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A NEW ROLE FOR L-ASCORBIC ACID: MICHAEL DONOR TO α,β-UNSATURATED CARBONYL COMPOUNDS[1]

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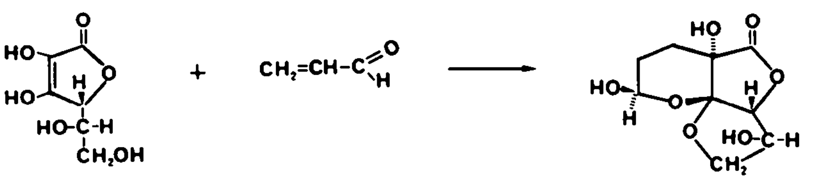
Abstract - In a novel Michael-type reaction L-ascorbic acid (1) undergoes addition to acrolein to give the tricyclic hemiacetal lactone 3. The constitution and relative configuration of 3 was studied by a combination of NMR and IR spectroscopy. Ultimately, the structure of 3 was determined by X-ray crystallography. The absolute stereochemistry follows from the known chirality of C-4 and C-5 of L-ascorbic acid. A mechanism for the reaction, including its steric course, is proposed. Methyl vinyl ketone reacts with 1 in a similar fashion to give diketo lactone derivative 4. Upon the action of methanolic hydrogen chloride on 4 one anomer, the tricyclic methyl ketal lactone 5, forms. Structure 5 is closely related to 3. Synthetic and possible biological applications of the new reaction are discussed.

Recently one of us and his associates found that L-ascorbic acid (1) undergoes enediol acetal formation with 1,2-dicarbonyl compounds like methylglyoxal and their vinylogues *e.g.*, 4-keto-2-pentenal.[2-4]

In contrast, we now observed that acrolein reacts with ascorbic acid in water to give a crystalline product that shows no olefinic protons and carbons, respectively, in the ^1H and ^{13}C NMR spectrum. A single carbonyl band appears at 1780 cm^{-1} in the IR and a signal at 175 ppm in the ^{13}C NMR spectrum. These values suggest the presence of a saturated lactone. The elemental analysis of the primary product (m.p. 114-116°) corresponds to the monohydrate of a 1:1 adduct between acrolein and 1. However, the adduct can be dehydrated to product 3 (m.p. 150-152°) *via* the ternary azeotrope with ethanol and benzene. Elemental analysis confirms that 3 is the 1:1 adduct.

Both 3 and its monohydrate show identical ^{13}C NMR spectra in DMSO-d_6 (Fig. 3). A noticeable feature is that most of the major signals appear as narrowly spaced "doublets" in the proton decoupled spectrum. This



1 3

points to the presence of two closely related isomers in solution. Indeed, mutarotation of 3 can be observed in DMSO (Fig. 4) which is indicative of an equilibrium between isomers of 3 in solution. These findings are consistent with a cyclic hemiacetal moiety that arises from the aldehyde group of acrolein.

Single crystals of 3 were obtained by recrystallization from methanol-ether, thus a structure determination by X-ray crystallography became feasible. Details of this study are described in the following paragraph.

X-Ray Crystallography. The compound 3 crystallizes in the monoclinic space group $P2_1$ with a = 6.293(3) A, b = 7.526(5) A, c = 10.318(4) A, β = 101.8(1)° and Z = 2. 802 independent reflections were collected on a NICOLET P3F automatic X-ray diffractometer using CuKα radiation with a graphite monochromator on the incident beam. The structure was solved using the symbolic addition procedure[5] for non-centrosymmetric crystals. The coordinates and thermal factors for each C and O atom were refined by full-matrix least-squares methods using program[6] ORFXLS3. The function minimized by the least-squares procedure was $\Sigma w(|F_o|-|F_c|)^2$, where the weights, w (derived from estimated standard deviations of observed intensity) were calculated according to Gilardi[7]. All 802 data were used in the refinement. Hydrogen atoms were located in a difference map calculated after several cycles of refinement on just the C and O atoms and their positional parameters were then also refined. The hydrogens on the hydroxyl oxygens did not refine well in that the resultant O-H distances and COH angles were unreasonable.

A subsequent difference map showed good positions for the hydrogens on O-5 and O-9 and indicated a disordered hydrogen on O-12. Structure factors calculated using these hydrogen parameters give final R factors, where $R = \frac{\Sigma||F_o|-|F_c||}{\Sigma|F_o|}$ and $R_w = \left[\frac{\Sigma w(|F_o|-|F_c|)^2}{wF_o^2}\right]^{1/2}$, of 3.24% for R and 3.94% for R_w. Table 1 lists the final refined coordinates and B_{eq} values for the non-hydrogen atoms, the refined coordinates for the hydrogens bonded to carbon atoms, and the difference map coordinates for the hydroxyl hydrogens. Anisotropic thermal parameters for the non-hydrogen atoms and a comparison between observed and calculated structure factors are available from the authors.

The X-ray analysis has elucidated the structural formula of the addition product to be that shown in the stereodiagram in Fig. 1, drawn by computer from the experimentally determined coordinates.[8] The molecule has three fused rings, one 6-membered ring and two 5-membered rings. This is consistent with the structure of the cyclic hemiacetal 3,6-ketal of 2-(3-oxopropyl)-3-oxo-L-gulonolactone (3). The relative configurations at the five chiral centers were established by the X-ray analysis. The absolute configuration was deduced by using the known chirality of atoms C-4 and C-5 of ascorbic acid as a reference to the new chiral centers. We choose the rational name of 1,3,7-trioxa-8-oxo-(5S,9S,12R)-trihydroxy-(2R,6R)-tricyclo-$[4.3.2.0^{2,6}.0^{2,9}]$ dodecane. This numbering was used throughout the text. However, upon our request Chemical Abstracts suggested the following name: (3S,3aR,5aS,6R)-hexahydro-3,5a,8-trihydroxy-2H,5H-furo-[3',2':2,3]furo-[3,4-b]pyran-5-one.

Bond distances and angles which are illustrated in Fig. 2 fall within expected values. The angles around C-2, C-6 and C-9 indicate that the fused ring system was able to form without significant strain. The 6-membered ring has a somewhat flattened chair conformation. The ring fusion at the 5-membered and 6-membered ring junction is *cis* (the O-3, C-2, C-9, O-9 torsion angle is 34.1(5)°). Both 5-membered rings are in an envelope conformation and are twisted about the C-2, C-6 bond such that these atoms from the "flaps"; C-6 is out of the plane formed by C-2, O-3, C-4 and C-5 in one of the rings and C-2 is out of the plane formed by C-6, C-7, C-8 and C-9 in the other five-membered ring. The ring junction between the two 5-membered rings is also *cis* (the H-6, C-6, C-2, O-1 torsion angle is -31.2(5)°). Crystal packing is influenced by the presence of 3 intermolecular hydrogen bonds. O-5 is a donor

Table 1. Fractional Coordinates and Equivalent Isotropic Thermal Parameters with e.s.d.'s in Parentheses.

Atom	x	y	z	B_{eq}
O(1)	0.3529(4)	0.5957(7)	0.1958(2)	2.5(1)
C(2)	0.2379(5)	0.7594	0.1806(3)	2.3(1)
O(3)	0.1204(4)	0.7605(7)	0.0505(2)	2.9(1)
C(4)	0.2057(6)	0.8843(9)	-0.0302(4)	3.5(2)
C(5)	0.4037(7)	0.9697(8)	0.0552(4)	2.9(1)
O(5)	0.6029(4)	0.8954(7)	0.0337(3)	3.1(1)
C(6)	0.3816(6)	0.9252(7)	0.1955(4)	2.8(1)
O(7)	0.2476(4)	1.0638(6)	0.2375(3)	3.3(2)
C(8)	0.0714(7)	0.9907(9)	0.2762(4)	3.0(1)
O(8)	-0.0665(5)	1.0844(7)	0.3054(3)	3.9(2)
C(9)	0.0834(5)	0.7917(8)	0.2762(4)	2.6(1)
O(9)	-0.1240(4)	0.7134(7)	0.2319(3)	2.9(1)
C(10)	0.1752(7)	0.7223(9)	0.4137(4)	3.1(1)
C(11)	0.2473(7)	0.5295(8)	0.4059(4)	3.3(2)
C(12)	0.4265(6)	0.5204(8)	0.3256(4)	3.2(1)
O(12)	0.6042(4)	0.6154(8)	0.3970(3)	3.9(2)

$$B_{eq} = \frac{4}{3} \sum_i \sum_j \beta_{ij}\, \bar{a}_i \cdot \bar{a}_j$$

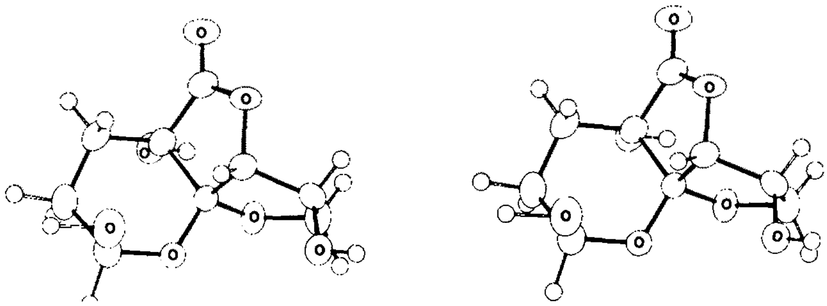


Fig. 1. Stereodiagram of the structural formula and stereoconfiguration of 3 as determined by X-ray diffraction.

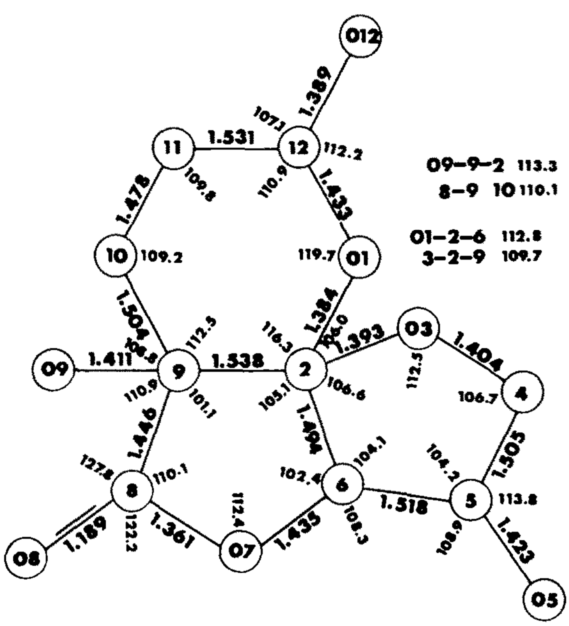


Fig. 2. Bond lengths (e.s.d. 0.003 Å) and bond angles (e.s.d. 0.5°) in 3.

Table 2. Fractional Coordinates for H Atoms with e.s.d.'s in Parentheses.

Atom	x	y	z
H(4)	0.248(9)	0.811(8)	-0.113(6)
H(4)	0.082(8)	0.979(8)	-0.063(6)
H(5)	0.410(8)	1.103(9)	0.053(5)
H(6)	0.531(8)	0.907(9)	0.261(5)
H(10)	0.302(9)	0.801(9)	0.452(5)
H(10)	0.065(7)	0.736(9)	0.471(5)
H(11)	0.120(8)	0.450(9)	0.373(5)
H(11)	0.286(8)	0.478(8)	0.506(6)
H(12)	0.479(8)	0.377(9)	0.306(5)
H(O5)	.635	.945	-.023
H(O9)	-.174	.754	.159
H(O12)	.682	.616	.325
	.664	.591	.478

to the ring oxygen O-1 with the H•••O distance at 2.10 A, the O•••O distance at 2.84 A an the O-H•••O angle at 166.7°. O-9 is a donor to O-5 with an H•••O distance of 1.99 A, and O•••O distance of 2.72 A and an O-H•••O angle of 151.4°. The hydrogen on O-12 appears to be disordered between two positions. In one of its disordered positions it does not form any hydrogen bonds. In the other position it is a donor to a hydrogen bond to O-9 with an H•••O distance of 1.84 A, an O•••O distance of 2.74 A and an O-H•••O angle of 153.0°. The only other intermolecular approach less than a van der Waals' separation is between C-2 and O-5 at 3.14 A.

DISCUSSION

After the structure of the tricyclic lactone 3 was unequivocally determined we were able to confirm and complete the interpretation of its spectra.

All major signals in the ^{13}C NMR spectrum have been assigned to the two anomers of 3. The minor peaks can be attributed to a small amount of bicyclic lactone free aldehyde which is also formed in solution (Fig. 3). The fact that 3 and its monohydrate have identical NMR spectra in DMSO-d_6 indicates that the water molecule is loosely bound to 3, since the DMSO-water interactions are stronger than the intermolecular association of water and compound 3.

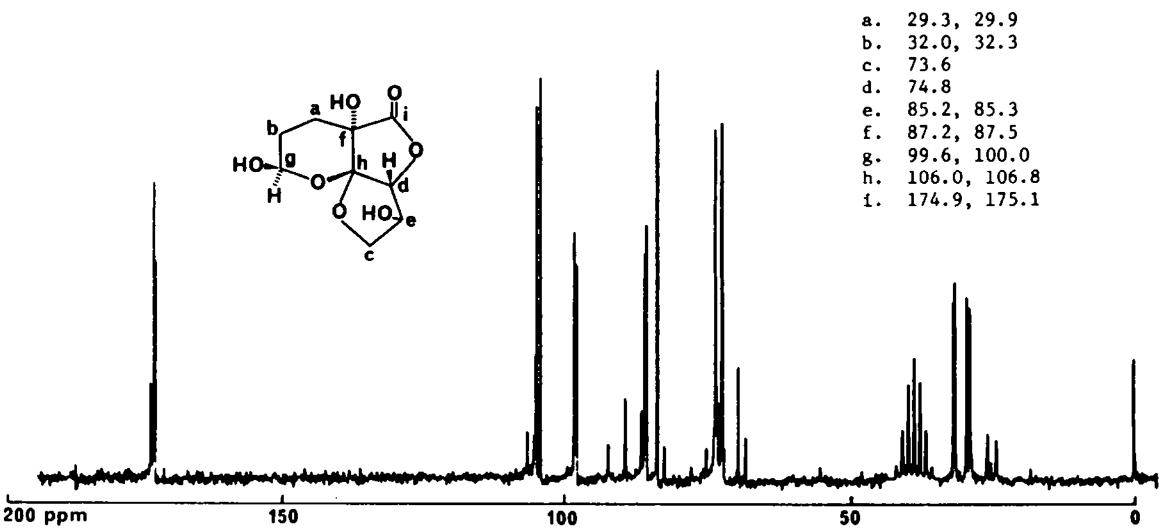


Fig. 3. Proton decoupled ^{13}C NMR spectrum of 3 in DMSO-d_6.

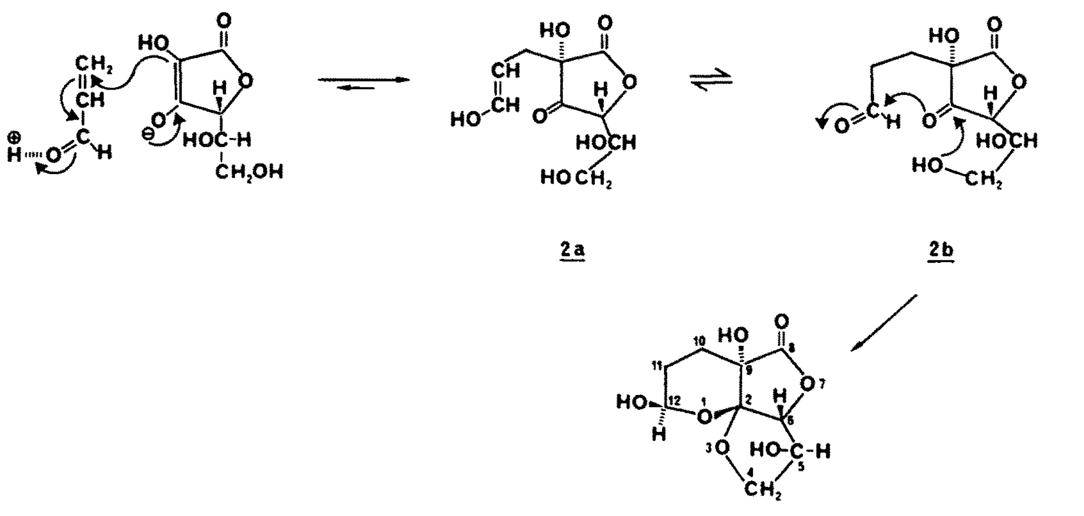
The ^{13}C NMR spectrum of the monohydrate in D_2O shows the same pattern with "doublet" signals. There are only two significant ($\geq$ 2 ppm) downfield shifts: for C-5 and the lactone carbonyl resonance. This allows the conclusion that the C-5 hydroxyl is involved as a donor in hydrogen bonding to the hydrate molecule.

The ^1H NMR spectra of 3 and its monohydrate in DMSO-d_6 are identical except for the size of the water peak. The C-6 proton appears as a sharp singlet at δ 4.5. Due to the rigid geometry of the two fused five-membered rings coupling to the C-5 proton is negligible. The C-12 hemiacetal proton appears as a broadened singlet at δ 5.6. This peak partly overlaps with a hydroxyl proton resonance.

The mutarotation of 3 cannot be observed in methanol. We assume that the equilibrium is reached rapidly and thereby escapes detection. In DMSO, however, the change of rotation can be followed (Fig. 4). The measurements were taken at 365 nm, because the rotation is larger at shorter wavelengths. Time zero refers to the time the solvent is added to 3. Since no measurement is possible before the sample is completely dissolved, the optical rotation of pure 3 at t = 0 cannot be determined experimentally. The initial reading (t = 4.5) is negative but changes rapidly towards positive values. The negative rotation could be due to a transient levorotatory species - maybe the free aldehyde - which forms in the process of establishing the anomeric equilibrium.

Scheme 1 outlines a possible reaction mechanism for the formation of 3. L-ascorbate-3-anion can be regarded as an ambident nucleophile with two potentially reactive sites: O-3 and C-2. In our case the π-electron density around C-2 is sufficiently increased to make C-2 the nucleophile which can attack the conjugated double bond of acrolein leading to the keto aldehyde 2b (*via* 2a). This is essentially a Michael reaction wherein ascorbic acid acts as the donor. A concerted nucleophilic attack of O-6 upon C-3 and O-3 upon the aldehyde carbon of 2b should lead to the tricyclic product 3. Concerning the steric course of the reaction one can infer from the configuration of the product that acrolein approaches C-2 of ascorbic acid from the side opposite to C-5 and C-6. Hence the addition is stereoselective. The ultimate ring closure involving C-3 and O-6 of ascorbic acid leads to the thermodynamically favored *cis*-junction of the two five-membered rings. Furthermore, in the crystal lattice, the hemiacetal hydroxyl prefers an equatorial position away from the ketofuranose ring.

It is noteworthy that the Michael addition



2a

2b

3

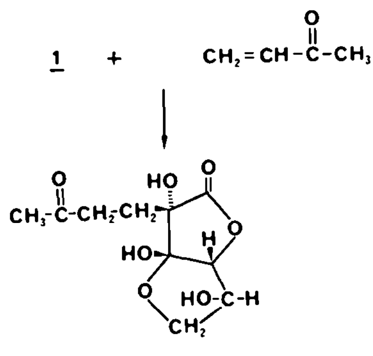
Scheme 1

step takes place at pH ~ 4 of ascorbic acid. No basic catalyst is necessary. Since water was used as a solvent, the dissociation of 1 provides sufficient ascorbate-3-anions to act as "starters". As the reaction progresses, the various equilibria are shifted towards more dissociation and formation of the stable tricyclic product 3.

We could not find any example in the literature where ascorbic acid plays the role of a Michael donor. There are, however, two somewhat analogous reactions. The formation of ascorbigen: L-ascorbic acid was alkylated[9] at C-2 with 3-(hydroxymethyl)indol in aqueous solution at pH 4. Earlier Jackson *et al.*[10] had reported on the C-2 benzylation of L-ascorbic acid. In both examples a subsequent cyclization between O-6 and C-3 to form a hemiketal was observed. A similar bicyclic lactone intermediate was described in a recent paper on the synthesis of L-ascorbic acid 2-O-phosphate.[11] The crystalline dimer of dehydro-L-ascorbic acid, a pentacyclic structure, also contains keto-furanose rings.[12] These examples underscore the tendency towards hemiketal formation between O-6 and a carbonyl group on C-3.

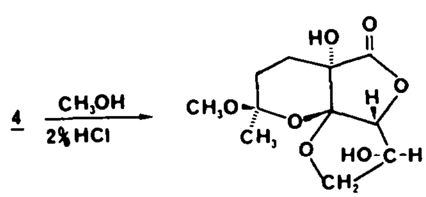
Methyl vinyl ketone as acceptor. Once the reaction with acrolein was clarified, we proceeded to investigate the scope of this new reaction with ascorbic acid. Methyl vinyl ketone was chosen to serve as Michael acceptor.

A crystalline compound could be isolated that perfectly analyzed for $C_{10}H_{14}O_7$ which corresponds to the 1:1 adduct of ascorbic acid and the α,β-unsaturated ketone. In principle, either the 2-(3-oxobutyl)-3-oxo-L-gulonolactone, or its cyclic hemiketal 4 could have been formed. There are two carbonyl bands in the IR spectrum (1700 cm^{-1}, 1760 cm^{-1}). The ^{13}C NMR spectrum in D_2O (Fig. 5) shows only one ketone carbonyl resonance at 214.7 ppm in addition to the lactone carbonyl at 177.9 ppm. This is consistent with structure 4. A hemiketal carbon is indicated by the signal at 108.3 ppm. In the ^1H NMR spectrum (DMSO-d_6) the sharp singlet at δ 2.1 belongs to the methyl protons adjacent to a keto function; the singlet at δ 4.4 can be ascribed to the C-4 proton. Two hydroxyl proton resonances at δ 5.6 and 6.8 disappear completely upon exchange with D_2O. The preference for cyclization between O-6 and C-3 of 1 is confirmed by the exclusive formation of product 4. The reaction takes place in aqueous ascorbic acid solution at pH ~ 4, or in a phosphate buffer at pH 7.4.



4

Glycosidation (2% HCl in methanol) of 4 affords a crystalline product which lacks the carbonyl absorption at 1700 cm^{-1} in the IR spectrum. In the ^{13}C NMR spectrum (Fig. 6) there is no signal for a keto carbonyl but the resonance at 173.8 ppm indicates the lactone carbonyl. Two ketal carbons (112.8 ppm and 114.8 ppm) are present instead. The ^1H NMR spectrum shows a sharp singlet at δ 3.7 corresponding to a methoxyl group; another sharp methyl peak appears at δ 1.4. The spectra and the correct elemental analysis for $C_{11}H_{16}O_7$ are in perfect agreement with structure 5, the tricyclic methyl ketal lactone.



5

Structure 5 is closely related to compound 3. We are basing our configurational assignments on analogies with 3. The configuration at the methyl ketal carbon was deduced from

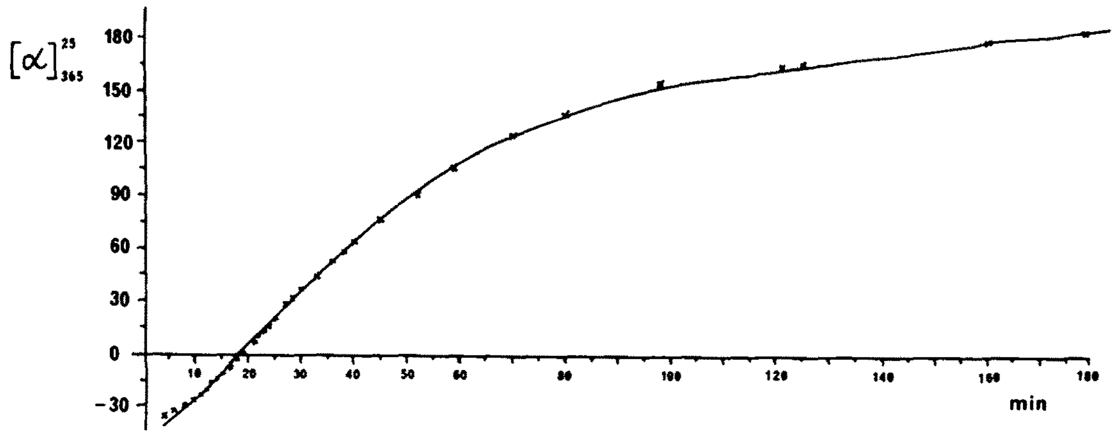


Fig. 4. Mutarotation of 3 in DMSO (c, 1.5).

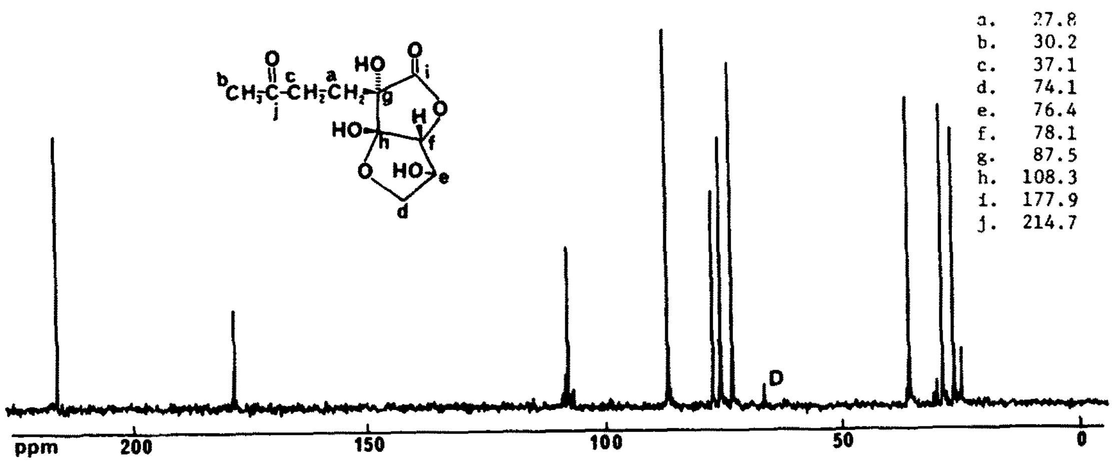


Fig. 5. Proton decoupled ^{13}C NMR spectrum of 4 in D_2O (D:p-dioxane reference).

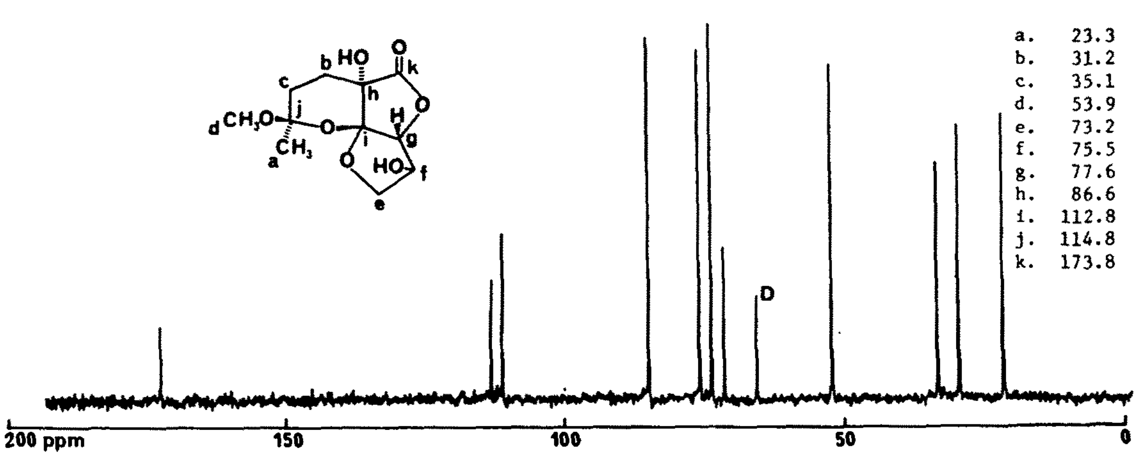


Fig. 6. Proton decoupled ^{13}C NMR spectrum of 5 in D_2O (D:p-dioxane reference).

Dreiding models and especially space filling Catalin models of the two possible anomers of 5. The methoxyl group in β-position is much preferred sterically. In the tetrahydropyran ring assuming a twist-boat conformation the β-methoxy group is placed in equatorial position. The ^{13}C NMR spectrum of the crude material clearly shows that 5 is the major product contaminated by some unreacted 4.

Similar attempts to convert 3 into a methyl glycoside resulted in a syrupy mixture of products which shows three distinct peaks in the methoxy region (δ 3.4-3.6) of the ^{1}H NMR spectrum. After partial chromatographic separation spectral data of the individual fractions suggest that two anomeric C-12 methyl glycosides were formed together with a dimethyl acetal-methyl ketal derived from the aldehyde form of 3. The separation and purification of these products is in progress.

Conclusions. This is the first report on L-ascorbic acid as a novel powerful Michael carbanion donor towards two reactive α,β-unsaturated carbonyl compounds. Further studies are already under way to evaluate the scope of this reaction. We are interested in using biologically significant α,β-unsaturated aldehydes like 4-hydroxypentenal as acceptors. Assuming that the Michael addition is reversible *in vivo* an adduct with 4-hydroxypentenal could have cancerostatic activity.[13] Unsaturated nitriles and esters, and also quinones, shall be investigated as potential Michael acceptors.

The fact that the reaction with acrolein takes place under almost physiological conditions suggests that ascorbic acid might be useful as a detoxifying agent *in vivo*. Acrolein is a metabolite of the widely used anticancer agent cyclophosphamide. Protection against some toxic side-effects which have been linked to acrolein was achieved by co-administration of sodium 2-mercaptoethane sulfonate[14] or N-acetylcysteine.[15] In both cases a free sulfhydryl group is believed to react with the double bond of acrolein to form a non-toxic addition compound. Since 3 proved to be a non-toxic substance[16], a similar protection might be possible by co-administration of cyclophosphamide and L-ascorbic acid.

In addition, compound 4 in the diketo form appears to be set up for a Robinson annellation leading to a cyclohexenone derivative with a chiral tertiary hydroxyl. The side chain or the lactone ring of such a potential structure could be transformed into other functionalities. We shall try to explore the use of 4 as a new chiral intermediate for syntheses.

EXPERIMENTAL

Melting points were determined on an Electrothermal melting point apparatus and are uncorrected. IR spectra were recorded on a Beckman IR 10 spectrophotometer. A Perkin-Elmer Model 141 M Polarimeter was used for the optical rotation measurements. ^{13}C NMR spectra were taken on a Varian CFT-20 spectrometer at 20 MHz. The ^{1}H NMR spectra were recorded on a Varian EM-360 (60 MHz) spectrometer and on a Varian CFT-20 spectrometer at 80 MHz. Elemental analysis were performed by Galbraith Laboratories, Inc., Knoxville, Tennessee. L-(+)-ascorbic acid, acrolein (97%) and methyl vinyl ketone (technical grade) were purchased from Aldrich and used without further purification. A standard solution of Tillmans' reagent (TR) *i.e.*, 2,6-dichloroindophenol sodium salt,[17] was used for the titration of 1.

Monohydrate of 3. To a stirred solution of 20 g (0.11 mol) ascorbic acid (1) in 100 ml H_2O under a N_2 atmosphere 5.8 g (0.10 mol) acrolein was added dropwise. Throughout and after the addition the temperature was not allowed to rise above 25°. A white precipitate usually formed within two hours. If not, crystallization was induced by seeding. The suspension was stirred at room temperature until titration (with TR solution) of aliquots taken from the supernatant liquid indicated a quantitative consumption of 1. After being kept in the refrigerator overnight the solid was filtered, washed and dried. 13 g monohydrate of 3, m.p. 111-113° was obtained. Recrystallization from 95% ethanol gave transparent prisms, m.p. 114-116°. The supernatant liquid was freeze-dried and the residual solid recrystallized from 95% ethanol to afford an additional crop (5.1 g) of the monohydrate. Total yield: 18.1 g, 72%. Anal. Calcd. for $C_9H_{12}O_7 \cdot H_2O$: C, 43.24; H, 5.60; O, 51.16. Found: C, 43.37; H, 5.71; O, 51.03.

1,3,7-Trioxa-8-oxo-(5S,9S,12R)-trihydroxy-(2R,6R)-tricyclo-[4.3.2.0^{2,6}.0^{2,9}]-dodecane (3). 10 g (0.04 mol) monohydrate of 3 was dissolved in 140 ml hot absolute ethanol, 50 ml dry benzene was added and the mixture was distilled. After removal of the ternary and binary azeotrope the residual dry ethanol solution was concentrated to about 60 ml. Compound 3 crystallized as white needles, m.p. 150-152°. Yield: 7g, 75%. IR (KBr), 3580-3100 (broad, ν_{OH}), 2960 and 1780 cm^{-1}. ^{1}H NMR (DMSO-d_6, 80 MHz) δ 1.7-2.4 (m, 4H), 3.8-4.3 (m, 3H), 4.45 (s, 1H), 5.58 (br s, 2H, partially disappears upon addition of D_2O), 6.4-6.8 (~2H, D_2O exchangeable peaks). ^{13}C NMR (DMSO-d_6): see Fig. 3. $[\alpha]_D^{25}$ = +34.4° ± 0.3° (c, 1 in methanol). Mutarotation (c, 1.5 in DMSO): $[\alpha]_{365}^{23}$ = -36° → + 184° (4.5 min → 3 hrs); see Fig. 4. Anal. Calcd. for $C_9H_{12}O_7$: C, 46.55 H, 5.17 O, 48.28. Found: C, 46.34 H, 5.26 O, 48.05.

Acetalization of 3. 1.6 g (7 mmol) of 3 in 25 ml 2% methanolic hydrogen chloride was stirred at room temperature for 8 hours. The solution was neutralized with powdered silver carbonate, filtered and dried (Na_2SO_4). The methanol was removed *in vacuo*. The residue, a colorless syrup, was subjected to column chromatography on neutral alumina with benzene-ethanol (3:1) as eluent. TLC on alumina sheets (using the same eluent) indicated that two materials with R_f 0.25 and R_f 0.13 had been separated. ^1H NMR spectra of the two isolated syrupy products showed three methoxy methyls (δ 3.4, 3,5, 3.6) for one material (R_f 0.25), while the other product (Rf 0.13) had one methoxy peak at δ 3.5. So far the purification and characterization has not been completed.

3,6-Hemiketal of 2-(3-oxobutyl)-3-oxo-L-gulonolactone (4). It was found advantageous to introduce methyl vinyl ketone in the vapor phase following a method by DeBoer.[18] 20 g (0.11 mol) ascorbic acid (1) was dissolved in 100 ml H_2O. Dry nitrogen gas (flow rate ~40 ml/min) was bubbled through 8.8 g (0.12 mol) methyl vinyl ketone and then introduced into the ascorbic acid solution through a fritted disc. The temperature was kept below 25°. After about 10 hours all the ketone - except for a small residue of non-volatile material- had been transferred. Stirring of the homogeneous reaction mixture at 0° was continued until titration of aliquots indicated a constant amount (26%) of unreacted 1. Hence the conversion of 1 was 74%. Some unreacted methyl vinyl ketone had to be removed *in vacuo* before the reaction mixture was freeze-dried. The crude product was extracted with hot ethyl acetate (ratio: 10 ml per 1 g). A white crystalline solid separated from the extract. After cooling and filtration 13.7 g of product 4 were obtained. The yield was 66% based on 1 consumed. Recrystallization from ethyl acetate yielded transparent crystals, m.p. 134-136°. Direct recrystallization of the crude product from ethyl acetate gave pure 4 in a somewhat lower yield. IR (KBr) 3380, 3260, 3010, 2950, 2900, 1760 and 1700 cm^{-1}. ^1H NMR (DMSO-d_6, 80 MHz) δ 1.87 (m, 2H), 2.07 (s, 3H), 2.5 (m, 2H), 3.8-4.2 (m, 3H), 4.43 (s, 1H), 5.62 (br s, 2H), 6.79 (s, 1H). The signals at 5.62 and 6.79 disappear completely upon addition of D_2O. ^{13}C NMR (D_2O): See Fig. 5. $[\alpha]_D^{25}$ = +41° ± 0.3° (c, 1 in methanol). Anal. Calcd. for $C_{10}H_{14}O_7$: C, 48.78; H, 5.69; O, 45.53. Found: C, 48.91; H, 5.77; O, 45.32.

1,3,7-Trioxa-8-oxo-(5S,9S)-dihydroxy-12R(?)-methoxy-12R(?)-methyl-(2R,6R)-tricyclo-[4.3.2.0^{2,6}.0^{2,9}]-dodecane (5). 1.5 g (6.1 mmol) of 4 was dissolved in 25 ml anhydrous methanol containing 2% hydrogen chloride and stirred for 7 hours at room temperature. The yellow solution was neutralized with powdered silver carbonate, cooled, filtered and dried (Na_2SO_4). Removal of the solvent *in vacuo* gave a syrup which partly crystallized. The crude product was recrystallized twice from ethyl acetate to afford 0.5 g (31%) of pure 5, colorless crystals, m.p. 154-156°. IR (KBr): 3440, 3330, 2950 and 1730 cm^{-1}. ^1H NMR (DMSO-d_6, 60 MHz) δ 1.40 (s, 3H), 1.6-2.3 (m, 4H), 3.68 (s, 3H), 3.8-4.3 (m, 3H), 4.43 (s, 1H), 5.3 and 5.82 (D_2O exchangeable protons). ^{13}C NMR (D_2O) see Fig. 6. $[\alpha]_D^{25}$ = +53.7° ± 0.3° (c, 1 in methanol). Anal. Calcd. for $C_{11}H_{16}O_7$: C, 50.77; H, 6.15; O, 43.08. Found: C, 50.69; H, 6.24; O, 43.17.

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