NMR SPECTROSCOPIC STUDY OF PRODUCTS OF ASCORBIGEN REACTION IN ACID MEDIUM

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1D and 2D NMR spectroscopy was used to differentiate between 2′-[(indol-3″-yl)methyl] ascorbigen, 2′-[2″-[(indol-3″-yl)methylindol-3″-yl]methyl]ascorbigen and their isomers as the products formed when ascorbigen reacts in acid medium. Signals in the ^1H and ^{13}C NMR spectra of these compounds were fully assigned.

In a previous report it was shown that in acid medium ascorbigen, namely 2-C-[indol-3-yl)methyl]-α-L-threo-D-glycero-3-hexulofuranosono-1,4-lactone (I), a substance found in cabbage and other Cruciferae, liberates L-ascorbic acid to afford compounds in which two or three indolylmethyl groups are due to one ascorbic acid fragment [1]. It was concluded from ^1H NMR, mass spectroscopy, and high-performance liquid chromatography data that 2′-[(indol-3″-yl)methyl] ascorbigen (IIa) and 2′-[2″-[indol-3″-yl)methylindol-3″-ene]methyl] ascorbigen (IIIa) were formed in acid medium by means of successive addition of the (indol-3-yl)methyl group to the ascorbigen (I) molecule [1].

It is well known, however, that electrophilic substitution reactions in 3-alkylinodoles proceed via the formation of intermediate 3,3-disubstituted indolenines with subsequent migration of one of the substituents at position 2 (acid-catalyzed Planche rearrangement) [2]. In the case of ascorbigen I the Planche rearrangement should proceed via the intermediate cations IV, V or VI. The possibility cannot be excluded, therefore, that instead of compounds IIa and IIIa isomeric structures IIb and IIIb-d, whose mass spectra and ^1H NMR spectra will be of a similar type, may be formed. It is most unlikely that type IIc isomers, which have an end 2-substituted indole will result, since in the PMR spectra of ascorbigen acidic reaction products the proton signal from the five-membered indol ring is observed in low field [1] (see scheme on the following page).

The aim of the current work is to analyze in detail the ^1H and ^{13}C NMR spectra of the products of ascorbigen reaction in acid medium and to establish the structure of these compounds.

Ascorbigen I. Initially, a full assignment was made of signals in the ^1H and ^{13}C NMR spectra of the starting compound, namely ascorbigen I (Tables 1 and 2).

Proton signals of the ascorbic acid radical corresponded to previously published data [3]. Some ambiguity arose in assigning the 4′-H and 7′-H proton signals in the spectrum of the indole part of ascorbigen I. An approach adopted in an earlier communication [4] was used to resolve this problem: using the high-field shift of the $C_{(7')}$ carbon atom (111-116 ppm), the position of the 7′-H signal was determined from the heteronuclear $^1H-^{13}C$ chemical shift-correlation spectrum (Fig. 1); then the remaining indole proton signals were assigned using $^1H-\{^1H\}$ double resonances.

In addition, the unambiguous assignment of the 4′-H and 7′-H proton signals was arrived at from the nuclear Overhauser effect (NOE) value of the indole protons for selective saturation of indolylmethyl group signals using differential spectroscopy [5]. As is clear from Fig. 2, selective saturation of indolylmethyl protons in ascorbigen I increases the strength of the 4′-H and 2′-H signals.

In order to assign the signals in the ^{13}C NMR spectrum of ascorbigen I we used the heteronuclear correlation of ^1H and ^{13}C chemical shifts (HETCOR) with direct (for protonated carbons) [6] and long-range (for quaternary carbons) [7] $^1H-^{13}C$ coupling constants (Table 2).

So, as Tables 1 and 2 indicate, the ^1H and ^{13}C chemical shifts of ascorbigen were in line with published data for 3-substituted indoles [8, 9].

Institute for Research into New Antibiotics, Russian Academy of Medical Sciences, Moscow 119867. Translated from Khimiya Geterotsiklicheskikh Soedinenii, No. 3, pp. 353-359, March, 1993. Original article submitted June 29, 1992.

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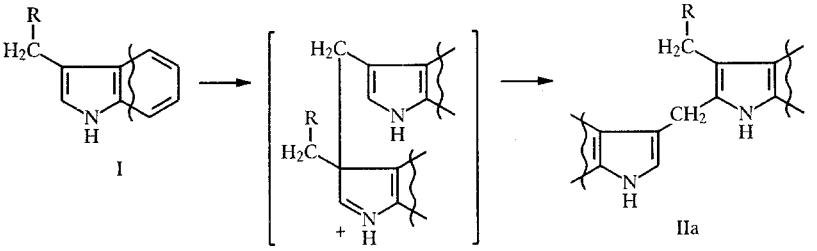
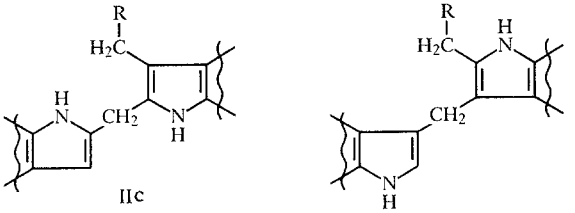
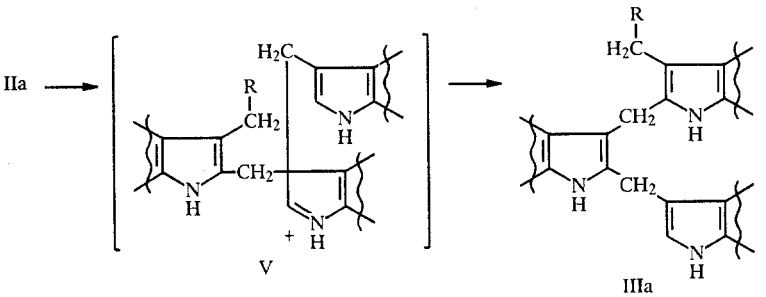


I IV IIa

or

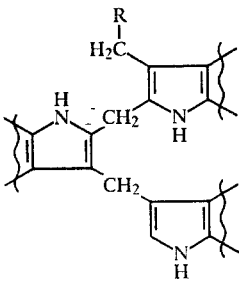


IIc IIb

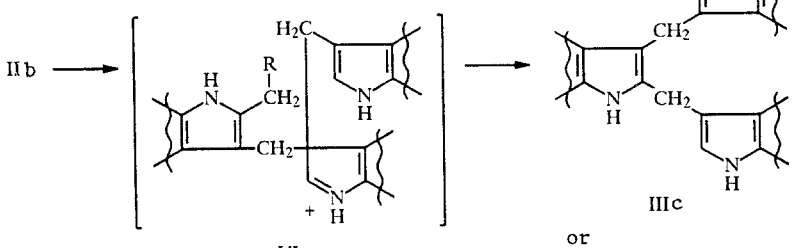


IIa V IIIa

or

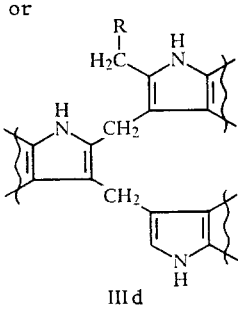


IIIb



IIb VI IIIc

or



IIId

I—IV R=

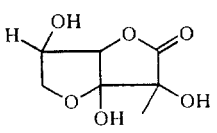


TABLE 1. ^{1}H NMR Spectra Data for the Test Compounds

Proton	I		IIa		IIIa	
	δ, ppm*	J, Hz	δ, ppm*	J, Hz	δ, ppm*	J, Hz
4-H	3,78	J_{45} = 0,7	4,18		4,16	J_{45} = 0,8
5-H	4,20	J_{56a} = 3,3; J_{56b} = 5,7	4,29	J_{56a} = 3,4; J_{56b} = 5,8	4,29	J_{56a} = 3,3; J_{56b} = 5,8
6*a*-H	3,99	J_{6a6b} = 9,7	4,04	J_{6a6b} = 9,7	4,04	J_{6a6b} = 9,7
6*b*-H	4,12		4,18		4,17	
$CH_{2'}$	3,23 3,40	J = 14,2; $J_{2'}$ = 0,7	3,38 3,41	J = 14,8	3,41	
2'-H	7,20					
4'-H	7,62	$J_{4'5'}$ = 8,0; $J_{4'6'}$ = 1,0	7,62	$J_{4'5'}$ = 7,9; $J_{4'6'}$ = 0,9	7,58	
5'-H	6,97	$J_{4'7'}$ = 1,0; $J_{5'6'}$ = 7,1	6,91	$J_{4'7'}$ = 0,9; $J_{5'6'}$ = 7,3	**	
6'-H	7,03	$J_{5'7'}$ = 1,0; $J_{6'7'}$ = 8,1	6,94	$J_{5'7'}$ = 0,9; $J_{6'7'}$ = 8,2	**	
7'-H	7,31		7,16		**	
$CH_{2''}$			4,29		4,32	
2''-H			7,00			
4''-H			7,38	$J_{4''5''}$ = 8,0; $J_{4''6''}$ = 1,0	7,22	$J_{4''5''}$ = 8,0; $J_{4''6''}$ = 1,2
5''-H			6,91	$J_{4''7''}$ = 1,0; $J_{5''6''}$ = 7,2	6,84	$J_{4''7''}$ = 0,9
6''-H			7,04	$J_{5''7''}$ = 0,9; $J_{6''7''}$ = 8,1	6,96	$J_{5''6''}$ = 7,1; $J_{5''7''}$ = 0,9
7''-H			7,31		7,24	$J_{6''7''}$ = 8,2
$CH_{2'''}$					4,21	
2'''-H					7,00	
4'''-H					7,33	$J_{4'''5'''}$ = 7,7; $J_{4'''6'''}$ = 1,1
5'''-H					6,88	$J_{4'''7'''}$ = 1,0; $J_{5'''6'''}$ = 7,1
6'''-H					7,05	$J_{5'''7'''}$ = 0,9; $J_{6'''7'''}$ = 8,1
7'''-H					7,30	

*Chemical shifts measured relative to TMS as internal standard.

**Signals were observed at 6.86-6.89 ppm and their assignment was ambiguous.

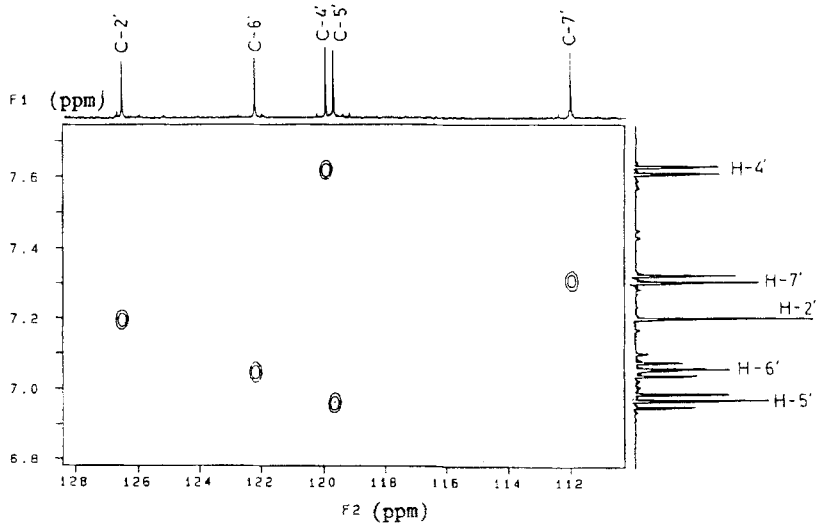


Fig. 1. Heteronuclear ^{1}H–^{13}C chemical shift-correlation spectrum of ascorbigen I (only aromatic region of the spectrum shown).

TABLE 2. ^{13}C Chemical Shifts (CD_3OD, 49.00 ppm) of Test Compounds

Carbon	I	IIa	IIIa	Carbon	IIa	IIIa
$C_{(1)}$	178,74	178,46	178,44	CH_{2}''	23,67	22,60
$C_{(2)}$	80,92	80,79	80,70	$C_{(2'')}$	124,07	137,11
$C_{(3)}$	108,68	109,01	109,00	$C_{(3'')}$	113,58	108,43
$C_{(4)}$	88,32	88,10	88,03	$C_{(4'')}$	119,58	118,98
$C_{(5)}$	75,51	75,61	75,60	$C_{(5'')}$	119,63	119,63
$C_{(6)}$	75,51	76,03	76,03	$C_{(6'')}$	122,33	121,57
CH_{2}'	31,82	31,39	31,50	$C_{(7'')}$	112,14	111,55
$C_{(2')}$	126,59	139,08	139,18	$C_{(7a'')}$	138,25	137,05
$C_{(3')}$	107,50	104,19	103,90	$C_{(3a'')}$	128,73	130,16
$C_{(4')}$	119,99	119,92	119,75	CH_{2}'''		23,48
$C_{(5')}$	119,73	119,49	119,78	$C_{(2''')}$		124,10
$C_{(6')}$	122,25	121,42	121,21	$C_{(3''')}$		113,88
$C_{(7')}$	112,04	111,38	111,36	$C_{(4''')}$		119,55
$C_{(7a')}$	137,53	136,99	136,64	$C_{(5''')}$		119,44
$C_{(3a')}$	129,09	130,55	130,48	$C_{(6''')}$		122,42
				$C_{(7''')}$		112,26
				$C_{(7a''')}$		138,23
				$C_{(3a''')}$		128,39

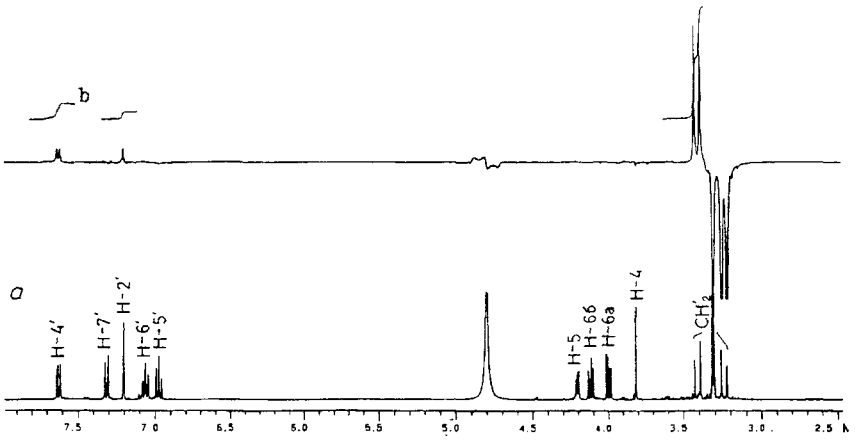


Fig. 2. NOE measurement for aromatic protons in ascorbigen I: a) ^1H NMR spectrum of ascorbigen I; b) the differential spectrum obtained by deducting spectrum *a* from the spectrum recorded for selective saturation of the $CH_{2'}$ group proton.

Compound IIa. In the ^1H and ^{13}C NMR spectra of compound IIa (Tables 1 and 2) signals were observed for an ascorbic acid radical, two methylene groups and two indole rings. The spectra of the ascorbic acid radical were fully in line with the data obtained for ascorbigen I.

The approaches described above for ascorbigen I were used to interpret signals in the ^1H and ^{13}C NMR spectra of the indole rings. Protons were assigned for each of the indole rings from the heteronuclear $^1H-^{13}C$ chemical shift-correlation spectrum and the proton homonuclear chemical shift-correlation spectrum (COSY) [10]. In view of the fact that low-intensity crossspeaks were observed between the 2″-H (7.00 ppm) signal and the methylene group signal at 4.29 ppm in the COSY spectrum, the latter could be unambiguously assigned to the $CH_{2''}$ group.

To determine the arrangement of indole rings in the IIa molecule and to assign the quaternary carbon signals we recorded the long-range heteronuclear $^1H-^{13}C$ chemical shift-correlation spectrum (Fig. 3). As can be seen from the spectrum, the $CH_{2'}$ group protons correlated with $C_{(3a')}$ (130.55 ppm), while the $CH_{2''}$ protons correlated with $C_{(3a'')}$ (128.73 ppm). From this data the compound IIa structure can be corroborated unambiguously and isomeric structure IIb can be discounted.

Compound IIIa. Signals were displayed in the ^1H and ^{13}C NMR spectra of compound IIIa (Tables 1 and 2) for an ascorbic acid radical, three methylene groups, and three indole rings. The position of the resonance signals of the ascorbic acid group protons and carbons was in line with data for compounds I and IIa.

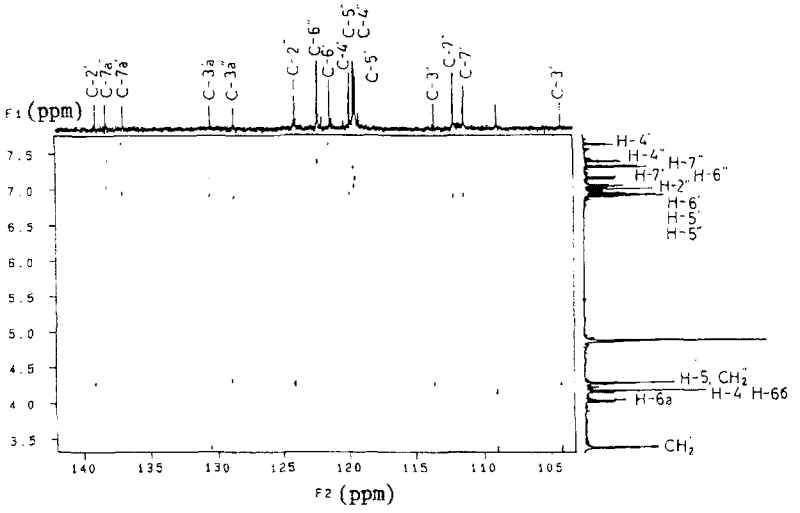
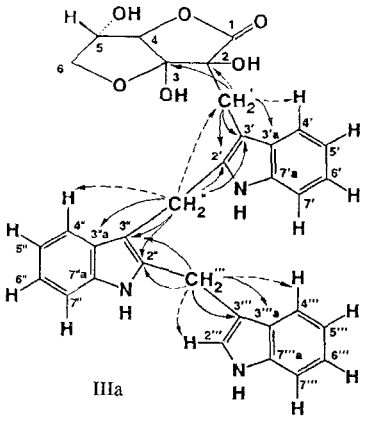


Fig. 3. Long-range heteronuclear $^{1}H-^{13}C$ chemical shift-correlation spectrum ($^{n}J_{C-H}$ = 8.0 Hz) of compound IIa (only aromatic region of spectrum shown along ^{13}C chemical shift axis).



IIIa

Fig. 4. Experimental results of measuring NOE and long-range heteronuclear $^{1}H-^{13}C$ chemical shift correlation in compound IIIa (broken line and arrow depict protons between which NOE was observed; continuous line and arrow depict presence of crosspeaks between ^{1}H and ^{13}C in the heteronuclear correlation spectrum).

In the COSY spectrum of compound IIa low-intensity crossspeaks were observed between the protons at 7.00 ppm (2‴-H) and one of the methylene groups at 4.21 ppm ($CH_{2''}$). The remaining methylene group signals were assigned using NOE measurement experiments (results shown in Fig. 4). Selective irradiation of each of the methylene groups increased the strength of the signals in the aromatic region of the spectrum (4′-H, 4″-H, 4‴-H, respectively); this enabled structures IIIb-d to be discounted. Having thus established the position of the 4′-H, 4″-H, and 4‴-H signals, the COSY spectrum of compound IIIa could be used to make unambiguous assignments for the 5″-H, 5‴-H, 6″-H, 6‴-H, 7″-H, and 7‴-H signals (Table 1). The 5′-H, 6′-H, and 7′-H proton signals were observed in the narrow spectral region between 6.86 and 6.89 ppm and their assignment was ambiguous.

Using the heteronuclear $^{1}H-^{13}C$ chemical shift-correlation spectrum it was possible to assign the indole ring CH signals with the exception of $C_{(5')}$ and $C_{(6')}$. The position of these signals was determined using the selective polarization transfer of the 4′-H proton via long-range $^{1}H-^{13}C$ coupling constants [11].

Experiments on the long-range heteronuclear $^1H-^{13}C$ chemical shift correlation (Fig. 4) were used to assign the quaternary carbon atom signals. As the correlation peak of different methylene group protons corresponded to the $C_{(3a)}$ carbon of each indole ring, this served as additional confirmation of structure IIIa. The position of the $C_{(2')}$, $C_{(2'')}$, $C_{(7a')}$, $C_{(7a'')}$, and $C_{(7a''')}$ signals was established using the selective polarization transfer of the $CH_{2'}$, $CH_{2''}$, 4′-H, 6″-H, and 6‴-H protons via long-range $^1H-^{13}C$ coupling constants.

The absence of compounds IIb and IIIb-d from the products of ascorbigen reaction in acid medium is in agreement with the notion that, like the benzyl radical, the (indol-3-yl)methyl group is more inclined to migrate than the alkyl substituent in intermediate IV. In the case of the intermediate indolenine derivative V the less bulky and sterically hindered [(indol-3)-yl]methyl radical, which is not linked to the ascorbic acid moiety, is the one that migrates. At the same time the possibility cannot be excluded that the indole ring is directly alkylated at position 2 in compounds I and IIa with subsequent formation of compounds IIa and IIIa, respectively.

EXPERIMENTAL

Ascorbigen I was synthesized using the technique described in [12], and compounds IIa and IIIa were obtained using the method outlined in [1].

^1H and ^{13}C NMR spectra were recorded on a Varian VXR-400 spectrometer under pulse conditions using quadrature detection at an operating frequency of 400 and 100.6 MHz for ^1H and ^{13}C, respectively. Spectra were taken using solutions of the test compounds in methanol-D_4; ^1H chemical shifts were measured relative to internal standard TMS. ^{13}C chemical shifts are given relative to the solvent signal (49.00 ppm).

The following experimental conditions applied in registering ^1H NMR spectra: pulse deflection angle 70°, 90°-pulse duration 30.5 μsec, data access time 3.5 sec, delay between pulses 5 sec. In recording ^{13}C NMR spectra for complete quenching of spin–spin coupling with protons the observation pulse detection angle was 45°, the 90°-pulse duration was 17.4 μsec, the data access time was 1.1 sec, and the delay between pulses was 2 sec.

2D proton homonuclear chemical shift-correlation and heteronuclear $^1H-^{13}C$ chemical shift-correlation spectra were recorded using the COSY [10] and HETCOR [6, 7] pulse sequences, which form part of the spectrometer program. Nuclear Overhauser effects were measured using the NOEDIF pulse sequence [5] on non-degassed samples. ^{13}C NMR spectra with selective proton polarization transfer via long-range $^1H-^{13}C$ coupling constants were recorded using the INEPTL pulse sequence [11].

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