A Peptide–Sugar Crystalline Complex containing a Very Strong Hydrogen Bond

Robert O. Gould, Paul Taylor, Katherine M. Varvill, and Malcolm D. Walkinshaw*†

Department of Chemistry, University of Edinburgh, Edinburgh EH9 3JJ, Scotland, U.K.

The *X*-ray crystal structure of a complex of galacturonic acid and tryptophan methyl ester shows a laminar packing arrangement in which hydrophobic bilayers of amino acid sandwich hexapyranose monolayers; the lattice allows 50 : 50 occupancy of α- and β-galactopyranose anomers and dimer pairs of galacturonic acid molecules are formed by means of a very strong hydrogen bond of 2.43 Å between the hemiprotonated carboxy groups.

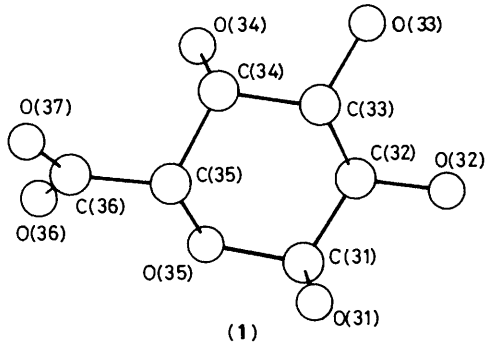
Hexapyranose residues are known to act as recognition sites on cell surfaces.[1] Sugar binding proteins also serve as receptors which regulate bacterial chemotaxis.[2] Such proteins are essential components of a number of active transport systems.[3] The crystal structure of the galacturonic acid, GalA (**1**), and tryptophan methyl ester, TryOMe (**2**), complex, provides a unique high resolution model to examine the interaction between sugars and peptides.

Crystals were prepared by slow evaporation of an aqueous mixture of TryOMe·HCl and GalA. Thin lamellar crystals (m.p. 139 °C, with decomposition) diffracted *X*-rays well.‡ Crystallographic refinement shows that the stoicheiometric composition is:

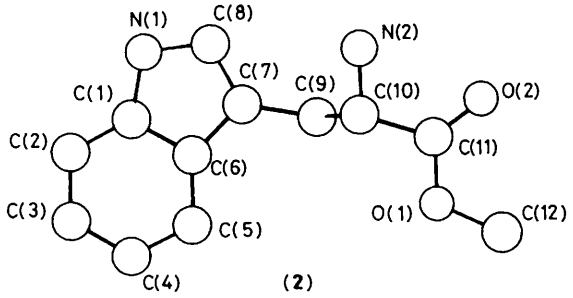
$$4\text{TryOMe}\cdot 1\alpha\text{-GalA}\cdot 1\beta\text{-GalA}\cdot 3\text{HCl}\cdot 2\text{H}_2\text{O}.$$

On drying, the lamellar plates deformed into saddle or cylindrical shapes.

Bond lengths and angles of TryOMe are similar to those in related structures.[4] The two crystallographically independent molecules also adopt similar conformations: (molecule 1, molecule 2) C(12)–O(1)–C(11)–O(2) = 1.3°, 1.0°; O(1)–C(11)–C(10)–C(9) = −61.9°, −63.2°; N(2)–C(10)–C(9)–C(7) = 62.4°, 53.6°. As in all aromatic peptide structures, the χ_2 angle [C(10)–C(9)–C(7)–C(8) = −81.2°, −91.2°] does not deviate far from 90°. The galacturonic molecule adopts a standard chair conformation with bond lengths and angles in the expected ranges. The conformation of the carboxy group is such that O(37) is *cis* to the ring oxygen O(35) [O(37)–C(36)–C(35)–O(35) = 0.1°].



(1)



(2)

† *Present address:* Preclinical Research, Pharmaceutical Division, Sandoz AG, CH-4002 Basel, Switzerland.

‡ *Crystal data:* $2(C_6H_{10}O_7)\cdot 4(C_{12}H_{14}N_2O_2)\cdot 3HCl\cdot 2H_2O$, M = 1406.6, monoclinic, a = 18.903(6), b = 9.549(3), c = 20.810(7) Å, β = 115.82(2)°, U = 3381 Å^3, Z = 2, D_c = 1.38 g cm^{-3}, space group $C2$. Structure solved[7] using 2499 independent data with $I > 2\sigma(I)$ and refined[8] to R = 0.063 for 461 parameters. Atomic co-ordinates, bond lengths and angles, and thermal parameters have been deposited at the Cambridge Crystallographic Data Centre. See Notice to Authors, Issue No. 1, 1986.

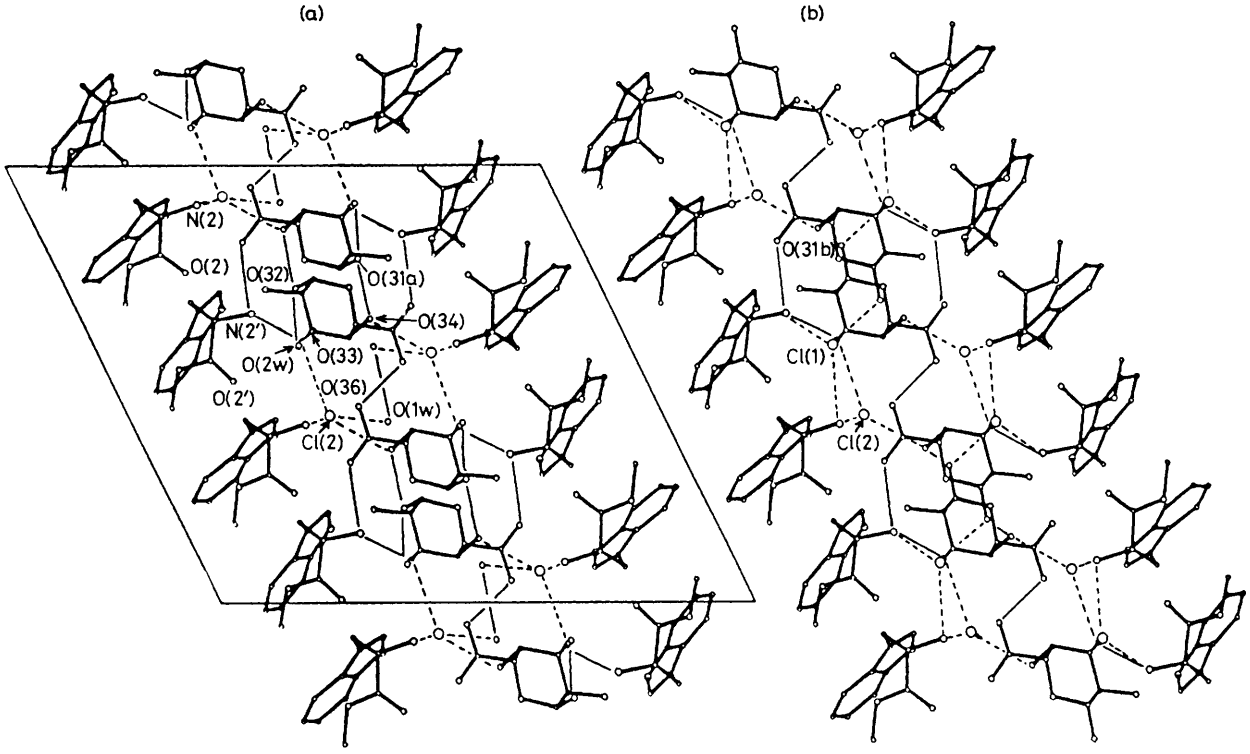


Figure 1. Projection of the two possible structures along [010]. Large circles represent chloride ions. Hydrogen bonds are indicated: ——, for all O · · · N and O · · · O less than 3.0 Å and ---- for all Cl · · · O and Cl · · · N less than 3.3 Å. (a) α-Galacturonate layer, water rich. (b) β-Galacturonate layer, chloride rich.

Table 1. Intermolecular contacts (Å).

α and β structures		β structure only		α structure only	
O(36) · · · O(36)	2.431	O(31b) · · · O(33)	2.984	O(31a) · · · O(34)	2.814
O(32) · · · O(37)	3.080	O(31b) · · · O(34)	2.789	O(31a) · · · N(1')	2.985
O(2) · · · O(32)	3.170			O(31a) · · · O(2W)	2.901
N(2') · · · O(33)	2.754				
N(2') · · · O(37)	2.872				
N(2') · · · O(35)	2.958				
N(2) · · · O(37)	3.024				
N(1') · · · O(32)	3.065				
N(2') · · · O(32)	3.174				
Cl(2) · · · N(1)	3.239	Cl(1) · · · N(2)	3.073	Cl(2) · · · O(1W)	3.362
Cl(2) · · · H(1N)	2.179	Cl(1) · · · H(11N)	2.086	O(2W) · · · N(2)	3.032
Cl(2) · · · N(2)	3.230	Cl(1) · · · N(2')	3.239	O(2W) · · · N(2')	3.085
Cl(2) · · · H(12N)	2.327	Cl(1) · · · H(22N)	2.194	O(2W) · · · O(1W)	3.056
Cl(2) · · · O(33)	3.127	Cl(1) · · · O(31b)	3.289	O(1W) · · · O(1W)	2.969
Cl(2) · · · H(33O)	2.249	Cl(1) · · · O(2')	3.791	O(1W) · · · O(36)	3.126
Cl(2) · · · O(34)	3.040				
Cl(2) · · · H(34O)	2.308				

The crystal stoicheiometry is consistent with two mutually exclusive but equally probable arrangements: either α-GalA [with O(1) axial], one chloride ion [Cl(2)], and two water molecules [O(1W), O(2W)] (Figure 1a), or, β-GalA [with O(1) equatorial], two chloride ions, and no water (Figure 1b).

Both arrangements show the structure composed of bilayer sheets of TryOMe which sandwich the hydrophilic chloride, water, and sugar monolayer. The lamellar crystal shape indicates that the direction of slowest growth [001] is perpendicular to the sheets.

The sugar monolayer is held together by three sugar–sugar hydrogen bonds, the most important being the short [O(36) · · · O(36) = 2.431 Å] carboxy · · · carboxy interaction.[5] The positive charge of the two fully protonated amine groups is balanced by 1.5 chloride ions and the hemiprotonated carboxy group. Thus, rather than the expected salt bridge between the acid carboxy function and protonated amines, as found in other peptide complexes,[6] the strongest interaction energy is from hydrogen bonded sugar dimers. Other so called 'very strong hydrogen bonds' have O · · · O distances as short as 2.41 Å.

There are seven direct sugar–peptide hydrogen bonds common to both the α and β structures, most of which involve the protonated amine groups (Table 1). The strongest interactions between the sugar monolayer and the peptide bilayer are, however, likely to be those involving chloride

co-ordination. The fully occupied Cl(2) ion is four co-ordinate with approximately tetrahedral geometry and bridges two oxygen ligands from two different galacturonate molecules, and two nitrogen ligands from two different peptide molecules. As with the half occupied chloride [Cl(1)], both the indole nitrogen and the amine nitrogen are involved in co-ordination.

It is reasonable to suggest that the interactions found in this structure, particularly the involvement of bridging chloride ions, may be used as a model for the specific interactions between sugars and peptides occurring in biological systems.

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