

**PURDIE REAGENT-INDUCED C<sub>(5)</sub>-EPIMERIZATION  
OF D-GALACTURONIC ACID DERIVATIVES  
IN THE PRESENCE OF METHYL SULPHIDE\***

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Methylation of methyl (methyl  $\alpha$ -D-galactopyranosid)uronate (*I*) with Purdie reagent (methyl iodide and silver oxide) in the presence of methyl sulphide gives, in addition to the expected methyl (methyl 2,3,4-tri-O-methyl- $\alpha$ -D-galactopyranosid)uronate (*II*), the olefinic saccharide *IV* resulting from  $\beta$ -elimination and methyl (methyl 2,3,4-tri-O-methyl- $\beta$ -L-altropyranosid)uronate(*III*) from readdition of a proton to the configurationally unstable carbanion formed as an intermediate during the E1cB process. Compounds *III* and *IV* were also formed when *II* was treated with Purdie reagent in the presence of methyl sulphide. The conversion mechanism of *II* to *III* was confirmed by treatment of *II* in [O—<sup>2</sup>H] methanol with Purdie reagent in the presence of methyl sulphide. The unequal reactivity of methyl D-galacturonate and D-galactose derivatives in the eliminations following the E1cB mechanism was explained in terms of different electron distribution calculated by the CNDO/2 method.

Alkylation of alcohols with Purdie reagent is promoted by methyl sulphide added to the reaction mixture<sup>1</sup>. In cases where the standard Purdie methylation of carbohydrates bearing the bulky trityl group yielded only partially methylated products<sup>2,3</sup> the addition of methyl sulphide resulted<sup>1,3</sup> in a complete methylation. The addition of methyl sulphide enabled (*cf.*<sup>3</sup>) a total methylation of methyl 4-O-acetyl-3-O-benzyl-6-O-trityl- $\alpha$ -D-glucopyranoside which, because of the presence of an O-acetyl group, could not be methylated with a more efficient method in a strongly alkaline medium.

The isolation of methyl (methyl 2-O-benzyl-4-deoxy-3-O-methyl- $\beta$ -L-*threo*-hex-4-enopyranosid)uronate from the methylation<sup>4</sup> of methyl (methyl 2-O-benzyl- $\alpha$ -D-galactopyranosid)uronate was inconsistent with results<sup>5</sup> according to which the esterified uronic acid derivatives treated with CH<sub>3</sub>I and Ag<sub>2</sub>O were reported not to undergo degradation by elimination. Since in the above-mentioned experiments<sup>4</sup> a catalytic amount of methyl sulphide was added to the reaction mixture it seemed interesting to ascertain whether the catalyst was responsible for the formation of the  $\beta$ -elimination product. Therefore, methyl (methyl  $\alpha$ -D-galactopyranosid)uronate (*I*)

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was methylated with a) Purdie reagent, b) Purdie reagent in the presence of a catalytic amount of methyl sulphide, and the respective reactions were monitored by thin-layer chromatography. Methylation a) was a very slow reaction leading to the expected methyl (methyl 2,3,4-tri-O-methyl- $\alpha$ -D-galactopyranosid)uronate (*II*). The presence of  $\beta$ -elimination products in the reaction mixture was not detected.

Methylation in the presence of the catalyst was a faster process and after a few hours TLC of the reaction mixture showed that simultaneously with *II* and the partially methylated 4-deoxyhexuronosides, two by-products, both moving faster than *II*, were also formed. The by-product having the highest  $R_F$  was detected with dilute  $\text{KMnO}_4$  solution. After the reaction was complete the main portion of *II* was crystallized and the by-products were isolated by chromatography of the mother liquor. The substance detected with  $\text{KMnO}_4$  was identical with the authentic<sup>6</sup> methyl (methyl 4-deoxy-2,3-di-O-methyl- $\beta$ -L-threo-hex-4-enopyranosid)uronate (*IV*). Isolation of *IV* proved that the presence of methyl sulphide in the reaction mixture, resulting in the modification (activation) of  $\text{Ag}_2\text{O}$  to give a stronger base<sup>1</sup>, was responsible for the  $\beta$ -elimination and, therefore, the formation of the olefin described previously<sup>4</sup> was associated with the presence of methyl sulphide.

The formulation of the second by-product as methyl (methyl 2,3,4-tri-O-methyl- $\beta$ -L-altropyranosid)uronate (*III*) was based on the spectral evidence. Its mass spectrum, comparable to that of methyl (methyl 2,3,4-tri-O-methyl- $\alpha$ -D-glucopyranosid)uronate<sup>7</sup>, showed, in agreement with the elemental composition, the substance to be a methyl (methyl 2,3,4-tri-O-methyl-hexopyranosid)uronate. The assignment of the absolute configuration at  $\text{C}_{(5)}$  was based on CD measurements revealing a negative Cotton effect at 210 nm. The investigation of glycosides of D-uronic acids allowed Listowsky and coworkers<sup>8</sup> to demonstrate that substances of this structural feature display a positive Cotton effect at 210 nm. On the other hand, it has been found by others<sup>9,10</sup> that substances containing L-guluronic acid show a negative Cotton effect in the same region. Consequently, the negative Cotton effect at 210 nm determined for *III* proved that this substance belonged to the L-series. The L-*altro* configuration was assigned on the basis of <sup>1</sup>H-NMR measurements (Table I). From the  $J_{4,5}$  and  $J_{2,3}$  values found for *III* at 25°C follows that these are time-averaged coupling constants<sup>11-13</sup> of a mixture of conformers. The coupling constant  $J_{4,5}$  decreased and  $J_{2,3}$  increased on cooling and at -100°C they reached approximately 1 and 9 Hz, respectively. These values corresponded to a diaxial arrangement of H-2 and H-3 and diequatorial or axial-equatorial arrangement of H-4 and H-5 which, in the L-hexopyranosiduronate series was consistent with the L-*altro* or L-*ido* configuration. Carbohydrates of these series are known to exist in both (<sup>4</sup> $\text{C}_1$  and <sup>1</sup> $\text{C}_4$ ) chair conformations and the mere fact that the coupling constants of *III* were found to be temperature dependent suggested that the substance was a mixture of conformers. The L-*ido* configuration for *III* was ruled out since the coupling constant  $J_{4,5}$  considerably varied with temperature, this being inconsistent with a change expected for an L-*ido*

TABLE I  
Chemical Shifts ( $\delta$ -scale) and Coupling Constants (Hz)

Compound	Solvent	Chemical shifts <sup>a</sup>										Constants			
		H-1	H-2	H-3	H-4	H-5	COOCH <sub>3</sub>	CH <sub>3</sub>	J <sub>1,2</sub>	J <sub>2,3</sub>	J <sub>3,4</sub>	J <sub>4,5</sub>			
<i>II</i>	CDCl <sub>3</sub>	5.00 d	—	3.65 bt <sup>b</sup>	—	4.09 bt	4.35 d	3.83	3.45; 3.53; 3.51 <sup>c</sup>	2.6	d	d	1.4		
<i>II</i>	(CD <sub>3</sub> ) <sub>2</sub> CO	4.86 d	3.50 q	3.56 q	4.05 bt	4.30 d	3.71	3.32; 3.39; 3.42; 3.46	2.4	9.5	2.2	1.6			
<i>III</i>	CDCl <sub>3</sub>	4.82 d	3.60 q	3.83 q	4.04 q	4.36 d	3.78	3.48; <sup>c</sup> 3.53; 3.55	2.8	8.2	2.8	4.2			
<i>III</i>	(CD <sub>3</sub> ) <sub>2</sub> CO	4.72 d	3.50 q	3.76 q	3.89 q	4.26 d	3.70	3.36; 3.38; 3.42; 3.45	2.5	7.5	2.7	5.0			
<i>V</i>	CDCl <sub>3</sub>	4.86 d	3.40	—	3.70 m	4.05 d	3.81	3.44; 3.50; 3.52; 3.62	3.2	d	d	9.5			

<sup>a</sup> Observed multiplicities: d doublet; t triplet; bt broadened triplet; q quartet; m multiplet; <sup>b</sup> deceptive simple second-order spectrum; <sup>c</sup> 6 proton singlet; <sup>d</sup> first-order coupling constants were not observed.

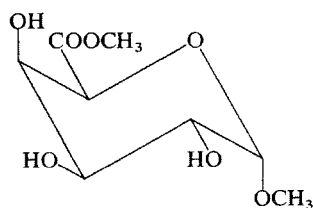
compound where the population of conformers in the mixture is in favour of the  ${}^4C_1(L)$  conformer. (The arrangement of H-4 and H-5 for idose in the  ${}^4C_1(L)$  conformation is axial-equatorial and equatorial-axial in the  ${}^1C_4(L)$  conformation. Consequently, one would predict a small change of  $J_{4,5}$  during the "freezing-out" of one conformer. On the other hand, H-4 and H-5 in an *altro* compound in  ${}^4C_1(L)$  or  ${}^1C_4(L)$  conformation are diequatorially or diaxially oriented. The considerable variation of  $J_{4,5}$  with the varying temperature found for the substance under investigation was in accord with such an arrangement). The exclusion of the *L-ido* configuration for *III* i.e. the fact that during the formation of *III* from *II* the inversion of the configuration took place exclusively at  $C_{(5)}$ , was supported by the mechanistic isotope-exchange study showing the presence of  ${}^2H$  at  $C_{(5)}$ . An inversion of the configuration both at  $C_{(4)}$  and  $C_{(5)}$  can occur *via* a carbanion intermediate; after its stabilization resulting in the formation of the *L-ido* isomer, the deuterium incorporation would be observed at both centres of asymmetry. The mass spectrum of *IIIa*, isolated from the conversion of *II* to *IIIa* carried out in  $[O-{}^2H]$  methanol showed the presence of  ${}^2H$  exclusively at  $C_{(5)}$ .

When reacting *II* with  $Ag_2O$  and 10 molar equivalents of methyl iodide and methyl sulphide, i.e.  $(CH_3)_3S^{(+)}I^{(-)}$  in 1,2-dimethoxyethane for 24 hours an equilibrium between *II* and the formed *III* and *IV* was established, as shown by TLC, and compounds *III* and *IV* were isolated by chromatography in  $\sim 8\%$  yield. Some *III*, but not *IV*, was also formed from *II* and methyl sulphide and silver oxide in methanol, i.e. in the absence of  $CH_3I$ . No reaction was observed, however, when *II* was treated with  $(CH_3)_3S^{(+)}I^{(-)}$  in 1,2-dimethoxyethane in the absence of  $Ag_2O$ .

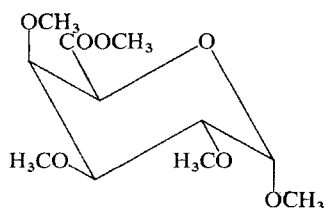
Preliminary experiments involving the same relative amount of Purdie reagent, methyl sulphide and *II* in methanol showed that a non-specific destruction of the reaction mixture components took place. The destruction was probably promoted by the presence of a relatively large amount of water resulting from the methylation of the solvent. When *II* was treated with an equimolar amount of those reagents in methanol the equilibrium between the starting *II* and the formed *III* settled faster ( $\sim 4$  hours), compared with the same reaction in 1,2-dimethoxyethane and the decomposition was less pronounced. Extension of the reaction time (20 hours) resulted in a higher yield of the olefin *IV* (elimination of the  $C_{(4)}$  substituent from both *II* and the formed *III*) but apparently had no effect upon the final ratio of *II* to *III*.

When methyl (methyl 2,3,4-tri-O-methyl- $\alpha$ -D-glucopyranosid)uronate (*V*) was treated with  $(CH_3)_3S^{(+)}I^{(-)}$  and  $Ag_2O$  in 1,2-dimethoxyethane neither the olefin *IV* nor any compound that could be isomeric with the starting material was formed, as showed by TLC and gas chromatography<sup>14</sup>.

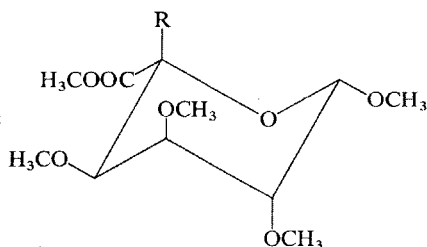
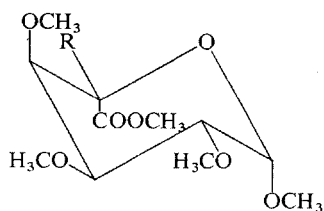
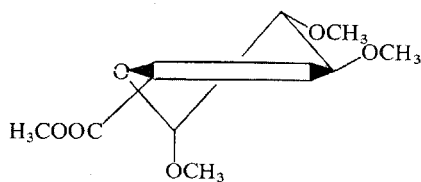
The elimination of substituents at  $C_{(4)}$  in *cis* and *trans* systems (the case of D-glucuronic and D-galacturonic acids) in substrates treated with potassium hydroxide has been studied<sup>15</sup> and no significant difference in behaviour of these two types



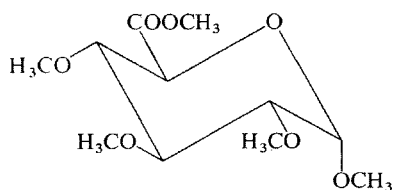
I



II

III, R = <sup>1</sup>HIIIa, R = <sup>2</sup>H

IV



V

of compounds was found. The difference in the behaviour of *II* and *V* treated with  $(\text{CH}_3)_3\text{S}^{(+)}\text{I}^{(-)}$  and  $\text{Ag}_2\text{O}$  showed the more pronounced capability of *II* to undergo eliminations caused by stereoelectronic effects encountered when sufficiently mild base was applied. Since both *II* and *V* easily undergo  $\beta$ -elimination reaction, the driving force for which is the acidic nature of H-5, the difference in the susceptibility towards basic reagents cannot be observed when the base is either too strong<sup>15,16</sup> or the C<sub>(4)</sub> substituent is a very good leaving group<sup>17</sup>. Our results are in agreement with those of Schmidt and Neukom who reported the formation of a higher proportion of olefinic substances from the *D-galacto* than from the *D-gluco* compound when a series of methyl (methylhexopyranosid)uronates was acetylated with sodium acetate and acetic anhydride<sup>18</sup>.

The formation of *III* and *IV* from *II* by a weak base can be explained by the E1cB mechanism of  $\beta$ -elimination in uronic acid derivatives<sup>16</sup> according to which this process is assumed to proceed in two steps. In the first step carbanion (*A*, *B*) is gene-

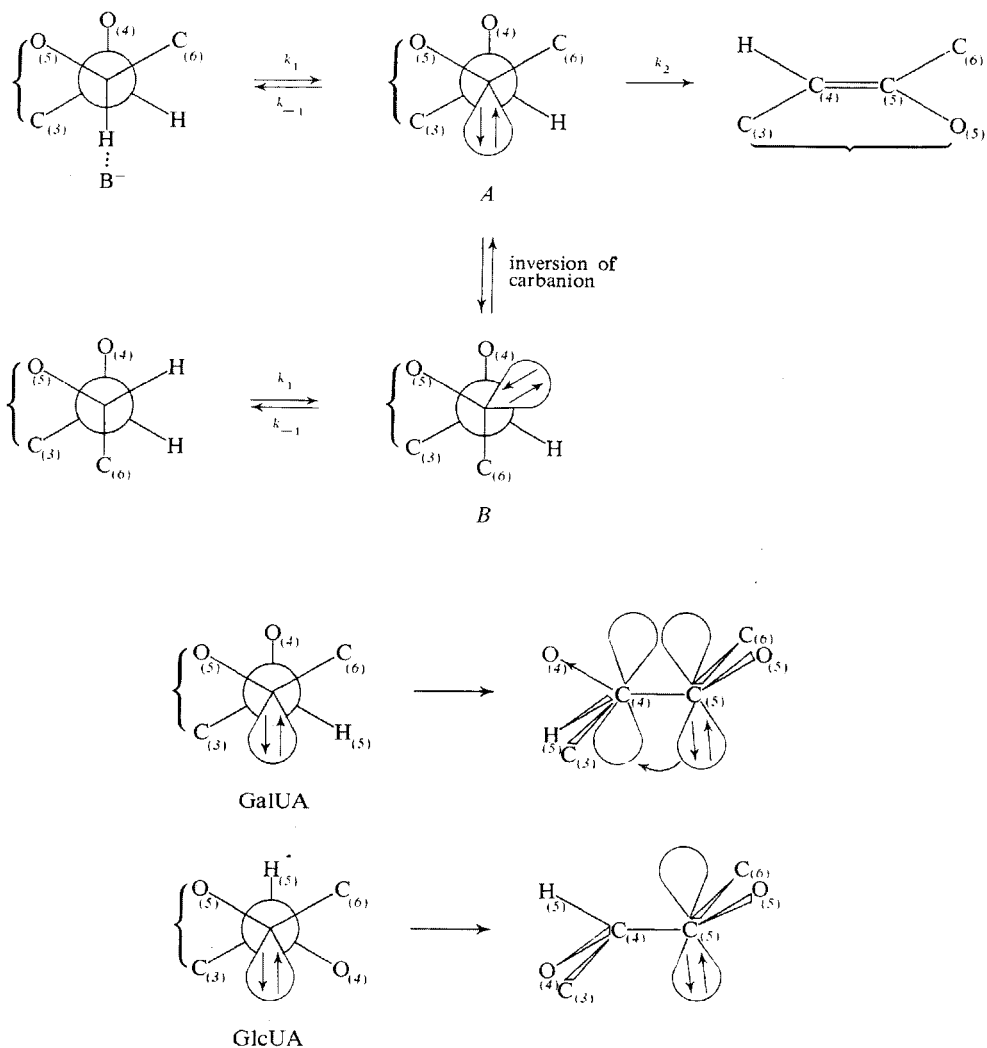
rated in a relatively high concentration. Although carbanions are supposed to be short-lived particles their relative stability was demonstrated *e.g.* by the labeling by tritium at  $C_{(5)}$  of L-guluronic acid units formed during the enzymic  $C_{(5)}$ -epimerization of polymannuronic acid<sup>19</sup> the initial step of which is also the abstraction of H-5. The scission of the  $C_{(5)}$ -H bond in *II* by a weak base, not found with aldoses, was due to the presence of a methoxycarbonyl group. We have studied its effect upon the electron distribution by the CNDO/2 method in its original parametrization<sup>20</sup>. The geometrical parameters for all molecules under study were the same and taken from the X-ray structures<sup>21</sup>. Since other papers<sup>22,23</sup> concerning the electron distributions during 1,2-eliminations showed that the most significant changes in the electron distributions occur at atoms Y—C—C—H, our attention was focussed mainly on the distribution at those sites. According to the disturbance theory<sup>24</sup> the extent of the interaction of the base with the atom of hydrogen, *i.e.* the rate of the carbanion formation, is proportional to the frontier electron density in the lowest unoccupied (LU) molecular orbital (MO). Table II shows charges at the sites in question and the frontier electron densities at the atom of hydrogen. The changes in the electron distributions at the atom of hydrogen, follow from the presented values. These are most pronounced in the case of H-5, which becomes more positive, and the carbon atom  $C_{(5)}$  more negative. The variations in the electron distribution are even more pronounced when expressed in terms of frontier electron density values, higher by two orders of magnitude in the case of D-galacturonic acid, explaining thus the unreactivity of D-galactose. Table II lists also the  $P_H^{LU}$  charges for both anomers of D-galactose and D-galacturonic acid from which follows the different reactivity of the two anomeric forms.

The observed different reactivity of D-galacturonic and D-glucuronic acid derivatives can be explained in the following way. The just formed electron pair of the carbanion of D-galacturonic acid is *trans* towards the leaving group and is able to interact with

TABLE II

Charges  $Q[e]$  at H-5,  $C_{(4)}$ ,  $C_{(5)}$ ,  $O_{(4)}$ , and Frontier Electron Densities  $P_H^{LU}$  of Hydrogen at LUMO for Both Anomers of D-Galactose and D-Galacturonic Acid Calculated by CNDO/2 Method

Compound	$Q_H$	$Q_{C_{(4)}}$	$Q_{C_{(5)}}$	$Q_{O_{(4)}}$	$P_H^{LU}$
$\alpha$ -D-Gal	+0.0044	0.1095	0.1219	-0.2483	0.0008
$\beta$ -D-Gal	+0.0003	0.1093	0.1211	-0.2470	0.0008
$\alpha$ -D-GalUA	+0.0207	0.0610	0.1335	-0.2488	0.0987
$\beta$ -D-GalUA	+0.0160	0.0608	0.1326	-0.2474	0.1014



the antibonding orbital  $\sigma^*$  of the C<sub>(4)</sub>—O<sub>(4)</sub> bond; it is localized, due to the high electronegativity of oxygen, at carbon C<sub>(5)</sub>. This interaction stabilizes the carbanion and lowers the activation energy of its formation since it results in strengthening of the C<sub>(4)</sub>—C<sub>(5)</sub> and weakening of the C<sub>(4)</sub>—O<sub>(4)</sub> bonds. In the case of D-glucuronic acid the interaction of the electron pair would be significantly less pronounced, the activation energy of the formation of the carbanion would be considerably higher and the carbanion, even if it were formed, would be less stable due to the mutual orientation of the electron pair and  $\sigma^*$  of the C<sub>(4)</sub>—O<sub>(4)</sub> bond. The consequence of this interaction can be seen when comparing the energies of the individual bonds in

the carbanions. The values  $E_{AB}$  (Table III) required for this purpose were obtained from the energy distributions in the CNDO/2 method according to<sup>25</sup>, characterizing the energies of the bonds linking the atoms  $C_{(4)}$ ,  $C_{(5)}$ , and  $O_{(4)}$ .

In the process described, the carbanion *A* underwent an inversion through a flat conformation<sup>26</sup> resulting in the inverted configuration at  $C_{(5)}$  (*B*). The reversible addition of a proton occurred at both faces of the carbanion and led to the formation of two substances one of which was the starting material with the *D-galacto* configuration whilst the other was its  $C_{(5)}$  diastereoisomer. The final ratio of the two products thus formed depended upon the equilibrium proportion of the two carbanions and also on the ratio of the rate constants ( $k_1$ ,  $k_{-1}$ ,  $k'_1$ ,  $k'_{-1}$ ). The higher stability of the carbanion *A* together with the higher value of  $k_{-1}$  manifested itself in the higher conversion of *III* to *II*, proved by TLC. In the second step of the reaction the  $C_{(4)}$  substituent was split off and the double bond between  $C_{(4)}$  and  $C_{(5)}$  was formed. Since the  $OCH_3$  is a poor leaving group,  $k_2 \ll k_1$  and therefore the yield of the olefin was low.

To verify the above-discussed mechanism, *II* was treated with  $(CH_3)_3 S^{(+)}I^{(-)}$  and  $Ag_2O$  in 1,2-dimethoxyethane containing a small amount of  $^2H_2O$ . Should the above-mentioned two-step mechanism be correct then, after the deuteration of the conjugate acid  $BH^+$  and subsequent stabilization of the carbanion, at least some *II* and *III* ought to be deuterium-labelled at  $C_{(5)}$ . The reaction product *IIIa* and the material having the same chromatographic mobility as the starting material (now assumed to be a mixture of *D-galacto*- $C_{(5)}$ - $^1H$  and *D-galacto*- $C_{(5)}$ - $^2H$ ), were isolated from the reaction mixture containing a small amount of  $^2H_2O$ . Mass spectrometry showed that 4 and 37% of *II* and *IIIa*, respectively, contained deuterium at  $C_{(5)}$ . The addition of  $^1H$  or  $^2H$  in the system considered was governed by statistical chance and depended, thus, upon the amount of  $^2H_2O$  present. A higher degree of deuteration at  $C_{(5)}$  of *II* and *IIIa* could not be achieved by addition of a larger amount of  $^2H_2O$  as this resulted in a nonspecific decomposition of the starting material (or of the intermediates of the process) and no pure compounds could be isolated

TABLE III

$E_{AB}$  Values for Anomeric *D-Galacturonic* Acids and their Carbanions *A* [kcal/mol] Obtained by CNDO/2 Calculations

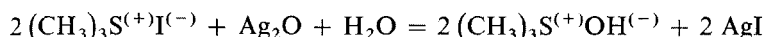
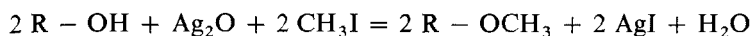
Compound	$E_{C_{(4)}C_{(5)}}^a$	$E_{C_{(4)}C_{(5)}}$	$E_{C_{(4)}O_{(4)}}^a$	$E_{C_{(4)}O_{(4)}}$
$\alpha$ - <i>D</i> -GalUA	-677.953	-655.914	-619.154	-627.972
$\beta$ - <i>D</i> -GalUA	-678.013	-655.786	-619.630	-628.124

<sup>a</sup> Carbanion



from the reaction mixture. With [O—<sup>2</sup>H]methanol as solvent the degree of deuterium incorporation at C<sub>(5)</sub> of *II* and *IIIa* (shown by mass spectrometry) was 25 and 96%, respectively, proving thus a carbanion being the intermediate in the described C<sub>(5)</sub>-epimerization.

The equilibrium ratio of *II*, *III*, and *IV* was the same regardless whether the reaction was carried out in pure 1,2-dimethoxyethane or in that solvent containing a small amount of water. Therefore it seems unlikely that trimethylsulphonium hydroxide, which can be formed in the system due to the presence of water (either added or liberated during methylation), (CH<sub>3</sub>)<sub>3</sub>S<sup>(+)</sup>I<sup>(-)</sup>



was the base responsible for the side reactions observed during the methylation of some D-galacturonic acid derivatives with Purdie reagent in the presence of methyl sulphide. Trimethylsulphonium hydroxide, as a very strong base, may have been responsible for the nonspecific decomposition of the uronic acid components in the reaction mixture observed when a larger amount of water was present. Hence, the possibility of modification ("activation") of silver oxide *e.g.* by complex formation<sup>1</sup> with methyl sulphide, to give a new base, stronger than Ag<sub>2</sub>O itself, cannot be excluded. The complex appears to be formed more readily from (CH<sub>3</sub>)<sub>3</sub>S<sup>(+)</sup>I<sup>(-)</sup> and Ag<sub>2</sub>O than from (CH<sub>3</sub>)<sub>2</sub>S and Ag<sub>2</sub>O; however, for the formation of the stronger base the presence of methyl iodide is not necessary (in the presence of (CH<sub>3</sub>)<sub>3</sub>S<sup>(+)</sup>I<sup>(-)</sup> and Ag<sub>2</sub>O *II* was converted to *III* and *IV*, in the absence of CH<sub>3</sub>I a trace amount of *III*, but not *IV*, was still formed; in the absence of (CH<sub>3</sub>)<sub>2</sub>S no side reaction was observed). Supposedly the presence of methyl iodide was important for proceeding of the side reactions mainly in the early stage since, obviously, the complex was formed faster or before the alkylation took place. This was supported by the fact that the conversion of *II* treated with (CH<sub>3</sub>)<sub>3</sub>S<sup>(+)</sup>I<sup>(-)</sup> and Ag<sub>2</sub>O to *III* and *IV* was observed regardless of the solvent although in methanol the small amount of methyl iodide, formed as a result of the collapse of (CH<sub>3</sub>)<sub>3</sub>S<sup>(+)</sup>I<sup>(-)</sup> into its components, must have necessarily been consumed for the methylation of the solvent.

The described conversion of methyl (methyl 2,3,4-tri-O-methyl- $\alpha$ -D-galactopyranosid)uronate to the corresponding *L-althro* derivative can be utilized in the synthesis<sup>27</sup> of other L-altruronic acid derivatives which, otherwise, would be accessible only with difficulty.

## EXPERIMENTAL

Melting points were determined on a Kofler hot-stage. Optical rotations were measured with a Perkin-Elmer polarimeter model 141. The 70 eV mass spectra were obtained as described<sup>7</sup>, the <sup>1</sup>H-NMR spectra were taken with a Tesla BS 485 B spectrometer at 80 MHz (internal standard

tetramethylsilane). The spectra of *III* were measured between +50 to -100°C. The CD spectra of methanol solution were measured with a Jouan CD Dichrograph Model 185 at room temperature. The concentration of the solutions was ~1 mg/ml. The quantum-chemical calculations were carried out with a CDC 3300 computer at the United Nations Computing Center, Bratislava. Thin-layer chromatography (TLC) was performed on Silica gel G and preparative chromatography on dry-packed silica gel (0.1–0.15 mm) columns employing tetrachloromethane–acetone mixtures: A 9 : 4, B 7 : 1, C 4 : 1, D 12 : 1. Prior to packing the silica gel was equilibrated with 40% (v/w) of the mobile phase instead of the recommended<sup>28</sup> 10%.

Detection was effected by charring with 5% sulphuric acid in ethanol. Olefinic components were located by spraying with 0.1% potassium permanganate in acetone. Methyl sulphide and 1,2-dimethoxyethane were dried<sup>29</sup> and freshly distilled. Methyl iodide was purified<sup>29</sup> and stored in contact with mercury and anhydrous calcium chloride. Silver oxide was freshly prepared<sup>30</sup> and, when used in isotope-exchange reactions, dried under diminished pressure.

The degree of deuterium incorporation at C<sub>(5)</sub> in *IIIa* was determined by mass spectrometry. Its mass spectrum was compared with that of methyl (methyl 2,3,4-tri-O-methyl- $\alpha$ -D-glucopyranosiduronate<sup>7</sup>). The degree of deuteration was calculated from the comparison of the peak intensities of ions containing deuterium at C<sub>(5)</sub>, *i.e.* from those shifted by one mass unit upwards compared with those containing an atom of hydrogen at C<sub>(5)</sub> (ions A<sub>1</sub>, A<sub>2</sub>, E<sub>1</sub>, E<sub>2</sub>, D<sub>1</sub>, and K<sub>2</sub>). Solutions were concentrated under diminished pressure below 40°C.

#### Methylation of Methyl (Methyl $\alpha$ -D-Galactopyranosid)uronate (*I*)

a) Silver oxide (4 g) and methyl iodide (10 ml) were added to a suspension of *I* (1 g) in 1,2-dimethoxyethane (10 ml) and the mixture was shaken in the dark. Fresh portions of Ag<sub>2</sub>O (1 g) were added every day and after 3 days the mixture containing two products ( $R_F$  0.2 and 0.4, solvent A) was filtered, the solids washed with chloroform and the filtrate combined with the washings was concentrated. The partially methylated product was remethylated as described above to give a syrup which partially crystallized. The main product *II* (0.6 g), obtained by recrystallization from ether–isopropyl ether, melted at 71–73°C, (*ref.*<sup>31</sup>) *m.p.* 72°C. The mother liquor containing, as showed by TLC, *II*, product resulting from incomplete methylation of *I* and no other by-products, was concentrated and chromatographed to give a further crop of *II* (0.3 g, total yield 75.6%). The slower moving material, as showed by mass spectrometry, was an unresolved mixture of di-O-methyl derivatives of *I*.

b) Silver oxide (2 g), methyl iodide (30 ml) and methyl sulphide (0.15 ml) were added to a suspension of *I* (3 g) in 1,2-dimethoxyethane. The mixture was shaken in the dark and a fresh portion of silver oxide (3 g) was added after 24 h. The reaction course was periodically checked by TLC in solvent A. The reaction mixture was worked up after 48 h and the partially methylated product was remethylated with fresh portions of silver oxide (3 g) every 24 h for 3 days. After this time the partially methylated derivatives of *I* and of methyl (methyl 4-deoxy- $\beta$ -L-threo-hex-4-enopyranosid)uronate ( $R_F$  0.3, 0.35) were no more present. The reaction mixture contained one major ( $R_F$  0.4) and two minor products ( $R_F$  0.45, 0.6); the faster moving one was also detected with the KMnO<sub>4</sub> spray. The mixture was worked up, the major product crystallized (1.28 g) and the mother liquor chromatographed on a column of silica gel (100 . 3.8 cm) with solvent B. The olefin *IV* (616 mg, 17.3%),  $[\alpha]_D^{22} + 227^\circ$ , lit. (*cf.*<sup>7</sup>)  $[\alpha]_D + 241^\circ$ , lit.<sup>32</sup>  $[\alpha]_D + 189^\circ$  was eluted first. Its mass<sup>33</sup> and <sup>1</sup>H-NMR (*cf.*<sup>6</sup>) spectral data were identical with those reported. Elution with solvent C gave the *L-altro* derivative *III* (85 mg, 2.4%),  $[\alpha]_D^{22} + 102^\circ$  (*c* 1, methanol). For C<sub>11</sub>H<sub>20</sub>O<sub>7</sub> (264.3) calculated: 49.99% C, 7.63% H, 58.72% CH<sub>3</sub>O; found: 49.80% C, 7.44% H, 58.90% CH<sub>3</sub>O. The mass spectrum confirmed that *III* was a methyl (methyl 2,3,4-tri-O-methyl hexopyranosid)uro-

nate and the CD spectrum showed a negative Cotton effect at 210 nm. For <sup>1</sup>H-NMR data see Table I. Further elution with solvent C gave an additional amount of *II* (1.07 g, total yield 65.9%).

#### Treatment of *II* with CH<sub>3</sub>I, Ag<sub>2</sub>O and (CH<sub>3</sub>)<sub>2</sub>S

a) *In 1,2-dimethoxyethane*: Compound *II* (1 g, 3.78 mmol) in 1,2-dimethoxyethane (20 ml) was treated with CH<sub>3</sub>I (2.4 ml, 38 mmol), (CH<sub>3</sub>)<sub>2</sub>S (2.8 ml, 38 mmol) and dried Ag<sub>2</sub>O (2 g), and the mixture was shaken in the dark. The composition of the reaction mixture was checked every hour by TLC in solvent C. The presence of *III* and *IV* was first detected after 3 h. A fresh portion of Ag<sub>2</sub>O (1 g) was added after 3 hours and after a total period of 24 h, at which time the ratio of the reaction mixture components appeared to be constant, the mixture containing *II*, *III*, and *IV* (*R<sub>F</sub>* 0.3, 0.35 and 0.5) was worked up and chromatographed on a column of silica gel (65 × 3.4 cm) as described above. In the total recovery of 95.9% the yields of the respective substances participated as follows: *IV*, 69.2 mg (0.298 mmol, 7.9%), *III*, 85.3 mg (0.316 mmol, 8.5%); *II*, 796 mg (3.014 mmol, 79.7%).

b) *In methanol*: Methyl iodide (0.24 ml, 3.8 mmol), methyl sulphide (0.28 ml, 3.8 mmol) and silver oxide (dried, 1 g) were added to a solution of *II* (1 g, 3.78 mmol) in methanol (15 ml). The mixture was shaken in the dark and its composition was checked by TLC as above. Detectable amounts of *III* and *IV* were present after 1/2 h and 2 h, respectively. After 4 h, when the ratio of *II*, *III*, and *IV* was settled and the base-line of the thin-layer chromatogram was detected with both reagents, the mixture was filtered, the solids washed with methanol and the filtrate, combined with the washings, was concentrated. Chromatography of the residue afforded 66.3 mg (0.285 mmol, 7.5%) of the olefin *IV*, 76.6 mg (0.28 mmol, 7.7%) of the *L-altro* derivative *III*, and 590.8 mg (2.235 mmol, 59.1%) of the starting *II*; total recovery 74.3%.

c) *In 1,2-dimethoxyethane containing <sup>2</sup>H<sub>2</sub>O*: Compound *II* (0.1 g, 0.38 mmol) added to a mixture of 1,2-dimethoxyethane (2 ml), (CH<sub>3</sub>)<sub>2</sub>S (0.28 ml, 3.8 mmol), CH<sub>3</sub>I (0.24 ml, 3.8 mmol), Ag<sub>2</sub>O (dried, 0.1 g), and <sup>2</sup>H<sub>2</sub>O (50 μl) was shaken in the dark for 48 h with fresh portions of <sup>2</sup>H<sub>2</sub>O (50 μl) after 5 and 24 h (total amount of <sup>2</sup>H<sub>2</sub>O 150 μl, 7.5 mmol). The composition of the reaction mixture, as shown by TLC, was similar to *a*) and after work-up and chromatography the mass spectra of the isolated *II* and *IIIa* were taken. The deuterium incorporation at C<sub>(5)</sub> found for *II* and *IIIa* were ~ 4 and 37%, respectively. In an attempt to increase the degree of deuteration 0.5 ml (25 mmol) of <sup>2</sup>H<sub>2</sub>O was added into the same reaction mixture. TLC run after 1 h showed the absence of *II*, *III* and *IV*, while an intense spot, detected with both reagents, was present on the base-line.

d) *In [O-<sup>2</sup>H]methanol*: Compound *II* (50 mg, 0.19 mmol) was added to a mixture of CH<sub>3</sub>I (0.0125 ml, 0.2 mmol), Ag<sub>2</sub>O (0.05 g), (CH<sub>3</sub>)<sub>2</sub>S (0.015 ml, 0.2 mmol) in [O-<sup>2</sup>H] methanol (0.825 ml, 20 mmol), and shaken in the dark for 3 h. The composition of the reaction mixture, as shown by TLC, was similar to *b*) and, since there was a strong spot on the base-line detectable with both reagents, the mixture was worked up. Mass spectrometry of *II* and *IIIa*, isolated by chromatography, revealed the degree of deuteration to be 25 and 96%, respectively.

#### Treatment of *V* with (CH<sub>3</sub>)<sub>2</sub>S, CH<sub>3</sub>I, and Ag<sub>2</sub>O

The starting material (100 mg) in [O-<sup>2</sup>H]methanol (1.5 ml) was treated as described for the α-D-galacto compound (*d*). After 4 h TLC (solvent D) displayed *V* to be the sole mobile component of the mixture and revealed an intense spot at the base-line detectable with both reagents. Mass spectrum of *V*, recovered by chromatography, showed that the starting compound was not deuterized at C<sub>(5)</sub>.

## Conversion of III to II

Methyl (methyl 2,3,4-tri-O-methyl- $\beta$ -L-altropyranosid)uronate in methanol was treated with an equimolar amount of  $(\text{CH}_3)_2\text{S}$ ,  $\text{CH}_3\text{I}$  and  $\text{Ag}_2\text{O}$ . TLC (solvent C) showed that after 5–6 h the settled ratio between the starting material and the formed  $\alpha$ -D-galacto derivative was  $\sim 1:1$ . A slight spot was detected on the base-line and, in addition to traces of the olefin IV, an unidentified minor product ( $R_f$  0.15) was also found.

## REFERENCES

1. Bannister B., Kováč P.: Presented at the VII. International Symposium on Carbohydrate Chemistry, Bratislava, August 1974.
2. Adams M. H., Reeves F. R., Goebel W. F.: *J. Biol. Chem.* **140**, 653 (1941).
3. Kováčik V., Kováč P.: *Chem. Zvesti* **27**, 662 (1973).
4. Kováč P.: *Carbohyd. Res.* **22**, 464 (1972).
5. Aspinall G. O., Barron P. E.: *Can. J. Chem.* **50**, 2203 (1972).
6. Kováč P., Hirsch J., Kováčik V.: *Carbohyd. Res.* **32**, 360 (1974).
7. Kováčik V., Kováč P.: *Org. Mass. Spectrom.* **9**, 172 (1974).
8. Listowsky I., Englard S., Avigad G.: *Biochemistry* **8**, 1781 (1969).
9. Morris E. R., Rees D. A., Thom D.: *J. Chem. Soc., Chem. Comm.* **1973**, 245.
10. Grant G. T., Morris E. R., Rees D. A., Smith P. J. C., Thom D.: *FEBS (Fed. Eur. Biochem. Soc.) Lett.* **32**, 195 (1973).
11. Anet F. A. L.: *J. Amer. Chem. Soc.* **84**, 1053 (1962).
12. Feltkamp H., Franklin N. C.: *J. Amer. Chem. Soc.* **87**, 1616 (1965).
13. Durette P. L., Horton D., Bhacca N. S.: *Carbohyd. Res.* **10**, 565 (1969).
14. Anderle D., Kováč P., Hirsch J.: *J. Chromatogr.* **105**, 206 (1975).
15. Kiss J., Burkhardt F.: *Helv. Chim. Acta* **53** 1000 (1970).
16. BeMiller J. N., Kumari G. V.: *Carbohyd. Res.* **25**, 419 (1972).
17. Kiss J.: *Advan. Carbohyd. Chem.* **29**, 229 (1974).
18. Schmidt H. W. H., Neukom H.: *Tetrahedron Lett.* **1969**, 2011.
19. Haug A., Larsen B.: *Carbohyd. Res.* **20**, 225 (1971).
20. Pople J. A., Beveridge B. L. in the book: *Approximative Molecular Orbital Theory*. McGraw-Hill, New York 1970.
21. Chu S., Jeffrey G. A.: *Acta Crystallogr., Ser. B* **24**, 830 (1968).
22. Hao H., Fujimoto H., Fukui K.: *Bull. Chem. Soc. Jap.* **42**, 1256 (1969).
23. Tvaroška I., Klimo V., Valko L.: *Tetrahedron* **30**, 3275 (1974).
24. Fukui K., Hao H., Fujimoto H.: *Bull. Chem. Soc. Jap.* **42**, 348 (1969).
25. Fischer H., Kollmar H.: *Theor. Chim. Acta* **16**, 163 (1970).
26. Wolfe S.: *Accounts Chem. Res.* **5**, 102 (1972).
27. Kováč P., Hirsch J., Tvaroška I., Palovčík R., Kováčik V., Sticzay T.: *This Journal*, in press.
28. Loev B., Goodman M. M.: *Chem. Ind. (London)*. **1967**, 2026.
29. Perrin D. D., Armarego W. L. F., Perrin D. R. in the book: *Purification of Laboratory Chemicals*. Pergamon Press, Oxford 1966.
30. Hirst E. L., Percival E.: *Methods Carbohyd. Chem.* **2**, 145 (1963).
31. Luckett S., Smith F.: *J. Chem. Soc.* **1940**, 1506.
32. Hashimoto H., Sekiyama T., Sakai H., Yoshimura J.: *Bull. Chem. Soc. Jap.* **44**, 235 (1971).
33. Kováčik V., Kováč P.: *Anal. Biochem.* **64**, 45 (1975).

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