INDOLE 3-HYDROXYMETHYLATION AND THE SYNTHESIS OF ASCORBIGENS

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The ascorbigen 2-C-[(indolyl-3)methyl]-β-L-threo-L-glycero-3-hexylofuranosono-1,4-lactone and its derivatives are produced by condensation of 3-hydroxymethylindole and its derivatives with L-ascorbic acid at pH 3-5 [2, 4, 5]. Ascorbigens are known to have pronounced immunomodulating properties [1], so the development of routes for synthesizing these compounds is of great potential value. A number of methods exist for the preparation of 3-hydroxymethylindoles, which are key compounds in ascorbigen synthesis. The most convenient method is based on the reduction of the corresponding 3-formylindoles, using $NaBH_4$ [6]. The preparation of ascorbigens by the reaction of indoles with formaldehyde and L-ascorbic acid has also been described, though the yields of these reaction were not stated [4]. The aim of the present work was to compare methods for preparing 3-hydroxymethylindoles, and to use them for synthesizing the corresponding ascorbigens. Since 3-hydroxymethylindoles are relatively unstable, easily forming polyindolemethanes in acidic conditions [3], our approach was to use these compounds directly in the ascorbigen synthesis reactions, estimating the 3-hydroxymethylation reactions in terms of the yield of the corresponding ascorbigens.

Indole (Ia) 3-hydroxymethylation was carried out by boiling indole with paraformaldehyde in methanol in the presence of sodium methanoate (method A). 3-Hydroxymethylindole yields were quantitative, and condensation with L-ascorbic acid produced the ascorbigen with a yield of 62%, calculated from the indole input. The method used for the preparation of 2-methylascorbigen is of special interest, because of the inconvenience of the methods for preparing 2-methyl-3-formylindole [7]. 3-Hydroxymethylation of 2-methylindole (Id) in the same conditions as used for indole resulted in the production of 2-methyl-3-hydroxymethylindole (IId), which was converted to 2'-methylascorbigen (IIId) without preliminary purification, with a yield of 5%, calculated from the indole input. The structure of IIId was confirmed by PMR, IR and UV spectroscopy, and by mass spectrometry. 5-Bromo-3-hydroxymethylindole (IIe) was also prepared by the direct 3-hydroxymethylation of 5-bromoindole (Ie), and was condensed with ascorbic acid without preliminary purification, to produce 5-bromoascorbigen (IIIe) with a yield of 10%, calculated from the indole input.

Thus, good 3-hydroxymethylindole yields were obtained from the reaction with paraformaldehyde in the presence of sodium methanoate only for the N-unsubstituted indole, and this route is useful only for the synthesis of 2-methyl-3-hydroxymethylindole IId. It was interesting that N-substituted indole 3-hydroxymethylation did not take place in these conditions. This may be because formaldehyde interacts with N-deprotonated indole anions in alkaline conditions; these anions cannot form in N-substituted indoles.

When 3-hydroxymethylation of indoles is carried out in acid conditions (method B), it is important to remember that 3-hydroxymethylindoles do not exist in acid conditions, because they easily form di- and polyinbdolylmethanes with cleavage of formaldehyde [3]. In the present experiments, 3-hydroxymethylindoles were taken up by ascorbic acid, which thus acts both as a catalyst of 3-hydroxymethylation and as a trap for the resulting 3-hydroxymethylindoles. The yields were in all cases below 10% (calculated from the indole input), though the reaction worked both with N-unsubstituted (Ia, d, e) and N-substituted (Ib, c) indoles. The structures of the resulting ascorbigens (IIIa-e) were confirmed by PMR spectroscopy. HPLC analysis was used to show that compounds IIIa-e were identical to previous ascorbigen preparations.

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MATERIALS AND METHODS

IR spectra were taken on a Perkin–Elmer IR-283 apparatus in KBr tablets, and UV spectra were taken using a Specord UV VIS spectrophotometer in ethanol (0.02 mg/ml). PMR spectra were taken on a Bruker WH-360 apparatus at a working frequency of 360 MHz, using tetramethylsilan as the internal standard. Signals were assigned by the double resonance method. Low resolution electron bombardment mass spectra were recorded using a Varian MAT-311A apparatus by direct sample injection with an ionizing electron energy of 70 eV, a cathode emission current of 1 mA, and an accelerating voltage of 3 kV. TLC was carried out using Silufol UV-254, and preparative chromatography was carried out using Silicagel LSL 5/40 μm (Chemapol). Chromatograms were run on plates (20 × 20 cm) with a non-attached layer 1 mm thick, and were examined in UV light, or were sprayed (Silufol) with Ehrlich's reagent.

3-Hydroxymethylindole (IIa)

Metallic sodium (2.3 g, 0.1 mole) was added to 120 ml methanol with stirring, after which indole (11.7 g, 0.1 mole) and paraform (4 g, 0.13 mole formaldehyde) were added; the mix was boiled with stirring for 8 h. The reaction was then cooled, and was filtered to remove unreacted indole. The filtrate was evaporated to dryness, and 200 ml of water was added to the residue, and the resulting suspension was extracted with chloroform (3 × 70 ml); the extract was washed with water (3 × 30 ml), dried over Na_2SO_4, and evaporated to dryness. The yield was 14.70 g (quantitative) of a dark-colored oily substance. TLC analysis gave a single spot with R_f = 0.33 (in chloroform). The PMR spectrum (in $CDCl_3$) was: 7.65 d (1H), 7.20-7.80 m (3H), 6.76 s (1H, H-2, indole ring protons), 4.52 s (2H, protons of the exocyclic methyl group).

Similar methods were used to prepare compounds IId and IIe, which were used in the L-ascorbic acid condensation reaction without purification.

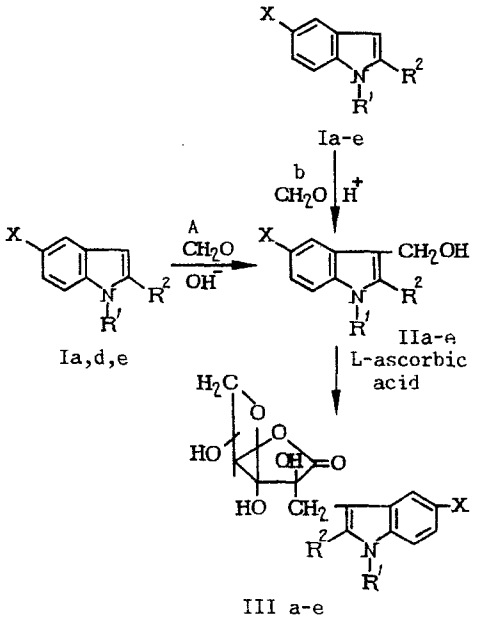
2-C-[(2-methylindolyl-3)methyl]-β-L-threo-L-glycero-3-hexylofuranosono-1,4-lactone (2'-methylascorbigen) (IIId)

Method A

A feshly-prepared solution of 0.60 g (3.70 mmoles) of 2-methyl-3-hydroxymethylindole IId was added to a solution of 0.82 g (4.65 mmoles) of L-ascorbic acid in 20 ml citrate-phosphate buffer, pH 4.0, at 20°C with mixing. The reaction mixture was filtered after mixing for 3 h, and the filtrate was extracted with ethyl acetate (2 × 20 ml), and the extract was dried over Na_2SO_4 and evaporated to dryness. The material was chromatographed on Silicagel (in ethyl acetate), and the fraction with R_f = 0.52 was collected by elution with ethyl acetate and evaporated to dryness. This method yielded 60 mg (5.0% in terms of 2-methylindole) of an amorphous colorless substance with R_f = 0.63 in ethyl acetate, RT = 3.36 min. IR spectral data were: $\nu_{C=O}$ 1790 cm^{-1}. The UV spectral data were: [λ_{max} nm, (lg ε)]: 255 (1.87), 275 (1.31), 282 (1.33), 289 (1.28). Mass spectroscopy gave m/z (%): 81 (7), 82 (2), 83 (7), 84 (24), 87 (7), 100 (7), 101 (14), 102 (46), 103 (14), 115 (16), 116 (50), 117 (7), 128 (16), 129 (4), 130 (7), 131 (6), 142 (25), 143 (100), 144 (70), 145 (8), 146 (2), 149 (5), 176 (3), 274 (3), 275 (2), 319 (2) (M^+). The PMR spectrum in C_5D_5N gave (δ in ppm, J in Hz): 5.02 s (1H, H-4), 4.72 dd (1H, $J_{5,6A}$ 4.0, $J_{5,6a}$ 6.1, H-5), 4.48 dd (1H, $J_{6a,6b}$ 9.1, H-6), 4.32 dd (1H, H-6, protons of the ascorbic acid residue), 3.91 s (2H, protons of the extracyclic methylene group), 2.59 s (3H, protons of the indole 2'-methyl group). The signals from indole protons overlapped with those of the solvent, and are not given here. The PMR spectrum in CD_3OD gave (δ in ppm, J in Hz): 7.57 d (1H), 7.19 d (1H), 6.97 m (1H), 6.90 (1H, protons of the indole ring), 4.25 ddd (1H, H-5), 4.28-4.02 m (2H), 4.01 dd (1H, H-6, protons of the ascorbic acid residue), 3.35-3.10 m (protons of the exocyclic methylene group overlapping with solvent signals), 2.35 s (3H, protons of the indole 2'-methyl group).

Method B

A solution of 1.5 g (8.50 mmoles) of L-ascorbic acid in 10 ml of water and 0.2 g paraform (6.5 mmoles formaldehyde) were added to a solution of 0.66 g (5.00 mmoles) of 2-methylindole in 10 ml ethanol. The mixture was boiled with stirring for 5 h, and was then filtered, and the filtrate was evaporated to half its initial volume. Water (200 ml) was added to the



residue, and by-products were extracted with chloroform (6 × 50 ml). The aqueous fraction was extracted with ethyl acetate (2 × 100 ml), and the ethyl acetate extract was washed with water (2 × 20 ml), dried over Na_2SO_4, passed through a layer of activated charcoal, and evaporated to dryness. This produced a yellow foam, which was chromatographed on Silicagel in ethyl acetate, and a fraction with R_f = 0.52 was eluted with ethyl acetate and evaporated to dryness. The yield was 50 mg (3.1% calculated for 2-methylindole) of an amorphous colorless substance with R_f = 0.63 in ethyl acetate and RT = 3.36 min.

Compounds IIIa, b, c, and d were prepared by similar methods. The yields of compound IIIa were 62% and 6% by methods A and B respectively, 11% for IIIb, 2% for IIIc, and 10% and 7% for IIId by methods A and B (calculated for the corresponding indole).

RT values for compounds IIIa, b, c, and d were 3.20, 3.73, 4.32, and 4 min respectively.

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