FORMATION OF AROMATIC COMPOUNDS FROM CARBOHYDRATES

PART I. REACTION OF D-GLUCURONIC ACID, D-GALACTURONIC ACID, D-XYLOSE, AND L-ARABINOSE IN SLIGHTLY ACIDIC, AQUEOUS SOLUTION*

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(Received September 16th, 1971; accepted for publication, October 1st, 1971)

ABSTRACT

The reaction of D-glucuronic acid in aqueous solutions of pH 3.5 and 4.5 at 96° yielded 2-furaldehyde (**1**), 2-furoic acid (**12**), 2,3-dihydroxy-2-cyclopenten-1-one (reductic acid) (**2**), 1,2-dihydroxybenzene (**7**), 1,2,3-trihydroxybenzene (**8**), 2,3-dihydroxytoluene (**10**), 2,3-dihydroxybenzoic acid (**9**), 2,3-dihydroxyacetophenone (**11**), 3,8-dihydroxy-2-methylchromone (**3**), 5,6,7,8-tetrahydro-3,5-dihydroxy-2-methyl-8-oxobenzopyrone (**4**), and 3-acetyl-1-oxocyclohexane-2,3,6-triol (**18**). The last two compounds were shown to be precursors of **3** and **11**, respectively. Two compounds of formula $C_{10}H_8O_5$ (**5**, **6**) were also isolated. From the reaction of D-galacturonic acid at pH 4.5, most of the above compounds were isolated, but in lower yields, and from D-xylose, compounds **1**, **3**, **4**, **6**, **10**, **11**, and $C_{10}H_8O_4$ were obtained. L-Arabinose yielded the same main components as D-xylose (except for the absence of **6**) but in lower yields.

INTRODUCTION

Cellulose products undergo degradation and yellowing during storage and use, and these ageing processes are accelerated at elevated temperature and by exposure to the atmosphere. At least in the case of highly refined, bleached pulps and products, such a change in quality primarily involves transformations of the carbohydrate constituents. In spite of many and extensive investigations, the mechanism of colour formation is almost unknown.

These earlier studies, summarised in the more-recent publications of Rapson and Corbi[2] and of Beélik[3], show, among other things, that water-soluble mono- and oligo-saccharides, as well as their degradation products 2-furaldehyde (**1**) and its 5-hydroxymethyl derivative, are formed by heat-ageing of pulp. Filter papers and pulp sheets have been impregnated with various carbohydrates of low molecular weight and some potential products of their degradation, and the contribution of these compounds to the yellowing by heat-ageing has been measured[2,3]. Pentoses produce more colour than hexoses, and uronic acids cause more-severe yellowing than aldoses.

*Dedicated to Professor M. Stacey, C.B.E., F.R.S., in honour of his 65th birthday.

Aldoses form acids during ageing[3] and thus accelerate the degradation and yellowing. It was also shown that isomerisation of aldoses occurs under the moderately acid conditions of heat-ageing, and it was pointed out that the enediol intermediates involved are also key intermediates in the formation of 2-furaldehydes[3]. The latter compounds have long been regarded as the main precursors of colour by degradation of carbohydrates.

Recently, the isomerisation of D-glucuronic acid in neutral, aqueous solution into various uronic and hexulosonic acids was reported[4]. This isomerisation occurs more readily and completely down the carbon chain than for monosaccharides. The 4-*O*-methyl derivative of D-glucuronic acid, the important plant-xylan constituent, is reported to be still more readily isomerised[5]. We have found[6] that D-glucuronic acid gives a similar isomerisation pattern in the pH range (3–6) characteristic for ageing, as it does at pH 7. Saccharides of low molecular weight, furan derivatives, and a considerable proportion of uronic acids were found in ethanol extracts of various aged pulps[6,7]. The presence of small amounts of very active colour-precursors, namely reductic acid (2,3-dihydroxy-2-cyclopenten-1-one) (**2**) and some phenolic and/or enolic compounds were also detected[6].

In the present investigation, we have studied the formation of furan derivatives, reductic acid, and other non-carbohydrate compounds of low molecular weight from D-glucuronic and D-galacturonic acids, and from D-xylose and L-arabinose in weakly acidic, aqueous solutions, in order to obtain more knowledge of colour formation and the precursors involved during ageing. The formation of 2-furaldehyde (**1**) and its 5-hydroxymethyl derivative by treatment of pentoses and hexoses, respectively, with acid is well known[8]. The formation of 2-furaldehyde (**1**), 5-formyl-2-furoic acid, and reductic acid (**2**) by acid treatment of certain uronic acids has also been much studied, most recently by Feather and Harris[9]. Compound **2** is also one of the major products obtained by treatment of some keto-glycosides[10] with acid. Aso[11] reported the isolation of small amounts of a benzenoid derivative, 3,8-dihydroxy-2-methylchromone (**3**), after treating alginic acid and other polyuronides in an autoclave at 160°.

RESULTS AND DISCUSSION

In initial, small-scale experiments, aqueous solutions of D-glucuronic acid at pH values in the range 2–8 were kept at 96°. Extraction of the reaction mixtures with ethyl acetate gave (paper chromatography and t.l.c.), *inter alia*, furan derivatives, reductic acid, and a series of compounds giving positive reactions with reagents for phenolic and carbonyl compounds. The maximal yield of the phenolic compounds was obtained at pH ~4. The aqueous phase contained uronic and ulosonic acids, reductic acid, and a group of non-carbohydrate components showing characteristic colours with sugar reagents (in particular with the ketose reagent resorcinol–hydrogen chloride). Some of the last compounds, previously found in the extract of aged pulps[6], were readily transformed into yellow or brown products when a paper chromatogram

of them was heated or exposed to light. Those compounds that are labile and difficult to purify will be discussed later.

The main interest in this investigation has been devoted to the more-lipophilic products, mainly found in the ethyl acetate extract. In a larger scale experiment (*A*), D-glucuronic acid was treated at pH 3.5 and 96° for 48 h under aerobic conditions, the pH being kept constant by continuous addition of sodium hydroxide. The ethyl acetate extract, after fractionation, gave the compounds listed in Table I. These compounds included those previously isolated from hexuronic acids, namely 2-furaldehyde (**1**), reductic acid (**2**), and 3,8-dihydroxy-2-methylchromone (**3**), as well as other compounds (**4–12**). The yields of products were low, which is understandable, since these compounds were shown to react further to yield coloured products. A good illustration of this was given when a t.l.c. plate, made to test the purity of the reductic acid and some of the other compounds (chromones and catechols) isolated, was left unsprayed for one day in the laboratory. The compounds, which immediately gave weakly coloured spots (probably caused by chelate formation with traces of iron in the silica gel), were converted into dark spots of various colours, apparently representing oxidation and polymerisation products.

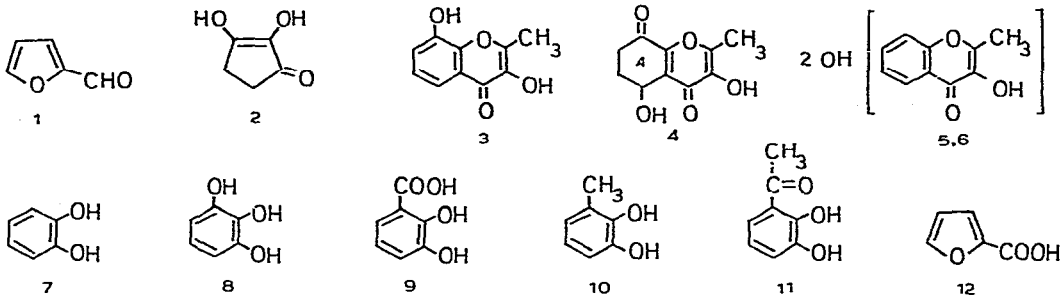
TABLE I

COMPOUNDS ISOLATED AFTER TREATMENT[a] OF D-GLUCURONIC ACID

Compound	*M.p. (degrees)*	R_F *(t.l.c.)*		*Colour with ferric chloride*	*Yields (%)*	
		Solvents a	b		A *pH 3.5*	B *pH 4.5*
1		0.95	0.74		0.4	[b]
2	212–213	0.2[c]	0	white	2.8	2.3
3	228–230	0.47	0.34	violet	3.4	3.0
4	190–192	0.3[c]	0.1[c]	brick-red	1.7	0.8
5	223–224	0.6[c]	0.3[c]	green	0.7	0.9
6	254–256 (dec.)	0.57	0.41	reddish-violet	0.2	0.8
7	103–105	0.56	0.49	bluish-black	0.05	[b]
8	129–131	0.20	0.28	black	[b]	0.05
9	200–204	0.6[c]	0.3[c]	blue	0.1	[b]
10		0.70	0.57	black	0.05	[b]
11	96–98	0.91	0.67	greyish-violet	0.3	0.2
12	129–130	0.77	0.4[c]	yellow	0.2	[b]

[a]For 48 h at 96°, at pH 3.5 and 4.5. [b]Detected, but yields not determined. [c]Tailing.

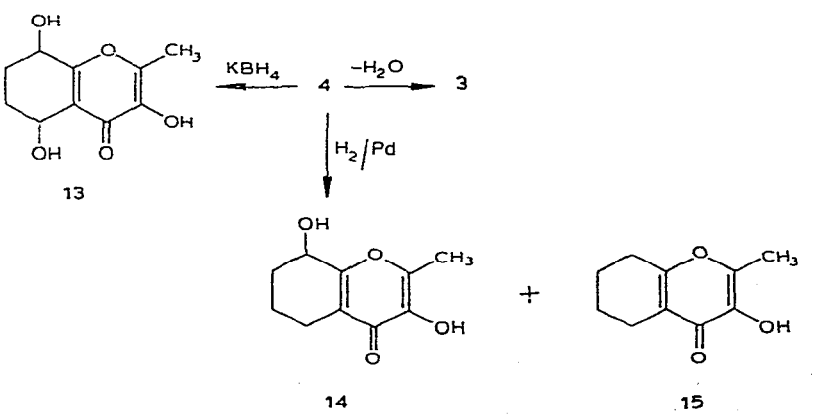
Another experiment (*B*) was performed in acetate buffer at pH 4.5 and under nitrogen. The ethyl acetate extract was fractionated on Sephadex LH-20 and contained all the compounds identified in Expt. *A*, as well as some minor, un-identified products. The yields of the major compounds were similar, with two exceptions (Table I); the amount of the sensitive compound **4** was lower (perhaps because of the extraction procedure) and the amount of **6** was higher.



Compound **3**, the major non-carbohydrate component in the reaction product, showed a molecular ion of 192 in its mass spectrum, consistent with its assignment as 2-methyl-3,8-dihydroxychromone. The m.p. for **3** and its diacetate were identical with those previously reported[11].

Compound **4** was found to be a precursor of **3**, yielding ~50% of **3** upon treatment for 48 h at 96° in aqueous acetate buffer at pH 4.5. In the preliminary communication[1], a structure (6,7,8,8a-tetrahydro-3,8a-dihydroxy-2-methyl-8-oxobenzopyrone) was suggested for this compound, but further studies now indicate the structure 5,6,7,8-tetrahydro-3,5-dihydroxy-2-methyl-8-oxobenzopyrone (**4**). The mass spectrum of **4** shows a molecular ion of 210 and the elemental analysis corresponds to $C_{10}H_{10}O_5$, *i.e.* compound **3**-H_2O. Treatment of **4** with acetic anhydride–pyridine gave a mixture of the acetates of compounds **3** and **4**. With acetic anhydride–hydrogen chloride, only the acetate of **4** was obtained. Borohydride reduction of **4** gave **13**, containing three hydroxyl groups ($C_{10}H_{12}O_5$; m.p. 162–165°). Catalytic hydrogenation of **4** (over 5% palladium-on-carbon) gave two compounds that were apparently products of allylic hydrogenolysis. The main product **14** ($C_{10}H_{12}O_4$; m.p. 168–169°) contained two hydroxyl groups, whereas the second compound (**15**) possessed one hydroxyl group.

Compound **4** and its reaction products have some properties similar to those



Scheme 1

of maltol (**16**). On t.l.c. plates, **16**, **4**, **13**, **14**, and **15** gave the same red colours with ferric chloride. Compounds **13**, **14**, and maltol (**16**) had λ_{max} 278 nm in ethanol, and λ_{max} 320 nm in alkaline ethanol; the corresponding maxima for **15** are at 273 and 317, respectively). These data, along with the n.m.r. data for these compounds and their acetates (Table II) indicate that the maltol ring-system is present in compounds **4**, **13**, **14** (and **15**). The keto group in ring *A* of **4** must be at C-8, since treatment of **4** under weakly acidic conditions gives **3** which has a phenolic group at C-8. The establishment of a hydroxyl group at C-5 in **4** was deduced from the reactions of **4** (shown in scheme 1) and from the n.m.r. data assigned to the products isolated. In the n.m.r. spectrum of the reduction product **13**, there are two one-proton triplets at τ 5.50 and 5.22 in methanol-d_4. The magnitude of the chemical shift values indicates protons on carbon atoms bonded to hydroxyl groups. Two such triplets could only result from a structure having one of the hydroxyl groups at C-8, as previously deduced, and the other at C-5, where they are adjacent to methylene groups. Further support for the location of the hydroxyl group at position C-5 in **13** (and **4**) is given by the n.m.r. data of compound **14**, in which the latter hydroxyl group has been removed. This modification of the structure results in a new two-proton multiplet for methylene protons (τ 7.4–7.6; methanol-d_4), well separated from the four-proton multiplet (τ 7.9–8.2) assigned to H-6,6′ and H-7,7′. Therefore, the ring *A* hydroxyl-group of the precursor **4** must be at C-5. Acid-catalysed elimination of the hydroxyl group, followed by enolisation of the 8-keto group, would give **3**.

TABLE II

FIRST-ORDER CHEMICAL SHIFTS[a] OF COMPOUNDS **4**, **13**, **14**, AND THEIR ACETATES

Compound	*Solvent*	*Chemical Shifts*[b] (τ)					
		H-5	*H-5′*	*H-6,6′,7,7′*	*H-8*	*Methyl*[c]	*Acetyl*[c]
4	Me_2SO-d_6	5.00 t		7.3–8.1 m		7.70	
4	Pyridine-d_5	4.54 t		6.6–7.9 m		7.74	
Acetylated **4**	Chloroform-*d*	3.80 t		7.0–7.6 m		7.69	7.81,7.99
13	Methanol-d_4	5.22 t		7.9–8.2 m	5.50 t	7.68	
13	Pyridine-d_5	4.78 t		7.6–8.1 m	5.30 t	7.77	
Acetylated **13**	Chloroform-*d*	4.02 t		7.8–8.1 m	4.25 t	7.71	7.79,8.00,7.87
14	Methanol-d_4		7.4–7.6 m	7.9–8.2 m	5.48 t	7.63	
Acetylated **14**	Chloroform-*d*		7.3–8.2 m	7.3–8.2 m	4.25 t	7.68	7.76,7.88

[a]Data taken from spectra measured at 60 MHz. [b]Observed multiplicities: m, multiplet; t, triplet. [c]Three-proton singlets.

Two other bicyclic compounds, **5** and **6**, were isolated. They showed great similarities with **3** in their mass-spectrometric fragmentation pattern, as well as in their u.v. and i.r. spectra. Mass spectrometry and elemental analysis showed that each had the formula $C_{10}H_8O_5$, and n.m.r. studies of the two compounds and their crystalline acetates supported the conclusion that they are chromones similar to **3**,

but having one more hydroxyl group. The structures of these compounds are being further studied.

Compounds **7–12** were identified, on the basis of spectroscopic data (variously i.r., m.s., and n.m.r.) and by comparison with authentic compounds, as 1,2-dihydroxybenzene (**7**), 1,2,3-trihydroxybenzene (**8**), 2,3-dihydroxybenzoic acid (**9**), 2,3-dihydroxytoluene (**10**), 2,3-dihydroxyacetophenone (**11**), and 2-furoic acid (**12**). The formation of **7** by treatment of D-glucose under strongly alkaline conditions has been reported by Enkvist[12].

Treatment of D-galacturonic acid at pH 4.5 (as in Expt. *B*) gave, with the exception of 2-furoic acid (**12**), the same compounds as had been isolated from D-glucuronic acid (Table III). All compounds except **4** were obtained in lower yield. It is difficult to draw any conclusions as to whether the lower yields have any structural significance.

TABLE III

COMPOUNDS ISOLATED[a] FROM D-GALACTURONIC ACID AND D-XYLOSE

Compound	*Yield* (%)		*Compound*	*Yield* (%)	
	D-*GalUA*	D-*Xyl*		D-*GalUA*	D-*Xyl*
1	[b]	0.7	**8**	0.05	
2	[b]		**9**	0.05	
3	1.3	0.6	**10**	[b]	[b]
4	1.0	0.2	**11**	0.05	0.2
5	0.2		**12**		
6	0.1	0.05	**17**		0.3[c]
7	[b]				

[a]After treatment for 48 h in aqueous acetate buffer at pH 4.5.[b]Detected in small amount, but not quantitatively determined. [c]M.p. 186–191°; R_F 0.83 (Solvent *a*); brown colour with $FeCl_3$.

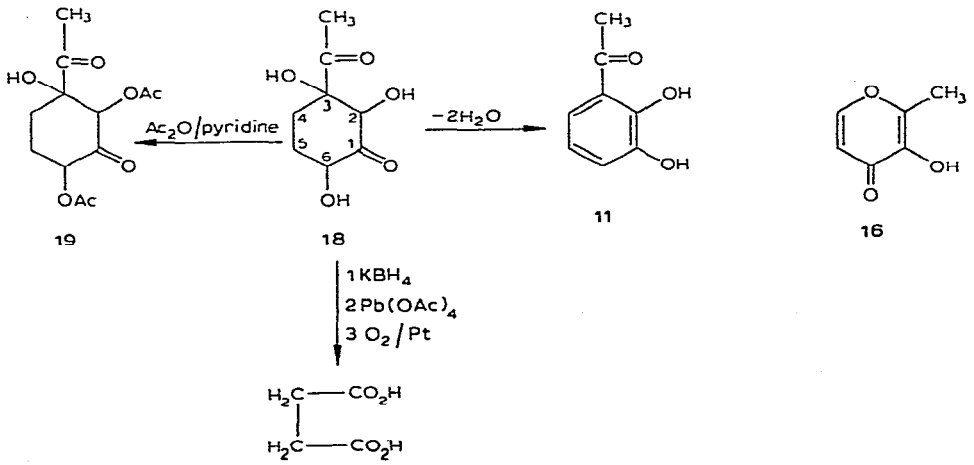
In a small-scale experiment on 4-*O*-methyl-D-glucuronic acid (conditions as in Expt. *B*), the products, which were only partly fractionated, included **1**, **2**, **3**, **4**, **7**, **8**, and **10**. Likewise D-xylose gave the products shown in Table III. Reductic acid (**2**) and some of the minor components obtained from D-glucuronic acid were not detected. Perhaps the most-notable observation is that one of the two $C_{10}H_8O_5$ compounds, namely **5**, was not detected, but another compound (**17**, $C_{10}H_8O_4$) was isolated. L-Arabinose yielded the same main components as D-xylose (an exception was that the chromone **6** could not be detected), but in lower yields (compare the difference between D-galacturonic and D-glucuronic acid). Compounds **3** and **4** were isolated in ~0.1% yield, and compound **17** was present in traces only.

The compound remaining in the aqueous phase from Expt. *A*, after ethyl acetate extraction, were fractionated on Dowex-50(H^+) resin. Although not completely separated, at least when the fractionation was carried out on a preparative scale, the components in the mixture were eluted in the following order: (*a*) uronic acids and

small amounts of pentoses, (*b*) small amounts of pentuloses, (*c*) a group of non-carbohydrate components which formed characteristic colours when treated with sugar reagents, and (*d*) a fraction containing reductic acid (the amount of which was added to the part isolated from the ethyl acetate phase to give the yield recorded in Table I). Finally, small amounts of more lipophilic components (mainly compounds **4** and **12**) were eluted with aqueous ethanol. Our interest was concentrated on a further study of the compounds present in fraction *c*. To isolate these compounds in larger amounts, it was later found to be more convenient to first extract with butanone. Subsequently, chromatography on cellulose and silicic acid columns, followed by preparative t.l.c., gave small amounts of the components which were highly unstable and difficult to isolate in pure form.

However, one component (**18**) was obtained in crystalline form (m.p. 149–150°). Mass spectrometry and elemental analysis indicated the formula $C_8H_{12}O_5$. Furthermore, it was found that, on sublimation, **18** was transformed into 2,3-dihydroxyacetophenone (**11**, 70%). This finding is of conclusive importance for the structural determination of the compound. When heated in an aqueous solution at pH 4.5 and 96°, **18** gave *ca.* 50% of **11**. Compound **18** formed a crystalline acetate (**19**), for which elemental analysis, and n.m.r. and i.r. data indicated one hydroxyl and two *O*-acetyl groups. The fact that one of the hydroxyl groups of **18** was difficult to acetylate indicates that it is tertiary.

The n.m.r. data of compounds **18** and **19** (Table IV) and the fact that succinic acid (**20**) was obtained from **18** after borohydride reduction, and lead tetraacetate and catalytic oxidation indicate that **18** must be 3-acetyl-1-oxocyclohexane-2,3,6-triol rather than 3-acetyl-1-oxocyclohexane-2,3,5-triol, as tentatively suggested[1]. The n.m.r. spectra of **18** and **19** each contain a one-proton singlet consistent with a hydroxyl group at position 2. The fact that succinic acid is formed in the degradation of **18** excludes the presence of a hydroxyl group at position 5. The fact that the other



19 18 20

TABLE IV

FIRST-ORDER CHEMICAL SHIFTS[a] OF COMPOUND **18** AND ITS ACETATE **19**

Compound	*Solvent*	*Chemical Shifts*[b] (τ)					
		H-2	*OH-3*	*H-4,4',5,5'*	*H-6*	*Ac-3*	*AcO-2,6*
18	Methanol-d_4	5.44 s	5.36 s[c]	7.5–8.1 m	5.57 t (*J* 8 Hz)	7.69	
19	Chloroform-*d*	4.34 s	5.95 s	7.3–7.8 m	4.40 t (*J* 8 Hz)	7.72	7.88–7.98

[a]Data taken from spectra measured at 60 MHz. [b]Observed multiplicities: m, multiplet; s, singlet; t, triplet. [c]Three hydroxyl protons (OH-3), together with OH-2 and OH-6).

one-proton signal is a triplet, with a shift similar to that of H-2, indicates that it must be located adjacent to the ring carbonyl, *i.e.* position 6, which accordingly is also the position of the hydroxyl group in formula **18.**

It is most likely that many of the compounds isolated from the treatment of uronic acids and pentoses under these slightly acidic conditions are intermediates in colour formation during heating and ageing of cellulosic material containing xylans or other polysaccharides having uronic acids and pentoses as structural units.

Studies, which will be published elsewhere, indicate that reductic acid and some of the phenolic compounds are more-important colour precursors than are 2-furaldehydes. It is reasonable to assume that transformations through similar compounds, as shown in the present investigation, are also of importance in the degradation of plant polysaccharides in Nature, for instance in the slow humification of *Sphagnum* mosses into peat. These mosses are rather rich in uronic acids [13], but low in the content of aromatic lignin[14,15]. The conditions in a peat bog are unfavourable for microbial reactions and the pH is slightly acidic.

The mechanisms involved in the formation of phenolic compounds from uronic acids and pentoses under slightly acidic conditions are complex, and obviously include transformation of the carbohydrates, fragmentation, recombination of fragments, and ring-closure to produce non-aromatic intermediates, two of which (**4** and **18**) have been isolated in the present investigation. Without discussing the mechanisms further at the present stage, one might point out the similarity between the structures of the latter compounds and intermediates in the biogenetic (or modelled) synthesis of phenolic compounds. One example is the recently reported, biogenetically modelled synthesis of methyl 6-hydroxy-2,4-dioxo-6-phenylcyclohexanecarboxylate (by cyclisation of a 3,5,7-triketo-ester), which was readily aromatised by acid-catalysed dehydration[16].

EXPERIMENTAL

General. — Concentrations were carried out at diminished pressure below 40°. The melting points are corrected.

Paper chromotograms (Whatman No. 1 paper) were run in 6:2:1 1-butanol–acetic acid–water, or butanone saturated with water. T.l.c. was performed on Silica Gel HF_{254} (Merck) plates with (*a*) 9:1 dichloromethane–acetic acid, (*b*) 4:1 dichloromethane–acetonitrile, and (*c*) 3:2 dichloromethane–acetone. Silicic acid (100 mesh, Mallinckrodt), Sephadex LH-20, and Dowex-50W x8 (H^+, 50–100 mesh) were used for column chromatography. Distilled solvents were used for the preparative column and t.l.c. separations.

Standard spray reagents were used for sugars, carbonyl compounds, alditols, and phenols (or enols), namely (*a*) *p*-anisidine hydrochloride, (*b*), resorcinol–hydrochloric acid, (*c*) silver nitrate–sodium hydroxide, (*d*) 2,4-dinitrophenylhydrazine, (*e*) periodate–benzidine, (*f*) diazotised sulphanilic acid, and (*g*) ferric chloride. Spots on papers and t.l.c. plates were also studied in u.v. light (366 nm) before spraying. Acetates and products that were unreactive towards the above reagents were detected (t.l.c. plates) with 25% sulphuric acid (and heating) or with iodine vapour. N.m.r. spectra were recorded at 60 MHz. Chemical shifts are given in τ units (tetramethylsilane, internal standard). Mass spectra were recorded on a Perkin–Elmer 270 spectrometer.

The sublimations (or distillation) were done at 0.5 mmHg in an electrically heated tube. The tube was connected to a cooled trap, when 2-furaldehyde was collected. The reference samples of 2-furaldehyde (**1**), 2-furoic acid (**12**) (m.p. 129–130°), 1,2-dihydroxybenzene (**7**) (m.p. 103–105°), 1,2,3-trihydroxybenzene (**8**) (m.p. 132–133°), and 2,3-dihydroxytoluene (**10**) (m.p. 63–66°) were commercial ones. The sample of reductic acid (m.p. 211–212°) had been previously isolated at this laboratory[10]. The reference sample of 2,3-dihydroxyacetophenone (m.p. 96–98°) was prepared, in low yield, from pyrocatechol, as previously described[17], and 2,3-dihydroxybenzoic acid (m.p. 200–204°) was obtained in 60% yield by oxidation of compound **3** with hydrogen peroxide[11].

Treatment of D-*glucuronic acid.* — In a series of small-scale experiments, D-glucuronolactone (20 mg) in water, or standard buffer solutions (0.6 ml) in the pH range *ca.* 2–8, was heated for various times at 96°. After the reaction, the products in the dark-brown solutions were partitioned between water and ethyl acetate. The two fractions were studied chromatographically to determine the approximate yields of various reaction products. The aqueous phases contained uronic and ulosonic acids and their lactones, small amounts of monosaccharides, and various non-carbohydrate components.

The ethyl acetate extracts contained a series of compounds giving positive reactions towards the sprays *a*, *d*, *f*, and *g*. The maximal yield of these lipophilic products was obtained at pH ~4 and on treatment for 2 days. Many components in the aqueous and ethyl acetate phases were obviously active intermediates in the colour formation, because they readily gave coloured spots when paper chromatograms and t.l.c. plates were heated or exposed to light.

Experiment A. — D-Glucurono-6,3-lactone (40.00 g) in water (1.52 l) was heated at 96° for 48 h in a flask having a reflux condenser. M Sodium hydroxide was

added initially, and periodically afterwards, to adjust the pH to 3.5; a total of 91 ml was required. The cooled, brown solution (with some precipitate) was extracted with ethyl acetate (5 × 1.50 l), and the combined extracts were dried (Na_2SO_4) and evaporated. The resulting syrup (7.48 g) was fractionated on a silicic acid column (4.5 × 120 cm), using 4:1 dichloromethane–acetonitrile. Nine fractions were collected and are listed below in order of elution. The weight of each fraction and its main constituents are given in brackets; the total yields of the main compounds purified from the various fractions are given in Table I.

Fraction I (0.22 g; **1**); compound **1** was isolated by vacuum distillation.

Fraction II (0.23 g; **11** and **12**); the two components were separated by preparative t.l.c., and a part of each was purified by sublimation.

Fraction III (0.07 g; **10**); compound **10** was purified by sublimation.

Fraction IV (0.30 g; **5**); compound **5** crystallised directly upon evaporation, and was purified by recrystallisation from ethanol.

Fraction V (0.66 g; **5**, **6**, and **9**); the three main components were obtained in pure state after another separation on a silicic acid column (2 × 90 cm), using 9:1 dichloromethane–acetonitrile, followed by sub-fractionation on t.l.c. plates with solvent *a*. Part of **6** was recrystallised from ethanol, and **9** was completely purified by sublimation.

Fraction VI (1.65 g; **6**, **7**, and **3**); the main component was **3**, which crystallised directly and was recrystallised from ethanol. The other two components were fractionated by sublimation.

Fraction VII (1.26 g; **3** and **4**); the main component was **3**, obtained directly by crystallisation. Compound **4** was isolated from the mother liquor by preparative t.l.c. (solvent *a*) and crystallised from ethanol.

Fraction VIII (0.52 g; **4**); **4** was obtained from this fraction (which also contained condensation products and various minor, low-molecular components) by direct crystallisation from ethanol, and from the mother liquor by t.l.c.

Fraction IX (1.82 g; **2** and **8**); from this fraction (containing considerable amounts of high-molecular material), **2** was obtained by crystallisation from 2:1 ethanol–acetone. A small amount of **8** was detected in the mother liquor.

The aqueous fraction was concentrated to 150 ml and placed on a column (4.7 × 120 cm) of Dowex-50 (H^+) resin and eluted with water. The fractions were studied by paper chromatography. The components were eluted in the following order: (*a*) uronic and ulosonic acids and their lactones, as well as small amounts of pentoses; (*b*) small amounts of pentoses, together with tailing from previous fractions; (*c*) (4.8 g) a group of non-carbohydrate components, together with tailing from previous fractions and some condensation products; and (*d*) (1.2 g) reductic acid (**2**), together with condensation products. Finally, elution with 50% aqueous ethanol gave a fraction (*e*) (0.7 g) containing condensation products and small amounts of compounds **4** and **12**. Fractions *a* and *b* were not further studied in the present investigation. The amount of **2** in fraction *d* was obtained by sublimation of an aliquot. The amounts of **4** and **12** in fraction *e* were estimated by preparative t.l.c. (solvent *a*) and

sublimation, respectively, on aliquots. The additional amounts of the three compounds found are included in the total yields given in Table I.

Fraction *c* was placed on a column (3 × 87 cm) of cellulose (Whatman Standard Grade, 60 mesh) and eluted with butanone saturated with water. Three main fractions were collected: (*a*) (0.85 g) containing some compounds that formed colours with the spray reagent *a* and *b*; (*b*) mainly D-glucuronic acid and isomers; and (*c*) (0.29 g) containing two components, giving similar colour reactions as the compounds in fraction *a*. Fraction *a* was sub-fractionated on a silicic acid column (2 × 65 cm), using 3:2 dichloromethane–acetone as eluant. Some components were destroyed during this procedure, but two compounds were enriched in separate fractions. However, by paper chromatography in butanone saturated with water, they had identical R_F values (0.61). They were further purified by preparative t.l.c. (solvent *c*). One of the compounds (R_F 0.27, solvent *c*, brown with spray *a* and violet with spray *b*) was very labile and not identified. There are indications that it reacts further to coloured products *via* two of the components present in fraction *c* from the cellulose column (R_F values 0.22 and 0.29, respectively, on paper chromatography in butanone saturated with water). The other compound (**18**) (R_F 0.35 in solvent *c*, yellow with spray *a* and weakly brown with spray *b*) was obtained crystalline and characterised as described below. All components described here with R_F values have also been detected in ethanol extracts of aged pulps[6].

In a later experiment, in order to obtain more of these labile compounds, the aqueous solution from a similar experiment as Expt. *A*, after the batchwise extraction with ethyl acetate, was extracted with butanone (5 × 1 litre), and the latter extract, after evaporation, was worked up by cellulose and silicic acid chromatography as described above.

Experiment B. — (*a*) D-Glucurono-6,3-lactone (40.00 g) in a 0.5M acetate buffer (pH 4.5) (1.5 l) was treated for 48 h at 96°, with a stream of nitrogen bubbling through the solution. The reaction mixture was continuously extracted for 2 days with ethyl acetate (2 × 1.5 l) and then for 1 day with butanone (1.5 l), yielding syrups of 5.96 and 2.73 g, respectively. The latter extract contained insignificant amounts of the compounds given in Table I, and low yields of the labile, more-hydrophilic compounds described under Expt. *A*, and was discarded. The ethyl acetate extract, which was dried well *in vacuo* after evaporation, and therefore was almost free from **1**, was fractionated on a Sephadex LH-20 column (4.7 × 110 cm). Eight main fractions were collected by elution with water, and a final one using ethanol as eluant. The weights of these fractions and their main components are given below. The total yields of compounds isolated in Expt. *B* are given in Table I. They were purified essentially as in Expt. *A*, if not otherwise stated: *I* (1.10 g; **2** and **4**); the two compounds were separated on a silicic acid column, using 4:1 dichloromethane–acetonitrile. *II* (0.48 g; **2**). *III* (0.16 g; **12** and unidentified components). *IV* (0.09 g; **11**). *V* (0.23 g; **7** and **8**); the two compounds were readily separated by sublimation; very characteristic for compound **8**, also when present in small amounts in a complex mixture, is the red colour on t.l.c. with 1% vanillin in conc. sulphuric acid (and heating). *VI* (0.14 g; **10**

and an unknown compound with R_F 0.40 and greyish black with reagent *g*). *VII* (0.62 g; **3**). *VIII* (0.42 g; **3** and **5**). *IX* (eluted with ethanol; 0.90 g; **3**, **5**, and **6**).

(*b*) D-Galacturonic acid (20.00 g) was treated in 0.5M acetate buffer (pH 4.5) (750 ml) and worked up as described in (*a*). The ethyl acetate extract amounted to 1.82 g. The compounds identified and the yields of those isolated in pure form are given in Table III.

(*c*) 4-*O*-Methyl-D-glucuronic acid (0.20 g), prepared from a birch xylan[18], in a 0.5M acetate buffer (pH 4.5) (8 ml), was treated as described in (*a*). The reaction mixture was then extracted with ethyl acetate (3 × 10 ml), and the combined extracts were evaporated to dryness to give a residue (*ca.* 25 mg) which was partly fractionated by sublimation. The fractions were studied by t.l.c. (solvents *a* and *b*), and the following compounds were detected: **1**, **2**, **3**, **4**, **7**, **8**, and **10**.

(*d*) D-Xylose (40 g) in a 0.5M acetate buffer (pH 4.5) (1.52 l) was treated as (*a*), and the product worked up essentially as in Expt. *A*. The ethyl acetate extract was 2.22 g; the yields are given in Table III. In one fraction (0.21 g), **11** was accompanied by compound **17** which was not detected in the products from the uronic acids. Compounds **11** and **17** were separated on a silicic acid column (1.5 × 60 cm), using 97:3 benzene–methanol. Compound **17** was recrystallised from ethanol.

In other experiments, solutions of D-xylose and L-arabinose (10.00 g each) in 0.5M acetate (pH 4.5) buffer (380 ml) were heated and worked up essentially as (*a*). The ethyl acetate extracts amounted to 0.55 and 0.23 g, respectively. T.l.c. studies of the fractions from Sephadex chromatography revealed no major components other than those found in the previous experiment with D-xylose (see Table III). An exception was that **6** could not be detected from L-arabinose. Compound **17** was found in traces only. From L-arabinose, **3** and **4** were isolated in pure form (yield, *ca.* 0.1%).

Characterization and identification of compounds **3**, **4**, **5**, **6**, **17**, *and* **18** (Some data are given in the Tables or in the text). — *2-Methyl-3,8-dihydroxychromone* (**3**). The physical data for **3** and its acetate, which was prepared using acetic anhydride–pyridine (1:1) at 35° for 15 min, were identical with lit. values[11].

Anal. Calc. for $C_{10}H_8O_4$: C, 62.5; H, 4.2. Found for **3**: C, 62.3; H, 4.2. Calc. for $C_{14}H_{12}O_6$: C, 60.9; H, 4.31; O, 34.8. Found for **3**-acetate: C, 60.7; H, 4.41; O, 34.8.

5,6,7,8-Tetrahydro-3-5-dihydroxy-2-methyl-8-oxo-benzopyrone (**4**). The compound was obtained as light-yellow crystals (strong yellow fluorescence on t.l.c. plates in u.v. light), with no optical rotation; λ_{max} (EtOH): 233 (ε 18,600), 260 (inflection, 5500), 330 (3150), and (alkaline EtOH) 255 (35,000), 285 (inflection, 5500), 415 nm (5300); ν_{max}^{KBr} strong bands at 1680, 1600, and 1560 cm^{-1}. Mass spectrum: *m/e* 39 (10% base peak), 43(90), 53(15), 55(20), 65(12), 66(10), 69(15), 93(40), 94(16), 97(10), 98(14), 121(60), 122(19), 126(12), 153(12), 154(45), 164(30), 192(100), 193(12), 209(15), 210(80, M^+), 211(11).

Anal. Calc. for $C_{10}H_{10}O_5$: C, 57.1; H, 4.8. Found: C, 56.7; H, 4.7.

Treatment with acetic anhydride–pyridine (1:1) at 35° for 15 min, or at 4° for 16 h, gave a mixture of the acetates of **3** and **4**. Acetylation with acetic anhydride–conc. hydrochloric acid (99:1) at 4° for 16 h yielded exclusively the acetate of **4**. This was

purified by t.l.c. using benzene–methanol (97:3), but was not obtained crystalline. Treatment of **4** (50 mg) in acetate buffer of pH 4.5 (5 ml) at 96° for 48 h yielded *ca.* 50% of compound **3**, which was isolated by t.l.c. (solvent *a*), and identified by its chromatographic properties, m.p., and i.r. and mass spectra.

Borohydride reduction of **4** (40 mg) in aqueous solution yielded 5,6,7,8-tetrahydro-3,5,8-trihydroxy-2-methylbenzopyrone (**13**), which crystallised spontaneously. The compound, purified by sublimation, was colourless, m.p. 170–171°; λ_{max} (EtOH): 232 (inflection, ε 15,500), 278 (6750), and (alkaline EtOH) 227 (18,500), 320 nm (6100); ν_{max}^{KBr}: strong bands at 1650, 1600, and 1570 cm^{-1}. Mass spectrum: *m/e* 39 (21% base peak) 41(12), 42(18), 43(90), 44(24), 45(58), 50(18), 51(24), 52(22), 55(15), 60(24), 62(10), 67(16), 75(10), 76(11), 77(28), 78(100), 79(11), 95(20), 105(14), 123(48), 166(8), 176(9), 181(19), 184(11), 194(33), 212(10, M^+).

Anal. Calc. for $C_{10}H_{12}O_5$: C, 56.6; H, 5.6. Found: C, 56.4; H, 5.8.

A sample of **4** (32 mg; 0.152 mmole) in ethanol (15 ml) was shaken at 22° in a hydrogen atmosphere in the presence of 5% palladium-on-carbon; the uptake of hydrogen was very slow. After 3 h, the treatment was interrupted (total uptake of hydrogen; 0.305 mmole). T.l.c. showed that no **4** was present; the major product was **14**, together with a small amount of **15**. Compounds **13**, **14**, **15**, and maltol (**16**) gave almost identical colours when sprayed with spray *g*, namely first wine-red and then brick-red. They had the following R_F values on t.l.c. (solvent *a*): **13**, 0.19; **14**, 0.60; **15**, 0.92; and **16**, 0.90. Compounds **14** and **15** were separated by t.l.c. (solvent *a*), and 23 and 3 mg, respectively, were obtained in a pure state.

5,6,7,8-Tetrahydro-3,8-dihydroxy-2-methylbenzopyrone (**14**), recrystallised from ethanol, had m.p. 168–170°, λ_{max} (EtOH): 215 (inflection, ε 12,100), 249 (inflection, 5600), 278 (7200), and (alkaline EtOH) 222 (18,000) and 320 nm (6200); ν_{max}^{KBr}: strong bands at 1650, 1600, and 1580 cm^{-1}. Mass spectrum: *m/e* 39 (21% base peak), 41(19), 43(90), 54(28), 77(13), 79(12), 83(28), 97(50), 107(11), 125(13), 140(12), 153(100), 154(12), 167(12), 177(12), 179(32), 180(10), 195(13), 196(90, M^+), 197(13).

Anal. Calc. for $C_{10}H_{12}O_4$: C, 61.2; H, 6.1. Found: C, 61.5; H, 6.4.

5,6,7,8-Tetrahydro-3-hydroxy-2-methylbenzopyrone (**15**). The mode of formation, the chromatographic properties, and the u.v. and mass spectra indicate that this is the structure of compound **15**. The compound had λ_{max} (EtOH): 213 (inflection, ε 10,100), 254 (inflection, 5900), 273 (6800), and (alkaline EtOH) 226 (15,000), and 317 nm (5400). Mass spectrum: *m/e* 39 (15% base peak) 41(18), 43(45), 53(11), 55(20), 57(11), 67(10), 69(10), 77(11), 79(12), 81(14), 91(8), 109(9), 123(6), 149(11), 165(25), 179(100), 180(85, M^+), 181(11).

For comparison, the u.v. data of maltol (**16**) are: λ_{max} (EtOH) 278 nm (ε 9600), and (alkaline EtOH) 320 nm (ε12,000).

Compound **5**, obtained as light-yellow crystals after sublimation and recrystallisation from ethanol, had m.p. 223–224°.

Anal. Calc. for $C_{10}H_8O_5$: C, 57.7; H, 3.9. Found: C, 57.2; H, 3.9.

Compound **6**, obtained as light-yellow crystals after sublimation and recrystallisation from ethanol, had m.p. 254–256° (dec.).

Anal. Calc. for $C_{10}H_8O_5$: C, 57.7; H, 3.9. Found: C, 57.2; H, 3.7.

Compound **17**, obtained as reddish-yellow crystals after recrystallisation from ethanol, had m.p. 186–191°, λ_{max} (EtOH): 243 (ε 3520) and 353 nm (ε 13,280); ν_{max}^{KBr}: strong bands at 3140, 1665, and 1590 cm^{-1}. Mass spectrum: *m/e* 39 (13% base peak), 43(42), 51(12), 52(22), 53(9), 63(9), 65(21), 80(9), 108(6), 121(93), 122(11), 136(1), 163(2), 177(3), 192(100, M^+), 193(14).

Anal. Calc. for $C_{10}H_8O_4$: C, 62.5; H, 4.2. Found: C, 62.6; H, 4.2.

3-Acetyl-1-oxocyclohexane-2,3,6-triol (**18**), when recrystallised from ethanol–ethyl acetate (1:1), had m.p. 149–150°; ν_{max}^{KBr} strong bands at 3450, 1720, and 1700 cm^{-1}. Mass spectrum: *m/e* 39 (20% base peak), 41(25), 42(19), 43(96), 44(19), 45(19), 53(27), 55(43), 57(30), 69(16), 71(21), 73(23), 81(15), 85(43), 86(10), 99(45), 100(10), 109(23), 103(12), 127(100), 128(11), 145(36), 153(3), 170(2), 188(3, M^+).

Anal. Calc. for $C_8H_{12}O_5$: C, 51.1; H, 6.4. Found: C, 51.4; H, 6.5.

Compound **18** (30 mg) was acetylated with acetic anhydride and pyridine (1:1) at 35° for 15 min, and the acetate (**19**), when recrystallised from ethanol, had m.p. 167–168°; ν_{max}^{KBr} strong bands at 3500, 1750, 1730, and 1715 cm^{-1}.

Anal. Calc. for $C_{12}H_{16}O_7$: C, 52.9; H, 5.9. Found: C, 52.6; H, 5.8.

Attempted sublimation of **18** (30 mg) gave 2,3-dihydroxyacetophenone (**11**) in 70% yield, which was identical with an authentic sample by t.l.c., m.p., and i.r., n.m.r., and mass spectrometry. Treatment of compound **18** in acetate buffer (pH 4.5) at 96° for 48 h yielded *ca.* 50% of compound **11**.

Compound **18** (16 mg) was reduced with aqueous sodium borohydride and the product, after treatment with Dowex-50 (H^+) resin and removal of boric acid by methanol distillation, was oxidised with lead tetraacetate[19] (750 mg) in pyridine (4 ml) at 22° for 2 h. To the reaction mixture were then added a few drops of water and oxalic acid (1600 mg), the precipitate was filtered off, the filtrate evaporated to dryness and the residue extracted with ethyl acetate (3 × 25 ml). The latter extract was evaporated, the residue was dissolved in water (10 ml) to which was added sodium carbonate (50 mg) and platinum catalyst (40 mg, 5% on carbon), and the mixture was treated with a stream of finely divided oxygen at 60° for 2 h. The product, obtained by treatment of the filtered solution with Dowex-50 (H^+) resin and evaporation, was shown by paper electrophoresis (pH 4.5; buffer, 2:3:30 pyridine–acetic acid–water; detection with bromophenol after heating) to contain mainly succinic acid. The main part of the product was dissolved in methanol (5 ml), to which was added two drops of conc. sulphuric acid, and the mixture was heated for 2 h in a boiling water-bath. Then, water (10 ml) was added, and the product was extracted with ethyl acetate. It was shown by g.l.c. (Perkin–Elmer 900, column of 3% OV 1) that the extract contained mainly a product having the same retention times as methyl succinate. The structure was further proved by its mass spectrum, being identical with the mass spectrum of an authentic sample: *m/e* 43 (13% base peak), 44(19), 45(13), 55(84), 59(59), 87(25), 114(27), 115(100), 146(1, M^+).

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