2200, 3320, 1730 1680—1580
NMR (solvent, pH)		(D_2O, pH = 5)		
^1H δ (ppm):	H_2—C_6	2.79 (br, d, 2H, *J* = 2 Hz)		
	H—C_5	3.85 (br, t, 1H)		
	H—C_4	4.33 (br, s, 1H)		
		(D_2O, pH = 1)	(CF_3COOH)	(D_2O)
	H_2—C_6	3.28 (ABX, 2H)	3.53—3.93 (m, 2H)	3.04—3.44 (ABX, 2H)
	H—C_5	4.28 (oct. 1H)	4.6 —4.87 (m, 1H)	4.11—4.28 (oct. 1H)
	H—C_4	4.94 (d, 1H)	5.07 (d, 1H, *J* = 2)	4.45 (d, 1H)
	NH_3^+		7.17 (3H)	
^{13}C (ppm):	C_1		176.34	174.8
	C_2		156.18	114.16
	C_3		120.98	174.6
	C_4		80.16	79.8
	C_5		67.82	67.83
	C_6		45.2	43.06

[+] Sample of *I* prepared by Scheme 1.

TABLE II

^{13}C NMR spectral data of ascorbic acid (AA) and 6-amino-6-deoxi-ascorbic acid (NH_2—AA) prepared by scheme I.

	C_1	C_2	C_3	C_4	C_5	C_6
AA (pD = 2.5)	174.29	118.7	157.1	77.22	69.98	63.19
AA (pD = 7.2)	178.21	114.21	176.17	79.35	70.63	63.73
NH_2—AA	174.8	114.16	174.6	79.8	67.83	43.06

HBr/HOAc, but the product was not characterized. With CBr_4—Ph_3P we obtained an oily product which was not crystallized even after purification by column chromatography. The reaction of *VI* with sodium azide failed. We obtained a product which did not contain nitrogen. Investigation is still in progress. Another method used for the preparation of *II* used the 5,6-anhydro derivative of iso-ascorbic acid *VIII* (Scheme 2).

The reaction of *VIII* with sodium azide was monitored by TLC and UV spectroscopy. After 18 hours the reaction mixture was treated by cationic ion exchange resin, followed by catalytic hydrogenation to give 6-amino-6-deoxy-iso-ascorbic acid *II*.

EXPERIMENTAL

The IR spectra were recorded on a Perkin-Elmer Infracord Model 257 G and the UV spectra on a Pye Unicam SP8-100 instrument. The ^1H NMR spectra were run on a JOEL 90 Q spectrometer with TMS as internal standard; chemical shifts were given in ppm-values (δ). The ^{13}C NMR spectra were recorded on a JEOL SX 90 Q spectrometer with TMS as internal standard. TLC was carried out on commercial plates (Merck, Kieselgel F_{254}), followed by detection with iodine vapour or by spraying with molybdenphosphoric acid (5% solution in ethanol). The solvent systems for chromatography were:

(A) *n*-butanol-acetic acid-water = 4 : 1 : 5 (upper layer)

(B) *n*-propanol-1N ammonium hydroxyde-acetone = 30 : 20 : 2

(C) benzene-acetic acid = 1 : 1

Column chromatography was performed on silica gel 0.063—0.2 mm (»Kemika«).

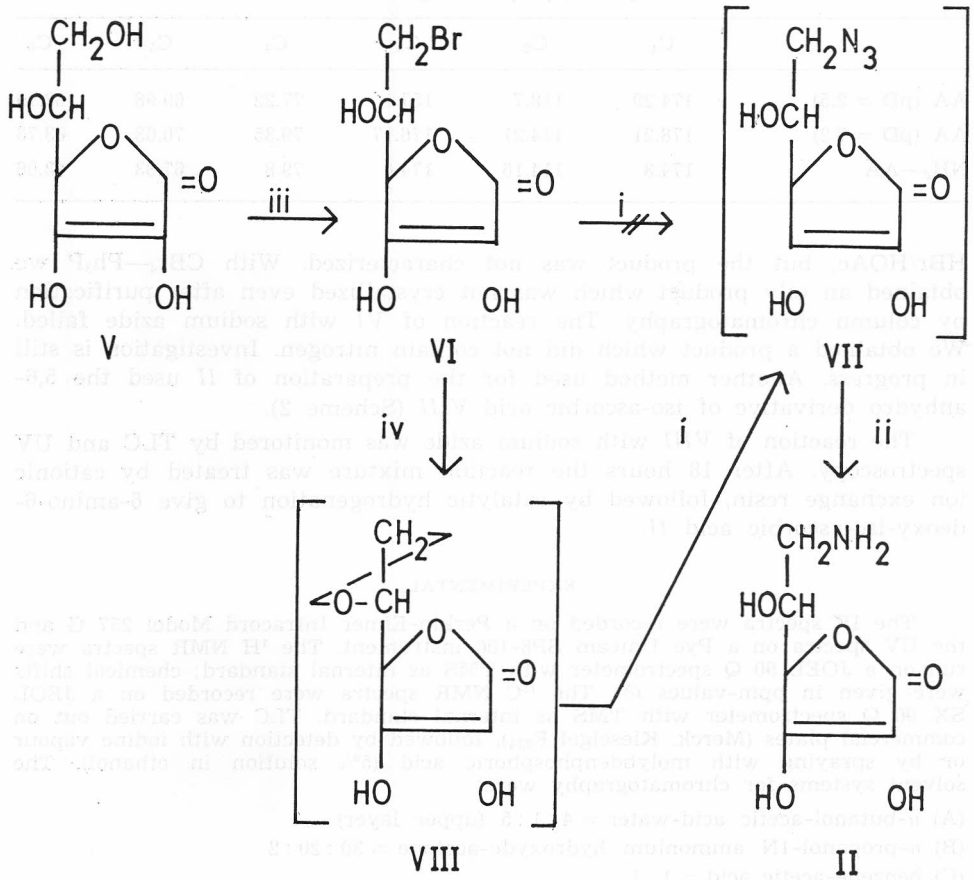
Optical rotations were measured on a KARL ZEISS POLAMAT A polarimeter.

Melting points were determined on a FISHER-JONS apparatous, the values are uncorredsted.

6-Amino-6-deoxy-ascorbic Acid (I)

To a stirred solution of 6-deoxy-ascorbic acid (5 g, 20.9 mmol) dissolved in water-cellosolve (1 : 1) (33.8 ml) sodium azide (2. g, 30.76 mmol) was added, and the mixture was heated at 80 °C for 3 hours. Then, an equal portion of NaN_3 was added and heating was continued for an additional 3 hours. The solution was kept at 25 °C overnight. The ion exchange resin Amberlyst 15 (46 ml in H^+ form) was added and the reaction mixture stirred for one hour. The resin was separated by filtration, washed with water (4 × 34 ml) and the combined filtrate and washings evaporated under reduced pressure to a smaller volume (50 ml). The catalyst 5% Pd/C (1.2 g, 0.564 mmol) was added, and the reaction mixture hydrogenated for 7 hours under 3.5 bar of H_2 at 25 °C. The catalyst was filtered off, washed with water (7 ml), and 20% sodium hydroxide solution was added until pH = 5.4. The

SCHEME 2



i = NaN_3

ii = H_2, Pd/C

iii = CBr_4 – Ph_3P

iv = Na_2CO_3

solution was concentrated under reduced pressure to a smaller volume (7 ml). After stirring for 3 hours and standing in refrigerator overnight, the precipitate was filtered, washed with water (1 ml) and dried. The yield was 3.8 g of crude product (32% purity by I_2 titration). The crude product was recrystallized from water (30 ml) to yield 6-amino-6-deoxy-ascorbic acid (*I*), (0.58 g).

m. p. = 210 °C (decomp.), $[\alpha]_D^{25} = 102.8$ (c 0.687, H_2O).

UV: $\lambda_{max} = 265$ nm, $\varepsilon = 18072$ M^{-1} cm^{-1}, $A_{1\,cm}^{1\%} = 1032$.

IR: KBr, ν (cm^{-1}): 3310, 1730, 1590—1560 (broad), 1510.

^{13}C NMR (D_2O); δ (ppm): 43.06 (C_6), 66.82 (C_5), 79.79 (C_4), 174.6 (C_3), 114.17 (C_2), 174.83 (C_1).

^1H NMR (D_2O); δ (ppm): 4.94 (d, H—C_4), 4.57 (oct. H—C_5), 3.78 (ABX 2H—C_6).

6-Bromo-6-deoxy-iso-ascorbic Acid (VI)

To a solution of isoascorbic acid (*V*) (1 g, 5.68 mmol) and triphenylphosphine (2.985 g, 11.35 mmol) in DMF (17 ml), a solution of CBr_4 (1.98 g, 5.97 mmol) in DMF (5.75 ml) was added dropwise at —5 °C and the mixture was stirred for 10 hours at room temperature. The solvent was evaporated and coevaporated with benzene under reduced pressure, and the residue was slurred in a mixture of benzene (20 ml) and water (80 ml). The separated aqueous layer was again extracted with benzene (4 × 20 ml), followed by ethylacetate (10 × 20 ml). The combined ethylacetate extracts were treated with carbon, filtered, dried ($MgSO_4$), and evaporated to yield *VI* as an oily ethyl-acetate solvate (0.8 g).

$[\alpha]_D^{25} = -7.3$ (c 1.042, H_2O).

TLC $R_f = 0.775$, (system A).

UV; $\lambda_{max} = 365$ nm, $\varepsilon = 12934$, $A_{1\,cm}^{1\%} = 541.13$.

IR ν (cm^{-1}): 3500—3000 (broad), 1750—1730 (br), 1670.

^1H NMR; δ (ppm): 4.88 (d, H—C_4), 3.4 (m, 2H—C_6), 2.77 (d, H—C_5), ethyl acetate: 1.08 (t, CH_3—CH_2—), 4.09 (m, CH_3—CH_2—), 1.92 (s, CH_3CO—).

^{13}C NMR; δ (ppm): 178.39 (C_1), 161.12 (C_3), 123.76 (C_2), 82.68.
(D_2O): (C_4), 72.24 (C_5), 37.7 (C_6).
($CH_3COOCH_2CH_3$): 18.96 (d), 26.24 (a), 78.13 (c), 170.54 (b).

a b c d

Anal: $C_6H_7O_5Br \cdot CH_3COC_2H_5$ (311.46) calc'd.: Br 25.66%
found: Br 25.4 %

6-Amino-6-deoxy-iso-ascorbic Acid (II)

To a solution of sodium carbonate (1.04 g, 9.8 mmol) in water (6.75 ml) a solution of 6-bromo-6-deoxy-iso-ascorbic acid (1 g, 5.68 mmol) in water (1.3 ml) was added dropwise at 20 °C followed by sodium azide (0.4 g, 6.15 mmol). The solution was then stirred for 12 hours. A sample showed the presence of a new spot on TLC with $R_f = 0.65$ (solvent A). Ion exchange resin Amberlyst 15 (11.75 ml in H^+ form) was added to the solution and it was stirred for 1 hour. The resin was filtered off, washed with water (4 × 7.5 ml), and the combined filtrate concentrated under reduced pressure to 10 ml. To the concentrate 5% Pd/C (0.2 g, 0.094 mmol) was added and the mixture was hydrogenated at 3.5 bar and 25 °C for 7 hours. The catalyst was filtered off, washed with water (1.2 ml) and the filtrate was evaporated to dryness (0.77 g). The crude product was crystallized from water (3 ml) to yield *II* (0.16 g, 15.9%).

m. p. = 215 °C (decomp.)

$[\alpha]_D^{20} = 75.15$ (c 0.678, H_2O)

Anal. $C_6H_9O_5N$ (175.15) calc'd.: N 8.0%
found: N 7.9%

UV; $\lambda_{max} = 266$ nm, $\varepsilon = 13572$, $A_{1\,cm}^{1\%} = 755$.

IR; KBr, ν (cm^{-1}): 3200—3000 (broad), 2745, 1695, 1630, 1590—1500, 1075.

^1H NMR ($CF_3COOD + D_2O$); δ (ppm): 4.98 (d, H—C_4), 4.6 (m, H—C_5), 3.51 (d, 2H—C_6).

^{13}C NMR ($CF_3COOH + D_2O$); δ (ppm): 42.5 (C_6), 69.02 (C_5), 79.01 (C_4), 154.74 (C_3), 120.65 (C_2), 174.83 (C_1).

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The ^1H and ^{13}C NMR spectra were recorded by the NMR Service of the »Ruđer Bošković« Institute.

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SAŽETAK

Priprava 6-amino-6-deoksi derivata askorbinske i izo-askorbinske kiseline

B. Šušković

Pripravljeni su 6-amino-6-deoksi derivati askorbinske i izo-askorbinske kiseline serijom nukleofilnih supstitucija na C_6-atomu. U redu izo-askorbinske kiseline dušik je uveden u položaj C_6 preko pretpostavljenog 5,6-epoksi derivata izo-askorbinske kiseline.