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Preliminary communication

Conjugation of L-ascorbic acid and D-glucose

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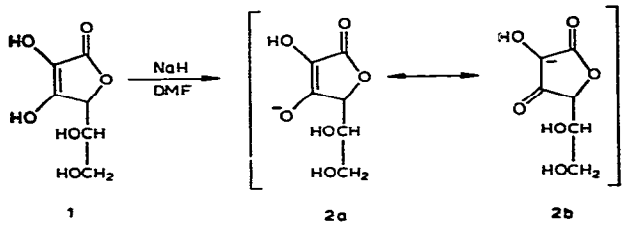
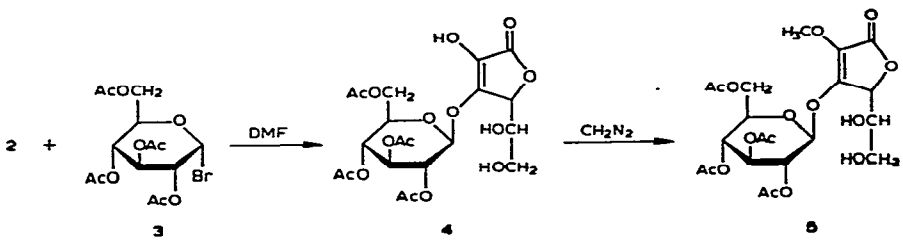
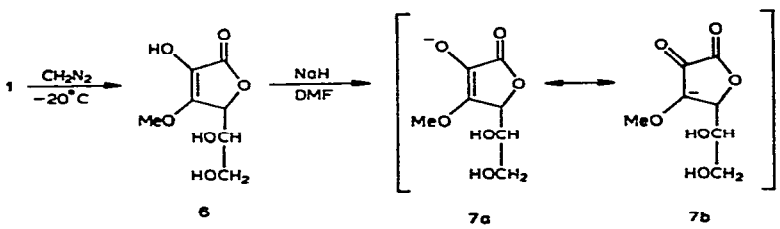
The biochemical activity of L-ascorbic acid (vitamin C) (**1**) has led to the synthesis of numerous analogs and derivatives of this compound. From a different viewpoint, **1** has been considered a useful synthetic precursor to many molecules of potential biological utility on account of its inherent, varied chemical functionality. Thus, L-threonolactone has been prepared[1] from **1**. Also, Jones *et al.*[2] have succeeded in transforming **1** into 3-hexuloses, and Brimacombe *et al.*[3] have attempted to synthesize spirolactones from **1**. As part of a program directed toward the transformation of **1** into complex carbohydrates, we report the synthesis and the proof of structure of the coupling product of **1** with 2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranosyl bromide (**3**). Another stimulus for the present study was the potential biological activity of the coupling product; indeed, **1** exists in many natural materials in the form of a more complicated compound, namely ascorbigen, which exhibits antiscorbutic activity and which releases **1** on hydrolysis. Ascorbigen was first isolated[4] pure from cabbage and savoy and synthesized[5] from **1** and 3-(hydroxymethyl)indole.

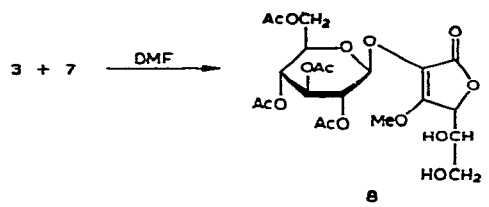
In the present work, the coupling was accomplished by treatment of **3** with L-ascorbate anion (**2**) in *N,N*-dimethylformamide at room temperature for 8 h. 3-*O*-(2,3,4,6-Tetra-*O*-acetyl-β-D-glucopyranosyl)-L-ascorbic acid (**4**) was obtained in 22% yield* by extraction and subsequent chromatography on silica gel. The major product was 2,3,4,6-tetra-*O*-acetyl-D-glucose[6] (39%), and the starting material **3** was also recovered in 10% yield. When coupling was performed by using conditions of phase-transfer catalysis (compound **3**, sodium ascorbate, tetra-*n*-butylammonium bromide, and benzene), **4** was obtained in a lower yield (16%). The ^{1}H-n.m.r. spectrum (60 MHz) of **4** in chloroform-*d* showed a one-proton doublet at τ 4.30 ($J_{1',2'}$ 7.0 Hz) attributable to the anomeric proton (H-1′). The large coupling constant is consistent with the β-glycosidic linkage. The i.r. spectrum (Nujol) indicated that **4** has an α,β-unsaturated lactone ring [1770, 1740 (overlap with acetate carbonyl groups), 1660 cm^{-1}]. The u.v. spectrum provided further evidence for the presence of a conjugated system in **4** (λ_{max}^{MeOH} 227 nm). A positive (green) ferric chloride test in methanol indicated the presence of the enolic hydroxyl group. On the basis of the foregoing data, the coupling product was assigned the structure shown by formula **4**. However, the compound could not be obtained completely pure, because decomposition occurred in

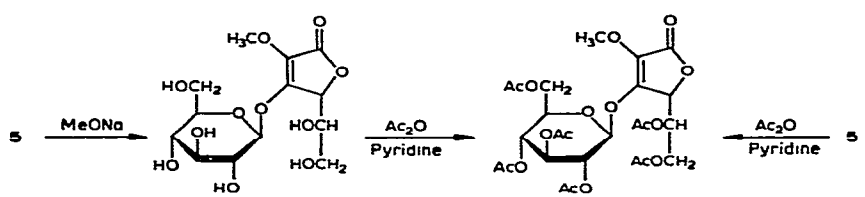
*Partial decomposition occurred during chromatography.

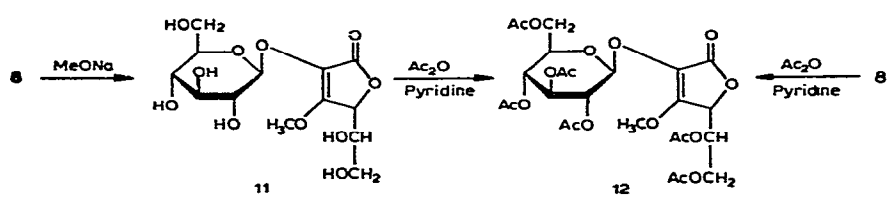












DMF = *N,N*-dimethylformamide

solution. The decomposition is presumably attributable to the autohydrolysis of the glycosidic linkage caused by the acidic proton of the hydroxyl group at C-2. This hydroxyl group could be protected by treatment of **4** with diazomethane to yield 2-*O*-methyl-3-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl)-L-ascorbic acid (**5**) almost quantitatively; m.p. 143.5–144°, $[\alpha]_D^{23}$ –10.5° (*c* 0.20, methanol). *Anal.* Calc. for $C_{21}H_{28}O_{15}$: C, 48.47; H, 5.42. Found: C, 48.21; H, 5.69. The spectral data indicated that **5** has a β-glycosidic linkage and an α,β-unsaturated lactone ring; ^{1}H-n.m.r. data: ($CDCl_3$, 100 MHz): τ 4.27 (1H, d, $J_{1',2'}$ 7.0 Hz, H-1'), 5.22 (1H, s, H-4), 6.06 (3H, s, OCH_3), 7.91, 7.93, and 7.94 (12H, $OCOCH_3$); ^{13}C-n.m.r. data: (p.p.m. downfield from Me_4Si, $CDCl_3$, 15.09 MHz): δ 170.6, 169.9 ($OCOCH_3$), 169.4 (C-1), 152.9 (C-3), 123.9 (C-2), 98.7 (C-1'), 75.9 (C-4), and 20.6 ($OCOCH_3$); ν_{max}^{KBr} 3410, 1770, 1740, and 1660 cm^{-1}; λ_{max}^{MeOH} 227 nm. Compound **5** showed a negative ferric chloride test and released formaldehyde[7] on periodate oxidation; these results indicated that the two enolic hydroxyl groups were substituted and that vicinal hydroxyl groups were present at C-5 and C-6.

Although the coupling under the present conditions was expected to occur at O-3 of **1** on the basis of the different acidities of the hydroxyl groups on C-2 and C-3, in order to prove the coupling position unambiguously, 3-*O*-methyl-2-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl)-L-ascorbic acid (**8**) was synthesized. Selective methylation of **1** with diazomethane below –20° provided 3-*O*-methyl-L-ascorbic acid[8] (**6**). Treatment of **3** with 3-*O*-methyl-L-ascorbate anion (**7**) in *N,N*-dimethylformamide at room temperature for 10 h gave **8** as an amorphous solid in 17% yield; $[\alpha]_D^{23}$ –7.5° (*c* 0.20, methanol); ^{1}H-n.m.r. data: ($CDCl_3$, 100 MHz): τ 4.67 (1H, d, $J_{1',2'}$ 6.9 Hz, H-1'), 5.26 (1H, d, $J_{4,5}$ 1.6 Hz, H-4), 5.81 (3H, s, OCH_3), 7.87, 7.92, 7.96, and 7.99 (12H, $OCOCH_3$); ^{13}C-n.m.r. data: (downfield from Me_4Si, $CDCl_3$, 15.09 MHz): δ 170.5, 170.0, 169.5 ($OCOCH_3$), 168.6 (C-1), 160.5 (C-3), 117.8 (C-2), 98.6 (C-1'), 76.0 (C-4), and 20.6 ($OCOCH_3$); ν_{max}^{KBr} 3430, 1775, 1750, and 1680 cm^{-1}; λ_{max}^{MeOH} 229 nm. *Anal.* Calc. for $C_{21}H_{28}O_{15}$: C, 48.47; H, 5.42. Found: C, 48.68; H, 5.29. Compound **8** showed also a negative ferric chloride test and released formaldehyde on periodate oxidation. The characterization of compound **8** thus corroborated the assignment of the structure of the regioisomer **5**.

As **2** and **7** have ambident nucleophilic characters, and **2a** and **2b** (ref. 9) and **7a** and **7b** (ref. 10) were proposed as resonance structures, respectively, *C*-glycosidation might also have been expected. However, *C*-glycosidation products were not detected under the reaction conditions employed in the present study, a result which presumably can be explained by effects of the medium. In general, *C*-alkylation is favored in media of high hydrogen-bonding power[11] and *O*-alkylation predominates in such polar aprotic solvents as *N,N*-dimethylformamide and dimethyl sulfoxide[12]. This effect of the medium has been observed previously[13] in benzylation of **1**.

To afford a deacetylated conjugate, compound **5** was treated with sodium methoxide at room temperature. The reaction was complete within 20 min to provide syrupy 3-*O*-(β-D-glucopyranosyl)-2-*O*-methyl-L-ascorbic acid (**9**). Decomposition occurred when the reaction time was prolonged. It is known that enol glycosides are usually sensitive to alkali[14]. The ^{1}H-n.m.r., i.r., and u.v. spectra of **9** were consistent with the assigned structure;

^{1}H-n.m.r. data: (methanol-d_4, 60 MHz): τ 4.53 (1H, d, $J_{1',2'}$ 6.2 Hz, H-1'), 5.02 (1H, d, $J_{4,5}$ 1.2 Hz, H-4), and 5.74 (3 H, s, OCH_3); ν_{max}^{film} 3380, 1775, 1745, and 1680 cm^{-1}; λ_{max}^{MeOH} 230 nm. Reacetylation of **9** with acetic anhydride in pyridine gave 5,6-di-*O*-acetyl-2-*O*-methyl-3-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl)-L-ascorbic acid (**10**) in 84% yield as an amorphous solid; $[\alpha]_D^{23}$ −45.8° (*c* 0.24, chloroform); ^{1}H-n.m.r. data: ($CDCl_3$, 60 MHz): τ 4.37 (1H, d, $J_{1',2'}$ 7.0 Hz, H-1'), 5.12 (1H, d, $J_{4,5}$ 1.8 Hz, H-4), 6.06 (3H, s, OCH_3), 7.76, 7.90, and 7.95 (18H, $OCOCH_3$); ν_{max}^{film} 1775, 1760, and 1690 cm^{-1}; λ_{max}^{MeOH} 229 nm. *Anal.* Calc. for $C_{25}H_{32}O_{17}$: C, 49.67; H, 5.34. Found: C, 49.64; H, 5.44. Similarly, deacetylation of **8** yielded 2-*O*-(β-D-glucopyranosyl-3-*O*-methyl-L-ascorbic acid (**11**); ^{1}H-n.m.r. data: (methanol-d_4, 60 MHz): τ 4.95 (1H, $J_{1',2'}$ 6.0 Hz, H-1') and 5.69 (3H, s, OCH_3); ν_{max}^{film} 3340, 1765, 1740, and 1655 cm^{-1}; λ_{max}^{MeOH} 232 nm. Reacetylation of **11** gave 5,6-di-*O*-acetyl-3-*O*-methyl-2-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl)-L-ascorbic acid (**12**) in 86% yield; m.p. 117–118°, $[\alpha]_D^{23}$ −66.7° (*c* 0.18, chloroform); ^{1}H-n.m.r. data: ($CDCl_3$, 60 MHz): τ 4.72 (1H, d, $J_{1',2'}$ 6.9 Hz, H-1'), 5.20 (1H, d, $J_{4,5}$ 2.5 Hz, H-4), 6.22 (3H, s, OCH_3), 7.87, 7.91, 7.93, 7.95, and 7.98 ($OCOCH_3$); ν_{max}^{film} 1770, 1750, and 1680 cm^{-1}; λ_{max}^{MeOH} 232 nm. *Anal.* Calc. for $C_{25}H_{32}O_{17}$: C, 49.67; H, 5.34. Found: C, 49.38; H, 5.31. Acetylation of **5** and **8** also provided **10** and **12**, respectively.

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