Dissociation constants of D-galacturonic and D-glucuronic acid and their *O*-methyl derivatives

R. KOHN and P. KOVÁČ

Institute of Chemistry, Slovak Academy of Sciences, 809 33 Bratislava

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Dissociation constants (pK, 20°C) of D-galacturonic acid, D-glucuronic acid, their *O*-methyl derivatives, as well as of the corresponding 4,5-unsaturated substance have been determined by means of discontinuous potentiometric titration. The found pK values have been interpreted from the point of view of inductive and mesomeric effects, and nonbonding interactions. It is demonstrated that the effect of the methoxy substitution at various positions of D-galacturonic acid on its acidity has an additive character.

Методом скачкообразного потенциометрического титрования определены константы диссоциации (pK, 20°C) D-галактуроновой и D-глюкуроновой кислот, их *O*-метилпроизводных и соответствующей 4,5-ненасыщенной уроновой кислоты. Полученные значения pK объяснялись с точки зрения индукционных и мезомерных эффектов и дисперсионных взаимодействий. Была доказана аддитивность влияния заместителя —OCH_3 на отдельных углеродных атомах D-галактуроновой кислоты на ее силу.

D-Galacturonic acid is the main constituent of pectin, a large amount of which is present in plants. D-Glucuronic acid is an abundant component of numerous natural polysaccharides; it is very important in metabolic processes occurring in living organisms [1]. 4-Deoxy-β-L-*threo*-hex-4-enopyranosyluronic acid is formed in the process of digestion of animals and man by the action of enzymes of microbial origin such as pectin lyase and pectate lyase [2—5], as well as by analogous base-catalyzed depolymerization of pectin [6]. This 4,5-unsaturated acid occurs among the degradation products of these processes either as a monomeric unit or as the end-unit of oligomeric fragments of pectin.

Detailed information about various properties of uronic acids and their derivatives is important in structural research on acid polysaccharides, and to understand

more deeply the physicochemical properties and biological functions of these substances. The objective of the present work was to determine the dissociation constants of D-galacturonic and D-glucuronic acid, a number of their *O*-methyl derivatives, and of the corresponding 4,5-unsaturated uronic acid.

Experimental

D-Galacturonic acid (*I*) was obtained from Fluka, A.G. (Switzerland) and from Nutritional Biochemicals (USA). D-Glucuronic acid (*VII*) was a product of Sigma Chemical Corp. (Grade I, USA) and BDH Chemicals (England).

Methyl (methyl α-D-galactopyranosid)uronate [7], its 4-*O*-methyl- [8], 2,3-di-*O*-methyl- [9], 2,4-di-*O*-methyl- [10], 2,3,4-tri-*O*-methyl- [11] derivatives, methyl (methyl 2,3,4-tri-*O*-methyl-α-D-glucopyranosid)uronate [12], and methyl (methyl 4-deoxy--β-L-*threo*-hex-4-enopyranosid)uronate [13] were prepared and characterized as described previously. Other chemicals were of reagent grade purity. The used 0.05 M sodium hydroxide solution was carbonate-free. The buffers were obtained from SEVAC (Prague; pH 4.00, 7.00), Merck (pH 4.00, 7.00), and Radiometer (Copenhagen; pH 6.50); the given pH values (±0.02) hold for 20°C. Freshly boiled redistilled water showing specific conductivity less than 2×10^{-4} S·m^{-1} was used.

Potentiometric titrations were carried out using a Radelkis OP-205 (Budapest) instrument equipped with a glass (Radiometer, type G 202 B) and a calomel (SCE) electrode.

Determination of the dissociation constant

Solutions of free acids were prepared by treatment of the solutions of the corresponding methyl esters at a concentration of 0.010—0.012 equiv. $COOCH_3$/l with 0.020—0.024 M sodium hydroxide for 18 h at room temperature. Air was excluded during this operation. To assure the absence of any lactone, D-glucuronic acid was treated in the same manner. With the exclusion of atmospheric carbon dioxide, the resulting solutions were percolated through a column of Dowex 50WX2 (H^+ form) and the obtained solutions of acids were used immediately for the determination of the dissociation constant.

The calculation of the dissociation constant K was based on the data obtained by discontinuous potentiometric titration. The 3.00 mM solutions of the acids were titrated step-wise at 20±0.1°C with 0.05 M sodium hydroxide without the addition of any electrolyte. The ionic strength of the solution and its concentration was not kept constant during this operation. The function of the glass electrode was checked at the beginning and at the end of each potentiometric titration with the aid of buffers. The levelled-off potential of the indication electrode was read 5 min after the addition of a portion of the sodium hydroxide solution.

The dissociation constants K were calculated according to eqns (*1*) and (*2*)

$$pK = pH - \log \frac{[RCOO^-]}{[RCOOH]} \qquad (1)$$

$$[RCOO^-] = [B] + [H^+] - [OH^-] \qquad (2)$$

where $[B]$ is the concentration of the salt formed by the addition of sodium hydroxide. The proton concentration $[H^+]$ was calculated from the pH values. In order to simplify the calculations it was assumed that in these highly diluted solutions $a_{H^+} \doteq [H^+]$. Under these experimental conditions the member $[OH^-]$ could be neglected.

The checking calculations of the thermodynamic dissociation constant (pK_a) were made according to eqn (*3*)

$$pK_a = pH - \log \frac{a_{RCOO^-}}{a_{RCOOH}} = pH - \log \frac{[RCOO^-]\,\gamma_1}{[RCOOH]\,\gamma_2} \qquad (3)$$

where γ_1 and γ_2 are the single-ion activity coefficients for the altering ionic strength I. When $[RCOO^-]$ and [RCOOH] were calculated, in contrast to the preceding procedure, the change of the concentration as well as the single-ion activity coefficient γ_{H^+} (converting pH to $[H^+]$) were taken into account.

The dissociation constants pK and pK_a were calculated for 10 different degrees of ionization α, starting from the initial value up to $\alpha = 0.85$.

Results and discussion

Verification of the working procedure

It is known [14] that under alkaline conditions uronic acids may undergo oxidative degradation. Therefore, we have run experiments with D-galacturonic acid and methyl ester of its α-methyl glycoside and found that under the above-described conditions of deesterification with base no degradation occurs, *i.e.* the process had no effect upon the dissociation constant determined.

Since some of the compounds under investigation were available only in very small quantities, the dissociation constants were calculated from the data obtained by common discontinuous potentiometric titration. The first calculations were done according to eqns (*1*) and (*2*), not taking into account the change of the ionic strength and of the concentration during the process of neutralization. Fig. 1 shows the pK values found for D-galacturonic acid and its *O*-methyl derivatives *vs.* degree of ionization α. It is obvious that in a relatively wide range of α, corresponding to the degree of neutralization *DN* 0.2—0.75, the pK remains practically constant (within the experimental error ± 0.01).

In some cases the thermodynamic dissociation constant pK_a was also calculated using the exact procedure (eqn (*3*)), tabulated values γ_i calculated according to Debye—Hückel theory were used [15]. Since the effective diameter of the

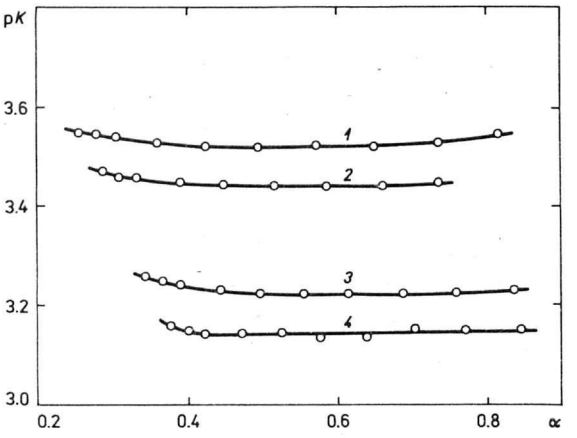

Fig. 1. Dissociation constants (pK) of D-galacturonic acid and its O-methyl derivatives *vs.* the degree of ionization α.
1. D-Galacturonic acid; *2.* methyl 2,3-di-O-methyl-α-D-galactopyranosiduronic acid; *3.* methyl 4-O-methyl-α-D-galactopyranosiduronic acid; *4.* methyl 2,3,4-tri-O-methyl-α-D-galactopyranosiduronic acid.

hydrated uronic acid anion (a_i') has not yet been determined it was problematic to decide what values should be used for this parameter. Finally, we have decided to calculate pK_a using the values known for a small anion (acetic acid, $a_i' = 0.45$ nm) and a bulky anion (diphenylacetic acid, $a_i' = 0.80$ nm). Since very diluted solutions were used the activity coefficient of the undissociated uronic acid (γ_2) was considered to be equal to unity. The results showed that pK and pK_a differed within the experimental error, *e.g.* for $\alpha = 0.5$ the pK was lower only by 0.01 than pK_a. Consequently, the very good agreement of pK and pK_a, together with the constant value of pK in a relatively wide range of α, justify the use of the described experimental technique and the simplified calculation of the dissociation constant of uronic acids described above.

Dissociation constants pK

Dissociation constants pK found for the compounds under investigation are given in Table 1. The shown differences (ΔpK) caused by substitution refer to the corresponding unsubstituted uronic acid; ΔpK for the 4,5-unsaturated derivative *IX* refers to the pK of *I*.

The pK values for *I* and *VII* are mean values of 15 measurements; for other substances mean values of 3—5 analyses are given. The mean values were

Table 1

Dissociation constants (pK) of uronic acids and their derivatives ([COOH] = 3.00 mequiv./l)

Compound	Acid	pK (20°C)	ΔpK
I	D-Galacturonic	3.51 ± 0.01	—
II	Methyl α-D-galactopyranosiduronic	3.51 ± 0.01	0.00
III	Methyl 4-*O*-methyl-α-D-galactopyranosiduronic	3.22 ± 0.01	− 0.29
IV	Methyl 2,4-di-*O*-methyl-α-D-galactopyranosiduronic	3.22 ± 0.01	− 0.29
V	Methyl 2,3-di-*O*-methyl-α-D-galactopyranosiduronic	3.42 ± 0.01	− 0.09
VI	Methyl 2,3,4-tri-*O*-methyl-α-D-galactopyranosiduronic	3.13 ± 0.01	− 0.38
VII	D-Glucuronic	3.28 ± 0.01	—
VIII	Methyl 2,3,4-tri-*O*-methyl-α-D-glucopyranosiduronic	3.03 ± 0.02	− 0.25
IX	Methyl 4-deoxy-β-L-*threo*-hex-4-enopyranosiduronic	3.10 ± 0.01	− 0.41

calculated from the constant pK values ($\alpha = 0.5$). In order to achieve maximum accuracy pK values for uronic acids as well as pH of buffers obtained from different suppliers were also determined. The found differences in pH and pK were within ± 0.01.

D-*Galacturonic acid and its O-methyl derivatives*

The pK values found for *I*—*VI* make it possible to evaluate the effect of substitution with a methoxyl group of the hydroxyl group at various sites (C-1—C-4) of the uronic acid moiety upon its dissociation constant.

It can be seen from the pK found for D-galacturonic acid (*I*) and its α-methyl glycoside *II* that the methoxyl group at C-1 has no effect upon the dissociation constant of the acid. The same holds for the methoxyl group at C-2, which is evident from the fact that the same pK value was found for the 4-*O*-methyl and 2,4-di-*O*-methyl derivative (*III* and *IV*). Taking this into account, pK of *V* characterizes the effect of the methoxyl group at C-3. The effect of the substitution is most pronounced when it is at C-4, *i.e.* at the position closest to the carboxyl group as in *III*. The lowest pK value was found for the fully methylated D-galacturonic acid derivative *VI*. The fact that the sum of Δ pK of 2,3-di-*O*-methyl and 4-*O*-methyl derivative is equal to that of ΔpK of 2,3,4-tri-*O*-methyl derivative proves the additive character of the substitution around the tetrahydropyran ring upon the acidity of D-galacturonic acid *O*-methyl derivatives. The further away from the carboxyl group is the substituent, the less pronounced is this effect. For the substitution on the individual carbon atoms it holds

C-X	C-1	C-2	C-3	C-4
ΔpK	0.00	0.00	− 0.09	− 0.29

The observed increased acidity of D-galacturonic acid derivatives as a result of the methylation of the hydroxyl group located close to the carboxyl group is in agreement with the general rules of organic chemistry. For instance, pK_a (25°C) 3.90 and 3.48 [16] are reported for glycolic acid and its *O*-methyl derivative (methoxyacetic acid), respectively. Since the negative inductive effects ($-I_s$) of a hydroxyl and methoxyl group are very similar [17] the overall increased acidity of *O*-methyl D-galacturonic acid derivatives must be caused also by other factors resulting from the alkylation.

Methyl 4-deoxy-β-L-threo-hex-4-enopyranosiduronic acid

The double bond in the neighbourhood of the carboxyl group increases the acidity of the substance, compared to the acidity of the parent uronic acid ($\Delta pK = -0.41$), as a result of higher electronegativity of the sp^2 hybridized carbon atoms. The negative inductive effect of the enolacetal bond exceeds its mesomeric effect which, in turn, hinders the splitting off of a proton from the carboxylic function. Another cause of the higher acidity is the stabilization of the formed anion by mesomeric effect [18]. For comparison, pK_a values (25°C) found for propionic and acrylic acid are 4.88 and 4.26, respectively [18].

D-*Glucuronic acid and its 2,3,4-tri-O-methyl derivative*

It is known that D-glucuronic acid is a stronger acid than is D-galacturonic acid. The dissociation constants reported by various authors for these two uronic acids differ a little, since different experimental conditions have been used for the determination. The following p*K* values for D-glucuronic acid can be found in the literature: 3.33 [19], 3.20 [21], 3.24 (25°C) [14], 3.23 (25°C) [23]; the value determined during this work: 3.28 ± 0.01 (20°C) (Table 1). For D-galacturonic acid the following p*K* have been determined: 3.42 (27°C) [20], 3.42 [21], 3.49 (23.6°C) [22], 3.47 (25°C) [23]; the value found during this work: 3.51 ± 0.01 (20°C) (Table 1).

Although the p*K* values determined by various authors differ somewhat they show that D-glucuronic acid is a stronger acid than is D-galacturonic acid. The difference between the dissociation constants of the two acids found during this work ($\Delta pK = -0.23$) is in good agreement with the results obtained by *Haug* and *Larsen* ($\Delta pK = -0.22$) [21] and by *Holvik* and *Høiland* ($\Delta pK = -0.24$) [23]. Our objective was mainly to find the difference between the p*K* values of D-glucuronic and D-galacturonic acid and some derivatives thereof and to interpret them.

D-Galactopyranuronic and D-glucopyranuronic acid (in $^4C_1$ conformation) differ from each other in the orientation of the hydroxyl group at C-4. This difference may result in different nonbonding interactions (intramolecular hydrogen bonding) between this hydroxyl group, the carboxyl group, and/or the oxygen atom in the tetrahydropyran ring; in this way the dissociation of the uronic acid may be affected. The different rate of hydrolysis of α-glycosides of D-glucuronic and D-galacturonic acid has been explained in an analogous manner [1]. Similar difference in acidity has been also found for another pair of uronic acids: D-mannuronic ($^4C_1$ conformation) and L-guluronic acid ($^1C_4$ conformation). The former is the stronger acid (pK 3.38 [21]) of the two and has, similarly as D-glucuronic acid, the HO-4 equatorially oriented and *trans* towards the carboxyl group. L-Guluronic acid (pK 3.65 [21]) has HO-4 axially oriented and *cis* towards the acid function.

In order to eliminate the effect of hydrogen bonding upon the dissociation of the acids pK for the fully methylated derivatives *VI* and *VIII* have been determined. The results (Table 1) show that, although the found difference in pK is smaller (Δp$K = -0.10$), the D-glucuronic acid derivative still shows stronger acidity than does the D-*galacto* isomer.

The possibility for nonbonding interactions was examined also by means of Courtauld models. Although they showed several possibilities for intramolecular hydrogen bonding no unambiguous conclusion, which would be in accordance with the rules of dissociation of other organic acids, could be made. We assume therefore, that the difference in the dissociation constants of D-galacturonic and D-glucuronic acid is mainly owing to orbital interactions in the system of atoms C-6—C-4. The more pronounced tendency of D-galacturonic acid derivatives to undergo β-elimination reactions, compared to the D-*gluco* analogues, was explained in a similar manner [11]. Further work, including quantum chemical calculations, is needed in order to make the final conclusion about the complex factors causing different acidity of the two isomeric uronic acids.

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